ENVIRONMENTAL CONTAMINATION FROM

CLANDESTINE DRUG MANUFACTURE

By

AUSTIN LEE CIESIELSKI

Bachelor of Arts in Chemistry and Biochemistry
Coe College
Cedar Rapids, Iowa
2015

Master of Science in Forensic Science
Oklahoma State University
Tulsa, OK
2017

ENVIRONMENTAL CONTAMINATION FROM

CLANDESTINE DRUG MANUFACTURE

Dissertation Approved:

Jarrad R. Wagner, Ph.D., F-ABFT

Dissertation Adviser

David Wallace, Ph.D.

Ronald Thrasher, Ph.D.

Lara K. Maxwell, DVM, Ph.D., DACVCP

ACKNOWLEDGEMENTS

While many people have helped me along my academic journey, I would like to give my thanks to a few of those who have devoted significant portions of time to help me learn and be a successful researcher. First, my advisor, Dr. Jarrad Wagner, who has not only assisted in the lab, helping me work through complex extractions or syntheses, but who has also provided support to me in my personnel life. I would also like to thank my committee members. Dr. David Wallace has been a great resource for talking through experimental designs that can be supported by statistical analyses. Dr. Ronald Thrasher has been a continued source of comedic relief and emotional support during the stress-filled process of becoming a doctoral candidate and defending my work. Dr. Lara Maxwell not only presented me with questions that challenged me to apply all my knowledge, thus making me critically think about my research, but she also allowed me to enter the Veterinary Biomedical Sciences (VBSC) program and was flexible with me as I took forensic science-based course work in anticipation for the start of the Forensic Science Ph.D. program. To all of my committee members, I would like to thank you for your great flexibility as I transferred from the VBSC program, to the Forensic Sciences program. Being the first person to pass through this program, there were many unknowns we had to face together, all of which were further complicated by the COVID-19 pandemic. Lastly, I would like to thank all those from CDC-NIOSH who have helped me throughout my research. Drs. John Snawder, Marissa Alexander-Scott, and Jerry Smith all provided assistance in collection and analysis of the One Pot surface and ambient air samples. When I became a CDC-NIOSH fellow, Drs. John Snawder and Marissa Alexander-Scott provided me with continued learning opportunities during my travels to the CDC-NIOSH Taft Laboratory in Cincinnati, OH. To everyone who has helped me along this journey, I would like to say thank you and your assistance was greatly appreciated.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: AUSTIN LEE CIESIELSKI

Date of Degree: MAY, 2020

Title of Study: ENVIRONMENTAL CONTAMINATION FROM CLANDESTINE
        DRUG MANUFACTURE

Major Field: FORENSIC SCIENCE

Abstract: Drug abuse and drug seizures are on the rise across the United States. As drug prevalence increases, the public is more likely to come into contact with drugs, drug-contaminated locations, or sites of drug production. These exposures can lead to adverse health effects. To assess the severity of health effects stemming from sites of drug use and production, the compounds contaminating these locations need to be identified and quantitated. This research sought to assess the identity and amount of environmental contamination generated by sites of drug production. To accomplish this, wastewater, ambient air, and surfaces were all tested for contaminants generated by One Pot methamphetamine labs. Methods were also developed to examine contaminants generated by fentanyl and fentanyl analog synthesis and tablet formulation. With the amount of environmental contamination generated by One Pot methamphetamine labs better understood, more educated risk assessments can be developed regarding the safety and well-being of those living within or near former and current One Pot methamphetamine labs. Additionally, the development of methods to determine surface contamination within sites of fentanyl synthesis and tableting will assist in the development of effective decontamination procedures and will provide information regarding the level of hazards present within these sites.

TABLE OF CONTENTS

Chapter   Page

TABLE OF CONTENTS

Chapter   Page

LIST OF TABLES

Table   Page

LIST OF TABLES

Table   Page

LIST OF FIGURES

Figure   Page

LIST OF FIGURES

Figure   Page

LIST OF FIGURES

Figure   Page

CHAPTER I

INTRODUCTION

Abuse of illicit drugs is a growing problem in the United States. In 2015, an estimated

10.1% (27.1 million people) of the population ages 12 and older reported abusing illicit drugs,

while in 2017, this number grew to 11.2% (30.5 million people).[1,2] Along with this increase in

drug abuse, drug seizures have also been on the rise, particularly for the stimulant

methamphetamine and the synthetic opioid fentanyl. In 2015, the National Forensic Laboratory

Information System (NFLIS), an information gathering entity for the United States Drug

Enforcement Administration (DEA), reported methamphetamine seizures to account for 17.61%

of drug seizures reported while fentanyl accounted 0.91%; in 2017, these numbers grew to

21.99% and 3.57%, respectively.[3,4]

As drug abuse and seizures rise, the chance of people coming in contact with illicit drugs,

drug-contaminated materials or locations, and/or hazardous waste associated with drugs and their

production increases. Exposures of this type can lead to adverse health effects for those who

encounter them, the severity of which is dependent on the type and length of exposure. In the

case of methamphetamine, which is the most commonly produced illicit drug in the United States,

exposure to contaminated locations may put people in contact with not only the drug itself, but

also with a wide range of volatile organic compounds (VOCs), acids, bases, and reactive metals,

depending on the production method used.[5] These contaminants can be introduced to the

environment in a number of ways: illegal dumping into water sources, volatilization leading to air

contamination, and settling of contaminants from the air resulting in surface contamination. Because

contaminants can be introduced to the environment in so many different ways, several methods must

be developed to identify what contaminants are present in different environmental medias (i.e. water,

air, and surfaces) and at what concentration these contaminants are present. Without such methods,

people may unknowingly be exposed to contaminants stemming from methamphetamine production

and waste, which can lead to wide range of adverse health effects. Health effects from acute

exposures to methamphetamine lab-related contaminants can range widely, from minor irritation

resulting from dermal exposure to dilute hydrogen chloride gas to respiratory failure and death

resulting from the inhalation of a high concentration of ammonia gas.[6,7] Health effects from chronic

exposure to methamphetamine lab contaminants are unknown, though an increased chance of cancer

development is suspected.[8]

　　　　While sites of methamphetamine production pose major health risks to those who encounter

or live near them, fentanyl production labs have not yet been observed in the United States, with the

drug being mostly manufactured and then smuggled into the country from China and Mexico.[5] Even

though production sites are not yet of concern in the United States, fentanyl itself poses a significant

health risk due to its high potency. A lethal dose of fentanyl in humans has been determined to be 2

mg, which is approximately the size of the date stamped on a penny (Figure 1).[9,10] With such a small

amount of drug being potentially fatal, the ability to identify environmental contamination stemming

from fentanyl before people come in contact with it is critical. Not only is identification crucial, but

the ability to detect the drug at low concentrations is necessitated due to its high potency. This task is

made further arduous by the number of fentanyl analogs (fentalogs) being used illicitly by people who

are trying to circumvent law enforcement and find unscheduled substances that provide "highs"

similar to those obtained through the use of other opioids. Many of these fentalogs have a similar

chemical structure to fentanyl, but are slightly modified by the addition, subtraction, or substitution of

functional groups. These alterations can drastically alter the potency of the fentalogs, with some

having lower potencies than fentanyl (butyrylfentanyl, 0.3x the potency) and some having higher

potencies than fentanyl (carfentanil, 100x the potency).[11] Since environmental contamination

resulting from any of these fentalogs can have lethal ramifications, a method is needed to identify as

many of these analogs as possible at concentrations below those deemed safe for opioid-naïve

individuals.

Figure 1. A lethal dose (2 mg) of fentanyl powder, which is comparable in size to the date stamped on a penny. Image taken from the DEA.[10]

As outlined above, environmental contamination from methamphetamine production and

waste sites, as well as from fentanyl-contaminated sites can have major adverse health consequences.

Methods need to be developed to identify these compounds from the different environmental matrices

they may contaminate, such as water, air, and surfaces. Not only do methods need to identify what

contaminants are present in these matrices, but they need to be able to quantitate how much of each

contaminant is present in order estimate how hazardous a contaminated site may be. The studies laid

out in this dissertation aim to fulfill these needs, with an overarching hypothesis that environmental

hazards associated with clandestine drug production and use can be identified and quantitated by

analysis of adjacent contaminated water sources, ambient air, and household surfaces. In the first

study, wastewater was collected and analyzed for the presence of byproducts from One Pot methamphetamine labs. This study built upon a previous proof-of-concept study that showed the potential of wastewater as a means to identify One Pot methamphetamine waste (Green MK, Ciesielski AL, Wagner JR, Unpublished data, February 2020) and applied that work to a real-world setting. In the second study, ambient air was collected from One Pot methamphetamine labs in order to determine the identity and concentration of volatile compounds contaminating the air within these locations. This study then performed stand-off detection to assess how far these air contaminants could be observed in measurable amounts, therefore determining a contamination zone associated with One Pot methamphetamine production. In the third study, the amount of surface contamination resulting from One Pot methamphetamine production was assessed, as well as the effectiveness of a simple water decontamination to remediate these production sites. In the fourth study, the initial method development was performed to optimize a surface swab technique to capture fentanyl and fentalogs from surfaces and quantitate them at levels as low as or lower than expected remediation values. This method was then applied to multiple surface materials commonly found in households to determine the efficiency of the method at capturing the drugs from these different surface materials. The results of these four studies will advance the detection capability for contaminants stemming from clandestine drug manufacture and use. These results will also provide the groundwork for decontamination studies that look to remediate locations of drug production and use by providing the identities and concentrations of contaminants associated with One Pot methamphetamine labs and sites of fentanyl use, as well as the analytical methods with which to make these determinations. With this information, public health officials will be better able to make policies to enhance the safety of people living in or near sites of drug use and production, as well as set realistic and achievable remediation levels for the decontamination and re-inhabitance of drug-contaminated sites.

CHAPTER II

DETECTION OF ONE POT METHAMPHETAMINE LABORATORIES
VIA WASTEWATER SAMPLING

2.1 Introduction

The illicit production and use of methamphetamine is a problem that the United States

has been combating for over 50 years.[12] As new legislation has been adopted to prevent

methamphetamine production and hinder its availability for use, methamphetamine producers

have developed new methods of production to circumvent existing legislation. According to the

DEA, the current favorite method of methamphetamine production throughout the United States

is the One Pot method, which accounted for 86% of all clandestine methamphetamine laboratory

seizures in 2016.[13]

The One Pot method is a variation of older lithium-ammonia reduction methods that

simplifies methamphetamine production to a single reaction vessel, which is commonly a plastic

bottle. Lithium-ammonia reduction methods of methamphetamine production, such as the One

Pot method, use lithium as an electron source to reduce the hydroxyl group on pseudoephedrine

or ephedrine, forming methamphetamine. Ammonia acts as a solvent for the electrons, carrying

them to the pseudoephedrine molecules.[14] While older lithium-ammonia reduction methods used

liquid anhydrous ammonia to carry the electrons, in the One Pot method, ammonia gas is

generated *in situ* by combining sodium hydroxide and ammonium nitrate.

One reason the One Pot method has become the favorite method for methamphetamine production is its simplicity. To make a One Pot methamphetamine lab, the chemicals previously described can simply be added to a plastic bottle with camp fuel, diethyl ether, or some other organic solvent and then allowed to react or "cook"; little chemistry knowledge is required to produce the methamphetamine. Additionally, the materials used to perform this process can be easily purchased at convenient stores without raising the suspicion of law enforcement that illicit methamphetamine production is occurring. Upon completion of the One Pot methamphetamine cook, the producer is left with solid waste, liquid waste, and the desired methamphetamine powder. The solid and liquid waste may be disposed of by throwing it in municipal trash, burning it, or dumping it down a drain.

In the United States, 76% of residencies are connected to public wastewater systems.[15] If waste from a clandestine One Pot methamphetamine lab is dumped down a drain in a residence, it will enter the wastewater system and may then be collected and analyzed for chemical markers unique to One Pot methamphetamine production without the necessity of obtaining a search warrant.[16] Such analyses were proven successful by the Oklahoma State University Forensic Toxicology and Trace Laboratory (OSU-FTTL) during a proof-of-concept study funded by the National Institute of Justice (NIJ) during FY16 (Green MK, Ciesielski AL, Wagner JR, Unpublished data, February 2020). This study seeks to build upon that proof-of-concept study by increasing the sensitivity of the methods developed in the 2016 study and then applying them to field applications. To do this, wastewater samples were collected from municipalities in South Carolina, Georgia and Oklahoma, and were analyzed via solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the presence of waste products from One Pot methamphetamine labs. These waste products include: methamphetamine, pseudoephedrine/ephedrine, and an over-reduced methamphetamine byproduct known as 1-(1,4-cyclohexadienyl)-2-methylaminopropane (CMP). The major

methamphetamine metabolite amphetamine was also monitored to assist in differentiating methamphetamine production from methamphetamine use.

The aim of this study is to assess the capability of wastewater analysis in determining areas where One Pot methamphetamine waste is being dumped into the public wastewater system. Additionally, this research seeks to differentiate methamphetamine and pseudoephedrine found in wastewater as being from One Pot methamphetamine waste or from an individual who is using methamphetamine and then excreting into the public wastewater system. Completion of this work will provide law enforcement an additional tool to proactively identify and seize One Pot methamphetamine labs while also providing environmental protection agencies the ability to assess the amount of methamphetamine lab-related chemicals present in wastewater so they can perform appropriate treatment processes and ensure the removal of these chemicals before releasing treated wastewater back into the environment.

2.2 Review of the Literature

Wastewater epidemiology is the use of wastewater as a means to anonymously obtain information about a select population through analytical measurements of biomarkers found in the wastewater.[17] First introduced in 2001 by Christian Daughton, wastewater epidemiology is based on the idea that biomarkers related to a person's health and product use are excreted from the body as people use the bathroom.[18] After being excreted from the body, these biomarkers are introduced to a wastewater system, where they can be captured during collection of a water sample and analyzed for in a lab. The results of these analyses can give researchers an idea about such things as diseases circulating in a community based on observations of specific virus strains in the wastewater, the frequency of diet choices based on food-specific biomarkers such as phytoestrogens formed with a plant-based diet, or the amount of drug use occurring in city based on the presence of drugs and their metabolites.[19–21]

When monitoring drug use in a given population, wastewater epidemiologists monitor wastewater for not only the drug of interest, but also its metabolites. This assists in differentiating drug use from drug disposal, as the later situation would not have metabolites present in the sample. For example, if a wastewater epidemiologist was monitoring methamphetamine use in a population, they would test the wastewater samples that were collected for the presence of methamphetamine and amphetamine, as a dose of methamphetamine is excreted as approximately 50% unchanged drug and 10-20% as the demethylated metabolite amphetamine; these percentages can change based on the pH of the urine, with lower pH values resulting in higher unchanged-drug excretion percentages, but rarely is amphetamine completely absent from urine following methamphetamine use.[22,23] If a wastewater sample is found to have a high methamphetamine concentration and no amphetamine present, this is suggestive that the methamphetamine present is due to dumping of the drug into the wastewater system, and not from use within the population.

This idea of drug dumping can be further supported if byproducts formed during methamphetamine production are monitored for during analysis, as all routes of methamphetamine production have their own unique set of byproducts present in the powdered drug and in the waste generated during production.[24–26] The DEA currently uses these route-specific byproducts to determine how seized methamphetamine samples are produced, but this type of approach has not been applied to wastewater epidemiology work.[27] A proof-of-concept study was previously performed to show the feasibility of such work, but the idea of using methamphetamine-specific byproducts to assess the amount of production occurring in a population has yet to be used in an application-type study (Green MK, Ciesielski AL, Wagner JR, Unpublished data, February 2020).

In the United States, the most common route of methamphetamine production is the One Pot method.[5] This route of methamphetamine production has several characteristic byproducts, including unreduced ephedrine or pseudoephedrine, 1,2-dimethyl-3-phenylaziridine, and 1-(1,4-

cyclohexadienyl)-2-methylaminopropane (CMP).[28] In addition to these route-specific

byproducts, One Pot methamphetamine "cooks" as they are known, also have many other

byproducts that may be hazardous to human health, such as organic solvents, sodium hydroxide,

lithium hydroxide, ammonia gas, sulfuric acid, hydrogen chloride, and lithium metal. These

byproducts can be abundant in One Pot methamphetamine labs, with some reports claiming that

for every pound of methamphetamine produced via a One Pot cook, 5-7 pounds of waste is also

generated.[29] In order to prevent detection of their clandestine operations, methamphetamine

manufacturers dispose of this lab waste in any way they can, including throwing in the trash,

burning it, or dumping it into the wastewater system.

　　　If One Pot byproducts and waste materials are dumped into the wastewater system, they

can lead to many adverse health effects. The organic solvents associated with One Pot

methamphetamine labs are commonly camp fuels (white gas or light petroleum distillate) and/or

diethyl ether. Camp fuel is made up of many different cyclic and straight chained hydrocarbons,

generally ranging in size $C_5 - C_9$.[30] When introduced to the wastewater system, hydrocarbons are

more resilient than other organic carbons, such as fats, that may be present, which means

microbes present in the wastewater system may not be able to degrade them before the

hydrocarbons reach a wastewater treatment plant (WWTP).[31] If the hydrocarbons do reach a

WWTP, they may be subjected to a chlorination process, which is commonly used to disinfect

wastewater prior to it being released back into the environment. Chlorination of these

hydrocarbons can lead to the formation of chloroalkanes, many of which are not readily

decomposed in nature, can bioaccumulate in exposed wildlife, and are potentially carcinogenic to

humans.[32–34] As shown in Figure 2, if these chloroalkanes are released into the environment

following wastewater treatment, they can lead to multiple routes of exposure for humans,

including inhalational exposures if they volatilize, oral exposures if they enter the ground water

and make it into a drinking water source, and dermal exposure if they enter a water source used

for washing.[35]
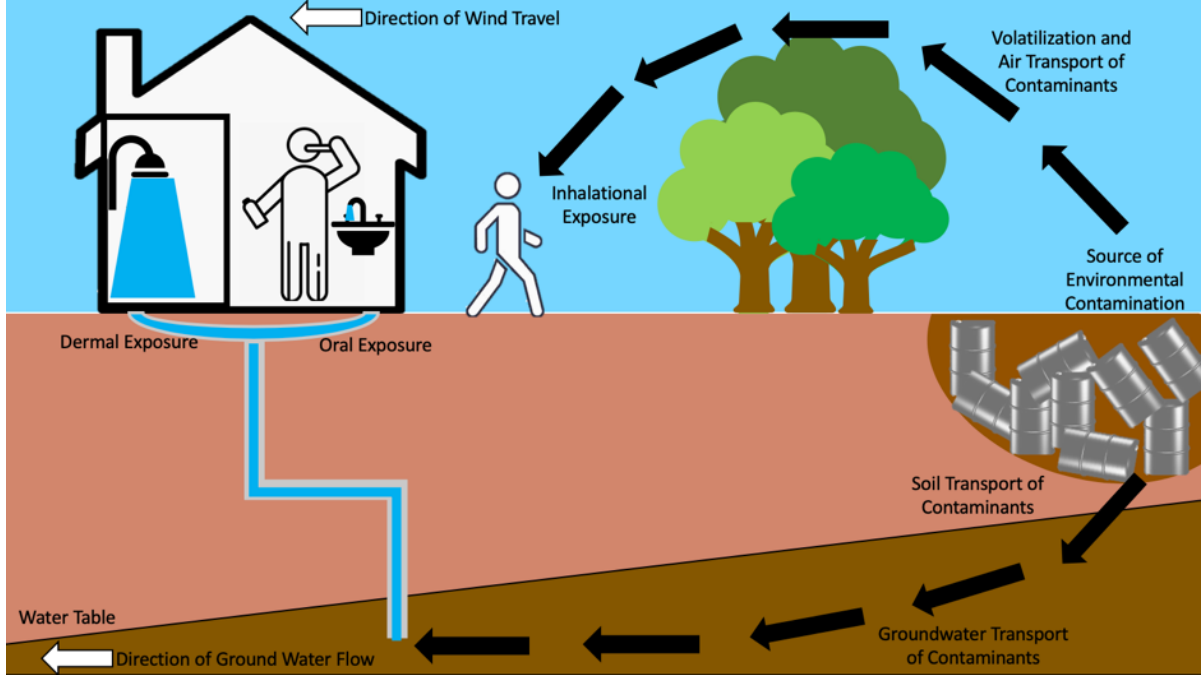
Figure 2. If solvents dumped into the wastewater system are released to the environment after treatment, they can lead to inhalational, oral, and dermal exposures. Image recreated based on Casarett & Doull's Toxicology: The Basic Science of Poisons.[35]

In addition to hydrocarbons, a large portion of the waste from One Pot methamphetamine cooks stems from the sludge, which is the solid waste formed at the bottom of a cook. The sludge is made up of unreacted sodium hydroxide and ammonium nitrate, as well as sodium nitrate that forms due to the chemical reaction between these two compounds (Equation 1) and lithium hydroxide that forms during the breakdown of lithium. Sodium hydroxide, lithium hydroxide, and ammonia are all alkaline by nature and can lead to health complications to those who come in contact with them. Sodium hydroxide, commonly referred to as lye, and lithium hydroxide are caustic chemicals that can lead to tissue damage if people come in contact with them.[36] Repeated introduction of sodium hydroxide and lithium hydroxide to drain pipes can cause damage to occur to wastewater piping through corrosion and from heat that occurs when sodium hydroxide comes into contact with water, which can soften PVC pipes.[37] Ammonia as a gas can cause swelling and

fluids to accumulate in the respiratory tract, leading to difficulty breathing, and can causes

olfactory fatigue, causing individuals to be unable to smell the chemical, putting them at greater

risk for unknowingly being exposed to hazardous levels of the gas. Additionally, ammonia

readily dissolves in water, forming ammonium hydroxide, which can damage drain pipes and is

only efficiently removed by tertiary wastewater treatment processes, which aren't employed by

all WWTP.[7,38] Dissolved ammonia also increases the oxygen demand on the wastewater system's

microbiome, which oxidize ammonia to nitrate (Equation 2).[39,40]

Equation 1. Balanced chemical reaction between ammonium nitrate and sodium hydroxide, forming sodium nitrate, ammonia, and water.

$$NH_4NO_3 + NaOH \rightarrow NaNO_3 + NH_3 + H_2O$$
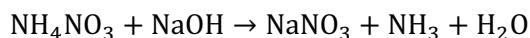
Equation 2. Aerobic conversion of ammonia to nitrate in the wastewater system

$$2\,NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$
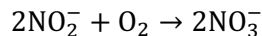
The reduction of dissolved oxygen in wastewater that can occur due to the introduction of

ammonia from One Pot waste can facilitate the growth of additional anaerobic microbes. Some

of these anaerobic microbes may obtain energy through the reduction of sulfur-containing

compounds, thus leading to the production of another harmful compound, hydrogen sulfide.

Hydrogen sulfide is a flammable, color-less gas that smells like rotten eggs. While hydrogen

sulfide can be detected in the air by the human nose at concentrations between 0.0005-0.3 parts

per million (ppm), the gas can quickly lead to olfactory fatigue and prevent people from

identifying its presence.[41,42] Hydrogen sulfide is commonly associated with WWTP, and is found

in most wastewater at levels of 1-5 ppm, with the air directly above the wastewater having higher

concentrations due to the volatility of hydrogen sulfide.[43] The Center for Disease Control and

Prevention's National Institute for Occupational Safety and Health (CDC-NIOSH) has

determined the relative exposure limit (REL) for hydrogen sulfide to be 10 ppm with a 10-minute ceiling.[42] This means workers should not be exposed to 10 ppm hydrogen sulfide for longer than 10-minute periods of time throughout an 8-hour work day. With hydrogen sulfide levels already at 1-5 ppm in normal wastewater, the addition of ammonia from One Pot waste can lead to hydrogen sulfide levels that quickly approach the REL. Additionally, if hydrogen sulfide escapes the wastewater, it can remain in the surrounding atmosphere for as much as 42 days, leading to possible extended exposure times for those in the affected area.[43]

Another source of hydrogen sulfide gas that stems from One Pot methamphetamine lab waste is the introduction of hydrogen chloride (HCl) to the wastewater system. HCl gas, which is used to precipitate methamphetamine out of solution during the final steps of a One Pot cook, is highly soluble in water, producing hydrochloric acid. When in the presence of sulfur-containing compounds, hydrochloric acid can react and lead to the formation of hydrogen sulfide gas.[44] While the level of hydrogen sulfide gas generated due to One Pot waste will likely not result in a level near the immediately dangerous to life and health (IDLH) value of 100 ppm, it may still result in adverse health effects to those who are exposed, such as eye/nose/throat irritation, headaches, memory and balance problems, and fatigue.[42,43] Additionally, hydrogen sulfide is known to cause corrosion and damage to wastewater pipes, potentially leading to additional environmental contamination if a wastewater pipe breaks.[45]

While One Pot methamphetamine waste introduces numerous contaminants to the public wastewater system, research has not yet been done to quantitate these contaminants and track them back to their source. In order to perform such a feat, an understanding of the workings of a wastewater system is first needed. In the United States, the most common type of wastewater system is a gravity-fed system (Figure 3).[46] With a gravity-fed wastewater system, wastewater is deposited into a pipe from a residence; this pipe has a negative slope, ranging between 8-30 inches per 100 feet of pipe that allows water to flow passively downhill and assists in preventing solids from depositing within the pipe.[46] This pipe continues its downward gradient until it

reaches a WWTP or a lift station. A lift station is a concrete basin containing a pump that

captures wastewater from a gravity-pipe. When a specific volume of water is captured within the

basin, the pump activates and brings a portion of the water to another gravity-pipe that is situated

at a higher elevation, where it is discharged and once again flows passively downhill until it

reaches another lift station or a WWTP. The location and frequency of lift stations are dependent

on many factors, such as cost to bury a gravity-pipe at a given depth, the water table of a location,

and the frost line of the location.[46] Also, multiple gravity-pipes may deposit wastewater into a

single lift station and multiple gravity-pipes may combine into a single pipe upstream from a lift

station, creating a complex web of wastewater pipes that make backtracking to the source of a

contaminant difficult.
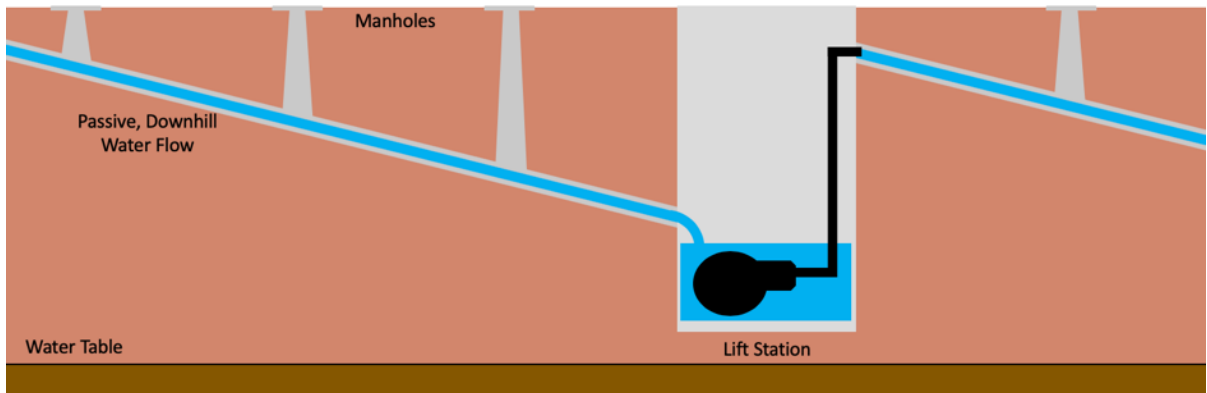
Figure 3. Gravity fed wastewater system. Water flows passively downhill until it reaches a lift station. The lift station pumps the water into another gravity-pipe at a higher elevation, where it then flows passively downhill again. This is repeated until the wastewater reaches a wastewater treatment plant. Image recreated based on the Red Run web site.[47]

Once wastewater reaches a WWTP, it must undergo treatment before it is released into

the environment. The types of treatment performed depend on multiple factors, including the

type of contaminants expected in the wastewater and the standards governing bodies have put in

place regarding cleanliness of the effluent released by the WWTP. Almost all WWTP must, at a

minimum, remove particulates, organics, and pathogenic bacteria from the wastewater, reduce

nitrates and phosphates to reasonable levels, and neutralize waste stemming from industries and

contaminants.[48] The regulations WWTPs must meet can fall under stream standards or effluent

standards. Stream standards are those that limit the amount of contaminants that can be released

into a body of water, such as dissolved oxygen, pH, contaminants, and turbidity. Effluent

standards are those that are related to the quality of water being released by the WWTP, such as

biochemical oxygen demand (BOD), suspended solids, and pH.[48] In other words, stream

standards determine the level of contaminants that are allowable in a body of water that the

WWTP releases its effluent into while effluent standards determine the level of contaminant that

are allowable in the effluent itself.

      Regardless of the standards that must be met, in the United States wastewater almost

always undergoes two levels of treatment, with some locations performing a third level of

treatment as well (Figure 4). The first level of treatment (primary treatment) removes

approximately 60% of suspended solids and 35% of BOD; BOD is a measurement of the amount

of organic matter present in the wastewater and is determined by the amount of oxygen needed by

microbes during decomposition.[49] Primary treatment is commonly comprised of screens,

grinders, grit chambers, and clarifiers. First, screens separate large, non-dissolvable materials,

such as branches or clothing, from the wastewater before it enters a grinder, or comminutor. The

grinder shreds any debris that may have passed through the screen and then the wastewater flows

into a grit chamber, where its flow rate is drastically slowed so ground debris can settle out of the

water and to the bottom of the chamber where it can be removed. After the grit chamber, the

wastewater flows to the primary clarifier, where the wastewater is held for a pre-determined

about of time. During the holding process, fats and grease float to the top of the clarifier where

they are removed with a skimmer while solids settle to the bottom and are pumped out of the

clarifier. Once the holding time has passed, the wastewater moves on to the second level of

treatment (secondary treatment).[48]
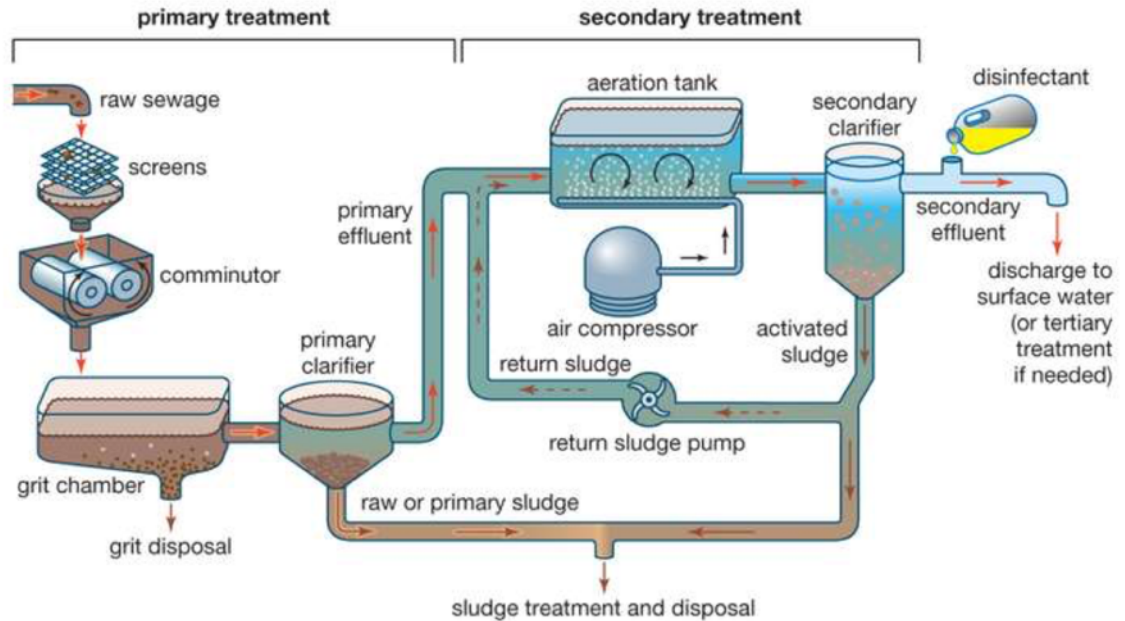
Figure 4. Schematic outlining some of the primary and secondary wastewater treatment processes that can be performed at a WWTP. Image taken from Encyclopaedia Britannica.[48]

During secondary treatment, 85% of the remaining suspended solids and BOD are removed from the wastewater, leaving less than 6% of the starting suspend solids and less than 56% of the starting BOD remaining in the wastewater. Additionally, secondary treatment removes soluble organic matter, which cannot be done during primary treatment. Much of secondary treatment relies on the use of microbes to decompose the organic matter within the wastewater, a process that is accomplished in one of four ways: trickling filter, activated sludge, oxidation pond or rotating biological contacter. In the trickling filter approach, a bed of stones inoculated with microbes is continually sprayed with wastewater. As the wastewater flows through the stones, the microbes absorb nutrients from the waste, thus decomposing the organics present in the wastewater and lowering the BOD. After flowing through the stones, the wastewater is collected in a secondary clarifier that works in the same manner as the primary clarifier; after a period of holding, the water is allowed to move on to the third step of treatment or goes to disinfection.

The second option for secondary treatment is activated sludge, which is the option shown in the schematic above (Figure 4). During activated sludge treatment, wastewater flows into an aeration tank, where it is held for a given period of time. During its retention, oxygen is bubbled into the aeration tank from the bottom, promoting microbial growth. As in trickle filtering, the microbes degrade the organics in the wastewater, which is then released to secondary clarifier.[48]

The third option for secondary treatment is an oxidation pond. To treat wastewater with an oxidation pond, the water is released into a shallow lagoon, where it is held and algae is allowed to grow on its surface. The algae use sunlight and inorganic compounds from the microbes within the lagoon to produce oxygen, which it releases into the water, thus feeding the microbes in a symbiotic relationship. After a period of holding, the water is filtered to remove the algae before it is sent to a secondary clarifier.[48]

The fourth option for secondary treatment is a rotating biological contacter. The contactor is a series of plastic disks mounted on a shaft that transverses a settlement pond. The disks are partially submerged in the settlement pond and partially exposed to the air; as the shaft turns, the disks are alternately submerged and re-exposed to the air, thus promoting microbial growth. During their periods of submersion in the wastewater, the microbes are able to collect nutrients from the dissolved organic material. They then use the oxygen they receive while exposed to the air to metabolize the organics and remove them from the wastewater. After a period of retention, the water is released to a secondary clarifier.[48]

If the environment that the treated wastewater is release into is considered vulnerable, such as an endangered ecosystem or an area such as the Gulf of Mexico where large algae blooms cause annual dead zones, a third treatment process (tertiary treatment) may be necessary before disinfection takes place. Tertiary treatment of wastewater can be any series of effluent polishing, plant nutrient removal, or land treatment and generally results in 99% removal of suspended solids and BOD. During effluent polishing, wastewater from secondary treatment is filtered through a series of sand and gravel to capture any remaining organics that may be present. This

water can also be subjected to plant nutrient removal, which consists of removal of phosphorus and nitrogen-containing compounds, thus limiting the amount of plant and algae growth that can result from water released from the WWTP. Phosphates are removed by chemical precipitation of the phosphorus-containing contaminants, while nitrogen-containing compounds must be converted to nitrates by microbes and then to nitrogen gas by a specific species of microbes added to the tertiary treatment containment tanks within the WWTP. The last type of tertiary treatment is land treatment, where secondary treatment wastewater is disinfected and then released on land, where soil, sand, clay, and gravel can act as natural filters to finish removing organic compounds before the water mixes with the groundwater. While highly effective at removing solids and BOD from water, tertiary wastewater treatment is only use when necessitated by the environment, due to its cost, which can be over double the cost of primary and secondary treatments.[48]

The final step in a WWTP before the water can be released to the environment is disinfection. Disinfection is used to destroy pathogens and protect the health of humans and animals that may be exposed to the treated wastewater. To disinfect the wastewater, it is mixed with chlorine gas or sodium hypochlorite for a given period of time. Another, newer technique for disinfection is through the use of ultraviolet (UV) radiation, where the treated water is forced through a small pipe with windows that contain UV lights at wavelength of 185-254 nm.[50] The UV lights have enough energy to cause mutations in pathogenic DNA, leading the pathogens to be unable to perform their necessary cellular functions and/or replication and leading to death. UV radiation has the advantage of not adding chemicals to the water that is about to be released into the environment, thus lowering the amount of contaminants released with the treated water.

While wastewater treatment does remove much of the organic content from wastewater, concern has been raised about the effectiveness of WWTP to remove pharmaceuticals and illicit drugs from the wastewater before its release into the environment. Several studies have followed wastewater through a WWTP and analyzed for the presence of prescription and over-the-counter pharmaceuticals, as well as illicit drugs and their metabolites. Many of the drugs tested for were

still observed in the wastewater effluent following treatment. Additionally, almost all the drugs observed in the wastewater effluent were also observed in the river downstream from where the wastewater was discharged.[51,52] One research group went a step further and tested the river water supplying a Spanish town with drinking water. Not only did they find illicit drugs and/or their metabolites in the river water supplying the drinking water treatment plant, but they were able to observe drugs following each treatment step used by the drinking water plant. Their findings included amphetamine-type compounds, with the exception of methylenedioxymethamphetamine (MDMA), being removed by the pre-chlorination and sand filtration step, cocaine being completely removed by the activated carbon filtration step, MDMA being removed post-chlorination, and the benzoylecgonine remaining detectable in the final drinking water at levels between 45-130 ng/L.[53]

Better treatment techniques are needed to assist in the removal of drugs and metabolites from wastewater and from drinking water, however, another approach to this issue would be to focus on reducing the amount of drugs being introduced into the wastewater system. One way to accomplish this would be to follow wastewater contamination upstream to its approximate source, where law enforcement could then use classical investigative techniques, such as observations and trash-pulls, to determine the exact location of the contamination. While there is no reasonable expectation of privacy once something is dumped into the wastewater system, and thus no search warrant is needed to collect and analyze such a sample, some groups, such as the Sewage Analysis CORe group Europe (SCORE) have developed ethical guidelines to discourage groups from performing such tactics.[16,54] These ethical guidelines were established to prevent research groups from abusing the data they collected from wastewater and singling out individual cities or even neighborhoods about the increased drug usage observed there. While in agreement with this ethical approach, this research doesn't aim to single out individuals or groups of people, but rather simply identify locations where drug production is occurring in an attempt to minimize the amount of contaminants released into the environment.

Therefore, the focus of this research was to apply a previously developed, proof-of-concept study to a real world application. Wastewater samples were collected from various municipalities in Oklahoma, South Carolina, and Georgia and analyzed with liquid chromatography tandem mass spectrometry (LC-MS/MS) for the presence of methamphetamine, pseudoephedrine, amphetamine, and the over-reduced byproduct from One Pot methamphetamine cooks known as 1-(1,4-cyclohexadienyl)-2-methylaminopropane (CMP). This research aimed to use data collected from these samples to identify locations where One Pot methamphetamine labs were being actively produced and their waste was being dumped into the public wastewater system to assist law enforcement on where to focus their anti-drug efforts, as well as inform environmental protection agencies as the amount of methamphetamine-related waste being introduced to the public wastewater system.

2.3 Methodology
2.3.1 Reagents and materials

All reagents and materials except Nanopure^{TM} water were purchased from commercial suppliers; Nanopure^{TM} water was obtained through the use of a Barnstead^{TM} Nanopure^{TM} Diamond laboratory water system (Thermo Scientific, Waltham, MA). Hydrochloric acid (37%) was purchased from VWR Analytical (VWR, Sugar Land, TX). Ammonium formate was purchased from Alfa Aesar (Alfa Aesar, Ward Hill, MA). Formic acid was purchased from EDM (EDM Millipore Corp, Billerica, MA). LC-MS grade methanol was purchased from JT Baker$^{®}$ (Avantor Performance Materials Inc., Center Valley, PA). Ammonium hydroxide was purchased from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA).

Amphetamine, Amphetamine-d$_6$, Methamphetamine, Methamphetamine-d$_5$, and 1S,2S(+)-Pseudoephedrine standards were all purchased at a concentration of 1 mg/mL in methanol from Cerilliant (Cerilliant Corp, Round Rock, TX). Pseudoephedrine-d$_3$ HCl standard

was also bought from Cerilliant at a concentration of 100 µg/mL in methanol. CMP-HCl

standard was purchased at a concentration of 1 mg/mL in methanol from Cayman (Cayman

Chemical, Ann Arbor, MI).

2.3.2 Sample collection

Wastewater samples were collected in South Carolina (SC), Georgia (GA), and

Oklahoma (OK). All samples collected from SC/GA were shipped overnight on ice to the

Oklahoma State University Forensic Toxicology and Trace Laboratory (OSU-FTTL) in Tulsa,

OK for analysis; these samples were designated SC followed by a number corresponding to their

order of collection. Samples collected in OK were immediately taken to the OSU-FTTL for

analysis following collection; these samples were designated OK followed by a number

corresponding to their order of collection. Samples were collected from an array of wastewater

entities, including lift stations, sanitary sewer lines accessed via manhole covers, and wastewater

treatment plants. Sampling locations were a mixture of convenient locations, used to find

evidence of methamphetamine use or production in select neighborhoods, as well as strategically

planned sampling of areas that had former One Pot methamphetamine laboratories. Collection

procedures differed slightly between SC/GA and OK and are summarized below.

2.3.2.1 South Carolina and Georgia samples

Samples SC 1-73 were collected using either an ISCO 3700 or ISCO 6700 automated

portable sampler (Teledyne ISCO, Lincoln, NE) drawing wastewater by peristaltic pump through

sanitary-grade Tygon® 3350 silicone tubing (Saint-Gobain, Malvern, PA) into 1000 mL

polypropylene bottles (See Figure 5). Samples were classified as either grab samples or

composite samples. For grab sampling, 500 mL of water was collected all at once in a single

bottle. For composite sampling, 15 mL of water was collected hourly over 24 hours with all 24

aliquots being deposited into a single bottle. Prior to analysis, grab and composite samples were transferred to 250 mL Nalgene high-density polyethylene bottles (Nalge Nunc International, Penfield, NY), cooled on ice, and then shipped overnight to the OSU-FTTL for laboratory analysis.

Figure 5. ISCO 3700 autosampler with silicon tubing. Water is moved by peristaltic pump from the wastewater system into polypropylene bottles housed within the body of the autosampler.

SC 1-18 are neighborhood lift station grab samples collected from over one-third of the lift stations across a city of 30,000 residents. Neighborhoods represented by these samples range from dozens to hundreds of occupancies, with sample SC 2 being a mixed residential-industrial lift station that primarily serves an industrial park.   Gravity-fed lift stations receiving water primarily from residencies are denoted as "neighborhood" lift stations in Table 8, while "multiple neighborhood" lift stations are situated further downstream and receive wastewater from forced mains exiting "neighborhood" lift stations, as well as residencies and business that feed into the

forced mains. For example, the SC 13 lift station includes feeder flows from SC 8, 10, 12, 14, 15, 16, 17, 18, plus additional neighborhood lift stations not sampled.

SC 19-28 are grab samples that were all collected on the same day: SC 19, 20 and 21 are from 3 lift stations, each handling roughly one-third of the flows from a city of 30,000 and SC 22 is the confluence of these flows as they arrive at a WWTP. SC 23 was collected from a wastewater line at the same WWTP from a second city of approximately 20,000 residents. The SC 24 grab sample was taken from the primary WWTP influent line, consisting of a wastewater flow of approximately 13 million gallons per day, which includes the two previously mentioned cities, plus a few additional connected wastewater customers. This wastewater is mechanically aerated for microbial digestion in a large open basin for approximately 1-2 days, flowing continuously over a weir (SC 25), which is acts as a small, adjustable dam that limits the volume and flow rate of wastewater released from the aeration basin. After leaving the first aeration basin, the wastewater flows into a second aeration basin (SC 26) of equal size for an additional 2-3 days before flowing to secondary clarifiers (SC 27) and a post-final treatment, composed of chlorine shock and removal. SC 28 was collected after final treatment and immediately prior to the effluent being discharged to an adjacent river. Samples SC 22-28 are shown in Figure 6.

Figure 6. SC 22-28 grab samples collected from a county WWTP. The left three samples were collected prior to wastewater treatment, the next three were collected during different steps of the treatment process, and the right-most sample was taken post-treatment.

SC 29-35 are 24-hr composite samples taken over a week from a gravity-fed lift station primarily contributed to by a county detention center, with sampling beginning on a Tuesday and concluding on a Monday.

SC 36-46 are 24-hr composite samples taken from a municipality of approximately 5,000 residents. SC 36-40 are from two residential lift stations, with SC 36-38 containing the site of One Pot methamphetamine lab within the previous calendar year. SC 39 was taken from spillage observed and contained within the ISCO autosampler housing, arising from the overfill of SC 36-38 collection bottles (See Figure 7). SC 41-46 are composite samples from the small municipality WWTP inlet, which includes flows from other lift stations in addition to the two sampled.

Figure 7. Spillage from SC 36-38 was contained within the autosampler housing, collected, and reported as SC 39.

SC 47-51 are 24-hr composites from a lift station collecting from many thousands-of-residencies. SC 51 sample was an attempt to establish a sample location on a hundreds-of-

residencies gravity-fed neighborhood wastewater line, just prior to its tie-in with the lift station's high-flow influent line. This attempt proved unsuccessful, as the sampler's inlet tubing was observed being swept downstream in the high-flow wastewater upon return a week later and the composite sample's precise origin is unknown.

SC 52-56 are 24-hr composite samples from a storm sewer access mistaken for a sanitary sewer manhole at a city park; the storm sewer manhole cover was within ten meters of the sanitary sewer manhole cover. As can be seen in Figure 8, collected storm water presented observable clarity in comparison to sewage water samples.

Figure 8. Collected stormwater samples (left) show greater visual clarity than wastewater samples (right).

As shown in Figure 9, SC 57-69 are 24-hr composite samples from a manhole at the end of a one-city-block headwaters gravity line connected to approximately 30 residencies. SC 73 is a grab sample from a small manhole, shown in Figure 10, accessed at the midpoint of this city block, noticeably too shallow and narrow to house the wastewater sampler for composite collection.

Figure 9. ISCO 3700 autosampler positioned inside the manhole at the headwater gravity line where composite samples SC 57-69 were collected. Headwater gravity line was located at the end of a residential block and served approximately 30 residencies with no other known feeders.

Figure 10. Shallow manhole used to obtain grab sample SC 73. The manhole was located one-half block upstream from the location where SC 57-69 were collected.

SC 70-72 were collected from a manhole containing joining gravity flows from several dozen residencies. SC 70 and 71 were taken from 5-day immersions of a polypropylene ALLWIK Absorbent Sock (Brady SPC, Milwaukee, WI) and 2 industrial sorbent pads (New Pig Corporation, Tipton, PA), respectively. These sorbents are shown in Figure 11.

Figure 11. Polypropylene industrial sorbent sock (black) and industrial sorbent pads (yellow) used to collect SC 70 and 71. The left image shows the sorbents prior to being submerged in wastewater and the right image shows the sorbents following retrieval.

2.3.2.2 Oklahoma samples

Samples OK 1-32 were all grab samples and were collected by lowering a clean container into the wastewater until the container became full. Once full, the container was brought back to the surface and approximately one liter of sample was poured into 1 L Nalgene wide-mouth high-density polyethylene bottles (Thermo Fisher Scientific, Waltham, MA). The collection container was rinsed three times with water prior to collecting additional samples. Samples were placed on ice while additional samples were collected. Once all samples had been collected, they were brought to the OSU-FTTL and immediately underwent cleanup procedures.

2.3.3 Sample extraction

Upon receipt at the OSU-FTTL, samples were kept on ice until undergoing a cleanup procedure. If samples were not going to be cleaned the day of arrival in the lab, they were frozen at -20°C until the day prior to cleanup, at which time they were permitted to slowly thaw in a 4°C refrigerator.

To remove debris from the wastewater samples, 35 mL of sample was added to two 50 mL conical-bottom centrifuge tubes (VWR, Sugar Land, TX) (70 mL total). The tubes were then centrifuged at 2800 RCF for 8 minutes. After centrifugation, the samples were poured through a coffee filter (Farmer Bros. Co, Ft. Worth, TX) into a graduated cylinder until 50 mL of sample had been collected. The cleaned samples were then transferred to 250 mL TraceClean wide mouth amber glass jars (VWR, Sugar Land, TX) and stored in a 4°C refrigerator overnight.

SPE was used to extract and concentrate methamphetamine, pseudoephedrine, CMP, and amphetamine from the wastewater samples. For SPE, the following materials were utilized: Oasis MCX 3 cc (60 mg, 30 µm) cartridges (Waters Corporation, Milford, MA), VacElut 20 Manifold (Agilent Technologies, Santa Clara, CA), CEREX 48 Flow Control and CEREX 48 Sample Concentrator (SPEware Corporation, Baldwin Park, CA) (See Figure 12). The following solutions were utilized: internal standard Mix (1000 ng/mL solution of all three deuterated internal standards in LC-MS grade water), 10 mM hydrochloric acid (HCl) solution prepared with 37% HCl and LC-MS grade water, LC-MS grade methanol, ACS grade ammonium hydroxide and Mobile Phase A. Table 1 outlines the solid phase extraction procedure. Briefly, for every sample, 5 µL of internal standard mix and 20 mL of 10 mM hydrochloric acid solution were added to 50 mL of sample. SPE cartridges were loaded into the CEREX 48 Flow Control unit and conditioned prior to being moved to the VacElut 20 manifold for sample addition. After sample addition, the cartridges were returned to the CEREX 48 Flow Control unit for a rinse step and then the cartridges were dried under positive pressure for 20 minutes at approximately 80 psi.

After being vacuum dried, elution buffer was added and the eluent was collected into labeled 8 mL plastic test tubes. Samples were dried to complete dryness under nitrogen at 40°C in the CEREX 48 Sample Concentrator. Mobile phase A was used as reconstitution buffer and was added to each test tube. Following thorough vortexing, every sample was transferred to a 1 mL amber LC injection vial for instrumental analysis.

Figure 12. CEREX 48 Flow Control Unit (left), CEREX 48 Sample Concentrator (middle), and VacElut 20 manifold (right) used for SPE.

Table 1. SPE procedure.

SPE Step	Parameter
Sample Preparation	50 mL Wastewater Sample
	5 μL Internal Standard Mix
	20 mL 10 mM HCl
Condition	2 mL LC-MS grade methanol
	2 mL 10 mM HCl
	2 mL 10 mM HCl
Sample Addition	70 mL sample, internal standard, and HCl mixture
Rinse	2 mL 10 mM HCl
Cartridge Dry Down	20 min at ~80 psi
Elution	2 mL 2% ammonium hydroxide in methanol
Elution Dry Down	Under nitrogen at 40°C
Reconstitution	200 μL Mobile Phase A

2.3.4 Liquid chromatography tandem mass spectrometry

Shimadzu 20-series UFLC pumps paired with a Sciex 4000 QTRAP® MS/MS was used for the LC-MS/MS analysis (See Figure 13).

Figure 13. LC-MS/MS instrumentation setup at the OSU-FTTL. Shimadzu 20-series UFLC pumps paired with a Sciex 4000 QTRAP® MS/MS.

For liquid chromatography, chromatographic separation was achieved with a Chromegabond WR C18 5 µm column (15 cm x 2.1 mm) (ES Industries, Inc., West Berlin, NJ) with a Restek Raptor Biphenyl 2.7µm guard cartridge (5 x 3.0 mm) (Restek Corporation, Bellefonte, PA). Mobile Phase A (MPA) consisted of 2 mM ammonium formate and 0.1% formic acid in LC-MS grade water, while Mobile Phase B (MPB) consisted of 2 mM ammonium formate and 0.1% formic acid in LC-MS grade methanol. The LC had a total flow rate of 0.400 mL/min. Mobile Phase B concentration was held at 27.5% for the first 5 minutes of the sample run, and was then increased to 100% for 1.5 minutes, and was then decreased to 27.5% for 1.5 minutes for a total run time of 8 minutes (Table 2). All changes in mobile phase B concentration were set to immediately occur and end with no ramp. Injections were set at 1 µL and the oven temperature was set to 30ºC.

Table 2. LC gradient. Elapsed time is the time from sample injection. Changes in mobile phase concentration were set to occur immediately.

Elapsed Time (min)	% MPA	% MPB
0.00	72.5	27.5
5.00	72.5	27.5
5.01	0.0	100.0
6.50	0.0	100.0
6.51	72.5	27.5
8.00	72.5	27.5

For mass spectrometry, Table 3 shows the ion transitions and mass spectrometer voltage parameters for the compounds of interest: methamphetamine, pseudoephedrine, amphetamine, and CMP. Additionally, there are three deuterated internal standards, methamphetamine-d_5, pseudoephedrine-d_3, and amphetamine-d_6. Since this method cannot differentiate the diastereomers pseudoephedrine and ephedrine, all values reported for pseudoephedrine may also be contributed to by the presence of ephedrine. Pseudoephedrine is the only compound named for simplicity.

Table 3. Mass Spectrometer Parameters. Target analytes Methamphetamine, Pseudoephedrine, Amphetamine, and CMP were identified using two mass ion fragments each. Internal standards Methamphetamine-d_5, Pseudoephedrine-d_3, and Amphetamine-d_6 were identified using one mass ion fragment each. The values listed in column "Q1" are the precursor mass-to-charge ratio (m/z) for each compound. The values listed in column "Q3 Mass" are unique fragment ion m/z for each compound. The columns labeled "DP", "CE", and "CXP" refer to the voltages utilized for declustering potential, collision energy, and collision energy speed, respectively.

Compound	Q1 (m/z)	Q3 (m/z)	DP (volts)	CE (volts)	CXP (volts)	RT (min)
Methamphetamine	150.1	91.0	56	25	14	3.03
	150.1	119.0	56	15	4	
Methamphetamine-d_5	155.0	91.1	60	20	4	3.03
Pseudoephedrine	166.1	91.1	46	39	12	2.24
	166.1	132.9	46	31	20	
Pseudoephedrine-d_3	169.2	151.0	26	21	26	2.24
Amphetamine	136.2	119.0	36	13	18	2.89
	136.2	91.0	36	25	14	
Amphetamine-d_6	142.1	125.1	41	13	6	2.89
CMP	152.2	79.1	41	27	12	4.23
	152.2	77.1	41	45	0	

Trueness of the compound identity was confirmed through comparing the areas of the two MRM transitions, resulting in an identification or ID ratio, also known as an MRM ratio. Every "Q1" and "Q3" m/z pairing generated a chromatographic peak. MRM ratios for each compound, with the exception of internal standards, were calculated by dividing the peak area of the second pairing of each compound by the peak area of the first pairing. To build an acceptable MRM ratio range, the ratios observed in every calibrator were averaged. For results to be accepted, the MRM ratio had to be within ±20% of the MRM ratio average, using two decimal points for the percentage value.

2.3.5 Method validation

Since the SPE-LC-MS/MS method used for this research was modified from a previously validated SPE-LC-MS/MS method (Green MK, Ciesielski AL, Wagner JR, Unpublished data, February 2020), only a mini-validation was performed on the calibration model to assess linearity of the calibration curve, as well as the accuracy and precision of each calibrator in the calibration model.

The quantitation ratios, the ratio of the larger MRM transition area to the internal standard transition area, from the calibrators that met the identification criteria were plotted versus concentration. After the data were plotted, they were fitted with a line of best fit, and weightings were adjusted to assure the best correlation, or highest R^2 value. The R^2 for this line was required to be greater than 0.9. For the calibration points to be included in this study, they had to have an accuracy and precision (%CV) within ±20% when applied to the line of best fit. The lower limit of quantitation (LLOQ) was permitted to be within ±30% for both accuracy and precision, though its instrument response had to be at least five times greater than the response of a blank. The linear range tested for all non-internal standard compounds in the LC-MS/MS method contained calibrator at the following concentrations: 300, 200, 100, 50, and 1 ng/L.

Three replicates of the calibration curve were extracted and concentration values were calculated for each calibrator. Accuracy for each calibrator was calculated by averaging the concentration of the six replicates and dividing that average by the expected concentration of that calibrator and then multiplying by 100 (Equation 3). Precision for each calibrator was calculated by dividing the standard deviation by the average observed concentration, subtracting that value from 1 and multiplying by 100 (Equation 3). R^2 values for each calibration curve were obtained after applying a line of best fit, and all values averaged for a given compound. All concentration values were obtained utilizing MultiQuant™ software (SCIEX, Foster City, CA), which is specifically designed for LC-MS/MS result analysis. All other values and statistical parameters were obtained by utilizing Microsoft Excel (Microsoft Corporation, Redmond, WA).

Equation 3. Calculation of calibrator accuracy and precision.

$$\text{Accuracy (\%)} = \left(\frac{\text{Avg Conc}}{\text{Expected Conc}}\right) \times 100$$

$$\text{Precision (\%)} = \left(1 - \left(\frac{\text{Std Dev}}{\text{Avg Conc}}\right)\right) \times 100$$

2.4 Results
2.4.1 Method validation

Table 4 through Table 7 demonstrate the accuracy and precision for all calibrator levels of methamphetamine, pseudoephedrine, amphetamine, and CMP while Figure 14 shows the calibration curve graphically; all values fell within the acceptance criteria of ±20%. The "Average" column refers to the average concentration, in ng/L, of the 3 replicate runs. Accuracy and precision are reported as percentages, with 100% considered to be absolute. Any value below or above true accuracy or precision is considered a suppression or enhancement of calibrator concentration, respectively. The LLOQ was determined to be 1 ng/L for methamphetamine, pseudoephedrine, and amphetamine and 50 ng/L for CMP and there were no observed peaks in

the extracted blank samples. In subsequent sections, any value outside the calibration range are

estimates based on the slope of each line of best fit, but must meet identification criteria to be

reported. The line of best fit for methamphetamine, amphetamine, and CMP was determined to

be a linear fit with $1/x^2$ weighting. The line of best fit for pseudoephedrine was determined to be

a linear fit with $1/y^2$ weighting.

Table 4. Methamphetamine linearity results. ND=Not detected.

Methamphetamine Calibrator (ng/L)	Average	Overall Accuracy	Overall Precision
300	316	105%	94%
200	209	104%	97%
100	105	105%	95%
50	41.2	82%	98%
1	1.03	103%	96%
Blank	ND		
R^2	0.997		

Table 5. Pseudoephedrine linearity results. ND=Not detected.

Pseudoephedrine Calibrator (ng/L)	Average	Overall Accuracy	Overall Precision
300	308	103%	97%
200	197	99%	99%
100	100	100%	99%
50	49.1	98%	94%
1	1.01	101%	92%
Blank	ND		
R^2	0.999		

Table 6. Amphetamine linearity results. ND=Not detected.

Amphetamine Calibrator (ng/L)	Average	Overall Accuracy	Overall Precision
300	304	101%	95%
200	202	101%	99%
100	100	100%	98%
50	50.1	100%	99%
1	1.00	100%	100%
Blank	ND		
R²	0.998		

Table 7. CMP linearity results. ND=Not detected.

CMP Calibrator (ng/L)	Average	Overall Accuracy	Overall Precision
300	296	99%	95%
200	202	101%	96%
100	101	101%	97%
50	49.6	99%	98%
Blank	ND		
R²	0.995		
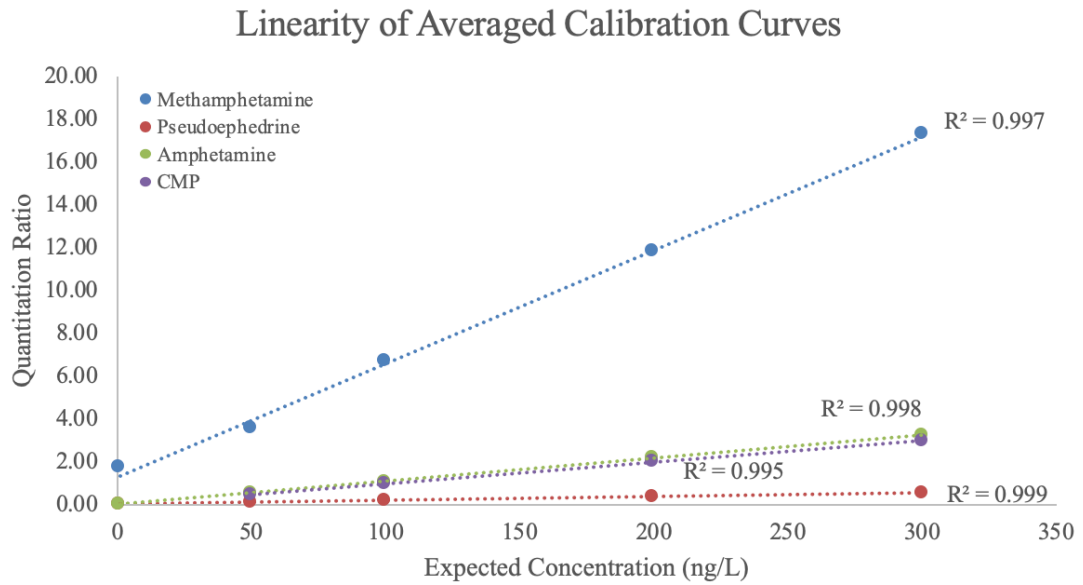
Figure 14. Averaged linear calibration curves obtained by plotting the quantitation ratio vs the expected concentration.

2.4.2 Wastewater analysis
2.4.2.1 South Carolina and Georgia samples

Four shipments of wastewater samples were sent from SC/GA to the OSU-FTTL between May 1, 2017 and December 6, 2017. In total, 73 samples were collected from municipalities in SC and GA and analyzed at the OSU-FTTL. Table 8 summarizes the findings from the SC/GA wastewater samples. Analysis of SC 1-18 was conducted May 19, SC 19-28 on June 21, SC 29-46 on October 11 and SC 47-73 on December 6. Analysis dates are separated by lines on Table 8. Concentrations above 300 ng/L exceed the upper limit of quantitation (ULOQ) for the method and are therefore outside of the validated analytical range. Values provided that are above the ULOQ are estimates based on the equation of the line of best fit derived from the calibrators of each drug and are provided for convenience. While samples above the ULOQ could have been diluted and reanalyzed to accurately quantitate the amount of drug within each sample, the semi-quantitative values obtained were deemed sufficient to identify methamphetamine use or production.

Table 8. Concentration of methamphetamine (meth), pseudoephedrine (pseudo), CMP, and amphetamine (amp) observed in wastewater samples collected in select SC/GA municipalities. All concentrations listed in ng/L. Concentrations above 300 ng/L exceed the ULOQ for the method and are estimates provided for convenience.

	Sample Demographics			Observed Drug Concentration (ng/L)			
Sample Number	Collection Site	Grab/ Composite		Meth	Pseudo	CMP	Amp
SC 1	College campus Lift Station (LS)	Grab		2	587	0	499
SC 2	Multiple-neighborhood LS	Grab		760	0	0	188
SC 3	Neighborhood LS	Grab		701	399	0	256
SC 4	Neighborhood LS	Grab		22	16	0	1155
SC 5	Multiple-neighborhood LS	Grab		2286	465	0	435
SC 6	Neighborhood LS	Grab		946	989	0	899
SC 7	Neighborhood LS	Grab		23	1219	0	174
SC 8	Multiple-neighborhood LS	Grab		26	1482	0	231
SC 9	Neighborhood LS	Grab		93	1640	0	244
SC 10	Neighborhood LS	Grab		16	2128	0	387
SC 11	Neighborhood LS	Grab		26	640	0	678

Analysis Date: 5/19/2017

	SC 12	Neighborhood LS	Grab	4905	2298	0	729
	SC 13	Multiple-neighborhood LS	Grab	1426	2062	0	420
	SC 14	Neighborhood LS	Grab	49	444	0	0
	SC 15	Neighborhood LS	Grab	0	3158	0	0
	SC 16	Neighborhood LS	Grab	117	484	0	0
	SC 17	Neighborhood LS	Grab	0	662	0	253
	SC 18	Neighborhood LS	Grab	9739	1377	0	1006
Analysis Date:6/21/2017	SC 19	Multiple-neighborhood LS	Grab	295	0	0	140
	SC 20	Multiple-neighborhood LS	Grab	441	949	0	204
	SC 21	Multiple-neighborhood LS	Grab	446	762	0	218
	SC 22	WWTP inlet feedstream - 30,000 residents	Grab	280	345	0	119
	SC 23	WWTP inlet feedstream - 20,000 residents	Grab	952	1255	0	530
	SC 24	WWTP inlet	Grab	654	527	0	258
	SC 25	WWTP Aeration Basin #1 Weir Outflow	Grab	127	80	0	1
	SC 26	WWTP Aeration Basin #2 Weir Outflow	Grab	0	0	0	0
	SC 27	WWTP Secondary Clarifier Outflow	Grab	0	0	0	0
	SC 28	WWTP Post Chlorine Removal (outfall)	Grab	0	0	0	0
Analysis Date: 10/11/2017	SC 29	Detention Center LS Tue	Composite	909	94	0	167
	SC 30	Detention Center LS Wed	Composite	788	0	0	108
	SC 31	Detention Center LS Thu	Composite	1132	0	0	239
	SC 32	Detention Center LS Fri	Composite	523	126	0	106
	SC 33	Detention Center LS Sat	Composite	612	351	0	141
	SC 34	Detention Center LS Sun	Composite	990	366	0	408
	SC 35	Detention Center LS Mon	Composite	615	481	0	168
	SC 36	Neighborhood LS	Composite	712	1568	0	572
	SC 37	Neighborhood LS	Composite	933	1618	0	647
	SC 38	Neighborhood LS	Composite	747	954	0	843
	SC 39	Neighborhood LS	Composite	873	1450	0	498
	SC 40	Neighborhood LS	Composite	434	1823	0	494
	SC 41	WWTP inlet feedstream - 5,000 residents	Composite	430	556	0	191
	SC 42	WWTP inlet feedstream - 5,000 residents	Composite	406	474	0	184
	SC 43	WWTP inlet feedstream - 5,000 residents	Composite	392	446	0	164
	SC 44	WWTP inlet feedstream - 5,000 residents	Composite	598	628	0	231
	SC 45	WWTP inlet feedstream - 5,000 residents	Composite	666	424	0	274
	SC 46	WWTP inlet feedstream - 5,000 residents	Composite	529	621	0	222
	SC 47	Multiple-neighborhood LS - Tue	Composite	3447	810	0	643
	SC 48	Multiple-neighborhood LS - Thu	Composite	1830	1017	0	419
	SC 49	Multiple-neighborhood LS - Fri	Composite	1265	494	0	287
	SC 50	Multiple-neighborhood LS - Sat	Composite	1413	1027	0	399
	SC 51	Multiple-neighborhood LS	Composite	752	391	0	212

SC 52	Neighborhood stormwater manhole - Tue	Composite	310	13	0	53
SC 53	Neighborhood stormwater manhole - Wed	Composite	181	7	0	30
SC 54	Neighborhood stormwater manhole - Thu	Composite	177	16	0	27
SC 55	Neighborhood stormwater manhole - Fri	Composite	278	17	0	32
SC 56	Neighborhood stormwater manhole - Sat	Composite	183	0	0	28
SC 57	one city block - Wed	Composite	9346	28	0	1092
SC 58	one city block - Thu	Composite	6497	14	0	99
SC 59	one city block - Fri	Composite	5983	1425	0	155
SC 60	one city block - Sat	Composite	9604	1570	0	937
SC 61	one city block - Sun	Composite	7329	89	0	747
SC 62	one city block - Mon	Composite	6044	1863	0	758
SC 63	one city block - Tue	Composite	6145	134	0	430
SC 64	one city block - Wed	Composite	8601	37	0	939
SC 65	one city block - Thu	Composite	5042	90	0	993
SC 66	one city block - Fri	Composite	16330	25	0	2692
SC 67	one city block - Sat	Composite	16950	34	0	3218
SC 68	one city block - Sun	Composite	39980	49	0	7580
SC 69	one city block - Mon	Composite	6589	14	0	1337
SC 70	neighborhood manhole	Composite	569	1397	0	228
SC 71	neighborhood manhole	Composite	650	1544	0	224
SC 72	neighborhood manhole	Grab	2437	717	0	327
SC 73	1/2 city block	Grab	10150	0	0	201

Table 9 summarizes the number of SC/GA samples positive for the four compounds of interest and the frequency of each compound's presence. Of the 73 samples analyzed from select SC/GA municipalities, 68 (93%) were positive for methamphetamine, 64 (88%) were positive for pseudoephedrine, 0 (0%) were positive for CMP, and 67 (92%) were positive for amphetamine.

Table 9. Total number and frequency of the 73 wastewater samples from SC/GA positive for methamphetamine, pseudoephedrine, CMP, and amphetamine.

	Meth	Pseudo	CMP	Amp
Number of Positives	68	64	0	67
Frequency of Samples Positive (%)	93	88	0	92

2.4.2.2 Oklahoma samples

A total of 32 wastewater samples were collected in OK and analyzed at the OSU-FTTL between July 19, 2017 and September 27, 2017. Table 10 summarizes the findings from the OK wastewater samples. Analysis of OK 1-10 was conducted on July 19, OK 11-18 on August 3, and OK 19-32 on September 27. Analysis dates are separated by lines in the table. Concentrations above 300 ng/L exceed the ULOQ for the method and are therefore outside of the validated analytical range. Concentrations denoted with an asterisk were below the LLOQ obtained on the day of analysis but met all other criteria for being designated a peak. Any values provided that fell above the ULOQ or below the LLOQ values were outside the validated analytical measurement range and are therefore provide for convenience.

Table 11 summarizes the number of OK samples positive for the four compounds of interest and the frequency of each compound's presence. Of the 32 samples analyzed from OK, 30 (94%) were positive for methamphetamine, 25 (78%) were positive for pseudoephedrine, 5 (16%) were positive for CMP, and 29 (91%) were positive for amphetamine. While statistics could be used to compare the frequency of positives between SC/GA and OK, it was decided that this was not necessary. The frequency of positives was meant to be a quick, easy to read summary of the results showing there was a large percentage of samples positive for drugs and was not meant to be a comparison between the two communities, as the sampling approaches and the type of locations (residential vs commercial) varied greatly between the two states.

Table 10. Concentration of methamphetamine (meth), pseudoephedrine (pseudo), CMP, and amphetamine (amp) observed in wastewater samples collected in OK. All concentrations listed in ng/L. Concentrations above 300 ng/L exceed the ULOQ for the method and are estimates provided for convenience. Concentrations listed with asterisks are below the LLOQ but meet all other criteria for being designated a peak.

	Sample Demographics			Observed Drug Concentration (ng/L)			
	Sample Number	Collection Site	Grab/ Composite	Meth	Pseudo	CMP	Amp
Analysis Date: 7/19/2017	OK 1	West Bank (WB) Lift Station	Grab	1463	2209	0	209
	OK 2	WB Manhole (MH) 1: Western Pines Apt.	Grab	3235	364	0	462
	OK 3	WB MH2: W. 24th St.	Grab	291	0	0	8*
	OK 4	WB MH3: Eugene Field Elementary	Grab	719	1*	0	61
	OK 5	WB MH4: W. 21st St.	Grab	1011	58	0	81
	OK 6	New Block (NB) Lift Station	Grab	4159	759	4*	579
	OK 7	NB MH1: Old Jail	Grab	1338	0	0	1141
	OK 8	NB MH2: Wassco Bottling Co.	Grab	4572	489	6*	597
	OK 9	NB MH3: Orcutt Machine and Oil Tools	Grab	2062	0	0	240
	OK 10	NB MH4: S. 38th W. Ave.	Grab	6080	2186	17*	1281
Analysis Date: 8/3/2017	OK 11	South Lewis (SL) Lift Station	Grab	2388	1990	19*	508
	OK 12	SL MH1: Citiplex Towers Parking Lot	Grab	860	4310	0	198
	OK 13	SL MH2:	Grab	183	246	0	1093
	OK 14	SL MH3: Deerfield Estates Apt.	Grab	0	4560	0	0
	OK 15	SL MH4:	Grab	0	28	0	0
	OK 16	SL MH5	Grab	2814	2602	0	632
	OK 17	SL MH6: Wal-Mart Parking Lot	Grab	136	0	0	0
	OK 18	SL MH7: River Spirit Casino Hotel	Grab	2388	74	13*	864
Analysis Date: 9/27/2017	OK 19	Clark Park	Grab	10*	0	0	5*
	OK 20	117th E. Pl and 2nd St. S.	Grab	1459	0	0	89
	OK 21	Across from Continental Carbonic Products	Grab	2354	373	0	221
	OK 22	S. 122nd E. Ave and E. 4th Pl. S.	Grab	4151	2936	0	489
	OK 23	Aspen Manufactured Homes	Grab	10040	1886	0	5493
	OK 24	Knights Inn	Grab	4109	220	0	1201
	OK 25	Daylight Donut Flour Co.	Grab	782	162	0	168
	OK 26	Ridgeview Apt.	Grab	1346	0	0	43
	OK 27	Mingo Creek across from Meadowbrook Apt.	Grab	3475	1380	0	710
	OK 28	Mingo Creek across from E. 7th St. S.	Grab	431	405	0	115
	OK 29	S. 103rd E. Ave between 12th St. and 14th St.	Grab	8873	113	0	2505
	OK 30	S. 105th E. Ave between 12th St. and 14th St.	Grab	1391	160	0	424
	OK 31	Greenleaf Wholesale Flowers	Grab	3691	142	0	432
	OK 32	Sierra Pointe Apt.	Grab	1108	11	0	230

Table 11. Total number and frequency of the 32 wastewater samples from OK positive for methamphetamine, pseudoephedrine, CMP, and amphetamine.

	Meth	**Pseudo**	**CMP***	**Amp**
Number of Positives	30	25	5	29
Frequency of Samples Positive (%)	94	78	16	91

*All CMP positives were below the LLOQ of 50 ng/L but met all other criteria to be designated a positive peak.

2.5 Discussion

2.5.1 Method validation

Results of the linearity study performed on the SPE-LC-MS/MS method used to extract methamphetamine, pseudoephedrine, amphetamine, and CMP from 50 mL wastewater samples found the method to be successful and robust. Methamphetamine, pseudoephedrine, and amphetamine had linear quantitative ranges of 1-300 ng/L; the 1 ng/L CMP calibrator was not able to be differentiated from the background of the instrument so it could not be included as part of CMP's linear quantitative range. CMP had a linear quantitative range of 50-300 ng/L. All accuracy and precision values tested during linearity fell within the allowable ±20%, with most falling within ±5% of the true value (Table 4 – Table 7).

2.5.2 Wastewater analysis

In total, 105 wastewater samples were collected and analyzed for the presence of methamphetamine, pseudoephedrine, CMP, and amphetamine. Of the 73 samples collected in SC/GA, none were positive for CMP. Of the 32 samples collected in OK, 5 were positive for CMP, though the concentration of CMP in these samples were below the LLOQ of 50 ng/L. From the data collected, it can be seen that wastewater collection systems in SC, GA and OK routinely contain levels of methamphetamine and pseudoephedrine in the range of nanograms per milliliter and would likely, or almost certainly obfuscate use of these 2 targets as indicators of One Pot methamphetamine lab waste being deposited into the wastewater system.

The presence of routine nanogram per milliliter levels of methamphetamine may likely arises from methamphetamine users, a theory further supported by the robust presence of known metabolite amphetamine found alongside the higher values for methamphetamine. However, nanogram per milliliter presence of pseudoephedrine is likely to be from legitimate, over-the-counter consumption of pseudoephedrine and not illicit One Pot production of methamphetamine. In fact, the highest levels of methamphetamine in this study corresponded to very low pseudoephedrine levels and is therefore suggestive the high amount of methamphetamine consumption did not come from a One Pot lab. Initially it was expected that an observed increased value in methamphetamine and pseudoephedrine concentrations in wastewater might indicate the presence of a clandestine laboratory; however, early observations of these values do not support this concept. It seems that a substantial amount of the observed methamphetamine and pseudoephedrine concentrations were probably due to excretion of used methamphetamine product as well as pseudoephedrine as a decongestant, as there were fairly high background levels discovered in field samples. Furthermore, these values that did not appear to rise and fall together, as would be expect if waste from a One Pot lab was flowing through a wastewater system. Additionally, the low amount of observed CMP, which is a signature of One Pot methamphetamine labs, indicates that the production of the excreted methamphetamine took place via a route that does not include CMP as a signature compound, such as the P2P route, which is commonly used in Mexican super-labs.

2.5.2.1 South Carolina and Georgia samples

As can be seen in Table 9, methamphetamine, pseudoephedrine, and amphetamine were all present in approximately 90% of the wastewater samples collected in SC/GA. When comparing the concentration of methamphetamine to the concentration of amphetamine observed in the 73 samples from SC/GA, the data suggests that methamphetamine was of biological origin,

as opposed to from One Pot methamphetamine lab waste. When used by humans, methamphetamine is excreted as 50% unmetabolized drug and 10-20% as the metabolite amphetamine, a trend observed in a majority of the SC/GA samples.[22] To add to the theory that the observed methamphetamine was of biological origin, amphetamine has been shown to absent from One Pot methamphetamine cooks, which is the predominate method of methamphetamine production within the United States.[5,28] Also, the lack of the One Pot byproduct CMP further suggests the methamphetamine and pseudoephedrine observed in the SC/GA wastewater samples was not from illicit production in One Pot labs.

Some lift station grab samples presented spikes ranging well above the ULOD of 300 nanograms per liter and appear to range into the nanogram per milliliter level, as seen in samples SC 5, 12, 18, 72 and 73. A study by Oyler et al. reported peak methamphetamine urine concentrations to be greater than 6,000 μg/L from an administered dose of 20 mg.[55] Therefore, one 250 mL urination, dilution into 500 gallons ($\approx$1900 L) of wastewater would yield a concentration of 800 nanograms per liter, which is similar to the concentrations observed from the grab sample data. However, the National Highway Traffic Safety Administration (NHTSA) reported in 2004 for methamphetamine that the "common abused doses are 100-1000 mg/day, and up to 5000 mg/day in chronic binge use."[22] While the commonly abused dose reported by the NHTSA suggests a higher concentration of methamphetamine may be being excreted into the wastewater than what was observed in this study, many other factors can impact the concentration of methamphetamine observed in each sample, such as the volume of the lift station where the grab sample was collected, how many times that lift station had been pumped out prior to sampling, and if a methamphetamine user was on a binge or if they had crashed and were not using at the time of collection.

For composite sampling, the highest 24-hr methamphetamine concentrations were seen in SC 66-68, taken from a manhole at the end of a one-city-block headwaters gravity line connected

to approximately 30 residencies. Composite samples were taken from this location over a 13-day

period, with samples SC 66-68 taken over the second weekend of sampling. Methamphetamine

concentrations from SC 66-68 are greatly elevated when compared to the other samples taken

from this location over the 13 days of collection, suggesting a resident(s) were likely binge using

methamphetamine over the weekend or a guest had visited one of the residencies and was a

methamphetamine user. The low levels of pseudoephedrine and lack of CMP suggest the

consumed methamphetamine was not of One Pot origin, but rather it was likely

methamphetamine smuggled into the United States from Mexico, which is produced by the P2P

method and doesn't require pseudoephedrine or produce CMP as a byproduct.[5,24]

SC/GA samples collected from WWTPs showed attenuation of methamphetamine,

pseudoephedrine, and amphetamine as they underwent applied sewage treatment methods. SC 22-

24, grab samples from the influent to a large WWTP serving 50,000+ residents, and SC 41-46,

composite samples from the influent to a small WWTP serving 5,000 residents, reported

consistent methamphetamine concentrations of several hundred nanograms per liter. Both

methamphetamine and pseudoephedrine grab sample levels in SC 24 at the large WWTP influent

are consistent with a mixture of its two feeder flows, SC 22 and 23. Stepwise attenuation of the

monitored chemical compounds by activated sludge microbial processes across the large WWTP

is evident when comparing the influent values (SC 24) to Aeration Basin #1 exit weir values (SC

25) and Aeration Basin #2 exit weir values (SC 26). These samples clearly show a reduction in

methamphetamine, pseudoephedrine, and amphetamine following each wastewater treatment step

utilized by the large WWTP, with all three becoming undetectable following the second aeration

basin.

Deployment of the automated sampler into manhole chimneys, where possible, proved

challenging and required some adaptions to achieve hanging suspension of the sampler and

successful inlet tube immersion below the wastewater flow. Exploration of alternatives to

achieve reduced setup time behind traffic barricades and easier retrieval included manhole

deployment of low cost industrial spill control sorbent pads and a sorbent sock. These sorbent materials were deployed into flowing wastewater and left for several days to investigate simpler sample collection methods, as seen in SC 70 and 71. While these sorbents were able to collect methamphetamine, pseudoephedrine, and amphetamine from the wastewater, further research needs to be completed to determine the efficiency of which the sorbents capture drug from wastewater, and how much drug is left in the sorbent after being rung out so the captured wastewater can be analyzed.

In-manhole sampler deployment in a stormwater access point initially mistaken for a wastewater access proved to yield valuable data (SC 52-56). The data suggest a background level of contamination in the low hundreds of nanograms per liter may exist for the neighborhood, arising from surface water runoff from roofs, cars, driveways, streets, etc. and may possibly include dilution by groundwater infiltration as well, as is typical of both sewer and stormwater collection systems. Evidence of this low hundreds of nanograms per liter background level suggests the linear quantitative range developed for this research may be too low to differentiate methamphetamine use from production. As background levels approached the ULOQ, the presence of any additional methamphetamine, whether from use or production, pushed many of the results above the ULOQ, therefore making them estimated values that may be inaccurate. If the analytical method was detuned, or made less sensitive and therefore reduced the sensitivity to point where the concentration of methamphetamine observed due use fell towards the LLOQ while the concentration from production was towards the ULOQ, differentiating these sources may be more practical than the attempt made during this study.

2.5.2.2 Oklahoma samples

The first and second set of samples collected in OK represents a snapshot of the communities sampled. These 18 samples were taken from easily accessible manhole covers and

wastewater lift stations around OK to get an idea of the methamphetamine, pseudoephedrine, CMP, and amphetamine levels in the wastewater collection system. The third set of samples collected (OK 19-32) were from areas that local law enforcement has historically found larger numbers of One Pot methamphetamine labs when compared to other areas of OK. As can be seen in Table 10, all the samples collected from these areas were positive for methamphetamine and amphetamine, and all but one was positive for pseudoephedrine. None of the samples collected during the third OK collection were positive for CMP, suggesting the methamphetamine observed in the samples was from methamphetamine use and not from One Pot waste. Two samples collected during the third OK collection showed the potential of using this technique as a way of identifying residencies where methamphetamine is being used. OK 29 and OK 30 were collected from consecutive manhole locations along the same wastewater gravity main. OK 29 had an additional 34 houses feeding the gravity line as compared to OK 30. OK 29 contained six times the concentration of methamphetamine and amphetamine as OK 30, suggesting a methamphetamine user among the 34 residencies upstream of the manhole where OK 29 was collected. While the goal of this study was to identify locations where methamphetamine is being produced via the One Pot, declining domestic methamphetamine production has made this goal difficult to achieve.[5] However, the ability of this method to identify locations where methamphetamine is being used may still be of importance for law enforcement agencies.

As can be seen in Table 11, methamphetamine and amphetamine were present in approximately 90% of the wastewater samples collected in OK, with amphetamine present in every sample that contained methamphetamine, except for one sample. Pseudoephedrine was present in under 80%, and CMP was suggestively present in 15% of the samples. When comparing the concentration of methamphetamine to the concentration of amphetamine observed in the 32 samples from OK, the data suggests that methamphetamine use, as opposed to the dumping of waste from a One Pot methamphetamine lab, was observed in all but 5 of the wastewater samples. The 27 samples that are suggestive of methamphetamine use contained the

methamphetamine metabolite amphetamine and no measurable CMP, suggesting that no One Pot methamphetamine lab waste was present in these samples. However, the remaining 5 samples did contain CMP peaks. While all 5 CMP peaks were below the LLOQ of 50 ng/L, they met all other criteria for being designated a positive peak, including correct retention times, ion ratios, and an instrumental response over 5x that which was present in any blanks. While a currently active study at the OSU-FTTL (unpublished) has shown CMP to be at least partially excreted by humans as unmetabolized drug, of 168 urine samples analyzed that have previously been reported positive for methamphetamine, only 2 have tested positive for CMP, suggesting either CMP is metabolized to large extent or few people are using One Pot methamphetamine. Without definitive proof as to the extent of CMP metabolism, the source of the very low CMP levels detected in this study is difficult to assess and may be suggestive of either the presence of waste from a One Pot methamphetamine lab or possibly CMP excreted in urine. In either case, the presence of CMP suggests the presence of a One Pot methamphetamine lab, with the CMP either resulting from the lab waste or from excretion after use of methamphetamine produced via the One Pot method.

The low number of CMP positives, and thus assumed One Pot methamphetamine labs, observed in this study can perhaps be explained by the current trends in methamphetamine use and production. According to the DEA, methamphetamine use is on the rise, due to the high availability of methamphetamine coming into the United States from Mexico and the record low prices of the drug.[13] Because of the current ease in obtaining low-cost methamphetamine from Mexico, many methamphetamine users have switched from producing their own methamphetamine to purchasing from dealers that have had the drug smuggled into the United States by the Mexican cartels. The current influx of cheap methamphetamine, alongside tighter state regulations on methamphetamine precursors, has led to a 16-year low in domestic methamphetamine production.[13]

While current domestic production of methamphetamine is low, intelligence suggests that Mexican cartels are beginning to focus their efforts on methamphetamine distribution down the east coast of the United States.[13] It is the belief of the DEA that as new customers begin using methamphetamine, the price will begin to rise and this rise in methamphetamine price will lead to more people once again producing methamphetamine themselves.[13] If domestic methamphetamine production increases, this research could prove to be beneficial in the identification of clandestine One Pot methamphetamine labs. Currently, this research has shown that wastewater can be used to identify areas of methamphetamine use, even narrowing the location of use down to as little as 15 residencies.

Future work is needed to assess the metabolic fate of CMP if it is going to be used as a marker for One Pot methamphetamine production. If CMP is heavily metabolized in the human body, than its presence in wastewater strongly suggests the presence of an active One Pot methamphetamine lab in the area that is dumping lab waste into the wastewater system. If CMP is poorly metabolized in the human body, its presence may be suggestive of a One Pot methamphetamine lab or somebody using methamphetamine produced in a One Pot lab. Regardless of the extent of CMP metabolism, its presence does suggest One Pot methamphetamine production is occurring somewhere, making it an optimal target to backtrack to its source. Even if a One Pot methamphetamine lab is not found by this type of wastewater tracking, a methamphetamine user may be caught and may be willing to give up their supplier, ultimately leading to the removal of a hazardous One Pot methamphetamine lab and reducing the amount of environmental contamination stemming from it.

CHAPTER III

AMBIENT AIR MONITORING OF ONE POT METHAMPHETAMINE
LABORATORIES

3.1 Introduction

With the production of methamphetamine comes the production of hazardous

byproducts, which can negatively impact the health of those exposed. Exposures can come in the

form of dermal, oral, or inhalational exposures, all which have different pharmacokinetics,

making health effects stemming from these exposures difficult to predict.[56] In addition, the

identity and concentrations of many of the byproducts formed and released into the environment

from methamphetamine production are unknown, making health impact predictions nearly

impossible. This is especially true for One Pot methamphetamine labs, which have not had their

volatile byproducts assessed.

While the identity of many of the volatile byproducts from One Pot methamphetamine

labs are unknown, the identity of others are obvious due to their integral part in the conversion of

pseudoephedrine to methamphetamine. One such volatile byproduct is ammonia gas, which is

generated within the One Pot reaction vessel by combining sodium hydroxide and ammonium

nitrate. During a One Pot reaction, the ammonia gas bubbles through an organic solvent,

stripping lithium metal of electrons, which are then used to reduce the pseudoephedrine or

ephedrine to methamphetamine.

Another known volatile byproduct generated during a One Pot methamphetamine cook is HCl gas. HCl gas is bubbled into the organic solvent after the cook has come to completion, causing the methamphetamine to precipitate out of the organic solvent as a hydrochloride salt so it can be recovered in a usable form. Both ammonia gas and HCl gas are classified as corrosive gases that pose a significant health hazard to people who are exposed to them without the proper personal protective equipment (PPE).[57,58] To add to this health hazard, the organic solvents used during a One Pot cook are volatile, causing them to readily become a gas that can be inhaled and cause numerous health effects, including respiratory irritation, pulmonary edema, liver and neurological damage, and loss of coordination in people within and near clandestine methamphetamine labs.[59] Methamphetamine itself can also be released in a gaseous state during a One Pot methamphetamine cook, adding yet another respiratory hazard to those who are within close proximity of a One Pot cook.[60]

While the identity of several of the volatile byproducts formed during a One Pot methamphetamine cook are known, no attempt has been undertaken to identify or quantitate any other byproducts formed during a One Pot methamphetamine cook. This study aimed to do just that. For this study, One Pot methamphetamine cooks were performed in a garden shed to simulate the environment that methamphetamine may be produced in these small-yield clandestine labs. Air monitoring was performed inside the garden shed, as well as from varying distance downwind of the cook site to identify gases released during a One Pot methamphetamine cook and concentrations present. The goal of this study was to capture ambient air from within a site of One Pot methamphetamine production and identify and quantitate the volatile compounds present within the captured air, as well as monitor how far the volatile byproducts from a One Pot methamphetamine lab could be detected, thus establishing a contamination zone in which people may be exposed to One Pot methamphetamine lab byproducts.

3.2 Review of the Literature

Methamphetamine was first synthesized in 1893 by the Japanese pharmacologist

Nagayoshi Nagai by reducing ephedrine, which he isolated from plants of the *Ephedra* family.[61]

Since then, chemist have developed numerous routes of production for the drug, all incorporating

different precursors and reactants (See Figure 15).[62] Of the many routes of production, two types

of syntheses have been primarily used in the United States: the P2P method and the

pseudoephedrine/ephedrine method. The P2P method uses phenyl-2-propanone (P2P) as a

precursor, which is subjected to an amination and a reduction process, resulting in the formation

of methamphetamine. The pseudoephedrine/ephedrine method uses pseudoephedrine or

ephedrine as a precursor, which is then reduced to form methamphetamine. As shown in Figure

15, both types of syntheses can be performed in numerous ways.
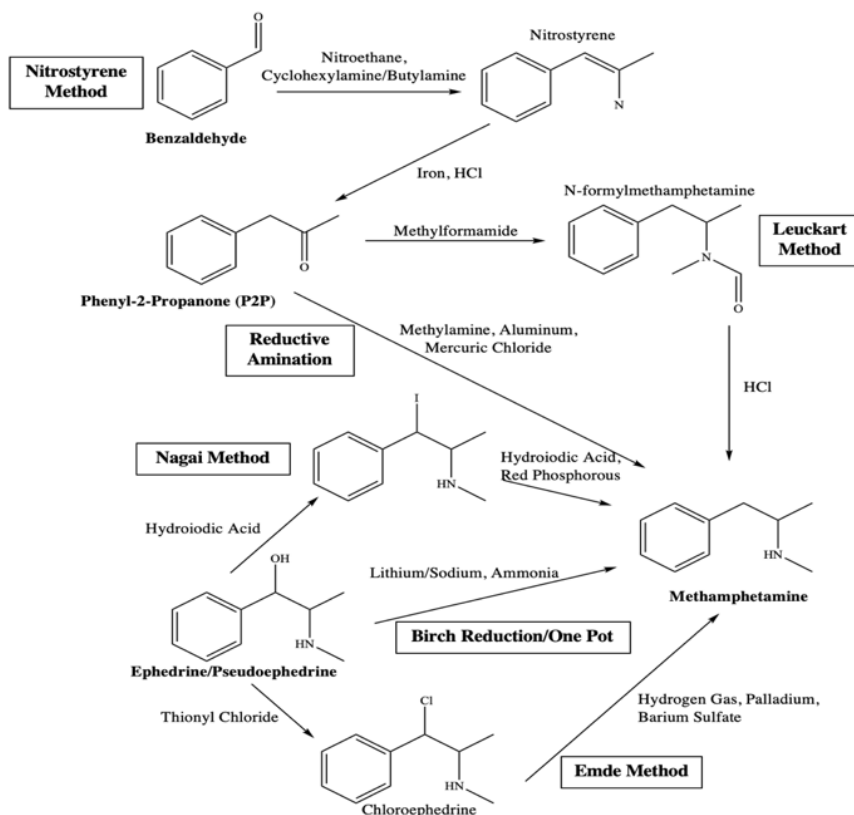
Figure 15. An overview of several popular routes of methamphetamine production. Image recreated based on Mat
Desa and Ismail.[62]

Sites of illicit methamphetamine manufacture first began appearing in the United States following the passing of the Controlled Substances Act of 1970, which made all amphetamine-type compounds schedule II drugs.[63] After becoming scheduled, sources of pharmaceutical methamphetamine became scarce, leading groups of people to search for a way to produce the drug themselves. The first such way was the P2P method, which combined phenyl-2-propanone with either methylamine in a reductive amination reaction to form methamphetamine or formic acid and methylformide in an amination reaction, followed by a reduction reaction with hydrochloric acid to form methamphetamine (See Figure 15).[62] Regardless of the exact synthesis method performed, hazardous contaminants were introduced into the air surrounding the methamphetamine lab, including formic acid and the heavy metals lead and mercury.[64]

All of these air contaminants can cause adverse health effects by different mechanisms. Formic acid is a corrosive chemical that can directly damage cells when coming in contact with them. This can lead to irritation of the respiratory tract, confusion, or breathlessness and wheezing.[65] The lead particles contaminating the air within a P2P methamphetamine lab are small enough that, when inhaled, 90% are maintained in the lungs, and ultimately absorbed into the blood where they can lead to adverse health effects; the primary physiological effect of lead is neuropathy.[66] Mercury vapors are readily diffuse from the lungs into the bloodstream and are quickly distributed throughout the body, having their greatest impact on the central nervous system (CNS), leading to a characteristic triad of symptoms including excitability, tremors, and gingivitis (Mad Hatter's Disease).[66] While these volatilized byproducts from P2P methamphetamine labs may have led to adverse health effects in those performing the cooks, the symptoms would have likely appeared slowly over time and gone unnoticed until later in life. Additionally, an epidemic of methamphetamine use had swept the United States, leading to an increased demand for the drug, which clandestine manufacturers were eager to capitalize on, regardless of the potential health implications.

With the widespread use of methamphetamine sweeping across the United States, law enforcement began cracking down on the P2P labs used to produce the illicit drug, leading to the eventual scheduling of phenyl-2-propanone in February 1980.[61] With this precursor compound more difficult to obtain, clandestine chemist began experimenting with new ways to produce methamphetamine, leading to an increased interest in the pseudoephedrine/ephedrine method.

The first pseudoephedrine/ephedrine methamphetamine lab seizure in the United States occurred in 1982.[61] This lab was using the Red-P method, which combines pseudoephedrine or ephedrine with red phosphorus and hydriodic acid, forming a iodo-ephedrine intermediate product, before fulling reducing to methamphetamine (See Figure 15).[67,68] Unlike the P2P method, which resulted in a racemic mixture of potent d-methamphetamine and it's far less potent l-isomer, pseudoephedrine/ephedrine methods of methamphetamine production form solely the more potent d-isomer, as long as l-ephedrine or d-pseudoephedrine are used as the starting material.[67,69] As with the P2P method, the Red-P method introduces several hazardous contaminants into the ambient air, leading to the potential of adverse health effects for anyone exposed to a lab environment. Some of these contaminants include hydroiodic acid, HCl, phosphoric acid, various VOCs, white phosphorus, and phosphine gas.[64]

Of the Red-P byproducts introduced to the air within a Red-P methamphetamine lab, at least three are acids. All three acid byproducts covered here, hydroiodic, HCl, and phosphoric, are corrosive compounds that may lead to eye and respiratory damage if an individual is exposed to them.[70–72] While hydroiodic acid and HCl are volatile acids that readily partition into water vapor present in ambient air and thus pose a realistic inhalational hazard, phosphoric acid in fairly non-volatile unless heated; however, the heating of phosphoric acid is part of a Red-P methamphetamine cook, so phosphoric acid is included as an air contaminant here as it may be present in the air during an active Red-P cook.[67,72]

While the three acids mentioned above are found in almost all Red-P methamphetamine labs, the VOCs present in these labs are highly variable, with solvents such as methanol, ethanol,

isopropyl alcohol, camp fuel, naphtha, acetone, toluene, and ether all potentially present. The organic solvents found in Red-P lab serve two purposes: first, for extracting pseudoephedrine from cold medication if bulk powder is not being used, and second, to extract the methamphetamine from the aqueous cook after the pH has been increased to around 11 following completion of the reduction reaction.[73] The toxicity of VOCs vary greatly among different classes of VOCs, as well as with a single class of VOCs. For example, the molecular structures of the straight chain, saturated hydrocarbons hexane (C_6H_{14}) and heptane (C_7H_{16}) differ by only a CH_2 entity, however, hexane is much more toxic. This is because it is more volatile, a smaller molecule (can more readily cross membranes), and can cause peripheral neuropathy, as well as CNS neuropathies.[74] In general, four factors determine a VOCs toxicity, including its carbon number, the number of double and triple bonds present, its structural configuration (straight-chain, branched, cyclic), and what functional groups are present.[35] While the toxicological effects of VOC exposure vary according to what VOC is present, most VOC inhalational exposures lead to irritation of the respiratory tract following acute, low concentration exposures and can lead to loss of consciousness following acute, high concentration exposures.[75] Chronic VOC exposure can lead to degeneration of the white matter in the CNS, cardiac dysrhythmias, and pulmonary edema.[35,75] Additionally, VOC concentrations can be exacerbated in methamphetamine labs, as often times the labs are sealed by those producing the drug to prevent odors from escaping, which may alert law enforcement to the illicit activity being performed.[76]

The last two Red-P byproducts covered by this review are the phosphorous-containing compounds white phosphorous and phosphine gas. During a Red-P cook, the heating of the reaction may cause the red phosphorous used to convert to white phosphorous, which is the most unstable, volatile, and hazardous form of phosphorous.[77,78] Due to its spontaneous ignitability in ambient air, perhaps the greatest hazard associated with white phosphorus exposure is the severe burns it causes when individuals come in contact with it or its fumes. These burns occur immediately following an exposure and heal slowly, with the eyes, respiratory tract, skin, and GI

tract all potentially being impacted. A three-staged time course has been documented for individuals subjected to severe white phosphorus exposures. The first stage, which occurs between minutes and eight hours following the exposure, symptoms such as lacrimation, burning, vomiting, and coughing are pronounced. If the burns received from the exposure are severe enough, the exposed individual may succumb to shock with 24-48 hour. The second phase begins as the first phase ends and can last eight hours to three days; this phase is dubbed the asymptomatic phase. The third phase begins four to eight following the second phase and may include multiple organ failure, CNS damage, or death.[79]

The second phosphorous-containing byproduct released by a Red-P methamphetamine lab is phosphine gas. Phosphine gas is potentially fatal byproduct produced when red phosphorus is heated in the presence of moisture.[80] Exposure to the gas can lead to dizziness, convulsions, irregular heart rate, vomiting, and pulmonary edema. Symptoms of phosphine gas exposure may not be observed immediately in those who were exposed, with some symptoms not progressing until 72 hours after exposure.[81] Phosphine gas is heavier than air, which means it will settle to the low-lying areas within a Red-P methamphetamine lab.[82] This settled phosphine gas may be disturbed as individuals walk through a Red-P methamphetamine lab, resuspending it and leading to an exposure.[83] Additionally, phosphine is gas is highly explosive; when allowed to settle in low-lying areas of a methamphetamine lab, it may be exposed to sparks from low-sitting power outlets or appliances, leading to an explosive hazard to accompany the health hazard posed by this methamphetamine lab byproduct.[82]

As with P2P methamphetamine production, over time, law enforcement began to regulate the chemicals needed to perform a Red-P methamphetamine cook. In 1988, congress passed the Chemical Diversion and Trafficking Act, which gave the DEA authority to regulate chemicals needed in clandestine drug manufacture and impose criminal sanctions on those misusing these chemicals without preventing the general population from obtaining them for legitimate use.[84] Chemicals needed in for the synthesis of methamphetamine using the Red-P method added to this

list included hydriodic acid, red phosphorus, hypophosphorous acid, and iodine. Additionally, the Comprehensive Methamphetamine Control Act of 1996 restricted the purchase and importation of the pseudoephedrine and ephedrine bulk powder commonly used in Red-P methamphetamine labs, making it difficult to obtain the precursor chemicals necessary to make methamphetamine. While over-the-counter cold medication could be used for Red-P methamphetamine production, it necessitates an extraction step to separate the pseudoephedrine from the inert ingredients before performing the reduction reaction.[85] In order to continue making methamphetamine without alerting law enforcement, clandestine chemists once again changed their production method, this time to the Birch reduction method.

The Birch reduction, or "Nazi," method of methamphetamine production combines pseudoephedrine with lithium metal and liquid anhydrous ammonia; the ammonia acts as a solvent to carry electrons stripped from the lithium to the pseudoephedrine, which is then reduced to methamphetamine. After restrictions were placed on many of the Red-P precursor chemicals, the Birch reduction became a popular route of methamphetamine production, as all of its precursor chemicals were easily obtainable without drawing attention from law enforcement. The lithium used was generally cut out of batteries and the liquid anhydrous ammonia could be bought or stolen from farmers who use it to add nutrients to their fields. Additionally, the pseudoephedrine didn't need to be in the form of pure powder or extracted from cold medication like in the Red-P method; rather the cold medication only had to be ground up and added to the reaction, allowing for easier methamphetamine production. Other chemicals used in a Birch reduction methamphetamine lab include organic solvents and HCl gas, which is usually generated by reacting sulfuric acid with sodium chloride (Equation 4). These chemicals and the hazards associated with exposure to them were detailed above during discussion about the byproducts from Red-P methamphetamine labs.

Equation 4. Balanced chemical reaction between sulfuric acid and sodium chloride forming hydrogen chloride and sodium sulfate.

$$H_2SO_4 + 2\ NaCl \rightarrow 2\ HCl + Na_2SO_4$$
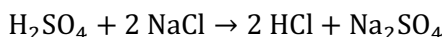
During a Birch reduction methamphetamine cook, several chemicals are released into the environment, some at levels that are immediately dangerous to the life and health of those exposed.[86] In a study by Martyny et al. characterizing the amount of airborne and surface contamination present in Red-P and Birch reduction methamphetamine labs, the group found ammonia levels within Birch reduction labs to exceed the IDLH values developed by CDC-NIOSH. Peak ammonia concentrations in the Birch reduction methamphetamine labs examined were found to be 410 ppm, with peak levels within breathing zones to be 370 ppm;[86] the IDLH for ammonia gas is 300 ppm.[87] Ammonia is hydroscopic, so it will seek out moisture in the nearby environment. If an individual is exposed to ammonia, it will readily dissolve in moisture-rich areas of the individual's body, including the eyes, nose, and mouth, rapidly converting to the corrosive, alkaline compound ammonium hydroxide.[87] When introduced to the body, ammonium hydroxide can lead to irritation of the exposed area, burning/rashes, breathlessness, and corrosive damage. Not only is ammonia a hazard within Birch reduction methamphetamine labs, but the producers of such labs generally don't transport the chemical appropriately, resulting in potential exposures whenever a transportation vessel, such as an old propane tank or bucket, fail and release the chemical into the environment (Figure 16).

By 2005, law enforcement and legislators had once again worked to develop laws that limited the availability of methamphetamine precursor chemicals. The Combat Methamphetamine Act of 2005 put further restrictions on the amount of pseudoephedrine that could be purchased by an individual, allowing 3.6 g to be purchased per day and 9 g to be purchased per month. Additionally, a photo ID became required for purchasing.[88] These restrictions on the precursor material pseudoephedrine dealt a major blow to methamphetamine

manufacturers, as Red-P methamphetamine cooks generally used around 150 g of pseudoephedrine while Birch reduction cooks generally used around 30 g of pseudoephedrine.[89,90] While some methamphetamine producers relied on "smurfs" to purchase pseudoephedrine for them in exchange for money or drugs, others began using a new methamphetamine synthesis that required less starting material called the One Pot.

Figure 16. Left.) A propane tank used to illicitly transport anhydrous ammonia. The blue discoloration of the bronze fitting is indicative of the presence of ammonia. **Right.)** A vehicle illicitly transporting anhydrous ammonia in a propane tank when the tank's structural integrity became compromised, resulting in a plume of ammonia being released into the environment. Images adapted from the Clandestine Laboratory Safety Certificate Program Student Manual.[73]

As mentioned previously, the One Pot method of methamphetamine production utilizes ammonia and lithium to reduce pseudoephedrine to methamphetamine. The One Pot is a modified Birch reduction cook, where, instead of using liquid anhydrous ammonia, ammonia gas is generated within the reaction vessel. Being similar to a Birch reduction methamphetamine cook, the One Pot is predicted to have similar volatile byproducts, however, no previous studies were found in the literature. Without examining the volatile byproducts released from a One Pot methamphetamine lab, there is no information to develop accurate and effective safety protocols. Previous studies, such as the study by Martyny et al., showed the need to not only identify what compounds are present in the air surrounding methamphetamine labs, but also the amount of each compound present.[86] While some compounds, such as ammonia and HCl, are simple irritants at

low concentrations, exposure to higher concentrations of these same chemicals may lead to permanent health complications and even death.

Some of the byproducts assumed to be released from a One Pot methamphetamine lab include ammonia gas, HCl gas, and various VOCs, the identity of which depends on the organic solvent chosen by the person performing the One Pot. Additionally, volatilized methamphetamine is expected to be observed in the air within sites of One Pot methamphetamine production. As a freebase, methamphetamine is highly volatile, causing it to contaminate the ambient air within and around a site of production.[91] This volatilized methamphetamine is released in varying concentrations by P2P, Red-P, and Birch reduction cooks at concentrations that vary between cook types and even between cooks of the same type.[86,92]

In naïve methamphetamine users, CNS stimulation can occur with doses as low as 0.07 mg/kg.[93] In Red-P labs, methamphetamine concentrations in the air have been observed as high as 5500 $\mu g/m^3$ of air and in Birch reduction labs, methamphetamine concentrations have been observed as high as 680 $\mu g/m^3$ of air.[86] With an average adult weighing 62 kg and having a tidal respiratory volume of 0.0005 m^3, exposure of times of 1.5 and 12.5 hours, respectively, can lead to CNS stimulation, with children being affected at a much quicker rate.[94,95] Additionally, volatilized methamphetamine can settle to low-lying areas within a lab, putting children at an increased risk for exposure and can re-volatilized if the area is disturbed by people moving within the lab. This puts children at further risk of being exposed to methamphetamine, whether or not the location is an active methamphetamine lab. While the amount of volatilized methamphetamine released by Red-P and Birch reduction labs has been documents, it is currently unknown how much methamphetamine is released into the air within a One Pot methamphetamine lab.[86]

This review of the literature has outlined many of the airborne contaminants that stem from methamphetamine laboratories. While methamphetamine can be made via several

production routes, much research has been performed to determine the hazards associated with these different production routes and at what concentration they are present. While the One Pot is currently the number one route of methamphetamine production within the United States, the air contamination resulting from these cooks has yet to be characterized.[5] Without knowing what compounds are contaminating the air within and around a One Pot methamphetamine lab, and at what concentration these contaminants are present, public safety officials cannot make knowledgeable choices regarding the safety precautions that should be taken before entering these locations, the amount and type of decontamination that should be applied to these locations, and the health risks associated with those who come across or live near these lab sites. The goal of this research is to examine the air within a site of One Pot methamphetamine production, as well as perform standoff detection to monitor the air at varying distances from the site to determine the identity and concentration of air contaminants, such as ammonia gas, VOCs, and methamphetamine present. Completion of these goals will allow public safety officials to better understand what hazards are present with a One Pot methamphetamine lab, how far those hazards are able to drift from the site of production, and what the long-term health effects may be for those living near these sites of production.

3.3 Methodology

3.3.1 Reagents and materials

To best replicate a One Pot methamphetamine lab that would be found in an illicit clandestine lab, all reagents but lithium and pseudoephedrine were purchased from a local supermarket; lithium ribbon was purchased from Sigma-Aldrich (Sigma-Aldrich Corp, St. Louis, MO) while a mixture of ground pseudoephedrine and ephedrine tablets were obtained from a government source. Ammonium nitrate was obtained from instant cold compress packs (GoGoods.com, Inc., Columbia, MD). Sodium hydroxide was obtained from Drain Out® Crystal

Clog Remover (Summit Brands, Fort Wayne, IN). The organic solvents used included Prestone®

Premium Starting Fluid (Prestone Products Corporation, Chicago, IL) and Coleman® Camp Fuel

(Coleman Company, Wichita, KS). Equate® mineral oil and Great Value™ iodized salt were

purchased from Wal-Mart (Wal-Mart Stores, Inc., Bentonville, AR). Sulfuric acid was obtained

from Rooto Professional Drain Opener (Rooto Corporation, Howell, MI). While the

aforementioned brands of household chemicals were used for One Pot methamphetamine

production during this project, this in no way implies these are the only brands used during illicit

methamphetamine production nor does it endorse their use.

The remaining materials were for laboratory analysis performed by CDC-NIOSH at the

Taft Laboratory in Cincinnati, OH. Methamphetamine reference standards were purchased at a

concentration of 1 mg/mL in methanol from Cerilliant (Cerilliant Corporation, Round Rock, TX).

Methamphetamine-BSA conjugates and monoclonal antibodies for methamphetamine were

purchased from Arista Biologicals (Arista Biologicals Inc, Allentown, PA). A Milli-Q® Integral

system (Millipore Corporation, Billerica, MA) was used to obtain water filtered at 18 MΩ.

Microspheres were purchased from Luminex (Luminex Corporation, Austin, TX). Activation

buffer, wash buffer, storage/blocking buffer, and HEPES were supplied by Sigma (Sigma

Chemical Co, St. Louis, MO). Biotin-labeled anti-mouse IgG, 1-ethyl-3-(3-dimethyl-

aminopropyl)-carbodiimide hydrochloride, and N-hydroxysulfosuccinimide sodium salt were

purchased from Pierce Biotechnology (Pierce Biotechnology Inc, Rockford, IL). StreptavidinR-

phycoerythrin was purchased from Molecular Probes (Molecular Probes, Eugene, OR). PBS

containing 0.1% Triton™ X-100 was obtained from Mallinckrodt (Mallinckrodt Specialty

Chemical Company, Paris, KY).

3.3.2 One Pot methamphetamine cooks

Six One Pot methamphetamine cooks were performed from Tuesday, November 28, 2017 through Thursday, November 30, 2017 at the Oklahoma State University Fire Research and Training Center in Stillwater, OK. Of the 6 One Pot methamphetamine cooks performed, 3 used starting fluid (ethyl ether) as an organic solvent and 3 used camp fuel (light petroleum distillate) as an organic solvent. In order to best replicate a One Pot methamphetamine lab that would be used for illicit production, a modified cook recipe known to be used by clandestine chemists was followed and the lab was set up in a plastic garden shed to mimic a common site of clandestine methamphetamine manufacture (Figure 17).[96] For safety, researchers performing the cooks were dressed in level B protection (Figure 18), which included Tychem 2000 SFR chemically protective suits (DuPont, Wilmington, DE) worn over Workrite® FR thermally protective jumpsuits (Workrite Uniform Company, Nashville, TN), HazProof® chemically protective boots (Tingley Rubber Corp, Piscataway, NJ) and a Scott™ self-contained breathing apparatuses (SCBA) (3M, Saint Paul, MN). Due to the high fire-hazard associated with One Pot methamphetamine production, a firefighter was placed on standby in turnout gear during all One Pot cooks. Turnout gear included fire-resistant coat and overalls, a SCBA, a hardhat with face shield, and with a primed water hose (Figure 19).

For the One Pots, 600 mg of ground pseudoephedrine was added to a 32 oz plastic bottle. The contents of a 6"x9" instant cold compress pack (GoGoods.com, Inc, Columbia, MD) (ammonium nitrate) were then added to the plastic bottle, followed by either 2.5 cans of starting fluid or 600 mL of camp fuel. Before the starting fluid could be added to the plastic bottle, the cans needed to be depressurized. This was done by inverting the cans while holding down the dispenser button until air no longer expelled. The bottom was then pierced with a bottle opener so the solvent could be added to the plastic bottle. After the addition of the organic solvent, a single capful of sodium hydroxide was added to the cook bottle (Figure 20), followed by

approximately 6 mL of water, which catalyze the production of ammonia gas. Six 0.5 g strips of

lithium ribbon were then added to the plastic bottle and the bottle was capped.

Figure 17. The set up for the One Pot methamphetamine cooks performed. Cooks were performed in a plastic garden shed to simulate what a real cook environment may be like.

Figure 18. Level B protective suit, including chemically and thermally protective suits and a self-contained breathing apparatus

Figure 19. To mitigate fire hazards associated with One Pot methamphetamine labs, a trained firefighter was on standby in turnout gear with a primed hose throughout the duration of the cooks.

Figure 20. Sodium hydroxide was added to the One Pot methamphetamine cook in capful aliquots as deemed necessary for each individual reaction.

Once the bottle was capped, it was swirled to allow the added water to interact with the ammonium nitrate and sodium hydroxide, generating ammonia gas. The One Pot was placed in a ring stand to prevent tipping and was allowed to react, or "roll", for 1 hour. Every 5 minutes, the lid to the bottle was opened slightly to depressurize, or "burp", the reaction, allowing some ammonia gas to be released. If the reaction slowed and the rolling ceased, another capful of sodium hydroxide was added to the bottle during the next burping step.

After 1 hour of rolling, the cap was slowly removed from the bottle, fully releasing the ammonia gas from the bottle. Using forceps, the lithium strips were removed from the One Pot and placed under mineral oil to mitigate flammability. The solvent from the One Pot was then poured through two coffee filters (Farmer Bros Co, Ft. Worth, TX) into a clean, one-pint Mason jar (Kerr Glass Manufacturing Corp, Lancaster, PA). The plastic cook bottle was then rinsed with an additional 200 mL of organic solvent, and that additional solvent was also poured through the coffee filters into the Mason jar. After the organic solvent had been filtered into the Mason jar, one inch of iodized salt was added to a hydrogen chloride gas generator, comprised of a clean, 20 oz plastic bottle with a hose protruding from the cap (Figure 21). Approximately 9 mL of Rooto Professional Drain Opener (sulfuric acid) was added to the bottle and the cap was quickly screwed on. The salt/sulfuric acid mixture generated HCl gas, which was bubbled into the filtered organic solvent from the One Pot, causing the powdered methamphetamine salts to precipitate out of solution.

Figure 21. A typical hydrogen chloride gas generator, comprised of a plastic bottle with a hole in the cap and a hose protruding from the hole.

Following precipitation, the salts were separated from the organic solvent via vacuum filtration. The salts were then air dried at ambient conditions to allow for any remaining solvent to evaporate. The resulting salts were then subjected to a NIK Public Safety Narcotics Identification System presumptive colorimetric test (NIK Public Safety Inc, Jacksonville, FL) as well as Raman spectrometry to demonstrate successful conversion of pseudoephedrine to methamphetamine. The NIK colorimetric test used was Test U: Methamphetamine or MDMA (Ecstasy), which turns dark purple when methamphetamine is present (Figure 22). The Raman spectrometer used for field identification of methamphetamine was a FirstDefender™ RMX RX2863 Raman spectrometer (Thermo Scientific, Waltham, MA) and a sample spectrum obtained from this instrument can be seen in Figure 23. Once the production of methamphetamine had been confirmed, all fractions of the One Pot lab, including the liquid waste, solid waste, and the salts were disposed of in a fire-control study, conducted under the guidance of the Oklahoma Bureau of Narcotics and Dangerous Drugs (OBNDD).

Figure 22. An example of a positive NIK Public Safety Narcotics Identification System presumptive colorimetric test. Test U: Methamphetamine and MDMA was used to identify the presence of methamphetamine in the salts produced by each One Pot.
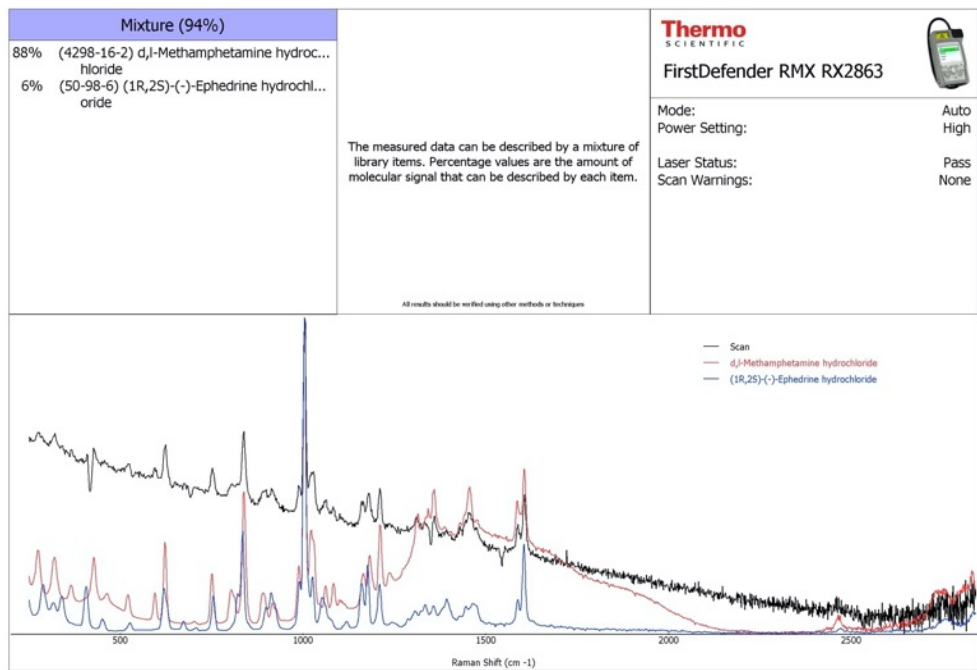
Figure 23. Raman spectrum obtained from analysis of salts produced during a One Pot methamphetamine cook. The black line is the spectrum obtained from the salts, the red line is the library spectrum for d/l-methamphetamine-HCl, and the blue line is the library spectrum for (1R,2S)-(-)-ephedrine-HCl.

3.3.3 Intra-shed air sampling

Air was collected from inside the cook shed before, during and after the One Pot methamphetamine cooks and analyzed to identify and quantitate volatilized methamphetamine and VOCs expelled from the One Pots during methamphetamine production. Air samples were collected using battery-powered, active air samplers provided by CDC-NIOSH as well as vacuum canister-style passive air samplers provided by Entech Instruments (Entech Instruments, Simi Valley, CA). The active air samplers were utilized for all three ether One Pots and the last two camp fuel One Pots. The passive air samplers were utilized for the first two ether One Pots and the first two camp fuel One Pots.

3.3.3.1 Active air sampling

Prior beginning the One Pot methamphetamine cooks, the five SKC Airchek 2000 active air samplers (SKC Inc, Eighty Four, PA) were each fitted with a 37 mm x 2 µm polytetrafluoroethylene (PTFE) filter and the pumps were set to draw in air at a rate of 1.5 L/min. The PTFE filters were used to trap volatilized methamphetamine found in the air that was pulled through the active air samplers. Four of the active samplers were set up inside the shed in two locations while the fifth sampler was fitted to one of the researchers performing the One Pot methamphetamine cook (Figure 24). As shown in Figure 25, the two locations housing the other four samplers were to the left of active One Pot and behind and to the right of the researchers. The active air sampler fitted to the researcher and one active air sampler from each of the other two locations within the shed were turned on and began pulling air through the filter immediately prior to starting a One Pot cook. The remaining two active air samplers, one from each location within the shed, were turned on and began pulling air through the filter after the One Pot was filtered, just prior to assembly of the HCl gas generator. Two PTFE filters were treated as field blanks; one was briefly exposed to the environment near the shed before any One Pot

methamphetamine cooks were performed and the second was exposed to the environment near the shed 30 minutes after the last One Pot methamphetamine cook had concluded. During the final ether One Pot methamphetamine cook, the pumps containing filters 25 and 26 were not turned on, resulting in no data collection from these filters. Additionally, the pumps containing filters 7 and 8 were actively sampling during the first camp fuel bottle failure. These pumps remained on throughout the duration of the cook that resulted in the bottle failure, as well as the second camp fuel cook, which was performed immediately following the bottle failure. Table 12 summarizes the sampler location, the time each active sample pump was on, and the average flow rate of the air being pulled through the sampler.

Figure 24. An SKC Airchek 2000 active air sampler fitted to a researcher as they performed a One Pot methamphetamine cook.

Figure 25. Locations of 4 out of 5 active air samplers. Two samplers were placed in each location designated by the orange circles. Two samplers were located left of the active One Pot methamphetamine lab and two samplers were located behind and to the right of the researchers performing the One Pot methamphetamine cooks.

Table 12. Active air pump locations and volumetric sampling information.

	Filter Number	Pump S/N	Start Time	End Time	Pump Run Time (min)	Avg. Total Flow Rate (mL/min)	Total Volume Sampled (L)	Pump Location	Activated Period	Cook Type
Cook 2	1	59755	14:25	16:42	140	1490.25	210.00	Front Left	Full	Camp Fuel
	2	50876	14:26	16:42	135	1483.85	200.70	Back Right	Full	Camp Fuel
	3	34665	14:23	16:41	138	1486.95	206.20	Researcher	Full	Camp Fuel
	4	34643	16:00	16:42	37	1482.00	68.00	Front Left	Salting Out	Camp Fuel
	5	34747	16:01	16:42	37	1512.65	56.00	Back Right	Salting Out	Camp Fuel
	6	-	-	-	-	-	-	Field Blank	Flash Exposure	Field Blank
Cook 3	7	59755	9:22	10:43	71	1510.50	107.25	Front Left	Full	Camp Fuel
	8	50876	9:23	10:43	70	1498.60	104.90	Back Right	Full	Camp Fuel
	9	34665	9:23	10:22	58	1507.40	87.43	Researcher	Full	Camp Fuel
	10	34643	12:20	12:32	14	1450.65	20.31	Front Left	Salting Out	Camp Fuel
	11	34747	12:14	12:32	18	1496.05	26.93	Back Right	Salting Out	Camp Fuel
Cook 4	12	34665	14:17	16:11	114	1506.15	171.70	Researcher	Full	Ether
	13	59755	14:15	16:08	111	1511.80	167.81	Front Left	Full	Ether
	14	50876	14:15	16:08	111	1506.55	167.23	Back Right	Full	Ether
	15	34747	15:41	16:08	31	1629.55	50.52	Back Right	Salting Out	Ether
	16	34643	15:41	16:06	29	1492.85	43.00	Front Left	Salting Out	Ether
Cook 5	17	59755	9:26	11:16	104	1508.90	156.93	Back Right	Full	Camp Fuel
	18	50876	9:26	11:16	104	1513.55	157.41	Front Left	Full	Camp Fuel
	19	34665	9:24	11:17	113	1404.70	158.73	Researcher	Full	Camp Fuel
	20	34643	10:58	11:16	13	1499.70	19.50	Back Right	Salting Out	Camp Fuel
	21	34747	10:58	11:16	13	1629.20	21.18	Front Left	Salting Out	Camp Fuel
Cook 6	22	59755	13:04	14:38	94	1491.05	140.15	Front Left	Full	Ether
	23	50876	13:04	14:38	94	1490.35	140.09	Back Right	Full	Ether
	24	34665	13:04	13:13	9	1226.10	11.03	Researcher	Full	Ether
	25	34643	-	-	-	-	-	Front Left	-	Ether
	26	34747	-	-	-	-	-	Back Right	-	Ether
	27	-	-	-	-	-	-	Field Blank	Flash Exposure	Field Blank

Upon completion of the One Pot methamphetamine cooks, the PTFE filters were

removed from the active air samplers and sent to the CDC-NIOSH Taft Laboratory in Cincinnati,

OH for quantitative analysis of the volatilized methamphetamine collected from the air samples.

At CDC-NIOSH, methamphetamine trapped on the PTFE filters was extracted with 2 mL of

phosphate-buffered saline (PBS) containing Triton[TM] X-100 as a surfactant (wetting buffer) and

then analyzed using a fluorescence covalent microbead immunosorbent assay (FCMIA)

developed by the Luminex Corporation (Luminex Corporation, Austin, TX).[97,98] The FCMIA

method used to analyze the samples was first developed by Smith et al. in 2010 and is

summarized in Figure 26.[99] Briefly, methamphetamine calibrators were prepared at 15, 7.5, 3.75,

1.88, 0.94, 0.46, 0.23, and 0 ng/mL in wetting buffer diluted 1/3 with storage/blocking buffer

(PBS, 1% Bovine serum Albumin (BSA), 0.05% sodium azide, pH=7.4). Fifty microliters of

methamphetamine conjugated microspheres at a concentration of 1×10^5 microspheres/mL in

storage/blocking buffer were added to the wells of a 1.2 µm filter membrane microtiter plate

(Merck Millipore Co, Burlington, MA) and the liquid was aspirated via a Millipore vacuum

manifold. After the wells were dried, 50 µL of the calibrators or methamphetamine-PSB-

Triton[TM] X-100 solutions were added to the wells, along with 50 µL of primary anti-

methamphetamine antibodies at a 1/250,000 dilution in storage/blocking buffer. The

microspheres, primary antibodies, and samples were then allowed to incubate at 37°C for 30

minutes on a microplate shaker in the absence of light.

After incubation, the wells of the microtiter plate were washed three times with wash

buffer (PBS, 138 mM sodium chloride, 2.7 mM potassium chloride, 0.05% Tween® 20). Next, 50

µL of 5 µg/mL biotin labeled, anti-mouse IgG in storage/blocking buffer was added to the wells,

and the plate was again allowed to incubate at 37°C for 30 minutes on a microplate shaker in the

absence of light. Following the second incubation, the wells were once again washed three times

with wash buffer and 50 µL of 4 µg/mL streptavidin R-PE reporter in storage/blocking buffer was

added to the wells. The plates were then incubated a third time at 37°C for 30 minutes on a

microplate shaker in the absence of light.
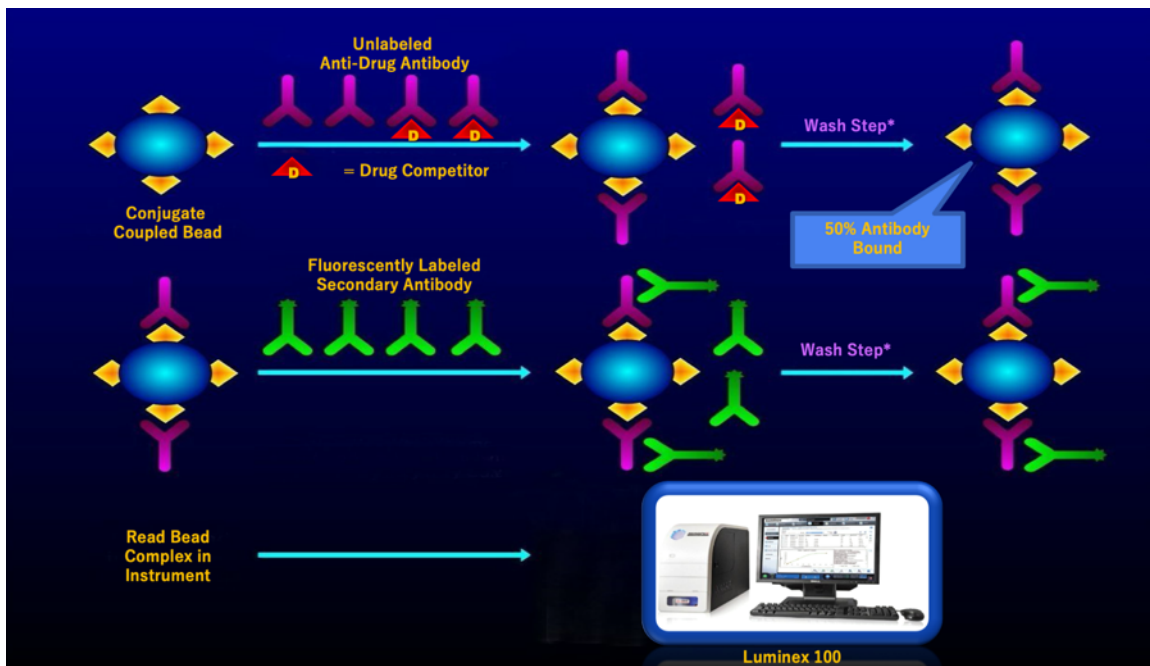
Figure 26. Schematic of the FCMIA used to quantitate methamphetamine captured on the PTFE filters during active air sampling. **Top.)** Methamphetamine present in samples competes with conjugated microspheres to bind primary antibodies. All antibodies not bound to microspheres are washed away. **Middle.)** A fluorescently labeled secondary antibody binds the primary antibody. All antibodies not bound are washed away. **Bottom.)** Microbead-antibody complex is loaded onto a LUMINEX 100 and fluorescence in measured. Because FCMIA is a competitive assay, the more methamphetamine present in the sample, the less fluorescence is observed by the LUMINEX 100 instrument.

Following the final incubation, the wells were washed three times with wash buffer and

the microspheres were resuspended in 100 μL of wash buffer. The microtiter plate was then

shaken vigorously for 1 minute to disperse the microspheres and the plate was loaded onto the

autosampler of the LUMINEX 100 instrument. The LUMINEX 100 was programmed to collect

data from 100 microspheres per sample and report the median fluorescence intensity (MFI) of the

microsphere-drug conjugate-primary anti-drug conjugate IgG antibody-secondary anti-IgG-

biotin-avidin complex. Since the FCMIA is a competitive immunoassay, the more

methamphetamine present in the sample loaded into the microtiter plate well, the less the sample

fluoresced. All samples were run in duplicate and the concentration reported was the average of the two results.

3.3.3.2 Passive air sampling

Passive air sampling was achieved using vacuum canister-style samplers provided by Entech Instruments. Ten air samplers were utilized for each of the four One Pot methamphetamine cooks monitored. The 10 samplers utilized included 3 Silonite™ treated, 1 L vacuum MiniCans™, 2 helium diffusion samplers (HDS), 4 Diffusive Sorbent Pens (DSP), and one 40 mL screw-top vial grab sampler. Each researcher performing the One Pot methamphetamine cooks had a HDS sampler clipped to the upper-left strap of the SCBA air tank harness and set of DSP samplers, one packed with Carbo Pack X and one packed with Tenax TA sorbent, clipped to the upper-right strap of the SCBA air tank harness. An example of the passive samplers can be seen in Figure 27.

Figure 27. Passive air monitors provided by Entech Instruments for capturing air contaminants during One Pot cooks. **a.)** 1L Minican™ vacuum canister, **b.)** Helium diffusion sampler (HDS), **c.)** A set of diffusive sorbent pens (DSPs), one packed with Carbo Pack X and one packed with Tenax TA sorbent, and **d.)** a 40 mL screw-top grab sampler.

The 3 samplers clipped to each researcher passively collected air at breathing level throughout the duration of each One Pot cook. The 1 L MiniCan™ vacuum canister grab samples were collected at 3 points throughout the One Pot methamphetamine cooks: prior to the start of

the cooks, after the cooks were complete but before salting out began, and after the methamphetamine salts had been separated from the post-salt solvent via filtration. The 40 mL screw-top vial grab sampler was left open to the environment on a shelf in the back-left of the cook shed, approximately 1 m from the ground; it was opened just prior to assembling the HCl gas generator and was capped following filtration of the methamphetamine salts from the post-salt solvent. Table 13 summarizes the sampler demographics and the time each sampler was allowed to collect air for.

Upon completion of the One Pot methamphetamine cooks, all passive air samplers were sent to Entech Instruments for quantitative, semi-quantitative, and qualitative analysis by gas chromatography mass spectrometry (GC-MS). The 1 L MiniCans^{TM} and the HDS personal monitors were analyzed using an Entech 7200 preconcentrator and 7650-M autosampler coupled to an Agilent 6890 GC instrument with an Agilent 5973 MS (Agilent Technologies, Santa Clara, CA). This instrumental set up is shown in Figure 28. The Entech 7200 preconcentrator concentrated 100 mL of the air from the 1 L MiniCans^{TM} to a volume of 1 µL, resulting in a 100,000 fold concentration of the sample. For the HDS samplers, the preconcentrator concentrated the entire 16 mL volume of the sampler into 10 mL, resulting in a 1.6 fold concentration of the sample.

Chromatographic separation was achieved with a DB-1 column (60 m x 0.320 mm x 1 µm) from Agilent. The GC was operated in splitless mode. Injection volumes were 1 µL for the 1 L MiniCans^{TM} and 10 µL for the HDS personal monitors. The column oven was programmed as follows: start at 35°C and hold for 5 minutes, increase to 95°C at 6°C/min, increase to 140°C at 10°C/min, and finally increase to 220°C at 15°C/min for a total run time of 24.83 minutes. The MS was set to scan from 29-280 m/z at 3 scans per second.

Table 13. Passive air sampler demographics and air collection time.

	Sampler Type	Serial Number	Period Sampled	Time Sampled	Cook Type
Cook 1	HDS Personal Monitor	4001100	Full Cook	2.5 hours	Ether
	DSP Tenax TA	811-0000038	Full Cook	2.5 hours	Ether
	DSP Carbo Pack X	838-0000211	Full Cook	2.5 hours	Ether
	HDS Personal Monitor	4001101	Full Cook	2.5 hours	Ether
	DSP Tenax TA	811-0000037	Full Cook	2.5 hours	Ether
	DSP Carbo Pack X	838-0000214	Full Cook	2.5 hours	Ether
	MiniCan™ Grab Sample 1	3611	Before Cook	1 minutes	Ether
	MiniCan™ Grab Sample 2	3622	During Cook	28 seconds	Ether
	MiniCan™ Grab Sample 3	3621	After Cook	30 seconds	Ether
	40mL Vial	1	During Cook	Salt to filter*	Ether
Cook 2	HDS Personal Monitor	4001103	Full Cook	2 hours	Camp Fuel
	DSP Tenax TA	811-0000039	Full Cook	2 hours	Camp Fuel
	DSP Carbo Pack X	8380000210	Full Cook	2 hours	Camp Fuel
	HDS Personal Monitor	4001098	Full Cook	2 hours	Camp Fuel
	DSP Tenax TA	811-0000042	Full Cook	2 hours	Camp Fuel
	DSP Carbo Pack X	838-0000215	Full Cook	2 hours	Camp Fuel
	MiniCan™ Grab Sample 1	3612	Before Cook	30 seconds	Camp Fuel
	MiniCan™ Grab Sample 2	3623	During Cook	30 seconds	Camp Fuel
	MiniCan™ Grab Sample 3	3624	After Cook	30 seconds	Camp Fuel
	40mL Vial	2	During Cook	Salt to filter*	Camp Fuel
Cook 3	HDS Personal Monitor	4001106	Full Cook	30 minutes	Camp Fuel
	DSP Tenax TA	811000041	Full Cook	30 minutes	Camp Fuel
	DSP Carbo Pack X	8380000216	Full Cook	30 minutes	Camp Fuel
	HDS Personal Monitor	4001105	Full Cook	30 minutes	Camp Fuel
	DSP Tenax TA	8110000036	Full Cook	30 minutes	Camp Fuel
	DSP Carbo Pack X	8380000209	Full Cook	30 minutes	Camp Fuel
	MiniCan™ Grab Sample 1	3614	Before Cook	30 seconds	Camp Fuel
	MiniCan™ Grab Sample 2	3616	During Cook	30 seconds	Camp Fuel
	MiniCan™ Grab Sample 3	3620	After Cook	30 seconds	Camp Fuel
	40mL Vial	3	During Cook	Salt to filter*	Camp Fuel
Cook 4	HDS Personal Monitor	4001107	Full Cook	2 hours	Ether
	DSP Tenax TA	8110000035	Full Cook	2 hours	Ether
	DSP Carbo Pack X	8380000213	Full Cook	2 hours	Ether
	HDS Personal Monitor	4001099	Full Cook	2 hours	Ether
	DSP Tenax TA	8110000040	Full Cook	2 hours	Ether
	DSP Carbo Pack X	8380000212	Full Cook	2 hours	Ether
	MiniCan™ Grab Sample 1	3618	Before Cook	30 seconds	Ether
	MiniCan™ Grab Sample 2	3615	During Cook	30 seconds	Ether
	MiniCan™ Grab Sample 3	3621	After Cook	30 seconds	Ether
	40mL Vial	4	During Cook	Salt to filter*	Ether

*The container was opened when salting out the methamphetamine free base from the solvent with the HCl acid gas generator. It was closed when the solid methamphetamine salts were filtered.
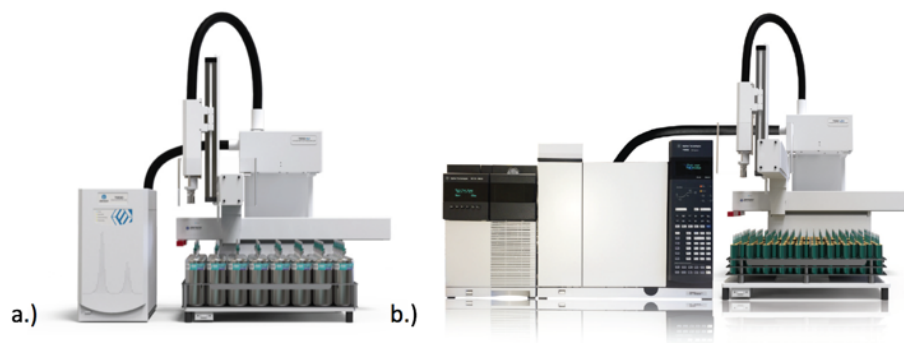
Figure 28. Instrumental set up for analysis of the 1 L MiniCans™ and HDS personal monitors. **a.)** The Entech 7200 preconcentratior with the 1 L MiniCans™ loaded. **b.)** The Entech 7200 preconcentrator with the HDS personal monitors loaded and a coupled Agilent GC-MS.

For the DSPs, thermal desorption was used to strip the analytes from the Carbo Pack X and Tenax TA sorbents and introduce them to the GC-MS for analysis. Prior to instrumental analysis, the Carbo Pack X DSPs were spiked with an internal standard mix using an Entech 4200 Sorbent Pen Spiking System. The Tenax TA DSPs were not spiked with an internal standard mix so all the compounds collected and analyzed from these DSPs were qualitative only. The DSPs were then loaded on to the Entech 5800 Sorbent Pen Desorption Unit that was coupled to an Agilent 7890B GC and an Agilent 5977A MS. This instrumentation is shown in Figure 29.
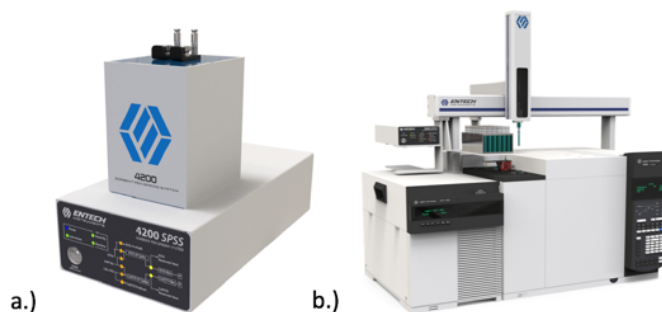
Figure 29. Instrumental set up for analysis of the DSPs, including **a.)** the Entech 4200 Sorbent Pen Spiking System and **b.)** the Entech 5800 Sorbent Pen Desorption Unit coupled to an Agilent GC-MS.

Due to the difference in sorbent binding properties and the compounds captured by the two different sorbent types, desorption conditions and chromatographic separation were different for the Carbo Pack X and the Tenax TA sorbents. For the Carbo Pack X sorbent, desorption was achieved by first preheating the DSP at 350°C for 2 minutes, and then desorbing the DSP at 300°C for 5 minutes. Chromatographic separation was achieved with a DB1 pre-column (5 m x 0.530 mm x 0.5 µm) from Agilent followed by a DB-1 column (60 m x 0.320 mm x 1 µm), also from Agilent. The GC was operated in split mode with a 25:1 split ratio. The column oven was programmed as follows: start at 35°C and hold for 5 minutes, increase to 150°C at 10°C/min, then increase to 210°C at 15°C/min and hold for 9.5 minutes, for a total run time of 30 minutes. The MS was set to scan from 34-450 m/z at 3 scans per second. For the Tenax TA sorbent, desorption was achieved by first preheating the DSP at 260°C for 2 minutes and then desorbing the DSP at 260°C for 5 minutes. Chromatographic separation was achieved with a Silonite™ coated 0.6 m filmless tubing pre-column followed by a DB-5ms Ultra Inert column (30 m x 0.25 mm x 0.5 µm) from Agilent. The GC was operated in split mode with a 10:1 split ratio. The column oven was programmed as follows: start at 35°C and hold for 5 minutes, then increase to 300°C at 10°C/min and hold for 6.5 minutes for a total run time of 38 minutes. The MS was set to scan from 34-450 m/z at 3 scans per second.

To analyze the air captured by the 40 mL screw-top vial, internal standards were spiked into the sample through the cap liner. Once internal standards had been added, a headspace sorbent pen (HSP) was inserted into the cap and created a seal with the cap liner. The samples were then evacuated to less than 0.01 atm through the HSP via a Vial Evacuation Tool and placed into a 5600 Sorbent Pen Extraction System (SPES) by Entech Instruments. In the SPES, the air samples were subjected to Vacuum Assisted Sorbent Extraction (VASE), which comprised of extracting the air sample into the HSP by placing the sample under vacuum for 20 hours at 35°C. After VASE, the HSP was removed from the vial and analyzed by an Entech 5800 Sorbent Pen Desorption Unit coupled to an Agilent 7890B GC and an Agilent 5977A MS.

Desorption of the HSPs was achieved by first preheating them at 260°C for 2 minutes and then desorbing them at 260°C for 5 minutes. Chromatographic separation was achieved with a Silonite™ coated 0.6 m filmless tubing pre-column followed by a DB-5ms Ultra Inert column (30 m x 0.25 mm x 0.5 μm) from Agilent. The GC was operated in split mode with a 10:1 split ratio. The column oven was programmed as follows: start at 35°C and hold for 5 minutes, then increase to 300°C at 10°C/min and hold for 6.5 minutes for a total run time of 38 minutes. The MS was set to scan from 34-450 m/z at 3 scans per second.

3.3.4 Standoff air monitoring

Standoff air monitoring was performed during each cook to assess the amount of ammonia gas and VOCs that could be detected at various distance from the site of One Pot methamphetamine production. HCl was also monitored for, but the sensor installed on the Los Gatos portable integrated cavity output spectrometer (ICOS) (ABB-Los Gatos Research, San Jose, CA) malfunctioned, preventing HCl levels from being recorded. Instrumentation used during standoff air monitoring included the Los Gatos portable ICOS, the Geospatial Measurement of Air Pollution (GMAP) vehicle, sensor pods (SPods), and a forward looking infrared (FLIR) camera. The GMAP vehicle, SPods, and FLIR camera were all provided by the United States Environmental Protection Agency's National Enforcement Investigations Center (EPA-NEIC, Denver, CO).

3.3.4.1 Ammonia monitoring

Ammonia gas detection was achieved by the GMAP vehicle and the Los Gatos ICOS. The GMAP vehicle, shown in Figure 30, is a mobile vehicle equipped with instrumentation meant to aid in industrial gas leak detection and repair. On-board instrumentation includes: an integrative cavity output spectrometer (ICOS) for analysis of methane (CH_4) and carbon dioxide

(CO2); a differential ultraviolet absorption spectrometer (DUVAS) for analysis of benzene, toluene, ethylbenzene, and xylenes (collectively called BTEX compounds); a photo ionization detector (PID) for analysis of volatile organic compounds (VOCs); a global positioning system (GPS) connected to Google Earth Pro (GEP) for sample mapping; a compact meteorological station to monitor wind speed and direction; and an air canister collection mechanism. When the GMAP vehicle is moving, collected samples can be mapped on GEP to aid in source determination for monitored air contaminants. When the GMAP vehicle is stationary, collected samples can be used to develop polar plots to map areas of differing air contaminant concentrations in order to locate the source of the effluents. If conditions are ideal, the stationary GMAP vehicle can also be used to estimate the rate of air effluent emissions from a source.

Figure 30. The GMAP vehicle operated by EPA-NEIC. On-board instrumentation includes an ICOS, PID, DUVAS, GPS, a meteorological station, and an air canister collection mechanism.

While none of the on-board instrumentation included in the GMAP vehicle were routinely used for ammonia analysis, the DUVAS was able to detect the gas, as it had been calibrated for ammonia measurement during factory assembly. Although able to detect ammonia

gas, this detection was qualitative in nature for three reasons. First, the DUVAS was not field calibrated due to the lack of a calibration standard. Second, the DUVAS did not have a heated sample line, which could lead to moisture accumulation and inhibit ammonia from reaching the instrument's detector. Third, the DUVAS had stainless steel fittings, which are commonly pitted and provide absorption sites for the polar ammonia molecules to adhere to as they enter the instrumentation, inhibiting the ammonia from reaching the instrument's detector. The GMAP vehicle parked approximately 15 m downwind of the One Pot cook site and the DUVAS was used to monitor for ammonia gas during all One Pot methamphetamine cooks. The GMAP vehicle's location during each One Pot cook is summarized in Table 14 and shown in Figure 31.

A portable ICOS by Los Gatos Research was also used to detect and quantitate ammonia plumes generated by the One Pot methamphetamine labs, with a quantitative range of $0.01 - 200$ ppm ($7-139,000$ μg ammonia/m^3 air). The ICOS internal optical cavity utilizes continuous-wave lasers to data-log low ppm-level ammonia using an external diaphragm vacuum pump, all which could be powered via battery packs. Due to its mobility, the ICOS could be easily deployed at various distances downwind of the One Pot cook site and moved as the wind was observed to shift (Figure 32), or it could be placed in the back of a rental car, which drove laps around the Oklahoma State University Fire Research and Training Center during times of active One Pot methamphetamine production (Figure 33).

Table 14. GMAP vehicle and SPod sampling events and locations (See Figure 31).

Data Set	Cook	GMAP Location	SPod 25 m Site	SPod 50 m Site	SPod 75 m Site	SPod 100 m Site
1	Ether #1	A	25a	50a	75a	100a
2	Camp Fuel #1	B	25b	50b	75b	100b
3	Camp Fuel Fail #1	C	25b	50b	75b	100b
4	Camp Fuel #2	D	25b	50b	75b	100b
5	Ether #2	C	25b	50b	75b	100b
6	Camp Fuel Fail #2	E	25c	50c	75c	100c
7	Ether #3	E	25c	50c	75c	100c

Figure 31. GMAP vehicle and SPod sampling locations at the Oklahoma State University Fire Research and Training Center. Red pins represent the GMAP vehicle locations while the blue, orange, and yellow pins represent the SPod locations (See Table 14). The red house icon denotes the One Pot production site.

Figure 32. Wind sock seen above ICOS (in yellow cart) which was deployed 100 m from the cook site (out of view to left of frame).

Figure 33. Configuration of battery-powered ICOS (larger yellow in foreground), vacuum pump (gray), battery pack (smaller yellow to right) as positioned in moving vehicle during data-logging. Trace background ammonia detected from vehicle exhaust was dampened or eliminated by opening all vehicle windows when vehicle was in motion or by shutting off the engine when parked.

3.3.4.2 VOC monitoring

Several PIDs were used to accomplish standoff monitoring for VOCs. The first PID was installed on the GMAP vehicle, allowing it to assess the level of VOCs that could be measured approximately 15 m from the site of One Pot production (Table 14 and Figure 31). The PID on-board the GMAP vehicle had a standard 10.6 eV lamp that was calibrated to isobutylene, so all VOC concentrations are reported as parts per billion (ppb) isobutylene.

All other PIDs used for the standoff monitoring of VOCs were SPods provided by EPA-NEIC (Figure 34) The SPods were developed as a stationary, fence line-type instrument to monitor for gas leaks and other unwanted releases of VOCs. The SPods contain a PID with a 10.6 eV lamp, as well as a meteorological station to monitor wind speed and direction. The solar-powered, SPods have the capability to develop polar plots in the same manner as the GMAP vehicle, though this was not incorporated into this particular study. For this study, 4 SPods were deployed downwind of the One Pot methamphetamine cooks at graduated distances from the cook shed. Three of the SPods were those developed by the EPA's Office of Research and Development (EPA-ORD, Research Triangle Park, NC) and the fourth SPod is a commercially available device developed by SenSevere Environmental Sensors (SenSevere LLC, Pittsburgh, PA). The standoff distances that the SPods were placed in-line from the cook shed were 25, 50, 75, and 100 m. As with the GMAP vehicle, the SPods were deployed during all 6 One Pot methamphetamine cooks. The three SPods developed by EPA rotated between the 25, 50, and 75 m location, with each SPod being deployed at each distance during one day of sampling; the SenSevere SPod was always deployed at 100 m. Table 14 and Figure 31 summarize the SPod deployment locations during the One Pot methamphetamine cooks.

Figure 34. Three SPods deployed at 50, 75, and 100 m from the One Pot methamphetamine cook shed.

The final piece of equipment used for standoff monitoring of VOCs was a FLIR camera. The camera used was a GF320 infrared imaging video camera designed by FLIR Systems (FLIR Systems Inc, Wilsonville, OR) (Figure 35). The FLIR was used to visualize the VOCs being emitted by the One Pot methamphetamine cooks. This was accomplished by use of a bandpass filter in the camera that limited the image to a 3.2-3.4 μm section of the IR spectrum. Use of the bandpass filter ensured only compounds with sufficient IR signals at the desired range were visible by the camera, and the filter used encompassed the range of greatest signal intensity for most VOCs.[100] To enhance the visualization of the VOCs, the FLIR camera was set to high sensitivity mode, in which part of the image is subtracted in order to better highlight movements, such as VOCs being emitted from the One Pot methamphetamine cook. This background subtraction is a function of the gas concentration being visualized, the path length between the

gas plume and the camera, and the temperature difference between the gas plume and the background.[100]

Figure 35. GF320 FLIR camera designed by FLIR Systems Inc.

3.4 Results

3.4.1 One Pot methamphetamine cooks

Of the 6 One Pot methamphetamine cooks performed, 2 resulted in bottle failures, where the plastic bottle used as a reaction vessel ruptured, spewing organic solvent and other One Pot methamphetamine lab-related byproducts throughout the cook site. Both bottle failures were camp fuel cooks. Following the bottle failure that occurred during the second camp fuel cook (Figure 36), the cook site was cleaned and reset and a new One Pot was started, still using camp fuel as an organic solvent. Following the second bottle failure (Figure 37), a new One Pot could not be performed, as no more pseudoephedrine was available. Instead, the contents of the failed One Pot was dumped into a 64 oz. rectangular, deep dish Glad food storage container (Glad Products Company, Oakland, CA) along with the entire contents of a new instant cold compress pack and a capful of sodium hydroxide and the conversion of pseudoephedrine to

methamphetamine was continued (Figure 38). To burp this One Pot, the lid was taken off the

Glad container and the solid found at the bottom of the One Pot was stirred with a plastic spatula.

All other procedures mirrored that of the other One Pot methamphetamine cooks

Figure 36. A hole formed in the One Pot reaction vessel of the second camp fuel cook due to repeated expansion and relaxation of the plastic.

Figure 37. A hole formed in the One Pot reaction vessel of the final camp fuel cook due to repeated expansion and relaxation of the plastic.

Figure 38. A One Pot methamphetamine lab performed in a 64 oz. Glad food storage container after the original bottle failed during the final camp fuel cook.

3.4.2 Intra-shed air sampling
3.4.2.1 Active air sampling

Of the 27 PTFE filters sent to the CDC-NIOSH laboratory in Cincinnati, OH, 26 were analyzed for the presence of methamphetamine. Filter number 1 was sacrificed to verify the quantitative method, and therefore no data were obtained from this filter. The concentration of methamphetamine captured by each filter is given in Table 15, with the concentration of methamphetamine listed in micrograms of methamphetamine per cubic meter of air.

Statistical analysis was performed on the results of the active air samplers. All statistical analyses were performed by use of Microsoft Excel (Microsoft Corporation, Redmond, WA). An analysis of variance (ANOVA) was used to compare the methamphetamine captured by each sampler within the cook site to get an idea of the distribution of volatilized methamphetamine within the site. A second ANOVA was also used to compare the amount of methamphetamine volatilized prior to and during the salting out process between the two organic solvents used. This second ANOVA was used to examine when the most methamphetamine was volatilized during the One Pot, and if there was a difference between the amount volatilized during each time

point between the two solvents used. Finally, a t-test was performed to compare the total amount of methamphetamine captured by the active air samplers during the ether One Pot cooks and the camp fuel One Pot cooks. This aimed to determine if the solvent type used changed the amount of methamphetamine volatilized during One Pot production. No statistically significant differences were observed with any of these analyses with $\alpha=0.05$.

Table 15. The concentration of methamphetamine captured by the PTFE filters loaded into the SKC Airchek 2000 active air samplers.

	Filter Number	Pump Location	Activated Period	Cook Type	Methamphetamine Conc. ($\mu g/m^3$)
Cook 2	1	Front Left	Full	Camp Fuel	NA
	2	Back Right	Full	Camp Fuel	0.31
	3	Researcher	Full	Camp Fuel	0.87
	4	Front Left	Salting Out	Camp Fuel	3.51
	5	Back Right	Salting Out	Camp Fuel	2.41
	6	Field Blank	Flash Exposure	Field Blank	0.00
Cook 3	7	Front Left	Full	Camp Fuel	2.01
	8	Back Right	Full	Camp Fuel	1.68
	9	Researcher	Full	Camp Fuel	1.35
	10	Front Left	Salting Out	Camp Fuel	0.52
	11	Back Right	Salting Out	Camp Fuel	0.02
Cook 4	12	Researcher	Full	Ether	3.59
	13	Front Left	Full	Ether	0.69
	14	Back Right	Full	Ether	0.40
	15	Back Right	Salting Out	Ether	12.75
	16	Front Left	Salting Out	Ether	2.49
Cook 5	17	Back Right	Full	Camp Fuel	12.75
	18	Front Left	Full	Camp Fuel	1.75
	19	Researcher	Full	Camp Fuel	3.18
	20	Back Right	Salting Out	Camp Fuel	0.31
	21	Front Left	Salting Out	Camp Fuel	1.25
Cook 6	22	Front Left	Full	Ether	2.43
	23	Back Right	Full	Ether	1.43
	24	Researcher	Full	Ether	0.06
	25	Front Left	Salting Out	Ether	0.00
	26	Back Right	Salting Out	Ether	0.00
	27	Field Blank	Flash Exposure	Field Blank	0.00

3.4.2.2 Passive air sampling

All passive air samplers were provided by and analyzed via GC-MS by Entech

Instruments. Due to a lack of chemical reference standards available, observed chromatographic

peaks were identified based on a mass spectral library search using the NIST mass spectra data

base instead of the standard practice of comparing an unknown peak to a known reference

standard. Of the 82 compounds quantitated by Entech Instruments' all of them were included in

the EPA's TO-14A, TO-15, or BTEX analytical methods for collecting and identifying VOCs in

stainless steel canisters (Table 16);[101,102] all other compounds were reported as qualitative only,

though some could be semi-quantified through estimating their concentrations by comparing the

compounds peak area to the peak area from a structurally similar compound included in the 82

quantified compounds. Methamphetamine and structurally similar byproducts produced by a One

Pot cook were not identified in the chromatograms of any of the passive air samples collected

during this study.

Table 16. The 82 compounds quantitated by Entech Instruments' GC-MS method. All 82 compounds are included in the EPA's TO-14A, TO-15, or BTEX analytical methods.

Compound	Chemical Formula	CAS No.	TO-14A	TO-15	BTEX
1,1-Dichloroethane	$C_2H_4Cl_2$	107-06-2	X	X	
1,1-Dichloroethene	$C_2H_2Cl_2$	75-35-4	X	X	
1,1,1-Trichloroethane	$C_2H_3Cl_3$	71-55-6	X	X	
1,1,1,2-Tetrachloroethane	$C_2H_2Cl_4$	630-20-6	X	X	
1,1,2-Trichloroethane	$C_2H_3Cl_3$	79-00-5	X	X	
1,1,2,2-Tetrachloroethane	$C_2H_2Cl_4$	79-34-5	X	X	
1,2-Dibromoethane	$C_2H_4Br_2$	106-93-4	X	X	
1,2-Dichlorobenzene	$C_6H_4Cl_2$	95-50-1	X	X	
1,2-Dichloroethane	$C_2H_4Cl_2$	107-06-2	X	X	
1,2-Dichloropropane	$C_3H_6Cl_2$	78-87-5	X	X	
1,2,4-Trichlorobenzene	$C_6H_3Cl_3$	120-82-1	X	X	
1,2,4-Trimethylbenzene	C_9H_{12}	95-63-6	X	X	
1,3-Butadiene	C_4H_6	106-99-0		X	
1,3-Dichlorobenzene	$C_6H_4Cl_2$	541-73-1	X	X	
1,3,5-Trimethylbenzene	C_9H_{12}	108-67-8	X	X	
1,4-Dichlorobenzene	$C_6H_4Cl_2$	106-46-7	X	X	
1,4-Dioxane	$C_4H_8O_2$	123-91-1		X	

Name	Formula	CAS			
2-Butanone	C_4H_8O	78-93-3		X	
2-Chloroprene	C_4H_5Cl	126-99-8		X	
2-Hexanone	$C_6H_{12}O$	591-78-6		X	
2,2,4-Trimethylpentane	C_8H_{18}	540-84-1		X	
4-Ethyltoluene	C_9H_{12}	622-96-8	X	X	
4-Methyl-2-pentanone	$C_6H_{12}O$	108-10-1		X	
Acetone	C_3H_6O	67-64-1		X	
Acetonitrile	C_2H_3N	75-05-8		X	
Acrolein	C_3H_4O	107-02-8		X	
Acrylonitrile	C_3H_3N	107-13-1	X	X	
Allyl Chloride	C_3H_5Cl	107-05-1	X	X	
Benzene	C_6H_6	71-43-2	X	X	X
Benzyl Chloride	C_7H_7Cl	100-44-7	X	X	
Bromodichloromethane	$CHBrCl_2$	75-27-4	X	X	
Bromoethene	C_2H_3Br	593-60-2		X	
Bromoform	$CHBr_3$	75-25-2		X	
Bromomethane	CH_3Br	74-83-9	X	X	
Carbon Disulfide	CS_2	75-15-0		X	
Carbon Tetrachloride	CCl_4	56-23-5	X	X	
Chlorobenzene	C_6H_5Cl	108-90-7	X	X	
Chloroethane	C_2H_5Cl	75-00-3	X	X	
Chloroform	$CHCl_3$	67-66-3	X	X	
Chloromethane	CH_3Cl	74-87-3	X	X	
cis-1,2-Dichloroethene	$C_2H_2Cl_2$	156-59-2	X	X	
cis-1,3-Dichloropropene	$C_3H_4Cl_2$	10061-01-5	X	X	
Cumene	C_9H_{12}	98-82-8		X	
Cyclohexane	C_6H_{12}	110-82-7		X	
Di-isopropyl Ether	$C_6H_{14}O$	108-20-3		X	
Dibromochloromethane	$CHBr_2Cl$	124-48-1	X	X	
Dichlorodifluoromethane	CCl_2F_2	75-71-8	X	X	
Dichlorotetrafluoroethane	$C_2Cl_2F_4$	76-12-2	X	X	
Ethanol	C_2H_6O	64-17-5		X	
Ethyl Acetate	$C_4H_8O_2$	141-78-6		X	
Ethyl tert-Butyl Ether	$C_6H_{14}O$	637-92-3		X	
Ethylbenzene	C_8H_{10}	100-41-4	X	X	X
Heptane	C_7H_{16}	142-82-5		X	
Hexachlorobutadiene	C_4Cl_6	87-68-3	X	X	
Hexane	C_6H_{14}	110-54-3	X	X	
Isopropyl Alcohol	C_3H_8O	67-63-0		X	
m,p-Xylene	C_8H_{10}	108-38-3, 106-42-3	X	X	X
Methyl Methacrylate	$C_5H_8O_2$	80-62-6		X	
Methyl tert-Butyl Ether	$C_5H_{12}O$	1634-04-4		X	
Methylene Chloride	CH_2Cl_2	75-09-2	X	X	
n-Butyl Benzene	$C_{10}H_{14}$	104-51-8			X
n-Propylbenzene	C_9H_{12}	103-65-1		X	X
Naphthalene	$C_{10}H_8$	91-20-3		X	
o-Chlorotoluene	C_7H_7Cl	95-49-8	X	X	

o-Cymene	C$_{10}$H$_{14}$	527-84-4			X
o-Xylene	C$_8$H$_{10}$	95-47-6	X	X	X
Propene	C$_3$H$_6$	115-07-1		X	
sec-Butyl Benzene	C$_{10}$H$_{14}$	135-98-8			X
Styrene	C$_8$H$_8$	100-42-5	X	X	
tert-Amyl Methyl Ether	C$_6$H$_{14}$O	994-05-8		X	
tert-Butanol	C$_4$H$_{10}$O	75-65-0		X	
tert-Butylbenzene	C$_{10}$H$_{14}$	98-06-6			X
Tetrachloroethene	C$_2$Cl$_4$	127-18-4	X	X	
Tetrahydrofuran	C$_4$H$_8$O	109-99-9		X	
Toluene	C$_7$H$_8$	108-88-3	X	X	X
trans-1,2-Dichloroethene	C$_2$H$_2$Cl$_2$	156-60-5	X	X	
trans-1,3-Dichloropropene	C$_3$H$_4$Cl$_2$	10061-02-6	X	X	
Trichloroethene	C$_2$HCl$_3$	79-01-6	X	X	
Trichlorofluoromethane	CCl$_3$F	75-69-4	X	X	
Trichlorotrifluoroethane	C$_2$Cl$_3$F$_3$	76-13-1	X	X	
Vinyl Acetate	C$_4$H$_6$O$_2$	108-05-4		X	
Vinyl Chloride	C$_2$H$_3$Cl	75-01-4	X	X	

For the 1 L MiniCans^{TM}, 21 quantifiable compounds were identified in the 12 air samples. A summary of the compounds quantified and their concentration in ppb air is shown in Table 17. Each MiniCan^{TM} air sample was given a name that follows the following template: Day-Cook-Time, so sample D1-C1-Pre means the sample was collected on the first day, during the first cook, prior to the cook being performed. As described in section 3.3.3.2 Passive air sampling, the 1 L MiniCan^{TM} grab samples were collected at 3 points throughout the One Pot methamphetamine cooks: "Pre" vacuum grabs were taken prior to the start of the cooks, "Mid" indicates after the cook reaction was complete but before salting out began, and "Post" means after the methamphetamine salts had been separated from the post-salt solvent via filtration.

The concentrations of all compounds collected in a single MiniCan^{TM} sample were summed to give the total concentration of VOCs present in the air. These total VOC concentrations were then compared statistically by use of an analysis of variance (ANOVA), with the collection time point acting as the independent variable. Table 18 shows the total and average VOC concentrations observed from each collection time point, as well as the fractional total and average VOC concentrations collected during One Pot methamphetamine cooks with each

organic solvent type. The ANOVA results are summarized by superscript letters in Table 18; the

total VOC concentration was the greatest mid-cook, followed by post-cook, and then the lowest

pre-cook. While the concentration observed mid-cook was significantly greater than the

concentrations observed pre and post-cook, the concentrations observed post-cook were not

statistically different than those observed pre-cook.

Table 17. Summary of the quantifiable VOCs collected with the 12 MiniCans^{TM}. All concentrations are in ppb air.

Cook	Ether 1			Camp Fuel 1			Fail 1/Camp Fuel 2			Ether 2		
Description (Day, Cook, Time)	D1-C1-Pre	D1-C1-Mid	D1-C1-Post	D1-C2-Pre	D1-C2-Mid	D1-C2-Post	D2-C1-Pre	D2-C1-Mid	D2-C1-Post	D2-C2-Pre	D2-C2-Mid	D2-C2-Post
1,2-Dimethyl cyclohexane	-	-	-	-	-	-	-	1.84	-	-	-	-
1,3-Dimethylcyclopentane	-	-	-	-	-	-	-	1.31	-	-	-	-
2-Methyl butane	-	-	-	-	823.19	2.61	-	633.03	195.65	1.62	-	-
2-Methyl heptane	-	-	-	-	-	-	-	10.32	1.47	-	-	-
2-Methyl pentane	-	-	-	-	0.75	-	-	1.70	-	-	-	-
3-Ethyl-2-pentanol	-	-	-	-	-	-	-	-	0.69	-	-	-
3-Methyl hexane	-	0.55	0.78	-	-	-	-	1.26	-	-	-	-
3,3-Dimethyl hexane	-	-	-	-	-	-	-	0.71	-	-	-	-
Acetone	0.80	1.09	0.89	3.13	-	3.10	0.98	-	-	-	0.59	0.69
Cyclohexane	-	-	1.20	-	133.26	4.57	-	585.36	43.05	1.66	-	-
Ethyl ether	-	83.78	31.28	-	-	-	-	-	-	-	84.18	7.06
Heptane	-	54.30	88.63	-	45.14	4.08	-	213.95	20.70	1.13	10.03	20.19
Hexane	-	-	-	-	2.67	-	-	7.65	0.73	-	-	-
Isopropyl Alcohol	-	3.21	-	-	-	-	-	1.04	-	-	-	-
Isopropylcyclobutane	-	-	-	-	-	-	-	1.11	-	-	-	-
Methyl cyclohexane	-	0.75	1.26	-	3.58	-	-	18.72	1.79	-	-	-
Pentane	-	-	-	-	16.14	-	-	15.91	4.43	-	-	-
Propane	-	-	-	-	6.26	0.52	-	2.61	-	-	-	-
Propyl cyclohexane	-	-	-	-	-	-	-	0.61	-	-	-	-
Total VOCs	0.08	143.68	124.04	3.13	1030.99	14.88	0.98	1497.13	268.51	4.41	94.80	27.94

Table 18. Total and average VOC concentrations collected with MiniCan^{TM} samplers during One Pot methamphetamine cooks. Fractional totals and fractional averages are the total and average VOC concentrations observed while performing a One Pot methamphetamine cook with the respective organic solvent. All concentrations are in ppb air.

Collection Point	Pre-Cook		Mid-Cook		Post-Cook	
Total VOC	9.32		2766.60		435.37	
Average VOC (±SD)	2.33 (±1.74)[b]		691.65 (±688.10)[a]		108.84 (±117.05)[b]	
Solvent	*Ether*	*Camp Fuel*	*Ether*	*Camp Fuel*	*Ether*	*Camp Fuel*
Fractional Total VOC	5.21	4.11	238.48	2528.12	151.98	283.39
Fractional Average VOC (±SD)	2.61 (±2.55)	2.06 (±1.52)	119.24 (±34.56)	1264.06 (±329.61)	75.99 (±67.95)	141.70 (±179.34)

*Means denoted with the same letter are not significantly different at $\alpha=0.05$.

The gas chromatograms produced from analysis of the 12 MiniCan™ samples are shown in Figure 39 – Figure 42. For all 4 sets of chromatograms, the pre-cook sample chromatogram is shown on top (black), the mid-cook sample chromatogram is shown in the middle (blue), and the post-cook sample chromatogram is shown on the bottom (red). Each peak on the chromatogram is designated with a number that corresponds with the key at the bottom of the figure. The serial number listed above each chromatogram corresponds to the serial number of the MiniCan™, listed in Table 13.
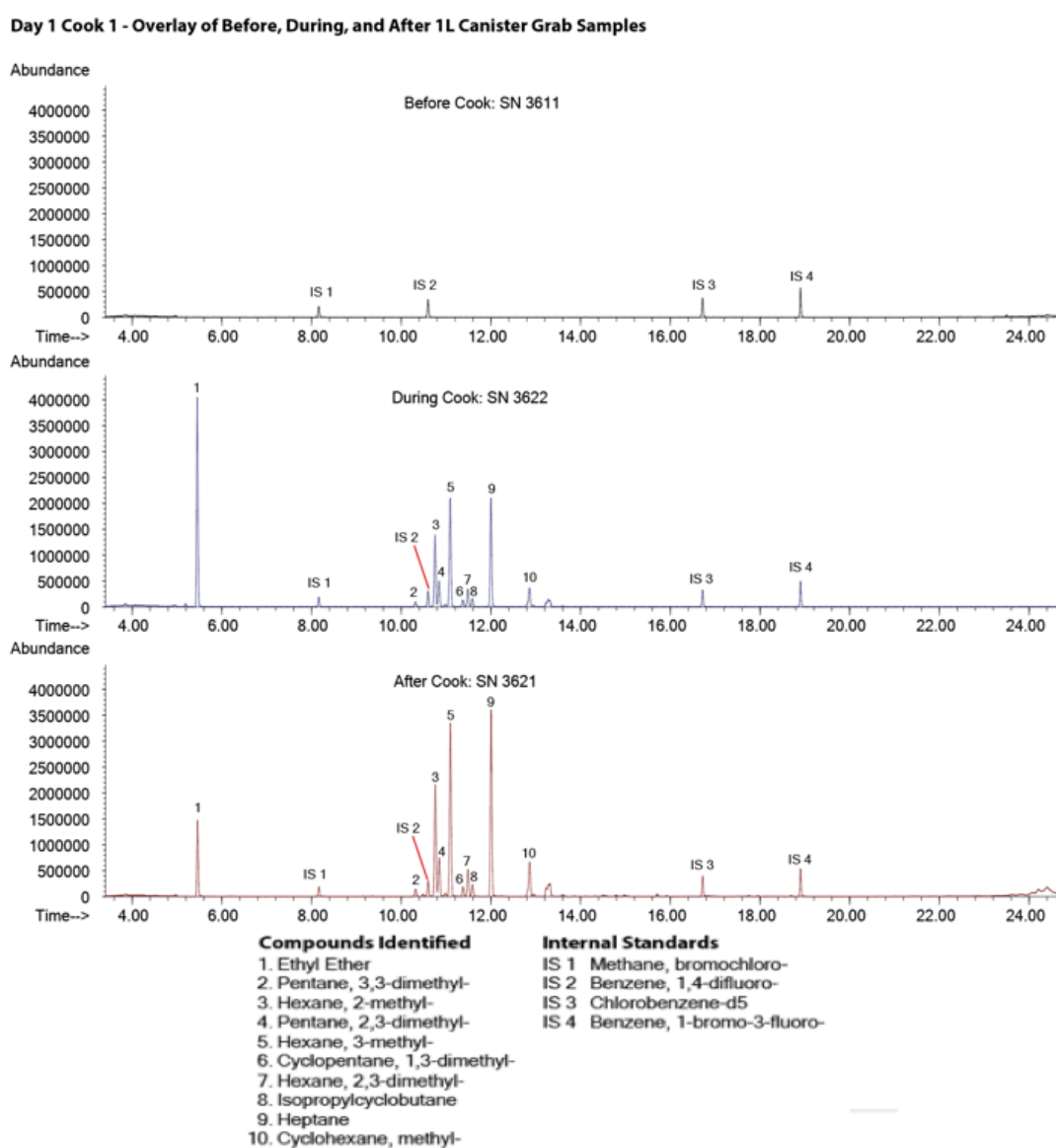
Figure 39. Gas chromatograms from the 3 MiniCan™ air samples taken during the first ether cook.

Figure 40. Gas chromatograms from the 3 MiniCan™ air samples taken during the first camp fuel cook.

Day 2 Cook 1 - Overlay of Before, During, and After 1L Canister Grab Samples

Compounds Identified
1. Propane
2. Isobutane
3. Butane
4. Butane, 2-methyl-
5. Pentane
6. n-Hexane
7. Pentane, 2,2-dimethyl-
8. Cyclopentane, methyl-
9. Pentane, 2,4-dimethyl-
10. Butane, 2,2,3-trimethyl-
11. Pentane, 3,3-dimethyl-
12. Cyclohexane
13. Hexane, 2-methyl-
14. Pentane, 2,3-dimethyl-
15. Hexane, 3-methyl-
16. Cyclopentane, 1,3-dimethyl-, cis-
17. Cyclopentane, 1,3-dimethyl-
18. Isopropylcyclobutane
19. Heptane
20. Cyclohexane, methyl-
21. Hexane, 2,5-dimethyl-
22. Hexane, 2,4-dimethyl-
23. Heptane, 2-methyl-
24. Cyclohexane, 1,3-dimethyl-, cis-
25. Cyclohexane, 1,3-dimethyl-, cis-
26. Octane
27. Cyclohexane, 1,3-dimethyl-, trans-
28. Octane, 2-methyl-
29. Octane, 3-methyl-
30. Nonane
31. Decane

Internal Standards
IS 1 Methane, bromochloro-
IS 2 Benzene, 1,4-difluoro-
IS 3 Chlorobenzene-d5
IS 4 Benzene, 1-bromo-3-fluoro-

Figure 41. Gas chromatograms from the 3 MiniCan™ air samples taken during the second camp fuel cook/first bottle failure. The top sample was taken prior to the first camp fuel bottle failure, the middle sample was taken following the bottle failure but prior to salting out during the re-do camp fuel cook, and the bottom sample was taken following conclusion of re-do camp fuel cook.

Compounds Identified
1. Ethyl ether
2. Hexane, 2-methyl-
3. Pentane, 2,3-dimethyl-
4. Hexane, 3-methyl-
5. Heptane
6. Cyclohexane, methyl-
7. Octane

Internal Standards
IS 1 Methane, bromochloro-
IS 2 Benzene, 1,4-difluoro-
IS 3 Chlorobenzene-d5
IS 4 Benzene, 1-bromo-3-fluoro-

Figure 42. Gas chromatograms from the 3 MiniCan™ air samples taken during the second ether cook.

While the MiniCan™ air samples captured 21 quantifiable VOCs during the One Pot methamphetamine cooks, the helium diffusion samplers only captured 11 quantifiable VOCs during the cooks. A summary of the VOCs captured by the 8 body-worn HDS personal monitors and their observed concentrations in ppb are shown in Table 19. Each HDS air sample was given a name that follows the following template: Day-Cook-Person, so sample D1-C1-P1 means the sample was collected on the first day, during the first cook, and researcher 1 had the sampler

clipped to their SCBA harness. During this project, researcher 1 handled the One Pot for the

majority of the time while researcher 2 set up lab supplies (funnels, filters, bottles, etc.) and

handed equipment and reagents to researcher 1 when needed.

Table 19. Summary of the quantifiable VOCs collected with the 8 HDS personal monitors. All concentrations are in ppb air.

Cook	Ether 1		Camp Fuel 1		Fail 1/Camp Fuel 2		Ether 2	
Description (Day, Cook, Person)	D1-C1-P1	D1-C1-P2	D1-C2-P1	D1-C2-P2	D2-C1-P1	D2-C1-P2	D2-C2-P1	D2-C2-P2
2-Methyl Butane	-	-	4027	4846	4572	6785	-	-
Acetone	142	88	-	-	-	-	274	180
Cyclohexane	-	-	835	865	2054	2986	-	-
Ethyl Ether	2840	2008	-	-	-	-	2785	4185
Heptane	1055	766	272	269	963	1299	884	1574
Hexane	-	-	-	-	132	-	-	-
Isopropyl Alcohol	-	-	-	-	69	-	-	-
Methyl Cyclohexane	-	-	-	-	89	-	-	-
Methylene Chloride	-	-	-	-	420	150	-	-
Pentane	-	-	129	156	164	259	-	-
Trichloroethene	-	-	-	162	1421	517	-	-
Total VOCs	4037	2861	5263	6298	9883	11996	3943	5938

For statistical analysis, two separate t-tests were run on data from Table 19. The first t-

test was used to compare the difference in the total amount of VOCs the two researchers were

exposed to, thus assessing if handling the One Pot methamphetamine cook led to a greater

exposure to VOCs than simply being in the vicinity of it. The second t-test was used to compare

the total amount of VOCs released by ether One Pots compared to the camp fuel One Pots. The

results of both t-tests are summarized in Table 20; for both t-tests, each HDS personal monitor

was treated as a data set, so each treatment group had four sets of data for comparison. At

$\alpha=0.05$, there was no significant difference in the amount of VOC exposure between the two

researchers performing the One Pot methamphetamine cook, however, there was a significant

difference in the amount of VOC exposure that occurred when ether was used as an organic

solvent when compared to camp fuel as an organic solvent.

Table 20. Summary of t-tests performed on HDS personal monitor VOC data. There was no significant difference in the amount of VOC exposure the two researchers were subjected to, but there was a significant difference in the amount of VOCs released by the ether One Pots when compared to the camp fuel One Pots.

HDS Sample	Average VOC Conc. (±SD)	p-value
Researcher 1	5782 (±2800)	0.689
Researcher 2	6774 (±3808)	
Ether One Pots	4195 (±1279)	0.049
Camp Fuel One Pots	8360 (±3130)	

The gas chromatograms produced from analysis of the 8 HDS personal monitors are

shown in Figure 43 – Figure 46. For all 4 sets of chromatograms, researcher 1 is shown on top

(black) and researcher 2 is shown on bottom (blue). Each peak on the chromatogram is

designated with a number that corresponds with the key at the bottom of the figure. The serial

number listed above each chromatogram corresponds to the serial number of the HDS personal

monitor, listed in Table 13.

While the sorbents in the MiniCans[TM] and HDS personal monitors are designed to

capture a wide range of VOCs, the Carbo Pack X sorbent from the DSPs was designed to be more

specific and better capture the aromatic hydrocarbon BTEX compounds. Due to this, quantitative

analysis of these DSPs was only focused on BTEX compounds and not the 82 quantifiable

compounds from the MiniCan[TM] and HDS analyses. Results of the DSP analyses are summarized

in Table 21.

Day 1 Cook 1 HDS

Figure 43. Gas chromatograms from the 2 HDS personal air samplers worn during the first ether cook, one sampler clipped to SCBA gear of each researcher.

Figure 44. Gas chromatograms from the 2 HDS personal air samplers worn during the first camp fuel cook, one sampler clipped to SCBA gear of each researcher.

Day 2 Cook 1 HDS

Abundance

Person 1: SN 4001106

Time--> 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00

Abundance

Person 2: SN 4001105

Time--> 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00
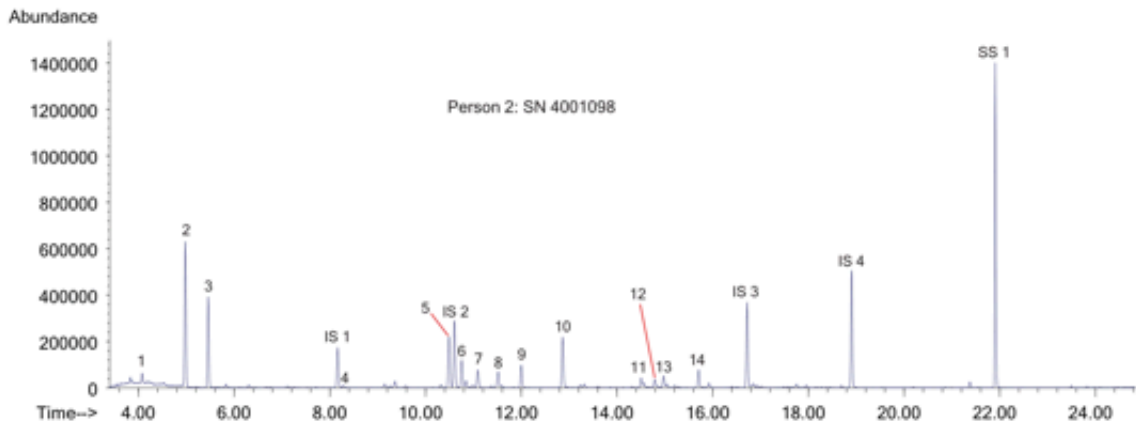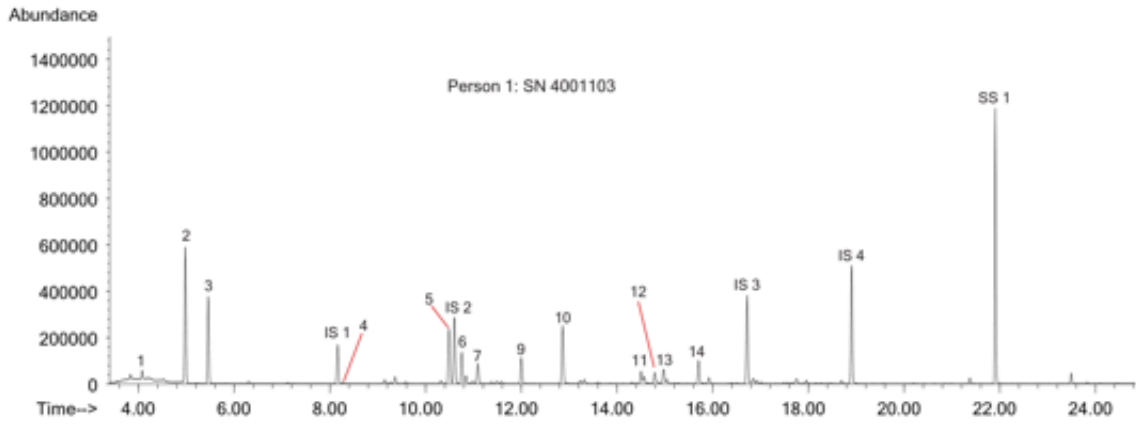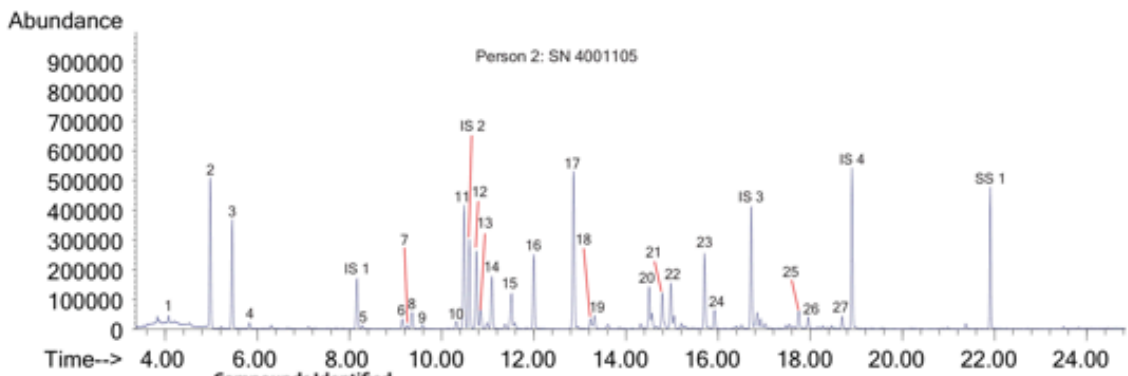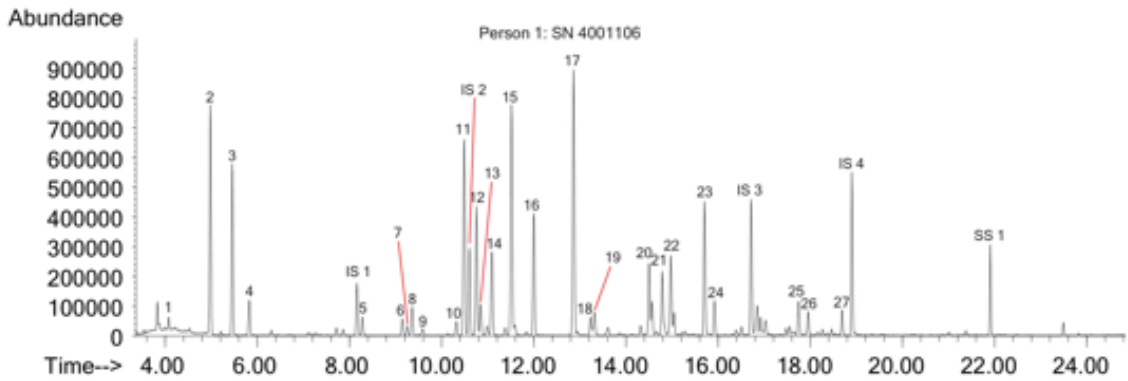
Compounds Identified

1. Butane
2. Butane, 2-methyl-
3. Pentane
4. Methylene chloride
5. n-Hexane
6. Pentane, 2,2-dimethyl-
7. Cyclopentane, methyl-
8. Pentane, 2,4-dimethyl-
9. Butane, 2,2,3-trimethyl-
10. Pentane, 3,3-dimethyl-
11. Cyclohexane
12. Hexane, 2-methyl-

13. Pentane, 2,3-dimethyl-
14. Hexane, 3-methyl-
15. Trichloroethylene
16. Heptane
17. Cyclohexane, methyl-
18. Hexane, 2,5-dimethyl-
19. Hexane, 2,4-dimethyl-
20. Heptane, 2-methyl-
21. Cyclohexane, 1,3-dimethyl-, cis-
22. Cyclohexane, 1,3-dimethyl-, cis-
23. Octane
24. Cyclohexane, 1,3-dimethyl-, trans-

25. Octane, 2-methyl-
26. Octane, 3-methyl-
27. Nonane

Internal Standards

IS 1 Methane, bromochloro-
IS 2 Benzene, 1,4-difluoro-
IS 3 Chlorobenzene-d5
IS 4 Benzene, 1-bromo-3-fluoro-

Surrogate Standard

SS 1 5-Bromo-2-fluorotoluene

Figure 45. Gas chromatograms from the 2 HDS personal air samplers worn throughout the first bottle failure and the second camp fuel cook, one sampler clipped to SCBA gear of each researcher.

Compounds Identified
1. Acetone
2. Ethyl ether
3. Hexane, 2-methyl-
4. Pentane, 2,3-dimethyl-
5. Hexane, 3-methyl-
6. Heptane
7. Cyclohexane, methyl-

Internal Standards
IS 1 Methane, bromochloro-
IS 2 Benzene, 1,4-difluoro-
IS 3 Chlorobenzene-d5
IS 4 Benzene, 1-bromo-3-fluoro-

Surrogate Standard
SS 1 5-Bromo-2-fluorotoluene
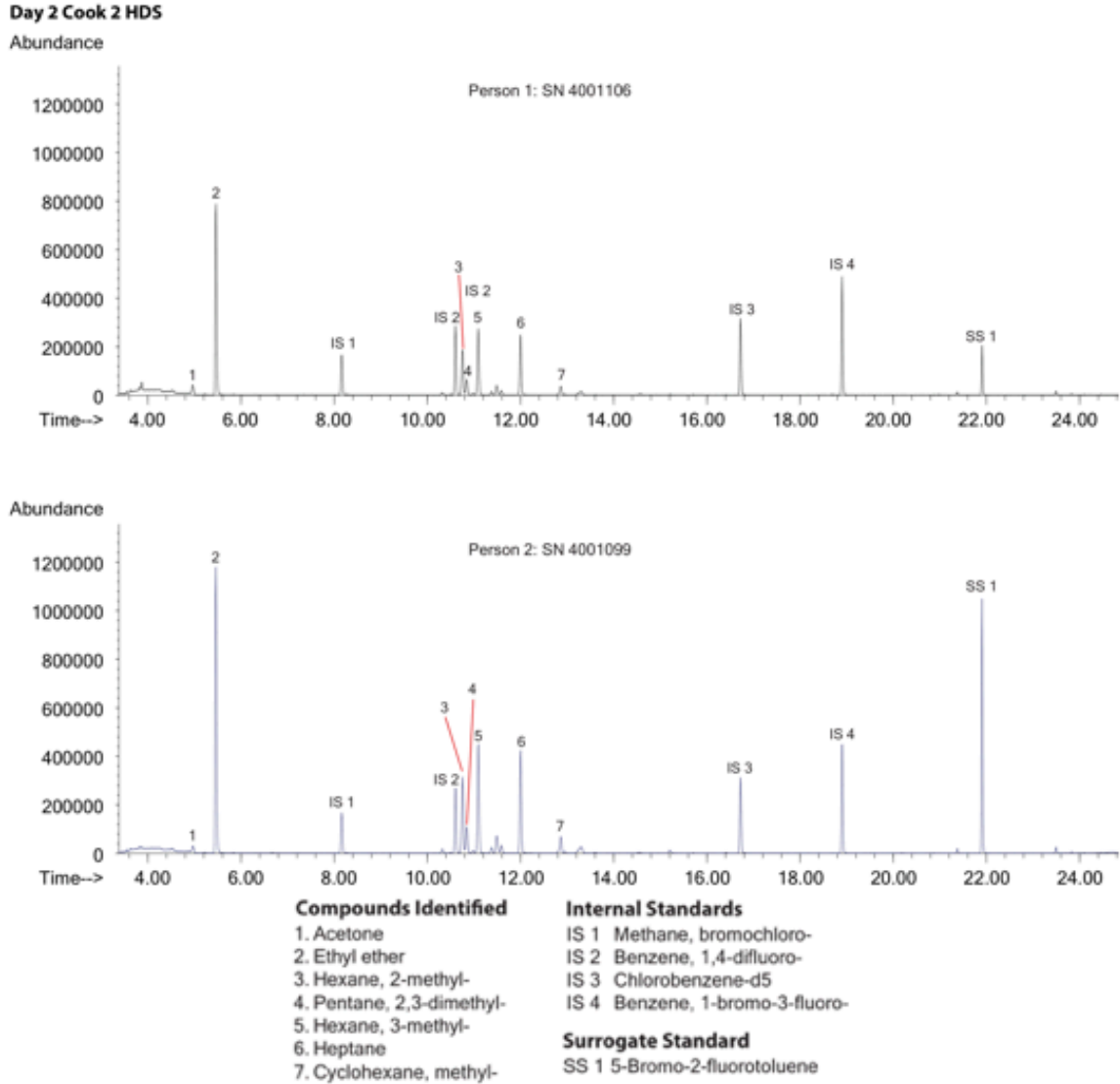
Figure 46. Gas chromatograms from the 2 HDS personal air samplers worn during the second ether cook, one sampler clipped to SCBA gear of each researcher.

Table 21. Summary of the quantifiable BTEX compounds collected with the 8 DSP monitors. All concentrations are in ppb air.

Cook	Ether 1		Camp Fuel 1		Fail 1/Camp Fuel 2		Ether 2	
Description (Day, Cook, Person)	D1-C1-P1	D1-C1-P2	D1-C2-P1	D1-C2-P2	D2-C1-P1	D2-C1-P2	D2-C2-P1	D2-C2-P2
Toluene	0.81	-	0.24	-	0.30	0.26	-	0.23
Benzene	-	-	0.27	-	-	-	-	0.33
Total BTEX	0.81	0.00	0.51	0.00	0.30	0.26	0.00	0.56

For statistical analysis, two separate t-tests were run on data from Table 21. The first t-test was used to compare the difference in the total amount of BTEX compounds the two researchers were exposed to, thus assessing if handling the One Pot methamphetamine cook led to a greater BTEX exposure than simply being in the vicinity of it. The second t-test was used to compare the total amount of BTEX compounds released by ether One Pots compared to the camp fuel One Pots. The results of both t-tests are summarized in Table 22. At $\alpha=0.05$, there was no significant difference in the amount of BTEX exposure between the two researchers performing the One Pot methamphetamine cook nor was there a significant difference in BTEX compounds released by the One Pot methamphetamine cooks when ether was used as an organic solvent when compared to camp fuel as an organic solvent.

Table 22. Summary of t-tests performed on DSP BTEX data. There was no significant difference in the amount of BTEX exposure the two researchers were subjected to, nor was there a significant difference in the amount of BTEX compounds released by the ether One Pots when compared to the camp fuel One Pots. Concentration in ppb.

DSP Sample	Average BTEX Conc. (±SD)	p-value
Researcher 1	0.41 (±0.34)	0.392
Researcher 2	0.21 (±0.27)	
Ether One Pots	0.27 (±0.21)	0.755
Camp Fuel One Pots	0.34 (±0.41)	

Though the Carbo Pack X sorbent in one set of DSPs is designed to capture BTEX compounds, other VOCs were also captured with these passive air sampling devices. This can be seen by observing the gas chromatograms associated with these samplers in Figure 47 – Figure 50. For all 4 sets of chromatograms, researcher 1 is shown on top (black) and researcher 2 is shown on bottom (blue). Each peak on the chromatogram is designated with a number that corresponds with the key at the bottom of the figure. The serial number listed above each chromatogram corresponds to the serial number of the HDS personal monitor, listed in Table 13.

Figure 47. Gas chromatograms from the 2 Carbo Pack X DSP samplers worn during the first ether cook.

Day 1 Cook 2 DSP Carbo Pack X
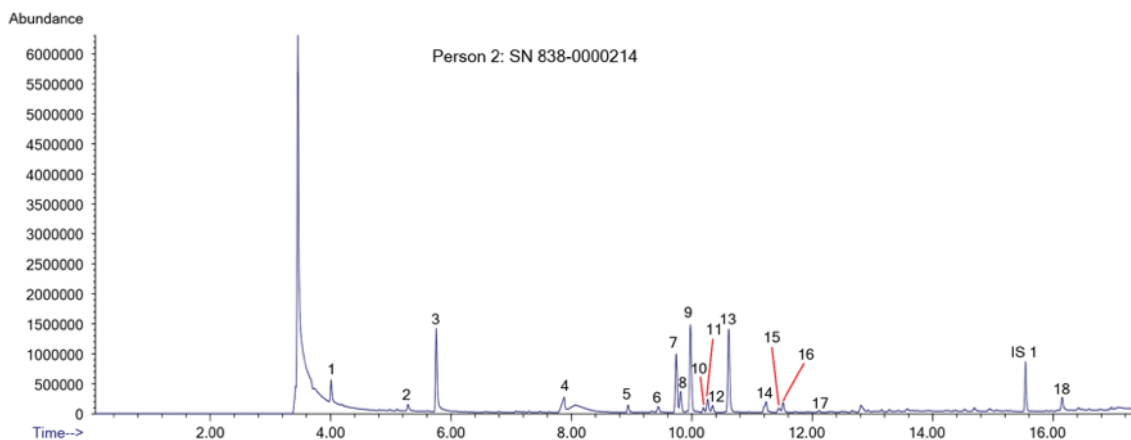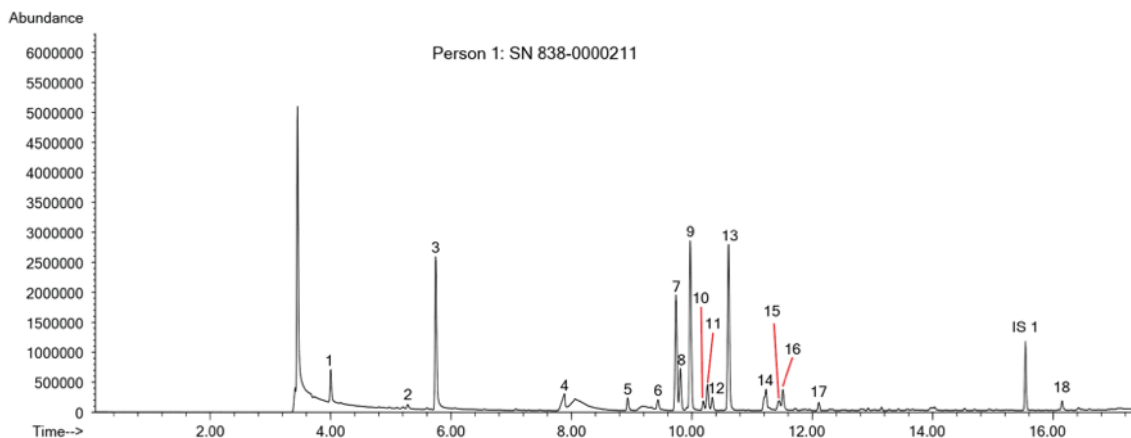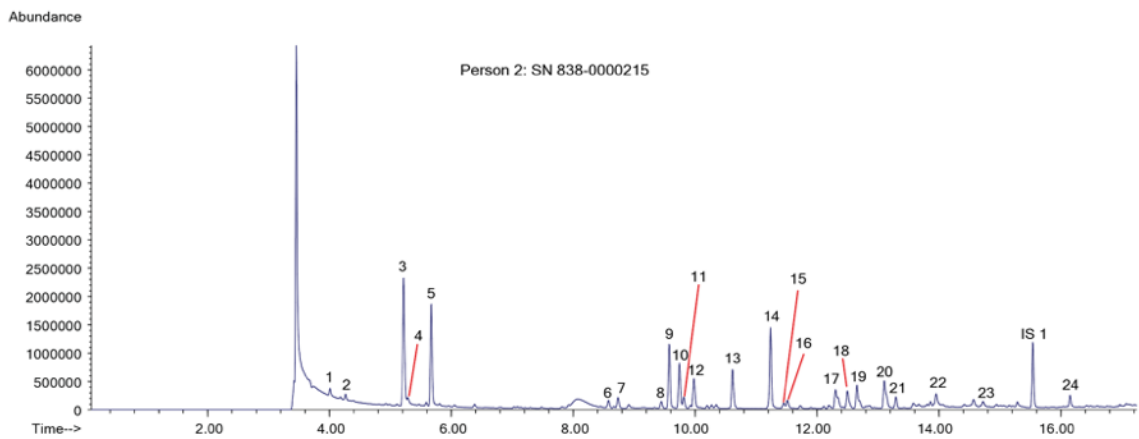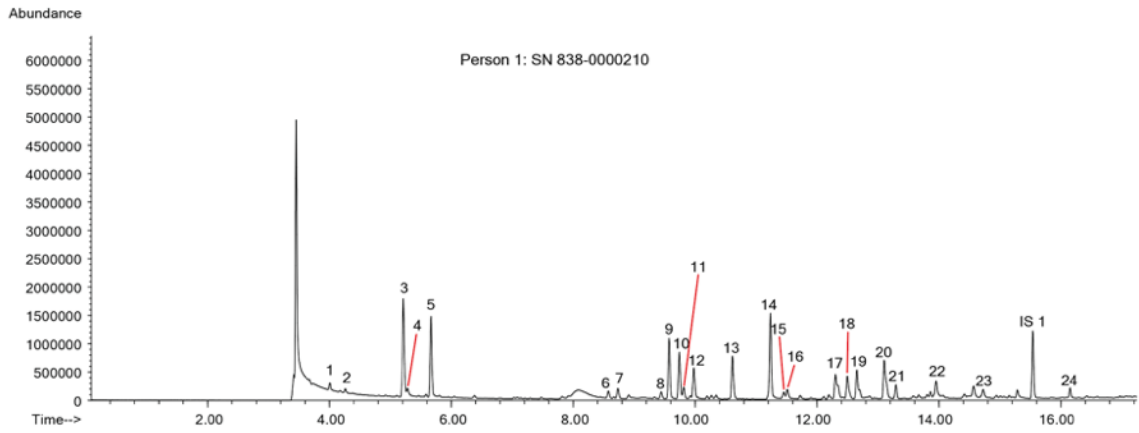
Compounds Identified
1. Acetaldehyde
2. Butane
3. Butane, 2-methyl-
4. Acetone
5. Pentane
6. Pentane, 2,2-dimethyl-
7. Pentane, 2,4-dimethyl-
8. Pentane, 3,3-dimethyl-
9. Cyclohexane
10. Hexane, 2-methyl-
11. Pentane, 2,3-dimethyl-
12. Hexane, 3-methyl-

13. Heptane
14. Cyclohexane, methyl-
15. Hexane, 2,5-dimethyl-
16. Hexane, 2,4-dimethyl-
17. Heptane, 2-methyl-
18. Heptane, 3-methyl-
19. Cyclohexane, 1,4-dimethyl-
20. Octane
21. Cyclohexane, 1,4-dimethyl-, trans-
22. Cyclohexane, ethyl-
23. Octane, 3-methyl-
24. Benzaldehyde

Internal Standards
IS 1 Benzene, 1-bromo-3-fluoro-

Figure 48. Gas chromatograms from the 2 Carbo Pack X DSP samplers worn during the first camp fuel cook.

Figure 49. Gas chromatograms from the 2 Carbo Pack X DSP samplers worn during the first bottle failure and second camp fuel cook.
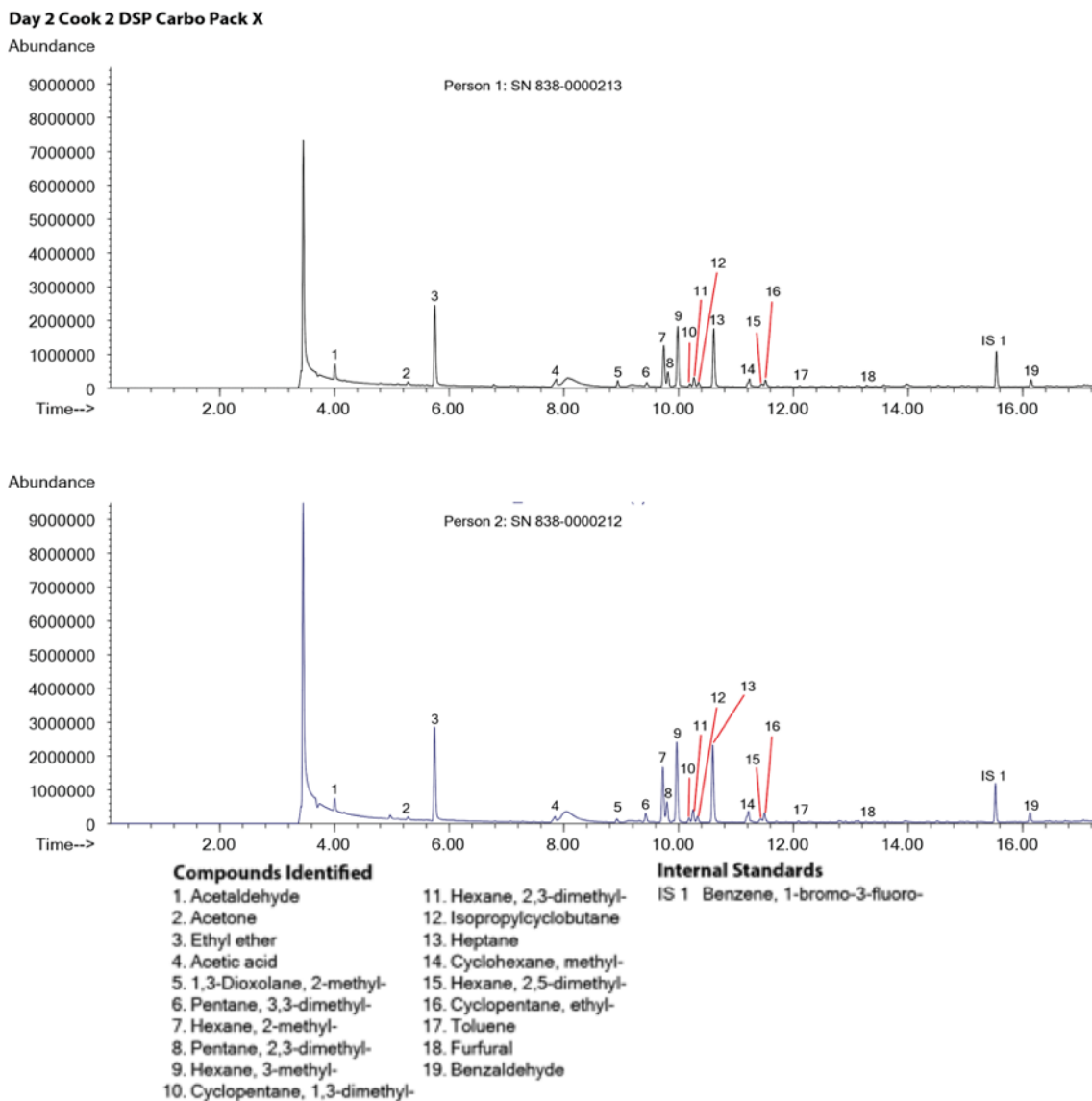
Figure 50. Gas chromatograms from the 2 Carbo Pack X DSP samplers worn during the second ether cook.

While the Carbo Pack X DSPs are optimal for the collection and analysis of BTEX compounds, the Tenax TA DSPs are designed for the collection and analysis of VOCs with higher boiling points, such as long-chain or polyunsaturated hydrocarbons. The Tenax TA DSPs used during this study were used solely in a qualitative manner. The identified chromatograms associated with these Tenax TA DSPs are shown in Figure 51 – Figure 54. For all 4 sets of chromatograms, researcher 1 is shown on top (black) and researcher 2 is shown on bottom (blue). Each peak on the chromatogram is designated with a number that corresponds with the key at the

bottom of the figure. The serial number listed above each chromatogram corresponds to the serial number of the HDS personal monitor, listed in Table 13.

Day 1 Cook 1 DSP Tenax

Person 1: SN 811-0000038

Person 2: 811-0000037

Compounds Identified

1. Ethyl ether
2. Hexane, 2-methyl-
3. Pentane, 2,3-dimethyl-
4. Hexane, 3-methyl-
5. Cyclopentane, 1,3-dimethyl-
6. Hexane, 2,3-dimethyl-
7. Isopropylcyclobutane
8. Heptane
9. Cyclohexane, methyl-
10. Hexane, 2,5-dimethyl-
11. Cyclopentane, ethyl-
12. 2(3H)-Furanone, 5-ethyldihydro-5-methyl-
13. Nonanal
14. Decanal
15. 2-Pentadecanone
16. n-Hexadecanoic acid
17. Octadecanoic acid
18. Bis(2-ethylhexyl) phthalate

Figure 51. Gas chromatograms from the 2 Tenax TA DSP samplers worn during the first ether cook.

Figure 52. Gas chromatograms from the 2 Tenax TA DSP samplers worn during the first camp fuel cook.

Figure 53. Gas chromatograms from the 2 Tenax TA DSP samplers worn during the first bottle failure and second camp fuel cook.

Figure 54. Gas chromatograms from the 2 Tenax TA DSP samplers worn during the second ether cook.

As noted above in section 3.3.3.2 Passive air sampling, the 40 mL screw-top vial grab sample was opened to the environment on a shelf in the back of the cook shed just prior to assembling the HCl gas generator and was capped following filtration of the methamphetamine salts. As with the Tenax TA DSP, the 40 mL screw-top grab samples were analyzed as qualitative only. As these screw-top vials were left sitting open in the atmosphere, any VOCs that were in the air could settle into the vial, with those present at the highest concentration most likely to fill the vial. The chromatograms for the 40 mL screw-top grab samples are shown in

Figure 55 – Figure 58. Each peak on the chromatogram is designated with a number that corresponds with the key at the bottom of the figure. The serial number listed above each chromatogram corresponds to the serial number of the HDS personal monitor, listed in Table 13.

Day 1 Cook 1 VASE on Empty Vial
VASE performed on Empty Vial (Salt to Filter*)

Empty Vial #1: HSP SN 411-0000178

Compounds Identified

1. Ethyl ether
2. Acetic Acid
3. 1,3-Dioxolane, 2-methyl-
4. Pentane, 3,3-dimethyl-
5. Hexane, 2-methyl-
6. Pentane, 2,3-dimethyl-
7. Hexane, 3-methyl-
8. Cyclopentane, 1,3-dimethyl-
9. Hexane, 2,3-dimethyl-
10. Isopropylcyclobutane
11. Heptane
12. Hexane, 2,2-dimethyl-
13. Cyclohexane, methyl-
14. Hexane, 2,5-dimethyl-
15. Hexane, 2,4-dimethyl-
16. Cyclopentane, ethyl-
17. Benzene, 1-chloro-4-(trifluoromethyl)-
18. Nonanal
19. Benzoic acid
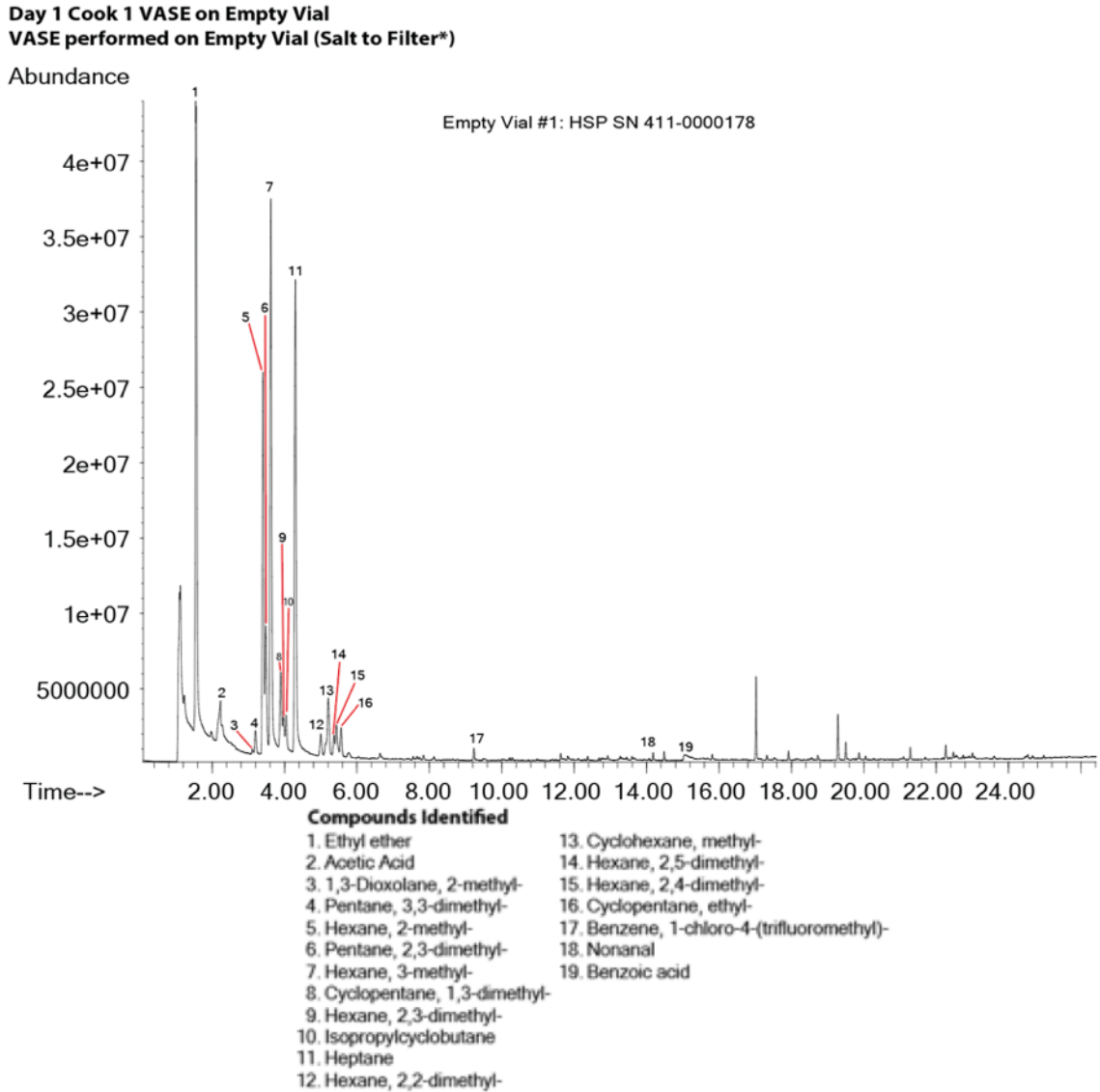
Figure 55. Gas chromatogram from the 40 mL screw-top vial opened prior to salting out the first ether cook.

Figure 56. Gas chromatogram from the 40 mL screw-top vial opened prior to salting out the first camp fuel cook.

Day 2 Cook 1 VASE on Empty Vial
VASE performed on Empty Vial (Salt to Filter*)

Abundance
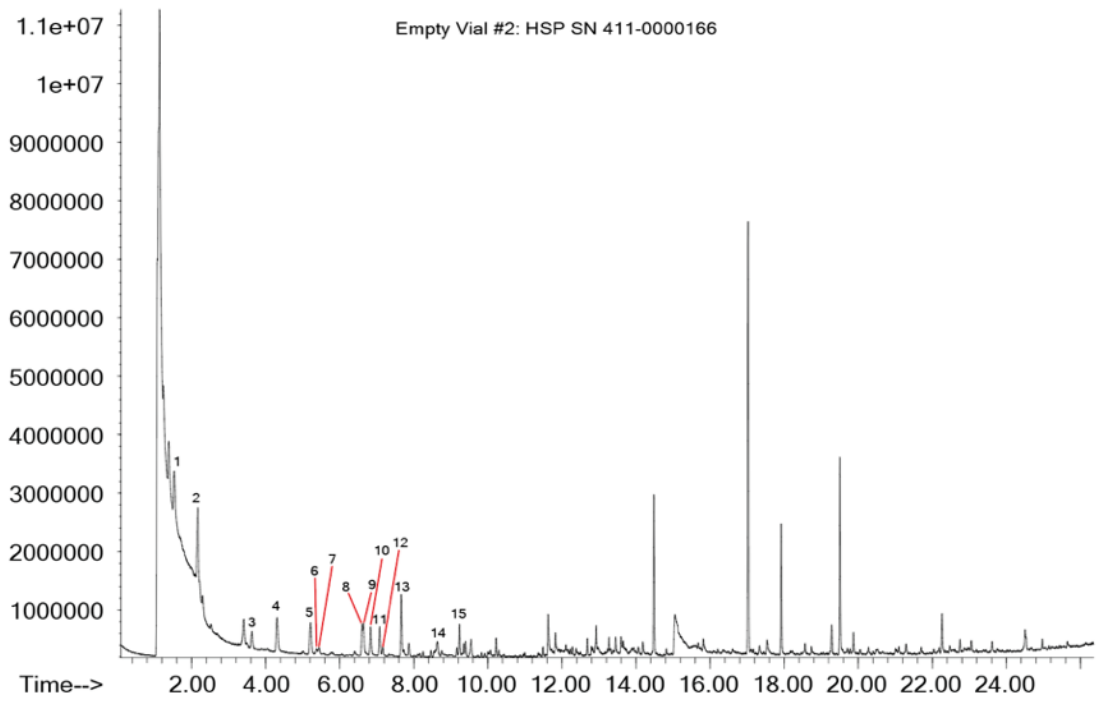
Empty Vial #3: HSP SN 411-0000157
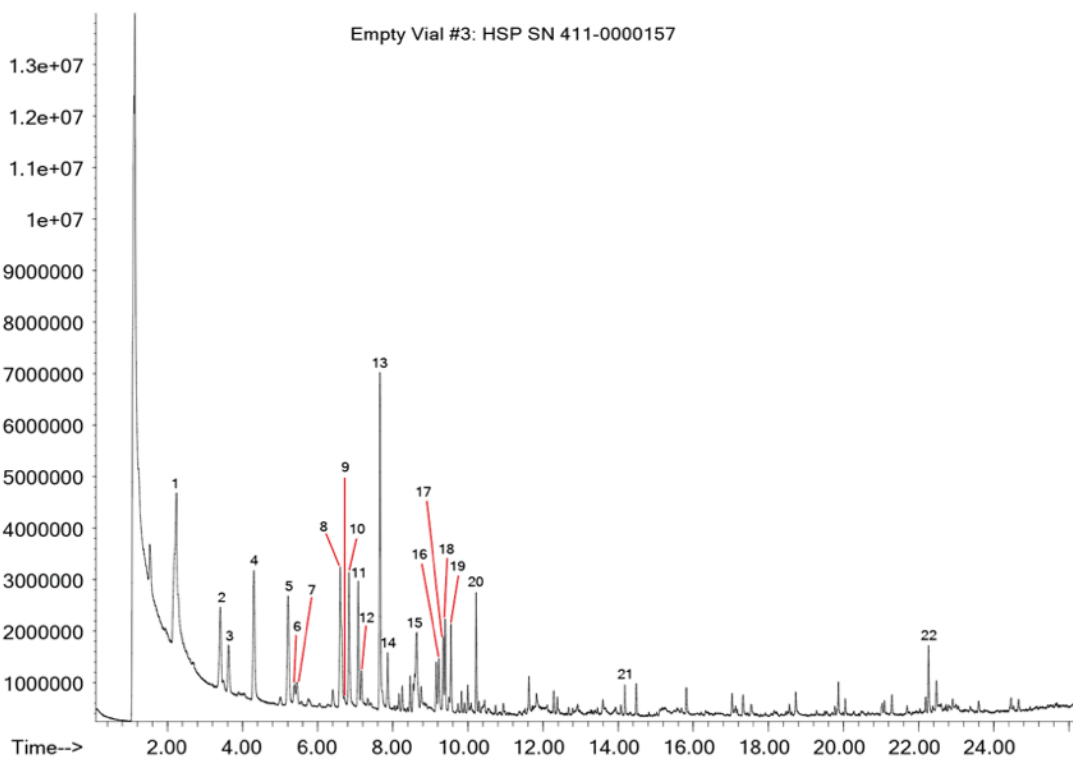
Compounds Identified

1. Acetic Acid
2. Hexane, 2-methyl-
3. Hexane, 3-methyl-
4. Heptane
5. Cyclohexane, methyl-
6. Hexane, 2,5-dimethyl-
7. Hexane, 2,4-dimethyl-
8. Heptane, 2-methyl-
9. Toluene
10. Heptane, 3-methyl-
11. Cyclohexane, 1,4-dimethyl-
12. Cyclohexane, 1,4-dimethyl-, trans-
13. Octane
14. Cyclohexane, 1,3-dimethyl-
15. Cyclohexane, ethyl-
16. Benzene, 1-chloro-4-(trifluoromethyl)-
17. Octane, 4-methyl-
18. Octane, 2-methyl-
19. Octane, 3-methyl-
20. Nonane
21. Nonanal
22. Hexadecane

Figure 57. Gas chromatogram from the 40 mL screw-top vial opened prior to salting out the second camp fuel cook.

Day 2 Cook 2 VASE on Empty Vial
VASE performed on Empty Vial (Salt to Filter*)

Empty Vial #4: HSP SN 411-0000154

Compounds Identified

1. Ethyl ether
2. Hexane, 2-methyl-
3. Pentane, 2,3-dimethyl-
4. Hexane, 3-methyl-
5. Cyclopentane, 1,3-dimethyl-
6. Hexane, 2,3-dimethyl-
7. Isopropylcyclobutane
8. Heptane
9. Hexane, 2,2-dimethyl-
10. Cyclohexane, methyl-
11. Hexane, 2,5-dimethyl-
12. Hexane, 2,4-dimethyl-
13. Cyclopentane, ethyl-
14. Benzene, 1-chloro-4-(trifluoromethyl)-
15. Camphene
16. Benzaldehyde
17. Isopropyl palmitate

Figure 58. Gas chromatogram from the 40 mL screw-top vial opened prior to salting out the second ether cook.

3.4.3 Standoff air monitoring

3.4.3.1 Ammonia monitoring

While the DUVAS carried onboard the GMAP vehicle was designed for analysis of BTEX compounds, this study utilized it for analysis of ammonia. The GMAP vehicle took ammonia measurements approximately 15 meters from the cook shed, though the DUVAS was not always activated at the start of a One Pot, but rather it was sometimes activated before or after the initiation of the One Pot. Due to the DUVAS not being optimized for ammonia

114

measurements, all ammonia measurements made by it were considered qualitative only. Figure

59 shows the trends in ammonia measurements taken by the DUVAS for all seven One Pot events

(3 ether cooks, 3 camp fuel cooks, and the first bottle failure). Figure 59 b-g show the lack of

optimization for the DUVAS in measuring ammonia, as the instrument observed negative

ammonia concentrations. While the concentrations listed on the y-axis of Figure 59 are not

reliable, the trend in measured ammonia can be used qualitatively to examine when plumes of

ammonia gas were observed by the instrumentation and for how long they remained in the

analyzed environment.

Figure 60 – Figure 65 show the ammonia concentrations measured by the Los Gatos

ICOS Gas Analyzer during 5 of the One Pot methamphetamine cooks at various standoff

distances; for reference purposes, previous research has shown Oklahoma to have an average

ambient background ammonia concentration of 1.8 ppb.[103] Spikes in ammonia concentration that

occurred during burp events are observed in several of the instrument readouts (Figure 60).

When the ICOS was loaded into the rental car and drove around the OSU Fire Research and

Training Center, the rental car speed varied from slow speeds of 3-5 mph to a maximum of 10-20

mph; instrument readouts obtained while driving around the training center are shown in Figure

63 – Figure 65. ICOS distances from the cook shed and field notes summarized in Table 23.

a) First ether cook

b) First camp fuel cook

c) First bottle failure

d) Second camp fuel cook

e) Second ether cook

f) Second bottle failure/Third camp fuel cook

g) Third ether cook
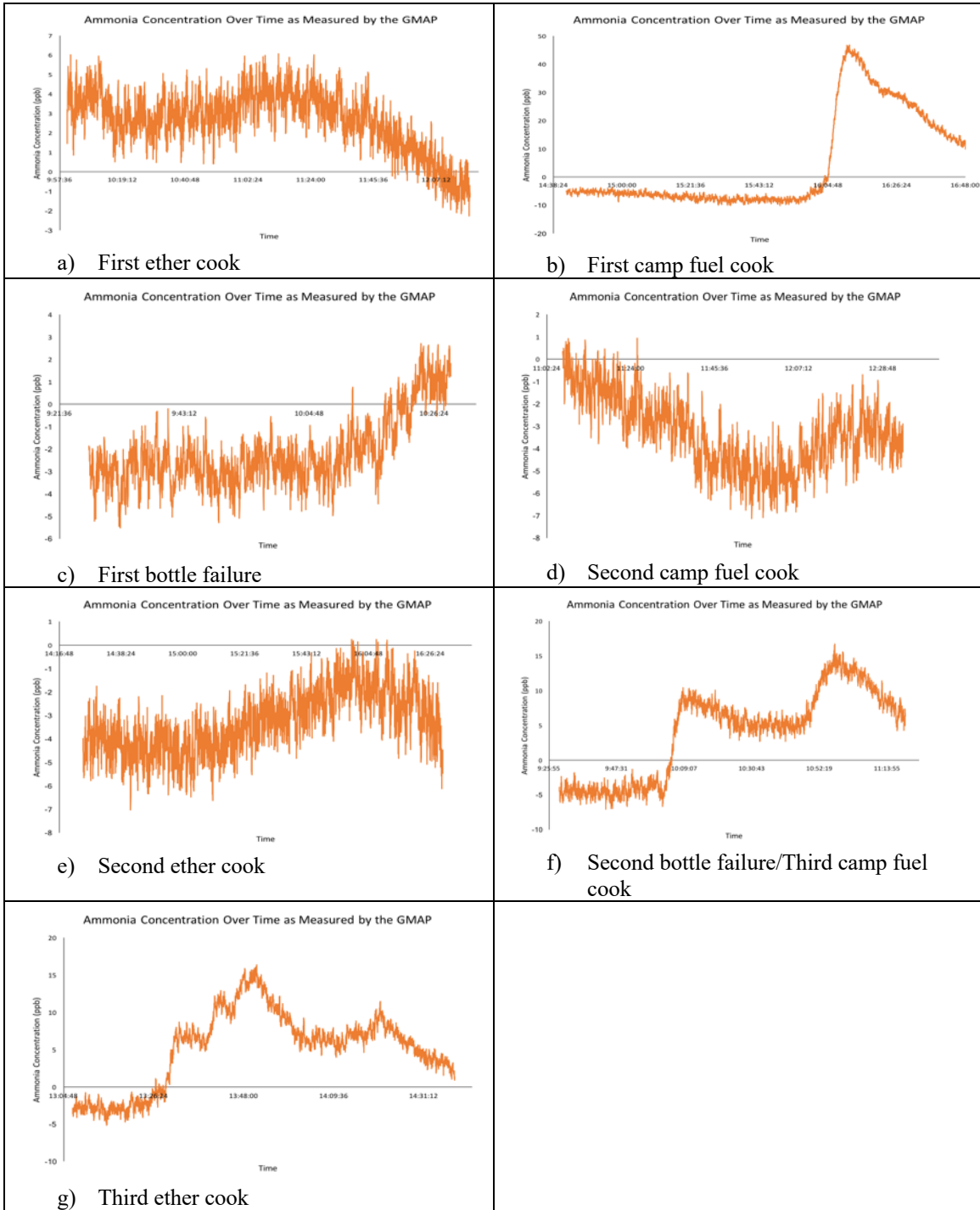
Figure 59. Ammonia concentrations measured by the DUVAS at 15m during each One Pot cook. All measurements are qualitative only and reported ammonia concentrations are provided only to examine trends in ammonia measurements.
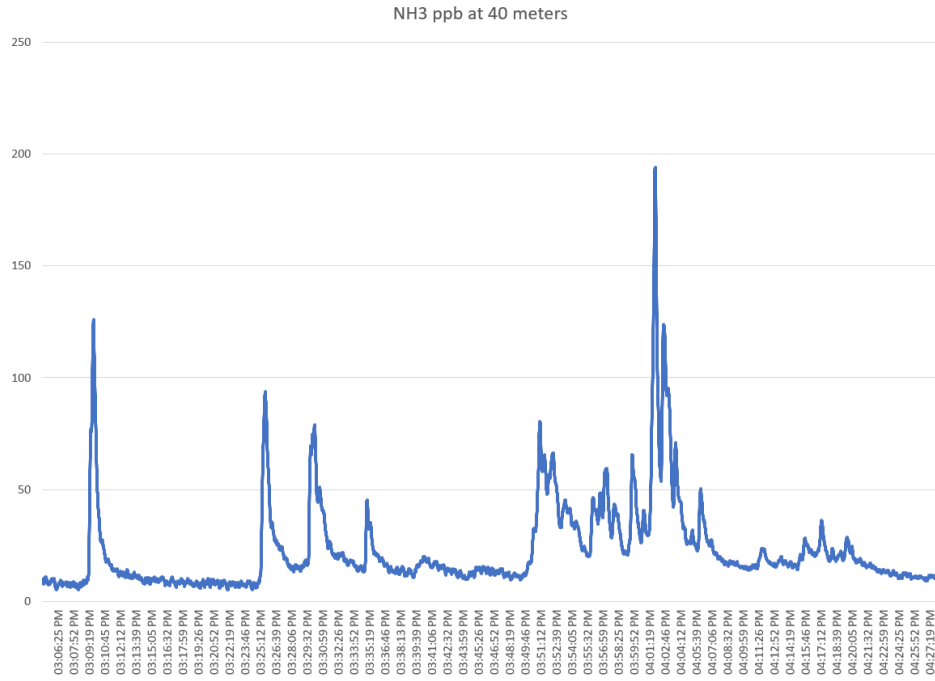
Figure 60. ICOS ammonia readings taken during the Tuesday afternoon camp fuel cook at 40 meters from the cook shed. One Pot burps are evident at 5 minute intervals beginning 3:25 pm.
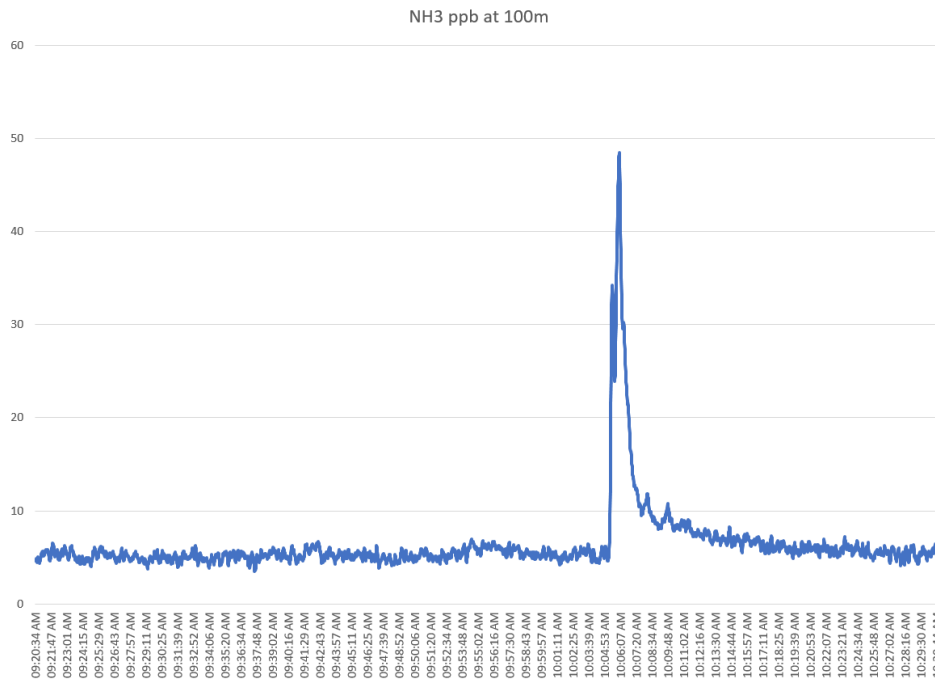
Figure 61. ICOS ammonia readings taken during the Wednesday morning camp fuel cook at 100 meters from the cook shed. The spike in ammonia concentration observed at 10:06 am corresponds to the bottle failure that occurred during this cook.

117
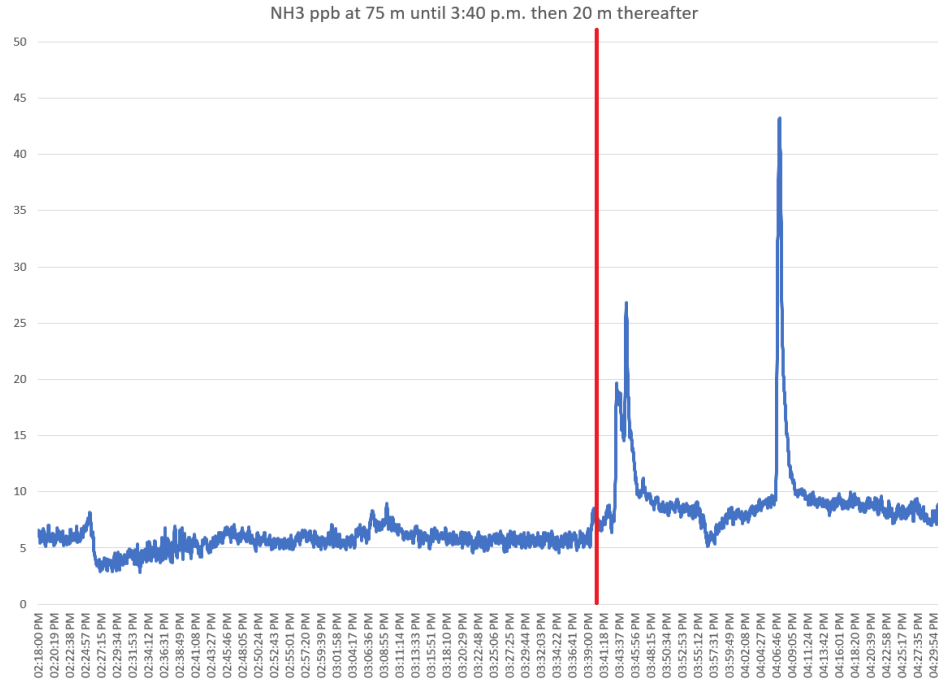
Figure 62. ICOS ammonia readings taken during the Wednesday afternoon ether cook at 75 meters from the cook shed. Red line marks ICOS relocation to 20 meters downwind of shed.
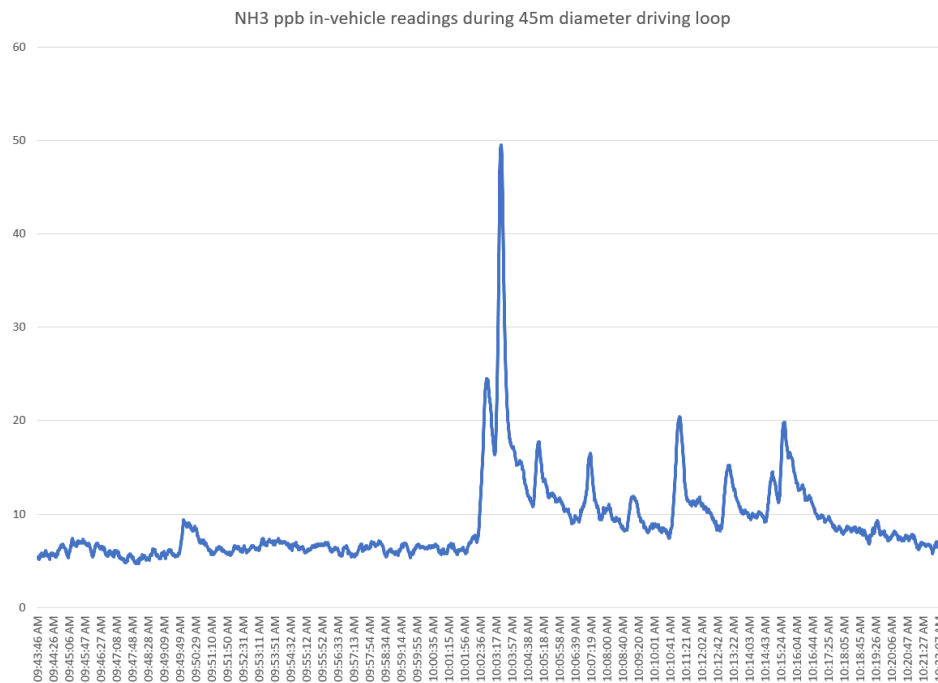
Figure 63. ICOS ammonia readings taken during the Thursday morning camp fuel cook while loaded in a rental vehicle and driven in circles of 45 meters in diameter downwind of the cook shed. Ammonia spikes became pronounced after the bottle failure and cook recovery to a food storage container that occurred shortly after 10 am. Peaks arose as the moving vehicle approached, and passed the live cook shed. Each circuit lap was approximately 2 minutes at low speed.
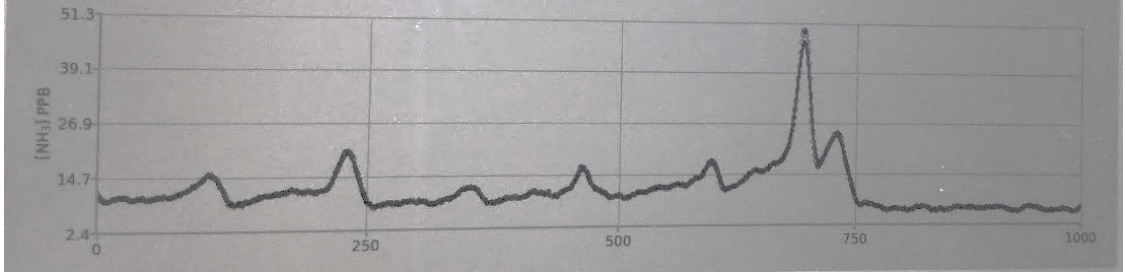
Figure 64. Ammonia peaks captured during 6 vehicle passes during the Thursday morning camp fuel cook.
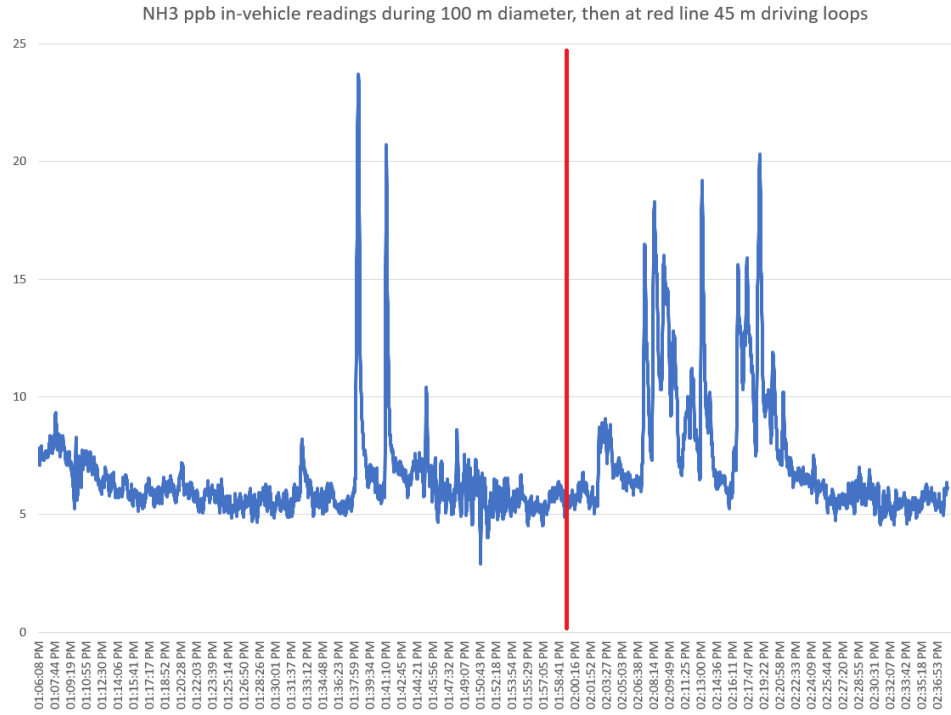
Figure 65. ICOS ammonia readings taken during the Thursday afternoon ether cook while loaded in a rental vehicle and driven in circles of 100 meters in diameter downwind of the cook shed. Circles later shortened to 45 meters in diameter at 1:59 pm (red line).

Table 23. Summary of ICOS distances from the cook shed and field notes.

Date	Cook type	Chronology	ICOS standoff	Notes
Nov 28 Tuesday PM	Camp Fuel	Cook start approx. 2:40 pm	40 m	Burp spikes evident
Nov 29 Wednesday AM	Camp Fuel	Cook start time approx. 9:25-9:41 am then bottle failure 10:04 am; fresh camp fuel cook from approximately 11:00 am – 12:30 pm	100 m	Bottle failure prominent spike
Nov 29 Wednesday PM	Ether	Cook start approx. 2:10 pm	75 m from cook start until 3:40 pm, then relocated to 20 m	ICOS turn on at 2:18 pm. Wind shift predicated move to 20 m
Nov 30 Thursday AM	Camp Fuel	9:30 am cook begun - bottle failure Camp Fuel cook, salvaged to food storage container and continued	Moving vehicle with 45 m diameter laps	Driven loops downwind of cook shed
Nov 30 Thursday PM	Ether	Cook start about 1:05 pm	Moving vehicle with 100 m diameter laps, laps later shortened to 45 m.	Driven loops downwind of cook shed

3.4.3.2 VOC monitoring

The GMAP vehicle's PID and the SPods were utilized for analysis of VOCs that were released during the One Pot methamphetamine cooks. The 3 EPA-ORD SPods reported PID data as raw analog-to-digital converter counts (DAQ counts) while the SenSevere SPod and the GMAP vehicle's PID reported data in ppb isobutylene. Data files collected by the SPod designated as SPod C during this study were corrupted, resulting in the loss of data from this SPod. Since the identity of the VOCs measured by the PIDs were not known and were likely comprised of numerous compounds, a correction factor could not be applied to the results and no true quantitative value could be determined during this research. However, a semi-quantitative value can be assigned to the data by comparing the instrument responses of the same units (i.e.

comparing the EPA SPod data to each other and comparing the GMAP vehicle's PID data to the

SenSevere SPod data).

For the PID installed on the GMAP vehicle, VOCs were identified at concentrations

between 14 and 2854 ppb isobutylene from a distance of 15 m from the cook shed. For the

SPods, at 25 m the VOCs were identified between 411 and 6030 DAQ counts, at 50 m the VOCs

were identified between 126 and 2697 DAQ counts, at 75 m the VOCs were identified between

117 and 594 DAQ counts, and at 100 m the VOCs were identified between 57.5 and 67.6 ppb

isobutylene. Table 24 shows the average, minimum, and maximum VOC concentrations

measured by the GMAP and SPod PIDs during each One Pot methamphetamine cook. Figure 66

– Figure 72 show the plots of the VOC concentrations measured over time by the GMAP vehicle

and SPod PIDs during each One Pot methamphetamine cook.

Table 24. The average, minimum, and maximum VOC concentrations measured by the GMAP vehicle and SPod PIDs during each One Pot methamphetamine cook. Data files collected by SPod C were corrupt, resulting in the loss of data from this SPod.

		Sampling Event						
		Ether 1	Camp Fuel 1	Camp Fuel Fail 1	Camp Fuel 2	Ether 2	Camp Fuel Fail 2	Ether 3
GMAP VOC Concentration (ppb Isobutylene)	**Average**	378	703	504	304	245	229	70
	Maximum	991	1850	2041	402	468	2854	284
	Minimum	278	522	373	267	216	115	35
25 m SPod VOC Concentration (DAQ Counts)	**Average**	973	1058	1005	798	546	-	-
	Maximum	2430	5737	6030	2131	1054	-	-
	Minimum	905	884	804	743	411	-	-
50 m SPod VOC Concentration (DAQ Counts)	**Average**	737	691	400	162	137	730	926
	Maximum	1072	2697	2134	200	153	2041	1076
	Minimum	682	638	280	133	126	624	897
75 m SPod VOC Concentration (DAQ Counts)	**Average**	-	-	-	-	-	137	461
	Maximum	-	-	-	-	-	162	594
	Minimum	-	-	-	-	-	117	374
100 m SPod VOC Concentration (ppb Isobutylene)	**Average**	64.4	64.6	60.7	57.9	58.5	63.1	63.6
	Maximum	65.5	67.6	65.8	58.6	59.0	65.2	64.3
	Minimum	63.8	64.1	59.6	57.5	58.0	60.4	63.0

a) GMAP vehicle PID – 15 m

b) EPA-ORD SPods – 25 m (black) and 50 m (orange)

c) SenSevere SPod – 100 m
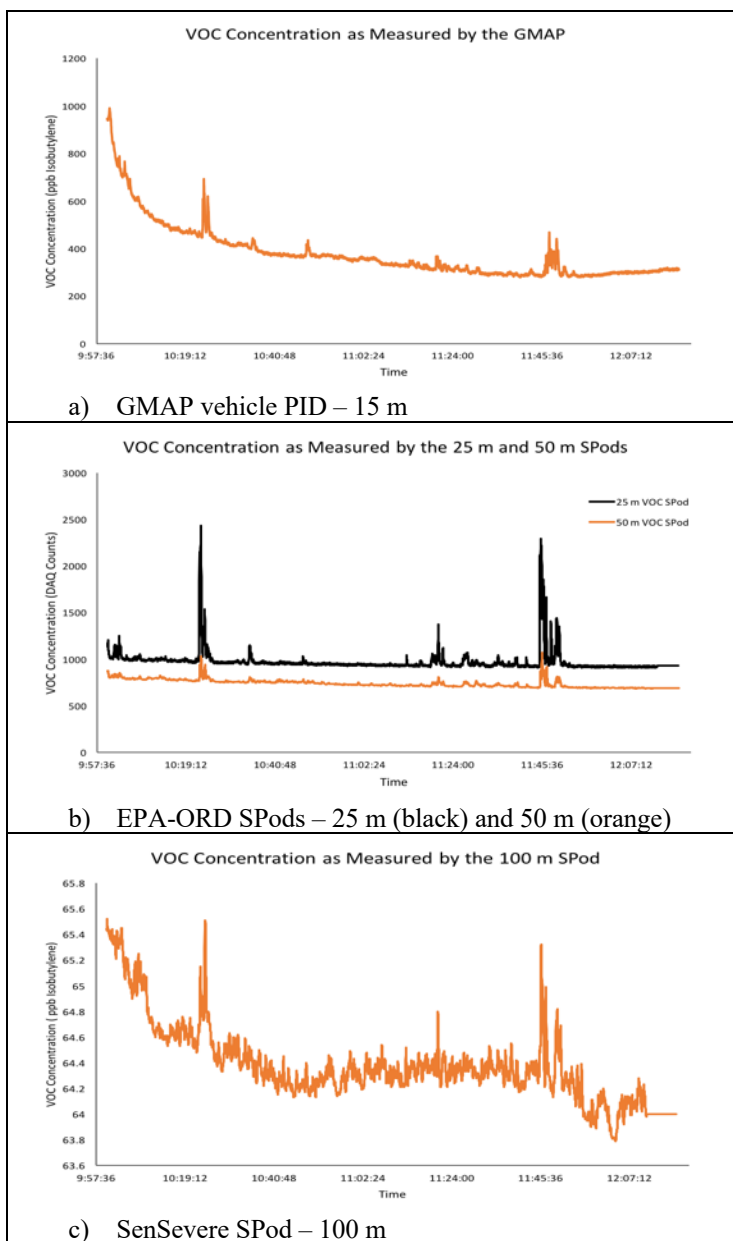
Figure 66. VOC concentrations measured over time during the first ether One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

a) GMAP vehicle PID – 15 m

b) EPA-ORD SPods – 25 m (black) and 50 m (orange)

c) SenSevere SPod – 100 m
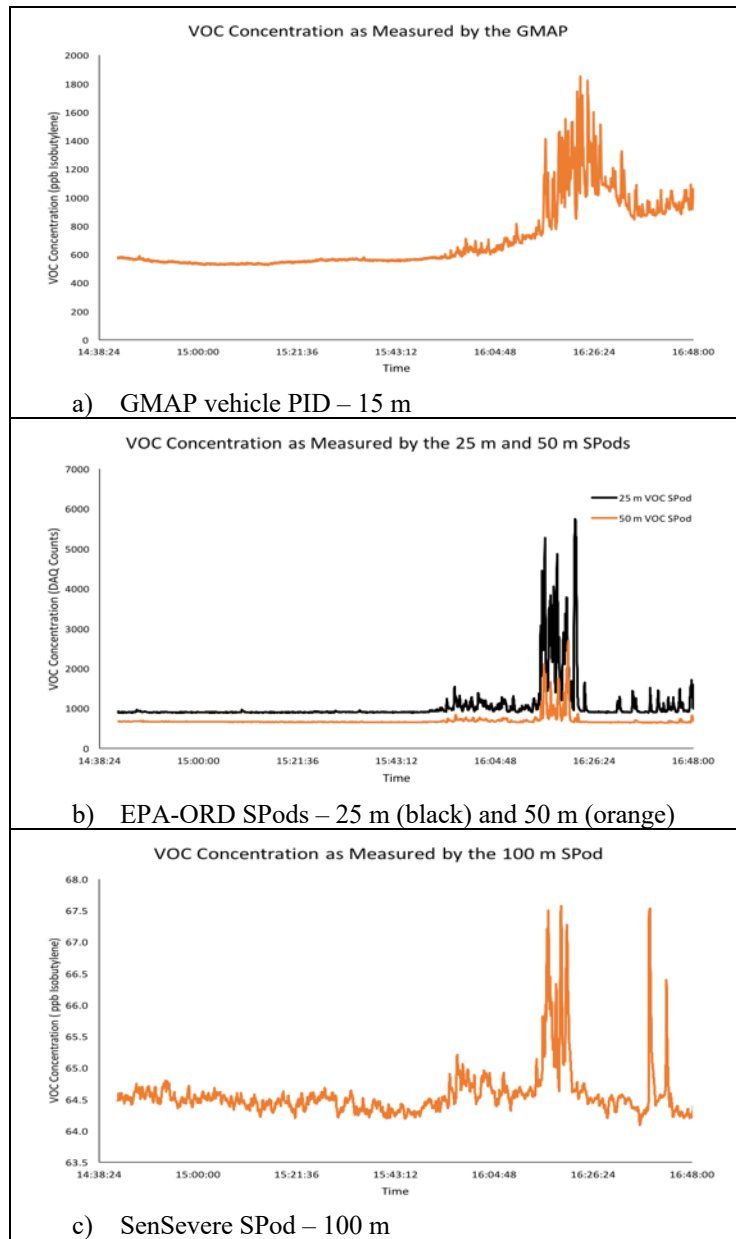
Figure 67. VOC concentrations measured over time during the first camp fuel One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

a) GMAP vehicle PID – 15 m

b) EPA-ORD SPods – 25 m (black) and 50 m (orange)

c) SenSevere SPod – 100 m
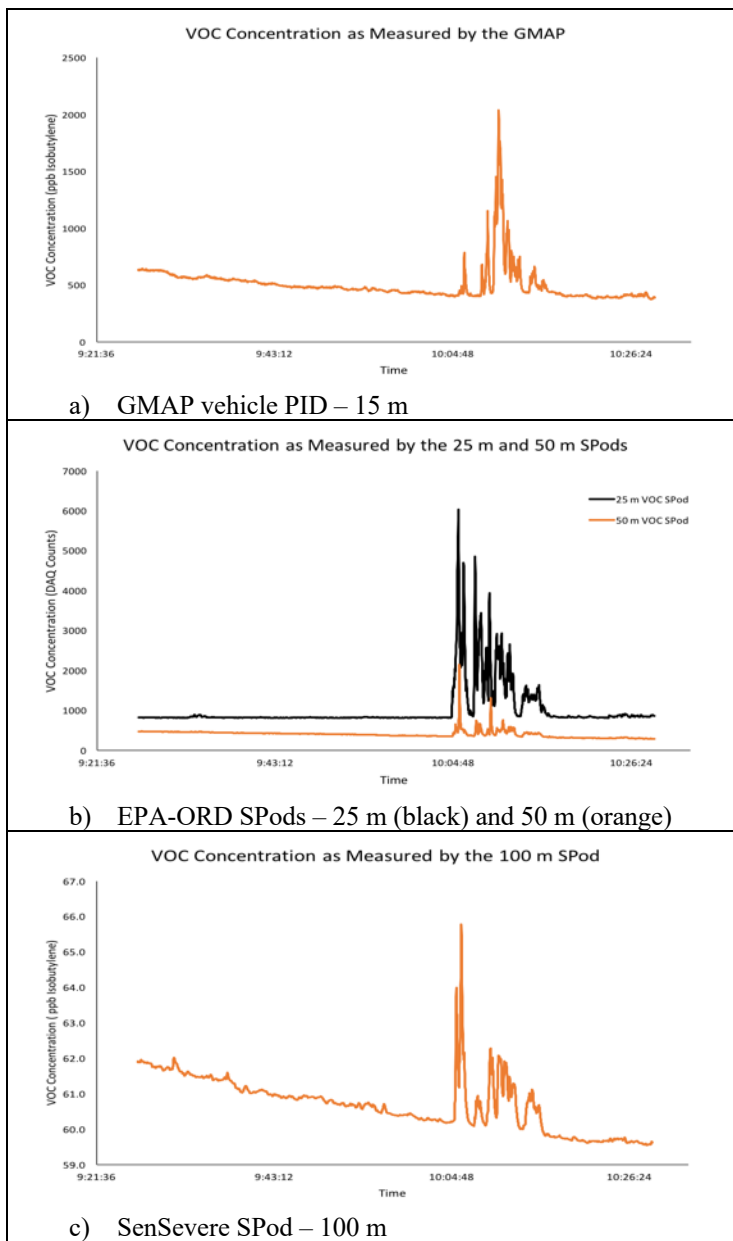
Figure 68. VOC concentrations measured over time during the first bottle failure. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

a) GMAP vehicle PID – 15 m

b) EPA-ORD SPods – 25 m (black) and 50 m (orange)

c) SenSevere SPod – 100 m
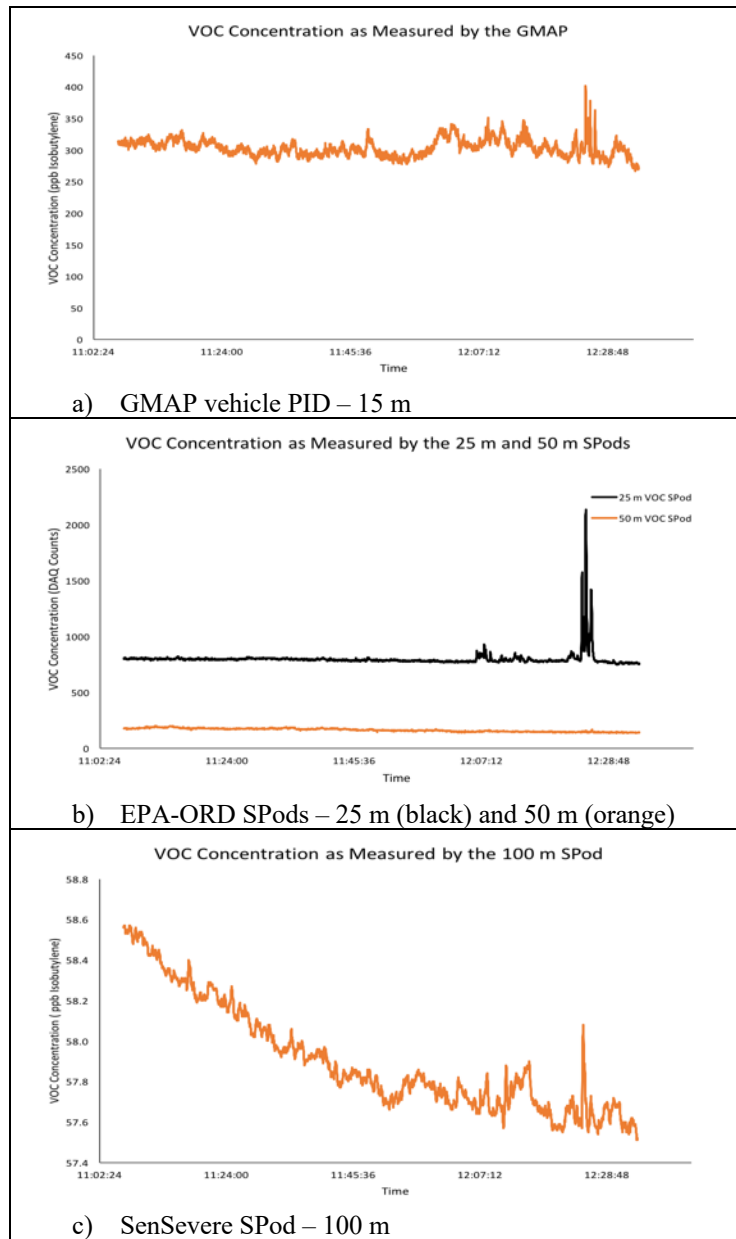
Figure 69. VOC concentrations measured over time during the second camp fuel One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

a)   GMAP vehicle PID – 15 m

b)   EPA-ORD SPods – 25 m (black) and 50 m (orange)

c)   SenSevere SPod – 100 m
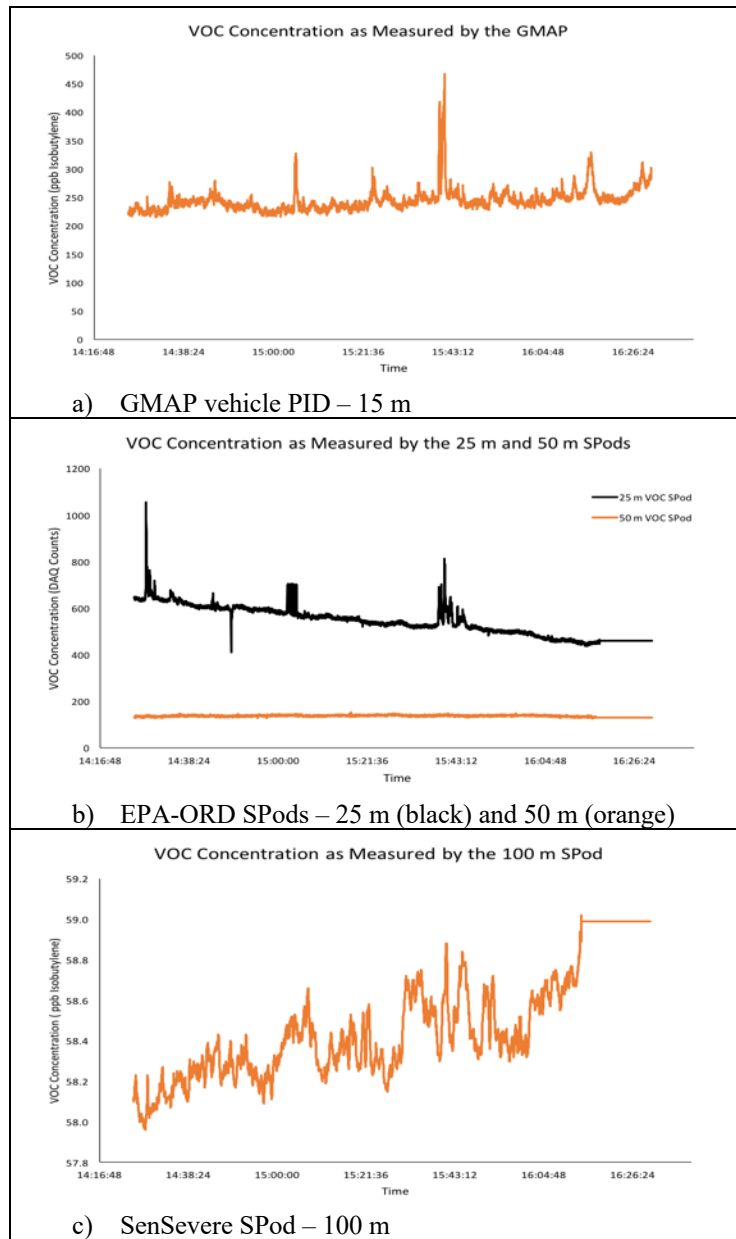
Figure 70. VOC concentrations measured over time during the second ether One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

a) GMAP vehicle PID – 15 m

b) EPA-ORD SPods – 25 m (black) and 50 m (orange)

c) SenSevere SPod – 100 m
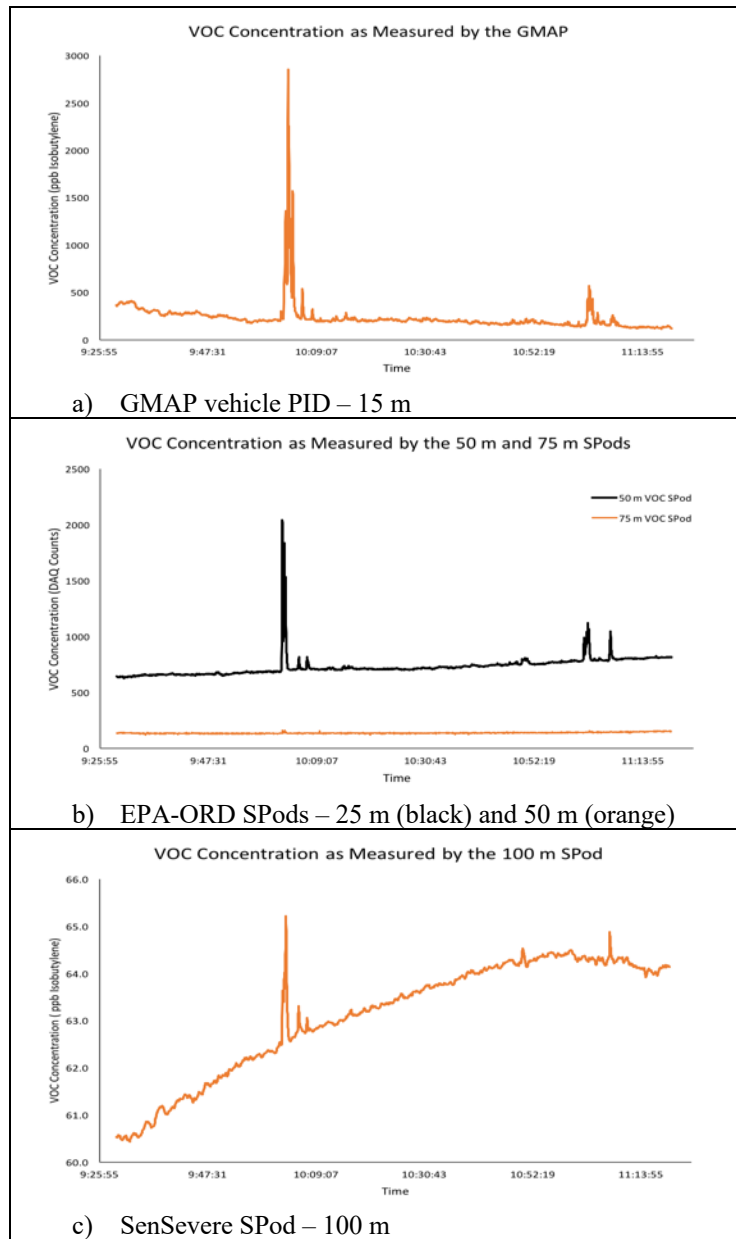
Figure 71. VOC concentrations measured over time during the second bottle failure/third camp fuel One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.
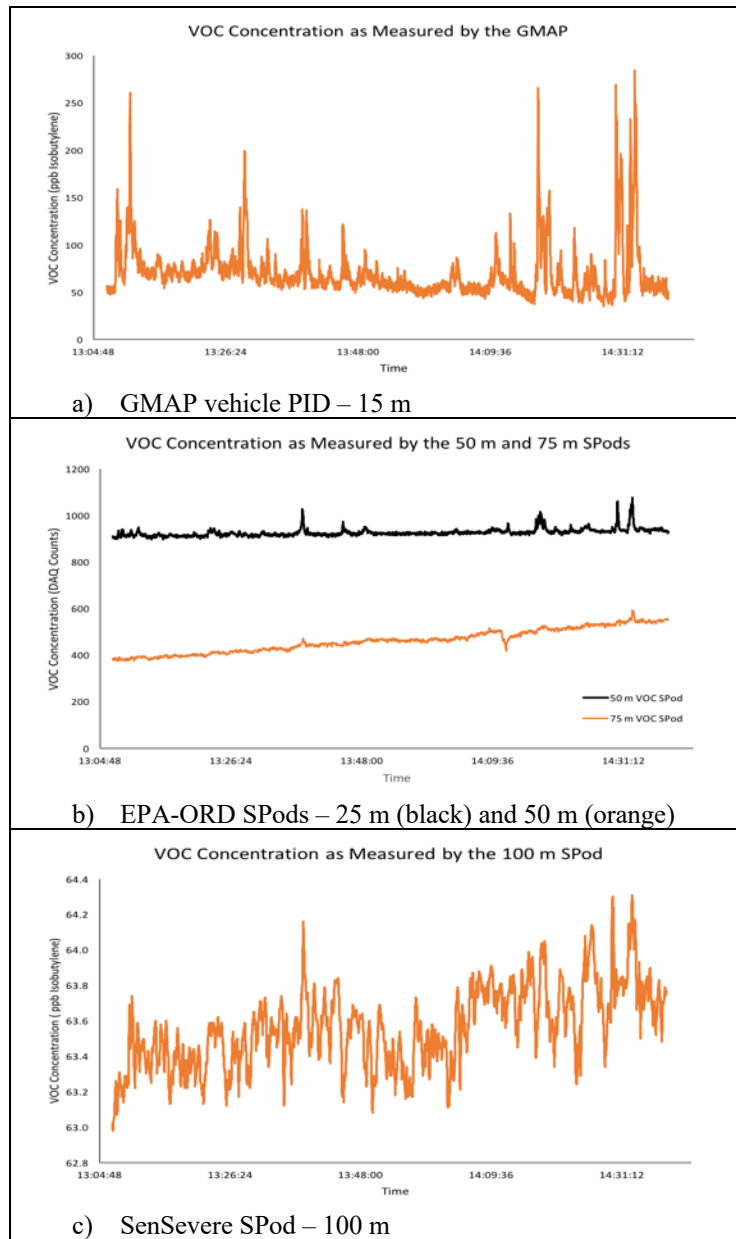
Figure 72. VOC concentrations measured over time during the third ether One Pot methamphetamine cook. **a.)** Measurements from the GMAP vehicle PID. **b.)** Measurements from the two working EPA-ORD SPods. **c.)** Measurements from the SenSevere SPod.

The maximum observed VOC concentrations were compared statistically using two separate two-way ANOVAs. The first compared the max VOC concentrations observed by the GMAP vehicle, which was parked 15 m from the One Pot, and the SenSevere SPod, which was set up 100 m from the One Pot. This two-way ANOVA looked at the difference in VOC concentrations observed between these two PIDs at various distances, as well as how the max

VOC concentrations differed between the two solvent types used. The two-way ANOVA found significant interaction (p=0.002) between the solvent type used and the distance from the One Pot that the PID was deployed, meaning the main effects of solvent type and distance cannot be examined individually. In other words, by having a significant interaction, the question of which solvent type results in a greater release of VOCs cannot be answered without also examining the distance the VOC plume is being measured from. This comparison can be completed by running four separate t-tests on the data (Table 25): the first comparing ether VOC concentrations at 15 and 100 m, the second comparing camp fuel VOC concentrations at 15 and 100 m, the third comparing ether and camp fuel VOC concentrations at 15 m, and the fourth comparing ether and camp fuel VOC concentrations at 100 m. The first t-test found no significant difference (p=0.071) in the in the VOC concentration observed during the ether One Pot methamphetamine cooks at 15 m when compared to those at 100 m. The second t-test found a significant difference (p=0.002) in the VOC concentration observed during the camp fuel One Pot methamphetamine cooks at 15 m when compared to those at 100 m. The third t-test found a significant difference (p=0.011) in the VOC concentration observed between the ether and camp fuel One Pot methamphetamine cooks at 15 m. The fourth t-test found no significant difference (p=0.199) in the VOC concentration observed between the ether and camp fuel One Pot methamphetamine cooks at 100 m.

Table 25. Summary of the four t-tests used to examine the significant interactions observed between the max VOC concentrations measured during the ether and camp fuel One Pot methamphetamine cooks at 15 and 100 m. Each row and each column represent a separate t-test, with the corresponding p-value recorded at the end of the row or the bottom column. VOC concentrations in ppb isobutylene.

Mean (±SD)	15 m	100 m	*p-value*
Ether	581 (±367)	62.9 (±3.5)	0.071
Camp Fuel	2248 (±533)	66.2 (±1.3)	0.002
p-value	0.011	0.199	

The second two-way ANOVA was also used to compare the max VOC concentrations observed during the One Pot methamphetamine cooks using ether and camp fuel, but it was used to compare the concentrations collected at 25 and 50 m with the EPA-ORD SPods. Since the SPod located 75 m from the cook site only had one set of data (Table 24), it was not included in the ANOVA. Additionally, since the 25 m SPod only contained two sets of data for both the ether and camp fuel One Pot methamphetamine cooks, the two max VOC concentrations recorded for each cook type were averaged to give a third data point for their respective solvent type at 25 m. As with the previous two-way ANOVA, there was significant interaction (p=0.001) between the solvent used and the distance from the cook site that the SPod was deployed. This interaction made it so the main effects of solvent type and distance on the max VOC concentration could not be examined individually so the same four t-tests were used to compare the max VOC concentrations obtained with both solvent types used at the two distances measurements were taken from. The results are summarized in Table 26. The first t-test found no significant difference (p=0.124) in the in the VOC concentration observed during the ether One Pot methamphetamine cooks at 25 m when compared to those at 50 m. The second t-test found a significant difference (p=0.001) in the VOC concentration observed during the camp fuel One Pot methamphetamine cooks at 25 m when compared to those at 50 m. The third t-test found a significant difference (p=0.001) in the VOC concentration observed between the ether and camp fuel One Pot methamphetamine cooks at 25 m. The fourth t-test found a significant difference (p=0.015) in the VOC concentration observed between the ether and camp fuel One Pot methamphetamine cooks at 50 m.

Table 26. Summary of the four t-tests used to examine the significant interactions observed between the max VOC concentrations measured during the ether and camp fuel One Pot methamphetamine cooks at 25 and 50 m. Each row and each column represent a separate t-test, with the corresponding p-value recorded at the end of the row or the bottom column. VOC concentrations in DAQ counts.

Mean (±SD)	25 m	50 m	*p-value*
Ether	1742 (±688)	767 (±532)	0.124
Camp Fuel	5884 (±147)	2291 (±355)	0.001
p-value	0.001	0.015	

The use of a FLIR camera allowed for real-time visualization of the VOCs released during the One Pot methamphetamine cook. While videos cannot be inserted into this document, Figure 73 show stills taken from a video captured by the FLIR camera. These stills illustrate the release of VOCs during a One Pot burp, highlighting how quickly an environment can fill with these compounds. Other videos (not shown here) demonstrate VOCs escaping through the small cracks in the ceiling, wall panels, and flooring, demonstrating the ease of VOCs to escape a site of One Pot production and contaminate adjacent environments.

Figure 73. Still shots taken from a video shot by the FLIR camera during a One Pot burp. **a.)** a One Pot as it is burped. **b.)** a One Pot one second after it was burped. **c.)** a One Pot six seconds after it was burped.

3.5 Discussion

3.5.1 One Pot methamphetamine cooks

Of the six One Pot methamphetamine cooks planned during this research, two resulted in bottle failures. A bottle failure occurs when the repeated stress and relaxation cycles the bottle is subjected too due to over-pressurization and burping, respectively, cause the structural integrity

of the bottle to fail, leading to leak. Many times, it is a bottle failure that leads to the flash fires commonly associated with One Pot methamphetamine production, as the failure releases a jet of flammable organic solvent, while simultaneously exposing lithium to air, which contains humidity that can cause the lithium to ignite, thus igniting the jet of organic solvent. In this case, both bottle failures occurred during One Pot methamphetamine cooks that used camp fuel as an organic solvent. Additionally, both bottle failures were likely the result of over-addition of sodium hydroxide. During the bottle failure cooks, a lack of "rolling" (visible bubbling and agitation) was observed so additional cap-fulls of sodium hydroxide were added during burping to drive the production of ammonia gas. The additional sodium hydroxide likely led to a greater formation of ammonia gas then desired, thus over-pressurizing the plastic bottles, ultimately leading to the compromised structural integrity of the reaction vessels, as shown in Figure 36 and Figure 37. While one of the camp fuel cooks were restarted and successfully carried out, there was not enough material to restart the second bottle failure. This resulted in only two camp fuel cooks, making statistical analysis difficult, though the bottle failures themselves also provided an additional look at environmental contamination stemming from a common event that occurs during One Pot methamphetamine production that was not intentionally built into this project.

3.5.2 Intra-shed air sampling
3.5.2.1 Active air sampling

While the literature has shown the salting out process to be the point in time that a majority of the volatilized methamphetamine is released in to the air, that was not the case in this study.[86] There was no significant difference in the amount of volatilized methamphetamine captured from the air by the active air samplers that were on for the duration of the cook versus those turned on during salting out. When examining the data of the five One Pot methamphetamine cooks where active air sampling was performed, two of the One Pot

methamphetamine cooks seem to agree with the literature, two seem to oppose the literature, and one is inconclusive. Cooks 2 and 4 both agree with the literature, with the active air samplers capturing more volatilized methamphetamine during the salting out process then during the rest of the cook (Table 15). Cooks 3 and 5 were not supported by the literature, likely due to the bottle failures that occurred during these One Pots. The second set of air samplers were not activated during cook 6, resulting in no data being collected during salting out of this One Pot.

When compared to the literature, the One Pot method had less volatilized methamphetamine present in the air than older methods of One Pot methamphetamine production. The One Pot methamphetamine cooks performed produced an average volatilized methamphetamine concentration of 11 $\mu g/m^3$ of air. A study by Martyny et al. examining the environmental contamination resulting from Red-P and Birch reduction methamphetamine cooks found average volatilized methamphetamine concentrations to be 760 and 170 $\mu g/m^3$ air respectively.[86] This may be explained by the amount of pseudoephedrine each cook starts with, and thus the amount of methamphetamine being produced by each cook method. Red-P cooks have been reported to start with 150 g of pseudoephedrine while Birch reduction cooks have been reported to start with 30 g of pseudoephedine;[89,90] the One Pot methamphetamine cooks performed during this project only used 0.6 g of pseudoephedrine. Performing the One Pots with a larger amount of pseudoephedrine, such as the ~12 g suggested by many of the One Pot manufacturers on online forums, may result in a similar amount of volatilized methamphetamine contaminating the air, as the 20-fold increase in starting material may generate volatilized methamphetamine concentrations closer to 220 $\mu g/m^3$.

3.5.2.2 Passive air sampling

In total, five different passive air sampling devices were used in this study. The 1 L MiniCans[TM] were used to collect samples before, during, and after the One Pot methamphetamine

cooks to observe what VOCs were present and how quickly they dissipated following completion of the One Pot. The HDS and two DSP samplers were worn at breathing level throughout the One Pot methamphetamine cooks to examine the level of VOC exposure individuals within these labs are exposed to. The 40 mL grab sample was allowed to equilibrate with the ambient air and provided an air sample representative of the air immediately adjacent to the jar in which the sample was collected.

A trend observed with the 1 L MiniCan™ sample data is that the concentration of VOCs captured throughout the camp fuel One Pot methamphetamine cooks quickly dissipated following completion of the cook while the concentration of VOCs captured throughout the ether One Pot methamphetamine cooks didn't dissipate as quickly (Table 17). For the two camp fuel cooks that passive air sampling was performed during, the concentration of VOCs present post-cook was, on average, 10% of that present mid-cook. For the two ether cooks that passive air sampling was performed during, the concentration of VOCs present post-cook was, on average, 58% of that present mid-cook. It is unclear why VOCs diffused out of the cook site at a slower rate during the ether cooks when compared to the camp fuel cooks, though the presence of the automotive starting fluid cans that had their bottoms pierced within the cook site may have been a continual source of VOCs throughout the One Pot but this cannot be said with certainty; the metal cans containing camp fuel were able to be capped during those One Pot methamphetamine cooks.

Of the three time points that the MiniCan™ samples were collected, the mid-cook samples contained the greatest concentration of VOCs (Table 18). This was expected, as mid-cook is when the One Pot is being burped and filtered, exposing the organic solvent used to the ambient air. Of interest, the camp fuel released a larger concentration of VOCs into the air than the ether did. All four pre-cook samples contained similar VOC concentrations, with a majority of the VOCs observed resulting from environmental background while the VOCs observed in the pre-cook sample from the second ether cook was due to the bottle failure during the preceding camp fuel cook, which spewed organic solvent throughout the cook site and was still evaporating

at the time of the pre-cook sample. For the mid-cook samples, it can be seen that camp fuel released, on average, 10x the amount of VOCs as ether did; the VOC concentration in the post-cook samples was 2x greater in the camp fuel samples than the ether samples.

This increased release of VOCs from the camp fuel One Pot methamphetamine cooks was further corroborated by the HDS personal monitors. These samplers found the air at breathing level to contain a significantly greater concentration of VOCs when camp fuel was used as the organic solvent as opposed to ether (Table 20). The results from analysis of these samplers also gave in site into the difference in exposure people who handle a One Pot methamphetamine lab have compared to those who are simply in the same room as one. Since one researcher handled the One Pot for the majority of the cook while the other got lab supplies set up for subsequent steps of the cook, their HDS monitors could be compared to see whether one researcher was exposed to more VOCs than the other. Based on the average VOC concentration captured by the HDS, both researchers were exposed to similar concentrations of VOCs. This means individuals who are within close proximity to an active One Pot, such as those who may be living in a house where One Pots are produced, are exposed to a similar level of VOCs as those who are actively manufacturing methamphetamine in this manner.

While the sorbent used in the MiniCan^{TM} and HDS samplers was designed to capture a wide range of VOCs, the sorbents of the DSPs was designed to capture a narrower range of VOCs. The first set of DSPs used Carbo Pack X as a sorbent and was designed to capture BTEX compounds, which are a class of compounds with known carcinogenic properties. The only BTEX compounds identified and quantitated from the Carbo Pack X DSPs were toluene and benzene. During the first ether and camp fuel cooks, only the researcher handling the One Pot had quantifiable toluene and/or benzene captured in their DSP while the first bottle failure, both researchers were exposed to toluene (Table 21). During the second ether cook, the DSP of the researcher setting up the lab equipment captured toluene and benzene, though it is likely that the badges containing the DSPs got mixed up and it was actually the researcher who handled the One

Pot that was exposed. While BTEX exposure are of concern due to their potential health risks, the levels observed in this study do not appear to be toxicologically important. A review of BTEX studies examining environmental BTEX concentrations found benzene and toluene to have average outdoor concentrations of 1.5-6.95 and 7.17-26.9 $\mu g/m^3$ respectively.[104] The levels of benzene and toluene exposure observed in this study were, respectively, 5x and 8x lower than the average outdoor concentrations, making the risk of BTEX exposure from One Pot methamphetamine labs minimal.

The second set of DSPs used Tenax TA as a sorbent, which is designed to capture VOCs with higher boiling points, such as long-chain or polyunsaturated hydrocarbons. The Tenax TA DSPs were qualitative passive air samplers used to identify some of the larger VOCs present in the air. As was expected, many more VOCs were captured during the camp fuel cooks than during the ether cooks, as camp fuel is made up of a large number of organic solvents that readily volatilize (Figure 51 – Figure 54). Many of the VOCs captured by the Tenax TA DSPs were methylated alkanes and saturated fatty aldehydes, which are neurotoxic and potentially carcinogenic, respectively.[105,106] The same compounds also identified in the 40 mL grab samples, though these samples saw a more even distribution of VOC species between the ether and camp fuel One Pot methamphetamine cooks (Figure 55 – Figure 58). While knowing the VOC species present as contaminants in the air is important, a flaw in this study is the lack of a field blank for the Tenax TA DSPs, as well as the 40 mL grab samples. A field blank was not obtained due to the limited number of passive air samplers provided for the project. A field blank, if taken before any One Pot production occurred, would have provided the identity of any VOCs present as background contaminants. Comparison of the VOCs that are present in both the camp fuel and ether One Pot cooks may provide an idea of potential background compounds, but these cannot be definitely attributed to the background or to the solvents without a field blank sample.

3.5.3 Standoff air monitoring
3.5.3.1 Ammonia monitoring

The DUVAS installed on the GMAP vehicle was not ideal for ammonia detection for

three reasons. First, the DUVAS was not calibrated in the field for ammonia prior to collecting

samples so the instrument sensitivity and specificity could not be verified. Second, the DUVAS

varied from standard ammonia sampling practices by not having a heated sampling line, which

can cause condensation to form in the inlet of the instrument. Since ammonia is hydroscopic, it

will seek out moisture in DUVAS sample line, preventing it from reaching the DUVAS sample

chamber and being analyzed. Third, the DUVAS had stainless steel fitting throughout the

instrument, which is not recommended for ammonia analysis. Stainless steel is pitted, which

provides absorption sites for the polar ammonia molecules to adhere to, thus preventing them

from reaching the sample chamber. Because of these three issues, the ammonia concentration

calculated by the DUVAS was considered unreliable and the data were treated as qualitative only.

Although the GMAP vehicle was parked within 15 m of the cook site during all 6 One

Pot cooks and both bottle failures, the DUVAS only obtained an increased instrumental response

for the first camp fuel cook, the second bottle failure/third camp fuel cook, and the third ether

cook (Figure 59 a, f, and g). While this lack of ammonia detection may be due to the three

reasons talked about above, it is more likely that ammonia plume detection by the DUVAS is

very dependent on wind direction, with the instrument response observed in Figure 59 a, c, d, and

e simply the depicting signal noise from the DUVAS.

During the first camp fuel cook (Figure 59 b), the concentration of ammonia gas

observed was stable from 14:38-16:04. This was the point in time in which the One Pot reaction

vessel remained closed to the environment, with the exception of the depressurizing burps that

were performed every 5 minutes. At 16:04, the One Pot reaction vessel was opened and the

solvent containing methamphetamine freebase was filtered, releasing the ammonia gas from the

cook into the atmosphere. This plume of ammonia gas was readily observed by the DUVAS, and the gradual decline in ammonia gas concentration can be observed as the ammonia diffused out of the shed and into the surrounding environment.

The next successful measurement of ammonia by the DUVAS was during the second bottle failure/third camp fuel cook (Figure 59 f). In this figure, two separate increases in ammonia concentration can be observed. The first increase occurred around 10:00 and corresponds to the release of ammonia during the actual bottle failure event. A slight decrease in ammonia concentration is then observed as the contents of the failed One Pot were added to a plastic food saver and the One Pot cook was completed and the ammonia that was released during the bottle failure diffused throughout the atmosphere. The second increase in ammonia concentration occurred around 10:52. This is when the lid was removed from the plastic food saver so the One Pot could be filtered. Of interest is that the ammonia level never returned to its baseline concentration, but rather remained elevated following the bottle failure and throughout the resultant One Pot cook. This was likely due to the negligible winds and the relatively high humidity on the day of this One Pot methamphetamine cook, which was 76%; ammonia would have been attracted to the excess moisture in the air, causing it to diffuse slower than on a drier day. With the elevated ammonia concentration in the air following the bottle failure, the ammonia released during the filtration of the organic solvent acted in an additive manner, further elevating the concentration of ammonia gas present. These results suggest that in humid environments, ammonia gas may be present in higher concentrations within One Pot methamphetamine labs, as it doesn't diffuse away as quickly and is allowed to build up within the site of production. This can lead to not only increased health hazards within and around a One Pot lab, but also may cause the ammonia concentration to reach its lower explosive limit of 15% and create a fire hazard.[87]

The final successful ammonia measurement with the DUVAS occurred during the third ether cook (Figure 59 g). Four increases in ammonia concentration were observed during the

cook around 13:28, 13:38, 13:43, and 14:15. The first three increases in ammonia were a result of burping the One Pot. This was the only One Pot methamphetamine cook performed during this research in which the release of ammonia gas during burping could be observed by the DUVAS, and even then, the ammonia plumes were not observed every 5 minutes when the One Pot was burped. The detection of ammonia during these burps is likely due to negligible winds during the third ether One Pot and the optimal positioning of the GMAP vehicle with respect to the cook shed. What can be observed during the burping events is an additive effect of burping on ammonia concentration. In other words, more ammonia was released during each burping event then could be diffused out of the shed between burping events, causing the concentration of ammonia gas to continue to grow until the wind speed increased and changed direction after the 13:43 burping event. This was the same phenomena observed during the second bottle failure/third camp fuel cook and was likely a result of the negligible winds and high humidity during the day. When the wind did pick up 13:55, the observed ammonia concentration quickly decreased until the One Pot reaction vessel was opened for filtration around 14:10, causing a final increase in the observed ammonia concentration.

While the DUVAS was not ideal for ammonia detection, the ICOS Gas Analyzer from Los Gatos Research was specifically designed for ammonia detection and quantitation. The ICOS proved to much more sensitive to ammonia gas, detecting quantifiable plumes of ammonia from as far as 100 m from the cook site, as well as detecting it wall being loaded in a moving vehicle. Rhythmic spikes in ammonia were observed during several of the One Pot cooks (Figure 61), which were resultant of the burping of the reaction. Being able to detect these rhythmic spikes in ammonia would be important in a field deployable instrument used by law enforcement. By seeing repeated ammonia spikes, it would be suggestive of the opening and closing of an ammonia-generating device, such as a One Pot methamphetamine lab, and not of a legitimate ammonia source, such as a household cleaner.

In addition to being able to detect ammonia released during One Pot burping events, the ICOS was also able to detect ammonia while being loaded in a vehicle and driven around the training center (Figure 63 and Figure 64). In these figures, increases in ammonia concentration are observed as the rental vehicle moves closer to the site of One Pot production and the concentration is observed to decrease as the vehicle moves away. In this manner, the ICOS could act as a sort of sonar to get an idea of where ammonia is being released. If deployed by law enforcement in a neighborhood, this technique may not be able to determine a single home as the source of ammonia emission, but it may be able to narrow the search range to only a couple of houses. Additionally, this ICOS sampler also comes with an HCl gas detector that could assist in this sonar idea; the HCl gas detector was not used in this research, as the one equipped on the instrument used was malfunctioning. If elevated ammonia concentrations were observed followed by elevated HCl concentrations, this would be suggestive of One Pot methamphetamine production and may provide enough suggestive evidence for law enforcement to obtain a warrant to search a home for One Pot methamphetamine labs, and if present, law enforcement would be able to seize them, reducing the amount of contamination being released into the surrounding environment.

3.5.3.2 VOC monitoring

PIDs work well for the standoff detection of VOCs, as they are sensitive instrumentation that are capable of observing most VOCs being released into the environment. The downside of using PIDs is that they are not specific, meaning they cannot differentiate between the compounds they are ionizing, and thus detecting. Due to this, the PID results are reported as total VOC concentration. In general, higher concentrations of VOCs were observed during the One Pot methamphetamine cooks that used camp fuel as an organic solvent, as opposed to ether. This is to be expected, as PIDs generally have a better response to long saturated hydrocarbons than to

short saturated hydrocarbons.[107] Camp fuel is a mixture of many saturated hydrocarbons, most of which have molecular structures that range from 5 to over 11 carbons in length.[108] The starting fluid used for the ether One Pot methamphetamine cooks is composed of 40-70% ethyl ether and 25-60% heptane.[109] While the ethyl ether and heptane can be observed with a PID, they will generate a lower detector response that the long, saturated hydrocarbons from the camp fuel.

While the camp fuel cooks resulted in a greater concentration of VOCs being observed by the PIDs, more frequent spikes in VOC concentration were observed during the ether cooks (Figure 66 vs. Figure 67). The larger number of spikes is likely due to the greater volatility of ether when compared to camp fuel, allowing more ether to be released from the One Pot during burping. While more spikes in the VOC concentration were observed in the ether cooks, the camp fuel cooks had a larger concentration of VOCs present when a spike was observed, which was usually during filtration of the One Pot.

Figure 66 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs during the first ether One Pot methamphetamine cook. The high VOC concentration observed at the beginning of the cook with the GMAP and SenSevere SPod is due to the loss of starting fluid during the depressurization of the cans. This increase can also be observed with the EPA-ORD SPod, but at a lesser extent. The large spike in VOCs observed near 10:25 correspond to the start of the first ether cook and the large spike near 11:45 correspond to filtration of the One Pot, just prior to salting out the methamphetamine. During both of these times, solvent is sitting in containers that are open to the environment, thus allowing more VOCs to be dispersed into the air than is observed during the rest of the cook. The spikes in VOC concentration during salting out is not due to the presence of HCl gas, as HCl does not readily ionize in PIDs, so the instrument cannot detect it.[107] The small spikes observed around 10:35 and 10:50 correspond to times when sodium hydroxide was added to the One Pot. During these events, the cap was removed from the One Pot so sodium hydroxide could be added to the reaction, allowing any VOCs that were in the headspace of the reaction to escape into the environment. The PIDs were able to detect plumes of

VOCs could be detected as far as 100 m downwind of the One Pot, though the burping events could not be detected at this distance. This suggests anyone living within 100 m of a One Pot methamphetamine lab could be subjected to environmental exposures from related chemicals. While the concentration of these compounds is likely low 100 m from their source, the health effects of repeated, low-level exposure to methamphetamine lab-related chemicals is currently unknown.[110]

Figure 67 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs during the first camp fuel One Pot methamphetamine cook. The concentration of VOCs present in the atmosphere begin increasing around 16:00, which is when the One Pot was filtered and salting out began. No other spikes in VOC concentration were observed during this One Pot, however, the baseline level of VOCs in the atmosphere was elevated slightly when compared to the ether One Pot performed earlier that morning. The absence of spikes in VOC concentration during burping events throughout the cook is attributed to the volatility of camp fuel when compared to ether. Ether is more volatile than camp fuel, causing more of this solvent to be released during burping than when camp fuel is used. The increased baseline level of VOCs is likely due to a decrease in wind speed observed during the afternoon of the camp fuel cook. The gentler breeze present during the camp fuel One Pot would cause the air, and thus the VOCs, to move less and would allow more of the VOCs to interact with the PID detector, resulting in a slightly elevated baseline level of VOCs.

Figure 68 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs during the first One Pot bottle failure. The large spike in VOC concentration around 10:10 is consistent with when the structural integrity of the bottle was compromised, causing camp fuel to be sprayed into the air. Following the bottle failure, another camp fuel One Pot methamphetamine cook was immediately started. The VOC concentrations measured by the GMAP vehicle and SPod PIDs during this second camp fuel cook can be seen in Figure 69. The GMAP vehicle's PID observed no apparent spike in the VOC concentration during the second

camp fuel cook, but the 25 m EPA-ORD SPod PID observed a large spike in VOC concentrations around 12:25 and the 100 m SenSevere SPod PID observed a small spike in VOC concentration at the same time point. Prior to the second camp fuel cook, the GMAP vehicle was moved to better position it downwind of the One Pot cook shed, however the wind direction changed before the second One Pot began and the GMAP was not moved again. Wind shift is therefore the most likely explanation for the GMAP PID not detecting VOCs emitted by the second camp fuel One Pot, but the EPA-ORD SPod at 25 m was still able to detect an increased concentration of VOCs during the filtration of the One Pot. The decrease in VOC concentration observed by the SenSevere SPod over the first 45 minutes of the second camp fuel One Pot is likely due to a raised background level of VOCs from the bottle failure that occurred immediately prior to the successful camp fuel cook. The SenSevere SPod was positioned far enough from the cook shed that it was able to detect smaller changes in VOCs than the SPods positioned close to the cook shed, which had larger concentrations of VOCs in the surrounding environment, masking small changes in the VOC concentration.

Figure 70 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs during the second ether cook. The PIDs were activated after the cans of starting fluid were depressurized, so the immediate increase in VOC concentration observed in the first ether cook (Figure 66) was not observed by the GMAP PID in this cook. As the VOCs from depressurization of the starting fluid cans diffused outwards from the cook shed, a spike in VOC concentration was observed by the 25 m EPA-ORD SPod. Due to the low level of VOCs present in the air during the second ether cook, small spikes in VOC concentration could be observed with the GMAP PID and the 25 m EPA-ORD SPod at several time points, such as around 15:05 and 15:21. These small spikes in VOC concentration correspond to times when the One Pot methamphetamine lab was burped. The large spike in VOC concentration observed around 15:40 was when the One Pot was filtered and the organic solvent was in a container open to the

atmosphere. For the second ether One Pot methamphetamine cook, no spike in VOC

concentration was observed with the SenSevere SPod located 100 m from the cook shed.

Figure 71 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs

during the second One Pot bottle failure/third camp fuel cook. As with the first bottle failure, a

large spike in VOC concentration is observed at the time point in which the structural integrity of

the bottle was compromised; this is seen around 10:00. Unlike the first bottle failure, after the

second bottle failure, the contents of the One Pot methamphetamine lab were dumped into a food

storage container, along with another instant cold pack and additional sodium hydroxide, and the

methamphetamine cook was allowed to continue. A second spike in VOC concentration is

observed around 11:00, which is when this modified One Pot was filtered and the organic solvent

was left open to the environment. The spikes in VOC concentration from the bottle failure and

the filtration process could be observed as far as 100 m from the cook site.

Figure 72 shows the VOC concentrations measured by the GMAP vehicle and SPod PIDs

during the third ether One Pot methamphetamine cook. As with the second ether cook, the

GMAP PID was not activated until the starting fluid cans had been depressurized, thus missing

the large, initial VOC spike. Also, as with the second ether cook, the background VOC

concentrations observed during the third ether cook were relatively low, enabling the spikes in

VOC concentration corresponding to the burping of the One Pot to be observed by the GMAP

vehicle's PID and the EPA-ORD SPods located at 50 m. No spikes in VOC concentration were

observed at 75 or 100 m during the third ether One Pot methamphetamine cook.

To supplement the VOC data collected with the GMAP vehicle and SPod PIDs, a

forward looking infrared (FLIR) video camera was used to visualize the VOCs released during

production of the One Pot methamphetamine labs. Figure 73 shows still photographs taken from

a video captured by the FLIR camera as two of the researchers hold and burp a One Pot. The first

photograph was taken right as the One Pot was burped and a small cloud of VOCs can be

observed beginning to surround the One Pot. In the second photograph, taken one second after

initiation of the burp, the cloud of VOCs can be seen surrounding the researcher's hands and forearms. In the third paragraph, the cloud of VOCs can be seen engulfing the researchers and is filling the headspace of the cook shed. Other videos from the FLIR camera allowed the researchers to visualize VOCs seen escaping the shed through cracks in the floor and walls, as well as rapid VOC diffusion once they escaped the shed and were mixed into the atmosphere by the wind.

By visualizing the plumes of VOCs released by the One Pot, a better understanding for how and where the VOCs move upon their release from the One Pot could be grasped. Of interest was the amount of VOCs that remained at breathing height until they were carried away by a breeze. HazMat training courses emphasis that most VOCs are heavier than air and will therefore settle in low-lying areas and can potentially cause health problems or pose a fire risk.[73] While it is true that VOCs will eventually settle in low-lying areas, the visualizations achieved with the FLIR camera showed that many of the VOCs will also linger at breathing level for some time before they finally settle to these low-lying areas. This could put anyone who enters a site of One Pot methamphetamine production at risk for potentially high levels of VOC exposure, even if they are not in a low-lying area.

CHAPTER IV

IDENTIFICATION AND REMEDIATION OF SURFACE CONTAMINATION FROM ONE POT METHAMPHETAMINE LABORATORIES

4.1 Introduction

As previously stated, the One Pot method is the most prominent route of methamphetamine production in the United States.[111] As with any route of methamphetamine production, the One Pot can cause environmental contamination in numerous ways, including the dumping of hazardous byproducts into the water system, the release of volatile compounds into the ambient air, and the contamination of surfaces within a site of methamphetamine production. For individuals who may inhabit a current or former site of One Pot methamphetamine production, residual methamphetamine that may have been volatilized during One Pot production can settle on surfaces, potentially leading to a drug exposure.

Typical exposures associated with former methamphetamine labs include dermal, inhalational, and oral exposures. Studies have shown methamphetamine can penetrate human skin, with absorption times varying depending on the material of the contaminated surface, as well as the moisture and pH of the skin.[112,113] Additionally, normal household activities, such as walking, can cause resuspension of methamphetamine to occur, leading to the potential of inhalational exposures.[114] The potential for oral exposures is greatest in children, who tend to have oral fixations and may put contaminated items in their mouths.[115]

Previous work by Martyny et al. examined the surface contamination associated with older routes of methamphetamine production, namely the Red-P and Birch reduction methods. That study found surface contamination levels to range from 0.1 – 860 µg/100 cm^2 for Red-P labs and 0.1 – 160 µg/100 cm^2 for Birch reduction labs after controlled production.[86] In seized former methamphetamine labs, Martyny et al. found average surface methamphetamine levels to be 499 µg/100 cm^2, exposure to which is equivalent to a dose of 0.38 mg/kg-day methamphetamine in infants;[86,93] CNS stimulation can occur in naïve users at levels as low as 0.07 mg/kg.[93] While the level of methamphetamine found in Red-P and Birch reduction labs can be quite high, these types of labs are designed to produce large amounts of methamphetamine when compared to One Pot methamphetamine labs, which typically produce less than 2 oz. of the drug.[111] Because of the lower amount of methamphetamine produced in One Pot labs, it is assumed these labs result in less surface contamination, though this theory has not been examined.

With contamination levels in methamphetamine production sites potentially reaching levels that can cause adverse health effects, remediation of these sites is important before allowing people to re-inhabit them. In the United States, there is no national standardized remediation practice or value. Individual states have set their own remediation levels that all fall between 0.05 – 1.5 µg/100 cm^2, however, they have not designated a decontamination method required to reach these remediation levels.[116] The EPA suggests throwing out items made mostly of porous materials (couches, chairs, etc.) and cleaning non-porous surfaces with soap and water.[116] No data has been published on the effectiveness of this cleaning technique for remediation of One Pot methamphetamine labs.

With the One Pot being the most common route of methamphetamine production in the United States, the amount of surface contamination associated with these labs needs to be examined. Additionally, a simple decontaminate technique needs to be assessed for effective remediation of former methamphetamine labs. This study sought to determine the level of

methamphetamine surface contamination observed following One Pot production and then tested the use of water as a simple decontamination agent for use in clandestine One Pot methamphetamine labs. Knowing the level of surface contamination within One Pot methamphetamine labs will be important in understanding the risks associated with entering and/or living in these clandestine drug labs while examination of the effectiveness of a simple decontamination technique will give confidence in the remediation techniques used at these former sites of methamphetamine production.

4.2 Review of the Literature

It is common knowledge that when methamphetamine is smoked or produced in a clandestine lab, some volatilized methamphetamine will be released into the environment and can settle on surfaces within the location of use or production. How much methamphetamine is released varies due to multiple factors, such as the amount of methamphetamine being smoked, the efficiency of the person smoking, the production method being used, and the amount of methamphetamine being produced.

When it comes to methamphetamine use, studies show that approximately two thirds of those who abuse the drug do so by either smoking or snorting it, and of those people, two thirds of them prefer to smoke methamphetamine over snorting it, though this number can vary over time and depending on geographical location.[117,118] When smoked, approximately 50% of the methamphetamine placed in a pipe will remain in the pipe while the other 50% is aerosolized. Of the methamphetamine that aerosolizes, 67 – 90% is taken into the body, leaving the remaining 10 – 23% in the atmosphere.[119,120] Based on those numbers, if a typical dose of 40 – 60 mg of methamphetamine is loaded into a pipe and smoked, 2 – 6.9 mg will be released into the environment and allowed to settle on surfaces within the site of use.[121] Previous studies have shown that this aerosolized methamphetamine tends to settle uniformly within the site of use,

leading to contamination levels in the range of 0.02 µg/100 cm^2 throughout a site of

methamphetamine use;[122] this contamination level may vary depending on the number of people

smoking, the size of the location smoking is occurring in, as well as the ventilation within the site,

which may cause areas of increased methamphetamine surface contamination due to movement

of air within the site. For reference, during remediation of former methamphetamine labs, most

states require the amount of surface methamphetamine contamination to be below 0.05 – 1.5

µg/100 cm^2, meaning a single smoked dose of methamphetamine can lead to 1.3 – 40% of the

total allowable methamphetamine surface contamination within a site.[116]

While smoking methamphetamine tends to leave a uniform, low-level contamination on

surfaces, the methamphetamine contamination from production is far greater and decreases as

you move outward from the source. In a study by Martyny et al. that examined the amount of

methamphetamine surface contamination generated by controlled Red-P and Birch reduction

cooks, it was found that following a Red-P cook, surfaces could be contaminated with as much as

860 µg of methamphetamine per 100 cm^2 surface while following a Birch reduction cook,

surfaces could be contaminated by as much as 160 µg of methamphetamine per 100 cm^2 surface.

This study took surface wipe samples at various distances from the site of the methamphetamine

cook and found that as they moved away from the cook site, the level of methamphetamine

concentration dramatically decreased (Table 27).[86] However, even the samples collected furthest

from the methamphetamine cook had concentrations of 0.1 µg/100 cm^2 or greater, which is the

decontamination value established by most states within the United States.[116] The study goes on

to state that, "virtually all surfaces within a structure were found to be contaminated above 0.1

µg/100 cm^{2}after a single cook," meaning one methamphetamine cook aerosolized enough

methamphetamine to contaminate an entire site above the allowable remediation values set up by

most state legislations.[86] While this study is considered by many to be the "gold standard" study

in examining methamphetamine lab-related contamination, it only focused on Red-P and Birch

reduction cooks, both of which are rarely seen in the United States today. Similar work remains

to be performed for One Pot methamphetamine labs.

Table 27. Surface methamphetamine concentrations collected with surface wipe sampling following controlled Red-P and Birch reduction methamphetamine cooks. Table reconstructed from data published by Martyny et al.[86]

Distance from Cook (m)	Number of Samples	Mean (μg/100 cm^2)	Range (μg/100 cm^2)
Red-P cooks	n=5		
< 2	14	100.9	0.1 – 860.0
2 – 4	11	40.7	0.8 – 45.0
> 4	4	21.7	11.6 – 31.0
Birch reduction cooks	n=3		
< 2	8	25.2	0.1 – 160.0
2 – 4	8	1.0	0.2 – 2.3
> 4	8	0.4	0.1 – 1.2

The amount of methamphetamine surface contamination discussed above was collected

after single methamphetamine cooks. In a real clandestine lab, many cooks would be performed

over time in the same area, likely with no clean up or decontamination steps implemented

between cooks. This would cause a buildup of methamphetamine surface contamination, leading

to levels much greater than those shown in Table 27. In addition to the controlled cooks

performed, Martyny et al. was able to take surface samples from within 14 seized

methamphetamine labs and found 82 of the 97 samples taken were positive for methamphetamine

at a level of 0.01 μg/100 cm^2 or greater, with mean and max concentrations of 511 and 16,000

μg/100 cm^2, respectively.[86] Many of the locations tested by Martyny were areas that could not be

contaminated by chemicals simply falling on a surface, but rather they were location such as

ceiling fans, ceilings, and air vents which would require aerosolization of the methamphetamine

to become contaminated. Additionally, many kitchen appliances, such as the refrigerators and

microwaves within these sites, were highly contaminated with methamphetamine. The presence

of large amounts of surface methamphetamine contamination within kitchens can lead to the

contamination of food and beverages, which can ultimately lead to oral exposures of methamphetamine for those living within these methamphetamine labs.

When methamphetamine is introduced to the body orally, it has a bioavailability of 67% and peak effects are reached in 180 minutes.[123] On average, children 2-5 years of age weigh around 15 kg;[124] with CNS stimulation occurring due to methamphetamine exposure at blood levels as low as 0.07 mg/kg, an average child would need to have 1.05 mg of methamphetamine in their blood stream to begin experiencing effects.[93] Based on these numbers, a child would have to ingest 1.57 mg of methamphetamine before CNS stimulation occurs. Martyny reported an average methamphetamine contamination level of 511 µg/100 cm^2, meaning 300 cm^2 of surface area within a home would contain enough methamphetamine to cause a 2-5 year old child to potentially have CNS stimulation.[86] When you consider that food, dishes, and toys all may be contaminated and could potentially transfer methamphetamine to a child through oral exposures, it wouldn't be hard for a child to accumulate 1.05 mg of methamphetamine in their blood.

In addition to oral methamphetamine exposures, people living within current or former methamphetamine labs can have inhalational exposures. While Chapter III of this dissertation examined the amount of methamphetamine released by labs into the air during a cook, the inhalational exposures being talked about here are related to resuspension of surface methamphetamine contamination. A study by VanDyke et al. examined the resuspension of methamphetamine for 24 hours following a Red-P cook. During this study, 2 Red-P methamphetamine cooks were performed on day one, and then testing occurred 13, 16, and 18 hours following the second cook, with no activity occurring within the site during the first 13 hours, a medium level of activity occurring the next 3 hours, and a high level of activity occurring the last 2 hours. This study found airborne methamphetamine levels in the area adjacent to the cook site to fall from an average of 640 µg/m^3 following the Red-P cooks to 70 µg/m^3 after the site had been left undisturbed for 13 hours. When medium levels of activity were performed in

this location (walking, sitting/standing from furniture, opening/closing cabinet doors), the airborne methamphetamine level rose to 170 μg/m^3 and when high levels of activity were performed (crawling, vacuuming, fluffing pillows, horseplay) the level rose to 210 μg/m^3.[125]

What is shown by VanDyke et al. is that most household activities will resuspend methamphetamine surface contamination, allowing it to be inhaled. When inhaled, methamphetamine has a 90% bioavailability and peak effects are reached in 18 minutes.[123] This means that an average 2-5 year old child can observe CNS stimulation after inhaling 1.17 mg of methamphetamine. An average 2-5 year old child has a tidal volume of 90 – 120 mL and a breath rate of 25 breaths per minute, meaning they take in 2.25 – 3 L of air every minute.[126] At this breathing rate, if the child is doing moderate activity within a contaminated home, such as walking through it, they can inhale between 382 – 510 μg of methamphetamine per minute, which is 32.6 – 43.5% of the dosage needed to cause CNS stimulation. If the child is playing or crawling around on the carpet, not only will the resuspend more methamphetamine then they would while walking, but they will also increase their respiration rate, leading CNS stimulation sooner.

Yet another route of exposure that can occur within current and former methamphetamine labs is dermal exposures. Studies by Salocks et al. examined the amount of methamphetamine that was able to cross skin (both epidermis and dermis) following contact with contaminated surfaces. They found that moisture, pH, and time of contact all played a major factor in the amount of methamphetamine that could cross the skin, with moist surfaces, low skin pH levels, and increased contact time leading to the greatest methamphetamine exposure.[112,113] Regardless of these three factors, it was found that at least a small fraction of methamphetamine (>0.1%) crossed the skin nearly immediately following contact with a contaminated surface. Even when skin was held in contact with a contaminated surface for only 15 seconds, methamphetamine was

able to penetrate epidermal layer and could not be completely removed with washing, allowing it to completely penetrate the skin samples used over time.[112]

From the VanDyke et al. study, surface wipes and vacuum samples found that surfaces throughout a site of methamphetamine production are contaminated with the drug. This includes locations children may play, such as on furniture or the carpet.[125] By coming in contact with these surfaces, children are subjected to dermal methamphetamine exposure. The more the children are allowed to play, the greater their exposure is, as they will be in contact with the contaminated surfaces for greater periods of time, and if they begin to sweat, moisture can cause more methamphetamine to be captured on the skin, while opened pores can provide an easier pathway for the drug to cross the skin.[112] This is especially true for infants, who spend more time crawling around and having more surface area in contact with contaminated surfaces than toddlers who are able to walk and run.

While additional routes of exposure do exist for those living in current and former methamphetamine labs, such as introduction through mucous membranes or open wounds, the oral, inhalational, and dermal routes of exposure discussed above make up a majority of the events leading to methamphetamine entering the body. Due to multiple routes of methamphetamine exposure and the variability in the amount of methamphetamine that can enter the body with each route of exposure, it is extremely difficult to estimate the total amount of methamphetamine that is entering the body of somebody living in these contaminated sites without performing urinary or blood drug testing over time. To add additional difficulty to this task, each method of methamphetamine production can result in different levels of surface contamination, and the most common route of production in the United States, the One Pot method, has not been thoroughly examined to determine the amount of surface contamination generated by this production method.[86]

It has been shown that surface methamphetamine contamination can be reduced through the use of numerous cleaning practices. The most commonly used cleaning agents include

bleach, OxiClean, 409®, and soap with water. While all of these have been shown to remove methamphetamine from surfaces with greater than 90% efficiency, they have only assessed their efficiencies in laboratory settings or on contaminated clothing;[127–129] their efficiencies at removing methamphetamine contamination from a former site of One Pot production have not been assessed. For the decontamination of former methamphetamine labs, the EPA advises against the use of bleach, as the byproducts produced have not been fully characterized and the use of acid in methamphetamine labs can result in the formation of chlorine gas if it comes in contact with bleach.[116] OxiClean, a peroxide-based cleaner, is not recommended by the EPA for decontamination of former methamphetamine labs due to the unknown byproducts that may be generated by its use.[116] 409® is a quaternary amine-based multipurpose household cleaner. While effective at removing methamphetamine surface contamination, it is impractical to use on a large-scale cleanup due to its link to the formation of occupational asthma in individuals working with it.[130] The use of soap and water has proven effective for removing surface methamphetamine contamination without forming hazardous byproducts and is currently the only decontamination method supported by the EPA for use in former methamphetamine labs.[116,128]

While supported by the EPA, the use of soap and water has not been tested by the scientific community as a decontamination solution in former One Pot methamphetamine laboratories. Before this decontamination solution is tested, the amount of surface contamination generated from a One Pot methamphetamine lab must first be investigated. This research seeks to do just that. Six One Pot methamphetamine labs were performed in a plastic garden shed to simulate a real lab environment. Following One Pot methamphetamine production, the site was swabbed and the resulting samples were tested in near-real time with lateral flow immunoassays to determine the presence or absence of methamphetamine. The production site was then cleaned by simply washing with water and the level of methamphetamine contamination was reassessed. Following the final One Pot methamphetamine cook, the samples tested by lateral flow immunoassay were sent to the CDC-NIOSH Taft laboratory in Cincinnati, OH for quantitative

analysis. This research was performed to complete two separate goals. The first was to determine what the level of surface methamphetamine contamination was following One Pot production. The second was to assess the effectiveness of a simple water wash in removing methamphetamine from the surfaces within these labs. Completion of this research will give others a better idea of the contamination level within former One Pot methamphetamine labs, and thus the risk individuals living within these sites may be in. Additionally, it will give insight into the effectiveness of a simplified decontamination technique, resulting in either confidence that a former One Pot methamphetamine lab can be remediated in this manner, or that additional work must be performed to find a suitable decontamination technique.

4.3 Methodology

4.3.1 Reagents and materials

This research and the ambient air monitoring research outlined in Chapter III of this dissertation were performed concurrently. All reagents and materials listed in section 3.3.1 Reagents and materials were also used in this research.

4.3.2 One Pot methamphetamine cooks

The six One Pot methamphetamine cooks performed during this research to assess the level of surface contamination generated by them are the same cooks outlined in section 3.3.2 One Pot methamphetamine cooks.

4.3.3 Surface wiping

To assess the level of methamphetamine contamination generated by the One Pot cooks, surface swabbing was performed before and after methamphetamine production, as well as

following a wet decontamination of the cook site and researchers; the wet decontamination consisted of simply being hosed off with water. Surface swab samples were collected from 11 locations within the cook shed, 2 locations directly outside the cook shed, and the arms, legs, chest, SCBA mask, and air tanks of the researchers (Figure 74 and Figure 75). Sampling locations from within the shed included the three walls, the ceiling, the floor, the table where the cooks took place, and a table that was used to hold additional supplies. All sampling sites were approximately 100 cm^2 and were sampled by wiping in a N-pattern, then a Z-pattern, and then another N-pattern. Samples were collected with a sterile cotton swab (Puritan Medical Products Company LLC, Guilford, ME) wetted with phosphate-buffered saline (PBS) containing Triton^{TM} X-100 added as a surfactant (wetting buffer). Gloves were changed between each sampling site to avoid cross contamination.
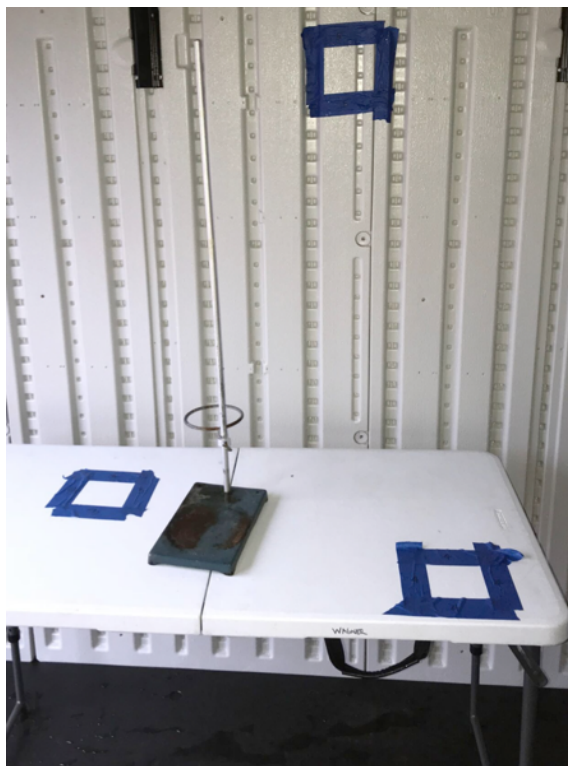
Figure 74. Three of eleven 10 x 10 cm (100 cm^2) sampling locations selected within the One Pot methamphetamine cook shed.

Figure 75. Surface contamination sampling of one of the researchers prior to beginning a cook. One of the sample locations directly outside the shed can be observed in the background.

4.3.4 Competitive lateral flow immunoassays

Surface swab samples were analyzed in near-real time by use of competitive lateral flow immunoassays (LFIAs). The LFIAs used in this research (Figure 76) were developed an provided by the Division of Applied Research and Technology at CDC-NIOSH (Cincinnati, OH) and the sampling methodology used was previously reported.[131] Briefly, the sample is loaded onto the sample pad and is drawn through the LFIA, towards a wicking pad by capillary action. When the sample crosses through the conjugate pad, any methamphetamine present will interact with gold nanoparticle-labeled anti-methamphetamine antibodies. The free antibodies and methamphetamine-antibody complexes will continue moving towards the wicking pad until they come in contact with the test line, which is spiked with methamphetamine-bovine serum albumin (BSA) conjugates. Free antibody will bind the methamphetamine-BSA conjugates at the test line,

causing a concentration of gold nanoparticles, which appear red in color due to their strong absorption of light at 520 nm.[132] If the antibody is bound to methamphetamine, it will not bind the BSA conjugates at the test line, preventing the red color from appearing. The control line of the LFIA contains a secondary antibody to the gold nanoparticle-labeled anti-methamphetamine antibody, causing it to capture the excess antibodies present in the LFIA and a red line to form during every test. The control line is used as a check to ensure the LFIA is operating correctly.
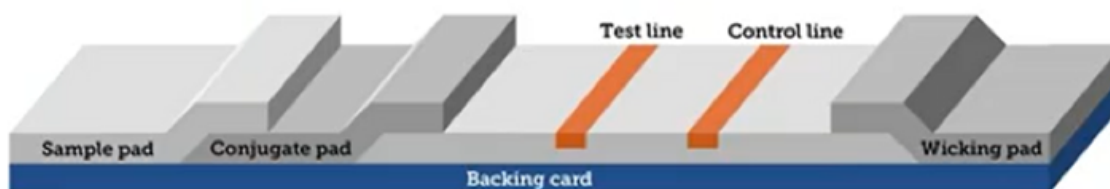
Figure 76. Schematic of a LFIA. Sample is introduced to the sample pad and migrates towards the wicking pad by capillary action. Sample containing methamphetamine will bind the gold nanoparticle-antibody conjugates, preventing them from binding to methamphetamine-BSA conjugates at the test line, resulting in the absence of a red line. Samples not containing methamphetamine will allow the gold nanoparticle-antibody conjugates to bind the methamphetamine-BSA conjugates at the test line, resulting in the presence of a red line. The control line contains a secondary antibody to the gold nanoparticle-antibody conjugate and will always result in a red line if the LFIA is working properly.

After swabbing a surface, the tip of the cotton swab was broken off into a tube containing 1 mL of wetting buffer. The solution was allowed to sit for several minutes, and then three drops were added to the sample chamber of the LFIA by a 0.5 mL transfer pipette. The LFIA was allowed to develop for 3-5 minutes and was then read. If two lines were clearly visible, the sample was classified as negative for methamphetamine, and if only one line was clearly visible, the sample was classified as positive for methamphetamine (Figure 77). If one line was clearly visible and the second was visible but faint in appearance, the sample was classified as a trace positive. The reported limit of detection of the LFIAs used was 50 ng/100 cm^2.

Figure 77. Competitive lateral flow immunoassay cassette developed by CDC-NIOSH for detection of methamphetamine at a concentration of 50 ng/100 cm^2. Sample 1A was taken pre-cook and sample 1B was taken from the same location post-cook. Two red lines indicate the absence of methamphetamine in the sample. One red line indicates the presence of methamphetamine in the sample.

4.3.5 Fluorescent covalent microbead immunosorbent assay

Following completion of the One Pot methamphetamine cooks, the remaining wetting buffer from the samples analyzed with LFIAs was sent to the CDC-NIOSH Taft Laboratory in Cincinnati, OH for quantitative analysis by FCMIA. This assay was covered in detail in section 3.3.3.1 Active air sampling were it was used to quantitate the amount of methamphetamine captured on PTFE filters during active air sampling. The methodology only differs between the PFTE filters and LFIA wetting buffer in the initial step. While methamphetamine had to be extracted from the PFTE filter with 2 mL of wetting buffer, the LFIA wetting buffer sample had no preparatory step. After the microspheres were added to 1.2 μm filter membrane microtiter plate and the liquid was aspirated, 50 μL of the FLIA wetting buffer sample was added to the wells and the methodology proceeded as previously described.

4.4 Results

In total, 96 surface wipe samples were collected and analyzed: 23 pre-cook, 41 post-cook, 27 post-decontamination (post-decon), and 5 others. Pre-cook samples were collected prior to any methamphetamine production, post-cook samples were collected following each methamphetamine cook, and post-decon samples were collected after the researchers and/or cook shed were hosed with water. The 5 samples from the other category included 2 field blanks, and 3 samples used to test the clothing of sample collectors at the end of the week, ensuring they were not contaminated with methamphetamine. All statistical analyses were performed by use of Microsoft Excel (Microsoft Corporation, Redmond, WA).

4.4.1 Competitive lateral flow immunoassays

The sample location and individual results for the 96 LFIAs are shown in Table 28. Table 29 summarizes the overall LFIA results. All LFIAs run on the pre-cook and other category samples were negative. For the post-cook samples, 23 were positive, 5 were trace positives, and 13 samples were negative. For the post-decon samples, 2 were positive, 3 were trace positives, and 22 were negative. Samples were considered trace positives when a faint line was observed at the test site of the LFIA, but the line was not bright enough to be deemed a true negative. LFIA results were analyzed by a Chi square test of independence, where H_0 stated the LFIA results were independent of the time point in which the sample was collected. For this test, trace positives were considered positives and the number of positive and negative LFIA observed during pre-cook, post-cook, and post-decon collection points were compared to the expected number of positive and negatives. The X^2 value was 34.96; with 2 degrees of freedom, the p-value was <0.001 so H_0 was rejected. This means the number of positive and negative LFIAs was dependent on the time point in which they were collected.

Table 28. Results of the LFIA and FCMIA performed on the surface swab samples. For all 96 samples, the vial of liquid sample was able to be analyzed by both assays.

	Sample ID	Sample Location	Sample Time	LFIA Results	FCMIA Results (ng/100 cm²)	Cook Type
	LFIA 1A	Left Side Shelf	Pre-Cook	Negative	0.15	Ether
	LFIA 1B	Left Side Shelf	Post-Cook	Positive	6.20	Ether
	LFIA 2A	Right Side Shelf	Pre-Cook	Negative	0.14	Ether
	LFIA 2B	Right Side Shelf	Post-Cook	Positive	7.94	Ether
	LFIA 3A	Left Front Table	Pre-Cook	Negative	0.26	Ether
	LFIA 3B	Left Front Table	Post-Cook	Negative	257.69	Ether
	LFIA 4A	Right Front Table	Pre-Cook	Negative	1.36	Ether
	LFIA 4B	Right Front Table	Post-Cook	Positive	130.82	Ether
	LFIA 5A	Left Wall	Pre-Cook	Negative	0.60	Ether
	LFIA 5B	Left Wall	Post-Cook	Trace	3.92	Ether
	LFIA 6A	Back Table	Pre-Cook	Negative	0.83	Ether
	LFIA 6B	Back Table	Post-Cook	Positive	9.04	Ether
	LFIA 7A	Back Wall	Pre-Cook	Negative	0.50	Ether
	LFIA 7B	Back Wall	Post-Cook	Positive	1.35	Ether
	LFIA 8A	Right Wall	Pre-Cook	Negative	0.31	Ether
	LFIA 8B	Right Wall	Post-Cook	Positive	2.05	Ether
	LFIA 9A	Floor Back	Pre-Cook	Negative	0.57	Ether
	LFIA 9B	Floor Back	Post-Cook	Positive	1.00	Ether
Cook 1	LFIA 10A	Ceiling	Pre-Cook	Negative	0.29	Ether
	LFIA 10B	Ceiling	Post-Cook	Positive	1.98	Ether
	LFIA 11A	Floor Front	Pre-Cook	Negative	0.53	Ether
	LFIA 11B	Floor Front	Post-Cook	Positive	4.34	Ether
	LFIA 12A	Left Door	Pre-Cook	Negative	0.39	Ether
	LFIA 12B	Left Door	Post-Cook	Positive	2.57	Ether
	LFIA 13A	Right Door	Pre-Cook	Negative	2.41	Ether
	LFIA 13B	Right Door	Post-Cook	Negative	0.10	Ether
	LFIA 14A	Front Right Arm-Researcher 2	Pre-Cook	Negative	0.12	Ether
	LFIA 14B	Front Right Arm-Researcher 2	Post-Cook	Negative	1.59	Ether
	LFIA 15A	Front Left Arm-Researcher 2	Pre-Cook	Negative	0.26	Ether
	LFIA 15B	Front Left Arm-Researcher 2	Post-Cook	Negative	0.83	Ether
	LFIA 16A	Back Right Leg-Researcher 2	Pre-Cook	Negative	0.21	Ether
	LFIA 16B	Back Right Leg-Researcher 2	Post-Cook	Negative	0.76	Ether
	LFIA 17A	Back Left Leg-Researcher 2	Pre-Cook	Negative	0.21	Ether
	LFIA 17B	Back Left Leg-Researcher 2	Post-Cook	Positive	2.48	Ether
	LFIA 18A	Back Head-Researcher 2	Pre-Cook	Negative	0.16	Ether
	LFIA 18B	Back Head-Researcher 2	Post-Cook	Negative	0.68	Ether
	LFIA 19A	Front Right Arm-Researcher 1	Pre-Cook	Negative	0.15	Ether
	LFIA 19B	Front Right Arm-Researcher 1	Post-Cook	Positive	6.52	Ether

	Sample ID	Location	Timing	Result	Value	Solvent
	LFIA 20A	Front Left Arm-Researcher 1	Pre-Cook	Negative	0.19	Ether
	LFIA 20B	Front Left Arm-Researcher 1	Post-Cook	Positive	2.41	Ether
	LFIA 21A	Back Right Leg-Researcher 1	Pre-Cook	Negative	0.15	Ether
	LFIA 21B	Back Right Leg-Researcher 1	Post-Cook	Positive	16.32	Ether
	LFIA 22A	Back Left Leg-Researcher 1	Pre-Cook	Negative	0.13	Ether
	LFIA 22B	Back Left Leg-Researcher 1	Post-Cook	Positive	3.91	Ether
	LFIA 23A	Face Mask-Researcher 1	Pre-Cook	Negative	0.14	Ether
	LFIA 23B	Face Mask-Researcher 1	Post-Cook	Positive	7.30	Ether
	LFIA 24A	Field Blank	-	Negative	0.13	Ether
	LFIA 25B	Field Blank	-	Negative	0.12	Ether
Cook 2	LFIA 29A	Front Right Arm-Researcher 3	Post-Cook	Negative	1.18	Camp Fuel
	LFIA 30A	Front Left Arm-Researcher 3	Post-Cook	Negative	0.67	Camp Fuel
	LFIA 31A	Back Left Leg-Researcher 3	Post-Cook	Negative	0.41	Camp Fuel
	LFIA 32A	Back Right Leg-Researcher 3	Post-Cook	Negative	0.64	Camp Fuel
	LFIA 33A	Forehead-Researcher 3	Post-Cook	Positive	5.26	Camp Fuel
	LFIA 34A	Front Right Arm-Researcher 4	Post-Cook	Positive	4.10	Camp Fuel
	LFIA 35A	Front Left Arm-Researcher 4	Post-Cook	Trace	4.90	Camp Fuel
	LFIA 36A	Back Left Leg-Researcher 4	Post-Cook	Negative	1.76	Camp Fuel
	LFIA 37A	Back Right Leg-Researcher 4	Post-Cook	Negative	3.55	Camp Fuel
	LFIA 38A	Face Mask-Researcher 4	Post-Cook	Positive	9.84	Camp Fuel
Cook 3	LFIA 29B	Air Tank-Researcher 1	Post-Decon	Negative	0.45	Camp Fuel
	LFIA 30B	Left Side Shelf	Post-Decon	Negative	1.13	Camp Fuel
	LFIA 31B	Right Side Shelf	Post-Decon	Negative	0.62	Camp Fuel
	LFIA 32B	Left Front Table	Post-Decon	Trace	4.30	Camp Fuel
	LFIA 33B	Right Front Table	Post-Decon	Positive	45.67	Camp Fuel
	LFIA 34B	Left Wall	Post-Decon	Negative	1.32	Camp Fuel
	LFIA 35B	Back Table	Post-Decon	Negative	3.17	Camp Fuel
	LFIA 36B	Back Wall	Post-Decon	Negative	0.65	Camp Fuel
	LFIA 37B	Right Wall	Post-Decon	Negative	1.04	Camp Fuel
	LFIA 38B	Floor Back	Post-Decon	Negative	1.31	Camp Fuel
	LFIA 39B	Ceiling	Post-Decon	Negative	1.71	Camp Fuel
	LFIA 40B	Floor Front	Post-Decon	Negative	2.47	Camp Fuel
	LFIA 41B	Left Door	Post-Decon	Negative	1.29	Camp Fuel
	LFIA 42B	Right Door	Post-Decon	Negative	1.10	Camp Fuel
	LFIA 39A	Front Right Leg-Researcher 2	Post-Cook	Positive	52.38	Camp Fuel
	LFIA 40A	Front Right Leg-Researcher 2	Post-Decon	Negative	1.67	Camp Fuel
Cook 4	LFIA 41A	Air Tank-Researcher 1	Post-Decon	Negative	1.49	Ether
	LFIA 42A	Air Tank-Researcher 4	Post-Decon	Negative	1.99	Ether
	LFIA 43A	Chest-Researcher 3	Post-Cook	Positive	15.45	Ether
	LFIA 44A	Right Front Table	Post-Cook	Positive	264.35	Ether
	LFIA 43B	Chest-Researcher 3	Post-Decon	Negative	1.05	Ether
	LFIA 45A	Left wall	Post-Cook	Trace	8.60	Ether
	LFIA 46A	Left door	Post-Cook	Trace	9.86	Ether

	LFIA 44B	Right Front Table	Post-Decon	Positive	37.20	Camp Fuel
	LFIA 45B	Left Wall (clean)	Post-Decon	Negative	1.53	Camp Fuel
	LFIA 46B	Left Door	Post-Decon	Negative	1.70	Camp Fuel
Cook 5	LFIA 47A	Air Tank-Researcher 1	Post-Decon	Negative	0.52	Camp Fuel
	LFIA 48A	Air Tank-Researcher 4	Post-Decon	Negative	0.27	Camp Fuel
	LFIA 48B	Air tank-Researcher 4	Post-Cook	Trace	5.53	Camp Fuel
	LFIA 49A	Chest-Researcher 3	Post-Decon	Trace	22.17	Camp Fuel
	LFIA 49B	Chest-Researcher 3	Post-Cook	Negative	1.62	Camp Fuel
	LFIA 50A	Belt on Air Tank-Researcher 1	Post-Cook	Positive	21.26	Ether
	LFIA 50B	Belt on Air Tank-Researcher 1	Post-Decon	Negative	2.50	Ether
Cook 6	LFIA 51A	OBNDD Suit	-	Negative	1.62	Ether
	LFIA 51B	Right Front Table	Post-Decon	Trace	15.43	Ether
	LFIA 52A	Left Wall	Post-Decon	Negative	0.85	Ether
	LFIA 28A	Jeans-Sample Collector 1	-	Negative	0.35	Ether
	LFIA 28B	Jeans-Sample Collector 2	-	Negative	0.39	Ether

Table 29. Summary of the LFIA results. Pre-cook samples were collected prior to methamphetamine production, post-cook samples were collected after methamphetamine production, and post-decon samples were collected after hosing the researchers and the cook shed with water. Trace positives had a faint line present at the test site of the LFIA, but the line was not bright enough to be deemed a true negative.

	Positive	**Trace**	**Negative**	**Total**
Pre-Cook	0	0	23	23
Post-Cook	23	5	13	41
Post-Decon	2	3	22	27
Other	0	0	5	5
Total	25	8	63	96

4.4.2 Fluorescent covalent microbead immunosorbent assay

The sample location and individual quantitative results for the 96 samples analyzed by FCMIA are shown in Table 28. Table 30 summarizes the overall FCMIA quantitative results after adjusting for outliers. Pre-cook samples had a maximum methamphetamine concentration of 0.83 ng/100 cm^2 and a minimum methamphetamine concentration of 0.12 ng/100 cm^2, with a mean concentration of 0.30 ± 0.20 ng/100 cm^2. Post-cook samples had a maximum methamphetamine concentration of 16.32 ng/100 cm^2 and a minimum methamphetamine concentration of 0.10 ng/100 cm^2, with a mean concentration of 4.35 ± 4.05 ng/100 cm^2. Post-

decon samples had a maximum methamphetamine concentration of 4.30 ng/100 cm^2 and a

minimum methamphetamine concentration of 0.27 ng/100 cm^2, with a mean concentration of

1.48 ± 0.93 ng/100 cm^2. After correcting for outliers, the concentration of methamphetamine

observed pre-cook, post-cook, and post-decon were compared with an ANOVA. No statistical

difference was found between MA concentrations observed pre-cook and post-decon while a

statistical difference was found between MA concentrations observed post-cook and pre-cook as

well as post-cook and post-decon. Points were considered outliers if they were 1.5 inter quartile

ranges above the third quartile concentration within their respective collection period. The

amount of surface contamination collected following the ether One Pot methamphetamine cooks

and camp fuel methamphetamine cooks was compared with a t-test. Following the ether One Pot

cooks, there was an average surface contamination of 4.98 ± 4.42 ng/100 cm^2. Following the

camp fuel One Pot cooks, there was an average surface contamination of 3.29 ± 2.81 ng/100 cm^2.

There was no statistical difference in the surface contamination generated by use of either solvent

type.

Table 30. Summary of the FCMIA quantitative data. All concentrations are in ng/100 cm^2.

	Pre-Cook	**Post-Cook**	**Post-Decon**
Max Conc	0.83	16.32	4.30
Min Conc	0.12	0.41	0.27
Mean Conc	0.29 (±0.20)[b]	4.42 (±4.00)[a]	1.48 (±0.93)[b]

*2 means with the same letter are not significantly different at α=0.05.

4.5 Discussion

4.5.1 Competitive lateral flow immunoassays

The lateral flow immunoassays were successfully able to identify locations of surface

methamphetamine contamination following all six One Pot cooks and the two bottle failures.

While claiming to have a limit of detection of 50 ng/100 cm^2, it was found that the LFIA were

able to consistently detect MA at concentrations as low as 5 ng/100 cm^2. In fact, if using 5

ng/100 cm^2 as the limit of detection, the LFIA reported only one false negative (LFIA 3B). The

false negative result was collected from the sample site located on the table where the One Pots

were being performed. The false negative likely represents experimenter error and not an error in

the analysis.

While only one false negative was reported, 13 samples classified as positive or trace

positive had methamphetamine concentrations of under 5 ng/100 cm^2, suggesting the LFIA's

sensitivity was over 10x greater than advertised. While beneficial for this research, which

generated an average surface methamphetamine contamination of only 4.42 ng/100 cm^2, these

LFIA may be overly sensitive for commercial application. Most states have a remediation

standard of 50-1500 ng of methamphetamine per 100 cm^2 of surface area.[116] By testing surface

contamination with a LFIA that has a limit of detection of closer to 5 ng/100 cm^2, companies may

perform more rigorous cleaning techniques, which will cost the company, and the individual

getting the site cleaned, more money than needed to just meet the state mandated remediation

level. Additionally, trying to clean a former methamphetamine production site to a surface

contamination level of 5 ng/100 cm^2 may be impractical due to the amount of methamphetamine

that may be recirculated through airways causing reoccurring contamination, as well as any

methamphetamine that may leach out of materials such as drywall, paint, and wood.

4.5.2 Fluorescent covalent microbead immunosorbent assay

In total, the quantitative FCMIA results had 9 outliers: 5 were from the post-cook

samples and 4 were from the post-decon samples. All of the outliers but 2 were collected from

the two sample locations where the One Pot failures occurred. It is believed that when bottle

ruptured, it covered the sample locations in camp fuel, depositing a large amount of

methamphetamine on the sample sights. While the sites were washed by hosing with water, camp

fuel is highly hydrophobic and may have left a thin film on the sample site, protecting the

methamphetamine from being washed away during the decontamination process. The use of

soap, as suggested by EPA, may have aided in decontaminating these sites by breaking down the

organic solvent and allowing the water to wash the deposited MA off the contaminated sites.[116]

The remaining 2 outliers were collected from the PPE of the researchers. One of the outliers was

on the leg of a researcher who was sprayed with camp fuel during the first bottle failure and the

second outlier was on the belt of the oxygen tank harness, which was likely exposed as the

researcher leaned against the table to work with the One Pot. The concentration of MA observed

from the outliers ranged from $21.26 - 264.69$ ng/100 cm^2 in the post-cook samples and $15.43 -$

45.67 ng/100 cm^2 in the post-decon samples.

When the amount of surface methamphetamine contamination observed during this

research was compared to the amount observed by Martyny et al. during the Red-P and Birch

reduction methods, less methamphetamine was released by the One Pot than these two older

methods of production. At a distance of less than 2 m from the cook, which was the distance all

of the One Pot surface samples were collected from during this study, Red-P cooks had a mean

concentration of 100,900 ng/100 cm^2, Birch reduction cooks had a mean concentration of 25,200

ng/100 cm^2, and the One Pot cooks had a mean concentration of 4.42 ng/100 cm^2.[86] This

difference in MA surface contamination levels likely stems from the amount of pseudoephedrine

the cooks began with, as Red-P cooks have been reported to start with 150 g of pseudoephedrine,

Birch Reduction cooks with 30 g of pseudoephedrine, and the One Pots performed in this study

only used 0.6 g of pseudoephedrine.[89,90] By starting with less pseudoephedrine than other

methods, the One Pot produces less methamphetamine, and thus does not generate as much

surface contamination. Due to the relatively small size of One Pot methamphetamine cooks,

clandestine labs that use this method generally contain multiple reaction vessels, with reports of

labs containing as many as 100 One Pots.[133] Due to this, surface sampling from actual locations

of clandestine One Pot MA manufacturing may provide surface contamination results that closer resemble those found in the Martyny et al. study.

CHAPTER V

DEVELOPMENT OF ANALYTICAL METHODS FOR THE DETECTION OF SURFACE CONTAMINATION IN LOCATIONS OF FENTANYL USE AND PRODUCTION

5.1 Introduction

Fentanyl is a schedule II synthetic opioid used for the treatment of chronic pain, such as in terminal cancer patients or people that have developed high opioid tolerances.[134,135] Fentanyl acts as an agonist on the μ-receptor and has potency 50-100x that of morphine, but unlike morphine, fentanyl is highly lipophilic, allowing it to easily cross the blood-brain barrier and quickly produce effects, such as euphoria and respiratory depression.[134,136,137] The potency of fentanyl has led to increased abuse in the United States, and with the increase in abuse has come an increase in the number of overdose-related fatalities. Overdose-related fatalities due to fentanyl usage have increased from approximately 3000 in 2013 to over 20,000 in 2016, making fentanyl overdose-related fatalities the leading cause of death in Americans under the age of 50.[138]

With fentanyl overdose-related fatalities on the rise, much work has been put into examining the absorption rate and bioavailability of fentanyl and fentanyl analogs, herein referred to collectively as fentalogs, through various routes of exposure.[135,136,139] One potential route of exposure that is of primary concern to public health officials is residual powders that may be

found on surfaces within locations of opioid use and/or production.

Due to the high potency of fentalogs, small quantities of residual powder may be sufficient to lead to opioid intoxication and even death.[140,141] Because of this safety concern, the establishment of remediation guidelines for locations of fentanyl use and production, similar to those established for the illicit stimulant methamphetamine, have been highly sought after.[142] Currently there is not enough toxicological data to develop meaningful dose-response type remediation guidelines, though some locations are still proceeding with the implementation of remediation guidelines that are not supported by toxicological data. These locations are requiring fentanyl sites to be decontaminated to levels below detection, which is considered 100 ng/100 cm^2 for many labs.[143,144]

In order to set such remediation guidelines, a method to collect these drugs from surfaces and accurately quantitate them must first be developed. Methods such as the NMAM 9106, 9109, and 9111 have been developed for collection of methamphetamine from surfaces, but to date, no such methods have been developed for the collection of fentalogs.[145] The goal of this research was to develop and validate a surface swab method to capture 17 fentalogs and 10 common adulterants and use it to assess the extraction efficiency of these compounds on 11 common household surfaces. Extraction efficiencies for each analyte were evaluated by the concentration of analyte recovered from the surface, as determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Following development of an optimized surface swabbing technique, this research examined its effectiveness at capturing the same 27 analytes from 11 commonly encountered household surface materials of varying degrees of porousness. By developing an optimized surface swabbing technique for capturing and quantitating fentalogs from common household surfaces, this research seeks to provide a feasible lower limit of detection for these fentalogs when collected from various surfaces, therefore assisting in the establishment of an achievable remediation level in fentanyl-contaminated locations.

5.2 Review of the Literature

While methamphetamine is the most common illicitly produced drug in the United States, opioids are currently gaining the most attention due to the number of overdose deaths attributed to their abuse.[111] In 2017, there were 70,237 drug overdose deaths, 47,600 (67.8%) of which were the result of opioid abuse.[146] Of these 47,600 opioid-related overdose deaths, synthetic opioids other than methadone, which include fentanyl and fentanyl analogs, accounted for 28,466 (59.8%).[147] This means synthetic opioids other than methadone accounted for 40.5% of all drug overdose deaths in the United States in 2017, and as the availability of prescription opioids begins to decrease due increased regulation of opioid prescribing practices, the number of people turning to the illicit purchasing and use of synthetic opioids is expected to increase.[111]

The synthetic opioid fentanyl is a schedule II drug used to treat extreme chronic pain or used to treat individuals who have become tolerant to other, less potent opioids.[134,135] It acts on the μ-opioid receptor, which is responsible for relaying pain signals, regulating respiratory functions, thermoregulation of the body, GI motility, and hormone secretion, with little interaction occurring at the δ- or κ-opioid receptors.[148,149] This can be seen by examining the inhibitory constant (K_i) of fentanyl versus the gold standard pain relieve opioid, morphine (Table 31).[149] The K_i value is a measurement of a drug's affinity for a receptor, obtained by examining the amount of drug needed to dissociate a radiolabeled ligand from a receptor; lower K_i values equate to higher binding affinities. The high affinity of fentanyl for the μ-opioid receptor makes it a highly potent pain killer, with studies reporting it to be 50-100 times more potent than morphine.[136]

Table 31. Comparison of K_i values for morphine and fentanyl at the major opioid receptors. Table reconstructed with data published by Chen et al.[149]

Drug	μ_1	μ_2	δ	κ_1
Morphine	0.260	8.6	358	52
Fentanyl	0.007	6.5	1140	242

While the high affinity of fentanyl for the μ-opioid receptor does make it a potent analgesic, it also causes fentanyl to be highly addictive. The μ-opioid receptor has been shown to be responsible for the physical dependence opioid-addicted individuals develop, as well as the euphoric effect they seek from drug usage, with μ_1 receptor stimulation resulting in euphoria and analgesic effects, whereas the μ_2 receptor is responsible for euphoria and the commonly observed side-effects of opioid intoxication (respiratory depression, reduced GI motility, and physical dependence).[150–152] As people begin to abuse opioids, they can develop tolerance to the drug, which occurs by desensitization and down-regulation of the opioid receeoptors.[153] Desensitization is the uncoupling of the opioid receptor from its signaling pathway, preventing downstream cellular signaling form occurring. For opioid receptors, G-protein coupled receptor kinases phosphorylate the receptor, causing the associated G-protein to uncouple from the receptor, therefore preventing the opioid receptor from sending cellular messaging.[153–155] Desensitized opioid receptors can quickly be resensitized if the concentration of opioids surrounding the cell decreases; this is accomplished by phosphatases, which removes the phosphorylation put in place by G-protein coupled receptor kinases.[155] If the opioid G-protein coupled receptor remains phosphorylated, arrestin proteins will eventually bind the receptor and signal the cell to internalize it via endocytosis. By internalizing the opioid receptor and reducing the total number of receptors present on the cells membrane, the cell is down-regulating its response to opioids.

As opioid-addicted individuals develop tolerance, they begin seeking additional or more potent drugs. For many people, this means turning to the use of illicit drugs, whether in the form of pills or powders. Interviews conducted by law enforcement found that most opioid-addicted individuals would purchase the cheapest available opioids, whether they were diverted prescription pills or heroin, as both would provide the desired euphoric effect.[156] In 2014, fentanyl began to appear in illicit drug markets across the United States, and due to its high

potency, could be mixed with fillers and pressed into counterfeit prescription pills or mixed with

heroin in order to provide a cheaper opioid that produced similar euphoria for its users.[157,158]

After 2014, counterfeit prescription pills made in this manner became increasingly more popular,

as a kilogram of fentanyl could be purchased from China for $3,500 at 90[+]% purity and then

pressed into pills that contained 1-2 mg of drug.[158] These counterfeit pills can then be sold at less

than the illicit market price of $1 per milligram of oxycodone, causing buyers to be more willing

to purchase the counterfeit pills and netting the drug dealers a greater profit than they would make

selling real prescription opioids (Table 32).[159] In 2019, fentanyl-containing counterfeit

prescription pills held an average of 1.7 mg of fentanyl, which is equivalent to 113 mg of

oxycodone;[160,161] 1 kg of fentanyl would make 588, 235 pills at this concentration.

Table 32. Potential profit generated by sale of fentanyl-containing counterfeit prescription pills. Table recreated with data published by the DEA.[158]

Amount of Fentanyl per Pill	Price per Pill	Price per Pill	Price per Pill
	$10	$15	$20
2 mg (500,000 pills)	$5 million	$7.5 million	$10 million
1.5 mg (666,666 pills)	$6.6 million	$9.9 million	$13.3 million
1 mg (1,000,000 pills)	$10 million	$15 million	$20 million

As the use and sale of fentanyl rose following its surge onto the United States illicit drug

market in 2014, law enforcement began enforcing tighter regulations on fentanyl prescriptions

and focused their efforts on reducing the amount of fentanyl entering the country. To avoid law

enforcement detection, and to get around the Controlled Substances Act that classifies fentanyl as

a schedule II drug, clandestine chemists began developing fentanyl analogs (fentalogs).

Fentalogs have the same structural backbone as fentanyl with slight modifications that make them

just dissimilar enough that they do not fall under the same federal scheduling as fentanyl itself.

This fentanyl backbone contains four major functional groups: the aniline ring, the piperidine

ring, the N-alkyl chain, and the amide group (Figure 78). A modification to any of these

functional groups not only changes the structure of the molecule, but also its chemical properties,

such as potency, volatility, and solubility.[140] While there are hundreds of known fentanyl

analogs, only 10-20 have been commonly encountered in the United States.[5,111,160] The structure

and potencies relative to fentanyl of several of these fentalogs are given in Figure 79.
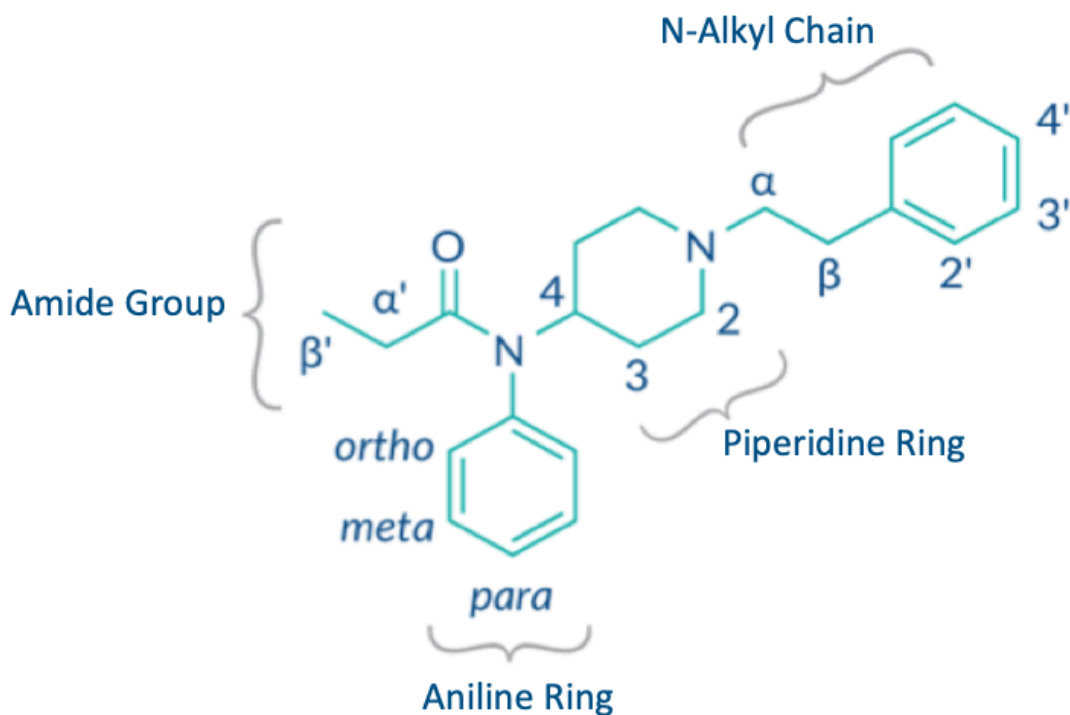
Figure 78. Functional groups making up the fentanyl structure. All locations denoted with numerals and/or Greek lettering can be substituted to produce a fentalog. Image recreated from Cayman Chemical.[162]

Fentanyl	Methoxyacetylfentanyl
Rel. Potency: 1	Rel. Potency: 0.3
Carfentanil	(±)-*cis*-3-Methylfentanyl
Rel. Potency: 30-100	Rel. Potency: 0.2 (- isomer), 20 (+ isomer)
Sufentanyl	Butyrylfentanyl
Rel. Potency: 10	Rel. Potency: 0.03-0.13
Remifentanil	Cyclopropylfentanyl
Rel. Potency: 1	Rel. Potency: 3
Acetylfentanyl	Furanylfentanyl
Rel. Potency: 0.3	Rel. Potency: 7
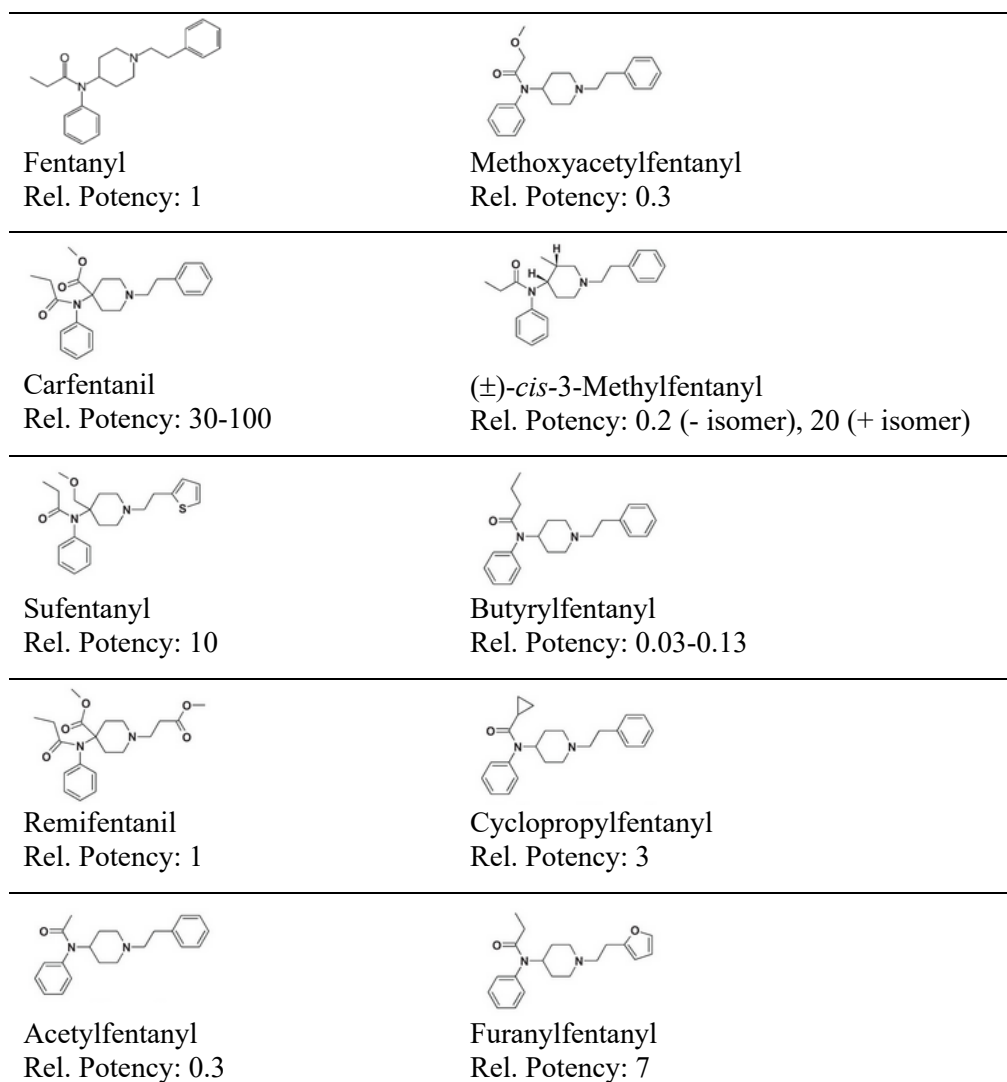
Figure 79. Structure and potency relative to fentanyl for several fentalogs. Potency data published by Wilde et al.[163]

Due to the potency of fentanyl and it analogs, environmental contamination stemming from them are a current source of major public health concerns. As with the surface methamphetamine contamination, surface fentanyl/fentalog contamination can come from various sources, such as from production, tableting, cutting, and use. To date, there has only been one known instance of fentanyl production within the United States; George Marquardt established a laboratory in Goddard, Kansas in the early 1990's, where he synthesized the opioid to be sold on the United States' east coast.[164] Most of the fentanyl entering the United States is manufactured in China and Mexico, with the DEA suspecting Ph.D. level chemists being recruited to produce

the opioid due to the time-intensive, difficult synthesis process.[111] Because clandestine

production of fentanyl has not occurred in the United States at the same level as with

methamphetamine, little research has been performed in regard to the level of surface

contamination generated by these labs. A study by Van Nimmen, Poels, and Veulemans did

examine the amount of inhalational and dermal exposure four works were subjected to during

three weeks of pharmaceutical fentanyl production. During this study, it was found that over a

third of the air samples collected at the breathing level of the fours workers followed throughout

the study contained fentanyl at levels above the 8-hr time weight average (8-hr TWA) of 100

ng/m^3, which is the amount of fentanyl a person is permitted to be exposed to over an 8-hr work

period. Dermal exposures were expressed as the loading rate of fentanyl onto the hands of the

four participants; exposures ranged from 0.02 – 1090 ng/cm^2/h, with the two workers

synthesizing the fentanyl showing the greatest exposure.[165] In the absence of an allowable dermal

exposure limit, the pharmaceutical company where this research took place set its own guideline

that allows an individual to be exposed to equivalent dose of fentanyl dermally as they would be

via inhalation. For this company, the allowable surface contamination limit for fentanyl was 1

ng/100 cm^2, which was well exceeded based on the amount of fentanyl contamination observed

on the 200 cm^2 section of hand that was swabbed and analyzed from the four workers.[166]

While pharmaceutical production companies tend to follow "Good Manufacturing

Practices and have a higher cleanliness standard than clandestine drug manufacturing labs, the

data published by Van Nimmen, Poels, and Veulemans suggest even these locations have levels

of contamination above that deemed unacceptable by the Occupational Safety and Health

Administration (OSHA). And while sites of drug production have historically had low level

surface contamination within them (nanogram to microgram range), sites of pill pressing

operations have visible levels of surface contamination that can likely be measured in milligrams

or even grams (Figure 80).[86] While visual inspection of these site is enough to observe a grossly

contaminated location requiring a Level A protective suit to enter, including a fully-

encapsulating, air tight suit with a SCBA as a source of oxygen, no research has been done to attempt to quantitate the amount of contamination actually present within a pill pressing operation.
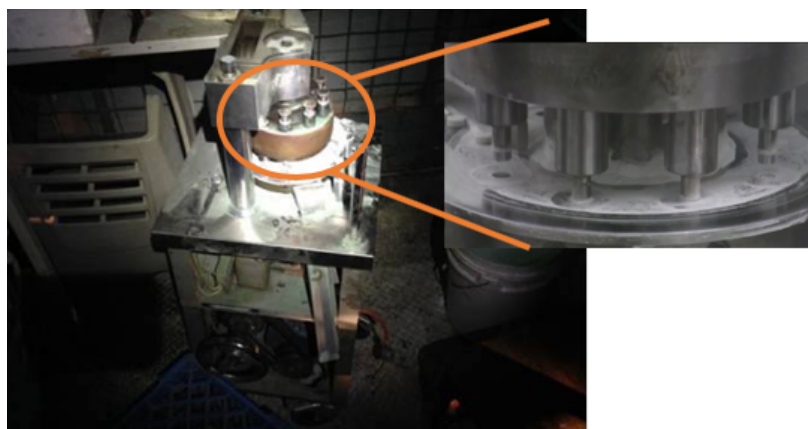
Figure 80. A clandestine pill pressing operation with visible surface contamination around the press and on the floor. Inset image is a close up of the press itself, showing a high level of contamination that could become airborne during operation and settle in locations throughout the site.

Another area of potential gross drug contamination is an area where drug cutting occurs. Drug cutting is the dilution of a drug with an adulterant to increase the overall mass of the product to be sold. For example, fentanyl is normally cut with heroin and sugars and then sold as heroin.[160] By mixing these compounds, the overall mass of the drug being sold can be increased greatly, and by selling it as heroin, which has lower potency than the fentanyl present in the mixture, buyers aren't aware they are receiving a more potent drug mixed with a cheap sugar. Cutting of drugs can occur in multiple different ways: mixing/chopping with a knife or credit card, stirring in a bowl, mixing in a concrete mixer, or combining in a blender/coffee grinder/food processer. Regardless of how its mixed, powder is likely to be left on surfaces, the potency of which can be variable. Outside of pharmaceutical settings, when drugs are mixed, they may not be done so efficiently. The fentanyl powder being mixed with sugar might not evenly distribute throughout the sample, as the fentanyl may have moisture or electrostatic interactions holding it together as clumps instead of as small flakes. This means section of the powder may contain "hot

spots," which are areas of high fentanyl concentration while other areas will contain little to no fentanyl and only sugar. This idea is shown schematically in Figure 81 in regards to mixing performed prior to tableting; in this example, the active substance (pink) would be fentanyl and the tablet matrix (green) would be the sugar or other adulterants. This inefficient mixing can complicate surface sampling as a site may appear visually contaminated but have no fentanyl present while another sight may only have a few small specs of powder visible, but the powder is pure fentanyl making it highly contaminated.
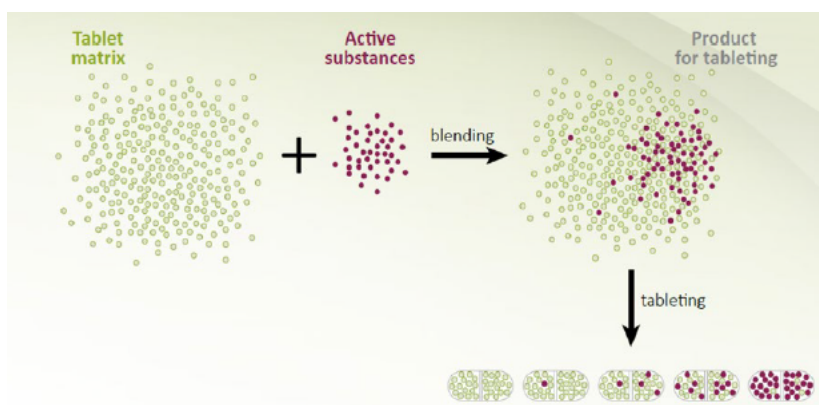
Figure 81. Schematic showing the potential of inefficient mixing during cutting of fentanyl. The active substance (pink) can be thought of as fentanyl and the tableting matrix (green) as sugars. Image taken from the DEA.[5]

Although the total contamination generated by the cutting and mixing of drugs in a clandestine operation has not been assessed in the literature, there have been assessments of the contamination generated by mixing of antineoplastic drugs in hospital pharmacies. One such study by Sessink et al. measured the amount of surface contamination generated while preparing mixtures of cyclophosphamide, ifosfamide, and 5-fluorouracil according to OSHA standards. Surface contamination was assessed in the biological safety cabinets where drugs were opened and mixed, as well as on counters where drugs were placed after mixing. Maximum contamination levels varied from $5.03 - 17.19$ ng/100 cm^2 in biological safety cabinets and $14.19 - 228.7$ ng/100 cm^2 on counters (Table 33).[167] These reported surface contamination levels are likely much lower than what would be observed in a clandestine cutting/mixing operation, as

clandestine operations are generally mixing large quantities of powders instead of small volumes of solutions containing dissolved drug and clandestine operations are not subjected to the safety and cleanliness regulations enforced on the hospital pharmacies by OSHA.

Table 33. Maximum concentrations of antineoplastic drugs captured from biological safety cabinets and counters within hospital pharmacies following mixing of drugs. Table recreated with data published by Sessink et al.[167]

	Cyclophosphamide	Ifosfamide	5-Fluorouracil
Biosafety Cabinet	17.19	5.03	17.2
Counter	122.27	14.19	228.7

Another study by Sisco, Najarro, and Burns examined the level of surface contamination present within forensic labs and evidence receiving rooms. These locations handle large volumes of numerous illicit substances, and although good laboratory safety protocols are implemented, surface contamination still occurs. Fentanyl, which is usually handled with great care due to its high potency, was found in 62% of the surface samples collected, ranging in concentrations between 0.008 – 54.968 ng/100 cm^2.[168] As with the hospital pharmacy contamination discussed above, forensic labs likely contain less surface contamination than a clandestine operation, due to their use of fumigation hoods, proper cleaning protocols, and safety mandates.

The final source of environmental contamination of fentanyl comes from its use. Fentanyl can be introduced to the body in many ways, including injections, smoking, snorting, oral ingestion, transdermal patches, sublingually, or vaporizing and inhaling the fumes (chasing the dragon).[169] While all of these routes of administration can lead to some degree of surface contamination, no research has examined the extent of this contamination. Additionally, how common each route of administration is varies among the user's demographics, further complicating the question of how much environmental contamination actually stems from illicit use.[170]

As within locations of methamphetamine production, which was discussed in previous chapters, locations of fentanyl processing and use put people at risk for oral, inhalational, and

dermal exposures. When fentanyl (pKa=8.8) is introduced to the body orally, it ionizes in the stomach, delaying absorption until the drug reaches the more alkaline environment of the intestines.[140] Upon absorption into the blood, fentanyl undergoes extensive first-pass metabolism by CYP 3A4, causing 2/3 of the absorbed fentanyl to be metabolized to inactive metabolites.[136,171] Although only a small fraction of fentanyl that reaches the gastrointestinal tract makes it to the bloodstream where it can induce an effect, its highly lipophilic nature causes it to be rapidly absorbed by membranes, such as the oral mucosa in the mouth, thus bypassing the first-pass metabolism of the liver;[136] sublingual and transmucosal absorption of fentanyl can lead to 50-76% bioavailability of fentanyl.[172] The lipophilic nature of fentanyl also causes it to be quickly distributed to tissues. It is able to readily cross the blood-brain barrier and interact with μ-opioid receptors.[136] Because of its lipophilic nature, low-dose oral exposures to fentanyl may result in more drug being absorbed in the mouth than reaching the gastrointestinal tract, leading to higher fentanyl blood levels and effects. With opioid naïve individuals demonstrating opioid intoxication symptoms following exposure to as little as 100 μg of fentanyl, even low-level environmental contamination can pose a risk to public health.[173]

While oral exposure can lead to a majority of the fentanyl to not reach systemic circulation, inhalation of fentanyl results in a much greater bioavailability of the drug. Absorption through the mucous membranes of the nasal passageway can result in 89% bioavailability while 90[+]% of the fentanyl that reaches the lungs is absorbed into the blood stream.[172,174] While fentanyl does have a low vapor pressure, meaning it will not readily volatilize, various factors within clandestine sites could cause the powder to become airborne, such as the operation of a pill press, cutting/mixing of powders, activation of fans or ventilation systems, and quick movements.[175] Due to its high bioavailability following inhalation, any airborne fentanyl can be can be dangerous to those who encounter it. Consider a standard adult male that has a tidal respiratory volume of 0.0005 m³ and respiratory rate of 12 – 20 breaths per

minute, the concentration of airborne fentanyl needed to reach a dose of 100 µg can be calculated

(Equation 5).[95,176] This calculation can be performed for various exposure times to assess to

health risk a contaminated site may poses for those within it (Table 34). It should be noted that

the values that the values displayed in Table 34 are rough approximations, as they are calculated

assuming 100% absorption and bioavailability of the inhaled fentanyl, and loss of drug due to

metabolism was neglected.

Equation 5. Equation used to calculate the concentration of airborne fentanyl (mg/m^3) needed to reach a desired dose. Equation is simplified and assumes 100% absorption and bioavailability and neglects drug loss due to metabolism.

$$\frac{\text{Desired Drug Dose (mg)}}{(\text{Exposure Time (min)} \times \text{Tidal Volume (m}^3) \times \text{Respiratory Rate (min}^{-1}))} = \text{Airborne Fentanyl Conc. } (^{mg}/_{m^3})$$

Table 34. The airborne fentanyl concentration (mg/m^3) required to achieve a dose of 100 µg over a given period of time at different respiratory rates.

Respiratory Rate (Breaths/Min)	Exposure Time						
	Instant	1 Min.	5 Min.	10 Min.	15 Min.	30 Min.	60 Min.
12	200.00	16.67	3.33	1.67	1.11	0.56	0.28
20	200.00	10.00	2.00	1.00	0.67	0.33	0.17

Based on the values shown in Table 34, the fentanyl concentration in the air would need

to be sufficiently high to lead to opioid intoxication. The study by Van Nimmen, Poels, and

Veulemans that examined the amount of surface and airborne fentanyl contamination within a

pharmaceutical production company found maximum airborne fentanyl contamination to be

0.013 mg/m^3.[165] This maximum airborne concentration is less than 1/10 of the concentration

needed to reach a 100 µg dose in 60 minutes, but the synthesis of fentanyl during this project was

performed in an optimal environment and not in a clandestine lab that is free of safety

regulations.

While oral and inhalational exposures pose a risk to public health, dermal exposures to

fentanyl are not as dangerous. Although fentanyl is a small, highly lipophilic molecule and many

pharmaceutical sources of fentanyl are produced for transdermal use, these patches, which are optimized for drug delivery, still requires 3-13 hours to deliver enough fentanyl to reach therapeutic blood levels.[139] At this rate of absorption, both palms would need to be completely covered with fentanyl for over 14 minutes to obtain a 100 μg dose of the opioid.[139] Dermal absorption of fentanyl can be increased if the skin it comes in contact with is moist, or if alcohols (i.e. hand sanitizers) are introduced to the skin.[9] Perhaps the greatest risk with dermal exposure to fentanyl is its transferability. In other words, fentanyl transfers from object to object fairly easily. Contaminated skin and clothing could cause fentanyl to spread to additional locations and contact with mucous membranes around the mouth and eyes may lead to adverse health effects. This risk can be mitigated by thorough washing of contaminated skin with soap and water.[9]

Although fentanyl can be washed from the skin and from PPE with soap and water, this just removes the drug and does not degrade it. Because of this, soap and water cannot be used as a decontamination agent in sites of fentanyl contamination as is suggested in sites of methamphetamine production.[116] Doing so would simply create a pool of contaminated water that would need to be contained and disposed of in a safe manner. Products that degrade fentanyl to inactive products have been examined lately, with the peracetic acid formulation known as Dahlgren Green showing >99.9% decontamination efficiency for fentanyl and carfentanil within 5 minutes of application.[177] Dahlgren Green combines paracetyl borate, a surfactant, and a pH buffer together, generating peracetic acid which can oxidize compounds, degrading them at the chemical level.[178] Peracetic acid itself is highly unstable and exists in constant state of equilibrium flux, where it will break down to acetic acid and hydrogen peroxide and then reform peracetic acid and water (Figure 82).[179] By generating peracetic acid with Dahlgren Green, it increases the shelf life of the decontamination agent and prevents the need to transfer large amounts of acid to sites of fentanyl contamination.[178]
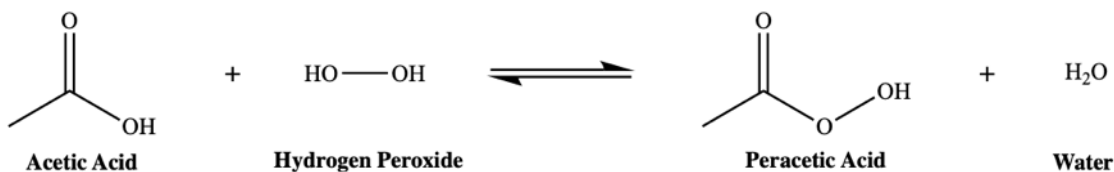
Figure 82. Equilibrium reaction of a peracetic acid in water solution.

While this decontamination agent shows promise, it does still have several drawbacks. First, when used to decontaminate fentanyl and carfentanil, both compounds were still present on the decontaminated surface in quantifiable amounts, meaning there was still potential for an exposure to occur.[180] Second, Dahlgren Green may not be appropriate for use in all locations. One study that examined its use made mention of it leaving a sticky residue on surfaces following decontamination and that its smell was overbearing to those working with it.[180] Dahlgren is a series of three proprietary chemicals that, when combined, generate peracetic acid.[178] Peracetic acid is known to cause lacrimation and upper respiratory irritation within 3 minutes at concentrations of 5 ppm. It is also known to be corrosive to skin and eyes, making it unideal for a location that people may have to enter without PPE.[181] The final drawback of Dahlgren Green is that it has only been tested against fentanyl and carfentanil.[177] While its success at degrading these two compounds is promising, it cannot be assumed that it will be as successful at degrading other fentalogs. Other fentalogs may not be as readily oxidized or they may be oxidized to fentalogs of greater potency.

Due to the drawbacks of Dahlgren Green, other decontamination agents need to be investigated for their efficiency at degrading fentanyl and fentalogs. Before other decontamination techniques can be assessed, methodology needs to be developed to capture fentanyl and fentalogs from surfaces, identify them, and quantitate the amount present. Such methodology can be used to determine what fentalogs are present and at what concentration prior to and following decontamination, thus providing insight into the effectiveness of a decontamination technique and the safety of a remediated location. Such methodology has been

developed for determining the level of methamphetamine surface contamination, but not for fentanyl or its analogs.[145]

When developing a methodology to detect surface contamination, one major question that gets presented is how clean is clean enough? In other words, how low should drug concentrations be before a location is considered remediated and safe for individuals to reinhabit. For methamphetamine, this remediation value ranged from 0.05-1.5 μg/100 cm^2 and was based on research that examined the amount of methamphetamine that may be present in contaminated sites and how that concentration would impact those most susceptible to the drug, such as children.[93,116] Due to a lack of data examining the amount of fentanyl contamination within sites, this approach is currently not possible, leading some locations to set remediation levels as the limit of detection for the surface swab technique and analytical instrumentation being used;[143] for many labs, this limit is 100 ng/100 cm^2.[144]

This limit of detection-type approach to decontamination was originally used when establishing remediation levels in former methamphetamine labs, with state legislators and research toxicologists quoted saying, "the belief was that in the face of an unknown risk to crawling infants, the process of reducing levels of known contaminants to the lowest practical levels using current available methods and processes made sense."[122] As this is the current attitude towards remediation of fentanyl contaminated locations, it is important that the techniques used to identify and quantitate fentalogs from surfaces be specific and sensitive. This research aims to develop such a method.

In this research, a surface swabbing technique was developed to identify and quantify 17 fentalogs and 10 common adulterants from surfaces using liquid chromatography tandem mass spectrometry. The surface swabbing technique was optimized on a non-porous surface to achieve the greatest extraction efficiencies possible from the tested surface. Once the swabbing methodology was optimized, the entire technique was validated based on guidelines set out by the Scientific Working Group for Forensic Toxicology (SWGTOX).[182] Following validation, the

method was used to examine the extraction efficiency of all 27 compounds on 11 different surface materials commonly encountered in homes to begin assessing how well different drugs can be captured from various surface materials. While a nationally accepted decontamination method for removing fentanyl and fentalogs from surfaces is currently unavailable, this research seeks to develop the sampling and analytical methodology needed to assist in its development.[9] By having a sensitive surface sampling method that is able to identify not only fentanyl, but 17 total fentalogs, when a decontamination technique is developed, its efficiency can quickly be assessed and it can be implemented to remediate sites of fentanyl environmental contamination.

5.3 Methodology
5.3.1 Reagents and materials

All reagents and materials except for Nanopure™ water were purchased from commercial suppliers; Nanopure™ water was obtained through the use of a Barnstead™ Nanopure™ Diamond laboratory water system (Thermo Scientific, Waltham, MA). Ammonium formate (99% crystalline) was purchased from Alfa Aesar (Ward Hill, MA). ACS grade formic acid was purchased from EDM (EDM Millipore Corp, Billerica, MA). HPLC grade methanol was purchased from Optima® (Thermo Fisher Scientific, Waltham, MA). ACS grade ammonium hydroxide and isopropyl alcohol were purchased from Fisher Scientific (Thermo Fisher Scientific, Waltham, MA).

A majority of the swabs and wipes used during this study were donated by the CDC-NIOSH Taft Laboratory. The polyester swabs (25-806 1PD), cotton swabs (806-WC), and rayon swabs (25-800 R 50) were from Puritan® (Puritan Medical Products Company LLC, Guilford, ME). The anti-static foam swabs (1017396) were from Sciex (Sciex, Framingham, MA). The 2 x 2" cotton gauze (22-362-178) was from Fisher. Kimwipes® (34155) were from Kimberly-Clark (Kimberly-Clark Worldwide, Inc, Roswell, GA). Fifty grade Whatman® paper (1450 055) was

from GE (GE Healthcare, Chicago, IL). The MX908™ Trace Sampling Swabs (415-00042) were

from 908Devices (908 Devices, Boston, MA). The Smiths Detection Nomex AE Mode Manual

Swab 500DT (6821201-B) were from Smiths Detection (Smiths Detection, Edgewood, MD).

The jumbo cotton balls (543577) were from Walgreens (Walgreens Co, Deerfield, IL). The Q-

tip® cotton swabs were from Unilever (Unilever, Trumbull, CT). The sterile nylon swab

applicators were from Copan (Copan Diagnostics, Corona, CA). The Vectra® Alpha® Nu

TX1069 9 x 9" wipers (03-232993) were from ITW Texwipe (ITW Texwipe, Kernersville, NC).

Approximately 10 x 10 cm surface coupons made of unfinished plywood, galvanized

steel, glass, laminate flooring, flat white, vinyl floor tile, and latex-based painted drywall surface

coupons were donated by the EPA's Homeland Security & Materials and Management Division.

Other surfaces obtained for testing include a lab bench made of black, phenolic resin (T.90.0.0)

(Mott Manufacturing Ltd, Brantford, ON, Canada), Greecian white marble tile (M S

International, Inc, Orange, CA), golden oak flooring (Heritage Mill Wood Flooring, Johnson

City, TN), concrete cap block (Home Depot, Atlanta, GA), and a white high-density polyethylene

table (Model#: 80726) (Lifetime, Knoxville, TN), all with surface areas of approximately 100

cm^2.

The following analytical reference standards and isotopically labeled internal standards

were obtained from the CDC Traceable Opioid Reference Material kit through Cerilliant

(Cerilliant Corp, Round Rock, TX) at a concentration of 1 mg/mL in methanol: 4-ANPP, 4-

ANPP-$^{13}C_6$, 4'methylacetylfentanyl HCl, 4'methylacetylfentanyl-$^{13}C_6$ HCl, acetylfentanyl,

acetylfentanyl-$^{13}C_6$, acrylfentanyl HCl, acrylfentanyl-$^{13}C_6$ HCl, benzylfentanyl HCl,

benzylfentanyl-$^{13}C_6$ HCl, (±)-β-hydroxythiofentanyl HCl, (±)-β-hydroxythiofentanyl-$^{13}C_6$ HCl,

butyrylfentanyl, butyrylfentanyl-$^{13}C_6$, carfentanil oxalate, carfentanil-$^{13}C_6$ oxalate, (±)-*cis*-3-

methylfentanyl HCl, 3-methylfentanyl-$^{13}C_6$ HCl, cyclopropylfentanyl, cyclopropylfentanyl-$^{13}C_6$

HCl, fentanyl, fentanyl-$^{13}C_6$, furanylfentanyl HCl, furanylfentanyl-$^{13}C_6$, methoxyacetylfentanyl

HCl, methoxyacetylfentanyl-$^{13}C_6$ HCl, *para*-fluorobutyrylfentanyl, *para*-fluorobutyrylfentanyl-

$^{13}C_6$, *para*-fluorofentanyl, *para*-fluorofentanyl-$^{13}C_6$, remifentanil HCl, remifentanil-$^{13}C_6$ HCl, U-47700, U-47700-$^{13}C_3^{15}N_2$, U-48800 HCl, U-48800-$^{13}C_3^{15}N_2$ HCl, U-49900, U-49900-$^{13}C_5$, valerylfentanyl HCl, and valerylfentanyl-$^{13}C_6$ HCl. Additionally, alprazolam, cocaine, cocaine-d_3, heroin, heroin-d_9, hydrocodone, naloxone, oxycodone, oxycodone-d_6, and sufentanyl were purchased from Cerilliant at a concentration of 1 mg/mL in methanol. Alprazolam-d_5, hydrocodone-d_3, naloxone-d_5, and sufentanyl-d_5 were purchased from Cerilliant at a concentration of 100 µg/mL in methanol.

5.3.2 Surface spiking and swabbing

To assess extraction efficiencies during each step of the research, a 100 cm^2 section of surface material was spiked with 20 µL of a 500 ng/mL solution containing the 27 compounds of interest in methanol, resulting in 10 ng of each compound present on the 100 cm^2 surface. Spiking was accomplished using a 2-20 µL variable pipettor (VWR International LLC, Radnor, PA) and moving in a "N" motion from the left side of the test site towards the right side, and then a "S" motion from the bottom of the test side towards the top. After spiking the surface, the methanol was allowed to evaporate before swabbing began.

Prior to swabbing the spiked surface, the swab was moistened with a wetting solvent. The test sites were swabbed 3 times with the moistened swab, following a "S-N-S" pattern, where the first "S" pattern started in the top left corner and ended in the bottom right corner, the "N" pattern started in the bottom left corner and ended in the top right corner, and the second "S" pattern started in the bottom right corner and ended in the top left corner. After swabbing the surface, the swab was placed in an empty 8 mL plastic test tube and the tube was capped. Once all swabs had been collected, 996 µL of extraction buffer was added to the tube, followed by 4 µL of a 2.5 µg/mL internal standard solution made up from the 27 isotopically labeled internal standards in methanol. Following addition of the extraction buffer, the samples were agitated for

a period of time before 650 μL of the extraction buffer was transferred to a 1.5 mL

microcentrifuge tube. The samples were centrifuged at 13,000 rpm for 10 minutes and then 500

μL of the supernatant was transferred to a 1 mL amber injection vial for LC-MS/MS analysis.

5.3.3 Surface swab method optimization

The initial conditions used for the surface swabbing method are denoted with asterisks in

Table 35. The initial method used methanol as a wetting solvent, as fentanyl is believed to be

readily soluble in alcohols and a cotton swab as the swab, as these are commonly used in the

practice of surface swabbing. [9,144,182,183] The initial extraction buffer used was a 50:50 mix of

MPA:MPB and the initial agitation parameters were vortexing at 1000 rpm for 5 minutes.

Method optimization for collecting fentalogs from a solid, non-porous surface took place

in seven steps: wetting solvent selection, wetting solvent modifier selection, addition of organics

to wetting solvent, swab material selection, extraction buffer selection, selection of agitation type,

and selection of agitation length. The variables tested during each optimization step are

summarized in Table 35. The three wetting solvents tested were water, isopropyl alcohol (IPA),

and methanol. The five wetting solvent modifiers tested were no modifier, addition of 1% formic

acid, addition of 1% ammonium hydroxide, a 90:10 mixture of water:methanol, and a 90:10

mixture of water:IPA. The organic additions tested were no organic addition, 30% methanol in

acidified water, and 30% isopropyl alcohol in acidified water. Thirteen swab types were tested,

including polyester swabs, cotton swabs, anti-static foam mass spectrometer cleaning swabs, 2 x

2" cotton gauze, Rayon swabs, Kimwipes, Whatman Filter paper (50 grade), MX908 Trace

Sampling wipe, Smith Nomex AE Mode wipe, cotton balls, Q-Tips, nylon swabs, and Vectra[®]

Alpha[®] Nu wipes. To prevent complete absorption of the extraction buffer during extraction, the

Kimwipes, cotton balls, and Vectra[®] Alpha[®] Nu wipes had to be trimmed prior to swabbing; the

Kimwipes were trimmed to approximately 11 x 5 cm, the cotton balls were cut into quarters, and

the Vectra® Alpha® Nu wipes were trimmed to 5 x 2.5 cm. The three extraction buffers tested were 98:2 mobile phase A (MPA):mobile phase B (MPB), 50:50 MPA:MPB, and MPB. The types of agitation included no agitation, sonication, vortexing at 1000 rpm, and sonication followed by vortexing at 1000 rpm. After determining the best form of agitation, the length of time the samples were agitated was tested at 30 seconds, 5 minutes, and 10 minutes of agitation.

An *n* of 5 was used for each variable tested and a blank extraction of clean methanol was performed with each variable to eliminate any bias that may originate from contaminated surfaces. Following each optimization step, the extraction efficiency for the variables tested were compared with a one-way ANOVA using Microsoft Excel (Microsoft Corporation, Redmond, WA), with $\alpha=0.05$ for all statistical tests. After identifying which variable had the best extraction efficiency, that variable was carried forward into the next optimization step. If no variable was found to be statistically more efficient at extracting the compounds of interest than the others, the variable carried into the next phase of testing was chosen based on which variable had the greatest mean extraction efficiency for the largest number of compounds.

Table 35. Extraction optimization steps performed and variables associated with each step. Initial method parameters denoted with an asterisks.

Test	Variables
Wetting Solvents	Water
	Isopropyl Alcohol
	Methanol[*]
Wetting Solvent Modifiers	No Modifier[*]
	1% Formic Acid
	1% Ammonium Hydroxide
	90:10 Water:Methanol
	90:10 Water:Isopropyl Alcohol
Organic Addition to Wetting Solvent	No Organic Solvent[*]
	70:30 Water:Methanol with 1% Formic Acid
	70:30 Water:Isopropyl Alcohol with 1% Formic Acid
Swabs	Polyester Swab
	Cotton Swab[*]
	Foam Mass Spectrometer Cleaning Swab
	2 x 2" Cotton Gauze
	Rayon Swab
	Kimwipe
	Whatman Filter Paper (50 Grade)
	MX908 Trace Sampling Wipe
	Smith Nomex AE Mode Wipe
	Q-Tip
	Cotton Ball
	Nylon Swab
	Vectra® Alpha® Nu Wipes
Extraction Buffers	98:2 Mobile Phase A:Mobile Phase B
	50:50 Mobile Phase A:Mobile Phase B[*]
	Mobile Phase B
Agitation Types	No Agitation
	Sonication
	Vortexing[*]
	Sonication then Vortexing
Agitation Times	30 Seconds
	5 Minutes[*]
	10 Minutes

5.3.4 Liquid chromatography tandem mass spectrometry

A set of Shimadzu Prominence 20 Series UFLC pumps (Shimadzu Scientific Instruments, Inc, Columbia, MD) paired with a Sciex 4000 QTRAP® MS/MS was used for LC-MS/MS analysis. Chromatographic separation was achieved with a Chromegabond WR C18 5 μm column (15 cm x 2.1 mm) (ES Industries, Inc, West Berlin, NJ) with a Restek Raptor Biphenyl 2.7 μm guard cartridge (5 x 3.0 mm) (Restek Corporation, Bellefonte, PA). MPA consisted of 10 mM ammonium formate and 0.1% formic acid in water while MPB consisted of 0.1% formic acid in methanol. The LC gradient is summarized in Table 36 and is as follows: 5% MPB at time 0, increased to 13% MPB over 0.50 minutes, increased to 50% MPB over 9.50 minutes, increased to 95% MPB over 1.25 minutes, held at 95% MPB for 1.50 minutes, decreased to 5% MPB over 0.10 minutes, held at 5% MPB for 2.15 minutes for a total run time of 15.00 minutes. Injections were set at 5 μL and the LC had a flow rate of 0.4000 mL/min. The column oven was set at 40°C.

Table 36. LC gradient. Elapsed time is the time from injection. Gradient time is the time amount of time in which the LC is changing from one mobile phase setting to the next.

Elapsed Time (min)	Gradient Time (min)	% MPA	% MPB
0.00	0.00	95	5
0.50	0.50	87	13
10.00	9.50	50	50
11.25	1.25	5	95
12.75	1.50	5	95
12.85	0.10	95	5
15.00	2.15	95	5

Table 37 shows the optimized ion transitions and mass spectrometer parameters for detecting the compounds of interest. Compounds were identified through multiple reaction monitoring (MRM), where the compounds of interest were monitored for two different ion transitions and the isotopically labeled internal standards were monitored for a single ion

transition. Identities of the compounds of interest were further confirmed through MRM ratios.

A MRM ratio range was established by averaging the MRM ratio for every calibrator and

allowing compounds to be within ±20% of this averaged MRM ratio. Compound identity was

also confirmed by retention time (RT) and relative retention time comparisons. Quantitation was

achieved by plotting the ratio of the largest ion transition peak area to the internal standard peak

area versus the known concentration of an external calibration curve to derive a linear line of best

fit.

Table 37. Mass spectrometer parameters, including compound retention times (RT), optimized precursor and product ions, declustering potentials (DP), collision energies (CE), and collision cell exit potentials (CXP).

Analyte	RT (min)	Precursor Ion (m/z)	Product Ions (m/z)	DP (v)	CE (v)	CXP (v)
4-ANPP	10.54	281.1	188.0, 104.9	66	25, 43	8, 16
4-ANPP -$^{13}C_6$	10.53	287.2	188.1	46	25	8
4'Methylacetylfentanyl HCl	11.66	337.1	119.0, 202.0	66	47, 31	18, 10
4'Methylacetylfentanyl-$^{13}C_6$ HCl	11.66	343.2	202.1	61	33	8
Acetylfentanyl	10.08	323.2	104.9, 188.0	81	53, 31	16, 8
Acetylfentanyl-$^{13}C_6$	10.07	329.2	188.1	71	33	8
Acrylfentanyl HCl	11.25	335.2	188.1, 104.9	56	31 53	8, 16
Acrylfentanyl-$^{13}C_6$ HCl	11.24	341.2	188.1	86	31	8
Alprazolam	13.13	309.1	281.1, 205.2	76	35, 51	14, 8
Alprazolam-d_5	13.12	314.1	286.2	71	37	14
Benzylfentanyl HCl	11.21	323.1	91.9, 174.0	81	55, 31	14, 8
Benzylfentanyl-$^{13}C_6$ HCl	11.20	329.1	91.0	56	63	14
(±)-β-Hydroxythiofentanyl HCl	10.11	359.2	341.1, 192.0	61	23, 33	16, 8
(±)-β-Hydroxythiofentanyl-$^{13}C_6$ HCl	10.10	365.1	347.1	66	23	14
Butyrylfentanyl	12.59	351.2	188.0, 104.9	81	33, 57	8, 16
Butyrylfentanyl-$^{13}C_6$	12.58	357.1	188.0	101	33	8
Carfentanil Oxalate	12.06	395.2	335.2, 112.9	66	25, 41	16, 18
Carfentanil-$^{13}C_6$ Oxalate	12.06	401.3	341.1	66	27	16
(±)-*cis*-3-Methylfentanyl HCl	12.35	351.2	202.1, 104.9	66	33, 55	10, 16
(±)-*cis*-3-Methylfentanyl-$^{13}C_6$ HCl	12.35	357.1	202.1	96	35	8
Cocaine	8.81	304.1	182.1, 77.0	66	27, 87	8, 10
Cocaine-d_3	8.81	307.1	185.0	71	27	8
Cyclopropylfentanyl	12.21	349.1	188.1, 105.0	66	33, 55	8, 16
Cyclopropylfentanyl-$^{13}C_6$ HCl	12.20	355.0	188.2	106	33	8
Fentanyl	11.51	337.2	105.0, 188.2	71	53, 31	16, 8
Fentanyl-$^{13}C_6$	11.51	343.2	188.1	71	33	8

Furanylfentanyl HCl	11.86	375.2	188.1, 104.9	86	31, 57	8, 16
Furanylfentanyl-$^{13}C_6$ HCl	11.86	381.2	188.1	76	33	16
Heroin	8.48	370.2	165.0, 268.0	86	63, 37	6, 14
Heroin-d_9	8.41	379.1	272.0	66	41	12
Hydrocodone	5.35	300.1	199.1, 128.1	81	41, 75	8, 20
Hydrocodone-d_3	5.33	303.1	199.0	71	41	8
Methoxyacetylfentanyl HCl	9.72	353.2	188.2, 105.0	71	31, 53	10, 16
Methoxyacetylfentanyl-$^{13}C_6$ HCl	9.71	359.2	188.1	66	31	8
Naloxone	4.42	328.1	310.1, 212.0	51	27, 55	14, 10
Naloxone-d_5	4.37	333.1	315.1	81	27	18
Oxycodone	5.04	316.1	298.1, 241.2	86	27, 41	16, 20
Oxycodone-d_6	4.98	322.1	304.2	66	29	16
para-Fluorobutyrylfentanyl	12.62	369.2	188.0, 104.9	71	53, 63	8, 16
para-Fluorobutyrylfentanyl-$^{13}C_6$	12.62	375.2	194.1	61	35	8
para-Fluorofentanyl	11.57	355.2	188.0, 104.9	41	33, 55	8, 16
para-Fluorofentanyl-$^{13}C_6$	11.56	361.2	194.1	81	35	8
Remifentanil HCl	9.19	377.2	113.0, 228.0	71	41, 29	18, 10
Remifentanil-$^{13}C_6$ HCl	9.18	383.2	112.9	71	41	18
Sufentanil	12.76	387.1	238.2, 111.1	66	27, 53	12, 18
Sufentanil-d_5	12.76	392.2	238.0	61	27	12
U-47700	11.78	329.1	283.9, 173.0	56	25, 43	14, 10
U-47700-$^{13}C_2,^{15}N_2$	11.78	334.0	285.9	61	25	14
U-48800 HCl	12.76	343.0	297.9, 218.0	51	25, 41	16, 10
U-48800-$^{13}C_3,^{15}N_2$ HCl	12.76	348.1	300.0	46	25	16
U-49900	12.26	357.1	283.9, 172.9	81	27, 47	14, 28
U-49900-$^{13}C_5$	12.26	362.1	285.1	56	27	14
Valerylfentanyl HCl	12.91	365.2	188.0, 104.9	71	35, 59	8, 16
Valerylfentanyl-$^{13}C_6$ HCl	12.91	371.2	188.1	71	35	8

5.3.5 Method validation

Validation of the surface swab with LC-MS/MS methodology was performed following guidelines set by SWGTOX. Eight validation studies were performed, including calibration model/linearity, limit of detection (LOD)/lower limit of quantitation (LLOQ), accuracy and precision, carryover, matrix effects/recovery efficiency/process efficiency, interference, and stability. Calibration model, accuracy, and precision tests were completed with drug standard spiked into extraction buffer, as this is how the calibration curve and quality control samples are prepared during day-to-day use. Matrix effects, recovery efficiency, process efficiency, and

interference tests were completed with a mix of spiked extraction buffer samples and samples swabbed from a black, phenolic resin lab counter top (T.90.0.0). Carryover and stability tests were completed with swabbed samples, as these are how environmental samples will be submitted to the lab.

5.3.5.1 Calibration model/linearity

To determine the linear range of the method, calibrators at 8 concentration levels (0.05, 0.1, 0.5, 1, 5, 10, 15, and 25 ng/100 cm^2) were analyzed by 7 replicates spread out over 7 days. The quantitation ratio of each compound from each calibrator was plotted against the actual concentration and the plot was fitted with a line of best fit. The line of best fit could be weighted but it was required to have a R^2 value of greater than 0.9. For a concentration to be included in the calibration model, it had to have an accuracy and a precision (%CV) value within ±20% when the concentration calculated by the model was compared to the true concentration; the LLOQ was permitted to have accuracy and precision values of ±30%.

5.3.5.2 Limit of detection/lower limit of quantitation

While the limit of detection (LOD) and LLOQ can be assessed separately and assigned different values, this method assessed them together, resulting in the LOD and LLOQ being the same value. To assess the LOD/LLOQ, three replicates of the proposed LOD/LLOQ were analyzed on three separate runs, resulting in a total of 9 data points. All nine data points had to meet identification and quantification criteria for the tested concentration to be deemed the LOD/LLOQ. The tested LOD/LLOQ concentration for all 27 compounds was 0.05 ng/100 cm^2.

5.3.5.3 Accuracy and precision

Accuracy testing assessed how close a value reported by the methodology is to the true value while precision testing assessed the variability within the methodology. Two quality control (QC) points were used to assess the accuracy and precision of the assay, one with a concentration in the upper half of the calibration range and one with a concentration in the lower half of the calibration range. The high-concentration QC (QC A) had a concentration of 15 ng/100 cm^2 while the low-concentration QC (QC B) had a concentration of 1 ng/100 cm^2. Each QC was analyzed four times a day for six days, with the first two analyses occurring during the morning and the second two analyses occurring in the afternoon for a total of 24 analyses over 12 runs. The resulting accuracy and precision values were required to be within ±20% of the true value to be deemed acceptable.

Accuracy was assessed by calculating the daily mean concentration and comparing that to the expected concentration to obtain the daily mean accuracy percentage. The daily mean accuracy percentages were then averaged to obtain the average accuracy percentage for each analyte in the assay at both QC values. The average accuracy percentage for QC A and QC B could then be averaged to obtain an overall accuracy for the method.

Precision of the assay was assessed in five ways: within-run, between runs, within-laboratory, interday, and intraday. Within-run precision examined the amount of variability observed between samples analyzed within the same run (i.e. within the morning or within the afternoon run). Between-run precision examined the amount of variability observed between all 12 runs performed. Within-laboratory precision examined the amount of variability observed during normal use of the assay/analytical instrumentation. Interday precision examined the total variability observed between test days. Intraday precision examined the variability observed between all samples analyzed within the same day

5.3.5.4 Carryover

Carryover assessed the amount of each compound observed in a blank sample analyzed directly after a sample of high concentration. In this case, carryover was evaluated by analyzing blanks injected after samples spiked at 250 ng/100 cm^2 (10x the highest concentration in the calibration curve). This test was repeated six consecutive times to ensure a buildup of analytes did not occur with repeated, high concentration injections. Carryover was considered acceptable if peak areas observed in the blanks were less than 25% of the peak areas at the LOD.

5.3.5.5 Matrix effects, recovery efficiency, and process efficiency

Matrix effects (ME), recovery efficiency (RE), and process efficiency (PE) are a set of three tests run concurrently to assess sources of signal loss or gain throughout the methodology. ME assesses ion suppression or enhancement that stems from the matrix the sample is injected onto the instrument in. RE assesses the method's ability to recover drugs from the tested, non-porous surface. PE assesses the methods overall efficiency at collecting drug from a surface, recovering it from the swab used, and then overcoming ion suppression/enhancement on the instrument; PE takes into account the ME and RE of the methodology.

To perform the ME study, the analytical results of 6 samples consisting of analytes that were "neat" in methanol (no extraction, sample set 1) were compared to 6 individual blank methanol samples that were extracted and then fortified with analytes after extraction (sample set 2). ME were calculated by dividing the average quantitation ratio of the samples fortified after extraction by the average quantitation ratio of the analyte in neat solution and multiplying by 100 (Equation 6).

Equation 6. Calculation of matrix effects. Set 1=neat samples. Set 2=samples fortified post-extraction.

$$ME\ (\%) = \frac{Set\ 2}{Set\ 1} \times\ 100$$

A 10 ng/100 cm^2 sample was used in this experiment. To prepare the neat samples (sample set 1), all 27 analytes were mixed and diluted in methanol at a concentration of 10 ng/100 cm^2. For sample set 2, blank methanol was spiked on a non-porous surface, allowed to evaporate, and then extracted as unknown samples would be. Following extraction, the resulting sample was spiked at a concentration of 10 ng/100 cm^2. Currently, there is no defined limit for acceptable matrix effects. For this study, a range of 50-150% was considered acceptable.

To perform the RE study, the analytical results of 6 samples that had the analytes of interest spiked on the tested, non-porous surface and then subjected to the full extraction method (sample set 3) were compared to sample set 2 from the ME study. RE was calculated by dividing the average quantitation ratio of the full extraction samples by the average quantitation ratio of the samples fortified after extraction and then multiplying by 100 (Equation 7).

Equation 7. Calculation of recovery efficiency. Set 2=samples fortified post-extraction. Set 3=samples subjected to the full extraction process.

$$RE\ (\%) = \frac{Set\ 3}{Set\ 2} \times\ 100$$

A 10 ng/100 cm^2 sample was used in this experiment. Sample set 2 preparation is explained in the ME section above. For sample set 3, 20 μL of a 500 ng/mL solution comprised on all 27 analytes of interest in methanol was spiked on a 10 cm x 10 cm non-porous surface, resulting in 10 ng of each analyte being deposited in the 100 cm^2 area. The methanol was allowed to evaporate and then the area was swabbed and extracted.

Currently, there is no defined limit for acceptable recovery efficiency. Additionally, it is unreasonable to expect 100% efficiency, as many factors can negatively impact the recovery of

drugs from a surface. Due to these issues, no pass/fail criteria was set for recovery efficiency, though a goal of at least 50% recovery was established for all analytes in the assay.

To perform the PE study, , sample set 3 from the RE study was compared to sample set 1 from the ME study. PE was calculated by dividing the average quantitation ratio of the full extraction samples by the average quantitation ratio of the of the neat samples and then multiplying by 100 (Equation 8). Sample set 1 preparation is explained in the ME section above and sample set 3 preparation is explained in the RE section above.

Equation 8. Calculation of process efficiency. Set 1=neat samples. Set 3=samples subjected to the full extraction process.

$$PE\ (\%) = \frac{Set\ 3}{Set\ 1} \times 100$$

Currently, there is no defined limit for acceptable process efficiency. Additionally, it is unreasonable to expect 100% efficiency, as many factors can negatively impact the process, including low recovery efficiency and the potential for matrix effects. Due to these issues, no pass/fail criteria was set for process efficiency, though a goal of at least 50% was established for all analytes in the assay.

5.3.5.6 Interference

The interference study examined the method's ability to sustain selectivity and trueness in the presences of high concentrations of numerous other compounds, many from the same class of drugs or with structural similarities to those incorporated into the method. While it is not possible to test every potential interfering substance, care was taken to evaluate relevant materials. While some interfering materials will become apparent in the matrix effects studies, it is useful to determine the effects of common drug adulterants, such as supplements, over-the-counter medications, and other illicit drugs that might be present during field work and may affect

the analytical measurements being performed in the lab. Additionally, several drug metabolites

were investigated as interfering agents. While drug metabolites will not be found in locations of

illicit drug production, their chemical structure is highly similar to that of the analytes of interest,

allowing for the robustness of the methodology to be further challenged and give greater

confidence in the selectivity and trueness of the method. The interferents used for this study are

shown in Table 38 and were all spiked on the test surface at 500 ng; all potentially interfering

substances were evaluated in the same injection.

Table 38. Analytes used as potential interferents and their drug classifications.

Analyte	Drug Class
6-MAM	Opioid
Acetaminophen	Analgesic
Amphetamine	Stimulant
Benzoylecgonine	Stimulant
Buprenorphine	Opioid
Caffeine	Stimulant
Cathinone	Stimulant
Codeine	Opioid
Cyclobenzaprine	Muscle Relaxant
Diazepam	Benzodiazepine
Diphenhydramine	Antihistamine
EDDP	Opioid
Gabapentin	Anticonvulsant
Hydromorphone	Opioid
Ibuprofen	NSAID
Ketamine	Anesthetic
Lorazepam	Benzodiazepine
MDMA	Stimulant
Methadone	Opioid
Methamphetamine	Stimulant
Methcathinone	Stimulant
Morphine	Opioid
Norcarfentanil	Opioid
Norfentanyl	Opioid
Norhydrocodone	Opioid
Noroxycodone	Opioid
O-desmethyltramadol	Opioid
Oxymorphone	Opioid
Ritalinic Acid	Stimulant
Salicylic Acid	Analgesic
Tramadol	Opioid

To perform the interference study, three sample sets were spiked on a 10 x 10 cm non-porous test surface, with each set containing six samples. Set 1 was spiked with 10 ng of the 27 compounds of interest, as well as 500 ng of the interference mix. Set 2 was spiked with 10 ng of the analytes of interest but not the 500 ng of interference mix. The calculated concentrations of set 1could be compared with those of set 2 to determine if ion suppression/enhancement occurred due to the presence of the interfering compounds. Set 3 was spiked with 500 ng of interference only. This set was used to determine the presence/absence of false positive from the interfering substances. The mean calculated concentrations for each sample set were compared, with passing criteria set so that sample set 1 was required to be within ±25% of sample set 2 and no false positives were observed in sample set 3.

5.3.5.7 Stability

Stability studies were used to determine the stability of each compound on the swab after swabbing a surface and in solution following extraction of the swab. All stability samples were performed in replicates of six. The average calculated concentration for each compound at each tested time point was compared to the average calculated concentration for each compound at time 0. The compound was considered to be stable if it stayed within ±30% of the concentration observed at time 0.

Stability of the compounds on the swab were assessed with the swab stored at room temperature (20°C), in the refrigerator (4°C), in the freezer (-20°C), and after shipping. Room temperature stability was assessed at 0, 24, and 48 hours. Refrigerator stability was assessed at 0, 36, and 72 hours. Freezer stability was assessed at 0, 72, and 144 hours. Shipping stability was assessed after shipping wipes to the OSU-FTTL from an offsite location, which took approximately 120 hours.

Stability of the analytes following extraction were assessed with the sample stored in the LC autosampler (4°C), in the refrigerator (4°C), and the freezer (-20°C). Autosampler stability was assessed at 0, 12, and 24 hours, with the same set of 6 samples reinjected at each time point without recapping the injection vial. Refrigerator stability was assessed at 0, 36, and 72 hours. Freezer stability was assessed at 0, 72, and 144 hours.

5.3.6 Assessment of multi-surface extraction efficiencies

To extraction efficiency of the surface swab method was assessed on 11 commonly encountered household surfaces: lab bench (phenolic resin), marble tile, painted drywall (flat white, latex based), galvanized steel, vinyl floor tile, unfinished plywood, glass, laminate, golden oak flooring, concrete cap block, and high density polyethylene (HDPE) table. All surfaces were spiked with 10 ng of drug and swabbed as explained in section 5.3.2 Surface spiking and swabbing. The swabbing method used was the final, optimized method developed in section 5.3.3 Surface swab method optimization. An *n* of 5 was used for each surface type swabbed and a blank coupon of each surface material was spiked with clean methanol and swabbed alongside the others to eliminate any bias that may originate from previously contaminated surfaces. Results were compared with a one-way ANOVA with $\alpha=0.05$.

5.4 Results
5.4.1 Surface swab method optimization

Results of the surface swab optimization steps for extracting fentalogs from a non-porous surface are shown below. For all results tables, the means and standard deviations have units of ng/100 cm². Additionally, results within the same row that are denoted with the same superscript letter are not significantly different at $\alpha=0.05$. Intravariable extraction efficiencies are shown on the last row of each results table under "Average Extraction Efficiency". For each test,

whichever variable had the greatest extraction efficiency for the largest number of compounds was carried over into the next phase of the optimization.

5.4.1.1 Wetting solvent selection

The results of the wetting solvent selection test are summarized in Table 39. For all 27 compounds of interest, water resulted in a significantly higher extraction efficiency than methanol or IPA. To assess the intravariable extraction efficiency for the 27 compounds of interest, the mean recovered drug concentrations for all three variables were compared with an ANOVA. These results are shown on the last line of Table 39. As with the individual compounds, the overall extraction efficiency was best when water was used as a wetting solvent.

Table 39. Mean (±SD) recovered drug concentrations (ng/100 cm^2) for each compound of interest from the wetting solvent selection test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	Water	Methanol	IPA
4-ANPP	0.72 (±0.14)[a]	0.40 (±0.09)[b]	0.37 (±0.06)[b]
4'Methylacetylfentanyl HCl	2.04 (±0.38)[a]	0.95 (±0.18)[b]	0.78 (±0.13)[b]
Acetylfentanyl	2.75 (±0.36)[a]	1.03 (±0.17)[b]	0.79 (±0.14)[b]
Acrylfentanyl HCl	1.94 (±0.31)[a]	0.86 (±0.16)[b]	0.70 (±0.12)[b]
Alprazolam	3.89 (±0.48)[a]	1.00 (±0.13)[b]	0.76 (±0.12)[b]
Benzylfentanyl HCl	2.95 (±0.39)[a]	0.95 (±0.18)[b]	0.74 (±0.11)[b]
(±)-β-Hydroxythiofentanyl HCl	3.32 (±0.42)[a]	1.01 (±0.18)[b]	0.78 (±0.11)[b]
Butyrylfentanyl	1.65 (±0.26)[a]	0.88 (±0.18)[b]	0.73 (±0.12)[b]
Carfentanil Oxalate	1.77 (±0.28)[a]	0.88 (±0.15)[b]	0.72 (±0.10)[b]
(±)-*cis*-3-Methylfentanyl HCl	2.02 (±0.37)[a]	0.89 (±0.15)[b]	0.71 (±0.12)[b]
Cocaine	4.28 (±0.50)[a]	1.13 (±0.16)[b]	0.96 (±0.12)[b]
Cyclopropylfentanyl	1.75 (±0.26)[a]	0.90 (±0.13)[b]	0.74 (±0.12)[b]
Fentanyl	2.20 (±0.36)[a]	0.94 (±0.12)[b]	0.74 (±0.12)[b]
Furanylfentanyl HCl	1.34 (±0.22)[a]	0.91 (±0.16)[b]	0.72 (±0.11)[b]
Heroin	4.49 (±0.48)[a]	1.21 (±0.16)[b]	0.94 (±0.17)[b]
Hydrocodone	4.56 (±0.70)[a]	1.33 (±0.21)[b]	1.06 (±0.17)[b]
Methoxyacetylfentanyl HCl	3.35 (±0.46)[a]	1.02 (±0.17)[b]	0.81 (±0.12)[b]
Naloxone	5.18 (±0.50)[a]	1.14 (±0.15)[b]	0.87 (±0.12)[b]
Oxycodone	5.12 (±0.64)[a]	1.34 (±0.20)[b]	1.23 (±0.22)[b]
para-Fluorobutyrylfentanyl	1.33 (±0.22)[a]	0.84 (±0.14)[b]	0.72 (±0.15)[b]
para-Fluorofentanyl	1.75 (±0.31)[a]	0.93 (±0.15)[b]	0.75 (±0.14)[b]
Remifentanil HCl	4.82 (±0.59)[a]	0.98 (±0.14)[b]	0.77 (±0.13)[b]
Sufentanyl	1.62 (±0.31)[a]	0.88 (±0.16)[b]	0.69 (±0.10)[b]
U-47700	2.40 (±0.36)[a]	0.92 (±0.15)[b]	0.74 (±0.13)[b]
U-48800 HCl	2.48 (±0.43)[a]	0.95 (±0.17)[b]	0.77 (±0.09)[b]
U-49900	2.61 (±0.37)[a]	0.83 (±0.14)[b]	0.70 (±0.12)[b]
Valerylfentanyl HCl	1.09 (±0.18)[a]	0.90 (±0.13)[b]	0.75 (±0.11)[b]
Average Extraction Efficiency	2.72 (±1.32)[a]	0.96 (±0.18)[b]	0.78 (±0.15)[b]

5.4.1.2 Wetting solvent modifier selection

The results of the addition of modifiers to the wetting solvent test are summarized in Table 40. For 26 of the 27 compounds of interest, water acidified with 1% formic acid achieved the greatest extraction efficiency. The greatest extraction efficiency for alprazolam was achieved with water made alkaline with 1% ammonium hydroxide, though the mean extracted concentration was not significantly different from that achieved with acidified water. When the

intravariable extraction efficiencies were compared, water acidified with 1% formic acid had the

greatest extraction efficiency, water made alkaline with 1% ammonium hydroxide had the next

greatest extraction efficiency, and water with no modifiers, water with 10% methanol and water

with 10% IPA all had the lowest extraction efficiencies.

Table 40. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the wetting solvent modifier test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	Water	1% Formic Acid	1% Ammonium Hydroxide	90:10 Water: Methanol	90:10 Water:IPA
4-ANPP	0.28 (±0.02)[c]	1.65 (±0.25)[a]	0.80 (±0.15)[b]	0.39 (±0.03)[c]	0.31 (±0.02)[c]
4'Methylacetylfentanyl HCl	1.74 (±0.30)[c]	3.76 (±0.34)[a]	2.67 (±0.51)[b]	1.79 (±0.17)[c]	1.72 (±0.23)[c]
Acetylfentanyl	2.22 (±0.39)[c]	4.11 (±0.37)[a]	2.86 (±0.44)[b]	2.18 (±0.20)[c]	2.14 (±0.26)[c]
Acrylfentanyl HCl	1.32 (±0.24)[c]	3.52 (±0.35)[a]	2.05 (±0.38)[b]	1.40 (±0.18)[c]	1.31 (±0.16)[c]
Alprazolam	3.16 (±0.38)[b]	4.21 (±0.36)[a]	4.35 (±0.37)[a]	3.22 (±0.36)[b]	3.39 (±0.40)[b]
Benzylfentanyl HCl	2.31 (±0.38)[b]	4.30 (±0.39)[a]	2.73 (±0.55)[b]	2.27 (±0.22)[b]	2.21 (±0.23)[b]
(±)-β-Hydroxythiofentanyl HCl	2.56 (±0.49)[c]	4.31 (±0.46)[a]	3.44 (±0.42)[b]	2.51 (±0.35)[c]	2.48 (±0.25)[c]
Butyrylfentanyl	1.48 (±0.29)[c]	3.48 (±0.30)[a]	2.17 (±0.53)[b]	1.46 (±0.14)[c]	1.40 (±0.18)[c]
Carfentanil Oxalate	1.49 (±0.25)[c]	3.51 (±0.35)[a]	2.64 (±0.48)[b]	1.52 (±0.16)[c]	1.44 (±0.13)[c]
(±)-*cis*-3-Methylfentanyl HCl	1.66 (±0.29)[c]	3.68 (±0.27)[a]	2.26 (±0.63)[b]	1.59 (±0.16)[c]	1.51 (±0.17)[c]
Cocaine	3.43 (±0.54)[b]	4.84 (±0.44)[a]	3.63 (±0.37)[b]	3.42 (±0.36)[b]	3.56 (±0.31)[b]
Cyclopropylfentanyl	1.51 (±0.26)[c]	3.41 (±0.33)[a]	2.06 (±0.50)[b]	1.50 (±0.19)[c]	1.38 (±0.16)[c]
Fentanyl	1.79 (±0.35)[c]	3.82 (±0.31)[a]	2.35 (±0.45)[b]	1.74 (±0.17)[c]	1.67 (±0.20)[c]
Furanylfentanyl HCl	1.04 (±0.19)[c]	2.92 (±0.28)[a]	1.85 (±0.42)[b]	1.04 (±0.10)[c]	0.97 (±0.12)[c]
Heroin	3.39 (±0.41)[b]	4.78 (±0.45)[a]	3.08 (±0.55)[b]	3.40 (±0.45)[b]	3.51 (±0.25)[b]
Hydrocodone	3.55 (±0.42)[c]	4.64 (±0.42)[a]	4.06 (±0.29)[b]	3.57 (±0.31)[c]	3.81 (±0.24)[b,c]
Methoxyacetylfentanyl HCl	2.74 (±0.46)[c]	4.55 (±0.38)[a]	3.53 (±0.41)[b]	2.69 (±0.28)[c]	2.70 (±0.26)[c]
Naloxone	3.64 (±0.52)[b]	5.06 (±0.56)[a]	3.77 (±0.42)[b]	3.87 (±0.28)[b]	4.14 (±0.41)[b]
Oxycodone	4.19 (±0.74)[b]	5.51 (±0.63)[a]	4.38 (±0.53)[b]	4.13 (±0.35)[b]	4.49 (±0.39)[b]
para-Fluorobutyrylfentanyl	1.24 (±0.36)[c]	3.19 (±0.27)[a]	1.99 (±0.42)[b]	1.18 (±0.10)[c]	1.19 (±0.18)[c]
para-Fluorofentanyl	1.53 (±0.31)[c]	3.49 (±0.26)[a]	2.22 (±0.52)[b]	1.50 (±0.16)[c]	1.43 (±0.15)[c]
Remifentanil HCl	3.60 (±0.51)[c]	5.09 (±0.50)[a]	4.42 (±0.38)[b]	3.65 (±0.47)[c]	3.91 (±0.27)[b,c]
Sufentanyl	1.30 (±0.36)[c]	3.33 (±0.36)[a]	2.16 (±0.46)[b]	1.24 (±0.15)[c]	1.19 (±0.14)[c]
U-47700	2.07 (±0.31)[c]	4.10 (±0.33)[a]	3.17 (±0.52)[b]	2.07 (±0.22)[c]	2.20 (±0.20)[c]
U-48800 HCl	2.23 (±0.52)[c]	4.14 (±0.34)[a]	3.43 (±0.53)[b]	2.22 (±0.24)[c]	2.38 (±0.24)[c]
U-49900	1.89 (±0.36)[b]	3.96 (±0.37)[a]	2.22 (±0.55)[b]	2.07 (±0.22)[b]	2.10 (±0.23)[b]
Valerylfentanyl HCl	1.01 (±0.26)[c]	2.72 (±0.23)[a]	1.69 (±0.37)[b]	1.01 (±0.08)[c]	0.99 (±0.14)[c]
Average Extraction Efficiency	2.16 (±1.00)[c]	3.93 (±0.83)[a]	2.81 (±0.92)[b]	2.17 (±1.00)[c]	2.20 (±1.12)[c]

5.4.1.3 Organic addition selection

While the addition of 10% organic solvent was examined during the wetting solvent modifier test, it was not tested in combination with the addition of 1% formic acid. Additionally, a slightly higher organic solvent percentage was used during this test in an attempt to capture a greater amount of the more lipophilic fentalogs. The results of the addition of organic solvents to the acidified wetting solvent test are summarized in Table 41. There was no significant difference between extraction efficiencies for any of the 27 compounds of interest when acidified water, 30% methanol in acidified water, or 30% IPA in acidified water were used as the wetting solvent. However, when the intravariable extraction efficiencies were compared, there was a significant difference between acidified water and 30% IPA in acidified water; intravariable extraction efficiency of 30% methanol in acidified water was not significantly different from acidified water or 30% IPA in acidified water. Since acidified water without an organic solvent addition had the largest intravariable extraction efficiency for all 27 compounds of interested, though not statistically significant, it was determined to be the best wetting solvent and was therefore carried into the next phase of the surface swab method optimization.

Table 41. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the organic addition test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	No Organic	70:30 Water:Methanol with 1% Formic Acid	70:30 Water:IPA with 1% Formic Acid
4-ANPP	1.22 (±0.28)[a]	0.97 (±0.28)[a]	1.15 (±0.23)[a]
4'Methylacetylfentanyl HCl	2.80 (±0.42)[a]	2.26 (±0.53)[a]	2.52 (±0.39)[a]
Acetylfentanyl	3.21 (±0.47)[a]	2.74 (±0.56)[a]	3.06 (±0.44)[a]
Acrylfentanyl HCl	2.53 (±0.44)[a]	2.14 (±0.51)[a]	2.32 (±0.40)[a]
Alprazolam	3.53 (±0.50)[a]	2.92 (±0.60)[a]	3.13 (±0.40)[a]
Benzylfentanyl HCl	3.41 (±0.38)[a]	2.91 (±0.63)[a]	3.09 (±0.41)[a]
(±)-β-Hydroxythiofentanyl HCl	3.63 (±0.56)[a]	3.17 (±0.59)[a]	3.40 (±0.48)[a]
Butyrylfentanyl	2.41 (±0.43)[a]	1.88 (±0.49)[a]	2.13 (±0.49)[a]
Carfentanil Oxalate	2.29 (±0.43)[a]	1.91 (±0.51)[a]	2.00 (±0.35)[a]
(±)-*cis*-3-Methylfentanyl HCl	2.49 (±0.43)[a]	1.97 (±0.52)[a]	2.08 (±0.38)[a]
Cocaine	3.53 (±0.46)[a]	3.16 (±0.51)[a]	3.41 (±0.34)[a]
Cyclopropylfentanyl	2.45 (±0.41)[a]	2.10 (±0.51)[a]	2.17 (±0.39)[a]
Fentanyl	2.75 (±0.42)[a]	2.37 (±0.54)[a]	2.53 (±0.46)[a]
Furanylfentanyl HCl	2.04 (±0.38)[a]	1.65 (±0.50)[a]	1.88 (±0.34)[a]
Heroin	3.63 (±0.43)[a]	3.14 (±0.58)[a]	3.38 (±0.28)[a]
Hydrocodone	3.46 (±0.39)[a]	2.97 (±0.44)[a]	3.22 (±0.37)[a]
Methoxyacetylfentanyl HCl	3.19 (±0.49)[a]	2.78 (±0.59)[a]	3.02 (±0.31)[a]
Naloxone	3.59 (±0.54)[a]	3.06 (±0.63)[a]	3.21 (±0.31)[a]
Oxycodone	3.76 (±0.48)[a]	3.13 (±0.62)[a]	3.34 (±0.40)[a]
para-Fluorobutyrylfentanyl	1.92 (±0.45)[a]	1.55 (±0.52)[a]	1.63 (±0.25)[a]
para-Fluorofentanyl	2.63 (±0.42)[a]	2.12 (±0.53)[a]	2.30 (±0.35)[a]
Remifentanil HCl	3.77 (±0.49)[a]	3.24 (±0.63)[a]	3.45 (±0.36)[a]
Sufentanyl	2.72 (±1.22)[a]	2.17 (±1.21)[a]	1.98 (±0.37)[a]
U-47700	2.64 (±0.39)[a]	2.17 (±0.47)[a]	2.49 (±0.33)[a]
U-48800 HCl	3.35 (±0.92)[a]	2.64 (±0.88)[a]	2.82 (±0.49)[a]
U-49900	2.98 (±0.46)[a]	2.50 (±0.53)[a]	2.71 (±0.37)[a]
Valerylfentanyl HCl	1.70 (±0.57)[a]	1.38 (±0.63)[a]	1.43 (±0.35)[a]
Average Extraction Efficiency	2.88 (±0.68)[a]	2.41 (±0.62)[b]	2.59 (±0.66)[a,b]

5.4.1.4 Swab selection

The results of the swab selection test are summarized in Table 42. Any values without standard deviations only had a single swab capture enough drug to be quantitated. Values reported as "N/A" had no swabs capture enough drug to be quantitated. Of the 13 swab types tested, Kimwipes, Whatman filter paper, and cotton balls were significantly better at collecting all 27 analytes than the other 10 swab types. While not statistically significant, Kimwipes had a the

best extraction efficiency of the three best swabs for 15 of the 27 compounds of interest and had

the best average extraction efficiency so it was deemed the overall best swab type to use for

collecting fentalogs from the non-porous surface.

Table 42. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the swab selection test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	Polyester Swab	Cotton Swab	Foam Swab	Cotton Gauze	Rayon Swab	Kimwipe	Whatman Paper
4-ANPP	1.04 (±0.32)[d]	1.15 (±0.28)[c,d]	0.89 (±0.14)[d]	0.69 (±0.26)[d]	0.06 (±0.02)[e]	2.15 (±0.90)[a]	1.58 (±0.40)[b,c]
4'Methylacetylfentanyl HCl	2.17 (±0.32)[c,d]	2.61 (±0.21)[c]	2.11 (±0.71)[c,d]	1.81 (±0.67)[d]	0.11 (±0.06)[e]	4.28 (±0.90)[a]	3.71 (±0.46)[a,b]
Acetylfentanyl	2.76 (±0.18)[c,d]	2.84 (±0.22)[c,d]	2.41 (±0.69)[d,e]	2.00 (±0.81)[e]	0.18 (±0.10)[f]	4.63 (±0.97)[a]	4.38 (±0.45)[a]
Acrylfentanyl HCl	2.02 (±0.26)[d,e]	2.22 (±0.19)[d,e]	1.99 (±0.53)[d,e]	1.69 (±0.72)[e]	0.11 (±0.05)[f]	3.82 (±0.94)[a]	3.31 (±0.40)[a,b]
Alprazolam	3.33 (±0.30)[b]	3.42 (±0.37)[b]	5.24 (±0.45)[a]	2.32 (±0.94)[c]	0.17 (±0.08)[d]	4.85 (±1.14)[a]	4.82 (±0.71)[a]
Benzylfentanyl HCl	2.91 (±0.22)[c,d]	2.96 (±0.28)[b,c,d]	2.42 (±0.68)[d,e]	2.06 (±0.85)[e]	0.16 (±0.09)[f]	4.80 (±1.06)[a]	4.51 (±0.52)[a]
(±)-β-Hydroxythiofentanyl HCl	3.22 (±0.23)[b,c]	3.12 (±0.37)[c]	2.37 (±0.63)[d]	2.10 (±0.92)[d]	0.25 (±0.12)[e]	4.81 (±1.01)[a]	4.71 (±0.54)[a]
Butyrylfentanyl	1.99 (±0.32)[c]	2.51 (±0.30)[c]	2.06 (±0.62)[c]	1.87 (±0.53)[c]	0.09 (±0.04)[d]	4.44 (±0.90)[a]	3.56 (±0.66)[b]
Carfentanil Oxalate	2.16 (±0.14)[d,e]	2.43 (±0.15)[c,d,e]	2.07 (±0.73)[d,e]	1.84 (±0.65)[e]	0.13 (±0.03)[f]	4.08 (±0.80)[a]	3.56 (±0.52)[a,b]
(±)-cis-3-Methylfentanyl HCl	2.13 (±0.30)[c,d]	2.52 (±0.24)[c,d]	2.15 (±0.66)[c,d]	1.93 (±0.74)[d]	0.11 (±0.04)[e]	4.68 (±1.06)[a]	3.87 (±0.43)[b]
Cocaine	3.98 (±0.24)[b]	3.76 (±0.35)[b]	2.97 (±0.77)[c,d]	2.31 (±1.02)[d]	0.29 (±0.18)[e]	5.38 (±1.04)[a]	5.70 (±0.63)[a]
Cyclopropylfentanyl	2.03 (±0.21)[c]	2.47 (±0.18)[c]	2.22 (±0.77)[c]	1.95 (±0.70)[c]	0.10 (±0.05)[d]	4.71 (±0.95)[a]	3.73 (±0.54)[b]
Fentanyl	2.36 (±0.24)[d,e]	2.59 (±0.21)[d]	2.22 (±0.73)[d,e]	1.93 (±0.75)[e]	0.12 (±0.05)[f]	4.33 (±0.94)[a]	3.83 (±0.38)[a,b]
Furanylfentanyl HCl	1.51 (±0.16)[d]	1.88 (±0.16)[d]	1.92 (±0.57)[d]	1.55 (±0.54)[d]	0.09 (±0.03)[e]	3.47 (±0.77)[a]	2.70 (±0.40)[c]
Heroin	4.12 (±0.33)[b]	3.74 (±0.44)[b,c]	3.00 (±0.66)[c,d]	2.30 (±0.95)[d]	0.31 (±0.20)[e]	5.40 (±1.30)[a]	5.58 (±0.71)[a]
Hydrocodone	4.10 (±0.37)[c]	3.76 (±0.53)[c,d]	2.93 (±0.59)[d,e]	2.27 (±1.06)[e]	0.41 (±0.20)[f]	5.56 (±1.19)[a]	5.82 (±0.74)[a]
Methoxyacetylfentanyl HCl	3.46 (±0.15)[b,c]	3.37 (±0.32)[c,d]	2.69 (±0.70)[d,e]	2.21 (±0.96)[e]	0.21 (±0.13)[f]	5.29 (±1.22)[a]	5.11 (±0.44)[a]
Naloxone	4.19 (±0.16)[c]	3.88 (±0.59)[c,d]	3.12 (±0.52)[d,e]	2.35 (±1.12)[e]	0.51 (±0.27)[f]	5.46 (±1.31)[a,b]	5.55 (±0.68)[a]
Oxycodone	4.46 (±0.34)[c]	4.24 (±0.31)[c,d]	3.27 (±0.79)[d,e]	2.46 (±1.10)[e]	0.49 (±0.25)[f]	5.76 (±1.28)[a,b]	6.08 (±0.77)[a]
para-Fluorobutyrylfentanyl	1.42 (±0.20)[e]	2.06 (±0.28)[d]	1.83 (±0.70)[d,e]	1.40 (±0.40)[e]	0.07 (±0.03)[f]	3.57 (±0.60)[a]	2.84 (±0.56)[b]
para-Fluorofentanyl	2.00 (±0.24)[d,e]	2.38 (±0.14)[d]	2.01 (±0.69)[d,e]	1.75 (±0.66)[e]	0.09 (±0.04)[f]	4.05 (±0.89)[a]	3.48 (±0.47)[a,b]
Remifentanil HCl	4.11 (±0.26)[c]	3.78 (±0.55)[c,d]	2.98 (±0.59)[d,e]	2.32 (±1.05)[e]	0.25 (±0.17)[f]	5.23 (±1.21)[a,b]	5.32 (±0.72)[a]
Sufentanil	1.77 (±0.48)[e]	2.80 (±0.92)[c,d]	2.42 (±1.00)[d,e]	1.67 (±0.60)[e]	0.18[f]	3.94 (±0.69)[b]	3.48 (±0.70)[b,c]
U-47700	2.41 (±0.25)[c,d]	3.04 (±0.26)[b,c]	2.06 (±0.68)[d]	1.99 (±0.72)[d]	0.13 (±0.07)[e]	4.55 (±0.98)[a]	4.10 (±0.46)[a]
U-48800 HCl	2.07 (±0.35)[d,e]	3.35 (±0.56)[b,c]	2.13 (±0.64)[d,e]	1.68 (±0.48)[e]	N/A[f]	3.96 (±0.65)[b]	3.81 (±0.62)[b]
U-49900	2.24 (±0.34)[c,d]	2.56 (±0.47)[c]	1.80 (±0.37)[d]	1.74 (±0.78)[d]	0.11 (±0.06)[e]	3.83 (±1.09)[a]	3.21 (±0.57)[a,b]
Valerylfentanyl HCl	1.17 (±0.26)[f]	1.86 (±0.34)[d,e]	1.60 (±0.62)[e,f]	1.31 (±0.29)[f]	0.11[g]	2.86 (±0.59)[a,b]	2.20 (±0.60)[c,d]
Average Extraction Efficiency	2.63 (±1.01)[c,d]	1.15 (±0.28)[c,d]	2.40 (±0.77)[d]	1.91 (±0.39)[e]	0.19 (±0.12)[f]	4.44 (±0.85)[a]	4.10 (±1.15)[a]

Table 42 Continued. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the swab selection test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	908 Wipe	Smith Wipe	Cotton Ball	Q-Tip	Nylon Swab	Vectra® Wipe
4-ANPP	N/A[e]	1.15 (±0.21)[c,d]	1.65 (±0.56)[b]	0.1[e]	N/A[e]	1.36 (±0.80)[c,d]
4'Methylacetylfentanyl HCl	0.06 (±0.01)[e]	3.38 (±0.43)[b]	4.18 (±0.79)[a]	2.63 (±0.43)[c]	N/A[e]	2.58 (±1.32)[c,d]
Acetylfentanyl	0.07 (±0.02)[f]	3.69 (±0.35)[b]	4.52 (±0.78)[a]	3.19 (±0.59)[b,c]	N/A[f]	3.01 (±1.47)[b,c,d]
Acrylfentanyl HCl	0.05 (±0.00)[f]	2.88 (±0.28)[b,c]	3.70 (±0.64)[a]	2.33 (±0.26)[c,d]	N/A[f]	2.42 (±1.28)[c,d]
Alprazolam	0.09 (±0.03)[d]	3.36 (±0.18)[b]	5.14 (±0.80)[a]	3.50 (±0.52)[b]	0.06 (±0.01)[d]	3.17 (±1.47)[b,c]
Benzylfentanyl HCl	0.06 (±0.01)[f]	3.66 (±0.34)[b]	4.66 (±0.84)[a]	3.31 (±0.58)[b,c]	0.05[f]	3.13 (±1.55)[b,c,d]
(±)-β-Hydroxythiofentanyl HCl	0.1[e]	3.83 (±0.31)[b]	4.81 (±0.76)[a]	3.40 (±0.59)[b,c]	N/A[e]	3.51 (±1.77)[b,c]
Butyrylfentanyl	0.06 (±0.01)[d]	3.44 (±0.52)[b]	4.35 (±0.75)[a]	2.51 (±0.56)[c]	N/A[d]	2.48 (±1.46)[c]
Carfentanil Oxalate	0.14[f]	2.97 (±0.46)[b,c]	3.96 (±0.60)[a]	2.52 (±0.53)[c,d]	N/A[f]	2.29 (±1.17)[c,d,e]
(±)-cis-3-Methylfentanyl HCl	0.06 (±0.01)[e]	3.61 (±0.47)[b]	4.80 (±0.75)[a]	2.68 (±0.50)[c]	N/A[e]	2.64 (±1.58)[c,d]
Cocaine	N/A[e]	4.37 (±0.36)[b]	5.33 (±0.83)[a]	4.15 (±0.64)[b]	N/A[e]	3.55 (±1.52)[b,c]
Cyclopropylfentanyl	0.06 (±0.02)[d]	3.46 (±0.61)[b]	4.62 (±0.84)[a]	2.52 (±0.53)[c]	N/A[d]	2.55 (±1.46)[c]
Fentanyl	0.07 (±0.02)[f]	3.42 (±0.44)[b,c]	4.31 (±0.70)[a]	2.79 (±0.46)[c,d]	N/A[f]	2.63 (±1.40)[c,d,e]
Furanylfentanyl HCl	0.06 (±0.02)[e]	2.83 (±0.51)[b,c]	3.27 (±0.60)[a,b]	1.84 (±0.36)[d]	N/A[e]	2.02 (±1.03)d
Heroin	0.13[e]	4.48 (±0.41)[b]	5.35 (±0.79)[a]	4.14 (±0.60)[b]	N/A[e]	3.95 (±1.66)[b,c]
Hydrocodone	0.11[f]	4.53 (±0.38)[b,c]	5.24 (±0.70)[a,b]	4.19 (±0.62)[c]	N/A[f]	3.77 (±1.42)[c,d]
Methoxyacetylfentanyl HCl	0.07 (±0.02)[f]	4.16 (±0.37)[b]	5.06 (±0.83)[a]	3.71 (±0.59)[b,c]	N/A[f]	3.08 (±1.46)[c,d,e]
Naloxone	N/A[f]	4.64 (±0.58)[b,c]	5.08 (±0.72)[a,b]	3.88 (±0.62)[c,d]	N/A[f]	3.69 (±1.48)[c,d]
Oxycodone	0.08 (±0.02)[f]	4.90 (±0.85)[b,c]	5.50 (±0.79)[a,b]	4.38 (±0.70)[c]	0.06 (±0.01)[f]	3.96 (±1.52)[c,d]
para-Fluorobutyrylfentanyl	0.07[f]	2.70 (±0.67)[b,c]	3.62 (±0.69)[a]	1.91 (±0.36)[d,e]	N/A[f]	2.07 (±1.09)[c,d,e]
para-Fluorofentanyl	0.06 (±0.02)[f]	3.09 (±0.46)[b,c]	3.89 (±0.65)[a]	2.50 (±0.44)[c,d]	N/A[f]	2.57 (±1.37)[c,d]
Remifentanil HCl	0.07 (±0.01)[f]	4.42 (±0.49)[b,c]	5.22 (±0.72)[a,b]	4.07 (±0.58)[c]	0.05[f]	3.74 (±1.55)[c,d]
Sufentanyl	N/A[f]	3.03 (±1.02)[c,d]	5.15 (±1.20)[a]	2.41 (±0.49)[d,e]	N/A[f]	2.19 (±0.99)[d,e]
U-47700	0.08 (±0.02)[e]	3.18 (±0.30)[b]	4.47 (±0.72)[a]	2.98 (±0.48)[b,c]	0.05 (±0.00)[e]	2.52 (±1.23)[b,c,d]
U-48800 HCl	N/A[f]	3.04 (±0.47)[c]	4.87 (±0.72)[a]	2.77 (±0.51)[c]	N/A[f]	2.79 (±1.24)[c,d]
U-49900	0.06 (±0.01)[e]	2.72 (±0.11)[b,c]	3.63 (±0.49)[a]	2.64 (±0.28)[b,c]	0.05[e]	2.77 (±1.34)[b,c]
Valerylfentanyl HCl	N/A[g]	2.53 (±0.58)[b,c]	3.32 (±0.57)[a]	1.56 (±0.35)[e,f]	N/A[g]	1.70 (±0.96)[d,e,f]
Average Extraction Efficiency	0.08 (±0.02)[f]	3.46 (±0.81)[b]	4.43 (±0.86)[a]	2.91 (±0.96)[c]	0.05 (±0.01)[f]	2.82 (±0.69)[c]

5.4.1.5 Extraction buffer selection

The results of the extraction buffer selection test are summarized in Table 43. MPB had the greatest extraction efficiency for all 27 compounds of interest, though it was only significantly different from 98:2 MPA:MPB and 50:50 MPA:MPB in 15 of the 27 compounds. When examining the intravariable extraction efficiencies, MPB did have a significantly greater overall extraction efficiency when compared to both 98:2 MPA:MPB and 50:50 MPA:MPB.

Table 43. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the extraction buffer test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	98:2 MPA:MPB	50:50 MPA:MPB	MPB
4-ANPP	2.13 (±0.33)[a]	2.16 (±0.41)[a]	2.79 (±0.73)[a]
4'Methylacetylfentanyl HCl	3.94 (±0.42)[b]	3.98 (±0.80)[b]	4.91 (±0.49)[a]
Acetylfentanyl	4.48 (±0.48)[b]	4.46 (±0.78)[b]	5.44 (±0.55)[a]
Acrylfentanyl HCl	3.66 (±0.38)[b]	3.60 (±0.67)[b]	4.52 (±0.62)[a]
Alprazolam	4.52 (±0.43)[b]	4.65 (±0.83)[a,b]	5.45 (±0.46)[a]
Benzylfentanyl HCl	4.58 (±0.55)[b]	4.55 (±0.77)[b]	5.48 (±0.59)[a]
(±)-β-Hydroxythiofentanyl HCl	4.78 (±0.55)[a,b]	4.72 (±0.88)[b]	5.68 (±0.50)[a]
Butyrylfentanyl	3.81 (±0.36)[b]	4.15 (±0.97)[a,b]	4.82 (±0.66)[a]
Carfentanil Oxalate	3.69 (±0.24)[b]	3.69 (±0.63)[b]	4.64 (±0.61)[a]
(±)-*cis*-3-Methylfentanyl HCl	4.17 (±0.31)[b]	4.19 (±0.97)[b]	5.15 (±0.64)[a]
Cocaine	5.33 (±0.67)[b]	5.32 (±0.86)[b]	6.31 (±0.43)[a]
Cyclopropylfentanyl	3.92 (±0.29)[b]	3.99 (±1.03)[b]	5.03 (±0.60)[a]
Fentanyl	3.99 (±0.34)[b]	4.05 (±0.80)[b]	4.97 (±0.70)[a]
Furanylfentanyl HCl	3.06 (±0.38)[b]	3.08 (±0.64)[b]	3.91 (±0.70)[a]
Heroin	5.56 (±0.89)[a]	5.46 (±0.83)[a]	6.43 (±0.57)[a]
Hydrocodone	5.40 (±0.63)[b]	5.35 (±0.87)[b]	6.32 (±0.26)[a]
Methoxyacetylfentanyl HCl	5.12 (±0.57)[b]	5.00 (±0.87)[b]	6.04 (±0.42)[a]
Naloxone	5.51 (±0.75)[a,b]	5.33 (±0.90)[b]	6.50 (±0.59)[a]
Oxycodone	6.24 (±0.73)[a,b]	5.74 (±1.01)[b]	7.20 (±0.39)[a]
para-Fluorobutyrylfentanyl	2.88 (±0.33)[b]	3.14 (±0.88)[a,b]	4.15 (±0.87)[a]
para-Fluorofentanyl	3.73 (±0.33)[b]	3.85 (±0.77)[b]	4.68 (±0.61)[a]
Remifentanil HCl	5.50 (±0.67)[b]	5.44 (±0.87)[b]	6.47 (±0.49)[a]
Sufentanyl	2.94 (±0.44)[a]	3.05 (±0.88)[a]	4.20 (±1.62)[a]
U-47700	4.40 (±0.49)[a,b]	4.31 (±0.71)[b]	5.21 (±0.61)[a]
U-48800 HCl	3.66 (±0.45)[a]	4.04 (±1.03)[a]	4.56 (±0.96)[a]
U-49900	4.03 (±0.49)[a]	4.05 (±0.69)[a]	4.72 (±0.80)[a]
Valerylfentanyl HCl	2.59 (±0.21)[a]	2.94 (±0.93)[a]	3.56 (±0.78)[a]
Average Extraction Efficiency	4.21 (±1.01)[b]	4.23 (±0.90)[b]	5.15 (±1.01)[a]

The results of the agitation type test are summarized in Table 44. The use of sonication or vortexing both resulted in the greatest extraction efficiency, while sonication followed by vortexing lead to an extraction efficiency that was not significantly different from no agitation being performed. When looking at the average extraction efficiency, sonication had a slightly greater efficiency than vortexing, though the difference was not statistically significant.

Table 44. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the agitation type test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	No Agitation	Sonication	Vortexing	Sonication then Vortexing
4-ANPP	2.64 (±0.31)[b]	3.34 (±0.17)[a]	3.31 (±0.20)[a]	2.78 (±0.28)[b]
4'Methylacetylfentanyl HCl	4.88 (±0.67)[c]	6.01 (±0.61)[a]	5.91 (±0.53)[a,b]	5.17 (±0.55)[b,c]
Acetylfentanyl	5.76 (±0.93)[b]	7.17 (±0.53)[a]	7.15 (±0.67)[a]	6.16 (±0.83)[a,b]
Acrylfentanyl HCl	4.46 (±0.58)[b]	5.53 (±0.49)[a]	5.58 (±0.43)[a]	4.67 (±0.60)[b]
Alprazolam	5.41 (±1.04)[b]	6.56 (±0.57)[a]	6.74 (±0.37)[a]	6.02 (±0.51)[a,b]
Benzylfentanyl HCl	5.58 (±0.83)[c]	6.84 (±0.58)[a,b]	6.97 (±0.60)[a]	5.95 (±0.81)[b,c]
(±)-β-Hydroxythiofentanyl HCl	6.05 (±0.90)[c]	7.48 (±0.65)[a]	7.41 (±0.61)[a,b]	6.40 (±0.94)[b,c]
Butyrylfentanyl	5.04 (±0.69)[b]	6.09 (±0.57)[a]	6.28 (±0.57)[a]	5.15 (±0.64)[b]
Carfentanil Oxalate	4.01 (±0.54)[b]	4.93 (±0.46)[a]	4.91 (±0.37)[a]	4.23 (±0.52)[b]
(±)-*cis*-3-Methylfentanyl HCl	4.92 (±0.68)[b]	6.18 (±0.79)[a]	6.28 (±0.40)[a]	5.43 (±0.69)[a,b]
Cocaine	5.76 (±0.93)[c]	7.08 (±0.58)[a]	6.99 (±0.59)[a,b]	6.05 (±0.82)[b,c]
Cyclopropylfentanyl	5.20 (±0.61)[c]	6.41 (±0.82)[a,b]	6.76 (±0.64)[a]	5.57 (±0.71)[b,c]
Fentanyl	5.02 (±0.61)[c]	6.10 (±0.55)[a,b]	6.30 (±0.53)[a]	5.41 (±0.65)[b,c]
Furanylfentanyl HCl	3.85 (±0.54)[c]	4.77 (±0.58)[a,b]	4.82 (±0.44)[a]	4.13 (±0.46)[b,c]
Heroin	6.00 (±1.05)[b]	7.47 (±0.70)[a]	7.29 (±0.53)[a]	6.43 (±0.92)[a,b]
Hydrocodone	5.87 (±1.05)[b]	7.12 (±0.63)[a]	6.97 (±0.76)[a]	6.18 (±0.77)[a,b]
Methoxyacetylfentanyl HCl	5.58 (±0.79)[b]	6.96 (±0.75)[a]	6.86 (±0.57)[a]	6.00 (±0.81)[a,b]
Naloxone	5.74 (±1.06)[b]	7.08 (±0.58)[a]	7.01 (±0.45)[a]	6.31 (±0.96)[a,b]
Oxycodone	6.02 (±1.17)[b]	7.68 (±0.82)[a]	7.58 (±0.63)[a]	6.80 (±0.93)[a,b]
para-Fluorobutyrylfentanyl	3.86 (±0.28)[b]	4.71 (±0.29)[a]	4.40 (±0.35)[a]	3.93 (±0.38)[b]
para-Fluorofentanyl	5.05 (±0.99)[b]	6.19 (±0.76)[a]	6.30 (±0.67)[a]	5.39 (±0.59)[a,b]
Remifentanil HCl	6.04 (±0.92)[b]	7.35 (±0.57)[a]	7.39 (±0.71)[a]	6.42 (±0.77)[a,b]
Sufentanyl	4.07 (±0.60)[b,c]	5.11 (±0.43)[a]	4.41 (±0.27)[b]	3.73 (±0.27)[c]
U-47700	4.25 (±0.66)[b]	5.22 (±0.48)[a]	5.22 (±0.40)[a]	4.51 (±0.42)[b]
U-48800 HCl	4.84 (±0.66)[b]	6.12 (±0.36)[a]	5.68 (±0.47)[a]	4.87 (±0.35)[b]
U-49900	3.83 (±0.66)[b]	4.94 (±0.32)[a]	4.97 (±0.52)[a]	4.23 (±0.33)[b]
Valerylfentanyl HCl	3.06 (±0.49)[b]	3.87 (±0.58)[a]	3.80 (±0.35)[a]	3.18 (±0.38)[b]
Average Extraction Efficiency	4.92 (±0.95)[b]	6.09 (±1.15)[a]	6.05 (±1.19)[a]	5.23 (±1.08)[b]

5.4.1.7 Agitation time selection

The results of the agitation time test are summarized in Table 45. While there was no significant difference in extraction efficiency for any of the 27 compounds of interest at 30 seconds, 5 minutes, and 10 minutes of agitation time, there was a trend of increasing extraction efficiency with increased agitation time.

Table 45. Mean (±SD) extracted concentration (ng/100 cm^2) for each compound of interest from the agitation time test. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Analyte	30 Seconds	5 Minutes	10 Minutes
4-ANPP	3.46 (±1.04)[a]	3.51 (±0.35)[a]	3.41 (±0.26)[a]
4'Methylacetylfentanyl HCl	5.59 (±1.55)[a]	5.72 (±0.49)[a]	5.73 (±0.33)[a]
Acetylfentanyl	7.09 (±2.05)[a]	7.07 (±0.57)[a]	7.18 (±0.36)[a]
Acrylfentanyl HCl	5.45 (±1.50)[a]	5.52 (±0.38)[a]	5.56 (±0.31)[a]
Alprazolam	6.94 (±1.99)[a]	7.11 (±0.64)[a]	7.03 (±0.58)[a]
Benzylfentanyl HCl	6.98 (±2.00)[a]	7.02 (±0.62)[a]	7.06 (±0.49)[a]
(±)-β-Hydroxythiofentanyl HCl	7.52 (±2.27)[a]	7.47 (±0.60)[a]	7.58 (±0.62)[a]
Butyrylfentanyl	5.68 (±1.45)[a]	5.93 (±0.70)[a]	6.18 (±0.40)[a]
Carfentanil Oxalate	4.69 (±1.26)[a]	4.85 (±0.37)[a]	4.93 (±0.35)[a]
(±)-*cis*-3-Methylfentanyl HCl	5.64 (±1.44)[a]	6.32 (±0.74)[a]	6.35 (±0.68)[a]
Cocaine	7.36 (±2.32)[a]	7.24 (±0.70)[a]	7.32 (±0.74)[a]
Cyclopropylfentanyl	5.93 (±1.62)[a]	6.17 (±0.53)[a]	6.37 (±0.43)[a]
Fentanyl	5.93 (±1.76)[a]	5.97 (±0.52)[a]	6.23 (±0.28)[a]
Furanylfentanyl HCl	4.20 (±1.04)[a]	4.50 (±0.45)[a]	4.55 (±0.28)[a]
Heroin	8.00 (±2.69)[a]	7.68 (±0.67)[a]	8.13 (±0.88)[a]
Hydrocodone	7.29 (±2.40)[a]	7.70 (±0.77)[a]	7.52 (±0.76)[a]
Methoxyacetylfentanyl HCl	6.99 (±2.14)[a]	6.97 (±0.60)[a]	7.25 (±0.65)[a]
Naloxone	7.55 (±2.28)[a]	7.66 (±0.66)[a]	7.86 (±0.77)[a]
Oxycodone	7.66 (±2.27)[a]	7.93 (±0.80)[a]	8.25 (±0.96)[a]
para-Fluorobutyrylfentanyl	4.03 (±1.09)[a]	4.14 (±0.34)[a]	4.35 (±0.35)[a]
para-Fluorofentanyl	5.71 (±1.58)[a]	5.83 (±0.58)[a]	5.94 (±0.25)[a]
Remifentanil HCl	7.73 (±2.39)[a]	7.66 (±0.68)[a]	7.84 (±0.74)[a]
Sufentanyl	3.93 (±1.22)[a]	3.76 (±0.43)[a]	3.68 (±0.94)[a]
U-47700	5.30 (±1.45)[a]	5.47 (±0.40)[a]	5.55 (±0.45)[a]
U-48800 HCl	5.96 (±1.66)[a]	5.80 (±0.44)[a]	6.03 (±0.58)[a]
U-49900	5.59 (±1.74)[a]	5.65 (±0.75)[a]	5.76 (±0.49)[a]
Valerylfentanyl HCl	3.48 (±0.94)[a]	3.57 (±0.47)[a]	3.77 (±0.40)[a]
Average Extraction Efficiency	5.99 (±1.38)[a]	6.08 (±1.36)[a]	6.20 (±1.40)[a]

5.4.2 Method validation

5.4.2.1 Calibration model/linearity

During assessment of the calibration model, all analytes were tested with the following calibrator concentrations: 0.05, 0.1, 0.5, 1, 5, 10, 15, and 25 ng/100 cm^2. Based on the results of the test, the reportable range of each analyte spans from the LLOQ of 0.05 ng/100 cm^2 to the ULOQ of 25 ng/100 cm^2. Integration parameters, including peak smoothing, ion ratios and allowances, curve fit and weighting, and R^2 values are summarized in Table 46. The fit type of all analytes was linear with 1/x^2 weighting and all calibration curves had R^2 values of >0.99.

Linearity accuracy and precision results are summarized Table 47 and Table 48, respectively. The calculated concentration of all calibrators fell within ±20% of the expected value and all identification criteria were met for each calibration point. Additionally, precision values were within ±20% for all calibration points; precision values are expressed as percent imprecision, so a value of 0% would represent absolute precision.

Table 46. Calibration model parameters for each compound, including the amount of smoothing applied to the chromatographic peaks, the observed MRM ion ratio and allowance, the curve fit type and weighting, and the R^2 value of the resulting calibration curve.

Analyte	Gaussian Smoothing	Ion Ratio	% Ratio Allowance	Fit Type	Weight	R^2
4-ANPP	2.0	0.97	20	Linear	$1/x^2$	0.999
4'Methylacetylfentanyl HCl	0.5	0.74	20	Linear	$1/x^2$	0.998
Acetylfentanyl	1.0	0.94	20	Linear	$1/x^2$	0.999
Acrylfentanyl HCl	0.5	1.00	20	Linear	$1/x^2$	0.998
Alprazolam	1.0	0.54	20	Linear	$1/x^2$	0.999
Benzylfentanyl HCl	1.0	0.67	20	Linear	$1/x^2$	0.998
(±)-β-Hydroxythiofentanyl HCl	1.0	0.50	20	Linear	$1/x^2$	0.999
Butyrylfentanyl	0.5	0.75	20	Linear	$1/x^2$	0.997
Carfentanil Oxalate	0.5	0.49	20	Linear	$1/x^2$	0.999
(±)-*cis*-3-Methylfentanyl HCl	0.0	0.77	20	Linear	$1/x^2$	0.997
Cocaine	2.0	0.26	20	Linear	$1/x^2$	0.999
Cyclopropylfentanyl	0.5	0.92	20	Linear	$1/x^2$	0.997
Fentanyl	0.5	0.97	20	Linear	$1/x^2$	0.998
Furanylfentanyl HCl	0.5	0.90	20	Linear	$1/x^2$	0.999
Heroin	2.0	0.89	20	Linear	$1/x^2$	0.998
Hydrocodone	2.0	0.37	20	Linear	$1/x^2$	0.999
Methoxyacetylfentanyl HCl	1.0	1.03	20	Linear	$1/x^2$	0.998
Naloxone	3.0	0.16	20	Linear	$1/x^2$	0.999
Oxycodone	2.0	0.18	20	Linear	$1/x^2$	0.998
para-Fluorobutyrylfentanyl	0.5	0.16	20	Linear	$1/x^2$	0.997
para-Fluorofentanyl	0.5	0.96	20	Linear	$1/x^2$	0.998
Remifentanil HCl	2.0	0.63	20	Linear	$1/x^2$	0.999
Sufentanyl	0.5	0.64	20	Linear	$1/x^2$	0.992
U-47700	0.5	0.41	20	Linear	$1/x^2$	0.998
U-48800 HCl	0.5	0.27	20	Linear	$1/x^2$	0.994
U-49900	0.5	0.38	20	Linear	$1/x^2$	0.998
Valerylfentanyl HCl	0.5	0.83	20	Linear	$1/x^2$	0.994

Table 47. Accuracy results for each calibration point. All accuracies are within ±20% of the true values.

	Accuracy														
	25 ng/100 cm^2		15 ng/100 cm^2		10 ng/100 cm^2		5 ng/100 cm^2		1 ng/100 cm^2		0.5 ng/100 cm^2		0.1 ng/100 cm^2		0.05 ng/100 cm^2	
Analyte	Average		Average		Average		Average		Average		Average		Average		Average	
4-ANPP	24.89	Good	15.10	Good	9.94	Good	4.92	Good	1.02	Good	0.50	Good	0.10	Good	0.05	Good
4'Methylacetylfentanyl	24.66	Good	14.95	Good	10.00	Good	4.96	Good	1.03	Good	0.48	Good	0.11	Good	0.05	4'Good
Acetylfentanyl	24.41	Good	14.78	Good	9.92	Good	5.01	Good	1.03	Good	0.49	Good	0.11	Good	0.05	Good
Acrylfentanyl	24.00	Good	14.77	Good	10.08	Good	4.92	Good	1.02	Good	0.51	Good	0.11	Good	0.05	Acrylf
Alprazolam	24.61	Good	14.84	Good	10.07	Good	4.93	Good	1.00	Good	0.50	Good	0.11	Good	0.05	Alprood
Benzylfentanyl	24.01	Good	15.03	Good	9.91	Good	4.98	Good	1.02	Good	0.50	Good	0.11	Good	0.05	Benzyl
(±)-β-Hydroxythiofentanyl	24.63	Good	14.67	Good	10.06	Good	4.92	Good	1.02	Good	0.50	Good	0.11	Good	0.05	(±)ood
Butyrylfentanyl	23.94	Good	14.83	Good	9.69	Good	5.11	Good	1.03	Good	0.50	Good	0.11	Good	0.05	Butood
Carfentanil	24.58	Good	15.03	Good	9.89	Good	4.84	Good	1.04	Good	0.50	Good	0.10	Good	0.05	Carood
(±)-*cis*-3-Methylfentanyl	23.67	Good	14.69	Good	10.22	Good	4.87	Good	1.03	Good	0.50	Good	0.11	Good	0.05	(±)-*cis*
Cocaine	24.53	Good	14.91	Good	9.99	Good	5.01	Good	1.02	Good	0.49	Good	0.11	Good	0.05	Coood
Cyclopropylfentanyl	24.07	Good	14.97	Good	10.14	Good	4.97	Good	1.01	Good	0.50	Good	0.11	Good	0.05	Cyood
Fentanyl	24.56	Good	15.13	Good	10.22	Good	4.84	Good	1.01	Good	0.49	Good	0.11	Good	0.05	Good
Furanylfentanyl	24.28	Good	14.69	Good	9.97	Good	4.97	Good	1.01	Good	0.50	Good	0.11	Good	0.05	Furood
Heroin	23.72	Good	14.85	Good	9.95	Good	4.88	Good	1.04	Good	0.52	Good	0.10	Good	0.05	Heroin
Hydrocodone	24.47	Good	14.96	Good	9.99	Good	4.91	Good	1.01	Good	0.49	Good	0.11	Good	0.05	Hyood
Methoxyacetylfentanyl	24.13	Good	15.02	Good	10.06	Good	4.98	Good	1.02	Good	0.50	Good	0.10	Good	0.05	Good
Naloxone	24.45	Good	14.84	Good	9.87	Good	5.02	Good	1.02	Good	0.50	Good	0.10	Good	0.05	Naood
Oxycodone	24.31	Good	14.95	Good	10.00	Good	5.01	Good	1.01	Good	0.50	Good	0.11	Good	0.05	Good
para-Fluorobutyrylfentanyl	24.07	Good	15.30	Good	9.73	Good	5.00	Good	1.04	Good	0.48	Good	0.11	Good	0.05	*para*ood
para-Fluorofentanyl	23.98	Good	14.85	Good	10.36	Good	4.86	Good	1.04	Good	0.50	Good	0.10	Good	0.05	*para*ood
Remifentanil	24.52	Good	14.99	Good	10.18	Good	4.97	Good	1.01	Good	0.49	Good	0.10	Good	0.05	Good
Sufentanyl	24.30	Good	14.68	Good	9.76	Good	5.02	Good	1.13	Good	0.47	Good	0.11	Good	0.05	Suood
U-47700	24.90	Good	14.95	Good	10.19	Good	4.91	Good	1.01	Good	0.51	Good	0.10	Good	0.05	Good
U-48800	24.84	Good	14.52	Good	9.62	Good	5.01	Good	1.11	Good	0.46	Good	0.11	Good	0.05	Good
U-49900	25.03	Good	14.60	Good	10.04	Good	4.99	Good	1.01	Good	0.49	Good	0.11	Good	0.05	Good
Valerylfentanyl	23.75	Good	15.33	Good	9.53	Good	4.90	Good	1.10	Good	0.49	Good	0.11	Good	0.05	Good

Table 48. Precision results for each calibration point. Results reported as percent imprecision. All precision values are within ±20% of the true values.

	Precision														
	25 ng/100 cm^2		15 ng/100 cm^2		10 ng/100 cm^2		5 ng/100 cm^2		1 ng/100 cm^2		0.5 ng/100 cm^2		0.1 ng/100 cm^2		0.05 ng/100 cm^2	
Analyte	Average		Average		Average		Average		Average		Average		Average		Average	
4-ANPP	4.13%	Good	1.57%	Good	3.15%	Good	3.48%	Good	4.51%	Good	3.69%	Good	4.89%	Good	2.24%	Good
4'Methylacetylfentanyl	5.04%	Good	2.96%	Good	6.06%	Good	4.91%	Good	6.16%	Good	6.83%	Good	9.40%	Good	5.39%	Good
Acetylfentanyl	5.03%	Good	3.88%	Good	3.92%	Good	5.24%	Good	6.07%	Good	6.03%	Good	4.31%	Good	1.55%	Good
Acrylfentanyl	4.47%	Good	2.42%	Good	5.62%	Good	5.84%	Good	5.72%	Good	3.13%	Good	5.06%	Good	2.34%	Good
Alprazolam	4.76%	Good	2.00%	Good	3.25%	Good	4.61%	Good	7.16%	Good	5.07%	Good	6.26%	Good	2.95%	Good
Benzylfentanyl	4.49%	Good	3.44%	Good	3.93%	Good	5.09%	Good	6.15%	Good	3.70%	Good	6.59%	Good	3.48%	Good
(±)-β-Hydroxythiofentanyl	5.23%	Good	2.71%	Good	2.11%	Good	4.14%	Good	5.97%	Good	4.98%	Good	3.62%	Good	1.70%	Good
Butyrylfentanyl	7.51%	Good	3.11%	Good	4.32%	Good	6.21%	Good	7.67%	Good	8.54%	Good	7.17%	Good	3.27%	Good
Carfentanil	5.06%	Good	3.48%	Good	4.21%	Good	3.54%	Good	7.37%	Good	3.73%	Good	6.55%	Good	4.18%	Good
(±)-cis-3-Methylfentanyl	5.83%	Good	4.04%	Good	5.77%	Good	9.07%	Good	6.43%	Good	8.06%	Good	4.56%	Good	2.38%	Good
Cocaine	3.64%	Good	3.71%	Good	2.75%	Good	3.45%	Good	5.69%	Good	4.93%	Good	10.56%	Good	5.13%	Good
Cyclopropylfentanyl	7.38%	Good	3.62%	Good	3.45%	Good	4.05%	Good	5.50%	Good	7.56%	Good	6.37%	Good	2.63%	Good
Fentanyl	4.85%	Good	2.45%	Good	3.73%	Good	3.84%	Good	5.44%	Good	4.40%	Good	6.06%	Good	2.83%	Good
Furanylfentanyl	6.06%	Good	3.41%	Good	1.70%	Good	4.36%	Good	6.45%	Good	5.62%	Good	6.68%	Good	3.61%	Good
Heroin	4.06%	Good	2.97%	Good	3.69%	Good	5.86%	Good	7.76%	Good	5.04%	Good	9.62%	Good	4.71%	Good
Hydrocodone	4.78%	Good	2.31%	Good	4.07%	Good	3.86%	Good	7.12%	Good	3.87%	Good	5.36%	Good	3.14%	Good
Methoxyacetylfentanyl	5.34%	Good	2.80%	Good	1.90%	Good	3.96%	Good	6.01%	Good	4.36%	Good	4.82%	Good	2.26%	Good
Naloxone	5.23%	Good	1.14%	Good	4.42%	Good	3.23%	Good	6.91%	Good	4.61%	Good	4.09%	Good	2.28%	Good
Oxycodone	6.19%	Good	2.79%	Good	3.26%	Good	3.77%	Good	4.06%	Good	4.34%	Good	7.93%	Good	11.09%	Good
p-Fluorobutyrylfentanyl	6.39%	Good	3.64%	Good	3.03%	Good	5.07%	Good	4.10%	Good	9.94%	Good	12.06%	Good	6.13%	Good
p-Fluorofentanyl	5.66%	Good	3.58%	Good	4.58%	Good	5.36%	Good	7.52%	Good	5.38%	Good	7.04%	Good	3.80%	Good
Remifentanil	4.53%	Good	2.01%	Good	3.14%	Good	3.43%	Good	4.38%	Good	2.93%	Good	6.03%	Good	3.25%	Good
Sufentanyl	7.20%	Good	11.13%	Good	5.33%	Good	11.02%	Good	8.64%	Good	10.35%	Good	8.45%	Good	4.06%	Good
U-47700	5.51%	Good	2.33%	Good	5.08%	Good	5.37%	Good	4.20%	Good	3.04%	Good	5.71%	Good	2.88%	Good
U-48800	10.46%	Good	6.52%	Good	8.11%	Good	10.11%	Good	7.63%	Good	5.96%	Good	6.27%	Good	3.29%	Good
U-49900	7.10%	Good	2.54%	Good	2.96%	Good	4.44%	Good	9.18%	Good	5.98%	Good	9.40%	Good	5.17%	Good
Valerylfentanyl	5.09%	Good	9.02%	Good	4.80%	Good	5.46%	Good	12.12%	Good	4.65%	Good	7.06%	Good	6.18%	Good

5.4.2.2 Limit of detection/lower limit of quantitation

LOD/LLOQ values are summarized in Table 49. For all 27 analytes, the 0.05 ng/100 cm^2 calibration point met identification criteria during the 9 replicates performed over 3 days. The signal-to-noise ratio observed for all analytes was above 5 and peaks could be easily differentiated and integrated when compared to the instrumental background.

Table 49. Average calculated concentration (ng/100 cm^2) and peak area for each compound at the LOD/LLOQ.

Analyte	0.05 ng/100 cm^2	
	Average Concentration	**Average Peak Area**
4-ANPP	0.05	3325
4'Methylacetylfentanyl HCl	0.05	4585
Acetylfentanyl	0.05	3475
Acrylfentanyl HCl	0.05	3631
Alprazolam	0.05	2805
Benzylfentanyl HCl	0.05	5641
(±)-β-Hydroxythiofentanyl HCl	0.05	2361
Butyrylfentanyl	0.05	3514
Carfentanil Oxalate	0.05	2387
(±)-*cis*-3-Methylfentanyl HCl	0.05	3206
Cocaine	0.05	6721
Cyclopropylfentanyl	0.05	3205
Fentanyl	0.05	4191
Furanylfentanyl HCl	0.05	3173
Heroin	0.05	298
Hydrocodone	0.05	680
Methoxyacetylfentanyl HCl	0.05	2924
Naloxone	0.05	1206
Oxycodone	0.05	937
para-Fluorobutyrylfentanyl	0.05	2076
para-Fluorofentanyl	0.05	3139
Remifentanil HCl	0.05	1602
Sufentanyl	0.05	3905
U-47700	0.05	3779
U-48800 HCl	0.05	5583
U-49900	0.05	3001
Valerylfentanyl HCl	0.05	4043

5.4.2.3 Accuracy and precision

Accuracy and precision of the methodology was assessed with the use of two quality control (QC) samples. The concentration of QC A was approaching the ULOQ and the

concentration of QC B was approaching the LLOQ, thus assessing accuracy and precision at both

end of the calibration range. The results of the accuracy test are summarized in Table 50. All

accuracy values fell within 80-120%. Precision results are summarized in Table 51. Precision is

reported as the percent of imprecision, so a value of 0% means the method always reported the

true concentration. All precision values fell within ±20%.

Table 50. Accuracy results for the high-concentration and low concentration quality control samples. Overall accuracy is the average observed accuracy for the compounds. All accuracies are within ±20% of the true values.

	Accuracy		
Analyte	QC A	QC B	Overall
4-ANPP	91%	96%	94%
4'Methylacetylfentanyl	92%	100%	96%
Acetylfentanyl	88%	93%	91%
Acrylfentanyl	90%	95%	92%
Alprazolam	93%	100%	97%
Benzylfentanyl	88%	96%	92%
(±)-β-Hydroxythiofentanyl	86%	91%	88%
Butyrylfentanyl	91%	98%	95%
Carfentanil	102%	110%	106%
(±)-*cis*-3-Methylfentanyl	95%	103%	99%
Cocaine	98%	106%	102%
Cyclopropylfentanyl	90%	98%	94%
Fentanyl	89%	97%	93%
Furanylfentanyl	90%	96%	93%
Heroin	102%	109%	105%
Hydrocodone	104%	113%	109%
Methoxyacetylfentanyl	102%	109%	106%
Naloxone	109%	117%	113%
Oxycodone	105%	112%	108%
para-Fluorobutyrylfentanyl	101%	107%	104%
para-Fluorofentanyl	85%	91%	88%
Remifentanil	101%	108%	105%
Sufentanyl	100%	106%	103%
U-47700	106%	114%	110%
U-48800	93%	100%	96%
U-49900	95%	101%	98%
Valerylfentanyl	100%	108%	104%

Table 51. Precision results for the high-concentration and low concentration quality control samples. Results reported as percent imprecision. All precision values are within ±20% of the true values.

Analyte	Within-Run Precision		Between Run Precision		Intraday Precision		Interday Precision		Within-Laboratory Precision	
	QCA	QCB	QCA	QCB	QCA	QCB	QCA	QCB	QCA	QCB
4-ANPP	4.76%	4.88%	5.94%	3.72%	4.89%	1.38%	1.89%	4.92%	7.08%	7.07%
4'Methylacetylfentanyl	5.65%	5.92%	4.36%	2.97%	1.75%	2.95%	4.63%	6.96%	7.51%	8.65%
Acetylfentanyl	4.36%	6.08%	0.06%	0.05%	5.67%	1.58%	0.80%	4.70%	7.11%	7.85%
Acrylfentanyl	4.52%	7.06%	0.06%	0.04%	4.52%	2.27%	3.68%	6.44%	7.38%	9.28%
Alprazolam	5.32%	6.56%	4.93%	5.19%	3.18%	2.34%	2.67%	5.17%	6.75%	8.67%
Benzylfentanyl	4.68%	6.42%	4.51%	3.45%	3.07%	2.96%	3.92%	7.35%	6.83%	9.30%
(±)-β-Hydroxythiofentanyl	4.49%	6.27%	4.92%	5.15%	3.75%	2.62%	3.43%	2.70%	6.78%	7.31%
Butyrylfentanyl	6.74%	9.22%	0.10%	0.07%	8.64%	1.87%	5.53%	5.12%	9.46%	10.71%
Carfentanil	4.78%	6.99%	0.07%	0.06%	6.38%	2.43%	3.53%	5.82%	7.14%	9.42%
(±)-*cis*-3-Methylfentanyl	6.01%	7.56%	4.25%	9.12%	0.13%	7.39%	7.75%	5.73%	9.81%	12.02%
Cocaine	5.40%	7.07%	4.52%	5.06%	2.43%	0.77%	3.76%	5.13%	7.01%	8.77%
Cyclopropylfentanyl	6.20%	6.00%	4.18%	6.74%	1.34%	5.24%	8.24%	2.51%	10.22%	8.35%
Fentanyl	3.95%	6.01%	0.05%	0.05%	3.96%	3.44%	2.32%	6.86%	6.06%	9.75%
Furanylfentanyl	5.34%	6.37%	0.05%	0.04%	2.90%	2.56%	3.36%	5.40%	6.95%	7.94%
Heroin	6.57%	8.74%	7.51%	6.39%	5.90%	1.60%	3.37%	2.92%	9.45%	9.35%
Hydrocodone	6.19%	5.67%	7.21%	5.20%	5.73%	3.31%	3.52%	5.77%	9.14%	8.74%
Methoxyacetylfentanyl	5.85%	6.77%	4.79%	5.94%	2.42%	3.51%	4.63%	4.90%	7.84%	9.06%
Naloxone	4.82%	7.18%	0.05%	0.04%	4.17%	3.51%	4.58%	6.08%	7.85%	8.72%
Oxycodone	2.72%	7.99%	0.06%	0.05%	6.12%	2.04%	1.73%	5.35%	6.92%	9.40%
para-Fluorobutyrylfentanyl	7.72%	7.89%	10.75%	8.20%	9.26%	6.00%	4.90%	5.36%	11.02%	11.27%
para-Fluorofentanyl	5.18%	7.10%	4.68%	3.69%	2.92%	3.41%	2.86%	6.85%	6.59%	9.26%
Remifentanil	5.70%	6.33%	5.26%	3.77%	3.38%	2.41%	5.67%	7.94%	8.72%	9.86%
Sufentanyl	18.17%	19.38%	0.17%	0.14%	11.43%	1.14%	8.26%	1.39%	19.82%	19.30%
U-47700	4.51%	6.52%	0.04%	0.05%	3.00%	2.54%	4.72%	7.49%	7.18%	10.25%
U-48800	10.32%	16.62%	13.53%	10.17%	11.39%	5.89%	8.87%	4.58%	12.55%	14.85%
U-49900	4.77%	6.41%	6.00%	2.30%	4.95%	3.90%	2.15%	4.73%	7.21%	6.94%
Valerylfentanyl	8.09%	12.00%	14.03%	11.63%	12.81%	7.96%	9.76%	7.05%	11.60%	12.55%

5.4.2.4 Carryover

As shown in Table 52, there was no carryover detected when a sample spiked at 10x the ULOQ was repeatedly injected followed by blanks. These results show the method is considered free of carryover, although blanks will still be included in the analysis for quality control purposes. This is especially important when running environmental samples, as the concentration of these samples could range in the mg/100 cm^2, a concentration in which carryover would most definitely be observed.

Table 52. Observed carryover in blanks following injections of a sample spiked at 250 ng/100 cm^2.

Analyte	Average Peak Area %*
4-ANPP	0.00
4'Methylacetylfentanyl HCl	0.00
Acetylfentanyl	0.00
Acrylfentanyl HCl	0.00
Alprazolam	0.00
Benzylfentanyl HCl	0.00
(±)-β-Hydroxythiofentanyl HCl	0.00
Butyrylfentanyl	0.00
Carfentanil Oxalate	0.00
(±)-*cis*-3-Methylfentanyl HCl	0.00
Cocaine	0.00
Cyclopropylfentanyl	0.00
Fentanyl	0.00
Furanylfentanyl HCl	0.00
Heroin	0.00
Hydrocodone	0.00
Methoxyacetylfentanyl HCl	0.00
Naloxone	0.00
Oxycodone	0.00
para-Fluorobutyrylfentanyl	0.00
para-Fluorofentanyl	0.00
Remifentanil HCl	0.00
Sufentanyl	0.00
U-47700	0.00
U-48800 HCl	0.00
U-49900	0.00
Valerylfentanyl HCl	0.00

*Peak area of blank compared to peak area of LOD/LLOQ

5.4.2.5 Matrix effects, recovery efficiency, and process efficiency

      ME, RE, and PE results are summarized in Table 53. While the ME allowance set for this research was ±50%, only one analyte fell outside a range of ±30%, therefore the ME observed during this methodology was deemed acceptable. For RE, 23 of the 27 compounds being analyzed for fell below the goal of 50% recovery. Although not ideal, only 3 analytes fell below 40% and none had less than 30% recovery. With the limit of detection established at 0.05 ng/100 cm^2 for all analytes, a 30% recovery was deemed acceptable as the method still provided adequate sensitivity to these low-recovery compounds. For PE, all but 5 analytes exceeded the goal of 50% PE, and all but 1 exceeded 40% PE, which was deemed acceptable, as the sensitivity of the assay still exceeds toxicologically relevant surface drug levels, even for analytes with lower PE.

Table 53. Calculated ME, RE, and PE of the method. All values reported as percent of spiked analyte.

Analyte	Matrix Effect	Recovery Efficiency	Process Efficiency
4-ANPP	118.48	39.13	46.36
4'Methylacetylfentanyl HCl	119.51	49.36	59.00
Acetylfentanyl	121.34	56.64	68.73
Acrylfentanyl HCl	123.17	47.52	58.54
Alprazolam	122.37	55.57	68.00
Benzylfentanyl HCl	124.36	55.69	69.26
(±)-β-Hydroxythiofentanyl HCl	124.38	59.91	74.51
Butyrylfentanyl	129.47	42.59	55.14
Carfentanil Oxalate	115.90	47.04	54.52
(±)-*cis*-3-Methylfentanyl HCl	126.09	44.96	56.68
Cocaine	129.36	64.83	83.86
Cyclopropylfentanyl	130.08	43.42	56.48
Fentanyl	123.21	49.63	61.15
Furanylfentanyl HCl	119.27	41.30	49.26
Heroin	121.32	68.28	82.84
Hydrocodone	118.45	71.21	84.35
Methoxyacetylfentanyl HCl	125.88	60.82	76.56
Naloxone	120.22	72.18	86.77
Oxycodone	122.01	73.30	89.44
para-Fluorobutyrylfentanyl	120.78	37.75	45.60
para-Fluorofentanyl	123.20	46.05	56.73
Remifentanil HCl	122.92	66.68	81.97
Sufentanyl	114.29	46.23	52.84
U-47700	123.35	52.57	64.85
U-48800 HCl	114.78	53.57	61.49
U-49900	118.57	53.65	63.62
Valerylfentanyl HCl	114.31	34.53	39.47

5.4.2.6 Interference

Results of the interference study are shown in Table 54. All but one compound passed the interference study with a mean calculated concentration of ±25%; the presence of one of the interference test compounds caused suppression of hydrocodone at levels of greater than 65%. However, because hydrocodone has an overall high extraction efficiency, it was still detected at levels similar to those of compounds with low recovery efficiency that were not highly impacted by the presence of the interferents so the suppression was deemed acceptable. Additionally, no false positives were observed in the samples containing only the interference mix.

Table 54. Results of the interference study. Percent difference is calculated by dividing the "with interferent" concentration by the "without interferent" concentration, multiplying by 100, and then subtracting that percentage from 100. Negative values depict ion enhancement occurred.

| Analyte | Mean Calculated Concentration (ng/100 cm^2) | | |
	Without Interferents	With Interferents	Percent Difference
4-ANPP	3.84	3.53	8.15
4'Methylacetylfentanyl HCl	8.29	7.86	5.18
Acetylfentanyl	8.11	7.97	1.69
Acrylfentanyl HCl	7.02	6.74	3.99
Alprazolam	8.09	7.92	2.11
Benzylfentanyl HCl	7.37	7.25	1.59
(±)-β-Hydroxythiofentanyl HCl	9.24	9.36	-1.27
Butyrylfentanyl	5.95	5.76	3.21
Carfentanil Oxalate	6.59	6.32	4.08
(±)-*cis*-3-Methylfentanyl HCl	7.66	7.47	2.51
Cocaine	8.61	9.22	-7.03
Cyclopropylfentanyl	6.59	6.26	5.02
Fentanyl	7.49	7.24	3.36
Furanylfentanyl HCl	5.81	5.51	5.17
Heroin	9.75	10.59	-8.63
Hydrocodone	10.12	3.29	67.48
Methoxyacetylfentanyl HCl	8.97	8.83	1.54
Naloxone	10.99	10.38	5.60
Oxycodone	12.62	14.19	-12.42
para-Fluorobutyrylfentanyl	5.92	5.05	14.56
para-Fluorofentanyl	5.82	5.53	4.95
Remifentanil HCl	10.02	10.35	-3.21
Sufentanyl	7.34	6.30	14.13
U-47700	8.47	8.31	1.83
U-48800 HCl	8.09	7.87	2.68
U-49900	7.31	7.19	1.59
Valerylfentanyl HCl	5.26	4.75	9.70

5.4.2.7 Stability

Stabilities of the 27 compounds while captured on the wipe are summarized in Table 55. While on the wipe, all compounds showed stability over the entire time course of the study. Stabilities of the 27 compounds following extraction are summarized in Table 56. Following extraction, all compounds but sufentanyl showed stability over the entire time course of the study; sufentanyl was only stable for 12 hours when stored in the autosampler and 36 hours when stored in the refrigerator.

Table 55. Compound stability on the wipe following surface swabbing. Stability was assessed while wipes were stored at room temperature (20ºC), refrigerator (4ºC), freezer (-20ºC), and after shipping. Stability reported in hours.

Analyte	Room Temp	Refrigerator	Freezer	Shipped
4-ANPP	48	72	144	120
4'Methylacetylfentanyl HCl	48	72	144	120
Acetylfentanyl	48	72	144	120
Acrylfentanyl HCl	48	72	144	120
Alprazolam	48	72	144	120
Benzylfentanyl HCl	48	72	144	120
(±)-β-Hydroxythiofentanyl HCl	48	72	144	120
Butyrylfentanyl	48	72	144	120
Carfentanil Oxalate	48	72	144	120
(±)-*cis*-3-Methylfentanyl HCl	48	72	144	120
Cocaine	48	72	144	120
Cyclopropylfentanyl	48	72	144	120
Fentanyl	48	72	144	120
Furanylfentanyl HCl	48	72	144	120
Heroin	48	72	144	120
Hydrocodone	48	72	144	120
Methoxyacetylfentanyl HCl	48	72	144	120
Naloxone	48	72	144	120
Oxycodone	48	72	144	120
para-Fluorobutyrylfentanyl	48	72	144	120
para-Fluorofentanyl	48	72	144	120
Remifentanil HCl	48	72	144	120
Sufentanyl	48	72	144	120
U-47700	48	72	144	120
U-48800 HCl	48	72	144	120
U-49900	48	72	144	120
Valerylfentanyl HCl	48	72	144	120

Table 56. Compound stability following extraction. Stability was assessed while samples were stored in the LC autosampler (4°C), refrigerator (4°C), and freezer (-20°C). Stability reported in hours.

Analyte	Autosampler	Refrigerator	Freezer
4-ANPP	24	72	144
4'Methylacetylfentanyl HCl	24	72	144
Acetylfentanyl	24	72	144
Acrylfentanyl HCl	24	72	144
Alprazolam	24	72	144
Benzylfentanyl HCl	24	72	144
(±)-β-Hydroxythiofentanyl HCl	24	72	144
Butyrylfentanyl	24	72	144
Carfentanil Oxalate	24	72	144
(±)-*cis*-3-Methylfentanyl HCl	24	72	144
Cocaine	24	72	144
Cyclopropylfentanyl	24	72	144
Fentanyl	24	72	144
Furanylfentanyl HCl	24	72	144
Heroin	24	72	144
Hydrocodone	24	72	144
Methoxyacetylfentanyl HCl	24	72	144
Naloxone	24	72	144
Oxycodone	24	72	144
para-Fluorobutyrylfentanyl	24	72	144
para-Fluorofentanyl	24	72	144
Remifentanil HCl	24	72	144
Sufentanyl	12	36	144
U-47700	24	72	144
U-48800 HCl	24	72	144
U-49900	24	72	144
Valerylfentanyl HCl	24	72	144

5.4.3 Assessment of multi-surface extraction efficiencies

The amount of drug recovery following the swabbing of the 11 household surfaces is summarized in Table 57. For all results, the means and standard deviations have units of ng/100 cm^2. Additionally, results within the same row that are denoted with the same superscript letter are not significantly different at α=0.05. The average amount drug recovered from each surface material is summarized in the last row of the results table.

Table 57. Mean (±SD) recovered drug amount (ng/100 cm^2) for each compound of interest from each surface type. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Compound	Lab Bench	Marble Tile	Painted Drywall	Stainless Steel	Vinyl Tile	Plywood
4-ANPP	2.57 (±0.38)[b]	1.30 (±0.54)[c,d]	0.43 (±0.09)[e,f]	4.12 (±1.39)[a]	3.61 (±0.99)[a]	0.26 (±0.07)[e,f]
4'Methylacetylfentanyl HCl	4.63 (±0.34)[c]	2.35 (±0.83)[e]	1.10 (±0.16)[f]	7.05 (±1.05)[a]	5.98 (±0.69)[b]	0.29 (±0.08)[f,g]
Acetylfentanyl	5.08 (±0.30)[c]	2.12 (±0.93)[f]	1.25 (±0.14)[g]	7.07 (±0.66)[a]	6.26 (±0.63)[b]	0.30 (±0.10)[h]
Acrylfentanyl HCl	3.81 (±0.32)[b]	2.04 (±0.76)[d,e]	0.89 (±0.13)[f]	5.96 (±1.13)[a]	5.35 (±0.84)[a]	0.29 (±0.07)[f]
Alprazolam	5.61 (±0.22)[c]	3.23 (±0.77)[d]	1.72 (±0.16)[e]	7.44 (±0.74)[b]	6.88 (±0.76)[b]	0.33 (±0.08)[g]
Benzylfentanyl HCl	5.01 (±0.38)[c]	2.25 (±0.92)[e]	0.97 (±0.20)[f]	7.02 (±1.20)[a]	6.05 (±0.70)[b]	0.29 (±0.07)[f]
(±)-β-Hydroxythiofentanyl HCl	5.31 (±0.39)[b]	2.04 (±1.04)[d,e]	1.37 (±0.19)[e,f]	6.95 (±0.94)[a]	6.18 (±0.48)[a]	0.25 (±0.09)[g,h]
Butyrylfentanyl	4.26 (±0.33)[c]	2.40 (±0.63)[e,f]	0.88 (±0.16)[g]	6.69 (±1.02)[a]	5.88 (±0.82)[b]	0.30 (±0.07)[g]
Carfentanil Oxalate	4.70 (±0.30)[c]	2.65 (±0.79)[d]	1.05 (±0.20)[e]	7.64 (±0.84)[a]	6.68 (±0.63)[b]	0.36 (±0.12)[e,f]
(±)-*cis*-3-Methylfentanyl HCl	4.75 (±0.39)[b]	2.20 (±0.72)[c]	0.88 (±0.18)[d,e]	7.87 (±1.51)[a]	5.50 (±0.89)[b]	0.33 (±0.09)[e]
Cocaine	6.94 (±0.51)[b]	2.91 (±1.15)[e]	2.05 (±0.17)[e,f]	8.85 (±1.10)[a]	8.33 (±0.68)[a]	0.44 (±0.14)[g,h]
Cyclopropylfentanyl	4.41 (±0.51)[c]	2.45 (±0.60)[e,f]	0.88 (±0.15)[g]	7.38 (±0.67)[a]	6.40 (±0.66)[b]	0.32 (±0.10)[g]
Fentanyl	4.51 (±0.31)[c]	2.22 (±0.79)[e,f]	0.98 (±0.16)[g]	6.92 (±0.89)[a]	6.04 (±0.71)[b]	0.28 (±0.08)[g,h]
Furanylfentanyl HCl	3.31 (±0.24)[b]	2.13 (±0.73)[c]	0.76 (±0.15)[d,e]	6.00 (±1.05)[a]	5.18 (±0.91)[a]	0.28 (±0.07)[e]
Heroin	7.32 (±0.49)[b]	2.84 (±1.24)[d,e]	1.87 (±0.39)[e,f]	8.85 (±1.79)[a]	7.35 (±0.94)[b]	0.40 (±0.14)[g]
Hydrocodone	7.58 (±0.90)[b]	2.76 (±1.52)[c,d]	2.26 (±0.28)[d,e]	9.16 (±1.23)[a]	7.80 (±0.69)[b]	0.46 (±0.15)[f]
Methoxyacetylfentanyl HCl	6.54 (±0.56)[b,c]	2.55 (±0.98)[e,f]	2.04 (±0.31)[f,g]	8.87 (±1.33)[a]	7.43 (±0.82)[b]	0.36 (±0.08)[h]
Naloxone	7.69 (±0.56)[a,b]	1.75 (±1.00)[e]	2.61 (±0.28)[d,e]	8.27 (±1.44)[a]	6.61 (±0.85)[b]	0.44 (±0.14)[f]
Oxycodone	9.30 (±1.05)[a,b]	3.57 (±1.76)[d]	3.28 (±0.27)[d]	10.78 (±1.75)[a]	9.74 (±0.82)[a,b]	0.00 (±0.00)[e]
para-Fluorobutyrylfentanyl	3.73 (±0.32)[c]	2.47 (±0.77)[d]	0.79 (±0.12)[e,f]	6.61 (±1.01)[a]	5.78 (±0.67)[b]	0.28 (±0.08)[f]
para-Fluorofentanyl	3.82 (±0.33)[c]	2.41 (±0.81)[e]	0.89 (±0.17)[g]	6.65 (±0.24)[a]	5.70 (±0.51)[b]	0.25 (±0.06)[h]
Remifentanil HCl	6.98 (±0.78)[b]	2.41 (±1.27)[e]	2.27 (±0.22)[e]	8.35 (±1.33)[a]	7.19 (±0.78)[b]	0.38 (±0.12)[f]
Sufentanyl	5.31 (±1.76)[b]	2.25 (±0.71)[c,d]	0.74 (±0.10)[d,e]	7.37 (±2.83)[a]	5.86 (±2.02)[a,b]	0.22 (±0.07)[e]
U-47700	5.27 (±0.60)[b]	2.10 (±0.90)[c,d]	1.30 (±0.29)[d]	7.60 (±1.00)[a]	5.41 (±1.02)[b]	0.36 (±0.11)[e]
U-48800 HCl	5.63 (±1.04)[b]	2.29 (±0.70)[c,d,e]	1.22 (±0.14)[e,f,g]	7.48 (±2.27)[a]	5.60 (±1.32)[b]	0.26 (±0.09)[g]
U-49900	3.42 (±1.01)[b]	1.27 (±0.79)[c,d]	0.70 (±0.26)[d,e,f]	4.77 (±1.50)[a]	3.17 (±1.14)[b]	0.31 (±0.09)[e,f]
Valerylfentanyl HCl	3.42 (±0.34)[b]	2.36 (±0.63)[c]	0.74 (±0.15)[d,e]	6.01 (±1.27)[a]	5.22 (±0.82)[a]	0.30 (±0.08)[e]
Average Recovery	5.22 (±1.61)[c]	2.35 (±0.49)[f]	1.33 (±0.69)[g]	7.32 (±1.37)[a]	6.19 (±1.32)[b]	0.31 (±0.09)[h]

Table 57 Continued. Mean (±SD) recovered drug amount (ng/100 cm^2) for each compound of interest from each surface type. Results within the same row denoted with the same superscript letter are not significantly different at α=0.05.

Compound	Glass	Laminate	Oak Flooring	Concrete	HDPE
4-ANPP	0.85 (±0.51)^{d,e}	0.34 (±0.05)^{e,f}	1.00 (±0.32)^{d,e}	0.02 (±0.05)^{f}	2.04 (±0.38)^{b,c}
4'Methylacetylfentanyl HCl	2.85 (±1.36)^{d,e}	0.59 (±0.14)^{f,g}	2.07 (±0.48)^{e}	0.13 (±0.10)^{g}	3.36 (±0.58)^{d}
Acetylfentanyl	2.94 (±1.29)^{e}	0.66 (±0.15)^{g,h}	2.43 (±0.54)^{e,f}	0.11 (±0.09)^{h}	4.24 (±0.55)^{d}
Acrylfentanyl HCl	2.73 (±1.27)^{c,d}	0.55 (±0.16)^{f}	1.74 (±0.38)^{e}	0.13 (±0.09)^{f}	3.19 (±0.52)^{b,c}
Alprazolam	8.49 (±0.54)^{a}	1.05 (±0.14)^{f}	2.04 (±0.26)^{e}	0.25 (±0.09)^{g}	6.09 (±0.58)^{c}
Benzylfentanyl HCl	3.54 (±1.58)^{d}	0.74 (±0.28)^{f}	2.57 (±0.62)^{e}	0.15 (±0.10)^{f}	3.12 (±0.57)^{d,e}
(±)-β-Hydroxythiofentanyl HCl	3.85 (±1.42)^{c}	1.01 (±0.24)^{f,g}	2.73 (±0.57)^{d}	0.12 (±0.11)^{h}	5.06 (±0.56)^{b}
Butyrylfentanyl	2.82 (±1.19)^{d,e}	0.56 (±0.14)^{g}	1.83 (±0.46)^{f}	0.14 (±0.09)^{g}	3.20 (±0.52)^{d}
Carfentanil Oxalate	3.93 (±1.45)^{c}	0.82 (±0.23)^{e,f}	2.08 (±0.56)^{d}	0.17 (±0.11)^{f}	4.24 (±0.72)^{c}
(±)-cis-3-Methylfentanyl HCl	1.80 (±1.15)^{c,d}	0.34 (±0.14)^{e}	2.48 (±0.50)^{c}	0.11 (±0.08)^{e}	2.73 (±0.73)^{c}
Cocaine	8.88 (±0.81)^{a}	1.22 (±0.17)^{f,g}	3.87 (±0.90)^{d}	0.23 (±0.07)^{h}	4.81 (±0.70)^{c}
Cyclopropylfentanyl	3.16 (±1.34)^{d,e}	0.56 (±0.11)^{g}	1.81 (±0.45)^{f}	0.13 (±0.08)^{g}	3.61 (±0.64)^{d}
Fentanyl	2.85 (±1.27)^{d,e}	0.57 (±0.16)^{g,h}	2.02 (±0.58)^{f}	0.12 (±0.09)^{h}	3.39 (±0.64)^{d}
Furanylfentanyl HCl	2.88 (±1.22)^{b,c}	0.49 (±0.13)^{d,e}	1.19 (±0.34)^{d}	0.13 (±0.10)^{e}	3.32 (±0.61)^{b}
Heroin	4.29 (±2.36)^{c}	1.00 (±0.27)^{f,g}	3.62 (±1.02)^{c,d}	0.20 (±0.12)^{g}	6.34 (±0.55)^{b}
Hydrocodone	3.85 (±2.27)^{c}	1.01 (±0.18)^{e,f}	3.75 (±0.94)^{c}	0.19 (±0.08)^{f}	7.24 (±0.76)^{b}
Methoxyacetylfentanyl HCl	3.92 (±1.90)^{d}	0.93 (±0.23)^{g,h}	3.50 (±0.83)^{d,e}	0.14 (±0.09)^{h}	5.94 (±0.70)^{c}
Naloxone	3.29 (±2.03)^{d}	1.65 (±0.40)^{e}	4.82 (±0.87)^{c}	0.00 (±0.00)^{f}	7.18 (±0.63)^{a,b}
Oxycodone	6.21 (±2.46)^{c}	0.00 (±0.00)^{e}	5.62 (±1.24)^{c}	0.00 (±0.00)^{e}	8.66 (±1.12)^{b}
$para$-Fluorobutyrylfentanyl	2.88 (±1.29)^{d}	0.52 (±0.10)^{e,f}	1.20 (±0.42)^{e}	0.11 (±0.08)^{f}	2.96 (±0.56)^{c,d}
$para$-Fluorofentanyl	2.82 (±1.12)^{d,e}	0.46 (±0.12)^{g,h}	1.61 (±0.33)^{f}	0.11 (±0.07)^{h}	3.25 (±0.50)^{c,d}
Remifentanil HCl	5.47 (±1.49)^{c}	1.38 (±0.42)^{e,f}	4.23 (±0.90)^{d}	0.18 (±0.11)^{f}	6.30 (±0.89)^{b,c}
Sufentanyl	2.74 (±1.21)^{c}	0.60 (±0.24)^{d,e}	2.30 (±1.25)^{c,d}	0.11 (±0.09)^{e}	3.32 (±1.12)^{c}
U-47700	1.29 (±0.79)^{d}	0.30 (±0.12)^{e}	2.88 (±0.68)^{c}	0.13 (±0.09)^{e}	2.83 (±0.55)^{c}
U-48800 HCl	1.67 (±1.04)^{d,e,f}	0.33 (±0.09)^{f,g}	2.95 (±1.22)^{c,d}	0.14 (±0.08)^{g}	3.49 (±1.22)^{c}
U-49900	1.16 (±0.51)^{c,d,e}	0.28 (±0.20)^{e,f}	2.92 (±0.64)^{b}	0.09 (±0.07)^{f}	1.71 (±0.37)^{c}
Valerylfentanyl HCl	2.74 (±1.12)^{b,c}	0.48 (±0.09)^{d,e}	1.32 (±0.48)^{d}	0.13 (±0.09)^{e}	2.42 (±0.62)^{c}
Average Recovery	3.48 (±1.91)^{e}	0.68 (±0.37)^{h}	2.61 (±1.15)^{f}	0.13 (±0.06)^{h}	4.22 (±1.78)^{d}

The average amount of drug recovered from the 11 surface types ranged from 0.13 ($\pm$0.06) – 7.32 ($\pm$1.37) ng/100 cm^2, with galvanized steel having the greatest overall drug recovery, followed by vinyl floor tile, lab benchtop, HDPE table, glass, golden oak flooring, marble tile, latex-based painted drywall, laminate flooring, unfinished plywood, and the concrete cap block (Table 58). Galvanized steel had the greatest drug recovery for all drugs but alprazolam and cocaine, both of which had higher recoveries from glass. Oxycodone and naloxone were the only compounds not recovered from all surfaces; oxycodone was not recovered in quantifiable amounts in any of the plywood, laminate, or concrete samples and naloxone was not recovered in quantifiable amounts in any of the concrete samples.

Table 58. Average drug recovered ($\pm$SD) in ng/100 cm2 for each material tested. Results in descending order.

Surface Material	Mean Drug Recovery ($\pm$SD)
Galvanized Steel	7.32 ($\pm$1.37)
Vinyl Floor Tile	6.19 ($\pm$1.32)
Lab Benchtop	5.22 ($\pm$1.61)
HDPE Table	4.22 ($\pm$1.78)
Glass	3.48 ($\pm$1.91)
Golden Oak Flooring	2.61 ($\pm$1.15)
Marble Tile	2.35 ($\pm$0.49)
Painted Drywall	1.33 ($\pm$0.69)
Laminate Flooring	0.68 ($\pm$0.37)
Plywood	0.31 ($\pm$0.09)
Concrete Cap Block	0.13 ($\pm$0.06)

5.5 Discussion
5.5.1 Surface swab method optimization

The final optimized surface swab method used an 11 x 5 cm Kimwipe, wetted with 1% formic acid in water to swab a 10 x 10 cm surface in an S-N-S pattern. Upon completion of swabbing, the Kimwipe was placed in an 8 mL test tube with 996 µL of 1% formic acid in methanol (MPB) and 4 µL of internal standard solution. This tube was sonicated for 10 minutes and then 650 µL of supernatant was transferred to a 1.5 mL microcentrifuge tube. The tube was

centrifuged for 10 minutes at 13,000 rpm and then 500 µL of supernatant was transferred to a 1 mL amber injection vial for LC-MS/MS analysis.

While methanol did not work well as a wetting solvent to capture the fentalogs off the non-porous surface, the methanol-based MPB did work well to extract the fentalogs off of the Kimwipe used during the extraction procedure, which is explained by methanol's weaker adsorbent properties and stronger desorbent properties. Previous studies suggest compounds are adsorbed at the hydrogen binding sites of cellulose-based materials, such as Kimwipes or cotton swabs. [183] The hydrogen binding sites are formed between the hydroxyl groups on the cellulose material and the solvent surrounding the fibers.[184] Since water has a greater hydrogen bonding ability than methanol, more hydrogen bonds can be formed, thus creating more adsorption sites along the cellulose fibers.[185] These hydrogen bonds are further strengthened by addition of formic acid, making the acidified water the preferred solvent for adsorption of the compounds of interest onto the wipe.[186] Introducing the Kimwipe to the methanolic extraction buffer reduces the amount of hydrogen bonding occurring between the cellulose wipes and the solvent, causing the compounds of interest to be desorbed from the wipe and released into the extraction buffer.[187]

The average extraction efficiency of this surface swab method on the non-porous lab bench used was 62.0 (±14.0)%, with a range of 34.1 (±2.6) – 82.5 (±9.6)% for the 27 compounds of interest, with oxycodone having the greatest extraction efficiency and 4-ANPP having the lowest extraction efficiency. The low extraction efficiency of 4-ANPP may be due to its high vapor pressure, which could result in loss of the drug from the surface into the air as it volatilizes.[188]

5.5.2 Method validation

The method validation was successful for all tests performed. The linear range for all 27 compounds was 0.05-25 ng/100 cm^2, providing excellent sensitivity for an environmental

sampling technique and the overall methodology was proven to be robust. The sensitivity of the analytical method is not only important for identifying low amounts of the highly potent fentalogs, but it can also make up for shortcoming in the extraction efficiency of the swab method. With an average extraction efficiency of 62%, this method would be able to detect and quantitate most of the fentalogs at a contamination level of 0.08 ng/100 cm^2. Carfentanil, being the most potent of the currently known and commonly encountered fentalogs, has an estimated intravenous effective dose for analgesia in adults of 2 μg, which is 25,000 x more drug than is detectable with this method.[9] Additionally, the most likely routes of exposure in locations contaminated with fentanyl are inhalational for adults and oral for children, both of which result in a lower bioavailability of the drug then if given intravenously.[174,189] Based on this, the current method should prove to be sufficiently sensitive when legislation regarding remediation levels for fentalogs is finally drafted.

5.5.3 Assessment of multi-surface extraction efficiencies

The final step in this research was to examine how well the 27 compounds of interest could be recovered from common household surfaces. The method was optimized on the non-porous lab bench, as it was designed to be chemically resistant and to be easily cleaned following each surface swab test.[190] While great for designing a surface swab method, most locations having fentanyl contamination will not have a phenolic lab bench present to sample from. As expected, the more porous surfaces, such as concrete and plywood, as well as the highly pitted surfaces, such as laminate, had the worst amount of drug recovery. This is likely due to compounds being absorbed into the material or being deposited into surface pits where the swab was unable to capture them. While the overall drug recovery was low in the porous and pitted surfaces, all compounds but oxycodone and naloxone were still recovered in some quantifiable amount from every surface type. Since such a low concentration of each compound was spiked

on the surfaces to begin with, the ability to capture and detect almost all of them from each surface material gives confidence that this methodology will be able to determine the effectiveness of decontamination efforts as they are developed and provide reliable safety information regarding the level of drug contamination within a formerly contaminated location.

CHAPTER VI

CONCLUSION

As the rate of drug abuse continues to rise in the United States, the risk of environmental exposures to drugs or the chemicals used to produce them also increases. This research sought to assess the levels of contamination stemming from two particular drugs, methamphetamine and fentanyl. These drugs were chosen as methamphetamine is the most commonly produced illicit drug in the United States, while fentanyl is one of the most potent drugs available and has increased in prevalence at an alarming rate. The overarching hypothesis of this research was that environmental hazards associated with clandestine drug production and use can be identified and quantitated by analysis of adjacent contaminated water sources, ambient air, and household surfaces.

The goal of the first study, found in Chapter II, was to assess the capability of wastewater analysis in determining areas where One Pot methamphetamine waste is being dumped into the public wastewater system. Using the wastewater system to identify locations of methamphetamine waste dumping proved difficult, as nanogram per milliliter concentrations of methamphetamine, amphetamine, and pseudoephedrine were routinely observed. CMP, the byproduct used to identify One Pot methamphetamine production was only found in a small percentage of samples, and even in the few samples containing CMP, it was below the LLOQ of the method, preventing quantitation. Although this research was unable to identify locations of methamphetamine production, it was able to identify locations of use, even narrowing the

source of methamphetamine use to as little as 15 residencies. These results show the potential of using wastewater monitoring to reduce the amount of methamphetamine contamination being introduced to the environment, whether by sites of One Pot production or by excretion following methamphetamine use.

The goal of the second study, found in Chapter III, was to examine the air within a site of One Pot methamphetamine production, as well as perform standoff detection to monitor the air at varying distances from the site to determine the identity and concentration of air contaminants, such as ammonia gas, VOCs, and methamphetamine present. Active air sampling from within a One Pot methamphetamine lab captured volatilized methamphetamine, allowing the amount of airborne contamination to be assessed. Additionally, passive air monitoring within a One Pot methamphetamine lab collected VOCs, the identity and concentration of which could be compared between One Pot cooks performed with two different organic solvents to determine how the air quality with these labs differs depending on the solvent being used. Standoff detection of VOCs and ammonia gas were successful at identifying spikes in the concentration of these compounds as the One Pots were burped, and following bottle failures. Portable instrumentation was also successfully loaded into a vehicle and was able to see increases in ammonia concentration as the vehicle neared the site of One Pot methamphetamine production, presenting the possibility of incorporating this research into the vehicles of narcotics agents as they scope out locations of potential methamphetamine production. These results show the amount of airborne contamination released by One Pot methamphetamine labs and how the species and concentration of airborne contaminants differ with the solvent used during production. They also established a detection range for several of these airborne contaminants, which can be used to assess how far these contaminants may travel and how many people may have been exposed to these One Pot methamphetamine lab-related contaminants.

The goal of the third study, found in Chapter IV, was to determine what the level of surface methamphetamine contamination was following One Pot production and to assess the

effectiveness of a simple water wash in removing methamphetamine from the surfaces within

these labs. Analysis of surface methamphetamine contamination collected immediately following

One Pot production found the levels of contamination to be 3-4 orders of magnitude lower than

the levels observed in older routes of methamphetamine production. Part of this discrepancy

likely was the result of the lower amount of pseudoephedrine used in One Pot production when

compared to older methods. Additionally, this research only assessed the amount of surface

contamination present following a single One Pot and then the site was decontaminated. In an

actual One Pot methamphetamine lab, many One Pots will likely be produced before the

manufacturer is caught and decontamination crews can remediate the site, allowing for a buildup

of contamination on the surfaces of an actual site of production. As for remediation, a simple

water wash proved effective in removing methamphetamine surface contamination from the

surfaces of the cook site and the PPE of the researchers as long as a bottle failure hadn't occurred.

The presence of excess organic solvent following a bottle failure hindered the ability of water to

wash methamphetamine from the surfaces, resulting in residual contamination following the

decontamination process. One limit of this section of research was the location of the One Pot

methamphetamine cooks. All cooks were done in a plastic shed, which could easily be hosed out.

Such an approach would likely not be feasible in a home or other dwelling in which One Pot

methamphetamine production is common. In these locations, surfaces would need to be scrubbed

with a brush wetted with soap and water, and, due to the porousness of some building materials,

the decontamination process would likely need to be repeated several times as methamphetamine

continued to leach from the surfaces.

The goal of the fourth study, found in Chapter V, was to develop and validate a surface

swab method to capture 17 fentalogs and 10 common adulterants and use it to assess the

extraction efficiency of these compounds on 11 common household surfaces. The developed

surface swab method was successfully validated against SWGTOX guidelines and was able to

collect and quantitate 25 of the 27 compounds off all 11 surfaces; oxycodone was not collected

from plywood, laminate or concrete and naloxone was not recovered from concrete. These results have laid the groundwork for remediation of fentalog contamination. This methodology can be used to assess the levels of fentalog contamination within a site to determine the hazard level associated with it, as well as assess the efficiency of fentalog decontamination methods that may be developed in the future.

These four studies together have examined the broad range of environmental contamination that stems from illicit drug production and use. While the amount of contamination stemming from one clandestine lab may vary from the amount generated by another, the techniques developed by this research will be able to assess the hazards associated with the lab and assist in determining the correct form of remediation needed. Moving forward, there is still much work needed to be done to expand on this research. One project would be to assess the metabolic fate of the One Pot methamphetamine byproduct CMP. While used as a marker for methamphetamine production in this research, it is unknown if it excreted from the body as unchanged drug. If CMP is greatly metabolized prior to being excreted, its presence in wastewater would provide evidence of One Pot methamphetamine production, but if it is excreted primarily as unchanged drug, its presence in wastewater may not be enough to differentiate methamphetamine use from production at a given location. Additionally, a studies such as those outlined in Chapters III and IV need to be completed for sites of fentalog production and tableting. By assessing the air and surface contamination levels in sites of fentalog production and tableting, better decisions can be made by law enforcement, emergency first responders, and public safety officials when entering these types of locations. This knowledge will also assist in the development of decontamination methods, as by knowing the amount of contamination present, researchers can better develop methods optimized to remediate that level of fentalog contamination.

REFERENCES

1.   Bose J, Hedden SL, Lipari RN, Park-Lee E, Porter JD, Pemberton MR. *Key Substance Use and Mental Health Indicators in the United States: Results from the 2015 National Survey on Drug Use and Health*. Rockville, MD: Center for Behavioral Health Statistics and Quality; 2016:74. http://www.samhsa.gov/data/release/2015-national-survey-drug-use-and-health-nsduh-releases.

2.   Bose J, Hedden SL, Lipari RN, Park-Lee E. *Key Substance Use and Mental Health Indicators in the United States: Results from the 2017 National Survey on Drug Use and Health*. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2018:124. https://www.samhsa.gov/data/report/2017-nsduh-annual-national-report.

3.   *National Forensic Laboratory Information System: 2015 Annual Report*. Springfield, VA: Drug Enforcement Administration; 2016:32. https://www.nflis.deadiversion.usdoj.gov/DesktopModules/ReportDownloads/Reports/NFLIS2015AR.pdf.

4.   *National Forensic Laboratory Information System 2017 Annual Report*. Springfield, VA: Drug Enforcement Administration; 2018:32. https://www.nflis.deadiversion.usdoj.gov/DesktopModules/ReportDownloads/Reports/NFLIS-Drug-AR2017.pdf.

5.   Dhillon. *2018 National Drug Threat Assessment*. DEA https://www.dea.gov/sites/default/files/2018-11/DIR-032-18%202018%20NDTA%20final%20low%20resolution.pdf.

6.   ATSDR - Medical Management Guidelines (MMGs): Hydrogen Chloride. CDC Web site. https://www.atsdr.cdc.gov/mmg/mmg.asp?id=758&tid=147. Published October 21, 2014. Accessed January 29, 2020.

7.   ATSDR - Medical Management Guidelines (MMGs): Ammonia. CDC Web site. https://www.atsdr.cdc.gov/mmg/mmg.asp?id=7&tid=2. Published October 21, 2014. Accessed January 29, 2020.

8.   Watanabe-Galloway S, Ryan S, Hansen K, Hullsiek B, Muli V, Malone AC. Effects of Methamphetamine Abuse Beyond Individual Users. *Journal of Psychoactive Drugs*. 2009;41(3):241-248. doi:10.1080/02791072.2009.10400534

9.   *Fact Sheet for OSCs: Fentanyl and Fentanyl Analogs*. EPA; 2018.

10.   Galleries: Fentanyl. DEA Web site. https://www.dea.gov/galleries/drug-images/fentanyl. Accessed January 30, 2020.

11.   Wilde M, Pichini S, Pacifici R, et al. Metabolic Pathways and Potencies of New Fentanyl Analogs. *Front Pharmacol*. 2019;10:238. doi:10.3389/fphar.2019.00238

12.   Gonzales R, Mooney L, Rawson RA. The Methamphetamine Problem in the United States. *Annual Review of Public Health*. 2010;31(1):385-398. doi:10.1146/annurev.publhealth.012809.103600

13.   *2017 National Drug Threat Assessment*. U.S. Department of Justice - Drug Enforcement Administration; 2017:67-82. https://www.dea.gov/docs/DIR-040-17_2017-NDTA.pdf.

14.   Person EC, Meyer JA, Vyvyan JR. Structural determination of the principal byproduct of the lithium-ammonia reduction method of methamphetamine manufacture. *Journal of Forensic Sciences*. 2005;50(1):1-9. doi:10.1520/JFS2003389

15.   Wastewater treatment (% population connected). OECD Web site. http://stats.oecd.org/index.aspx?DataSetCode=water_treat. Published 2015. Accessed December 21, 2017.

16.   *People v. Electronic Plating Company*.(Appellate Court of Illinois, First District, Fourth Division 1997). http://caselaw.findlaw.com/il-court-of-appeals/1076769.html. Accessed December 21, 2017.

17.   Lorenzo M, Picó Y. Wastewater-based epidemiology: current status and future prospects. *Current Opinion in Environmental Science & Health*. 2019;9:77-84. doi:10.1016/j.coesh.2019.05.007

18.   Daughton CG. Illicit Drugs in Municipal Sewage. In: *Pharmaceuticals and Care Products in the Environment*. Vol 791. ACS Symposium Series. American Chemical Society; 2001:348-364. doi:10.1021/bk-2001-0791.ch020

19.   Bisseux M, Colombet J, Mirand A, et al. Monitoring human enteric viruses in wastewater and relevance to infections encountered in the clinical setting: a one-year experiment in central France, 2014 to 2015. *Euro Surveill*. 2018;23(7). doi:10.2807/1560-7917.ES.2018.23.7.17-00237

20.   Venkatesan AK, Chen J, Driver E, Gushgari A, Halden RU. Assessing the Potential To Monitor Plant-Based Diet Trends in Communities Using a Wastewater-Based Epidemiology Approach. In: *Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets*. Vol 1319. ACS Symposium Series. American Chemical Society; 2019:187-198. doi:10.1021/bk-2019-1319.ch010

21.   *Wastewater Analysis and Drugs: A European Multi-City Study*. European Monitoring Centre for Drugs and Drug Addiction; 2018:10.

22.   Couper FJ, Logan BK. *Drugs and Human Performance Fact Sheets*. Washington, DC: National Highway Traffic Safety Administration; 2004:61-66.

23. Merves ML, Moore KA. Amphetamines/Sympathomimetic Amines. In: *Principles of Forensic Toxicology*. 4th ed. Washington, DC: AACC Press; 2013:353-370.

24. Stojanovska N, Fu S, Tahtouh M, Kelly T, Beavis A, Kirkbride KP. A review of impurity profiling and synthetic route of manufacture of methylamphetamine, 3,4-methylenedioxymethylamphetamine, amphetamine, dimethylamphetamine and p-methoxyamphetamine. *Forensic Science International*. 2013;224(1–3):8-26. doi:10.1016/j.forsciint.2012.10.040

25. Kunalan V, Kerr WJ, NicDaéid N. Clarification of route specific impurities found in methylamphetamine synthesised using the Birch method. *Forensic Science International*. 2012;223(1–3):321-329. doi:10.1016/j.forsciint.2012.10.008

26. Kunalan V, Kerr WJ, Daéid NN. Investigation of the reaction impurities associated with methylamphetamine synthesized using the Nagai method. *Anal Chem*. 2012;84:5744-5752. doi:10.1021/ac3009302

27. *International Narcotics Control Strategy Report - Volume 1: Drug and Chemical Control*. Bureau for International Narcotics and Law Enforcement Affairs; 2016:44. http://link.springer.com/10.1007/BF02900343. Accessed July 16, 2018.

28. Ciesielski AL, Green MK, Wagner JR. Characterization of One Pot Methamphetamine Laboratories Using GC-MS and LC-MS/MS. *J Forensic Chem*. [in Press]

29. Bartos L. Meth Production is Toxic to Communities (methtoxic). University of Nebraska-Lincoln Web site. http://lancaster.unl.edu/family/methtoxic.shtml. Published July 2005. Accessed April 14, 2016.

30. Poschen D. *Coleman Camp Fuel*. Wichita, KS: HOC Industries, Inc; 7AD:7.

31. Farrington JW, Quinn JG. Petroleum Hydrocarbons and Fatty Acids in Wastewater Effluents. *Journal (Water Pollution Control Federation)*. 1973;45(4):704-712.

32. Belkin S. Biodegradation of haloalkanes. *Biodegradation*. 1992;3:299-313. doi:10.1007/BF00129090

33. Hussain HH, El-Alali A, Jiries AG, Aharonian GW. Chemical evaluation of waste produced by a Jordanian University: a case study. *Waste Manage Res*. 2000;18:94-98.

34. Swedish Pollutant Release and Transfer Register - Chloro-alkanes. Swedish Environmental Protection Agency Web site. https://utslappisiffror.naturvardsverket.se/en/Substances/Chlorinated-organic-substances/Chloro-alkanes/. Published December 23, 2019. Accessed February 3, 2020.

35. Bruckner JV, Warren DA. Chapter 24: Toxic Effects of Solvents and Vapors. In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. 6th ed. New York, NY: McGraw-Hill; 2001:869-916.

36. *Sodium Hydroxide, Solid*. Waltham, MA: Fisher Scientific; 2008. https://fscimage.fishersci.com/msds/21300.htm. Accessed February 1, 2020.

37. Robyn. Using Drain Cleaners. ASAP Drain Guys and Plumbing Web site. https://www.asapdrainandplumbing.com/using-drain-cleaners/. Published November 14, 2017. Accessed February 1, 2020.

38. Thornton & Grooms Heating, Cooling, Plumbing, and More. Thornton & Grooms Web site. https://www.thorntonandgrooms.com. Accessed February 1, 2020.

39. Ruiz G, Jeison D, Chamy R. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Research*. 2003;37(6):1371-1377. doi:10.1016/S0043-1354(02)00475-X

40. Karri RR, Sahu JN, Chimmiri V. Critical review of abatement of ammonia from wastewater. *Journal of Molecular Liquids*. 2018;261:21-31. doi:10.1016/j.molliq.2018.03.120

41. ATSDR - ToxFAQs™: Hydrogen Sulfide Carbonyl Sulfide. https://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=388&tid=67. Accessed February 3, 2020.

42. Hydrogen Sulfide. In: *NIOSH Pocket Guide to Chemical Hazards*. 3rd ed. Cincinnati, OH: NIOSH Publications; 2007:170.

43. *ATSDR Public Health Statement: Hydrogen Sulfide*. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 2016:7. https://www.atsdr.cdc.gov/ToxProfiles/tp114-c1-b.pdf.

44. Chemical Suicides: The Risk to Emergency Responders. CHEMM Web site. https://chemm.nlm.nih.gov/chemicalsuicide.htm. Published June 26, 2019. Accessed February 3, 2020.

45. *Hydrogen Sulfide Corrosion in Wastewater Collection and Treatment Systems*. Washington, DC: US EPA; 1991. https://nepis.epa.gov/Exe/ZyNET.exe/P100Q4BV.txt?ZyActionD=ZyDocument&Client=EPA&Index=1991%20Thru%201994&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C91THRU94%5CTXT%5C00000033%5CP100Q4BV.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=2#. Accessed February 3, 2020.

46. *Collection Systems Technology Fact Sheet: Sewers, Conventaional Gravity*. Washington, DC: US EPA; 2002. https://nepis.epa.gov/Exe/ZyNET.exe/P10053D9.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000021%5CP10053D9.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i4

25&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackD
esc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL#.

47.   Gravity Sewer Systems. Red Run Web site.
       https://redrundrain.wordpress.com/2014/02/06/gravity-sewer-systems/. Published
       February 6, 2014.

48.   Nathanson JA, Ambulkar A. Wastewater treatment. Encyclopedia Britannica Web site.
       https://www.britannica.com/technology/wastewater-treatment. Accessed February 3, 2020.

49.   Biological Oxygen Demand (BOD) and Water. USGS Web site.
       https://www.usgs.gov/special-topic/water-science-school/science/biological-oxygen-
       demand-bod-and-water?qt-science_center_objects=0#qt-science_center_objects. Accessed
       February 3, 2020.

50.   Killing Bacteria with UV Light. LightTech Web site. https://www.light-
       sources.com/blog/killing-bacteria-with-uv-light/. Published May 17, 2016. Accessed
       February 3, 2020.

51.   Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The removal of pharmaceuticals, personal
       care products, endocrine disruptors and illicit drugs during wastewater treatment and its
       impact on the quality of receiving waters. *Water Research*. 2009;43(2):363-380.
       doi:10.1016/j.watres.2008.10.047

52.   Gros M, Petrović M, Ginebreda A, Barceló D. Removal of pharmaceuticals during
       wastewater treatment and environmental risk assessment using hazard indexes.
       Environment International. 2010;36(1):15-26. doi:10.1016/j.envint.2009.09.002

53.   Zuccato E, Castiglioni S. Illicit drugs in the environment. *Proc R Soc A*.
       2009;367(1904):3965-3978. doi:10.1098/rsta.2009.0107

54.   Prichard J, Hall W, Zuccato E, et al. Ethical research guidelines for wastewater-based
       epidemiology and related fields. March 2016. http://score-cost.eu/wp-
       content/uploads/sites/118/2016/11/WBE-ethical-guidelines-FINAL-March-2016-.pdf.
       Accessed August 17, 2018.

55.   Oyler JM, Cone EJ, Joseph RE, Moolchan ET, Huestis MA. Duration of Detectable
       Methamphetamine and Amphetamine Excretion in Urine after Controlled Oral
       Administration of Methamphetamine to Humans. *Clin Chem*. 2002;48(10):1703-1714.

56.   Prah J, Ashley D, Blount B, et al. Dermal, Oral, and Inhalation Pharmacokinetics of
       Methyl Tertiary Butyl Ether (MTBE) in Human Volunteers. *Toxicol Sci*. 2004;77(2):195-
       205. doi:10.1093/toxsci/kfh009

57.   *Ammonia*. Radnor, PA: Airgas USA, LLC; 2017:1-12.

58.   *Hydrogen Chloride*. Radnor, PA: Airgas USA, LLC; 2016:1-12.

59.   Volatile Organic Compounds' Impact on Indoor Air Quality. US EPA Web site.
       https://www.epa.gov/indoor-air-quality-iaq/volatile-organic-compounds-impact-indoor-
       air-quality. Published August 18, 2014. Accessed February 9, 2020.

60.   J. McKenzie E, M. Miskelly G, G. Butler PA. Detection of methamphetamine in indoor air using dynamic solid phase microextraction: a supplementary method to surface wipe sampling. *Analytical Methods*. 2013;5(20):5418-5424. doi:10.1039/C3AY40537K

61.   Weisheit R, White WL. *Methamphetamine: Its History, Pharmacology and Treatment*. Center City, MN: Hazelden Publishing; 2009. https://books.google.com/books?id=YqZkw-apxVAC. Accessed April 3, 2016.

62.   Mat Desa WNS, Ismail D. Impurity Profiling of Amphetamine and Methamphetamine Using Gas Chromatography Mass Spectrometry (GCMS) Harmonised Methods. *JSM*. 2017;46(1):149-156. doi:10.17576/jsm-2017-4601-19

63.   Methamphetamine Overview : Origin and History | Methoide. http://methoide.fcm.arizona.edu/infocenter/index.cfm?stid=164. Accessed April 19, 2016.

64.   Owens CV, Mason MM, Marr D. Remediation of Manufactured Methamphetamine in Clandestine Laboratories. A Literature Review. *J Chem Health Saf*. 2017;24(5):23-37. doi:10.1016/j.jchas.2017.01.004

65.   *Formic Acid*. London, England: Public Health England; 2019.

66.   Goyer RA, Clarkson TW. Chapter 23: Toxic Effects of Metals. In: *Casarett and Doull's Toxicology: The Basic Science of Poisons*. 6th ed. New York, NY: McGraw-Hill; 2001:811-867.

67.   Skinner HF. Methamphetamine synthesis via HI/red phosphorous reduction of ephedrine. *Forensic Sci Int*. 1990;48(2):128-134. doi:10.1016/0379-0738(90)90104-7

68.   David GE, Coxon A, Frew RD, Hayman AR. Isotope fractionation during precipitation of methamphetamine HCl and discrimination of seized forensic samples. *Forensic Science International*. 2010;200(1-3):123-129. doi:10.1016/j.forsciint.2010.03.043

69.   Methamphetamine drug profile. EMCDDA Web site. http://www.emcdda.europa.eu/publications/drug-profiles/methamphetamine. Published January 8, 2015. Accessed February 10, 2020.

70.   *Material Safety Data Sheet Hydroiodic Acid*. Badlapur District, Maharashtra, India: OMKAR http://www.omkarchemicals.com/msds%20final/Iodine/Hydro%20iodic%20Acid.pdf. Accessed February 10, 2020.

71.   *Hydrochloric Acid (Hydrogen Chloride)*. US EPA; 2000. https://www.epa.gov/sites/production/files/2016-09/documents/hydrochloric-acid.pdf. Accessed February 10, 2020.

72.   *Phosphoric Acid*. Phillipsburg, NJ: Mallinckrodt Baker Inc; 2000. https://www.atmos.umd.edu/~russ/MSDS/phosphoric_acid.htm. Accessed February 10, 2020.

73.   *Clandestine Laboratory Safety Certificate Program Student Manual*. 13th ed. Folsom, CA: National Environmental Systems

74. Buddrick O, Jones OAH, Morrison PD, Small DM. Heptane as a less toxic option than hexane for the separation of vitamin E from food products using normal phase HPLC. *RSC Adv*. 2013;3(46):24063. doi:10.1039/c3ra44442b

75. Brown KW, Armstrong TJ. Hydrocarbon Inhalation. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2020. http://www.ncbi.nlm.nih.gov/books/NBK470289/. Accessed February 9, 2020.

76. Miller MA, Kozel NJ. Methamphetamine Abuse: Epidemiologic Issues and Implications. 1991. doi:10.1037/e496202006-001

77. Kaley KB, Salocks C. *Red Phosphorus*. California: California Office of Environmental Health Hazard Assessment; 2003:2. https://oehha.ca.gov/media/downloads/risk-assessment/fact-sheet/red20phosphorus20fact20sheet20meth20labs2010270327.pdf.

78. Phosphorus. Royal Society of Chemistry Web site. https://www.rsc.org/periodic-table/element/15/phosphorus. Accessed February 11, 2020.

79. White Phosphorus. CDC-NIOSH Web site. https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750025.html. Published September 27, 2018. Accessed February 11, 2020.

80. Fu S, Song P, Liu X. Chapter 19: Thermal and flame retardancy properties of thermoplastics/natural fiber biocomposites. In: Fan M, Fu F, eds. *Advanced High Strength Natural Fibre Composites in Construction*. Woodhead Publishing; 2017:479-508. doi:10.1016/B978-0-08-100411-1.00019-4

81. Kaley KB, Salocks C. *Phosphine*. California: California Office of Environmental Health Hazard Assessment; 2003:2. https://oehha.ca.gov/media/downloads/risk-assessment/fact-sheet/phosphine20fact20sheet20meth20labs2010270327.pdf.

82. Phosphine. CDC-NIOSH Web site. https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750035.html. Published September 27, 2018. Accessed February 11, 2020.

83. Burgess JL. Phosphine Exposure from a Methamphetamine Laboratory Investigation. *Journal of Toxicology: Clinical Toxicology*. 2001;39(2):165-168. doi:10.1081/CLT-100103833

84. List I and List II Chemicals. DEA Web site. https://www.deadiversion.usdoj.gov/chem_prog/34chems.htm. Accessed February 12, 2020.

85. *Comprehensive Methamphetamine Control Act of 1996, 110*. Vol 3099.; 1996.

86. Martyny JW, Arbuckle SL, McCammon CS, Esswein EJ, Erb N, Van Dyke M. Chemical concentrations and contamination associated with clandestine methamphetamine laboratories. *Journal of Chemical Health and Safety*. 2007;14(4):40-52. doi:10.1016/j.jchas.2007.01.012

87.   Ammonia. CDC-NIOSH Web site.
       https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750013.html. Published
       September 27, 2018. Accessed February 12, 2020.

88.   *Combat Methamphetamine Epidemic Act of 2005, 120.* Vol 257.
       http://www.deadiversion.usdoj.gov/meth/pl109_177.pdf. Accessed April 13, 2016.

89.   Making Methamphetamine at home. Text Files Web site.
       http://www.textfiles.com/uploads/methmethod.txt. Accessed March 13, 2019.

90.   Clean M. The Complete Birch. Erowid Web site.
       https://erowid.org/archive/rhodium/chemistry/birch.mrclean.html. Accessed March 13,
       2019.

91.   Lim Abdullah AF, Miskelly GM. Recoveries of trace pseudoephedrine and
       methamphetamine residues from impermeable household surfaces: Implications for
       sampling methods used during remediation of clandestine methamphetamine laboratories.
       Talanta. 2010;81(1):455-461. doi:10.1016/j.talanta.2009.12.025

92.   Wright J, Edwards J, Walker GS. Methamphetamine Contamination in Homes –
       Contamination and Risk Levels. *Healthy Housing*. November 2016:12.
       doi:10.4225/50/581074e2913db

93.   Hammon TL, Griffin S. Support for selection of a methamphetamine cleanup standard in
       Colorado. *Regulatory Toxicology and Pharmacology*. 2007;48(1):102-114.
       doi:10.1016/j.yrtph.2007.02.002

94.   Walpole SC, Prieto-Merino D, Edwards P, Cleland J, Stevens G, Roberts I. The weight of
       nations: an estimation of adult human biomass. *BMC Public Health*. 2012;12(1):439.
       doi:10.1186/1471-2458-12-439

95.   Hallett S, Ashurst JV. Physiology, Tidal Volume. In: *StatPearls*. Treasure Island, FL:
       StatPearls Publishing; 2019. http://www.ncbi.nlm.nih.gov/books/NBK482502/. Accessed
       September 9, 2019.

96.   Mighty, Lord. How to make methamphetamine.
       http://mirror.bagelwood.com/textfiles/uploads/metanf.txt. Accessed September 11, 2016.

97.   Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR. Advanced multiplexed
       analysis with the FlowMetrixTM system. *Clinical Chemistry*. 1997;43(9):1749-1756.

98.   Oliver KG, Kettman JR, Fulton RJ. Multiplexed Analysis of Human Cytokines by Use of
       the FlowMetrix System. *Clinical Chemistry*. 1998;44(9):2057-2060.

99.   Smith J, Sammons D, Robertson S, Biagini R, Snawder J. Measurement of multiple drugs
       in urine, water, and on surfaces using fluorescence covalent microbead immunosorbent
       assay. *Toxicology Mechanisms & Methods*. 2010;20(9):587-593.
       doi:10.3109/15376516.2010.518172

100. *User's Manual: FLIR GF2xx Series*. Wilsonville, OR: FLIR Systems https://www.flir.com/globalassets/imported-assets/document/flir-gf3xx-series-user-manual.pdf.

101. Manning JA, Burckle JO, Hedges S, McElroy FF. *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Second Edition: Method TO-14A*. Cincinnati, OH: US EPA; 1999. https://www3.epa.gov/ttnamti1/files/ambient/airtox/to-14ar.pdf.

102. Manning JA, Burckle JO, Hedges S, McElroy FF. *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Second Edition: Method TO-15*. Cincinnati, OH: US EPA; 2015. https://19january2017snapshot.epa.gov/sites/production/files/2016-03/documents/to-15_vocs_2015_line_numbers_0.pdf.

103. Sather ME, Mathew J, Nguyen N, et al. Baseline ambient gaseous ammonia concentrations in the Four Corners area and eastern Oklahoma, USA. *J Environ Monit*. 2008;10(11):1319-1325. doi:10.1039/b807984f

104. Bolden AL, Kwiatkowski CF, Colborn T. New Look at BTEX: Are Ambient Levels a Problem? *Environ Sci Technol*. 2015;49(9):5261-5276. doi:10.1021/es505316f

105. *Toxicity Values for the VPH/EPH/APH Methodology*. Boston, MA: Massachusetts Department of Environmental Protection; 2003:65. https://www.mass.gov/files/documents/2016/08/ok/tphtox03.pdf.

106. Rizzo WB. Fatty Aldehyde and Fatty Alcohol Metabolism: Review and Importance for Epidermal Structure and Function. *Biochim Biophys Acta*. 2014;1841(3):377-389. doi:10.1016/j.bbalip.2013.09.001

107. *The PID Handbook: Theory and Application of Direct-Reading Photoionization Detectors (PIDs)*. 3rd ed. San Jose, CA: RAE Systems Inc; 2013. http://www.raesystems.com/customer-care/resource-center/pid-handbook. Accessed March 12, 2018.

108. *A Pocket Guide to Accelerant Evidence Collection*. 2nd ed. Lowell, MA: International Association of Arson Investigators; 1999. http://www.interfire.org/res_file/aec_20ig.asp. Accessed March 19, 2018.

109. Health and Safety Information on Household Products- Prestone Engine Starting Fluid. Household Product Database. https://hpd.nlm.nih.gov/cgi-bin/household/brands?tbl=brands&id=16007004. Published September 2017. Accessed March 12, 2018.

110. Scott MS, Dedel K. Clandestine methamphetamine labs. 2006. doi:10.1037/e486802008-001

111. *2019 National Drug Threat Assessment*. Springfield, VA: Drug Enforcement Administration; 2019. https://www.dea.gov/sites/default/files/2020-01/2019-NDTA-final-01-14-2020_Low_Web-DIR-007-20_2019.pdf.

112. Salocks CB, Hui X, Lamel S, et al. Dermal exposure to methamphetamine hydrochloride contaminated residential surfaces II. Skin surface contact and dermal transfer relationship. *Food and Chemical Toxicology*. 2014;66:1-6. doi:10.1016/j.fct.2013.12.044

113. Salocks CB, Hui X, Lamel S, Qiao P, Sanborn JR, Maibach HI. Dermal exposure to methamphetamine hydrochloride contaminated residential surfaces: Surface pH values, volatility, and in vitro human skin. *Food and Chemical Toxicology*. 2012;50(12):4436-4440. doi:10.1016/j.fct.2012.08.002

114. Owens CV, Mason MM, Marr D. Remediation of Manufactured Methamphetamine in Clandestine Laboratories. A Literature Review. *J Chem Health Saf*. 2017;24(5):23-37. doi:10.1016/j.jchas.2017.01.004

115. Hoffman K, Webster TF, Sjödin A, Stapleton HM. Toddler's behavior and its impacts on exposure to polybrominated diphenyl ethers. *Journal of Exposure Science and Environmental Epidemiology*. 2017;27(2):193-197. doi:10.1038/jes.2016.11

116. *Voluntary Guidelines for Methamphetamine Laboratory Cleanup*. United States Environmental Protection Agency; 2013:1-48. https://www.epa.gov/sites/production/files/documents/meth_lab_guidelines.pdf. Accessed September 13, 2016.

117. Simon SL, Richardson K, Dacey J, et al. A Comparison of Patterns of Methamphetamine and Cocaine Use. *Journal of Addictive Diseases*. 2001;21(1):35-44. doi:10.1300/J069v21n01_04

118. Lopez RB, Onyemekwu C, Hart CL, Ochsner KN, Kober H. Boundary Conditions of Methamphetamine Craving. *Exp Clin Psychopharmacol*. 2015;23(6):436-444. doi:10.1037/pha0000049

119. Harris DS, Boxenbaum H, Everhart ET, Sequeira G, Mendelson JE, Jones RT. The bioavailability of intranasal and smoked methamphetamine. *Clinical Pharmacology & Therapeutics*. 2003;74(5):475-486. doi:10.1016/j.clpt.2003.08.002

120. Cook CE, Jeffcoat AR, Hill JM, et al. Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metab Dispos*. 1993;21(4):717-723.

121. Kish SJ. Pharmacologic mechanisms of crystal meth. *CMAJ*. 2008;178(13):1679-1682. doi:10.1503/cmaj.071675

122. Martyny JW, Arbuckle SL, McCammon CS, Erb N, Van Dyke M. Methamphetamine contamination on environmental surfaces caused by simulated smoking of methamphetamine. *Journal of Chemical Health and Safety*. 2008;15(5):25-31. doi:10.1016/j.jchas.2008.02.004

123. Cruickshank CC, Dyer KR. A review of the clinical pharmacology of methamphetamine. *Addiction*. 2009;104(7):1085-1099. doi:10.1111/j.1360-0443.2009.02564.x

124. *Stature-for-Age and Weight-for-Age Percentiles: 2 to 20 Years: Boys*. Atlanta, GA: CDC; 2000. https://www.cdc.gov/growthcharts/data/set1clinical/cj41l021.pdf.

125. VanDyke M, Erb N, Arbuckle S, Martyny J. A 24-Hour Study to Investigate Persistent Chemical Exposures Associated with Clandestine Methamphetamine Laboratories. *Journal of Occupational and Environmental Hygiene*. 2008;6(2):82-89. doi:10.1080/15459620802590116

126. Paediatric Intensive Care - Clinical Practie Guideline: Paediatric Ventilation Guidelines. 2010. http://www.health.gov.fj/wp-content/uploads/2014/05/Ventilation-Guidelines-for-PICU_Oct-2010.pdf.

127. Nakayama M. Chemical Interaction of Bleach and Methamphetmaine: A Study of Degradation and Transformation Effects. 2002.

128. Serrano KA, Martyny JW, Kofford S, Contreras JR, Dyke MVV. Decontamination of Clothing and Building Materials Associated with the Clandestine Production of Methamphetamine. *Journal of Occupational and Environmental Hygiene*. 2012;9(3):185-197. doi:10.1080/15459624.2012.660096

129. Sisco E, Najarro M, Burns A. Quantifying the effectiveness of cleaning agents at removing drugs from laboratory benches and floor tiles. *Forensic Chemistry*. 2019;12:1-7. doi:10.1016/j.forc.2018.11.002

130. *Quaternary Ammonium Compounds in Cleaning Products: Health & Safety Information for Health Professionals*. New York, NY: Mount Sinai Selikoff Centers for Occupatonal Health; :9. https://med.nyu.edu/pophealth/sites/default/files/pophealth/QACs%20Info%20for%20Physicians_18.pdf.

131. Snawder JE, Striley CA, Esswein EJ, et al. Use of Direct Reading Surface Sampling Methods for Site Characterization and Remediation of Methamphetamine Contaminated Properties. *Journal of ASTM International*. 2011;8(6):11. doi:10.1520/JAI103481

132. Introduction to Lateral Flow Rapid Test Diagnostics. nanoComposix. https://nanocomposix.com/pages/introduction-to-lateral-flow-rapid-test-diagnostics. Accessed March 1, 2020.

133. Bryan E. Over 100 Meth "Reaction Vessels" Found In 1 House, Mannford Police Say. News on 6 Web site. http://www.newson6.com/story/40237818/100-meth-labs-found-in-1-house-mannford-police-say. Published April 2, 2019. Accessed June 3, 2019.

134. *Fentanyl*. Drug Enforcement Administration; 2018:1.

135. Jeal W, Benfield P. Transdermal Fentanyl. *Drugs*. 1997;53(1):109-138. doi:10.2165/00003495-199753010-00011

136. Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. *Neuropharmacology*. 2018;134:121-132. doi:10.1016/j.neuropharm.2017.10.016

137. Kerrigan S, Goldberger BA. Chapter 17: Opioids. In: *Principles of Forensic Toxicology*. 4th ed. Washington, DC: AACC Press; 2013:271-292.

138. Prevention of Occupational Exposure to Fentanyl and Other Opioids. Presented at the: Awareness Training Tool; March 2018; NIH.

139. Moss MJ, Warrick BJ, Nelson LS, et al. ACMT and AACT position statement: preventing occupational fentanyl and fentanyl analog exposure to emergency responders. *Clinical Toxicology*. 2018;56(4):297-300. doi:10.1080/15563650.2017.1373782

140. Concheiro M, Chesser R, Pardi J, Cooper G. Postmortem Toxicology of New Synthetic Opioids. *Front Pharmacol*. 2018;9. doi:10.3389/fphar.2018.01210

141. Higashikawa Y, Suzuki S. Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and its related compounds. VI. Structure-analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues. *Forensic Toxicol*. 2008;26(1):1-5. doi:10.1007/s11419-007-0039-1

142. Meth Lab Cleanup Standards, Clandestine Drug Lab Laws, DEA Clandestine Drug Lab Registry & EPA Guidelines. Meth Lab Cleanup Company Web site. https://www.methlabcleanup.com/Meth-Lab-Cleanup-Standards.html. Accessed September 3, 2019.

143. Quirk, Melendez, Arambula, et al. *AB-1596 Hazardous Substances: Contaminated Property: Fentanyl Cleanup.*; 2019. https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201920200AB1596. Accessed July 24, 2019.

144. Sanchez R, Thronson S, Frias L. *Protocol for Fentanyl Contaminated Properties*. Orange County Health Care Agency; 2018. https://www.methlabcleanup.com/Orange%20County%20DRAFT%20Fentanyl%20Protocol%205-7-18.pdf. Accessed July 18, 2019.

145. NIOSH Manual of Analytical Methods (NMAM), 5th Edition. December 2017. https://www.cdc.gov/niosh/nmam/pdfs/NMAM_5thEd_EBook.pdf. Accessed July 24, 2019.

146. Overdose Death Rates. NIH Web site. https://www.drugabuse.gov/related-topics/trends-statistics/overdose-death-rates. Published January 29, 2019. Accessed March 2, 2020.

147. Scholl L, Seth P, Kariisa M, Wilson N, Baldwin G. Drug and Opioid-Involved Overdose Deaths — United States, 2013–2017. *MMWR Morb Mortal Wkly Rep*. 2018;67(51-52):1419-1427. doi:10.15585/mmwr.mm675152e1

148. Toll L, Borsodi A, Bruchas M, et al. Opioid receptors: Introduction. IUPHAR/BPS Guide to Pharmacology Web site. https://www.guidetopharmacology.org/GRAC/FamilyIntroductionForward?familyId=50. Published October 8, 2015. Accessed September 12, 2019.

149. Chen J-C, Smith ER, Cahill M, Cohen R, Fishman JB. The opioid receptor binding of dezocine, morphine, fentanyl, butorphanol and nalbuphine. *Life Sciences*. 1993;52(4):389-396. doi:10.1016/0024-3205(93)90152-S

150. How does the opioid system control pain, reward and addictive behavior? ScienceDaily Web site. https://www.sciencedaily.com/releases/2007/10/071014163647.htm. Published October 15, 2007. Accessed March 3, 2020.

151. Kerrigan S, Goldberger BA. Opioids. In: *Principles of Forensic Toxicology*. 4th ed. Washington, DC: AACC Press; 2013:271-292.

152. Dietis N, Rowbotham DJ, Lambert DG. Opioid receptor subtypes: fact or artifact? *Br J Anaesth*. 2011;107(1):8-18. doi:10.1093/bja/aer115

153. Mehta V, Langford RM. Acute pain management for opioid dependent patients. *Anaesthesia*. 2006;61(3):269-276. doi:10.1111/j.1365-2044.2005.04503.x

154. Allouche S, Noble F, Marie N. Opioid receptor desensitization: mechanisms and its link to tolerance. *Front Pharmacol*. 2014;5:1-21. doi:10.3389/fphar.2014.00280

155. Williams JT, Ingram SL, Henderson G, et al. Regulation of μ-Opioid Receptors: Desensitization, Phosphorylation, Internalization, and Tolerance. *Pharmacol Rev*. 2013;65(1):223-254. doi:10.1124/pr.112.005942

156. *2013 National Drug Threat Assessment*. Springfield, VA: Drug Enforcement Administration; 2013. https://www.dea.gov/sites/default/files/2018-07/DIR-017-13%20NDTA%20Summary%20final.pdf.

157. Katz J. Short Answers to Hard Questions About the Opioid Crisis. *The New York Times*. https://www.nytimes.com/interactive/2017/08/03/upshot/opioid-drug-overdose-epidemic.html, https://www.nytimes.com/interactive/2017/08/03/upshot/opioid-drug-overdose-epidemic.html. Published August 3, 2017. Accessed March 3, 2020.

158. *Counterfeit Precription Pills Containing Fentanyls: A Global Threat*. Springfield, VA: Drug Enforcement Administration; 2016:10.

159. *Oxycodone (Trade Names: Tylox®, Percodan®, OxyContin®)*. Springfield, VA: Drug Enforcement Administration; 2019:1.

160. *Fentanyl Signature Profiling Program Report: October 2019*. Springfield, VA: Drug Enforcement Administration; 2019:3.

161. Opioid Conversion Chart. January 2015. https://olh.ie/wp-content/uploads/2014/09/Opioid-Conversion-Chart-2015.pdf. Accessed March 3, 2020.

162. Standardized Naming of Substituted Fentanyls. Cayman Chemical Web site. https://www.caymanchem.com/news/standardized-naming-of-substituted-fentanyls. Published January 16, 2018. Accessed March 3, 2020.

163. Wilde M, Pichini S, Pacifici R, et al. Metabolic Pathways and Potencies of New Fentanyl Analogs. *Front Pharmacol*. 2019;10. doi:10.3389/fphar.2019.00238

164. Costantini C, Foster D. The Walter White of Wichita. Fusion Web site. http://interactive.fusion.net/death-by-fentanyl/the-walter-white-of-wichita.html. Accessed March 3, 2020.

165. Van Nimmen NFJ, Poels KLC, Veulemans H a. F. Identification of Exposure Pathways for Opioid Narcotic Analgesics in Pharmaceutical Production Workers. *Ann Occup Hyg*. 2006;50(7):665-677. doi:10.1093/annhyg/mel028

166. Van Nimmen NFJ, Veulemans HAF. Development and validation of a highly sensitive gas chromatographic–mass spectrometric screening method for the simultaneous determination of nanogram levels of fentanyl, sufentanil and alfentanil in air and surface contamination wipes. *Journal of Chromatography A*. 2004;1035(2):249-259. doi:10.1016/j.chroma.2004.02.074

167. Sessink PJ, Connor TH, Jorgenson JA, Tyler TG. Reduction in surface contamination with antineoplastic drugs in 22 hospital pharmacies in the US following implementation of a closed-system drug transfer device. *J Oncol Pharm Pract*. 2011;17(1):39-48. doi:10.1177/1078155210361431

168. Sisco E, Najarro M, Burns A. A snapshot of drug background levels on surfaces in a forensic laboratory. *Forensic Chemistry*. 2018;11:47-57. doi:10.1016/j.forc.2018.09.001

169. *Drugs of Abuse: A DEA Resource Guide*. Springfield, VA: Drug Enforcement Administration; 2017:94. https://www.dea.gov/sites/default/files/sites/getsmartaboutdrugs.com/files/publications/DoA_2017Ed_Updated_6.16.17.pdf#page=40. Accessed March 4, 2020.

170. Gasior M, Bond M, Malamut R. Routes of abuse of prescription opioid analgesics: a review and assessment of the potential impact of abuse-deterrent formulations. *Postgraduate Medicine*. 2016;128(1):85-96. doi:10.1080/00325481.2016.1120642

171. Labroo RB, Paine MF, Thummel KE, Kharasch ED. Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: Implications for interindividual variability in disposition, efficacy, and drug interactions. *Drug Metab Dispos*. 1997;25(9):1072-1080. doi:10.0090-9556/97/2509-1072-1080$02.00/0

172. Fentanyl. Drug Bank Web site. https://www.drugbank.ca/drugs/DB00813. Published March 3, 2020. Accessed March 4, 2020.

173. Fentanyl: Incapacitating Agent. CDC-NIOSH Web site. https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750022.html. Published September 27, 2018. Accessed March 4, 2020.

174. Mather LE, Woodhouse A, Ward ME, Farr SJ, Rubsamen RA, Eltherington LG. Pulmonary administration of aerosolised fentanyl: pharmacokinetic analysis of systemic delivery. *British Journal of Clinical Pharmacology*. 1998;46(1):37-43. doi:10.1046/j.1365-2125.1998.00035.x

175. Gupta PK, Ganesan K, Gutch PK, Manral L, Dubey DK. Vapor Pressure and Enthalpy of Vaporization of Fentanyl. *J Chem Eng Data*. 2008;53(3):841-845. doi:10.1021/je7005067

176. Lindh WQ, Pooler M, Tamparo CD, Dahl BM. *Delmar's Comprehensive Medical Assisting: Administrative and Clinical Competencies*. Cengage Learning; 2009.

177. Durnal E. Fentanyl Decontamination Studies. Presented at the: EPA Decontamination Research and Development Conference; May 8, 2018; Washington, DC. https://www.epa.gov/sites/production/files/2018-11/documents/decon_presentation_011.pdf.

178. Dahlgren Decon. CRNE Tech Index Web site. http://www.cbrnetechindex.com/Print/4605/first-line-technology-llc/dahlgren-decon. Accessed September 9, 2019.

179. Block P. The Decomposition Kinetics of Peracetic Acid and Hydrogen Peroxide in Municipal Wastewaters. *proc water environ fed*. 2016;2016(10):555-563. doi:10.2175/193864716819707265

180. Sisco E, Najarro M, Burns A. Quantifying the effectiveness of cleaning agents at removing drugs from laboratory benches and floor tiles. *Forensic Chemistry*. 2019;12:1-7. doi:10.1016/j.forc.2018.11.002

181. Levels NRC (US) C on AEG. *Peracetic Acid Acute Exposure Guideline Levels*. Vol 8. Washington, DC: National Academies Press; 2010. https://www.ncbi.nlm.nih.gov/books/NBK220001/. Accessed March 4, 2020.

182. Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. *J Anal Toxicol*. 2013;37(7):452-474. doi:10.1093/jat/bkt054

183. Takagai Y, Kubota T, Kobayashi H, Tashiro T, Takahashi A, Igarashi S. Adsorption and Desorption Properties of trans-Resveratrol on Cellulose Cotton. *Anal Sci*. 2005;21(2):183-186. doi:10.2116/analsci.21.183

184. Mantanis GI, Young RA, Rowell RM. Swelling of compressed cellulose fiber webs in organic liquids. *Cellulose*.:1-22.

185. Venables DS, Schmuttenmaer CA. Spectroscopy and dynamics of mixtures of water with acetone, acetonitrile, and methanol. *The Journal of Chemical Physics*. 2000;113(24):11222-11236. doi:10.1063/1.1328072

186. Marushkevich K, Khriachtchev L, Räsänen M. Hydrogen Bonding between Formic Acid and Water: Complete Stabilization of the Intrinsically Unstable Conformer. *J Phys Chem A*. 2007;111(11):2040-2042. doi:10.1021/jp070363m

187. Przybysz P, Dubowik M, Kucner MA, Przybysz K, Przybysz Buzała K. Contribution of Hydrogen Bonds to Paper Strength Properties. *PLoS One*. 2016;11(5). doi:10.1371/journal.pone.0155809

188. Chemistry Dashboard: Piperidinone, 1-(2-phenylethyl)-. US EPA Web site. https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID30192826#properties. Accessed March 5, 2020.

189. Streisand JB, Varvel JR, Stanski DR, et al. Absorption and bioavailability of oral transmucosal fentnayl citrate. *Anesthesiology*. 1991;75(2):223-229.

190. Cappello A. Phenolic vs Epoxy Resin Work Surface: Which One Is Best? Work Station Industries Web site. http://resources.workstationindustries.com/blog/phenolic-vs-epoxy-resin-work-surface-which-one-is-best. Published April 27, 2016. Accessed March 5, 2020.

VITA

Austin Lee Ciesielski

Candidate for the Degree of

Doctor of Philosophy

Dissertation: ENVIRONMENTAL CONTAMINATION FROM CLANDESTINE
DRUG MANUFACTURE

Major Field: Forensic Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Forensic Science at
Oklahoma State University, Stillwater, Oklahoma in May, 2020.

Completed the requirements for the Master of Science in Forensic Science at Oklahoma
State University, Stillwater, Oklahoma in May, 2017.

Completed the requirements for the Bachelor of Arts in Chemistry and Biochemistry at
Coe College, Cedar Rapids, Iowa in May, 2015.

Experience:

Oklahoma State University Forensic Toxicology and Trace Lab, Aug. 2015 – Present

Integrated Toxicology General and Technical Supervisor, Nov. 2017 – Present

Basic Clandestine Laboratory Safety and Site Safety Officer Training, Oct. 2016

Level A Training for High-Hazard Clandestine Labs, Dec. 2019

CDC Development & Evaluation of Detection Methods for Illicit Drugs Fellowship
recipient, CDC-NIOSH, May 2019 – April 2020

Professional Memberships:

Student member of the American Academy of Forensic Sciences, Feb. 2017-Present

Student member of the Society of Forensic Toxicologists, May 2017-Present