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OPTIMAL EXTRACTION OF FENTANYL VOLATILE ORGANIC COMPOUNDS FOR THE DEVELOPMENT OF CANINE TRAINING AID MIMICS

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by

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This dissertation, written by Leann Forte, and entitled, Optimal Extraction of Fentanyl Volatile Organic Compounds for the Development of Canine Training Aid Mimics, having been approved in respect to style and intellectual content, is referred to you for judgment.

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DEDICATION

To my parents Wayne and Stacey Forte and my grandparents David and Meryl Schwalb

and Ernie and Margert Forte

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ABSTRACT OF THE DISSERTATION

OPTIMAL EXTRACTION OF FENTANYL VOLATILE ORGANIC COMPOUNDS FOR THE DEVELOPMENT OF CANINE TRAINING AID MIMICS

by

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Fentanyl is found to be 10 to 100 times more potent than morphine, which means a very small amount can cause an overdose. According to the National Center for Health Statistics (NCHS) and the Center of Disease Control and Prevention (CDC), in 2020 there was approximately 92,000 deaths caused by drug overdose with 56,000 of those deaths being attributed to the use of fentanyl or a fentanyl related material. [1]For this reason, it is imperative that law enforcement have ways to safely detect fentanyl both in field and in laboratory environments. One of the most effective ways of detecting narcotics in field is with the use of canines; however, currently there is no safe and reliable way to train canines to detect fentanyl. The first step in developing a safe method for canines to detect fentanyl is to determine what compound are present in the headspace of fentanyl that a canine could possibly associate with the odor of fentanyl. This project utilized solid phase microextraction gas-chromatography mass spectrometry (SPME-GC-MS) to collect and analyze the compounds that compose the vapor profile from reference grade fentanyl. This resulted in the detection and identification of nine compounds including heptane, styrene,

benzaldehyde, aniline, benzyl alcohol, benzeneacetylaldehyde, N-phenylpropanamide (NPPA), N-phenethyl-4-pipeeridinone (NPP) and 1-pehethyl-4-propionyloxypiperidine, with NPPA being the most abundant compound.

The degradation of fentanyl was also investigated through a series of experiments using field-relevant environmental conditions, examining degradation due to exposure to oxidation, humidity, and heat. Based on the results, NPPA was found to be the most notable product of degradation in all testing environments. There was also a significant increase in styrene after fentanyl was initially subjected to the 40 °C environment for the first week, though this was followed by a decrease in styrene abundance in following weeks. With a deeper understanding of the fentanyl headspace now known it is possible to move into mimic development. A mimic is defined as a training aid that utilizes the headspace compounds to imitate the odor of target material. The results indicated that there was sufficient evidence to consider NPPA as the test odorant for the development of a novel training aid mimic for fentanyl. Following the choice of odorant, three mimic preparations were developed, and the shelf-life and active usage lifetime of the novel aids were determined. These results revealed the shelf-life of the mimic to be one month and an active usage lifetime of least 6 hours. Finally, canine olfactory detection testing was conducted on three mimic preparations determine the validity of NPPA as a training aid for fentanyl. Two groups of canines were utilized; Group 1 included canines previously trained to detect fentanyl using the actual material and Group 2 included operational drug detection canines without prior training with fentanyl. The results from the canine tests, show that most of the canines correctly detected the substance to which they were trained;

however, the results showed a lack in positive alerts to any of the NPPA mimic preparations presented to the canines.

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ACRONYMS AND ABBREVIATION

1-PEP	1-phenethylpyridinium
1-PPO	1-phenethyl-1*H*-pyridin-2-one
1-SPO	1-styryl-1*H*-pyridin-2-one
4-ANPP	4-Anilino-N-Phenethyl-Piperidine
(A/E)	Analyte to Internal Standard Ratio
ALS	Automated Liquid Sampling
ASTM	American Society for Testing and Materials
CDC	Center of Disease Control
COMPS	Controlled Odor Mimi Permeation Systems
CBP	U.S. Customs and Border Protection
DEA	Drug Enforcement Agency
DHA	Dynamic Headspace Analysis
EI	Electron Impact
ESIS	Externally Sampled Internal Standard
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
HSSE	Headspace Sorptive Extraction
IMS	Ion Mobility Spectrometry
IR	Infrared
KCl	Potassium Chloride
LOD	Limit of Detection
LSD	Lysergic Acid Diethylamide

MDMA	3,4-methelenedioxy-methamphetamine
MHE	Multiple Headspace Extraction
MOE	Main Olfactory Epithelium
MS	Mass Spectrometry
MSPE	Magnetic Solid Phase Extraction
NACSW	National Association of Canine Scent Work
NCHS	National Center for Health Statistics
NIST	National Institute of Standards and Technology
NPP	*N*-phenethyl-4-piperidone
NPPA	*N*-pheylpropanamide
NPV	Negative Predictive Value
NRL	Naval Research Laboratory
ORs	Olfactory Receptors
PDMS/CAR/DVB	polydimethylsiloxane/carboxen/divinylbenzene
PPV	Positive Predictive Value
RH	Relative Humidity
SAMHSA	Substance Abuse and Mental Health Services Administration
SAR	Hong Kong Special Autonomous Region
SDME	Single Drop Microextraction
SHA	Static Headspace Analysis
SIE	Single Ion Extract
SIM	Single Ion Monitoring
SPME	Solid Phase Microextraction

SVOCs	Semi-Volatile Organic Compounds
TCCA	Trichloroisocyanuric acid
TCOs	Transnational Criminal Organizations
UN	United Nations
UNODC	United Nations Office on Drugs and Crime
US	United States
USA	United States of America
VOCs	Volatile Organic Compounds
VVOCs	Very Volatile Organic Compound

1. INTRODUCTION

Recently fentanyl has become one of the most abused drugs in the United States.[2] On January 13th, 2022, at the Sport and Medical Sciences Academy in Hartford, CT, three 7th-grade boys got access to fentanyl inside their school.[3] Police thought that one of the three boys was responsible for bringing 40 bags of the drug into the school. This encounter led to the death of a 13-year-old boy when he ingested a fatal dose. As a result of this incident, the school has decided to implement random bag checks as well as supply the school nurses with Narcan (an opioid antagonist that blocks the receptor site of the drug in the brain).[4] The presence of this fatal drug has changed, not only the life of that 13-year-old boy's family, but the lives of all that attend the Sport and Medical Sciences Academy.

On January 15th, 2022, officers from the Bakersfield Police Department responded to a call at the residence of John Lawson and Gabriella Goldberg.[5] The parents were desperate to help their 1-year-old son, who was blue and not breathing. When the police arrived, they successfully revived the baby boy and transported him to the local hospital. It was not until later that the police learned that a fentanyl overdose caused the boy's symptoms because of exposure to drug paraphernalia that was not only found all around his house but also in his crib. Luckily, he survived and is now in the custody of child protective services.

According to the Center for Disease Control (CDC), since 2013, fentanyl or fentanyl analog-related overdoses have increased from under 5 to 20 deaths per 100,000 standard population making this the leading cause of drug overdoses in the United States.[6] Fentanyl is 10 to 100 times more potent than morphine, meaning about 2 mg can cause an overdose.[7] As seen in the stories of the unnamed 13-year-old middle school student and the unnamed 1-year-old boy, this drug has no boundaries regarding age or demographics.

The illicit fentanyl and fentanyl analogs at the heart of these overdoses begin their journey in China, where it is manufactured in illegal labs.[8] From there, it also smuggled into our country through the mail or our ports of entry. An NBC News article written in 2021 stated that federal agents positioned at the border in El Paso, Texas, have seen a 4,000% increase in fentanyl seizures from 2018 to 2021.[9] This increase is attributed to fentanyl becoming the drug of choice for cartels. This increase in illegal trafficking is because of the high profits, higher potency, and smaller size, making it easier to transport clandestinely into the United States. As the amount of fentanyl coming into the United States rises, it becomes more and more dangerous to those who abuse drugs, especially those unaware of what it is or who do not know what they are taking. It also becoming increasingly dangerous for the law enforcement officers who are investigating crime scenes where fentanyl may be present. The question is now raised, how can law enforcement safely detect fentanyl in field? The answer using canines trained to detect fentanyl based on its unique vapor profile.

As a result of their highly acute olfactory system, canines have been employed for hundreds of years to detect odors of interest. Canines have been used to detect odors such as explosives, controlled substances, human scent, firearms, currency, mass storage devices, pests, ignitable liquids, invasive species, diseases such as cancer, and agriculture.[10] Since their sense of smell is 10,000 to 100,000 more sensitive than a human's sense of smell, canines are highly selective.[10] This means they can detect the single target odor in many other non-target odors. Coupled with their sense of smell is their ability to learn through operant conditioning, making canines an integral law enforcement tool utilized by police, military, search and rescue, medical, and assistance/service functions worldwide.[10] However, in the current climate canines are not being used to their full potential for the detection of fentanyl because of the health risks and safety issues fentanyl imposes.

The present research will discuss the development of a canine training aid mimic for fentanyl. Using a training aid has several benefits, including providing a safe environment for canines to train by eliminating the health hazards that arise when using the physical drug. The developed canine training aid will also allow for daily training, improving detection and minimizing the need for legal possession paperwork and the hassle of transporting the controlled substance. In the case of fentanyl, a training aid will benefit law enforcement by providing them with a safe and reliable method of training their canines on a dangerous substance. Training aids mimics are made of a non-illicit and non-hazardous compound or mixture of compounds that mimic the odor of a substance.[11] These compounds can be extracted from the headspace of the controlled substance using solid phase microextraction (SPME) followed by analysis via Gas Chromatography-Mass Spectrometry (GCMS). The main goal of the presented research is to determine the compounds the make up the vapor profile of fentanyl using SPME-GC-MS and which of those compounds is considered the major compound. Furthermore, this major compound will then be optimized into a controlled odor mimic permeation system for the development of a field deployable canine training aid. Finally, verification of the COMPS will begin with the use of scent detection canines.

2. LITERATURE REVIEW

2.1 Drugs

2.1.1 Current Drug Use in America and The Opioid Crisis

Beginning in the late 20th century and early 21st century, the drug epidemic in the US started to skyrocket, with overdose deaths increasing by 137% in 2000. This increase in fatalities in 2000 also included a 200% increase in opioid overdose deaths which signified the start of the opioid crisis.[12] This was considered the first wave of the opioid epidemic due to the increased use of prescription opioids.[13] By 2008, it was estimated that in persons 12 or older 7 million were dependent on illicit drugs with 1.7 million people dependent on opioids.[14]

Moving into 2010, the second wave of the opioid crisis began with increased heroin abuse.[13] It was estimated that around 9 million Americans 12 or older addicted to an illegal drug including cocaine, heroin, and psychotherapeutics.[15] Shortly after the second wave, a third wave in the opioid crisis began in 2013 with a rise in the misuse and abuse of illegally produced fentanyl. As the number of users and overdose deaths continued to increase, the United States entered the fourth wave of the opioid crisis in 2019. During this time, there was an increase in the misuse use of illegally man-made produced opioids such as fentanyl combined with other drugs such as heroin and cocaine.[13] The impact the opioid crisis has had on society can be seen in Figure 1.[13]

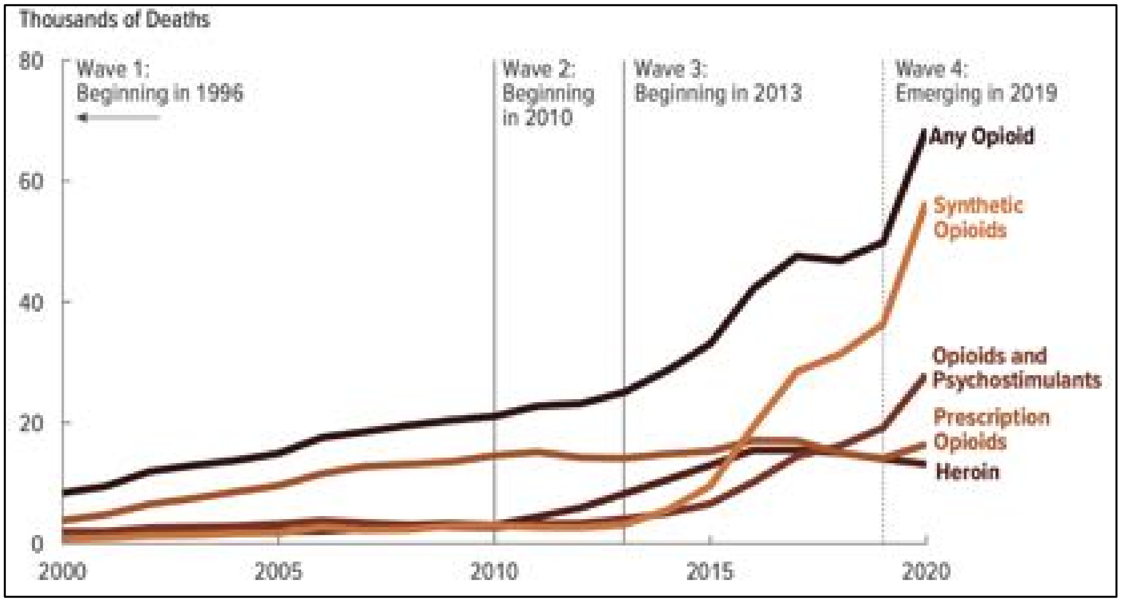

Figure 1. Overdose deaths involving any opioid from 2000 to 2020 representing all four waves of the opioid epidemic.

*Data was gathered using the information on the CDC WONDER database, Centers for Disease Control and Prevention, National Center for Health Statistics, "About Multiple Cause of Death 1999–2020" (accessed March 28, 2023), https://wonder.cdc.gov/mcd-icd10.html. See www.cbo.gov/ publication/58221#data.

In May 2022, the CDC released provisional data regarding overdose rates from 2020 to 2021. It is estimated that in 2021 106,622 overdose deaths occurred in the US, which was a 15 % increase from 2020.[16] The data also showed that overdose rates involving any opioids increased from 68,000 to 80,000, while deaths involving synthetic opioids, mainly fentanyl, increased from 56,000 to 71,000. Based on all the data published by NCHS and the CDC, there is overwhelming proof showing that since 2015 fentanyl has been the driving force for overdose deaths in the US.[2] It is also important to note that while overdose deaths result from prescription (pharmaceutical) fentanyl, the major problem lies with illicit fentanyl.

To help combat the opioid crisis sweeping across the United States, the federal government passed several laws between 2016 and 2018. The first law passed was The Comprehensive Addiction and Recovery Act in 2016. The passing of this law expanded the prevention and educational efforts with respect to the use of opioids, the availability of naloxone to law enforcement, resources to treat incarcerated individuals, expanded disposal sites for unwanted medications, launched both evidence-based and medication-based treatment programs, and strengthened drug monitoring programs.[17] Following this law, the 21st Century Cures Act was passed in 2016.[13] The final law passed was the Substance Use-Disorder Prevention, followed by the Promotes Opioid Recovery and Treatment for Patients and Communities Act in 2018 allowing for more available treatment for those suffering from addiction and expanding the availability of naloxone.[13] Other measures taken by the federal government include changing how opioid prescriptions are filled, lessening the availability of legal and illegal opioids, and having the postal service transmit electronic data to Customs and Border Protection on anything arriving into the US through international mail. After these three laws were passed and the regulations put into place the use of prescription opioids continued to fall. However, the use and misuse of illegal opioids have risen and are projected to continue growing.[13]

2.2. Fentanyl

2.2.1 Introduction to Fentanyl

Fentanyl (N-(1-(2-phenethyl)-4-piperidinyl-N-phenyl-propanamide)) is a synthetic opioid with a potency 100 times greater than that of morphine. It was first synthesized in 1960 by Dr. Paul Janssen and is used to treat severe short-term and chronic pain in cancer patients or as an anesthetic.[18,19] Since fentanyl has a valid medical use, it is classified as a Schedule II drug by the Controlled Substances Act. When a doctor prescribes fentanyl, it can be administered intravenously, as a patch, or a lozenge.[20] Illegally used fentanyl is seen more in overdose deaths and can be sold as a powder, on blotter paper, nasal sprays, or pressed into pills that resemble other opioid medications (as seen in Figure 2A).[21,22,23] Illegal fentanyl used as an adulterant in other drugs such as heroin, cocaine, and methamphetamine.[24] Since it only takes a small amount of fentanyl to produce a high or even cause an overdose, mixing it with other drugs can cause a problem since the user may not know it is present in what they purchased.[24]

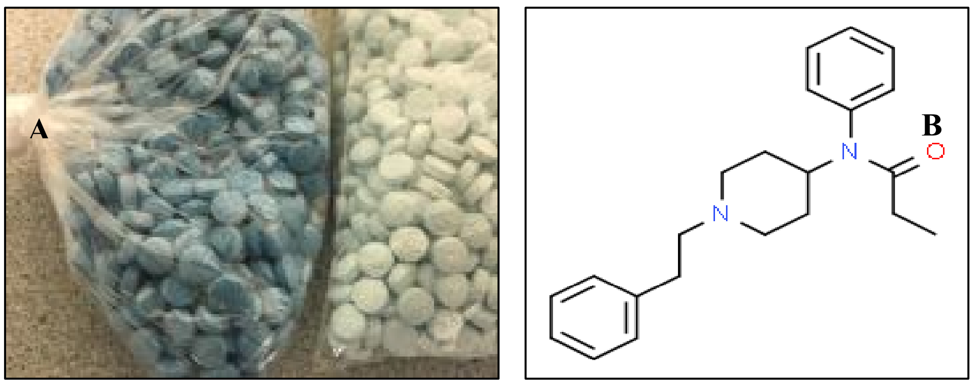

*Figure 2. **A** represents an example of pressed counterfeit fentanyl pills, while **B** represents the chemical structure of fentanyl.*

2.2.2 Fentanyl's Synthetic Pathways

As previously stated, fentanyl was first synthesized by Janssen Pharmaceutical from *N*-benzyl-4-piperidione.[25] The pathway for this synthesis is seen in Figure 3 (license ID 1361825-2) and shows: *N*-benzyl-4-piperidione (**1**) was then condensed to aniline to give the corresponding Schiff base (**2**).[27] The double bond obtained in the imine (**2**) was reduced using lithium aluminum hydride to get 1-benzyl-4-anilinopiperidine (**3**), which was then acylated using propanoic anhydride. This resulted in 1-benzyl-4-*N*-propinoyl-anilinopiperidine (**4**), which underwent debenzylation using standard H_2-Pd/C conditions to obtain norfentnayl. The norfentnayl (**5**) was then N-alkylated by 2-phenylethylchloride to get fentanyl (**6**).[26]

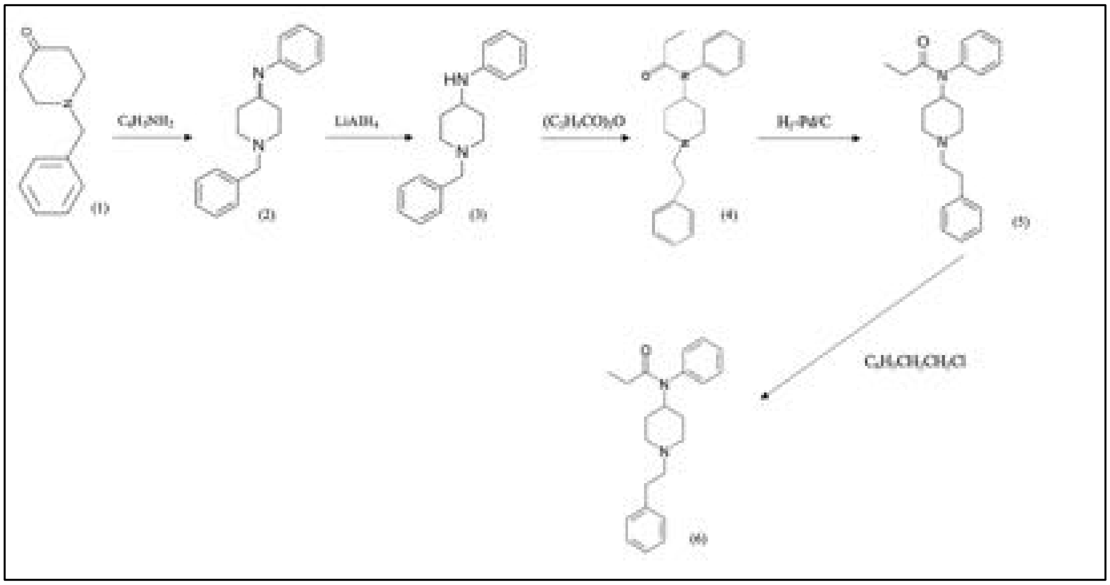

Figure 3.Represents the first synthetic pathway for Fentanyl done by Janssen Pharmaceuticals using N-benzyl-4-piperidone as a starting material.

In 1980 another major synthetic pathway for fentanyl was introduced called the Siegfried method (Figure 4).[27] This method used phenethyl-tosylate, phenethyl-bromide, or piperidone to make the *N*-phenethyl-4-piperidone (NPP) precursor. The NPP (**1**) is then reacted with aniline (**2**) to give an imine derivative (**3**). The imine derivative is then reduced to 4-Anilino-N-Phenethyl-Piperidine (4-ANPP). Following this, the 4-ANPP is reacted with propionyl chloride to give fentanyl (**4**).[28] Since these synthetic pathways have become public knowledge, others have discovered different routes; however, the Janssen and Siegfried methods remain the most well-known and utilized for synthesizing fentanyl.

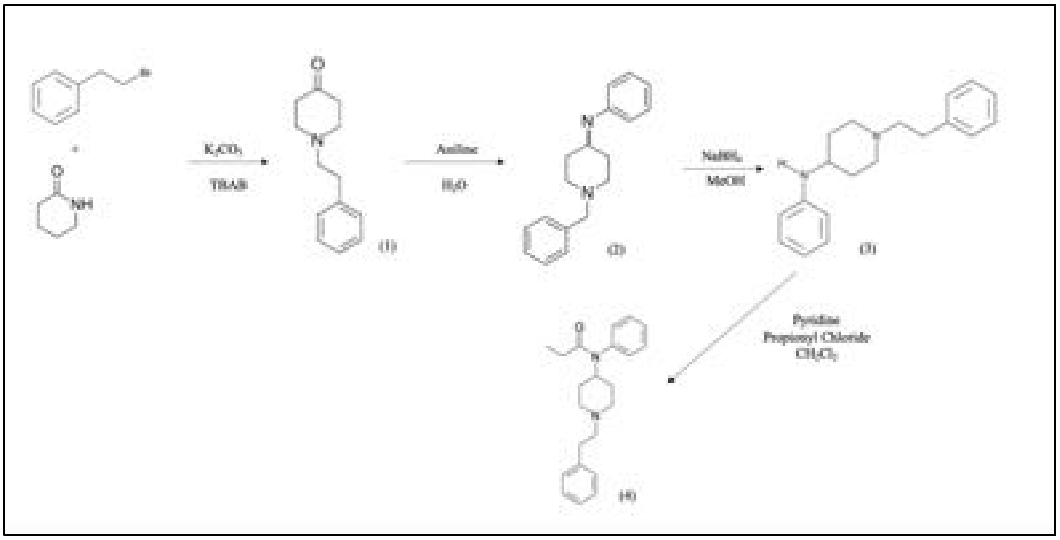

Figure 4. Represents the Siegfried synthetic pathway for Fentanyl using N-phenyl-4-piperidone (made by phenethyl-tosylate, phenethyl-bromide, or piperidone) as a starting material.

2.2.3 Fentanyl Degradation

Fentanyl degrades by multiple pathways. Bazley et al. published a review paper outlining the scientific research, including thermal degradation and oxidative degradation, to discover the degradation products of fentanyl.[29]

2.2.3.1 Thermal Degradation

Thermal degradation is the process of decomposing a chemical compound by heat. Figure 5 (license ID 1361825-1) shows the degradative pathways of fentanyl discovered in three studies by Garg et al. Manral et al. and Nishikawa.[29–32] The first study to identify any degradation products of fentanyl due to thermal degradation was completed by Garg et al.[29,30] In this study, fentanyl powder was heated to 350 °C for 5 minutes, producing five degradation products. These products were identified using the National Institute of Standards and Technology (NIST) mass spectral library as *N*-phenylpropanaminde (NPPA) (**11**), norfentanyl (**12**), 1-phenethylpyridinium, salt (1-PEP (**13**)), 1-styryl-1*H*-pyridin-2-one (1-SPO (**14**)) and 1-phenethyl-1*H*-pyridin-2-one (1-PPO (**15**)). In 2012

Nishikawa investigated fentanyl pyrolysis using pyrolysis gas-chromatography mass spectrometry (Py-GCMS) on transdermal fentanyl patches and heroin-fentanyl mixtures from 200 °C to 450 °C in 50 °C increments.[29,31] Six degradation products, pyridine (**16**), styrene (**17**), benzaldehyde (**20**), aniline (**19**), phenylacetaldehyde (**18**), and *N*-pheylpropanamide (**11**), were detected at all six temperatures while 3-methylpyridne (**21**) was seen only at 450 °C. Nishikawa also detected several different degradation products of fentanyl hydrochloride under both aerobic and anaerobic conditions at 750 °C. *N*-phenylpropanamide (**11**), pyridine (**16**), styrene (**17**), phenylacetaldehyde (**18**), and benzaldehyde (**20**) were products detected under all conditions, while aniline (**19**), (2-chloroethyl)benzene (**23**) and despropionyl fentanyl were detected under anaerobic conditions and benzyl chloride (**25**) and norfentanyl (**12**) were detected under aerobic conditions. Using high-performance liquid chromatography (HPLC), Manral et al. discovered compounds at 500 °C, including *N*-phenthyl-1,2,5,6-tertahydropyridine (**22**) and *N*-phenylpropanamide (**11**), while at 750 °C 3-methylindole (26) and phenylisocyanate (27).[29,32] In all the research conducted using thermal degradation, the most common and consistent compound seen was *N*-phenylpropanamide.

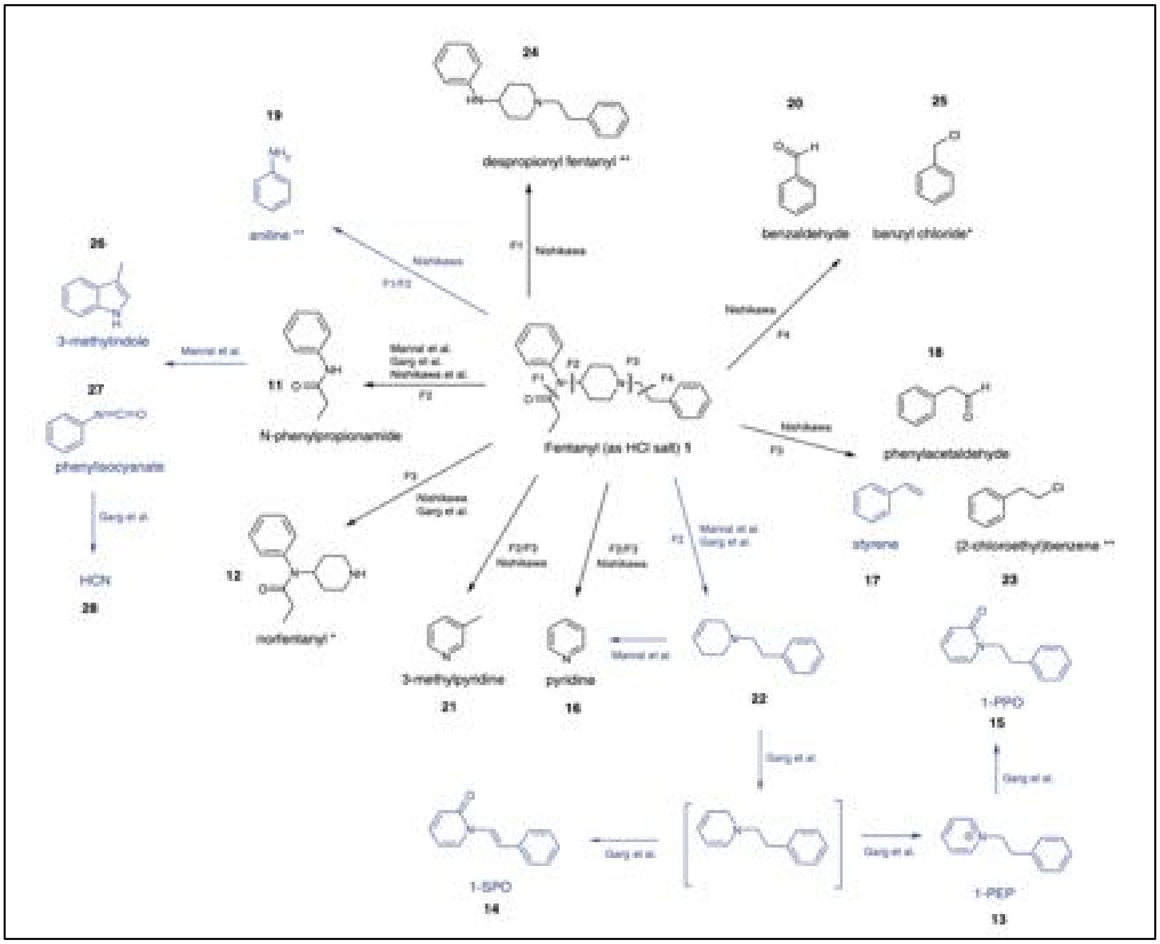

Figure 5. All known degradative pathways for fentanyl discovered through thermal degradation by Garg et al. Manral et al., and Nishikawa.

Based on these studies, fragmentation for primary pyrolytic products is thought to occur at the C-N bond at the 4-piperidine position. At this position, Manral et al. and Nishikawa believe there is an elimination of the propionanilide group and the β-hydrogen on the piperidine ring. Garg et al. believed the same process takes place but is solvent-mediated.[29–32] Several proposed mechanisms distinguish between secondary and primary pyrolytic products. For example, Garg et al. propose that 1-PEP, 1-SPO, and 1-PPO are produced due to nitrogen oxidation of *N*-phenethyl-1,2,5,6-tetrahydropyridine followed by protonation and rapid dehydration.[31] It is also suggested by Manral et al. that the formation of pyridine through the unsaturated piperidine ring of *N*-phenethyl-1,2,5,6-tetrahydropyridine as well as intramolecular rearrangement of *N*-phenylpropanamide produces the secondary products 3-methylindole and phenylisocyanate. Hydrogen cyanide is also thought to be produced from phenylisocyante at 750°C. [32]

2.2.3.2 Oxidative Degradation

Oxidation is the second most common pathway of degradation for pharmaceuticals.[33] Figure 6 (license ID 1361825-1) shows the oxidative pathways in fentanyl that were discovered in two different studies by Qi et al and Xu et al.[29,34,35]

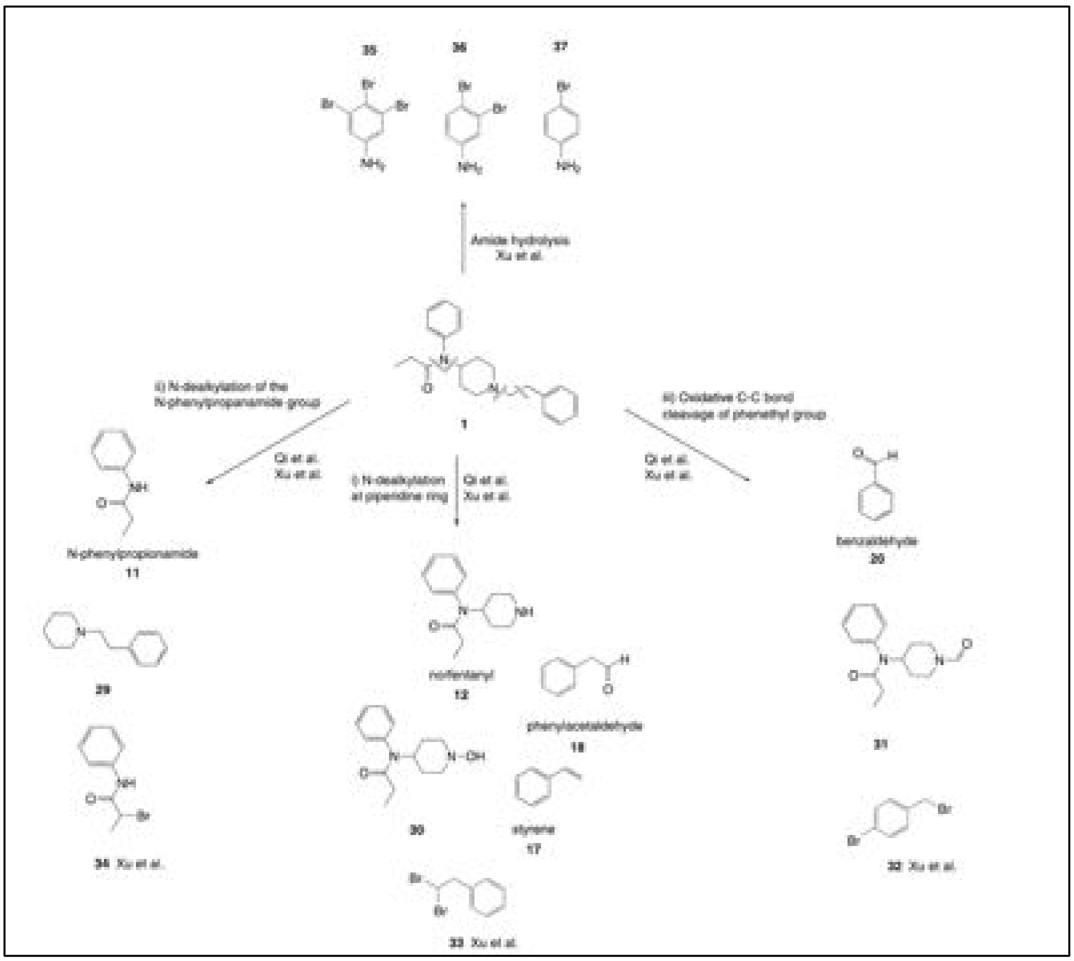

Figure 6. Degradative pathways for fentanyl discovered through oxidative degradation by Qi et al. and Xu et al.

In 2011, Qi et al. published an article investigating the oxidative degradation of fentanyl using peroxide and hypochlorite solutions.[34] The possible degradation products found when using an aqueous peroxide solution were identified as phenethylene (styrene (**17**)), benzaldehyde (**20**), benzenemethanol, benzeneacetaldehyde, N-phenylpropanamide (**11**), N-Phenyl-N-(4-piperidinyl) propenamide (**30**), N-(1-formylpiperidine-4-ys)-N-phenylpropanamide (**31**) and fentanyl. While using thricholorcyanuric acid produced benzaldehyde (**20**), benzonitrile, 2-chlorobenzaldehyde, 4-chlorobenzaldehyde, 2-chlororbenzyl chloride, 2,6-dichlorobenzyl chloride, N-phenylpropanamide (**11**), 4-chloropropionanilide and propanil. Xu et al. used a mixture of sodium bromide and sodium sulphite to induce oxidative degradation of fentanyl in dichloromethane.[35] The degradation compounds found and identified were 2-dibromo-ethylbenzene (**33**), 1-bromomethyl-4-bromobenzene (**32**), N-phenylpropanamide (**11**), N-phenyl-2-bropropionamide (**34**), 3,4-dibromoaniline (**36**), 2,4,6-tribomoaniline (**37**) and 4-bromoaniline (**35**).

Based on the work done by Qi et al., three different degradation pathways of fentanyl caused by oxidative degradation were proposed.[34] The first pathway begins with N-dealkylation at the piperidine ring, forming styrene and a secondary amine, which can then be oxidized into an oxime. The second pathway is when N-dealkylation occurs at the amide site. The final pathway is the oxidation of the carbon atom located next to the nitrogen in the piperidine ring leading to the cleavage of the C-C bond. While Xu et al. proposes four pathways where the first and second pathways are consistent with that of Qi et al.[35] The third pathway is the oxidation of the α-C next to the benzene ring, while the fourth pathway is the further hydrolysis of the amide and bromination reaction.[35]

There are other many causes of degradation such as photo- and acid/base degradation. However, thermal, and oxidative degradation can provide data that is most applicable to degradation that occurs under normal environmental conditions. Since the bulk of illegal drugs spend most of the time being transported and exposed to the environment forcing thermal and/or oxidative degradation under the correct parameters may provide insight on what compounds can be produced during degradation under environmental conditions. This can make it easter to discern what degradation compounds may be helpful for identification purpose through instrumental use or even canine scent detection.

2.2.4 Fentanyl Analogs

An analog is a drug with a structure and physical properties similar to the parent drug but can have different chemical and biological properties.[36] Illegal fentanyl analogs may be produced clandestinely by modifying the chemical structure in specific locations; this allows for "new" drugs to be marketed and sold without immediate legal implications.[37] In 1986, there were several fentanyl analogs such as remifentanil, alfentanil, sufentanil, carfentanil, and thiafentanul were approved by the US (United States) government for medicinal use in both humans and animals.[38] As with fentanyl, these analogs have a medical purpose, resulting in a schedule II classification.[39] The threat of analogs was not taken seriously until they became the cause of overdoses. The first analog to cause multiple overdoses was alpha-methylfentanyl in California in 1979, followed by 16 overdoses in Pennsylvania in 1984 due to 3-methylfentanyl.[40,41] Due to these fatal occurrences, the US government passed the Federal Analogue Act in 1986. This act stated that any federal law should treat an analog of a controlled substance for human consumption as a Schedule I controlled substance however, this act is not easy to enforce since it is difficult to prove that a substance is similar in structure and intended for human consumption, such as in the case of the United States vs. Damon Forbes.[42] From 1979 to 2018, approximately 27 fentanyl analogs have been introduced to the illegal drug market.[43] The most popular fentanyl analogs, their DEA schedule and the date they were scheduled can be seen in Table 1.[48] As previously stated, a new drug must be investigated based on the eight-factor criteria to determine its proper DEA schedule. However, temporary scheduling helps control a dangerous drug during this investigation. When temporary scheduling began, it lasted one year and could extend for six months; however, in 2012, that period increased to three years.[44] This has been a primary tool in combating the appearance of new synthetic drugs, which is seen as a back-and-forth game between law enforcement agencies and illicit drug manufacturers.[44] This means that the illicit drug manufacturers are producing new analogs as the old analogs are being scheduled.

Table 1. The most used fentanyl analogs and their DEA Schedules Adapted from Armenian et al.

Fentanyl Analog	DEA Schedule	Date of Scheduling
Alpha-methylfentanyl	Schedule I	9/22/1981
Sufentanil*	Schedule II	5/25/1984
3-methylfentanyl	Schedule I	9/22/1986
Alfentanil*	Schedule II	1/23/1987
3-methylthiofentanyl	Schedule I	5/29/1987
Acetyl-alpha-methylfentanyl	Schedule I	5/29/1987
Alpha-methylthiofentanyl	Schedule I	5/29/1987
Beta-hydroxyfentanyl	Schedule I	5/29/1987
Para-fluorofentanyl	Schedule I	5/29/1987
thiofentanyl	Schedule I	5/29/1987
Beta-hydroxy-3-methylfentanyl	Schedule I	1/8/1988
Carfentanil*	Schedule II	10/28/1988
Remifentanil*	Schedule II	11/5/1996
Acetyl fentanyl	Schedule I	7/17/2015
Beta-hydroxythiofentanyl	Schedule I	5/12/2016
Butyryl fentanyl	Schedule I	5/12/2016
AH-7921	Schedule I	5/15/2016
Thiafentanil*	Schedule II	8/26/2016
U-47700	Schedule I	11/14/2016
Furanyl fentanyl	Schedule I	11/29/2016
4-fluoroisobutyryl fentanyl	Schedule I	5/3/2017
MT-45	NS	-

* Represents the schedule II drugs meaning these drugs still have several medicinal uses in both human and veterinary medicines

NS means that the drug is not scheduled.

2.2.5 Origins of Illicit Fentanyl

Since fentanyl has become a rising epidemic, it is essential to know the origin of the illegal supply. This will help understand how people are getting the drug and what measures need to be implemented to decrease its flow into the United States. Based on evidence collected by the DEA, the three major countries responsible for the supply of fentanyl into the US are China, Mexico, and India.

As seen in Figure 7, China is currently the primary source of all trafficked fentanyl and fentanyl-related materials in the United States.[45] Fentanyl is produced at low cost in China and shipped into the United States via the international postal system or express consignment courier.[8] However, with laws and controls placed on fentanyl by the US government the Chinese suppliers have devised alternative ways of getting fentanyl into the country. To combat these laws the suppliers in China began shipping directly to transnational criminal organizations (TCOs) in Mexico, Canada, and the Caribbean in the form of fully processed powder or precursors for the cartels to "assemble" the drug. Once the TCOs have possession, fentanyl will either be mixed into heroin or cocaine or pressed into pills with industrial pill pressers, which can then be purchased on the dark market. Since fentanyl travels mainly through the postal system, once it gets into the US, it can become difficult for government agencies to address the threat at the ports of entry because of the varying legal status of the substances and the large amount of parcel traffic through international mail. In 2018 Beijing and the Hong Kong Special Autonomous Region (SAR) placed restrictions on the precursors 4-anilino-N-phenethyl-4-piperidine (ANPP) and N-phenethyl-4-piperidone (NPP), limiting their shipment to the Mexican TCOs.[8] These restrictions were carried out in 2019 when China officially began controlling all forms of fentanyl. These new measures taken by the Chinese government have significant potential to limit the production of fentanyl and trafficking from China.

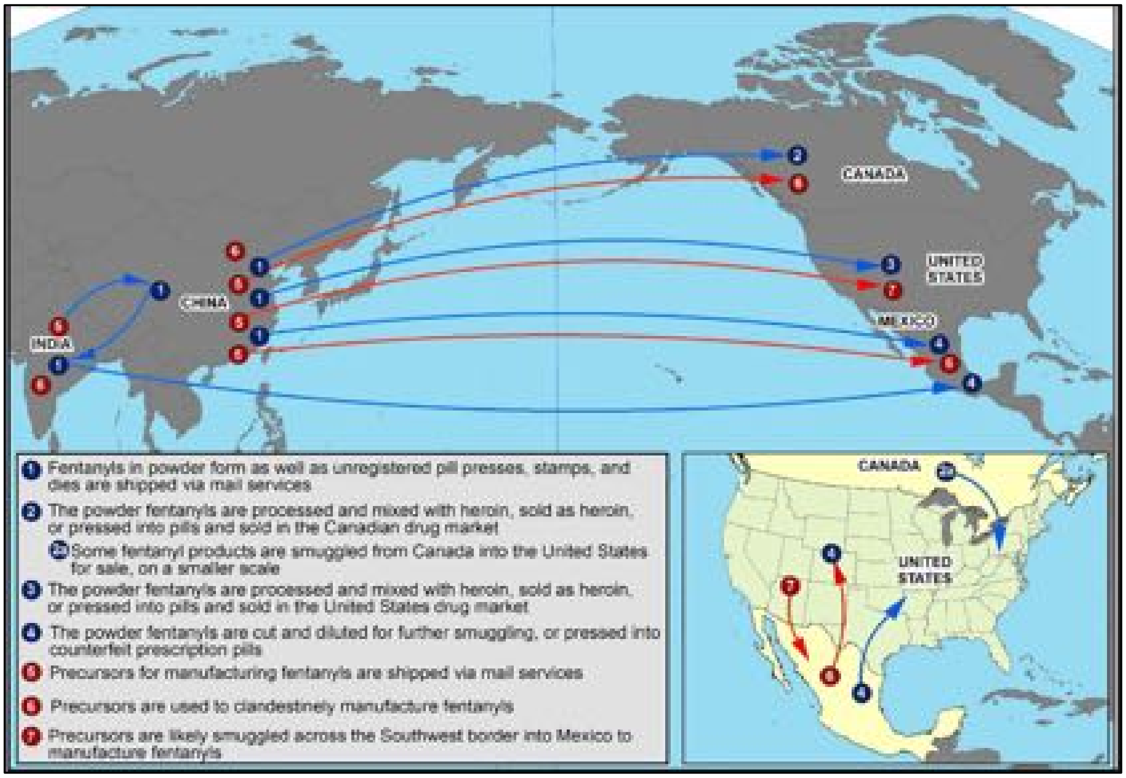

Figure 7. Flow of fentanyl into the United States from China, India, and Canada.

Not only does Mexico receive fentanyl and fentanyl-related material from China, but they have started to produce their own. In recent years, clandestine laboratories in Mexico have begun to use sophisticated processing methods to make their supply of fentanyl.[45] Since Mexico is the primary producer of heroin, when fentanyl is trafficked from Mexico to the US it is primarily mixed into heroin. Mexico has also been increasingly producing wholesale quantities of fentanyl pressed into pills to smuggle into the US.

Finally, starting in 2018, the production of fentanyl precursors began to shift from China to India because of China's new regulations on NPP and ANPP. Due to this shift in manufacturing, Indian nationals associated with the Sinaloa cartel supply the organization with NPP and ANPP. This resulted in the organizations' Chinese affiliates synthesizing fentanyl, which was then trafficked from India to Mexico.[45] With the production of fentanyl being transferred from China to India, the flow of fentanyl into the US will now become more diversified but the amount being trafficked will not likely be impacted and will remain the same.

2.2.6 Field Detection of Fentanyl

Since fentanyl is 100 times more potent than morphine, it takes a minimal amount to cause an overdose. This puts first responders and officers posted at the US ports of entry in immediate danger. To help protect them, the CDC provided key safety recommendations for when illicit drugs are present. These recommendations include wearing gloves, respiratory protection, and avoiding any actions that can cause the illicit drugs to become airborne. They also recommended not touching their eyes, nose, or mouth after touching a surface contaminated with illicit drugs and washing hands with soap and water after working in a contaminated area.[46]

While understanding the standardization and safety measures needed when handling illicit drugs is essential, it is also important to know and understand the field detection techniques available for law enforcement. These techniques include using portable instrumentation for detection, such as portable ion mobility spectrometry (IMS). IMS works by wiping a substrate across an item, such as a package or clothing, to collect residue of the suspected target substance (drugs, explosives, etc.). This substrate is then placed into a heated anvil where the temperature is raised to approximately 200 °C to volatilize the sample. Vaporous analytes are then mixed with gas phase ions and produce product ions. These product ions are then pulsed through an electric field of 200 V/cm, down the drift tube where they are separated based on their drift time.[47] In a study by John Wang, a portable IMS was used to identify fentanyl and seven analogs in less than 10 seconds within a 10 to 50 nanogram level.[48] Another study completed by Verkouteren et al. used 11 different fentanyl samples including fentanyl, acetyl fentanyl and carfentanil to evaluate the use of IMS as a detection method. Authors found that all 11 compounds could be individually detected with in a 1-10 ng LOD. IMS can be helpful when deployed at airports, shipping yards, and the US border because of its high sensitivity, portability, efficient speed, and trainability. Other forms of field detection for fentanyl include portable infrared spectroscopy (IR) which gives insight into the molecular structure of an unknown compound based on its interaction with the infrared light. Based on a study completed by Ramsay et al. using portable IR, fentanyl was identified in 10% of the 425 opioid samples with a 7% standard deviation making it a suitable option for field detection of fentanyl.[49] Some advantages to using portable IR include the durability of the instrument and the fast analysis speed while some disadvantages include a lack of spectral library information, data output formats.[50] There is also the use of colorimetric testing, where it has been found that the eosin Y test which is a mixture of the Marquis and Scott Tests will show a positive result for fentanyl if the color turns pink.[51] Colorimetric testing is widely used because of its accuracy, rapid response, ease of use and low cost. However, colorimetric tests are non-specific, cannot determine the individual components of a mixture, and some reagents can be prone to light and temperature sensitivity.[52] While all of these techniques have their own individual disadvantages, they do share one major disadvantage in common, they all require the user to come in contact with fentanyl.

Portable Raman Spectroscopy is one of the very few techniques that does not require the user to come into contact with fentanyl. This technique currently being used by law enforcement as a "point and shoot" detection of fentanyl. When using Raman spectroscopy, the interaction between the laser beam and the sample molecule will result in light scattering. Most of the photons will be elastically scattered (Rayleigh scattering), while a small amount of photons will be inelastically scattered (Raman scattering). The resulting plot will then give the intensity of Raman scattered light as a function of the frequency difference from the incident radiation, also called Raman shifts. These shifts will then provide information about vibrational and rotational transitions unique to every molecule.[53] Using portable Raman in 2018, Sherman et al. analyzed 52 known positive street fentanyl samples and determined the limit of detention to be 25 μg/mL.[54] Another study conducted by Gozdzialski et al. determined that portable Raman was successful in identifying and quantifying fentanyl by determining that their samples contained fentanyl concentrations between 12%-17% w/w.[49] Raman spectroscopy is non-destructive, highly selective, allows samples to be analyzed through translucent containers, and requires little to no sample preparation; however, it lacks the ability to detect obscured contraband, such as hidden under floorboards, behind drawers, or in other enclosed areas. Finally, Raman would fail to detect samples enclosed in darker or non-translucent containers since they would absorb the lasers energy. To combat the drawbacks of portable instrumentation, canines can be used as a biological field detection tool for fentanyl; however, because of the health risks and high potency fentanyl imposes, canines are not being used to their full potential.

2.3 Canines

2.3.1 History of Canine Use in Law Enforcement

In the 1970s, the United States began to use canines regularly in law enforcement. Today canines are used for various tasks, such as detecting drugs, explosives, arson/ignitable liquids, currency, firearms, and even human/non-human diseases.[10] This raises the important question, what makes canines superior in odor detection? Examining the canines' olfactory system closely is important to answer that question.

2.3.2 The Process of Olfaction in Canines

Olfaction, or the sense of smell, allows mammals to process and discriminate chemical compounds (odorants) from their environments.[55] Olfaction is also very important because it provides essential information for sustaining life, such as communication, locating food, finding mates, and even avoiding danger; making sense of smell is one of the most critical ways mammals interact with their environments.[56]

Just as with humans, when canines inhale, air enters through their nostrils; however, unlike humans, their nasal passage is split into two pathways separated by a septum.[57] In these passages, the volatile compounds in air travel through currents created by folds of bone called turbinates. As these volatile particles travel through the turbinates, they are filtered into the respiratory or olfactory epithelium. The olfactory epithelium receives approximately 12-13 % of each breath which is composed of small molecules that have not attached themselves to the mucous layer of the turbinates.[57,58]

In canines and most mammals, within the main olfactory epithelium (MOE), are olfactory receptors (ORs) which are bipolar transduction neurons that help to transmit olfactory information to the brain and are consistent with the ability to recognize a wide variety of diverse odorants.[59,60] Once inhalation has occurred and the compounds have been split between the pathways, the olfactory system will begin signal transduction to the brain which begins at the receptor-odorant interface. Within each OR cilia are extracellular portions that bind directly to the odorant and once the odorant is bound a signal will be transmitted to the olfactory bulb.[57] Once in the olfactory bulb, each odorant is recognized by a unique combination of activated ORs where, it will be recognized by on or many ORs.[10] This allows for one single receptor to recognize many different odors of even one odor be recognized by many other receptors.[61] Furthermore, the olfactory system's ability to recognize and characterize an odor is greatly increased based on a canine's previous familiarity with the odor or even an odor that is chemically similar. This allows a greater characterization and generalization of odors.[62] The full canine olfactory system can be seen in Figure 8.[62]

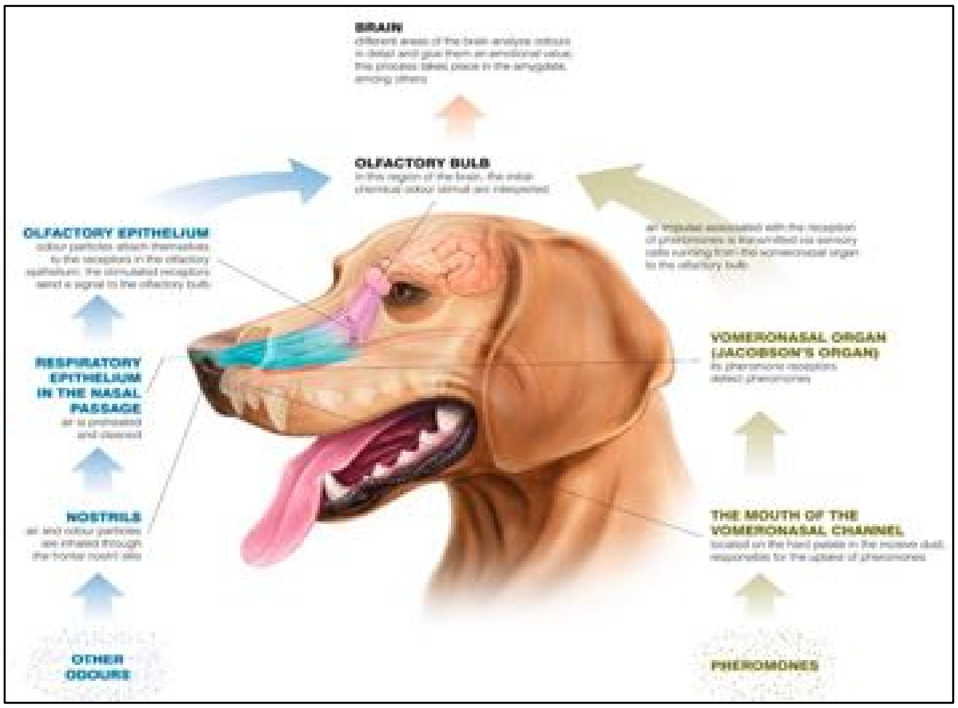

Figure 8.Complete process of canine olfaction.

Compared to humans, the canine olfactory system is vastly superior. The canine olfactory epithelium has a larger surface area and approximately 30 % more ORs.[10] Humans are known to have around 5 million ORs, while canines have about 200-300 million ORs. Canines also have approximately 1100 receptor genes compared to humans, who have 802 receptor genes.[57] This increases the sensitivity in canines and the selectivity allowing them to recognize a much larger variety of odors compared to humans. Due to their high selectivity, sensitivity, power of discrimination and low false positive rate canines remain the preferred option for law enforcement when compared to analytical instrumentation such as gas chromatography, liquid chromatography, and mass spectrometry. Furthermore, canines have higher detection speeds, the ability to follow a scent to its source, the ability to detect an odor without interference from the environment and the capability to function without the needs of instruments such as power, solvents, or columns.[62,63] A detailed comparison of canines to analytical instrumentation can be seen in Table 2 (license # 5562040348742)).[64] This makes canine odor detection a critical tool for military and law enforcement operations.

Table 2. Comparison of detector canines and analytical instrumentation (adapted from Furton et al.)

Aspect	Instrumentation	Canine
Duty Cycle	Theoretically can operate for 24 hours	Approximately a 1-hour search duration (depending on conditioning and environmental conditions)
Calibration Standards	Can be run simultaneously	Run individually
Operator/handler influence	Less of a factor	A potential Factor (depending on canine/handler training)
Introduction of novel targets	Time consuming	Rapid
Environmental conditions	Less affected	May adversely affect (ex. high temperature limiting search time)
Instrument lifetime	Generally, up to 10 years or more	Generally, 6-8 years
State of scientific knowledge	Well known	Known but still emerging
Selectivity (from interferences)	Sometimes problematic	Very good
Sensitivity	Very good	Very good
Overall speed of detection	Varies but generally slower	Rapid (in-real time)
Mobility/portability	Has increased in the last 10 years but still emerging	Very versatile
Capability of remote guidance and integration	Currently limited	Straightforward
Scent to source	Difficult with the current technology	Natural and quick
Intrusiveness	Varies based on mobility and size	Often innocuous (breed dependent)
Initial Cost	Between $20,000-$60,000	Approximately $10,000
Annual Cost	Around $5,000 (for a service contract)	Approximately $3,000 (for vet and food)

2.4 Collection Methods for Volatile Organic Compounds

Volatile organic compounds (VOCs) have a high vapor pressure and low water solubility. VOCs are low molecular weight compounds with compositions that allow them to evaporate under normal standard temperature and pressure.[64,65] VOCs can be identified as a compound with an initial boiling point less than or equal to 250 ºC when measured at the standard pressure of 101.3 kPa.[66] Many of the commonly known VOCs are manufactured and naturally occurring compounds used in producing paints, pharmaceuticals, and refrigerants, preservatives, and perfumes. VOCs are emitted into the gas phase by solid or liquid samples and usually contain hydrogen, oxygen, fluorine, chlorine, bromide, sulfur, or nitrogen.[66] They can then be further categorized into three classes by the ease at which they are emitted. These classes include very volatile organic compounds (VVOCs), volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs). [66]

Just as with any type of sample VOCs have various types of collection and analysis methods. Some collection methods for VOCs include the collection of whole air using different containers, such as steel canisters.[67] Collecting volatiles using a steel canister can be performed either sub-atmospheric (passive) or pressurized using a pump. This type of collection vessel allows for duplicate analyses and no effects from moisture or degradation. However, it can require a complicated sampling apparatus and rigorous cleaning is needed between samplings. It is also possible to collect VOCs by preconcentrating them onto a sorbent. These include the use of tubes packed with sorbents such as Tenax which is or trapping medium to high boiling point VOCs. One of the most widely used methods for collecting VOCs is headspace extraction. This includes the use of static headspace (SHA), dynamic headspace (DHA), single drop microextraction (SDME), headspace sorptive extraction (HSSE).

SHA is known to be one of, if not the simplest methods for headspace extraction. When using SHA, the sample is placed into a closed vessel, such as a vial, and allowed reach equilibrium. It is possible to heat during this step to drive off more VOCs but it not necessary to heat for equilibrium to be reached. This equilibration period depends on many factors including the temperature, vapor pressure and concentration of the sample as well as the phase ratio. A sample is considered to be at equilibrium when the concentration of vapor phase and the solid/liquid phase are no changing.[68] One example of SHA is gas tight syringe extraction where, once equilibration is completed an aliquot of the sample is taken and injected directly into the inlet of the instrument being used. A schematic of gas tight syringe SHA can be seen in Figure 9.

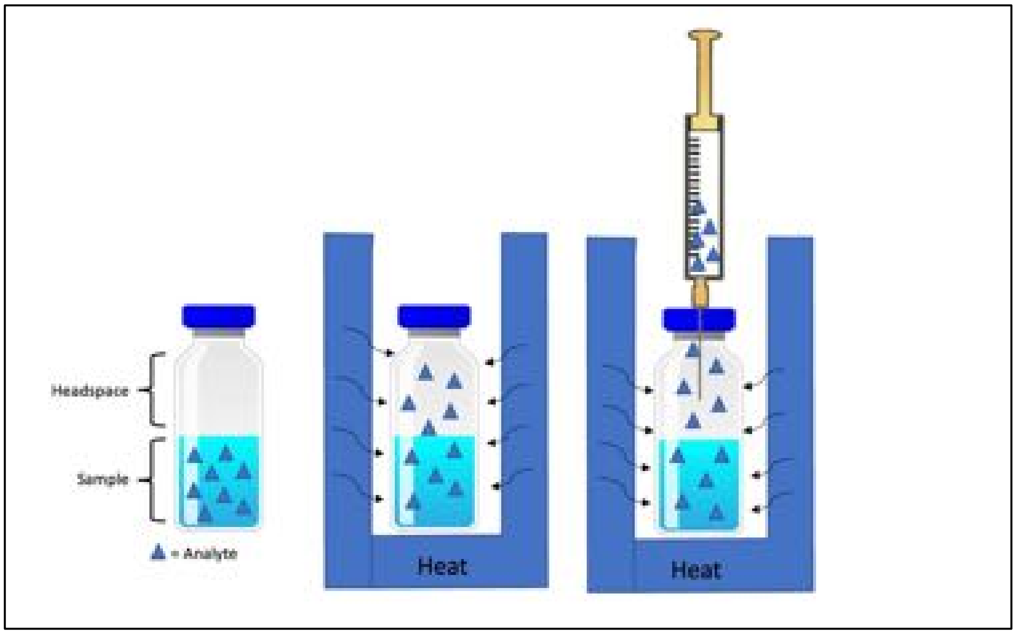

Figure 9. Schematic for a gas tight syringe static headspace sampling.

Another example of SHA is SPME, where instead of drawing up a sample of headspace into a syringe the analytes are adsorbed/absorbed and preconcentrated onto a silica fiber coated with a polymer. The polymer coating is chosen based on the sample being analyzed. The fiber is then directly injected into the inlet of the GC when the analytes are desorbed and analyzed.

There are several advantages to using SHA including quick and simple sample preparation, not solvent interferences, and a low risk of artifacts.[69] However, when compared to other headspace techniques SHA lacks sensitivity for compounds with lower volatility.[70] In particular, SPME is known to be relatively straight forward when compared to other methods as well and cheaper. The efficacy of SPME is dependent on the conditions of the extraction as well as the complexity of the sample matrix.[71] However one major drawback to using SPME is poor reproducibility.

Unlike SHA, with DHA, most of if not all of the vapors from the headspace are dynamically pulled or pushed into a canister, a sorbent coated tube, or directly into the inlet of the GC.[72] One of the most widely used methods of DHA is purge and trap where, a constant flow of gas is introduced over the sample, the vapors from the headspace then travel up the out of the outlet, and the analytes will be trapped. The trap used will typically consist of a cartridge that is packed with an adsorbent or can be a cool spot in the column or more commonly a thermal desorption tube. This trap will release the analytes using thermal desorption. A basic schematic of purge and trap DHA can be seen in Figure 10. Another form of DHA used is membrane extracted with sorbent interface (MESI). When using MESI as a headspace technique the volatiles from the sample pass through a membrane into a flow of carrier gas. While passing through the membrane the volatiles will partition accordingly and be trapped onto a sorbent or cryo trap. Once this is completed just as with other forms of DHA the trap is heated and the analytes are desorbed into the GC.[71]

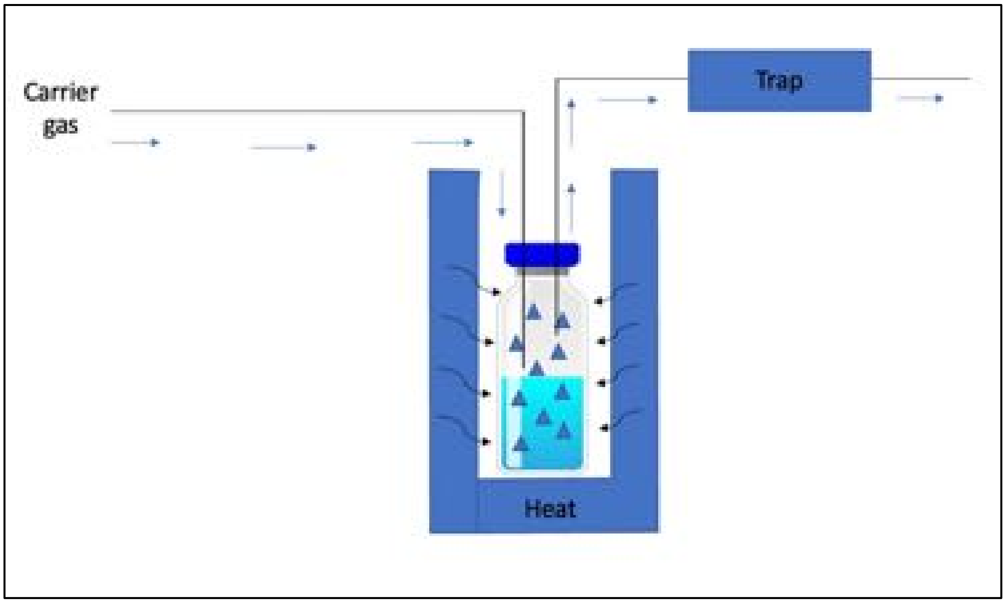

Figure 10. Schematic for a purge and trap dynamic headspace sampling.

Since DHA also allows for a larger amount of sample or even the entire sample over time to be delivered to the GC it provides better sensitivity and detection limits when analyzing samples in trace amounts.[71] However, DHA has been shown to be less sensitive for less volatile substances as well as poor repeatability and recovery.[71] Finally, in respect to purge and trap, when using a strong absorption trap DHA has been seen to generate artifacts from liable compounds due to the high temperatures needed for adequate desorption.[68]

HSSE is a headspace technique that follows the same basic principle of SPME but used a coated stir bar/rod or magnet rather than a silica fiber. In this technique the stir bar/rod or magnet is placed in the sample and then volatiles will adsorb onto the coated stir bar. Unlike SPME there is a larger surface area for the volatiles to adsorb, allowing for lower partition coefficients and better sensitivity. However, since there are very limited commercially available sample apparatuses for HSSE it is not a widely used method.

While SPME and HSSE uses a coated silica fused fiber/stir bar to extract headspace components, SDME exposes a syringe needle to the headspace with a single drop of solvent suspended on its end.[73] The solvent chosen will depend highly on its vapor pressure since the volatiles present in the headspace that have a higher affinity to the solvent will then be absorbed. Once extraction is complete, the drop of solvent will be drawn back into the syringe and injected into the GC. While this method is highly selective it has a limited application since there is a low number of suitable solvents.[74]

There are various types of headspace techniques where the choice of technique used depends on the information wanted as well as the instrumentation available. A summary comparison of the advantages and disadvantages of all the techniques discussed can be seen in Table 6. Based on the information seen in Table 3, (license # 1366665-1) when compared to the other techniques, SPME, just like DHA, offers a preconcentration step.[71] Finally, compared to the other techniques, SPME allows for the selection of the fiber coating based on the analytes polarity and molecular weight to allow for higher selectivity and better extraction efficiency while having the benefit of being low cost. SPME was chosen for the research discussed herein because of its ability to detect trace amounts of VOCs such as those seen in solid fentanyl as well as its ability to be brought into the field for sampling and be non-contact.

Table 3.Main advantages and disadvantages of the discussed headspace techniques (adapted from Wojnowski et al).

Technique	Main Advantages	Main Disadvantages
SHA	• Relatively straightforward • No sorbent • Wide range of applications, including screening	• No preconcentration stage • Applicable only to very volatile and volatile substances • Nonselective
DHA	• Effective enrichment stage • Short extraction time	• Low extraction efficiency for less volatile substances • Low recovery • Poor repeatability
SPME	• Straight forward procedure • Relatively inexpensive equipment • Good recovery • Fiber coating can be tailored to the analytes by polarity and molecular weight • Effective preconcentration step	• Relatively small surface area or volume of liquid coating on the fiber • Less useful for the determination of very volatile substances • Sorption is highly dependent on the extraction temperature
SDME	• High selectivity	• Limited applications due to a relatively small number of adequate solvents
HSSE	• Compatible with a majority of available GC devices • Large sorptive area	• Poor reproducibility • Few commercially available solutions

2.5 Odor/Training Aid Chemistry

Just as with many other environmental, pharmaceutical and food products the illegal substances detector canines are trained to find are made up of VOCs. These VOCs can also be referred to as odorants or the compounds that the canines associate with the target material's odor. It is from these odorants that canines have come to identify and detect their targets.[75] A single target can be composed of numerous odorants making it important to determine which one(s) a canine is associating with the target. To make this determination the first step is to extract the odorants from the target using one of several different techniques such as headspace analysis. Since this extraction is being done in the vapor phase it can be affected by parameters such as temperature and vapor pressure (volatility). Temperature can have a major effect on how much of the odorants will be available in the headspace for analysis. For example, it has been seen that higher temperatures will allow for more odorants to be released into the gas phase.[76] Vapor pressure can affect extraction because compounds with a higher vapor pressure will be readily emitted into the vapor phase, while compounds with a lower vapor pressure will not be as easily emitted into the vapor phase.[65] Once extracted, GC-MS or other analytical techniques, such as IMS, can be used to can help to determine which odorants in the headspace the canines may associate with the target odor. Using scent detector canines can help to determine what odorant(s) in the headspace simulate the odor of the target material. That odorant(s) can then be developed into one of several types of training aids.

While canines can be trained on the target material it can be challenging because the target material can be difficult to access or dangerous to use. To combat this, trainers, have the option of using various types of training aids that may or may not contain the target material. One common type of training aid is a pseudo-odor, also referred to as a mimic. Mimic development initially starts by identifying the major chemical components that comprise the headspace of the target material. These headspace compounds are also defined as the odorants that make up the odor of the target material. Mimics have several advantages, such as being used as a training material for substances that are regulated, making canine training safer and more efficient and providing access to substances to a larger number of canine teams. However, the use of mimics can also have drawbacks including producing a larger amount of odor than the target material, which can not only be hazardous to the canine but can also alter their perception of the odor and influence their threshold.[11] For example, when developing a mimic for MDMA Macias et al. found that varying the levels of piperonal (odorant of MDMA) in the training aid varied the canine's threshold and affected their ability to detect the aid.[77] Furthermore, mimics use materials that help to absorb and regulate the odor release of the headspace components, such as cellulose, alumina, and polymer bags. These materials also produce odor which can change the perception of the target odor. Using blanks and negative controls can help train canines off these odors during their training sessions to combat this. Finally, the diffusion rate of the odorants from the materials used in mimic production can be affected by the compounds vapor pressure, polarity, and molecular weight. While mimics are a popular option for canine training there are various other training aid options.

To combat the disadvantages of mimics while still allowing for the access that they provide, a variety of or non-pseudo training aids have been developed. The first alternative training aid to mimics is a dilution, where a small amount of the target material is mixed into a large amount of an inert solid or dissolved into an inert liquid. While dilutions provide easy-to-make nontoxic training aids, the diffusion rate of the target material from the matrix can be affected by vapor pressure, polarity and molecular weight of the target material.[11] Encapsulations are another type of non-pseudo training aid where a trace amount of the target material is placed into a matrix. Unlike dilutions, encapsulations utilize devices such as microspheres to contain the target material/matrix mixture. While encapsulations help render the materials safe, the interactions between the matrix and the target material can alter the odor profile.[11] Unlike a dilution or encapsulation, ad/absorption allows for the safe use of a target material for training by ad/absorbing its odor onto a substrate. To create this type of training aid, a substrate such as cotton is exposed to the headspace of the target material. It can absorb or adsorb the compounds in the odor profile during this exposure. These compounds are then released over time. This training aid has been used to help lower canine thresholds and make hazardous materials safer to transport; however, drawbacks include each compound interacting differently with the substrate making one matrix not applicable for all targets. The final type of non-pseudo-type training aid is liquid extractions which remove the components of the odor profile from the target material using a solvent. The target material is removed, and the solvent is used for canine training. Little research has been done using this type of training aid, but what has been done shows high selectivity and sensitivity once canines are fully trained.[11,78,79]

While training aids generally have their advantages, there are major considerations that need to be taken when developing a new training aid. These considerations include the lack of knowledge connecting odor production, odor detection, and canine behavioral identification.[11] This can cause difficulty in determining what targets must be selected for training aids, as canines may perceive odor differently. Another thing to consider when developing a training aid is the matrix effects caused by production materials such as plastic bags and sorbent materials surrounding the target odor. Since these matrices may produce large amounts of odor, they can interact or compete with the target odor, altering its perception. These interactions can be based on the chemical properties of the target, such as vapor pressure, polarity, solubility, and molecular weight, and how they influence the targets release from the matrix.[11] Finally, it is important to understand canine threshold, or the minimum concentration of odor detected, and how it will affect the perception of the training aid. Canine thresholds are dependent on the target vapor pressure and concentration and have been shown to vary across targets.[80] Canine thresholds can be manipulated through training and exposure to decreasing amounts of the target odor. Developing a new training aid is a complex process that requires an intricate knowledge of the target material’s physical properties such as its vapor pressure, solubility and even its molecular weight. The research discussed herein will outline the process of developing a training aid mimic for fentanyl based on its physical properties as well as past literature that explored training aid mimic development.

2.5.1 Previous Research in Training Aid Mimic Development

In 2002, a training aid mimic was developed for cocaine by Furton et al.[81] In this experiment, the authors used SPME-GC-MS to discover headspace components that make up the vapor profile of street cocaine. It was determined that the headspace components of cocaine included methyl benzoate, benzoic acid, methyl *trans*-cinnamate, anhydroecgonine methyl ester, *trans*-cinnamic acid, and ecgonine methyl ester. These compounds were then tested using cocaine-trained substance detection canines in a field trial. For these trials, 9-inch round galvanized steel boxes with six small holes were used to hold the test samples and placed into a temperature and humidity-controlled area. Solutions containing different amounts of cocaine and the headspace components, were prepared in chloroform and the samples equilibrated in the boxes for 20 minutes before the test. The tests were run double-blind so the dog handlers could not influence their canines to alert to the correct target.[81] The results of the canines field tests using 15 cocaine-trained detector dogs can be seen in Table 4 (license #5566100947911).[81] Based on the results it was determined that the only compound in the headspace of cocaine to elicit a positive response was methyl benzoate at levels 1 to 10 μg, however; when the canines were tested on any other byproduct or cocaine itself no alerts were recorded.[81] The results from this study align with literature that proves instead of canines alerting to the parent compound as a whole (cocaine), they are alerting to a compound or mixture of compounds present in the vapor profile of the parent compound (methyl benzoate).

Table 4. Results of detector-dog test of cocaine samples (cocaine HCl and Cocaine base) and the cocaine headspace components (adapted from Furton et al).

Amount of cocaine spiked on the substrate	Amount of byproducts spiked on the substrate	No alert canine	Alert by canine
0	0	8	0
0.1 mg cocaine HCl	10 µg ecgonine	8	0
0.1 mg cocaine HCl	10 µg ecgonine methyl ester	8	0
0.1 mg cocaine HCl	10 µg benzoic acid	8	0
0.1 mg cocaine HCl	0	11	0
0.1 mg cocaine HCl	1 µg methyl benzoate	7	4
0.1 mg cocaine HCl	10 µg methyl benzoate	2	9
1 g cocaine HCl	0	9	1
0	0	7	0
0.1 mg cocaine base	10 µg trans-cinnamic acid	7	0
0.1 mg cocaine base	10 µg methyl trans-cinnamate	7	0
0.1 mg cocaine base	10 µg ethyl benzoate	7	0
0.1 mg cocaine base	1 µg methyl benzoate	7	3
0.1 mg cocaine base	10 µg methyl benzoate	1	8
1 g cocaine base	0	11	1

Vu conducted a similar study in 2001 to determine the active odor of methamphetamine.[82] In this study, 12 samples of methamphetamine were investigated using headspace SPME, including illicit samples, methamphetamine base, and reference-grade methamphetamine. When comparing 9 of the 12 samples, seven compounds were found in common, including 1-choloro-P2P, 1-phenyl-1,2-propanedione, 1-phenyl-2-propanone, benzaldehyde, acetic acid, 3-phenyl-3-buten-2-one and acetophenone as seen in Table 4 (license # 1364266-)1.[82]

Table 5. List of the seven common compounds found in the headspace of 9 out of the 12 methamphetamine headspace samples.

	1-chloro-P2P	1-phenyl-1,2-propanedione	1-phenyl-2-propanone	Benzaldehyde	Acetic Acid	3-pheyl-2-buten-2-one	Acetophenone
135328A1	++++	+	++++	+	+	-	-
135328B1	+	+	++++	+	-	-	-
135328C1	+	+	++++	++	+	-	-
135328D1	++++	+++	++	++++	+	-	-
113544_1	++++	+++	+++	+++	+	-	-
134677_1	+	+	++++	-	-	-	-
13512511	-	+	++++	+	-	+	-
13512521	+	+++	++++	++++	++	-	-
135255_1	+++	++	++++	+++	+	-	-
I4s3_1g	...	...	-	-	...	...	...
Base11	...	...	+++	-			
P2P11	...	...	++++	+	...	...	...

As seen in Table 4, 1-phenyl-2-propanone and benzaldehyde were the most commonly occurring compounds; however, benzaldehyde was chosen as a test odorant since 1-phenyl-2-propanone is a controlled substance and challenging to access. Investigations were done using substance detection canines not previously exposed to methamphetamine. Once trained to detect benzaldehyde, the canines were able to locate illicit methamphetamine. Furthermore, when three additional untrained canines were trained on benzaldehyde, they located concealed methamphetamine 20 out of 22 times. These results indicated then when trained using benzaldehyde, canines could identify methamphetamine.

The studies completed by Furton et al. and Vu successfully utilized SPME-GC/MS to determine the headspace components of their respective parent compounds to determine their odorants. While both studies utilized canine field trials to verify their analytical findings, neither study used canines, both trained and not trained on their target material in the same study which would have helped to achieve a greater understanding the odorant.

Like these studies, the research discussed herein will use SPME-GC-MS to determine the compounds that make up the vapor profile of fentanyl to determine the possible odorant(s); however, unlike these studies, this research will use groups of canines both previously trained on fentanyl and untrained on fentanyl.

3. METHODOLOGY

3.1 Gas Chromatography-Mass Spectrometry (GC-MS)

A gas chromatograph (GC) consists of an inlet/injector port, a silica-coated capillary column, control equipment for a carrier gas, an oven, and a detector such as a flame ionization detector, an electron capture detector, or a mass spectrometer (MS) and a computer for data acquisition as seen in Figure 11 (license # 1364267-1).[80]

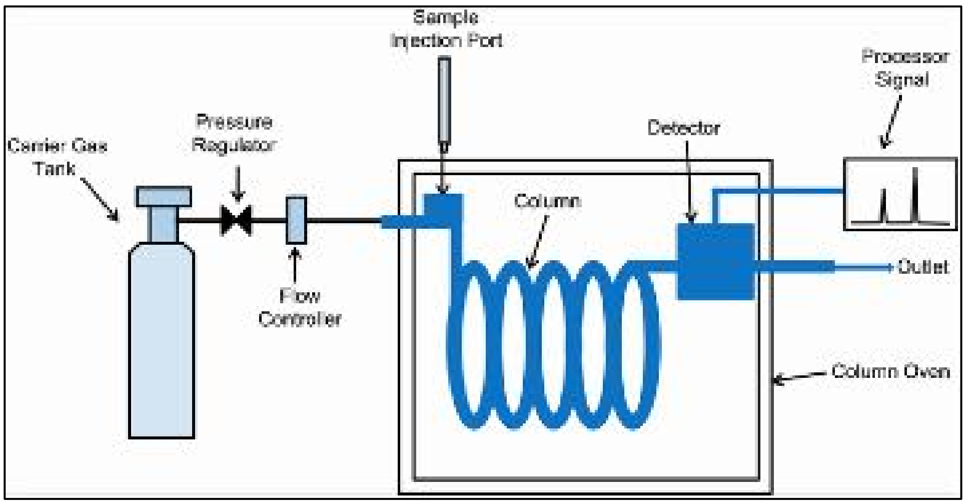

Figure 11. Schematic for a basic gas chromatograph.

A sample is vaporized into the gas phase when introduced into the inlet. For this vaporization to happen, the injection port must be held at a temperature higher than the boiling points of the compounds in the mixture. Once vaporized, the analyte is propelled through the column via a carrier gas. As the sample goes through the column, it will also move in and out of the stationary phase. Compounds with a lower affinity to the stationary phase will elute faster than those with a higher affinity for the stationary phase. The oven temperature will also affect this separation, where increasing the temperature allows compounds with lower boiling points to be eluted first. The elution of these compounds is then referred to as their retention time. Once through the column, the components will proceed to the detector.[83] Several different detectors can be used when separating a mixture with GC. These include flame ionization, thermal conductivity, electron capture, atomic emission, chemiluminescence, photoionization, and mass spectrometry. Table 6 lists typical GC detectors, the samples they are most suited to detect, and their limits of detection.

Table 6. Typical gas chromatography detectors and their limits of detection.

Type of Detector	Applicable Samples	Detection Limit
Mass Spectrometer (MS)	Tunable for any sample	0.25 to 100 pg
Flame Ionization (FID)	Hydrocarbons	1 pg/s
Thermal Conductivity (TCD)	Universal	500 pg/ml
Electron Capture (ECD)	Halogenated Hydrocarbons	5 fg/s
Atomic Emission (AED)	Element-selective	1 pg
Chemiluminescence (CS)	Oxidizing Reagent	Dark current of PMT
Photoionization (PID)	Vapor and gaseous compounds	0.002 to 0.02 ug/L

In the analytical chemistry field, using GC with an MS as seen in Figure 12 (license # 1364268-1), is considered an invaluable tool for detection.[83] MS can detect and identify compounds by separating them based on their mass-to-charge ratios. When an unknown sample is introduced to the MS, it is first ionized using techniques such as electron impact, chemical ionization, field ionization, matrix-assisted laser desorption ionization, inductively coupled plasma, and fast atom bombardment. In the research presented in this dissertation, electron impact (EI) ionization was used. Following ionization, the ions are then sent to the mass analyzer to be separated by their mass-to-charge ratios where they are then sent to the detector. When using MS, the ions can be detected using an electron multiplier, a faraday cup, or a photomultiplier conversion dynode. Once in the detector the ions will create a signal that will be sent to the computer for analysis.[83] While instrumental analysis is a vital part of any analytical examination it is important to first prepare the test sample using one of several techniques. With respect to the research presented here all samples will be extracted using headspace analysis, more specifically SPME.

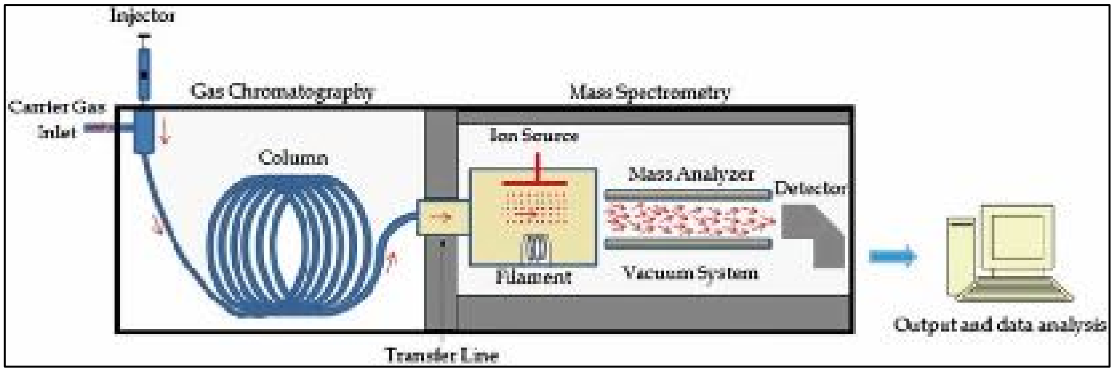

Figure 12. Diagram of a gas chromatograph-mass spectrometer.

3.2 Solid Phase Microextraction (SPME)

SPME was invented in 1989 by Janusz Pawliszyn. Since its inception, it has been widely used in many fields of analytical chemistry, from forensic science to environmental and food analysis.[84] SPME is a semi-quantitative, non-destructive technique used to sample the volatile organic compounds emitted by the target analyte. SPME has come to be a commonly used sampling methods because it allows for extracting and, pre-concentration, at one time.

The SPME apparatus a straightforward design taking the form of a modified syringe. It is comprised of a fiber holder assembly containing a 1-2 cm long, retractable SPME fiber. The fiber is a thin, fused-silica fiber coated with a liquid polymer, a solid sorbent, or a combination of both. The fiber is housed in a hollow needle that allows for its protection and is used to pierce the septum easily. The device itself operates simply, using the adjustable needle guide depth guide; the appropriate length is chosen, and the needle is then pierced into the septum and retracted by pressing the plunger down and exposing the fiber to the sample.[84] The apparatus can be seen in Figure 13 (license # 1366666-1).[85]

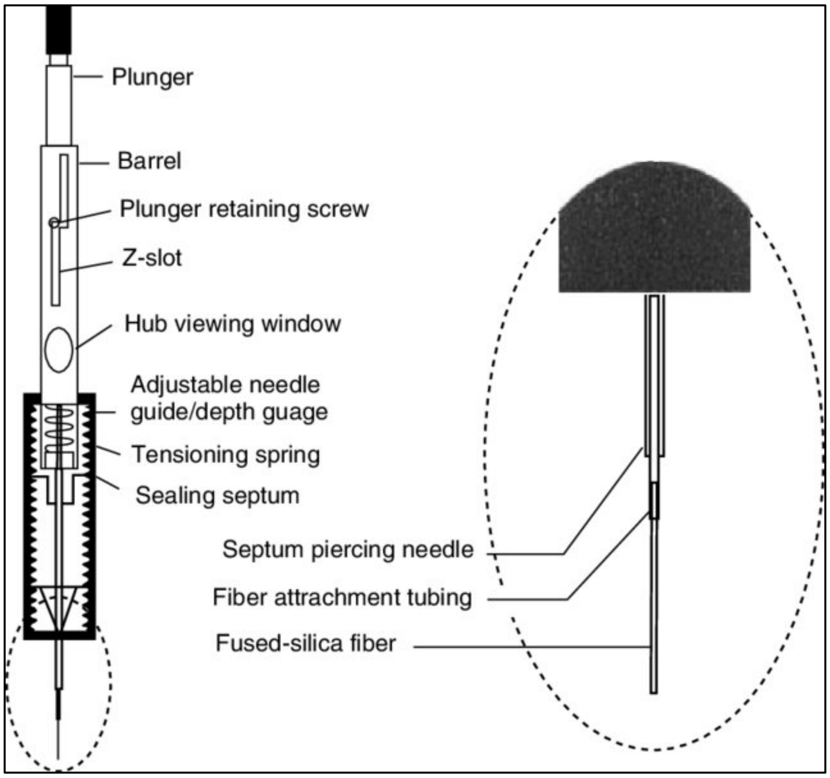

Figure 13. Schematic of a commercially available SPME apparatus.

SPME can be performed by one of two methods; direct immersion SPME, in which the fiber is directly inserted into the liquid sample, or headspace SPME, in which the fiber is exposed to the headspace of the sample, as seen in Figure 14. The theoretical principles of headspace SPME can be broken down into a three-phased system which includes a condensed phase (solid or liquid sample), a gas phase or headspace, and an extractant phase or a sorbent on a solid support which can be seen depicted in Figure 15.[86] The extractant phase is sent by the evaporation of analytes from the sample towards the extractive coating on the fiber. At the same time, the mass transport is controlled by the concentration gradient that is generated among the phases. The sorbent coating will then extract the target and non-target compounds until it reaches equilibrium. Once extraction is completed the analytes are desorbed of the fiber by exposing it to the inlet of the GC. The target analytes can be analyzed via analytical instrumentation such as GC-MS. Headspace SPME is preferred when analyzing volatile or semi-volatile organic compounds. As previously stated, SPME is a non-exhaustive technique that offers little to no solvent use, improved sample cleanup, and quick extraction/analysis time as well as the ability to be used for the extraction of trace amounts of samples.

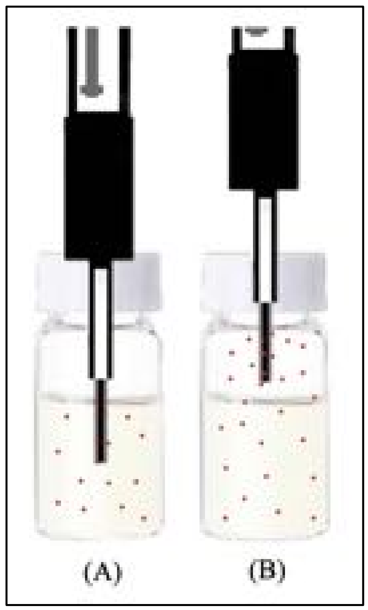

Figure 14. Direct immersion SPME (A) vs. headspace SPME (B).

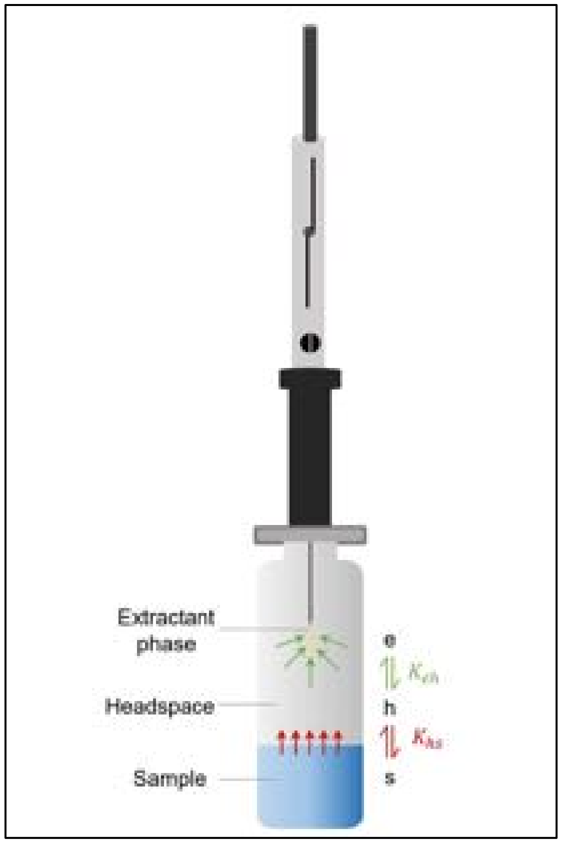

Figure 15. schematic of the three-phase system for SPME. Red is the sample headspace distribution, and green is the headspace extractant phase.

The extraction time is considered optimum when it yields the highest amount of target compounds adsorbed onto the fiber; however, the extraction kinetics, are influenced by several factors, such as the fiber coating and thickness. While fibers with thicker coatings do have a higher sample capacity and have shown higher recoveries; however, it has also been seen that as the fiber thickness increases, the required extraction length may increase as well.[87] The type of coating used will affect the selectivity of extraction, and equilibration time. Fibers with a polar coating will be used to extract polar compounds, while fibers with a non-polar coating will be used to extract non-polar compounds. The molecular mass of the compounds will also influence the coating used. The most used fiber coating is polydimethylsiloxane/carboxen/divinylbenzene (PDMS/CAR/DVB) because it is not only bipolar but can detect an extensive range of molecular masses. Table 7 shows a complete list of fiber coatings and their operating parameters.[84]

Table 7. Complete list of fiber coatings and their operating parameters.

Fiber coating and thickness	Recommended applications	Mechanism	MW	Polarity
100 µm PDMS	Volatiles	Absorbent	60-275	Non-polar
30 µm PDMS	Non-polar semi-volatiles	Absorbent	80-500	Non-polar
7 µm PDMS	Non-polar high molecular weight compounds	Absorbent	125-600	Non-polar
60 µm PEG	Alcohols and polar compounds	Absorbent	40-275	Polar
85 µm PA	Polar semi-volatiles	Absorbent	80-300	Polar
75 µm/85 µm CAR/PDMS	Gases and low molecular weight compounds	Adsorbent	30-225	Bipolar
65 µm PDMS/DVB	Volatiles, amines and nitro-aromatic compounds	Adsorbent	50-300	Bipolar
60 µm PDMS/DVB	Amines, nitroaromatic and polar compounds (HPLC use only)	Adsorbent	50-300	Bipolar
50/30 µm DVB/CAR/PDMS on a StableFlex fiber	Flavor compounds, volatiles and semi-volatiles, C3-C20	Adsorbent	40-275	Bipolar
50/30 µm DVB/CAR/PDMS on a 2 cm StableFlex fiber	Trace compound analysis	Adsorbent	40-275	Bipolar

3.3 Data analysis

During field testing, canines can exhibit a positive alert which is when they properly detect a target, a false positive which is when they incorrectly alert to a blank/distractor, a true negative which is when they correctly miss a blank/distractor, or a false negative is when they incorrectly miss a target. However, it is crucial to know the likelihood of a canine's alert being a true positive or a true negative. This is accomplished using the Predictive Value test, split into calculating Positive Predictive Values (PPV) and Negative Predictive Values (NPV). This method will test the likelihood of the canine responses being true positive or negative based on the values' prevalence, sensitivity, and specificity. When analyzing the results of this test, the closer the value is to 100%, the greater the likelihood that the value is true. Equations 2 and 3 represent how to calculate the NPV and PPV values. [88]

$$PPV = \frac{True\ Posive}{(True\ Postive + True\ Negative)}$$

Equation 1 Positive predictive value test

$$NPV = \frac{True\ Negative}{(True\ Negative + False\ Negative)}$$

Equation 2 Negative predictive value test

4. SIGNIFICANCE OF RESEARCH AND RESEARCH TASKS

As previously stated, the high potency of fentanyl makes field detection of fentanyl dangerous for law enforcement. Since such a small dose or exposure can cause an overdose, using traditional infield detection methods can be risky since it requires contact. Using canines can help alleviate those risks; however, training canines on fentanyl still comes with risks to the canine and to the handler during training. In the past, the development of safe canine training aids, such as those made for cocaine by Furton et al. and methamphetamine by Vu , paved the way for canines to safely be trained on controlled substances.[81,82] Using a similar strategy, this research proposes the development of a novel, safe canine training aid for fentanyl. This training aid can benefit everyday society and the forensic science community. These advantages include a safer and more efficient way to detect fentanyl in the field, as well as the increase in seizures of illegal fentanyl at the US ports of entry as well as police drug raids. The increase in seizures can also potentially result in a decrease in fentanyl overdoses. The research discussed here was completed through five different tasks.

1. The verification of a GC-MS method for the detection of a fentanyl liquid standard
2. The optimization of a SPME method for the detection of the headspace components of solid reference grade fentanyl
    a. Extraction time optimization
    b. Extraction temperature optimization
    c. Equilibration time optimization
3. The effects of passive degradation on reference-grade fentanyl

4. The formation of a controlled odor mimic permeation system (COMPS)
    a. Odor depletion with storage
    b. Odor depletion without storage
5. The verification of the fentanyl training aid mimic using scent detection canines

5. TASK 1: VERIFICATION OF A GC-MS METHOD FOR THE DETECTION OF A FENTANYL LIQUID STANDARD

5.1 Introduction

Investigation into a new compound or one that has not been commonly studied traditionally begins with method development using an analytical standard. In recent years the efforts to detect fentanyl have mainly been for toxicological purposes in death investigations. This means that fentanyl detection has been conducted by Liquid Chromatography-Mass Spectrometry (LC-MS), with the sample being urine, saliva, or blood. Since fentanyl has a high boiling point of 466°C, it is not easily analyzed using GC-MS; however, in 2008, Manral et al. developed a GC-MS method using a PB-5 capillary column for the detection of fentanyl.[89] The method developed by Manral et al. was modified for the detection of fentanyl in this work discussed below.[89]

5.2 Materials and Methods

5.2.1 Materials

Liquid 1000 ppm fentanyl solution in methanol analytical standard was purchased from Cayman Chemical (Ann Arbor, MI) (no information about the synthesis of fentanyl was provided by the company as it was considered proprietary information). The liquid standard was diluted further with methanol to 100 ppm for analysis. An Agilent 6890/5973N GC-MS system was used (Santa Clara, CA).

5.2.2 Methods

Since fentanyl is a nonpolar molecule, this method requires a nonpolar column. Based on the method developed by Manral et al., an HP-5MS 30-meter (0.25 mm x 0.25 μm) column was chosen.[89] The temperature program for the oven and the GC inlet were chosen based on the original parameters used in the Manral et al. method and can be seen in Table 8 . The scan range for the MS was set to *m/z* 40-300 to allow for a full-range scan of mass fragments and was operated in single ion monitoring (SIM) mode to detect fentanyl's major ions due to interferences from background noise. SIM mode was used to help increase the resolution of the peak since fentanyl has a high boiling point of 466°C and is a relatively large molecule with a molecular weight of 336.5 g/mol. Finally, an injection volume of 1 µL was used through Automated Liquid Sampling (ALS). Following the detection and identification of fentanyl, 10, 20, 30, 40, and 50 ppm dilutions of the analytical standard was made for external calibration to determine the limit of detection.

Table 8. GC-MS parameters used to detect a fentanyl liquid standard.

GC-MS Method Parameters	
Instrument	Agilent 6890/5973N GC-MS System
Column	30-meter DB-5
Split	10:1
Inlet Temperature	300°C
Oven	100°C to 250°C at 30°C/min (10 min hold) ramped 250°C to 270°C at 10°C/min
MS Transfer line	300°C
SIM	Ions 146, 189,245

5.3 Results and Discussion

As previously stated, 1 µL of a 100-ppm fentanyl liquid standard was injected into the GC. Since fentanyl is a large molecule with a high boiling point, it takes more energy or heat to vaporize the sample for GC analysis. For this purpose, the inlet temperature was set at 300 °C, which was also in line with the method developed by Marnal et al. In this method, a 10:1 split was used, the temperature program began at 100 °C and ramped to 270 °C at 30 °C/min. Once the temperature reached 270 °C, it ramped to 300 °C at 10

°C/min, where it was then held at 300 °C for 10 min. As previously stated, the MS was operated in SIM mode for the detection of fentanyl starting 2.5 minutes into run and until its completion using *m/z* 146,189, and 245.[90] The chromatograph for the detection of fentanyl can be seen in Figure 16. Using SIM/SCAN allowed the detection and identification of fentanyl using the NIST MS library with a retention time of 9.533 minutes and a total run time of 17 minutes.

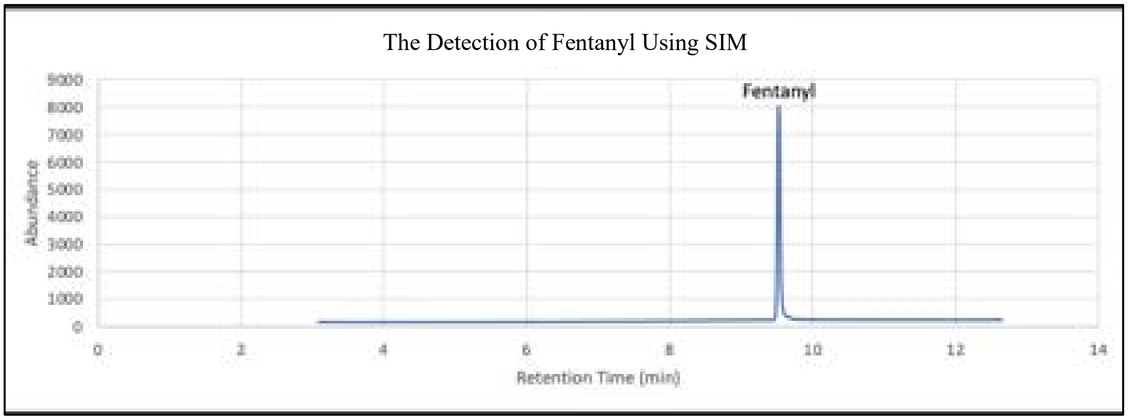

Figure 16. Chromatogram representing the detection of the fentanyl analytical standard using SIM mode.

Once the method development was successful, it was important to determine the limit of detection (LOD). In this task, the LOD was found using an external calibration curve. The average peak area was then plotted against the concentration to give the calibration curve in Figure 17. Once plotted, a linear trend line was added to the graph to provide the equation of the line and the value for linear regression (R^2). As seen in Figure 17, the R^2 value was 0.9973, which means this model had excellent linearity. Using the data provided by the calibration curve, the LOD of the fentanyl liquid standard was calculated in excel by using Equation 3, where σ is the standard deviation of the peak areas and m is the slope of the line.

$$LOD = \frac{3(\sigma)}{m}$$

Equation 3. Calculation of the limit of detection.

Using this method, the LOD was determined to be 2.8 ppm.

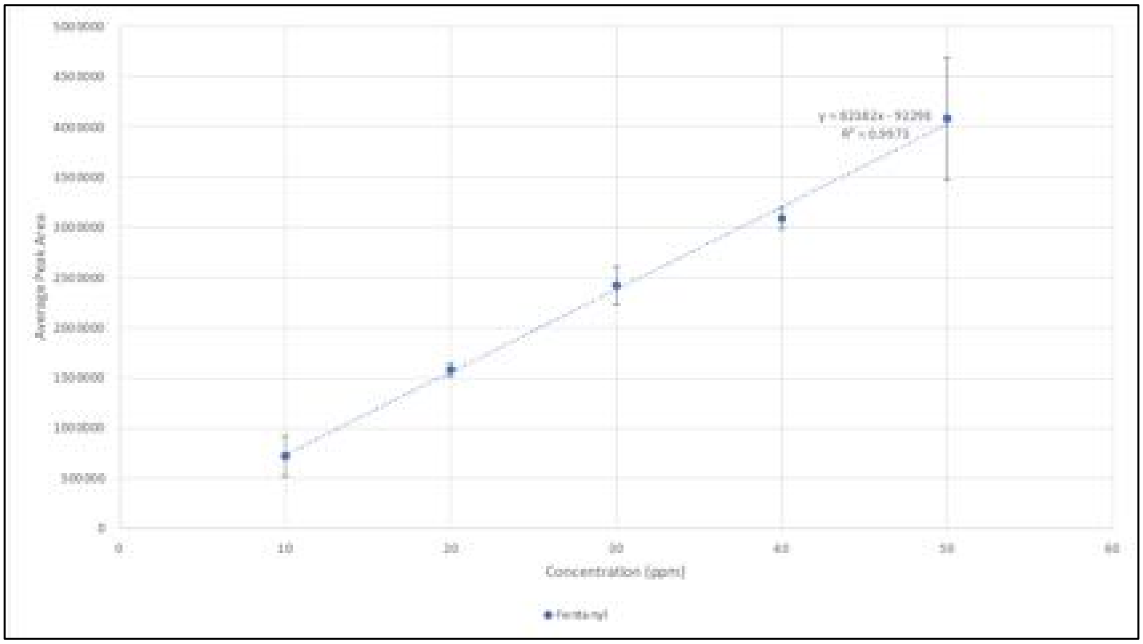

Figure 17. External calibration curve of a liquid fentanyl standard.

5.4 Conclusions

Based on the results from task one a GC-MS method using automated liquid sampling was successfully verified to detect a fentanyl liquid standard. This method included the use the SIM/SCAN mode, which successfully detected and identified fentanyl based on its major ions, m/z 146,189 and 245, with a retention time of 9.5 min. Using the successfully developed method, the resulting calibration curve showed excellent linearity with an R2 value of 0.9973 as well as good accuracy, precision, and reproducibility for concentrations. Finally, using the data provided by the calibration curve, the LOD of the fentanyl liquid standard was calculated in excel and was determined to be 2.8 ppm.

6. TASK 2: THE DEVELOPMENT OF A SPME METHOD FOR THE DETECTION OF THE HEADSPACE COMPONENTS OF SOLID REFERENCE GRADE FENTANYL

6.1 Introduction

When a detector canine alerts to a known target, such as cocaine or methamphetamine, it is not the drug they are detecting but one or more VOCs emitted by the parent compound. The detected VOC(s) to which they alert is called the active odorant. Several studies have been done to determine the active odorant(s) for some of the most used drugs, such as cocaine, methamphetamine, and MDMA. These studies determined that rather than alerting to the drug itself, the canines were alerting to methyl benzoate, benzaldehyde, and piperonal, respectively.[81,82,91] Detecting and identifying its VOC profile is the first step in training aid development. For this task, a SPME-GC-MS method was developed and optimized for the identification of the VOCs emitted by fentanyl. This task was split into three subtasks. Task 2a is the optimization of extraction temperature. Task 2b is the optimization of extraction time, and Task 2c is the optimization of equilibration time. Finally, a GC-MS method developed by the Naval Research Laboratory (NRL) was used to analyze the analytes extracted onto the fiber.

6.2 Materials and Methods

6.2.1 Materials

Reference-grade fentanyl was purchased from Cayman Chemicals (Ann Arbor, MI) (the company provided no information regarding the synthesis; it was considered proprietary information). Heptane, NPP (N-Phenethyl-4-piperidione), and 99.8% anhydrous acetonitrile were purchased from Sigma-Aldrich (St Louis, MO). NPPA (N-Phenylpropanamide) and (2,3-dimethylphenyl)-ethanone were purchased from Toronto

Research Chemicals (Toronto, ON, Canada). Finally, 20 mL VOA sampling vials were purchased from Fisher Scientific (Pittsburg, PA). All chemicals used in this experiment were used and received without further purification or modification.[92]

6.2.2 Safety Precautions for Handling Fentanyl

Handling of solid fentanyl was completed by two trained personnel: one was the handler, and the other was the observer. During handling, the handler and observer wore all proper personal protective equipment (PPE), including lab coats, gloves, goggles, and facemasks. While working in a ventilated hood, using a spatula, the handler transferred the solid fentanyl into a VOA glass vial with properly fitted septa lids. During this, the observer supervised with naloxone on hand in case of emergency. Once the glass vials were sealed, the fentanyl samples were not removed or manipulated during sampling, and all vials were kept closed. After the fentanyl was transferred into the vials, alcohol wipes were used to clean the preparation station to ensure no fentanyl residue remained. All used wipes and gloves were disposed of as hazardous waste upon completion.[92]

6.2.3 Pure Fentanyl Headspace Sampling

To adequately generate the headspace profile of reference-grade fentanyl, approximately 5 mg of fentanyl was placed in 20 mL VOA sampling vials with septa-fitted lids. To properly develop an extraction method for the detection of fentanyl's headspace profile, the SPME extraction time and temperature, as well as sample equilibration time, were investigated. Optimal headspace generation was achieved by allowing the fentanyl sample to equilibrate in the closed vial at room temperature (21 °C ± 3 °C) for 24 hours before sampling. The vial containing fentanyl was placed into a heating block and equilibrated at 35 °C for 30 minutes before a four-hour SPME extraction. The SPME extraction was accomplished using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber.[92]

For separation and detection of the VOCs present in the headspace profile of reference-grade fentanyl, an 8890/5977 Agilent GC-MS (Agilent Technologies, Santa Clara, CA) system was used equipped with a 15 m x 0.25 mm i.d x 0.25 μm HP-5MS column from Agilent technologies. After extraction, the analytes were desorbed off the SPME fiber at 260 °C for three minutes with a split ratio of 10:1 and a 2 mL/min flow rate. he GC oven had a starting temperature of 40 °C, where it was held for 30 seconds, and then increased to 240 °C at 30 °C/min, where it was held for 3 minutes. The mass scan range was kept constant at *m/z* 40-300, and the mass transfer line to the MS was set to 240 °C. The resulting compounds were preliminarily identified using the NIST Mass Spectral Library. All major compounds were further confirmed through comparison to standard solutions in acetonitrile prepared at a concentration of 50 ppm.[92]

6.2.4 Detection of the Headspace Components in the Liquid Standard

To determine if the headspace components can be seen in the liquid standard, SIM was used. The SPME method developed in section 6.2.3 was converted into an ALS method to analyze a 1000 ppm liquid standard using SIM. SIM was used to detect the major ions of the headspace components, which included ions 57, 79, 91, 93, 104, 106, 112 and 170, while ignoring the major ions for fentanyl. A concentration of 1000 ppm was used because any lower of a concentration would not adequately detect any of the headspace compounds even when using SIM.

6.3 Results and Discussion

6.3.1 SPME Method Development

To detect the compounds in the headspace profile of fentanyl, an SPME method was developed. The first parameter that was investigated was extraction time. For this investigation, extraction times of 30 minutes, 2 hours, and 4 hours were tested at a temperature of 35 °C. Figure 18 shows the peak areas of three VOCs in fentanyl's headspace, which were chosen to represent a highly volatile, intermediately volatile, and semi-volatile compound; however, each investigated parameter considered the complete vapor profile for each data point. In this task, benzaldehyde was selected as the highly volatile compound, NPPA was chosen as the intermediately volatile compound, and NPP was selected as the semi-volatile compound. Based on the results seen in Figure 18, it was determined that the maximum extraction time of 4 hours was needed to extract the compound with lower volatility adequately. For this reason, 4 hours was chosen as the extraction time for this SPME method.[92] A longer extraction time was not explored because an extraction longer than 4 hours could become problematic for field sampling.

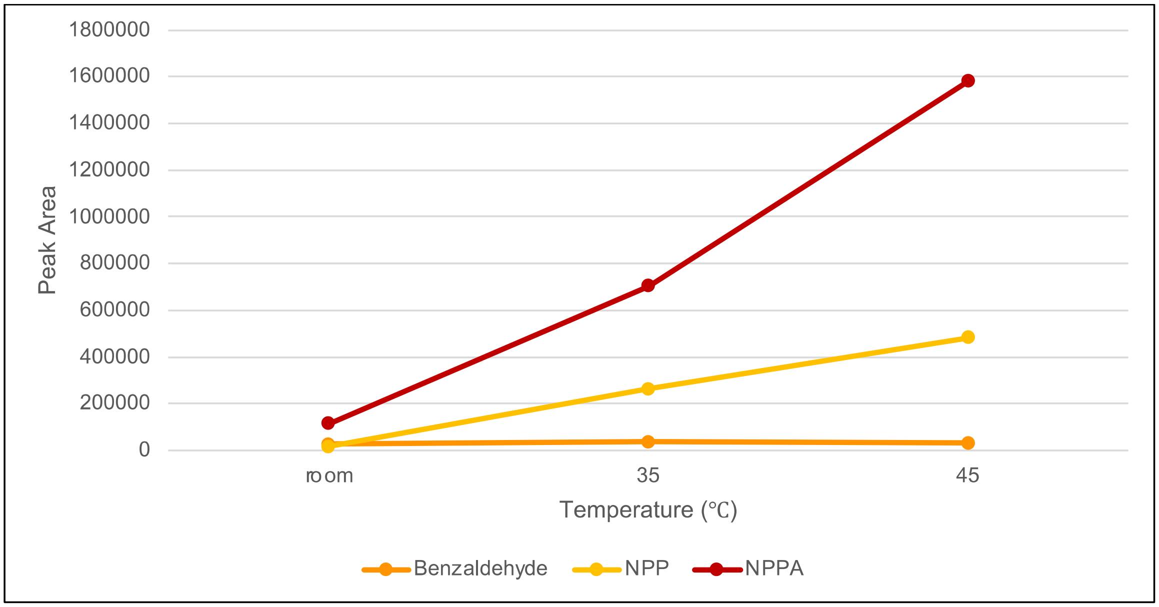

Figure 18. Average peak areas of three VOCs with high, intermediate, and low volatility at the tested extraction times (n=2).

Following the investigation of extraction time, the following parameter to be investigated was extraction temperature. Using the chosen 4-hour extraction time, three different temperatures, 22.7 °C (room temperature), 35 °C, and 45 °C, were tested. Figure 19 compares the same three selected VOCs at the different tested temperatures. Based on the results, as the temperature increases, the abundance of the analytes also increases; however, when looking at the entire profile, it was seen that increasing the temperature did not provide the detection of additional analytes. For this reason and to avoid thermal degradation of the sample a moderate temperature of 35 °C was chosen for extraction.[92]

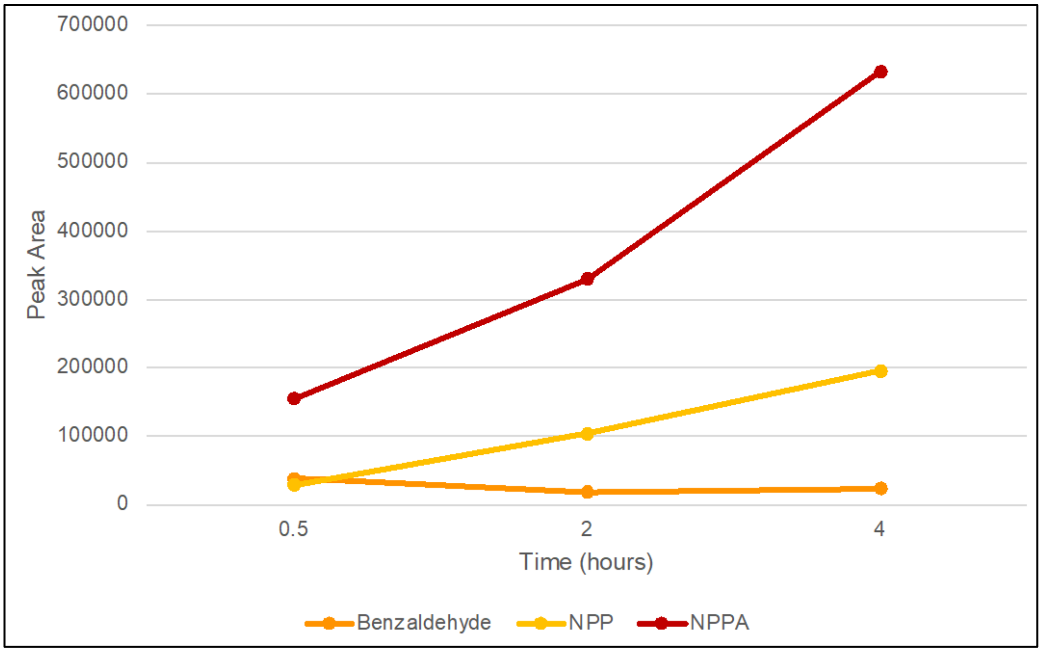

Figure 19. Average peak areas of the selected VOCs in the headspace of reference-grade fentanyl at three different extraction temperatures (n=2).

The last SPME parameter to be investigated was the sample equilibration time. Four different equilibration times were tested which included: 0, 30, 60, and 120 minutes. The results of this investigation can be seen in Figure 20. Based on the data there was a decrease in the peak area of the low and intermediately volatile compounds from 0 to 30 minutes of equilibration. Once the abundance of analytes begins to decrease, as seen with the low and intermediately volatile compounds from 0 to 30 minutes, it can compromise the quality of the headspace profile received from the sample. For this reason, 30 minutes was chosen as the optimal extraction time to extract all low, intermediate, and high volatile compounds.[92]

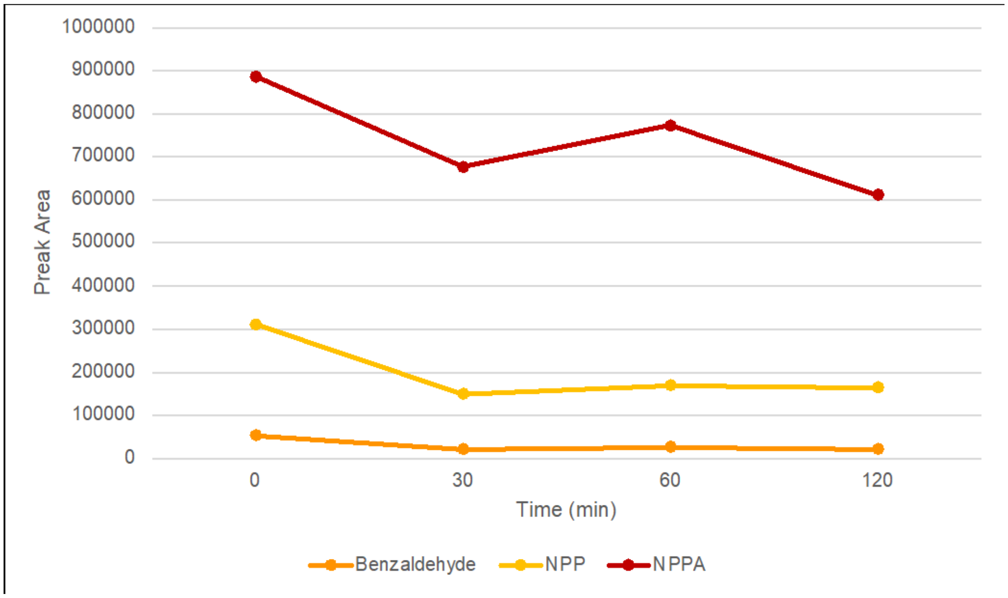

Figure 20. Average peak areas of three volatile compounds in the headspace of fentanyl at the tested equilibration times (n=2).

6.3.2 Headspace of Pure Reference Grade Fentanyl

Using the chosen extraction parameters, including a 4-hour extraction and 30-minute sample equilibration at 35°C, several VOCs were detected in the headspace. Using the NIST mass spectral library, these compounds were preliminarily identified as heptane, benzaldehyde, styrene, aniline, benzyl alcohol, benzeneacetaldehyde, NPPA, NPP, and 1-phenethyl-4-propinloxypiperidine. A chromatogram of the headspace profile of fentanyl can be seen in Figure 21 and the peak identities can be seen in Table 9.

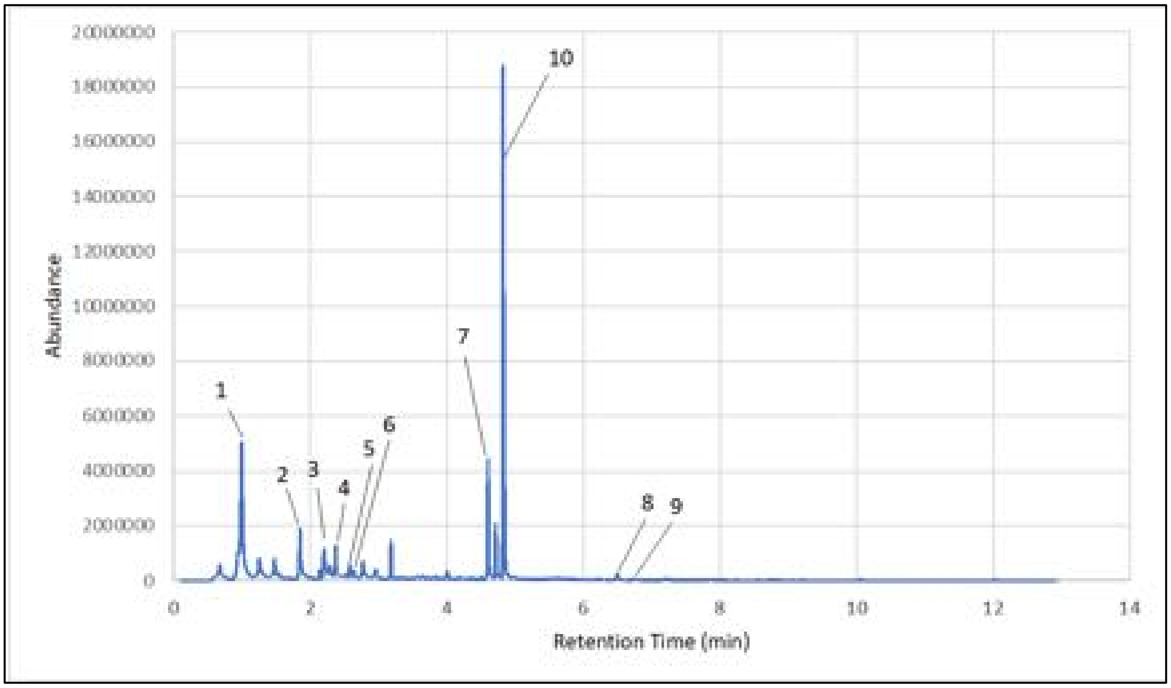

Figure 21. A chromatogram representing the headspace profile of reference-grade fentanyl.

Table 9. Peak identities, retention times, and major ions for the chromatogram in Figure 21.

Peak number	Compound	Majo r Ion	Retention Time
1	Heptane	57	0.921
2	Styrene	104	1.821
3	Benzaldehyde	106	2.187
4	Aniline	93	2.276
5	Benzyl Alcohol	79	2.566
6	Benzeneacetylaldehyde	91	2.632
7	NPPA	93	4.598
8	NPP	112	5.731
9	1-Phenethyl-4-Propinoloxypiperide	170	5.892
10	Siloxane	-	-

After preliminarily identifying the compounds using the NIST mass spectral library, the identities were confirmed by comparing retention times and major ions to external standards, except for peak 9, which was not commercially available. The peak at approximately 5 minutes was identified as a siloxane isomer which can be attributed to the SPME fiber. NPP and aniline being present in the headspace profile of fentanyl were understandable since both are known precursors in the synthesis of fentanyl. While NPPA and 1-phenthyl-4-propionyloxypiperidine are known chemical markers in the Janssen and Siegfried synthesis processes.[28,29] Although benzaldehyde and styrene cannot be attributed to synthesis, they have been identified as degradation products.[30] Since heptane, benzyl alcohol, and benzeneacetylaldehyde cannot be attributed to either synthesis or degradation, it is likely that their presence is due to manufacturing and/or packaging. Finally, using the information gained in Task 1, it was determined that fentanyl could not be seen in its headspace. It can be hypothesized that the major compounds which make up the unique vapor signature come from the synthesis, degradation of fentanyl as well as the packaging and manufacturing.

In addition to analyzing the headspace of reference-grade fentanyl, the headspaces of reference-grade fentanyl hydrochloride and fentanyl citrate were also investigated. The chromatograms for fentanyl hydrochloride and fentanyl citrate were then compared to that of reference-grade fentanyl (Figure 21) to determine if they shared any of the compounds identified in Table 9. Based on the results seen in Figure 22, reference-grade fentanyl and fentanyl hydrochloride had relatively similar headspace profiles. For example, they shared NPP, NPPA and benzaldehyde in common; however, fentanyl citrate did not share any compounds in common with either reference-grade fentanyl or fentanyl citrate.

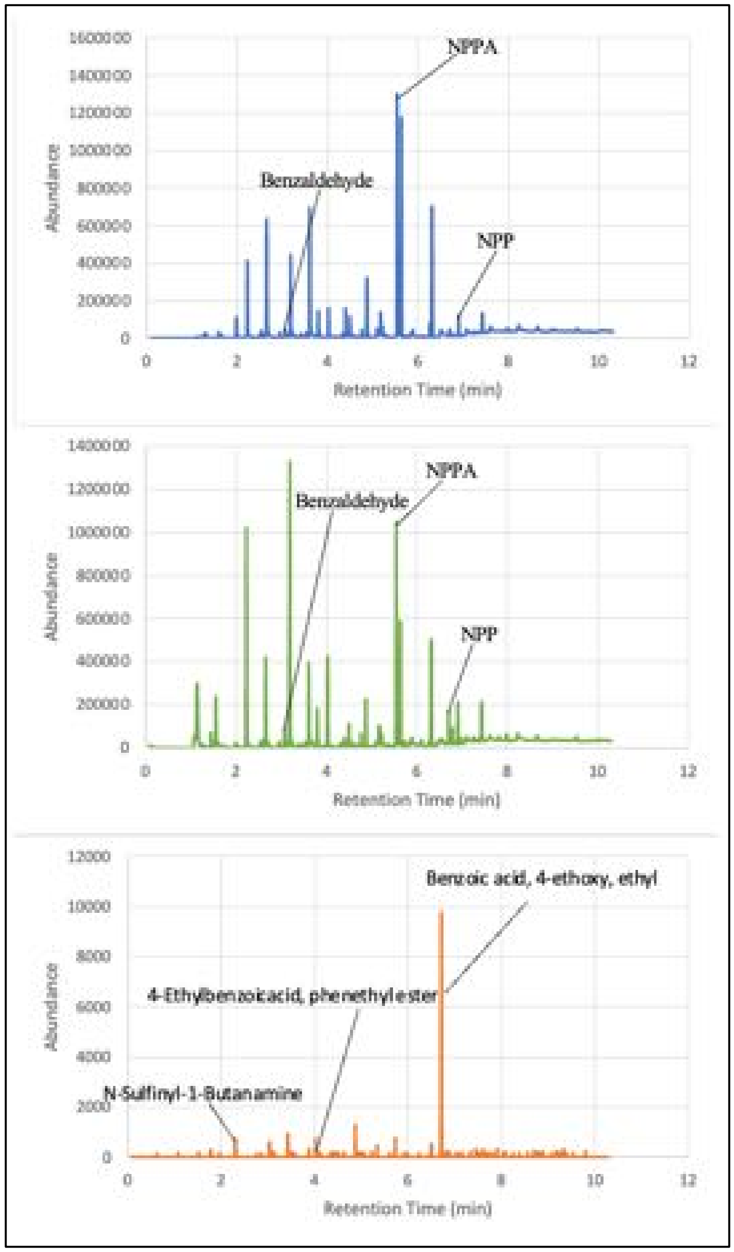

Figure 22. Comparison of the headspace profiles of reference grade fentanyl (blue), fentanyl hydrochloride (green) and fentanyl citrate (orange).

The method verified above was used to determine if the headspace compounds can be seen in the liquid standard using the major ions for the headspace compounds. Figure 23 shows that five out of the nine headspace compounds can be seen in the liquid standard when using SIM to detect their major ions (seen in Table 9) and at a high concentration (1000 ppm).[92]

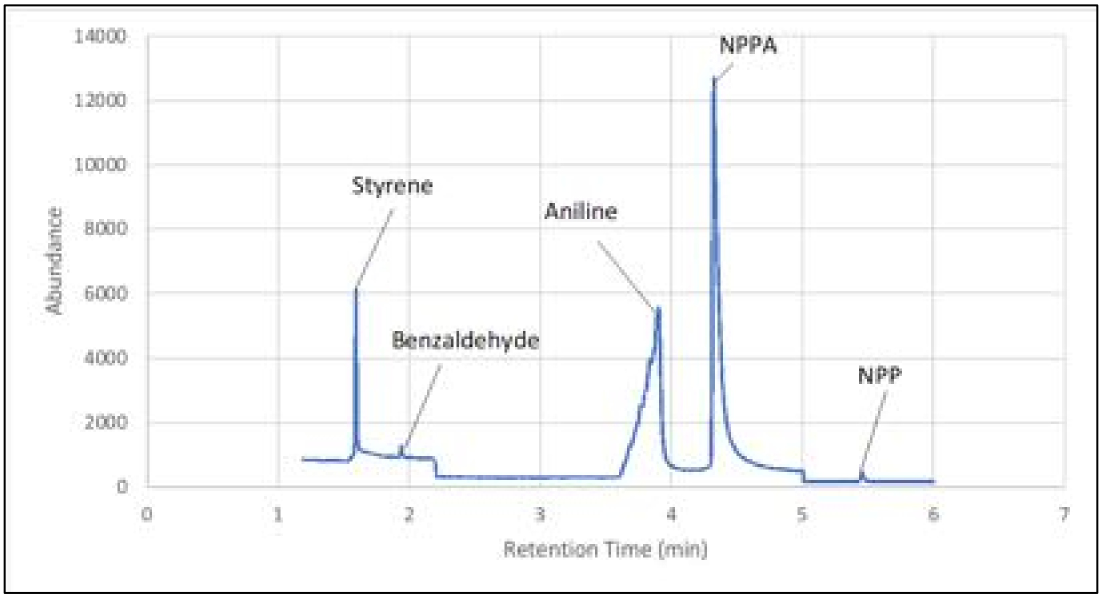

Figure 23. SIM chromatogram showing the detection of the tracked components in a fentanyl liquid.

6.4 Conclusions

A SPME method was successfully developed and used to determine the compounds in the headspace profile of reference-grade fentanyl. This resulted in the detection and identification of heptane, benzaldehyde, aniline, NPP, NPPA, benzyl alcohol, benzeneacetylaldehyde, and 1-phenethyl-4-propinoloxypiperidine with NPPA being identified as the major compound. It was hypothesized that benzaldehyde and styrene were products of degradation, while aniline and NPP were both known precursors in the synthesis of fentanyl. It was also concluded that NPPA and 1-phenethyl-4-propinoloxypiperidine were detected as they are known chemical markers for both the Janssen and Siegfried synthetic routes, while heptane, benzyl alcohol, benzeneacetylaldehyde most likely came from manufacturing or packaging. Furthermore, the headspace profiles of fentanyl hydrochloride and fentanyl citrate were compared to that of reference-grade fentanyl. Upon analysis it was seen that fentanyl hydrochloride had relatively similar headspace profiles when compared to reference-grade fentanyl. However, fentanyl citrate did not share any compounds in common with the headspace of either reference-grade fentanyl or fentanyl hydrochloride. Finally, it was concluded that five out of the nine identified compounds can be seen in the fentanyl liquid standard but only at high concentrations. Further investigation into the degradation of fentanyl will help to provide further information on the headspace of fentanyl as well as any additional analytes that can be used for identification.

7. TASK 3: THE EFFECTS OF PASSIVE DEGRADATION ON THE HEADSPACE OF SOLID REFERENCE GRADE FENTANYL

7.1 Introduction

A full investigation into the headspace profile of fentanyl can help provide a better understanding of its compounds. This can allow for the development of non-contact detection methods, such as a safe canine training aid mimic. This will, in turn, provide safer procedures for the field detection of fentanyl. Based on the data acquired in task two, the headspace profile of fentanyl was determined to be composed of heptane, styrene, benzaldehyde, benzyl alcohol, benzeneacetylaldehyde, NPPA, NPP, and 1-phenethyl-4-propionyloxypiperidine. Based on prior research conducted it was concluded that the verified headspace components; NPPA, benzaldehyde, and styrene are products of degradation.[30,32,34,35,93] The experiment discussed herein describes the process of reference-grade fentanyl undergoing passive degradation. Eight environments were investigated using different humidity, temperature, and either nitrogen or house air. Following preliminary experiments to stabilize the environments, the fentanyl samples were exposed to these environments, and the headspace profile was investigated for 28 days. After environmental exposure, headspace was analyzed using the method developed in Task 2. The results from this experiment will provide data that gives a better understanding on how degradation affects the headspace profile in respect to the known products of degradation as well as give insight to the origin of the remaining compounds. Finally, this investigation provided more information for which compounds are viable options as odorants for a canine training aid mimic.

7.2 Materials and Methods

7.2.1 Materials

Reference-grade solid fentanyl was purchased from Cayman Chemical (Ann Arbor, MI) (the company provided no information regarding the synthesis; it was considered proprietary information). Acetanilide, desiccant, and potassium chloride (KCl) were all purchased from Sigma Aldrich (St. Louis, MO). All chemicals used in this experiment were received without further modification or purification. 20 mL VOA sampling vials were purchased from Fisher Scientific (Pittsburgh, PA). Disposable glove bags ranging in size from 27″ x 17″ to 32″ x 4″ were purchased from Sigma Aldrich (St. Louis, MO). Thermochron Ibutton humidity and temperature monitors were purchased from Ibuttonlink Technologies (Whitewater, WI).[94]

7.2.2 Safety precautions for handling fentanyl

Handling of solid fentanyl was completed by two trained personnel in which, one was the handler, and the other was the observer. During handling, the handler and observer wore all proper personal protective equipment (PPE), including lab coats, gloves, goggles, and facemasks. While working in a ventilated hood, using a spatula, the handler transferred the solid fentanyl into a VOA glass vial with properly fitted septa lids. During this, the observer supervised with Naloxone on hand in case of emergency. Once the glass vials were sealed, the fentanyl samples were not removed or manipulated during sampling, and all vials were kept closed. After the fentanyl was transferred into the vials, alcohol wipes were used to clean the preparation station to ensure no fentanyl residue remained. For the environmental studies, proper care was taken to ensure no fentanyl residue was transferred onto the glove bag. All used wipes and gloves were disposed of as hazardous waste upon completion.[94]

7.2.3 Environmental Set Up

The fentanyl samples were exposed to eight different environments to understand better how passive degradative stresses affect the headspace profile of fentanyl. The environments were made by manipulating humidity, temperature, and air supply. Table 10 lists the environmental parameters that were selected to mimic the possible conditions illicit fentanyl could encounter during storage and transportation. Experiments took place in disposable glove bags with zipper closures to stabilize each environment for an extended time.

Table 10. Environments generated for the study.

	Humidity	Temperature	Air Supply
A	Dry	Ambient	Nitrogen
B	Dry	Ambient	Air
C	Dry	40°C	Nitrogen
D	Dry	40°C	Air
E	Humid	Ambient	Nitrogen
F	Humid	Ambient	Air
G	Humid	40°C	Nitrogen
H	Humid	40°C	Air

To achieve the lowest possible humidity needed in the dry condition in environments A to D, 100 grams of desiccant was placed into a ceramic dish and added to the glove bag. A Thermochron Ibutton sensor was also put into the bag to track the humidity and temperature. The sensor was set to take a reading once every hour for 24 hours (this was done in triplicate). A 2.5 mL KCl saturated in deionized water salt solution was placed inside the glove bag to generate the highest possible humidity for humid environments E to H. Again, a Thermochron Ibutton was used to track humidity and temperature. The sensor was set to take a reading once every 24 hours (this was done in triplicate). For the 40 °C condition needed for environments C to D and G to H, the samples were placed in a sand bath and heated to 40 °C. For the ambient condition, the laboratory temperature ranged between 22 °C and 25 °C. Finally, the glove bags were filled with UHP nitrogen or house air to accomplish the inert or oxygenated conditions respectively.[94] The set up can be seen in Figure 24 A-C.

7.2.4 Sampling and Analysis of Fentanyl Headspace

To generate the headspace profile of reference-grade fentanyl as it degrades, 5 mg of fentanyl was placed into a 20 mL VOA sampling vial equipped with a septum. Before exposure to each environment, headspace analysis was conducted and labeled as day zero to obtain a baseline. Three samples were exposed (meaning the vials were open) to each environment for seven days, after which the vials were closed and allowed to equilibrate at room temperature (21 °C ± 3 °C) for 24 hours before sampling. The headspace of these fentanyl samples was extracted and analyzed using the method developed in Task 2. For extraction, the samples were placed into a heating block to equilibrate at 35 °C for 30 minutes. A 4-hour extraction followed this at 35 °C with a DVB/CAR/PDMS SPME fiber. To improve the measurements reproducibility, an externally sampled internal standard (ESIS) was used.[95] For the ESIS, acetanilide was selected due to its structural similarity and similar molecular weight to NPPA, a known headspace component of fentanyl. 5 mg of acetanilide was added to a VOA sampling vial with a septum and allowed to equilibrate for 24 hours at room temperature before sampling. Before sampling the fentanyl headspace, the SPME fiber was inserted into the ESIS for a 2 min extraction at room temperature. Following extraction, the samples were immediately re-exposed to their respective environments. This process repeated until the samples were degraded for 28 days.

After extraction, the samples were analyzed using an 8890/5977 GC-MS system equipped with a 15 m x 25 mm i.d. x 0.25 μm, HP-5MS column (Agilent Technologies, Santa Clara, CA). The analytes were desorbed from the fiber by exposing them to the GC inlet for 3 minutes at 260 °C for 3 min with a 10:1 split ratio and a 2 mL/min flow rate. After a solvent delay of 30 seconds, the GC column oven was increased to 40 °C and then held for 30 seconds, where it then ramped to 240 °C at 30/min, where it was held at 240 °C for 3 min. The mass scan range used for this method was *m/z* 40-300. The analytes of interest that desorbed from the SMPE fiber were quantified by dividing the peak area of the ESIS by the peak area of each analyte, which resulted in the analyte to internal standard (A/E) ratio.[94] Sampling set up can be seen in Figure 24 D.

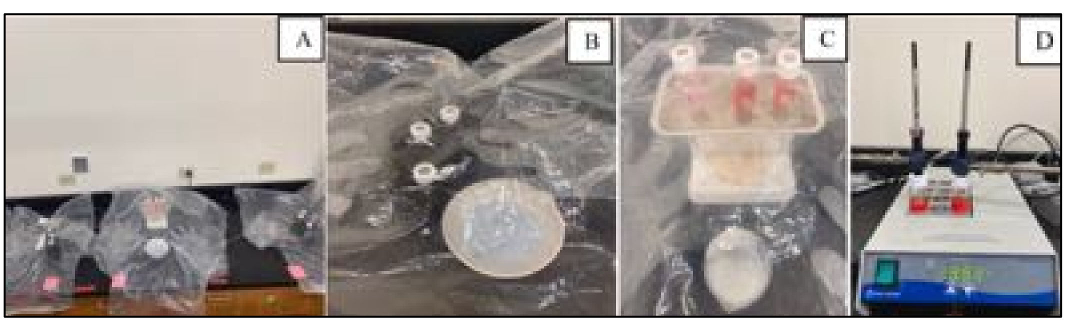

Figure 24. (A) Experimental setup for the environments investigated before sample exposure. (B) close up of environment A. (C) close up of environment G. (D) SPME sampling setup.

7.3 Results and Discussion

7.3.1 Environmental Setup

Before exposing fentanyl samples to the environments, it was essential to ensure that the humid and dry samples were stable. It was also important to know how long it took both environments to stabilize and what the %RH was. To achieve this, a Thermochron Ibutton set to take a measurement once every hour for 24 hours was placed into glove bags of dry and humid environments for an inert and house air supply. The first environment investigated was the dry inert environment. Based on the results seen in Figure 25, the lowest humidity was seen for trial one was seen at hour 5, trial two it is seen at hour 1, and trial three it is seen at hour four. However, to ensure equilibrium, the bags sat for 12 hours before introducing samples. While the results in Table 11 shows that after 12 hours, the average %RH for the dry inert environment is 5.791%.

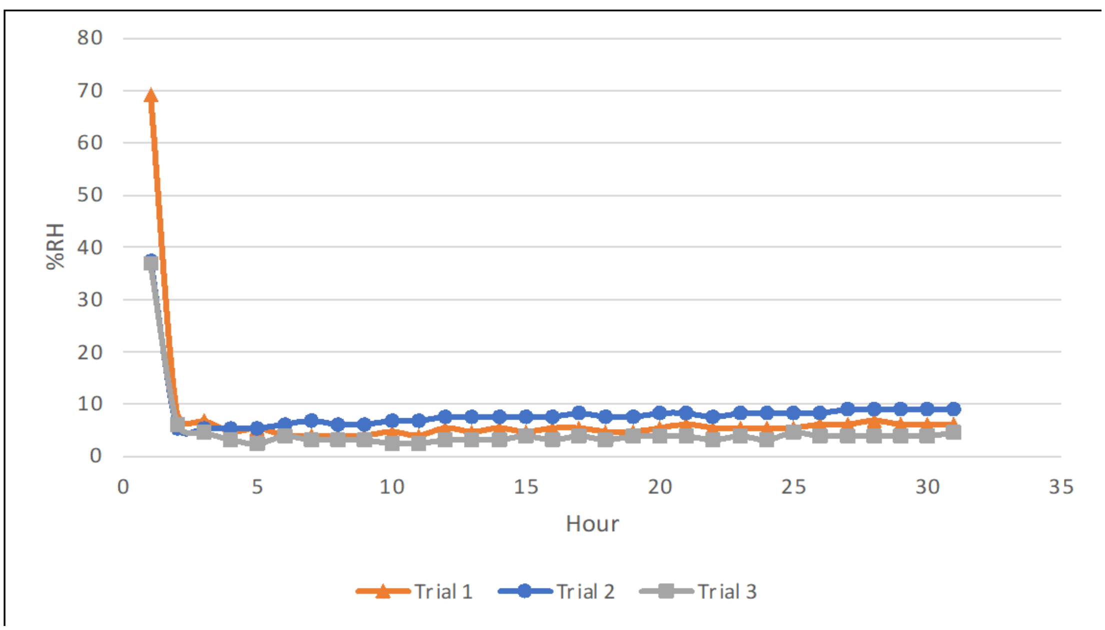

Figure 25. Data representing the readings for humidity stabilization in the dry inert environment.

Table 11. Average %RH for the dry inert environment.

Trial	AVG %RH
1	5.509 %
2	8.116 %
3	3.748 %
AVG %RH Trial 1-3	5.791 %
%RSD Trials 1-3	37.946 %

The following environment to be investigated was the dry house-air environment.

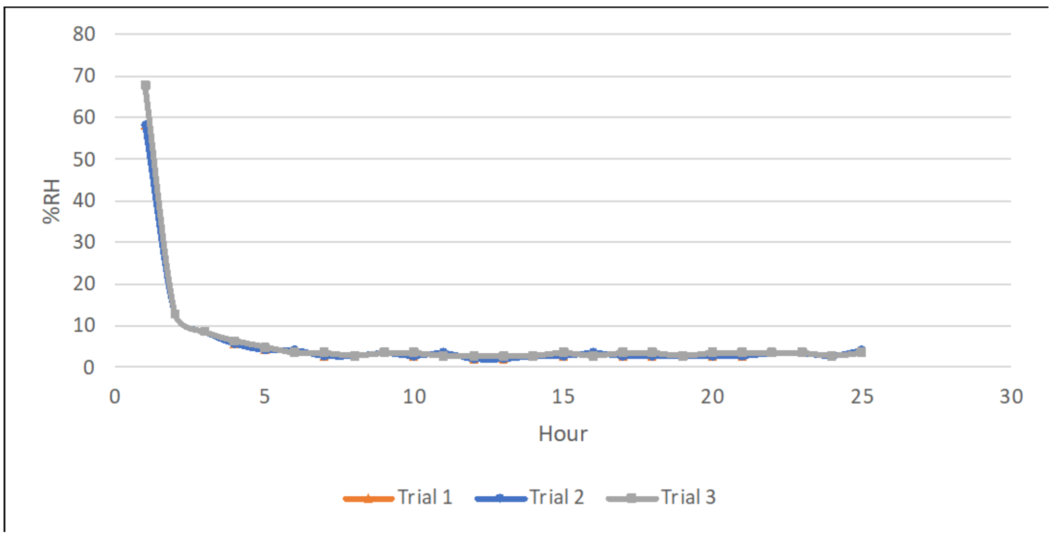

Figure 26. Data representing the reading for humidity stabilization in the dry house-air environment.

Based on the results in Figure 26, the lowest humidity for trial one was seen at hour 12, trial two at hour 12, and trial three at hour 8. To ensure equilibrium, the bags sat for 12 hours before introducing samples. These results align with those found when analyzing the dry inert environment in Figure 25. While the results from Table 12 shows that the average %RH that was achieved after that 12-hour period was 3.080%. Based on the results in Figure 27, the highest humidity for trial one was at hour 25, trial two at hour 19, and trial three at hour 22. To ensure equilibrium, the bags sat for 24 hours before introducing samples. The data in Table 13 shows that after the 24-hour stabilization period, the average %RH obtained by the humid inert environment is 80.033 %.

Table 12. Average %RH for the dry house-air environment.

Trial	AVG %RH
1	3.008 %
2	3.008 %
3	3.223 %
AVG %RH Trial 1-3	3.080 %
%RSD Trials 1-3	4.028 %

Following the dry-hour air environment, the humid inert environment was investigated.

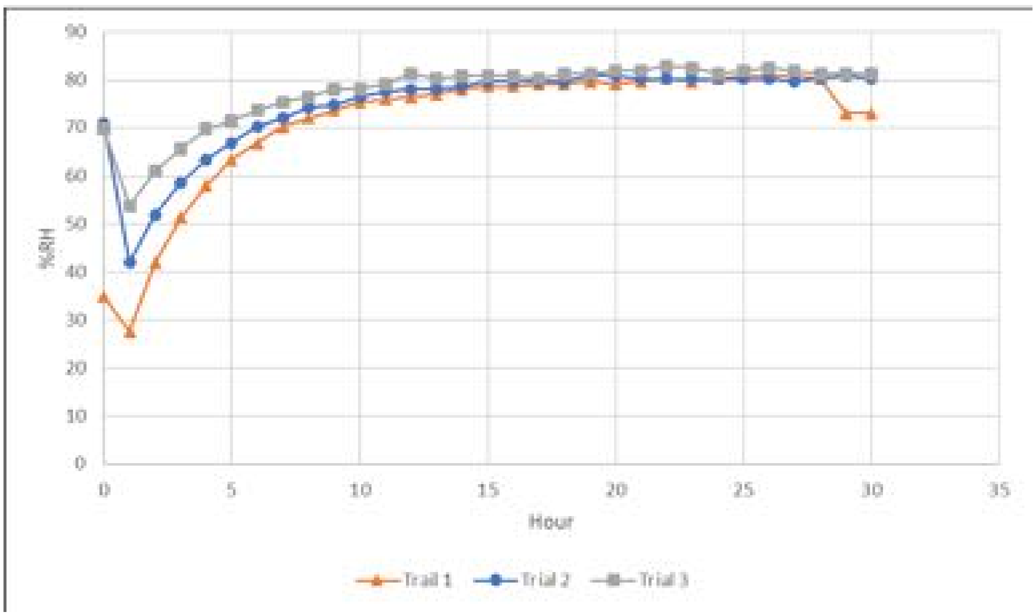

Figure 27. Data representing the readings for the humidity stabilization in the humid inert environment.

Table 13. Average %RH for the humid inert environment.

Trial	AVG %RH
1	78.720 %
2	79.916 %
3	81.482 %
AVG %RH Trial 1-3	80.033 %
%RSD Trials 1-3	1.741 %

The last environment to be investigated was the humid house-air environment.

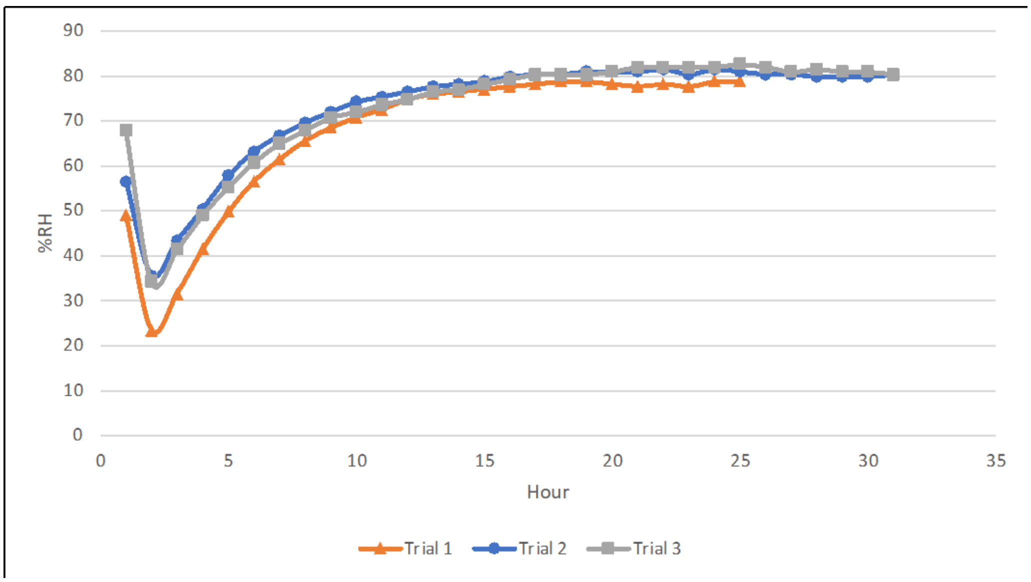

Figure 28. Data representing readings for the humidity stabilizations in the humid house-air environment.

Based on the results in Figure 28, the highest humidity for trial one was seen at hour 17, trial two at 24, and trial three at hour 20. To ensure equilibrium, the bags sat for 24 hours before introducing samples. The data gathered through the investigation of this environment aligns with the data collected in the study of the humid inert environment. After 24 hours, when the environment was fully stabilized, the average %, RH, seen in Table 14 was found to be 79.190 %.

Table 14. Average %RH for the humid house-air environment.

Trial	AVG %RH
1	79.748 %
2	80.168 %
3	80.654 %
AVG %RH Trial 1-3	79.190 %
%RSD Trials 1-3	2.688 %

Based on the data gathered, it is evident that for the dry environments, 12 hours was needed for the humidity to stabilize to between 3-5 %RH. At the same time, the humid environments required 24 hours for the humidity to stabilize to 70-80 %RH. However, for simplicity and ease, all environments were given 24 hours to stabilize before the initial sample introduction. The short time the glove bag is open to retrieve and re-introduce the samples from the environment has a negligent effect. This can be seen in Figure 29 and Figure 30 where the red circle indicates the samples were added to the bag, and within one measurement, the environment was stable.

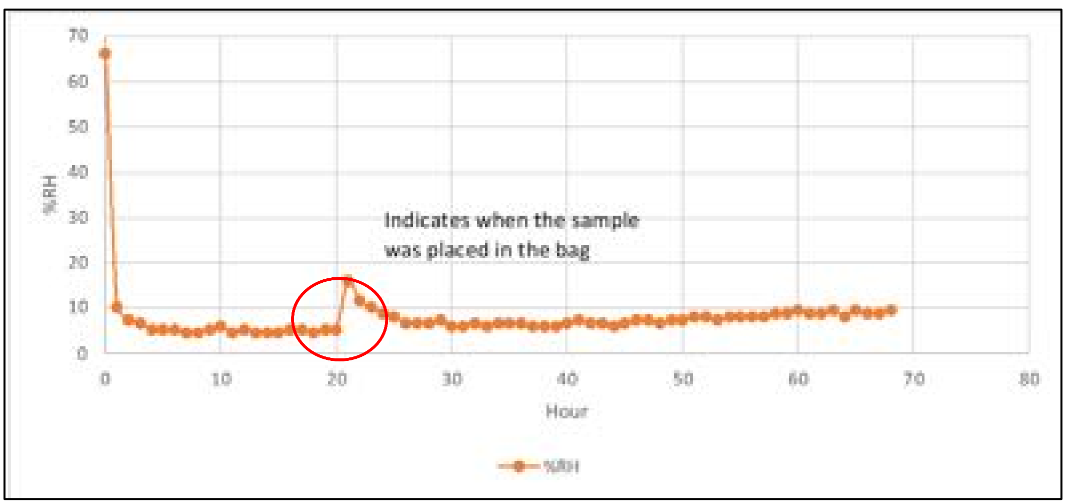

Figure 29. Data representing a single humidity decrease reading.

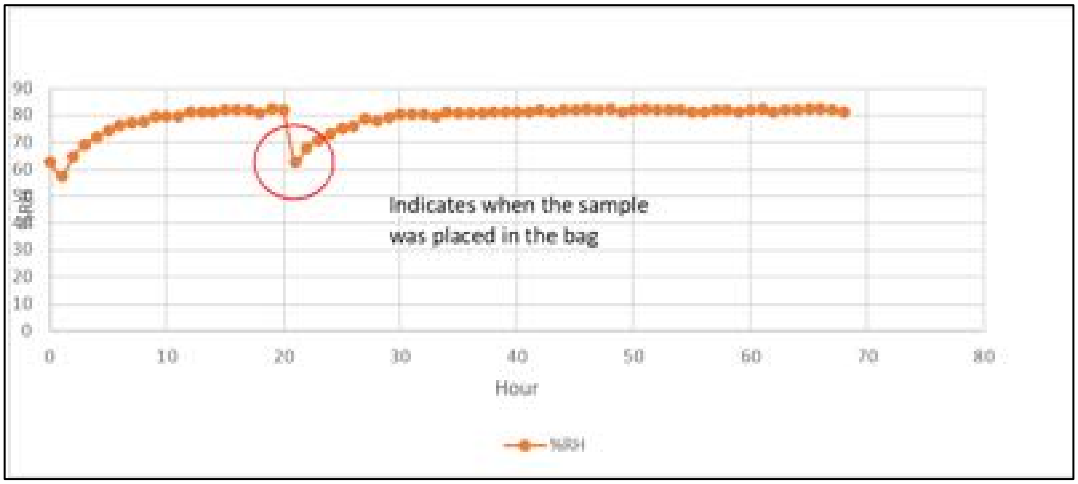

Figure 30. Data representing single humidity increase reading.

7.3.2 Sampling and Analysis of Fentanyl Headspace

7.3.2.1 The Effects of Oxygen, Moisture, and Heat on Fentanyl Headspace

During this task, an externally sampled internal standard (ESIS) was introduced for better accuracy, precision, and reproducibility. For this study, acetanilide was chosen as the ESIS because of its similarity in structure and mass to the major headspace component NPPA. Based on previous work done by MacCrehan et al. using the ESIS, it was important to optimize its extraction using SPME. This work stressed the idea of using short extraction times for an ESIS to not overload the fiber before sampling the analyte while allowing the fiber to adsorb enough vapor for later quantification.[96] Therefore, 5 mg of acetanilide was added to a VOA sampling vial and equilibrated at room temperature for 24 hours before SPME extraction. Four SPME extraction times were investigated at room temperature, 30 seconds, 1 minute, 1.5 minutes, and 2 minutes, and the data can be seen in Figure 31.

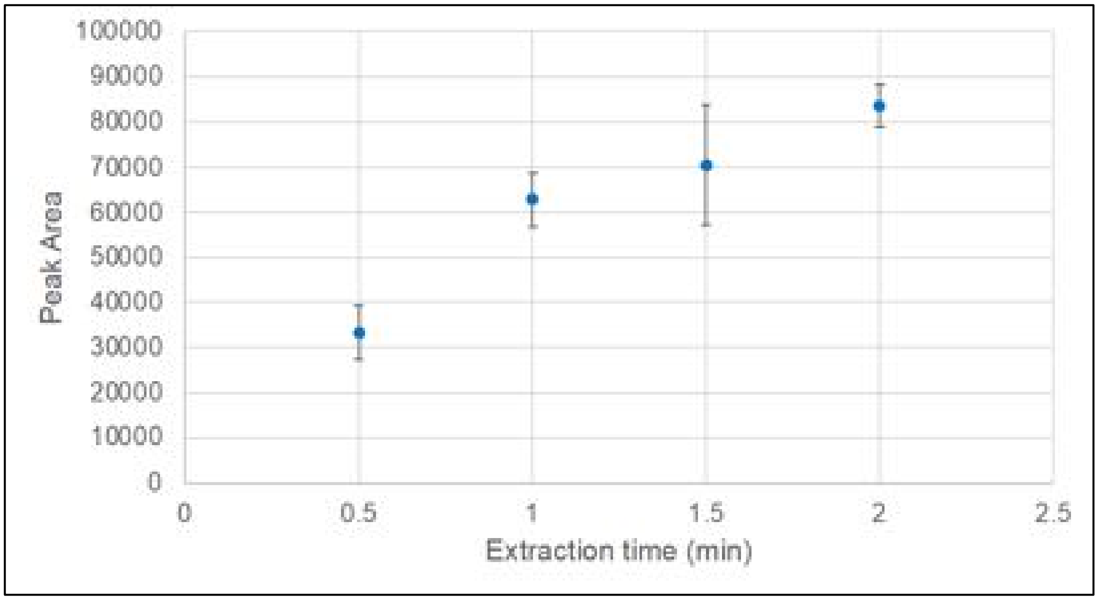

Figure 31.Data for ESIS extraction optimization (n=3).

Based on the data seen in Figure 31, a 2-minute SPME extraction time provided the best response and reproducibility. The extraction time stopped at 2 minutes limit the chance of overloading the fiber while allowing for an adequate extraction of the ESIS to provide good reproducibility based on the principles discussed by MacChrean et al.[96] For this reason, a 2-minute SPME extraction was chosen for the ESIS.

Fentanyl samples were placed into eight different environments to replicate conditions that illicit fentanyl may encounter during transportation from clandestine labs. The study examined the effects the environmental impact had on the headspace of fentanyl over 28 days. Analyses of the sample were performed weekly using SPME to determine the headspace profile over time. The VOCs detected and identified in this study match those found in the study conducted in Task 2.

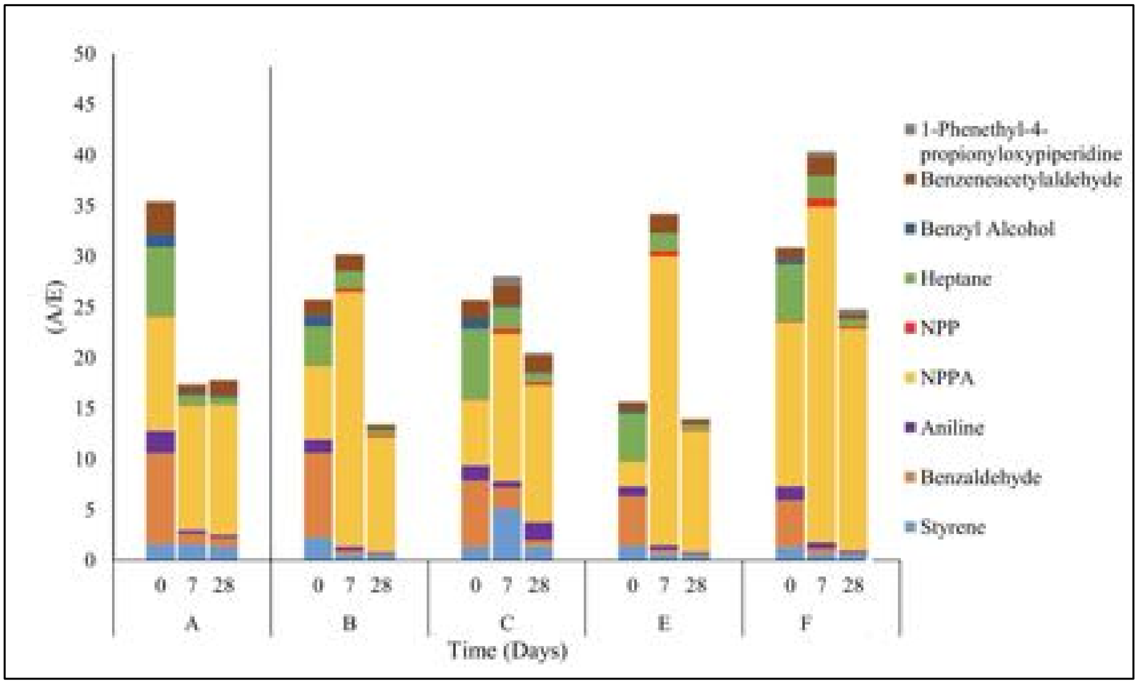

Figure 32. Comparison of headspace analysis of fentanyl over 28 days exposed to various environments (n=3). Environment A is used as a base environment to compare changes in humidity, oxygen, and heat.

The most significant changes in the headspace signature during degradation can be seen between Day 0 (fresh fentanyl) to Day 7 and Day 7 to Day 28. For this reason, those data points will be focused on as they provide the most critical information. However, the data for all days can be seen in Appendix A. As seen in Figure 32, from Day 0 to Day 7 in Environment A, NPPA and styrene remain constant while all other VOCs decrease. However, from Day 7 to Day 28, NPPA, benzaldehyde, and styrene remain constant while the other compounds decrease. For simplicity purposes, Environment A, whose parameters where dry, ambient and nitrogen gas, will be used as a base environment for which all other environments will be compared. The statistical differences between the environments and within the environments will be determined using 2-tailed t-tests. The results can be found in Appendix B and C. Based on the results seen in Figure 32, the introduction of oxygen, heat, and humidity resulted in a significant increase in NPPA in Environments B, C, and

E. By Day 28 of the degradation process, NPPA is the most abundant compound in the headspace while all the other compounds decreased. In Environment E, NPPA increased from Day 0 to 7 and, by Day 28, was the most dominant analyte while all other compounds decreased. The same can be said for Environment F with one difference: an increase in benzeneacetylaldehyde. When increasing the heat to 40 °C, styrene increased significantly from Day 0 to 7 compared to Environment A. This indicates that styrene is a product of thermal degradation which aligns with previously reported literature[30–33]. No statistically significant changes were seen for NPPA between or within the same environment (data for this can be seen in Appendix B and C). Most of the significant differences were seen in the lower abundant analytes.

7.3.2.2 Effects of Moisture and Oxygen on Fentanyl at an Elevated Temperature

A further investigation into styrene as a thermal degradant of fentanyl took place by changing the moisture and air supply of the environments but keeping the heat constant. To monitor the changes, this change was compared to Environment C, the base environment. When humidity was increased, or house air was used (G and D), there was an increase in the abundance of styrene seen from Day 0 to 7, and in Environment D, there was also an increase in benzeneacetylaldehyde, which decreased by Day 28. When both house air and humidity were used simultaneously (H), the same trends were noticed but with a higher abundance. Similarly, to Environment A, most VOCs decreased by Day 28 except for NPPA, which remained the most abundant analyte in the headspace. These results can be seen depicted in Figure 33. No statistically significant changes were seen for NPPA between or within the same environment. Most of the significant differences were seen in the lower abundant analytes.

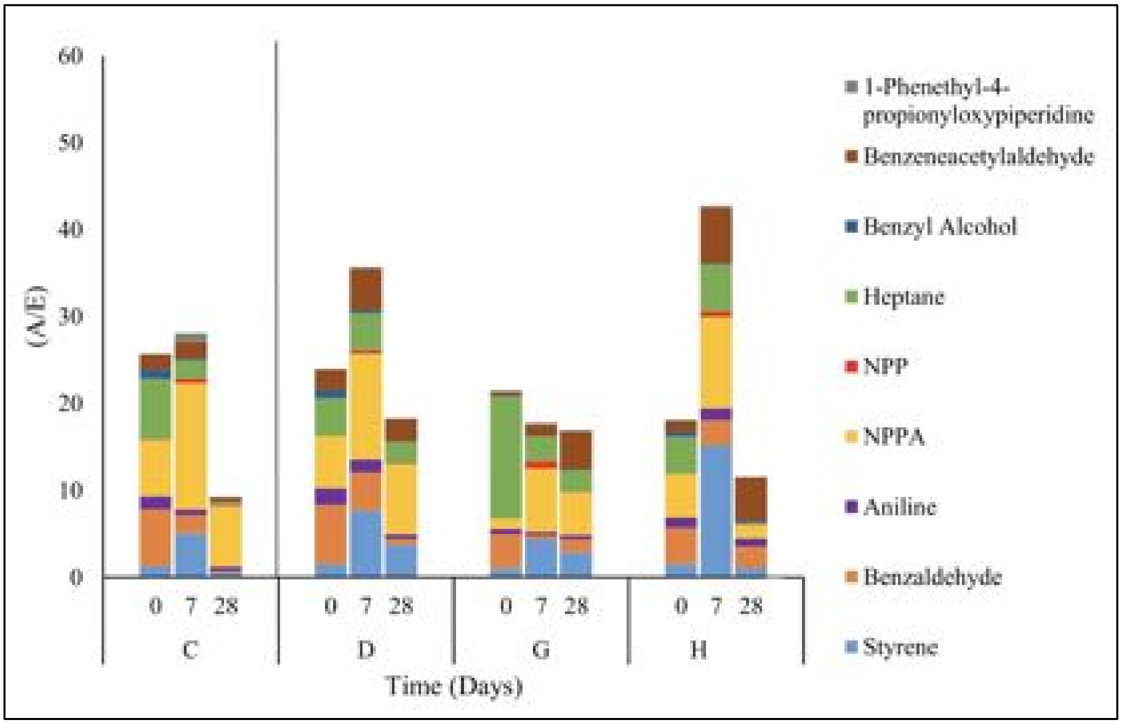

Figure 33. Comparison of headspace analysis of fentanyl over 28 days exposed to various environments (n=3). C is used as a base environment to compare changes due to humidity and oxygen while heat remains the same.

7.3.2.3 Change in Significant Degradants

Once the headspace analysis was completed, it was seen that NPPA was the most abundant headspace component throughout the study, and styrene was found to be a product of thermal degradation. Based on a publication by Qi et al., styrene is formed through oxidative cleavage of the C—N bond of the piperidine ring in fentanyl[35], and NPPA is a known degradant and a chemical marker for the synthetic route of fentanyl.[97] NPPA is formed via fragmentation by β-elimination, with water being one of the driving forces. Based on the results seen in Figure 34 the production of NPPA is constant in dry environments. Significant increases in NPPA, however, can be seen from Day 0 to 7 in the humid Environments E and F. When the temperature was increased in Environments G and H, the abundance of styrene increased however the of styrene was constant for all the ambient environments, there was a notable increase between Days 0 and 7. Both NPPA and styrene increased from Day 0 to 7, decreased from Day 7 to 14, and remained relatively constant until Day 28

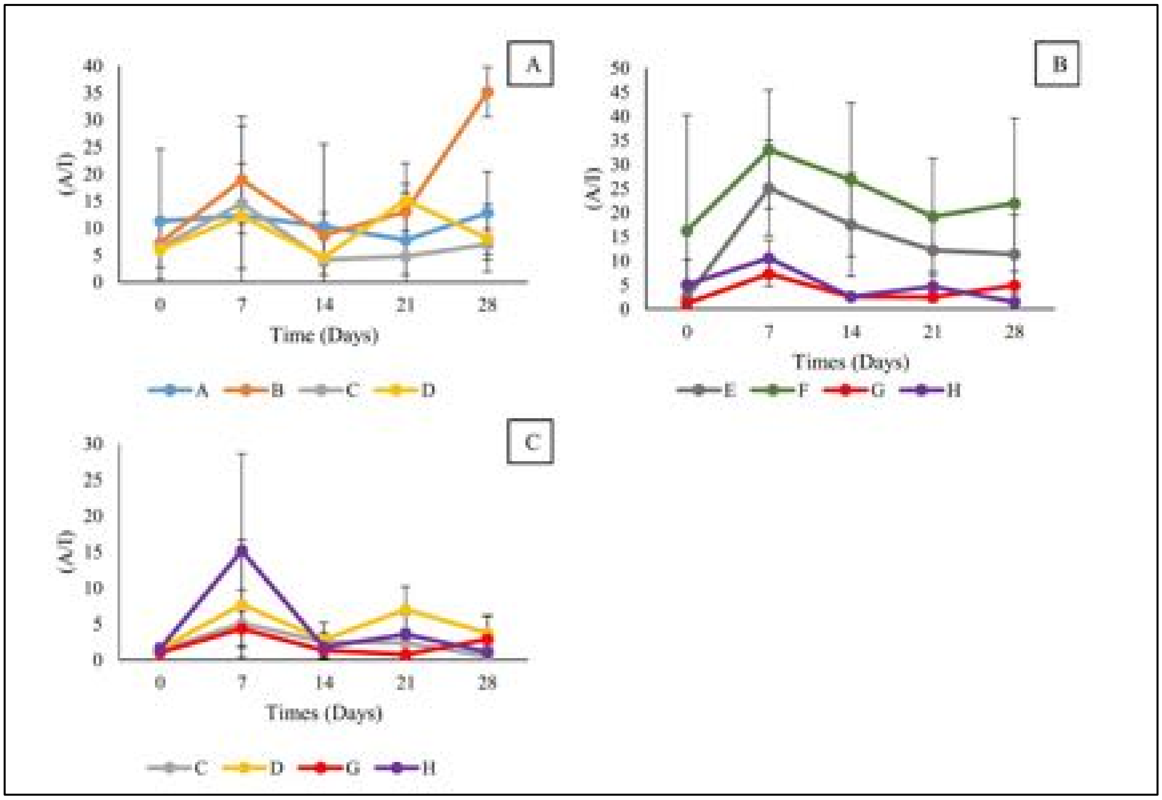

Figure 34. Changes in NPPA in dry environments (A) and humid environments (B) and change of styrene in environments at 40 ℃ (C).

7.4 Conclusions

Fentanyl samples were exposed to eight different environments to determine their effects on the headspace profile of fentanyl as it degrades. Based on the environmental impact on fentanyl's headspace, NPPA was seen as a prominent product of degradation throughout the entire study, which also aligns with data found in the literature. Furthermore, styrene was found to be a product of degradation in the environments where the heat was increased to 40 °C. Finally, as see in in past literature, benzaldehyde was also seen as a product of degradation, while all other compounds were suspected to be residual reagents of synthesis or from the manufacturing process. While styrene and benzaldehyde were identified as a product of degradation, this study validates the use of NPPA as a target analyte for the non-contact detection of fentanyl since it is prominent in every environment.

8: TASK 4: FORMATION OF A CONTROLLED ODOR MIMIC PERMIATION SYSTEM (COMPS)

8.1 Introduction

Several characteristics must be obtained to call a training aid "ideal." These characteristics include it being safe to handle and having the ability to pose no health risks to the canine. The training aid also needs to generate detectable levels of the odorants necessary for a proper alert and a suitable lifetime/shelf life.[82,98,99] In 2007, Furton and Harper developed the Controlled Odor Mimic Permeation System (COMPS) which allows a training aid to have the ideal characteristics described above.[100] Typically, a COMPS consist of the target odorant and an substrate material sealed into a polymer bag which regulates the amount of odor released. COMPS training aids are field deployable and releases reproducible and know amounts of odor. Once the training aid was constructed, it was crucial to determine how long it can be used. This study was done through SPME-GC-MS analysis which was broken down into two studies: a study to imitate regular use of the mimic where it was removed from its storage container at regular intervals and a study to estimate shelf life of the mimic where the mimics were not stored and constantly exposed to the environment.

8.2 Materials and Methods

8.2.1 Materials

N-Pheneylpropanamide was purchased from Cayman Chemical (Ann Arbor, MI). Alumina 80-200 mesh was purchased from Fisher Scientific (Waltham, MA). 3x2 in 2ML and 1 ML LDPE bags, as well as 2 oz metal screw top tins, were purchased by ULINE (Pleasant Prairie, WI). CAR/DVB/PDMS SPME fibers were purchased from SUPLECO

(Bellefonte, PA). 20 mL VOA sampling vials with a septum were purchased from Restek (Bellefonte, PA).

8.2.2 Mimic Development

To develop a training aid mimic using NPPA, it was important to ensure the level of NPPA emitted from the mimic was consistent with the level emitted from fentanyl at room temperature. To make this determination three mimic preparations were created and their NPPA levels were compared to that of 5 mg of bulk fentanyl. The standard operating procedure (SOP) for creating commercially available 3,4-Methylenedioxy-methamphetamine (MDMA) training aids developed in the Dr. Kenneth G. Furton research lab at Florida International University was implemented for the development of Mimic Preparation 1. The SOP indicated using a 1:4 ratio of odorant to alumina powder. For Mimic Preparation 1, 20 mg of NPPA to 80 mg of alumina powder were added to a 40 mL VOA vial and then shaken to ensure a homogenous mixture. After shaking, the mixture was added to a 2x3 in, 2 MIL LDPE COMPS bag and heat sealed. Unlike Mimic Preparation 1, Mimic Preparations 2 and 3 consisted of neat 60 mg of NPPA placed into a 1 MIL 2x3 in LDPE heat-sealed bag and a 2 oz screw top tin with perforated holes, respectively. A table detailing the mimic preparations can be seen in Table 15.

Table 15.The three different mimic preparations prepared.

Preparation	Contents	Container
1	20 mg NPPA, 80 mg Alumina	2x3 in 2 MIL LDPE bag
2	60 mg NPPA	2x3 in 1 MIL LDPE bag
3	60 mg NPPA	2 oz screw top metal tin with holes

To test the level of NPPA emitted from the three mimics, SPME was used to extract the headspace, which was then analyzed using an Agilent 8890-5977B GC-MS system. The samples were heat-sealed in bags and placed into a 20 mL VOA sampling vial, and the screw top tins were put into a 500 mL Teflon jar and allowed to equilibrate for 30 minutes at 35 °C, respectively. Unlike the previous SPME extractions done in this project, since the number of samples was large, this experiment used a shorter extraction time increase efficiency. While the equilibration time and extraction temperature from the method in Task 2 remained the same, four different extraction times were investigated 5, 10, 15, and 30 minutes. The headspace was extracted using a CAR/DVB/PDMA SPME fiber and then exposed to acetanilide as an externally sampled internal standard for 2 min. The fibers were then analyzed using the GC-MS method from Task 2.

8.2.3 Determination of Odor Depletion with Storage

Once the mimic had an NPPA peak in the same range as reference-grade fentanyl, an investigation was completed in triplicate to determine for how many active training hours the mimic could be used. For this study, active training was defined as a 15-minute period where the mimic was removed from its storage container (mylar bag) and the training aid was analyzed for 6 hours or 24 15-minute sessions. This study was designed to imitate regular use of a training aid where the aid was kept in storage when it was not being used and taken out of storage in regular intervals to mimic training. To complete this experiment, three mimics were made and placed into a 20 mL VOA vial. The sample was left to equilibrate for 30 minutes at 35°C and was then extracted using a CAR/DVB/PDMA SPME fiber for 15 minutes at 35°C. The fiber was then exposed to acetanilide as an externally sampled internal standard for 2 min. After extraction, the mimics were placed back into their Mylar bag storage container. The fibers were then analyzed using the GC-MS method from Task 2. Each mimic was sampled once a day for 24 days until 24 15-minute sessions had been completed.

8.2.4 Determination of Odor Depletion without Storage

In addition to investigating the odor depletion with storage, the odor depletion without storage was investigated. For this experiment, the mimics were tested in triplicate. The three mimics were hung in the laboratory for constant exposure to the environment. The mimics were sampled once a week starting with week 0 (the day of production) for one month using the exact sampling and analysis methods described in section 8.2.3. After the mimics were sampled, they were hung back up in the laboratory environment until their next sampling. This study was designed to estimate a shelf life of the mimic so the user knows when a new aid should be used,

8.3 Results and Discussion

8.3.1 Mimic Development

As previously stated, the extraction time for this study was shortened from 4 hours to help effectivity and efficiently process the large sample volume. While the equilibration time and extraction temperature remained the same four different extraction times were investigated 5, 10, 15, and 30 minutes. Based on the data in Figure 35, 15 minutes showed a higher response for both runs resulting in a 15-minute extraction time being chosen.

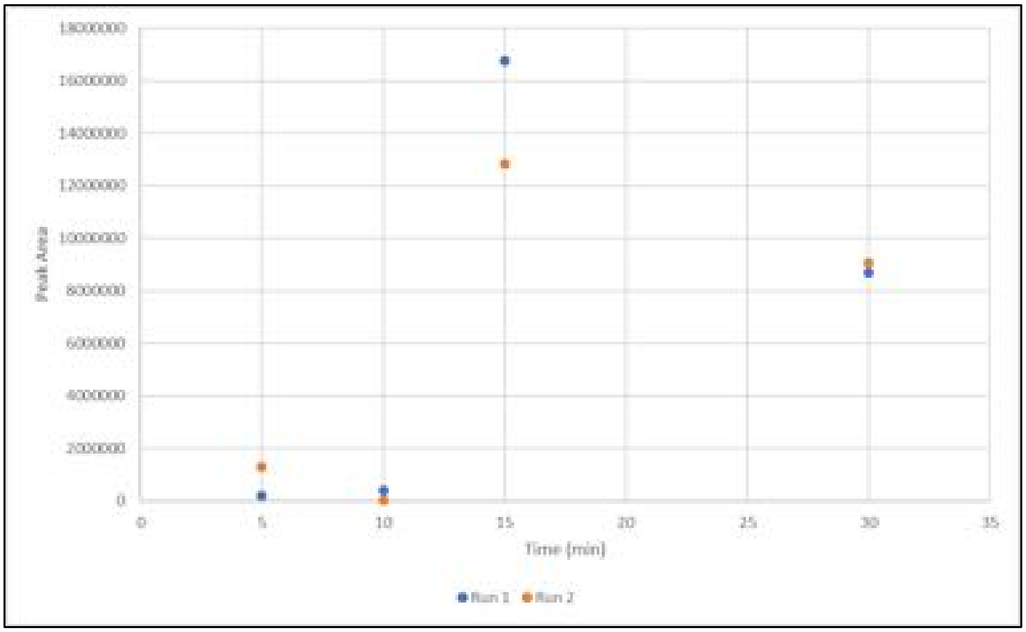

Figure 35. Extraction time optimization for analyzing the fentanyl mimic (n=2).

For this project, three mimic preparations were created to develop a final novel training aid mimic for fentanyl. The headspace of these three preparations was then investigated to determine if the intensity of NPPA emitted by the mimic was similar to the intensity of NPPA emitted by fentanyl. Based on the data seen in Figure 36, Preparation 1 produced an (A/E) value for NPPA most similar to that of bulk fentanyl. This means, when compared to the NPPA odor that fentanyl emits, preparation 1 is more like the NPPA odor than preparations 2 and 3. It is important to note that unlike the 5 mg of bulk fentanyl, preparation 1 and 2, which were prepared in 20 mL vials preparation 3 was sampled in a 500 mL Teflon jar. Following this analysis, all following experiments were conducted using Preparation 1.

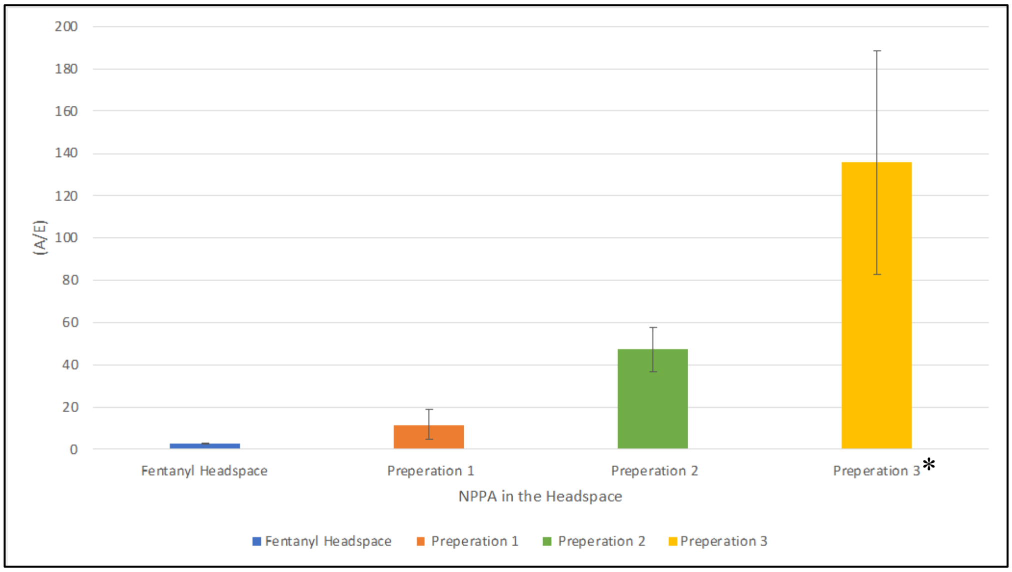

*Figure 36. Comparison of the NPPA (A/E) in the headspace of the mimic preparations to the NPPA (A/E) in the headspace of bulk fentanyl. * Preparation 3 was sampled in a different volume chamber then the other samples.*

8.3.2 Results for the Determination of Odor Depletion with Storage

When training canines, it is important to know how long the training materials can be used before they need to be disposed. For this purpose, a study was completed to determine how many training sessions the fentanyl training aid mimic can be used. This study defined a singular training session as 15 minutes, and the training aid was analyzed for 6 hours or 24 15-minute sessions. When the mimic preparations were not being sampled, they were stored in mylar bags in at room-temperature allowing them to replenish their odor. The number of usable training sessions for three fentanyl training aid mimics was investigated, starting with Session 0 or immediately after production. The results seen in Figure 37 show that over 6 hours or 24 15-minute training sessions, the average (A/E) stays relatively constant. There was a less than 1 % decrease in odor between sample Session 0 and sample Session 24. This minimal decrease in odor can be caused by mimic's ability to replenish its odor while it is being stored in-between sample sessions.

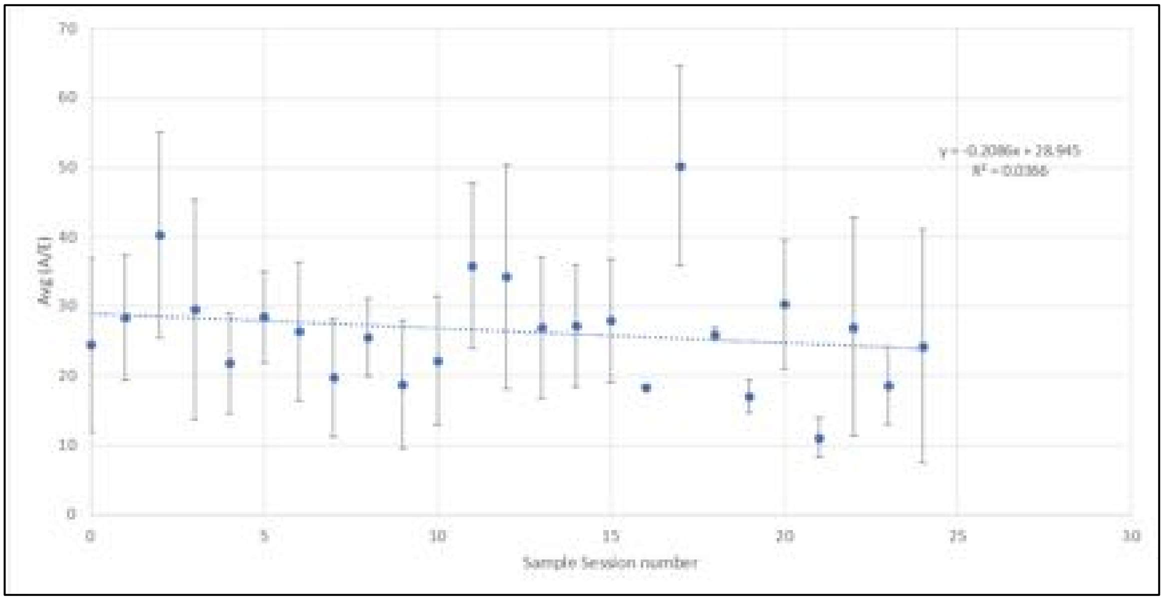

Figure 37.Data representing the results for mimic odor depletion with storage (n=3).

8.3.3 Results for the Odor Depletion without Storage

The final study in this task was investigating the shelf life of the mimic preparations without storage. Unlike the investigation into the active training lifetime, the mimics preparations in this experiment were not placed into a storage container between sample sessions to replenish their odor. The results from this study are seen in Figure 38 and show that by the end of one month, there was significant odor depletion. When calculated from week zero to week four, there was a greater than 78 % decrease in odor. This means that after one month of being consistently exposed to the lab environment, 78 % of the odor had evaporated.

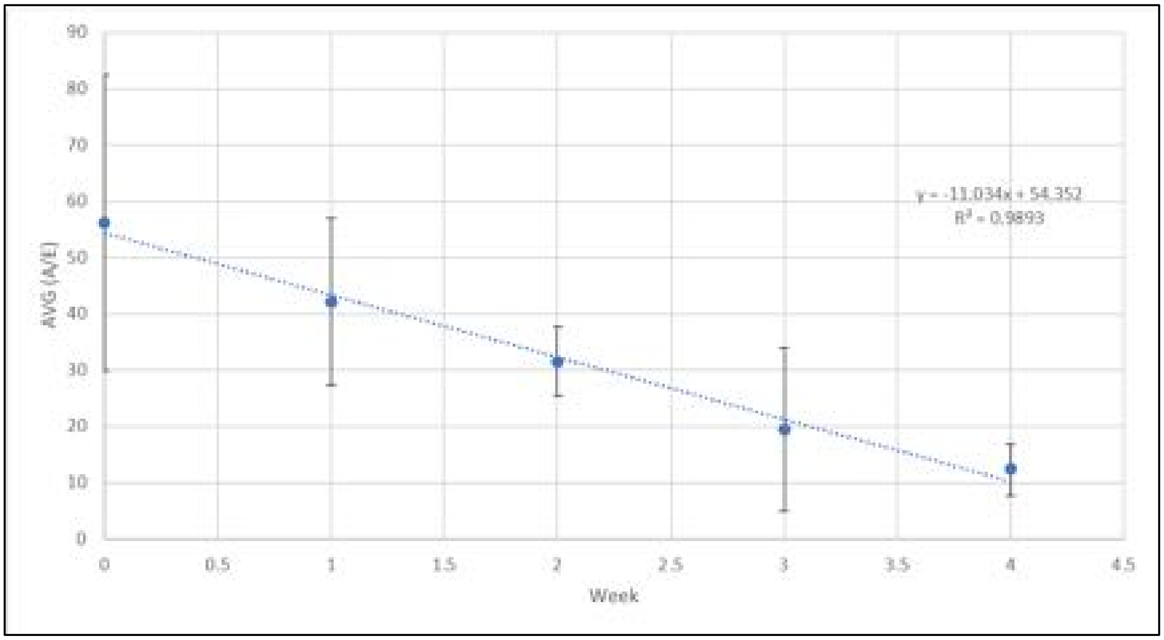

Figure 38.Results from the shelf-life study taking place over one month (n = 3).

8.4 Conclusion

In this task a COMPS for a novel fentanyl canine training aid mimic was optimized. The first step in this process was ensuring that the mimic produced an NPPA peak with a similar intensity to the NPPA peak in the headspace of fentanyl. It was concluded that when the headspace of Mimic Preparations 1-3 was compared to the headspace of bulk fentanyl, Mimic Preparation 1 produced the most similar abundance, in (A/E) ratio, of NPPA compared to the 5 mg fentanyl sample. A headspace investigation using SPME-GC-MS to determine how many training sessions the mimic can be used and how long it takes for the odor to be fully depleted. The results of these studies showed that the mimic is viable for the full 6 hours, or 24 15-minute training sessions, that were investigated. If fact, there was less than a 1 % odor decrease between sample session 0 and sample session 24. It was also concluded that after 4 weeks of constant exposure to the lab environment, there was greater than 78 % decrease in odor. This means that the mimic had a shelf life of one month when not properly stored and a lifetime of at least 6 hours when it is in active use for training.

9. TASK 5: THE VERIFICATION OF THE FENTANYL TRAINING AID MIMIC USING SCENT DETECTION CANINES

9.1 Introduction

To this day, law enforcement still use canines to perform various tasks such as detecting drugs, explosives, arson/ignitable liquid, firearms, currency, and missing persons.[10] Training a detector canine to perform these tasks can be done by imprinting them on a specific odor(s) through the target material or a mimic. In this case, the target material is the illicit substance, while a mimic is a material that emits a similar odor as the target material.[101] Training canines on fentanyl has been limited due to the health risks it poses to handlers and canines and the ability to find training materials. For this reason, a novel, safe, fentanyl canine training aid has been developed. This task aims to begin the verification of the novel fentanyl training aid mimic developed in the previous task using substance detection canines. This will be done using odor recognition tests (ORTs) which are intended to show whether the canines can identify the odor on which they were trained, as well as any targets which they associate to that odor. The ORTs for these trials will include the novel training aid and positive control, blanks, and several distractors (a non-target odor the canines are not trained to alert to) to validate that the training aid will not cause false alerts. These ORTs will be performed using two populations of canines those who have been previously trained on fentanyl and those who have not been previously trained on fentanyl.

9.2 Materials and Methods

9.2.1 Materials

Fentanyl was purchased from Cayman Chemical (Ann Arbor, MI). N-phenylpropanamide (NPPA) was purchased from BLDPharm (Shanghai, China) and Toronto Research Chemicals (Toronto, Canada), and alumina 80-200 mesh was purchased from Fisher Scientific (Waltham, MA). Acetanilide, S-tert-butyl-4-hydroxyanisole (BHA), and O-Toluic acid were purchased from Sigma Aldrich (St, Lois, MO). SPME CAR/DVB/PDMS fibers were purchased from Supelco INC. (Bellefonte, PA). The 40 mL and 20 mL VOA sampling vials were purchased from Restek (Bellefonte, PA). The 3x3 in and 2x3 in 2 MIL and 1 MIL LDPE bags and the 2 oz metal screw top tins were purchased from Uline (Pleasant Prairie, WI). The 2x2 in gauze pads were purchased from Dukal Corporation (Ronkonkoma, NY). 55 mm cellulose chromatography circular filter papers were purchased from Whatman (Maidstone, United Kingdom). The peanut butter dog treats were purchased from Old Mother Hubbard Baking Company (Tewksbury, MA), while the mint dog treats were purchased from Pedigree (McLean, VA). The strawberry hand lotion was purchased from Bath and Body Works (Columbus, OH).

9.2.2 Canine Field Trials

To determine the validity of NPPA as the odorant of fentanyl, three canine trials were conducted with a total of 31 canines. Of these 31, 26 canines (NF 1-26) were not previously trained on fentanyl and were provided by both civilian and government agencies while the remaining five canines (F 1-5) were previously trained on fentanyl by a government agency. Each canines used participated in one of three trials (Table 16). All three canine trials will be discussed in further detail in sections 9.2.2.1 to 9.2.2.3. All canine trial protocols were reviewed and approved by the Florida International University Animal Care and Use Committee (IACUC-21-015-CR02)

Table 16. Canine trial experimental breakdown.

Canine	Canine trial	Trained material	Who trained the canine
NF1	1	Fentanyl Positive Control	Agency's trainer
NF2	1	Fentanyl Positive Control	Agency's trainer
NF3	1	Fentanyl Positive Control	Agency's trainer
NF4	1	Fentanyl Positive Control	Handler
NF5	1	Fentanyl Positive Control	Handler
NF6	1	Fentanyl Positive Control	Handler
NF7	1	Fentanyl Positive Control	Handler
NF8	1	Fentanyl Positive Control	Handler
NF9	1	Mimic	Handler
NF10	1	Mimic	Handler
NF11	1	Mimic	Handler
NF12	1	Mimic	Handler
NF13	1	Mimic	Handler
NF14	1	Mimic	Handler
NF15	1	Mimic	Handler
NF16	1	Mimic	Handler
NF17	2	Mimic	Agency's trainer
NF18	2	Mimic	Agency's trainer
NF19	2	Fentanyl	Agency's trainer
NF20	2	Fentanyl	Agency's trainer
NF21	2	Mimic	Agency's trainer
NF22	2	Mimic	Agency's trainer
NF23	2	Mimic	Agency's trainer
NF24	2	Fentanyl	Agency's trainer
NF25	2	Mimic	Agency's trainer
NF26	2	Mimic	Agency's trainer
F1	3	Fentanyl	Agency's trainer
F2	3	Fentanyl	Agency's trainer
F3	3	Fentanyl	Agency's trainer
F4	3	Fentanyl	Agency's trainer
F5	3	Fentanyl	Agency's trainer

9.2.2.1 Training and Trials for Civilian Non-Fentanyl Trained Canines

As previously stated, 16 canines not previously trained to detect fentanyl were used for this study. Of these 16 canines, 10 were volunteers from the NACSW. The NACSW is an organization that was created to bring detector canine training to civilians and their dogs for competitions and sport. Canines participating in competitions through the NACSW can earn titles including NW1, NW2, NW3, ELT1, ELT2, and ELT3, whose award depends on canine performance during the competition.[102] For example, an NW3 title is awarded when a canine scores 100 with no more than three false alerts in a single competition. For this study, it was requested that canine volunteers possess a title of NW3 or higher. The remaining six civilian canines were provided and chosen by a private agency which sells scent detection canines. Once all canines were chosen, the population was evenly split, so half were trained on the fentanyl training aid mimic, and half were trained on the fentanyl positive control. All handlers and/or trainers were informed of their assigned odor and mailed their training materials. The canines from NACSW were trained by their handlers using guidelines provided by FIU (seen in Appendix F). The training stage of this study took place six weeks before the ORT. The important information such as breed, age, and years of experience for the non-fentanyl trained canines NF1-16 can be seen in Table 17.

Table 17. Important information about civilian non-fentanyl trained canines NF1-16.

Canine	Breed	Age	Experience	Training Material
NF1	Beagle	1 year	1 year	Fentanyl soak
NF2	Beagle	1 year	1 year	Fentanyl soak
NF3	Beagle	1 year	1 year	Fentanyl soak
NF4	Cocker Mix	7 years	Nose Work 3	Fentanyl soak
NF5	Jack Russell Terrier	9 years	Nose Work 3	Fentanyl soak
NF6	Labrador	6.5 years	Nose Work 3	Fentanyl soak
NF7	Shepherd	3 years	Nose Work 3	Fentanyl soak
NF8	Chocolate Lab	7 years	Nose Work 3	Fentanyl soak
NF9	Shih Tzu	10 years	Elite 1	Mimic
NF10	Pyrenees/Cattle Dog Mix	7 Years	Nose Work 2	Mimic
NF11*				Mimic
NF12*				Mimic
NF13*				Mimic
NF14*				Mimic
NF15*				Mimic
NF16*				Mimic

**It is important to note that while canines NF11-16 were assigned the training aid mimic as their odor, no data or metrics were gathered since they dropped out of the study before the ORT.*

Since this study used civilian scent detection canines that were not previously trained on fentanyl and were restricted from obtaining or working with actual fentanyl for both legal and safety concerns; however, they needed to be supplied with a positive control that was known to emit the vapor profile of fentanyl identified in Task 2. For this purpose, a fentanyl odor soak was developed. An odor soak is defined as a training aid in which the vapor profile of the target material is soaked onto a sorbent substrate.[103] To make the odor soak, 5 mg of reference-grade fentanyl was placed into a 20 mL VOA sampling vial, which was then placed open into a 16 oz glass canning jar. Once the fentanyl sample was placed in the jar, four 2x2 in cotton gauze pads or 55 mm circular cellulose chromatography filter papers were hung from the top of the jar, as seen in Figure 39. The jar was closed, and the headspace was left to soak into the gauze pads for three days at 35 °C.

Figure 39. Schematic for making the fentanyl odor soaks.

Following the three-day soaking period, the gauze pads were removed and placed into 3x3 in, 2 MIL LDPE bags and tested for the presence of the compounds in the fentanyl headspace using the SPME-GC-MS. The sealed odor soaks were placed into a 20 mL VOA vial, where it was allowed to equilibrate for 30 minutes at 35 °C. Once at equilibrium, the headspace was extracted for 15 minutes at 35 °C using a CAR/DVB/PDMS SPME fiber. After extraction, the fibers were exposed to acetanilide, which was used as an ESIS, for 2 min. The fibers were then inserted into the GC inlet at 260 °C for a 3-minute desorption with a 10:1 split ratio and a 2 mL/min flow. The GC had a 15 m HP5-MS column (250 µm x 0.25 µm) with the following parameters. The oven started at 40 °C and was held for 30 seconds; the temperature then ramped to 240 °C at 30 °C/min. The transfer line to the MS was held at 240 °C with a mass scan range of *m/z* 40-300. This process was repeated with 5 mg of reference-grade fentanyl to compare the intensity of the NPPA peak. This method of extraction and analysis was also previously described previously herein.

These trials were performed inside a 2000-square-foot temperature-controlled facility to limit contamination and environmental effects. The ORT consisted of nine scent lineups, each containing six boxes, as seen in Figure 40. Lines 1, 2, 4, 5, 6, 7, and 9 consisted of one fentanyl-related material, three blanks, and two distractors, while Lines 3 and 8 consisted of three blanks and three distractors. The blanks with this study were blank odor soaks, blank gauze pads, and alumina powder. While the distractors were prepared as odor soaks of sharpie, dog treats, nitrile gloves, and strawberry-scented lotion.

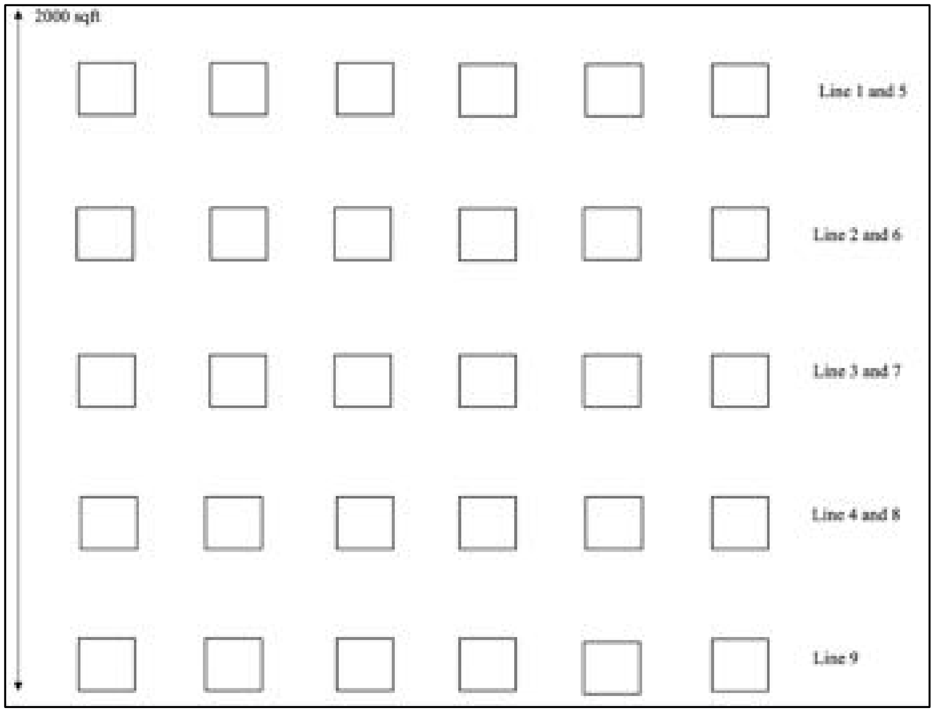

Figure 40. ORT set up for the canine field trials of canines NF-16.

The materials were placed into 2 oz metal screw top tins with perforated holes. These tins were then placed into open cardboard boxes and arranged into scent lineups based on the matrices seen in Appendix G. The location of all targets, blanks, and distractors was randomized for each canine to avoid introduction bias. Due to the space provided, canine teams began by running Lines 1-4 first, followed by Lines 5-9. The handlers were instructed to run their canines down each line and to allow their canines to perform their searches. This trial was a double-blind study where neither the canine handler nor the person recording the data knew the location of the targets. Only the person who set up the trial knew the contents of the lineups and was informed the handler if their canine's alert was right or wrong. If the alert was correct, a reward was given; if the alert was wrong, the canine was instructed to move on. The response was recorded as a positive alert if the canine was correct. If it was wrong, the response was recorded as a false alert.

9.2.2.2 Training and *Trials for Law Enforcement Working Non-Fentanyl Trained Canines*

This study also utilized operational working canines, which were not previously trained on fentanyl, but had previous experience in detection of other drugs, and will be referred to as NF17-26. Prior to any training a base line trial took place to assess to the canine's ability and visually represent the results of the training process. For this trial three canines were assigned reference-grade fentanyl as their trained odor and seven canines were assigned the mimic as their trained odor, and canine trainer conducted the training. They were originally sent the optimized COMPS, blanks, and distractors to begin training in a Training Aid Delivery Device (TADD), seen in Figure 41. However, when training began on the COMPS in the TADDS, the canines could not detect it. Based on this behavior, it was determined that the initial odor threshold (the lowest concentration the odor could be detected) for NPPA was higher than what the COMPS were emitting.

Figure 41. Training Aid Delivery Device (TADD).[104]

To remedy this threshold issue, the canines were trained on 30 mg of pure NPPA in glass vials. Once they successfully learned this odor, they moved onto training with Mimic Preparation 2 (60 mg of NPPA in a 1 MIL LDPE bag). Finally, once they were fully trained on that their threshold was once again lowered to Mimic Preparation one (the NPPA COMPS). The important information such as age, breed, years of experience, and training material for the canines NF17-26 can be seen in Table 18.

Table 18. Important information about canines NF17-26.

Canine	Breed	Age	Years of Experience	Training Material
NF17	Belgium Malinois	4 years	3 years	Mimic
NF18	Dutch Shepard	5 years	3 years	Mimic
NF19	Belgium Malinois	3 years	1 year	Fentanyl
NF20	Dutch Shepard	2.5 years	1 year	Fentanyl
NF21	Dutch Shepard	2.5 years	1 year	Mimic
NF22	Belgium Malinois	8 years	7 years	Mimic
NF23	German Shepard	2 years	1 year	Mimic
NF24	German Shepard	3 years	<1 year	Fentanyl
NF25	Dutch Shepard	3 years	1 year	Mimic
NF26*	German Shepard	4 years	2 Years	Mimic

**NF26 only participated in the pretrial, they were removed from the post trail due to an overactive alert.*

Unlike Trial 1, this trial used six, 6-arm scent wheels and example of which can be seen in Figure 42. All materials were placed into TADDS and the TADDS were placed into the canisters on the wheels. Wheel 1, 2, 5 and 6 consisted of a fentanyl related material, three blanks, and two distractors, while Wheel 3 consisted of three blanks and three distractors. Since these canines were not previously trained on fentanyl Wheel 4 consisted of a cocaine positive control, three blanks and two distractors. This helped to ascertain the canine's performance since they were all certified-on cocaine. In this trial the blanks consisted of blank mimics, while the distractors consisted of dog food, nitrile gloves, dog treats, O-toluic acid, beta hydroxybutanoic acid (BHA) and 5 additional distractors provided by the agency. The full ORT set up can be seen in Appendix H. The handlers were instructed to run their canines around each wheel and to allow their canines to perform their searches. This trial was a double-blind study where neither the canine handler nor the person recording the data knew the location of the targets. Only the person who set up the trial knew the contents of the lineups and was informed the handler if their canine's alert was right or wrong. If the alert was correct, a reward was given; if the alert was wrong, the canine was instructed to move on.

Figure 42. Six arm training scent wheel.[105]

9.2.2.3 Field Studies for Canines Previously Trained to Detect Fentanyl

For this study, five canines previously trained on to detect fentanyl citrate were used provided by a government agency and will be referenced as F1-5. The important information such as age, breed, and time in service for canines F1-5 can be seen in Table 19.

Table 19. Important information for fentanyl trained canines F1-5.

Canine	Breed	Age	Years in Experience	Training Material
F1	German Shepherd	1.5 years	3 months	Fentanyl citrate
F2	German Shepherd	1.5 years	4 months	Fentanyl citrate
F3	German Shepherd	2 years 1 month	9 months	Fentanyl citrate
F4	German Shepherd	1 year 7 months	5 months	Fentanyl citrate
F5	German Shepherd	1 year 4 months	4 months	Fentanyl citrate

As with the field studies run for the civilian canines, the trials took place inside a climate-controlled facility to limit contamination and environmental effects. All canines in this study were chosen by the trainers and assigned the mimic as their target odor. Since these canines were trained on fentanyl, there was no need for an imprinting period like the one described above. Instead of using an FIU-provided positive control, they used their training material, fentanyl citrate, as the positive control. For this ORT lines 1, 2, 4, 5, 6 and 8 contained one fentanyl related material, two distractors and three blanks, while lines 3 and 7 contained three distractors and three blanks. The blanks were comprised of empty heat-sealed, 2x3 in, 2 MIL LDPE bags and blank odor soaks in heat-sealed, 2x3 in, 2 MIL LDPE bags. The distractors for this study were prepared as odor soaks and included sharpie, dog food, dog treats, nitrile gloves, coconut-scented soap, acetone, orange peels, and strawberry lotion. All blank and distractor odor soaks were prepared and packaged as previously described.

For this study, the agency first received Mimic Preparation 1 (20 mg NPPA to 80 mg alumina in a 2x3 2 MIL LDPE bag); however, during the trial, the canines did not respond to the presence of Mimic Preparation 1. For this reason and upon request of the agency, they were given Preparations 2 (60 mg of NPPA in a 2x3 in 1 MIL LDPE bag) and 3 (60 mg of NPPA in a 2 oz metal screw top tin with perforated holes) to increase the NPPA odor delivered to the canines during a second trial. The materials were placed into a screw top metal tin with perforated holes. This tin was then placed into a can built into a wooden box, as seen in Figure 43.

Figure 43. Material containment used to present the odor to canines F1-5 during the field study.

To perform the ORT, lineup matrices were provided by FIU (Appendix I) and replicated using eight lines of six containments. An example of one of these lines can be seen in Figure 44. The location of all targets, blanks, and distractors was randomized for each canine to avoid introduction bias. The handlers were instructed to run their canines down each line and to allow their canines to perform their searches typically. This trial was a double-blind study where neither the canine handler nor the person recording the data knew the location of the targets. Only the person who set up the trial knew the contents of the lineups and was informed the handler if their canine's alert was right or wrong. If the alert was correct, a reward was given; if the alert was wrong, the canine was instructed to move on. The response was recorded as a positive alert if the canine was correct. If it was wrong, the response was recorded as a false alert.

Figure 44.Example of one scent lineup constructed for the ORT for canines F1-5.

9.3 Results and Discussion

9.3.1 Development of a Fentanyl Positive Control

Since non-fentanyl-trained civilian canines were being used in this study, a suitable positive control needed to be developed for both training and field studies. For this reason, a fentanyl odor soak was developed. The headspace of fentanyl was soaked onto both a 2x2 in sterile gauze pad and 55 mm filter paper to determine which substrate would better absorb the vapor profile. Based on the data seen in Figure 45 the gauze absorbed a larger abundance of each of the headspace components when compared to the filter paper. For this reason, cotton gauze was chosen as the substrate for the fentanyl odor soaks going forward.

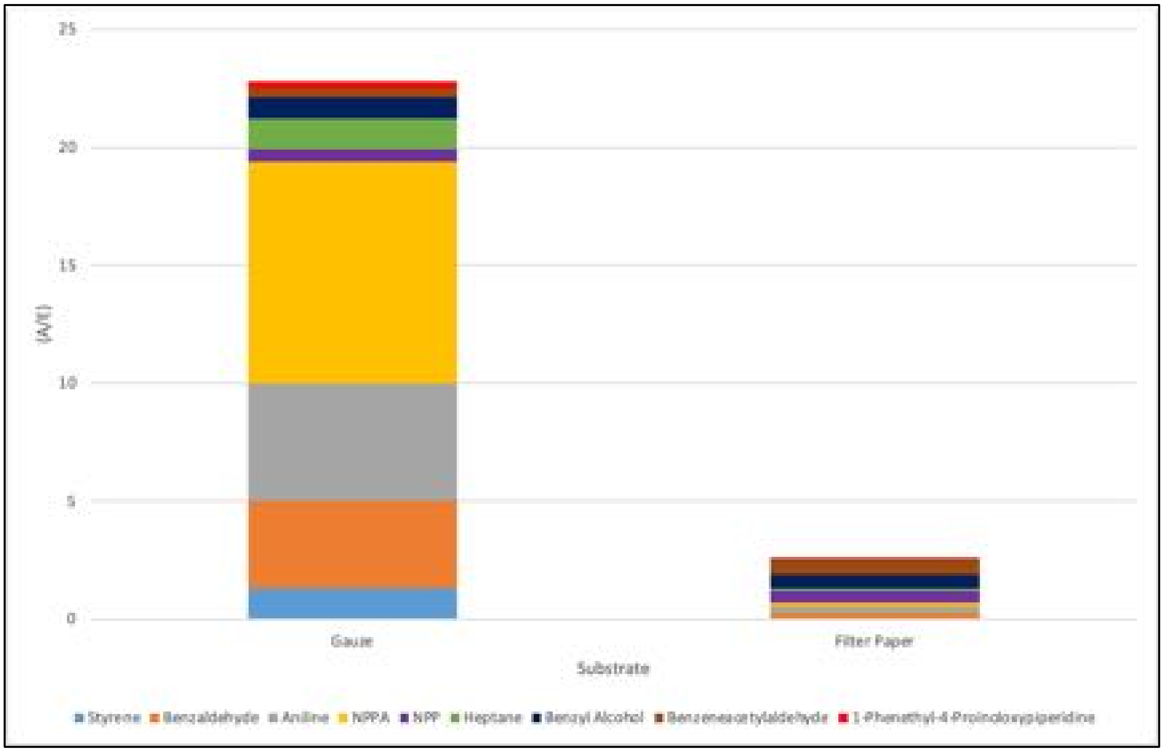

Figure 45. Fentanyl headspace soak Gauze vs. Filter paper (n=3),

Since the major component for the fentanyl training aid mimic was NPPA, it is also essential to compare the relative abundance (A/E) of NPPA in the headspace of the odor soak to that of fentanyl. Figure 46 shows that the abundance of NPPA in the fentanyl headspace 60% greater than that of the odor soak, though it still had a readily detectable amount of NPPA.

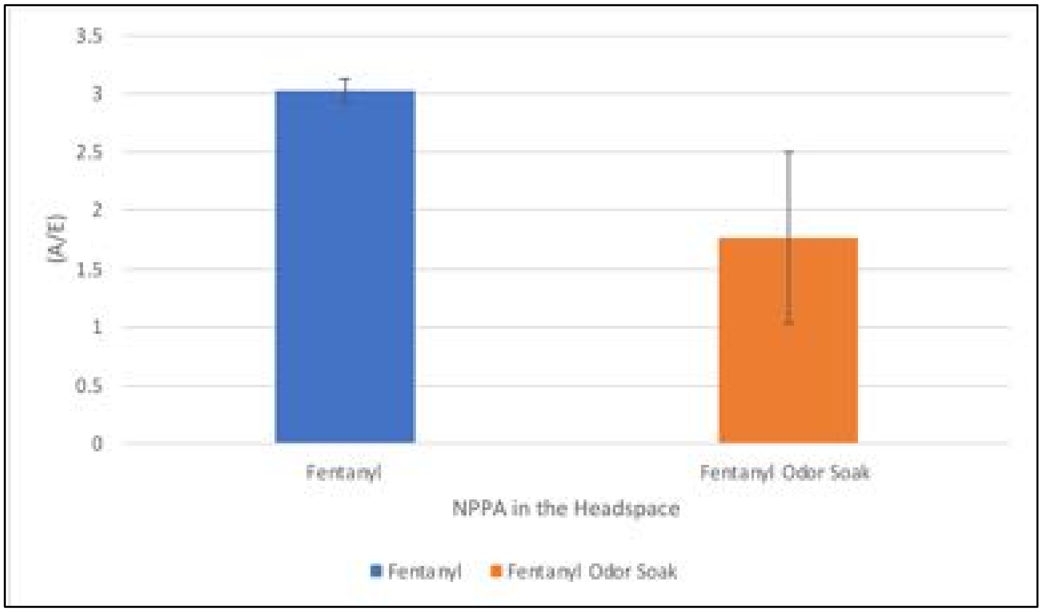

Figure 46. Comparison of the (A/E) of NPPA in the fentanyl headspace to the (A/E) of NPPA in the headspace of the fentanyl odor soak.

Finally, since the odor soak was used for imprinting, it was important to determine how many training sessions it would be viable. Like the mimic, the odor soak was investigated for 6 hours or 24 fifteen-minute training sessions with storage in between sample sessions. The results in Figure 47 show that even when being stored in between training sessions the odor soak should not be used for more than 15 minute or training session. This is evident by the soak having an average abundance in (A/E) of approximately 30 for Session 0 and then rapidly dropping to below the detection limit after Session 1 and remining at below the detection limit for the following 23 sessions.

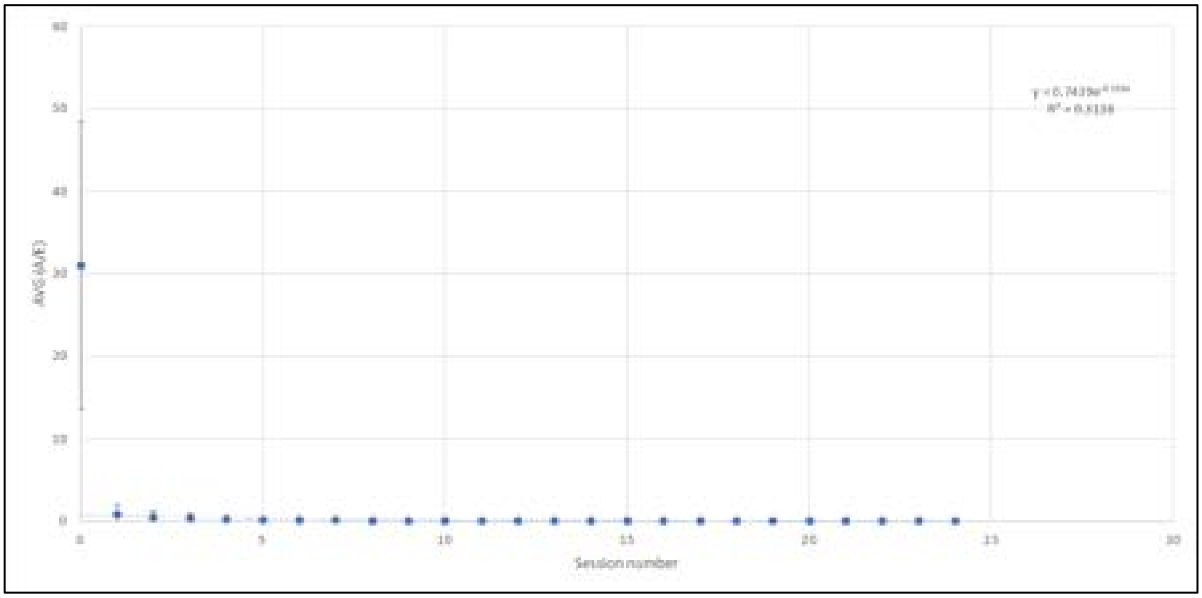

Figure 47. Results odor depletion with storage of the fentanyl odor soak.

9.3.2 Field Studies for Non-Fentanyl Trained Canines

9.3.2.1 Civilian Non-Fentanyl Trained Canines

As previously stated during field trials canines can exhibit a positive alert which is when they properly detect a target, a false positive which is when they incorrectly alert to a blank/distractor, a true negative which is when they correctly miss a blank/distractor, or a false negative is when they incorrectly miss a target. Furthermore, the number of positives and negatives can then be displayed as percentages to ascertain how a canine how a canine’s performance throughout the whole trial. As previously stated, the civilian non-fentanyl trained canines performed a trial consisting of nine scent lineups, each with six boxes. Each canine investigated the scent of 54 boxes, seven of which contained a target (a fentanyl-related material – mimic or odor soak) and 47 of which contained a non-target (blank or distractor). The results of this trial can be seen in Table 20.

Table 20.Data representing canine performance for civilian non-fentanyl trained canines NF1-16.

Canine	Positive Alert Rate	False Alert Rate	PPV	NPV
NF1	4%	4%	4%	88%
NF2	0%	0%	0%	87%
NF3	6%	2%	6%	90%
NF4	0%	10%	0%	84%
NF5	8%	6%	9%	93%
NF6	4%	2%	5%	89%
NF7	0%	10%	0%	84%
NF8	2%	12%	3%	86%
NF9*	0%	2%	0%	86%
NF10*	6%	8%	7%	91%
NF11**				
NF12**				
NF13**				
NF14**				
NF15**				
NF16**				
Average	3%	6%	3%	88%

**These canines in red represent those who were trained on the mimic.*

***It is important to note that canines NF11-16 have no data because they dropped out of the trial prior to the ORT.*

Based on the data in Table 20, canines NF1-10 had an average positive alert rate of 3% and an average false alert rate of 6%. The data in Table 20 also show that canines NF1-10 had an average PPV of 3% and an average NPV of 88%. This meant that there was a 3% chance that a positive alert was a true positive and an 88% chance that a negative (no response) being a true negative. Complete ORT data can be seen in Appendix I. The lack of positive alerts or high PPV values in this study could be due to the canines' ability to detect their training material. This means that the canines were not literate in their trained odors. This could be due to the lack of a central trainer being used. Using a central training would ensure that all trainers would be using the same training techniques and their canines would all be fully and properly trained on their assigned odor. As previously stated, the canines in this population were trained by their handlers rather than a certified trainer, which could have affected the canine's ability to detect their assigned odor. This canine's inability to detect the NPPA mimic as well as the odor soak can also be due to canines having a higher threshold for NPPA then the mimics and the odor soak were releasing.

9.3.2.2 Field Studies for Law Enforcement Working Non-Fentanyl Trained Canines

As previously stated, canines NF17-26 were provided by a government agency and were not previously trained to detect fentanyl. For their trial, three canines were trained to detect reference-grade fentanyl and seven were trained to detect the NPPA COMPS using TADDS. For this study 2 trials took place a pre-trial which occurred before the canines were trained on their assigned odors and a post-trial which occurred after the canines were trained on their assigned odors. Both trials consisted of six, 6-arm scent wheels where TADDS were placed into each canister. Each canine investigated 36 TADDs, five of which contained a target, with the remainder containing blanks and distractors. The results for the pre-trial can be seen in Table 21.

Table 21. Pre-trial data for working canines not previously trained on fentanyl NF17-26.

Canine	Positive Alert Rate	Negative Alert Rate	PPV	NPV
NF17	0%	3%	0%	90%
NF18	20%	0%	3%	94%
NF19	20%	3%	3%	94%
NF20	20%	3%	0%	93%
NF21	20%	0%	4%	93%
NF22	0%	3%	0%	91%
NF23	20%	0%	3%	94%
NF24	20%	3%	4%	93%
NF25	20%	10%	3%	94%
NF26	40%	3%	6%	91%
Average	18%	7%	3%	93%

Based on the data seen in Table 21, canines NF17-26 had an average positive alert rate of 18% to the fentanyl related material and an average 7% negative alert rate. It can also be seen that on the pre-trial the canines had an average 3% PPV and 93% NPV. This mean there was a 3% likelihood that a positive alert was a true positive and a 93% chance that a negative alert was a true negative. Following the pre-trial the canines were trained on their assigned odors. While the canines being trained on fentanyl did not appear to have any issues the canines being trained on the NPPA COMPS appeared to have issues detecting the compound in low amounts and needed to have the initial amount they were trained on increased. This is commonly referred to a high canine threshold or canines needed a larger amount of odor to detect than what the aid is emitting. As training progressed the amount of NPPA given to the canines was lowered to allow their threshold to reach the odor being emitted by the COMPS. Once the odor threshold was sufficiently lowered and all canines were properly trained on their assigned odors a post-trial was completed. The results for this trial can be seen in Table 22.

Table 22.post-trial data for working canines not previously trained on fentanyl NF17-26.

Canine	Positive Alert Rate	Negative Alert Rate	PPV	NPV
NF17	60%	3%	9%	94%
NF18	60%	0%	9%	94%
NF19	60%	3%	9%	94%
NF20	60%	3%	9%	94%
NF21	60%	0%	9%	94%
NF22	60%	3%	10%	93%
NF23	60%	0%	9%	94%
NF24	60%	3%	9%	94%
NF25	60%	10%	10%	93%
NF26*				
Average	60%	3%	9%	94%

*Canine NF26 was disqualified from the post-trail by the trainer due to having an overactive alert.

Based on the results seen in Table 22 canines NF17-25 had had an average positive alert rate of 60% for the overall trial and an average 3% negative alert rate. However, when taking a closer look at the positive alert rate as seen in Table 23 the canines were only detecting the material in which they were trained to as well as the cocaine positive control. It can also be seen that in the post-trial the canines had an average 9% PPV and 94% NPV. This mean there was a 9% likelihood that a positive alert was a true positive and a 94% chance that a negative alert was a true negative. Full ORT data can be seen in Appendix K.

Table 23. A closer look at the positive alert rates for the post-trial data for working canines not previously trained on fentanyl NF17-26.

Canine	Trained Material	Positive Alert rate (mimic)	Positive alert rate (fentanyl)	Positive alert rate (cocaine(
NF17	Mimic	100%	0%	100%
NF18	Mimic	100%	0%	100%
NF19	Fentanyl	0%	100%	100%
NF20	Fentanyl	0%	100%	100%
NF21	Mimic	100%	0%	100%
NF22	Mimic	100%	0%	100%
NF23	Mimic	100%	0%	100%
NF24	Fentanyl	0%	100%	100%
NF25	Mimic	100%	0%	100%
NF26*				

**Canine NF26 was disqualified from the post-trail by the trainer due to having an overactive alert.*

While the response of the canines did generally improve from the pre-trial to the post trail the results did not provide sufficient evidence that NPPA is the odorant for fentanyl. This can be seen by the fact that while a canine can be trained on NPPA it cannot find fentanyl and if it is trained on fentanyl, it cannot find NPPA.

9.3.3 Field Studies for Fentanyl-Trained Canines

Following the field study with non-fentanyl-trained canines, an additional field study was conducted with five canines previously trained on fentanyl citrate. Table 24 represents the results from the canine field study conducted with canines F1-5. Based on the results all five canines had a 6% positive alert rate for the trail since they were only able to locate their positive control and a 0% negative alert rate. Furthermore, they all had 7% PPV and a 93% NPV. This means that there was a 7% likelihood that a positive alert was a true positive and a 93% chance that a negative alert was a true negative. Full ORT data can be seen in Appendix L.

Table 24. Field study results for fentanyl trained canines F1-5.

Canine	Positive Alert Rate	Negative Alert Rate	PPV	NPV
F1	6%	0%	7%	93%
F2	6%	0%	7%	93%
F3	6%	0%	7%	93%
F4	6%	0%	7%	93%
F4	6%	0%	7%	93%
Average	6%	0%	7%	93%

Since these canines were trained on fentanyl citrate and did not give a positive response during their trial, it was critical to investigate the headspace of fentanyl citrate. When the headspace of fentanyl citrate was analyzed, which can be seen in Figure 22, it was concluded that NPPA was not present. The lack of NPPA in the headspace in their training material can account for the lack of positive results during the field trial.

9.4 Conclusion

This task began the verification process of the NPPA COMPS developed in Task 4 as a training aid for the detection of fentanyl. This was done by conducting canine field studies with 26 canines not previously trained for the detection of fentanyl (16 civilian and 10 law enforcement working) and five canines that were previously trained using fentanyl citrate. The ORT for the civilian canines not previously trained showed an inability for these canines to alert to their trained material, possibly due to canines having a high threshold for NPPA. The trial with the operational law enforcement canines showed that while a canine can be trained on NPPA it cannot find fentanyl and if it is trained on fentanyl, it cannot find NPPA. This could be due canines having a high threshold for NPPA or because NPPA is not the compound in the headspace in fentanyl that elicits a response from a canine properly trained on fentanyl. Finally, the canines previously trained on fentanyl (F1-5) did not show positive results likely due to their training material fentanyl citrate instead of fentanyl, as the two were shown to have very different headspace profiles, and no NPPA was detected from the headspace of the fentanyl citrate.

10. CONCLUSIONS

With the growing fentanyl epidemic in the US this high potency puts law enforcement at risk every day. This makes a non-contact detection method vital for the safety of our first responders and other law enforcement. Canines have historically been used to detect contraband such as drugs, explosives, and firearms using olfaction. This accomplished by training canines on the odor of these targets using a training aid. In this case, because fentanyl is so potent, using the target material would be hazardous. Thus, the experiments discussed here outlined the development of a novel training aid mimic for fentanyl to provide a safe way to train canines on fentanyl and, in turn, provide a safe non-contact way for law enforcement to detect fentanyl in field.

The experiments discussed in this study centered around the use of a GC-MS; however, there is minimal peer-reviewed literature discussing the detection of fentanyl using GC-MS. The one method developed to detect fentanyl using GC-MS was written in an article published in 2008 by Manral et al.[89] Since this method was published over 10 years ago it was important to validate its ability to detect fentanyl. Using the same instrumental parameters including column, temperature ramp, injection volume, flow rate and split ratio fentanyl was detected with a retention time of 9.53 minutes and an LOD of 2.8 ppm.

When developing a new training aid mimic, fully understanding the targeted compounds headspace or vapor profile is important. This implies that it is crucial to develop an optimized SPME method to detect the compounds that compose the headspace of the targeted material, which in this study is fentanyl. For this study a CAR/DVB/PDMS

SPME fiber used, and the equilibration time, extraction time, and extraction temperature were optimized. These optimized parameters resulted in a 30-minute equilibration time and a 4-hour extraction time at 35 °C. When using both the optimized extraction method, as well as a GC-MS method provided by NRL, nine compounds were detected and verified in the fentanyl headspace, including heptane, styrene, benzaldehyde, benzyl alcohol, benzeneacetylaldehyde, NPPA, NPP, and 1-phenethyl-4-propinolxypiperidine, with NPPA being the major headspace component. This information about the headspace of fentanyl later played a critical role in developing a non-contact detection method.

As previously stated, when developing a new training aid mimic, it is vital to fully understand the compound's headspace. In Task 2, the components of fentanyl's headspace were detected and verified using 50 ppm analytical standards. It was important to determine if these components resulted from degradation, synthesis, the manufacturing process, or the container. This would help to narrow down which of the identified compounds was more likely to be present in the headspace for a longer amount of time and thus be more available for both instrumental and canine detection. To accomplish this, reference-grade fentanyl samples underwent passive degradation using varying environmental conditions. They included room and heated temperatures, dry and humid atmospheres, and oxygen-rich and nitrogen-rich environments. This experiment showed that NPPA was a product of degradation in all environments, while styrene was a product of thermal degradation. All other headspace compounds were attributed to the synthesis, manufacturing, or packaging of fentanyl. Based on the results seen in this task and task 2, NPPA was chosen as the headspace compound that would be used in the development of a novel training aid canine mimic.

COMPS allow training aids to be field deployable and easy to use. For this experiment, three different preparations were made to compare the abundance of NPPA in the training aid to that in 5 mg of bulk fentanyl. The preparation (Preparation 1) with the peak abundance (A/E) most like 5 mg of bulk fentanyl was then used for further optimization. Since it was found that Preparation 1 had an NPPA content most similar to that of 5 mg of bulk fentanyl a SPME-GC-MS investigation was done to determine what would happen to the headspace over time when the mimic was stored between samplings and when it was not stored between samplings. These results showed that when stored between sessions, there was less than 1% odor depletion from sample Session 0 to sample Session 24 when the mimic was stored in a mylar bag between sample sessions. This means the training aid mimic can be used for a minimum of 6 hours when stored. However, this 6-hour time period was chosen to imitate the approximate amount of time a canine would train in one month and further studies would need to be done to investigate a longer time. When the training aids were not stored, there was greater than 78% odor depletion from week 0 to week 4. This means that the training aid mimic can only be used for one month when it is not stored.

With the training aid mimic COMPS successfully developed, it was important to investigate the validity of the COMPS with odor detection canines. In this study, both canines previously trained on fentanyl and not previously trained on fentanyl were used. The results from the canine trials showed that canines could easily be trained to detect fentanyl. However, canines trained on fentanyl, fentanyl citrate or the fentanyl odor soak could not detect the NPPA COMPS. Furthermore, it was evident that canines have difficulty being trained on NPPA possibly due to a high odor threshold. However, even when that threshold is manipulated to train canines on NPPA they could not detect fentanyl. Based on these results NPPA is not the odorant that would elicit a response from fentanyl trained canines.

Some future directions this study can take include using headspace compound mixtures to make a more robust mimic. While NPPA was identified as the major headspace compound, combining some of the lesser abundant compounds with NPPA may increase the chance of a canine alert as well as help with generalization. Based on previous data it is assumed that degradation products would best mimic the odor of fentanyl. For this reason, mixtures of known and confirmed degradation products will be investigated with canine previously trained on fentanyl. Once it is determined which determined which mixture or single compound produces an alert that mixture or compound will be analytically investigated using SPME-CG-MS to ensure the mimic is emitting a similar vapor content for those compounds as 5 mg of bulk fentanyl. Once the correct ratio of compounds is discovered based on the analytical data canine trials will be conducted to verify the new mimic. Furthermore, a more in-depth investigation needs to be done on the fentanyl analogs with 27 analogs scheduled by the DEA, it is vital to understand the headspace profile of each analog and look for compounds in common.

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APPENDICIES

Appendix A: Raw data for all eight environments in the study of passive degradation on reference grade fentanyl

Table 25.Average (A/E) and RSD values for Condition A.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.54503	14.7634	1.55413	19.6084	1.81023	23.2285	1.43208	14.9776	1.29550	16.5567
Benzaldehyde	9.02605	38.3698	1.06435	11.1095	0.76364	8.61886	0.86845	33.5649	0.91638	17.4948
Aniline	2.19070	70.3080	0.35194	17.8843	0.23307	26.9357	0.18332	44.3042	0.30462	24.6682
NPPA	11.2391	119.211	12.2752	150.077	10.3141	148.144	7.76069	134.932	12.7765	59.8546
NPP	0.06366	54.3471	0.06175	91.9977	0.02864	103.583	0.03228	140.430	0.03565	140.791
Heptane	6.84147	46.6450	0.97571	18.3550	0.88851	19.3690	0.72550	19.4477	0.71658	19.6419
Benzyl Alcohol	1.24040	39.1845	0.12129	38.1141	0.09646	24.2852	0.13629	11.5157	0.16325	26.5514
Benzeneacetylaldehyde	3.21135	51.2339	0.85717	37.4172	0.71589	21.2177	0.99623	6.37915	1.54000	24.7553
1-Phenethyl-4-propionyloxypiperidine	0.07945	128.877	0.13176	110.643	0.08116	112.003	0.08459	150.964	0.06618	153.508

Table 26.Average (A/E) and RSD values for Condition B.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	2.22845	17.4431	1.38149	18.2624	1.331263	52.0832	1.32693	12.0530	0.78903	20.2698
Benzaldehyde	8.38908	30.7224	1.22769	17.1006	0.723168	49.3497	0.79061	3.91719	0.38445	8.05555
Aniline	1.27266	22.4550	0.55556	5.66237	0.360824	53.9757	0.25687	26.2051	0.43650	15.4210
NPPA	7.25454	61.9121	18.9384	52.5213	8.696419	46.8857	13.0191	35.3106	35.2043	13.0584
NPP	0.02268	95.6469	0.02913	73.9595	0.008981	20.5265	0.01707	710.692	0.11472	105.771
Heptane	3.93651	80.9899	0.98681	2.08286	0.626488	54.4046	0.63890	16.3224	0.75542	13.8047
Benzyl Alcohol	1.02318	32.9345	0.21598	4.19527	0.099279	51.6412	0.10491	15.0935	0.08349	18.9660
Benzeneacetylaldehyde	1.56272	85.5440	1.13630	4.75501	0.450747	59.0098	0.61074	31.3327	1.57627	12.1402
1-Phenethyl-4-propionyloxypiperidine	0.07402	23.0780	0.08235	38.6152	0.033440	23.32672	0.07430	129.116	0.14979	64.0453

Table 27.Average (A/E) and RSD values for Condition C.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.29066	32.3086	5.05855	92.0134	2.508652	52.6238	2.35901	9.38577	0.48299	45.8416
Benzaldehyde	6.54473	11.6037	2.11153	33.1344	0.575409	15.0166	1.94777	5.56704	0.24704	43.8921
Aniline	1.51492	34.8549	0.63761	28.2398	0.525867	22.1449	0.29224	187.667	0.46852	117.058
NPPA	6.42336	3.89826	14.5292	25.4030	4.138416	26.6860	4.78197	58.8698	6.95425	40.4808
NPP	0.08015	49.4422	0.49458	43.8402	0.064673	29.3582	0.07787	72.4097	0.15668	35.9888
Heptane	6.97915	37.1453	2.09920	37.7148	1.056835	71.8706	1.21120	16.7982	0.26258	77.4828
Benzyl Alcohol	1.02771	7.59329	0.18257	35.6206	0.057835	14.3876	0.13075	7.22327	0.03887	24.2963
Benzeneacetylaldehyde	1.77827	22.9790	1.99907	17.5505	0.625292	25.7734	1.45609	25.6535	0.55604	67.1780
1-Phenethyl-4-propionyloxypiperidine	0.05741	38.6953	0.95892	44.6669	0.250566	35.2019	0.12927	42.6036	0.11917	46.2137

Table 28.Average (A/E) and RSD values for Condition D.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.4975	53.3639	7.6848	116.710	2.77226	87.3132	6.9866	34.8326	3.7418	65.038
Benzaldehyde	6.8557	40.2968	4.3829	70.5748	0.86303	65.9736	2.5660	8.37494	0.6495	33.085
Aniline	1.8823	45.4487	1.4664	73.5853	0.83618	68.6411	1.0821	17.7340	0.5573	34.428
NPPA	5.9988	89.1929	12.196	79.5956	4.57715	70.5144	15.179	40.8617	8.1035	76.540
NPP	0.0258	81.9558	0.3727	81.8257	0.04346	46.9451	0.1355	18.2176	0.1048	23.555
Heptane	4.3287	53.5882	4.2643	72.1900	1.38644	95.3284	3.7563	78.7391	2.3907	123.71
Benzyl Alcohol	0.9793	54.4015	0.4011	78.3563	0.08896	64.2774	0.2767	5.36292	0.0723	20.504
Benzeneacetylaldehyde	2.3563	71.3071	4.7225	56.5343	1.57212	80.9281	6.2535	19.4190	2.5901	46.883
1-Phenethyl-4-propionyloxypiperidine	0.0258	58.7248	0.2021	82.0130	0.05348	44.4565	0.0799	33.0129	0.05982	44.130

Table 29.Average (A/E) and RSD values for Condition E.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.38645	37.8231	0.59506	27.6615	0.50454	33.4321	0.39046	24.7106	0.5419	36.2711
Benzaldehyde	4.92393	4.42217	0.33000	22.6757	0.18170	19.3101	0.12929	25.7756	0.1015	26.4366
Aniline	0.94530	61.2473	0.42838	5.12115	0.27575	19.8371	0.19485	16.2164	0.1712	27.4360
NPPA	2.42577	53.9897	25.1100	39.4394	17.5225	61.0054	12.2390	60.6165	11.348	72.2927
NPP	0.03088	57.9625	0.36519	82.1425	0.27726	27.0287	0.29525	23.5042	0.1404	24.3960
Heptane	4.77311	32.7720	1.64025	41.6778	0.84301	53.5333	0.53457	40.0262	0.5405	47.3076
Benzyl Alcohol	0.30395	48.6442	0.03512	6.88404	0.02004	18.6479	0.01888	24.7178	0.0180	40.3023
Benzeneacetylaldehyde	0.80831	35.7458	1.59791	17.0824	0.77073	29.2794	0.56790	27.8854	0.4642	35.2732
1-Phenethyl-4-propionyloxypiperidine	0.04702	19.5335	0.10506	86.0636	0.08770	117.196	0.13231	127.522	0.0912	121.029

Table 30.Average (A/E) and RSD values for Condition F.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.2509	7.52632	0.6172	15.4068	0.5626	1.8032	0.4897	6.3145	0.5521	13.854
Benzaldehyde	4.6640	24.3801	0.5254	37.0454	0.2302	4.1864	0.1574	15.712	0.1032	16.005
Aniline	1.3454	46.8991	0.6162	12.4509	0.3812	20.659	0.2796	20.824	0.2511	37.109
NPPA	16.211	148.311	33.108	37.2089	26.834	59.393	19.171	63.051	21.906	80.791
NPP	0.1230	141.252	0.8755	94.2003	0.6401	92.548	0.3954	58.283	0.2224	63.989
Heptane	5.6257	35.6061	2.1636	55.3314	1.0677	20.488	0.8310	37.007	0.7129	29.088
Benzyl Alcohol	0.5023	73.9298	0.0432	25.5342	0.0251	8.2349	0.0202	8.7601	0.0141	19.722
Benzeneacetylaldehyde	1.0865	46.4147	1.9088	38.4797	0.9532	3.9201	0.6732	10.333	0.4737	8.1963
1-Phenethyl-4-propionyloxypiperidine	0.0693	67.7279	0.5025	123.419	0.7068	138.25	0.6874	129.26	0.4979	130.27

Table 31.Average (A/E) and RSD values for Condition G.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.0245	7.5384	4.3177	54.532	1.3182	119.050	0.7541	50.199	2.8546	111.90
Benzaldehyde	3.9256	86.122	0.5004	60.411	0.4372	36.1421	0.4329	59.863	1.4999	78.147
Aniline	0.6199	66.954	0.3781	31.508	0.2539	22.2929	0.2289	38.890	0.4977	45.487
NPPA	1.1969	94.547	7.2783	34.614	2.5592	22.6087	2.4553	38.570	4.8812	60.234
NPP	0.0210	122.71	0.8816	55.528	0.3542	97.2538	0.1187	52.300	0.0905	45.024
Heptane	14.056	65.703	2.8499	57.548	0.9283	49.9035	0.7742	73.166	2.4715	98.007
Benzyl Alcohol	0.1645	58.993	0.0412	48.972	0.0404	39.1420	0.0510	62.666	0.3473	136.78
Benzeneacetylaldehyde	0.3894	23.809	1.1926	58.595	1.1087	30.1265	0.9990	63.519	4.4328	101.11
1-Phenethyl-4-propionyloxypiperidine	0.0292	139.45	0.2947	63.776	0.1854	105.877	0.0539	48.696	0.0329	55.701

Table 32.Average (A/E) and RSD values for Condition H.

Compound	Fresh	RSD Fresh	7 days	RSD 7 days	14 days	RSD 14 days	21 days	RSD 21 days	28 days	RSD 28 days
IS (Acetanilide)	1	0	1	0	1	0	1	0	1	0
Styrene	1.5354	30.283	15.156	88.885	1.7852	91.584	3.5441	77.618	1.0792	70.488
Benzaldehyde	4.1688	6.8862	2.8783	120.52	0.8781	21.339	2.9245	72.102	2.4817	14.480
Aniline	1.1518	45.510	1.3906	45.795	0.6873	45.127	0.8111	52.477	0.9252	33.344
NPPA	5.0527	103.04	10.537	35.948	2.5820	13.634	4.6662	66.943	1.4550	23.337
NPP	0.0285	32.092	0.6248	34.539	0.0871	34.042	0.0769	32.714	0.0563	60.823
Heptane	4.2451	55.854	5.3619	60.543	0.8881	39.603	1.0278	106.28	0.2414	43.648
Benzyl Alcohol	0.6031	72.747	0.1593	101.06	0.0865	29.351	0.1341	39.868	0.2869	26.812
Benzeneacetylaldehyde	1.2706	71.623	6.3348	91.491	3.2832	51.276	6.3622	62.074	4.9980	24.473
1-Phenethyl-4-propionyloxypiperidine	0.02827	22.922	0.3050	20.056	0.0381	24.352	0.0324	52.721	0.0196	97.214

Appendix B: Two tailed T-Tests for the comparison between the environmental conditions used in the passive degradation of reference grade fentanyl.

Table 33.Statistical analysis comparing Condition A to Condition E.

Days	Compound	Condition A			Condition E			P Value
Fresh	Styrene	1.282778	1.655055	1.697274	0.789161	1.598943	1.771275	0.656105
	Benzaldehyde	11.88527	5.17509	10.01781	5.032943	4.673211	5.065641	0.110001
	Aniline	3.88654	0.878607	1.806975	1.606406	0.700915	0.528598	0.260075
	NPPA	5.361372	26.5718	1.784298	2.362379	3.765991	1.148955	0.320174
	NPP	0.062639	0.098758	0.029585	0.023637	0.051276	0.017745	0.218782
	Heptane	8.487651	3.163322	8.873449	5.69225	2.966974	5.660129	0.370468
	Benzyl Alcohol	1.618074	0.692035	1.411109	0.449828	0.307861	0.154188	0.033133
	Benzeneacetylaldehyde	5.078586	2.581204	1.974264	0.873835	1.058863	0.492239	0.067368
	1-Phenethyl-4-propionyloxypiperidine	0.025551	0.197546	0.015267	0.04811	0.055621	0.037346	0.613882
7	Styrene	1.680942	1.206462	1.77499	0.40741	0.662722	0.715051	0.008673
	Benzaldehyde	1.170228	0.936753	1.086082	0.255656	0.405307	0.329041	0.000812
	Aniline	0.283953	0.408183	0.363684	0.424387	0.452048	0.408721	0.11793
	NPPA	1.213497	33.54179	2.070461	19.42401	36.54532	19.36086	0.347748
	NPP	0.017863	0.125924	0.041477	0.161552	0.709673	0.224444	0.160226
	Heptane	0.768926	1.080981	1.077234	0.863263	1.908099	2.14939	0.178701
	Benzyl Alcohol	0.127334	0.164214	0.072344	0.037414	0.035352	0.032595	0.032152
	Benzeneacetylaldehyde	1.001083	1.08075	0.489694	1.282361	1.778928	1.730156	0.038413
	1-Phenethyl-4-propionyloxypiperidine	0.03611	0.299569	0.059629	0.036214	0.207477	0.071517	0.800837
14	Styrene	1.68337	1.467779	2.279549	0.327462	0.522837	0.663329	0.007535
	Benzaldehyde	0.76842	0.695563	0.826937	0.141932	0.194914	0.208275	0.000174
	Aniline	0.178829	0.301841	0.218544	0.212599	0.306337	0.308323	0.424798
	NPPA	1.688705	27.95622	1.297374	7.680202	28.89473	15.9926	0.539819
	NPP	0.011468	0.062914	0.011563	0.210858	0.358515	0.262413	0.005916
	Heptane	0.738752	0.850287	1.076517	0.373199	0.869833	1.273173	0.866859
	Benzyl Alcohol	0.115327	0.103832	0.070242	0.015845	0.021289	0.023006	0.005059
	Benzeneacetylaldehyde	0.861419	0.727918	0.558341	0.515173	0.854358	0.941694	0.745738
	1-Phenethyl-4-propionyloxypiperidine	0.040529	0.185298	0.017662	0.013151	0.204963	0.045005	0.938144
21	Styrene	1.17586	1.291876	1.82853	0.280377	0.430669	0.460348	0.007526
	Benzaldehyde	0.899418	1.04277	1.460481	0.091029	0.144916	0.151946	0.004047
	Aniline	0.21307	0.245473	0.091423	0.16052	0.222616	0.201475	0.829707
	NPPA	2.712944	19.80018	0.768972	6.132634	20.49545	10.08897	0.578177
	NPP	0.005787	0.084636	0.006433	0.271145	0.370483	0.237984	0.005411
	Heptane	0.563427	0.792187	0.820894	0.303403	0.556366	0.736811	0.266395
	Benzyl Alcohol	0.153976	0.124002	0.130912	0.015581	0.016856	0.02423	0.000242
	Benzeneacetylaldehyde	0.997085	0.932259	1.059353	0.387192	0.683934	0.634039	0.012346
	1-Phenethyl-4-propionyloxypiperidine	0.01083	0.23206	0.010894	0.010829	0.324972	0.061106	0.716091
28	Styrene	1.278748	1.08988	1.517883	0.320235	0.610785	0.69489	0.01094
	Benzaldehyde	1.037961	0.734696	0.976501	0.070535	0.116833	0.117213	0.000968
	Aniline	0.390872	0.253273	0.269725	0.117138	0.194804	0.20177	0.059614
	NPPA	9.173027	21.56	7.596731	4.323723	20.36439	9.355912	0.836189
	NPP	0.003575	0.093502	0.009884	0.125716	0.179624	0.116025	0.040472
	Heptane	0.569743	0.850331	0.729692	0.264158	0.588788	0.768749	0.355236
	Benzyl Alcohol	0.170109	0.116888	0.202764	0.009761	0.023343	0.021108	0.004619
	Benzeneacetylaldehyde	1.596449	1.133699	1.889874	0.275346	0.551658	0.565792	0.010901
	1-Phenethyl-4-propionyloxypiperidine	0.004922	0.183467	0.01017	0.009439	0.216879	0.04744	0.786723

P value < 0.05 values are statically different
No Statistical Difference

Table 34.Statistical analysis comparing Condition A to Condition F.

Days	Compound	Condition A			Condition F			P Value
Fresh	Styrene	1.282778	1.655055	1.697274	1.232149	1.35304	1.167572	0.107914
	Benzaldehyde	11.88527	5.17509	10.01781	5.833897	3.56277	4.5956	0.10691
	Aniline	3.88654	0.878607	1.806975	0.839394	1.144471	2.052437	0.428754
	NPPA	5.361372	26.5718	1.784298	0.938597	3.772591	43.92689	0.769938
	NPP	0.062639	0.098758	0.029585	0.024258	0.021133	0.320402	0.595702
	Heptane	8.487651	3.163322	8.873449	7.750786	3.772274	5.354182	0.606059
	Benzyl Alcohol	1.618074	0.692035	1.411109	0.139335	0.470693	0.880759	0.103052
	Benzeneacetylaldehyde	5.078586	2.581204	1.974264	0.492296	1.364999	1.390186	0.099071
	1-Phenethyl-4-propionyloxypiperidine	0.025551	0.197546	0.015267	0.046065	0.038541	0.123699	0.885105
7	Styrene	1.680942	1.206462	1.77499	0.713844	0.618016	0.521784	0.007104
	Benzaldehyde	1.170228	0.936753	1.086082	0.726789	0.335936	0.515604	0.01512
	Aniline	0.283953	0.408183	0.363684	0.638532	0.53132	0.680566	0.009923
	NPPA	1.213497	33.54179	2.070461	20.9272	32.92703	45.51739	0.17836
	NPP	0.017863	0.125924	0.041477	0.1821	0.657231	1.787613	0.163351
	Heptane	0.768926	1.080981	1.077234	3.549617	1.367343	1.58315	0.164978
	Benzyl Alcohol	0.127334	0.164214	0.072344	0.053796	0.031666	0.044388	0.046777
	Benzeneacetylaldehyde	1.001083	1.08075	0.489694	2.754997	1.381322	1.597498	0.085988
	1-Phenethyl-4-propionyloxypiperidine	0.03611	0.299569	0.059629	0.087891	0.204402	1.21549	0.370376
14	Styrene	1.68337	1.467779	2.279549	0.551855	0.571968	0.564249	0.006804
	Benzaldehyde	0.76842	0.695563	0.826937	0.219271	0.237208	0.234364	0.000156
	Aniline	0.178829	0.301841	0.218544	0.307442	0.372136	0.464175	0.06343
	NPPA	1.688705	27.95622	1.297374	10.3094	28.08239	42.11226	0.264701
	NPP	0.011468	0.062914	0.011563	0.213736	0.39028	1.316687	0.148677
	Heptane	0.738752	0.850287	1.076517	1.147386	0.820294	1.235487	0.327264
	Benzyl Alcohol	0.115327	0.103832	0.070242	0.027493	0.024523	0.023503	0.006295
	Benzeneacetylaldehyde	0.861419	0.727918	0.558341	0.936912	0.995987	0.926803	0.058308
	1-Phenethyl-4-propionyloxypiperidine	0.040529	0.185298	0.017662	0.036531	0.255862	1.828218	0.331469
21	Styrene	1.17586	1.291876	1.82853	0.506514	0.454042	0.50863	0.009523
	Benzaldehyde	0.899418	1.04277	1.460481	0.170012	0.128983	0.173465	0.004442
	Aniline	0.21307	0.245473	0.091423	0.272975	0.225095	0.340999	0.170227
	NPPA	2.712944	19.80018	0.768972	6.614811	20.17182	30.72917	0.284138
	NPP	0.005787	0.084636	0.006433	0.226915	0.006776	0.658102	0.241843
	Heptane	0.563427	0.792187	0.820894	1.147386	0.53309	0.812747	0.617611
	Benzyl Alcohol	0.153976	0.124002	0.130912	0.022085	0.020053	0.018553	0.00022
	Benzeneacetylaldehyde	0.997085	0.932259	1.059353	0.711952	0.592904	0.714789	0.004034
	1-Phenethyl-4-propionyloxypiperidine	0.01083	0.23206	0.010894	0.028902	0.335226	1.698202	0.309459
28	Styrene	1.278748	1.08988	1.517883	0.547509	0.524642	0.674367	0.005724
	Benzaldehyde	1.037961	0.734696	0.976501	0.094067	0.093291	0.122285	0.000945
	Aniline	0.390872	0.253273	0.269725	0.19355	0.201227	0.358683	0.482366
	NPPA	9.173027	21.56	7.596731	5.37899	19.75957	40.57985	0.458178
	NPP	0.003575	0.093502	0.009884	0.110345	0.174394	0.382586	0.098694
	Heptane	0.569743	0.850331	0.729692	0.908527	0.49549	0.734873	0.981214
	Benzyl Alcohol	0.170109	0.116888	0.202764	0.012219	0.012937	0.017382	0.004017
	Benzeneacetylaldehyde	1.596449	1.133699	1.889874	0.444035	0.459535	0.517688	0.008527
	1-Phenethyl-4-propionyloxypiperidine	0.004922	0.183467	0.01017	0.020863	0.236387	1.236479	0.318306

P value < 0.05 values are statically different
No Statistical Difference

Table 35.Statistical analysis comparing Condition A to Condition B.

Days	Compound	Condition A			Condition B			P Value
Fresh	Styrene	1.282778	1.655055	1.697274	1.834861	2.612098	2.238417	0.058405
	Benzaldehyde	11.88527	5.17509	10.01781	5.652295	8.745012	10.76996	0.810894
	Aniline	3.88654	0.878607	1.806975	0.948047	1.486317	1.383632	0.367492
	NPPA	5.361372	26.5718	1.784298	2.185552	10.73881	8.839279	0.650837
	NPP	0.062639	0.098758	0.029585	0.006141	0.047247	0.01466	0.157202
	Heptane	8.487651	3.163322	8.873449	0.255254	5.804497	5.749785	0.32716
	Benzyl Alcohol	1.618074	0.692035	1.411109	0.637501	1.260659	1.171399	0.559265
	Benzeneacetylaldehyde	5.078586	2.581204	1.974264	0.092299	2.704712	1.891159	0.249238
	1-Phenethyl-4-propionyloxypiperidine	0.025551	0.197546	0.015267	0.055284	0.088729	0.078055	0.932149
7	Styrene	1.680942	1.206462	1.77499	1.219613	1.672191	1.252673	0.491829
	Benzaldehyde	1.170228	0.936753	1.086082	1.337158	0.985642	1.360288	0.305466
	Aniline	0.283953	0.408183	0.363684	0.524324	0.555125	0.587236	0.007426
	NPPA	1.213497	33.54179	2.070461	7.729463	22.37337	26.71237	0.610809
	NPP	0.017863	0.125924	0.041477	0.005489	0.04766	0.034246	0.405044
	Heptane	0.768926	1.080981	1.077234	0.964295	1.004568	0.991569	0.920223
	Benzyl Alcohol	0.127334	0.164214	0.072344	0.208936	0.226203	0.212803	0.025327
	Benzeneacetylaldehyde	1.001083	1.08075	0.489694	1.113313	1.198035	1.09758	0.211354
	1-Phenethyl-4-propionyloxypiperidine	0.03611	0.299569	0.059629	0.045692	0.102503	0.098857	0.59694
14	Styrene	1.68337	1.467779	2.279549	1.38869	1.994129	0.610971	0.364117
	Benzaldehyde	0.76842	0.695563	0.826937	0.861857	0.989888	0.317761	0.856244
	Aniline	0.178829	0.301841	0.218544	0.470882	0.475637	0.135954	0.340375
	NPPA	1.688705	27.95622	1.297374	4.925505	13.02323	8.140526	0.867982
	NPP	0.011468	0.062914	0.011563	0.009786	0.010286	0.006872	0.315803
	Heptane	0.738752	0.850287	1.076517	0.667939	0.944706	0.26682	0.300326
	Benzyl Alcohol	0.115327	0.103832	0.070242	0.124989	0.132607	0.040242	0.935292
	Benzeneacetylaldehyde	0.861419	0.727918	0.558341	0.533951	0.665186	0.153107	0.208167
	1-Phenethyl-4-propionyloxypiperidine	0.040529	0.185298	0.017662	0.042151	0.031068	0.027101	0.416199
21	Styrene	1.17586	1.291876	1.82853	1.108606	1.775734	1.096463	0.744684
	Benzaldehyde	0.899418	1.04277	1.460481	1.455898	1.131966	0.626852	0.841738
	Aniline	0.21307	0.245473	0.091423	0.269933	0.289108	0.211585	0.232839
	NPPA	2.712944	19.80018	0.768972	9.079377	13.93937	16.0387	0.456619
	NPP	0.005787	0.084636	0.006433	0.007746	0.017414	0.026061	0.599405
	Heptane	0.563427	0.792187	0.820894	0.643308	0.723353	0.550042	0.416338
	Benzyl Alcohol	0.153976	0.124002	0.130912	0.128925	0.120262	0.065561	0.223557
	Benzeneacetylaldehyde	0.997085	0.932259	1.059353	0.691692	0.736638	0.403905	0.025156
	1-Phenethyl-4-propionyloxypiperidine	0.01083	0.23206	0.010894	0.033629	0.054751	0.134533	0.90371
28	Styrene	1.278748	1.08988	1.517883	0.690152	0.973557	0.703401	0.03054
	Benzaldehyde	1.037961	0.734696	0.976501	0.416887	0.35519	0.38129	0.004857
	Aniline	0.390872	0.253273	0.269725	0.359029	0.480611	0.46989	0.086271
	NPPA	9.173027	21.56	7.596731	30.36752	35.72881	39.51685	0.012123
	NPP	0.003575	0.093502	0.009884	0.004005	0.095713	0.244445	0.355874
	Heptane	0.569743	0.850331	0.729692	0.640795	0.844685	0.78078	0.720554
	Benzyl Alcohol	0.170109	0.116888	0.202764	0.069314	0.100582	0.080586	0.040196
	Benzeneacetylaldehyde	1.596449	1.133699	1.889874	1.582315	1.764543	1.38196	0.890056
	1-Phenethyl-4-propionyloxypiperidine	0.004922	0.183467	0.01017	0.069582	0.123738	0.256075	0.358575

P value < 0.05 values are statically different
No Statistical Difference

Table 36.Statistical analysis comparing Condition A to Condition C.

Days	Compound	Condition A			Condition C			P Value
Fresh	Styrene	1.282778	1.655055	1.697274	0.785526	1.345208	2.361892	0.925914
	Benzaldehyde	11.88527	5.17509	10.01781	5.889243	4.706239	9.971852	0.443951
	Aniline	3.88654	0.878607	1.806975	2.529269	0.912351	2.205375	0.776885
	NPPA	5.361372	26.5718	1.784298	5.28054	1.043705	11.67217	0.563407
	NPP	0.062639	0.098758	0.029585	0.008167	0.019997	0.04925	0.181182
	Heptane	8.487651	3.163322	8.873449	2.063958	6.699761	4.222643	0.331875
	Benzyl Alcohol	1.618074	0.692035	1.411109	1.122713	0.389519	1.425669	0.5646
	Benzeneacetylaldehyde	5.078586	2.581204	1.974264	3.952726	0.603217	2.513116	0.563051
	1-Phenethyl-4-propionyloxypiperidine	0.025551	0.197546	0.015267	0.014108	0.020483	0.043026	0.420659
7	Styrene	1.680942	1.206462	1.77499	17.8902	4.109435	1.054935	0.302232
	Benzaldehyde	1.170228	0.936753	1.086082	4.665219	1.158257	7.325479	0.136895
	Aniline	0.283953	0.408183	0.363684	2.59054	0.438829	1.36999	0.148665
	NPPA	1.213497	33.54179	2.070461	23.36391	7.459722	5.766514	0.995101
	NPP	0.017863	0.125924	0.041477	0.721527	0.155709	0.241097	0.15753
	Heptane	0.768926	1.080981	1.077234	7.580138	3.715795	1.497047	0.138439
	Benzyl Alcohol	0.127334	0.164214	0.072344	0.361635	0.108453	0.733376	0.201784
	Benzeneacetylaldehyde	1.001083	1.08075	0.489694	6.607677	1.667437	5.892664	0.067504
	1-Phenethyl-4-propionyloxypiperidine	0.03611	0.299569	0.059629	0.393507	0.11052	0.102388	0.610283
14	Styrene	1.68337	1.467779	2.279549	1.729129	1.048171	5.539487	0.534833
	Benzaldehyde	0.76842	0.695563	0.826937	0.871933	0.289263	1.427911	0.778864
	Aniline	0.178829	0.301841	0.218544	1.271975	0.185851	1.05074	0.144678
	NPPA	1.688705	27.95622	1.297374	3.458846	2.057485	8.215134	0.559183
	NPP	0.011468	0.062914	0.011563	0.048193	0.021116	0.061102	0.515329
	Heptane	0.738752	0.850287	1.076517	0.363296	0.91735	2.878676	0.55285
	Benzyl Alcohol	0.115327	0.103832	0.070242	0.105034	0.025463	0.136387	0.84365
	Benzeneacetylaldehyde	0.861419	0.727918	0.558341	1.243278	0.496536	2.976548	0.311508
	1-Phenethyl-4-propionyloxypiperidine	0.040529	0.185298	0.017662	0.077665	0.030132	0.052658	0.636779
21	Styrene	1.17586	1.291876	1.82853	3.619987	7.358441	9.981556	0.040275
	Benzaldehyde	0.899418	1.04277	1.460481	2.647221	3.68267	4.011264	0.006483
	Aniline	0.21307	0.245473	0.091423	0.720835	1.039598	1.485933	0.016633
	NPPA	2.712944	19.80018	0.768972	7.293922	18.68555	19.55794	0.362392
	NPP	0.005787	0.084636	0.006433	0.084478	0.149142	0.173037	0.050079
	Heptane	0.563427	0.792187	0.820894	0.726208	4.766599	5.776393	0.121317
	Benzyl Alcohol	0.153976	0.124002	0.130912	0.253276	0.287343	0.289653	0.000696
	Benzeneacetylaldehyde	0.997085	0.932259	1.059353	4.189904	7.088715	7.481965	0.007173
	1-Phenethyl-4-propionyloxypiperidine	0.01083	0.23206	0.010894	0.03698	0.09122	0.111727	0.955058
28	Styrene	1.278748	1.08988	1.517883	1.141098	5.964016	4.120395	0.157876
	Benzaldehyde	1.037961	0.734696	0.976501	0.402112	0.78959	0.75693	0.159833
	Aniline	0.390872	0.253273	0.269725	0.34044	0.62683	0.704919	0.100844
	NPPA	9.173027	21.56	7.596731	3.153224	15.06103	6.096333	0.457241
	NPP	0.003575	0.093502	0.009884	0.080704	0.130057	0.103751	0.098866
	Heptane	0.569743	0.850331	0.729692	0.136699	5.739896	1.295701	0.382888
	Benzyl Alcohol	0.170109	0.116888	0.202764	0.055896	0.076577	0.084679	0.026409
	Benzeneacetylaldehyde	1.596449	1.133699	1.889874	1.23907	3.590648	2.940881	0.226181
	1-Phenethyl-4-propionyloxypiperidine	0.004922	0.183467	0.01017	0.03944	0.089652	0.050392	0.921496

P value < 0.05 values are statically different
No Statistical Difference

Table 37.Statistical analysis comparing Condition C to Condition G.

Days	Compound	Condition C			Condition G			P Value
Fresh	Styrene	0.785526	1.345208	2.361892	1.111757	0.964661	1.005591	0.367702
	Benzaldehyde	5.889243	4.706239	9.971852	7.82748	2.084508	1.880343	0.310086
	Aniline	2.529269	0.912351	2.205375	1.092526	0.452345	0.316724	0.083014
	NPPA	5.28054	1.043705	11.67217	2.469998	0.815808	0.304138	0.202935
	NPP	0.008167	0.019997	0.04925	0.050467	0.010486	0.002188	0.81712
	Heptane	2.063958	6.699761	4.222643	21.88293	3.869626	16.54456	0.150717
	Benzyl Alcohol	1.122713	0.389519	1.425669	1.948351	0.242669	0.056342	0.750627
	Benzeneacetylaldehyde	3.952726	0.603217	2.513116	0.43308	0.452251	0.28527	0.11301
	1-Phenethyl-4-propionyloxypiperidine	0.014108	0.020483	0.043026	0.076188	0.008361	0.003152	0.899967
7	Styrene	17.8902	4.109435	1.054935	1.671446	5.100604	6.181056	0.563522
	Benzaldehyde	4.665219	1.158257	7.325479	0.848565	0.303817	0.348953	0.096491
	Aniline	2.59054	0.438829	1.36999	0.515608	0.304921	0.313865	0.157516
	NPPA	23.36391	7.459722	5.766514	5.45378	10.15287	6.228349	0.443521
	NPP	0.721527	0.155709	0.241097	0.977694	1.316074	0.351177	0.201227
	Heptane	7.580138	3.715795	1.497047	2.179808	1.656596	4.720127	0.521739
	Benzyl Alcohol	0.361635	0.108453	0.733376	0.064492	0.148115	0.143863	0.198742
	Benzeneacetylaldehyde	6.607677	1.667437	5.892664	1.997684	0.741651	0.838664	0.091082
	1-Phenethyl-4-propionyloxypiperidine	0.393507	0.11052	0.102388	0.463902	0.328084	0.092353	0.556895
14	Styrene	1.729129	1.048171	5.539487	0.283675	3.124136	0.547077	0.431956
	Benzaldehyde	0.871933	0.289263	1.427911	0.573264	0.263891	0.474452	0.280027
	Aniline	1.271975	0.185851	1.05074	0.318885	0.214763	0.228277	0.155298
	NPPA	3.458846	2.057485	8.215134	1.95343	3.106153	2.618268	0.346537
	NPP	0.048193	0.021116	0.061102	0.74888	0.200957	0.113163	0.193724
	Heptane	0.363296	0.91735	2.878676	0.399056	1.262757	1.126258	0.602164
	Benzyl Alcohol	0.105034	0.025463	0.136387	0.052644	0.022548	0.046039	0.229338
	Benzeneacetylaldehyde	1.243278	0.496536	2.976548	1.368067	0.731832	1.227008	0.574913
	1-Phenethyl-4-propionyloxypiperidine	0.077665	0.030132	0.052658	0.410869	0.093062	0.052309	0.312186
21	Styrene	3.619987	7.358441	9.981556	0.333514	0.861294	1.067523	0.028485
	Benzaldehyde	2.647221	3.68267	4.011264	0.719882	0.215869	0.363053	0.002325
	Aniline	0.720835	1.039598	1.485933	0.321184	0.143532	0.222032	0.020015
	NPPA	7.293922	18.68555	19.55794	2.098187	1.739848	3.520126	0.033176
	NPP	0.084478	0.149142	0.173037	0.143423	0.164826	0.048124	0.725958
	Heptane	0.726208	4.766599	5.776393	0.493909	0.402588	1.426234	0.131624
	Benzyl Alcohol	0.253276	0.287343	0.289653	0.085333	0.022086	0.045615	0.000498
	Benzeneacetylaldehyde	4.189904	7.088715	7.481965	1.670963	0.409818	0.916482	0.008819
	1-Phenethyl-4-propionyloxypiperidine	0.03698	0.09122	0.111727	0.0596	0.077338	0.025354	0.392392
28	Styrene	1.141098	5.964016	4.120395	6.517693	1.39793	0.648301	0.721439
	Benzaldehyde	0.402112	0.78959	0.75693	2.84548	0.700363	0.953942	0.284088
	Aniline	0.34044	0.62683	0.704919	0.751147	0.426708	0.315364	0.74528
	NPPA	3.153224	15.06103	6.096333	8.246284	2.809191	3.58813	0.46178
	NPP	0.080704	0.130057	0.103751	0.137619	0.065343	0.068754	0.631606
	Heptane	0.136699	5.739896	1.295701	5.229618	1.495059	0.689864	0.97257
	Benzyl Alcohol	0.055896	0.076577	0.084679	0.895844	0.065102	0.081034	0.373101
	Benzeneacetylaldehyde	1.23907	3.590648	2.940881	9.608739	1.855262	1.834505	0.52971
	1-Phenethyl-4-propionyloxypiperidine	0.03944	0.089652	0.050392	0.054132	0.022223	0.022475	0.221116

P value < 0.05 values are statically different
No Statistical Difference

Table 38.Statistical analysis comparing Condition C to Condition F.

Days	Compound	Condition C			Condition F			P Value
Fresh	Styrene	0.785526	1.345208	2.361892	1.069528	1.999503	1.537289	0.94681
	Benzaldehyde	5.889243	4.706239	9.971852	3.838342	4.356487	4.311604	0.169132
	Aniline	2.529269	0.912351	2.205375	0.604243	1.649013	1.202217	0.275818
	NPPA	5.28054	1.043705	11.67217	0.779685	3.527044	10.85161	0.837019
	NPP	0.008167	0.019997	0.04925	0.018013	0.033356	0.034403	0.844419
	Heptane	2.063958	6.699761	4.222643	2.084795	3.868631	6.781884	0.967241
	Benzyl Alcohol	1.122713	0.389519	1.425669	0.097095	0.834712	0.877542	0.398586
	Benzeneacetylaldehyde	3.952726	0.603217	2.513116	0.272787	2.055026	1.484252	0.380802
	1-Phenethyl-4-propionyloxypiperidine	0.014108	0.020483	0.043026	0.033682	0.030042	0.02109	0.813788
7	Styrene	17.8902	4.109435	1.054935	4.995812	10.03528	30.43749	0.468742
	Benzaldehyde	4.665219	1.158257	7.325479	0.789221	0.962851	6.882834	0.604908
	Aniline	2.59054	0.438829	1.36999	0.811648	1.287523	2.072756	0.921583
	NPPA	23.36391	7.459722	5.766514	7.647355	9.139058	14.82597	0.796376
	NPP	0.721527	0.155709	0.241097	0.420167	0.850295	0.604056	0.307528
	Heptane	7.580138	3.715795	1.497047	2.660421	4.462132	8.96315	0.692749
	Benzyl Alcohol	0.361635	0.108453	0.733376	0.05695	0.076131	0.345018	0.301354
	Benzeneacetylaldehyde	6.607677	1.667437	5.892664	2.729185	3.254988	13.02045	0.684254
	1-Phenethyl-4-propionyloxypiperidine	0.393507	0.11052	0.102388	0.283349	0.374057	0.257603	0.370367
14	Styrene	1.729129	1.048171	5.539487	0.624074	3.655075	1.076683	0.589809
	Benzaldehyde	0.871933	0.289263	1.427911	0.721144	1.085639	0.827805	0.967159
	Aniline	1.271975	0.185851	1.05074	0.390203	1.009117	0.66278	0.712939
	NPPA	3.458846	2.057485	8.215134	2.475034	2.975155	2.295867	0.347148
	NPP	0.048193	0.021116	0.061102	0.065166	0.075388	0.120895	0.103532
	Heptane	0.363296	0.91735	2.878676	0.532794	1.236167	0.895553	0.562289
	Benzyl Alcohol	0.105034	0.025463	0.136387	0.076698	0.115417	0.067562	0.950169
	Benzeneacetylaldehyde	1.243278	0.496536	2.976548	2.425327	5.222946	2.201486	0.232864
	1-Phenethyl-4-propionyloxypiperidine	0.077665	0.030132	0.052658	0.030592	0.035399	0.048553	0.357801
21	Styrene	3.619987	7.358441	9.981556	0.78378	3.563219	6.285556	0.230338
	Benzaldehyde	2.647221	3.68267	4.011264	5.297183	1.264298	2.212276	0.705116
	Aniline	0.720835	1.039598	1.485933	0.327611	1.129307	0.976596	0.459127
	NPPA	7.293922	18.68555	19.55794	3.004446	2.724691	8.269678	0.072705
	NPP	0.084478	0.149142	0.173037	0.059252	0.065878	0.105804	0.124303
	Heptane	0.726208	4.766599	5.776393	0.283761	0.517744	2.282174	0.176986
	Benzyl Alcohol	0.253276	0.287343	0.289653	0.072453	0.16769	0.162186	0.012464
	Benzeneacetylaldehyde	4.189904	7.088715	7.481965	2.29072	6.619215	10.17689	0.967458
	1-Phenethyl-4-propionyloxypiperidine	0.03698	0.09122	0.111727	0.024553	0.020761	0.052138	0.123379
28	Styrene	1.141098	5.964016	4.120395	0.454161	1.926285	0.857303	0.144769
	Benzaldehyde	0.402112	0.78959	0.75693	2.082338	2.584064	2.778874	0.001625
	Aniline	0.34044	0.62683	0.704919	0.601819	1.216317	0.957656	0.15436
	NPPA	3.153224	15.06103	6.096333	1.539251	1.08134	1.744672	0.137381
	NPP	0.080704	0.130057	0.103751	0.04583	0.028579	0.094669	0.117794
	Heptane	0.136699	5.739896	1.295701	0.166346	0.196076	0.361922	0.276875
	Benzyl Alcohol	0.055896	0.076577	0.084679	0.219547	0.370776	0.270533	0.009018
	Benzeneacetylaldehyde	1.23907	3.590648	2.940881	4.033627	6.373956	4.586699	0.072791
	1-Phenethyl-4-propionyloxypiperidine	0.03944	0.089652	0.050392	0.011811	0.005695	0.041372	0.099351

P value < 0.05 values are statically different
No Statistical Difference

Table 39.Statistical analysis comparing Condition C to Condition D.

Days	Compound	Condition C			Condition D			P Value
Fresh	Styrene	0.785526	1.345208	2.361892	1.411678	0.826543	1.633766	0.711283
	Benzaldehyde	5.889243	4.706239	9.971852	5.958571	7.402656	6.272964	0.860002
	Aniline	2.529269	0.912351	2.205375	0.941173	1.623151	1.980456	0.561121
	NPPA	5.28054	1.043705	11.67217	6.524914	6.138141	6.607037	0.897437
	NPP	0.008167	0.019997	0.04925	0.12211	0.074983	0.04336	0.104169
	Heptane	2.063958	6.699761	4.222643	4.697847	9.798331	6.441293	0.257429
	Benzyl Alcohol	1.122713	0.389519	1.425669	0.937612	1.073688	1.071847	0.883779
	Benzeneacetylaldehyde	3.952726	0.603217	2.513116	1.327818	1.881878	2.12514	0.593618
	1-Phenethyl-4-propionyloxypiperidine	0.014108	0.020483	0.043026	0.082928	0.046981	0.042335	0.112239
7	Styrene	17.8902	4.109435	1.054935	4.883222	0.494147	9.798297	0.67591
	Benzaldehyde	4.665219	1.158257	7.325479	2.017934	2.853261	1.463397	0.282567
	Aniline	2.59054	0.438829	1.36999	0.516124	0.55224	0.844486	0.259681
	NPPA	23.36391	7.459722	5.766514	18.6962	11.67122	13.22031	0.717103
	NPP	0.721527	0.155709	0.241097	0.744926	0.365884	0.372952	0.603027
	Heptane	7.580138	3.715795	1.497047	2.321953	1.219976	2.755676	0.303454
	Benzyl Alcohol	0.361635	0.108453	0.733376	0.154934	0.256871	0.135933	0.303584
	Benzeneacetylaldehyde	6.607677	1.667437	5.892664	1.616338	2.075426	2.30546	0.154694
	1-Phenethyl-4-propionyloxypiperidine	0.393507	0.11052	0.102388	1.423934	0.58053	0.872306	0.046209
14	Styrene	1.729129	1.048171	5.539487	0.98449	3.248558	3.29291	0.876504
	Benzaldehyde	0.871933	0.289263	1.427911	0.506119	0.547883	0.672227	0.435803
	Aniline	1.271975	0.185851	1.05074	0.427156	0.496146	0.654301	0.41068
	NPPA	3.458846	2.057485	8.215134	3.345742	5.399856	3.669652	0.83463
	NPP	0.048193	0.021116	0.061102	0.048159	0.085419	0.060441	0.258057
	Heptane	0.363296	0.91735	2.878676	0.430145	1.901562	0.838799	0.727035
	Benzyl Alcohol	0.105034	0.025463	0.136387	0.059509	0.065193	0.048805	0.403645
	Benzeneacetylaldehyde	1.243278	0.496536	2.976548	0.460653	0.632495	0.78273	0.270132
	1-Phenethyl-4-propionyloxypiperidine	0.077665	0.030132	0.052658	0.148718	0.301233	0.301749	0.020179
21	Styrene	3.619987	7.358441	9.981556	2.119088	0.568763	4.389207	0.098107
	Benzaldehyde	2.647221	3.68267	4.011264	2.070787	0.879808	3.560833	0.219516
	Aniline	0.720835	1.039598	1.485933	0.418156	0.211712	0.246872	0.026747
	NPPA	7.293922	18.68555	19.55794	8.697922	2.388518	3.259495	0.078154
	NPP	0.084478	0.149142	0.173037	0.124843	0.062972	0.04581	0.181656
	Heptane	0.726208	4.766599	5.776393	1.188593	0.501767	1.943264	0.18645
	Benzyl Alcohol	0.253276	0.287343	0.289653	0.14912	0.067951	0.175205	0.013179
	Benzeneacetylaldehyde	4.189904	7.088715	7.481965	2.318642	0.573506	1.476123	0.014172
	1-Phenethyl-4-propionyloxypiperidine	0.03698	0.09122	0.111727	0.095463	0.121004	0.171357	0.19299
28	Styrene	1.141098	5.964016	4.120395	0.460732	0.273552	0.714695	0.082049
	Benzaldehyde	0.402112	0.78959	0.75693	0.278192	0.126448	0.336499	0.044286
	Aniline	0.34044	0.62683	0.704919	0.187282	0.117753	1.100553	0.804177
	NPPA	3.153224	15.06103	6.096333	10.19611	5.126496	5.540149	0.784631
	NPP	0.080704	0.130057	0.103751	0.128392	0.221619	0.120044	0.218377
	Heptane	0.136699	5.739896	1.295701	0.105204	0.190222	0.492342	0.281647
	Benzyl Alcohol	0.055896	0.076577	0.084679	0.039607	0.029084	0.047932	0.029958
	Benzeneacetylaldehyde	1.23907	3.590648	2.940881	0.484581	0.223399	0.960152	0.050173
	1-Phenethyl-4-propionyloxypiperidine	0.03944	0.089652	0.050392	0.055629	0.148779	0.15312	0.167661

P value < 0.05 values are statically different
No Statistical Difference

Appendix C: Two tailed T-Test comparing the days withing a single environment for the passive degradation of reference grade fentanyl.

Table 40.Statistical analysis comparing the days withing condition A.

Condition A								
Days	Compund							P- Value
Fresh to Day 7	Styrene	1.28277804	1.65505535	1.69727395	1.68094178	1.20646204	1.77499038	0.96897082
	Benzaldehyd	11.885273	5.17509025	10.0178137	1.17022806	0.93675255	1.08608192	0.0164083
	Aniline	3.88653997	0.87860712	1.80697546	0.28395305	0.40818277	0.36368422	0.10771427
	NPPA	5.36137181	26.5717957	1.78429821	1.21349653	33.5417864	2.07046103	0.94099102
	NPP	0.06263882	0.09875798	0.02958512	0.01786277	0.12592388	0.04147685	0.96279406
	Heptane	8.48765056	3.16332154	8.87344923	0.76892626	1.08098086	1.0772338	0.03357756
	Benzyl Alcoh	1.61807446	0.69203505	1.41110864	0.12733378	0.16421367	0.07234404	0.01653763
	Benzeneacet	5.07858595	2.58120353	1.97426449	1.00108319	1.08074988	0.48969406	0.07178596
	1-Phenethyl-	0.02555138	0.19754584	0.01526655	0.03610958	0.29956885	0.05962866	0.6378069
Day 7 to Day 14	Styrene	1.68094178	1.20646204	1.77499038	1.68337008	1.46777947	2.27954939	0.44114437
	Benzaldehyd	1.17022806	0.93675255	1.08608192	0.76842048	0.69556305	0.8269366	0.01832114
	Aniline	0.28395305	0.40818277	0.36368422	0.17882904	0.30184095	0.21854376	0.08149614
	NPPA	1.21349653	33.5417864	2.07046103	1.68870501	27.9562241	1.29737369	0.89400307
	NPP	0.01786277	0.12592388	0.04147685	0.01146829	0.06291354	0.0115628	0.42155388
	Heptane	0.76892626	1.08098086	1.0772338	0.73875174	0.85028651	1.07651736	0.57598801
	Benzyl Alcoh	0.12733378	0.16421367	0.07234404	0.11532704	0.10383227	0.07024235	0.4533078
	Benzeneacet	1.00108319	1.08074988	0.48969406	0.86141884	0.72791813	0.55834145	0.52839453
	1-Phenethyl-	0.03610958	0.29956885	0.05962866	0.04052914	0.18529775	0.01766201	0.63680029
Day 14 to Day 21	Styrene	1.68337008	1.46777947	2.27954939	1.17586025	1.291876	1.82852951	0.29645625
	Benzaldehyd	0.76842048	0.69556305	0.8269366	0.89941772	1.04277032	1.46048122	0.09821281
	Aniline	0.17882904	0.30184095	0.21854376	0.21306992	0.24547334	0.09142287	0.44848214
	NPPA	1.68870501	27.9562241	1.29737369	2.71294366	19.8001824	0.76897178	0.82302936
	NPP	0.01146829	0.06291354	0.0115628	0.00578673	0.08463649	0.00643315	0.91304711
	Heptane	0.73875174	0.85028651	1.07651736	0.56342672	0.79218655	0.82089391	0.27333541
	Benzyl Alcoh	0.11532704	0.10383227	0.07024235	0.15397603	0.12400248	0.13091158	0.07071832
	Benzeneacet	0.86141884	0.72791813	0.55834145	0.99708499	0.93225936	1.05935332	0.0420122
	1-Phenethyl-	0.04052914	0.18529775	0.01766201	0.01083027	0.23206044	0.01089427	0.97156778
Day 21 to Day 28	Styrene	1.17586025	1.291876	1.82852951	1.27874827	1.08987957	1.51788251	0.59395071
	Benzaldehyd	0.89941772	1.04277032	1.46048122	1.03796098	0.73469636	0.97650077	0.320092
	Aniline	0.21306992	0.24547334	0.09142287	0.39087177	0.25327261	0.26972494	0.13042236
	NPPA	2.71294366	19.8001824	0.76897178	9.17302724	21.5600029	7.59673116	0.53955169
	NPP	0.00578673	0.08463649	0.00643315	0.00357477	0.09350227	0.00988419	0.93541291
	Heptane	0.56342672	0.79218655	0.82089391	0.56974292	0.85033061	0.72969244	0.94197134
	Benzyl Alcoh	0.15397603	0.12400248	0.13091158	0.17010878	0.11688849	0.20276389	0.3684362
	Benzeneacet	0.99708499	0.93225936	1.05935332	1.59644897	1.13369905	1.88987356	0.07144752
	1-Phenethyl-	0.01083027	0.23206044	0.01089427	0.00492229	0.18346708	0.01017039	0.85462175
Fresh to Day 28	Styrene	1.28277804	1.65505535	1.69727395	1.27874827	1.08987957	1.51788251	0.23959936
	Benzaldehyd	11.885273	5.17509025	10.0178137	1.03796098	0.73469636	0.97650077	0.01545713
	Aniline	3.88653997	0.87860712	1.80697546	0.39087177	0.25327261	0.26972494	0.10152044
	NPPA	5.36137181	26.5717957	1.78429821	9.17302724	21.5600029	7.59673116	0.87133888
	NPP	0.06263882	0.09875798	0.02958512	0.00357477	0.09350227	0.00988419	0.47076424
	Heptane	8.48765056	3.16332154	8.87344923	0.56974292	0.85033061	0.72969244	0.02934849
	Benzyl Alcoh	1.61807446	0.69203505	1.41110864	0.17010878	0.11688849	0.20276389	0.01872513
	Benzeneacet	5.07858595	2.58120353	1.97426449	1.59644897	1.13369905	1.88987356	0.16167138
	1-Phenethyl-	0.02555138	0.19754584	0.01526655	0.00492229	0.18346708	0.01017039	0.88114438

P value < 0.05 values are statiscally different	
No Statistical Difference	

Table 41.Statistical analysis comparing the days withing condition B.

Condition B								
Days	Compund							P- Value
	Styrene	1.83486121	2.61209771	2.2384168	1.21961347	1.67219114	1.25267347	0.0340002
	Benzaldehyd	5.65229502	8.74501187	10.7699594	1.33715793	0.98564218	1.36028777	0.00866836
	Aniline	0.94804694	1.48631713	1.38363248	0.52432428	0.55512544	0.5872357	0.01244595
	NPPA	2.18555236	10.7388103	8.83927869	7.7294627	22.3733655	26.712372	0.13730884
Fresh to Day :	NPP	0.00614093	0.04724658	0.01466047	0.00548901	0.04766024	0.03424646	0.73336352
	Heptane	0.25525391	5.80449667	5.74978453	0.96429488	1.00456811	0.9915686	0.18431463
	Benzyl Alcoh	0.6375006	1.26065916	1.17139869	0.20893646	0.22620255	0.21280336	0.01428983
	Benzeneacet	0.09229855	2.70471247	1.89115937	1.11331262	1.19803459	1.09757963	0.61031573
	1-Phenethyl-	0.05528441	0.08872893	0.07805531	0.04569157	0.1025025	0.09885737	0.70988617
	Styrene	1.21961347	1.67219114	1.25267347	1.38868964	1.99412896	0.6109706	0.91182112
	Benzaldehyd	1.33715793	0.98564218	1.36028777	0.86185707	0.98988826	0.31776055	0.10243126
	Aniline	0.52432428	0.55512544	0.5872357	0.47088217	0.47563656	0.13595412	0.16249571
	NPPA	7.7294627	22.3733655	26.712372	4.92550516	13.0232277	8.14052557	0.17424646
ay 7 to Day 1	NPP	0.00548901	0.04766024	0.03424646	0.00978613	0.01028577	0.00687225	0.1818294
	Heptane	0.96429488	1.00456811	0.9915686	0.66793868	0.94470599	0.26681977	0.14159837
	Benzyl Alcoh	0.20893646	0.22620255	0.21280336	0.12498907	0.1326067	0.04024249	0.0178048
	Benzeneacet	1.11331262	1.19803459	1.09757963	0.53395075	0.66518593	0.15310673	0.01192232
	1-Phenethyl-	0.04569157	0.1025025	0.09885737	0.04215142	0.03106796	0.02710121	0.06085644
	Styrene	1.38868964	1.99412896	0.6109706	1.10860592	1.77573428	1.09646294	0.99292608
	Benzaldehyd	0.86185707	0.98988826	0.31776055	1.45589759	1.13196605	0.62685156	0.33377343
	Aniline	0.47088217	0.47563656	0.13595412	0.26993291	0.28910844	0.21158493	0.41654951
	NPPA	4.92550516	13.0232277	8.14052557	9.07937706	13.9393683	16.0386999	0.2392503
ay 14 to Day :	NPP	0.00978613	0.01028577	0.00687225	0.00774588	0.01741411	0.02606124	0.20807101
	Heptane	0.66793868	0.94470599	0.26681977	0.64330782	0.72335348	0.55004162	0.95418805
	Benzyl Alcoh	0.12498907	0.1326067	0.04024249	0.12892521	0.12026162	0.06556119	0.88197242
	Benzeneacet	0.53395075	0.66518593	0.15310673	0.69169227	0.73663805	0.40390474	0.4372696
	1-Phenethyl-	0.04215142	0.03106796	0.02710121	0.03362911	0.05475101	0.13453255	0.25854873
	Styrene	1.10860592	1.77573428	1.09646294	0.6901523	0.97355699	0.70340063	0.09096786
	Benzaldehyd	1.45589759	1.13196605	0.62685156	0.41688725	0.35519033	0.38129	0.04683539
	Aniline	0.26993291	0.28910844	0.21158493	0.35902866	0.48061109	0.46988994	0.01662567
	NPPA	9.07937706	13.9393683	16.0386999	30.3675222	35.7288094	39.5168471	0.00272766
ay 21 to Day :	NPP	0.00774588	0.01741411	0.02606124	0.00400506	0.09571332	0.24444502	0.2369188
	Heptane	0.64330782	0.72335348	0.55004162	0.64079487	0.84468542	0.78078004	0.21100643
	Benzyl Alcoh	0.12892521	0.12026162	0.06556119	0.06931369	0.10058172	0.0805862	0.3822199
	Benzeneacet	0.69169227	0.73663805	0.40390474	1.5823148	1.76454309	1.38195998	0.00313874
	1-Phenethyl-	0.03362911	0.05475101	0.13453255	0.06958223	0.12373772	0.25607535	0.29919402
	Styrene	1.83486121	2.61209771	2.2384168	0.6901523	0.97355699	0.70340063	0.00404951
	Benzaldehyd	5.65229502	8.74501187	10.7699594	0.41688725	0.35519033	0.38129	0.00577254
	Aniline	0.94804694	1.48631713	1.38363248	0.35902866	0.48061109	0.46988994	0.00785709
	NPPA	2.18555236	10.7388103	8.83927869	30.3675222	35.7288094	39.5168471	0.00166362
resh to Day 2	NPP	0.00614093	0.04724658	0.01466047	0.00400506	0.09571332	0.24444502	0.26555332
	Heptane	0.25525391	5.80449667	5.74978453	0.64079487	0.84468542	0.78078004	0.15918897
	Benzyl Alcoh	0.6375006	1.26065916	1.17139869	0.06931369	0.10058172	0.0805862	0.0084946
	Benzeneacet	0.09229855	2.70471247	1.89115937	1.5823148	1.76454309	1.38195998	0.98696742
	1-Phenethyl-	0.05528441	0.08872893	0.07805531	0.06958223	0.12373772	0.25607535	0.24927924

P value < 0.05 values are statiscally different
No Statistical Difference

Table 42.Statistical analysis comparing the days withing condition C.

Condition C								
Days	Compund							P- Value
Fresh to Day 7	Styrene	0.78552608	1.34520835	2.36189233	17.8901977	4.10943453	1.05493486	0.29979838
	Benzaldehyd	5.88924289	4.70623925	9.97185155	4.66521894	1.15825652	7.32547932	0.36008956
	Aniline	2.52926852	0.91235131	2.2053752	2.59054043	0.43882898	1.36998983	0.62855351
	NPPA	5.28054035	1.04370465	11.6721697	23.3639111	7.45972212	5.76651437	0.38766267
	NPP	0.0081666	0.01999701	0.04924959	0.72152669	0.15570947	0.2410969	0.12078916
	Heptane	2.06395847	6.6997606	4.22264274	7.58013764	3.71579486	1.49704726	0.97827979
	Benzyl Alcoh	1.12271251	0.38951896	1.42566884	0.36163527	0.10845329	0.7333759	0.18079046
	Benzeneacet	3.95272605	0.60321688	2.51311582	6.60767733	1.66743739	5.89266405	0.26370166
	1-Phenethyl-	0.01410813	0.02048328	0.0430261	0.39350717	0.11052016	0.10238813	0.14058635
Day 7 to Day 14	Styrene	17.8901977	4.10943453	1.05493486	1.72912945	1.04817131	5.539487	0.41153147
	Benzaldehyd	4.66521894	1.15825652	7.32547932	0.87193288	0.28926327	1.42791125	0.12460714
	Aniline	2.59054043	0.43882898	1.36998983	1.27197518	0.18585071	1.05074046	0.42225557
	NPPA	23.3639111	7.45972212	5.76651437	3.45884648	2.05748542	8.21513424	0.26657413
	NPP	0.72152669	0.15570947	0.2410969	0.04819263	0.02111565	0.06110164	0.13549607
	Heptane	7.58013764	3.71579486	1.49704726	0.36329557	0.91734999	2.87867569	0.21099819
	Benzyl Alcoh	0.36163527	0.10845329	0.7333759	0.10503431	0.02546276	0.13638664	0.16580612
	Benzeneacet	6.60767733	1.66743739	5.89266405	1.24327818	0.49653565	2.97654752	0.13878453
	1-Phenethyl-	0.39350717	0.11052016	0.10238813	0.07766523	0.03013159	0.05265821	0.19901649
Day 14 to Day 21	Styrene	1.72912945	1.04817131	5.539487	3.6199871	7.35844128	9.98155591	0.14282138
	Benzaldehyd	0.87193288	0.28926327	1.42791125	2.64722078	3.68267029	4.01126357	0.0079878
	Aniline	1.27197518	0.18585071	1.05074046	0.7208351	1.03959803	1.48593334	0.57081825
	NPPA	3.45884648	2.05748542	8.21513424	7.29392237	18.6855514	19.5579434	0.07220209
	NPP	0.04819263	0.02111565	0.06110164	0.0844779	0.14914181	0.17303656	0.03353836
	Heptane	0.36329557	0.91734999	2.87867569	0.72620789	4.76659906	5.77639259	0.24058993
	Benzyl Alcoh	0.10503431	0.02546276	0.13638664	0.25327638	0.28734308	0.28965288	0.00585187
	Benzeneacet	1.24327818	0.49653565	2.97654752	4.18990419	7.08871525	7.48196549	0.02117861
	1-Phenethyl-	0.07766523	0.03013159	0.05265821	0.03698022	0.0912199	0.11172691	0.36891877
Day 21 to Day 28	Styrene	3.6199871	7.35844128	9.98155591	1.14109791	5.9640163	4.12039547	0.23443732
	Benzaldehyd	2.64722078	3.68267029	4.01126357	0.40211221	0.78958951	0.75692955	0.00286379
	Aniline	0.7208351	1.03959803	1.48593334	0.34044008	0.62683016	0.70491914	0.10182919
	NPPA	7.29392237	18.6855514	19.5579434	3.15322429	15.0610335	6.09633256	0.25519316
	NPP	0.0844779	0.14914181	0.17303656	0.08070381	0.1300568	0.10375106	0.36450485
	Heptane	0.72620789	4.76659906	5.77639259	0.13669913	5.73989631	1.29570132	0.58485051
	Benzyl Alcoh	0.25327638	0.28734308	0.28965288	0.05589625	0.07657689	0.08467878	0.00014908
	Benzeneacet	4.18990419	7.08871525	7.48196549	1.23906991	3.5906482	2.94088132	0.04304959
	1-Phenethyl-	0.03698022	0.0912199	0.11172691	0.03944044	0.08965196	0.05039183	0.49716412
Fresh to Day 28	Styrene	0.78552608	1.34520835	2.36189233	1.14109791	5.9640163	4.12039547	0.20372865
	Benzaldehyd	5.88924289	4.70623925	9.97185155	0.40211221	0.78958951	0.75692955	0.01785223
	Aniline	2.52926852	0.91235131	2.2053752	0.34044008	0.62683016	0.70491914	0.05895752
	NPPA	5.28054035	1.04370465	11.6721697	3.15322429	15.0610335	6.09633256	0.6793125
	NPP	0.0081666	0.01999701	0.04924959	0.08070381	0.1300568	0.10375106	0.01358238
	Heptane	2.06395847	6.6997606	4.22264274	0.13669913	5.73989631	1.29570132	0.42232212
	Benzyl Alcoh	1.12271251	0.38951896	1.42566884	0.05589625	0.07657689	0.08467878	0.04207992
	Benzeneacet	3.95272605	0.60321688	2.51311582	1.23906991	3.5906482	2.94088132	0.85462486
	1-Phenethyl-	0.01410813	0.02048328	0.0430261	0.03944044	0.08965196	0.05039183	0.12571403

P value < 0.05 values are statiscally different
No Statistical Difference

Table 43.Statistical analysis comparing the days withing condition D.

Condition D								
Days	Compund							P- Value
	Styrene	1.41167783	0.82654338	1.63376551	4.88322207	0.49414707	9.79829664	0.23506729
	Benzaldehyd	5.95857062	7.402656	6.27296352	2.01793431	2.85326127	1.46339666	0.00174632
	Aniline	0.94117279	1.62315095	1.98045582	0.51612374	0.5522404	0.84448596	0.05278116
	NPPA	6.52491395	6.13814119	6.60703733	18.6961971	11.671224	13.2203134	0.01918275
Fresh to Day	NPP	0.12211027	0.07498309	0.04336032	0.74492594	0.36588436	0.37295199	0.03118004
	Heptane	4.69784717	9.79833059	6.44129269	2.32195333	1.21997647	2.75567605	0.03558792
	Benzyl Alcoh	0.93761187	1.07368764	1.07184712	0.15493399	0.25687092	0.13593338	0.0001348
	Benzeneacet	1.32781776	1.8818777	2.12513973	1.61633833	2.07542591	2.30545967	0.51686813
	1-Phenethyl-	0.08292759	0.04698089	0.04233505	1.42393378	0.58053026	0.87230611	0.02195229
	Styrene	4.88322207	0.49414707	9.79829664	0.98449007	3.248558	3.2929097	0.41295836
	Benzaldehyd	2.01793431	2.85326127	1.46339666	0.50611915	0.54788307	0.67222653	0.01953382
	Aniline	0.51612374	0.5522404	0.84448596	0.42715584	0.49614558	0.65430096	0.41776874
	NPPA	18.6961971	11.671224	13.2203134	3.34574217	5.39985607	3.6696523	0.00950727
ay 7 to Day 1	NPP	0.74492594	0.36588436	0.37295199	0.04815887	0.08541864	0.0604415	0.02675273
	Heptane	2.32195333	1.21997647	2.75567605	0.43014473	1.90156248	0.83879916	0.17519742
	Benzyl Alcoh	0.15493399	0.25687092	0.13593338	0.05950858	0.0651925	0.04880457	0.0300642
	Benzeneacet	1.61633833	2.07542591	2.30545967	0.46065298	0.63249485	0.78273033	0.0035186
	1-Phenethyl-	1.42393378	0.58053026	0.87230611	0.1487176	0.30123319	0.30174907	0.04853436
	Styrene	0.98449007	3.248558	3.2929097	2.1190878	0.56876323	4.38920669	0.91683554
	Benzaldehyd	0.50611915	0.54788307	0.67222653	2.07078723	0.87980842	3.56083338	0.10938527
	Aniline	0.42715584	0.49614558	0.65430096	0.41815649	0.21171214	0.24687205	0.06527754
	NPPA	3.34574217	5.39985607	3.6696523	8.69792247	2.38851836	3.25949493	0.77187807
ay 14 to Day	NPP	0.04815887	0.08541864	0.0604415	0.12484279	0.06297234	0.04581049	0.64310028
	Heptane	0.43014473	1.90156248	0.83879916	1.18859349	0.50176655	1.94326449	0.81107298
	Benzyl Alcoh	0.05950858	0.0651925	0.04880457	0.14912043	0.0679508	0.17520489	0.08926072
	Benzeneacet	0.46065298	0.63249485	0.78273033	2.31864204	0.57350552	1.47612285	0.18025025
	1-Phenethyl-	0.1487176	0.30123319	0.30174907	0.09546287	0.12100401	0.17135694	0.09454961
	Styrene	2.1190878	0.56876323	4.38920669	0.46073238	0.27355225	0.7146948	0.16826412
	Benzaldehyd	2.07078723	0.87980842	3.56083338	0.27819151	0.12644775	0.33649862	0.0687953
	Aniline	0.41815649	0.21171214	0.24687205	0.18728242	0.11775315	1.10055321	0.61424201
	NPPA	8.69792247	2.38851836	3.25949493	10.1961133	5.12649628	5.54014872	0.44344682
ay 21 to Day	NPP	0.12484279	0.06297234	0.04581049	0.12839202	0.22161922	0.12004432	0.12317149
	Heptane	1.18859349	0.50176655	1.94326449	0.10520425	0.19022161	0.49234166	0.09336204
	Benzyl Alcoh	0.14912043	0.0679508	0.17520489	0.03960693	0.02908444	0.0479319	0.04853781
	Benzeneacet	2.31864204	0.57350552	1.47612285	0.48458115	0.22339858	0.96015168	0.17590329
	1-Phenethyl-	0.09546287	0.12100401	0.17135694	0.05562949	0.14877871	0.1531199	0.80767412
	Styrene	1.41167783	0.82654338	1.63376551	0.46073238	0.27355225	0.7146948	0.04143123
	Benzaldehyd	5.95857062	7.402656	6.27296352	0.27819151	0.12644775	0.33649862	0.00014206
	Aniline	0.94117279	1.62315095	1.98045582	0.18728242	0.11775315	1.10055321	0.07593857
	NPPA	6.52491395	6.13814119	6.60703733	10.1961133	5.12649628	5.54014872	0.76122229
resh to Day 2	NPP	0.12211027	0.07498309	0.04336032	0.12839202	0.22161922	0.12004432	0.12678026
	Heptane	4.69784717	9.79833059	6.44129269	0.10520425	0.19022161	0.49234166	0.01104318
	Benzyl Alcoh	0.93761187	1.07368764	1.07184712	0.03960693	0.02908444	0.0479319	2.6253E-05
	Benzeneacet	1.32781776	1.8818777	2.12513973	0.48458115	0.22339858	0.96015168	0.01871772
	1-Phenethyl-	0.08292759	0.04698089	0.04233505	0.05562949	0.14877871	0.1531199	0.14602095

P value < 0.05 values are statiscally different	
No Statistical Difference	

Table 44.Statistical analysis comparing the days withing condition E.

Condition E								
Days	Compund							P- Value
Fresh to Day 7	Styrene	0.78916106	1.59894331	1.77127463	0.40741	0.662722	0.715051	0.0672007
	Benzaldehyd	5.03294254	4.67321128	5.06564065	0.255656	0.405307	0.329041	4.1832E-06
	Aniline	1.60640574	0.70091541	0.5285981	0.424387	0.452048	0.408721	0.19716474
	NPPA	2.36237908	3.76599059	1.14895515	19.42401	36.54532	19.36086	0.01705753
	NPP	0.02363705	0.05127634	0.01774522	0.161552	0.709673	0.224444	0.12622817
	Heptane	5.69225026	2.96697374	5.66012916	0.863263	1.908099	2.14939	0.03357872
	Benzyl Alcoh	0.44982838	0.30786128	0.15418832	0.037414	0.035352	0.032595	0.03455314
	Benzeneacet	0.87383542	1.05886281	0.49223944	1.282361	1.778928	1.730156	0.02646318
	1-Phenethyl-	0.04810993	0.05562114	0.03734583	0.036214	0.207477	0.071517	0.33073506
Day 7 to Day 14	Styrene	0.40741	0.662722	0.715051	0.32746156	0.5228372	0.66332864	0.54230152
	Benzaldehyd	0.255656	0.405307	0.329041	0.141932	0.19491435	0.20827462	0.03594856
	Aniline	0.424387	0.452048	0.408721	0.21259943	0.30633652	0.30832327	0.01094284
	NPPA	19.42401	36.54532	19.36086	7.6802016	28.8947309	15.9926027	0.41812378
	NPP	0.161552	0.709673	0.224444	0.2108578	0.3585154	0.26241278	0.64799829
	Heptane	0.863263	1.908099	2.14939	0.37319884	0.86983274	1.27317331	0.16525523
	Benzyl Alcoh	0.037414	0.035352	0.032595	0.01584526	0.02128851	0.02300557	0.00422099
	Benzeneacet	1.282361	1.778928	1.730156	0.51517349	0.85435777	0.94169351	0.01561386
	1-Phenethyl-	0.036214	0.207477	0.071517	0.01315107	0.20496295	0.0450047	0.83688224
Day 14 to Day 21	Styrene	0.32746156	0.5228372	0.66332864	0.28037749	0.43066938	0.46034832	0.36675356
	Benzaldehyd	0.141932	0.19491435	0.20827462	0.09102885	0.14491615	0.15194634	0.13392545
	Aniline	0.21259943	0.30633652	0.30832327	0.16051991	0.22261605	0.20147504	0.09080143
	NPPA	7.6802016	28.8947309	15.9926027	6.13263377	20.4954532	10.0889699	0.52064779
	NPP	0.2108578	0.3585154	0.26241278	0.27114458	0.37048323	0.23798358	0.7996945
	Heptane	0.37319884	0.86983274	1.27317331	0.30340301	0.55636638	0.73681116	0.34866873
	Benzyl Alcoh	0.01584526	0.02128851	0.02300557	0.01558126	0.01685619	0.02422991	0.75435482
	Benzeneacet	0.51517349	0.85435777	0.94169351	0.38719202	0.6839345	0.63403945	0.27320899
	1-Phenethyl-	0.01315107	0.20496295	0.0450047	0.01082852	0.32497207	0.06110588	0.71577236
Day 21 to Day 28	Styrene	0.28037749	0.43066938	0.46034832	0.32023488	0.61078529	0.69488984	0.29692921
	Benzaldehyd	0.09102885	0.14491615	0.15194634	0.07053502	0.11683267	0.11721259	0.32388531
	Aniline	0.16051991	0.22261605	0.20147504	0.11713789	0.19480445	0.20176962	0.50960018
	NPPA	6.13263377	20.4954532	10.0889699	4.32372336	20.3643906	9.35591187	0.89577755
	NPP	0.27114458	0.37048323	0.23798358	0.12571553	0.1796235	0.11602534	0.0263849
	Heptane	0.30340301	0.55636638	0.73681116	0.26415833	0.58878783	0.76874914	0.96763212
	Benzyl Alcoh	0.01558126	0.01685619	0.02422991	0.00976077	0.02334346	0.02110801	0.87779593
	Benzeneacet	0.38719202	0.6839345	0.63403945	0.2753458	0.5516575	0.56579227	0.47352344
	1-Phenethyl-	0.01082852	0.32497207	0.06110588	0.00943889	0.21687882	0.04743992	0.74221348
Fresh to Day 28	Styrene	0.78916106	1.59894331	1.77127463	0.32023488	0.61078529	0.69488984	0.05930849
	Benzaldehyd	5.03294254	4.67321128	5.06564065	0.07053502	0.11683267	0.11721259	2.8428E-06
	Aniline	1.60640574	0.70091541	0.5285981	0.11713789	0.19480445	0.20176962	0.08220347
	NPPA	2.36237908	3.76599059	1.14895515	4.32372336	20.3643906	9.35591187	0.1363719
	NPP	0.02363705	0.05127634	0.01774522	0.12571553	0.1796235	0.11602534	0.00799287
	Heptane	5.69225026	2.96697374	5.66012916	0.26415833	0.58878783	0.76874914	0.00984266
	Benzyl Alcoh	0.44982838	0.30786128	0.15418832	0.00976077	0.02334346	0.02110801	0.02870433
	Benzeneacet	0.87383542	1.05886281	0.49223944	0.2753458	0.5516575	0.56579227	0.14721914
	1-Phenethyl-	0.04810993	0.05562114	0.03734583	0.00943889	0.21687882	0.04743992	0.52745097

P value < 0.05 values are statiscally different
No Statistical Difference

Table 45.Statistical analysis comparing the days withing condition F.

		Condition F						
Days	Compund							P- Value
	Styrene	1.23214906	1.35304037	1.16757184	0.7138443	0.61801588	0.52178391	0.00123172
	Benzaldehyd	5.8338972	3.56277029	4.59559954	0.72678929	0.33593604	0.51560399	0.00341792
	Aniline	0.83939361	1.14447094	2.05243665	0.63853247	0.5313198	0.68056603	0.1180797
	NPPA	0.93859693	3.77259087	43.9268859	20.9272007	32.9270298	45.517395	0.33907532
Fresh to Day	NPP	0.02425764	0.02113346	0.32040228	0.18209982	0.6572311	1.78761259	0.19616811
	Heptane	7.75078626	3.77227355	5.35418161	3.54961702	1.36734313	1.58315019	0.0623482
	Benzyl Alcoh	0.13933507	0.4706933	0.88075859	0.05379649	0.03166595	0.04438788	0.10195794
	Benzeneacet	0.49229609	1.36499878	1.39018606	2.75499667	1.3813219	1.59749785	0.18529564
	1-Phenethyl-	0.04606471	0.03854069	0.12369897	0.08789091	0.20440193	1.21549049	0.29414903
	Styrene	0.7138443	0.61801588	0.52178391	0.55185521	0.57196827	0.5642493	0.37824509
	Benzaldehyd	0.72678929	0.33593604	0.51560399	0.21927071	0.23720758	0.23436444	0.0590531
	Aniline	0.63853247	0.5313198	0.68056603	0.30744168	0.37213633	0.46417483	0.02074426
	NPPA	20.9272007	32.9270298	45.517395	10.3093981	28.0823877	42.1122587	0.61714032
ay 7 to Day 1	NPP	0.18209982	0.6572311	1.78761259	0.21373631	0.39027965	1.31668672	0.70855296
	Heptane	3.54961702	1.36734313	1.58315019	1.14738615	0.82029443	1.2354872	0.19437062
	Benzyl Alcoh	0.05379649	0.03166595	0.04438788	0.02749345	0.02452272	0.02350347	0.0500062
	Benzeneacet	2.75499667	1.3813219	1.59749785	0.93691234	0.99598682	0.926803	0.08826326
	1-Phenethyl-	0.08789091	0.20440193	1.21549049	0.03653148	0.25586179	1.82821791	0.77508866
	Styrene	0.55185521	0.57196827	0.5642493	0.50651373	0.45404152	0.50863016	0.01779663
	Benzaldehyd	0.21927071	0.23720758	0.23436444	0.1700121	0.12898261	0.17346536	0.00898662
	Aniline	0.30744168	0.37213633	0.46417483	0.2729752	0.22509531	0.34099919	0.14696367
	NPPA	10.3093981	28.0823877	42.1122587	6.6148108	20.171815	30.7291653	0.54330234
ay 14 to Day	NPP	0.21373631	0.39027965	1.31668672	0.22691452	0.00677579	0.65810227	0.43089504
	Heptane	1.14738615	0.82029443	1.2354872	1.14738615	0.53309023	0.81274702	0.33854094
	Benzyl Alcoh	0.02749345	0.02452272	0.02350347	0.02208453	0.02005275	0.01855348	0.03487485
	Benzeneacet	0.93691234	0.99598682	0.926803	0.71195198	0.59290363	0.71478875	0.00356321
	1-Phenethyl-	0.03653148	0.25586179	1.82821791	0.02890239	0.33522579	1.69820233	0.98089721
	Styrene	0.50651373	0.45404152	0.50863016	0.54750871	0.5246421	0.67436679	0.13741479
	Benzaldehyd	0.1700121	0.12898261	0.17346536	0.09406686	0.09329121	0.12228496	0.03420394
	Aniline	0.2729752	0.22509531	0.34099919	0.19354998	0.20122708	0.35868271	0.67620096
	NPPA	6.6148108	20.171815	30.7291653	5.37899042	19.7595683	40.5798456	0.83594314
ay 21 to Day	NPP	0.22691452	0.00677579	0.65810227	0.11034537	0.17439376	0.38258587	0.73746842
	Heptane	1.14738615	0.53309023	0.81274702	0.90852741	0.49548974	0.73487336	0.61065486
	Benzyl Alcoh	0.02208453	0.02005275	0.01855348	0.01221879	0.01293733	0.01738166	0.03400076
	Benzeneacet	0.71195198	0.59290363	0.71478875	0.44403517	0.45953546	0.51768834	0.01228776
	1-Phenethyl-	0.02890239	0.33522579	1.69820233	0.02086304	0.23638704	1.23647893	0.78026543
	Styrene	1.23214906	1.35304037	1.16757184	0.54750871	0.5246421	0.67436679	0.00073066
	Benzaldehyd	5.8338972	3.56277029	4.59559954	0.09406686	0.09329121	0.12228496	0.00225616
	Aniline	0.83939361	1.14447094	2.05243665	0.19354998	0.20122708	0.35868271	0.04108338
	NPPA	0.93859693	3.77259087	43.9268859	5.37899042	19.7595683	40.5798456	0.75774026
resh to Day 2	NPP	0.02425764	0.02113346	0.32040228	0.11034537	0.17439376	0.38258587	0.47893048
	Heptane	7.75078626	3.77227355	5.35418161	0.90852741	0.49548974	0.73487336	0.01341936
	Benzyl Alcoh	0.13933507	0.4706933	0.88075859	0.01221879	0.01293733	0.01738166	0.08752589
	Benzeneacet	0.49229609	1.36499878	1.39018606	0.44403517	0.45953546	0.51768834	0.10891004
	1-Phenethyl-	0.04606471	0.03854069	0.12369897	0.02086304	0.23638704	1.23647893	0.31748772

P value < 0.05 values are staticsally different
No Statistical Difference

Table 46.Statistical analysis comparing the days withing condition G.

Condition G								
Days	Compund							P- Value
Fresh to Day 7	Styrene	1.11175669	0.96466134	1.00559127	1.67144639	5.1006037	6.18105617	0.07282526
	Benzaldehyd	7.82748027	2.08450778	1.8803426	0.84856481	0.30381732	0.34895316	0.15450754
	Aniline	1.09252642	0.45234539	0.31672356	0.51560763	0.30492075	0.31386453	0.38528562
	NPPA	2.46999769	0.81580774	0.30413796	5.45377952	10.1528662	6.22834857	0.01887834
	NPP	0.05046662	0.01048591	0.00218834	0.97769368	1.31607361	0.35117746	0.03838247
	Heptane	21.8829344	3.86962558	16.5445626	2.17980808	1.65659596	4.72012689	0.10684591
	Benzyl Alcoh	1.94835077	0.24266933	0.05634201	0.06449232	0.14811505	0.14386266	0.35464445
	Benzeneacet	0.43307967	0.45225074	0.28527004	1.99768367	0.74165132	0.83866393	0.11988527
	1-Phenethyl-	0.07618839	0.00836138	0.00315163	0.46390178	0.32808368	0.09235305	0.07508884
Day 7 to Day 14	Styrene	1.67144639	5.1006037	6.18105617	0.28367545	3.12413574	0.54707738	0.14025542
	Benzaldehyd	0.84856481	0.30381732	0.34895316	0.57326442	0.2638914	0.47445186	0.76420541
	Aniline	0.51560763	0.30492075	0.31386453	0.318885	0.21476306	0.22827655	0.17838769
	NPPA	5.45377952	10.1528662	6.22834857	1.95343006	3.10615332	2.61826815	0.03411857
	NPP	0.97769368	1.31607361	0.35117746	0.74888033	0.20095656	0.11316328	0.20177505
	Heptane	2.17980808	1.65659596	4.72012689	0.39905551	1.26275711	1.12625838	0.12220116
	Benzyl Alcoh	0.06449232	0.14811505	0.14386266	0.05264368	0.02254846	0.04603897	0.05225296
	Benzeneacet	1.99768367	0.74165132	0.83866393	1.36806692	0.73183155	1.22700826	0.86065487
	1-Phenethyl-	0.46390178	0.32808368	0.09235305	0.41086864	0.09306207	0.05230908	0.52423962
Day 14 to Day 21	Styrene	0.28367545	3.12413574	0.54707738	0.33351367	0.86129404	1.06752316	0.57765268
	Benzaldehyd	0.57326442	0.2638914	0.47445186	0.71988167	0.21586921	0.36305334	0.98173776
	Aniline	0.318885	0.21476306	0.22827655	0.32118362	0.14353212	0.22203153	0.70187298
	NPPA	1.95343006	3.10615332	2.61826815	2.0981867	1.73984803	3.52012562	0.87546646
	NPP	0.74888033	0.20095656	0.11316328	0.1434231	0.16482616	0.04812362	0.30859353
	Heptane	0.39905551	1.26275711	1.12625838	0.49390914	0.40258807	1.42623384	0.73234504
	Benzyl Alcoh	0.05264368	0.02254846	0.04603897	0.08533308	0.02208612	0.0456153	0.63381923
	Benzeneacet	1.36806692	0.73183155	1.22700826	1.67096284	0.4098181	0.91648196	0.80384516
	1-Phenethyl-	0.41086864	0.09306207	0.05230908	0.05959964	0.07733825	0.02535419	0.31486629
Day 21 to Day 28	Styrene	0.33351367	0.86129404	1.06752316	6.51769281	1.39792956	0.64830091	0.3212566
	Benzaldehyd	0.71988167	0.21586921	0.36305334	2.84547957	0.70036334	0.95394174	0.19852643
	Aniline	0.32118362	0.14353212	0.22203153	0.75114704	0.42670806	0.31536388	0.12816984
	NPPA	2.0981867	1.73984803	3.52012562	8.24628359	2.80919105	3.58813039	0.24472232
	NPP	0.1434231	0.16482616	0.04812362	0.13761853	0.06534277	0.06875351	0.54665788
	Heptane	0.49390914	0.40258807	1.42623384	5.22961765	1.4950594	0.68986409	0.30276232
	Benzyl Alcoh	0.08533308	0.02208612	0.0456153	0.89584444	0.06510246	0.08103354	0.34177107
	Benzeneacet	1.67096284	0.4098181	0.91648196	9.60873935	1.85526164	1.83450461	0.25922243
	1-Phenethyl-	0.05959964	0.07733825	0.02535419	0.05413153	0.02222308	0.02247539	0.31832691
Fresh to Day 28	Styrene	1.11175669	0.96466134	1.00559127	6.51769281	1.39792956	0.64830091	0.37798868
	Benzaldehyd	7.82748027	2.08450778	1.8803426	2.84547957	0.70036334	0.95394174	0.30406179
	Aniline	1.09252642	0.45234539	0.31672356	0.75114704	0.42670806	0.31536388	0.67573085
	NPPA	2.46999769	0.81580774	0.30413796	8.24628359	2.80919105	3.58813039	0.11277378
	NPP	0.05046662	0.01048591	0.00218834	0.13761853	0.06534277	0.06875351	0.06711842
	Heptane	21.8829344	3.86962558	16.5445626	5.22961765	1.4950594	0.68986409	0.10298546
	Benzyl Alcoh	1.94835077	0.24266933	0.05634201	0.89584444	0.06510246	0.08103354	0.57641615
	Benzeneacet	0.43307967	0.45225074	0.28527004	9.60873935	1.85526164	1.83450461	0.19337047
	1-Phenethyl-	0.07618839	0.00836138	0.00315163	0.05413153	0.02222308	0.02247539	0.89263046

P value < 0.05 values are staticsally different	
No Statistical Difference	

Table 47.Statistical analysis comparing the days withing condition H.

Condition H								
Days	Compund							P- Value
	Styrene	1.06952784	1.99950298	1.53728856	4.99581162	10.035279	30.4374856	0.15498575
	Benzaldehyd	3.83834154	4.35648726	4.31160445	0.78922116	0.96285089	6.88283412	0.55574187
	Aniline	0.60424322	1.64901313	1.20221657	0.81164777	1.28752267	2.07275571	0.64237432
	NPPA	0.77968522	3.52704359	10.8516095	7.6473553	9.13905779	14.8259701	0.21413097
Fresh to Day	NPP	0.0180129	0.03335579	0.03440285	0.42016675	0.85029462	0.60405616	0.00876954
	Heptane	2.08479512	3.8686312	6.78188383	2.66042056	4.46213196	8.96315008	0.65552402
	Benzyl Alcoh	0.09709492	0.83471211	0.87754181	0.05694951	0.07613078	0.34501845	0.17542186
	Benzeneacet	0.27278652	2.05502601	1.48425228	2.72918506	3.25498783	13.0204466	0.20921292
	1-Phenethyl-	0.03368231	0.0300423	0.02108954	0.28334883	0.37405671	0.25760349	0.0014633
	Styrene	4.99581162	10.035279	30.4374856	0.62407415	3.65507469	1.07668253	0.16309539
	Benzaldehyd	0.78922116	0.96285089	6.88283412	0.72114405	1.08563856	0.82780495	0.37512429
	Aniline	0.81164777	1.28752267	2.07275571	0.39020263	1.00911711	0.66278005	0.16062719
	NPPA	7.6473553	9.13905779	14.8259701	2.47503432	2.97515548	2.29586693	0.02231981
ay 7 to Day 1	NPP	0.42016675	0.85029462	0.60405616	0.06516628	0.0753878	0.12089467	0.01289799
	Heptane	2.66042056	4.46213196	8.96315008	0.53279379	1.23616666	0.89555273	0.07656441
	Benzyl Alcoh	0.05694951	0.07613078	0.34501845	0.07669779	0.11541686	0.06756163	0.48245642
	Benzeneacet	2.72918506	3.25498783	13.0204466	2.42532719	5.22294606	2.20148615	0.43060136
	1-Phenethyl-	0.28334883	0.37405671	0.25760349	0.03059214	0.03539865	0.04855294	0.00171743
	Styrene	0.62407415	3.65507469	1.07668253	0.78377986	3.5632193	6.28555607	0.39501002
	Benzaldehyd	0.72114405	1.08563856	0.82780495	5.29718332	1.26429846	2.21227567	0.16938833
	Aniline	0.39020263	1.00911711	0.66278005	0.32761136	1.12930703	0.97659617	0.7047598
	NPPA	2.47503432	2.97515548	2.29586693	3.00444618	2.72469129	8.26967776	0.31481231
ay 14 to Day	NPP	0.06516628	0.0753878	0.12089467	0.05925179	0.06587785	0.10580426	0.67421428
	Heptane	0.53279379	1.23616666	0.89555273	0.28376076	0.51774408	2.28217359	0.84330875
	Benzyl Alcoh	0.07669779	0.11541686	0.06756163	0.07245281	0.16768986	0.1621856	0.23651826
	Benzeneacet	2.42532719	5.22294606	2.20148615	2.29072033	6.61921503	10.1768897	0.28201765
	1-Phenethyl-	0.03059214	0.03539865	0.04855294	0.02455328	0.02076119	0.05213823	0.63924305
	Styrene	0.78377986	3.5632193	6.28555607	0.4541612	1.92628452	0.85730324	0.20902623
	Benzaldehyd	5.29718332	1.26429846	2.21227567	2.0823377	2.58406417	2.77887395	0.73804595
	Aniline	0.32761136	1.12930703	0.97659617	0.60181855	1.21631703	0.95765596	0.72608832
	NPPA	3.00444618	2.72469129	8.26967776	1.53925144	1.0813396	1.74467163	0.15142389
ay 21 to Day	NPP	0.05925179	0.06587785	0.10580426	0.04583028	0.02857878	0.09466854	0.44838807
	Heptane	0.28376076	0.51774408	2.28217359	0.16634568	0.19607568	0.36192219	0.28239923
	Benzyl Alcoh	0.07245281	0.16768986	0.1621856	0.21954732	0.37077574	0.27053332	0.04756114
	Benzeneacet	2.29072033	6.61921503	10.1768897	4.03362705	6.37395636	4.5866992	0.59824199
	1-Phenethyl-	0.02455328	0.02076119	0.05213823	0.01181122	0.00569471	0.04137163	0.43403719
	Styrene	1.06952784	1.99950298	1.53728856	0.4541612	1.92628452	0.85730324	0.42556959
	Benzaldehyd	3.83834154	4.35648726	4.31160445	2.0823377	2.58406417	2.77887395	0.00314553
	Aniline	0.60424322	1.64901313	1.20221657	0.60181855	1.21631703	0.95765596	0.55397428
	NPPA	0.77968522	3.52704359	10.8516095	1.53925144	1.0813396	1.74467163	0.29832985
resh to Day 2	NPP	0.0180129	0.03335579	0.03440285	0.04583028	0.02857878	0.09466854	0.24678576
	Heptane	2.08479512	3.8686312	6.78188383	0.16634568	0.19607568	0.36192219	0.04316737
	Benzyl Alcoh	0.09709492	0.83471211	0.87754181	0.21954732	0.37077574	0.27053332	0.28630702
	Benzeneacet	0.27278652	2.05502601	1.48425228	4.03362705	6.37395636	4.5866992	0.01332217
	1-Phenethyl-	0.03368231	0.0300423	0.02108954	0.01181122	0.00569471	0.04137163	0.49865959

P value < 0.05 values are staticsally different	
No Statistical Difference	

Appendix D: Raw data for the active training study for both the training aid mimic and the fentanyl odor soak.

Table 48.Raw data for all 24 15-minute session for the mimic analysis.

mimic												
Session	Trail 1 PA		IS	A/I 1	Trail 2 PA	IS	A/I 2	Trial 3 PA	IS	A/I 3	AVG A/I	SD
0	3669723		153226	23.9464778	6020862	182914	37.2899942	2029222	164945	12.302416	24.5129626	12.5034124
1	5784723		198135	29.1958665	4039509	211466	19.1024042	6756815	182291	37.0660921	28.4547876	9.00474429
2	4014071		167985	23.8954133	7044573	159196	44.195419	7130321	139282	52.6297081	40.2401505	14.7648294
3	2367084		129345	18.3005451	5171494	126889	40.7560466				29.5282958	15.8784374
4	10820402		372469	29.0504767	5448244	373663	14.5806745	6248508	289622	21.053707	21.5616194	7.23534061
5	8278929		240637	34.404223	6492803	322555	21.3691882	7717867	242732	29.7359536	28.5031876	6.60477966
6	8796627		260478	33.6674381	4082712	269180	15.1559581	7099481	232847	30.4897136	26.41271	9.89863102
7	5600122		283902	19.7255461	8759741	253829	34.5104027	5600122	283902	19.7255461	19.7255461	8.5360409
8	7241872		371367	19.5005803	8666872	325764	26.6047568	8459102	277202	30.5160208	25.5404526	5.58431195
9	3610233		245549	14.714684	3091353	251121	12.310213	7559742	258203	29.2782888	18.7677286	9.18246327
10	4626810		283372	16.3276894	10206275	311148	32.8019945	3767939	218018	17.3244324	22.1680388	9.22411569
11	5785803		253394	22.8174424	10218629	264892	38.5765859	8511030	184628	46.0982625	35.8307636	11.8808165
12	7514040		219086	34.2503168	4905225	270768	18.1159701	7138054	141628	50.402138	34.2561416	16.1430847
13	8614210		290130	29.6908627	11621075	326919	35.5472609	6567207	418839	15.6795499	26.9725579	10.2089848
14	5195352		249715	20.8051258	6565131	272026	23.9884384	11102011	298902	37.1593733	27.2209790	8.7226327
15	5771288		323099	17.8622275	8483124	266559	31.8245642	7409397	216848	34.1686208	27.9518707	8.81605418
16	4747458		258130	18.3917328	5013148	274166	18.2850828	4254160	229368	18.5473128	18.4080427	0.13182355
17	5874357		110355	53.231453	5768573	166828	34.5776668	7418084	117708	63.0210691	50.2767296	14.4500718
18	8970831		354268	25.3221601	7407152	291164	25.4397934	7654180	282224	27.1209394	25.9609643	1.00638028
19	7846909		396964	19.7673064	6278818	412316	15.2280958	3982708	246510	16.1749949	17.0567990	2.39463817
20	5771746		287731	20.0595209	11268887	297527	37.8751744	7571150	227080	33.2097509	30.381482	9.23843637
21	4253650		298479	14.2033001	3132630	305885	10.2412018	4016329	459831	8.74196341	11.0622718	2.82181697
22	2348849		219997	10.6812775	9070068	215776	42.0731129	5606577	198946	28.1955735	26.9833213	15.7309885
23	3978823		271024	14.6807036	5615210	224020	25.0656638	2962449	183045	16.1842662	18.6435445	5.61229785
24	1968197		249375	8.01282009	9101001	219482	41.4658395	9649339	414812	23.2809125	24.2865324	16.7448758
Instrument did not save the data												

Table 49.Raw data for all 24 15-minute session for the fentanyl odor soak analysis.

Fentanyl Odor Soak											
Session	Trail 1 PA	IS	A/I 1	Trail 2 PA	IS	A/I 2	Trial 3 PA	IS	A/I 3	AVG AI	SD
0	50457	1633	30.8983466	39341	2858	13.7652204	275065	5657	48.6238289	31.0957986	17.430143
1	25935	105169	0.24660309	19359	104690	0.18491738	199994	92639	2.15885318	0.86345788	1.12226914
2	15561	157372	0.09888036	10668	153226	0.06962265	207654	152556	1.36116574	0.50988958	0.7373719
3	11537	145272	0.07941654	6618	141990	0.04660892	120022	123401	0.97261773	0.36621439	0.52541682
4	15586	287476	0.0542167	23324	296921	0.07855288	148096	242377	0.61101507	0.24792822	0.31467779
5	11756	242558	0.04846676	5564	230776	0.02410996	121830	240617	0.50632333	0.19296668	0.27164794
6	4096	223115	0.0183418	7284	213447	0.03412557	111825	221878	0.50399319	0.18548685	0.27594745
7	7672	254667	0.03012562	8304	220110	0.03772659	85523	203760	0.41972419	0.16252546	0.22277305
8	6542	220393	0.02968334	6468	214254	0.03018847	41943	221981	0.1889486	0.08294014	0.09180637
9	2952	173657	0.01699903	2496	164036	0.01521008	49320	164945	0.29900876	0.11040596	0.16331727
10	3750	209148	0.01792989	2434	239912	0.01014539	30881	185112	0.16682333	0.0649662	0.08829669
11	3251	182914	0.01777338				24643	157987	0.15598119	0.05791819	0.09772768
12	4498	199070	0.02259507	3255	169644	0.01918724	44678	139386	0.32053434	0.12077222	0.17300747
13	7437	197758	0.03760657	5587	253823	0.0220114	37510	205747	0.18231129	0.08064309	0.08839185
14	5218	197163	0.02646541	6344	199854	0.03174317	29241	184731	0.15828962	0.07216607	0.07463185
15	3478	265626	0.0130936	7232	203892	0.03546976	12296	131490	0.09351281	0.04735872	0.04150691
16	4480	186307	0.02404633	3034	197010	0.01540023	22817	136060	0.16784507	0.06909721	0.08562735
17	0	125347	0	0	161908	0	23462	112808	0.2079817	0.06932723	0.12007829
18	0	246685	0	0	221030	0	26139	173265	0.1508614	0.05028713	0.08709987
19	0	27517	0	0	288707	0	22810	191774	0.11894209	0.03964736	0.06867125
20	0	289936	0	0	267804	0	16702	182566	0.09148472	0.03049491	0.05281873
21				0	250359	0	12815	175436	0.07304658	0.03652329	0.05165173
22	0	194011	0	0	193236	0	11219	126812	0.08846955	0.04423477	0.06255742
23	0	192202	0	0	194198	0	11085	136470	0.08122664	0.04061332	0.05743591
24	0	178584	0	0	169878	0	12173	120178	0.10129562	0.05147781	0.07280061
Instrument did not save the data											

Appendix E: Raw data for the depletion study for both the training aid mimic.

Table 50.Raw data for four weeks of mimic odor depletion.

mimic											
Week	A1	I1	(A/I)1	A2	I2	(A/I)2	A3	I3	(A/I)3	AVG (A/I)	STDV
0	6069737	90392	67.1490508	8731863	116183	75.1561158	2891472	110595	26.1446901	56.1499522	26.2919206
1	8358994	215457	38.7965766	5872122	201043	29.2082888	9327771	160277	58.1978138	42.0675597	14.7689755
2	2917638	98063	29.7526896	3726802	97132	38.3684265	1122360	42644	26.3193946	31.4801369	6.20751171
3	4684319	133955	34.9693479	1554065	235967	6.5859421	2179098	130562	16.6901396	19.4151432	14.3865793
4	2331005	244926	9.5171807	2867452	293215	9.77934962	4110889	233308	17.6200087	12.305513	4.60435465

Appendix F: FIU training guidelines provided to NACSW canine handler volunteers.

Guidelines for introducing new odors:

<u>Segment 1- Odor Imprinting</u> (2-4 weeks out from testing date)

- **PLEASE USE THE ODOR PACKET AS PROVIDED. DO NOT REMOVE THE GAUZE FROM THE PLASTIC BARRIER.**
- **IF YOUR CANINE HAS AN ACTIVE ALERT (BITTING, PAWING OR MOUTHING) YOU CANNOT PARTICIPATE IN THIS STUDY BUT MAY BE USEFUL FOR OTHER STUDIES IN THE FUTURE**

- Keep environment simple and basic for odor imprinting.
- Use care in handling of odor:
    - Use gloves when handling the plastic barriers.
        - Make sure to use different gloves when handling blanks and targets.
    - Remove packet from silver bag when using odor.
    - After training, place packet back in silver bag and seal,
        - Please store them in a cool ventilated area when training is not in process.
            - If you have mason jars it would be fine if you want to store them in there
    - Note packets in bag marked 'blank' – careful not to contaminate (do not handle after handling odor and do not store blank bag next to or with odor bags)

<u>PROCESS</u>

- Closed, but ventilate boxes.
- Run your dog.
- Reward first sign very quickly of interest in novelty odor. As dog gets repeated repetitions, the interest and response will increase. Take care not to flip box for toy reward dogs.
- Once you are seeing odor recognition, move to segment 2

**We are recommending pairing with primary rather than another odor to further differentiate the STOs (Study Target Odors) from K9NW odors. It is not imperative but knowing that many of you are competing with your dogs, this may provide more context for the dog. Alternatively, you may however pair the 'new' odor (STO) with a K9NW odor, just the same way you introduced Anise or Clove*

*** You could also pair in a separate tin next to the STO tin, but still reward externally to avoid disturbance.*

****Do not expect the dog's response to a new target odor to look exactly the same as their K9NW sport odors at first introduction.*

<u>Segment 2 - Odor Recognition</u> (1-2 weeks out from testing date)

- Boxes (closed)
- Expand environments.
- This phase you will need to balance fast payment to further cement importance of new odor with delayed payment for indication behavior.

<u>Segment 3 - Searches</u> (2 weeks out from testing date; you should have a solid month or more of searches with good odor recognition before test date) **(OPTIONAL)**

- Hides outside of boxes in new environments.
- Vehicles, Interiors, Exteriors
- Proceed like you would with any new K9NW odor.
- Use your training log. Please note especially environmental conditions, falses and misses.

<u>Troubleshooting, FAQs & Comments</u>
Please email amy@k9nosework.com with any concerns and we will add to this list.

- Out of odor
    - Please contact Leann Forte for new odor (lfort016@fiu.edu)

- **Frequency of training sessions**
    - **Shorter and frequent. Perhaps 5 min each with a minimum of 4 times per week. Alternatively, up to two 5 min sessions at different times of day at minimum of three times per week. The more frequent (daily) the better but not practical for everyone**.

- Training locations and contamination: Concerns with training in K9NW locations. We wouldn't recommend immediately duplicating a K9NW search that has fresh lingering odor with an STO search in same hide locations. It is also important to train in non K9NW locations to cement isolated recognition. It may be helpful to separate your training sessions and not work K9NW odors in back-to-back sessions with STO sessions. The more you do to keep context clear the better and it should also help you not accidentally touch or contaminate the two odor types.
- You have a total of 6 weeks for training. If more time is needed, please contact me.

Please get together and train with others. You will need to verify your training with other observers and hide setters.

Appendix G: Lineup matrices used for civilian canines not previously trained on fentanyl NF 1-10.

Table 51.Line up matrix for canine NF1.

NF1	1	2	3	4	5	6
LINE 1	D	P4	I5	G4	F	B8
LINE 2	I8	I7	E	H2	A2	B11
LINE 3	B4	I1	G1	F	B16	D
LINE 4	I5	F	P1	B9	I9	H2
LINE 5	E	B7	A3	D	I11	B5
LINE 6	B13	P2	I2	H3	G3	C
LINE 7	I10	B15	B6	F	P3	H5
LINE 8	G2	B2	C	H4	B3	B10
LINE 9	H1	B15	B12	H3	A1	B14

Table 52.Line up matrix for canine NF2.

NF2	1	2	3	4	5	6
LINE 1	G1	C	B1	P	I1	H (E)
LINE 2	B2	A1	D	I2	I3	H (F)
LINE 3	E	B3	G	B10	H(C)	I5
LINE 4	P	I6	H (D)	B4	I7	F
LINE 5	I8	H (E)	C	B11	B5	A2
LINE 6	H1	G3	D	I10	B2	P
LINE 7	H2	P	I11	E	B12	B7
LINE 8	B8	G4	B13	B14	F	H3
LINE 9	B17	C	A3	B9	B16	H4

Table 53.Line up matrix for canine NF3.

NF3	1	2	3	4	5	6
LINE 1	I11	C	B6	H5	P	G3
LINE 2	A1	H4	I6	B9	HI	I2
LINE 3	G	E	F	B12	B13	I3
LINE 4	B1	P	I10	H3	I7	D
LINE 5	B12	D	B10	G	F	P
LINE 6	I5	D	B10	G	F	P
LINE 7	P	H1	C	B11	I1	B14
LINE 8	B13	H3	B7	B5	G	H5
LINE 9	H2	A2	B3	B8	B2	E

Table 54.Line up matrix for canine NF4.

NF4	1	2	3	4	5	6
LINE 1	B	D-L	P-soak	B	B	D-E
LINE 2	D-F	B	B	D-H	P-mimic	B
LINE 3	D-D	B	D-C	B	B	B
LINE 4	B	P-dilute	D-M	D-L	B	B
LINE 5	D-L	B	B	P-mimic	D-K	B
LINE 6	D-J	B	D-H	B	B	P-soak
LINE 7	B	D-C	D-J	P-soak	B	B
LINE 8	B	B	B	D-H	B	D-C
LINE 9	B	D-K	A-mimic	B	D-J	B

Table 55.Line up matrix for canine NF5.

NF5	1	2	3	4	5	6
LINE 1	D-L	B	P-Soak	B	D-E	B
LINE 2	B	B	D-F	D-H	B	P-mimic
LINE 3	B	D-D	D-C	B	B	B
LINE 4	B	P-dilute	D-M	B	B	D-L
LINE 5	P-mimic	B	B	D-K	D-L	B
LINE 6	B	P-soak	D-J	B	B	D-H
LINE 7	D-C	B	P-soak	B	D-J	B
LINE 8	D-H	B	B	B	D-C	B
LINE 9	D-K	A-mimic	B	D-J	B	B

Table 56.Line up matrix for canine NF6.

NF6	1	2	3	4	5	6
LINE 1	D-E	P-Soak	B	B	D-L	B
LINE 2	P-mimic	B	D-F	B	B	D-H
LINE 3	D-D	B	B	B	B	D-C
LINE 4	D-L	P-dilute	B	B	D-M	B
LINE 5	B	A-mimic	D-L	D-K	B	B
LINE 6	P-soak	D-H	B	B	D-J	B
LINE 7	B	B	D-C	B	P-soak	D-J
LINE 8	B	D-H	D-C	B	B	B
LINE 9	B	B	D-K	B	D-J	A-mimic

Table 57.Line up matrix for canine NF7.

NF7	1	2	3	4	5	6
LINE 1	P-Soak	D-L	B	D-E	B	B
LINE 2	D-F	P-mimic	B	D-H	B	B
LINE 3	B	D-D	D-C	B	B	B
LINE 4	B	D-L	P-dilute	B	B	D-M
LINE 5	D-L	B	A-mimic	B	B	D-K
LINE 6	D-H	B	B	P-soak	D-J	B
LINE 7	D-J	p-soak	B	D-B	B	B
LINE 8	B	B	D-H	B	B	D-C
LINE 9	D-K	B	B	A-mimic	B	D-J

Table 58.Line up matrix for canine NF8.

NF8	1	2	3	4	5	6
LINE 1	B	B	D-L	B	D-E	P-Soak
LINE 2	B	D-H	D-F	P-mimic	B	B
LINE 3	B	B	B	D-D	B	D-C
LINE 4	D-M	D-L	B	B	P-dilute	B
LINE 5	D-L	B	A-mimic	B	B	D-K
LINE 6	D-H	B	B	P-soak	D-J	B
LINE 7	D-J	p-soak	B	D-B	B	B
LINE 8	B	B	D-H	B	B	D-C
LINE 9	D-K	B	B	A-mimic	B	D-J

Table 59.Line up matrix for canine NF9.

NF9	1	2	3	4	5	6
LINE 1	B	B	B	D-L	P-Soak	D-E
LINE 2	D-H	D-F	B	B	B	P-mimic
LINE 3	B	D-C	D-D	B	B	B
LINE 4	P-dilute	B	B	D-M	B	D-L
LINE 5	D-L	B	B	B	P-mimic	D-K
LINE 6	P-soak	D-H	B	B	D-J	B
LINE 7	B	D-H	B	D-J	B	P-soak
LINE 8	B	B	B	B	D-C	D-H
LINE 9	A-mimic	D-K	B	B	B	D-J

Table 60.Line up matrix for canine NF10.

NF10	1	2	3	4	5	6
LINE 1	B	B	B	D-L	P-Soak	D-E
LINE 2	D-H	D-F	B	B	B	P-mimic
LINE 3	B	D-C	D-D	B	B	B
LINE 4	P-dilute	B	B	D-M	B	D-L
LINE 5	D-L	B	B	B	P-mimic	D-K
LINE 6	P-soak	D-H	B	B	D-J	B
LINE 7	B	D-H	B	D-J	B	P-soak
LINE 8	B	B	B	B	D-C	D-H
LINE 9	A-mimic	D-K	B	B	B	D-J

Appendix H: Full ORT set up for the operational law enforcement canines who were not previously trained on fentanyl (NF17-26).

Table 61.Full ORT set up for canines NF17-26.

Wheel 1					
1	2	3	4	5	6
Mimic 1	D6	Blank	Blank	D2	Blank

Wheel 2					
1	2	3	4	5	6
CHP-1	D1	Blank	D5	Blank	Fentanyl 1

Wheel 3					
1	2	3	4	5	6
Blank	D3-1	Blank	CHP-5	Blank	CHP-4

Wheel 4					
1	2	3	4	5	6
D3-2	Cocaine	D4	Blank	D6	Blank

Wheel 5					
1	2	3	4	5	6
Blank	Blank	CHP-3	Mimic 2	Blank	CHP-2

Wheel 6					
1	2	3	4	5	6
Blank	D2	Fentanyl 2	Blank	CHP-5	Blank

Appendix I: Lineup matrices used for canines previously trained on fentanyl F1-5.

Table 62.Lineup matrix for canine F1.

F1	1	2	3	4	5	6
LINE 1	B1-1	Mimic-1	D1-1	B1-2	D3-1	B2-1
LINE 2	D2-1	B2-2	CBP 35g Control	B1-3	B2-3	D4-1
LINE 3	B2-4	B1-4	D5-1	B1-5	D6-1	B2-5
LINE 4	D7-1	B2-6	B1-6	CBP 5 g Control	D8-1	B1-7
LINE 5	B1-8	D9-1	B2-8	D1-2	B2-7	Mimic-2
LINE 6	D4-2	B2-8	CBP 35 Control	B1-9	D6-2	B2-9
LINE 7	D3-2	B1-10	B2-10	D5-2	B1-11	B2-11
LINE 8	B1-12	D7-2	B2-12	B1-13	Mimic-3	D9-2

Table 63.Lineup matrix for canine F2.

F2	1	2	3	4	5	6
LINE 1	Mimic-1	B2-1	D3-1	B1-1	B1-2	D1-1
LINE 2	B2-2	CBP 35g Control	D2-1	B2-3	D4-1	B1-3
LINE 3	B2-5	D6-1	B2-4	B1-4	B1-5	D5-1
LINE 4	B1-7	D8-1	CBP 5 g Control	B1-6	B2-6	D7-1
LINE 5	D9-1	B1-8	D1-2	B2-8	Mimic-2	B2-7
LINE 6	D6-2	B2-9	D4-2	B1-9	B2-8	CBP 35g Control
LINE 7	B2-11	D3-2	B2-10	D5-2	B1-11	B1-10
LINE 8	D9-2	B1-13	D7-2	Mimic-3	B2-12	B1-12

Table 64.Lineup matrix for canine F3.

F3	1	2	3	4	5	6
LINE 1	D1-1	B1-2	B1-1	D3-1	B2-1	Mimic-1
LINE 2	B1-3	D4-1	B2-3	D2-1	CBP 35g Control	B2-2
LINE 3	D5-1	B2-5	B1-4	B2-4	D6-1	B1-5
LINE 4	D7-1	B2-6	B1-6	D8-1	CBP 5 g Control	B1-7
LINE 5	B2-7	Mimic-2	B2-8	D1-2	B1-8	D9-1
LINE 6	CBP 35g Control	B2-8	B1-9	D4-2	B2-9	D6-2
LINE 7	B2-10	B2-11	D5-2	B1-10	D3-2	B1-11
LINE 8	B1-12	B2-12	Mimic-3	D7-2	B1-13	D9-2

Table 65.Lineup matrix for canine F4.

F4	1	2	3	4	5	6
LINE 1	B2-1	D3-1	Mimci-1	D1-1	B1-1	B1-2
LINE 2	D4-1	B2-2	B1-3	CBP 35g Control	D2-1	B2-3
LINE 3	B1-4	D6-1	B2-5	D5-1	B2-4	B1-5
LINE 4	B2-6	D7-1	B1-6	B1-7	D8-1	CBP 5 g Control
LINE 5	D1-2	B2-7	B1-8	D9-1	Mimic-2	B2-8
LINE 6	B2-9	CBP 35g Control	D6-2	D4-2	B2-8	B1-9
LINE 7	D5-2	B2-11	B2-10	B1-11	D3-2	B1-10
LINE 8	D7-2	Mimic-3	B1-13	D9-2	B2-12	B1-12

Table 66.Lineup matrix for canine F5.

F5	1	2	3	4	5	6
LINE 1	D3-1	B1-1	B2-1	D1-1	B1-2	Mimic-1
LINE 2	CBP 35g Control	D4-1	B2-2	B1-3	B2-3	D2-1
LINE 3	B1-5	B2-5	D5-1	B2-4	D6-1	B1-4
LINE 4	D8-1	CBP 5 g Control	D7-1	B1-7	B2-6	B1-6
LINE 5	Mimic-2	D9-1	B1-8	B2-8	D1-2	B2-7
LINE 6	B2-8	D6-2	B2-9	B1-9	CBP 35g Control	D4-2
LINE 7	B1-11	D3-2	B1-10	B2-11	D5-2	B2-10
LINE 8	B2-12	D9-2	Mimic-3	D7-2	B1-12	B1-13

Appendix J: Full ORT data for the field trials of civilian canines not previously trained on fentanyl canines NF1-10.

Table 67.Full ORT field trial results for canine NF1.

<u>Trial data</u>
K9 Code: NF-1 K9 Age:1 Year

Breed: Beagle Experience: 1 Year

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	A	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	I	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Table 68.Full ORT field trial results for canine NF2.

Trial data

K9 Code: NF-2 K9 Age: 1 Year

Breed: Beagle Experience: 1 Year

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	I	N	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	I	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Table 69.Full ORT field trial results for canine NF3.

Trial data

K9 Code: NF-3 K9 Age: 1 Year

Breed: Beagle Experience: 1 Year

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	I	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	I

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	I	N	N	N	A

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	I	N	N	N	A

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	I

Table 70.Full ORT field trial results for canine NF4.

Trial data

K9 Code: NF-4 K9 Age: 7 Years

Breed: Cocker Mix Experience: NW3

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Table 71.Full ORT field trial results for canine NF5.

Trial data

K9 Code: NF-5 K9 Age: 9 Years

Breed: Jack Russell Terrier Experience: NW3

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Table 72.Full ORT field trial results for canine NF6.

Trial data

K9 Code: NF-6 K9 Age: 6.5 Years

Breed: Labrador Experience: NW3

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Table 73.Full ORT field trial results for canine NF7.

Trial data

K9 Code: NF-7 K9 Age: 3 Years

Breed: German Shepherd Experience: NW3

K9 Response:

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Table 74.Full ORT field trial results for canine NF8.

Trial data

K9 Code: NF-8

K9 Age: 7 Years

Breed: Chocolate Lab

Experience: NW3

K9 Response:

Line 1	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 2	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Line 3	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 4-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 5-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 6-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 7-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 8-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Line 9-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Table 75.Full ORT field trial results for canine NF9.

Trial data

K9 Code: NF-9 — K9 Age: 10 Years

Breed: Shih Tzu — Experience: Elite 1

K9 Response:

Line 1	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 2	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 3	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 4-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 5-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Line 6-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 7-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 8-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 9-	**1**	**2**	**3**	**4**	**5**	**6**
Response: A=alert, I=interest, N= no response	N	A	N	N	N	N

Table 76.Full ORT field trial results for canine NF10.

Trial data

K9 Code: NF-10 K9 Age: 7 Years

Breed: Pyrenees/Cattle Dog Mix Experience: NW2

K9 Response

Line 1	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	A	N

Line 2	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	A	N	N	N

Line 3	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	A

Line 4-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	A	N	N	N	N	N

Line 5-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	A	N

Line 6-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 7-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	A	N	N

Line 8-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Line 9-	1	2	3	4	5	6
Response: A=alert, I=interest, N= no response	N	N	N	N	N	N

Appendix K: Full ORT data for canines NF17-25 who are operational law enforcement canines who were not previously trained on fentanyl.

Table 77.Pre-trial ORT data for canine NF17.

Pre-trial Data	03.27.2023				
K9 Code:	NF-17		K9 Age:	4	
Breed:	MAL		Experience:	3	
Wheel 1					
1	2	3	4	5	6
A	N	N	N	A	N
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 3					
1	2	3	4	5	6
N	N	I	N	N	N
Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 78. Pre-trial ORT data for canine NF18.

Pre-trial Data	03.27.2023				
K9 Code:	NF-18		K9 Age:	5	
Breed:	Dutch		Experience:	3	

Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	I	A	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 79. Pre-trial ORT data for canine NF19.

Pre-trial Data 03.27.2023					
K9 Code:	NF-19		K9 Age:	3	
Breed:	Mal		Experience:	1	
Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	N
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	N	A	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 80.Pre-trial ORT data for canine NF20.

Pre-trial Data 03.27.2023					
K9 Code:	NF-20		K9 Age:	2 1/2	
Breed:	Dutch Shep		Experience:	1	

Wheel 1					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	A	N	N	A	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	I	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	A	N

Table 81. Pre-trial ORT data for canine NF21.

Pre-trial Data	03.27.2023				
K9 Code:	NF-21		K9 Age:	2 1/2	
Breed:	Shepard		Experience:	1	

Wheel 1					
1	2	3	4	5	6
N	A	A	I	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 3					
1	2	3	4	5	6
A	N	N	N	I	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 82. Pre-trial ORT data for canine NF22.

Pre-trial Data	03.27.2023				
K9 Code:	NF-22		K9 Age:	8	
Breed:	Mal		Experience:	7	
Wheel 1					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 83. Pre-trial ORT data for canine NF23.

Pre-trial Data	03.27.2023				
K9 Code:	NF-23		K9 Age:	2	
Breed:	Shepard		Experience:	1	

Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	A	N

Table 84. Pre-trial ORT data for canine NF24.

Pre-trial Data	03.27.2023				
K9 Code:	NF-24		K9 Age:	3	
Breed:	German Shepard		Experience:	< 1 yr	
Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	A
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 3					
1	2	3	4	5	6
N	N	A	N	N	N
Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	A
Wheel 5					
1	2	3	4	5	6
N	A	N	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	A

Table 85. Pre-trial ORT data for canine NF25.

Pre-trial Data 03.27.2023					
K9 Code:	NF-25		K9 Age:	3	
Breed:	Dutch		Experience:	1	

Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	I	N	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 86.Pre-trial ORT data for canine NF26.

Pre-trial Data	03.27.2023				
K9 Code:	NF-26		K9 Age:	4	
Breed:	Shepard		Experience:	2	

Wheel 1					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	A

Wheel 6					
1	2	3	4	5	6
N	N	N	A	N	N

Table 87.Post-trial ORT data for canine NF17.

K9 Code:	NF-17		K9 Age:	4	
Breed:	MAL		Experience:	3	

Wheel 1					
1	2	3	4	5	6
A	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	I	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 6					
1	2	3	4	5	6
A	N	N	I*	N	N

Table 88.Post-trial ORT data for canine NF18.

K9 Code:	NF-18		K9 Age:	5	
Breed:	Dutch		Experience:	3	

Wheel 1					
1	2	3	4	5	6
A	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 89.Post-trial ORT data for canine NF19.

K9 Code:	NF-19		K9 Age:	3	
Breed:	Mal		Experience:	1	

Wheel 1					
1	2	3	4	5	6
I	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	A

Wheel 3					
1	2	3	4	5	6
N	I*	N	A	N	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	I*	I*	N

Wheel 6					
1	2	3	4	5	6
N	N	A	N	N	N

Table 90.Post-trial ORT data for canine NF20.

K9 Code:	NF-20		K9 Age:	2 1/2	
Breed:	Dutch Shep		Experience:	1	

Wheel 1					
1	2	3	4	5	6
A	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	I*	N

Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 6					
1	2	3	4	5	6
N	N	N	I	N	N

Table 91.Post-trial ORT data for canine NF21.

K9 Code:	NF-21		K9 Age:	2 1/2	
Breed:	Dutch Shepard		Experience:	1	
Wheel 1					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	A
Wheel 3					
1	2	3	4	5	6
N	A	N	N	N	N
Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	I	N	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	A	N	A	N

Table 92.Post-trial ORT data for canine NF22.

K9 Code:	NF-22		K9 Age:	8	
Breed:	Mal		Experience:	7	

Wheel 1					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 2					
1	2	3	4	5	6
I	N	N	N	N	N

Wheel 3					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	A	N	N	N

Wheel 6					
1	2	3	4	5	6
N	A	N	N	N	N

Table 93.Post-trial ORT data for canine NF23.

K9 Code:	NF-23		K9 Age:	2	
Breed:	German Shepard		Experience:	1	
Wheel 1					
1	2	3	4	5	6
A	N	N	N	N	N
Wheel 2					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 3					
1	2	3	4	5	6
N	N	N	N	N	N
Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	N	N	A	N	N
Wheel 6					
1	2	3	4	5	6
N	N	N	N	N	N

Table 94.Post-trial ORT data for canine NF24.

K9 Code:	NF-24		K9 Age:	3	
Breed:	German Shepard		Experience:	< 1 yr	
Wheel 1					
1	2	3	4	5	6
N	N	A	N	N	N
Wheel 2					
1	2	3	4	5	6
N	N	N	I	N	A
Wheel 3					
1	2	3	4	5	6
N	N	I	N	N	N
Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N
Wheel 5					
1	2	3	4	5	6
N	N	I	N	N	N
Wheel 6					
1	2	3	4	5	6
N	N	A	N	N	N

Table 95.Post-trial ORT data for canine NF25.

K9 Code:	NF-25		K9 Age:	3	
Breed:	Dutch		Experience:	1	

Wheel 1					
1	2	3	4	5	6
A	N	N	N	N	N

Wheel 2					
1	2	3	4	5	6
N	N	N	N	I*	N

Wheel 3					
1	2	3	4	5	6
N	N	I	N	A	N

Wheel 4					
1	2	3	4	5	6
N	A	N	N	N	N

Wheel 5					
1	2	3	4	5	6
N	N	N	A	N	N

Wheel 6					
1	2	3	4	5	6
A	N	N	I	N	N

Appendix L: Full ORT data for the field trials of canines previously trained on fentanyl F1-5.

Table 96.Full ORT results for canine F1 using preparation 2.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220734 (Meky) FIU ID: F1

Breed: German Shepherd

K9 Age: 1 year, 5 months

K9 Time in service: 3 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 1 - F.I.U. Mimic 1

Line 1

Location in line	1	2	3	4	5	6
Response: A=alert,	MIMIC 1	B2-1	D3-1	B1-1	B1-2	D1-1
I=interest, N= no response	**N**	**N**	N	N	N	N

Line 2

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-2	CBP 35g CONTROL	D2-1	B2-3	D8-1	B1-3
I=interest, N= no response	N	A	N	N	N	N

Line 3

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-5	D6-1	B2-4	B1-4	B1-5	D5-1
I=interest, N= no response	N	N	N	N	N	N

Line 4

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-7	D8-1	CBP 5g CONTROL	B1-6	B2-6	D7-1
I=interest, N= no response	N	N	A	N	N	N

Line 5

Location in line	1	2	3	4	5	6
Response: A=alert,	D9-1	B1-8	D1-2	B2-8	MIMIC 2	B2-7
I=interest, N= no response	N	N	N	N	**N**	**N**

Line 6

Location in line	1	2	3	4	5	6
Response: A=alert,	D6-2	B2-9	D4-2	B1-9	B2-8	CBP 35g CONTROL
I=interest, N= no response	N	N	N	N	N	A

Line 7

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-11	D3-2	B2-10	D5-2	B1-11	B1-10
I=interest, N= no response	N	N	N	N	N	N

Line 8

Location in line	1	2	3	4	5	6
Response: A=alert,	D9-2	B1-13	D7-2	MIMIC 3	B2-12	B1-12
I=interest, N= no response	N	N	N	**N**	**N**	N

Table 97.Full ORT results for canine F2 using preparation 2.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220670 (Deki) HU ID: F2

Breed: German Shepherd

K9 Age: 1 year, 5 months

K9 Time in service: 4 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 1 - F.T.D. Mimic 1

Line 1

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-1	MIMIC 1	D1-1	B1-2	D3-1	B2-1
I=interest, N= no response	N	M	N	N	N	N

Line 2

Location in line	1	2	3	4	5	6
Response: A=alert,	D2-1	B2-2	CBP 5g CONTROL	B1-3	B2-3	D4-1
I=interest, N= no response	N	N	A	N	N	N

Line 3

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-4	B1-4	D5-1	B1-5	D6-1	B2-5
I=interest, N= no response	N	N	N	N	N	N

Line 4

Location in line	1	2	3	4	5	6
Response: A=alert,	D7-1	B2-6	B1-6	CBP 5g CONTROL	D8-1	B1-7
I=interest, N= no response	N	N	N	A	N	N

Line 5

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-8	D9-1	B2-8	D1-2	B2-7	MIMIC 2
I=interest, N= no response	N	N	N	I	N	M

Line 6

Location in line	1	2	3	4	5	6
Response: A=alert,	D8-2	B2-8	CBP 5g CONTROL	B1-9	D6-2	B2-9
I=interest, N= no response	N	N	A	N	N	N

Line 7

Location in line	1	2	3	4	5	6
Response: A=alert,	D3-2	B1-10	B2-10	D5-2	B1-11	B2-11
I=interest, N= no response	N	N	N	N	I	N

Line 8

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-12	D7-2	B2-12	B1-13	MIMIC 3	D9-2
I=interest, N= no response	N	N	N	N	M	N

Table 98.Full ORT results for canine F3 using preparation 2.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220304 (Boy) FIU ID: F3

Breed: German Shepherd

K9 Age: 2 years, 1 month

K9 Time in service: 9 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 1 - F.I.U. Mimic 1

Line 1

Location in line	1	2	3	4	5	6
Response: A=alert,	D1-1	B1-2	B1-1	D3-1	B2-1	MIMIC 1
I=interest, N= no response	**N**	**N**	N	N	N	**N**

Line 2

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-3	D4-1	B2-3	D2-1	CBP 10g CONTROL	B2-2
I=interest, N= no response	N	N	N	N	A	N

Line 3

Location in line	1	2	3	4	5	6
Response: A=alert,	D5-1	B2-5	B1-4	B2-4	D6-1	B1-5
I=interest, N= no response	N	N	N	N	I	N

Line 4

Location in line	1	2	3	4	5	6
Response: A=alert,	D7-1	B2-6	B1-6	D8-1	CBP 5g CONTROL	B1-7
I=interest, N= no response	N	N	N	N	A	N

Line 5

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-7	MIMIC 2	B2-8	D1-2	B1-8	D9-1
I=interest, N= no response	N	**N**	N	N	**N**	**N**

Line 6

Location in line	1	2	3	4	5	6
Response: A=alert,	CBP 10g CONTROL	B2-8	B1-9	D4-2	B2-9	D6-2
I=interest, N= no response	A	N	N	N	N	N

Line 7

Location in line	1	2	3	4	5	6
Response: A=alert,	B2-10	B2-11	D5-2	B1-10	D3-2	B1-11
I=interest, N= no response	N	N	N	N	N	N

Line 8

Location in line	1	2	3	4	5	6
Response: A=alert,	B1-12	B2-12	MIMIC 3	D7-2	B1-13	D9-2
I=interest, N= no response	N	N	**N**	**N**	**N**	N

Table 99.Full ORT results for canine F4 using preparation 2.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220599 (Ojkac) [illegible]

Breed: German Shepherd

K9 Age: 1 year, 7 months

K9 Time in service: 5 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 1 - F.I.U. Mimic 1

Line 1

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-1	MIMIC 1	D1-1	B1-2	D3-1	B2-1
I = interest, N = no response	N	[illegible]	N	N	N	N

Line 2

Location in line	1	2	3	4	5	6
Response: A = alert,	D2-1	B2-2	CBP 35g CONTROL	B1-3	B2-3	D4-1
I = interest, N = no response	N	N	A	N	N	N

Line 3

Location in line	1	2	3	4	5	6
Response: A = alert,	B2-4	B1-4	D5-1	B1-5	D6-1	B2-5
I = interest, N = no response	N	N	N	N	N	N

Line 4

Location in line	1	2	3	4	5	6
Response: A = alert,	D7-1	B2-6	B1-6	CBP 5g CONTROL	D8-1	B1-7
I = interest, N = no response	N	N	N	A	N	N

Line 5

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-8	D9-1	B2-8	D1-2	B2-7	MIMIC 2
I = interest, N = no response	N	N	N	N	I	[illegible]

Line 6

Location in line	1	2	3	4	5	6
Response: A = alert,	D4-2	B2-8	CBP 35g CONTROL	B1-9	D6-2	B2-9
I = interest, N = no response	N	N	A	N	N	N

Line 7

Location in line	1	2	3	4	5	6
Response: A = alert,	D3-2	B1-10	B2-10	D5-2	B1-11	B2-11
I = interest, N = no response	N	N	N	N	N	N

Line 8

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-12	D7-2	B2-12	B1-13	MIMIC 3	D9-2
I = interest, N = no response	N	N	N	N	[illegible]	N

Table 100.Full ORT results for canine F5 using preparation 2.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220664 (Tobi) [illegible]

Breed: German Shepherd

K9 Age: 1 year, 4 months

K9 Time in service: 4 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 1 - F.I.U. Mimic 1

Line 1						
Location in line	1	2	3	4	5	6
Response: A=alert,	MIMIC 1	B2-1	D3-1	B1-1	B1-2	D1-1
I=interest, N= no response	[illegible]	[illegible]	N	N	N	[illegible]
Line 2						
Location in line	1	2	3	4	5	6
Response: A=alert,	B2-2	CBP 35g CONTROL	D2-1	B2-3	D4-1	B1-3
I=interest, N= no response	N	A	N	N	N	N
Line 3						
Location in line	1	2	3	4	5	6
Response: A=alert,	B2-5	D6-1	B2-4	B1-4	B1-5	D5-1
I=interest, N= no response	N	N	N	N	N	N
Line 4						
Location in line	1	2	3	4	5	6
Response: A=alert,	B1-7	D8-1	CBP 5g CONTROL	B1-6	B2-6	D7-1
I=interest, N= no response	N	N	A	N	N	N
Line 5						
Location in line	1	2	3	4	5	6
Response: A=alert,	D9-1	B1-8	D1-2	B2-8	MIMIC 2	B2-7
I=interest, N= no response	N	[illegible]	N	N	[illegible]	[illegible]
Line 6						
Location in line	1	2	3	4	5	6
Response: A=alert,	D6-2	B2-9	D4-2	B1-9	B2-8	CBP 35g CONTROL
I=interest, N= no response	N	N	N	N	N	A
Line 7						
Location in line	1	2	3	4	5	6
Response: A=alert,	B2-11	D3-2	B2-10	D5-2	B1-11	B1-10
I=interest, N= no response	N	N	N	N	N	N
Line 8						
Location in line	1	2	3	4	5	6
Response: A=alert,	D9-2	B1-13	D7-2	MIMIC 3	B2-12	B1-12
I=interest, N= no response	N	N	[illegible]	[illegible]	[illegible]	N

Table 101.Full ORT results for canine F4 using preparation 3.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220599 (Qkac)   FLU ID: 14

Breed: German Shepherd

K9 Age: 1 year, 7 months

K9 Time in service: 5 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 2 - F.L.U. Mimic 2

Line 1

Location in line	1	2	3	4	5	6
Response: A = alert,	MIMIC 1	B2-1	D3-1	B1-1	B1-2	D1-1
I = interest, N = no response	N	[illegible]	N	N	N	N

Line 2

Location in line	1	2	3	4	5	6
Response: A = alert,	B2-2	CBP 25g CONTROL	D2-1	B2-3	D4-1	B1-3
I = interest, N = no response	N	A	N	N	N	N

Line 3

Location in line	1	2	3	4	5	6
Response: A = alert,	B2-5	D6-1	B2-4	B1-4	B1-5	D5-1
I = interest, N = no response	N	N	N	N	N	N

Line 4

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-7	D8-1	CBP 5g CONTROL	B1-6	B2-6	D7-1
I = interest, N = no response	N	N	A	N	N	N

Line 5

Location in line	1	2	3	4	5	6
Response: A = alert,	D9-1	B1-8	D1-2	B2-8	MIMIC 2	B2-7
I = interest, N = no response	N	N	N	N	N	[illegible]

Line 6

Location in line	1	2	3	4	5	6
Response: A = alert,	D6-2	B2-9	D4-2	B1-9	B2-8	CBP 25g CONTROL
I = interest, N = no response	N	N	N	N	N	A

Line 7

Location in line	1	2	3	4	5	6
Response: A = alert,	B2-11	D3-2	B2-10	D5-2	B1-11	B1-10
I = interest, N = no response	N	N	N	N	N	N

Line 8

Location in line	1	2	3	4	5	6
Response: A = alert,	D9-2	B1-13	D7-2	MIMIC 3	B2-12	B1-12
I = interest, N = no response	N	N	N	N	[illegible]	N

Table 102.Full ORT results for canine F5 using preparation 3.

Field Operations Canine Academy
Pseudo Fentanyl Trial

Data Log Sheet

K9 ID: 220664 (Tobi)   FEU ID:F5

Breed: German Shepherd

K9 Age: 1 year, 4 months

K9 Time in service: 4 months

Previous training on Fentanyl: Yes
K9 response to training aid:

Trial 2 - F.E.U. Mimic 2

Line 1

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-1	MIMIC-1	D1-1	B1-2	D3-1	B2-1
I = interest, N = no response	N	[illegible]	N	N	N	N

Line 2

Location in line	1	2	3	4	5	6
Response: A = alert,	D2-1	B2-2	CBP 35g CONTROL	B1-3	B2-3	D4-1
I = interest, N = no response	N	N	A	N	N	N

Line 3

Location in line	1	2	3	4	5	6
Response: A = alert,	B2-4	B1-4	D5-1	B1-5	D6-1	B2-5
I = interest, N = no response	N	N	N	N	N	N

Line 4

Location in line	1	2	3	4	5	6
Response: A = alert,	D7-1	B2-6	B1-6	CBP 5g CONTROL	D8-1	B1-7
I = interest, N = no response	N	N	N	A	N	N

Line 5

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-8	D9-1	B2-8	D1-2	B2-7	MIMIC-2
I = interest, N = no response	N	N	N	N	N	I

Line 6

Location in line	1	2	3	4	5	6
Response: A = alert,	D4-2	B2-8	CBP 35g CONTROL	B1-9	D6-2	B2-9
I = interest, N = no response	N	N	A	N	N	N

Line 7

Location in line	1	2	3	4	5	6
Response: A = alert,	D3-2	B1-10	B2-10	D5-2	B1-11	B2-11
I = interest, N = no response	N	N	N	N	N	N

Line 8

Location in line	1	2	3	4	5	6
Response: A = alert,	B1-12	D7-2	B2-12	B1-13	MIMIC-3	D9-2
I = interest, N = no response	N	N	N	N	[illegible]	N

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PUBLICATIONS AND PRESENTATIONS

Forte, Leann; Furton, Kenneth G (2020, February 17-22) Optimal Extraction of Fentanyl VOCs for the Development of Canine Training Aid Mimics (conference presentation) 2020 *American Academy of Forensic Science Meeting*, Anaheim, CA, United States

Forte, Leann; Furton, Kenneth G (2020, May) Optimal Extraction of Fentanyl VOCs for the Development of Canine Training Aid Mimics, 2020 *International Association of Forensic Science Meeting*, Sydney, Australia (Abstract Accepted, Meeting Canceled)

Forte, Leann; Vaughan Stephanie; DeGreeff, Lauryn; Holness, Howard; Furton, Kenneth G (2021, February 15-19) Effects of Degradative Stress on Vapor Analysis of Fentanyl (conference presentation) 2021 *American Academy of Forensic Science Meeting*, Huston, TX, United States (held virtually)

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