11-11-2010

Development of a Dynamic Headspace Concentration Technique for the Non-Contact Sampling of Human Odor Samples and the Creation of Canine Training Aids

Lauryn E. DeGreeff
Florida International University, ldegreeff@hotmail.com

DOI: 10.25148/etd.FI10120312

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DEVELOPMENT OF A DYNAMIC HEADSPACE CONCENTRATION TECHNIQUE FOR THE NON-CONTACT SAMPLING OF HUMAN ODOR SAMPLES AND THE CREATION OF CANINE TRAINING AIDS

A dissertation submitted in partial fulfillment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

by

Lauryn E. DeGreeff

2010

To: Dean Kenneth Furton

College of Arts and Sciences

This dissertation, written by Lauryn E. DeGreeff, and entitled Development of a Dynamic Headspace Concentration Technique for the Non-Contact Sampling of Human Odor Samples and the Creation of Canine Training Aids, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Yong Cai

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Stephan Rose

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DeEtta Mills

_____
David Chatfield

_____
Kenneth Furton, Major Professor

Date of Defense: November 11, 2010

The dissertation of Lauryn E. DeGreeff is approved.

_____
Dean Kenneth Furton
College of Arts and Sciences

_____
Interim Dean Kevin O'Shea
University Graduate School

Florida International University, 2010

DEDICATION

I dedicate this work to my mother, Marilyn Burns, and my father, Louis DeGreeff. To my mother, for supporting me through my breakdowns as well as my triumphs. To my father, for being the wise one to whom I can always turn. Without their ongoing support and patience, the completion of this work would not have been possible.

ACKNOWLEDGMENTS

I would like to thank my major professor and my dissertation committee. I thank Dr. Kenneth Furton, my major professor, for his guidance over the last four years. I appreciate the time, patience and opportunities he has given me during my stay at Florida International University. I also thank my committee members, Drs. DeEtta Mills, Yong Cai, David Chatfield, and Stephan Rose for offering their time and valued opinions and advice.

I would also like to thank those who have collaborated with me during this project. I thank Susan Stejskal and Barbara Weakley-Jones for maintaining their excitement for my work and always offering their assistance and resources. I thank the Miami-Dade Medical Examiner's Office, particularly Larry Cameron, Bruce Hyma and the staff of the morgue, as well as the owner and staff of Borden Cremation Services for their assistance and access to their resources during my project. I also thank the Technical Support Working Group for their financial contribution to my project.

I would like to thank all of the members of my research group for being the sounding boards for my ideas and contributing to the evolution of my research and myself as a chemist. I would like to give a special thanks to my lab manager, Howard Holness, without his many hours of helping and listening none of this work would have been completed.

Last but certainly not least, I would like to thank my friends and family for all of the support they have given to me. I especially thank my mother and father for their mental, emotional and financial support through my many, many years of school, Megan Bottegal and Michael Macias for encouraging and supporting me through the good times

and bad, and Heather Nelson for always listening, whether or not she understood, and for making my life more fun. I love you all. Thank you.

ABSTRACT OF THE DISSERTATION

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Lauryn E. DeGreeff

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Professor Kenneth Furton, Major Professor

Human scent and human remains detection canines are used to locate living or deceased humans under many circumstances. Human scent canines locate individual humans on the basis of their unique scent profile, while human remains detection canines locate the general scent of decomposing human remains.

Scent evidence is often collected by law enforcement agencies using a Scent Transfer Unit, a dynamic headspace concentration device. The goals of this research were to evaluate the STU-100 for the collection of human scent samples, and to apply this method to the collection of living and deceased human samples, and to the creation of canine training aids.

The airflow rate and collection material used with the STU-100 were evaluated using a novel scent delivery method. Controlled Odor Mimic Permeation Systems were created containing representative standard compounds delivered at known rates, improving the reproducibility of optimization experiments.

Flow rates and collection materials were compared. Higher air flow rates usually yielded significantly less total volatile compounds due to compound breakthrough through the collection material. Collection from polymer and cellulose-based materials demonstrated that the molecular backbone of the material is a factor in the trapping and releasing of compounds. The weave of the material also affects compound collection, as those materials with a tighter weave demonstrated enhanced collection efficiencies.

Using the optimized method, volatiles were efficiently collected from living and deceased humans. Replicates of the living human samples showed good reproducibility; however, the odor profiles from individuals were not always distinguishable from one another. Analysis of the human remains samples revealed similarity in the type and ratio of compounds.

Two types of prototype training aids were developed utilizing combinations of pure compounds as well as volatiles from actual human samples concentrated onto sorbents, which were subsequently used in field tests. The pseudo scent aids had moderate success in field tests, and the Odor pad aids had significant success. This research demonstrates that the STU-100 is a valuable tool for dog handlers and as a field instrument; however, modifications are warranted in order to improve its performance as a method for instrumental detection.

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1. INTRODUCTION

1.1. Scent and Scenting Canines

Canines have been used as scent detectors for thousands of years.[1] The earliest detector canines were used to locate prey when hunting with their masters. The canine's ability to hunt prey comes naturally; all dogs, domesticated and wild, have a natural drive to hunt and a keen ability to locate their prey on the basis of the particular scent given off by the prey object.[2] The use of scent canines has evolved from merely a hunting tool to a detection device used by many government agencies, law enforcement agencies, and private industries. Current uses of scent dogs include, but are not limited to, the detection of drugs, bombs, accelerants, humans (living and deceased), agricultural products, currency, melanoma, and pests.[3] Canines have the potential to detect almost anything that gives off an odor.

Scent of varying composition and strength is emitted from all living organisms through metabolism, respiration, and glandular secretions. Volatile compounds are generated from these processes and released as gaseous vapors which provide the odor detected by the canine. Just as these processes are unique to each individual, each individual will have a unique scent,[4] which, with a canine's natural drive to hunt or track prey, gives it its capacity for following specific animals for lengths of time and over diverse terrain.

The most common legal dispute regarding canine scent evidence is the use of canines to track or trail living human scent, and has been disputed for more than one hundred years. The court system of Alabama first addressed scent and scent canines as

early as 1893 in *Hodge v. State*, in which a trained canine followed the scent of a suspect from the crime scene to the home of the suspect. For the first time, such evidence was deemed admissible in a United States court.[5]

Shortly thereafter in 1896, evidence from a human tracking canine was admitted in *State v. Hall*, but with the additional stipulation that the canine in question have a reasonable amount of training and experience.[6] Several years later, however, in a 1903 case in Nebraska, the state ruled in *Brott v. State* that canine evidence was inadmissible for being too error-ridden and subjective.[7] From these early rulings until today, canine scent evidence has been repeatedly challenged by the courts.

Today, for scientific scent evidence to be admitted in court, the scientific technique must pass either a *Kelly-Frye* hearing or a *Daubert* hearing depending upon the state in which the case is tried. The state of California conducted a *Kelly-Frye* hearing in 2005 to determine if scent evidence collected with the Scent Transfer Unit (STU-100), a scent evidence collection device, met the standard of reliability for *Kelly-Frye*. The STU-100 evidence was found to be admissible.[8]

Other examples of court rulings include *People v. Mitchell* (2003), where the court ruled against the admission of scent identification line-ups as evidence in the California Court of Appeals because the scientific basis for such practices had yet to be supported by data,[9] and *People v. Willis* (2004) where scent was collected from a piece of evidence with the Scent Transfer Unit. The scent was collected onto a gauze pad, and the pad was presented to a bloodhound, which subsequently trailed and located the suspect. The scent evidence was found to be admissible, but was later reversed on appeal.[10]

The greatest challenges to admitting human scent evidence to the courts are the reliability and experience of the canine and canine handler and the science behind human scent identification.[11] There is some currently published research, but there is a dearth of knowledge and published research regarding the composition, stability, uniqueness, and collection of human scent. To overcome the continuing question of scent evidence admissibility to the courts more research must be published. The STU-100 and similar devices need further research and improvement.

1.2. Canine Olfaction

Odor molecules are small, volatile compounds, each with a different structure. Mammals have highly developed olfactory systems capable of high powers of discrimination allowing small differences in chemical structure yield different perceived odors.[12] The odor molecules enter the nose and dissolve into the mucus layer where they are captured by the cilia and where the olfactory receptors in the epithelial lining of the nasal cavity are located. The mucus layer contains small, proteins that bind and move the odor molecules across the mucus layer to the receptors. The odorant receptors mediate odor reception by triggering olfactory neurons. The binding of the odorant to the olfactory receptors creates an action potential in the neurons through a G-protein-mediated reaction, or an IP3- (inositol 1,4,5-triposphate) mediated reaction. The action potential causes the signal to travel to the olfactory bulb in the brain, where the signal is transmitted to the olfactory cortex. The olfactory cortex sends information to higher areas of the brain for odor discrimination or to the limbic system for emotional and

physiological responses. The brain interprets the intensity of the particular odor based on the number of receptors that are stimulated to the action potential.[12,13]

When canines breathe, air flows into their mouth and nose with less than the required upward velocity for air to reach their olfactory area. When canines are detecting a scent, however, they inhale sharply, or sniff, to create an eddy-current that carries the air further upward into their olfaction area the upper portion of their nose, where the scent is detected.[14]

Canine olfactory systems are especially sensitive compared to that of humans. Canine elongated snouts have bony cavities, called turbinates, which increase their internal surface area and provide for a greater number of olfactory receptors,[14,15] Canines have 50 times more olfactory receptor cells than humans. The olfactory bulb-volume of canines is 600 times greater than that of humans and takes up 35% of the canine brain as compared to only 5% of the human brain.[3] Their olfactory systems are adapted to discriminate among very low quantities of many types of odorants,[4] even among single odors in a mixture.[17] Canines have olfactory receptors lining both sides of their nasal cavity, which allows them to locate the origin of a scent by discriminating between intensity differences on either side of their nasal cavity.[14] For these reasons, along with their other capabilities, canines are optimal scent detectors.

1.3. Scent Delivery, Collection and Analysis

1.3.1. Human Scent Collection

When human scent is collected from a crime scene, a piece of evidence, or a suspect, the odor is presented to a canine. Investigators then hope the canine will recognize the scent and begin trailing, leading them to the victim, a suspect, the crime scene, or additional evidence.

Investigators may collect scent evidence in several ways: First is the direct approach where investigators present the actual scent source to the canine. Alternatively, one may use the swiping or absorption methods, in which a piece of material or gauze pad is swiped over the surface of the scent article or left in contact with the scent article for a period of time. In a similar method, a suspect may be asked to hold gauze or a metal bar for a period of time, which is then presented to the canine. A benefit of the swiping and absorption techniques is that the scented material can be stored for future use. The drawback of all three, methods, however, is the risk of destroying or contaminating other evidence.[18] Local police and federal agencies eliminate this problem by employing a scent "vacuum" device that collects and concentrates the scent onto a piece of material without contact with the actual piece of evidence.

The Scent Transfer Unit

The Scent Transfer Unit (STU-100) is a field-portable, dynamic-airflow, collection device for the concentration of volatiles from scent samples onto a sorbent material. The STU-100 is a simple device consisting of a small vacuum pump attached to a Teflon-coated hood designed to hold a piece of material (Figure 1). The hood has been modified with a stainless steel plate to hold a 2'x2' piece of collection material.[19] When the STU-100 is swept over the subject or object of interest, air is drawn toward the device, concentrating any VOC's present onto the sorbent material at the face. The gauze pad is removed and may be presented to the canine in order to initiate a search or returned to a laboratory for analysis and/or storage. The use of the STU-100, compared to other methods, is unobtrusive and does not disturb or destroy evidence.

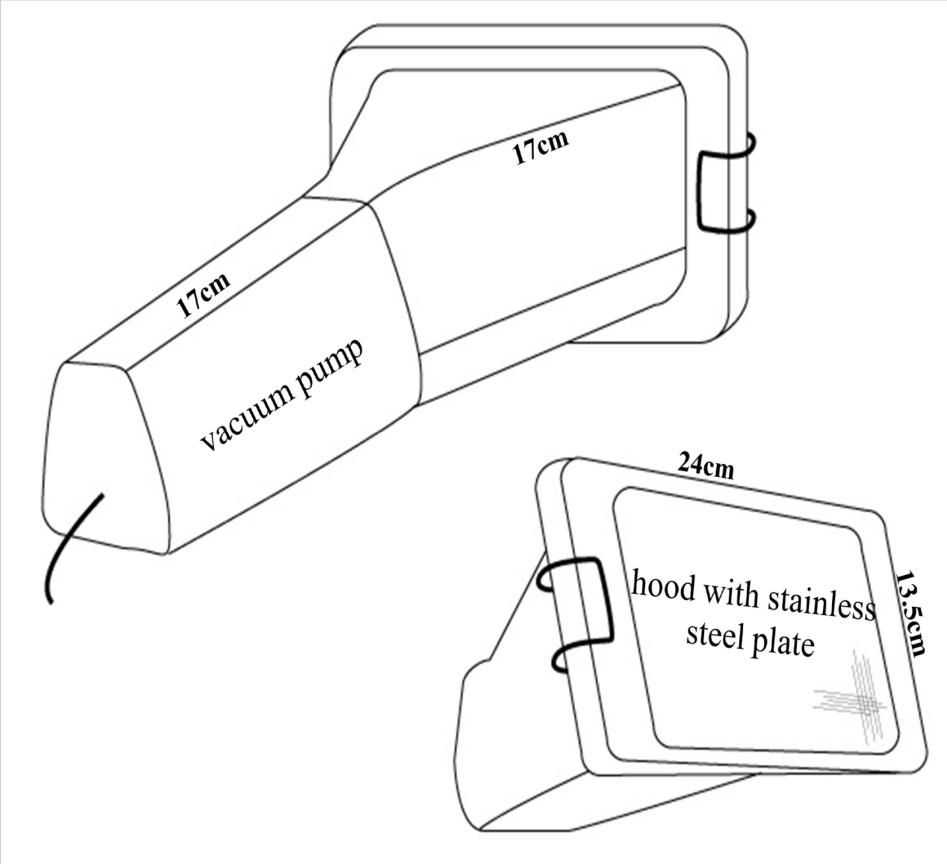


Figure 1. Diagram of Scent Transfer Unit

The STU-100 was developed in the late 1980s by William Tolhurst, a veteran dog handler and trainer. The original, larger scent transfer machine developed by Tolhurst required a 110V power supply and was heavy and cumbersome. That model was revised and reduced in size to the 12V model currently in use, which is lighter and field-ready.[20] It has been employed by hundreds of police and federal agencies in the United States since its patent in 1998.[21] Nonetheless, minimal work has been done to test it analytically or to optimize the device.

The Federal Bureau of Investigation (FBI) and the Southern California Bloodhound Coalition were the first organizations to scientifically assess the STU-100. They first addressed the issue of cross-contamination between samplings taken with the STU-100 by cleaning with isopropanol swabs between consecutive samplings. Following cleaning there was no evidence of cross contamination of the samplings, either analytically or with the canines.[18]

It was further demonstrated that the STU-100 was capable of collecting human scent from post-blast debris: A bomb was detonated and scent pads were collected from the post-blast debris using the STU-100. The scent pads were presented to twelve canine teams, which were asked to trail to the person who had handled the bomb before detonation. Of the twelve canines, 78.3% trailed to the correct person with no false positives.[18]

Curran et al. conducted a similar study using the STU-100 to collect human scent from post-blast debris of a C4 roadside bomb and a peroxide bomb, separately detonated. The explosive devices were handled by a human subject, detonated, and the debris was recovered. The scent evidence was collected from the debris with the STU-100, and the gauze pads were presented to canine teams. All twelve teams trailed correctly for the peroxide bomb, and 72% correctly identified the subject with no false positives; 91% of the canine teams trailed correctly for the C4 device, and 54% made a correct identification, with two false positives.[22] Both research groups demonstrated that trained canines can accurately trail and identify the correct subject from evidence collected with the STU-100, even under unusual or extreme situations.

Eckenrode et al. evaluated the STU-100 analytically to determine the efficiency of scent collection using Johnson and Johnson gauze pads (the same gauze pads used by police agencies and the FBI) for collection. The group evaluated the adsorption and desorption VOCs to gauze pads. The VOC mixtures used were volatiles in drinking water and toxic organic compounds, but not necessarily known human scent compounds. To evaluate desorption of VOCs from the pads, the VOC mixture (volatiles in drinking water) was injected into the middle of the pad and sealed in a Tedlar bag. Compounds were released into the headspace of the bag during a period of equilibration. The headspace was removed from the bag and analyzed using GC/MS. The gauze pads successfully released the VOCs into the headspace.[23]

To evaluate adsorption, a gauze pad was placed onto the STU-100. Samples were collected at the highest vacuum flow-rate setting from a stream of gas containing VOCs (toxic organic compound mixture). The pads were removed from the STU-100 and placed into Tedlar bags and allowed to equilibrate. The headspace was collected and analyzed. Fifteen of 39 target compounds were detected suggesting that the high flow-rate reduced collection efficiency,[23] though multiple flow-rates were not tested. While desorption and adsorption properties of the collection material were addressed, STU-100 performance itself was not evaluated, nor were evaluations conducted using known human scent compounds.

Fletcher evaluated STU-100 flow rate and collection materials using ten known human scent compounds of different functional groups. High and low flow rates were evaluated, along with several collection materials including Johnson and Johnson gauze

pads, cotton pads, and a carbon-coated foam. Analytes were delivered to the STU-100 using an air pump. The collection material was placed into a Tedlar bag where it was allowed to equilibrate before it was concentrated and analyzed. Results showed that the low flow-rate yielded the highest analyte recovery, and the gauze out-performed the other collection media. Yet, even under optimal conditions, only five of the ten analytes were detected.[24] Since only the highest and lowest flow rates were evaluated, it would also enhance the research to evaluate a middle flow rate and to sample with the vacuum off (i.e., no flow rate). Finally, alternative non-cotton-based materials should be assessed.

Other studies using the STU-100 were performed to evaluate the best collection material and flow-rate for sampling: Prada et al. used actual human-hand odor samples to evaluate the STU-100. It was determined that collection with the STU-100 yielded less total mass and variety of compounds compared to direct contact methods.[19] Hudson conducted controlled experiments using standard compound samples spiked onto stainless steel bars to simulate hand odor. The ability of the STU-100 to collect samples at different flow rates and with different collection materials were compared. Three materials (King's cotton, Dukal gauze, and Johnson and Johnson gauze) and a variety of flow rates were compared. There was no significant difference among flow rates for the Dukal and Johnson and Johnson gauze, however, the higher flow rates for the King's cotton collected significantly greater mass. It was not documented whether all compounds, or a select few, were collected at each flow rate and with each material.[25]

In summary, the further analytical evaluation of the STU-100 using a wider variety of sorbent materials and flow rates is necessary. Also, an improved the method of

standard compound delivery would be beneficial, since human scent VOCs do not necessarily flow from the body and thus using an air pump as a VOC delivery system is not be realistic, nor is spiking compounds directly onto the collection material. A static method of standard compound delivery would be more realistic. It is also necessary to use continue standard compounds during the evaluation, as opposed to actual human samples as standards are more easily controlled. Additionally, the application of the STU-100 as a forensic tool should be evaluated for uses beyond the collection of living human scent for canines.

Collection Materials

Volatile organic compounds, such as those in human scent, tend to be preserved well in textiles because of their porous nature, but the extent of the ability to trap or release VOCs depends on type of material used.[16] In the United States, the FBI uses Johnson and Johnson gauze pads, a blend of cotton with rayon and polyester. Other scent-collection protocols use alternative collection materials to collect and store of human scent. The Dutch National Police use King's Cotton, a pure cotton gauze, while other research studies with human scent canines have used Dukal cotton gauze.[26,27]

The materials to be evaluated in this study include two gauzes: Dukal cotton gauze, Johnson and Johnson gauze (a cotton blend), as well as three pure, non-dyed, one-ply materials: cotton, rayon and polyester.

Cotton

A single cotton fiber consists of a single cell grown from the epidermis of the cotton seed. As many as twenty seeds are found inside each seed-pod, or boll. At maturity, the boll opens to expose the mass of cotton fibers, which can be as many as 150,000 or more fibers per boll.[28] Each fiber is mostly of cellulose, typically 94%, but the cellulose content may vary as a result of variations in soil, growing climate, and cotton varietal.[29] The remaining 6% of the fiber is composed of proteins, pectic material, minerals, and organic molecules. Most of the non-cellulosic material, however, is removed during processing.[28] The basic structure of cellulose is the $C_6H_{10}O_5$ unit, or D-glucose. The cellulose polymer is a linear chain of β-glucose linked at the 1 and 4 positions (Figure 2).[30]

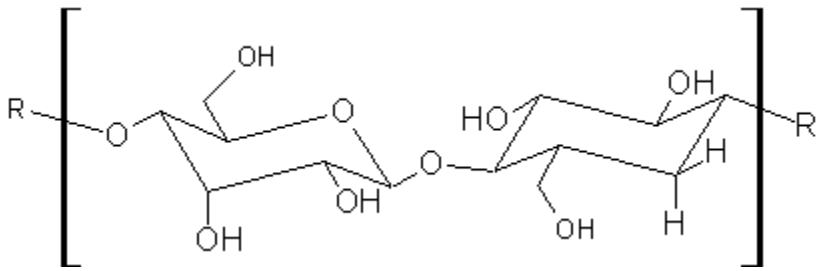


Figure 2: Molecular structure of cellulose.

The outermost region of the cotton fiber is the cuticle, which protects the fiber from oxidation and is made primarily of waxes and pectic material. Immediately inside the cuticle is the primary wall laid during the first phase of growth. The primary wall is composed of fine cellulose fibrils, and it is within the mesh of these fibrils that impurities are found, including pectic and fatty substances. A secondary wall is laid during the second phase of growth and consists of many cellulose fibril layers laid upon the inside

of the primary wall. At maturity, this wall makes up 90% of the total mass of the fiber. In the middle of the secondary wall is the lumen, an opening that runs through the length of the fiber.[30]

Rayon

Rayon refers to any fiber made of regenerated cellulose, of which there are there are three main types: viscose, cuprammonium and saponified cellulose acetate. Viscose rayon is made from cotton lint or wood pulp.[31] To produce viscose, the raw material is soaked in alkali, which dissolves the cellulose, and then is treated with carbon disulfide (Figure 3). Because of the similar cellulosic backbone, the chemical properties of cotton and rayon tend to be similar.[30]

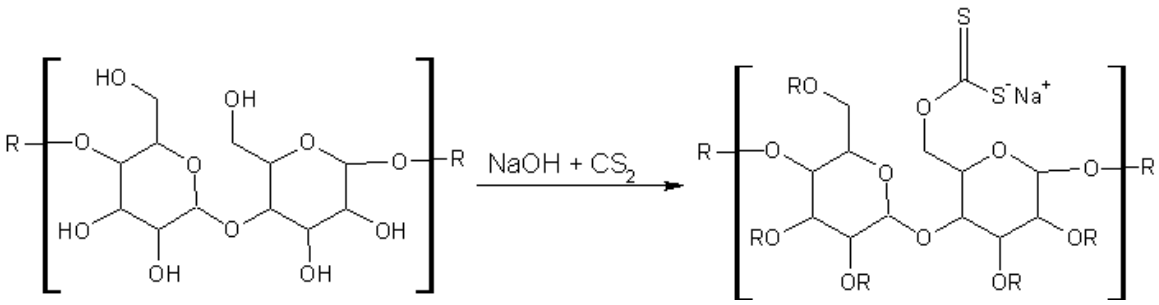


Figure 3: Synthesis of rayon.

Polyester

Polyester is a synthetic, long-chain, polymer fiber. By definition, to be considered polyester, the polymer must be 85% by weight of a substituted ester,[31] hence the name "poly" and "ester." The polyester polymer is formed from an esterification reaction between a bifunctional carboxylic hydroxyl group and a bifunctional alcohol hydroxyl group (Figure 4). In order for the polymer chain to be linear, it is required for the precursors to be bifunctional. If it is not linear, the polymer will no longer have fiber quality. The most common starting products to produce polyester are ethylene glycol and terephthalic acid or dimethyl terephthalate.[32]

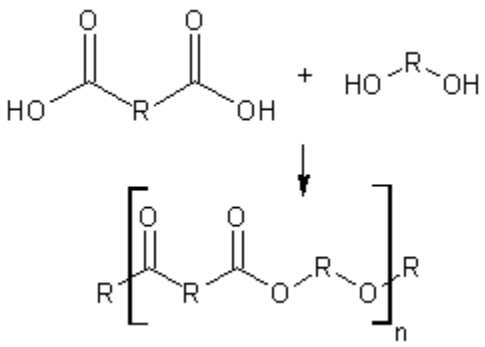


Figure 4: Esterification process to yield the polyester polymer.

The choice of collection material is important because the material may affect the trapping or releasing of the compounds being analyzed. Hudson spiked compounds onto Johnson and Johnson gauze, Dukal gauze, and King's cotton to determine the ability of the material to release the compounds.[25] The mixture of compounds spiked onto the

materials included a variety of functional groups previously documented in human scent. It was determined that significantly more total compounds were released by the Johnson and Johnson gauze than by Dukal gauze or King's cotton. Additionally, significantly greater amounts of esters and aliphatics/aromatics were released than ketones and alcohols. It was suggested that ketones and alcohols were found in lesser quantities because they were not released as readily from the pure cotton materials (King's and Dukal) given the polar-polar interaction between the compounds and the cellulosic backbone of the cotton material. Johnson and Johnson material, on the other hand, is not made entirely of cellulosic material so polar compounds may be more readily released. Hudson observed comparable results with human hand odor samples.[25]

Prada et al. compared additional classes of materials (cotton, polyester, rayon, and wool) for sample collection using actual human hand samples. They recovered a greater variety of functional groups from the samples collected with cotton and rayon as compared to polyester; polyester, however, appeared to be optimal for collecting acids. No standard compounds were sampled to verify results.[19] Further studies should be done using delivery of static standard classes of compounds in a controlled setting.

1.3.2. Scent Delivery

Controlled Odor Mimic Permeation Systems (COMPS) are used to deliver standard compounds in a reliable and reproducible manner at controlled rates to a canine or to a dynamic airflow collection device. Harper developed COMPS as canine training

aids for explosive and drug detection canines. Target drugs or explosive odors were spiked onto sterile gauze and placed into a permeable, polymer bag and heat sealed. The target odors diffused through the plastic membrane at a known and reproducible rate to be used as training aids for a canine.[33] In this research, the COMPS contained compounds previously reported human scent VOCs, and are used not as canine training aids, but as a means of introducing a flow of compounds to the STU-100 at controlled rates.

1.3.3. Scent Analysis: SPME-GC/MS

Solid phase microextraction (SPME) with gas chromatography/mass spectrometry (GC/MS) has been widely employed since the early 1990s for collection live human scent and human remains volatiles. Many researchers have used a collection medium such as gauze or an article of clothing for their collection research.[19,26-34-36] Researchers collected odor onto a medium, then placed the medium into a sealed container. The headspace in the container and could be sampled using SPME.

Other novel approaches for human scent collection using SPME have been designed. Gallagher et al. and Ostrovskaya et al. placed glass funnels over the skin of the arm, then used SPME to sample the volatiles emanating directly from the skin.[37,38] Zhang et al. designed an active sampling device into which one's hand and forearm are placed. Air from the chamber containing the hand was passed to a second chamber where SPME was used to collect the volatiles.[39]

SPME-GC/MS has also been used on several occasions to collect VOCs from human remains. Hoffman et al. used SPME-GC/MS to characterize the VOCs in the

headspace of decomposing human tissue and blood.[39] Awan et al. used on-fiber derivatization to recover cadaverine and putrescine from samples of rotten meat, vegetables, and cheese.[40]

Solid Phase Microextraction

The implementation of solid phase microextraction or SPME was first published in 1990 by Arthur and Pawliszyn. SPME was conceived as an alternative for classical solid phase extraction (SPE). Solid phase microextraction is advantageous because it eliminates the use of solvents and minimizes extraction time compared to SPE. The SPME fiber was designed to fit easily into a gas chromatography injector port without modification.[41] It is suitable for the extraction of analytes from any clean aqueous sample containing volatile or semi-volatile analytes or from analytes in the headspace of any solid or liquid sample. SPME can be used for a large range of analyte concentrations, from parts per billion (ppb) to low parts per hundred (pph) ranges;[42] however, highly polar and ionic analytes cannot be extracted without derivatization.[43]

The SPME apparatus consists of the fiber and holder. The fiber is made of fused silica coated with an absorbent polymer attached to a thin metal rod and protected by a metal sheath to prevent the coating from being stripped when not in use. The metal rod is attached to a spring, and the whole is secured inside a holder resembling a modified syringe (Figure 5).[42]

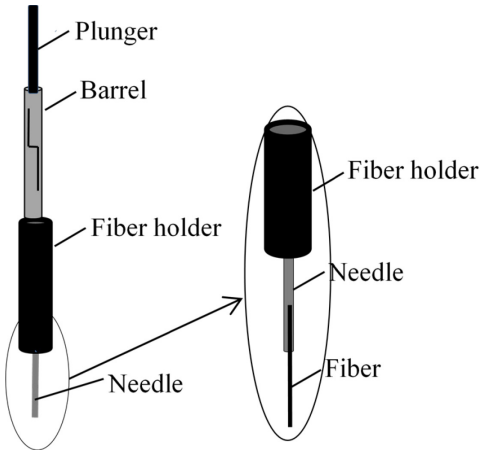


Figure 5: Solid phase microextraction (SPME) apparatus.

Extraction of analytes using SPME is a two part process. First, the fiber is introduced to the sample, the sample analytes partition between the fiber coating and the sample, the fiber is then removed from the sample, and is placed directly into the injection port of the instrument where the analytes are desorbed with gas chromatography. The analytes are thermally desorbed into the injector port, or using HPLC, the analytes are desorbed by a solvent.[42]

In thermal desorption, the high temperature of the injector port decreases the partition coefficient between the fiber coating and the carrier gas so the fiber can no longer retain analytes. The carrier gas then assists in removal of analytes and transports them to the column for separation.[42]

The fiber either can be immersed directly into an aqueous sample or into the headspace where it is left for a pre-determined amount of time while the analytes are extracted onto the fiber. During extraction, the equilibrium between three phases must be considered:

Between the fiber and the headspace: $K_{fh} = C_f/C_h$ Eq. 1

Between the headspace and the solution: $K_{hs} = C_h/C_s$ Eq. 2

Between the fiber and the solution: $K_{fs} = C_f/C_s$ Eq. 3

where K is the distribution constant and C is the concentration of the analyte in each phase. The recovery of analytes is proportional to the overall equilibrium of the three phases and represented by:

$$C_o V_s = C_h^{\infty} V_h + C_s^{\infty} V_s + C_f^{\infty} V_f \qquad \text{Eq. 4}$$

where C_0 =initial concentration, V = volume, and C^{∞}= equilibrium concentration.

Then the number of moles of analyte in the fiber coating, n_f, is given by the following:

$$n_f = \frac{K_{fs} V_f V_s C_o}{K_{fs} V_f + K_{hs} V_h + V_s} \qquad \text{Eq. 5}$$

The terms in the denominator represent the analyte capacity for each of the three phases.[42]

The previous equations suggests that increasing the volume of fiber coating by increasing either the thickness or length of the fiber will improve its sensitivity, however this will also affect the ability of the analytes to move into and out of the coating which, in turn, increases the equilibration time. The best approach for improving sensitivity is by increasing the K_{fs} term by selecting the appropriate fiber coating for the system being sampled or by altering the extraction temperature. Increasing the extraction temperature increases the sensitivity for high boiling compounds but lowers the sensitivity of low boiling compounds, and may also affect K_{hs}.[42]

There are a number of fiber coatings from which to select depending on the analyte(s) of interest. Currently, SUPELCO is the sole manufacturer of SPME fibers. Fiber coating phases available are included in Table 1.

Table 1: SPME fiber coatings commercially available by SUPELCO.[44]

Phase	Analytes
Polydimethylsiloxane (PDMS)	Non-polar
Polyacrylate (PA)	Polar
Carboxen/Polydimethylsiloxane (CAR/PDMS)	Highly volatile analytes
Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	Moderately polar, amines, alcohols
Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)	General use, C3-C20 or MW 40-275
Carbowax (CW)	Moderately polar

Polydimethylsiloxane (PDMS), polyacrylate (PA), and Carbowax (CW) are liquid phases, while divinylbenzene (DVB) and carboxen (CAR) are porous particles that must be suspended in one of the liquid phases. The most common nonpolar phase fiber coating is PDMS and it is available in three thicknesses; the thicker phases extract a greater number of analytes but require a longer extraction time. Thicker phases are recommended for analysis of larger analytes. Polyacrylate (PA) and Carbowax (CW) are more polar phases. Polyacrylate is a solid at room temperature, making the movement of the analytes between the sample and the fiber coating slower, thus increasing extraction times and requiring higher temperatures for extraction. Divinylbenzene (DVB) is a porous particle that retains analytes in its pores. The addition of DVB to CW increases the polarity of the coating, while the addition of DVB to PDMS allows for better retention of small analytes. Carboxen (CAR) is also a porous particle, and when

combined with PDMS, results in a bipolar phase with improved retention of small analytes.[42]

Gas Chromatography and Mass Spectrometry

Chromatography is the separation of an analyte mixture based on the distribution and affinity of the analytes between two phases, a mobile phase and a stationary phase. When a sample is introduced into a chromatographic system, the sample components are transported by the mobile phase. As they move through the system, the sample components come into contact with the stationary phase and partition between the stationary and mobile phases. While the components are in the stationary phase, they are no longer moving forward. The amount of time an analyte spends in either phase is related to its affinity for that phase. Gas and liquid chromatography are both common today. Liquid chromatography (LC) uses a liquid mobile phase while gas chromatography (GC) uses a gas mobile phase.

There are two types of gas chromatography: gas-solid chromatography (GSC), which has a solid stationary phase, and gas liquid chromatography (GLC), which has a liquid stationary phase. GLC may be carried out either in a packed column with liquid-coated particles, or an open tubular column with liquid coated walls. Of these, GLC with an open tubular column is most widely used today for analysis.[45]

The gas mobile phase must be inert as to not influence sensitivity, nonflammable, and inexpensive. Hydrogen, helium, and nitrogen are most commonly used as the mobile

phase. Nitrogen yields the most efficient separation because of its higher molecular mass and low diffusion coefficient; however, runs times with nitrogen tend to be longer because of the low velocity required. Hydrogen may be used, though it is flammable and reactive, thus extra precautions are required. Helium is used in most cases because it lacks these drawbacks cited with the other gases.[45]

The GC stationary phases are cross-linked and bonded to the wall of a column so they are not eluted with the mobile phase. An optimum stationary phase should have a low vapor pressure, be thermally and chemically stable, and have a low viscosity. Phases may range from non-polar to polar. Polar phases have functional groups that specifically interact with analytes. The elution order of analytes from the column depends on the volatility of the analytes and their interactions with such functional groups. Non-polar phases have no functional groups to interact with the analytes; instead they are retained by dispersive forces and will be separated by volatility, thus those with similar boiling points cannot be separated.[45] An example of commercially available stationary phases are given in Table 2.

Table 2: Commercially available GC stationary phases.[43]			
Phase	**Name(s) (Agilent)**	**Polarity**	**Use**
100% Dimethyl-polysiloxane	DB-1, DB-5	Least polar	Bp separations for solvents and petroleum products
5% Diphenyl-95% dimethyl-polysiloxane	DB-5.625, DB-5ht	Nonpolar	Bp separations for aromatics, and environmental samples
20% Diphenyl-80% dimethyl-polysiloxane	----------	Slightly polar	Volatile compounds
35% Diphenyl-65% dimethyl-polysiloxane	DB-35	Intermediate polarity	Pesticides, PCBs, amines
14% Cyanopropylphenyl–86% dimethyl-polysiloxane	DB-1701	Intermediate polarity	Pesticides, PCBs, alcohols, oxygenated compounds
100% Trifluoropropylmethyl-polysiloxane	DB-210, DB-200	Selective for lone pairs	Environmental samples, solvents, freons
50% Diphenyl-50% dimethyl polysiloxane	DB-17, DB-608	Intermediate polarity	Triglycerides, phthalic acid esters, PAHs
65% Diphenyl-35% dimethyl polysiloxane	----------	Medium polarity	Triglycerides, free fatty acids, terpenes
50% Cyanoprophylphenyl-50% dimethyl-polysiloxane	DB-225	Polar	Fatty acids methyl esters, carbohydrates
Polyethyleneglycol	DB-Wax	Polar	Fatty acids methyl esters, terpenes, acids, amine, solvents
90% Biscyanopropyl-105 phenylcyanopropyl-polysiloxane	----------	Very polar	Cis/trans isomers

When the sample is initially injected into the GC, it must be delivered to the column in reproducible quantities as not to exceed the capacity of the column or the linear range of the detector. The sample must reach the column with minimal spreading and in the same composition it had initially. While samples can be solids, liquids or gases, the solids and liquids must be easily vaporized and be thermally stable.[46]

A split injection is most common, as it prevents overloading of the column and improves peak shape. As its name implies, only a portion of the sample and carrier gas

mixture go to the column, while the rest go to waste. Because a portion of the sample goes to waste, sensitivity is not maximized, and the technique is prone to mass discrimination. When sensitivity is of the greatest importance, as the case of trace analysis, splitless injection may be used.[45]

Separation occurs because analytes rapidly distribute themselves between stationary and mobile phases; this distribution depends on the distribution coefficient, K, which depends on the type of analyte, mobile or stationary phase, and temperature. Analyte separation is given by:

$$K = {C_s}/{C_m} \qquad \text{Eq. 6}$$

where C_s is the concentration of the analyte in the stationary phase and C_m is the concentration of the analyte in the mobile phase. Components in a sample will only be separated if their K values are different. To improve separation between two chemical species, variables should be altered to change the K value for either component. Varying the mobile phase does not affect separation; either the stationary phase must be changed (by changing the column) or the temperature must be adjusted.[46]

When the temperature of the column oven is increased, more solute is in the vapor phase causing the solute to migrate. If the temperature is too high, too much solute will be in the vapor phase, and the separation between analyte peaks will be poor. When the temperature of the column oven is lowered, more solute will be in the liquid phase, which increases separation. If the temperature is too low, little solute will be in the vapor phase and analytes will migrate slower and increase analysis time and broaden peaks.

Isothermal runs may be used for samples with few analytes, but for samples with many peaks, a temperature gradient must be used for optimum peak-shape and separation.[45]

In GC/MS, the GC separates and transports analytes to the mass spectrometer (MS), where the analytes are ionized and identified based on their mass. A mass spectrometer consists of a sample inlet (the GC column in the case of GC/MS), an ionization source, mass analyzers to separate ions, a detector to measure abundance of compound fragments, and a data processor. The MS produces fragment ions and separates such ions, detects the ions and measures their abundance, and then processes the signal.[46]

The first step in mass spectrometry is the ionization of gas-phase molecules, which yields either the molecular ion, M^+, or a radical cation.

$$M + e^- \rightarrow M^{\bullet} + 2e^- \qquad \text{Eq. 7}$$

The radical cation may undergo further fragmentation as a result of the excitation of rotational and vibrational energy levels caused by excess energy. Like-molecules will fragment into predictable patterns as long as the ionization energy remains the same. The fragmentation pattern of a molecule can be used to generate information about the parent molecule's identity and structure. The mass-to-charge ratio (m/z) of each fragment is measured, and the fragments are then plotted as m/z vs. frequency of occurrence / intensity.[43,45]

Electron ionization (EI), chemical ionization (CI), or field ionization (FI) may be used for gas-phase ionization. Analytes must be volatile and thermally stable for all three techniques. Electron impact is the most common ionization technique for organic

samples. It is considered a "hard" technique, meaning it produces a great amount of fragmentation, and the molecular ion is not usually seen. In EI, a heated filament produces electrons that are accelerated toward an anode.[45] The electron beam interacts with outer electrons of analyte molecules causing the molecules to absorb energy and lose an electron. Energy is set at 70eV in all commercial instruments; this energy must remain consistent to get reproducible fragmentation patterns.[43] Only about 10eV is required to ionize the majority of organic molecules, thus the large amount of excess energy produces the large amount of fragmentation seen in EI.[45]

Chemical ionization utilizes exothermic chemical reactions between a reagent gas and the analytes to ionize the analytes. The method CI is considered a "soft" ionization technique; there is little fragmentation of the parent ion, and the molecular ion is commonly present.[43]

Field ionization is another soft ionization technique that uses intense electric fields to ionize analytes. The amount of energy transferred to the molecule is less than 1eV, consequently there is low excess energy and little to no fragmentation.[45]

The mass analyzer separates the fragment ions based on mass-to-charge ratio using electric or magnetic fields. Quadrupole and ion-trap instruments are most commonly used, and are both are derived from the principle of separating ions by the stability of their trajectory as they move through oscillating electric fields. An ion traps uses an oscillating electric field to trap ions by applying a resonant frequency with a circular electrode. The top and bottom of the electrode are capped to trap the ions (Figure 6). The resonant frequency is adjusted to cause ions of a specific mass to be

expelled.[45] Once expelled, the ion moves to the detector. The resonant frequency is adjusted over a certain range to measure all masses within the range of interest.

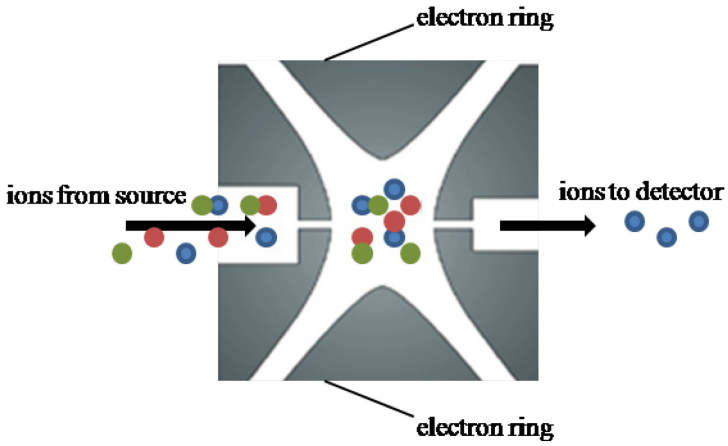


Figure 6: Schematic of ions passing through ion trap.

A gas chromatography instrument routinely is directly coupled to the mass spectrometer by inserting the GC column directly into the ion source. Quadrupole and ion trap instruments can handle 1mL/min and 3mL/min, which is compatible with normal capillary columns for GC.[43] The MS is kept under high vacuum to prevent analyte ions from colliding with gas molecules before they reach the injector. If collisions were to occur, the ion's trajectory would be altered, causing it to hit the wall of the trap or quadruple and lose its charge, and never makes it to the detector. For this reason, the column enters through a series of vacuum-sealed chambers with elaborate pumping systems to go from atmospheric pressure to high vacuum.[45]

2. LIVING HUMAN SCENT RESEARCH

2.1. Introduction

2.1.1. Human scent

Human skin

Human skin is a multilayered organ that performs a variety of the body's functions from protection to maintaining homeostasis. There are three layers of the skin, the epidermis-the outermost layer, the dermis-the middle layer, and the hypodermis-the innermost layer, which work together to carry out all of the functions of the skin. The epidermis is primarily a barrier functioning to prevent foreign bodies from entering the body and protecting it against ultraviolet light. The dermis is the main structural component of skin, and the main site of the vasculature, nerves, and lymphatic systems. The hypodermis, or subcutaneous layer, is mainly used for fat storage.[48]

New skin cells in the deeper layers multiply and push toward the surface and older cells on the surface are replaced by deeper cells, allowing the skin to continually renews itself as it sheds dead cells from the surface. As skin cells are pushed upward, they flatten and decrease in size as they move towards the surface.[14] Once on the skin's surface, cells are shed as rafts.[48]

Structures or appendages in the epidermis perform the skin's many duties. Appendages include hair follicles, eccrine and apocrine sweat glands, and sebaceous glands. Skin secretions produced by the skin glands, include sweat, oil, mucus and other glandular secretions. Eccrine glands release sweat to regulate body temperature. Water

in sweat is carried by blood vessels to the surface of the epidermis, where it is released through the eccrine glands and evaporates to cool the body. Eccrine glands cover the entire body but are concentrated on the forehead, palms, soles of the feet and axilla (armpits).[48] Eccrine sweat contains inorganic ions, lactate, urea, ammonia, amino acids, and proteins. The composition varies among individuals and may be altered because of disease, drugs or diet.[49]

Sebaceous glands are most often associated with hair follicles, and are found all over the body with a concentration on the face, scalp, upper trunk and pubic area. Some sebaceous glands are found independent of the hair follicles in select locations, including the eyelids, the nipples and the genitals. Only the palms of the hands and the soles of the feet completely lack sebaceous glands.[50,51]

Sebaceous glands secrete sebum which is composed of squalene, cholesterol, cholesterol esters, wax esters, and triglycerides. Hair follicles associated with sebaceous glands tend to harbor bacteria that create enzymes to break down triglycerides in the sebum releasing free fatty acids to the skin's surface.[51.52] Sebum from the sebaceous glands is distributed over the body, including the palms and soles, through movement and mixing with other gland secretions.[3]

Secretions from the apocrine sweat glands are most commonly associated with body malodor.[48,49] Apocrine glands are found at the base of hair follicles in limited regions of the body, such as the axilla, nipples, navel and genitals. Women tend to have a greater number of apocrine glands but these glands are more active in men than they are in women.[52]

Sweat from the apocrine glands is secreted in response to emotional stress. Apocrine sweat contains proteins, carbohydrates, and ammonia; it is sterile and odorless when it exits the glands, but when it makes contact with bacteria on the skin, an odor is produced.[48]

The body's secretions provide a warm, moist environment for bacteria to grow and reproduce, which it does so in abundance. The human body is covered with bacteria that is associated with the production of human odor. The greatest abundance of the bacteria is around the face, neck, axilla, groin and feet. Individuals differ in the quantity and type of bacteria on the skin. Bacteria resident on the body includes: *Gram positive cocci, aerobic diphtheroids, acne baccillia,* and *Staphyloccocus aureus*. Additionally the axilla region will house *Staphylococcus albus, Corynebacteria, Aerobacteria aerogenes*, and *Sarcina lutea*.[48]

Production and Transmission of Human Scent

Researchers at the National Institute for Medical Research in London filmed a warm, microbe-filled air current surrounding the human body. In their study, heat produced by the body warms the surrounding air, creating a current of air filled with microbes and dead skin cells. It has been estimated that the body's air current contains four to five times as many microbes as normal air. This air current can escape through clothing. When a glove was placed over the hand, the air flow slowed at first, but then eventually appeared to escape through the glove at a normal rate.[53]

A human regularly sheds about 40,000 skin cells per minute in the form of skin rafts. Each skin raft is made up of one to a few skin cells. Skin rafts are approximately 14 μm in diameter and weigh approximately 0.07 μg. Rafts are flat and rounded, easily lifted from the body and moved by flowing air. The smaller rafts can be carried longer distances by air currents while larger rafts will land closer to the body. Each skin raft can carry up to four microbes.[48] These bacteria decompose the skin secretions into low molecular weight, volatile compounds, generating a vapor[54] that continues to be emitted as long as there are nutrients and moisture available. Theoretically, each individual has a unique collection of bacteria, producing a unique vapor composition, and thus a unique odor.[48] A person's unique odor is deposited in the environment as the air current around the body transports skin rafts and their bacteria through it.[53]

Human Scent Composition

Numerous studies have been conducted to identify the volatile organic compounds of human scent. Depending on the sampling and extraction methods and the area of the body sampled, a vast variety of compounds have been identified. Functional groups identified include many short-to-medium chain and branched aldehydes,[19,35,37,38,55-58] hydrocarbons,[19,35,37,55-57] alcohols,[19,34-36,38,55-57] fatty acids and fatty acid esters,[19,35,38,55,57] and a limited variety of ketones[19,35,37,38,55,57] and nitrogen-containing compounds.[35,55]

Most studies have focused on apocrine-gland secretions in the axilla[26,34,59-62] as they contribute largely to body malodor. It has been suggested that the precursors of odorous compounds are transported to the surface of the skin by carrier proteins where they interact with the microflora in this region to create odor.[50,63] Upon initial collection, apocrine sweat is odorless, however, when sweat is treated with N-acyl-glutamine aminoacylase (from *Corynebacteria*) it acquires a pungent odor. It is believed that the *Corynebacteria* convert longer, branched fatty acids originating from triacylglycerol in the sebum to shorter, volatile fatty acids generating an odor.[64-66] It is estimated that *Corynebacteria* makes up about 80% of microbes found in the axilla region.[52] Males tend to have a greater amount of *Corynebacteria*,[69] and with more active apocrine glands in males, it may explain the stronger body odor generally associated with male perspiration.

Sampling methods and areas have varied. The most common method has been to wipe sweat from an area of the body with gauze or a similar material, then to extract the sweat from the material.[26,34,59-61] Another method has been to extract the sweat directly from a piece of clothing worn by the subject.[54,68] Such methods have tended to recover mostly short-chain fatty acids, in particular, the C_6-C_{11} straight-chain and branched acids,[54,59-61] iso-valeric acid,[54,69] and (E)-3-methyl-2-hexenoic acid[60,61] have been major contributors. The components from chemically extracted sweat samples are not necessarily the VOC's that freely emanate from the body.

Non-axillary odor is produced from different biological processes and is distinct from axillary odor. The warm and moist environment of the palms of the hands creates

an excellent environment for bacteria growth and odor production. The hands of human subjects tend to be more forensically relevant as their skin cells and odor are transferred by touch. A number of studies have sampled VOC's emanating from the hands or forearms and have found that the compounds produced from the hands differ from those of the axilla with fewer acids recovered, and the greater recovery of compounds from other functional groups. Table 3 below includes a selection of compounds commonly seen in such hand-odor studies. Of the many compounds listed in Table 3, tetradecane, octanal, nonanal, decanal, 6-methyl-5-hepten-2-one and dodecanoic acid are most the commonly reported in the published research on this subject.

Table 3. Selected human scent compounds extracted from the headspace of the hands or forearms. Only compounds published in two or more studies are listed below.			
Hydrocarbons	Reference	**Acids**	Reference
Heptane	55,57	Acetic acid	38,55
Nonane	35,55	Propanoic acid	38,55
Decane	37,55	Butanoic acid	19,38
Dodecane	19,35	Hexanoic acid	19,38,55
Tridecane	19,35	Heptanoic acid	19,55
Tetradecane	19,35-37,55,56	Octanoic acid	19,38,55
Pentadecane	19,37,55,56	Decanoic acid	19,55,57
Hexadecane	19,37,55,56	Dodecanoic acid	19,35,38,55,57
Heptadecane	19,55	Pentadecanoic acid	55,57
Octodecane	19,55	Hexadecanoic acid	55,57
Cyclohexadecane	19,55	Heptadecanoic acid	55,57
Heneicosane	19,55	Octadecanoic acid	55,57
Docosane	19,55,57	Benzoic acid	55,57
Toluene	55,57	Lactic acid	55,57
3-Octene	19,55	**Alcohols**	
Benzene, 1,3,5-trimethyl-	19,35	Benzyl alcohol	19,35,36,38,56
Aldehydes		4-Hexen-1-ol	55,57
2-Methylpropanal	55,57	1-Hepten-3-ol	55,57
Heptanal	19,35,55,57	1-Octen-3-ol	38,55
Octanal	19,35,37,55,57	Phenol	19, 36,38,55
2-Octanal	19,35	Phenylethyl alcohol	19,55
Nonanal	19,35-38,55,57	Nonanol	19,55
2-Nonanal	19,35	2-Furanmethanol	19,35
Decanal	19,35-38,55,56	**Esters**	
Undecanal	19,35,36,38	Methoxy acetic acid, dodecyl ester	38
Dodecanal	19,36,38,55	Butanoic acid, methylester	19,55
Benzaldehyde	19,35,38,55	Hexanedioic acid, dimethyl ester	19,35,55
2-Furaldehyde	35,36	Octanoic acid, methylester	38,55
Ketones		Nonanoic acid, methylester	19,35,55
Butanone	55,57	Dodecanoic acid, methylester	19,35
2-Pentanone	55,57	Methylhexadecanoic acid, methyl ester	55,57
3-Pentanone	55,57	Octadecenoic acid, methyl ester	55,57
2-Decanone	19,55	2-Hydroxybenzoic acid, phenylmethyl ester	55,57
6-Methyl-5-hepten-2-one	19, 35,37,38,55,57	Pentanedioic acid, ester	55,57
Geranyl acetone	19,35,38,55	14-Methylpentadecanoic acid, methyl ester	55,57

Uniqueness of Human Scent

Odor can be influenced by many factors including endogenous sources such as diet, disease, menstrual cycle in women, age, living conditions, as well as exogenous sources such as fragrances, soaps, and lotions. The terminology used by the human scent research group at Florida International University describes odor as primary, secondary, or tertiary, depending upon the odor source. Tertiary odor results from exogenous sources such as lotions or perfumes, while secondary odor is derived from endogenous sources such as diet, disease or environment. Primary odor, of interest in this research, is comprised of one's individual odor that is stable over time and is not affected by endogenous or exogenous sources.[26]

It has been known anecdotally for more than 100 years that each human has a unique scent. As early as 1887, Romanes documented, in his famous article in *Nature,* that each person has a unique scent.[70] He wrote:

> "The whole body of a man exhales a peculiar or individual odor which a dog can recognize as that of his master amid a crowd of other persons."

Since then, law enforcement has used canines, most commonly bloodhounds, to locate persons based on a trail of their individual odor.

Studies have shown that trained canines are capable of discriminating between trails laid by different people and locating a single subject when an abundance of distracter odors are present.[71,72] Canines may also be used in a "match-to-sample" situation in which they are asked to match a suspect's odor from a number of different odors. Studies have further proven that canines have moderate-to-high success at

discriminating and matching human odors in such scenarios[73,74,75] even when the odors have been aged for a length of time.[76]

Other experiments with trained canines have been designed to determine if canines discriminate on the basis of genetically-based differences in odor or with environmental factors, such as diet, soaps, detergents, and the like. Harvey et al. and Hepper tested canines' ability to discriminate between monozygotic twins living together and apart and non-related persons.[77,78] The studies of both Harvey et al. and Hepper showed that the canines were not able to discriminate between the monozygotic twins, though they did have success in discriminating between heterozygotic twins. Harvey et al. showed that the environmental cues only slightly increased the canine's ability to distinguish between the monozygotic twins (not a statistically significant increase). Hepper's results, however, indicated that the canines were capable of distinguishing between twins only living apart. Both studies, nevertheless, pointed to a genetic basis for human scent individuality.

Research suggests that the genetic basis for the production of individual odortype may be based on the chromosomal region known as the major histocompatibility complex (MHC). The MHC is a chromosomal region found in all vertebrates and is involved in immunologic recognition. The products of the MHC are proteins that combine with carbohydrates to form glycoproteins, which are typically found in the outer membranes of cell.[79] Genes in the MHC are highly mutatable, yielding a large number of possible alleles and making it highly unlikely for two individuals to possess identical MHC types.[79,80]

Lewis Thomas proposed in 1976 that the MHC is associated with individual odortype. He established that olfactory mating preferences are likely driven by genetic differences in the highly polymorphic H-2 region of the MHC in mice.[80] Thomas's group observed that male and female mice will select only mates of different H-2 types. The mice that were made to be genetically identical, except for differences in the H-2 region, tended to nest together. Conversely, mice that were genetically similar in all manners including identical H-2 regions tended to avoid nesting together. It was also established that male and female mice could be trained to distinguish between mice with differences in the H-2 region.[79]

The highly polymorphic region of the MHC in humans, equivalent to the H-2 region in mice, is known as the HLA (human leukocyte antigen) region. The HLA is known for its importance during human organ transplant, particularly kidney transplants and skin grafts, where it is important for the donor and the recipient to have similar HLA types to prevent the recipient's immune system from attacking and rejecting the transplanted tissue.[79] The HLA region may be associated with human odortype differences. Wobst et al. examined MHC-related differences in human urine, saliva and sweat and showed that MHC molecules could be found on body surfaces associated with these fluids.[81] Eggert et al. analyzed volatiles in human urine by GC/MS and demonstrated some association between odor components and MHC type.[82]

The MHC molecules themselves are too large and their vapor pressure is too low to be odorous. It has been proposed that the MHC molecules found on the body's surface may be broken down by the body's microflora into smaller, more volatile molecules to

create odor[81] or that the larger soluble MHC molecules may be associated with smaller more volatile molecules that are selectively transported through the body to be release as odorants.[81,83,84]

2.1.2. Human Scent Detection Canines

Human scent detection canines are used in a many ways and in multiple settings, including trailing and tracking, search and rescue, evidence search and scent line-ups. They may be trained on generic human scent, as are search and rescue dogs, or on individual human scent, as trailing dogs are.

Search and rescue dogs are trained on generalized human scent, which means they are trained to find the scent of any living person in a given area. These canines find lost or missing individuals by locating a scent and moving in the direction of increasing intensity. They may be trained in one or several specialties, including wilderness searches to locate missing persons in remote areas, disaster search and rescue, avalanche rescue, or water searches.[2]

Tracking and trailing dogs both are trained to follow a trail beginning at the last known location of a person of interest or from a scent article associated with that person. Tracking dogs follow a combination of human odor and ground disturbance keeping their head close to the ground and following the trail footfall to footfall. Trailing dogs may be pre-scented on a specific person or may follow the freshest trail. Trailing dogs follow odor plumes of a specific human scent in addition to ground disturbance odor. They tend

to trail with their heads up, nose to the wind, and do not necessarily follow the trail, footfall to footfall. Trailing dogs are typically pre-scented on a scent object or last known location.[85]

Harvey and Harvey demonstrated the ability of human scent detection canines to accurately trail individual humans through different environments based on scent pads collected using the STU-100. Eight bloodhounds (novice and expert) were used on five different trails. The trails were between 0.5 and 1.5 miles with a "Y" shaped pattern, requiring the canine to make a decision between turning left or right. The trails were aged for twenty-four hours prior to introduction to the canines. The trailing environments included a local park, a college campus and a downtown, urban area, all with a high amount of foot traffic making trail contamination highly probable. Harvey found that 96% of the veteran canines and 77.5% of all canines successfully completed the trials, demonstrating the ability of trained canines to discriminate and follow individual people based on scent.[71]

Some human scent discrimination canines are trained for use in scent line-ups, conducted primarily in Europe. Results of tests by scent discrimination canines in scent line-ups have been used in European criminal investigation and as evidence in court. The basic principle of the test is for the canine to match the odor of a target scent article to that of a suspect from an array of distracter odors.[73]

Two basic systems are used: the "tube retrieving system" in Western Europe (Netherlands, Germany, Denmark, and Belgium) and the "cloth responding system" in Eastern Europe (Poland, Russia, and Hungary). The specific format of the test varies

with jurisdiction. In the tube retrieving system, an object from a crime scene is collected to be used as the scent article. The odors of the suspect and the distracters are collected by having the suspect hold stainless steel tubes in his hands for two to five minutes. A canine is presented with the scent article, and asked to search a line-up of the stainless steel tubes for the matching odor.[73]

With the cloth responding system, a cotton material is used as the scent article. This material is placed on or wrapped around a piece of evidence, and foil is wrapped around it to maximize contact and enhance odor absorption. The odor of the suspect and distracters is also collected by having the suspect hold the cloth for fifteen minutes, the materials are then sealed in glass jars that are placed in holders in a line-up. The canine is presented with the cloth from the scent article and asked to match the odor to that in the scent line-up.[73]

Schoon has scientifically tested the ability of trained canines to accurately complete a scent identification line-up and assessed the validity of the scent identification line-ups as a forensic tool. On the basis of the completion of her analysis, she determined that the scent line-ups surpass the standard for reliability for use as a forensic tool.[74]

As discussed previously, a variety of research has been carried out on the uniqueness of human scent and canines' ability to match human scent; however, further research with human scent canines would enhance the use of these canines in the field and in court. The Scientific Working Group for Dog and Orthogonal Detector Guidelines (SWGDOG) is a group consisting of experts from local, state, federal and international agencies acting to establish best practice guidelines for detector canines. The SWGDOG

subcommittee on research and technology strongly encourages further research to be carried out on a number of topics regarding detection canines and odor, including the collection and storage of human scent and the components of human scent (Appendix 1).[85]

2.2. Materials

2.2.1. Dissipation Experiments

Dissipation experiments were carried out in an open container using three sorbent materials, including Dukal brand, sterile, 2"x2", 8ply gauze pads (DUKAL Corporation Syosset, NY, USA), bleached, desized, mercerized cotton print cloth, and spun polyester type 54, (Test Fabrics Inc., West Pittston, PA). The Dukal gauze was packaged in the form of a 2"x2" square. The other materials were cut to mimic this size and shape. A single piece of Dukal gauze was used, however, three layers of the cotton and polyester materials were utilized in order to prevent the bleeding of the compounds through the material. It should be noted that the mass of a single piece of Dukal gauze approximately equals the mass of three pieces of cotton or polyester material.

The standard compounds commonly found as components of human scent[35] used in this study included decanal, 99%; dodecane, anhydrous, 99+%; geranyl acetone, 96%; nonanal, 95%; methyl caprylate (octanoic acid, methyl ester), 99%; furfuryl alcohol, 99%; 2-furladehyde, 99% (Sigma-Aldrich, Inc., St. Louis, MO, USA); 6-methyl-5-hepten-2-one, 99%; dimethyl adipate (hexanedioic acid, dimethyl ester), 99+% (Acros

Organics, NJ, USA); liquefied phenol, 90% w/w (Fisher Chemical, Fair Lawn, NJ, USA); and tetradecane, 99+% (Aldrich Chemical Company, Inc., Milwaukee, WI, USA). The compounds from the list represent the functional groups associated with human scent compounds: ketones, aldehydes, alcohols, aliphatics and fatty acid methyl esters.

Dissipation of the compounds through permeable bags was measured. The material containing the compounds of interest was sealed into either high density, polypropylene bags, 3”x4”x2MIL or low density, polyethylene bags, 3”x3”x1.5MIL (Veripak, Atlanta, GA). Gravimetric analysis was carried out using a Mettler Toledo XS205 Dual Range balance (Mettler-Toledo Inc., Columbus, OH, USA).

2.2.2. Background Elimination

A human scent collection chamber was designed for odor collection. Several filters were compared to optimize VOC removal, including the Filtrete Air cleaning Filter, Ultra Allergen, 20”x20”x1” (3M Construction and Home, St. Paul, MN), WINIX Replacement Carbon Pre-Filters, and WINIX Replacement HEPA Filter (WINIX Inc., Hoffman Estates, IL). The filters were evaluated using five standard compounds commonly found as components in human scent, including furfuryl alcohol, 2-furladehyde; 6-methyl-5-hepten-2-one, hexanedioic acid, dimethyl ester; and tetradecane.

The air in the chamber was analyzed using solid phase micro extraction (SPME) with 50/30mm divinylbenzne/carboxen on polydimethylsiloxane fibers (SUPELCO, Bellefonte, PA, USA). The compounds were thermally desorbed from the SPME fibers

and analyzed on a Varian CP-3800 gas chromatogram with a Saturn 2000 MS/MS ion trap (Varian, Inc., Walnut Creek, CA, USA). The column used was an Agilent HP-5MS, 30m x 0.25mm (i.d.) with a phase thickness of 0.25mm (Agilent Technologies, Inc., Santa Clara, CA, USA).

Following the evaluation of the filtration system, air samples were taken using the Scent Transfer Unit (STU-100) (Big T LLC, Haw River, NC). Scent samples were collected onto Dukal brand sterile gauze pads. Following sample collection, the gauze was stored in 10mL, clear, screw top vials with PRFE/Silicone septa (SUPELCO, Bellefonte, PA, USA).

2.2.3. Optimization of the Scent Transfer Unit

The collection and release of compounds onto four types of material were compared: Dukal brand, sterile, 2”x2”, 8ply gauze pads (DUKAL Corporation Syosset, NY, USA), bleached, desized, mercerized cotton print cloth, spun polyester type 54, viscose rayon cloth (Test Fabrics Inc., West Pittston) and Johnson and Johnson brand, sterile 2”x2” gauze pads (Johnson and Johnson, Skillman, NJ). For the purpose of cleaning the materials, HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ) was used.

For sampling, the materials were placed into either high density, polypropylene clear bags, 3”x4”x2MIL, or low density, polyethylene clear bags, 3”x3”x1.5MIL. Five standard compounds commonly found as components in human scent were spiked onto

the material before placing the material to be sampled into the permeable bags. These compounds included furfuryl alcohol, 2-furladehyde, 6-methyl-5-hepten-2-one, hexanedioic acid, dimethyl ester; and tetradecane. Samples were collected using the STU-100. Following sampling, the materials were removed from the STU-100, placed into the same 10 mL, clear, screw top glass vials, and analyzed using solid phase micro extraction with gas chromatography/mass spectrometry.

2.2.4. Human Subject Sampling

Scent samples were taken from human subjects using the STU-100. Before sampling, the participants were asked to wash and rinse their hands and forearms with Natural, Clear Olive Oil Soap (Life of the Party, North Brunswick, NJ). The scent samples were collected onto Johnson and Johnson brand gauze and placed into clear glass vials following collection. Extraction and analysis was performed using SPME-GC/MS.

2.3. Methodology

2.3.1. Dissipation Study of Representative Compounds

Open Dissipation

The dissipation rates of eleven standard compounds representing five functional groups were determined by gravimetric analysis (Table 4). Before the addition of the standard compounds, the total mass of the material alone was determined using an analytical balance, then 25 μL of each compound was spiked onto each of the materials and placed into a weigh boat. The materials included a single piece of clean Dukal brand gauze, three layers of cotton material, or three layers of polyester material. Compounds with similar functional groups were grouped together on the same piece of material. A negative control for each material was also created in the same manner. Immediately after adding the compounds to the material, the material was weighed again. The mass continued to be recorded over time, and the amount of compound remaining versus time was plotted. The rate of dissipation was determined by the slope of the line.

Table 4. Compounds used in dissipation experiments.

Functional Group	Compound
Aliphatics	Tetradecane
	Dodecane
Aldehydes	Nonanal
	Decanal
	2-Furaldehyde
Ketones	6-Methyl-5-hepten-2-one
	Geranyl acetone
Alcohols	Phenol
	Furfuryl alcohol
Fatty acid esters	Octanoic acid, methyl ester
	Hexanedioic acid, dimethyl ester

Closed Dissipation

The dissipation rate of each compound from the material was again determined by gravimetric analysis, however the materials were sealed into permeable polymer bags. The mass of both the material of interest and polymer bag were initially measured using an analytical balance, then 25 μL of each compound was spiked onto a piece of material, which were then sealed into separate polymer bags. Each compound was spiked onto the material separately. A negative control was made in the same manner by sealing a clean piece of material into a bag with no compound. The high density, polypropylene bags and low density, polyethylene bags were compared employing the same materials as previously used with the open dissipation experiments. The rate of dissipation was determined in the same manner as in the open air dissipation.

2.3.2. Background Elimination

Description of the Human Scent Collection Chamber

A human scent collection chamber was designed to reduce background contamination during human scent sampling experiments. An enclosure large enough to sample a single human was built in such a way as to utilize positive pressure air flow (Figure 7). A metal cover was attached and sealed securely to the top of the chamber, while the other walls of the chamber allowed small amounts of air to pass. A section of the metal cover was removed and replaced with a grating. A filter was placed over the grating and a forced induction device over that. As clean air enters the chamber, it passes

through a series of filters, removing a portion of the unwanted volatiles from the air. The contaminated air is then forced out through the small openings along the walls of the chamber via positive air flow.

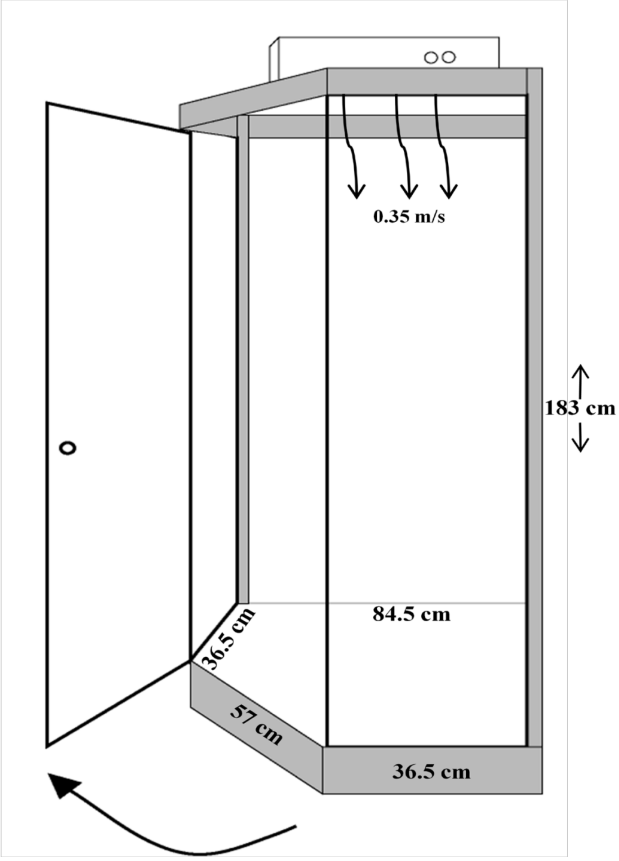


Figure 7. Schematic of Human Scent Collection Chamber.

SPME Exposure Times

It was imperative to determine the optimal exposure time of the SPME fiber in the chamber for further experimentation. Several known human scent compounds were artificially introduced into the chamber using COMPS containing compounds spiked onto Dukal gauze pads sealed into a low density, polyethylene bag. The COMPS were placed

under the forced induction device and over the metal grating (no filter was used). Air from the forced induction device carrying the human scent compounds entered the chamber for thirty minutes. The device was then turned off and the SPME fiber was exposed for a given amount of time. The SPME fibers were held in place by ring stands approximately 18 inches above the floor of the chamber.

Filter Selection

Three types of filters including an air cleaning filter, an activated carbon filter, and a HEPA filter as well as a positive control using no filters were tested to minimize the entry of contaminants into the chamber. The effectiveness of the filters was evaluated using COMPS positioned under the forced induction device and above the filters. As a result of the air flowing from the forced induction device, the scent compounds were forced through the filters and into the chamber. The relative amounts of compound entering the chamber were determined using SPME-GC/MS.

Validation of the Human Scent Collection Chamber

For validation, a series of blank samples were taken inside and outside of the chamber using the STU-100. The STU-100 was run for one minute on the medium airflow setting while collecting the odorants onto Dukal brand sterile gauze. The fan on the chamber was run for six hours prior to collection to remove contaminants from the air

contained inside of the chamber. The odor content was analyzed using SPME-GC/MS, and the type and amount of human scent compounds collected were compared.

2.3.3. Optimization of the Scent Transfer Unit

Though the materials used for odor collection are sterile when they are removed from the package, they still contain human scent VOC's, thus prior to sampling, all collection materials were analytically cleaned with methanol and were baked for 45 minutes at 105ºC. The SPME-GC/MS analysis was used to determine if there were any remaining human scent VOC's on the materials. Materials that were demonstrated to be predominately free of human scent compounds were used for further experimentation.

For the sampling of standard compounds, a cleaned piece of the material of interest was placed on the face of the STU-100 and secured by a stainless steel plate. The compounds were sampled by holding the STU-100 one inch above the COMPS for a given amount of time. All sampling with the STU-100 took place in the human scent collection chamber. The filtration system on the chamber was run for a minimum of two hours before sampling in order to remove any contaminants previously remaining inside of the chamber.

For sample collection, each of four air flow speeds was tested for each of collection material. The flow rates tested were taken from settings on the STU-100 unit and included: high (9), medium (5), low (0) or no air flow. The materials tested included Dukal cotton gauze, Johnson and Johnson gauze (composed of a mixture of rayon, cotton and polyester), mercerized cotton, Dacron polyester and viscose rayon. To determine

whether a greater number of material layers would enhance or diminish scent collection, multiple layers of material were also tested. Layers of the collection material were placed on top of one another and onto the STU-100 for sampling. The rate of air flow through the STU-100 was measured using an anemometer for each material / flow rate combination.

2.3.4. Human Subject Sampling

Odors from four human subjects, two males and two females, were collected using two layers of Johnson and Johnson gauze at low (0) flow rate. The scent samples were taken from the palm of one hand of each subject. Before sampling, the subject was asked to wash his/her hands with a fragrance-free soap and allow them to air dry. The subject sampled him/herself inside of the human scent collection chamber for one minute. The subject was instructed to pick up the STU-100 with one hand and sample the palm of the other. The same hand was sampled for each replicate sample, and the subject was instructed to not touch anything between replicate samples.

2.3.5. Extraction and Analysis

Following sample collection, the materials were immediately removed from the STU-100, placed into 10 mL, screw top vials and allowed to equilibrate over night. Following equilibration, the headspace was sampled for 21 hours using DVB/CAR/PDMS SPME fibers. The compounds were thermally desorbed from the

SPME fibers and analyzed on the GC/MS with a HP-5MS column. The injector temperature was held at 250ºC and the column oven was held at 40ºC for five minutes, and then increased to 250ºC over 23 minutes.

For quantitation, calibration curves were created using 10, 20, 50 and 100ppm solutions of standard compounds in dichloromethane. The concentration v. area counts were plotted for each compound separately. An example is given in

Figure 8.

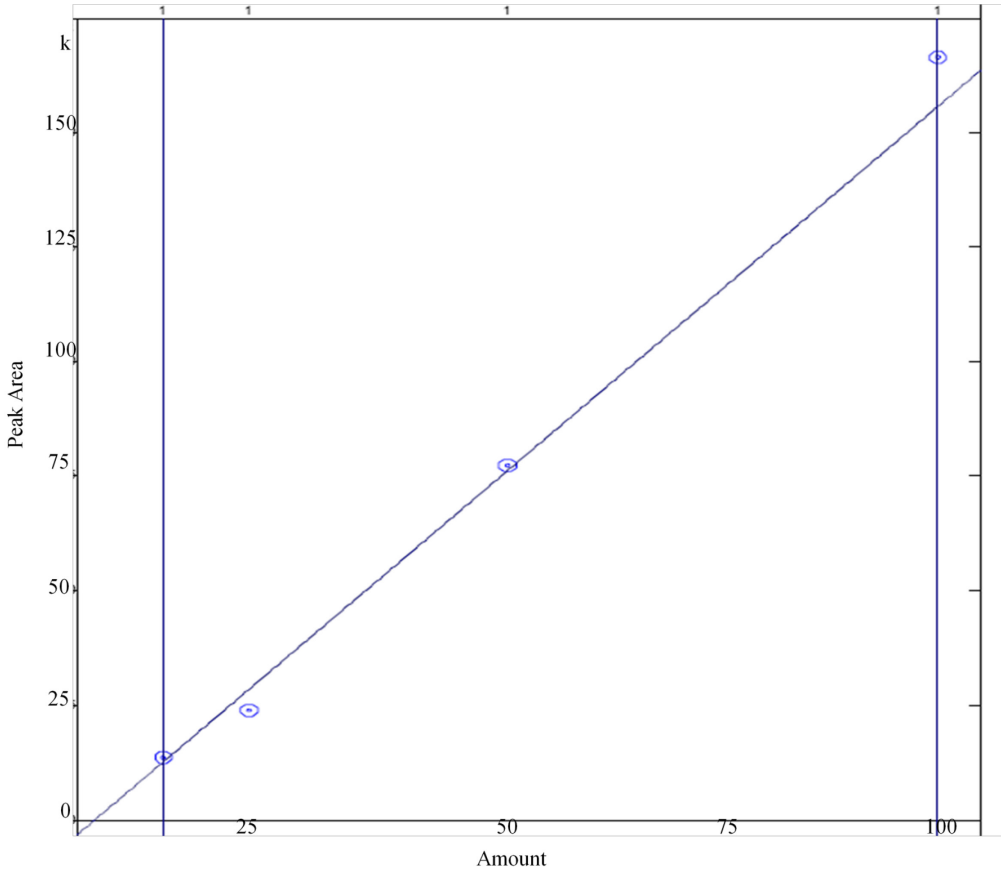


Figure 8. Calibration curve for 6-methyl-5-hepten-2-one. (R^2 = 0.998)

2.3.6. Methods for Statistical Evaluation

The source of variation between samples was evaluated using analysis of variance (ANOVA). The ANOVA is used to test whether altering the controlled factor, such as flow rate or material, produces a significant difference in the amount of compound collected compared to differences found in replicate samples. One-way ANOVA is used to determine whether variation between samples is the result of random error in measurement or to a single controlled factor. Two-way ANOVA is used when two factors are compared during an experiment.[86]

A Student t-test is used to determine whether the difference between two outcomes is the result of random variation or because they are actually different. A paired t-test is used when comparing matched samples and is given by the following equation:

$$t = \bar{d}\sqrt{n}s_d \quad \text{Eq. 8}$$

where $\bar{d}$ is the mean of the differences between the pairs, n is the number of paired results, and s_d is the standard deviation of the differences between the pairs.[86]

Cluster analysis is used to group sets of objects on the basis of overall similarity, and was used to determine dissimilarity of the replicate samples. A cluster tree or dendrogram was created using Minitab 15 Statistical Software (Minitab Inc., State College, PA). The y-axis measures the similarity of the observations on the x-axis, with 100 being exactly the same and zero being completely dissimilar. The observations on

the x-axis were the replicate samples. The more similar the replicate samples are to one another, the more reproducible.[86]

Principle Component Analysis (PCA) was also applied to the data using Minitab 15 Statistical Software to compare the similarity between human subject samples in amount and type of VOCs detected from human subject samples. PCA is used to generate orthogonal variables which reduce large amounts of correlated data by finding linear combinations, or principal components, describing the original variables. The first and second principle components account for a majority of the variation in the data. When the first two principle components are plotted, the axes represent the greatest variation in one direction (principle component 1, PC1) versus the next greatest variation in the other direction (PC2). The resulting plot thus reduces the original data by representing it in only two dimensions, when using the first two components only.[86]

2.4. Results / Discussion

2.4.1. Dissipation Study of Representative Compounds

Open Dissipation

The rate at which particular compounds evaporates from a material was measured using gravimetric analysis. Standard compounds representing human scent compounds were spiked onto different materials and, as evaporation occurred, the amount of compound remaining was plotted against the time in minutes. A best-fit line was fitted to the linear section of each graph. The slope of such line was considered to be the rate of

dissipation which was determined for each functional group and triplicate was averaged (Figure 9a-c). The dissipation rates in mg/min for each functional and material combination are given in Figure 10.

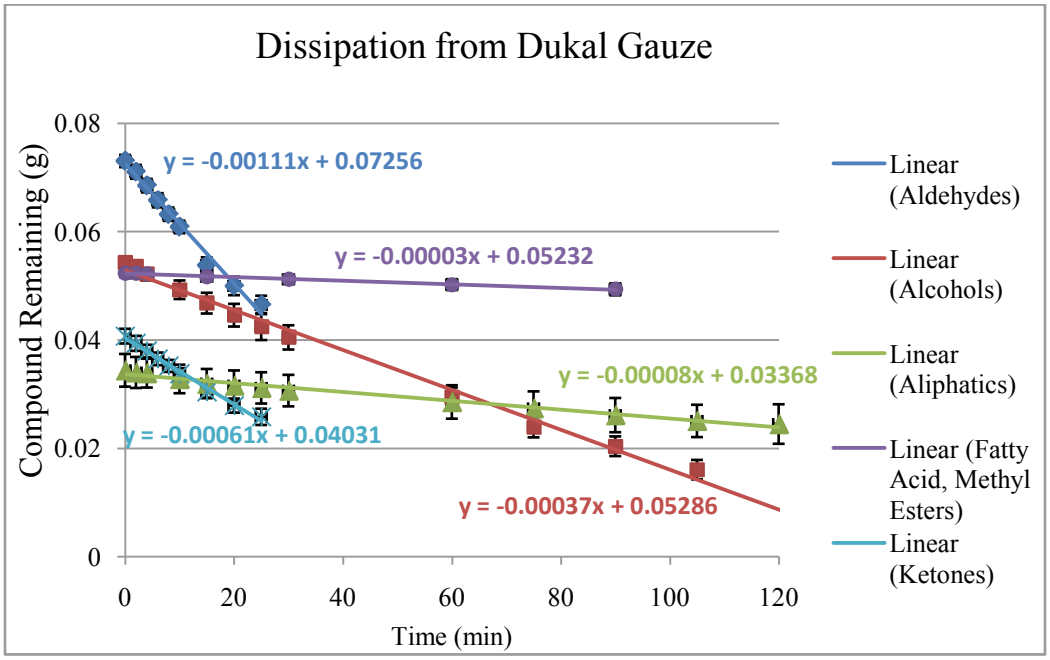


Figure 9a. Dissipation of standard compounds from Dukal gauze in an open container. Standard compounds are separated by functional group. Only the linear portion of the lines are included.

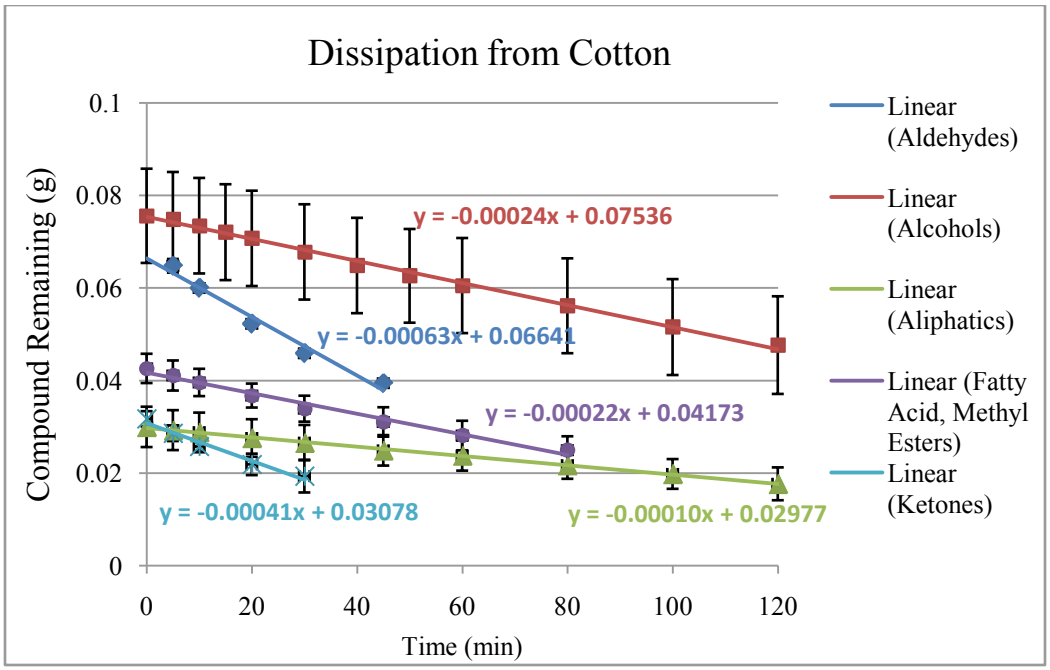


Figure 9b. Dissipation of standard compounds from cotton in an open container. Standard compounds are separated by functional group. Only the linear portion of the lines are included.

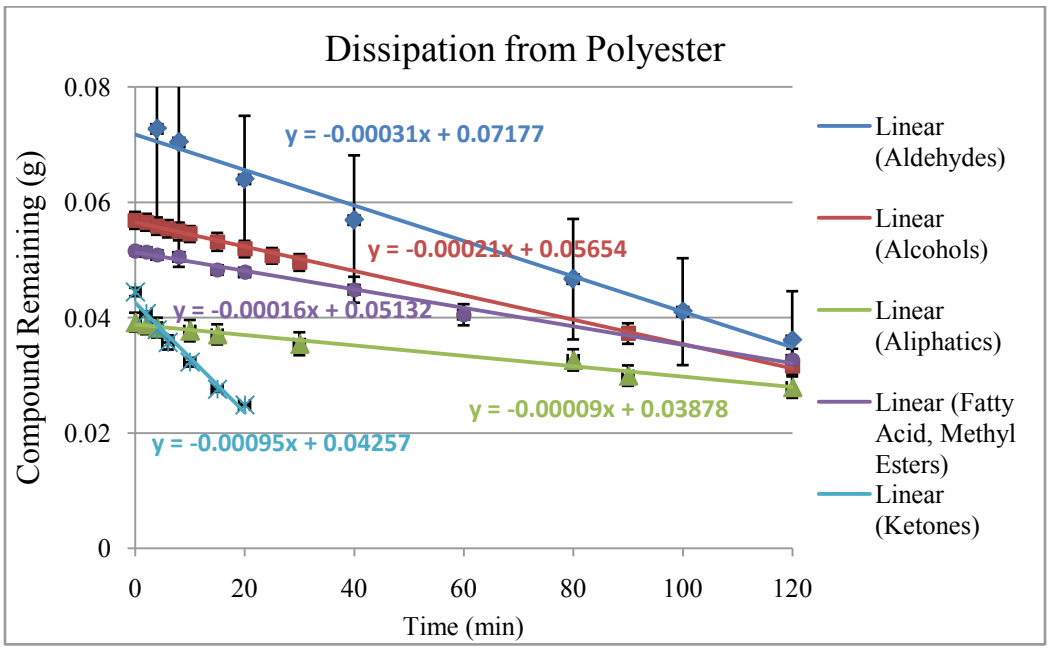


Figure 9c. Dissipation of standard compounds from polyester in an open container. Standard compounds are separated by functional group. Only the linear portion of the lines are included.

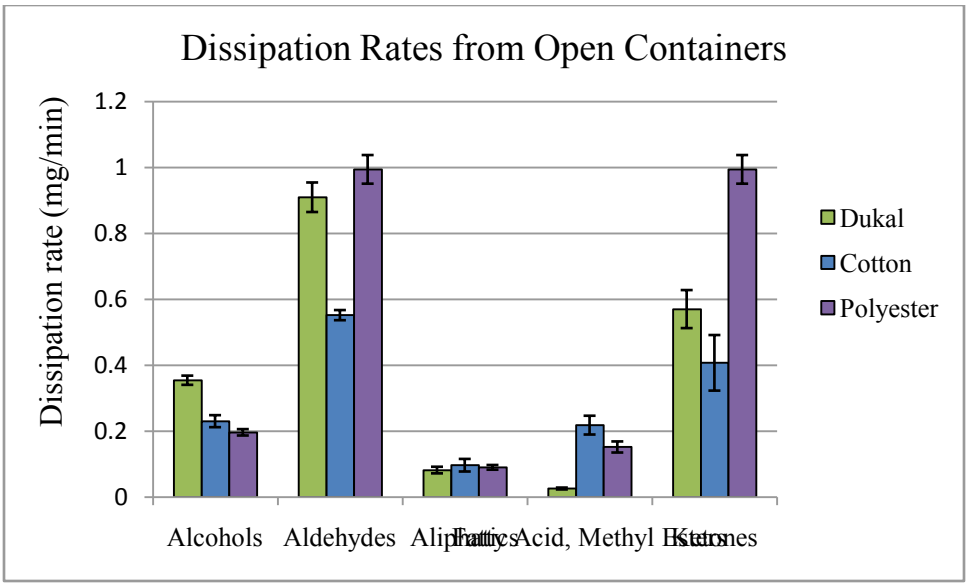


Figure 10. Comparison of the dissipation rates for compounds grouped by functional group from the three materials, Dukal Gauze, polyester material and cotton material.

Two-way ANOVA was performed to examine the affects of material and compound functional group on dissipation rate. It was determined that the dissipation rates were statistically different among the types of materials and functional groups. However, the difference in rates between functional groups was much larger than between materials (variation with functional groups: $F_{calc} = 457.0$, $F_{crit} = 2.690$; variation with materials: $F_{calc} = 20.86$, $F_{crit} = 3.316$). The ANOVA results suggest that the rate of dissipation in open containers is governed more by the volatility of the compound, and is less affected by interactions between the compound and the material.

Closed Container Dissipation / Creation of COMPS

Controlled Odor Mimic Permeation Systems (COMPS) were created to deliver standard compounds to the STU-100 at controlled rates. To ensure constant and reproducible rates of compound released by the COMPS, the dissipation rates were determined by gravimetric analysis. In a second study of dissipation rates, the materials were sealed into permeable bags and the dissipation rates were determined as the compounds evaporated from the materials and through the permeable bags. Initially, dissipation rates were measured for each compound and material combination through 1.5MIL, low density, polyethylene bags (Figure 11). The rates of dissipation were calculated in the same manner as previously discussed and compared with two-way ANOVA. Again, it was found that there was less variation among materials (F_{calc} = 5.993, F_{crit} = 3.493), but great variation among compounds (F_{calc} = 33.51, F_{crit} = 2.348). The ANOVA results indicate that the dissipation rates are governed by the volatility of the compound as well as the compound's ability to pass through the pores of the permeable polymer bags which is influenced by the molecular structure and size of the compound. As it was determined that the type of material has less affect on the rate of dissipation, Dukal cotton gauze was used exclusively, as to simplify the rest of the experiment.

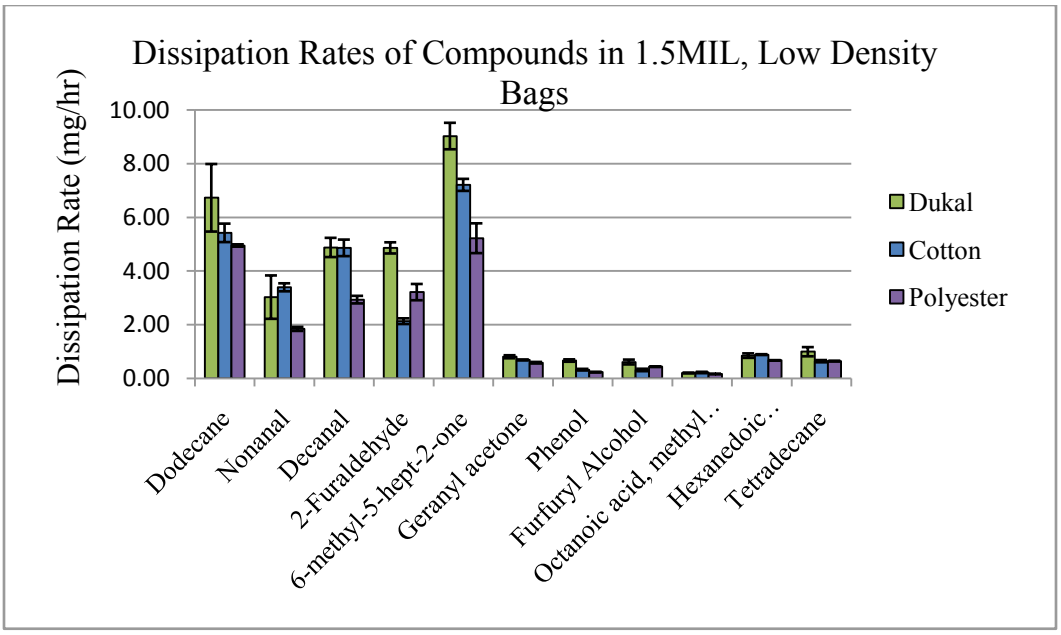


Figure 11. Comparison of dissipation rates of selected compounds from three materials through low density, polyethylene, permeable bags.

The dissipation rates of each compound from the Dukal gauze are listed in Table 5 from the lowest rate of dissipation (octanoic acid, 0.207 mg/min) to the highest rate (6-methyl-5-hepten-2-one, 9.033 mg/min). The difference between the lowest and highest rates was nearly two orders of magnitude. Figure 12 graphically expresses the range of dissipation rates between the different compounds (error bars are contained within the data points for all compounds with the exception of nonanal, decanal and 6-methyl-5-hepten-2-one). For the purposes of future experimentation, it is advantageous to minimize the difference between dissipation rates, making the rates of all compounds as similar as possible. This assures that approximately the same amount of compound is coming from the permeable bag in a given amount of time for any compound, which is valuable during sampling as it minimizes variables.

Table 5. Dissipation rates of standard compounds from low density, 1.5MIL polyethylene bags, listed slowest to fastest.			
	Compound	**Rate (mg/min)**	
1	Octanoic acid, methyl ester	0.20700	**slowest**
2	Furfuryl alcohol	0.61600	
3	Phenol	0.67100	
4	Geranyl acetone	0.81200	
5	Hexanedioic acid, dimethyl ester	0.85467	
6	Tetradecane	1.00000	
7	Nonanal	3.03300	
8	2-Furaldehyde	4.86700	
9	Decanal	4.88300	
10	Dodecane	6.73300	
11	6-Methyl-5-hepten-2-one	9.03300	**fastest**

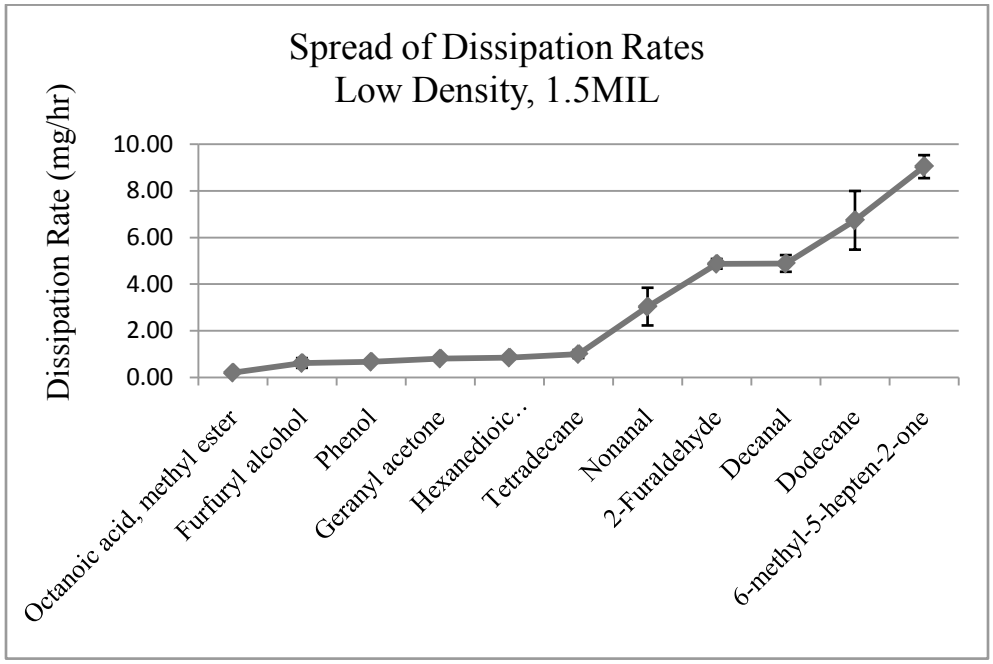


Figure 12. Comparison of dissipation rates of each compound through a low density, polyethylene, permeable bag.

The same method was repeated, however the compounds were spiked onto gauze and sealed into 2MIL, high density, polypropylene bags and 3MIL, low density, polyethylene bags. The type of permeable bag was altered to potentially reduce the differences between dissipation rates of the different compounds. Again, the rates of dissipation were determined and compared (Figure 13). There was no significant difference between the dissipation rates of the compounds through the 1.5MIL and 3MIL low density bags and thus no improvement in the range of dissipation rates. However, for some compounds there were significant differences between the rates through the low and high density bags (Table 6).

Table 6. Outcome of paired T-test (two-tailed) comparing dissipation rates through various permeable bags.

	T_{crit}	T_{calc}	**Significant difference? (yes/no)**
LD,1.5MIL v. LD,3MIL	2.78	2.55	No
LD,1.5MIL v. HD,2MIL	2.57	3.49	Yes

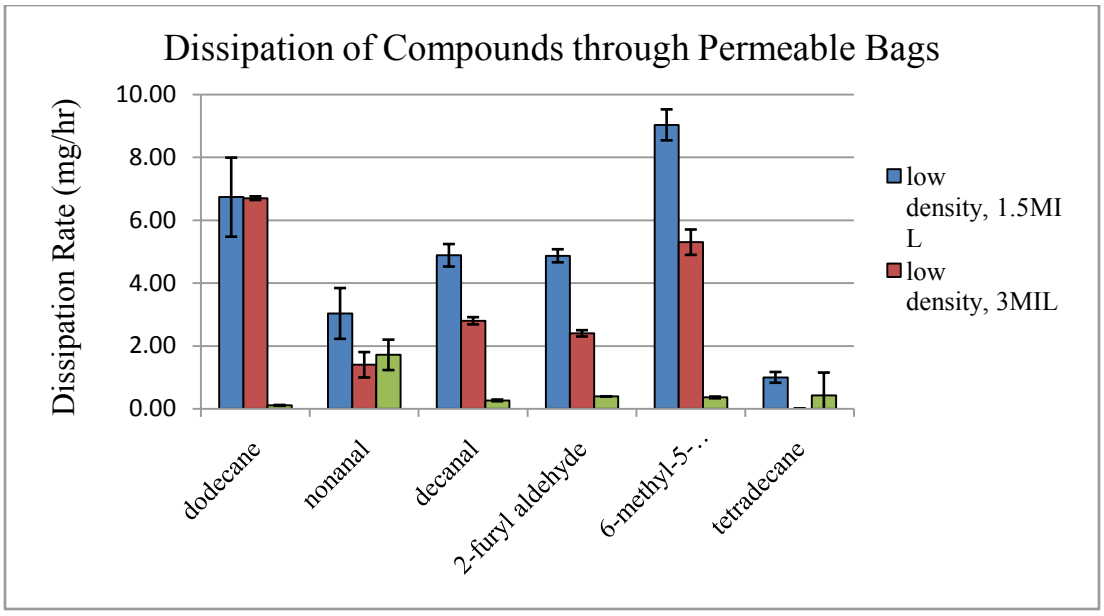


Figure 13. Comparison of dissipation rates of selected compounds through low density, 1.5MIL; low density, 3MIL; and high density, 2MIL permeable bags.

On the basis of the results, the optimal permeable bag was selected for each compound individually. The selection of the optimal bag was based on minimizing of the range of dissipation rates between compounds. The optimal bag for each compound is given in Table 7. Using these permeation conditions, the range of dissipation rates was reduced from nearly two orders of magnitude to less than one order of magnitude (Figure 14) with the exception of nonanal. Nonanal gave particularly high dissipation rates with all three bag types.

Table 7. Dissipation rates of standard compounds from low density, 1.5MIL polyethylene bags, and high density, 2MIL polypropylene bags, listed slowest to fastest.			
Low Density 1.5MIL (LD) or High Density 2MIL (HD)			
	Compound	**Rate (mg/min)**	**LD or HD**
1	Dodecane	0.108	HD
2	Octanoic acid, methyl ester	0.21	LD
3	Decanal	0.267	HD
4	6-Methyl-5-hepten-2-one	0.361	HD
5	2-Furaldehyde	0.394	HD
6	Tetradecane	0.422	HD
7	Furfuryl alcohol	0.616	LD
8	Phenol	0.671	LD
9	Geranyl acetone	0.812	LD
10	Hexanedioic acid, dimethyl ester	0.85467	LD
11	Nonanal	1.716	HD

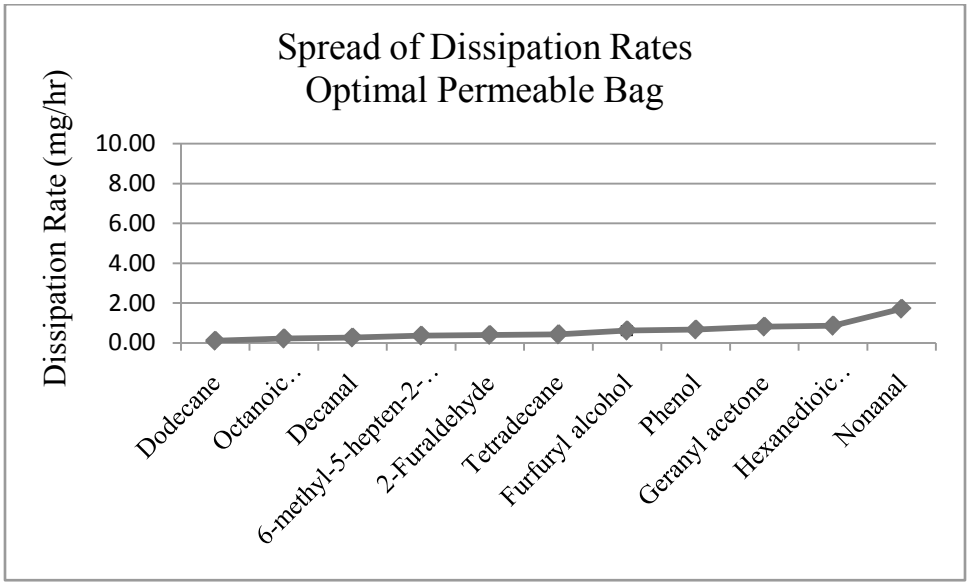


Figure 14. Comparison of dissipation rates through optimal permeable bags (either low density, 1.5MIL or high density 2MIL). **Error bars contained within data points**

In a final attempt to minimize the difference in dissipation rates for future experimentation, a single compound was selected from each functional group. These compounds are given below (8). They have previously been reported in the literature as

human scent compounds and have been shown to have good resolution and sensitivity on the GC/MS system employed (Figure 15).

Table 8. Standard compounds chosen for study.			
Compound	**Functional Group**	**Molecular Weight**	**Literature cited**
6-Methyl-5-hepten-2-one	Ketone	126.2	3,4,5,6,11,16
2-Furaldehyde	Aldehyde	96.08	4,16
Tetradecane	Aliphatic	198.39	4,6,11,16
Furfuryl alcohol	Alcohol	98.1	4,16
Hexanedioic acid, dimethyl ester	Fatty acid, methyl ester	174.19	4,6,16

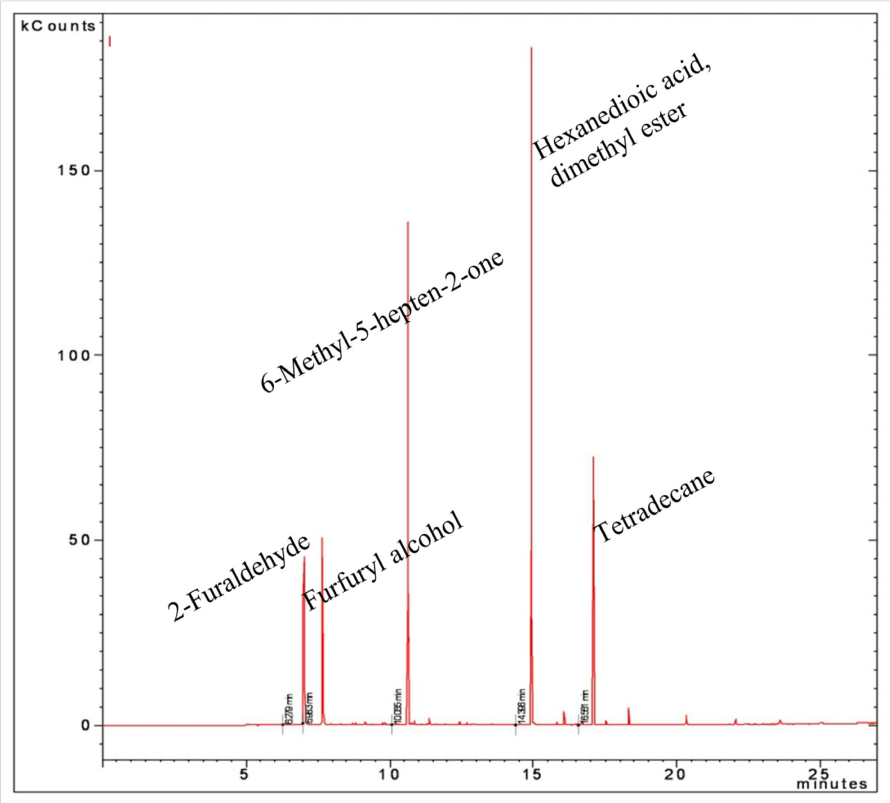


Figure 15. Chromatogram of five standard compounds selected for further experimentation.

2.4.2. Background Elimination

To improve reproducibility during scent collection and decrease background contamination, the human scent collection was designed and optimized. Optimization of the human scent collection chamber was carried out using standard compounds. The standard compounds were enclosed in COMPS bags which were placed outside the chamber and under the forced induction device, forcing the compounds into the chamber.

Fiber Exposure Times

For odor collection from inside of the human scent collection chamber, SPME fibers were placed into the chamber and held in place by ring stands. Volatiles were collected by exposing the fibers for varying lengths of time; 1, 3, 6, 18 and 24 hours. The quantity of each of the compounds collected from inside the chamber for each exposure length are depicted in Figure 16. On the basis of the results a fiber exposure time of 18 hours was used for further analysis.

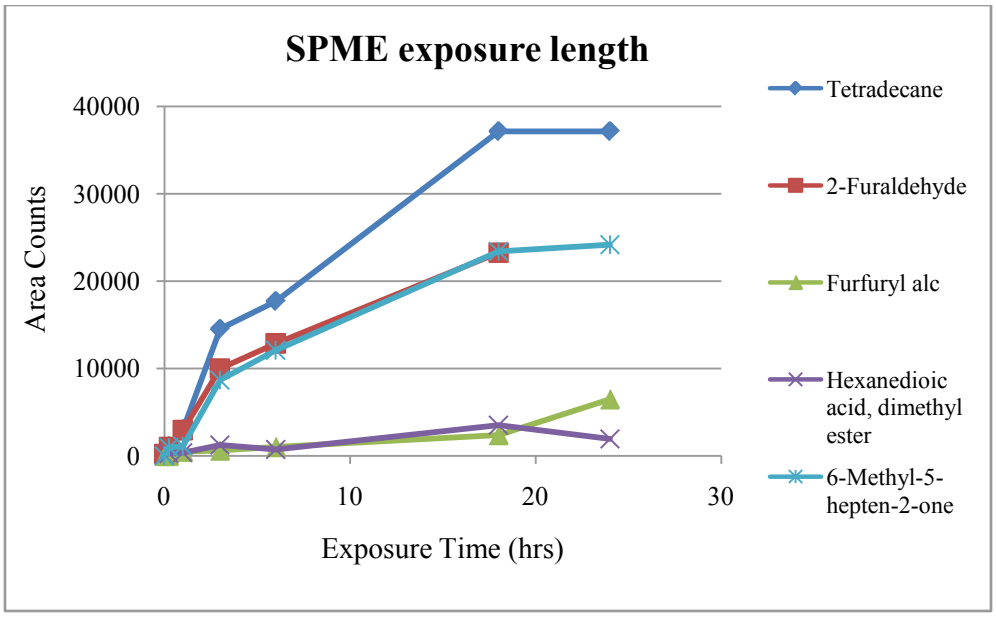


Figure 16. Quantity of compound collected by SPME fibers at varying exposure lengths.

Filter Selection

A series of filters used for the human scent collection chamber were tested and compared based on their ability to remove VOCs from the air passing into the chamber. Three types of filters were tested including a Filtrete air cleaning filter, a carbon filter and a HEPA filter. Standard compounds were used for testing in the same manner as previously discussed. A negative control test was also run using no filter. The best possible filter was chosen on the basis of the results in Figure 17. The amount of scent collected among all treatments differed significantly (Single Factor ANOVA: $F_{calc} = 5.42$, $F_{crit} = 5.14$). The air filter alone reduced a large amount of compounds compared to the control (Single Factor ANOVA: $F_{calc} = 26.8$, $F_{crit} = 5.99$). However, the quantity of furfuryl alcohol was not significantly reduced. The addition of the carbon filter to the air filter further reduced all compounds including a significant reduction in furfuryl alcohol.

The HEPA filter in combination with the air filter actually increased the amount of compounds present in the chamber compared to the air filter alone. This is possibly a result of an abundance of such compounds previously present in the HEPA filter at the time of purchase. Based on these results, the air filter in combination with the carbon filter was used in further experimentation.

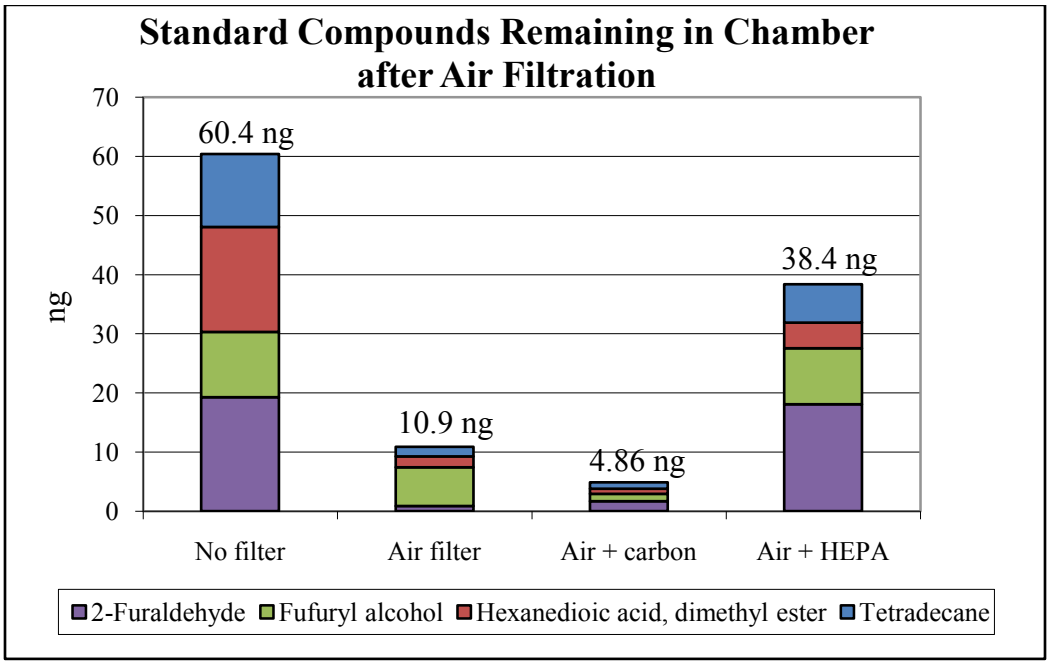


Figure 17. Quantity of selected compounds collected inside of the chamber after passing through a series of filters.

Validation of Human Scent Collection Chamber

The STU-100 was used to collect scent samples from the environment inside and immediately outside the human scent collection chamber. Both sets of samples were taken indoors in a laboratory setting under the same environmental conditions. Before

sampling, the chamber was run on high for six hours using the carbon and air cleaning filters to remove contaminates from inside the chamber. All samples were taken consecutively. The samples taken immediately outside of the chamber contained ten known human scent compounds, as listed in Figure 18, while samples taken inside the chamber in the same fashion contained only six human scent compounds. There was also a significant reduction (approximately 66%) in the total amount of human scent compounds inside the chamber. The human scent collection chamber successfully removed a significant quantity of human scent-related volatiles found in the air and thus enhanced non-contact dynamic airflow sampling by decreasing background contamination.

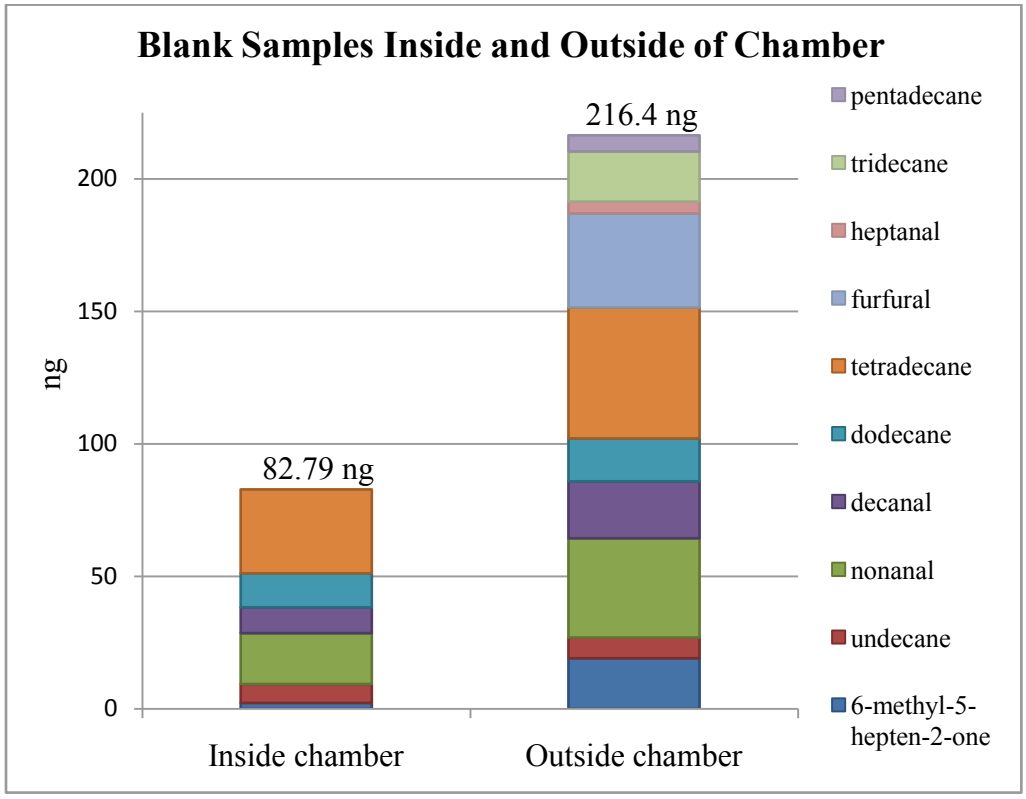


Figure 18. Bar graph representing the total amount of compounds collected inside and immediately outside the Human Scent Collection Chamber.

A cluster analysis was performed to compare the similarity of the volatile background profiles of the same four replicate samples collected both inside and outside the chamber. Figure 19 is a dendrogram representing such analysis. The more similar the profiles of two samples, the lower they are connected in the dendrogram. The most similar samples are Ch1, Ch2, and Ch3, the three samples taken from inside the chamber. While it did not group with the other three, the fourth sample from inside the chamber (Ch4) was far more similar to Ch1, Ch 2, and Ch3 than it was to samples Out1 and Out4 from outside the chamber. Samples Out1 and Out4 were the least similar to the entire group and to each other. The dendrogram indicates that the samples taken inside the chamber were more similar to one another, and thus more reproducible, than the replicates taken outside of the chamber. This confirms the importance of the use of the human scent collection chamber during experimentation indicating that a more reproducible background environment is obtained using the chamber than simply sampling a subject indoors.

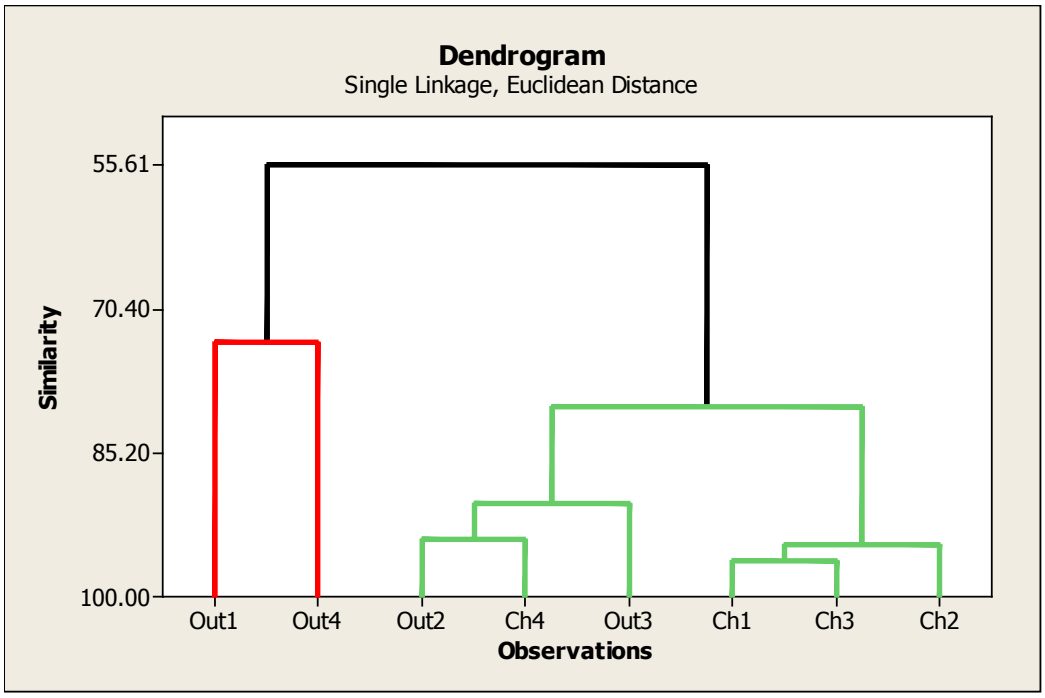


Figure 19. Dendrogram representing the similarity of replicate samples taken inside and immediately outside the Human Scent Collection Chamber.

2.4.3. Optimization of the Scent Transfer Unit

Flow rate / Material

The flow rate of the air drawn past the material and into the STU-100 was measured for each setting/material combination, i.e. flow rates 0, 5, 9 and off with materials polyester, rayon, cotton, Johnson and Johnson gauze, and Dukal gauze. Macroscopically, the material making up the Dukal gauze has the most open weave, followed by the polyester and rayon materials which have relatively open weaves, the cotton material which has an intermediate weave, and then Johnson and Johnson which has the tightest or closest weave (Figure 20). In Figure 21, a pattern can be observed relating the weave of the material to the obstruction of airflow into the STU-100. The

Johnson and Johnson gauze impeded the airflow most effectively while Dukal gauze allowed for the greatest airflow into the STU-100.

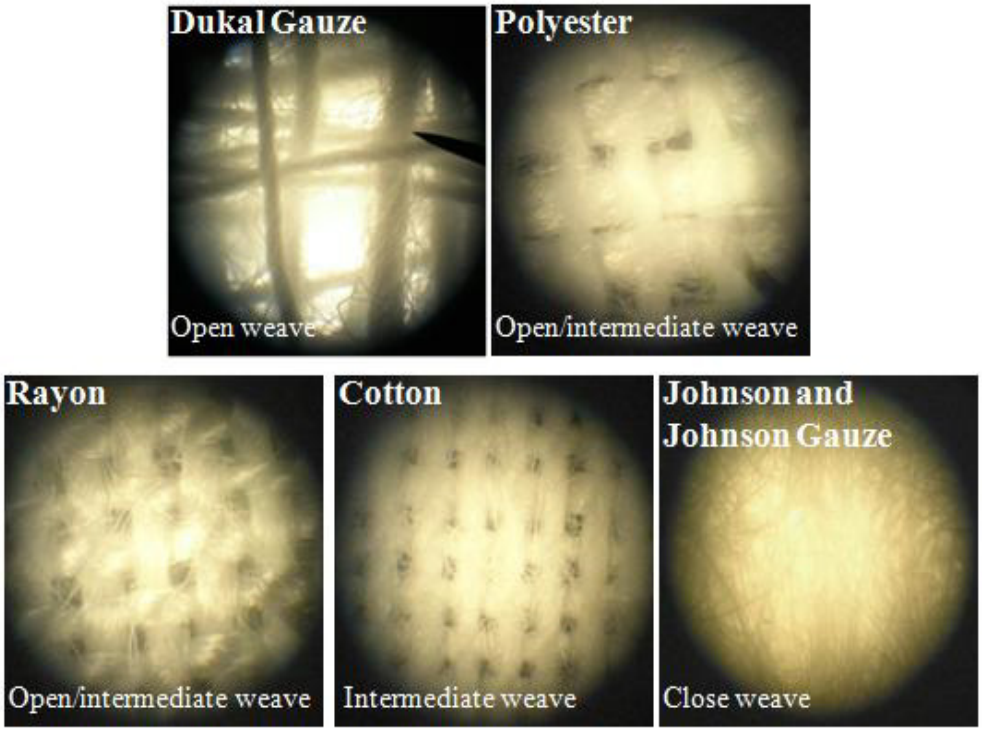


Figure 20. Photographs of collection material taken at 4x magnification.

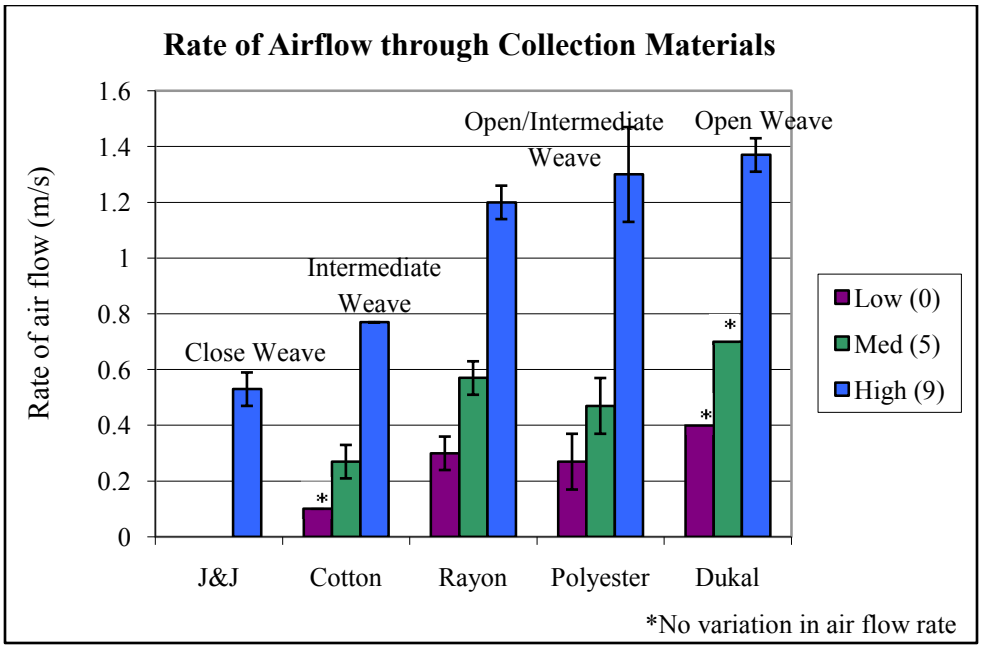


Figure 21. Rate of airflow into the STU-100 for each material / flow rate combination.

The total amounts of the standard compounds collected from the headspace of each material after sampling with the STU-100 at different airflow settings are shown in Figure 22. In general, the STU-100 used with no vacuum yielded the fewest compounds recovered from the headspace of all materials. The low (0) and medium (5) flow rate settings performed marginally better than the highest setting (9), but there was no significant difference among settings. The breakthrough of compounds through the collection material during sampling most likely played a role in the lesser amount of compound collected at the higher flow rate. The breakthrough of compounds through material refers to the fact that at the higher flow rate, the compounds are drawn quickly past the collection material without being deposited onto the material, and are thus lost to the environment.

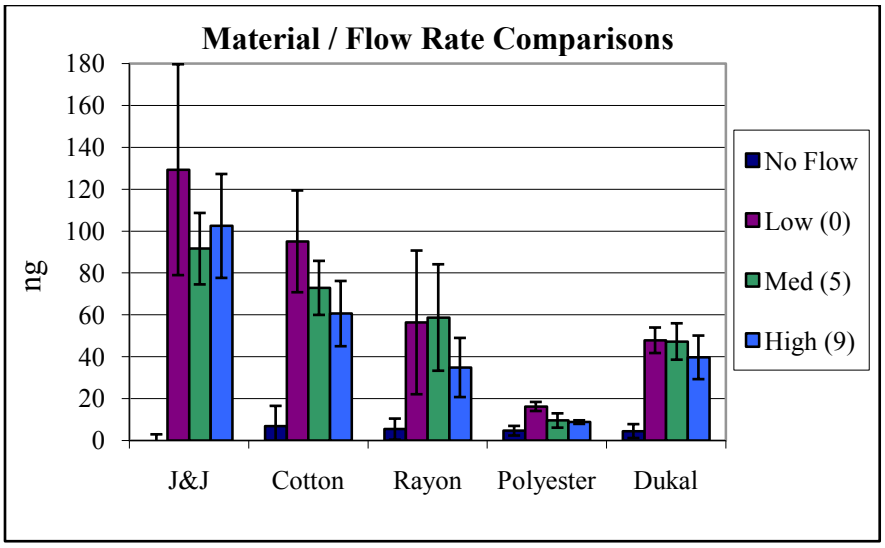


Figure 22. Average total amount of standard compound collected for each material / flow rate combination, in triplicate.

Table 9 lists the amount of each standard compound recovered at each material/flow rate combination. When comparing collection material performance, overall, the polyester material trapped and/or released the fewest compounds compared to the other four materials. Across the four tested airflows, the complete suite of VOCs was not recovered from any material from any material evaluated. All compounds were collected from the Johnson and Johnson gauze except at no flow rate, from the Dukal gauze except tetradecane at no and low flow rates, and from the cotton material except tetradecane at the low flow rate. The furfuryl alcohol and 6-methyl-5-hepten-2-one were not seen at any flow rate using the polyester material. This difference may be related to the molecular structure of polyester since it differs from that of the other materials. Polyester contains a long chain synthetic polymer backbone held together by ester bonds with no free –OH groups, while cotton has a cellulosic backbone containing many free hydroxyl groups and rayon is a synthetic cellulose-based material structurally similar to cotton. The Dukal brand gauze is made wholly of cotton and Johnson and Johnson brand

gauze is a blend of cotton, rayon and polyester. The data here suggest that the trapping or the release of the compounds may be related to the molecular structure of the collection material; however collection is also likely affected by additional factors.

Table 9. Quantity (ng) of standard compound collected at each material/flow rate combination (in triplicate, plus or minus one standard deviation).

J&J	No Flow	Speed 0	Speed 5	Speed 9
2-Furaldehyde	-	0.19 ±3.69	1.39 ±1.34	0.56 ±1.26
Furfuryl alcohol	-	2.60 ±3.19	2.15 ±0.75	1.01 ±1.45
6-Methyl-5-hepten-2-one	1.58 ±0.27	2.95 ±1.26	3.87 ±2.29	1.52 ±0.70
Hexanedioic acid, dimethyl ester	2.94 ±1.46	90.05 ±46.2	61.10 ±16.3	68.75±24.7
Tetradecane	0.59 ±1.81	33.52 ±19.4	23.09 ±4.34	30.60 ±0.71
Cotton	**No Flow**	**Speed 0**	**Speed 5**	**Speed 9**
2-Furaldehyde	0.61 ±0.83	2.16 ±0.97	1.12 ±0.11	0.33 ±0.35
Furfuryl alcohol	1.83 ±3.17	11.24 ±1.24	9.00 ±0.98	6.60 ±0.51
6-Methyl-5-hepten-2-one	0.65 ±1.13	6.33 ±1.44	6.14 ±0.31	3.28 ±2.99
Hexanedioic acid, dimethyl ester	1.37 ±1.58	75.35 ±34.2	55.86 ±25.2	50.38±13.7
Tetradecane	2.32 ±3.29	-	0.74 ±3.43	-
Rayon	**No Flow**	**Speed 0**	**Speed 5**	**Speed 9**
2-Furaldehyde	-	-	1.38 ±2.39	2.92 ±2.54
Furfuryl alcohol	-	0.97 ±1.69	0.95 ±1.65	-
6-Methyl-5-hepten-2-one	0.61 ±1.05	-	-	-
Hexanedioic acid, dimethyl ester	-	39.78 ±23.1	26.47 ±5.90	19.88±15.3
Tetradecane	4.81 ±0.93	15.63 ±7.23	29.91 ±11.1	12.04±1.66
Polyester	**No Flow**	**Speed 0**	**Speed 5**	**Speed 9**
2-Furaldehyde	4.66 ±0.61	4.55 ±0.32	4.53 ±0.30	0.43 ±0.44
Furfuryl alcohol	-	-	-	-
6-Methyl-5-hepten-2-one	-	-	-	-
Hexanedioic acid, dimethyl ester	-	5.39 ±0.42	0.82 ±0.48	5.17 ±0.50
Tetradecane	-	6.30 ±2.10	4.19 ±3.37	3.17 ±0.53
Dukal	**No Flow**	**Speed 0**	**Speed 5**	**Speed 9**
2-Furaldehyde	0.15 ±0.52	0.85 ±0.30	0.54 ±0.52	0.18 ±0.26
Furfuryl alcohol	-	5.17 ±0.50	5.28 ±0.10	4.45 ±0.06
6-Methyl-5-hepten-2-one	1.71 ±0.37	2.06 ±0.86	4.76 ±1.08	0.90 ±0.33
Hexanedioic acid, dimethyl ester	2.62 ±2.65	39.77 ±5.76	34.62 ±6.41	28.86±9.39
Tetradecane	-	-	2.07 ±5.74	5.30 ±4.44

When Figure 21 and Figure 22 are directly compared, it could be suggested that the amount of compound trapped/released is also closely related the measureable flow rate of air into the STU-100. For example, the Johnson and Johnson gauze impedes airflow to the greatest extent and also traps/releases a greater amount of compounds among the five materials tested. It can be concluded that the molecular structure as well as the weave of the material has an effect on its ability to trap and release volatile compounds using the STU-100.

In summary, the Johnson and Johnson gauze was the most effective material to trap/release compounds tested; however, no one material collected the total suite of VOCs at every flow rate measured. A greater amount of the 2-furaldehyde and furfuryl alcohol was collected by the cotton material and the Dukal gauze. This result could be related to the hydrogen bonding between the aldehyde and the alcohol, and the free hydroxyl groups on the cellulosic backbone of the cotton materials. For this reason, both flow rate and collection material must be taken into consideration when using the STU-100 in the field.

Material Layering

It was seen in the previous section that the material with the tightest weave, and thus the greatest propensity to impede airflow into the STU-100, yielded the greatest total amount of standard human scent compounds in the headspace. For this reason, multiple layers of each of the different collection materials were tested to determine if additional

material layers, and thus additional reduction of the airflow, would further improve scent collection. One, three and six layers of polyester, cotton or Dukal were tested as well as one, two and four material layers of the Johnson and Johnson gauze. Because of the thickness of the Johnson and Johnson gauze, it was not possible to effectively use more than four layers at a time. The samples were collected in triplicate at the low flow rate (0), as this was previously determined to be the optimal flow rate. The rate of airflow through one, two and four layers of the Johnson and Johnson gauze was measured and determined to be less than 0.1 m/s for all samples as were the airflow rates for three and six layers of the other three materials.

The greatest total amount of compound was collected using two layers of the Dukal gauze (Figure 23). As seen previously, when comparing the single material layers, the greatest total quantity was recovered from the Johnson and Johnson gauze. However, when multiple material layers were applied, the Dukal gauze out-performed the Johnson and Johnson gauze. A high amount of compound was also recovered from the two layers of polyester; however, the reproducibility was poor. These results may, again, be related to airflow, although too low to be measured (< 0.1 m/s). For instance, a single layer of Dukal gauze does not trap/release as many compounds as a single layer of Johnson and Johnson gauze as a result of compound breakthrough, but the two layers of Johnson and Johnson gauze likely blocked airflow to such a degree that less volatiles compared to two layers of Dukal gauze.

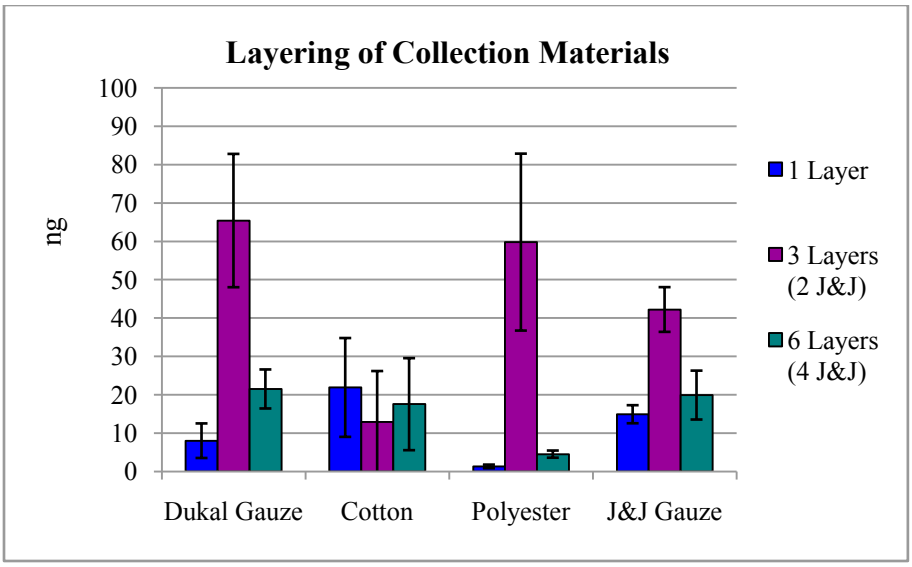


Figure 23. Averaged total amount of standard compound collected for each material / layer number combination carried out in triplicate.

The amount of compound collected onto the intermediate number of layers was determined to be significantly greater than the amount collected onto the other layers for all materials except cotton (Table 10). This can be explained because a single layer of material is more prone to compound breakthrough during the sampling process as air containing VOCs is swept quickly passed the material before volatiles can be deposited. Two/three layers of material slows the air flow, preventing such breakthrough, and increases the surface area onto which compounds can be trapped, thus increasing the quantity recovered. Increasing the number of layers beyond this, increases surface area but also impedes airflow to such a degree that a lower quantity is collected onto the material.

Table 10. Use of ANOVA (two factor without replication) to determine the variation between quantity of compounds recovered from different numbers of material layers.		
	F_{calc}	**Significant difference?**
Dukal	10.312	yes
Cotton	0.837	no
Polyester	11.965	yes
J&J	84.615	yes

2.4.4. Human Subject Sampling

Scent profiles were successfully acquired in triplicate from four individuals, two males and two females (M1, M2, F1, F2), using the Johnson and Johnson gauze pad as collection material and the lowest flow rate setting with the STU-100, previously shown to trap/release the greatest amount of standard compounds in single layer tests. Seven compounds previously reported to be human scent constituents were detected among the human subjects sampled and are listed in Table 11. The compounds listed in Table 11are color coded to correspond to Figure 24 which visually depicts the relative ratio patterns of the seven collected VOCs. There are both qualitative and quantitative similarities and variations among the samples collected from the four subjects.

Table 11. Human scent compounds detected in human subject samples.												
Compound	**F1a**	**F1b**	**F1c**	**F2a**	**F2b**	**F2c**	**M1a**	**M1b**	**M1c**	**M2a**	**M2b**	**M2c**
Undecane (19)				x	x	x						
Dodecane (19,35)				x	x	x	x		x			
Tetradecane (19,35-37,55,56)							x	x		x	x	
Heptadecane (19,55)										x	x	
2-Furaldehyde (35,36)	x	x		x	x	x						
Hexanedioic acid, dimethyl ester (19,35,36)	x	x	x	x	x	x	x		x			
Geranyl acetone (19,35,38,55)				x	x	x	x	x		x	x	X

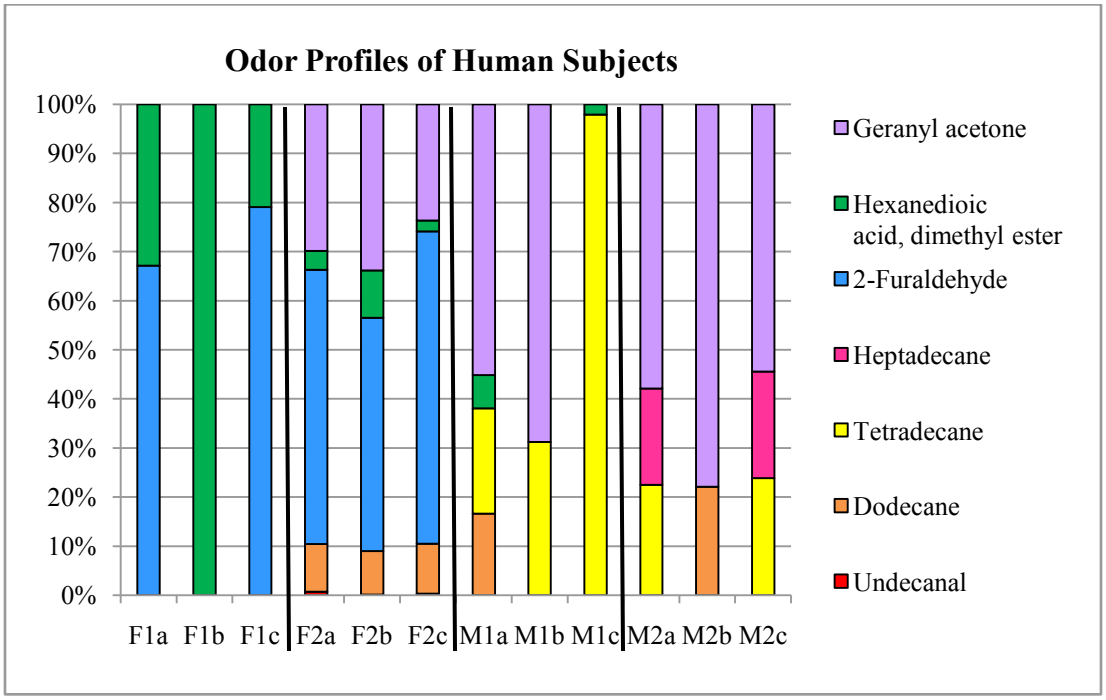


Figure 24. Relative ratios of compounds collected from the palms of four human subjects using the STU-100.

Principle Component Analysis, or PCA, was conducted to reduce the multivariate data collected from the scent profiles of the four human subjects. The first two principle components were plotted below in two dimensions (Figure 25). The three replicate samples taken from F1 and F2 group together well, and are well separated from the other groups thus indicating that the profiles are unique and reproducible. M1 and M2, however, overlap with one another, though are still separated from F1 and F2. The profiles of M1 and M2 would not be considered distinguishable from one another. It should be noted that a greater total quantity of scent compounds were collected from the female samples indicating that increasing the quantity of scent collected could improve reproducibility and distinguishablity of human scent samples with the STU-100. This could be accomplished by further improving extraction or collection efficiency or improving instrument sensitivity.

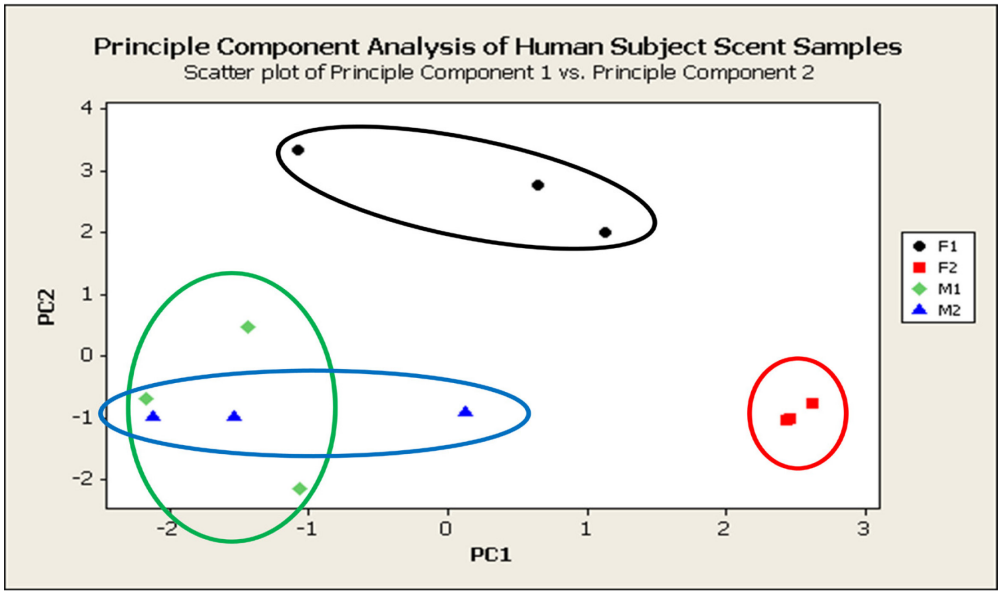


Figure 25. PCA plot representing the similarity between triplicate samples of the scent profiles of four human subjects.

2.5. Conclusions

Experiments were conducted to scientifically evaluate the Scent Transfer Unit-100 (STU-100) as a collection and concentration tool for human scent, and to study the utility of five differing sorbent mediums for the collection of volatile organic compounds previously reported to be present in human scent. For this study, a series of COMPS (Controlled Odor Mimic Permeation System) were developed to deliver five standard compounds to the STU-100 at controlled rates. To reduce background contamination during sampling, a human scent collection chamber was also designed using positive air flow, forcing clean air into the chamber and pushing out contaminated air. It was determined that the chamber enhanced non-contact, dynamic airflow sampling by decreasing the amount of background contamination thereby improving the reproducibility of replicate samples.

Using the human scent collection chamber, volatiles were collected by the STU-100 onto five collection materials at four air flow rates. It was found that the collection material utilized affected the quantity of compound recovered in such a way that materials with a tighter weave tended to collect a greater amount of the compounds, as the greater airflow restriction results in less compound lost due to breakthrough of the compounds through the collection material. However, it should be noted that the fiber chemistry of the collection material also played a role in the recovery of compounds, as the polyester polymer was not as efficient at trapping/releasing certain compounds compared to the cellulose-based cotton and rayon materials.

Changes in the flow rate settings on the device influenced the quantity of VOC's collected, with higher flow rates generally yielding lesser amounts of VOC's, again, likely due to compound breakthrough. When the airflow through the STU-100 is high, either due to a high flow rate setting on the STU-100 or because of the weave of the collection material, VOC's may be swept past the material instead of being trapped by the collection material because of the force of the vacuum.

To further impede compound breakthrough, the layering of collection material was tested. When multiple layers of a single material were used, the greatest amount of compound recovered occurred when using an intermediate number of material layers.

Overall, the polyester material trapped/released the least amount of standard human scent compounds, whereas Johnson and Johnson and Dukal trapped/released the greatest amount of compounds. For single layered materials, Johnson and Johnson gauze collected the greatest amount of compounds overall but showed some variation when comparing individual compounds and flow rates. For the layered materials, three layers of the Dukal gauze trapped/released the greatest amount of compounds.

Following optimization, non-contact dynamic airflow sampling using the STU-100 was successfully applied to the collection of VOC's from the palms of four human subjects. SPME-GC/MS analysis of the samples revealed VOC profiles for each subject. As can be seen in the PCA analysis, the reproducibility between replicate samples with the resulting VOC profiles was good, but confirmation of the individuality of human scent profiles could be improved with improved instrumental detection limits and collection / extraction efficiency.

In the future, similar sampling devices should be carefully evaluated before use in the field as there are significant variations in collection efficiencies among material(s) and flow rates.

3. DECEASED HUMAN SCENT RESEARCH

3.1 Introduction

3.1.1. Human Remains Detector Canines

Human remains detector (HRD) canines, also known as cadaver dogs or victim recovery dogs, are trained to locate human remains (as opposed to animal remains) including whole bodies, body parts, tissue, blood, bone and decomposition fluids. Well-trained HRD canines are capable of locating very small quantities of remains as well as remains that have been submerged in water or buried. The HRD canines differ from human scent canines in that they locate the general scent of decomposing human remains, while human scent canines attempt to locate the unique scent of an individual person. Since all human bodies undergo the similar processes during decomposition, albeit at different rates, the odor is the similar between people for each stage of decomposition, unlike the unique odor of individual live humans.

The specialty of HRD canines evolved from search and rescue. Search-and-rescue dogs are trained to locate living humans, often in wilderness or disaster settings. While working with their search and rescue dogs, handlers noticed that the canines would lose the scent path if the living person had expired, as the change from living human odor to deceased human odor was unfamiliar to these canines.[87] The first canine to be trained exclusively for human remains detection was a Labrador named Pearl that worked with the New York State police. Pearl was first trained in 1974 to assist in a homicide investigation involving multiple victims in a large wilderness area. Pearl's first find was a body buried four feet deep.[16] Today, several decades after Pearl's first success, hundreds of search dog units and volunteers utilize these specially trained HRD canines.

Human remains detection canines use a method called air-scenting when they locate remains, which entails the canine utilizing a scent cone to locate the scent source. A scent cone is formed as scent molecules diffuse away from the scent source in the air into a cone. When a canine enters a scent cone, it moves side-to-side and from low scent concentration to high concentration to the scent source located at the apex of the cone.[16,88]

A scent cone may be altered by weather conditions and other factors making it more difficult for a canine to locate the scent source. Optimal operating conditions include a light breeze to circulate the scent and cool temperatures (40° – 60° F) over moist and loose soil.[88,89] Higher temperatures tend to be detrimental to a search for two reasons. First, when the ground is warm a greater amount of scent is released, and when the air is warm scent rises more quickly. When air temperatures are higher than the ground temperature, the odor will rise too quickly and not be dispersed efficiently.[90] Also, when temperatures are very high, a canine will pant to dissipate heat, and when a canine pants, it cannot sniff efficiently and may have trouble locating an odor.[87]

Wind is the most important factor affecting scent dispersal. Wind of any speed or direction will change the shape of the scent cone. High winds cause the scent to be dispersed too rapidly creating a low concentration of odor molecules in the air, while very low or no winds cause the scent to be dispersed too slowly, causing the scent only to be detected very close to the source.[90]

Soil condition is another factor in the search for buried remains. Loose soil allows a greater amount of odor to permeate the air. Tightly packed and clay soils trap

odor under the surface, preventing or retarding scent dispersal.[90] When bodies are buried under very tightly packed soil, the odor may move laterally through loose gravel beneath dense crust and be released from cracks made by plant roots. This condition makes it difficult for a scent canine to pinpoint the actual location of buried remains.[87]

Several studies have been published that focus on the capability of HRD canines, including the use of canines to locate extremely small or old scent sources such as human teeth, scattered remains, old graves, and materials that had indirect contact with remains materials. These studies show that HRD canines are adept at locating minimal quantities of odor including buried and aged remains. The canines' performances are affected, however, by training, familiarity with the scent source, and environmental conditions.[87,91-93]

3.1.2. Canine Training and Training Aids

A majority of HRD canine handlers are civilians and not directly associated with any law enforcement or government agency. There are no centralized organizations that have established training guidelines and provides trainings as is found with drug and explosive detecting canines, for example, and thus even with the many groups associated with canine human remains detection, there are no universally accepted methods for training. While some research has been published on this topic, it is minimal. The SWGDOG organization has developed best practice guidelines for human remains

detection canines in an attempt to improve their performance and reliability (Appendix 2).[85]

In real life scenarios, scent sources for training aids range from fresh bodies to putrefied bodies in the height of odor production to ancient skeletal remains. The odor source may be a whole body, body parts, tissue or blood. For canines to locate all types and ages of human remains, it is imperative that handlers use as many different training aids as possible. Training aids commonly include human bone, gauze that has been soaked in decomposition fluid, blood, adipocere, grave dirt, and articles or clothing previously in contact with remains.[16,88,92] These training aids are difficult to obtain because of access and legal restrictions and are potential biohazards. Human flesh is the best scent source and can be decomposed to different levels; however, it is particularly difficult to obtain and has the greatest number of legal restrictions.[16]

Chemical pseudo scents may be used as training aids as an alternative to actual human remains. Putrescine and cadaverine are particularly odorous compounds formed during the decomposition process and are commonly found in pseudo scent mixtures. While these compounds may be easier to obtain, they are hazardous chemicals that must be handled with care. Cadaverine and putrescine are known to be found in all decaying organic matter[93] and have also been found in human saliva.[94] Also, these mixtures likely do not completely represent the entire odor picture of human remains as there have been no scientific studies showing which odor or combinations of odors are of interest to HRD canines.

The SWGDOG subcommittee on Research and Technology has created a list of research needs for the detection canine community. In their document (Appendix 1), SWGDOG considers the need for the development of reliable training aids to be critical, particularly for HRD canines. More research must be done to create training aids that are easily and legally obtainable, are non-hazardous, are easy to work with and reusable, and represent the whole odor picture for the canines.[85]

The current research will utilize the STU-100 to provide more information about the signature compounds from the decomposition of human bodies, which could lead to the formulation of a more effective pseudo scent or the use of the STU-100 as an investigative tool or as a tool for canine handlers. Scent transfer units are already possessed by local police departments and federal agencies. The application of such a collection device could potentially be used to confirm the alert of an HRD canine when the body is no longer present, or could be used to create odor pads for canines in the same manner as is already done for the human scent canines.

3.1.3. Human Decomposition and Odor Production

Taphonomy is the study of the decomposition process of an organism. Forensic taphonomy includes the estimation of postmortem interval, the determination of cause and manner of death and aiding in the location of clandestine graves,[95] including reconstruction of events leading up to and following death.[96]

Decomposition can be observed as early as four minutes after death. As soon as the heart stops pumping, the cells in the body are deprived of oxygen, which causes a rise in carbon dioxide in the blood and a decrease in internal pH. Waste builds up in the cells, poisons them, and causes cell death. At the same time, cellular enzymes break down the cell by a process known as autolysis.[97]

There are two stages of autolysis. The first stage is the early reversible stage where ATP is no longer produced causing the termination of molecular synthesis and mitochondrial activity. The pH in the body is lowered as a result of the anaerobic fermentation of pyruvic acid to lactic acid, impeding protein synthesis. The second stage is the late irreversible stage where proteins and enzymes begin to denature, causing the cell membranes to breakdown.[96]

Autolysis begins a cascade of other events that lead to the body's complete decomposition. In short, after autolysis begins, the anaerobic breakdown of the body's macromolecules (carbohydrates, lipids and proteins) begins because of the action of bacteria in the digestive and respiratory systems. The breakdown of molecules leads to color changes and bloating as gases begin to build up in the body. As the gases expand in the body, the skin loses its integrity and ruptures, which reintroduces oxygen allowing for aerobic activities to resume and further break down the body to skeletonization.[95]

Preliminary changes to the body occur within the first few hours after death. These changes are *algor mortis*, *livor mortis* and *rigor mortis*. *Algor mortis* refers to the reduction of the body temperature that marks the end of the body's metabolic processes.

The body cools until the temperature is at equilibrium with the surrounding environment.[98]

Livor mortis refers to the settling or pooling of blood as a result of gravity, which produces a red or purple discoloration. The pooled blood is first red, but turns to purple as hemoglobin loses oxygen atoms. *Livor* occurs as early as one hour after death and finishes, or becomes fixed, after about ten hours.[98]

Rigor mortis refers to the tendency of the body's muscles and joints to become rigid beginning with the eyes, neck and jaw and spreading through the rest of the body. After death, the muscle cells begin to lose integrity, which allows an influx of calcium into these cells. Since calcium is responsible for the contraction of muscle fibers, as it flows into the muscle cells the muscle shortens and becomes rigid. The muscles remain contracted because the ATP that usually pumps calcium out of the cell is no longer available. Rigor will dissipate as the muscle tissue degrades and the muscle fibers become detached from one another. Rigor generally occurs within the first hours of death and passes after 24 to 48 hours.[96]

There are considered to be five stages of decomposition:

Stage 1.) Fresh: few exterior changes to the body, including the paling of the skin that results from the lack of oxygenated blood and the appearance of algor, livor, and rigor mortis. Internal decomposition begins as a result of bacterial action and autolysis. Stage one is also characterized by early skin slippage caused by autolysis at the dermal-epidermal junction. A subtle odor is detectable by canines but not by humans.

Stage 2.) Bloating or Putrefaction: the body creates an anaerobic environment that favors bacterial growth in the gut and bowel. Bacteria break down larger molecules causing the body to swell because of the internal production of gases. Black discoloration or a greenish tint may appear beneath the skin. The black discoloration of the skin is caused by the formation of a black precipitate during the breakdown of hemoglobin. A greenish discoloration is the result of the deanimation of amino acids. During stage two, odor is detectable by both canines and humans.

Stage 3.) Decay: the skin ruptures releasing the gases that have built up inside, reintroducing oxygen to the body. This stage is the height of odor production and soft tissue loss.

Stage 4.) Liquefaction: the body begins to lose its integrity as the organs liquefy and the bones become visible. The body begins to dry out and odor is reduced.

Stage 5.) Skeletonization: the body's decay rate slows greatly as the last of the soft tissue decays. A slight, musty odor remains for some time. Bone may remain intact for many years or it may slowly be broken down by decalcification, dissolution by acid or by scavenger activity.[16,96,98]

The rate of decomposition is affected by many factors, including the condition of the body before death, manner of death, clothing or wrappings around the body, location of the body after death and insect and animal activity. Age, height, weight, gender and fitness level affect the rate of decomposition as the surface area of the body and the amount of fatty acids present alter the rate.[98]

Clothing may slow decomposition by providing protection for the body or may increase the rate by creating a dark enclosed area for maggots to reside and rapidly breakdown tissue.[99] Wounds or injuries inflicted to the body before death increase the rate of decomposition as they provide additional entry sites for insects and bacteria. A body decomposing outdoors in a warm environment will decompose faster than a body kept indoors or refrigerated. There will be an increased amount of insect activity outdoors which, with warmer temperatures, greatly increases the rate of decomposition.[98] Generally, higher temperatures promote decomposition; however, very high temperatures may cause enzymes to denature, decelerating decomposition.[96] Low temperatures slow or even halt decomposition by retarding autolysis, and slowing the rate of bacterial breakdown of proteins and insect consumption of tissue.[100] Deeply buried bodies decay at a much slower rate than bodies exposed to the environment. Burial restricts animal and insect access to the body, and soil tends to act as a solar barrier creating cooler temperatures with fewer fluctuations. Moisture from the soil encourages the growth of microorganisms, again increasing the rate of decomposition. A body submerged in water decomposes slower due to cooler temperatures and lack of sunlight.[96]

A body left in an extremely warm, dry environment, may undergo mummification as a result of desiccation and dehydration of the tissue. Adipocere, a grayish-white, cheese-like substance may form on the body in a cool, moist environment because of the saponification of lipids.[98] Moist or very humid environments encourage adipocere formation because water reduces the oxidation rate of fatty acids allowing more of the fatty acids to be converted to adipocere.[96]

The body's macromolecules, proteins, lipids, and carbohydrates, degrade into smaller, simpler molecules and gases during decomposition. Proteins undergo proteolysis where they are denatured into their component amino acids by bacterial enzymes. Amino acids may then undergo deamination, decarboxylation, or desulfhydration. Desulfhydration is responsible for the production of dimethyl disulfide and other foul-smelling sulfide compounds. The loss of an amine and a hydrogen during deamination causes an accumulation of ammonia. The deanimation of L-phenylalanine is responsible for the greenish tint of the skin during Stage 2 decomposition. Decarboxylation is carried out by bacterial enzyme activity, primarily by *Enterobacteriaceae,* such as *Clostridium* and *Lactobacillus*, yielding the production of carbon dioxide and biogenic amines.[98] The amino acids, ornithine, lysine, histidine, tyrosine, tryptophan and phenylalanine are transformed into putrescine, cadaverine, histamine, tyramine, tryptamine and phenylethylamine by decarboxylation, respectively. Two of such amines, cadaverine and putrescine, are particularly odorous molecules and may be partially responsible for detection by HRD canines.[95]

The body's adipose tissue is predominantly composed of triacylglycerols (triester glycerol and three long-chain fatty acids). Tissue lipases hydrolyze these lipids into saturated and unsaturated fatty acids. The fatty acids most commonly include: oleic, palmitric, linleic, stearic, myristic, palmitoleic and vaccenic. In an aerobic environment, the unsaturated fatty acids are oxidized to aldehydes and ketones. In an anaerobic environment, the unsaturated fatty acids are saturated.[98]

Carbohyrdrates are broken down into glucose monomers by microorganisms. In an aerobic environment, the glucose monomers are converted into organic acids, and then further decomposed into carbon dioxide and water. In an anaerobic environment, the monomers are converted into butyric and acetic acids and related alcohols. Bacterial carbohydrate fermentation will produce methane, hydrogen sulfide and hydrogen gases. [98]

3.1.4. Human Remains VOC's

Only a few research groups have attempted to characterize the volatile organic compounds emanating from human remains. A condensed list of compounds recovered from such groups is included in Table 12. The list contains a huge variety of compounds and functional groups including, acids and acid esters, alcohols, halogens, ketones, aldehydes, cyclic hydrocarbons, sulfides and nitrogen-containing compounds. The best represented functional group is cyclic hydrocarbons, with toluene and p-xylene being reported the most regularly. Additionally, dimethyl disulfide and tetrachloroethylene are particularly widely reported in the literature.

Table 12. VOCs from human remains documented in peer-reviewed literature.					
	Reference	**Reported as human scent**		**Reference**	**Reported as human scent**
Acids/esters			**Aldehydes**		
Propanoic acid	39	Y	2-Hexanal	39	N
Butanoic acid	4,39,101	Y	Hexanal	4,39,102	N
Butanic acid, ethyl ester	39,102	N	Benzaldehyde	4,39,102	Y
Hexanoic acid	4,39	Y	2,4-Heptadienal	39	N
Pentanoic acid	4	N	2-Heptenal*	39	N
Heptanoic acid	4,101	Y	Heptanal	4,39	Y
Nonanoic acid	4	N	2-Octenal	39	Y
Octanoic acid	4	Y	Octanal	39	Y
Butanoic acid, butyl ester	39	N	2,4-Nonadienal	39	N
Hexanoic acid, ethyl ester	39	N	2-Nonenal	39,103	Y
Hexanoic acid, pentyl ester	39	N	Nonanal	39,101	Y
Hexanoic acid, hexyl ester	39	N	3-Methyl butanal	105	N
Hexadecanoic acid, methyl ester	103,104	N	Decanal	103,105	Y
Propanoic acid, 2methyl, ethyl ester	102	N	Pentanal	102	N
Acetic acid, propyl ester	102	N	**Cyclic Hydrocarbons**		
Alcohols			Toluene	39,102-104	Y
1-Pentanol	4,39,102	N	p-Xylene	36,102-105	N
1-Hexanol	39,102	N	o-Xylene	103,104	N
1-Octen-3-ol	39	Y	m-Xylene	102	N
1-Hexanol, 2-ethyl	36,105	N	Indole	39	N
1-Octanol	39	N	2-Pentyl-furan	4,39	N
2-Propanol	105	N	Ethyl benzene	102,103	N
Phenol	101,105	Y	Styrene	103-105	N
Phenol, 4-methyl	105	N	1-methyl-2-ethyl benzene	103,104	N
Benzenemethanol, α, α, dimethyl	103,104	N	Methyl benzene	105	N
Ethanol	102,105	N	1,2,3-Trimethyl benzene	102,105	N
1-Butanol	102	N	Di-limonene	102,105	N
Halogens			2-Ethyl-1,4-dimethyl benzene	105	N
Tetrachloroethylene	39,103-105	N	Naphthalene	104,105	N
Carbon tetrachloroide	103,104	N	1-Methylethenyl benzene	105	N
1,1,2-trichloro-1,2,2-trifluoro ethane	104	N	Benzene	102,104	N
Trichloromonofluoro Methane	103,104	N	1-Methoxy propyl benzene	104	N
Dichlorodifluoro Methane	103,104	N	1,2,4-Trimethyl benzene	102	N
Chloroform	103,104	N	1,3,5-Trimethyl benzene	102	Y
Dichlorotetrafluoro Ethane	103,104	N	1-Ethyl, 3-methyl benzene	102	N
1,1-dichloro-1-fluoroethane	104	N	Propyl benzene	102	N
Trichloroethene	103,104	N	**Sulfides**		
Ketone			Dimethyl disulfide	4,39,102-105	N
Cyclohexanone	39,102	N	Dimethyl sulfide	105	N
2-Heptanone	39,102	N	Dimethyl trisulfide	102-105	N
2-Propanone	102,103,105	N	Methyl ethyl disulfide	102,105	N
1-Phenyl ethanone	105	N	Carbon disulfide	102-104	N
2-Butanone	102,105	N	**N-containing compounds**		
2-Nonanone	105	N	Trimethylamine	4	N
3-Pentanone	102	Y	Methenamine	103-104	N
2-Pentanone	102	Y	Benzonitrile	103	N
6-Methyl-5-hepten-2-one	101	Y			

Statheropoulos et al. (2005 and 2007) measured the VOCs from several deceased human bodies in the early stages of decomposition using thermal desorption (TD) GC/MS. The VOCs were extracted using triple sorbent tubes. The contents of the tubes were thermally desorbed into a GC/MS and separated using cryogenic focusing. In the first study, only two bodies were sampled[102] and, in the second study, only a single body was sampled.[105] The most prominent compound found in both studies was dimethyl disulfide. Other sulfides, ketones, and benzene derivatives were also frequently occurring. It should be noted that neither cadaverine nor putrescine were detected.[102,105]

Vass et al. (2004 and 2008) also sampled the VOCs associated with four buried human bodies using TD/GC/MS with triple sorbent tubes and cryogenic focusing for extraction and analysis in attempt to initiate a "Decompositional Odor Analysis Database." In both studies, over 400 VOCs were recovered including eight compound categories: cyclic and non-cyclic hydrocarbon, nitrogen compounds, oxygen compounds, acids/esters, halogens, sulfur compounds and other. Vass et al. also noted a number of fluorinated compounds, suggesting that the presence of fluorine is the result of the liberation of fluorine previously ingested with drinking water. Again, it should be noted that neither cadaverine nor putrescine were recovered.[103,104]

Hoffman et al. attempted to identify the VOCs in various types of human tissues. The types of tissues chosen for sampling are commonly used as training aids for HRD canines including blood, blood clot, placenta, muscle, testicle, skin, body fat, adipocere and bone. Each tissue sample was placed into separate vials, the headspace was extracted by SPME and analyzed by GC/MS. Classes of compounds recovered from the samples

included acids, alcohols, aldehydes, halogens, aromatics, ketones and sulfides. The group found both qualitative similarities and differences in the VOCs recovered from the various tissue types.[39] This evidence substantiates the idea that while it is possible to train a relatively effective HRD canine on a limited number of scent sources, HRD canines should be trained on a variety of odor sources for the best remains recovery.

Vass et al. was the only group to compare VOCs from human remains to animal remains. Human and animal bones, aged 5-9 years, were placed in Tedlar bags. The vapor within the bags was collected and analyzed. The odor profiles between the different animals were unique in ratio and specific compound (Table 13).[104] These results support the notion that HRD canines are capable of selectively locating human remains in preference to animal remains.

Table 13. Volatiles found associated with human and animal bones.

	Human	Dog	Deer	Pig
Carbon tetrachloride	x			
Toluene	x	x	x	
1,1,2-trichloro-1,2,2-trifluoro ethane	x	x		
Tetrachloroethene		x	x	
Trichloromonofluoromethane		x	x	
1,4-Dimethyl benzene	x	x		
Naphthalene		x		
Benzene	x	x	x	
Dichlorodifluoromethane		x	x	
Chloroform		x		
Ethyl benzene	x	x		
Decanal	x	x	x	x
Nonanal	x	x	x	
Hexane	x		x	
Benzenemethanol, α,α, dimethyl	x	x		
1-Ethyl, 2-methyl benzene			x	
Hexadecanoic acid, methyl ester	x		x	
1,2-Benzenedicarboxylic acid, diethyl ester	x			
Undecane		x		

3.2 Materials

3.2.1. Standard Compound Sampling and Optimization of the STU

Six standard compounds previously cited as human remains volatiles (Table 11) were used to optimize the Scent Transfer Unit (STU-100) for the collection of human remains odor, including n-butyric acid, 99+% (Acros Organics, NJ), heptanoic acid, 99%; 6-methyl-5-hepten-2-one, 99%; liquefied phenol; dimethyl disulfide and nonanal, 95% (Sigma-Aldrich Co, St. Louis MO).

The standard compounds were delivered to the STU-100 using Controlled Odor Mimic Permeation Systems (COMPS) which were prepared by spiking 25μL of a single compound onto Dukal brand, sterile, 2"x2", 8 ply gauze pads (DUKAL Corporation, Syossett, NY). The gauze pads were sealed into low density, polyethylene bags, 3"x3"x1.5MIL or high density, polypropylene bags, 3"x3"x2MIL (Veripak, Atlanta, GA).

Solid phase micro extraction (SPME) was used for the extraction of the compounds from the headspace. Four types of SPME fibers were trialed, including grey, 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS); blue, 65 μm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB); white, 85 μm Polyacrylate (PA); and black, 75 μm Polydimethylsiloxane/Carboxen (PDMS/CAR) (SUPELCO, Bellefonte, PA). Analysis was conducted using a Varian Ion Trap CP-3800 Gas Chromatograph / Saturn 2000 MS/MS (Varian Inc., Walnut Creek, CA) with a DB-225MS column (Agilent Technologies, Santa Clara, CA). For sampling, a standard solution of the above listed compounds was spiked onto Dukal gauze and sealed into

10mL, screw top, glass vials with PRFE/Silicone septa (SUPELCO, Bellefonte, PA). The solvent used was dichloromethane (Pharmco-AAPER, Brookfield, CT).

Odor collection with the STU-100 was optimized using three types of collection materials; spun polyester type 54 (Test Fabrics Inc., West Pittston, PA); Johnson and Johnson brand, sterile 2"x2" gauze pads (Johnson and Johnson, Skillman, NJ) and Dukal brand, sterile, cotton gauze. After sampling, the collection material was returned to cleaned 10mL clear, screw top glass. Sample vials and collection materials were cleaned using HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ).

3.2.2. Remains Sampling

Human remains samples were collected from two locations, the Miami-Dade County Medical Examiner in Miami, FL and Borden Cremation Service in Louisville, KY. For comparison purposes, animal remains and living humans were sampled, as well. The animal products sampled are included in Table 14. All animal samples, with the exception of the canine, were obtained from Publix Grocery Stores in Miami Beach, FL or were donated by a local restaurant. The participants for the living human odor samples were required to wash and rinse the hands and forearms with Natural, Clear Olive Oil Soap (Life of the Party, North Brunswick, NJ) prior to sampling.

Table 14. Animal remains obtained for sampling with STU-100.		
Sample Type	**Location Obtained**	**Condition of sample prior to sampling**
Canine 1	Borden Pet Crematory	Fresh
Canine 2	Borden Pet Crematory	Fresh
Ahi tuna	Local restaurant	Moderately spoiled
Whole skinless chicken	Local restaurant	Extremely spoiled
Lamb chops	Local restaurant	Moderately spoiled
Pork chop	Publix Grocery	Fresh
Beef steak	Publix Grocery	Fresh
Hamburger	Publix Grocery	Fresh

All scent samples were taken with the STU-100. Prior to sampling, the STU-100 was cleaned with a sterile alcohol pad (Fisher Healthcare, Fairlawn, NJ). The collection material used was Dukal brand gauze with polyester material and placed into clear glass vials following collection. Extraction and analysis was performed using SPME-GC/MS with a DVB/CAR/PDMS SPME fiber and a DB-225MS GC column.

3.2.3. Creation of Training Aids

Odor pad training aids

Canine training aids were made by collecting target odor with the STU-100 onto Dukal cotton gauze and polyester material. For the initial set of experiments, the odor pads were sealed into low density, 1.5MIL, polyethylene bags, and then sealed again into aluminized, moisture barrier bags (3M, St. Paul, MN). For further experiments the odor pads were placed directly into the aluminized bags and sealed or were sealed into glass spice jars with plastic sifter lids (Bed, Bath and Beyond Inc.).

Pseudo scent

Chemical-based canine training aids were created using a solution of the most commonly occurring VOCs found in human remains, as determined during previous sampling. Three formulations were made and the contents are listed below (Table 15) (all compounds were purchased from Sigma-Aldrich Co, St. Louis, MO). For all formulations, reagent grade dichloromethane was used as the solvent.

Table 15. Compounds included in pseudo scent canine training aid mixture.

Compound	Mixture 1	Mixture 2	Mixture 3
Toluene	X	X	
Acetic Acid	X	X	
1,2,3-Trimethylbenzene	X	X	
Styrene	X	X	
p-xylene	X	X	
o-xylene	X	X	
2-Furaldehyde	X	X	
6-Methyl-5-hepten-2-one	X	X	
Tridecane	X	X	
2-Ethyl-1-hexanol	X	X	
Benzaldehyde	X	X	
Nonanal	X	X	
2-2-Methoxyethoxy ethanol	X	X	
1-Octanol	X	X	
Benzonitrile	X	X	
Benzoic acid, methyl ester	X	X	
Decanal	X	X	
Pentadecane	X	X	
Hexadecane	X	X	
Heptadecane	X	X	
Benzyl alcohol	X	X	
Phenol	X	X	
Octanoic acid	X	X	
Cadaverine		X	X
Putrescine		X	X

The training aids were made by spiking the compound mixtures Dukal cotton gauze. For the first set of experiments the gauze was sealed into low density, 1.5MIL, polyethylene bags, which were in turn sealed into aluminized, moisture barrier bags. In the second set of experiments, the gauze pads were sealed only into the aluminized bags. In the final experiments, the pads were placed into glass jars.

3.3. Methodology

3.3.1. Controlled Odor Mimic Permeation Systems

Controlled Odor Mimic Permeation Systems, or COMPS, were created in the same manner as that for the living human odor compounds (Chapter 2). Twenty-five μL of each compound was spiked onto a piece of Dukal cotton gauze and sealed into a polymer bag. The dissipation rate for each compound was measured for both the low density, polyethylene bags and the high density, polypropylene bags. The dissipation rates were determined by gravimetric analysis, as done previously.

3.3.2. Selection of Fiber Chemistry / Exposure Time

A variety of SPME fiber chemistries and fiber exposure times were evaluated using the standard compounds. Twenty-five μL of a 100ppm solution containing the six standard compounds in dichloromethane was spiked onto a Dukal pad, placed into a 10mL glass vial, and left to equilibrate overnight at room temperature. Grey, blue, white and black SPME fibers were inserted into the vials and exposed for 5, 10, 20, 45, 90, and

180 minutes. The quantity of each compound collected for each fiber was plotted versus time (minutes) and the optimum fiber type was chosen. This experiment was repeated with only the grey fiber by increasing the exposure times to 0.5, 1, 2, 6, 20, and 24 hours; however, using the STU-100 with COMPS. Again, the quantity of each compound was plotted against time (hours) and the optimum exposure time was selected.

3.3.3. Optimization of the Scent Transfer Unit

For the sampling process, COMPS were created as described above. Twenty-five μL of each compound was spiked onto Dukal gauze and placed into the appropriate permeable bag. All of the compounds that were to be sealed in the low density, permeable bags (nonanal, phenol, heptanoic acid, butyric acid, and 6-methyl-5-hepten-2-one) were spiked onto the same piece of gauze and sealed into a single low density, permeable bag. The dimethyl disulfide was spiked onto a separate piece of Dukal gauze and sealed into a high density, permeable bag. The COMPS were set aside for two hours, until the dissipation rates became steady (i.e., the linear portion of the dissipation graph was reached).

For sample collection, a cleaned piece of the material of interest was placed on the face of the STU-100. Prior to sampling, the material was analytically cleaned with methanol and baked. The STU-100 was held approximately one inch above the COMPS for 60 second. After sampling, the collection material was removed and returned to a clean, 10mL glass vials where it was allowed to equilibrate overnight at room

temperature. The sampling was repeated in triplicate and blank samples were created in the same manner by sampling COMPS containing no compounds.

Flow rate / Material

A single piece of Dukal gauze, two layers of the Johnson and Johnson gauze, or three layers of polyester material cut into 2"x2" pieces were evaluated. For each collection material, four flow rates were tested: no flow (STU-100 off), low (0), med (5) and high (9). The collection material was removed and placed into a clean, 10mL vial for extraction.

Material Layering

To determine whether a combination of material types would enhance or impede scent collection, multiple layers of material were assessed. Layers of the collection material were placed on top of one another and onto the STU-100, Dukal gauze with polyester material or Johnson and Johnson gauze with polyester material. The flow rate of the STU-100 was run at the low (0) and medium (5) flow rates. Following sampling, the layers of materials were removed from the STU-100 and placed into a single vial for extraction.

Extraction and Analysis

The extraction and analysis procedure employed was previously optimized for the analysis of living human scent samples. After collection, the samples were allowed to equilibrate overnight before the headspace was sampled using solid phase microextraction (SPME). Divinylbenzne/Carboxen/Polydimethylsilocane SPME fibers were used to sample the headspace for 21 hours at room temperature. Following extraction, the fibers were thermally desorbed into a Varian Ion Trap GC/MS with a DB-225MS with a split ratio of 10:1 and a column flow rate of 1.0mL/min. The column temperature was held at 40°C for two minutes and then increased to 220°C at 7°C/minute.

For quatitation, calibration curves were created using 5, 10, 20, and 50ppm solutions of standard compounds. The solutions consisted of a mixture of all standard compounds of interest in dichloromethane. The concentration v. area counts were plotted for each standard separately. An example is given in Figure 26.

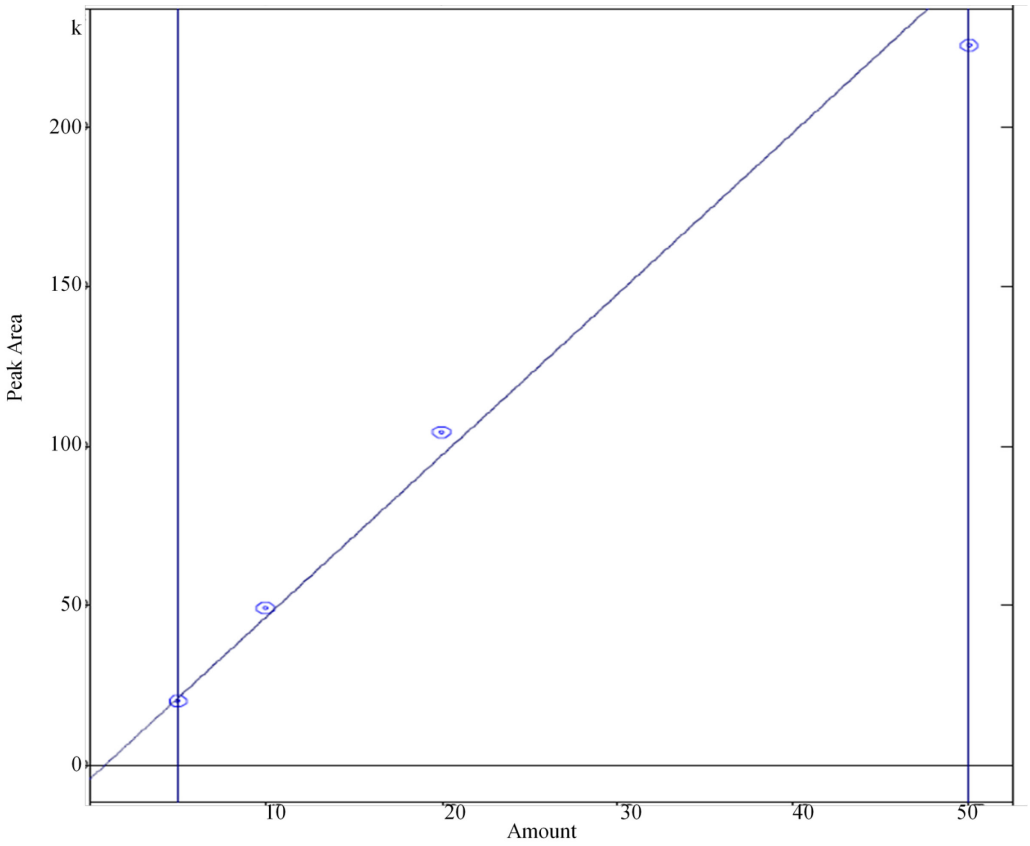


Figure 26. Calibration curve for dimethyl, disulfide. (R^2 = 0.993)

3.3.4. Remains Sampling

Sampling protocol: Human remains

Human remains odor was collected with the STU-100 maintained one to four inches from the subject for 60 seconds during collection. The lowest flow rate setting (0) was used with layered Dukal gauze and polyester as the collection materials.

Human remains samples were collected from the Miami-Dade County Medical Examiner in Miami, FL and from Borden Cremation Service in Louisville, KY. All of the bodies were estimated to be in stage one or stage two decomposition. Samples were collected from the length of the body. Any clothing and/or coverings present on the body were removed when possible. All samples were collected in triplicate.

Extraction and Analysis Method Optimization

Compounds were initially extracted from the headspace of the samples using SPME at room temperature with an exposure time twenty-one hours. The analytes on the SPME fiber were thermally desorbed into the injector port of the GC/MS with a 10:1 split ratio. The extraction and gas chromatography methods are given below (Table 16).

Table 16. Extraction and GC parameters before method optimization.

SPME extraction time	21 hours
SPME extraction temp	Room temperature
Injection temp	200° C
Split ratio	10:1
Column flow rate	1.0 mL/min
Temperature program	40°C, hold 2 min 7°C/min to 220°C

The initial extraction and analysis method was optimized using a small mixture of compounds. However, actual remains samples contain many more VOCs, thus the extraction and analysis methods were re-optimized to improve collection of the full array of compounds. On the basis of compounds initially detected in the samples as well as compounds documented in the literature, a solution of twenty-nine standard compounds

associated with human remains odor was prepared for optimization (Table 17). The final extraction and analysis methods are given in Table 18 below.

Table 17. Standard compounds composing human remains odor mixture.	
Undecane	Decanal
Tridecane	Benzaldehyde
Tetradecane	2-Furaldehyde
Pentadecane	6-Methyl-5-hepten-2-one
Hexadecane	5,9-Undecadien-2-one, 6,10 dimethyl
Heptadecane	Toluene
Phenol	1,2,3-Trimethyl benzene
Furfuryl alcohol	Styrene
1-Octanol	1,4-Dimethyl benzene (p-xylene)
Benzyl alcohol	1,2-Dimethyl benzene (o-xylene)
2-Ethyl-1-hexanol	Butyric acid
2-2-Methoxyethoxy ethanol	Acetic acid
Hexanal	Octanoic acid
Nonanal	Benzoic acid, methyl ester
Benzonitrile	2-Methyl propanoic acid
Dimethyl disulfide	Hexanedioic acid, dimethyl ester
Dimethyl trisulfide	Octanoic acid, methyl ester
Chloroform	

Table 18. Extraction and GC parameters after method optimization.		
	Extraction 1	Extraction 2
SPME extraction time	30 min	21 hours
SPME extraction temp	Room temperature	70°C ± 5°C
Injection temp	200°C	200°C
Split ratio	10:1	Splitless
Column flow rate	1.0 mL/min	1.0 mL/min
Temperature program	40°C, hold 2 min 5°C/min to 80°C 15°C/min to 220°C	40°C, hold 2 min 7°C/min to 85°C 3°C/min to 95°C 7°C/min to 220°C

SPME bias / SPME exposure times

Since a lengthy exposure time (21 hours) was used for SPME extraction, an experiment was designed to determine whether some compounds are were being lost because of SPME bias. Ten μL of a 200 ppm standard compound solution was spiked onto Dukal gauze and polyester material. The materials were placed into a 10mL, glass vial and allowed to equilibrate over night. Compounds were extracted from the headspace using SPME exposed for varying lengths of time (15min, 1hr, 3hr, and 21hr). If bias was occurring because of SPME extraction, some compounds would be seen at the shorter exposure times, but not at the 21 hour exposure time.

The SPME exposure times for the extraction of the early eluting compounds from the standard compound solution were varied once again. Shorter exposure times (5min, 15min, 30min, 1hr, 2hr, and 4hr) were compared to the longer exposure time (21hr).

Split v. splitless injection

Next, the injection method was altered to improve the sensitivity of the analysis. Split (10:1) and splitless injection methods were compared. Again, 10μL of the 200ppm solution was spiked onto gauze. The compounds were extracted from the headspace with SPME for 21 hours at room temperature.

Heating samples

In order to potentially increase the quantity of VOCs in the headspace, sample vials were heated to varying temperatures. Again, the standard compound mix was spiked onto gauze and placed into the vials. The vials were heated during equilibration and extraction periods to 90, 70, 60, 50, 37 (body temperature), and 25°C (room temperature). This was conducted for both long SPME fiber exposure times of twenty-one hours, as well as for shorter exposure times of thirty minutes.

Living human samples

For comparison purposes, samples were taken from living humans using the STU-100. The procedure for living human scent sampled was the same as previously employed. Eight subjects, four male and four female, were sampled with the STU-100 on the low flow rate setting (0) and with the Dukal gauze and polyester as collection materials. Before sampling, the subject was first asked to wash his/her hands with a fragrance-free soap. The palms of the subject were sampled three times consecutively, inside of the human scent collection chamber. The subject was instructed not to touch anything between replicates. Blank samples were collected in the same manner, in the human scent collection chamber, prior to human scent collection.

Animal remains samples

The sampling of the canine remains took place at the Borden Pet Crematory & Memorial Center in Louisville, KY. The two canine samples were collected in the same manner as the human remains. The other animal remains samples were sampled outdoors and were placed into Tupperware containers covered with a mesh grating held on by zip ties. The remains were sampled several hours after removal from storage in a freezer. Blank samples were taken of the containers before sampling, and fresh containers were used with each new sample.

3.3.5. Methods of Statistical Evaluation

For statistical analysis ANOVA and PCA were used again as a measure of variance associated with experimental variables and to depict similarity between odor profiles. For the analysis of the results of the canine trials, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated. The PPV gives the probability that a positive answer is the correct answer. In other terms, it is the probability that when a canine makes an alert, the alert is correct. This is calculated by the number of true positives divided by the number of true positives plus false positives, i.e.

$$\boldsymbol{PPV} = \frac{\boldsymbol{True\ Pos.}}{\boldsymbol{True\ Pos.+False\ Pos.}} \qquad \text{Eq. 9}$$

Conversely, the NPV gives the likelihood that a negative response is a correct, or that when a canine is non-responsive to an aid, the non-response is correct and the aid does not contain an odor of interest. The NPV is calculated by the number of true negatives divided by the number of true negatives and false negatives, i.e.

$$\boldsymbol{NPV = \frac{True\ Neg.}{True\ Neg.+False\ Neg.}} \quad \text{Eq. 10}$$

3.3.6. Canine Training Aids

Creation of training aids

Pseudo scent training aids

Three pseudo scent mixtures were created (Table 15), the first consisting of the most commonly occurring VOC's in human remains odor, as determined by previous analysis, the second including cadaverine and putrescine in addition to this mixture, and the third mixture consisting only of cadaverine and putrescine. All solutions were prepared to 10ppm with dichloromethane as the solvent.

Initially, 100µL of the solutions were spiked separately onto Dukal gauze. The solvent was allowed to evaporate for one minute before sealing the gauze pads into low density, polyethylene, permeable bags and then into separate aluminized bags. Based on initial canine trials, it was determined that the quantity of scent initially used was not enough for the canine's to detect, thus for future experimentation, 100µL of 60ppm solutions were spiked onto the Dukal gauze and the gauze pads were sealed directly into the aluminized bags or placed into glass jars. The polyethylene, permeable bags were not

used again as it was believed that not enough scent was escaping through the bags for the canines to detect. Further studies were carried out altering the concentration of pseudo scent on the gauze pad and will be discussed below.

Odor pad training aids

Another set of training aids was created using scent collected with the STU-100. Dukal gauze was used as the collection material used, and sampling was conducted for one minute on the lowest flow rate setting. The STU-100 was held one to four inches above the remains. Scent samples were taken from freshly deceased bodies located at Borden Cremation Services. Other scent sources used included fresh canine remains (obtained from Borden Pet Cemetery), human cremains (obtained from Borden Cremation Services) and gauze containing decomposition fluid, adipocere, bone residue, or blood. The decomposition fluid, adipocere, bone residue and blood samples were provided by the Kentucky Office of the Medical Examiner in the form of gauze pads soaked in the above mediums and placed into glass jars. The odor remaining in the jars was collected with the STU-100 by placing it directly over the opening of the jars. The jars were stored below freezing temperature between uses.

For the initial canine trials, the collection material was removed from the STU-100 and sealed into the polyethylene, permeable bags, which were then sealed into aluminized bags. For supplementary canine trials, the odor samples were either sealed directly into the aluminized bags or placed into glass jars.

Canine trials

General set-up

For the testing of the training aids with canines, the training aids were stored in glass jars or aluminized bags, as discussed previously. In each canine trial, a row (or rows) of ten cement blocks were placed outdoors on a paved surface approximately five feet apart. The training aids were placed inside of each block and left uncovered. Each block contained a training aid, a blank or was left empty. For the training aids contained in the aluminized bags, the gauze was removed from the bag and placed directly into the cement block. Extra caution was required to prevent the canines from moving the cement blocks and exposing the gauze pads. For the training aids contained in the glass jars, the outer lid was removed, exposing a plastic, perforated lid. The canines could smell the odor inside of the jar, but were not able to make contact with the gauze pad itself even in the case that the cement block was moved.

For each trial, a positive control was run to be sure that the canines were working properly. The handler was asked to choose a training aid to which the canine was known to alert. Any canine that did not alert to the positive control was eliminated from the trial.

Individual trials set ups

Trial 1: Pseudo scent and Odor pad training aids: Preliminary trials

Four sets of ten cement blocks were placed out, each containing a piece of gauze. Training aid odor sources for the Odor pad included fresh human remains, canine remains and cremains. Training aids were also made from the three pseudo scent mixtures listed in Table 15 by spiking 100μL of a 10ppm solution (1μg) onto a Dukal gauze pad. The solvent was allowed to evaporate for one minute before sealing the pads in to permeable bags.

Day 1: All training aids were sealed into low density, polyethylene, permeable bags and subsequently sealed into aluminized bags. The training aids were made one day prior to the trials and were stored indoors at room temperature. The training aids were placed into the four cement block line-ups in the following manner (Table 19). Four canines were run on Day 1. Canines 1 and 4 were considered novices, while canines 2 and 3 were considered experts. The experience level of the canine (i.e. expert or novice) was dictated by the handler before the trial.

Table 19. Training aid set up for Trial 1, Day1.

	Set 1	*Set 2*	*Set 3*	*Set 4*
Block 1	-	-	Pseudo I	-
Block 2	-	-	-	-
Block 3	Fresh remains 1	-	Pseudo II	-
Block 4	-	Canine remains	-	-
Block 5	-	-	Positive control	-
Block 6	-	Blank gauze	-	Pseudo III
Block 7	-	Positive control	-	Positive control
Block 8	-	-	-	-
Block 9	Fresh remains 2	-	-	-
Block 10	-	-	-	Cremains

Day 2, Part 1: New training aids were made from the same scent sources for Day 2. However, this time the permeable bags were not used, as to increase the quantity of scent available to the canines. Again, the training aids were set up in four rows of ten blocks according to Table 20. Canines 2, 3 and 4 were used again along with four additional canines (Canines 5-8). Canines 5, 7 and 8 were novices, and canine 6 was considered an expert.

Table 20. Training aid set up for Trial 1, Day 2, Part 1.

Block #	*Set 1*	*Set 2*	*Set 3*	*Set 4*
Block 1	-	-	Pseudo I	-
Block 2	-	Fresh remains 1	-	-
Block 3	Positive control	-	-	Pseudo III
Block 4	-	-	-	-
Block 5	-	-	-	-
Block 6	-	-	Pseudo II	Cremains
Block 7	-	Canine remains	-	-
Block 8	-	Blank	Fresh remains 1	-
Block 9	-	-	-	-
Block 10	-	-	-	-

Day 2, Part 2: In order to further increase the available odor, multiple gauze pads containing the same odor were placed in a single block. A single set of ten blocks were run. Block 1 contained four scent pads from Fresh remains 1 and one pad from Fresh remains 2; a total of five scent pads. Block 8 contained six scent pads containing Pseudo II. The other blocks contained gauze with no odor. Only the canines that previously responded correctly to the positive control were used.

Trial 2: Pseudo scent

A trial was prepared to assess the canines' interest in the three pseudo scent mixtures. In the first trial, 1µg of the pseudo scent solutions were spiked onto gauze, but the canines did not respond; however when six pads were placed together in a block, the canines alerted. For this reason, in Trial 2, a greater amount of the compound mixtures

were spiked onto Dukal gauze. One hundred-µL of a 60ppm solution (6µg) of each pseudo scent mixture was spiked onto separate gauze pads. The solvent was allowed to evaporate for one minute, and then the pads were sealed into aluminized bags. A blank pad was prepared by spiking the gauze with the solvent alone.

The training aids were placed in ten a row of ten cement blocks according to Table 21. Six canines were tested including two experts and four novices. The training aids were prepared, then immediately shipped and placed into a freezer until use (about one week).

Table 21. Training aid set up for Trial 2.

Block #	*Contents*
Block 1	Pseudo III
Block 2	-
Block 3	Pseudo I
Block 4	Blank
Block 5	-
Block 6	-
Block 7	-
Block 8	-
Block 9	-
Block 10	Pseudo II

Trial 3: Pseudo scent: Concentration

Training aids were prepared from a 60ppm solution of Pseudo II. Three-µg, 6µg, 12µg, and 24µg of the solution were spiked onto Dukal gauze pads. The solvent was

allowed to evaporate for five minutes before sealing the pads into separate aluminized bags. A blank was also prepared with only dichloromethane and placed into an aluminized bag. The training aids were prepared, then immediately shipped and placed into a freezer until use (about one week).

The training aids were placed in one set of ten blocks in the following manner (Table 22). The cement blocks not containing training aids were left empty. The same six canines were used for this trial as were used in Trial 2.

Table 22. Training aid set up for Trial 3.

Block #	*Contents*
Block 1	12µg
Block 2	-
Block 3	3µg
Block 4	-
Block 5	Blank
Block 6	-
Block 7	6µg
Block 8	-
Block 9	24µg
Block 10	-

Trial 4: Pseudo scent: Aluminized bags v. glass jars

Two sets of training aids were made by spiking 50, 100, and 200µL of a 200ppm (10, 20, and 40µg respectively) of Pseudo II solution onto separate pieces of Dukal gauze. Two blanks were also prepared by spiking 120µL of dichloromethane onto Dukal

gauze pads. One set of training aids were sealed into aluminized bags and the second set was placed into glass jars. After preparation, the training aids were immediately shipped to the location of the trial and stored in a freezer until use (less than one week).

Two sets of ten blocks were placed in rows. One set containing the training aids removed from aluminized bags and the other set containing the training aids in glass jars. The same six canines were used as in Trial 2.

Trial 5: Odor pad: Life time of scent in open jars

In order to determine how long a detectable quantity of scent would remain on a training aid exposed to the environment, a series of trials were conducted over a 24 hour period. Scent samples of decomposition fluid on gauze were taken with the STU-100. Two sets were taken using three gauze pads each. Blanks were also prepared by taking a scent sample of an empty jar. Each pad was placed into glass jars as done previously and all jars were immediately shipped to the trial location and stored in a freezer until use (less than one week).

A set of ten blocks was set up according to Table 23. The first trial was run immediately after opening the jars. Additional trials were run two, twelve, and twenty-four hours after the initial opening. Initially, five canines, two expert and three novice canines, were used. For the additional trials, three of the five canines were used, including two experts and one novice canine.

Table 23. Training aid set up for Trial 5.

Block #	*Contents*
Block 1	-
Block 2	Decomp 1
Block 3	-
Block 4	Blank 1
Block 5	-
Block 6	Blank 2
Block 7	-
Block 8	-
Block 9	Decomp 2
Block 10	-

Trial 6: Odor pad: Collection method

Six training aids were prepared by collecting odor with the STU-100 and were placed in separate glass jars in a single line-up of ten cement blocks. The scent source was a piece of gauze soaked in decomposition fluids. The blank sample was prepared by sampling over an empty jar of the same type. The number of pads per training aid and the length of collection with the STU-100 were varied (Table 24) as a method of varying the scent quantity on the gauze pads. Following preparation, the training aids were immediately shipped to the training location and stored below freezing until use less than a week later. Six canines were used, two experts and four novice.

Table 24. Training aids created for Trial 6.

Sample	*Number of gauze pads*	*Length of collection*
1	1	1 min
2	3	1 min
3	6	1 min
4	1	5 min
5	1	10 min
Blank	3	1 min

Trial 7: Odor pad: Assortment of scent sources

Five training aids and one blank were prepared using the STU-100. For each training aid, scent was collected onto a single Dukal gauze pad over a period of three minutes. The scent sources consisted of gauze material that had been soaked in decomposition fluid, soaked in blood, wiped over bone, wiped over a freshly deceased body, or wiped over adipocere. All the scent sources were stored in separate glass jars below freezing temperatures.

Ten cement blocks were placed in a line-up containing the five training aids, blank gauze and empty jars (Table 25). Eight canines were used, three novice, two intermediate and three expert.

Table 25. Training aid set up for Trial 7.

Block #	*Contents*
Block 1	Empty jar
Block 2	Blood
Block 3	Fresh remains
Block 4	Decomp fluid
Block 5	Adipocere
Block 6	Blank
Block 7	Bone
Block 8	Empty jar
Block 9	Empty jar
Block 10	Empty jar

Population Study:

A population study was conducted to evaluate the response of trained HRD canines to the previously created training aids. The study was carried out utilizing a number of canines, from novice to expert, training by different handlers and maintained under different agencies. The participating canine / handler teams included in the study are listed in Table 26 along with the estimated level of expertise, and type of positive control used during evaluation.

Table 26. Canine / handler teams, experience level of such teams, and type of positive controls used in population study.			
Handler	**Canine**	**Experience**	**+ Control**
Handler B	B1	Expert	Gauze with decomp fluid
	B2	Novice	Gauze with decomp fluid
	B3	Intermediate	Gauze with decomp fluid
	B4	Intermediate	Gauze with decomp fluid
	B5	Expert	Gauze with decomp fluid
Handler MI	MI1	Expert	Decomposed arm (bone and tissue)
	MI2	Expert	Decomposed arm (bone and tissue)
Handler MA	MA1	Expert	Blood and grave dirt
	MA2	Intermediate	Blood and grave dirt
	MA3	Expert	Blood and grave dirt
	MA4	Expert	Blood and grave dirt
Handler CH	CH1	Intermediate	Dried blood
	CH2	Expert	Dried blood
	CH3	Expert	Dried blood
	CH4	Novice	Dried blood
	CH5	Expert	Dried blood
	CH6	Novice	Dried blood
	CH7	Intermediate	Dried blood
Handler AM	AM1	Expert	Liquified flesh
	AM2	Expert	Liquified flesh
	AM3	Intermediate	Liquified flesh
	AM4	Novice	Liquified flesh
	AM5	Expert	Liquified flesh
	AM6	Novice	Liquified flesh
	AM7	Novice	Liquified flesh
	AM8	Expert	Liquified flesh

Each group was sent two identical sets of training aids. One set packaged in aluminized bags and the other set in glass jars. The training aids were prepared using the STU-100 on the lowest flow rate for 3 minutes. A single Dukal gauze pad was used for each aid. The odor sources included a blank, two distracters and two human remains sources. The human remains sources were decomposition fluid on gauze and a freshly

deceased body. The distracter sources included the remains of a whole chicken and live human. The blank was prepared by sampling an empty glass jar. The training aids were labeled A-E, and the label for each training aid was determined by a random number generator for each training aid set. Immediately after the preparation of the training aids, the kits were mailed to each participating group with instructions to keep the kit in a freezer until use.

Each group of canines involved in the trial was given specific instructions regarding trial set up. These instructions followed the same methodology as the previous canine trials. Data sheets were provided for each handler to fill out during the trial. Examples of the instructions and data sheets are given in Appendices 3 and 4.

3.4. Results / Discussion

3.4.1. Standard Compound Selection

Six standard compounds, previously documented to be present in human remains odor, were chosen to represent human remains volatiles (Table 27). All compounds show good chromatographic separation; however, the peak shape for the acids is poor, as they tend to thermally decompose during injection (Figure 27). Dimethyl trisulfide was initially included in the standard compound mix, but was removed for reasons to be discussed below.

Table 27. Standard compounds used in study.			
Compound	**Functional Group**	**Molecular Weight**	**Literature cited**
6-Methyl-5-hepten-2-one	Ketone	126.2	ST
Nonanal	Aldehyde	142.2	HR5, ST
Phenol	Alcohol	94.11	HR1. ST
Heptanoic acid	Acid	130.2	F2, ST
Butyric acid	Acid	88.11	HR5, F2, ST
Dimethyl Disulfide	Sulfide	94.2	1HR1-5, F2

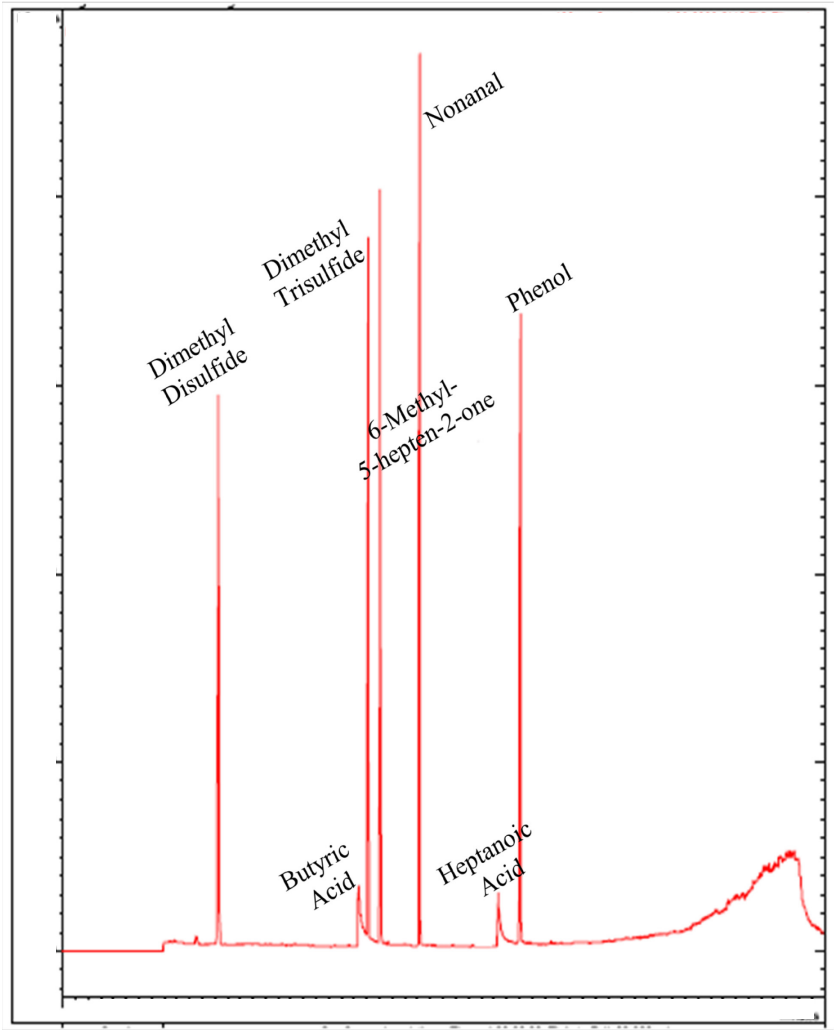
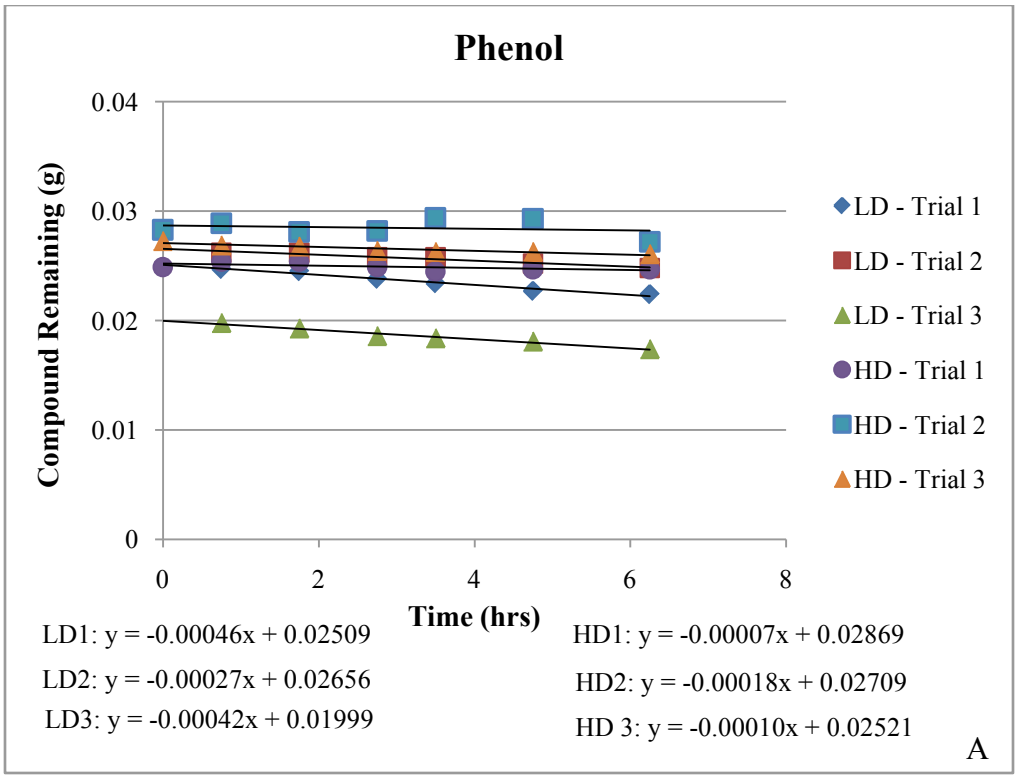
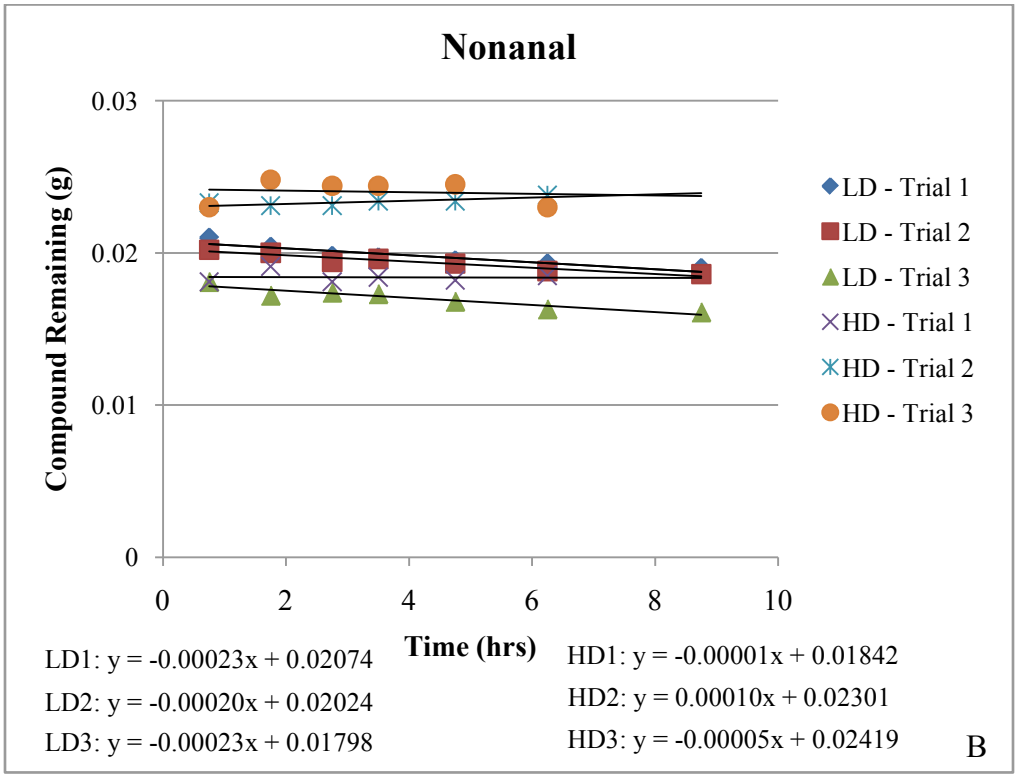


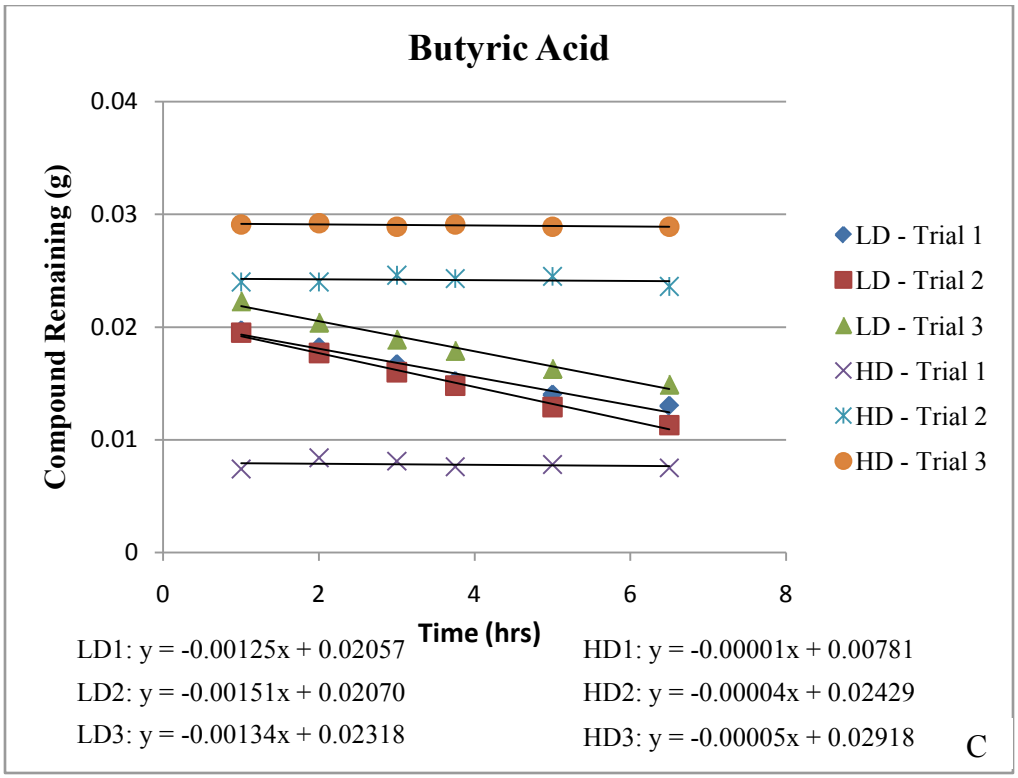
Figure 27. Chromatogram of compounds chosen for study.

3.4.2. Controlled Odor Mimic Permeation Systems

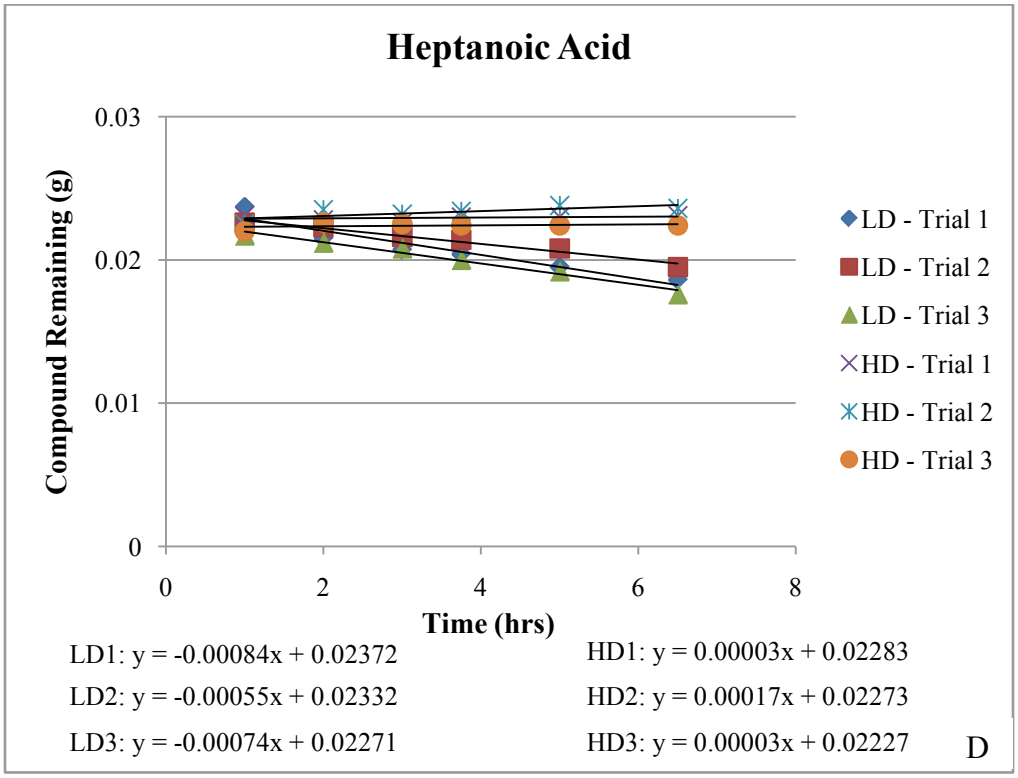
The STU-100 was optimized for human remains scent collection using COMPS created in such a manner to reliably and reproducibly deliver representative human remains compounds to the STU-100 at known rates. The compounds were spiked onto gauze and sealed into permeable bags. Initially, two sets of COMPS were created for each compound, with one set sealed in low density, permeable bags and the other in high density, permeable bags. After spiking 25μL of each compound onto the gauze, the changing mass of the gauze/bag was measured over time. The amount of compound remaining was plotted against time in minutes for each compound, and a best fit line was fitted to the linear section of the graph, the slope of which is the rate of dissipation (Figure 28a-f). The dissipation rate for each compound was determined for both types of bags in triplicate and averaged (Table 28).


Phenol
Compound Remaining (g)
0.04
0.03
0.02
0.01
0
0
2
4
6
8
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.00046x + 0.02509
LD2: y = -0.00027x + 0.02656
LD3: y = -0.00042x + 0.01999
HD1: y = -0.00007x + 0.02869
HD2: y = -0.00018x + 0.02709
HD 3: y = -0.00010x + 0.02521
A

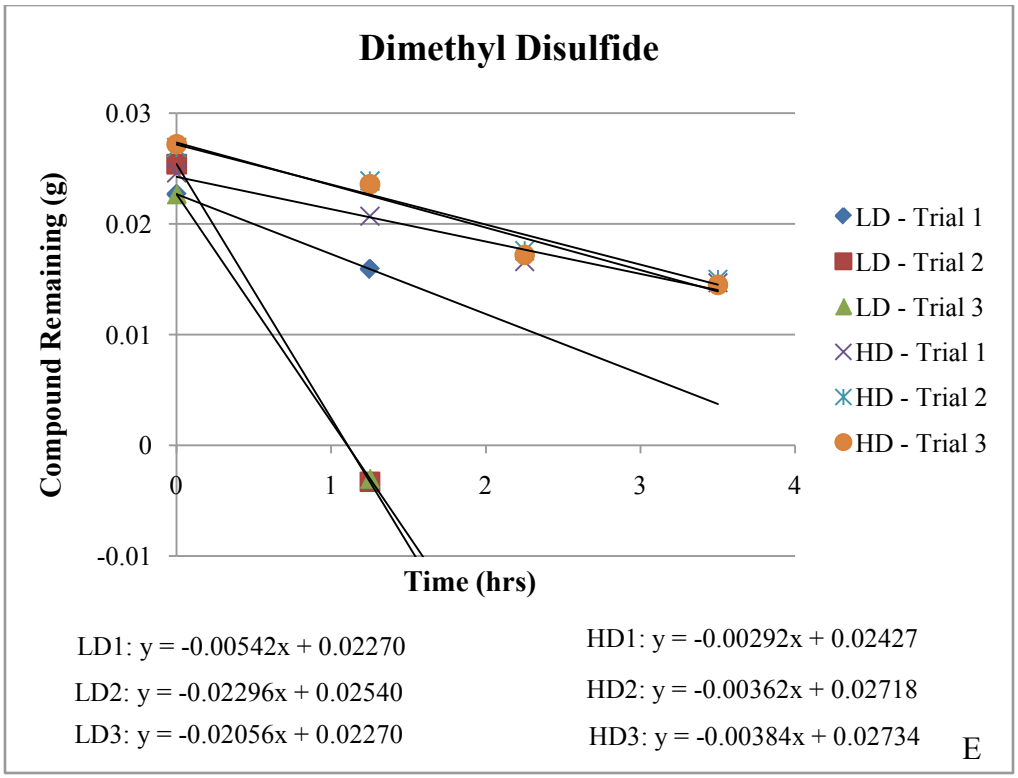

Nonanal
Compound Remaining (g)
0.03
0.02
0.01
0
0
2
4
6
8
10
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.00023x + 0.02074
LD2: y = -0.00020x + 0.02024
LD3: y = -0.00023x + 0.01798
HD1: y = -0.00001x + 0.01842
HD2: y = 0.00010x + 0.02301
HD3: y = -0.00005x + 0.02419
B


Butyric Acid
Compound Remaining (g)
0.04
0.03
0.02
0.01
0
0
2
4
6
8
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.00125x + 0.02057
LD2: y = -0.00151x + 0.02070
LD3: y = -0.00134x + 0.02318
HD1: y = -0.00001x + 0.00781
HD2: y = -0.00004x + 0.02429
HD3: y = -0.00005x + 0.02918

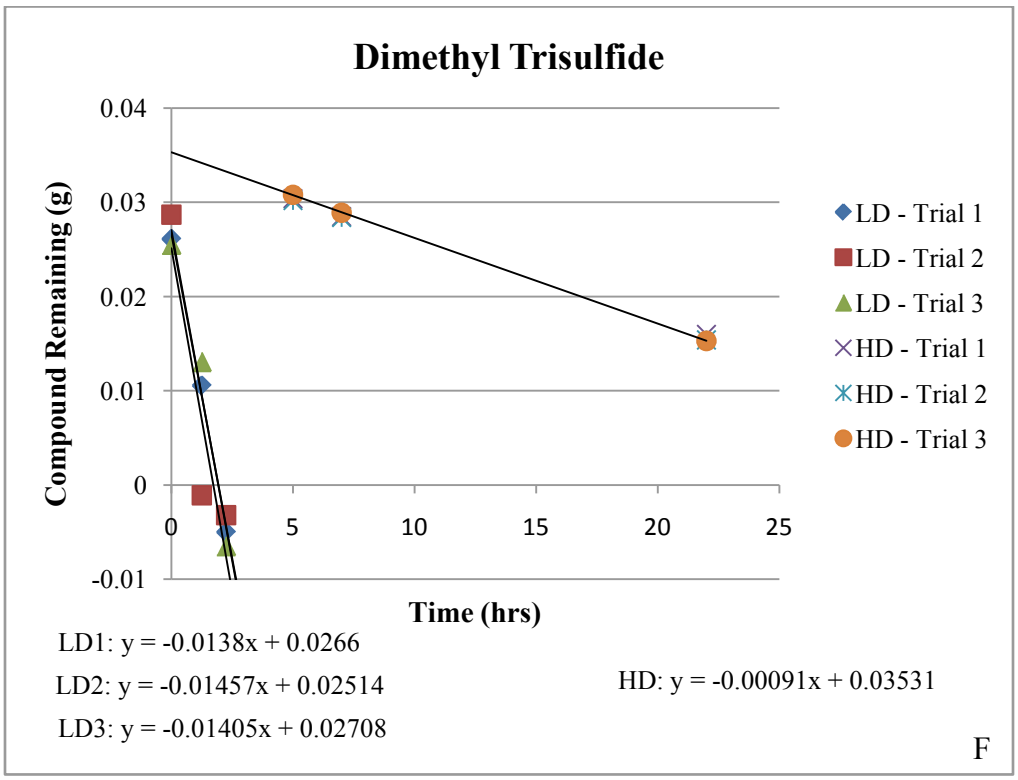
C


Heptanoic Acid
Compound Remaining (g)
0.03
0.02
0.01
0
0
2
4
6
8
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.00084x + 0.02372
LD2: y = -0.00055x + 0.02332
LD3: y = -0.00074x + 0.02271
HD1: y = 0.00003x + 0.02283
HD2: y = 0.00017x + 0.02273
HD3: y = 0.00003x + 0.02227

D


Dimethyl Disulfide
Compound Remaining (g)
0.03
0.02
0.01
0
-0.01
0
1
2
3
4
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.00542x + 0.02270
LD2: y = -0.02296x + 0.02540
LD3: y = -0.02056x + 0.02270
HD1: y = -0.00292x + 0.02427
HD2: y = -0.00362x + 0.02718
HD3: y = -0.00384x + 0.02734

E


Dimethyl Trisulfide
Compound Remaining (g)
0.04
0.03
0.02
0.01
0
-0.01
0
5
10
15
20
25
Time (hrs)
LD - Trial 1
LD - Trial 2
LD - Trial 3
HD - Trial 1
HD - Trial 2
HD - Trial 3
LD1: y = -0.0138x + 0.0266
LD2: y = -0.01457x + 0.02514
LD3: y = -0.01405x + 0.02708
HD: y = -0.00091x + 0.03531

F

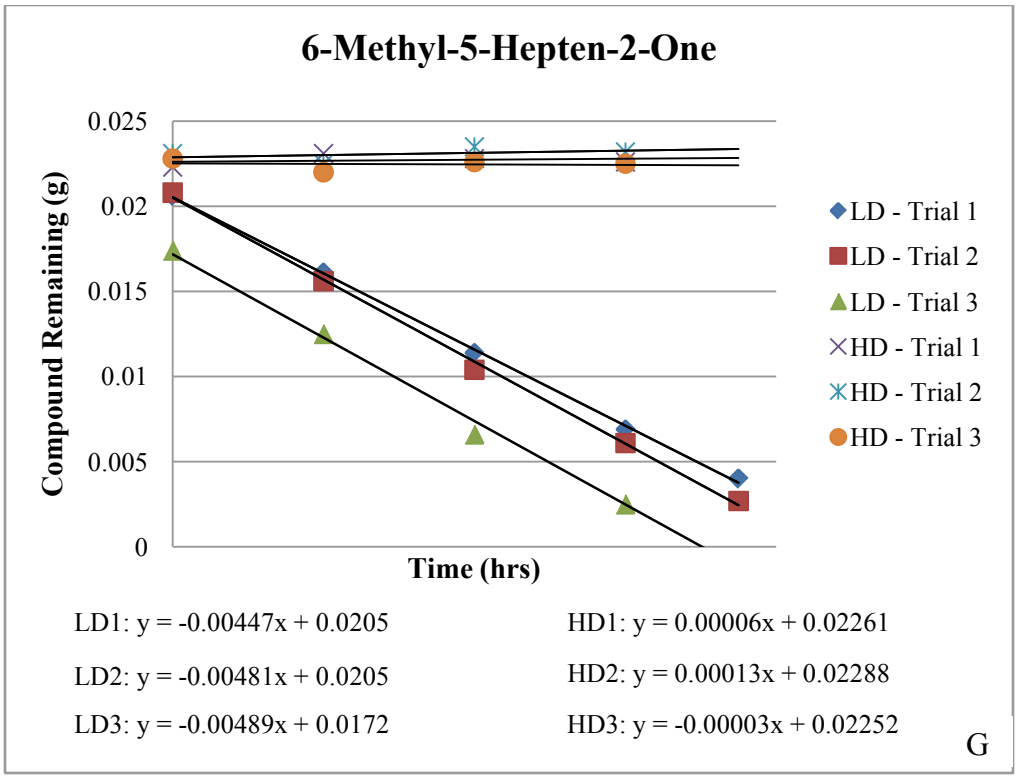


Figure 28a-f. Dissipation rates of standard compounds; a.) phenol; b.) nonanal; c.) butyric acid; d.) heptanoic acid; e.) dimethyl disulfide; f.) dimethyl trisulfide; g.) 6-methyl-5-hepten-2-one.

Table 28. Dissipation rates of standard compounds through high and low density, permeable bags in g/hr.

Compound	LD (avg)	LD (std dev)	HD (avg)	HD (std dev)
Nonanal	*0.00022*	0.00002	-0.00001	0.00008
Phenol	*0.00038*	0.00010	0.00012	0.00006
Heptanoic acid	*0.00071*	0.00015	-0.00008	0.00008
Butyric acid	*0.00258*	0.00223	0.00003	0.00002
Dimethyl disulfide	0.01631	0.00951	*0.00346*	0.00048
6-Methyl-5-hepten-2-one	*0.00473*	0.00023	-0.00005	0.00008
Dimethyl trisulfide	0.01414	0.00039	*0.00091*	0.00000

To keep the difference between dissipation rates less than approximately one order of magnitude, the low density polymer, bags were chosen for nonanal, phenol, heptanoic acid, butyric acid and 6-methyl-5-hepten-2-one and the high density, polymer

bags were chosen for dimethyl disulfide and dimethyl trisulfide. With the exception of dimethyl trisulfide, the dissipation rates of all the compounds were steady (i.e. the graph is linear) at the two hour point. Dimethyl trisulfide did not reach a constant rate of dissipation until at least five hours. For that reason, and because a similar sulfide compound was already represented in the group of standard compounds, dimethyl trisulfide was eliminated from further experimentation with COMPS. For future sampling, the COMPS were left to equilibrate for two hours before sampling with the STU-100.

3.4.3. Selection of Fiber Chemistry / Exposure Time

Four types of SPME fibers were evaluated using a standard compound mix spiked onto gauze and sealed into 10mL vials. The compounds were extracted from the headspace for times varying from five minutes to 180 minutes. The quantity of each compound recovered from the headspace is illustrated in

Figure 29. Phenol was not collected at any exposure time using the blue and black fibers except in extremely small quantities. A greater amount of phenol was able to be recovered using the grey and white fibers; however, the quantity recovered was still minimal. Little or no 6-methyl-5-hepten-2-one and nonanal were recovered from the headspace using the black fiber. For these reasons, the black and blue fibers were eliminated. The grey and white fibers performed to about the same level; however, at its greatest extraction time (20min), the total amount of all compounds collected using the

grey fiber surpassed that of the white fiber. The grey fiber was thus chosen for further evaluations.

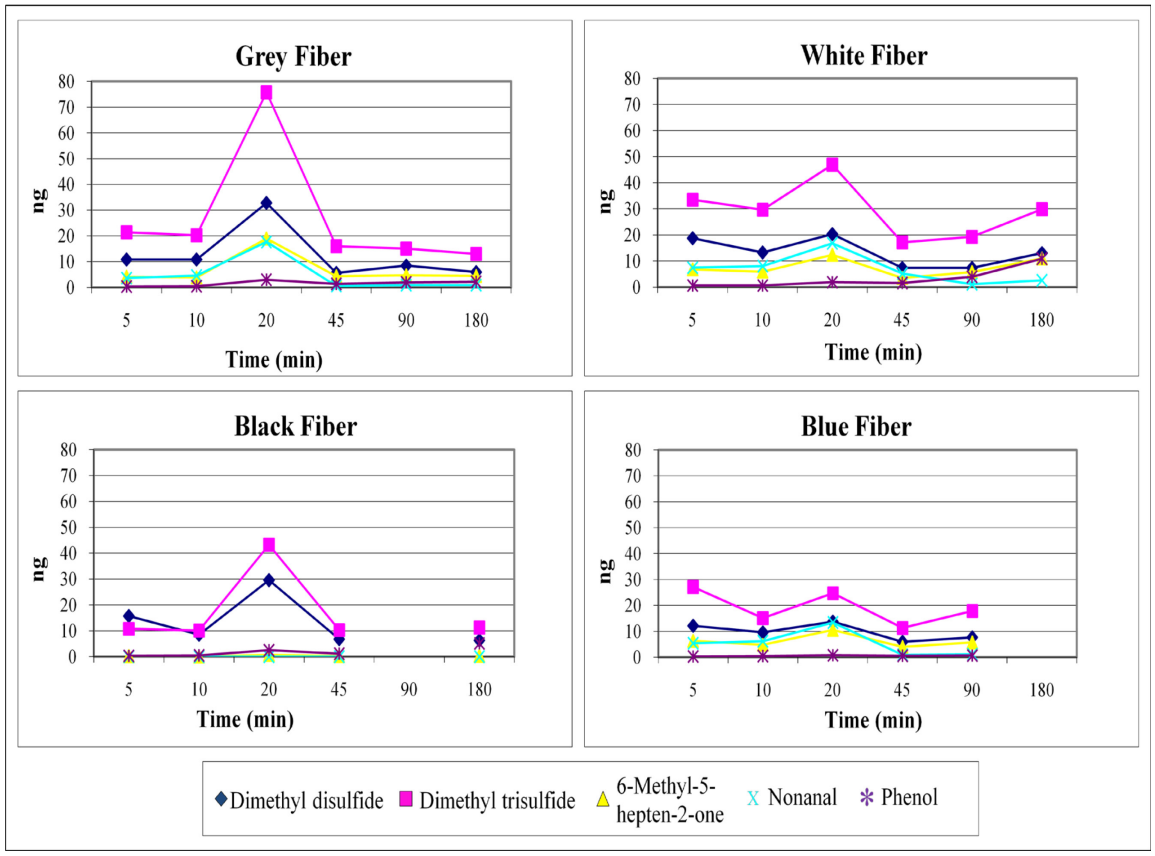


Figure 29. Quantity of standard compounds collected from different SPME fibers at varying exposure lengths.

Fiber exposure times were examined again using only the grey fiber. This time samples were collected with the STU-100 using COMPS and a larger range of extraction times were evaluated as it was previously shown that longer extraction times were optimal for the extraction of human scent volatiles from gauze. The results are given in Figure 30. The compounds in the headspace reached equilibrium between the headspace

and the fiber sometime after six hours and before twenty, thus twenty hours was chosen for the extraction time.

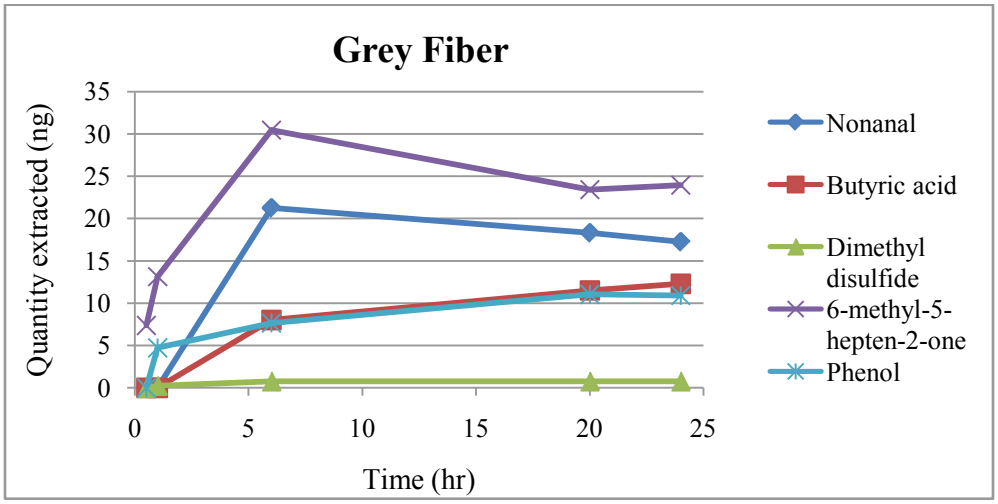


Figure 30. Quantity of standard compound recovered using the grey SPME fiber at varying exposure lengths.

3.4.4. Optimization of the Scent Transfer Unit

Flow rate / Material

The quantities of standard compounds collected by the STU-100 at each flow rate/ material combination were compared (Figure 31). The STU-100 used with no vacuum yielded the least amount of compounds in the headspace, confirming that little volatiles can be passively collected onto material (with no contact). Dimethyl disulfide was not detected in any circumstance, likely because of its extremely high volatility (vapor pressure = 22mmHg). There was no statistical difference between the flow rates using the polyester material, as determined by ANOVA (Table 29). For the Dukal and Johnson and Johnson gauzes, the quantities of compound collected at each flow rate were

significantly different. In particular, for the Dukal gauze the amount collected was much higher for the low (0) flow rate than for the high (9) flow rate. This is likely the result of compound breakthrough, which tends to be more prominent in the Dukal gauze than the Johnson and Johnson gauze, as Dukal gauze has a more open weave allowing more volatiles to pass through without being deposited.

Table 29. Use of ANOVA (two factor without replication) to determine the variation between quantity of compounds recovered from different numbers of material layers.

	F_{calc} (F_{crit} =3.490)	Significant difference?
Dukal	7.769	yes
Polyester	1.213	No
J&J	6.048	Yes

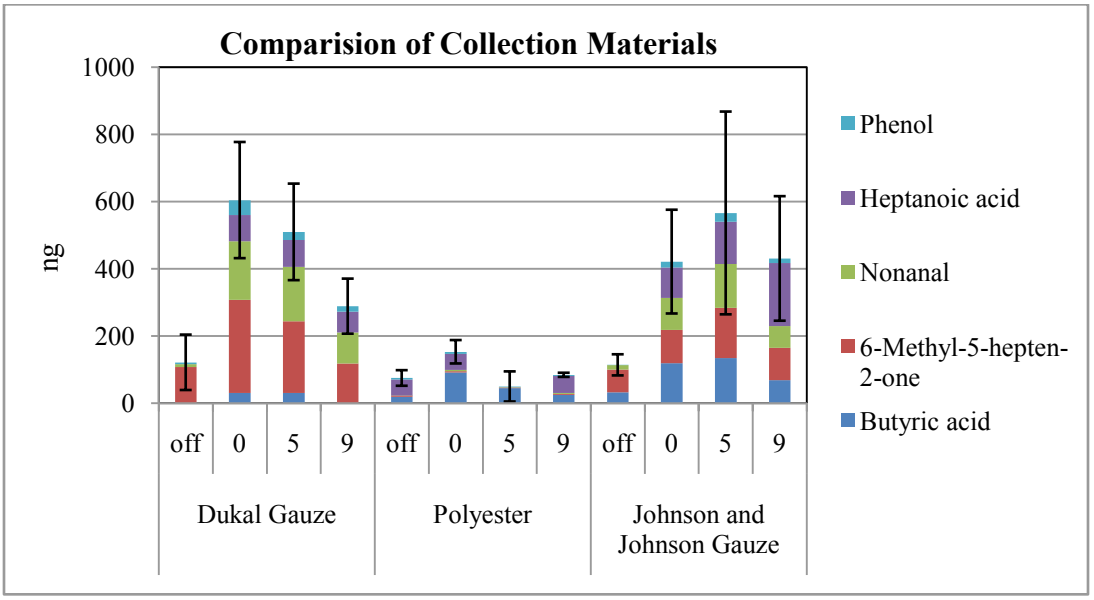


Figure 31. Quantity of compounds recovered from three collection materials.

The least amounts of standard compounds were collected from the polyester at all flow rates. For the polyester, the presence of the ketone was below detection limits at all flow rates, and only minimal amounts of the alcohol and aldehyde were collected. Only the acids were trapped/released in moderate quantities with the polyester material. There are likely several reasons for the low quantity of compounds seen in the headspace of the polyester. As hypothesized previously, it is likely that the thicker gauze materials prevented the breakthrough of the compounds compared to the polyester. The three layers of the polyester material were used for collection may not have been thick enough to prevent the compounds from being pulled past the collection materials before being deposited. Additionally, the polyester material is structurally different at a molecular level, compared to the gauze materials, which may also affect its ability to trap and/or release compounds. The Dukal brand gauze is made entirely of cotton, which has a cellulose backbone containing many free hydroxyl groups. The Johnson and Johnson gauze is a cotton blend, containing rayon (a man-made cellulose-based material), polyester, and cotton. Polyester contains a cellular backbone of a long chain synthetic polymer held together by ester bonds with no free hydroxyl groups. The structural differences likely affect the ability of a material to trap and/or release compounds.

Material layering

Because the polyester material showed some promise for the collection of acids, it was layered with the gauze materials for collection by the STU-100. The total quantity of

standard compounds collected in this manner is represented in Figure 32. The STU-100 collection with the Dukal gauze and polyester material showed little changed compared to the Dukal gauze alone across all flow rates except for a slight increase in the collection of the butyric acid. The butyric acid was not detectable from the Dukal gauze alone at some flow rates, but with the addition of the polyester material, butyric acid could be collected at all flow rates. The combination of the Johnson and Johnson gauze and polyester yielded a consistent increase in the amount of heptanoic acid collected across all flow rates, however this yielded a decrease in the quantity of all other compounds. This could be due to competition for binding sites either on the material itself or the SPME fiber. The Dukal gauze / polyester combination was used at the low flow rate for further sample collections with the STU-100

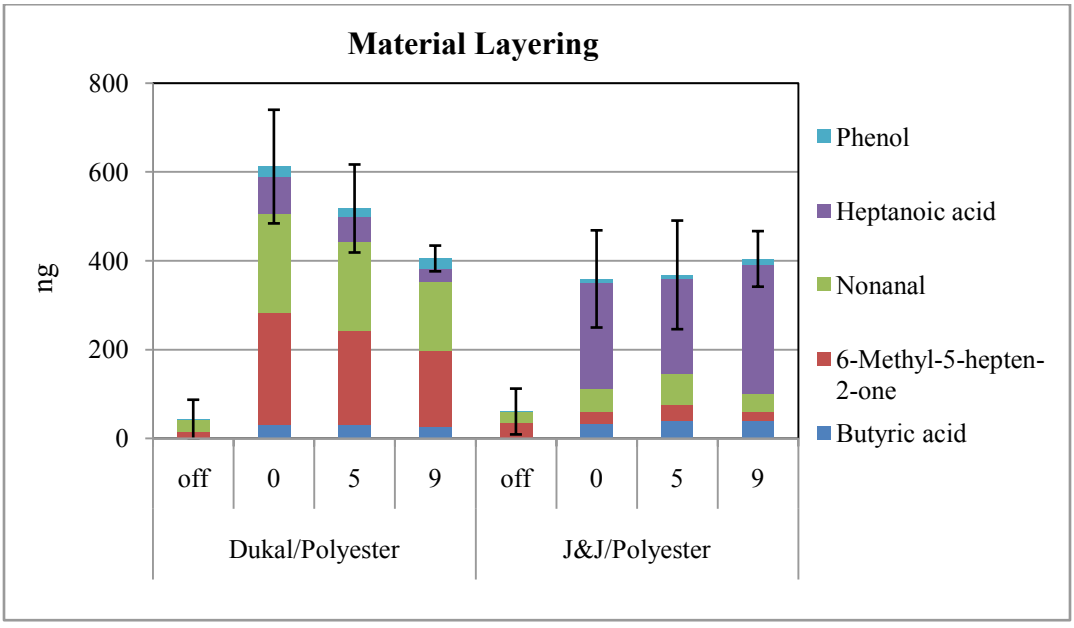


Figure 32. Quantity of compounds recovered from two material combinations.

3.4.5. Identification of Human Remains Odorants

Project discussion

A study was conducted to determine the type and ratio of VOCs from human remains using the STU-100 as a collection and concentration tool. To do so, a number of deceased human bodies were sampled with the STU-100 using the previously optimized method. The type and ratio of compounds, also known as the odor profile, were compared. Compounds that remained constant in all deceased subject profiles were considered signature compounds.

Initial results

The first set of human remains samples were taken from the Miami-Dade morgue. All of the subjects sampled were in stage one or two decomposition and had all clothing, body bags, etc. removed whenever possible. Table 30 lists the compounds collected from the first eight subjects sampled. All compounds listed were confirmed by MS library and retention time based on standard compounds. The subjects estimated to be in stage one decomposition, or fresh, are indicated by F, and those subjects estimated to be in stage two decomposition are indicated by D (decomposed).

Table 30. Compounds recovered from human remains samples collected with the STU-100.								
Compound	**D1**	**D2**	**D3**	**F1**	**F2**	**F3**	**F4**	**F5**
Acetic acid	X	X	X	X	X	X	X	X
Hexanal	X					X		
Tridecane	X			X	X	X		
2-Ethyl-1-hexanol	X	X	X	X	X	X	X	X
Benzaldehyde	X	X	X	X	X	X	X	X
1-Octanol	X							
Nonanal	X	X	X	X	X	X	X	X
Tetradecane	X		X	X	X	X		
Decanal	X	X	X	X	X	X	X	X
Pentadecane	X		X	X	X	X		
Hexadecane	X		X	X	X	X		
Phenol	X			X	X	X		
Heptadecane	X			X	X	X		
Octanal	X					X		

Of the fourteen compounds identified, five were detected in all eight samples, including acetic acid, 2-ethyl-1-hexanol, benzaldehyde, nonanal, and decanal. Other predominant compounds included tetradecane, pentadecane and hexadecane. When compared to compounds seen in previously published literature, one would expect to see such compounds as trichloroethylene, dimethyl disulfide and trisulfide and p-xylene but none of which were detected. Further optimization of the extraction and analysis methods should be done to enhance volatile collection, and will be discussed below.

Method Optimization

Some compounds that one would expect to be associated with human remains VOCs were not recovered in initial experiments. It was unknown if this is because the compounds were simply not present on the collection material, were not extracted from the headspace, or were lost for some other reason. Attempts to further optimize the extraction and injection method were made in order to minimize such issues.

SPME bias / SPME fiber exposure time

The first issue that was considered was that of SPME bias. Because of the lengthy exposure time used, it was possible that bias was occurring due to competition for binding sites on the fibers, causing the loss of potentially important compounds. This was not initially considered during optimization because only a few standard compounds were analyzed and no issues were observed, but in actual samples where there are many more compounds present, bias may become more of an issue as there are a wider variety of molecules competing for binding sites on the SPME fiber. If bias was occurring due to long fiber exposure times, one would expect the presence of certain compounds at the shorter extraction times that would be lost at longer extraction times. A standard mixture of a wider variety compounds was spiked onto gauze and the headspace was extracted for varying lengths of time. The quantities of compounds recovered for each extraction time were compared (Figure 33).

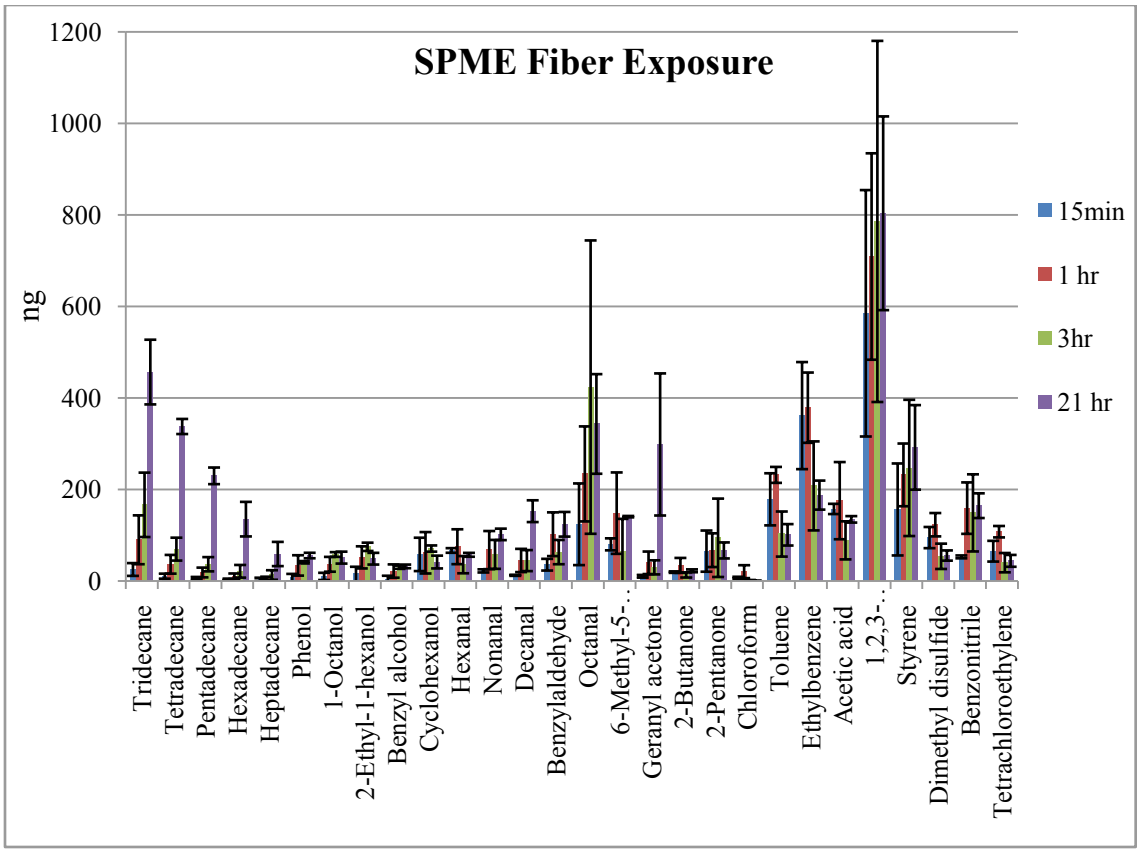


Figure 33. Quantity of compounds recovered by SPME at varying exposure times.

On the basis of the results in Figure 33, chloroform, toluene, ethylbenzene and dimethyl disulfide showed significantly higher responses at the shorter exposure times. Thus, the absence of compounds, such as dimethyl disulfide, from remains samples may be the result of long SPME exposure times and not caused by the nonexistence of the compound from the actual samples or its high vapor pressure. The use of shorter exposure times for future extractions was considered.

Split v. splitless injection

In an attempt to improve compound recovery, split and splitless GC injection methods were compared using a large standard compound mixture. The liquid mixture was injected into the GC under both split (10:1) and splitless conditions, and the chromatograms were compared. The chromatograms from split ad splitless injections of a 50ppm liquid solution are compared in Figure 34. The same was repeated using actual human remains samples collected by the STU-100 and extracted by SPME (Figure 35).

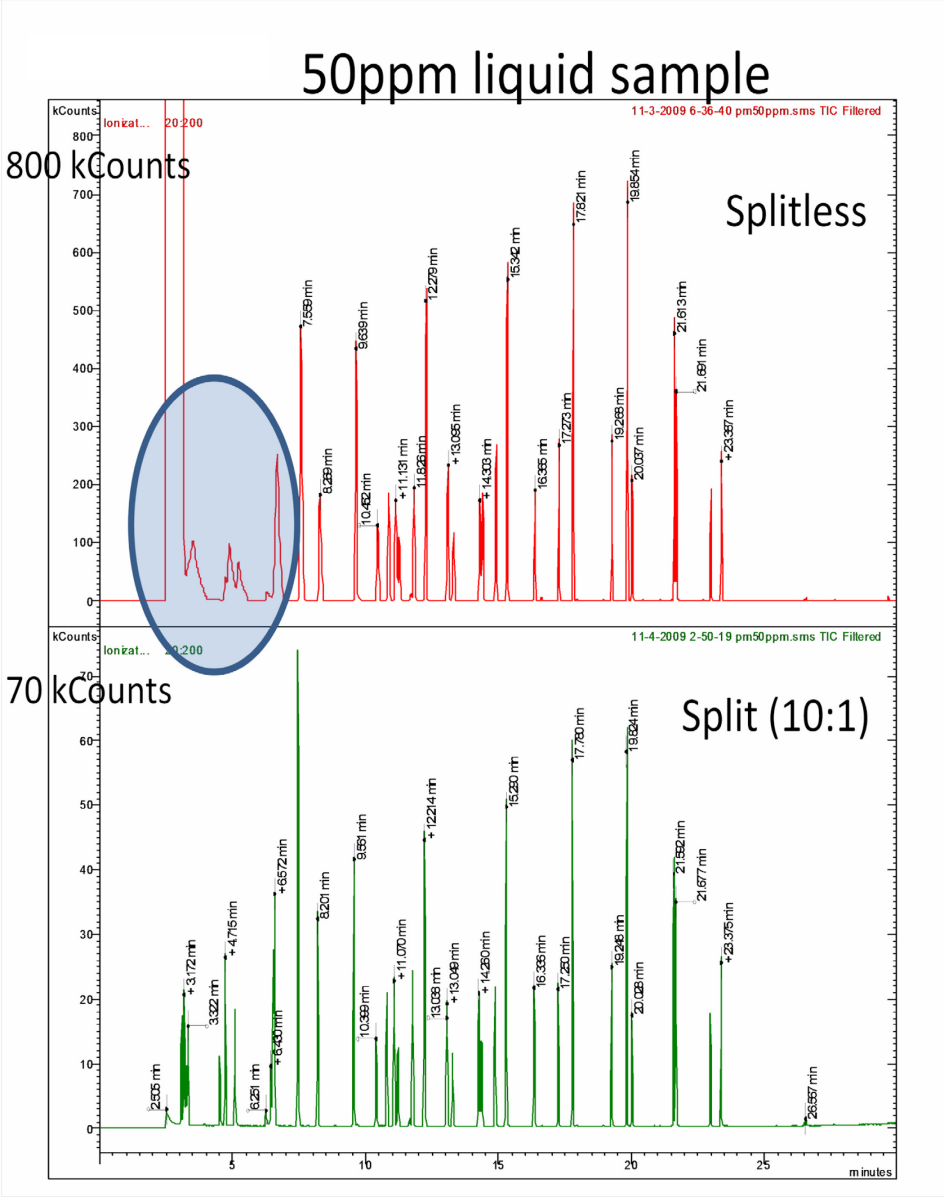


Figure 34. Chromatograms comparing the split and splitless injection of a 50ppm standard mixture.

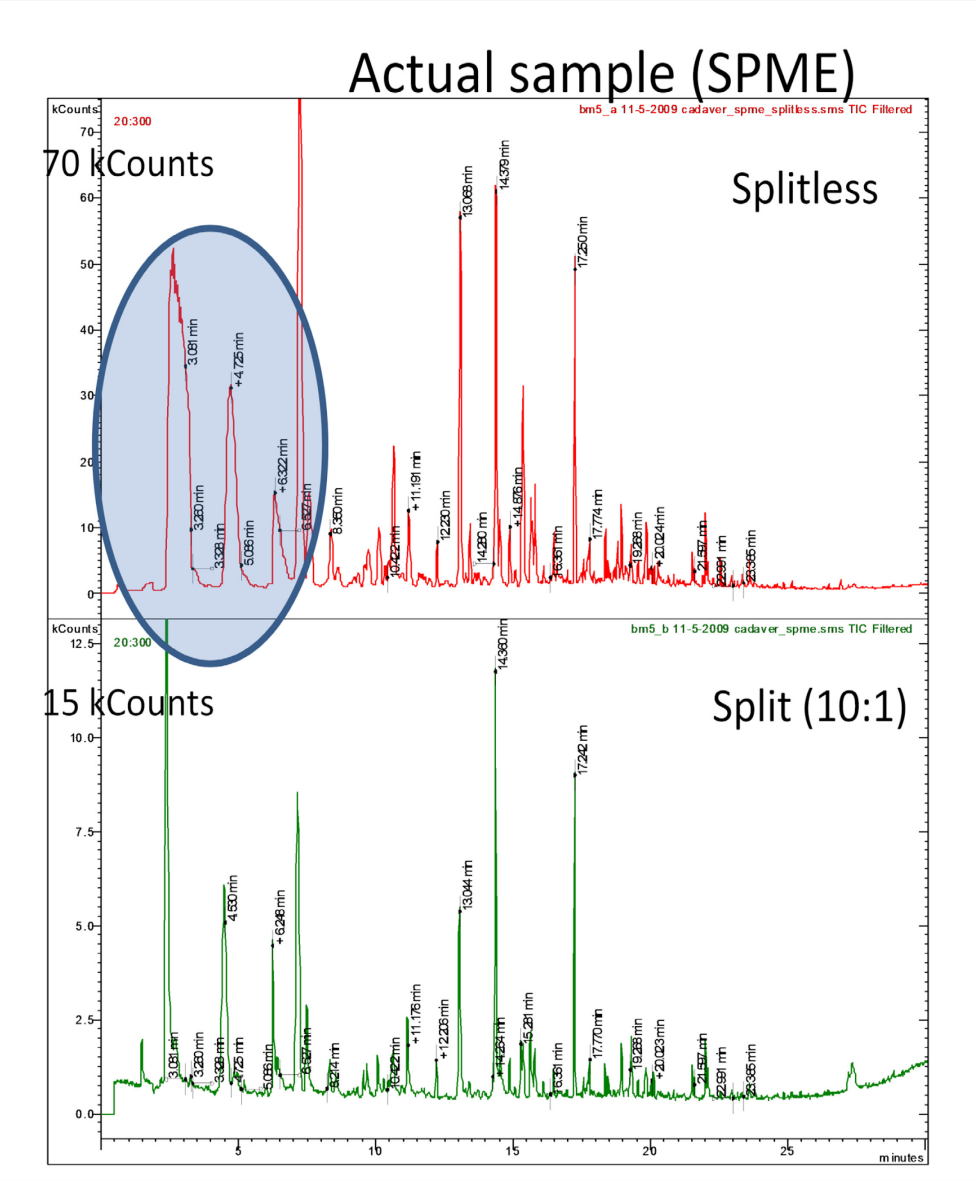


Figure 35. Chromatograms comparing split and splitless injection of human remains samples extracted by SPME.

The peak heights were about an order of magnitude larger using splitless injection yielding improved sensitivity. Along with the improved sensitivity, however, comes the loss of early eluting compounds due to peak broadening, causing detection issues for these compounds. The early eluting compounds also tend to be the compounds lost with longer exposure times, and thus for this reason, two sampling methods were created. For further experimentation, all samples were analyzed with first a shorter exposure time (30min) and split injection (10:1) for improved detection, followed by a longer exposure time and splitless injection for improved sensitivity. The early eluting compounds to be extracted with the shorter exposure time and the later eluting compounds to be extracted with the longer exposure time are listed in Table 31.

Table 31. Compounds used in the standard mixture.

30min exposure	20 hour exposure	
Chloroform	Butyric acid	Tetradecane
Toluene	1,2,3-Trimethyl benzene	Benzonitrile
Dimethyl disulfide	Dimethyl disulfide	Dimethyl adipate
Acetic acid	2-Furaldehyde	Benzoic acid, methyl ester
Hexanal	6-Methyl-5-hepten-2-one	Decanal
o-Xylene	Tridecane	Pentadecane
p-Xylene	2-Ethyl-1-hexanol	Benzyl alcohol
Undecane	Furfuryl alcohol	Hexadecane
Styrene	Octanoic acid, methyl ester	Phenol
2-Methyl propanoic acid	Benzaldehyde	Octanoic acid
	Nonanal	Heptadecane
	2-2-Methoxyethoxy ethanol	Geranyl acetone
	1-Octanol	

Heating samples

Vials containing the collection material were heated to increase the amount of VOCs in the headspace. The standard compound mix was spiked onto Dukal gauze and closed into the sample vials. The sample vials were heated over night, during equilibration and extraction. This was duplicated for both short and long exposure times in triplicate. For the thirty minute exposure, the vials were heated to 37°C (body temperature, ± 3°C) and 50°C (± 3°C) and compared to vials left at room temperature (24°C ± 2°). On the basis of the results in Figure 36, there is no significant difference between the quantities of compounds collected at each of the temperatures. This is likely because these compounds are already highly volatile, and heating yields no further improvement.

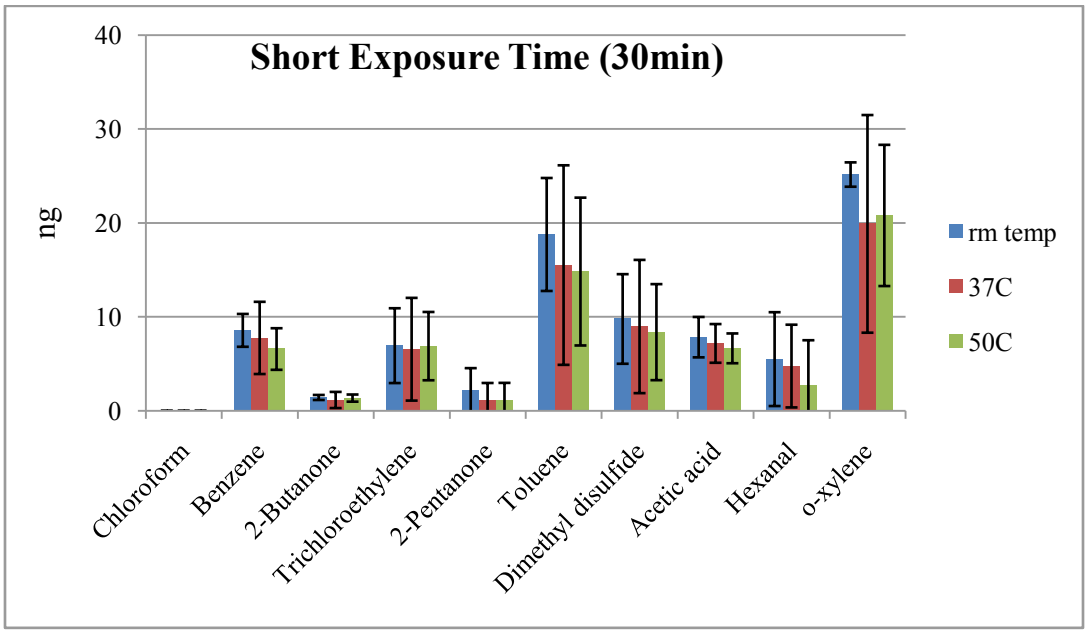


Figure 36. Total quantity of compound recovered from a 30 min extraction when sampled are heated during equilibration and extraction.

For the longer exposure times, sample vials were heated to 37°C (body temperature), 50°C, 60°C, 70°C, and 90°C (± 3°C) and compared to vials left at room temperature (23°C ± 1°). The vials heated to 90°C yielded the greatest quantity of compound for the majority of the compound (Figure 37); however, there was a reduction in a few of the early eluters at the higher temperatures. For this reason, the temperature of 70°C was chosen for use with the 20-hour extractions during future experimentation.

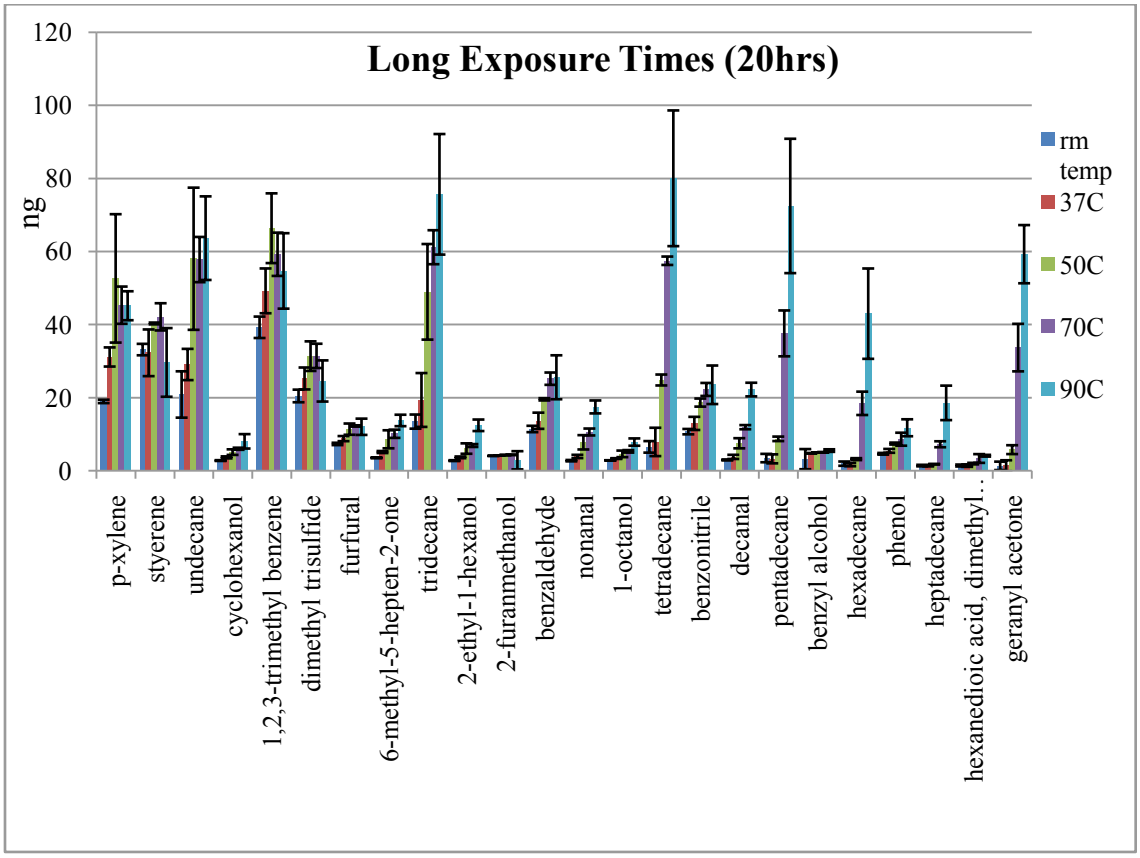


Figure 37. Total quantity of compound recovered from a 20 hour extraction when sampled are heated during equilibration and extraction.

Final results

Miami-Dade Medical Examiner

Twenty-one deceased human bodies from the Miami-Dade morgue were sampled using the STU-100 and the previously optimized collection, extraction and analysis parameters. The compounds recovered and the numbers of occurrences are listed in Table 32. All compounds were confirmed by the MS library and retention time comparison to standard compounds, with the exception of the compounds listed with asterisks (*), which were not able to be confirmed with standard compounds Sixteen of the compounds listed below were identified in at least 19 of the 21 samples (90%).

Table 32. Compounds recovered from human remains samples using optimized extraction and analysis methods.	
Compound	**# of occurrences**
Acetic acid	21
Styrene	21
2-Furaldehyde	21
6-Methyl-5-hepten-2-one	21
2-Ethyl-1-hexanol	21
Benzaldehyde	21
Nonanal	21
1-Octanol	21
Benzonitrile	21
Decanal	21
Pentadecane	21
Hexadecane	21
Phenol	21
Heptadecane	21
1,2,3-Trimethylbenzene	20
Benzyl alcohol	19
Tridecane	17
Toluene	16
Dimethyl trisulfide	15
2-Butoxy ethanol*	14
Naphthalene*	13
Tetradecane	12
2-Methyl propanoic acid	12
Undecanoic acid, 10 methyl-methyl ester*	11
5-Methyl-2-(1-methylethyl)-cyclohexanol*	10
Furfuryl alcohol	9
2-Pentadecyn-1-ol*	9
1-4-(1-Hydroxy-1-methylethyl)phenyl ethanone*	8
Methoxy phenyl oxime*	7
1,2,3,4-tetramethyl benzene*	6
2-(2-methyoxyethoxy)ethanol*	6
Decanoic acid, methyl ester*	5
Hexanoic acid*	4
Geranyl acetone	3
2-(2-(2-Ethyoxyethoxy) ethoxy-ethanol*	3
7-Octen-2-ol, 2,6 dimethyl*	3

The optimized extraction and analysis methods allowed for a greater variety of compounds to be recovered, including dimethyl trisulfide and toluene, which had been expected to be present. Trichloroethylene, p-xylene, and dimethyl disulfide were still not recovered from the samples though they are documented in multiple sources to be comments of remains odor. The compounds may have not been seen because they were not trapped or released by the collection material, because they may have been lost during the extraction/analysis process, or they were simply not present. Dimethyl disulfide and trichloroethylene have particularly high vapor pressures (22mmHg and 75mmHg) and thus may dissipate to the surroundings before they can be captured. It should also be noted that compounds listed in other publications were sampled from either bodies in stages four and five of decomposition[103,104] or from only body parts,[39] thus the scent compounds may not be the same as collected here.

The compounds collected from stage one and stage two decomposition samples were compared in type and ratio (Figure 38). Only the compounds found in 66% or more of the samples were considered in the comparison. Each color bar in the graph represents a different compound and the thickness of the bar represents the relative quantity. This type of graph is known as a scent profile. The scent profiles from each group of samples were nearly identical, indicating that odor of stage one and stage two decomposition are similar. These preliminary results suggest the existence of universal compounds in the odor of recently deceased human material which could eventually be used as an indicator of the existence of a deceased body or as the basis for creating new training aids.

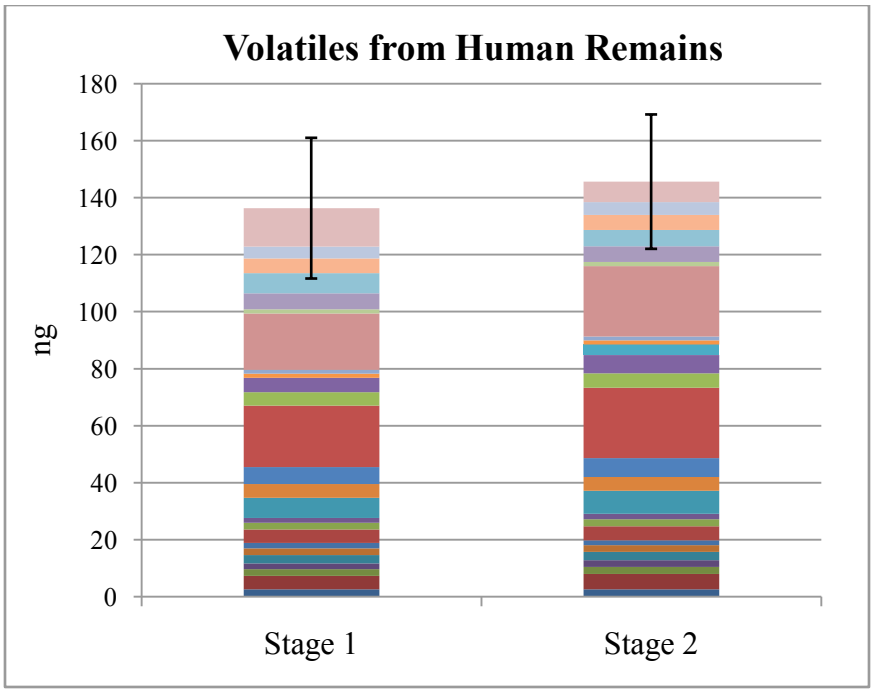


Figure 38. Odor profiles collected from human remains in stage one and stage two decomposition.

Borden Crematory

The same procedure was used to collect samples from human remains in a separate location, Borden Crematory. The bodies sampled in this second location were all freshly deceased (Stage 1 decomposition). Samples from six subjects were collected and fourteen compounds were identified in five or more of the samples (83%) (Table 33).

Table 33. Compounds recovered from human remains collected at Borden Crematory.	
Compound	**# of occurrences**
Styrene	6
Tridecane	6
2-Ethyl-1-hexanol	6
Benzaldehyde	6
2-2-Methoxyethoxy ethanol	6
Tetradecane	6
Benzoic acid, methyl ester	6
Decanal	6
Pentadecane	6
Phenol	6
Octanoic acid	6
Heptadecane	6
Isobornyl acetate*	6
Naphthalene*	5
Undecane	4
1,2,3,-Trimethyl benezene	4
Geranyl acetone	4
2-Hexyl-1-octanol*	4
2-Butyl-1-octanol*	4
2-Ethyl hexanoic acid*	4
6-Methyl-5-hepten-2-one	3
Nonanal	3
Benzonitrile	3
Undecanoic acid*	3
2-Hexyl-1-decanol*	3
Undecane	2
2-Hexyl ethanol*	2
Acetic acid	1
2-Ethylhexyl tetradecyl ester, oxalic acid*	1
5-Methyl-2-1-methylethyl cyclohexanol*	1
Methyl salicylate*	1
2-Decenal*	1
2-Methyl undcanethiol*	1
1,1-Oxybis-octane*	1

The purpose of collecting samples from a second location was to eliminate volatiles that may be due to background odor. Because the first group of samples was collected at a morgue, it was impossible to subtract out the background odor, because a blank sample taken will contain many of the same volatiles as human remains are present at all times. The background volatiles at the crematory are unique compared to those from the morgue. The background VOCs from the morgue are most likely the result of cleaning agents and other chemicals used for preparation and processing of the bodies for autopsy, while the background VOCs from the crematory are likely due to the process of cremation. Background compounds were eliminated from analysis by comparing the VOCs present in location one (morgue) to those from location two (crematory). Table 34 lists all of the compounds recovered from at least 66% of the samples from location one and location two separately. The sets of samples from the two locations were found to have 13 compounds in common (highlighted in the table below). Using only the thirteen common compounds, the scent profiles from the two locations were compared and appeared very similar (Figure 39).

Table 34. Comparison of compounds collected from human remains sampled in two locations.		
Compound	**Crem**	**ME**
Toluene		x
Acetic acid		x
o-Xylene		x
p-Xylene		x
Styrene	x	x
Undecane	x	
1,2,3-Trimethyl benezene	x	x
Dimethyl trisulfide		x
2-Furaldehyde		x
6-Methyl-5-hepten-2-one		x
Tridecane	x	x
2-Ethyl-1-hexanol	x	x
Benzaldehyde	x	x
Nonanal		x
2-2-Methoxyethoxy ethanol	x	x
1-Octanol		x
Tetradecane	x	
Benzonitrile		x
Benzoic acid, methyl ester	x	x
Decanal	x	x
Pentadecane	x	x
Benzyl alcohol		x
Hexadecane		x
Phenol	x	x
Octanoic acid	x	x
Heptadecane	x	x
Geranyl acetone	x	
Isobornyl acetate*	x	
2-Hexyl-1-octanol*	x	
Naphthalene*	x	x
2-Butyl-1-octanol*	x	
2-Ethyl hexanoic acid*	x	
2-Butoxy ethanol*		x

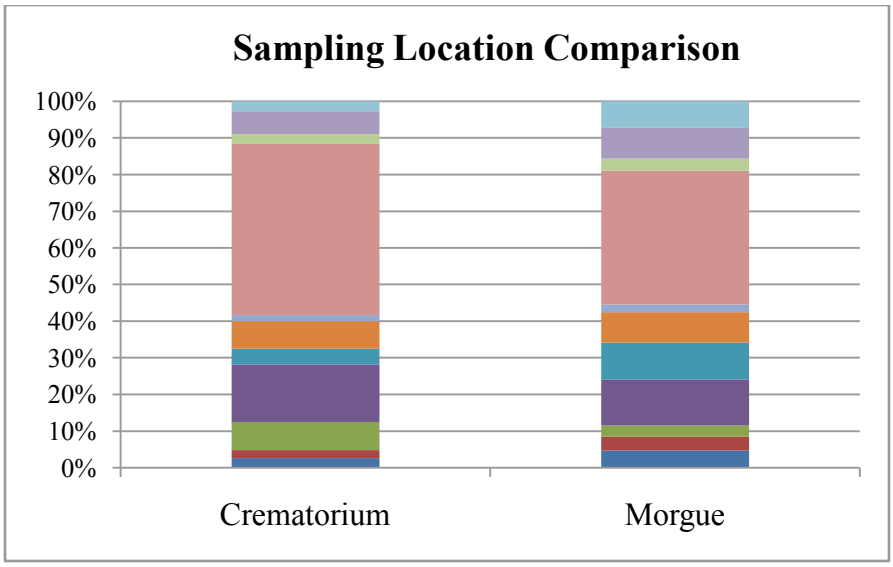


Figure 39. Odor profiles of human remains sampled in two locations.

Several benzene-derivatives were included in the thirteen compounds. Cyclic hydrocarbons are one of the most prevalent classes of compounds associated with human remains odor. Styrene,[102,104,105] 1,2,3-trimethyl benzene,[102,105] and naphthalene[104,105] all have been previously reported as being associated with human remains. Substituted benzenes are likely produced during the decomposition process due to the microbial modification of various root compounds. In Vass et al., styrene and naphthalene were detected consistently throughout the decomposition process.[104]

Many oxygenated compounds are also often associated with remains odor. During decomposition, the unsaturated fatty acids from adipose tissue are converted to aldehydes and ketones by oxidation under aerobic conditions.[98] Benzaldehyde has been reported as a component of both living[19,35,38,55] and deceased[4,39,102] human odor, as well

as associate with the blood of lung cancer patients.[106] Decanal has been reported in living[19,35-38,55,57] and deceased[103-105] human scent, as well.

Tridecane, pentadecane, heptadecane and other straight chain alkanes have not been documented as a human remains odor component. They have, however, been identified as components of living human scent in a number of sources.[19,35,37,55,56]

The alcohol 2-ethyl-1-hexanol has been reported by several groups as being associated with human remains odor[39,105] as well the odor from pig decomposition.[107] Phenol was also previously identified in human[105] and pig remains,[107] as well as a common component of human scent.[19,36,38,55] 2-2-Methoxyethoxy ethanol was also recovered in this study, but has not been reported as a component of human odor, living or deceased.

Octanoic acid was identified as a component of decomposition fluid odor[3] and as a component of living human scent.[19,38,55] Octanoic acid and other organic acids are formed by the conversion of glucose monomers from carbohydrates under aerobic conditions. Other acids such as butyric acid and propionic acid, which are often associated with human remains odor but were not identified in this research, are formed in later stages of decomposition when the body provides an anaerobic environment.[100] The bodies in their research had not reached this stage of decomposition, explaining why such compounds were not detected.

Benzoic acid, methyl ester has not been reported in the literature, although other acid esters are common.[39,102-104] Benzoic acid, methyl ester was detected as a component of pig remains odor.[107]

Live humans

Samples from living humans were collected, extracted and analyzed in the same manner as those from the human remains. The odor profiles for living and deceased humans were compared (Figure 40). The lettering F and M indicate the female and male subjects from the living human study, while Mor and Cr indicate randomly selected remains samples from the morgue and crematorium, respectively. There are a number of similar compounds between the living and deceased human samples, as to be expected. The deceased samples have a greater variety of compounds compared to individual living human samples, but are more similar to one another than the living human samples. The living human odor samples all contain geranyl acetone which is not found in any of the remains samples. This is in agreement with the literature as this compound has not been previously reported as a decomposition odor compound, but it has been previously reported as a living human scent component in several sources.[19,35,38,55]

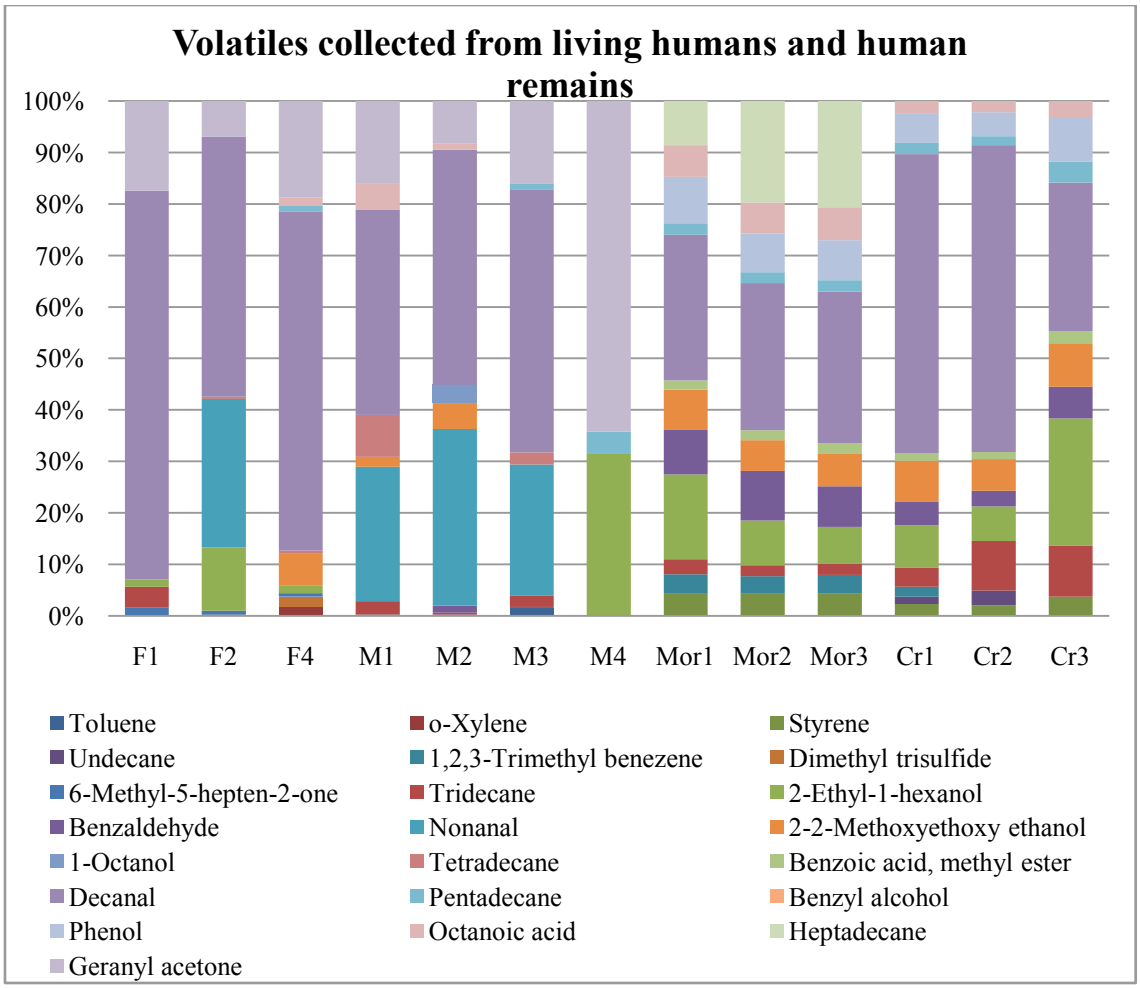


Figure 40. Volatiles collected from living human and human remains odor.

The living samples show more between subjects variation, depicted in the PCA plot (Figure 41). The remains samples tend to group more tightly and thus are more similar to one another than the living samples. This trend is to be expected as it has been shown that living humans have individualized or unique odor while deceased humans have generalized odor.

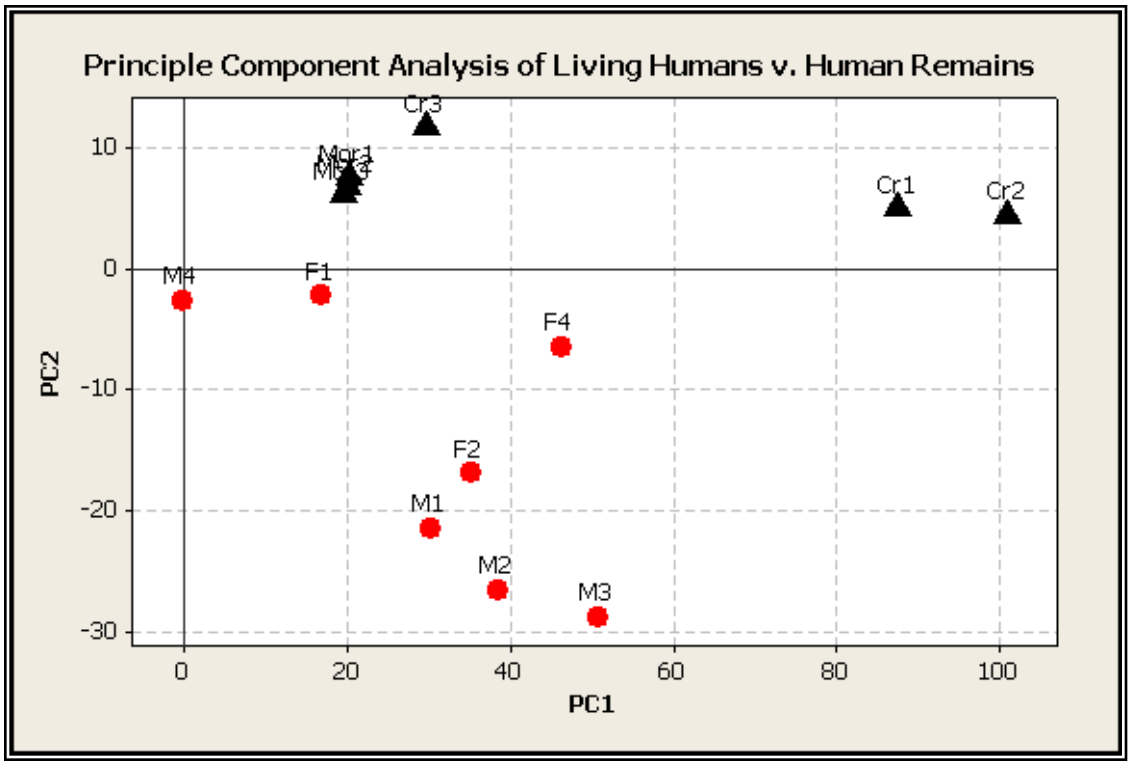


Figure 41. Plot of principle components representing the similarity between human remains and living human odors.

Animal remains

Odor samples from animal remains were also collected with the STU-100 and extracted and analyzed in the same manner. The odor profiles of several animals and four randomly selected human remains samples were compared (Figure 42). There were many similarities between the profiles of all remains; however, no one compound was found in all types of samples. Styrene and benzoic acid, methyl ester were the only two compounds found in all human remains samples, but not in any animal remains samples. These results indicate that the odor from human remains is different than that of animal remains. This is substantiated by the plot of the principle component analysis of animal remains volatiles vs. human remains volatiles (Figure 43). The human remains samples

are far separated from the other animals in the plot. This is to be expected as it is known that well-trained HRD canines will pass by animal remains in a search to solely locate the human remains.

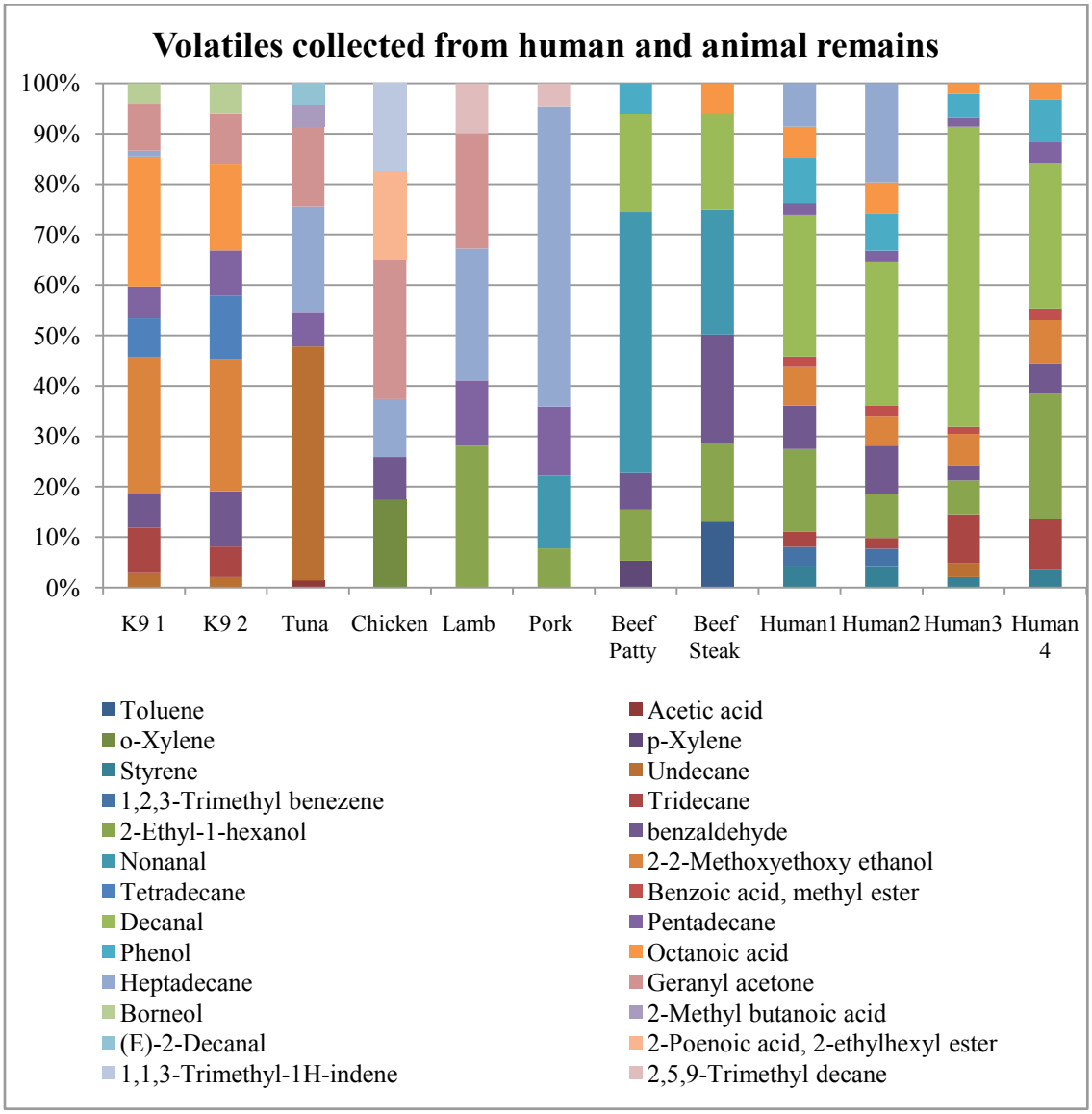


Figure 42. Odor profiles collected from the remains of animals and humans.

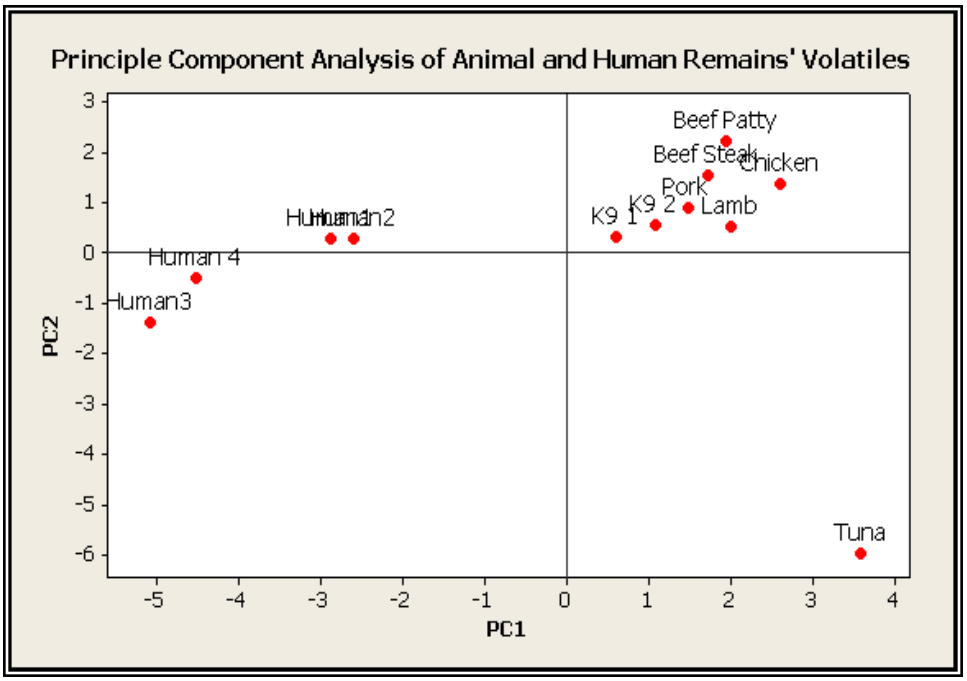


Figure 43. Plot of principle components representing the similarity between animal remains and human remains odors.

3.4.6. Canine Training Aids / Canine Trials

Trial 1: Pseudo scent and Odor pad training aids: Preliminary trials

Day 1: Both pseudo scent and odor pad training aids were tested during the preliminary canine trials. On Day 1, the samples were sealed into permeable bags which were then stored in aluminized bags. During the trial, the training aids were removed from the aluminized bags and placed into four line-ups of ten cement blocks. The training aids included odors that were collected with the STU-100 and were from two different fresh human remains, canine remains, and cremated human remains (cremains), as well as three pseudo scent variations. Three positive controls and a blank (gauze with

no odor) were also placed in the blocks. The canine's responses to each block are given in Table 35.

Table 35. Canine responses to training aids in Trial 1, Day 1.

Block # (Set.Block)	***Contents***	***K9 1 (novice)***	***K9 2 (advanced)***	***K9 3 (advanced)***	***K9 4 (novice)***	***Total***
1.3	Fresh remains 1	0	A	A	0	2/4
1.9	Fresh remains 2	0	0	A	I	1(2)/4
2.4	Canine remains	I	0	0	A	1(2)/4
2.6	Blank gauze	0	A	A	A	3/4
2.7	Positive control	A	A	A	A	4/4
3.1	Pseudo I	0	0	0	0	0/4
3.3	Pseudo II	0	0	0	0	0/4
3.5	Positive control	0	A	A	A	3/4
4.6	Pseudo III	0	0	A	A	2/4
4.7	Positive control	A	A	A	A	4/4
4.10	Cremains	0	0	0	0	0/4
	False Alerts	3	4	3	3	13/120
		(A = Alert, I = Interest, 0 = No Response)				

The total number of false alerts possible was determined by the total possible number of alerts (total number of alerts = total number of blocks x number of canines) subtracted by the number of possible correct alerts (number of possible correct alerts = number of blocks containing aids x number of canines). The blank gauze was considered a possible false alert, so 40 blocks times 4 canines equals 160 possible alerts. Ten blocks contain aids, times four canines equals 40 possible correct alerts, thus 160 - 40 = 120 possible false alerts.

All canines except for one alerted on the three positive controls; the other canine alerted on two of the three. Correctly alerting to the positive controls indicates that the canines were trained properly and ready to work. Three of the four canines alerted on the blank prepared from clean gauze, suggesting possible contamination. No interest was shown for Pseudo I or Pseudo II, however two canines alerted on Pseudo III. Pseudo III contained only a solution of cadaverine and putrescine. Training aids containing such compounds are commonly used early in HRD canine training. It is possible that the two canines that alerted to Pseudo III had previously been trained on cadaverine and putrescine. Two of four canines alerted or showed interest to the fresh remains samples in both instances. This substantiates the use of the odor pad training aids. Only thirteen false alerts were made by the canines out of a possible 120.

Day 2, Part1: For the next set of canine trials, fresh training aids were made from the same scent sources. In hopes to increase the concentration of available scent, the scent pads were not placed in the permeation bags. Extra precautions were taken to prevent any contamination of the blank. The results of this trial still demonstrated interest by the canines for the use of the odor pad training aids, but showed no improvement over Day 1 (Table 36). Canines 6 and 7 did not alert to the positive control, and thus were not included in the results. The two canines that alerted to the cremation remains (K9 5 and K9 8) were the only two canines of the group that had previously been trained on cremains. Two false alerts were made by only one of the included canines.

Table 36. Canine responses to training aids in Trial 1, Day 2, Part 1.

Block # (set.block)	***Contents***	***K9 2 (adv)***	***K9 3 (adv)***	***K9 4 (nov)***	***K9 5 (nov)***	***K9 6 (adv)***	***K9 7 (nov)***	***K9 8 (nov)***	***Total***
1.3	Positive control	A	A	A	A	0	0	A	5/5
2.2	Fresh remains 1	A	0	0	0	X	X	0	1/5
2.7	Canine remains	0	0	A	0	X	X	0	1/5
2.8	Blank gauze	0	0	0	0	X	X	0	0/5
3.1	PseudoI	0	0	0	0	X	X	0	0/5
3.6	PseudoII	0	0	0	0	X	X	0	0/5
3.8	Fresh remains 2	0	A	0	0	X	X	0	1/5
4.3	PseudoIII	0	0	0	0	X	X	0	0/5
4.6	Cremains	0	0	0	A	X	X	A	2/5
	FalseAlerts	0	0	0	0	X	X	2	2/30
		(A = Alert, I = Interest, 0 = No Response)							

Day 2, Part 2: In order to further increase the available scent concentration, multiple gauze pads with similar odors were placed together in the cement blocks. One set of ten blocks containing two training aids was used. The two training aids were comprised of five odor pads of odor pad training aids from the fresh remains and six pads containing Pseudo II. Only the canines that had correctly alerted to the positive control at the beginning of the previous run were used. Of these five canines, three alerted and one showed interest for the block containing the pseudo scent, and all alerted to the block containing the fresh remains odor.

These results indicate that the odors of both the pseudo scent-based and the odor pad training aids are recognizable to trained canines; however the concentration of odor and the packaging the training aids need to further be examined.

Trial 2: Pseudo Scent

Three pseudo scent formulations were used to make training aids. A single row of ten blocks were set up containing such training aids, and the responses of HRD canines to the line-up were recorded (Table 37). All of the six canines evaluated alerted to Pseudo II, the formulation containing the compounds previously recovered during analysis of human remains samples and the biogenic amines, cadaverine and putrescine. The line-up also included a training aid containing a solution of only cadaverine and putrescine (Pseudo III) and an aid containing just the mixture of human remains volatiles (Pseudo I). The canines did not alert to either of these, nor did they alert to the blank. All canines did, however, correctly alert to the positive control prior to running the line-up.

Table 37. Canine responses to training aids in Trial 2.

Block #	***Contents***	***Response***
1	Pseudo III	0/6
3	Pseudo I	0/6
4	Blank	0/6
10	Pseudo II	6/6
	False alerts	0/42

Trial 3: Pseudo scent: Concentration

A single run of ten blocks were set up with training aids containing Pseudo II in varying concentrations. Of the six canines that were used for the trial, only one alerted

on one (12μg of Pseudo II) of the four possible training aids (Table 38). All canines alerted correctly to the positive control in a separate line-up, and no false alerts were made. The results conflict with the results from Trial 2 where all six canines tested alerted to the blocks containing Pseudo II.

Table 38. Canine responses to training aids in Trial 3.

Block #	***Contents***	***Response***
1	12μg	1/6
3	3μg	0/6
5	Blank	0/6
7	6μg	0/6
9	24μg	0/6
	False alerts	0/36

Trial 4: Pseudo scent: Aluminized bags v. glass jars

It was hypothesized after Trial 3, that odor could have been lost through the aluminized bags, decreasing the potency of the training aids. For this reason, the aluminized bags were compared to an alternative storage container, glass jars. Two sets of ten cement blocks were used, the first set containing training aids made from varying concentrations of Pseudo II stored in aluminized bags and the other set containing training aids stored in glass jars. The training aids in the aluminized bags yielded a single canine alert and the aids in the glass jars yielded three canine alerts (Table 39). All canines correctly alerted to the positive control prior to the trial and none of the canines made false alerts. The glass jars improved canine response to the training aids, but not

significantly. Also, there was no correlation between odor concentration and canine response.

Table 39. Canine responses to training aids in Trial 4.

Block # (set.block)	***Contents***	***Response***
1.1	Bag: 10μg	1/6
1.3	Bag: blank	0/6
1.5	Bag: 20μg	0/6
1.7	Bag: 40μg	0/6
2.2	Jar: 10μg	1/6
2.4	Jar: blank	0/6
2.6	Jar: 20μg	0/6
2.8	Jar: 40μg	2/6
	False alerts	0/84

Trial 5: Odor pad: Life time of scent in open jars

When canine trials are being conducted the time lapse between the start of the first canine run and the last canine run may be as long as several hours depending upon number of canines being used, among other factors. It is important to confirm that the odor concentration of the scent source is still at a high enough to be detected by the final canine as easily as the first canine. An experiment to determine the life time of an odor source in an open container was carried out with trained HRD canines. Two training aids were made with the scent collected by the STU-100 from gauze soaked in decomposition fluid, as well as, two blanks. All samples were set out in a line-up of ten cement blocks for 24 hours and run at different time intervals. At time zero, the five canines alerted to both of the training aids and did not alert to the blanks (Table 40). After 24 hours, there

were still three alerts to the training aids out of a possible six, a 50% rate of detection. There were no false alerts during any run. To insure consistency in odor concentration for further canine trials, it was suggested that the jars not be left out for more than twelve hours, which is more than enough time necessary to carry out a trial with many canines.

Table 40. Canine responses to training aids in Trial 5.

Block #	***Contents***	***Run 1 (0 hr)***	***Run 2 (2 hrs)***	***Run 3 (12 hrs)***	***Run 4 (24hrs)***
2	Decomp 1	5/5	3/3	3/3	2/3
4	Blank 1	0/5	0/3	0/3	0/3
6	Blank 2	0/5	0/3	0/3	0/3
9	Decomp 2	5/5	3/3	2/3	1/3
	False alerts	0/40	0/24	0/24	0/24

Trial 6: Odor pad: Collection methods

Similar to Trial 3, where the concentration of pseudo scent odor on the scent pad was varied, the number of scent pads and length of scent collection was varied in an attempt to modify the amount of available odor on the odor pad. Training aids made from 1, 3, and 6 gauze pads, collected for 1, 5, and 10 minutes were placed in a line-up of ten cement blocks. All canines positively alerted to all training aids (with the exception of the blank) (Table 41). While this was a positive result for the use of odor pad training aids, it did not yield any additional information about the lower detection limits of the canines utilizing such aids.

Table 41. Canine responses to training aids in Trial 6.

Sample	***Number of gauze pads***	***Length of collection***	***Response***
Decomp	1	1 min	6/6
Decomp	3	1 min	6/6
Decomp	6	1 min	6/6
Decomp	1	5 min	6/6
Decomp	1	10 min	6/6
Blank	3	1 min	0/6
		False alerts	0/36

Trial 7: Odor pad: Assortment of scent sources

In the previous trials, all of the odor pad training aids were made directly from freshly deceased remains or from gauze soaked in decomposition fluid. It is important for HRD canines to be exposed to a diverse range of odors during training. In order to show that the STU-100 could be used to create training aids of different odors, aids were made from the scent of gauze soaked in/wiped across several different mediums. Eight canines were run on five different training aids and one blank (Table 42). All of the canines alerted to the scent pads made from blood, fresh remains and decomposition fluid. Five of the eight canines alerted to the adipocere scent pad. The three canines that did not alert were the three novice dogs. Only two canines (advanced and intermediate) alerted to the bone sample; however, upon arrival, the jar containing the gauze with bone substance had come open, thus less odor was available to be collected by the STU-100 and therefore less odor was available to the canines. Two canines falsely alerted to the

blank, but no other false alerts were made. These results show that the STU-100 can be used for making of training aids from any type of scent source.

Table 42. Canine responses to training aids in Trial 7.

Block #	***Contents***	***Response***
2	Blood	8/8
3	Fresh Remains	8/8
4	Decomp Fluid	8/8
5	Adipocere	5/8
6	Blank	2/8
7	Bone*	2/8
	False alerts	2/40

Population study

A final set of field tests were carried out to assess canine response to a series of Odor pad training aids packaged in both glass jars and aluminized bags placed in separate line-ups. The canines that were used in this trial had various levels and types of training and experience (Table 26). The odor sources included freshly deceased human remains and decomposition fluid, as well as the odors of live humans and chicken remains, and blank gauze pads.

The responses of twenty-six canines under five different trainers/handlers were evaluated (Table 43 and Table 44). In the case of the training aids in glass jars, eleven of the twenty-six canines alerted (one canine showed interest) to the training aid made from the freshly deceased body, and six alerted (one showed interest) to the aid made from the odor of decomposition fluid. The number of alerts to scent pads with remains odor was

significantly greater than would be expected by chance, as only two canines alerted to the blank samples.

Table 43. Results from population study; training aids in jars.

Handler	***Canine***	***Blank***	***Live Human***	***Chicken Remains***	***Freshly Deceased***	***Decomp Fluid***
Handler B	B1	A	0	0	A	A
	B2	0	0	A	A	A
	B3	A	0	A	0	0
	B4	0	0	0	A	A
	B5	0	0	0	A	A
Handler MI	MI1	0	0	A	A	0
	MI2	0	A	I	0	0
Handler MA	MA1	0	0	0	0	0
	MA2	0	A	0	0	A
	MA3	0	A	0	0	0
	MA4	0	0	A	0	0
Handler CH	CH1	0	0	0	0	A
	CH2	0	0	0	0	0
	CH3	0	0	0	0	0
	CH4	0	0	0	0	0
	CH5	0	0	0	A	0
	CH6	0	0	0	0	0
	CH7	0	0	0	0	0
Handler AM	AM1	0	0	0	A	I
	AM2	0	0	0	I	0
	AM3	0	0	A	A	0
	AM4	0	0	0	0	0
	AM5	0	0	0	0	0
	AM6	0	0	0	A	0
	AM7	0	0	0	A	0
	AM8	I	0	0	A	0
Total	26	2(3)/26	2/26	5(6)/26	11(12)/26	6(7)/26
		(A = Alert, I = Interest, 0 = No Response)				

The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated based on the canine responses listed in Table 43. The PPV and NPV values were calculated excluding the responses to the scent pads made from live human and animal remains because some forms training or cross-training may consider a canine to alert to such odors correct while others may not. The PPV for the training aids in the jars was 86%; in other words 86% of the canine alerts were correct. The NPV was 41%, meaning that 41% of the time that a canine did not give a response, the non-response was correct. The chance that a canine gives a correct positive alert (PPV) is based on the quality of the scent source (the training aids in question) and the training of the canine. The chance that a canine gives a correct non-response (NPV) is an issue of training and not related to the quality of the training aids. A PPV of 86% indicates that odors from the training aids created in this study were reasonably recognizable to human remains canines. A NPV of 41% is low; however, this could be improved with familiarity to the training aids and trial set up.

The training aids stored in the aluminized bags yielded poorer results compared to those stored in the glass jars. Only three canines alerted to the aids made from remains odor, compared to seven canines that alerted to the blanks, and the PPV for this set of training aids was only 53% (NPV = 30%), indicating that the odor was likely not fully contained inside the aluminized bags, allowing the scent to dissipate from the inside of the bags during storage or to modify the scent picture. The high number of positive responses to the blanks may indicate possible cross-contamination when the aluminized bags are stored next to one another.

Table 44. Results from population study; training aids in aluminized bags.

Handler	***Canine***	***Blank***	***Live Human***	***Chicken Remains***	***Freshly Deceased***	***Decomp Fluid***
Handler B	B1	0	0	A	0	A
	B2	0	0	0	0	0
	B3	0	0	0	0	0
	B4	0	0	0	A	0
	B5	0	0	0	0	0
Handler MI	MI1	0	0	0	0	A
	MI2	A	A	0	0	0
Handler MA	MA1	A	0	0	0	0
	MA2	A	0	0	A	A
	MA3	0	0	A	0	0
	MA4	A	0	0	0	A
Handler CH	CH1	0	0	0	0	0
	CH2	0	0	0	0	0
	CH3	0	0	0	0	0
	CH4	0	0	0	0	0
	CH5	0	0	0	0	0
	CH6	0	0	0	0	0
	CH7	0	0	0	I	0
Handler AM	AM1	A	0	I	0	0
	AM2	0	I	0	0	0
	AM3	A	0	0	0	0
	AM4	0	0	A	0	0
	AM5	0	I	0	A	0
	AM6	0	A	0	0	0
	AM7	0	0	A	0	0
	AM8	A	I	0	0	0
Total	26	7/26	2(5)/26	4(5)/26	3(4)/26	4/26
		(A = Alert, I = Interest, 0 = No Response)				

The live human and animal remains odors were used as distracters that may elicit responses from HRD canines that have been cross-trained on live human scent or from novice canines. For the training aids stored in jars, only two canines alerted on the live scent, the same rate as false alerts on the blank; however five canines alerted on the chicken remains (three intermediate, two expert). These canines may need additional training to prevent alerts on the generic odor of decomposition, as opposed to the odor of *human* decomposition. For the training aids stored in the aluminized bags, the number of alerts on both the living human and animal remains was less than the number of false alerts on the blank.

No significant differences were found between the responses of expert canines compared to the novice canines, as might have been expected. The PPV of expert and novice canines were 89% and 100%, and the NPV were 40% and 43%, respectively, indicating that the canines' responses to the training aids are likely more affected by the manner of training and type of training aid encountered during training than the amount of training. For instance, Handler B is the only handler in the study that uses scent line-ups in regular training, and Handler B's dogs gave the highest rate of positive responses to the remains training aids. Handler AM also uses scent line-ups in training, but only in the beginning stages of training and for remedial work. These canines also gave a relatively high number of correct alerts. Other handlers only use line-ups only occasionally or not at all.

The types of training aids used during regular training may have also affected the canine response. As mentioned previously, it is imperative that the quantity and type of

scent source is varied regularly during training. The available odor from the scent pads is relatively low compared actual tissue or body parts. Canines that were already accustomed to lesser quantities of odor would likely perform better during these trials, compared to canines that have only been trained on large quantities of odor. Handlers B and AM use a wide variety of scent sources and quantities, while Handler MI only uses tissue, bones and body parts yielding greater amounts of available scent. Such differences may affect the canines' performances.

The trends are consistent with SWGDOG recommendations. In the Human Remains Detection document (Appendix 2), SWGDOG best practices recommend that both odor recognition tests (scent line-ups) and comprehensive assessments (training aids hidden or similar scenario) be used during training. SWGDOG also recommends that the types of training aids include a wide variety of human remains' odor sources and levels of decomposition.[85]

If using the Odor pad training aids, the amount of available odor could be easily increased and decreased by adding and removing scent pads, thus they have the potential to be used to improve canines' responses to lower quantities of odor, and the type of odor can be altered by collecting scent pads from various remains sources. To further improve and understand the potential of these training aids, canine handlers should incorporate the Odor pad training aids in their regular training, followed by further testing of the canines.

3.5. Conclusions

As was previously carried out with living human scent volatiles, the Scent Transfer Unit was optimized for the collection of human remains volatiles. Controlled Odor Mimic Permeation Systems (COMPS) were created using six compounds representative of the compounds previously documented in human remains odor, and were used to reliably and reproducibly deliver the standard compounds at controlled rates to the STU-100 during the optimization process. As with living human scent, the collection material and flow rate for the STU-100 were optimized. The results were similar to those found previously. It was concluded that the molecular structure as well as the weave structure of the collection material affects the amount of compound trapped/released. The greatest amount of total compound was recovered from the cotton based-materials (Johnson and Johnson and Dukal gauzes); however, the polyester material showed potential for the collection of acids. The polyester material was layered with the gauze materials to enhance collection of all compounds. It was established that the greatest amount of compounds were recovered using the Dukal gauze / polyester material at the low flow rate.

After optimizing the collection method with standard compounds, the sample extraction and analysis methods were optimized for actual samples. The SPME exposure time, split v. splitless sample injection and sample heating were examined. On the basis of the results obtained from these experiments, a method requiring two extractions per sample was developed, where the samples are first extracted using a shorter fiber exposure time (30 min) at room temperature and split injection (10:1) for the improved

detection of the early eluting compounds, followed by a longer (20 hour) extraction at 70°C with splitless injection for improved sensitivity of the later eluting compounds.

The optimized collection, extraction and analysis methods were applied to the sampling of human remains. Deceased bodies were sampled from two locations. The VOCs collected from both locations were compared and thirteen compounds were found in common. The ratios and quantities of such compounds were consistent between the two sampling locations as well, indicating that these compounds are significant to picture of human remains odor.

The odor profiles of the human remains samples were compared to those of living human and animal remains samples collected and analyzed in the same manner. While there were many compounds in common between the living and deceased human samples, the complete odor profiles differed. The living human samples showed greater variation between samples compared to the deceased human samples which were more similar to one another, as statistically depicted by PCA. Similarly, human and animal remains samples were compared. The human odor profiles were easily separated from the animal profiles in PCA plot. The results of the comparisons of the odor from living human, human remains and animal remains are consistent with the capabilities of trained HRD canines.

On the basis of the optimization of the STU-100 and the identification of human remains volatiles, two sets of potential canine training aids were created. Chemical-based, pseudo scent training aids were created using a mixture of the volatile compounds previously identified from the remains samples with the biogenic amines, cadaverine and

putrescine. Canine trials were arranged to assess the interest of HRD canines in such aids. During these trials, the canines showed moderate, however inconsistent interest in the training aids. For better results, the formulation of the pseudo scent mixture should be further improved.

Odor pad training aids were also created using the STU-100 for the collection the human remains odor onto a gauze pad. The gauze pads containing the odor of interest was removed from the STU-100 and stored, to be used as a canine training aid. The Odor pad training aids showed great potential when tested with HRD canines, as the majority canines showed interest in the aids at different concentration and from different odor sources in every scenario tested. These training aids should be further developed for use in the field.

4. CONCLUSIONS

The Scent Transfer Unit or STU-100 is currently used in the United States by hundreds of law enforcement agencies as a method of scent collection for use with human scent canines. Nevertheless, there has been a limited amount of research on its capabilities and optimization. In this research the collection material and flow rate of the STU-100 was optimized. The issues of compound breakthrough as well as the importance of the structural differences between collection materials were studied. The total amount of compounds recovered from the polyester material was significantly less than from the cellulose-based materials except for acids, suggesting that the interaction between the VOCs and the material chemistry plays a role in the trapping and releasing of compounds. Even more significantly, the weave of the material affects the amount of compound collected, as those materials with a tighter weave tend to enhance collection compared to those with a looser weave. The tighter weave reduces the rate of the airflow past the material and into the STU-100, causing less VOC's to be carried past the collection material and lost. Higher air flow rates generally yielded less total VOCs also due to compound breakthrough. Thus, collection with lower flow rates was more successful. For future sample collection with any dynamic airflow type of device, the collection material and flow rates should be carefully evaluated prior to use as there may be significant variations in collection efficiencies.

The STU-100 was also optimized for the collection of human remains volatiles, a novel application. The results of the optimization were similar to that of the living human scent; however the polyester material showed potential for the collection of acids,

and thus was chosen to be layered with the cotton-based gauze materials to improve the collection of acids, an important class of compound in human remains odor.

A novel scent delivery system was designed in order to reliably and reproducibly disperse standard compounds to the STU-100 during optimization. The Controlled Odor Mimic Permeation Systems (COMPS) have been previously utilized as a method for delivering explosive or drug odor to canines during training. By applying a similar system to human odor VOCs, the standard compounds were delivered to the STU-100 at known rates, improving the reproducibility of optimization experiments.

The optimized sampling method was applied to the collection of living and deceased human odor. Analysis of the living human scent samples showed good reproducibility between replicate samples, however the replicate odor profiles from the individuals were not distinguishable from one another in all cases. The analysis of the samples from the deceased humans revealed a number of compounds common to all samples. The ratios of these compounds also remained constant between the samples.

On the basis of data gathered using the STU-100, canine training aids were created. The human remains detection canine community is in need of improved of training aids that are easily and legally obtainable, non-hazardous, reusable, and represent the entire odor picture of human remains. Two types of potential canines training aids were created in an attempt to fulfill the requirements currently demanded by HRD canine handlers. One type is a chemical-based pseudo scent and the other consists of odor pads made from STU odor collection.

The pseudo scent training aids were created using the compounds recovered earlier during the sampling of human remains. The results from the canine trails with these aids were only moderately successful. In the present state of development, the pseudo scent-based training aids may be useful for reinforcement and threshold testing, but is not yet sufficient as a replacement for actual human remains. To improve the canine interest in training aids of this nature, a better knowledge of the scent picture of human remains is required.

The odor pad training aids were more successful when tested with the HRD canines. This type of training aid can be created from any type of scent source, allowing for the diversity in training aid odor necessary to train a successful HRD canine. The lower limit detection of canine can be improved by using different amounts of available scent, which can also be accomplished with these aids by changing the collection length and/or number of pads used. These training aids can be created by any police department, agency or university with access to a dynamic headspace scent collection/concentration device, then shipped to and stored by the canine handler with no legal, biohazard or disposal issues. Also, since these odor pads yielded reliable results with the canine teams and have simplified odor profiles compared to actual human remains, they are useful in focusing the signature chemicals for human remains detection. Because of the great potential for this type of training aids, further development should be carried out to improve durability and to examine the storage and usage limits.

Overall, this research has demonstrated the utility of dynamic headspace concentration for identifying characteristic volatile organic compounds in living and

deceased human odors, as well as preparing novel training aids for human remains detection.

LIST OF REFERENCES

1. Furton, Kenneth G; Myers, Lawrence J. The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. Talanta 2001; 54; 487-500.

2. Snovak, Angela Eaton. Guide to search and rescue dogs. Barron's; New York: 2004

3. Lorenzo, Norma; Wan, TianLang; Harper, Ross J; Hsu, Ya-Li; Chow, Michael; Rose, Stefan; Furton, Kenneth G. Laboratory and field experiments used to identify *Canis lupus var. familiaris* active odor signature chemicals from drugs, explosives, and humans. Analytical Bioanalytical Chemistry 2003; 376; 1212-1224.

4. Gerritsen, Resi; Haak, Ruud. K9 professional tracking. Detselig Enterprises Ltd, Canada: 2001.

5. *Hodge v. State*, 98 Ala. 10 (Ala. 1893).

6. *State v. Hall*, 4 Ohio Dec. 147,148 (Ohio Misc. 1896).

7. *Brott v. State*, 70 Neb. 395 (Neb. 1903).

8. *State of California v. Benigno Salcido*, 110 Cal. App. 4th 379 (2005).

9. *People v. Jeffrey Dewayne Mitchell*, 110 Cal. App. 4th 772 (2003).

10. *People v. Ryan Willis*, 115 Cal. App4th 379 (2004).

11. Prada, Paola A; Furton, Kenneth G. Human scent detection: A review of its developments and forensic applications. Revista de Ciencias Forenses 2008; 1(2); 81-87.

12. Buck, Linda B. Unraveling the sense of smell. Angewandte Chemie International Edition 2005; 44; 6128-6140.

13. Zhihua, Zou; Buck, Linda B. Combinatorial effects of odorant mixes in olfactory cortex. Science 2006; 311 (5766); 1477-1481.

14. Pearsall, Milo D; Verbruggen, Hugo. Scent: Training to track, search and rescue. Alpine Publications Inc., Loveland, Colorado: 1982.

15. Kaldenback, Jan. K9 scent detection. Detselig Enterprises Ltd, Alberta, Canada: 1998.

16. Rebmann, Andrew; David, Edward; Sorg, Marcella H. Cadaver dog handbook. CRC Press; Washington D.C.: 2000.

17. Lesniak, Anna; Walczak, Marta; Jezierski, Tadeusz; Sacharczuk, Mariusz; Gawkowski, Maciej; Jaszczak, Kazimierz. Canine olfactory receptor gene polymorphism and its relation to odor detection performance by sniffer dogs. Journal of Heredity 2008; 99(5); 518-527.

18. Stockham, Rex A.; Slavin, Dennis L.; Kift, William. Specialized use of human scent in criminal investigations. Forensic Science Communications 2004; 6(3).

19. Prada, Paola; Curran, Allison M.; Furton, Kenneth G. The evaluation of human hand odor volatiles on various textiles: A comparison between contact and non-contact sampling methods. Journal of Forensic Sciences 2009; 55(1); 50-55.
20. http://stu100.com. Big T LLC. Accessed May 17, 2010.

21. Tolhurst, William D.; Harris, Lawrence R. United States Patent, # Des. 397,051. August 18, 1998.

22. Curran, Allison M.; Prada, Paola A.; Furton, Kenneth G. Canine human scent identifications with post-blast debris collected from improvised explosive devices. Forensic Science International 2010; 199(1-3); 103-108.

23. Eckenrode, Brian A.; Ramsey, Scott A.; Stockham, Rex A.; VanBerkel, Gary J.; Asano, Keiji G.; Wolf, Dennis A. Performance evaluation of the Scent Transfer Unit ^{TM} (STU-100) for organic compound collection and release. Journal of Forensic Science 2006. 51(4); 780-789.

24. Fletcher, Kendra S. Optimization and development of a human scent collection method. Master's Thesis: Uniformed Services University of Health Sciences, Department of Preventative Medicine and Biometrics; 2007.

25. Hudson, Davia Tamar. Variables affecting the collection and preservation of human scent components through instrumental and biological evaluations. Doctoral Dissertation: Florida International University, Department of Chemistry and Biochemistry; 2009.

26. Curran, Allison M.; Rabin, Scott I.; Furton, Kenneth G. Analysis of the uniqueness and persistence of human scent. Forensic Science Communications 2005; 7(2).

27. Wirks-Brown, Jessica S.; Furton, Kenneth G. An evaluation of volatile organic compounds from biological specimens over time. American Academy of Forensic Science 62nd Annual Scientific Meeting. Feb 2010; Seattle, WA.

28. Cook, J. Gordon. Handbook of textile fibers: Natural fibres, V. I. Merrow Publishing Co. Ltd, England: 1984.

29. Peters, R.H. Textile chemistry: Volume II: Impurities in fibres; purification of fibres. Elsevier Publishing Company, New York: 1967.

30. Peters, R.H. Textile chemistry: Volume I: The chemistry of fibres. Elsevier Publishing Company, New York: 1963.

31. Cook, J. Gordon. Handbook of textile fibers: Man-made fibres, V. II. Merrow Publishing Co. Ltd, England: 1984.

32. Ludewig, Hermann. Polyester fibres: Chemistry and technology. Wily Interscience, New York: 1964.

33. Harper, Ross James. Improving the scientific reliability of biological detection of explosives by *Canis familiaris*: Active odour signatures and their implications. Doctoral Dissertation: Florida International University, Department of Chemistry and Biochemistry; 2005.

34. Curran, Allison M.; Rabin, Scott I.; Prada, Paola A.; Furton, Kenneth G. Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. Journal of Chemical Ecology 2005. 31(7); 1607-1619.

35. Curran, Allison M.; Ramirez, Carlos F.; Schoon, Adee A.; Furton, Kenneth G. The frequency of occurrence and discriminatory power of compounds found in

human scent across a population determined by SPME-GC/MS. Journal of Chromatography B; 2006. 32(8); 1617-1623.

36. Hudson, Davia T.; Curran, Allison M.; Furton, Kenneth G. The stability of collected human scent under various environmental conditions. Journal of Forensic Sciences 2009; 54(6); 1270-1276.

37. Ostrovskaya, Asira; Landa, Peter A.; Sokolinsky, Marina; Rosalia, Anthony D.; Maes, Daniel. Study and identification of volatile compounds from human skin. Journal of Cosmetic Science 2002; 53; 147-148.

38. Gallagher, M.; Wysocki, C. J.; Leyden, J. J.; Spielman, A. I.; Sun, X.; Preti, G. Analyses of volatile organic compounds from human skin. Cutaneous Biology 2008; 159; 780-791.

39. Hoffman, Erin M.; Curran, Allison M.; Dulgerian, Nishan; Stockham, Rex A.; Eckenrode, Brian A. Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Science International 2009; 186; 6-13.

40. Awan, M. Ali; Fleet, I.; Thomas, C. L. Paul. Determination of biogenic diamines with a vaporization derivatisation approach using solid-phase microextraction gas chromatography-mass spectrometry. Food Chemistry 2008; 111; 462-468.

41. Arthur, Catherine L.; Pawliszyn, Janusz. Solid phase microextraction with thermal desorption using fused silica optical fibers. Analytical Chemistry 1990; 62; 2145-2148.

42. SchreppersWercinski, Sue Ann. Solid phase microextraction: A practical guide. Marcel Dekker, Inc.; New York: 1999.

43. Hubschmann, Hans-Joachim. Handbook of GC/MS. Wiley-VCH, Germany: 2009.

44. www.sigmaaldrich.com. Sigma-aldrich. Accessed May 17, 2010.

45. Robards, K.; Haddad, P.R.; Jackson, P.E. Principles and practice of modern chromatographic methods. Elsevier, New York: 2004.

46. deHoffman, Edmond; Stroobant, Vincent. Mass Spectrometry: Principles and application, 3rd edition. John Wiley and Sons, Ltd; England: 2007.

47. Chu, David H. Chapter 7. Development and structure of skin. Eds. Wolff K; Goldsmith L.A.; Katz S.I.; Gilchrest B.; Paller A.S.; Leffell D.J. *Fitzpatrick's Dermatology in General Medicine, 7ed*. McGraw-Hill; 2007.

48. Syrotuck, William G. Scent and the scenting dog. Barkleigh Productions; Mechanicsburg, PA: 2000.

49. Hurley, Harry J. Chapter 3. The eccrine sweat glands: Structure and Function. Eds. Freinkel, Ruth K.; Woodley, David T. *The Biology of the Skin.* The Parthenon Publishing Group; New York: 2001.

50. Nicolaides, N. Skin Lipids: Their biochemical uniqueness. Science 1974; 186(4158); 19-26.

51. Nelson, Amanda M.; Thiboutot, Diane M. Chapter 77. Anatomy of the Sebaceous Gland. Eds. Wolff K; Goldsmith L.A.; Katz S.I.; Gilchrest B.; Paller A.S.; Leffell D.J. *Fitzpatrick's Dermatology in General Medicine, 7ed*. McGraw-Hill; 2007.

52. Stoddart, D. Michael. The scented ape. Cambridge University Press; New York: 1990.

53. Doyle, Christine. The secret cloud that surrounds us. Family Health 1970; 29; 33-35.

54. Kanda, F.; Yagi, E.; Fukuda, M.; Nakajima, K.; Ohta, T.; Nakata, O. Elucidation of chemical compounds responsible for foot malodour. British Journal of Dermatology 1990; 122; 771-776.

55. Bernier, Ulrich R.; Kline, Daniel L.; Barnard, Donald R.; Schreck, Carl E.; Yost, Richard A. Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate attractants

for the yellow fever mosquito (*Aedes aegypti*). Analytical Chemistry 2000; 72; 747-756.

56. Zhang, Zhou-Min; Cai, Ji-Jin; Ruan, Gui-Hua; Li, Gong-Ke. The study of fingerprint characteristics of the emanations from human arm skin using the orginal sampling system by SPME-GC/MS. Journal of Chromatography B 2005; 822; 244-252.

57. Bernier, Ulrich R.; Kline, Daniel L.; Schreck, Carl E.; Yost, Richard A.; Barnard, Donald R. Chemical analysis of human skin emanations: Comparison of volatiles from humans that differ in attraction of *Aedes aegypti*. Journal of the American Mosquito Control Association 2002; 18(3); 186-195.

58. Roberts, Craig S.; Gosling, L. Morris; Spector, Tim D.; Miller, Paul; Penn, Dustin J.; Petrie, Marion. Body odor similarity in noncohabiting twins. Chemical Senses 2005; 30; 651-656.

59. Munk, Signe; Munch, Petra; Stahnke, Louise; Adler-Nissen, Jens; Schieberle, Peter. Primary odorants of laundry soiled with sweat/sebum: Influence of lipase on the odor profile. Journal of Surfactants and Detergents 2000; 3(4); 505-515.

60. Zeng, Xiao-Nong; Leyden, James J.; Spielman, Andrew I.; Preti, George. Analysis of characteristic human female axillary odors: Qualitative comparison to males. Journal of Chemical Ecology 1996; 22(2); 237-257.

61. Zeng, Xiao-Nong; Leyden, James J.; Lawley, Hentry J.; Sawano, Biyohito; Nohara, Isao; Preti, George. Analysis of characteristic odors from human male axillae. Journal of Chemical Ecology 1991; 14(7); 1469-1489.

62. Penn, Dustin J.; Oberzaucher, Elisabeth; Grammer, Karl; Fischer, Gottfried; Soini, Helena A.; Wiesler, Donald; Novotny, Milos V.; Dixon, Sarah J.; Xu, Yun; Brereton, Richard G. Individual and gender fingerprints in human body odor. Interface 2007; 4; 331-340.

63. Li, Weiwei; Lui, Min. Method of preventing malodor. United States Patent Application #20020192171; 2002.

64. James, A.G.; Casey, J.; Hyliands, D.; Mycock, G. Fatty acid metabolism by cutaneous bacteria and its role in axillary malodour. World Journal of Microbiology and Biotechnology 2004; 20: 787-793.

65. Natsch, Andreas; Derrer, Samuel; Flachsmann, Felix; Schmid, Joachim. A broad diversity of volatile carboxylic acids, released by a bacterial aminoacylase from axilla secretions, as candidate molecules for the determination of human-body odor type. Chemistry and Biodiversity 2006; 3; 1-20.

66. Kuhn, Fabian; Natsch, Andreas. Body odour of monozygotic human twins: A common pattern of odorant carboxylic acids released by bacterial aminoacylase from acilla secretions contributing to an ingerited body odor type. Interface 2009; 6; 377-392.

67. Leyden, J.J.; McGinley, K.J.; Holzle, E.; Labows, J.N.; Kligman, A.M. The microbiology of the human axilla and its relationship to axillary odor. Journal of Investigative Dermatology 1981; 77; 413-416.

68. Haze, Shinichiro; Gozu, Yoko; Nakamura, Shoji; Kohno, Yoshiyuki; Sawano, Kiyohito; Ohta, Hideaki; Yamazaki, Kazuo. 2-Nonenal newly found in human body odor tends to increase with aging. Journal of Investigative Dermatology 2001; 116(4); 520-524.

69. Amoore, J. Specific anosmia and the concept of primary odor. Chemical Senses and Flavors 1977; 2; 267-281.

70. Romanes, George J. Experiments on the sense of smell in dogs. Nature 1887; 36; 273-274.

71. Harvey, Lisa M.; Harvey, Jeffrey W. Reliability of bloodhounds in criminal investigations. Jounal of Forensic Science 2003; 48(4); 811-816.

72. Hepper, Peter G.; Wells, Deborah L. How many footsteps do dogs need to determine the direction of an odour trail. Chemical Senses 2005; 30; 291-298.

73. Schoon, G.A.A. Scent identification lineups by dogs (*Canis familiaris*): Experimental design and forensic application. Applied Animal Behaviour Science 1996; 46; 257-267.

74. Schoon, Gertrud A. A. A first assessment of the reliability of an improved scent identification line-up. Journal of Forensic Sciences 1998; 43(1); 70-75.

75. Settle, Ray H.; Sommerville, Barbara A.; Mccormick, James; Broom, Donald M. Human scent matching using specially trained dogs. Animal Behavior 1994; 48; 1443-1448.

76. Schoon, G. A. A. The effect of the ageing of crime scene objects on the results of scent identification line-ups using trained dogs. Forensic Science International 2005; 147; 43-47.

77. Harvey, Lisa M.; Harvey, Serina J.; Hom, Michele; Perna, Avi; Salib, John. The use of bloodhounds in determining the impact of genetics and the environment on the expression of human odortype. Journal of Forensic Sciences 2006; 51(5); 1109-1114.

78. Hepper, Peter G. The discrimination of human odour by the dog. Perception 1988; 17(4); 549-554.

79. Beauchamp, Gary K.; Yamazaki, Kunio; Boyse, Edward A. The chemosensory recognition of genetic individuality. Scientific American 1985; 253; 86-92.

80. Yamazaki, K.; Boyse, E. A.; Mike, V.; Thaler, H. T.; Mathieson, B. J.; Abbott, J.; Boyse, J.; Zayas, Z. A.; Thomas, L. Control of mating preferences in mice by genes in the major histocompatibility complex. Journal of Experimental Medicine 1976; 144; 1324-1335.

81. Wobst, Birgit; Zavazava, Nicholas; Luszyk, Dagmar; Lange, Katrin; Ussat, Sandra; Eggert, Frank; Ferstl, Roman; Muller-Ruchholtz, Wolfgang. Molecular forms of soluble HLA in body fluids: Potential determinants of body odor cues. Genetica 1999; 104; 275-283.

82. Eggert, Frank; Luszyk, Dagmar; Haberkorn, Klaus; Wobst, Brian; Vostrowsky, Otto; Westphal, Eckhard; Bestmann, Hnas Jurgen; Muller-Ruchholtz, Wolfgang; Fersl, Roman. The major histocompatibility complex and the chemosensory signaling of individuality in humans. Genetica 1999; 104; 265-273.

83. Singh, Prim B. Chemosensation and genetic individuality. Reproduction 2001; 121; 529-539.

84. Ferstl, Roman; Eggert, Frank; Muller-Ruchholtz, Wolfgang. Major histocompatibility complex-associated disorders. Nephrol Dial Transplant 1998; 13; 1117-1119.

85. http://www.swgdog.org. Scientific Working Group for Dog and Orthagonal Detector Guidelines. Accessed June 14, 2010.

86. Miller, James N; Miller, Jane C. Statistics and chemometrics for analytical chemsitry, 5th ed. Pearson Education Limited; New York 2005.

87. Page, Douglas. Is forensic science going to the dogs? Forensic Magazine 2008; 5(5); 33-40.

88. Lasseter, Alanna E.; Jacobi, Keith P.; Farley, Ricky; Hensel, Lee. Cadaver dog handler team capabilities in the recovery of buried human remains in the southeastern United States. Journal of Forensic Science 2003; 48(3); 617-621.

89. France, Diane L.; Griffin, Tom J.; Swanburg, Jack G.; Lindemann, John W.; Davenport, G. Clark; Trammell, Vickey; Armbrust, Celcilia T.; Kondratieff, Boris; Nelson, Al; Castellano, Kim; Hopkins, Dick. A multidisciplinary approach to the detection of clandestine graves. Journal of Forensic Science 1992; 37(6); 1445-1457.

90. Sorg, Marcella H.; David, Edward; Rebmann, Andrew J. Cadaver dogs, taphonomy and postmortem interval. Eds. Reichs, Kathleen J. *Advances in the Identification of Human Remains. Forensic Osteology, 2nd Ed.* Thomas Books; 1998; 120-144.

91. Cablk, Mary; Sagebiel, John. Field capability of dogs to locate individual human teeth. Journal of Forensic Science. *In Press.*

92. Komar, Debra. The use of cadaver dogs in locating scattered, scavenged human remains: Preliminary field test results. Journal of Forensic Science 1999; 44(2); 405-408.

93. Oesterheleg, L.; Krober, S.; Rottmann, K.; Willhoft, J.; Braun, C.; Thies, N.; Puschel, K.; Silkenath J.; Gehl, A. Cadaver dogs – A study on detection of contaminated carpet squares. Forensic Science International 2008; 174; 35-39.

94. Cooke, M.; Leeves, N.; White C. Time profile of putrescine, cadaverine, indole, and skatole in human saliva. Archives of Oral Biology 2003; 48; 323-327.

95. Oxenham, Marc. Forensic approaches to death, disaster and abuse. Australian Academic Press; 2008.

96. Haglund, William D.; Sorg, Marcella H. Forensic taphonomy: The postmortem fate of human remains. CRC Press; Boca Raton 1997.

97. Bull, Ian D.; Berstan, Robert; Vass, Arpad; Evershed, Richard P. Identification of a disinterred grave by molecular and stable isotope analysis. Science and Justice 2009. 49; 142-149.

98. Hunter, John; Cox, Margaret. Forensic archaeology: Advances in theory and practice. Routledge:; New York 2005.

99. Tibbett, Mark; Carter, David O. Soil analysis in forensic taphonomy: Chemical and biological effects of buried human remains. CRC Press 2008.

100. Vass, Arpad A.; Bass, William M.; Wolt, Jeffery D.; Foss, John E.; Ammons, John T. Time since death determinations of human cadavers using soil solution. Journal of Forensic Sciences 1992; 37(5); 1236-1253.

101. Tolliver, Samantha Shirelle. Identification of *Canis familiaris* signature odor chemicals in human remains using derivatization solid-phase microextraction / gas chromatography / mass spectrometry. Master's thesis: Florida International University; Department of Chemistry and Biochemistry 2005.

102. Statheropoulos, M; Spiliopoulou C.; Agapiou, A. A study of volatile organic compounds evolved from the decaying human body. Forensic Science International 2005. 153: 147-155.

103. Vass, Arpad A.; Smith, Rob R.; Thompson, Cyril V; Burnett, Michael N; Wolf, Dennis; Synstelien, Jennifer A; Dulgerian, Nishan; Eckenrode, Brian. Decompositional odor analysis database. Journal of Forensic Science 2004; 49; 760-769.

104. Vass, Arpad A; Smith, Rob; Thompson, Cyril V; Burnett, Michael; Dulgerian, Nishan; Eckenrode, Brian. Odor analysis of decomposing buried human remains. Journal of Forensic Science 2008; 53(2); 384-391.

105. Statheropoulos, M; Agapiou, A; Spiliopoulous, C; Pallis, G.C; Sianos, E. Environmental aspects of VOCs evolved in the early stages of human decomposition. Science of the Total Environment 2007; 385; 221-227.

106. Deng, Chunhui; Zhang, Xiangmin; Li, Ning. Investigation of volatile biomarkers in lung cancer blood using solid phase microextraction and capillary gas chromatography-mass spectrometry. Journal of Chromatography B 2004; 808; 269-277.

107. Dekeirsschieter, J; Verheggen, F.J; Gohy, M; Hubrecht, F; Bourguignon, L; Lognay, G.; Haubruge, E. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* L.) in different biotopes. Forensic Science International 2009; 189; 46-53.

APPENDICES

Appendix 1: Copy of the approved SWGDOG Guidelines for SC7, Research and Technology. Highlighted portions directly pertain to this research.

SWGDOG SC7 – RESEARCH & TECHNOLOGY

Posted for Public Comment 1/3/07 - 3/3/2007. Approved by Membership 3/12/2007.

Statement of Purpose:

The mission of the SWGDOG subcommittee on Research and Technology is to identify research and technological approaches, topics, and findings that are relevant to the detection canine and orthogonal detector (primarily instrumental detection) communities. This subcommittee is also charged with identifying areas in need of engagement by the scientific community and topics that should be the focus of the next generation of research efforts.

The Research and Technology Subcommittee serves as a clearinghouse for the available scientific literature regarding detector dogs and orthogonal detectors making available a searchable database of up-to-date publications and encouraging research in areas where gaps exist in the knowledge base or detection capabilities. In addition, this subcommittee will utilize the latest scientific information to make recommendation on best practices to other SWGDOG subcommittees. In the course of its work, this subcommittee will identify topics that need clarification and those that would benefit from a newer, more scientific approach. Research on various topics is expected to focus on facilitating all aspects of detection work and increasing cost-effectiveness of the relevant programs. Additionally, this subcommittee will outline key research concerns and, or project areas with the intent of establishing potential collaborative relationships between researchers and operational personnel, and identifying potential areas of funding.

1. RECOMMENDED RESEARCH

Below are recommended research topics based on feedback from the community and SWGDOG members and review of the available literature. The following topics are proposed and rated for the desirability of research and potential funding allocation using the following criteria: CRITICAL (potential mission stoppage); ESSENTIAL (can still do the job but this makes it better); ENHANCING (job can still be done but this is nice to have around) or DESIRABLE (wish list). ***This four-point rating scale was added after the public comment period and thus SWGDOG is particularly interested in public comments on the proposed ratings as well as comments on additional areas to be included. Please submit comments at www.swgdog.org.***

1.1. **Identification/quantification of target odorants.** This area focuses on identifying chemicals available to canines from target materials under different conditions and developing and critically evaluating surrogate continuation aids (also referred to as pseudos, simulants, calibrants, mimics) with similar physicochemical properties to real target materials.

1.1.1. Identification of odorant chemicals present in and above targets (particularly human remains, explosives and drugs) including novel applications (chemical, microbes, etc.). ESSENTIAL

1.1.2. Evaluation of changes in odorant(s) over time and environmental conditions. ENHANCING

1.1.3. Evaluation of changes in perception of the odor as a function of changes in concentration of the odorant(s). ENHANCING

1.1.4. Evaluation of optimal storage and handling practices (including containers) to prevent cross-contamination of training aids. ESSENTIAL

1.1.5. Development of methods for monitoring levels of contamination of aids. Identify when training aids are contaminated, how long it takes to dissipate the contamination odor off the pure odor training aid. CRITICAL

1.1.6. Identification of odor chemicals in non target materials that can potentially trigger false alerts (particularly for drugs, explosives and humans remains).

ENHANCING

1.1.7. Evaluation of dissipation of odorant(s) after removal of targets. How soon can you reuse an area – time for dissipation/ decomposition of residual odors? ESSENTIAL

1.1.8. Develop a scientifically valid odor list for testing detector dogs (particularly explosives). CRITICAL

1.1.9. Development of reliable surrogate continuation aids (particularly for drugs, explosives and human remains). These must provide controlled delivery of chemicals to allow for an assessment of threshold variance, but not to be used for certification purposes. (canine and possibly equipment calibration). This also pertains to emerging threats. CRITICAL

1.2. Research on olfaction- Focused on laboratory research, either chemical or behavioral.

For example, the question regarding the limitation of tracking would best be considered under "dog performance" and not under olfaction. (as supporting section 1.1)

1.2.1. Development of aids ENHANCING

1.2.1.1. Identifying the optimal numbers, amounts and identities of target odors (particularly for explosives and human remains).

1.2.1.2. Test improved training aids/proficiency test delivering reliable controlled odor amounts (critical evaluation between training aids and real materials).

1.2.1.3. Develop and scientifically validate non-hazardous training materials. Minimize risks and provide reliable amounts of target odors.

1.2.1.4. Determine the ability to trap and release target odors fo r collection materials and develop "intelligent" materials that are odor specific.

1.2.2. Determination of thresholds ENHANCING

1.2.3. Comparison of detectors ESSENTIAL

1.2.3.1. Critical comparisons of capabilities of certified detector dogs and electronic noses to reliably detect target odors in the presence of

interfering (distractor) odors.

1.2.3.2. Quantifying cost effectiveness of canine search teams over human searchers (with and without using instruments).

1.2.3.3. Comparison of standoff capabilities of canines and instrumentation

1.2.3.4. Listing of complementary instrumentation for application with canines.

1.2.3.5. Comparison of dogs to other biological detection entities.

1.3. Research on Learning. This section will include actual experimentation on training methodologies, types of reinforcement, relationship between training and operations performance and questions on generalization and concept formation. The following topics are proposed: ENHANCING

1.3.1. Research on the effectiveness of training aids. Does extensive experience with the training aid help or hinder the later detection of the real odor? CRITICAL

1.3.2. What is the optimal way to utilize training aids? Start easy (e.g., most volatile) or start hard (e.g., least volatile). Start with mixture of odors ("cocktail" or "beef stew" approach) or with individual odors. ESSENTIAL

1.3.3. Masking effects and training to overcome them. ESSENTIAL

1.3.4. Memory for previously trained odors. DESIRABLE

1.3.5. Effects of extinction on olfactory search and detection. ESSENTIAL

1.3.6. Context effect. ENHANCING

1.3.7. Search images (history: define in more detail). ESSENTIAL

1.3.8. Generalization versus concept formation on the response to novel odors. ESSENTIAL

1.3.9. Reinforcement effects, the effects and side effects of negative reinforcement. DESIRABLE

1.4.9.1. Food versus Play reinforcement. Increasing selection pool

1.3.10. Effects of reinforcement schedules on performance. ESSENTIAL

1.3.11. Effects of odor quantity on detection. Is there really a difference in training on 10 g. of TNT versus 10000 g. of TNT? And if so, what and why?

ENHANCING ESSENTIAL

1.3.12. Effects of additional cues on target detection (such as the odor of the human placing the target and the odor of newly dug holes). ESSENTIAL

1.4. Dog Performance – An important goal when training working dogs is to determine the

performance envelope of the dogs so that there is a correct understanding of the ir capabilities and limitations. Only when we know how the dogs are presently working will we be able to determine the effectiveness of new manipulations. Basically, the goal is to obtain a clear understanding of how the current working dogs actually work and what variables affect their probability of detection. Some of the most important variables to be considered in this topic are:

1.4.1. Environmental variables ENHANCING

1.4.1.1. Temperature and humidity

1.4.1.2. Type of terrain

1.4.1.3. Effects of wind and rain

1.4.1.4. Effects of time since target was planted.

1.4.1.5. Effects of target micro- niche, buried, in trees, under water etc. (With a focus on interactive effects)

1.4.1.6. Characterization of structures of odor plumes

1.4.2. Behavioral variables ENHANCING

1.4.2.1. Maximum and optimal search time

1.4.2.2. Effects of target density

1.4.2.3. Effects of knowledge of the area being searched, previous experience in the same area

1.4.3. Trainer/handler variables ENHANCING

1.4.3.1. On versus off-leash for the probability of detection

1.4.3.2. Reinforcement history

1.4.4. REST/RASCO/MEDDS etc. (Remote Explosive Scent Tracing). Independent scientific evaluations of the capabilities and limits of the REST systems.

ESSENTIAL

1.4.5. Physical and physiological function of the dog as related to performance. ESSENTIAL

1.5. Selection, Development, and Early Experience. This is a somewhat related collection of topics. The overall goal is to determine how to optimize the development of detector dogs. Suggested topics include:

1.5.1. Early olfactory experience and later detection of that odor ENHANCING

1.5.2. Does environmental enrichment help prepare dogs for harsh and different environments? ENHANCING

1.5.3. Rearing in a kennel versus home environment- which is better? DESIRABLE

1.5.4. What is required during development to get a good working dog? ESSENTIAL

1.6. Veterinary issues

1.6.1. Orthopedic problems DESIRABLE

1.6.2. Breed problems etc. DESIRABLE

1.6.3. Evaluation of transmitting thermometer to determine heat stress in dogs. One handler/supervisor can immediately see on a receiver the internal body temperature of all the dogs and determine if any are becoming hyperthermic or hypothermic. ENHANCING

1.7. Human scent

1.7.1. Determine the optimal materials and procedures for the collection and storage of human scent. CRITICAL

1.7.2. Quantify the influence of environmental factors (particularly time) on human scent composition and detection (incorporate into optimize training protocols). ESSENTIAL

1.7.3. Evaluate which chemicals make human scent unique and the influence/correlation to state of health and genetic factors (MHC influence). ENHANCING

1.7.4. Evaluate what components of human scent dogs use to detect live humans. ENHANCING

1.7.5. Quantify the amount of human scent required for dogs to trail and to identify. ENHANCING

1.7.6. Conduct critical evaluations of the limitation of human scent dogs (aged trails, versus fresh trails, no scent article, large contamination) CRITICAL

1.7.7. Evaluate the difference between live and deceased human scent and the timing and chemicals characteristic of human remains. ENHANCING

1.7.8. Critically evaluate contamination issue (If humans shed skin cells 24/7 from their entire bodies, for example, does a pair of gloves stop the human odor from transferring to the training aids?) CRITICAL (also included in the training aids section)

2. POTENTIAL FUNDING SOURCES

The table below lists some potential funding agencies including contact persons and the foci/interests of the agencies.

Agency Website Foci

NIJ www.ojp.usdoj.gov/nij State & Local Law Enforcement

TSWG www.tswg.gov Combating terrorism

DARPA www.darpa.mil Stealthy sensors

NIH www.nih.gov Basic science

HSARPA http://www.hsarpasbir.com Security/First responders

CBP http://www.cbp.gov Customs and border protection

ONR http://www.onr.navy.mil Warfare and combating terrorism

DHS/S&T www.dhs.gov/scienceandtechnology Technology to protect the homeland

APPENDIX 7-1 - DATABASE OF PUBLISHED LITERATURE

Using a collect list of key words and topic areas, a detailed literature database has been constructed using Reference manager and will be made available on www.swgdog.org. The database includes reviewed journal articles, edited chapters and technical reports

with explanation of how these reports may be requested. There will also be a selected list of books focusing on those with an underlying scientific basis and detailed references

Appendix 2: Copy of the approved SWGDOG Guidelines for SC8 Substance Dogs: Human Remains. Highlighted portions directly pertain to this research.

SWGDOG SC8– SUBSTANCE DETECTOR DOGS
Human Remains Detection (HRD)
Land and Water

Posted for Public Comment 1/14/09 – 3/14/09. Approved by the membership 9/15/09.

Statement of purpose: To provide recommended best practice guidelines for training, certification and documentation pertaining to human remains (cadaver) detection canines on land and /or water. The following guidelines pertain to land and water or a combination of both applications.

1. INITIAL TRAINING

1.1. The canine trainer shall be competent in human remains detection and utilize a structured curriculum with specific training and learning objectives.

1.2. The training course shall include training on the complete spectrum of human remains at varying stages of decomposition. All training aids shall be treated as biohazardous material. The procurement, use, handling, storage and disposal of training aids shall be in compliance with applicable local, state and federal requirements. Examples of training aids include the following:

1.2.1. Human blood (fresh and old).

1.2.2. Human decomposition material (tissue, adipocere, wet and dry bone, body fluids).

1.2.3. Burned human tissue.

1.3. The quantity and type of substances used shall be dependent on the region, mission and operational deployment needs of the canine team.

1.4. Training shall include exposing the canine to a variety of different types of searches, locations and environments.

1.5. The training shall include varying quantities of target odors, containers and varying lengths of placement time.

1.6. The canine shall be trained to perform an effective independent search on or off lead without excessive handler guidance.

1.7. Handler/trainer training shall include the following:

1.7.1. Search planning, techniques, tactics and equipment.

1.7.2. Dog handling techniques.

1.7.3. First aid for dog and handler.

1.7.4. National Incident Management System (NIMS) (ICS 100 and 200, IS 700) courses are available online.

1.7.5. Additional training as specified by local, state and federal requirements.

1.7.6. Proper use, handling, storage and disposal of biohazardous materials.

1.7.7. Legal aspects and courtroom testimony as outlined in Sub Committee 6's document.

1.7.8. Crime scene/evidence preservation/and record keeping.

1.7.9. In addition, water safety shall be included for HRD water teams.

1.8. The initial training should continue until the HRD canine team is certified or deemed unacceptable.

2. CERTIFICATION

2.1. Certification for HRD canines shall be comprised of a comprehensive assessment together with either an odor recognition assessment or a double-blind assessment, or both as outlined in SWGDOG General Guidelines.

2.1.1. Odor recognition assessment

2.1.1.1. The handler shall be advised of the parameters of the search.

2.1.1.2. The handler shall know the number of target objects, but not the placement.

2.1.1.3. The evaluating official shall know the desired outcome of the search.

2.1.2. Comprehensive assessment

2.1.2.1. The handler shall be advised of the parameters of the search, yet shall not know the desired outcome.

2.1.2.2. The handler shall not know the number or placement of the target objects.

2.1.2.3. The evaluating official shall know the desired outcome of the search.

2.1.2.4. The assessments shall include a blank search.

2.1.3. Double-blind assessment

2.1.3.1. No participant or observer present at the assessment location(s) shall be aware of the parameters of the search.

2.2. Ideally, the certification shall be designed in a manner that resembles searches conducted in the canine team's normal operational environment.

2.3. The test shall be designed to evaluate:

2.3.1. The canine's ability to recognize the odor.

2.3.2. The canine's ability to respond to the odor.

2.3.3. The handler's ability to recognize the canine's alert.

2.3.4. The handler's ability to articulate where the material is located.

2.4. For successful certification, the canine team shall achieve a 90% confirmed alert rate with no false alerts.

2.5. A canine team that fails the certification process shall complete a corrective action plan before making another attempt to certify.

3. MAINTENANCE TRAINING

3.1. Maintenance training is meant to sustain and enhance the performance of the handler, canine and the canine team.

3.2. In training, situations are purposely sought where the capabilities of the canine and handler are challenged within the operational environments for which the team may be deployed.

3.3. Routine maintenance training is essential in order to maintain mission readiness. A canine team shall spend a minimum of 16 hours per month in routine land and/or water training to maintain the proficiency level of the team.

3.4. The canine team shall conduct regular objective-oriented training sufficient to maintain and enhance operational proficiency. Maintenance training shall include the following:

3.4.1. Routine training, conducted solely by the handler to maintain the canine's proficiency and to reinforce odor recognition, is an acceptable form of training but must be combined with supervised training on a regular basis.

3.4.2. Supervised training, conducted by a competent trainer other than the handler, in order to improve performance, identify and correct training deficiencies and perform proficiency assessments is considered a best practice.

4. TRAINING AIDS

4.1. Training shall be done on actual human remains in varying stages of decomposition to conform to best practices.

4.1.1. The source of the training aids shall be reliable and documented.

4.2. The training aids shall be labeled and packaged in a manner safe for both the handler and canine throughout training.

4.2.1. Each label shall contain, at minimum, the type of training aid, a biohazard label and the date the training aid was acquired.

4.3. Each training aid shall be properly stored (either frozen, air dried, or refrigerated) and secured in a safe manner.

4.4. Each training aid shall be maintained in a manner to avoid loss, destruction and cross contamination.

4.5. Handling and care of training aids shall include the following:

4.5.1. Each training aid shall be handled in accordance with biohazard safety standards for proper handling, storage and disposal.

4.5.2. Each training aid shall be rotated on a regular basis, evaluated to determine the level of decomposition and replaced if contaminated.

4.5.3. Storage of training aids shall be in a manner that prevents odor and physical contamination, i.e., each range of decomposing cadaver materials should be stored in separate containers.

4.6. Disposal and or destruction of the training aids shall follow local, state or federal guidelines pertaining to biohazardous materials.

5. RECORDS AND DOCUMENT MANAGEMENT

5.1 The handler/organization/agency shall maintain training, and/or deployment/utilization records. Documents shall be retained in accordance with federal, state and unit guidelines. Records may include but are not limited to the following data:

5.1.1. Training records shall include:

5.1.1.1. Name of handler and canine.

5.1.1.2. Date and time training was conducted.

5.1.1.3. The trainer's name and position.

5.1.1.4. Type and amount of training aid used.

5.1.1.5. Height and/or depth of the hide.

5.1.1.6. Location where training took place.

5.1.1.7. Type of training (wilderness, disaster, land, water, buried, etc.).

5.1.1.8. The training objective (to frame the result of the training scenario).

5.1.1.9. Additional information may include: weather conditions, terrain.

5.1.1.10. Other information as required by the organization and/or agency.

5.1.1.11. Set Time

5.1.2. Deployment and utilization records shall include:

5.1.2.1. Name of handler and canine.

5.1.2.2. Date and time of deployment.

5.1.2.3. Location of deployment.

5.1.2.4. Requesting agency.

5.1.2.5. Length of search.

5.1.2.6. Description of search.

5.1.2.7. Type of search (wilderness, disaster, water, etc.).

5.1.2.8. Results of search.

5.1.2.9. Location of a positive find, using GPS coordinates (when available).

5.1.2.10. Other information as required by the organization and/or agency.

5.1.3. Certification records

5.1.3.1.Name of canine and handler

5.1.3.2.Date team certified

5.1.3.3.Certification authority, i.e., agency, professional organization, and/or individual(s)

5.1.3.4.The standard or guideline under which the canine team is certified

5.1.3.5. Name of individual(s) awarding certification.

5.1.3.6.Search area types included in certification assessment

5.1.3.7.Type and amount of materials included in certification assessment

5.1.3.8.Location of certification

5.1.3.9.Set Time

6. USE OF RECORDS AND DOCUMENTATION

6.1. Records may be discoverable in court proceedings and may become evidence of the canine team's reliability. Record retention policy shall be determined by department/organization guidelines

6.2. Training records are necessary to illustrate the type and amount of training that the team has experienced before and after certification

6.3. Confirmed operational outcomes can be used as a factor in determining capability

6.4. Unconfirmed operational outcomes shall not be used as a factor in determining capability in that they do not correctly evaluate a canine team's proficiency (i.e., residual odor can be present or concealment may preclude discovery)

LAND APPLICATIONS

7. *INITIAL HRD DETECTION TRAINING ON LAND* shall include exposing the canine to a variety of different types of search locations and environments including the following variables:

7.1. Ground surface.

7.2. Elevated position not to exceed 2 meters (≈6 ft).

7.3. Buried at least 15 to 61 centimeters (6 to 24 inches) depending on soil composition.

8. CANINE TEAM CERTIFICATION

8.1. Parameters of the test: The test area shall not be an area that is normally used for daily or routine training of the canine team.

8.1.1. Prior to the start of the certification, the handler will inform the evaluator how the canine will respond when the target odor is detected.

8.1.2. The human remains detector canine shall be tested on at least two of the suggested materials in the complete spectrum of materials as identified in sections 1.2.1 and 1.2.2.

8.1.3. Recommended minimum quantities of materials for certification shall be set in accordance with mission requirements.

8.1.4. Placement of the aids shall include the following:

8.1.4.1. Ground surface.

8.1.4.2. Elevated position not to exceed 2 meters (≈6 ft).

8.1.4.3. Buried 15 to 61 centimeters (6 to 24 inches) depending on soil composition.

8.1.5. The test shall include blank areas containing freshly disturbed soil uncontaminated by human remains.

8.1.6. Animal remains distractors shall be included in at least one search area.

8.1.7. The certification shall include scenarios resembling searches within the normal operational environment. The test shall include at least four individual search areas with a minimum of one blank area, from at least two of the categories listed below. Individual search areas may contain multiple target odors. The test shall be designed to evaluate the canine's ability to recognize the odor, respond to the odor and the handler's ability to interpret this alert. Search categories and suggested maximum search times utilized in certifications are listed below:

8.1.7.1. Wilderness searches shall cover a minimum of 4050 m^2 (≈1 acre) in 30 minutes/acre depending on the scent quantity and source.

8.1.7.2. Urban searches shall cover a minimum of 4050 m^2 (≈1 acre) in 30 minutes. The area searched and search time may vary depending on the scent quantity and source.

8.1.7.3. Building/structure searches shall cover a minimum 93 m^2 (≈1000 sq. ft.) in 30 minutes. The area searched and search time may vary depending on the scent quantity

and source. Vehicle searches (interior and exterior) shall cover a minimum of three to six vehicles. Search time should be three minutes per vehicle.

8.1.7.4. Disaster area search time may be dictated by the difficulty of the scenario.

8.1.8. The minimum set time of training aids shall be no less than 30 minutes and no more than 24 hours. The maximum set time may be extended as dictated by the mission of the agency.

8.1.9. For successful certification, the canine team shall achieve at least a 90% confirmed alert rate for certification, with no false alerts.

8.2. Use of distractors

8.2.1. Natural distractors are normally present and vary depending on the certification area.

8.2.2. Care must be taken not to place artificial distractions in a manner that causes contamination with the test substance odor. Target odors should not be placed near areas with decomposed human waste.

9. MAINTENANCE TRAINING FOR HRD – LAND shall include the following components:

9.1. A variety of locations, environmental conditions and times of day.

9.2. A variety of training aid amounts and the full spectrum of decomposition of those training aids.

9.3. A variety of heights, depths, containers and distraction odors.

9.4. A variety of types of searches including wilderness, disaster, vehicles, buildings, open areas and shoreline (based on mission specific requirements).

9.5. A varied duration of search times.

9.6. A variety of search area sizes.

9.7. A variety of blank searches.

9.8. A variety of searches that include animal distractors.

WATER APPLICATIONS

10. INITIAL HRD TRAINING ON WATER shall include exposing the canine to a variety of different types of search locations and environments including the following variables:

10.1. Shoreline searches.

10.2. Shallow, deep, still and swift running water from a watercraft.

10.3. Cadaver material at varying depths of water.

10.4. Blank areas which do not include human remains but may include animal remains.

10.5. Empty unused training aid containers.

10.6. Varying quantities of target odors, containers and lengths of time of placement.

11. CANINE TEAM CERTIFICATION

11.1. Parameters of the test: The test area shall not be an area that is normally used for daily or routine training of the canine team.

11.1.1. Prior to testing on water, the canine team shall successfully perform an odor recognition test on land.

11.1.2. Proofing/verification of the certification area should be conducted prior to the actual certification using a certified canine team who is not participating in the certification. This practice is designed to show that the trained odor is present in the target locations and nowhere else.

11.1.3. Prior to the start of the certification, the handler will articulate to the evaluator the canine's alert to the target odor.

11.1.4. Handlers are required to wear personal flotation devices (PFD) when on a boat, pier or near the water. PFD is optional for the canine.

11.1.5. The human remains detector canine shall be tested on at least two of the suggested materials in the complete spectrum of materials as identified in sections 1.2.1. and 1.2.2.

11.1.6. Recommended quantities of materials for certification shall be no less than 30 grams (1oz).

11.1.7. Placement of the aids shall include all of the following:

11.1.7.1. Shoreline assessment no less than 46 meters (50 yd) in length, no more than 4 meters (≈12 ft) from shore, no greater than 1 meter (≈3ft) in depth and spending no longer than 15 minutes to search the area.

11.1.7.2. Boat assessment: in calm water (lake or pond) no less than 90 x 90 meters (≈100 by 100 yd) assessment area with the area divided into four quadrants. Scent material shall be placed in a depth of between 3.0 to 3.5 meters (≈10 to 11 ft) in one of the quadrants. The canine's response shall be within a radius of 2 meters (≈6 ft) of the highest concentration of the target odor. Search time in the boat shall be no more than 45 minutes per 90 meter² area.

11.1.7.3. The training aids shall be placed no less than 30 minutes prior to testing.

11.1.7.4. All training aids shall be removed at completion of certification.

11.1.8. Ideally, the test shall be designed in a manner to resemble searches within the normal operational environment.

11.1.9. The test shall be designed to evaluate:

11.1.9.1. The canine's ability to recognize the odor.

11.1.9.2. The canine's ability to respond to the odor.

11.1.9.3. The handler's ability to interpret the canine's alert.

11.1.9.4. The handler's ability to articulate where the submerged material is located.

11.1.10. For successful certification, the canine team shall achieve a 90% confirmed alert rate and no false alerts.

12. MAINTENANCE TRAINING FOR HRD – WATER shall include:

12.1. A variety of locations, environmental conditions, and times of day.

12.2. A variety of training aid amounts and the full spectrum of decomposition.

12.3. A variety of depths, containers and distraction odors.

12.4. A variety of types of searches to include all types of water (still, slow-moving and fast-flowing water).

12.5. A varied duration of search times.

12.6. A variety of search area sizes.

12.7. A variety of blank searches

Appendix 3: Instructions to canine handlers for training aid trials

Prior to testing:

- **Keep training aids in freezer until use.** Remove aids from freezer at least 15min prior to use.
- Fill out the top half of the "K9 Data Sheet"
    - The canine/handler name and information will not be included in any publications or reported in any manner

Test set up: Positive and Negative controls

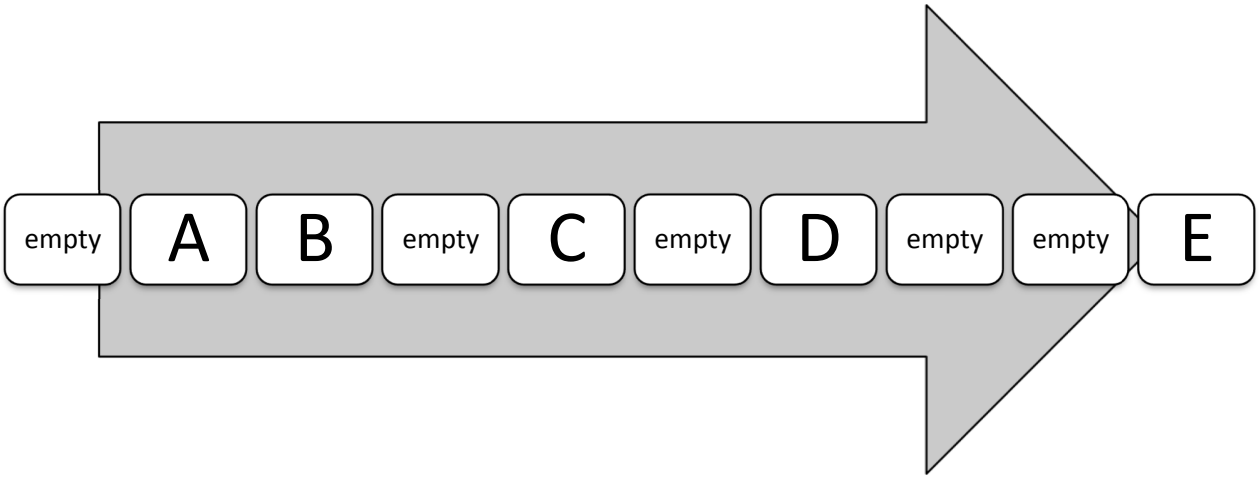
- Set out and run the dogs on the two negative controls provided and a positive control of your choice
    - For the positive control, use a training aid to which the canine is known to alert
        - The positive control is used to show that the canine is ready to work
    - For the negative controls, remove the lid from the jar and set out. Open the bag by cutting with scissors and empty the gauze pad into position.
        - The negative controls are used to get the dog used to the type of samples he/she will be seeing during the experiment
        - Negative controls can be reused, however handle with fresh gloves to prevent cross-contamination

Test set up: Aluminized bags

- Set up 10 containers in a scent line-up as pictured below.
    - There will be five empty containers and five with training aids A-E
    - Use cement blocks, baskets, cages etc to conceal and protect the training aids
    - Containers should be five feet apart
- Wear gloves during set up and change if gauze is touched
- Open bag with scissors and empty the gauze pad into its correct position in/under the container
    - When opening, be careful to not cut the gauze pad inside
    - After set up, do not touch gauze pad unless absolutely necessary!!!! If it absolutely must be touched, wear gloves provided and change gloves between touching more than one pad to prevent cross contamination!!!!!

Test set up: Glass jars

- Set up another row of 10 containers as pictured below.
    - There will be five empty containers and five with training aids A-E
    - Containers should be five feet apart
- Wear gloves during set up and change if gauze is touched
- Remove screw caps from glass jars
    - Do not open jars until set up is complete!
    - Only remove screw caps, do not remove the snap-on cap with holes
    - Place the glass jars in/under the containers



Canine testing:

- Run one dog at a time
    - Do not let other canine/handlers watch the runs whenever possible
    - Dogs/handlers may take as much time as necessary
- Record results on table in "K9 Data Sheet"
    - Record the dog's response as an "alert", "interest" or "no response"
    - Remember the 5 samples will include some combination of blanks, distracters and samples. The dogs will most likely not respond to all 5 aids. Also, individual dogs may respond differently and still be correct.

After testing:

- Add any additional comments to "K9 Data Sheet" that may be relevant (ie. change in canine behavior, problem with training aid, change in weather conditions, etc.).
- Return data sheets in envelope provided as soon as possible.
- Email or call me if you have any questions or would like to know the results:

Appendix 4: Example data sheets for canine handlers for use during training aid trials.

Date/time of assessment: _____ Location: _____

Canine name: _____ Handler name: _____

Canine age: _____ Canine breed: _____

Years of canine experience: _____ Certified in HRD: yes / no

Type of certification: -

_____

Date of last certification: _____ Certification frequency:_____

Canine alerted correctly to positive control before assessment? yes / no

Type of positive control used:

_____

Sample	Alert	Interest	No Response
A			
B			
C			
D			
E			

Weather conditions (temp, humidity, rain, etc.):

Additional notes to assessor:

VITA

LAURYN E. DEGREEFF

August 25, 1981	Born, Canton, Ohio
1999 - 2000	Emory University Atlanta, GA
2000 - 2003	B.A. Anthropology New York University New York City, NY
2002	Internship Office of the Chief Medical Examiner New York City, NY
2004 - 2006	B.S. Chemistry Chapman University Orange, CA
2004	Provost's List Chapman University Orange, CA
2005 - 2006	Research Assistant Chapman University Orange, CA
2006	High Achievement in the Study of Chemistry Chapman University Orange, CA
2006 – 2010	Research Assistant Florida International University Miami, Florida
2007-2009	President / Vice-President Chemistry Graduate Student Organization Florida International University Miami, Florida

PRESENTATIONS

DeGreeff LE, Furton KG. Collection and determination of human remains volatiles by non-contact, dynamic airflow. Presented at the American Chemical Society Regional Conference; Tampa, FL; May 2010.

DeGreeff LE, Furton KG. Determination of the odor signature of human remains using non-contact, dynamic airflow sampling. Presented at the Pittsburg Conference; Orlando, FL; March 2010.

DeGreeff LE, Furton KG. Collection of human remains volatiles by non-contact, dynamic airflow sampling. Presented at the 62nd American Academy of Forensic Science Conference; Seattle, WA; February 2010.

DeGreeff LE, Curran AM, Furton KG. Optimization of the Scent Transfer Unit (STU-100) for the non-contact sampling of human scent volatile compounds. Presented at the 61st American Academy of Forensic Science Conference; Denver, CO; February 2009.

DeGreeff LE, Herran S, Furton KG. The development of the human scent collection for the minimization of environmental contamination during non-contact human scent sampling. Presented at the 60th American Academy of Forensic Science Conference; Washington DC; February 2008.

DeGreeff LE, Kim CS. The effect of particle size on copper uptake to iron oxyhydroxide nanoparticles as a function of pH. Presented at the 17th Annual Graduate Women in Science Conference; Orange, CA; March 2006.

DeGreeff LE, DeBruyn WJ. The photo-production of acetone from dissolved organic matter in seawater. Presented at the American Chemical Society Western Regional Conference; Anaheim, CA; January 2006.

DeGreeff LE, Kim CS. The effect of particle size on copper uptake to iron oxyhydroxide nanoparticles as a function of pH. Presented at the American Chemical Society Western Regional Conference; Anaheim, CA; January 2006.

McKee MA, DeGreeff LE, Kim CS. Reactivity of iron oxyhydroxide nanoparticles with As(V), Cu(II), Hg(II), and Zn(II) as a function of particle size. Presented at the 231st American Chemical Society National Conference; Atlanta, GA; March 2006