Anal Bioanal Chem (2011) 401:1295–1307
DOI 10.1007/s00216-011-5167-0

ORIGINAL PAPER

Collection and identification of human remains volatiles by non-contact, dynamic airflow sampling and SPME-GC/MS using various sorbent materials

Lauryn E. DeGreeff · Kenneth G. Furton

Received: 30 March 2011 / Revised: 1 June 2011 / Accepted: 5 June 2011 / Published online: 22 June 2011

Abstract Human remains detection canines are used in locating deceased humans in diverse scenarios and environments based on odor produced during the decay process of the human body. It has been established that human remains detection canines are capable of locating human remains specifically, as opposed to living humans or animal remains, thus suggesting a difference in odor between the different sources. This work explores the collection and determination of such odors using a dynamic headspace concentration device. The airflow rate and three sorbent materials—Dukal cotton gauze, Johnson & Johnson cotton-blend gauze, and polyester material—used for odor collection were evaluated using standard compounds. It was determined that higher airflow rates and openly woven material, e.g., Dukal cotton gauze, yielded significantly less total volatile compounds due to compound breakthrough through the sorbent material. Collection from polymer- and cellulose-based materials demonstrated that the molecular backbone of the material is a factor in compound collection as well. Volatiles, including cyclic and straight-chain hydrocarbons, organic acids, sulfides, aldehydes, ketones, and alcohols, were collected from a population of 27 deceased bodies from two collection locations. The common compounds between the subjects were compared and the odor profiles were determined. These odor profiles were compared with those of animal remains and living human subjects collected in the same manner. Principal component analysis showed that the odor profiles of the three sample types were distinct.

Keywords Forensic/toxicology · Human remains · Odor · Detection canines · Odor sampling

L. E. DeGreeff · K. G. Furton
International Forensic Research Institute,
Department of Chemistry and Biochemistry,
Florida International University,
11200 S.W. 8th Street,
Miami, FL 33199, USA

K. G. Furton (✉)
College of Arts and Sciences, Florida International University,
ECS 450, 11200 SW 8th Street,
Miami, FL 33199, USA
e-mail: furtonk@fiu.edu

Introduction

Human remains detection (HRD) canines or cadaver dogs are trained to locate human remains including whole bodies, body parts, tissue, blood, bone, and decomposition fluids based on the odor produced by the decomposition of such remains. Well-trained HRD canines are adept at locating fresh remains, shortly after death, remains that have been submerged in water or buried, and remains in very small quantities.

The process of decomposition begins as early as 4 min after death. As soon as the heart stops pumping, the cells in the body are deprived of oxygen, causing 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 [1].

Autolysis initiates a cascade of other events leading to the body's complete decomposition. Following autolysis, the anaerobic breakdown of the body's macromolecules (carbohydrates, lipids, and proteins) begins due to the action of bacteria in the digestive and respiratory systems. The breakdown of molecules leads to color changes in the skin and bloating of the body as gases begin to build up in

the body. As the gases expand internally, the skin loses its integrity and ruptures, reintroducing oxygen and allowing for aerobic activities to resume and further break down the body to skeletonization [2].

The body's macromolecules—proteins, lipids, and carbohydrates—degrade into smaller, simple 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 [2]. The loss of an amine and a hydrogen during deamination causes an accumulation of ammonia. The deamination of L-phenylalanine is responsible for the greenish tint of the skin during early to mid-level decomposition. Decarboxylation is carried out by bacterial enzyme activity, primarily *Enterobacteriaceae*, yielding the production of carbon dioxide and biogenic amines [3] including cadaverine and putrescine from ornithine and lysine, respectively. Cadaverine and putrescine are particularly odorous molecules and may be partially responsible for detection by HRD canines [2].

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 found include oleic, palmitic, linoleic, stearic, myristic, palmitoleic, and vaccenic. In an aerobic environment, the unsaturated fatty acids are oxidized to aldehydes and ketones, and in an anaerobic environment, the unsaturated fatty acids are saturated [3].

The body's carbohydrates 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 produces methane, hydrogen sulfide, and hydrogen gases [3].

Only a few research groups have attempted to characterize the volatile organic compounds emanating from human remains. A condensed list of compounds identified in human remains by different research groups is included in Table 1. 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 with the highest frequency; dimethyl disulfide, a particularly odorous compound, was widely reported in the literature as well.

Table 1 VOCs from human remains documented in peer-reviewed literature

Compounds	Reference
Acids/esters	
Propanoic acid	[8]
Butanoic acid	[8–10]
Butanoic acid, ethyl ester	[4, 8]
Hexanoic acid	[8, 9]
Pentanoic acid	[9]
Heptanoic acid	[9, 10]
Nonanoic acid	[9]
Octanoic acid	[9]
Formic acid	[11]
Acetic acid	[11]
Propriolic acid	[11]
Ethyl acetate	[11]
Butanoic acid, butyl ester	[8]
Hexanoic acid, ethyl ester	[8]
Hexanoic acid, pentyl ester	[8]
Hexanoic acid, hexyl ester	[8]
Hexadecanoic acid, methyl ester	[6, 7]
Propanoic acid, 2-methyl–, ethyl ester	[4]
Acetic acid, propyl ester	[4]
Propanoic acid, ethyl ester	[11]
Alcohols	
1-Pentanol	[4, 8, 9]
1-Hexanol	[4, 8, 11]
1-Octen-3-ol	[8]
1-Hexanol, 2-ethyl	[5]
1-Octanol	[8]
2-Propanol	[5]
Phenol	[5, 10]
Phenol, 4-methyl	[5]
Benzenemethanol, α,α,dimethyl-	[6, 7]
Ethanol	[4, 5]
1-Butanol	[4]
1-Undecanol	[11]
1-Octanol, 6-methyl	[11]
1-Nonanol	[11]
1-Propanol, 2-amino	[11]
Halogens	
Trichloroethene	[6, 7]
Carbon tetrachloroide	[6, 7]
1,1,2-Trichloro-1,2,2-trifluoro ethane	[6]
Trichloromonofluoro-methane	[6, 7]
Dichlorodifluoromethane	[6, 7]
Chloroform	[6, 7]
Dichlorotetrafluoroethane	[6, 7]
1,1-Dichloro-1-fluoroethane	[7]
Tetrachloroethene	[6–8]
Isopropyl sulfonyl chloride	[11]

Table 1 (continued)

Compounds	Reference
Ketone/ether	
Cyclohexanone	[4, 8]
2-Heptanone	[4, 8]
2-Propanone	[4, 5, 7]
1-Phenyl ethanone	[5]
2-Butanone	[4, 5]
2-Nonanone	[5]
3-Pentanone	[4]
2-Pentanone	[4]
6-Methyl-5-hepten-2-one	[10]
3-Buten-2-one	[11]
Dimethyl ester	[11]
Aldehydes	
2-Hexanal	[8]
Hexanal	[4, 8, 9]
Benzaldehyde	[4, 8, 9]
2,4-Heptadienal	[8]
2-Heptenal	[8]
Heptanal	[8, 9]
2-Octenal	[8]
Octanal	[8]
2,4-Nonadienal	[8]
2-Nonenal	[7, 8]
Nonanal	[8, 10]
3-Methyl butanal	[5]
Decanal	[5, 7, 11]
Pentanal	[4]
Cyclic hydrocarbons	
Toluene	[4, 6–8, 11]
p-Xylene	[4–8]
o-Xylene	[6, 7]
m-Xylene	[4]
Indole	[8]
2-Pentyl-furan	[8, 9, 11]
Ethyl benzene	[4, 7]
Styrene	[5–7]
1-Methyl-2-ethyl benzene	[6, 7]
Methyl benzene	[5]
1,2,3-Trimethyl benzene	[4, 5]
d-Limonene	[4, 5]
2-Ethyl-1,4-dimethyl benzene	[5]
Naphthalene	[5, 6]
1-Methylethenyl benzene	[5]
Benzene	[4, 6]
1-Methoxy propyl benzene	[6]
1,2,4-Trimethyl benzene	[4]
1,3,5-Trimethyl benzene	[4]
1-Ethyl, 3-methyl benzene	[4]
Propyl benzene	[4]

Table 1 (continued)

Compounds	Reference
Straight-chain hydrocarbons	
Butane, 2-methyl	[11]
Isobutane	[11]
1,3-Butadiene, 2 methyl	[11]
Pentane	[11]
2-Pentene, 2-methyl	[11]
Hexane	[11]
Hexane, 2-methyl	[11]
Hexane, 3-methyl	[11]
Heptane, 2-methyl	[11]
Heptene, 2,6-dimethyl	[11]
Sulfides	
Dimethyl disulfide	[4–9]
Dimethyl sulfide	[5]
Dimethyl trisulfide	[4–7]
Methyl ethyl disulfide	[4, 5]
Carbon disulfide	[4, 6, 7]
N-containing compounds	
Trimethylamine	[9]
Methenamine	[6, 7]
Benzonitrile	[7]
Ethanamine, *N*-ethyl	[11]
Methanediamine, tetra methyl	[11]

Statheropoulos et al. measured the volatile organic compounds (VOCs) from several deceased human bodies in the early stages of decomposition using thermal desorption (TD) GC/MS. Two bodies (estimated time of death, 3–4 weeks) were sampled [4] in the first study and a single body (estimated time of death, 3 days) in the second study [5]. The most prominent compound found in both studies was dimethyl disulfide. Other sulfides, ketones, and benzene derivatives were also frequently occurring [4, 5].

Vass et al. also sampled the VOCs associated with four buried human bodies (estimated time of death 1+ years) using TD/GC/MS. 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 others. Vass et al. [6, 7] also noted a number of fluorinated compounds, suggesting that the presence of fluorine is due to the liberation of fluorine previously ingested with drinking water.

Hoffman et al. [8] attempted to identify the VOCs in samples of various types of decomposed human tissues, 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 solid phase microextraction (SPME) and analyzed by GC/MS. The 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.

A final study by Caldwell et al. [11] analyzed the VOCs from air and SPME samples collected from one buried and one hanging body. Many additional compounds were recovered that were not previously documented, including a number of straight-chain hydrocarbons, alcohols, and acids. As seen in Table 1, there is a huge discrepancy in the reported compounds between different research groups. This may be due to the differences in sample collection or preparation, but can also be attributed to the extremely small sample sizes used by the above listed authors. A larger sample size would allow researchers to select the compounds common to all human remains in order to focus on the important compounds. Of all the above listed studies, only nine whole human bodies have been sampled. This work is the first to report VOCs obtained from a population of 27 individuals from multiple locations to determine similar volatiles.

Additionally, many of the bodies previously sampled were buried, and all decomposed outdoors, in an uncontrolled environment. The burial of the body adds the possible effect of the soil, pH, moisture, microorganisms, etc., on the scent picture. The goal of this work was to determine the basic scent picture of human remains with as few alterations from the environment as possible. This was accomplished by sampling unburied bodies provided by both a morgue and a crematorium

In this study, a dynamic headspace concentration device was employed for scent collection. The Scent Transfer Unit (STU-100) used in this study is a field-portable, dynamic airflow collection device for non-contact scent sampling designed to concentrate human scent onto a scent pad and was originally designed to be used in conjunction with human scent detection dogs. It is a simple device consisting of a small vacuum pump attached to a Teflon-coated hood designed to hold a piece of material. The hood has been modified with a stainless steel plate to hold 2×2-in. piece of collection material [12]. When the STU-100 is swept over the subject or object of interest, air is drawn toward the device, collecting any VOCs present in the sample matrix to the gauze pad at the face. The gauze pad is then removed and may be presented to the canine in order to initiate a search. The STU-100, compared with other devices, is unobtrusive and does not disturb or destroy evidence.

A number of studies have been conducted evaluating the performance of STU-100 for the collection of living human scent [13–15]. This is the first peer-reviewed research applying non-contact, dynamic headspace sampling to the collection and evaluation of human remains. The use of such a device for human remains odor collection has the potential to not only identify the volatiles making up human remains odor but also improve canine training and training aids.

Materials/methods

Sampling of standard compounds

Controlled odor mimic permeation systems (COMPS) were fabricated to deliver standard compounds to a dynamic airflow collection device at controlled rates. Such devices have previously been used to deliver standard compounds for the dynamic headspace collection of living human scent volatiles [16]. The COMPS were prepared by spiking 25 μL of a single compound onto Dukal brand, sterile 2 × 2-in. eight-ply gauze pads (Dukal Corporation, Syossett, NY, USA). The gauze pads were immediately sealed into low-density polyethylene bags, 3 in. × 3 in. × 1.5 MIL (Veripak, Atlanta, GA, USA). The volatiles then dissipated from the permeable bags at reproducible rates.

The five standard compounds previously cited as human remains volatiles and representing a range of functional groups were used, including *n*-butyric acid, 99+% (Acros Organics, NJ, USA); heptanoic acid, 99%; 6-methyl-5-hepten-2-one, 99%; liquefied phenol; and nonanal, 95% (Sigma-Aldrich Co., St. Louis MO, USA) [10].

Standard compound sampling was conducted using the STU-100 (Big T LLC, Haw River, NC, USA). For the sampling of the standard compounds, a cleaned piece of the selected sorbent material was placed on the front of the STU-100. Prior to sampling, the material was analytically cleaned with HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ, USA) and baked in an isothermal oven for 45–60 min at 105 °C according to previously established protocols [12]. Cleaned materials were selected at random and analyzed by SPME-GC/MS to confirm that the materials were free of interfering VOCs.

For sampling, the STU-100 was held approximately 1 in. above the COMPS. Collection was carried out for 60 s in triplicate. A blank was prepared in the same manner by sampling COMPS containing no compounds. After sampling, the collection material was removed and returned to a cleaned, 10-mL, clear screw top glass vial with PTFE/Silicone septa (SUPELCO, Bellefonte, PA, USA). Four air flow speeds/settings were tested—high (9), medium (5), low (0) and no air flow—as well as three collection materials: Dukal brand sterile, 2 × 2-in. eight-ply gauze pads, spun polyester (Test Fabrics Inc., West Pittston, PA,

USA), and Johnson & Johnson brand sterile 2 × 2-in. gauze pads (Skillman, NJ, USA).

To determine whether multiple layers of the same collection material enhance or impede scent collection, multiple layers of each material were also assessed. Layers of the collection material were placed on top of one another and onto the STU-100. 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 and analysis.

The extraction and analysis procedure employed was previously optimized for the analysis of living human scent samples [16]. The samples were allowed to equilibrate overnight before the headspace was sampled using SPME. Divinylbenzne/Carboxen/PDMS SPME fibers (SUPELCO) were used to sample the headspace for 21 h. Following extraction, the fibers were thermally desorbed into a Varian Ion Trap GC/MS (CP-3800 Gas Chromatograph/Saturn 2000 MS/MS, Varian Inc., Walnut Creek, CA, USA). The column used was a DB-225MS from Agilent Technologies. Quantitative analysis was performed using external calibration curves for each compound of interest.

Sampling of human/animal remains

Human and animal remains from various sources were sampled in triplicate with the STU-100. Prior to sampling, and between each sample, the STU-100 was cleaned with sterile alcohol prep pads (Fisher HealthCare, Fair Lawn, NJ, USA). A similar cleaning procedure was shown to remove VOCs from the STU-100 between samplings by Stockham et al. [17]. The STU-100 was maintained 1 to 4 in. inches from the subject for 60 s 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's Office in Miami, FL, and from Borden Cremation Service in Louisville, KY. All of the bodies were estimated to be in early to mid-stage of decomposition based on the classification system in *Forensic Taphonomy* [18]. 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.

Animal remains samples were collected from meat products purchased at Publix Grocery Stores in Miami Beach, FL, or were donated by a local restaurant, with the exception of the canine remains. 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 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 off the empty containers previous to remains sampling. Fresh containers were used with each new sample. All animal remains sampled are listed in Table 2.

For comparison purposes, volatiles were also collected from living human subjects using the STU-100. The procedure for living human scent sampling was adopted from a method previously employed by DeGreeff et al. [16]. 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. The palms of the subjects' hands were sampled following washing with a fragrance-free soap (Natural, Clear Olive Oil Soap, Life of the Party, North Brunswick, NJ, USA). The palms of each subject were sampled three times and the subjects were instructed to avoid touching anything between replicate sampling. Blank samples were collected in the same manner prior to human scent collection.

Samples were extracted and analyzed by SPME-GC/MS as per the conditions stated in Table 3. All samples were analyzed first with a shorter exposure time (30 min) and split injection for the improved detection of the early

Table 2 Animal remains obtained for sampling with STU-100

Sample type	Location obtained	Condition of sample prior to sampling based on observation
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

Table 3 Extraction and GC parameters

	Extraction 1	Extraction 2
SPME extraction time	30 min	20 h
SPME extraction temp	Room temperature	70±5 °C
Injection temp (°C)	200	200
Split ratio	10:1	Splitless
Column flow rate (mL/min)	1.0	1.0
Temperature program	40 °C, hold 2 min	40 °C, hold 2 min
	5 °C/min to 80 °C	7 °C/min to 85 °C
	15 °C/min to 220 °C	3 °C/min to 95 °C
		7 °C/min to 220 °C

eluting analytes, followed by a longer extraction (20 h) at 70 °C with a splitless injection for improved sensitivity of the later eluting analytes. Extraction times and temperatures were previously optimized by DeGreeff [19]. Compounds were identified using the MS library and confirmed using retention time and spectra matches to standard compounds.

Statistical analysis

The source of variation between samples was evaluated using ANOVA. ANOVA can assess whether altering the controlled factor, such as flow rate or material, produces a significant difference in the amount of compound collected compared with the differences found in replicate samples. Two-way ANOVA is used when two factors may influence the outcome of an experiment [20].

PCA or principal component analysis was also applied to the data using Unscrambler X 10.0 software by Camo to compare the amount and type of VOCs detected from the scent samples. PCA is used to reduce large amounts of correlated data by finding linear combinations, or principal components, describing the original variables. The first and second principal components account for a majority of the variation in the data. When the first two principal components are plotted, the axes represent the greatest variation in one direction (principal 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 [20].

Results/discussion

STU-100 optimization

The quantities of the standard compounds collected by the STU-100 at each flow rate/collection material combination are shown in Fig. 1. The STU-100 used with no vacuum yielded the least amount of compounds in the headspace. This confirms that few volatiles can be passively collected onto the material (with no contact). Dimethyl disulfide was not detected in any circumstance likely because of its extremely high volatility (vapor pressure=22 mmHg). There was no statistical difference between the flow rates using the polyester material, as determined by ANOVA (Table 4). For the Dukal and Johnson & 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 at the low (0) flow rate than at the high (9) flow rate. This is likely due to compound breakthrough which tends to be more prominent in the Dukal gauze than the Johnson & Johnson gauze. The Dukal gauze has a more open weave, allowing more volatiles to pass through without being deposited. This becomes increasingly evident at higher flow rates [16].

The lowest amounts of the standard compounds were collected on the polyester at all flow rates. For the polyester, the presence of the ketone was below the detection limits at all flow rates. Furthermore, only minimal

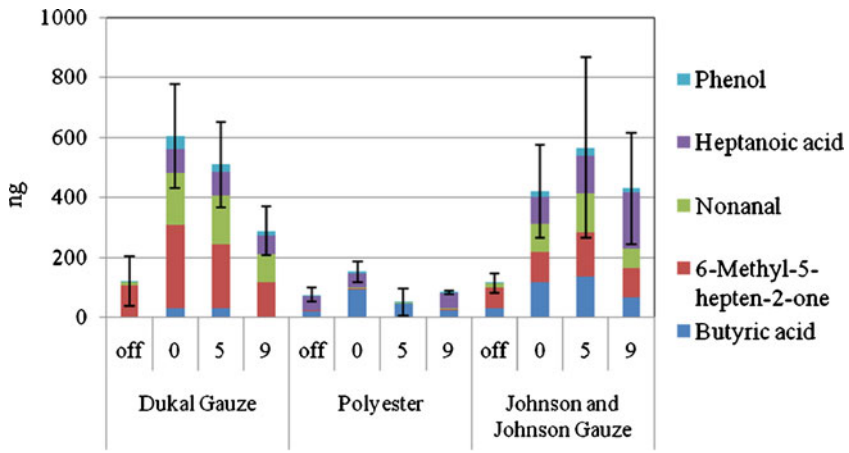

Fig. 1 Quantity of standard compounds collected by the STU-100 at different flow rate and collection material combinations

Table 4 Use of ANOVA (two factors without replication) to determine variations between the quantity of compounds recovered at varying flow rates from several sorbent materials

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

amounts of the alcohol and aldehyde were seen, and only the acids were collected in moderate quantities. There are several potential reasons for the low quantity of compounds seen in the headspace of the polyester. As hypothesized previously by DeGreeff et al. [16], it is likely that the thicker gauze materials prevented the breakthrough of the compounds compared with the polyester. Three layers of the polyester material were used for collection, which 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 with 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 & Johnson gauze is a cotton blend containing rayon (a man-made cellulose-based material), polyester, and cotton. The structural backbone of polyester consists of a long-chain synthetic polymer held together by ester bonds with no free hydroxyl groups. Thus, the bonding or non-bonding of compounds to the material backbones likely affects compound collection. If an analyte binds too tightly to the material, it will not enter the headspace readily and will not be seen during analysis. Alternatively, if an analyte does not have any binding affinity for the collection material, it will not be collected onto the material and, again, not be seen during analysis.

Because the polyester material showed some promise for the collection of acids, the polyester material was layered with the gauze materials for collection by the STU-100. The total quantity of standard compounds collected in this manner is presented in Fig. 2. The STU-100 collection with the Dukal gauze and polyester material showed mostly no change compared with the Dukal gauze alone across all flow rates. However, there was 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; however, with the addition of the polyester material, butyric acid could be collected at all flow rates. The Johnson & Johnson gauze and polyester combination yielded a consistent increase in the amount of heptanoic acid collected at all flow rates, causing the decrease in the quantity of all other compounds. This could be due to competition for binding sites either on the collection material itself or the on the SPME fiber. The Dukal gauze/polyester combination was used at the low flow rate for further sample collections with the STU-100

Human remains collection

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 to be signature compounds.

The odor from 21 deceased human bodies was collected from the Miami-Dade morgue using the STU-100. The compounds recovered and the numbers of occurrences are listed in Table 5. Some compounds (as referred to in the table footnote) were not confirmed with standard compounds; all others were confirmed by the MS library and

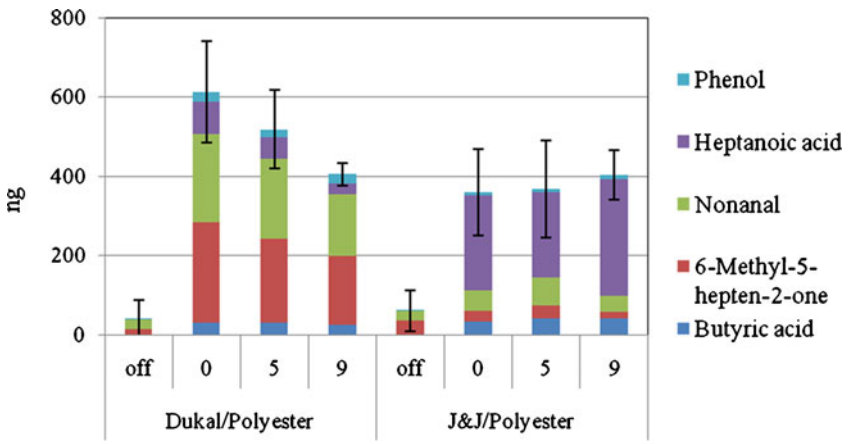

Fig. 2 Quantity of standard compounds collected by the STU-100 onto layered collection materials

Table 5 Compounds recovered from human remains samples

Compound	No. of occurrences		Compound common to both locations
	Morgue (*n*/21)	Crematorium (*n*/6)	
Acetic acid	21	1	X
Styrene	21	6	X
2-Furaldehyde	21		
6-Methyl-5-hepten-2-one	21	3	X
2-Ethyl-1-hexanol	21	6	X
Benzaldehyde	21	6	X
Nonanal	21	3	X
1-Octanol	21		
Benzonitrile	21	3	X
Decanal	21	6	X
Pentadecane	21	6	X
Hexadecane	21		
Phenol	21	6	X
Heptadecane	21	6	X
2-(2-Methoxyethoxy)ethanol	21	6	X
Benzoic acid methyl ester	21	6	X
1,2,3-Trimethylbenzene	20	4	X
Benzyl alcohol	19		
Octanoic acid	19	6	X
Tridecane	17	6	X
Toluene	16		
Dimethyl trisulfide	15		
2-Butoxy ethanol[a]	14		
Naphthalene[a]	13	5	X
Tetradecane	12	6	X
2-Methyl propanoic acid	12		
Undecanoic acid, 10-methyl-methyl ester[a]	11		
5-Methyl-2-(1-methylethyl)-cyclohexanol[a]	10	1	X
Furfuryl alcohol	9		
2-Pentadecyn-1-ol[a]	9		
1-[4-(1-Hydroxy-1-methylethyl)phenyl] ethanone[a]	8		
Methoxy phenyl oxime[a]	7		
1,2,3,4-tetramethyl benzene[a]	6		
Decanoic acid, methyl ester[a]	5		
Hexanoic acid[a]	4		
Geranyl acetone	3	4	X
2-(2-Ethoxyethoxy) ethanol[a]	3		
7-Octen-2-ol, 2,6 dimethyl[a]	3		
Isobornyl acetate[a]	0	6	
Undecane	0	4	
2-Hexyl-1-octanol[a]	0	4	
2-Butyl-1-octanol[a]	0	4	
2-Ethyl hexanoic acid[a]	0	4	
Undecanoic acid	0	3	
2-Hexyl-1-decanol[a]	0	3	
2-Hexyl ethanol	0	2	
2-Ethylhexyl tetradecyl ester, oxalic acid[a]	0	1	
5-Methyl-2,1-methylethyl cyclohexanol[a]	0	1	
Methyl salicylate[a]	0	1	
2-Decanal	0	1	

[a] Matched by mass spectral library but not confirmed by standard; compounds in italics were found in high frequency (<66%)

retention time comparison to standard compounds. Sixteen of the compounds listed below were common to at least 19 of the 21 cadaver samples (90%).

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. Samples from six subjects were collected and 14 compounds were identified in five or more of the samples (83%, Table 5).

A second set of samples were collected from an alternative location because the background air samples in the morgue location were inundated with volatiles from human decomposition, and thus, though field blanks were collected, they could not be used to eliminate compounds due to background odor. This was remedied by sampling in a second, and distinctly different, location. The second set of samples was taken from a crematorium. The background VOCs from the morgue are most likely due to cleaning agents and other chemicals used for the preparation and processing of the bodies for autopsy, while the background VOCs from the crematory are likely due to the process of cremation; thus, volatiles at the crematory are unique compared with those from the morgue. Background compounds were eliminated from the analysis by comparing the VOCs present in location 1 (morgue) with those from location 2 (crematory). Compounds that were unique to either of the locations were removed as they were considered background volatiles. Similar compounds were considered significant to the human remains odor profile.

The sets of samples from the two sampling locations were found to have 13 compounds in common (highlighted in the table below). Figure 3 represents the scent profile of the crematorium and morgue subjects using the 13 compounds in common. Each color bar in the graph represents a different compound, and the thickness of the bar represents

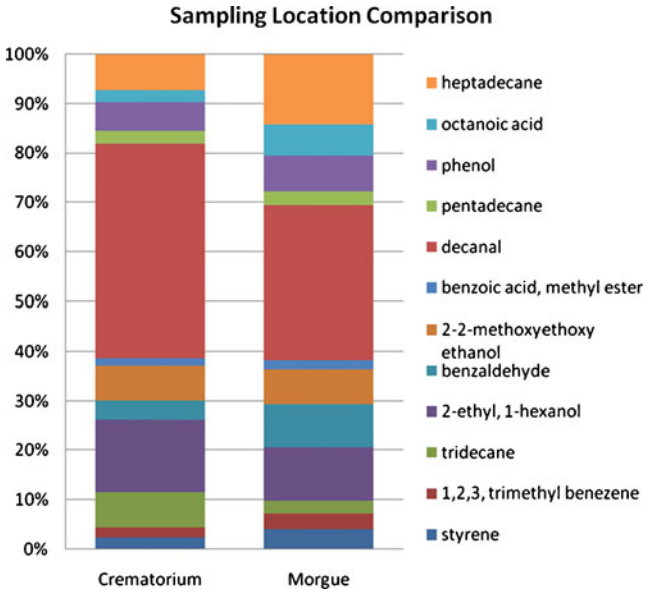

Fig. 3 Odor profiles of human remains sampled in two locations

the relative quantity of such compound. The scent profiles from each group were visually similar, indicating that the decomposition process follows the same pathway for all human bodies in the same stage of decomposition, producing similar sets of compounds; however, minor differences may be seen due to the unique composition of individual bodies. The preliminary results suggest the existence of universal compounds in the odor of human remains, which could eventually be used as an indicator of the existence of a deceased body or as the basis for creating new training aids.

Several benzene derivatives were included in the 13 compounds. Cyclic hydrocarbons are one of the most prevalent classes of compounds associated with human remains odor. Styrene [5–7], 1,2,3-trimethyl benzene [4, 5], and naphthalene [5, 6] 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. [6], styrene and naphthalene were detected consistently throughout the decomposition process.

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 [3]. Benzaldehyde has been reported as a component of both living [21–23] and deceased [4, 8, 9] human odor, as well as associated with the blood of lung cancer patients [24]. Decanal has been reported in living [12, 21–23, 25–27] and deceased [5, 7, 11] human scent, as well.

Tridecane, pentadecane, and heptadecane have not been documented in the literature on human remains odor; however, other straight-chain hydrocarbons were previously reported by Caldwell et al. [11]. The three have been repeatedly identified as components of living human scent in a number of sources [12, 21, 22, 26, 28].

2-Ethyl-1-hexanol has been reported by several groups as being associated with human remains odor [5, 8] as well the odor from pig decomposition [29]. The other alcohol detected in this study, phenol, was previously identified in human [5] and pig remains [29]. Phenol has been documented as a common component of human scent [12, 22, 23, 25]. 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 previously identified as a component of decomposition fluid odor [9] and as a component of living human scent [12, 22, 23]. Octanoic acid and other organic acids are formed by the conversion of glucose monomers from carbohydrates under aerobic conditions [3]. Benzoic acid methyl ester has not been reported in the literature as a product of human decomposition, although other acid esters are common [4, 6–8]. Benzoic acid methyl ester was detected as a component of pig remains odor [29].

Of the 13 compounds determined in this study to be significant to the human remains odor profile, eight compounds were reported in previous studies. Other compounds, such as *p*-xylene and dimethyl disulfide, were not recovered in this study, although they are documented in multiple sources to be constituents of remains odor [4–9]. Tentative explanations for these missing compounds include: (1) The compounds were not trapped or released by the collection material. Dimethyl disulfide has a particularly high vapor pressures (22 mmHg) and thus may dissipate to the surroundings before they can be captured. (2) They may have been lost during the extraction/analysis process. (3) They were simply not present due to the sampling conditions (i.e., the bodies in this study were sampled indoors away from environmental factors), due to the differences in stage of decomposition compared with other studies, or were associated with background volatiles in the outdoor locations as few blanks and/or negative controls were considered in many of the studies.

Comparison samples—live human scent and animal remains

Samples from living humans were collected, extracted, and analyzed in the same manner as those from the human remains. All samples were analyzed in triplicate, and only the compounds common to at least two of the replicate samples were included in the data. The odor profiles of the two groups of samples were compared (Fig. 4). “Liv” represents the subjects from the living human study, while “Mor” and “Cr” are randomly selected human remains samples. There are a number of similar compounds between

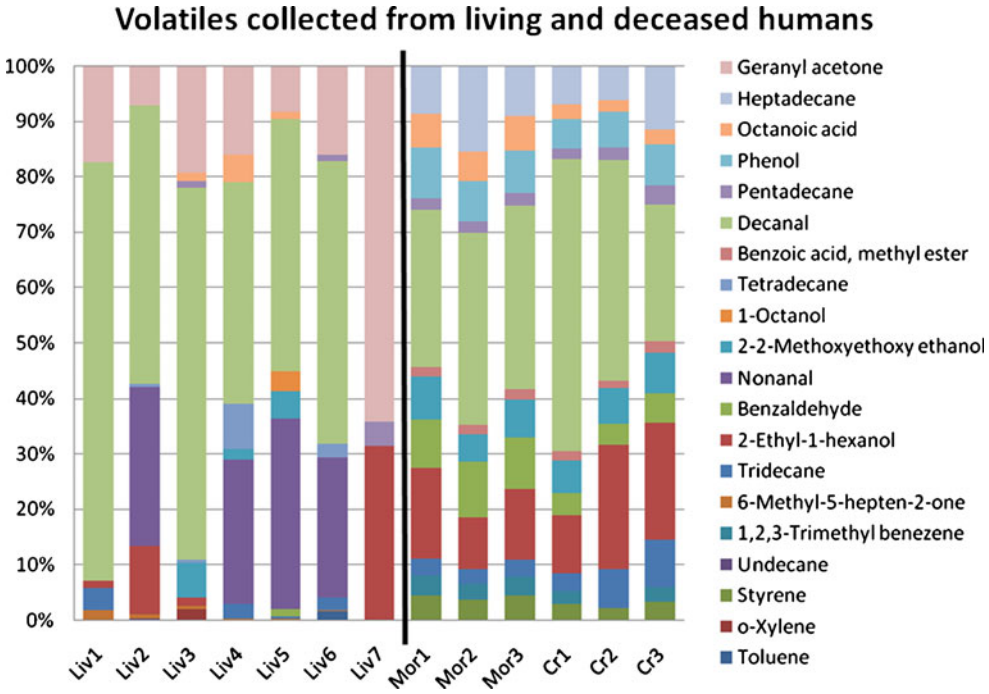

Fig. 4 Volatiles collected from living human and human remains odor. *Liv1–7* living subjects, *Mor1–3* human remains collected from morgue, *Cr1–3* human remains collected from crematorium

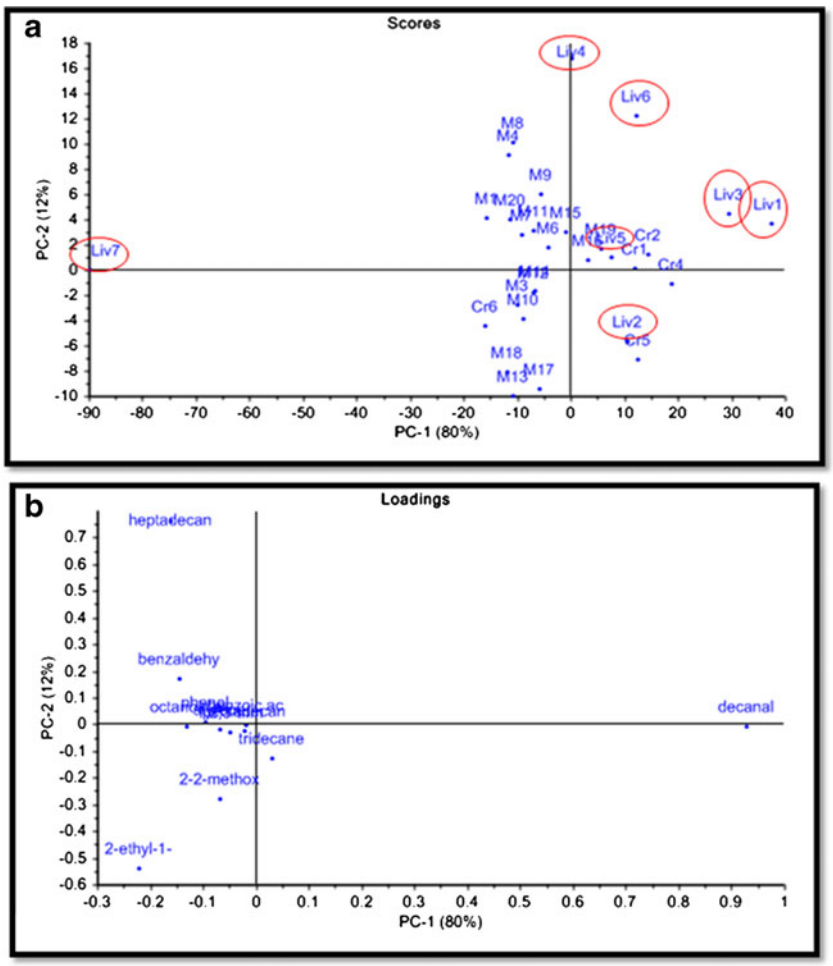

Fig. 5 **a** PCA plot representing the maximum variance between living and deceased human samples. **b** Loadings plot of the above PCA

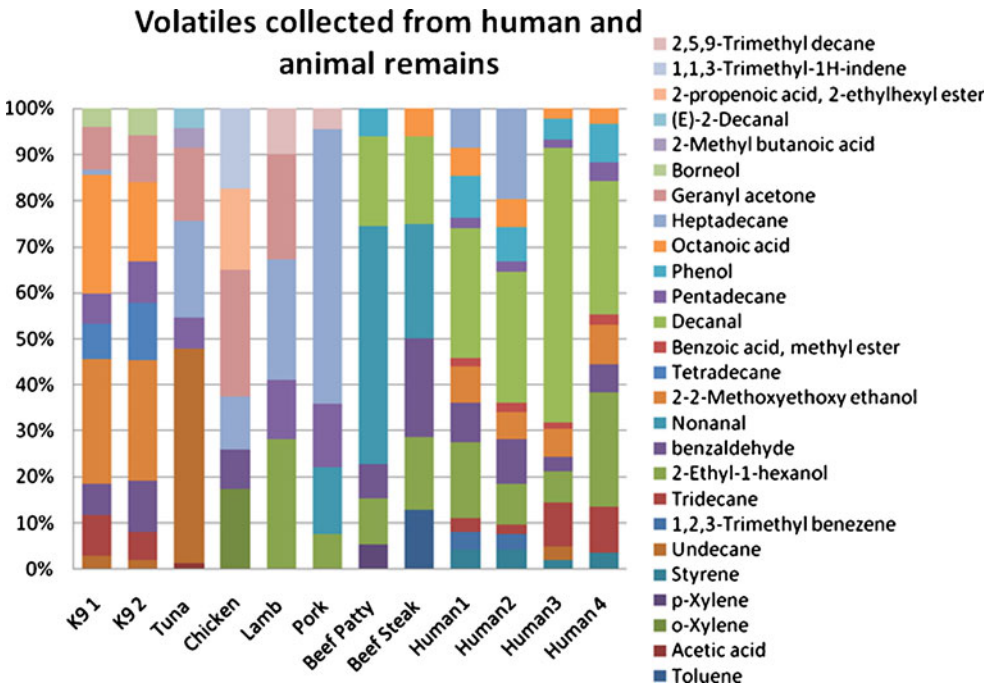

Fig. 6 Odor profiles collected from the remains of animals and humans

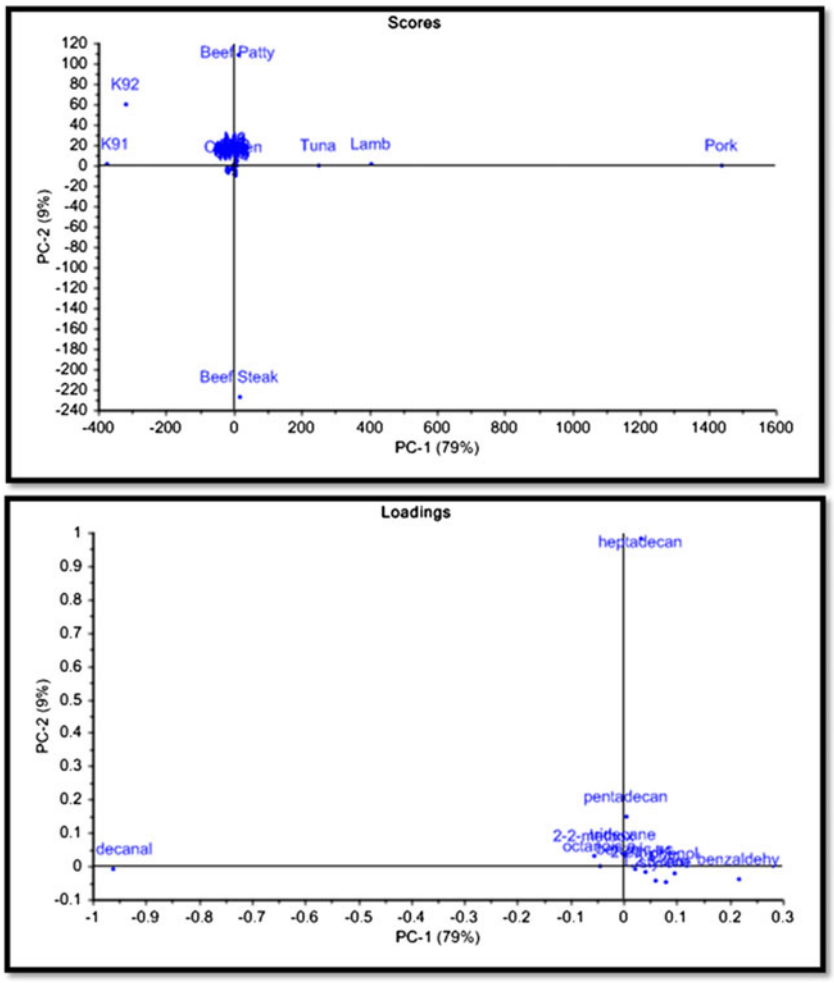

Fig. 7 **a** PCA plot representing the maximum variance between deceased animal and human samples. **b** Loadings plot of the above PCA

the living and deceased human samples. However, the deceased samples have a greater number of compounds compared with the living samples. There are several compounds unique to each group. For example, all of the living humans scent samples contain geranyl acetone (5,9-Undecadien-2-one, 6,10-dimethyl), which is not found in any of the human 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, however, been previously reported as a living human scent component in several sources [12, 21–23]. Additionally, 6-methyl-5-hepten-2-one, nonanal, and tetradecane were detected in the living human scent samples (compounds were not detected in background), but were excluded from the deceased human odor samples as they were determined to be artifacts of the sampling location background. Styrene, benzoic acid methyl ester, phenol, and heptadecane were all detected in at least 66% of the human remains odor samples, but were not detected in any of the living human scent samples.

Additionally, the living samples show greater variation between subjects. This can be seen in the PCA plot (Fig. 5). The human remains samples tend to group more tightly than the living samples. This trend is to be expected as it is known that each living human has a unique odor while deceased humans have general odor.

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 were compared (Fig. 6). There were many similarities between the profiles of all remains. No one compound was found in all remains 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. This indicates that the odor from human remains is different from that of animals. This is substantiated by the plot of the principal component analysis of animal remains volatiles versus human remains volatiles (Fig. 7). 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 locate solely the human remains. Additionally, it can be seen that the two samples from canine remains are grouped close together.

Conclusions

Non-contact, dynamic headspace concentration was employed using the STU-100 for the collection of human remains volatiles. The sorbent materials and the flow rate of the STU-100 were assessed, and it was concluded that the molecular structure as well as the weave characteristics of the collection material affect the amount of compound trapped/released. The greatest amount of total compound was recovered from the cotton-based materials (Johnson & 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.

Following the evaluation of sorbent materials and flow rates, deceased bodies were sampled from two locations. The VOCs collected from both locations were compared, and 13 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 with those of living human and animal remains samples collected and analyzed in the same manner. Both qualitative similarities and differences were found in all types of samples. While there were many compounds in common between the living and deceased human samples, the complete odor profiles differed. Similarly, human and animal remains samples were compared. The human odor profiles were easily separated from the animal profiles in the PCA plot.

This research has demonstrated the utility of dynamic headspace concentration for identifying characteristic volatile organic compounds in deceased human odors. The STU-100 delivers consistent and reproducible samples that can potentially be used as a tool for canine handlers and as a field instrument.

Acknowledgments The authors would like to thank Dr. Abuzar Kabir for his thoughtful insights and contributions in the preparation of this manuscript. The authors would also like to thank Barbara Weakley-Jones, the Office of the Miami-Dade Medical Examiner, and Borden Cremation Services for access to their resources for this research.

References

1. Bull ID, Berstan R, Vass A, Evershed RP (2009) Identification of a disinterred grave by molecular and stable isotope analysis. Sci Justice 49:142–149
2. Oxenham M (2008) Forensic approaches to death, disaster and abuse. Australian Academic Press, Bowen Hills
3. Hunter J, Cox M (2005) Forensic archaeology: advances in theory and practice. Routledge, New York
4. Statheropoulous M, Spiliopoulou C, Apapiou A (2005) A study of volatile organic compounds evolved from the decaying human body. Forensic Sci Int 153:147–155

5. Statheropoulous M, Apapiou A, Spiliopoulou C, Pallis GC, Sianos E (2007) Environmental aspects of VOCs evolved in early stages of human decomposition. Sci Total Envir 385:221–227
6. Vass AA, Smith RR, Thompson CV, Burnett MN, Dulgerian N, Eckenrode BA (2008) Odor analysis of decomposing buried human remains. J Forensic Sci 53:384–390
7. Vass AA, Smith RR, Thompson CV, Burnett MN, Wolff DA, Synstelien JA, Dulgerian N, Eckenrode BA (2004) Decompositional odor database. J Forensic Sci 49:760–769
8. Hoffman EM, Curran AM, Dulgerian N, Stockham RA, Eckenrode BA (2009) Characterization of the volatile organic compounds present in the headspace of decomposing human remains. Forensic Sci Int 186:6–13
9. Lorenzo N, Wan TL, Harper RJ, Hsu YL, Chow M, Rose S, Furton KG (2003) Laboratory and field experiments used to identify *Canis lupus* var. *familiaris* active odor signature chemicals from drugs, explosives and humans. Anal Bioanal Chem 376:1212–1224
10. Tolliver SS (2005) 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
11. Caldwell PT, Beussman DJ, Tipple CA, Dulgerian N, Eckenrode BA (2011) SPME and whole air sampling comparisons of human decomposition odor. 63rd American Academy of Forensic Sciences Annual Meeting
12. Prada PA, Curran AM, Furton KG (2011) The evaluation of human hand odor volatiles on various textiles: a comparison between contact and noncontact sampling methods. J Forensic Sci. Accessed 11 Apr 2011
13. Curran AM, Prada PA, Furton KG (2010) Canine human scent identifications with post-blast debris collected from improvised explosive devices. Forensic Sci Int 199:103–108
14. Stockham RA, Slavin DL, Kift W (2004) Survivability of human scent. Forensic Sci Comm 6(4)
15. Harvey LM, Harvey JW (2003) Reliability of bloodhounds in criminal investigations. J Forensic Sci 48:811–816
16. DeGreeff LE, Curran AM, Furton KG (2011) Evaluation of selected sorbent materials for the collection of volatile organic compounds related to human scent using non-contact sampling mode. Forensic Sci Int 209:133–142
17. Stockham RA, Slavin DL, Kift W (2004) Specialized use of human scent in criminal investigations. Forensic Sci Comm 6(3)
18. Haglund WD, Sorg MH (1997) Forensic taphonomy: the postmortem fate of human remains. CRC, Boca Raton
19. DeGreeff LD (2010) Development of a dynamic headspace concentration technique for the non-contact sample of human odor samples and the creation of canine training aids. PhD dissertation, Florida International University, Department of Chemistry and Biochemistry
20. Miller JN, Miller JC (2005) Statistics and chemometrics for analytical chemistry, 5th edn. Pearson Education Limited, New York
21. Curran AM, Ramirez CF, Schoon AA, Furton KG (2006) The frequency of occurrence and discriminatory power of compounds found in human scent across a population determined by SPME-GC/MS. J Chrom B 3298:1617–1623
22. Gallagher M, Wysocki CJ, Leyden JJ, Spielman AI, Sun X, Preti G (2008) Analyses of volatile organic compounds from human skin. Cutaneous Bio 159:780–791
23. Bernier UR, Kline DL, Barnard DR, Schreck CE, Yost RA (2000) 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*). Anal Chem 72:747–756
24. Deng C, Zhang X, Li N (2004) Investigation of volatile biomarkers in lung cancer blood using solid phase microextraction and capillary gas chromatography–mass spectrometry. J Chrom B 808:269–277
25. Hudson DT, Curran AM, Furton KG (2009) The stability of collected human scent under various environmental conditions. J Forensic Sci 54:1270–1276
26. Ostrovskaya A, Landa PA, Sokolinsky M, Rosalia AD, Maes D (2002) Study and identification of volatile compounds from human skin. J Cosmetic Sci 53:147–148
27. Bernier UR, Kline DL, Schreck CE, Yost RA, Barnard DR (2002) Chemical analysis of human skin emanations: comparison of volatiles from humans that differ in attraction of *Aedes aegypti*. J Amer Mosquito Control Assoc 18:186–195
28. Zhang ZM, Cai JJ, Ruan GH, Li GK (2005) The study of fingerprint characteristics of the emanations from human arm skin using original sampling system by SPME-GC/MS. J Chrom B 822:244–252
29. Dekeirsschieter J, Verheggen FJ, Gohy M, Hubrecht M, Bourguignon L, Lognay G, Haubruge E (2009) Cadaveric volatile organic compounds released by decaying pig carcasses in different biotopes. Forensic Sci Int 189:46–53