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Characterization of the volatile organic compounds present in the headspace of decomposing human remains

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ABSTRACT

Law enforcement agencies frequently use canines trained to detect the odor of human decomposition to aid in determining the location of clandestine burials and human remains deposited or scattered on the surface. However, few studies attempt to identify the specific volatile organic compounds (VOCs) that elicit an appropriate response from victim recovery (VR) canines. Solid-phase microextraction (SPME) was combined with gas chromatography–mass spectrometry (GC–MS) to identify the VOCs released into the headspace associated with 14 separate tissue samples of human remains previously used for VR canine training. The headspace was found to contain various classes of VOCs, including acids, alcohols, aldehydes, halogens, aromatic hydrocarbons, ketones, and sulfides. Analysis of the data indicates that the VOCs associated with human decomposition share similarities across regions of the body and across types of tissue. However, sufficient differences exist to warrant VR canine testing to identify potential mimic odor chemical profiles that can be used as training aids. The resulting data will assist in the identification of the most suitable mixture and relative concentrations of VOCs to appropriately train VR canines.

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1. Introduction

A search for buried homicide victims is a time-consuming process. Complications can be attributed to the physical and chemical changes that occur at the burial site over time. In addition, there can be an unlimited number of potential burial locations available to the offender. In 2003 the FBI conducted an internal study which identified physical markers and common characteristics of clandestine burial sites using information collected over a 10-year period from 1993 to 2003 [1]. The study revealed the following physical characteristics regarding located burial sites that contained human remains: (1) the average age of a burial site at the time of the search was 4–6 years, (2) typical burials varied in depth from 1.5 to 2.5 feet (0.46–0.76 m), (3) burial sites were located a short distance off of infrequently traveled roads or pathways, (4) clandestine burial sites were located approximately 10 feet (3.0 m) away from the closest large tree and were typically surrounded by bushes or heavy foliage, and (5) the corpse was usually clothed or wrapped in plastic and faced down. While some of these physical characteristics or visual cues may help direct an investigation, they can also be misleading. Investigators often employ supplemental search techniques to increase their confidence in locating a clandestine burial site.

A variety of specialized search techniques employing botanists, entomologists, geologists (employing ground penetrating radar) and forensic anthropologists are often used to narrow the scope of a search. In many cases information from informants is used to further reduce the search area. Physical characteristics, statistical data, and special search strategies often help identify specific search areas, but supplemental search techniques, such as using victim recovery (VR) canines, are the tool most often utilized to pinpoint clandestine burial sites prior to engaging in costly excavations. Canines are used because of their proven relative accuracy, their sensitivity and selectivity, their ability to be rapidly deployed and cover a large area; and their relatively long search or working cycle [2–4].

Searches conducted by the FBI for clandestine burial sites usually result from requests by other law enforcement agencies when their investigative leads or resources have been exhausted. Often many months and even years have elapsed for some search requests, and the burial site physical characteristics that normally can be advantageous are essentially nonexistent. As a result, investigators have relied almost exclusively on chemical detection strategies involving canines. Out of 11 searches for clandestine

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burials using canine teams conducted by the FBI in 2005–2006 where the age of the burial site averaged 38 months, no recoveries resulted that could be credited to the work of the canine teams [5]. These results bring into question whether the search locations were adequately identified through investigative efforts or whether the canines had been trained on an appropriate odor chemical profile that would enable them to detect the aged burial site.

The odor chemicals that elicit an alert by VR canines are suspected to be composed of a variety of volatile organic compounds (VOCs). The identity and relative concentrations of the components in the appropriate VOC profile are a matter of speculation. Furthermore, it is not known whether one chemical component, several components, or the whole VOC profile establishes the VR canine's particular olfactory "match" that elicits a trained response. A previous study to determine dominant odor chemicals emanating from explosives for use in developing canine training aids reported a wide variability in the measured odor chemicals for smokeless powders. Canine testing indicated that there was no single low explosive component that elicited responses in the majority of the canines, suggesting that canines should be trained on multiple smokeless powders to ensure that effective odor chemical profile generalization is achieved [6]. These data also suggest that canines trained on limited varieties of smokeless powders will lead to canines able only to reliably detect the specific commercial brands from which they were trained. This information may prove significant for VR canine trainers using a limited variety of human remains for training aids.

Developing training aids to achieve odor chemical profile generalization capabilities in VR canines requires a more exact understanding of the VOC profiles associated with the decomposition of human remains. Several attempts have been made to correlate the odor chemical profile utilized by VR canines by conducting tests with mimics. For example, in field tests, VR canine trainers reported that their canines alerted when they used 1,5-pentanediamine (cadaverine), 1,4-butanediamine (putrescine), indole, and 3-methyl indole (skatole) as training aids and presented each of these in the single pure chemical form [2]. Various studies have identified VOCs released by decomposing human remains [2,7], and there is new research into the chemical composition of buried human remains and the changes in the surface VOC profile over time [8] indicating some correlation to the stage of decomposition and odor emanation [9]. However, mixtures of VOCs in controlled relative ratios to simulate the odor profile of decomposing human remains have not been used in canine training to test their effectiveness in producing canines capable of finding human remains.

Many VR canine trainers obtain human remains from local medical examiners, morgues, or other canine trainers, but little attention has been paid to consistency with regard to decomposing human tissue type, temperature, quantity, moisture content, and decomposition time. In any burial, including a clandestine one, the biochemical stages and rates of human decomposition determine the VOCs generated. Detection of these VOCs is influenced heavily by such factors as temperature, moisture level, oxygen content or availability, and contact with immediate surroundings such as the soil. Soil is a complex matrix comprised of organic material, minerals and salts, as well as organic and aqueous solutions that may be acidic, neutral, or alkaline. In addition, soil type and the presence of ground water as well as anaerobic and aerobic microorganisms can greatly affect the rate of biochemical decomposition and the observed VOC byproducts [10]. The process of soft-tissue decomposition itself (i.e., the decomposing body) can modify the localized burial microenvironment in terms of microbiological load, pH, moisture, or changes in redox status and can affect the VOCs generated [11]. As such, the training aids obtained by VR canine trainers may not be sufficient to simulate decomposed human remains that have been subjected to varying environmental influences and may not provide the proper odor chemical profile to ensure adequate generalization.

It is generally known throughout the forensic community that many VOCs emanate from the decomposition of human remains either buried or on the surface. A strategic goal of this research is to narrow the scope of identified VOCs in an effort to determine the appropriate odor chemicals required to train VR canines. This study provides a VOC analysis using SPME of the headspace above 14 different and random decomposing human remains tissue types that have previously been used as VR canine training materials. Further research is being conducted to determine if a correlation exists between these VOCs and the VOCs contained in and released from the soil in and around clandestine burials. This work is part of an on-going long-term FBI research strategy directed at the development and improvement of VR canine performance and the development of portable detection instrumentation.

2. Materials and methods

The human tissue samples evaluated in this study were collected from VR canine trainers who were actively using them as training aids. All of the human tissue samples used in this study were legally and ethically obtained in accordance with state and federal regulations and were not destroyed but were appropriately returned to their sources. The 14 samples consisted of tissue from a blood clot, a blood clot from a placenta, blood, muscle, a testicle, skin, body fat attached to skin, adipocere (1), adipocere (2), adipose or fat tissue, vertebra bone, bone (1), bone (2), and teeth. The samples were stored in 10 mL glass vials and sealed with Teflon-faced white septa caps (Supelco, Bellefonte, PA). Prior to use, the vials were cleaned with acetone and heated for 24 h at 200 °C to remove any compounds present initially inside the vials. The vials were then analyzed using the method described below (blank) prior to inserting the decomposition sample. All of the samples were stored in these sealed vials in a cold room held at 7 °C and 45% relative humidity. Prior to analysis the samples were allowed to equilibrate for 4 h at room temperature (25 °C and 50–55% relative humidity). The maximum amount of time the samples were at room temperature was 48 h. The headspace above the human tissues was sampled at room temperature using polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm solid-phase microextraction (SPME) fibers (Supelco, Bellefonte, PA) on the basis of previously reported data [2]. Each human remains sample was exposed to the PDMS/DVB fiber first for 20 min and then for 40 min (static SPME) to determine the effect of exposure time and the VOC uptake from headspace constituents. After headspace extraction, each sample was analyzed via gas chromatography–mass spectrometry (GC–MS).

The GC–MS systems employed for these analyses were based on the 6890 GC and the 5973 mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA). The GC–MS systems were equipped with a DB5-MS capillary column (J&W Scientific, Agilent Technologies, Palo Alto, CA), 15 m in length, 0.25 mm ID, and 0.25 μm film thickness, and a 0.75 mm ID SPME injection port liner operated in splitless mode at 250 °C. Helium carrier gas was set to 1.5 mL/min flow rate and the GC oven was held at an initial temperature of 35 °C for 1 min, ramped to 80 °C at 3 °C/min, then to 120 °C at 10 °C/min, and finally to 260 °C at 40 °C/min. The total run time for the analysis method was 23.5 min. The Agilent 5973 MSD was scanned from 50 to 550 amu at a rate of 2.94 scans/s.

This qualitative method was based on a previously reported GC–MS method which analyzed the VOCs released from the decomposition of human remains in shallow burial sites [10]. Compounds were tentatively identified using the NIST02 mass spectral library, extracted ion chromatograms, and/or chemical standard comparison.

3. Results and discussion

3.1. SPME extraction time determination

This study used equilibrium headspace SPME with GC–MS to identify the VOCs emanating from a variety of tissue types obtained from actual training aid samples. Fourteen samples were analyzed using standard SPME methodology under generally controlled aerobic conditions. The headspace above all of the human remains samples was analyzed and evaluated initially using both 20 and 40 min SPME extraction times. Two extraction times with the PDMS/DVB SPME fibers were used to determine if there were any measurable qualitative effects or differences due to the fiber

extraction time. These samples had not been previously characterized in terms of their VOC profile; therefore it was necessary to determine if a longer extraction time would reveal the presence of additional compounds due to low vapor pressure and/or higher molecular weight components. It was found that when employing either a 20 or 40 min extraction time, the total ion current ratios were the same, and 33 compounds were measured. A measurable quantitative difference between the two extraction times resulted in an improved signal-to-noise ratio for all analytes at the 40 min exposure time. However, because other compounds were not measured at the longer extraction time, the 20 min extraction time was selected for all subsequent analyses to improve analytical expediency. The total ion current chromatographic area reproducibility of this analytical method for measurements performed using the 20 min extraction time in triplicate (intraday) over the course of these experiments (approximately 8 weeks) averaged about 9% for the 33 detected components.

3.2. Comparison of human remains samples

VR canines are trained to locate and alert to odor from decomposing human remains, often with the assumption that the odor of human remains can be generalized. This means that canines trained on sniffing (sampling) the VOC profile generated from any piece of decomposing human tissue should alert to an entire decomposing body or parts of a human body that may have been scattered by carnivore or environmental action. Table 1 lists the 33 VOCs detected in the headspace across the 14 human remains samples, ordered by class and molecular weight. Most of these VOCs are superscripted with a reference to the literature where they have been previously reported from research involving both live humans as well as decomposing human remains. This cross-referencing provides a measure of corroboration to the data presented in this table. The 33 headspace components are divided into 7 groups by chemical classes that include acids/acid esters, alcohols, aldehydes, halogens, aromatic hydrocarbons, ketones, and sulfides. No 2 samples generated the same VOC profile; however, across the 14 different human remains samples there are qualitative similarities and differences. The data in Table 1 suggest that generalization may prove problematic unless more tissue types are represented in the training aid or a tissue type is selected that comprises a majority of the VOCs measured.

The tabulated data indicate that fat tissue (22 VOCs) is followed by muscle and bone (19 VOCs), adipocere (18 VOCs), and blood (12 VOCs) when based simply on the number of compounds generated by each particular tissue type. From an analytical perspective, the greater number of potential differentiators, i.e., VOCs, provides greater statistical confidence in a result; however, it still has not been determined whether the canine response depends on the presence of a specific target odor chemical, or a profile of target odor chemicals, or on a specific set of VOCs, or on the entire VOC profile. A qualitative assessment and comparison of previously reported data on the VOCs emanating from buried remains indicated that the compounds identified here represent a subset of an overall and long-term VOC profile [8]. This information is not comprehensive because surface (i.e., air and environmental factors) and subsurface (i.e., soil biochemistry) effects are not addressed in these experiments. Because these tissue types have not been exposed to soil, other matrices, or the environment, these effects will need to be studied and more clearly delineated before any guidelines can be established.

Table 2 lists the 33 VOCs in order of average chromatographic retention time and demonstrates the frequency of occurrence (H = high [67–100%], M = medium [33–66%], and L = low [<33%]) of these headspace compounds across the 14 samples of human remains. Of the 33 compounds, there were 17 low-frequency compounds, and 15 medium-frequency compounds, and *p*-Xylene was the sole high-frequency compound measured. None of the compounds extracted in the headspace were found in all of the human remains samples. It is interesting to note that more than half (approximately 55%) of the compounds extracted were found to be present overall in the high- and medium-frequency ranges. The two major compound classes observed, in terms of frequency, were alcohols and aldehydes.

3.3. Specific tissue-type analysis – muscle

Figs. 1, 2, 3 and 4 illustrate the total ion current chromatograms produced from the headspace analysis of human muscle, fat, bone, and blood samples, respectively. Figs. 5, 6, 7 and 8 show pie charts illustrating the distribution of the chemical classes measured in the headspace of the muscle, fat, bone, and blood samples, respectively. In Fig. 1, the headspace of human muscle was shown to contain acid esters, alcohols, and aldehydes, with a few aromatic hydrocarbons and a ketone. Statheropoulos et al. [7] found that the breakdown of muscle tissue yields volatile fatty acids; however, in this study only the esters were detected. This may be due to the effect from the extent of decomposition time on the human remains VOC profile. The research by Statheropoulos targeted human decomposition from hours and days to a few months whereas the present research targeted a time frame of several weeks. Volatile fatty acids may not have been observed in the current study because their concentrations were below the detection limit of the GC–MS. Lorenzo et al. did report the presence of fatty acid esters in a muscle sample [2]. As shown in Fig. 5, muscle tissue was found to decompose predominantly into aldehydes which was noted in other reports (see citations in Table 1), but these other reports did not specifically list muscle breakdown as the cause.

Statheropoulos et al. [7] and Inoue et al. [12] both reported that toluene had been found in the headspace of adipose tissue, brain, skeletal muscle, liver and kidney. In the current study, toluene and *p*-xylene were found in many of the different tissue types, including muscle.

3.4. Specific tissue-type analysis – adipose

Qualitative differences were observed in the headspace VOC composition among the four adipose (fat or primarily triglycerides) tissue samples analyzed. The headspace of two adipose tissue samples (with and without skin attached) and two adipocere (primarily saturated fatty acids) tissue samples contained compounds such as acid esters, alcohols, aldehydes, aromatic hydrocarbons, a ketone, and a sulfide. Vass et al. previously reported that decomposing adipose tissue contains such compounds as indole, skatole, putrescine, cadaverine, and fatty acids [13]. In this study indole was detected in the headspace of only one of the four adipose tissue samples. It is noted that fatty acids/acid esters were detected in the headspace of all four of the human adipose samples. Body fat with skin contained pentanoic acid, hexanoic acid–ethyl ester, hexanoic acid–pentyl ester, and hexanoic acid–hexyl ester. Adipose tissue contained butanoic acid, butanoic ethyl ester, butanoic butyl ester, and hexanoic acid–ethyl ester. Putrescine and cadaverine, both highly volatile polar compounds, were not detected in any of the 14 human remains samples analyzed via the method used in this study. Similar studies have also reported the nondetection of these compounds [8].

3.5. Specific tissue-type analysis – adipocere

A significant amount of research has been performed in an attempt to understand adipocere formation and how determin-

Table 1
Thirty-three VOCs measured in the headspace of decomposing human tissue types, ordered by class and molecular weight.

Compound classification	MW ($g\ mol^{-1}$)	Sample type													
		Blood clot	Blood clot/ placenta	Blood	Muscle	Testicle	Skin	Body fat attached to skin	Adipocere (1)	Adipocere (2)	Fat	Bone vertebra	Bone (1)	Bone (2)	Three teeth
Acid/acid esters															
Propanoic acid [2]	74.08								×					×	
Butanoic acid [2]	88.10			×						×					
Pentanoic acid [2]	102.13							×			×				
Butanoic acid, ethyl ester [7]	116.16									×					
Hexanoic acid [2]	116.16										×				
Butanoic acid, butyl ester [2,8]	144.21									×	×				
Hexanoic acid, ethyl ester [2,8]	144.21				×			×		×	×				
Hexanoic acid, pentyl ester [2,8]	186.29				×			×			×			×	
Hexanoic acid, hexyl ester [2,8]	200.32				×			×							
Alcohols															
1-Pentanol [2,7]	88.51								×		×	×	×	×	×
1-Hexanol [21]	102.17			×	×	×		×		×	×			×	
1-Octen-3-ol [23]	128.21			×	×			×		×	×			×	
1-Hexanol, 2-ethyl- [7]	130.23			×	×	×		×			×	×		×	
1-Octanol [21]	130.23			×	×			×			×			×	
Aldehydes															
2-Hexenal	98.14				×						×			×	
Hexanal [2,7,20,21]	100.16			×	×					×	×	×	×	×	
Benzaldehyde [2,7]	106.12		×	×	×	×				×				×	
2,4-Heptadienal	110.15									×	×				
2-Heptenal	112.17										×				
Heptanal [2,7,21]	114.19				×					×	×		×	×	
2-Octenal	126.20				×					×	×			×	
Octanal [20–22]	128.21				×			×		×	×		×	×	
2,4-Nonadienal [20]	138.21				×						×			×	
2-Nonenal [20,21]	140.22				×					×	×			×	
Nonanal [20,21]	144.25			×	×			×			×		×	×	
Halogen															
Tetrachloroethylene	165.83	×	×	×		×	×		×			×	×		×
Aromatic hydrocarbons															
Toluene [7,20]	92.14			×	×		×	×	×						×
p-Xylene [7]	106.17	×	×	×	×	×	×	×	×	×	×	×	×	×	
Indole [2]	117.15	×	×			×				×					
2-Pentyl-furan [2]	138.21					×		×	×	×	×	×	×	×	×
Ketone															
Cyclohexanone [7]	98.14			×								×			×
2-Heptanone [7]	114.19				×	×	×	×		×				×	
Sulfide															
Dimethyl disulfide [2,7]	94.20		×			×	×	×		×		×	×		×

Note: citation indicates compound specifically listed in the reference.

ing its presence or absence can be used in forensic investigations. Adipocere formation is of interest to forensic scientists because it can preserve the remains it encases, thereby slowing down the decomposition process. Adipocere forms from the fatty tissue in a body. Adipose tissue is composed mainly of triglycerides that, during decomposition, undergo hydrolysis to yield free fatty acids. Hydrogenation of the free fatty acids yields
saturated fatty acids that, under the right conditions, form into adipocere. The effect of the burial environment [14], the effect of the soil type [15], and the effect of the burial method [16] have all been studied. In the present VOC analysis of adipocere-based training aids, adipocere (1) was found to contain butanoic acid and adipocere (2) contained pentanoic acid, hexanoic acid, butanoic acid–butyl ester, and hexanoic acid–ethyl ester.

3.6. Specific tissue-type analysis – bone and blood

Bones undergo a process of decomposition known as diagenesis. The organic collagen and inorganic components break down, resulting in the production or generation of a relatively narrow variety of VOC [13]. The headspace of the three bone samples, including one known to have come from vertebra, had the following VOCs in common: several aldehydes, an alcohol, a halogen, an aromatic hydrocarbon, and a sulfide. In other preliminary studies, aldehydes have been measured in the headspace above a variety of different bones from both humans and animals [17].

The headspace of the blood clot, the blood clot from a placenta, and the pure blood sample contained aromatic hydrocarbons and a halogen in common (see Table 1). Blood is rich in proteins as well

Table 2
Frequency of occurrence for the headspace VOCs detected in human tissue decomposition samples.

Retention time (min)	Compound name	Frequency (%)	Overall frequency (H, M, L)
1.38	Propanoic acid	14	L
1.45	Dimethyl disulfide	57	M
1.69	Toluene	43	M
1.77	1-Pentanol	43	M
2.18	Tetrachloroethylene	64	M
2.18	Hexanal	50	M
2.20	Butanoic acid, ethyl ester	7	L
2.54	Butanoic acid	14	L
3.14	2-Hexenal	21	L
3.41	*p*-Xylene	93	H
3.59	1-Hexanol	50	M
3.93	Cyclohexanone	21	L
3.99	2-Heptanone	43	M
4.35	Heptanal	36	M
4.76	Pentanoic acid	14	L
6.01	Benzaldehyde	42	M
6.05	2-Heptenal	7	L
6.94	1-Octen-3-ol	43	M
7.20	2-Pentyl-furan	64	M
7.58	Butanoic acid, butyl ester	14	L
7.66	Hexanoic acid, ethyl ester	29	M
7.77	Octanal	43	M
8.00	2,4-Heptadienal	14	L
8.67	Hexanoic acid	7	L
8.83	1-Hexanol, 2-ethyl-	50	M
9.93	2-Octenal	29	L
10.63	1-Octanol	36	M
11.97	Nonanal	43	M
14.34	2-Nonenal	29	L
16.65	2,4-Nonadienal	21	L
18.55	Indole	29	L
18.76	Hexanoic acid, pentyl ester	29	M
20.43	Hexanoic acid, hexyl ester	14	L

as a host of other compounds. When proteins decompose they undergo proteolysis, whereby the protein is broken down via enzymatic processes into proteoses, peptones, polypeptides, and amino acids. As the process continues further, substances such as indole and skatole are produced [10]. The blood clot and blood clot from placenta samples both were found to generate indole; however, skatole was not identified in the headspace of any of the three blood samples. The presence of aldehydes was identified in the headspace of two of the blood samples. The blood clot from a placenta contained benzaldehyde, and the "pure" blood sample contained both hexanal and nonanal. Benzaldehyde and hexanal have been previously reported (through SPME–GC–MS analysis) to be present in the headspace of blood; however, nonanal has not been detected [18]. Another study that examined the compounds present in the headspace of blood samples did not report the presence of aldehydes [19] but did report the presence of toluene. In the current study, toluene was detected only in the "pure" blood sample. The lack of consistency across these studies is to be expected because of the variety of the aforementioned variables involved in these analyses that may have influenced the samples.

3.7. Specific tissue-type analysis – fluid

A previous study by Lorenzo et al. [2] that used SPME to analyze the headspace of human decomposition fluid (pooled) surrounding a cadaver reported the presence of cadaverine, putrescine, hexanal, *p*-cresol, indole, 3-methyl-indole, dimethyl-sulfide and fatty acids. In the current study, the presence of hexanal was detected in the headspace of the human blood, muscle, adipocere (2), and all three human bone samples. Indole also was present in the testicle

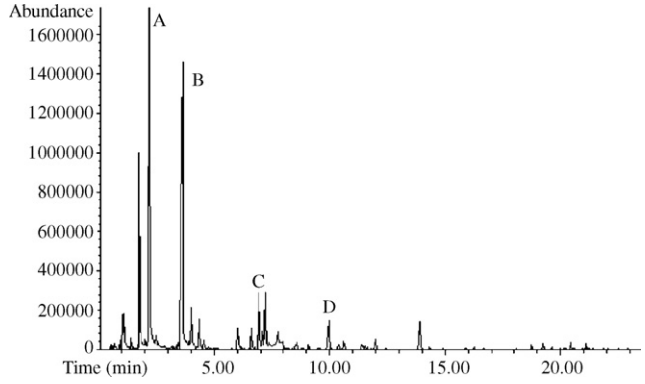

Fig. 1. Total ion current chromatogram of VOCs present in the headspace of muscle tissue with major components identified as: (A) hexanal; (B) *p*-xylene; (C) 1-octen-3-ol; (D) 2-octenal.

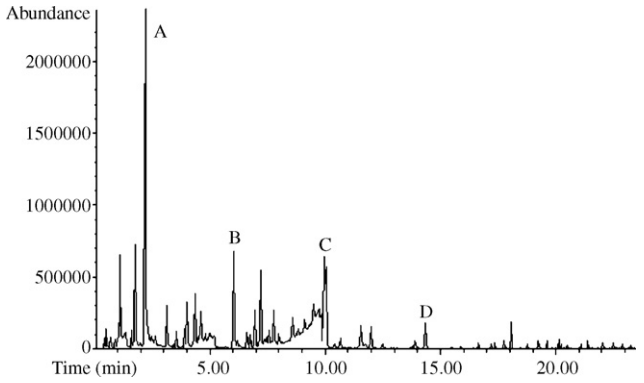

Fig. 2. Total ion current chromatogram of the VOCs present in the headspace of fat tissue with major components identified as: (A) hexanal; (B) 2-heptenal; (C) 2-octenal; (D) 2-nonenal.

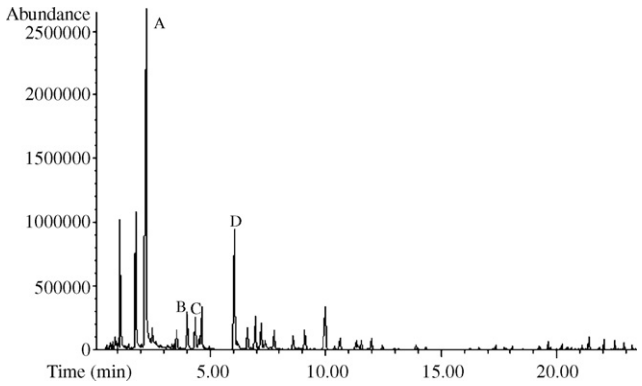

Fig. 3. Total ion current chromatogram of the VOCs present in the headspace of bone tissue with major components identified as: (A) hexanal; (B) 2-heptanone; (C) heptanal; (D) benzaldehyde.

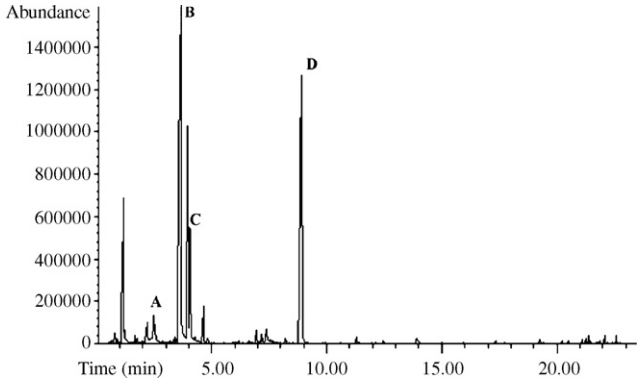

Fig. 4. Total ion current chromatogram of VOCs present in the headspace of blood with the major components identified as: (A) butanoic acid; (B) 1-hexanol; (C) cyclohexanone; (D) 1-hexanol, 2-ethyl.

(organ) tissue and adipocere (2) samples. The blood clot from the placenta, testicle tissue, skin, human fat with skin tissue, adipocere (2), vertebra bone, as well as bone (2) all contained dimethyl disulfide. The headspace evaluation of the three human teeth also identified dimethyl disulfide, and this compound generated the largest signal-to-noise ratio in the total ion current chromatographic profile for these samples.

3.8. Specific tissue type analysis – other considerations

The samples analyzed in this study were collected postmortem and placed in separate containers. It is safe to assume that they have continued to decompose without the influence of environmental conditions that would have otherwise affected buried human remains or remains left outdoors for surface decomposition. It is important to note that the conditions of the experiments described here more closely parallel aerobic decomposition rather than the more likely anaerobic scenario encountered with buried human remains. The biochemistry and the rate of decomposition for these two scenarios have been found, as expected, to be quite

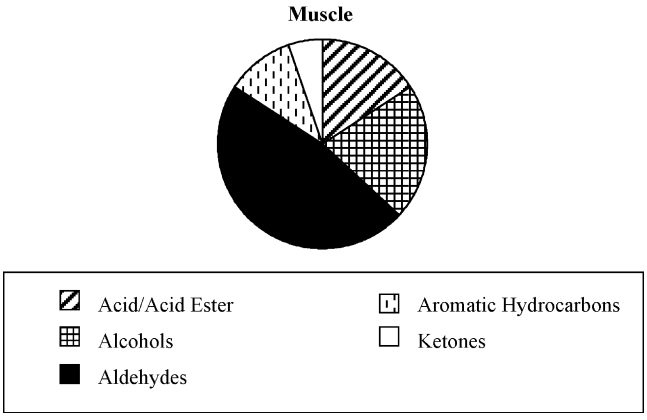

Fig. 5. Distribution of chemical classes in a muscle sample.

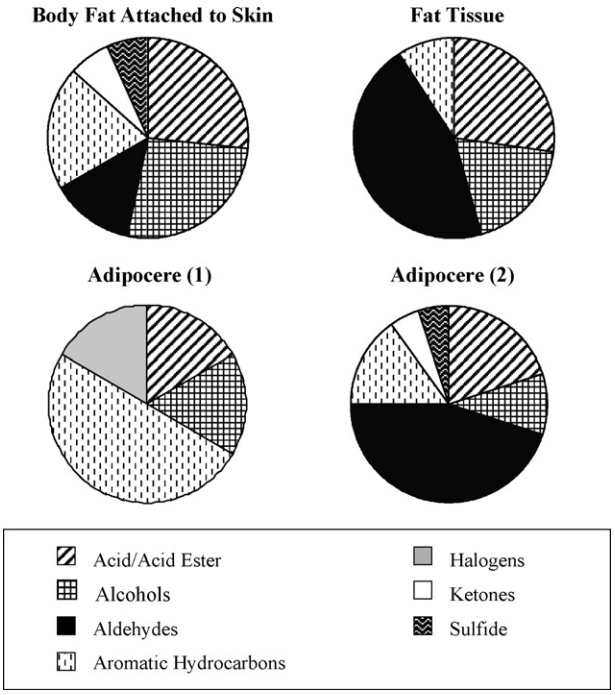

Fig. 6. Distribution of chemical classes among human fat samples.

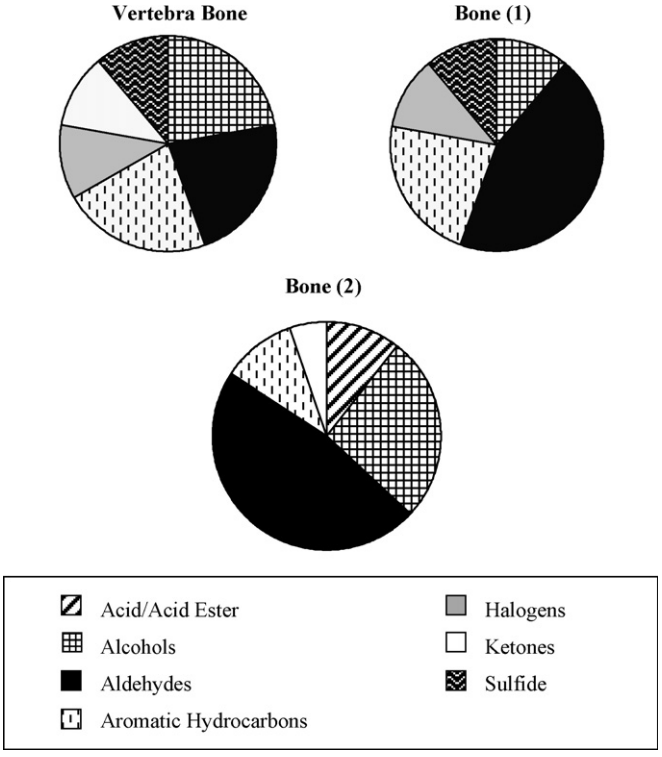

Fig. 7. Distribution of chemical classes among bone samples.

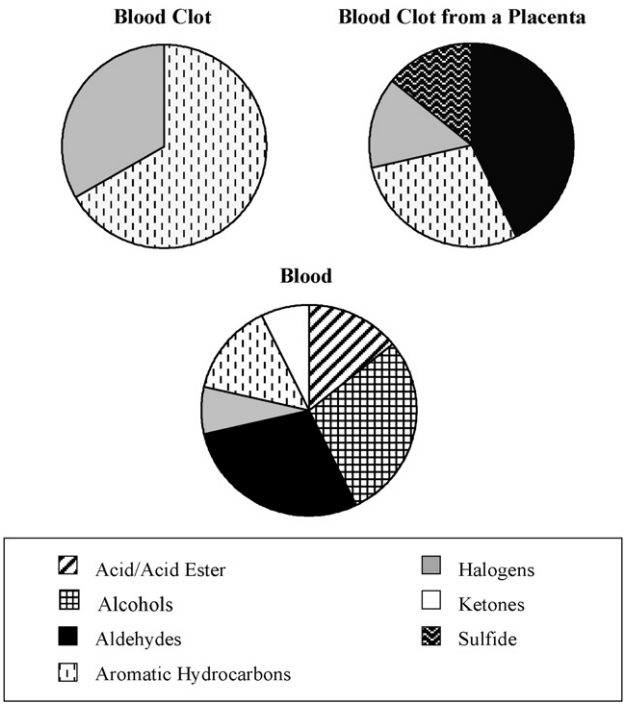

Fig. 8. Distribution of chemical classes among human blood samples.

different. This study was an important first step in studying tissue types for the purpose of understanding the VOC profiles currently used by a short list of VR canine trainers.

4. Conclusions

The 14 human remains samples evaluated in this study revealed 33 VOCs that included acids/acid esters, alcohols, aldehydes, halogens, aromatic hydrocarbons, ketones, and sulfides. Among the headspace VOCs were 1 high-, 15 medium-, and 17 low-frequency compounds based on appearance in the total ion current chromatogram profiles. The results from this study support the premise that odors released by human decomposition share similarities across the body regions and types represented; however, there are enough differences to warrant an examination or determination of the proper tissue type(s) that would provide the highest number of target odor chemicals for VR canine training purposes. It is important to recall that the samples evaluated in this study were canine training aid samples obtained from operational VR canine teams and may illustrate the need for improved information tracking on the "state of decay" beyond the general decomposition material type to enable more meaningful conclusions from subsequent analyses. For example, it would be useful for the bone samples to have information including type of bone, intact or fragment, dry or wet, etc.

Currently there is no standard selection process for human remains samples used for VR canine training aids. The results from this study indicate that when only specific human tissue types are used as training aids, the VOC profile presented to the canine may be limited and not provide a sufficient spectrum of target odor chemicals to ensure odor generalization. It was also found that no unique set of VOCs in the profiles of all of the samples could be used. Combining various tissue types for purposes of generalizing the odor chemical profile for training aids may prove beneficial. Correlation of the tissue-type composites used as training aids with the canine testing results are expected to be important in determining the VOC profiles that are eliciting a desired response.

Future research needs to be performed to provide a more comprehensive VOCs measurement and to verify that this analytical approach is adequate. This study used equilibrium headspace SPME to collect the VOCs emanating from a variety of tissue types. More exhaustive extraction techniques such as non-destructive purge-and-trap or direct headspace methods should be compared and contrasted with these results. Using supplemental analytical methods will help to assure that all of the VOCs from these tissue types are being measured adequately by SPME alone.

The eventual definition of the key odor chemicals used by VR canines to detect aged human buried remains will improve the overall ability of the canines to accurately locate clandestine burial sites. Determining this select group of odor chemicals may be useful to recommend a tissue selection criterion (to ensure odor generalization), to assist with the creation of a human remains mimic for training VR canines and as possible indicator compounds for field portable detection or sensor systems intended to complement canines searching for clandestine burials.

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