J Forensic Sci, March 2008, Vol. 53, No. 2
doi: 10.1111/j.1556-4029.2008.00680.x
Available online at: www.blackwell-synergy.com

Arpad A. Vass,[1] *Ph.D.; Rob R. Smith,*[2] *B.S.; Cyril V. Thompson,*[2] *M.S.; Michael N. Burnett,*[2] *Ph.D.; Nishan Dulgerian,*[3] *M.S.; and Brian A. Eckenrode,*[4] *Ph.D.*

Odor Analysis of Decomposing Buried Human Remains*

ABSTRACT: This study, conducted at the University of Tennessee's Anthropological Research Facility (ARF), lists and ranks the primary chemical constituents which define the odor of decomposition of human remains as detected at the soil surface of shallow burial sites. Triple sorbent traps were used to collect air samples in the field and revealed eight major classes of chemicals which now contain 478 specific volatile compounds associated with burial decomposition. Samples were analyzed using gas chromatography-mass spectrometry (GC-MS) and were collected below and above the body, and at the soil surface of 1.5–3.5 ft. (0.46–1.07 m) deep burial sites of four individuals over a 4-year time span. New data were incorporated into the previously established Decompositional Odor Analysis (DOA) Database providing identification, chemical trends, and semi-quantitation of chemicals for evaluation. This research identifies the ''odor signatures'' unique to the decomposition of buried human remains with projected ramifications on human remains detection canine training procedures and in the development of field portable analytical instruments which can be used to locate human remains in shallow burial sites.

KEYWORDS: forensic science, human decomposition, odor analysis, human remains detection canines, buried bodies, clandestine graves

This paper is the second of a multi-part research series which is attempting to identify the volatile chemical components of human burial decomposition. In Part I (1), the Decompositional Odor Analysis (DOA) Database was established. Four burial sites were established at the University of Tennessee's Anthropological Research Facility (ARF) with a sampling strategy, which allowed for the collection of volatile compounds below and above the body, and at the surface of these sites which had different burial depths and where burial occurred at different times of the year. Chemical classes were identified, separated into individual compounds relative to their abundance, and correlated to the time of their evolution in relation to environmental factors which illustrated the complexity of human burial decomposition.

The primary goal of the current phase of this study was to expand and query the DOA Database to define the chemical fingerprint at the surface of burial sites produced by volatile compounds during the decomposition process of human remains over a span of many years. While still a challenging endeavor, understanding the chemistry of human decay processes in shallow burial sites can provide specific data necessary for the development of reliable field detection instrumentation capable of locating clandestine burial sites. Additionally, determining the volatile chemical signature emanating from a burial site has projected ramifications on human remains detection (HRD) canine training procedures and perhaps even entomological scent attractants.

Clandestine burial sites (defined herein as an illicit act where human remains are buried in hastily dug, shallow graves) have always been difficult to locate, especially when the burial sites are several years old and vegetation has begun to repopulate the area. The ability of HRD canines to detect these sites, while poorly understood, uncharacterized, and unstandardized, is nevertheless impressive (2–14). Their ability to locate as little as 5–15 mg of human tissue, blood, or bone, either buried, on the surface, or elevated above the ground, still exceeds the ability of our best instrumentation. Additional verbal reports of their ability to identify cremains, graves over 100 years old, and minute amounts of human material (even when masked) nearly defy explanation.

Given their reported abilities, HRD canines are still not the final answer for grave detection today primarily because of a lack of standardization in their training procedures and a poor understanding of their scenting capabilities. The DOA Database has shown that the odor of decomposition changes over time, therefore, an HRD canine trained only on tissue will potentially have a more difficult time detecting bone material since the lack of tissue has caused the composition of the odor signature to change. If a canine is trained only on tissue, do you use fresh autopsy material, tissue in active decay or mummified, dry tissue, or a combination of these materials? These are important questions, since the volatile compounds that the canine is sensing as discriminators to differentiate human from nonhuman, fresh from old, or even live from dead subjects, are currently unknown.

A secondary purpose of this study was to begin addressing these issues while the primary focus was to determine the specific volatile signatures associated with a burial decompositional event. This goal required: (1) identifying the key volatile organic compounds (VOCs) that emanate from the ground which are reliable, reproducible, and available for detection; (2) determining the concentration

[1]Oak Ridge National Laboratory, Life Sciences Division, Oak Ridge, TN 37831-6101.
[2]Oak Ridge National Laboratory, Chemical Sciences Division, Oak Ridge, TN 37831-6101.
[3]Federal Bureau of Investigation, Laboratory Division, Evidence Response Team Unit, Quantico, VA 22135.
[4]Federal Bureau of Investigation, Laboratory Division, Counterterrorism and Forensic Science Research Unit, Quantico, VA 22135.

*This work was funded by the Federal Bureau of Investigation, Counterterrorism and Forensic Science Research Unit. The submitted manuscript has been authorized by a contractor of the U.S. Government under contract No. DE-ACO5-00OR22725. Accordingly, the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or to allow others to do so, for U.S. Government purposes.

Received 3 April 2007; and in revised form 9 Sept. 2007; accepted 15 Sept. 2007.

of the VOCs found in the air directly above the burial site; (3) establishing a timeline of when these VOCs are liberated; (4) when they are the most abundant; and (5) assessing (ranking) which chemicals are the most significant and persistent.

Identification of these compounds as well as knowledge of the required range of sensitivity is vital for the development of analytical instrumentation and sensors comprising a cost-effective, portable burial detection device. Additionally, identification of these compounds with subsequent verification by HRD canines will provide a basis by which the HRD canine handlers can begin standardizing and optimizing their training procedures.

The DOA Database contains a record of the VOCs as they are produced near the body and follows their migration upwards, through the soil column, to the surface. Because of the magnitude of the database, only those VOCs detected at the ground/air interface will be discussed in this report. The current database spans the first 4 years of burial decomposition, but also includes data obtained from a 16-year-old burial containing only skeletonized remains and was used to determine the longevity and persistence of these compounds.

Materials and Methods

Triple Sorbent Traps

Triple sorbent traps (TSTs) were composed of 14 mm (0.55 in) sections of Carbotrap, Carbotrap-C, Carbosieve S-III sorbents (Supelco, Bellefonte, PA) packed (in that order) in the center of a 76 mm × 6 mm OD × 4 mm ID (2.99 in × 0.24 in OD × 0.13 in i.d.) stainless steel tube. The tube was stamped with a number and an arrow indicating the sampling flow direction (Fig. 1). Triple sorbent traps were cleaned prior to spiking by heating at 380°C for 3–4 h with a flow of helium at 50–100 mL/min. A stainless steel manifold designed to accept 18 traps was used to process the traps in a batch fashion. The clean traps were spiked with either 10 ng or 25 ng of bromobenzene as a performance verification standard and stored in a freezer at −18°C until use. The mean and standard deviation were calculated for the bromobenzene signals. Points lying outside 3 sigma of the mean were considered outliers and eliminated from the bromobenzene analysis. The mean and standard deviation were then calculated for the remaining samples. Samples containing bromobenzene in amounts within 1 sigma of the mean (68%) were selected for the dataset and further analysis. Traps were sealed during storage and transport using ¼ in (6.35 mm) stainless steel Swagelok® nuts and caps.

Bromobenzene peak detection during sample analyses confirms that the instrument is working properly and allows for analyte comparisons of different TSTs based on the amount of bromobenzene detected. Assuming a response factor of one for all compounds compared to bromobenzene, an estimate of the analyte mass present on any given TST can be calculated. Knowing the air volume sampled allows for an estimation of the analyte concentration in the sampled zone.

Spiking Method

The spiking method was a static dilution technique utilizing 250 mL amber dilution bottles equipped with screw-on vapor-lock valved caps maintained at 70°C to 90°C throughout the spiking process. A master stock bottle (250 μg/mL) was prepared by spiking 42 μL of neat bromobenzene through the push button vapor-lock cap. This was allowed to equilibrate using magnetic stirring with 10 glass beads for at least 10 min after which time 250 μL of the

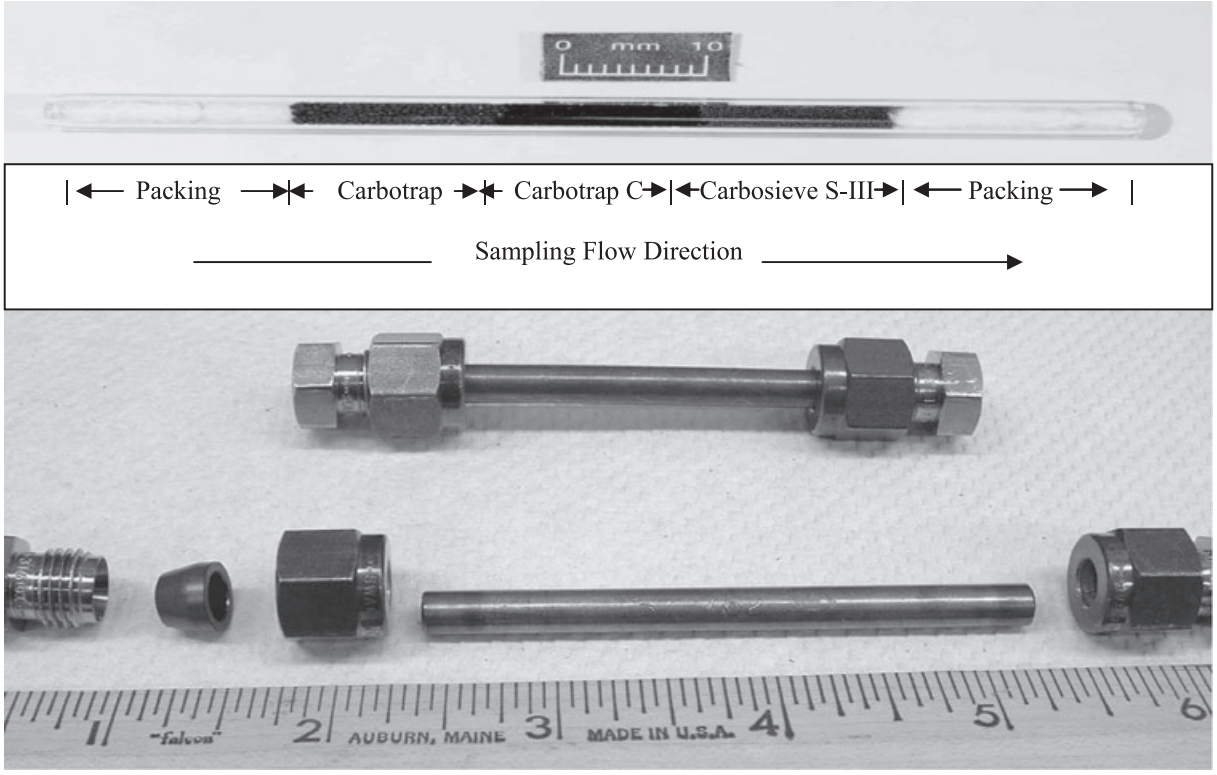

FIG. 1—*Sample acquisition using triple sorbent traps (TSTs) which is based on three sorbent materials with unidirectional sampling and reverse flow thermal desorption allowing sample volumes ranging from mLs to hundreds of Ls.*

master stock was removed using a gas-tight syringe and transferred to another 250 mL amber dilution bottle to make a working standard (250 ng/mL). The working standard was again allowed to equilibrate as described previously before proceeding to the spiking step. During the trap spiking step, the TST was connected on the downstream side of a split/splitless inlet on a Hewlett-Packard 5890 series II gas chromatograph (Santa Clara, CA) maintained at 90°C with a helium flow of about 90 mL/min through the trap. Both the split vent and the purge vent valves were closed during spiking. Each trap was spiked with 100 μL of the working standard using a gas-tight syringe with a Pressure-Lok® valve injected through the injection port septum to effect a mass of loading of 25 ng per trap. At least one trap from each batch was analyzed by TD/GC/MS to confirm the spiking efficiency and trap purity for the batch.

Sampling Tubes for Burial Sites

Three tube designs were used in this study. The initial tube design consisted of 18 in (457 mm) sections of ¾ in (19 mm) OD stainless steel tubing perforated with ¼ in (6.35 mm) diameter holes alternating between a vertical and horizontal alignment through the longitudinal axis of the tube. One end of the tube was welded shut and connected to a 2 ft (0.61 m) section of ¼ in (6.4 mm) OD stainless steel tubing through a 90° elbow fitting at the other. The ¼ in (6.4 mm) OD stainless steel tubing was terminated with a union fitting sealed with a cap.

Subsequent tubes for vapor sampling of graves in the study were constructed of ¾ in (19 mm) OD and 1½ in (38 mm) OD stainless steel tubing. The larger diameter tubes (2.9 L volume) were each fabricated from a 10 ft (3.05 m) section of 1-½ in OD × 0.065 in (38 mm OD × 1.65 mm) wall thickness stainless steel tubing by bending a 2½ ft (0.76 m) vertical arm at each end. This design left a 5 ft (1.53 m) horizontal section in the middle of the tube. The smaller diameter tubes (0.4 L volume) were each fabricated from a 6 ft (1.8 m) section of ¾ in OD × 0.035 in (19 mm OD × 0.09 mm) wall thickness stainless steel tubing by bending 8 in (20.32 cm) vertical arms at each end. This left a 4½ ft (1.37 m) horizontal section in the middle of the tube. Each tube was rinsed in hexane and methanol and heated to 200°C by passing air from a heat gun through the tube. The tube was sampled with a TST to ensure cleanliness and then 42 holes were drilled through the horizontal section of the tube. The holes were on 2 in and 2½ in (5.08 cm and 6.35 cm) centers for the small and large tubes, respectively, and were alternated between a vertical and horizontal alignment. Swagelok® reducers 1½ in x ¼ in or ¾ in x ¼ in (38 mm × 6.4 mm × 19 mm × 6.4 mm) were placed on each end of the tubes for sampling ports (1).

Sampling Hood

A high volume sampling hood 18 in × 54 in × 10 in (45.72 cm × 137.16 cm × 25.4 cm) was also fabricated from 1/16 in (1.6 mm) stainless steel, with one ¼ in (6.35 mm) Swagelok® fitting in the center of the hood for sampling. The total interior volume of the hood, when placed on the grave, was *c*. 130 L. The hood surface was covered with a series of electrically insulated wires which were used to provide resistive heating when connected to a variable output 120 VAC power transformer. Hood sampling events occurred with the hood heated to generally around 32–38°C which was sufficient to prevent water vapor condensation. Early sampling experiments showed that water condensation interfered with the vapor sample integrity which required a modification of the earlier design.

Chemicals

Analytical grade chemicals, including bromobenzene, were purchased from Sigma Chemical Corporation (St. Louis, MO).

Instrumentation

Thermal desorption analyses of the TST samples acquired during the study were conducted on a Hewlett-Packard (Agilent) 5890/5972 GC-MS. The instrument was modified for thermal desorption of TST samples by inserting a three-way valve into the helium inlet line. One leg of the valve was routed to the GC carrier gas inlet and the other leg was routed to the upstream side of the TST when mounted on the GC inlet. The GC inlet was modified to accept a TST by welding an inlet septum nut to a male ¼ in (6.35 mm) Swagelok® fitting. Flow during desorption was directed through the septum by piercing the septum with a short (*c*. 2 in [50.8 mm]) section of 0.53 mm i.d. aluminum-clad uncoated guard column. The helium flow was routed through the TST during desorption and through the GC carrier gas inlet during analysis. The GC was equipped with a Restek Crossbond® Rtx®-1PONA column (Bellefonte, PA) (100 m, 0.25 mm ID, 0.5 μm d_f) for the analytical separations.

Operating Conditions for the HP Instrument—The cryofocusing technique used a short loop (between 5 and 6 in long [12.7 and 15.24 cm]) of 1/16 in (1.6 mm) OD stainless steel tubing (0.030 in ID [0.76 mm]) connected to a low dead volume 1/16 in (1.6 mm) stainless steel "tee" connector. The "tee" outlet was either to an atmospheric vent (during desorption) or when this was closed, to the analytical column. The cryofocusing loop was immersed in liquid nitrogen during desorption.

Operating Conditions for the HP 5890 GC/MS—Thermal desorption; helium carrier at 40 mL/min vented to the atmosphere after the cryofocusing loop; coil heater used to heat TST to 350°C during a 5-min desorption period; GC split and septum purge flows blocked off during desorption; GC parameters include the following.

Initial temperature, 35°C; initial hold time, 7.0 min; initial rate, 10°C/min; second temperature, 45°C; second hold time, 5.0 min; second rate, 1.5°C/min; third temperature, 70°C; third hold time, 5 min; third rate, 3°C/min; final temperature, 250°C; final hold time, 5 min; injector temperature, 250°C; detector temperature, 280°C; 4-min acquisition delay scanned from 35–550 *m/z*.

Sampling

A four-port manifold was constructed using Parker fittings, toggle valves, and Porter fine metering valves to allow independent control of four different TST samples. A Gast Model DOA-P104B-AA diaphragm pump (Benton Harbor, MI) was used to provide vacuum to the manifold.

Triple sorbent traps were connected to the sampling manifold via sections of ¼ in (6.35 mm) PTFE tubing terminated on either end with silicone tubing. Flow rates were determined before and after sampling using an ADM 3000 Flowmeter from J & W Scientific® (Folsom, CA). Triple sorbent traps were connected to the sampling ports of either the sampling tubes or the sampling hoods with ¼ in (6.35 mm) tube fittings. Sample start times and stop times were recorded and used with the average flow rate to calculate the total sample volume.

Sampling did not occur on a regular basis and was primarily determined by weather conditions, time of year and manpower availability. Estimated sampling rates were weekly during the warmer months and monthly during the cooler months.

Bone Vapor Sampling

Two samples each of pig, human, dog, and deer femora and humeri, skeletonized between 5 and 9 years previously, were used for this study and were obtained from the University of Tennessee's zooarcheological and forensic collections. Bone vapor emanating from these skeletal elements were individually captured using a dedicated Tedlar® bag which had been cut open at one corner to allow for bone insertion. After placing the specified bone in the bag it was sealed by means of a rod clamped between two pieces of 1 in × 2 in (2.54 cm × 5.08 cm) poplar wood. Each piece of poplar had been routed lengthwise using a ½ in (12.7 mm) diameter semi-circular bit to a depth of 0.469 in (11.9 mm). Consequently, when the smoothed Tedlar® bag is wrapped around the top and bottom side of the rod, it is sealed along four lines of pressure running the length of the bag opening. Bags so sealed have been shown to maintain a seal for months at a time (Fig. 2).

The headspace inside the bag is allowed to equilibrate for 3 days before sampling. A specified volume was then drawn from the valved port of the bag through a TST. In most cases, each bone was analyzed twice. Control blanks, Tedlar® bags containing no skeletal elements, were processed in a manner identical to those with skeletal elements present. Compounds identified in these control blanks were not used as discriminatory components of the bone odor study.

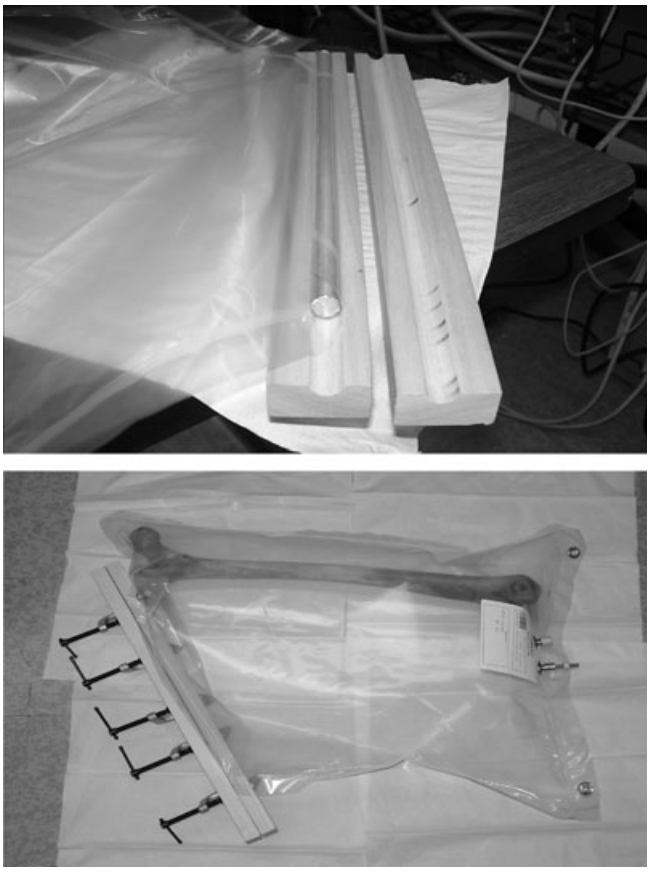

FIG. 2—*Tedlar® bag sealing device for bone vapor sampling.*

Data Reduction

GC-MS data was transferred to a satellite PC for processing. The HPCHEM method was used to calculate the peaks and areas using the quantitation database generated from aggregate data reviews. After identification and quantitation was complete, the data was reviewed manually using the QEdit Quant Result feature of the HPCHEM software. In this review, individual compounds were identified, if present in the sample, and the total ion chromatogram (TIC) quantitated manually. Unknowns with a peak height of greater than 50,000 were also reviewed and either added to the quantitation database or listed as unidentified unknowns if no identification was possible from the software database. When the QEdit review was complete, the results were saved, and both a file and screen summary report were generated. The screen report was saved as a text file, which was then imported into Microsoft Excel®. Only the columns containing the compound name, retention time, and integrated area were used. This file was saved back into the same text file name. The file was then imported into the Microsoft Access® database, where it was associated with the experimental data from the sample.

Results

In the final tally, 478 separate compounds were detected and identified as volatile or semi-volatile components of the burial decompositional process. Extremely light volatile compounds, such as ammonia, hydrogen, carbon dioxide, and methane which have been reported as decomposition products of soft tissue (2) were not detectable with the current methodology, but could also contribute to the odor during early decomposition.

Of these 478 compounds, 30 were identified as key markers of human decomposition which were detectable at the soil surface (Table 1). These 30 compounds were produced at the corpse level (below body) at various concentrations and migrated upwards through the soil column unmodified by bacteria, potentially indicating that varying soil types and their resident microbial populations would not influence the presence, liberation, or detection of these compounds. One compound, carbon tetrachloride, slightly increased in abundance as it migrated upwards through the soil column, possibly indicating microbial transformation of a base compound into carbon tetrachloride. Of the 30 key compounds detected in a burial situation, 19 were also detected when collecting TST air samples above corpses decaying on the surface (unburied), confirming the hypothesis that they are originating from the corpse. Expected compounds not detected during surface decomposition are identified in Table 1, potentially indicating that these compounds are formed during strictly anaerobic processes or require transformation of a base compound by soil microflora. Many of these 30 compounds have also been identified in other forensic case studies involving odor analysis of advanced human decomposition (15) indicating that these compounds are not unique to American populations or produced solely by our testing methodologies.

The complexity of decompositional events requires that the comparison of time elapsed and the degree of decomposition be viewed in Accumulated Degree Days (ADDs) (16,17). ADDs are an accumulation of average daily temperatures in degrees Celsius over the course of the decomposition cycle. When referring to burial situations, the term BADD is used and refers to the Burial Accumulated Degree Days (1), indicating the accumulated average daily temperature in the burial vault. Since the evolution of these compounds is cyclic (1), Table 1 provides the BADDs when the surface

TABLE 1—*Surface detection of compounds liberated from burial decompositional events ranked in order of perceived importance.*

Rank	Compound	Concentration (ppt*)		Compound Detection BADD† Range		BADDs Indicating Prominent Abundance Maxima	Compound also Detected in Odor of Specified Mammalian Bone
		Max.	Control Maximum	Earliest	Latest		
1	Carbon tetrachloride	83	5	309	>18,000	1,976; 4,286	Human
2	Toluene	218	9	300	>18,000	762; 2,834; 6,276	Human, deer, dog
3	Ethane, 1,1,2-trichloro-1,2,2-trifluoro	122	5	309	16,932	535; 3,956	Human, dog
4	Tetrachloroethene‡	148	7	309	>18,000	762; 4,886	Deer, dog
5	Naphthalene‡	229	2	309	>18,000	762; 4,011	Dog
6	Trichloromonofluoromethane‡	120	8	309	>18,000	535; 3,956	Deer, dog
7	Dimethyl disulfide	58	1	309	16,932	791; 3,701; 6,646	
8	1,4 dimethyl benzene	176	4	475	>18,000	769; 3,856	Human, dog
9	Benzene	98	4	309	>18,000	1,012; 6,647	Human, deer, dog
10	Dichlorodifluoromethane	109	n.d.	343	16,932	590; 4,011	Deer, dog
11	1,2 dimethyl benzene	287	5	343	>18,000	762; 3,956	
12	Chloroform‡	83	9	309	16,932	762; 6,276	Dog
13	Ethyl benzene	102	4	309	>18,000	762; 2,307; 3,956	Human, dog
14	Styrene‡	29	n.d.	308	>18,000	762; 4,010	
15	Dimethyl trisulfide	76	n.d.	309	16,932	762; 6,647	
16	Decanal	36	5	475	>18,000	475; 3,956	Human, deer, dog, pig
17	Sulfur dioxide‡	11,373	3	343	7,280	697; 3,859; 4,432	
18	Nonanal	10	3	400	>18,000	2,459; 4,010	Human, deer, dog
19	Carbon disulfide	34	n.d.	309	>18,000	2,697; 4,182	
20	Hexane	117	3	590	7,280	791; 2,855; 3,701	Human, deer
21	Benzenemethanol, alpha, alpha, dimethyl	66	n.d.	167	7,280	2,856; 4,286	Human, dog
22	Trichloroethene‡	7	n.d.	309	4,653	1,011; 3,659; 4,432	
23	1-ethyl, 2-methyl benzene	80	1	536	4,653	762; 3,956	Deer
24	1-methoxypropyl benzene‡	10	n.d.	309	3,896	3,850	
25	Hexadecanoic acid, methyl ester‡	296	n.d.	762	4,432	762; 4,286	
26	1,2 Benzenedicarboxylic acid, diethyl ester	91	n.d.	536	7,280	6,276	Human, deer
27	Undecane	178	n.d.	535	>18,000	762; 4,653	Human
28	Methenamine	382	n.d.	476	7,280	2,963; 4,653	Dog
29	Dichlorotetrafluoroethane‡	15	n.d.	309	4,432	590; 4,011	
30	1,1-dichloro-1-fluoroethane‡	4	n.d.	309	3,896	3,850	

n.d., none detected.
*ppt, parts per trillion.
†BADD, burial accumulated degree days.
‡Not detected during surface decomposition.

concentrations for each compound were most prominent and also lists the BADD range during which detection occurred. As a rule of thumb, BADD values for eastern Tennessee, U.S.A., average 5,234 BADDs/year.

The results can also be segregated into three distinct groups (Table 2). Group 1 includes those compounds that are detected throughout the burial decomposition process (both soft tissue decomposition and diagenesis—the breakdown of mineral and collagen in bone). They are present in the test burial site that is over 16 years old and tend to be the least cyclic in their evolution. The group includes benzene derivatives and the most prominent halogen compounds and aldehydes. Group 2 includes those chemicals that only appear early in the decomposition process. They typically are only detectable for a little over 1 year (~7,300

TABLE 2—*Groups of compounds which are detected on the surface during a burial decomposition process.*

Group 1. Surface Compounds which are Detected Throughout the Burial Decomposition Process	Group 2. Surface Compounds which are Found only Early in the Burial Decomposition Process (<7,300 BADDs)	Group 3. Surface Compounds which Persist Until all Soft Tissue Is Gone (<18,000 BADDs)
Compound	Compound	Compound
Ethyl benzene	Trichloroethene	Dichlorodifluoromethane
Toluene	1-methoxypropyl benzene	Dimethyl disulfide
Tetrachloroethene	Sulfur dioxide	Ethane, 1,1,2-trichloro-1,2,2-trifluoro
1,4 dimethyl benzene	Hexadecanoic acid, methyl ester	Chloroform
Carbon tetrachloride	Dichlorotetrafluoroethane	Dimethyl trisulfide
1,2 dimethyl benzene	Hexane	
Naphthalene	1,1-Dichloro-1-fluoroethane	
Styrene	1-Ethyl, 2-methyl benzene	
Benzene	Benzenemethanol, alpha, alpha, dimethyl	
Nonanal	Methenamine	
Decanal	1,2 Benzenedicarboxylic acid, diethyl ester	
Trichloromonofluoromethane		
Carbon disulfide		
Undecane		

BADDs) and tend to be the most cyclic. This group includes esters, certain benzene derivatives, and some of the halogen compounds. As has been previously reported (1), a recently buried corpse will have no surface detectable volatile compounds associated with the decompositional process for up to 17 days. Group 3 compounds are those which are consistently present as long as soft tissue remains (including mummified tissue) on the corpse (up to nearly 18,000 BADDs). This group includes many of the sulfur and halogenated compounds. It is interesting to note that of these 30 compounds, 24 of them peak within the first year (5,234 BADDs).

The analysis of clean bone (bone with no tissue adhering to the surface) is considered a very important and concurrently developing component of this study since many burials are not discovered for many years and only skeletal material is recovered. Skeletal elements selected were femora and humeri from pig, human, dog, and deer species. These particular species were chosen since they are most commonly found in outdoor settings and are of sufficient size to survive environmental conditions for many years.

When analyzing bone in the absence of any tissue, 72 compounds were detected and identified, 12 of which were recognized as important markers for burial decomposition (Table 1). Results of this study show that the odor of each type of bone is quite unique in terms of ratios of very specific component classes—aldehydes, ketones, alcohols, and amides (Fig. 3)—and could alone be used for identification purposes. Class components considered important for differentiation and which were combined to determine relative class ratios include: 2-propanone, 2-decanone, and 2-nonanone (ketones); acetamide, *N*,*N*-dimethyl (amides); hexanal, heptanal, nonanal, octanal, pentanal, decanal, and butanal (aldehydes); and phenol in combination with 1-pentanol, 1-heptanol, 1-hexanol, and ethanol (alcohols). While many additional compounds were detected in the skeletal elements used for this study, the class compounds listed above are considered the most important for differentiation of these species and several of these are also important in headspace analysis over burials—most notably nonanal indicating that bone odor can indeed contribute to the overall odor associated with burial decomposition. Note in Table 1 that carbon tetrachloride and

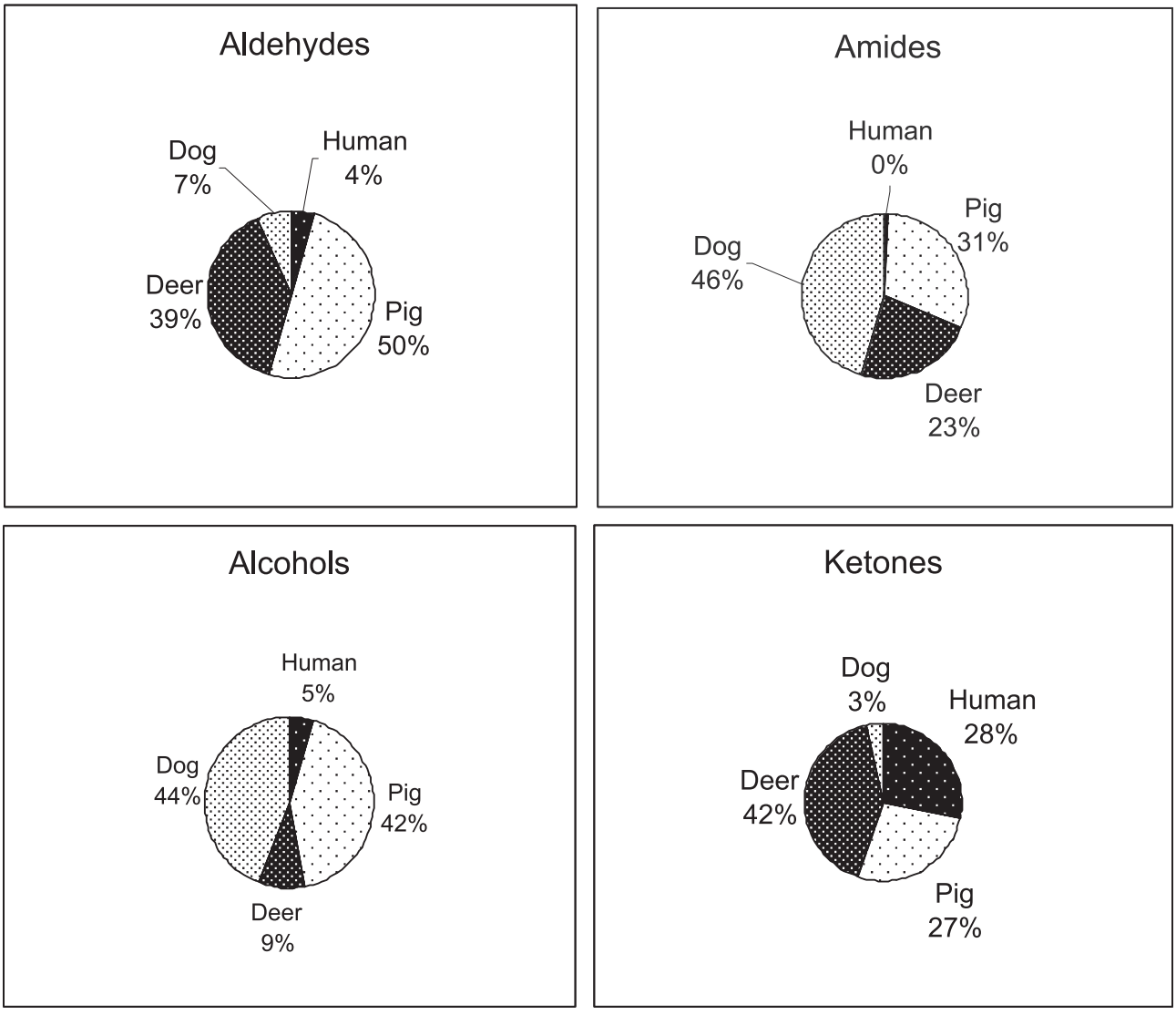

FIG. 3—*Pie charts illustrating differences in bone odor composition among various species of common mammals.*

undecane are unique for being found in human bone but not in any of the other mammalian species used in this study.

Discussion

As previously discussed (1), many factors associated specifically with burials affect the decomposition rate and the evolution of chemicals. These include:

1. temperature, which is a function primarily of altitude and latitude;
2. the presence of water—from the body itself, the environment, or air moisture (water is important because it serves as a diluent and solvent for polar molecules);
3. acidity or alkalinity (pH), which affects intracellular chemical reactions and microbial activity; and
4. the decreased availability of oxygen from deep burials, being submerged, or at high altitudes, all of which slow the decomposition process due to retardation of oxidative processes.

Similar to the evolution of volatile fatty acids and inorganic components in the soil underneath decompositional events (18), the cyclic production of the 30 key volatile components detected at the surface of graves can not be attributed solely to temperature, implying that temperature is not the only significant environmental factor in burial decomposition (1). Other, less well studied aspects of burial decomposition include the soil type, lack of insects, water pooling from poor drainage, the season of burial, the state of the decedent, and whether the decedent is wrapped or clothed (19–21). While not every aspect of the burial environment could be studied in this research, a multiplicity of variables (varying depths, state of the decedent, age of burial, and season of burial) were included in this study. In light of these factors, the perceived importance (ranking) of the compounds (Table 1) in the database (as tools for the development of analytical instruments that can be used to detect clandestine burials and for improving HRD canine training procedures) was determined utilizing a decision tree based upon levels of the following factors:

1. Reproducibility of detection (between burials and regardless of depth).
2. Detection of the compound as a component of human bone odor.
3. Abundance of the compound.
4. Longevity of detection.
5. Background control concentrations.
6. Whether the compounds were detected in surface decompositional events.
7. Whether the compounds were detected in relevant areas other than the University of Tennessee's decay research facility (e.g., Noble, GA; morgues, forensic cases submitted to our laboratory, reports from other researchers).
8. Uniqueness of the compound.
9. Chemical class trends.
10. Effects of the environment (temperature, moisture, barometric pressure).

It is interesting to note that many of the 30 compounds in Table 1 are not very unique and can in fact be found in many outdoor samples taken virtually anywhere. Many of the common compounds selected for the list were detected in small amounts in background control samples obtained from a variety of sources which included trip blanks, soil blanks, empty burial pit blanks, instrument blanks, outdoor samples in various locations around Knoxville, TN, air blanks, morgue blanks, and hood blanks. The levels of detected compounds in the blanks were dependent on traffic movement, wind direction, nearness to urban centers, weather conditions and time of day—to mention but a few of the variables. The results reported in Table 1 include only those compounds detected at the soil surface—only what would be available for analytical instruments and HRD canines to detect. Compounds in this list were only included if a large, definitive trend was observed where the concentration below and above the corpses was higher than what was measured in the hood samples and if the concentrations below and above the corpses were greatly elevated above background controls, indicating that the corpse was indeed the source of the compound. However, it must be noted that even though control sample concentrations at the surface were typically less than what was observed in the hood samples once decomposition had begun, on occasion some control compounds showed elevated, transient concentration spikes for unknown reasons and this must be taken into account when developing analytical instrumentation to detect clandestine burial sites.

This study has shown that, for the most part, human decompositional end-products are not very unique in the chemical world. Included in Table 1 are the maximum surface concentrations of each compound from both the test and control samples observed during the course of this study. Test sample maximums were always higher than the corresponding control maximums. Since each test compound began at a level equal to, or below, those seen for control samples, no minimum value for each test compound is reported. To provide some guidance for future development of analytical detection instrumentation, it is suggested that the maximum values for each compound in the control samples represent the minimum compound concentrations.

The design of this study for the purpose of characterizing the odor at the soil/air interface of a burial necessitated the use of capture hoods to minimize dilutional effects of blowing wind and air streaming. To determine the true equilibrated concentration of each compound, the capture hoods were left on the grave generally overnight and, in a few cases, for several days prior to sampling. This type of static sampling methodology could have artificially increased the maximum concentration value for compounds in the key list and must be taken into account when developing instrumentation to determine concentration levels during real-time analyses.

The true value of this list for clandestine burial site detection purposes is understanding which compounds will be present at what time during the decompositional process (BADDs), their relative abundance, and most importantly, their relative ratios compared to the other compounds in the list at similar times.

Based on the results depicted in Table 2, it is anticipated that at least three different sets of standards could be formulated to test the various phases of burial decomposition. The next logical progression in this study will be to develop/modify analytical instrumentation which can detect a significant proportion of these 30 compounds in the specified range of concentrations and chemical groupings. In addition, HRD trained canines could be tested to determine their alerting responses to these 30 compounds, individually and in combination ratios specified in the DOA Database.

Success in this undertaking will allow for the further development of analytical tools, methodologies, and portable sensors, calibrated for burial detection, which are able to assist the investigator in rapidly, cost effectively, and accurately locating clandestine burial sites. This research could also advance our understanding of canines' scenting ability and allow for the development of training aids capable of enhancing the canines' performance and reliability.

The current version of the database is presently being archived at the Federal Bureau of Investigation, Counterterrorism and Forensic Science Research Unit, Quantico, Virginia. The database includes all detectable compounds, relative abundances, chemical trends, experimental information, methodologies, and weather data related to sampling.

Acknowledgments

We gratefully acknowledge the University of Tennessee's Department of Anthropology (Drs. Richard and Lee Jantz and Dr. Walter Klippel) for allowing us access to the Anthropological Research Facility and for allowing use of skeletal material for this study, and Rex A. Stockham, M.F.S, Federal Bureau of Investigation, Quantico, VA for his support of this research and valuable guidance from his field experiences. We also wish to thank Janet L. Besanceney, Suzan Ferguson, and John Randle (SARTECH canine handlers) for their time spent demonstrating and explaining HRD training procedures, capabilities, and techniques.

References

1. Vass AA, Smith RR, Thompson CV, Burnett MN, Wolf DA, Synstelien JA, et al. Decompositional odor analysis database. J Forensic Sci 2004;49(4):760–9.
2. Killam EW. The detection of human remains. Springfield IL: Charles C. Thomas, 1990.
3. Komar D. The use of cadaver dogs in locating scattered, scavenged human remains: preliminary field test results. J Forensic Sci 1999;44(2):405–8.
4. O'block R, Doere S, True N. The benefits of canine squads. J Police Sci Admin 1979;7(2):155–60.
5. Department of the Treasury. Law enforcement canine training. Washington: US Printing Office, 1993.
6. Chapman S. Police dogs in North America. Springfield, IL: Charles C. Thomas, 1990.
7. Hoving GL. Buried body search technology. Identification News 1986;2:3–15.
8. Kalmus H. The discrimination by the nose of the dog of individual human odours and in particular the odours of twins. Brit J Anim Behav 1955;3(1):25–31.
9. Kristofeck W. A Study of attitudes, knowledge and utilization of canine teams by the Louisville Division of Police. Louisville, KY: University of Louisville, 1991.
10. Lilly J, Puckett M. Social control and dogs: a sociohistorical analysis. Crime Delinquency 1997;43(2):123–47.
11. Pearsall M, Verbruggen H. Scent detection. New York: Barkleigh Productions, 1982.
12. Schall R. An evaluation of the animal-behavior theory for earthquake prediction. Calif Geol 1988;41(2):16–21.
13. Syrotuck W. Scent and the scenting dog. New York: Barkleigh Productions, 1977.
14. Lorenzo N, Wan TL, Harper RJ, Hsu YL, Chow M, Rose S, et al. Laboratory and field experiments used to identify *Canis lupus* var. *familiaris* active odor signature chemicals from drugs, explosives, and humans. Anal Bioanal Chem 2003;376:1212–24.
15. Statheropoulos M, Spiliopoulos C, Agapiou A. A study of volatile organic compounds evolved from the decaying human body. Forensic Sci Int 2005;153:147–55.
16. Soil Survey Staff. Soil taxonomy, agriculture handbook no. 436, Chapter 4. Washington: US Printing Office, 1975.
17. Edwards R, Chaney B, Bergman M. Temperature developmental units for insects. Pest & Crop Newsletter 1987;2:5–6.
18. Vass AA, Bass WM, Wolt JD, Foss JE, Ammons JT. Time since death determinations of human cadavers using soil solution. J Forensic Sci 1992;37(5):1236–53.
19. Clark MA, Worrell MB, Pless JE. Postmortem changes in soft tissue. In: Haglund WD, Sorg MH, editors. Forensic taphonomy: the postmortem fate of human remains. Boca Raton, FL: CRC Press, 1997;151–60.
20. Turner B, Wiltshire P. Experimental validation of forensic evidence: a study of the decomposition of buried pigs in a heavy clay soil. Forensic Sci Int 1999;101:113–22.
21. Vass AA. Beyond the grave—understanding human decomposition. Microbiology Today 2001;28:190–2.

Additional information and reprint requests:
Arpad A. Vass, Ph.D.
Oak Ridge National Laboratory
1 Bethel Valley Road
P.O. Box 2008
X-10, 4500S, MS 6101, Room E-148
Oak Ridge, TN 37831-6101
E-mail: vassaa@ornl.gov