Law Enforcement Sensitive

FORENSIC REPORT
Orange County, Orlando, Florida
Preliminary Report #2

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SUBJECT: Orange County Sheriff's Department case number OCSO#08-069208. Preliminary results of various tests to determine the possibility of a decompositional event.

DATE: September 24, 2008

On July 24, 2008 Investigator Mike Vincent of the Orange County Sheriff's Department sent us a carpet sample from the trunk of a 1998 Pontiac Sunfire (FL License number G63-XV) to determine if the odor present on the sample and in the trunk of the vehicle was indicative of a decompositional event. Additional air samples, collected by Investigator Vincent on triple sorbent traps (TSTs), were sent to us on September 3, 2008 and included air samples from the vehicle interior and composite air samples from all the items identified in the trunk compartment. These samples were sent to us in reference to research we have been performing since 2002 in an attempt to identify the chemical composition of human decomposition odor. This research has resulted in the development of a Decompositional Odor Analysis database (References 1-2).

Odor Analysis

The initial carpet sample was enclosed in a sealed metal can. A preliminary analysis was performed by collecting a small (0.8 ml) sample of air from the can and injecting the air into a gas chromatograph/mass spectrometer. Several compounds were observed in this sample (primarily chloroform); however, it was deemed necessary to concentrate the sample in order improve the sensitivity for the lower abundance compounds.

The sample was removed from the metal can and placed in a Tedlar bag for several days at 35 degrees C and allowed to off-gas into the Tedlar bag. The analytes from several ml of gas from the Tedlar bag were concentrated by cryo-focusing them with liquid nitrogen at the head of a gas chromatograph/mass spectrometer. Preliminary results of this analysis are summarized in Table 1. Compounds in the sample were tentatively identified by mass spectral library match. Standards of the tentatively identified compounds have not yet been run. It is important to note that gasoline was found in the vehicle trunk which presented a significant chromatographic signal and overlapped with approximately 41% of the chemicals typically observed in decompositional events.

Previous reports of a decomposing pizza found in the vehicle trunk were not true and this was incorrectly stated in the previous report. An MSDS sheet, listing the ingredients of BlueStar, was obtained and evaluated by a chemist (Dr. Michael Burnett, Oak Ridge National Laboratory). His conclusion was that the components of the product could not have contributed to the chemical signature obtained in these analyses. Additional samples which have been analyzed and are being summarized in this report include:

1) control trunk carpet sections obtained from three Pontiac Sunfire vehicles (two from 1998 and one from 1996 – the carpet samples were all a similar color and type as the Florida vehicle) located in a junkyard in Knoxville, TN and placed in a Tedlar bag at 35 degrees C for two days (collected by Dr. David Glasgow, ORNL);
2) a piece of pizza (mushroom and pepperoni) in the original pizza box which was allowed to decompose for several days at the University of Tennessee's Decay Research Facility and then placed in a Tedlar bag at 35 degrees C (included as a control even though no pizza was actually found in the Florida vehicle). It is interesting to note that no maggots were found on the pizza;
3) composite air samples of all the items found in the trunk of the Florida vehicle collected on triple sorbent traps (TSTs),
4) air samples from the Florida vehicle interior compartment collected on TSTs,
5) Laser Induced Breakdown Spectroscopy (LIBS) results from the Florida trunk sample,
6) air from the laboratory at ORNL where the Florida trunk carpet sample was prepared and stored (control);
7) TST Florida trip blank (control);
8) TST air sample from garage where Florida vehicle is stored (control);
9) Volatile fatty acid analysis (not yet complete) which will also be used to assist in the determination of a decompositional event and is the best means of obtaining a post-mortem interval (PMI) in this instance in lieu of entomological evidence.

Of the 51 chemicals identified from the Florida trunk carpet sample, (many gasoline components detected are not listed in the Table), 41 (80%) are consistent with decompositional events. Only seventeen of these overlap with known or possible gasoline constituents leaving 24 compounds (59 %) found in the decompositional odor analysis database potentially unaccounted for. It is important to note that this sample was removed from the trunk and eventually isolated in a Tedlar bag so the compounds which were detected in this analysis were off-gassing from the carpet sample and did not include vapors that may been in the air of the trunk.

Nine compounds identified in the control carpet samples from the junkyard were also detected in the Florida trunk carpet samples. These were all in trace amounts. Only four of these could not be attributed to possible gasoline vapor components – one of these compounds was chloroform, but was only detected in trace amounts in the control carpet sample.

Only six compounds identified in the pizza, which were also detected in the Florida trunk sample, could not be attributed to gasoline vapor components. Of these six compounds, four were detected in the control carpet samples from the junkyard.

Laboratory air where the sample was stored and processed was sampled to determine if the room contained any chemical components which might have contributed to the odor detected in the Florida trunk carpet sample. None were detected.

The TST trip blank contained a number of additional compounds unrelated to what was found in the Florida car trunk. In order to keep Table 1 as simple as possible not all compounds are listed, but some of the additional compounds detected in this sample included: butane, 2-butene, 2-methyl butane, 1-pentene, 1-hexene, pentanal, 1-heptene, heptane, 1-octene, heptanal and substituted benzenes.

The Florida Forensics Unit Garage TST air sample was composed of primarily gasoline vapors (not all components listed in Table 1), freons and degreasers (tetrachloroethene), all commonly found in garages. No chloroform or sulfur containing compounds were detected in spite of the fact that TSTs could potentially concentrate more material than would direct sampling of the headspace of the carpet sample in a Tedlar bag (10 mLs were injected). The TST pump was calibrated to collect 500mL/minute and 77 minutes were collected so the spectrum would have represented approximately 39 L of air. Freons detected included trichlorofluoromethane, dichlorofluoroethane and trichlorotrifluoroethane. Some of the additional compounds detected in this sample included: 2-methyl butane, 2-methyl butene, 2,3 dimethyl butane, 2-methyl pentane, cyclohexane, methyl cyclopentane, 3-methyl hexane, cyclopentane, heptane, 3-methyl 2-hexane, methylcyclohexane, 1,2 dimethyl benzene (xylenes) and styrene.

The TST air sampling from the Florida vehicle interior showed common hydrocarbons and significant amounts of xylenes present [69 minutes collection time (34.5 L of air)]. Not all compounds are listed in Table 1. Some additional compounds detected in this sample included: substituted benzenes, pentanes, heptanes, heptenes and 2-propanol.

The composite air sampling of the trash bag contents [72 minutes collection time (36 L of air)] showed that compounds were present which represented primarily ubiquitous hydrocarbons, many of which are also found in gasoline (not all listed in Table 1). Dichlorofluoroethane and tetrachloroethene (also seen in the garage air sample) were detected in low concentrations. Some of the additional compounds detected in this sample include: cyclopropane, dimethyl pentane, substituted hexanes, substituted naphthalenes, cyclopentane, 2-heptene, cyclohexane and heptanal.

Air sampling summary

Out of 24 compounds detected in the Florida trunk sample which did not overlap with known or possible gasoline constituents, 16 (67 %) known to be associated with human decompositional events were detected in the odor signature from the Florida trunk carpet sample whose source could not be potentially linked to any of the controls which were analyzed. These included 2-methyl furan, acetic acid methyl ester, butanoic acid methyl ester, carbon disulfide, carbon tetrachloride, chloroethane, chloroform, chloromethane, decanal, dichloroethene, dichloromethane, dimethyl trisulfide, dimethyl disulfide, hexanol, methanethiol and trichloroethene. Of these 16 compounds, seven were significant human decomposition chemicals listed in the database. Only five of these

seven compounds are being used to draw conclusions about the possibility of a decompositional event occurring. Decanal and trichloroethene were dropped from this list because they were only detected in trace amounts. These five compounds are:

Carbon disulfide
- appears very early in human decomposition (<100 ADDs[a])
- appears in both aerobic and anaerobic decomposition

Carbon tetrachloride
- appears very early in human decomposition (<100 ADDs)
- appears in both aerobic and anaerobic decomposition
- potentially a human specific marker (not seen in select animal remains)

Chloroform
- appears very early in human decomposition (<100 ADDs)
- primarily detected in deprived oxygen (anaerobic) decompositions

Dimethyl trisulfide
- appears very early in human decomposition (<100 ADDs)
- appears in both aerobic and anaerobic decomposition

Dimethyl disulfide
- appears very early in human decomposition (<100 ADDs)
- appears in both aerobic and anaerobic decomposition

[a] ADD – accumulated degree day (an accumulation of average daily temperatures))

This assumes that all the control samples contributed equally to the odor signature in the trunk (which is unlikely) and does not take into account that numerous compounds in the controls were detected in trace amounts and that some were potentially highly concentrated by the TST collection procedure. [For example, fluorinated compounds were detected in the Forensics Unit Garage, but were not detected in the Florida vehicle trunk sample potentially indicating very little, if any, contribution of the garage air to the trunk signature].

TSTs collected from the Florida trunk were analyzed even though the trunk liner had been removed and the odor would not have been representative of what was originally present at the time of vehicle discovery. Even with the liner removed, sulfur containing compounds such as carbon disulfide, carbon tetrachloride, chloroform and dimethyl disulfide were still detectable in trace amounts. The trunk was the only location where all these types of compounds were detected.

Common fluorinated compounds usually associated with human decomposition were not detected in the Florida trunk sample. It is possible, although this has not been studied, that a 3 year-old child may not have had sufficient time (many years) to ingest enough fluorinated compounds for them to be incorporated into tissue and then to appear in the decompositional breakdown of soft tissue and bone. Additionally, several of the identified compounds are typically associated with anaerobic decomposition. While the actual significance of this not known, it indicates that any type of decompositional event that might have been associated with the odor in the trunk of the car could have occurred under deprived oxygen conditions.

Laser-Induced Breakdown Spectroscopy (LIBS) for elemental analysis

LIBS analysis was also performed on the both the Florida trunk carpet sample and the control samples. As decomposition progresses, various inorganic elements found in human tissue, such as magnesium (Mg), calcium (Ca), iron (Fe) and sodium (Na) are found in increasing concentrations (over time) in drainage from decompositional events. This technique was utilized to determine if known inorganic components of decompositional events were elevated over the controls and also to determine if the concentration ratios of these elements could be used to determine a rough post-mortem interval (reference 3).

Introduction to LIBS

LIBS provides rapid multielemental microanalysis of bulk samples (solid, liquid, gas, aerosol) in the parts-per-million (ppm) range with little or no sample preparation which has been widely demonstrated. In this technique, a laser vaporizes a small volume of sample material with sufficient energy for optical excitation of the elemental species in the resultant sample plume. The vaporized species then undergo de-excitation and optical emission on a microsecond time scale, and time-dependent ultraviolet-visible spectroscopy fingerprints the elements associated with the spectral peaks. LIBS is typically a surface analytical technique, with each laser pulse vaporizing microgram or submicrogram sample masses. However, the rapidity of sampling (typically 10 Hz laser repetition rate) and the ability to scan a sample surface, provides sufficient statistics for bulk sampling.

The greatest advantage of LIBS is its capability for remote chemical analysis of samples with minimal handling and little or no sample preparation, which minimizes generation of waste to the microgram per pulse of ablated material. The instrumentation and operation of a LIBS system is simpler than some of the more sensitive techniques, and analysis times on the order of minutes, make it more amenable for real-time analysis of chemical processes. Although calibration standards are required for quantitative analysis, the generation of a single calibration curve will suffice for analysis of samples in a similar matrix.

In the analysis of the carpet samples that were obtained, care was taken to scan the sample consistently. A laser beam of 532 nm wavelength was focused onto the surface of the Florida trunk carpet sample and control carpet samples collecting the excited light using collection optics that were delivered to a spectrometer which detected specific band wavelengths. These wavelengths corresponded to between 260 nm to 300 nm for Mg, between 390 to 420 nm for Ca, between 570 to 610 nm for Na, and between 240 to 280 nm for Fe. The data were collected in multiples of five and compared to each other. All spectral information was very consistent within the multiples of spectra that were captured and analyzed. It is interesting to note that every element known to be associated with a decompositional event (that could be detected using this technique) was elevated over control values. [Non metals such as potassium, chloride, etc. have a greater ionization potential than metallic and semi-metallic elements and could not be detected using the current laser system].

The LIBS spectra of Mg, Ca, Na, and Fe for the control carpet and sample carpet are shown in Figures 1-4.

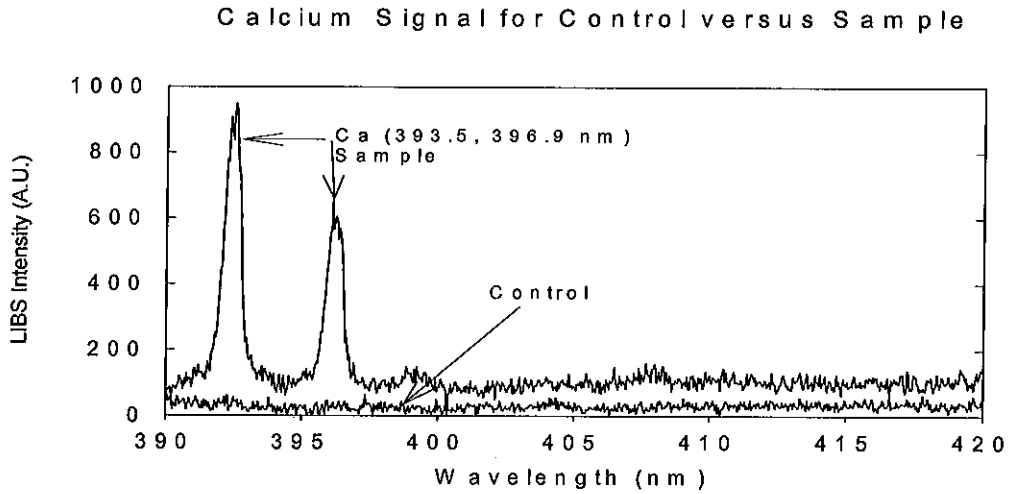

Figure 1. Spectra of control carpet and sample carpet for the presence of Calcium (Ca).

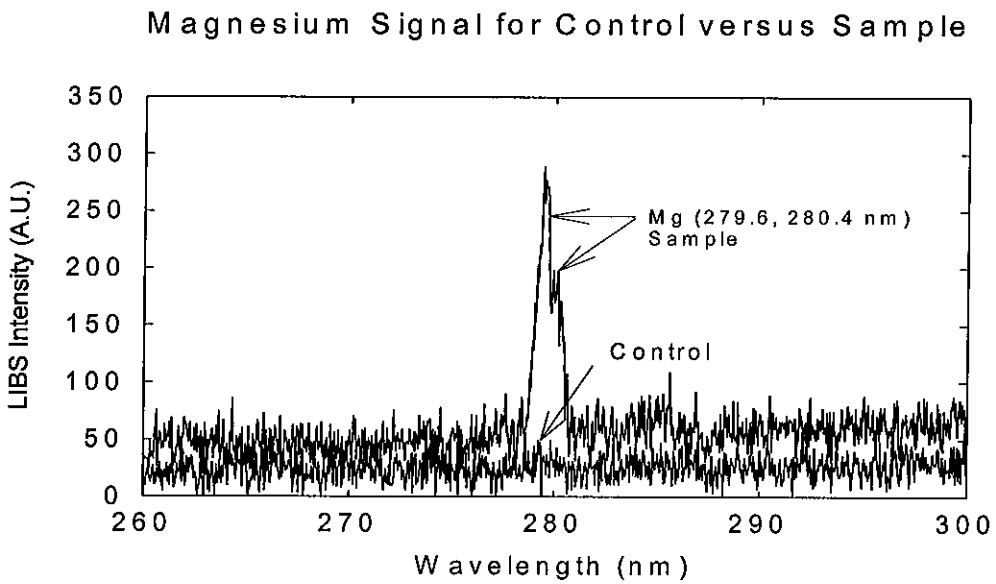

Figure 2. Spectra of control carpet and sample carpet for the presence of Magnesium (Mg).

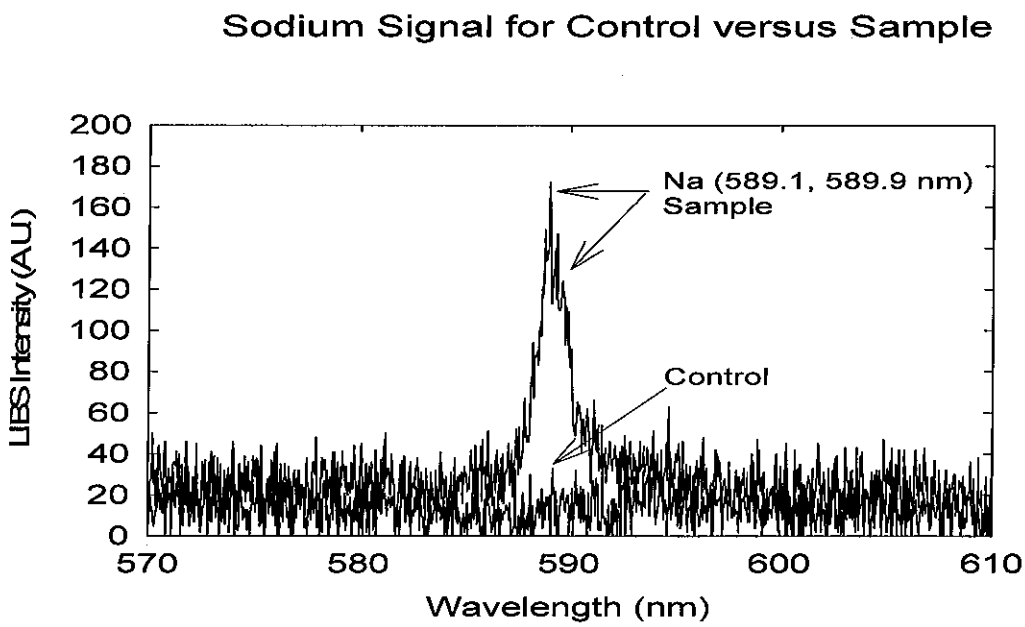

Figure 3. Spectra of control carpet and sample carpet for the presence of Sodium (Na).

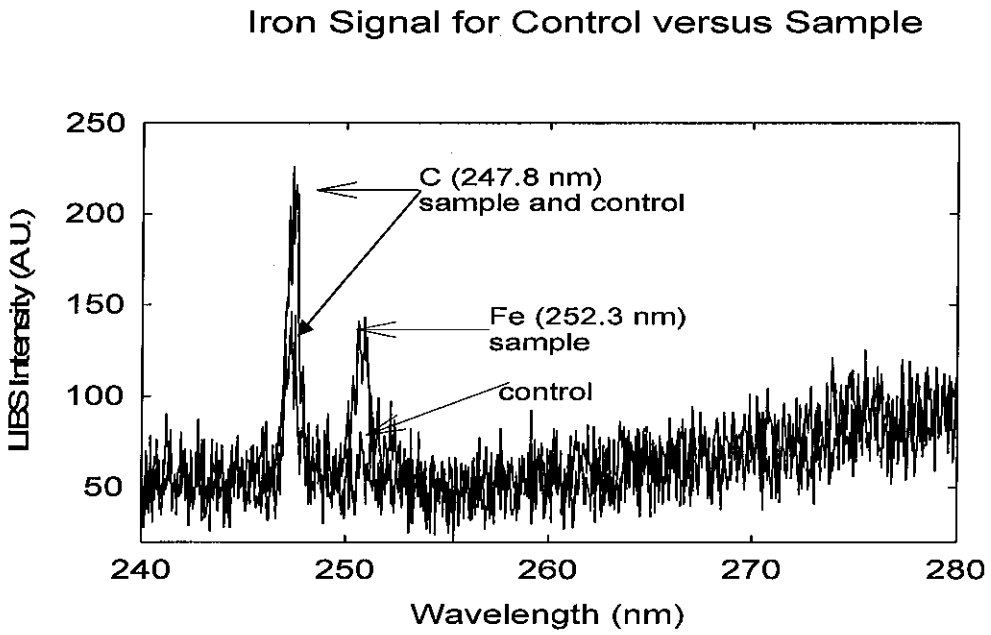

Figure 4. Spectra of control carpet and sample carpet for the presence of Iron (Fe). Carbon - (C).

Post-mortem interval determinations in this instance are best carried out using volatile fatty acids (VFAs), but can be done in a crude fashion using inorganic elements. The 5:1 ratio of calcium:magnesium typically occurs at 90 Accumulated Degree Days (ADDs). An ADD is simply the accumulation of average daily temperatures (in Celsius) over time. The exact temperatures inside the vehicle trunk are not known. If one assumes it would be quite warm – Florida in the summer (~95 degrees F or 35 degrees C), this equates to approximately 2.6 days of decomposition. At 2.6 days, the levels of sodium should be much higher than what was detected using LIBS. This points to a PMI of less than 2.6 days, but the error estimation for sodium is quite high as one approaches the beginning of decomposition. The low concentration of sodium also indicates almost no contribution to the sample from the BlueStar product. Low amounts of carbon (C) also indicate a recent PMI.

While these are still preliminary results (specific retention times must be confirmed with standards, specific compounds should be quantitated and VFA analyses need to be completed), both odor analysis and LIBS results appear to be quite consistent with a decompositional event having occurred in the trunk of the vehicle. Of particular interest is the large concentration of chloroform (not a common ingredient in commercial products) and the presence of sulfur containing compounds in the Florida trunk carpet sample which are particularly characteristic of decompositional events.

Finally, we wish to discuss the possibility that these chemical signatures are of human origin. The decomposition odor products of animal remains (dog, deer and pig - primarily bone) have been studied since these are the most commonly encountered in outdoor environments. These animals can be distinguished from human remains by the percentages of certain classes of chemicals (ketones, amides, aldehydes and alcohols). While the direct correlation of animal bone to early human decomposition may not be exact, the results are interesting and potentially point to a human origin as the source of the scent. Table 2 shows which compounds were detected in the Florida trunk sample and compares them to what is known about animal decomposition.

Table 2

Compound Class	Compound	Detected in FL trunk Tedlar bag sample?	Detected in selected animal remains?	Detected in human remains	Approximate ADD when first detected in human remains
Ketone	2-propanone	yes	Yes (pig only)	yes	230
Ketone	2-decanone	no	yes	yes	1976 (late)
Ketone	2-nonanone	no	yes	yes	4011 (late)
Amide	**Acetamide, N,N-dimethyl**	no	yes	no	
Aldehyde	hexanal	no	yes	yes	1698 (late and low concentration
Aldehyde	heptanal	no	yes	yes	1698 (late and low concentration
Aldehyde	nonanal	no	yes	yes	400 (very low concentration)
Aldehyde	octanal	no	yes	yes	4011 (late)
Aldehyde	pentanal	no	yes	yes	4011 (late)
Aldehyde	decanal	yes	yes	yes	475
Aldehyde	butanal	Yes (trace)	Yes (pig only)	yes	Aerobic decomposition product only
Alcohol	Phenol	no	Yes (dog only)	yes	200 (primarily aerobic decomposition product)
Alcohol	1-pentanol	no	yes	yes	1307
Alcohol	**1-heptanol**	no	yes	no	
Alcohol	1-hexanol	yes	yes (pig only	yes	536
Alcohol	ethanol	yes	Yes (pig only)	yes	1307 (gasoline component)

While not conclusive, these data indicate that:

1) Nearly all the compounds present in early human decomposition were detected in the trunk samples if their concentrations were high enough to detect. Carbon tetrachloride was also detected and is a human specific marker with these animal sets.
2) Compounds that have been detected in these select animal remains and not in humans were not detected in the Florida trunk carpet sample (1-heptanol, Acetamide, N,N-dimethyl). This is also suggestive of a human decompositional event.
3) Compounds associated with anaerobic decomposition are seen supporting a possible deprived oxygen type of decompositional event.
4) Compounds with a late ADD were not detected supporting an early stage decompositional event.

Conclusions

The correlation between all the techniques, the comparison to what is known about the decomposition of human and animal remains, indications of early decomposition products and the presence of the five key major compounds associated with human decomposition (primarily the sulfur containing compounds) **indicates that a portion of the total odor signature identified in the Florida vehicle trunk is consistent with a decompositional event that could be of human origin.** Additional components that made up a portion of the total odor signature included gasoline constituents and an unusually large concentration of chloroform - far greater than what is typically seen in human decomposition.

These results still do not rule out the remote possibility that an unusual variety of products or materials (not present in the trunk at the time of vehicle discovery) may have had some contribution to the overall chemical signature.

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