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# Establishing the volatile profile of pig carcasses as analogues for human decomposition during the early postmortem period

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## Abstract

Following a mass disaster, it is important that victims are rapidly located as the chances of survival decrease greatly after approximately 48 h. Urban search and rescue (USAR) teams may use a range of tools to assist their efforts but detector dogs still remain one of the most effective search tools to locate victims of mass disasters. USAR teams can choose to deploy human scent dogs (trained to locate living victims) or human remains detection (HRD) dogs (trained to locate deceased victims). However, little is known about the variation between live human scent and postmortem human remains scent and the timeframe during which one type of scent transitions to the other. The aim of the current study was to measure the change in the scent profile of human decomposition analogues during the first 72 h postmortem by measuring the volatile organic compounds (VOCs) that comprise the odour. Three pig carcasses (*Sus scrofa domesticus* L.) were placed on a soil surface and allowed to decompose under natural conditions. Decomposition odour was sampled frequently up to 75 h postmortem and analysed using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). A total of 105 postmortem VOCs were identified during the early postmortem period. The VOC profile during the early postmortem period was highly dynamic,

changing both hourly and daily. A transition period was observed after 43 h postmortem, where the VOC profile appeared to shift from a distinct antemortem odour to a more generalised postmortem odour. These findings are important in informing USAR teams and their use of detector dogs for disaster victim recovery.

**Keywords:** Mass spectrometry, Chromatography, Forensic pathology, Analytical chemistry, Chemistry

## 1. Introduction

Locating victims following a mass disaster is a task of critical importance for ethical, social, and religious reasons [1, 2, 3]. Mass disasters can refer to natural disasters such as earthquakes, tsunamis and hurricanes, or man-made disasters often associated with acts of terrorism. Once safe to do so, urban search and rescue (USAR) teams will enter the disaster scene with the intent to locate, extricate and medically assist living victims. USAR teams will commonly use hearing, vision, sensitive microphones, geophones, cameras (e.g. infrared, visible and thermal) and detector dogs to locate the living [4, 5]. Despite advances in technology, detector dogs are still considered the most valuable search tool in disaster recovery scenes as they can more rapidly search large areas and track an odour to source [6, 7, 8].

Search and rescue dogs (sometimes referred to as disaster search dogs) are trained to detect and indicate live or deceased victims in debris that results from natural and man-made disasters [9]. Depending on the disaster event, terrain being searched, and the availability of search and rescue dogs at a scene, USAR teams may deploy human scent dogs (trained to locate living victims) and human remains detection (HRD) dogs (trained to locate deceased victims), rather than deploying dual-trained dogs to find both. Determining which dogs to deploy should be based on the search strategy and the likelihood of locating survivors. However, little is known about the variation between live human scent and postmortem human remains scent and the timeframe during which one type of scent transitions to the other [4, 10, 11]. This can confound the ability of search dogs to successfully locate an entrapped victim, whether alive or deceased.

In a forensic context, research on human scent dogs has focused on tracking, trailing, and scent-identification line-up canines and their ability to distinguish individual body odours of live humans [12, 13]. An individual's unique odour during life is determined by many factors including genetics, diet, metabolism, and environmental and lifestyle factors [14]. Whether or not this scent is detectable following a mass disaster is still unknown since the medical condition of an entrapped victim may resemble an individual who is fasting, stressed or

severely injured [5, 15]. Research in this area has therefore focused on detecting the volatile organic compounds (VOCs) that comprise the odour of expired air, blood and urine from victims entrapped beneath rubble and debris, as these are consistently present [4, 5, 15, 16].

The decomposition odour following death has also been extensively investigated in a forensic context, predominantly to identify the VOCs used by HRD dogs and insects to locate decomposing remains [6, 7, 10, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31] and to identify alternative search techniques for locating victim remains [1, 3, 4, 5, 32]. Most studies that use whole cadavers/ carcasses chemically profile the complete decomposition process, from autolysis to putrefaction and liquefaction, through to the final stages of skeletonisation or dry remains [10, 17, 18, 19, 20, 21, 22, 23, 26, 29, 30, 31, 33, 34]. The decomposition odour throughout this timeframe is important to understand in the context of missing persons or victims of homicide that may have been missing for weeks, months or years. In the context of mass disasters, however, USAR operations are most critical in the first 72 h since the survival rate of victims dramatically declines during the first 48 h following the disaster [4, 15]. Although the human body can survive without food for more than 3 weeks, it can only survive 3–7 days without water and approximately 4 min without air [4, 35] hence the need for rapid search and recovery strategies.

Of the numerous decomposition studies carried out to date, only a few have investigated the early postmortem interval (PMI) (i.e. 0–72 h) [1, 2, 3]. With the exception of the study by Statheropoulos et al. [2] in which duplicate VOC samples were collected at 0, 4, 8 and 24 h from a body with a PMI estimate of 3 days, all other studies that have investigated the VOC profile of the early postmortem period have only collected VOC samples once daily during this timeframe [1, 2]. This provides a ‘snapshot’ of the detectable VOC profile at fixed points during the first 72 h but does not advance our understanding of how the VOC profile changes during this time. Such knowledge could assist detection of disaster victims by ensuring USAR teams deploy the search dog best suited to the task of recovery.

The aim of the current study was to measure the change in the VOC profile of human decomposition analogues during the first 72 h postmortem. The authors have previously shown the importance of using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) to characterise decomposition odour [21, 22, 23, 27, 28, 30, 31]. GC×GC-TOFMS provides many noteworthy benefits for peak detection in decomposition odour analysis that is beneficial due to the wide dynamic range and complexity of the VOC profile. Hence, a more comprehensive chemical profile can be developed for decomposition odour using GC×GC-TOFMS, including superior detection of

many trace compounds that may prove to be important markers for HRD dogs. This is the first study to apply GC×GC-TOFMS, including its superior deconvolution power, to the investigation of the VOC profile at repeated time points throughout the early postmortem period when disaster victim recovery is most critical. This study also represents the first characterisation of early postmortem VOC profiles from whole replicate carcasses ( $n = 3$ ), as studies have previously only used single or duplicate carcass(es) or human cadaver(s). The application of multivariate statistics could therefore be applied to this data in contrast with existing literature. The implementation of multivariate statistics was anticipated to allow better extraction of meaningful data from the complex multivariate data generated by GC×GC-TOFMS, and to generate a more detailed characterisation of the VOC profile throughout the early postmortem period. The intent of this study was to provide new information to USAR teams and canine handlers about the timeframe during which the decomposition scent becomes detectable.

## 2. Materials and methods

### 2.1. Experimental design

Three domestic pig carcasses (*Sus scrofa domesticus* L.) weighing approximately 60 kg each were used as analogues for human decomposition. After researching the vendor to confirm they followed established animal welfare guidelines, the carcasses were purchased postmortem from Hawkesbury Valley Meat Processors, a licensed abattoir in Sydney, NSW, Australia on March 3, 2015. Following the guidelines of the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edn. 2013) (<http://www.nhmrc.gov.au/guidelines-publications/ea28>), animal ethics approval was not required, as the experimental subjects were not killed specifically for the purposes of the research and were purchased postmortem. All pigs were killed using captive-headbolt, the standard procedure employed in Australian abattoirs. The carcasses were wrapped in a large polyethylene tarpaulin and transported to the field site within an hour of death where they were placed directly on the soil surface approximately 3 m apart.

The field site for this study is privately owned by the University of Technology Sydney (UTS) and has been approved for research and educational purposes. The field site, an open Eucalyptus woodland, is located in Western Sydney, NSW, Australia (33° 38S, 150° 39E). The soils in the area consist of sandy clay loam topsoil underlain by a highly weathered bedrock (saprolite) layer forming on shale/siltstone/sandstone bedrock. The topsoil at the study location is mostly acidic and ranges between pH 5.5–6.5 throughout the year.

The three carcasses were placed on their side on the soil surface and covered with wire mesh anti-scavenging cages when samples were not being collected.

A control site was established approximately 20 m from the pig carcasses to collect background VOC samples of the surrounding vegetation and soil. The trial was conducted during the summer months from March 3–March 6, 2015 (i.e. ~0–72 h postmortem). Decomposition of the pig carcasses was observed twice daily. The decomposition stage of the carcasses was adapted from observations made by Payne [36]. Decomposition was reported in both experimental days and accumulated degree hours (ADH). ADH was calculated by taking the sum of the average hourly temperatures within the specified time period [37]. Weather data was recorded throughout the study using a Hobo Weather Station equipped with a Hobo U30 No Remote Communication data logger (OneTemp, Marlestone, NSW, Australia) which recorded hourly measurements of the ambient temperature ( $^{\circ}\text{C}$ ), rainfall (mm), relative humidity (%), solar radiation ( $\text{W}/\text{m}^2$ ), wind speed (m/s), wind direction ( $\emptyset$ ), and gust speed (m/s).

Experimental logistics dictated the sampling frequency of this study. Pig carcasses can only be purchased from the abattoir at the end of the day (as excess stock) and were collected at approximately 16:15 on March 3, 2015. Following transport to the field site, the first VOC sample collection (referred to hereafter as 1 h postmortem) commenced at 17:20. The intent of this initial sample collection was to provide a baseline VOC profile of the odour for each carcass soon after death (and before any physical signs of decomposition were apparent) for comparison with subsequent VOC profiles as decomposition proceeded. VOC sampling was only conducted during daylight hours. Hence, VOC samples were collected repeatedly from each carcass on Day 0 (1 h postmortem), Day 1 (17, 19, 21 and 23 h postmortem), Day 2 (43, 45, 47 and 49 h postmortem) and Day 3 (69, 71, 73 and 75 h postmortem).

## 2.2. VOC sample collection

VOC sample collection involved placing a stainless steel hood over each of the carcasses or control site and allowing the headspace to accumulate for 15 min [20]. The headspace was sampled dynamically by attaching an ACTI-VOC low flow air sampling pump (Markes international Ltd., Llantrisant, UK) to one end of a sorbent tube containing Tenax TA and Carbograph 5DT (Markes International Ltd.) and attaching the other end of the sorbent tube to a sampling port on the stainless steel hood (see [20] for a schematic). Air was pumped into the sorbent tube at a flow rate of 100 mL/min for 10 min to obtain a 1 L sample of the headspace above the pig carcass or control site. In accordance with the EPA Method (TO-17)[38], all tubes were sealed with brass storage-caps, wrapped in aluminium foil and placed in a glass air-tight container for transportation to the laboratory. A field blank sample was collected on each experimental day prior to the first sample collection to account for natural

variation in the atmospheric VOC profile. Field blanks were collected by uncapping a blank tube for 30 s at the field site and then re-capping the tube as documented in previous studies [22, 23, 30, 39]. The field blank tubes were stored with the experimental sorbent tubes to account for contamination or artefacts that may have resulted during the storage period.

### 2.3. VOC sample analysis

Prior to analysis, an eVol® XR handheld automated analytical syringe (SGE Analytical Science, Weatherill Park BC, NSW, Australia) was used to inject an internal standard consisting of 2 µl of 150 ppm bromobenzene (GC grade, Sigma-Aldrich, Castle Hill, NSW, Australia) in methanol (HPLC Grade, Sigma-Aldrich) onto each sorbent tube to allow for normalisation. Thermal desorption of the sorbent tubes was performed using a Markes Unity 2 Thermal Desorber and Series 2 ULTRA multi-tube autosampler (Markes International Ltd.). Each sorbent tube was thermally desorbed at 300 °C for 4 min and collected onto a general purpose cold trap (TenaxTA/Carbograph 1TD) at -10 °C. Secondary desorption was performed at 300 °C for 3 min with a 20 mL/min split flow. Following thermal desorption, a reconditioning method of 330 °C for 30 min was performed on each sorbent tube.

A 1 m uncoated fused silica transfer line (Markes International Ltd.) held at 120 °C was used to connect the thermal desorption unit to a Pegasus® 4D GC×GC-TOFMS (LECO, Castle Hill, NSW, Australia) via an Ultimate Union Kit (Agilent Technologies, Mulgrave, NSW, Australia). The first dimension (<sup>1</sup>D) column was a 30 m × 0.250 mm inner diameter (ID), 1.40 µm film thickness Rxi®-624Sil MS column (Restek Corporation, Bellefonte, PA, USA) and the second dimension (<sup>2</sup>D) column was a 2 m × 0.250 mm ID, 0.50 µm film thickness Stabilwax® column (Restek Corporation). The <sup>1</sup>D and <sup>2</sup>D columns were joined using a SilTite µ-Union (SGE Analytical Science). A constant flow of helium carrier gas (high purity, BOC, Sydney, NSW, Australia) was used at a rate of 1.00 mL/min. The <sup>1</sup>D oven was initially held at 35 °C for 5 min, then increased to 240 °C at a rate of 5 °C/min, and held for a further 5 min. The modulator temperature offset was +5 °C and the <sup>2</sup>D oven temperature offset was +15 °C. The modulation period was 5 s with a 1 s hot pulse. The MS transfer line was maintained at 250 °C. The mass range was between 29–450 amu with an acquisition rate of 100 spectra/s. The temperature of the ion source was 200 °C, the electron ionisation energy was 70 eV, and a 200 V offset above the optimised detector voltage was used.

### 2.4. Data processing

Data processing was carried out using ChromaTOF® (version 4.51.6.0; LECO). A signal-to-noise (*S/N*) ratio of 150 was set with a baseline offset of 0.8. Peak

width in the  $^1\text{D}$  was 30 s while peak width in the  $^2\text{D}$  was 0.15 s. A list of compounds was created through mass spectral matching with the National Institute of Standards and Technology (NIST) Mass Spectral Library using a match threshold of 80%.

The Statistical Compare software feature within ChromaTOF<sup>®</sup> was subsequently used to perform peak alignment between samples using a mass spectral match threshold of 60%. Samples were input into Statistical Compare and separated into two classes: experimental ( $n = 39$ ) and control ( $n = 13$ ). Statistical Compare was also used to perform peak re-searching at a  $S/N$  of 20. During alignment, analytes were only retained if found in at least 5 samples out of the 52 total samples or if found in 7% of the samples within a class. Following alignment, the analyte peak areas (calculated using unique mass) were normalised using the bromobenzene internal standard peak area. The resulting peak table was further processed in Microsoft Excel where chromatographic artefacts were removed.

Fisher ratios (i.e. the ratio of between-class variance to within-class variance) were calculated for each compound using the Statistical Compare software feature. A Fisher Ratio threshold was chosen using a critical F value ( $F_{\text{crit}}$ ) of 4.03. This threshold was determined as demonstrated in previous studies [27, 40, 41] and compounds above this value were considered to be compounds of interest. Those compounds that exhibited Fisher Ratios below  $F_{\text{crit}}$  were deleted. Compounds were also deleted if they were the result of column or sorbent bleed. Principal component analysis (PCA) was performed using The Unscrambler<sup>®</sup> X (version 10.3; CAMO Software, Oslo, Norway). Prior to PCA analysis, the data was pre-processed in The Unscrambler<sup>®</sup> X. Pre-processing steps included mean centering, variance scaling, and unit vector normalisation [42, 43]. Following PCA, the data was verified to contain no outliers by means of the Hotelling's  $T^2$  95% confidence limit.

### 3. Results and discussion

#### 3.1. Weather conditions

This study was conducted during the Australian summer from March 3–March 6, 2015. The mean temperature during this time was 21.8 °C with a minimum temperature of 13.0 °C and a maximum temperature of 30.5 °C recorded. The humidity ranged from 25.8% – 99.5%, with an average humidity of 75.2%. Solar radiation varied between 0.6  $\text{W}/\text{m}^2$ –591.9  $\text{W}/\text{m}^2$ , with an average solar radiation of 85.2  $\text{W}/\text{m}^2$ . There was no recorded rainfall during the early postmortem period and wind speed and gust speed did not exceed 0 m/s.

### 3.2. Decomposition stages

On day 0 at 1 h (ADH 27.0), all three carcasses were characterised as being in the fresh stage of decomposition. On day 1 between 17–23 h (ADH 377.8–572.4), two of the three carcasses were characterised as being in the bloat stage of decomposition while the third carcass remained in the fresh stage of decomposition. On day 2 from 43–49 h (ADH 910.7–1071.0), all three carcasses were characterised as being in the bloat stage of decomposition. On day 3 from 69–75 h (ADH 1411.7–1514.1), all three carcasses were characterised as transitioning from bloat to active decay, whereby some liquefaction was observed and the bloating of carcasses began to subside. These stages of decomposition are consistent with prior decomposition studies during the summer months in this environment [21, 22, 30, 39].

### 3.3. Identification of postmortem VOCs

This study used GC×GC-TOFMS to analyse decomposition odour in the early postmortem period. GC×GC-TOFMS is a superior separation technique that is suited to analysing complex decomposition odour profiles [21, 22, 23, 27, 28, 31]. The addition of a <sup>2</sup>D column increases peak capacity by allowing a greater degree of separation between analytes that may have otherwise co-eluted in traditional one-dimensional analyses [44]. GC×GC-TOMS also produces enhanced sensitivity. These qualities make GC×GC-TOFMS a better suited separation technique for complex decomposition odour [21]. The use of GC×GC-TOFMS in this study allowed for the identification of 466 components during the early postmortem period before further processing.

After sorbent bleed, column bleed, and compounds that did not meet the Fisher ratio threshold ( $F_{crit} = 4.03$ ) were deleted, 108 compounds of interest were identified. These 108 compounds were assigned to one or more compound classes including alcohols, aldehydes, aromatics, carboxylic acids, esters, ethers, hydrocarbons, ketones, nitrogen-containing compounds, sulfur-containing compounds or other. Of the 108 compounds of interest, three compounds (nonadecane, cyclohexylbenzene, and 2,4,6-trimethyloctane) were found in higher concentrations in the control samples than in the experimental samples and were removed. Therefore, 105 compounds were identified and included in the final list of postmortem VOCs (Table 1).

Overall, nitrogen-containing compounds represented the highest proportion of VOCs detected (25 of the total 105 postmortem VOCs), and were detected in all postmortem samples. The nitrogen-containing compound, benzonitrile, was detected at all sampling points during the early postmortem period. Sulfur-containing compounds represented the second highest proportion of VOCs detected (24 compounds of the total 105 postmortem VOCs), and were also



**Table 1.** Tentative VOCs identified during the early postmortem period. Decomposition odour literature references have been given for previously identified VOCs.

Volatile organic compounds (VOCs)	Hours postmortem												
	1	17	19	21	23	43	45	47	49	69	71	73	75
<b>Sulfur-containing</b>													
1,2,4-Trithiolane [3]						▲	▲	▲	▲	▲	▲	▲	▲
2,4-Dithiapentane [3,20, 39]		▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Aminomethanesulfonic acid			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	
Carbon disulfide [10, 17, 23, 32, 49, 50]		▲	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲
Dimethyl sulfide [2, 7, 10, 17, 19, 20, 32, 50]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Dimethyl sulfone [3]		▲			▲	▲	▲	▲	▲	▲	▲	▲	▲
Dimethyl Sulfoxide [3]								▲	▲	▲	▲	▲	▲
Dimethyl trisulfide [2, 10, 17, 32, 49]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Disulfide, dimethyl [2, 7, 8, 10, 17, 19, 20, 24, 26, 32]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Disulfide, methyl (methylthio) methyl			▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Hydrogen sulfide								▲	▲	▲	▲	▲	▲
Methane, isothiocyanato-	▲	▲	▲			▲	▲	▲	▲	▲	▲	▲	▲
Methanesulfonic acid, ethyl ester								▲	▲	▲	▲	▲	▲
Methanesulfonic acid, methyl ester [3]								▲	▲	▲	▲	▲	▲
Methanesulfonylacetic acid	▲		▲	▲	▲		▲	▲	▲	▲	▲	▲	▲
Methanethioamide, N,N-dimethyl-								▲	▲	▲	▲	▲	▲
Methanethiol [1, 3, 19, 24]			▲			▲	▲	▲	▲	▲	▲	▲	▲
Methyl ethyl disulfide [2, 3]									▲	▲	▲	▲	▲
Methyl thiolacetate [24]						▲		▲	▲	▲	▲	▲	▲
p-Dithiane-2,5-diol								▲	▲	▲	▲	▲	▲
Sulfur dioxide [10, 17, 19, 32]		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Sulfurous acid, dimethyl ester	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Thiocyanic acid, methyl ester	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Thiophene, 3-methyl-								▲	▲	▲	▲	▲	▲
<b>Alcohol</b>													
1,5-Hexadien-3-ol	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
1-Butanol, 3-methyl- [30]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
1-Hexanol [3, 7, 18, 32, 46, 49]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
1-Pentanol [7, 10, 18, 19, 32, 46, 49]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
1-Propanol [18, 31, 39]						▲			▲	▲	▲	▲	▲
1-Propanol, 2-methyl- [3, 18, 19, 32, 46]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
2-Butanol, (R)-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
2-Pentanol [3, 32]									▲	▲	▲	▲	▲
3-Hexen-1-ol, (Z)-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲

(Continued)

**Table 1.** (Continued)

Volatile organic compounds (VOCs)	Hours postmortem												
	1	17	19	21	23	43	45	47	49	69	71	73	75
Ethanol [3, 18, 19, 32, 49]		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Isopropyl Alcohol		▲	▲	▲	▲	▲	▲	▲			▲		▲
p-Cresol [2, 18, 19, 31, 39]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
p-Dithiane-2,5-diol								▲	▲	▲	▲	▲	▲
Phenylethyl Alcohol					▲	▲	▲	▲	▲	▲	▲	▲	▲
<b>Aldehyde</b>													
2-Heptenal, (Z)-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
2-Hexenal, (E)-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
2-n-Butylacrolein					▲		▲	▲	▲	▲	▲	▲	▲
2-Propenal									▲	▲	▲	▲	▲
Butanal [3, 10, 19, 50]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Butanal, 2-methyl-	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Butanal, 3-methyl- [2, 3]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Formaldehyde		▲								▲			▲
Formamide, N,N-dibutyl-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲		▲
Formamide, N,N-dimethyl- [3, 18, 19]						▲	▲	▲	▲	▲	▲	▲	▲
Hexanal [6, 7, 8, 10, 18, 32, 33, 50]								▲	▲	▲	▲	▲	▲
Methacrolein	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Nonanal [6, 7, 10, 17, 18, 49, 50]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Propanal [18]							▲	▲	▲	▲	▲	▲	▲
Propanal, 2-methyl-	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
<b>Aromatic</b>													
1,3,5-Triazine							▲	▲	▲	▲	▲	▲	▲
1,3-Diazine										▲	▲	▲	▲
5H-1-Pyridine	▲			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Benzonitrile [17, 18, 19, 26, 31]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Benzoic acid, methyl ester	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Furan, 2-ethyl- [32, 39]					▲			▲	▲	▲	▲	▲	▲
Methanamine, N-(phenylmethylene)-									▲		▲	▲	▲
p-Cresol [2, 18, 19, 31, 39]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Phenylethyl Alcohol					▲	▲	▲	▲	▲	▲	▲	▲	▲
Pyrazine, 2,6-dimethyl- [18]								▲	▲	▲	▲	▲	▲
Pyrazine, methyl- [18]					▲	▲			▲	▲	▲	▲	▲
Thiophene, 3-methyl-								▲	▲	▲	▲	▲	▲
trans-calamenene	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
<b>Carboxylic acid</b>													

(Continued)

Table 1. (Continued)

Volatile organic compounds (VOCs)	Hours postmortem												
	1	17	19	21	23	43	45	47	49	69	71	73	75
Butanoic acid [7, 8, 18, 19, 31]				▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Butanoic acid, 2-methyl- [10, 18, 19, 32]	▲				▲			▲	▲	▲	▲	▲	▲
Butanoic acid, 3-methyl- [18, 19]	▲				▲		▲	▲	▲	▲	▲	▲	▲
Methanesulfonylacetic acid	▲		▲	▲	▲		▲	▲	▲	▲	▲	▲	▲
Pentanoic acid [7, 8, 19, 31, 33]					▲			▲	▲	▲	▲	▲	▲
Propanoic acid [7, 8, 31, 33]								▲	▲	▲	▲	▲	▲
Propanoic acid, 2-methyl-					▲			▲	▲	▲	▲	▲	▲
<b>Ester</b>													
3-Hexen-1-ol, acetate, (Z)-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Acetic acid, methyl ester [39]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Benzoic acid, methyl ester	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Butanoic acid, 2-methyl-, ethyl ester [32]									▲		▲	▲	▲
Butanoic acid, butyl ester [7, 19]						▲		▲		▲	▲	▲	▲
Butanoic acid, ethyl ester [7, 32]						▲	▲	▲	▲	▲	▲	▲	▲
Butanoic acid, propyl ester										▲	▲	▲	▲
Carbonic acid, dimethyl ester						▲		▲	▲	▲	▲	▲	▲
Diethyl azodicarboxylate							▲			▲	▲	▲	
Ethyl Acetate [18, 49]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Formic acid, propyl ester							▲	▲	▲	▲	▲	▲	▲
Methanesulfonic acid, ethyl ester								▲	▲	▲	▲	▲	▲
Methanesulfonic acid, methyl ester [3]								▲	▲	▲	▲	▲	▲
Methyl dimethylcarbamate						▲		▲	▲	▲	▲	▲	▲
n-Propyl acetate						▲		▲	▲	▲	▲	▲	▲
Propanoic acid, ethyl ester [18]	▲	▲	▲			▲	▲	▲	▲	▲	▲	▲	▲
Sulfurous acid, dimethyl ester	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
<b>Ether</b>													
1,3-Dioxolane, 2-methyl-								▲	▲	▲	▲	▲	▲
2,2-Dimethoxybutane [30]				▲		▲	▲	▲	▲	▲	▲	▲	▲
Propane, 2,2-dimethoxy-	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
<b>Hydrocarbon</b>													
1,3,5,7-Cyclooctatetraene	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
á-Pinene	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Aromandendrene	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
n-Hexane			▲	▲					▲	▲	▲		▲
<b>Ketone</b>													
2,3-Butanedione	▲	▲		▲	▲	▲		▲			▲	▲	▲

(Continued)

**Table 1.** (Continued)

Volatile organic compounds (VOCs)	Hours postmortem												
	1	17	19	21	23	43	45	47	49	69	71	73	75
2,3-Pentanedione	▲	▲	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲
2,6,6-Trimethyl-2-cyclohexene-1,4-dione					▲		▲	▲	▲	▲	▲	▲	▲
2-Butanone [2, 3, 31, 32, 50]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
3-Octanone [18, 31]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Acetyl valeryl	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Hydrazinecarboxamide	▲	▲				▲				▲			
Methane, isocyanato-	▲		▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Methyl thiolacetate [24]						▲		▲	▲	▲	▲	▲	▲
Methyl vinyl ketone	▲	▲	▲		▲	▲	▲		▲				▲
N,N-Dimethylacetamide [10, 19]			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Propanamide, N,N-dimethyl-								▲	▲	▲	▲	▲	▲
<b>Nitrogen-containing</b>													
1,3,5-Triazine							▲	▲	▲	▲	▲	▲	▲
1,3-Diazine										▲	▲	▲	▲
5H-1-Pyridine	▲			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Acetonitrile, (dimethylamino)-						▲		▲	▲	▲	▲	▲	▲
Aminomethanesulfonic acid			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Benzonitrile [17, 18, 19, 26, 31]	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Cyanamide, dimethyl-								▲	▲	▲	▲	▲	▲
Diethyl azodicarboxylate							▲			▲	▲	▲	
Ethylenimine					▲	▲	▲	▲	▲	▲	▲	▲	▲
Formamide, N,N-dibutyl-	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲		▲
Formamide, N,N-dimethyl- [3, 18, 19]						▲	▲	▲	▲	▲	▲	▲	▲
Hydrazinecarboxamide	▲	▲				▲				▲			
Methanamine, N-(phenylmethylene)-									▲		▲	▲	▲
Methane, isocyanato-	▲		▲		▲	▲	▲	▲	▲	▲	▲	▲	▲
Methane, isothiocyanato-	▲	▲	▲			▲	▲	▲	▲	▲	▲	▲	▲
Methanethioamide, N,N-dimethyl-								▲	▲	▲	▲	▲	▲
Methenamine [10, 17, 39, 50]		▲			▲	▲	▲	▲	▲	▲	▲	▲	▲
Methyl dimethylcarbamate						▲		▲	▲	▲	▲	▲	▲
Methylamine, N,N-dimethyl-			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
N,N-Dimethylacetamide [10, 19]			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
Propanamide, N,N-dimethyl-								▲	▲	▲	▲	▲	▲
Pyrazine, 2,6-dimethyl- [18]								▲	▲	▲	▲	▲	▲
Pyrazine, methyl- [18]					▲	▲			▲	▲	▲	▲	▲
Thiocyanic acid, methyl ester	▲	▲	▲		▲	▲	▲	▲	▲	▲	▲	▲	▲

(Continued)

**Table 1.** (Continued)

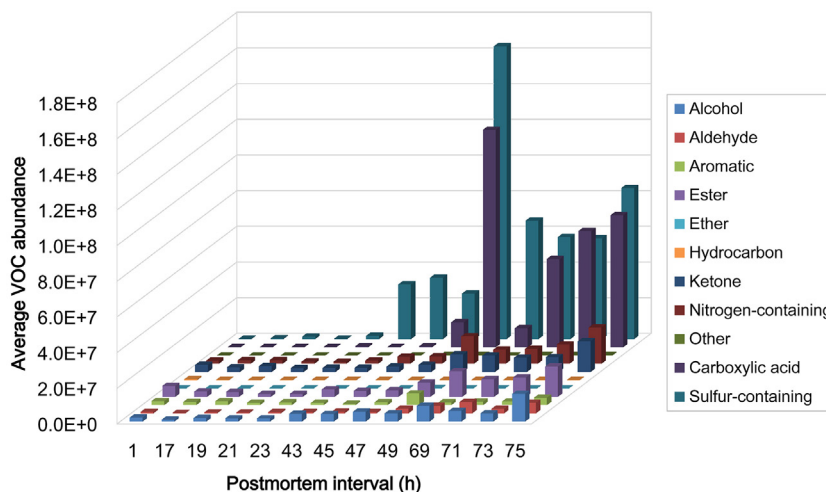
Volatile organic compounds (VOCs)	Hours postmortem												
	1	17	19	21	23	43	45	47	49	69	71	73	75
<b>Other</b>													
Aminomethanesulfonic acid			▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
tert-Butyl Hydroperoxide								▲	▲	▲	▲	▲	▲

detected in all postmortem samples. Specifically, dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) were detected at all sampling points. Esters were the third highest proportion of VOCs detected (17 of the total 105 postmortem VOCs), and were detected in all postmortem samples. Cis-3-hexenyl acetate, methyl acetate, methyl benzoate and ethyl acetate were detected at all sampling points. Ethers represented the lowest proportion of VOCs detected (3 of 105 postmortem VOCs). The ethers detected were 2-methyl-1,3-dioxolane, 2,2-dimethoxybutane and 2,2-dimethoxypropane. The detection of ethers in this study was likely due to the increased peak capacity, sensitivity and selectivity afforded by GC×GC-TOFMS as previous literature has only identified ethers when GC×GC-TOFMS has been used [18, 21, 22, 26, 30, 45].

### 3.4. Prominent classes and VOCs in the postmortem profile

The VOC profile of the early postmortem period is highly dynamic, with the average VOC abundance of different compound classes shifting considerably between sampling days (Fig. 1). The most abundant compound class detected immediately after death (i.e. 1 h postmortem) was the esters, primarily attributed to the compounds cis-3-hexenyl acetate, ethyl acetate and methyl acetate. On day 1 (17–23 h) the most abundant classes were esters (primarily attributed to cis-3-hexenyl acetate and methyl acetate) and ketones (primarily attributed to 2-butanone). On day 2 (43–49 h) the most abundant class was the sulfur-containing compounds (primarily DMDS, DMTS, sulfur dioxide and methyl sulfocyanate). On day 3 (69–75 h) the most abundant classes were sulfur-containing compounds (primarily DMDS and DMTS) and carboxylic acids (primarily propanoic acid and butanoic acid).

The experimental design of this study involved collecting multiple decomposition odour samples per experimental day. This led to the ability to observe trends in the average abundance of individual postmortem VOCs within experimental days. Sulfur-containing compounds have previously been reported as one of the most abundant classes of VOCs in decomposition odour, especially during the early

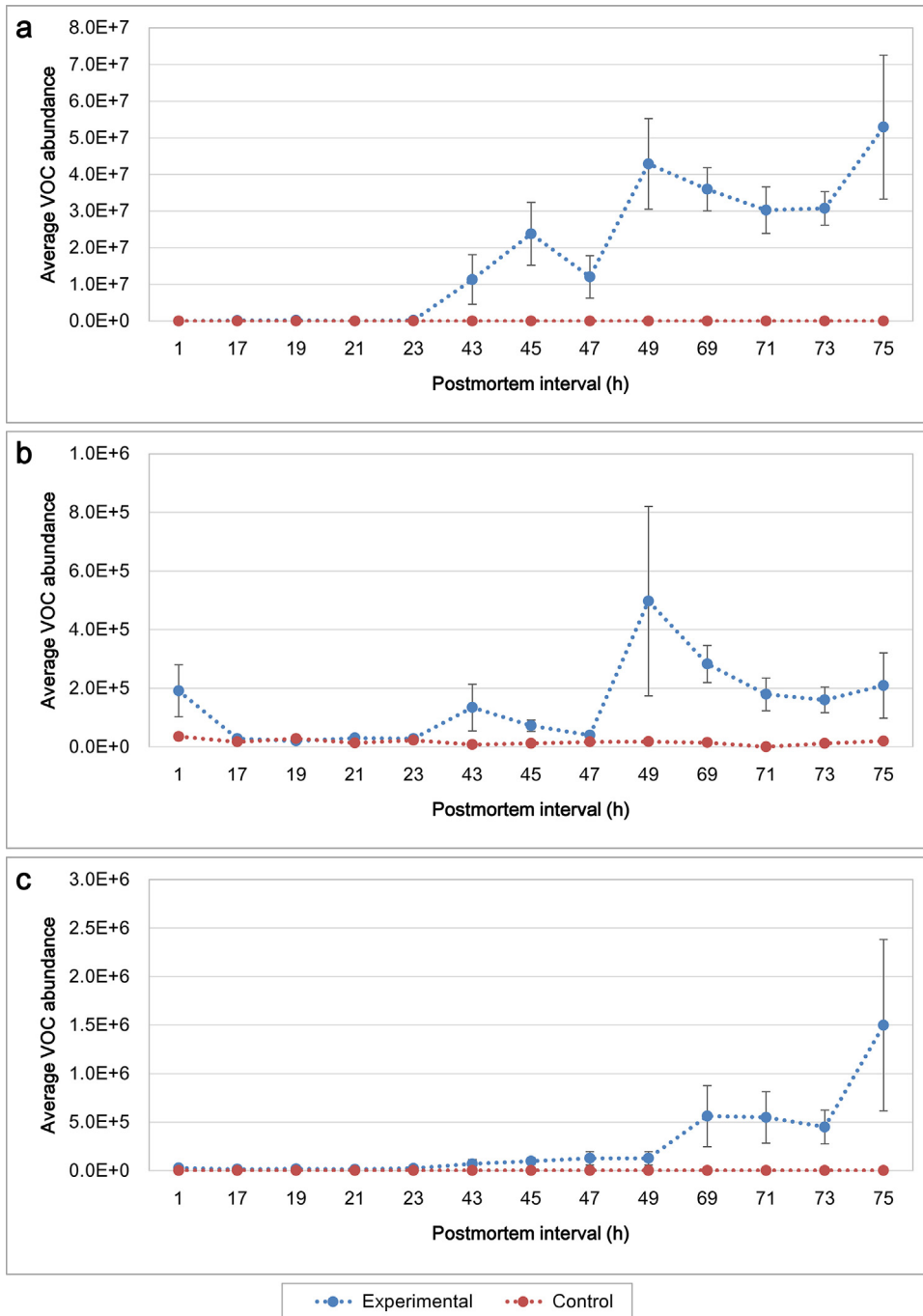


**Fig. 1.** Average VOC abundance of compound classes during the early postmortem period.

postmortem period [17]. The liberation of sulfur-containing VOCs is most likely due to the breakdown of proteins during decomposition [32] and can also be contributed from bacteria involved in the decomposition process [46]. Sulfur-containing compounds such as DMDS and DMTS have been reported as important signalling compounds for the entomological habitation of the carcasses [46, 47]. Intra-day measurements from this study indicated that the profile of DMDS is dynamic, steadily increasing in abundance between 23 h – 75 h postmortem with some fluctuations (Fig. 2a). Similar trends in abundance of DMDS during the early postmortem period have previously been observed by Statheropoulos et al. [3].

Benzonitrile, a nitrogen-containing compound, has previously been reported as a VOC present in decomposition odour during active decay and skeletonisation [17, 19, 29, 39]. The presence of benzonitrile during decomposition is likely due to the degradation of proteins and amino acids. Benzonitrile has previously been reported as a product of the degradation of the phenylalanine amino acid [46]. Intra-day measurements from this study indicated that benzonitrile exhibited a dynamic profile during the early postmortem period, peaking in abundance at 49 h postmortem before steadily declining (Fig. 2b).

The alcohol 2-methyl-1-propanol has also been reported as a VOC present in the headspace and soil surrounding decomposing human and pig remains [3, 19, 20, 32, 39]. Alcohols result from the degradation of carbohydrates in the body with some contribution from bacteria such as *Clostridia* [19, 32, 48]. Intra-day measurements from our study indicated that 2-methyl-1-propanol has a dynamic profile during the early postmortem period, steadily increasing in abundance from 23 h postmortem and reaching a maximum at 75 h postmortem (Fig. 2c).



**Fig. 2.** Average VOC abundance of a) dimethyl disulphide (DMDS) b) benzonitrile and c) 2-methyl-1-propanol during the early postmortem period (error bars represent standard error based on  $n = 3$  replicates).

### 3.5. Transition of the postmortem VOC profile

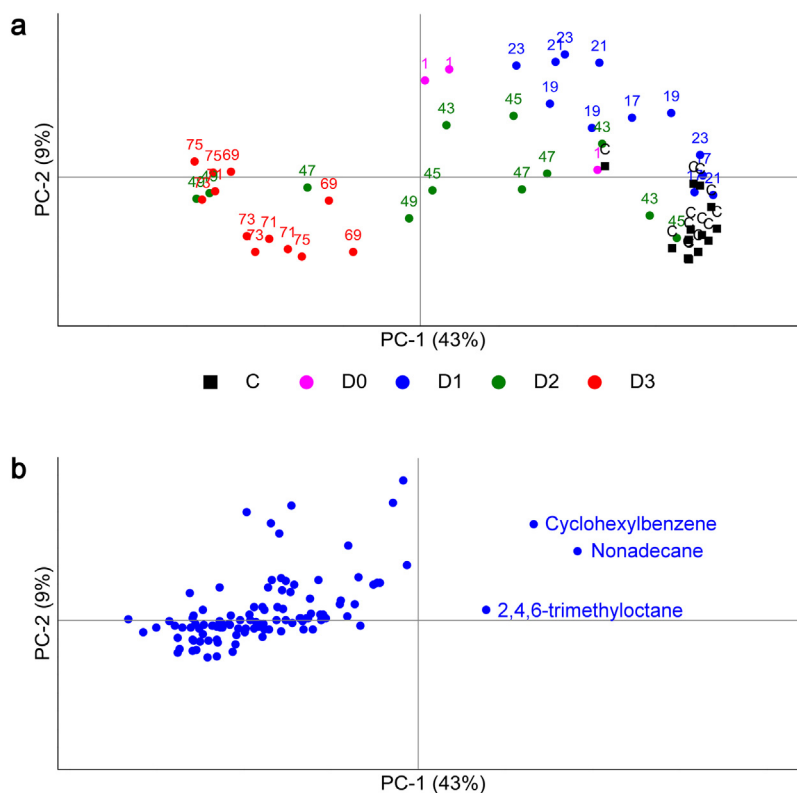
This study included the use of multiple replicate pig carcasses ( $n = 3$ ) that were sampled several times per experimental day. This allowed for the use of appropriate statistical methods leading to better characterisation of inter- and intra-carcass variation of postmortem VOCs ultimately resulting in more reliable data and trends. Pig carcasses are typically used in forensic studies where ethical restrictions preclude the use of human remains [18, 19]. Pigs are preferred as analogues over other animals because of their similarities to humans, including internal anatomy, lack of fur on the skin, gastrointestinal bacteria, muscle and tissue structure, and progression of decomposition [1]. The use of pig carcasses in decomposition odour research can be beneficial to allow for appropriate method development prior to proceeding with the use of human remains. In the context of this study, the use of replicate human remains would not have been realistic as multiple body donations on the same day were unlikely.

While bar graphs and compound lists are an effective way of presenting postmortem VOC data when performing initial screening [1], without further data transformation it is difficult to extract the most relevant information from the large data set produced by the GC×GC-TOFMS analysis. Hence, principal component analysis (PCA) was performed as an additional step in this study to extrapolate the most pertinent information from the postmortem VOC data.

Principal component analysis is a statistical technique that allows for the elucidation of relationships between variables by reducing the dimensionality of the data [42]. Given that decomposition odour is a complex profile, the relationship between individual variables may not be immediately obvious. Hence by reducing the data to principal components a greater depth of the relationships can be investigated.

The PCA scores plot (Fig. 3a) of decomposition odour in the early postmortem period revealed grouping of sampling hours. Intra-day variability resulted in the spread between samples collected on a single sampling day. The group that exhibited the least extent of variation was the control samples, which could be differentiated from the majority of postmortem samples. This was to be expected as control samples represented background environmental samples without the presence of a carcass and hence should be relatively comparable in their VOC profile. The samples collected 1 h postmortem did not form a distinct group. During this early stage of decomposition it is most likely that the postmortem VOCs closely resemble antemortem odour, which may be unique to each carcass. The samples collected between 17–23 h (Day 1) appeared to be differentiated from samples collected on all other days with the exception of two samples that overlapped with the control samples. The samples collected





**Fig. 3.** PCA plot of calculated a) scores (postmortem interval in h) and b) loadings during the early postmortem period (showing relevant compounds only).

between 43–49 h (Day 2) did not form distinct clusters but started to spread from the Day 1 VOC profiles towards the Day 3 VOC profiles. This suggests that 43–49 h postmortem is the transition period in decomposition whereby the VOC profile shifts from an antemortem odour to a postmortem odour. By Day 3, the VOC profiles were more closely grouped suggesting comparable postmortem VOC profiles that may be indicative of decomposition odour.

PCA loadings plots assist in identifying VOCs that contribute to the spread of points over the PCA scores plot. The PCA loadings plots (Fig. 3b) of decomposition odour during the early postmortem period demonstrated that nonadecane, cyclohexylbenzene and 2,4,6-trimethyloctane contributed significantly to the spread of scores of control samples on day 0 and day 1. However, on days 2 and 3 the loadings were closely grouped and the compound names were barely legible because many compounds were contributing to the spread of day 2 and 3 scores. This observation is important as it highlights the necessity of using GC×GC to analyse decomposition odour when it has transitioned from an antemortem odour to a more complex postmortem odour after the first 24 h. Using traditional one-dimensional GC would not provide sufficient chromatographic resolution of all the compounds identified at this stage of decomposition.

These findings are important when considering the use of scent-detection dogs during disaster victim search and recovery. It is clear that the VOC profile during the early postmortem period is dynamic and changes both hourly and daily. Notably, the period on day 2 whereby the VOC profile transitioned to a complex postmortem odour could have potential ramifications on the choice of scent-detection dogs deployed to a disaster scene. Given that the early postmortem odour appears to more closely resemble an antemortem odour until day 2, the use of human scent dogs may be more effective during the first 24 h following a mass disaster. While HRD dogs may be more effective as a search tool after 48 h when the VOC profile more closely resembles decomposition odour.

As this is the first study to use replicate carcasses and sampling points to profile the VOC profile during the early postmortem period, this study must be repeated before recommendations can be provided. The use of pig remains was ideal for optimising the method and will be utilised to carry out a replicate study during the same season to identify inter-year variation. The study will also be expanded to incorporate human remains to determine the similarities and/or differences between the postmortem VOCs produced by human and pig remains in the same microenvironment. It is recognised that the postmortem VOC profile identified in this study is specific to our geographical location and numerous environmental variables may impact the transition from antemortem to postmortem odour. For this reason, the authors encourage this study to be repeated in other climates in order to provide recommendations for canine handlers globally.

#### 4. Conclusions

The aim of this study was to measure the change in the VOC profile of pig carcasses as decomposition analogues for human remains during the early postmortem period by sampling regularly up to 75 h postmortem. A total of 105 postmortem VOCs were identified during the early postmortem period using GC×GC-TOFMS analysis. These compounds were from a range of classes including alcohols, aldehydes, aromatics, carboxylic acids, esters, ethers, hydrocarbons, ketones, nitrogen-containing compounds, sulfur-containing compounds or other. The abundance of the compound classes shifted hourly and daily during the early postmortem period. Individual postmortem VOCs also demonstrated a dynamic profile during this period. The use of multivariate analysis allowed for a clear transition in postmortem VOC profiles to be distinguished. The results of this study provide new information about the transition of VOCs soon after death and particularly during the timeframe when disaster response is critical. With repeat analyses, such information can inform

USAR teams on the optimal use of detector dogs to locate victims, both alive and deceased, following a mass disaster.

## Declarations

### Author contribution statement

Prue Armstrong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Katie D. Nizio, Katelynn A. Perrault: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shari L. Forbes: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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