The validation of human decomposition fluid as a cadaver-detection dog training aid

by

REBECCA C. BUIS

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student:

Date: 23 May, 2016

In Loving Memory of

Sharon G. Buis

Missing May 23, 2014

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Abstract

Cadaver-detection dogs are used by police services to locate human remains. Due to ethical restrictions, the dogs are not trained using human cadavers, but rather, using pseudo-scents or human tissues, such as blood and decomposition fluid. However, the chemical profile of these training aids has not been comprehensively investigated and their accuracy as substitutes for decomposed remains has not been determined. The aim of this study was to validate human decomposition fluid as a training aid for cadaver-detection dogs. The study examined the odour profile of decomposition fluid, including the changes in the profile over time (aged for one year) and under different storage conditions (room temperature, refrigerator and freezer) in order to determine the optimal conditions for its use as a training aid. The study also examined the dogs' sensitivity to decomposition fluid and compared their responses with the chemical odour profiles.

The odour profile of the decomposition fluid was collected using Solid-Phase Micro-Extraction (SPME) and analysed using Comprehensive Two Dimensional Gas Chromatography—Time of Flight Mass Spectrometry (GC×GC-TOFMS). The volatile organic compounds (VOCs) identified in decomposition fluid were compared to the VOCs reported in the literature for human cadaver decomposition odour. A wide range of characteristic decomposition VOCs were identified in the decomposition fluid. While individual VOCs were not comparable to human remains, the compound class proportions of the odour profiles were deemed similar. Variable odour profiles were observed under different storage conditions; room temperature and refrigeration were suitable, but freezing was not recommended for sample storage.

The decomposition fluid was also serially diluted to 1 part-per-trillion to determine the sensitivity of cadaver-detection dogs to this training aid. The samples were presented to three cadaver-detection dog teams under standard indoor training conditions over 14 training sessions. The dogs were capable of detecting the 1 part-per-trillion dilutions after several exposures to the fluid. The samples were subsequently analysed using SPME-GC×GC-TOFMS to determine the odour profile for all dilution levels. A range of VOCs were detected, although their abundances decreased in the lowest dilutions. The results of this study suggest that decomposition fluid closely mimics the odour profile of a decomposing cadaver and is a suitable training aid for cadaver-detection dogs when stored appropriately.

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Related Publications

Buis R, Rust L, Nizio K, Rai T, Stuart B, Forbes S. (2015). Investigating the sensitivity of cadaver-detection dogs to decomposition fluid. *Journal of Forensic Identification*, 65(6): 985-997.

Rust L, **Buis R**. (2015). The Scent of a Crime. *Australasian Science*, 36(7), 16-18.

Chapter 1:

Introduction

Chapter 1: Introduction

1.1 Cadaver-detection Dogs

Dogs are used in a variety of roles in police work, including general patrol, detection of drugs and explosives, search and rescue, and locating cadavers. When a person goes missing, police will often call out canine search teams to rapidly search large areas of land [1]. These canine teams consist of both search and rescue (SAR) dogs and cadaver-detection dogs. Most SAR dogs follow the scent trail of a live human, which is unique to the individual [2-6]. A cadaver-detection dog is trained for a different type of "scent work", in which they are taught to recognize and alert to the scent of decomposing remains, rather than that of living individuals [1, 7-9]. Cadaver-detection dogs are used in a number of scenarios, including helping to locate missing persons, suspected homicides where a body has been buried or otherwise hidden, and to locate bodies or body parts in mass disasters such as earthquakes, tsunamis and airplane crashes [10-12].

The first recorded use of cadaver-detection dogs was reported in the UK in the 1960s, during the search of Saddleworth Moor for the bodies of the children murdered by Ian Brady and Myra Hindley (*Moors Murderers*) [13]. Two bodies were discovered during this initial search, and when the case was re-opened in the 1980s, the new cadaver-detection dog search teams found an additional two victims. These dogs were SAR police dogs that had been cross-trained to also recognize the scent of decomposition [13, 14].

In 1974, the New York State Police obtained the first dog trained exclusively for cadaver detection. She was involved in the detection of several buried bodies from a multiple homicide in Oneida County [15]. By 1977, and as a result of these successes, cadaver dogs were widely accepted as a method for detecting clandestine graves. The Connecticut State Police instituted the first training program for cadaver dogs, initially with a focus on detecting surface-deposited bodies, but soon included the detection of buried remains [15].

Today, cadaver dogs remain a specialised service, and not all police departments can afford to train such dogs. However, large police departments often host several specialised dog and handler teams, which can be loaned to smaller departments as needed [15]. There is also a wide variety of

accredited volunteer search and rescue teams with SAR, cadaver-detection, and dual-trained dogs, such as the SE Alaska Dogs Organized for Ground Search in the United States (SEADOGS) [16], the Canadian Search Dog Association (CSDA) [17], the National Search and Rescue Dog Association (NSARDA) in the UK [18], and Search and Rescue Dogs Australia (SARDA) [19] who can be called upon to perform cadaver searches.

1.1.1 Detection of Decomposition Odour

Globally, there is no standardised method for detecting human remains. The cadaver dog is often deployed as part of a detection team, involving police officers, geophysicists, archaeologists, anthropologists, biologists, and entomologists. However, the dog is usually the first search method employed, as it is the least intrusive method of rapidly searching a scene [8, 20]. A search can be a long, difficult and time-consuming process, especially when a large area of difficult terrain must be covered. A well-trained cadaver-detection dog can cover the ground in a fraction of the time it would take a search team, while preserving the integrity of the scene [1, 8, 15]. The dogs are taught to detect decomposition by-products from various stages of decomposition, including gases and liquids, adipocere, and even mummified remains [21].

When the scent molecules from these by-products (i.e. Volatile Organic Compounds, or VOCs) are dispersed into the air, they form a heavily concentrated “scent pool” around the remains (Figure 1-1) [15, 21]. The concentration gradient reduces as the VOCs disperse throughout the air and the scent becomes fainter as it moves away from the body. This is known as a “scent cone” [15, 21]. Even buried bodies produce scent cones, although there is some delay in their release as the scent molecules must permeate through the soil column [22, 23].

Figure 1-1: Primary scent cone (adapted from Rebmann *et al*, 2000 [15])

Cadaver-detection dogs detect these scents by passing the inhaled air over specialised olfactory receptor cells in their nasal passages. Every scent sets off a unique pattern of receptor cells, firing messages to the dog's brain, allowing it to individualize many more scents than a human is capable of detecting. In this way, a handler can train a dog to recognize specific scents [15]. The dog is trained to follow the scent from the edge of the cone, working back and forth across an area, until it reaches the most concentrated area above or around the remains. When it reaches this point, it will "alert", often laying down (passive) or barking (active) to notify the handler of the find [15].

There are a number of variables that can interfere with a scent cone, creating difficulties for cadaver-detection dogs. Environmental factors include strong winds, rough terrain, vegetation, and the weather. Strong winds can disperse the VOCs beyond the dog's ability to detect the scent molecules, and intermittent or changing winds can create discontinuous and distorted scent cones, making it difficult for the dog to home in on the source. Heat and humidity can cause the scent cone to rise vertically, preventing detection by the dog unless it is very close to the source (Figure 1-2) [8, 15, 21].

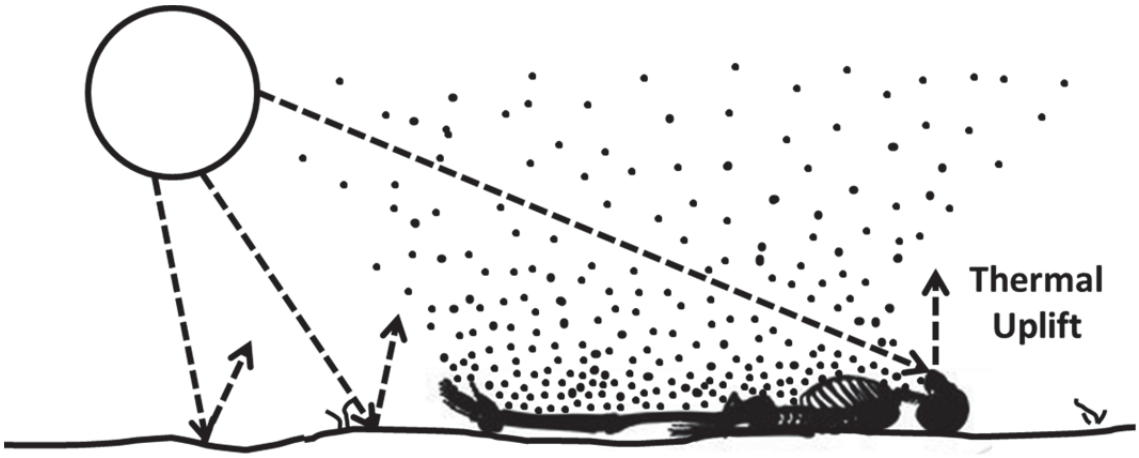

Figure 1-2: Vertical scent cone created by heat (adapted from Rebmann *et al*, 2000 [15])

Moist air, such as fog or high humidity, or precipitation can suppress the formation of a scent cone, with the potential for rain to wash the scent away from the source. In theory, a cadaver-detection dog could still find the remains by tracking the scent through the water, although this tends to refer to large or still bodies of water [8, 21]. Vegetation such as trees or bushes, rough terrain, buildings, and other barriers can disrupt the scent cone, as well. Scent molecules can be carried over such obstacles, and pool around them, creating an area with a falsely high concentration of VOCs [15, 21], as shown in Figure 1-3.

Figure 1-3: Secondary scent pool caused by a natural barrier (adapted from Rebmann *et al*, 2000 [15]), 1) the primary scent pool, 2) the secondary scent pool

A handler must be aware of these potential distortions in order to interpret the dog's signals accurately. Handlers should be aware of the way the dog tracks air-borne scents and must consider these variables when planning a search strategy. The dog can follow the scent cone, but

the handler must know how to analyse the environment and conditions in which the dog is working in order to maximize the efficiency and effectiveness of the search [15, 21].

Notably, a body does not necessarily need to be present in order for a cadaver dog to detect the presence of decomposition odour. If a body has been left in one place for even as little as 10 minutes, it leaves behind an odour, known as "residual scent" [24, 25]. Cadaver-detection dogs are able to detect transient graves, where a body was buried [25], then exhumed and relocated, as well as blood spatter and small amounts of body fluids [8, 26]. If a body is not present at the scene, the presence of trace evidence such as the blood spatter or putrefactive liquid can provide much-needed physical evidence for the link between the crime scene, victim, and perpetrator.

The significance that this evidence can have in a court of law places pressure on the search team, police officers and prosecutors to ensure that this evidence is accurate, and they must ensure that the methods by which this evidence was obtained are both legally and scientifically sound. However, concerns have been raised, most notably by American, Dutch and Polish courts, regarding the extent of the cadaver dogs' abilities and the time frames and environments in which they are truly effective for detecting remains and residual evidence [27-34].

1.1.2 Effectiveness and Limitations

Cadaver dogs have been a forensic tool for over 50 years, but there is little scientific research into their effectiveness and limitations. Unlike DNA analysis, cadaver dogs have not been scientifically validated and thus, their evidence in court is often viewed with caution [21, 26, 28]. The first scientific study regarding cadaver dogs was undertaken in 1999, nearly 40 years after the first use of cadaver dogs in forensic investigations. These studies focused on the physical abilities of the dogs under unusual or extreme conditions, such as in snow and extreme cold (-30^{o}C) or in water [1]. Further studies investigated the dogs' capabilities in the dark [15], in moorland environments with high levels of other decomposing matter [14] and also tested their ability to detect 20 year old burials in extreme heat (30^{o}C) [7]. Recent studies have also investigated the cadaver-detection dog's ability to distinguish between different animal species [14], small remnants of human remains such as teeth, grave soil, charred remains, and blood residue [25, 35-38], and residual decomposition scent on carpet fibres or gauze pads [9, 24].

Most of these studies have provided only preliminary results, and the true extent of the cadaver dog's abilities remains undetermined. It is currently estimated that a dog's sense of smell is a thousand times more sensitive than humans [39], but few studies have calibrated the degree of sensitivity. The compounds that a dog uses to discern the scent of decomposition are unknown, as are the concentrations necessary for a dog to detect these compounds. There have been numerous police investigations in which the validity of evidence obtained by the cadaver dog cannot be proven or refuted because of this lack of scientific data, the most notable of which have been the Madeline McCann case in 2007 [40] and the Casey Anthony case in 2011 [41]. The dogs rely heavily on their handlers for direction, and even with a well-trained dog, a poorly trained handler can cause many difficulties during an investigation [21]. Therefore, it is essential that the cadaver-detection dogs' abilities are scientifically validated to uphold their credibility as investigative tools in court.

1.1.3 Training and Training Aids

When training cadaver-detection dogs, it is essential to train the dogs using the materials that they will be tasked with locating during a search. The dogs may be required, over the course of their service, to locate a wide variety of odours from fresh bodies, bodies in various stages of decomposition, skeletal remains, as well as body parts, tissues, or residual scents in soil or on other surfaces [9]. Therefore, it is imperative to train the dogs on a wide variety of materials, including cadavers, body parts, tissues, blood, bones and teeth. They should also be exposed to remains from a wide variety of environmental conditions and locations, such as aged or charred bones, buried bodies, scattered remains, or sites where a body has been removed and only residual scents and grave soil remain [9, 15, 24, 35].

Due to legal and ethical restrictions, human cadavers cannot be used to train cadaver-detection dogs. Instead, many police departments use porcine remains, adipocere, grave soil, or partial human remains, such as bones, blood, and decomposition fluid [1, 7, 9, 15]. Pigs are commonly used as human analogues in many forensic studies [42-46], but recent studies have suggested that the VOCs produced by a porcine tissue are distinctly different from those of decomposed human remains [47-50]. DeGreef and Furton [47], Cablk *et al* [48], and Vass [49] originally discovered approximately 18 compounds reported to be unique to the odour profile of human remains, but

several additional studies identified most of these compounds in porcine remains using more advanced instrumentation [50-53]. Rosier *et al* [50] identified 8 compounds that could separate pig and human decomposition from other animals, and a further 5 esters which could be unique to human remains. However, this is not a clear distinction, since a number of other studies equally claim that it is not yet possible to distinguish between human and animal remains, either chemically or using detection dogs [14, 54], and further research needs to be conducted.

Since agencies cannot be certain of the similarity between human and porcine decomposition odour profiles, many choose not to train their dogs on porcine remains. Access to human cadavers for use as training aids can be highly restricted, and can be considered biohazardous, which limits the availability of items that can be used as cadaver-detection dog training aids [24]. Instead, agencies often use synthetic substitutes for the scent of decomposition. However, the creation of these pseudo-scents did not involve a careful chemical analysis of decomposition odour; instead, the developers compiled a number of compounds thought to be present in decomposition odour, such as putrescine and cadaverine [55, 56]. Later investigations of these pseudo-scents by Stadler *et al* in 2012 [57], and by Tipple *et al* in 2014 [58] indicated that the chemical composition of these training aids are oversimplifications of the complex and dynamic profile of decomposed remains. In fact, cadaver-detection dogs that had been trained only on human remains were not able to locate the synthetic scents [58]. They concluded that these synthetic training aids were not sufficient, and until an appropriate synthetic training aid can be developed, should only be used in combination with other natural training aids, if at all [57, 58].

Given the issues with using porcine remains and synthetic scents, dog handlers are also concerned with the validity of the human tissues currently being used. Once the training aid has been removed from the local environment (i.e. tissues removed from the body), the absence of many of the decomposition variables may alter the VOC profile produced by the sample, especially if it is stored in a refrigerator and re-used, as is common for most training aids. The subsequent degradation of tissues when stored as training aids may not represent true decomposition, and may produce different odour profiles than when they are part of a whole body system.

Human tissues such as decomposition fluid, which is the fluid produced by the breakdown of the body's internal tissues and organs, need to be fully validated as training aids for cadaver-detection dogs. Studies investigating the dogs' responses to various training aids found that the dogs are

typically able to detect decomposition fluid to a dilution level of 10^{-7}[59], and were capable of detecting samples from the mid-bloat and late active decay stages with more ease than samples from the rest of the decomposition period [59]. They could detect carpet squares contaminated with decomposition odour aged up to 65 days [24], and fresh gauze pads saturated with the odour of fresh human remains, cremated human remains, decomposition fluid, adipocere and blood with almost 90% accuracy [9]. Cadaver-detection dogs are reported to be able to locate human teeth within a 100 m^2 space with an 80% accuracy rate [35], detect 1 µL of blood diluted up to 4000 times [36], and gravesoil samples with 93% accuracy [25]. However, it has not yet been determined whether the odour profiles of these training aids accurately resemble the odour profile of decomposed human remains. An accurate determination of the sensitivity levels of cadaver-detection dogs to their various training aids has not been carried out to date.

In summary, there are three main problems in the use of training aids for cadaver-detection dogs: the use of porcine remains and pseudo-scents, neither of which may produce accurate representations of human decomposition odour; the lack of clarity regarding the accuracy of the odour profile produced by human tissues used as training aids; and a lack of knowledge regarding the sensitivity of cadaver-detection dogs to decomposition fluid. This study endeavours to define the cadaver dogs' abilities, applications and limitations in a scientific manner, and to offer insight into the functional methodology of the dogs. The project focuses specifically on the validation of human decomposition fluid as a training aid, and is undertaken with the aim of determining the sensitivity and limitations of cadaver-detection dogs and the optimal ageing and storage conditions for these training aids by police.

1.2 Cadaver Decomposition

Decomposition chemistry is the study of the natural biochemical processes that result in the degradation of a species' remains into its elemental components [60]. Post-mortem changes in the body are well-documented and the overall processes of decomposition have been well-researched. The decomposition process is often reported by its stages, namely fresh, bloat, active decay, advanced decay, dry and remains [42, 60-63].

During the fresh stage of decay (Figure 1-4a), after the heart has stopped, the lack of oxygen causes the cells to self-digest, a process referred to as autolysis [56, 64]. When blood circulation stops, *livor mortis* develops within the first few hours; blood pools in the lower portions of the body, and the rest of the body appears translucent and drained due to the lack of fluids [46]. The body also undergoes *algor mortis*, or body cooling, until it reaches the temperature of the surrounding environment [46, 57]. Within the first 12 hours after death, *rigor mortis*, or the stiffening of the muscles, also develops. Depending on the temperature of the surrounding environment, *rigor mortis* will disappear within 48 hours of death [46, 57]. Flies lay eggs on the body, which hatch into larvae that begin to consume the remains. The larvae drive the removal of soft tissue when vertebrate scavengers are absent [42, 60]. Since there is no oxygen circulating through the body, the intestinal and respiratory tracts become ideal environments for anaerobic microbes, which break down the carbohydrates, lipids and proteins in the body into organic acids, fatty acids, and gases. These gases expand the body, transitioning into the bloat stage of decomposition [60, 65].

During the bloat stage, the enzymes and bacteria present in the stomach and intestines of the body break down the surrounding tissues in a process known as putrefaction [64]. The by-products of this degradation process produce the odours, distension, and blue-green discolouration of the abdomen associated with the bloat stage (depicted in Fig 1-4b, below). The body becomes inflated and distorted by gases. The body's internal bacteria and other microbes break down tissue macromolecules into smaller components, which form putrefactive liquid during this stage of decomposition. This putrefactive liquid, also known as decomposition or purge fluid, is the fluid that forms as the body's internal structures liquefy and is purged from the mouth, nose, anus and other openings (such as wounds) that may be present on the body [46], by the gases that have formed as a by-product of the decomposition process. Eventually, the bloating and the consumption of the soft tissues by fly larvae cause the skin to rupture. This releases the gases, causing the body to collapse, and allows oxygen to return to the body cavity [66]. This oxygen-rich environment is ideal for aerobic microbes and fly larvae, which begin to consume a larger portion of the soft tissue on the cadaver [60, 61].

The active decay stage is characterized by a rapid loss of body mass, caused by the fly larvae and microbes digesting the soft tissues of the remains [62, 67]. Figure 1-4c highlights the early period of active decay. As this stage progresses, there is a substantial release of cadaveric materials and

fluids into the soil via the purging decomposition fluid, and this overload of nutrients, minerals, soil carbons and increased pH can be toxic to the vegetation surrounding the remains. The area of immediate nutrient influx and dead vegetation is referred to as a Cadaver Decomposition Island (CDI). The cadaver itself often shows signs of desiccation and colour changes during the active decay stage. The extremities of the body, such as the limbs, nose, ears and lips tend to dry out, demonstrating a brown, leathery appearance [63]. The head and abdomen may change from blue-green to a black discolouration. This black discolouration is due to the iron content in degraded blood haemoglobin combining with the hydrogen sulphide produced by bacteria to form iron sulphide [46]. Active decay continues until the larval masses have migrated away from the cadaver to pupate [60].

This migration often coincides with the commencement of the advanced decay stage, where the majority of the cadaveric material has been consumed by insects and scavengers, and their activity around the cadaver is dramatically decreased (Figure 1-4d). There is little change in the cadaver mass loss, and minimal decomposition appears to be occurring. The majority of the soft tissues of the cadaver have been consumed, although the CDI around the body is still distinct, and the soil shows an increase in carbon, nitrogen and other nutrient content during the advanced decay stage [66, 68, 69].

It is often difficult to identify the transition from the advanced decay stage to the dry and skeletal remains stages [42]. The dry stage is usually indicated by mummification of any remaining soft tissues and skin. Only bones, skin, hair or fur, and other keratinized body parts, like nails, are left to bleach in the sun, and slowly degrade [60, 70] (Figure 1-4e). There is also increased plant growth at the edges of the CDI, and the remains stage is usually indicated by the growth of plants inside the CDI [60, 71]. Entomological activity is greatly decreased during this stage, as the majority of nutrients and moisture have already been depleted by insects, scavengers, soil microbes and plant growth during the previous stages of decomposition [60].

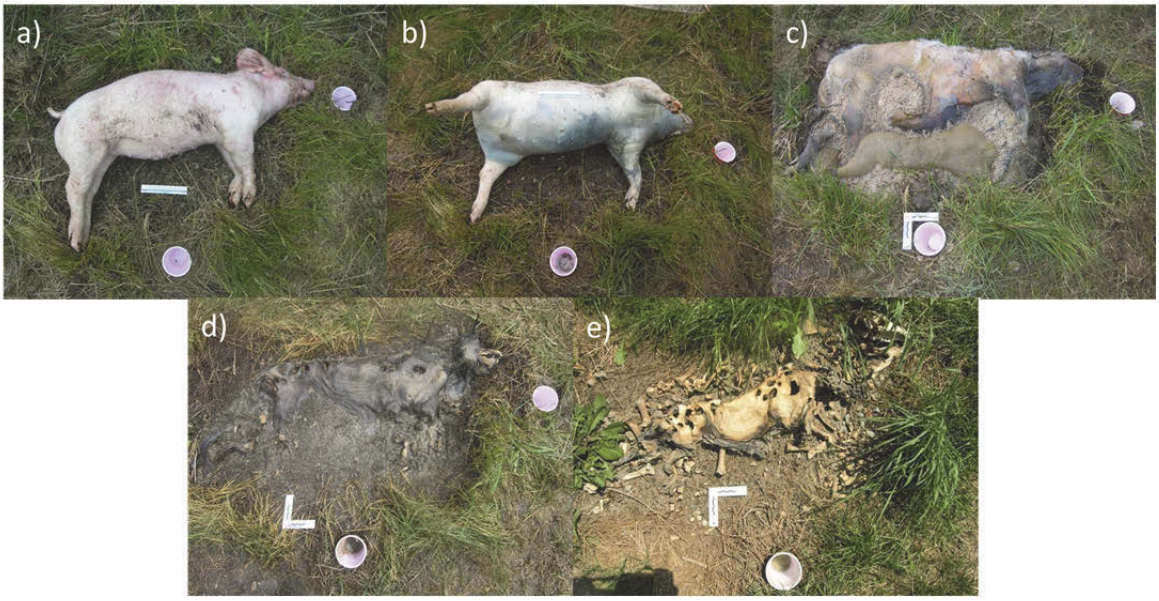

Figure 1-4: Stages of decomposition, as demonstrated by porcine remains: a) fresh, b) bloat, c) active decay, d) advanced decay, e) dry/remains (Photographs by Katelynn Perrault)

While decomposition typically follows the same general stages, the time it takes for the body to undergo these changes is highly dependent on environmental conditions, such as temperature, soil conditions (soil type, moisture, or pH), rainfall, insect and scavenger access, and humidity. Variables of the body itself, such as any trauma that has been inflicted, the size and weight of the body, and the presence and type of clothing may affect decomposition. Whether the body was buried, the depth of the burial, whether it was deposited on the surface, and the type of surface are variables that must also be considered. The body position and location, whether the body is indoors, outdoors, or in a vehicle, any potential exposure to chemicals, and many other variables must all be taken into consideration when trying to predict the rate at which decomposition has occurred. All of these factors affect the rates of decomposition, and are specific to the area of deposition, known as a microenvironment. Small changes in soil type, pH or water content, the type of vegetation present, the amount of shade in the area, or the type of insects or scavengers present, will also affect the decomposition process [60, 72-74].

Many studies have investigated decomposition in different environments in order to understand how these variables affect the rate of decomposition [23, 60, 66, 71, 74-79]. There are several decomposition parameters that researchers can control, such as the soil type, restricting insect

and scavenger access, or ensuring the body decomposes in specific soil conditions, but there are many variables which cannot be controlled.

The purpose of the current study is to chemically profile decomposition fluid in the absence of these parameters, since this is the form used to train cadaver-detection dogs. It is, however, recognized that the dogs typically search in environments where these variables impact decomposition, and they must therefore be considered when conducting a search. This chapter will examine the chemical processes which produce decomposition fluid and the accompanying gases used by the cadaver-detection dogs to locate remains. The variables impacting decomposition will not be discussed here, as they have been extensively reported elsewhere [23, 60, 66, 71, 73-80].

1.3 The Chemistry of Decomposition

As decomposition progresses, the body's organs and tissue macromolecules are broken down into smaller components, forming decomposition fluid and gaseous by-products [56]. The human body is comprised of approximately 64% water, 20% proteins, 10% fat, 5% minerals, and 1% carbohydrates [23], and it is the by-products of the degradation of these carbohydrates, proteins, and lipids that form the majority of the gases that will be further examined in this study. This is a new area of study, however, and not yet well characterized, with the earliest published studies regarding the chemistry of the degradation pathways and the formation of gaseous by-products appearing around the turn of the millennium [56, 65, 74, 81].

1.3.1 Protein Degradation

During decomposition, cells release enzymes called proteases, which degrade proteins into proteoses, peptones, polypeptides and amino acids. These, in turn, are degraded by microbes into nitrogen-containing compounds (such as ammonia), alcohols (diols and thiols), organic acids, and sulphides (Figure 1-5) [82]. Sulphides are the most common by-products of amino acid degradation; dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) are the most consistently reported decomposition VOCs [22, 47-49, 78, 81, 83-88]. Degraded proteins are also

believed to be responsible for the production of hydrogen sulphide, methane and ammonia [82], which are thought to contribute to the scent that humans associate with decomposition [8].

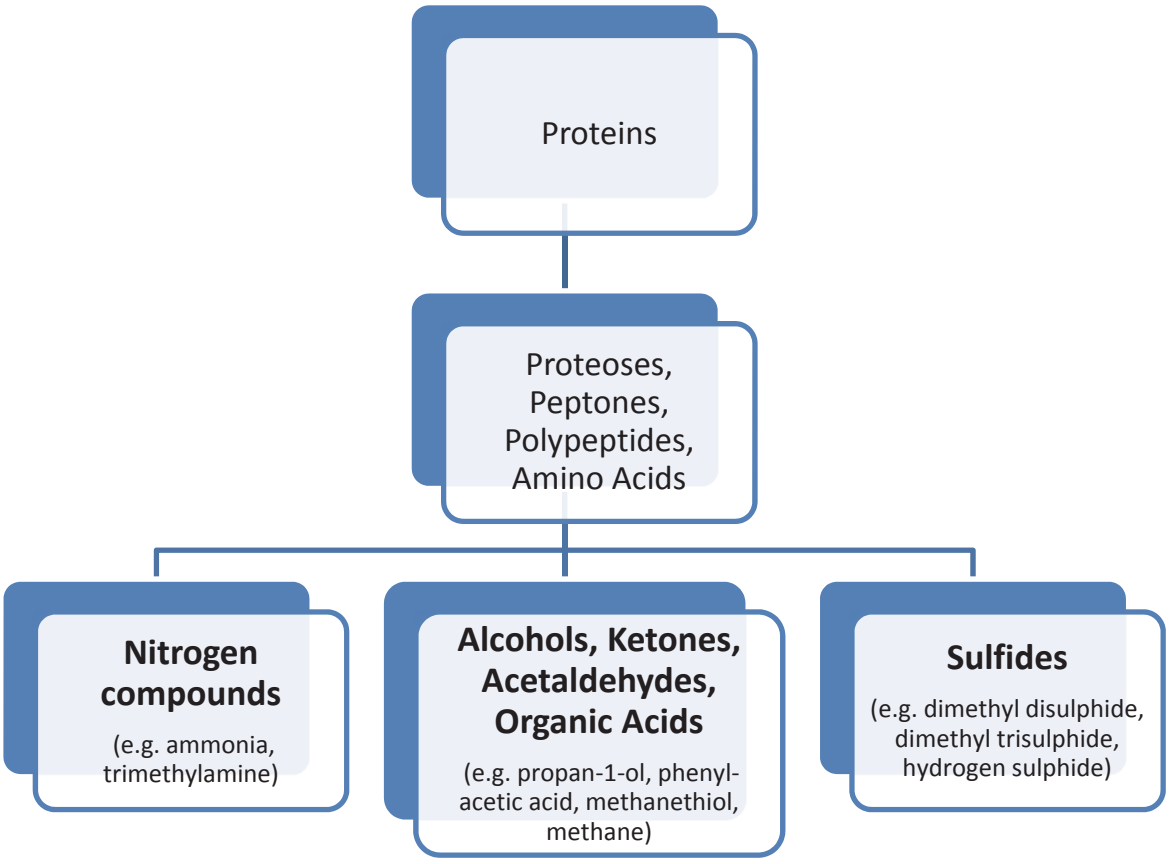

Figure 1-5: Protein degradation pathways during the decomposition of soft tissue (adapted from Dent *et al*, 2004 [74])

Proteins also break down into putrescine and cadaverine, which are the compounds believed to be responsible for the strong, distinct odour of decomposition [56], however, no studies investigating the volatiles produced by decomposition have detected either of these compounds in the headspace of decomposed remains [22, 24, 56, 78, 81, 83, 85, 89-91]. Tryptophan, an amino acid that often functions as a neurotransmitter, decomposes into indole [92], a 6-membered benzene ring attached to a 5-membered, nitrogen-containing pyrrole ring. It is found throughout the decomposition process, although the levels of indole tend to increase over time, and are highest during advanced decomposition [93]. In addition to being found in decomposition fluid and gases, indole is often found as a component of faecal matter, flower scents, and even perfumes [83, 94].

The decomposition of proteins is highly dependent on variables such as soil microbiology, moisture, and temperatures. It is not a uniform process throughout the body, as the first organs to decompose are usually the intestines and pancreas, due to high levels of gut flora. This is followed by proteins in the brain, liver and kidneys, and finally, the proteins throughout the rest of the body [46, 56]. This may affect the succession of gases in the decomposition odour profile, as the different organs and tissues degrade and produce these odours at differing times.

1.3.2 Lipid Degradation

Lipids degrade into alcohols, ketones and carboxylic acids, although these processes can be complex. Lipids are predominantly comprised of triglycerides, which degrade into glycerol and fatty acids, such as the unsaturated fatty acids oleic and linoleic acid. These undergo hydrogenation into saturated fatty acids, including stearic and palmitic acid, which form a large proportion of decomposition fluid, although they have low volatility. It is the oxidation of these acids which can form the volatile aldehydes, ketones and alcohols that are associated with decomposition odour (Figure 1-6) [74, 82, 86].

In an aerobic environment, unsaturated fatty acids leach from the body first, while the saturated fats accumulate in the skin and fatty tissues, turning a dark yellow colour [46]. The unsaturated fatty acids break down into aldehydes and ketones, and with sufficient air and moisture, eventually the saturated fatty tissue will yield aldehydes and ketones [74].

An anaerobic environment will leach some unsaturated fatty acids, but in general, the fatty acids will be hydrogenated into myristic, palmitic and stearic acids, and eventually, adipocere will form in the body [46, 74, 82]. Adipocere is a waxy, soap-like substance that forms in the fatty tissues of the body, and can preserve internal organs [46, 82]. Adipocere can also degrade to produce volatiles such as butanoic acid, pentanoic acid, and hexanoic acid-ethyl ester [83].

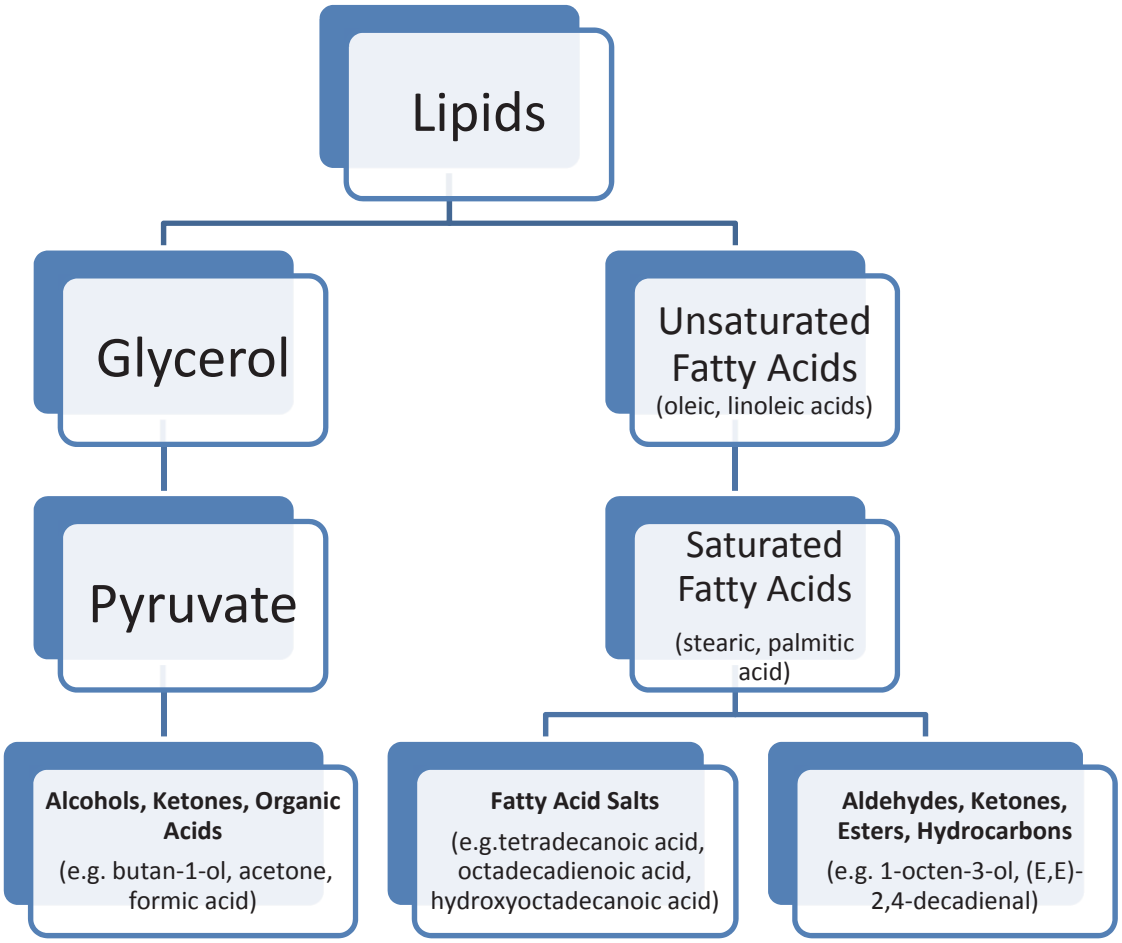

Figure 1-6: Lipid degradation pathways during the decomposition of soft tissue (adapted from Dent *et al*, 2004 [74])

The exact fatty acid content of a body varies from person to person, but the main components tend to be $C_{18:1}$ (oleic acid), $C_{18:2}$ (linoleic acid), $C_{16:1}$ (palmitoleic acid), and $C_{16:0}$ (palmitic acid) [46, 95]. Cholesterol, a steroid lipid found in mammalian cells, and squalene, an unsaturated, branched hydrocarbon present in the integumentary system (i.e. the hair, nails, and skin [96]), are also found in putrefactive liquid [97]. They are released from the cell membranes as the cell degrades during autolysis. Squalene, in particular, rapidly degrades to aldehydes and ketones [98]. However, the exact composition of the odour profile produced by the decomposition of lipids is highly dependent on the source materials and the burial or surface deposition conditions of the body [83].

1.3.3 Carbohydrate Degradation

The oxidation of carbohydrates forms alcohols, ketones and carboxylic acids and is also dependent on the burial or surface deposition characteristics surrounding the body. Under aerobic conditions, most carbohydrates (sugars) are broken down into glucose monomers, which oxidize completely into carbon dioxide, water, methane, hydrogen and hydrogen sulphide (Figure 1-7), while a few oxidize incompletely and form organic acids and alcohols [46, 74, 82].

Under partial or full anaerobic conditions, both bacteria and fungi decompose the carbohydrates. Since fungi and bacteria have different mechanisms for the decomposition process, the end products will depend on the availability of free oxygen in the environment. The presence of some oxygen (a partially aerobic environment, such as a shallow burial) will allow the fungi to degrade the sugars into organic acids such as glucuronic acid, citric acid, or oxalic acid, while the bacteria will degrade the sugars into acetaldehyde, carbon dioxide, water, etc, in an aerobic environment [56]. The complete absence of oxygen (such as in a deep burial) will cause the bacteria to ferment, leading to the formation of methane, hydrogen sulphide, various alcohols (e.g. ethanol or propanol), and organic acids such as lactic acid, and butanoic acid [46, 56, 74, 82].

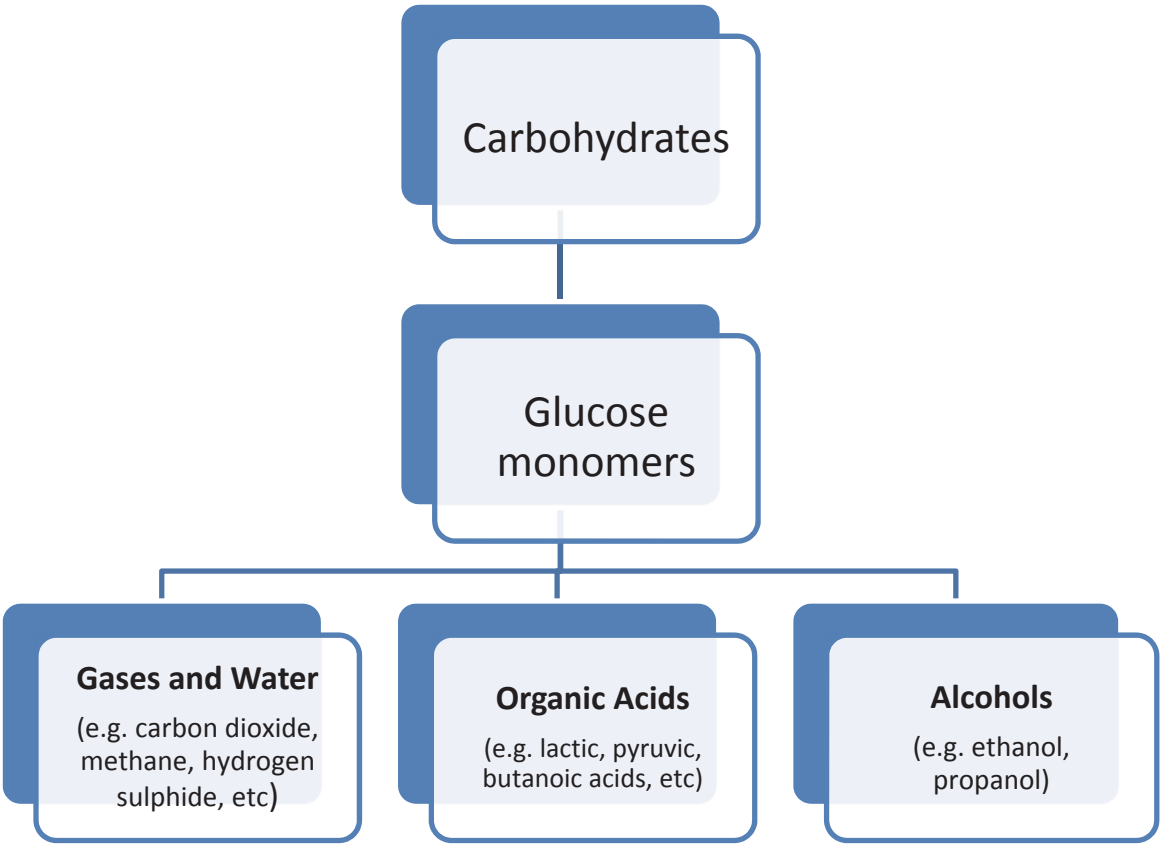

Figure 1-7: Carbohydrate degradation pathways during the decomposition of soft tissue (adapted from Dent *et al*, 2004 [74])

While the decomposition pathways for these macromolecules are generally known, the understanding of human decomposition processes remains highly reliant on the understanding of the surrounding environment. One cannot assume that an aerobic or anaerobic, acidic or alkaline environment will always produce the same compounds. The initial composition of the remains and the numerous variables at the location (soil type, humidity, water access, temperature, insect and scavenger access, etc) will all play a role in the development of the decompositional by-products that can be found at any burial or deposition site [46, 56].

1.3.4 Volatile Organic Compounds (VOCs) Produced by Decomposition

The breakdown of protein, lipid and carbohydrate macromolecules during the decomposition process releases gaseous by-products known as volatile organic compounds (VOCs). VOCs are defined as chemical compounds with low boiling points and high vapour pressures, which prefer to remain in a gaseous state. It is these volatile components that form part of the characteristic odour that can be associated with a decomposing cadaver.

The exact composition of decomposition odour, including the succession and rate of formation (known as an odour profile) has been under examination since the turn of the millennium [22, 49, 51, 52, 78, 82-88, 90, 99, 100, 101]. Decomposition odour has been studied to determine the post-mortem interval of decomposed remains based on the composition of VOCs at the time of discovery, and to scientifically determine the VOCs that cadaver-detection dogs use to locate human remains [22, 78, 81, 83-88]. The exact number of compounds within the odour profile of decomposition varies widely within the literature, as some studies have identified as few as 30-35 VOCs released during the decomposition of the body [83, 85], while other studies have identified over 470 VOCs produced throughout the decomposition process [22]. The majority of these 470 compounds are believed to be environmental, deriving from sources such as soil microbes and decomposing vegetation [99, 100, 52, 54, 101].

The trends and patterns with which all of these compounds are produced also vary within the literature, but there are several classes of compounds that have been generally identified in decomposition odour, including sulphides (which are the most consistently reported compound class), aldehydes, ketones, alcohols, hydrocarbons, and occasionally, chlorinated and fluorinated compounds [22, 47, 48, 81, 84-87].

The degradation of proteins, carbohydrates and lipids provide the main sources for these compounds, as described in Sections 1.3.1, 1.3.2, and 1.3.3, but the specific compound and amount of each compound may vary depending on the source material. Aromatics, aldehydes, ketones and alcohols are produced by several of the degradation pathways [15, 22, 48, 49, 78, 81, 83, 85-88, 102, 103]. Sulphides are generally formed during the enzymatic breakdown of sulphur-containing amino acids in skin, creating thiols and polysulphides such as dimethyl sulphide,

dimethyl disulphide, and dimethyl trisulphide [47, 48, 78, 83, 84, 86-88]. Hydrocarbons are produced mainly by lipid degradation [78]. Nitrogenous compounds such as amines are formed through the breakdown of proteins, but are not sufficiently volatile for traditional gas analysis methods to detect [84]. Halogenated compounds are not natural components of the body, but may be detected in the body due to their presence in drinking water [81, 82].

Understanding the degradation pathways and the mechanisms by which the decomposition odour profile is produced improves the knowledge base regarding the odour-active compounds within the VOC profile and expands the understanding of a variety of areas under forensic study. These compounds act as attractants to many carrion insects that colonize remains [70, 78, 104-106]. Understanding the patterns, successions and formation rates of the VOCs would improve knowledge surrounding the insect succession patterns which entomologists use to estimate long post-mortem intervals (PMIs). A fully characterized decomposition odour profile could also be used to develop instrumentation that would detect biomarkers of decomposition [50, 90, 107], and could be used to help locate human remains in a search situation. Finally, cadaver-detection dogs, which are the focus of this study, also use this odour profile to search for and locate human remains [15].

1.4 Collection and Analysis of Decomposition VOCs

Decomposition odour has been studied extensively in the environmental and food sciences areas, to examine issues such as levels of air pollutants, the scent of landfills, waste water treatment and to monitor food and water quality [108-114]. The application of this chemistry to forensic science is a recent addition, and was initially applied to entomology [115-118], and more recently applied to the area of canines and cadaver detection [22, 78, 81, 83, 88].

There are a wide variety of methods available for collecting and analysing decomposition VOCs, and the collection technique used may affect the number and types of VOCs collected and detected. Most methods require sample preparation, such as solvent desorption of activated charcoal strips [103] and glass and Teflon cartridges [86]. Sorbent tubes and solid-phase micro-extraction (SPME) require minimal sample preparation, and are easy to use in a field setting; as a result, they are the most commonly reported sample collection techniques for decomposition

VOCs [*Sorbent tubes*: 22, 51, 53, 78, 80, 81, 84,-88, 101, 102, 119, 120. *SPME*: 48, 52, 58, 83, 99, 115, 121-124].

1.4.1 Sorbent Tubes

A sorbent tube is a stainless steel, hollow tube that is packed with one or more sorbents capable of trapping a range of volatile compounds. The type of sorbent must be chosen based on the target compounds of interest. This can prove problematic for non-target analysis where the chemical properties of the compounds can vary considerably and are not always known prior to collection. There is currently no standard method for decomposition VOC analysis, and as a result, sorbents that are able to collect the widest range of expected compounds, such as Tenax TA / Carbograph 5TD (C_3-C_{30}, Markes International, Ltd), are used for decomposition odour profiling [22, 51-53, 78, 81, 85, 86, 102, 120, 125-127].

The advantage to sorbent tubes is the minimal equipment required for sampling, which is ideal for fieldwork situations, where there may not be electricity or room for elaborate equipment [52]. When air is allowed to pass through the tube, either passively or drawn through by a pump, the VOCs adsorb onto the sorbent. The VOCs in the air are concentrated and trapped in the tube, which can be sealed and transported to the laboratory for instrumental analysis.

In the laboratory, the samples are introduced onto the gas chromatograph- mass spectrometer (GC-MS) using thermal desorption (TD). A specialised unit is required for sorbent tubes, called a thermal desorber, or TD unit (Figure 1-8). Tubes are heated inside an oven under a flow of inert carrier gas. The high temperatures of the ovens (300^{o}C) causes the sorbent tubes to release, or desorb VOCs , which are carried off the sorbent by the carrier gas, and focused on an electrically-cooled cold trap (around 15 °C). The trap focuses the compounds for injection. The compounds are desorbed again from the cold trap, and the sample is injected onto the column of the GC-MS [128].

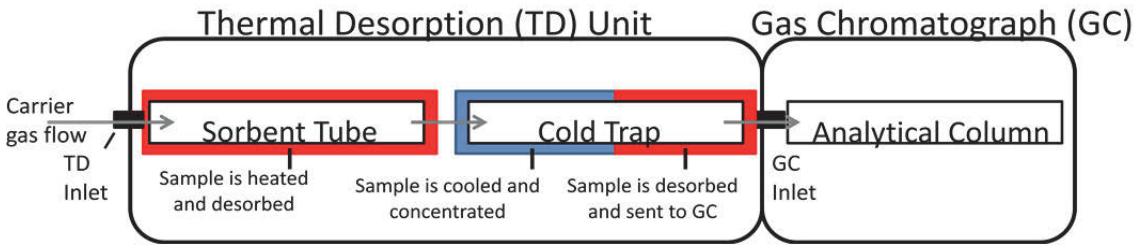

Figure 1-8: A schematic of the Thermal Desorption (TD) Unit for desorbing and concentrating sorbent tube samples

The use of TD sorbent tubes offers several advantages, including minimal sample preparation- which reduces the time required for sample collection and analysis, and *in situ* analysis of VOCs- minimizing the risk of sample loss from transport and storage [128]. Although it is possible to re-collect desorbed samples on some instruments, for most researchers, the sample is only available for a single analysis, and cannot be re-analysed if lost [80].

The chromatograms produced using TD sorbent tubes typically show fewer artefacts than other VOC sample collection methods. As there is no solvent required to desorb the samples, there is also no solvent peak, and minimal column bleed with the potential to overwhelm the chromatogram and mask other compound peaks [128]. This is advantageous for the analysis of complex mixtures like decomposition odour [52]. It is also believed that since sorbent tubes can hold a variety of sorbents, this method is able to collect a wider and more discriminatory VOC profile [53, 126]. With this active method of sampling, however, there is the potential for the lighter volatiles to be drawn through the tubes too rapidly to adhere to the sorbent, and as a result, the method may be biased towards heavier VOCs. Also, TD units can be prohibitively expensive, and unless a laboratory conducts frequent VOC analysis, they are unlikely to have a TD unit; as a result, many scientists prefer to use solid-phase micro-extraction for the analysis of decomposition odour [48, 83, 115, 122].

1.4.2 Solid-Phase Micro-Extraction (SPME)

Solid-phase micro-extraction (SPME) is a passive method of volatile collection. A fibre coated with a sorbent is exposed to the sample. As with sorbent tubes, the type of sorbent must be chosen based on the types of compounds that are to be collected. The length of time that the fibre is

exposed to the sample is also important, since SPME fibres work on the principle of competitive sorption. This means that the heavier volatiles may displace the lighter volatiles from the fibre as exposure time increases [128]. There is minimal contamination or loss of sample and when analysed, the peak shapes in the chromatograms are superior to those produced using a solvent desorption sample preparation step, since there is often little difference between the volatility of the solvent and the target VOCs [128]. SPME fibres are very fragile and were originally developed as a method for rapid analysis of samples within the laboratory. They can be a challenge to use in the field, since this fragility is exacerbated by non-ideal conditions such as wind, rain or extreme heat [125]. The samples, like sorbent tubes, can only be analysed once, due to the thermal desorption step required to remove the compounds from the sorbent. SPME does, however, provide high quality results, and is both quick and easy to use, which can be important when there are a large number of samples to analyse.

Thermal desorption is also used to analyse SPME fibres, however, the sample is injected directly into the inlet of the GC-MS rather than using a TD unit. There is no need for specialised equipment to desorb and focus the compounds, since they are already pre-concentrated on the fibre [128]. The inlet is heated to around 200^{o}C in order to desorb the sample, and the carrier gas in the inlet then carries the sample directly to the column of the GC-MS. An auto-sampler can be purchased to automate the process, which is ideal for a large number of samples, but it is not required for the analysis of VOCs collected using SPME fibres.

Decomposition studies that use SPME [48, 52, 83, 122, 124] typically identify different compounds than studies using sorbent tubes [22, 50, 52, 53, 81, 84, 85, 87, 88, 125, 126], and this is likely due to the different sorbent types and methods by which the VOCs are trapped. It is currently believed that SPME traps lighter VOCs, has greater compound specificity, and is better in lab-based applications, while sorbent tubes trap a wider range of heavier compounds, and are a more robust collection method, ideal for use in the field. A direct comparison of the two methods by Perrault *et al* [52] found that the odour profiles collected from grave soil using sorbent tubes and SPME were complementary. Each method collected a unique subset of compounds in the decomposition odour, and the use of both methods in tandem broadened the range of analytes identified. Overall, however, Perrault *et al* [52] found that sorbent tubes collected more decomposition-unique compounds when compared to the control samples than SPME.

Since decompositional studies often compare their results to those provided in the literature, comparisons are made between studies using different sampling techniques. This sort of comparison will demonstrate variations in results, which may be attributed to decomposition variables, but may in fact be due to these different collection techniques. Therefore, researchers must be aware of the bias that may be introduced to their results by their choice of sample collection technique.

1.4.3 Gas Chromatography — Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is currently the most common method used for decomposition VOC analysis, although as yet, there is no standardisation with respect to the column, temperature or other parameters used [22, 47-49, 78, 81, 83, 84, 86, 87, 99, 103, 121, 122, 127, 128]. Gas chromatography is based on the principles of the relative polarity and boiling points of compounds. Inside the GC-MS oven (Figure 1-9) is a capillary column, between 10 m and 60 m in length, coated with a polymer stationary phase. The length of the column and the type of stationary phase used is chosen based on the type of compounds that are to be analysed. The column is attached to a temperature-controlled inlet where samples are injected and desorbed from the SPME fibres (Fig 1-9). The column is mounted inside an oven, where the temperature is controlled by the computer software. When samples are injected into the inlet, they are swept onto the column and along the stationary phase by an inert carrier gas (usually helium, hydrogen, or nitrogen). The chemical properties of the compounds cause different interactions with the stationary phase. Usually, depending on the type of stationary phase, larger and less volatile compounds will be retained by the stationary phase for a longer time and at lower temperatures, while the lighter and more volatile compounds will be released from the stationary phase and carried through the column to the detector as the temperature inside the oven increases [130]. Since the analytes elute from the column at different times (relative retention time) based on the chemistry of the stationary phase and the temperature of the oven, the analysis is highly dependent on determining optimal GC parameters which will give an ideal separation of analytes [130].

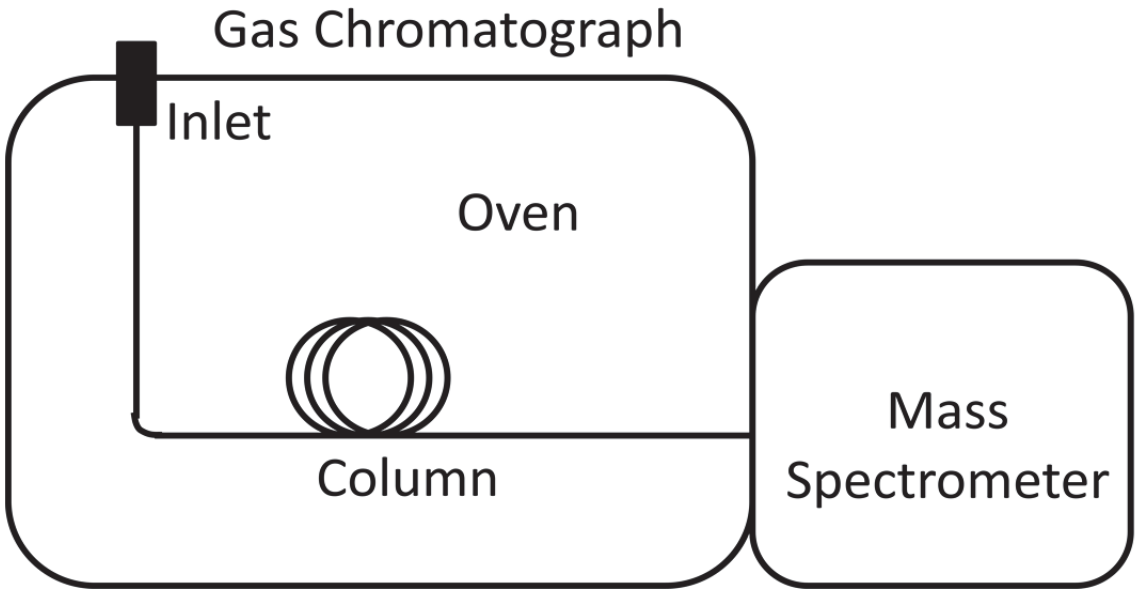

Figure 1-9: A schematic of a GC-MS instrument (adapted from Perrault, 2015 [80])

There are several types of detectors that can be coupled with a GC, such as a flame ionisation detector (FID) or an electron capture detector (ECD), but a mass spectrometer is the most common detector for non-targeted analysis [80]. Compounds are bombarded with a stream of electrons, causing fragmentation into positively charged ions. The ions are transferred to a detector, which counts the number of ions of a certain mass (known as relative abundance). This data is compiled into a two-dimensional chromatogram - a series of peaks showing relative retention times versus relative ion abundance. The identity and relative amount of a compound present in the sample can be determined from the chromatogram and mass spectrum [130]. The chromatogram can be manipulated to display the mass spectra scans of various peaks, and peak identifications can be made based on matches to existing mass spectral libraries, such as those available from the National Institute of Standards and Technology (NIST), or by confirming their retention times using standards.

Problems are encountered in GC-MS when compounds have similar retention times; the compounds can co-elute and demonstrate similar properties in the mass detector, which leads to compound misidentification or the masking of one compound by another [87, 130]. GC-MS also has a limited peak capacity and sensitivity when a large number of compounds are present [87], making it difficult to separate compounds with a polarity different to that of the stationary phase used (restricted specificity). For example, the GC-MS can separate either short or long chain acids,

both of which are present in decomposition odour, but not in a single column, due to their opposing polarities [86]. These problems become increasingly challenging as sample complexity increases, as is the case with decomposition odour.

1.5 Comprehensive Two-Dimensional Gas Chromatography — Time-of-Flight Mass Spectrometry (GC×GC-TOFMS)

In order to account for sample complexity, researchers in the field of decomposition chemistry have begun to utilise comprehensive two-dimensional gas chromatography—time-of-flight mass spectrometry (GC×GC-TOFMS) to analyse decomposition VOCs. This is a highly novel technique in forensic science, and only a few groups have used it worldwide [51-54, 80, 86- 88, 126, 127, 131-135].

GC×GC is a multidimensional separation technique, which means that it uses two mechanisms (in this case, two columns) for analyte separation combined in one system. The separation of the analytes in the first column (first dimension) must therefore be preserved so that they are not re-combined in the second separation (second dimension). The 'comprehensive' part of the instrumentation title indicates that these separations require the presence of the entire sample, or a representative portion of it, so that it can present a complete compositional analysis of the sample [136]. This is opposed to a heart-cutting GC×GC system, which only passes a targeted subset of the sample on to the second dimension column, and only analyses those few, targeted components of the sample [136]. Since the compounds present in decomposition fluid odour profiles are relatively unknown, it is essential to analyse the entire sample, and gain an understanding of its complete composition.

The GC×GC system applies the same basic principles as one-dimensional GC, except with the addition of a short secondary column, secondary oven, and a modulator (Figure 1-10) [87, 131]. The short secondary column (usually 2 m or less in length) contains a different stationary phase to the first column, which allows the samples to undergo two independent separation processes. The secondary column is housed in a secondary, smaller oven, where the temperature is offset by 5-

20^{o}C. A modulator joins the two columns and it is this modulator that, when combined with the change in polarity of the second dimension column, allows for the greater separation of the compounds [80, 87, 131].

There are various types of modulators, usually categorised as either pneumatic modulators, which are based on a valve or flow function [137-140] or thermal modulators, which use a heater or a cryogenic system to trap analytes from the first dimension column [141-144]. The GC×GC-TOFMS system in this study used a cryogenic-based thermal modulator. As the compounds elute from the first column, they are focused using purified nitrogen gas that has been chilled by liquid nitrogen cold jets in the modulator. The modulator "freezes" or traps the eluting compounds in small time increments (1-5 seconds), known as a modulation period or P_M. The eluting compounds are heated and injected or "pulsed" onto the second column, a process referred to as "modulation" [145]. The second dimension column is much shorter than the first dimension so that each pulse can be separated before the next one is injected onto the column. The change in stationary phase of the second column and increase in temperature allows compounds that co-eluted on the first column to separate based on the different polarities and boiling points. Modulation produces both a first dimension and second dimension retention time for each compound, however, the time for a GC×GC analysis is comparable to that of a GC-MS, since this modulation process occurs simultaneously to the first dimension separation [80, 87, 145]. The secondary column and modulation process refine the separation of the compounds on the column and greatly reduces co-elution, providing cleaner and more accurate spectra [88, 145].

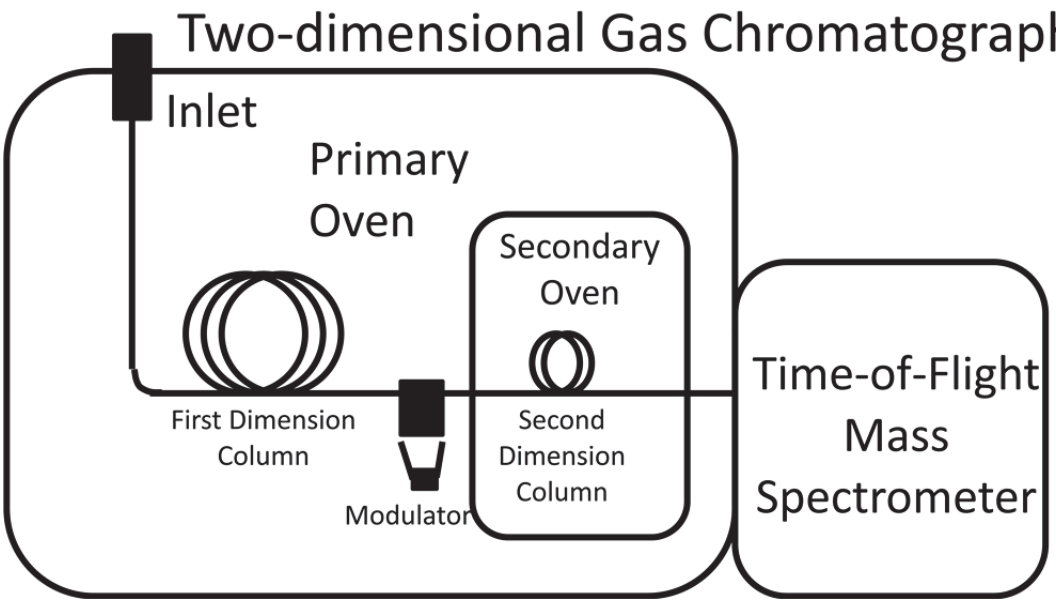

Figure 1-10: A schematic of a GC×GC-TOFMS system (adapted from Perrault, 2015 [80])

The second major advantage of GC×GC is the addition of the time-of-flight mass spectrometer (TOFMS). GC×GC produces a large number of very narrow peaks, with peaks eluting from the secondary column with a width of 100-400 ms at the base. The detector used must therefore be able to collect data at a minimum rate of 50 Hz. The traditional detectors used in GC-MS are quadrupole mass spectrometers, which scans through all of the ions, looking for the specific mass-to-charge ratio (*m/z*) ion range that has been set by the operator by continuously altering its voltage. They are not sufficiently sensitive or rapid to collect the level of data produced by the GC×GC. The TOFMS, however, can reach up to 1000 Hz, and is a non-scanning detector, which detects all the ions simultaneously, rather than constantly altering its voltage to locate specific *m/z* values. This prevents mass skewing, which is the change in the relative intensity of analyte peaks because the concentration is constantly changing as a continuous detector. TOFMS instead scans the sample and locates new ions in the eluting sample [146]. The large amount of data that the TOFMS is capable of collecting also allows for the deconvolution of co-eluting peaks in the software, where partially co-eluting analytes can be clearly resolved into two separate, identifiable compound peaks [147]. Both the potential for deconvolution and the prevention of mass skewing enhance the accuracy of analyte identification using TOFMS, and produce a higher peak capacity. It is therefore an ideal choice for GC×GC [88, 148].

The TOFMS bombards the compounds with electrons, producing fragmented ions that drift through the flight tube towards the detector [149]. Since the kinetic energy is the same, the fragmented ions separate based on mass, with the lighter ions moving faster than the larger, heavier ions. It separates and identifies compounds based on the predictable pattern of their arrival times at the detector [149]. A three-dimensional data set is produced and visualized in the form of a chromatogram (Figure 1-11) which shows the compounds' retention times on both the first and second columns, as well as the relative ion abundance. The relative ion abundance is plotted as the z-axis on three-dimensional plots (known as a contour plot), or, in the case of two-dimensional (surface) plots, as a colour scale, where blue is the background noise, and the increase in the intensity of the colour (green to yellow to red) indicates an increase in relative ion abundance for that particular compound peak. Identification of compounds is completed in a similar manner to the GC-MS, using both mass spectral data and retention indices from the first dimension retention times, or standards, which provides higher confidence in identification results [147].

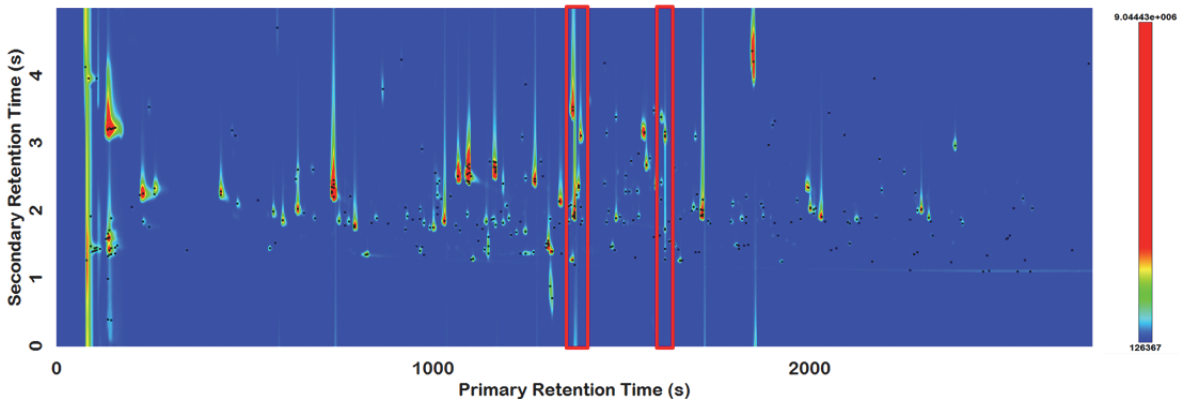

Figure 1-11: A GC×GC-TOFMS surface plot chromatogram of decomposition fluid headspace VOCs. Compounds (indicated by black dots) are separated in two dimensions, according to the two column properties. Intensity of the colour (blue to green to yellow to red) indicates the abundance of the compound. The second dimension separation of numerous co-eluting compounds in the first dimension can be observed in the red boxes

GC×GC-TOFMS provides enhanced peak capacity, which in turn improves resolution and the separation power for peaks. The GC×GC is capable of separating mixtures with hundreds or thousands of compounds more effectively than GC-MS due to the additional selectivity provided by the second column [80]. The second dimension separation also removes interfering chemical

signals, such as column bleed, which decreases background noise and improves the sensitivity. A standard GC-MS has a limit of detection (LOD) between 100-0.1 ng, or 1 part-per-million to 1 part-per-trillion [130]. The sensitivity of the GC×GC-TOFMS is increased by a factor of 10 [150], although this has yet to be proven for decomposition VOCs.

Overall, GC×GC is a valuable technique for separating complex mixtures particularly in the areas of food chemistry, environmental studies and more recently forensic science [49,78, 81, 83, 86, 88, 108-118]. Its application to decomposition odour profiling will assist in providing a more comprehensive VOC profile and a better understanding of the VOCs used by cadaver-detection dogs when detecting the odour of human remains.

1.6 Research Aims and Objectives

The purpose of this study is to validate human decomposition fluid as a training aid for cadaver-detection dogs by chemically profiling the odour using GC×GC-TOFMS. This will involve determining whether there are sufficient types and numbers of VOCs between the odour profiles produced by decomposition fluid and human cadavers for the purposes of cadaver-detection dog training. A sufficient number of VOCs means that the major compounds and compound types reported for human remains and are also detected in human decomposition fluid.

The odour profile of human decomposition fluid will be compared to the odour of decomposing human remains (based on a literature review). The study will investigate several variables, including the optimal storage conditions under which the training aid can be maintained and the change in the odour profile over time. This research will assist in determining the conditions and length of time that decomposition fluid can be satisfactorily used as a training aid for cadaver-detection dog training.

This study also aims to link the odour profile of decomposition fluid to the response of cadaver-detection dogs. It aims to determine the key VOCs present in decomposition fluid that produce a positive alert. It will also determine the sensitivity of the dogs to decomposition fluid as a training aid. The sensitivity of the dogs will be compared to the detection limits of the analytical

instrumentation, and examine the possibility that the dogs' sensitivity to decomposition odour will increase as they become more familiar with decomposition fluid as a training aid.

Ultimately, the results from this study will form the basis for validating the use of human decomposition fluid as a cadaver-detection dog training aid, and will help to streamline and standardize cadaver-detection dog training procedures. This will allow for the testimony provided by a cadaver-detection dog handler to be presented as a reliable form of evidence in court.

Chapter 2:

Method Optimisation

Chapter 2: Method Optimisation

2.1 Introduction

The sampling technique used to collect the odour of decomposition will have an effect on the types of VOCs captured. While the use of SPME and sorbent tubes is represented extensively in the literature [*Sorbent tubes*: 22, 51, 53, 78, 80, 81, 84,-88, 101, 102, 119, 120. *SPME*: 48, 52, 58, 83, 99, 115, 121-124], each technique has generally been used individually. Studies that use either sorbent tubes or SPME for their sample collection will often compare their results to those provided by both methods in the literature, leading to discrepancies between the types and numbers of compounds reported. This can be problematic since the types of sorbents available for each technique, although capable of collecting the same general range of compounds, are not equivalent to each other. This leads to the potential for biased results due to the use of a single collection technique and sorbent type. The different manners of collection for the two techniques, and their preferential suitability to either the laboratory (SPME) or field work (sorbent tubes) could lead to the discrepancies reported in the published literature [51, 80].

Only one study to date has compared the two techniques directly for the same set of decomposition odour samples [51]. The study determined that both SPME and sorbent tubes collected approximately the same proportions of unique compounds, and that an equal proportion of the VOC profile was also collected by both techniques. There were, however, variations in the number of compounds within each compound class that the collection techniques captured. For example, the sorbent tubes captured a greater proportion of alcohols and sulphur-containing compounds, while SPME trapped more esters and hydrocarbons. The study also determined that while each technique had its advantages and disadvantages, sorbent tubes were optimal for *in situ* analysis in the field, with minimal loss of compounds due to transport and storage, while SPME was better suited to a laboratory setting, especially when specialised instrumentation is not available for desorbing the compounds from the sorbent tubes.

Traditionally, these collection methods have been followed with an analysis of the samples by gas chromatography-mass spectrometry (GC-MS) [22, 48, 49, 78, 81, 83-85, 103, 124, 151], but this

method suffers from several limitations, including insufficient peak capacity, an inability to manage dynamic range, and restricted selectivity in one dimension when analysing complex mixtures such as decomposition odour (see Section 1.4.3) [86, 87, 101]. Given these limitations, the use of comprehensive two-dimensional gas chromatography—time-of-flight mass spectrometry (GC×GC-TOFMS or GC×GC) has become more popular in recent years [54, 58, 86-88, 124, 125]. The GC×GC provides increased peak capacity, which in turn, improves peak resolution. This means that compounds in complex mixtures such as decomposition odour can be separated more effectively. The addition of the second dimension column also decreases background noise and improves the sensitivity of the technique [101]. Further, the TOFMS provides a higher sensitivity to VOCs, as well as a higher scan speed, which is necessary to collect the large amount of data produced by the GC×GC separation. It also prevents mass skewing of the data, which increases the accuracy of compound identification [102]. Only one study [101] has carried out a direct comparison of GC-MS and GC×GC-TOFMS for decomposition odour analysis, and determined that, in addition to the above mentioned advantages, the range of new software features available provided many new and improved possibilities for data handling and analysis. Overall, the comprehensive screening approach of GC×GC-TOFMS makes it the preferred method for the non-targeted analysis of decomposition VOCs [101].

Since decomposition fluid produces a complex odour profile and contains a wide range of compounds and classes, it is important that the method chosen collects a broad range of VOCs. In order to gain a true understanding of the complex nature of the training aids, and the capabilities of the dogs when detecting these samples, it was essential to separate and identify the VOCs present in decomposition fluid. The GC×GC-TOFMS was chosen as the preferred method for this type of sample analysis, however, a technique for collecting the VOCs needed to be optimised. The use of both collection techniques in tandem was considered too time-consuming for this study. Hence, sorbent tubes and SPME were compared to determine which technique collected the widest range of decomposition volatiles based on the conditions under which a cadaver-detection dog could reasonably be expected to detect their training aids. Training conditions typically involve a closed facility with minimal weathering effects, such as solar radiation, air flow or precipitation, and a broad temperature range, between 10 ^{o}C and 40 ^{o}C.

2.2 Materials and Methods

2.2.1 Decomposition Fluid Samples

The decomposition fluid samples used in this study were obtained from bodies donated to the UTS Surgical and Anatomical Science Facility (SASF). The samples were collected in the same manner as the training aids used by the New South Wales (NSW) Police Dog Unit. The ethical use of tissues from bodies donated to science is covered under the *NSW Anatomy Act of 1977* [152] and is provided by the donor's consent to donate their body.

Due to the limited availability of donors, a whole cadaver could not be used to obtain samples. Instead, un-embalmed, refrigerated limbs were used to produce the decomposition fluid for this study. Information regarding the age, gender and PMI of the donors were not available due to privacy policies. The limbs and tissue samples were placed in a fume hood at room temperature for 5 to 10 days. The decomposition fluid produced by all of the donors was collected by the Manager of SASF and stored in a single glass specimen vial in the refrigerator at 4 ^{o}C, regardless of age or gender or tissue type. When the study was complete, the fluid samples were disposed of according to the UTS biohazardous waste disposal procedures.

2.2.2 Sorbent Tubes and Thermal Desorption

Since sorbent tubes are a dynamic sample collection method, a purge and trap system was created to collect the headspace from decomposition fluid. The system involved a Pyrex filtration flask, placed on a heating block, with air drawn through the system at a constant rate by a FLEC Air Pump FL-1001 Chematec (Markes International, Llantrisant, UK). The filtration flask was rinsed with detergent, water, deionised water, methanol and acetone and then heated for 12 hours at 100^{o}C prior to each use in order to remove any residual compounds present in the flask. The rubber septum and Teflon tubing were also rinsed with methanol and acetone and allowed to dry before use.

A blank of the laboratory air and a system blank were collected. The laboratory air blank was collected by pumping the ambient laboratory air through a sorbent tube. The system blank was collected from the glass system before an optimised volume of decomposition fluid (acclimated to

room temperature for 30 minutes at 20 ^{o}C) was deposited in the base of the 500 mL flask, which was then sealed with a pierced rubber septum. Teflon tubing attached the system to the sorbent tubes containing Tenax TA/Carbograph 5TD (Supelco, Bellefonte, PA) and the pump (Figure 2-1). Tenax TA is suitable for capturing compounds in the C_7 – C_{30} range, and is reportedly best for collecting aromatics, semi-volatiles, non-polar and polar compounds, while the Carbograph 5TD collects VOCs in the C_3– C_7 range. The combination of sorbents is used to increase the range of compounds that can be collected [22, 81, 88]. Headspace was allowed to accumulate before sample collection. Before the sample was analysed using GC×GC-TOFMS, a 5µL bromobenzene (150ng/mL; Fisher Scientific, Victoria, Australia) internal standard was injected directly onto each tube using an eVol® XR hand-held automated analytical syringe (SGE Analytical Science, Sydney, Australia).

During the optimisation process, a headspace accumulation time of 15, 30, 45 and 60 minutes were investigated, along with sample collection times of 5, 7, 10, and 12 minutes. The flow rate was varied from 50 to 125 mL/min, and different volumes of decomposition fluid were tested, including 3, 5, and 10 mL. The temperature of the sample was also varied from 25 to 80 ^{o}C to determine the optimal sample extraction temperature. The results of the sorbent tube optimisation for decomposition fluid are reported in Section 2.3.1.

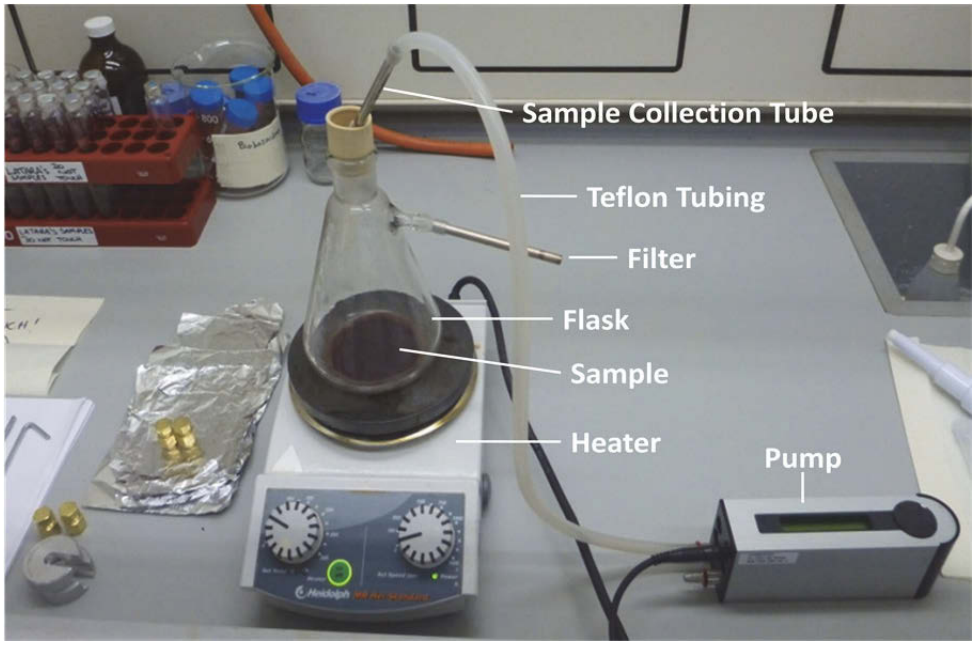

Figure 2-1: The purge and trap system for trapping VOCs on sorbent tubes

2.2.3 Solid-Phase Micro-Extraction (SPME)

Since SPME is a passive method of sample collection, the VOCs produced by decomposition fluid could be sampled from glass vials. Prior to sampling, the 20 mL glass headspace vials (Supelco, Bellefonte, PA) were rinsed with detergent, deionised water, methanol and acetone, and heated overnight at 100^{o}C to remove any residual compounds in the vials. An empty vial was analysed on each sampling day to ensure they were clean before decomposition samples were added.

The decomposition samples were removed from the refrigerator and allowed to equilibrate to room temperature (30 minutes at 20^{o}C). One (1) mL of decomposition fluid was placed in each vial, with 5 µL of 150 ng/µL bromobenzene (Fisher Scientific) added to the vial as an internal standard. Three control vials with only 5 µL bromobenzene internal standard were also prepared. The samples were placed in a block heater in a fume hood and allowed to accumulate headspace for an optimised period (Figure 2-2).

The SPME fibre was encased in a sampling syringe with a thin metal rod that pierced the septum of the headspace vials. The fibre was exposed to the headspace of the vial for the desired time, and to allow the VOCs to adsorb onto the sorbent. The fibre was then retracted into the syringe and transferred to the instument for immediate analysis.

During the optimisation process, the ideal fibre (sorbent) type was investigated, based on the three most common types of fibre used in decomposition research, namely PDMS (a non-polar absorbent fibre), PDMS/DVB and PDMS/DVB/CAR (bi-polar adsorbent fibres). Sample collection (fibre exposure) times between 5 and 60 minutes were investigated, as were sample temperatures from 25 to 80 ^{o}C. The inlet desorption temperature was varied between 150 ^{o}C and 250 ^{o}C. The volume of sample was also varied from 0.5 to 5 mL to determine the optimal sample volume for VOC production. The results of the SPME optimisation for decomposition fluid are reported in Section 2.3.2.

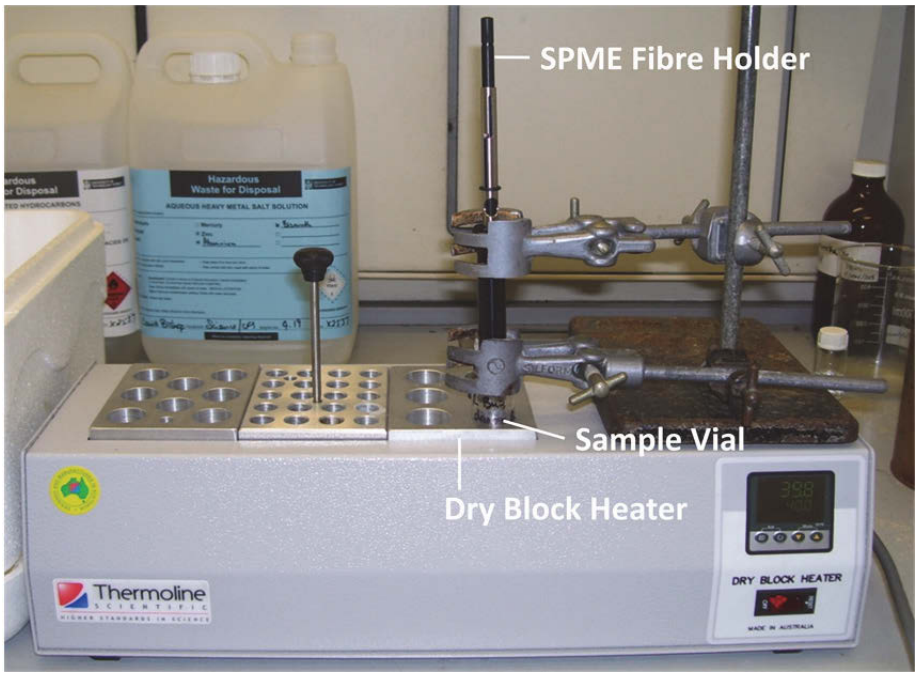

Figure 2-2: SPME fibre sampling from 20 mL glass headspace vial with decomposition fluid in block heater

2.2.4 One-dimensional and Two-dimensional Gas Chromatography — Mass Spectrometry

At the beginning of this study, the GC×GC-TOFMS was still being installed, and the initial optimisation of fibre type, temperature and exposure time for SPME was carried out using an Agilent 6890N GC coupled with a 5973N Mass Selective Detector (Agilent Technologies, Mulgrave, VIC, Australia). The column used was a low polarity DB-VRX capillary column (30 m length x 250 µm diameter x 1.40 µm film thickness, Agilent Technologies), which was designed for optimal VOC resolution, with a helium carrier gas flow rate of 100 mL/min. The data was compiled in ChemStation (version E.02.02.1431, Agilent Technologies), and library searches were performed with the 2005 National Institute of Standards and Technology (NIST) mass spectral library, using a minimum match criteria of 70%. The method proposed by Hoffman *et al* [83] was used for this determination.

Once the GC×GC-TOFMS (Pegasus® 4D, LECO Australia, Castle Hill, NSW, Australia) was available for use, the method optimisation was transferred to, modified, and completed using this instrument. Due to the limited number of studies that used GC×GC for decomposition research [57, 86, 87], there is no standard method for analysing these VOCs; each methodology was therefore tested and modified to determine the ideal parameters for use on the laboratory instrument, and for both sorbent tubes and SPME in this study. Parameters that were optimised included oven temperature and ramping rates, secondary oven and modulator temperature offsets, modulation period, and mass detector collection speed. SPME also required the optimisation of desorption time and temperature in the inlet of the GC×GC.

Sorbent tubes were thermally desorbed using a Unity 2 Thermal Desorber (Markes International, Ltd, Gold River, California, USA) coupled with a Series 2 ULTRA™ multi-tube autosampler (Markes International, Ltd). The TD unit connected to the GC×GC-TOFMS via a 1 m uncoated, fused silica transfer line (Markes International, Ltd), held at 120 ^{o}C. The GC×GC system contained a mid-polar Rxi®-624Sil MS (30 m x 250 µm internal diameter x 1.40 µm film thickness) first dimension column and a polar Stabilwax® (2 m × 25 µm internal diameter x 0.50 µm film thickness) second dimension column (Restek Corporation, Sydney, NSW, Australia). This column set was determined to be ideal for decomposition VOC analysis by GC×GC–TOFMS [127]. The first column was joined to the TD transfer line using an Ultimate Union kit (Agilent Technologies) and to the second column using a SilTite™ µ-Union (SGE Analytical Science, Ringwood, VIC, Australia). The carrier gas was helium, with a flow rate of 1.00 mL/min.

Data analysis was performed using ChromaTOF® software (version 4.50.8.0, LECO Australia). Baseline tracking was performed with an 80 % offset and automatic baseline smoothing. A 30 s peak width in the first dimension and a 0.15 s peak width in the second dimension were used. A signal-to-noise ratio (S/N) of 150 was used, and a minimum library match factor of 80% was required using the NIST 2011 mass spectral library.

Following data processing in either ChemStation (GC-MS) or ChromaTOF® (GC×GC), compound lists were compiled in Microsoft Excel 2007. Compounds were sorted into classes (sulphur-containing, nitrogen-containing, alcohols, aromatics, esters, ethers, ketones, aldehydes, carboxylic acids, hydrocarbons, halogenated, and other) for further analysis.

2.3 Results

2.3.1 Sorbent Tubes and Thermal Desorption

The sorbent tubes were initially conditioned at 300^{o}C for 5 min, as per the manufacturer's instructions, and then a series of optimisation parameters were investigated. These parameters were adapted from methods reported in the literature that employed sorbent tubes for collection of decomposition odour [22, 57, 78, 81, 85-87, 102, 119, 120].

Headspace accumulation was carried out for 15, 30, 45 or 60 minutes before sample collection began, while holding all other parameters constant, as described in the method used by Stadler *et al* [57]. After the headspace accumulation time was optimised, sample collection flow rates of 50 mL/min, 75 mL/min, 100 mL/min, or 125 mL/min were examined, while holding all other parameters constant. Following this, sample collection times were varied from 5 minutes to 12 minutes and the headspace was collected at room temperature (25 ^{o}C), 35 ^{o}C, 40 ^{o}C and 80 ^{o}C. Sample volumes of 3 mL, 5 mL, and 10 mL were also tested to determine the minimum amount required to produce a comprehensive decomposition VOC profile. When each variable was tested, the other variables of interest were fixed at a constant value. This was not an ideal optimisation method, but was the only way to determine the effect of each variable on the overall method, and to determine the optimal parameter for the variable under investigation. A control sample was collected from the laboratory air to determine background contamination, and a system blank was collected before the decomposition fluid was added to the flask.

Optimal sample collection parameters required a 30 minute headspace accumulation time, a flow rate of 100 mL/min, a sampling time of 10 minutes at a temperature of 80 ^{o}C, and a sample volume of 10 mL, as summarized in Table 2-1. The 15 minute accumulation time did not provide a wide range of VOCs, and the peak intensity was relatively low. The 30 and 45 minute headspace accumulation times provided intense compound peaks, and a broad volatile spectrum, while the 60 minute accumulation time demonstrated a loss of several early, small-chain compounds present in the 30 and 45 minute accumulation time chromatograms. Since the 30 minute accumulation time provided one of the widest and most intense VOC profiles in the shortest amount of time, it was chosen as the optimal headspace accumulation time. Flow rates of 50 mL/min and 75 mL/min also collected fewer VOCs than those provided by 100 mL/min and 125

mL/min. Since 100 mL/min is more common in the literature, and there was no distinct increase in peak intensity or number of VOCs with the 125 mL/min flow rate, 100 mL/min was chosen as the optimal pumping rate for sample collection. The lowest sample collection times, as in the case of the previous parameters, did not collect a sufficient VOC profile, while the 10 and 12 minute sample collection times were very similar. Hence, 10 minutes was chosen as the more efficient sample collection time. The lower sample temperatures tested (25 ^{o}C, 30 ^{o}C, and 40 ^{o}C) were unable to produce well resolved peaks in the sorbent tube system. The system required a high temperature (80 ^{o}C) to collect a comprehensive decomposition odour profile. With a smaller flask or a more efficient system, the 3 or 5 mL sample volume might have been sufficient, but the purge-and-trap system used in this study required 10 mL of fluid to produce sufficient headspace in the 500 mL filtration flask.

There were, however, difficulties in obtaining proper vacuum pressure with this purge-and-trap system. This led to difficulties in obtaining clean system blanks, even after trying a range of cleaning methods, oven bake times and temperatures, and pumping the system for several minutes before collecting the system blank and depositing the samples. A glove cabinet with a flow of inert gas, which could possibly eliminate this contamination, was not available for use during this study. Hence, even after optimisation, the use of sorbent tubes was not deemed satisfactory for this study (further discussion provided in Section 2.3.4).

Table 2-1: Parameters optimised for sorbent tube VOC collection. Those highlighted in red are the optimal parameters

Headspace Accumulation Time (min)	Flow Rate (mL/min)	Sample Collection Time (min)	Sample Temperature (^{o}C)	Volume of Fluid (mL)
15	50	5	25	3
30	75	7	30	5
45	100	10	40	10
60	125	12	80	

2.3.2 Solid-Phase Micro-Extraction

Three different SPME fibre phases were tested using the Hoffman *et al* [83] method for GC-MS: polydimethylsiloxane (PDMS), polydimethylsiloxanedivinylbenzene (PDMS/DVB), and polydimethylsiloxane-divinylbenzene-carboxen (PDMS/DVB/CAR) (Supelco, Bellefonte, PA). The fibres were conditioned (20 minutes at 220^{o}C) before optimisation. The headspace was accumulated for 15, 30 and 45 minutes, respectively. Each fibre was exposed to the static headspace for 5, 15, 30, 45 or 60 minutes at room temperature (Table 2-2). GC×GC-TOFMS inlet temperatures of 150 ^{o}C, 200 ^{o}C and 250 ^{o}C were also tested. Samples were heated to 25 ^{o}C, 35^{o}C, 40^{o}C and 80 ^{o}C using a dry heating block (Thermoline Scientific, Australia); these temperatures were chosen based on published articles which had used SPME previously for decomposition VOC collection [48, 83, 99, 115, 121-123]. After headspace extraction, each sample was initially analysed by GC-MS. This analysis ruled out the use of the PDMS fibre, since the few peaks produced by this fibre were difficult to distinguish from the baseline, and the internal standard produced a peak with poor intensity and peak tailing. However, there were no notable differences between the PDMS/DVB and the PDMS/DVB/CAR fibres using GC-MS.

Once the method optimisation procedure was moved to the GC×GC-TOFMS, the PDMS/DVB and PDMS/DVB/CAR fibres were tested again. Chromatograms produced by each fibre were overlapped in ChromaTOF®, and the PDMS/DVB/CAR fibre demonstrated a wider range of volatiles with greater peak intensity than the PDMS/DVB fibre. Once the fibre type was optimised, headspace accumulation time, sample temperatures, fibre exposure times, fluid volumes and GC inlet temperatures were tested. Optimal VOC collection, as in the case of sorbent tubes, required a headspace accumulation time of 30 minutes; the 15 minute accumulation did not collect sufficient VOCs, while the 45 minute accumulation time produced similar results to the 30 minute accumulation time. A sample temperature of 35 ^{o}C was determined to be optimal, since room temperature did not produce sufficient VOCs, 80 ^{o}C denatured the sample, and there was no significant advantage to selecting the 40 ^{o}C sample temperature over 35 ^{o}C. The 0.5 mL sample volume did not produce sufficient headspace, but all of the other volumes tested produced comprehensive decomposition odour profiles; a sample volume of 1 mL was chosen as the most sustainable volume throughout the study. A fibre exposure time of 45 minutes was optimal for this study, since the lower exposure times did not collect as many VOCs, while the 60 minute exposure time led to the loss of some of the lighter VOCs due to the competitive sorption of the fibre phase

(discussed in section 2.4). The ideal inlet temperature for SPME was 250 °C, and fibres were desorbed for 5 minutes in the inlet to prevent carry-over (contamination) from previous samples. The SPME parameters investigated, including those chosen as optimal, are summarised in Table 2-2.

Table 2-2: The parameters tested for SPME VOC collection. Those highlighted in red indicate the optimal parameters

Fibre Type	Headspace Accumulation Time (min)	Sample Temperature (°C)	Fibre Exposure Time (min)	Volume of Fluid (mL)	Inlet Temperature (°C)
PDMS	15	25	5	0.5	150
PDMS/DVB	30	35	15	1	200
PDMS/DVB/CAR	45	40	30	1.5	250
		80	45	3	
			60	5	

2.3.3 Comprehensive Two-dimensional Gas Chromatography — Time of Flight Mass Spectrometry

While the method proposed by Hoffman *et al* [83] was suitable for GC-MS analysis, it did not adequately resolve all compounds when transferred to the GC×GC-TOFMS instrument. The GC×GC methods used by Brasseur *et al* [87], Dekeirsschieter *et al* [86], and Stadler *et al* [57] were therefore tested and modified. Several different primary oven temperatures and ramping methods, secondary oven offsets, modulator offsets, and modulation periods were tested using each of these methods. Ultimately, a new methodology was adapted for this study from Stadler *et al* [57], with further optimisation of the oven temperatures, ramping rates, secondary oven offset, modulator offset, modulation pulse, hot and cold jets, and mass detector collection speed to account for the difference between the sorbent tubes used by Stadler *et a*l [57] and the SPME fibres ultimately used in the current study. Changing the oven ramping rates from 5 °C/min for the entire run to 7 °C/min for the first third of the run and 5 °C/min for the rest of the run shortened the overall analysis time for the GC×GC-TOFMS. This allowed the lighter volatiles to elute more

rapidly at the beginning of the chromatogram, while reducing the separation and elution rates for the complex decomposition VOC mixture in the middle to the end of the run, as well as minimizing the wrap-around effect (the overlap of peaks from different P_Ms). Increasing the secondary oven offset, decreasing the modulator offset, and increasing the P_M all increased the separation capability of the second dimension column and minimized the wrap-around effect. Daily mass calibrations and tuning were performed using perfluorotributylamine (PFTBA). The final methodology is summarized in Table 2-3. The method was optimal for both sorbent tubes and SPME.

Table 2-3: Summary of the optimised GC×GC-TOFMS method developed for the odour profiling of decomposition fluid

Parameter	Setting
Front inlet	Splitless, 3 mL/min, 250°C
Oven 1	35°C/5 min -> 7°C/min -> 120°C -> 5°C/min-> 240°C/5 min
Oven 2	10°C offset
Modulator	5°C offset
Modulation Period	5 sec Cold jets- 1.50 sec Hot pulse- 1.00 sec
Transfer Line	250°C
Source Temperature	200°
Electron Ionization Voltage	-70 V
Data Acquisition Rate	100 spectra/sec
Mass Range	29-450 amu
Detector Voltage	1500V

2.3.4 A Comparison of Sorbent Tubes and Solid-Phase Micro-Extraction

The aim of this optimisation process was to determine which collection method was better suited to collecting a wide range of decomposition VOCs under the laboratory conditions available. All the compounds produced by each method were therefore compiled and classified. The overall proportions of the VOC profile collected by each method, as well as the types and proportions of individual compound classes collected by each method were examined. As demonstrated in Figures 2-3 and 2-4, the sorbent tubes and SPME collected the same types of compounds, and

approximately the same proportions of these compounds. Sorbent tubes collected higher proportions of aldehydes, and hydrocarbons (Figure 2-3), while SPME collected slightly higher proportions of alcohols, esters and nitrogen-containing compounds (Figure 2-4).

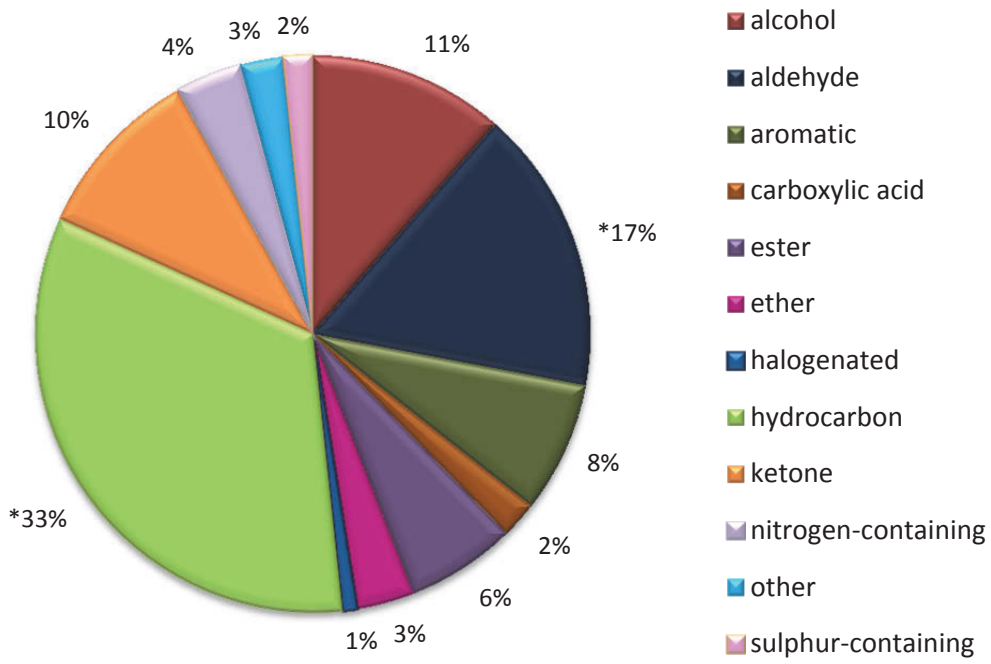

Figure 2-3: Proportion of compound classes collected by sorbent tubes. The proportionately larger classes collected by sorbent tubes compared to SPME are indicated with an asterisk (*)

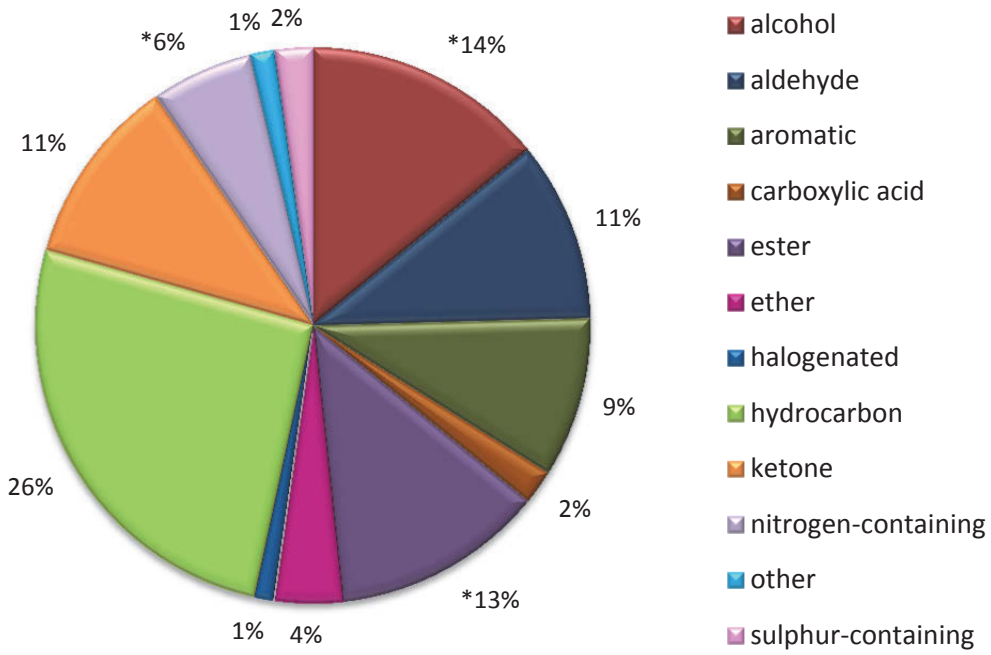

Figure 2-4: Proportion of compound classes collected by SPME. The proportionately larger classes collected by SPME compared to sorbent tubes are indicated with an asterisk (*)

When comparing the overall proportions of the odour profile that each method collected, it is evident that SPME collected a larger portion of unique compounds (Figure 2-5). Approximately 45% of the VOCs present in the odour of decomposition were collected only by SPME, while 12% of the VOCs present in the odour of decomposition were collected only by sorbent tubes. When combined with the number of compounds collected by both methods (i.e. 43% of the overall decomposition VOC profile), SPME collected 88% of the entire VOC profile, while sorbent tubes only collected 55% of the VOC profile detected.

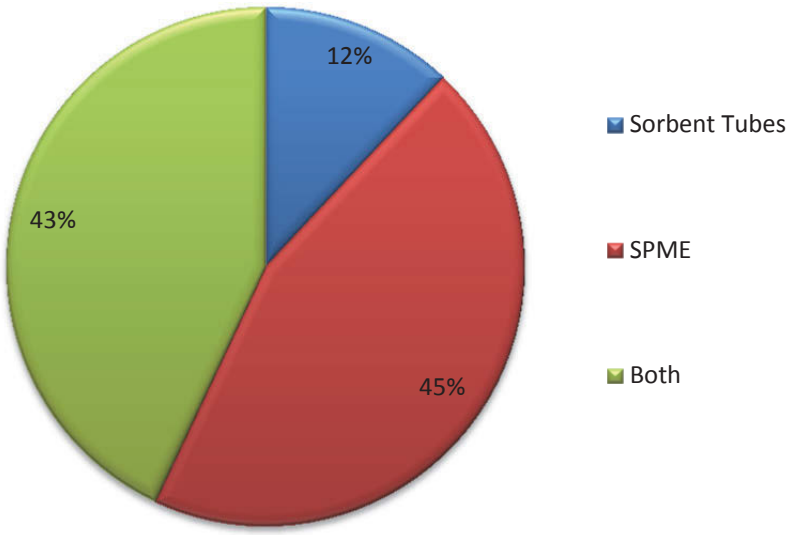

Figure 2-5: Proportion of compounds collected by each sample collection method

2.4 Discussion

The complexity of decomposition odour requires methods that can collect and analyse the wide range of compounds present in the sample. Due to the complex nature of decomposition fluid, it was essential to use GC×GC-TOFMS for optimal sample analysis, and to compare the use of sorbent tubes and SPME in order to determine which technique collected the widest range of decomposition volatiles under the laboratory conditions used in this study.

The sorbent tube system created for this study was based on a schematic of a system created by Brasseur *et al* [87]. A filter was placed at the entrance to the system to filter contaminated air before it reached either the sample or the sorbent tube. In spite of this precaution, the system blanks collected prior to sampling showed the presence of contaminants on every sampling day. The laboratory air blank, the filter, and the samples themselves did not contain any of these contaminants when they were analysed along with the system blank. Pumping air through the system for half an hour before collecting the system blank and samples did not result in a clean system blank. It was theorized that the rubber septum and Teflon tubing used as part of the system may not have created a sufficient vacuum to prevent contaminants from entering the system in other ways. However, this does not explain why the laboratory air blank did not contain

any of the contaminants identified in the system blank. A glove box with a flow of inert gas through the system could have been a potential method for avoiding this problem and obtaining a clean system blank. However, a glove cabinet was not available for use at the time of this experiment.

The poor vacuum pressure is more likely to be the reason for the poor sample collection. The system required high temperatures (80 ^{o}C), high pumping rates (100 mL/min), and relatively long sample collection times for the size of the container (500 mL filtration flask) in order to collect sufficient headspace on the sorbent tubes for GC×GC-TOFMS detection. If the system had been better sealed, it is likely that the air flow through the system would have been sufficient to collect the VOCs without a high flow rate or sample temperature. The high temperature caused denaturing of the samples after every 3rd replicate (i.e. approximately every 30 min), and one sampling session (i.e. approximately 10 sorbent tubes) would require 30-50 mL of decomposition fluid. Given the limited number of donors available, this was not a sustainable sample volume. This sample temperature was also far higher than the temperatures cadaver-detection dogs would experience during their training sessions or in the field, and these sampling conditions would not give an accurate representation of the VOCs the dogs are expected to detect.

Sorbent tubes are a popular method for decomposition odour profile collection, with more than 15 different studies reporting their use in the past decade [22, 51, 53, 54, 57, 78, 80, 81, 84-87, 101, 102, 119, 120, 125, 126, 131-133]. The combination of Tenax TA and Carbograph 5TD allows for the collection of compounds in the C_3– C_{30} range, and covers a wide range of compound classes. Hence, this combination is often used in decomposition odour analysis [22, 51, 81, 88]. Forbes and Perrault [51] reported that this combination of sorbent phases does not collect carboxylic acids, and while the current study was able to detect carboxylic acids, they represented a very small proportion of the overall profile (i.e. 2%). The study reported that the sorbent tubes collected many of the corresponding esters for these acids, which compares to the results seen in the current study, although the overall proportion of carboxylic acids and esters collected by sorbent tubes was relatively small in both studies. When compared to the SPME results, sorbent tubes collected a larger proportion of hydrocarbons and aldehydes, which is likely due to the extended carbon range; with a range up to C_{30}, the sorbent was capable of trapping the longer-chain compounds that SPME was not able to collect.

It was hypothesized that the sorbent tubes would collect more decomposition VOCs than SPME due to the wider sorbent carbon range and the dynamic method of sample collection. However, sorbent tubes collected fewer VOCs; representing approximately 55% of the overall VOC profile detected, which was likely due to the issues experienced with the sampling system. Sorbent tubes are hardy, good for transporting and storing samples with minimal sample loss, and convenient to use, especially when coupled with an auto-sampler. However, they were not well suited to the small scale, indoor laboratory sample collection method that was used in this study.

The SPME set-up in this study required temperatures and sample volumes that more closely resembled a situation where cadaver-detection dogs might be required to detect decomposition fluid. A temperature of 35 ^{o}C may be considered high, but it is not uncommon, especially in outdoor Sydney, Australia in the spring and summer months. This temperature also prevented denaturing of the samples. Only 1 mL of fluid in a 20 mL glass vial was required for successful detection and identification of decomposition VOCs using GC×GC-TOFMS. This was a reasonable volume of fluid, which could be sustained throughout the study. The overall system set-up also required less space within a laboratory fume hood, which was ideal for the busy laboratory space in which sample collection occurred.

The SPME fibre phase had a large influence on the type and range of compounds collected. Most studies that use SPME for decomposition VOC collection have used a PDMS/DVB fibre [48, 51, 53, 83, 122, 124]. This is because the PDMS/DVB fibre collects volatiles with a molecular weight range of 50-300, and is particularly good at collecting amines and other nitrogen-containing compounds [51]. This study found that the PDMS/DVB/CAR fibre was better suited to the collection of a wide range of volatiles, since it collected a range of compounds from C_3-C_{20} (which, although less than the Tenax TA/Carbograph 5TD sorbent tubes, is still a wide range for SPME fibre sorbents), and a molecular weight range of 40-275. The addition of the carboxen on the fibre allows for the recovery of the lighter sulphides and oxygenated compounds. A PDMS/DVB fibre also has a low recovery rate for alcohols and sulphides, and the addition of the carboxen sorbent helps to counter this problem [51]. When compared to the sorbent tubes, SPME collected a larger percentage of alcohols, esters and nitrogen-containing compounds in the decomposition VOC profile. The recovery of carboxylic acids was still low, but the larger number of long-chain esters and minimal increase in the number of ketones is a result of the competitive sorption of the SPME fibre (discussed in section 1.3.2), where the heavier, or longer-chain VOCs displace the lighter

VOCs over time. With an exposure time of 45 minutes, the PDMS/DVB/CAR fibre was able to collect and concentrate some of the heavier VOCs that would not be collected by the 10 minute sampling time of the sorbent tubes. The addition of the carboxen to the PDMS/DVB fibre widened the range of compounds that could be collected by a single fibre, which allows for more compounds to be collected while still ensuring a high throughput of samples. The use of PDMS/DVB/CAR fibres for SPME also ensured that a larger proportion of the overall decomposition odour profile was collected (88% of the entire VOC profile).

Neither method was able to directly mimic the conditions under which the dogs locate their training aids (i.e. room temperature). SPME was able to more closely mimic these conditions, since it collected VOCs at 35 ^{o}C from the same sample volumes typically used as training aids (i.e. 1 mL). SPME was also able to pre-concentrate a wider range of decomposition VOCs. This was ideal for GC×GC-TOFMS analysis, since it increased the potential for identifying trace quantities of compounds within the overall decomposition profile.

2.5 Conclusions

The difficulty with obtaining a clean system blank with the sorbent tube system, the high sample temperatures and volumes required for adequate VOC collection, and the fact that sorbent tubes collected a smaller proportion of the overall VOC profile indicated that the purge-and-trap system was not an ideal method for the present study. Since SPME required smaller volumes of fluid and could collect a broader range of decomposition VOCs under conditions similar to those employed during the training of cadaver-detection dogs, it was determined to be the optimal method for sample collection in this study.

An optimal method for the GC×GC-TOFMS was adapted from Stadler *et al*, 2012 [57]. The modifications made to the method allowed for a greater separation of decomposition VOCs in both the first and second dimension.

Chapter 3:

Odour Profiling of Human Decomposition Fluid

Chapter 3: Odour Profiling of Human Decomposition Fluid

3.1 Introduction

Decomposition fluid (also known as purge fluid or putrefactive liquid) is the liquid formed during the autolysis and putrefaction of a cadaver during the bloat and active decay stages of decomposition. It is formed due to the degradation of the body's tissues and organs by enzymes and chemical reactions (autolysis) and by internal bacterial communities (putrefaction) [57, 64]. These enzymes and bacteria break down the carbohydrates, lipids, and proteins in the body to produce a variety of substances, some of which are gaseous by-products of these reactions, such as VOCs [60]. These VOCs produce the odour profile of decomposition which insects and cadaver-detection dogs (among other animals) use to locate decomposed remains [15, 22, 70, 78, 104-106].

In a natural environment, the decomposition fluids and gases produced by autolysis and putrefaction would remain with the cadaver. Since each organ and tissue decomposes at different rates [56], and each contain different proportions of carbohydrates, lipids and proteins, the composition of the fluid and the odour profile changes according to each influx of new materials [23, 83]. However, as in the case of the decomposition fluid samples used in this study, if the fluid has been removed from the body system, it may produce alternative odour profiles. There is no influx of new materials to alter the profile of the fluid as the body continues to decompose, and instead, the sample simply degrades the materials present in the fluid, which could lead to a training aid profile that does not represent the odour profile of a decomposing cadaver. Currently, it is not known whether the removal of a sample of fluid from the body system will alter the VOC profile produced by the fluid. Since decomposition is a dynamic process and influenced by a wide range of variables (see Section 1.2), it is hypothesized that removing the sample from the body, and subsequently from these variables, may affect the profile significantly. It is also unknown how the original proportions of muscle mass or fatty tissues in the body may affect the proportions of

lipids, carbohydrates and proteins in decomposition fluid, which in turn, could alter the proportions of the VOCs and compound classes present in the odour profile.

Although a large amount of research still needs to be conducted in this field, it is known that cadaver-detection dogs are able to identify a range of materials as "decomposition odour" including human remains, and various tissue and organ samples that are used as training aids. The mechanism by which the cadaver-detection dogs locate this scent is currently unknown, and it is believed that there are several key compounds or a set of distinct compounds that cadaver-detection dogs associate with decomposition odour. However, without a clear understanding of the odour profiles produced by decomposing remains and the effects that different decomposition variables will have on the odour profile, it is not clear whether these dogs are being trained and utilized to the best of their abilities. There are currently no universally accepted training methods or aids, or accreditation guidelines for these dogs, and as a result, the Scientific Working Group for Dog and Orthogonal Detector Guidelines (SWGDOG, [153]) was created to help establish these guidelines for detector dogs, including cadaver-detection dogs. The SWGDOG considers the need for the development of reliable training aids to be critical, and these training aids must be easily and legally obtainable, non-hazardous, easy to use, re-useable, and representative of the whole decomposition odour [153].

Thus, this chapter of the thesis aims to validate human decomposition fluid as a training aid from a chemistry perspective. This will be achieved by profiling the odour of human decomposition fluid as a cadaver-detection dog training aid and comparing this profile to that of decomposing human remains, in order to determine whether it represents a similar cadaveric odour profile. The experiments will determine the best storage conditions under which the training aid can be maintained, and study the change in the profile over time under these storage conditions. The purpose of these experiments is to identify a point at which the odour profile may no longer resemble that of decomposition odour, in order to determine the point at which the samples should no longer be reused. This study will also directly examine the chemical profile of a series of aged and diluted training samples, that have been presented to the dogs as training aids, and will determine any trends in the changes of the odour profiles, so that these results can be compared directly to the results obtained from the dogs during training sessions (see Chapter 4). This study aims to determine the reliability and usefulness of human decomposition fluid as a training aid,

and to provide a basis for the study of decomposition odour profiles as they relate to the development of optimal training aids for cadaver-detection dogs.

3.2 Materials and Methods

3.2.1 Decomposition Samples

The decomposition fluid samples in this study were collected from de-boned arm, thigh and abdominal tissue samples, in the manner outlined in Section 2.2.1. The fluid was collected in a glass specimen jar and stored at room temperature until there was sufficient fluid for the entire study, at which point it was sub-sampled into glass headspace vials. The amount of tissue available for decomposition limited the amount of fluid available for the study, and it was decided that the 1 mL samples of fluid would be re-used three times over the course of the study (i.e. for Day 0, 49 and 120). A layer of parafilm was placed over the caps of the vials between each use to prevent the loss of VOCs through the hole in the septum caused by the SPME fibre.

3.2.2. Comparison of Decomposition Fluid and Human Cadaveric Odour Profiles

In order to determine the validity of using decomposition fluid as a training aid, a comparison of the profile of the training aid and that of a human cadaver must be made. Since many cadaver dog handlers store their decomposition fluid training aids at room temperature, and the dogs are most often asked to locate the aids at room temperature, the decomposition fluid profile for this study was also obtained from fluid stored at room temperature.

Six (6) 1 mL aliquots of decomposition fluid were placed in 20 mL glass headspace vials, which had been cleaned and baked at 100 ^{o}C for 3 hours, and allowed to cool to room temperature. An internal standard of 5 µL of 20 ppm bromobenzene was added to each vial. The vials were analysed using the method outlined in Sections 2.3.2 and 2.3.3.

At the time of this study, the Australian Facility for Taphonomic Experimental Research (AFTER; a facility that allows the use of cadavers to study the processes of decomposition) was not yet open in Sydney, Australia. As a result, the odour profile results from the decomposition fluid could not be directly compared to that of a decomposing human cadaver in the same environment as originally planned. Instead, the results were compared to the literature for VOC profiles of decomposed human remains. While not ideal, it was the only alternative in the timeframe of this study, due to delays in the opening of AFTER. A literature review for all human decomposition research articles was carried out [22, 47- 49, 51, 81, 83-85, 102, 119, 120, 122, 129, 154], and a list was compiled of all the VOCs reported therein. This list was then compared to the list of compounds produced by the decomposition fluid training aids in this study.

3.2.3 Storage and Ageing Trials

Since decomposition fluid can be difficult to obtain, the cadaver-detection dog handlers and training coordinators often store and re-use the samples for months, or even years, until new samples become available. However, there is no standard protocol for the storage and handling of the training samples, and these variables would likely have an effect on how long the decomposition fluid could be used as a viable training aid. This portion of the study examined the changes to the odour profile of the training aid as it aged under various storage conditions to determine the optimal timeframe for using the training aid from a chemical perspective.

The 63 mL of decomposition fluid collected for the storage and ageing trials was sub-sampled into 1 mL aliquots in 20 mL glass headspace vials, which had been cleaned, baked at 100 ^{o}C for 3 hours, and allowed to cool. The vials were divided between three storage conditions: room temperature (20 ^{o}C), refrigerator (4 ^{o}C), and freezer (-20 ^{o}C). Each storage condition contained three replicates per sampling day, as well as a control vial containing 1 mL of saline solution (Figure 3-1). This gave a total of 21 sample vials and 7 control vials per storage condition, which were each sampled 3 times over the course of the study. Five (5) µL of 20 ppm bromobenzene was added as an internal standard to all of the samples and controls.

Figure 3-1: The decomposition fluid samples (left) and controls (right) in 20 mL glass headspace vials for the room temperature storage condition study

The fridge and freezer samples were allowed to acclimate to room temperature before sampling occurred. The method used for sample collection and analysis has been previously outlined in Sections 2.3.2 and 2.3.3. The VOCs were sampled once per week for the first two months of the study (Days 0, 7, 14, 21, 28, 35, 42, 49, and 56), every two weeks for the next two months (Days 70, 84, 98, 112), and once per month for the remainder of the year (Days 140, 170, 200, 230, 260, 290, 320, and 350). Day 0 samples were placed in their storage conditions for 3 hours in order to allow the storage conditions to have an effect on the fresh fluid before sampling was undertaken.

Due to ongoing issues with the GC×GC-TOFMS and the time-sensitive nature of this trial, Days 28, 35, 42, 49, 56, 70, 112, 170, 200, and 260 were not able to be collected and analysed during the original trial. As this represented half of the samples in the study, a second storage and ageing trial was repeated to represent these missing samples. However, due to time constraints, this second trial was only able to recover samples up to Day 70 (Days 28, 35, 42, 49, 56 and 70), for a total of 17 out of 21 original samples.

3.2.4 Dilution and Ageing Trials

Samples of decomposition fluid were also collected, serially diluted and aged for use in training sessions with the cadaver-detection dogs (see Chapter 4). The odour profiles of these training aids were chemically analysed to determine the limit of detection of the GC×GC-TOFMS. This trial also

allowed a comparison of the VOC profiles to the results obtained from the sensitivity trials with the cadaver-detection dog teams.

Twelve (12) 100 mL volumetric flasks were cleaned and baked overnight at 100 ^{o}C. When removed from the oven, they were immediately capped, allowed to cool to room temperature, and filled with 50 mL of tap water. Tap water was chosen because it more accurately represents the type of water that may be used to dilute decomposition fluid at a crime scene (i.e. rain water, hand washing, etc).

Ten (10) mL of stock solution (i.e. undiluted decomposition fluid) was placed in the first volumetric flask, tap water was added to the mark, and the flask inverted several times to mix the solution, forming the 10^{-1} solution. Ten (10) mL of the 10^{-1} solution was placed in the second volumetric flask, tap water added to the mark, and the solution mixed to form the 10^{-2} solution. This serial dilution process was repeated until a dilution level of 10^{-12} was achieved, producing a total of 13 samples (i.e. one stock sample and 12 dilutions).

Forty (40) mL of the fluid was divided between two sets of glass scintillation vials (cleaned and baked at 100 ^{o}C for 3 hours). One set of samples was taken to the training sessions with the dogs (described in Section 4.2.2), and left with the training co-ordinator for future training sessions. The second set remained in the laboratory for chemical analysis, and as a back-up set of samples for the training sessions, if required. Excess fluid was disposed of according to the UTS biohazardous waste policy.

Two sets of dilutions were also aged up to 2 years (24 months) under refrigerated conditions (see section 3.2.3) and presented to the dogs during training sessions (Section 4.2.2). Dilutions were aged for 6, 9, 12, 15, 18, 21 and 24 months. After each dog training session, the unused diluted samples were returned to the laboratory and chemically profiled using the SPME-GC×GC-TOFMS methods outlined in Sections 2.3.2 and 2.3.3.

3.2.5 Data Analysis

The program used for data analysis was ChromaTOF® version 4.51.6.0 (LECO, Australia). The baseline offset was 0.8, with automatic smoothing. The expected peak width in the first dimension

was 25 s, and the second dimension had an expected peak width of 0.3. The signal-to-noise ratio (*S/N*) was 150, and it was used with a minimum similarity match of 800 to the 2011 National Institute of Standards and Technology (NIST) mass spectral library database for all of the trials. Peak areas were calculated using unique mass and the Statistical Compare software feature within ChromaTOF® was used to align the peaks.

The decomposition fluid samples used for comparison with the literature were entered as a single class in Statistical Compare and normalised against the internal standard. Samples for the storage trial, ageing trials, and dilution samples were also entered into Statistical Compare in individual classes for controls, storage (i.e. room temperature, fridge, and freezer categories), age (i.e. classes categorised by day or month), and dilution (i.e. classes characterized by dilution level). The peaks in the chromatograms for the samples in these categories were aligned using Statistical Compare. The analyte match criteria were as follows: a mass threshold of 10, a minimum similarity match of 800, a maximum retention time separation of 1 s in the first dimension, and a maximum retention time difference of 0.6 s in the second dimension. A minimum analyte filter of 75% was applied for each compound, which means that a compound must be present in 75% of the samples within a class to be included in the compound list. For example, in the room temperature samples, which had 17 sampling days, 3 replicates per day for a total of 51 samples, a compound had to be present in a minimum of 39 samples to be included in the compound list.

After a peak was identified using the initial data processing method, Statistical Compare then performed a second peak search of the samples using a lower, user-defined *S/N* (in this study, 20) to identify compounds that were not found using the initial peak search function with a *S/N* of 150. This function searches for peaks that have been identified in most samples at this particular retention time (in both the first and second dimension) with an *S/N* of 150, but were not detected in other samples because the peak *S/N* at this particular retention time was below 150, even although the peak was present in the sample.

The Fisher ratio was then calculated for each compound, and compared to a critical *F* value. The Fisher ratio is the ratio of between-class variance to within-class variance, and is a simple method for identifying significant differences between classes for each compound [155]. If the Fisher ratio was greater than the critical *F* value (i.e 3.056 for the storage conditions), the compound was

considered a decomposition VOC. This process generated a list of significant decomposition compounds from within the compound list compiled by the Statistical Compare process.

The significant compounds for each trial in Statistical Compare were exported into Microsoft Excel for further data processing. In Excel, compounds known to be artefacts of column bleed (i.e. siloxanes, silanols or phthalates) were removed, and controls were manually subtracted from samples. The high sensitivity of the GC×GC-TOFMS means that these artefacts and column bleed are more likely to be detected, and caution must be applied when classifying compounds as decomposition by-products. Finally, the compounds were sorted into 12 classes: sulphur-containing, nitrogen-containing, alcohols, aldehydes, aromatic, carboxylic acids, esters, ethers, halogenated, hydrocarbons, ketones, or other. While the mechanism of their production via the decomposition process is currently unknown, halogenated compounds were included as a category, rather than removing them as artefacts from the internal standard. Some previous studies have reported halogens as biomarkers of decomposition [22, 49, 81], and they cannot be explicitly excluded as decomposition by-products. If necessary, compounds were sorted into two or more compound classes, based on their structure; for example, furan is both an aromatic compound and an ether. The peak areas for the compounds were summed for each class, and the data was graphed accordingly.

Both the unclassified (but otherwise processed data with removal of column bleed, etc) and the classified compound data from Microsoft Excel were loaded into The Unscrambler® X, version 10.3 (Camo Software, Oslo, Norway). The data was normalised (unit vector normalisation), mean-centred, and scaled (by standard deviation) using The Unscrambler® X prior to principal component analysis (PCA). PCA reduces the dimensionality and complexity of large data sets, and allows them to be visualized on graphs as “scores” and “loadings” plots, making it easier to visualize the relationships within the data. Ultimately, the PCA plots allow for the visualisation of the differences between data within a data set, or, in this case, between compounds, compound classes, storage conditions, sample days, sample ages, dilution levels or sample classes.

PCA takes all of the variables present in the data (such as sampling days, types of compounds, and the amount of each compound present in each sample) and transforms them into principal components (PCs). The program generated seven different PCs for these data sets, and the data within each data set was calculated as a set of values or “scores” along the PCs. On the PCA plot,

these scores can be visually compared based on their distribution across the PCs; closely clustered scores demonstrate little to no differentiation, and points which are farther from each other on a PC have a higher degree of differentiation. Each PC axis also displays an axis loading percentage, which indicates how much of the variance within the data has been characterized by that particular PC. The highest variance is always found on the first PC axis, and then each subsequent PC explains the lesser variances in the data. For example, a PC-1 axis loading of 30% indicates that 30% of the variance within the data can be explained by the first principal component. The scores points can also be compared to the loadings plot, which shows the variables (the compounds or compound classes) that have influenced the differentiation of the points seen within the scores plot. For example, if PC-1 demonstrates a high degree of differentiation between Day 0 and Day 7 in the scores plot, the corresponding PC in the loadings plot will show which compounds have the most influence on this differentiation.

The plots used in this chapter report only the first two PCs, since they highlight the majority of the differentiation between the samples within each data set. PCs three through seven did not demonstrate enough differentiation to improve the results of the first two PCs, and thus, were not included in the data presented herein. Loadings plots have also been included in the results sections to show the compounds and/or compound classes that had the most influence on the discrimination of each data set.

Several of the plots also contain correlation loadings, which are shown as concentric ovals on the plot. Any compounds located in the outer ring of the ovals are compounds of significance, and have had the most influence on the variance between scores along that PC. Some of the data has been displayed in PCA bi-plots, which overlap the scores and loadings plots so that the influence of the loadings on the scores can be more easily examined.

3.3 Results

This section reports the results for three distinct chemical profiling trials. The first trial is a comparison of the human decomposition fluid odour profile compiled in this study with the human cadaveric odour profiles reported in the literature, in order to determine whether decomposition fluid accurately represents the odour profile of decomposed remains. The second

trial examines the odour profile of the decomposition fluid stored under different conditions over time to determine the optimal conditions for storing and maintaining decomposition fluid as a training aid from a chemical perspective. The third trial examines the chemical profile of training samples that have been serially diluted and aged in the refrigerator to mimic the conditions under which cadaver-detection dogs are exposed to decomposition fluid as a training aid. This last trial allowed for a direct comparison of the chemical odour profile to the results of the dog training sessions outlined in Chapter 4, providing an estimation of the limit of detection of GC×GC-TOFMS and the degree of sensitivity of the cadaver-detection dogs to decomposition fluid.

Due to the large number of VOCs produced by decomposition fluid, it was not possible to create calibration curves using standards of each compound to produce a quantitative analysis in this study. Instead, all compounds were assigned a ratio to the internal standard to provide a semi-quantitative analysis, and as a result, only the proportion or abundance of compounds and compound classes will be reported herein.

3.3.1 Comparison of Decompositon Fluid and Human Cadaveric Odour Profiles

When comparing the odour profiles of human decomposition fluid and human cadavers, only the compounds reported in the literature could be used for the compilation of human cadaveric odour profiles. A total of 15 peer-reviewed articles were found, and although several studies reported more than 400 compounds using GC-MS, many did not list the compounds detected in the odour profiles. A total of 236 compounds were identified within the literature. In comparison, the GC×GC-TOFMS analysis detected 1438 compounds in the odour profile of human decomposition fluid. For the full lists of compounds, see Appendices A and B. When these lists of compounds were compared, only 10% of the compounds were identified in both the human cadaveric odour profile and the human decomposition fluid profile. Approximately 5% of the compounds were unique to the odour profile of cadavers described in the literature, while almost 85% of the compounds were unique to the odour profile of decomposition fluid analysed in this study. There were several compounds which were not included in the comparison because they were not present in both lists, but were analogous (e.g. 2,2-dimethyl hexane and 2,4-dimethyl hexane). The

compound lists were therefore classified and sorted to investigate the similarities between the proportions of compound classes within the odour profiles as this was deemed a better method of comparing decomposition odour profiles (Figure 3-2).

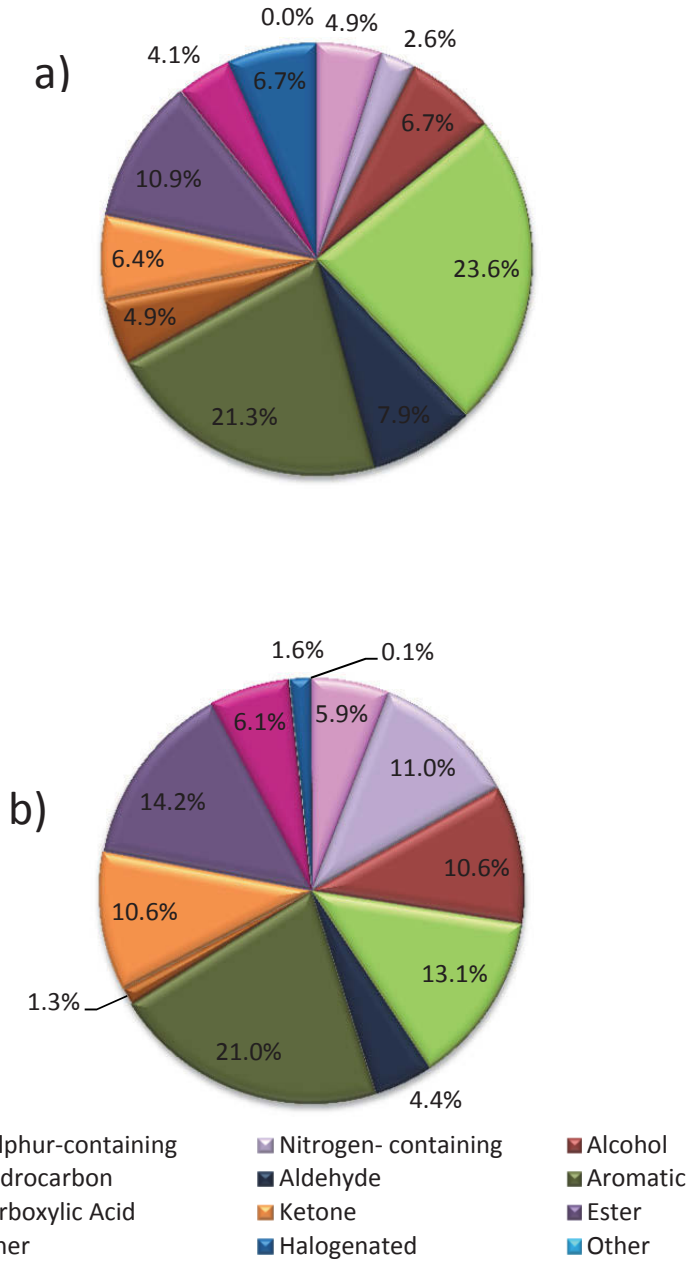

Figure 3-2: The proportion of compound classes found in a) the odour profile of human cadaver decomposition, as reported in the literature, and b) the odour profile of human decomposition fluid, as determined in this study

When these compound lists were classified and the compound class profiles were compared, the odour profiles demonstrated a greater similarity to each other than when comparing individual decomposition VOCs; the majority of the compound classes identified in human decomposition fluid were within 5% of the proportions reported in human remains . The proportions of the majority of compound classes were similar, although the literature reported a higher proportion of hydrocarbons, aldehydes, carboxylic acids, and esters for human remains, while the decomposition fluid produced a larger proportion of sulphur- and nitrogen-containing compounds, alcohols, ketones, and ethers. The literature also reported a large number of halogens, but no 'other' compounds (those compounds which were unable to be classified as per the previous classification categories), while decomposition fluid produced a small proportion of halogenated and 'other' compounds (Figure 3-2).

3.3.2 Storage and Ageing Trials

The odour profiles for the three storage conditions over the course of the year were analysed using PCA (Figure 3-3). While the odour profiles were initially similar (Figure 3-3a), the profiles changed as the samples aged. The odour profiles of the samples stored and aged at room temperature and in the refrigerator remained similar until the last few months of the trial. However, the odour profile of the samples stored and aged in the freezer appeared to change after the first two months, and in the final months of the trial, the profile is clearly distinct from the initial odour profile.

When examining the loadings plot for the storage conditions (Figure 3-3b), the correlation ellipse demonstrated that the differentiation between the room temperature and refrigerator samples resulted from changes in the proportions of benzene, à-myrcene, and several ketones, including octanone and hexanone, within the samples. The compounds that caused the largest differentiation between the samples stored in the freezer and the other two storage conditions were various benzenes, undecane and tridecane. The samples stored and aged in the refrigerator produced the largest number of compounds (1235), while the samples stored and aged in the freezer produced the lowest number of compounds (1002).

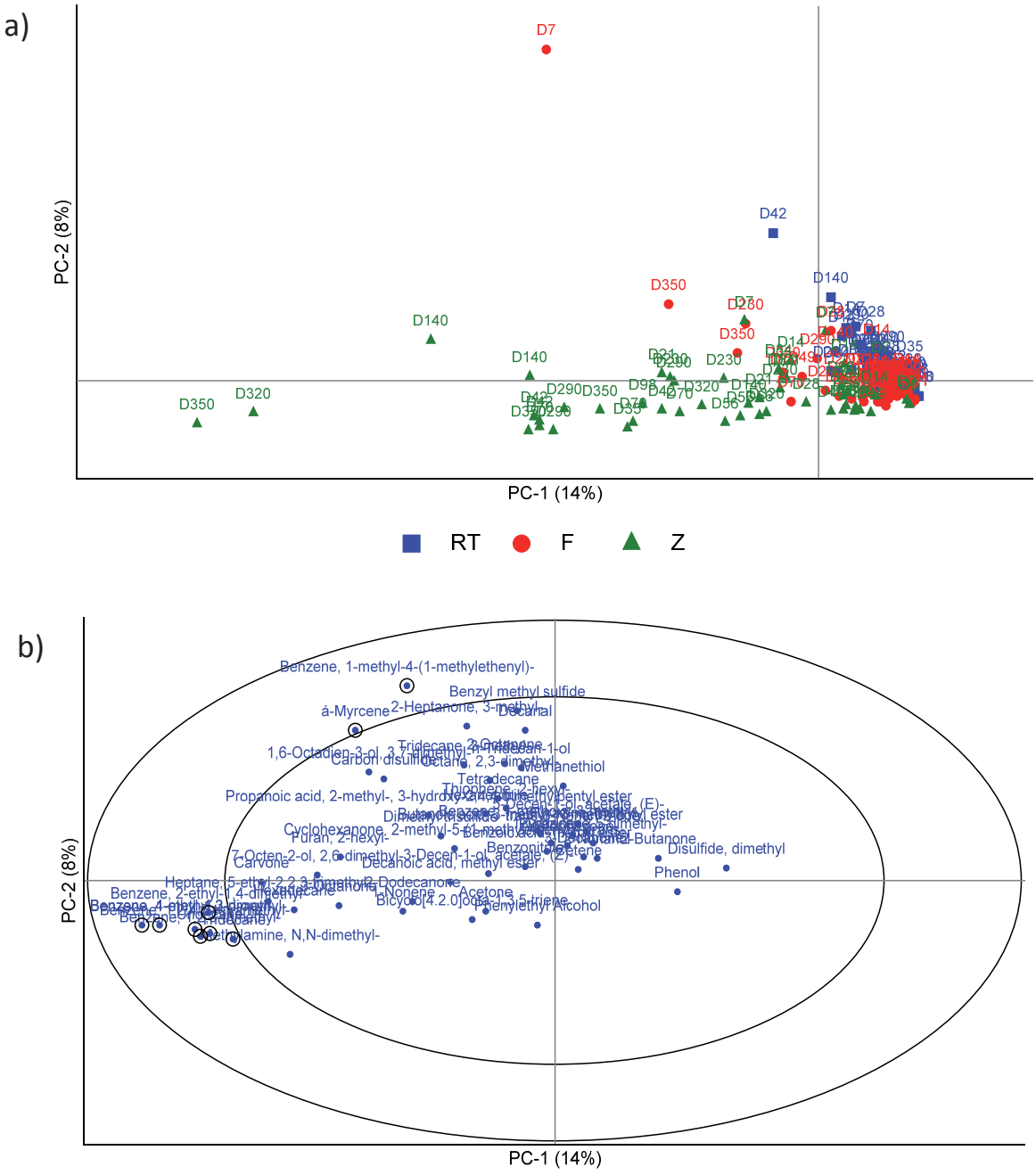

Figure 3-3: PCA plots for the storage and ageing trial: a) Scores plot showing the differentiation between the samples stored and aged: at room temperature (RT, blue), in the refrigerator (F, red) and in the freezer (Z, green), b) Loadings plot indicating the VOCs influencing the differentiation between storage conditions as the samples aged

When the VOCs that were considered significant (see Section 3.2.5 for calculation of significance) in the storage condition trial were sorted by compound class, the differences between the three storage conditions became clearer (Figure 3-4). Overall, aromatic compounds form the largest proportion of the decomposition fluid odour profile for all three storage conditions. However, the odour profile for samples stored at room temperature contained higher proportions of esters and nitrogen-containing compounds than the other two storage conditions. The samples stored in the refrigerator produced more ketones, hydrocarbons and alcohols than the other two storage conditions. The samples stored in the freezer produced the highest proportion of halogenated compounds, aldehydes and sulphur-containing compounds. Overall, a very low proportion of carboxylic acids, halogenated, and other compounds were identified as significant VOCs in the odour profile of decomposition fluid for any of the three storage conditions.

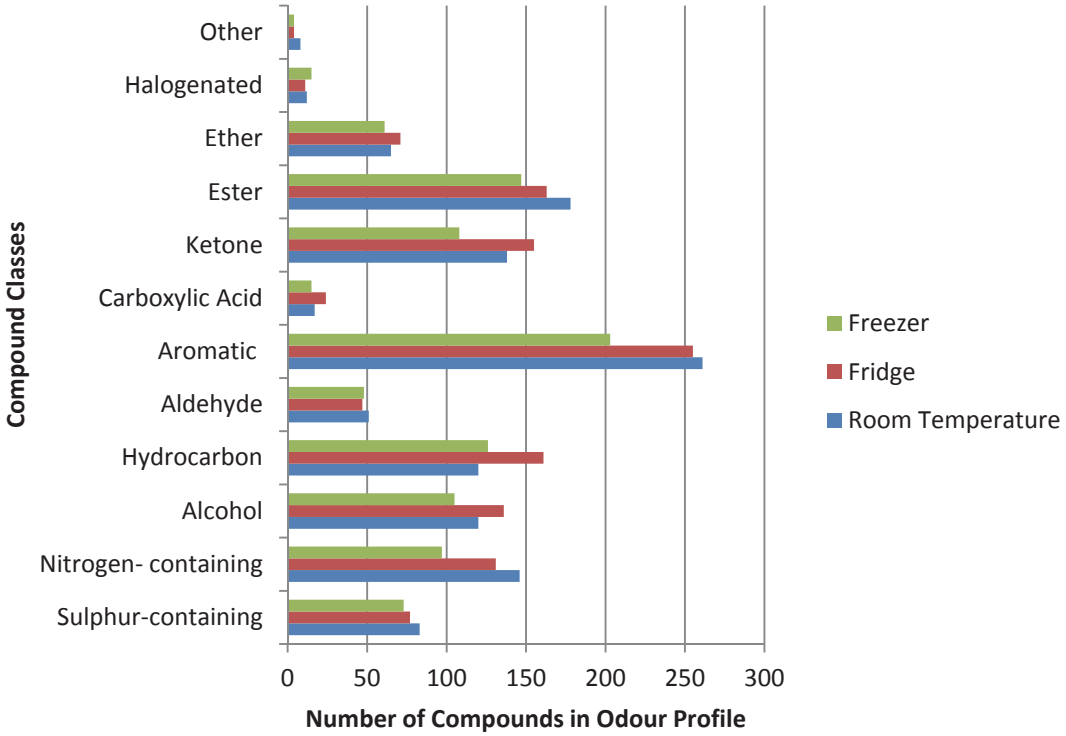

Figure 3-4: A comparison of the number of compounds present within the overall odour profile of decomposition fluid, categorised by compound class between each storage condition

Figure 3-5 highlights the changes to each compound class for the three storage conditions throughout the ageing trial and demonstrates a large proportion of alcohols and aromatics in all of

the samples. For samples stored and aged at room temperature (Figure 3-5a), alcohols and aromatics comprises approximately 50% of the entire VOC profile initially, but this proportion decreases over time. A trend in ketone production was observed, with a peak in ketones towards the end of the initial odour profile (represented by Days 0-21), and again towards the end of the trial (represented by Days 230-290). A relatively large proportion of sulphur-containing compounds were also observed throughout the room temperature trial, although their concentration varied widely from sampling day to sampling day.

The samples stored and aged in the refrigerator (Figure 3-5b) also show a large proportion of alcohols and aromatics, although their abundance does not decrease as evidently over time as in the samples stored and aged at room temperature. A large proportion of ketones were also observed in the samples stored and aged in the refrigerator, and although the abundance is low during the initial profiling period, the proportion of ketones within the odour profile increases steadily as the samples age. The sulphur-containing compounds present in the refrigerated samples demonstrate the opposite trend to the ketones, and decrease as time progresses.

A larger proportion of alcohols were observed in the odour profiles of the samples stored and aged in the freezer compared to the other two storage conditions (Figure 3-5c). In fact, alcohols and aromatics comprise over 90% of the Day 0 odour profile, and over 50% of the odour profile for all the other days, except Day 42. The proportion of alcohols and aromatics decreases over time for the samples aged in the freezer, but not as noticeably as for the samples aged at room temperature and in the refrigerator. A smaller proportion of ketones were identified in the samples and remained constant throughout the freezer trial. Sulphur-containing compounds were identified in the frozen samples, and increased in abundance several times throughout the aging profile (e.g. on Days 21, 42, 84, and 320). There was also a greater proportion of hydrocarbons in the freezer odour profiles that increased in abundance on the same days (Days 21, 42, 84, and 320) as the sulphur-containing compounds.

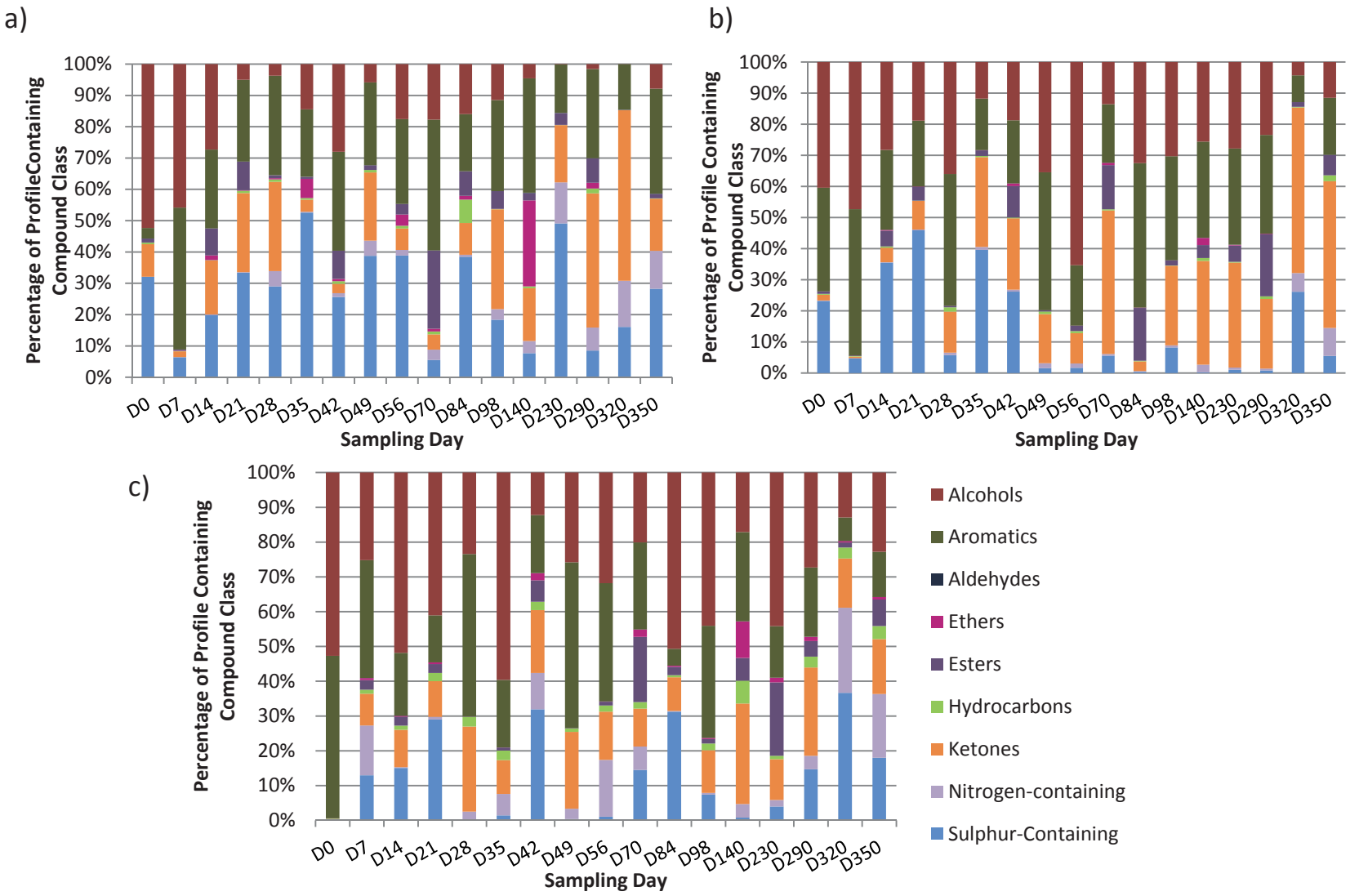

Figure 3-5: Variation in the proportions of compounds by compound class as decomposition fluid samples aged (Day 0 to Day 350): a) at room temperature, b) in the refrigerator, and c) in the freezer

3.3.2.1 Decomposition Fluid Stored and Aged at Room Temperature

By separating the storage condition results into their respective ageing studies, a more detailed investigation of the changes within the odour profile could be carried out. An initial PCA plot of all days and compounds was produced for each storage condition, but demonstrated minimal differentiation (see Appendix C). Once a 95% confidence interval was applied to the results, and the outliers were removed, a more apparent ageing pattern was observed (See Figure C2 in Appendix C).

An examination of all compounds present in the samples revealed that only 7% of the compounds identified in the odour profile during the room temperature trial were present in 80% or more of the samples. For example, 2-decanol appeared in all 51 samples stored and aged at room temperature, while indane appeared in 41, or 80%, of the samples stored at room temperature. Dimethyl disulphide and dimethyl trisulphide, two of the most commonly reported decomposition VOCs, were found in 96% and 94% of the samples, respectively. A complete list of the most common VOCs found in the samples stored and aged at room temperature can be found in Appendix D.

Under room temperature storage conditions, the PCA results showed minimal variations in the odour profile over time. The changes were mostly grouped by age (Days 0-21, initial profile; Days 28-56, early ageing; Days 70-140, mid-ageing; Days 230-250, late ageing period), although some days did show distinct differentiation from the general composition of their age grouping. Since the scores and loadings plots do not provide any additional information, the plots are not included here and can be found in Appendix C. Due to the large number of significant compounds identified in this study, the correlations plot, which explained some of the trends in the scores plot, was also very difficult to read (see Appendix C, Figure C2). This made it difficult to determine which compounds were influencing the differentiation of the ageing samples due to the fact that PCA plots made from large data sets may not give sufficient differentiation between sample groups, even when differences exist. As a result, PCA was performed on the sum of the compound classes in order to reduce the number of compounds in the analysis [87, 88, 125]. The compounds were sorted into their respective compound classes (sulphur-containing, nitrogen-containing, alcohols, aldehydes, aromatic, carboxylic acids, esters, ethers, halogenated, hydrocarbons, ketones, or other; Figure 3-6). PCA was therefore performed to show the ageing of the samples when

influenced by the differentiation of compound classes (Figure 3-7), rather than the individual compounds.

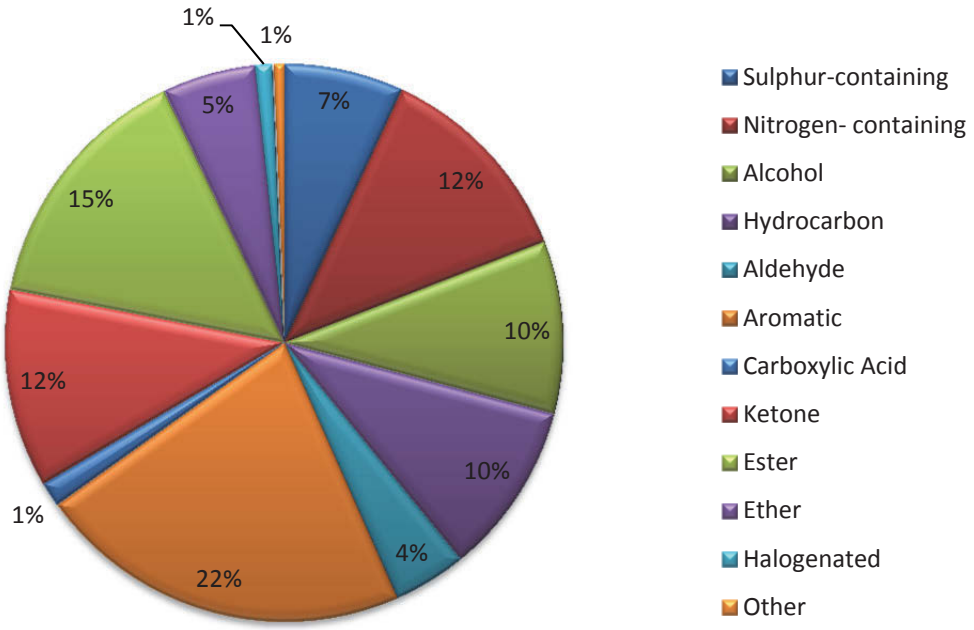

Figure 3-6: Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored at room temperature for a year

Overall, alcohols, aromatics, esters, hydrocarbons, ketones, and nitrogen-containing compounds comprised the majority of the odour profile for samples stored and aged at room temperature (Figure 3-6). When this data was input for PCA, the ageing pattern of the profile was more evident. In Figure 3-7a, the intitial odour profile (Days 0-21) and early ageing profile (Days 28-56) represent distinct groups, but the profile demonstrates greater variance as the samples age (Days 70-350). The loadings plot (Figure 3-7b), shows that aldehydes, aromatics, carboxylic acids, ethers, esters, and nitrogen-containing compounds have the most influence on the variance between the odour profiles. The bi-plot (Figure 3-7c) demonstrates that the initial profile contains larger proportions of halogens, ketones and sulphur-containing compounds, while aldehydes, aromatics, and nitrogen-containing compounds influence the change in the odour profile as the samples age. The samples in the mid- to late ageing period contain more aldehydes, aromatics, carboxylic acids, ethers and esters, allowing the samples aged for these periods to be differentiated from the initial and early ageing periods.

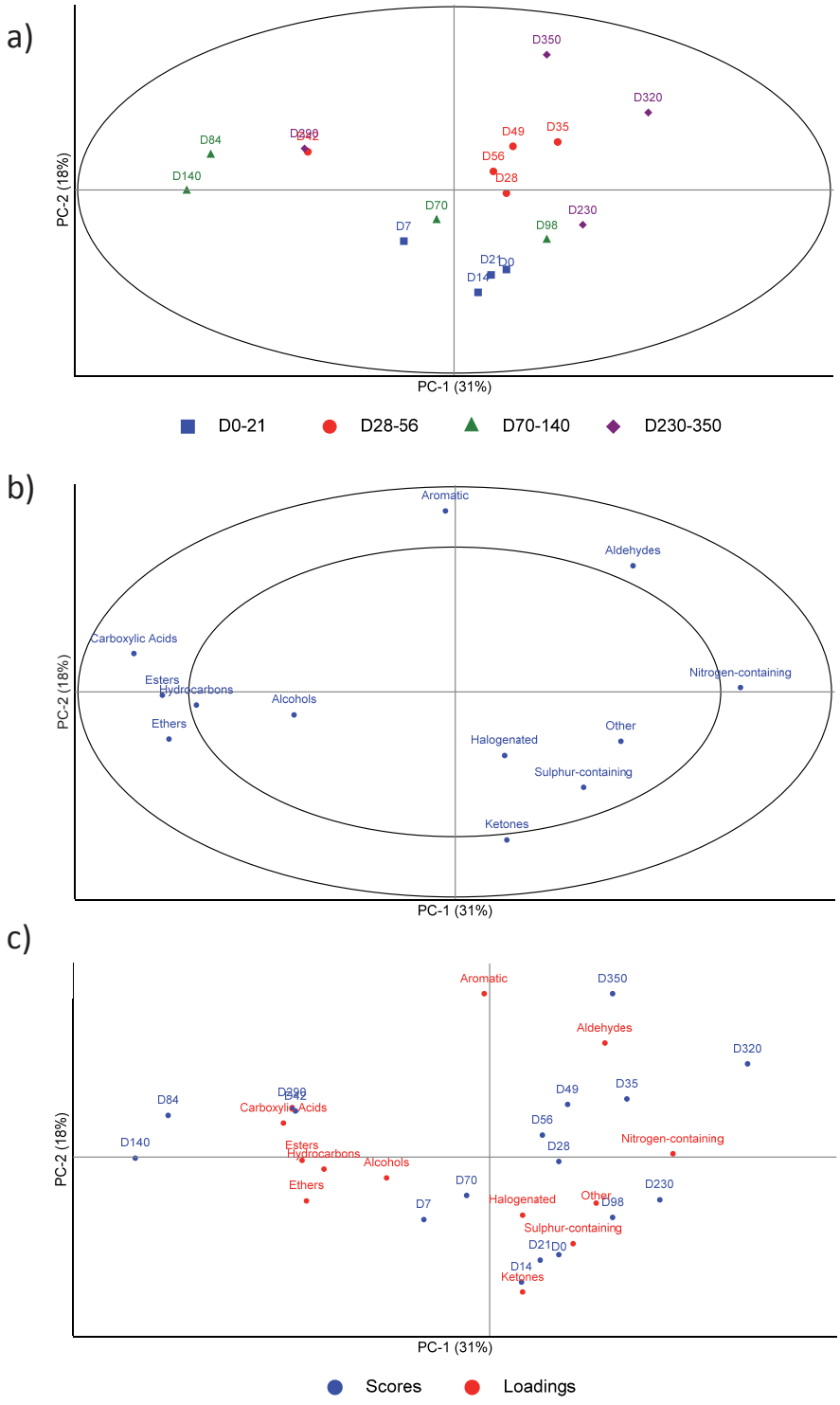

Figure 3-7: PCA plots for the odour of decomposition fluid stored and aged at room temperature, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores

Separating the compound classifications into sampling days (Figure 3-8) demonstrates the same trends observed in Figure 3-7c. Alcohols, aromatics, ketones, and nitrogen-containing compounds comprise the largest proportion of the odour profile each day for samples stored and aged at room temperature. On Day 56, alcohols are at their highest abundance in the mid-ageing period, and are consistently present in the odour profile throughout the ageing process. The aromatics, like the alcohols, are consistently produced throughout the trial, with higher proportions evident in the early (Days 0-21) to mid-ageing period (Days 35-56) and later in the trial (Days 320-350). Ketones were also consistently identified with higher abundances during the initial profiling days, and again around Day 230. The nitrogen-containing compounds were most abundant in the early to mid-ageing period, while the sulphur-containing compounds were most abundant during the initial profile. Carboxylic acids, ethers, and esters were prevalent during the mid and late ageing periods, as was seen in Figure 3-7c, influencing the odour profiles for Days 84-140 and Day 290.

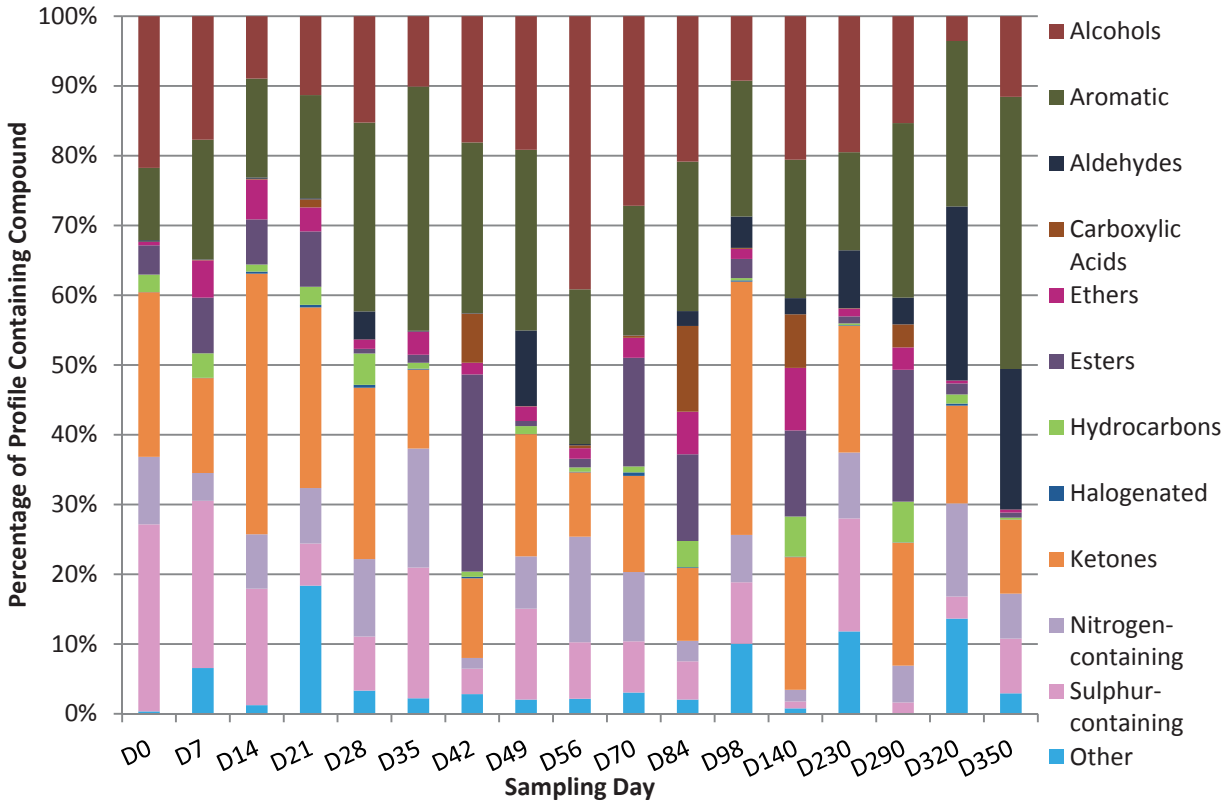

Figure 3-8: Day-to-day change in the proportion of compound classes present in the odour profile of decomposition fluid as it aged at room temperature

3.3.2.2 Decomposition Fluid Stored and Aged in the Refrigerator

The patterns in the odour profiles of samples aged in the refrigerator were harder to distinguish than those seen in the samples stored and aged at room temperature. Even with the removal of the outliers from the initial PCA results (Appendix E), the ageing pattern is less evident when all significant VOCs identified throughout the refrigeration study are included. The odour profiles for the initial early ageing period demonstrated some clustering, but the samples for the remainder of the ageing trial showed a considerable amount of variance (See Appendix E, Figure E2). VOCs were therefore sorted into their compound classes to highlight any discernible trends (Figure 3-9).

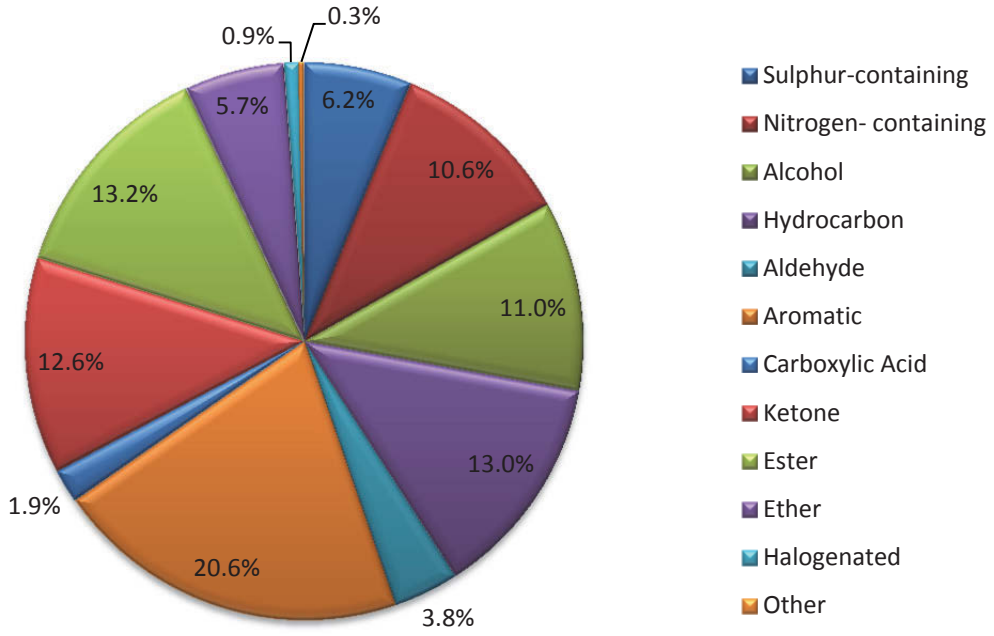

Figure 3-9: Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored in the refrigerator for a year

The odour profiles for samples stored and aged in the refrigerator demonstrated a high proportion of alcohols, aromatics, esters, hydrocarbons, ketones, and nitrogen-containing compounds (Figure 3-9). However, the PCA plot of the compound classes did not show a clear distinction between the ageing periods (Figure 3-10a), although the odour profile during the initial ageing period is distinct from the odour profile during the later ageing periods. The loadings plot (Figure 3-10b) indicates that alcohols, aldehydes, aromatics, carboxylic acids, esters, and sulphur-containing compounds all contribute to the differentiation between samples. An examination of the bi-plot (Figure 3-10c) and the bar graph (Figure 3-11) indicates that the number of alcohols and aromatics increase as

the samples age. Figure 3-11 also indicates that aldehydes were low in abundance overall, but slowly increased as the samples aged. Carboxylic acids demonstrated various increases in abundance throughout the sampling period, but were rarely identified by the end of the ageing trial. The esters steadily increased in proportion, and were most abundant during the mid and late ageing periods. Ketones comprised a moderate proportion of the odour profiles for each of the samples, with a peak in abundance observed in the mid-ageing period (Day 98, Figure 3-11), followed by a decline until the trial ended. Nitrogen- and sulphur-containing compounds were prevalent in the initial profile, declined rapidly by the middle of the early ageing period, and were almost entirely absent by the end of the study.

An examination of the individual compounds present in the samples revealed that only 10% of the compounds identified in the samples stored and aged in the refrigerator were present in 80% or more of the samples. For example, N,N-dimethyl-methylamine appeared in all 51 samples stored and aged in the refrigerator, while 2-nonanone appeared in 92%, of the refrigerated samples. Dimethyl disulphide and dimethyl trisulphide both appeared in 96% of the samples, consistent with the samples stored and aged at room temperature. A complete list of the most common VOCs found in the samples stored and aged in the refrigerator can be found in Appendix F.

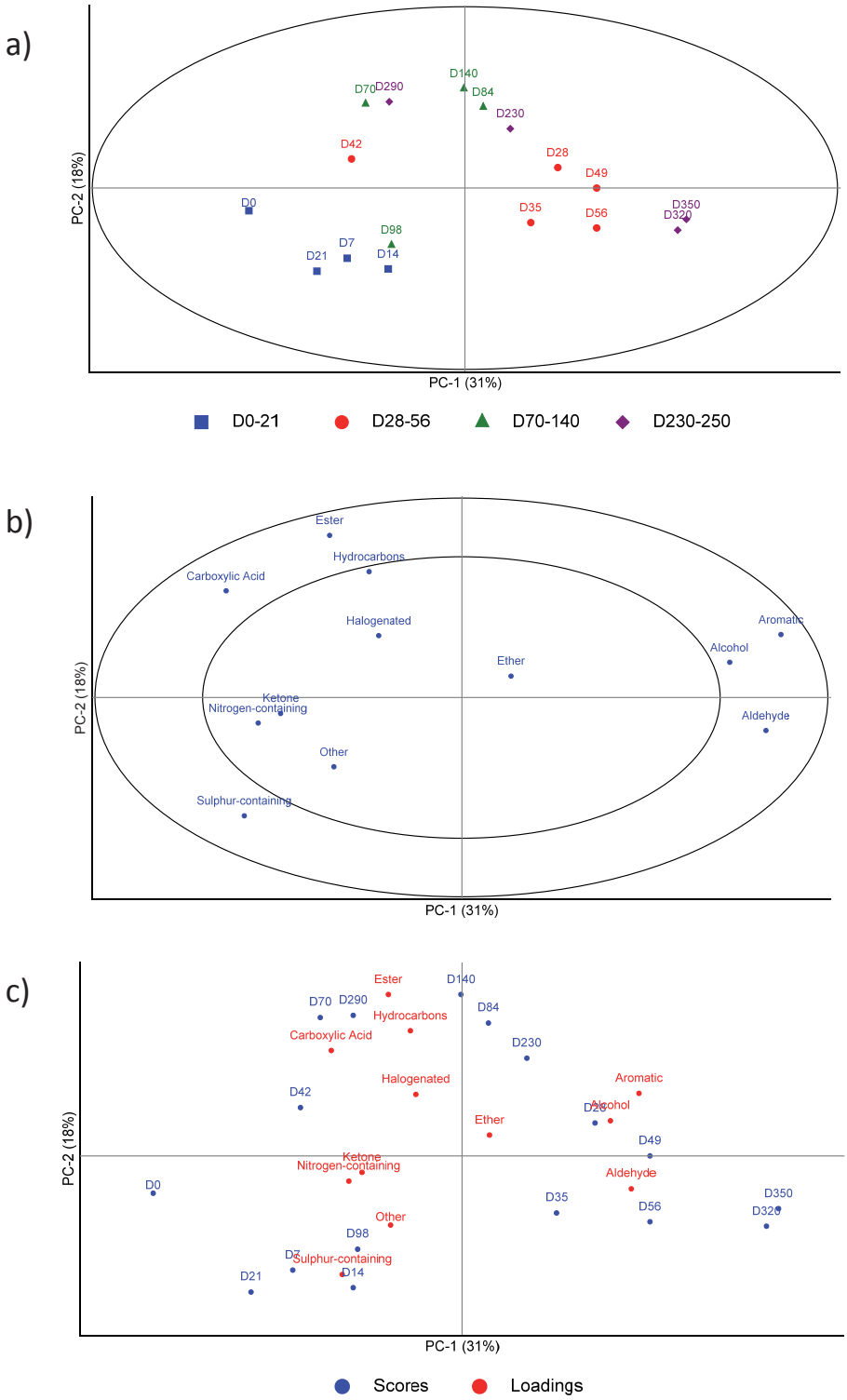

Figure 3-10: PCA plots for the odour of decomposition fluid stored and aged in the refrigerator, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores

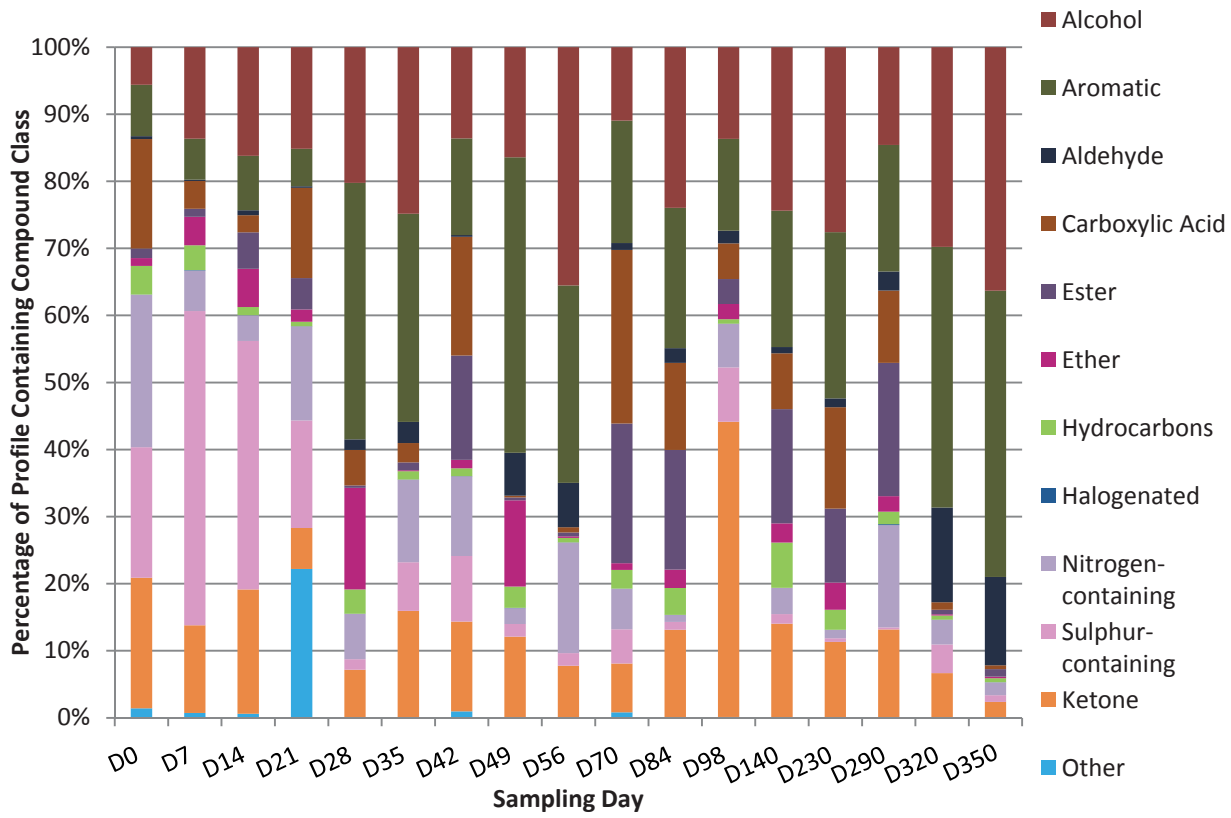

Figure 3-11: Day-to-day change in the proportion of compound classes present in the odour profile of decomposition fluid as it aged in the refrigerator

3.3.2.3 Decomposition Fluid Stored and Aged in the Freezer

Of the three storage conditions investigated in this study, the trends were most evident for samples stored and aged in the freezer (see Figure 3-12 and Appendix G). Samples in the initial and early ageing periods were distinctly grouped. The mid-ageing period (Days 70-140) demonstrated a high level of variation between sampling days, while the late ageing period (Days 2330-350) demonstrated clustering with some variation towards the end of the study (Figure 3-12a). The loadings plot (Figure 3-12b) is difficult to interpret due to the high number of VOCs identified, however, compounds such as benzenes, hexenol, ethanone, and limonene influenced the sample differentiation along PC-1, while other benzene compounds, butanoic acids and indenes influenced the profile variations along PC-2.

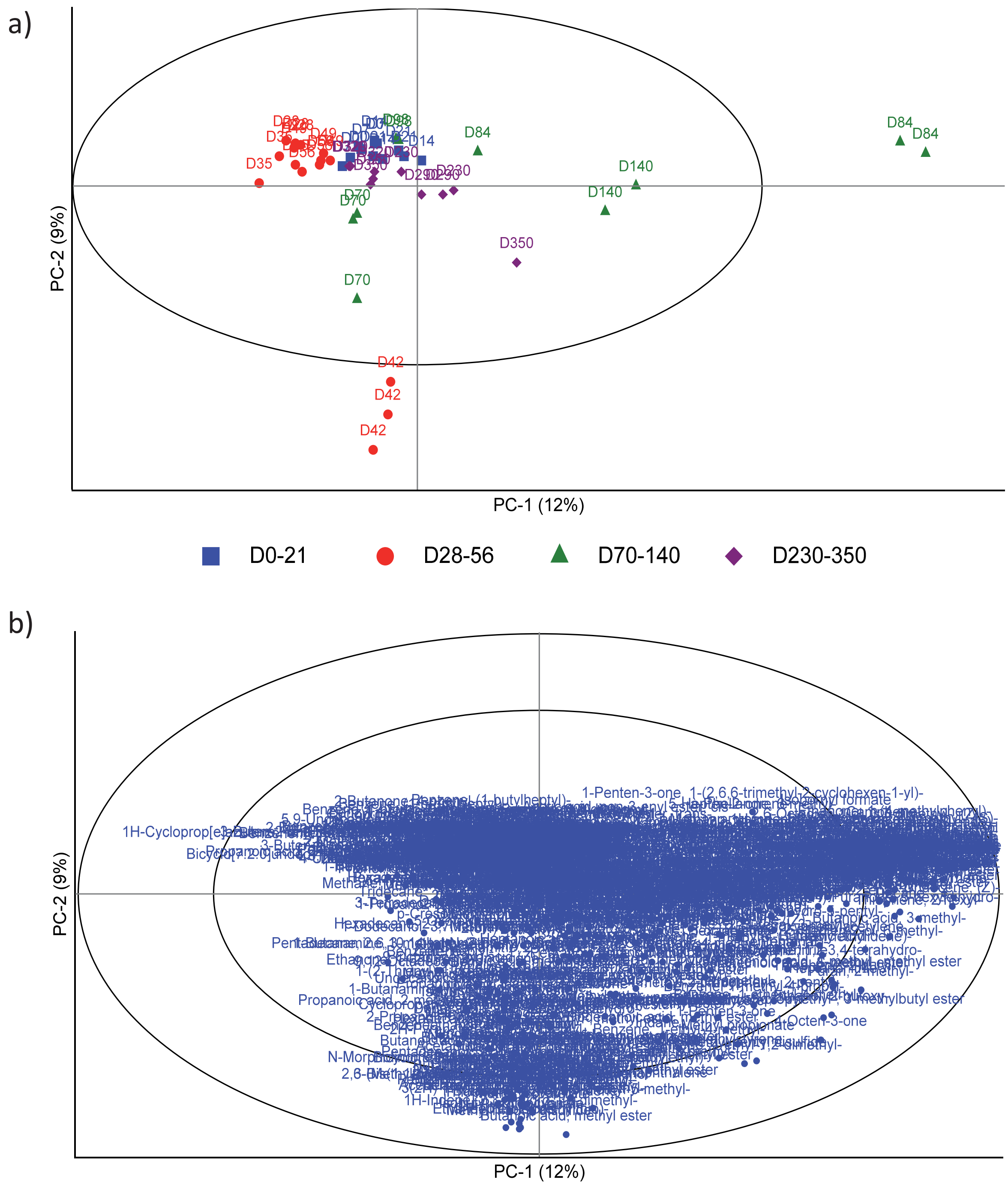

Figure 3-12: PCA plots for the odour of decomposition fluid stored and aged in the freezer, with outliers removed: a) Scores plot sorted by days and all compounds, original outliers removed, 95% CI, b) Loadings plot demonstrating the VOCs influencing the variations among the samples in plot A

An examination of the individual compounds present in the samples revealed that only 9% of all compounds identified in the samples stored and aged in the freezer were present in 80% or more of the samples. Dimethyl disulphide and dimethyl trisulphide both appeared in 100% of the samples (consistent with samples stored and aged at room temperature and in the refrigerator), while hexadecane appeared in 80% of the samples. A complete list of the most common VOCs found in the samples stored and aged in the freezer can be found in Appendix H.

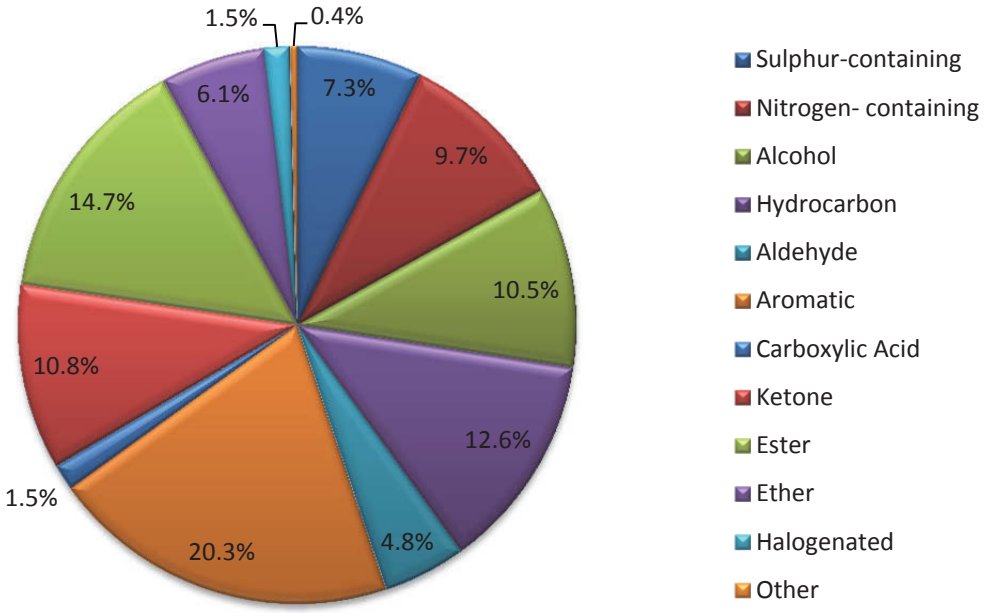

Figure 3-13: Overall proportions of compound classes present in the odour profile of decomposition fluid when samples have been stored in the freezer for a year

When the significant VOCs were sorted into compound classes, they demonstrated a high proportion of aromatics, alcohols, esters, hydrocarbons, and ketones in the overall odour profile for samples stored and aged in the freezer (Figure 3-13). The PCA plot (Figure 3-14a) shows that the initial profile is distinct from the other three ageing periods, but there is no clear distinction between the early, mid, and late ageing periods.

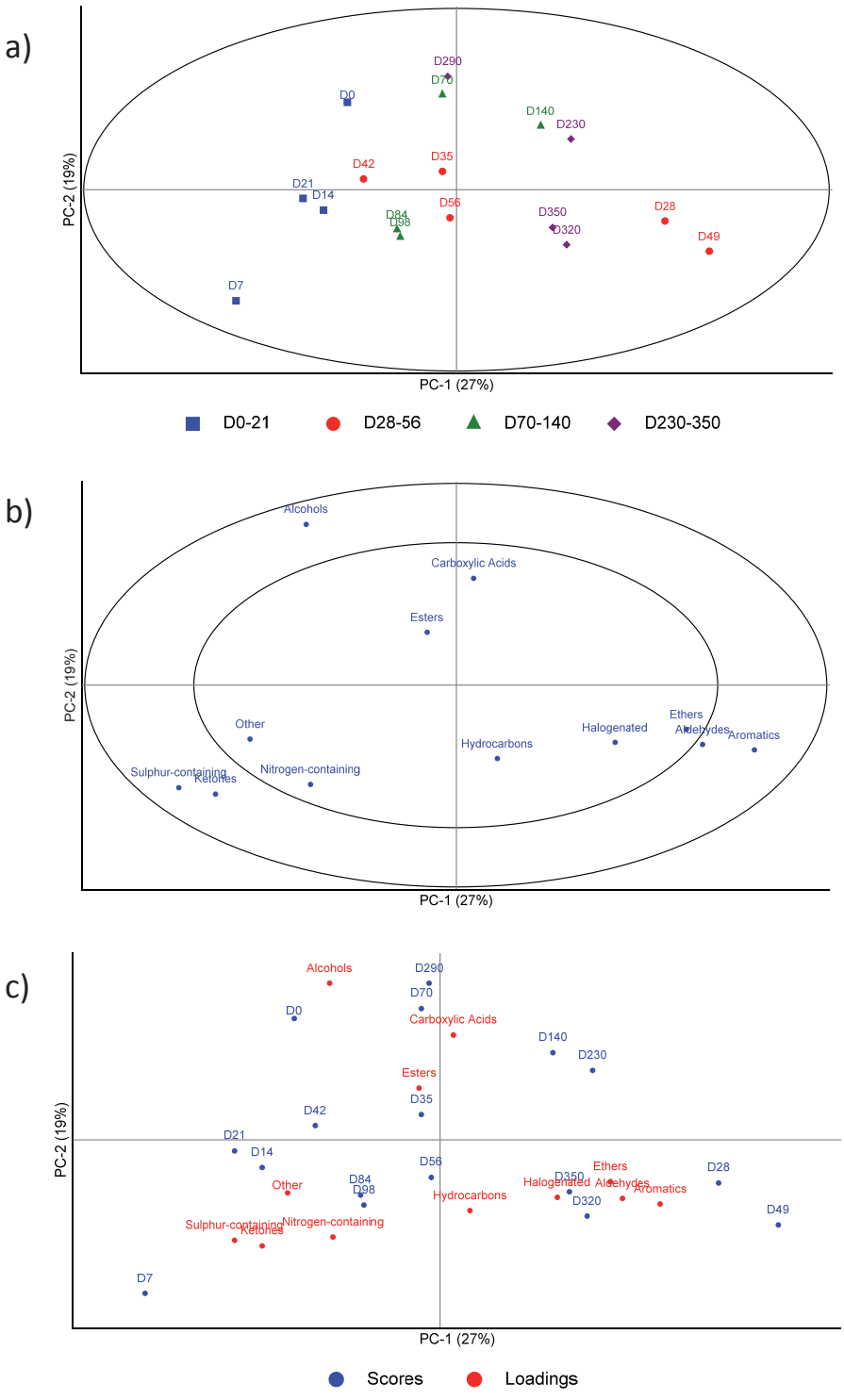

Figure 3-14: PCA plots for the odour of decomposition fluid stored and aged in the freezer, sorted by compound class: a) Scores plot with 95% CI, b) loadings plot with correlation loadings ellipse, c) bi-plot overlapping A and B to show the influence of the loadings on the scores

The loadings plot (Figure 3-14b) indicates that the alcohols, aldehydes, aromatics, ketones and sulphur-containing compounds had the greatest influence on the differentiation of the odour profiles. A comparison of the bi-plot (Figure 3-14c) and the bar graph (Figure 3-15) indicate that the alcohols have a large influence on the overall profile. The alcohols form a high proportion of the odour profile initially, again at the beginning of the mid-ageing period, and during the late ageing period. Aldehydes form only a small proportion of the overall odour profile, but they have a large influence on the differentiation of the late ageing period samples from the other sampling days. Ketones and sulphur-containing compounds are largely present in the initial profile, and again during the late ageing period, although typically in less abundance.

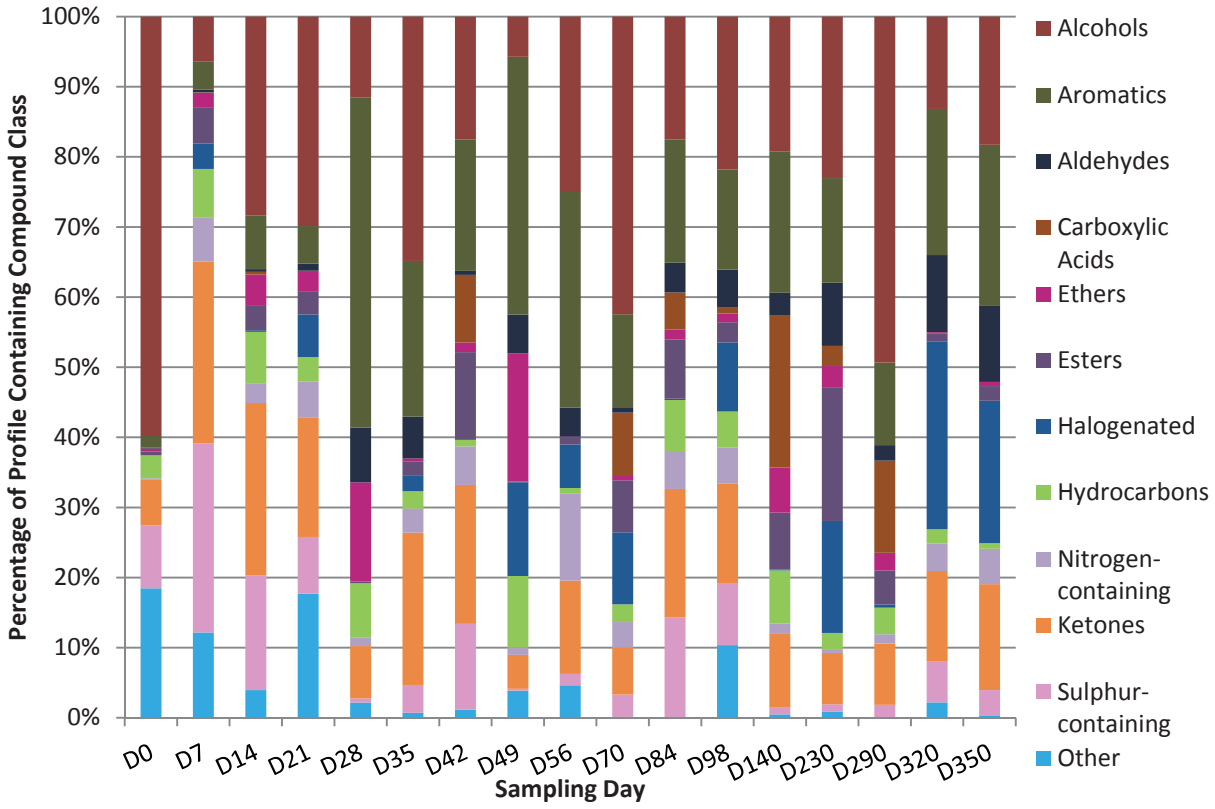

Figure 3-15: Day-to-day change in the proportion of compound classes present in the odour profile of decomposition fluid as it aged in the freezer

3.3.3 Dilution and Ageing Trials

Based on the preliminary odour profile results from the storage and ageing trials, it was decided that the study should be extended and compared to the dogs' responses. The samples stored in the refrigerator were brought to room temperature and diluted for each of the dog training sessions so that the chemical profiles identified in the storage and ageing trials could be compared to the sensitivity of the cadaver-detection dogs. Since the profile was still recognisable as "decomposition odour" after 12 months of ageing in the refrigerator, the trial was also extended to determine the odour profile of training aids used for up to 24 months.

When the significant VOCs of all of the aged and diluted decomposition fluid samples (from 3 months to 24 months) were analysed, it was discovered that 30% of all compounds identified in the dilutions were present in 80% or more of the samples. For example, hexadecane appeared in all 124 diluted samples, while 3-pentanone appeared in 101, or 82%, of the samples. A complete list of the most common VOCs found in the diluted and aged training samples can be found in Appendix I.

The significant VOCs were input for PCA (Figure 3-16). The stock sample and 10^{-1}, 10^{-2} and 10^{-3} dilutions could be differentiated along PC-1 based on their odour profiles; however, dilutions of 10^{-4} and below could not be discriminated from each other (Figure 3-16a). Dilutions below this level tended to cluster based on age, with the 6-month, 9-month and 12-month aged samples being separated along PC-2. Notably, the 3-month diluted samples demonstrated a different odour profile to the 6, 9, and 12-month samples, but could not be differentiated from samples aged beyond 12 months and up to two years. The loadings plot (Figure 3-16b) indicates that the stock, 10^{-1} and 10^{-2} samples are differentiated from the lower dilutions by an abundance of pyrazines, benzenes, and nonanone. The 6, 9, and 12- month samples were differentiated because of higher levels of undecenes and decanes in the odour profiles. In PCA, however, a 21% cumulative differentiation between both the first and second dimensions indicates a low overall differentiation between the samples [80]. The responses of the cadaver-detection dogs (reported in Chapter 4) indicated that the diluted samples still contained decomposition odour, even when highly dilute, suggesting that the instrumentation was not sufficiently sensitive to detect the many trace decomposition VOCs present in the samples below dilutions of 10^{-3}.

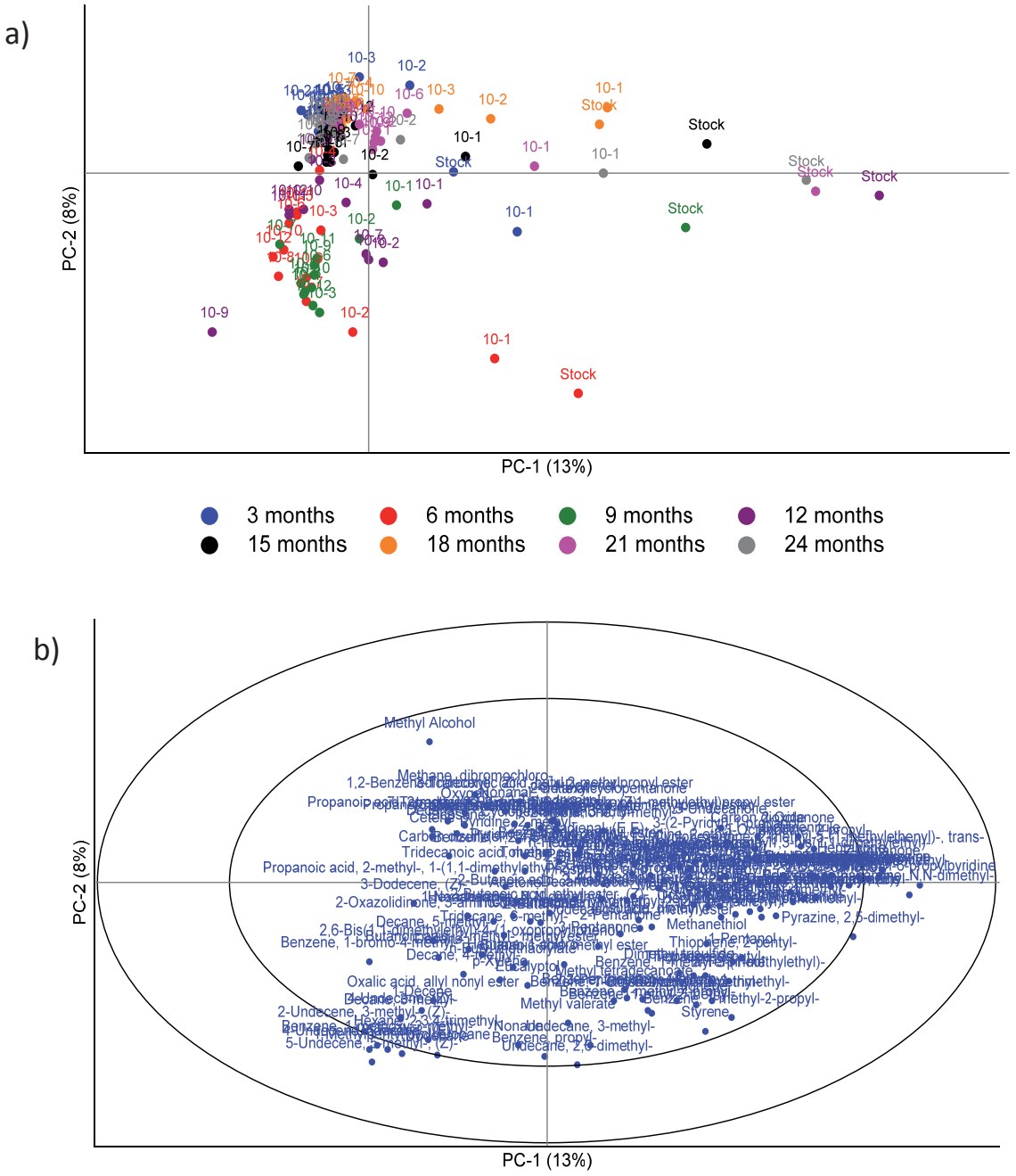

Figure 3-16: PCA plots for the odour profile of diluted and aged decomposition fluid, with all significant compounds present: a) Scores plot displaying all dilution levels and ages of samples, b) loadings plot demonstrating significant VOCs influencing differentiation of samples in plot A

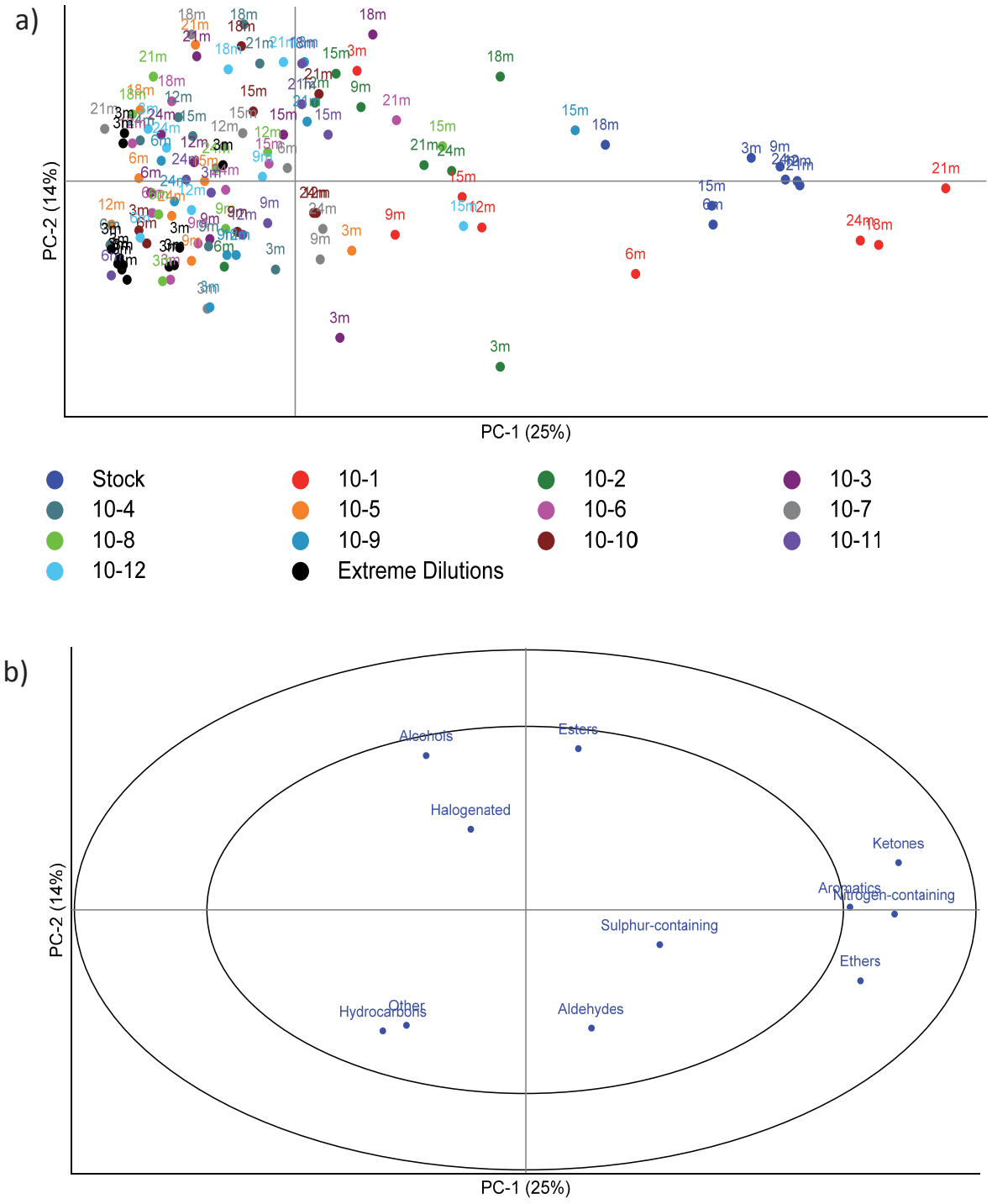

Figure 3-17: PCA plots for the odour profile of diluted and aged decomposition fluid, sorted by compound class: a) scores plot displaying separation of aged samples by dilution level, b) loadings plot demonstrating the compound classes influencing the differentiation in plot A.

Similar to the storage and ageing trials, it is easier to see trends in the diluted and aged samples when the VOCs are sorted by compound classes. A PCA plot of the dilutions, separated by compound class (Figure 3-17a) indicates that the aromatics, ethers, ketones, and nitrogen-containing compounds had a noticeable effect on the differentiation between the stock, 10^{-1}, 10^{-2} and 10^{-3} dilutions, and the more diluted samples (Figure 3-17b). There is little distinction between the different ages of the fluid samples in this plot, however, these trends can be more clearly seen when the samples are analysed using bar graphs (see Figures 3-18 to 3-25).

3.3.3.1 Dilutions Aged for 3, 6, 9 and 12 Months

In the diluted samples aged for 3 months (Figure 3-18), the proportion of 'other' compounds, such as anhydrous hexanoic acid, as well as alcohols increased as the compounds became more dilute. The nitrogen-containing compounds were lost from the profile early in the dilution series, while aromatic compounds decreased in abundance as the samples were diluted.

In the diluted samples aged for 6 months (Figure 3-19), the proportions of hydrocarbons increased, but the alcohols, aromatics and ketones decreased as the samples were diluted. Hydrocarbons comprised over 50% of the odour profile in the samples diluted beyond 10^{-5} in the 6 month aged samples. The diluted samples aged for 9 months (Figure 3-20) also showed a decrease in alcohols, aromatics and sulphur-containing compounds. The sulphur-containing compounds were not detected in the lowest dilutions. However, there was an increase in the proportions of esters and hydrocarbons in the odour profiles as the samples were diluted. The alcohols, hydrocarbons and ketones increased in abundance as the diluted samples were aged for 12 months (Figure 3-21), accompanied by a decrease in abundance of aromatics, halogens and 'other' compounds.

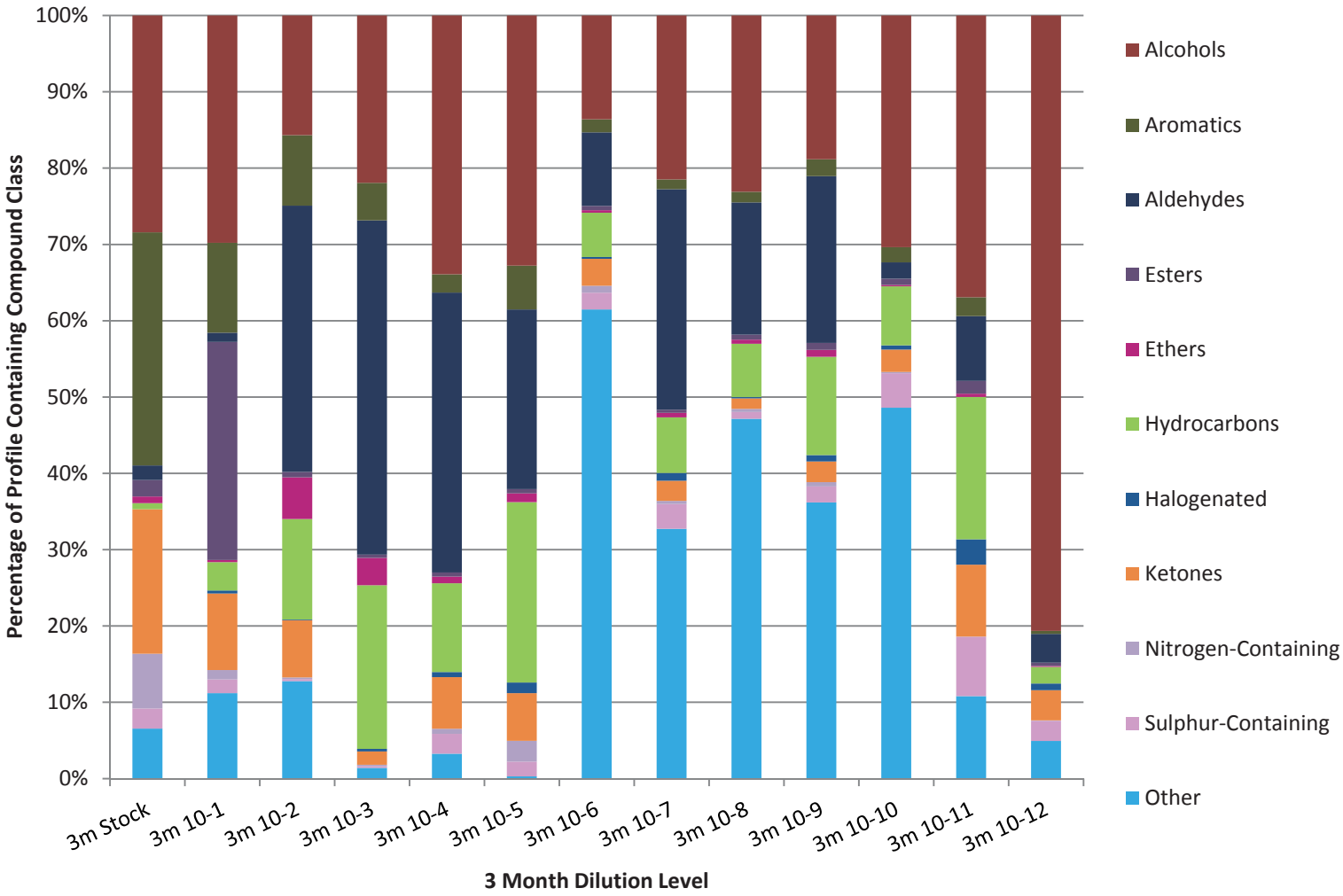

Figure 3-18: Proportion of compound classes present in the odour profile of 3 month aged and diluted decomposition fluid

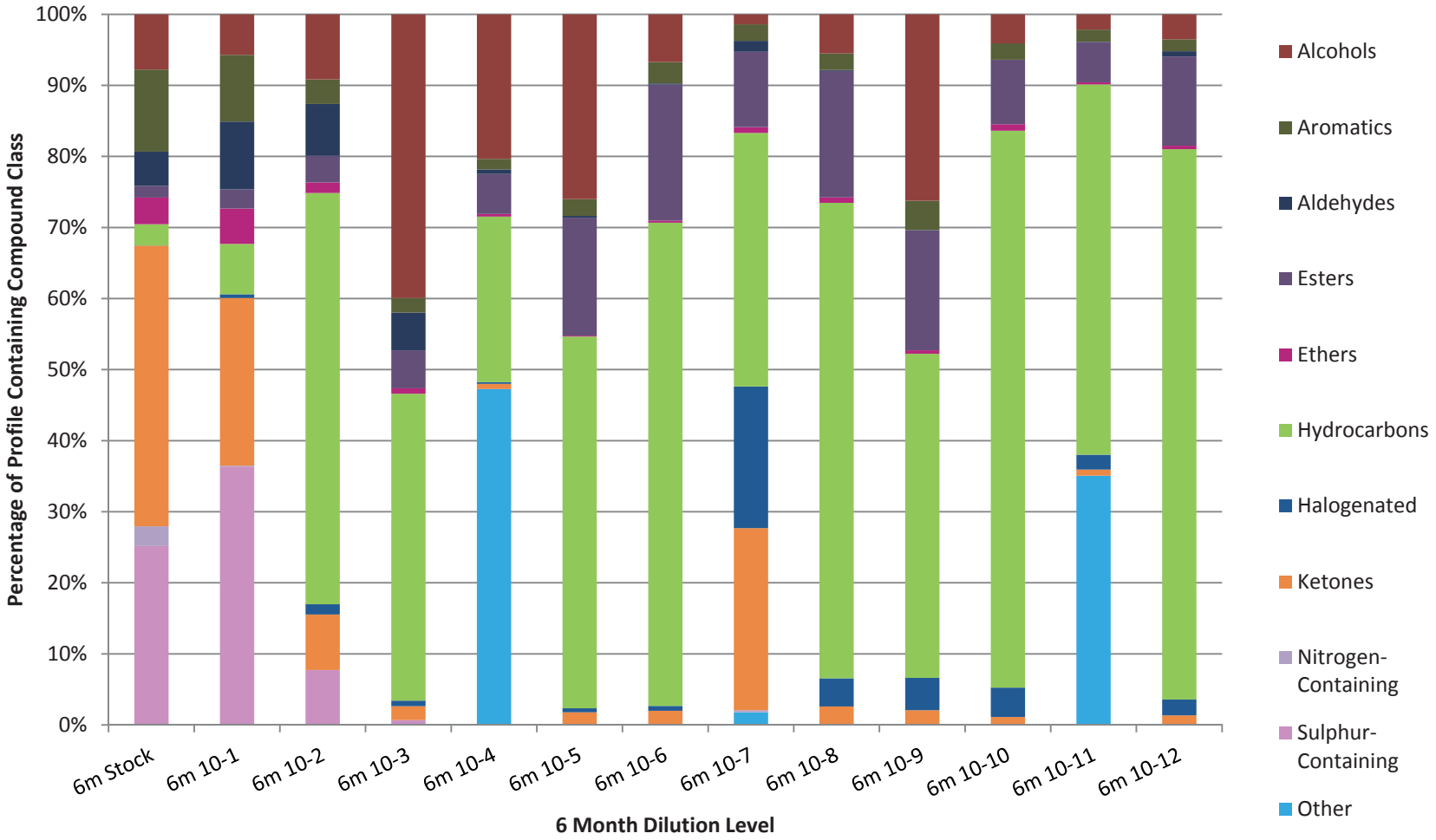

Figure 3-19: Proportion of compound classes present in the odour profile of 6 month aged and diluted decomposition fluid

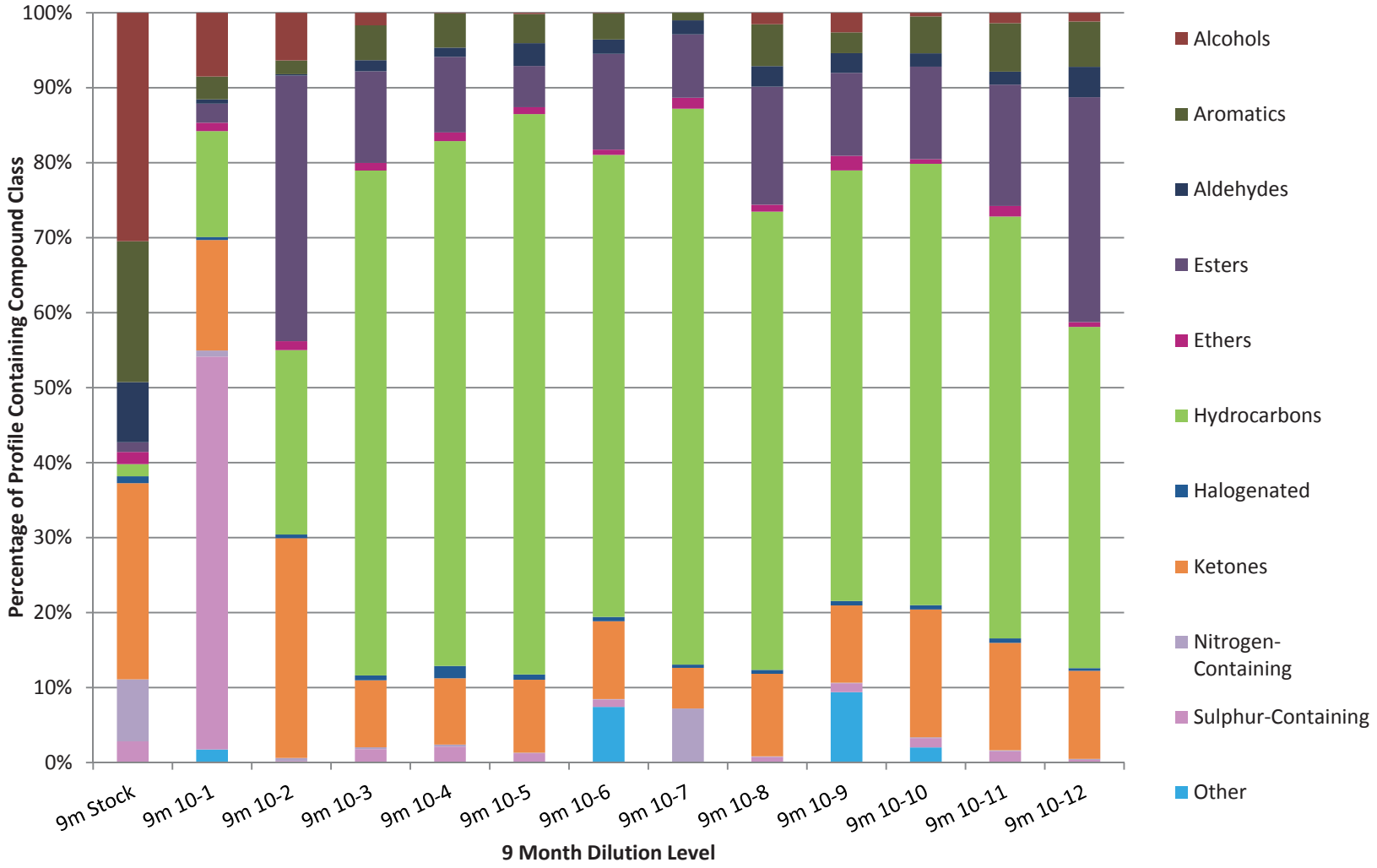

Figure 3-20: Proportion of compound classes present in the odour profile of 9 month aged and diluted decomposition fluid

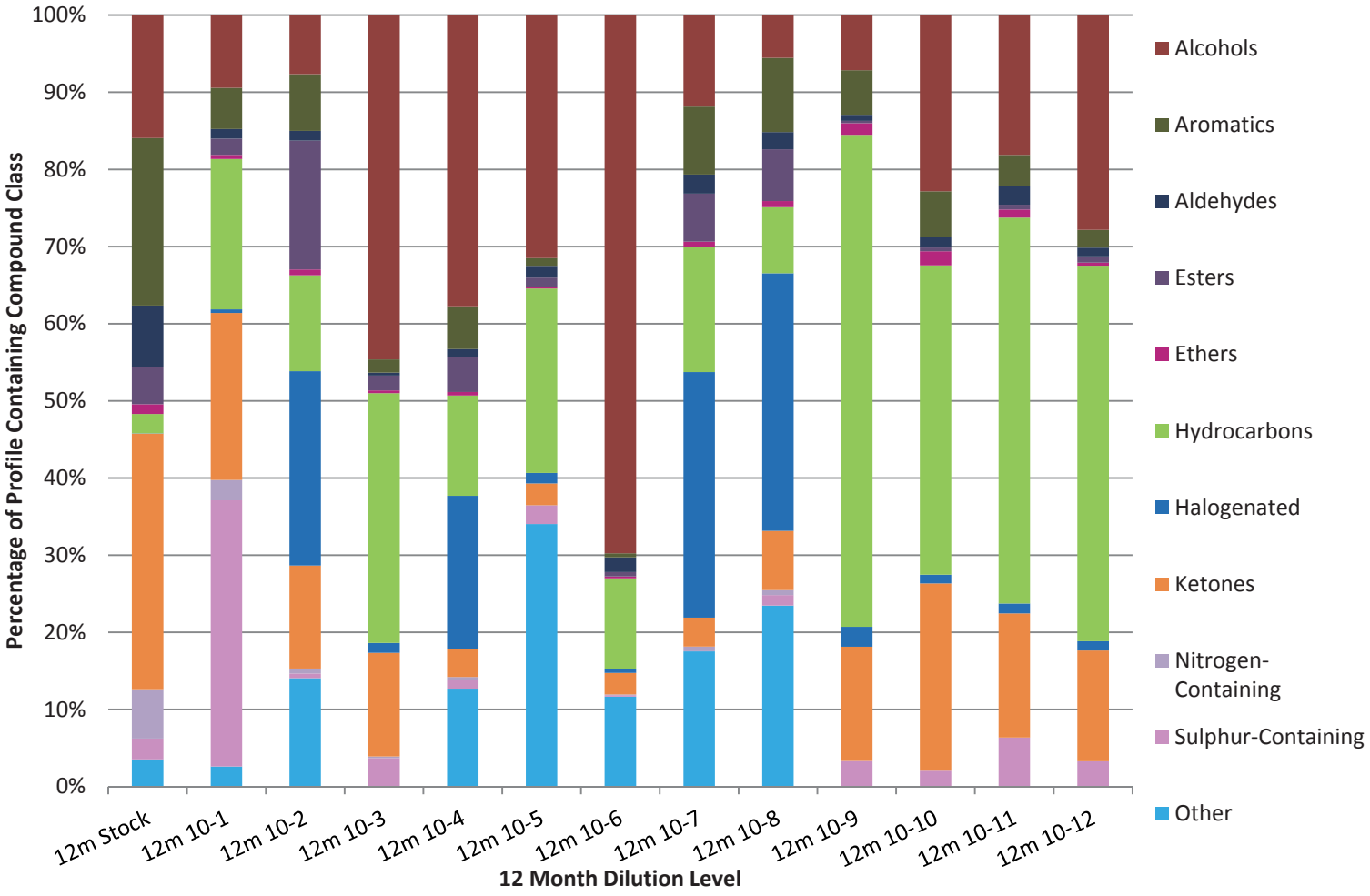

Figure 3-21: Proportion of compound classes present in the odour profile of 12 month aged and diluted decomposition fluid

3.3.3.2 Dilutions Aged for 15, 18, 21 and 24 Months

As the samples aged beyond 12 months, the odour profile continued to change dynamically. There were a large proportion of alcohols present in the diluted samples aged for 15 months (Figure 3-22). The sulphur-containing compounds disappeared from the profile early in the dilution series, while the proportions of aromatics and 'other' compounds increased.

The diluted samples aged for 18 months (Figure 3-23) also demonstrated an increase in the proportion of alcohols in the odour profile as the samples were diluted. The samples demonstrated large proportions of esters and ketones, although, unlike all of the previous dilution series, there were few hydrocarbons present in this set of odour profiles.

The sulphur-containing compounds in the diluted samples aged for 21 months (Figure 3-24) decreased noticeably in abundance after the 10^{-2} dilution, but there were still some sulphur-containing compounds present in the lowest (10^{-12}) dilution. There was also an increase in the abundance of alcohols, while the aromatics formed a small, decreasing proportion of the odour profile. These samples also contained a relatively high abundance of halogens and ketones, when compared to the other dilution series.

The oldest samples, aged for 24 months (Figure 3-25), contained large proportions of alcohols and hydrocarbons. The ketones decreased as the samples were diluted, while the nitrogen- and sulphur-containing compounds were lost from the profile early in the dilution series.

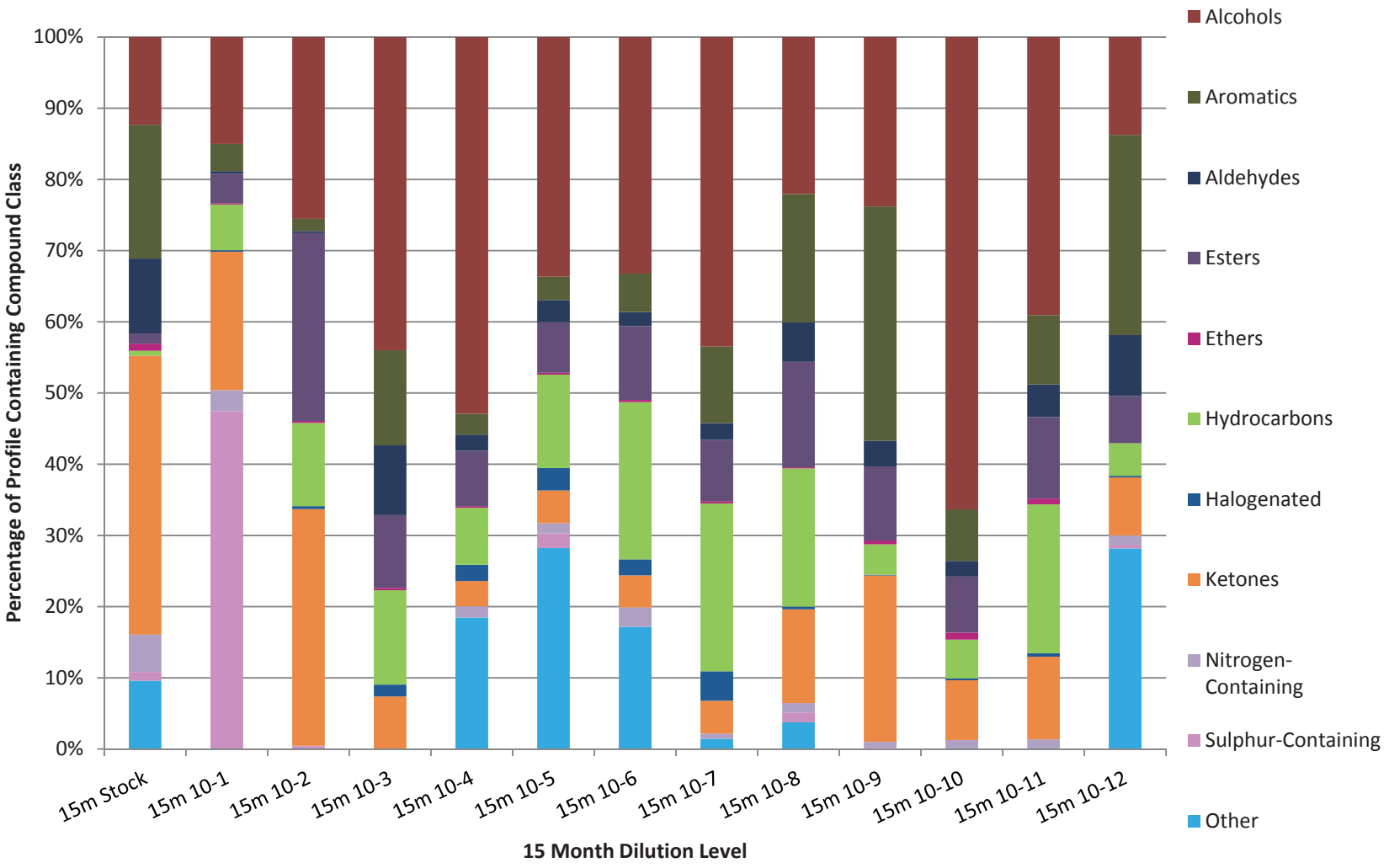

Figure 3-22: Proportion of compound classes present in the odour profile of 15 month aged and diluted decomposition fluid

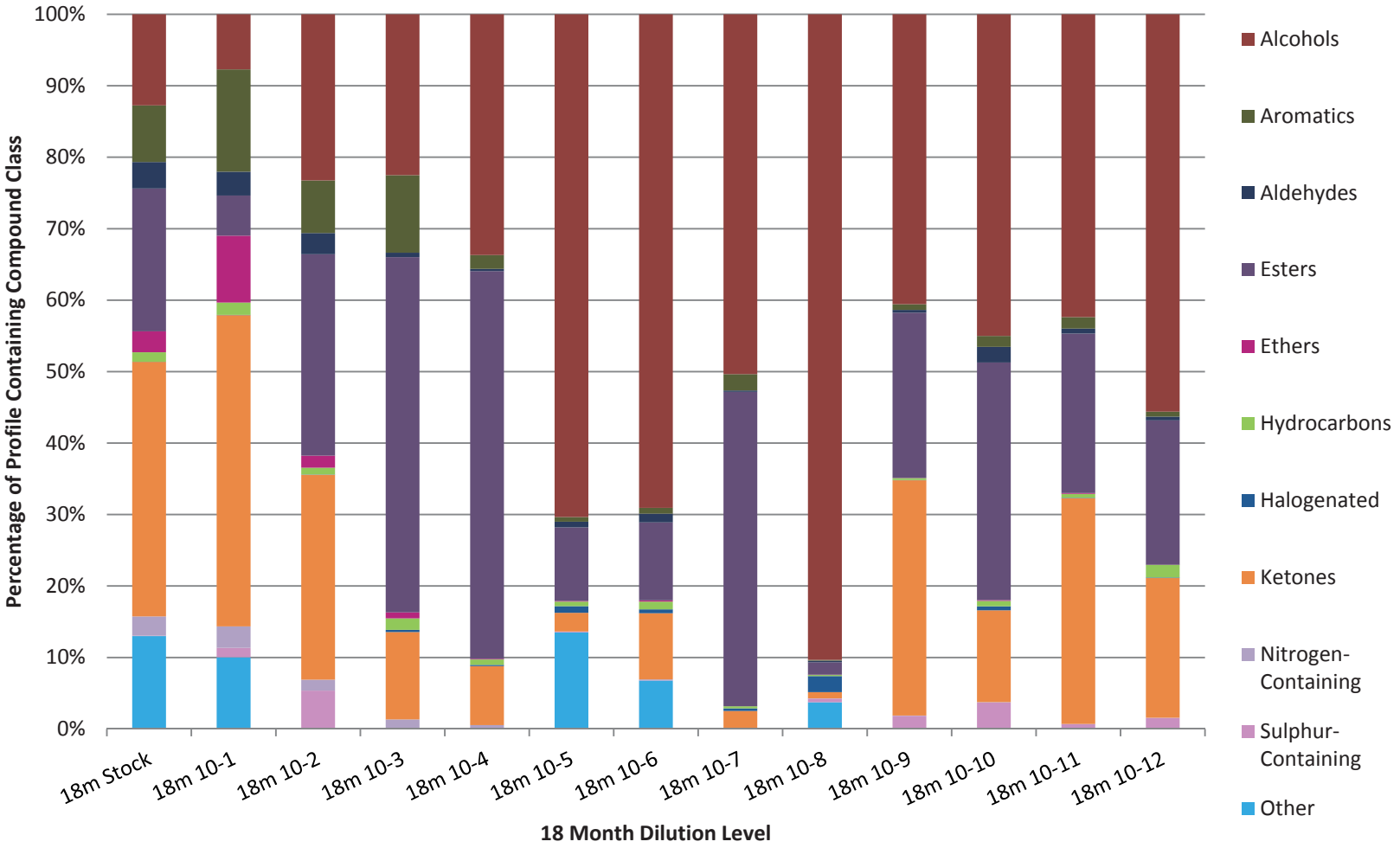

Figure 3-23: Proportion of compound classes present in the odour profile of 18 month aged and diluted decomposition fluid

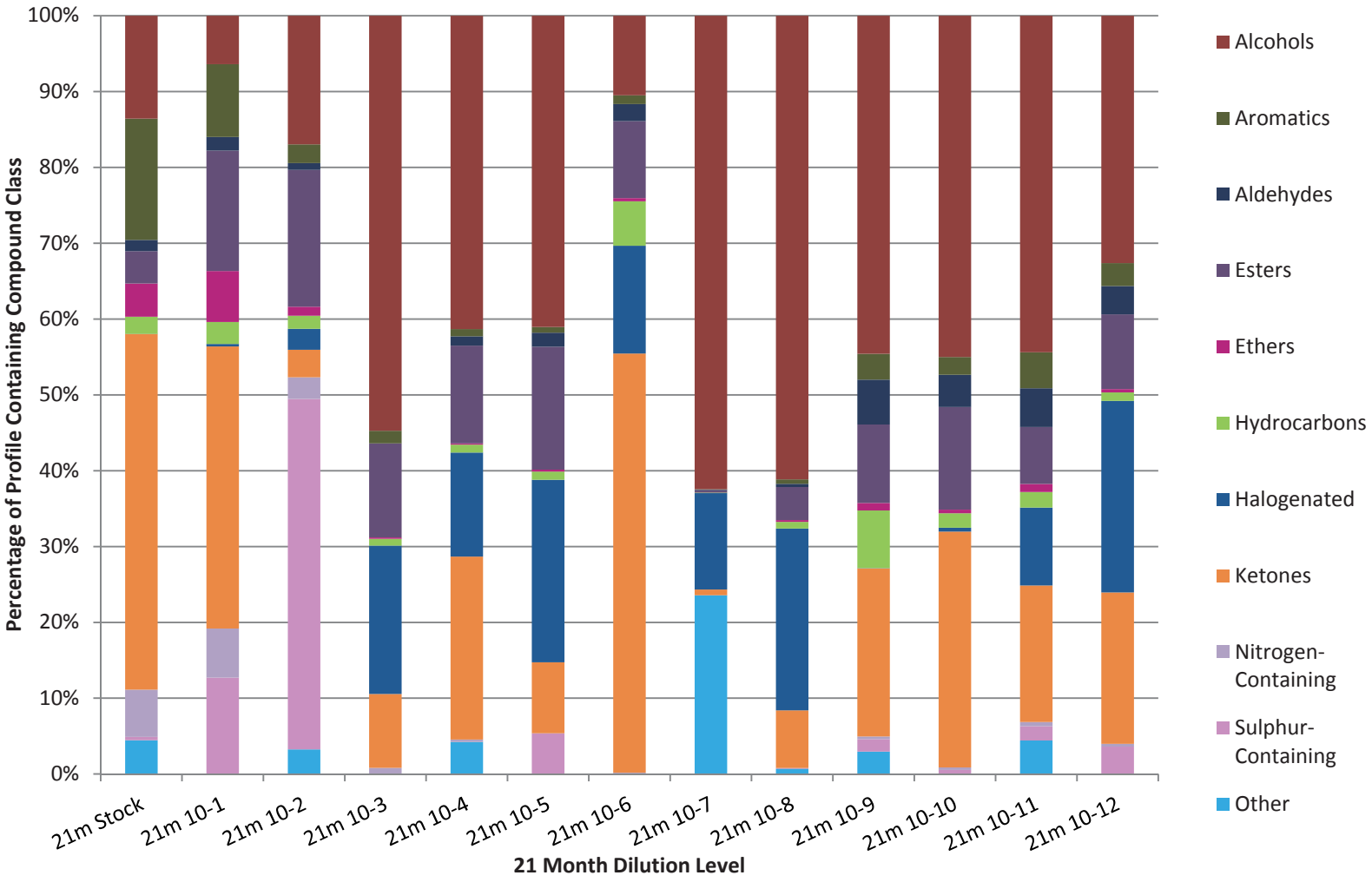

Figure 3-24: Proportion of compound classes present in the odour profile of 21 month aged and diluted decomposition fluid

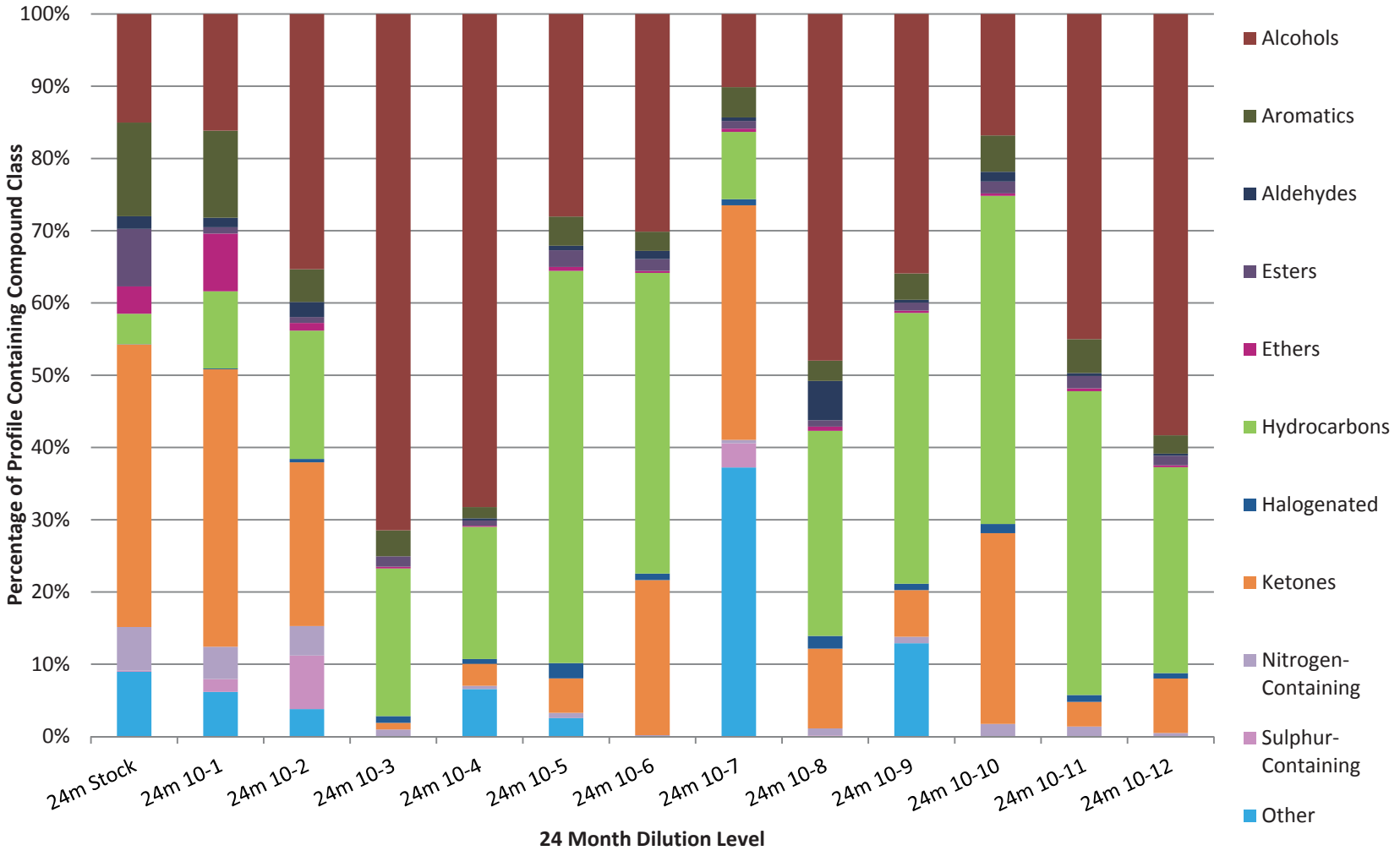

Figure 3-25: Proportion of compound classes present in the odour profile of 24 month aged and diluted decomposition fluid

In general, the alcohols and hydrocarbons increased in proportion within the profiles as the samples were diluted. The aldehydes, aromatics, esters, ketones, and sulphur-containing compounds generally decreased in proportion within the odour profile as the samples were diluted. The ethers, halogens and nitrogen-containing compounds were generally low in abundance, but did not change noticeably in abundance within the profiles. While the alcohols, aromatics, hydrocarbons and ketones comprised the majority of the odour profiles for most of the dilution series profiles, there was little consistency to the pattern of compound class changes within the odour profile of decomposition fluid both as it was diluted, and as those samples aged.

As the dilutions aged (from Figure 3-18 to Figure 3-25), there was an increase in the proportion of alcohols. The proportion of aromatic compounds remained relatively steady throughout the ageing process, but the aldehydes decreased and were not detected within the odour profile by 12 months. Esters were high in abundance in the 6 (Figure 3-19), 9 (Figure 3-20) and 18 (Figure 3-23) month samples, and then decreased in abundance towards the end of the trial. Ethers were low in abundance throughout the ageing profile. Hydrocarbons formed a large proportion of the profile from 6-12 months (Figure 3-19, 3-20 and 3-21), and increased in abundance again at 24 months (Figure 3-25). Halogenated compounds were highest in abundance in the 12 and 21 month samples (Figures 3-21 and 3-24). Ketones increased steadily in proportion over time, as did the 'other' compounds. Nitrogen-containing compounds formed a small proportion of the profile throughout, although they increased in abundance as the sample aged to 24 months (Figure 3-25), while the sulphur-containing compounds demonstrated the opposite, with a maximum abundance between 6 and 15 months (Figures 3-19 to 3-22) but were negligible by the end of the trial at 24 months (Figure 3-25).

In general, as the fluid was diluted (shown by the individual bar graphs in Figures 3-18 to 3-25), there was a general loss of the low abundance compound classes, and the proportions of the alcohols and hydrocarbons tended to increase as the samples were diluted.

3.4 Discussion

3.4.1 Comparison of Decomposition Fluid and Human Cadaveric Odour Profiles

This trial aimed to chemically profile the odour of decomposition fluid as a cadaver-detection dog training aid, and to compare the profile to that reported for decomposing human cadavers in the literature. This was carried out to determine whether the odour profile of the fluid is sufficiently similar to decomposed remains to be considered a valid substitute when training the dogs.

A direct comparison between the compounds listed in the literature and the compounds identified in this study revealed a large discrepancy between the data. The comparison identified only a 10% overlap between the compounds reported for decomposed remains and identified for decomposition fluid, which could not be considered similar in terms of their odour profiles. Although several studies reported identifying more than 400 compounds in their decomposition odour profiles, not all studies listed all of the compounds identified. Approximately 200 compounds were listed in the literature, which represents only half of the number of VOCs claimed to have been identified for decomposed remains. The current study identified more than 1400 compounds for decomposition fluid, making it difficult to conduct a direct comparison with the literature. This was particularly the case for the large number of analogous compounds, which could have resulted from differences in the variables surrounding the decomposition processes for the bodies and the body tissues in each of the studies.

The comparison of the proportion of compound classes in the odour profiles was more representative. This classification system could account for variations in the number of compounds produced, and for analogous compounds, unlike the comparisons of the VOCs. The proportions of compound classes demonstrated a greater similarity between the profiles, suggesting that the odour profiles produced by the decomposition fluid could be considered a valid representation of decomposed remains.

There were some variations in the compound-classified odour profiles from the literature, which could be accounted for through the types of studies that were conducted. Many of the studies reported in the literature used different types of sample collection techniques than those used in this study, such as liquid extractions and liquid injections [119, 129], headspace injections [49],

sorbent tubes [22, 50, 81, 85, 120], or different types of SPME fibres [83, 84, 122]. Some of the studies investigated the composition of decomposition by-products in the laboratory [51, 83, 119, 129], or involved long-term studies of decomposing bodies at a field site [22, 49, 81], while others collected single samples from bodies available during case studies [47, 85, 120, 122]. Several studies investigated the live scent of humans, which could reasonably be produced by the recently deceased [135, 154] and victims of unknown status, such as those trapped in the wreckage of an earthquake [85, 102, 120, 135].

It is unknown how the original, living scent of a person affects the decomposition odour profile, or how the composition of their body may affect it. It is well-documented that humans produce unique scents, which tracking canines can use to identify and locate people when they are alive [156-162]. It has also recently been reported that this scent changes to a more generic decomposition odour profile as the decomposition process progresses [163], but it is unclear how much of the body's original, unique scent remains in the decomposition odour profile. All of these variables could readily affect the types of VOCs produced, collected and analysed for the odour profiles reported in the literature.

This study used cadaveric tissues with no bones or internal organs present in the samples to produce decomposition fluid, and collected samples of the odour profile from different stages throughout the decomposition process. Thus, the decomposition fluid more closely mimics that produced by dismembered limbs, since many of the internal bacteria and enzymes would be unavailable to decompose these detached body parts. The absence of internal organs, as well as the potential changes in the odour profile during the different stages at which it was collected, may have affected the proportion of compound classes identified in the decomposition fluid. It is possible that the different ratios of tissues to organs and bones, as well as the presence of gut bacteria in a decomposing cadaver would cause the body to produce a slightly different odour profile than that produced by the individual tissues when removed from the body to form decomposition fluid. Due to the logistics of the experiments, the decomposition fluid was collected from the donors and then aged separately from the cadavers. It is therefore unknown whether the samples of aged decomposition fluid would produce the same odour at, for example day 54, as the odour produced by a cadaver that had been decomposing intact for 54 days. A more rigorous procedure would have collected the decomposition fluid as it was produced by an aging

cadaver. With the recent opening of the AFTER facility, an appropriate comparison of the odour profiles could be completed with a future study.

Further, the studies reported in the literature predominantly used GC-MS to analyse and identify the compounds present in their samples, while this study used a more advanced technique for odour profile analysis (i.e. GC×GC-TOFMS). Given the limitations of GC-MS for analysing complex matrices (discussed in Chapter 2), it is possible that the differences in compounds identified in the decomposition fluid profile are due to the increased accuracy, sensitivity, and separation capacity of the GC×GC-TOFMS. The increased number of compounds in this study when compared to previous studies can be predominantly attributed to the advantages of this method; for example, it is difficult to detect trace level compounds using GC-MS, but these same compounds could be detected with the increased sensitivity of the GC×GC. It is also possible that compounds were misidentified, or masked by co-eluting compounds in the GC-MS profile, but these same compounds were better resolved and accurately identified by the GC×GC-TOFMS [101].

Overall, human decomposition fluid is one of the best representations of a decomposing human cadaver. It is certainly a better training aid than the synthetic scents currently available (see Section 1.1.3), which contain fewer than 10 compounds, most of which are amines and ketones [57, 58]. The synthetic scents do not represent the complexity of the decomposition odour profile that has been demonstrated in this study. Decomposition fluid may also be better than other training aids, such as blood or bone, which would have odour profiles unique to the particular tissue used. If alternative training aids are used, whether single-tissue or synthetic scents, it is recommended that they be employed in conjunction with decomposition fluid in order to ensure that the cadaver-detection dogs are exposed to the widest spectrum of the decomposition odour profile.

Any future studies in this area should compare the decomposition fluid collected from a cadaver (not just tissues) to the odour profile produced by a cadaver *in situ*. This would allow for a direct comparison between the odour profiles of decomposition fluid and decomposed remains in the same environment and was the original intent of this study. It would also provide a better comparison between the odour profiles of decomposition fluid in a controlled training area (where the effects of environmental variables such as air flow, solar radiation, temperature, and precipitation are controlled and minimized), to that of a body present in an environment with a

large number of decomposition variables, such as soil type, water content, scent barriers, fluctuating temperatures, and changes in air flow, where a cadaver-detection dog could reasonably be tasked with searching (see Sections 1.1.1 and 1.2).

3.4.2 Storage and Ageing Trials

This trial examined the typical storage and handling conditions used by the NSW Police Dog Unit for their training aids, in order to determine the validity of the decomposition fluid when it has been stored under these conditions for a period of time. It also aimed to determine the conditions which would maximize the validity and longevity of the training aid.

In general, the samples stored and aged at room temperature and in the refrigerator showed minimal variation in the odour profile as it aged, until the last few months (i.e. Days 230 to 350, or 9-12 months) of the sampling period. However, the odour profile for samples stored in the freezer began to change after the first month. This was not anticipated, since the low temperatures of the freezer should, theoretically, halt the degradation process. It was assumed that the temperature in the freezer would not be optimal for bacteria and other micro-organisms that drive the decomposition process. It was originally hypothesized that the odour profile for samples stored at room temperature would change the most of all the storage conditions, since room temperature is within the optimal range that most decomposition bacteria function. It is likely that since the sealed vials limited the oxygen present for the degradation reactions, the aerobic micro-organisms driving the degradation were ultimately out-competed by anaerobic bacteria. These anaerobic bacteria, combined with the much lower temperatures of the freezer, which could have inhibited both the aerobic and anaerobic bacteria, may have been sufficient to cause the distinct profile variation observed in the samples stored in the freezer.

The samples stored and aged in the freezer also contained a larger abundance of aldehydes, ethers, and sulphur-containing compounds, while the samples stored and aged in the refrigerator contained more alcohols, hydrocarbons, ketones, and carboxylic acids. The samples stored and aged at room temperature contained a large proportion of aromatics, esters, nitrogen-containing and other compounds. Throughout the ageing process, there were a range of trends within each of the compound classes, and across each storage condition. Aside from a high proportion of

alcohols and aromatics in each storage condition, there were no clear trends for any of the compound classes across any of the storage conditions. The causes of these differences are not well understood, since the chemical processes involved in decomposition have not been extensively studied. The decomposition of tissues, organs and large macromolecules into various compound classes has been studied [74], but the exact route of formation for each individual compound is not clear. Therefore, it is difficult to determine what mechanism is causing the production of higher proportions of one type of compound class over another in each sample or storage condition.

However, the different temperatures of the storage conditions are likely the most influential factor for the changes in the decomposition pathways that create different proportions of compound classes in each storage profile. The variations in the profiles as they age may result from the degradation pathways created by the main variables that influence decomposition (i.e. temperature, oxygen availability and moisture content within the samples) [73, 74, 164]. The temperature variations would drive the rate of decomposition [63], while oxygen availability would determine which type of degradation occurs and thus, affect the rate of decomposition [74]. Moisture content would determine how much of the sample could be degraded, since many of these degradation reactions require water for hydrolysis and decomposition of soft tissue, as evidenced by the mummification of bodies when moisture is absent [165]. The low oxygen and low temperatures in the samples stored and aged in the freezer may have encouraged the anaerobic degradation pathways to start immediately, while the relatively warmer temperatures of the refrigerator and room temperature may have caused the aerobic processes to occur first, later switching to anaerobic-driven processes. Most of the literature describing the odour profile of decomposing humans is either an entire profile compiled over a long-term study, or is a single ‘snap-shot’ of the odour at a particular point in time. Hence, it is difficult to accurately compare the trends reported in the literature to the trends observed in the decomposition fluid profile or to determine the specific causes for these chemical variations [51-54, 78, 80, 86, 87, 124-127, 131-134, 166].

According to Janaway *et al* [23], the decompositional by-products will reflect the amount of proteins, lipids and carbohydrates ratios originally present in the body. Therefore, the high proportions of alcohols and aromatics in all of the profiles can be attributed to the fact that they are common by-products of the decomposition of carbohydrates, lipids and proteins. Alcohols are

also more likely to be produced by the fermentation process that decomposition will undergo in low-oxygen environments [74]. An oxygen-abundant environment will produce more acids, so the relatively warmer conditions for the samples stored in the refrigerator and at room temperature may have encouraged the aerobic degradation processes, which resulted in relatively higher proportions of carboxylic acids in the odour profile, while the cool temperatures of the freezer may have encouraged the anaerobic bacteria to degrade the samples instead.

Carboxylic acids are also produced by enteric flora [56], and the lack of internal organs in the decomposition process for the creation of the fluid in this study could account for the overall low proportions of acids in all three storage condition profiles. The deamination of proteins (amino acids) release nitrogen-containing compounds and sulphides [74]. Since proteins are found in a variety of tissues, and different tissues degrade at different rates, the various abundances of nitrogen- and sulphur-containing compounds in the odour profile could be attributed to the degradation of these different proteins as the samples aged. Lipids are also slowly degraded as the samples decompose. The lipids present in the body are mainly triglycerides [74], which degrade into glycerol and a range of saturated and unsaturated fatty acids. Fatty acids can further degrade into water and various gases. Depending on the environment, they can also form adipocere, or can form aldehydes and ketones, which would explain the slow increase in aldehydes and ketones over time in all of the samples.

The loss of some of these compound classes over time may have been due to the repeated use of the sample vials. It is possible that some of the compounds were only produced at detectable levels early in the decomposition process, but may have been present in very trace concentrations as the samples aged. Due to ongoing issues with the instrumentation, several sampling days could not be collected during the original ageing trial. The second trial, conducted at the end of the year to account for the missing samples, required a fresh set of decomposition samples. These new samples may have contained different proportions of compounds, and therefore, produced different proportions of compound classes in the odour profile. This could account for some of the variations in the compound class trends observed throughout the ageing profile. Unfortunately, without repeating the study again, wherein the same set of samples could be used for the entire year, the effect this second set of samples had on the results presented here cannot be determined.

It is evident that the odour profile of the decomposition fluid does change over time, but the overall lack of differentiation of the samples (all less than 50%) indicates that even after a year, the odour profile produced by the samples could still be considered "decomposition odour." The variation in the sample profiles suggests that cadaver-detection dogs should be presented with samples from different stages of the ageing process to ensure that they are familiar with a wide spectrum of possible decomposition VOCs. This also applies to the storage conditions. Storing samples at room temperature or in the refrigerator minimized the variation in the odour profile over time, and is therefore recommended for maximizing the longevity of decomposition fluid as a training aid. However, handlers who have the potential to be called to work in sub-zero temperatures may benefit from presenting their dogs with samples that have been stored in the freezer. Frozen samples can prepare the dog for working with bodies that are frozen or buried in snow, and are producing low concentrations of VOCs, while samples that have been frozen and thawed could also help the dogs locate bodies that are subjected to the freeze-thaw cycle in cold climates.

The samples stored at room temperature in this study were stored in a temperature-controlled laboratory, where the temperature rarely fluctuated above or below 20 ^{o}C. A dog handler working out of their vehicle, or in a building where temperature is not as strictly controlled, may find that higher room or environmental temperatures denature their samples and that they cannot continue to use the samples for an entire year. This phenomenon was seen during the training sessions in this study (described in Chapter 4), where a handler had transported decomposition fluid samples in his vehicle, hoping to be able to train with his cadaver-detection dog while on operational deployment. The temperatures that week were recorded in the high 30s (^{o}C), and the samples, upon return, were clotted, beginning to grow a black mold, and smelled musty (pers.comm. LSC D. Cole). Thus, handlers should be encouraged to use their discretion when storing, handling, and using decomposition fluid as a training aid for long periods of time, or under unusual climatic conditions.

Ideally, the handler should have access to fresh samples of decomposition fluid on a regular basis. However, the limited availability of these samples and the ethical and legal restrictions surrounding their collection can make this difficult. It is therefore recommended to collect new samples whenever possible, but samples can be kept, when stored appropriately, for up to a year. This study did not identify a point at which the odour profile of the training aid changed distinctly

enough to no longer qualify as “decomposition odour,” and hence, further work in this area of study should focus on continuing to monitor the profile of decomposition fluid as it ages, especially at room temperature or in refrigerated storage conditions. It is possible that the training aids can be considered valid for longer than 12 months, and further research may determine a specific point at which the fluid is no longer useful as a training aid.

3.4.3 Dilution and Ageing Trials

The final trial examined the odour profile of serially diluted decomposition fluid that was also provided to cadaver-detection dogs during training (described in Chapter 4), to determine the capability of the GC×GC-TOFMS used in this study and compare it to the sensitivity levels of the cadaver-detection dogs.

To test the sensitivity, samples of decomposition fluid were diluted from stock to 10^{-12}. A decomposition odour profile was still recognisable in the GC×GC profiles at 12 months in the storage and ageing trials, and the samples stored at room temperature and in the refrigerator produced more ideal decomposition odour profiles. Therefore, the ageing portion of the dilution trial was extended to 24 months, and the decomposition odour profile of training aids stored in the refrigerator and brought to room temperature for training sessions was examined. Freezer samples were not used, since, in Australia, cadaver-detection dogs are not likely to require training on samples which have undergone a freeze-thaw cycle. Although the GC×GC-TOFMS was able to detect compounds in the lowest dilution range, it appears that many of the trace decomposition VOCs were not detected below the 10^{-4} dilution level as these samples could not be statistically differentiated based on their odour profile.

The proportions of compound classes present in the odour profile of decomposition fluid as it ages can be related to the degradation of the carbohydrates, lipids, and proteins present in the original sample. The large proportions of alcohols and aromatics can be attributed to the fact that they are produced as a degradation by-product of all three components of the fluid. The ethers form only a small proportion of the profile as it ages, although they are a by-product of decomposing carbohydrates. They are rarely reported in the literature studies that have used GC-MS [87], but have been reported in studies using GC×GC [80, 86]. It is hypothesized that they are produced at

trace concentrations which are not detectable using GC-MS, but are detectable with the enhanced sensitivity of the GC×GC. A similar hypothesis is proposed for the low abundance of carboxylic acids present in the odour profile of the diluted samples, although it is also possible that the acids are not present due to poor interactions between their functional group and the phenyl compounds in the stationary phase of the GC column [80], or because they are produced by enteric bacteria [80], which were missing during this study. The proportions of sulphur-containing compounds can be attributed to the various degradation rates for the proteins present in the fluid, while the general increase in ketones over time is due to the slow degradation of lipids [74].

As the fluid was diluted, there was a general decrease in the number of compounds present in the sample, which is to be expected, and some of the less abundant compound classes were lost. However, there was no consistent trend observed for the diluted samples. The abundance of alcohols and hydrocarbons generally increased as the profile was diluted, but the prevalence of all other compound classes varied greatly from dilution series to dilution series. There are several potential reasons for these inconsistencies. For example, the sample may have been exposed to oxygen when it was sampled for training aids or sub-sampled into headspace vials, which could have affected the manner in which the compounds degraded, as could the variations in temperature when the samples were stored (refrigerator) versus when they were used at the training site (ambient temperature).

There were also two sets of samples that were aged, diluted, and used as training aids, based on their availability and the age of the sample set when a training day could be arranged. One set was used for the 6, 9, 12 and 21 month training days, and one was used for the 3, 15, 18, and 24 month training days. The different sample sets, collected from various donors at different times during the study, could account for some of the variations seen in the profiles, especially in the PCA plot (Figure 3-16, page 82), where several of the 6, 9, and 12 month diluted samples were differentiated from the other dilutions.

It is apparent that handling and storing a sample in the same manner each time will not necessarily produce the same odour profile. There are far too many variables affecting the decomposition process and the degradation of the samples, including the source materials, the collection and sampling processes, the handling and storage methods, the temperatures and other environmental conditions at the training site to be able to reproduce an identical odour profile

each time a sample or set of samples is used. A handler should therefore attempt to minimize these variables, but also recognise that a real operational scenario is likely to include many of these variables. The low overall differentiation between all of the diluted samples (21%), especially those diluted lower than a 10^{-4} dilution, indicates that the odour profile produced by all of these samples still represents decomposition and, as will be shown in Section 4.4, can be detected and recognised as such by the cadaver-detection dogs. The effect of these variations in profile on the detection ability of the dogs is discussed in more depth in Section 4.4.

Overall, when the VOC lists from the diluted, stored and aged samples were compiled, there were 178 compounds that could be found in over 80% of all the samples (see complete list in Appendix J). Of these 178 compounds, 35 were found in 95% or more of the samples, and are reported in Table 3-1.

Table 3-1: The 35 compounds found in 95% or more of the stored, aged, and diluted decomposition fluid samples.

Compound Name	Percentage of Samples Containing Compound
2-Undecanol	100.00%
Benzene, 1-ethenyl-4-ethyl-	100.00%
p-Xylene	100.00%
Styrene	100.00%
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	99.19%
Undecane	99.19%
2-Decanol	99.02%
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	98.39%
7-Tetradecene, (E)-	98.39%
Methyl Alcohol	98.39%
Octanal	98.39%
2-Heptanone	98.21%
Isobornyl formate	98.04%
2-Decanone	97.72%
2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)-	97.58%
Hexanal	97.58%
Oxalic acid, allyl nonyl ester	97.58%
Trichloromethane	97.58%
Disulfide, dimethyl	97.39%
2-Undecanone	97.06%
2-Octanone	96.94%
Nonanal	96.77%
Acetophenone	96.73%
Dimethyl trisulfide	96.73%
2-Heptanone, 3-methyl-	96.08%
3-Octanone	96.08%
4-Decene, 2,2-dimethyl-, (E)-	96.08%
à-Phellandrene	96.08%
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	96.08%
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	96.08%
o-Cymene	96.08%
Pyrazine, 2,5-dimethyl-	96.08%
Methyl tetradecanoate	95.42%
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	95.16%
à-Terpineol	95.10%

This highlights the inadequacy of synthetic scents, which contain only 10 compounds [57, 58], none of which were found at detectable levels by the GC×GC in the odour profile of human decomposition fluid. While it is not clear how any of these compounds affect the cadaver-detection dogs' ability to locate decomposition odour, it is hypothesized that some combination of these compounds causes the dogs to recognize and alert to decomposition odour. The trends of formation for these compounds and the dynamic manner in which they change throughout the decomposition process should be studied in greater detail to better understand how decomposition odour is produced over time. These compounds could also be tested individually, or in various combinations during dog training sessions in order to observe the dogs' responses to these compounds, and to determine whether they represent the key decomposition VOCs recognized by cadaver-detection dogs. Unfortunately, this was not possible during the timeframe of this study.

Since there is currently no individual compound, set of compounds, or even pattern of compound classes within the odour profile that clearly distinguishes "decomposition odour" or indicates which VOCs the dogs associate with decomposition odour, further research into this field is required. A closer examination of the major compounds, especially those determined to be common to over 80% of the decomposition samples in this study, as well as a better method for discrimination between compounds in the data processing stage would help to clarify which combination of compounds or compound classes are creating the distinct odour associated with decomposition. Investigating specific compounds or sets of compounds that are present as the samples age would also prove beneficial, as this information could be used to create a range of more accurate and selective training aids for the cadaver-detection dogs, which would be easier for the dog handlers to obtain and use during training sessions.

3.5 Conclusions

The aims of this human decomposition fluid validation study were to profile the odour of the fluid in its function as a cadaver-detection dog training aid, and to compare the profile to that of a decomposing human cadaver. The project also examined the typical storage and handling conditions used by police dog units in order to maximize the longevity of the training aid. Finally,

this section of the study profiled the training samples provided to the dogs in order to determine the chemical compounds present in the samples and compare the sensitivity of our chemical detector (i.e. GC×GC-TOFMS) with biological detectors (i.e. cadaver-detection dogs).

While the study was limited by an inability to compare the odour profile of human decomposition fluid directly to the odour profile of a decomposing human cadaver, there were sufficient similarities between the compound classes in the odour profiles to consider human decomposition fluid a valid representation of a human cadaver, and thus, an acceptable training aid for cadaver-detection dogs.

The odour profile of the training aids did change over time, but the overall low differentiation of the samples indicates that even after a year, the odour profile produced by the samples could still be considered "decomposition odour." New samples should still be collected whenever possible, given the difficulty in obtaining human decomposition fluid, but dogs should also be exposed to aged samples in order to maximize their sensitivity to the odour of late-stage decomposition. Samples should be stored at room temperature or in the refrigerator, although, depending on the climate of the area where a handler works, the handler should use their discretion with regards to potentially using samples that have been stored at high room temperatures or in the freezer.

This study was not able to identify a single compound, specific set of compounds, or even pattern of compound classes within the odour profile that distinguishes "decomposition odour" or indicates what the dogs are using to identify decomposition odour. However, it was able to identify a suite of 35 compounds that were detected in 95% of the samples analysed. Further study of these compounds may highlight several key VOCs that the dogs target. There was no clear pattern to the odour profiles produced by the diluted and aged samples, and this is likely due to the large number of decomposition variables that can affect each individual sample. The low overall differentiation between the samples, no matter their storage condition, age, or dilution level, indicates that decomposition fluid still produces an odour profile that the dogs would be able to recognise as "decomposition."

There is still much research that should be carried out to study the changes to the profile of the fluid, the degradation of the odour profile over time, and the relationship between the chemical profile of decomposition fluid and a dog's positive alerts. However, the findings of this study

support the hypothesis that human decomposition fluid can be considered a viable training aid for cadaver-detection dogs from a chemical perspective.

Chapter 4:

Cadaver-detection Dog Trials

Chapter 4: Cadaver-detection Dog Trials

4.1 Introduction

Human tissues such as decomposition fluid have not yet been fully validated as training aids for cadaver-detection dogs. Prior studies investigating the dogs' responses to various training aids found that the dogs are able to detect diluted volumes of decomposition fluid to 10^{-7} [59], carpet squares contaminated with decomposition odour [24], gauze pads saturated with the odour of fresh human remains, cremated human remains, decomposition fluid, adipocere and blood [9], human teeth [35], 1 µL of blood diluted up to 4000x [36], and various gravesoil samples [25]. However, it has not yet been determined whether the odour profiles of these training aids accurately reflect the odour profile of decomposed human remains.

The aim of this trial was to determine the ability of cadaver-detection dog teams to detect small volumes and concentrations of decomposition fluid, and to investigate whether their sensitivity was enhanced with regular training sessions. This study was carried out with the intention of validating one of the training aids used by police canine handlers in Australia (i.e. human decomposition fluid). The use of validated training aids allows handlers to document the limits of their dogs' abilities and provide scientific evidence of their capabilities in court [167]. To date, there have been no reported studies that have attempted to systematically calibrate the dogs' sensitivity to decomposition fluid or determine whether that sensitivity increases with regular exposure to the specific training aid.

Previous studies involving decomposition odour as training aids have used objects exposed to cadavers for specified time intervals [7, 24] or have used fixed concentrations of training aids [36, 122]. A preliminary study by the author [59] demonstrated that cadaver-detection dogs in the UK were capable of detecting dilutions of up to 10^{-7} (i.e. 1 part-per-ten-million) of decomposition fluid.

It is important to clarify that the aim of this project was not to increase the dogs' sensitivity to the training aids. A dog trained to react to extremely dilute volumes of fluid would raise some of the same issues that have been found in Low Copy Number DNA, specifically in the case of secondary

transfer and legitimate contact [168]. Since dogs have not been reliably proven to be able to distinguish between human and animal remains [9, 14, 48, 49, 51, 88], it is unclear whether a dog trained to alert to extremely dilute traces of decomposition odour would alert to the trail of a scavenger who has carried off a decomposing animal carcass. It is equally uncertain whether the dog might alert to the residual scent of a carcass that had lain on soil several months or even years before, or if traces of decomposition odour on the hands of someone who touched spoiled meat might cause the dogs to respond. It comes down to a question of practicality; how much residual odour is required for the alert to be relevant? Training the dogs to an extremely high level of sensitivity would raise more questions than could be answered in an investigation, and the likelihood of the dogs being required to detect such minute concentrations of decomposition fluid at a scene would be rare.

The aim of this research was therefore to determine the current sensitivity levels of cadaver-detection dogs to decomposition fluid as a training aid, and to determine whether that sensitivity increases with regular exposure to these training aids (for example, whether a dog with several years of training experience is more sensitive to the fluid than a dog newly exposed to the training aid). It was of interest to determine the smallest levels of decomposition odour that the dogs detect under normal circumstances. This knowledge could help to clarify situations such as the Madeleine McCann or Casey Anthony cases, where the dogs reportedly alerted to trace amounts of decomposition odours in the vehicles which were purportedly used to transfer their bodies [40, 41]. Even if the bodies of Madeleine or Caylee had been in the vehicles, would there have been sufficient residual scent for the dogs to detect it several days later? If the handlers in these cases had a better scientific understanding of the detection capabilities of their dogs, these questions could have been answered, thus enhancing their credibility in court.

Linking the responses of the dogs to the chemical odour profiles of decomposition fluid (as discussed in Sections 3.3.3 and 3.4.3) will also provide a better understanding of the mechanism by which cadaver-detection dogs locate decomposed remains. If a comprehensive decomposition odour profile can be characterized for a wide range of volumes and ages of fluid, these chemical results can be linked to the responses of the dogs during their training sessions.

Ultimately, a greater understanding of the key chemical compounds that the dogs use to locate remains in various stages of decomposition would in turn allow for the development of accurate

synthetic training aids, and avoid the legal, ethical and biohazard issues surrounding the use of human remains as cadaver-detection dog training aids (discussed in Section 1.1.3). However, this represents the long-term goal of a much larger research program. The focus of this study was to determine the baseline sensitivity level for the cadaver-detection dogs used by the NSW Police Dog Unit as part of the validation process for the use of human decomposition fluid as a cadaver-detection dog training aid.

4.2 Materials and Methods

4.2.1 Decomposition Fluid Samples

Human decomposition fluid samples were obtained from the UTS SASF as outlined in Section 2.2.1. Samples were collected from de-boned upper arm, thigh or abdominal tissue samples obtained from newly donated, un-embalmed bodies. The tissues were decomposed at room temperature for 5-7 days before decomposition fluid was collected, stored in glass specimen jars, and refrigerated at 4 ^{o}C. Due to limited availability, samples were often used for several training sessions before new material could be obtained.

A previous study by the author had used decomposition fluid diluted to 10^{-7} [59], and so the intent of the current study was to increase the dilution levels beyond that already tested. The stock decomposition fluid was diluted to 10^{-12} (i.e. 1 part-per-trillion) by serially diluting 10 mL of fluid in 100 mL volumetric flasks containing tap water. Tap water was chosen as the dilutant rather than distilled water or saline solution because it more accurately mimicked the use of rain water, which could dilute the scent at a real scene. This serial dilution method produced a total of 13 samples (Fig 4-1, below) for use during training sessions with the cadaver-detection dog teams. In order to best mimic the conditions under which the training aid would be used and stored by the NSW Police Dog Unit, the samples were stored in the fridge in 20 mL glass scintillation vials between training sessions.

Fresh fluid indicates fluid used within 3 months of tissue decomposition. There were 6 trials that used fresh fluid to test the cadaver-detection dogs' sensitivity levels. A set of fresh fluid dilutions was also diluted to 10^{-24} (1 part-per-septillion, giving a total of 25 samples) and presented to the

dogs. This was carried out in an attempt to determine their lowest sensitivity levels when the dogs were responding positively to all of the previous dilutions presented to them.

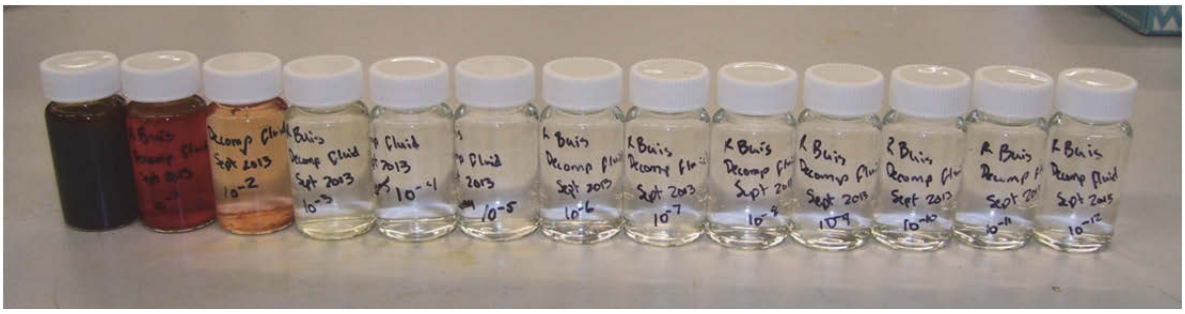

Figure 4-1: Serially diluted decomposition fluid samples for dog trials

Dilutions of the decomposition fluid were also aged up to 2 years (24 months) and presented to the dogs during training sessions. This examined the dogs' ability to locate aged remains or areas where a body may have been located while decomposing over time, such as in cases of scavenged and scattered remains, or post-mortem relocation of a body. Dilutions were aged for 6, 9, 12, 15, 18, 21 and 24 months.

The unused portions of the diluted samples were returned to the laboratory after each dog training session and chemically profiled using the SPME-GC×GC-TOFMS method outlined in Chapter 2 and discussed in Chapter 3.

4.2.2 Training Sessions

Training sessions were completed at the NSW Police Dog Unit's training facility, which was set up for scent-training and accreditation testing for a variety of detection dogs and handlers. At the time this study commenced, there were only four accredited cadaver-detection dog teams (i.e. one dog and one handler per team) in Australia and all of them were based with the NSW Police Dog Unit, although more have been trained and accredited since. Training sessions were held approximately every 6-8 weeks; although the exact number of dog/handler teams varied depending on the teams' availability. Hence, each trial typically involved two or three teams. While the teams had previously trained on blood, bone, and grave soil, decomposition fluid had not been used as a training aid prior to this study.

Concrete bricks (cinder blocks) were placed in a standard scent line-up formation around the training facility (Figure 4-2). The author of this thesis (the researcher) placed one (1) mL of each of the diluted decomposition fluid samples in 1 L metal cans with vented lids, and each can was placed randomly inside a concrete brick in the line-up (Figure 4-3). The training co-ordinator was aware of the location of the target odours, but the handlers were blind to the location of the sample-containing cans.

Figure 4-2: Concrete bricks in a standard scent training line-up

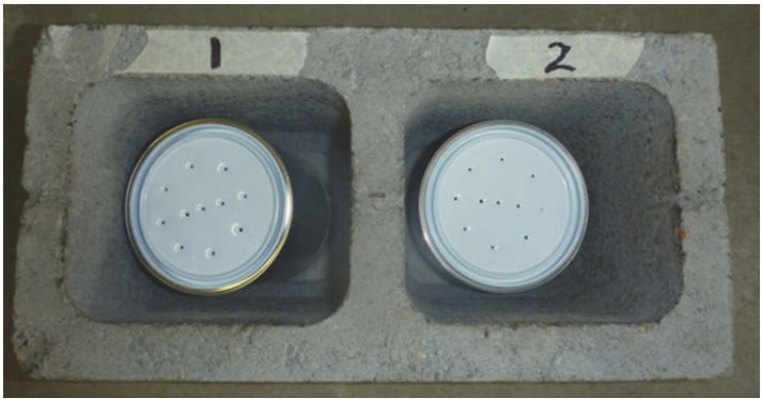

Figure 4-3: Sample cans inside the scent line-up bricks

Cans without a target odour contained a “distractor” item, which is a common object that could be present at a scene, and to which the dogs should not alert. The distractor odours in these trials included potting soil, eucalyptus leaves, salt, powdered drinking chocolate, empty aluminium cans, wooden ice cream sticks, and cotton pads. Cans containing 1 mL of tap water acted as both a distractor odour and as the control for the diluted decomposition fluid samples. While there were

approximately 40 distractor items present for each trial, only 6-7 of the target odours were placed out per run, so as to minimize the cross-contamination of target scents within the line-up.

Each team conducted an initial search of the 48 cans containing the first six target odours during the training session, before the cans were rearranged and a second set of seven target odours was placed out for all of the teams to perform a second scent line-up across the 48 cans. Between each team's scent line-up, the researcher changed the lids on all of the target cans, and all of the cans (both target and distractor) were wiped clean using fresh pieces of paper towel and rearranged randomly among the concrete bricks to prevent the next dog from alerting to the scent of the previous dog, rather than the target odours. The trial concluded when the scent line-ups were completed, or when it was determined that the dogs were losing interest, and any further trials would merely reinforce a location-alert rather than a positive alert to the scent itself. Due to time constraints, the dogs were only able to complete the trials using each sample set once. The dogs were given a play reward for a correct indication by the training coordinator, rather than the handlers or the researcher, in order to minimize bias and to reinforce proper training procedures. After each team completed their run, the results were discussed by the researcher and the training coordinator to ensure that the responses observed during the trial were properly categorised.

Dog and handler teams were evaluated on the response given by the dog and on the handler's interpretation of that response. There were four potential responses, which are summarized in Table 4-1: positive alert (the dog correctly alerts to the target odour and the handler correctly calls the alert), partial positive alert (the dog displays a behavior change at the target odour, but does not give a true positive alert), false positive alert (the dog alerts to a distractor odour, and the handler incorrectly calls the alert), and false negative response (the dog does not alert to the target odour, or the dog alerts to the target odour but the alert is not recognized by the handler). The dogs were trained to use a passive alert known as a "freeze," where the dog crouches in place, with their nose pointed at the location of the target odour.

Table 4-1: Summary of the classifications for dog responses to target odours

Response Type	Sample Present	Dog gives an alert	Handler calls alert
Positive	✓	✓	✓
Partial Positive	✓	~ (behaviour changes)	X
False Positive	X	✓	✓
False Negative	✓	X	X

Initial training sessions began with the strongest dilutions, placed in descending order throughout the line-up. When the dogs were able to successfully locate these samples, the more dilute samples were introduced. Once the dogs demonstrated an aptitude for locating the lower dilutions when the cans were placed in order of strongest to most dilute samples, the samples were placed in random dilution order. Additionally, once the dogs were able to successfully locate the initial dilution volumes (i.e. 1.0 mL), smaller volumes of sample (i.e. 0.2 mL, 0.1mL) were introduced.

4.2.3 Data Analysis

The data collected during the dog trials was compiled and analysed in Microsoft Excel 2007. Chemical odour profile data displayed in section 4.3.4 was compiled using ChromaTOF®, and further analysed in Microsoft Excel 2007, as outlined in Sections 3.2.4 and 3.2.5.

4.3 Results

During this study, there were only four accredited cadaver-detection dog teams in Australia. One dog was retired shortly after the current study began, leaving only three teams available throughout this study, although the number of teams present for each trial varied based on the

availability of these teams on training days. The results recorded below are presented as percentages (e.g. 50% of the dogs responded positively to the particular dilution), because this allowed for a comparison of results across all of the trials, even when one of the teams was unable to attend a training session.

4.3.1 Fresh Diluted Decomposition Fluid

Figure 4-4 displays the results obtained during the first six trials with the cadaver-detection dog teams. Trial 1 represented the first time the dogs were exposed to decomposition fluid as a training aid. Figure 4-4 indicates a strong positive response by each team to the stock solution and first dilution, but thereafter, the teams had difficulty detecting the lower dilutions. During Trial 2, each team's response was much stronger and they were able to locate more of the target odours. By Trial 3, the teams were responding to the dilutions with confidence, and it was decided to randomize the order of dilutions for Trial 4. The strong responses to most of the lowest dilutions obtained during trial 4 led to the decision to reduce the volume of decomposition fluid from 1.0 mL to 0.2 mL in the next set of trials.

Dilution Level	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
stock	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
10^{-1}	100.00%	100.00%	100.00%	100.00%	50.00%	66.66%
10^{-2}	50.00%	100.00%	100.00%	100.00%	50.00%	33.33%
10^{-3}	50.00%	100.00%	100.00%	100.00%	50.00%	66.66%
10^{-4}	50.00%	100.00%	66.66%	100.00%	50.00%	66.66%
10^{-5}	50.00%	66.66%	100.00%	100.00%	50.00%	66.66%
10^{-6}	0.00%	33.33%	100.00%	50.00%	100.00%	66.66%
10^{-7}	0.00%	100.00%	100.00%	100.00%	100.00%	33.33%
10^{-8}	0.00%	100.00%	66.66%	100.00%	100.00%	33.33%
10^{-9}	0.00%	100.00%	100.00%	100.00%	50.00%	66.66%
10^{-10}	0.00%	100.00%	100.00%	50.00%	100.00%	66.66%
10^{-11}	0.00%	66.66%	33.33%	100.00%	100.00%	66.66%
10^{-12}	0.00%	33.33%	66.66%	100.00%	100.00%	66.66%

Figure 4-4: The percentage of dogs responding correctly to dilute samples of decomposition fluid in the fresh fluid trials (Trials 1-6). Green indicates that all dogs correctly located the sample, red indicates that no dogs correctly located the sample, and shadesof orange indicate the degree to which the dogs were able to locate the sample.

When the volume of decomposition fluid was reduced, one team had some difficulty locating the target odours during the first line-up in Trial 5, as demonstrated in Figure 4-4. However, during the second set of dilutions in Trial 5, both teams were able to locate most of the reduced volumes with minimal difficulty. Trial 6 exhibits an improvement for those two teams as they responded to the lower dilutions with more consistency. The third team had difficulty locating the target odours during Trial 6, which may be related to their absence from training and operational deployment for the two training sessions prior to this trial (i.e. Trials 4 and 5). As a result, the cadaver-detection dog team had not been exposed to the scent of decomposition fluid for several months. However, subsequent trials including aged samples (shown in Figure 4-7) demonstrated an improved response by this team following consistent exposure to the training aid. Overall, as summarized in Figure 4-5, the recovery rates for fresh fluid was relatively high, with an average of 77% correct sample recovery.

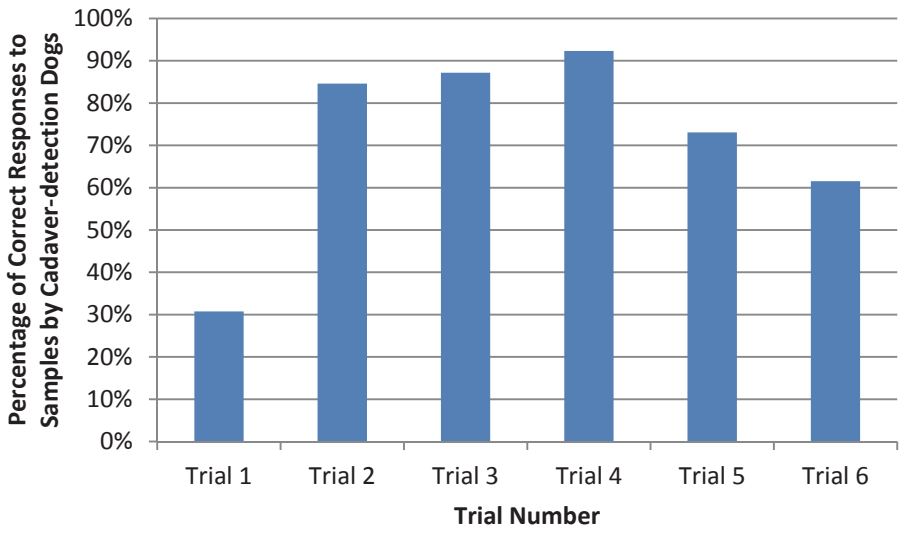

Figure 4-5: Percentage detection rates of diluted samples for Trials 1-6 (fresh fluid)

4.3.2 Aged Diluted Decomposition Fluid

In this second half of the dog trials (Trials 7-14), the emphasis was on the dogs' ability to locate fluid that had been aged for various lengths of time. Figure 4-6 summarizes the cadaver-detection dog team responses to diluted decomposition fluid that had been aged for up to 24 months (2 years).

During this ageing trial, the volume of decomposition fluid placed out for the dogs was halved to 0.1 mL of sample. Similar to the fresh fluid trial (3 months aged), the dogs correctly located 80% of the samples. This trend of high recoveries continued over the next few trials (6-12 months aged). However, there was a distinct decrease in the dogs' ability to recover the 15 month aged samples during Trial 11 (a comparison of these results to the odour profiles of the samples can be found in Section 4.3.4). This initially appeared to be the limit of their capability to detect aged decomposition fluid; however, the older dilutions were still presented to the dogs in Trials 12-14. In Trial 12, the dogs returned to recovery rates of over 80% for all of the following aged samples (18-24 months). Overall, the recovery rates for the aged, diluted fluid were slightly higher than for the fresh, diluted fluid (Figure 4-7), with an average correct sample recovery rate of 81%.

Dilution Level	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
stock	100.00%	100.00%	100.00%	100.00%	50.00%	100.00%	100.00%	100.00%
10^{-1}	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%
10^{-2}	100.00%	100.00%	0.00%	100.00%	50.00%	100.00%	100.00%	100.00%
10^{-3}	50.00%	100.00%	100.00%	100.00%	100.00%	66.66%	100.00%	100.00%
10^{-4}	100.00%	100.00%	50.00%	100.00%	0.00%	100.00%	50.00%	50.00%
10^{-5}	100.00%	100.00%	100.00%	100.00%	50.00%	66.66%	100.00%	50.00%
10^{-6}	100.00%	100.00%	50.00%	66.66%	0.00%	100.00%	50.00%	100.00%
10^{-7}	50.00%	100.00%	100.00%	100.00%	0.00%	100.00%	100.00%	50.00%
10^{-8}	50.00%	100.00%	50.00%	100.00%	0.00%	100.00%	100.00%	100.00%
10^{-9}	100.00%	100.00%	100.00%	100.00%	0.00%	100.00%	100.00%	100.00%
10^{-10}	0.00%	100.00%	50.00%	100.00%	50.00%	100.00%	100.00%	100.00%
10^{-11}	100.00%	100.00%	100.00%	100.00%	50.00%	66.66%	50.00%	100.00%
10^{-12}	100.00%	100.00%	100.00%	33.33%	100.00%	50.00%	50.00%	50.00%

Figure 4-6: The percentage of dogs responding correctly to dilute samples of decomposition fluid in the aged fluid trials (Trials 7-14). Green indicates that all dogs correctly located the sample; red indicates that no dogs correctly located the sample, and shades of orange indicate the degree to which the dogs were able to locate the sample

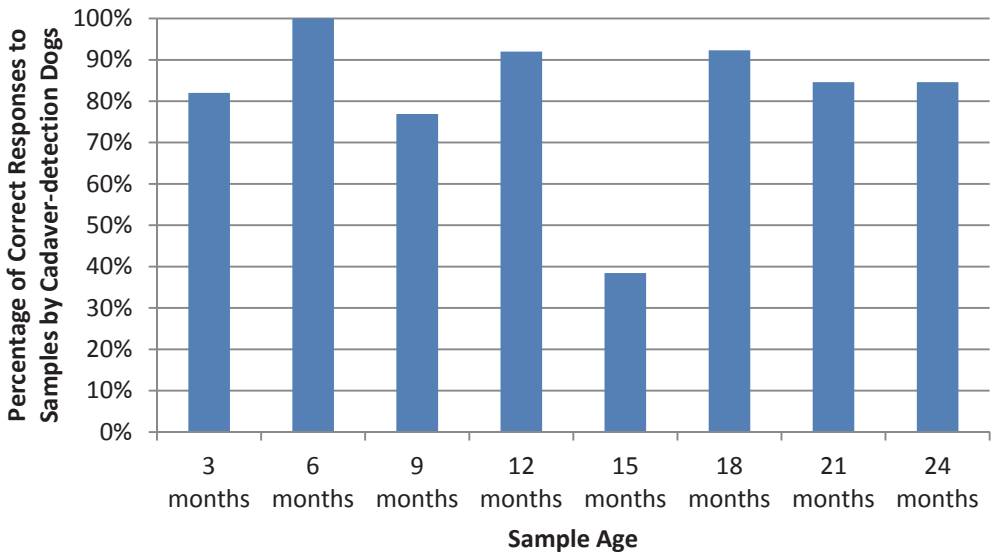

Figure 4-7: Percentage detection rates of diluted samples for Trials 7-14 (aged fluid)

4.3.3 Correct vs. Incorrect Responses

An analysis of the number of the cadaver-detection dog teams' correct and incorrect responses to the target odours over the course of the 14 trials is shown in Figure 4-8. There are relatively few incorrect (partial positive, false positive or false negative) responses to the dilutions, and this trend remained consistent throughout both the fresh and aged decomposition fluid trials. There was an increase in incorrect responses when a dog had not attended the previous training session, as can be seen in Team A for Trial 12, or Team C for Trials 6 and 12 (Figure 4-8). There is no overall increasing trend in the number of incorrect responses, which is an ideal outcome for this study. However, there is also no decreasing trend in the number of incorrect responses, although it is hypothesized that this could improve with ongoing training sessions. Most of the incorrect responses were due to false positive alerts to distractor odours, usually foil or cotton wool.

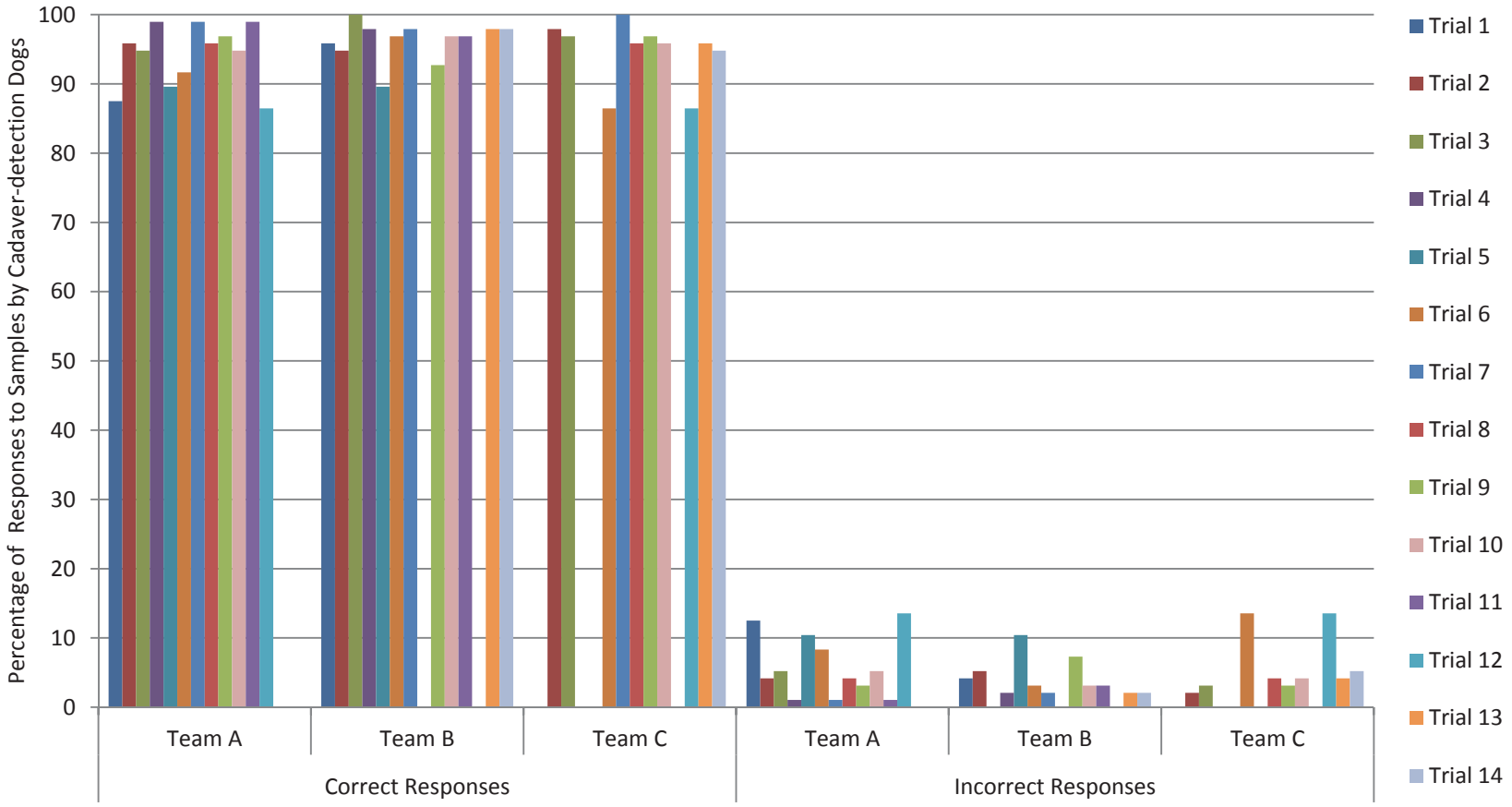

Figure 4-8: A comparison of correct and incorrect responses in all 14 trials. Note: any missing bars indicate that the team was not available for that particular trial

4.3.4 Comparison of Odour Profiles to Dog Responses

The chemical odour profiles obtained from the aged training samples (described in Section 3.3.3) are compared to the responses given by the dogs for each sample set. The variations identified in the odour profiles may provide some explanation for the dogs' responses to each sample.

When presented with the dilutions aged for 3 months, the dogs had difficulty detecting the 10^{-3}, 10^{-7}, 10^{-8}, and 10^{-10} samples (Figure 4-9b). In the profiles for these samples (Figure 4-9a), there is an increase in the alcohols and other compound classes, when compared to the other 3-month dilution profiles. In the final trial for this study (Trial 14), with the agreement of the training coordinator, samples of fresh fluid (i.e. aged up to 3 months) were diluted beyond 10^{-12} for one training session only. The 25 x 0.1 mL fresh fluid samples were placed in decreasing order from stock to 10^{-24}, in a series of 4 runs. The initial decrease in dilutions confused one of the dogs (shown in their responses to 10^{-13} and 10^{-14} in Figure 4-9b), but the dogs had little difficulty detecting the further dilutions, down to 10^{-21}. The responses to the 10^{-22} and 10^{-23} dilutions were poor, with only one of the dogs locating them, but surprisingly, all three dogs could locate the 10^{-24}, or 1 part-per-septillion dilution (Figure 4-9b). The odour profiles for these 'extreme' dilutions are also shown in Figure 4-10a and while they demonstrate similar classes of compounds to the less dilute samples, the proportions of compound classes varies considerably. Notably, as the dilution level decreased, the proportion of 'other' compounds increased considerably, comprising more than 50% of the odour profile for the 10^{-14}, 10^{-18}, 10^{-22}, 10^{-23} and 10^{-24} dilutions. Regardless, the dogs produced positive alerts for most of these samples.

All three dogs had no difficulty detecting the 6 month samples (Figure 4-10b), even although there were variations in the chemical profile (Figure 4-10a), such as an increase in hydrocarbons and a decrease in ketones as the sample was diluted. The 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10} samples proved difficult for at least one of the dogs to detect in the 9 month ageing trial (Figure 4-11b). In this case, there was no general trend that caused these reactions, instead, changes in the proportions of sulphur-containing compounds, hydrocarbons, other, esters, and ketones respectively, were noted in the samples that dogs had difficulty locating. An increase in the alcohol proportion of the 10^{-6} and 10^{-12} samples was observed for the 12 month sample profiles (Figure 4-12a) when compared to the dog trial results (Figure 4-12b).

At least one, although occasionally both, of the dogs present for this trial had difficulty detecting the stock, 10^{-2}, and all of the 10^{-4} to 10^{-12} samples in the 15 month trial (Figure 4-13b). When each of the sample profiles were compared (Figure 4-13a), there was a general increase in the proportions of alcohols, ketones, aromatics, and hydrocarbons in the samples, as well as a decrease in the proportion of sulphur-containing compounds. Variations in the proportion of sulphur-containing and ester compounds, as well as an increase in the alcohols also appeared in the 10^{-3}, 10^{-5}, and 10^{-11} samples for the 18 month trials (Figure 4-14a), which the dogs had difficulty detecting (Figure 4-14b).

In the 21 month samples, the odour profiles for the 10^{-4}, 10^{-6}, 10^{-11}, and 10^{-12} samples (Figure 4-15a) indicated variations in the alcohol, sulphur-containing and halogenated compound classes which may be responsible for the one of the dogs' difficulties in locating theses samples (Figure 4-15b). In the last trial, one of the dogs had difficulty locating the 10^{-4}, 10^{-5}, 10^{-7} and 10^{-12} samples after being aged for 24 months (Figure 4-16b). The profiles for these four samples indicated a decrease in sulphur-containing compounds, an increase in ketones, hydrocarbons, and other compounds, and variations in the alcohols when compared to the other samples in that trial (Figure 4-16a). Overall, changes to the proportions of alcohols, sulphur-containing and other compound classes in the odour profiles appear to be the main differences between the samples that the dogs were unable to locate and those to which they responded correctly.

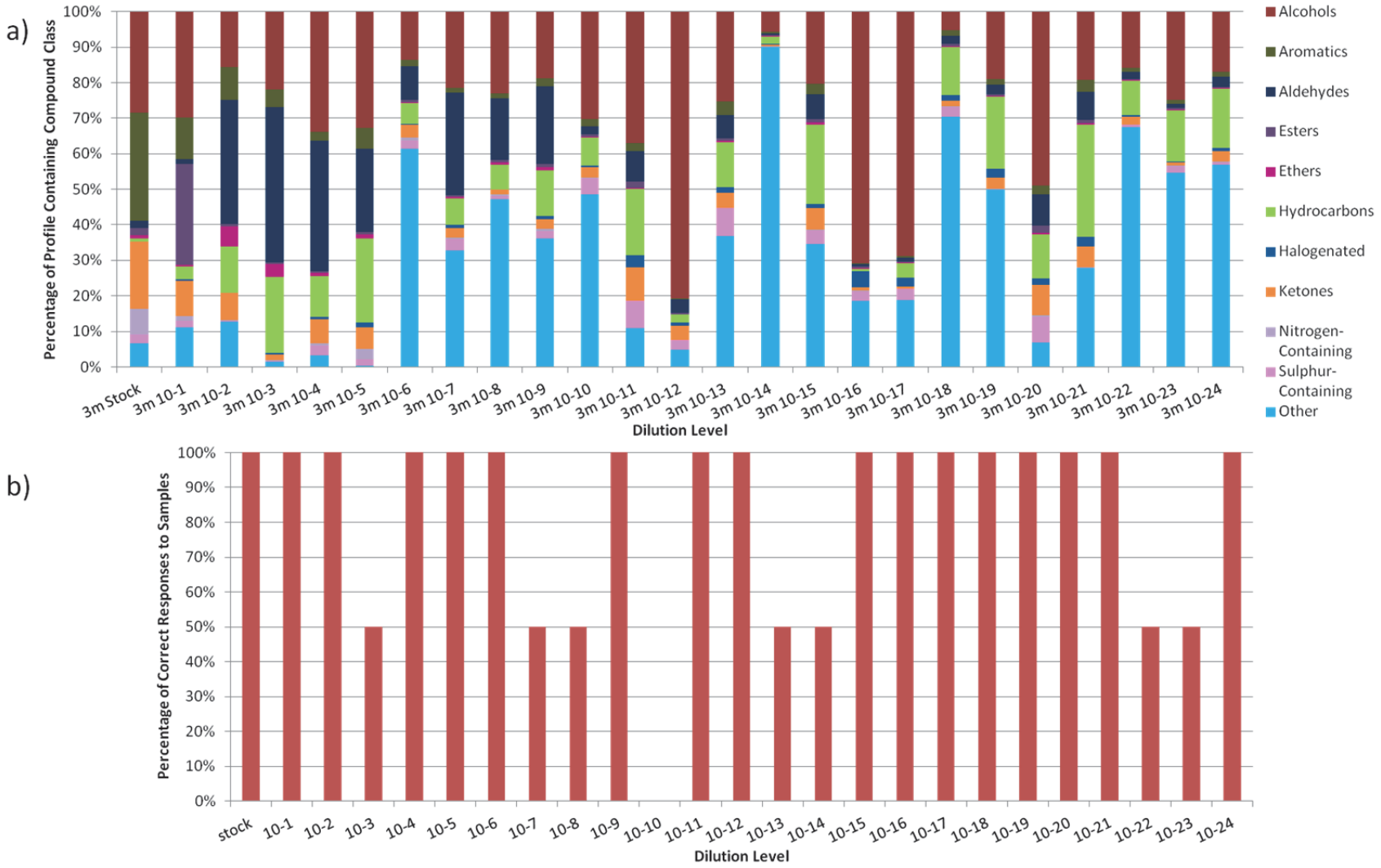

Figure 4-9: The comparison of chemical profiles to dog detection rates for the 3 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

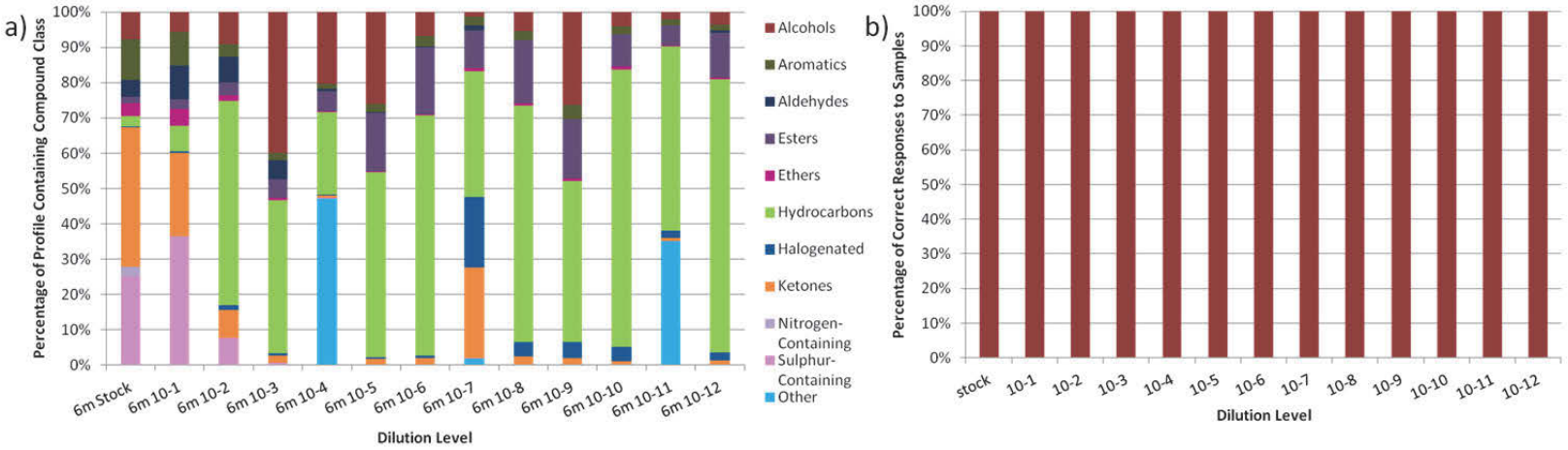

Figure 4-10: The comparison of chemical profiles to dog detection rates for the 6 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

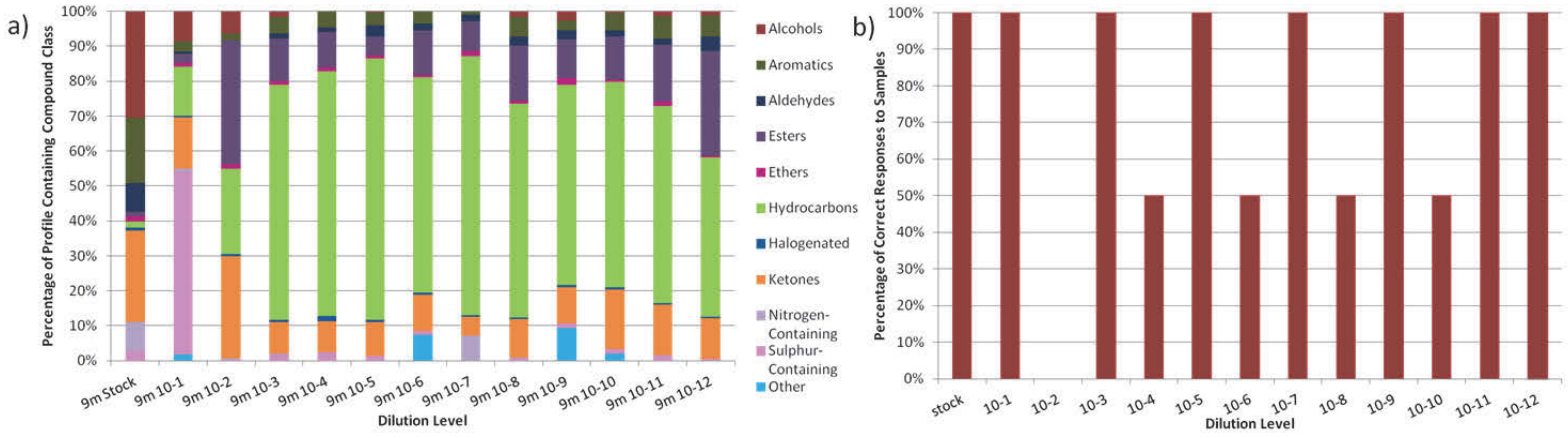

Figure 4-11: The comparison of chemical profiles to dog detection rates for the 9 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

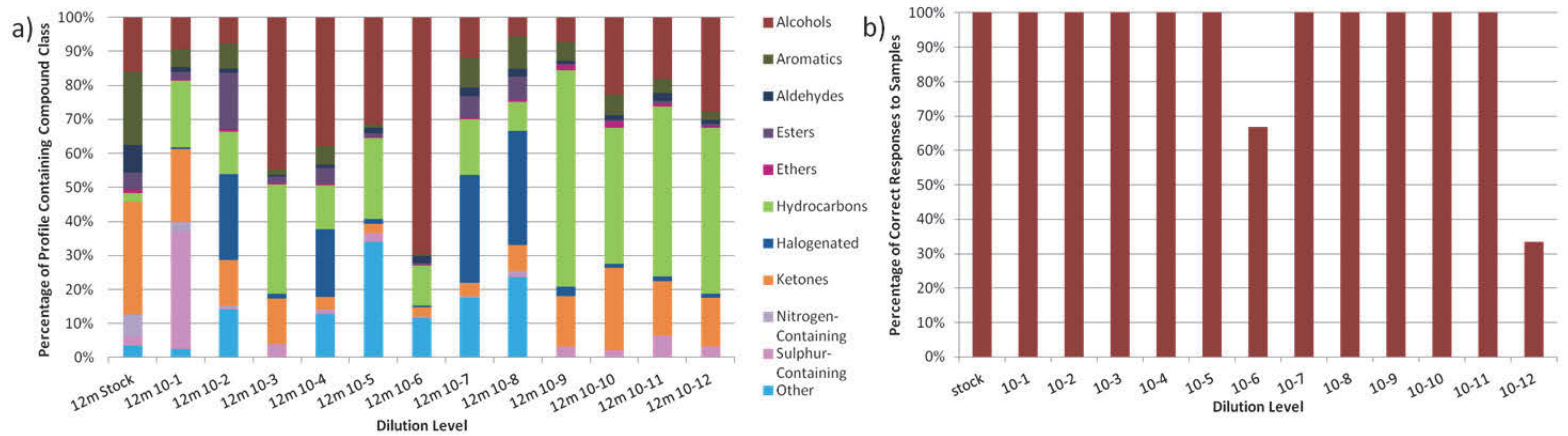

Figure 4-12: The comparison of chemical profiles to dog detection rates for the 12 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

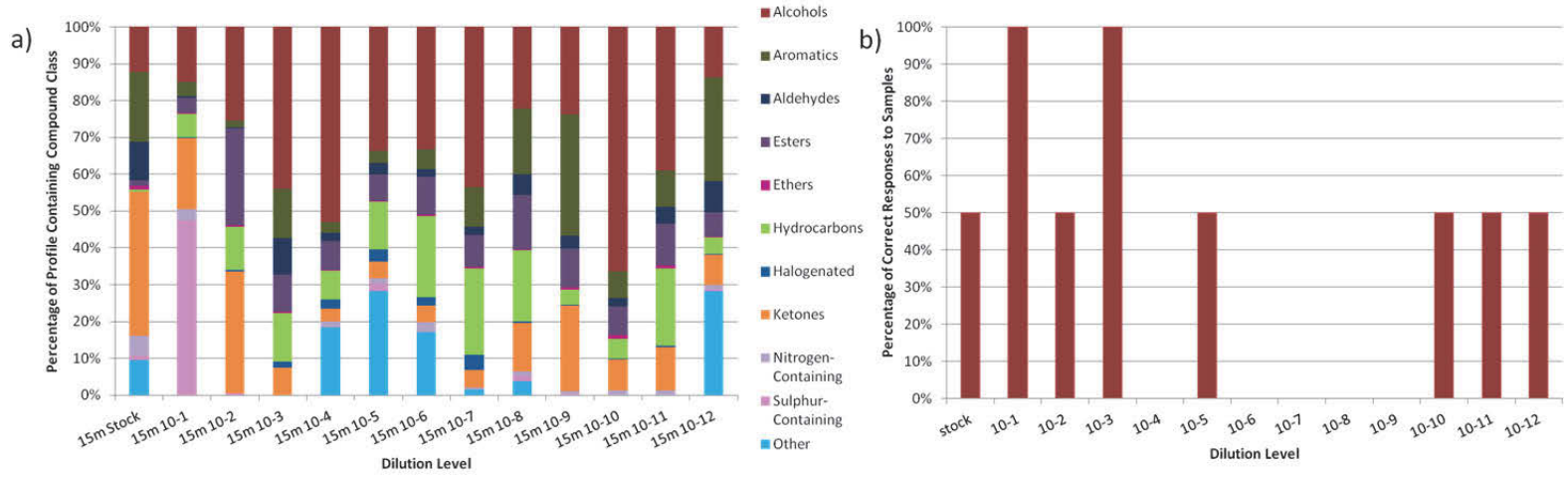

Figure 4-13: The comparison of chemical profiles to dog detection rates for the 15 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

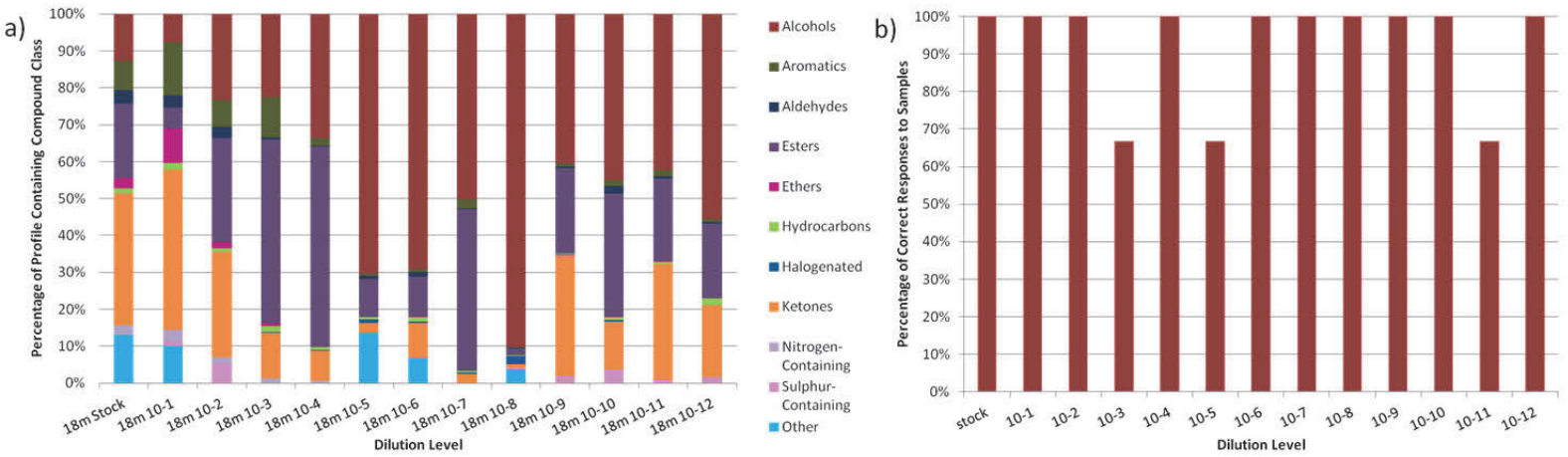

Figure 4-14: The comparison of chemical profiles to dog detection rates for the 18 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

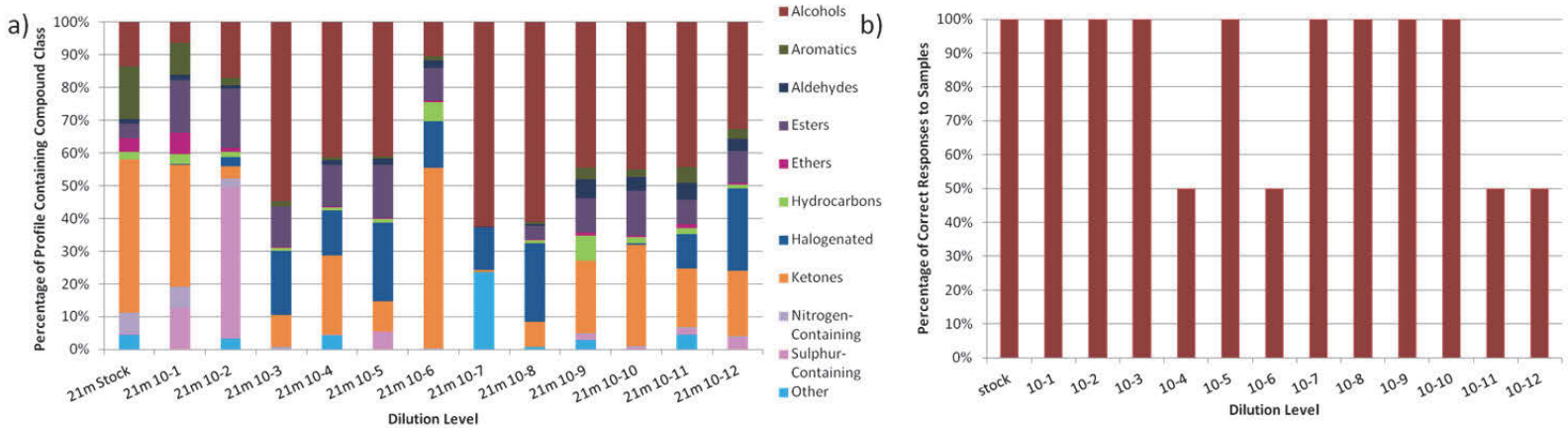

Figure 4-15: The comparison of chemical profiles to dog detection rates for the 21 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

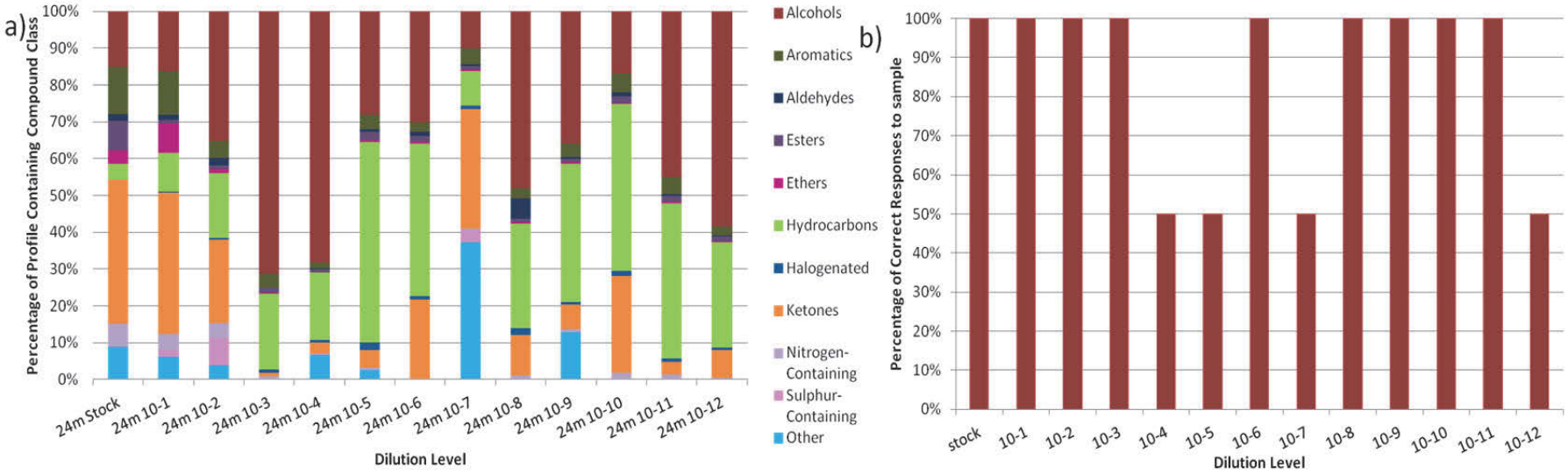

Figure 4-16: The comparison of chemical profiles to dog detection rates for the 21 month aged and diluted samples: a) chemical compound class proportions, b) dog detection rates

4.4 Discussion

4.4.1 Fresh and Aged Dilutions of Decomposition Fluid

In order to examine the dogs' sensitivity to decomposition fluid, a systematic approach was required, and a dilution series was chosen as the method of delivery for this particular study. A preliminary study by the author [59] demonstrated that the dogs were capable of detecting dilutions of up to 10^{-7} (i.e. 1 part-per-ten-million) and therefore, the dilutions in this study were further reduced to 10^{-12} (i.e. 1 part-per-trillion). This represents the lowest detection limit reported in the literature for cadaver-detection dog teams using decomposition fluid as a training aid.

A 1.0 mL sample of decomposition fluid was the initially tested sample volume during the training sessions, since the police canine handlers in this study had previously used 1.0 mL of blood as a training aid. While Trial 1 represented the dogs' first exposure to decomposition fluid as a training aid, their positive responses to the stock sample and first dilutions were expected since the experimenters could also smell decomposition odour for the 10^{-1} and the 10^{-2} dilutions. The dogs did demonstrate their enhanced olfactory sensitivity, however, by alerting to dilutions as low as 10^{-5}. This was further evidenced when all three teams responded positively to most of the lower dilutions during Trial 2, and when, by the end of Trial 4, the teams were consistently able to locate the lowest dilutions. Given these results, the decision was made to lower the volume of fluid in order to test the dogs' sensitivity to smaller aliquots of the training aid. A study by Lorenzo *et al* [122] indicated that their dogs were reliably able to detect 250 µL (0.25 mL) of stock decomposition fluid, and so, a volume of 200 µL (0.2 mL) was chosen for the dilution series in Trials 5 and 6.

Team A was able to detect all dilutions at the reduced volume during Trial 5 (see Figure 4-4), while Team B had difficulty locating several of the mid-level dilutions, but not the lowest dilution, at the reduced volume. Since there were only two dogs available for this particular trial, it is unclear whether this occurred in response to the lowered volumes, or whether other factors were involved. The lowered volume may also explain the difficulty for Team C when locating the lowest dilutions after a 2-month absence from training and deployment (Trial 6, Figure 4-4).

After a discussion with the training coordinator, it was decided that the 0.2 mL volume of decomposition fluid was no longer a challenge for the cadaver-detection dogs, and that the volume would be decreased once again. Hence, at the beginning of the ageing trials (Trial 7), the volume of sample was reduced to 0.1 mL, and the cans were placed in random order. Similar to the decrease from 1 mL to 0.2 mL, the dogs were initially confused by the decrease in volume (and, likely, a decrease in the amount of odour present), but they were soon able to consistently detect the samples, and only one sample (10^{-10}) was missed entirely by the dogs.

The teams demonstrated a strong positive response to almost all of the aged samples presented to them. Studies by Oesterhelweg *et al* [24] and Lasseter *et al* [7] determined that dogs could detect decomposition residue for up to 65 days (approximately 2 months), and remains that had been skeletonised over 20 years prior to their trials. These results indicate that the cadaver-detection dogs can be expected to detect aged decomposition odour, and that the decomposition odour is still recognizable years later. However, this does not explain the poor results the dogs provided to the 15 month aged and diluted samples in this study. This was the oldest sample the dogs had ever been exposed to at the time of its introduction during training. It is possible that the odour had changed distinctly at this point (discussed in Section 4.4.3), and the dogs required the introduction of this new version of the odour profile as a training aid in order to continue to recognize it as decomposition fluid in the later samples of aged fluid (i.e. 18-24 month samples). Whether there is truly a change in the profile, or if there was another reason for the poor response to the 15 month aged samples, this reinforces the need for multiple training aids from various stages of the decomposition process. The change in the odour profile of decomposing remains as they age will have an effect on the ability of the dogs to recognize and alert to their target odours. They need to be exposed to the widest possible variety of odour profiles in their training aids in order to be effective in the field.

In the case of the 'extreme' dilutions, the ability of the dogs to detect the 10^{-24} dilution was astounding. To put this number in perspective, the training aids they had previously detected were diluted to 10^{-12}, or 1 part-per-trillion, which is the equivalent of one drop of decomposition fluid in 20 Olympic-sized swimming pools. A 10^{-24} dilution is 1 part-per-septillion, which is significantly more dilute than 10^{-12}, demonstrating the dogs' superior olfactory capabilities. There were several problems with this trial, however, that meant this result could not be validated. The distractor cans used in this trial had been re-used for several previous trials that day, which may have given

the dogs other indicators, such as trace amounts of dog saliva in the distractor cans, which allowed them to locate the new sample cans amongst them. This trial was also run by a training-coordinator-in-training, and due to his inexperience, he may have inadvertently telegraphed the target odour locations to the dogs. Overall, all of the teams demonstrated strong responses to decomposition fluid, and their sensitivity to the diluted samples was enhanced as they were regularly exposed to the training aid.

4.4.2 Correct vs. Incorrect Responses

When comparing correct and incorrect responses, the incorrect responses were minimal and appeared to be mostly influenced by the dogs' mannerisms and method of searching. The cadaver-detection dog in Team A was a highly energetic English springer spaniel who demonstrated a higher number of false negative responses than the other teams. This was attributed to her rapid method of searching; she would often 'skip' cans in her rush to scent the next can in the line-up, leading to a false negative response. Dog B was also an English springer spaniel but she demonstrated a slow and methodical search method; this included 'self-checking' behaviour, where she would scent the adjacent cans again before alerting to her target can. As a result, she produced a higher number of false positive responses. The cadaver-detection dog in Team C was a long-haired German shepherd with a quick and methodical manner of searching. He demonstrated the lowest number of false responses before his two-month absence from training, but this error rate increased considerably after he returned (see Trial 6 in Figure 4-4, and Trial 12 in Figure 4-6). This suggests that the dogs require regular exposure to decomposition fluid in order to maintain their sensitivity to the lower dilutions of decomposition fluid.

The most common false responses were false positives to foil and cotton wool; however, there is no clear indication of why this occurred. It is recommended that future research investigate and profile these distractor items and compare the profiles to those of decomposition odour. A comparison of chemical profiles could indicate whether they produce sufficiently similar odour compounds to confuse the dogs, or whether further investigation into the reasons for false positive responses is required.

Due to the small number of teams and trials, the response and recovery rate data cannot be analysed for statistical significance. The important information obtained from this data set is that the correct responses for each team are considerably greater than the incorrect responses, and that the number and pattern of incorrect responses to decomposition fluid appear to be mostly dependent on the dogs' scenting method. However, with further research in this field, statistically significant data regarding these observed trends could be obtained.

It should be noted that the cadaver-detection dogs are a living detection system, and as such, will not yield entirely reproducible results. The dogs are not aware that they are being tasked with looking for human remains. They are simply looking for the source of the smell that will lead to a play reward. For this reason, there will always be variations in their responses from session to session, especially if one of the dogs is not in the mood to search or play, or has missed training recently. This does not mean that they are ineffective search tools; it is a side-effect of having to deal with a living detection system.

4.4.3 Comparison of Odour Profiles to Dog Responses

When directly comparing the responses of the dogs to the odour profile produced by each dilution, it can be difficult to relate the two based on specific compounds that may be causing the responses. It is more relevant to examine the changes to the compound classes in the samples that one or more of the dogs failed to locate, and compare to those samples that they were able to locate. As explained in Section 3.3.3, even the GC×GC, which is the most sensitive analytical instrument currently available for this type of research, was unable detect many of the trace decomposition VOCs that may have assisted to distinguish between the odour profiles of the dilutions beyond 10^{-4} using PCA. However, the dogs were regularly able to recognise the decomposition odour of samples diluted to 10^{-12}, demonstrating that the dogs are far more sensitive than the analytical equipment currently available. In spite of the occasional difficulties that a living detection system like a dog may cause, they are still the fastest, least intrusive, and most sensitive method for locating human remains.

Each sample set produced a different odour profile, and each of those profiles changed differently when diluted. The only consistency was a decrease in the number and abundance of compounds

present in the samples as the fluid was diluted (Section 3.3.3), although there was a general increase in the proportion of alcohols and a decrease in the proportion of sulphur-containing compounds as the samples were diluted. Variations were also observed in the proportions of ketones, esters and other compounds in the samples that the dogs had difficulty detecting. It is interesting to note that despite the large number of compounds produced and the wide variations in the profiles throughout the dilution and ageing of the compounds, neither cadaverine nor putrescine were detected in the odour profile of any sample. This clearly contradicts the theory proposed by Gill-King [56] and Killam [8], which hypothesise that the cadaver-detection dogs use these two compounds to locate decomposing remains. These two compounds also represent a proportion of the pseudo-scents currently produced as synthetic training aids for human decomposition. It is clear that pseudo-scents, which contain fewer than 10 compounds overall [57], and mostly amines and ketones, are not accurate representations of the incredibly complex and dynamic odour of decomposition. Until a range of more accurate synthetic scents can be developed, the current pseudo-scents should only be used (if at all) in conjunction with real human tissues whenever possible. The dogs need to be presented with a variety of training aids from different stages of decomposition and from different levels of dilution in order to make the most of their capabilities in the field.

Given the variations in the odour profiles as the samples were diluted and aged, it is unlikely that the dogs use a single compound to determine the presence or absence of decomposition. There was no single compound class that could uniquely identify decomposition odour, or even a specific ratio of compound classes that would demonstrate this profile. Based on the ability of the dogs to locate the samples even when the proportions of compound classes in the diluted and aged samples changed distinctly from the stock sample odour profile, the dogs appear to use a combination of compounds to identify decomposition, but it is not yet clear what compounds, or even compound classes these may be, although further investigation should be made into the 35 compounds determined to be present in over 95% of the decomposition samples in this study (Section 3.4.3). Overall, the dogs located the majority of the samples in the majority of the trials, including the ‘extreme’ dilutions using the fresh fluid in the last trial. Even the 18 month aged samples, which were chemically quite different from the other samples (Figure 4-14a) were located with a 92% recovery rate. However, the 15 month samples (Figure 4-13a), which all the dogs had difficulty locating, demonstrated odour profiles similar to the 3 month and 24 month

aged samples (Figure 4-9a and 4-16a). Therefore, it is difficult to explain, based on the odour profile, why the dogs were unable to locate some of these samples, and suggests that a variable other than (or in addition to) the chemical profile is causing the dogs' false responses to their training aids.

Future investigations into the cadaver-detection dogs' sensitivity levels should continue with lowered volumes of decomposition fluid, as well as with decomposition fluid that has been aged up to several years. Lower dilutions and volumes of fluid should be trialed in order to determine the limit of sensitivity for each cadaver-detection dog as this could not be confirmed in this study, given that they alerted to extremely dilute decomposition fluid samples. The trials should also be carried out under non-standard training conditions, such as an outdoor mock crime scene, in order to determine whether variables such as rain or wind will affect the dogs' sensitivity levels in operational scenarios. Where possible, the results from tests under these conditions should also be linked to the chemical odour profiles in order to determine the types of compounds influencing the dogs' ability to locate decomposition odour. This may prove difficult, however, given the challenge of identifying the key VOCs with the current limits of detection for the GC×GC-TOFMS, which is the most sensitive instrumentation available for decomposition odour analysis at this time. This type of study will require instrumentation that is sufficiently sensitive to detect the VOCs in such trace concentrations as are found in the extreme dilutions, and as such, it may not yet be possible to link the odour profiles of extremely dilute decomposition fluid samples to the responses obtained from cadaver-detection dog training sessions.

4.5 Conclusions

The aim of this study was to examine the dogs' capability to detect the odour of decomposition fluid at diluted concentrations, and to determine if their sensitivity increased with consistent exposure to the training aid. After regular exposure to the decomposition fluid, the cadaver-detection dog teams were able to reliably detect a range of dilutions and volumes with the lowest being 0.1 mL of 1-part-per-trillion (10^{-12}) decomposition fluid, which had been aged for 24 months. The dogs were also able to detect dilutions of 1 part-per-septillion (10^{-24}) of fresh decomposition

fluid, although this trial was not repeated, and cannot be considered reliable without validation. These represent the lowest sensitivity levels reported in the scientific literature to date.

It is possible that the dogs rely mainly on the alcohols and sulphur-containing compounds within the odour profile in order to identify and locate decomposing remains. However, there is no consistency to these results, and there are indications that the false responses the dogs give are not caused solely by changes to the odour profiles of their training aids. While further testing is required to determine the exact limits of the dogs' sensitivity to decomposition fluid, these results do support the use of human decomposition fluid as a valid training aid for cadaver-detection dog teams in Australia.

Chapter 5:

Conclusions and Future Works

Chapter 5: Conclusions and Future Works

5.1 Summary of Thesis

While cadaver-detection dogs have been used by police forces for decades, the investigation of their abilities and limitations in different environmental conditions and with different types of training aids has only been studied since the late 1990s. Decomposition odour profiling is also a relatively new field of research that has only been studied since the turn of the millennium. Information regarding both of these fields is increasing, but few studies have attempted to link the findings to date. Studies have investigated the odour profiles of decomposing human or pig remains [22, 49, 57, 78, 80, 81, 86, 87, and more), different types of training aids (such as gauze pads [7]), the longevity of the target odour [24], and the accuracy and recovery rates of the dogs when exposed to decomposition odour samples [25]. To add to the limited knowledge base in this field, the current study aimed to validate human decomposition fluid as a training aid used for cadaver-detection dogs in Australia and elsewhere in the world. This aim was achieved by developing VOC collection and analysis methods for the odour profile of decomposition fluid, investigating the odour profile of the training aid when stored under various conditions and for different periods of time, and comparing the odour profiles of the diluted training aids to the responses given by the cadaver-detection dogs during training sessions.

In Chapter 2, a method for the collection and analysis of decomposition VOCs was developed and reported. Using SPME fibres to collect the VOCs from decomposition fluid proved to be a faster and more practical method when compared to the purge-and-trap system designed for collecting VOCs using sorbent tubes. The SPME collection technique used a smaller volume of decomposition fluid, and collected a wider range of volatile compounds from the overall decomposition fluid profile. The PDMS/DVB/CAR fibre was identified as the optimal fibre for the study. A method for sample analysis using GC×GC-TOFMS was adapted from Stadler *et al* [57], providing a high level of sensitivity and separation of analytes.

Following optimisation, SPME-GC×GC-TOFMS was subsequently used to profile the odour of human decomposition fluid as a cadaver-detection dog training aid. The number and type of VOCs

produced by decomposition fluid were compared to the VOCs reported in the literature for decomposing human remains (Chapter 3). The decomposition fluid samples used in this study were not easily compared to the profile of decomposing cadavers in the literature. The difference in VOCs reported may have resulted from the lack of a whole body for the production of the decomposition fluid, especially the lack of intestinal bacteria that initiate the decomposition process; this absence may have altered the profile of the decomposition fluid when compared to a decomposing cadaver. The variation could also be attributed to the use of different sample collection and analysis techniques in the literature. Different techniques, such as SPME and sorbent tubes have been shown to collect different proportions of compounds depending on the sample collection method. Furthermore, the use of GC-MS has been shown to suffer limitations, such as co-elution and masking of compounds, and GC×GC-TOFMS is now considered the preferred method for optimal separation and identification of decomposition VOCs. However, when the proportions of compound classes (rather than individual compounds) within the decomposition fluid profile were compared to those reported in the literature, the profiles demonstrated greater similarity. The overall proportions of compound classes suggested that decomposition fluid is an accurate representation of the profile of a decomposing cadaver, and is more accurate than the commercially available synthetic scents.

This study also examined the storage conditions under which the training aid can be maintained as a valid and accurate representation of the odour of human remains. The change in the profile over time was studied to determine a point at which the odour profile might no longer resemble that of a human cadaver and lose its value as a training aid (Chapter 3). Although there were no obvious trends in the changes to the profile of the fluid as it aged under different storage conditions, the VOC profile still resembled decomposition odour after 12 months. The room temperature and refrigerated storage conditions were determined to be preferable for storing decomposition fluid compared to the freezer, where the odour profile changed considerably as the samples aged. However, cadaver-dog handlers should use their discretion when storing decomposition fluid samples for long periods of time, depending on the environmental conditions under which they are required to work.

As in the case of the storage and ageing samples, there were no obvious trends seen in the changes to the odour profiles of the diluted and aged training samples (Chapter 3). While the overall number and abundance of compounds decreased as the samples were diluted, there was

no consistency regarding which compound classes were lost. Despite the enhanced sensitivity of GC×GC-TOFMS, which was able to detect compounds even in the 1 part-per-trillion samples, the samples could not be statistically differentiated below the level of 10^{-4} dilutions (1 part-per-ten-thousand). Given the positive results of the storage and ageing trials, the study was extended to 24 months and applied to the cadaver-detection dog trials. Using samples stored in the refrigerator and brought to room temperature for the trials, these diluted and aged samples were presented to the dogs during training sessions in order to be able to directly compare the odour profiles to the cadaver-detection dog responses (Chapter 4).

This study investigated the sensitivity of the dogs to decomposition fluid as a training aid, comparing their sensitivity to the detection limits of the GC×GC-TOFMS. The possibility that the dogs' sensitivity to decomposition odour would increase as they became more familiar with their training aids was investigated through regular exposure to increasingly small volumes of more dilute samples of decomposition fluid (Chapter 4). The findings demonstrated that the cadaver-detection dogs were far more sensitive than GC×GC-TOFMS. The VOC profile could not easily be detected in samples below 10^{-4}; however, the dogs were regularly able to detect samples diluted to 10^{-12}, and on one occasion, diluted to 10^{-24}. As the dogs became more familiar with their training aids through regular exposure, they appeared to become more sensitive to the training aids, and were able to locate the diluted samples with greater confidence in the later trials. It was shown that the cadaver-detection dogs did require regular exposure during training sessions to maintain their sensitivity to decomposition fluid as a training aid.

Chapter 4 of the thesis also aimed to link the VOC profile of decomposition fluid to the alerts obtained from training sessions with cadaver-detection dogs. The aim was to identify the VOCs present in the decomposition fluid samples to determine which VOCs were consistently present and may represent the VOCs that the cadaver-detection dogs use to locate human remains. However, it was not possible to determine whether there was a single compound or set of compounds that the dogs used to identify decomposition odour or that caused their false responses. Although the alcohols and the sulphur-containing compound proportions varied in a number of the samples to which the dogs falsely alerted, a consistent pattern was not observed, and therefore, it is likely that some other variable is also involved in producing false responses. This study found that the dogs' personalities and the regularity of their training had an influence on the type of false responses given. While the dogs may not be faultlessly reliable detection

systems because they are living beings, they are still the fastest, least intrusive, and most sensitive detection method for locating decomposing remains. Overall, the cadaver-detection dogs in this trial demonstrated high recovery rates and a low number of false responses, regardless of the age or dilution level of the decomposition fluid provided for their training.

The results from this study have validated the use of human decomposition fluid as a cadaver-detection dog training aid in Australia, but further validation is required. There are still many knowledge gaps in the field of decomposition odour profiling, in determining cadaver-detection dog capabilities, and in understanding the impact of decomposition VOCs on cadaver-detection dog alerts. Both of these fields are new and developing areas of research, and there are many future studies that should be undertaken to further develop this knowledge, some of which will be proposed below.

5.2 Future Recommendations

The decomposition fluid samples used in this study were provided by the UTS SASF, and were produced from decomposing sections of tissue from various parts of the body, rather than from decomposing cadavers. Although the tissues were not embalmed, they were often taken from specimens that had been refrigerated prior to sampling. This may have caused some of the bacteria that would have decomposed the tissue samples naturally to have been removed by the refrigeration process, or the bacteria may not have been present in the original tissue due to the location from which the samples were dissected (i.e. no gut flora present to decompose a sample of arm tissue). The tissue samples were also collected from several donors over the course of the study. Hence, the variations in the VOC profiles, when compared to the VOC profiles reported in the literature (discussed in Chapter 3) may have been due to the variations in tissue types, variations in the original donor body proportions (a larger donor may have provided more fatty tissue, and therefore a larger proportion of lipids, for degradation, etc), or to the lack of a complete body system while decomposition occurred. Therefore, it is recommended that future studies use human cadavers for the production of decomposition fluid, if possible.

A direct comparison between the odour profile of decomposition fluid produced by a cadaver and the odour profile released from the cadaver should also be undertaken. This would decrease the

potential for variations in VOC profile caused by multiple donors and incomplete tissue samples. It is recommended that GC×GC-TOFMS be used for any future research in decomposition odour profiling, due to its enhanced sensitivity and separation capabilities when compared to the GC-MS.

It would also be valuable to study the changes to the profile of decomposition fluid aged for more than a year. When stored under refrigerated or room temperature conditions, there is potential that the VOC profile will still resemble decomposition odour beyond this time frame. Such a study would assist in identifying a point at which the decomposition fluid samples no longer resemble the odour profile of the original source, and is therefore no longer viable as a training aid. Personal communication with local police dog handlers has indicated that decomposition fluid may need to be stored for more than a year due to the difficulty in accessing it as a training aid.

Future research with the cadaver-detection dogs should focus on examining the causes for their false responses by examining the odour profiles produced by the distractor items they alert on (i.e. aluminium foil). It may be that these items produce similar VOCs to the target odours. If shown to be correct, more rigorous avoidance training may be needed for these items in the future. Alternatively, it is possible that the VOCs in the distractor items are distinct from the target odour, and there is a different cause for false responses which needs to be elucidated.

The odour profiles of the decomposition fluid training samples that the dogs are able to locate should be further compared to the profiles of the training samples which they are unable to locate, using a consistent set of training samples. This may allow for a more comprehensive profile to be determined, which would assist in determining the key compounds or compound classes that cause the dogs' responses. If these compounds can be identified, the dynamic changes to these compounds throughout the decomposition process can be specifically studied, eventually leading to the production of a range of synthetic scents which accurately mimic the decomposition process.

Finally, additional testing with regards to the dilution of samples presented to the dogs is recommended. With the presentation of lower and lower dilution levels, the dogs will eventually reach a point where they are unable to distinguish the diluted decomposition fluid from the tap water controls, which would indicate their lowest limits of detection. These sensitivity tests should also be performed in a variety of outdoor and/or mock scenes, in order to determine how variables such as temperature, humidity or wind speed will affect their sensitivity levels.

Determination of the sensitivity levels of cadaver-detection dogs will assist handlers in understanding the capabilities of their dogs, and form a baseline for testing their capabilities under extreme conditions, such as in cases where a body has been buried for many years, encased in cement, deposited in water, etc.

These results provide the first connection between the odour profiling research completed over the last decade and the training procedures used for the cadaver-detection dogs. It is hoped that these results will provide a baseline for further research in this field, where researchers can begin to clarify the manner in which the cadaver-detection dogs identify and locate decomposing matter. This would allow for the development of a more accurate range of synthetic scents, which could be made widely available for cadaver-detection dog handlers; these, in turn, could help to standardize training procedures for cadaver-detection dogs worldwide. With the standardization of training procedures, the results provided by these dogs can be validated, and presented as a reliable form of evidence in court.

FINIS

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Appendices

Appendix A:

List of Significant VOCs Obtained from Human Decomposition Fluid

Appendix A: List of Significant VOCs Obtained from Human Decomposition Fluid

*VOCs listed in *red* are those in common with the VOCs found in the literature

(-)-Carvone
(1R,2R,3R,5S)-(-)-Isopinocampheol
(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
(2S,3S)-(-)-3-Propyloxiranemethanol
(3E,5Z)-1,3,5-Undecatriene
(3Z,5E)-1,3,5-Undecatriene
(5-Ethylcyclopent-1-enyl)methanol
(E)-1-Phenyl-1-butene
(E)-2-Pentenenitrile
(E)-4-Oxohex-2-enal
(E,E,E)-2,4,6-Octatriene
(S)-(+)-2',3'-Dideoxyribonolactone
(Z)-Dodec-5-en-4-olide
[1,1'-Biphenyl]-2-amine
1-(2,4-Dimethyl-furan-3-yl)-ethanone
1-(2-Thienyl)-1-propanone
1-(Methylthio)-3-pentanone
1,10-Undecadiene
1,11-Dodecadiene
1,13-Tetradecadiene
1,1'-Bicyclopropyl, 1,1'-dimethyl-
1,2,3-Trithiolane
1,2,5-Trimethylpyrrole
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
1,2-Dimethylcyclopropanecarboxylic acid
1,2-Dithiolane
1,2-Heptadiene
1,2-Propanedione, 1-phenyl-
1,3,3,4-Tetramethyl-2-oxabicyclo[2.2.0]hexane
1,3,5,7-Cyclooctatetraene
1,3,5-Cycloheptatriene
1,3,5-Cycloheptatriene, 7,7-dimethyl-
1,3,5-Heptatriene, (E,E)-
1,3,7-Octatriene, 3,7-dimethyl-
1,3-Benzodioxole
1,3-Cyclododecadiene, (E,Z)-
1,3-Cyclohexadiene, 1,3,5,5-tetramethyl-
1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-
1,3-Cyclohexadiene, 5-ethyl-
1,3-Diazine
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-
1,3-Dioxolane, 2-heptyl-4-methyl-
1,3-Heptadiene, 5,5-dimethyl-
1,3-Nonadiene, (E)-
1,3-Octadiene
1,3-Oxathiane
1,3-Pentadiene
1,3-Pentadiene, (Z)-
1,3-Propanediol
1,3-trans,5-cis-Octatriene
1,4-Cyclohex-2-enedione
1,4-Cyclohexadiene, 1,2-dimethyl-
1,4-Cyclohexadiene, 1-ethyl-
1,4-Dioxane
1,4-Heptadiene
1,4-Octadiene
1,4-Undecadiene, (E)-
1,5,9-Decatriene, 2,3,5,8-tetramethyl-
1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-
1,5-Cyclooctadiene, 1,3-dimethyl-
1,5-Hexadien-3-ol
1,5-Hexadiene, 2,5-dimethyl-
1,5-Hexadiene, 2,5-dimethyl-3-methylene-
1,6,11-Dodecatriene, (Z)-
1,6-Dioxaspiro[4.4]nonane, 2-ethyl-
1,6-Octadien-3-ol, 3,7-dimethyl-
1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
1,6-Octadiene, 3,7-dimethyl-, (S)-
1,7-Octadien-3-ol, 2,6-dimethyl-
1,8-Nonadiene
1,9-Decadiyne
1,E-11,Z-13-Octadecatriene

10-Dodecyn-1-ol
10-Heneicosene (c,t)
10-Methyldodec-3-en-4-olide
10-Undecen-1-ol
11-Dodecenol
11-Tetradecen-1-ol, acetate, (Z)-
13-Docosenoic acid, methyl ester
1-Adamantanol
1-Bromo-3,7-dimethyl-2,6-octadiene
1-Butanamine
1-Butanamine, 2-methyl-
1-Butanamine, 3-methyl-
1-Butanamine, 3-methyl-N-(2-phenylethylidene)-
1-Butanamine, 3-methyl-N-(3-methylbutylidene)-
1-Butanamine, N-(phenylmethylene)-
1-Butanamine, N-butyl-
1-Butanamine, N-butylidene-
1-Butanol
1-Butanol, 2-methyl-, (S)-
1-Butanol, 3-methoxy-
1-Butanol, 3-methyl-
1-Butanol, 3-methyl-, acetate
1-Butanol, 3-methyl-, propanoate
1-Butanone, 1-phenyl-
1-Butanone, 3-methyl-1-phenyl-
1-Cyclohexene-1-methanol
1-Decanol
1-Decene
1-Decyne
1-Docosene
1-Dodecanamine, N,N-dimethyl-
1-Dodecanol, 3,7,11-trimethyl-
1-Dodecen-3-yne
1-Dodecene
1-Eicosanol
1H,1H,2H,2H-Perfluorooctan-1-ol
1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4à,4aá,7bà)]-
1-Heptanol
1-Hepten-3-ol
1-Heptene
1-Heptene, 3-methyl-
1-Heptene, 4-methyl-
1-Hexadecanol
1-Hexanethiol
1-Hexanol
1-Hexanol, 2-ethyl-
1-Hexanone, 1-(2-thienyl)-
1-Hexanone, 1-phenyl-
1-Hexen-3-yne, 2-methyl-
1-Hexene
1-Hexene, 2,4,4-triethyl-
1-Hexene, 3,5-dimethyl-
1-Hexene, 3-methyl-
1-Hexene, 5-methyl-
1H-Indene, 1-ethylidene-
1H-Indene, 2,3-dihydro-1,1-dimethyl-
1H-Indene, 2,3-dihydro-1,6-dimethyl-
1H-Indene, 2,3-dihydro-4,7-dimethyl-
1H-Indene, 2,3-dihydro-4-methyl-
1H-Indole, 3-methyl-
1H-Indole, 5-methyl-
1H-Indole, 6-methyl-
1H-Pyrrole, 1-butyl-
1H-Pyrrole, 2-ethyl-
1H-Pyrrole, 2-ethyl-4-methyl-
1H-Pyrrole, 3,4-diethyl-2-methyl-
1H-Pyrrole, 3-ethyl-2,4-dimethyl-
1H-Pyrrole, 3-methyl-
1-Iodo-2-methylundecane
1-Methoxy-3-(2-hydroxyethyl)nonane
1-Methyl-4-(1-methylethyl)-cyclohexane
1-Methylcycloheptene
1-Methyldecahydronaphthalene
1-Nonanol
1-Nonen-3-ol
1-Nonen-4-ol
1-Nonene
1-Nonene, 4,6,8-trimethyl-
1-Octanamine
1-Octanol
1-Octanol, 2-butyl-
1-Octen-3-ol
1-Octen-3-one

1-Octen-4-yne
1-Octene
1-Octene, 3,7-dimethyl-
1-Octyn-3-ol, 4-ethyl-
1-Pentadecanamine, N,N-dimethyl-
1-Pentanethiol
1-pentanol
1-Pentanol
1-Pentanol, 4-methyl-
1-Penten-3-ol
1-Penten-3-one
1-Penten-3-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
1-pentene
1-Phenyl-2-butanone
1-Propanamine, 2-methyl-N-(2-methylpropylidene)
1-Propanamine, 3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-, S-oxide
1-Propanol
1-Propanol, 2,2'-oxybis-
1-Propanol, 2-methyl-
1-Propanol, 3-(methylthio)-
1-Propanol, 3,3'-oxybis-
1-Propanone, 1-phenyl-
1-Propyne, 3-phenyl-
1-Pyrrolidinecarboxaldehyde
1-Tetradecyne
1-Tridecanamine, N,N-dimethyl-
1-Undecanamine, N,N-dimethyl-
1-Undecanol
1-Undecene
2-(2-Bromoethyl)-1,3-dioxolane
2(3H)-Furanone, 5-butyldihydro-
2(3H)-Furanone, 5-ethenyldihydro-5-methyl-
2(3H)-Furanone, 5-ethyldihydro-
2(3H)-Furanone, 5-heptyldihydro-
2(3H)-Furanone, 5-hexyldihydro-
2(3H)-Furanone, dihydro-3,5-dimethyl-
2(3H)-Furanone, dihydro-3-methyl-
2(3H)-Furanone, dihydro-5-(2-octenyl)-, (Z)-
2(3H)-Furanone, dihydro-5-methyl-
2(3H)-Furanone, dihydro-5-pentyl-
2(3H)-Furanone, dihydro-5-propyl-
2(3H)-Thiophenone, dihydro-5-methyl-
2-(3-Methylbutyl)-3,5-dimethylpyrazine
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
2(5H)-Furanone, 5,5-dimethyl-
2(5H)-Furanone, 5-ethyl-
2(5H)-Furanone, 5-methyl-
2,2,7,7-Tetramethyloctane
2,3,3-Trimethyl-1-hexene
2,3,5-Trimethyl-6-butylpyrazine
2,3,5-Trimethyl-6-ethylyrazine
2,3,5-Trimethyl-6-propylpyrazine
2,3,6-Trimethylacetophenone
2,3-Butanediol
2,3-Butanediol, [S-(R*,R*)]-
2,3-Butanedione
2,3-Dimethyl-5-n-propylpyrazine
2,3-Dimethylphenyl isocyanate
2,3-Hexanedione
2,3-Nonadiene
2,3-Octanediol
2,3-Octanedione
2,3-Pentanedione
2,4,4-Trimethyl-1-pentanol
2,4,6-Cycloheptatrien-1-one
2,4,7,9-Tetramethyl-5-decyn-4,7-diol
2,4-Decadienal, (E,E)-
2,4-Decadienoic acid, methyl ester, (E,Z)-
2,4-Dimethylstyrene
2,4-Dithiapentane
2,4-Heptadienal, (E,E)-
2,4-Hexadienal, (E,E)-
2,4-Hexadienoic acid, methyl ester
2,4-Nonadienal
2,4-Nonadienal, (E,E)-
2,4-Nonadiene, (E,E)-
2,4-Octadienal, (E,E)-
2,4-Octadiene
2,4-Undecadien-1-ol
2,5-Furandione, 3,4-dimethyl-
2,5-Octadiene
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol
2,6-Dimethyl-1,3,5,7-octatetraene, E,E-

2,6-Dimethyldecane
2,6-Nonadienal, (E,Z)-
2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-
2,7-Octadien-1-ol
2-Acetyl-1-pyrroline
2-Acetylthiazole
2-Butanol
2-Butanol, 1-methoxy-
2-Butanol, 3-methyl-
2-Butanone
2-Butanone, 3-methyl-
2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
2-Butanone, 4-phenyl-
2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1 -yl)-, (E)-
2-Butenal
2-Butenal, 2-ethyl-
2-Butenal, 2-methyl-
2-Butenal, 2-methyl-, (E)-
2-Butenal, 3-methyl-
2-Butenoic acid, 2-methyl-, 1-methylethyl ester, (Z)-
2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-
2-Butenoic acid, 2-methyl-, 3-methylbutyl ester, (E)-
2-Butenoic acid, 2-methyl-, propyl ester, (E)-
2-Butenoic acid, 3-amino-, methyl ester
2-Butenoic acid, 3-methyl-, 2-methylpropyl ester
2-Butenoic acid, 3-methyl-, butyl ester
2-Butenoic acid, 3-methyl-, methyl ester
2-Butenoic acid, methyl ester, (E)-
2-Butyl-1-decene
2-Butyl-3-methylpyrazine
2-Chloro-4-fluorobenzonitrile
2-Cyclohexen-1-ol, 3-bromo-
2-Cyclohexen-1-one, 2-methyl-
2-Cyclohexen-1-one, 3,5-dimethyl-
2-Cyclohexen-1-one, 3-methyl-
2-Cyclopenten-1-one
2-Cyclopenten-1-one, 2,3-dimethyl-
2-Cyclopenten-1-one, 2-pentyl-
2-Decanol
2-Decanol, methyl ether
2-Decanone
2-Decen-1-ol
2-Decenal, (Z)-
2-Decenoic acid, methyl ester, (E)-
2-Dodecanol
2-Dodecanol, 2-methyl-
2-Dodecanone
2-Ethylidenecyclohexanone
2-Furanmethanethiol, 5-methyl-
2-Furanmethanol, 5-ethenyltetrahydro-à,à, 5-trimethyl-, cis-
2-Furanmethanol, tetrahydro-
2-Heptanol
2-Heptanol, (S)-
2-Heptanol, 3-methyl-
2-Heptanol, 5-methyl-
2-Heptanol, 6-methyl-
2-Heptanone
2-Heptanone, 3-methyl-
2-Heptanone, 4,6-dimethyl-
2-Heptanone, 5-methyl-
2-Heptanone, 6-methyl-
2-Heptenal, (Z)-
2-Heptenal, 2-methyl-
2-Hexadecene, 2,6,10,14-tetramethyl-
2-Hexanol
2-Hexanol, 2,5-dimethyl-, (S)-
2-Hexanol, methyl ether
2-Hexanone
2-Hexanone oxime
2-Hexanone, 3,4-dimethyl-
2-Hexanone, 3-methyl-
2-Hexanone, 4-methyl-
2-Hexanone, 5-methyl-
2-Hexanoylfuran
2-Hexen-1-ol, acetate, (Z)-
2-Hexenal
2-Hexenal, (E)-
2-Hexenal, 2-ethyl-
2-Hexene, 3,4,4-trimethyl-
2-Hexene, 3,5,5-trimethyl-
2-Hexenoic acid, methyl ester

2H-Pyran, tetrahydro-2-methyl-
2H-Pyran-2-one, 5,6-dihydro-6-pentyl-
2H-Pyran-2-one, 6-ethyltetrahydro-
2H-Pyran-2-one, 6-heptyltetrahydro-
2H-Pyran-2-one, tetrahydro-
2H-Pyran-2-one, tetrahydro-6-methyl-
2H-Pyran-2-one, tetrahydro-6-pentyl-
2H-Pyran-2-one, tetrahydro-6-propyl-
2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-
2-Isoamyl-6-methylpyrazine
2-Isobutyl-3-methylpyrazine
2-Isobutyl-4-methylpyridine
2-Isobutylthiazole
2-Isopropyl-5-methylhex-2-enal
2-Isopropylpyrazine
2-Methoxy-5-methylphenol
2-Methyl-1,6-dioxaspiro[4.4]nonane
2-Methyl-3-(methylthio) furan
2-Methyl-3-isopropylpyrazine
2-Methyl-3-propylpyrazine
2-Methyl-6-propylpyridine
2-methylester butanoic acid
2-methylheptane
2-methyloctacosane
2-Methylphenylacetylene
2-Naphthalenol
2-n-Butyl furan
2-n-Butylacrolein
2-n-Heptylfuran
2-n-Hexylcyclopentanone
2-n-Octylfuran
2-Nonanol
2-Nonanone
2-Nonen-4-one
2-Nonenal, (E)-
2-Octanol, (S)-
2-Octanol, 2-methyl-
2-Octanol, 8,8-dimethoxy-2,6-dimethyl-
2-Octanone
2-Octen-1-ol, (E)-
2-Octen-4-one
2-Octenal, (E)-
2-Octene
2-Octene, (Z)-
2-Octenoic acid, methyl ester, (E)-
2-Octyn-1-ol
2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)
2-Oxetanone, 4-methyl-
2-Pentadecanol
2-Pentanol
2-Pentanol, 4-methyl-
2-Pentanol, acetate
2-Pentanone
2-Pentanone, 3-methyl-
2-Penten-1-ol, (Z)-
2-Pentenal, (E)-
2-Pentene, 4-bromo-
2-Pentene, 4-methyl-, (Z)-
2-Pentenenitrile
2-Pentenoic acid, methyl ester
2-Pentylcyclopentanone
2-Phenethyl-á-phenylpropionate
2-Phenylpropenal
2-Piperidinone
2-Propanol, 1-(2-butoxy-1-methylethoxy)-
2-Propanol, 1-butoxy-
2-Propanol, 1-methoxy-
2-Propanone, 1-methoxy-
2-Propenal
2-Propenal, 2-methyl-3-phenyl-
2-Propenenitrile, 3-phenyl-, (E)-
2-Propenoic acid, butyl ester
2-Propylcyclohexanol
2-Pyrrolidin-1-yl-bicyclo[3.2.1]octan-8-ol
2-Pyrrolidinone
2-Sec-Butylcyclohexanone
2-Tetradecanol
2-Tetradecanone
2-Thiophenecarboxaldehyde
2-Tridecene, (Z)-
2-Undecanol
2-Undecanone
2-Undecanone, 6,10-dimethyl-
2-Undecenal, E-

2-Undecene, 3-methyl-, (E)-
2-Undecene, 3-methyl-, (Z)-
3(2H)-Thiophenone, dihydro-2-methyl-
3-(2-Pyridyl)-1-propanol
3-(4-Methylpent-3-enyl)thiophene
3-(Methylthio)-2-butanone
3-(Methylthio)propanoic acid methyl ester
3(N,N-Dimethylmyristylammonio) propanesulfonate
3,3-Dimethylacryloyl chloride
3,4-Hexanedione
3,5,9-Undecatrien-2-one, 6,10-dimethyl-, (E,E)-
3,5-Dimethyl-2-octanone
3,5-Octadien-2-one
3,5-Octadien-2-one, (E,E)-
3,5-Octadiene, (Z,Z)-
3,6-Dipropyl-2,5-dimethylpyrazine
3-[(Z)-1-Butenyl]-4-vinylcyclopentene
3-Buten-1-ol, 3-methyl-
3-Buten-2-ol, 2-methyl-
3-Buten-2-one, 3-methyl-
3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-
3-Buten-2-one, 4-(2,2-dimethyl-6-methylenecyclohexyl)-
3-Buten-2-one, 4-phenyl-, (E)-
3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-
3-Decanol
3-Decanone
3-Decen-1-ol, acetate, (Z)-
3-Decen-2-one
3-Dodecanone
3-Dodecen-1-yne, (Z)-
3-Dodecene, (Z)-
3-Ethyl-1,5-octadiene
3-Ethylcyclopentanone
3-Heptanone
3-Heptanone, 2-methyl-
3-Heptanone, 5-methylene-
3-Hepten-2-one
3-Heptene, 2,6-dimethyl-
3-Heptenoic acid, methyl ester, (E)-
3-Hexanol
3-Hexanol, 5-methyl-
3-Hexanone
3-Hexanone, 2,4-dimethyl-
3-Hexanone, 2,5-dimethyl-4-nitro-
3-Hexanone, 2-methyl-
3-Hexanone, 4-methyl-
3-Hexanone, 5-methyl-
3-Hexen-1-ol
3-Hexen-1-ol, acetate, (Z)-
3-Hexen-2-one
3-Hexen-2-one, 5-methyl-
3-Hexenoic acid, methyl ester, (E)-
3-Hexenoic acid, methyl ester, (Z)-
3-Hydroxy-3-methyl-2-butanone
3-Hydroxyphenylacetylene
3-Methoxybut-1-ene
3-Methyl,2-butenoic acid, isopropyl ester
3-Methyl-2-butenoic acid, 2-pentyl ester
3-Methyl-3-cyclohexen-1-one
3-Nonanol
3-Nonanone
3-Nonen-2-one
3-Nonyne
3-Octanol
3-Octanone
3-Octen-2-one
3-Octen-5-yne, (Z)-
3-Octene, (Z)-
3-Octene, 4-ethyl-
3-Octenoic acid, methyl ester, (Z)-
3-Octyne
3-Pentanol
3-Pentanol, 2-methyl-
3-Pentanone
3-Pentanone, 2-methyl-
3-Penten-1-ol, 4-methyl-
3-Penten-2-one, (E)-
3-Penten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
3-Penten-2-one, 4-methyl-
3-Pentenoic acid, 4-methyl-
3-Pentenoic acid, 4-methyl-, methyl ester
3-Phenylpropanol

3-Tetradecanone
3-Tetradecene, (E)-
3-Thiopheneacetonitrile
3-Thiophenecarboxaldehyde
3-Tridecene, (E)-
3-Tridecene, (Z)-
3-Undecanone
3-Undecene, (Z)-
3-Undecene, 10-methyl-
4'-(2-Methylpropyl)acetophenone
4,4-Dimethylpent-2-enal
4,7-Methano-1H-indenol, hexahydro-
4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-
4-Cyclohexyl-1-butanol
4-Decene, 2,2-dimethyl-, (E)-
4-Decenoic acid, methyl ester, Z-
4-Ethyl-2-hexynal
4-Ethylcyclohexanol
4-Heptanol
4-Heptanone
4-Heptanone, 3-methyl-
4-Hepten-1-ol
4-Heptenal, (Z)-
4-Heptenoic acid, methyl ester, (E)-
4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, acetate
4-Hexen-2-one, 3-methyl-
4-Hydroxy-3-hexanone
4-Isobutylpyrimidine
4-Methyl-1,5-Heptadiene
4-Methyl-1-heptyn-3-ol
4-Methyl-2-hexanol
4-Nonanone
4-Nonene, 5-methyl-
4-Nonenoic acid, methyl ester
4-Octen-3-one
4-Octenoic acid, methyl ester
4-Penten-2-one, 4-methyl-
4-Tetradecene, (Z)-
4-Tridecanone
4-Undecanone
4-Undecene, (Z)-
4-Undecene, 10-methyl-, (E)-
4-Undecene, 3-methyl-, (E)-
4-Undecene, 5-methyl-, (Z)-
5,6,7,8-Tetrahydroindolizine
5,6-Epoxyhexanol-1
5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-
5,9-Undecadien-2-ol, 6,10-dimethyl-
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-
5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-
5-Decen-1-ol, acetate, (E)-
5-Dodecene, (E)-
5-Ethyl-2-furaldehyde
5-Ethyldecane
5H-1-Pyrindine
5-Hepten-2-ol, 6-methyl-
5-Hepten-2-one, 6-methyl-
5-Hexen-2-one
5-Hexen-2-one, 5-methyl-3-methylene-
5-Hexenoic acid, methyl ester
5-Hydroxy-4-octanone
5-Methyl-2-phenyl-2-hexenal
5-Methyl-2-thiophenecarboxaldehyde
5-Oxotetrahydrofuran-2-carboxylic acid
5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester
5-Tetradecene, (E)-
5-Tridecene, (E)-
5-Undecen-3-yne, (Z)-
6-Amino-1-hexanol, N,N-dimethyl-, methyl ether
6-Hepten-3-ol, 4-methyl-
6-Hepten-3-one, 4-methyl-
6-Methyl-2-Heptanol, acetate
6-Methyl-3,5-heptadiene-2-one
6-Octen-1-ol, 3,7-dimethyl-, formate
6-Oxabicyclo[3.1.0]hexan-2-one
6-Tridecen-4-yne, (E)-
7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin
7-Hexadecene, (Z)-
7-Hexadecenoic acid, methyl ester, (Z)-
7-Methyl-1,2,3,5,8,8a-hexahydronaphthalene
7-Octen-2-ol, 2,6-dimethyl-
7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-
7-Pentadecanone
7-Tetradecene, (E)-

8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-
9,10-Diazatricyclo[4.4.0.0(2,8)]dec-9-ene
9,12,15-Octadecatrienal
9,12-Octadecadienoic acid, methyl ester
9,12-Octadecadienoic acid, methyl ester, (E,E)-
9-Decen-1-ol, methyl ether
9-Heptadecanone
9-Hexadecenoic acid
9-Octadecene, 1-methoxy-, (E)-
9-Octadecenoic acid (Z)-, methyl ester
à Isomethyl ionone
á-Bisabolene
Acetaldehyde
Acetamide
Acetamide, 2-(4-morpholyl)-N-(4,5,6,7-tetrahydro-2-cyano-benzothien-3-yl)-
Acetamide, N-(2-methylpropyl)-
Acetamide, N-butyl-
Acetamide, N-methyl-
Acetic acid
Acetic acid, 1,7,7-trimethyl-bicyclo [2.2.1]hept-2-yl ester
Acetic acid, 2-phenylethyl ester
Acetic acid, butyl ester
Acetic acid, hexyl ester
Acetic acid, hydroxy-
Acetic acid, methyl ester
Acetic acid, non-3-enyl ester, cis-
Acetic acid, phenyl ester
Acetic acid, trifluoro-, 3,7-dimethyloctyl ester
Acetic acid, undec-2-enyl ester
Acetoin
Acetone
Acetonitrile, (dimethylamino)-
Acetophenone
Acetyl valeryl
á-Ethylphenethyl alcohol
à-Ionone
à-Isopropylbenzyl alcohol
Amantadine
à-Methylstyrene
Aminomethanesulfonic acid
Amyl nitrite
á-Myrcene
Aniline, N-benzyloxycarbonyl-4-bromo-
Anisole
à-Phellandrene
á-Phenylethyl butyrate
à-Pinene
Aromandendrene
à-Terpineol
Aziridine, 1-ethenyl-
Aziridine, 2-phenyl-
Azulene
Benzaldehyde
Benzaldehyde, 3-methyl-
Benzaldehyde, 4-(methylthio)-
Benzaldehyde, 4-ethyl-
Benzaldehyde, 4-methyl-
Benzaldehyde, 4-pentyl-
Benzenamine, 4-butyl-
Benzene
benzene methanol,
Benzene, (1,1,4,6,6-pentamethylheptyl)-
Benzene, (1,1-dimethyldecyl)-
Benzene, (1,1-dimethylpropyl)-
Benzene, (1-butylheptyl)-
Benzene, (1-butylnonyl)-
Benzene, (1-butyloctyl)-
Benzene, (1-ethyldecyl)-
Benzene, (1-ethylnonyl)-
Benzene, (1-ethyloctyl)-
Benzene, (1-methylbutyl)-
Benzene, (1-methylethyl)-
Benzene, (1-methylpropyl)-
Benzene, (1-pentylheptyl)-
Benzene, (1-pentyloctyl)-
Benzene, (1-propyldecyl)-
Benzene, (1-propylheptyl)-
Benzene, (1-propylnonyl)-
Benzene, (2-chloroethyl)-
Benzene, (2-methoxyethyl)-
Benzene, (methoxymethyl)-
Benzene, (methylthio)-
Benzene, [2-(methylthio)ethyl]-

Benzene, 1,2,3,4-tetramethyl-
Benzene, 1,2,3-trimethyl-
Benzene, 1,2,4,5-tetramethyl-
Benzene, 1,2-dimethoxy-
Benzene, 1,3-bis(1,1-dimethylethyl)-
Benzene, 1,3-diethenyl-
Benzene, 1,3-diethyl-5-methyl-
Benzene, 1,3-dimethoxy-
Benzene, 1,3-dimethyl-
Benzene, 1,4-dimethoxy-
Benzene, 1-bromo-2-ethyl-
Benzene, 1-ethenyl-2-methyl-
Benzene, 1-ethenyl-4-ethyl-
Benzene, 1-ethenyl-4-methoxy-
Benzene, 1-ethenyl-4-methyl-
Benzene, 1-ethyl-2,3-dimethyl-
Benzene, 1-ethyl-2-methyl-
Benzene, 1-ethyl-3,5-dimethyl-
Benzene, 1-ethyl-3-methyl-
Benzene, 1-ethyl-4-methoxy-
Benzene, 1-ethyl-4-methyl-
Benzene, 1-heptenyl-
Benzene, 1-methoxy-3-methyl-
Benzene, 1-methoxy-4-methyl-
Benzene, 1-methyl-3-(1-methylethyl)-
Benzene, 1-methyl-3-propyl-
Benzene, 1-methyl-4-(1-methylethenyl)-
Benzene, 1-methyl-4-(1-methylpropyl)-
Benzene, 1-methyl-4-butyl
Benzene, 1-methyl-4-propyl-
Benzene, 1-octenyl-
Benzene, 1-propynyl-
Benzene, 2,4-diethyl-1-methyl-
Benzene, 2-ethyl-1,4-dimethyl-
Benzene, 2-propenyl-
Benzene, 4-ethyl-1,2-dimethyl-
Benzene, butyl-
Benzene, cyclopropyl-
Benzene, heptyl-
Benzene, hexyl-
Benzene, octyl-
Benzene, pentyl-
Benzene, propyl-
Benzeneacetaldehyde
Benzeneacetaldehyde, à-ethylidene-
Benzeneacetic acid, ethyl ester
Benzeneacetic acid, methyl ester
Benzenecarbothioic acid, S-ethyl ester
Benzenecarbothioic acid, S-methyl ester
Benzeneethanal, 4-[1,1-dimethylethyl]-
Benzeneethanamine
Benzeneethanamine, N-(phenylmethylene)-
Benzeneethanol, 4-hydroxy-
Benzeneethanol, á-methyl-
Benzeneethanol, à-methyl-
Benzenemethanamine, N-(phenylmethylene)-
Benzenemethanamine, N,N-dimethyl-
Benzenemethanethiol
Benzenemethanimine
Benzenemethanol, à,à,4-trimethyl-
Benzenemethanol, à,à-dimethyl-
Benzenemethanol, à-methyl-, (R)-
Benzenemethanol, à-methyl-, (S)-
Benzenepropanoic acid 1-methylethyl ester
Benzenepropanoic acid, 2-butyl ester
Benzenepropanoic acid, 2-methylpropyl ester
Benzenepropanoic acid, ethyl ester
Benzenepropanoic acid, methyl ester
Benzenepropanoic acid, pentyl ester
Benzo[b]thiophene
Benzocyclobuten-1(2H)-one
Benzofuran
Benzofuran, 2,3-dihydro-
Benzoic acid, 1-methylethyl ester
Benzoic acid, 2-hydroxy-, 2-methylbutyl ester
Benzoic acid, 2-hydroxy-, pentyl ester
Benzoic acid, 2-methylpropyl ester
Benzoic acid, ethyl ester
Benzoic acid, methyl ester
Benzonitrile
Benzonitrile, 3-chloro-2-fluoro-
Benzophenone
Benzothiazole
Benzoxazole, 2-methyl-

Benzoylformic acid
Benzyl alcohol
Benzyl methyl disulfide
Benzyl methyl ketone
Benzyl methyl sulfide
Benzyl nitrile
Bicyclo[10.1.0]tridec-1-ene
Bicyclo[2.1.1]hex-2-ene, 2-ethenyl-
Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, (1R-endo)-
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-
Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl-
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
Bicyclo[3.1.0]hexane, 6-methylene-
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-
Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-
Bicyclo[4.2.0]octa-1,3,5-triene
Bicyclo[4.2.0]octa-1,3,5-triene, 7-methyl-
Bicyclo[7.1.0]decane
Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-
Biphenyl
Bornyl acetate
Butanal
Butanal, 2-methyl-
Butanal, 3-methyl-
Butanamide
Butanamide, 3-methyl-
Butane, 1-bromo-2-methyl-
Butane, 1-isothiocyanato-
Butane, 1-isothiocyanato-3-methyl-
Butane, 1-methoxy-
Butane, 1-methoxy-3-methyl-
Butanedioic acid, dimethyl ester
Butanenitrile
Butanenitrile, 2-methyl-
Butanenitrile, 3-methyl-
Butanethioic acid, S-methyl ester
Butanoic acid
Butanoic acid, 1-methylethyl ester
Butanoic acid, 1-methylhexyl ester
Butanoic acid, 1-methylpropyl ester
Butanoic acid, 2-methyl-
Butanoic acid, 2-methyl-, 2-methylpropyl ester
Butanoic acid, 2-methyl-, ethyl ester
Butanoic acid, 2-methyl-, methyl ester
Butanoic acid, 2-methyl-, pentyl ester
Butanoic acid, 2-methylbutyl ester
Butanoic acid, 2-methylene-, methyl ester
Butanoic acid, 2-methylpropyl ester
Butanoic acid, 3-hydroxy-, methyl ester
Butanoic acid, 3-hydroxy-, methyl ester, (S)-
Butanoic acid, 3-hydroxy-3-methyl-, methyl ester
Butanoic acid, 3-methyl-
Butanoic acid, 3-methyl-, 1-methylethyl ester
Butanoic acid, 3-methyl-, 3-methylbutyl ester
Butanoic acid, 3-methyl-, butyl ester
Butanoic acid, 3-methyl-, ethyl ester
Butanoic acid, 3-methyl-, hexyl ester
Butanoic acid, 3-methyl-, phenylmethyl ester
Butanoic acid, 3-methyl-, propyl ester
Butanoic acid, 3-methylbutyl ester
Butanoic acid, anhydride
Butanoic acid, butyl ester
Butanoic acid, ethyl ester
Butanoic acid, hexyl ester
Butanoic acid, methyl ester
Butanoic acid, propyl ester
Butyl 2-methylbutanoate
Butyl aldoxime, 3-methyl-, anti
Butyl aldoxime, 3-methyl-, syn-
Butyl caprylate
Butyl myristate
Butylated Hydroxytoluene
Butyrolactone
Camphene
Carbamic acid, monoammonium salt
Carbamic acid, phenyl ester
Carbamimidothioic acid, methyl ester
Carbon dioxide
Carbon disulfide

Carbonic acid, dimethyl ester
Carbonodithioic acid, S,S-dimethyl ester
Carbonotrithioic acid, dimethyl ester
Carvone
Caryophyllene
ç-Dodecalactone
Cetene
Cinnamaldehyde, (E)-
cis,cis-2,7-Nonadiene
cis-10-Heptadecenoic acid, methyl ester
cis-2-Methyl-7-octadecene
cis-4-Decenal
cis-5-Dodecenoic acid, methyl ester
cis-7-Tetradecen-1-ol
cis-Hept-4-enol
Citronellol
Copaene
Coumarin
Creosol
ç-Terpinene
Cyclobuta[1,2-d:3,4-d']bis[1,3]dioxole, tetrahydro-, (3aà,3bà,6aà,6bà)-
Cyclobutanecarboxylic acid, 2-propenyl ester
Cyclodecane
Cyclodecane, octyl-
Cyclohexane
Cyclohexane, (1-methylpropyl)-
Cyclohexane, 1,1,3,5-tetramethyl-, trans-
Cyclohexane, 1,1,3-trimethyl-
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-
Cyclohexane, 1-ethenyl-2-methyl-, trans-
Cyclohexane, 1-ethyl-2-methyl-, trans-
Cyclohexane, 1-isopropyl-1-methyl-
Cyclohexane, 1-methyl-3-(1-methylethyl)-
Cyclohexane, 1-methyl-3-pentyl-
Cyclohexane, 1-methyl-3-propyl-
Cyclohexane, 1-methyl-4-(1-methylbutyl)-
Cyclohexane, butyl-
Cyclohexane, decyl-
Cyclohexane, hexyl-
Cyclohexane, isothiocyanato-
Cyclohexane, octyl-
Cyclohexanecarboxylic acid isopropyl ester
Cyclohexanecarboxylic acid, methyl ester
Cyclohexanemethanol, 2-methyl-
Cyclohexanol
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate
Cyclohexanol, 2,4-dimethyl-
Cyclohexanol, 2-methyl-, cis-
Cyclohexanol, 2-methyl-, trans-
Cyclohexanol, 2-methyl-5-(1-methylethenyl)-
Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1à,2á,5à)-
Cyclohexanol, 4-methyl-
Cyclohexanol, 4-methyl-, cis-
Cyclohexanone
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-
Cyclohexanone, 2-propyl-
Cyclohexanone, 4-ethyl-
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-
Cyclohexene, 1-methyl-4-(1-methylethylidene)-
Cyclohexene, 4-methyl-3-(1-methylethylidene)-
Cyclononene
Cyclooctane, 1,4-dimethyl-, cis-
Cyclopentane, (2-methylpropylidene)-
Cyclopentane, 1-methyl-3-(2-methylpropyl)-
Cyclopentane, butyl-
Cyclopentane, methyl-
Cyclopentanepropanol, 2-methylene-
Cyclopentanone
Cyclopentanone, 2-ethyl-
Cyclopentene, 1-butyl-
Cyclopentene,1-heptyl-
Cyclopentene,1-hexyl-
Cyclopropane, (2-methylenebutyl)-
Cyclopropane, 1-heptyl-2-methyl-
Cyclopropane, 1-methyl-2-(3-methylpentyl)-
Cyclopropane, ethylidene-
Cyclopropane, hexylidene-
Cyclopropane, octyl-
Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester

Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-
Decane
Decane, 2,3,5,8-tetramethyl-
Decane, 2,5-dimethyl-
Decane, 2-methyl-
Decane, 3,4-dimethyl-
Decane, 3-methyl-
Decane, 4-ethyl-
Decane, 5-methyl-
Decanenitrile
Decanoic acid, ethyl ester
Decanoic acid, methyl ester
Decanoic acid, propyl ester
Dehydromevalonic lactone
Di(1,2,5-oxadiazolo)[3,4-b:3,4-E]pyrazine, 4,8-diacetyl-
Diethyl azodicarboxylate
Dihydro-2(3H)-thiophenone
Dihydrocarvyl acetate
Dimethyl sulfide
Dimethyl sulfone
Dimethyl Sulfoxide
Dimethyl trisulfide
Dimethylamine
Diphenyl ether
Diphosphoric acid, diisooctyl ester
Dispiro[2.1.2.4]undecane, 8-methylene-
Disulfide, dimethyl
Disulfide, isopentyl methyl
Disulfide, methyl (methylthio)methyl
Disulfide, methyl phenyl
Disulfide, methyl propyl
Di-trimethylsilyl peroxide
dl-Mevalonic acid lactone
Dodecanal
Dodecane
Dodecane, 2,6,10-trimethyl-
Dodecane, 2,6,11-trimethyl-
Dodecane, 2,7,10-trimethyl-
Dodecane, 2-methyl-
Dodecanenitrile
Dodecanoic acid, 1-methylethyl ester
Dodecanoic acid, ethenyl ester
Dodecanoic acid, ethyl ester
Dodecanoic acid, methyl ester
Dodecanoic acid, propyl ester
Dotriacontane
E-1,6-Undecadiene
E-1,9-Dodecadiene
Eicosane
Ethane
Ethane, 1,1-bis(methylthio)-
Ethane, 1,2-bis(methylthio)-
Ethane, 1,2-dicyclopropyl-
Ethane, hexachloro-
Ethanol
Ethanol, 2-(2-ethoxyethoxy)-
Ethanol, 2-(dodecyloxy)-
Ethanol, 2-(methylthio)-
Ethanol, 2-butoxy-
Ethanol, 2-chloro-, acetate
Ethanol, 2-ethoxy-
Ethanone, 1-(1H-pyrrol-2-yl)-
Ethanone, 1-(2-aminophenyl)-
Ethanone, 1-(2-furanyl)-
Ethanone, 1-(2-hydroxyphenyl)-
Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-
Ethanone, 1-(2-pyridinyl)-
Ethanone, 1-(3-methylphenyl)-
Ethanone, 1-(3-thienyl)-
Ethanone, 1-(4-methylphenyl)-
Ethanone, 1-[4-(1-methylethyl)phenyl]-
Ethanone, 1-cyclohexyl-
Ethene, 1,2-bis(methylthio)-
Ethyl 5-methylhexanoate
Ethyl 9-decenoate
Ethyl tiglate
Ethylbenzene
Ethylene, 1,2-dichloro-, (Z)-
Eucalyptol
Formaldehyde
Formamide, N,N-dibutyl-
Formamide, N,N-diethyl-
Formamide, N,N-dimethyl-

Formamide, N-phenyl-
Formic acid, 2-phenylethyl ester
Formic acid, hexyl ester
Furan
Furan, 2-(1-pentenyl)-, (E)-
Furan, 2-(1-pentenyl)-, (Z)-
Furan, 2-(2-propenyl)-
Furan, 2,3-dihydro-
Furan, 2-[(methyldithio)methyl]-
Furan, 2-butyltetrahydro-
Furan, 2-ethyl-
Furan, 2-ethyl-5-methyl-
Furan, 2-hexyl-
Furan, 2-methyl-
Furan, 2-pentyl-
Furan, 2-propyl-
Furan, 3-pentyl-
Furan, 4-methyl-2-propyl-
Furan, tetrahydro-2,5-dimethyl-
Furfural
Geraniol
Glycerin
Heneicosane
Heptacosane
Heptadecane
Heptadecane, 2-methyl-
Heptadecane, 3-methyl-
Heptadecanoic acid, methyl ester
Heptanal
Heptane
Heptane, 1-(methylthio)-
Heptane, 2,2,4,6,6-pentamethyl-
Heptane, 2,2-dimethyl-
Heptane, 2,4-dimethyl-
Heptane, 5-ethyl-2,2,3-trimethyl-
Heptanethioic acid, S-methyl ester
Heptanoic acid
Heptanoic acid, 2,6-dimethyl-, methyl ester
Heptanoic acid, ethyl ester
Heptanoic acid, methyl ester
Heptanonitrile
Heptanophenone
Hexacosane
Hexadecanal
Hexadecane
Hexadecane, 2,6,10,14-tetramethyl-
Hexadecanenitrile
Hexadecanoic acid, 15-methyl-, methyl ester
Hexadecanoic acid, ethyl ester
Hexadecanoic acid, methyl ester
Hexanal
Hexanal, 2-ethyl-
Hexanal, 5-methyl-
Hexanamide
Hexane, 1-(methylthio)-
Hexane, 1-methoxy-
Hexane, 1-nitro-
Hexane, 2,2,3-trimethyl-
Hexane, 2,2-dimethyl-
Hexane, 2,3,4-trimethyl-
Hexane, 2,4-dimethyl-
Hexane, 2-methyl-
Hexane, 3,3-dimethyl-
Hexane, 3-bromo-
Hexane, 3-methyl-
Hexane, 4-ethyl-2-methyl-
Hexanenitrile
Hexanethioic acid, S-methyl ester
Hexanoic acid
Hexanoic acid, 1-methylethyl ester
Hexanoic acid, 2-ethyl-, methyl ester
Hexanoic acid, 2-ethylhexyl ester
Hexanoic acid, 2-methylpropyl ester
Hexanoic acid, 2-tetrahydrofurylmethyl ester
Hexanoic acid, 5-methyl-, methyl ester
Hexanoic acid, anhydride
Hexanoic acid, butyl ester
Hexanoic acid, hexyl ester
Hexanoic acid, methyl ester
Homosalate
Hydrazinecarboxamide
Hydrocinnamic acid
Hydrocoumarin
Hydrogen sulfide

Ibuprofen methyl ester
Indan, 1-methyl-
Indane
Indole
Isoamyl cyanide
Isoborneol
Isobornyl formate
Isobutyl isothiocyanate
Isobutyl isovalerate
Isobutyl laurate
Isobutylamine
Isobutyronitrile
Isophorone
Isopropyl Alcohol
Isopropyl myristate
Isopropyl palmitate
Isopropyl phenyl ketone
Isopropyl tiglate
Lauryl acetate
Levomenthol
Limonene
l-Menthone
Longifolene
Mercaptoacetone
Methacrolein
Methacrylic anhydride
Methanamine, N-(phenylmethylene)-
Methanamine, N-heptylidene-
Methanaminium, 1-carboxy-N,N,N-trimethyl-, hydroxide, inner salt
Methane, (methylsulfinyl)(methylthio)-
Methane, bromochloro-
Methanesulfonyl chloride
Methanethiol
Methenamine
Methional
Methyl 2-(methylthio)acetate
Methyl 2-furoate
Methyl 3-(methylthio)-(E)-2-propenoate
Methyl 4-(methylthio)butyrate
Methyl 4,8-dimethylnonanoate
Methyl 4-oxododecanoate
Methyl 6-methyl heptanoate
Methyl 6-methyloctanoate
Methyl Alcohol
Methyl anthranilate
Methyl dimethylcarbamate
Methyl ethyl disulfide
Methyl formate
Methyl Isobutyl Ketone
Methyl isocyanide
Methyl isopropyl disulphide
Methyl isovalerate
Methyl methacrylate
Methyl n-butyl disulfide
Methyl n-hexyl disulfide
Methyl n-nonyl sulphide
Methyl n-octyl sulfide
Methyl pentyl disulfide
Methyl propionate
Methyl salicylate
Methyl sec-butyl disulphide
Methyl tetradecanoate
Methyl thiolacetate
Methyl tiglate
Methyl valerate
Methyl vinyl ketone
Methylal
Methylamine, N,N-dimethyl-
Methylene chloride
Methyltartronic acid
Methylthio-2-propanone
Morpholine, 4-octadecyl-
Myristic acid vinyl ester
N-(3-Methylbutyl)acetamide
N,N-Dimethylacetamide
n-Amyl isovalerate
Naphthalene
Naphthalene, 1,2,3,4-tetrahydro-
Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-
Naphthalene, 1,2,3,4-tetrahydro-1,8-dimethyl-
Naphthalene, 1-methyl-
Naphthalene, 2-methyl-
n-Butyl methacrylate
n-Butyl tiglate

n-Butyl-á-phenylpropionate
n-Butylethylenediamine
n-Capric acid isobutyl ester
n-Caproic acid vinyl ester
n-Caprylic acid isobutyl ester
Neodihydrocarveol
n-Hexadecanoic acid
n-Hexane
Nitric oxide
Nitroxide, bis(1,1-dimethylethyl)
N-Methylvaleramide
N-Morpholinomethyl-isopropyl-sulfide
n-Nonylcyclohexane
n-Octanoic acid isopropyl ester
Nona-3,5-dien-2-one
Nonadecane
Nonanal
Nonane
Nonane, 2,2,4,4,6,8,8-heptamethyl-
Nonane, 2-methyl-
Nonanenitrile
Nonanoic acid, ethyl ester
Nonanoic acid, methyl ester
n-Pentylpyrazine
n-Propyl acetate
n-Tridecan-1-ol
Octacosane
Octadecane
Octadecane, 2-methyl-
Octanal
Octane
Octane, 1,1'-oxybis-
Octane, 2,3-dimethyl-
Octane, 2,4,6-trimethyl-
Octane, 2,6-dimethyl-
Octane, 3,5-dimethyl-
Octane, 3-ethyl-
Octane, 4-ethyl-
Octane, 4-methyl-
Octanenitrile
Octanethioic acid, S-methyl ester
Octanoic acid
Octanoic acid, 2-methyl-, methyl ester
Octanoic acid, 3-methylbutyl ester
Octanoic acid, 4-methyl-, methyl ester
Octanoic acid, ethyl ester
Octanoic acid, methyl ester
o-Cymene
Oleic Acid
Oxalic acid, allyl hexadecyl ester
Oxalic acid, allyl nonyl ester
Oxalic acid, isobutyl nonyl ester
Oxazole, trimethyl-
Oxepine, 2,7-dimethyl-
Oxime-, methoxy-phenyl-
Oxirane, (methoxymethyl)-
Oxirane, 2-ethyl-2-methyl-
Oxirane, 2-methyl-2-phenyl-
Oxirane, 3-hydroxypropyl-
Oxirane, dodecyl-
Oxirane, tetradecyl-
Oxygen
o-Xylene
Pantolactone
p-Cresol
p-Cymene
Pent-3-enylamine
Pentadecane
Pentadecane, 2,6,10,14-tetramethyl-
Pentadecanenitrile
Pentadecanoic acid, 14-methyl-, methyl ester
Pentadecanoic acid, methyl ester
Pentanal
Pentanal, 2,2-dimethyl-
Pentanal, 2-methyl-
Pentane
Pentane, 1-(methylthio)-
Pentane, 2,3-dimethyl-
Pentane, 2,4-dimethyl-
Pentane, 2-methyl-
Pentane, 3-bromo-
Pentane, 3-bromo-3-methyl-
Pentane, 3-methyl-
Pentanenitrile

Pentanoic acid
Pentanoic acid, 1,1-dimethylpropyl ester
Pentanoic acid, 10-undecenyl ester
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester
Pentanoic acid, 2-hydroxy-4-methyl-, methyl ester
Pentanoic acid, 2-methyl-, methyl ester
Pentanoic acid, 2-methylpropyl ester
Pentanoic acid, 2-phenylethyl ester
Pentanoic acid, 3-hydroxy-, methyl ester
Pentanoic acid, 3-methylbutyl ester
Pentanoic acid, 4-methyl-
Pentanoic acid, 4-methyl-, ethyl ester
Pentanoic acid, 4-methyl-, methyl ester
Pentanoic acid, 4-methyl-, pentyl ester
Pentanoic acid, ethyl ester
Pentanoic acid, octyl ester
Pentanoic acid, pentyl ester
Pentyl (E)-2-methylbut-2-enoate
Phenacyl thiocyanate
Phenol
Phenol, 2-(methylthio)-
Phenol, 2,4-bis(1,1-dimethylethyl)-
Phenol, 2-chloro-
Phenol, 2-ethyl-
Phenol, 2-methoxy-
Phenol, 2-methyl-
Phenol, 3-methyl-
Phenol, 3-methyl-4-(methylthio)-
Phenol, 4-ethyl-
Phenol, 4-pentyl-
Phenylethyl Alcohol
Phosphonic acid, (p-hydroxyphenyl)-
Phosphonofluoridic acid, methyl-, nonyl ester
Piperidine
p-Menthane, 2,3-dibromo-8-phenyl-
p-Pentylaniline
Propanal
Propanal, 2-methyl-
Propanamide
Propanamide, 2-methyl-
Propane, 1,1-dimethoxy-2-methyl-
Propane, 1,3-bis(methylthio)-
Propanenitrile, 3-(methylthio)-
Propanoic acid
Propanoic acid, 1-methylethyl ester
Propanoic acid, 2,2-dimethyl-, 2-phenylethyl ester
Propanoic acid, 2-methyl-
Propanoic acid, 2-methyl-, 1-methylethyl ester
Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester
Propanoic acid, 2-methyl-, 2-methylbutyl ester
Propanoic acid, 2-methyl-, 2-methylpropyl ester
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester
Propanoic acid, 2-methyl-, 3-methylbutyl ester
Propanoic acid, 2-methyl-, anhydride
Propanoic acid, 2-methyl-, butyl ester
Propanoic acid, 2-methyl-, ethyl ester
Propanoic acid, 2-methyl-, methyl ester
Propanoic acid, 2-methyl-, phenylmethyl ester
Propanoic acid, 2-methyl-, propyl ester
Propanoic acid, 2-methylpropyl ester
Propanoic acid, 2-phenylethyl ester
Propanoic acid, 3-amino-2-methyl-
Propanoic acid, 3-hydroxy-, methyl ester
Propanoic acid, 3-methoxy-, methyl ester
Propanoic acid, butyl ester
Propanoic acid, pentyl ester
Propyl octanoate
Pulegone
p-Xylene
Pyrazine
Pyrazine, (2-methylpropyl)-
Pyrazine, 2,3-dimethyl-
Pyrazine, 2,3-dimethyl-5-(2-methylpropyl)-
Pyrazine, 2,5-dimethyl-
Pyrazine, 2,5-dimethyl-3-propyl-
Pyrazine, 2,6-diethyl-
Pyrazine, 2,6-dimethyl-
Pyrazine, 2-butyl-3,5-dimethyl-
Pyrazine, 2-ethenyl-6-methyl-
Pyrazine, 2-ethyl-3,5-dimethyl-
Pyrazine, 2-ethyl-5-methyl-
Pyrazine, 2-ethyl-6-methyl-
Pyrazine, 2-methyl-5-propyl-

Pyrazine, 3,5-dimethyl-2-propyl-
Pyrazine, 3-ethyl-2,5-dimethyl-
Pyrazine, ethyl-
Pyrazine, methyl-
Pyrazine, tetramethyl-
Pyrazine, trimethyl-
Pyridine
Pyridine, 1-acetyl-1,2,3,4-tetrahydro-
Pyridine, 2-(2-methylpropyl)-
Pyridine, 2,3,4,5-tetrahydro-
Pyridine, 2,3,6-trimethyl-
Pyridine, 2,3-dimethyl-
Pyridine, 2,4,6-trimethyl-
Pyridine, 2,4-dimethyl-
Pyridine, 2,5-dimethyl-
Pyridine, 2,6-dimethyl-
Pyridine, 2-butyl-
Pyridine, 2-ethyl-
Pyridine, 2-ethyl-4,6-dimethyl-
Pyridine, 2-ethyl-5-methyl-
Pyridine, 2-ethyl-6-methyl-
Pyridine, 2-methyl-
Pyridine, 2-pentyl-
Pyridine, 2-propyl-
Pyridine, 3-(methylthio)-
Pyridine, 3,5-dimethyl-
Pyridine, 3-butyl-
Pyridine, 3-ethyl-
Pyridine, 3-methyl-
Pyridine, 3-phenyl-
Pyridine, 3-propyl-
Pyridine, 4-pentyl-
Pyridine, 5-ethyl-2-methyl-
Pyridine, 5-hexyl-2-methyl-
Pyrimidine, 5-methyl-
Pyrrole
Pyrrolidine, 1-acetyl-
Quinazoline
Quinazoline, 4-methyl-
Quinoline, 4-methyl-
Quinoxaline
sec-Butyl (E)-2-methylbut-2-enoate
S-Methyl propanethioate
Spiro[2.4]heptan-5-one
Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-
Spiro[cyclopropane-1,2'-[6.7]diazabicyclo[3.2.2]non-6-ene]
Squalene
Styrene
Sulfide, methyl 1-methyl-2-butenyl
Sulfur dioxide
Sulfurous acid, butyl octyl ester
Sulfurous acid, dipentyl ester
Terpinen-4-ol
Tetrachloroethylene
Tetracosane
Tetradecane
Tetradecane, 1-iodo-
Tetradecane, 3-methyl-
Tetradecane, 6,9-dimethyl-
Tetradecanoic acid
Tetradecanoic acid, ethyl ester
Tetradecanoic acid, propyl ester
Tetrahydro[2,2']bifuranyl-5-one
Tetrahydrofuran
Tetrahydrofuran-2-one, 5-[1-hydroxyhexyl]-
Tetrahydrofurfuryl chloride
Tetrasulfide, dimethyl
Thiazole
Thiazole, 5-ethyl-2-methyl-
Thiazole, tetrahydro-
Thiirane
Thiirane, methyl-
Thiocyanic acid, methyl ester
Thiophene
Thiophene, 2-butyl-
Thiophene, 2-ethyl-
Thiophene, 2-heptyl-
Thiophene, 2-hexyl-
Thiophene, 2-methyl-
Thiophene, 2-octyl-
Thiophene, 2-pentyl-
Thiophene, 2-propyl-
Thiophene, 3-ethyl-

Thiophene, 3-methyl-
Thujopsene-I3
Toluene
Topotecan
trans, trans-Octa-2,4-dienyl acetate
trans,trans-2,9-Undecadiene
trans-1-Butenylcyclopentane
trans-2-(2-Pentenyl)furan
trans-2-Methyl-4-hexen-3-ol
trans-3-Nonen-2-one
trans-à-Bergamotene
trans-á-Ionone
trans-á-Ocimene
trans-Farnesol
Trichloromethane
Tridecane
Tridecane, 2-methyl-2-phenyl-
Tridecane, 3-methyl-
Tridecane, 3-methylene-
Tridecanoic acid, 12-methyl-, methyl ester
Tridecanoic acid, methyl ester
Undecanal
Undecane
Undecane, 2,6-dimethyl-
Undecane, 2-methyl-
Undecane, 3,5-dimethyl-
Undecane, 3-methyl-
Undecane, 4,8-dimethyl-
Undecane, 4-methyl-
Undecanoic acid
Undecanoic acid, 11-bromo-, methyl ester
Undecanoic acid, methyl ester
Undecanol-3
Valeric acid, 2-pentadecyl ester
Valeric acid, 2-tridecyl ester
Valeric acid, dodecyl ester
Z-1,6-Undecadiene
Z-1,8-Dodecadiene

Appendix B:

List of Significant VOCs Obtained from Human Cadaver Decomposition Literature

Appendix B: List of Significant VOCs Obtained from Human Cadaver Decomposition Literature

*VOCs listed in *red* are those in common with the VOCs found in human decomposition fluid

a,a dimethyl 1-hexanol, 2-ethyl benzaldehyde
(E)-2-nonenal
1,1,2,3-tetramethylcyclohexane
1,1,2-trichloro-1,2,2-trifluoroethane
1,1-dichloro-1-fluoroethane
1,2,3-trimethyl benzene
1,2,4-trimethyl(1-α)cyclohexane
1,2,4-trimethylbenzene
1,2-benzenedicarboxylic acid, diethyl ester
1,2-dichloroethene
1,2-diethylbenzene
1,2-dimethylbenzene
1,3,5-trimethylbenzene
1,3,5-trimethylcyclohexane
1,3-diethenylbenzene
1,3-dimethylcyclohexane
1,3-pentadiene
1,4,-dimethylcyclohexane
1,4-dichlorobenzene
1-butanol
1-chloro-nonane
1-ethenyl-4-ethylbenzene
1-ethyl, 2-methylbenzene
1-ethyl,4-methylbenzene
1-ethyl-2-methylbenzene
1-ethyl-3-methyl benzene
1-ethyl-4-methylcyclohexane
1-ethyl-4-methylcyclohexane
1-heptene
1-hexanol
1-hexene
1H-pyrrole
1-methoxypropyl benzene
1-methyl napthalene
1-methyl-2-(1-methylethyl)benzene
1-methyl-2-ethyl benzene
1-methylethenylbenzene
1-octanol
1-octen-3-ol
1-phenyl ethanone
1-propene
1-undecene
2,2,3,3-tetramethylbutane
2,2,3-trimethylbutane
2,2-dimethylbutane
2,2-dimethylhexane
2,3,3-trimethylpentane
2,3,4-trimethylpentane
2,3-dihydro-1-H-indene
2,3-dihydro-2-methyl-1-H-indene
2,3-dimethylbutane
2,3-dimethylhexane
2,3-dimethylpentane
2,4,dimethylheptane
2,4-dimethyl pentane
2,4-dimethylthiane, S,S-dioxide
2,4-dimethylthiophene
2,4-heptadienal
2,4-nonadienal
2,5-dimethylfuran
2-butanone
2-butylfuran
2-ethoxy-2-methylpropane
2-ethyl-1,4-dimethylbenzene
2-ethyl-1-hexanol
2-ethyl-5-methylfuran
2-ethylfuran
2-furancarboxaldehyde
2-furanmethanol
2-heptanone

2-hexanone
2-hexenal
2-methyl butanal
2-methyl butane
2-methyl furan
2-methyl pentane
2-methyl propanal
2-methyl-1-pentanol
2-methyl-1-pentene
2-methyl-1-propanol
2-methyl-1-propene
2-methyl-1-propenylbenzene
2-methylhexane
2-methyloctane
2-methylpentane
2-nonanone
2-octenal
2-octene
2-pentanone
2-pentene
2-pentylfuran
2-propanol
2-propanone (acetone)
2-β-pinene
3,6-dimethyloctane
3-ethyl-1-pentene
3-heptanone
3-hexanone
3-methyl butanal
3-methyl hexane
3-methyl pentane
3-methyl-1-butanol
3-methyl-2-heptanone
3-methylheptane
3-methylhexane-2-one
3-methyloctane
3-octene
3-pentanone
4-heptanone
4-methyl-2-pentene
4-methylheptane
4-methylphenol
5-methyl-1-heptene
5-methyl-3-hexanone
6,10-dimethyl-5,9-undecadien-2-one
6-methyl-5-hepten-2-one
7-hexadecenoic acid-methyl ester
acetic acid, ethyl ester
acetic acid, methyl ester
acetic acid, propyl ester
acetic acid-phenylmethyl ester
benzaldehyde
benzene
benzothiazole
benzyl alcohol
butanal
butanoic acid
butanoic acid, butyl ester
butanoic acid, ethyl ester
c4-benzene (benzene)
carbon disulfide
carbon oxide sulfide
carbon tetrachloride
carbonic acid, dimethyl ester
chlorocyclohexane
chlorodifluoromethane
chloroform
chloromethane
cyclohexane
cyclohexanol, allyl isothiocyanate
cyclohexanone
cyclohexene
cyclopentanetridecanoic acid- methyl ester
decanal
decane
decanoic acid-methyl ester
dichlorodifluoromethane
dichloromethane
dichlorotetrafluoroethane
di-limonene
dimethyldisulfide
dimethylsulfide
dimethyltrisulfide
dodecane

dodecanoic acid
dodecanoic acid, 10-methyl, methyl ester
dodecanoic acid-methyl ester
Ethanal (acetaldehyde)
ethanol
ethylbenzene
furancarboxylic acid-methyl ester
heptadecane
heptanal
heptane
heptanoic acid
hexadecane
hexadecanoic acid, methyl ester
hexanal
hexane
hexanedioic acid- dimethyl ester
hexanoic acid
hexanoic acid, ethyl ester
hexanoic acid, hexyl ester
hexanoic acid, pentyl ester
hexanoic acid-methyl ester
hydroxystearic acid
indole
isoprene
methenamine
methyl benzene (toluene)
methyl ethyl disulfide
methyl propyl disulfide
methyl tetradecanoate
methyl-2-propenyldisulfide
methylcyclohexane
methylcyclopentane
m-xylene
naphthalene
n-octylacetate
nonanal
nonane
nonanoic acid
nonanoic acid-methyl ester
octanal
octane
octanoic acid
octanoic acid-methyl ester
oleic acid
o-xylene
palmitic/hexadecanoic acid
pentadecanoic acid-methyl ester
pentanal
pentane
pentanoic acid
phellandrene
phenol
propanedioic acid-methyl ester
propanoic acid
propanoic acid, 2-methyl, ethyl ester
propyl benzene
p-xylene
pyridine
stearic/octadecanoic acid
styrene
sulfur dioxide
tetrachloroethylene
tetradecanal
tetradecane
tetradecanoic acid
tetramethylhexane
trichloroethane
trichloroethene
trichloroethylene
trichloromonofluoromethane
tridecane
tridecanoic acid-methyl ester
trimethylamine
trimethyldecane
undecanal
undecane
undecanoic acid-methyl ester
α,α-dimethyl benzenemethanol
α-pinene
α-terpinene
α-terpinolene
γ-terpinene

Appendix C:

PCA Plots for Samples Stored and Aged at Room Temperature

Appendix C: PCA Plots for Samples Stored and Aged at Room Temperature

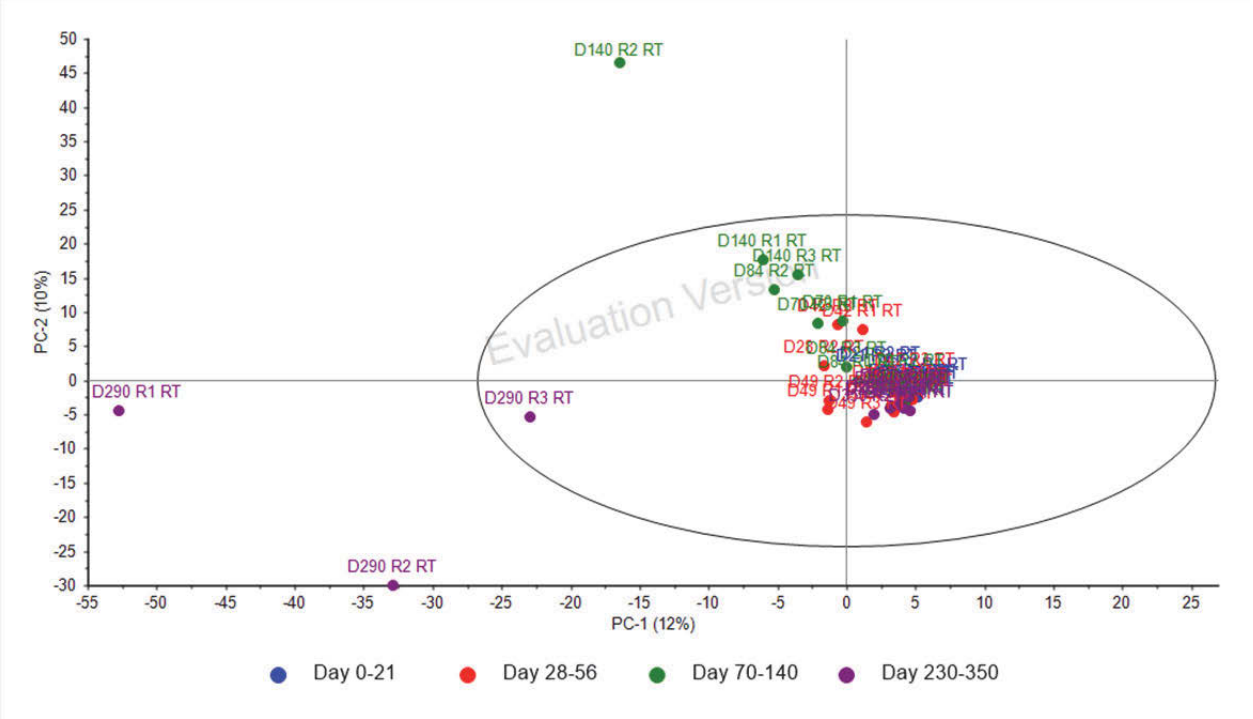

Figure C1: The original PCA plot for samples stored and aged at room temperature, with a 95% CI

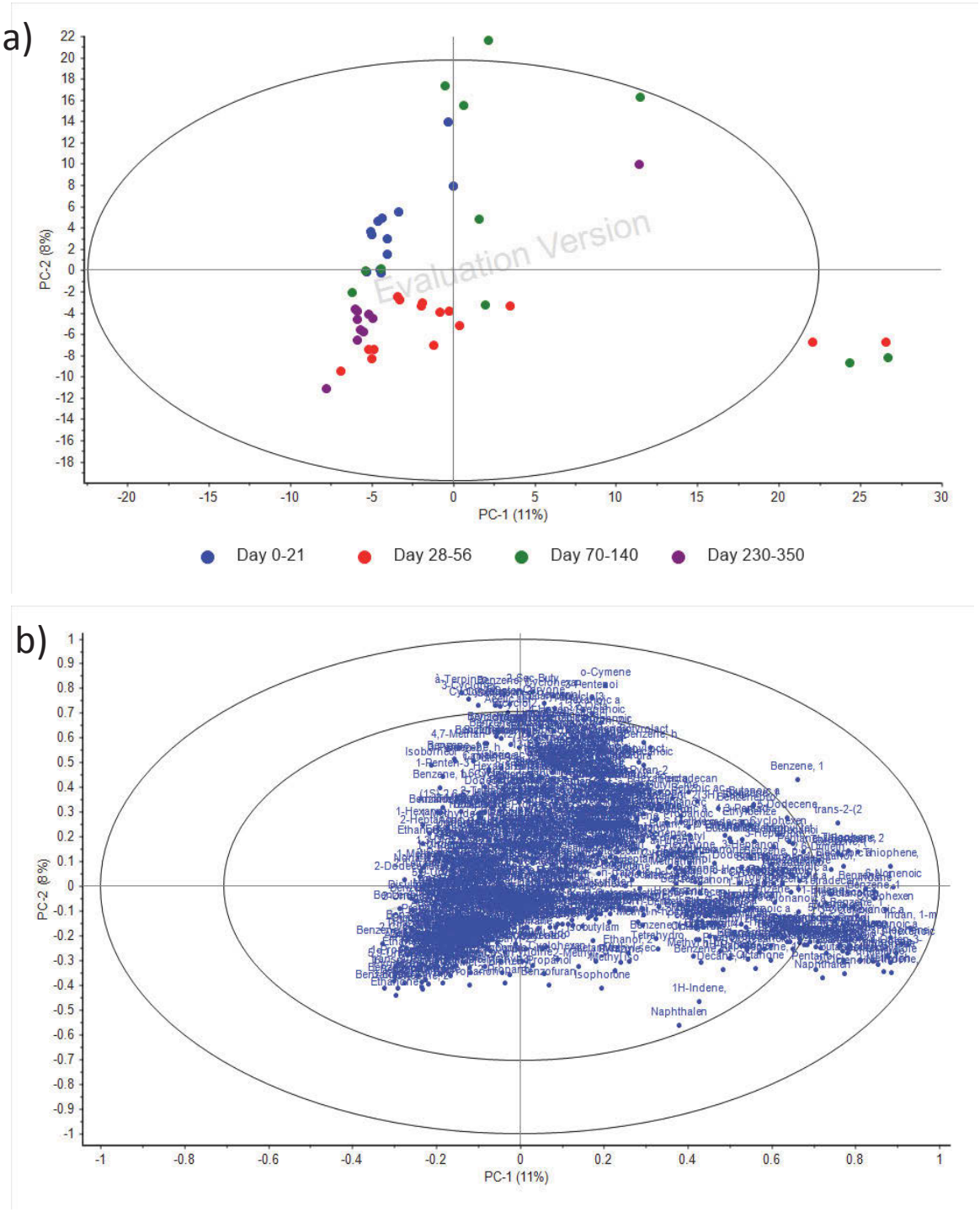

Figure C2: PCA plots for the odour profile of decomposition fluid stored at room temperature: a) Scores plot, with 95% C showing the variations between samples, b) Loadings plot demonstrating the compounds influencing the variations in Plot A. Note: the large numbers of labelled compounds on the plot make it difficult to read. The outliers from Figure C1 have been removed for this plot

Appendix D:

Predominant VOCs Found in Samples Stored and Aged at Room Temperature

Appendix D: Predominant VOCs Found in Samples Stored and Aged at Room Temperature

Compound Name	% of Samples Containing VOC
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	80.39%
1-Decene	100.00%
1-Hexadecanol	84.31%
1-Hexanol, 2-ethyl-	86.27%
2(3H)-Furanone, 5-heptyldihydro-	82.35%
2-Butanone	84.31%
2-Decanol	100.00%
2-Decanone	98.04%
2-Decenal, (Z)-	88.24%
2-Dodecanol	84.31%
2-Dodecanone	98.04%
2-Heptanone	98.04%
2-Heptanone, 3-methyl-	100.00%
2-Hexadecene, 2,6,10,14-tetramethyl-	86.27%
2-Nonanone	94.12%
2-Octanone	96.08%
2-Pentanone	90.20%
2-Undecanone	98.04%
2-Undecanone, 6,10-dimethyl-	84.31%
5-Decen-1-ol, acetate, (E)-	82.35%
5-Hepten-2-one, 6-methyl-	82.35%
7-Octen-2-ol, 2,6-dimethyl-	80.39%
Acetone	88.24%
Acetophenone	98.04%
à-Terpineol	90.20%
Benzene, 1-ethyl-3-methyl-	80.39%
Benzeneacetic acid, methyl ester	88.24%
Benzonitrile	96.08%
Benzyl methyl ketone	84.31%
Bicyclo[4.2.0]octa-1,3,5-triene	86.27%
Carbon dioxide	80.39%
Carbon disulfide	84.31%
Cetene	92.16%

Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	92.16%
Cyclopentane, butyl-	82.35%
Decanoic acid, methyl ester	98.04%
Dimethyl trisulfide	94.12%
Disulfide, dimethyl	96.08%
Disulfide, methyl (methylthio)methyl	92.16%
Dodecanal	88.24%
E-1,6-Undecadiene	82.35%
Furan, 2-pentyl-	94.12%
Heptanonitrile	94.12%
Hexadecanoic acid, methyl ester	86.27%
Hexanoic acid, methyl ester	80.39%
Indane	80.39%
Methanethiol	92.16%
Methyl tetradecanoate	94.12%
Methylamine, N,N-dimethyl-	82.35%
Nonanenitrile	82.35%
Octanenitrile	88.24%
Octanoic acid, methyl ester	88.24%
o-Cymene	96.08%
p-Cresol	82.35%
Propanoic acid, 2-methyl-, methyl ester	80.39%
Pyrazine, 2,5-dimethyl-	96.08%
Pyrazine, methyl-	88.24%
Pyrazine, trimethyl-	92.16%
Pyridine	80.39%
Pyridine, 2,6-dimethyl-	88.24%
Thiophene, 2-pentyl-	80.39%
trans, trans-Octa-2,4-dienyl acetate	84.31%
Undecanoic acid, methyl ester	88.24%

Appendix E:

PCA Plots for Samples Stored and Aged in the Refrigerator

Appendix E: PCA Plots for Samples Stored and Aged in the Refrigerator

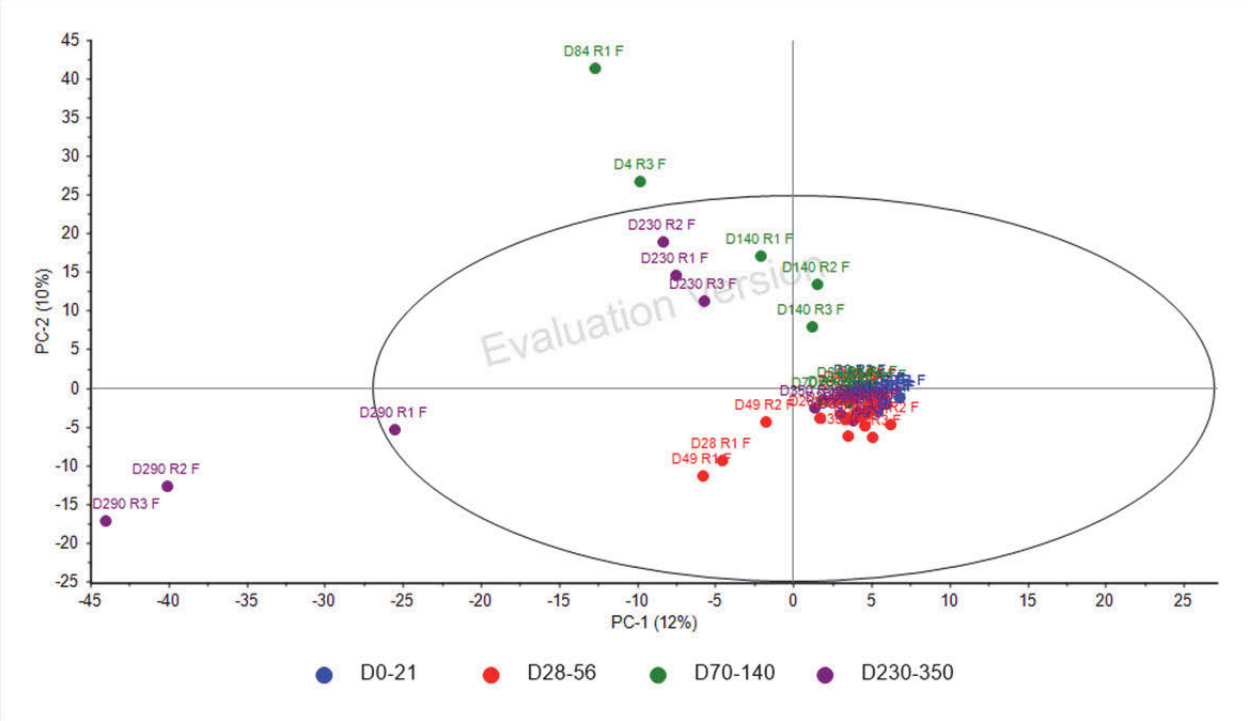

Figure E1: The original PCA plot for samples stored and aged in the refrigerator with a 95% CI.

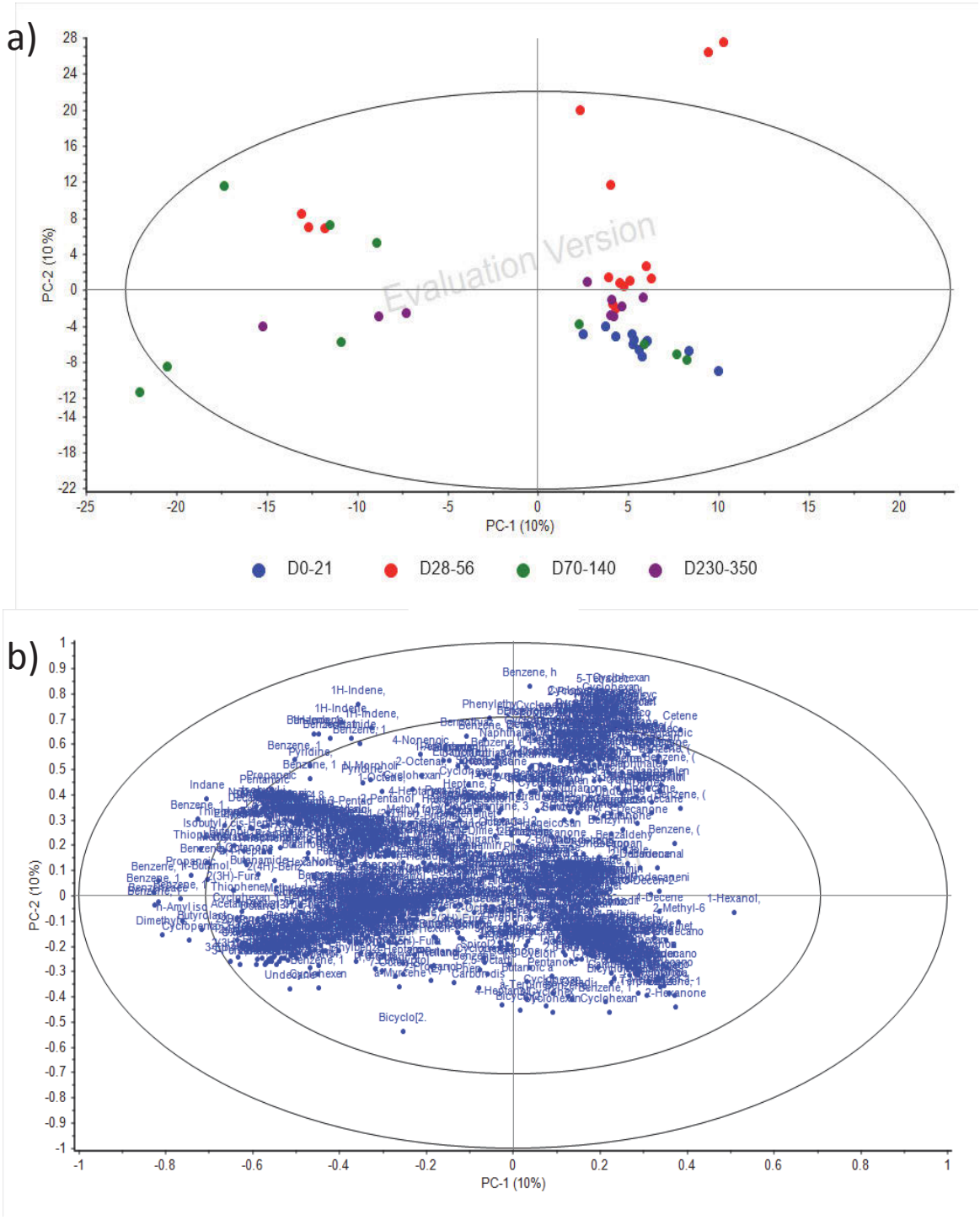

Figure E2: PCA plots for the odour of decomposition fluid stored in the refrigerator: a) Scores plot sorted by days and all compounds, with original outliers removed and a 95% CI, demonstrating variations between samples, B) Loadings plot, indicating compounds influencing the variations in Plot A

Appendix F:

Predominant VOCs Found in Samples Stored and Aged in the Refrigerator

Appendix F: Predominant VOCs Found in Samples Stored and Aged in the Refrigerator

Compound Name	% of Samples Containing VOC
1,13-Tetradecadiene	96.08%
1,3-Nonadiene, (E)-	82.35%
1,6-Octadien-3-ol, 3,7-dimethyl-	82.35%
1H-Indene, 2,3-dihydro-4,7-dimethyl-	80.39%
1H-Indene, 2,3-dihydro-4-methyl-	90.20%
1-Octanol	86.27%
1-Octanol, 2-butyl-	88.24%
1-Undecanol	84.31%
2-Decanol	98.04%
2-Decanone	96.08%
2-Dodecanone	94.12%
2-Furanmethanethiol, 5-methyl-	84.31%
2-Heptanol	80.39%
2-Heptanone	98.04%
2-Heptanone, 3-methyl-	96.08%
2-Heptanone, 4,6-dimethyl-	82.35%
2-Heptanone, 6-methyl-	96.08%
2-Hexanone	80.39%
2-Nonanol	84.31%
2-Nonanone	92.16%
2-Nonenal, (E)-	86.27%
2-Octanone	96.08%
2-Pentanol	90.20%
2-Pentanone	88.24%
2-Pentenoic acid, methyl ester	80.39%
3,5-Octadien-2-one, (E,E)-	84.31%
3-Decen-1-ol, acetate, (Z)-	80.39%
3-Decen-2-one	80.39%
3-Hexen-1-ol, acetate, (Z)-	90.20%
3-Octanol	86.27%
3-Octanone	96.08%
3-Penten-2-one, (E)-	82.35%

3-Tridecene, (E)-	82.35%
4-Decenoic acid, methyl ester, Z-	86.27%
4-Heptanone	80.39%
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	96.08%
7-Hexadecenoic acid, methyl ester, (Z)-	80.39%
9-Decen-1-ol, methyl ether	90.20%
Acetophenone	92.16%
Anisole	84.31%
à-Phellandrene	96.08%
Aromandendrene	84.31%
Benzaldehyde	90.20%
Benzaldehyde, 4-ethyl-	86.27%
Benzene, 1,2,4,5-tetramethyl-	80.39%
Benzene, 1-ethyl-2,3-dimethyl-	92.16%
Benzene, 1-ethyl-2-methyl-	84.31%
Benzene, 1-methoxy-4-methyl-	80.39%
Benzene, 1-methyl-3-(1-methylethyl)-	92.16%
Benzene, 1-methyl-3-propyl-	80.39%
Benzene, 1-methyl-4-(1-methylethenyl)-	84.31%
Benzene, 1-methyl-4-propyl-	86.27%
Benzeneacetaldehyde	88.24%
Benzeneacetic acid, methyl ester	96.08%
Benzeneethanol, à-methyl-	82.35%
Benzoic acid, methyl ester	96.08%
Benzyl alcohol	92.16%
Benzyl methyl ketone	82.35%
Cetene	80.39%
Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	88.24%
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	92.16%
Decanoic acid, methyl ester	96.08%
Dimethyl trisulfide	96.08%
Disulfide, dimethyl	96.08%
Disulfide, methyl (methylthio)methyl	80.39%
Eicosane	80.39%
Furan, 2-pentyl-	94.12%
Heptadecane	82.35%
Heptanonitrile	94.12%
Hexanenitrile	82.35%
Hexanoic acid, methyl ester	80.39%
Methanethiol	92.16%
Methyl tetradecanoate	100.00%
Methylamine, N,N-dimethyl-	100.00%

Naphthalene	82.35%
Naphthalene, 1-methyl-	80.39%
Naphthalene, 2-methyl-	80.39%
Nonane, 2,2,4,4,6,8,8-heptamethyl-	82.35%
n-Tridecan-1-ol	80.39%
Octane, 2,4,6-trimethyl-	88.24%
Octanenitrile	80.39%
Pentadecanoic acid, 14-methyl-, methyl ester	92.16%
Pentadecanoic acid, methyl ester	82.35%
Pentanal, 2-methyl-	82.35%
Phenylethyl Alcohol	92.16%
Propanoic acid, 2-methyl-, methyl ester	80.39%
Pyrazine, 2,5-dimethyl-	98.04%
Pyrazine, methyl-	80.39%
Pyrazine, trimethyl-	92.16%
Thiocyanic acid, methyl ester	84.31%
Thiophene, 2-pentyl-	86.27%
trans-3-Nonen-2-one	94.12%
Tridecane, 3-methyl-	84.31%

Appendix G:

PCA Plots for Samples Stored and Aged in the Freezer

Appendix G: PCA Plots for Samples Stored and Aged in the Freezer

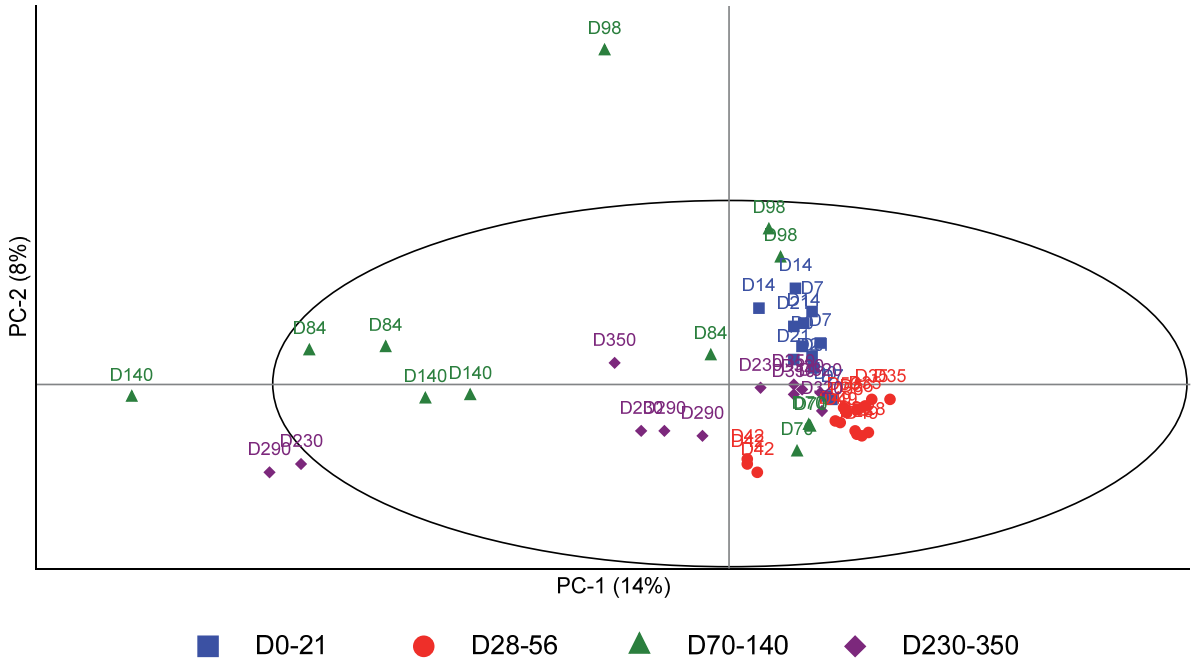

Figure G1: Original PCA scores plot of samples stored and aged in the freezer. This plot includes all of the freezer sample data and a 95% CI.

Appendix H:

Predominant VOCs Found in Samples Stored and Aged in the Freezer

Appendix H: Predominant VOCs Found in Samples Stored and Aged in the Freezer

Compound Name	% of Samples Containing VOC
1,13-Tetradecadiene	80.39%
1,3,5,7-Cyclooctatetraene	94.12%
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	82.35%
1,3-Nonadiene, (E)-	82.35%
1,4-Undecadiene, (E)-	84.31%
1,6-Octadien-3-ol, 3,7-dimethyl-	86.27%
1-Decene	80.39%
1-Hexanol, 2-ethyl-	86.27%
1-Nonanol	92.16%
2-Decanone	100.00%
2-Dodecanone	94.12%
2-Heptanol	82.35%
2-Heptanone	100.00%
2-Heptanone, 3-methyl-	92.16%
2-Heptanone, 6-methyl-	90.20%
2-Methyl-6-propylpyridine	80.39%
2-Nonanone	94.12%
2-Octanone	98.04%
2-Pentanone	80.39%
2-Phenylpropenal	86.27%
2-Undecanol	100.00%
2-Undecanone	96.08%
3,5-Octadien-2-one, (E,E)-	92.16%
3-Decanone	86.27%
3-Decen-1-ol, acetate, (Z)-	88.24%
3-Decen-2-one	80.39%
3-Hexen-1-ol, acetate, (Z)-	90.20%
3-Penten-2-one, (E)-	82.35%
3-Undecanone	90.20%
4-Decene, 2,2-dimethyl-, (E)-	96.08%
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	84.31%
5-Hepten-2-one, 6-methyl-	86.27%

7-Octen-2-ol, 2,6-dimethyl-	88.24%
Acetophenone	100.00%
Anisole	82.35%
à-Terpineol	100.00%
Benzaldehyde	96.08%
Benzaldehyde, 4-ethyl-	80.39%
Benzene, 1-methyl-4-(1-methylethenyl)-	96.08%
Benzene, 4-ethyl-1,2-dimethyl-	92.16%
Benzeneacetaldehyde	86.27%
Benzoic acid, methyl ester	90.20%
Benzonitrile	82.35%
Benzyl alcohol	90.20%
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	96.08%
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	96.08%
Decanoic acid, methyl ester	80.39%
Dimethyl trisulfide	100.00%
Disulfide, dimethyl	100.00%
Disulfide, methyl (methylthio)methyl	86.27%
Dodecanoic acid, methyl ester	80.39%
Furan, 2-pentyl-	90.20%
Heptanonitrile	90.20%
Hexadecane	80.39%
Isobornyl formate	98.04%
Methanethiol	90.20%
Methyl tetradecanoate	92.16%
Methylamine, N,N-dimethyl-	88.24%
Nonane, 2,2,4,4,6,8,8-heptamethyl-	82.35%
Octanenitrile	80.39%
Oxirane, dodecyl-	84.31%
p-Cresol	88.24%
Phenylethyl Alcohol	92.16%
Pyrazine, 2,5-dimethyl-	94.12%
Pyrazine, methyl-	80.39%
Pyrazine, trimethyl-	92.16%
Pyridine	88.24%
Pyridine, 2-methyl-	84.31%
Pyrrole	86.27%
Thiocyanic acid, methyl ester	80.39%
Thiophene, 2-pentyl-	96.08%

Appendix I:

Predominant VOCs Found in Diluted Samples of Decomposition Fluid

Appendix I: Predominant VOCs Found in Diluted Samples of Decomposition Fluid

Compound Name	% of Samples Containing VOCs
1-Decene	91.13%
1-Hepten-6-one, 2-methyl-	87.10%
1-Hexanol, 2-ethyl-	99.19%
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	98.39%
2-Decanone	96.77%
2-Dodecanone	93.55%
2-Heptanone	96.77%
2-Hexene, 5,5-dimethyl-, (Z)-	82.26%
2-Nonanone	88.71%
2-Octanone	97.58%
2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)-	97.58%
3-Dodecene, (Z)-	90.32%
3-Heptanone	84.68%
3-Pentanone	82.26%
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	92.74%
7-Tetradecene, (E)-	98.39%
Acetone	98.39%
Benzene, 1-bromo-4-methyl-	91.13%
Benzene, 1-ethenyl-4-ethyl-	100.00%
Benzene, 1-ethyl-3-methyl-	99.19%
Benzene, 1-methoxy-3-methyl-	86.29%
Benzene, 1-methyl-3-(1-methylethyl)-	83.06%
Benzene, propyl-	84.68%
Carbon disulfide	86.29%
Cetene	93.55%
Decanal	93.55%
Decane, 3-methyl-	91.94%
Dodecanal	96.77%
Eicosane	94.35%
Furan, 2-pentyl-	88.71%
Heptanal	90.32%
Heptane, 2,2,4,6,6-pentamethyl-	87.10%
Hexadecane	100.00%

Hexanal	97.58%
Hexanoic acid, methyl ester	82.26%
Limonene	84.68%
Methyl Alcohol	98.39%
n-Butyl methacrylate	85.48%
Nonanal	96.77%
Octanal	98.39%
Oxalic acid, allyl nonyl ester	97.58%
Oxygen	83.06%
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	99.19%
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	95.16%
p-Xylene	100.00%
Styrene	100.00%
Tetradecane	94.35%
Toluene	83.06%
Trichloromethane	97.58%
Tridecanoic acid, methyl ester	90.32%
Undecane	99.19%
Undecane, 2,6-dimethyl-	88.71%

Appendix J:

Predominant Compounds Found in Human Decomposition Fluid

Appendix J: Predominant Compounds Found in Human Decomposition Fluid

Compound Name	% of Samples Containing Compound
2-Undecanol	100.00%
Benzene, 1-ethenyl-4-ethyl-	100.00%
p-Xylene	100.00%
Styrene	100.00%
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	99.19%
Undecane	99.19%
2-Decanol	99.02%
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	98.39%
7-Tetradecene, (E)-	98.39%
Methyl Alcohol	98.39%
Octanal	98.39%
2-Heptanone	98.21%
Isobornyl formate	98.04%
2-Decanone	97.72%
2-Oxazolidinone, 3-amino-5-(4-morpholinylmethyl)-	97.58%
Hexanal	97.58%
Oxalic acid, allyl nonyl ester	97.58%
Trichloromethane	97.58%
Disulfide, dimethyl	97.39%
2-Undecanone	97.06%
2-Octanone	96.94%
Nonanal	96.77%
Acetophenone	96.73%
Dimethyl trisulfide	96.73%
2-Heptanone, 3-methyl-	96.08%
3-Octanone	96.08%
4-Decene, 2,2-dimethyl-, (E)-	96.08%
à-Phellandrene	96.08%
Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	96.08%
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	96.08%
o-Cymene	96.08%
Pyrazine, 2,5-dimethyl-	96.08%
Methyl tetradecanoate	95.42%
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	95.16%

à-Terpineol	95.10%
2-Dodecanone	94.96%
Tetradecane	94.35%
1,3,5,7-Cyclooctatetraene	94.12%
trans-3-Nonen-2-one	94.12%
Decanal	93.55%
Acetone	93.31%
2-Heptanone, 6-methyl-	93.14%
Benzaldehyde	93.14%
Benzoic acid, methyl ester	93.14%
Heptanonitrile	92.81%
Dodecanal	92.50%
2-Nonanone	92.28%
1-Nonanol	92.16%
Benzene, 1-ethyl-2,3-dimethyl-	92.16%
Benzene, 4-ethyl-1,2-dimethyl-	92.16%
Benzeneacetic acid, methyl ester	92.16%
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	92.16%
Pentadecanoic acid, 14-methyl-, methyl ester	92.16%
Phenylethyl Alcohol	92.16%
Pyrazine, trimethyl-	92.16%
Decane, 3-methyl-	91.94%
Furan, 2-pentyl-	91.79%
Decanoic acid, methyl ester	91.50%
Methanethiol	91.50%
Benzyl alcohol	91.18%
Benzene, 1-bromo-4-methyl-	91.13%
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	91.04%
1-Hexanol, 2-ethyl-	90.58%
1-Decene	90.51%
3-Dodecene, (Z)-	90.32%
Heptanal	90.32%
Tridecanoic acid, methyl ester	90.32%
1H-Indene, 2,3-dihydro-4-methyl-	90.20%
2-Pentanol	90.20%
3-Hexen-1-ol, acetate, (Z)-	90.20%
3-Undecanone	90.20%
9-Decen-1-ol, methyl ether	90.20%
Benzene, 1-methyl-4-(1-methylethenyl)-	90.20%
Hexadecane	90.20%
Methylamine, N,N-dimethyl-	90.20%
Benzene, 1-ethyl-3-methyl-	89.79%

Benzonitrile	89.22%
Undecane, 2,6-dimethyl-	88.71%
Cetene	88.70%
1,13-Tetradecadiene	88.24%
1-Octanol, 2-butyl-	88.24%
2-Decenal, (Z)-	88.24%
3,5-Octadien-2-one, (E,E)-	88.24%
Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	88.24%
Octane, 2,4,6-trimethyl-	88.24%
Octanoic acid, methyl ester	88.24%
Pyridine, 2,6-dimethyl-	88.24%
Undecanoic acid, methyl ester	88.24%
Benzene, 1-methyl-3-(1-methylethyl)-	87.61%
Thiophene, 2-pentyl-	87.58%
Eicosane	87.37%
Benzeneacetaldehyde	87.25%
1-Hepten-6-one, 2-methyl-	87.10%
Heptane, 2,2,4,6,6-pentamethyl-	87.10%
Benzene, 1-methoxy-3-methyl-	86.29%
1-Octanol	86.27%
2-Hexadecene, 2,6,10,14-tetramethyl-	86.27%
2-Nonenal, (E)-	86.27%
2-Phenylpropenal	86.27%
3-Decanone	86.27%
3-Octanol	86.27%
4-Decenoic acid, methyl ester, Z-	86.27%
Benzene, 1-methyl-4-propyl-	86.27%
Bicyclo[4.2.0]octa-1,3,5-triene	86.27%
Disulfide, methyl (methylthio)methyl	86.27%
Hexadecanoic acid, methyl ester	86.27%
Pyrrole	86.27%
2-Pentanone	86.27%
n-Butyl methacrylate	85.48%
Carbon disulfide	85.30%
p-Cresol	85.29%
3-Heptanone	84.68%
Benzene, propyl-	84.68%
Limonene	84.68%
1,4-Undecadiene, (E)-	84.31%
1,6-Octadien-3-ol, 3,7-dimethyl-	84.31%
1-Hexadecanol	84.31%
1-Undecanol	84.31%

2-Butanone	84.31%
2-Dodecanol	84.31%
2-Furanmethanethiol, 5-methyl-	84.31%
2-Nonanol	84.31%
2-Undecanone, 6,10-dimethyl-	84.31%
3-Decen-1-ol, acetate, (Z)-	84.31%
5-Hepten-2-one, 6-methyl-	84.31%
7-Octen-2-ol, 2,6-dimethyl-	84.31%
Aromandendrene	84.31%
Benzene, 1-ethyl-2-methyl-	84.31%
Oxirane, dodecyl-	84.31%
Pyridine	84.31%
Pyridine, 2-methyl-	84.31%
trans, trans-Octa-2,4-dienyl acetate	84.31%
Tridecane, 3-methyl-	84.31%
Benzaldehyde, 4-ethyl-	83.33%
Anisole	83.33%
Benzyl methyl ketone	83.33%
Oxygen	83.06%
Toluene	83.06%
Octanenitrile	83.01%
Pyrazine, methyl-	83.01%
1,3-Nonadiene, (E)-	82.35%
2(3H)-Furanone, 5-heptyldihydro-	82.35%
2-Heptanone, 4,6-dimethyl-	82.35%
3-Penten-2-one, (E)-	82.35%
3-Tridecene, (E)-	82.35%
5-Decen-1-ol, acetate, (E)-	82.35%
Benzeneethanol, à-methyl-	82.35%
Cyclopentane, butyl-	82.35%
E-1,6-Undecadiene	82.35%
Heptadecane	82.35%
Hexanenitrile	82.35%
Naphthalene	82.35%
Nonane, 2,2,4,4,6,8,8-heptamethyl-	82.35%
Nonanenitrile	82.35%
Pentadecanoic acid, methyl ester	82.35%
Pentanal, 2-methyl-	82.35%
Thiocyanic acid, methyl ester	82.35%
2-Hexene, 5,5-dimethyl-, (Z)-	82.26%
3-Pentanone	82.26%
1,3-Dioxolane, 2-(3-bromo-5,5,5-trichloro-2,2-dimethylpentyl)-	81.37%

2-Heptanol	81.37%
Hexanoic acid, methyl ester	81.01%
1H-Indene, 2,3-dihydro-4,7-dimethyl-	80.39%
2-Hexanone	80.39%
2-Methyl-6-propylpyridine	80.39%
2-Pentenoic acid, methyl ester	80.39%
3-Decen-2-one	80.39%
4-Heptanone	80.39%
7-Hexadecenoic acid, methyl ester, (Z)-	80.39%
Benzene, 1,2,4,5-tetramethyl-	80.39%
Benzene, 1-methoxy-4-methyl-	80.39%
Benzene, 1-methyl-3-propyl-	80.39%
Dodecanoic acid, methyl ester	80.39%
Indane	80.39%
Naphthalene, 1-methyl-	80.39%
Naphthalene, 2-methyl-	80.39%
n-Tridecan-1-ol	80.39%
Propanoic acid, 2-methyl-, methyl ester	80.39%