UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

COMPARAISON DE L'ODEUR DE DÉCOMPOSITION ENTRE LES CADAVRES ET LES RESTES HUMAINS UTILISÉS COMME AIDES À LA DÉTECTION DE CADAVRES

COMPARING THE DECOMPOSITION ODOUR BETWEEN CADAVERS AND HUMAN REMAINS USED AS CADAVER DETECTION DOG TRAINING AIDS

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Résumé

Les chiens de détection de cadavres (CDD) sont des chiens spécialisés d'importance forensique pouvant détecter les composés organiques volatils (COV) liés à la décomposition. Leurs capacités olfactives exceptionnelles leur permettent de détecter des restes humains et/ou des objets associés à des restes humains qui peuvent passer inaperçus au nez humain. Pour atteindre un taux de réussite élevé dans leur recherche, la formation des CDD doit impliquer une exposition répétée à l'odeur de décomposition sous la forme d'outils d'entrainement. En fonction de leur disponibilité et de leur facilité d'accès, les organisations qui forment les CDD utilisent des formulations chimiques, des restes d'animaux et/ou des restes humains comme outils d'entrainement. Présentement, la Police provinciale de l'Ontario (OPP) utilise des membres amputés (en particulier des pieds) obtenus à la suite de chirurgies consenties effectuées sur des patients diabétiques comme outils d'entrainement des CDD. Les connaissances sur le profil volatil des membres inférieurs/pieds amputés et leur pertinence en tant qu'alternative aux autres restes humains (par exemple, sang, tissus mous, os, etc.) qui sont couramment utilisés pour l'entraînement des CDD sont limitées. Ainsi, cette étude a pour but de déterminer le profil des COV des membres inférieurs amputés et le comparer au profil des COV des cadavres se décomposant dans un environnement canadien extérieur, afin de déterminer leur validité à des fins de formation des CDD. Les COV ont été collectés à l'aide de tubes sorbants et analysés par chromatographie en phase gazeuse bidimensionnelle couplé à la spectrométrie de masse à temps de vol (GC × GC – TOFMS). De plus, la réponse des CDD aux outils d'entrainement et aux cadavres a également été enregistrée pour comprendre leur réponse dans le contexte de la détection des COV. Plusieurs COV liés à la décomposition mentionnée dans la littérature appartenant à des classes telles que les acides, les alcools, les aldéhydes, les aromatiques, les aliphatiques cycliques, les esters et analogues, les éthers, les halogènes, les cétones, les aliphatiques linéaires, les COV contenant de l'azote et du soufre ont été identifiés. Sur la base du profil des COV et des taux de détection élevés des CDD (allant de 78 % à 100 %), cette étude a conclu que les membres inférieurs amputés sont un substitut approprié comme outils d'entraînement pour les CDD. Il s'agit d'une considération importante pour les organisations qui dépendent actuellement de l'utilisation de restes d'animaux en raison de la difficulté à

accéder aux restes humains. Les auteurs proposent que ceux-ci soient utilisés en tant qu'outils d'entrainement par les organisations de formation CDD à travers le monde avec une éthique appropriée et le consentement des donneurs. Une considération doit être faite en termes de persistance de l'anesthésique utilisé pendant la procédure d'amputation, puisque cette étude a révélé la présence d'anesthésique - Sévoflurane dans les outils d'entraînement des années après l'ablation chirurgicale des membres. Des études supplémentaires sont nécessaires pour établir la durée pendant laquelle les membres amputés peuvent être stockés et utilisés, puisque cette étude n'a enregistré qu'une variabilité subtile due au vieillissement et aux conditions de stockage. De plus, cette étude a déterminé que d'autres outils d'entraînement, tels que les dents n'étaient pas jugées adaptées à l'entraînement des CDD et que les CDD avaient besoin d'une formation accrue sur les outils d'entraînement ayant été enterrées, car ceux-ci étaient difficiles à localiser.

Certificate of authorship and originality

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.

I also certify that the thesis has been written by me, Rushali Dargan. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged.

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

Rushali Dargan

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Date: 29th November 2022

Dedication

This thesis is dedicated to my supportive parents:

Gp. Cpt. Ravi Dargan

Mrs. Reena Dargan

and my loving sister:

Ms. Rishika Dargan

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List of Abbreviations and Symbols

^{1}D	First dimension
1D	One-dimensional
^{2}D	Second dimension
2D	Two-dimensional
3D	Three-dimensional
ADD	Accumulated degree days
AFTER	Australian Facility for Taphonomic Experimental Research
ATP	Adenosine triphosphate
CDD	Cadaver detection dogs
CDI	Cadaver decomposition island
DMDS	Dimethyl disulphide
DMS	Dimethyl sulphide
DMTS	Dimethyl trisulphide
DNA	Deoxyribonucleic acid
DOA	Decomposition odour analysis
ED	Experimental day
FARF	Forensic Anthropology Research Facility
GC	Gas chromatography
GC×GC	Comprehensive two-dimensional gas chromatography
GPR	Ground-penetrating radar

HS-SPME	Headspace solid phase microextraction
ID	Internal diameter
IUPAC	International Union of Pure and Applied Chemistry
KADD	Kelvin accumulated degree days
MS	Mass spectrometry
m/z	Mass/charge ratio
NIST	National Institute of Standards and Technology
ODORS	OPP Decomposing Odor Research Site
OSAC	Organisation of Scientific Area Committees
OPP	Ontario Provincial Police
PC	Principal component
PCA	Principal component analysis
PMI	Postmortem interval
REST[ES]	*Recherche en Sciences Thanatologiques [Expérimentales et Sociales]*
S/N	Signal/noise ratio
TD	Thermal desorption
TIC	Total ion chromatogram
TOF	Time-of-flight
TOFMS	Time-of-flight mass spectrometry
UQTR	*Université du Québec à Trois-Rivières*
VOC	Volatile organic compound

VR	Victim recovery

Publications

Review article:

Dargan R, Samson C, Burr WS, Daoust B and Forbes SL (2022) Validating the Use of Amputated Limbs Used as Cadaver Detection Dog Training Aids. Front. Anal. Sci. 2:934639. https://doi.org/10.3389/frans.2022.934639

Research article:

Dargan, R, Forbes, SL. Cadaver-detection dogs: A review of their capabilities and the volatile organic compound profile of their associated training aids. WIREs Forensic Sci. 2021; 3:e1409. https://doi.org/10.1002/wfs2.1409

Abstract

Cadaver detection dogs (CDDs) are specialised scenting dogs of forensic relevance that can detect decomposition-related volatile organic compounds (VOCs). Their exceptional olfactory capabilities allow them to detect human remains and/or objects associated with human remains which may go unrecognised by the human nose. To achieve a high success rate in their search, CDD training must involve repeated exposure to decomposition odour in the form of training aids. Depending upon their availability and ease of access, organizations that train CDDs use chemical formulations, animal remains, and/or human remains as training aids. Currently, the Ontario Provincial Police (OPP) uses amputated limbs (specifically feet) obtained from consented surgeries performed on diabetic patients as a CDD training aid. There is limited knowledge about the volatile profile of amputated lower limbs/feet and their appropriateness as an alternative to other human remains (e.g. blood, soft tissue, bone, etc.) that are commonly used for CDD training. Thus, this study investigated the VOC profile of amputated lower limbs and compared it to the VOC profile of cadavers decomposing in an outdoor Canadian environment in order to determine their validity for CDD training purposes. VOCs were collected using sorbent tubes and analysed with comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC–TOFMS). In addition, the response of CDDs to these training aids and cadavers was also recorded to understand their response in the context of the VOC profile detected. Several previously reported decomposition-related VOCs belonging to classes including acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs were identified. Based on the VOC profile and high CDD detection rates (ranging from 78% – 100%) this study concluded that amputated lower limbs are a suitable substitute as CDD training aids. This is an important consideration for organisations that currently rely on the use of animal remains due to the difficulty in accessing human remains. The author proposes that this novel training aid can be incorporated by CDD training organisations across the globe with appropriate ethics and donor consent. One consideration must be made in terms of persistence of the anaesthetic used during amputation procedures since this study found the presence of anaesthetic – specifically sevoflurane in the CDD training aids upto 2.5 years after the surgical removal of limbs. There is a need for further studies to establish the duration for

which amputated limbs can be stored and used since this study recorded only subtle variability owing to ageing and storage conditions. Furthermore, this study determined that other forms of training aids such as teeth were not deemed suited for CDD training and that CDDs needed increased training on buried training aids as these were difficult to locate.

Summary of thesis outline

The aim of this study was to advance the knowledge in the field of human remains decomposition with regard to cadaver detection dog (CDD) training aids used by the Ontario Provincial Police (OPP), and cadavers decomposing in the outdoor Canadian environment. The ultimate objective was to draw a comparison between human remains decomposing in the two scenarios to establish the validity of using amputated lower limbs/feet as novel CDD training aids. The need for validating a new CDD training aid arose as a result of ethical constraints in acquiring human remains and the questionable suitability of using animal remains and chemical formulations as CDD training aids. Thus, this research attempted to add value to the field of CDD training by validating of use of amputated lower limbs as novel CDD training aids by comparing the VOC profiles of training aids to that of decomposing cadavers and observing CDD responses to both.

The first chapter of this thesis is a background and literature review of the process of decomposition, CDDs and their training. The second chapter is a summary of the analytical methods used in the current study. The remaining chapters in this thesis are a compilation of three individual studies written in three chapters and followed by a chapter that draws parallels among the three studies. The third chapter presents analytical results obtained from the first study by analysing CDD training aids used by the OPP Canine Unit. The fourth chapter presents analytical results obtained from the second study by analysing decomposing cadavers in an outdoor Canadian environment. The fifth chapter is focused on presenting CDD responses when they were exposed to their training aids during the third study. The sixth chapter is a discussion targeted towards comparing the analytical outcomes of training aids and cadavers from Chapters 3 and 4, respectively. Additionally this chapter also attempts to view the CDD responses (recorded in Chapter 5) in the context of the analytical outcomes. The last chapter of this thesis presents key findings of this study in the form of a conclusion and future work that should be undertaken to advance the current knowledge in the field.

Chapter 1: INTRODUCTION

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Death and events that occur after death have been a topic of spiritual and scientific curiosity over centuries. In the recent past, multiple studies have been conducted to understand thanatology, a multidisciplinary scientific field associated with death [1]. Contributions from the expertise of a multitude of fields such as chemistry, entomology, archaeology, anthropology, biology, geology, sociology, and psychology have enhanced our current understanding of death. The process, patterns and rates of decomposition, time since death, cause and manner of death, location and identification of the deceased, and even, the legal, the psychological and the spiritual aspect of death is better understood [2].

Among the many thanatological studies, the focus on the field of taphonomy has grown over the last 40 years. Taphonomic studies aim to understand the postmortem changes and the complex relationship of the decomposing remains with its surrounding environment. The term 'taphonomy' was coined by Efremov in 1940. He defined taphonomy as the study of the transition of all organic matter from the remains into the earth's surface as a geological record [3]. In its pioneer stages, taphonomy included the study of prehistoric human remains and it was closely associated with palaeontology, prehistoric archaeozoology, and archaeology [4]. Eventually, its application was extended to include the study of human remains in a forensic context [5]. In 1997, Haglund and Sorg determined the goals of forensic taphonomy as: estimating time since death or postmortem interval (PMI) and circumstances of death; determining cause and manner of death; reconstructing events after death; and differentiating perimortem (at the time of death) changes from postmortem (following death) changes [6]. Establishing these goals implied that the factors driving the changes during decomposition, and the correlation between ecology and decomposition still needed to be studied within the framework of forensic taphonomy [7; 5]. Therefore, forensic taphonomy, as it is understood today, is a field that relates to events occurring between death and recovery of decomposing remains [8].

Much like thanatology, forensic taphonomy encompasses several disciplines, of which, decomposition chemists have focused on understanding the odorous compounds that are released into the environment, thus forming the 'odour of death' evolved during the

process of decomposition [9]. ‘Odour of death’ as potential evidence in the forensic scenario was brought to public light through *The State of Florida vs. Casey Marie Anthony* case [10]. On 15th July 2008, three year old Caylee Anthony was reported missing by her grandmother – Cindy Anthony who had not seen Caylee in 30 days. Later, Cindy also reported an unpleasant odour in her daughter’s vehicle. Among other pieces of evidence, this unpleasant odour became a crucial piece of evidence and experts were brought in to test for decomposition-related compounds in the vehicle. The expert witness for the prosecution testified that five decomposition-related compounds were revealed in the analytical results. In contrast, the expert witness for the defence stated that there was no scientifically validated instrumentation method to detect decomposition-related compounds. Additionally, he argued that the five compounds mentioned by the prosecution’s expert witness could be from alternative sources such as a garbage bag that was found in the vehicle [10]. Since 2008, there has been a significant contribution to ‘odour of death’ studies to identify the key decomposition compounds, and to standardise procedures intending to reduce the variability in results.

The primary application of the ‘odour of death’ is to police fieldwork in facilitating the search and detection of human remains with the help of cadaver detection dogs (CDDs). Dogs are able to differentiate and even locate odour sources, this innate feature is beneficial to police in search operations [11]. CDD alerts as evidence were accepted in court long before analytical results from decomposition odour. One of the earliest testimonies based on CDD evidence which was accepted was *The People of the State of Illinois vs. Montano* (1990). This was a first-of-its-kind case where the jury pronounced the offender guilty based on a CDD alert even in the absence of the victim’s cadaver [12]. Since then there have been multiple cases where CDD evidence has been introduced, admitted and at times questioned [13; 11]. On one hand, ‘odour of death’ in the form of a CDD response has been admitted in multiple cases however, it still lacks standardisation at a global level [11]. At the same time, the credibility and admissibility of ‘odour of death’ as analytical scientific evidence has been challenged in court and this is predominantly because it is not well studied [11]. Research such as the current study is aimed at contributing to the knowledge base of the community with a hope to eventually standardise analytical aspects which would make ‘odour of death’ more recognised evidence in court. Additionally, the current research can contribute towards eventually reforming cadaver dog training protocols and adding value to the standardisation goal.

To understand the 'odour of death', the process of decomposition, changes that occur to a body after death, and the factors that cause and influence these changes must be understood.

1.1 Terrestrial decomposition process

Carter described the terrestrial decomposition process to follow a sigmoidal (S-shaped) pattern where it progresses rapidly in the beginning with the loss of most soft tissue and then at a much slower or almost stagnant rate as time passes [14]. Predominantly, the decomposition process is about the interaction between the decomposing remains and their ecological surrounding. Both the ecology and the remains influence each other and determine how the decomposition process will progress. During decomposition, a series of complex physiochemical and biological changes occurring in the remains causes a release of organic material into the surroundings. This influx of nutrients, carbon and water creates a distinctly visible high concentration island of fertility, known as a cadaver decomposition island (CDI), around the remains [7]. The initial pulse of new matter in the CDI is degenerative to vegetation but it also supports new plant life eventually [7]. Thus, the process of decomposition is prime to nutrient cycling and maintaining an ecological balance. The influence of decomposing remains on the environment is not one-way as multiple environmental factors govern this process and have an impact on the rate, end stage, and products produced during decomposition. These environmental factors along with some intrinsic factors (factors pertaining to the remains itself) that affect decomposition are discussed in section 1.1.1

1.1.1 Factors affecting decomposition

There are intrinsic (internal bodily) and extrinsic (external environmental) factors that influence the rate of decomposition. The following flowchart (Figure 1.1) lists some of the extrinsic and intrinsic factors significant to decomposition [15; 7; 16-19].

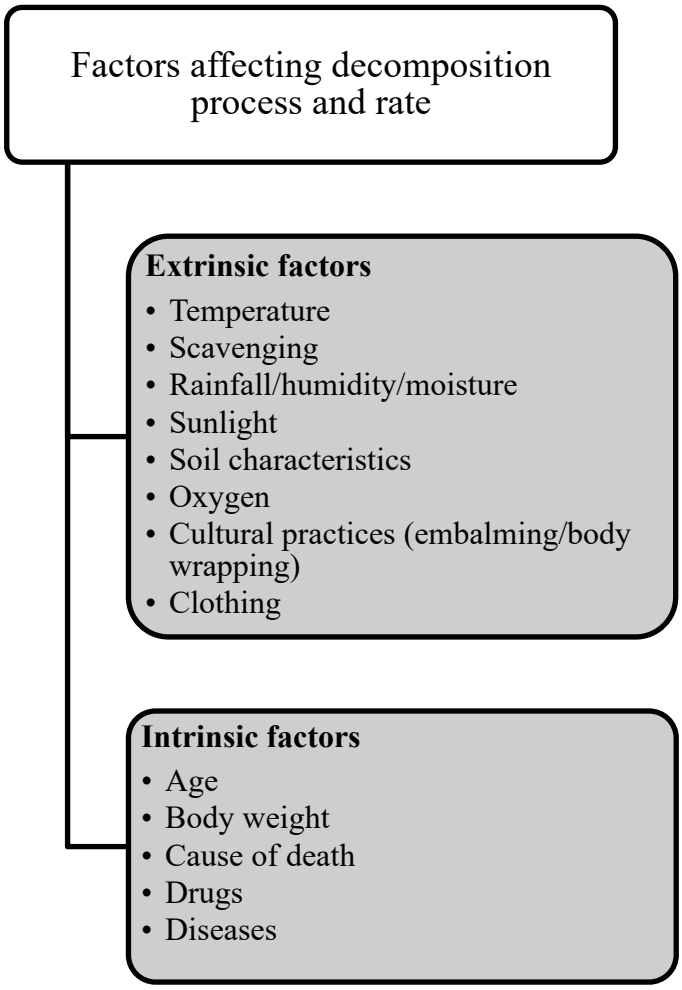


Figure 1.1: List of extrinsic and intrinsic factors that can affect the rate and process of decomposition [15; 7; 16-19].

Among these listed factors, temperature (both ambient and physiological) is one of the most significant to influence the rate of decomposition [20]. Higher temperatures accelerate the rate of a chemical reaction and thus decomposition, which is a chain of complex biochemical reactions, should increase with an increase in temperature [21]. Freezing temperatures are known to restrict the activity of enzymes, insects, and microbes. Thus, the decomposition slows down significantly in sub-zero temperatures [22-25]. Since ambient temperature is considered of utmost importance to decomposition, accumulated degree days (ADD) is a preferred scale to study the state and progression of decomposition. ADD in a forensic taphonomy context is the sum of average daily temperatures on consecutive days under consideration for a decomposition event and starting from the day of death or deposition [26].

Vertebrate and invertebrate activity accelerates the process of soft tissue decomposition. When local fauna and insects have access to a decomposing body, considerable soft tissue

can be lost rapidly [27-29]. In contrast, remains can become mummified and remain intact for months when vertebrate and invertebrate scavengers are excluded from a decomposition environment [30].

Moisture is a variable that favours decomposition when at optimum levels as it is necessary for bacterial and insect activity [17]. High levels of moisture in the environment can saturate the remains which slows down decomposition as insects can be drowned and the remains are prone to form adipocere (adipocere formation elaborated in section 1.1.4) [31; 32]. Conversely, a dry environment dehydrates the remains and impairs bacterial proliferation and insect activity, which ultimately causes mummification of tissue [15; 17].

Several soil characteristics such as soil texture, porosity, pH, temperature, oxygen content, and moisture content influence the soil microbial activity which can also impact the rate of decomposition for both surface and burial decomposition scenarios [25]. Reportedly, acidic soil (podzol, pH 4.6) promotes the growth of plants and fungi around the remains and can result in decomposition three times faster than alkaline soils (rendzina, pH 7.8) [33; 17]. Additionally, lower temperatures, high moisture (due to less evaporation), and limited oxygen supply observed during burial scenarios favours better preservation than surface decomposition [34; 24; 17].

Clothing or body wrapping has a dual influence on the decomposition rate. They can provide an ideal warm environment for insects to survive by acting as a barrier against predators and rain. However, in some instances, clothing may inhibit insects from colonising by hindering their access to the cadaver [35; 36].

Indoor scenarios protect the remains from wild scavengers and direct sunlight. This environment typically slows down the process of insect colonisation and exposes the remains to heating or cooling cycles throughout the day thus altering the decomposition process [37]. Remains discovered indoors after a period of time are often mummified and can show signs of scavenging if domestic pets or unhygienic conditions (e.g. rodents) are found within the home.

Apart from environmental factors, intrinsic factors such as body size and weight alter the decomposition rates. A study using pig and piglet carcasses indicated that piglet carcasses decomposed 2.82 times faster than large pig carcasses [38]. Another intrinsic factor, age

at the time of death, correlates to the bone mass and density which can impact the decomposition process. Infants and juveniles have low bone mass, and elderly individuals have low bone density thus, their bones will degrade more quickly and can even become completely mineralised [16]. Conversely, a study reported that decelerated decomposition was observed in babies due to simplified gut microflora [39]. The condition of the individual at the time of death can also dictate events after death such as a wound or a trauma around the time of death, which provides an additional opening for flies to lay eggs [40]. Thus, these areas show accelerated decomposition much like natural orifices on the human body e.g. genitals, mouth and nose [41]. Other factors like the quantity and nature of drugs present in an individual's body at the time of death can also affect the insect activity following death. Even commonly used drugs such as paracetamol are known to influence the development of insects [42]. Studies by Goff et al. on the effects of cocaine and heroin on the rate of development in Sarcophagidae demonstrated that larvae develop more rapidly if reared on the liver or spleen of rabbits that had been killed by a lethal dose of cocaine or heroin [43; 44]. Effect of drugs impacts the insect activity which ultimately can affect the rate of decomposition.

These key factors do not act in isolation, since the manner in which decomposition will progress is an outcome of the interactive effect of all or multiple factors. Thus, since there can be innumerable combinations of microenvironments possible at the site of decomposition, it becomes vital to replicate taphonomic studies across varying environments. This can help attain comparative and standardised data considering all the variations possible. Hence, conducting a taphonomic study in the Canadian environment, more specifically in a region of the Québec province, with different weather conditions year round, was one of the foci of the current study.

Regardless of the rate at which decomposition progresses, there are typical changes occurring in the remains that manifest as early and late postmortem changes through alteration in the physical appearance of the cadaver. Based on the postmortem changes, decomposition is divided into various stages. These stages are an overlapping continuum that can occur at varying times after death depending on the physiological factors and the environment at the decomposition site.

1.1.2 Stages of decomposition

Even though decomposition is a continuum, it can be divided into several visually distinct stages identified as: fresh, bloat, active decay, advanced decay and dry/skeletal remains. These stages are based on the macroscopic postmortem changes and insect succession patterns [45; 46]. At a given instance, different parts of the body can be in different stages of decomposition. Hence, an overlap between each of the stages within the same body is plausible [47; 48]. The various stages and their characteristic features are described in Table 1.1.

Table 1.1: Characteristic features of various decomposition stages

Stages	**Characteristic Features**
Fresh	Minimal macroscopic changes [49]. Early postmortem changes such as algor mortis, rigor mortis and livor mortis begins [24].
Bloat	Marbling and skin slippage commonly appear [49]. Skin discolouration may occur as an effect of livor mortis and marbling [50]. Putrefaction gases accumulate and cause swelling of internal organs and soft tissue [51]. This stage lasts until the gases are released from the orifices and ruptured skin leading to the deflation of the corpse [14; 52].
Active decay	Accelerated decay rates, multiple skin ruptures along with continued marbling and skin slippage. High entomological activity with larval masses feeding on the corpse [53]. Hair loss, blackish skin discolouration, soft tissue liquefaction with frothing and dryness in limbs [54]. Discolouration and leathery appearance of any exposed bone [49]. Intense cadaveric odour [49]. Release of liquified cadaveric material into the soil environment causing a sudden influx of nutrients, minerals and carbon content. This could impact the vegetation in the surrounding area and formation of a

	nutrient rich and vegetation deprived area surrounding the cadaver, also known as a Cadaver Decomposition Island (CDI) [14].
Advanced decay	Reduction in rate of decomposition [55]. Most cadaveric mass is lost as most of the soft tissue is either consumed by the larvae or liquefied [49]. Odour decreases in intensity [49].
Dry/Skeletal Remains	Desiccation of any remaining soft tissue causing mummification. Appearance of this leathery mummified tissue is synonymous with arrested decay stage [14; 56]. Degradation of bones, cartilage and keratin rich tissues such as hair, fur and nails occurs slowly over time [57; 14]. The plants may begin to regrow around the remains, especially within the nutrient rich CDI area [14; 21].

Through these stages of decomposition, the cadaver undergoes multiple postmortem changes at the cellular level that are manifested in the visual appearance of the cadaver. These postmortem changes have been discussed in section 1.1.3

1.1.3 Postmortem changes

One of the first evident postmortem changes is thermoregulation or algor mortis where the body temperature gradually equilibrates with ambient temperature. This phenomenon is commonly used to estimate PMI, but can be subject to a certain degree of error as estimation is based on a 'normal' body temperature that can vary between individuals [56]. Additionally, PMI can be estimated only during the temperature transition period. Once the ambient temperature is reached by the body, it is no longer possible to predict when the body commenced cooling.

Simultaneously, a reversible muscle stiffening phenomenon known as rigor mortis or rigidity can occur due to cross linking of muscle fibres after depletion of adenosine triphosphate (ATP) [56]. Rigor mortis occurs in all muscle tissues, but it first becomes evident in small muscle clusters including the jaw, eyelids and fingers, and then across the rest of the body. Rigor mortis is extremely variable and can occur approximately 2 –

6 h postmortem extending to 24 – 82 h [4]. Over time with the progression of decomposition and the breakdown of proteins in muscles, rigor mortis is lost [58].

Livor mortis or lividity is a physical phenomenon which is characterised by dark discolouration due to the pooling of blood under gravitational forces in the lower parts of the body. This results in an appearance of 'pallor' or pale skin colouration in regions that do not have a supply of circulating blood, while the other regions of the body develop a dark red/purple colouration due to the deposition of blood under gravity [56]. Lividity can appear as early as 30 min postmortem but is commonly visible between 2 – 4 h postmortem [4]. Over a period of time it becomes 'fixed' but this duration is highly variable, and therefore, it is not widely accepted for estimating PMI.

At the cellular level, two major processes – autolysis and putrefaction, drive the process of decomposition. Autolysis, the self-destruction of cells, commences when the oxygen supply decreases significantly as a result of the cessation of the cardiovascular system. This leads to the development of an anaerobic environment that promotes anaerobic glycolysis and releases carbon dioxide (CO_2) and lactic acid creating low pH levels within the cadaver [59; 21]. Low pH levels cause rupture and breakdown of the cell membrane releasing membrane-bound cell organelles called lysosomes [60; 61]. Lysosomes contain hydrolytic enzymes which degrade protein, carbohydrates, nucleic acids and lipids in cells and organs [20; 22]. Autolysis becomes visible in the form of necrotic tissue, cloudy appearance of cornea and liquefaction of splenic pulp.

Autolysis initiates putrefaction which is a microbe-driven process that can occur simultaneously with or towards the end of autolysis [20; 62; 63]. Microbes that escape into the intestines from the gut via the blood vessels cause haemolysis. Haemolysis along with haemoglobin degradation become visible as a green colouration in a 'marbling' pattern along the veins [62; 63; 59]. Eventually, putrefaction becomes visible as a blackish discolouration of the tissue when the bacteria produce hydrogen sulphide that reacts with iron in blood haemoglobin to produce iron sulphide [24]. Putrefaction also results in the accumulation of gases such as hydrogen sulphide (H_2S), carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3), sulphur dioxide (SO_2) and hydrogen (H_2) which cause bloating [64; 65]. The internal organs decompose at differential rates as putrefaction occurs first in muscle and fibrous tissues compared to connective tissue [66]. As a result, larynx-trachea, stomach, intestines, spleen, mesentery, liver, pancreas, adrenal medulla and

pregnant uterus are the first to decompose (within hours to days after death). Other organs including heart, lungs, kidneys, oesophagus, diaphragm, blood vessels, urinary bladder, prostate, uterus, skin, muscle-tendon structures and bones take much longer to decompose (days to years) [18]. Another externally visible phenomenon that can result from either autolysis or putrefaction is skin slippage where the epidermal layer separates and slips away from the dermis [34]. Since this occurs commonly in the hand and feet area, it is also known as 'degloving'.

These visual postmortem changes are driven by complex chemical reactions that cause breakdown of larger biomacromolecules into their simpler forms. This involves a series of chemical reactions or a pathway that is dependent on the nature of macromolecule and environmental conditions (such as presence or absence of oxygen, etc.).

1.1.4 Biochemical pathway

At the elemental level, the human body is made of carbon, hydrogen, nitrogen, oxygen, calcium, sodium, potassium, iron, copper, sulphur, phosphorous, magnesium and chlorine. These elements are comprised as 64% water, 20% proteins, 10% lipids, 5% minerals, and 1% carbohydrates which are the building blocks of cells in the human body. As a consequence of enzymatic- and microbial-driven decomposition, the complex molecules of nucleic acid (in DNA), proteins, lipids and carbohydrates break down to simpler molecules [62].

Protein degradation or proteolysis is facilitated by protease enzymes yielding proteoses, peptones and polypeptides as degradation products. These molecules further breakdown to form amino acids. Depending on their composition, amino acids could further degrade via processes such as deamination, decarboxylation or desulphydration [20; 24; 63]. Decarboxylation of specific amino acids such as ornithine and lysine results in the formation of putrescine and cadaverine which have been reported as significant decomposition by-products [20; 64; 67; 24]. Desulphydration of sulphur-containing amino acids results in the production of hydrogen sulphide, sulphides, ammonia, thiols, pyruvic acid [24; 63] and two commonly reported decomposition compounds: dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) [68; 69]. A schematic representation of protein degradation is depicted in Figure 1.2. Proteolysis occurs at varying rates depending on moisture, temperature, bacterial action and tissue type.

Degradation occurs first in soft tissues (neural and epithelial) and then in integuments and connective tissues [23; 24]. As a result, decomposition tends to occur first in the liver, brain and kidney and then in the rest of the body. Keratin and collagen take much longer to degrade compared to other proteins. As a result, tooth enamel, bones, cartilage, nails, hair and fur are present even beyond late decomposition stages [70; 24; 63].

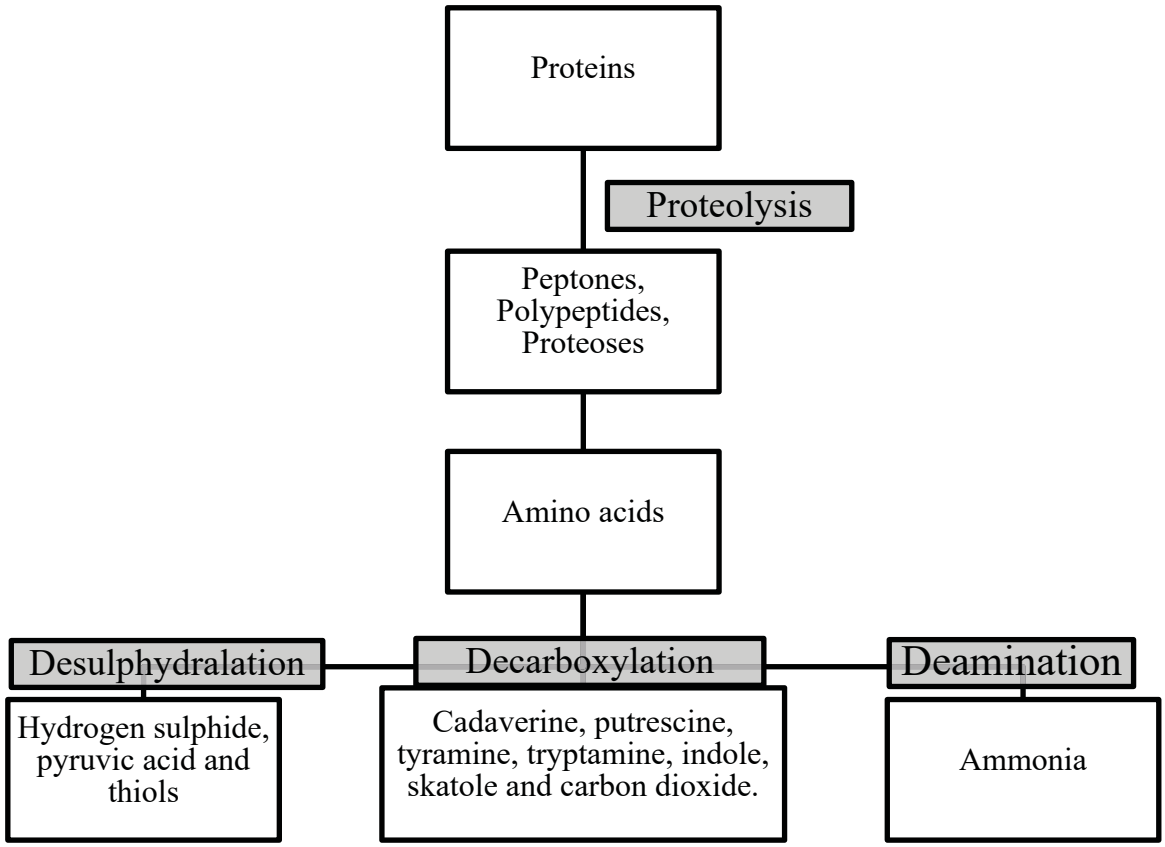


Figure 1.2: Schematic representation of protein degradation.

Adipose tissue in the human body is made up of 80% lipids, 5% protein and 15% water. The lipids are degraded by a hydrolysis process in the presence of lipase enzymes which results in the production of saturated and unsaturated fatty acids. The second step in their degradation process is carried out by bacterial enzymes and is dependent on the availability of oxygen [24]. The process of oxidation in the presence of oxygen yields aldehydes and ketones. In the absence of oxygen, the conversion of unsaturated fatty acids (such as linoleic, palmitoleic and oleic acid) to saturated fatty acids (such as palmitic, stearic and myristic acid) occurs via hydrogenation. As hydrolysis and hydrogenation continue, the formation of the saturated fatty acids increases. Additionally, hydroxy fatty acids can be formed during the process which remains as adipocere [23; 71; 24; 63; 72]. Sometimes fatty acids can form fatty acid salts with sodium and potassium ions as

additional constituents of adipocere [25]. Adipocere is a greyish-white waxy substance that is formed during the decomposition of fatty tissue [24; 61]. Adipocere formation is a late postmortem phenomenon which is observed in some cadavers under appropriate conditions such as the presence of high moisture content, anaerobic environment, warm temperature and a mildly alkaline pH [73]. Adipocere is resistant to further putrefaction and therefore it can preserve internal organs. Under specific conditions, adipocere may further degrade to produce butanoic acid, pentanoic acid and hexanoic acid that can be detected as volatile compounds in the environment [68]. A schematic representation of lipid degradation is depicted in Figure 1.3.

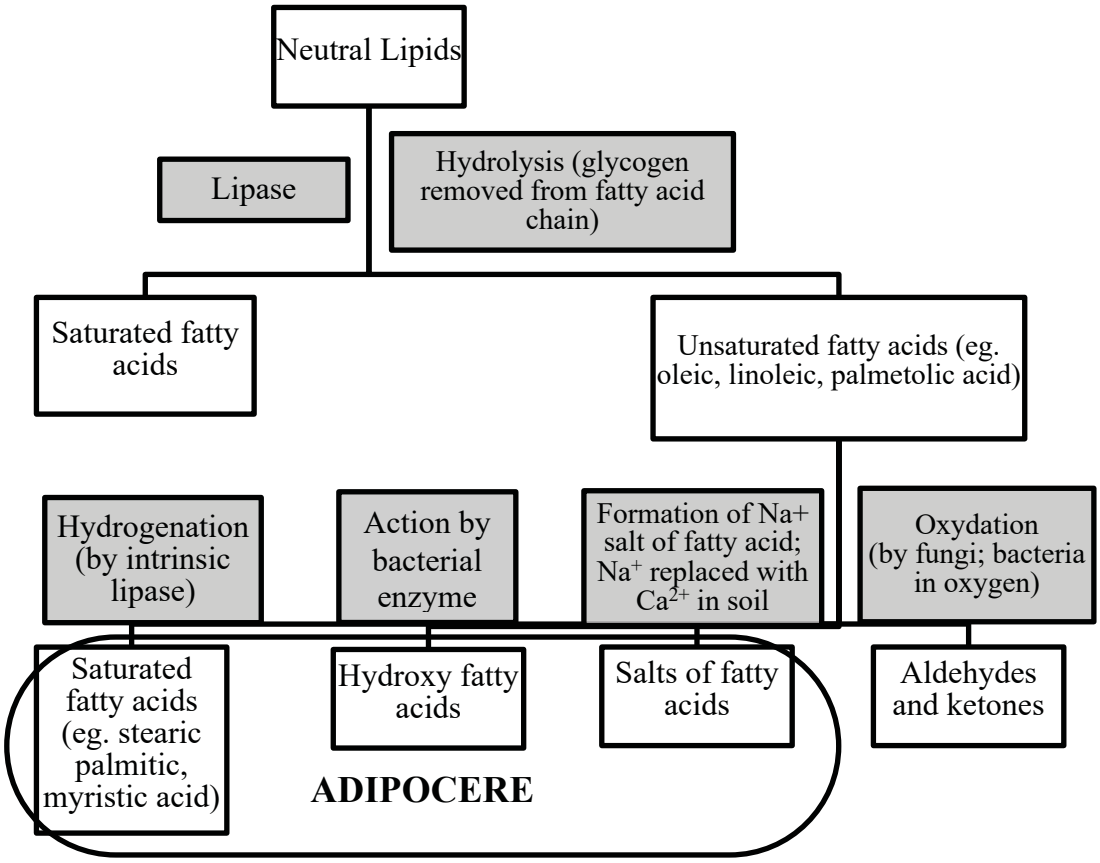


Figure 1.3: Schematic representation of lipid degradation.

Carbohydrates are another significant macromolecule in the human body. Glycogen, a complex carbohydrate is a major source of stored energy. At the time of death, carbohydrate degradation results in a variety of products depending on the microbial species (bacteria vs. fungi) involved in the process of degradation and the availability of oxygen (aerobic vs. anaerobic conditions). Carbohydrates are preferred energy sources for bacteria, thus, their degradation begins early during the process of decomposition. Complex carbohydrates such as glycogen is broken down into glucose (its monomer).

Glucose can subsequently degrade via different pathways. Under aerobic conditions, bacteria completely degrade glucose into carbon dioxide, water, methane and hydrogen via organic intermediates such as pyruvic acid, lactic acid and acetaldehyde. Under anaerobic conditions, alcohols (ethanol, methanol, propanol and butanol), organic acids (lactic acid, butyric acid and acetic acid), and acetone are produced [74; 23; 75; 61; 76]. Similarly, in the presence of fungi under anaerobic conditions, organic acids such as glucuronic acid, citric acid and oxalic acid are produced [23; 61]. Decarboxylation of glucose in the presence of yeast produces ethanol via an acetaldehyde intermediate [77; 75]. A schematic representation of carbohydrate degradation is depicted in Figure 1.4.

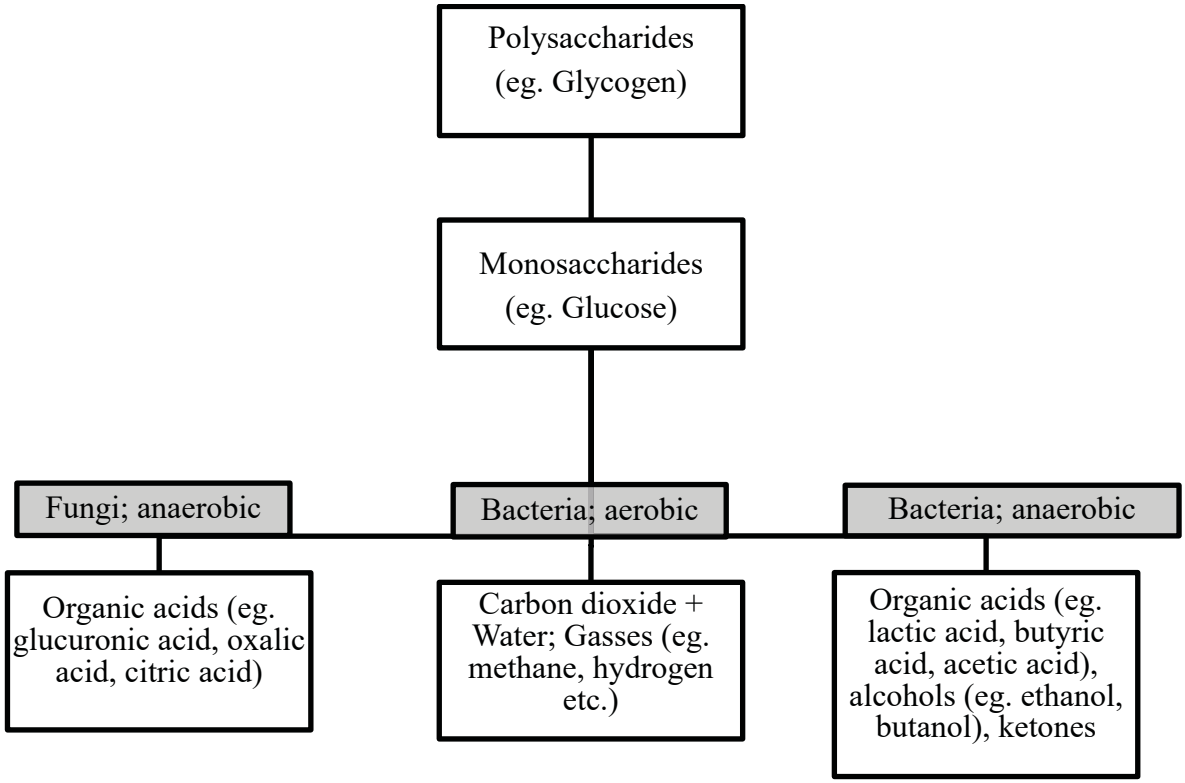


Figure 1.4: Schematic representation of carbohydrate degradation.

Apart from proteins, lipids and carbohydrates, DNA and its constituent molecules present in most cells of the body undergo degradation in the presence of nuclease enzymes. This results in the formation of nucleotides and nucleosides. The ribose sugar in nucleotides and nucleosides follow the same degradation pathway as carbohydrates. The purine and pyrimidine bases lead to generation of xanthine and hypoxanthine. These bases can be detected in vitreous humour for PMI estimation [78]. The phosphorus (from nucleotides) is present in the surrounding soil either in their oxidised form (for acidic soil) or in their inorganic form associated with iron, aluminium, magnesium and calcium [25]. Nitrogen

from the bases then enters the soil in the surrounding environment to become a part of the nutrient cycle.

Degradation of biomacromolecules results in the production of a plethora of products and by-products. In their simplest form, these exist as volatile gases such as CO_2, H_2S, CH_4, NH_3, SO_2, H_2 and volatile organic compounds (VOCs) which is discussed further below. The biochemistry of these VOCs has been studied to determine the macromolecules from which they originated and to understand the chemistry of decomposition. Generally, it is assumed that lipids and sugars are the source of oxygenated compounds; amino acids are a source of aromatic compounds, nitrogen-containing compounds and sulphur-containing compounds; and, nucleic acids are also a source for some nitrogen-containing compounds [79].

1.2 Decomposition odour

The volatile organic compounds or VOCs resulting from the degradation of biomacromolecules comprise decomposition odour, often colloquially referred to as the 'odour of death'. To determine the VOC profile of decomposition odour, studies have used either humans or human analogues [80; 81]. The choice of subjects depends on several factors such as the aim of the research, the area available for study, the feasibility of using human remains, and numerous other factors. A recent review that compared human and human analogues for decomposition studies concluded that in general, it was easier to control the confounding factors (e.g. weight, diet, etc. of the subjects), and replicate studies involving human analogues such as pigs but, the choice of samples relies on the objective of the study [81]. Among all human analogues used (pigs, mice, rats, rabbits, deer, cows, chicken), pigs have been the most accepted subjects to replace cadavers in taphonomic studies [82]. This is predominantly because they are readily available, and they have anatomical similarities with humans. The fat-to-muscle ratio, tissue density, hair distribution around the body, diet and immunological system of the two species show appreciable similarities [83; 84; 81; 82]. However, even with such anatomical similarities, for studies specific to decomposition odour, dissimilarities have been reported in VOC profiles of human and pig remains [85-87]. These could be largely attributed to variability in gut microflora and bacterial load in the abdominal cavity which impacts the microbial-driven decomposition pattern and rate [86; 82]. Thus, it was

concluded that pigs can be excellent for the pilot phase of a study as they have a lower cost of purchase and maintenance, shorter life cycle time to mature into an adult, require a relatively smaller outdoor facility to conduct studies, are less confronting in public opinion, and have fewer ethical restrictions associated with their use [88]. However, when the goal of studying decomposition VOCs is to identify the 'key' compounds or markers associated with human decomposition odour, cadavers are a preferred choice of subjects over pigs. Thus, cadavers were the preferred choice of subjects in the current study where human remains odour had to be analysed and studied. The research interest in the field of decomposition odour or VOCs that comprise the decomposition odour analysis is ever-increasing, primarily due to the ecological and forensic relevance (discussed in sections 1.2.1 and 1.2.2, respectively) of decomposition odour.

1.2.1 Ecological relevance of decomposition odour

Like most other naturally occurring elements of nature, decomposition plays a critical role in ecological sustainability. In a way, decomposition odour is contributory in maintaining an ecological balance through the 'recycling' of decomposing remains. VOCs and the odour associated with decomposition may go undetected by the human nose however, it innately attracts some fauna, such as small and large vertebrate species including rodents, birds and canids to the remains. Some of these species are scavengers and decomposing remains serve as their nutritional source. The scavengers drive the decomposition process forward as discussed in section 1.1.1. Additionally, some necrophagous invertebrates such as carrion flies and beetles are innately attracted to the body also. The presence of decomposition VOCs in the environment acts as a signal to necrophagous invertebrates. This signal works by 'attracting' the flies to the remains. It can also act as a stimulus to initiate mating and/or oviposition [89]. The presence of decomposition odour in the form of specific VOCs indicates the presence of decomposing remains in the environment which interests necrophagous invertebrates as they support larval growth and the insects' life cycle. Flies that arrive on the decomposing remains resulting from 'attraction' to VOCs, tend to lay egg masses on the remains during the early stages of decomposition in areas of soft tissue or orifices on the remains for the larvae to feed easily [90; 89; 9]. This drives the process of decomposition forwards.

1.2.2 Forensic relevance of decomposition odour

The arrival of the insect species is influenced by the presence of VOCs and these VOCs vary with stages of decomposition thus, influencing the arrival of specific invertebrate species. The arrival of different species is successive and follows a fairly predictable sequence [91]. This knowledge is used to the advantage of forensic entomologists that use insect succession and the period of the insect life cycle to predict the PMI in known environmental conditions.

However, the greatest application of decomposition odour is in the search and detection of decomposing remains. Some canids, specifically *Canis familiaris* or domestic dogs, can be trained to locate and identify 'decomposition odour hot spots' thus, indicating a potential crime scene in a forensic context. Under the principle of *Corpus deliciti*, finding a corpse becomes crucial evidence to prove a crime in court, thus decomposition odour as evidence is crucial in indicating the relevant location of a deceased individual [92]. On humanitarian grounds, it is a moral responsibility to locate and respectfully treat potentially missing remains or even reunite families with remains of their loved ones enabling some form of closure.

Detection of decomposition odour involves identifying the presence of decomposition-related VOCs in the environment. Some vertebrates, and specifically dogs can smell decomposition odour, this capability when tamed adequately becomes a powerful tool to mankind. Over the years, dogs have been domesticated to an extent that they can be trained to detect decomposition odour in a scenario involving searching and locating missing persons and/or human remains [93]. This capability of dogs is predominantly used by law enforcement organisations that deploy trained dogs to locate decomposed bodies. Another less commonly used technique of decomposition odour detection is by analysing for the presence of compounds in air or soil that are typically decomposition-related dominant in a decomposition scenario. A major reason for analytically determining the decomposition-related compounds is to identify what dogs' might be potentially alerting to when they indicate the presence of this odour. Thus, analytical analysis is instrumental in filling the gaps in our current knowledge.

1.3 Analytical detection of decomposition odour

While dogs are extremely reliable for fieldwork, they can not tell us the VOCs that they recognise as decomposition-related and thus chemical analysis is necessary to establish this. In decomposition studies, a primary matrix can include human cadavers, human analogues such as domestic pigs (*Sus scrofa domesticus* L.), human organs, decomposing human tissue, blood, fat tissue and bones. A secondary matrix can result from the migration of VOCs from the primary matrix to the surrounding environment. Thus, for VOC analysis a secondary matrix such as air, soil, water, or textiles in contact with the remains are commonly studied [94-97]. The resulting VOCs can vary with the type of secondary matrix investigated for the study. A comparison of VOCs from the air and soil matrix surrounding decomposing pig remains identified more VOCs in soil samples potentially due to microbial activity in the soil, as well as greater VOC retention in soil and dispersion of VOCs in the air due to wind and evaporation [98].

The analysis of VOCs released during the process of decomposition involves several steps including: sample collection and/or sample preparation; separation of target compounds from a mixture; and, detection of target compounds. Sample collection and preparation involves trapping the VOCs before injecting them for separation. A popular method for VOC separation is by gas chromatography (GC). The compounds separated by the GC are transferred to a detector for analyte detection. Mass spectrometry (MS) is the most popular detection method used for non-targeted compound detection and identification in decomposition studies [79].

1.3.1 Sample collection and injection

Sample collection for analysis of VOCs involves extracting them from their environment. This is accomplished by immobilising the VOCs on a solid adsorbent material and subsequently releasing them for analysis. Headspace solid phase microextraction (HS–SPME) with fibres, and sorbent tubes packed with sorbent material are two of the more prominent VOC collection equipment that have been used in environmental VOC sampling [9]. Alternatively, gauze pads and cartridges containing adsorbent material have been used to collect VOCs in some decomposition-related studies [99; 72].

The use of HS–SPME compared to sorbent tubes depends on the nature of the matrix being studied. As HS–SPME requires tissue samples to be placed in vials before collection, they are only suitable when working with small tissue samples. In contrast, sorbent tubes can be used when collecting VOCs from larger remains such as cadavers. While there are multiple types of adsorbent materials that sorbent tubes can be packed with, a dual combination of TenaxTA/Carbograph 5TD has been previously used for VOC collection from decomposing remains [94; 96; 87]. Tenax TA material is suitable for collection of C_6 – C_{30} of aromatics, semi-volatiles, nonpolar and polar compounds, while the Carbograph collects VOCs in the C_3 – C_8 range. A quartz wool plug is generally added prior to sorbent material to avoid their direct exposure to the atmosphere [100]. Figure 1.5 is a schematic representation of sorbent tubed used in the current study.

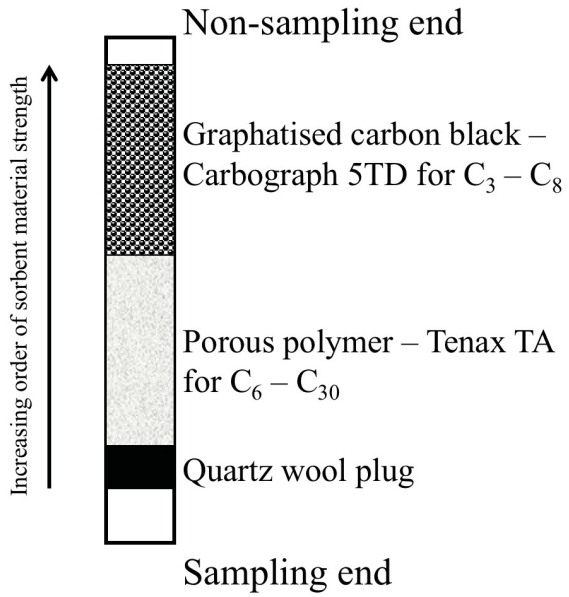


Figure 1.5: Schematic representation of the dual sorbent material (Tenax TA/Carbograph 5TD) tube used in the current study.

Once the VOCs are collected on the adsorbent material of sorbent tubes, they can be desorbed by thermal desorption, concentrating them on a cold trap, and re-desorbing them under high temperatures (~300 °C). The use of thermal desorption along with the concentration step is preferred over conventional solvent extraction methods as it reduces the possibility of contamination and loss of analytes, as well as reduces analysis time while providing a higher sensitivity [101; 79]. The desorbed VOCs are subsequently injected into the column of a gas chromatography instrument for separation and further analysis.

1.3.2 Gas chromatography

Gas chromatography (GC) is an analytical separation technique used to separate a volatile or semi-volatile mixture. As defined by World Health Organisation, volatiles are compounds that have boiling points roughly in the range of 50 to 250 °C, while semi-volatiles have boiling points roughly in the range of 250 °C to 400 °C [102]. Separation of compounds in gas chromatography occurs only in the gaseous phase however, the sample introduced can be either gaseous or liquid. Thus, the first step in separation is to vaporise the sample if it was introduced as a liquid. This step is eliminated when samples are collected on sorbent tubes and introduced by thermal desorption. The volatile compounds are carried from the injection port into the column using an inert carrier gas such as helium, argon, nitrogen, or hydrogen which comprises the mobile phase [103]. Chromatographic columns (usually 10 – 60 m) are capillary columns lined with a polymer or viscous liquid stationary phase that have low resistance to mobile phase flow and allow for fast and efficient separation [104]. In a capillary column, separation of the components in the mixture occurs on the basis of difference in migration time. The mixture interacts with the stationary phase in the column as it moves along with the carrier gas. These interactions allow components to be retained in the column. The duration of retention is different for various components depending on analyte type, stationary phase type, column temperature, type of carrier gas and flow rate of carrier gas [103]. Thus, each of the components in the mixture elute at different times from the column and are subsequently introduced to the detector. The output of GC is a graphical representation called a gas chromatogram with peaks that represent resolved compounds in a mixture.

Over the years, one-dimensional GC has evolved into comprehensive two-dimensional gas chromatography (GC×GC) schematically represented in Figure 1.6. GC×GC utilises two columns of different polarity, the primary or first dimension (^{1}D) column (usually 30 – 60 m length × 0.25 mm internal diameter) and a short secondary or second dimension (^{2}D) column (usually 0.5 – 3 m length × 0.10 – 0.18 mm internal diameter for non-MS detectors, and 0.18 – 0.25 mm internal diameter for MS detectors). Separation in each of the columns occurs independent of each other also known as orthogonal separation. A thermal modulator or flow modulator is placed between the two columns that enables controlled flow of analytes into the ^{2}D column, thus allowing enough time for analytes to separate there. Depending on the modulation period (usually between 3 – 8 s), analytes

eluting from ^{1}D are concentrated into a pulse at the modulator before being introduced into ^{2}D and being sliced further [105; 106]. The thermal modulation time and temperature is adjusted so that each pulse is sliced into three to four fractions and then separated in ^{2}D before the introduction of the following pulse in the modulator. The greatest advantage of GC×GC is that it separates peaks that would otherwise coelute in a one-dimensional gas chromatographic separation. GC×GC has better peak capacity (maximum number of theoretically possible peaks) and better separation power and peak resolution [107; 108].

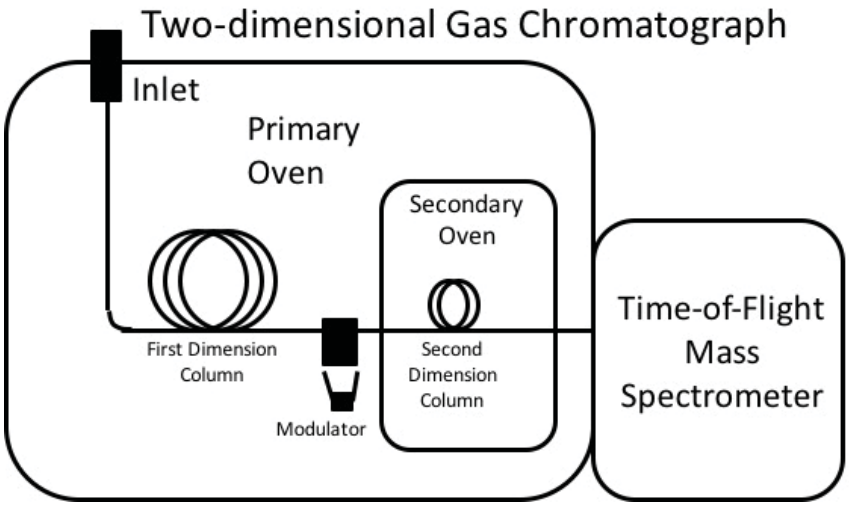


Figure 1.6: Schematic representation of GC×GC system (adapted from [94]).

1.3.3 Mass spectrometry

Mass spectrometry (MS) is a sensitive and specific technique used to identify the components of a mixture qualitatively and/or quantitatively. A typical mass spectrometer works by carrying out eluate introduction, ionisation and ion detection. The eluate from the chromatograph chamber is introduced into the ionisation chamber in the mass spectrometer via a heated transfer line. The molecules entering the ionising chamber are bombarded with high energy (~ 70 eV) electrons. This converts the molecular species into ionic species. During this procedure, owing to the excessive internal energy, molecules break down into fragments. Fragmentation is molecule specific and gives rise to a mass spectrum (relative amount vs. mass/charge ratio) of that molecule. The mass analyser in the mass spectrometer estimates the mass/charge ratio (m/z) for the identification of unknown compounds [109]. As an example, the mass spectra of bromobenzene has been represented in Figure 1.7 showing each of the fragments of bromobenzene with their m/z ratios on the X-axis and relative amount on the Y-axis.

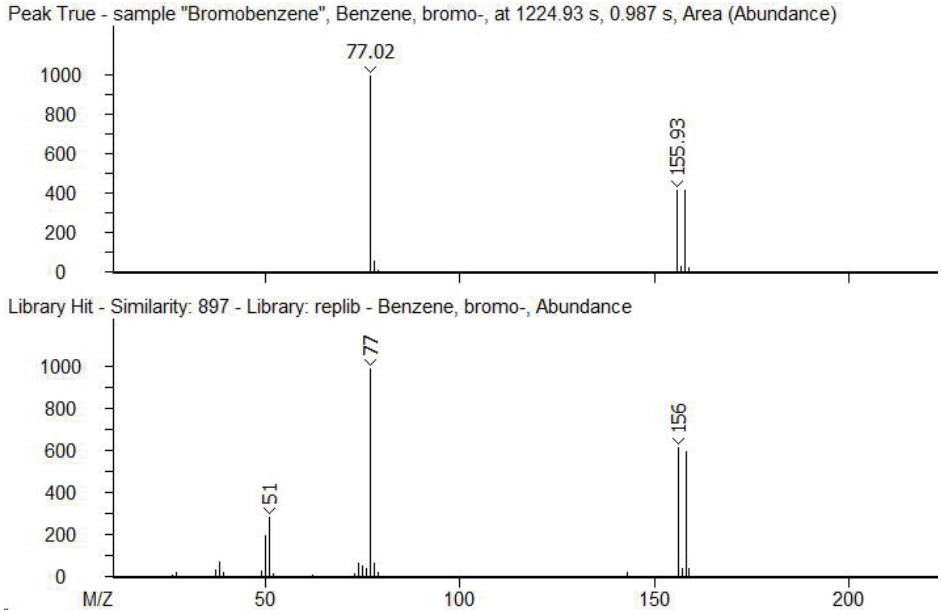


Figure 1.7: Mass spectra of Bromobenzene.

A non-conventional mass spectrometer, a time-of-flight (TOF) schematically represented in Figure 1.8. A TOF mass spectrometer (TOFMS) works by estimating the time taken by the ion to travel from the ion source to the detector which will be proportional to its mass when parameters including kinetic energy, charge and distance travelled by each of the ions is kept the same. TOFMS is beneficial as it measures all m/z simultaneously and allows for higher acquisition rates which is essential for GC×GC as the modulator produces narrow peaks, and the detector must be efficient at scanning those peaks at an adequate rate [110].

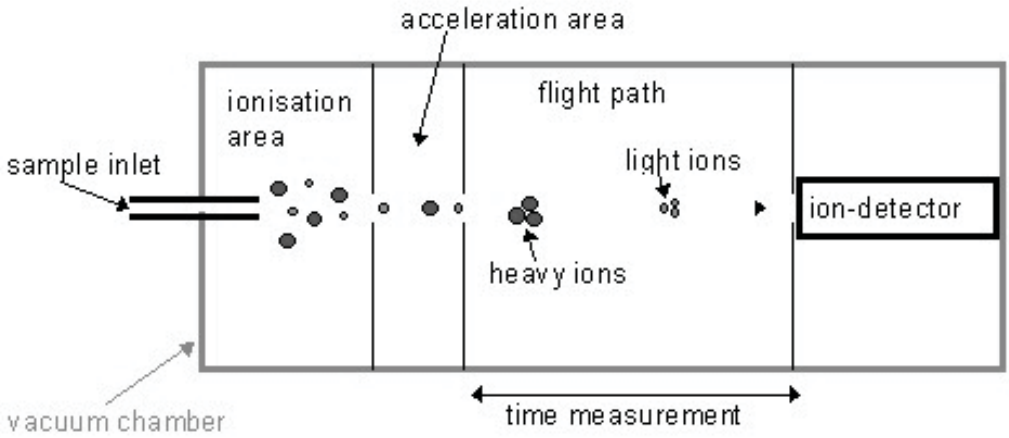


Figure 1.8: Schematic representation of Time-of-Flight Mass Spectrometer (TOFMS) (adapted from [111]).

1.3.4 Analytical output

The analytical output from any chromatograph is called a chromatogram. A chromatogram of a GC–MS analysis is in the form of a total ion chromatogram (TIC) with peaks presented on the X- and Y-axis coordinate system. The X-axis represents the retention time or the time since the start of the chromatographic analysis taken by each analyte to be detected. The Y-axis represents the intensity or sum of the masses of all fragments scanned at a particular retention time. In the case of a GC×GC–TOFMS, since the analytes are separated on two different columns, the TIC has two retention times along the X-axis plotted against the peak intensity on the Y-axis (Figure 1.9). The output of a GC×GC–TOFMS can also be viewed as a 3D surface plot or a 3D contour plot (top view of a 3D surface plot) (Figure 1.10). As shown in Figure 1.10, the first dimension (primary column) retention time is along the X-axis, the second dimension (secondary column) retention time is along the Y-axis, and peak intensity is along the Z-axis. The coloured intensity scale represents the peak height which indicates the analyte concentration.

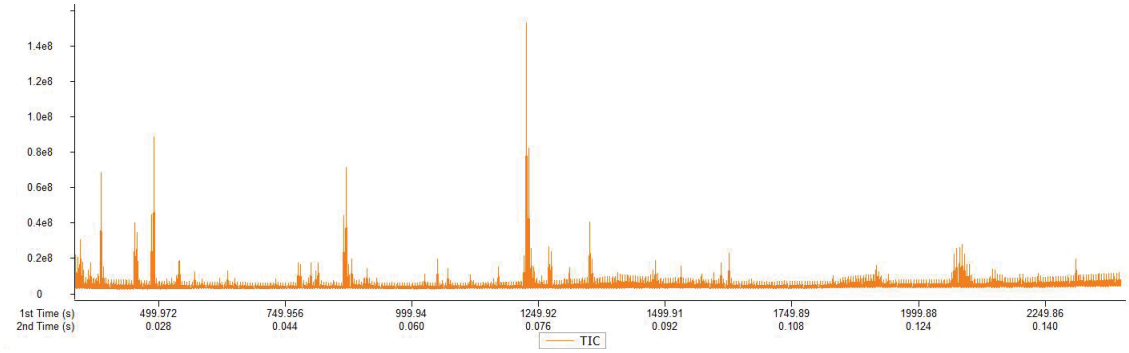


Figure 1.9: Total ion chromatogram (TIC) of a GC×GC–TOFMS analysis.

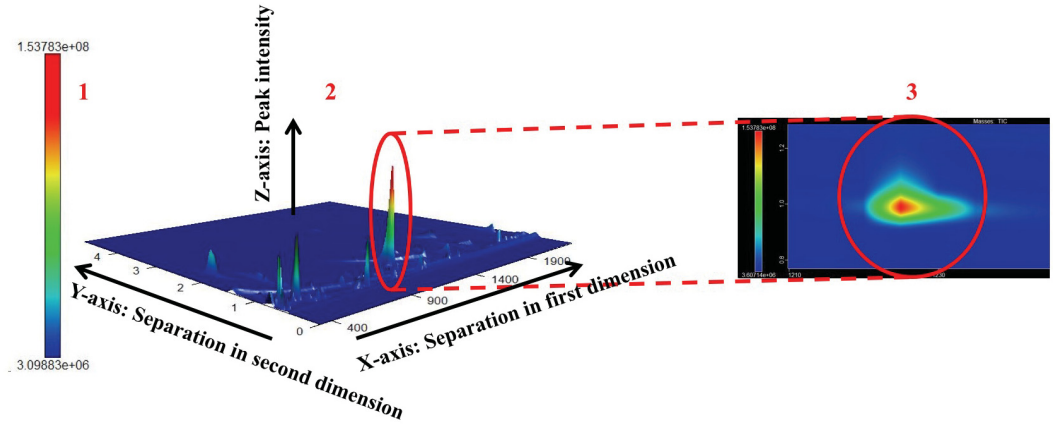


Figure 1.10: Analytical output of GC×GC–TOFMS showing 1) the colour intensity scale for peaks 2) 3D surface plot with peaks 3) magnified 3D contour plot of a peak.

At the end of the analysis, the compounds significant to decomposition are identified. With the current knowledge, it can not be said if these are the compounds that CDDs identify as decomposition-related [112]. Nevertheless, if these compounds are found similar across multiple decomposition studies in varying environments, it can be concluded that the dogs are constantly exposed to them in a decomposition event thus, making them compounds of interest for further research. The following section elaborates on decomposition odour detection by dogs.

1.4 Biological detection of decomposition odour

The term 'biological detectors' is used to describe plants and animals that can be modified biologically or behaviourally to indicate the presence of a 'target odour' [113; 114]. Over thousands of years, humans have been able to fully domesticate dogs, and both humans and dogs have adapted in a way to be able to communicate with each other through signalling [115; 116]. Additionally, dogs can detect and even differentiate between very similar odours making them good biological detectors. Their exceptional capability has aided police work in tracking humans, search and rescue, detection of drugs, currency, explosives and human remains [117]. A well-trained dog can cover larger and difficult terrain search areas in a much shorter time than humans on foot [93]. Dogs are fast, efficient, reliable, specific, sensitive, compact and relatively inexpensive which makes them a preferred detection tool over other biological or analytical tools [114].

1.4.1 Olfaction in dogs

Much like how humans perceive any smell, dogs respond to any odour via the process of chemoreception where the interaction of chemical compounds (odour molecules) and receptors produces an electrical signal that can be interpreted by their brain [118]. Even with this basic similarity in how humans and dogs identify the smell, dogs' olfaction or sense of smell is reported to be about 10,000 to 100,000 times more sensitive than humans [119]. As the air is inhaled, the warm and humidified environment of the nostrils and respiratory epithelium moistens it for increased efficiency of gaseous exchange in the lungs [120]. Unlike humans, the anatomy of a dog's nose is designed to split the inhaled air into two separate pathways for smelling (olfaction) and breathing (respiration) (Figure 1.11). While most of the air enters the lungs for breathing, some part enters a specialised chamber called the olfactory recess which is located at the rear of the nose below the eyes [121; 122]. The olfactory recess is lined with epithelial cells with numerous neurons. Each of these cells has finger-like projections called cilia that have odour receptors. The odour receptors are specific and act as binding sites for their respective odour molecules. Electrical signals generated from the receptor-chemical reaction are transmitted to the olfactory bulb [121; 122]. The signal is then sent to the olfactory cortex of the brain where it is interpreted in combination with signals received from all the individual odour receptors to identify a particular odour source. A unique combination of receptors is activated in a dog's brain depending on the odour source [121; 122]. Sniffing of odour molecules is made more efficient through the use of side slits when exhaling air as opposed to the regular nostrils which are used only for inhalation. The air is exhaled outward and downward which restricts the mixing of exhaled and fresh air meant to be inhaled (Figure 1.12) [122].

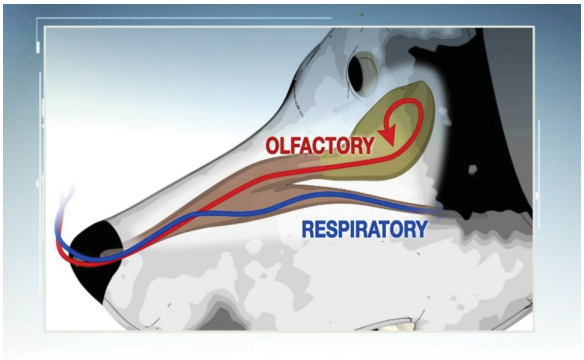


Figure 1.11: Smelling (olfaction) and breathing (respiration) pathways in dogs (adapted from [123]).

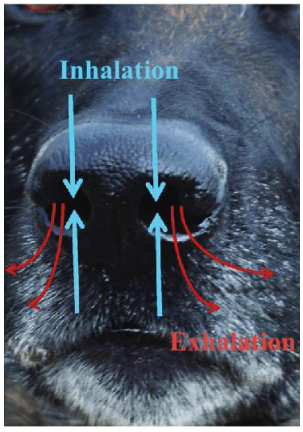


Figure 1.12: Inhalation and exhalation pattern in dogs (adapted and modified from [119]).

Dogs also have an accessory olfactory organ or the vomeronasal gland located at the roof of their mouth. This organ plays a key role in detecting pheromones for sexual activity [124]. It is suggested that sometimes, these organs may also aid in odour detection especially when the odour source is in the water. Dogs have been observed to take 'licks' of water which is slightly different to the manner in which they drink water. This form of 'licking' potentially lets some of the odour molecules into the vomeronasal organ [122].

1.4.2 Specialised detection dogs

Largely, all dogs have a well developed olfactory system compared to humans with some variations in odour detection capability among various dog breeds [125; 126]. However, this olfactory capability of dogs' is not helpful to humans unless the odour is 'learned' (behaviour developed with experience as a result of repeated exposure to the same situation, contrary to innate behaviour). This requires continuous exposure and recognition to the target odour and can be achieved through constant training. Training allows the dogs to detect, perceive, discriminate and identify the target odour. Dogs that have undergone rigorous training and those that are deemed capable of locating a target odour are known as specialised detection dogs [127]. Based on the nature of the target odour, specialised dogs have been trained to locate drugs, explosives, currency, contrabands, accelerants, live human scent, human remains and even some diseases such as cancer, parkinson's etc. [128-130]. Depending on the number of target odours a dog is trained on, they could be referred to as single profile (trained on only one target odour) or cross trained (trained on more than one target odour). A study comparing the performance difference among single profile and cross trained dogs did not report any

significant impact on dog's performance based on the number of odour profiles they were trained to identify [131]. Nevertheless, few other factors could impact dogs' performance such as handler skills, dog-handler relationship, training protocols, training material used, and dogs' physical and behavioural attributes. Enhanced performance and reliable true responses can be attained through appropriate selection and training of detection dogs [113]. A well trained detection dog can be deployed for quick field screening in disaster scenarios, at border security control, or during public searches. In a forensic context, detection dog searches in any scenario is treated as a preliminary search before the results are analytically confirmed. One such detection dog used regularly by law enforcement officials is a cadaver detection dog (CDD). CDDs are trained to locate decomposition odour from cadavers, human remains or objects that are associated with a decomposition scenario [112]. CDDs are sometimes referred to as human remains detection (HRDs) dogs or victim recovery (VR) dogs in some parts of the world. Their ability to indicate and respond to the presence of decomposition odour in a scenario makes CDDs also known as biological detectors of decomposition odour.

1.4.3 Cadaver detection dogs

Working dogs are chosen based on their physical and behavioural traits to fulfil requirements relating to the nature of their work and the geography of the location [132; 133]. Generally, for HRD work, attributes such as body build and size, heat tolerance to long searches, sex, good stamina and ability to adapt to change are considered fitting [93; 134]. A dog that can work independently while cooperating with its handler, one that is highly motivated, and has an adequate 'play-drive' (willingness to indulge in play) and desire to attain a reward should be selected [113]. Once a suitable dog has been identified, it undergoes substantial training to be qualified in detecting decomposition odour. During field searches, CDDs can be expected to locate victims ranging diversely in their stage of decomposition. The individual could have died a few hours ago and may be considered 'fresh' or could be human remains dating several hundred years. The fresh versus the skeletonised or mummified remains can have varying odour profiles and since CDDs are essentially identifying volatile compounds present in the decomposition odour, they must be trained on diverse decomposition odour profiles [112]. Hence, the primary method of training a CDD is to expose the dog to the wide range of material with decomposition odour, which is dynamic in nature through various decomposition stages [112].

1.4.4 Cadaver detection dog training aids

There is a wide range of training aids available for use, typically they can be synthetic or natural depending on their source of origin. The type of training aid that will be adopted for training purposes is largely dependent on the dog handler or the organisation's ability to ethically procure and store human remains training aids [93]. Synthetic training aids in the form of chemical formulations are easy to acquire, but it has been reported that they are over-simplified and not comparable to the human decomposition odour [112]. Natural training aids such as human blood, human tissue, soil or textile associated with decomposing human remains, and decomposition fluid are the most desirable [112]. Generally, human remains sources including human tissue and human decomposition fluid are preferred over animal tissue as dogs are reported to be able to differentiate between the odour from both of these sources, however, it can be challenging to ethically acquire human tissues [135; 68; 136; 112]. It is found that soil from sites of decomposition might not be able to retain all decomposition-related compounds over time and thus, it must be used with caution [112]. As for blood, it is best suited to train blood detection dogs specifically but can be used in combination with other natural training aids to train CDDs [137; 112].

The use of blood and decomposition fluid has been validated for use as CDD training aids however, this has not been done for all training aid types [136; 137]. An example of one unvalidated alternative to natural CDD training aids is voluntarily donated limbs by patients undergoing amputation surgeries. Such training aids are currently used by the Ontario Provincial Police (OPP) Canine Unit. These limbs have not been validated as a cadaver-dog training aid since their VOC profile has not been studied prior to the current research. The greatest advantage of using amputated lower limbs/feet is that they are more easily and ethically available as compared to any cadaveric material thus, their validation as an alternative could prove to be revolutionary in the field of CDD training.

1.5 Research aims and objectives

A working dog's competency is only as good as its training and thus, in recent years, there has been a constant effort to find alternative CDD training material to improve their training protocol, ultimately enhancing their search capability. While cadavers, cadaveric tissue and cadaveric decomposition fluid can be the most ideal training aid, they are not

easily available for use. To work around this problem, the OPP Canine Unit has collaborated with Queen's University, Kingston and Kingston General Hospital to obtain voluntarily donated amputated lower limbs/feet following surgery of diabetic patients. The odour profile of these limbs has not been studied before, and thus, their resemblance to the odour profile of cadavers is not understood. CDDs are vital to the justice system which expects them to be proficient and assumes that they are trained on appropriate training aids. Hence, this study will analyse the VOC profile of CDD training aids used by the OPP Canine Unit and compare them to the VOC profile of cadavers in order to validate (or otherwise) their use as CDD training aids in the future. This study will also record CDD responses on the CDD training aids and cadavers in an outdoor scenario to associate the dogs' responses to the VOCs that are evolved during decomposition. Thus, the dual approach of this study involves conducting analytical experimentation and recording observational data from biological detection via CDD responses on two different sources – CDD training aids and cadavers. The objectives of this thesis are:

1) To validate the analytical method (using previously optimised methods in [94; 96; 87] on TD–GC×GC–TOFMS for collection and analysis of VOCs in an air matrix from cadavers and CDD training aids (Chapter 2)

2) To analyse the VOC profile of various CDD training aids used by the OPP Canine Unit. The different type of training aids includes amputated lower limbs/feet, fresh blood samples deposited on gauze pads, and teeth donated by a dentist. The effect of storage conditions and ageing on VOC change over a period of time will be studied by analysing the same training aids repeatedly over a span of 18 months. This will help identify significant VOCs in CDD training aids and VOC changes that can occur because of their indoor storage conditions and handling by dog handlers (Chapter 3).

3) To analyse the VOC profile of cadavers in a taphonomic facility – *Recherche en Sciences Thanatologiques [Expérimentales et Sociales]* (REST[ES]). The decomposition process in a Québec climate will be studied from donors. This will identify VOCs significant to the human decomposition process occurring in a natural outdoor environment (Chapter 4).

4) To record observations from CDD responses to CDD training aids and cadavers during their indoor and outdoor training sessions around the same time when VOCs are collected from these samples. This will help understand any change in their response that can occur if the decomposition odour perceived by them changes over time in the case of CDD training aids, and compare their responses to cadavers with that of training aids (Chapter 5).

5) To compare the VOC profile of CDD training aids used by the OPP Canine Unit to the VOC profile of cadavers. Additionally, to view CDD observations in context to VOC profile results. This will help validate or otherwise, the use of these training aids, particularly amputated lower limbs/feet for training CDD. (Chapter 6).

The ideology behind conducting the current study is to have dog observations carried out in sync with a chemical analysis thus, enabling the detection of decomposition-related VOCs which could be of interest to the dogs. The primary focus is on amputated lower limbs/feet which continue to decompose as they are necrotic tissue, and the differences or similarities in their decomposition in comparison to cadaveric decomposition processes. It is hypothesised that since the type of microbes in necrotic tissue is different from gut microflora, there could be differences in the VOCs evolved during decomposition. This research will aim to establish and explain if these differences were significant or insignificant enough to disregard or otherwise accept the use of amputated lower limbs/feet. Any variance in VOC profiles will be established by determining a list of VOCs detected in the CDD training aids and cadavers. If the variance is found to be insignificant, the use of amputated lower limbs/feet for CDD training purposes can be validated.

Chapter 2: ANALYTICAL METHOD VALIDATION

Chapter 2: ANALYTICAL METHOD VALIDATION

2.1 Introduction

Much like the classic 1D GC method building process, GC×GC method development requires a comparative assessment of chromatograms that are altered with any change in the analytical method. However, developing a suitable method for GC×GC separation is slightly more complex than regular 1D GC separation due to the additional column contribution in ^{2}D. Generally, method development is trial and error based, at the end of which, parameters that allow for improved peak resolution, peak separation, and peak shape are identified and adopted [138]. Developing a new method on material with no previously established analytical procedure is a multistep process that involves selecting chromatographic conditions, developing an analytical approach, sample preparation, method optimisation, and method validation [139]. These steps rely on several factors such as the physicochemical properties of the sample, nature of the sample, location of sample collection, etc. For instance, the method for VOC collection from human tissue in a vial varies greatly from that of cadavers in an outdoor environment [140]. With analytical optimisation, multiple factors govern the separation efficiency during chromatography. These include column diameter, column film thickness, column length, carrier gas nature, carrier gas flow rate, inlet pressure, column phase combination for the primary and secondary column, column temperature, stationary phase, modulator type, modulation period, and acquisition rate [138; 139]. A decade old study emphasised the fact that all these factors have a complex interplay as they are interrelated, and changing one or multiple of these factors has a direct impact on elution temperatures, peak width, and separation resolution, ultimately impacting the efficiency of the chromatographic method [141]. Thus, the analytical parameters must be considered carefully during method development.

Addressing every parameter during the method development process for sample collection and analysis is time-consuming and laborious. However, when previously established and tested methods exist, multiple steps can be incorporated as-is and thus, the task can be reduced to the last method optimisation or validation step. For the current study, since decomposition odour had been previously studied, analytical parameters

were adopted from previous studies conducted in Australia using GC×GC to analyse decomposition VOCs [94; 142; 96; 87]. These studies were chosen as reference studies primarily because they were based on cadaver decomposition in an outdoor scenario which aligned with the major objective of the current study. Additionally, they provided a detailed account of suitable parameters for sample analysis on an instrument configuration similar to the one used in the current study. Nonetheless, some method validation still needed to be conducted as reference studies and the current study varied in the nature of samples being analysed. The samples in the previous studies were either cadavers or whole pig remains however, the current study was conducted on cadavers and human remains used as cadaver dog training aids. Thus, this additional factor of variability in the size of cadavers and human remains needed to be addressed during sample collection. Moreover, for sample analysis, the reference studies used a Markes Unity 2 Thermal Desorber (Markes International Ltd., Llantrisant, UK) coupled with a LECO Pegasus® 4D TOFMS LECO (LECO Corporation, Castle Hill, NSW, Australia). A similar (but not the same) instrument – Markes TD 100-xr (Markes International Ltd.) coupled with a Pegasus® BT 4D TOFMS (LECO Corporation, Mississauga, Ontario, Canada) was available for the current study. The most significant difference between the two instruments was the BenchTOF (BT) configuration which has a higher acquisition rate and sensitivity. Some of the other differences included a 50 ˚C higher ion source temperature and a thinner film of the secondary column compared to the older version of the instrument used in Australia. With the baseline information on all the analytical parameters from previous studies and instrument configurations, steps were taken to validate and adopt or suitably modify them for the current research. This chapter discusses the process of method validation adopted in this study.

2.2 Methods

2.2.1 Sample collection and introduction

In general, the collection of VOCs from the air matrix is a two-step process: accumulation of air in the headspace above the remains and collection on a sorbent material. During optimisation of this step, three major parameters were considered: the size of the samples (which impacted the method used for accumulating air to generate a headspace above the

sample); the duration during which air was allowed to accumulate (accumulation period); and the volume and the rate at which the air sample must be collected.

Following this step, the VOCs from the sorbent material were extracted and introduced into the analytical system for further analysis. This was achieved by heating the sorbent material to thermally desorb the VOCs. Much like sample collection parameters, there are several thermal desorption parameters (such as the volume sample injected) that govern the outcome of an analysis. Sample collection and thermal desorption (sample introduction) aspects that were adopted for the current study are further discussed below.

2.2.1.1 Sorbent tubes

Two sorbent materials Tenax TA and Carbograph 5TD embedded within stainless steel tubes (Markes International Ltd.) were used for the VOC collection. These tubes were stored using brass caps which were replaced with diffLock (diffusion-locking) caps when they were introduced into the system for analysis (Figure 2.1).

Figure 2.1: Stainless steel sorbent tubes sealed with brass caps (bottom) and diffLock caps (top).

2.2.1.2 Collection pumps

Two commercially available air sample collection pumps were tested, these included: a manual hand-held air sampling pump EASY-VOC (Markes International Ltd.) (Figure 2.2) and a battery-operated low flow air sampling pump ACTI-VOC (Markes International Ltd.) (Figure 2.3). When using EASY-VOC to sample, five consecutive air sample draws of 100 ml each were collected. The ACTI-VOC can operate at constant pressure and constant flow mode of which, a constant flow of 100 mL/min was used as it was the choice of flow rate in the reference studies [94; 96; 87]. The ACTI-VOC pump was calibrated at the required constant flow as per the instructions in the user manual provided by the manufacturer.

Figure 2.2: Hand-held EASY-VOC air sampling pump.

Figure 2.3: ACTI-VOC pump with flat bladed screwdriver used to operate the pump.

2.2.1.3 Air accumulation and collection volume

Prior to drawing the air onto the sorbent tubes, the air was accumulated by placing an aluminium hood over the decomposing remains. This process allowed for VOCs of interest to achieve a sufficient vapour pressure and be present in the air matrix for eventual collection onto the sorbent tubes. For accumulating air above cadavers, an aluminium hood was placed over the cadaver for 15 min. This protocol was adopted from previous studies for sampling cadavers using an aluminium hood with dimensions 190 cm × 120 cm × 70 cm (Figure 2.4). For the part of the study involving CDD training aids, the protocol was slightly modified and an aluminium hood of dimensions 38 cm × 38 cm × 38 cm (Figure 2.5) was placed over the training aids for 10 min during which, the air was

accumulated above the remains. At the end of the accumulation step, 500 mL of air sample was collected.

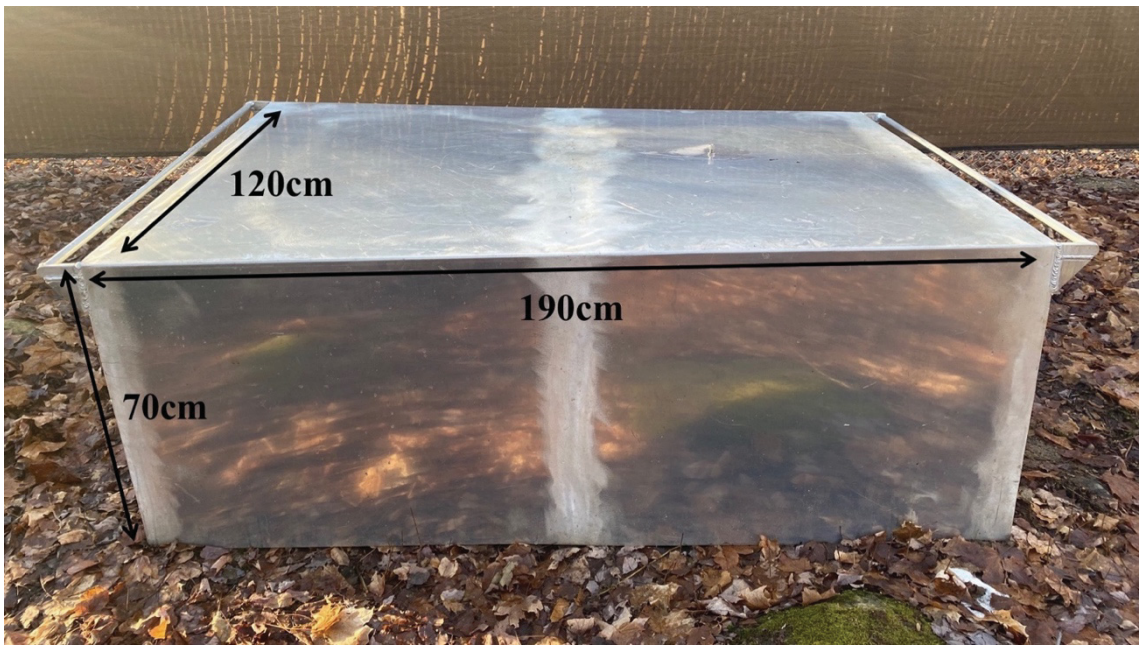


Figure 2.4: Aluminium hood used for accumulating air in the headspace above cadavers.

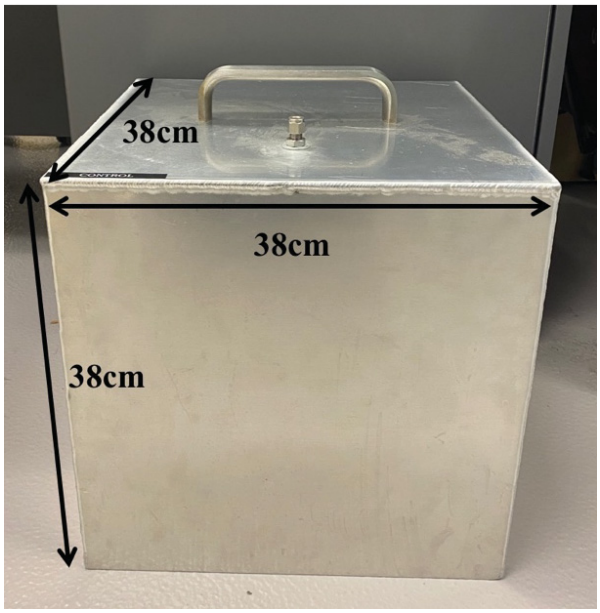


Figure 2.5: Aluminium hood used for accumulating air in the headspace above cadaver detection dog training aids.

2.2.1.4 Sample collection method

Each sample collection tube is uniquely identified with a number (Figure 2.6.3) and at the time of sample collection, this was noted to identify the sample. The aluminium hood of suitable size was placed over the cadavers or CDD training aids for a suitable period as

indicated in section 2.2.1.3 (Figure 2.6). The tubes also have an arrow (Figure 2.6.4) which must point in the direction of air sample collection (arrow pointing upwards or towards the non-sampling end of the tube when the tube is attached to the aluminium hood). For sample collection, the brass caps were removed, the sampling end of the tube was attached to a sampling port (Swagelok Company, Ohio, US) (Figure 2.6.1) on the aluminium hood, and the non-sampling end was attached to the ACTI-VOC pump (Figure 2.6.2). The air sample was drawn for 5 min at the rate of 100 mL/min to collect the desired 500 mL of sample. All tubes were sealed with brass storage caps after collection, wrapped in aluminium foil and placed in sealed mason jars before being transported to the laboratory. The sorbent tubes were stored at 4 ˚C until the sample analysis was performed. The brass caps were replaced with diffLok caps before they were placed in the thermal desorption unit. These caps allow the trapped VOCs to desorb for introduction into the system for chromatographic analysis. All the samples were collected as triplicates and control samples were collected to account for background VOCs and to identify any contamination or artifacts that could have arisen during transport and storage. The hoods were wiped down with acetone (ACS grade, Thermo Fisher Scientific, Waltham, MA, USA) between VOC collection from two cadavers or CDD training aids.

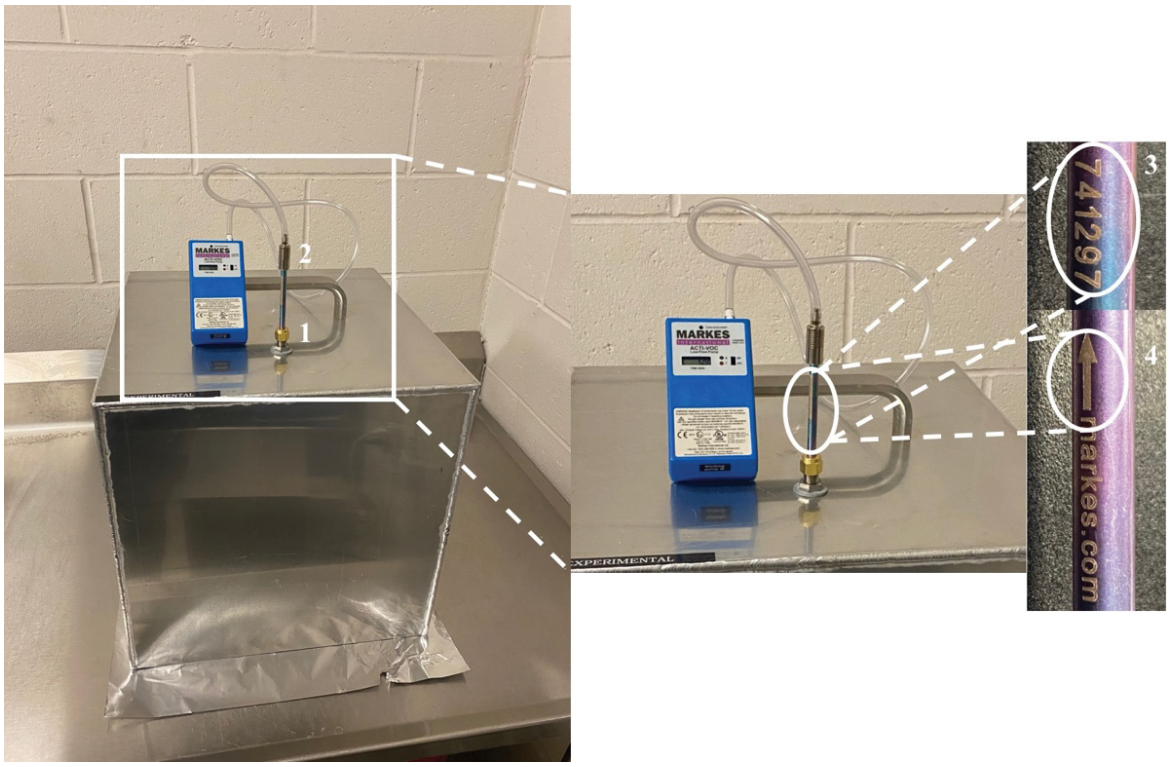


Figure 2.6: Sample collection tube attached to the aluminium hood at its 1) sampling end; and to an ACTI-VOC pump at its 2) non-sampling end. 3) The unique

identification number of the tube. 4) Arrow pointing in the direction of air sample collection and pointing towards the non-sampling end of the tube.

2.2.1.5 Sample introduction by thermal desorption

The thermal desorption unit used in the current study was a Markes TD 100-xr multi-tube autosampler (Markes International Ltd.). The parameters adopted for the current study were derived from reference studies. Each sorbent tube was heated to 300 ˚C for 4 min to allow thermal desorption of the compounds before being collected onto a general-purpose cold trap at -10 ˚C. The trap was desorbed at 300 ˚C for 3 min at a desorption flow rate of 20 mL/min. The reference studies desorbed the cold trap with no split in the samples. The split ratio used can have an impact on chromatographic separation thus, during optimisation of the thermal desorption method, a split ratio of 10:1 and no split were analysed. These split ratios were achieved by adjusting the flow rates through the trap and the split vent. A split ratio of 10:1 on the trap implied that only one part of ten parts of the entire VOC sample from the trap was injected for chromatographic analysis. In the current study, the remaining part of the un-injected sample was recollected on the same tube to save the sample in the event of instrument errors or malfunction.

2.2.2 Analytical analysis

2.2.2.1 Column choice

The reference studies reported using a 30 m × 0.250 mm inner diameter (ID) × 1.40 µm film thickness Rxi®-624Sil MS column (Restek Corporation) as the ^{1}D column, and a 2 m × 0.250 mm ID × 0.5 µm film thickness Stabilwax® column (Restek Corporation) as the ^{2}D column. When obtaining columns for the current instrument, the manufacturers supplied the desired columns as the reference studies with the same column configurations except for the film thickness of the secondary Stabilwax® column (Restek Corporation). Due to the manufacturer's availability, the column provided had a film thickness of 0.25 µm instead of 0.5 µm and thus, the secondary column had reduced film thickness compared to those used in the reference studies.

2.2.2.2 Carrier gas pressure

Helium has been the choice of carrier gas over hydrogen for GC×GC–TOFMS analysis thus, it was used in the current study [107]. The current instrument operated on constant

pressure mode. During optimisation for the constant inlet carrier gas pressure, the analysis was conducted at two different inlet pressures: 7.8 psi (~1mL/min at 35 ˚C) and 17.8 psi (~2 mL/min at 35 ˚C). A Restek EZMC® method translator and flow calculator software (Restek Corporation) was used to calculate the inlet pressure and its associated flow rate based on the column configurations and initial column temperature.

2.2.2.3 Temperature program

The GC oven program is an extremely important parameter for any chromatographic separation [138]. The temperature program for the current study was adopted from the reference study as it allowed adequate separation in the ^{1}D and ^{2}D. The initial temperature of the ^{1}D oven was set to 35 ˚C and held at this temperature for 5 min before increasing at a rate of 5 ˚C/min to 240 ˚C where it was held for a further 5 min. The offset for the modulator was 5 ˚C and the offset for the ^{2}D column was 15 ˚C. In the current study, the maximum temperature was set to 230 ˚C instead of 240 ˚C to avoid exceeding the maximum temperature of the current column set. The temperature program also allows for the secondary oven (temperature offset relative to the primary GC oven) and the modulator temperature (temperature offset relative to the secondary oven) settings. The offset for the modulator was set to 5 ˚C and the offset for the ^{2}D column was 15 ˚C as recommended by the LECO Pegasus BT ChromaTOF software (LECO Corporation).

2.2.2.4 Modulation

Similar to the reference studies, a thermal modulation system with liquid nitrogen coolant was used in the current study. The two parameters that impact a chromatographic separation are the duration of modulation and the pulse time of hot and cold jets. The modulation period can generally range from 3 - 8 seconds. The reference studies used a modulation period of 5 s with a 1 s hot pulse and 1.5 s cold pulse time. During optimisation of the modulation period, 5 s and 8 s durations were tested.

2.2.2.5 Detector parameters

A TOFMS detector was used with the ion source held at 250 ˚C which was 50 ˚C higher than that used in the reference studies as 250 ˚C was deemed optimum for the current instrument by the manufacturers. A target mass acquisition range of 29 – 450 amu was considered optimum for environmental decomposition VOCs and the electron ionisation

energy at 70 eV was adopted in the current study. The acquisition rate used previously was 100 spectra/s which was considered insufficient for the current instrument and the manufacturer suggested an acquisition rate of 180 – 230 spectra/s. Thus, 200 spectra/s was tested and adopted.

2.2.3 Standards

2.2.3.1 Internal Standard

An internal standard consisting of 0.2µL of 50 ppm bromobenzene (GC grade, Sigma-Aldrich, St. Louis, MO, USA) in methanol (HPLC Grade, Sigma-Aldrich) was injected using an eVol® XR handheld automated analytical syringe (SGE Analytical Science, Weatherill Park BC, NSW, Australia) onto each sorbent tube before analysis.

2.2.3.2 Decomposition Standards

0.2 µL of 10 ppm concentration of three standard mixes (Restek Corporation) was injected into the sorbent tubes and analysed. These standards allowed for retention time comparisons between standard compounds and those present in the unknown samples. The samples were injected once every six months to observe any shift in retention times. The compounds present in each of the three standard mixes and their retention times have been summarised in Table 2.1 Over the course of the study, the major peaks of all the standards appeared within ± 5 s in ^{1}D and ± 0.25 s in ^{2}D from the retention values listed below.

Table 2.1: Standard compounds analysed and their retention times using the current method and instrumentation.

Standards	**Retention time (^{1}D and ^{2}D) (s)**
Standard mix 1	
Ethyl Acetate	389.979, 0.712
Methyl acetate	229.989, 0.623
3-Methyl-2-butanone	534.97, 0.726
Methyl Isobutyl Ketone	759.955, 0.725
2-Pentanone	614.964, 0.769
Butyrolactone	1344.92, 1.685

2-Heptanone	1144.93, 0.671
Standard mix 2	
1-Butanol	589.966, 0.992
2-Butanol	414.977, 1.029
1-Pentanol	864.948, 1.109
3-Pentanol	669.961, 0.958
2-Methylfuran	334.982, 0.697
2-Pentylfuran	1324.92, 0.733
Indole	2149.87, 2.675
Dimethyl disulphide	724.957, 0.718
Dimethyl trisulphide (DMTS)	1319.92, 0.963
Dimethyl sulphide	199.991, 0.552
Standard mix 8	
3-Methyl butanal	514.971, 0.632
Pentanal	629.964, 0.753
Hexanal	909.946, 0.750
Octanal	1404.91, 0.764
2-Octenal	1559.9, 0.850
Nonanal	1624.9, 0.773
Decanal	1829.89, 0.783
Heptanal	1164.93, 0.661

2.2.4 Quality Control

A system was incorporated during the analysis to ensure acceptable analytical results and ensure the proper functioning of the instruments. These included conducting the repeated instrumental check, cleaning or reconditioning the sorbent tubes prior to re-use, running blank sorbent tubes, and collecting controls for each of the samples. This has been elaborated in the following mentioned points:

- The constant flow rate of the ACTI-VOC pump set to 100ml/min was checked once a month using a flow cytometer.

- Daily checks to identify any leaks in the system were conducted as per the LECO protocols of a Pegasus® BT 4D GC×GC-TOFMS (LECO, Mississauga, Ontario, Canada) instrument.

- The thermal modulation cold jets were defrosted at the end of running the analysis for the day. LECO recommends defrosting the jets at the end of a 24 hour run time and in the current study, the jets were defrosted overnight and checked prior to starting the analysis the following day.

- The sample collection sorbent tubes were cleaned or reconditioned following analysis and stored for re-use. The reconditioning was carried out at 330 ˚C for 30 mins. Random batch testing was performed and recorded as a blank run. For tubes with direct injections of standards, reconditioning was carried out for 60 mins to ensure the removal of analytes. Tubes stored without use for 3 months or longer were reconditioned again prior to use.

- If the blank tube runs had more analyte peaks than a usual blank run, the trap was cleaned, if needed, the column was conditioned and then another blank was run.

- Controls were collected and analysed for each of the samples which corresponded to either storage containers in case of CDD training aids or environmental background odour in case of cadavers in the outdoor study.

2.2.5 Data analysis

ChromaTOF® (version 5.51.6.0; LECO) was used for data processing with a 150 signal-to-noise (S/N) ratio. The National Institute of Standards and Technology (NIST) Mass Spectral Library was used to establish a list of compounds with a mass spectral match threshold of 70%. Further statistical analysis was conducted by previously written custom R programming scripts which can be accessed using the link: https://github.com/wesleyburr/GCxGC_Amputated_Limbs/. These were used to merge samples and analyse them in accordance with the control samples which were suitably edited for the current sample sets [143]. Figure 2.7 is a graphical explanation of the steps These scripts along with the data repository are available on GitHub and can be accessed using the link: https://github.com/RushaliDargan/Decomposition.

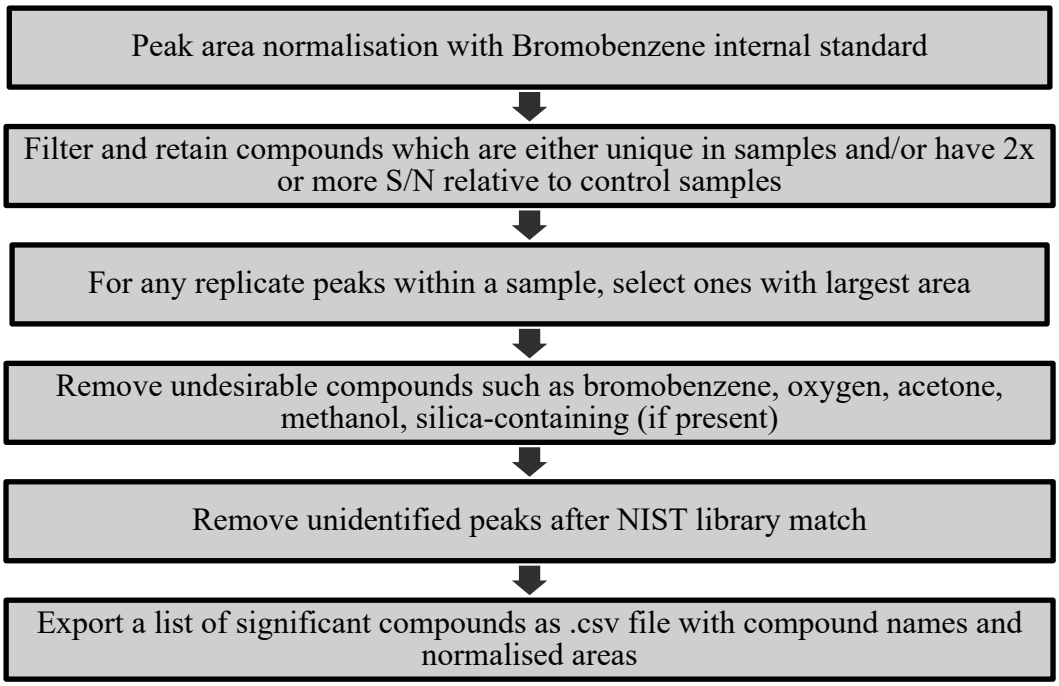


Figure 2.7: Flowchart representing steps involved in data analysis with custom R scripts.

The Principal component analysis (PCA) was carried out for data visualisation using the Unscrambler (Version 11 CAMO software) for VOCs identified as significant in the samples after the data analysis step.

2.3 Results and discussion

2.3.1 Sample collection and introduction

The sorbent material used to trap VOCs can be used as a solid phase microextraction (SPME) fibre or packed within tubes. The stainless steel sorbent tubes were preferred over SPME fibre in this study as the thin, delicate and fragile nature of the fibre makes it inappropriate for use in an outdoor environment [144]. The preferred dual sorbent material packed in stainless steel tubes was Tenax TA/Carbograph 5TD as Tenax TA material is suitable for the collection of C_6 – C_{30} of aromatics, semi-volatiles, nonpolar and polar compounds, while Carbograph 5TD collects VOCs in the C_3 – C_8 range and the use of this material has been recommended for decomposition VOCs [94].

For extracting the analytes trapped inside the sorbent tubes, the thermal desorption method is recommended over solvent extraction as it increases sensitivity and efficiency, improves reproducibility, and avoids interference from the solvent which could cause

contamination and loss of analytes [145]. Thus, the thermal desorption method was used for the current study.

The use of pumps for sample collection is an active air sampling method. As an alternative to using pumps, the sorbent tubes can be used for passive air sampling where the VOCs from the external environment defuse onto the sorbent material in the tube. Passive air sampling was not conducted as the uptake of analytes solely depends on their diffusion rate, thus, heavier and low volatility analytes might not be collected as effectively [146]. Additionally, passive sampling takes longer than active sampling. During the method optimisation phase of this study, the air was drawn from above the jars used to store CDD training aid samples using an EASY-VOC (without the air sample accumulation step) and using an ACTI-VOC from the headspace above the training aid samples (with the sample accumulation step via an aluminium hood). The EASY-VOC samples were injected in splitless mode while the ACTI-VOC samples were injected with a 10:1 split ratio at the cold trap. The chromatographic output of decomposition VOCs collected from the air matrix using EASY-VOC (without sample accumulation and injection in splitless mode) versus ACTI-VOC (with sample accumulation and injection in 10:1 split mode) is shown in Figures 2.8 and 2.9.

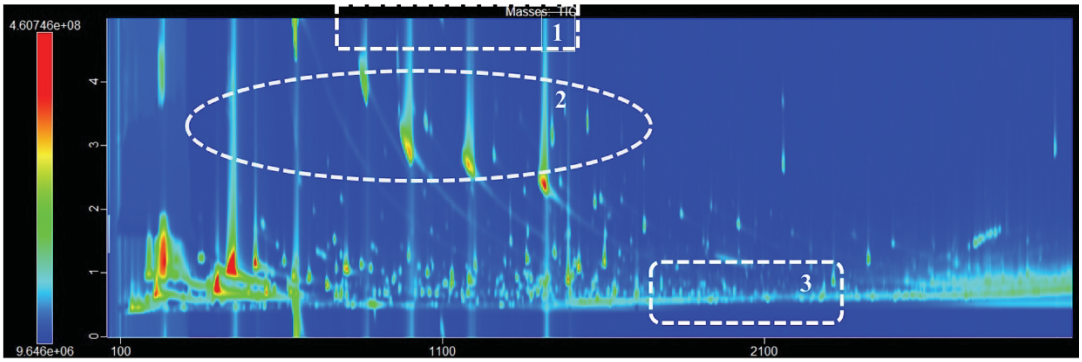


Figure 2.8: 2D chromatogram of decomposition VOCs collected from air matrix using EASY-VOC (without air sample accumulation step; splitless injection) showing 1) wraparound, 2) banding and peak broadening, and 3) poor separation in 1D.

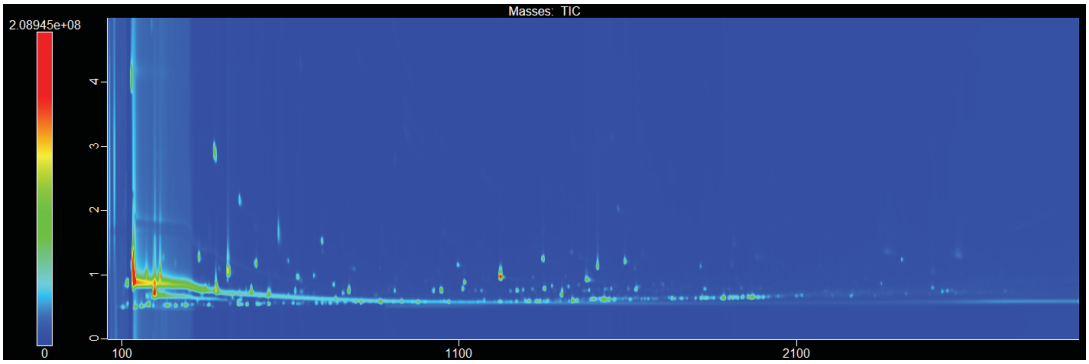


Figure 2.9: 2D chromatogram of decomposition VOCs collected from air matrix using ACTI- VOC (with air sample accumulation step; 10:1 split ratio injection) showing reduced wraparound and tailing, and improved 1D separation.

As depicted in Figures 2.8 and 2.9, for the same human remains sample when all the other analytical parameters remained the same, chromatographic separation improved when decomposition VOCs from the air were collected using an ACTI-VOC and injection with a split mode as compared to using EASY-VOC without the accumulation step and in splitless injection mode. Figure 2.8 shows several chromatographic separation issues such as wrap-around (when retention time of peaks in ^{2}D exceeds the modulation period), peak broadening and banding. Wrap around is not desirable as it can interfere by coeluting with compounds that are introduced in the ^{2}D in the following modulation period [147]. Similarly, peak broadening is not a desirable chromatographic feature as it adversely impacts the peak resolution [148]. In this sample, these chromatographic faults resulted due to overload or high injection volume of the sample causing poor separation during analysis. This was predominantly because the samples were overly concentrated [149] when the air was collected directly from the storage jars and the entire sample was injected for chromatographic separation in the splitless mode which concentrates the columns and reduces the separation resolution. These issues were resolved when a hood was placed above the remains and the air was allowed to homogenise during the air sample accumulation step and injection was made in split mode – with a 10:1 split ratio at the cold trap. Additionally, when comparing the EASY-VOC and ACTI-VOC sample collection pumps, the manufacturers determined that the performance and compound reproducibility of EASY-VOC had < 5% relative standard deviation compared to the ACTI-VOC flow pump [150]. However, one drawback of using EASY-VOC is that the rate at which the air sample is collected is not consistent since it relies on the skill of the experimenter [151]. Thus, the ACTI-VOC pump along with a sample accumulation step and sample injection at a 10:1 ratio was a preferred technique for the current study.

A 15 min time period for accumulating air in the headspace of cadavers was used in the reference studies and thus was adopted in the current research. The reduced accumulation time of 10 min was chosen for CDD training aids because of the size difference of the aluminium hoods used for these sample types. The hood used for the training aids was much smaller than that used for cadavers and thus it required less time for the air in its headspace to homogenise. Additionally, some of the training aids had an intense odour and longer periods of VOC accumulation had the potential to over concentrate the sorbent tubes during the VOC collection step.

A sample collection volume of 500 mL was considered suitable for the available headspace above the training aids due to the smaller aluminium hoods compared to those used for cadavers. Additionally, due to the intense odour of some of the training aids previously mentioned, collecting 1 L of the sample could also overload the sorbent tube. Thus, for consistency, 500 mL of air sample was collected from the headspace of both the sample types – CDD training aids and cadavers.

2.3.2 Analytical analysis

The three analytical parameters tested during method optimisation were constant pressure value, modulation period and detector spectra acquisition rate. GC×GC–TOFMS can operate either at constant pressure or constant flow modes. The constant flow mode allows the flow to be constant throughout the run while the inlet pressure increases with an increase in temperature [152]. In the constant pressure mode, the inlet pressure stays the same while the flow decreases with an increase in temperature [152]. Since the current instrument operated at constant pressure, the two constant inlet pressure values tested were 7.8 psi vs. 17.8 psi. 7.8 psi was chosen as it corresponded to ~1 mL/min flow rates at 35 ˚C which was the set constant carrier gas flow rate in the reference studies. 17.8 psi which corresponded to ~2 mL/min flow rates at 35 ˚C was chosen because the Restek EZMC® method translator and flow calculator software (Restek Corporation) suggested that 1.4 mL/min to 2 mL/min were optimum carrier gas flow rates for the current column set configuration. The 2D chromatographic outputs and total ion chromatograms (TIC) of decomposition VOCs analysed at 7.8 psi vs. 17.8 psi constant inlet pressure modes are shown in Figures 2.10 and 2.11, respectively.

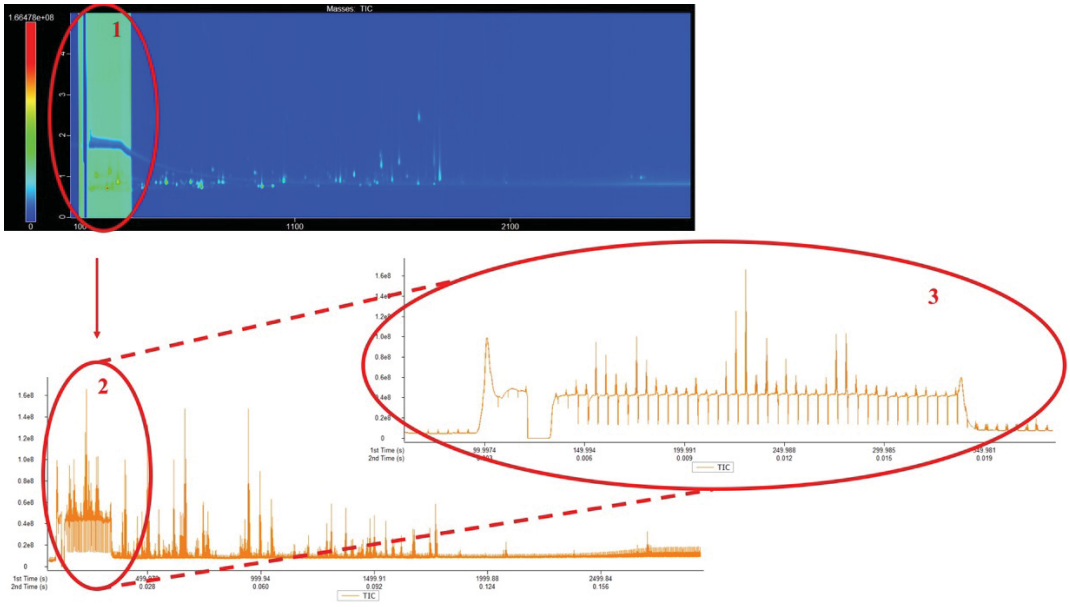


Figure 2.10: 1) 2D chromatogram (top left) and 2) total ion chromatogram (bottom left) of decomposition VOCs collected from air matrix analysed at 7.8 psi constant inlet pressure (~1mL/min at 35 ˚C) showing 3) magnified image of poor baseline**.**

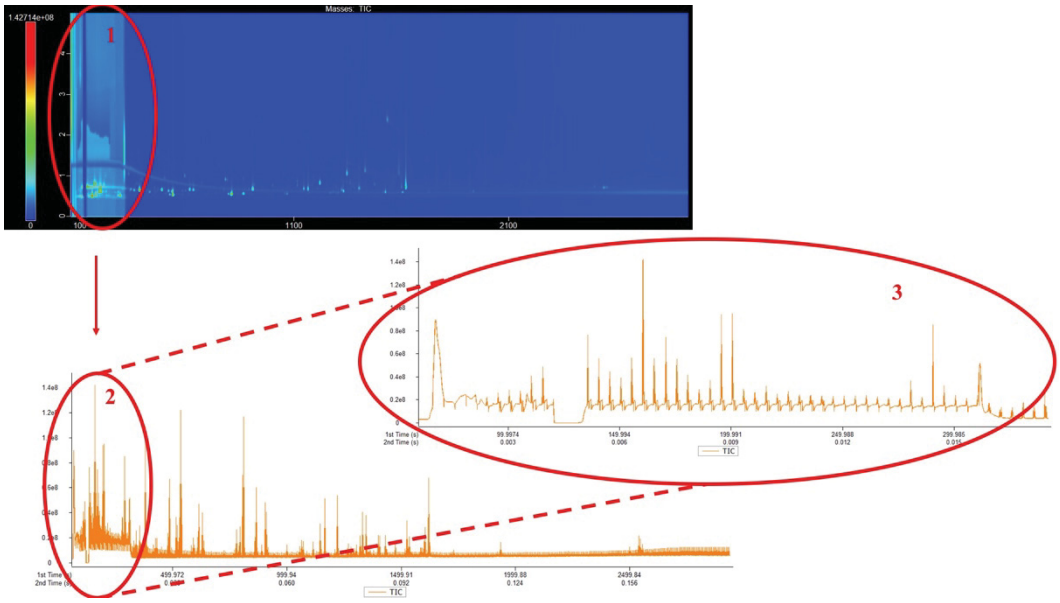


Figure 2.11: 1) 2D chromatogram (top left) and 2) total ion chromatogram (bottom left) of decomposition VOCs collected from air matrix analysed at 17.8 psi constant inlet pressure (~2mL/min at 35 ˚C) showing 3) magnified image of improved baseline.

As depicted in the magnified image of the total ion chromatogram (#3 of Figures 2.10 and Figure 2.11, there were more baseline disturbances observed when the sample was analysed at 7.8 psi compared to 17.8 psi. Thus, 17.8 psi was preferred for the current study as it showed an improved baseline.

The samples were analysed with a 5 s and 8 s modulation period. A 5 s modulation was recommended by the reference studies, while the 8 s modulation period was tested to improve the wrap around peaks observed in some of the samples. The analysis with an 8 s modulation resolved the wraparound issue however it reduced the separation resolution along the ^{1}D (Figure 2.12). The 8 s modulation was thus deemed too long. Longer modulation periods have additional drawbacks as they have fewer slices, a tendency to overload ^{2}D and they also utilise excessive coolant (liquid N_2 in the current instrument) [147]. Even though the 5 s modulation showed wrap around for some compounds, since they did not majorly interfere with compounds in the following modulation period, the wrap around during the 5 s modulation period was accepted over an ineffective separation in the ^{1}D using an 8 s modulation. Thus, the 5 s modulation period (Figure 2.13) was adopted from prior reference studies. The samples were also run at 6 s and 7 s modulation however, this did not enhance the separation compared to 5 s modulation (results not shown).

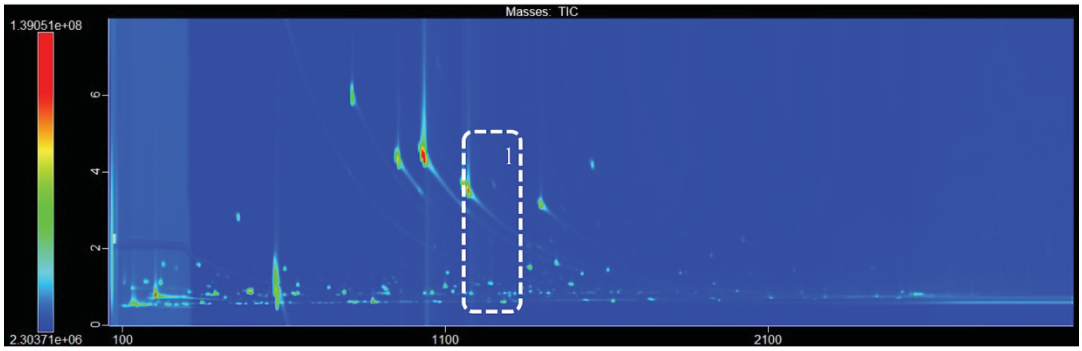


Figure 2.12: 2D chromatogram of decomposition VOCs analysed with an 8s modulation period showing 1) poor separation in the ^{1}D.

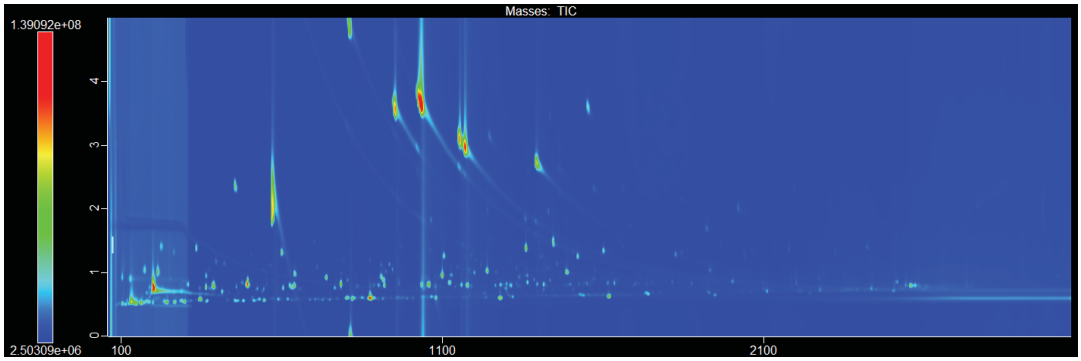


Figure 2.13: 2D chromatogram of decomposition VOCs analysed with a 5s modulation period showing better separation in the ^{1}D.

Finally, as mentioned in section 2.2.2.5, 200 spectra/s was selected over 100 spectra/s used in the reference studies.

2.4 Conclusion

The analytical method for the current study was successfully validated with parameters directly accepted or modified from previously published studies [94; 96; 87] to suit the requirements of the current samples and instrument type. In summary, once the headspace was accumulated under the aluminium hood, an ACTI-VOC low flow air sampling pump (Markes International Ltd., Llantrisant, UK) was used to collect air samples onto Tenax TA/Carbograph 5TD sorbent tubes (Markes International Ltd., Llantrisant, UK). The pump was set to draw air at a constant flow rate of 100 mL/min and a 500 mL sample was collected on every sorbent tube with sampling in triplicates. All the tubes were sealed with brass storage caps, wrapped in aluminium foil and placed inside mason jars for transportation to the laboratory. The sorbent tubes were stored at 4 ˚C until analysed.

An internal standard consisting of 0.2µl of 50 ppm bromobenzene (GC grade, Sigma-Aldrich) in methanol (HPLC Grade, Sigma-Aldrich) was injected using an eVol® XR handheld automated analytical syringe (SGE Analytical Science, Wetherill Park BC, NSW, Australia) onto each sorbent tube before analysis. A Markes TD 100-xr multi-tube autosampler (Markes International Ltd., Llantrisant, UK) was used to thermally desorb the VOCs from the sorbent tubes. Each sorbent tube was heated to 300 °C for 4 min to allow thermal desorption of the compounds before being collected onto a general purpose cold trap at -10 °C. The trap was desorbed at 300 °C for 3 min at a desorption flow rate of 20 mL/min with a 10:1 split. After the sorbent tubes had undergone thermal desorption, they were reconditioned for 30 min at 330 ˚C for subsequent re-use. The thermal desorption unit was connected to a Pegasus® BT 4D GC×GC–TOFMS (LECO, Mississauga, Ontario, Canada) using a transfer line. A 30 m × 0.250 mm inner diameter (ID) × 1.40 µm film thickness Rxi®-624Sil MS column (Restek Corporation, Bellefonte, PA, USA) was used as the ^{1}D column, and a 2 m × 0.250 mm ID × 0.25 µm film thickness Stabilwax® column (Restek Corporation, Bellefonte, PA, USA) was used as the second dimension column. Helium (high purity, Praxair Canada Inc., Trois-Rivieres, Québec, Canada) was used as the carrier gas at a constant pressure of 17.8 psi. The ^{1}D oven was set to 35 ˚C and held at this temperature for 5 min before increasing at a rate of 5 ˚C/min to 230 ˚C where it was held for a further 5 min. The offset for the modulator was 15 ˚C and the offset for the ^{2}D column was 5 ˚C. The modulation period

was 5 s with a 1 s hot pulse. The transfer line between the ^{2}D column and the MS was held at 250 ˚C. An acquisition rate of 200 spectra/s was used to target a mass acquisition range of 29 – 450 amu. The ion source was held at 250 °C, the electron ionization energy was 70 eV.

2.5 Limitations and future recommendations

To be able to compare research outcomes with previously published studies, there was a conscious effort to keep as many parameters as possible similar to prior studies. However, a downside to this is that the study may be influenced by a potential design bias when selecting parameters for sample collection and analysis. Thus, this could result in selecting several aspects such as the size of the donor aluminium hood, choice of sorbent tubes, columns, instruments, and method for normalisation based on internal standards that have been previously reported to be suitable for decomposition VOC analysis. Since no attempt was made to assess or modify any of these, there may have been a bias introduced when establishing study design for the current study.

Using the optimised method, it was observed that there was scope for improvement in the chromatographic separation along the ^{1}D. For one sample in particular, the peaks had a slightly lower separation resolution likely due to column overload (Figure 2.14). At the time of method optimisation there was limited access to samples and such an issue was not encountered. This resolution problem can easily be resolved by reducing the temperature ramp and/or increasing the split ratio. Figure 2.15 depicts a 2D chromatogram of decomposition VOCs when the sample was analysed using a 4˚C/min temperature ramp and injected at a 20:1 split ratio. The optimised method, which used a 5˚C/min temperature ramp and injected at a 10:1 split ratio (Figure 2.14), still identified all the peaks however, it needed additional user cross verification and could not solely rely on the software assigned identification.

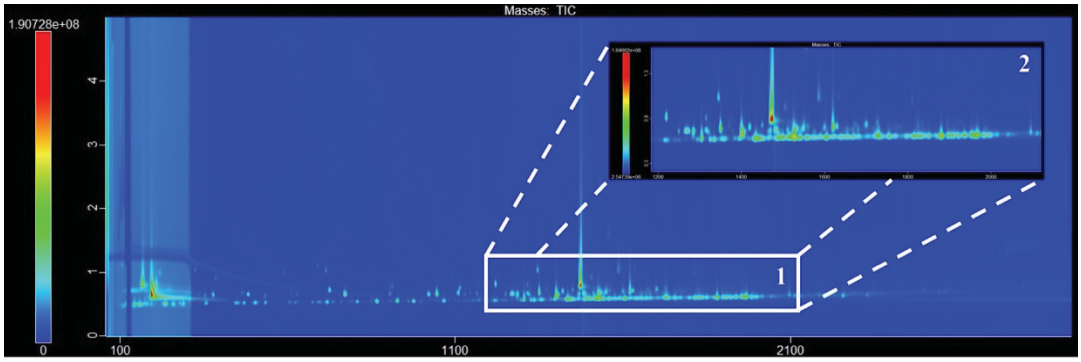


Figure 2.14: 2D chromatogram of decomposition VOCs showing separation using a 5 ˚C/min temperature ramp when the sample was injected at 10:1 split.

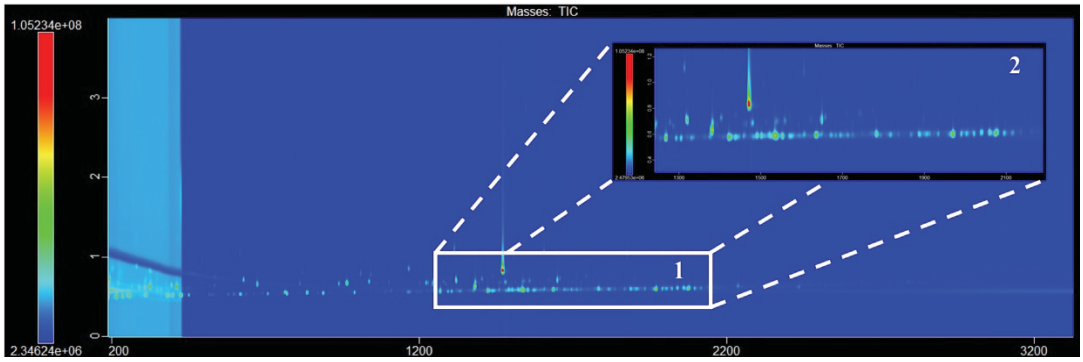


Figure 2.15: 2D chromatogram of decomposition VOCs showing separation using a 4 ˚C/min temperature ramp when the sample was injected at 20:1 split.

Another factor that can be taken into consideration is the acceptability of the peak abundance in blanks. This becomes particularly important in quantitative analysis. Incorporating these recommendations in future studies will enhance the chromatographic resolution and produce results with a higher accuracy in the field of decomposition VOC analysis.

Chapter 3: VOLATILE PROFILE OF CADAVER DETECTION DOG TRAINING AIDS

Chapter 3: VOLATILE PROFILE OF CADAVER DETECTION DOG TRAINING AIDS

3.1 Introduction

Cadaver detection dog (CDD) training aids are odorous materials used as an alternative source to the decomposition odour of cadavers [112]. These training materials are crucial as cadavers are rarely available for training. When the CDDs are trained on the available training material, they recognise the 'known' odour of their training material to identify this target odour during field searches. Thus, the dog handlers aim to use training materials that closely resemble the cadaveric odour [153]. Since odour is comprised of volatile and semi-volatile compounds, the knowledge of decomposition VOCs in CDD training materials can be vital to dog handlers as this can help them identify and maintain appropriate training material [154]. As mentioned previously in section 1.4.4, the training aids can be natural or synthetic in nature.

Previously, the use of several natural training aids such as decomposition fluid, blood, human remains, and soil surrounding decomposing remains has been scientifically recognised and a recent review by the author of this thesis summarised the various VOCs detected in CDD training aids [136; 137; 112]. Among all studies, one significant study was conducted by Hoffman et al. (2009) to detect VOCs across 14 human remains samples specifically used as CDD training aids [68]. The analysis from blood, placenta, muscle, testicle, skin, bone, teeth, fat and adipocere samples revealed that even though human decomposition odour had similarities across various tissue types, there were also dissimilarities suggesting none of the training materials were ideal in isolation to others for CDD training. The researchers concluded that if CDDs were presented with VOCs from only one tissue type during their training, they would not be exposed to the entire spectrum of VOCs that comprise the decomposition odour and thus, these CDDs would be limited in their field detection capability. It was suggested that combining various tissue types during training as opposed to training the dogs on a specific tissue type can help achieve the desired outcomes. This way of training has generally been accepted among dog handlers and researchers in the field of decomposition odour analysis.

One of the greatest challenges faced by organisations that train CDDs is the difficulty to obtain human remains since the acquisition of cadaveric tissue has numerous legal and ethical aspects associated with it. As an alternative to human remains, there has been a recent proposal to use voluntarily donated amputated lower limbs/feet following surgical removal from diabetic patients. Since these are voluntarily donated by living patients, the legal and ethical aspects are considerably easier. Certain police organisations (such as the Ontario Provincial Police known to the researcher) have adopted the use of amputated lower limbs/feet in their regular CDD training sessions. The use of amputated lower limbs/feet as a substitute training material has been previously mentioned in one other research thesis [155]. However, to the current knowledge of the researcher of this study, there have been no studies to establish their VOC profile or validate their use as a CDD training aid. Prior studies conducted on diabetic limbs have been limited to analysing the VOCs resulting from bacterial species (grown under in vitro conditions) that infect diabetic foot ulcers [156; 157]. Thus, the primary focus of the current project was to chemically analyse the amputated lower limbs/feet being used as CDD training material by the Ontario Provincial Police (OPP) Canine Unit. This could help validate their use as alternative training material for CDDs in the future. The OPP Canine Unit in collaboration with Queen's University, Kingston collects feet (including bone, skin and muscle tissue) following amputation surgeries on diabetic patients which are conducted at Kingston General Hospital. Since this project was conducted predominantly for the OPP Canine Unit, some of their other training materials (including blood and teeth) were also chemically analysed to gain a better understanding of the VOCs that their CDDs are exposed to regularly. The current chapter presents results from the VOC profile analysis of CDD training aids used by the OPP Canine Unit using GC×GC–TOFMS.

3.2 Methods

3.2.1 Ethics

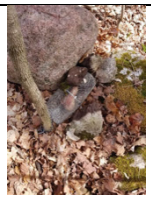
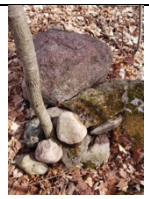
The human research ethics approval for working with human remains in the form of CDD training aids was obtained from *le comité d'éthique de la recherche avec des êtres humain* at *Université du Québec à Trois-Rivières* (UQTR) with the certificate number CER-19-261-07.12.

3.2.2 Samples

The samples used in the current study were all CDD training aids used by the OPP Canine Unit. These were either stored outdoors at a woodland site maintained by the OPP Canine Unit called the OPP Decomposing Odor Research Site (ODORS), or indoors at room temperature or in a refrigerator or freezer. For this study, the samples were classified as foot, bone, tissue, blood and teeth. The foot training aids contained tissues along with bones while the other categories of samples either had only tissue, blood or teeth and thus were classified that way. Not all bone training aids were dry bones since most of them still had soft tissue attached to them. Regardless, they were recognised as bones since this classification was adopted based on the description provided by the OPP Canine Unit. Especially, in instances where the samples were stored in PVC pipes which could not be opened for cross verification, the researcher had to rely on the provided information. It must be noted that the foot, bone and tissue samples all originated from the amputated lower limbs/feet of diabetic patients, while the teeth samples were a donation to the OPP Canine Unit by a dentist, and the blood (deposited on gauze) samples were donated by the dog handlers thus, the blood was not of cadaveric origin. The amputated lower limbs and/or feet were obtained from voluntary donations following surgeries of diabetic patients conducted in September 2017, December 2017, January 2019 and July 2020. Thus, the OPP Canine Unit obtained training aids in three distinct batches – in late 2017, early 2019 and mid 2020. Table 3.1 presents all the training aid samples available for this study along with their description and images. All images were either taken by the researcher or collected from a report on ODORS generated by the OPP Canine Unit [158].

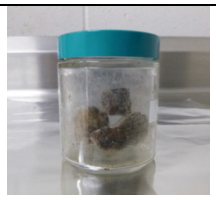
Table 3.1: List of CDD training aids that were analysed in the current study along with their description and images.

Sr. no.	**Training aid name**	**Date on which the sample was obtained by the OPP Canine Unit**	**Sample Description**	**Image**
AMPUTATED LOWER LIMBS TRAINING AIDS – comprises of foot stored outdoors and indoors, bone and tissue training aids				

	Foot stored outdoors - contains entire sections of the amputated lower limb bone along with tissue.			
1.	Foot S	24th January 2019	A foot placed at ODORS to decompose.	
2.	Foot R	24th January 2019	Foot with 4 toes placed at ODORS hidden between rocks in a cage.	 
3.	Foot B	24th January 2019	A foot with 5 toes + A foot without toes buried at ODORS.	 
	Foot stored indoors - contains entire sections of the amputated lower limb bone along with tissue.			
4.	Foot 1	2nd October 2017	Foot with toes stored in a PVC pipe within a zip lock bag.	
5.	Foot 2	2nd October 2017	Foot with bones stored in PVC pipe within a glass jar. This was wrapped in gauze to absorb the liquefied decomposition fluid.	

6.	Foot 3	11th December 2017	Skin and muscle stored in PVC pipe within a glass jar (unclear if any bone present along with tissue).	
7.	Foot 4	11th December 2017	Cross section of a leg stored in PVC pipe within a glass jar.	
8.	Foot 5	11th December 2017	Foot without toes stored in a glass jar.	
9.	Foot 6	24th January 2019	Cross section of a leg stored in PVC pipe within a glass jar.	
10.	Foot 7	24th January 2019	Cross section of a leg stored in PVC pipe within a glass jar.	
11.	Foot 8	24th January 2019	Cross section of a leg stored in a PVC pipe within a glass jar.	

12.	Foot 9	24th January 2019	Cross section of a leg stored in a PVC pipe within a glass jar.	
	Bone – Contains bone with little to no tissue attached to it.			
13.	Bone 1	2nd October 2017	Ankle bone stored in a small Teflon lid glass jar.	
14.	Bone 2	2nd October 2017	Heel and foot bones stored in a glass jar.	
15.	Bone 3	8th December 2017	Bone stored in a PVC pipe within a glass jar.	
16.	Bone 4	8th July 2020	Left foot bones stored in a glass jar. This was generated from Foot S sample when brought indoors.	

17.	Bone 5	8th July 2020	Leg section of tibia/fibula stored in a glass jar. This was generated from Foot S sample when brought indoors.	
18.	Bone 6	8th July 2020	Small ankle bone in a small Teflon lid glass jar	
19.	Bone 7	8th July 2020	Small ankle bone in a small Teflon lid glass jar	
	Tissue – Contains tissue without any bones.			
20.	Tissue 1	2nd October 2017	Tissue from foot stored in a small Teflon lid glass jar	
21.	Tissue 2	2nd October 2017	Skin and flesh tissue stored in a small Teflon lid glass jar	
22.	Tissue 3	8th July 2020	Mummified skin in a small Teflon lid glass jar	
OTHER TRAINING AIDS – comprises of Blood and Teeth				
	Blood- Contains fresh blood donated by living individuals deposited on a gauze			

23.	Blood 1	November 2017	Blood on gauze stored in a small Teflon lid glass jar	
24.	Blood 2	November 2017	Blood on gauze stored in a small Teflon lid glass jar	
25.	Blood 3	22nd February 2021	Blood on gauze stored in a small Teflon lid glass jar	
	Teeth – Contains teeth samples with almost no organic matter			
26.	Teeth 1	Prior to 2017	A couple of teeth donated, few years old and stored in a small Teflon lid glass jar	

3.2.3 Experimental design

The VOCs from most of the training aids were collected indoors in a room with a metallic table. Prior to sample collection, the table was cleane d with acetone (ACS grade, Thermo Fisher Scientific) and a sheet of aluminium foil was spread over it. This cleaning protocol was followed between collecting samples from two different training aids along with observing a 10 min waiting period to allow for ventilation in the room. This was practised to ensure that the VOCs from one sample were not carried over to the next.

The VOC samples from training aids were collected repeatedly from the same training aids during the months of September 2019, December 2019, July 2020, October 2020, March 2021 and May 2021, based on their availability during the visits to the OPP Canine Unit. The samples collected in the month of September 2019 were only used for optimising the analytical method. Control samples were also collected in the same sample

collection room from storage containers without the training aids. All the training aid samples which were used to study VOC profiles (via GC×GC-TOFMS analysis) used in the current study have been summarized in Table 3.2. This table also presents training aid storage information during the month of VOC collection. The storage information for samples collected in the month of September 2019 was unavailable. Generally, if any sample was stored in a refrigerator or a freezer, it was left at room temperature for about 8 – 24 h before VOC collection.

Table 3.2: List indicates the months when VOCs were collected and analysed from CDD training aids along with the storage condition information for the respective months.

Sr. no.	**Training aid Name**	**Month of VOC collection and analysis**					
		September 2019	**December 2019**	**July 2020**	**October 2020**	**March 2021**	**May 2021**
	Foot stored outdoor						
1.	Foot S						
2.	Foot R	✓					
3.	Foot B	✓					
	Foot stored indoor						
4.	Foot 1						
5.	Foot 2						
6.	Foot 3						
7.	Foot 4						
8.	Foot 5	✓					
9.	Foot 6						
10.	Foot 7						
11.	Foot 8						
12.	Foot 9						
	Bone						
13.	Bone 1	✓					
14.	Bone 2						
15.	Bone 3	✓					
16.	Bone 4						
17.	Bone 5						

18.	Bone 6						
19.	Bone 7						
	Tissue						
20.	Tissue 1						
21.	Tissue 2						
22.	Tissue 3						
	Blood						
23.	Blood 1						
24.	Blood 2						
25.	Blood 3						
	Teeth						
26.	Teeth 1						

Key for Table 3.2

	Stored outdoors
	Stored outdoors but sampled both outdoors and indoors (after exhuming the remains)
	Stored in refrigerator
	Stored at room temperature
	Stored in freezer

3.2.4 Analytical method

All the steps for VOC accumulation, collection and analysis have been detailed in Chapter 2 and remained the same.

The VOC collection method was slightly modified for one of the foot training aids placed at ODORS and hidden beneath the rocks in a cage (Foot #R). During VOC collection for this training aid, the hood could not be placed over the rocks as its size was not sufficient, therefore, the accumulation step was skipped, and VOCs were directly collected on the sorbent tube.

3.2.5 Data analysis

Statistical analysis was performed based on methods described in section 2.2.5 of Chapter 2. The R scripts were suitably edited for each of the training aid types (foot stored outdoors, foot stored indoors, bone, tissue, blood, teeth) and are available on GitHub which can be accessed using the link: https://github.com/RushaliDargan/Decomposition/tree/main/CDD_training_aids

The R codes resulted in VOCs that were significant to each training aid sample relative to controls. The VOCs were then classified into compound classes based on classes reported in the literature as – acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues (analogues include anhydrides), ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs. The VOCs and their classes were then analysed for abundance, relative class concentration and any variability in VOC occurring due to ageing and storage conditions of the training aids.

3.3 Results

Analysis of six different types of CDD training aids (foot stored outdoors, foot stored indoors, bone, tissue, blood, teeth) used by the OPP Canine Unit and studied over a span of 1.5 years resulted in 85 samples whose VOCs were analysed. In all of the samples analysed, a total of 2026 VOCs were detected however, the number of VOCs per sample varied greatly as it ranged from eight VOCs (in Teeth #1 October 2020) to 430 VOCs (in Foot #5 December 2019). The detected VOCs were classified into one of the following classes – acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs. Each of these classes have been previously identified as a VOC class that comprises decomposition odour [135; 71; 159; 160; 68; 85; 161; 162; 96; 163; 87].

3.3.1 Training aid VOC profiles: Compound class abundance and prominent VOCs

This section elaborates on the compound class abundance and prominent compounds (most frequently detected) across all CDD training aid types used by the OPP Canine Unit. Figure 3.1 represents the class abundance of the 2026 VOCs detected in this study.

This figure highlights that aromatics were the most abundant class (n = 342; 17%) followed by linear aliphatics (n = 278; 14%), esters and analogues (n = 262; 13%), alcohols (n = 216; 11%), nitrogen-containing (n = 183; 9%), ketones (n = 177; 9%), cyclic aliphatics (n = 174; 9%), halogen-containing (n = 162; 8%), ethers (n = 92; 5%), aldehydes (n = 58; 3%), acids (n = 46; 2%) and sulphur-containing VOCs (n = 36; 2%) VOCs. Here 'n' denotes the total number of VOCs in the compound class while the % value represents the percentage of total VOCs present in a compound class for CDD training aids.

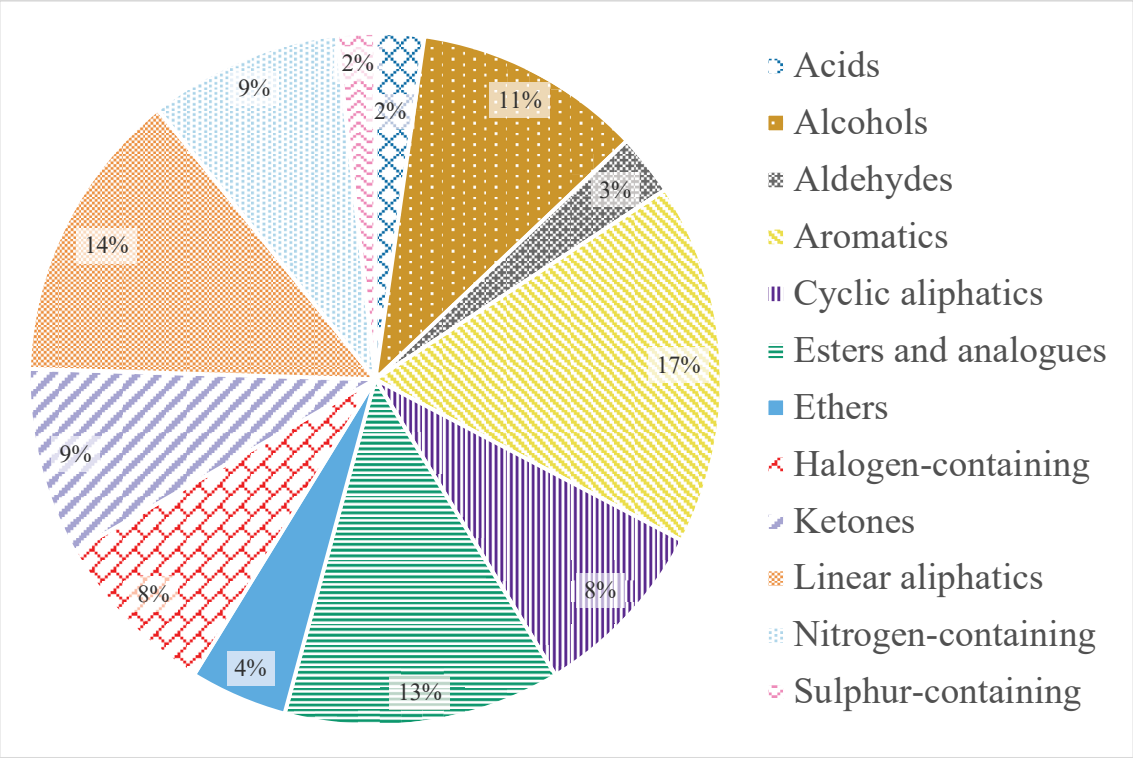


Figure 3.1: Abundance of VOCs (represented as percentage of total VOCs) per compound class for CDD training aids.

Of the 2026 VOCs detected, Table 3.3 presents the 18 most prominently occurring VOCs detected across the 85 samples generated from the six training aid types. These VOCs were detected in at least 30% of samples (26 or more samples) ranging up to 79%. These compounds are relevant because they are VOCs that the CDDs are constantly exposed to during their training sessions. The 18 VOCs listed in Table 3.3 belonged to the following classes – acids, alcohols, aldehydes, aromatics, esters and analogues, nitrogen-containing, and sulphur-containing VOCs. Ethers, ketones, cyclic aliphatics and linear aliphatics were not found among the 18 VOCs. One additional halogen-containing compound – sevoflurane, was identified as a commonly occurring VOC, however, sevoflurane is not

known to be a decomposition-related compound. A potential source of sevoflurane in these samples is discussed later in section 3.4.3.1. Most of the other prominent VOCs detected in amputated lower limbs/feet CDD training aids have been previously reported in the literature as human decomposition odour-related VOCs. Among all of the commonly occurring VOCs, 2,6-dimethyl pyridine, 2-methyl pyridine and dimethyl trisulphide (DMTS) were detected most frequently and will be discussed later in section 3.4.2.

Table 3.3: 18 prominent VOCs detected in over 30% of samples analysed from CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	**Volatile organic compound**	**Compound class**	**Percentage of samples in which the VOC was detected**	**Previously reported in literature as human decomposition odour-related**
1.	2,6-Dimethyl pyridine (2,6-Lutidine)	Aromatic	79%	
2.	2-Methyl pyridine	Aromatic	67%	[136]
3.	Dimethyl trisulphide (DMTS)	Sulphur-containing	67%	[135; 71; 159; 160; 99; 161; 136]
4.	4-Methyl pyrimidine	Aromatic	64%	
5.	Methenamine	Nitrogen-containing	61%	[135; 160; 136]
6.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohol	47%	[164; 136; 165]
7.	Methyl pyrazine	Aromatic	48%	[136]
8.	Pyridine	Aromatic	48%	[162; 164; 136; 165]
9.	2,5-Dimethyl pyrazine	Aromatic	42%	[164; 136]
10.	Methyl thiocyanate	Ester and analogue	41%	[136]
11.	Sevoflurane	Halogen-containing	38%	None – likely not human

				decomposition odour-related.
12.	Trimethylamine	Nitrogen-containing	36%	[136]
13.	Thiazole	Aromatic	36%	[136]
14.	3-Methyl-butanoic acid	Acid	35%	[136]
15.	2-Methyl-propanoic acid	Acid	33%	[99; 136]
16.	Acetamide	Nitrogen-containing	32%	[136]
17.	5-Methyl-2-furancarboxaldehyde	Aldehyde	31%	
18.	Trimethyl pyrazine	Aromatic	31%	[164; 136]

3.3.1.1 Outdoor foot

There were three outdoor foot training aids – one decomposing on the surface (Foot #S), one hidden between rocks (Foot #R) and one buried (Foot #B), all of which were sampled in this study.

Foot #S

Foot #S was analysed only in the month of July 2020 since it was eventually brought indoors and re-identified and sampled as Bone #4 and #5. The VOC profile of Foot S revealed 74 VOCs that were significantly higher in the training aid compared to the control samples. The aromatic class (24% of total no. of VOCs) was the most abundant in this sample followed by alcohols (15% of total no. of VOCs) and nitrogen-containing VOCs (12% of total no. of VOCs). Sulphur-containing VOCs (3% of total no. of VOCs) and acids (1% of total no. of VOCs) were the least abundant class to be detected.

Foot #R

Foot #R was analysed in the months of July 2020, October 2020 and May 2021 thus, generating three samples from this training aid. The VOC profile of the three Foot R samples revealed 25 – 35 VOCs that were significantly higher in the training aid compared to the control samples. Overall, halogen-containing VOCs (18% of total no. of VOCs) and linear aliphatic VOCs (16% of total no. of VOCs) were the most abundant.

Aldehydes and sulphur-containing VOCs (2% of total no. of VOCs), and acids (1% of total no. of VOCs) were the least abundant classes to be detected in this training aid.

Foot #B

Foot #B was analysed in the months of July 2020, October 2020 and May 2021 thus, generating three samples from this training aid. The VOC profile of the three Foot B samples revealed 11 – 36 VOCs that were significantly higher in the training aid compared to the control samples. Overall, the aromatic class (21% of total no. of VOCs) was the most abundant in this sample followed by alcohols (15% of total no. of VOCs). Sulphur-containing VOCs and ethers (2% of total no. of VOCs each) were the least abundant classes, while acids were not detected. In May 2021, this training aid was exhumed and sampled indoors along with the usual outdoor site of sampling. During this trial, only 11 VOCs were identified as significant to the training aid when it was buried while 78 VOCs were identified as significant when this training aid was exhumed and sampled indoors.

Of the seven samples generated from analysing the three foot training aids in the outdoor scenario, 194 different VOCs were identified as being significantly higher in the samples compared to the controls. The 194 VOCs belonged to all classes of compounds (indicated in Figure 3.2) including acids (n = 2; 0 – 3%) , alcohols (n = 22; 3 – 27%), aldehydes (n = 9; 0 – 8%), aromatics (n = 37; 14 – 36%), cyclic aliphatics (n = 19; 3 – 14%), ester and analogues (n = 12; 0 – 14%), ethers (n = 6; 0 – 6%), halogen-containing (n = 23; 0 – 26%), ketones (n = 14; 3 – 11%), linear aliphatics (n = 25; 6 – 23%) nitrogen-containing (n =21; 8 – 16%), and sulphur-containing VOCs (n = 4; 0 – 4%). Here ‘n’ denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a compound class for foot training aids stored outdoors. Thus, as evident in Figure 3.2, aromatics and alcohols were the most abundant classes while ethers, sulphur-containing VOCs and acids, were the least abundant classes in the outdoor foot training aids.

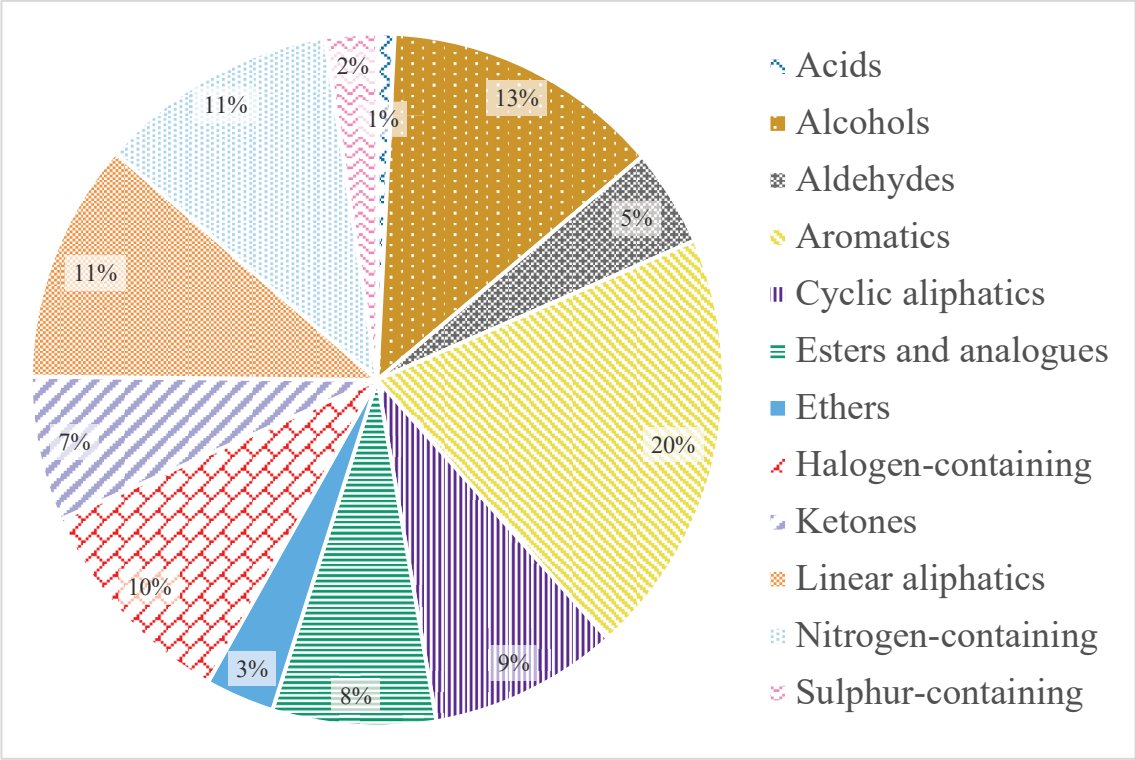


Figure 3.2: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for foot training aids stored outdoors.

Of the 194 VOCs detected, Table 3.4 presents the 12 most prominently occurring VOCs detected across the seven samples generated from analysing the foot samples decomposing in the outdoor environment. These VOCs were detected in at least 40% of samples (three or more samples) ranging up to 57%.

Table 3.4: 12 most prominent VOCs detected in over 40% of foot samples stored in an outdoor environment and used by the OPP Canine Unit as CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature as human decomposition odour-related
1.	1-Octen-3-ol	Alcohol	57%	[80]
2.	Methyl thiocyanate	Ester and analogues	57%	[136]

3.	1,1,2,2-Tetrafluoro-propane	Halogen-containing	43%	
4.	2-Methyl-1,3,6-trioxocane	Cyclic aliphatics	43%	
5.	2-Pentylfuran	Aromatic	43%	[68; 136]
6.	2-Propen-1-ol	Alcohol	43%	
7.	Camphene	Aromatic	43%	[136]
8.	Diethyltoluamide	Nitrogen-containing	43%	
9.	Dimethyl disulphide	Aromatic	43%	[135; 71; 159; 160; 68; 80; 164; 136; 165]
10.	Isobutyl 3-hydroxy-2,2,4-trimethylpentanoate	Ester and analogues	43%	
11.	Isothiocyanatomethane	Nitrogen-containing	43%	
12.	α-Pinene	Cyclic aliphatics	43%	[71; 136; 166]

3.3.1.2 Indoor foot

There were nine foot training aids stored indoors. A detailed account of the year they were obtained as training aids, the storage conditions and the months they were sampled can be found in Tables 3.1 and 3.2.

Foot #1 and #2

Foot #1 and #2 were obtained from amputation surgery conducted in 2017 and these were analysed in the months of December 2019, July 2020, October 2020, March 2021 and May 2021 thus, generating five samples for each of the two training aids. Throughout the duration of the trial, the two training aids were stored in a refrigerator. The VOC profile of the five Foot #1 samples revealed 30 – 204 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the five Foot #2 samples revealed 59 – 296 VOCs significant in the training aid compared to the control samples. Overall, for both Foot #1 and #2, aromatics (28% – 25% of total no. of VOCs) were the

most abundant class, while acids (1 – 2% of total no. of VOCs) were the least abundant class to be detected.

Foot #3 and #4

Foot #3 and #4 were obtained from amputation surgery conducted in 2017 and these were analysed in the months of March 2021 and May 2021 thus, generating two samples for each of the two training aids. Throughout the duration of the trial, the two training aids were stored in a freezer. The VOC profile of the two Foot #3 samples revealed 138 – 182 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the two Foot #4 samples revealed 216 – 298 VOCs as statistically significant in the training aid compared to the control samples. Overall, for both Foot #3 and #4, aromatics (22 – 26% of total no. of VOCs) and alcohols (12 – 14% of total no. of VOCs) were the most abundant classes, while sulphur-containing VOCs (2% of the total no. of VOCs) were the least abundant class to be detected.

Foot #5

Foot #5 was obtained from amputation surgery conducted in 2017 and these were analysed in the month of December 2019, July 2020, October 2020, March 2021 and May 2021 thus, generating five samples. Throughout the duration of the trial, Foot #5 was stored at room temperature. The VOC profile of the five Foot #5 samples revealed 23 – 430 VOCs as statistically significant in the training aid compared to the control samples. Overall, aromatics (19% of total no. of VOCs), and esters and analogues (15% of total no. of VOCs) were the most abundant classes, while ethers (4% of total no. of VOCs), acids (3% of total no. of VOCs) and aldehydes (2% of total no. of VOCs) were the least abundant classes to be detected.

Foot #6 and #7

Foot #6 and #7 were obtained from amputation surgery conducted in 2019 and these were analysed in the months of March 2021 and May 2021 thus, generating two samples for each of the two training aids. Throughout the duration of the trial, the two training aids were stored in a refrigerator. The VOC profile of the two Foot #6 samples revealed 67 – 109 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the two Foot #7 samples revealed 67 – 69 VOCs as statistically significant in the training aid compared to the control samples. Overall, for both Foot #6 and #7, aromatics (20 – 24% of total no. of VOCs) and cyclic aliphatics (13 – 10% of

total no. of VOCs) were the most abundant classes, while acids (5% of total no. of VOCs), ethers (4% of total no. of VOCs) and aldehydes (1% of total no. of VOCs) were the least abundant classes to be detected.

Foot #8 and #9

Foot #8 and #9 were obtained from amputation surgery conducted in 2019 and these were analysed in the months of October 2020, March 2021 and May 2021 thus, generating three samples for each of the two training aids. The two training aids were stored in a freezer prior to the October 2020 trial and then in a refrigerator prior to March and May 2021 trials. The VOC profile of the three Foot #8 samples revealed 66 – 196 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the three Foot #9 samples revealed 64 – 171 VOCs as statistically significant in the training aid compared to the control samples. Overall, for both Foot #8 and #9, aromatics (19% of total no. of VOCs) and alcohols (15 – 16% of total no. of VOCs) were the most abundant classes, while ethers (2 – 4% of total no. of VOCs) were the least abundant class to be detected.

The 29 samples generated from analysing the nine feet training aids stored indoors had 1491 different VOCs which were identified as statistically significant in the samples compared to the controls. The 1491 VOCs belonged to all classes of compounds (indicated in Figure 3.3) including acids (n = 36; 0 – 13%), alcohols (n = 159; 3 – 20%), aldehydes (n = 41; 0 – 6%), aromatics (n = 271; 11 – 38%), cyclic aliphatics (n = 125; 2 – 18%), ester and analogues (n = 175; 0 – 21%), ethers (n = 63; 0 – 8%), halogen-containing (n = 115; 1 – 20%), ketones (n = 131; 0 – 14%), linear aliphatics (n = 206; 4 – 25%), nitrogen-containing (n =139; 3 – 17%), and sulphur-containing VOCs (n = 30; 0 – 10%). Here ‘n’ denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a compound class for foot training aids stored indoors. Thus, aromatics followed by alcohols and linear aliphatics were the most abundant classes while ethers, aldehydes, acids and sulphur-containing VOCs were the least abundant classes in the foot training aids stored indoors.

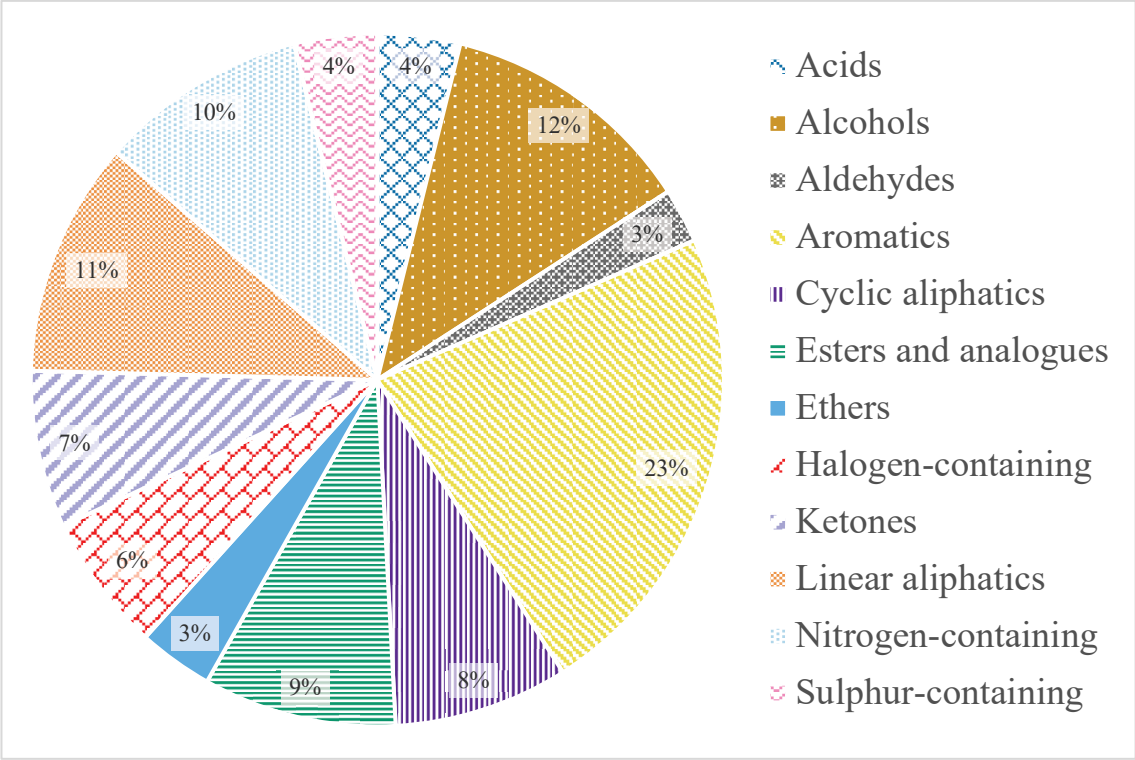


Figure 3.3: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for foot training aids stored indoors.

Of the 1491 VOCs detected, Table 3.5 presents the 12 most prominently occurring VOCs detected across the 29 samples generated from analysing the foot samples stored in indoor conditions. These VOCs were detected in at least 50% of samples (15 or more samples) ranging up to 93%.

Table 3.5: 12 prominent VOCs detected in over 50% of foot samples stored in an indoor environment and used by the OPP Canine Unit as CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature as human decomposition odour-related
1.	Dimethyl trisulphide (DMTS)	Sulphur-containing	93%	[135; 71; 159; 160; 99; 161; 136]

2.	2,6-Dimethyl pyridine (2,6-Lutidine)	Nitrogen-containing	90%	
3.	2-Methyl pyridine	Nitrogen-containing	79%	[136]
4.	Methenamine	Nitrogen-containing	79%	[135; 160; 136]
5.	4-Methyl pyrimidine	Nitrogen-containing	76%	
6.	Methyl pyrazine	Nitrogen-containing	72%	[136]
7.	2,5-Dimethyl pyrazine	Nitrogen-containing	69%	[164; 136]
8.	Pyridine	Nitrogen-containing	69%	[162; 164; 136; 165]
9.	Sevoflurane	Halogen-containing	69%	None – likely not human decomposition odour-related.
10.	Methyl thiocyanate	Ester and analogue	65%	[136]
11.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohol	59%	[164; 136; 165]
12.	3-Methyl-butanoic acid	Acid	52%	[136]

3.3.1.3 Bone

There were seven bone training aids analysed during the study and a detailed account of the year they were obtained as training aids, the storage conditions, and the months they were sampled can be found in Tables 3.1 and 3.2.

Bone #1 and #2

Bone #1 and #2 were obtained from amputation surgery conducted in 2017 and these were analysed in the months of December 2019, July 2020, October 2020, March 2021 and May 2021 thus, generating five samples for each of the two training aids. Throughout the duration of the trial, Bone #2 was stored at room temperature while Bone #1 was

initially stored at room temperature and then moved to the refrigerator prior to analysis in March 2021. The VOC profile of the five Bone #1 samples revealed 80 – 125 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the five Bone #2 samples revealed 54 – 159 VOCs as statistically significant in the training aid compared to the control samples. Overall, aromatics (20% of total no. of VOCs) were the most abundant class in Bone #1, while esters and analogues (30% of total no. of VOCs) dominated the VOC classes for Bone #2. Sulphur-containing compounds (1 – 4% of total no. of VOCs), acids (2 – 4% of total no. of VOCs) and aldehydes (2 – 3% of total no. of VOCs) were the least abundant class in the two training aids.

Bone #3

Bone #3 was obtained from amputation surgery conducted in 2017 and was analysed in the months of March 2021 and May 2021 thus, generating two samples. Throughout the duration of the trial, Bone #3 was stored in a freezer. The VOC profile of the two Bone #3 samples revealed 94 – 170 VOCs as statistically significant in the training aid compared to the control samples. Aromatics (17% of total no. of VOCs) were the most abundant classes in Bone #3, while ethers (4% of total no. of VOCs) and sulphur-containing VOCs (2% of total no. of VOCs) were the least abundant classes.

Bone #4 and #5

Bone #4 and #5 were obtained from amputation surgery conducted in 2020. Initially, they were decomposing in an outdoor environment and identified as Foot #S, but prior to sampling in October 2020, they were brought indoors and stored at room temperature. The two training aids were analysed in the months of October 2020, March 2021 and May 2021 thus, generating three samples for each of the two training aids. Throughout the duration of the trial, they were stored at room temperature. The VOC profile of the three Bone #4 samples revealed 110 – 155 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the five Bone #5 samples revealed 56 – 110 VOCs as statistically significant in the training aid compared to the control samples. Overall, esters and analogues (30% of total no. of VOCs) dominated the VOC classes in Bone #4, while aromatics (21% of total no. of VOCs) were the most abundant class in Bone #5. Ether (3% of total no. of VOCs) and aldehydes (2% of total no. of VOCs) were the least abundant classes in both Bone #4 and #5.

Bone #6 and #7

Bone #6 and #7 were obtained from amputation surgery conducted in 2020 and these were analysed in the months of March 2021 and May 2021 thus, generating two samples for each of the two training aids. Throughout the duration of the trial, Bone #6 and #7 were stored at room temperature. The VOC profile of the two Bone #6 samples revealed 16 – 77 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the two Bone #7 samples revealed 25 – 60 VOCs as statistically significant in the training aid compared to the control samples. Overall, aromatics (30 – 34% of total no. of VOCs) were the most abundant class, while sulphur-containing VOCs (0 – 2% of total no. of VOCs) and acids (0 – 1% of total no. of VOCs) were the least abundant classes for these two training aids.

The 22 samples generated from analysing the seven bone training aids produced 1019 different VOCs which were identified as statistically significant in the samples compared to the controls. The 1019 VOCs belonged to all classes of compounds (indicated in Figure 3.4) including acids (n = 27; 0 – 13%), alcohols (n = 116; 5 – 20%), aldehydes (n = 31; 0 – 6%), aromatics (n = 164; 10 – 40%), cyclic aliphatics (n = 81; 2 – 14%), ester and analogues (n = 146; 0 – 43%), ethers (n = 43; 0 – 10%), halogen-containing (n = 81; 0 – 14%), ketones (n = 81; 0 – 14%), linear aliphatics (n = 137; 2 – 31%), nitrogen-containing (n = 91; 4 – 21%), and sulphur-containing VOCs (n = 21; 0 – 9%). Here ‘n’ denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a class for bone training aid. Thus, aromatics followed by esters and analogues, alcohols, and nitrogen-containing VOCs were the most abundant classes while aldehydes, sulphur-containing, acids and ethers were the least abundant classes in bone training aids.

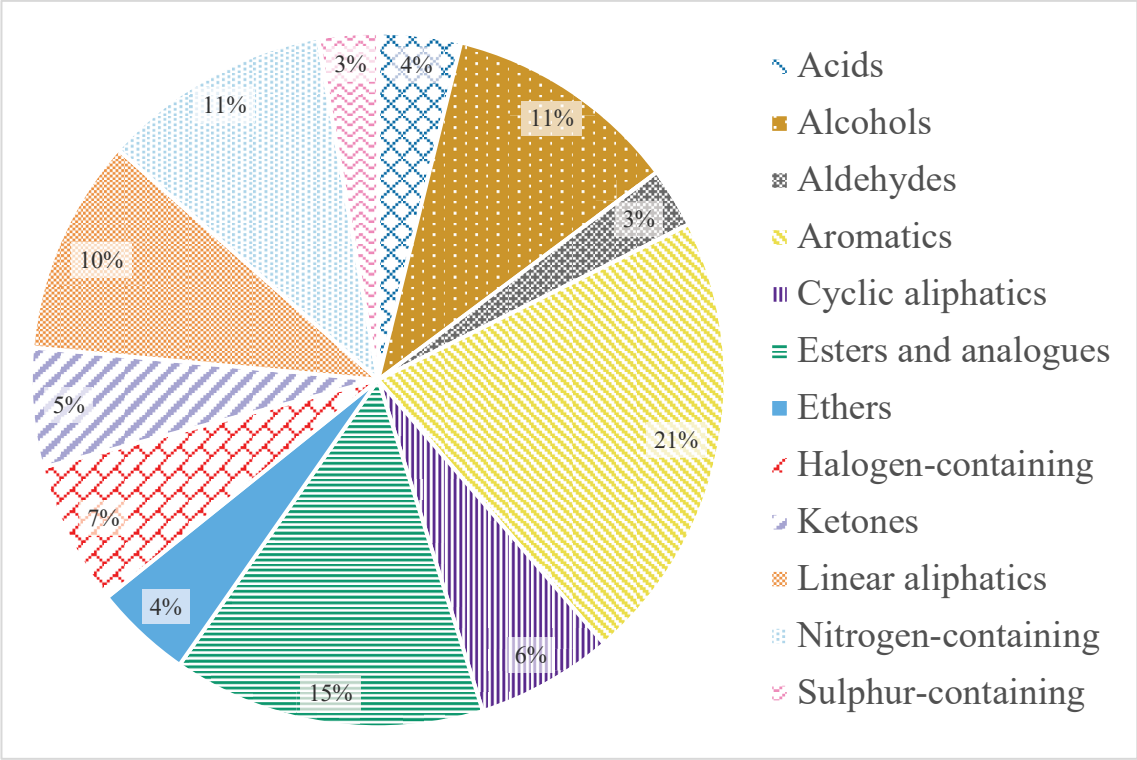


Figure 3.4: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for bone training aid.

Of the 1019 VOCs detected, Table 3.6 presents the 13 most prominently occurring VOCs detected across the 29 samples generated from analysing bone training aids. These VOCs were detected in at least 40% of samples (nine or more samples) ranging up to 82%.

Table 3.6: 13 prominent VOCs detected in over 40% of bone samples used by the OPP Canine Unit as CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature as human decomposition odour-related
1.	2,6-Dimethyl pyridine (2,6-Lutidine)	Aromatic	82%	
2.	Methenamine	Nitrogen-containing	73%	[135; 160; 136]

3.	Dimethyl trisulphide (DMTS)	Sulphur-containing	59%	[135; 71; 159; 160; 99; 161; 136]
4.	2-Methyl pyridine	Aromatic	59%	[136]
5.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohol	55%	[164; 136; 165]
6.	4-Methyl pyrimidine	Aromatic	55%	
7.	Sevoflurane	Halogen-containing	55%	None – likely not human decomposition odour-related.
8.	2-Methyl-propanoic acid	Acid	50%	[136]
9.	3-Methyl-butanoic acid	Acid	45%	[136]
10.	Isopropyl acetate	Esters and analogues	41%	
11.	3-Sulfo-L-alanine	Nitrogen-containing	41%	
12.	Methyl pyrazine	Aromatic	41%	[136]
13.	Thiazole	Aromatic	41%	[136]

3.3.1.4 Tissue

There were three tissue training aids analysed during the study and a detailed account of the year they were obtained as training aids, the storage conditions, and the months they were sampled can be found in Tables 3.1 and 3.2.

Tissue #1 and #2

Tissue #1 and #2 were obtained from amputation surgery conducted in 2017 and these were analysed in the months of December 2019, July 2020, October 2020, March 2021 and May 2021 thus, generating five samples for each of the two training aids. Throughout the duration of the trial, Tissue #1 was stored at room temperature, while Tissue #2 was initially stored at room temperature and then moved to the refrigerator prior to analysis in March 2021. The VOC profile of the five Tissue #1 samples revealed 30 – 142 VOCs

as statistically significant in the training aid compared to the control samples. The VOC profile of the five Tissue #2 samples revealed 31 – 135 VOCs as statistically significant in the training aid compared to the control samples. Overall, for both Tissue #1 and #2, aromatics (27 – 25% of total no. of VOCs) were the most abundant class, while aldehydes (3 – 4% of total no. of VOCs), acids (1 – 2% of total no. of VOCs) and sulphur-containing VOCs (1 – 2% of total no. of VOCs) were the least abundant classes to be detected.

Tissue #3

Tissue #3 was obtained from amputation surgery conducted in 2020 and analysed in the months of March 2021 and May 2021 thus, generating two samples for each of the two training aids. Throughout the duration of the trial, Tissue #3 was stored at room temperature. The VOC profile of the two Tissue #3 samples revealed 51 – 59 VOCs as statistically significant in the training aid compared to the control samples. Aromatics (32% of total no. of VOCs) and cyclic aliphatics (17% of total no. of VOCs) were the most abundant classes, while aldehydes (2% of total no. of VOCs) and sulphur-containing VOCs (2% of total no. of VOCs) were the least abundant classes to be detected.

The 12 samples generated from analysing the three tissue training aids produced 560 different VOCs which were identified as statistically significant in the samples compared to the controls. The 560 VOCs belonged to all classes of compounds (indicated in Figure 3.5) including acids (n = 12; 0 – 8%), alcohols (n = 60; 2 – 17%), aldehydes (n = 18; 0 – 7%), aromatics (n = 119; 20 – 38%), cyclic aliphatics (n = 46; 0 – 24%), ester and analogues (n = 58; 2 – 13%), ethers (n = 25; 0 – 9%), halogen-containing (n = 31; 0 – 12%), ketones (n = 44; 2 – 10%), linear aliphatics (n = 90; 3 – 19%), nitrogen-containing (n = 52; 7 – 35%), and sulphur-containing VOCs (n = 5; 0 – 3%). Here ‘n’ denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a class for tissue training aid. Thus, aromatics followed by nitrogen-containing and linear aliphatics VOCs were the most abundant classes while acids, aldehydes, sulphur-containing and ethers were the least abundant classes in tissue training aids.

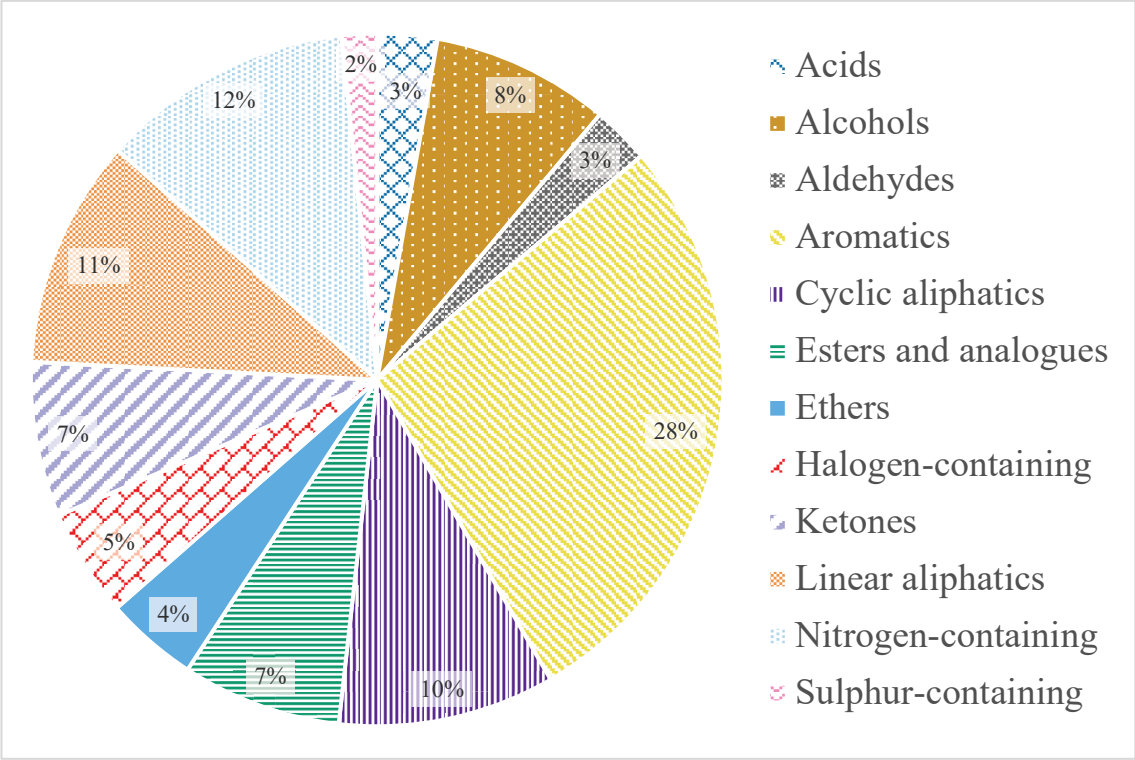


Figure 3.5: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for tissue training aid.

Of the 560 VOCs detected, Table 3.7 presents the 21 most prominently occurring VOCs detected across the 12 samples generated from analysing tissue training aids. These VOCs were detected in at least 40% of samples (five or more samples) ranging up to 100%.

Table 3.7: 21 prominent VOCs detected in over 40% of tissue samples used by the OPP Canine Unit as CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature as human decomposition odour-related
1.	4-Methyl pyrimidine	Aromatic	100%	
2.	2,6-Dimethyl pyridine (2,6-Lutidine)	Aromatic	92%	
3.	2-Methyl pyridine	Aromatic	92%	

4.	2,4,6-Trimethyl pyridine	Aromatic	75%	
5.	Dimethyl trisulphide (DMTS)	Sulphur-containing	75%	[135; 71; 159; 160; 99; 161; 136]
6.	Pyridine	Aromatic	75%	[162; 164; 136; 165]
7.	Trimethylamine	Nitrogen-containing	67%	[136]
8.	Methenamine	Nitrogen-containing	58%	[135; 160; 136]
9.	Methyl pyrazine	Aromatic	58%	[136]
10.	Acetamide	Nitrogen-containing	50%	[136]
11.	1H-1,2,4-Triazole	Cyclic aliphatics	42%	
12.	2,3-Dimethyl pyrazine	Aromatic	42%	[136]
13.	2,4-Dimethyl pyridine	Aromatic	42%	
14.	2,5-Dimethyl pyrazine	Aromatic	42%	[164; 136]
15.	2,5-Dimethyl-1H-pyrrole	Aromatic	42%	
16.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohols	42%	[164; 136; 165]
17.	5-Methyl-2-furancarboxaldehyde	Aldehyde	42%	
18.	Heptanonitrile	Nitrogen-containing	42%	[136]
19.	Methyl isocyanide	Nitrogen-containing	42%	[136]
20.	Trimethyl pyrazine	Aromatic	42%	[164; 136]
21.	Thiazole	Aromatic	42%	[136]

3.3.1.5 Blood

There were three blood training aids analysed during the study and a detailed account of the year they were obtained as training aids, the storage conditions, and the months they were sampled can be found in Tables 3.1 and 3.2.

Blood #1 and #2

Blood #1 and #2 were obtained in 2017 and these were analysed in the months of December 2019, July 2020, October 2020, March 2021 and May 2021 thus, generating five samples for each of the two training aids. Throughout the duration of the trial, Blood #2 was stored at room temperature while Blood #1 was initially stored at room temperature and then moved to the refrigerator prior to analysis in March 2021. The VOC profile of the five Blood #1 samples revealed 30 – 142 VOCs as statistically significant in the training aid compared to the control samples. The VOC profile of the five Blood #2 samples revealed 24 – 159 VOCs as statistically significant in the training aid compared to the control samples. Overall, for both Blood #1 and #2, aromatics (19 – 29% of total no. of VOCs) were the most abundant class, while sulphur-containing VOCs (2 – 4% of total no. of VOCs), and acids and aldehydes (0 – 4% of total no. of VOCs) were the least abundant classes to be detected.

Blood #3

Blood #3 was obtained in 2021 and analysed in the months of March 2021 and May 2021 thus, generating two samples for each of the two training aids. Throughout the duration of the trial, Blood #3 was stored at room temperature. The VOC profile of the two Blood #3 samples revealed 27 – 55 VOCs as statistically significant in the training aid compared to the control samples. Aromatics (21% of total no. of VOCs) and linear aliphatics (18% of total no. of VOCs) were the most abundant classes, while acids (2% of total no. of VOCs) were the least abundant class to be detected.

The 12 samples generated from analysing the three blood training aids had 603 different VOCs which were identified as statistically significant in the samples compared to the controls. The 603 VOCs belonged to all classes of compounds (indicated in Figure 3.6) including acids (n = 13; 0 – 7%), alcohols (n = 65; 0 – 18%), aldehydes (n = 22; 0 – 7%), aromatics (n = 115; 12 – 46%), cyclic aliphatics (n = 56; 0 – 16%), ester and analogues (n = 57; 0 – 12%), ethers (n = 26; 1 – 11%), halogen-containing (n = 44; 0 – 16%), ketones

(n = 58; 3 – 12%), linear aliphatics (n = 78; 3 – 22%), nitrogen-containing (n = 56; 0 – 25%), and sulphur-containing VOCs (n = 13; 0 – 7%). Here 'n' denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a class for blood training aid. Thus, aromatics followed by linear aliphatics, cyclic aliphatics and alcohols were the most abundant classes while aldehydes, sulphur-containing and acids were the least abundant classes in blood training aids.

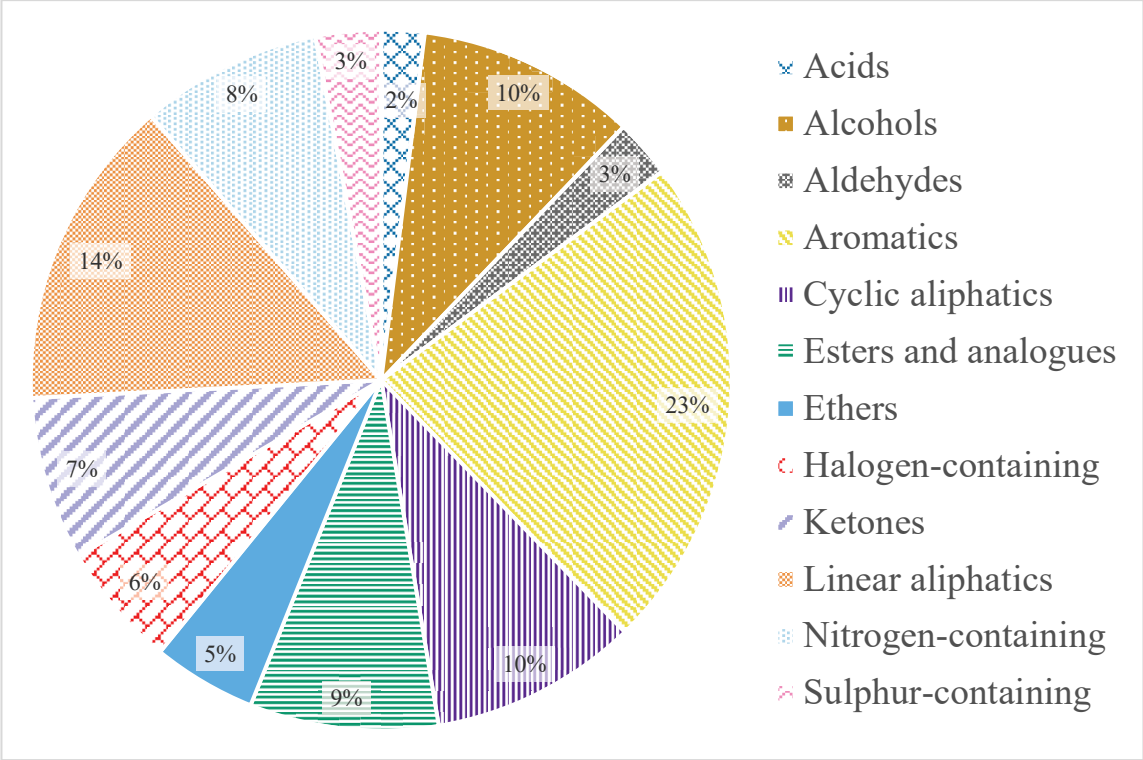


Figure 3.6: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for blood training aid.

Of the 603 VOCs detected, Table 3.8 presents the 15 most prominently occurring VOCs detected across the 12 samples generated from analysing blood training aids. These VOCs were detected in at least 40% of samples (five or more samples) ranging up to 92%.

Table 3.8: 15 prominent VOCs detected in over 40% of blood samples used by the OPP Canine Unit as CDD training aids during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC	Previously reported in literature as human blood or human

			was detected	**decomposition odour-related**
1.	2,6-Dimethyl pyridine (2,6-Lutidine)	Aromatic	92%	[137]
2.	2-Methyl pyridine	Aromatic	67%	[137]
3.	2,5-Dimethyl pyrazine	Aromatic	67%	[137]
4.	4-Methyl pyrimidine	Aromatic	67%	[137]
5.	2-Isobutyl-3-methyl pyrazine	Aromatic	58%	[137]
6.	Dimethyl trisulphide (DMTS)	Sulphur-containing	58%	[135; 71; 159; 160; 99; 161; 136; 137]
7.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohol	50%	[164; 136; 165; 137]
8.	Isobutyronitrile	Nitrogen-containing	50%	
9.	Pyridine	Aromatic	50%	[162; 164; 136; 165; 137]
10.	1,2,3-Trimethyl-cyclohexane	Cyclic aliphatics	42%	
11.	3-Methyl-butanoic acid	Acid	42%	[136; 137]
12.	Methenamine	Nitrogen-containing	42%	[135; 160; 136]
13.	Methylacrylonitrile	Nitrogen-containing	42%	
14.	Pentane	Linear aliphatics	42%	
15.	Trimethyl pyrazine	Aromatic	42%	[137]

3.3.1.6 Teeth

There was one set of teeth training aids analysed during the study and a detailed account of the year they were obtained as training aids, the storage conditions, and the months they were sampled can be found in Tables 3.1 and 3.2.

Teeth #1

Teeth #1 was obtained sometime in 2017 or prior and was analysed in the months of December 2019, July 2020 and October 2020 thus, generating three samples. Throughout the duration of the trial, it was stored at room temperature. The VOC profile revealed 8 – 200 VOCs as statistically significant in the training aid compared to the control samples. A total of 262 different statistically significant VOCs were detected. The remaining VOCs were detected only in one of the three samples. The 262 VOCs belonged to all classes of compounds (indicated in Figure 3.7) including acids (n = 4; 0 – 2%), alcohols (n = 20; 3 – 25%), aldehydes (n = 9; 0 – 4%), aromatics (n = 71; 13 – 30%), cyclic aliphatics (n = 24; 8 – 25%), ester and analogues (n = 21; 0 – 10%), ethers (n = 7; 0 – 3%), halogen-containing (n = 18; 7 – 13%), ketones (n = 22; 0 – 10%), linear aliphatics (n = 41; 0 – 25%), nitrogen-containing (n = 23; 3 – 25%), and sulphur-containing VOCs (n = 2; 0 – 2%). Here 'n' denotes the total number of VOCs in the compound class while the % value represents the average percentage of VOCs present in a class for the teeth training aid. Thus, aromatics followed by linear aliphatics were the most abundant classes while aldehydes, ethers, sulphur-containing VOCs, and acids were the least abundant classes in teeth training aids.

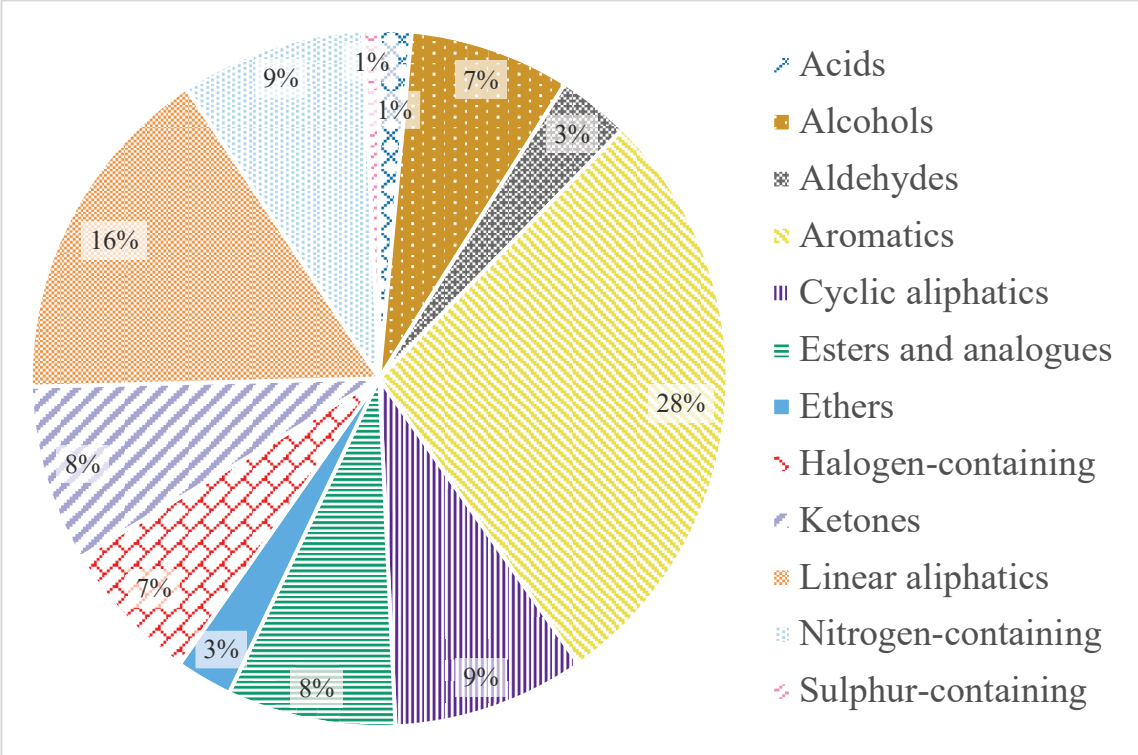


Figure 3.7: Abundance of average number of VOCs (represented as percentage of total VOCs) per compound class for the teeth training aid.

Of the 262 VOCs detected, Table 3.9 presents six VOCs that were detected in two of the three samples collected from the one set of teeth used as CDD training aids by OPP. Thus, these six VOCs were detected in over 67% (2 of 3) of the samples.

Table 3.9: Six prominent VOCs detected in over 67% of teeth used by the OPP Canine Unit as CDD training aids and analysed during multiple trials conducted between December 2019 to May 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature as human decomposition odour-related
1.	1,3-Diethyl-benzene	Aromatic	67%	
2.	1,6,7-Trimethyl-naphthalene	Aromatic	67%	
3.	2-Methyl-1,1'-biphenyl	Aromatic	67%	
4.	4-Ethyl-3-heptene	Linear aliphatics	67%	
5.	Aniline	Aromatic	67%	[167]
6.	Octahydro-pentalene	Linear aliphatics	67%	

3.3.2 Normalised area based concentrations

The detected VOCs in each of the samples were normalised using an internal standard – bromobenzene. This semi-quantitative approach was used to study the relative class concentration of compound classes within each of the training aids and the change in VOC concentration over the duration of this study in some prominent VOCs which is discussed further in this section.

3.3.2.1 Relative class concentration

Figure 3.8 represents the overall average relative class concentrations of the compound classes detected in CDD training aids. This figure highlights that among all the samples, alcohol followed by nitrogen-containing VOCs and aromatics had the highest relative class concentrations. Acids, linear aliphatics, esters and analogues, ketones, halogen, and

sulphur-containing VOCs had an average relative class concentration. The remaining classes including cyclic aliphatic VOCs, aldehydes and ethers had the least relative class concentration.

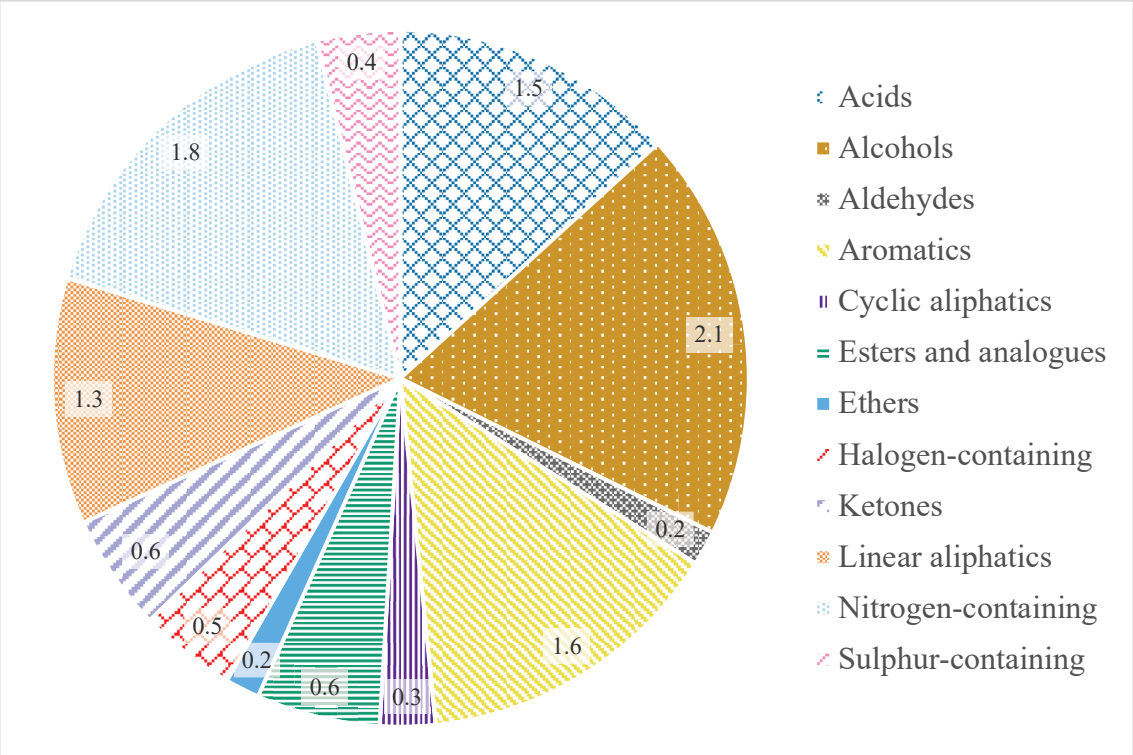


Figure 3.8: Relative compound class concentration determined by average normalised area of the VOCs in the classes detected in CDD training aids during multiple trials conducted between December 2019 to May 2021.

Figure 3.9 represents the average concentration of the compound classes detected in each of the training aids across several samples analysed during the multiple trials which were conducted between December 2019 to May 2021. Overall, the foot samples which were stored indoors had the highest relative class concentration of all compound classes compared to any other type of training aids followed by bone, teeth, tissue, blood and foot training aids stored outdoors. The relative class concentration of training aids from 2017 in most training aid types (foot stored indoors, tissue and blood) was higher compared to the most recent samples from 2019. For example, the average relative class concentration of Foot #1 – #5, Tissue #1 – #2 and Blood #1 – #2 (all of which were relatively aged) was higher than that of recent training aids Foot #6 – #9, Tissue #3 and Blood #3, respectively. In the case of bone, the aged and recent training aids all showed approximately the same average relative class concentration. Training aid comparisons and interpretations are further discussed in section 3.4.

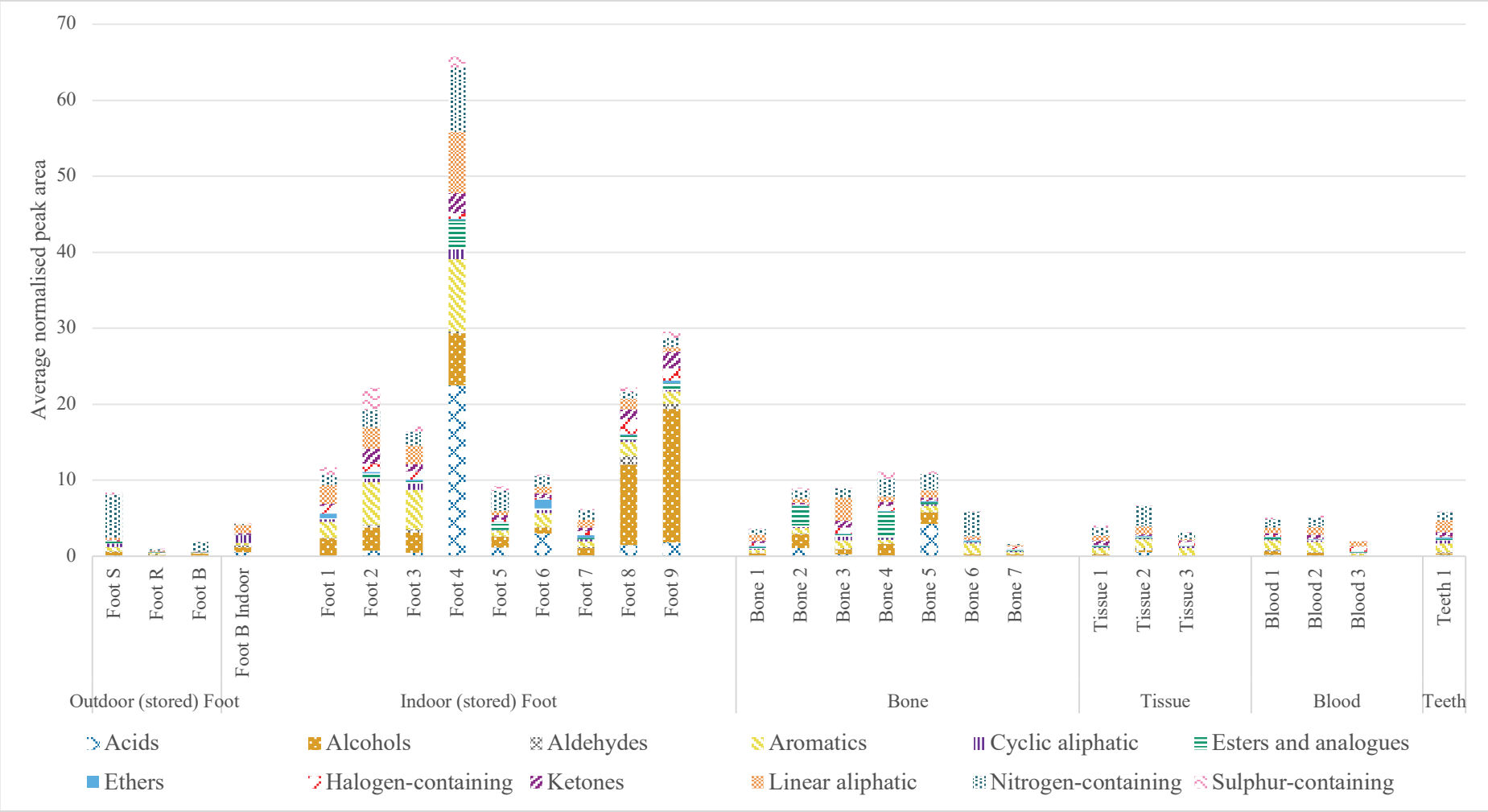


Figure 3.9: Sum of average normalised areas indicating the relative class concentrations for all compound classes in each CDD training aid analysed during multiple trials conducted between December 2019 to May 2021.

3.3.2.2 Change over time

To understand the change over the duration of the study period, the change in normalised areas of the three most prominently occurring VOCs – 2,6-dimethyl pyridine; dimethyl trisulphide; and 2-methyl pyridine were visualised. These have been represented in Figures 3.10, 3.11, and 3.12, respectively. As determined from these figures, there was no trend observed for any of the three VOCs, however, the relative class concentrations were reduced during the months of December 2019 and October 2020, while they were relatively higher in the remaining months. A potential explanation for this trend is discussed in section 3.4

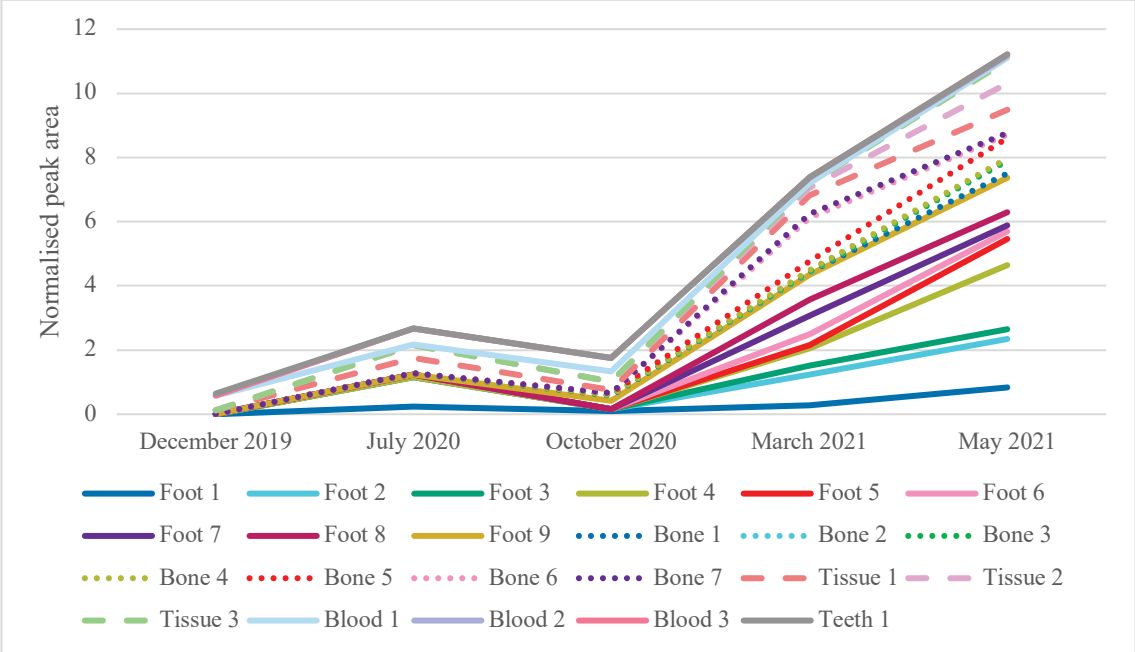


Figure 3.10: Trend of 2,6-dimethyl pyridine (2,6-lutidine) in CDD training aids over a span of 1.5 years observed with samples collected in December 2019, July 2020, October 2020, March 2021 and May 2021.

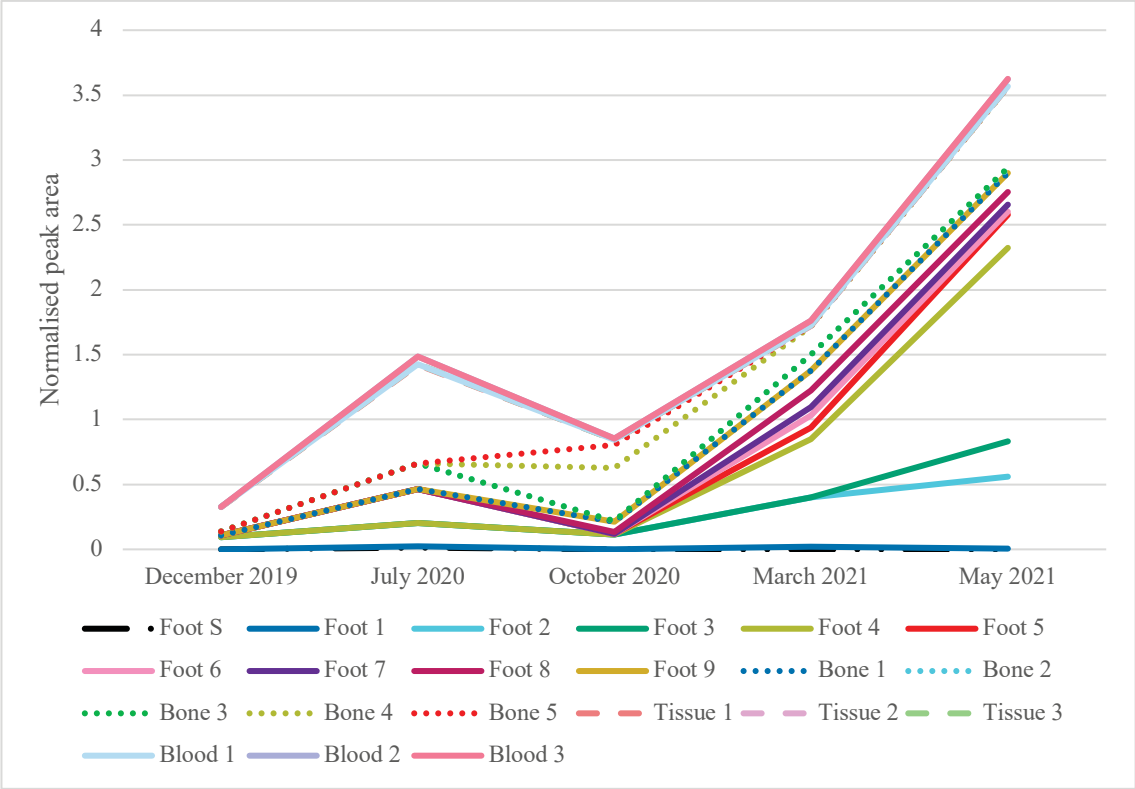


Figure 3.11: Trend of dimethyl trisulphide (DMTS) in CDD training aids over a span of 1.5 years observed with samples collected in December 2019, July 2020, October 2020, March 2021 and May 2021.

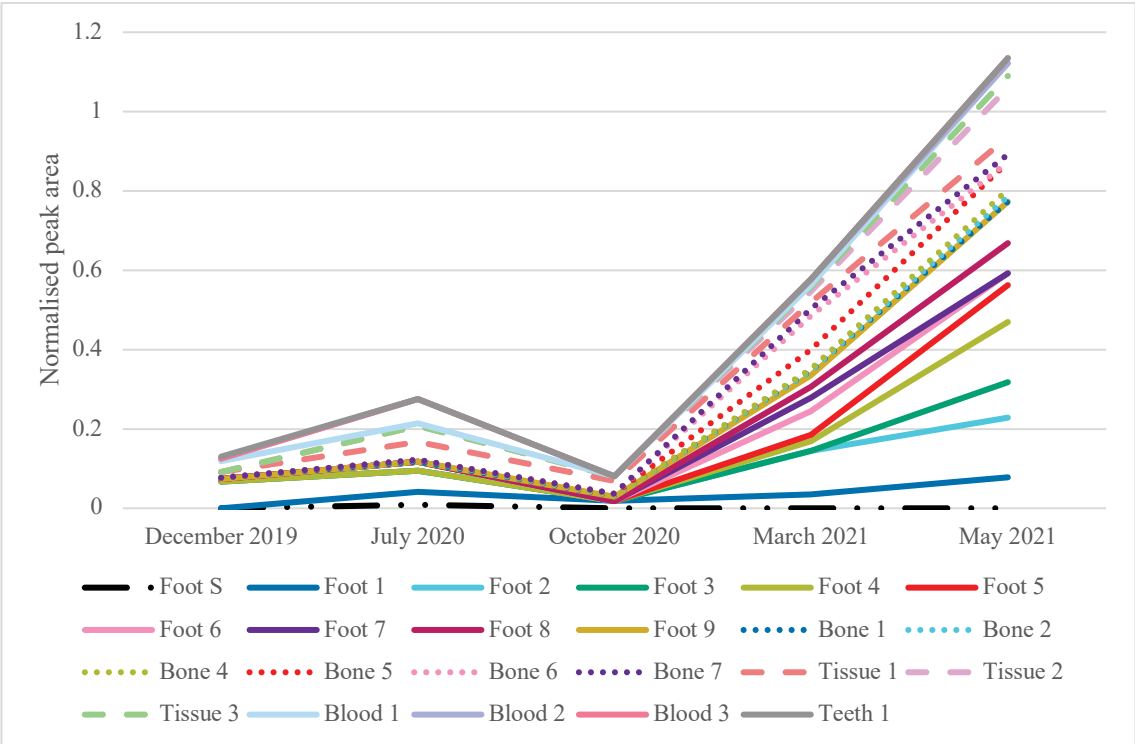


Figure 3.12: Trend of 2-methyl pyridine in CDD training aids over a span of 1.5 years observed with samples collected in December 2019, July 2020, October 2020, March 2021 and May 2021.

3.3.3 Impact of ageing and storage conditions

Principal component analysis (PCA) was performed to cluster and allow for dimension reduction of training aids to understand the variability (if any) due to ageing and storage conditions of the foot samples (stored indoors) and bone training aids. Tissue, teeth and blood samples did not have sufficient data points for ageing or storage sample groups. Additionally, these were missing one or more of the three storage conditions thus, they were not included in the data analysis. In contrast, both the foot samples stored indoors and bones had all three storage conditions along with aged and relatively recent samples thus, they were included in the current analysis. PCAs were constructed using normalised areas for the VOCs identified as most prominent in the two types of training aids (foot stored indoors and bones). This included VOCs that occurred in over 50% of the foot samples stored indoors and over 40% of bone samples, all of which have been listed in Tables 3.5 and 3.6, respectively. The construction of PCAs was based on normalised areas of prominent VOCs only to avoid over-weighting of significant VOCs (occurring only in a few samples) in the analysis.

3.3.3.1 Ageing

Foot

Figure 3.13 represents a PCA based on 12 VOCs (detected in at least 50% of the foot samples stored indoors) to attempt to explain the variability due to the ageing of these samples. For this, the 29 samples from nine foot training aids were grouped into two sample sets – training aids obtained in 2017 (Foot #1 – #5) that had aged for longer than those obtained in 2019 (Foot #6 – #9). The resulting PCA highlighted 98% and 1% of the explained variance along PC-1 and PC-2, respectively (cumulative: 99%). The associated loadings plots (represented as a biplot in Figure 3.14) were used to determine which VOCs were important to the construction of specific principal components (PCs) and samples. This PCA elucidated that training aids obtained in 2017 and 2019 had variability predominantly along PC-1. As evident from the loadings, this variance was owing to the presence of 3-methyl-1-butanol and sevoflurane that dominated 2019 samples and most specifically Foot #9 (May 2021). The training aids obtained in 2017 had subtle variability along PC-2 and of these samples, in particular, extremes were observed for Foot #4 and #2 (May 2021) owing to the relatively high abundance of 2,6-dimethyl pyridine, dimethyl sulphide and methenamine compared to the remaining 2017 samples. Thus, the 2017 and

2019 training aid samples had high inter-sample set variability and the 2019 samples distributed along PC-1 had greater intra-sample set variability compared to those obtained in 2017. The PCA also highlights a cluster of samples, generally belonging to the early sampling months of December 2019, July 2020, and October 2020, while the samples that became increasingly variable from the cluster belonged to the later sampling months of March 2021 and May 2021.

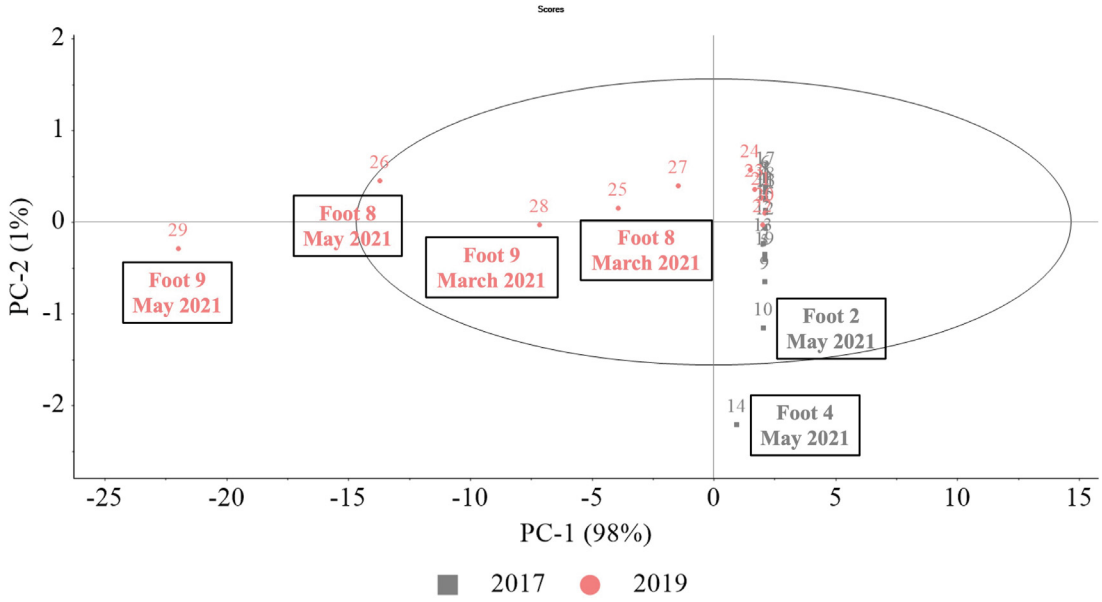


Figure 3.13: (Ageing of foot) PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 12 prominent VOCs in the nine foot training aids (Foot #1 – #9) stored indoors generating 29 samples (or data points in the above PCA) from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent the year OPP acquired these as training aids, grey squares for the year 2017 samples and pink circles for the year 2019).

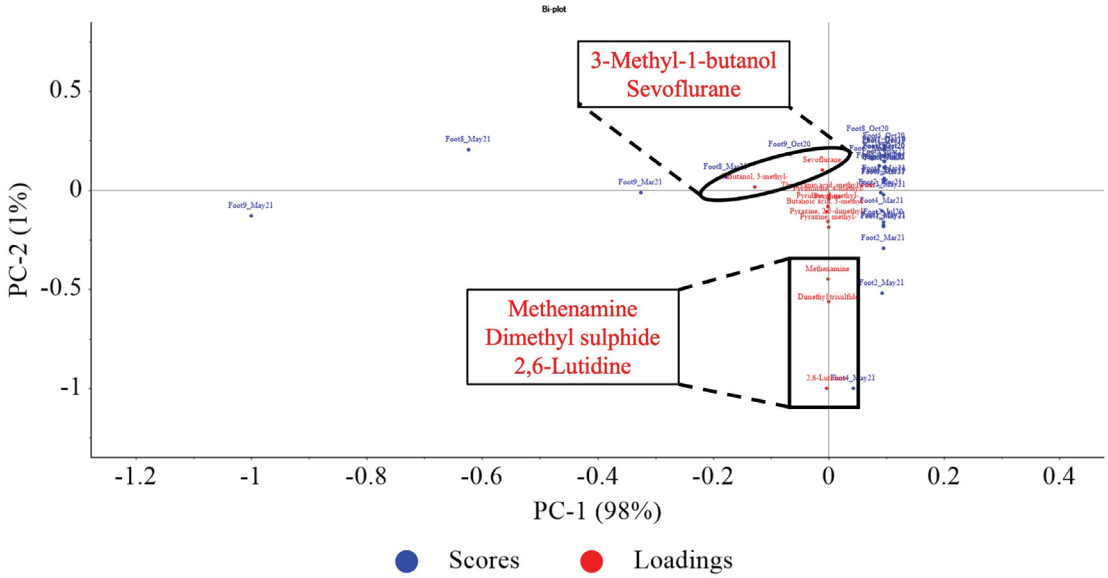


Figure 3.14: (Ageing of foot) PCA biplot for PC-1, PC-2 of the nine foot training aids (Foot #1 – #9) stored indoors generating 29 samples from analyses conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, blue circles represent scores [foot samples] and red circles represent loading [VOCs]).

To further confirm the inter-sample set variability between 2017 and 2019 samples, the extreme loadings of Foot #9 and #8 both from March and May 2021 were removed and a new PCA was constructed. This is represented in Figure 3.15 where PC-1 and PC-2 have 51% and 32% of the explained variance, respectively, a dramatic difference from the 98% and 1% of Figure 3.13. The reduced explained variance value implies that the four extreme samples which were removed dominated the variability. Even with this, some variability between the foot training aids stored indoors persisted due to the presence of specific VOCs (as elaborated in the previous paragraph) that dominated each of the sample sets.

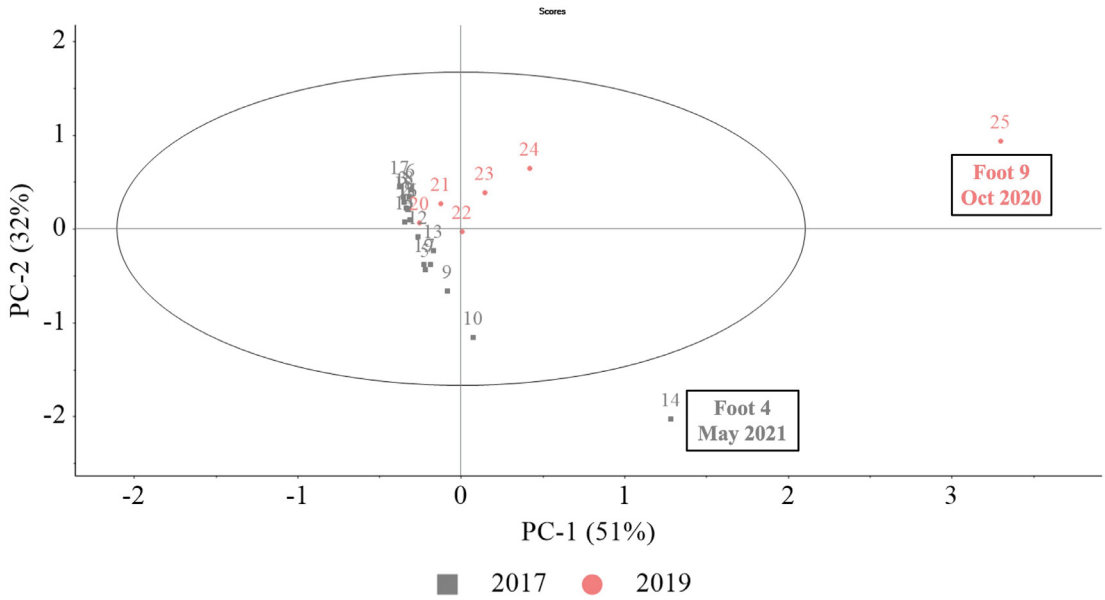


Figure 3.15: (Ageing of foot) PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 12 prominent VOCs in 25 samples (after removing Foot #9 and #8 – March 2021 and May 2021) generated from the nine foot training aids stored indoors (or data points in the above PCA) obtained from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent the year OPP acquired these as training aids, grey squares for the year 2017 samples and pink circles for the year 2019).

Bone

Figure 3.16 represents a PCA based on 13 VOCs (detected in at least 40% of the bone samples) to understand the variability due to the ageing of bones. For this, the 22 samples from seven bone training aids were grouped into two sample sets – training aids obtained in 2017 (Bone #1 – #3) that had aged for longer than those obtained in 2020 (Bone #4 – #7). The resulting PCA showed 61%, 17%, 8%, 6% and 4% of explained variance along PC-1, PC-2, PC-3, PC-4 and PC-5, respectively (cumulative: 96%). The variability between the two sample sets was distinct along PC-5 (4% variance – represented in Figure 3.15). The associated loadings plots (represented as a biplot in Figure 3.17) were used to determine which VOCs were important to the construction of specific PCs and samples. This PCA elucidated that the 2017 training aids visible along the negative PC-5 were dominant in sevoflurane and 3-methyl-1-butanol. The 2020 training aids visible along positive PC-5 were dominant in dimethyl trisulphide, 3-methyl-butanoic acid and 3-sulfo-L-alanine. Thus, the two sample sets were found to be subtly variable as the explained variance along PC-5 was only 4%. Some variability was also observed along PC-1 where, 2-methyl-propanoic acid was relatively dominant in two of 2017 samples – Bone 2 (July

2020 and May 2021) compared to two of the 2020 samples – Bone 5 (March 2021 and May 2021).

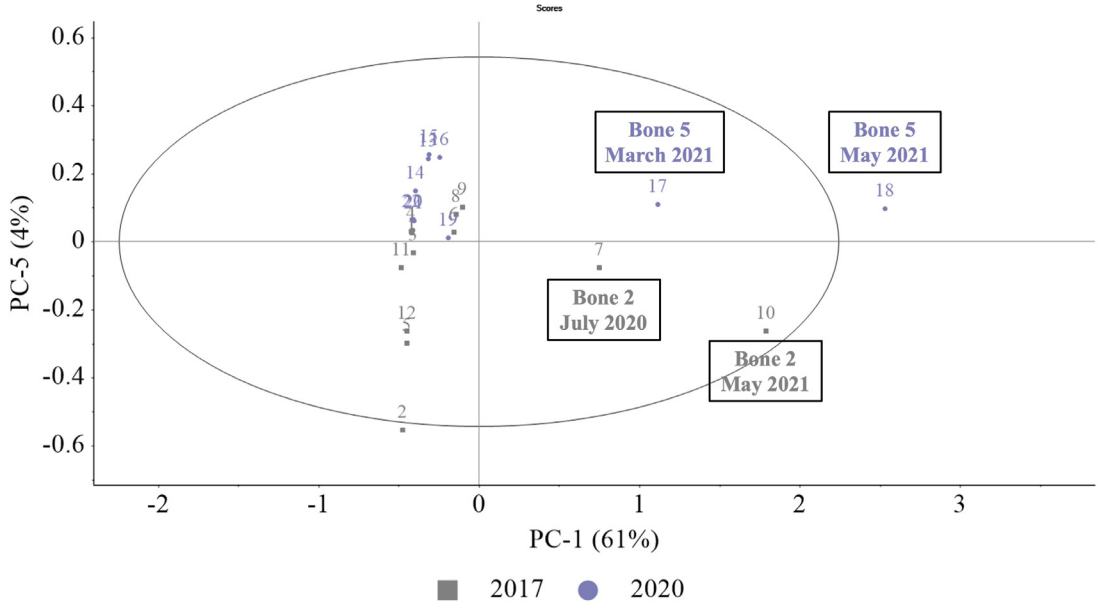


Figure 3.16: (Ageing of bone) PCA scores plot for PC-1, PC-5. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 13 prominent VOCs in 22 samples (or data points in the above PCA) generated from seven bone training aids (Bone #1 – #7) from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent the year OPP acquired these as training aids, grey squares for the year 2017 samples and lavender circles for the year 2020).

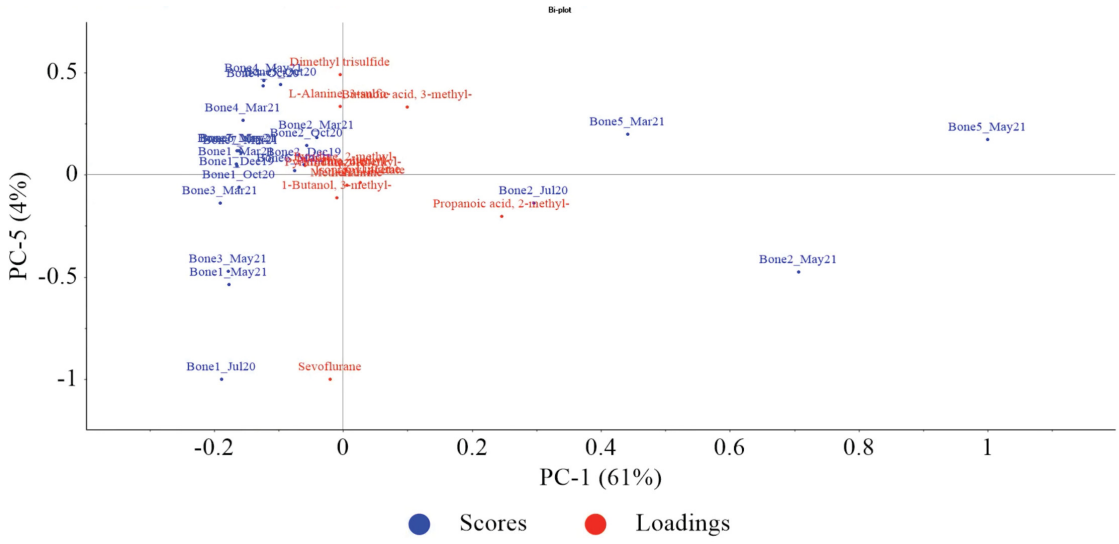


Figure 3.17: (Ageing of bone) PCA biplot for PC-1, PC-5 of the seven bone training aids (Bone #1 – #7) stored indoors generating 22 samples from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, blue circles represent scores [bone samples] and red circles represent loading [VOCs]).

3.3.3.2 Storage

PCA for storage was performed only on the 2017 training aids – Foot #1 – #5 (for foot samples stored indoors) and Bone #1 – #3 (for bone) to determine the variation between storage conditions (room temperature, freezer and refrigerator). The 2017 samples were chosen as they included all three storage conditions, while 2019 (in the case of foot) and 2020 (in the case of bone) samples included mostly only one of the three storage conditions. Foot #8 and #9 were stored in the freezer in October 2020 however, their storage condition was changed to refrigerator prior to the following analysis in March 2021. Thus, analysing the 2019 foot samples would have only included two data points for freezer storage, eight for refrigerator storage and none for room temperature. Similarly, all the 2020 bone samples were only stored at room temperature thus there were no data points for freezer or refrigerator storage. Considering these factors, the less aged samples (2019/2020) for the two training aid types were not used to analyse the variability due to storage conditions.

Foot

Figure 3.18 represents the PCA of all 2017 foot samples stored indoors (19 samples from Foot #1 – #5) constructed to decompose the variability contributed by the storage conditions. Like ageing, the PCA was based on the top 12 most prominent VOCs listed in Table 3.5. In the resulting PCA, PC-1 had the highest explained variability of 67%, and PC-2 and PC-3 had an explained variability of 15% and 9% (cumulative: 91%). The associated loadings plots (represented as a biplot in Figure 3.19) were used to determine which VOCs were important to the construction of specific PCs and samples. The variability among room temperature stored samples and refrigerator samples were distinct along PC-1 and PC-3 (Figure 3.18). None of the other PCs showed any distinct variability among the three sample sets. The variability in room temperature and refrigerator stored samples was dictated by the dominance of 3-methyl-butanoic acid, methenamine, 3-methyl-1-butanol and DMTS in room temperature storage and methyl pyrazine, 2,6-dimethyl pyridine, 2,5-dimethyl pyrazine and sevoflurane in the refrigerator stored samples. No clustering was observed for the samples stored in the freezer however, relative to all samples, a sample stored in the freezer – Foot #4 (May 2021) was extreme.

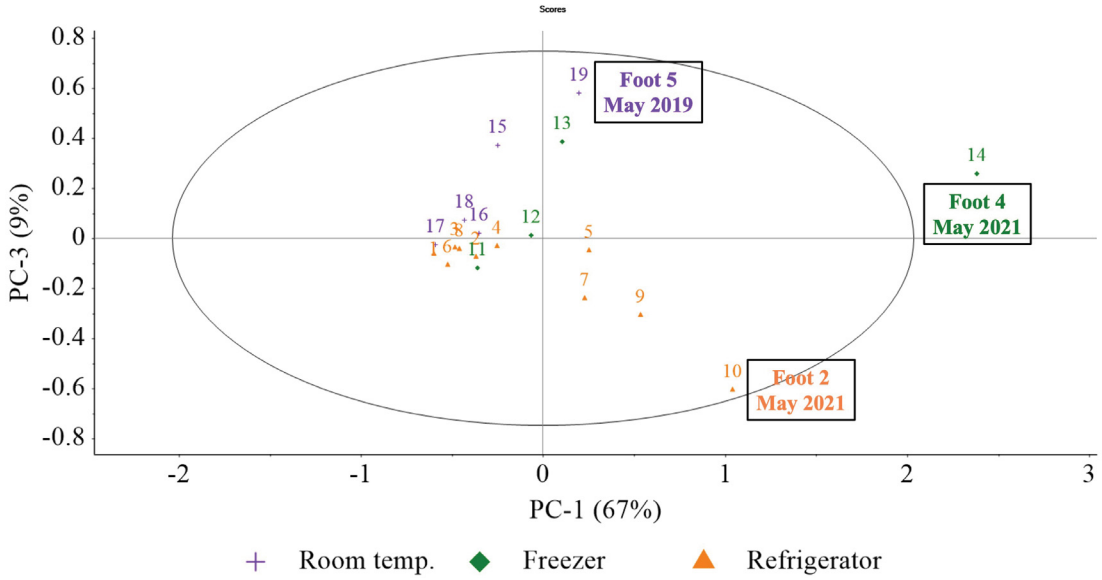


Figure 3.18: (Storage of foot) PCA scores plot for PC-1, PC-3. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of the 12 prominent VOCs in five foot training aids stored indoors (Foot #1 – #5) generating 19 samples (or data points in the above PCA) from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent storage condition of the samples, purple crosses for room temperature, green diamonds for freezer and orange triangles for refrigerator).

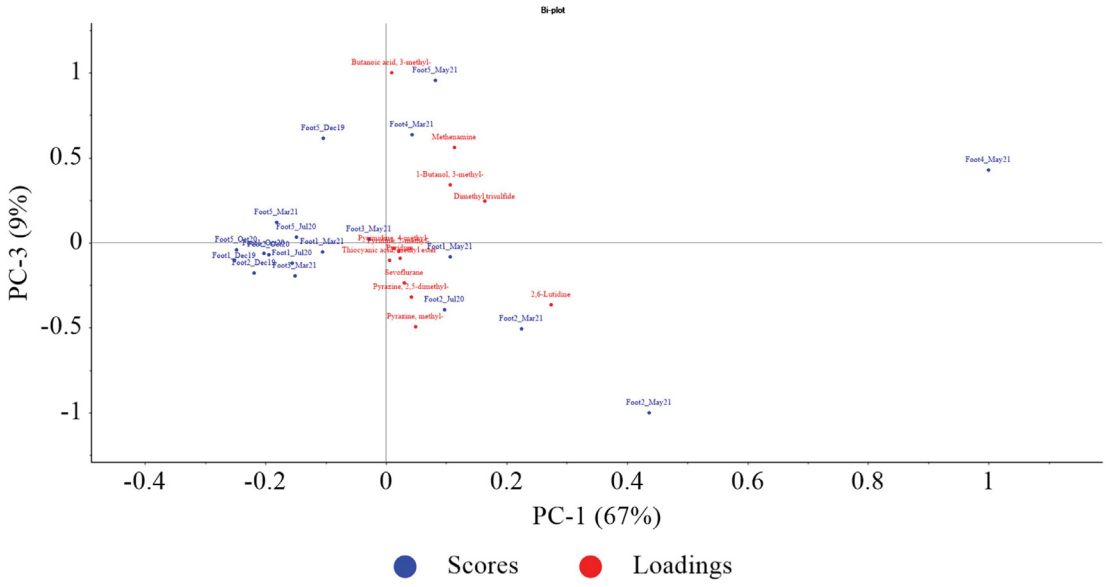


Figure 3.19: (Storage of foot) PCA biplot for PC-1, PC-3 of the five foot training aids (Foot #1 – #5) stored indoors generating 19 samples from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, blue circles represent scores [foot samples] and red circles represent loading [VOCs]).

Bone

Figure 3.20 (PC-1, PC-2) and 3.22 (PC-1, PC-5) represent the PCA of all 2017 bone training aids (12 samples from Bone #1 – #3), constructed to decompose the variability

contributed by the storage conditions. Like ageing, the PCAs were based on the top 13 most prominent VOCs listed in Table 3.6. For the resulting PCA, PC-1 had the highest explained variability of 72% followed by 16%, 7%, 3% and 2% for PCs 2, 3, 4 and 5 (cumulative: 100%). The associated loadings plots (represented as biplots in Figures 3.21 and 3.23) were used to determine which VOCs were important to the construction of specific PCs and samples. Figure 3.17 highlighted that samples stored at room temperature were variable along PC-1 compared to the other two storage conditions. This was due to the dominance of 2-methyl-propanoic acid and isopropyl acetate in room temperature samples compared to other storage conditions. Among the refrigerator and freezer stored samples, Bone #3 (March 2021) was most variable along PC-2 due to the dominant presence of 3-sulfo-L-alanine and sevoflurane in the samples relative to the remaining samples.

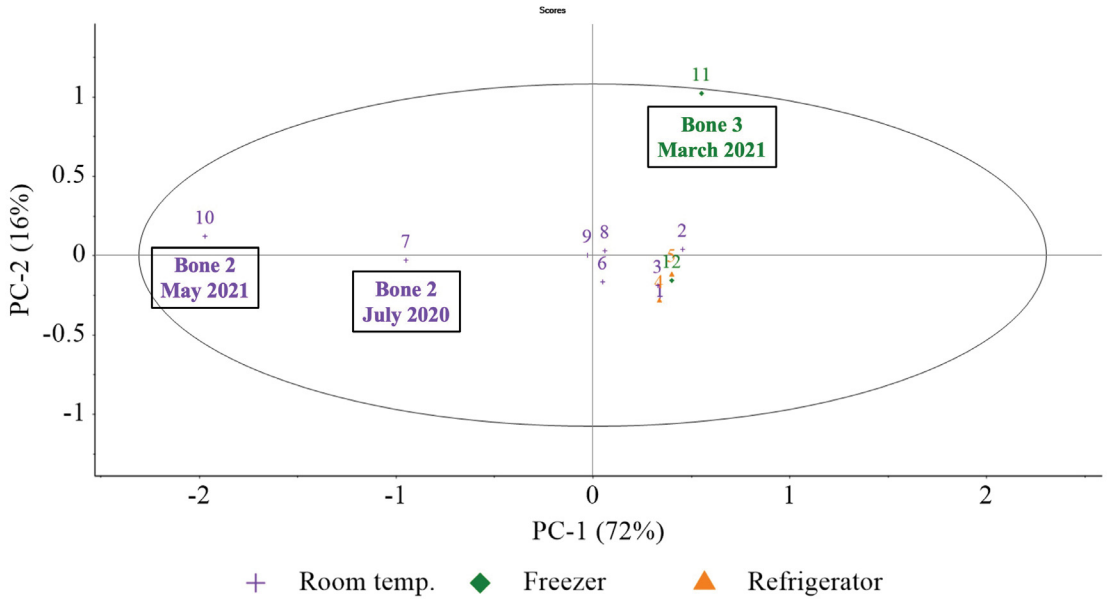


Figure 3.20: (Storage of bone) PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of the 13 prominent VOCs in three bone training aids (Bone#1 – #3) generating 12 samples (or data points in the above PCA) from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent storage condition of the samples, purple crosses for room temperature, green diamonds for freezer and orange triangles for refrigerator).

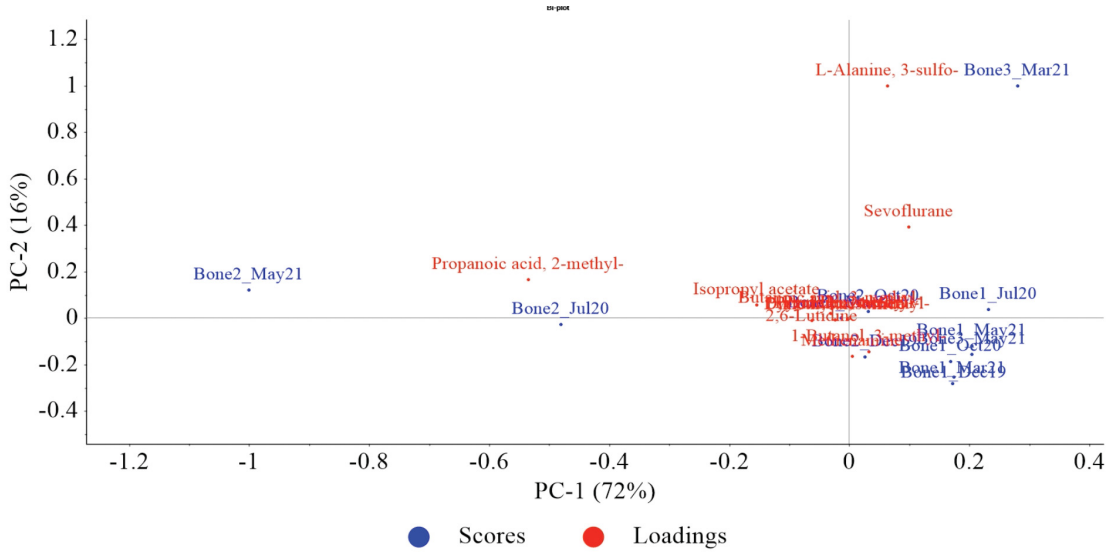


Figure 3.21: (Storage of bone) PCA biplot for PC-1, PC-2 of the three bone training aids (Bone #1 – #3) stored indoors generating 12 samples from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, blue circles represent scores [bone samples] and red circles represent loading [VOCs]).

The variability among all the three storage conditions became most distinct along PC-5 (represented in Figure 3.22) however, the variability was subtle since the explained variance along PC-5 was only 2%. This variability was due to the dominance of 3-methyl-butanol and 2,6-dimethyl pyridine in refrigerator and freezer samples and DMTS in room temperature samples.

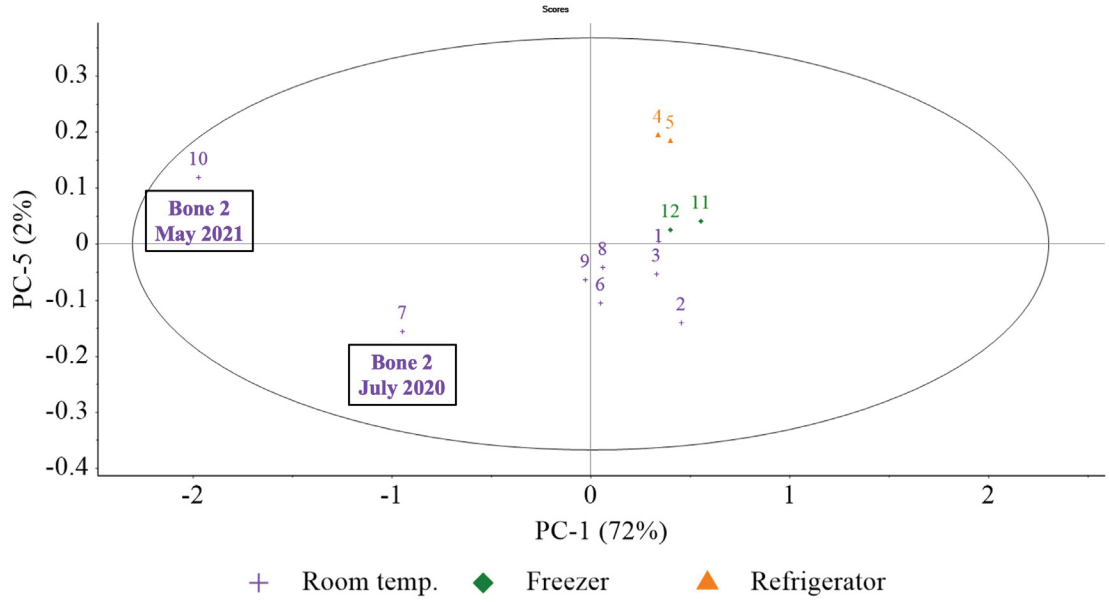


Figure 3.22: (Storage of bone) PCA scores plot for PC-1, PC-5. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of the 13 prominent VOCs in bone samples (Bone#1 – #3) generating 12 samples (or data points in the above PCA) from analysis conducted in five different months – December 2019,

July 2020, October 2020, March 2021 and May 2021. (Here, colour codes and symbols represent storage condition of the samples, purple crosses for room temperature, green diamonds for freezer and orange triangles for refrigerator).

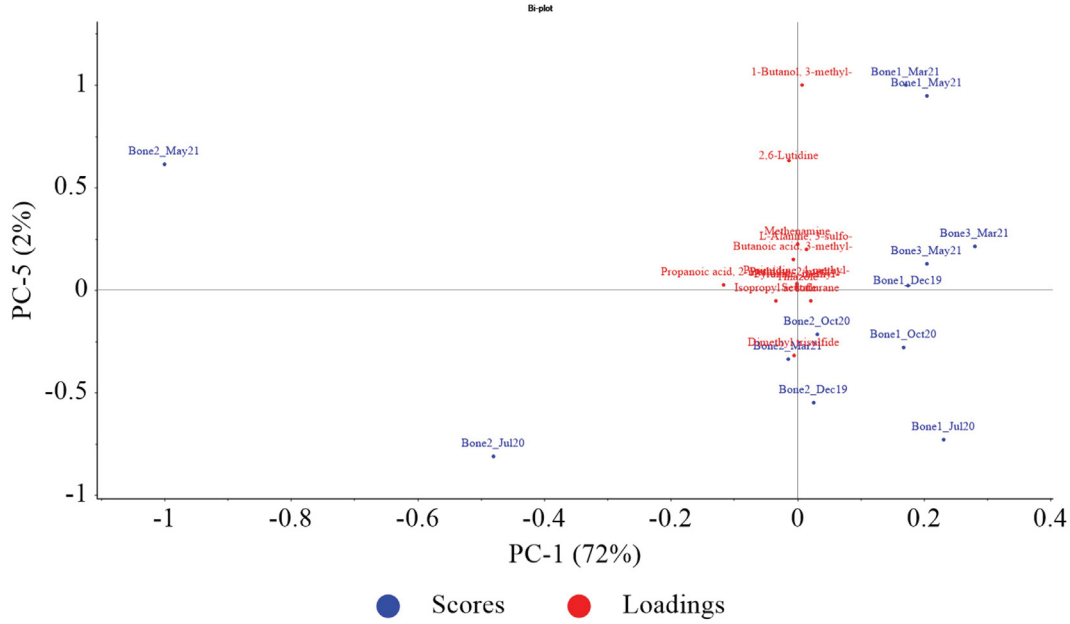


Figure 3.23: (Storage of bone) PCA biplot for PC-1, PC-5 of the three bone training aids (Bone #1 – #3) stored indoors generating 12 samples from analysis conducted in five different months – December 2019, July 2020, October 2020, March 2021 and May 2021. (Here, blue circles represent scores [bone samples] and red circles represent loading [VOCs]).

3.4 Discussion

3.4.1 Class abundance and relative class concentrations

All VOC classes mentioned previously were detected in each CDD training aid used by the OPP Canine Unit. The trends in class abundance (indicated in Figures 3.1 to 3.7) based on the number of VOCs per class largely remained similar across all training aid types with only subtle variations. This study could categorise the several classes of compounds into high, average and low abundance. Generally, aromatics, linear aliphatics, esters and analogues and alcohols showed high abundance, while nitrogen-containing VOCs, ketones, cyclic aliphatics and halogen-containing VOCs showed average abundance. The remaining classes ethers, aldehydes, acids and sulphur-containing VOCs showed low abundance. Similarly, the average relative class concentration (indicated in Figure 3.8) was similar where, aromatics and alcohols along with nitrogen-containing VOCs had high average relative class concentrations, while aldehydes, ethers, sulphur-

containing VOCs along with cyclic aliphatic VOCs had low average relative class concentrations. Thus, while the number of VOCs in the aromatic class was the highest, the relative class concentration of alcohols remained dominant. Similarly, while the fewest number of VOCs (indicating low class abundance) were present in ether, aldehydes, acids and sulphur-containing VOC classes, ethers had the lowest relative class concentration overall.

The relative class concentration based on normalised areas (Figure 3.9) indicated that foot samples stored indoors had the highest class concentration relative to other training aids. This could be due to the fact that foot samples stored indoors had an intense odour (perceived by the researcher) potentially owing to the presence of large amounts of organic matter and decomposition fluid (compared to other training aids stored indoors like bone, tissue, blood) along with their storage in small containers (compared to outdoor storage). The figure also highlights that bone had a higher relative class concentration compared to tissue. In the current study, only Bone #1, #6 and #7 were dry bones while Bone #2, #3, #4 and #5 still had minimal tissue and/or decomposition fluid present. Thus, the presence of tissue and decomposition fluid in the bone samples could have resulted in relatively higher class concentrations compared to tissue. Furthermore, it is evident from Figure 3.9 that the dry bones – Bone #1, #6 and #7 had a reduced relative class concentration compared to other wet bone training aids. This is consistent with observations made with foot training aids stored indoors relative to other samples. Thus, it can be concluded that a larger sample with a greater amount of organic matter (tissue) or decomposition fluid has a higher relative class concentration of VOC classes. Likewise, when comparing the tissue training aids, Tissue #3 had a lower relative VOC class concentration compared to Tissue #1 and #2 and this could be because Tissue #3 was a section of mummified skin, unlike Tissue #1 and #2 which were relatively wet samples. In the case of Blood, #3 had a lower number of VOCs and low relative VOC class concentration compared to Blood #1 and #2. This could be due to the fact that Blood #3 was a fresh blood sample while the other two were relatively degraded. A prior study conducted on the effect of weathering of blood used as a training aid reported that weathered blood samples had a reduced number of VOCs compared to fresh samples which were contrary to the results of the current study [137]. The prior study considered blood samples which were 0 – 1 day old as fresh while those older than a week were classed as weathered [167]. However, in the current study, Blood #3 which was already

over a week old the first time it was analysed and was considered fresh relative to Blood #1 and #2 (over two years old at the time of first analysis). Thus, variability in the duration from when the samples were collected and analysed could have led to varying results. Another reason for the difference could be that the blood samples were allowed to naturally weather in the prior study however, the samples in the current study were preserved and stored in the refrigerator or at room temperature. Among the foot training aids stored outdoors, the relative VOC class concentration of the foot decomposing on the surface (Foot #S) was higher than that of the one hidden in rock (Foot #R) or buried (Foot #B). Additionally, when the buried foot was exhumed and VOCs were collected indoors (Foot #B Indoor), a higher relative VOC class concentration was detected compared to when it was analysed in the buried scenario. This highlights that burying the training aid can reduce the VOC concentration detectable at the surface level. Furthermore, the aged samples generally had a higher relative class concentration of VOC classes relative to the less aged samples. This could be due to the fact that the training aids from 2017 are more degraded than the more recent samples from 2019 and 2020.

3.4.2 Three prominent VOCs in all CDD training aids

This study reported several specific compounds that were prevalent in CDD training aids used by the OPP Canine Unit (indicated in Table 3.3). 2,6-dimethyl pyridine (2,6-lutidine), 2-methyl pyridine and dimethyl trisulphide (DMTS) were identified as the three most statistically significant VOCs to CDD training aids. 2,6-dimethyl pyridine and 2-methyl pyridine are heterocyclic aromatic substituted pyridines. Pyridine and its many derivatives have previously been reported in decomposition odour and it has been suggested that pyridine can originate from vitamin B3 [85; 164; 165; 168]. 2,6-dimethyl pyridine has only been reported in human blood decomposition odour while 2-methyl pyridine has been reported both in human blood and decomposition fluid VOC profile [136; 137]. A majority of decomposition odour-related studies have determined that sulphur-containing VOCs such as dimethyl sulphide (DMS), dimethyl disulphide (DMDS) and DMTS are the most significant class of VOCs as they are repeatedly reported. They are responsible for imparting the characteristic foul decomposition smell, and are semiochemicals that attract forensically-relevant insects to the decomposing remains [135; 98; 169; 9]. Sulphur-containing compounds are produced during the bacterial-driven breakdown of amino acids that contain sulphur (e.g. cysteine,

methionine) [72]. Thus, the presence of DMTS in all of the amputated lower limbs/feet training aids in the current study is significant when comparing their odour profile to cadavers.

The trends in the change in relative concentration (based on normalised areas) of 2,6-dimethyl pyridine, 2-methyl pyridine and DMTS (Figure 3.10 – 3.12) revealed relatively higher concentrations in the months of July 2020, March 2021 and May 2021 compared to December 2019 and October 2020. This could be due to higher ambient temperatures in the months of July 2020, March 2021 and May 2021 in the sample collection room. July coincides with the Canadian summer while October is the Canadian autumn, and December and October are relatively cooler. A limitation of this study was that ambient temperature data was not collected during these trials (recommended for future studies) however, at the time of sample collection, even though the samples were collected indoors, the room was not thermally regulated in December 2019 and October 2020 which would have led to colder temperatures indoors compared to July 2020, March 2021 and May 2021 when the room was heated. No steady trends in VOC concentrations were observed in the current study over a span of 1.5 years, it is possible that future studies focusing on longer study durations may be able to identify a trend.

3.4.3 VOCs in amputated lower limbs/feet

The most significant training aids in the current study included the foot, bone and tissue all of which originated from the surgically removed amputated lower limbs/feet of diabetic patients. Among all the training aids originating from amputated lower limbs (foot, tissue and bone), the feet that were decomposing outdoors had the least number of prominent VOCs (only one – methyl thiocyanate) which was consistent with the commonly detected VOCs in the training aids' VOCs (Table 3.3), while foot samples stored indoors had the most VOCs in common. The difference in VOCs detected in indoor versus outdoor storage even though they both originated from amputated lower limbs could be due to the difference in the decomposition process since factors such as ambient temperature, storage conditions, etc. varied greatly in the two scenarios [165; 163] thus, potentially leading to different VOC profiles. One other reason for not detecting some VOCs in the outdoor samples could be that the air inside the storage container became concentrated with VOCs while the air surrounding the outdoor training aids is constantly

dissipated. Furthermore, the Foot #S decomposing on the surface had the largest number of detected VOCs compared to Foot #R and #B which were hidden in rocks and buried, respectively. The lesser VOCs detected in Foot #R could be attributed to variability in the sample collection method whereby the air accumulation step was skipped since the hood could not be placed over the rocks and the researcher refrained from disturbing the training aid for sample collection. The Foot #B had fewer VOCs detected when it was buried than when it was exhumed and sampled, therefore, emphasising the fact that concealing the training aids can reduce the detectable odour and this can be a way to challenge CDD during their outdoor searches, eventually helping to improve their capability. The variation in buried and surface training aids could be due to variable decomposition process caused by concealing decomposition remains as reported previously [170]. Furthermore, 75 VOCs were detected in Foot #S when it was sampled outdoors in July 2020, whereas when it was sampled indoors as Bone #4 and #5 in October 2020, 120 and 57 VOCs were detected, respectively. A reason for detecting a higher number of VOCs in Bone #4 could be that it was being sampled indoors and also that it had a lot of organic matter (toes/finger tissue) still present while Bone #5 had some organic matter but was relatively dry. Thus when comparing VOCs in Foot #S and Bone #4, where both samples had organic material present, it is likely that the sample location had an impact on VOCs being detected. Thus, there is a difference in the VOC profile of samples based on the location of sample collection (indoor vs. outdoor).

3.4.3.1 Amputation procedure-related VOCs

One non-decomposition-related compound, sevoflurane, was identified in 69% of foot samples decomposing indoors and 55% of bone samples. Sevoflurane is a known anaesthetic and could not have been produced as a result of decomposition of the amputated lower limbs/feet. Its presence in the samples can be explained by the fact that the amputated lower limbs/feet were obtained by surgeries conducted on living patients prior to which, anaesthesia would have been administered and this compound persisted in the amputated lower limbs/feet long after they were being used as CDD training aids. Even though the foot samples decomposing outdoors and the tissue samples originated from amputated lower limbs, sevoflurane was not found to be significant in these training aids. Additionally, the PCAs constructed for storage conditions revealed that among other VOCs, sevoflurane was dominant in samples stored in the refrigerator and freezer relative to room temperature storage. Thus, storing the training aids at room temperature can be a

way of reducing the presence of sevoflurane which may not be a desirable compound in CDD training material as it is not decomposition odour-related. However, similar hypotheses can not be concluded with the ageing of training aids as sevoflurane was found to be dominant in aged samples from 2017 in the case of foot training aids and it was dominant in relatively recent samples from 2020 in bone training aids. Thus, law enforcement organisations that eventually adopt the use of amputated lower limbs/feet for CDD training must be aware that these training aids can contain amputation procedural-related VOCs (such as anaesthetics) which can persist in the training aids for several months to years.

3.4.3.2 Bacterial infection-related VOCs

Some compounds which have been reported to originate from bacterial species infecting diabetic foot ulcers were also detected in the current study however, these were not among the prominent compounds identified [156; 157]. These VOCs included 2-ethyl-1-hexanol, styrene and indole which are reportedly produced in the presence of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Some other compounds such as 3-trifloroacetoxydocecane, benzaldehyde and DMS were not detected in the amputated lower limbs/feet during the current study [156; 157]. This could be due to differences in analytical methods (GC×GC–TOFMS vs. GC-MS) or sample matrix (decomposing amputated lower limbs/feet vs. bacterial species grown in vitro) of the current study compared to previous studies. About 60 different VOCs originating from five different microbial species have been reported as potential biomarkers of diabetic wounds [171], a majority of these have also been reported in decomposition VOC profile and/or detected in CDD training aids in the current study.

3.4.4 VOCs in other CDD training aids

In addition to amputated lower limbs/feet, blood and teeth were also analysed since they were included in the training materials used by the OPP Canine Unit. Among prominent compounds identified for blood (in Table 3.8), 11 of the 15 identified VOCs have been previously reported in blood used as CDD training aids [137]. For the teeth training aids, aniline was the only VOC among the six prominent VOCs which was detected in 29% of all the samples analysed in the current study. None of the other five of the six VOCs reported as prominent to teeth in the current study (in Table 3.9) have been previously

reported in teeth samples or in human decomposition odour. This could be because the teeth did not have any organic matter left and hence, no decomposition-related VOCs were evolved and detected in the current study. Thus, the VOC profile of teeth samples used in this study was not found to be an ideal representation of the VOC profile of decomposing human remains. This deemed the teeth training aids as the least suited for CDDs.

3.4.5 Impact of ageing and storage conditions

The impact of ageing and storage conditions was analysed using the PCA clustering method based on prominent VOCs in the samples. The researcher acknowledges that the identification of prominent VOCs is subjective. In the current study, the selection of prominent VOCs was based on identifying VOCs that contributed towards the best explained variance. Additionally, an attempt was made to not disturb the spatial distribution of samples when the threshold of what was defined as prominent changed, as this would have otherwise impacted the interpretation of results. The method applied was then able to provide a list of approximately 10 – 30 most important VOCs.

Considering these factors, this study found that both ageing and storage conditions of the training aids can cause subtle variability in their VOC profile based on the PCAs constructed with two types of training aids analysed – foot samples stored indoors and bone. The variability in the sample sets was minimal due to ageing of bone, varying predominantly along PC-5 with 4% variance while for the foot samples stored indoors the variability due to ageing was extremely evident along PC-1 with 98% variance. The strong variance due to ageing in the foot samples was reduced even when the most influencing samples – Foot #8 and #9 (March and May 2021) were removed from the analysis, thus confirming the subtle variability.

The variance due to storage of foot training aids was visible along PC-3 with 9% variance resulting from room temperature stored samples that clustered separately from refrigerator stored samples. Similarly, for bone, the freezer and refrigerator samples clustered separately from the room temperature storage which were spread along positive PC-5 (with 2% variance). Thus, both the storage PCAs identified that the samples stored at room temperature were slightly different from refrigerator and freezer storage as they clustered separately along PCs with low explained variance. No distinct variability was

observed among refrigerator and freezer stored samples. One limitation in the storage based sample set classification was the lack of data points for freezer and refrigerator storage, especially in the case of bone training aids since a majority of the training aids were stored at room temperature. Additionally, all data points for foot room temperature storage came from the same training aid (Foot #5), for bone refrigerator data set came from Bone #1, while for bone freezer data set came from Bone #3 which could have also contributed towards closer clusters of the samples. Increasing the data set and having more samples in future studies will help improve data visualisation and potential clustering. Thus, the current study can not conclude that the clustering was based solely on storage conditions and not on the fact that the storage data points originated from the same training aid. Even if it is attributed to the storage condition, the variability was subtle considering the low explained variance values. Additionally, the current study could not conclude if one storage condition was favourable over the other since decomposition-related compounds were observed across all storage conditions. A reason for this could be that even though the samples are stored under their specific storage condition, they are often removed from storage and remain at ambient conditions for long durations (sometimes even days) thus, the decomposition process continues even when it may slow down due to colder temperatures [96] such as that of freezer or refrigerator. Regardless, further studies are needed to understand the entire picture of the contribution of ageing and storage on CDD training aids.

3.5 Conclusion

The aim of this study was to analyse the VOC profile of CDD training aids used by the OPP Canine Unit. 2026 VOCs were detected in the training aids samples analysed over a span of 1.5 years. All decomposition-related classes were identified in these training aids. When analysing all CDD training aids, the general trend for class abundance from highest to lowest was aromatics followed by linear aliphatics, esters and analogues, alcohols, nitrogen-containing, ketones, cyclic aliphatics, halogen-containing, ethers, aldehydes, acids and sulphur-containing VOCs. Likewise, the trend for relative class concentration was alcohols followed by nitrogen-containing VOCs, aromatics, acids, linear aliphatics, esters and analogues, ketones, halogen-containing VOCs, sulphur-containing VOCs, cyclic aliphatics, aldehydes and ethers. This study presents prominent compounds detected in all CDD training aids and individual training aid types in Tables 3.3 to 3.9.

Several decomposition-related compounds were detected in amputated lower limbs/feet and blood training aids however, teeth had the least number of decomposition-related compounds in its list of compounds identified as prominent. The detection of VOCs was found to be influenced by factors such as location of sample collection (indoor vs. outdoor), site of decomposition (surface vs. burial) and presence of organic matter (dry vs. wet samples). One prominent non-decomposition-related VOC – sevoflurane was detected in foot and bone training aids (both originating from amputated lower limbs) which was not detected in blood or teeth samples. Sevoflurane, an anaesthetic potentially used during amputation surgeries, was detectable years after the amputated lower limbs were being used for CDD training. It is of significance to note that if amputated lower limbs/feet are used as CDD training aids, the CDD could potentially be exposed to some amputation procedure-related VOCs. The presence of sevoflurane may be reduced if the training aids are stored at room temperature. This study found subtle variability with the ageing of foot and bone training aids owing to their storage conditions, however, further research is needed since this variability was not distinct.

Chapter 4: VOLATILE PROFILE OF REST[ES] DONORS

Chapter 4: VOLATILE PROFILE OF REST[ES] DONORS

4.1 Introduction

Decomposition odour is comprised of the VOCs evolved as end-products and by-products of decomposition due to the breakdown of biomolecules through varying biochemical pathways. As elaborated in section 1.1.1 both the decomposition process and the VOCs resulting from it depend on several factors. All these factors – temperature, rainfall, scavengers etc. vary with geographical location and contribute to the variability of the decomposition VOC profile. A recent study comparing the VOCs evolved during decomposition of pigs in different continents concluded that the decomposition VOC profile varied significantly in North America (Ontario) compared to those observed in Europe and Australia [172]. This was likely due to the lower average temperature of Ontario when compared to the location of study in Europe and Australia which had higher average temperatures. The difference was notably observed in the chemical profile of acetic acid which was not readily detected in North America [172]. This emphasises the fact that there is a need to repeatedly study the decomposition of remains in various environments since results from one environment type can not be extrapolated to another.

When establishing the study design for decomposition odour analysis, multiple factors need to be considered such as the subject (humans vs. human analogues); location (outdoor vs. morgue, surface vs. burial); and sample matrix (air vs. soil VOC analysis) for the study. Only three studies to date have analysed VOCs with the same parameters as in the current study [173; 96; 87]. These included analysis of VOCs in the air matrix above human remains decomposing in an outdoor environment. Hence, a literature review of the three studies with a study design most similar to the current one was conducted. There are several other decomposition studies in the literature, however, these have either used human analogues; were conducted in a morgue; analysed burial scenarios; used soil as a matrix; or analysed VOCs in internal gas reservoirs.

Two of the three studies to investigate outdoor surface decomposition VOC in an air matrix, published in 2019 in Sydney, Australia were conducted at the Australian Facility for Taphonomic Experimental Research (AFTER). In 2019, Knobel et al. [87] compared decomposition VOC profiles of human and pig remains. Two summer and two winter

decomposition trials each with two human and two pig remains were conducted. The decomposition patterns and the variation in their VOC profiles (ultimately in their decomposition odour) showed significant differences existed between the two species and these differences were more significant during warmer months. Deo et al. [96] conducted a further study at AFTER to monitor the VOC profile of five donors across different climatic conditions. It was reported that in the presence of warmer initial temperatures, a higher variety of compound classes were detected, whereas cooler temperatures led to fewer decomposition VOCs with lower abundances however, there were no consistently recurring compounds. The third study by Stefanuto et al. [173] in 2015 was conducted at the Forensic Anthropology Research Facility (FARF) in Texas, USA. This study focused on comparing decomposition trends in humans and pigs, and statistical analysis of GC×GC-TOFMS data. Unlike the two studies conducted at AFTER where the trials were conducted for 1 – 3 months, the FARF study focused on studying decomposition for up to six experimental days.

Previously, in Canada, decomposition studies have used pig remains due to a lack of legal and ethical acquisition of human remains [174; 175; 69; 176-178]. These studies have focused on the entomological succession during decomposition, decomposition process or VOC analysis in the Canadian environment. Another retrospective study on human remains was conducted with data collected from law enforcement case files [39]. The study compared the decomposition process in different environments (outdoor surface, buried, indoor and water). The current study is the first outdoor decomposition odour analysis study to be conducted at the facility for Research in Experimental and Social Thanatology / *Recherche en Sciences Thanatologiques [Expérimentales et Sociales]* (REST[ES]), the only human decomposition facility in Canada to date. As per the Köppen-Geiger climatic classification [179], the humid continental climate in Québec, Canada is different from the humid subtropical climate of Sydney, Australia and Texas, USA. This study aimed to understand the decomposition process of cadavers in a natural southeastern Canadian environment while recording the VOCs.

4.1 Methods

4.1.1 Ethics

The ethics approval for the study conducted at REST[ES] was obtained from *le sous-comité d'éthique du laboratoire d'enseignement et de recherche en anatomie* at UQTR with the certificate number CER-09-148-06.05. All the cadavers (henceforth referred to as donors) in this study were obtained under the UQTR Body Donation Program wherein, consenting donors arrive at the UQTR morgue for study and research purposes, including at the REST[ES] facility.

4.1.2 Donors details

This study acquired eight donors through the body donation program of the UQTR teaching and research human anatomy laboratory. All the donors were refrigerated during transport to and once they arrived at the UQTR morgue. The details of each of the donors and their decomposition trials have been summarised in Table 4.1.

Table 4.1: Details of the donors received at REST[ES] for the 2020/2021 decomposition study trial.

Sr. no.	Donor ID.	Date of death	Cause of death	Sex	Age (yrs)	Weight (kg)	Date of Donor Arrival at REST[ES] (Day 0)	Trial duration	Season in Canada during placement of the donor
1.	2020-01	5th August 2020	Asphyxiation due to hanging.	Male	55	68	10th August 2020	87 days	Summer
2.	2020-02	7th August 2020	Hepatic cirrhosis, anuric renal insufficiency (i.e. kidney failure due to anuria).	Male	71	70	10th August 2020	87 days	Summer
3.	2020-03	27th September 2020	Parkinsonian syndrome.	Female	69	54	28th September 2020	38 days	Autumn
4.	2020-04	4th October 2020	Inferior STEMI [ST Elevation Myocardial Infarction], vascular dementia, multiple strokes, MCAS [Mass Cell Activation Syndrome].	Female	79	79.4	5th October 2020	31 days	Autumn

5.	2021-06	10th May 2021	Stage 4 Metastatic melanoma in lungs, brain, bones, muscles, kidneys, adrenal glands, and liver.	Male	77	79.5	11th May 2021	73 days	Spring
6.	2021-08	9th July 2021	Likely thrombosis in lower limb, dementia.	Female	93	60	10th July 2021	72 days	Summer
7.	2021-10	30th September 2021	Advanced lung neoplasia.	Male	78	56	2nd October 2021	34 days	Autumn
8.	2021-11	11th October 2021	Metastatic lung cancer.	Male	54	44	12th October 2021	30 days	Autumn

4.1.3 Experimental design

This study was conducted at the UQTR REST[ES] facility. The donors were transported from the UQTR morgue to REST[ES] by funeral directors licensed to transport human remains. REST[ES] is an outdoor human decomposition facility located in Bécancour, Québec with a mixed temperate forest dominated by maple and white spruce trees with a sandy loam soil texture.

Once the donors arrived at REST[ES], they were directly placed on the soil surface within a pre-determined plot size of 3 m × 3 m. Each plot was at least 4 m apart. Another plot in an unused area of the site was identified as the control site for the trial. The control site was used to collect control (i.e. background) VOC samples to differentiate the environmental VOCs from decomposition-related VOCs. Anti scavenging (Figure 4.1) cages were placed over the donors for the entire duration of the trial and only removed for the purpose of collecting samples. These cages discourage mammalian and avian scavengers while still allowing invertebrate insects to access the remains.

Figure 4.1: Anti scavenging cages placed on donors during the outdoor studies conducted at REST[ES].

Visual observations in the form of written notes and photographs were recorded at regular frequencies throughout the duration of the trial. The stage of decomposition was reported in both experimental days (ED) and Kelvin accumulated degree days (KADD) [28]. KADD on a particular day was calculated by taking the sum of the average daily

temperatures in the Kelvin scale (K) for the trial period until that day. Multiple prior decomposition studies have used ADD (temperatures in ˚C) [180; 181; 96] however, the Kelvin scale was preferred over ˚C as negative ˚C temperatures were observed during the trial which complicated the ADD calculation. A Hobo Weather Station equipped with a Hobo U30 No Remote Communication data logger (Onset Computer Corporation, Bourne, MA, USA) was used to record ambient conditions every 15 min. The recorded weather conditions included ambient temperature (˚C), rainfall (mm), relative humidity (%), solar radiation (W/m^2), wind speed (m/s), wind direction, and gust speed (m/s). Decomposition VOC samples were collected from each of the donors on varying days depending on their decomposition rates. Visual observations were made and photographs were also collected on several days during the trial.

4.1.3.1 Donor decomposition stages observations

Donors were classified into one or more decomposition stages on a given experimental day based on their visual appearance. The decomposition stages and the key taphonomic features used to classify the donor into a specific stage have been summarised in Table 4.2 and Table A 1 of Appendix B. The presence of one or more of these taphonomic features corresponding to each stage in one of the three donor body parts – head and neck; thorax and abdomen; and limbs resulted in the identification of the particular decomposition stage in the donor. Thus, since each of the three parts was classified into a stage separately, the donor could have existed in multiple stages at the same time, resulting in differential decomposition. Table 4.2 is a modified version of Table 1.1 in Chapter 1 which summarised widely accepted decomposition stages [30; 58; 20; 26; 14; 52; 24; 53; 63; 182; 54; 51]. This was accepted to be the closest approximation to the observations made in the current study which was specific to environmental conditions at REST[ES].

Table 4.2: Donor decomposition stages and their corresponding key taphonomic features used to identify stages in donors during the trial conducted at REST[ES] between August 2020 to November 2021.

Stages	**Stage defining key taphonomic features**
Fresh	No distinct macroscopic change since the time of death.
Bloat	Swelling of whole or parts of the corpse.
Active decay	Rapid loss of soft tissue.

	Skin desiccated in small patches with a defined outline. Intense marbling. Intense green colour of the abdomen.
Desiccation	Dry, dehydrated tissue covering larger patches on the skin. (These usually resulted when several small desiccated patches in the vicinity merged to form a larger portion of desiccated tissue)
Skeletonisation	Partial or complete exposure of bones.

4.1.4 Analytical method

All the steps for VOC accumulation, collection and analysis have been detailed in Chapter 2. Table 4.3 summarises the experimental days on which VOCs were collected from each of the donors in the current study. The experimental day (ED) 0 corresponds with the day the donor arrived at REST[ES] which was also the first day of the trial. The samples were collected depending on the donor, initially on a daily basis when the decomposition progressed rapidly, then once every 2 – 3 days, and finally weekly as the decomposition process slowed.

Table 4.3: Experimental day on which VOCs were collected from donors at REST[ES] during their decomposition trials conducted in studies between August 2020 and November 2021.

Sr. no.	**Donor ID.**	**Experimental days (ED)**
1.	2020-01	0, 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 26, 28, 30, 32, 35, 42, 49, 56, 65, 74, 81, 87
2.	2020-02	0, 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 26, 28, 30, 32, 35, 42, 49, 57, 65, 74, 81,87
3.	2020-03	0, 1, 2, 3, 4, 5, 7, 9, 12, 31
4.	2020-04	0, 1, 3, 4, 5, 7, 11, 14, 16, 19, 24, 32, 38
5.	2021-06	0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 17, 20, 24, 27, 29, 45, 51, 59, 66, 73
6.	2021-08	0, 1, 2, 3, 4, 5, 6, 9, 11, 13, 16, 18, 20, 24, 27, 31, 34, 39, 46, 53, 66, 72
7.	2021-10	0, 1, 2, 3, 4, 6, 9, 11, 13, 16, 18, 20, 24, 27, 30, 34

8.	2021-11	0, 1, 2, 3, 6, 8, 10, 14, 17, 20, 24, 27, 30

4.1.5 Data analysis

Statistical analysis was performed based on methods described in section 2.2.5. The R scripts were edited for each of the donors for their corresponding experimental days and are available on GitHub which can be accessed using the link: https://github.com/RushaliDargan/Decomposition/tree/main/REST%5BES%5D_donors

The R codes resulted in VOCs that were significant to each donor on each experimental day. The VOCs were then classified into VOC classes (acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs). The VOCs and their classes were compared for any variability in the VOC profile that may have occurred as decomposition progressed and across donors that were decomposing in warmer versus cooler average ambient temperatures. The stages were reclassified as – early, middle and late for the ease of statistical analysis. Here, the early stage lasted until the donors had a fresh appearance, the middle stage corresponded with the bloat and active decay stages and the late stage was any stage that occurred once the bloat and active decay stages had passed. This was done to better interpret any stage-dependent VOCs and avoid overlap since the donors showed differential decomposition for the majority of the trial duration. Table 4.4 represents the duration (in ED) during which the three decomposition stages were observed for each of the donors.

Table 4.4: Duration (in ED) for the three decomposition stages in eight donors studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021 with the season of donor placement mentioned in parenthesis.

Decomposition stage	**Experimental day (ED) of donors for each of the stages observed**							
	2020-01 (summer)	**2020-02 (summer)**	**2020-03 (autumn)**	**2020-04 (autumn)**	**2021-06 (spring)**	**2021-08 (summer)**	**2021-10 (autumn)**	**2021-11 (autumn)**

Early	Overall fresh appearance	N/A	0 – 1	0 – 9	0 – 2	0 – 3	0	0 – 1	0
Middle	Commence of bloat and active decay stage	1 – 23	2 – 16	10 – 38	3 – 31	4 – 27	1 – 18	2 – 34	1 – 30
Late	Post-bloat and post-active decay stage	24 – 87	17 – 87	N/A	N/A	28 – 73	19 – 72	N/A	N/A

4.2 Results

4.2.1 Weather Data

The human decomposition trials at REST[ES] were conducted between the 10th August 2020 to 11th November 2021 with eight donors. Since the trial duration for each of the donors was variable, weather conditions specific to each trial have been outlined in Table 4.5.

Table 4.5: Weather conditions during the human decomposition trials conducted at REST[ES] from August 2020 to November 2021.

Sr. no.	Donor ID.	Trial period	Weather conditions											
			Canadian season during donor placement	Avg. temp.		Temp. range (min – max)		Avg. Relative humidity (%)	Relative humidity range (min – max) (%)	Total rainfall (mm)	Avg. rainfall (mm)	Avg. solar radiation (W/m^2)	Avg. wind speed (m/s)	Avg. gust speed (m/s)
				(℃)	(K)	(℃)	(K)							
1.	2020-01	10th Aug – 5th Nov 20	Summer	11.5	284.7	-8.4 – 33.3	264.7 – 306.5	83.5	35.3 – 100	327.4	0	34.2	0	0.6
2.	2020-02	10th Aug – 5th Nov 20	Summer	11.5	284.7	-8.4 – 33.3	264.7 – 306.5	83.5	35.3 – 100	327.4	0	34.2	0	0.6
3.	2020-03	28th Sep – 5th Nov 20	Autumn	7.0	280.2	-8.4 – 24.9	264.7 – 298.1	83.6	35.3 – 100	201.8	0.1	26.4	0	0.8
4.	2020-04	5th Oct – 5th Nov 20	Autumn	5.8	279.0	-8.4 – 22.7	264.7 – 295.9	81.7	35.3 – 100	135.4	0	27.9	0	0.9
5.	2021-06	11th May – 23rd July 21	Spring	18.0	291.1	0.8 – 34.7	273.9 – 307.9	75.6	21.3 – 100	68.2	0	78.1	0	0.5

6.	2021-08	10th July – 20th Sep 21	Summer	19.5	292.7	7.5 – 33.1	280.7 – 306.2	82.3	30.3 – 100	16.2	0	48.4	0	0.2
7.	2021-10	2nd Oct – 5th Nov 21	Autumn	9.6	282.8	-2.8 – 22.3	270.3 – 295.5	85.8	41.8 – 100	9.8	0	23.1	0	0.6
8.	2021-11	12th Oct – 11th Nov 21	Autumn	8.0	281.2	-4.1 – 22.3	269.1 – 295.5	83.8	34.8 – 100	32.8	0	27.8	0	0.8

4.2.2 Donor decomposition stages

Donor 2020-01 (Summer 2020 placement)

Donor 2020-01 was already in active decay on ED 0 (KADD 293.3). Fresh stage was not observed in this donor. Active decay was distinct in the limbs due to intense marbling, in the head region on ED 2 (KADD 887.0) as a result of desiccated skin patches in the neck, followed by the abdomen on ED 3 (KADD 1181.8) due to intense green discolouration. The whole cadaver was at the peak of active decay by ED 8 (KADD 2645.6). The body developed mild bloat on ED 3 (KADD 1181.8), before entering full bloat on ED 6 (KADD 2062.9). The bloat and the active decay stage had completely subsided on ED 23 (KADD 6982.5). The earliest signs of the desiccation stage were evident in the head region on ED 4 (KADD 1475.1) and skeletonisation began as of ED 11 (KADD 3513.0). By ED 17 (KADD 5256.2), the donor was largely desiccated. The donor remained in the desiccation and skeletonisation stage until the end of the trial on ED 87 (KADD 25051.6). The duration (in KADD) of each of the stages has been graphically represented in Figure 4.2. Detailed decomposition changes in donor 2020-01 have been summarised in Table A 2 of Appendix B.

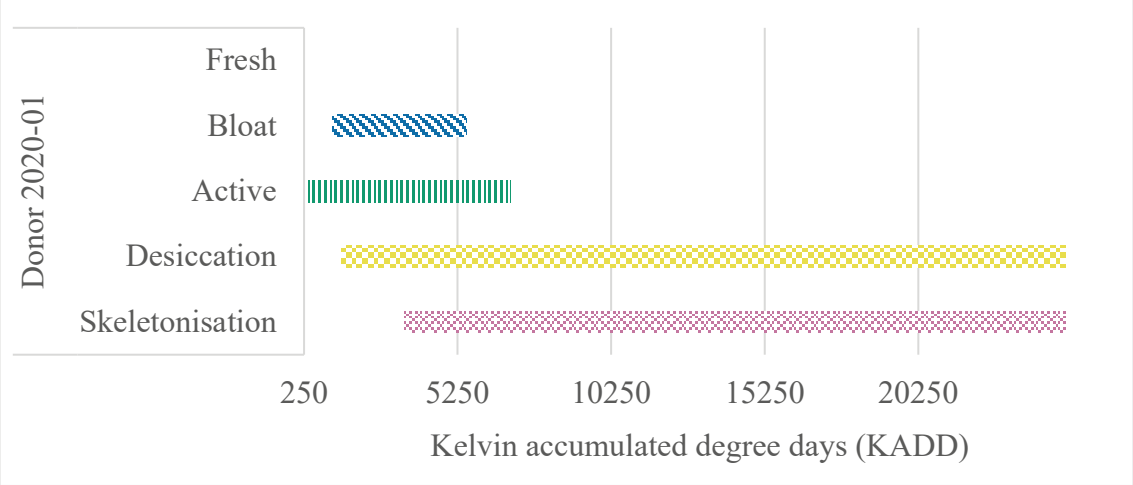


Figure 4.2: Duration of decomposition stages (against KADD) observed in donor 2020-01 during the decomposition trial conducted at REST[ES] between August 2020 to November 2020.

Donor 2020-02 (Summer 2020 placement)

Donor 2020-02 was in the fresh stage of decomposition on ED 0 (KADD 293.3) until ED 4 (KADD 1475.1) when the body developed mild bloat. A full bloat stage was observed on ED 8 (KADD 2645.6) and it completely subsided on ED 10 (KADD 3222.7). Active decay began first in the upper limbs potentially due to pre-existing (before arrival at REST[ES]) ruptures in the skin (reason not known) which attracted the flies and resulted in skin blistering due to larval activity as early as ED 2 (KADD 887.0). By ED 4 (KADD 1475.1), the head (showing larval activity) and trunk (showing desiccated skin patches) were in active decay. The whole body was at the peak of active decay by ED 8 (KADD 2645.6) which lasted until ED 16 (KADD 4970.3). The earliest signs of the desiccation stage were evident in the head region on ED 4 (KADD 1475.1) and skeletonisation began on ED 10 (KADD 3222.7). By ED 10 (KADD 3222.7) and ED 16 (KADD 4970.3), the head and whole body, respectively was largely desiccated. The donor remained in the desiccation and skeletonisation stage until the end of the trial on ED 87 (KADD 25051.6). The duration (in KADD) of each of the stages has been graphically represented in Figure 4.3. Detailed decomposition changes in donor 2020-02 have been summarised in Table A 2 of Appendix B.

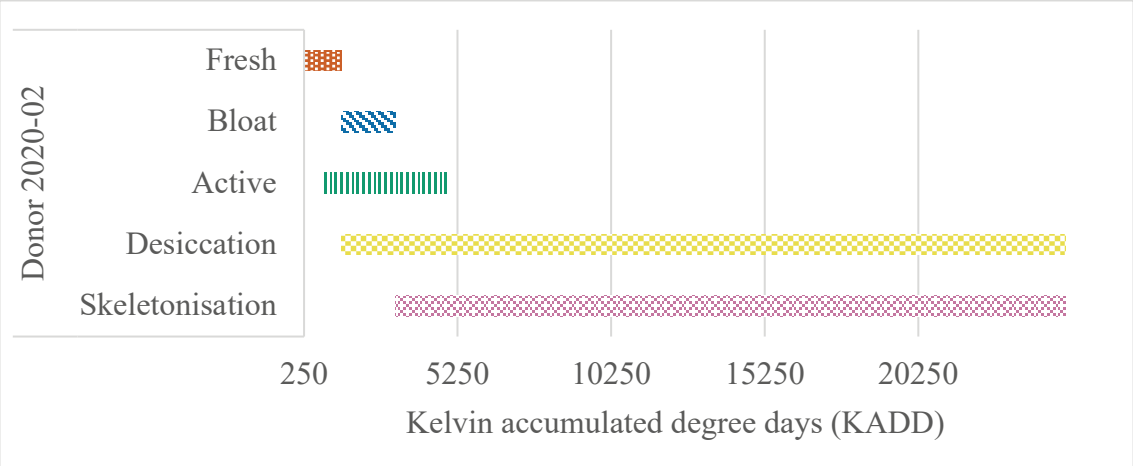


Figure 4.3: Duration of decomposition stages (against KADD) observed in donor 2020-02 during the decomposition trial conducted at REST[ES] between August 2020 to November 2020.

Donor 2020-03 (Autumn 2020 placement)

Donor 2020-03 was also in the fresh stage of decomposition on ED 0 (KADD 292.7). The donor developed mild bloat in the neck on ED 17 (KADD 5105.1) and mild bloat in the torso on ED 22 (KADD 6513.7) which subsided on ED 29 (KADD 8462.0). Active decay began on ED 9 (KADD 2854.8) when the abdomen developed an intense green colour and the head showed patches of desiccated skin in the lower lip. The desiccation stage began on ED 17 (KADD 3134.9). At the end of the observation period on ED 38 (KADD 10927.4), the donor was largely in the active decay stage. No skeletonization was observed in this donor within the trial duration since the trial was much shorter than donors placed in summer in the same year as it lasted only until the commencement of snowfall. The duration (in KADD) of each of the stages has been graphically represented in Figure 4.4. Detailed decomposition changes in donor 2020-03 have been summarised in Table A 3 of Appendix B.

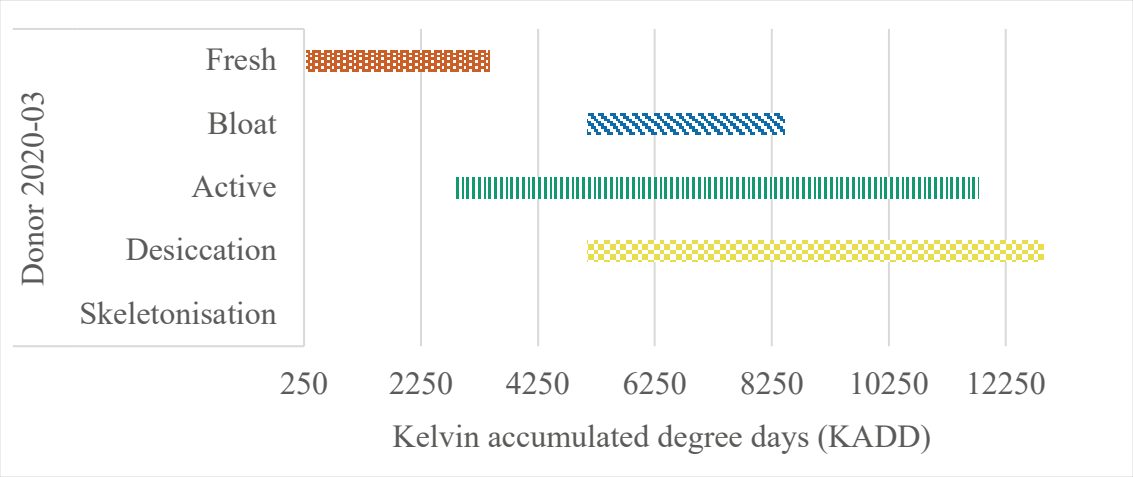


Figure 4.4: Duration of decomposition stages (against KADD) observed in donor 2020-03 during the decomposition trial conducted at REST[ES] between September 2020 to November 2020.

Donor 2020-04 (Autumn 2020 placement)

Donor 2020-04 was in the fresh stage of decomposition on ED 0 (KADD 283.6) until ED 4 (KADD 1415.1). Mild bloating was observed in the neck on ED 7 (KADD 2258.4) and by ED 8 (KADD 2537.4) the upper limbs had developed marbling and the abdomen had a prominent greenish colour. Active decay began on ED 3 (KADD 1134.5) in the trunk followed by the limbs on ED 4 (KADD 1415.1) as both these developed patches of desiccated skin and by ED 5 (KADD 1702.1) the head also entered active decay with egg masses present in the nostrils. The desiccation stage began on ED 22 (KADD 6461.5) in the face. At the end of the observation period on ED 31 (KADD 8927.0), the donor was largely in the active decay and desiccation stages with mild bloating in the neck. The donor did not show skeletonisation by the end of the trial period of this study since the trial was much shorter than donors placed in summer in the same year as it lasted only until the commencement of snowfall. The duration (in KADD) of each of the stages has been graphically represented in Figure 4.5. Detailed decomposition changes in donor 2020-04 have been summarised in Table A 3 of Appendix B.

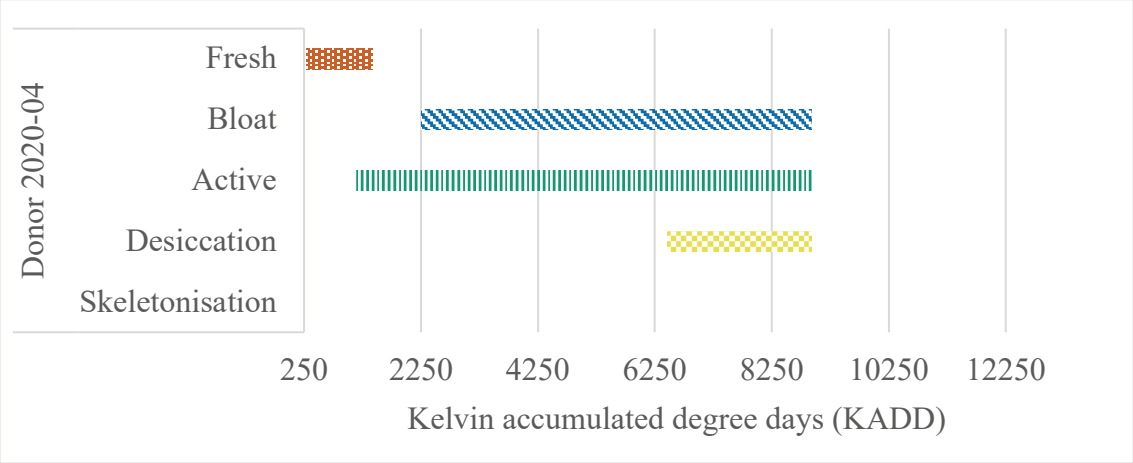


Figure 4.5: Duration of decomposition stages (against KADD) observed in donor 2020-04 during the decomposition trial conducted at REST[ES] between October 2020 to November 2020.

Donor 2021-06 (Spring 2021 placement)

Donor 2021-06 was in the fresh stage of decomposition on ED 0 (KADD 282.2) which lasted until ED 10 (KADD 3178.2) with the whole donor in bloat and active decay. Bloating started in the neck on ED 10 (KADD 3178.2) and the donor achieved maximum bloat on ED 12 (KADD 3763.5) in the head, neck, upper thorax and abdomen regions. The upper thorax showed signs of post-bloat on ED 14 (KADD 4339.6) and in the abdomen on ED 20 (KADD 6052.2). However, the entire bloat lasted until ED 24 (KADD 7212.9) when the lower limbs lost bloat too. The active decay stage began on ED 4 (KADD 4127.5) when patches of desiccated skin were visible in the neck and trunk region and this stage lasted until ED 27 (KADD 8104.7). The whole cadaver was at the peak of active decay by ED 11 (KADD 3473.9) when skin slippage and intense larval activity were visible in the head, upper thorax, upper limbs and areas around the genitals. Desiccation began in the face on ED 10 (KADD 3178.2) followed by the regions around the genitals and the upper thorax the following day on ED 11 (KADD 3473.9). Skeletonisation was visible by ED 17 (KADD 5199.4) when bones in the head region became visible. The donor remained in the desiccation and skeletonisation stage until the end of the trial on ED 73 (KADD 21546.2). The duration (in KADD) of each of the stages has been graphically represented in Figure 4.6. Detailed decomposition changes in donor 2021-06 have been summarised in Table A 2 of Appendix B.

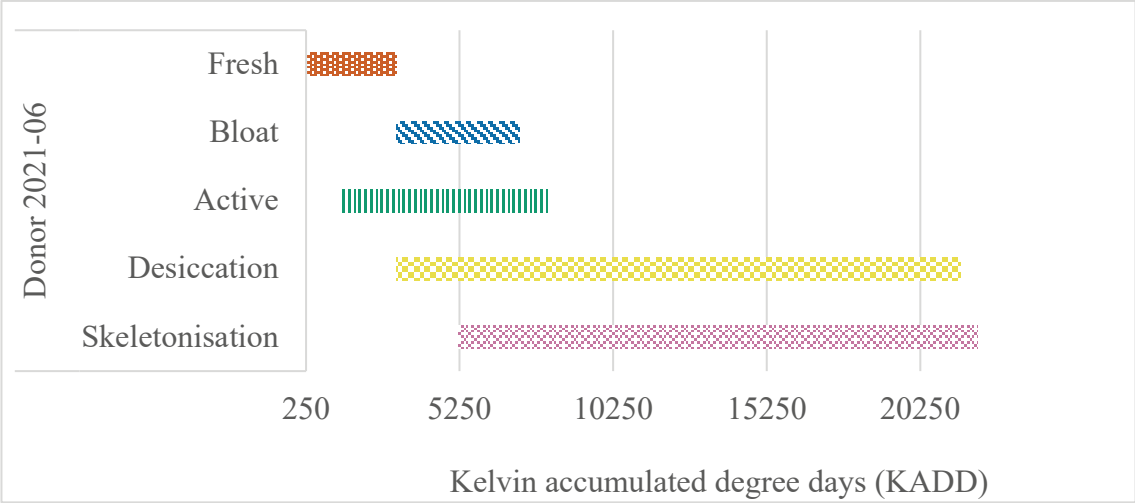


Figure 4.6: Duration of decomposition stages (against KADD) observed in Donor 2021-06 during the decomposition trial conducted at REST[ES] between May 2021 to July 2021.

Donor 2021-08 (Summer 2021 placement)

Donor 2021-08 was in the fresh stage of decomposition on ED 0 (KADD 291.1) until ED 2 (KADD 878.2) when egg masses were abundant and larval activity began in the nostrils initiating the active decay stage of decomposition in the head region. The bloat was observed on ED 3 (KADD 1171.5) in the abdomen, and it completely subsided on ED 10 (KADD 3234.4). By ED 4 (KADD 1465.7), larval activity and skin slippage were observed in the lower limbs, head, neck and upper thorax region thus, by this day, active decay was occurring in all regions of the donor. The whole body was at the peak of active decay by ED 5 (KADD 1762.3) and it lasted until ED 18 (KADD 5566.9). The earliest signs of the desiccation stage were evident in the head region and upper thorax on ED 5 (KADD 1762.3) and skeletonisation began as of ED 6 (KADD 2057.0) when the bone of the skull was largely visible. The donor remained in the desiccation and skeletonisation stage until the end of the trial on ED 72 (KADD 21359.1). The duration (in KADD) of each of the stages has been graphically represented in Figure 4.7. Detailed decomposition changes in donor 2021-08 have been summarised in Table A 2 of Appendix B.

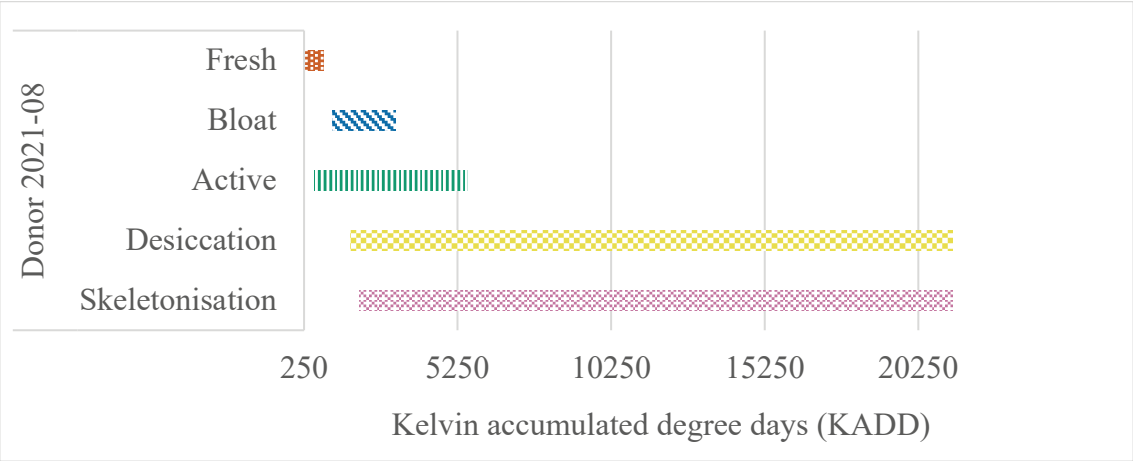


Figure 4.7: Duration of decomposition stages (against KADD) observed in donor 2021-08 during the decomposition trial conducted at REST[ES] between July 2021 to September 2021.

Donor 2021-10 (Autumn 2021 placement)

Donor 2021-10 was also in the fresh stage of decomposition on ED 0 (KADD 283.7) which lasted until ED 9 (KADD 2846.5). The donor did not show bloat during the trial period. Active decay began on ED 2 (KADD 850.4) with patches of desiccated skin in the upper thorax and greenish discolouration in the abdomen. This stage peaked by ED 13 (KADD 4000.6) when skin slippage and larval activity were observed in all regions of the donor. The desiccation stage began on ED 11 (KADD 3423.1) in the face and neck and skeletonisation was observed on ED 16 (KADD 4857.3) when bones from the skull became visible. At the end of the observation period on ED 34 (KADD 9887.3), the donor was largely in the desiccated and skeletonised stage, while some active decay stage was evident from the larval activity on the posterior side of the donor. No bloat stage was observed in this donor within the trial duration of the study. The duration (in KADD) of each of the stages has been graphically represented in Figure 4.8. Detailed decomposition changes in donor 2021-10 have been summarised in Table A 3 of Appendix B.

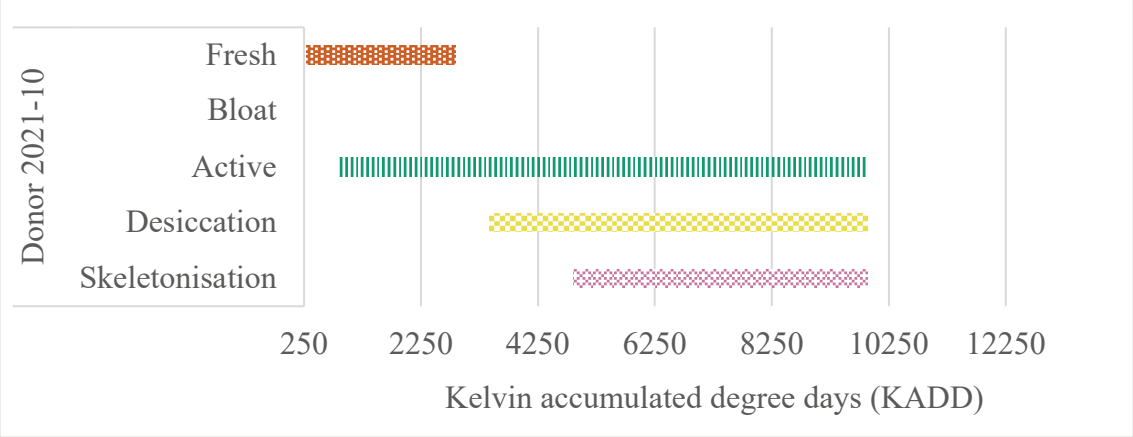


Figure 4.8: Duration of decomposition stages (against KADD) observed in donor 2021-10 during the decomposition trial conducted at REST[ES] between October 2021 to November 2021.

Donor 2021-11 (Autumn 2021 placement)

Donor 2021-11 was also in the fresh stage of decomposition on ED 0 (KADD 288.2) until ED 3 (KADD 1154.1) The donor showed a bloated abdomen since its arrival at the facility which eventually subsided by ED 24 (KADD 7040.8). Active decay began in the abdomen on ED 1 (KADD 576.6) followed by the face on ED 6 (KADD 2010.8). The desiccation stage began on ED 6 (KADD 2010.8) in the face. At the end of the observation period on ED 30 (KADD 8709.5), the donor was largely in the active decay stage with skin slippage in the limbs, larval activity in the face and greenish colouration in the abdomen along with desiccation in the face, limbs and area around the genitals. The donor did not show skeletonisation by the end of the trial period of this study. The duration (in KADD) of each of the stages has been graphically represented in Figure 4.9. Detailed decomposition changes in donor 2021-11 have been summarised in Table A 3 of Appendix B.

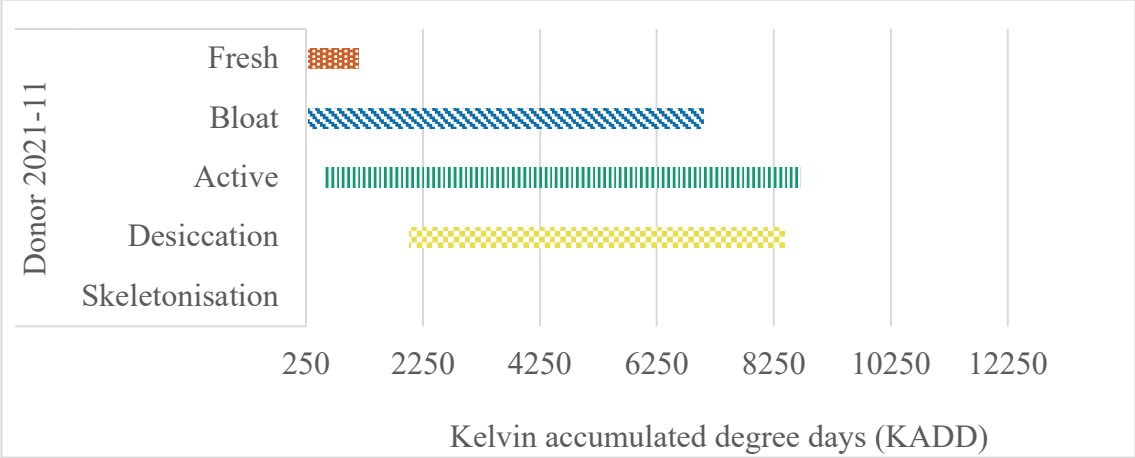


Figure 4.9: Duration of decomposition stages (against KADD) observed in donor 2021-11 during the decomposition trial conducted at REST[ES] between October 2021 to November 2021.

Based on decomposition observations, two types of donor decomposition stage progressions were observed. One where all five stages of decomposition were observed within the trial period and the donors had entirely transitioned from the bloat and active decay stage to the desiccated and/or skeletonised stage by the end of the trial period. In the other instance, the donors remained in the bloat and/or active decay stage of decomposition with signs of desiccation and/or skeletonisation at the end of the trial. The first instance was observed for donors 2020-01, 2020-02, 2021-06 and 2021-08, where the decomposition occurred in relatively warmer average ambient temperatures compared to the remaining donors. For the remaining donors – 2020-03, 2020-04, 2021-10 and

2021-11, decomposition occurred at relatively cooler average ambient temperatures and the trial was stopped once it was difficult to sample due to the presence of snow covering the bodies. Figure 4.10 summarises the average ambient temperature-dependent donor decomposition stages against duration (in KADD). Thus, for further statistical analysis of the results, the donors were categorised by the average ambient temperature at which their decomposition occurred. The differences in decomposition stages observed in the two donor categories have been further discussed in section 4.4.1

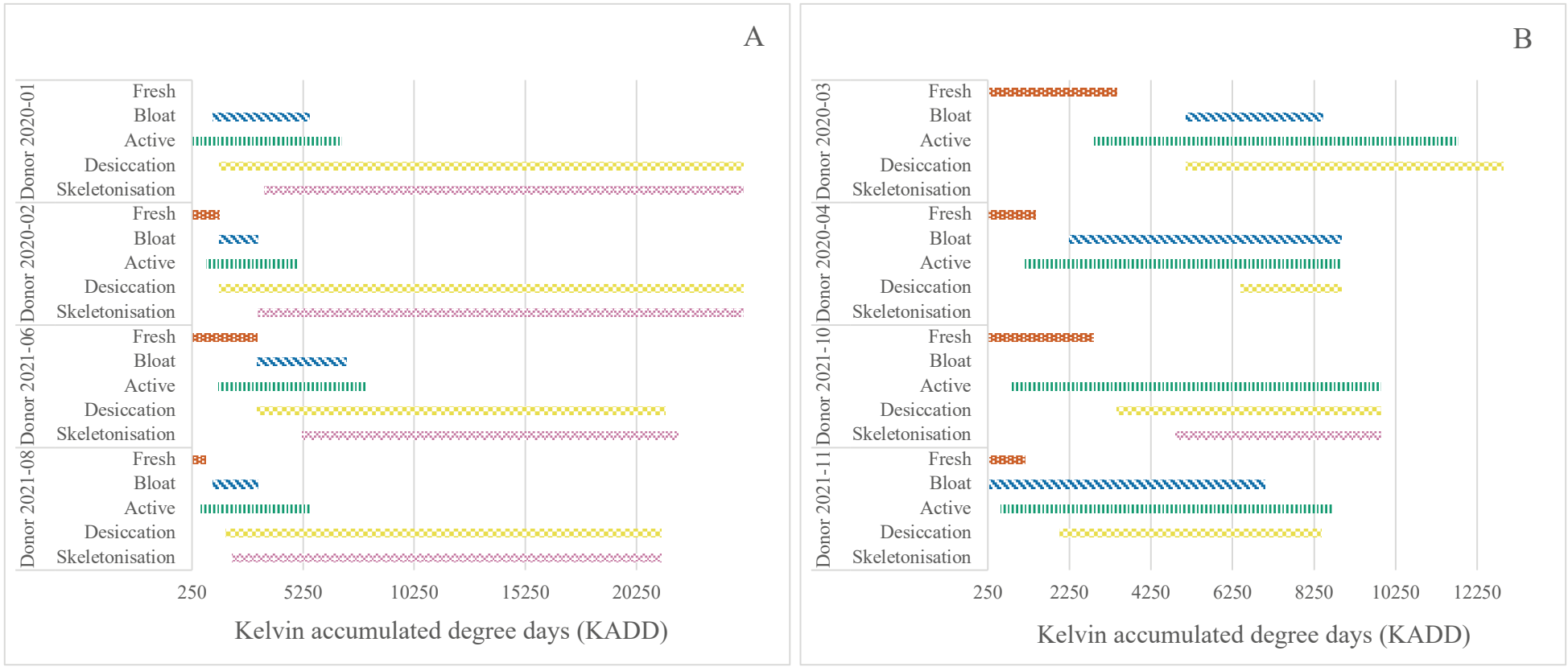


Figure 4.10: Duration of decomposition stages (against KADD) observed in donors decomposing at a) average warmer temperatures and b) average cooler temperatures during the decomposition trials conducted at REST[ES] between August 2020 to November 2021.

4.2.3 Donor decomposition VOC profiles

Analysis of the eight donors decomposing at REST[ES] during trials conducted between August 2020 to November 2021 resulted in the detection of 1412 VOCs. These were classified into one of the following classes – acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs. Each of the classes has been previously identified as a VOC class that comprises decomposition odour and also detected in CDD training aids [135; 71; 159; 160; 68; 85; 161; 162; 96; 163; 87]. The VOC class abundance (based on the number of VOCs), relative class concentration (based on normalised areas), prominent compounds (most frequently detected) across all donors and variability in donor groups (ambient temperature- and decomposition stage-dependent) by PCA are highlighted in this section.

4.2.3.1 Class abundance based on the number of VOCs detected

Table 4.6 represents the total number of VOCs and class abundance for each of the donors detected during their decomposition trial. From this table, it is observed that even though the number of VOCs detected in each of the donors was variable, higher VOCs were detected in donors decomposing at warmer average temperatures (2020-01, 2020-02, 2021-06, 2021-08) compared to those decomposing at cooler average temperatures (2020-03, 2020-04, 2021-10, 2021-11).

Table 4.6: Total number of VOCs and VOC class abundance (by number of VOCs) present in eight donors studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

Donor ID	**2020-01**	**2020-02**	**2020-03**	**2020-04**	**2021-06**	**2021-08**	**2021-10**	**2021-11**
Total no. of VOCs	841	921	249	147	624	578	313	289
Acids	30	34	5	3	21	25	11	12
Alcohols	88	119	23	7	82	72	34	26
Aldehydes	45	50	11	9	36	29	18	12
Aromatics	135	133	39	25	82	80	56	47

Cyclic aliphatics	46	38	14	13	28	28	12	20
Esters and analogues	125	118	25	15	92	77	21	19
Ethers	33	47	13	4	22	28	18	19
Halogen-containing	53	71	39	21	52	39	31	37
Ketones	82	99	17	10	57	54	19	18
Linear aliphatics	73	76	37	22	62	51	27	36
Nitrogen-containing	103	107	20	15	72	76	58	35
Sulphur-containing	28	29	6	3	18	19	8	8
Colour code key		Donor decomposing at warmer average ambient temperature.				Donor decomposing at cooler average ambient temperature.		

The average abundance (in percentage) of each of the classes for the eight donors in this study has been visually represented in Figure 4.11. From Table 4.6 and Figure 4.11 it is evident that aromatics were the most abundant class followed by esters and analogues, nitrogen-containing VOCs, and alcohols. Linear aliphatics, ketones, halogen-containing VOCs and aldehydes were of average abundance. Finally, cyclic aliphatics, ethers, acids and sulphur-containing VOCs were the least abundant. This trend largely remained consistent across individual donors decomposing at warmer average ambient temperatures. The trend varied slightly for donors decomposing at cooler average ambient temperatures. Instead, halogen-containing and linear aliphatics VOCs (in place of esters and analogues and alcohols) became one of the most abundant classes along with aromatics and nitrogen-containing VOCs.

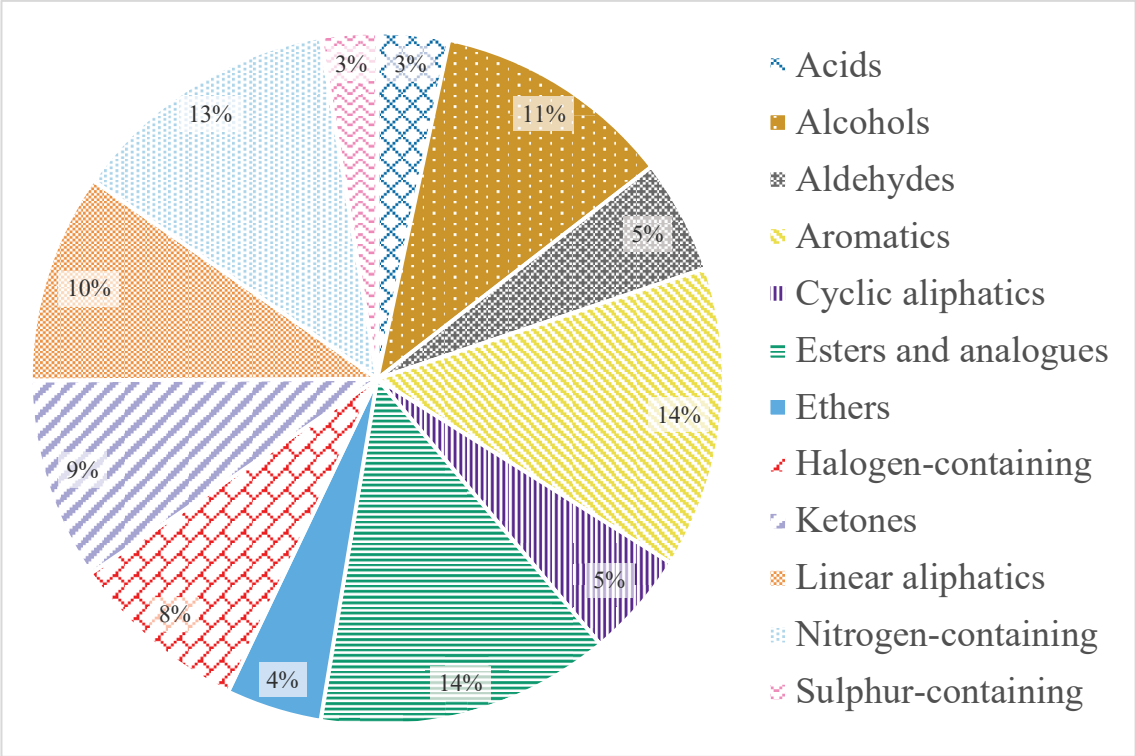


Figure 4.11: Average VOC class abundance by percentage of VOC comprising each class across eight donors studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

For statistical purposes, the decomposition stages were classified as early, middle and late (as elaborated in section 4.1.5). The number of VOCs detected in each of the stages for the eight donors has been summarised in Table 4.7. This table highlights that even though the number of VOCs varied across the stages for each of the donors, most VOCs were detected in the middle stage (during which the donors were in the bloat and/or active decay stage of decomposition), followed by the late and then early stages. Relative to other donors, the highest number of VOCs in the early stage were detected for donor 2020-03, even though this donor decomposed during cooler average ambient temperatures. One reason for this could be that the early (fresh) stage of donor 2020-03 was the longest up to ED 9 while most other donors had an early stage only from ED 0 – 4. Thus, this could highlight the fact that even though the donor appeared fresh, decomposition progressed internally which could have resulted in a greater number of VOCs. Donors 2020-03, 2020-04, 2021-10 and 2021-11 did not reach the late stage of decomposition during the trial period hence, the table does not indicate any detected VOCs in the late stage for these donors. Additionally, at the time of arrival at REST[ES] donor 2020-01 was already in active stage thus, no early (fresh) stage was observed in this donor.

Table 4.7: Number of VOCs detected in each decomposition stage for the eight donors studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

Decomposition stage		**2020-01**	**2020-02**	**2020-03**	**2020-04**	**2021-06**	**2021-08**	**2021-10**	**2021-11**
Early	Fresh	-	49	140	38	108	25	64	29
Middle	Commence of bloat and active decay stage	744	721	157	117	509	375	287	277
Late	Post bloat and post-active decay	374	581	-	-	330	358	-	-
Colour code key			Donor decomposing at warmer average ambient temperature.				Donor decomposing at cooler average ambient temperature.		

All classes of compounds were detected in the middle and late stages of decomposition however, the early stage was missing some classes in individual donors. In the early stage, sulphur-containing VOCs were not detected in three of the eight donors 2020-04, 2021-08 and 2021-10. Additionally, halogen- and nitrogen-containing VOCs were not detected in donor 2020-11, and cyclic aliphatics VOCs were not detected in donor 2021-08 and 2021-10. Figure 4.12 summarises the average class abundance observed in the three decomposition stages. This figure highlights that the early stage had the least abundance for all the classes, while the middle and the late stage generally had comparable abundance for all the classes. Distinct differences were observed in esters and analogues and nitrogen-containing compounds as these classes had a relatively higher abundance during the middle stage. Alcohols and ketones had a relatively higher abundance during the late stage of decomposition.

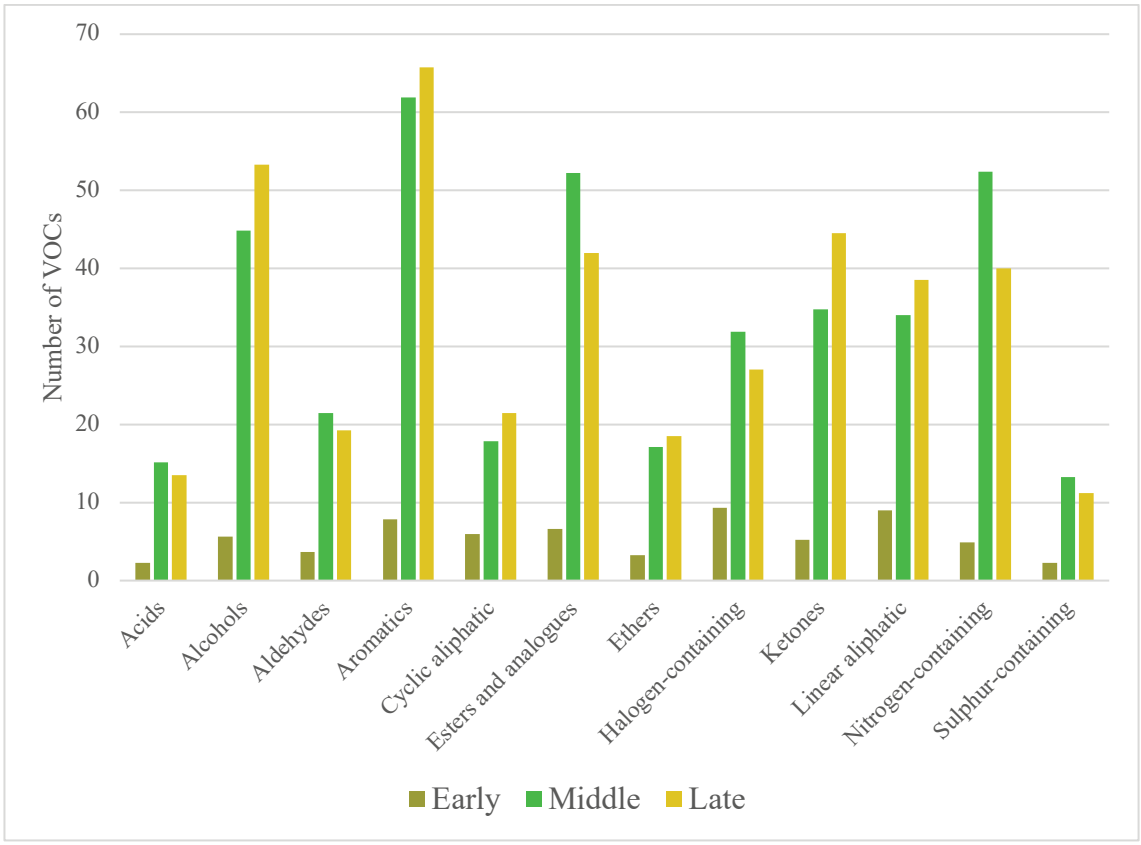


Figure 4.12: Average VOC class abundance across eight donors in three decomposition stages – early, middle and late studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

4.2.3.2 Relative class concentration based on normalised area

The detected VOCs in each of the samples were normalised using an internal standard – bromobenzene. This semi-quantitative approach was used to study the relative class concentration of compound classes for the donors. Figure 4.13 represents the relative class concentration averaged across the eight donors in this study. Nitrogen-containing VOCs had the highest relative class concentration followed by alcohols, acids, esters and analogues, aromatics, ketones, sulphur-containing VOCs, linear aliphatics, ethers, aldehydes, halogen-containing VOCs and finally, cyclic aliphatics had the lowest relative class concentration.

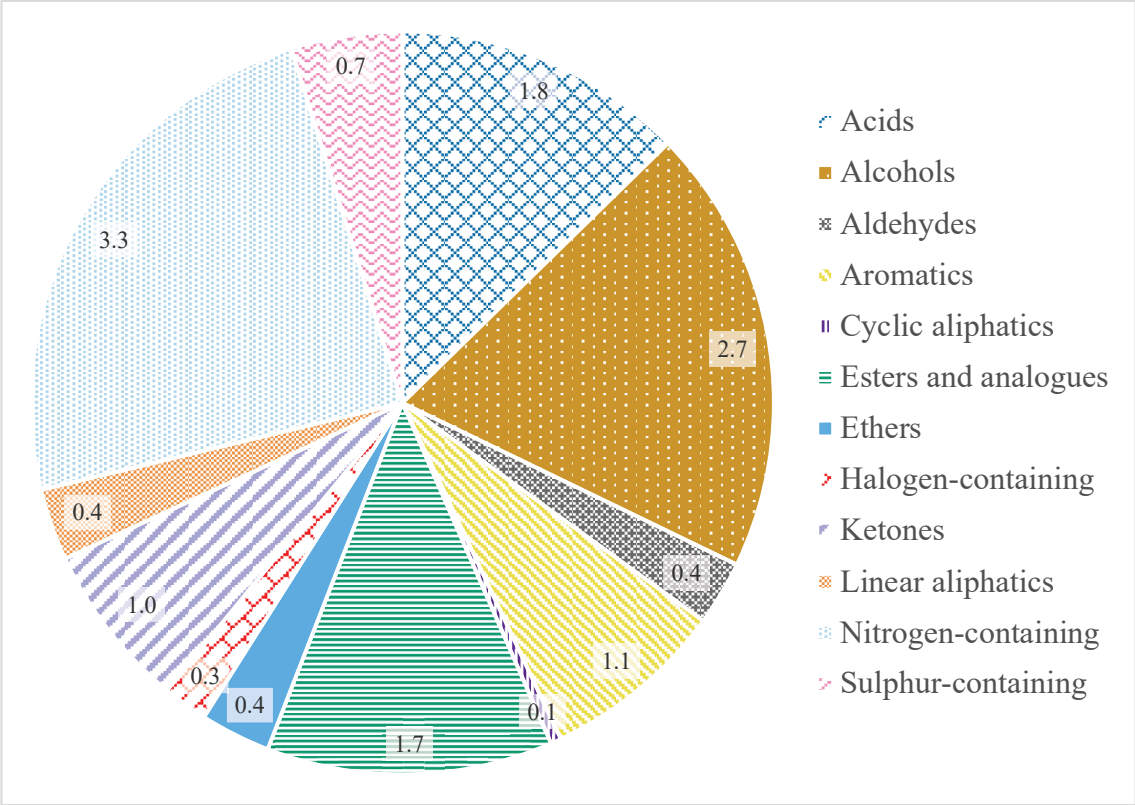


Figure 4.13: Relative class concentration averaged across eight donors studied during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

Much like the class abundance, the total average relative class concentrations of VOC classes were greater for donors decomposing during warmer average ambient temperatures than those decomposing during cooler average ambient temperatures. This has been represented in Figure 4.14.

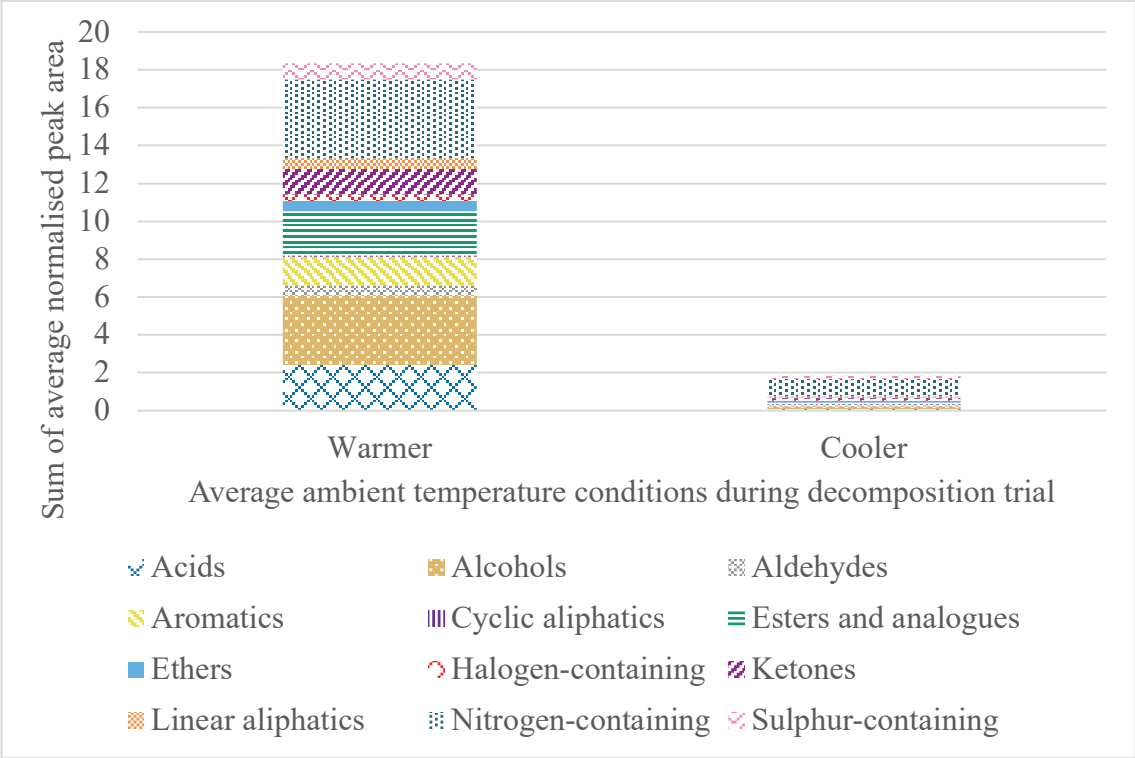


Figure 4.14: Relative class concentration averaged for four donors each decomposing during warmer average temperatures and cooler average temperatures during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

Figure 4.15 represents the average relative class concentration of individual donors. The relative class concentration was highest in donor 2020-01 followed by donors 2020-02, 2021-08, 2021-06, 2021-10, 2021-11, 2020-03 and lowest in donor 2020-04. Thus, reemphasing the fact that the total average relative class concentrations of VOCs were greater for donors decomposing during warmer average temperatures (former four mentioned) than those decomposing during cooler average temperatures (latter four mentioned). This has been discussed further in section 4.3.2.

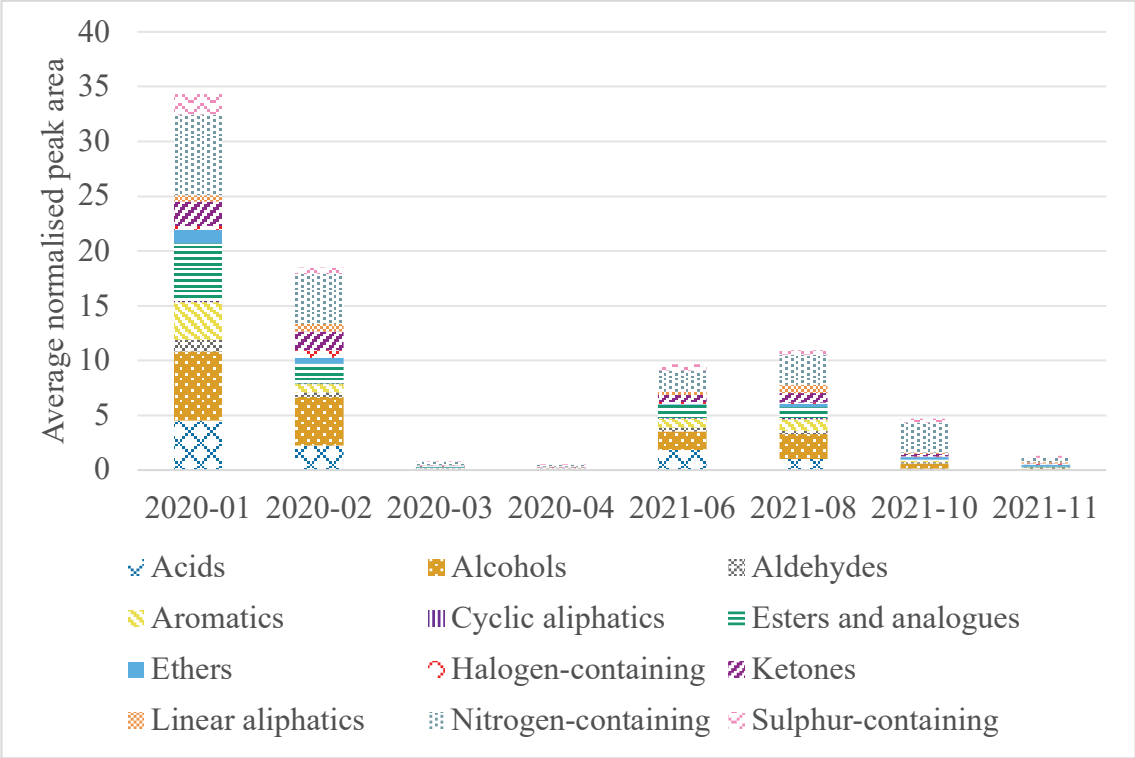


Figure 4.15: Relative class concentration of eight donors decomposing during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

Figures 4.16 shows the relative class concentration for the VOC classes across different decomposition stages averaged across the eight donors. In this instance too, like the class abundance, the early stage had the lowest relative class concentrations for each of the VOC classes compared to the middle and late stages. Compared to other classes, acids, alcohols and nitrogen-containing VOCs had slightly higher relative class concentration. When comparing the latter two stages, acids, aromatics, esters and analogues and nitrogen-containing compounds had a relatively higher relative class concentration during the middle stage, while halogen-containing VOCs had a slightly higher relative class concentration during the late stage. The relative class concentration of all the other classes was mostly comparable between the middle and late stages.

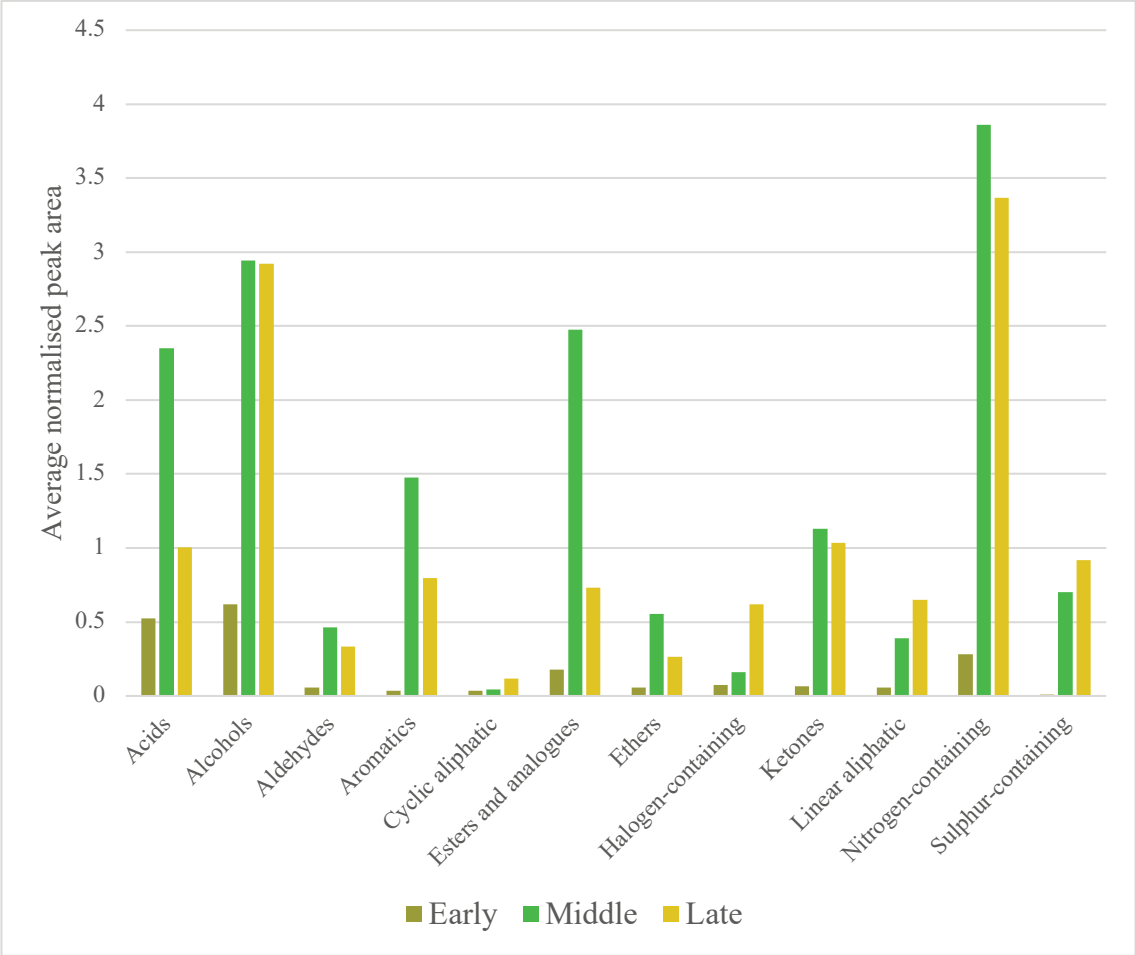


Figure 4.16: Relative class concentration averaged across eight donors studied for the three decomposition stages – early, middle and late during decomposition trials conducted at REST[ES] between August 2020 to November 2021.

4.2.3.3 Prominent and unique VOCs

Among the 1412 VOCs, 32 VOCs occurred in over 30% of the total samples analysed from eight donors. These were identified as the prominent donor decomposition-related VOCs in the current study and have been listed in Table 4.8. The 32 VOCs listed belonged to the following classes – acids, alcohols, aldehydes, aromatics, ester and analogues, ethers, ketones, nitrogen-containing, and sulphur-containing. Aldehydes, linear aliphatics, cyclic aliphatics and halogen-containing VOCs were not found among the 32 VOCs.

Table 4.8: 32 prominent VOCs detected in over 30% of samples analysed for eight donors at REST[ES] during trials conducted between August 2020 to November 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Stage of decomposition in which the VOC was detected in current study	Previously reported in literature as human decomposition odour-related	Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Stage of decomposition in which the VOC was detected in current study	Previously reported in literature as human decomposition odour-related
1.	Dimethyl trisulphide (DMTS)	Sulphur-containing	54%	Middle, late	[135; 71; 159; 160; 99; 161; 136]	17.	2,4-Dithiapentane	Sulphur-containing	36%	Middle	
2.	Methyl thiocyanate	Esters and analogues	48%	Early, middle, late	[136]	18.	2-Heptanol	Alcohols	34%	Middle, late	[136]
3.	Trimethylamine	Nitrogen-containing	44%	Middle, late	[136]	19.	3-Octanol	Alcohols	34%	Early, middle, late	[136]
4.	3-Methyl-3-buten-1-ol (Isoprenol)	Alcohols	43%	Middle, late		20.	Dimethyl sulfone	Sulphur-containing	34%	Middle	[136]
5.	2-Phenylethanol	Alcohols	43%	Middle, late	[136]	21.	2,6-Dimethyl pyrazine	Aromatics	34%	Middle	[164; 136]
6.	Trimethyl pyrazine	Aromatics	41%	Middle, late	[164; 136]	22.	2-Methyl-propanoic acid	Acids	33%	Middle	[99; 136]

7.	Methyl pyrazine	Aromatics	40%	Middle, late	[136]	23.	Tetramethyl pyrazine	Aromatics	33%	Middle, late	[162; 164]
8.	Dimethyl disulphide (DMDS)	Sulphur-containing	39%	Early, middle	[135; 71; 159; 160; 68; 80; 164; 136; 165]	24.	2-Butanol	Alcohols	33%	Middle, late	[136]
9.	4-Methylphenol (p-Cresol)	Alcohols	38%	Middle, late	[159]	25.	2,2-Dimethoxy-propane	Ethers	33%	Middle	
10.	3-Methyl-1-butanol (isoamyl alcohol)	Alcohols	38%	Early, middle	[164; 136; 165]	26.	2-Hexanol	Alcohols	33%	Middle, late	[136]
11.	3-Methyl-2-pentanone	Ketones	38%	Middle, late	[164; 136; 165]	27.	Butyl butyrate	Esters and analogues	32%	Middle	[68; 80; 136]
12.	3-Methyl-butanoic acid	Acids	37%	Middle, late	[136]	28.	4-Methyl-1-pentanol	Alcohols	31%	Middle	[136]
13.	Pyridine	Aromatics	37%	Middle	[162; 164; 136; 165]	29.	1-Methyl-1H-pyrrole	Aromatics	31%	Middle, late	
14.	2-Methyl-1-propanol	Alcohols	36%	Early, middle		30.	Dimethyl sulphide (DMS)	Sulphur-containing	31%	Early, middle	[161; 164; 136]
15.	2-Pentanol	Alcohols	36%	Middle, late	[162]	31.	1-Octanol	Alcohols	31%	Middle, late	[68; 136]

16.	3-Methyl pyridine	Aromatics	36%	Middle, late	[136]	32.	5-Ethyldihydro-2(3H)-furanone	Ester and analogues	30%	Middle	

4.2.3.4 Principal component analysis

PCA was performed to cluster and allow for dimension reduction of donors to understand the variability between donors due to warmer versus cooler average ambient temperatures during their decomposition trial. PCAs were also performed to understand variability through different decomposition stages – early, middle and late. Both these PCAs were constructed using normalised areas for the VOCs identified as most prominent in all donors (for average ambient temperature-dependent variability) or VOCs prominent in four donors each decomposing at warmer and cooler average temperatures (for donor stage-dependent variability). The resulting PCAs are represented and discussed further in this section.

4.2.3.4.1 Average ambient temperature-dependent PCA

Figure 4.17 represents a PCA based on 32 VOCs (listed in Table 4.8) which were detected in at least 30% (48 or more of the total 160) samples collected from all eight donors. This PCA was used to understand the variability due to variable average ambient temperatures (warmer vs. cooler) during the decomposition trials at REST[ES]. The eight donors were grouped into two sample sets – donors decomposing at warmer average ambient temperatures (Donor 2020-01, 2020-02, 2021-06 and 2021-08) and donors decomposing at cooler average ambient temperatures (Donor 2020-03, 2020-04, 2021-10 and 2021-11). The resulting PCA highlighted 37% and 22% of the explained variance along PC-1 and PC-2, respectively (cumulative: 59%). The associated loadings plots (represented in the biplot in Figure 4.18) were used to determine which VOCs were important to the construction of specific PCs and samples. This PCA elucidated that the donors decomposing at cooler average temperatures were tightly clustered, while those decomposing at warmer average temperatures were variable across PC-1 and PC-2. Since PC-1 and PC-2 had the highest combined explained variance, the VOCs involved in the construction of these PCs were dominant in warmer average ambient temperature donors. Thus, the associated PC-1 and PC-2 loadings plots were used to determine 14 of the 32 VOCs – trimethylamine, 3-methyl-butanoic acid, 3-methyl pyridine, 2,6-dimethyl pyrazine, 2-methyl-propanoic acid, 2-butanol, butyl butanoate, DMDS, trimethyl pyrazine, 3-methyl-1-butanol, DMTS, methyl thiocyanate and 2-methyl-1-propanol which were most dominant in donors decomposing at warmer average ambient temperature. The remaining 18 of the 32 VOCs were all still relatively significant in warmer temperature donors as most of the samples from donors decomposing at a cooler

temperature lay along the negative X-axis, while all the 32 loadings (VOCs) were along the positive X-axis (as evident in magnified X- and Y-axis origin in Figures 4.18). The distribution remained consistent along the remaining PCs – PC-3, PC-4, and PC-5 (with 17%, 10% and 7% of the explained variance, respectively) – where samples from donors decomposing at warmer temperatures were spread along the PCs, while those from donors decomposing at cooler temperatures were clustered around the X- and Y-axis origin. Thus, there was variability in the two sample sets resulting from the fact that all the VOCs identified as prominent to decomposition in this study were relatively more significant in donors decomposing at warmer temperatures than those at cooler temperatures.

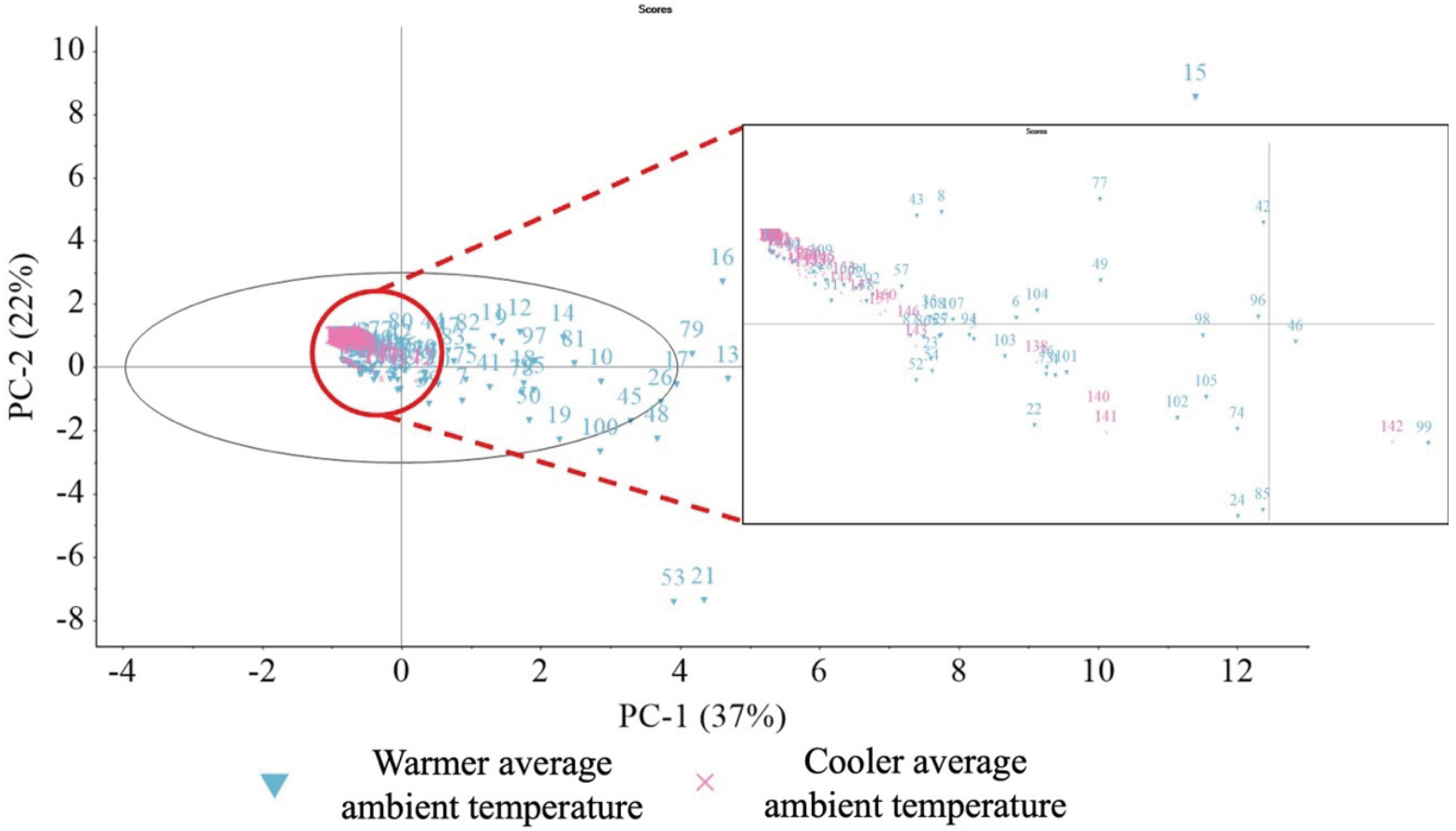


Figure 4.17: Average ambient temperature-dependent PCA scores plot for PC-1, PC-2 along with a magnified image of X- and Y-axis origin. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 32 prominent VOCs in over 30% of samples (or data points in the above PCA) collected from the eight donors at REST[ES] during decomposition trials conducted between August 2020 to November 2021. (Here, colour codes and symbols represent the average ambient temperatures during the trials, blue inverted triangles for donors decomposing at warmer average ambient temperatures and pink crosses for donors decomposing at cooler average ambient temperatures samples).

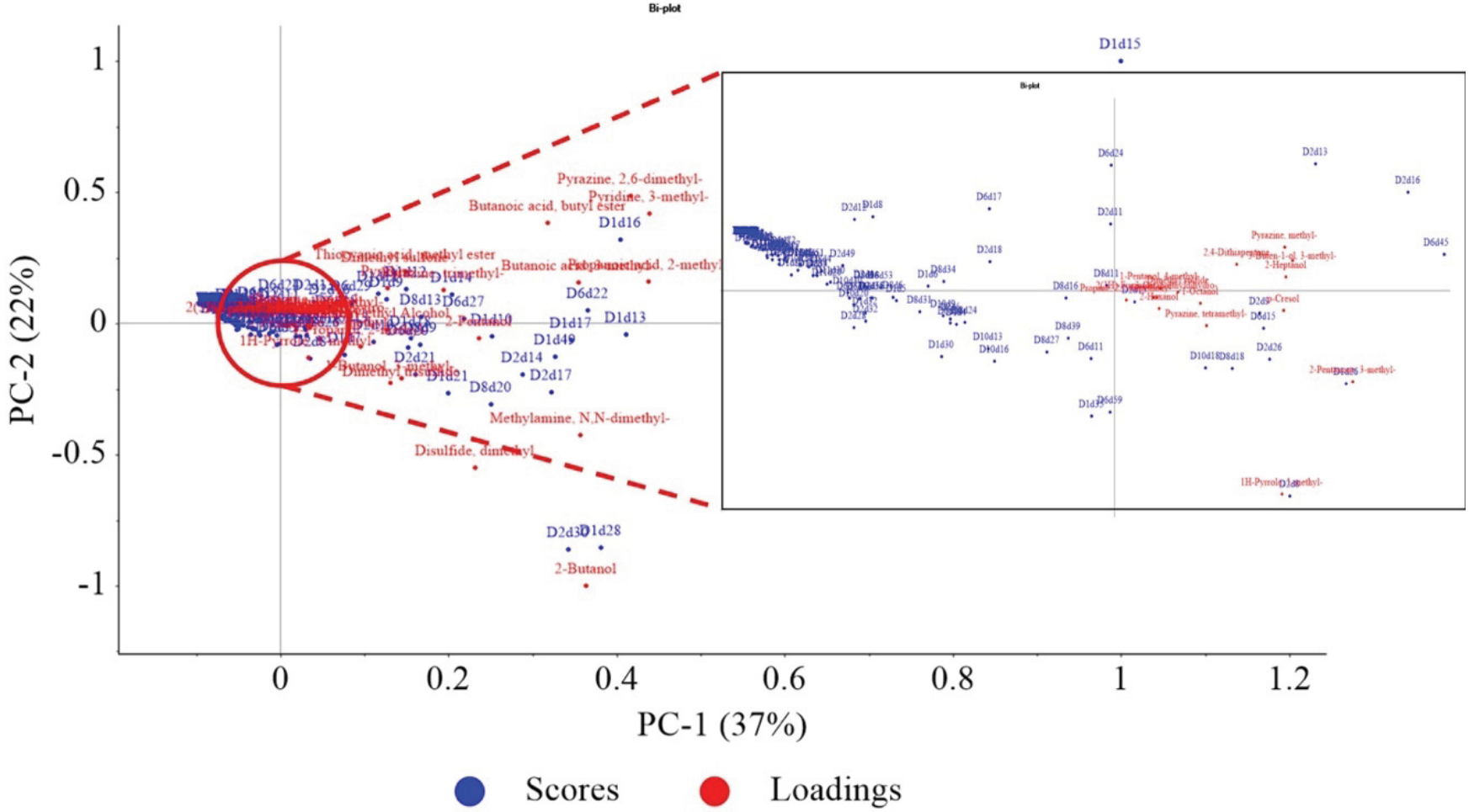


Figure 4.18: Average ambient temperature-dependent PCA biplot for PC-1, PC-2 along with a magnified image of X- and Y-axis origin for the eight donors at REST[ES] during decomposition trials conducted between August 2020 to November 2021. (Here, blue circles represent scores [donor samples] and red circles represent loading [VOCs]).

4.2.3.4.2 Donor decomposition stage-dependent PCA

Since there was variability observed in donors decomposing at warmer and cooler average ambient temperatures, two individual PCAs were constructed with the two donor sets (warmer and cooler average ambient temperatures) to understand the variability across decomposition stages.

Donors decomposing at warmer average temperatures

Figure 4.19 represents a PCA based on 22 VOCs (listed in Table 4.9) which were detected in at least 45% (50 or more of the total 109) samples collected from four donors – 2020-01, 2020-02, 2021-06 and 2021-08 which had decomposed at warmer average temperatures. The samples were grouped into three sample sets – early, middle and late decomposition stages. The resulting PCA highlighted 51% and 16% of the explained variance along PC-1 and PC-2, respectively (cumulative: 66%). The associated loadings plots (represented in a biplot in Figure 4.20) were used to determine which VOCs were important to the construction of specific PCs and samples. This PCA elucidated that the samples belonging to the early decomposition stage were tightly clustered close to the origin of the X- and Y-axis while the middle and late stage samples were found to be variable along the two PCs. PC-2 had the best explained variance for the two sample sets as samples belonging to the middle stages were distributed along the positive axes, while those belonging to the late stages were distributed along the negative axes. Thus, from the loadings associated with PC-2, it was determined that methyl thiocyanate; 2,6-dimethyl pyrazine; butyl butanoate, 2,4-dithiapentane, 3-methyl pyridine and 3-methyl-3-buten-ol were significant during the middle decomposition stage. Likewise, 2-butanol, 2-hexanol, 3-methyl-2-pentanone, tetramethyl pyrazine, DMTS, trimethylamine and 2-phenylethanol were significant during the late decomposition stage. Along the remaining PCs, the early stage samples remained most tightly clustered close to the origin of the X- and Y-axis followed by a relatively tighter cluster of late stage samples, compared to samples from the middle stage which were mostly spread along all PCs. A potential reason for observing such clustering has been discussed in section 4.3.4. No other variability in the sample groups (early vs. middle vs. late) was observed along the remaining PCs – PC-3, PC-4, PC-5 with 20%, 8% and 2% of the explained variance, respectively.

Table 4.9: 22 prominent VOCs detected in over 45% of donor samples collected from four donors decomposing at average warmer ambient temperatures during decomposition trials conducted between August 2020 to November 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature human decomposition odour-related
1.	Dimethyl trisulphide (DMTS)	Sulphur-containing	61%	[135; 71; 159; 160; 99; 161; 136]
2.	3-methyl-3-buten-1-ol	Alcohol	59%	
3.	2-Phenylethanol	Alcohol	59%	[136]
4.	Trimethyl pyrazine	Aromatic	59%	[164; 136]
5.	Methyl pyrazine	Aromatic	55%	[136]
6.	Methyl thiocyanate	Ester and analogues	55%	[136]
7.	3-Methyl pyridine	Aromatic	53%	[136]
8.	2-Pentanol	Alcohol	51%	[162]
9.	2,6-Dimethyl pyrazine	Aromatic	50%	[164; 136]
10.	2-Heptanol	Alcohol	50%	[136]
11.	3-Methyl-butanoic acid	Acid	50%	[136]
12.	3-Methyl-2-pentanone	Ketone	50%	[164; 136; 165]
13.	2,4-Dithiapentane	Sulphur-containing	49%	
14.	Tetramethyl pyrazine	Aromatic	49%	[162; 164]
15.	Trimethylamine	Nitrogen-containing	49%	[136]
16.	2-Butanol	Alcohol	48%	[136]
17.	3-Octanol	Alcohol	48%	[136]

18.	4-Methylphenol (p-Cresol)	Alcohol	48%	[159]
19.	2-Hexanol	Alcohol	47%	[136]
20.	Butyl butyrate	Ester and analogues	47%	[68; 80; 136]
21.	2-Methyl-propanoic acid	Acid	46%	[99; 136]
22.	4-Methyl-1-pentanol	Alcohol	46%	[136]

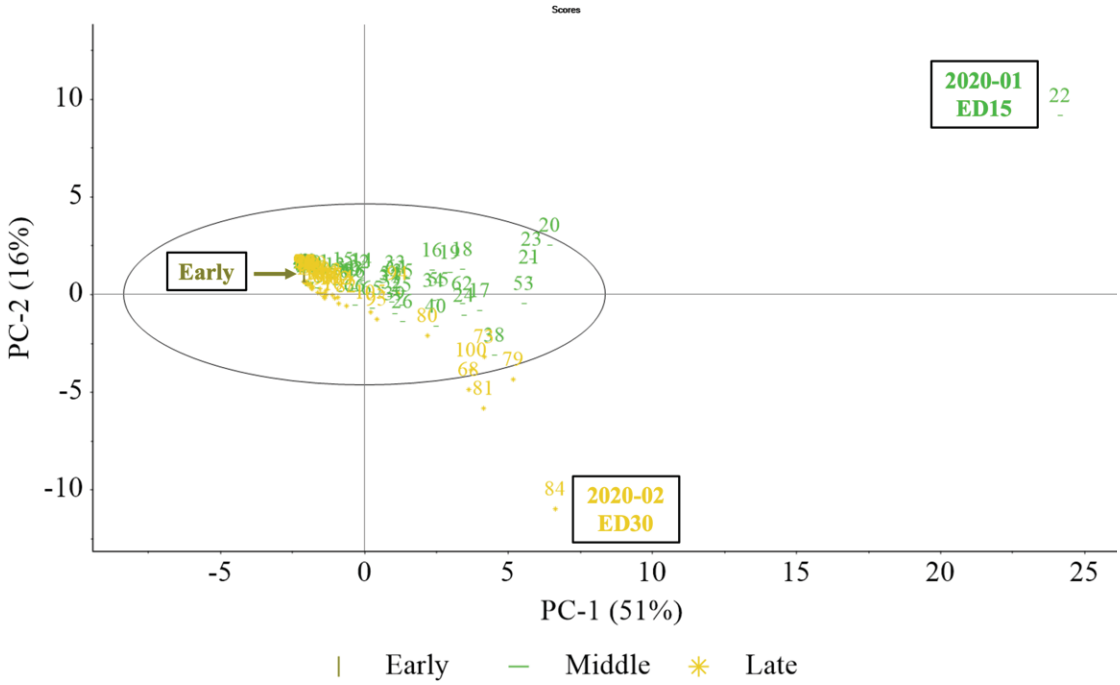


Figure 4.19: Stage-dependent PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 22 prominent VOCs in over 45% samples (or data points in the above PCA) collected from four donors at REST[ES] during decomposition trials conducted at warmer average ambient temperatures between August 2020 to November 2021. (Here, colour codes and symbols represent decomposition stages, brownish green vertical lines for early, light green horizontal line for middle and yellow stars for late).

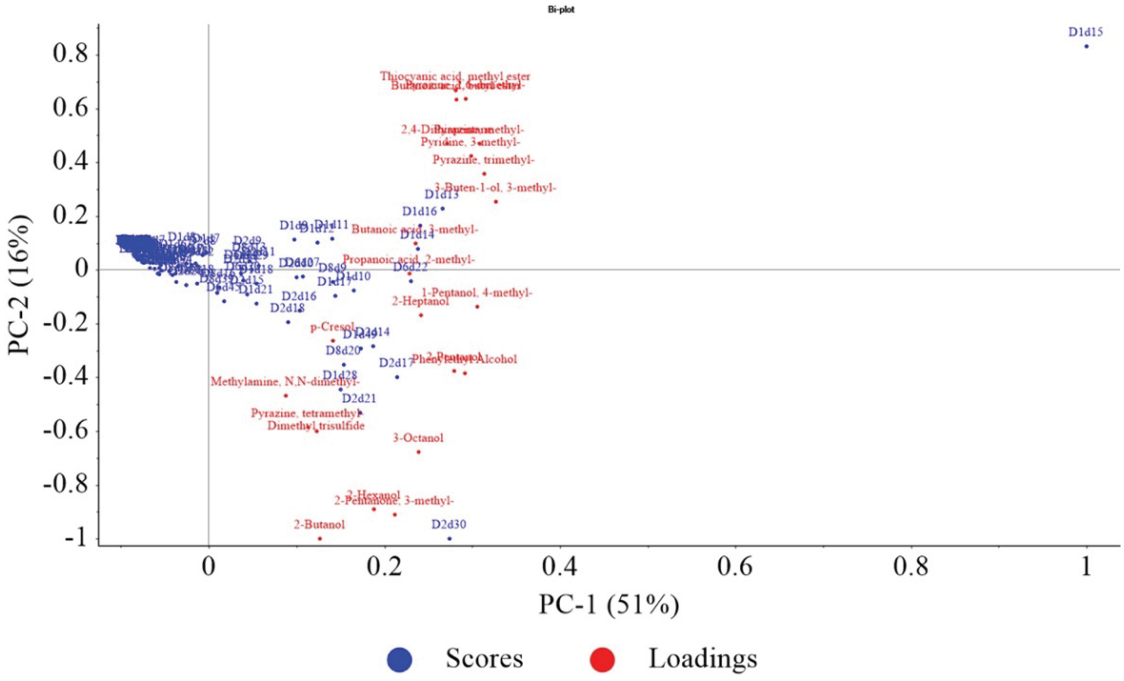


Figure 4.20: Stage-dependent PCA biplot for PC-1, PC-2 for four donors at REST[ES] during decomposition trials conducted at warmer average ambient temperatures between August 2020 to November 2021. (Here, blue circles represent scores [donor samples] and red circles represent loading [VOCs]).

Donors decomposing at average cooler temperatures

Figure 4.21 represents a PCA based on 16 VOCs (listed in Table 4.10) which were detected in at least 20% (11 or more of the total 51) samples collected from four donors – 2020-03, 2020-04, 2021-10 and 2021-11 which had decomposed at cooler average temperatures. The samples were grouped into two sample sets – early and middle. No late stage was observed in any of these four donors. The resulting PCA highlighted 72% and 19% of the explained variance along PC-1 and PC-2, respectively (cumulative: 91%). The associated loadings plots (represented in a biplot in Figure 4.22) were used to determine which VOCs were important to the construction of specific PCs and samples. This PCA elucidated that the early decomposition stage was tightly clustered close to the origin of the X- and Y-axis while the middle stage samples were found to be variable along the two PCs. Thus, the samples belonging to the early and middle stages of decomposition had variability. Five of the 16 VOCs – trimethylamine, DMDS, 3-methyl-1-butanol, 1,3,5-triazine and 2-methoxy-ethanol were the most significant loadings along PC-1 thus, these VOCs were most significant to the middle decomposition stage relative to the early stage. Some extreme loadings were observed and all of these belonged to the middle (peak active) decomposition stage observed in donor 2021-10. A potential reason for these extreme loadings has been discussed in section 4.4.1

Table 4.10: 16 prominent VOCs detected in over 20% of donor samples collected from four donors decomposing at cooler average ambient temperatures during decomposition trials conducted between August 2020 to November 2021.

Sr. no.	Volatile organic compound	Compound classes	Percentage of samples in which the VOC was detected	Previously reported in literature human decomposition odour-related
1.	Dimethyl disulphide (DMDS)	Sulphur-containing	43%	[135; 71; 159; 160; 68; 80; 164; 136; 165]
2.	Dimethyl trisulphide (DMTS)	Sulphur-containing	41%	[135; 71; 159; 160; 99; 161; 136]
3.	Trimethylamine	Nitrogen-containing	35%	[136]
4.	Methoxybenzene (anisole)	Ether	31%	
5.	Methyl thiocyanate	Ester and analogues	31%	[136]
6.	1-Methoxy-3-methyl-butane	Ether	29%	[136]
7.	3-Methyl-1-butanol	Alcohol	27%	[164; 136; 165]
8.	Dimethyl sulphide (DMS)	Sulphur-containing	27%	[159; 68; 136]
9.	3-Hexene	Linear-aliphatics	25%	
10.	Pyridine	Aromatic	25%	[162; 164; 136; 165]
11.	2-Methylpropane (Isobutane)	Linear aliphatics	24%	
12.	1-Propyloctyl-benzene	Aromatic	24%	
13.	1,3,5-Triazine	Nitrogen-containing	22%	
14.	2-Methoxy-ethanol	Alcohol	22%	
15.	2-Methyl-butane	Linear aliphatics	22%	

16.	Tetrachloroethylene	Halogen-containing	22%	[68; 80; 136]

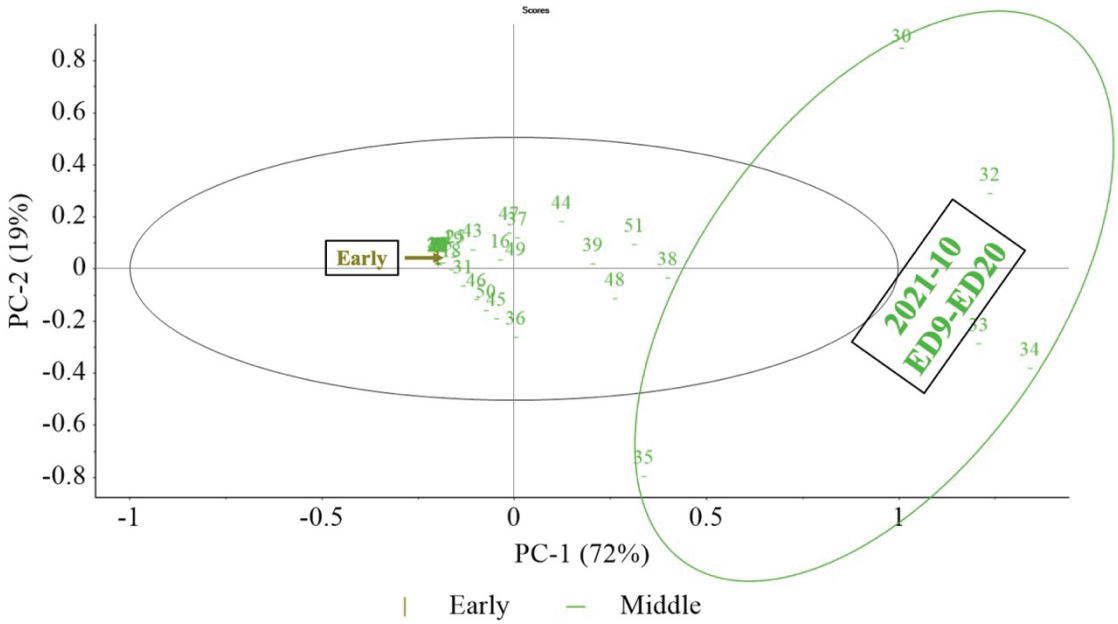


Figure 4.21: Stage-dependent PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 16 prominent VOCs in over 20% of samples (or data points in the above PCA) collected from four donors at REST[ES] during decomposition trials conducted at cooler average ambient temperatures between August 2020 to November 2021. (Here, colour codes and symbols represent decomposition stages, brownish green vertical lines for early and light green horizontal lines for middle).

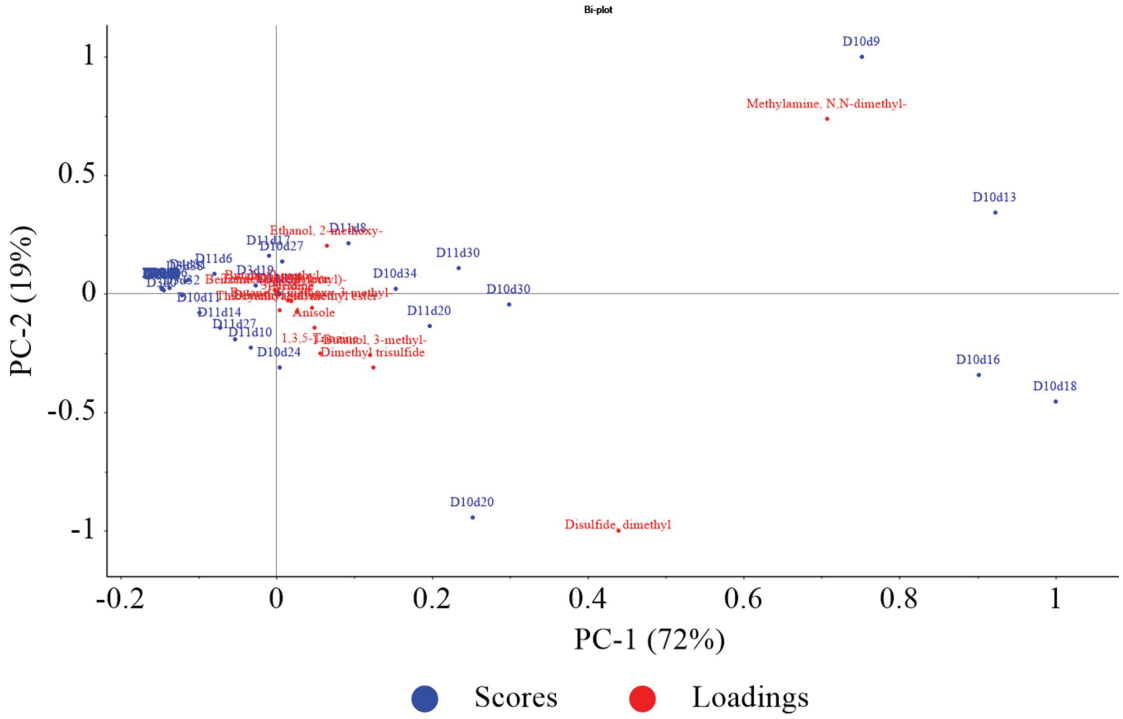


Figure 4.22: Stage-dependent PCA biplot for PC-1, PC-2 for four donors at REST[ES] during decomposition trials conducted at cooler average ambient temperatures between August 2020 to November 2021. (Here, blue circles represent scores [donor samples] and red circles represent loading [VOCs]; 'D' represents donor ID and 'd' represents the ED).

4.3 Discussion

4.3.1 Donor decomposition stage observations

This study identified differences in the decomposition progression of donors based on the average ambient temperatures during the months of decomposition thus, the donors were categorised into two types (warmer vs. cooler average ambient temperature donors). The donors decomposing at warmer average ambient temperatures (2020-01, 2020-02, 2021-06, 2021-08) arrived at REST[ES] during the Canadian summer or spring season and they entirely transitioned to the desiccation and/or skeletonisation stage at the end of their trial. In contrast, donors decomposing at cooler average ambient temperatures (2020-03, 2020-04, 2021-10, 2021-11) arrived at REST[ES] during the Canadian autumn season and they transitioned from the fresh stage and remained in one or more of the following four stages (bloat, active decay, desiccated, skeletonised) at the end of their trial. Other than ambient temperature, this difference in the progression of stages could be because the trial duration of donors decomposing at warmer average ambient temperatures lasted for over 2 months

(ED 72 – ED 87 or KADD 21359.1 – 25051.6 depending on the donor) compared to those at cooler average ambient temperatures which lasted for about a month (ED30 – ED38 or KADD 8709.5 – 10927.4 depending on the donor). However, regardless of the trial duration, the rate of decomposition at warmer average ambient temperatures was faster when comparing the progression through stages at the end of the first month of the decomposition trial for each of the donors. Further, the fresh stage lasted longer in donors decomposing at cooler average ambient temperatures (between ED 3 – ED 11 or KADD 1154.1 – 3415.6), while for three of the four donors (except donor 2021-06) decomposing at warmer average ambient temperatures it was much shorter (between ED 1 – ED 4 or KADD 590.9 – 1475.1). The fresh stage for donor 2021-06 lasted up to ED 10 (KADD 3178.2), longer than the other donors of its category (donors decomposing at warmer average ambient temperatures). This variability in donor 2021-06 could be due to the fact that its trial began in the Canadian spring season when the average daily temperatures were close to 13˚C, while the trial for the other donors started in the Canadian summer season when daily average temperatures were around 21˚C in the beginning (first week) of their trial. It has been previously determined that the initial temperature conditions have an impact on the process of decomposition and the VOCs released [96]. Additionally, the bloat stage was not as significant at cooler temperatures as most donors showed only mild bloating in the neck unlike donors decomposing at warmer temperatures that developed significant bloat in the abdomen soon after bloating in the face and neck. Further, when present, the bloat stage started much later (from the day of arrival of the donor at REST[ES]) which was the case for two of the four cooler temperature donors – 2020-03 and 2020-04. In another donor (2020-10) decomposing at a cooler average temperature, there were no signs of bloating during its entire trial period. Thus, the donors decomposing in months of relatively cooler average ambient temperatures had a slower rate of decomposition. Therefore, this study concludes that decomposition progresses rapidly when the ambient temperatures are higher. This is consistent with previously reported literature reporting the impact of ambient temperature on the decomposition process [181; 96; 87].

The current study recorded decomposition progression only as observations (qualitatively), no quantitative estimations such as using the concept of total body scores were used. Total body score estimations (specifically the method developed by Megyesi et al. [26]) have been used by numerous studies however, several studies have shown the

limitations of its application as it is geography specific [183; 184; 180; 185; 186]. The current study did not incorporate this method because, at the time of conducting the decomposition trials, a simultaneous study to develop a new scoring system for donors at REST[ES] was underway. Scoring the donors was not the primary objective of this research as it would not have contributed to understanding their VOC profiles or changes in VOC profiles through the decomposition stages. Thus, decomposition stages were purely observational and weighted against ambient temperatures in KADD.

Since the current study was conducted in an outdoor environment, the VOC collection and analysis at colder temperatures lasted only until the beginning of snowfall. Due to logistical limitations, the VOC collection was not resumed the following season when the snow melted. This limitation in the current study could be incorporated in future studies to understand the impact of snow on the process of decomposition.

4.3.2 Class abundance and relative class concentrations

This study reported the average class abundance (based on the number of VOCs) and relative class concentration (based on normalised areas) across individual donors and trends depending on the average ambient temperature conditions and stage of decomposition. Both the class abundance and the relative class concentration for all classes of VOCs reported in this study were higher for donors decomposing at warmer average ambient temperatures. When comparing class abundance, aromatics were the most abundant followed by esters and analogues, and nitrogen-containing VOCs. This is consistent with what has been reported in a prior study on human decomposition as aromatics were reported as most abundant in summer and esters were most abundant during winter in a study using human cadavers conducted by Knobel et al. [87]. Alcohols, linear aliphatics, ketones, halogen-containing VOCs and aldehydes were of average abundance. Finally, cyclic aliphatics, ethers, acids and sulphur-containing VOCs were the least abundant. This trend varied slightly for donors decomposing at cooler average ambient temperatures, as halogen-containing and linear aliphatics VOCs became one of the most abundant classes along with aromatics and nitrogen-containing VOCs. A prior study conducted on human analogues (pigs) has previously reported detecting a higher abundance of halogenated compounds during their decomposition trials conducted in the winter season (colder temperature) [87]. The trend in relative class concentration varied

across donors (indicated in Figure 4.15). A study comparing class trends between pig and human VOCs in Australia reported that hydrocarbons, alcohols and aromatics were prevalent during warmer temperatures, while esters, hydrocarbons and aldehydes were prevalent during cool ambient temperatures [87]. The warm and cool ambient temperatures in Australia and in the current study (in Québec, Canada) are not alike. Regardless, in the current study, from comparing both abundance and relative concentration class trends, nitrogen-containing VOCs, alcohols, esters and analogues, and aromatics were prevalent at warmer ambient temperatures, while abundance and relative concentration class trends were variable in cooler ambient temperatures. Overall, the average relative class concentration for all VOC classes was higher for donors decomposing at warmer average ambient temperatures. Among the donors decomposing at warmer ambient temperatures, donor 2020-01 had the highest average relative class concentration, while donor 2020-06 had the lowest average relative class concentration. This could be because of lower initial ambient temperatures when donor 2020-06 arrived at REST[ES] (discussed previously in section 4.4.1) relative to other donors decomposing at warmer average ambient temperatures. Among the donors decomposing at cooler average ambient temperatures, donor 2020-10 had the highest average relative class concentration, while donors 2020-03 and 2020-04 had the lowest average relative class concentration. This could be because donor 2020-10 was most advanced in its decomposition relative to all the other donors decomposing at cooler temperatures. Even at the last ED during the trial of this donor (ED 34 or KADD 9887.2) larval activity was extremely significant on the posterior side of the donor in contact with the soil surface. Moreover, this was the only donor decomposing at a cooler temperature that showed signs of skeletonisation by the end of its trial. This could also be the reason for the variability of donor 2020-10 relative to other donors decomposing at cooler temperatures as was evident in the PCA represented in Figure 4.21.

The stage-dependent class abundance and relative class concentrations were least in the early (fresh appearing) stage of decomposition compared to the middle (bloat and active decay) and late (post-bloat and post-active decay) stages. During the early stages following death, the decomposition process commences and the transition from perimortem to postmortem odour takes some time during which, decomposition VOCs may not be readily detected leading to a reduced VOC abundance [96]. This could also be the reason for fewer VOC classes in each of the donors during the early stage of

decomposition as shown by ethers, acids, cyclic aliphatics, sulphur-containing, nitrogen-containing and halogen-containing VOCs not being detected in one or more donors. When comparing the middle and late stages for class abundance, esters and analogues and nitrogen-containing VOCs were evidently higher during the middle stage, while alcohols and ketones had a higher abundance during the late stage of decomposition. Similarly, the relative class concentration of acids, aromatics, esters and analogues and nitrogen-containing VOCs was relatively higher during the middle stage, while halogen-containing VOCs and linear aliphatics had a higher relative class concentration during the late stage. All the remaining classes had more or less comparable class abundance and relative class concentrations for middle and late stages. Thus, from these observations, it can be concluded that esters and analogues and nitrogen-containing VOCs are significant during the bloat and active decay stage of decomposition relative to other stages (fresh, desiccated and skeletonised). Although not higher than the middle and late stages, acids, alcohols and nitrogen-containing VOCs had a high relative class concentration compared to other classes in early stage of decomposition. A prior study conducted in 2019 on human donors in Australia concluded that the class trends varied across individual donors and did not report any trends during early (ADD 0 – 300), middle (ADD 300 – 700) and late stages (ADD 700+) [96]. Since the number of compounds detected in decomposition studies is in the hundreds [98; 94; 136; 137], VOCs are classified into compound classes for ease of reporting them. One limitation of using compound classes is the discrepancy in classification between studies. For example, pyridine has been classified as a nitrogen-containing compound in a prior study [137] however, it was classified as an aromatic VOC in the current study. This limits the class comparison across studies by different authors. In the current study, the VOC classes were kept consistent between VOCs identified in Chapters 3 and 4 and a class priority order based on the rules of the IUPAC nomenclature [187] was maintained during the classification of VOCs. This aided classification of VOCs with multiple functional groups. This is further discussed in section 6.4.

4.3.3 Donor VOCs

Of the total 1412 VOCs identified in donors decomposing at REST[ES] in the current study, 32 VOCs were identified as prominent as they occurred in over 30% of samples (50 or more of 160 samples). 27 of the 32 prominent VOCs have been previously reported

in the literature as human decomposition odour-related and the studies that identified these VOCs has been summarised in Table 4.8. The five VOCs that have not previously been reported in human decomposition odour included 3-methyl-3-buten-1-ol, 4-methylphenol, 2,4-dithiapentane, 1-methyl-1H-pyrrole and 5-ethyldihydro-2(3H)-furanone. The former three VOCs – 3-methyl-3-buten-1-ol [162], 4-methylphenol [188] and 2,4-dithiapentane [85; 98] have been reported in animal remains decomposition odour. From these, 4-methylphenol can result from anaerobic catabolism of tyrosine by multiple microbial species [61] and 2,4-dithiapentane, a sulphur-containing VOC can be produced during degradation of proteins which could result in free sulphur-containing amino acids [85]. These can undergo further breakdown by microbial degradation under aerobic or anaerobic conditions to produce sulphur-containing VOCs [85]. Other than these three VOCs, the remaining two – 1-methyl-1H-pyrrole and 5-ethyldihydro-2(3H)-furanone have never been reported in decomposition odour. 1-methyl-1H-pyrrole is a pyrrole derivate and the pyrrole ring naturally occurs in haemoglobin, myoglobin, and vitamin B12 thus they can be produced during the degradation of these complexes [189]. Alternatively, pyrrole can also be produced by some bacteria [190]. 5-ethyldihydro-2(3H)-furanone is a lactone which can be produced during microbial biotransformation of hydroxy fatty acids [191]. Some other VOCs such as indole, phenol, o-xylene, and p-xylene [71; 159; 72; 98; 140; 97] have commonly been reported in the literature and frequently detected as decomposition-related VOCs. In the current study, these VOCs were not identified as prominent as they were present in less than 30% of the samples. Indole was detected in 27% of samples, phenol was in 6% of samples, o-xylene was in 7% of samples and p-xylene was in 1% of samples. One reason for not identifying these VOCs as prominent could be the difference in the analytical technique used in previous studies compared to that in the current study. A study conducted in Texas, USA which focused on the analysis of VOCs up to six days postmortem identified 58 VOCs as key to human decomposition VOC profile [173]. Of these 58, 32 have been detected in the current study and one – dimethyl sulfone was identified among the 58 VOCs and also identified as prominent in the current study.

4.3.4 Temperature- and stage-dependent PCA

Through the construction of PCAs, this study found that decomposition-related VOCs were dominant at warmer average ambient temperatures thus causing variability in donors

decomposing at warmer temperatures relative to those decomposing at cooler temperatures. This conclusion was drawn from a PCA based on 32 VOCs that were identified as prominent which occurred in 30% of decomposing donor samples. Of the total 1412 VOCs, 1332 were detected during warmer average ambient temperatures and 581 were detected in cooler average ambient temperatures (with overlap). At cooler average ambient temperatures 501 of the 581 VOCs were consistent with those detected at warmer average ambient temperatures. A majority of the 80 remaining VOCs from the 581 were detected in only 1 – 2 samples (except isoserine and 1-(ethenyloxy)-octadecane which were detected in four samples). Thus, there were no broadly detected cold temperature-specific VOCs. The variability in the PCA and decomposition VOC profile was only owing to the fact that the VOCs at warmer temperatures had higher values for the normalised area (implying greater relative class concentration).

The two PCAs produced to understand the variability between the decomposition stages highlighted that the early stage, when the donors appeared fresh, was most variable relative to the middle and late stages. This was due to the fact that the (prominent) VOCs used in the construction of these PCAs were either absent or not dominant in the early stages. The first PCA for donors decomposing at warmer average ambient temperature was generated with 22 (prominent) VOCs detected in over 45% of samples and therefore identified as prominent in the current study. This threshold of 45% was lowered to 20% when generating a PCA for donors decomposing at cooler temperatures because there were no VOCs that were present in over 45% of the samples. Thus, during the current study, there was no specific threshold for the number of samples that a VOC had to be present in to be identified as prominent, since this continued to change based on the sample set. The aim was to construct a PCA that could explain and visualise data while having a high explained variance. It was observed that even when the threshold value was changed, the spatial distribution of samples in the PCA more or less remained the same and did not impact the interpretation of the results. As a result, 22 VOCs were identified as prominent at warmer average ambient temperatures and 16 VOCs were identified as prominent at cooler average ambient temperatures. All of the 22 VOCs at warm temperatures were consistent with the list of 32 VOCs (Table 4.8) identified as significant to donor decomposition in the current study, while only 7 of the 16 VOCs at cooler temperatures were consistent with this list (Table 4.8).

In the PCA to study stage-dependent variability in donors decomposing at warmer average ambient temperature, the middle and late stages were found to be somewhat variable (16% explained variance along PC-2 in Figure 4.19). This was due to the dominance of methyl thiocyanate, 2-6 dimethy pyrazine, butyl butanoate, 2,4-dithiapentane, 3-methyl pyridine and 3-methyl-3-buten-1-ol during the middle decomposition stage. Simultaneously, 2-butanol, 2-hexanol and 3-methyl-2-pentanone were significant during the late decomposition stage. All of these compounds have been previously reported as human decomposition odour-related except 3-methyl-3-buten-1-ol and 2,4-dithiapentane as mentioned previously in this section. It was also observed that the early and late stage samples formed a tighter cluster around the axis origin relative to middle stage samples. This indicated that middle stage samples were the most variable potentially resulting from the accelerated decomposition process during this stage which could have resulted in an intra-stage variable VOC profile. In the PCA to study stage-dependent variability in donors decomposing at cooler average ambient temperature, early and middle stages were found to be variable (72% explained variance along PC-1 in Figure 4.21) due to dominance of trimethylamine, DMDS, 3-methyl-1-butanol, 1,3,5-triazine and 2-methoxy-ethanol in the middle decomposition stage relative to the early stage. From these, other than 1,3,5-triazine which has been reported in the animal decomposition odour [192], and 2-methoxy-ethanol which has never been reported as a decomposition VOC, the remaining VOCs have been reported in human decomposition odour. DMDS, a sulphur-containing compound is a highly reported decomposition VOC [71; 159; 165]. As elaborated earlier, sulphur-containing VOCs can be produced during the breakdown of sulphur-containing amino acids. 3-methyl-1-butanol, a higher alcohol has been reported to be a product of the biological conversion of leucine, an amino acid [193]. Trimethylamine is commonly reported as a product of the decomposition process and a recent study reported its usefulness in the estimation of the postmortem interval (PMI or time since death) [194]. In this study, the authors found that the concentration of trimethylamine steadily increased up to 35 hours after death however, this could not be validated in the current study as no more than one sample per donor could be collected within a PMI of 35 hours. Moreover, trimethylamine was not detected in the early stage in the current study. This could be due to the difference in the experimental design of the two studies since headspace from homogenised tissue samples decomposing in vitro were analysed by gas chromatography in the Li et al. study [194]. In the current study,

headspace from cadavers decomposing in an outdoor environment was analysed using GC × GC–TOFMS.

Thus, the PCAs indicate that the early decomposition stage (fresh) varies greatly from the middle stages (bloat and active decay). The late stage (post-bloat and post-active decay) is subtly variable from the middle stage since the explained variance was only 16% (in Figure 4.20). As discussed, these variabilities could be explained by specific VOCs that dominated in the middle and late stages of decomposition.

4.4 Conclusion

The aim of this study was to analyse the VOC profile of eight donors decomposing at REST[ES] during trials conducted between August 2020 to November 2021. This resulted in the identification of 1412 VOCs which were significant in samples compared to controls. All decomposition-related classes were identified in these training aids. The general trend for class abundance from highest to lowest was aromatics followed by esters and analogues, nitrogen-containing, alcohols, linear aliphatics, ketones, halogen-containing, aldehydes, cyclic aliphatics, ethers, acids and sulphur-containing VOCs. Likewise, the trend for relative class concentration was nitrogen-containing VOCs, alcohols, acids, esters and analogues, aromatics, ketones, sulphur-containing VOCs, linear aliphatics, ethers, aldehydes, halogen-containing VOCs and cyclic aliphatics. This study presents prominent compounds detected in donor decomposition (listed in Tables 4.8 to 4.10). This study found a variability in donors' decomposition VOC profile due to variability in average ambient temperature (warmer vs. cooler). VOC profile of donors also varied with decomposition stage with the middle stage (during bloat and active decay) being the most variable relative to early and late stages due to VOCs that dominated the middle stage.

Chapter 5: PERFORMANCE OF CADAVER DETECTION DOGS ON TRAINING AIDS

Chapter 5: PERFORMANCE OF CADAVER DETECTION DOGS ON TRAINING AIDS

5.1 Introduction

The search for and detection of human remains is a vital first step prior to their recovery and identification to establish a crime in a forensic context or to return the remains to the victims' beloved from a humanitarian perspective. Human remains detection methods can be destructive or non-destructive in nature [195]. Destructive search techniques such as probing the soil and digging using equipment can damage the remains and disturb the crime scene or the natural environment [196]. Thus, unless necessary these are less preferred over non-destructive methods such as geophysical search techniques (e.g. ground penetrating radar, magnetometers, metal detectors etc.) and the use of CDDs [196]. For many years, CDDs have been a valuable detection tool for law enforcement and volunteer organisations in locating human remains.

CDDs as a detection tool respond to decomposition VOCs by sniffing the air and passing it over specialised olfactory receptor cells in their nasal passages. Every odour source (such as a cadaver emitting decomposition odour) has a set of volatile compounds giving it a unique odour pattern that triggers a specific set of receptors which allows for differentiating between odour sources [93]. It is important to understand the existence and spread of VOCs or odour molecules in the air because it helps dogs locate the target odour source. Closer to the object, the VOCs accumulate densely above the odour source which is the point of origin forming a 'scent pool' [93]. They are eventually dispersed in the environment forming a concentration gradient (Figure 5.1) and in the presence of unidirectional wind, the VOCs spread in a cone manner, popularly known as a 'scent cone' (Figure 5.2). As the distance from the point of origin increases, the odour becomes fainter and diffused [93]. Once the dog detects the edge of a scent cone, it works backwards to find the source of the odour, at which trained dogs give an 'alert' such as a passive laying down or active barking to notify their handler [93]. Several environmental factors such as temperature, wind direction, and the presence of barriers (e.g. hills, trees etc.) also influence the concentration and spread of the odour molecules. Therefore, it is vital that a dog handler understands the principles of odour molecules' transmission in

the environment because a dog relies on its handler's capability to consider environmental factors and lead them to work efficiently during a search [93; 197]. Thus, dogs must be able to search independently while following instructions from their handler.

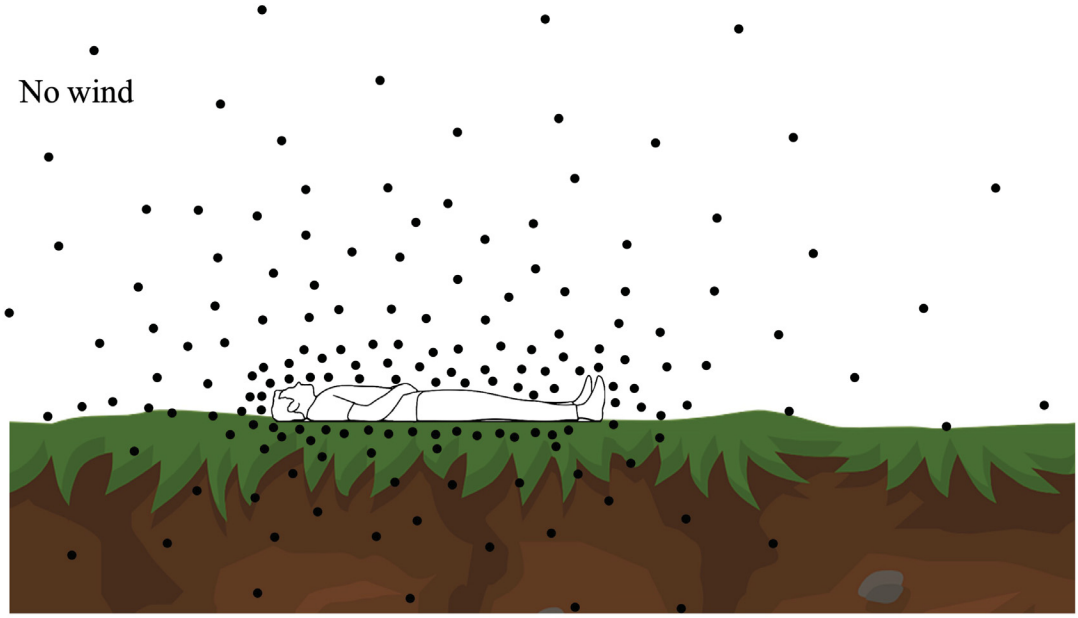


Figure 5.1: Scent pool around the remains showing concentration gradient of decomposition odour molecules with higher density around the source and reducing density away from the source in an outdoor environment with no wind (adapted from [93]).

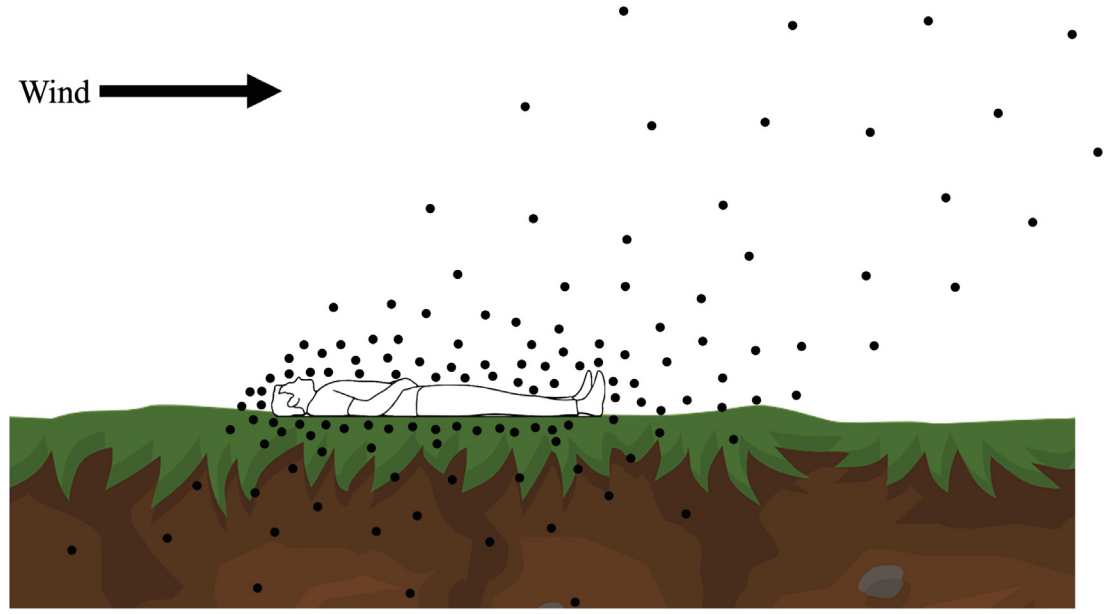


Figure 5.2: Scent cone showing a cone shaped concentration gradient of decomposition odour molecules with higher density around the source and reducing density away from the source in an outdoor environment with unidirectional wind (adapted from [93]).

The earliest records of the application of CDDs was in the 19th century to locate human remains of victims of the infamous 'Jack the Ripper' [198]. The greatest advantage of using CDDs over other non-destructive geophysical techniques is that they can search large areas in short time periods. Furthermore, they are functional in different terrain types which is important as the search scenarios can widely vary from indoor to outdoor (forests, grasslands, mountains or deserts) searches, or even within water [199]. There are case studies and research that have shown the success of CDDs in locating human remains in multiple scenarios. Komar (1999) was the first to deduce that colder temperatures did not impair CDDs ability to find a bone [200]. A study by Lasseter et al. (2003) [201] indicated that dogs could detect dry buried bones and further studies by Martin et al. (2012) [202] and Glavaš et al. (2019) [203] concluded their success in detecting historical gravesites. Riezzo et al. (2014) proved that dogs can detect blood at low concentrations with dilutions of ratios up to 1:1,000,000 [204]. In 2008, Fairgrieve found that a well trained CDD can locate a cremated human bone [205]. Finally, two separate studies by Oesterhelweg et al. (2008) and Alexander et al. (2015) highlighted that CDDs could identify residual odour in the absence of the remains [206; 131]. From all these studies, it is evident that CDDs can efficiently work in scenarios where the remains have been buried, hidden, concealed or burnt, making them an excellent and sensitive alternative to other search techniques.

The true capability of CDDs is still being uncovered as there are only limited scientific trials that have been conducted to date. Observations over the years have highlighted that a CDD is only as good as its training and the training of its handler. Thus, the training of CDDs is a major aspect that contributes towards their success as a search and detection tool. Despite this, there remains a major gap in the field of CDD training without any global standards. A few organizations such as The Organisation of Scientific Area Committees for Forensic Science (OSAC) Dogs & Sensors in the USA are focusing on establishing standards and guidelines related to training procedures. The OPP Canine Unit in Ontario, Canada has adapted training protocols from OSAC and adopted them suitably to meet the Canadian standards [199].

Currently, there is no training aid that is proven to encompass the entire odour profile of a decomposing cadaver thus, organisations use varying materials for training CDDs. Theoretically, cadavers would be a good training material for CDDs but they are not readily available. Some dog handlers may have access to cadavers at a human taphonomic

facility (known as a body farm). Anecdotally, such facilities with multiple decomposing cadavers in a limited area can be highly concentrated with decomposition odour and thus, overwhelming for CDDs [199]. Furthermore, in some instances, CDDs may be expected to search for human tissue, biological fluids or even dismembered remains as opposed to cadavers. For example, in a case that occurred between 2010 to 2017 in Ontario, Canada, several men from the gay community went missing and eventually, CDDs helped locate dismembered remains of victims that were concealed inside garden planters. Considering these instances and the difficulty of legally accessing cadavers, training CDDs on human remains other than cadavers is thought to be an appropriate alternative [199]. Presently, as elaborated in Chapter 3, amputated lower limbs/feet, teeth and blood are used as training aids for CDDs by the OPP Canine Unit. This chapter highlights the outcomes of CDD dog trials conducted with OPP Canine Unit training aids. Data was collected for three dog trials conducted as regular training sessions by the OPP Canine Unit in July 2020, March 2021 and May 2021. This study was conducted repeatedly with the same training aids to see if the CDD responses changed over time due to any potential change in the odour profile.

5.2 Methods

5.2.1 Ethics

The animal ethics approval for conducting an observational study with working dogs was obtained from *le comité de bons soins aux animaux* at UQTR with the certificate number 2020-S.F.2.

5.2.2 Cadaver detection dogs

During the three dog trials, 10 certified cadaver detection dogs were exposed to training aids in their typical training environment. The CddsThe number of dogs present during each of the trials varied greatly and depended upon their availability. A summary of the information of each of the dogs along with the training sessions where they were present is shown in Table 5.1.

Table 5.1: Details of CDDs that participated in the OPP Canine Unit training trials conducted in July 2020, March 2021 and May 2021.

Dog ID#	**Age (at the time of their first training session)**	**Gender**	**Breed**	**Years of experience in cadaver detection work**	**Training other than cadaver detection**	**Training session(s) when present**
Dog 1	4 years	Male	Labrador	3 years	None	July 2020; March 2021; May 2021
Dog 2	4 years	Female	Labrador	3 years	Search and rescue	July 2020
Dog 3	18 months	Male	Belgian Malinois	3 months	None	March 2021; May 2021
Dog 4	9.5 years	Male	Dutch Shepherd	7 years	Search and rescue	March 2021
Dog 5	6 years	Male	German Shepherd	5 years	General purpose	May 2021
Dog 6	8.5 years	Male	German Shepherd	5 years	General purpose	May 2021
Dog 7	6.5 years	Male	German Shepherd	5 years	General purpose	May 2021
Dog 8	3 years	Male	German Shepherd	2 years	General purpose	May 2021
Dog 9	8.5 years	Male	German Shepherd	6 years	General purpose	May 2021
Dog 10	2 years	Male	Spring Spaniel	6 months	None	May 2021

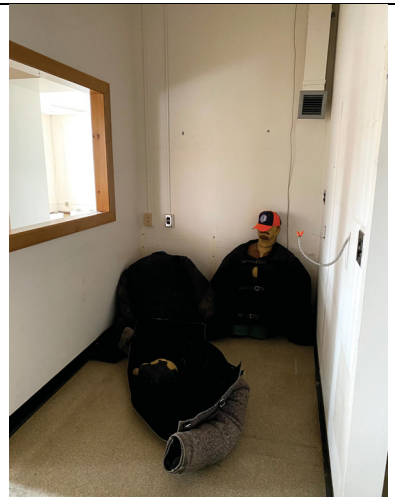
5.2.3 Training aids and training scenarios

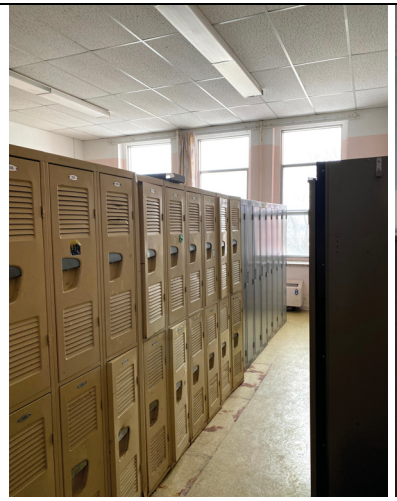
All training aids used in the current study belonged to the OPP Canine Unit. The CDDs in the current study had been previously exposed to the training aids prior to conducting the CDD trials. The training aids used were also samples from which VOCs were analysed, details of which have been reported in Chapter 3. The training aid details and their storage conditions have been previously summarised in Tables 3.1 and 3.2 of Chapter 3. The training aids that were stored indoors (in a freezer, refrigerator or at room temperature) were used for both indoor and outdoor trials while the samples stored outdoors at ODORS were used only for outdoor trials.

The indoor and the outdoor trials were conducted in a variety of scenarios all of which have been outlined in Table 5.2.

Table 5.2: Training scenarios present at the OPP Canine Unit which were used in the current study to conduct dog trials in July 2020, March 2021 and May 2021.

Training scenario	**Training aid location description**	**Images**
Carousel	The carousel is a training device with multiple numbered arms attached to a rotating centre disc. Odour sources are placed at the ends of these arms usually contained inside cans. Stainless steel shaker cans that had lids with small openings were used to hold odour sources in the current scenario.	 
Boxes	Wooden boxes with an opening at the top are used as training tools. Multiple boxes can be placed inside a room where each box holds a maximum of one odour source.	 

Wall with odour holes	A wooden wall-like structure with multiple numbered holes can be used as a training tool. Each of the holes is connected to a PVC pipe which may contain an odour source.	 
Human dummy	A human dummy as a training tool is a mannequin wearing clothes. The odour source can be hidden anywhere within the clothing.	

Locker room	A locker room search scenario is a room with multiple lockers and cupboards. The odour source can be hidden anywhere in this room.	 
Luggage room	A luggage room search scenario is a room with travel luggage and multiple other items such as sheets, tins and wooden boxes. The odour source can be hidden anywhere in this room.	

Apartment	An apartment search scenario is set up to resemble an apartment with a living room, kitchen, office, etc. with furnishings that would usually be found at a home. The odour source can be hidden in any room within the apartment.	  
Bite room	A bite room is a room used for training scenarios with random items such as large wooden tables, shelving racks etc. This room is used for conducting bite training with the dogs. The odour source can be hidden anywhere in this room.	

Outdoor search scenario	Outdoor scenarios can be grassland or woodland searches. These are areas present within the perimeter of the OPP Canine Unit.	

In all these scenarios, the target odour (i.e. OPP Canine Unit training aids) was hidden to avoid any visual cues to the handler or the CDD. The indoor training was conducted as double-blind trials where the dog handler and the observers present in the room were unaware of the training aid location. The experimenter (the OPP canine trainer in this study) that placed the training aids in the relevant locations was present outside the room (where the trial was conducted) to verify the responses called out by the handler without observing the search. The outdoor training was conducted as a single-blind trial where the dog handler was unaware of the location of the training aid however, the observers and the experimenter knew the location and were in the vicinity to confirm a correct response. Since this was an observational study, the researcher of this study was the observer while the protocol for conducting these trials was determined by the OPP canine trainer. As a part of regular training the CDD handlers or the canine trainer exposed the dogs to training aids multiple times to re-enforce CDD response to training aids however, during this study, only the first exposure CDD response was recorded. The response results from any follow-up exposures to the same training aid on that day were not used for statistical estimations to ensure no observer or handler bias was induced in the current study. Generally, during each trial, three to four target odours were placed at different locations and the CDDs were worked for no longer than a few minutes followed by breaks to avoid fatigue. The actual duration of search and break frequency given to the CDD was determined by its handler. Additionally, whenever the handler requested it, the CDDs were allowed blank runs in rooms with a similar layout but without a target odour such as in the case of an apartment search scenario. This was an opportunity for the CDDs to become accustomed to the search areas, such as furniture in an apartment, prior to the actual search.

5.2.4 Performance measures and data analysis

The CDD responses were recorded as:

1. True positive or hit – when the CDD correctly responded to the presence of the target odour (refer to Figure 5.3.).
2. Interest response – when the CDD showed a behavioural change and narrowed down the target odour but did not find its exact location.

3. False negative or miss – when the CDD did not give any response at a location where there was a target odour or where the handler failed to call the response.
4. False positive or false alarm – when the CDD incorrectly gave a positive response to a location without the target odour.
5. True negative or correct rejection – when the CDD correctly did not respond to a location without the target odour.

Figure 5.3: Example of a cadaver detection dog indicating a true positive alert to a training aid hidden behind the door.

In theory, a dog can have an infinite number of false positives and true negatives in a search, making further statistical calculations unworkable. Thus, for realism, researchers try to expose the dog to a finite number of opportunities to express false positives and true negatives. This can be achieved by placing distractor odours in close vicinity to the target odour. A distractor odour is any odour other than the target odour to which the dog is not trained to respond. In the current study, coffee, dog treats, seeds and other items were placed inside shaker cans of the carousel and under boxes other than the ones containing the target odour (i.e. the training aid).

Once the CDD responses were recorded, the following statistical measures were used to analyse the data:

- Detection rate (%) $= \frac{\text{Total number of true positive + true negatives}}{\textit{Total number of possible true outcomes}} \times 100$
- Interest response rate (%) $= \frac{\text{Total number of interest responses}}{\textit{Total number of possible outcomes}} \times 100$

- False response rate (%) = $\frac{\text{Total number of false negatives + false positives}}{Total\ number\ of\ possible\ false\ outcomes} \times 100$

Here, when calculating the response rate of the CDDs for each of the training aids, the total number of possible outcomes is equal to the total number of possible responses by the CDDs on the target odour and any distractor odours that were present during that trial. Apart from the statistical data, observational data was recorded and any additional incidents of false positives by the CDD to random objects or locations within a search area were recorded.

A Pearson correlation statistical test was performed to compare the detection rate of each CDD to the years of experience they had in decomposition odour detection. This test results in a Pearson correlation coefficient or r value. Here, r = 1 indicates a positive linear correlation; r = 0 indicates no correlation; r = -1 indicates a negative linear correlation. The p-value of the tests was also estimated and here, the p-value < 0.05 implied that the r value was statically significant. Additionally, a scatter plot was generated to graphically represent the correlation (if any) between the detection rate and years of experience of CDDs. The R^2 value for the trendline in the scatter plot was identified where an R^2 value closer to 1 would indicate a strong correlation between the two variables.

5.3 Results

The CDD responses from trials conducted in July 2020, March 2021 and May 2021 have been summarised in Table A 4, A 5 and A 6 of Appendix D respectively. The CDD responses (detection rate, interest rate, false response rate) for trials conducted in each of the three months have also been compiled and graphically represented in Figures 5.4 to 5.8. (*The response rate values are rounded to the nearest whole number).

Figure 5.4 represents the response rate results from trials conducted in July 2020 and May 2021 on CDD training aids decomposing outdoors at ODORS. There was no outdoor trial conducted in March 2021 due to the excessive amount of snow at ODORS. The outdoor trial on Foot #S was conducted only in July 2020 since during the month of March 2021 and May 2021, it was stored indoors and re-identified as Bone #4 and #5. The figure indicates that a 100% detection rate of CDDs was recorded for Foot #S and #R while only 33% interest response and 67% false response rate was recorded for Foot #B. Thus, none of the three dogs were able to detect Foot #B. Unlike Foot #S and #R which were decomposing on the surface, Foot #B was buried, and this could have added to the difficulty in detection.

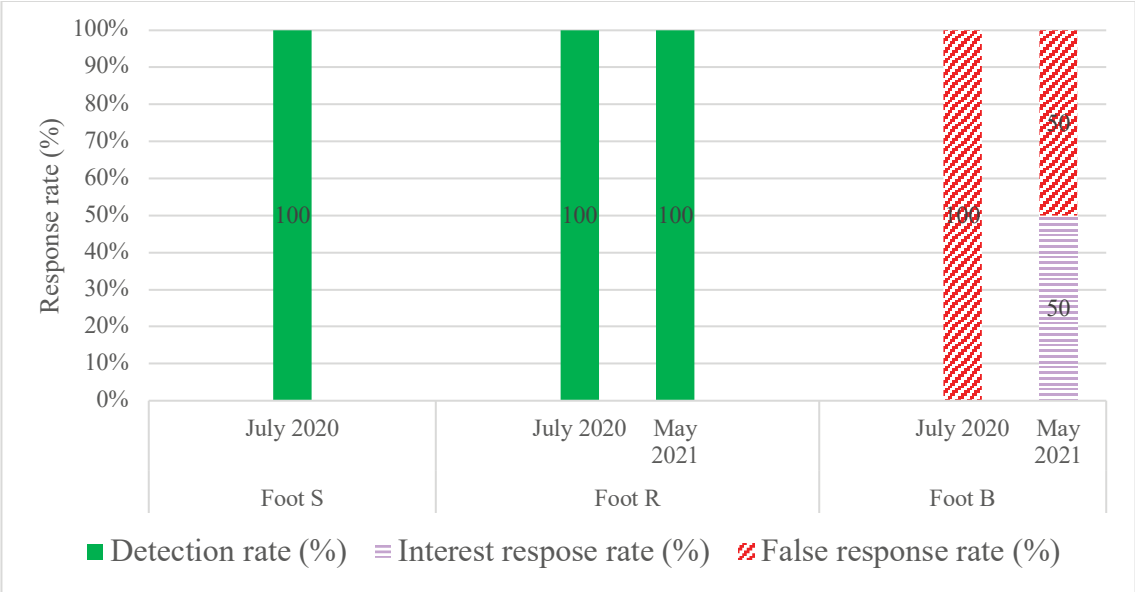


Figure 5.4: Response rate of CDDs on foot training aids stored outdoors at ODORS training aids during the trials conducted in July 2020 and May 2021.

Figure 5.5 presents the response rate results for foot training aids stored indoors (in a freezer, refrigerator or at room temperature). This figure indicates that for most of the foot training aids, a 100% detection rate was observed except for Foot #5 and #9. The cumulative results from trials conducted across all three months resulted in an overall 91% detection response rate for Foot #5 due to one of the two dogs indicating a false negative in the March 2021 trial and 78% for Foot #9 due to two of the seven dogs present indicating false negatives in the May 2021 trial. Additionally, a total of six false positive events in the May 2021 trial occurred at random locations in the training scenarios with two sets of training aids – Foot#5, and #8 and #9 combined. A plausible cause for these false positive results has been discussed later in this chapter in section 6.4.

Figure 5.5: Response rate of CDDs on foot training aids stored indoors during the trials conducted in July 2020, March 2021 and May 2021.

Figure 5.6 presents the response rate results for bone training aids stored indoors (in a freezer, refrigerator or at room temperature). This figure indicates that for most of the bone training aids, a 100% detection rate was observed except for Bone #1 and #4 which had an overall 89% detection rate. One CDD each showed interest in the vicinity of Bone #1 and #4 during searches for each of the training aids, however, failed to identify the exact locations. Bone #1 was a bone sample with some mould present on it while Bone #4 was a part of Foot #S sample which was brought and stored indoors on July 2020. Thus, as of the May 2021 trial, Bone #4 had been treated as an indoor training aid for over a year. In this study, no false negatives were recorded and one false positive event during the search for Bone #1 was recorded in the March 2021 trial.

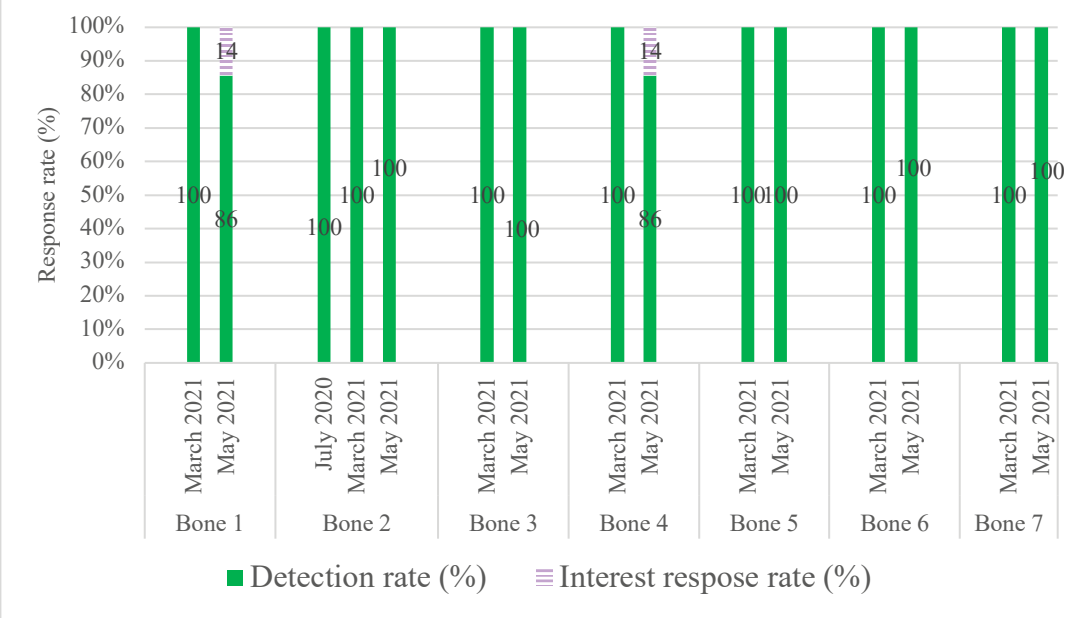


Figure 5.6: Response rate of CDDs on bone training aids during the trials conducted in July 2020, March 2021 and May 2021.

Figure 5.7 presents the response rate results for tissue training aids stored indoors (in a refrigerator or at room temperature). This figure indicates a 100% detection rate for Tissue #1 and #3 and an overall 91% detection rate on Tissue #2 due to failure to detect at the first attempt by one of the seven dogs in the May 2021 trial on this training aid. Tissue #1 and #2 were relatively wet tissue samples compared to Tissue #3 which was a dry mummified skin sample. It is significant to note that all the dogs that were exposed to Tissue #3 (the dry mummified skin) detected it. Additionally, one false positive event was recorded when Dog #1 indicated at a random location during the search for Tissue #2 in May 2021 which has been discussed later in section 5.4.

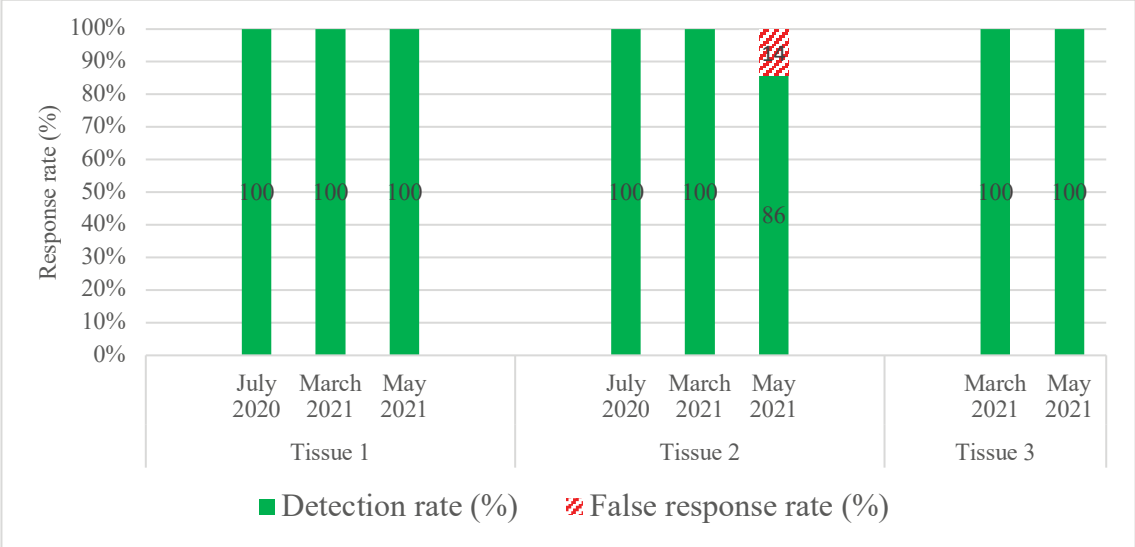


Figure 5.7: Response rate of CDDs on tissue training aids during the trials conducted in July 2020, March 2021 and May 2021.

Figure 5.8 presents the response rate results for blood training aids stored indoors (in a refrigerator or at room temperature). The cumulative results from trials conducted across all three months presented in this figure indicate an overall 82% detection rate on Blood #1, 90% for Blood #2 and 100% for Blood #3. Blood #1 and #2 were relatively degraded blood samples originally collected in 2017 compared to Blood #3 which was only about a week old during the March 2021 trial. Additionally, one false positive event was recorded when Dog #2 indicated at a random location during the search for Blood #2 in May 2021.

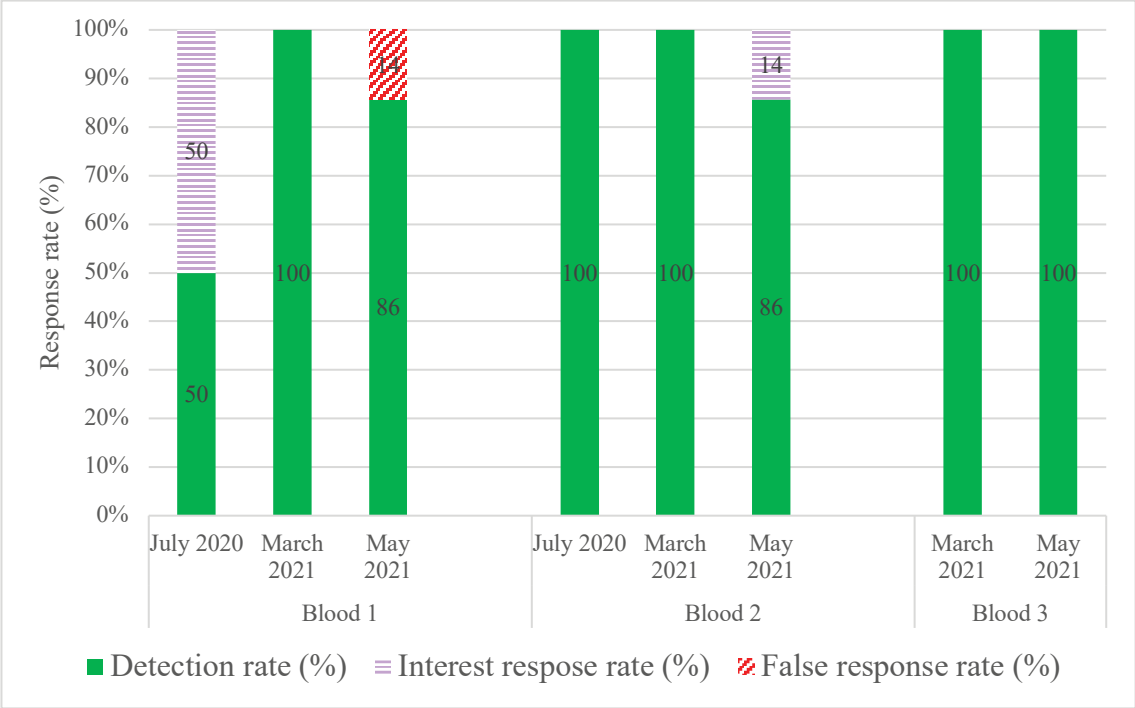


Figure 5.8: Response rate of CDDs on blood training aids during the trials conducted in July 2020, March 2021 and May 2021.

A Pearson correlation test was used to compare the inter-CDD detection rate to their corresponding years of experience. The Pearson correlation coefficient (r) was found to be 0.176, which is closer to 0 compared to 1 indicating no correlation. The p-value for this test was found to be = 0.625 which is > 0.05 thus implying that the r value was not significant. A scatter plot with a linear trendline in Figure 5.9 between detection rate and years of experience indicates a r^2 value = 0.031 which pointed towards a poor positive linear trendline. None of these values highlight any correlation between CDD detection rates and their years of experience as a trained cadaver detection dog.

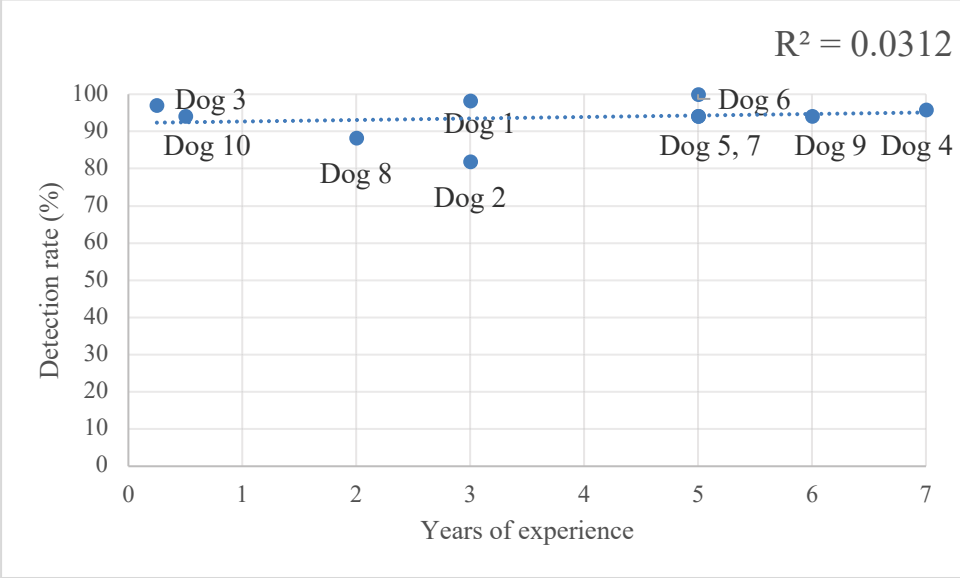


Figure 5.9: Scatter plot of detection rate vs. years of experience for each CDD present in the current study.

5.4 Discussion

The primary aim of the current study was to record the response rate of CDDs on training aids. This study predominantly focused on understanding the validity of the training aids to identify any training aids (originating from amputated limbs, blood or teeth) which could be less suitable for CDD training or became less suitable over time. Thus, the detection rate, interest response rate and false response rates of all the dogs on specific training aids were estimated. The interest responses were recorded separately and not included as a true response when estimating the detection rate. The study also compared the inter-dog detection capability based on their years of experience as trained CDD for which no significant correlation was found. It is widely accepted that detection dogs with repeated training have better detection capability. The absence of any correlation between

years of experience (or training) could be due to inter-dog differences, inter-breed differences and handler influence [207].

Overall, the detection rate of all the training aids ranged between 78% – 100% except for the buried foot sample where the interest response rate was only 33% and the detection rate was 0% owing to just one interest response from one of the three CDDs exposed to this training aid. These response rates have been further discussed later in this section. There were no false responses of CDDs on any of the distractors however, a total of nine false positive responses occurred in the search scenario with the target odour when the dogs falsely alerted at random locations or objects (not intentionally placed as distractor odour by the OPP canine trainer). Whenever such false positive events occurred, the OPP canine trainer recorded it and then the trainer asked the handler to continue searching even after the CDD gave a false positive response during the search. Since this study was observational in a regular dog training setting, all the search protocols were determined by the OPP canine trainer. Thus, the researcher (the observer of the study) recorded these false positive events however, these have not been used in statical calculations since the probability of such incidents occurring is infinite. The two search scenarios where these occurred were in the carousel and apartment search and they have been discussed later in this section.

One of the objectives of conducting repeated dog trials was to evaluate if the dog response changed over time due to any potential change in the odour profile that occurred. The response rates over time did not indicate any change in odour profile apparent to the CDDs' nose. However, this does not eliminate the possibility that the odour profile comprised of the VOCs did not change with time. This outcome simply highlighted that the change (if any) did not impact the CDDs' ability to locate the training aids.

For the samples present at the OPP ODORS site, a 100 % detection rate was recorded for Foot #S which was decomposing on the surface, however, this is based on one dog's response data collected in one trial (July 2020). Eventually, this foot was brought indoors and re-identified as Bone #4 and #5. The following dog trials which were conducted on Bone #4 and #5 resulted in 89% and 100% detection rates respectively and have been discussed further in this section. For Foot #R, which was also decomposing foot hidden among rocks, two of the two dogs (that is one dog each in July 2020 and May 2021) accurately responded to the training aid, resulting in a 100% detection rate. Among all

the training aids tested, Foot #B was not detected by any of the dogs and only one of the three dogs showed interest in the area resulting in a 33% interest response rate. Foot #B which was the only buried training aid in the current study was not correctly detected at any other instance which could be due to the reduced odour coming from the buried remains. In burial scenarios, dogs rely on the presence of decomposition odour (accumulated scent pool) at ground level to detect the remains [93]. A lack of odour at ground level can result in the buried target odour being undetectable which could have been the case in the current search scenario. To test this theory, the Foot #B sample was exhumed in May 2021 and the VOCs reanalysed indoors, results of which have been discussed in Chapter 3. As evident from these results, a greater number of decomposition-related VOCs were detected when Foot #B was exhumed and sampled indoors versus when it was analysed as a buried sample. While VOCs of the exhumed sample was tested, no dog trial was conducted on the exhumed Foot #B sample in this study however, a later dog trial study conducted in March 2022 by the current research group (results not published) revealed that three dogs exposed to the exhumed Foot #B successfully gave true positive responses. This suggests that burying human remains reduces the amount of detectable odour to the CDDs and that they needed to be trained further on the trace VOCs present in a buried scenario. Studies in the past have shown that dogs are able to correctly locate buried remains and grave sites [201-203; 199], thus it is likely that with training and continuous exposure, the dogs in the current study could also locate buried training aids. Among all the sample sets, the sample at ODORS had the least number of trials with only one, two and three CDDs in total being exposed to Foot #S, #R and #B, respectively. Further trials may need to be conducted to achieve statistically reliable results.

For all the foot training aids stored indoors, except Foot #5 and #9, a 100% detection rate was observed. The response rate on Foot #5 was 91% as one of the two CDDs in the March 2021 trial was not able to detect the presence of the training aid. A 78% detection rate was observed on Foot #9 as two CDDs were not able to locate the target odour during this search. During the May 2021 trial, Foot #8 and #9 were concealed in two separate rooms of the same apartment. During this search four of the seven CDDs present (Dogs #3, #5, #7 and #8) gave a false positive response at a third location inside the apartment after successfully locating the first target odour and while still searching for the second. In this instance, the trainer asked the handlers to disregard the alert and continue searching. Similarly, in the case of Foot #5, Dog #7 and #10 indicated a false positive

alert at another location of the apartment after true positive responses on Foot #5. It was suggested by the dog handlers that the ventilation in the room concentrated the air in the corner and the CDDs responded to that. The handlers concluded that this could be due to air becoming saturated and dissipated across the search area as a result of the training aids left in the room for a long period of time (hours). However, since the VOCs were not collected from this location in the apartment, this theory can not be confirmed.

Among the bone samples used as training aids, all seven either elicited true positive responses or interest was shown in their vicinity. The detection range for all bone samples ranged from 89% – 100%. The bone training aids used by the OPP Canine Unit are a combination of dry bones and bones with organic material (tissue). There was no difference observed in the CDDs response to dry versus wet bones. The two interest responses were recorded in the May 2021 trial when two CDDs showed interest in the area surrounding Bone #1 and #4 but could not identify the exact location of the target odour. Bone #1 is a relatively dry bone with mould on it while Bone #4 is a bone with some tissue still intact. Since all the other CDDs gave true positive responses on both these samples, it is less likely that the presence of mould on Bone #1 could have impacted the CDDs performance. Even if the presence of this mould altered the VOC profile, it did not hinder the other CDDs' ability to detect it. The mould was not characterised, and neither was its specific VOC profile studied however, this should be considered for future studies. While mould occurs on naturally decomposing human remains, the type of mould in the natural environment versus that in a jar in an indoor environment with limited oxygen can be different. This difference in the type of mould can ultimately affect the VOC profile since different moulds have different VOC profiles [208]. Thus, it can be significant to study the nature of mould that is developed on these training aids and those found naturally in the environment.

For the tissue samples, only one false negative response was observed for Tissue #2 which resulted in a 91% detection rate for this training aid. A 100% detection rate was observed for Tissue #1 and #3. Tissue #1 and #2 were relatively wet tissue samples compared to Tissue #3 which was a dry mummified skin sample. The results in this study did not highlight any differentiation in CDDs' capability to locate dry and wet tissue samples. The dry tissue sample (Tissue #3) had the same or higher detection rate compared to the other two wet tissue samples. When recording observations, the first response for each CDD was noted. Any following exposure to the same training aid during consecutive runs

on that day was not considered for statistical calculations. When the trial on Tissue #2 was conducted in May 2021, Dog #9 failed to alert to the target odour and the trial was recorded as a false positive. However, in the following run on the same day, Dog #9 indicated a true positive response on Tissue #2. Thus, a false negative response did not necessarily indicate a questionable capability of the CDD or indicate a lack of odour from the training aid (since all the other CDDs gave an alert). This only meant that the CDD failed to identify the odour at the moment. The consecutive positive response was not considered in the statistical calculation because the study was designed to be objective in nature. At the time of any consecutive runs, the dog handlers are generally aware of the location of the training aid and thus, the test is no longer a blind test. Single- or double-blind tests are essential because dogs are known to pick up cues from their handlers and surroundings which influences the outcomes of their responses [209; 115]. During the search conducted in July 2020, Dog#1 indicated a positive response to residual odour when searching for Tissue #2 as a result of a prior search conducted on Tissue #1 in the same room with a 30 min gap between the two searches. This was only recorded as an additional observation and not used for statistical calculations.

As of May 2021, Blood #1 and #2 training aids were much more degraded (over two years) than the Blood #3 training aid (three to five months). All the dogs gave either a true positive or interest response on all blood training aids in all trials except one dog which did not indicate on Blood #1 in the May 2021 trial. This false negative resulted from the CDD's alert not being recognised by the handler. The handler was not confident in the response of the CDD and thus did not call out the response however, the trainer at the end of the trial indicated that the area where the CDD showed a change in behaviour was where the target odour was located. This resulted in an 82% detection rate for Blood #1, 90% for Blood #2 and 100% for Blood #3. Thus, the detection rate of degraded blood was slightly lower than that of fresh blood. Additionally, in the July 2020 trial, Dog #2 gave a false positive response to Blood #2. This trial was being conducted in a room with a carousel and the handler was aware that one target odour was hidden in one of the arms of the shaker can. Dog #2 on entering the room gave a false response close to the entrance of the room, it did not attempt to approach the carousel. In this instance, the handler decided to direct the CDD to each of the shaker cans which led to a true positive response. Blood is used as a training aid for blood detection dogs and often for cadaver detection dogs since it is easily accessible through donations and consent to use. The blood

available in the current study was not cadaveric blood but rather donations from dog handlers. Nonetheless, prior studies have reported that dogs can detect both types of blood (cadaveric and donation from living individuals) with high accuracy rates [167; 137; 210].

Thus, the results of this study concluded that the CDDs were able to successfully locate all training aids except the buried sample. No change in CDD response over a period of time occurred due to any potential change in the odour profile of the training aids. One limitation of this study was the number of CDDs as only one or two dogs were available while conducting trials on each of the target odours during the July 2020 and March 2021 trial. This problem was resolved in the May 2021 trial with eight CDDs participating. Statistically significant results require repeated testing thus, the author suggests that a trial with a higher number of CDDs over repeat trials be conducted in future studies. It is evident that the use of CDDs as a forensic search tool is extremely useful however, it has been recommended that CDD responses should be combined with another search and detection technique (such as GPR or soil probe) or a second CDD [202]. This could have been helpful in the search for the buried training aid when one of the dogs showed interest in the area and soil probing may have helped release odorous compounds from the soil for easier access by the CDD. Additionally, a search of the ground with a GPR could have also indicated the buried material of interest.

5.5 Conclusion

Thus, the results of this study concluded that the CDDs were able to successfully locate all training aids with an overall detection rate ranging between 78% – 100% except for the buried sample with a 0% detection rate and 33% interest response rate. The false response rate in this study was low and ranged between 9% – 22% owing to the false negative responses where the CDDs failed to locate the training aids. There were no false positives on the distractor odour however, a total of nine false positive incidences were observed on random locations/objects across all three months. There was no difference observed in the detection rate of wet versus dry tissue samples however, the fresh blood (about three to five months degraded) had a 100% detection rate while the detection rate of degraded blood (over two years) ranged between 82% – 90%. There was no change observed in CDD response over a period of time due to any potential change in the odour

profile of the training aids. Additionally, no significant correlation was observed between the rate of detection and years of experience of CDDs.

Chapter 6: COMPARING VOC PROFILES AND CDD PERFORMANCE ON TRAINING AIDS AND REST[ES] DONORS

Chapter 6: COMPARING VOC PROFILES AND CDD PERFORMANCE ON TRAINING AIDS AND REST[ES] DONORS

A primary aim of this study was to validate the use of CDD training aids for training purposes. The previous three chapters of this thesis (3, 4 and 5) reported the VOC profiles of CDD training aids, VOC profiles of REST[ES] donors and CDD performance on CDD training aids. This chapter aims to discuss and compare the results previously reported in Chapters 3, 4 and 5 in an attempt to 1) draw parallels between the VOC profiles of CDD training aids and donors decomposing at REST[ES] and 2) place CDD performance results in a VOC context.

To the author's knowledge, this was the first study to draw a direct comparison between amputated lower limbs/feet and cadavers. All previous studies have analysed either human remains or cadavers independently and compared the resulting VOC profile with the literature. Previously, human remains-related studies have focused on VOC analysis of human organs, tissue, muscle, blood, placenta adipose tissue etc. [68; 164; 165; 168; 163]. There are multiple reasons that can influence and generate differences in VOC profiles of OPP training aids and cadavers. Some of these factors include the state of the tissue or cadaver, microbial species that influence decomposition, and storage and handling conditions. At the time of obtaining these sample sets, the OPP amputated lower limbs are in a necrotic condition (localised tissue death due to lack of blood flow and oxygen) which is contrary to the state of cadavers where the postmortem cell autolysis occurs (elaborated in section 1.1.3). Additionally, during this study, the cadavers were decomposing naturally in an outdoor environment with researchers only handling them during sample collection, while as described earlier (section 3.2.2) most of the OPP training aids were stored in containers (glass jars and PVC pipes) at different temperatures (refrigerator, freezer, room temperature) and they were regularly taken in and out of storage for CDD training purposes. As a result of this, while the cadavers went through the classic decomposition stages over the period of this study, there were no visual changes observed in the OPP training aids. Based on these factors it is hypothesised that while cadaveric decomposition will encompass all the decomposition VOCs through all the stages, the VOC profile of each of the individual OPP training aid might not entirely

represent the cadaveric VOC profile. Finally, studies have reported diabetic limbs are predominantly infected by *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* [156; 157; 211; 212] apart from other microbial species, while cadavers have a complex gut flora that along with environmental microbial species drive the decomposition process. The primary question that has been addressed in discussing this chapter is whether the impact of these multiple factors led to the VOC profiles of the two sample sets being more dissimilar than similar or vice versa. The objective was to determine if the VOC profile of CDD training aids and cadavers were comparable enough to deem them as validated training aids for CDDs.

6.1 Comparison of the VOC profiles of CDD training aids and donor decomposition at REST[ES]

6.1.1 VOC profiles of CDD training aids and REST[ES] donor samples

CDD training aids had a greater number of VOCs (2026) compared to those detected for REST[ES] donors (1412). This could be due to the fact that there was greater variability in CDD training aids in terms of training aid types (amputated lower limbs, blood and teeth) which were studied over a longer duration (1.5 years) relative to the REST[ES] donors who were studied for no longer than three months. Of the total VOCs detected, 956 VOCs were common between the two sample sets. Thus, 68% of the VOCs detected in REST[ES] donor samples were also detected in CDD training aids used by the OPP Canine Unit. 1070 additional VOCs detected in CDD training aids were not detected in REST[ES] donors however, 70% (or 744 VOCs) of these remaining 1070 were only detected in 1 – 2 samples from the total 85 samples collected in this study. When identifying VOCs associated with CDD training aids, a large number of VOCs that did not occur across multiple samples can be eliminated as being important to CDD training aids. The exact origin of less significant VOCs is unknown as several decomposition-related compounds can originate from unknown metabolic pathways [213]. The statistical analysis used in the current study was designed to only identify VOCs that were significant in the samples (CDD training aids or REST[ES] donors) compared to the control (background) odour. Thus, it can be assumed that the less frequently occurring VOCs (present in only a few samples) were significant to the specific samples only (CDD training aids or REST[ES] donors) and may have a decomposition-related origin

however, their exact metabolic pathway is unknown. This study identified VOCs that were prominent (occurring in over 30% of samples) in CDD training aids and REST[ES] donors (Tables 3.3 and 4.8). The 32 prominent compounds identified in REST[ES] donors were also detected in CDD training aids. Likewise, 17 of the 18 prominent compounds, except sevoflurane, which were identified in CDD training aids were likewise detected in REST[ES] donors.

When comparing the similarity in terms of the percentage of VOCs found common between the CDD training aid type (foot, bone, tissue, blood and teeth) and REST[ES] donors for each CDD training aid type, it was found that feet stored indoors had the most similarity followed by bone, blood, tissue, foot stored outdoors and finally, the teeth samples had the least similarity. The similarity, indicated by the percentage of VOCs detected in REST[ES] donor samples which were also detected in CDD training aids, have been summarised in Table 6.1. From this table, blood and teeth had 26% and 11% of VOCs, respectively, which were also found in the REST[ES] donor samples. Previous studies have reported VOCs in both blood and teeth for their use as CDD training material. While the VOCs detected during blood degradation have been studied extensively, the VOC profile of teeth has been reported in only one prior study [68]. The one study on teeth by Hoffman et al. reported the presence of 1-pentanol, tetrachloroethylene, toluene, 2-pentylfuran, cyclohexanone, and DMDS in their teeth samples. Of these, only DMDS was detected in the December 2019 trial in the current study. The limited VOC similarity between teeth samples and REST[ES] donors, and the absence of previously reported VOCs, could be attributed to the fact that the teeth used in the current study contained minimal organic material. Among CDD training aids originating from amputated lower limbs (foot, bone, tissue), feet stored indoors had the highest percentage of VOCs common with those detected in REST[ES] donors, followed by bone, tissue and finally foot stored outdoors. This is consistent with the trend observed previously for the abundance (number) of VOCs detected and the VOC relative class concentrations where, feet stored indoors had the highest number of VOCs and highest relative class concentration followed by bone, tissue and the least in foot stored outdoors. This has been reported in sections 3.3.1 and 3.3.2. Therefore, even though the samples had the same origin, as mentioned in Chapter 3, VOC collection location (outdoor vs. indoor) and/or the amount of organic matter (more in foot and bone and less in tissue samples) determines their VOC profiles. This can in turn impact the similarity of their

VOC profile with REST[ES] donor VOC profiles. Each CDD training aid (listed in Table 6.1) had a lower percentage of VOCs common with the REST[ES] donors compared to the percentage (68%) of all the CDD training aids combined. This indicates that if CDDs are trained on individual training aids, the number of VOCs of interest (in this case, the VOCs identified in REST[ES] donors) that they are exposed to will be reduced, compared to if they were exposed to all of the training aids consistently during their training sessions.

Table 6.1: Summary of similarity by total percentage of VOCs common between CDD training aids and REST[ES] donors analysed in the current study.

Training aid type	**Total no. of VOCs detected in the CDD training aid type**	**No. of VOCs common between CDD training aid and REST[ES] donors**	**Percentage (%) of VOCs common with CDD training aid and REST[ES] donors (No. of VOCs common between CDD training aid and REST[ES] donors / Total no. of VOCs detected in REST[ES] donors × 100)**
Outdoor (stored) foot	194	166	12%
Indoor (stored) foot	1491	782	55%
Bone	1019	597	42%
Tissue	560	309	21%
Blood	603	372	26%
Teeth	262	158	11%

When comparing only the list of prominent VOCs identified individually in the two sample sets, nine VOCs were common between the two sample sets. These included – DMTS, methyl thiocyanate, trimethylamine, trimethyl pyrazine, methyl pyrazine, 3-methyl-1-butanol, pyridine, 3-methyl-butanoic acid and 2-methyl-propanoic acid. All of these have been previously reported in both human and animal decomposition VOC profiles thus, they are not exclusive to decomposing human remains [188; 98; 214; 192].

These nine mentioned VOCs can be significant when identifying VOCs that CDDs potentially associate as decomposition-related as they were prominently present in both sample sets (CDD training aids and REST[ES] donors).

6.1.2 Class abundance and Relative class concentration trends in CDD training aids vs. REST[ES] donor samples

The abundance trend (based on the number of VOCs belonging to each class) of the two sample sets in increasing order of class abundance has been summarised in Figure 6.1. Overall the trend in the two sample sets was similar with some variations; linear aliphatics showed high abundance (> 10% of total VOCs) in CDD training aids, while they had medium abundance (5 – 10% of total VOCs) for REST[ES] donor samples. Likewise, nitrogen-containing VOCs showed a medium abundance in CDD training aids and a high abundance in REST[ES] donor samples. Other than these two classes, aldehydes had low abundance (< 5% of total VOCs) in CDD training aid classes and medium abundance in REST[ES] donor samples. The individual CDD training aid type was no different from the result of their overall abundance trend and each training aid type had more or less the same abundance trend as in REST[ES] donors.

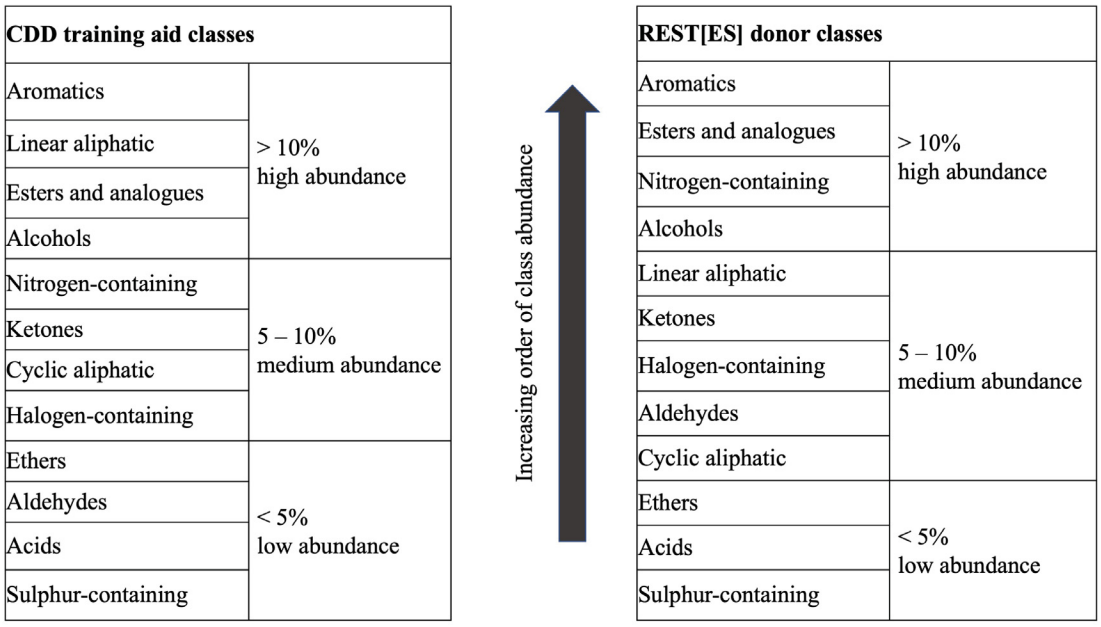


Figure 6.1: Summary of class abundance trends in CDD training aid and REST[ES] donor samples.

The relative class concentration (based on the average normalised area of VOCs belonging to each class) trends in the two sample sets – CDD training aid and REST[ES]

donor samples, varied as represented in Figure 6.2. Even considering the variability in this trend, the alcohol and nitrogen-containing VOC class had the highest relative class concentration for both sets of samples. Similarly, cyclic aliphatics, aldehydes and ethers had the lowest relative class concentration in both CDD training aids and REST[ES] donor samples. The relative concentration trend varied quite a bit for individual CDD training aid types compared to the trend observed in REST[ES] donors. In this regard, the relative class concentration trend of the foot training aids stored indoors was most similar to the trend observed in the REST[ES] donors, while trends in the foot stored outdoors, and teeth training aids were most variable followed by tissue and blood. This was due to the fact that the nitrogen-containing VOCs had an exceptionally large relative class concentration in the foot stored outdoors samples, while in teeth, linear aliphatics had a high relative class concentration along with acids and alcohols while sulphur-containing VOCs had a minimal relative class concentration. Trends relating to the tissue and blood training aids were variable from the REST[ES] donors since they had much lower relative concentrations of acids and alcohols and relatively higher concentrations of aromatic and linear aliphatic classes. Thus, based on the relative class concentration, the foot training aids stored indoors and bone may be the most ideal training aids. This is consistent with the fact that most bones still had tissue attached to them thus resembling the feet stored indoors which are comprised of both bones and tissue originating from amputated lower limbs.

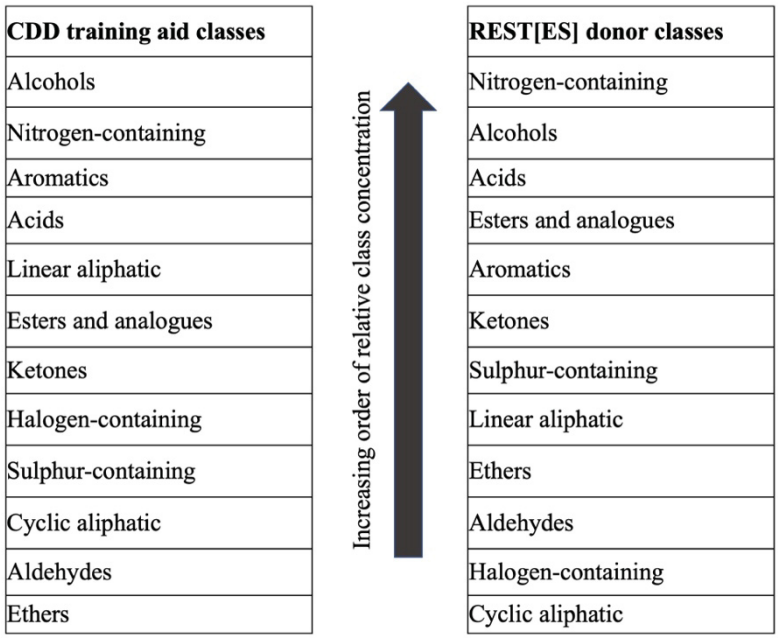


Figure 6.2: Summary of relative class concentration trends in CDD training aid and REST[ES] donor samples.

Thus, based on the percentage of VOCs detected in CDD training aids that were common with the REST[ES] donors, the class abundance trends and the relative concentration trends, it can be concluded that as hypothesised, it would be best if OPP exposed their CDDs to all training aids types except teeth. However, if they had to limit the number of training aids then the VOC profile of foot samples stored indoors was closest to that of the REST[ES] donors.

6.1.3 Principal component analysis of CDD training aids and REST[ES] donor samples

PCA was performed to cluster and allow for dimension reduction of all samples collected in this study to understand the variability between VOC profiles of CDD training aids and REST[ES] donors. Prior PCAs represented in Chapters 3 and 4 were used to understand intra-sample set variability – within CDD training aids due to ageing and storage in Chapter 3, and within REST[ES] donors due to ambient temperature and decomposition stages in Chapter 4. The PCA is constructed to understand variability between the two sample sets – CDD training aids and REST[ES] donors. Normalised areas for the VOCs listed in Tables 3.3 and 4.8 which were identified as prominent in all CDD training aids and REST[ES] donors were used to construct this PCA. Thus, the PCA was based on a total of 41 VOCs present in over 30% of CDD training aids and REST[ES] donor samples

which have been represented in Figure 6.3 and discussed further in this section. For this PCA, the samples were grouped into two sample sets – CDD training aids and REST[ES] donors. The resulting PCA highlighted 40%, 23%, 11%, 8%, 6%, 5% of the explained variance along PC-1, PC-2, PC-3, PC-4, PC-5 and PC-6, respectively (cumulative: 93%). The associated loadings plots (represented as a biplot in Figure 6.4) were used to determine which VOCs were important to the construction of specific PCs and samples. This PCA elucidated that most samples belonging to the two sample sets were clustered around the X- and Y-axis origin. The extreme loadings from CDD training aids were spread across PC-1, while those of REST[ES] donors were present along PC-2. This was the only variability observed in the two sample sets. No specific clustering or variability in the two sample sets was evident along any of the remaining PCs (PC-3 – PC-6). Foot #8 and #9 samples from March and May 2021 trials which were previously identified as the most variable samples among all CDD training aid samples were also identified as extreme loadings in the current PCA. From the loadings, 3-methyl-1-butanol dominated in Foot #8 and #9 samples from March and May 2021 trials causing their separation from the remaining samples. This is consistent with reporting in Chapter 3 from Figure 3.12 where the dominance of this VOC caused variability in the four samples mentioned (Foot #8 and #9 samples each from March and May 2021 trials). Likewise, ED 15 and ED 17 samples from donors 2020-01 and 2020-02, respectively were extreme along PC-2 thus these two samples were the most variable relative to all other REST[ES] donor samples. As evident from the loadings shown in the biplot associated with this PCA (Figure 6.4), this variability was caused by the dominance of methenamine, 3-methyl pyridine, and 2,6-dimethyl pyrazine in the two REST[ES] donor samples. ED 15 and ED 17 corresponded with the transition of the two donors from active decay into the desiccated stage. Methenamine has been previously detected during late stages of decomposition such as skeletonisation (in pigs) [98] and in (human) burials up to three years [135; 161]. However, it is not specific to the later decomposition stage as it has also been detected during the active decay stage [98]. Similarly, both 3-methyl pyridine and 2,6-dimethyl pyrazine have also been detected during the active decay stage in pigs [98]. In the current study, methenamine, 3-methyl pyridine, and 2,6-dimethyl pyrazine were detected in both the active decay and desiccated stage (a later decomposition stage), however, they were dominant in two of the four donors that saw a transition from active to the desiccated stage during their period of observation.

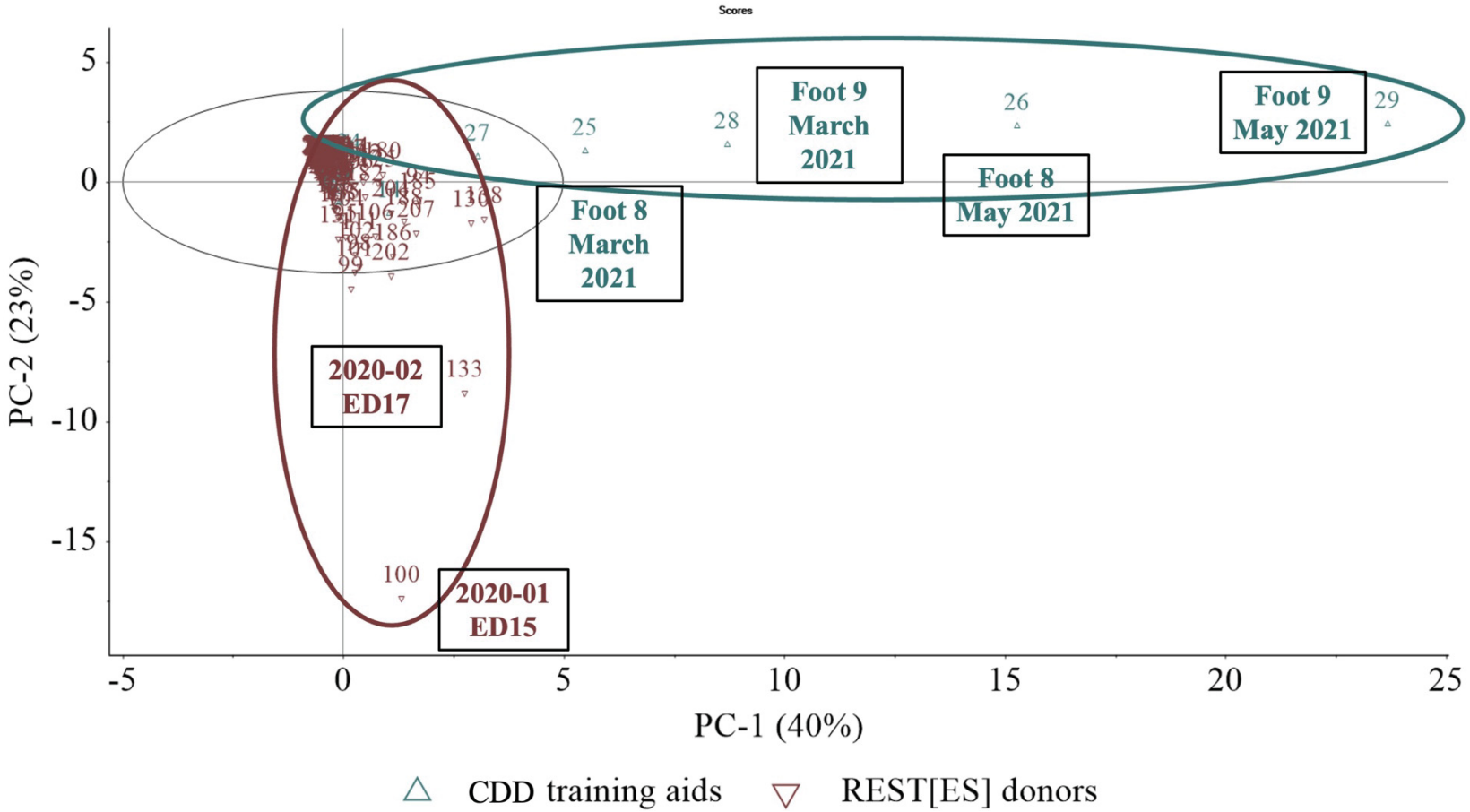


Figure 6.3: PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 41 prominent VOCs in over 30% of CDD training aid samples and REST[ES] donor samples (or data points in the above PCA) collected in the current study. (Here, colour codes and symbols represent sample set type, olive green triangles for CDD training aids and brown inverted triangles for REST[ES] donors; 'D' represents donor ID and 'd' represents the ED).

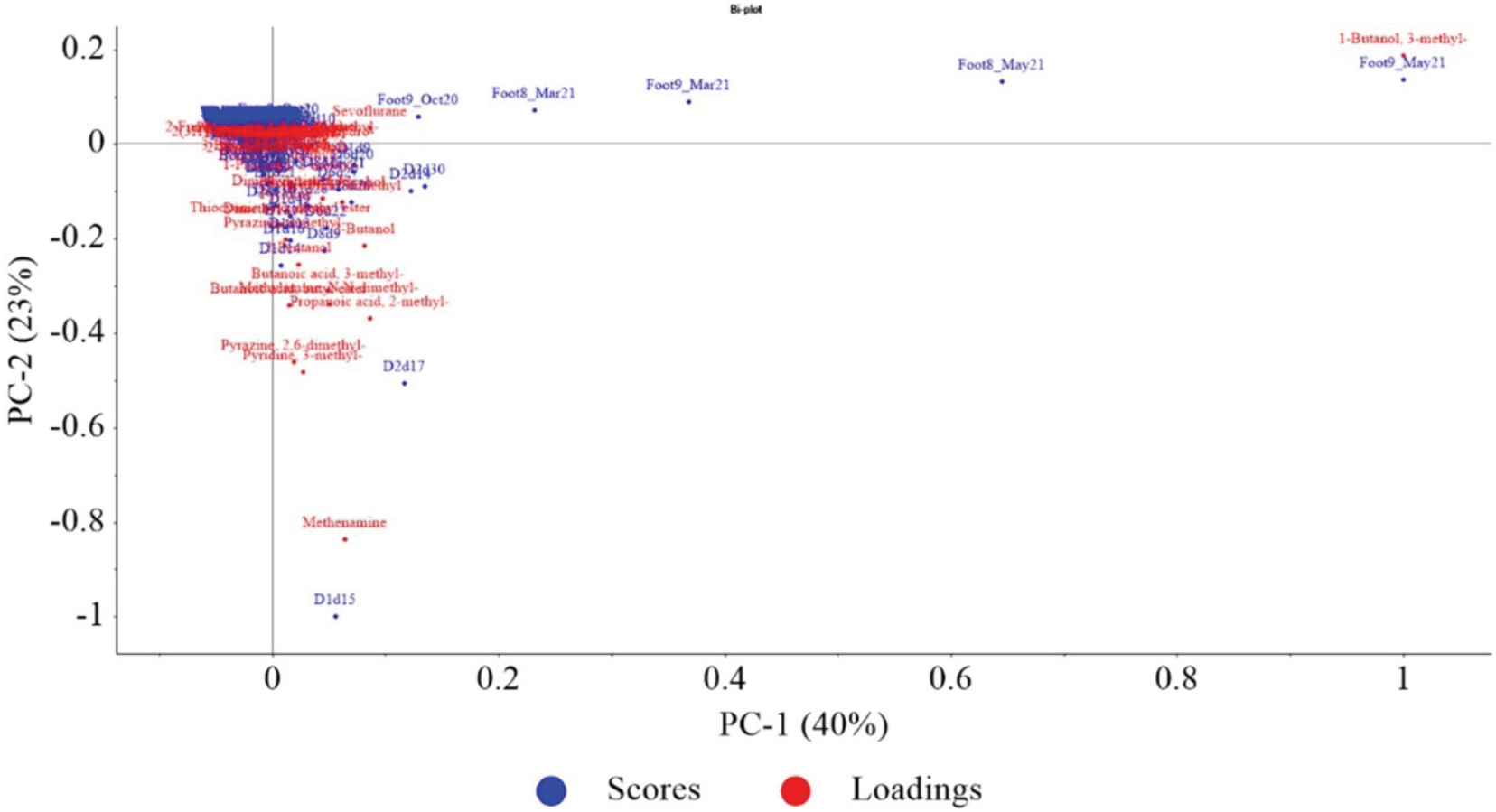


Figure 6.4: PCA biplot for PC-1, PC-2 for CDD training aids and REST[ES] donor samples collected in the current study. (Here, blue circles represent scores – CDD training aids and REST[ES]donor samples and red circles represent loadings – VOCs; 'D' represents donor ID and 'd' represents the ED).

An alternative PCA was reconstructed once the six extreme samples identified in the entire sample set were removed, to observe for any variability in the two sample sets without the influence of these samples. This PCA has been represented in Figure 6.5. As evident from this PCA, both the CDD training aids and REST[ES] donor samples were spread along PC-1 (34% of explained variance). No further variability in the two sample sets was observed along any other PCs including PC-2, PC-3, PC-4, PC-5 and PC-6 with 19%, 13%, 12%, 6%, and 3% of the explained variance, respectively. Since the REST[ES] donor samples were more spread along PC-1 compared to the CDD training aids, it can be concluded that these samples had greater intra-sample set variability. As evident from the loadings represented as biplots in Figure 6.6, this variability can be attributed to the dominance of trimethylamine, 2-butanol, 2-methyl-propanoic acid, DMDS and 3-methyl-butanoic acid in the REST[ES] donor samples. None of these VOCs are specific to human decomposition odour as they have been detected in pig decomposition VOC profiles in previous studies [188; 85; 72; 192]. Additionally, these VOCs were not entirely absent from CDD training aids although, they were not as significant. Thus, with the exception of the outlier samples identified in Figure 6.3, and considering the low variance along all PCs and the absence of separate clustering of the two sample sets, it can be concluded that there was no distinct variability found between the two sample sets based on VOCs that were prominent to each of the sample sets.

Thus, based on PCA, and contrary to what was initially hypothesised, the factors such as potential difference in microbial species and location of decomposition (indoor vs. outdoor) that could have caused variation in the VOCs profile did not have a significant enough impact to demonstrate variability in the two sample sets (CDD training aids and REST[ES] donors).

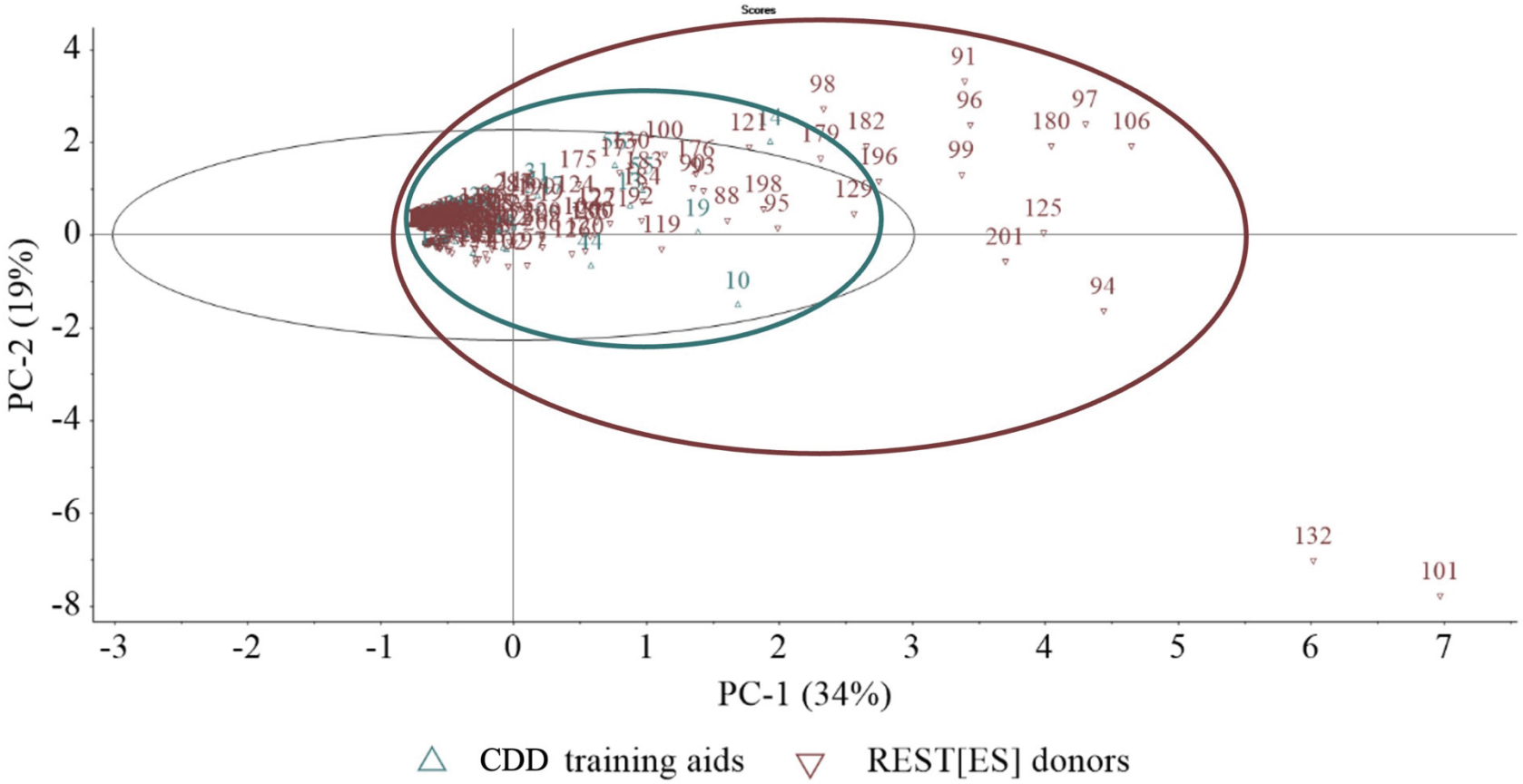


Figure 6.5: PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC×GC-TOFMS normalized peak area of 41 prominent VOCs in over 30% of CDD training aid samples and REST[ES] donor samples (or data points in the above PCA) after removing the six extreme loadings identified from PCA in Figure 6.4. (Here, colour codes and symbols represent sample set type, olive green triangles for CDD training aids and brown inverted triangles for REST[ES] donors).

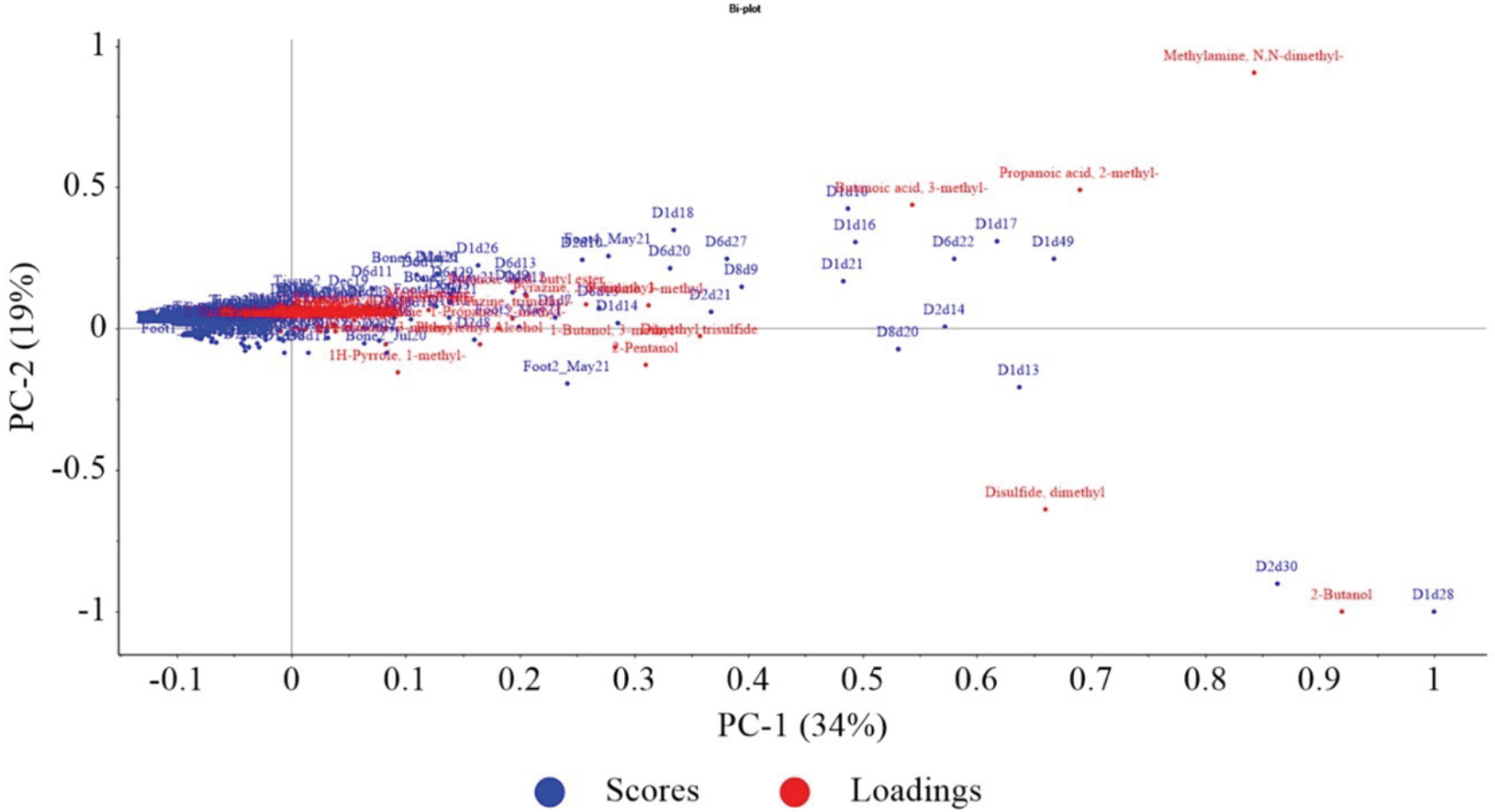


Figure 6.6: PCA biplot for PC-1, PC-2 for CDD training aids and REST[ES] donor samples after removing the six extreme loadings identified from PCA in Figure 6.4. (Here, blue circles represent scores – CDD training aids and REST[ES]donor samples and red circles represent loadings – VOCs).

6.2 VOC profile and CDD performance

Even though CDDs are a widely accepted tool in the search and detection of human remains, there is little knowledge about which VOCs they identify as decomposition-related. The current study recorded VOC profiles (analytical detection method) comprising decomposition odour in CDD training aids and REST[ES] donors and recorded CDD responses (biological detection method) to understand the VOCs the CDD could be responding to. Identifying the exact VOC or combination of VOCs that stimulate a neurological response in a CDD's brain was beyond the scope of this study however, identifying prominent VOCs in CDD training aids and REST[ES] donors was a significant outcome of the current study as CDDs are constantly exposed to both sample sets during their training and field searches. It is often reported that biological detection methods by dogs outperform analytical methods of detection [215] however, this requires estimation of the olfactory threshold for decomposition-related VOCs and detection limits of the instrument. Currently, there is a gap in the literature for the olfactory threshold of CDDs on the decomposition-related VOCs thus, evaluating if CDDs outperformed the current analytical was not an objective of this research. This section summarises CDD responses and prominent VOCs reported for each CDD training aid type. Additionally, this section presents CDD training conducted at REST[ES] on a fresh and a decomposed donor along with a list of VOCs detected in two donors at the time of this trial.

6.2.1 CDD training aid VOC profiles and CDD performance

When trying to understand which CDD training aid specific VOCs the CDD are exposed to during the training session, the list of prominent VOCs identified in this study and mentioned in Tables 3.3 to 3.9 is beneficial. Sevoflurane was the only prominent VOC detected in CDD training aids which was not detected in REST[ES] donors.

Among the three major categories of training aids (amputated lower limbs, blood and teeth), no prior scientific study had recorded CDDs' performance on amputated lower limbs, while the performance of dogs on blood as a training aid has been extensively recorded [216; 204; 167; 137; 210]. Only one prior study recorded dogs' performance on human teeth used as a training aid [217]. In that study, the authors reported a 78%, 61% and 20% recovery (detection) rate by three dogs on ten teeth placed 0.9 m apart within 10

m^2. No such CDD performance was recorded in the current study as teeth samples were not used as a training aid during the training session conducted at the OPP Canine Unit. This was because prior to the commencement of this study, CDD handlers had reported that their CDDs were struggling to locate the teeth samples and they had a poor detection rate. The VOC profile of teeth was analysed in the current study and as previously reported, only one (aniline) of the six VOCs found prominent in teeth has been previously reported in human decomposition odour. Additionally, teeth had the least similarity with VOCs detected in REST[ES] donors compared to any other training aid. Thus, the findings from the VOC analysis suggested that teeth were not ideal for training purposes which was consistent with CDD handler observations. As a result, the OPP Canine Unit decided to discontinue using teeth as a training aid.

Even though the performance of CDD on amputated lower limbs had never been recorded before, human remains have been used previously, and they are a widely accepted choice of CDD training aid. When using human remains as training aids, a study reported success rates of up to 94% by Royal Canadian Mounted Police handlers and dog teams [218]. An earlier study conducted as field searches to locate bones and gauze and clothing soaked with decayed material found recovery rates ranging between 55% − 95% with the lowest recovery rates occurring for dry old human bones. This could be because unlike human tissue, weathered (old) bones have limited organic matter which results in the reduced intensity of the VOCs [70], thus resulting in a less detectable odour compared to human soft tissue. In the current study, the detection rate ranged between 78% – 100% except for buried Foot #B which had a 0% detection rate when CDD trials were conducted in July 2020 and May 2021. The VOC profiles from these two trials (July 2020 and May 2021) suggested that only two VOCs – DMDS and methyl thiocyanate were detected as common between Foot #B and the list of prominent VOCs in REST[ES] donors. This could suggest the fact that even though DMDS is considered a significant decomposition VOC, it is not solely responsible for eliciting a positive detection response in CDDs. The overall detection rate of foot stored indoors and bone training aids were found to be the same (96%). This could be because the bones used in the current study as CDD training aids were not dry, most bones still had some soft tissue attached. This study observed only three of the seven bones as appearing dry – Bone #1, #6 and #7 and two of these dry bones (Bone #6 and #7) had a 100% detection rate, while Bone #1 had an 89% detection rate with one of the CDD showing interest but not being able to identify its exact location.

Thus, overall this study did not find poor detection rates for dry bone samples. This was consistent with the CDD responses on tissue samples as Tissue #3, which was a desiccated skin sample, had a 100% detection rate. Based on the VOC analysis, it was found that the wetness/dryness of the sample, determined by the presence of organic matter and decomposition fluid, impacted the number of VOCs detected and thus impacted the percentage similarity with the REST[ES] donor VOC profiles. However, this difference in the VOC profile did not seem to impact the CDD performance.

Blood VOC profiles and dog performance on blood used as a training aid have been reported in several studies previously [167; 137; 210]. A study by Chilcote et al. [167] reported the limit of detection for degraded blood by CDD to be one month for blood on concrete (porous) and one week for blood on varnished wood samples (non-porous). In the current study, CDDs were able to detect aged blood (3.5 years old in May 2021) on gauze (porous) surface with a detection rate of 86%. A major difference between the two studies was that blood was allowed to naturally weather in an outdoor environment in the Chilcote et al. study, while in the current study gauze with blood was stored in glass jars and at room temperature (except Blood #1 which stored in a refrigerator after March 2021). Another study found that CDDs were able to detect blood diluted over 2000 times easily on porous surfaces compared to non-porous surfaces [216]. Thus, for CDD training protocols it is suggested to store blood on gauze or other porous surfaces as opposed to directly in glass jars or non-porous storage conditions. Additional studies on blood used as a training aid have focused on understanding dog sensitivity to diluted blood and washed blood where the studies found CDDs could detect dilutions up to 1:1,000,000 [204] and without difficulty up to two washes [219]. Blood was not diluted or washed in the current study, it was treated as any other CDD training aid (amputated lower limbs, teeth) and stored indoors. As of the May 2021 trial, Blood #1 and #2 had aged for 3.5 years and had an 86% detection rate and Blood #3 was degraded for three months and had a 100% detection rate by CDDs. Thus, it was found that CDDs could detect fresh blood more readily compared to degraded blood. The VOC profile of fresh and degraded blood from the May 2021 trial suggested that even though a greater number of VOCs were detected in aged blood (Blood #1 and #2), the fresher blood #3 had more VOCs in common with the list of 32 VOCs identified as prominent in REST[ES] donors. Table 6.2 presents the VOCs that were identified as common with the REST[ES] donors' most prominent VOCs for each of the blood training aids in the May 2021 trial.

Table 6.2: List of VOCs detected in the May 2021 trial that were found common between CDD blood training aids and prominent VOCs in REST[ES] donors (listed in Table 4.8). (The VOCs highlighted in grey are common across all three blood training aids in the May 2021 trial).

Blood 1 (aged 3.5 years)	**Blood 2 (aged 3.5 years)**	**Blood 3 (3 months old)**
2-Pentanol	Dimethyl sulfone	2,4-Dithiapentane
3-Methyl-1-butanol	Dimethyl trisulphide (DMTS)	3-Methyl-1-butanol
3-Methyl-butanoic acid	Methyl thiocyanate	3-Methyl-2-pentanone
Dimethyl disulphide (DMDS)	Pyridine	3-Methyl-butanoic acid
Methyl thiocyanate	Trimethyl pyrazine	Dimethyl trisulphide (DMTS)
Pyridine		Methyl thiocyanate
		Pyridine

6.2.2 Fresh and decomposed REST[ES] donor VOC profiles and CDD performance

6.2.2.1 Method

Unlike the training sessions conducted with CDD training aids, only one CDD trial was conducted with certified donors at REST[ES] in October 2021. VOCs were also collected from the two donors tested – 2021-11, a relatively fresh donor on ED 8, and 2020-01, a decomposed donor on ED 801. The sample collection, analysis technique, data processing and statistical analysis method remained the same and have been previously detailed in Chapters 2 and 4. VOC analysis was conducted to generate a list of VOCs that were significant in the two donors and represented VOCs that the CDDs were being exposed to during the trial at REST[ES]. For the dog trial, eight certified CDDs were exposed to the two donors a day after VOC collection. Donor 2021-11 on ED 9 and remains (skull and femur) from donor 2020-01 on ED 802 were placed in forestland around REST[ES] and every dog-handler team was given an opportunity to locate the remains. Table 6.3 summarises information of each of the dogs present at this trial. Three (Dog #3, #7 and

#8) of the eight dogs present in the REST[ES] trial were also present at the training sessions discussed in Chapter 5.

Table 6.3: Details of CDDs that participated in the REST[ES] trial conducted in October 2021.

Dog ID#	**Age (at the time of their first trial)**	**Gender**	**Breed**	**Years of experience in cadaver detection work**	**Training other than cadaver detection**	**Donor it was exposed to during trial at REST[ES]**
Dog 3	18 months	Male	Belgian Malinois	3 months	None	Fresh; decomposed
Dog 7	6.5 years	Male	German Shepherd	5 years	General purpose	Fresh; decomposed
Dog 8	3 years	Male	German Shepherd	2 years	General purpose	Fresh; decomposed
Dog 11	4.5 years	Male	Labrador	1.5 years	Search and rescue	Fresh; decomposed
Dog 12	3 years	Male	Shepherd	2 years	General purpose	Fresh; decomposed
Dog 13	6 years	Female	Labrador	3 years	Search and rescue	Fresh; decomposed
Dog 14	4 years	Male	Labrador	2.5 years	None	Fresh; decomposed
Dog 15	4.5 years	Female	Labrador	3 years	None	Fresh; decomposed

6.2.2.2 Results and discussion

Table 6.4 summarises the VOCs that were identified as significant in the two donors (2021-11 and 2020-01) a day prior to the CDD trial conducted at REST[ES]. All of these VOCs were previously detected in one or more donors during the decomposition trials conducted at REST[ES] and discussed in Chapter 4. Thus, no new VOCs were identified on ED 801 of donor 2020-01. By ED 801 donor 2021-01 had largely skeletonised and

had minimal desiccated tissue attached to the bones. 3-methyl-1-butanol and DMDS were the two VOCs identified in REST[ES] donors and detected in donor 2020-01 more than a year later (ED 801). The other relatively fresher donor 2021-11 was predominately in the bloat/active decay stage as of ED 8. Five VOCs – trimethylamine, 3-methyl-1-butanol, DMDS, DMTS and DMS which were identified as prominent to REST[ES] donors were also detected in donor 2021-11 on ED 8. During the CDD trial, all eight CDDs had a 100% detection rate on both the fresh donor and the bones from the decomposed donor.

Table 6.4: List of VOCs sorted in decreasing order of their normalised areas which were identified as significant in donor 2021-11 on ED 8 and donor 2020-01 on ED 801 in October 2021. (The VOCs highlighted in grey are the compounds which were identified as prominent in REST[ES] donor in Table 4.8).

Donor 2021-11 ED 8 (fresh donor)	**Donor 2020-01 ED 801 (decomposed donor)**
Trimethylamine	Acetic anhydride
Ethyl-cyclopropane	2-Methyl-1-propene
Ethylenediamine	2-(1,1,3,3-tetramethylbutyl)- phenol
3-Methyl-1-butanol	Furan
3-Aminopropionitrile	Allantoic acid
1-Methoxy-3-methyl-butane	2-Hexanone
Dimethyl disulphide (DMDS)	3-Methyl-1-butanol
p-Xylene	1-Hexanol
2-Pentene	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate
2,2,4-Trimethyl-3-penten-1-ol	Disulphide, bis(1,1,3,3-tetramethylbutyl)
1-Ethyl-4-methyl-benzene	Dimethyl disulphide (DMDS)
Cyclohexane	3-Hexen-1-ol
3-Hexene	1-Penten-3-one
Phenyl trifluoromethyl ether	6-(4-chlorophenyl)tetrahydro-2-methyl-2H-1,2-oxazine
Dimethyl trisulphide (DMTS)	Prenol
Dimethyl sulphide (DMS)	2-Ethyl-1-butanol
Bis(1,1,3,3-tetramethylbutyl)-disulphide	

2,2-Difluoro-propane	
2,4-Dimethyl-hexane	
1-Penten-3-one	
[2,2,2-trifluoro-1-(trifluoromethyl)ethyl]- benzene	
6-(4-chlorophenyl)tetrahydro-2-methyl-2H-1,2-oxazine	
1,2,4,5-tetrafluoro-3-(trifluoromethyl)-benzene	
Methyl N-hydroxybenzenecarboximidoate	

Since this study was conducted only once, there is a need to repeat such a trial to validate the results obtained. The preliminary results suggest that sulphides and 3-methyl-1-butanol in both fresh and decomposed donors, along with trimethylamine in the fresh donor, can be of significance to the CDD when it attempts to locate decomposing human remains.

6.3 Impact of this study

A prime focus of this study was to understand if amputated lower limbs could be used as a CDD training aid. Based on the VOC profile and performance of CDDs, the current study validated the use of amputated lower limbs for CDD training purposes. Additionally, the current study found amputated lower limbs (especially feet stored indoors and bones) as a better alternative to blood and teeth CDD training aids since amputated lower limbs had more similarity with the VOCs detected in REST[ES] donors. This finding is important because current training aids have several issues, namely that human remains are difficult to ethically acquire [112], but the animal remains and chemical formulations (synthetic training aids) which are easy to acquire have been proven to not resemble human decomposition odour, making them substandard for training purposes [112]. With the validation of amputated lower limbs, the gap in the field of CDD training can be bridged and these can be ethically acquired with the consent of the living donor undergoing amputation surgery. Thus, amputated lower limbs which

would otherwise be incinerated can be utilised ethically for both research and training purposes. Even with the variable ageing and storage conditions of the amputated lower limbs, they were deemed suitable for use years after acquiring them. For example, Foot #1, #2 and #5 which were some of the oldest training aids to be acquired by the OPP Canine Unit in 2017 (October – December), when analysed in May 2021 had several VOCs (8, 21 and 14 VOCs respectively) that were common with the 32 VOCs prominently identified in the REST[ES] donors.

The field of decomposition odour has advanced immensely in an attempt to gain sufficient scientific backing since 2011 when it was first presented and heavily criticized as expert evidence in the criminal prosecution [10]. The decomposition VOC profile has been studied in great detail to understand its potential application in locating remains [220] and establishing PMI [165]. Since the ambient environment plays a crucial role in determining the decomposition process and hence the VOCs released during the process, it was vital to conduct a study in the Canadian environment. The current study became the first donor-based study in Canada to record the decomposition process and VOCs evolved during human decomposition. The research outcomes from this and future studies at REST[ES] can be used to develop and validate better training aids for CDD training. Additionally, acquiring information on the chemical composition of decomposition is the first step toward developing alternative electronic sensors and field detection devices to locate sites associated with decomposition events which are deemed too hazardous for CDD teams. Thus, this study has contributed by identifying significant VOCs that occur during the process of decomposition in Canada.

6.4 Limitations

One of the major limitations of this study was that the fate of the samples could not be determined by the researchers. Over the 1.5 years when CDD training aids were analysed, their fate (storage conditions and durations that they were left at ambient conditions and used as training aids) constantly changed. This likely impacted their VOC profile compared to if they were left undisturbed in the same storage condition for a period of time. However, this limitation did not impact the outcome of the current study as the aim was to analyse CDD training aids under their natural conditions without altering any treatment that they are subjected to by the OPP Canine Unit. The only impact this had on

the study was when handlers had acquired specific training aids for CDD training at the time of sample collection, thus making them unavailable during all five trials over the 1.5 year period. For one of the CDD training aids (Foot #R) which was hidden beneath rocks at the outdoor site, the air accumulation step using the hood was removed since the location of the sample did not allow placement of the hood over the training aids. This could have impacted the VOCs being detected but the researcher refrained from disturbing the training aid for sample collection. For the outdoor study conducted at REST[ES], donors decomposing at cooler temperatures could not be sampled once the snowfall commenced. Likewise, sample collection was not conducted on days of excessive rainfall and when rainwater pooled around donor 2020-04 making sampling difficult as it restricted placement of the hood over the donor.

Much like the availability of CDD training aids, the availability of dog-handler teams was beyond the control of the researchers. This resulted in five VOC analysis trials but only three CDD trials. Additionally, for the first two of the three CDD trials, only 2 – 3 dog-handler teams were available. This study successfully determined the VOCs that were prominent (recurring in samples) in specific CDD training aids and REST[ES] donors. However, this study could not determine which VOCs elicit a response from CDDs and those that they associate with decomposition odour. From a CDD perspective, identifying prominent VOCs in this study was a key outcome however, what the researcher determined as prominent is somewhat subjective. In cases where PCAs were constructed, the threshold for the minimum number of samples that a VOC must occur in was based on PCA with the best explained variance. Even though the prominent VOCs have been repeatedly reported and discussed in this thesis, the appendices A and C present the entire list of VOCs detected on each of the training aids and REST[ES] donors. Additionally, it is not necessary that the prominent compounds are the ones that CDDs relate to as decomposition odour. Further dog neurological studies must be conducted to establish this.

Considering the large number of VOCs that are detected, it will be quite a task to discuss every VOC individually. This is why most results in decomposition-based studies are based on compound classes. One limitation of using compound classes is that there is inconsistency in VOC classification among different studies (also discussed in Chapter 3). This becomes evident when one VOC has multiple functional groups thus, falling under several categorised. This study attempted to overcome the problem by resorting to

IUPAC rules of functional group priority for most VOCs. However, the researcher did still run into uncertainty and in these instances being consistent in classification was preferred. For example, aromatics were prioritised over any other functional groups and the researcher tried to remain consistent with this except in fluorine-containing VOCs which were categorised as halogen-containing VOCs. Since there are no standardised norms of classification decided, every researcher should remain consistent within their study, however, compound class based comparisons across different studies will not be entirely reliable. There is a need in the community to structure guidelines for consistent classification which would make drawing parallels across studies more reliable.

The statistical analysis of this study was conducted under the guidance of a statistician who wrote the R codes for this study. The identification of VOCs significant in samples over the controls was based on double or more S/N in the peaks. This factor of two (double) was decided based on discussions with the statistician. No attempts were made to increase this value to three (triple) or more. The results could look different if this was attempted since using a higher value implies making a selection of significant VOCs more stringent thus, reducing the number of VOCs retained after filtering.

6.5 Conclusions

To validate the use of CDD training aids used by the OPP Canine Unit, their VOC profiles were compared to REST[ES] donors in this chapter. After comparing the two sample sets, an overall 68% of the VOCs detected in REST[ES] donor samples were found common with those detected in the CDD training aids. Individual CDD training aids had a reduced percentage of VOCs in common with the donors. Thus, to expose a CDD to the majority of human decomposition-related VOCs produced by cadavers, they should be trained on all CDD training aid types available at the OPP Canine Unit (except teeth). All prominent VOCs detected in the donors were also detected in the CDD training aids. Hence, it can be concluded that presently the OPP Canine Unit expose their CDDs to significant decomposition VOCs during training with these training aids. This is further enhanced by exposing the CDD to whole cadavers and human remains during training at the REST[ES] facility. Among the three training aid types, teeth had the lowest number of VOCs common with those identified in REST[ES] donors, while amputated lower limbs and especially the foot samples stored indoors, had the highest number of VOCs in common.

In the CDD trials, it was found that the detection rates of CDDs when detecting amputated lower limbs stored indoors ranged between 78% – 100%. Among all amputated lower limbs stored outdoors, the CDDs had a 100% detection rate for training aids decomposing on the surface but they could not detect the buried training aid. Likewise, for blood training aid, the detection rates for aged blood were slightly lower (82% – 90%) than for fresher blood (100%). These results suggest that amputated lower limbs are an appropriate alternative to the training material such as blood and teeth which were also analysed in the current study.

Chapter 7: CONCLUSION AND FUTURE WORKS

Chapter 7: CONCLUSION AND FUTURE WORKS

7.1 Summary of research outcomes

This study was predominately focused on understanding if amputated lower limbs/feet could be used as a novel cadaver detection dog (CDD) training aid. There is a desire to find alternative sources of CDD training aids since the current training materials such as animal remains and chemical formulations are found to be inadequate in their representation of human decomposition odour. As a result, amputated lower limbs which are of human origin and can be legally and ethically accessed, as donors can consent to their donation, have become a desirable alternative CDD training aid under consideration by CDD handlers in Canada. Therefore, validating their use for CDD training by law enforcement agencies and civilian dog training organizations is a valuable outcome in the field of CDD training and human remains search and detection. The study design for the current research was adopted from prior studies based on validating CDD training aids [136; 137] and decomposition odour [94; 96; 87]. Since two training aids – decomposition fluid and blood, have been previously validated [136; 137] by recording both VOC profile data from instrumental analysis and observational data from CDD trials, the current study also focused on recording both aspects. Thus, in order to determine the validity of amputated lower limbs/feet, VOCs from CDD training aids (amputated lower limbs, blood and teeth) used by the Ontario Provincial Police (OPP) Canine Unit were chemically analysed, and VOCs from decomposing donors in an outdoor environment were also chemically analysed, and CDD responses to training aids and donors were recorded.

In Chapter 2, methods used for sample collection, sample analysis and data analysis were evaluated and reported. The analytical techniques for VOC collection from air matrix with subsequent analysis using GC×GC-TOFMS were adopted from prior decomposition studies [94; 96; 87]. The methods were validated and suitably modified for the current sample set and instrument configuration. The modification resulting from the validation step included using a smaller aluminium hood for air sample collection from CDD training aids, adding a 10 min accumulation for CDD training aids, reducing the air volume previously reported from 1 L to 500 mL, injecting samples with a 10:1 split from

the previously reported splitless mode and increasing the acquisition rate from 100 specta/s to 200 specta/s. In addition to the analysis parameters established in the previous decomposition reference studies, these modified parameters were optimised for conducting analysis in the current study. While these parameters worked well, it was observed that some parameters could still be altered to enhance the chromatographic resolution such as increasing the split or reducing the temperature ramp which further improved the separation. The data analysis was performed as a two step process, the first step was conducted through NIST library searches using ChromaTOF® software and the second step was achieved by running custom R programming scripts. During the second step, significant compounds were identified (having double or more S/N) in samples compared to controls (background).

Once the analytical methods were established, the VOC profile of all CDD training aid types used by the OPP Canine Unit were established through trials conducted over a span of 1.5 years, specifically during December 2019, July 2020, October 2020, March 2021 and May 2021 (Chapter 3). The CDD training aids that were sampled originated from amputated lower limbs (foot stored outdoors, foot stored indoors, bone, tissue), blood and teeth. The analysis resulted in the identification of 2026 VOCs from all decomposition-related classes including acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters and analogues, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulphur-containing VOCs. Of these, aromatics, linear aliphatics, esters and analogues, and alcohols had the highest abundance while ethers, aldehydes, acids and sulphur-containing VOCs had the least abundance. Similarly, alcohols, nitrogen-containing VOCs, and aromatics had the highest relative class concentration while cyclic aliphatics, aldehydes and ethers had the lowest relative class concentration for each training aid type. This chapter identified VOCs that were prominent (based on the number of samples they occurred in) to CDD training aids and also individual CDD training aid types. Among the 18 prominent VOCs in CDD training aids, 14 have been previously reported in the literature as human decomposition-related, 3 of the 4 remaining compounds can originate as a result of decomposition, while one VOC – sevoflurane, a known anaesthetic likely resulted from the amputation surgical procedure. The detection of VOCs was found to be influenced by factors such as the location of sample collection (indoor vs. outdoor), site of decomposition (surface vs. burial) and presence of organic matter (dry vs. wet samples). This chapter also recorded subtle variability resulting from

ageing (aged vs. recent training aids acquired in 2017 and 2019/2020) and storage conditions (room temperature, refrigerator and freezer) in foot stored indoors and bone training aids. This variability indicated in the PCAs was very subtle as evident from the low explained variance of principal components which were 32% and 4% for ageing, and 9% and 2% for storage of foot and bones, respectively. For storage conditions, room temperature stored samples were found to be variable compared to freezer or refrigerator storage. The subtle variabilities resulted from specific VOCs that dominated the sample groups and were discussed in the chapter at length. Further studies are needed to understand the entire picture of the contribution of ageing and storage on CDD training aids since the PCA data points (samples) in the current study were limited especially for storage conditions. For the same training aid analysed over 1.5 years, no variability in the relative concentration of the three most significant VOCs was observed. The trends indicated that the relative VOC concentration likely depended on the ambient temperature conditions during sample collection since greater normalised area values were obtained in warmer ambient temperatures than in cooler ambient temperatures.

In a parallel study detailed in Chapter 4, VOCs were analysed from donors decomposing in an outdoor environment at the REST[ES] facility located in Québec, Canada. This study was conducted for two reasons – to compare the resulting VOC profile of human cadavers with those of CDD training aids and to document the human decomposition process in Canada. This study is the first to report the decomposition process and VOC profile of eight donors decomposing in two different ambient weather conditions (warm and cool temperatures) in Canada. 32 VOCs occurring in over 30% of samples were identified as prominent to REST[ES] donors and of these, 26 VOCs have been previously reported in the literature as related to human decomposition. All of the prominent VOCs also occurred in the CDD training aid VOC profiles. The 1412 decomposition VOCs detected belonged to all compound classes mentioned previously and were detected in all donors. The class abundance trend in REST[ES] donors was similar to that observed in CDD training aids with aromatics, esters and analogues, nitrogen-containing VOCs and alcohols having the highest abundance while cyclic aliphatics, ethers, acids and sulphur-containing VOCs had the least abundance. Likewise, even for relative class concentration, the trends were similar with nitrogen-containing VOCs, alcohols, acids, esters and analogues, and aromatics having the highest relative class concentration while ethers, aldehydes, halogen-containing VOCs and cyclic aliphatics had the lowest relative class

concentration. The different ambient weather conditions resulted in variable relative class concentrations which were higher at warmer ambient temperatures. The ambient temperature also impacted the donor VOC profiles as a result of which some prominent VOCs were more dominant at warmer ambient temperatures and this caused the two sample groups (donors decomposing at warmer vs. cooler average ambient temperatures) to vary. The progression through decomposition stages was quicker when the average ambient temperatures were warmer. Variability was also identified in the VOC profiles owing to the decomposition stages where the middle (active decay and bloat) stage was most variable relative to the early (fresh) and late (desiccated and dry remains) stages.

The CDD trial sessions discussed in Chapter 5 were held in July 2020, March 2021 and May 2021 with 2, 3 and 7 certified CDDs in each of the trials, respectively. During these trials, the CDDs were exposed to the training aids in both indoor and outdoor search scenarios. The CDD training aids used in the trials were the same samples from which VOCs were collected except for teeth which were not incorporated in the CDD trials. The results indicated that the CDDs were able to successfully locate the majority of training aid types as the overall detection rate ranged from 78% – 100%, except for the buried sample which had a 0% detection rate (recorded twice) and 33% interest response rate (recorded once). The false response rate resulting from events when the CDD could not detect the training aid was low and ranged from 9% – 22%. There were no false positives on the distractor odour however, a total of nine false positives were observed on random locations/objects across all three trials. There was no difference observed in the detection rate of wet versus dry tissue samples, however, the fresh blood had a 100% detection rate while degraded blood had an 82% – 90% detection rate. There was no change observed in CDD response over a period of time and no significant correlation was observed between the rate of detection and years of experience of CDDs.

The last chapter (Chapter 6) comparing VOC profiles and CDD performance found similarities between the VOC profile of CDD training aids and REST[ES] donors in terms of class abundance, relative concentration and prominent VOCs. The VOC class abundance trend of individual training aid type closely resembled that of REST[ES] donors, while relative class concentration trends were somewhat variable. Foot stored indoors and bone training aids had relative class concentration trends that closely resembled that of REST[ES] donors. When comparing the VOC profile of individual training aids, foot samples stored indoors had the maximum number of VOCs (55%)

common with those detected in REST[ES] donors followed by bone, blood, tissue, and foot samples stored outdoors, while the least number of common VOCs were detected in teeth. The combined VOC profile from all CDD training aid types had the highest percentage (68%) of VOCs common with REST[ES] donors, thus implying that it would be preferable to expose CDDs to all training aid types in order to expose them to the maximum VOCs from the human decomposition odour spectrum. However, if OPP had to limit the number of training aids then the VOC profile of foot samples stored indoors was closest to that of the REST[ES] donors. Teeth were found to be the least ideal training aid based on VOC profile and CDD response reported by handlers. The presence of sevoflurane, the anaesthetic in CDD training aids and its absence from the REST[ES] donors did not seem to impact the CDD detection capability as evidenced by the 100 % donor detection rate of both fresh and decomposed donors at REST[ES].

Thus, based on the VOC profiles and CDD performance, it can be concluded that amputated lower limbs/feet can be used as an alternative training aid for CDD training purposes. Nonetheless, considering the limitations of this study (mentioned in Chapter 6) and the inability to answer questions beyond the scope of this research, the author recommends further research on this important topic.

7.2 Future works

A majority of the training aids used in this study came from amputated lower limbs which still contained organic matter. Only a few bones (3 of 7) were dry. Further study is needed to establish the validity of using dry human bones as training aids. It is reported (based on anecdotal evidence of the handlers) that CDDs have difficulty locating dry bone and this may be due to a lack of organic matter that they are more commonly trained on. Thus, constant training on dry remains can be useful to expose CDDs to the VOC profile of both dry and wet remains from amputated lower limbs.

This study found subtle variability owing to ageing and storage conditions however, the duration for which the amputated lower limbs could be used and the most ideal storage conditions could not be established. This was because decomposition-related VOCs and high CDD detection rates were obtained in both aged and fresher training aids and also under all storage conditions. Thus, it is recommended that further studies be conducted to understand the impact of ageing and storage conditions in more detail.

The study conducted at REST[ES] was limited to the spring, summer and autumn seasons in Canada. The winter season which is marked by the presence of snow lasts for five to six months per year during which donor decomposition was not studied. In a parallel study conducted by the author's research group, it was observed that donors continued to decompose while they were covered in snow because the snow acted much like an insulator (results not published yet). Thus, it is likely that VOCs are produced in winter as decomposition progresses. The current methods do not support the collection of VOCs in snow therefore, there is a need to develop new sample collection techniques to understand the VOCs evolved from the donors during winter and the VOCs that are able to permeate the snow for CDD detection.

The CDD trials at REST[ES] were conducted as a pilot study since only two donors were involved in the one trial conducted. There is a need to constantly expose the CDDs to cadavers as no form of training aid will be able to fully represent the cadaveric decomposition odour profile better than cadavers themselves. From a CDD perspective, having trained on smaller training aids and then being exposed to human cadavers in field searches can be overwhelming as observed in the current study where even with a 100% detection rate of the two REST[ES] donors, some CDDs were hesitant to approach the fresh donor. This could be attributed to the size of the donor which the CDDs are not used to experiencing during their training sessions. Thus, constant exposure to cadavers is recommended. This can only be achieved in a setting such as a human taphonomic research facility where law enforcement agencies do not have to undergo extensive paperwork themselves to have access to the cadavers. It is recommended that dog training agencies across the globe collaborate with human taphonomic facilities to provide their CDDs with the best possible training for the search and detection of human remains.

Even though this study identified significant VOCs, it could not be established which VOCs elicit a CDD response. This can not be achieved unless a neuroscientist is involved in recording a CDD brain activity which was beyond the scope and expertise of this study. Results from decomposition studies such as the current one require further canine neurological studies to understand VOCs that interest the CDDs. This could help to develop additional chemical training aids, or alternative biological detectors such as electronic noses and biosensors in the future to complement or even substitute for CDD searches when it is appropriate to do so.

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APPENDICES

APPENDIX A: VOCS DETECTED IN CDD TRAINING AIDS

- This appendix presents a list of 988 VOCs listed in the order of their reducing prominence (occurence in most samples). VOCs with the same prominence have been listed alphabetically.
- The 662 VOCs listed in blue are those that were also detected in REST[ES] donors.
- This list does not include VOCs that occurred only in 1 or 2 samples of the total 86 CDD training aid samples (85 samples from all CDD training aid types + 1 sample from buried foot samples indoor in May 2021).

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
1	2,6-Lutidine	Aromatics	77.91		89.66	81.82	91.67	91.67	33.33
2	Dimethyl trisulfide	Sulphur-containing	66.28	14.29	93.10	59.09	75.00	58.33	
3	Pyridine, 2-methyl-	Aromatics	66.28	14.29	79.31	59.09	91.67	66.67	33.33
4	Pyrimidine, 4-methyl-	Aromatics	62.79		75.86	54.55		66.67	
5	Methenamine	Nitrogen-containing	61.63		79.31	72.73	58.33	41.67	33.33
6	1-Butanol, 3-methyl-	Alcohols	47.67		58.62	54.55	41.67	50.00	
7	Pyrazine, methyl-	Aromatics	47.67	14.29	72.41	40.91	58.33	25.00	
8	Pyridine	Aromatics	47.67		68.97	27.27		50.00	
9	Pyrazine, 2,5-dimethyl-	Aromatics	41.86	14.29	68.97	9.09	41.67		
10	Thiocyanic acid, methyl ester	Esters and analogues	41.86	57.14	65.52	27.27	8.33	33.33	33.33
11	Sevoflurane	Halogen-containing	37.21		68.97	54.55			
12	Butanoic acid, 3-methyl-	Acids	36.05		51.72	45.45			
13	Methylamine, N,N-dimethyl-	Nitrogen-containing	36.05		44.83	31.82		25.00	
14	Thiazole	Aromatics	36.05	28.57	37.93	40.91	41.67	33.33	
15	Propanoic acid, 2-methyl-	Acids	33.72		44.83	50.00		33.33	
16	Acetamide	Nitrogen-containing	31.40		41.38	22.73		25.00	33.33
17	2-Furancarboxaldehyde, 5-methyl-	Aldehydes	30.23	28.57	31.03	27.27		25.00	33.33
18	Pyrazine, trimethyl-	Aromatics	30.23	14.29	48.28	4.55	41.67		
19	Aniline	Aromatics	29.07		41.38	22.73	33.33	16.67	66.67
20	1,3,5-Triazine	Nitrogen-containing	27.91	14.29	27.59	36.36	33.33	16.67	
21	L-Alanine, 3-sulfo-	Nitrogen-containing	27.91	14.29	31.03	40.91	25.00	8.33	33.33
22	Pyridine, 2,4,6-trimethyl-	Aromatics	27.91		34.48	4.55		33.33	
23	1H-Pyrrole, 1-methyl-	Aromatics	26.74	14.29	34.48	22.73	25.00		
24	Isobutyronitrile	Nitrogen-containing	26.74	14.29	31.03	13.64	25.00		33.33
25	Oxirane, 2-ethyl-2-methyl-	Ethers	26.74		24.14	31.82	33.33	33.33	33.33
26	Pyrazine	Aromatics	25.58		34.48	18.18	33.33	33.33	
27	Pyridine, 2,4-dimethyl-	Aromatics	25.58		37.93	4.55		33.33	33.33
28	Hexanenitrile	Nitrogen-containing	24.42		41.38	9.09	33.33	25.00	
29	Methylacrylonitrile	Nitrogen-containing	24.42	14.29	34.48		33.33		33.33
30	1H-Pyrrole, 2,5-dimethyl-	Aromatics	23.26		27.59	27.27		8.33	
31	Isopropyl acetate	Esters and analogues	23.26		37.93	40.91			
32	Phenol, 4-(1,1-dimethylpropyl)-	Alcohols	23.26		44.83	13.64	25.00		33.33
33	1-Heptanol	Alcohols	22.09	14.29	17.24	31.82	16.67	16.67	33.33
34	1,3-Diazine	Nitrogen-containing	22.09	14.29	31.03	13.64	25.00	25.00	
35	1,3,6-Trioxocane, 2-methyl-	Ethers	22.09	42.86	34.48	18.18	8.33	8.33	
36	1H-1,2,4-Triazole	Aromatics	22.09		34.48	4.55		16.67	33.33
37	2,2-Dimethoxybutane	Ethers	22.09		17.24	22.73			33.33
38	Acetaldehyde	Aldehydes	22.09	14.29	31.03	18.18	25.00	8.33	33.33
39	Butanenitrile	Nitrogen-containing	22.09		34.48	18.18	16.67	8.33	33.33
40	Disulfide, methyl (methylthio)methyl	Sulphur-containing	22.09		48.28	22.73			
41	Ethanol, 2-methoxy-	Alcohols	22.09		48.28	18.18		8.33	
42	Isothiazole, 3-methyl-	Aromatics	22.09		31.03	22.73	8.33	25.00	33.33
43	Phenylethyl Alcohol	Alcohols	22.09		37.93	18.18	16.67	16.67	
44	Propane, 2,2-dimethoxy-	Ethers	22.09		24.14	22.73	25.00		
45	1,4-Pentadien-3-one	Ketones	20.93		17.24	22.73			
46	2-Butenal, 2-methyl-	Aldehydes	20.93	14.29	34.48		16.67		33.33
47	2-Nonanone	Ketones	20.93		27.59	22.73	16.67	16.67	33.33
48	4-Methylthiazole	Aromatics	20.93		34.48	18.18	8.33	16.67	33.33
49	Pentanoic acid	Acids	20.93		17.24	27.27		16.67	
50	1-Butanol, 3-methyl-, acetate	Esters and analogues	19.77		31.03	36.36			

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
51	2-Propanone, 1-hydroxy-	Ketones	19.77		24.14	36.36		16.67	
52	Acetonitrile, hydroxy-	Nitrogen-containing	19.77		31.03	18.18			
53	Cyclohexane, 1,1,2,3-tetramethyl-	Cyclic aliphatic	19.77		27.59	27.27	8.33	8.33	
54	Methane, isothiocyanato-	Nitrogen-containing	19.77	42.86	17.24	22.73	8.33	16.67	33.33
55	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	Aromatics	19.77		31.03	22.73	25.00		
56	Phenol, 4-ethyl-	Alcohols	19.77		27.59	27.27	16.67	8.33	
57	1,1,4,5,6-Pentamethyl-2,3-dihydro-1H-indene	Aromatics	18.60		31.03	18.18			
58	1,2-Ethanediol, diformate	Esters and analogues	18.60		34.48	13.64			
59	3-Octanone	Ketones	18.60		34.48	13.64		16.67	
60	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	Cyclic aliphatic	18.60	28.57	17.24	18.18	8.33		33.33
61	Ethanol, 2,2'-oxybis-	Alcohols	18.60	28.57	27.59	18.18	8.33	8.33	
62	Ethyl formate	Esters and analogues	18.60	14.29	13.79	31.82	16.67	16.67	
63	Hexanoic acid	Acids	18.60		24.14	22.73			
64	Octane, 4-ethyl-	Linear aliphatic	18.60		10.34	22.73	25.00		
65	Propanoic acid	Acids	18.60		24.14	22.73	16.67	8.33	
66	Pyrazine, 2,3-dimethyl-	Aromatics	18.60		27.59			16.67	33.33
67	2-Chloroethanol	Alcohols	17.44	14.29	27.59	13.64			
68	2-Pentanol	Alcohols	17.44		20.69	22.73	8.33		
69	2,4-Dithiapentane	Sulphur-containing	17.44		31.03	18.18		16.67	
70	Acetonitrile, (dimethylamino)-	Nitrogen-containing	17.44	28.57	20.69	4.55		16.67	
71	Benzene, (1-butylheptyl)-	Aromatics	17.44	14.29	13.79	22.73		8.33	
72	Benzene, (1,3-dimethylbutyl)-	Aromatics	17.44		37.93	4.55		8.33	33.33
73	Bis-(ethoxycarbonyl)methoxymethyloxyiminomethane	Nitrogen-containing	17.44		13.79	27.27	16.67		
74	Dimethyl sulfide	Sulphur-containing	17.44		24.14	22.73	16.67		
75	Furan, 2-pentyl-	Aromatics	17.44	42.86	31.03	9.09	8.33		
76	Methallyl cyanide	Nitrogen-containing	17.44		24.14	4.55			33.33
77	p-Cresol	Alcohols	17.44	28.57	20.69	4.55		16.67	
78	Pentalene, octahydro-	Linear aliphatic	17.44		24.14	9.09	8.33		66.67
79	Propylene Glycol	Alcohols	17.44	14.29	34.48	4.55	8.33	8.33	33.33
80	β-Myrcene	Linear aliphatic	17.44	14.29	24.14	9.09	8.33	16.67	33.33
81	1-Methylbicyclo(4.4.0)decane(trans)	Cyclic aliphatic	16.28		6.90	22.73			33.33
82	1,4-Dioxan-2-ol	Alcohols	16.28		31.03	13.64	16.67		
83	3-Hexene	Linear aliphatic	16.28	28.57	20.69	9.09	16.67	16.67	
84	4-Piperidinone, 2,2,6,6-tetramethyl-	Ketones	16.28		27.59	4.55		8.33	
85	Butanenitrile, 3-methyl-	Nitrogen-containing	16.28		20.69	9.09		16.67	
86	Carbamic acid, N-[1,1-bis(trifluoromethyl)ethyl]-, 4-(1,1,3,3-tetramethylbutyl)phenyl ester	Halogen-containing	16.28		27.59	18.18		16.67	
87	Carbonyl sulfide	Sulphur-containing	16.28		24.14	27.27			
88	Ethanol, 2-(vinyloxy)-	Alcohols	16.28		34.48	13.64		8.33	
89	Formamide, N,N-dimethyl-	Nitrogen-containing	16.28		31.03	4.55	8.33	16.67	33.33
90	Heptanonitrile	Nitrogen-containing	16.28		27.59			8.33	
91	Methyl ethyl disulfide	Sulphur-containing	16.28		27.59	27.27			
92	Nonane, 3-methyl-	Linear aliphatic	16.28		17.24	9.09			33.33
93	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	Esters and analogues	16.28	42.86	17.24	22.73			33.33
94	Propanenitrile, 3-(dimethylamino)-	Nitrogen-containing	16.28		24.14	9.09		16.67	
95	Propanoic acid, 1-methylethyl ester	Esters and analogues	16.28		20.69	36.36			
96	Propanoic acid, 2-oxo-	Acids	16.28		20.69	18.18	16.67	8.33	33.33
97	Trichloroethylene	Halogen-containing	16.28	14.29	13.79	22.73	16.67	16.67	
98	1,2-Ethanediol, monoformate	Esters and analogues	15.12		20.69	22.73			
99	1,3-Cyclopentadiene	Cyclic aliphatic	15.12		20.69	4.55			33.33
100	1,4-Dioxane, 2,5-dimethyl-	Ethers	15.12		6.90	13.64			33.33
101	2-Pentanone, 3-methyl-	Ketones	15.12	14.29	27.59	9.09			
102	3-Carene	Cyclic aliphatic	15.12	28.57	20.69	4.55	16.67	8.33	33.33
103	9-Methylbicyclo[3.3.1]nonane	Cyclic aliphatic	15.12		20.69	13.64		8.33	
104	Benzene, (1-pentylheptyl)-	Aromatics	15.12		24.14	18.18	16.67		
105	Benzene, 1,3-diethyl-	Aromatics	15.12		20.69	13.64	8.33	8.33	66.67
106	Butanoic acid	Acids	15.12		34.48	9.09			
107	Caprolactam	Nitrogen-containing	15.12		24.14	18.18	8.33	8.33	
108	Cyclohexane, 1,3-dimethyl-	Cyclic aliphatic	15.12		10.34	9.09			
109	Dimethyl sulfone	Sulphur-containing	15.12		31.03	9.09		16.67	
110	Ethyl Acetate	Esters and analogues	15.12	14.29	6.90	18.18			

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
111	Furan, 2,5-dimethyl-	Aromatics	15.12		13.79	18.18		8.33	33.33
112	N-Nitrosodimethylamine	Nitrogen-containing	15.12		20.69	4.55			
113	Naphthalene, 1,2,3,4-tetrahydro-1,8-dimethyl-	Aromatics	15.12		31.03	13.64			33.33
114	Phenol, 2-methyl-	Alcohols	15.12	28.57	24.14	18.18			
115	Propanoic acid, 2-methyl-, ethyl ester	Esters and analogues	15.12		17.24	36.36			
116	1-Butanol, 2-methyl-	Alcohols	13.95		24.14	18.18	8.33		
117	1,1'-Biphenyl, 2-methyl-	Aromatics	13.95		17.24	13.64			66.67
118	2-Butanol	Alcohols	13.95		27.59	9.09			
119	2-Heptanol	Alcohols	13.95		17.24	31.82			
120	2-Pentene	Linear aliphatic	13.95	14.29	3.45	22.73			33.33
121	2-Propen-1-ol	Alcohols	13.95	42.86	17.24	4.55			33.33
122	2-Thiophenecarboxaldehyde	Aldehydes	13.95		13.79	22.73		8.33	
123	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	Alcohols	13.95		27.59	13.64			
124	5,9-Undecadien-2-one, 6,10-dimethyl-	Ketones	13.95		17.24	9.09	8.33		33.33
125	Benzene, (1-propylnonyl)-	Aromatics	13.95		20.69	22.73	8.33		
126	Benzene, (1-propyloctyl)-	Aromatics	13.95		10.34	22.73	16.67		
127	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	Ketones	13.95	28.57	31.03			8.33	
128	Butanenitrile, 2-methyl-	Nitrogen-containing	13.95		20.69	4.55		16.67	
129	Butanoic acid, 2-methyl-	Acids	13.95		17.24	27.27			
130	Butanoic acid, 3-methyl-, ethyl ester	Esters and analogues	13.95		13.79	36.36			
131	Ethanol, 2-(2-ethoxyethoxy)-	Alcohols	13.95	14.29	24.14	13.64		8.33	
132	Eucalyptol	Ethers	13.95		13.79	18.18	16.67	8.33	33.33
133	Glycolaldehyde dimer	Aldehydes	13.95		20.69	18.18		16.67	
134	Guanidine carbonate	Nitrogen-containing	13.95		17.24	18.18	16.67	8.33	
135	Methane, tribromo-	Halogen-containing	13.95	14.29	17.24	18.18	16.67		
136	Perfluorooctane	Halogen-containing	13.95		10.34	22.73		8.33	
137	Phenol, 4-(1-methylpropyl)-	Alcohols	13.95	14.29	24.14	13.64		8.33	
138	S-Methyl methanethiosulphonate	Sulphur-containing	13.95		17.24	31.82			
139	1-Nonylcycloheptane	Cyclic aliphatic	12.79		20.69	9.09			33.33
140	1-Propanol, 2-methyl-	Alcohols	12.79		24.14	18.18			
141	1-Propene, 2-methoxy-	Ethers	12.79		17.24	4.55			33.33
142	1,8,11-Heptadecatriene, (Z,Z)-	Linear aliphatic	12.79		13.79	27.27	8.33		
143	1H-Indene, 2,3-dihydro-1,1-dimethyl-	Aromatics	12.79		24.14	13.64	8.33		
144	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-	Aromatics	12.79		17.24	13.64			33.33
145	2-Hexanol	Alcohols	12.79		20.69	18.18	8.33		
146	2-n-Butyl furan	Aromatics	12.79	28.57	17.24	13.64			
147	3-Buten-1-ol, 3-methyl-	Alcohols	12.79		20.69				
148	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	Aromatics	12.79	14.29	13.79	18.18	8.33		
149	Acetic acid, butyl ester	Esters and analogues	12.79		10.34	22.73	8.33	8.33	33.33
150	benzene, 1,1'-(1-methylethylidene)bis[4-methyl-	Aromatics	12.79		17.24	9.09		8.33	33.33
151	Benzene, 1,4-dichloro-	Aromatics	12.79		24.14	4.55			33.33
152	Benzoxazole	Aromatics	12.79	14.29	17.24	4.55			
153	Butanoic acid, 2-methyl-, ethyl ester	Esters and analogues	12.79		10.34	36.36			
154	Butanoic acid, ethyl ester	Esters and analogues	12.79		13.79	31.82			
155	Butylated Hydroxytoluene	Aromatics	12.79		31.03	9.09			
156	Dimethyl ether	Ethers	12.79		27.59	4.55	8.33	8.33	
157	Disulfide, dimethyl	Sulphur-containing	12.79	42.86	20.69			8.33	33.33
158	Furan, 2-ethyl-	Aromatics	12.79	14.29	17.24	13.64	8.33		33.33
159	Hexane, 2,3,5-trimethyl-	Linear aliphatic	12.79		24.14	9.09	8.33	8.33	
160	Indole	Aromatics	12.79		24.14	9.09	8.33		33.33
161	Isopropyl butyrate	Esters and analogues	12.79		13.79	31.82			
162	Methyl isocyanide	Nitrogen-containing	12.79	14.29	3.45	9.09		16.67	
163	Propanoic acid, 2-methyl-, 1-methylethyl ester	Esters and analogues	12.79		17.24	27.27			
164	Pyrrole	Aromatics	12.79		10.34	13.64	16.67	16.67	33.33
165	trans-4a-Methyl-decahydronaphthalene	Aromatics	12.79		20.69	4.55		8.33	
166	1-Propene, 2-methyl-	Linear aliphatic	11.63	14.29	24.14	4.55	8.33		
167	1,2,4,5-Tetroxane, 3,3,6,6-tetramethyl-	Ethers	11.63	14.29	27.59	4.55			
168	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	Aromatics	11.63		31.03	4.55			
169	2-Cyclopenten-1-one, 2-methyl-	Ketones	11.63		10.34	4.55			33.33
170	2-Furanmethanol	Alcohols	11.63		13.79	13.64			

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
171	2-Hexene, 3-methyl-	Linear aliphatic	11.63		13.79	4.55		8.33	33.33
172	2-Hydroxy-iso-butyrophenone	Ketones	11.63		24.14	9.09			33.33
173	2-Octanone	Ketones	11.63	28.57	10.34	13.64	8.33	8.33	
174	2-Pentene, 3-methyl-	Linear aliphatic	11.63		10.34	27.27	8.33		
175	2(3H)-Furanone, dihydro-5-propyl-	Esters and analogues	11.63		3.45	22.73		8.33	33.33
176	2(5H)-Furanone	Esters and analogues	11.63		27.59	4.55		8.33	
177	2(5H)-Furanone, 3-methyl-	Esters and analogues	11.63		10.34	13.64		8.33	
178	3-Hexene, 2,2-dimethyl-	Linear aliphatic	11.63		20.69	9.09	8.33		33.33
179	3-Penten-2-one	Ketones	11.63	14.29	6.90	9.09			33.33
180	Anisole	Ethers	11.63		3.45	22.73	8.33		
181	Benzene, (1-butyloctyl)-	Aromatics	11.63	14.29	10.34	4.55			
182	Benzene, 1-ethyl-2,4,5-trimethyl-	Aromatics	11.63		27.59	4.55			33.33
183	Benzene, 2,4-dimethyl-1-(1-methylpropyl)-	Aromatics	11.63		20.69	13.64			33.33
184	Benzene, hexyl-	Aromatics	11.63	14.29	20.69	4.55		8.33	33.33
185	Benzonitrile	Nitrogen-containing	11.63			13.64			33.33
186	Butane, 1-methoxy-3-methyl-	Ethers	11.63	14.29	17.24	18.18			
187	Butanoic acid, 2-methyl-, 1-methylethyl ester	Esters and analogues	11.63		24.14	13.64			
188	Butanoic acid, 2-methyl-, propyl ester	Esters and analogues	11.63		6.90	36.36			
189	Butanoic acid, propyl ester	Esters and analogues	11.63		6.90	36.36			
190	Cyclohexane, 1-ethyl-2,3-dimethyl-	Cyclic aliphatic	11.63		24.14	9.09	8.33		
191	Cyclohexane, 1,2,3-trimethyl-	Cyclic aliphatic	11.63		6.90	4.55			
192	Cyclopentanone, 2-methyl-	Ketones	11.63		17.24	13.64			
193	Cyclopropane, ethylidene-	Cyclic aliphatic	11.63		3.45	13.64			
194	Ethanol, 2-(2-methoxyethoxy)-	Alcohols	11.63		24.14	4.55	8.33	8.33	
195	Ethanol, 2-propoxy-	Alcohols	11.63		17.24	18.18			33.33
196	Methyl methacrylate	Esters and analogues	11.63		24.14	4.55	8.33	8.33	
197	n-Propyl acetate	Esters and analogues	11.63		13.79	27.27			
198	Naphthalene, 1,2,3,4-tetrahydro-1-methyl-	Aromatics	11.63		17.24	9.09	8.33	8.33	33.33
199	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-	Aromatics	11.63		10.34	22.73			
200	Octane, 2,6-dimethyl-	Linear aliphatic	11.63		20.69	9.09			
201	Pentane	Linear aliphatic	11.63		3.45	9.09	8.33		33.33
202	Pyridine, 2-ethyl-	Aromatics	11.63		27.59				
203	Undecane, 2,6-dimethyl-	Linear aliphatic	11.63		17.24	13.64	8.33		33.33
204	1-Butene, 3-methyl-	Linear aliphatic	10.47		6.90	18.18		8.33	
205	1H-Pyrrole, 2-methyl-	Aromatics	10.47	28.57	10.34	9.09	8.33	8.33	
206	2-Ethynyl pyridine	Aromatics	10.47		10.34	9.09			
207	2-Furancarbonitrile	Nitrogen-containing	10.47	14.29	13.79		8.33		33.33
208	2-Heptanone, 6-methyl-	Ketones	10.47		6.90	9.09			
209	2-Isobutyl-3-methylpyrazine	Aromatics	10.47			4.55	8.33		
210	2-Propanol, 1-methoxy-	Alcohols	10.47		24.14	4.55		8.33	
211	2,5-Furandione, 3-methyl-	Esters and analogues	10.47		13.79	4.55			
212	3-Pentanol	Alcohols	10.47		17.24	13.64			
213	3-Penten-2-ol	Alcohols	10.47		24.14	4.55			
214	3-Pentenoic acid, 4-methyl-	Acids	10.47		17.24	13.64	8.33		
215	3,5-Difluorobenzaldehyde	Halogen-containing	10.47	14.29	10.34	9.09			33.33
216	3,5-Difluorophenol	Halogen-containing	10.47		20.69	4.55		8.33	33.33
217	6-Dodecanol	Alcohols	10.47		17.24	4.55			33.33
218	Acetoin	Ketones	10.47	14.29	13.79	13.64		8.33	
219	Aziridine, 1-methyl-	Nitrogen-containing	10.47		13.79	4.55		8.33	
220	Benzaldehyde, 2-hydroxy-	Aldehydes	10.47		13.79	13.64	8.33	8.33	
221	Benzene, (1-butylhexyl)-	Aromatics	10.47		10.34	4.55	8.33		33.33
222	Benzene, iodo-	Halogen-containing	10.47		6.90	9.09			33.33
223	Benzyl nitrile	Nitrogen-containing	10.47		17.24	4.55			
224	Bromoacetone	Ketones	10.47		3.45	9.09			33.33
225	Butanoic acid, 2-methylpropyl ester	Esters and analogues	10.47		17.24	9.09			
226	Butanoic acid, 2-oxo-, ethyl ester	Esters and analogues	10.47		13.79	9.09		8.33	
227	Cyclohexene, 3,3,5-trimethyl-	Cyclic aliphatic	10.47	28.57	10.34	9.09	8.33	8.33	
228	Dotriacontane	Linear aliphatic	10.47		10.34	9.09	8.33		
229	Formamide	Nitrogen-containing	10.47		24.14		8.33	8.33	
230	Formamide, N-cyclohexyl-	Nitrogen-containing	10.47		10.34	13.64			33.33

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231	Heptane, 2-methyl-	Linear aliphatic	10.47		17.24		8.33		33.33
232	Heptane, 2,4-dimethyl-	Linear aliphatic	10.47		6.90	4.55			33.33
233	Hexane, 2,3-dimethyl-	Linear aliphatic	10.47	14.29	10.34	9.09			
234	Hexanoic acid, 2-methyl-	Acids	10.47		13.79	22.73			
235	Hydrogen isocyanate	Nitrogen-containing	10.47		17.24	4.55			
236	Methane, dibromo-	Halogen-containing	10.47		17.24	18.18			
237	Methane, isocyanato-	Nitrogen-containing	10.47		13.79	9.09	8.33		
238	Methyl Isobutyl Ketone	Ketones	10.47		6.90	4.55			33.33
239	Nonane, 2,5-dimethyl-	Linear aliphatic	10.47		17.24	9.09			
240	Octane, 3,5-dimethyl-	Linear aliphatic	10.47		10.34	9.09	8.33		33.33
241	Phenol, 2-(1,1-dimethylethyl)-	Alcohols	10.47		20.69			8.33	
242	Phenol, p-tert-butyl-	Alcohols	10.47	14.29	13.79	9.09		8.33	33.33
243	Propanal	Aldehydes	10.47		10.34	4.55	8.33		33.33
244	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	Esters and analogues	10.47		17.24	9.09	8.33		33.33
245	Propanoic acid, 2,2-dimethyl-	Acids	10.47		20.69	9.09			
246	Spiro[3.6]deca-5,7-dien-1-one,5,9,9-trimethyl	Cyclic aliphatic	10.47		20.69	9.09		8.33	
247	1-Octen-3-ol	Alcohols	9.30	57.14	6.90	4.55			
248	1-Pentanol, 4-methyl-	Alcohols	9.30		17.24	13.64			
249	1,2-Benzenediol, o-(4-methoxybenzoyl)-o'-(2,2,3,3,4,4,4-heptafluorobutyryl)-	Halogen-containing	9.30		13.79	18.18			
250	1,2,3-Trifluoro-4-trifluoromethylbenzene	Halogen-containing	9.30		10.34	9.09			
251	1H-Indene, 2,3-dihydro-1,1,2,3,3-pentamethyl-	Aromatics	9.30		13.79	13.64			
252	1H-Indene, 2,3-dihydro-1,4,7-trimethyl-	Aromatics	9.30		27.59				
253	2-Butanone, 3,3-dimethyl-	Ketones	9.30		13.79	9.09			
254	2-Butene	Linear aliphatic	9.30		10.34	13.64			33.33
255	2-Octene, 4-ethyl-	Linear aliphatic	9.30		13.79	4.55			33.33
256	2-Propanol, 2-methyl-	Alcohols	9.30		17.24	4.55			
257	2,3-Butanedione	Ketones	9.30		17.24	9.09			33.33
258	2,4-Dimethylfuran	Aromatics	9.30	14.29	6.90	9.09	8.33	8.33	33.33
259	2H-Pyran-2-one, tetrahydro-	Ethers	9.30		10.34	9.09	8.33	8.33	
260	3-Furaldehyde	Aldehydes	9.30		10.34	4.55			
261	Acetic acid, hydroxy-	Acids	9.30		13.79	9.09	8.33		33.33
262	Benzene, 1-(1-methylethenyl)-2-(1-methylethyl)-	Aromatics	9.30		24.14			8.33	
263	Benzene, 1-ethyl-2,4-dimethyl-	Aromatics	9.30		17.24	4.55		8.33	33.33
264	Benzene, 1-ethyl-3-methyl-	Aromatics	9.30		10.34	18.18	8.33		
265	Benzene, 1,2,3,5-tetrafluoro-	Halogen-containing	9.30		17.24	4.55		8.33	33.33
266	Benzene, 1,4-dimethyl-2-(2-methylpropyl)-	Aromatics	9.30		20.69	9.09			
267	Benzene, 2-propenyl-	Aromatics	9.30		17.24			8.33	
268	Benzoic acid, ethyl ester	Esters and analogues	9.30		17.24	4.55		8.33	33.33
269	Butane,1,2,4-trichloro-heptafluoro-	Halogen-containing	9.30		17.24	4.55		8.33	33.33
270	Carbonic acid, octadecyl vinyl ester	Esters and analogues	9.30		10.34	13.64		8.33	33.33
271	Cyclohexane	Cyclic aliphatic	9.30	14.29		9.09			
272	Cyclohexane, 1,1,3-trimethyl-	Cyclic aliphatic	9.30		10.34	18.18		8.33	
273	Cyclohexene, 1-methyl-	Cyclic aliphatic	9.30		10.34	13.64	8.33		33.33
274	Cyclopentane, ethyl-	Cyclic aliphatic	9.30		24.14	4.55			
275	Cyclopentanone, 3-methyl-	Ketones	9.30	14.29	10.34	9.09			
276	Cyclopentene	Cyclic aliphatic	9.30		10.34	4.55	8.33		33.33
277	Diphenyl sulfide	Sulphur-containing	9.30	14.29	3.45		8.33		33.33
278	Ethanol, 2-phenoxy-	Alcohols	9.30		24.14	4.55			
279	Ethanol, 2,2,2-trifluoro-	Halogen-containing	9.30	14.29	13.79	4.55	8.33	8.33	
280	Ethylenimine	Nitrogen-containing	9.30		10.34	13.64			
281	Formaldehyde	Aldehydes	9.30	14.29	20.69			8.33	
282	Formamide, N,N-dibutyl-	Nitrogen-containing	9.30		6.90	9.09		8.33	33.33
283	Furan, 2-ethyl-5-methyl-	Aromatics	9.30		3.45	18.18	8.33	8.33	33.33
284	Isopropylcyclobutane	Cyclic aliphatic	9.30		10.34	4.55	8.33		
285	Octane, 1-chloro-	Halogen-containing	9.30		6.90	13.64		8.33	
286	p-Aminotoluene	Aromatics	9.30		13.79	9.09	8.33	8.33	
287	p-Cymene	Aromatics	9.30		10.34	9.09	8.33	8.33	
288	Pentane, 2,2-dimethyl-	Linear aliphatic	9.30		13.79	9.09	8.33		33.33
289	Propane, 1-(methylthio)-	Sulphur-containing	9.30		20.69	9.09			
290	Propanenitrile	Nitrogen-containing	9.30		6.90	27.27			

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291	Propanenitrile, 3-hydroxy-	Nitrogen-containing	9.30		20.69	9.09			
292	Propanoic acid, 2-methyl-, propyl ester	Esters and analogues	9.30			36.36			
293	Pyrazine, 3-ethyl-2,5-dimethyl-	Aromatics	9.30	14.29	13.79				
294	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso-	Ketones	9.30		13.79	9.09	8.33	8.33	
295	Trichloromonofluoromethane	Halogen-containing	9.30	14.29	13.79	9.09	8.33		
296	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	Cyclic aliphatic	9.30		13.79	9.09			
297	Undecane, 3-methyl-	Linear aliphatic	9.30		10.34	4.55			
298	1-Octene	Linear aliphatic	8.14		3.45	4.55			
299	1-Penten-3-ol	Alcohols	8.14	14.29	13.79				33.33
300	1-Pentene, 2-methyl-	Linear aliphatic	8.14		20.69	4.55			
301	1-Propanol	Alcohols	8.14		6.90	9.09			33.33
302	1-Propanol, 2,2'-oxybis-	Alcohols	8.14		17.24	9.09			
303	1,2-Ethanediol	Alcohols	8.14		10.34	18.18			
304	1,4-Dimethyladamantane #	Cyclic aliphatic	8.14		6.90	13.64			
305	1H-Indene, 2,3-dihydro-4,7-dimethyl-	Aromatics	8.14		20.69				33.33
306	2-Octene	Linear aliphatic	8.14		6.90	18.18			
307	2-Pentanone	Ketones	8.14		13.79	9.09			33.33
308	2-Propanol, 1,1'-oxybis-	Alcohols	8.14		10.34	9.09			
309	2-Vinylfuran	Aromatics	8.14		10.34	13.64			
310	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	Esters and analogues	8.14		17.24				
311	2(3H)-Furanone, dihydro-4-methyl-	Esters and analogues	8.14		6.90	4.55			
312	2H-Pyran-2-one	Ethers	8.14		10.34	18.18			
313	3-Octanol	Alcohols	8.14		13.79	9.09			
314	3-Octene, 2,2-dimethyl-	Linear aliphatic	8.14		6.90	9.09		8.33	33.33
315	3-Pentanone, 2,4-dimethyl-	Ketones	8.14		20.69				
316	3-Penten-2-one, 4-methyl-	Ketones	8.14		10.34	4.55			
317	5,6,7,8,9,10-Hexahydrobenzocyclooctene	Aromatics	8.14		24.14				
318	Benzene, (1-ethylnonyl)-	Aromatics	8.14		17.24	4.55	8.33		
319	Benzene, 1-ethenyl-2-methyl-	Aromatics	8.14		3.45	13.64	8.33	8.33	33.33
320	Benzene, 1-ethyl-3,5-dimethyl-	Aromatics	8.14		13.79	4.55	8.33	8.33	
321	Benzene, 1-methyl-4-butyl	Aromatics	8.14		10.34	4.55	8.33	8.33	33.33
322	Benzene, 2-ethyl-1,4-dimethyl-	Aromatics	8.14	14.29	13.79		8.33		33.33
323	Benzene, 4-ethenyl-1,2-dimethyl-	Aromatics	8.14		3.45	13.64	8.33		
324	Benzene, pentamethyl-	Aromatics	8.14		13.79	9.09	8.33		
325	Benzene, pentyl-	Aromatics	8.14		6.90	4.55	8.33		
326	Benzofuran, 2-methyl-	Aromatics	8.14	14.29	6.90	9.09	8.33	8.33	
327	Bis(dimethylamino)trifluorophosphorane	Halogen-containing	8.14		13.79	4.55	8.33	8.33	
328	Butane, 2,2-dimethyl-	Linear aliphatic	8.14		10.34	4.55	8.33	8.33	33.33
329	Butane, 2,2,3,3-tetramethyl-	Linear aliphatic	8.14		3.45	9.09		8.33	33.33
330	Butanoic acid, 3-methyl-, 1-methylethyl ester	Esters and analogues	8.14		17.24	9.09			
331	Carbohydrazide	Nitrogen-containing	8.14		6.90	13.64			
332	Carbon Tetrachloride	Halogen-containing	8.14		10.34	4.55		8.33	
333	Carbonic acid, eicosyl vinyl ester	Esters and analogues	8.14		6.90	13.64			
334	Carbonic acid, tetradecyl vinyl ester	Esters and analogues	8.14	14.29	10.34	9.09		8.33	
335	Cyclohexane, 1,2-dimethyl-	Cyclic aliphatic	8.14		20.69	4.55			
336	Cyclohexane, propyl-	Cyclic aliphatic	8.14		6.90	13.64			
337	Cyclohexene, 4-ethenyl-	Cyclic aliphatic	8.14		24.14				
338	Cyclooctane, 1,4-dimethyl-, cis-	Cyclic aliphatic	8.14		17.24		8.33		33.33
339	Cyclopropyl carbinol	Alcohols	8.14		3.45	13.64			
340	Diethyltoluamide	Nitrogen-containing	8.14	57.14	6.90	4.55			
341	Diisobutyl cellosolve	Linear aliphatic	8.14		13.79	4.55		8.33	33.33
342	Disulfide, bis(1,1,3,3-tetramethylbutyl)	Sulphur-containing	8.14		17.24	4.55	8.33		
343	Dodecane, 2,7,10-trimethyl-	Linear aliphatic	8.14		17.24	4.55	8.33		
344	Formic acid, butyl ester	Esters and analogues	8.14		13.79				
345	Heptane, 2,3-dimethyl-	Linear aliphatic	8.14		20.69	4.55			
346	Hydrazine	Nitrogen-containing	8.14		17.24	4.55	8.33		
347	Methanesulfonic acid, methyl ester	Esters and analogues	8.14		13.79	13.64			
348	Methyl propionate	Esters and analogues	8.14		13.79	9.09			33.33
349	Methylthio-2-propanone	Ketones	8.14		24.14				
350	Naphthalene, 1,6-dimethyl-	Aromatics	8.14		10.34	13.64	8.33		

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
351	Nitrous oxide	Nitrogen-containing	8.14		10.34	9.09		8.33	33.33
352	Nonane, 4,5-dimethyl-	Linear aliphatic	8.14			22.73	8.33		
353	Octane, 2,7-dimethyl-	Linear aliphatic	8.14		17.24	4.55			
354	Octane, 3-methyl-	Linear aliphatic	8.14		6.90	4.55	8.33		33.33
355	Pentane, 2,3,3-trimethyl-	Linear aliphatic	8.14	14.29	13.79		8.33	8.33	
356	Pentanoic acid, 4-methyl-	Acids	8.14		13.79	9.09			
357	Pentanoic acid, 4-methyl-, ethyl ester	Esters and analogues	8.14		6.90	22.73			
358	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	Alcohols	8.14		17.24	4.55	8.33		
359	Phthalic acid, 2-isopropylphenyl methyl ester	Esters and analogues	8.14	28.57	10.34		8.33		
360	Propanedioic acid	Acids	8.14		13.79	9.09			33.33
361	Propanoic acid, 2-methyl-, 3-methylbutyl ester	Esters and analogues	8.14		13.79	13.64			
362	Propanoic acid, 2-oxo-, methyl ester	Esters and analogues	8.14		10.34	4.55			33.33
363	Propanoic acid, ethyl ester	Esters and analogues	8.14			31.82			
364	Propanoic acid, propyl ester	Esters and analogues	8.14		3.45	27.27			
365	Pyrazine, 2,6-dimethyl-	Aromatics	8.14	14.29	13.79	4.55	8.33		
366	sec-Butyl acetate	Esters and analogues	8.14		6.90	22.73			
367	Benzene	Aromatics	6.98		13.79	4.55			
368	1-Nonanol	Alcohols	6.98		10.34				33.33
369	1-Octanol, 2-butyl-	Alcohols	6.98		6.90	13.64			
370	1-Penten-3-one	Ketones	6.98		13.79	4.55			
371	1-Propanol, 3-(methylthio)-	Alcohols	6.98		20.69				
372	1,1,1,4,4,4-Hexafluorobut-2-ene	Halogen-containing	6.98		13.79	9.09			
373	1,3-Dioxolane, 2,2-dimethyl-	Ethers	6.98		17.24				
374	1,3-Pentadiene	Linear aliphatic	6.98		6.90	9.09			
375	1,4-Pentadiene	Linear aliphatic	6.98		13.79	4.55			
376	12-Crown-4	Ethers	6.98		17.24				
377	1H-Pyrrole-2,5-dione	Ketones	6.98		10.34	9.09			
378	1H-Pyrrole, 3-methyl-	Aromatics	6.98		10.34	9.09			
379	2-Butanone, 3-methyl-	Ketones	6.98		13.79				
380	2-Butenal	Aldehydes	6.98		13.79				
381	2-Cyclopenten-1-one, 3-methyl-	Ketones	6.98		13.79	4.55			33.33
382	2-Ethylacrolein	Aldehydes	6.98		10.34				33.33
383	2-Ethylhexyl acrylate	Esters and analogues	6.98		6.90				
384	2-Pentanone, 5-hydroxy-	Ketones	6.98		17.24	4.55			
385	2-Pentenal	Aldehydes	6.98	28.57	3.45	4.55			
386	2-Propanol, 1,1,1,3,3,3-hexafluoro-	Halogen-containing	6.98		6.90				33.33
387	2-Propenoic acid, methyl ester	Esters and analogues	6.98		3.45	4.55			
388	2-Undecanethiol, 2-methyl-	Alcohols	6.98	14.29	6.90	4.55			
389	2(5H)-Furanone, 5,5-dimethyl-	Esters and analogues	6.98		10.34	4.55			
390	3-Buten-2-ol, 2-methyl-	Alcohols	6.98		3.45	4.55			
391	3-Heptene	Linear aliphatic	6.98	28.57	6.90	9.09			
392	3-Methyl-2-(2-methyl-2-butenyl)-furan	Aromatics	6.98	14.29		22.73			
393	4-Aminostyrene	Aromatics	6.98		10.34	4.55			
394	4-Cyclopentene-1,3-dione	Ketones	6.98		6.90	4.55			33.33
395	4-Penten-2-one, 4-methyl-	Ketones	6.98			9.09			33.33
396	Acetic acid, hydroxy-, methyl ester	Esters and analogues	6.98		10.34	9.09			
397	Acetic acid, phenylmethyl ester	Esters and analogues	6.98		6.90	9.09			33.33
398	Amylene hydrate	Alcohols	6.98		17.24	4.55			
399	Benzene, (1-propyldecyl)-	Aromatics	6.98	14.29	6.90	4.55			
400	Benzene, 1-ethyl-4-(2-methylpropyl)-	Aromatics	6.98		10.34	9.09			
401	Benzene, 1-methyl-	Aromatics	6.98		6.90	9.09			
402	Benzene, 1-methyl-2-propyl-	Aromatics	6.98		17.24	4.55			
403	Benzene, 1-methyl-3-(1-methylethyl)-	Aromatics	6.98		6.90	9.09			33.33
404	Benzene, 1-methyl-4-(1-methylpropyl)-	Aromatics	6.98		13.79				33.33
405	Benzene, 1,2-dichloro-	Aromatics	6.98	28.57	10.34				
406	Benzene, 1,2,3,4-tetrafluoro-	Halogen-containing	6.98	14.29	6.90	9.09			
407	Benzene, 1,2,4-triethyl-	Aromatics	6.98		10.34	13.64			
408	Benzene, 2,4-diethyl-1-methyl-	Aromatics	6.98		3.45	9.09			33.33
409	Benzoic acid, methyl ester	Esters and analogues	6.98			9.09			33.33
410	Butane, 1,1,3,4-tetrachloro-1,2,2,3,4,4-hexafluoro-	Halogen-containing	6.98		13.79	9.09			

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411	Butanoic acid, 2-methyl-, methyl ester	Esters and analogues	6.98		10.34	13.64			
412	Butanoic acid, butyl ester	Esters and analogues	6.98		6.90	18.18			
413	Camphene	Aromatics	6.98	42.86	6.90				
414	Carbamic acid, methyl ester	Esters and analogues	6.98		3.45				
415	4-hydroxy2-butanone	Alcohols	6.98		13.79	4.55			
416	Cyclohexane, 1-ethyl-2-methyl-	Cyclic aliphatic	6.98		13.79			8.33	
417	Cyclohexane, 1,2,4-trimethyl-	Cyclic aliphatic	6.98		10.34	9.09		8.33	
418	Cyclopentane, 1-methyl-3-(2-methylpropyl)-	Cyclic aliphatic	6.98		10.34	4.55		8.33	33.33
419	Cyclopentene, 1-methyl-	Cyclic aliphatic	6.98	14.29	3.45	13.64			33.33
420	Cyclopentene, 3-methyl-	Cyclic aliphatic	6.98		3.45	4.55			33.33
421	Cyclopropane, ethyl-	Cyclic aliphatic	6.98		6.90	4.55			
422	Decane, 2,6,8-trimethyl-	Linear aliphatic	6.98		6.90	4.55		8.33	33.33
423	Diacetyl sulphide	Sulphur-containing	6.98		10.34	4.55		8.33	
424	Diethyl Phthalate	Esters and analogues	6.98		10.34	4.55			33.33
425	Dodecane	Linear aliphatic	6.98		6.90	4.55		8.33	
426	Ethane	Linear aliphatic	6.98		13.79	4.55			
427	Ethane, iodo-	Halogen-containing	6.98		10.34	9.09			33.33
428	Ethane, nitro-	Nitrogen-containing	6.98		10.34	4.55			
429	Ethanol, 2-(2-butoxyethoxy)-	Alcohols	6.98		3.45	13.64		8.33	33.33
430	Ethene, ethoxy-	Ethers	6.98		6.90	4.55			
431	Formamide, N-methyl-	Nitrogen-containing	6.98		10.34	4.55		8.33	
432	Formic acid, propyl ester	Esters and analogues	6.98		17.24	4.55			
433	Furan, 2-methyl-	Aromatics	6.98		17.24		8.33		
434	Heptacosan-9-ol	Alcohols	6.98		10.34	9.09			33.33
435	Hexane, tetradecafluoro-	Halogen-containing	6.98	28.57		9.09		8.33	33.33
436	Hexanoic acid, 1-methylethyl ester	Esters and analogues	6.98		10.34	13.64			
437	Hydrazinecarboxamide	Nitrogen-containing	6.98		10.34	4.55	8.33	8.33	
438	Isoamyl cyanide	Nitrogen-containing	6.98		3.45	13.64	8.33	8.33	
439	l-Leucine, n-butoxycarbonyl-N-methyl-, undecyl ester	Esters and analogues	6.98		13.79	4.55			
440	Methane, oxybis[dichloro-	Ethers	6.98		6.90	4.55		8.33	
441	Methanesulfonyl chloride	Halogen-containing	6.98		10.34	9.09	8.33		
442	Methyl isobutyrate	Esters and analogues	6.98		10.34	9.09		8.33	
443	Naphthalene, 1,2,3,4-tetrahydro-1,4,6-trimethyl-	Aromatics	6.98		10.34	4.55			
444	Naphthalene, 1,2,3,4-tetrahydro-2,5,8-trimethyl-	Aromatics	6.98		17.24	4.55			
445	Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-	Aromatics	6.98		13.79	9.09			
446	o-Xylene	Aromatics	6.98	14.29	6.90	13.64			
447	Octane, 2,2,6-trimethyl-	Linear aliphatic	6.98		6.90			8.33	33.33
448	Octanoic acid	Acids	6.98		6.90	18.18			
449	Oleyl alcohol, trifluoroacetate	Halogen-containing	6.98		13.79	4.55			33.33
450	Oxime-, methoxy-phenyl-_	Nitrogen-containing	6.98		10.34	4.55		8.33	33.33
451	p-Xylene	Aromatics	6.98		10.34	4.55			
452	Pentane, 2-methyl-	Linear aliphatic	6.98		17.24		8.33		
453	Pentane, 2,3-dimethyl-	Linear aliphatic	6.98	28.57	3.45	13.64			
454	Phenol	Alcohols	6.98	14.29	3.45	13.64		8.33	
455	phenol, 2-(1,1,3,3-tetramethylbutyl)-	Alcohols	6.98		17.24	4.55			
456	Phenol, 2-ethyl-	Alcohols	6.98		13.79		8.33	8.33	
457	Propanedinitrile, cyclohexyl(2-methylcyclohexyl)-	Nitrogen-containing	6.98		10.34	4.55	8.33	8.33	
458	Propanoic acid, butyl ester	Esters and analogues	6.98			27.27			
459	Propene	Linear aliphatic	6.98		3.45	18.18	8.33		
460	Pyrazine, ethenyl-	Aromatics	6.98		10.34	9.09	8.33		
461	Pyrazine, tetramethyl-	Aromatics	6.98	14.29	17.24				
462	Pyridine, 2,3,6-trimethyl-	Aromatics	6.98		10.34	9.09		8.33	
463	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	Aromatics	6.98		17.24				33.33
464	Tetrachloroethylene	Halogen-containing	6.98		13.79	4.55		8.33	
465	Thiophene, 2-methyl-	Aromatics	6.98		13.79	4.55	8.33		
466	Triethylene glycol	Alcohols	6.98		6.90	4.55	8.33		
467	Tuaminoheptane	Nitrogen-containing	6.98		20.69				
468	Urea	Nitrogen-containing	6.98		17.24	4.55			
469	β-Phellandrene	Cyclic aliphatic	6.98		13.79		8.33		
470	γ-Terpinene	Aromatics	6.98		6.90	4.55			

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471	(Methylthio)-acetonitrile	Nitrogen-containing	5.81		13.79	4.55			
472	1-Decanol, 2-hexyl-	Alcohols	5.81		3.45	18.18			
473	1-Hydroxy-2-butanone	Ketones	5.81		10.34	4.55			
474	1-Octanamine, N-methyl-	Nitrogen-containing	5.81		3.45	9.09			
475	1-Octanol	Alcohols	5.81		6.90				
476	1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	Aromatics	5.81		6.90				33.33
477	1,2-Dipropylcyclopropene	Cyclic aliphatic	5.81		10.34	4.55			33.33
478	1,2-Ethanediol, monoacetate	Esters and analogues	5.81		13.79				
479	1,2,3-Trifluorobenzene	Halogen-containing	5.81		13.79	4.55			
480	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1 methylethyl)-, [1S-(1α,2α,3aβ,4α,5α,7aβ,8S*)]-	Cyclic aliphatic	5.81		13.79	4.55			
481	1,2,4-Trithiolane	Sulphur-containing	5.81		10.34	9.09			
482	1,3-Cyclohexadiene, 1,3,5,5,6,6-hexamethyl-	Cyclic aliphatic	5.81	14.29	13.79				
483	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	Alcohols	5.81		17.24				
484	1(2H)-Naphthalenone, 3,4-dihydro-3,3,6,8-tetramethyl-	Ketones	5.81		6.90	4.55			
485	11,14-Eicosadienoic acid	Acids	5.81		3.45	13.64			
486	12-Methylaminolauric acid	Acids	5.81		10.34	9.09			
487	1H-Indene, 1-chloro-2,3-dihydro-	Aromatics	5.81		13.79	4.55			
488	1H-Indene, 2,3-dihydro-1,6-dimethyl-	Aromatics	5.81		10.34				33.33
489	2-Butenal, 2-ethenyl-	Aldehydes	5.81		6.90	4.55			
490	2-Cyclopenten-1-one	Ketones	5.81		10.34	4.55			
491	2-Hexenal	Aldehydes	5.81		6.90	9.09			
492	2-Isopropylpyrazine	Aromatics	5.81		10.34				
493	2-Pentanol, 3-methyl-	Alcohols	5.81		3.45	18.18			
494	2-Pentanol, 4-methyl-	Alcohols	5.81		6.90	9.09			33.33
495	2-Propanol, 1-(2-methoxypropoxy)-	Alcohols	5.81		6.90	4.55			
496	2-Propanone, 1-(acetyloxy)-	Ketones	5.81		10.34	4.55			
497	2-Propanone, 1,1,1-trifluoro-	Halogen-containing	5.81		6.90	9.09			33.33
498	2,3-Dimethyl-3-heptene	Linear aliphatic	5.81		3.45	4.55			
499	2,5-Difluorobenzaldehyde	Halogen-containing	5.81		6.90	13.64			
500	2,6-Di-tert-butyl-4-hydroxy-4 methylcyclohexa-2,5-dien-1-one	Ketones	5.81		13.79	4.55			
501	2,6-Dimethylbicyclo[3.2.1]octane	Cyclic aliphatic	5.81		10.34	4.55			
502	2(3H)-Furanone, 5-methyl-	Esters and analogues	5.81	14.29	6.90	9.09			
503	2H-1,2-Oxazine, 6-(4-chlorophenyl)tetrahydro-2-methyl-	Aromatics	5.81	14.29	3.45	4.55			
504	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-	Aromatics	5.81		17.24				
505	3-Buten-1-ol	Alcohols	5.81		13.79				
506	3-Heptanone	Ketones	5.81	14.29	6.90				33.33
507	3-Hexanone	Ketones	5.81		6.90	9.09			
508	3-Methylheptyl acetate	Esters and analogues	5.81		6.90	13.64			
509	3,5-Dimethylcyclopentene	Cyclic aliphatic	5.81		3.45	4.55			33.33
510	3,5-Dithiahexanol 5,5-dioxide	Alcohols	5.81	28.57	3.45				
511	3',4'-Difluoroacetophenone	Halogen-containing	5.81		10.34	4.55			
512	4(1H)-Pyrimidinone, 6-methyl-	Ketones	5.81		6.90				33.33
513	5-Ethyldecane	Linear aliphatic	5.81		6.90	9.09			
514	5-Undecene	Linear aliphatic	5.81			4.55			33.33
515	Acetic acid	Acids	5.81		13.79				33.33
516	Acetic acid ethenyl ester	Esters and analogues	5.81	28.57	6.90				
517	Acetyl valeryl	Linear aliphatic	5.81		13.79	4.55			
518	Aminomethanesulfonic acid	Acids	5.81		13.79	4.55			
519	Benzaldehyde, 2-methyl-	Aldehydes	5.81		6.90	4.55			
520	Benzene, (1-propylheptyl)-	Aromatics	5.81			9.09			
521	Benzene, 1-chloro-4-(trifluoromethyl)-	Aromatics	5.81	14.29	6.90	4.55			33.33
522	Benzene, 1-ethyl-4-methyl-	Aromatics	5.81	14.29	3.45	13.64			
523	Benzene, 1,2,4,5-tetrafluoro-3-(trifluoromethyl)-	Halogen-containing	5.81		10.34				
524	Benzene, 1,2,4,5-tetramethyl-	Aromatics	5.81		6.90	4.55			33.33
525	Benzene, 1,3-dimethoxy-	Ethers	5.81		17.24				
526	Benzene, tert-butyl-	Aromatics	5.81		3.45				
527	Benzenemethanol, α,α-dimethyl-	Alcohols	5.81		6.90				
528	Benzestrol	Alcohols	5.81		13.79	4.55			
529	Benzoic acid, 2-methyl-, anhydride	Esters and analogues	5.81		6.90	4.55			
530	Benzyl methyl sulfide	Sulphur-containing	5.81		13.79				

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531	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	Cyclic aliphatic	5.81		6.90	9.09			
532	Butanal, 2-methyl-	Aldehydes	5.81		6.90	4.55			
533	Butane, 2-methyl-	Linear aliphatic	5.81		13.79				
534	Butanoic acid, 1-methylpropyl ester	Esters and analogues	5.81		6.90	13.64			
535	Butyl 2-methylbutanoate	Esters and analogues	5.81			22.73			
536	Carbonic acid, tridecyl vinyl ester	Esters and analogues	5.81	14.29	6.90	4.55			
537	Cyclodecane	Cyclic aliphatic	5.81		6.90	4.55			
538	Cyclohexane, (1-methylpropyl)-	Cyclic aliphatic	5.81		10.34	4.55			
539	Cyclohexane, 3-ethyl-5-methyl-1-propyl-	Cyclic aliphatic	5.81		6.90	4.55			
540	Cyclohexanol	Alcohols	5.81						
541	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	Esters and analogues	5.81		3.45	13.64			33.33
542	Cyclohexanone, 4-ethyl-	Ketones	5.81		13.79	4.55			
543	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	Ketones	5.81			9.09			33.33
544	Cyclohexene, 1,5,5-trimethyl-3-methylene-	Cyclic aliphatic	5.81			9.09			
545	Cyclopentane, 1,2,4-trimethyl-	Cyclic aliphatic	5.81		6.90	13.64			
546	Cyclopentane, hexyl-	Cyclic aliphatic	5.81		3.45	4.55			33.33
547	Cyclopropane, 1,2-dimethyl-, cis-	Cyclic aliphatic	5.81		10.34	9.09			
548	Decane	Linear aliphatic	5.81		10.34	9.09			
549	Decane, 2,5-dimethyl-	Linear aliphatic	5.81	14.29	10.34				
550	Diethyl azodicarboxylate	Nitrogen-containing	5.81	28.57	6.90	4.55			
551	Erythro-3-bromo-2-pentanol	Alcohols	5.81		10.34	4.55			
552	Ethane, (methylthio)-	Sulphur-containing	5.81		6.90	13.64			
553	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	Halogen-containing	5.81		10.34	4.55			
554	Ethanol, 2-butoxy-	Alcohols	5.81	14.29		9.09			33.33
555	Ethanol, 2,2'-oxybis-, dinitrate	Alcohols	5.81		13.79				
556	Ethanone, 1-(2-furanyl)-	Ketones	5.81		10.34				33.33
557	Furan, 2-propyl-	Aromatics	5.81		10.34	4.55			
558	Furan, 3-methyl-	Aromatics	5.81		10.34	9.09			
559	Furfural	Aromatics	5.81		13.79				
560	Heptane	Linear aliphatic	5.81	14.29	6.90				33.33
561	Heptane, 2,5-dimethyl-	Linear aliphatic	5.81		10.34				
562	Heptane, 2,6-dimethyl-	Linear aliphatic	5.81		3.45	4.55			
563	Heptane, 3-ethyl-2-methyl-	Linear aliphatic	5.81		6.90				
564	Heptane, hexadecafluoro-	Halogen-containing	5.81	14.29		13.64			33.33
565	Heptanoic acid	Acids	5.81		3.45	13.64			
566	Hexane, 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-	Halogen-containing	5.81		6.90	9.09			33.33
567	Hexane, 2,2,5-trimethyl-	Linear aliphatic	5.81		13.79				
568	Hexane, 2,5-dimethyl-	Linear aliphatic	5.81		3.45				
569	Hexane, 3,3-dimethyl-	Linear aliphatic	5.81		6.90	9.09			
570	Hexane, 3,4-bis(1,1-dimethylethyl)-2,2,5,5-tetramethyl-	Linear aliphatic	5.81		6.90	9.09			
571	Hexanoic acid, 2-ethyl-	Acids	5.81		10.34	9.09			
572	Hydrogen azide	Nitrogen-containing	5.81		10.34				
573	l-Alanine ethylamide	Nitrogen-containing	5.81		13.79				
574	Longifolene	Aromatics	5.81		10.34				
575	Manganese, acetylpentacarbonyl-, (OC-6-21)-	Ketones	5.81		6.90				
576	Methanethiol	Sulphur-containing	5.81		13.79				
577	Methyl 7,11,14-eicosatrienoate	Esters and analogues	5.81		6.90	13.64			
578	Methyl formate	Esters and analogues	5.81		3.45	13.64			
579	Methyl nitrite	Nitrogen-containing	5.81		6.90				33.33
580	Methyl salicylate	Esters and analogues	5.81		10.34	4.55			
581	Methyl valerate	Esters and analogues	5.81		3.45	18.18			
582	Naphthalene, 1,2,3,4-tetrahydro-2,7-dimethyl-	Aromatics	5.81		17.24				
583	Naphthalene, 1,6,7-trimethyl-	Aromatics	5.81		6.90	4.55			66.67
584	Naphthalene, 2-methyl-	Aromatics	5.81		13.79				
585	Naphthalene, decahydro-, trans-	Aromatics	5.81		10.34	4.55			33.33
586	Nitric oxide	Nitrogen-containing	5.81	14.29	13.79				
587	Octane, 2-methyl-	Linear aliphatic	5.81	14.29					
588	Octane, 4-methyl-	Linear aliphatic	5.81		10.34	4.55			
589	Oxalic acid, diallyl ester	Esters and analogues	5.81	14.29	6.90	9.09			
590	Oxetane, 3,3-dimethyl-	Ethers	5.81		13.79	4.55			

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591	Pentane, 2,2,4-trimethyl-	Linear aliphatic	5.81	14.29	3.45	4.55			
592	Phenol, 3-(1-methylethyl)-	Alcohols	5.81		13.79	4.55			
593	Phenylephrine	Aromatics	5.81		10.34	4.55			33.33
594	Propane, 2-iodo-	Halogen-containing	5.81	28.57	3.45	9.09			
595	Propanoic acid, 2-methyl-, 2-methylpropyl ester	Esters and analogues	5.81			22.73			
596	Propanoic acid, 2-methylpropyl ester	Esters and analogues	5.81			22.73			
597	Pyridine, 3-methyl-	Aromatics	5.81		6.90	13.64			
598	Pyridine, 5-ethenyl-2-methyl-	Aromatics	5.81		10.34	4.55			
599	Thiophene, 3-methyl-	Aromatics	5.81		6.90	9.09			
600	Thiourea, tetramethyl-	Nitrogen-containing	5.81		10.34				33.33
601	trans-Crotonyl alcohol	Alcohols	5.81		10.34	4.55			
602	Triethylamine	Nitrogen-containing	5.81		3.45				33.33
603	Undecane, 2-methyl-	Linear aliphatic	5.81		3.45				33.33
604	Undecane, 2,2-dimethyl-	Linear aliphatic	5.81		10.34	4.55			
605	α-Terpineol	Alcohols	5.81		10.34				
606	1-Butanol	Alcohols	4.65		10.34				
607	1-Decanol, 2-ethyl-	Alcohols	4.65		3.45	4.55			
608	1-Dodecanol, 3,7,11-trimethyl-	Alcohols	4.65		10.34	4.55			
609	1-Hexanol	Alcohols	4.65		6.90	4.55			
610	1-Hexene	Linear aliphatic	4.65		3.45	9.09			
611	1-Octen-3-one	Ketones	4.65		6.90	4.55			
612	1-Penten-3-one, 4-methyl-	Ketones	4.65		3.45	4.55			
613	1-Pentene, 2,4-dimethyl-	Linear aliphatic	4.65		6.90	9.09			
614	1-Pentene, 2,4,4-trimethyl-	Linear aliphatic	4.65		10.34				
615	1,1'-Biphenyl, 2,2'-diethyl-	Aromatics	4.65		6.90				33.33
616	1,1'-Biphenyl, 4-methyl-	Aromatics	4.65		3.45				33.33
617	1,1'-Biphenyl, 4,4'-difluoro-	Halogen-containing	4.65		6.90				33.33
618	1,2-Ethanediamine, N'-ethyl-N,N-dimethyl-	Nitrogen-containing	4.65		10.34				
619	1,2,4,4-Tetramethylcyclopentene	Cyclic aliphatic	4.65			4.55			
620	1,3-Cyclohexadiene	Cyclic aliphatic	4.65		3.45				
621	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	Cyclic aliphatic	4.65		13.79				
622	1,3-Dioxolan-2-one	Ketones	4.65		13.79				
623	1,3-Dioxolane, 2-ethyl-4-methyl-	Ethers	4.65			4.55			
624	1,3-Oxathiane	Sulphur-containing	4.65		13.79				
625	1,3,6-Trioxocane	Ethers	4.65		6.90	9.09			
626	1,3,8-p-Menthatriene	Cyclic aliphatic	4.65	14.29	3.45	9.09			
627	1,4-bis[2-(4-Methylphenyl)diazen-1-yl]piperazine	Nitrogen-containing	4.65		3.45	9.09			
628	1,5-Hexadiene, 3,3,4,4-tetrafluoro-	Halogen-containing	4.65		6.90				33.33
629	1H-Indene, 1-ethylidene-	Aromatics	4.65		3.45				
630	1H-Indene, 2,3-dihydro-1,5,7-trimethyl-	Aromatics	4.65		10.34	4.55			
631	1H-Indene, octahydro-	Aromatics	4.65		13.79				
632	1H-Indene, octahydro-, cis-	Aromatics	4.65		10.34				
633	1H-Pyrazolo[3,4-d]pyrimidin-4-amine	Nitrogen-containing	4.65			13.64			
634	2-Butanone	Ketones	4.65		10.34				33.33
635	2-Butene, 2-methyl-	Linear aliphatic	4.65		3.45	13.64			
636	2-Cyclohexen-1-one	Ketones	4.65		6.90	9.09			
637	2-Ethyl-1-dodecanol	Alcohols	4.65		6.90	4.55			
638	2-Hexanone, 5-methyl-	Ketones	4.65		3.45	9.09			
639	2-Hexene, 3,5-dimethyl-	Linear aliphatic	4.65		6.90				
640	2-Pentanol, acetate	Esters and analogues	4.65		10.34				
641	2-Propanol, 1-butoxy-	Alcohols	4.65		3.45	4.55			33.33
642	2-Undecanone	Ketones	4.65		6.90	4.55			
643	2,2,4,4-Tetramethyloctane	Linear aliphatic	4.65		3.45	4.55			
644	2,2'-Difluorobiphenyl	Halogen-containing	4.65	14.29	3.45	4.55			
645	2,3-Pentanedione	Ketones	4.65		6.90	4.55			
646	2,3,5-Trimethyl-6-ethylpyrazine	Aromatics	4.65		13.79				
647	2,4-Difluorophenol	Halogen-containing	4.65		10.34	4.55			
648	2,4,5-Trifluorobenzyl alcohol, 1-methylpropyl ether	Halogen-containing	4.65		3.45	9.09			
649	2,5-Hexanedione	Ketones	4.65		6.90	9.09			
650	3-Decene, 2,2-dimethyl-	Linear aliphatic	4.65		6.90	4.55			33.33

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651	3-Heptene, 2-methyl-	Linear aliphatic	4.65		6.90	9.09			
652	3-Hexen-2-one	Ketones	4.65		3.45	4.55			33.33
653	3-Hydroxy-3-methyl-2-butanone	Ketones	4.65		10.34				
654	3-Methyl-1,2-diazirine	Nitrogen-containing	4.65			18.18			
655	3-Methylcyclopentyl acetate	Esters and analogues	4.65		6.90	4.55			
656	4-Methyl-2-tert-octylphenol	Alcohols	4.65		10.34	4.55			
657	7-Heptadecyne, 17-chloro-	Halogen-containing	4.65		3.45				
658	Acenaphthene	Aromatics	4.65		10.34				33.33
659	Acetic acid, hydrazide	Nitrogen-containing	4.65		3.45	13.64			
660	Benzene, (1-pentyloctyl)-	Aromatics	4.65		3.45	4.55			33.33
661	Benzene, [2,2,2-trifluoro-1-(trifluoromethyl)ethyl]-	Halogen-containing	4.65		6.90				
662	Benzene, 1-methoxy-4-methyl-	Ethers	4.65		3.45	9.09			
663	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-	Aromatics	4.65		10.34	4.55			
664	Benzene, 1,3-diethyl-5-methyl-	Aromatics	4.65		3.45	9.09			33.33
665	Benzene, 1,3-dimethyl-5-(1-methylethyl)-	Aromatics	4.65			9.09			
666	Benzene, fluoro-	Halogen-containing	4.65		10.34	4.55			
667	Benzene, heptyl-	Aromatics	4.65		6.90	4.55			33.33
668	Benzene, n-butyl-	Aromatics	4.65	28.57	3.45				
669	Benzeneacetaldehyde	Aldehydes	4.65		10.34				
670	Benzeneacetonitrile, α-oxo-	Nitrogen-containing	4.65		3.45	9.09			
671	Benzeneethanol, β-ethenyl-α-phenyl-	Alcohols	4.65		10.34	4.55			
672	Benzothiazole	Aromatics	4.65		13.79				
673	Benzoyl fluoride	Halogen-containing	4.65		13.79				
674	Bicyclo[2.2.1]heptane, 2-ethyl-	Cyclic aliphatic	4.65		3.45	4.55			
675	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	Cyclic aliphatic	4.65		3.45	4.55			
676	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Cyclic aliphatic	4.65	14.29	6.90				33.33
677	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	Ketones	4.65	28.57	3.45	4.55			
678	Borane carbonyl	Linear aliphatic	4.65		10.34	4.55			
679	Butanal	Aldehydes	4.65		6.90				
680	Butanal, 3-methyl-	Aldehydes	4.65		3.45	4.55			
681	Butane	Linear aliphatic	4.65		3.45	4.55			33.33
682	Butanoic acid, 3-methyl-, 3-methylbutyl ester	Esters and analogues	4.65		13.79				
683	Carbon disulfide	Sulphur-containing	4.65		3.45	9.09			
684	Carbonic acid, dimethyl ester	Esters and analogues	4.65		6.90	4.55			
685	Cyclobutane, (1-methylethylidene)-	Cyclic aliphatic	4.65		3.45	4.55			33.33
686	Cyclohexane, ethyl-	Cyclic aliphatic	4.65	14.29	6.90				33.33
687	Cyclohexane, methyl-	Cyclic aliphatic	4.65		3.45	4.55			
688	Cyclohexene	Cyclic aliphatic	4.65	14.29	6.90				
689	Cyclohexene, 3-methyl-	Cyclic aliphatic	4.65		6.90				
690	Cyclohexene, 3,5,5-trimethyl-	Cyclic aliphatic	4.65	14.29	3.45				
691	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	Cyclic aliphatic	4.65		3.45	9.09			
692	Cyclohexene, 4-methyl-	Cyclic aliphatic	4.65		6.90	9.09			
693	Cyclooctane, methyl-	Cyclic aliphatic	4.65	14.29	6.90	4.55			
694	Cyclopentane, 1-ethyl-2-methyl-, cis-	Cyclic aliphatic	4.65		10.34				33.33
695	Cyclopropane, 1,1-dimethyl-	Cyclic aliphatic	4.65		3.45				
696	Cyclotridecane	Cyclic aliphatic	4.65		6.90	4.55			33.33
697	Decane, 3,6-dimethyl-	Linear aliphatic	4.65		10.34	4.55			
698	Decane, 3,8-dimethyl-	Linear aliphatic	4.65		13.79				
699	Disulfide, methyl propyl	Sulphur-containing	4.65		6.90	9.09			
700	dl-Alanyl-l-alanine	Nitrogen-containing	4.65		6.90	9.09			
701	Dodecane, 1-iodo-	Halogen-containing	4.65		6.90				
702	Eicosyl heptyl ether	Ethers	4.65		6.90	4.55			
703	Ethane, 1,2-dichloro-	Halogen-containing	4.65	14.29	3.45	4.55			
704	Ethane, 1,2-dimethoxy-	Ethers	4.65		6.90	4.55			
705	Ethanol, 2-ethoxy-	Alcohols	4.65		13.79				
706	Ethanone, 1-(4-fluorophenyl)-	Halogen-containing	4.65		6.90	4.55			
707	Ethene, iodo-	Halogen-containing	4.65		10.34	4.55			
708	Ether, 1-butylvinyl methyl	Ethers	4.65		3.45	4.55			
709	Fenchone	Ketones	4.65		13.79				
710	Furan	Aromatics	4.65		3.45	4.55			

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711	Furan, 2,3-dihydro-	Aromatics	4.65		6.90				
712	Heptadecane	Linear aliphatic	4.65		3.45	4.55			
713	Heptane, 1-chloro-	Halogen-containing	4.65		3.45	9.09			
714	Heptane, 3-methyl-	Linear aliphatic	4.65		6.90	4.55			
715	Hexacosane	Linear aliphatic	4.65		10.34				
716	Hexane, 2-methyl-	Linear aliphatic	4.65		10.34	4.55			
717	Hexane, 2,4-dimethyl-	Linear aliphatic	4.65		10.34				
718	Mesitylene	Aromatics	4.65		6.90				33.33
719	Methane, bromodichloro-	Halogen-containing	4.65		6.90				
720	Methanesulfonic anhydride	Sulphur-containing	4.65		10.34	4.55			
721	n-Hexane	Linear aliphatic	4.65		6.90	4.55			33.33
722	N,N-Dimethylacetamide	Nitrogen-containing	4.65		10.34	4.55			
723	N,N,O-Triacetylhydroxylamine	Nitrogen-containing	4.65		3.45	9.09			
724	Naphthalene, 1-ethyl-	Aromatics	4.65		3.45				
725	Naphthalene, 1,2,3,4-tetrahydro-2-methyl-	Aromatics	4.65		10.34				33.33
726	Naphthalene, 1,2,3,4-tetrahydro-5-methyl-	Aromatics	4.65						33.33
727	Naphthalene, 1,2,3,4-tetrahydro-6-methyl-	Aromatics	4.65		6.90	4.55			33.33
728	Naphthalene, 1,4-dimethyl-	Aromatics	4.65		3.45	4.55			33.33
729	Nonane	Linear aliphatic	4.65		3.45	4.55			33.33
730	Nonanoic acid	Acids	4.65	14.29		9.09			
731	o-Toluidine	Aromatics	4.65		13.79				
732	Octane, 2,3,7-trimethyl-	Linear aliphatic	4.65		6.90				
733	ortho tert-Butyl cyclohexyl acetate	Esters and analogues	4.65		6.90	9.09			
734	Pentanal	Aldehydes	4.65		10.34	4.55			
735	Pentane, 1-(methylthio)-	Sulphur-containing	4.65		13.79				
736	Pentanoic acid, ethyl ester	Esters and analogues	4.65			18.18			
737	Phenol, 2-(1-methylethyl)-	Alcohols	4.65		6.90				
738	Phenol, 3-fluoro-	Halogen-containing	4.65		6.90	4.55			
739	Phenol, 4-propyl-	Alcohols	4.65		10.34				
740	Propane, 2,2-difluoro-	Halogen-containing	4.65		6.90	9.09			
741	Propanoic acid, 2-methyl-, butyl ester	Esters and analogues	4.65			18.18			
742	Propanoic acid, anhydride	Esters and analogues	4.65		10.34	4.55			
743	Pyrazine, 2-ethyl-3,5-dimethyl-	Aromatics	4.65		10.34				
744	Pyrazine, 2-ethyl-6-methyl-	Aromatics	4.65		3.45				
745	Pyrazine, ethyl-	Aromatics	4.65		13.79				
746	R-(-)-1,2-propanediol	Alcohols	4.65		6.90	4.55			
747	S-Methyl 3-methylbutanethioate	Esters and analogues	4.65		13.79				
748	Stearic acid hydrazide	Nitrogen-containing	4.65	14.29	6.90	4.55			
749	Styrene	Aromatics	4.65		10.34	4.55			
750	Sulfur dioxide	Sulphur-containing	4.65	14.29	3.45	4.55			
751	Tetracosane	Linear aliphatic	4.65	14.29	3.45	9.09			
752	Tetrahydropyran	Ethers	4.65	14.29	6.90	4.55			
753	Thiophene	Aromatics	4.65		6.90	4.55			33.33
754	Thiophene, 3-ethyl-	Aromatics	4.65			9.09			
755	trans-β-Ionone	Ketones	4.65			9.09			
756	Undecane, 2,5-dimethyl-	Linear aliphatic	4.65		6.90	4.55			33.33
757	Undecane, 4,6-dimethyl-	Linear aliphatic	4.65		3.45	4.55			
758	Undecane, 4,7-dimethyl-	Linear aliphatic	4.65		6.90	4.55			
759	α-Methylstyrene	Aromatics	4.65		10.34	4.55			
760	α-Pinene	Aromatics	4.65	42.86	3.45				
761	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	Alcohols	3.49		3.45				
762	(Z)-2-Heptene	Linear aliphatic	3.49		6.90	4.55			
763	1-Butanol, 3-methyl-, propanoate	Esters and analogues	3.49		10.34				
764	1-Butene	Linear aliphatic	3.49		3.45	4.55			
765	1-Dodecanol	Alcohols	3.49		6.90	4.55			
766	1-Fluoro-3-(trifluoro-methyl)benzene	Halogen-containing	3.49		10.34				
767	1-Heptene	Linear aliphatic	3.49			4.55			
768	1-Heptene, 2-methyl-	Linear aliphatic	3.49		10.34				
769	1-Hexanol, 2-ethyl-	Alcohols	3.49		3.45	4.55			
770	1-Hexanol, 3-methyl-	Alcohols	3.49		10.34				

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771	1-Methyl-2-n-hexylbenzene	Aromatics	3.49		6.90				33.33
772	1-Octene, 3,7-dimethyl-	Linear aliphatic	3.49	14.29	3.45	4.55			
773	1-Pentanol	Alcohols	3.49		10.34				
774	1-Pentanol, 2-ethyl-4-methyl-	Alcohols	3.49	14.29	3.45				33.33
775	1-Propen-2-ol, formate	Esters and analogues	3.49		3.45	4.55			
776	1-Propylcyclopentene	Cyclic aliphatic	3.49			4.55			33.33
777	1,1-Dichloro-1-fluoroethane	Halogen-containing	3.49		3.45				
778	1,1'-Biphenyl, 3-methyl-	Aromatics	3.49		6.90	4.55			
779	1,2-Propanediol, 1-acetate	Esters and analogues	3.49		3.45	9.09			
780	1,2-Propanedione, 1-phenyl-	Ketones	3.49						
781	1,2,4-Trifluorobenzene	Halogen-containing	3.49		3.45				
782	1,2,4,5-Tetrazin-3-amine, 6-methyl-	Nitrogen-containing	3.49		6.90	4.55			
783	1,3-Dioxolane, 2-methyl-	Ethers	3.49		6.90				
784	1,3,2-Dioxaborolan-4-one, 2-ethyl-	Ketones	3.49		6.90				
785	1,4-Butanediol	Alcohols	3.49		6.90				
786	1,6-Octadien-3-ol, 3,7-dimethyl-, formate	Esters and analogues	3.49		6.90	4.55			
787	11-Tricosene	Linear aliphatic	3.49		3.45	4.55			
788	1H-Indene, 2,3-dihydro-1,2-dimethyl-	Aromatics	3.49		3.45	9.09			
789	1H-Indene, 2,3-dihydro-4-methyl-	Aromatics	3.49	14.29	3.45	4.55			
790	2-(Pentadec-14-en-1-yl)furan	Aromatics	3.49		0.00				
791	2-Amino-1,3-propanediol	Alcohols	3.49		6.90				
792	2-Butanone, 4-(dimethylamino)-3-methyl-	Ketones	3.49						33.33
793	2-Butene, 1-methoxy-	Esters and analogues	3.49		3.45				
794	2-Butene, 2,3-dimethyl-	Linear aliphatic	3.49		6.90				33.33
795	2-Fluoro-3-(trifluoromethyl)phenol	Halogen-containing	3.49		3.45	4.55			33.33
796	2-Heptanone	Ketones	3.49		6.90	4.55			
797	2-Heptanone, 7,7,7-trichloro-	Ketones	3.49			4.55			
798	2-Heptene	Linear aliphatic	3.49	14.29	3.45				
799	2-Methyl-3-isopropylpyrazine	Aromatics	3.49						
800	2-n-Heptylfuran	Aromatics	3.49			9.09			33.33
801	2-Octenal	Aldehydes	3.49		6.90	4.55			
802	2-Oxetanone, 4-methyl-	Ketones	3.49		10.34				
803	2-Pentanone, 4-hydroxy-	Ketones	3.49		3.45	9.09			
804	2-Pentanone, 4-hydroxy-4-methyl-	Ketones	3.49		3.45				
805	2-Pentene, 4-methyl-	Linear aliphatic	3.49			9.09			33.33
806	2-Propanol, 1-(2-butoxy-1-methylethoxy)-	Alcohols	3.49		3.45	4.55			
807	2-Propanol, 1-propoxy-	Alcohols	3.49		6.90				33.33
808	2-Propanol, 1,3-dichloro-	Alcohols	3.49		3.45	4.55			
809	2-Propenal	Aldehydes	3.49		6.90				
810	2-Propenenitrile	Nitrogen-containing	3.49		3.45	4.55			
811	2-Tetradecanone	Ketones	3.49		3.45	4.55			
812	2,2-Dimethylindene, 2,3-dihydro-	Aromatics	3.49		3.45				
813	2,2,3,3-Tetrafluoro-1-propanol	Halogen-containing	3.49		3.45	4.55			
814	2,2'-Dimethylbiphenyl	Aromatics	3.49		6.90				33.33
815	2,3-Butanediol	Alcohols	3.49		3.45	9.09			
816	2,4-Di-tert-butylphenol	Alcohols	3.49		6.90	4.55			
817	2,4-Difluorotoluene	Halogen-containing	3.49		6.90	4.55			
818	2,4-Dimethyl-1-heptene	Linear aliphatic	3.49		6.90	4.55			
819	2,5-Hexanediol, 2,5-dimethyl-	Alcohols	3.49		10.34				
820	2(3H)-Furanone, 5-butyldihydro-	Esters and analogues	3.49			9.09			33.33
821	2(3H)-Furanone, 5-ethyldihydro-	Esters and analogues	3.49		3.45	4.55			
822	2(3H)-Furanone, 5-ethyldihydro-5-methyl-	Esters and analogues	3.49			13.64			
823	2(3H)-Furanone, dihydro-5-methyl-	Esters and analogues	3.49		3.45	4.55			
824	3-(4-Isopropylphenyl)-2-methylpropionaldehyde	Aldehydes	3.49			4.55			
825	3-(Methylthio)-2-butanone	Ketones	3.49		10.34				
826	3-Aminopropionitrile	Nitrogen-containing	3.49	14.29	3.45	4.55			
827	3-Buten-2-one, 3-methyl-	Ketones	3.49		3.45				
828	3-Ethylcyclopentanone	Ketones	3.49		10.34				
829	3-Heptene, 4-ethyl-	Linear aliphatic	3.49		3.45				66.67
830	3-Heptene, 4-methyl-	Linear aliphatic	3.49		3.45	9.09			

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage CDD training aid samples in which VOC was detected	Percentage CDD outdoor stored foot training aid samples in which VOC was detected	Percentage CDD indoor stored foot training aid samples in which VOC was detected	Percentage CDD bone training aid samples in which VOC was detected	Percentage CDD tissue training aid samples in which VOC was detected	Percentage CDD blood training aid samples in which VOC was detected	Percentage CDD teeth training aid samples in which VOC was detected
831	3-Hexanone, 2-methyl-	Ketones	3.49		10.34				
832	3-Hexanone, 2,5-dimethyl-4-nitro-	Ketones	3.49		3.45	4.55			
833	3-Nonene	Linear aliphatic	3.49		3.45	4.55			
834	4-Cyanocyclohexene	Nitrogen-containing	3.49		6.90				
835	4-Ethylbenzoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	Esters and analogues	3.49		6.90				33.33
836	4-Octene	Linear aliphatic	3.49		6.90				
837	4,7-Methano-1H-inden-6-ol, 3a,4,5,6,7,7a-hexahydro-, acetate	Esters and analogues	3.49		3.45				
838	6-Oxa-bicyclo[3.1.0]hexan-3-one	Ketones	3.49		3.45	4.55			
839	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	Esters and analogues	3.49						33.33
840	7-Tetradecene	Linear aliphatic	3.49		3.45	4.55			
841	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Ketones	3.49		6.90				
842	Acetaldehyde, hydroxy-	Aldehydes	3.49						33.33
843	Acetic acid, anhydride with formic acid	Esters and analogues	3.49		6.90	4.55			
844	Acetone cyanohydrin	Nitrogen-containing	3.49		6.90				
845	Allantoic acid	Acids	3.49		6.90	4.55			
846	Benzaldehyde, 2,4-dimethyl-	Aldehydes	3.49		6.90	4.55			
847	Benzene, (1-butylnonyl)-	Aromatics	3.49		6.90				
848	Benzene, (1-ethyloctyl)-	Aromatics	3.49		3.45	9.09			
849	Benzene, (1-methylethyl)-	Aromatics	3.49		6.90	4.55			
850	Benzene, 1-chloro-3-(trifluoromethyl)-	Aromatics	3.49			4.55			
851	Benzene, 1-ethyl-2-methyl-	Aromatics	3.49		6.90	4.55			
852	Benzene, 1-methoxy-2-methyl-	Ethers	3.49		6.90				
853	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-	Aromatics	3.49	28.57					33.33
854	Benzene, 1,2,3-trimethyl-	Aromatics	3.49		3.45	4.55			
855	Benzene, 1,3-bis(1,1-dimethylethyl)-	Aromatics	3.49		10.34				
856	Benzene, 1,3-dichloro-	Aromatics	3.49		6.90				
857	Benzene, 1,3-dimethyl-	Aromatics	3.49		6.90				
858	Benzene, 2-ethyl-1,3-dimethyl-	Aromatics	3.49	14.29		4.55			33.33
859	Benzene, chloro-	Aromatics	3.49	14.29	3.45				
860	Benzene, pentafluoro-	Halogen-containing	3.49		6.90	4.55			
861	Benzenemethanol, 2-methyl-, acetate	Esters and analogues	3.49		3.45	9.09			
862	Benzonitrile, 2-methyl-	Nitrogen-containing	3.49		6.90	4.55			
863	Benzyl alcohol	Alcohols	3.49	14.29	6.90				
864	Benzyl methyl ketone	Ketones	3.49		10.34				
865	Bicyclo[2.1.0]pentane, 1,4-dimethyl-	Cyclic aliphatic	3.49		3.45	4.55			
866	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-	Cyclic aliphatic	3.49		6.90				
867	Biphenyl	Aromatics	3.49		6.90	4.55			
868	Butane, 1-methoxy-	Ethers	3.49			13.64			
869	Butanoic acid, 2-methyl-, 1-methylpropyl ester	Esters and analogues	3.49		3.45	9.09			
870	Butanoic acid, 2-methyl-, 3-methylbutyl ester	Esters and analogues	3.49		10.34				
871	Butanoic acid, 3-methyl-, butyl ester	Esters and analogues	3.49			13.64			
872	Butanoic acid, 3-methyl-, propyl ester	Esters and analogues	3.49		3.45	9.09			
873	Butyrolactone	Esters and analogues	3.49		3.45	4.55			33.33
874	1,3-Hexadiene	Linear aliphatic	3.49	14.29	3.45				
875	Cedrol	Alcohols	3.49		3.45				
876	cis-Thujopsene	Cyclic aliphatic	3.49		3.45	9.09			
877	cis,cis-1,6-Dimethylspiro[4.5]decane	Cyclic aliphatic	3.49		10.34				
878	Cyclohexane, 1,3,5-trimethyl-	Cyclic aliphatic	3.49		10.34				
879	Cyclohexane, hexyl-	Cyclic aliphatic	3.49		3.45	4.55			
880	Cyclohexane, isocyanato-	Nitrogen-containing	3.49		6.90	4.55			
881	Cyclohexane, isothiocyanato-	Nitrogen-containing	3.49		3.45				33.33
882	Cyclohexanepropanol-	Alcohols	3.49			9.09			
883	Cyclohexene, 1-butyl-	Cyclic aliphatic	3.49		10.34				
884	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	Ethers	3.49		6.90				33.33
885	Cyclopentane, 1-methyl-3-(1-methylethyl)-	Cyclic aliphatic	3.49		3.45				
886	Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester	Esters and analogues	3.49		3.45	9.09			
887	Cyclopentanone	Ketones	3.49		3.45	4.55			
888	Cyclopropane, 1,1-diethyl-	Cyclic aliphatic	3.49		6.90				
889	Decanal	Aldehydes	3.49		10.34				
890	Decane, 2-methyl-	Linear aliphatic	3.49			4.55			33.33

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891	Diazene, dimethyl-	Nitrogen-containing	3.49		3.45				33.33
892	Diethyl carbonate	Esters and analogues	3.49			9.09			
893	Diethyl fumarate	Esters and analogues	3.49		6.90	4.55			
894	Dimethylamine	Nitrogen-containing	3.49		3.45				33.33
895	Dodecane, 2-methyl-	Linear aliphatic	3.49		3.45				
896	Dodecane, 2,6,10-trimethyl-	Linear aliphatic	3.49		3.45	9.09			
897	Dodecane, 4,6-dimethyl-	Linear aliphatic	3.49		10.34				
898	Eicosane	Linear aliphatic	3.49	14.29	3.45	4.55			
899	Erythro-2-methyl-3,4-dibromo-2-butanol	Alcohols	3.49		6.90	4.55			
900	Ethane, pentafluoro-	Halogen-containing	3.49		6.90				33.33
901	Ethanedioic acid, dimethyl ester	Esters and analogues	3.49		6.90	4.55			
902	Ethanol	Alcohols	3.49		3.45				
903	Ethanol, 2,2-dichloro-	Alcohols	3.49		3.45	4.55			
904	Ethanone, 1-(2,4-difluorophenyl)-	Halogen-containing	3.49						33.33
905	Ethylbenzene	Aromatics	3.49		6.90	4.55			
906	Ethyllinalool	Alcohols	3.49			4.55			
907	Formic acid, 2-methylpropyl ester	Esters and analogues	3.49		10.34				
908	Furan, 2-hexyl-	Aromatics	3.49		6.90	4.55			
909	Glycerin	Alcohols	3.49		6.90				
910	Glycolaldehyde dimethyl acetal	Aldehydes	3.49		6.90	4.55			
911	Heptacosane	Linear aliphatic	3.49						
912	Heptanal	Aldehydes	3.49		10.34				
913	Heptane, 2,2,4-trimethyl-	Linear aliphatic	3.49		6.90	4.55			
914	Heptane, 2,2,4,6,6-pentamethyl-	Linear aliphatic	3.49		3.45				
915	Heptane, 4-methyl-	Linear aliphatic	3.49		6.90	4.55			
916	Hexadecane	Linear aliphatic	3.49		6.90				
917	Hexanal	Aldehydes	3.49		3.45	4.55			33.33
918	Hexanal, 2-ethyl-	Aldehydes	3.49		6.90	4.55			
919	Hexane, 3,4-dimethyl-	Linear aliphatic	3.49			4.55			
920	Indene	Aromatics	3.49		3.45	4.55			33.33
921	Isobutane	Linear aliphatic	3.49		6.90				33.33
922	Isobutyl isovalerate	Esters and analogues	3.49		6.90	4.55			
923	Isopropyl Alcohol	Alcohols	3.49		3.45	9.09			
924	Isopropyl palmitate	Esters and analogues	3.49		3.45	4.55			
925	Levomenthol	Alcohols	3.49		3.45	9.09			
926	Linalool	Alcohols	3.49			4.55			
927	Mandelamide	Nitrogen-containing	3.49			9.09			
928	Methacrolein	Aldehydes	3.49			9.09			
929	Methane, dibromochloro-	Halogen-containing	3.49		10.34				
930	Methanesulfonic acid, ethyl ester	Esters and analogues	3.49		3.45	9.09			
931	Methional	Aldehydes	3.49		10.34				
932	Methoxyacetyl chloride	Ethers	3.49		10.34				
933	Methyl glyoxal	Aldehydes	3.49		6.90	4.55			
934	Methyl thiolacetate	Esters and analogues	3.49		3.45	9.09			
935	Methyl trifluoroacetate	Halogen-containing	3.49		6.90	4.55			
936	Methyl vinyl ketone	Ketones	3.49		6.90				
937	Methylene chloride	Halogen-containing	3.49		3.45	4.55			
938	Methylenecyclopropane	Cyclic aliphatic	3.49		6.90				
939	Monoethanolamine	Alcohols	3.49		3.45				
940	Monomethyl carbonotrithioate	Nitrogen-containing	3.49		3.45	9.09			
941	N-α,N-ω-Di-cbz-L-arginine	Nitrogen-containing	3.49		6.90	4.55			
942	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	Aromatics	3.49		3.45				33.33
943	Naphthalene, 1,2,3,4-tetrahydro-5,6-dimethyl-	Aromatics	3.49		3.45				
944	Naphthalene, 1,2,3,4-tetrahydro-5,7-dimethyl-	Aromatics	3.49		6.90				
945	Naphthalene, 1,2,3,4-tetrahydro-6,7-dimethyl-	Aromatics	3.49		6.90	4.55			
946	Naphthalene, 2-(1-methylethyl)-	Aromatics	3.49		6.90				33.33
947	Naphtho[2,1-b]furan, dodecahydro-3a,6,6,9a-tetramethyl-	Aromatics	3.49		3.45	4.55			
948	Nonadecane	Linear aliphatic	3.49		6.90	4.55			
949	Nonanal	Aldehydes	3.49		6.90	4.55			
950	Nonane, 1-chloro-	Halogen-containing	3.49		6.90				

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951	Nonane, 2-methyl-	Linear aliphatic	3.49		6.90	4.55			
952	Nonane, 2-methyl-3-methylene-	Linear aliphatic	3.49		6.90	4.55			
953	Nonane, 2,6-dimethyl-	Linear aliphatic	3.49		6.90				
954	Nonane, 3,7-dimethyl-	Linear aliphatic	3.49		6.90				
955	Octadecane, 6-methyl-	Linear aliphatic	3.49		3.45				
956	Octane	Linear aliphatic	3.49		3.45	4.55			
957	Octane, 1,1'-oxybis-	Ethers	3.49		3.45	9.09			
958	Octane, 2,5-dimethyl-	Linear aliphatic	3.49		10.34				
959	Octane, 2,5,6-trimethyl-	Linear aliphatic	3.49		3.45				
960	Pentane, 1-nitro-	Nitrogen-containing	3.49		3.45	9.09			
961	Pentane, 1,1,2,2,3,3,4,4-octafluoro-	Halogen-containing	3.49		6.90	4.55			
962	Pentane, 2,4-dimethyl-	Linear aliphatic	3.49		6.90				
963	Pentane, 3-ethyl-2,2-dimethyl-	Linear aliphatic	3.49		3.45	4.55			
964	Pentanenitrile	Nitrogen-containing	3.49		6.90	4.55			
965	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	Esters and analogues	3.49			4.55			
966	Pentanoic acid, 4-methyl-, pentyl ester	Esters and analogues	3.49			13.64			
967	Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl)-	Alcohols	3.49			9.09			
968	Phenol, 2-chloro-	Alcohols	3.49		3.45	4.55			
969	Phenol, 2,4,6-tri-tert-butyl-	Alcohols	3.49	14.29	6.90				
970	Phenol, 4-fluoro-	Halogen-containing	3.49		3.45	4.55			
971	Propanal, 2-methyl-	Aldehydes	3.49	14.29	3.45				
972	Propane, 1,1-dimethoxy-	Ethers	3.49		3.45				
973	Propane, 1,1,2,2-tetrafluoro-	Halogen-containing	3.49	42.86					
974	Propanenitrile, 3-(methylthio)-	Nitrogen-containing	3.49		10.34				
975	Propanoic acid, 2-hydroxy-, methyl ester, (±)-	Esters and analogues	3.49		3.45				
976	Propanoic acid, 2-hydroxy-2-methyl-	Acids	3.49		6.90				
977	Pyrimidine, 4,6-dimethyl-	Aromatics	3.49		3.45				
978	Spiro[4.5]decane	Cyclic aliphatic	3.49			9.09			
979	Sulfurous acid, butyl dodecyl ester	Esters and analogues	3.49		10.34				
980	Tetradecane	Linear aliphatic	3.49			4.55			
981	Thiocyanic acid, 1,1,3-trimethyl-3-phenylbutyl ester	Esters and analogues	3.49			4.55			
982	Thiophene, 2-pentyl-	Aromatics	3.49		6.90				
983	Thiophene, 3,4-dimethyl-	Aromatics	3.49		6.90				
984	Toluene	Aromatics	3.49		10.34				
985	trans-Sesquisabinene hydrate	Alcohols	3.49		6.90				
986	trans, cis-2-Ethylbicyclo[4.4.0]decane	Cyclic aliphatic	3.49		6.90	4.55			
987	Trichloromethane	Halogen-containing	3.49	14.29	6.90				
988	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	Ethers	3.49		3.45	4.55			

APPENDIX B: REST[ES] DONOR DECOMPOSITION STAGES

This appendix contains images of decomposing cadavers. Reader discretion is advised.

Table A 1: Summary of visual observations used to classify REST[ES] donors in decomposition stages**.**

Stages	**Visible characteristics**		**Reference images**
	Taphonomic modification	**Entomological activity***	
Fresh	No macroscopic changes Pinkish/natural appearance of the skin colour. Early signs of appearance of greenish discolouration in the abdomen. Early signs of appearance of marbling visible from the greenish discoloration of blood vessels. Cracks or ruptures in skin. Algor mortis, rigor mortis and livor mortis may be present. Antemortem and perimortem modifications may be present. **Key feature**: No distinct macroscopic change since time of death	Beginning of insect arrival. Beginning of appearance of insect eggs.	
Bloat	Putrefaction gases accumulate and cause swelling of internal organs and soft tissue.	Insects and egg masses may be present	REDACTED Bloated abdomen with greenish colouration

	This stage lasts until the gasses are released from the orifices and ruptured skin leading to deflation of the corpse. Beginning of marbling visible from the green/black discoloration of blood vessels Blue/Purple appearance of skin colour. Greenish discolouration in the abdomen may be present. Skin slippage may be present. **Key feature**: Swelling of parts of the corpse.		
Active decay	Accelerated decay rates, multiple skin ruptures along with continued marbling and skin slippage. Hair loss, blackish skin discolouration, soft tissue liquefaction with frothing and dryness in limbs. Release of liquified cadaveric material. Appearance of desiccated tissue in patches **Key feature**: Rapid loss of soft tissue, skin desiccated in small patches, intense marbling and intense green colour of the abdomen.	High entomological activity with larval masses feeding on the corpse.	REDACTED Desiccated patches on skin REDACTED Skin slippage

			REDACTED Intense larval activity
Desiccation	Soft tissues that appear dry and dehydrated Skin discolouration ranging from orange to brown/black with a leather-like appearance Complete dehydration of the soft tissues which leads to the natural preservation of the body. *Desiccation was categorised towards the end of active decay in any region of the body. Any desiccation that occurred in patches was categorised as active decay. **Key feature**: Dry, dehydrated tissue coving larger than small patches in the skin.	Reduced entomological activity.	REDACTED Desiccated skin
Skeletonisation	Partial or complete exposure of bones Anatomical connections by ligaments and tendons may be maintained or lost. **Key feature**: Partial or complete exposure of bones.	Significantly less to no entomological activity.	REDACTED Bones exposed

*Entomological activity is not a taphonomic modification of the body, but rather an indicator of the "active or a later" stage of decomposition.

Table A 2: Decomposition stages and images of REST[ES] donors decomposing at warmer average ambient temperature**.**

	Donor 2020-01	**Donor 2020-02**	**Donor 2021-06**	**Donor 2021-08**
Week 1	REDACTED ED 0 – Active decay Livor mortis in the hands and legs Greenish marbling in legs Slight marbling in the chest and abdomen Bruising in the neck Minimal fly activity	REDACTED ED 0 – Fresh Livor mortis visible in the neck and partially in the torso Ruptured skin in both the lower arms Reddish appearance of neck and lower arms potentially from blood deposits in the skin	REDACTED ED 2 – Fresh Bluish appearance of fingernails Minimal fly activity	REDACTED ED 0 – Fresh Ruptured skin in both the left limb Skin peeling in lower half of left limb
Week 1	REDACTED ED 3 – Bloat and active decay Increased Marbling in neck, lower abdomen and legs	REDACTED ED 2 – Fresh and active decay	REDACTED ED 4 – Fresh and active decay Livor mortis visible in the posterior side of the donor	REDACTED ED 3 – Bloat and active decay Beginning of mild bloating in the abdomen

	Skin peeling from regions of the face Desiccated patches in the neck and regions around the eye Mild bloating in the torso Active decay in the face and limbs Egg masses in head, neck, ears and genital Visible and larvae fry activity	Egg masses and larval activity in the ruptured skin in both the lower arms Reddish appearance of neck and lower arms potentially from blood deposits in the skin	Bluish appearance of fingernails Desiccated patches of skin in the upper torso Greenish discolouration in the lower limbs and abdomen Egg masses in mouth, nose, hair, eyes, armpits and genital	Greenish discolouration in abdomen Intense larval activity in face and some larval activity in genitals and left lower limb Reddish marbling in limbs and upper thorax Egg masses in genitals, hair and lower left limbs
Week 1	REDACTED ED 4 – Bloat, active decay and desiccation Increased Marbling in neck, lower abdomen and legs Skin peeling from regions of the face Desiccation in face and neck Bloating in the torso	REDACTED ED 4 – Bloat, active decay and desiccation Beginning of mild bloating in the torso Beginning of desiccation in upper limbs Some desiccated brownish skin in the face and lower arms	REDACTED ED 6 – Fresh and active decay Livor mortis visible in the posterior side of the donor Bluish appearance of fingernails Desiccated patches of skin in the upper torso	REDACTED ED 5 – Bloat, active decay and desiccation Bloating in the abdomen Greenish discolouration in abdomen and upper limbs Egg masses in genitals and inner left thigh

	Active decay in the face and limbs Egg masses in head, neck, ears and genital Visible and larvae fry activity	Skin slippage in the face, arms and neck Hair detached from the head region Marbling in the feet, torso and legs	Greenish discolouration in the lower limbs and abdomen Egg masses in mouth, nose, hair, eyes, armpits and genital	Intense larval activity in head, face, neck, limbs and genitals Skin slippage in head, face, neck, limbs and genitals Decomposition fluids purging in head Beginning of desiccation in head and thorax CDI visible in the soil in contact with head
Week 1	REDACTED ED 6 – Bloat, active decay and desiccation Intense marbling in the legs and abdomen Leathery desiccated tissue in the face, head and upper torso Patches of desiccated skin in the lower torso region			REDACTED ED 6 – Bloat, active decay, desiccation and skeletonisation Skull bones becoming visible Bloating in the abdomen Greenish discolouration in abdomen and upper limbs

	Skin slippage in the torso, abdomen and upper arms Significant bloating in the abdomen and torso Excessive egg masses in the head, face and genitals Visible fly activity			Decomposition fluid covering the thorax of cadaver giving it a shiny appearance Intense larval activity in head, face, neck, limbs and genitals Skin slippage in limbs and genitals Desiccation in head, thorax and areas around genitals Marbling in torso and limbs CDI visible in the soil in contact with regions above the hips
Week 2	REDACTED ED 8 – Bloat, active decay and desiccation Marbling intensified in hands and visible in legs and abdomen	REDACTED ED 8 – Bloat, active decay and desiccation Marbling in the abdomen, legs and feet Skin slippage in legs	REDACTED ED 10 – Bloat, active decay and desiccation Desiccated skin in the face	REDACTED ED 10 – Active decay, desiccation and skeletonisation Skull, collar bones and bones of upper limbs visible

	Desiccated tissue in the face, head and area around genitals Skin slippage in the hands, torso, abdomen and legs Active decay in all regions of donor Bloat still visible Influx of liquefied products in the soil and beginning of CDI formation around the body Body hair detached from the torso Larvae masses especially in the head, face and genital area Visible fly activity	Peak of active decay in the head, face, hands, genital and torso Full bloat visible in the cadaver Influx of liquefied products in the soil and beginning of CDI formation around the body Intense larvae activity in the head, face, hands, torso in contact with the and genital area Some regions in the face and arms begin to show signs of leathery tissue and blackening of the skin surface Soapy fat deposits around the cadaver Visible fly and larvae activity	Desiccated skin patches in the torso and limbs Intense greenish discolouration in the torso Mild greenish discolouration in limbs Beginning of bloat in the neck Egg masses in the head and genitals Larval activity in mouth Intense fly activity Skin slippage in the thorax, neck and head	Bloating completely settled in cadaver Larval activity in most regions of cadaver Skin blistering in right lower limb Desiccation in thorax, abdomen, areas around genitals and limbs Desiccation patches in right lower limb Marbling in abdomen and left lower limbs Head hair detached from skull and present Fat deposits visible between legs A full CDI is visible
Week 2	REDACTED	REDACTED	REDACTED	

	ED 11 – Bloat, active decay, desiccation and skeletonisation Intensified marbling in hands Skin slippage in hands and legs Partial bloat visible in the abdomen Beginning of advanced decomposition in regions of the face Blackish brown skin appearance in the torso, abdomen, hands and legs Partial face bones visible CDI visible in the upper half of the cadaver Intense larvae activity in the genital, face and neck area	ED 10 – Active decay, desiccation and skeletonisation Skin slippage in lower legs and feet Active decay in the parts of head, neck, torso, legs and genital region Bloat lost in the cadaver Blackish brown skin appearance in the torso, abdomen, hands and legs Partial face and parts of hands in advanced decomposition Appearance of leathery skin in upper torso and legs Bone exposure in lower half of the hands and back of the head CDI visible in the upper half of the cadaver and in soil between the thighs	ED 12 – Bloat, active decay and desiccation Desiccated skin in the face, upper limbs, thorax and areas around genitals Desiccated skin patches in the torso and limbs Greenish discolouration in the torso and limbs Full bloat visible in the cadaver Egg masses in the head, hair and genitals Larval activity in head, face, neck and genitals Intense fly activity Skin slippage in the thorax, neck, head, inner thighs, area around genitals Some bodily hair lost while some still attached	

		Intense larvae activity in the genital, torso and neck area		
Week 3	REDACTED ED 18 – Active decay, desiccation and skeletonisation Skin slippage under the feet and fingers Active decay in the genital and torso region Bloat completely settled Blackish brown skin appearance in the whole cadaver with mummified whitish skin in the left thigh Advanced decomposition in face with exposure of jaws, partial skull and spine from behind the neck	REDACTED ED 16 – Desiccation and skeletonisation The whole cadaver transitioned into advanced decomposition Brownish leathery skin in hands, torso, legs, feet and head CDI clearly visible around the cadaver Exposure of skull, right clavicle, ribs and bones of arms and hands	REDACTED ED 14 – Bloat, active decay and desiccation Desiccated skin in the face, neck, upper limbs, thorax and areas around genitals Greenish discolouration in the torso and limbs Full bloat visible in the cadaver Some bloat lost in the neck and areas around the shoulder Egg masses in the head, hair and genitals Intense larval activity in head, face, neck and genitals Intense fly activity	REDACTED ED18 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, collar bones and bones of upper limbs visible Head hair detached from skull and present A full CDI visible

	CDI visible in the soil in contact with regions from head to knees Reduced fly activity		Skin slippage in the torso, upper and lower limbs and, area around genitals Some bodily hair lost while some still attached CDI visible in the soil in contact with head	
Week 3			REDACTED ED 17 – Bloat, active decay, desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Reddish marbling in the abdomen and limbs Decomposition fluid covering the cadaver surface giving it a shiny appearance Bloat visible in the cadaver	

			Some bloat lost in the neck and areas around the shoulder and abdomen Intense larval activity in genitals, armpits and posterior side of cadaver Skin slippage in lower limbs Some bodily hair lost while some still attached Some skull bone visible CDI visible in the soil in contact with head to knees	
Week 3			REDACTED ED 20 – Bloat, active decay, desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Reddish marbling in the abdomen and limbs	

			Bloat settled in the cadaver except for in the thighs Intense larval activity in genitals, armpits and posterior side of cadaver Skin slippage in lower limbs Some bodily hair lost while some still attached Some skull bone visible CDI visible in the soil in contact with head to knee	
Week 4	REDACTED ED 23 – Desiccation and skeletonisation Skin slippage under the feet and in hands Desiccated and mummified tissue present Skull and jaws exposed	REDACTED ED 21 – Desiccation and skeletonisation The whole cadaver transitioned into advanced decomposition Brownish leathery skin in hands, torso, legs, feet and head	REDACTED ED 27 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Reddish marbling in lower limbs, abdomen and upper limbs	REDACTED ED 23– Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible

	Fat deposits visible around the head, shoulders and between legs A full CDI visible	CDI clearly visible around the cadaver Exposure of skull, right clavicle, ribs and bones of arms and hands	Bloat completely settled in cadaver Decomposition fluid covering the cadaver surface giving it a shiny appearance Skin slippage in lower limbs Some bodily hair lost while some still attached Some skull bone visible Black colour of fingernails A full CDI visible	Head hair detached from skull and present A full CDI visible
Week 5	REDACTED ED 32 – Desiccation and skeletonisation Skin slippage under the feet and in hands Desiccated and mummified tissue present Skull and jaws exposed	REDACTED ED 32 – Desiccation and skeletonisation Brownish leathery skin in hands, torso, legs, feet and head Exposure of skull and bones of upper limbs	REDACTED ED 31 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Reddish marbling in limbs and abdomen changed to dark black brown coloration	REDACTED ED 31– Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, neck bones, collar bones, parts of rib cage, bones

	Liquified fat deposits visible around the head, shoulders and between legs A full CDI visible	Minimal larvae activity Dry white patches on skin surface A full CDI visible	Some bodily hair lost while some still attached Some skull bone visible A full CDI visible	of upper limbs and pelvic region visible Head hair detached from skull and present A full CDI visible
Week 6	REDACTED ED 39 – Desiccation and skeletonisation Skin slippage under the feet Desiccated and mummified tissue present Skull and jaws exposed Dry white patches on skin surface mostly in pelvic region A full CDI visible	REDACTED ED 39 – Desiccation and skeletonisation Desiccated and mummified tissue present Exposure of skull and bones of upper limbs Dry white patches on skin surface A full CDI visible		REDACTED ED 38 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, neck bones, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible Head hair detached from skull and present A full CDI visible
Week 7	REDACTED	REDACTED	REDACTED	REDACTED

	ED 44 – Desiccation and skeletonisation Skin slippage under the feet Desiccated and mummified tissue present Skull and jaws exposed Dry white patches on skin surface mostly in pelvic region A full CDI visible	ED 46 – Desiccation and skeletonisation Desiccated and mummified tissue present Exposure of skull and bones of upper limbs Dry white patches on skin surface A full CDI visible	ED 43 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Some skull bone visible Dry white patches on skin surface in head, neck, area around shoulders, torso and lower limbs Persistence of marbling as dark brown to black colouration A full CDI visible	ED 44 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, neck bones, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible Head hair detached from skull and present A full CDI visible
Week 8	REDACTED ED 53 – Desiccation and skeletonisation Skin slippage under the feet Desiccated and mummified tissue present	REDACTED ED 51 – Desiccation and skeletonisation Desiccated and mummified tissue present	REDACTED ED 51 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Some skull bone visible	REDACTED ED 51 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver

	Skull and jaws exposed Dry white patches on skin surface A full CDI visible	Exposure of skull and bones of upper limbs Whitish appearance of skin from rehydration following rains CDI visible but less distinctly from wet and dark soil because of rains present in vicinity	Increased dry white patches on skin surface in head, neck, area around shoulders, torso, upper and lower limbs Persistence of marbling as dark brown to black colouration A full CDI visible	Skull, neck bones, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible Head hair detached from skull and present A full CDI visible
Week 9	REDACTED ED 60 – Desiccation and skeletonisation Desiccated and mummified tissue present Skull and jaws exposed Dry white patches on skin surface CDI visible but partially covered by leaf litter	REDACTED ED 56 – Desiccation and skeletonisation Desiccated and mummified tissue present Exposure of skull and bones of upper limbs Dry white patches on skin surface CDI visible	REDACTED ED 59 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Some skull bone visible Whitish appearance of skin from rehydration following rains CDI visible	

Week 10	REDACTED ED 63 – Desiccation and skeletonisation Additional desiccated and mummified tissue present Skull and jaws exposed Dry white patches on skin surface CDI visible but partially covered by leaf litter	REDACTED ED 63 – Desiccation and skeletonisation Desiccated and mummified tissue present Exposure of skull and bones of upper limbs Dry white patches on skin surface CDI visible but partially covered by leaf litter		REDACTED ED 66 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver Skull, neck bones, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible Head hair detached from skull and present A full CDI visible
Week 11	REDACTED ED 70 – Desiccation and skeletonisation Additional desiccated and mummified tissue present	REDACTED ED 70 – Desiccation and skeletonisation Desiccated and mummified tissue present		REDACTED ED 72 – Desiccation and skeletonisation Desiccated tissue in most regions of the cadaver

	Dry white patches on skin surface Skull and jaws exposed CDI visible but partially covered by leaf litter	Exposure of skull and bones of upper limbs Dry white patches on skin surface CDI visible but partially covered by leaf litter		Skull, neck bones, collar bones, parts of rib cage, bones of upper limbs and pelvic region visible Head hair detached from skull and present

Table A 3: Decomposition stages and images of REST[ES] donors decomposing at cooler average ambient temperature.

	Donor 2020-03	**Donor 2020-04**	**Donor 2021-10**	**Donor 2021-11**
Week 1	REDACTED ED 0 – Fresh Rigor mortis Livor mortis in torso side, neck, back of head, inner thighs	REDACTED ED 0 – Fresh Beginning of marbling in the neck and abdomen Half of the right toe cut off Livor mortis in neck, shoulders and head	REDACTED ED 0 – Fresh Greenish discolouration on shoulders and abdomen Reddish spots on dorsal side of the palm	REDACTED ED 0 – Fresh and bloat Greenish discolouration in abdomen and thorax Mild bloat in abdomen Livor mortis in posterior side of entire cadaver
Week 1	REDACTED ED 4 – Fresh Rigor mortis passed in cadaver Appearance of marbling in abdomen and limbs Bluish colour in fingernails	REDACTED ED 5 – Active decay Egg masses in nostrils Desiccated skin patches in face, upper limbs and abdomen Reddish marbling in neck and upper limbs	REDACTED ED 2 – Fresh and active decay Greenish discolouration shoulders and abdomen Reddish spots on dorsal side of the palm	REDACTED ED 6 – Bloat, active decay and desiccation Intense greenish discolouration in abdomen and thorax Mild bloat in abdomen Desiccation in face

	Egg masses in nostrils and between legs		Desiccated skin patches on thorax Greenish marbling in lower limbs Egg masses in mouth and nostrils	Desiccated skin patches in torso and limbs Larval activity in face Skin peeling in face, neck, upper limbs and shoulder
Week 2	REDACTED ED 9 – Fresh and active decay Livor mortis still visible in the head, shoulders and torso close to the surface Appearance of desiccated skin patches in the face Marbling intensified around torso and abdomen Shrivelled skin in the fingers Egg masses in the mouth, nostrils, hair, armpit and genital region	REDACTED ED 7 – Bloat and active decay Minimal fly activity Mild bloat in neck Desiccated skin patches in face, upper limbs and abomen Reddish marbling in neck and upper limbs	REDACTED ED 11 – Active decay and desiccation Skin peeling in upper limbs, areas around genitals, thorax, shoulders and neck Desiccated skin in face, neck and thorax Desiccated skin patches in thorax Egg masses in genitals and head	REDACTED ED 10 – Bloat, active decay and desiccation Intense greenish discolouration in abdomen, thorax, and area around the shoulders Bloat in abdomen Desiccation in face, neck and area around the shoulders Desiccated skin patches in torso and limbs Larval activity in face, neck and genital region

			Larval activity in face, neck, thorax, upper limbs and genital area Greenish discolouration in abdomen, thorax, shoulders and upper limbs Marbling in dorsal side of palms	Skin peeling in face, neck, shoulder, upper limbs, lower limbs and genital region
Week 2	REDACTED ED 13 – Active decay Desiccated skin patches in face Greenish colour in abdomen Intense marbling in thighs, lower limbs and torso Skin peeling off in the face Eggs masses in nose, mouth, hair, neck, ear, armpits and between legs		REDACTED ED 13 – Active decay and desiccation Skin peeling in limbs, abdomen areas around genitals, thorax, shoulders and neck Desiccated skin in face, neck, thorax, area around the hips, limbs and around genitals	

	Larval activity in nostrils and between the lower limbs		Intense larval activity in face, neck, thorax, upper limbs and genital area Greenish discolouration in abdomen, thorax, shoulders and upper limbs Marbling in limbs	
Week 3	REDACTED ED 17 – Bloat, active decay and desiccation Livor mortis still visible in the head, shoulders and torso close to the surface Desiccated skin in the face Marbling in the neck, head, torso, abdomen Shrivelled skin in the fingers Skin ruptures in the torso and arm	REDACTED ED 18 – Bloat and active decay Head and face in active decay Patches of desiccated tissue present in the face and neck Yellowish appearance of face Egg and larvae present in the face Bloating in the neck	REDACTED ED 16 – Active decay, desiccation and skeletonization Desiccated tissue in most regions of the cadaver Intense larval activity in genitals and internal torso of the cadaver Continued larval activity in face and limbs Beginning of skull bone becoming visible	REDACTED ED 17 – Bloat, active decay and desiccation Intense greenish discolouration in abdomen and thorax Bloat in abdomen Desiccation in head, neck, upper limbs and lower limbs and torso in contact with the soil Larval activity in head, upper and lower limbs, genitals, torso

	Egg masses in the mouth, hair and torso in contact with the surface and genital region Larval activity in nostrils , neck and genitals Mild bloat in the neck		Darkened marbling in abdomen and limbs	in contact with soil and genital region Skin peeling in thorax, upper limbs, lower limbs and genital region
Week 4	REDACTED ED 25 – Bloat, active decay and desiccation Desiccated skin in parts of the face Marbling in the neck, head, torso, abdomen and arms Shrivelled skin in the fingers with some ruptures Skin slippage around nose and mouth Active decay in the face, head and arms	ED 22 – Bloat, active decay and desiccation Desiccation in face and neck Minimal fly activity Bloating in the neck Hard fat deposits	ED 25 – Active decay, desiccation and skeletonization Skull and pelvic bone almost entirely visible Marbling in lower limbs Leathery desiccated tissue visible externally in most regions of cadaver Intense larval activity internally and on the posterior side of the cadaver Some beard and head hair still attached to remaining facial tissue	REDACTED ED 24 – Active decay and desiccation Desiccated tissue in face, limbs, shoulders, thorax, around genital area Greenish discolouration in abdomen Skin slippage in limbs, thorax Intense larval activity in face, genitals, posterior side of the cadaver Egg masses around genital area

	Egg masses in hair Larval activity in head, armpits, genitals and in the posterior side of the donor Mild bloat in the torso			
Week 5		REDACTED ED 31 – Bloat, active decay and desiccation Desiccation in face, ears and neck Patches of desiccated tissue in thorax and limbs Decomposition fluid purging from ears Minimal fly activity Bloating in the neck Hard fat deposits Marbling in abdomen and limbs	REDACTED ED 34 – Active decay, desiccation and skeletonization Skull and pelvic bone almost entirely visible Leathery desiccated tissue visible externally in most regions of cadaver Larval activity continued internally and on the posterior side of the cadaver Some beard and head hair still attached to remaining facial tissue	REDACTED ED 30 – Active decay and desiccation Desiccated tissue in face, limbs, shoulders, thorax, around genital area Greenish discolouration in abdomen Skin slippage in limbs, thorax Continues larval activity in face, genitals, posterior side of the cadaver Egg masses around genital area

Week 6	ED 38 – Active decay and desiccation Desiccated skin in parts of the face which had advanced in decomposition more than other parts of the cadaver Marbling still present in torso, lower limbs Marbling still present on torso Larval activity in mouth, genitals, armpits and posterior side of the cadaver in contact with the soil			

APPENDIX C: VOCS DETECTED IN REST[ES] DONORS

- This appendix presents a list of 841 VOCs listed in the order of their reducing prominence (occurence in most samples). VOCs with the same prominence have been listed alphabetically.
- The 684 VOCs listed in red are those that were also detected in CDD training aids.
- This list does not include VOCs that occurred only in 1 or 2 samples of the total 160 samples from REST[ES] donors.

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage of donor samples in which VOC was detected	Percentage of donor 2020-01 samples in which VOC was detected	Percentage of donor 2020-02 samples in which VOC was detected	Percentage of donor 2020-03 samples in which VOC was detected	Percentage of donor 2020-04 samples in which VOC was detected	Percentage of donor 2021-06 samples in which VOC was detected	Percentage of donor 2021-08 samples in which VOC was detected	Percentage of donor 2021-10 samples in which VOC was detected	Percentage of donor 2021-11 samples in which VOC was detected
1	Dimethyl trisulfide	Sulphur-containing	54.38	67.74	41.94	14.29		64.00	72.73	56.25	76.92
2	Thiocyanic acid, methyl ester	Esters and analogues	47.50	58.06	45.16	7.14		68.00	50.00	56.25	46.15
3	Methylamine, N,N-dimethyl-	Nitrogen-containing	44.38	41.94	32.26	28.57	12.50	56.00	72.73	43.75	46.15
4	3-Buten-1-ol, 3-methyl-	Alcohols	43.13	61.29	54.84			44.00	77.27	31.25	
5	Phenylethyl Alcohol	Alcohols	43.13	67.74	64.52			36.00	63.64	31.25	
6	Pyrazine, trimethyl-	Aromatics	40.63	74.19	54.84			48.00	54.55	6.25	
7	Pyrazine, methyl-	Aromatics	40.00	61.29	58.06			40.00	59.09	25.00	
8	Disulfide, dimethyl	Sulphur-containing	38.75	25.81	22.58	21.43		40.00	68.18	56.25	76.92
9	p-Cresol	Alcohols	38.13	38.71	48.39	7.14		48.00	59.09	31.25	23.08
10	1-Butanol, 3-methyl-	Alcohols	37.50	35.48	32.26	14.29		40.00	68.18	37.50	46.15
11	2-Pentanone, 3-methyl-	Ketones	37.50	54.84	54.84			32.00	59.09	31.25	
12	Butanoic acid, 3-methyl-	Acids	36.88	58.06	48.39			44.00	45.45	31.25	
13	Pyridine	Aromatics	36.88	41.94	38.71			44.00	45.45	50.00	38.46
14	1-Propanol, 2-methyl-	Alcohols	36.25	48.39	45.16	21.43		28.00	59.09	18.75	23.08
15	2-Pentanol	Alcohols	36.25	38.71	51.61			52.00	68.18	12.50	
16	Pyridine, 3-methyl-	Aromatics	36.25	61.29	51.61			40.00	59.09		
17	2,4-Dithiapentane	Sulphur-containing	35.63	83.87	45.16			40.00	13.64	25.00	
18	2-Heptanol	Alcohols	34.38	64.52	51.61			32.00	45.45	6.25	
19	3-Octanol	Alcohols	34.38	51.61	61.29	7.14		40.00	31.82	6.25	7.69
20	Dimethyl sulfone	Sulphur-containing	34.38	48.39	41.94			40.00	40.91	37.50	15.38
21	Pyrazine, 2,6-dimethyl-	Aromatics	33.75	67.74	45.16			44.00	36.36		
22	Propanoic acid, 2-methyl-	Acids	33.13	54.84	45.16			36.00	45.45	18.75	
23	Pyrazine, tetramethyl-	Aromatics	33.13	54.84	51.61			32.00	54.55		
24	2-Butanol	Alcohols	32.50	51.61	51.61			28.00	59.09		
25	2-Hexanol	Alcohols	32.50	48.39	51.61			36.00	50.00	6.25	
26	Propane, 2,2-dimethoxy-	Ethers	32.50	45.16	38.71			28.00	45.45	31.25	30.77
27	Butanoic acid, butyl ester	Esters and analogues	31.88	67.74	29.03			60.00	27.27		
28	1-Pentanol, 4-methyl-	Alcohols	31.25	51.61	45.16			36.00	50.00		
29	1H-Pyrrole, 1-methyl-	Aromatics	31.25	45.16	48.39			32.00	31.82	37.50	
30	1-Octanol	Alcohols	30.63	54.84	54.84			24.00	40.91		
31	Dimethyl sulfide	Sulphur-containing	30.63	25.81	25.81			40.00	40.91	37.50	61.54
32	2(3H)-Furanone, 5-ethyldihydro-	Esters and analogues	30.00	38.71	38.71			20.00	45.45	25.00	38.46
33	2-Chloroethanol	Alcohols	29.38	61.29	29.03			36.00	27.27	25.00	
34	Pyridine, 2-methyl-	Aromatics	29.38	51.61	41.94			44.00	31.82		
35	Thiophene, 2-hexyl-	Sulphur-containing	29.38	54.84	58.06			20.00	31.82		
36	Acetoin	Ketones	28.75	64.52	58.06	7.14	12.50	20.00	4.55		
37	1,2-Ethanediamine, N'-ethyl-N,N-dimethyl-	Nitrogen-containing	28.13	35.48	22.58			48.00	50.00	25.00	
38	Butyl 2-methylbutanoate	Esters and analogues	28.13	48.39	45.16			40.00	27.27		
39	Propanal, 2-methyl-	Aldehydes	28.13	45.16	16.13	28.57		44.00	31.82	12.50	15.38
40	Pyrazine	Aromatics	28.13	32.26	38.71			32.00	45.45	25.00	7.69
41	2(3H)-Furanone, dihydro-5-methyl-	Esters and analogues	27.50	38.71	41.94	7.14		16.00	50.00		23.08
42	Dihydro-2(3H)-thiophenone	Ketones	27.50	51.61	45.16			28.00	31.82		
43	Indole	Aromatics	26.88	54.84	32.26			44.00	22.73		
44	Thiazole	Aromatics	26.25	51.61	41.94			28.00	18.18	12.50	
45	1,3,5-Triazine	Nitrogen-containing	25.63	32.26	38.71	7.14	12.50	12.00	22.73	37.50	23.08
46	Ethanol, 2-(methylthio)-	Alcohols	25.63	48.39	45.16			28.00	18.18	6.25	
47	1H-Pyrrole, 2-methyl-	Aromatics	25.00	32.26	25.81	7.14	12.50	28.00	31.82	12.50	30.77
48	Acetonitrile, (dimethylamino)-	Nitrogen-containing	25.00	35.48	29.03			32.00	36.36	25.00	
49	Butanoic acid, propyl ester	Esters and analogues	25.00	58.06	19.35			40.00	27.27		
50	Ethane	Linear aliphatic	25.00	51.61	51.61	7.14		16.00	9.09	6.25	

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage of donor samples in which VOC was detected	Percentage of donor 2020-01 samples in which VOC was detected	Percentage of donor 2020-02 samples in which VOC was detected	Percentage of donor 2020-03 samples in which VOC was detected	Percentage of donor 2020-04 samples in which VOC was detected	Percentage of donor 2021-06 samples in which VOC was detected	Percentage of donor 2021-08 samples in which VOC was detected	Percentage of donor 2021-10 samples in which VOC was detected	Percentage of donor 2021-11 samples in which VOC was detected
50	Ethane	Linear aliphatic	25.00	51.61	51.61	7.14		16.00	9.09	6.25	
51	Hydrazine	Nitrogen-containing	25.00	41.94	51.61	7.14	12.50	12.00	4.55	25.00	7.69
52	2-Undecanone	Ketones	24.38	58.06	48.39			16.00	9.09		
53	Methyl ethyl disulfide	Sulphur-containing	24.38	67.74	25.81			32.00	9.09		
54	Propanoic acid, 2-methyl-, ethyl ester	Esters and analogues	24.38	58.06	32.26			32.00	13.64		
55	Butanoic acid	Acids	23.75	32.26	25.81			36.00	36.36	6.25	15.38
56	Butanoic acid, 2-methyl-, propyl ester	Esters and analogues	23.75	48.39	45.16			24.00	13.64		
57	Isobutane	Linear aliphatic	23.75	19.35	16.13	7.14		40.00	22.73	31.25	46.15
58	n-Propyl acetate	Esters and analogues	23.75	51.61	32.26	7.14		32.00	13.64		
59	Oxirane, 2-ethyl-2-methyl-	Ethers	23.75	25.81	35.48	21.43	12.50	28.00	13.64	12.50	23.08
60	1-Nonanol	Alcohols	23.13	45.16	48.39			24.00	4.55	6.25	
61	2-Butanone, 3-methyl-	Ketones	23.13	25.81	29.03	7.14		24.00	50.00	6.25	7.69
62	Anisole	Ethers	23.13	19.35	22.58	21.43	12.50	16.00	18.18	43.75	38.46
63	Bis-(ethoxycarbonyl)methoxymethyloxyiminomethane	Nitrogen-containing	23.13	38.71	29.03			28.00	13.64	25.00	15.38
64	Butanoic acid, 3-methylbutyl ester	Esters and analogues	23.13	45.16	38.71			32.00	13.64		
65	Formamide, N,N-dimethyl-	Nitrogen-containing	23.13	38.71	29.03		12.50	40.00	4.55	25.00	
66	1,3-Diazine	Nitrogen-containing	22.50	41.94	32.26			24.00	13.64	25.00	
67	2-Propen-1-ol	Alcohols	22.50	35.48	25.81	7.14		24.00	22.73	18.75	15.38
68	Disulfide, methyl (methylthio)methyl	Sulphur-containing	22.50	61.29	22.58			20.00	9.09	18.75	
69	L-Alanine, 3-sulfo-	Nitrogen-containing	22.50	35.48	25.81	21.43	12.50	36.00	4.55		23.08
70	Methyl thiolacetate	Esters and analogues	22.50	48.39	29.03			24.00	13.64	12.50	7.69
71	Pyrazine, 3-ethyl-2,5-dimethyl-	Aromatics	22.50	48.39	19.35			28.00	36.36		
72	Ethylenimine	Nitrogen-containing	21.88	29.03	25.81			28.00	18.18	31.25	15.38
73	Propanoic acid, 2-methyl-, butyl ester	Esters and analogues	21.88	45.16	38.71			20.00	18.18		
74	2-Furancarbonitrile	Nitrogen-containing	21.25	45.16	32.26			12.00	13.64	25.00	
75	2-Hydroxy-3-pentanone	Ketones	21.25	35.48	35.48			32.00	18.18		
76	Acetonitrile, hydroxy-	Nitrogen-containing	21.25	35.48	32.26	7.14		16.00	27.27	12.50	
77	Butanoic acid, 2-methyl-, ethyl ester	Esters and analogues	21.25	51.61	29.03			24.00	13.64		
78	Butanoic acid, 3-methyl-, butyl ester	Esters and analogues	21.25	48.39	16.13			48.00	9.09		
79	Furan, 2-ethyl-	Aromatics	21.25	22.58	32.26			32.00	18.18	25.00	7.69
80	2-Nonanone	Ketones	20.63	25.81	29.03			40.00	27.27		
81	Butanoic acid, 2-methyl-, 3-methylbutyl ester	Esters and analogues	20.63	38.71	45.16			24.00	4.55		
82	Butanoic acid, 3-methyl-, ethyl ester	Esters and analogues	20.63	48.39	29.03			28.00	9.09		
83	Butanoic acid, ethyl ester	Esters and analogues	20.63	41.94	16.13			40.00	18.18		7.69
84	Formamide, N-methyl-	Nitrogen-containing	20.63	35.48	35.48			20.00	9.09	25.00	
85	Methane, isothiocyanato-	Nitrogen-containing	20.63	22.58	16.13			32.00	27.27	37.50	7.69
86	Methylthio-2-propanone	Ketones	20.63	41.94	38.71			16.00	18.18		
87	2-n-Butyl furan	Aromatics	20.00	3.23	41.94	7.14		24.00	27.27	12.50	23.08
88	Butane, 1-methoxy-3-methyl-	Ethers	20.00	22.58	25.81			8.00		43.75	61.54
89	Dimethyl ether	Ethers	20.00	19.35	12.90	28.57	12.50	32.00	18.18	18.75	15.38
90	Pentanoic acid	Acids	20.00	32.26	19.35			36.00	22.73		15.38
91	Pyrrole	Aromatics	20.00	35.48	29.03		12.50	12.00		31.25	23.08
92	1-Pentanol	Alcohols	19.38	38.71	38.71			16.00	4.55		15.38
93	Acetic acid, hydroxy-	Acids	19.38	29.03	29.03	14.29		20.00	18.18	6.25	7.69
94	Pentanoic acid, 4-methyl-, ethyl ester	Esters and analogues	19.38	51.61	22.58			24.00	9.09		
95	Benzaldehyde, 2-hydroxy-	Aldehydes	18.75	19.35	22.58			24.00	31.82	12.50	15.38
96	Ethanol, 2-methoxy-	Alcohols	18.75	16.13	25.81	7.14	12.50	16.00	9.09	31.25	30.77
97	Isobutyronitrile	Nitrogen-containing	18.75	35.48	35.48		12.50	8.00	18.18	6.25	
98	Pentanoic acid, butyl ester	Esters and analogues	18.75	38.71	32.26			20.00	13.64		
99	Prenol	Alcohols	18.75	45.16	29.03			24.00			7.69
100	3-Aminopropionitrile	Nitrogen-containing	18.13	22.58	29.03			12.00	18.18	25.00	15.38
101	Benzenemethanol, α-methyl-	Alcohols	18.13	35.48	51.61			4.00	4.55		
102	Ethane, nitro-	Nitrogen-containing	18.13	25.81	32.26			20.00	18.18	6.25	7.69
103	Ethanone, 1-(2-furanyl)-	Ketones	18.13	16.13	25.81	7.14		24.00	31.82	6.25	7.69
104	Methenamine	Nitrogen-containing	18.13	12.90	19.35	21.43	25.00	24.00	27.27	6.25	7.69
105	Propanoic acid	Acids	18.13	29.03	22.58			36.00	13.64	6.25	
106	Thiophene, 3-methyl-	Aromatics	18.13	22.58	35.48			28.00	13.64	6.25	
107	2,2-Dimethoxybutane	Ethers	17.50	54.84	35.48						
108	2,6-Lutidine	Aromatics	17.50	38.71	29.03			24.00	4.55		

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109	3-Hexene	Linear aliphatic	17.50	6.45	9.68	28.57	12.50	24.00	18.18	25.00	30.77
110	3-Pentenoic acid, 4-methyl-	Acids	17.50	6.45	38.71			28.00	31.82		
111	N,N,O-Triacetylhydroxylamine	Nitrogen-containing	17.50	35.48	19.35	7.14		4.00	27.27	12.50	7.69
112	Pentane	Linear aliphatic	17.50	29.03	32.26			20.00	13.64	6.25	
113	Propanoic acid, butyl ester	Esters and analogues	17.50	45.16	29.03			12.00	9.09		
114	1-Heptanol	Alcohols	16.88	35.48	41.94			12.00			
115	2-Octenal	Aldehydes	16.88	22.58	38.71			16.00	13.64	6.25	
116	3-Pentanol	Alcohols	16.88	12.90	25.81			28.00	36.36		
117	Pentanoic acid, ethyl ester	Esters and analogues	16.88	45.16	25.81			16.00	4.55		
118	Pyrazine, 2,3-dimethyl-	Aromatics	16.88	22.58	19.35			24.00	36.36		
119	1-Hexanol	Alcohols	16.25	19.35	32.26		12.50	16.00	18.18		7.69
120	Acetic acid, butyl ester	Esters and analogues	16.25	38.71	25.81			12.00	13.64		
121	Cyclopentane, butyl-	Cyclic aliphatic	16.25	16.13	32.26			8.00	40.91		
122	Propanoic acid, 1-methylethyl ester	Esters and analogues	16.25	35.48	16.13			24.00	18.18		
123	Urea, tetramethyl-	Nitrogen-containing	16.25	35.48	32.26	14.29		8.00	4.55		
124	(3aR,4R,8R,8aS)-3a,4,8a-Trimethyl-7-methylenedecahydro-4,8-methanoazulene-rel-	Cyclic aliphatic	15.63	35.48	19.35	14.29		8.00	18.18		
125	2-Heptanone, 6-methyl-	Ketones	15.63	9.68	16.13	21.43		24.00	27.27	12.50	
126	3-Carene	Cyclic aliphatic	15.63	25.81	19.35			20.00	18.18	6.25	7.69
127	5-Ethylcyclopent-1-enecarboxaldehyde	Aldehydes	15.63	29.03	41.94			8.00	4.55		
128	Aniline	Aromatics	15.63	29.03	19.35	7.14		16.00	13.64	12.50	
129	Benzene, 1-ethyl-4-methyl-	Aromatics	15.63	16.13	16.13	14.29		24.00	9.09	12.50	23.08
130	Butane, 2-methyl-	Linear aliphatic	15.63	6.45	9.68	14.29	12.50	8.00	31.82	18.75	38.46
131	Butanethioic acid, S-methyl ester	Esters and analogues	15.63	38.71	25.81			12.00	9.09		
132	Butyrolactone	Esters and analogues	15.63	29.03	29.03	7.14			27.27		
133	Furan, 3-methyl-	Aromatics	15.63	38.71	19.35		12.50	12.00	13.64		
134	Styrene	Aromatics	15.63	19.35	22.58	14.29	12.50	16.00	13.64	12.50	
135	4-Cyclopentene-1,3-dione	Ketones	15.00	19.35	25.81	7.14		20.00	9.09	12.50	
136	Aminomethanesulfonic acid	Acids	15.00	25.81	25.81			20.00		12.50	7.69
137	Furan, 2,5-dimethyl-	Aromatics	15.00	22.58	25.81			8.00	31.82		
138	Isopropyl acetate	Esters and analogues	15.00	22.58	25.81			24.00	13.64		
139	Isopropyl butyrate	Esters and analogues	15.00	32.26	12.90			28.00	13.64		
140	Methyl Isobutyl Ketone	Ketones	15.00	32.26	25.81			20.00	4.55		
141	Methyl isobutyrate	Esters and analogues	15.00	22.58	25.81	14.29		8.00		18.75	15.38
142	Propiolactone	Esters and analogues	15.00	16.13	16.13			16.00	13.64	31.25	15.38
143	S-Methyl 3-methylbutanethioate	Esters and analogues	15.00	45.16	22.58			12.00			
144	S-Methyl propanethioate	Esters and analogues	15.00	35.48	19.35			16.00	13.64		
145	1,2-Ethanediol	Alcohols	14.38	29.03	16.13			28.00	4.55		7.69
146	2-Propenoic acid, methyl ester	Esters and analogues	14.38	16.13	22.58	7.14	12.50	12.00	9.09	25.00	
147	3-Hexanone	Ketones	14.38		29.03			28.00	31.82		
148	Acetic acid, methyl ester	Esters and analogues	14.38	16.13	16.13	14.29	12.50	24.00	13.64	6.25	
149	Benzene, (1-butylheptyl)-	Aromatics	14.38	16.13	9.68	14.29	25.00	12.00	13.64	18.75	15.38
150	Butane, 1-methoxy-	Ethers	14.38		3.23	14.29		40.00	22.73	18.75	15.38
151	Cyclohexane	Cyclic aliphatic	14.38	16.13	25.81	14.29	25.00	8.00	4.55	12.50	7.69
152	Diethyl azodicarboxylate	Nitrogen-containing	14.38	22.58	19.35	21.43	12.50	8.00	9.09	6.25	7.69
153	Methylacrylonitrile	Nitrogen-containing	14.38	22.58	25.81			4.00	18.18	18.75	
154	Propanoic acid, 2-methyl-, 3-methylbutyl ester	Esters and analogues	14.38	35.48	35.48			4.00			
155	1-Propanol, 3-(methylthio)-	Alcohols	13.75	38.71	25.81			4.00	4.55		
156	2-Cyclohexen-1-one	Ketones	13.75	19.35	19.35	7.14		12.00	9.09	6.25	23.08
157	2-Propanone, 1-hydroxy-	Ketones	13.75	29.03	22.58			20.00	4.55		
158	2-Propenenitrile	Nitrogen-containing	13.75	6.45	12.90	7.14		16.00	9.09	18.75	46.15
159	Acetonitrile	Nitrogen-containing	13.75	22.58	16.13	14.29	12.50	4.00	27.27		
160	Benzene, 1,2,3-trimethyl-	Aromatics	13.75	16.13	25.81	14.29		12.00	4.55	12.50	7.69
161	Cyclopropane, ethylidene-	Cyclic aliphatic	13.75	9.68	19.35		12.50	12.00	22.73	12.50	15.38
162	Ethanol, 2-ethoxy-	Alcohols	13.75	22.58	12.90	14.29	12.50	4.00	9.09	25.00	7.69
163	Hexanoic acid, butyl ester	Esters and analogues	13.75	41.94				20.00	18.18		
164	Methional	Aldehydes	13.75	38.71	9.68			24.00	4.55		
165	Propanoic acid, propyl ester	Esters and analogues	13.75	25.81	12.90			20.00	22.73		
166	Tetradecane	Linear aliphatic	13.75	12.90	9.68	14.29	12.50	12.00	22.73	12.50	15.38
167	1-Octen-3-one	Ketones	13.13	19.35	16.13	7.14		16.00	9.09	12.50	7.69

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168	1,4-Dioxane	Ethers	13.13	16.13	9.68	21.43		24.00	9.09	12.50	
169	2-Pentanone, 5-hydroxy-	Ketones	13.13	12.90	9.68			24.00	31.82		7.69
170	3-Penten-2-ol	Alcohols	13.13	16.13	9.68			12.00	40.91	6.25	
171	3(2H)-Thiophenone, dihydro-2-methyl-	Ketones	13.13	22.58	35.48				13.64		
172	Ethane, 1,1-bis(methylthio)-	Sulphur-containing	13.13	41.94	25.81						
173	Ethanol, 2-butoxy-	Alcohols	13.13	6.45	3.23	7.14		24.00	27.27	25.00	7.69
174	Ethyl formate	Esters and analogues	13.13	32.26	12.90	7.14		8.00	4.55	12.50	7.69
175	Methyl formate	Esters and analogues	13.13	16.13	12.90	7.14		16.00	22.73	6.25	7.69
176	Oxazole	Aromatics	13.13	29.03	22.58				4.55	25.00	
177	1,3-Dioxolane	Ethers	12.50	3.23	9.68	7.14	25.00	20.00	9.09	12.50	30.77
178	1,3,6-Trioxocane, 2-methyl-	Ethers	12.50	32.26	12.90			4.00	13.64	6.25	7.69
179	2-Octene	Linear aliphatic	12.50	3.23	22.58			16.00	22.73	12.50	7.69
180	2H-Pyran-2-one, tetrahydro-	Ethers	12.50	16.13	9.68	7.14		8.00	18.18		38.46
181	3-Nonanone	Ketones	12.50	22.58	41.94						
182	Acetic anhydride	Esters and analogues	12.50	19.35	12.90	21.43	12.50		13.64	6.25	15.38
183	Cyanamide, dimethyl-	Nitrogen-containing	12.50	29.03	19.35				4.55	25.00	
184	Cyclohexene	Cyclic aliphatic	12.50	12.90	22.58	14.29	25.00	16.00	4.55		
185	γ-Dodecalactone	Esters and analogues	12.50	35.48	29.03						
186	2-Hexanone	Ketones	11.88	6.45	16.13			16.00	18.18	12.50	15.38
187	2(5H)-Furanone	Esters and analogues	11.88	22.58	9.68			20.00	9.09	12.50	
188	3-Hydroxy-3-methyl-2-butanone	Ketones	11.88	9.68	32.26			12.00	13.64		
189	3-Methylcyclopentyl acetate	Esters and analogues	11.88	9.68	6.45	7.14	25.00	28.00	4.55		23.08
190	Benzenepropanoic acid, ethyl ester	Esters and analogues	11.88	35.48	25.81						
191	Butanal, 2-methyl-	Aldehydes	11.88	25.81	6.45			16.00	18.18	6.25	
192	Butanenitrile	Nitrogen-containing	11.88	29.03	12.90	7.14		12.00	4.55	6.25	
193	Cyclopentene	Cyclic aliphatic	11.88	9.68	19.35			8.00	22.73	6.25	15.38
194	Diethyltoluamide	Nitrogen-containing	11.88	3.23	12.90	7.14		12.00	40.91		7.69
195	Ethane, iodo-	Halogen-containing	11.88	16.13	19.35	21.43			9.09	18.75	
196	Furan, 2-hexyl-	Aromatics	11.88	9.68	29.03			20.00	9.09		
197	Methyl dimethylcarbamate	Esters and analogues	11.88	19.35	19.35			8.00	4.55	25.00	
198	Monomethyl carbonotrithioate	Nitrogen-containing	11.88	38.71	22.58						
199	Nitric oxide	Nitrogen-containing	11.88	22.58	12.90			12.00	13.64	6.25	7.69
200	Propanoic acid, 2-methyl-, propyl ester	Esters and analogues	11.88	29.03	25.81			8.00			
201	Propanoic acid, 2-oxo-	Acids	11.88	6.45	6.45			20.00	36.36	12.50	
202	Propanoic acid, ethyl ester	Esters and analogues	11.88	38.71	12.90			8.00	4.55		
203	1-Butanol, 3-methyl-, acetate	Esters and analogues	11.25	35.48	16.13			8.00			
204	1,2,5-Thiadiazole	Aromatics	11.25	29.03	16.13				13.64	6.25	
205	2(3H)-Furanone, dihydro-3-methyl-	Esters and analogues	11.25		32.26			8.00	27.27		
206	2(3H)-Furanone, dihydro-5-propyl-	Esters and analogues	11.25	25.81	12.90		12.50	12.00	9.09		
207	3-Octanone	Ketones	11.25	12.90	16.13			4.00	18.18	25.00	
208	Benzene, (1-pentylheptyl)-	Aromatics	11.25	9.68	9.68	7.14		24.00	4.55	12.50	15.38
209	Benzene, (1-propyloctyl)-	Aromatics	11.25			21.43	25.00	12.00	13.64	25.00	23.08
210	Benzene, 1-chloro-4-(trifluoromethyl)-	Aromatics	11.25	12.90	6.45	7.14		16.00	9.09	18.75	15.38
211	Butanoic acid, 2-methylpropyl ester	Esters and analogues	11.25	22.58	12.90			16.00	13.64		
212	Cyclohexanol	Alcohols	11.25	6.45	41.94			12.00			
213	Formamide	Nitrogen-containing	11.25	29.03	9.68			12.00	4.55	12.50	
214	Methanesulfonyl chloride	Halogen-containing	11.25	25.81	22.58	7.14		8.00			
215	Oxime-, methoxy-phenyl-	Ethers	11.25	3.23	9.68			8.00	22.73	31.25	15.38
216	Phenol, 2-methyl-	Alcohols	11.25	25.81	25.81			4.00	4.55		
217	Phenol, 4-(1,1-dimethylpropyl)-	Alcohols	11.25	19.35	19.35			12.00	9.09	6.25	
218	Phenol, p-tert-butyl-	Alcohols	11.25	6.45	3.23	7.14		8.00	22.73	25.00	23.08
219	Propanenitrile	Nitrogen-containing	11.25	12.90	22.58	7.14	12.50	16.00	4.55		
220	1-Butanol, 3-methyl-, propanoate	Esters and analogues	10.63	25.81	16.13			16.00			
221	2-Hexanone, 5-methyl-	Ketones	10.63	9.68	32.26			12.00	4.55		
222	3-Penten-2-one	Ketones	10.63	22.58	16.13			8.00	9.09		7.69
223	5,9-Undecadien-2-one, 6,10-dimethyl-	Ketones	10.63	12.90	22.58	14.29		12.00	4.55		
224	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	Ketones	10.63	12.90	16.13	14.29	12.50	8.00	9.09	6.25	
225	Carbohydrazide	Nitrogen-containing	10.63	12.90	3.23		12.50	8.00	13.64	25.00	15.38
226	Heptane, hexadecafluoro-	Halogen-containing	10.63	9.68	19.35			12.00	18.18		7.69

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227	Tetrachloroethylene	Halogen-containing	10.63	3.23	6.45	35.71	37.50		13.64	12.50	7.69
228	Thiophene	Aromatics	10.63	12.90	9.68	7.14	12.50	4.00	22.73	6.25	7.69
229	1-Hepten-3-one	Ketones	10.00	25.81	19.35			8.00			
230	1-Octene	Linear aliphatic	10.00	16.13	12.90			12.00	4.55	12.50	7.69
231	1-Penten-3-one	Ketones	10.00		19.35			12.00	18.18	12.50	7.69
232	1,2,4-Trifluorobenzene	Halogen-containing	10.00	16.13	16.13	14.29	12.50	4.00	4.55		7.69
233	1,3-Propanediol	Alcohols	10.00	25.81	16.13			12.00			
234	1H-Pyrrole, 2,5-dimethyl-	Aromatics	10.00	16.13	9.68			16.00	13.64	6.25	
235	2-Octanone	Ketones	10.00	3.23	12.90	7.14	12.50	24.00	13.64		
236	2-Phenylpropenal	Aldehydes	10.00	29.03	16.13			8.00			
237	2-Thiophenecarboxaldehyde	Aldehydes	10.00	6.45	6.45			12.00	18.18	25.00	7.69
238	2,3-Butanedione	Ketones	10.00	6.45	12.90		12.50	24.00	9.09	6.25	
239	3-Furaldehyde	Aldehydes	10.00	12.90	16.13	7.14		4.00	9.09	12.50	7.69
240	3,5-Dithiahexanol 5,5-dioxide	Alcohols	10.00	19.35	22.58		12.50	4.00		6.25	
241	5-Hepten-2-one, 6-methyl-	Ketones	10.00	9.68	3.23			8.00	22.73	25.00	7.69
242	Acetonitrile,2-(methylimino)	Nitrogen-containing	10.00	29.03	6.45				9.09	18.75	
243	Benzene, (1-butyloctyl)-	Aromatics	10.00	12.90	12.90	7.14	12.50		4.55	12.50	23.08
244	Benzyl alcohol	Alcohols	10.00	19.35	12.90	7.14		4.00		25.00	
245	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	Cyclic aliphatic	10.00	6.45	19.35	7.14	12.50	12.00	9.09		7.69
246	Ethyl Acetate	Esters and analogues	10.00	22.58	9.68	14.29			4.55	12.50	7.69
247	Ethylenediamine	Nitrogen-containing	10.00	9.68				12.00	18.18	25.00	15.38
248	Formamide, N-methylthio	Nitrogen-containing	10.00	32.26	12.90				4.55	6.25	
249	Furan, 2-pentyl-	Aromatics	10.00	6.45	16.13			16.00	4.55	12.50	15.38
250	Pentane, 1,1,2,2,3,3,4,4-octafluoro-	Halogen-containing	10.00	3.23	12.90	28.57	12.50	12.00	9.09	6.25	
251	1-Pentene, 2,4,4-trimethyl-	Linear aliphatic	9.38	12.90	6.45		12.50	20.00	4.55	12.50	
252	2-Pentanone, 4-hydroxy-4-methyl-	Ketones	9.38	6.45	12.90			16.00	22.73		
253	Acetaldehyde	Aldehydes	9.38	12.90	12.90	7.14		20.00		6.25	
254	Acetamide, N-(2-methylpropyl)-	Nitrogen-containing	9.38	9.68	16.13			8.00	22.73		
255	Benzene, 1,2,4,5-tetrafluoro-3-(trifluoromethyl)-	Halogen-containing	9.38	6.45	12.90	7.14	12.50	12.00	4.55		23.08
256	Formic acid, butyl ester	Esters and analogues	9.38	16.13	16.13			8.00	13.64		
257	Furfural	Aromatics	9.38	3.23	9.68			12.00	13.64	6.25	30.77
258	Indane	Aromatics	9.38	19.35	9.68	7.14	12.50			12.50	15.38
259	Pentanoic acid, 4-methyl-	Acids	9.38	12.90	16.13			12.00	13.64		
260	α-Pinene	Aromatics	9.38	3.23	12.90	7.14	12.50	8.00	22.73		7.69
261	1-Propanol	Alcohols	8.75	12.90	6.45	7.14		4.00	13.64	12.50	7.69
262	1,1,1,4,4,4-Hexafluorobut-2-ene	Halogen-containing	8.75	9.68	9.68			8.00		18.75	23.08
263	1,3-Oxathiane	Sulphur-containing	8.75	22.58	6.45			12.00	9.09		
264	2-Butenal, 2-methyl-	Aldehydes	8.75	25.81	6.45	7.14		8.00	4.55		
265	2-Butenal, 3-methyl-	Aldehydes	8.75	22.58	16.13			8.00			
266	2-Cyclohexene-1-methanol, 2,6,6-trimethyl-	Alcohols	8.75	22.58	16.13			4.00	4.55		
267	2-Decanone	Ketones	8.75	12.90	22.58			4.00	9.09		
268	2-Ethylacrolein	Aldehydes	8.75	3.23	3.23	14.29	12.50	28.00	4.55	6.25	
269	2-Heptenal	Aldehydes	8.75	12.90	12.90			12.00	13.64		
270	2-Hexenal	Aldehydes	8.75	9.68	9.68		12.50	8.00	4.55	18.75	7.69
271	2(3H)-Furanone, 5-hexyldihydro-	Esters and analogues	8.75	12.90	25.81				9.09		
272	Aziridine, 1-ethenyl-	Nitrogen-containing	8.75	29.03	16.13						
273	Benzene, n-butyl-	Aromatics	8.75	12.90	19.35	14.29			4.55		7.69
274	Benzonitrile	Nitrogen-containing	8.75	6.45	6.45			12.00	4.55	18.75	23.08
275	Cyclohexane, methyl-	Cyclic aliphatic	8.75	6.45	6.45	14.29	12.50	20.00	9.09		
276	Cyclohexanone	Ketones	8.75	22.58	16.13	7.14	12.50				
277	Cyclohexene, 1-methyl-	Cyclic aliphatic	8.75	9.68	3.23		12.50	12.00	22.73		7.69
278	Cyclopentanone, 2-methyl-	Ketones	8.75	19.35	16.13	7.14		4.00			7.69
279	dl-Alanyl-l-alanine	Nitrogen-containing	8.75	3.23	6.45			20.00	13.64	18.75	
280	Ethane, 1,2-dichloro-	Halogen-containing	8.75	6.45	16.13			16.00		6.25	15.38
281	Ethanol, 2,2'-oxybis-	Alcohols	8.75	22.58	12.90			8.00	4.55		
282	Furan, 2-propyl-	Aromatics	8.75		16.13				27.27		23.08
283	Hexadecane	Linear aliphatic	8.75	6.45	12.90			16.00	18.18		
284	Methyl nitrite	Nitrogen-containing	8.75	9.68	12.90				9.09	18.75	15.38
285	Methylene chloride	Halogen-containing	8.75	6.45	6.45	14.29		28.00		6.25	

Sr. No.	Compound names (NIST libray hits)	Compound class	Percentage of donor samples in which VOC was detected	Percentage of donor 2020-01 samples in which VOC was detected	Percentage of donor 2020-02 samples in which VOC was detected	Percentage of donor 2020-03 samples in which VOC was detected	Percentage of donor 2020-04 samples in which VOC was detected	Percentage of donor 2021-06 samples in which VOC was detected	Percentage of donor 2021-08 samples in which VOC was detected	Percentage of donor 2021-10 samples in which VOC was detected	Percentage of donor 2021-11 samples in which VOC was detected
286	Pentanoic acid, 4-methyl-, pentyl ester	Esters and analogues	8.75	22.58	6.45			12.00	9.09		
287	β-Phellandrene	Cyclic aliphatic	8.75	12.90	19.35	7.14		8.00	4.55		
288	1-Ethylcyclopentene	Cyclic aliphatic	8.13	6.45	6.45			4.00	27.27	6.25	7.69
289	1-Octen-3-ol	Alcohols	8.13	19.35	16.13			8.00			
290	1,3-Octadiene	Linear aliphatic	8.13	19.35	16.13				4.55	6.25	
291	1H-Pyrrole, 1-pentyl-	Aromatics	8.13	29.03	12.90						
292	2-Cyclopenten-1-one, 2-methyl-	Ketones	8.13	12.90	6.45		12.50	16.00	9.09		
293	2-Octen-1-ol	Alcohols	8.13					8.00	31.82	12.50	15.38
294	2-Piperidinone	Ketones	8.13	32.26	9.68						
295	2,3-Pentanedione	Ketones	8.13	22.58	12.90			8.00			
296	3-Pentanone	Ketones	8.13	3.23	9.68			16.00	18.18	6.25	
297	Acetamide	Nitrogen-containing	8.13	29.03				12.00	4.55		
298	Acetic acid	Acids	8.13	3.23	6.45		12.50	8.00	13.64	12.50	15.38
299	Benzaldehyde, 2-methyl-	Aldehydes	8.13	19.35	12.90			4.00		6.25	7.69
300	Benzene, 1-ethyl-3-methyl-	Aromatics	8.13	9.68	16.13	7.14		8.00	9.09		
301	Butane, 1-chloro-	Halogen-containing	8.13	3.23	3.23	14.29		12.00	4.55	12.50	23.08
302	Carbonic acid, dimethyl ester	Esters and analogues	8.13	9.68	3.23	7.14		16.00	9.09	6.25	7.69
303	D-Limonene	Cyclic aliphatic	8.13	19.35	19.35				4.55		
304	Methane, isocyanato-	Nitrogen-containing	8.13	6.45	12.90				13.64	25.00	
305	Methane, nitro-	Nitrogen-containing	8.13	6.45	3.23	7.14		8.00	18.18		23.08
306	N-(3-Methylbutyl)acetamide	Nitrogen-containing	8.13	9.68	19.35				18.18		
307	Naphthalene	Aromatics	8.13	9.68	16.13				4.55	12.50	15.38
308	Nonadecane	Linear aliphatic	8.13	16.13	6.45			8.00	9.09		15.38
309	Pentadecane	Linear aliphatic	8.13	12.90	25.81	7.14					
310	Pentane, 1-chloro-	Halogen-containing	8.13	9.68	9.68			8.00	22.73		
311	Pentane, 3-methyl-	Linear aliphatic	8.13	3.23	6.45	14.29	12.50	12.00	9.09	6.25	7.69
312	Perfluorooctane	Halogen-containing	8.13	6.45	12.90	7.14	12.50	8.00	9.09		7.69
313	Phenol, 2-chloro-	Alcohols	8.13	3.23	12.90			20.00	13.64		
314	Propanoic acid, 3-amino-2-methyl-	Acids	8.13	16.13	6.45	7.14	12.50			18.75	7.69
315	Thiirane, methyl-	Sulphur-containing	8.13	19.35	12.90			8.00	4.55		
316	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	Cyclic aliphatic	8.13	3.23	12.90			16.00	9.09		15.38
317	α-Methylstyrene	Aromatics	8.13	16.13	12.90			4.00			23.08
318	(R)-(-)-3-Methyl-2-butanol	Alcohols	7.50	12.90	25.81						
319	2-Propanol, 2-methyl-	Alcohols	7.50	3.23	6.45			16.00	18.18		7.69
320	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	Alcohols	7.50	3.23	3.23	7.14	12.50	12.00		12.50	23.08
321	3-Undecanone	Ketones	7.50	6.45	32.26						
322	3,5-Difluorophenol	Halogen-containing	7.50	3.23	9.68			20.00	9.09		7.69
323	Benzene, 1-methoxy-2-methyl-	Ethers	7.50	16.13	12.90				13.64		
324	Benzeneacetaldehyde	Aldehydes	7.50	3.23	6.45			8.00	22.73	6.25	7.69
325	Cyclobutanol	Alcohols	7.50					12.00	9.09	18.75	30.77
326	Cyclopentanone	Ketones	7.50		3.23		12.50	8.00	18.18	18.75	7.69
327	Disulfide, bis(1,1,3,3-tetramethylbutyl)	Sulphur-containing	7.50	6.45	6.45			8.00	4.55	12.50	23.08
328	Disulfide, methyl propyl	Sulphur-containing	7.50	22.58	16.13						
329	Ethanol, 2-(vinyloxy)-	Alcohols	7.50	16.13	12.90			8.00	4.55		
330	Formaldehyde	Aldehydes	7.50	6.45	9.68			24.00		6.25	
331	Heptane, 2-methyl-	Linear aliphatic	7.50	9.68	3.23	21.43	25.00		9.09	6.25	
332	Hexane, 1,1,1,2,2,3,3,4,4,5,5,6,6-tridecafluoro-	Halogen-containing	7.50	9.68	3.23		12.50	4.00	4.55	6.25	30.77
333	Methyl isocyanide	Nitrogen-containing	7.50	6.45	16.13			8.00	4.55	6.25	7.69
334	N,N-Dimethylacetamide	Nitrogen-containing	7.50	25.81	9.68			4.00			
335	Nonane, 2,2,4,4,6,8,8-heptamethyl-	Linear aliphatic	7.50	12.90	9.68	7.14	12.50	4.00	4.55	6.25	
336	Pentane, 3-ethyl-2,2-dimethyl-	Linear aliphatic	7.50	12.90	16.13	7.14					15.38
337	Propanoic acid, anhydride	Esters and analogues	7.50	19.35	12.90			8.00			
338	Pyrazine, 3,5-dimethyl-2-propyl-	Aromatics	7.50	22.58	3.23			8.00	9.09		
339	Sulfur dioxide	Sulphur-containing	7.50	3.23	9.68		12.50	20.00	4.55		7.69
340	Undecanal	Aldehydes	7.50	12.90	9.68	7.14	12.50	8.00	4.55		
341	Undecane	Linear aliphatic	7.50	9.68	9.68	7.14	25.00	4.00	4.55		7.69
342	1-Pentene, 2,4-dimethyl-	Linear aliphatic	6.88	3.23	6.45		12.50	12.00	13.64		7.69
343	1,2-Dithiolane	Sulphur-containing	6.88	22.58	9.68				4.55		
344	2-Butanone, 3,3-dimethyl-	Ketones	6.88	6.45	12.90	7.14		12.00	4.55		

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345	2-Heptanone	Ketones	6.88	3.23	6.45			16.00	9.09	6.25	7.69
346	2-Heptanone, 5-methyl-	Ketones	6.88	3.23	32.26						
347	2-Hexen-1-ol	Alcohols	6.88		6.45	7.14		8.00	27.27		
348	2-Pentanol, 4-methyl-	Alcohols	6.88	25.81	9.68						
349	2,3,5-Trimethyl-6-ethylpyrazine	Aromatics	6.88	6.45	6.45			12.00	18.18		
350	Benzene, iodo-	Halogen-containing	6.88	3.23	12.90	14.29	12.50	4.00	4.55		7.69
351	Benzofuran	Aromatics	6.88	6.45	12.90	7.14	12.50	4.00			15.38
352	Benzoic acid, methyl ester	Esters and analogues	6.88		3.23	7.14		12.00	9.09	18.75	7.69
353	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	Ketones	6.88	3.23	12.90	14.29	25.00	4.00			7.69
354	Butanal, 3-methyl-	Aldehydes	6.88			7.14	25.00		13.64	6.25	30.77
355	Propionitrile	Nitrogen-containing	6.88	12.90	6.45				4.55	25.00	
356	Decane	Linear aliphatic	6.88	6.45	6.45	7.14			13.64	12.50	7.69
357	Heptanonitrile	Nitrogen-containing	6.88	32.26					4.55		
358	Hexanal, 2-ethyl-	Aldehydes	6.88		3.23			8.00	4.55	31.25	15.38
359	Methyl n-hexyl disulfide	Sulphur-containing	6.88	22.58	12.90						
360	n-Hexylmethylamine	Nitrogen-containing	6.88	9.68	19.35			4.00	4.55		
361	N-α,N-ω-Di-cbz-L-arginine	Nitrogen-containing	6.88	3.23	3.23	7.14	12.50	16.00	9.09		7.69
362	o-Xylene	Aromatics	6.88		3.23			16.00	18.18	12.50	
363	Octanoic acid, ethyl ester	Esters and analogues	6.88	35.48							
364	phenol, 2-(1,1,3,3-tetramethylbutyl)-	Alcohols	6.88	6.45	3.23	14.29	12.50	12.00	9.09		
365	Quinoline	Aromatics	6.88	19.35	16.13						
366	Thiourea, tetramethyl-	Nitrogen-containing	6.88	9.68	6.45			16.00	9.09		
367	trans-2-Nonenal	Aldehydes	6.88	22.58	12.90						
368	1-Decene	Linear aliphatic	6.25	6.45	6.45	7.14		8.00	4.55		15.38
369	1-Octanol, 2-butyl-	Alcohols	6.25	6.45	3.23	7.14		8.00		6.25	23.08
370	2-Butenal	Aldehydes	6.25	16.13	6.45		12.50	8.00			
371	2-Cyclohexen-1-one, 3-methyl-	Ketones	6.25	12.90	12.90			4.00	4.55		
372	2-Oxetanone, 4-methyl-	Ketones	6.25	6.45	3.23				4.55	37.50	
373	2-Propanol, 1,1,1-trichloro-2-methyl-	Alcohols	6.25		32.26						
374	2,3-Bis(trifluoromethyl)buta-1,3-diene	Halogen-containing	6.25	6.45	3.23		25.00	4.00	9.09	6.25	7.69
375	2,4-Di-tert-butylphenol	Alcohols	6.25	9.68	6.45				9.09	12.50	7.69
376	3-Methyl-2-(2-methyl-2-butenyl)-furan	Aromatics	6.25	3.23	9.68			12.00	13.64		
377	3-Octene	Linear aliphatic	6.25	6.45	3.23	7.14		8.00	9.09	12.50	
378	Acetic acid, phenyl ester	Esters and analogues	6.25	22.58	9.68						
379	Benzene, (1-propylnonyl)-	Aromatics	6.25	6.45	9.68	7.14		8.00		6.25	7.69
380	Benzene, pentyl-	Aromatics	6.25	12.90	6.45			8.00	4.55		7.69
381	Benzenecarbothioic acid, S-methyl ester	Esters and analogues	6.25	9.68	22.58						
382	Benzestrol	Alcohols	6.25	16.13	6.45			4.00	9.09		
383	Benzofuran, 2-methyl-	Aromatics	6.25	3.23	22.58	7.14		4.00			
384	Butane, 2,2,3,3-tetramethyl-	Linear aliphatic	6.25	3.23	12.90	7.14		12.00	4.55		
385	Butanenitrile, 3-methyl-	Nitrogen-containing	6.25	16.13	16.13						
386	Camphene	Aromatics	6.25	12.90		21.43	12.50				15.38
387	Cyclohexane, isothiocyanato-	Nitrogen-containing	6.25	6.45	6.45			4.00		18.75	15.38
388	Cyclopentane, ethyl-	Cyclic aliphatic	6.25	12.90	16.13				4.55		
389	Decanal	Aldehydes	6.25	6.45	3.23			4.00	18.18	12.50	
390	Dibutyl phthalate	Esters and analogues	6.25	12.90	12.90	7.14	12.50				
391	Dimethylamine	Nitrogen-containing	6.25	22.58	9.68						
392	Ethane, 1,2-dichloro-1,1,2,2-tetrafluoro-	Halogen-containing	6.25	9.68	9.68			12.00	4.55		
393	Ether, 1-butylvinyl methyl	Ethers	6.25	16.13	12.90	7.14					
394	Ethyl methanesulfinate	Sulphur-containing	6.25	32.26							
395	Heptane, 3-methyl-	Linear aliphatic	6.25	3.23	6.45	7.14	12.50	12.00		6.25	7.69
396	Hexane, 1-(methylthio)-	Sulphur-containing	6.25	22.58	9.68						
397	Naphthalene, 1-methyl-	Aromatics	6.25	12.90	12.90	7.14			4.55		
398	Octane, 1-chloro-	Halogen-containing	6.25	6.45	12.90	7.14		4.00	9.09		
399	Pentane, 1-methoxy-	Ethers	6.25	6.45	12.90	7.14		12.00			
400	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	Esters and analogues	6.25		3.23	14.29		4.00	9.09		30.77
401	Pentanoic acid, 3-methylbutyl ester	Esters and analogues	6.25	6.45	25.81						
402	Phenol, 2-methoxy-	Alcohols	6.25		9.68			4.00	27.27		
403	Propanal	Aldehydes	6.25	3.23	6.45	7.14		20.00			7.69

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404	Propane, 2,2-difluoro-	Halogen-containing	6.25		6.45	14.29		8.00	4.55	6.25	15.38
405	Pyrazine, 2-ethyl-6-methyl-	Aromatics	6.25	19.35	6.45			8.00			
406	Pyrimidine, 4-methyl-	Aromatics	6.25	16.13	12.90					6.25	
407	(3aR,4R,8R,8aS)-3a,4,7,8a-Tetramethyl-1,2,3,3a,4,5,8,8a-octahydro-4,8-methanoazulene	Cyclic aliphatic	5.63	22.58	3.23	7.14					
408	1-Hexanol, 2-ethyl-	Alcohols	5.63	6.45	9.68			4.00	13.64		
409	1-Penten-3-ol	Alcohols	5.63	9.68	16.13			4.00			
410	1,2,4,5-Tetrazin-3-amine, 6-methyl-	Nitrogen-containing	5.63	6.45	16.13					12.50	
411	1,3,5-Trifluorobenzene	Halogen-containing	5.63	3.23	9.68	14.29	12.50	4.00	4.55		
412	1,4-Dioxin, 2,3-dihydro-	Ethers	5.63	6.45	9.68	7.14		4.00	4.55		7.69
413	2-Hydroxy-3-hexanone	Ketones	5.63	3.23				16.00	18.18		
414	2-n-Heptylfuran	Aromatics	5.63		25.81	7.14					
415	2-Pentanol, 3-methyl-	Alcohols	5.63	3.23	19.35				9.09		
416	2-Propanol, 1,1,1,3,3,3-hexafluoro-	Halogen-containing	5.63	9.68	9.68	7.14				6.25	7.69
417	2(3H)-Furanone, 5-butyldihydro-	Esters and analogues	5.63		16.13			12.00	4.55		
418	2(3H)-Furanone, dihydro-5-pentyl-	Esters and analogues	5.63	6.45	12.90			12.00			
419	2H-1,2-Oxazine, 6-(4-chlorophenyl)tetrahydro-2-methyl-	Aromatics	5.63		3.23			8.00	18.18		15.38
420	3-Trifluoroacetoxypentadecane	Halogen-containing	5.63	12.90	3.23			4.00	4.55	6.25	7.69
421	Benzene, 1-methoxy-4-methyl-	Ethers	5.63		16.13				18.18		
422	Benzene, 1,4-dichloro-	Aromatics	5.63	9.68	6.45	14.29		4.00			7.69
423	Benzoxazole	Aromatics	5.63	12.90	12.90					6.25	
424	Butanoic acid, 1-methylpropyl ester	Esters and analogues	5.63	22.58	6.45						
425	Carbonyl sulfide	Sulphur-containing	5.63		6.45	7.14	12.50		4.55		30.77
426	Cyclopentanol	Alcohols	5.63	9.68	19.35						
427	Cyclopentene, 1-methyl-	Cyclic aliphatic	5.63	6.45	9.68	7.14		8.00			7.69
428	Cyclopentene, 3-methyl-	Cyclic aliphatic	5.63	3.23	9.68	7.14	12.50	8.00			7.69
429	Eucalyptol	Ethers	5.63	6.45	3.23			8.00	18.18		
430	Formic acid, propyl ester	Esters and analogues	5.63	19.35				8.00	4.55		
431	Glycine, ethyl ester	Esters and analogues	5.63	16.13	3.23			8.00	4.55		
432	Isothiazole	Aromatics	5.63	16.13	6.45			4.00	4.55		
433	Methanethiol	Sulphur-containing	5.63	12.90	6.45			8.00	4.55		
434	Octane, 2-methyl-	Linear aliphatic	5.63	3.23	9.68	7.14		4.00	13.64		
435	Oxirane, 2-methyl-2-(1-methylethyl)-	Ethers	5.63	6.45	9.68	7.14			9.09		7.69
436	p-Cymene	Aromatics	5.63	3.23	6.45				4.55	12.50	23.08
437	Phenol	Alcohols	5.63	3.23	12.90	7.14		8.00	4.55		
438	Phenol, 4-ethyl-	Alcohols	5.63	16.13	9.68				4.55		
439	Phthalic anhydride	Esters and analogues	5.63	9.68	6.45	21.43	12.50				
440	Propanoic acid, 2,2-dimethyl-	Acids	5.63	3.23	3.23				22.73		15.38
441	Propanoic acid, pentyl ester	Esters and analogues	5.63	16.13	12.90						
442	Thiirane	Sulphur-containing	5.63	6.45				20.00	4.55	6.25	
443	Trichloromonofluoromethane	Halogen-containing	5.63	6.45	9.68	7.14		4.00	4.55		7.69
444	β-Myrcene	Linear aliphatic	5.63	6.45	3.23	7.14	25.00	4.00	9.09		
445	1-Butene	Linear aliphatic	5.00	3.23	3.23	7.14	12.50	12.00	4.55		
446	1-Fluoro-3-(trifluoro-methyl)benzene	Halogen-containing	5.00					28.00			7.69
447	1-Propene, 2-methyl-	Linear aliphatic	5.00	12.90	6.45			8.00			
448	1-Tetradecene	Linear aliphatic	5.00	12.90	6.45			8.00			
449	1,1,1-Trifluoro-2-propanol	Halogen-containing	5.00	12.90	3.23	7.14				6.25	7.69
450	1,2,4,5-Tetroxane, 3,3,6,6-tetramethyl-	Ethers	5.00	3.23	6.45				9.09	12.50	7.69
451	1H-Isoindole-1,3(2H)-dione, 2-methyl-	Ketones	5.00	16.13	9.68						
452	2-Hexene, 3,5-dimethyl-	Linear aliphatic	5.00	3.23	3.23			8.00	4.55		23.08
453	2-Methyl-3-isopropylpyrazine	Aromatics	5.00	25.81							
454	2-Trifluoroacetoxydodecane	Halogen-containing	5.00	6.45	3.23	14.29			9.09	6.25	
455	2-Undecanethiol, 2-methyl-	Alcohols	5.00		6.45			16.00	9.09		
456	2,4-Dimethylfuran	Aromatics	5.00	19.35	3.23				4.55		
457	3-Decanone	Ketones	5.00		25.81						
458	3-Hexen-1-ol	Alcohols	5.00		16.13			4.00	4.55	6.25	
459	5-Hepten-2-one	Ketones	5.00	6.45	19.35						
460	Benzene, (1-butylhexyl)-	Aromatics	5.00		3.23			4.00		18.75	23.08
461	Benzene, (1-ethyldecyl)-	Aromatics	5.00	9.68	9.68		12.50	4.00			
462	Benzene, (1-methylethyl)-	Aromatics	5.00	6.45	3.23	14.29	12.50			6.25	7.69

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463	Benzene, 1-methyl-3-propyl-	Aromatics	5.00	12.90	6.45		12.50				7.69
464	Benzene, 1,2-dichloro-	Aromatics	5.00	6.45	3.23	7.14	12.50	8.00	4.55		
465	Benzene, 1,2-difluoro-	Halogen-containing	5.00		6.45	7.14	37.50	8.00			
466	Benzene, 1,3,5-tri-tert-butyl-	Aromatics	5.00	6.45	3.23	21.43	12.50				7.69
467	Benzene, hexyl-	Aromatics	5.00	12.90	6.45			4.00	4.55		
468	Benzenemethanol, α,α-dimethyl-	Alcohols	5.00	6.45	12.90			8.00			
469	Butane, 1,1,3,4-tetrachloro-1,2,2,3,4,4-hexafluoro-	Halogen-containing	5.00	6.45	3.23			12.00	9.09		
470	Butanenitrile, 2-methyl-	Nitrogen-containing	5.00	16.13			12.50	4.00		6.25	
471	Butanoic acid, 3-methyl-, 3-methylbutyl ester	Esters and analogues	5.00	9.68	3.23			16.00			
472	Carbamic acid, N-[1,1-bis(trifluoromethyl)ethyl]-, 4-(1,1,3,3-tetramethylbutyl)phenyl ester	Halogen-containing	5.00	3.23	3.23			16.00		12.50	
473	Carbon Tetrachloride	Halogen-containing	5.00	9.68	3.23		12.50	12.00			
474	Decanoic acid, ethyl ester	Esters and analogues	5.00	25.81							
475	Ethanol, 2-(methylamino)-	Alcohols	5.00	16.13	9.68						
476	Formamide, N,N-dibutyl-	Nitrogen-containing	5.00	12.90			12.50	8.00			7.69
477	Furan, 2-ethyl-5-methyl-	Aromatics	5.00	3.23	6.45				22.73		
478	Heptane	Linear aliphatic	5.00	9.68	9.68	7.14		4.00			
479	Phenyl trifluoromethyl ether	Halogen-containing	5.00		3.23	14.29		4.00	9.09		15.38
480	Pyridine, 2-ethyl-	Aromatics	5.00	22.58	3.23						
481	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	Aromatics	5.00		3.23		12.50	4.00	9.09	12.50	7.69
482	Thiophene, 2-pentyl-	Aromatics	5.00	6.45	3.23			8.00	9.09	6.25	
483	Tridecane	Linear aliphatic	5.00	3.23	9.68	7.14		8.00	4.55		
484	1-Hexene	Linear aliphatic	4.38		6.45	7.14		8.00		6.25	7.69
485	1H-Pyrrole, 1-butyl-	Aromatics	4.38		3.23			16.00	4.55		7.69
486	2-Butene	Linear aliphatic	4.38	9.68	3.23				4.55		15.38
487	2-Fluoro-3-(trifluoromethyl)phenol	Halogen-containing	4.38		3.23			4.00	9.09	6.25	15.38
488	2-Heptene	Linear aliphatic	4.38	3.23	3.23	14.29		8.00			7.69
489	2-Methyl-3-propylpyrazine	Aromatics	4.38		12.90				13.64		
490	2-n-Octylfuran	Aromatics	4.38	3.23	19.35						
491	2-Nonanol	Alcohols	4.38	6.45	16.13						
492	2-Tridecanone	Ketones	4.38	22.58							
493	2-Trifluoroacetoxypentadecane	Halogen-containing	4.38	6.45	3.23	14.29		4.00	4.55		
494	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	Esters and analogues	4.38	6.45	6.45	7.14		4.00	4.55		
495	2,3-Butanediol	Alcohols	4.38	9.68	9.68				4.55		
496	2,4-Difluorophenol	Halogen-containing	4.38		6.45			12.00	9.09		
497	3-(Methylthio)propyl acetate	Esters and analogues	4.38	22.58							
498	3,5-Difluorobenzaldehyde	Halogen-containing	4.38	3.23	9.68	14.29			4.55		
499	4-Penten-2-ol	Alcohols	4.38		9.68				18.18		
500	Acetic acid ethenyl ester	Esters and analogues	4.38	3.23			12.50	8.00	9.09		7.69
501	Acetophenone	Ketones	4.38	3.23	3.23			12.00		6.25	7.69
502	Benzene, (1-butylnonyl)-	Aromatics	4.38	6.45	3.23			4.00	4.55	6.25	7.69
503	Benzene, (1-propylheptyl)-	Aromatics	4.38		3.23					12.50	30.77
504	Benzene, [2,2,2-trifluoro-1-(trifluoromethyl)ethyl]-	Halogen-containing	4.38	6.45	3.23	7.14	12.50		4.55		7.69
505	Benzene, 1,2-dimethoxy-	Ethers	4.38		3.23				27.27		
506	Benzene, 1,2,4-trimethyl-	Aromatics	4.38	6.45	3.23	7.14		8.00	4.55		
507	Benzene, 1,3-dichloro-	Aromatics	4.38		12.90	7.14	12.50				7.69
508	Benzophenone	Ketones	4.38	6.45	12.90	7.14					
509	Benzyl nitrile	Nitrogen-containing	4.38	16.13	6.45						
510	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	Cyclic aliphatic	4.38	12.90		7.14		8.00			
511	Butanoic acid, 4-pentenyl ester	Esters and analogues	4.38		22.58						
512	Carbamic acid, monoammonium salt	Acids	4.38	12.90	9.68						
513	Diacetyl sulphide	Sulphur-containing	4.38	9.68	3.23	7.14	12.50				7.69
514	Dicyclopentadiene	Cyclic aliphatic	4.38	3.23	3.23			16.00	4.55		
515	Diethyl Phthalate	Esters and analogues	4.38	3.23	3.23	14.29	12.50	8.00			
516	Dodecanal	Aldehydes	4.38		6.45			12.00	9.09		
517	Ethanone, 1-(5-chloro-2-hydroxyphenyl)-	Ketones	4.38	16.13	6.45						
518	Furan, 2,3-dihydro-	Aromatics	4.38			7.14		8.00	13.64		7.69
519	Hexanoic acid	Acids	4.38	3.23	3.23			12.00	4.55		7.69
520	Hydrazinecarboxamide	Nitrogen-containing	4.38	9.68	6.45				9.09		
521	l-Alanine ethylamide	Nitrogen-containing	4.38	12.90	6.45			4.00			

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522	L-Cysteine sulfinic acid	Acids	4.38	3.23	9.68	7.14	12.50	4.00			
523	Monoethanolamine	Alcohols	4.38	3.23	12.90			4.00			7.69
524	N-Methyltaurine	Nitrogen-containing	4.38	3.23	6.45			8.00	4.55	6.25	
525	Nitrous oxide	Nitrogen-containing	4.38	9.68	9.68					6.25	
526	o-Cymene	Aromatics	4.38	9.68	3.23			8.00	4.55		
527	Peroxide, dimethyl	Ethers	4.38	6.45	16.13						
528	Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl)-	Alcohols	4.38		3.23	14.29		4.00		18.75	
529	Propanoic acid, 2-hydroxy-2-methyl-	Acids	4.38	3.23	9.68				13.64		
530	Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	Esters and analogues	4.38	3.23	3.23			12.00	9.09		
531	Thiophene, 2-methyl-	Aromatics	4.38					4.00		18.75	23.08
532	1-Nonene	Linear aliphatic	3.75	9.68	3.23				4.55		7.69
533	1-Octyn-3-ol, 4-ethyl-	Alcohols	3.75					24.00			
534	1-Propanol, 2-amino-, (±)-	Alcohols	3.75	9.68	9.68						
535	1,2-Butanediol	Alcohols	3.75	9.68	6.45			4.00			
536	1,3-Dioxolane, 2-methyl-	Ethers	3.75	6.45				4.00	4.55	6.25	7.69
537	1,4-Cyclohexadiene, 1-methyl-	Cyclic aliphatic	3.75	3.23	3.23				18.18		
538	1H-Indene, 1-ethylidene-	Aromatics	3.75	6.45	12.90						
539	2-Butylcyclopenanone	Ketones	3.75	19.35							
540	2-Furancarboxaldehyde, 5-methyl-	Aldehydes	3.75	6.45	12.90						
541	2-Methyl-2-azatricyclo[4.3.1.0(3,8)]decane	Nitrogen-containing	3.75	19.35							
542	2-Pentene	Linear aliphatic	3.75		3.23	7.14					30.77
543	2,6,6-Trimethylcyclohexa-1,4-dienecarbaldehyde	Aldehydes	3.75				12.50		22.73		
544	3-Hepten-2-one	Ketones	3.75	9.68	9.68						
545	3-Hexen-2-one	Ketones	3.75	16.13	3.23						
546	3,4-dimethylfuran	Aromatics	3.75	6.45	9.68			4.00			
547	Acetaldehyde, hydroxy-	Aldehydes	3.75		6.45	14.29	25.00				
548	Acetic acid, hydrazide	Nitrogen-containing	3.75	3.23				4.00		12.50	15.38
549	Benzene, chloro-	Aromatics	3.75		12.90				9.09		
550	Benzene, propoxy-	Ethers	3.75	12.90	3.23					6.25	
551	Benzene, propyl-	Aromatics	3.75	9.68	6.45		12.50				
552	Benzeneacetic acid, ethyl ester	Esters and analogues	3.75	19.35							
553	Butanal	Aldehydes	3.75		9.68			12.00			
554	Butanamide	Nitrogen-containing	3.75	19.35							
555	Butane	Linear aliphatic	3.75		6.45				18.18		
556	Butane, 1-isocyano-	Nitrogen-containing	3.75	3.23	3.23			4.00	9.09	6.25	
557	Butane,1,2,4-trichloro-heptafluoro-	Halogen-containing	3.75	6.45	9.68	7.14					
558	Butanoic acid, 2-methyl-	Acids	3.75	3.23	6.45			4.00	9.09		
559	Butanoic acid, 4-chloro-	Acids	3.75	3.23	3.23			8.00	9.09		
560	Butanoic acid, methyl ester	Esters and analogues	3.75	9.68				4.00	9.09		
561	Butanoic acid, phenyl ester	Esters and analogues	3.75	19.35							
562	Butylated Hydroxytoluene	Aromatics	3.75	3.23	6.45			4.00	9.09		
563	Carbamic acid, methyl-, ethyl ester	Esters and analogues	3.75	16.13	3.23						
564	Carbonic acid, eicosyl vinyl ester	Esters and analogues	3.75	3.23		21.43		8.00			
565	Cyclopentane, methyl-	Cyclic aliphatic	3.75	3.23	6.45			8.00	4.55		
566	Dotriacontane	Linear aliphatic	3.75	12.90	3.23			4.00			
567	Ethanamine, N,N-difluoro-	Halogen-containing	3.75	9.68	9.68						
568	Ethanol, 2,2,2-trifluoro-	Halogen-containing	3.75		6.45	7.14		8.00		6.25	
569	Ethyl Chloride	Halogen-containing	3.75		3.23			4.00		18.75	7.69
570	Furan, 2-methyl-	Aromatics	3.75	6.45	6.45		12.50		4.55		
571	Glycolaldehyde dimer	Aldehydes	3.75	6.45		21.43	12.50				
572	Heptacosane	Linear aliphatic	3.75	6.45	9.68				4.55		
573	Mesitylene	Aromatics	3.75	3.23	6.45	7.14			4.55		7.69
574	Methanesulfonamide, N,N-dimethyl-	Nitrogen-containing	3.75	12.90	6.45						
575	Methanesulfonyl fluoride	Halogen-containing	3.75	16.13	3.23						
576	Methylal	Ethers	3.75	9.68	6.45					6.25	
577	Nonane	Linear aliphatic	3.75	3.23			12.50	4.00	13.64		
578	Octanal	Aldehydes	3.75	6.45	6.45			8.00			
579	Octane	Linear aliphatic	3.75	3.23	3.23				9.09	6.25	7.69
580	Pentanal	Aldehydes	3.75	6.45	3.23		12.50	8.00			

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581	Pentanal, 2-methyl-	Aldehydes	3.75	9.68				8.00		6.25	
582	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	Esters and analogues	3.75	3.23	9.68	7.14	12.50				
583	Propanenitrile, 3-hydroxy-	Nitrogen-containing	3.75	3.23	3.23	7.14		8.00	4.55		
584	Pyridine, 3-ethyl-	Aromatics	3.75	19.35							
585	Toluene	Aromatics	3.75	3.23	3.23	7.14		8.00			7.69
586	(S)-(+)-1,2-Propanediol	Alcohols	3.13	9.68				4.00		6.25	
587	(Z)-Undec-6-en-2-one	Ketones	3.13	9.68	6.45						
588	1-Butanol, 2-methyl-	Alcohols	3.13	3.23	3.23				4.55	6.25	7.69
589	1-Heptanamine	Nitrogen-containing	3.13	3.23	6.45						15.38
590	1-Octanamine, N-methyl-	Nitrogen-containing	3.13	9.68	3.23			4.00			
591	1,2,3-Trifluorobenzene	Halogen-containing	3.13	12.90	3.23						
592	1,2,4-Trithiolane	Sulphur-containing	3.13	6.45	6.45				4.55		
593	1,3-Pentadiene	Linear aliphatic	3.13	6.45	3.23			4.00	4.55		
594	1,3,2-Dioxaborolan-4-one, 2-ethyl-	Ketones	3.13	6.45				4.00	9.09		
595	12-Methylaminolauric acid	Acids	3.13		3.23			4.00	9.09		7.69
596	1H-Pyrrole-2-ethanamine, 1-methyl-	Aromatics	3.13	6.45	3.23					12.50	
597	2-Amino-1,3-propanediol	Alcohols	3.13		3.23			4.00	13.64		
598	2-Butanone	Ketones	3.13	6.45	6.45					6.25	
599	2-Decen-1-ol	Alcohols	3.13		3.23			12.00	4.55		
600	2-Decenal	Aldehydes	3.13	3.23	12.90						
601	2-Propanamine	Nitrogen-containing	3.13		3.23			8.00	9.09		
602	2,3-Hexanedione	Ketones	3.13		9.68				9.09		
603	3-Methoxybut-1-ene	Ethers	3.13		6.45			4.00		6.25	7.69
604	3',4'-Difluoroacetophenone	Halogen-containing	3.13		9.68	7.14					7.69
605	4-Nonene	Linear aliphatic	3.13	3.23				8.00		6.25	7.69
606	Benzaldehyde	Aldehydes	3.13	6.45	6.45					6.25	
607	Benzene, (1-ethylnonyl)-	Aromatics	3.13		6.45	7.14	12.50			6.25	
608	Benzene, (1-pentyloctyl)-	Aromatics	3.13	6.45	9.68						
609	Benzene, 1-methyl-3-(1-methylethyl)-	Aromatics	3.13	9.68	3.23			4.00			
610	Benzene, 1,3-difluoro-	Halogen-containing	3.13		3.23				4.55	12.50	7.69
611	Benzenepropanoic acid 1-methylethyl ester	Esters and analogues	3.13	16.13							
612	Butanoic acid, 2-methyl-, methyl ester	Esters and analogues	3.13	3.23				4.00	4.55	12.50	
613	Caprolactam	Nitrogen-containing	3.13					12.00		6.25	7.69
614	Cyclohexane, 1-ethyl-2-methyl-	Cyclic aliphatic	3.13	3.23	3.23		12.50			6.25	7.69
615	Cyclohexane, ethyl-	Cyclic aliphatic	3.13	3.23		7.14		4.00	4.55	6.25	
616	Cyclohexanone, 4-ethyl-	Ketones	3.13		12.90			4.00			
617	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	Cyclic aliphatic	3.13	16.13							
618	Cyclohexene, 3,5,5-trimethyl-	Cyclic aliphatic	3.13	3.23	3.23			4.00	4.55		7.69
619	Cyclopentanone, 3-methyl-	Ketones	3.13		12.90			4.00			
620	Cyclopropane, ethyl-	Cyclic aliphatic	3.13	6.45				4.00	4.55		7.69
621	Dodecane	Linear aliphatic	3.13	3.23	6.45	7.14		4.00			
622	Eicosane	Linear aliphatic	3.13	3.23	6.45	14.29					
623	Erythro-3-bromo-2-pentanol	Alcohols	3.13	3.23				4.00	4.55	12.50	
624	Ethane, 1,1,2-trichloro-1,2,2-trifluoro-	Halogen-containing	3.13	6.45				12.00			
625	Ethanesulfonyl fluoride	Halogen-containing	3.13	9.68	6.45						
626	Formamide, N-phenyl-	Nitrogen-containing	3.13		16.13						
627	Formic acid, heptyl ester	Esters and analogues	3.13					12.00	4.55	6.25	
628	Fumaronitrile	Nitrogen-containing	3.13	9.68	3.23			4.00			
629	Heneicosane	Linear aliphatic	3.13	3.23	9.68	7.14					
630	Heptanal	Aldehydes	3.13	3.23	3.23	7.14		8.00			
631	Hexanal	Aldehydes	3.13	3.23	3.23			8.00	4.55		
632	Hexane, 2-methyl-	Linear aliphatic	3.13	9.68			12.50	4.00			
633	Hexane, 2,4-dimethyl-	Linear aliphatic	3.13	3.23	3.23		12.50				15.38
634	Hexanethioic acid, S-methyl ester	Esters and analogues	3.13	6.45	9.68						
635	Hexanoic acid, 2-methyl-	Acids	3.13		3.23			4.00	13.64		
636	Hordenine	Aromatics	3.13	3.23	3.23			4.00		6.25	7.69
637	Isopropyl Alcohol	Alcohols	3.13		6.45			4.00	9.09		
638	l-Leucine, n-butoxycarbonyl-N-methyl-, undecyl ester	Esters and analogues	3.13	3.23				4.00	9.09		7.69
639	Mequinol	Aromatics	3.13	9.68	6.45						

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640	Methane, diazo-	Nitrogen-containing	3.13	3.23	12.90						
641	Methane, iodo-	Halogen-containing	3.13	6.45	3.23			8.00			
642	Methanesulfonic acid, methyl ester	Esters and analogues	3.13	6.45	6.45				4.55		
643	Methyl isovalerate	Esters and analogues	3.13	6.45	3.23			4.00	4.55		
644	Methyltartronic acid	Acids	3.13	3.23	9.68						7.69
645	n-Hexane	Linear aliphatic	3.13	3.23	6.45			4.00		6.25	
646	Octane, 3-methyl-	Linear aliphatic	3.13			7.14		12.00	4.55		
647	Pentane, 2-methyl-	Linear aliphatic	3.13	9.68	3.23			4.00			
648	Pentanoic acid, 3-methyl-	Acids	3.13	6.45	6.45				4.55		
649	Phenol, 4-(1-methylpropyl)-	Alcohols	3.13	3.23	6.45			4.00	4.55		
650	Phenylephrine	Aromatics	3.13	9.68	3.23			4.00			
651	Propanedioic acid	Acids	3.13	6.45	3.23			4.00	4.55		
652	Propanoic acid, 2-methyl-, 2-phenylethyl ester	Esters and analogues	3.13	6.45	9.68						
653	Propanoic acid, 2-methyl-, pentyl ester	Esters and analogues	3.13		16.13						
654	Propanoic acid, phenyl ester	Esters and analogues	3.13	16.13							
655	Pyrazine, 2,5-dimethyl-	Aromatics	3.13		6.45					18.75	
656	Pyrazine, 3,5-diethyl-2-methyl-	Aromatics	3.13	9.68	3.23			4.00			
657	(E)-4,8-Dimethylnona-1,3,7-triene	Linear aliphatic	2.50					16.00			
658	1-Butanol	Alcohols	2.50	9.68	3.23						
659	1-Butene, 3-methyl-	Linear aliphatic	2.50	6.45				4.00	4.55		
660	1-Decanol, 2-ethyl-	Alcohols	2.50	3.23		7.14			4.55		7.69
661	1-Hexene, 4-methyl-	Linear aliphatic	2.50		3.23			12.00			
662	1-Propen-2-ol, acetate	Esters and analogues	2.50		6.45			8.00			
663	1-Tridecene	Linear aliphatic	2.50	12.90							
664	1,4-Dioxane, 2,5-dimethyl-	Ethers	2.50					4.00	9.09		7.69
665	1,4-Pentadiene	Linear aliphatic	2.50	3.23		14.29				6.25	
666	2-Acetyl-1-pyrroline	Aromatics	2.50	12.90							
667	2-Isopropylpyrazine	Aromatics	2.50	12.90							
668	2-Piperidinone, N-[4-bromo-n-butyl]-	Ketones	2.50						9.09		15.38
669	2-Propanone, 1,1,1-trifluoro-	Halogen-containing	2.50						9.09	6.25	7.69
670	2,3-Dimethyl-5-n-propylpyrazine	Aromatics	2.50	3.23	9.68						
671	2,5-Difluorobenzaldehyde	Halogen-containing	2.50				12.50	4.00	4.55	6.25	
672	2,5-Pyrrolidinedione, 1-methyl-	Ketones	2.50	9.68	3.23						
673	3-Heptanone	Ketones	2.50		9.68		12.50				
674	3-Hepten-1-ol	Alcohols	2.50		12.90						
675	3-Hexanone, 2,5-dimethyl-4-nitro-	Ketones	2.50		3.23			4.00	9.09		
676	3-Methyl-2-thiophenecarboxaldehyde	Aldehydes	2.50	12.90							
677	3-Octen-2-one	Ketones	2.50	3.23	9.68						
678	3-Penten-1-ol, 2,2,4-trimethyl-	Alcohols	2.50		3.23				4.55		15.38
679	4-Cyanocyclohexene	Nitrogen-containing	2.50	3.23	6.45			4.00			
680	4-Heptanone	Ketones	2.50		9.68				4.55		
681	4-Hydroxy-3-hexanone	Ketones	2.50	9.68	3.23						
682	4-Penten-2-one, 3-methyl-	Ketones	2.50	12.90							
683	Benzene, 1-fluoro-2-(trifluoromethyl)-	Halogen-containing	2.50		3.23	14.29	12.50				
684	Benzene, 1,2,3,4-tetrafluoro-	Halogen-containing	2.50	3.23	9.68						
685	Benzene, 1,3-dimethyl-	Aromatics	2.50					8.00		12.50	
686	Benzene, fluoro-	Halogen-containing	2.50	6.45	3.23			4.00			
687	Benzeneethanamine, 2-fluoro-β,3-dihydroxy-N-methyl-	Halogen-containing	2.50	3.23	6.45			4.00			
688	Benzothiazole	Aromatics	2.50	3.23	3.23			8.00			
689	Biphenyl	Aromatics	2.50	6.45		7.14		4.00			
690	Butane, 2,2-dimethyl-	Linear aliphatic	2.50	3.23				4.00	4.55		7.69
691	Butanoic acid, 2-propenyl ester	Esters and analogues	2.50	12.90							
692	Butanoic acid, 3-methyl-, propyl ester	Esters and analogues	2.50	6.45				8.00			
693	1,3-Hexadiene	Linear aliphatic	2.50		3.23				4.55	6.25	7.69
694	Carbamic acid, methyl ester	Esters and analogues	2.50	9.68	3.23						
695	Carbon disulfide	Sulphur-containing	2.50		6.45	7.14			4.55		
696	Cyanamide	Nitrogen-containing	2.50		9.68				4.55		
697	Cyanopyrazine	Aromatics	2.50	12.90							
698	Cyclohexane, 1,1,3-trimethyl-	Cyclic aliphatic	2.50	6.45	3.23						7.69

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699	Cyclooctane, methyl-	Cyclic aliphatic	2.50	3.23			12.50				15.38
700	Cyclopropane, 1-ethyl-2-methyl-, cis-	Cyclic aliphatic	2.50	6.45		14.29					
701	Cyclopropane, 1,1-dimethyl-	Cyclic aliphatic	2.50	3.23	3.23			4.00		6.25	
702	Diisobutyl cellosolve	Linear aliphatic	2.50	3.23	3.23					12.50	
703	Dimethyl Sulfoxide	Sulphur-containing	2.50	6.45	6.45						
704	Ethanol	Alcohols	2.50	6.45					9.09		
705	Ethanol, 2-(2-ethoxyethoxy)-	Alcohols	2.50		3.23			8.00	4.55		
706	Ethanol, 2,2-dichloro-	Alcohols	2.50		6.45				9.09		
707	Ethylene, 1,2-dichloro-	Halogen-containing	2.50		6.45				9.09		
708	Fenchone	Ketones	2.50	9.68	3.23						
709	Furan	Aromatics	2.50		3.23			8.00		6.25	
710	Heptacosan-9-ol	Alcohols	2.50		3.23	14.29	12.50				
711	Heptane, 1-chloro-	Halogen-containing	2.50	3.23	3.23	7.14					7.69
712	Hexane, 3-methyl-	Linear aliphatic	2.50	3.23	6.45			4.00			
713	Hydrogen isocyanate	Nitrogen-containing	2.50	9.68	3.23						
714	Isoquinoline	Aromatics	2.50		12.90						
715	Isoserine	Nitrogen-containing	2.50			7.14				12.50	7.69
716	Isoxazole, 5-methyl-	Aromatics	2.50	12.90							
717	Lactic acid	Acids	2.50	9.68	3.23						
718	Mercaptoacetone	Ketones	2.50	9.68	3.23						
719	Methanamine, N-(phenylmethylene)-	Nitrogen-containing	2.50	6.45	6.45						
720	Methane, tribromo-	Halogen-containing	2.50						4.55	12.50	7.69
721	Methyl propionate	Esters and analogues	2.50	3.23	3.23				4.55	6.25	
722	Methyl salicylate	Esters and analogues	2.50					16.00			
723	Methyl valerate	Esters and analogues	2.50	12.90							
724	Methyl vinyl ketone	Ketones	2.50	3.23	9.68						
725	Octadecane, 1-(ethenyloxy)-	Ethers	2.50							18.75	7.69
726	Pentane, 2,3-dimethyl-	Linear aliphatic	2.50	6.45	6.45						
727	Pentane, 2,3,4-trimethyl-	Linear aliphatic	2.50		3.23				13.64		
728	Phenol, 2-(1-methylethyl)-	Alcohols	2.50		6.45				9.09		
729	Phenylethyne	Aromatics	2.50	6.45	3.23					6.25	
730	Quinazoline	Aromatics	2.50		12.90						
731	Tetracosane	Linear aliphatic	2.50	3.23	9.68						
732	Tuaminoheptane	Nitrogen-containing	2.50					16.00			
733	β-Pinene	Aromatics	2.50	9.68	3.23						
734	(Butylamino)acetonitrile	Nitrogen-containing	1.88	9.68							
735	(Z)-3-Heptene	Linear aliphatic	1.88		3.23			4.00	4.55		
736	1-(2,4-Dihydroxyphenyl)-2-(4-methoxy-3-nitrophenyl)ethanone	Ketones	1.88	3.23	6.45						
737	1-Dodecanol	Alcohols	1.88	6.45	3.23						
738	1-Dodecanone, 2-(imidazol-1-yl)-1-(4-methoxyphenyl)-	Ethers	1.88		3.23	7.14		4.00			
739	1-Eicosanol	Alcohols	1.88		3.23	14.29					
740	1-Methyldodecylamine	Nitrogen-containing	1.88		6.45				4.55		
741	1-Nonen-3-ol	Alcohols	1.88	9.68							
742	1-Pentanol, 4-methyl-2-propyl-	Alcohols	1.88			7.14			4.55	6.25	
743	1-Propanesulfonyl chloride	Halogen-containing	1.88		3.23				9.09		
744	1-Propanone, 1-phenyl-	Ketones	1.88	6.45	3.23						
745	1,1'-Biphenyl, 2-methyl-	Aromatics	1.88	3.23	6.45						
746	1,13-Tetradecadiene	Linear aliphatic	1.88	6.45	3.23						
747	1,2-Ethanediamine, N,N,N'-trimethyl-	Nitrogen-containing	1.88	3.23				4.00		6.25	
748	1,2-Ethanediol, monoformate	Esters and analogues	1.88					8.00	4.55		
749	1,3,5-Triazine, hexahydro-1,3,5-trimethyl-	Nitrogen-containing	1.88	3.23	6.45						
750	1,6-Dioxaspiro[4.4]nonane, 2-ethyl-	Ethers	1.88						13.64		
751	2-Butene, 2-methyl-	Linear aliphatic	1.88	3.23	3.23				4.55		
752	2-Hexanone, 3-methyl-	Ketones	1.88		3.23				9.09		
753	2-Hexene	Linear aliphatic	1.88	3.23	6.45						
754	2-Hydrazinoethanol	Alcohols	1.88	9.68							
755	2-Pentadecanone	Ketones	1.88		9.68						
756	2-Pentanone	Ketones	1.88	9.68							
757	2-Pentene, 3-methyl-	Linear aliphatic	1.88					8.00		6.25	

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758	2-Propanone, 1-chloro-	Ketones	1.88			7.14		4.00	4.55		
759	2-Propenoic acid, ethyl ester	Esters and analogues	1.88	9.68							
760	2,3-Butanediol, [S-(R*,R*)]-	Alcohols	1.88	6.45	3.23						
761	2,3-Dehydro-1,8-cineole	Ethers	1.88	6.45	3.23						
762	2,3,5-Trimethyl-6-propylpyrazine	Aromatics	1.88					4.00	9.09		
763	2,4-Hexadienal, (E,E)-	Aldehydes	1.88		6.45					6.25	
764	2,4-Hexadiene, 2,5-dimethyl-	Linear aliphatic	1.88						13.64		
765	2,4-Nonadienal, (E,E)-	Aldehydes	1.88		9.68						
766	2,4,6-Trifluorobenzoyl chloride	Halogen-containing	1.88		3.23		12.50	4.00			
767	2H-Pyran-2-one, tetrahydro-6-methyl-	Ethers	1.88		3.23			8.00			
768	3-Hexanol	Alcohols	1.88		9.68						
769	3-Methyl-1,2-diazirine	Nitrogen-containing	1.88						4.55	6.25	7.69
770	3-Pentanone, 2-methyl-	Ketones	1.88	3.23	6.45						
771	3,4-Diacetylfurazan	Aromatics	1.88	3.23	6.45						
772	3,4-Difluorobenzaldehyde	Halogen-containing	1.88					4.00	9.09		
773	4-Carene, (1S,3R,6R)-(-)-	Cyclic aliphatic	1.88		3.23					6.25	7.69
774	5-Hepten-3-one, 5-methyl-	Ketones	1.88		9.68						
775	5-Methyl-2-thiophenecarboxaldehyde	Aldehydes	1.88	3.23					9.09		
776	5-Tridecene	Linear aliphatic	1.88					4.00	4.55		7.69
777	9-Octadecenoic acid (Z)-, methyl ester	Esters and analogues	1.88	3.23	3.23		12.50				
778	Acetamide, N-methyl-	Nitrogen-containing	1.88	9.68							
779	Allantoic acid	Acids	1.88					8.00	4.55		
780	Azodicarbonamide	Nitrogen-containing	1.88	3.23	6.45						
781	Benzene	Aromatics	1.88	3.23			12.50		4.55		
782	Benzene, (1-methyldecyl)-	Aromatics	1.88	3.23	6.45						
783	Benzene, 1-chloro-2-fluoro-	Aromatics	1.88				12.50		4.55		7.69
784	Benzene, 1-propenyl-	Aromatics	1.88	3.23	6.45						
785	benzene, 1,1'-(1-methylethylidene)bis[4-methyl-	Aromatics	1.88		3.23	7.14					7.69
786	Benzene, 1,1'-propylidenebis-	Aromatics	1.88					4.00	9.09		
787	Benzene, 1,2-difluoro-4-(trifluoromethyl)-	Halogen-containing	1.88	3.23	3.23			4.00			
788	Benzene, 1,2,3,5-tetrafluoro-	Halogen-containing	1.88					4.00		6.25	7.69
789	Benzene, 1,4-difluoro-	Halogen-containing	1.88			14.29		4.00			
790	Benzene,1-chloro-3,5-difluoro-	Halogen-containing	1.88			14.29		4.00			
791	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	Cyclic aliphatic	1.88					8.00		6.25	
792	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Cyclic aliphatic	1.88		3.23				9.09		
793	Bicyclo[4.2.0]octa-1,3,5-triene	Cyclic aliphatic	1.88		3.23					12.50	
794	Bis(2-chloroethyl) ether	Ethers	1.88		9.68						
795	Cyclobutylamine	Nitrogen-containing	1.88	6.45				4.00			
796	Cyclohexane, 1,3-dimethyl-, cis-	Cyclic aliphatic	1.88	3.23						6.25	7.69
797	Cyclohexane, methylene-	Cyclic aliphatic	1.88		6.45						7.69
798	Cyclopentane, (2-methylbutylidene)-	Cyclic aliphatic	1.88						13.64		
799	Cyclopropane, 1,2-dimethyl-, cis-	Cyclic aliphatic	1.88	3.23	3.23			4.00			
800	Cyclopropane, 1,2-dimethyl-, trans-	Cyclic aliphatic	1.88	6.45					4.55		
801	Cyclopropaneethanol	Alcohols	1.88						13.64		
802	Diazirine	Nitrogen-containing	1.88		6.45			4.00			
803	Diphenyl sulfide	Sulphur-containing	1.88		6.45	7.14					
804	Disulfide, bis[1-(methylthio)ethyl]	Sulphur-containing	1.88					12.00			
805	Ethane, (methylthio)-	Sulphur-containing	1.88	9.68							
806	Ethanone, 1-[6-(4-methyl-1,2,5-oxadiazol-3-yl)-4H-pyrazolo[3,4-c]-1,2,5-oxadiazol-4-yl]-	Ketones	1.88	3.23	3.23			4.00			
807	Ethene, ethoxy-	Ethers	1.88		3.23				4.55		7.69
808	Ethene, iodo-	Halogen-containing	1.88		9.68						
809	Ethylene oxide	Ethers	1.88	3.23	6.45						
810	Formamide, N-formyl-N-methyl-	Nitrogen-containing	1.88					8.00	4.55		
811	Furan, 2-butyltetrahydro-	Aromatics	1.88	3.23	3.23					6.25	
812	Furan, tetrahydro-2-methyl-	Aromatics	1.88		6.45	7.14					
813	Furan, tetrahydro-3-methyl-	Aromatics	1.88					8.00		6.25	
814	Hexacosane	Linear aliphatic	1.88	3.23		7.14		4.00			
815	Hexane, 3,3,4,4-tetrafluoro-	Halogen-containing	1.88	3.23	6.45						
816	Hexanoic acid, ethyl ester	Esters and analogues	1.88	6.45		7.14					

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817	Hydrazine, methyl-	Nitrogen-containing	1.88	3.23			12.50		4.55		
818	Indene	Aromatics	1.88	6.45							7.69
819	Indole, 3-methyl-	Aromatics	1.88	3.23	6.45						
820	Isobutyl acetate	Esters and analogues	1.88	6.45				4.00			
821	Mandelamide	Nitrogen-containing	1.88		6.45			4.00			
822	Manganese, acetylpentacarbonyl-, (OC-6-21)-	Linear aliphatic	1.88		3.23	7.14		4.00			
823	Meglumine	Alcohols	1.88		3.23				4.55	6.25	
824	Methanesulfonic anhydride	Sulphur-containing	1.88		6.45				4.55		
825	Methanethioamide, N,N-dimethyl-	Nitrogen-containing	1.88	3.23	6.45						
826	Methyl glyoxal	Aldehydes	1.88					8.00	4.55		
827	Methyl isopropyl disulphide	Sulphur-containing	1.88		9.68						
828	N-Nitrosodimethylamine	Nitrogen-containing	1.88	3.23				4.00		6.25	
829	Nonanal	Aldehydes	1.88	3.23					9.09		
830	Nonanenitrile	Nitrogen-containing	1.88	9.68							
831	p-Dioxin, 2,3-dihydro-5-methyl-	Ethers	1.88	6.45	3.23						
832	Pentane, 2,3,3-trimethyl-	Linear aliphatic	1.88	3.23	3.23						7.69
833	Phenol, 3-(1-methylethyl)-	Alcohols	1.88	3.23	3.23				4.55		
834	Propane, 1,1,2,2-tetrafluoro-	Halogen-containing	1.88		3.23	7.14	12.50				
835	Propanoic acid, 2-methyl-, anhydride	Esters and analogues	1.88					4.00	9.09		
836	Pyridine, 2,3-dimethyl-	Aromatics	1.88	6.45	3.23						
837	Pyrimidine, 2-methyl-	Aromatics	1.88	9.68							
838	Semicarbazide	Nitrogen-containing	1.88		9.68						
839	Sulfurous acid, butyl octyl ester	Esters and analogues	1.88		3.23					6.25	7.69
840	Thiocyanic acid, ethyl ester	Esters and analogues	1.88	9.68							
841	α-Terpineol	Alcohols	1.88		3.23						15.38

APPENDIX D: CDD RESPONSE RESULTS

The tables in this appendix indicate one of the five CDD response outcomes as observed during each trial. Additionally, a sixth category that states ‘absent’ has been indicated when that dog was not present for the trial conducted on the training aid.

Table A 4: Responses recorded during the dog trials conducted in July 2020 with two OPP cadaver dog-handler teams using training aids.

Sr. no.	Training aid ID#	Training location and description	CDD responses		Additional notes
			Dog 1	Dog 2	
1.	Foot S	Outdoor; foot placed on ground	Absent	True positive	
2.	Foot R	Outdoor; sample hidden in rocks.	Absent	True positive	
3.	Foot B	Outdoor; buried sample	Absent	False negative	Dog #2 did not indicate to the presence of the target odour.
4.	Foot 1	Indoor; inside an apartment among furniture	True positive	True positive	
5.	Foot 2	Indoor; apartment furniture	True positive	True positive	
6.	Foot 5	Indoor; inside a locker in locker room	True positive	True positive	
7.	Bone 2	Indoor; inside an apartment among the furniture	True positive	True positive	

8.	Tissue 1	Indoor; carousel	True positive	True positive	
9.	Tissue 2	Indoor; carousel	True positive	True positive	Dog #1 indicated a false positive on residual odour from Tissue #1 which was a prior search conducted in the same room before the true positive response.
10.	Blood 1	Indoor; carousel	True positive	Interest	
11.	Blood 2	Indoor; carousel	True positive	True positive	Dog #2 also indicated a false positive at a different location in the room before the true positive response.

Table A 5: Responses recorded during the dog trial conducted in March 2021 with three OPP cadaver dog-handler teams using training aids.

Sr. no.	Training aid ID#	Training location and description	CDD responses			Additional notes
			Dog 1	Dog 3	Dog 4	
1.	Foot 1	Indoor; boxes.	True positive	Absent	True positive	
2.	Foot 2	Indoor; inside bite room at a corner.	True positive	Absent	True positive	
3.	Foot 4	Indoor; inside a tin in a room filled with luggage bags.	Absent	True positive	True positive	
4.	Foot 5	Indoor; behind a door in a room	True positive	Absent	False negative	Dog #4 did not indicate to the presence of the target odour.
5.	Foot 6	Indoor; inside a dummy resembling a human figure.	True positive	Absent	True positive	
6.	Foot 8	Indoor; inside a bag in a room filled with luggage bags.	True positive	Absent	True positive	
7.	Foot 9	Indoor; inside a dummy resembling a human figure.	True positive	Absent	True positive	

8.	Bone 1	Indoor; inside a wooden drawer of an apartment placed among another furniture items.	Absent	True positive	True positive	Dog #3 also indicated a false positive at a different location in the room after the true positive response.
9.	Bone 2	Indoor; inside a locker in a locker room	True positive	Absent	True positive	
10.	Bone 3	Indoor; boxes	True positive	Absent	True positive	
11.	Bone 4	Indoor; boxes	True positive	Absent	True positive	
12.	Bone 5	Indoor; inside bite room in a corner	True positive	Absent	True positive	
13.	Bone 6	Indoor; carousel	Absent	Absent	True positive	
14.	Bone 7	Indoor; inside a drawer of a metallic locker in a locker room	Absent	True positive	True positive	
15.	Tissue 1	Indoor; inside a plastic crate placed one of the shelves of a metallic rack in bite room.	Absent	True positive	True positive	

16.	Tissue 2	Indoor; boxes	Absent	True positive	True positive	5 distractor odours were present: coffee, dog food, chewing gum, isopropyl alcohol on gauze.
17.	Tissue 3	Indoor; inside a PVC pipe behind wall with odour holes.	Absent	Absent	True positive	
18.	Blood 1	Indoor; carousel.	True positive	Absent	True positive	
19.	Blood 2	Indoor; inside a metallic Chester drawer.	Absent	True positive	Absent	
20.	Blood 3	Indoor; inside a drawer of a metallic locker in a locker room	Absent	True positive	True positive	

Table A 6: Responses recorded during the dog trial conducted in May 2021 with eight OPP cadaver dog-handler teams using training aids.

Sr. no.	**Training aid ID#**	**Training location and description**	**CDD responses**								**Additional notes**
			Dog 1	**Dog 3**	**Dog 5**	**Dog 6**	**Dog 7**	**Dog 8**	**Dog 9**	**Dog 10**	
1.	Foot R	Outdoor; sample hidden in rocks.	True positive	Absent	Absent	Absent	Absent	Absent	Absent	Absent	
2.	Foot B	Outdoor; buried sample	Interest	False negative	Absent	Absent	Absent	Absent	Absent	Absent	Dog #1 showed a change in behaviour while Dog #3 did not give any alert.
3.	Foot 1	Indoor; inside a file cabinet in an apartment	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	

4.	Foot 2	Indoor; behind boxes in room with boxes	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
5.	Foot 3	Indoor, wooden drawer in an apartment.	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
6.	Foot 4	Indoor, boxes	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
7.	Foot 5	Indoor; inside a locker in a room	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	Dogs #7 and #10 indicated a false positive response at different locations in the search room after the true positive response.

8.	Foot 6	Outdoor; backyard of a house	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
9.	Foot 7	Outdoor; grassland search	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
10.	Foot 8	Indoor; inside a vacuum cleaner in an apartment	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	Dogs #3, #5, #7 and #8 all indicated a false positive response between detecting Foot #8 and #9 at the same location in the search room where Foot #8 and #9 were hidden.
11.	Foot 9	Indoor; inside a locker in an apartment	Absent	False negative	False negative	True positive	True positive	True positive	True positive	True positive	

											Dogs #3 and #5 did not indicate to the presence of the target odour from Foot #9.
12.	Bone 1	Indoor; carousel.	Absent	True positive	True positive	True positive	Interest	True positive	True positive	True positive	Dog #7 showed change in behaviour but could not identify the location of odour source.
13.	Bone 2	Outdoor; in the front yard of a house.	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
14.	Bone 3	Indoor, boxes	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	

15.	Bone 4	Indoor; inside a locker on a hallway	Absent	True positive	True positive	True positive	True positive	Interest	True positive	True positive	
16.	Bone 5	Indoor; inside a PVC pipe behind wall with odour holes	Absent	True positive	True positive	True positive	True positive	True positive	True positive	True positive	
17.	Bone 6	Indoor; carousel	True positive	True positive	Absent	Absent	Absent	Absent	Absent	Absent	5 distractor odour were present: candy, crayon, dog treats, chapstick, whiteout
18.	Bone 7	Indoor; carousel	True positive	True positive	Absent	Absent	Absent	Absent	Absent	Absent	8 distractor odour were present: dishwashing

											soap, highlighter 1, cat food, bird feed, tennis ball, candle, highlighter 2, glue.
19.	Tissue 1	Indoor; carousel	True positive	True positive	Absent	Absent	Absent	Absent	Absent	Absent	8 distractor odour present: dishwashing soap, highlighter 1, cat food, bird feed, tennis ball, candle, highlighter 2, glue.
20.	Tissue 2	Indoor; carousel	Absent	True positive	True positive	True positive	True positive	True positive	False negative	True positive	Dog #9 did not indicate

											at the target location as his first response. During round 2 of the search when he was exposed to this sample again, he indicated a true positive response.
21.	Tissue 3	Indoor; carousel	True positive	True positive	Absent	Absent	Absent	Absent	Absent	Absent	5 distractor odours were present: candy, crayon, dog treats,

											chapstick, whiteout
22.	Blood 1	Indoor; inside a metallic locker in a locker room	Absent	True positive	True positive	True positive	True positive	False negative	True positive	True positive	Dog #8 handler did not call out the dog's response.
23.	Blood 2	Indoor; carousel whel.	Absent	True positive	True positive	True positive	True positive	True positive	True positive	Interest	Dog #10 showed a change in behaviour.
24.	Blood 3	Indoor; carousel	True positive	True positive	Absent	Absent	Absent	Absent	Absent	Absent	5 distractor odours were present: shaving cream, play doh, beef cubes, deodorant, empty can

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