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Using ethically sourced training aids for human remains detection dog training

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A B S T R A C T

Human remains detection dogs (HRDDs) play vital roles in forensic investigations and search and rescue missions by detecting decomposing human remains. However, there is a lack of standardized training protocols globally. This study evaluates various ethically sourced HRDD training aids, including amputated limbs, blood, and teeth used by the Ontario Provincial Police (OPP). Expanding on prior research, this study assesses amputated limbs stored outdoors, bone, tissue, blood, and teeth. The volatile organic compound (VOC) profiles of these aids are compared to cadavers decomposing at the Research in Experimental and Social Thanatology (REST) facility. The results highlighted that the combined VOC profile from all HRDD training aid types demonstrates a 68% similarity to REST cadavers, emphasizing the potential benefit of exposing HRDDs to a diverse range of training aids. This is because the similarities in VOC profiles of individual training aid types (amputated limbs stored indoors, bone, blood, tissue, amputated limbs stored outdoors, teeth) with REST cadavers were lower than 68%. Teeth (without organic matter) were identified as the least ideal training aid for enabling dogs to detect cadavers based on VOC profiles and HRDD responses. However, training on teeth may be required for operational needs, particularly when HRDDs need to locate teeth during field searches. This study also highlights the effectiveness of using amputated limbs and blood together as they comprise a majority of the 68% VOCs found similar to the REST cadavers and elicit desirable HRDD responses to decomposition odor.

1. Introduction

Human remains detection dogs (HRDDs) are specially trained canines that are used to locate and indicate the presence of decomposing human remains, including whole and parts of cadavers. The use of HRDDs is valuable in crime scene investigations, and search and rescue operations. HRDDs do not possess an inherent response to decomposing human remains odor, their capability is built over time through rigorous training [1]. Training HRDDs involves repeated exposure to target odors that is similar to their target decomposition odor and then rewarding them by positive reinforcement for a final trained response (sit/down/bark/stare around the odor source). This eventually leads to their ability to discriminate the distinct smell of human remains from other odors including background odor, live scent or distractor odors used during their training. Theoretically, cadavers would be a good training material for HRDDs but they are not readily available. Some dog handlers may have access to cadavers at a human taphonomic facility (known as a body farm) but these are mostly limited to North America. Anecdotally, such facilities with multiple decomposing cadavers in a limited area can be highly concentrated with decomposition odor and thus, overwhelming for HRDDs [2]. Therefore, training HRDDs on ethically and legally acquired alternative materials is a necessary requirement and deemed appropriate. The materials used to train HRDDs are referred to as HRDD training aids. These include synthetic chemical formulations

Abbreviations: HRDD, Human remains detection dogs; GC×GC-TOFMS, Two-dimensional gas chromatography time-of-flight mass spectrometry; OPP, Ontario Provincial Police; PCA, Principal component analysis; REST, Research in Experimental and Social Thanatology; VOC, Volatile organic compound.

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(pseudoscents), decomposing animal remains, decomposition fluid, blood, soil found in proximity to decomposing remains, teeth and objects that have been in contact with decomposing remains (clothing, gauze, soil etc.) usually obtained by HRDD handlers from a crime scene [3,4]. The advantages and disadvantages of various training aid types have been elaborated in Table 1. The exact material being used for training varies across the globe and depends primarily on their availability and the ease of acquiring ethically sourced material. Ethically sourced training aids ensure that the materials are obtained with dignity, respect, and legal compliance. This approach upholds the integrity of the training process, and respects the sensitivities surrounding the use of such materials. Even though some training aids (such as blood, and decomposition fluid) are considered valid [5,6], there is no standardized protocol that recommends a range of validated training aids for HRDDs.

Between 2010 and 2017 in Ontario, Canada, several men from the LGBTQ2S + community went missing and eventually, certified HRDDs which are used in Canada for homicide, missing person and mass disaster cases were deployed to locate dismembered remains of victims that were concealed inside garden planters [15]. Instances such as these prompted law enforcement agencies to find an ethical and legal framework to utilize human remains to train their HRDDs. Consequently, in recent years, the Ontario Provincial Police (OPP) in Ontario, Canada began using amputated limbs as HRDD training aids whose use was validated in a study by the authors of this article [16]. These ethically sourced limbs were obtained from voluntary donations of diabetic patients undergoing surgery, for training HRDDs at the OPP canine unit. OPP initially chose amputated limbs as they are readily available, ethically procured with the consent of living individuals, can be used and decomposed in controlled environments, and potentially encompass the decomposing odor. To the best of authors' knowledge, OPP is the only organization to be using amputated limbs from diabetic patients as a training aid. Before their use, much like other organizations across the globe, OPP used material (clothes, gauze etc.) that had been in contact with cadavers. Previously published literature gives a detailed account of commonly used HRDD training aids worldwide [3]. Validating the use of amputated limbs for training HRDDs could allow for standardized and relatively consistent odor profiles across multiple HRDD training agencies. Since this research was conducted in collaboration with the OPP Canine Unit, the primary objective of this study was to assess the suitability of their ethically sourced HRDD training aids including amputated limbs (classified as foot, bones, and tissue in this study), teeth, and blood (donated from living individuals) as training aids for HRDDs. This was achieved by direct comparison of odor comprised of the volatile organic compounds (VOCs) from HRDD training aids and cadavers (referred to as donors) decomposing in a natural outdoor woodland environment at the Research in Experimental and Social Thanatology (REST) facility in Québec, Canada. The previous study [16] by the authors focused solely on the amputated limbs (foot samples) stored indoors, while this communication includes additional components such as: foot samples (stored outdoors), tissue and bone from amputated limbs; blood; and teeth. The aim of this short communication is to compare the VOC profiles of the different HRDD training aids used by OPP with cadavers and report on their validity as training aids for HRDDs to locate cadavers and parts of cadavers in both fresh and decomposed stages.

Table 1
Advantages and disadvantages of commonly used HRDD training aids.

Types of HRDD Training Aids	Advantages	Disadvantages
Chemical formulations (pseudoscents)	Easily accessible Free from bio-hazard Composition can be customized.	Lack representation of compounds found in human decaying bodies.[7]
Decomposing animal remains	Diverse decomposition-related volatile signatures [8,9]. Easily available and cost effective compared to acquiring human remains.	Volatile compounds released differ among species and not fully representative of human decomposition. [10]
Blood	Easily sourced.	Limited in replicating the complexity of cadaveric odor.
	Effective in training blood detection dogs and instances where working dogs are required to locate blood [6].	Some agencies may not want to train HRDDs on blood alone as this may lead to false positive results.
Gravesoil	Representative of natural decomposition environment as it encompasses decomposition fluids	Risk of training on non-targeted chemical substances if control soil is not used [11]
Parts of cadaver	Closest to real-world situations with decomposition odors that can be used to improve dogs' cadaver detection skills.	Not comprehensive in covering the full spectrum of volatile compounds as in a whole cadaver [12] Limitation relating to acquisition, ethics, legislation, and biohazard.
Cadaver clothes	Accumulate cadaveric odors effectively as they are soaked with decomposition fluids.	The material of the cloth can have an impact on the odor (e.g. Cotton clothes are preferred due to better adsorption of cadaveric VOCs). Risk of potential contamination if clothes from different post-mortem intervals are used together. [13]
Gauze prepared using Scent Transfer Unit 100TM (STU-100)	Traps decaying corpse scent effectively. Represents 60 to 85 % of the total post-mortem VOC profile [14]	Limitations in replicating real-world conditions and scenarios.

2. Main text

2.1. Experimental design

VOC samples were collected from the headspace of both HRDD training aids used by the OPP Canine Unit (human remains) and REST donors (cadavers). In addition to analyzing VOCs, HRDD trials were also conducted to record dog responses to training aids and cadavers. In this study, the training aids were classified as foot (stored indoors or outdoors), bone (dry and wet), tissue (soft tissue – dry and wet), blood and teeth. The foot samples stored outdoors were decomposing on the surface, concealed between rocks, or buried. The foot training aids (stored indoors and outdoors) contained tissues along with bones while the other categories of samples either had only tissue, blood or teeth and thus were classified accordingly. Not all bone training aids were dry bones since most of them still had wet soft tissue attached to them. Regardless, they were recognized as bones since this classification was adopted based on the description provided by the OPP Canine Unit. Particularly, in instances where the training aids were stored in PVC pipes which could not be opened for cross-verification, the researcher had to rely on the information provided. It must be noted that all the training aids were donated voluntarily with consent to the OPP Canine Unit. This included the foot, bone and tissue training aids all originating from the donated amputated lower limbs/feet of diabetic patients undergoing surgical removal procedures, while the teeth were donated by a dentist, and the blood (deposited on gauze) training aids were donated by the dog handlers thus, neither the blood nor teeth was of cadaveric origin. The ethics certification to work with these training aids was obtained from *le comité d'éthique de la recherche avec des etres humain* at UQTR with the certificate number CER-19–261-07.12. VOC collection from HRDD training aids was performed in the months of December 2019, July 2020, October 2020, March 2021 and May 2021. The VOC analysis was conducted using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) which allows for enhanced peak capacity and better resolution compared to conventional gas chromatography-mass spectrometry (GC–MS), an essential requirement in forensic odor analysis [17]. Controls (background odor samples) were collected with each experimental sample within the same environment as the sample using their respective storage containers without the training aids. The statistical approach employed in the research was tailored to exclusively identify abundant VOCs present in the samples (either HRDD training aids or REST donors) in comparison to the respective control (background) odor. A detailed account of the VOC collection protocol, VOC analysis method, and statistical analysis can be found in a previous study by the authors [16].

The HRDDs were also exposed to the same training aids from which VOCs were analyzed, aiming to observe and record their responses. The ethics certification to work with HRDDs was obtained from *le comité de bons soins aux animaux* at UQTR with the certificate number 2020-S.F.2. Double-blind dog trials were conducted during OPP's routine canine maintenance training sessions in July 2020, March 2021, and May 2021. Fig. 1 of section 2.2 highlights additional details of dog trials and training aids used during the study. In some instances, the dogs were exposed to distractor odors (such as coffee, dog food, isopropyl alcohol on gauze, bird feed, ball etc.) in addition to their target odor (training aids). Training dogs on distractor odors, background or control odors improves their ability to discriminate target scents among other non-target odors, enhancing their reliability and accuracy in real-world detection scenarios [18]. Details of HRDD training scenarios can be found in a previous study by the authors [16]. The dog responses during these trials were recorded as true positive, true negative, false positive, false negative and interest response (when the dogs showed a behavioural change and narrowed down the target odor but did not alert on the exact location). These responses were then used to estimate (in percentage) the detection rate, interest response rate and false response rate indicated below.

- Detection rate (%) $= \frac{\text{Total number of true positive + true negatives}}{Totalnumberofpossibletrueoutcomes} \times 100$
- Interest response rate (%) $= \frac{\text{Total number of interest responses}}{Totalnumberofpossibleoutcomes} \times 100$
- False response rate (%) $= \frac{\text{Total number of false negatives + false positives}}{Totalnumberofpossiblefalseoutcomes} \times 100$

For the REST donors, VOC profiles were analyzed from eight REST donors decomposing in varying seasons along with background environment odor. The sample collection and analysis was carried out to a maximum of three months while the dog trial was only conducted once at REST in October 2021 with one fresh and one decomposed donor at day 9 and day 802 of decomposition, respectively. The ethics approval for working with cadavers at REST 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.

2.2. Results and discussion

Fig. 1 outlines HRDD responses recorded during the trials along with the number of HRDDs present during dog trials conducted with training aids and donors at REST, dog details, training aid details (including date

KEY	
(green)	True Positive
(hatched)	False negative
(dotted)	Interest

HRDD ID #		HRDD training aids																									REST donors	
		Amputated limbs stored outdoors			Amputated limbs stored indoor									Bone originating from amputated limbs							Tissue originating from amputated limbs			Blood donated by HRD handlers				
		Foot S (foot decomposing on surface)	Foot R (foot hidden in rocks)	Foot B (buried foot)	Foot 1	Foot 2	Foot 3	Foot 4	Foot 5	Foot 6	Foot 7	Foot 8	Foot 9	Bone 1 (dry bone)	Bone 2 (wet bone)	Bone 3 (wet bone)	Bone 4 (wet bone)	Bone 5 (wet bone)	Bone 6 (dry bone)	Bone 7 (dry bone)	Tissue 1 (wet soft tissue)	Tissue 2 (wet soft tissue)	Tissue 3 (desiccated skin)	Blood 1 (blood donated in Nov-17)	Blood 2 (blood donated in Nov-17)	Blood 3 (blood donated in Feb-21)	Fresh (ED = 9)	Decomposed (ED = 802)
	Date when the training aids was obtained by the OPP Canine Unit	8-Jul-20	24-Jan-19	24-Jan-19	02-Oct-17	02-Oct-17	11-Dec-17	11-Dec-17	11-Dec-17	24-Jan-19	24-Jan-19	24-Jan-19	24-Jan-19	02-Oct-17	02-Oct-17	11-Dec-17	8-Jul-20	8-Jul-20	8-Jul-20	8-Jul-20	02-Oct-17	02-Oct-17	8-Jul-20	Nov-17	Nov-17	22-Feb-21		
	Storage conditions	Outdoor	Outdoor	Outdoor	Refrigerator	Refrigerator	Freezer	Freezer	Room temp.	Refrigerator	Refrigerator	Freezer + Refrigerator	Freezer + Refrigerator	Room temp.+ Refrigerator	Room temp.	Freezer	Room temp.	Room temp.	Room temp.	Room temp.	Room temp.	Room temp. + Refrigerator	Room temp.	Room temp. + Refrigerator	Room temp.	Room temp.	Outdoor	Outdoor
Dog 1	(4 years; Male; Labrador)		May-21	May-21	Jul-20; Mar-21	Jul-20; Mar-21			Jul-20; Mar-21	Mar-21		Mar-21	Mar-21		Jul-20; Mar-21	Mar-21; May-21	Mar-21; May-21	Mar-21; May-21	May-21	May-21	Jul-20; May-21	Jul-20	May-21	Jul-20; Mar-21	Jul-20	May-21		
Dog 2	(4 years; Female; Labrador)	Jul-20	Jul-20	Jul-20	Jul-20	Jul-20			Jul-20						Jul-20						Jul-20	Jul-20		Jul-20	Jul-20			
Dog 3	(18 months; Male; Belgian Malinois)			May-21	May-21	May-21	May-21	Mar-21	May-21	May-21	May-21	May-21	May-21	Mar-21; May-21	May-21	May-21	May-21	May-21	May-21	Mar-21; May-21	Mar-21; May-21	Mar-21; May-21	May-21	May-21	Mar-21; May-21	Mar-21; May-21	Oct-21	Oct-21
Dog 4	(9.5 years; Male; Dutch Shepherd)				Mar-21	Mar-21		Mar-21	Mar-21	Mar-21		Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21	Mar-21		Mar-21		
Dog 5	(6 years; Male; German Shepherd)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21			
Dog 6	(8.5 years; Male; German Shepherd)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21			
Dog 7	(6.5 years; Male; German Shepherd)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21		Oct-21	Oct-21
Dog 8	(3 years; Male; German Shepherd)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21		Oct-21	Oct-21
Dog 9	(8.5 years; Male; German Shepherd)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21			
Dog 10	(2 years; Male; Spring Spaniel)				May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21	May-21				May-21		May-21	May-21			
Dog 11	(4.5 years; Male; Labrador)																										Oct-21	Oct-21
Dog 12	(3 years; Male; Shepherd)																										Oct-21	Oct-21
Dog 13	(6 years; Female; Labrador)																										Oct-21	Oct-21
Dog 14	(4 years; Male; Labrador)																										Oct-21	Oct-21
Dog 15	(4.5 years; Female; Labrador)																										Oct-21	Oct-21

Fig. 1. Summary of HRDD responses (indicated by colour-coded cells) recorded during dog trials conducted in July 2020, March 2021 and May 2021 at OPP, Ontario with dog training aids and the one dog trial conducted in October 2021 at REST facility with cadavers.

of acquisition and storage conditions), and months during which each of the dogs participated in the trial.

In addition to the dog trials results, the VOC results were also studied and these showed that 68 % of the VOCs detected in the REST donor samples were also detected in HRDD training aids used by the OPP Canine Unit. Thirty-two (32) prominent VOCs (occurring in over 30 % of samples) were identified in REST donors and these were also detected in HRDD training aids. Likewise, 17 of the 18 prominent VOCs (occurring in over 30 % of samples), except sevoflurane, were identified in HRDD training aids, and were detected in REST donors. 16 of these 17 VOCs have been detected in decomposition odor except 5-Methyl-2-furancarboxaldehyde however, 2-furancarboxaldehyde has also been reported in decomposition-related odor [5]. Sevoflurane, which was not detected in REST donors or the blood and teeth samples, is a known anaesthetic that was potentially used during the amputation surgeries. It was detectable years after amputation and when the amputated lower limbs were being used for HRDD training. As reported in the prior study [16], it is of significance to note that if amputated lower limbs/feet are used as HRDD training aids, the HRDD could potentially be exposed to some amputation procedure-related VOCs. During observation of the HRDD responses, it was concluded that the presence of sevoflurane, a type of anesthesia, does not negatively affect the performance of the dogs in carrying out their detection tasks. This was concluded because the HRDDs that had undergone training using amputated limbs exhibited a 100 % detection rate (estimated from dog responses recorded and represented in Fig. 1) for identifying both fresh and decomposed donors (present at REST). When comparing the list of 32 and 18 prominent VOCs identified individually in REST donors and HRDD training aids, respectively, nine VOCs (indicated in Table 2) were consistent among them.

All of these nine VOCs have been previously reported in both human and animal decomposition VOC profiles however, they are not specific to decomposition as they can have multiple other sources of origin. Regardless, it can be concluded that the high occurrence of these VOCs and their presence in both HRDD training aids and REST donors make these nine mentioned VOCs significant when identifying VOCs that HRDDs potentially associate as decomposition-related. There may be several other compounds that remain undetected using the current analytical method however, the undetectability of compounds does not imply their absence. While this study focused on recording volatile organic compound (VOC) profiles and HRDD responses to training aids and REST donors, identifying the exact VOC or combination of VOCs that stimulate a neurological response in a HRDD's brain was beyond the scope of this study. Though dogs often excel as biological detection compared to analytical methods (instrumentation) [26], the absence of olfactory threshold data for decomposition-related VOCs limits a direct comparison. Therefore, this research primarily reports HRDD responses and prominent VOCs, leaving the identification of exact VOCs that elicit certain responses in dogs to future studies.

Table 3 indicates that similarity in terms of the percentage of VOCs found common between the different HRDD training aid types (foot, bone, tissue, blood, teeth) and REST donors. This table highlights that the foot samples stored indoors had the most similarity (55 %) followed by bones (42 %), blood (26 %), tissue (21 %), foot samples stored outdoors (12 %) and finally, the teeth samples had the least similarity (11 %).

In the case of the foot decomposing outdoors, the foot sample decomposing on the surface had a greater number of VOCs (74) detected than those concealed by rock (up to 35 VOCs) or burial (up to 36 VOCs). In the buried foot, a greater number of VOCs (78) were detected when the buried foot was exhumed and sampled indoors versus when the headspace was collected above the grave. During the dog trials, HRDDs either showed interest only or failed to locate the buried foot sample. However, a later dog trial by the current research group (results not published) revealed that three dogs once exposed to the exhumed sample successfully produced correct final responses. This emphasizes the fact that concealing the training aids may reduce the detectable odor and this can be a way to challenge HRDDs during their outdoor searches eventually helping to improve their capability [27]. Among several other factors such as weather, decomposition stage etc. that influence the odor detection capability of dogs, concealment has also been reported to have an effect as it can restrict the amount of VOCs available for detection and modify the natural microbes present thus, influencing

Table 3
Summary of similarity by total percentage of VOCs common between HRDD training aids and REST donors analysed in the current study.

Training aid type	Total no. of VOCs detected in the HRD training aid type	No. of VOCs common between HRDD training aid and REST donors	Percentage (%) of VOCs common with HRDD training aid and REST donors (No. of VOCs common between HRDD training aid and REST donors / Total no. of VOCs detected in REST donors × 100) *Total no. of VOCs in REST donors = 1412
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 %

Table 2
List of nine prominent VOCs found common between individual HRDD training aids and REST donors.

	VOCs common with REST donors detected in individual HRDD training aids						Previously reported in literature as human decomposition odor-related
	Amputated limbs				Blood	Teeth	
	Foot stored indoors	Foot stored outdoors	Bone	Tissue			
Dimethyl trisulfide	Yes	Yes	Yes	Yes	Yes	No	[19–24,5]
Methyl thiocyanate	Yes	Yes	Yes	Yes	Yes	Yes	[5]
Trimethylamine	No	Yes	Yes	No	Yes	No	[5]
Trimethyl pyrazine	Yes	Yes	Yes	Yes	No	No	[8,5]
Methyl pyrazine	Yes	Yes	Yes	Yes	Yes	No	[5]
3-methyl-1-butanol	No	Yes	Yes	Yes	Yes	No	[8,5,9]
Pyridine	No	Yes	Yes	No	Yes	No	[25,8,5,9]
3-methyl-butanoic acid	No	Yes	Yes	No	No	No	[5]
2-Methyl-propanoic acid	No	Yes	Yes	No	Yes	No	[23,5]

the odor [2]. In bone and tissue training aids, the dryness (limited organic matter) or wetness (presence of organic matter) of the samples impacted the number of VOCs detected and in turn the number of VOCs common with REST donors. Additionally, the results in this study did not highlight any differentiation in HRDDs' capability to locate dry and wet samples as two dry bones (Bone #6 and #7) had a 100 % detection rate and one dry bone Bone #1 had an 89 % detection rate. This was consistent with the HRDDs responses on tissue samples as Tissue #3, which was a desiccated skin sample, had a 100 % detection rate. Thus, during the study, it was concluded that even though the VOC collection location (outdoor vs. indoor), site of decomposition (surface vs. burial), and/or the amount of organic matter (more in foot and wet bone samples and less in dry soft tissue sample) determined the VOC profile and its similarity with those of REST donors, the difference in VOC profile among the HRDD training aids originating from the same origin (amputated lower limbs) did not seem to impact the HRDD performance except in the case where the training aid was buried/concealed.

For blood training aids, even though the less degraded blood (Blood #3) had a reduced number of VOCs (up to 55 VOCs) compared to the more degraded blood (Blood #1 and #2) (up to 159 VOCs), the detection rates were higher for less degraded blood (Blood #3). As shown in Fig. 1, 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 HRDDs. This was potentially because the less degraded blood had more VOCs similar to the 32 prominent VOCs detected in REST donors compared to more degraded blood (shown in Table 4). Thus, it was found that HRDDs could detect less degraded blood (1 to 3 months degraded) more readily compared to highly degraded blood (2 to 3.5 years degraded).

For the teeth training aid, six prominent VOCs were identified from teeth analyzed in the current study of which, aniline was the only compound to be previously reported as decomposition-related [28]. None of the remaining five compounds (1,3-diethyl-benzene, 1,6,7-trimethyl-naphthalene, 2-methyl-1,1′-biphenyl, 4-ethyl-3-heptene, octahydro-pentalene) have been previously reported in teeth samples or human decomposition odor. This could be because the teeth analyzed did not contain any organic matter and hence, no soft-tissue decomposition-related VOCs were evolved and detected in the current study. The one study on teeth by Hoffman et al. [29] reported the presence of 1-pentanol, tetrachloroethylene, toluene, 2-pentylfuran, cyclohexanone, and dimethy disulfide (DMDS) in their teeth samples. Of these, only DMDS was detected in the December 2019 trial in the current study. While teeth may be the easiest to ethically acquire compared to other human remains as they can be obtained from consented donations of living individuals, the limited VOC similarity between teeth samples (without organic matter) and REST donors, the absence of any previously reported decomposition-related VOCs among the prominent teeth VOCs, and anecdotal reports from OPP canine handlers indicating that the HRDDs did not respond to the teeth used in training sessions deemed its VOC profile as unsuitable for training HRDDs whose operational requirement is to locate cadavers or parts of cadavers. These findings may differ if studies were conducted using a set of teeth with sufficient organic matter still present. Additionally, teeth as training aids may also be useful when the dogs' operational requirement is to locate teeth during field searches.

Our study also examined the VOC chemical class (acids, alcohols, aldehydes, aromatics, cyclic aliphatics, esters, ethers, halogen-containing, ketones, linear aliphatics, nitrogen-containing, and sulfur-containing VOCs) trends between HRDD training aids and REST donor samples. The abundance trend, ranked by the number of VOCs in each class, was largely similar between the two sets (refer to Fig. 2). Aromatic compounds were the most abundant, while ethers, acids and sulfur-containing compounds were the least abundant (<5% of total VOCs). In terms of relative class concentration, ranked by the average normalized area of compounds, variations were found in trends across different HRDD training aids. Regardless, overall alcohol and nitrogen-containing VOCs had the highest relative class concentration while, cyclic aliphatics, aldehydes, and ethers had the lowest relative class concentration in both HRDD training aids and REST donor samples (refer to Fig. 3). The foot training aids stored indoors and bones had relative class concentration trends that closely resembled REST donors. Additionally, foot samples stored indoors and wet bones (which still had organic content and soft tissue attached to them) originating from the amputated limbs were more effective in mirroring the VOC profile of REST donors when compared to dry tissue from amputated limbs, blood or teeth samples. Regardless, training on dry bone and blood is important given the need for HRDDs to locate these materials operationally. Overall, considering the common VOCs, class abundance, and concentration trends, it is recommended that HRDDs be exposed to all training aid types (foot, bone, and soft tissue from amputated limbs, and blood) except teeth containing minimal organic matter, which showed the least similarity to donor VOC profiles. Additionally, teeth are less likely to be located operationally in the absence of any other human remains material.

Principal component analysis (PCA) was used to examine VOC profile variations between HRDD training aids and REST donors. Following the removal of several (six of 85) extreme samples, the PCA (presented in Fig. 4) highlighted two key findings. Firstly, there was greater intra-sample set variability in REST donors compared to the HRDD training aid samples, even between HRDD training aids of different types (such as foot, bone, tissue, blood and teeth). This variability could be attributed to the dominance of trimethylamine, 2-butanol, 2-methylpropanoic acid, dimethyl disulfide, and 3-methylbutanoic acid in the REST donor samples. These VOCs were not entirely absent from HRDD training aids, although they were not as significant based on the distribution observed on the PCA biplot (Fig. 5). Secondly, 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 (HRDD training aids and REST donors). Thus, based on PCA, and contrary to what was initially hypothesized, 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 between the HRDD training aids types and REST donors. The findings from the PCA were consistent with the dog trials as the HRDDs did not seem to differentiate between odor profiles of their training aids and donors as indicated by the 100 % detection rate for identifying both fresh and decomposed donors (at REST). It was also observed that even though foot samples stored outdoors and teeth had low detection rates among HRDDs, they did not cluster separately in the PCAs. Additionally, during the trials, one HRDD, who had never been trained with any of the training aids used by the OPP, was exposed to these samples and donors, and provided a trained final response for both. It is worth noting that this aspect of the study was only conducted once in an outdoor setting, and was recorded as an additional

Table 4
List of VOCs detected in the May 2021 trial that were found common between HRDD blood training aids and prominent VOCs in REST donors. (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 trisulfide (DMTS)	3-Methyl-1-butanol
3-Methyl-butanoic acid	Methyl thiocyanate	3-Methyl-2-pentanone
Dimethyl disulfide (DMDS)	Pyridine	3-Methyl-butanoic acid
Methyl thiocyanate	Trimethyl pyrazine	Dimethyl trisulfide (DMTS)
Pyridine		Methyl thiocyanate Pyridine

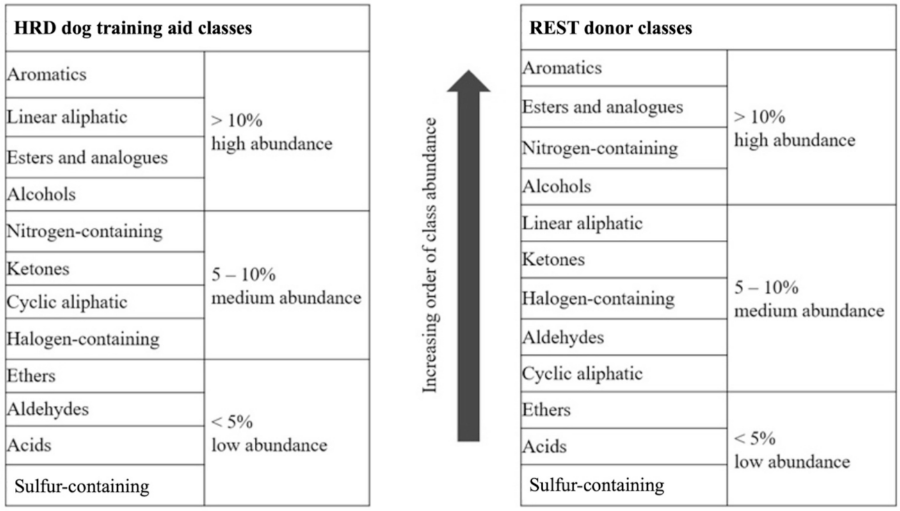

Fig. 2. Summary of class abundance trends in HRDD training aid and REST donor samples.

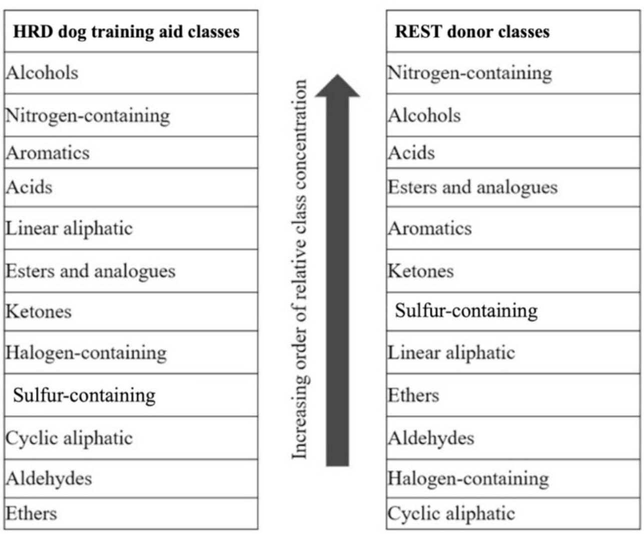

Fig. 3. Summary of relative class concentration trends in HRDD training aid and REST donor samples.

observation (not reported in the results shown in Fig. 1) during the study, thus, adding to the value of validating the training aids used in the current study. The authors recommend further studies be conducted on working dogs that have never been trained for human remains, as a majority of the dogs used in the trials had trained on and previously been exposed to the OPP training aids. Examining dogs lacking such exposure facilitates a better understanding of their innate capabilities, and training techniques, thereby minimizing potential biases from prior training. This method strengthens the credibility and applicability of results concerning human remains detection, spanning various scenarios and operational environments.

3. Conclusions

When comparing VOC profiles and HRDD performance, similarities were found between the VOC profile of HRDD training aids and REST donors in terms of class abundance, relative concentration and significant VOCs. The VOC class abundance trend of individual training aid type closely resembled that of REST donors, while relative class concentration trends were somewhat variable. Foot training aids stored indoors and bone training aids had relative class concentration trends that closely resembled those of REST 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 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 HRDD training aid types had the highest percentage (68 %) of VOCs common with REST donors, thus implying that it would be preferable to expose HRDDs to all training aid types (foot, bone, and soft tissue from amputated limbs, and blood) in order to expose them to the maximum VOCs from the human decomposition odor spectrum. However, if limited training aids are available, then the VOC profile of foot samples stored indoors was closest to that of the REST donors. Teeth (without organic matter) were found to be the least ideal training aid based on the VOC profile and HRDD response reported by handlers when HRDDs are expected to locate cadavers or parts of cadavers during field searches. Training on teeth may be necessary depending on operational requirements where HRDDs are required to locate teeth during field searches. The presence of sevoflurane, the anaesthetic in HRDD training aids and its absence from the REST donors did not impact the HRDD detection capability as evidenced by the 100 % donor detection rate of both fresh and decomposed donors at REST.

Ethics approval

The human research ethics approval for working with human remains in the form of HRDD 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.

The ethics approval for the study conducted at REST 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.

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.

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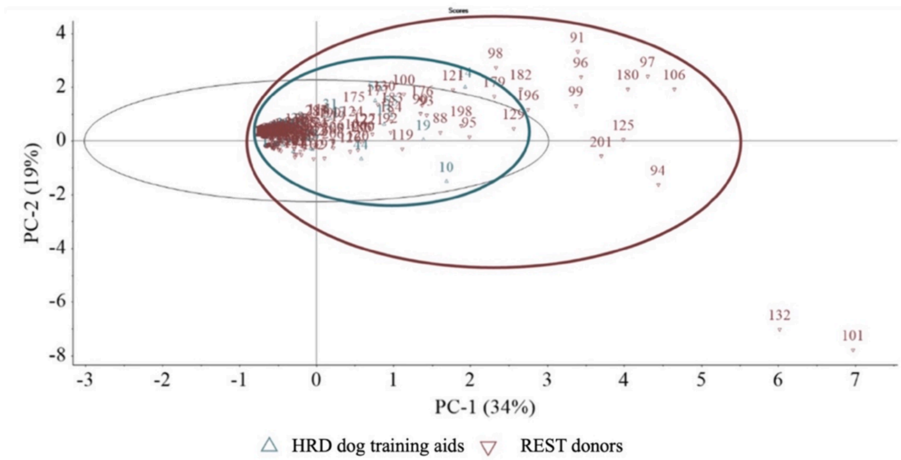

Fig. 4. PCA scores plot for PC-1, PC-2. PCA scores were calculated using the pre-processed GC × GC-TOFMS normalized peak area of prominent VOCs in HRDD training aid samples and REST donor samples (or data points in the above PCA) after removing the six extreme loadings. (Here, colour codes and symbols represent sample set type, olive green triangles for HRDD training aids and brown inverted triangles for REST donors).

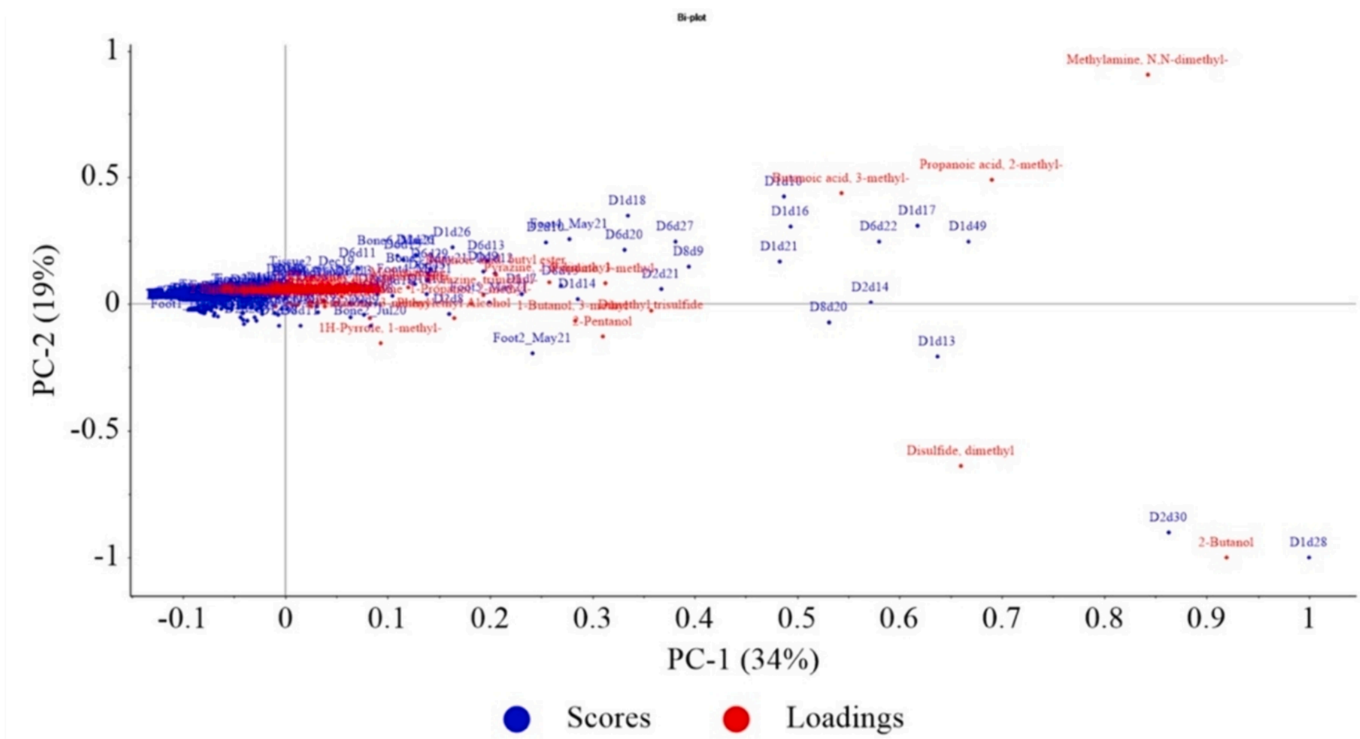

Fig. 5. PCA biplot for PC-1, PC-2 for HRDD training aids and REST donor samples after removing the six extreme loadings identified from PCA in Fig. 4. (Here, blue circles represent scores – HRDD training aids and REST donor samples and red circles represent loadings – VOCs).

Declaration of Generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work the author (RD) used Magic media on Canva in order to generate the image of a dog and blood on a gauze pad for the graphical abstract. After using this tool/service, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Rushali Dargan: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Darshil Patel:** Writing – review & editing, Visualization. **Wesley S. Burr:** Visualization, Software. **Benoit Daoust:** Writing – review & editing. **Clifford Samson:** Writing – review & editing, Resources. **Shari L. Forbes:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Clifford Samson reports a relationship with Ontario Provincial Police that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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