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Original Article

A Method for Controlled Odor Delivery in Olfactory Field-Testing

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Abstract

A widely recognized limitation in mammalian olfactory research is the lack of current methods for measuring odor availability (i.e., the quantifiable amount of odor presented and thus available for olfaction) of training or testing materials during behavioral or operational testing. This research utilized an existing technology known as Controlled Odor Mimic Permeation Systems (COMPS) to produce a reproducible, field-appropriate odor delivery method that can be analytically validated and quantified, akin to laboratory-based research methods, such as permeation devices that deliver a stable concentration of a specific chemical vapor for instrumental testing purposes. COMPS were created for 12 compounds across a range of carbon chain lengths and functional groups in such a way to produce similar permeation rates for all compounds. Using detection canines as a model, field-testing was performed to assess the efficacy of the method. Additionally headspace concentrations over time were measured as confirmation of odor availability using either externally sampled internal standard-solid phase microextraction-gas chromatography-mass spectrometry (ESIS-SPME-GC-MS) or collection onto a programmable temperature vaporizing (PTV) GC inlet with MS detection. Finally, lifetime usage was considered. An efficient method for producing and measuring reliable odor availabilities across various chemical functional groups was developed, addressing a noted gap in existing literature that will advance canine and other nonhuman mammal research testing.

Key words: canine olfaction testing, controlled odor mimic permeation system, odor availability, olfaction

Introduction

In laboratory settings, permeation tubes are often used to release constant, quantifiable amounts of a vapor for analytical purposes (Lucero 1971); however, such methods require closely controlled temperatures and airflow, making them impractical as a method to control odor availability (or the quantifiable amount of odor presented) for olfactory field-testing with canines or other mammals. For olfactory discrimination and similar olfactory studies, researchers have created their own olfactometers that deliver a given vapor *via* a controlled airstream (Pfaffmann et al. 1958; Slotnick et al. 1974; Johnston et al. 1994; Hall et al. 2016; Burton et al. 2019). Like a permeation tube system, odor can be increased or decreased by dilution in air. Although these instruments work well in controlled laboratory settings, the olfactometers cannot readily be used in field settings and require additional training of the animal to sample from the odor delivery port. It is highly desirable to create a system that provides the advantages of permeation tubes, which is also easily transported and adaptable to field conditions. In relation to canine

olfactory experiments, there is a well-cited scarcity of techniques for this purpose, which severely limits research and advances in the field (SWGDOG 2010; Lotspeich et al. 2012; Lazarowski et al. 2015; Hall et al. 2016; OSAC 2017). The current research provides a solution to this acknowledged gap in literature by addressing odor availability in olfactory perception experiments and applications.

Odor availability, or the amount or concentration of odor presented for olfaction, is a major topic of misconception among canine programs specifically, where the mass of the training material is often misconstrued as equivalent to its odor availability (SWGDOG 2010; Lotspeich et al. 2012; OSAC 2017). For example, the Scientific Working Group for Dog and Orthogonal detector Guidelines (SWGDOG) recommends using a minimum of 113.4 g (1/4 pound) of a substance for certification of an explosives detection canine (SWGDOG 2012). Additionally, many trainers and handlers colloquially refer to training on "trace" or "bulk" amounts of a target with little consideration of odor availability. Although mass does affect odor availability, the 2 concepts are not equivalent (Lotspeich et al. 2012). Moreover, other factors affect odor availability, such as vapor pressure, surface area of the material, container volume, molecular interactions with the container (e.g., wrapping/burial), temperature, humidity, rates of evaporation or sublimation, age of the material, and odor dispersion (Lotspeich et al. 2012; MacCrehan et al. 2012; Ewing et al. 2013; Papet 2016). Both SWGDOG and its successor organization, the Dogs and Sensors Subcommittee of the Organization of Scientific Area Committees (OSAC) under the National Institute of Standards and Technology (NIST), list research into odor availability as a priority need for advancing the field of canine olfaction (SWGDOG 2010; OSAC 2017). Such gaps in research lead to confusing and inappropriate training guidelines for working dogs and relevant research that can, in turn, result in deficiencies in detector performance.

Aside from training and operational considerations for working dogs, odor availability should also be controlled as a variable in olfactory and behavioral studies. It is essential to deliver known, quantifiable, and reproducible rates of odor release to subjects throughout a research study in order to obtain uniform, reproducible results. By disregarding odor availability, results often have limited applicability and interpretability, leading to overgeneralizations in the literature (Hallowell et al. 2012; Lotspeich et al. 2012; Lazarowski et al. 2015; Hall et al. 2016; Papet 2016). For example, variations in vapor pressure and odor intensity are very influential in odor perception by a canine as shown by Hallowell et al. (2012) in a study where canines trained to 9 cross-contaminated odors were only able to successfully locate the 2 or 3 most volatile compounds, an effect known as overshadowing (Papet 2016).

Other studies, although otherwise being well-designed, fail to control for differences in odor availability. Thus, a study that might have drawn useful conclusions to enhance current understanding of olfactory perception is limited without this experimental design consideration. For example, one experiment by Hall et al. (2016) evaluated canines' tendency to discriminate between chemically related alcohols increasing in carbon chain length. The authors, however, did not control for the decreasing vapor pressure (and thus decreasing odor availability) with increasing chain length. Results of the study showed that canines were better at discriminating alcohols with shorter carbon chains (higher vapor pressure and greater odor availability) than those with the longer chains, consequently making it unclear whether this finding was related simply to the odor availability differences between longer/shorter chain alcohols or to actual differences in olfactory perception (Hall et al. 2016). Similarly, the authors of a study testing canines trained to detect ammonium nitrate alone for their ability to locate similar ammonium nitrate-based odors recognized that the inability to match odor intensity between testing odor pairs could have influenced canine perception of those odors (Lazarowski et al. 2015).

One study by Lotspeich et al. (2012) attempted to evaluate odor availability of certain liquid explosives for the purpose of canine training. Noting that the concept of odor availability is widely misconstrued by practitioners in the field, the authors propose using a model for vapor generation to help determine odor availability based on factors such as container volume, sample amount, and temperature (Lotspeich et al. 2012). Although such research is a step in the right direction, it does not control odor availability between dissimilar odor chemicals and materials as would have been necessary in olfactory perception experiments similar to Hall et al. (2016) and Lazarowski et al. (2015), for example.

To address the issue of providing quantifiable amounts of odor in canine training within forensic settings, Furton and Harper (2008) designed Controlled Odor Mimic Permeation Systems (COMPS), which deliver odor at known and reproducible amounts. COMPS are a permeable polymer container stored inside a nonpermeable package and can, therefore, be used over multiple training or testing sessions with consistent levels of odor present. The permeable polymer container can be optimized for desired permeation rates and is adjustable per chemical compound or material. Additionally, COMPS are simple, disposable, and cost efficient. For example, Macias et al. (2010) used COMPS as a calibrant for canines trained to detect piperonal, providing for a consistent method of training between both individual canines and sessions. Although successful in controlling odor availability between sessions using the same material, this application did not address the need to control odor availability between various analytes being tested (in this case, piperonal and another narcotic training aid), which would allow for comparison between canine perception of multiple chemical compounds or materials.

The current study used COMPS to control odor availability across compounds of differing carbon chain lengths and functional groups and, thus, with highly differing vapor pressures in order to present canines with similar concentrations of odor throughout a battery of field tests. This application not only controls permeation rates during canine testing, it also matches permeation rates of various neat analytical compounds for use in research settings. In this way, it can be considered a method of fieldable permeation tubes. Further, headspace measurements were done to compare analyte vapor concentration from the COMPS, in addition to canine testing for proofs of concept.

Materials and methods

Experimental method

For this study, COMPS were created by spiking 5 µL of a neat liquid analytical standard onto a piece of gauze (DUKAL Corporation, 2" × 2", 12 ply) folded in half inside of a 2" × 3" low-density polyethylene (LDPE; Industrial Poly Bags; Uline) bag that was then heat sealed (Figure 1). LDPE bags of varying thicknesses were tested for each compound: 1, 2, 3, 4, 6, and 8 MIL. Compounds tested and their respective molecular structures and vapor pressures are given in Table 1. The compounds selected were acids of varying chain lengths and orientation (C4–C7, straight and branched) and 5-carbon compounds of differing functional groups, in addition to methyl benzoate. All 12 compounds were purchased from Sigma-Aldrich and were at least 99% pure.

Figure 1. Image of a COMPS to be placed inside of a barrier bag that will serve as primary containment.

Permeation rate, as determined by gravimetric analysis, was used as one measure of odor availability. For the purpose of this study and future canine testing, the goal was to select LPDE bag thicknesses to provide similar odor permeation rates for each compound in Table 1 despite variations in vapor pressure and molecular structure. COMPS were placed in a weigh boat on an analytical balance and the mass was recorded over time for a minimum of 4 h. When not being weighed, the COMPS were kept in a fume hood. Permeation rate in mass per unit time (mg/min) was then calculated as the slope of the mass as a function of time. All measurements were taken in replicates of 3 or more, subtracting the masses of the empty LPDE bag and unspiked gauze pad to obtain the compound mass. Additionally, negative controls (i.e., blank material) were measured in the same manner and showed no decrease in mass with time.

Headspace analysis and instrumentation

Headspace concentration was used as another measurement of odor availability. For this purpose, each COMPS was placed in a 1-pint metal sample container (Tri-Tech Forensics), which was then placed in a 1-gallon epoxy-lined metal sample container (Tri-Tech Forensics). The containers were then stored in an open fume hood. Samples of air were taken at 1 and 3 h, and a lid with a 1-cm hole was placed on the container only during sampling to minimize dilution of the sample with surrounding air. Sample collection was done through a whole-air sampling method. A 3/16" polytetrafluoroethylene (PTFE) tube was inserted into the hole of the lid to a depth of 10 cm, mimicking the approximate sample location during a canine sniff. A Grab Air Sample Pump (SKC Inc.) was then attached to the tubing and approximately 750 mL of the headspace was collected into a 1-L Tedlar bag (SKC Inc.). All samples were taken at room temperature and samples were immediately analyzed.

The air in the Tedlar bags was preconcentrated onto a cooled injection system (CIS; CIS-4, Gerstel, Inc.) by flowing 500 mL (50 mL/min for 10 min) onto a Tenax-filled CIS liner cooled to 0 °C. After trapping, analytes were rapidly thermally desorbed from the liner at 250 °C directly onto the column of a gas chromatograph-mass spectrometer (GC-MS; Agilent 7890A gas chromatograph/5975 mass selective detector). The GC column was a 30 m × 0.32 mm ID Rtx-Volatiles column (Restek Inc.) and the ionization source was in electron ionization (EI) mode at 70 eV. A split ratio of 10:1 was used with a constant flow rate of 2 mL/min. The GC column oven was initially held at 40 °C for 1 min, then the temperature was increased 30 °C/min to 240 °C, where it was held for an additional minute. The mass spectrometry acquisition range was set from 30 to 200 m/z. Quantification was done *via* comparison to an external calibration curve.

Headspace concentration was also measured at varying temperatures and relative humidities using pentanoic acid as a representative analyte. The same method was followed as for the samples taken at room temperature, except the metal containers were placed in a large environmental chamber (12' × 12' × 10') that allowed adjustment of temperature and relative humidity. The chamber contains an exhaust apparatus so that the air was purged between samples. Samples were taken at the following temperature and relative humidity combinations to mimic outdoor sampling conditions in the mid-Atlantic region: 20 °C at 20% RH, 26 °C at 40% RH, 32 °C at 60% RH, and 6 °C at 25% RH. For each condition, 4 replicates were sampled in the chamber together, placed at least 8 feet apart. Blanks were also taken under each testing condition.

For the purpose of field sampling, odor concentration above COMPS of varying LDPE bag thicknesses was determined using externally sampled internal standard-solid phase microextraction-gas chromatography-mass spectrometry (ESIS-SPME-GC-MS). This method, based on MacCrehan et al. (2011), uses a separately sampled internal standard in order to improve the reproducibility of SPME by accounting for fiber-to-fiber and day-to-day instrument variability. In this case, 100 µL of methyl salicylate (99%, Thermo Fisher Scientific) was pipetted into a 40-mL glass vial with a screw top cap with a PTFE/silicone septa (Sigma-Aldrich) and allowed to equilibrate for 18 h. Four different odor levels of COMPS were prepared using methyl benzoate by varying LDPE bag thickness according to Table 2. Each COMPS was placed separately in an open 8" × 6" × 4" cardboard box (Uline) and allowed to permeate for 1 h. The COMPS with no LDPE bag was allowed to permeate from an open box for only 5 min. These times were chosen to mimic the amount of time such odors would be allowed to permeate before canine trials. Next, polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR) fibers (Sigma-Aldrich) were exposed to the headspace of the methyl salicylate for 15 s, followed immediately by a 5-min extraction of the COMPS. SPME fiber extraction time was previously optimized, and a 5-min extraction was found to be optimal. The fibers were suspended 2 inches above the COMPS during the extraction. Fibers were desorbed at 250 °C onto a polar SolGel-WAX column (30 m × 0.25 mm ID, Trajan Scientific and Medical) in a GC-MS (Agilent 6890 gas chromatograph/5973 mass selective detector). A split ratio of 20:1 was used with a constant flow rate of 1 mL/min. The GC column oven temperature was increased from 40 to 180 °C at 20 °C/min, then to 240 °C at 10 °C/min, and finally to 260 °C at 20 °C/min. The ionization source was in EI mode at 70 eV and the mass spectrometry acquisition range was set from 45 to 450 m/z. All samples

Table 1. The compounds tested and relevant information, including structure and vapor pressure

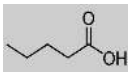
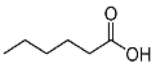
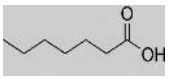
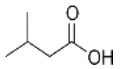
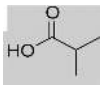
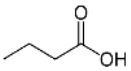
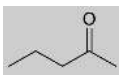
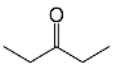
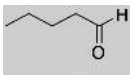
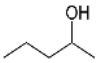
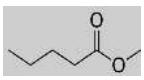
Compound	Structure	Elemental composition	Vapor pressure (mmHg at 25 °C)
Pentanoic acid		$C_5H_{10}O_2$	0.196
Hexanoic acid		$C_6H_{12}O_2$	0.0435
Heptanoic acid		$C_7H_{14}O_2$	0.0107
3-Methylbutanoic acid		$C_5H_{10}O_2$	0.44
2-Methylpropanoic acid		$C_4H_8O_2$	1.81
Butanoic acid		$C_4H_8O_2$	1.65
Pentan-2-one		$C_5H_{10}O$	35.4
Pentan-3-one		$C_5H_{10}O$	37.7
Pentanal		$C_5H_{10}O$	31.8
Pentan-1-ol		$C_5H_{12}O$	2.2
Methyl pentanoate		$C_6H_{12}O_2$	32.5
Methyl benzoate		$C_8H_8O_2$	0.38

Table 2. Approximate odor level restriction based on chosen LDPE bag thicknesses

	Approximate odor restriction	LDPE bag thickness
1	High	4 MIL, 2” × 3” inside aluminum bag (3” × 4”) with 1/8” hole
2	Medium	8 MIL, 2” × 2”
3	Low	4 MIL, 2” × 3”
4	Unrestricted	No bag

were analyzed in triplicate. Data is represented as a ratio between resulting peak areas of the analyte to the externally sampled internal standard, identified as A/E.

Lifetime testing

In addition to testing headspace concentration at various temperatures and humidities, it is also important to test lifetime of the COMPS. COMPS lifetime, that is, usage time, was estimated using the pentanoic acid COMPS. A single COMPS was sealed in a metalized barrier bag (3.5” × 4.5”), which functioned as primary containment, and then placed in a 16-oz. glass jar as secondary containment. Following a week of storage in this manner, the COMPS was removed from both primary and secondary storage and placed in the 1-gallon sample container for 1 h, and the headspace was sampled and analyzed by CIS-GC/MS using the previously described method. The COMPS was replaced in primary and secondary containment following sampling. This procedure was repeated daily to mimic 9 daily, 1-h canine training sessions. The headspace concentration was compared with a freshly made COMPS. All samples were prepared and analyzed in triplicate.

Canine olfactory trial

A canine olfactory test was completed as a proof of concept for COMPS odor delivery, testing canine discrimination between carboxylic acids compounds of varying chain lengths and branching. Seventeen canines were trained to detect pentanoic acid COMPS, prepared as described above and stored in a metalized barrier bag (3.5” × 4.5”) inside a 16-oz. glass jar when not in use. The canines were subsequently tested on their tendency to generalize or discriminate between pentanoic acid and other related acids in COMPS, including 2-methylpropanoic acid, 3-methylbutanoic acid, butanoic acid, hexanoic acid, and heptanoic acid.

The test was performed as a series of odor recognition tests (ORTs). The ORTs were comprised of a line of five 8” × 6” × 4” cardboard boxes (Uline), providing a uniform method of testing the canines’ ability to locate the trained materials. For testing, each ORT contained 1 target COMPS, 1 distractor odor, and 3 blanks. Distractor odors were one of the following, selected at random: limonene, cinnamaldehyde, α-amylcinnamaldehyde, citral, cuminaldehyde, pinene, β-caryophyllene, isoamyl acetate, nerolidol, eucalyptol, or phenol (98% pure, Sigma-Aldrich), all prepared as COMPS. Negative runs containing 1 distractor and 4 blanks were also included. To avoid introduction bias, the locations of all odors and distractors were assigned by a random number generator for each canine.

All trials were carried out as a double-blind testing scenario where neither the handler nor the 2 impartial evaluators knew the identity or location of the odors. Canine responses were recorded as one of the following: alert (i.e., a positive, correct response) or false alert (i.e., a positive, incorrect response). Alert rates and false alert rates were subsequently calculated as percent responses for all dogs.

Canine participants were volunteers from the National Association of Canine Scent, LLC (NACSW) a.k.a. K9 NoseWork. K9 NoseWork is an organization dedicated to providing domestic (pet) dogs with classes and competitions in scent detection using the essential oils birch, anise, and clove. Participants were instructed to train on the pentanoic acid in the same manner in which they train with their essential oils.

Ethical note

All trial protocols were reviewed and approved by the Florida International University Institutional Animal Care and Use Committee as well as the Navy Bureau of Medicine and Surgery.

Results and discussion

Permeation rates for single and binary odors

The major advantage for using COMPS in olfaction field testing is that they control the delivery of odorants at a known and constant rate, helping to correct differences across vapor pressures of various

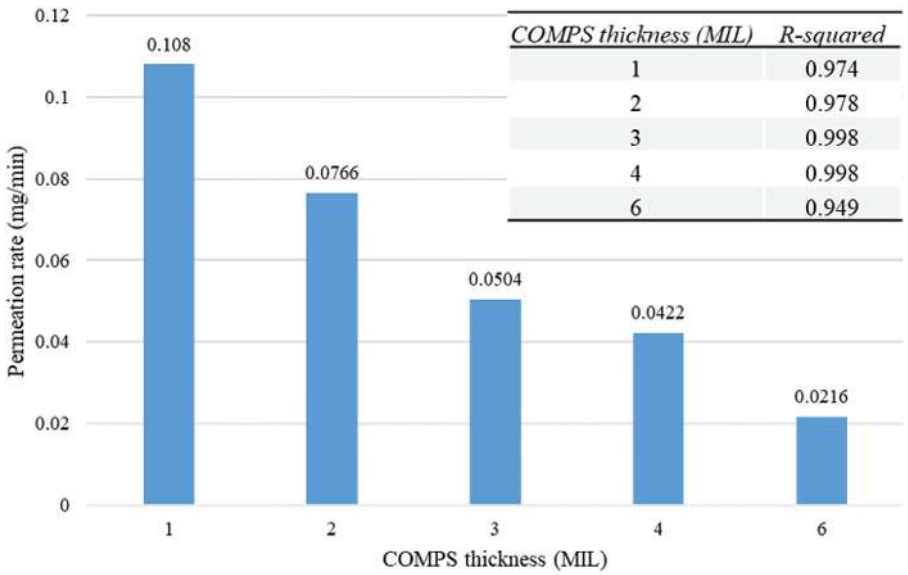


Figure 2. Permeation rates (mg/min) and *R*-squared values for all tested COMPS thicknesses (MIL) for methyl benzoate.

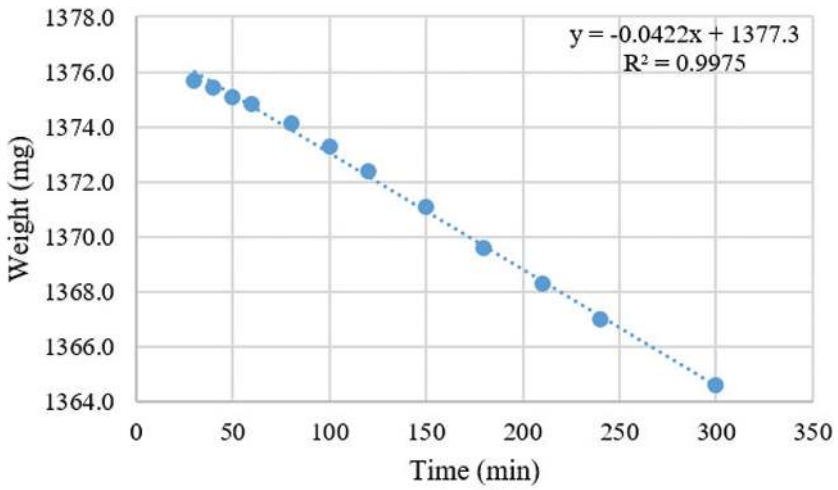


Figure 3. Permeation rate given by mass loss (mg) per time (min) for the selected COMPS bag thickness for methyl benzoate (4 MIL). Error bars representing 1 SD from the average are included in the marker.

chemicals to provide similar amounts of odor for all compounds. The proper thicknesses of LDPE bag (MIL) was determined by measuring odor dissipation using gravimetric analysis. For brevity, all individual compound test data is not shown, but an example using methyl benzoate is provided herein (Figure 2). Permeation rates (mg/min) are given for all 5 LDPE thicknesses tested, as well as *R*-squared values showing the line fit for each permeation rate, which were both used in COMPS selection. In this case, the 4-MIL bag was chosen (Figure 3) because it had both the best line fit of those tested (R^2 = 0.998) as well as a steady permeation rate of 0.0422 mg/min, which approximately matched that of all other compounds (see below).

The process for selecting LDPE bag thickness for methyl benzoate was repeated for each of the remaining 11 compounds. Figure 4A shows a comparison of vapor pressures for all tested compounds, highlighting the large variation within this group of chemicals, whereas Figure 4B compares the permeation rates using the selected bag thicknesses for each. The COMPS produced steady dissipation and odor permeation rates within approximately 5% of each other for most compounds. Equal rates of permeation could not be achieved for some compounds due to the limited range of discrete bag thicknesses available. In these cases, the value closest to the others that provided a steady, reproducible permeation rate was chosen. Notably, the relative standard deviation (RSD) was reduced from 138.0% among vapor pressures to 31.8% among permeation rates for all compounds using this method, indicating that COMPS were effective at controlling odor availability across a variety of different compounds.

A sample set of 10 individual compounds was chosen for vapor concentration measurement to confirm that the selected permeation rates resulted in consistent odor availability. The vapor concentration above each COMPS was quantified over time (at 1 and 3 h) to show that odor was released at a steady, reproducible rate (Table 3). The headspace concentration of pentanoic acid was steady over time, at approximately 0.48 ppm_v (±7.4%) after 1 h and 0.45 ppm_v (±14%) after 3 h. The majority of the acidic compounds produced vapor

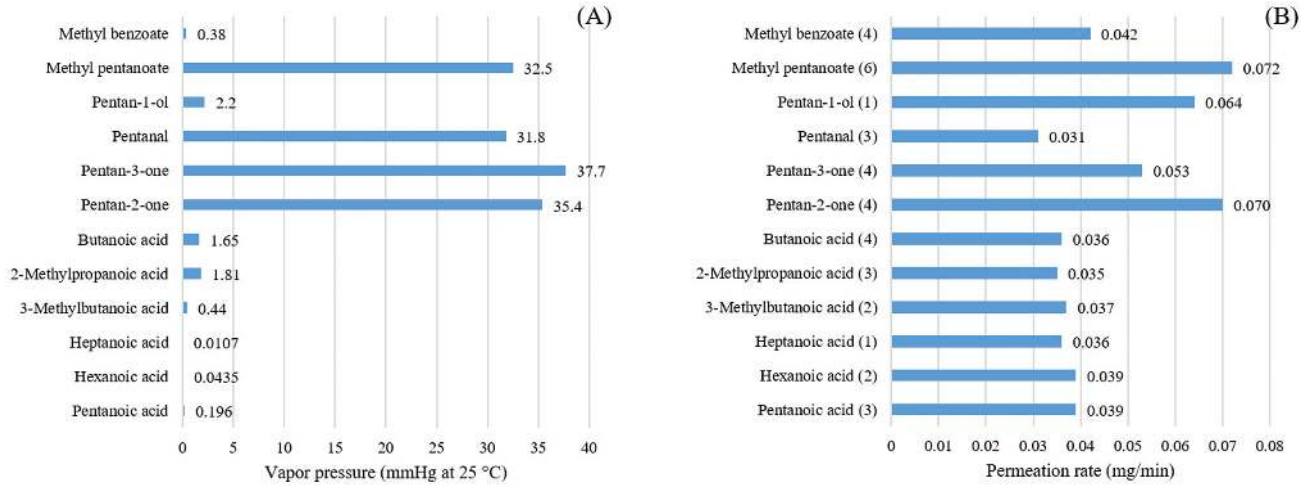


Figure 4. Variation in **(A)** vapor pressures (mmHg at 25 °C) for 12 tested compounds (RSD = 138.0%) compared with **(B)** permeation rates (mg/min) for 12 tested compounds (RSD = 31.8 %). Numbers in parenthesis show selected COMPS thickness in MIL.

Table 3. Headspace concentration (ppm_v) of 10 individual compounds in COMPS, collected after 1 and 3 h of dissipation

Compound	1 h (ppm_v)	3 h (ppm_v)
Pentanoic acid	0.479 ± 7.4 %	0.453 ± 14.3 %
Hexanoic acid	0.402 ± 93.5 %	0.428 ± 65.9 %
Heptanoic acid	0.0690 ± 6.6 %	0.0541 ± 9.0 %
3-Methylbutanoic acid	0.410 ± 19.3 %	0.765 ± 25.9 %
2-Methylpropanoic acid	1.46 ± 26.2 %	0.398 ± 37.9 %
Butanoic acid	0.960 ± 4.4 %	0.888 ± 29.5 %
Pentan-2-one	0.721 ± 6.6 %	0.491 ± 37.5 %
Pentanal	1.72 ± 38.1 %	0.371 ± 75.2 %
Pentan-1-ol	1.39 ± 71.2 %	0.736 ± 55.3 %
Methyl pentanoate	1.63 ± 33.9 %	0.991 ± 39.5 %

Error is expressed as relative standard deviation.

Table 4. Permeation rates for methyl benzoate contained in various thicknesses of COMPS (4 MIL and 8 MIL), plus a barrier bag to restrict vapor permeation (4 MIL w/1/8″ hole)

	Permeation rate (mg/min)	*R*-squared	Time of gravimetric analysis (h)
4 MIL	0.0422 ± 5.9 %	0.998	6
8 MIL	0.00499 ± 5.0 %	0.990	13.5
4 MIL w/1/8″ hole	0.000179 ± 17.7 %	0.972	318

Error is expressed as relative standard deviation.

concentrations similar to that of pentanoic acid, though some of the other functional groups deviated. Heptanoic acid yielded the lowest vapor concentration, although it is unclear whether this is due to an analytical issue, that is, sample degradation in the heated GC inlet, rather than a lack of compound dissipation from the COMPS. Four compounds (2-methylpropanoic acid, pentanal, pentan-1-ol, and methyl pentanoate) had higher vapor concentrations than pentanoic acid at 1 h and then depleted some at 3 h. There was a higher disparity in vapor concentration than in permeation rate due to other factors such as evaporation rate, diffusion rate, and surface adsorption. For example, 2 of the compounds (hexanoic acid and 3-methylbutanoic acid) increased their vapor concentrations from 1 to 3 h, likely due to one or more of these factors. However, when compared with the variation in vapor pressures, the discrepancy remains smaller for headspace concentrations. Thus, COMPS do assist in controlling odor availability.

Varying concentrations single odors

In addition to matching permeation rates between compounds, the permeation rate of methyl benzoate was manipulated to release odor at discrete levels using different bag thicknesses so that canine detection of varying concentrations could be tested. Three odor levels were developed using 4-MIL and 8-MIL bags, as well as a 4-MIL COMPS contained in a Mylar barrier bag with a 1/8” hole punched in this outer bag (leaving the LDPE bag intact) to further restrict vapor permeation. The resulting permeation rates (mg/min) decreased incrementally by approximately 1 order of magnitude from the 4-MIL bag at 0.0405 mg/min to the 8-MIL bag at 0.00499 mg/min and finally the 4-MIL bag with a 1/8” hole at 0.000179 mg/min (Table 4). As the permeation rates were lowered, it was necessary to extend the period of gravimetric analysis in order to obtain reliable data of the observed permeation rates, and those times are also noted in Table 4. These results show that COMPS can be used effectively to control permeation rates and, thus, increase or decrease odor availability as desired.

Odor availability above each of the 3 different bag thicknesses and a noncontained gauze was tested using ESIS-SPME-GC-MS to imitate field methods. As can be seen by Figure 5, the noncontained gauze produced the greatest analyte/externally sampled internal standard (A/E) ratio, whereas the 4 MIL with the 1/8” hole (i.e., the thickest LDPE bag) produced the least. With decreasing bag thickness, the amount of analyte recovered increased, which was the expected result. These results show that odor availability reflects the equivalent changes in permeation rate from Table 4, which are both controlled using selected LDPE bag thicknesses for the single compound methyl benzoate.

Lifetime determination

When considering canine training aids or other olfactory testing materials, it is important to determine lifetime, or use viability, as it can inform users how long the training or testing material will effectively

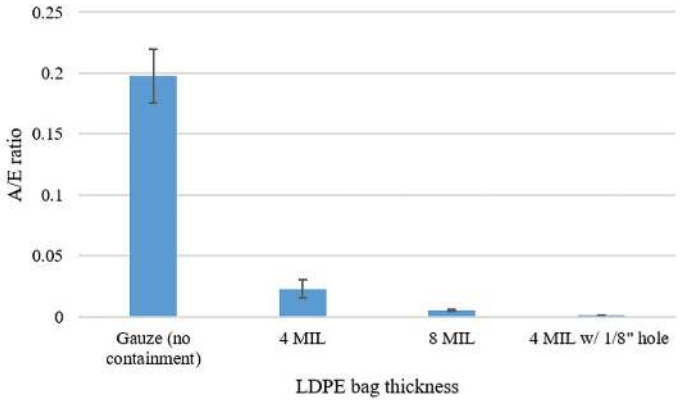


Figure 5. Average analyte/externally sampled internal standard ratio for 3 different LDPE bag thicknesses comprising methyl benzoate COMPS.

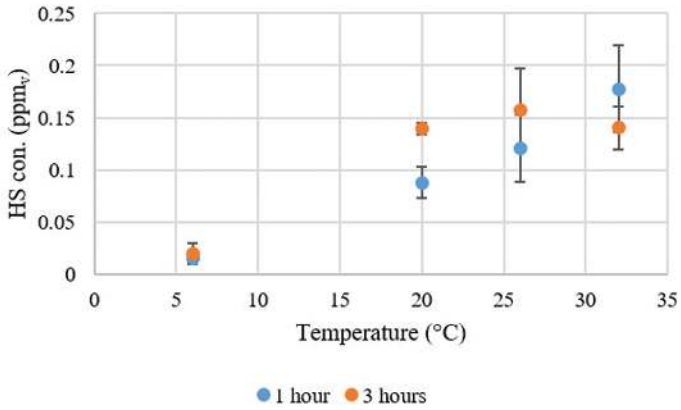


Figure 6. Headspace concentration of pentanoic acid COMPS after 1 and 3 h at varying temperatures. The relative humidities at each respective temperature were: 20% RH at 20 °C, 40% RH at 26 °C, 60% RH at 32 °C, and 25% RH at 6 °C. Error bars represent 1 SD from the average.

permeate at a given odor level. In this regard, 2 factors were studied, as described in the methods: (i) headspace concentration at varying temperature and humidities and (ii) headspace concentration after single hours of usage, mimicking the length of an average testing or training session. Pentanoic acid was used as a representative analyte for each of these factors because it is in both chemical groups studied (i.e., acids of varying lengths and 5-carbon compounds with differing functional groups).

The headspace concentration of pentanoic acid COMPS determined at differing temperature/relative humidity combinations is given in Figure 6. The conditions were chosen to mimic seasonal outdoor working conditions in the mid-Atlantic region. After 1 h, the concentration increased with increasing temperature as expected, meaning that under field conditions, more odor will be available as temperature is increased. The increase in odor availability was predictively linear (R^2 = 0.979) with increasing temperature for the 1-h test, indicating that changes in relative humidity had a minimal effect on odor availability. After 3 h of use, however, the odor from the COMPS held at higher temperatures decreased as the odor began to be depleted, indicating it is necessary to replace these more often when used at higher temperatures.

The lifetime usage of the COMPS was determined by measuring the headspace concentration following individual hours of "usage," which were designed to mimic daily, 1-h-long sessions of training or testing. COMPS were removed from their storage and the headspace concentration was measured daily for 1 h. The concentration of pentanoic acid was higher after the first hour than the fresh COMPS, likely due to adsorption or interaction of the compound with the bag while in storage (Figure 7). While in the barrier bag, the compound reaches equilibrium, which is disrupted when the COMPS is removed from its storage. Once removed from that closed system, the COMPS will resume releasing odor at the previously determined permeation rate. After the first hour, the concentration then remained consistent with that of the fresh COMPS through hour 7, after which it began decreasing. These results are consistent with emission trends of permeation tubes, which have 3 phases: saturation, steady state, and depletion (Lucero 1971). The saturation phase refers to the time from initial creation of the permeation tube or COMPS until equilibration of the analyte is reached, whereas depletion refers to the phase when analyte supply is exhausted. The phase between these two is known as the steady state, which provides the desired, consistent permeation rate of the analyte and is, thus, the only phase useful for measurement and experimentation in the field. It was, therefore, determined that the steady state phase for COMPS is between hours 1 and 7, indicating the viable time for use. Additionally, it is notable that standard deviation among replicates was generally lower during this time period, supporting that the steady state phase is more consistent across replicates as well as time.

These results are consistent with and can also help explain the trends observed in Table 3 and Figure 6, which measured the headspace concentration above single COMPS. Hour 1 can be considered the saturation phase, and it is thus expected to be slightly higher than

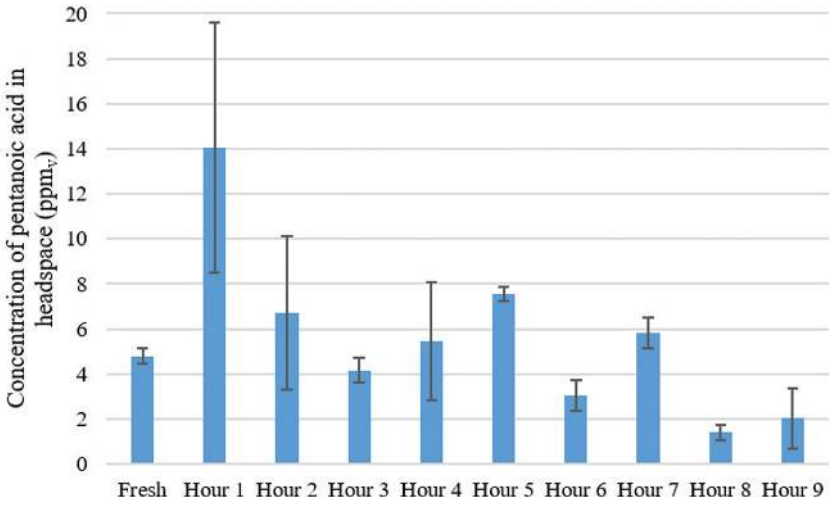


Figure 7. Headspace concentration of pentanoic acid above COMPS measured by the hour, mimicking 9 single hours of canine training.

Table 5. Canine (n = 17) data for trial. N = no alert; A = alert

Dog ID	2-Methylpropanoic acid	Butanoic acid	3-Methylbutanoic acid	Hexanoic acid	Heptanoic acid	Testing odors (out of 5)	Pentanoic acid (training odor; out of 4)	False alerts (out of 25)
TD101	N	N	A	N	N	1	4	0
TD102	A	A	N	N	N	2	4	0
TD103	N	A	A	N	N	2	4	0
TD104	A	N	N	N	N	1	4	1
TD105	A	N	A	A	A	4	4	1
TD106	N	N	A	A	A	3	4	2
TD107	A	A	A	N	N	3	4	1
TD108	N	N	N	N	N	0	4	4
TD109	A	N	A	A	N	3	4	2
TD110	N	N	N	N	N	0	4	2
TD111	A	N	N	A	N	2	4	2
TD201	N	A	A	A	N	3	4	2
TD202	A	N	N	N	N	1	3	3
TD203	N	N	A	N	N	1	3	6
TD204	N	A	N	N	N	1	4	3
TD209	N	N	N	N	A	1	4	3
TD213	A	A	N	N	N	2	4	3
Total	8	6	8	5	3	30	97%	8.0%

the steady state sample taken at hour 3. This was observed for all but 1 of the 10 single COMPS (3-methylbutanoic acid). These variations can again be explained by evaporation/diffusion rates or adsorption to the LDPE surface.

Canine validation

In an experiment similar to Hall et al. (2016), a proof-of-concept canine trial involving 17 dogs was conducted to test the COMPS training aids created (Table 5). Canines were trained to detect pentanoic acid and tested on 5 chemically related carboxylic acids, varying in length and branch conformation. The alert rate for the trained odor was 97%, whereas the alert rates for the testing compounds were all significantly lower, ranging from 18% to 47% (Figure 8). Discrimination increased with increasing carbon number difference, indicating a proportional relationship of perception between carbon difference and discrimination. Simply put, the more molecularly similar a chemical is to a trained odor, the easier it is for canines to generalize. These results were not due to chance alone because the canines were also asked to determine the presence or absence of their specific trained odor, and their capabilities were confirmed through validation testing.

These results agree with the results of Hall et al. (2016), which tested canine perception of alcohols of varying chain length. This previous study identified the same proportional trend, where increased carbon difference resulted in increased discrimination. However, Hall et al. identified the major weakness of their study as not being able to control for vapor pressure in odor delivery. The current study minimized this variable as a possible weakness by using COMPS to deliver all odors at a similar permeation rate (see Figure 4) and confirmed Hall's observations. It also addresses variables related to container volume and sample amount by regulating odor diffusion. This advance can be used for future odor delivery in canine trials, as well as trials with other species.

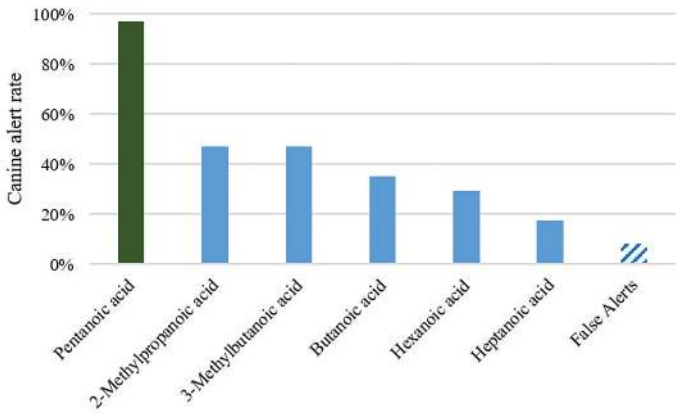


Figure 8. Results for canine trial testing generalization-discrimination between acidic compounds; pentanoic acid = training odor (n = 17).

Conclusion

A method was created for use in olfactory testing and related research based on COMPS, which effectively provided a system for comparable permeation rates of compounds across varying functional groups and chain lengths. Although COMPS have previously been used to regulate odor permeation of a single chemical compound or material for over the course of multiple training and testing sessions (Macias et al. 2010), this study greatly expanded the application by relating odor permeation among various chemicals. Furthermore, by characterizing COMPS using analytical experimentation, the technique can be applied as a method of fieldable permeation tubes for research involving olfaction and behavior in mammals.

LDPE bag thicknesses were chosen for 12 compounds based on permeation rate and *R*-squared values, which reflect consistency in permeation and diffusion of each compound despite varying vapor pressures. COMPS were also created for methyl benzoate with 4 distinct permeation rates depending on LDPE bag thickness, demonstrating that permeation rates can be selected to manipulate odor availability. Additionally, the headspace concentration was tested above single compounds, showing that at room temperature they can be reliably utilized between hours 1 and 7. Furthermore, lifetime usage was defined as an essential consideration for canine training materials.

These results are an advancement in canine olfaction research with implication to future research efforts. Although COMPS may not be appropriate to odorants involving complex mixtures or matrices (because the individual components may not permeate the barrier at equivalent rates), the results show that for simple odorants, it is possible to perform olfactory testing in a reliable, quantifiable manner with support of analytical measurements. Despite being widely recognized as a major limitation in canine olfactory research and training, this is the first time that an effective field-appropriate, analytical method of controlling odor availability across a large variety of chemicals for canine testing has been achieved. Future use of this method can improve canine research and behavioral or operational testing, ultimately advancing canine proficiency. It also has similar implications for other nonhuman mammal olfactory and behavioral testing.

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Conflict of interest statement

The authors have no conflict of interest to declare.

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