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Canine Generalization to Molecularly Similar Odors and Odor Mixtures

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14. ABSTRACT

The Naval Research Laboratory, in collaboration with the Naval Surface Warfare Center Indian Head EOD Technology Division and Florida International University performed research exploring the tendency for canines to generalize or discriminate similar odorants and odorant mixtures, referred to as the generalization-discrimination balance. The effects of molecular structure, mixture perception, and training on canine ability to generalize or discriminate between related target odors were studied. The effect of training was addressed by incrementally increasing the number of odorants as training continued. Controlled odor mimic permeation systems (COMPS) were used to provide consistent permeation rates across all odorants. Trial results indicated some tendency to increase generalization with increased training on multiple odorants, though only training on multiple functional groups increased generalization significantly. Best practice for detection canine training is to train each on a variety of materials and mixtures.

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Table of Contents

Table of figures

Table of Tables

EXECUTIVE SUMMARY

The Naval Research Laboratory, in collaboration with the Naval Surface Warfare Center Indian Head EOD Technology Division and Florida International University performed research exploring the tendency for canines to generalize or discriminate similar odorants and odorant mixtures, referred to as the generalization-discrimination balance. The effects of molecular structure, mixture perception, and training on canine ability to generalize or discriminate between related target odors were studied. Three sets of odorants were used in three separate experiments: (1) single target odorants of similar functional groups with differing carbon chain lengths, (2) single target odorants of similar carbon chain length but differing functional groups, and (3) binary odorant mixtures of both similar and dissimilar molecules. Within each odor set, the effect of training was addressed by incrementally increasing the number of odors each canine was trained to detect, and subsequently comparing the tendency of canines to generalize to like odorants as training continued. Initial laboratory analyses were carried out to ensure equivalent and reproducible odor availability.

Controlled odor mimic permeation systems (COMPS) were used to provide consistent permeation rates across all odorants. Once optimized, the COMPS were used for all canine training and ensuing detection trials. Canines in the single-odorant studies increased discrimination with increasing molecular dissimilarity. Canines in the binary mixture study, however, increased discrimination with molecular similarity because the individual components within a similar mixture are more difficult to separately identify. Trial results indicated some tendency to increase generalization with increased training on multiple odorants, though only training on multiple functional groups increased generalization significantly. Based on the results of this research, best practice for detection canine training is to train each canine on a variety of materials and mixtures. Further consideration of this topic will improve understanding of the canine olfaction processes, which could improve training and deployment proficiencies.

BACKGROUND / MOTIVATION

Canines are often deployed for the detection of substances, such as explosives, narcotics, humans (living and deceased), agricultural products, and wildlife. In such search capacities, canines are extremely adept at generalizing between like odor profiles of similar target odorants while simultaneously discriminating these profiles from large amounts of background or distracting odors. Generalization refers to when small differences between odorants are ignored, versus discrimination where those differences are accentuated. This selectivity is largely considered to be superior compared to instrumental detectors [1]. However, there is a dearth of fundamental research regarding canines' olfactory abilities concerning generalization and discrimination of similar odorants.

In the field, detection canines must generalize between target odorants of various origins, variants, and purities yet discriminate between target odorants and similar non-target or background odors. It is known that increased training on a target odorant increases canine ability to discriminate between that odorant and other like compounds [2]. For example, the odor of snapdragon flowers contains methyl benzoate, which is also the main odorant of cocaine. Cerreta et al. demonstrated that trained canines successfully discriminate between the two sources of methyl benzoate and will not alert to snapdragons when trained to cocaine [3]. While discrimination in such instances is important, it is also often necessary for canines to generalize between like odorants. In this example, canines should detect cocaine regardless of any manufacturing or cutting agent differences in the drug.

There are few peer-reviewed data available concerning canine generalization and discrimination of odorants. Cerna et al. revealed that when trained explosive detection canines previously trained to a single TNT source were expected to detect TNT of different origins, they did not readily generalize to alternative forms [4]. Similarly, another study showed that canines trained to only a single source of ammonium nitrate detected other variants of ammonium nitrate at a significantly lower rate than the trained variant [5]. These research studies demonstrate how an imbalance of generalization-discrimination in trained detection canines could be detrimental in the field. Understanding how to shift this balance can inform how canines are trained, thus increasing proficiency.

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The balance between generalization and discrimination in odor recognition is affected by several factors, including molecular structure, mixture perception, and training. The current study was designed to address each of these influences in turn. Initial detection canine testing explored perception of similar single-target odorant compounds. Structurally similar molecules may compete and activate overlapping olfactory receptors, making those compounds more difficult to discriminate [6]. In one study of odorant similarity, Cleland et al. showed that rats generalized between their trained odor, acetic acid, and propionic acid, another carboxylic acid with one more carbon than acetic acid [7]. A similar study in canines trained domestic dogs to pentanol and presented each with a two-choice system to determine the level of generalization and discrimination in alcohols of differing lengths of carbon chains. Similar to the study of rats, the canines showed increased discrimination with an increase in carbon atom differences between the trained odorant and the testing odorant [8]. Presenting canines with a series of odorants similar to their training odorant can test the extent to which canines generalize or discriminate. Such information is important for improving the performance of detection canines.

Additionally, research explored the perception of structurally similar and dissimilar compounds in simple binary odorant mixtures. Mixtures present many challenges for olfaction. The components of a mixture may be perceived as either individual odorants or as the mixture as a whole, a process determined by the way in which the odorants interact within the olfactory system [6, 9, 10]. For example, odorants perceived as dissimilar are more likely to be perceived independently within a mixture, while structurally similar compounds will be more difficult to distinguish when presented as individual components [9]. A study of canines trained to potassium chlorate alone found a deficiency in the detection of potassium chlorate mixtures [11]. In such cases, detection canines must be capable of identifying target odorants, irrespective of the odor mixture present.

Finally, the current study explored the effect of training and conditioning on the generalization-discrimination balance. It is possible to alter the perception of similar odorants through associative learning. Mishra et al. demonstrated that the generalization-discrimination balance can be shifted by human conditioning in larval *Drosophila* [12]. Similarly, Wright et al. (2008) used honeybees to show that those bees trained to a fixed-ratio binary odorant mixture generalized to different ratios of binary mixtures less than those initially trained to variable-ratio binary mixtures [13]. In the previously mentioned study of canines trained to potassium chlorate alone, training on mixtures

containing potassium chlorate in addition to potassium chlorate alone improved detection of the mixed materials [11]. This brings forth several questions not yet investigated. Does training on one mixture or compound over others result in a shift of the generalization-discrimination balance? Does increasing the number of mixtures or like-compounds for training further affect the balance? Understanding how training on various single- and mixed-target odors affects proficiency is essential for canine success in the field. Therefore, this research explores the concepts of molecular structure, mixture perception, and training as they relate to canine odor perception using a controlled method of odor delivery and three separate odor sets.

PART 1. OPTIMIZATION OF VAPOR DELIVERY

Methods

Study design – Prior to conducting any type of odor-based research with working dogs, it is imperative that all odorants provided to the canines for experimentation be delivered at known, quantifiable, and reproducible levels. There is often a misconception in canine research that the quantity or mass of bulk material presented to the canine is directly correlated to the odor vapor available to the canine. In fact, odor availability is related to the vapor concentration in a given container, and not necessarily the quantity of material in that container. Odor availability is also related to the rate of evaporation (or sublimation) for a particular compound, which is dictated by a number of factors including surface area of the bulk material or liquid surface, as only the surface molecules have the potential to escape. Interactions with the delivery medium and container material, as well as environmental conditions such as temperature and humidity, also influence vapor concentration and odor availability. To ensure that reproducible odor concentrations are presented to the canine throughout the trials, it is imperative that these factors are closely controlled.

In this study, a method was developed using Controlled Odor Mimic Permeation Systems (COMPS) [14] for the delivery of analyte odorants at similar concentrations from pure compounds of varying vapor pressures with training materials of standardized surface area. COMPS are efficient and simple to use; in this study they were comprised of a small quantity of pure compound spiked onto a gauze pad which was then sealed into a permeable bag [14]. The vapor from the compound then dissipated through the permeable bag at a controlled and measurable rate, allowing for reproducible odorant delivery over a period of hours. The dissipation rate was determined by

gravimetric analysis, where the mass loss is plotted versus time and the slope of the linear section of the line is the rate of permeation. This rate is dictated by the compound vapor pressure, the intermolecular interactions between the compound and the gauze material, and the thickness of the permeable bag. Permeable bags of varying thicknesses were utilized to control the permeation rate, allowing to match permeation rates between analytes of varying vapor pressures.

Data collection and analysis – All compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO) and were at least 99% pure. Compounds included pentanoic acid, isovaleric acid, isobutyric acid, butanoic acid, hexanoic acid, heptanoic acid, 1-pentanol, pentanal, 2-pentanone, 3-pentanone, methyl valerate, pyridine, and methyl benzoate. All COMPS were created by spiking 5 μL of the compound of interest onto a single piece of Dukal gauze (2"x2", 4 ply), folded in half, and placed into 2"x4" permeable bags of varying thicknesses (1, 2, 3, 4, or 6 MIL). The bags were then heat-sealed closed (Figure 1). For odor delivery of binary mixtures, two COMPS were prepared separately, then heat-sealed together, attaching the bags at the tops and bottoms.

Figure 1. Example of COMPS.

Permeation rates of each compound through the permeable bags were determined by gravimetric analysis. For this purpose, COMPS were placed in a weigh boat on an analytical balance, and the mass was recorded. The mass continued to be recorded periodically for at least four hours. When not being weighed, the COMPS were kept in a fume hood. The permeation rate was calculated as the slope of the line of mass vs. time (mg/min). All measurements were taken in replicates of three or more. Blank COMPS were also tested to confirm that no mass was lost over time.

Vaporous analyte was measured in the headspace of the COMPS by placing individual COMPS in 1 pint (0.47 L) metal sample containers (Tri-Tech Forensics), which were then placed into 1 gallon (3.8 L) epoxy-lined metal sample containers (Tri-Tech Forensics). To minimize collection of (and thus dilution by) the surrounding air, a lid with a 1 cm hole was briefly placed on the container only during sampling. For sampling, 3/16" PTFE tubing was inserted into the hole to a depth of approximately 10 cm to mimic the approximate sampling location during a canine sniff. Approximately 750 mL of the headspace was collected into 1 L Tedlar bags (SKC Inc.) using a Grab Air Sample Pump (SKC Inc.).

For analysis, the contents of the Tedlar bags were pre-concentrated onto a cooled injection system (CIS) (CIS-4, Gerstel, Inc.) that was in-line with the gas chromatograph / mass spectrometer (GC/MS) (Agilent 7890A GC / 5975 MS). The contents of the Tedlar bags were trapped at 50 mL/min for 10 minutes for a total of 500 mL sampled onto a Tenax-filled CIS liner cooled to 0 °C. Following trapping, analytes were rapidly thermally desorbed from the Tenax at 250 °C directly onto the head of the GC column. Analytes were separated with a 30 m x 0.32 mm i.d., Rtx-Volatiles column (Restek Inc.). The GC column oven was held at 40 ˚C for one minute. The temperature was then increased to 240 ˚C at 30˚C/min and held for an additional one min. Analytes were detected by the mass selective detector. Quantification was carried out by comparison to an external liquid calibration curve.

Headspace measurements were made for all analytes at room temperature after one and three hours in the sample container. In addition, the headspace from a single representative analyte (pentanoic acid) was measured at varying temperatures and humidities. This was done using the procedure described above, but placing the metal containers with the COMPS in a large environmental chamber (12' x 21' x 10'). The test chamber provides temperatures ranging from -34 to 85 °C and a relative humidity range of 10 – 95%. It also houses an exhaust apparatus that purges the air at 300 CFM between samples. Temperatures and humidities were chosen to mimic outdoor sampling conditions in the mid-Atlantic region, and included average room conditions of 20 °C at 20% RH, 26 °C at 40% RH, 32 °C at 60% RH, and 6 °C at 25% RH. For each condition, four replicates were placed in the chamber together but spaced at least 8 feet from one another in all directions. Blanks of the chamber were also taken at each temperature/humidity combination.

Further experiments were carried out to determine the optimal container/packaging to transport

the COMPS to the testing sites and for storage when not in use. All individual COMPS were first placed in a 4" x 6" mercury-metalized mylar barrier envelope consisting of an exterior layer of metalized polyethylene terephthalate (PET) and an interior layer of 2.5 MIL linear low-density polyethylene (LLDPE) (ESP Packaging LLC). Each envelope was both zip-sealed and heat-sealed closed. Multiple of the same COMPS type enclosed in the barrier bag were then stored in a single outer container. The two outer containers tested included a larger (7.5" x 11.5") sealed envelope and a glass canning-type jar (16 oz) with an unlined lid (Fillmore Container). Both were tested for permeability and odorant loss by creating a pentanoic acid COMPS which was first placed in the small inner envelope, and then further contained in the glass jar or larger envelope. To test for permeability, the entire apparatus was then placed in a 1 gallon metal sample container with a fitted lid. The container was stored under ambient conditions in a fume hood. The headspace within the sample container was analyzed daily using the methods described above. Container blanks were taken prior to placing the COMPS, and all measurements were taken in triplicate.

Finally, the "lifetime" or usage time of the COMPS was estimated using the pentanoic acid COMPS described above. A single pentanoic acid COMPS was sealed in the inner envelope, which was then closed in a glass jar for seven days. After one week, the COMPS was removed from both the outer and inner packaging and placed in the 1 gallon sample container for one hour (no lid), after which the headspace was sampled and analyzed in the manner described above. This was repeated daily to mimic the COMPS use for daily, one-hour training sessions. The headspace concentration after each hour of "use" was also compared to a freshly made COMPS. All samples were prepared and analyzed in triplicate.

Results

It is considered desirable to produce a known and constant amount of odorants for canine testing purposes. Because the compounds have variable vapor pressures, and thus produce variable headspace concentrations, the permeation rates (mass per unit time) were controlled using the COMPS devices. The appropriate thickness of the permeable bag making up the COMPS was selected so that all compounds would yield similar permeation rates. The permeation rate was determined by gravimetric analysis for each compound. Sample graphs of the mass of pentanoic acid lost through different bag thicknesses over time are given in Figure 2 and Figure 3. In the case of pentanoic acid, the 3 MIL bag yielded a steady rate of 0.037 mg/min that could be approximately matched by the other compounds of interest. Table 1 gives all the permeation rates measured for

each bag compound / bag thickness pair that was tested. By selecting the appropriate bag thickness, the permeation rates for many compounds were kept within 5% of that for the pentanoic acid. However, the permeation rate of five compounds deviated greater than 5%. This could not be avoided due to the limitation of discrete bag thickness values. In these cases, the bag thickness that provided the closest, reproducible permeation rate to pentanoic acid were chosen.

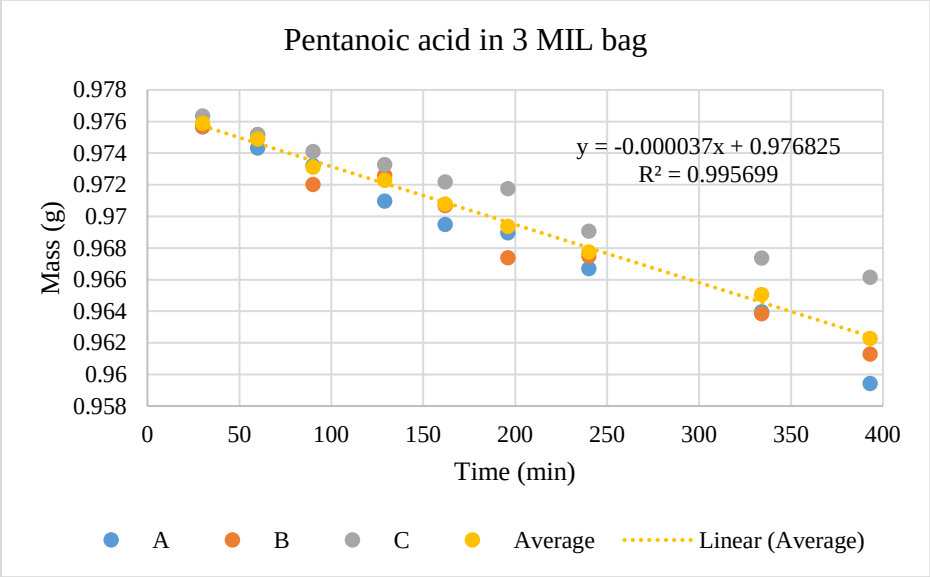

Figure 2. Loss of pentanoic acid on gauze through a 3 MIL permeable, polymer bag over time.

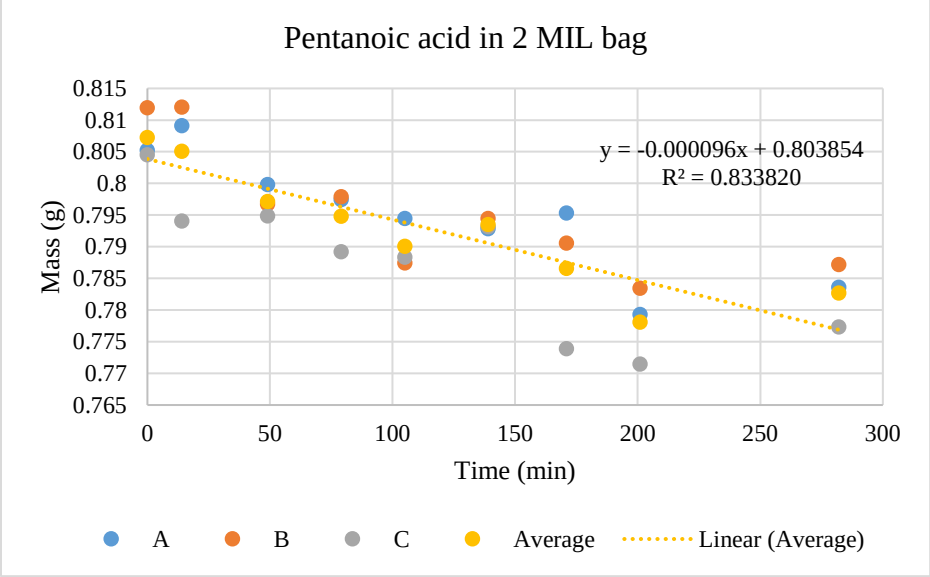

Figure 3. Loss of pentanoic acid on gauze through a 2 MIL permeable, polymer bag over time.

Table 1. Compound vapor pressures and permeation rates through selected bag polymer bag thickness.

Compound	***Vapor pressure (mmHg at 25 °C)***	***Selected bag thickness (MIL)***	***Permeation rate (mg/min)***
Pentanoic acid	0.196	3	0.037
Hexanoic acid	0.0435	2	0.039
Heptanoic acid	0.0107	1	0.036
Isovaleric acid	0.44	2	0.037
Isobutyric acid	1.81	3	0.035
Butanoic acid	1.65	4	0.036
2-Pentanone	35.4	4	0.070
3-Pentanone	37.7	4	0.053
Pentanal	31.8	3	0.031
Pentanol	2.2	1	0.064
Methyl valerate	32.5	6	0.072
Methyl benzoate	0.38	4	0.044
Pyridine	20.8	2	0.039

The headspace from all single COMPS was also quantified. The vapor concentration from each compound were measured after 1 and 3 hours. Results from all compounds are summarized in Table 2. The vapor concentration of pentanoic acid was approximately 0.48 ppmV after 1 hour and 0.45 ppmV after 3 hours. A majority of the acidic compounds yielded vapor concentrations similar to that of pentanoic acid. The one deviation, however, was heptanoic acid, which was quite a bit lower. It was suspected that this was due to the breakdown of the carbon chain in the heated inlet. Pentanal, pentanol, and methyl valerate all have higher vapor concentrations than pentanoic acid at 1 hour and showed some depletion at 3 hours. Headspace concentrations varied more than permeation rates because other factors such as evaporation and diffusion rate, and surface absorption vary with each compound. However, compared to the variation between vapor pressures of this set of the compounds, the disparity between vapor concentrations is much smaller, and thus the COMPS do help to control the amount of vapor delivered to the canine.

Table 2. Headspace concentration of individual compounds in COMPS. Headspace was collected after one hour and three hours of the COMPS being contained in a metal sample container.

Compound	***1 hour (ppmV)***	***3 hour (ppmV)***
Pentanoic acid	0.479 (± 0.0355)	0.453 (± 0.0648)
Hexanoic acid	0.402 (± 0.376)	0.428 (± 0.282)
Heptanoic acid	0.0690 (± 0.00457)	0.0541 (± 0.00488)
Isovaleric acid	0.410 (± 0.0792)	0.765(± 0.198)
Isobutyric acid	1.46 (± 0.383)	0.398 (± 0.151)
Butanoic acid	0.960 (± 0.0420)	0.888 (± 0.262)
2-Pentanone	0.721 (± 0.0476)	0.491 (± 0.184)
Pentanal	1.72 (± 0.656)	0.371 (± 0.279)
Pentanol	1.39 (± 0.989)	0.736 (± 0.407)
Methyl valerate	1.63 (± 0.553)	0.991 (± 0.391)

In addition to single odorants, binary odorant mixtures were created by attaching two individual COMPS to one another. The headspace concentrations from these binary training materials were also tested. Results are summarized in Figure 4. The amount of pentanoic acid was equivalent across all mixtures. The concentration of the second compound varied, but remained at concentrations similar to that of the pentanoic acid.

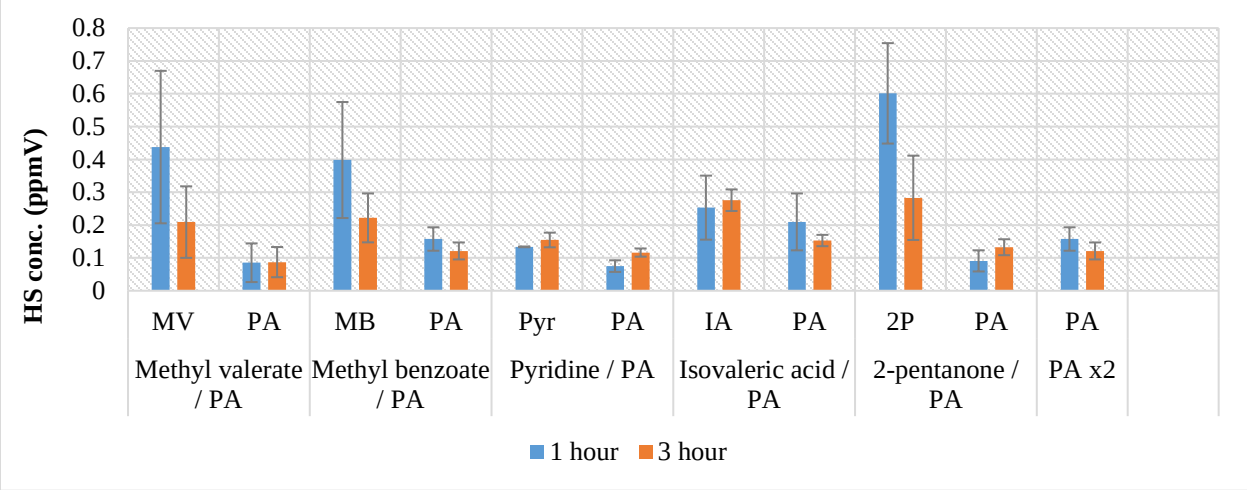

Figure 4. Headspace concentration of COMPS training aids for binary mixtures (PA = pentanoic acid), taken after one hour and three hours in metal sample containers.

As canine testing often takes place in outdoor or other locations where the environment cannot be controlled, the concentration of odorants from pentanoic acid COMPS was measured at varying temperatures and humidities to represent those that might be found in the Mid-Atlantic region. The headspace concentrations of pentanoic acid collected at varying temperatures are given in Figure

5. The increase in concentration with increasing temperature is linear after one hour in the sample container, but after three hours the amount in the headspace starts to decrease at the highest temperature (Figure 5). As the temperature is increased, the amount of odorants lost from the COMPS also increases per unit time, thus after three hours, less odor is available at the higher temperature compared to the lower temperatures as the pentanoic acid begins to be depleted. These results indicate that during canine olfaction testing, as expected, canines will experience a greater concentration as the environmental temperatures is increased. The training material will need to be changed more frequently at higher temperatures, as the amount of odorants depletes faster.

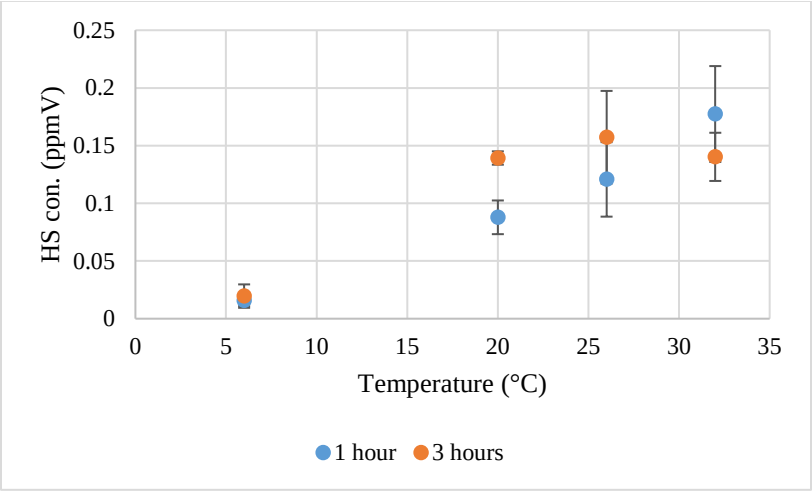

Figure 5. Headspace concentration of pentanoic acid after one and three hours in metal sample containers with increasing temperatures.

To preserve the integrity of the COMPS and prevent odorant loss during storage, all COMPS were stored in two layers of packaging: an inner barrier envelope and an outer wrapping / container. Two outer containers were tested to include an additional larger envelope and a 16 oz. glass mason-type jar. COMPS in both dual-layer packaging were held in and sampled daily for 4 days. The amount of pentanoic acid vapor that permeated out of the large envelope was significantly higher than the blank at Day 1 and increased on Day 2. The vapor was then mostly depleted by Day 3 and could not be detected at Day 4 (Figure 6, left). In contrast, pentanoic acid vapor permeating from the glass jars could only be detected from one replicate and never at a level significantly greater than the blanks (Figure 6, right). The glass jars were thus selected for future storage and transport.

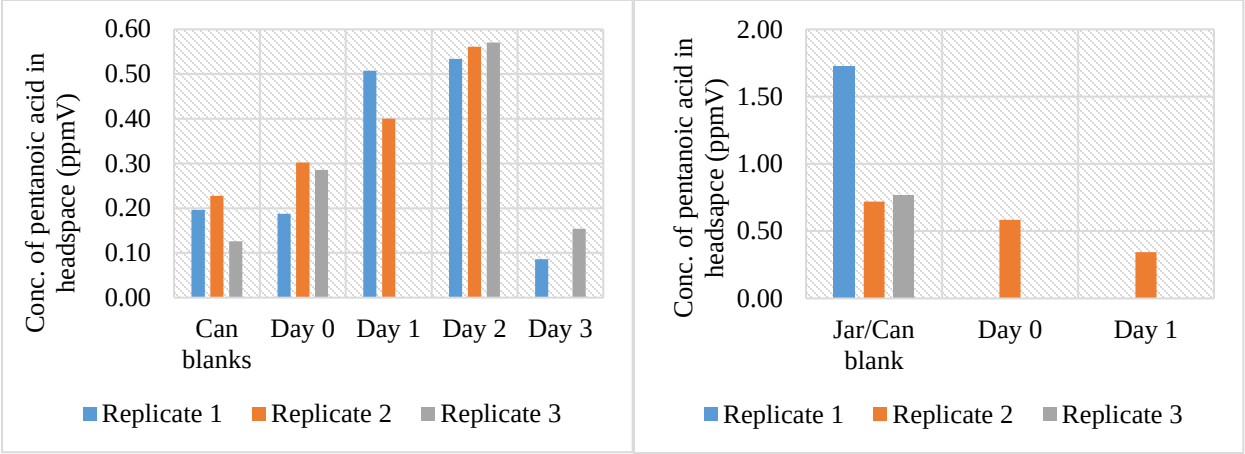

Figure 6. Concentration of pentanoic acid collected through two layers of packaging over four days of storage; (left) from barrier envelopes, and (right) from glass jars.

Upon determining the appropriate storage system, the lifetime of the COMPS were estimated in a manner to mimic daily training sessions. COMPS were removed from storage and their output was measured over one hour per day for a total of nine hours (over nine days) (Figure 7). Compared to the odorants released from fresh COMPS, results showed an initial increase in the amount of pentanoic vapor present at hour 1 (day 1). This is likely due to the vapor adsorbing to the exterior of the COMPS while in the envelope. After the first hour/day, the concentration remained similar to that of the fresh training aid for seven hours/days. After seven hours, the amount began to decrease, and would be considered no longer viable for use. Canine handlers were then instructed to dispose of COMPS training materials after six total hours of use.

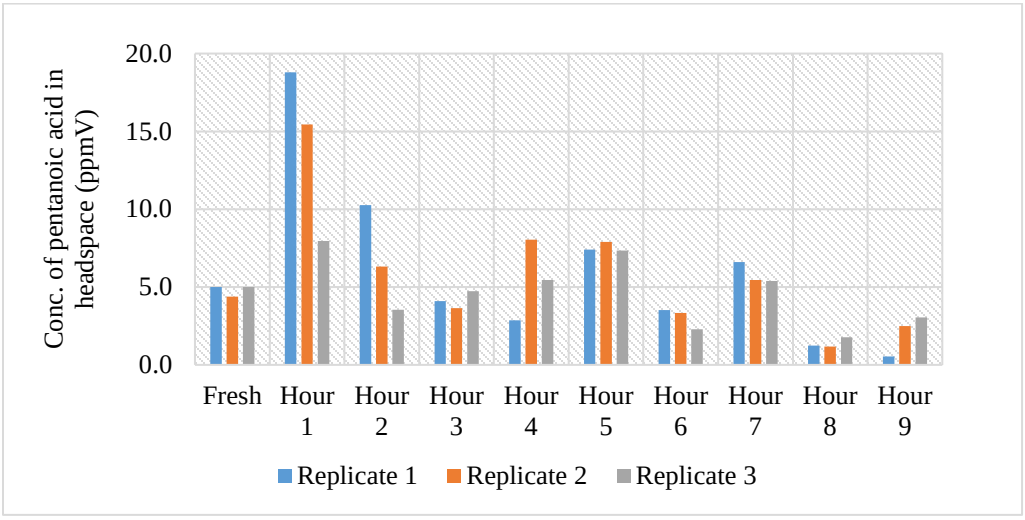

Figure 7. Concentration of pentanoic acid available after single hours of "usage".

PART 2. GENERALIZATION / DISCRIMINATION BETWEEN SIMILAR MOLECULES OF DIFFERING CARBON CHAIN LENGTHS

Methods

Study design – The research was carried out as a series of three sequential detection trials testing the canines' tendency to generalize from one acid to others that differ by carbon chain length (referred to as Odor Set 1; structures provided in Appendix A). Canines were initially trained to pentanoic acid. During the first trial, the canines were tested for proficiency at detecting the trained odorant, then were evaluated with other acids of differing lengths. Prior to the second trial, the canines were trained to a second acid. The second trial included proficiency testing on both trained odorants. Canines were then tested again on other acids. This was repeated for the third trial, after the canines had been trained on a third acid. The tendency of the canines to generalize or discriminate the untrained odorants was assessed following each subsequent training period.

Each trial utilized validation assessments to confirm canine proficiency for their respective trained odorant(s). For each of the trials, a minimum of four validation assessments was performed as a combination of searches and integrated odor recognition tests (to be described below). To be included in the final data, a canine had to detect at least 75% of the validation odors. Searches incorporated some combination of indoor container searches, room / furniture searches, or external vehicle searches. An example is shown in Figure 8. Each validation search contained only one or two target odorant(s) depending on the size of the search area, as well as blanks.

In addition to searches, odorant recognition tests (ORTs) were used as a test of the canine's ability to detect target odorant(s). ORTs provide a uniform method to determine a canine's ability to locate and identify a target material [15]. For each trial, the ORTs were comprised of a line of five 8 x 6 x 4" cardboard boxes (Figure 9). For integrated ORT validations, each set of 5 boxes consisted of 1 trained target odorant and 4 blanks. For ORTs testing untrained odors, each set of 5 boxes contained 1 target, 1 distractor, and 3 blanks. Negative runs consisting of 1 distractor and 4 blanks were also used. Distractor odorants were used in all testing scenarios, but were excluded from validation assessments.

Figure 8. Example of a canine performing an indoor container/area search.

Figure 9. An example of a canine participant completing an odorant recognition test (ORT). The ORT consisted of five boxes, each containing a target odorant, a distractor odorant, or a blank.

Odorants and odorant delivery – Training and testing odorants for each trial in Odor Set 1 are provided in Table 3. All canines were initially trained on pentanoic acid, and were instructed to continue training on pentanoic acid throughout all three trials. After the first trial, the canines were assigned randomly to one of two groups for second training odor assignments. One group was trained to heptanoic acid (compound with the greatest difference in chain length), and the other to isovaleric acid (compound with the branched structure of the same chain length of the trained odor). For the third trial, all canines were trained on both heptanoic and isovaleric acids. ORTs were designed as described previously, with each set of five boxes containing one testing odorant. Each ORT also contained one distractor odorant, which was one of the following, selected at random: limonene, cinnamaldehyde, α-amylcinnamaldehyde, citral, cuminaldehyde, pinene, eucalyptol, phenol, linalool, carvone, β-caryophyllene, isoamyl acetate, pinene, nerolidol, 3-

carene, furanmethanol, 2-pentylfuran, or farnesene. All COMPS were created prior to each trial, and were never reused.

Table 3. Odor Set 1 – Training and testing odorants for Trials 1-3.

	Trial 1	Trial 2	Trial 3
Training Odorant(s)	Pentanoic acid	Pentanoic acid, heptanoic acid (Group A only) OR isovaleric acid (Group B only)	Pentanoic acid, heptanoic acid AND isovaleric acid
Testing Odorant 1	Isovaleric acid	Isovaleric acid (Group A) OR heptanoic acid (Group B)	Butanoic acid
Testing Odorant 2	Heptanoic acid	Butanoic acid	Isobutyric acid
Testing Odorant 3	Butanoic acid	Isobutyric acid	Hexanoic acid
Testing Odorant 4	Isobutyric acid	Hexanoic acid	n/a
Testing Odorant 5	Hexanoic acid	n/a	n/a

Canine participants – A Cooperative Research and Development Agreement (CRADA) with the National Association of Canine Scent Work®, LLC (NACSW™), a.k.a. K9 Nose Work® [16], was established for all canine training and trials. K9 Nose Work is a sporting group for domestic (pet) dogs that offers classes and competitions in scent detection using essential oils (birch, anise, and clove). The group trains and tests in scenarios that mimic search and scent techniques for law enforcement and other related working dogs [16]. All handlers were instructed to train with the provided training odorant(s) "as usual," meaning that they should not alter methods and should continue training in the same manner in which they train with K9 Nose Work® odors.

Test integrity – All trials were conducted as double-blind assessments where neither the handler nor the evaluators knew the identity or location of the odorants within the ORTs. Within each ORT, the locations of the target, testing, and distractor odorants were assigned by a random number generator for individual canines. Prior to each canine, the odorants were rearranged according to the randomly assigned locations and testing areas were inspected, and cleaned if necessary, i.e. saliva, urine, etc. Canines and handlers that had not been tested were not permitted to observe other participants during testing. While "learning" of novel odorants could not be completely eliminated, it was minimized by presenting each novel odorant to a canine no more than once per trial.

Data collection and analysis – The two blind and impartial evaluators that observed each trial were selected because they were experienced in reading canine behavior during olfaction exercises and could therefore appropriately categorize canine responses as one of the following: positive alert, false alert, interest, or strong interest. Observed handler error(s) were also noted for any strong interest or false alert responses to assist in later analysis. All data was then compiled, and any canine that did not successfully locate 75% of the validation odorants or that had excessive false alerts was excluded from final results.

Statistical analyses of the data collected were made, using multiple methods to compare canine responses to training and testing odorants as well as responses between canine testing groups. Positive predictive value (PPV) is a measure of how frequently a canine alert is correct. Given as a percentage, a PPV closer to 100% means that there is a higher probability that a canine response is correct, i.e. not a false or missed alert.

$$\boldsymbol{PPV} = \frac{\boldsymbol{True\ positive}}{\boldsymbol{True\ positive + False\ positive}} \quad (\textit{Equation 1})$$

McNemar's test is used to compare nominal data with 2 by 2 contingency tables (example given in Table 4). It was used to describe consistency in canine responses across testing groups and testing odorants. Specifically, it defined the probability that an alert rate of a testing odorant was similar to an alert rate of a trained, or validation, odorant. McNemar's test calculations are based on the chi-square value, found using Equation 2. The chi-square value was then compared to the appropriate χ^2_{crit} value to determine significance at a specific level. If χ^2 is greater than χ^2_{crit}, the probability is considered statistically significant.

Table 4. A sample 2 by 2 contingency table used in McNemar's test calculations.

		Testing Odor	
		Y	N
Training odor	Y	a	b
	N	c	d

$$\boldsymbol{\chi^2} = \frac{(\boldsymbol{b-c})^2}{(\boldsymbol{b+c})} \quad (\textit{Equation 2})$$

The final statistical method used was the chi-square test of independence, which compares independent samples with discrete outcomes. The test was used both to compare the alert rates between testing groups of canines, and to compare the alert rates of all canines between trials. Specifically, it compared the responses to testing odors for Group A to Group B, then it compared the responses for each trial. The chi-square independence test used contingency tables to compare observed values versus expected values (example given in Table 5). Observed and expected values were calculated from the expected frequencies based on the sum of alerts and misses for all groups being considered. The chi-square value was calculated for each group using Equation 3 (O = observed, E = expected). This value was then compared to χ^2_{crit}. Again, if χ^2 was greater than χ^2_{crit}, the difference between responses for canine testing groups is considered statistically significant.

Table 5. A sample contingency table for Odor Sets 1 and 2 used in the chi-square of independence test. (N = sum of alerts and misses for both groups).

	Alerts	*Misses*	*Row total*
Group A			*x*
Group B			*y*
Column total	*a*	*b*	*N*

$$\chi^2 = \sum \frac{(O-E)^2}{E} \qquad (Equation\ 3)$$

Results

Due to scheduling conflicts, trial dates could not be arranged to have equal amounts of training time available between each. Canines had a minimum of 4 weeks of training, and a maximum of 10 weeks, with several trials being divided between two dates. Each trial took place in two locations. Trial dates and locations are listed in Table 6.

Table 6. Dates and locations of canine trials.

Trial	**Date**	**Location**
Trial 1	January 22	Charles County, MD
	February 25	Deerfield Beach, FL
Trial 2	March 4	Charles County, MD
	April 9 / 22	Deerfield Beach, FL
Trial 3	April 15 / May 20	Charles County, MD
	May 20 / 21	Deerfield Beach, FL

Trial 1 – In Trial 1, all canines were trained to pentanoic acid only. A total of 21 canines participated in this trial, though 4 were excluded due to either the unsuccessful location of 75% of the validation odorants or a high false alert rate. A summary of Trial 1 results are included in Figure 10; alerts for each canine in each trial are given in Tables B1-2; and all statistical results are provided in Appendix C. The canines included in the data had a 97% alert rate to the trained odorant, pentanoic acid. The alert rates were significantly lower for all testing odorants (Table D1), ranging from 18% to 47%, indicating minimal generalization to these odorants. However, the molecules that differed from the target odorant by only one or no carbons had the highest alert rates (i.e. isobutyric, isovaleric, and butanoic acids). The compound with the lowest alert rate (heptanoic acid) was the molecule most different from the target odorant. Heptanoic acid also had the lowest PPV at 69% (Table C1).

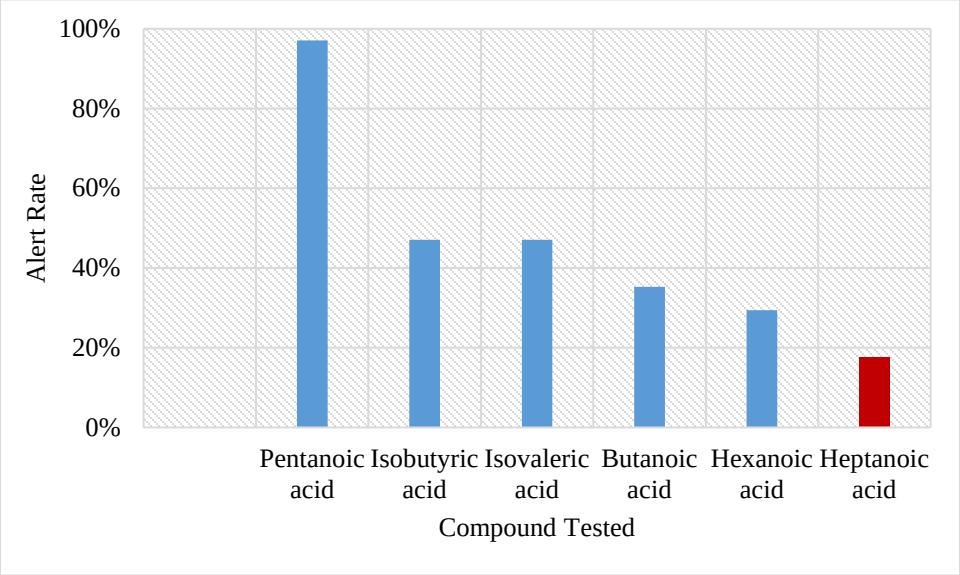

Figure 10. Results from Odor Set 1: Trial 1 – Alert rate for the validation odorant (pentanoic acid) compared to the novel odorants.

Trial 2 – In Trial 2, a total of 20 canines participated, though 5 were excluded due to either unsuccessful location of 75% of the validation odorants or a high number of false alerts. All canines were trained to pentanoic acid in addition to a second odorant. A summary of Trial 2 results are included in Figure 11. The alert rates for the respective validation odors decreased slightly to 91%. Examining the individual testing odorants in Figure 11, overall there was no increase in the tendency to generalize to the testing odorants. For isovaleric and hexanoic acids, alert rates

increased slightly from Trial 1 to Trial 2, while they decreased for butanoic and isobutyric acids. This same trend was seen in the PPV (Table C2). The chance of detection of butanoic and hexanoic acids was statistically different than that of the trained odorants (Table D2).

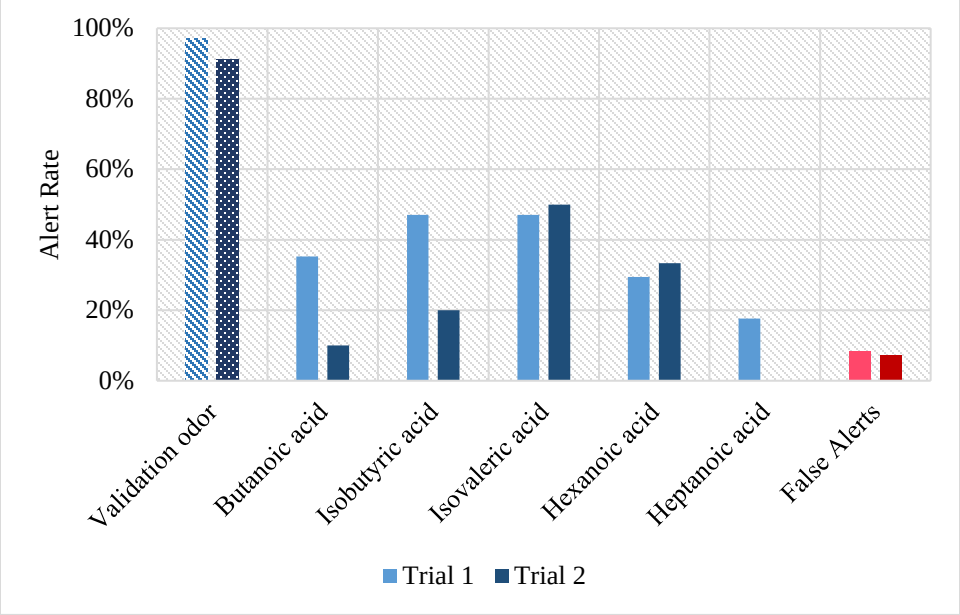

Figure 11. Results from Odor Set 1: Trials 1 and 2 – Alert rate for the validation odorants (heptanoic acid for Group A and isovaleric acid for Group B) compared to the novel odorants.

Trial 2 further explored the generalization-discrimination balance by addressing the effect of training on multiple compounds. Seven canines were trained on heptanoic acid (Group A) and 8 were trained on isovaleric acid (Group B), and alert rates are compared in Figure 12. Group A alerted to their training odorants 82% of the time, and Group B alerted to their training odorants 97% of the time. Notably, no canine in Group B alerted to heptanoic acid. Also, no canines alerted to more than 2 of the testing odorants, indicating that discrimination, not generalization, actually increased from Trial 1 to Trial 2. It was expected that for Trial 2, Group A would have a higher alert rate to hexanoic acid compared to Group B after being trained on pentanoic acid and heptanoic acid; however, this was not the case. Group B actually had a higher alert rate to hexanoic acid. Also, it was hypothesized that Group B would have more readily generalized to isobutyric acid after being trained on pentanoic acid and isovaleric acids. This was also not the case, as Group A actually had a higher alert rate to isobutyric acid. Overall, there was no tendency of one group to generalize or discriminate more than the other.

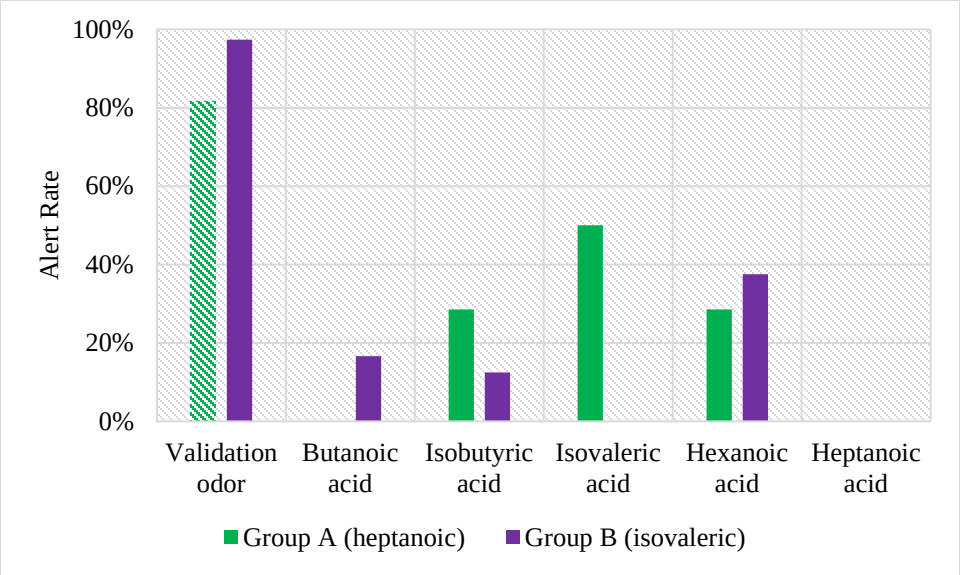

Figure 12. Results from Odor Set 1: Trial 2 – Alert rates for Group A (trained to heptanoic acid) compared to Group B (trained to isovaleric acid).

Trial 3 – A total of 21 canines participated in Trial 3, and 19 were included in the final results. The alert rate for trained odorants in Trial 3 decreased again to 89%. Detection of untrained odorants increased; however, only to levels near that of Trial 1 (Figure 13), though PPV for these odorants was constant through the trials (i.e. false alert rates decreased) (Table C3). Group A, which trained on heptanoic acid before isovaleric acid, demonstrated slightly higher alert rates to all testing odorants (Figure 14).

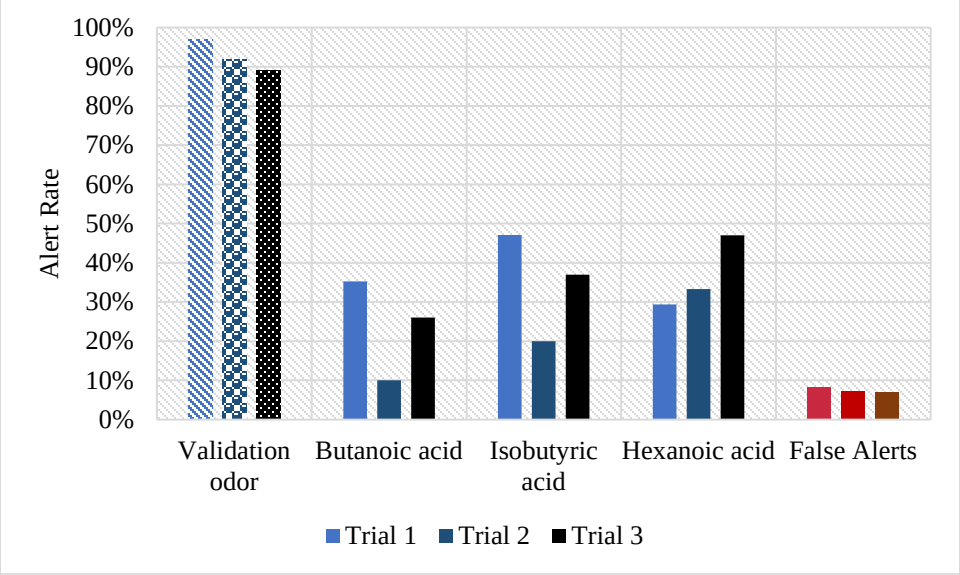

Figure 13. Results from Odor Set 1: Trials 1, 2, and 3 – Alert rate for the validation odorants (heptanoic and isovaleric acids for all dogs) compared to the novel odorants.

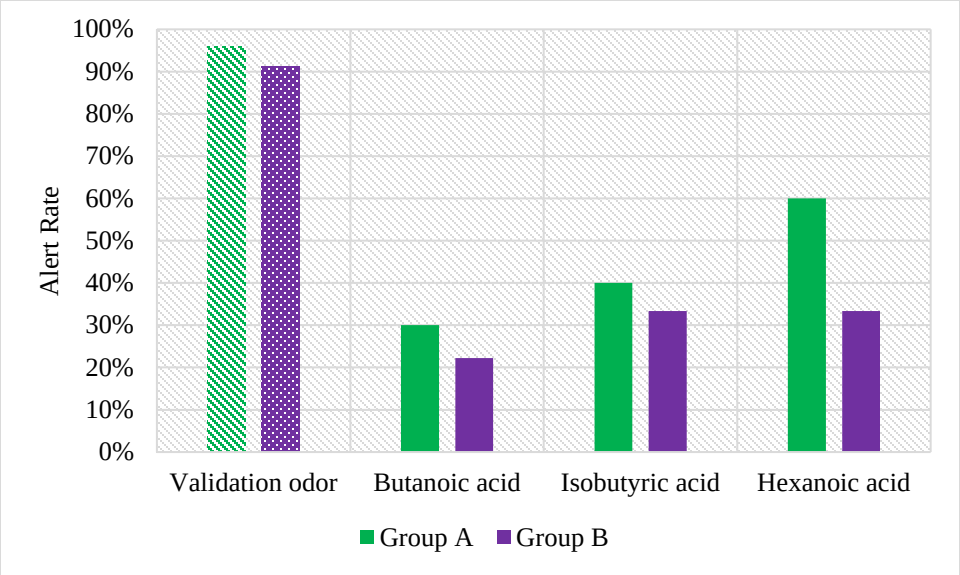

Figure 14. Results from Odor Set 1: Trial 3 – Alert rates for Group A (trained to heptanoic acid first, then isovaleric acid) compared to Group B (trained to isovaleric acid first, then heptanoic acid).

The combined alert rates were not above chance (50%) for any tested compound (only the alert rate for Group A on hexanoic acid was higher than chance, at 60%). There was an increase over the trials in the percent of canines that alerted to 1 or more testing odorant (35% in Trial 1, 60% in Trial 2, and finally 74% in Trial 3), as shown in Figure 15. Similarly, the percent of canines alerting to 2 or more testing odorants increased from Trial 1 (24%) to Trial 3 (32%). While some canines seem to generalize with additional training on varying length acids, this was not the case for all canines, and the type of training odorants (i.e. Group A vs. Group B) did not affect this trend (Table D3 and Table E4).

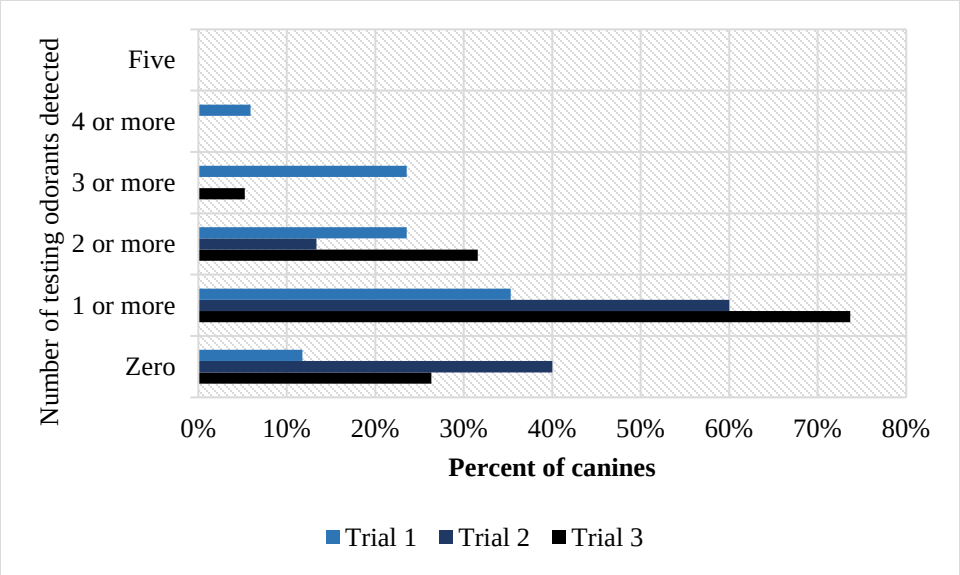

Figure 15. Results from Odor Set 1: Trials 1, 2, and 3 – Summary of the total number of testing odorants detected by canines.

Summary

Odor Set 1 explored canine generalization to similar compounds of differing carbon chain lengths and degree of branching. In Trial 1, the testing odorants with the highest alert rates were those most similar to the trained odorant in chain length, i.e. isobutyric, isovaleric, and butanoic acids, which differ in length from pentanoic acid by either one carbon or are branched. The compound with the lowest alert rate (heptanoic acid) was also the most different testing odorant compared to the training odorant, with two additional carbons. This initial data agrees with previous studies in rats and canines that found that these animals generalize between molecularly similar compounds.

After Trial 3, Group A demonstrated higher alert rates to the testing odorants after having lower overall rates in Trial 1. This increase in generalization for Group A only could indicate that training on the two most different testing odorants (i.e. pentanoic acid and heptanoic acid) aided in generalization when compared to Group B, which trained on more similar compounds, a branched and a straight chain acid of the same length (i.e. pentanoic and isovaleric acids). While some generalization did occur, the compounds canines generalized to seems to be based on individual preferences.

PART 3. GENERALIZATION / DISCRIMINATION BETWEEN MOLECULES OF DIFFERING FUNCTIONAL GROUPS

Methods

The research was carried out as a series of three separate detection trials used to test the canines' tendency to generalize from one five-carbon compound to others that differ only by functional group (referred to as Odor Set 2; structures provided in Appendix A). Each trial and all statistical analyses were carried out in the same manner as in Part 2. Training and testing odorants for each trial are given in Table 7.

Table 7. Odor Set 2 – Training and testing odorants for Trials 1-3.

	Trial 1	Trial 2	Trial 3
Training Odorant(s)	Pentanoic acid	Pentanoic acid, 2-pentanone (Group A only) OR pentanol (Group B only)	Pentanoic acid, 2-pentanone AND pentanol
Testing Odorant 1	2-Pentanone	Pentanol (Group A) OR 2-Pentanone (Group B)	3-Pentanone
Testing Odorant 2	Pentanol	3-Pentanone	Methyl valerate
Testing Odorant 3	3-Pentanone	Methyl valerate	Pentanal
Testing Odorant 4	Methyl valerate	Pentanal	n/a
Testing Odorant 5	Pentanal	n/a	n/a

Canines were initially trained and validated on pentanoic acid, then evaluated with compounds containing other functional groups. After the first trial, the canines were assigned randomly to one of two groups for second training odorant assignments. One group was trained to 2-pentanone, and the other to pentanol. For the third trial, all canines were trained on both 2-pentanone and pentanol. Canines were instructed to continue training on pentanoic acid throughout all trials. ORTs were designed as described previously, with each set of five boxes containing one testing odorant, or one target odorant and one distractor odorant, randomized for each canine. All COMPS were created prior to each trial, and were never reused. The tendency of canines to generalize or discriminate untrained odorants was assessed following each subsequent training period.

Results

Due to scheduling conflicts, trial dates could not be arranged so that canines would have equal amounts of training between each. Canines had a minimum of 4 weeks of training, and a maximum of 9 weeks, with one trial being divided between two dates. Each trial took place in two locations. Trial dates and locations are listed in Table 8.

Table 8. Dates and locations of canine trials.

Trial	Date	Location
Trial 1	March 5 / 10	Clearwater, FL
	March 18	Baltimore, MD
Trial 2	April 15	Baltimore, MD
	May 5	Clearwater, FL
Trial 3	May 13	Baltimore, MD
	June 23	Clearwater, FL

Trial 1 – A total of 17 canines performed Trial 1, with one canine excluded for unsuccessful location of 75% of the validation odorants. The alert rate for the validation odorant (pentanoic acid) was 98%. The alert rates for each compound can be seen in Figure 16, and data for each canine are given in Tables B4-5). Alert rates ranged from 6% for pentanol and 2-pentanone to 81% for methyl valerate. Canines were statistically more likely to detect methyl valerate and pentanal (Table D4), indicating that canines were more likely to generalize to these compounds than to pentanol and 2- and 3-pentanone. This was expected as methyl valerate and pentanal are the most structurally similar molecule of the tested compounds to pentanoic acid. Pentanol, on the other hand, is the only compound lacking a carboxyl group. It elicited the lowest alert rates and PPV, and is statistically different from the other testing compounds, except for 2-pentanone (Table C4 and Table D4).

Only one canine each alerted to pentanol and 2-pentanone. While they were not the same canine, both of these canines showed a relatively high degree of generalization across this trial, alerting to at least 3 of the testing odors. Many of the canines showed an ability to generalize, with 94% alerting to at least one testing odorant (Figure 17), though no single canine alerted to all of the testing odorants.

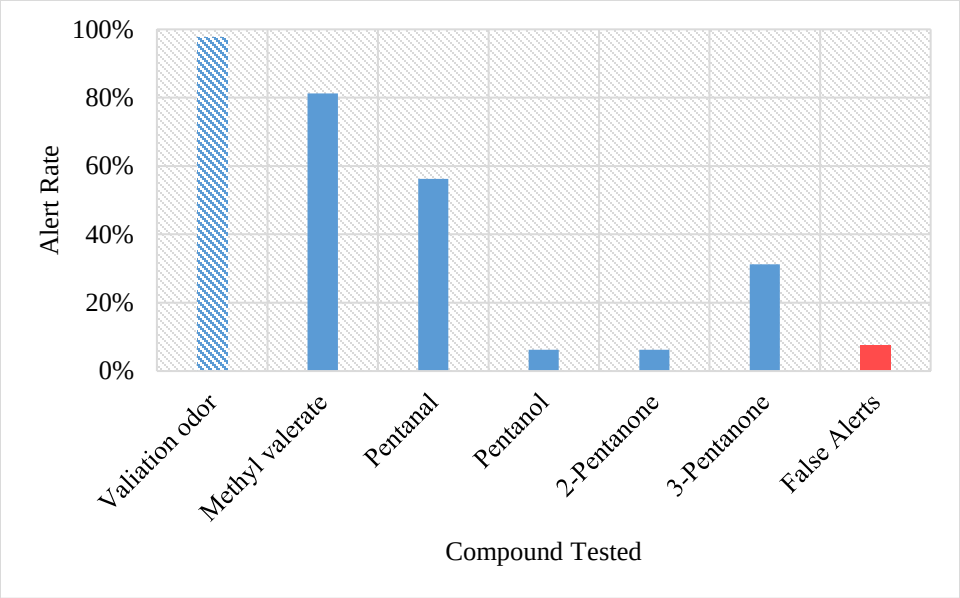

Figure 16. Results from Odor Set 2: Trial 1 – Alert rate for the validation odorant (pentanoic acid) compared to the novel odorants.

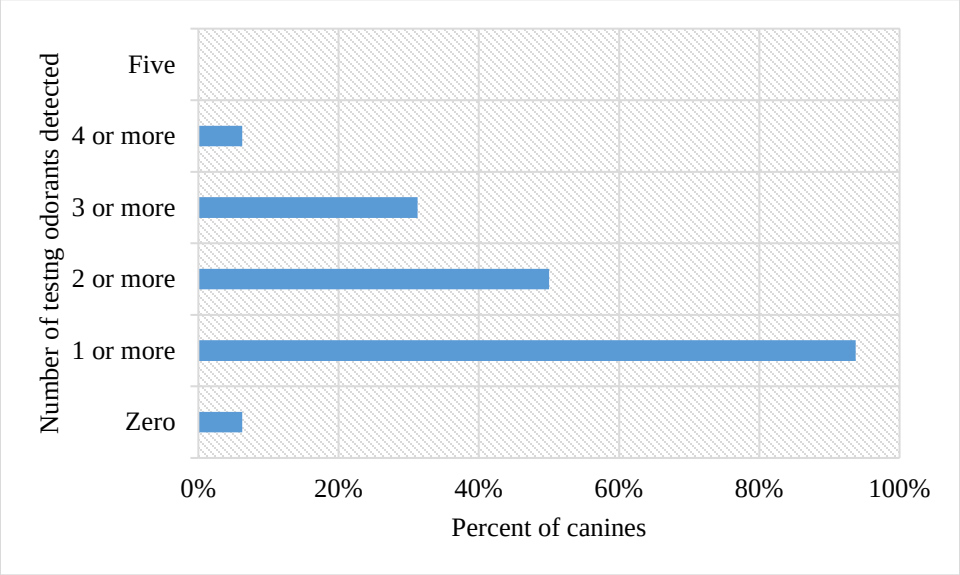

Figure 17. Results from Odor Set 2: Trial 1 – Summary of the total number of testing odorants detected by canines.

Trial 2 – Seventeen canines participated in Trial 2, and they were all included in the final results. The results are summarized in Figure 18. Compared to Trial 1, canines had a lower alert rate to both the validation odorants (85%) and to methyl valerate (65%), though the PPV was consistent (Table C5). However, generalization to all other compounds increased, with the exception of 3-

pentanone, whose alert rate remains significantly different from that of the training compound (Table D5).

A comparison of Group A (8 canines, trained to 2-pentanone) to Group B (9 canines, trained to pentanol) for Trial 2 is shown in Figure 19. There was no significant difference in the alert rates of Groups A and B, though some differences were observed. For example, all of the canines in Group B alerted to 2-pentanone, which Group A was trained to detect, and 88% of Group A alerted to pentanal compared with 67% of Group B. Figure 20 shows the number of testing odors that canines alerted to in Trial 2 as compared to Trial 1. The number of testing odors detected increased, with 100% of canines alerting to at least one testing odorant, and only one canine finding all testing odorants. Generalization appeared to increase with training to a second functional group.

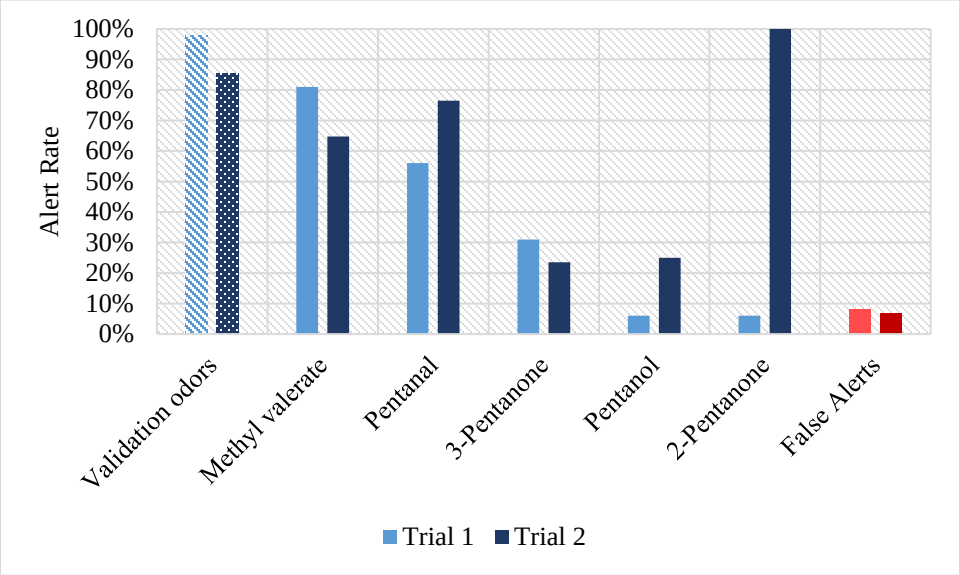

Figure 18. Results from Odor Set 2: Trials 1 and 2 – Alert rate for the validation odorants (2-pentanone for Group A and pentanol for Group B) compared to the novel odorants.

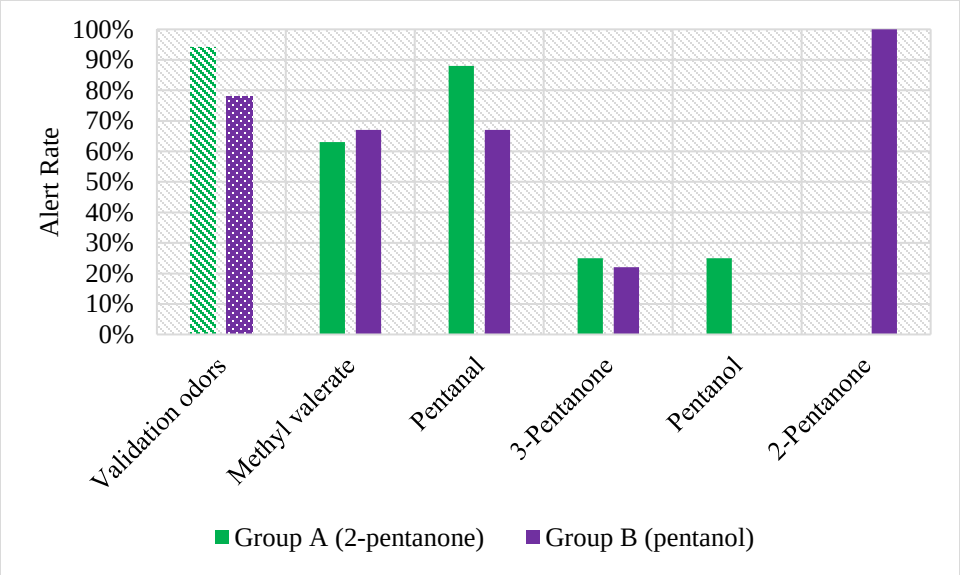

Figure 19. Results from Odor Set 2: Trial 2 – Alert rates for Group A (trained to 2-pentanone) compared to Group B (trained to pentanol).

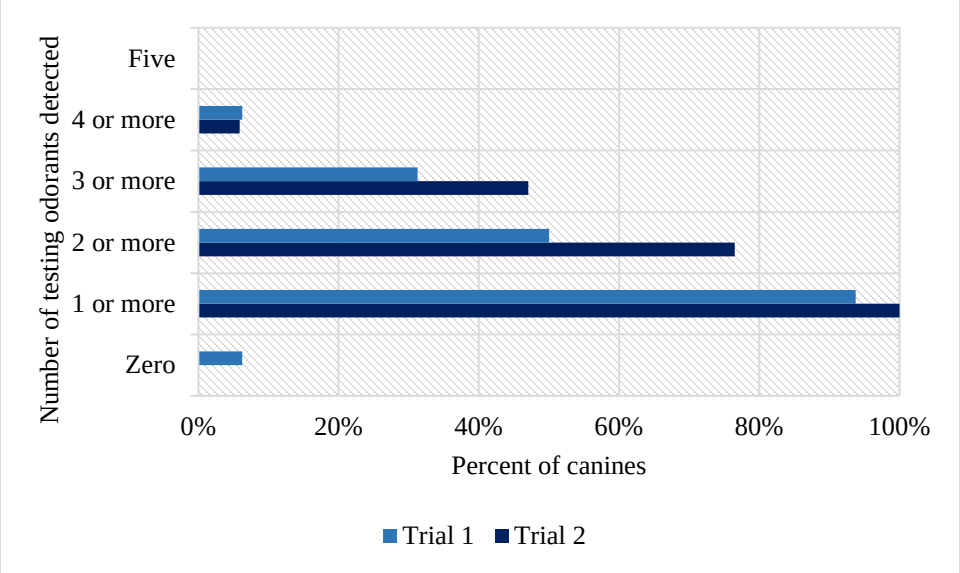

Figure 20. Results from Odor Set 2: Trials 1 and 2 – Summary of the total number of testing odorants detected by canines.

Trial 3 – Fifteen canines participated in Trial 3, all of which were included in the final results. A summary of the results can be seen in Figure 21. Detection of training odorants increased from Trial 2 to 92%, though this rate remains slightly lower than that of Trial 1. Canines were still most likely to detect methyl valerate and pentanal, but alert rates for all testing odorants in this trial increased compared to previous trials, and all were above chance, ranging from 73% to 100%.

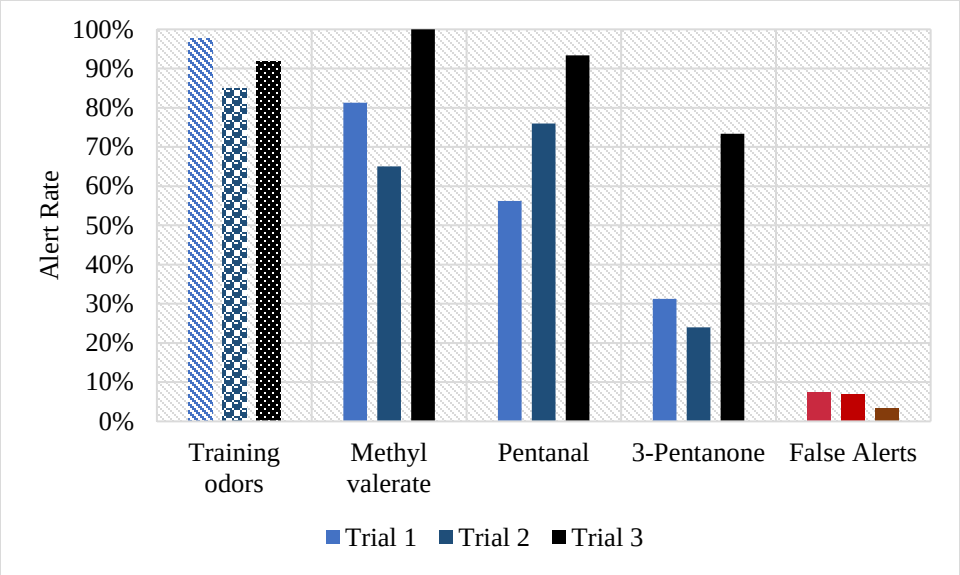

Figure 21. Results from Odor Set 2: Trials 1, 2, and 3 – Alert rate for the validation odorants (2-pentanone and pentanol for both groups) compared to the novel odorants.

All the PPVs for all testing odorants increased to at least 95% (Table C6). Figure 22 shows the percent of canines that detected testing odorants. It demonstrates that generalization increased over each trial, so that in Trial 3 100% of dogs detected 2 or more testing odorants.

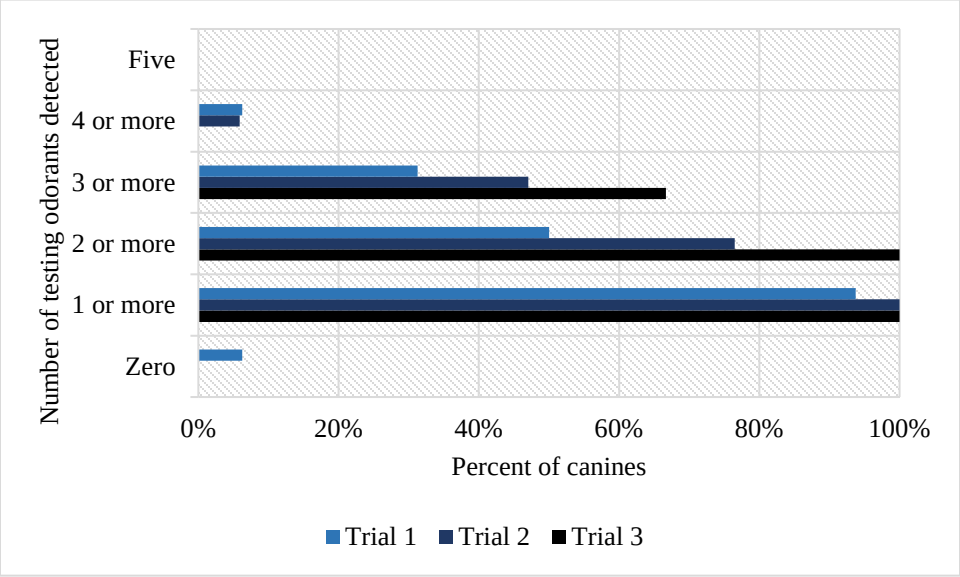

Figure 22. Results from Odor Set 2: Trials 1, 2, and 3 – Summary of the total number of testing odorants detected by canines.

A comparison of the proficiency of Group A compared to Group B can be seen in Figure 23. Group A, which trained on 2-pentanone before pentanol, maintained a higher rate of detection for 3-pentanone. This rate also increased compared to Trial 2. The opposite is true for Group B, which

trained on pentanol before 2-penanone. For this group, the alert rate for pentanal was higher than Group A's. This was not true for Trial 2. These results suggest that training odorant does affect the compound to which a canine will generalize.

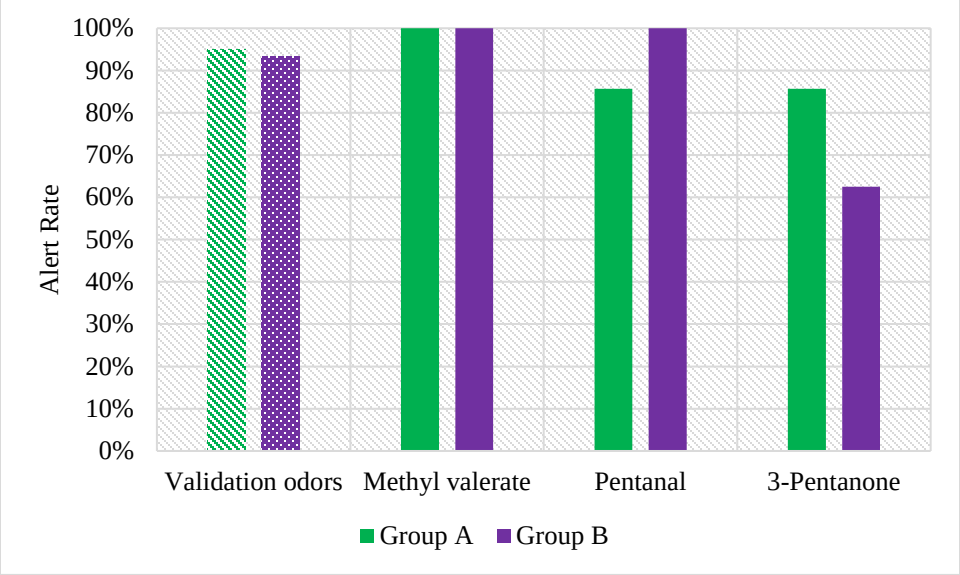

Figure 23. Results from Odor Set 2: Trial 3 – Alert rates for Group A (trained to 2-pentanone, then pentanol) compared to Group B (trained to pentanol, the 2-pentanone).

Summary

Odor Set 2 was designed to demonstrate generalization-discrimination between related compounds of differing functional groups. Canine responses were correlated to compound structure, specifically the presence and location of a carbonyl group on the molecule. Canines initially discriminated most between the carbonyl-containing compounds and pentanol. Methyl valerate was the most structurally similar testing odor, and had the highest alert rate after Trial 1. Pentanal had the second highest alert rate, and was the only other testing odorant with the carbonyl group on C-5. Generalization increased significantly from Trial 1 to Trial 3 for both groups (Table E5), and, in Trial 3, the alert rates were well above chance for all testing compounds with no statistical difference compared to the training odorants. These results indicate that training on target odorants with a variety of functional groups will aid in generalization for trained detection odorants.

PART 4. GENERALIZATION / DISCRIMINATION BETWEEN BINARY MIXTURES

Methods

The research was carried out as two sequential trials used to test the canines' tendency to generalize from one compound or simple mixture to other binary mixtures (referred to as Odor Set 3; structures provided in Appendix A). Trials were carried out in the same manner as in Parts 2 and 3, although only two trials were conducted. Statistical analysis was also done in the same manner. Each training and testing odorant was paired with pentanoic acid to create a binary mixture, presented in Table 9 (referred to as compound mixtures). All compounds in the mixture were delivered at similar odorant concentrations (see Part 1). Canines were initially split into three groups at random, one group being trained to pentanoic acid alone, another to the methyl benzoate mixture (structurally dissimilar), and the last to the methyl valerate mixture (structurally similar). Based on chemical structure and previous trial results discussed above, testing odorants were chosen on a continuum of most similar to pentanoic acid to least similar. The authors ranked the compounds, given in Table 10, with 1 being the most similar to pentanoic acid and 5 being the least similar. For the second trial, the canines in the first group continued to train to pentanoic acid alone, while the other two groups were trained on both methyl benzoate and methyl valerate mixtures.

In both trials, the canines were tested for proficiency at detecting the trained odorant mixture, then evaluated for capability of detecting other related binary mixtures. ORTs were designed as described previously, with each set of five boxes containing one testing mixture, or one target mixture and one distractor odorant, randomized for each canine. All COMPS were created prior to each trial, and were never reused. The level of generalization or discrimination to untrained odorants was assessed following each subsequent training period.

Table 9. Odor Set 3 – Training and testing odorants for Trials 1-2.

	Trial 1	Trial 2
Training Mixture(s)	Pentanoic acid alone (Group 1 only) OR methyl benzoate mixture (Group 2 only) OR methyl valerate mixture (Group 3 only)	Pentanoic acid alone (Group 1 only) OR methyl benzoate mixture (Group 2 and 3) AND methyl valerate mixture (Group 2 and 3 only)
Testing Mixture 1	Pentanoic acid alone (Groups 2 and 3)	Pentanoic acid alone (Groups 2 and 3)
Testing Mixture 2	Methyl benzoate mixture (Groups 1 and 3)	Methyl benzoate mixture (Group 1 only)
Testing Mixture 3	Methyl valerate mixture (Groups 1 and 2 only)	Methyl valerate mixture (Groups 1 only)
Testing Mixture 4	2-Pentanone mixture	2-Pentanone mixture
Testing Mixture 5	Isovaleric acid mixture	Isovaleric acid mixture
Testing Mixture 6	Pyridine mixture	Pyridine mixture

Table 10. Odorant compounds used in mixture with pentanoic acid ranked from most similar to pentanoic acid to least similar.

1	Methyl valerate
2	Isovaleric acid
3	2-Pentanone
4	Methyl benzoate
5	Pyridine

Results

Due to scheduling conflicts and emergency weather delays, trial dates had to be scheduled with unequal amounts of training time between each. Canines had a minimum of 5 weeks of training, and a maximum of 8 weeks, with each trial being divided between two dates. Each trial took place in two locations. Trial dates and locations are listed in Table 11.

Table 11. Dates and locations of canine trials.

Trial	Date	Location
Trial 1	July 22	Richmond, VA
	August 19	Gainesville, FL
Trial 2	August 26	Richmond, VA
	September 24 / October 14	Gainesville, FL

Trial 1 – A total of 32 canines participated in Trial 1, though two were excluded from the final results due to behavioral reasons (i.e. they were deemed to be not working by the evaluators). Group 1 had 10 canines, Group 2 had 13 canines, and Group 3 had 11 canines. The summary of

the results is presented in Figure 24, and alerts for each canine in each trial are given in Tables B7-8). The total alert rate to trained odorant mixtures was 95%, and alert rates to all testing mixtures was above chance (50%), ranging from 75% to 91%. There was no significant difference between alert rates to testing mixtures indicating a high tendency to generalize across all three groups. Figure 25 shows the number of testing mixtures to which the canines alerted. All canines generalized to at least two testing mixtures, while over half (53%) generalized to all testing mixtures.

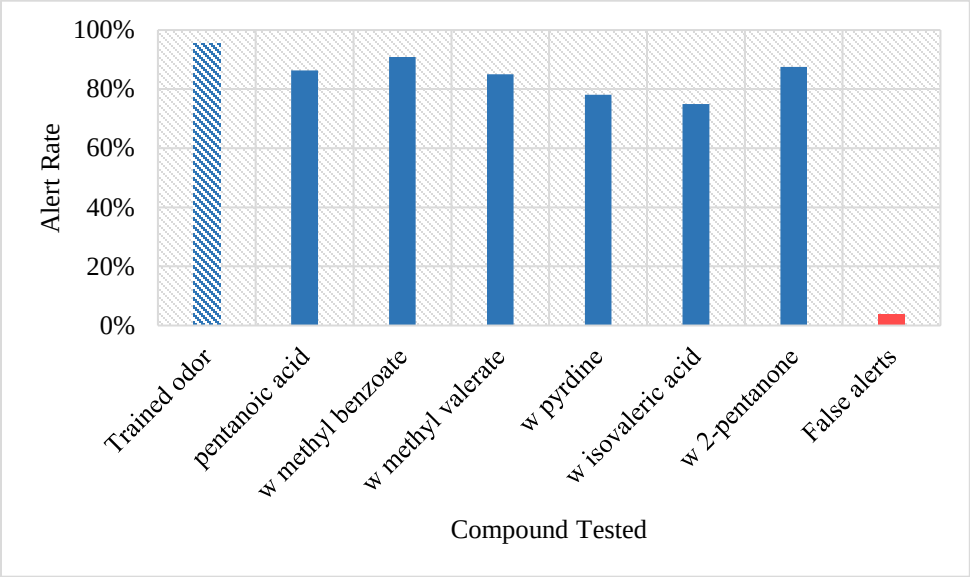

Figure 24. Results from Odor Set 3: Trial 1 – Alert rate for the validation mixtures (pentanoic acid alone for Group 1, methyl valerate with pentanoic acid for Group2, methyl benzoate with pentanoic acid for Group 3) compared to the novel mixtures.

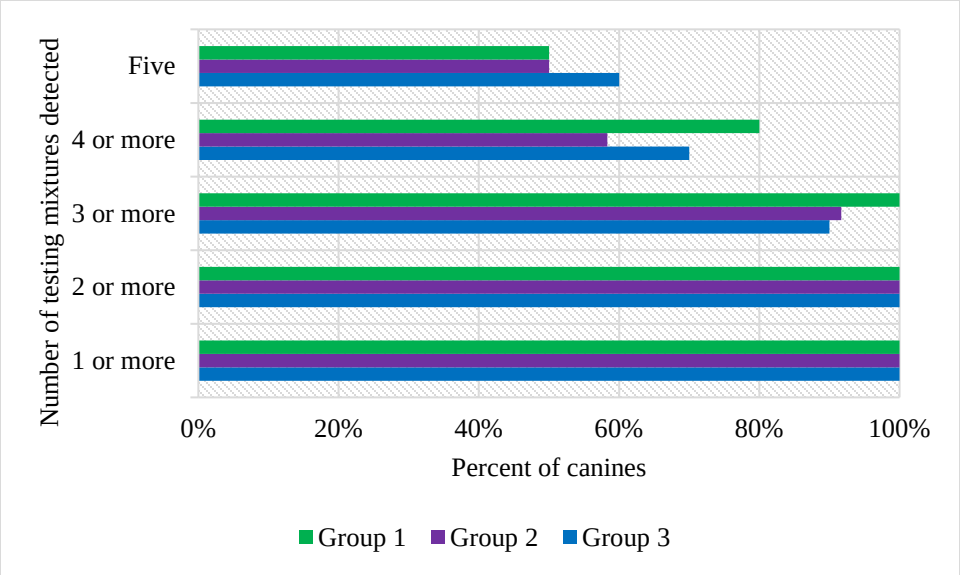

Figure 25. Results from Odor Set 3: Trial 1 – Summary of the total number of testing mixtures detected by canines.

A comparison of alert rates by group is shown in Figure 26. Canines in Group 1, trained only to pentanoic acid alone, were most likely to locate the mixtures containing a compound structurally dissimilar from pentanoic acid. When pentanoic acid was mixed with the most similar compound, methyl valerate, however, they were least likely to detect the mixture. Canines in Group 2 were trained on the methyl benzoate mixture, a structurally different chemical, and Group 3 was trained on the methyl valerate mixture, a structurally similar compound. Overall, Group 3 had a higher propensity to generalize than Group 2, as can be seen in Figure 25, and was better at detecting pentanoic acid alone (Figure 26).

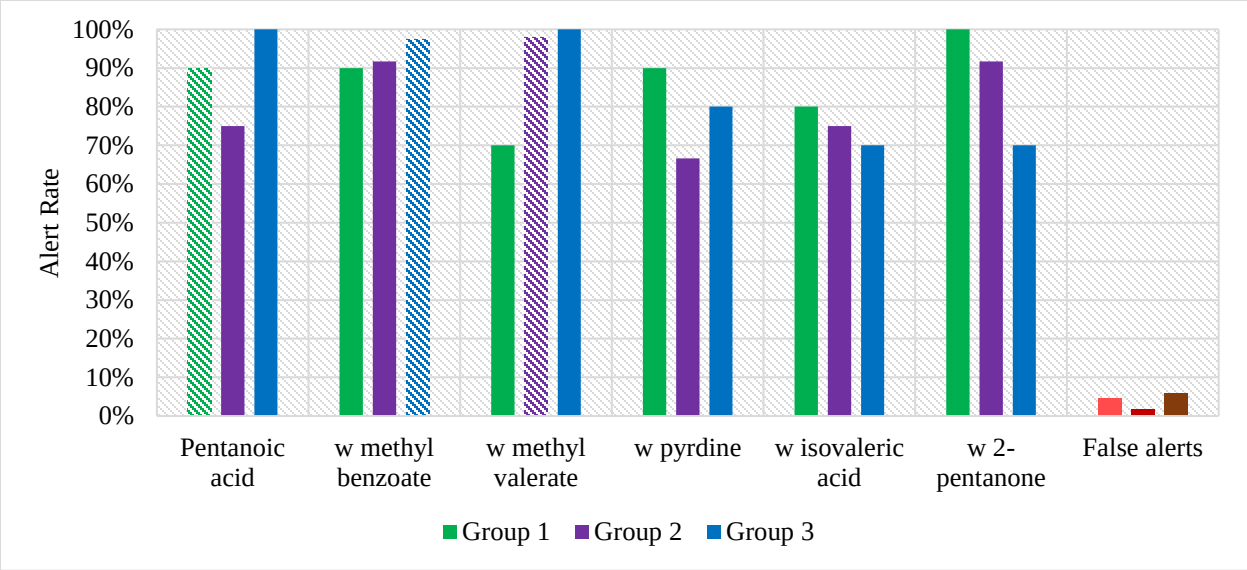

Figure 26. Results from Odor Set 3: Trial 1 – Alert rates for Group 1 (trained to pentanoic acid) compared to Group 2 (trained to methyl valerate with pentanoic acid) and Group 3 (trained to methyl benzoate with pentanoic acid).

Trial 2 – Thirty-four canines participated in Trial 2, and all were included in the final results. The alert rate to trained mixtures was 96%, equivalent to that of Trial 1. Alert rates for isovaleric acid, 2-pentanone, and methyl valerate mixtures (the most similar mixture components) all increased, while alert rates for methyl benzoate and pyridine mixtures (the least similar mixture components) decreased, as did the alert rate to pentanoic acid alone (Figure 27). The response to the pyridine mixture is significantly different from the validation mixtures. However, the changes were not significant for the remaining mixtures, and all detection rates remained well above chance (74-100%).

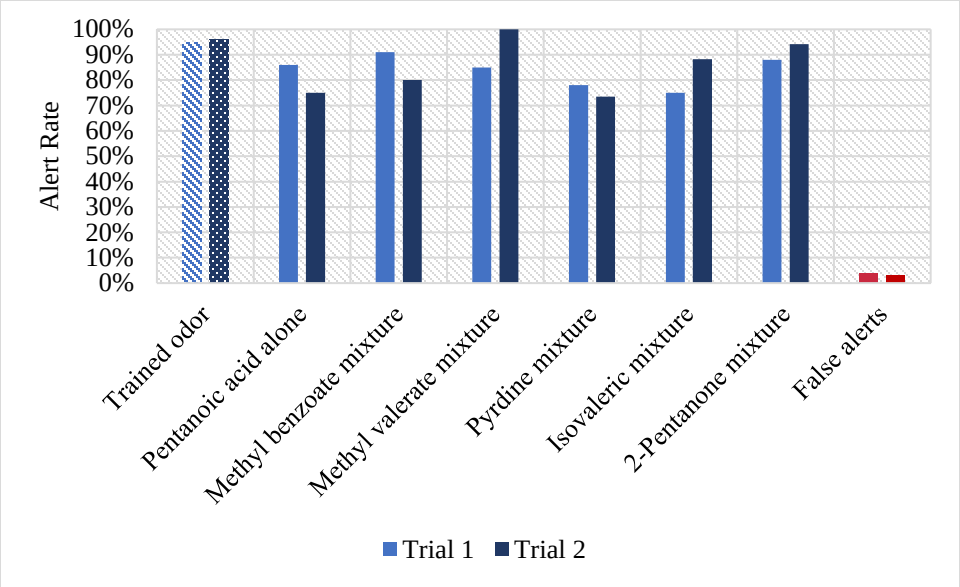

Figure 27. Results from Odor Set 3: Trials 1 and 2 – Alert rate for the validation mixtures (pentanoic acid alone for Group 1, methyl valerate with pentanoic acid and methyl benzoate with pentanoic acid for Groups 2 and 3) compared to the novel mixtures.

When comparing the separate groups (Figure 28), Group 1 detected the methyl valerate mixture at 100%, but the isovaleric acid mixture, the next most similar compound to pentanoic acid, only 70% of the time. This is likely because many canines generalized from pentanoic acid to methyl valerate, as was seen with Odor Set 2, but some had a more difficult time distinguishing pentanoic acid from isovaleric acid. They were also more likely to distinguish pentanoic acid in the pyridine mixture (90%), which is the most chemically different molecule tested. Group 2, overall, generalized more to all mixtures in Trial 2 compared to Trial 1, while alert rates for Group 3 canines only increased for isovaleric acid and 2-pentanone mixtures. Group 3 was also less proficient at detecting pentanoic acid alone, compared to their performance in Trial 1 and compared to Group 2. These results suggest that, for Group 2, training first on a structurally similar mixture (methyl valerate) and second on a structurally different mixture (methyl benzoate) aided in generalization compared to Group 3. Figure 29 demonstrates this point further. All canines in Groups 1 and 2 detected at least three testing mixtures, compared to 73% of Group 3. Group 1 generalized the most, with 80% of canines in that group finding at least 4 testing mixtures. No canines from Group 2 or 3 detected all 5 testing mixtures.

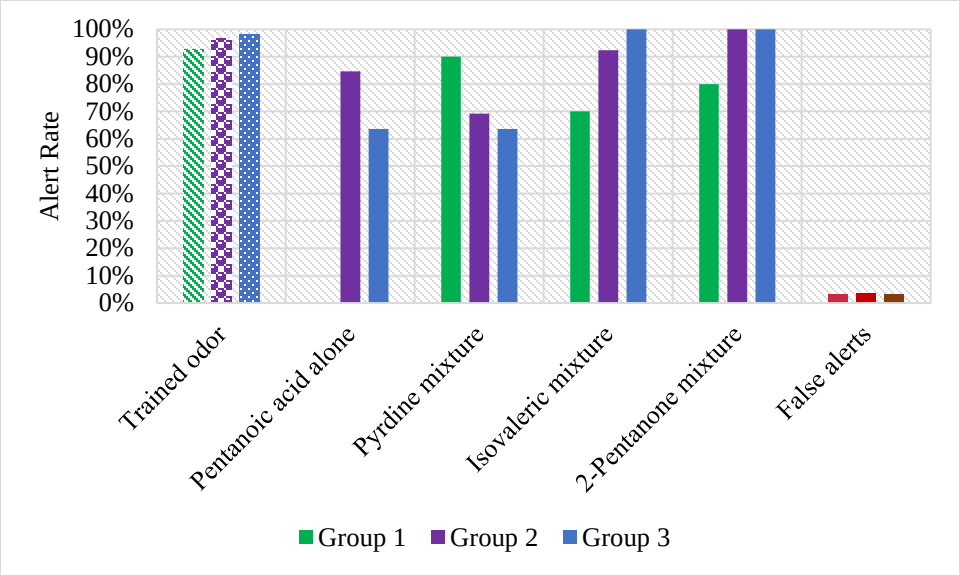

Figure 28. Results from Odor Set 3: Trial 2 – Alert rates for Group 1 (trained to pentanoic acid) compared to Group 2 (trained to methyl valerate with pentanoic acid first, then methyl benzoate with pentanoic acid) and Group 3 (trained to methyl benzoate with pentanoic acid, then methyl valerate with pentanoic acid).

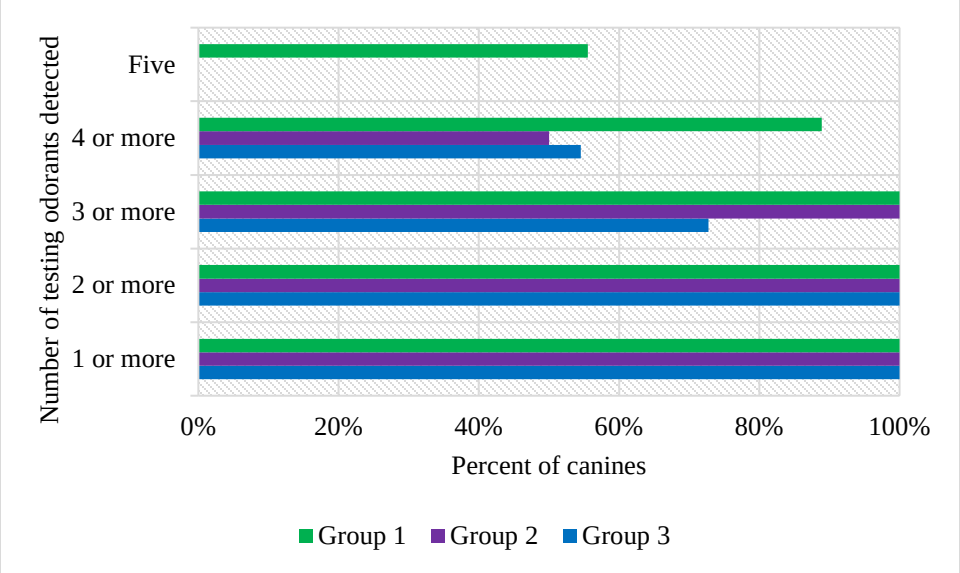

Figure 29. Results from Odor Set 3: Trial 2 – Summary of the total number of testing mixtures detected by canines.

Summary

Odor Set 3 utilized a series of simple binary mixtures to explore the canine generalization-discrimination balance. Canines trained to pentanoic acid alone detected all mixtures at rates above chance, though two of the testing mixtures were detected at rates lower than the trained odor (methyl valerate and isovaleric acid mixtures). Methyl valerate and isovaleric acid are similar structurally to pentanoic acid, so it was expected that these alert rates should be lower than for

dissimilar compounds. While this seem counterintuitive when considering the single odors tested in previous trials, in which canines were better able to generalize to structurally similar compounds, in fact simple binary mixtures are perceived differently by the olfactory system. In the case of mixtures, when two like components are together it is more difficult to separate and identify individual components, muddying the perception of the odor picture. In turn, it is easier to identify the single training odor when it is mixed with components that are structurally different. This is consistent with previous research exploring perception of binary mixtures [6, 9, 10].

Groups 2 and 3 were trained on binary mixtures. The results for Trial 1 agree with previous research that suggests that it is easier for olfaction processes to identify individual components of mixtures made of structurally different compounds [9]. In this case, it appears that it was more difficult for Group 2 (trained to methyl valerate mixture first) to identify the separate components of their trained mixture than Group 3 (trained to methyl benzoate mixture first). Trial 2 diverged from these results because Group 2 became more adept at generalization than Group 3, though both groups had high rates of detection for all testing mixtures.

The PPV for all compounds was greater than 95% in both Trials 1 and 2 (Tables C7-8). Additionally, only the pyridine mixture in Trial 2 showed an alert rate statistically different from the training mixtures (Table D8), and there was no statistically significant difference between trials or groups of canines (Tables E3 and E6). When considering these results together, it indicates that overall, canines show a significant tendency to generalize to simple binary mixtures containing at least one of their training odorants when odorants in the mixture are presented at similar concentrations.

DISCUSSION

Figure 30 displays a flow chart of work flow for the project, including Parts 1-4. It is provided as a summary for reference in the following discussion and conclusions.

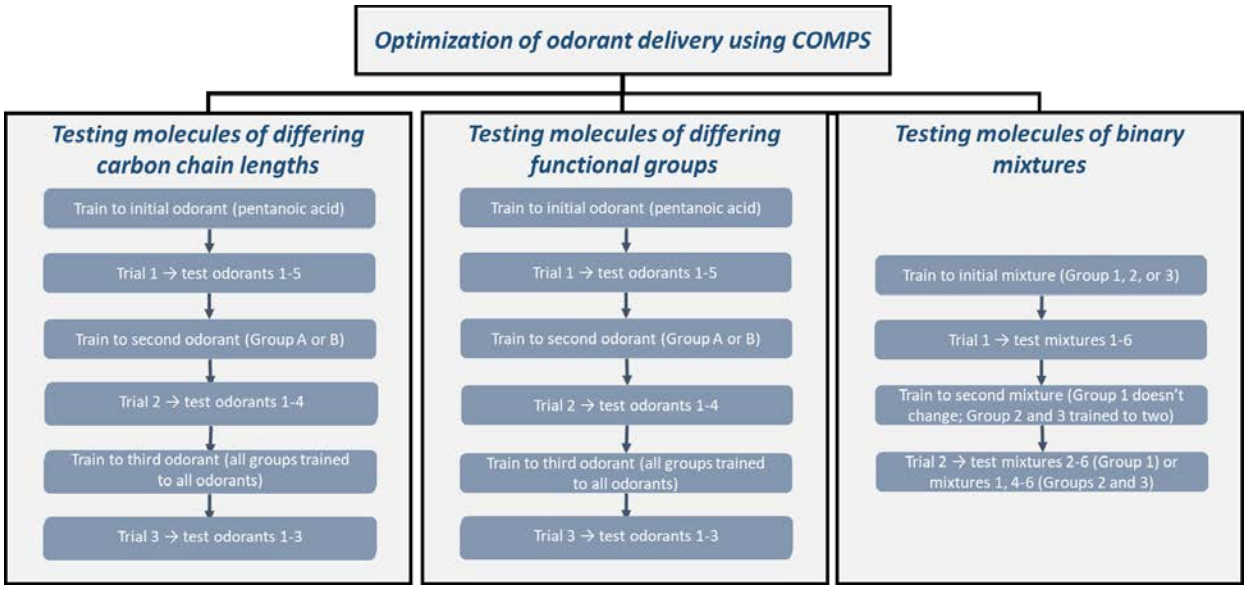

Figure 30. Summary depiction of project work flow.

Odor Set 1 vs. Odor Set 2

A comparison was done between Odor Sets 1 and 2 (Trial 3 only) to determine if canines had a tendency to generalize to one set of compounds over the other. Results are given in Figure 31. Canines in Odor Set 1 alerted to 37% of testing odors, while in Odor Set 2, canines alerted to 89% of testing odorants, a difference that is statistically significant (Table E7). Canines display a greater tendency to generalize across different compounds of the same carbon length, regardless of functional group. This is also supported by the results of Odor Set 1 (presented previously) where canines had a higher alert rate to compounds that differed from their training odorant by only one or no (i.e. branched) carbons. Varying the carbon chain length resulted in a higher tendency for discrimination. In Odor Set 2, the only compound without a carbonyl group consistently had lower alert rates than the other testing odorants. Since all of the odors tested in Odor Set 2 had the same length carbon chain, these results indicate that the carbonyl plays an important role in olfactory receptor binding, and that carbon chain length is very influential in the generalization-discrimination balance.

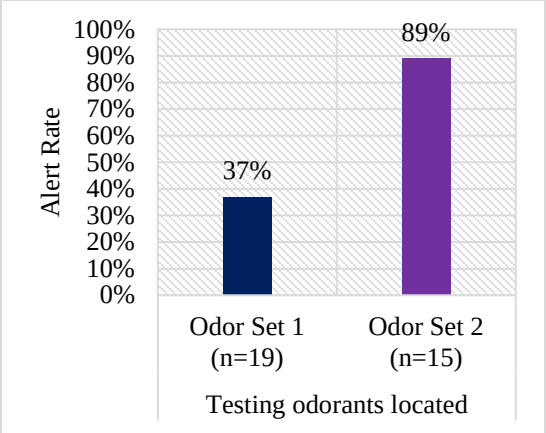

Figure 31. Number of testing odorants alerted to by canines in Odor Sets 1 and 2. (n=number of canines)

Canine Experience Comparison – Novice vs. expert

All participants were surveyed at the beginning of the study and asked to provide basic information about their canine, including breed, age, gender, and years of experience in odor detection activities. For data analysis purposes, canines were split into two groups based on years of experience (for Trial 1 in each odor set only). In the first analysis, shown in Figure 32, a canine was considered "novice" if it had less than two years of experience in odor detection, while those considered "expert" had at least two years of experience. Odor Sets 2 and 3 show increased generalization for expert canines, and the difference in detection of testing odorants between novice and expert canines for Odor Set 3 is statistically significant (Table E8). Because there were only two novice canines in Odor Set 2, it is difficult to draw conclusions about statistical significance.

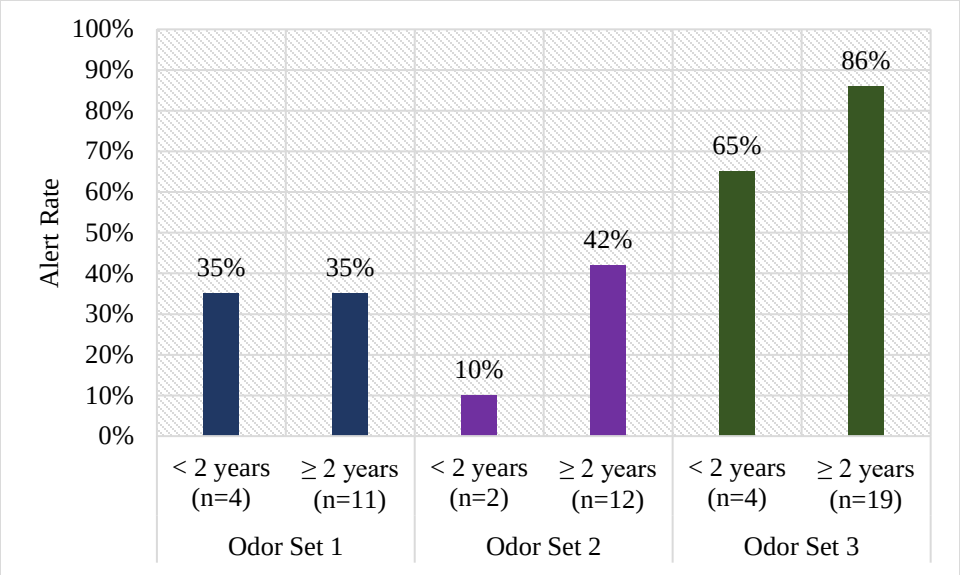

Figure 32. Detection rate of testing odorants by canines in each odor set. Canines with less than two years of experience in odor detection are considered novice, while canines with at least two years of experience are considered experts. (n=number of canines)

There were relatively few canine participants with less than two years of experience (10 out of 42 for all odor sets), so to bolster the statistical reliability of the results, a second set of analyses was performed. In this set, a "novice" was considered a canine with no more than 2.5 years of experience in odor detection. An "expert" canine had at least three years of experience. These results are shown in Figure 33 (and Table E9). All three odor sets show increased detection of testing odorants by expert canines compared to novice canines, though the difference is not statistically significant. These results suggest that more experienced canines in odor detection tend to generalize more than novice canines, regardless of the odor set. An increased number of novice canines would most likely support this data and further increase the statistical reliability of the results.

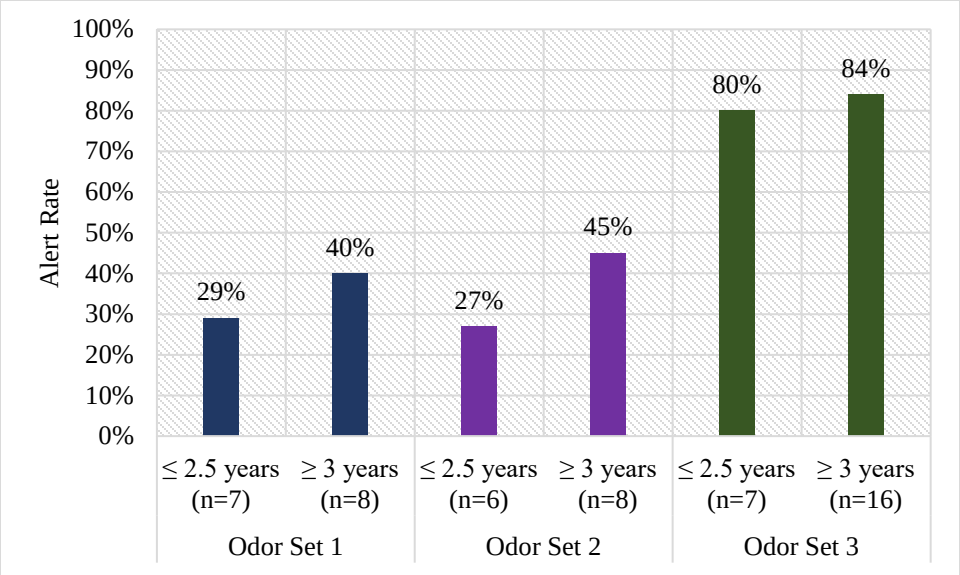

Figure 33. Detection rate of testing odorants by canines in each odor set. Canines with no more than 2.5 years of experience in odor detection are considered novice, while canines with at least three years of experience are considered experts. (n=number of canines)

Breed Comparison – Traditional detector dog breeds vs. non-traditional detector dog breeds

Canines were split into two groups based on traditional detector dog breeds versus non-traditional detector dog breeds for comparison (for Trial 1 in each odor set only). Traditional breeds included Labradors, German shepherds, spaniels (English springers and fields), Belgian manilois, and beagles. The results are presented in Figure 34. While canines from traditional breeds alerted to more testing odorants in Odor Sets 1 and 2, the difference is not significant (Table E10). Also, these canines alerted to slightly fewer testing odorants than non-traditional breeds in Odor Set 3. These results indicate that there is not a significant tendency for traditional detector dog breeds to generalize more or less than canines of other breeds.

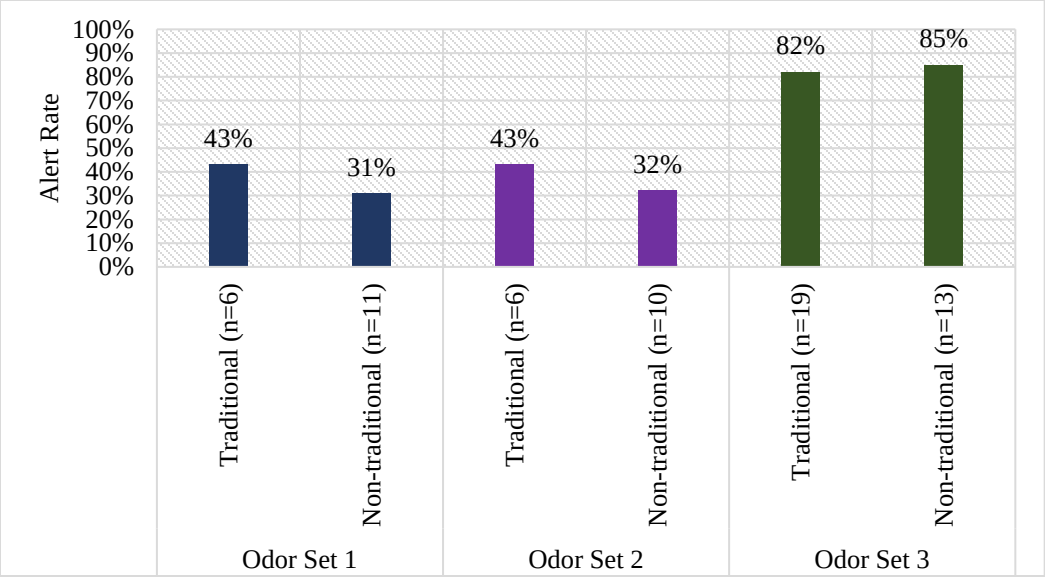

Figure 34. Detection rate of testing odorants by canines in each odor set. Canine detection is compared by breed, considering traditional detector dog breeds and non-traditional detector dog breeds. (n=number of canines)

CONCLUSIONS

Prior to beginning any canine training or testing, COMPS were optimized as a method for systematic odorant delivery. Through characterization of odorant permeation rates for pure compounds and vapor concentration available to canines over varying temperature/humidities, it was possible to develop consistent testing and training methods for detection canines that can be used for many training materials in several different environmental conditions. COMPS bag thicknesses were selected for all thirteen compounds used in this work and permeation rates were determined through gravimetric analysis. Bag thicknesses were chosen to be as similar as possible to pentanoic acid's permeation rate (0.037 mg/min in 3 MIL), allowing similar amounts of odorant availability for each compound despite varying vapor pressures. Additionally, these permeation rates were tested at varying environmental conditions, i.e. temperature and humidity. It is important to understand how these factors affect permeation rates of target compounds since they affect evaporation and diffusion rate, both of which will determine how much compound remains around the COMPS and is therefore available to a canine for detection.

To further evaluate the proper use of COMPS, packaging and lifetime was determined. It was concluded that COMPS should be stored and transported in dual-layer packaging with glass jars to prevent odorant depletion. Additionally, COMPS in this study should be discarded after seven

hours of training since vapor concentration decreases to an un-operable amount after that time. Utilizing COMPS for canine olfaction research allows vapor concentration to be reproducible and measurable despite variations. In turn, this informs proper utilization and handling of training materials, allowing for enhanced understanding of canine olfaction research and applications.

Once the COMPS were optimized, they were distributed for canine training and subsequently used in all detection trials. Odor Sets 1 and 2 studied sets of related single-odorant targets, while Odor Set 3 studied simple binary odorant mixtures. It was hypothesized that generalization between testing odorants would increase with increased training. This was true for all odor sets, though to varying degrees. Examining the statistical analyses (Appendix D) for canine responses to testing compounds in Odor Set 1, canines did not show a significant tendency to generalize. Responses to all testing odorants was significantly lower than responses to the training odorant in Trials 1 and 3, meaning that while some generalization did occur, there is not a great tendency for canines to generalize between molecules of varying carbon chain lengths.

The hypothesis that generalization would increase with increased training was true for Odor Set 2, which tested compounds of the same chain length with different functional groups. Detection rates for two of the five testing compounds were not significantly different from that of the trained odorant in Trial 1, meaning that canines generalized initially to those two compounds, methyl valerate and pentanal (Appendix C). By Trial 3, the canines were generalizing to all testing compounds. Odor Set 2 is the only odor set in which there was a significant difference in detection rates between groups trained with different odorants (Appendix E), meaning that training odorant was significant for canine generalization in this instance.

For Odor Set 3, generalization increased with training (i.e. from Trial 1 to Trial 2); however, the tendency to generalize was high initially, so the increase was not significant (Appendix D). The alert rate to the mixture containing pyridine (the most dissimilar compound) was significantly lower than to the training odors in Trial 2 (all others were statistically similar), implying that discrimination between the training odors and this mixture actually increased. Other trends can be seen for generalization based on the training odorant mixtures, but they are not statistically significant. Overall, results indicate that training on structurally similar mixtures is influential for generalization of binary compound mixtures.

It was hypothesized that canines would generalize more to compounds similar to their training odorant, and this was observed for the single odorant sets. Trends could be seen based on similarity of molecules, where canines were better able to generalize to testing odorants that were more similar to their training odorant. As dissimilarity increased, discrimination increased. It then logically followed that increasing the variety of functional groups to which canines were trained shifted the generalization-discrimination balance by encouraging generalization across multiple types of training odorants. More research is needed to define the underlying mechanisms of olfactory perception and understand how it can be altered through training. These results have important implications for trained detection canines, which generally train on a variety of structurally unrelated target odorants.

This research explored the concepts of odor perception in canine detection to determine how molecular structure, mixture perception, and training influence the generalization-discrimination balance. Overall, training on more odorants improves generalization, though certain trends can be seen depending on structural similarity of training and testing odorants. For example, the more chemically similar a compound is to the training odorant, the more likely a canine will be to generalize, except when in simple binary mixtures, where structurally similar compounds are more difficult to discriminate. Notably, it is easier for canines to generalize to compounds of different functional groups than to compounds of differing chain length. Also, canines with more experience in odor detection tend to generalize more than novice canines. Based on the results of these studies, best practice is to train canines on a variety of target materials and mixtures, and to continue maintenance training to increase experience. Additional research should be done to supplement these results and related topics in order to optimize the canine generalization-discrimination balance, improve training efficiency, and minimize false positives and negatives in the field.

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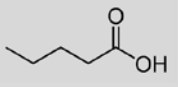
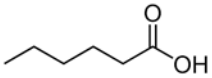
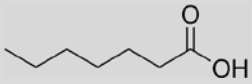
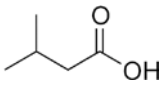
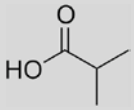
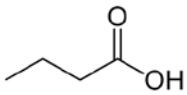
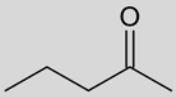
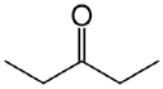
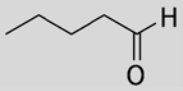
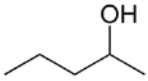
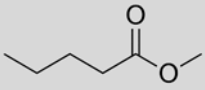
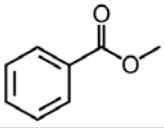
APPENDIX A

Table 1. Compound structures for odorants used in Odor Sets 1, 2, and 3.

Compound	***Odor Set (1, 2, or 3)***	***Structure***
Pentanoic acid	1, 2, 3	
Hexanoic acid	1	
Heptanoic acid	1	
Isovaleric acid	1, 3	
Isobutyric acid	1	
Butanoic acid	1	
2-Pentanone	2, 3	
3-Pentanone	2	
Pentanal	2	
Pentanol	2	
Methyl valerate	2, 3	
Methyl benzoate	3	
Pyridine	3	

APPENDIX B

Table 1. All data from Odor Set 1: Trial 1, excluding canines that did not qualify. (A = Alert, N = No response).

Dog ID	*Isobutyric acid*	*Butanoic acid*	*Isovaleric acid*	*Hexanoic acid*	*Heptanoic acid*	***Testing odorants (out of 5)***	***Pentanoic acid (training odorant; out of 4)***	***False alerts (out of 25 possible)***
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%***

Table 2. All data from Odor Set 1: Trial 2, excluding canines that did not qualify. (A = Alert, N = No response, n/a = validation odorant, dashes indicate a canine not run on that odorant).

Dog ID	*Isobutyric acid*	*Butanoic acid*	*Isovaleric acid*	*Hexanoic acid*	*Heptanoic acid*	***Testing odoantrs (out of 4)***	***Validation odorant (out of 4 or other indicated)***	***False alerts (out of 21 possible or other indicated)***
TD101	N	N	A	A	n/a	**2**	**4**	**1**
TD102	A	N	N	N	n/a	**1**	**3**	**0**
TD104	N	N	n/a	A	N	**1**	**4**	**1**
TD106	N	A	n/a	A	N	**2**	**4**	**1**
TD107	N	N	n/a	A	N	**1**	**4**	**1**
TD108	N	N	n/a	N	N	**0**	**4**	**3**
TD109	N	N	n/a	N	N	**0**	**4**	**0**
TD110	N	N	N	A	n/a	**1**	**4**	**0**
TD111	N	N	A	N	n/a	**1**	**4**	**3**
TD201	N	N	n/a	N	N	**0**	**4**	**1**
TD203	N	--	--	N	n/a	**0**	**5/7**	**1/20**
TD204	N	--	--	N	n/a	**0**	**6/8**	**3/17**
TD206	A	--	--	N	n/a	**1**	**5/7**	**4/21**
TD209	N	--	n/a	N	N	**0**	**6/6**	**2/26**
TD213	A	--	n/a	N	N	1	**7/7**	**2/26**
Total	***3***	***1***	***2***	***5***	***0***	***11***	***91%***	***7%***

Table 3. All data from Odor Set 1: Trial 3, excluding canines that did not qualify. (A = Alert, N = No response).

Dog ID	*Isobutyric acid*	*Butanoic acid*	*Hexanoic acid*	***Testing odorants (out of 3)***	***Validation odorant (out of 5 possible)***	***False alerts (out of 17 or 20* possible)***
TD101	N	A	N	**1**	**5**	**1**
TD103	A	A	N	**2**	**5**	**0**
TD104	N	N	N	**0**	**5**	**1**
TD105	N	N	A	**1**	**5**	**2**
TD106	N	A	A	**2**	**5**	**3**
TD107	N	N	N	**0**	**4**	**1**
TD108	N	N	N	**0**	**4**	**0**
TD109	A	N	A	**2**	**3**	**0**
TD110	N	N	A	**1**	**5**	**1**
TD111	A	A	A	**3**	**5**	**1**
TD201	N	N	A	**1**	**5**	**0**
TD202	N	N	N	**0**	**3**	**4**
TD203	A	N	A	**2**	**5**	**1**
TD204	A	N	A	**2**	**5**	**1**
TD206	N	N	N	**0**	**5**	**2**
TD208	N	A	N	**1**	**5**	**2**
TD209	A	N	N	**1**	**5**	**2**
TD210	A	N	N	**1**	**5**	**1**
TD213	N	N	A	**1**	**5**	**1**
Total	***7***	***5***	***9***	***21***	***89%***	***6%***

Table 4. All data from Odor Set 2: Trial 1, excluding canines that did not qualify. (A = Alert, N = No response).

Dog ID	*Methyl valerate*	*Pentanal*	*Pentanol*	*2-Pentanone*	*3-Pentanone*	***Testing odorants (out of 5)***	***Pentanoic acid (training odorant; out of 4)***	***False alerts (out of 25 possible)***
TD113	A	A	N	N	N	**2**	**4**	**1**
TD114	A	A	N	N	A	**3**	**4**	**2**
TD116	A	A	N	N	N	**2**	**4**	**1**
TD117	A	A	N	N	A	**3**	**4**	**0**
TD118	A	A	N	N	N	**2**	**4**	**0**
TD216	A	N	N	N	N	**1**	**4**	**2**
TD217	A	N	N	N	N	**1**	**4**	**1**
TD218	A	N	N	N	N	**1**	**4**	**3**
TD219	A	A	N	N	A	**3**	**4**	**3**
TD220	N	A	N	N	N	**1**	**4**	**4**
TD223	A	N	N	N	N	**1**	**4**	**3**
TD224	A	N	N	N	N	**1**	**4**	**2**
TD225	N	A	N	A	A	**3**	**4**	**2**
TD227	A	A	A	N	A	**4**	**4**	**2**
TD228	A	N	N	N	N	**1**	**4**	**3**
TD229	N	N	N	N	N	**0**	**3**	**1**
Total	***13***	***9***	***1***	***1***	***5***	***29***	***98%***	***8%***

Table 5. All data from Odor Set 2: Trial 2. (A = Alert, N = No response, n/a = validation odorant).

Dog ID	*Methyl valerate*	*Pentanal*	*Pentanol*	*2-Pentanone*	*3-Pentanone*	***Testing odorants (out of 4)***	***Validation odorant (out of 4)***	***False alerts (out of 25 or 21* possible)***
TD113	A	A	n/a	A	N	**3**	**4**	**4***
TD114	N	A	n/a	A	N	**2**	**3**	**3***
TD116	N	A	N	n/a	N	**1**	**4**	**3***
TD117	A	A	n/a	A	A	**4**	**4**	**0***
TD118	N	N	A	n/a	N	**1**	**3**	**2***
TD216	A	A	N	n/a	A	**3**	**4**	**0**
TD217	N	A	N	n/a	N	**1**	**4**	**1**
TD218	A	A	n/a	A	N	**3**	**2**	**1**
TD219	N	N	n/a	A	N	**1**	**4**	**2**
TD220	A	A	N	n/a	A	**3**	**4**	**0**
TD222	N	A	n/a	A	N	**2**	**2**	**2**
TD223	A	A	N	n/a	N	**2**	**4**	**0**
TD224	A	A	A	n/a	N	**3**	**3**	**1**
TD225	A	A	N	n/a	N	**2**	**4**	**3**
TD227	A	A	n/a	A	N	**3**	**3**	**2**
TD228	A	N	n/a	A	A	**3**	**3**	**2**
TD229	A	N	n/a	A	N	2	**3**	**1**
Total	***11***	***13***	***2***	***9***	***4***	***39***	***85%***	***7%***

Table 6. All data from Odor Set 2: Trial 3. (A = Alert, N = No response).

Dog ID	*Methyl valerate*	*Pentanal*	*3-Pentanone*	***Testing odorants (out of 3)***	***Validation odorant (out of 5* or 6 possible)***	***False alerts (out of 17* or 20 possible)***
TD113	A	A	A	**3**	**5***	**0***
TD114	A	A	A	**3**	**5***	**0***
TD116	A	A	A	**3**	**2***	**1***
TD117	A	A	A	**3**	**5***	**3***
TD118	A	A	A	**3**	**5***	**1***
TD216	A	A	A	**3**	**6**	**0**
TD217	A	N	A	**2**	**6**	**0**
TD218	A	A	N	**2**	**5**	**0**
TD219	A	A	N	**2**	**5**	**1**
TD220	A	A	N	**2**	**6**	**0**
TD222	A	A	N	**2**	**5**	**2**
TD223	A	A	A	**3**	**5**	**1**
TD224	A	A	A	**3**	**6**	**0**
TD227	A	A	A	**3**	**6**	**0**
TD229	A	A	A	**3**	**6**	**1**
Total	***15***	***14***	***11***	***40***	***92%***	***4%***

Table 7. All data from Odor Set 3: Trial 1, excluding canines that did not qualify. (A = Alert, N = No response, n/a = validation odorant).

Dog ID	*Pentanoic acid alone*	*Pentanoic acid + methyl benzoate*	*Pentanoic acid + methyl valerate*	*Pentanoic acid + pyridine*	*Pentanoic acid + isovaleric acid*	*Pentanoic acid + 2-pentanone*	***Testing odorants (out of 5)***	***Training odorant (out of 4)***	***False alerts (out of 25 possible)***
TD118	N	A	n/a	A	A	A	**4**	**4**	**0**
TD119	A	n/a	A	A	A	A	**5**	**4**	**1**
TD122	n/a	A	A	N	A	A	**4**	**4**	**0**
TD123	A	A	n/a	A	A	A	**5**	**4**	**0**
TD124	A	A	n/a	A	A	A	**5**	**4**	**0**
TD125	n/a	A	N	A	A	A	**4**	**3**	**0**
TD126	n/a	A	A	A	A	A	**5**	**4**	**3**
TD127	A	A	n/a	N	N	N	**2**	**4**	**1**
TD128	A	n/a	A	A	A	A	**5**	**4**	**0**
TD129	A	n/a	A	A	A	A	**5**	**4**	**1**
TD130	n/a	A	A	A	A	A	**5**	**4**	**0**
TD131	A	n/a	A	A	A	A	**5**	**4**	**1**
TD132	A	A	n/a	A	A	A	**5**	**4**	**1**
TD133	A	n/a	A	A	A	A	**5**	**4**	**0**
TD134	A	n/a	A	A	N	A	**4**	**4**	**1**
TD135	A	A	n/a	A	A	A	**5**	**4**	**1**
TD136	n/a	A	A	A	A	A	**5**	**4**	**2**
TD137	A	N	n/a	N	A	A	**3**	**4**	**0**
TD138	A	A	n/a	A	A	A	**5**	**4**	**1**
TD139	n/a	A	N	A	N	A	**3**	**4**	**2**

TD140	A	A	n/a	A	A	A	**5**	**4**	**2**
TD230	n/a	A	A	A	A	A	**5**	**4**	**1**
TD232	n/a	A	A	A	A	A	**5**	**3**	**0**
TD233	n/a	N	A	A	N	A	**3**	**3**	**5**
TD234	A	A	n/a	N	N	A	**3**	**4**	**1**
TD235	N	A	n/a	A	N	A	**3**	**4**	**0**
TD236	N	A	n/a	N	A	A	**3**	**3**	**0**
TD238	A	n/a	A	A	N	N	**3**	**4**	**2**
TD239	A	n/a	A	A	A	A	**5**	**4**	**5**
TD240	A	n/a	A	N	N	N	**2**	**4**	**5**
TD242	n/a	A	N	A	A	A	**4**	**3**	**2**
TD243	A	n/a	A	N	A	N	**3**	**3**	**3**
Total	***19***	***20***	***17***	***25***	***24***	***28***	***133***	***95%***	***4%***

Table 8. All data from Odor Set 3: Trial 2. (A = Alert, N = No response, n/a = validation odorant, dashes indicate a canine not run on that odorant).

Dog ID	*Pentanoic acid alone*	*Pentanoic acid + methyl benzoate*	*Pentanoic acid + methyl valerate*	*Pentanoic acid + pyridine*	*Pentanoic acid + isovaleric acid*	*Pentanoic acid + 2-pentanone*	***Testing odorants (out of 4 or 5*)***	***Training odorant (out of 4 or 6*)***	***False alerts (out of 29 or 33* possible)***
TD118	A	n/a	n/a	A	A	A	**4**	**4**	**0**
TD119	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD122	n/a	N	A	A	A	A	**4***	**4**	**2***
TD123	A	n/a	n/a	N	A	A	**3**	**4**	**1**
TD124	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD125	n/a	A	A	A	A	A	**5***	**4**	**0***
TD126	n/a	A	A	A	A	A	**5***	**4**	**4***
TD127	A	n/a	n/a	A	N	A	**3**	**4**	**1**
TD128	N	n/a	n/a	N	A	A	**2**	**4**	**1**
TD129	A	n/a	n/a	A	A	A	**4**	**4**	**0**
TD130	n/a	A	A	A	A	A	**5***	**4**	**0***
TD131	A	n/a	n/a	A	A	A	**4**	**4**	**0**
TD132	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD133	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD134	N	n/a	n/a	A	A	A	**3**	**3**	**1**
TD135	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD136	n/a	A	A	A	A	A	**5***	**4**	**1***
TD137	N	n/a	n/a	A	A	A	**3**	**4**	**0**
TD138	A	n/a	n/a	A	A	A	**4**	**4**	**1**
TD139	n/a	A	A	A	A	N	**4***	**3**	**1***
TD140	N	n/a	n/a	A	A	A	**3**	**4**	**2**

TD230	n/a	A	A	A	A	A	**5***	**3**	**0***
TD232	n/a	N	A	A	N	A	**3***	**4**	**1***
TD233	n/a	A	A	--	--	--	**2***	**3**	**0***
TD234	A	n/a	n/a	A	A	A	**4**	**6***	**2**
TD235	A	n/a	n/a	N	A	A	**3**	**6***	**2**
TD236	A	n/a	n/a	--	A	A	**3**	**6***	**0**
TD237	A	n/a	n/a	N	A	A	**3**	**4***	**0**
TD238	A	n/a	n/a	N	A	A	**3**	**6***	**2**
TD239	N	n/a	n/a	N	A	A	**2**	**6***	**1**
TD240	N	n/a	n/a	N	A	A	**2**	**6***	**1**
TD241	A	n/a	n/a	A	A	A	**4**	**6***	**1**
TD242	n/a	A	A	A	N	A	**4***	**4***	**1***
TD243	A	n/a	n/a	A	A	A	**4**	**6***	**1**
Total	***18***	***8***	***10***	***25***	***30***	***32***	***123***	***96%***	***3%***

APPENDIX C

Positive predictive values (PPVs) were calculated for each validation and testing odorant for each trial using Equation 1. True positive (alert) rates and false positive rates were compared for each odor. All canines were considered as a single group in each trial.

Table 1. Positive predictive values for Odor Set 1: Trial 1.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	97%	8.00%	**92%**
Butanoic acid	35%	8.00%	**82%**
Isobutyric acid	47%	8.00%	**85%**
Isovaleric acid	47%	8.00%	**85%**
Hexanoic acid	29%	8.00%	**79%**
Heptanoic acid	18%	8.00%	**69%**

Table 2. Positive predictive values for Odor Set 1: Trial 2.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	91%	7.00%	**93%**
Butanoic acid	10%	7.00%	**59%**
Isobutyric acid	20%	7.00%	**74%**
Isovaleric acid	50%	7.00%	**88%**
Hexanoic acid	33%	7.00%	**83%**
Heptanoic acid	0%	7.00%	**0%**

Table 3. Positive predictive values for Odor Set 1: Trial 3.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	89%	7.0%	**93%**
Butanoic acid	26%	7.0%	**79%**
Isobutyric acid	37%	7.0%	**84%**
Hexanoic acid	47%	7.0%	**87%**

Table 4. Positive predictive values for Odor Set 2: Trial 1.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	98%	8.00%	**92%**
Methyl valerate	81%	8.00%	**91%**
Pentanal	56%	8.00%	**88%**
Pentanol	6%	8.00%	**43%**
2-Pentanone	6%	8.00%	**43%**
3-Pentanone	31%	8.00%	**79%**

Table 5. Positive predictive values for Odor Set 2: Trial 2.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	85%	7.00%	**92%**
Methyl valerate	65%	7.00%	**90%**
Pentanal	76%	7.00%	**92%**
Pentanol	25%	7.00%	**78%**
2-Pentanone	100%	7.00%	**93%**
3-Pentanone	24%	7.00%	**77%**

Table 6. Positive predictive values for Odor Set 2: Trial 3.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	92%	4.0%	**96%**
Methyl valerate	100%	4.0%	**96%**
Pentanal	93%	4.0%	**96%**
3-Pentanone	73%	4.0%	**95%**

Table 7. Positive predictive values for Odor Set 3: Trial 1.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	95%	4.00%	**96%**
pentanoic acid	86%	4.00%	**96%**
w methyl benzoate	91%	4.00%	**96%**
w methyl valerate	85%	4.00%	**96%**
w pyrdine	78%	4.00%	**95%**
w isovaleric acid	75%	4.00%	**95%**
w 2-pentanone	88%	4.00%	**96%**

Table 8. Positive predictive values for Odor Set 3: Trial 2.

Target	*True positive rate*	*False positive rate*	***PPV***
Validation odorant	96%	3.00%	**97%**
pentanoic acid	75%	3.00%	**96%**
w methyl benzoate	80%	3.00%	**96%**
w methyl valerate	100%	3.00%	**97%**
w pyrdine	74%	3.00%	**96%**
w isovaleric acid	88%	3.00%	**97%**
w 2-pentanone	94%	3.00%	**97%**

APPENDIX D

Using the McNemar chi-square test, the probability of a canine detecting the testing odorant was compared to the probability of detecting the validation odorant. For each testing odorant within a trial, the canines were considered as one group. In order to pair the data, the number of correct alerts to the validation odorants was divided by the number of opportunities each dog had for detecting that odorant, either 4 or 5 or an average if not all dogs had the same number of opportunities. This helped to account for the higher number of validation tests compared to a single exposure to each testing odorant. Data was entered into 2 x 2 contingency tables (example provided in Table 4), and the χ^2 value was determined using Equation 2. To determine significance, χ^2 was compared to the χ^2_{crit} value for one degree of freedom at a 95% confidence level ($\chi^2_{crit} = 3.841$). If χ^2 was greater than χ^2_{crit}, the probability of a canine detecting a testing odorant was considered significantly different (*Sig diff*) from that of the validation odorants.

Table 1. McNemar chi-square test results for Odor Set 1: Trial 1.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**17**	**16.5**	**0.5**		
Butanoic acid	17	6	11	6.231	*Y*
Isobutyric acid	17	8	9	4.455	*Y*
Isovaleric acid	17	8	9	4.455	*Y*
Hexanoic acid	17	5	12	7.143	*Y*
Heptanoic acid	17	3	14	9.000	*Y*

Table 2. McNemar chi-square test results for Odor Set 1: Trial 2.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**15**	**11.2**	**3.8**		
Butanoic acid	10	1	9	4.455	*Y*
Isobutyric acid	3	3	0	2.000	*N*
Isovaleric acid	4	2	2	0.000	*N*
Hexanoic acid	15	5	10	5.333	*Y*
Heptanoic acid	8	0	8	3.600	*N*

Table 3. McNemar chi-square test results for Odor Set 1: Trial 3.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**19**	**17.8**	**1.2**		
Butanoic acid	19	5	14	9.000	*Y*
Isobutyric acid	19	7	12	7.143	*Y*
Hexanoic acid	19	9	10	5.333	*Y*

Table 4. McNemar chi-square test results for Odor Set 2: Trial 1.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**16**	**15.75**	**0.25**		
Methyl valerate	16	13	3	0.200	*N*
Pentanal	16	9	7	2.778	*N*
Pentanol	16	1	15	9.941	*Y*
2-Pentanone	16	1	15	9.941	*Y*
3-Pentanone	16	5	11	6.231	*Y*

Table 5. McNemar chi-square test results for Odor Set 2: Trial 2.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**17**	**14.5**	**2.5**		
Methyl valerate	17	11	6	2.000	*N*
Pentanal	17	13	4	0.667	*N*
Pentanol	8	2	6	2.000	*N*
2-Pentanone	9	9	0	2.000	*N*
3-Pentanone	17	4	13	8.067	*Y*

Table 6. McNemar chi-square test results for Odor Set 2: Trial 3.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**15**	**13.76**	**1.24**		
Methyl valerate	15	15	0	2.000	*N*
Pentanal	15	14	1	0.333	*N*
3-Pentanone	15	11	4	0.667	*N*

Table 7. McNemar chi-square test results for Odor Set 3: Trial 1.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**32**	**30.5**	**1.5**		
pentanoic acid	22	19	3	0.200	*N*
w methyl benzoate	22	20	2	0.000	*N*
w methyl valerate	20	17	3	0.200	*N*
w pyrdine	32	25	7	2.778	*N*
w isovaleric acid	32	24	8	3.600	*N*
w 2-pentanone	32	28	4	0.667	*N*

Table 8. McNemar chi-square test results for Odor Set 3: Trial 2.

Target	*Total tests*	*Yes*	*No*	χ^2	*Sig diff?*
Validation odorant	**34**	**32.26**	**1.74**		
pentanoic acid	24	18	6	2.000	*N*
w methyl benzoate	10	8	2	0.000	*N*
w methyl valerate	10	10	0	2.000	*N*
w pyrdine	34	25	9	4.455	*Y*
w isovaleric acid	34	30	4	0.667	*N*
w 2-pentanone	34	32	2	0.000	*N*

APPENDIX E

Using the chi-square test of independence, canine responses to testing odorants for each group was compared to each other (Tables 37-39). The data was entered into 2 x 2 contingency tables (example provided in Table 5). The χ^2_{crit} for one degree of freedom at a 95% confidence level (χ^2_{crit} = 3.84) was compared to the resulting χ^2 value for each trial. The χ^2 value was determined using Equation 3. Additional chi-square of independence testing was performed for canine responses to testing odorants for each trial compared by group (Tables 40-42) and by Odor Sets 1 and 2 (Table 43). Finally, the same statistical analysis was used to compare results by canine experience (Tables 44-45) and for traditional versus non-traditional detector dog breeds (Table 46). A significant difference (*Sig diff?*) indicates that there is a difference between the canine responses for each tested parameter.

Table 1. Chi-square of independence test results for Odor Set 1 comparing the distribution of responses for Group A to Group B canines for all trials.

	χ^2_{crit}	χ^2	*Sig diff?*
Trial 1	3.84	0.09	*N*
Trial 2	3.84	0.86	*N*
Trial 3	3.84	1.15	*N*

Table 2. Chi-square of independence test results for Odor Set 2 comparing the distribution of responses for Group A to Group B canines for all trials.

	χ^2_{crit}	χ^2	*Sig diff?*
Trial 1	3.84	1.35	*N*
Trial 2	3.84	1.34	*N*
Trial 3	3.84	0.10	*N*

Table 3. Chi-square of independence test results for Odor Set 3 comparing the distribution of responses for Group 1, Group 2, and Group 3 canines for all trials.

	χ^2_{crit}	χ^2	*Sig diff?*
Trial 1	3.84	0.74	*N*
Trial 2	3.84	1.29	*N*

Table 4. Chi-square of independence test results for Odor Set 1 comparing the distribution of canine responses for Trials 1, 2, and 3 for each group.

	χ^2_{crit}	χ^2	*Sig diff?*
Group A	3.84	1.51	*N*
Group B	3.84	3.38	*N*

Table 5. Chi-square of independence test results for Odor Set 2 comparing the distribution of canine responses for Trials 1, 2, and 3 for each group.

	χ^2_{crit}	χ^2	*Sig diff?*
Group A	3.84	12.97	*Y*
Group B	3.84	20.15	*Y*

Table 6. Chi-square of independence test results for Odor Set 3 comparing the distribution of canine responses for Trials 1 and 2 for each group.

	χ^2_{crit}	χ^2	*Sig diff?*
Group 1	3.84	0.25	*N*
Group 2	3.84	1.38	*N*
Group 3	3.84	0.08	*N*

Table 7. Chi-square of independence test results comparing canine responses to testing odorants for Odor Set 1 to Odor Set 2.

	χ^2_{crit}	χ^2	*Sig diff?*
Testing odorants located	3.84	28.34	*Y*

Table 8. Chi-square of independence test results comparing canine experience for each odor set. Canines were considered novice with less than 2 years of experience, and expert with at least 2 years of experience.

	χ^2_{crit}	χ^2	*Sig diff?*
Odor Set 1	3.84	0.00	*N*
Odor Set 2	3.84	3.68	*N*
Odor Set 3	3.84	5.23	*Y*

Table 9. Chi-square of independence test results comparing canine experience for each odor set. Canines were considered novice with less than 3 years of experience, and expert with at least 3 years of experience.

	χ^2_{crit}	χ^2	*Sig diff?*
Odor Set 1	3.84	1.08	*N*
Odor Set 2	3.84	2.47	*N*
Odor Set 3	3.84	0.24	*N*

Table 10. Chi-square of independence test results for traditional detector dog breeds versus non-traditional detector dog breeds for each odor set.

	χ^2_{crit}	χ^2	*Sig diff?*
Odor Set 1	3.84	1.31	*N*
Odor Set 2	3.84	1.04	*N*
Odor Set 3	3.84	0.17	*N*