

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
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1. REPORT DATE (DD-MM-YYYY) 10-08-2023		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 21-May-2021 - 31-Aug-2022	
4. TITLE AND SUBTITLE Final Report: Environmental Impact on Explosives Detection Dogs and Mitigation Strategies - Research in Physical Sciences			5a. CONTRACT NUMBER W911NF-21-2-0124		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHORS			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Texas Technical University Box 41035 349 Admin Bldg Lubbock, TX 79409 -1035			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 78018-HC-H.1		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Paola Tiedemann
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 806-834-0983

RPPR Final Report

as of 10-Aug-2023

Agency Code: 21XD

Proposal Number: 78018HCH

Agreement Number: W911NF-21-2-0124

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DUNS Number: 041367053

EIN: 756002622

Report Date: 30-Sep-2022

Date Received: 10-Aug-2023

Final Report for Period Beginning 21-May-2021 and Ending 31-Aug-2022

Title: Environmental Impact on Explosives Detection Dogs and Mitigation Strategies - Research in Physical Sciences

Begin Performance Period: 21-May-2021

End Performance Period: 31-Aug-2022

Report Term: 0-Other

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 3

STEM Participants: 3

Major Goals: This report covers contract No. W911NF2120124 encompassing Tasks 1-5 reflecting work over May 2021 – August 2022. The overall objective of this project was to (1) robustly characterize canine limits of detections under varying environmental conditions covering a wide range of explosive classes (smokeless powder, Composition 4 (C-4), TNT, and the chemical precursor ammonium nitrate), (2) robustly perform a chemical characterization of odor behavior from a variety of explosive classes under varying environmental conditions via instrumental analysis approaches, (3) evaluate their interactive effect in a simulated operational environmental setting and (4) systematically train and acclimatize dogs to mitigate environmental effects on performance.

Accomplishments: A total of five tasks were accomplished, ranging from Task 1 acquisition and training of eight detection canines from Chiron K9. Task 2 monitored and measured canine physiological parameter and canine detection limits for four energetic sources (AN, C4, TNT and SP) while controlling for odor concentration at standard room conditions. Task 3 was geared on the chemical characterization of odor behavior utilizing SPME-GC/MS to monitor explosive signatures across targeted environmental parameters. The purpose of Task 4 was to characterize canine detection sensitivity under a concurrent odor interactive effect in a simulated operational environment. Lastly, Task 5 implemented an acclimatization plan to mitigate performance under extreme conditions.

Training Opportunities: Nothing to Report

Results Dissemination: Conference lecture: "Explosives detection in varying environmental conditions", invited speaker, 4th Annual Working Dog Symposium, Department of Defense, April 5-8, 2022. Virtual Platform. Paola Tiedemann, Nathaniel Hall

Conference lecture: "The effect of environmental conditions and acclimatization on canine detection of explosives" Virtual Platform Annual Working Dog Symposium, Department of Defense Authors: Sarah A. Kane, Lauren Fernandez, Dr. Dillon E. Huff, Dr. Paola A. Prada-Tiedemann, Dr. Nathaniel J. Hall

RPPR Final Report
as of 10-Aug-2023

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

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Funding Support:

RPPR Final Report

as of 10-Aug-2023

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Environmental impacts on explosives detection dogs and mitigation strategies

Contract No: W911NF2120124

Prepared September 30, 2022

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Table of Contents

Task 1: Acquire and train explosives detection dogs.....	9
Task 2: Characterizing Canine Detection Limits Under Extreme Environmental Conditions	10
Abstract	10
Background	11
Methods and Materials	14
Participants	14
Animal welfare considerations.....	14
Experimental set-up	15
Room set up	15
Odorants	16
Data collection Equipment.....	17
Physiological Measures.....	19
Environmental conditions	21
Canine odor detection training and testing	21
Experimental Design.....	23
Statistical analysis	23
Results & Discussion	24
Threshold	24
Subcutaneous Temperature	27
Perceived Respiratory Effort.....	29
Heart Rate.....	30
Latency	30
Conclusions	32
Task 3: Characterizing odor behavior in extreme environments	35

Abstract	35
Introduction	36
Vapor Technology for Canine Detection	36
Extreme Environmental Conditions	36
Materials and methods	38
Chemicals and explosive samples	38
Solid Phase Microextraction (SPME) and Instrumental Analysis of Explosives	42
Environmental Chamber	43
Statistical Analysis	49
Results	49
Target VOC Concentrations	49
Discussion	66
SPME fiber extraction efficiency	66
SPME fiber selection and optimization	66
Environmental Chamber	67
Composition 4	67
Smokeless powder	68
Ammonium nitrate powder	68
Ammonium nitrate prill	68
TNT	68
PETN	69
Limits and future challenges	69
Conclusion	69
Task 4: Characterizing canine detection sensitivity and odor interactive effects in a simulated operational environment	71
Abstract	71

Methods and Materials	72
Participants.....	72
Animal welfare considerations.....	72
Experimental Set up	72
Odorants	73
Data collection equipment.....	73
Physiological Measures.....	75
Environmental conditions	76
Canine odor detection testing and training.....	77
Data Analysis	78
Results and Discussion.....	78
Factors affecting detection limit.....	80
Canine subcutaneous temperature and detection limit.....	81
Heart rate and detection limit.....	82
Latency and Inter-Olfactometer-Interval	82
Discussion and Conclusions.....	85
Supplemental Task: Instrumental Validation of explosive odor delivery method: Olfactometer dilution evaluation.....	87
Abstract	87
Chemicals and explosive samples.....	88
Olfactometer Design	88
Solid-Phase Microextraction (SPME) Sampling Procedure	90
Target VOC External Calibration	92
Statistical Analysis.....	96
Discussion	102
Composition 4.....	103

TNT	104
Ammonium nitrate prill.....	105
Double-based smokeless powder	105
Limitations and future challenges	105
Conclusion	106
Task 5: Acclimatization/mitigation training under extreme environments.....	107
Abstract	107
Introduction	108
Methods and Materials	108
Participants	108
Animal welfare considerations.....	108
Odorants	108
Data collection equipment.....	108
Environmental conditions	108
Canine acclimatization training and testing	109
Canine testing.....	110
Results and Discussion.....	110
Detection limit changes and acclimatization plan.....	110
Conclusions	112
References	114

Project Summary

This report covers contract No. W911NF2120124 encompassing Tasks 1-5 reflecting work over May 2021 – August 2022. The overall objective of this project was to (1) robustly characterize canine limits of detections under varying environmental conditions covering a wide range of explosive classes (smokeless powder, Composition 4 (C-4), TNT, and the chemical precursor ammonium nitrate), (2) robustly perform a chemical characterization of odor behavior from a variety of explosive classes under varying environmental conditions via instrumental analysis approaches, (3) evaluate their interactive effect in a simulated operational environmental setting and (4) systematically train and acclimatize dogs to mitigate environmental effects on performance.

Task 1 targeted the acquisition and training of eight detection canines from Chiron K9. These canines were exclusively used for all canine behavioral assessments presented in this report.

In Task 2, the purpose was to monitor and measure canine physiological parameters and canine detection limits for four energetic sources (AN, C4, TNT and SP) while controlling for odor concentration at standard room conditions with an air dilution olfactometer. Targeted environmental conditions were hot conditions at both high and low humidity as well as cold conditions at both high and low humidity for a total of four environmental parameters. Canine physiological parameters evaluated included subcutaneous temperature, perceived respiration effort (PRE) and latency to search. Threshold assessments at the high temperatures at both high and low humidity, as well as low temperature and high humidity all led to decrements for two odorants. The low temperature conditions with low humidity only impacted smokeless powder, and overall had the smallest magnitude impact on performance. Subcutaneous temperature measurements were highest in the hot and humid conditions in comparison to all other conditions; suggesting that heat stress could pose a higher risk in these environmental conditions. Perceived respiratory effort (PRE) also increased most in high temperature and high humidity conditions, followed by high temperature low humidity and standard conditions. PRE was less impacted in the cold conditions. Latency to initialize a trial also increased in the high temperature conditions (low and high humidity) compared to standard and cold conditions. Furthermore, longer latency to initiate a trial was associated with poorer detection thresholds. This suggests that lags in the dogs working behavior could be an important early cue that detection sensitivity maybe poorer, or at least non optimal.

Task 3 was geared on the chemical characterization of odor behavior utilizing solid phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS) to monitor explosive odor signatures across targeted environmental parameters. Selected explosives for this study were ammonium nitrate (powder and prill), double base smokeless powder, composition 4 (C4), 2,4,6-trinitrotoluene (TNT),

and pentaerythritol tetranitrate (PETN). These five explosive classes were subjected to four extreme environmental conditions (Hot/Humid, Hot/Dry, Cold/Humid, and Cold/Dry) to monitor chemical odor profiles. The presence of target volatile organic compounds (VOCs) were tested in two extraction modes: 1) headspace extraction procedures inside environmental chamber while explosive was under targeted environmental parameters and 2) outside environmental chamber taking the explosive out of the environmental parameter into standard laboratory conditions. Smokeless powder, ammonium nitrate (powder and prill), composition 4, TNT, and PETN mean concentrations were affected at all conditions as measured by each target VOC concentration in the headspace. Explosive target odor volatiles extracted outside the chamber in ambient conditions resulted in decreased concentrations for explosives exposed in hot conditions and increased concentrations for explosives exposed in cold conditions. Conversely, explosives exposed and extracted inside the environmental chamber resulted in expected physiochemical properties with increased concentration in hot conditions and decreased concentrations in cold conditions. The study depicts SPME-GC/MS as a valid approach to evaluate odor signatures from an array of explosive classes, providing optimized SPME fiber chemistry as well as chromatographic methods for successful detection of key odor volatiles. This study corroborates the need for evaluation of explosive target odors to provide validation of odor quality and advance the knowledge of odor fate under distinctive environmental conditions that may be encountered in typical operational settings.

The purpose of Task 4 was to characterize canine detection sensitivity under a concurrent odor interactive effect in a simulated operational environment. This task utilized an alternative forced choice test design in which target odor was always present in one of three olfactometers. The four odorants tested included: C4, double based smokeless powder, ammonium nitrate (prill form, agricultural grade) and TNT. Each canine was tested for one threshold session at each corresponding environmental parameter for each target odorant. The threshold testing was conducted as an alternative forced choice task with a 3-down 1-up descending staircase procedure. The results from this task suggest that decrements in detection performance appear to be largely, or at least importantly, impacted by behavioral/physiological effects on the canine rather than changing odor availability. This is exemplified by performance decrements occurring in high temperature conditions, especially in high temperature and high humidity conditions where odor availability was at its greatest. These results suggest the potential that canine acclimatization and conditioning could have on mitigating potential performance decrements, and lead into Task 5.

Task 5 implemented an acclimatization plan to mitigate performance decrement under extreme conditions. Based on the results from Task 4, the target odorant used for this study was C-4 under a high temperature and high humidity condition, given the significant decrement observed with this odor under these conditions. In this acclimatization plan, the treatment group (n=4) were physically fatigued under slowly

escalating environmental conditions, followed by ~20 mins of odor detection training. The control group (n=4) was petted for five minutes followed by the same odor detection task under standard environmental conditions. Both groups were then challenged under high temperature and high humidity conditions following 10 and 20 days of acclimatization training. The treatment group had a lower detection limit than the control group across both testing days under high temperature and high humidity. Further, treatment dogs showed improvement in behavioral measurements, such as latency to search, but not in physiological measures such as subcutaneous temperature. Together, these results suggest that 10 days of acclimatization to environmental conditions can help improve detection performance.

Task 1: Acquire and train explosives detection dogs

Eight detection canines were acquired and trained by Chiron K9 for detection work (see Figure 1: Participants for all canine testing. Top left: Dalton, Bello, Rocket, Dasty. Bottom Left: Zulu, Moni, Jack, Luna). All dogs were previously acquired by a federal program but were determined to be unfit for the needs of the program. The dogs were therefore made available for this research program.



Figure 1: Participants for all canine testing. Top left: Dalton, Bello, Rocket, Dasty. Bottom Left: Zulu, Moni, Jack, Luna

Task 2: Characterizing Canine Detection Limits Under Extreme Environmental Conditions

Abstract

Detection canines are deployed to detect explosives in a wide range of environmental conditions. These environmental conditions may have negative impacts on canine capabilities as a sensor. This study leveraged an air dilution olfactometer to present controlled odor concentrations of four different energetic materials (double base smokeless powder, C4, ammonium nitrate prill, TNT) to dogs working in a range of high temperature, standard, and low temperature conditions with high and low humidity conditions. The air dilution olfactometer controlled concentrations independent of environmental condition and dogs' detection threshold limits were measured using a descending staircase procedure. We measured dogs' threshold twice for each energetic under each environmental condition. Results indicated heterogeneity in effects based on energetic, but all odors were detected at the lowest concentrations under standard conditions. Smokeless powder detection was reduced under all environmental conditions compared to standard and was least detectable under high temperature and humidity conditions. AN detection was poorest under high temperature both high and low humidity conditions. C4 in contrast, was least detectable at low temperatures with high humidity. TNT detection was difficult under all conditions, so decrements were difficult to detect. Additional measures were also found to be associated with detection limits. Under high temperature conditions, correlations were observed between mean subcutaneous temperature and detection limits, such that dogs experiencing greater temperature increases showed poorer detection limits. In addition, dog's latency to sample the odor port from the onset of a trial was longest in the high temperature conditions. Further, longer latencies were predictive of poorer detection limits. Overall, dogs showed deficits in detection sensitivity limits under all environmental conditions for at least one energetic material when the concentration of that energetic material was not directly impacted by the environmental conditions. These results suggests that behavioral factors related to environmental exposure can have important impacts on canine detection sensitivity and should be considered in operational environments.

Background

Dogs have a highly developed olfactory system giving them keen odor detection capabilities (Kathryn A. Bamford, n.d.; Marchal et al., 2016). Humans have successfully harnessed this ability in the dog for scent detection tasks, including the detection of diseases (Guest, 2013; Jendryny et al., 2020), pathogens (Gottwald et al., 2020), human scent (Greatbatch et al., 2015), cancer (Gordon et al., 2008; McCulloch et al., 2006; Moser & McCulloch, 2010), narcotics (Lorenzo et al., 2003; Newlon, 1974), and explosives (Frank Jr et al., 2022; Furton & Myers, 2001; Gazit et al., 2021; Lazarowski & Dorman, 2014). Military, law enforcement, and other government agencies heavily rely on the detection capabilities of working dogs.

For decades, explosives detection dogs (EDD) have been one of the main tools for the detection of explosives (Harper & Furton, 2007). The remarkable capabilities of these dogs, including their mobility and sensitivity, make it possible to detect various munitions used in acts of terrorism or war in a timely and efficient manner. Previous studies have successfully shown that dogs can be trained to detect a wide variety of explosive materials with excellent sensitivity and specificity (Johnston et al., 1998; Lazarowski et al., 2015; Waggoner et al., 2022).

However, it is important to acknowledge the limitations of dogs' capabilities to detect specific concentrations of relevant target odorants. While it was found that dogs can detect miniscule amounts of accelerants from 5.0 to 0.005 μL (Kurz et al., 1994; Tindall & Lothridge, 1995), there are limitations to dogs' odor sensitivity. In an operational context, odor availability may be limited due to low volatility of the target (i.e., vapor pressure), small quantities of the target, or concealment/air restriction. Thus, quantifying canine limits of detection is an important research task, to better understand the limits at which canines can successfully operate as a reliable detection tool. However, limited data has been collected to date on canine limits of detection for a range of energetic materials.

Additionally, detection capability may vary substantially based on odorant source. There can be a wide range of vapor pressures for different energetic materials, such as ammonium nitrate (a common oxidizer) at 25C is 5.0×10^{-6} Torr, TNT at a vapor pressure of 3.0×10^{-6} Torr (Harper et al., 2005), whereas DNT has a vapor pressure of 2.63×10^{-4} (Östmark et al., 2012). A greater difference in vapor pressure can be seen in RDX (a component of C4), with a reported vapor pressure of 4.85×10^{-12} atm (3.69×10^{-9} Torr) (Ewing et al., 2013). Such substantial variation of vapor pressure suggests there could be important variations in detection canine capabilities, although to date, this has yet to be demonstrated in the scientific literature. Table 1 shows reported vapor pressure values at 25C of commonly used explosive materials, but it is important to note, that operational explosives contain several other volatiles due to manufacturing contaminants and degradation products that are likely key for canine odor detection (MacCrehan et al., 2020).

Table 1: Known vapor pressure of commonly used explosive materials

Odor	Vapor pressure (atm) at 25C	Reference
AN	6.58e-9	(Harper et al., 2005)
TNT	3.95e-9	(Harper et al., 2005)
RDX	4.85e-12	(Ewing et al., 2013)
TNX	1.61e-10	(Cundall et al., 1981)
PETN	1.84e-11	(Furton & Myers, 2001)
NG	5.95e-07	(Dionne et al., 1986)
DNT	3.46e-7	(Östmark et al., 2012)

Importantly, canine odor sensitivity is not only affected by varying vapor pressure. When explosive detection canines are deployed in an operational environment there are a multitude of environmental conditions that physically affect the dog and odor source (Farr et al., 2021). Previous work has found that weather conditions, air temperature, wind currents, relative humidity, and vapor pressure can affect how scent is dispersed and therefore odor availability, directly impacting canine detection (Cablak & Heaton, 2006; Mendel, Burns, et al., 2018; Mendel, Furton, et al., 2018).

Working dogs may need to be deployed in environments that reach temperatures higher than 40°C and lower than 0°C. Extreme conditions such as these pose a threat of heat exhaustion or hypothermia, impeding the dog's ability to perform odor detection work. While the standard range of canine temperature core body temperature is between 37.2°C and 39.2°C (Baker & Miller, 2013), previous studies have demonstrated the while working, dogs can exceed 40.6°C (O'Brien et al., 2017; Robbins et al., 2017). Temperatures of this severity have been shown to increase fatigue and cause clinical signs of heat exhaustion (Hall et al., 2021; Stanley, 1980). Furthermore, past studies have indicated that working in higher temperatures increases respiratory rate, due to the physical activity and need for cooling in extreme environmental conditions (Lopedote et al., 2020).

A dog's primary source of cooling is evaporative cooling through panting (Goldberg et al., 1981). Panting is the act of rapid breathing to increase the evaporation of water from the respiratory tract (Crawford Jr, 1962). During this process, substantially more air passes through the mouth of the dog instead of passing along the olfactory tract. Further, prior work has shown there is a decrease in detection accuracy when the respiratory rate/panting increases, due to the body's natural response to heat stress (Gazit & Terkel, 2003a).

However, the detection capabilities of dogs are not only affected by extreme heat. Cold stress and hypothermia are additional potential concerns for dogs working in cold environments. It is known that hypothermia stage one occurs for dogs experiencing core temperatures of 35° C or lower (Brodeur et al., 2017). During stage one hypothermia dogs will remain conscious, however continuous shivering will occur with signs of lethargy. This could lead to dogs experiencing early fatigue during detection work, eventually leading to increased difficulty with the detection task.

In addition, extreme environmental conditions are not only based on temperature, but also the humidity of the environment can drastically manipulate the way a specific temperature causes stress to the body (Carter & Hall, 2018; Potter et al., 2020; Powell, 2008). Relative humidity is the ratio of the amount of moisture content in the air relative to the amount that is possible at a given temperature (Torrecilla et al., 2008). When the relative humidity of an environment is high, the evaporation process necessary for thermoregulation is impeded, causing high temperatures to have greater stress effects. Previous studies have found greater heat stress affects in dogs working in extreme hot and humid environmental conditions (Horowitz & Nadel, 1984; Otto et al., 2017; Taylor et al., 2008). In addition, because humidity leaves water molecules against the skin, which could cause the low temperature environment to feel colder. This could potentially cause scent detection dogs to display hypothermic signs at a rapid rate while working in the field.

Given the range of prior studies highlighting correlational and some experimental effects of environmental conditions on detection dog performance, the goal for Task 2

of this project was to create a systematic evaluation of temperature and humidity effects on canine detection for a series of energetic materials. We manipulated temperature (40°C vs 0°C) and high and low humidity conditions (90/70% vs 40/50%) and measured canine physiological parameters and canine detection limits for four energetic sources while controlling for odor concentration with an air dilution olfactometer.

Methods and Materials

Participants

Participants of this study included eight altered adult working dogs of various breeds (Table 2).

All dogs were previously eliminated from working dog programs and had previous training and/or odor work experience. Detailed descriptions of each participant can be found in Table 2. Dogs received a minimum of two walks a day and obedience training to maintain appropriate enrichment and exercise during the duration of the study. The primary reinforcement for testing and training was dog treats and dog food. Therefore, one-quarter of the total food ration was fed to the dogs in the morning, and the remaining 75% was given as a second meal at the end of the day. Dogs received additional food throughout the day during walks and training. Lastly, all dogs had free access to water at all times.

Animal welfare considerations

All procedures utilized in the following experiments were approved by the Texas Tech University Institutional Animal Care and Use Committee (Protocol # 21051-07). While at the Canine Olfaction Lab, participants were housed in indoor kennels (2.43 x 1.22 m) located in a climate-controlled room with free access to either an outdoor kennel (2.43x 1.22 m) or a connected indoor kennel (2.43 x 1.22 m).

During testing in environmental conditions, participants were monitored to avoid hypothermia and hyperthermia. If the participant showed signs of distress, such as uncontrollable panting, excessive shivering, refusal to search for five consecutive trials, and laying down, then the dog was immediately removed from the chamber and the session was terminated. Furthermore, dogs were tested for a maximum of 40 minutes, after which dogs were removed from the chamber, and sessions were terminated.

Table 2: Participant descriptions

Dog	Approximate Age (Years)	Breed	Approximate weight (Kg)	Reproductive Status
Zulu	4	German Shorthaired pointer	22.68	Altered male
Luna	2	German Shorthaired pointer	27.67	Altered female
Moni	3	German Shorthaired pointer	24.49	Altered female
Dasty	3	Labrador retriever	27.21	Altered male
Jack	2	Labrador retriever	29.48	Altered male
Bello	3	German Shorthaired pointer	25.63	Altered male
Dalton	2	Labrador retriever	34.02	Altered male
Rocket	2	Labrador retriever	27.21	Altered male

Experimental set-up

Room set up

An environmental chamber located at the Canine Olfaction Lab measuring 3m x 3m was the primary testing and training environment during this study (see Figure 2). The chamber had built in HVAC and supplemental heater units to allow the room to reach temperatures below 0C and above 43C. Supplemental equipment, such as dehumidifiers and humidifiers, were placed in the room to control relative humidity conditions.

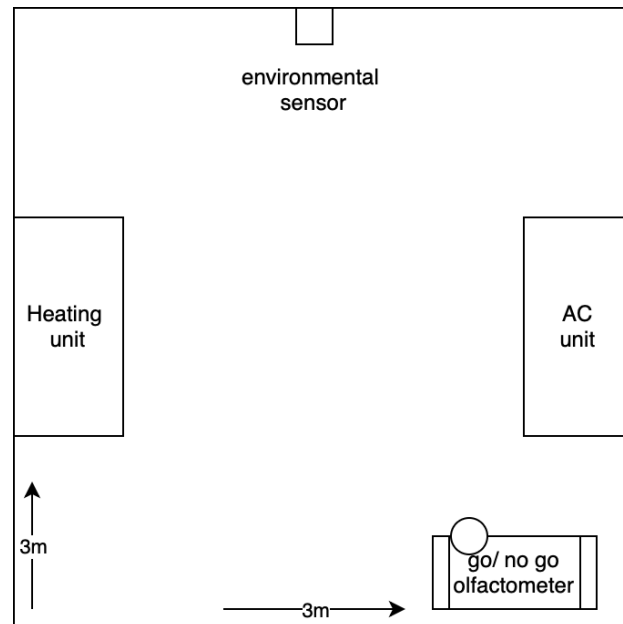


Figure 2: Schematic diagram of environmental chamber

Odorants

Four different energetic materials were used in this experiment for testing and training. The following odorants used in this experiment were double-base smokeless powder (DB SP), ammonium nitrate (AN), trinitrotoluene (TNT) and C4. Each of the odorants were placed into a heated water bath during training and testing to standardize odor concentration. Temperature of the water bath was dependent on the odor being used and was increased for less volatile odors to improve detection. All odors underwent systematic air dilutions during testing for baseline and experimental environmental conditions. Due to the variation of volatility of each material, flow rate used for dilution was dependent on odor (Table 3).

Samples of AN and TNT were placed in larger vials to increase the surface area of the odorant (see Table 3). Lastly, smokeless powder underwent dilution steps of 0.01, 0.00316, 0.001, 0.000316, and 0.0001. The remaining odorants underwent dilution steps of 1.0, 0.316, 0.1, 0.0316, and 0.01, due to dogs failing to reach qualification

criterion (>85% accuracy) at a dilution factor of 1%. Refer to Table 3 for detailed description of each odorant used in this experiment.

Table 3: Description of odorants

Odor	Mass (g)	Flow rate (L/min)	Temperature of water bath (°C)	Brand of energetic material	Vial size Diameter (cm)
Double-base smokeless powder	10g	10 L/min	30 °C	Hodgdon H335	2.54 cm
Ammonium nitrate	20g	3 L/min	40°C	Omni explosives	5.08 cm
Trinitrotoluene	10.5g	3 L/min	40°C	Omni explosives	5.08 cm
C4	8g	3 L/min	40°C	Omni explosives	2.54 cm

Data collection Equipment

Air Dilution Olfactometer

Participants were trained and tested using an automated go/ no-go air dilution olfactometer system (Figure 3). The system utilizes Alicat (Tucson, AZ, USA) gas mass airflow controllers to create a serial air dilution of the odorant being tested. A glass vial is placed in a water bath set at the desired temperature (30 – 40°C) for each specific odor being tested. The glass vial is attached to two Teflon (PTFE) tubes to allow for clean air introduction and displacement of odorant headspace into the system to begin a serial dilution. The odorant/ air mixture then travels through the mass airflow controllers and static mixers to create the desired air dilution. Lastly, the diluted air travels to the port to allow participants to sniff the odor. The total airflow delivered to the dog was dependent on the odor material and ranged from 3 L/min to 10L/min but was always consistent for all dilutions for the same odor. Refer to Figure 3 for a detailed diagram of the function and air flow of the apparatus.

The olfactometer was designed for go/no go testing and therefore delivered either clean air, or air containing volatiles of the target odorants to a stainless-steel odor port. The odor port was fitted with an exhaust fan, which exhausted odorant from the odor port during inter-trial intervals. The odor port also contained an infrared beam pair which detected canine nose entries into the port. Canine responses were measured by the infrared beam pair, in which dogs were trained to hold their nose in the port for 4 continuous seconds as a go response. A no go response was scored by the dog sampling the odor port (inserting their nose) followed by the removal of their nose for 4 consecutive seconds. Tones were used by the computer to indicate if a response was correct or incorrect to a handler that was blind to the presence/absence of the odor.

The correct tone was played when a participant would respond with a four second nose hold in the port when the port contained the diluted target (hit), or if the dog responded by inserting their nose into the port and disengaging from the port when the port contained clear air (correct rejection). Furthermore, an incorrect tone was played when participants would incorrectly hold their nose for four seconds in the port to a nontarget trial (false alert) or disengage from the port during a target trial (miss). A "Timeout" was recorded if the dog did not sample the odor port within 45s of a trial initiation tone and being prompted to search by the handler. Intertrial intervals were 30 seconds. For the last 10 seconds of the intertrial interval the olfactometer was triggered to present the odor of the next trial to ensure a stable concentration was presented prior to the dog sampling.

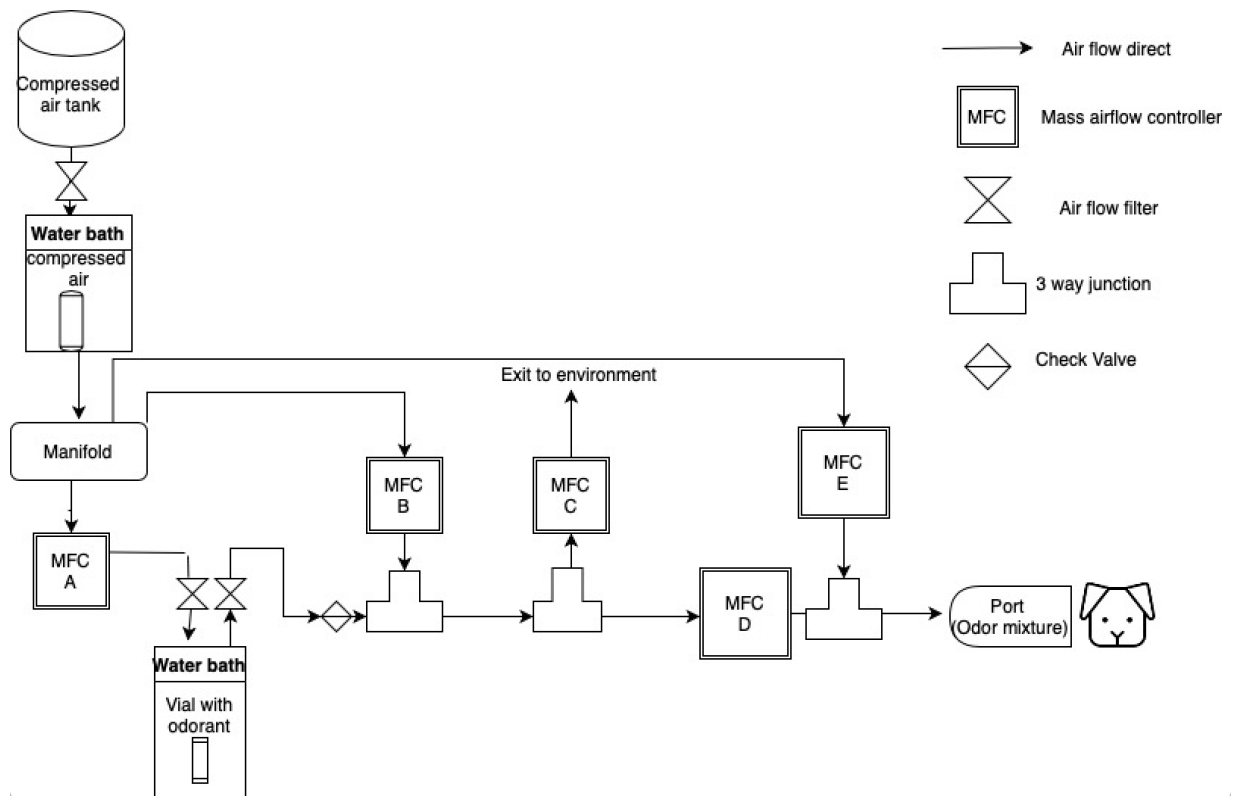


Figure 3: Diagram of mass air flow dilution olfactometer. The path of air flow is shown from left to right. Clean air travels through a warm water bath heated to replicate the same temperature of the odorant water bath. The clean air then travels to the manifold and is dispersed to the MFC A, MFC B and MFC E. Air from MFC A is pushed into the vial containing an odorant located in a separate water bath to collect the odorant from the vial and then pushed out of the vial to a three-way junction connected to MFC B. The air from the vial is mixed with clean air from the manifold the travels to the remaining mass air flow controllers. The odorant is systematically diluted by the mass air flow controllers and then pushed down the odor line to be delivered to the port.

Physiological Measures

Heart Rate.

Participants wore a Polar H10 heart rate monitor during each test session (see Figure 4). Mean 5 min heart rates were calculated for every 5 min of the testing session.

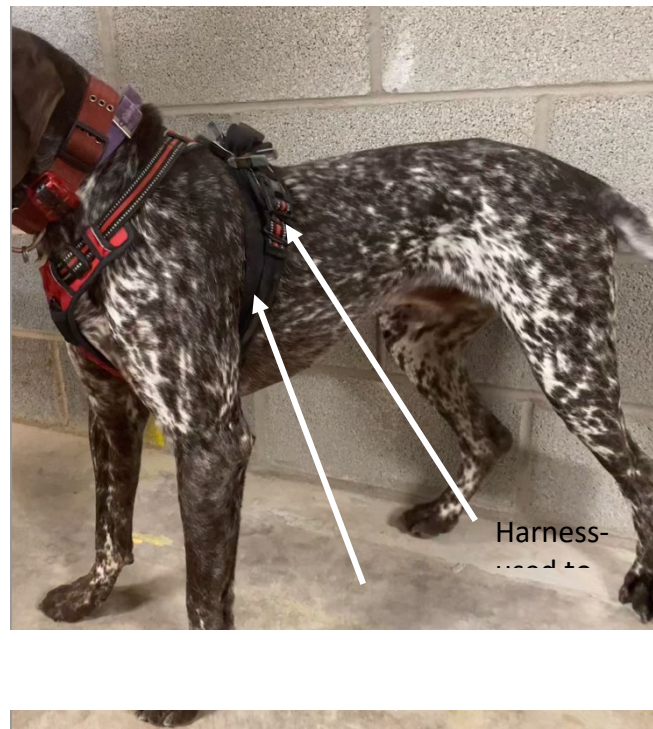


Figure 4: Canine with the Polar® Heart Rate monitor was clipped with alligator clips to keep it tight to the dog's body during testing

Subcutaneous Temperature.

Temperature of each participant was taken before entering the chamber and every five minutes during testing. Temperature was determined by scanning a HomeAgain TempScan 134.2 kHz ISO microchip (Hoskinson et al., 2014) located subcutaneously in the shoulder of the dog. The temperature was recorded by the handler.

Perceived respiration effort (PRE).

Videos were coded using BORIS (Friard & Gamba, 2016). Each video was coded at five-minute intervals (0,5,10,15,20, ect.) for the first five seconds of each interval. The first five second clip started at the start of the first trial. Intervals began after the first five second clip. However, for the video to meet criteria for coding the dog must be in sight of the camera with 75% of the body showing with the dog's nose outside of the odor port and the mouth must be visible. If a video clip did not meet criteria, the video was scanned for the nearest five second clip that met criteria from the specific interval. A PRE scale rating system described by Dechant et al. (in prep) was used to determine the respiration effort of each participant during the five second clip for each five-minute interval. Scoring for this scale ranges from 0 to 10. Zero being defined as

resting and breathing is an involuntary process, and ten being very heavy breathing and the chest and abdomen are expanding and collapsing violently. For inter-observer agreement, two coders scored the same 20% of the videos for PRE. Inter-class correlations were used to assess agreement.

Environmental conditions

Canine's detection sensitivity was tested twice (2x) for each odorant (4 odorants) in four different extreme environmental conditions (4x) and a standard condition. Details of the environmental conditions can be found in Table 4. Conditions were selected to match possible extremes encountered in an operational setting. A sensor was placed in the room that monitored temperature and humidity conditions throughout the testing session to ensure the environmental conditions remained within limits of variation.

Table 4: Environmental conditions of threshold assessment

Condition	Temperature °C (± 5 degrees)	Relative humidity ($\pm 5\%$)
Baseline	21 °C	50%
High temperature/ high humidity	40 °C	70%
High temperature/ low humidity	40°C	40%
Low temperature/ high humidity	0°C	90%
Low temperature/ low humidity	0°C	50%

Canine odor detection training and testing

Preliminary training

Dogs were previously trained to three explosive compounds using an automated 3-alternative line-up olfactometer. Previous research provides a detailed description of

the automated odor delivery system used for initial training (Aviles-Rosa et al., 2021). This odor system consists of three separate olfactometers equipped with six airflow valves and Teflon tubing to allow odor to flow to an opening or port for participants to sniff the odorant. Dogs were trained to detect double-base smokeless powder, AN, and C4.

Upon arrival at the Canine Olfaction lab dogs were trained to a go/no-go air dilution system, consisting of one port presenting either clean air or the odor dilution of the specific odor selected for training. Each dog received two sessions of 20 trials with each odor of the four odors (double-base smokeless powder, ammonium nitrate, trinitrotoluene, and C4) or the necessary number of sessions needed to achieve 85% accuracy or higher for two consecutive double-blinded sessions.

Canine odor threshold testing

Once participants reached criteria, all dogs were tested on a go/no go air dilution system with a descending staircase, three-down one-up procedure (DeChant & Hall, 2021; Leek, 2001). Following the descending staircase method, if the dog made three correct consecutive responses, the concentration of the odor was automatically lowered by a half-log step (see Figure 5 for an example). The lowering of concentration would continue until the dog made an incorrect response. If incorrect, the concentration was raised to the previous dilution step. Concentration was lowered or raised over the session until dogs showed 8 reversals in the direction of change of concentration. The session continued until dogs either reached 8 reversals, 40 min of training, or met a welfare criterion for discontinuing (described in *Animal Welfare Conditions*).

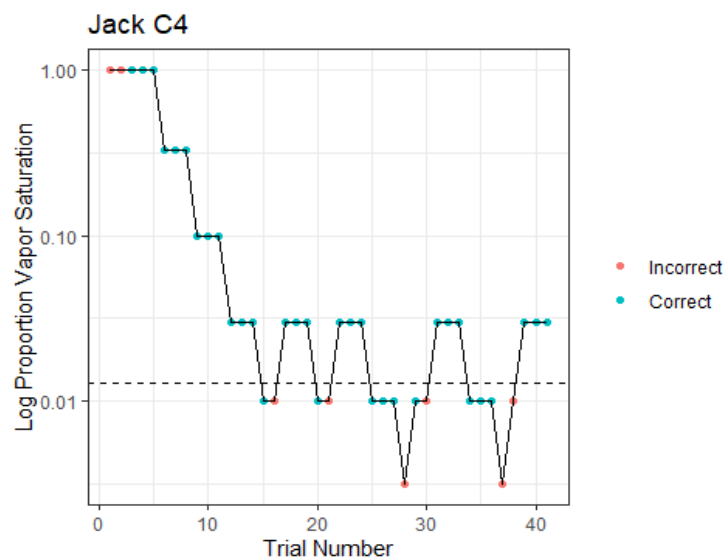


Figure 5: The dog Jack following the 3-down 1-up adaptive threshold procedure for C-4 in standard conditions. After three correct responses, the concentration was decreased by a half-log. If an incorrect response was recorded, the concentration was increased by a half-log. Testing continued until completing eight reversals or sixty trials. Threshold was calculated as the geometric mean of the last six reversal points.

Experimental Design

Dogs completed all testing for one odorant before proceeding to the next in the following order (SP, C4, AN, TNT). For each odor, dogs completed initial go/no go qualification training. After meeting qualification, dogs completed two threshold assessments at standard conditions. Dogs were then pseudo-randomly assigned such that half of the dogs started in the hot conditions (hot to cold) and half in the cold conditions (cold to hot). Dogs completed two threshold sessions per environmental condition before advancing to the next environmental condition. Dogs alternated between starting in hot or cold conditions when advancing from one target odorant to the next.

Statistical analysis

Threshold was calculated as the geometric mean of the last 6 (of 8) reversal points. If a dog did not complete 8 reversals due to early termination (due to welfare criterion, exceeding the session time, or failing to achieve three consecutive responses to decrease in concentration) the starting concentration was imputed as the missed reversal points to reflect poorer performance.

To evaluate the impact of environmental condition a mixed effect model was fit in which log transformed threshold was predicted by the odor, condition, and their interaction. A random intercept was fit for each dog. Fixed effects were evaluated with Anova from the car package (Fox & Weisberg, 2018a). Post hoc tests were conducted in which each environmental condition was compared to standard conditions.

To evaluate the impact of cutaneous temperature, heart rate, perceived respiratory effort, and latency, individual regression models tested the impact of each predictor variable on threshold detection. Each model included an interaction with odor to allow for effects to vary by odor. If an interaction was significant, regression models were fit for each target odor separately.

Results & Discussion

Threshold

Average threshold across all eight dogs for each odor averaged across all conditions is shown in Figure 6, which highlights large differences in sensitivity limits for the different energetics. Dogs showed highest sensitivity for SP followed by C-4, AN and TNT. A linear mixed effect model showed a main effect of odor type on threshold detection limits ($X^2=2,512$, $df=3$, $p<0.001$). Post hoc tests indicate there was a significant difference in sensitivity between all energetics except for between AN and TNT ($t=0.83$, $p=0.84$).

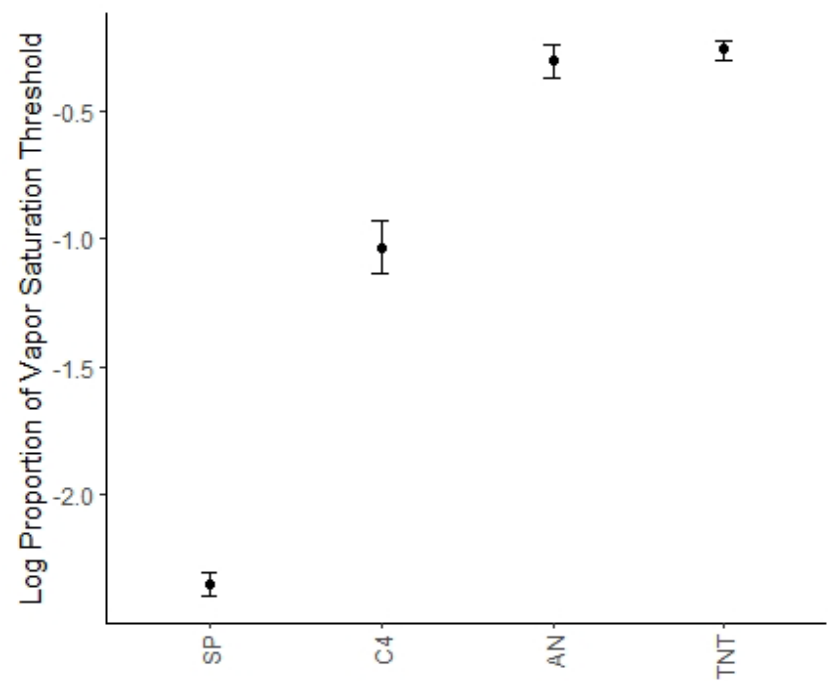


Figure 6: Average threshold of each odor. Error bars show 95% bootstrap estimated confidence intervals.

Table 5: Detection limit (log of vapor saturation) for each odor in each condition and 95% confidence intervals.

Odor		Standard	High Temp High Humid	High Temp Low Humid	Low Temp High Humid	Low Temp Low Humid
AN	Mean	-0.4511	-0.2321	-0.0907	-0.2686	-0.4636
	95% CI	-0.612, - 0.2897	-0.393, - 0.0707	-0.252, 0.0707	-0.430, -0.1072	-0.625, - 0.3023
C-4	Mean	-1.1848	-1.2181	-0.9844	-0.8170	-0.9716
	95% CI	-1.346, - 1.0235	-1.380, -1.0568	-1.146, - 0.8230	-0.978, - 0.6556	-1.133, - 0.8102
SP	Mean	-2.5939	-2.1563	-2.3230	-2.2918	-2.3751
	95% CI	-2.755, - 2.4325	-2.318, - 1.9950	-2.484, - 2.1617	-2.453, -2.1304	-2.536, - 2.2137
TNT	Mean	-0.2515	-0.2126	-0.2114	-0.2786	-0.3524
	95% CI	-0.413, - 0.0902	-0.374, - 0.0512	-0.373, - 0.0501	-0.440, - 0.1172	-0.514, - 0.1911

Figure 6 and Table 5 show the detection limits for each target odor under each environmental condition and standard conditions. A mixed effect model indicated that threshold was unrelated to breed ($X^2=0.11$, $df=1$, $p<0.74$). However, there was a significant odor by condition interaction ($X^2=38.45$, $df=12$, $p<0.001$) as well as main effects of odor ($X^2=2,922$, $df=3$, $p<0.001$) and condition ($X^2=28.02$, $df=4$, $p<0.001$).

To evaluate the odor by condition interaction, post hoc tests were conducted between conditions for each odor. All environmental conditions were compared to the standard condition as the control (see Table 5). All environmental conditions led to a decrement

in detection limits in conditions for at least one odorant. Importantly, performance was never better in an environmental condition in comparison to standard. High Temp High Humidity, High Temp Low Humidity, and Low Temp High Humidity all led to decrements for two odorants. Low Temp Low humidity only impacted smokeless powder, and overall had the smallest magnitude impact on performance.

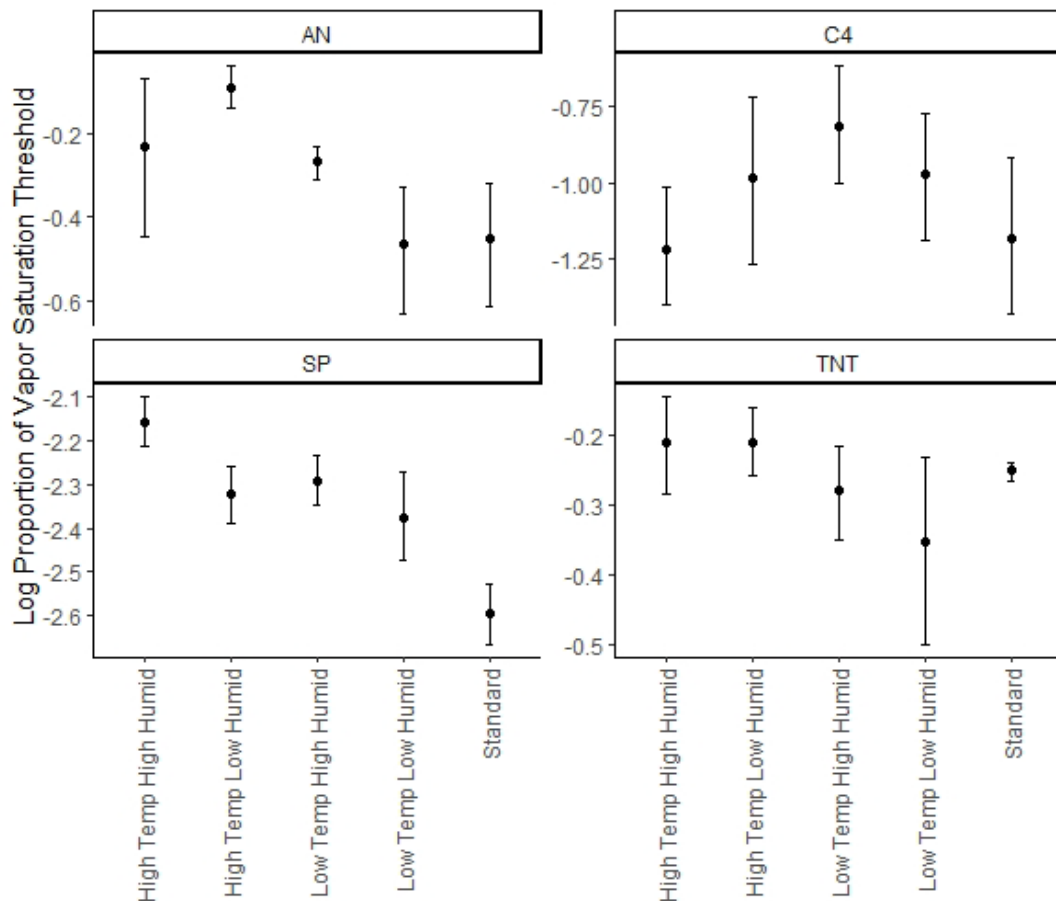


Figure 7: Threshold (Log proportion of vapor saturation) for each environmental condition and odor. Error bars show bootstrap estimated 95% confidence intervals.

Table 6: Threshold difference for each environment condition compared to standard condition.

Condition s	High Temp High Humid	High Temp Low Humid	Low Temp High Humid	Low Temp Low Humid

Odor:	t-ratio	p	t-ratio	p	t-ratio	p	t-ratio	p
AN	2.21	0.05	3.64	<0.001	1.84	0.09	-0.12	0.90
C-4	-0.03	0.73	2.02	0.06	3.71	<0.01	2.15	0.06
SP	4.42	<0.01	2.73	0.02	3.05	<0.01	2.21	0.03
TNT	0.39	0.78	0.40	0.78	-0.27	0.78	-1.02	0.78

Subcutaneous Temperature

Figure 8 shows the subcutaneous temperature across the first 20 min of the session. In the hot and standard conditions, temperature increased as dogs completed the threshold test. During the cold conditions, temperature decreased across sessions to below biologically plausible values, indicating the cutaneous sensor was susceptible to the room temperature. Therefore, analyses were limited to standard and high temperature conditions.

First, a linear mixed effect model analysis of differences in mean cutaneous temperatures between the three conditions indicate there was a significant difference in mean temp between conditions ($\chi^2=242$, $df=2$, $p<0.001$). Post hoc tests indicate that cutaneous temperature was highest in High Temperature High Humidity in comparison to High Temperature Low Humidity ($p<0.001$) and Standard conditions ($p<0.001$), indicating an important impact of humidity. Further, High Temperature Low Humidity also led to higher cutaneous temperatures compared to standard ($p<0.001$).

To evaluate the impact of mean subcutaneous temperature on threshold, a linear mixed effect model was fit in which threshold was predicted by odor, mean subcutaneous temperature and their interaction. There was a significant interaction between odor and mean temperature ($\chi^2=8.64$, $df=3$, $p=0.03$). To evaluate this interaction, a regression model was fit for each odorant. There was a significant relationship between mean cutaneous temperature and threshold from SP ($\chi^2=58.12$, $df=1$, $p<0.001$) and AN ($\chi^2=7.16$, $df=1$, $p=0.007$), but not for TNT or C4 ($p>0.05$; see Figure 9).

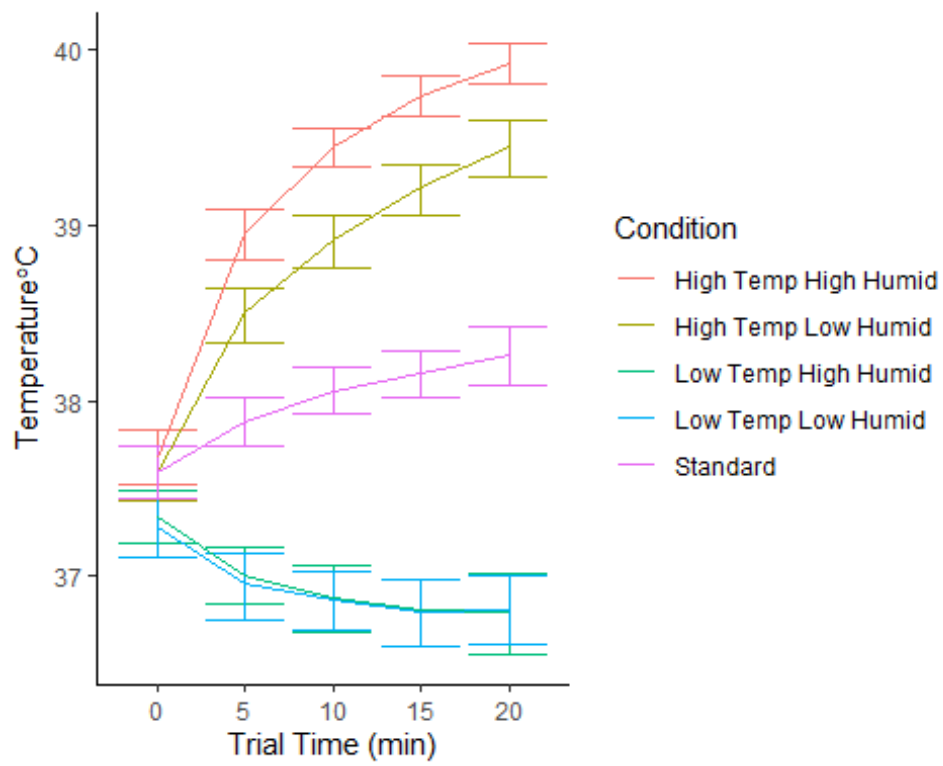


Figure 8: Implanted skin temperature during testing. Error bars show the 95% confidence interval.

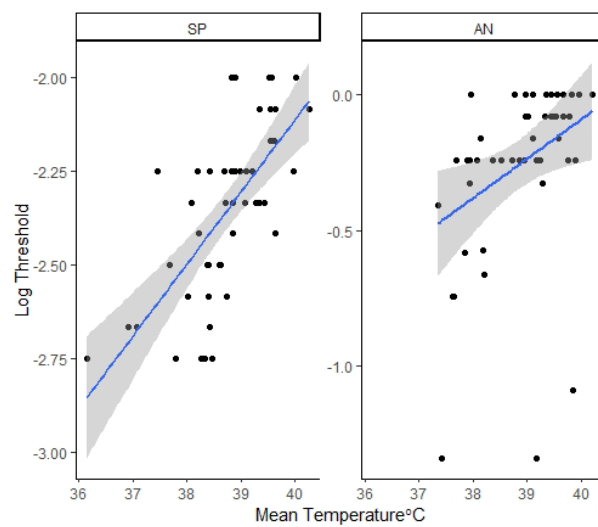


Figure 9: Threshold relation to mean subcutaneous temperature. Line shows the best fit regression.

Perceived Respiratory Effort

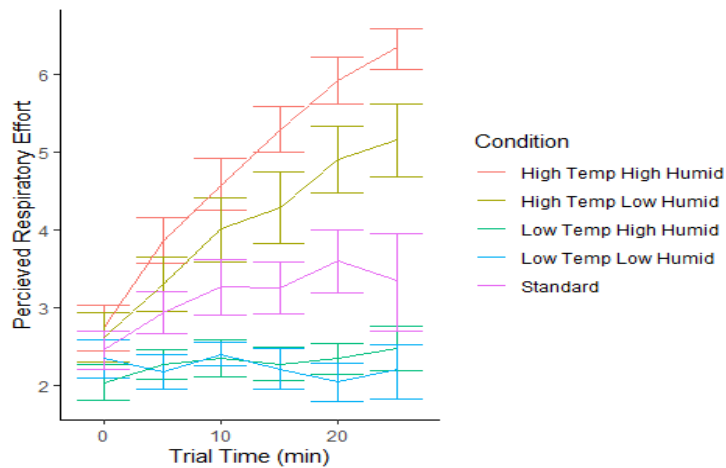


Figure 10: Perceived Respiratory Effort ratings during the threshold assessments. Error bars show the boot strap estimated 95% confidence intervals.

Dogs showed increased PRE score across the session during standard and hot conditions but was stable in the low temperature conditions (see Figure 10). Linear mixed effect model shows that PRE did differ between the environmental conditions ($\chi^2=948$, $df=4$, $p<0.001$). Post hoc tests indicate that there was a significant difference in PRE score between all conditions except for the two low temperature conditions ($t=0.38$, $p=0.99$).

To evaluate whether PRE was related to detection threshold, the log transformed threshold was predicted by odor, PRE score and their interaction. There was a significant interaction ($\chi^2=17.45$, $df=3$, $p<0.001$) between PRE and odor on threshold. Conducting separate analysis for each odor indicates that increased PRE led to higher threshold for AN ($\chi^2=4.71$, $df=1$, $p<0.03$), but for lower threshold for C4 ($\chi^2=7.28$, $df=1$, $p<0.01$; see Figure 11).

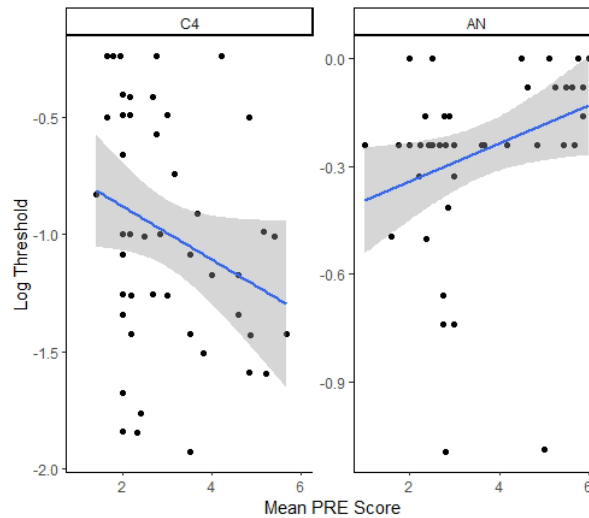


Figure 11: Relationship between PRE and Threshold.

Heart Rate

Continuous heart rate was recorded from the Polar H10 during the threshold condition. However, the data contained substantial movement associated artifacts during the session and data from several sessions were lost due to a dropped connection between the sensor and data recorder. Due to the high level of artifact and missing data, heart rate analysis was not performed.

Latency

Latency was scored as the time between the olfactometer initializing the trial with the start tone and the first nose poke into the odor port. Latency to initialize a trial did vary by environmental condition ($\chi^2=30.1$, $df=4$, $p<0.001$). Figure 12 and post-hoc tests shows the latency was highest in the two high temperature conditions but was similar in the low temperature and standard conditions.

Mean latency to initiate trials in a session was further related to threshold measured. A mixed effect model was fit in which threshold was predicted by the mean session latency, odor and their interaction. There was no significant interaction ($\chi^2=1.85$, $df=3$, $p=0.60$), but there was a main effect of odor ($\chi^2=2.762$, $df=3$, $p<0.001$) and latency ($\chi^2=14.26$, $df=1$, $p<0.001$). Figure 13 shows there was a positive relationship such that increases in latency (or delay in initiating a trial) was associated with higher (or poorer) thresholds. Thus, when dogs more quickly approached the odor port when indicated to do so, detection sensitivity was higher.

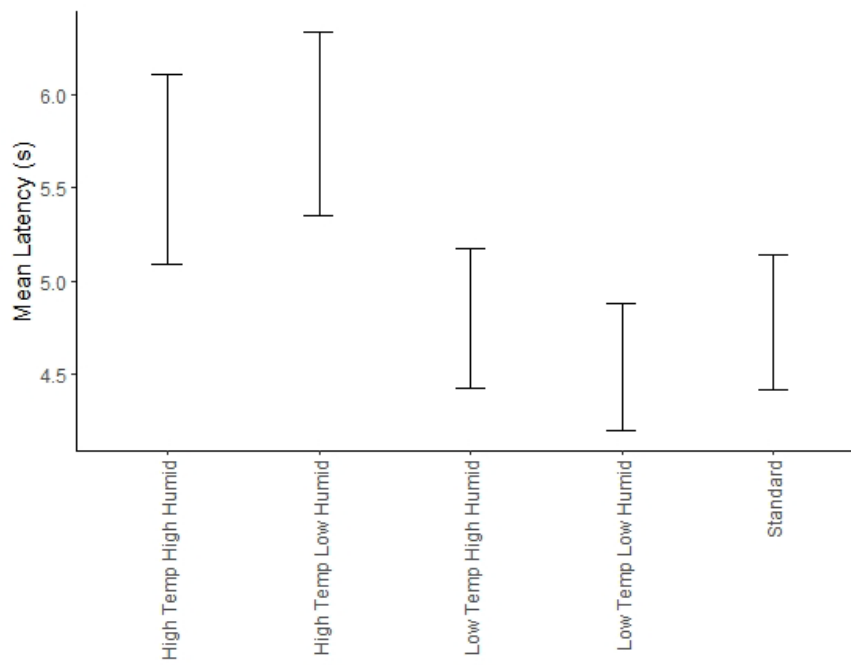


Figure 12: Latency to sample odor port by environmental condition. Error bars show the 95% bootstrap estimated confidence intervals.

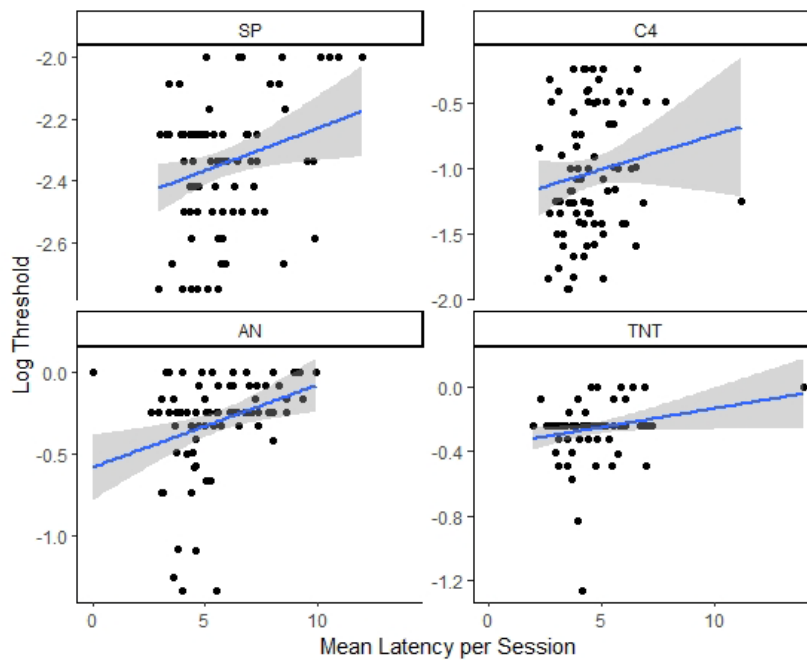


Figure 13: Relationship between mean latency to initiate a trial in a session with overall threshold.

Conclusions

Detection thresholds/sensitivity limits were one of the lowest/optimal for canines for each odor at standard temperature and humidity conditions (22 °C and 50% RH). Canines did not perform statistically better in any extreme environmental condition in comparison to standard conditions. Decrements in detection limits, however, were observed for at least one target odor for each of the extreme environmental conditions. Heat (high and low humidity) and high humid cold conditions had decrements for more target odors than the cold dry conditions. A decrement was only observed for Low temperature and low humidity conditions with smokeless powder, and this decrement was the smallest decrement in comparison to the other conditions, suggesting that although an effect maybe observed, this was the least operationally significant effect.

Interestingly, no effects between conditions were observed for TNT. This, however, may reflect a restriction of range to detect effects, because dogs' threshold was near the highest odor concentration that could be presented by the air dilution olfactometer, even under standard conditions. Thus, there was a restriction in the decrement that could be observed, because canines measured limits were already at or near the highest concentrations that could be presented by the air dilution olfactometer. Another unexpected finding of note is that dogs' performed unexpectedly well in the

high temperature and high humidity condition for C-4. This appears to be driven largely by two dogs that started in the cold conditions and worked up to the hot conditions, whereas dogs that started in the hot conditions showed more of the decrement expected. Due to the low sample size, formal analysis of these effects is not possible, but does suggest that good performance of dogs in this condition may in part reflect learning/training/acclimatization effects with the odor. This suggests further studies in which the order of conditions is randomized, or acclimation effects are specifically studied, are warranted.

There were also interesting relationships in the measured physiological data and detection sensitivity. Subcutaneous temperature measurements were highest in the hot and humid conditions in comparison to all other conditions; this indicates heat stress is at greatest risk in these environmental conditions. Further, an analysis of the dogs' mean temperature measured during the testing session was related to detection threshold for SP and AN, indicating that detection performance declined with greater increases in temperature. For example, for SP, a 1 °C increase in subcutaneous temperature was associated with a 0.19 change in log threshold, reflecting a potentially important impact of canine subcutaneous temperature on performance.

Perceived respiratory effort (PRE) also increased most in high temperature and high humidity conditions, followed by high temperature low humidity and standard conditions. PRE was little impacted in the cold conditions. There is also some conflicting evidence of the effect of PRE on threshold, such that overall, increases in perceived respiratory effort did lead to poorer detection limits for AN, which is to be expected based on prior work showing that respiratory distress can lead to poorer detection (Gazit & Terkel, 2003b). Notably, however, there was an opposite effect for C4. To better understand this discrepancy, further data is necessary. However, the negative relationship with C4 may be related to the unexpectedly good performance of a subset of dogs in the high temp high humid condition that ended with that condition (as noted above). Thus, additional analysis is necessary to confirm this trend, but does suggest additional data collection fully randomizing condition order with a larger sample would be a useful addition.

Latency to initialize a trial also increased in both high temperature conditions compared to standard and cold conditions. Furthermore, longer latency to initiate a trial was associated with poorer detection thresholds. This suggests that lags in the dogs working behavior could be an important early cue that detection sensitivity maybe poorer, or at least non optimal.

There are important considerations for the present study. First, odor concentration was maintained and controlled across environmental conditions with an air dilution olfactometer. In operational searches, the odor source itself will be exposed to the

extreme conditions and vary odor availability. This may change the effect observed such that although canine detection limits may change in high temperature and high humidity conditions, changes in odor availability may compensate minimizing overall performance decrements. Similarly, in cold conditions, even if canine detection capability remains similar, lower odor availability may change performance. Thus, additional evaluation in which odor source is allowed to change with environmental conditions is an important next step (Task 4).

Further, direct assessment of how odor availability changes for these energetic materials in varying environmental conditions would be an important next step (Task 3). This will help elucidate how odor availability changes in these conditions which adds an additional layer to predicting and understanding canine (or even electronic) detection of explosives in varying environmental conditions.

Overall, the results do suggest that subcutaneous temperature, search initiation (latency), respiratory effort and environmental conditions can be important predictors of changes in canine detection threshold (sensitivity) limits. Thus, canine handlers should be aware and attuned to these parameters to be able to adjust canine search tasks to limit potential search at reduced detection sensitivity.

Task 3: Characterizing odor behavior in extreme environments

Abstract

Biological detection systems, such as Explosive Detection Dogs (EDDs) are the most robust in-field detection system for explosives detection. EDDs have superior maneuverability, sensitivity, and environmental tolerance compared to electronic sensors. Due to increased capabilities in field readiness, EDDs work in inhospitable and variable extreme environments. Complexity of these environmental conditions can result in operational performance decrements by either changing target odor characteristics (chemical/physical properties) or impacting the sensor system itself. Analytical instrumentation to evaluate and characterize odor behavior in extreme environments from a wide range of explosive classes has not been studied to date. Knowledge of odor fate and availability are thus unknown across varying climatic parameters. Robust chemical characterization of odor behavior and instrumental optimization was conducted utilizing solid phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS) to monitor explosive odor signatures across targeted environmental parameters. Selected explosives for this study were ammonium nitrate (powder and prill), double base smokeless powder, composition 4 (C4), 2,4,6-trinitrotoluene (TNT), and pentaerythritol tetranitrate (PETN). These five explosive classes were subjected to four extreme environmental conditions (Hot/Humid, Hot/Dry, Cold/Humid, and Cold/Dry) to monitor chemical odor profiles. The presence of target volatile organic compounds (VOCs) was tested in two extraction modes: 1) headspace extraction procedures inside environmental chamber while explosive was under targeted environmental parameters and 2) outside environmental chamber taking the explosive out of the environmental parameter following odorant exposure right into standard laboratory conditions. Smokeless powder, ammonium nitrate (powder and prill), composition 4, TNT, and PETN mean concentrations were affected at all conditions as measured by each target VOC concentration in the headspace. Explosive target odor volatiles extracted outside the chamber in ambient conditions resulted in decreased concentrations for explosives exposed in hot conditions and increased concentrations for explosives exposed in cold conditions. Conversely, explosives exposed and extracted inside the environmental chamber resulted in expected physiochemical properties with increased concentration in hot conditions and decreased concentrations in cold conditions. This study implements instrumental analysis with dynamic headspace sampling to monitor the effects of extreme environmental conditions on the odor availability of key explosive odorants for EDDs.

Introduction

The complex nature of odor associated with odor transport, odor availability, and odor quality are important aspects for environmental impact when applied to odor chemistry. Odors are complex mixtures that contain a multitude of components that creates challenges to properly represent the odor profile of the target analytes of interest. The complexities add an additional layer of challenges for canine detection training aids for these complex mixtures (Schultz & DeGreeff, 2022). Degradation of odor over the course of time, evaporation loss (sublimation), or environmental conditions drastically impact the availability and quality of odor, presenting a major limitation to odor detection research. In the environment, temperature and humidity affect the overall consistency of odor and influences characterization of odor. Odor detection focuses on the emission of gaseous vapors from target materials that are released into the environment.

Vapor Technology for Canine Detection

There are a variety of instrumental techniques available for the trace detection of explosives (Lefferts & Castell, 2015; Makinen, Nousiainen, & Sillanpaa, 2011; Pellegrino, Holthoff, & Farrell, 2018; Wallin, Pettersson, Ostmark, & Hobro, 2009). Mass spectrometry (MS) is selected for the sensitivity to characterize the canine's odor environment and the dynamic chemical classes within the odor profile (Makinen et al., 2011). High performance liquid chromatography (HPLC), capillary electrophoresis (CE) coupled with fluorescence or electrochemical detection, and gas chromatography electron capture (GC/ECD) with luminescence has been used for trace explosive detection methods (Gopel, 2000). Ion mobility spectrometry (IMS) has been developed as a screening tool deployed in airports for the detection of trace explosives on the outside of luggage or paper tickets (Ewing, Atkinson, Eiceman, & Ewing, 2001).

While multiple instrumental techniques are widely used for explosive trace detection, the use of canine detection is still a pivotal tool for homeland security purposes (Furton et al., 2015). Canines offer a rugged, easily deployable, and highly mobile detection tool that offers advantages over laboratory instrumentation. Optimal field detection capabilities and instrumentation are essential to detecting an explosive threat prior to detonation. Difficult and complex environments yield to varying environmental conditions which can not only affect the sensor system (canine) but can also potentially change the target odorant itself.

Extreme Environmental Conditions

Examination of the effect of temperature and humidity on the headspace of explosives gives an insight into how climate can drastically affect canine detection training aids in various operational training environments. An increase in temperature and

humidity affects the overall vapor pressure availability of volatile organic compounds (VOCs) and can influence the dynamic range of detection for canines (L. DeGreeff, Katilie, Johnson, & Vaughan, 2021; Frank Lucas Steinkamp, Giordano, Collins, & Rose-Pehrsson, 2015). The influence of environmental conditions on odor availability is a significant component to vapor detection as greater signature VOCs increase the detection rate (L. DeGreeff et al., 2021). Conversely, some environmental conditions suppress or prohibit detection of explosives and fall below the minimum threshold for olfactory canine detection (L. DeGreeff et al., 2021).

Optimal canine detection training must take into consideration odor availability concepts which are influenced by external extreme environmental conditions. These environmental factors influence the dominant odorants available in the headspace of explosive samples based on changes within physiochemical properties of volatility (density, vapor pressure, or surface adsorptivity) (K. G. Furton & Myers, 2001). Explosives exposed to extreme environmental conditions that mimic operational conditions in the field (Figure 14) are approximated and identified on the Köppen-Geiger classification system: (1) Hot/Humid is correlated to equatorial rainforest, fully humid (Af) and equatorial monsoon (Am) areas; (2) Hot/Dry is steppe climate (BS) or desert climate (BW); (3) Cold/Humid is snow climate, fully humid (Df); (4) Cold/Dry is snow climate with dry summer or winter (Ds or Dw). Approximation of classifications is to visualize global climate patterns and correlate to operational conditions to characterize odor in extreme behavior. While there are limited studies evaluating canine physiological effects on the environment (I. Gazit, Goldblatt, & Terkel, 2005; Irit Gazit & Terkel, 2003), there is a gap on the chemical perspective of how odor changes when exposed to extreme environmental conditions.

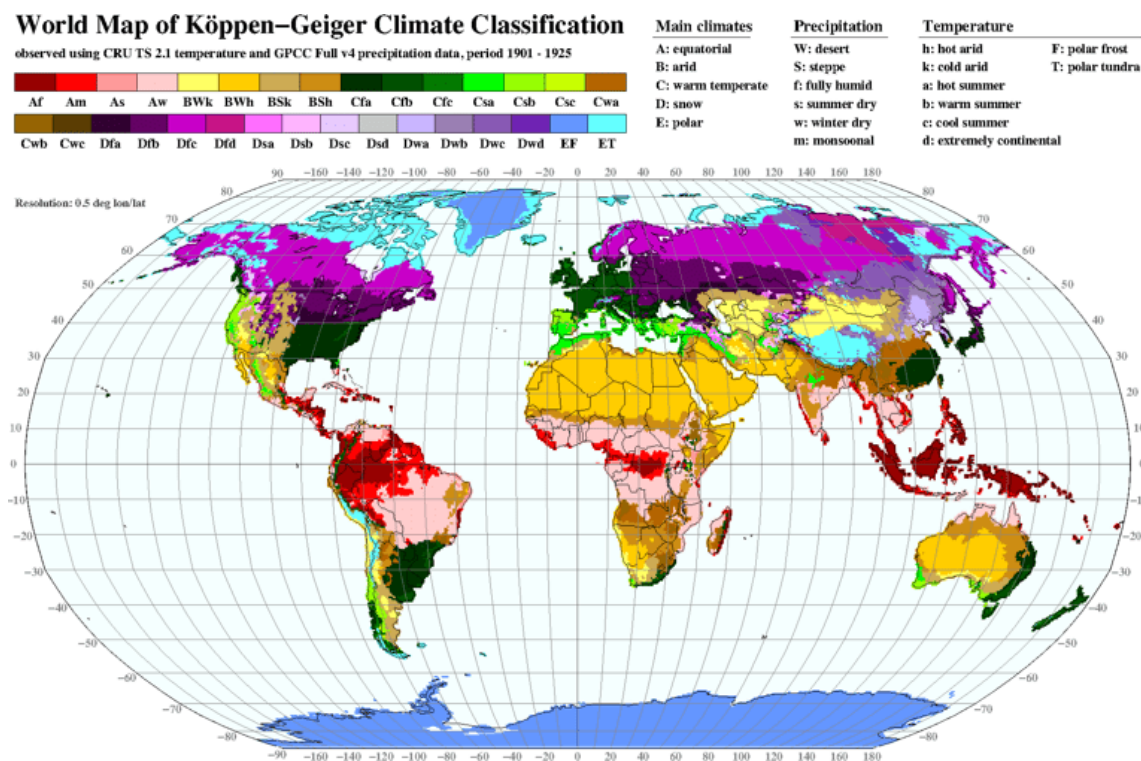


Figure 14: Köppen–Geiger Climate Classification World Map Köppen–Geiger climate type classification system. Permission obtained from: (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006). www.borntraeger-cramer.de/journals/metz

The aim of this task is to robustly characterize odor behavior of a wide range of explosive classes (smokeless powder, composition 4 (C4), trinitrotoluene (TNT), ammonium nitrate (AN (powder and prill form), and pentaerythritol tetranitrate (PETN) under varying environmental conditions via instrumental analyses, namely solid phase microextraction coupled with gas chromatography/mass spectrometry (SPME-GC/MS).

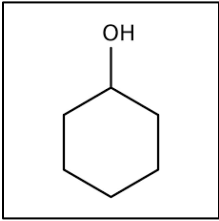
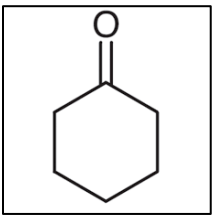
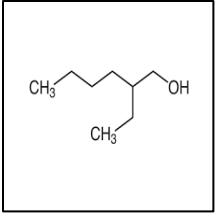
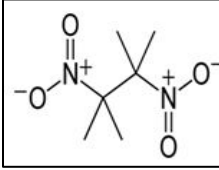
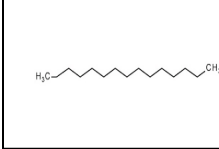
Materials and methods

Chemicals and explosive samples

Energetic materials used in this study were approved by Texas Tech University's Institutional Laboratory Safety Committee (ILSC Protocol #2110E1). Composition 4 (C4), ammonium nitrate (AN) prill, 2,4,6-trinitrotoluene (TNT), and pentaerythritol tetranitrate (PETN, 25 grain) were purchased from Omni Distribution, Inc (Marion, AR, USA). Double based smokeless powder #335 was purchased from Hodgdon Powder Co., Inc (Shawnee, KS, USA). Ammonium nitrate (AN) powder was purchased from Sigma-Aldrich (St. Louis, MO, USA). Calibration chemical standards used included diphenylamine (DPA), dibutyl phthalate (DBP), cyclohexanol, cyclohexanone, 2,3-dimethyl, 2,3-dinitrobutane (DMNB), pentadecane, hexanal, 1,3, dinitrobenzene (DNB), 2,4,6-trinitrotoluene (TNT), and 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)

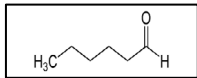
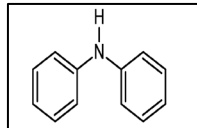
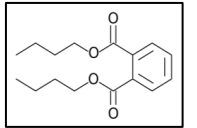
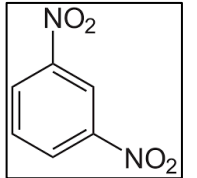
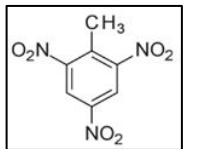
from Sigma-Aldrich (St. Louis, MO, USA). 2-ethyl-1-hexanol from Thermo Fisher Scientific (Waltham, MA, USA). Physiochemical properties of target VOCs are listed in Table 7 and Table 8.

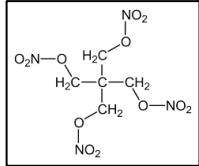
Table 7: Properties of target explosive volatiles in C-4. * Vapor pressure data obtained from PubChem database <https://pubchem.ncbi.nlm.nih.gov/>

Explosive	VOC	Molecular Weight (g/mol)	Vapor Pressure at 25 °C (Torr)*	Chemical Structure
Composition 4 (C-4)	Cyclohexanol	100.16	0.80	
	Cyclohexanone	98.14	4.33	
	2-ethyl, 1-hexanol	130.23	0.14	
	2,3-dimethyl-2,3-nitrobutane	176.17	0.0021	
	Pentadecane	212.41	0.00343	

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Table 8: Properties of target explosive volatiles: *Duplicate target VOCs found in multiple explosives were omitted. Ammonium nitrate powder omitted as 2-ethyl-1-hexanol is the only signature VOC. ** Vapor pressure data obtained from PubChem database <https://pubchem.ncbi.nlm.nih.gov/>

Explosive	VOC	Molecular Weight (g/mol)	Vapor Pressure at 25 °C (Torr) **	Chemical Structure
Ammonium nitrate prill*	Hexanal	100.16	11.26	
Double base smokeless powder	Diphenylamine	169.22	0.00067	
	Dibutyl phthalate	278.34	0.0000201	
2,4,6-trinitrotoluene (TNT)*	1,3-dinitrobenzene	168.11	0.0002	
	2,4,6-trinitrotoluene	227.13	0.00000802	
Pentaerythritol tetranitrate (PETN)	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	286.41	0.0085	

				
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Solid Phase Microextraction (SPME) and Instrumental Analysis of Explosives

Target odorants were extracted from the headspace of C4, ammonium nitrate (powder and prill), double based smokeless powder, TNT, and PETN via SPME coupled to GC-MS optimized detection method of the signature explosive volatiles. The analytical method measured VOC accumulation from tested odorants. Explosives were exposed under specified extreme environmental conditions; temperature and humidity (Table 9) to monitor vapor signature (Table 7 and Table 8).

Table 9: Environmental Chamber Conditions

Condition	Temperature (°C)	Relative Humidity (RH%)
Hot/Humid	40	70
Hot/Dry	40	40
Cold/Humid	0	90
Cold/Dry	0	50

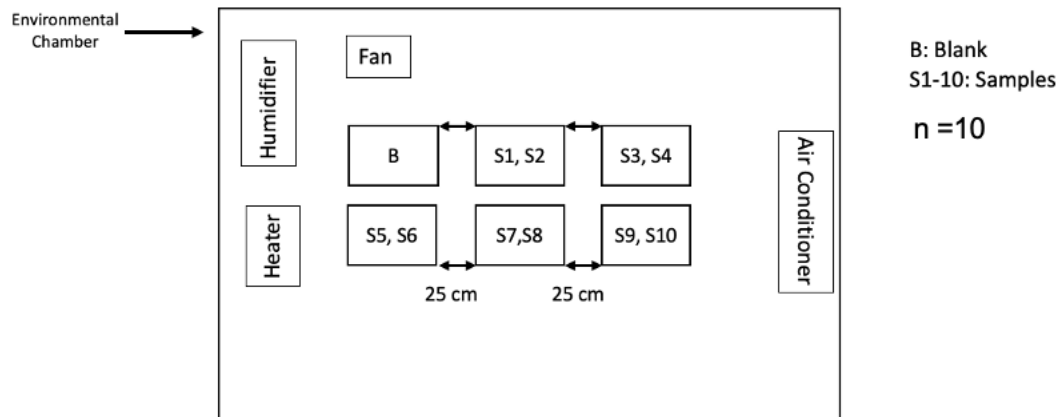
Each environmental condition and target odorant combination included a total of ten replicate samples. All chromatographic separations utilized an Agilent 7890A GC Series GC system, equipped with front and rear split/splitless injection ports, fitted with an HP5 30 m, 0.25 mm i.d., 0.25 mm capillary column. Helium was the carrier gas at a flow rate of 1 mL min⁻¹ at an average velocity of 37 cm s⁻¹. The mass spectrometer (MS) operated in electron ionization (EI) full scan mode from 45 to 550 amu, with a 3-min solvent delay. All compounds in the headspace were assigned based on mass spectral matches to the NIST 2017 mass spectral library.

Quantification of target VOC concentrations being extracted by the SPME fiber was performed via external calibration using a standard mix of signature VOCs within each explosive class. For the target VOCs of each explosive class, the calibration curve range between 1-100 mg/L demonstrated the fiber capacity to extract target analytes at trace concentrations in addition to higher concentrations quantified above 100ppm without losing linearity as observed with the linear dynamic range coefficients of > 0.98 . To approximate the amount of VOCs extracted by the SPME fiber, the slope of the line obtained in the calibration curve was used as a response factor for each of the analyzed compounds.

Environmental Chamber

Target explosive odorants were exposed to extreme environmental conditions using an environmental chamber housed in the Canine Olfaction Lab at Texas Tech University (Figure 15).

A

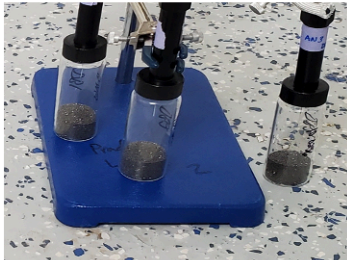


B

Inside Chamber for Environmental Exposure, Transfer to Lab for Extraction



Extractions performed
inside chamber after
exposure to
environmental conditions



Extractions performed
under laboratory room
conditions after
environmental exposure

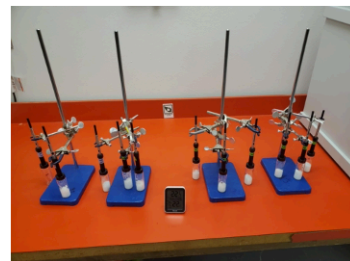


Figure 15: Environmental Chamber Schematic and Sampling Scheme. A) Environmental chamber schematic with sampling location within chamber. Samples ($n = 10$) and blank are included with spacing between samples. Chamber schematic includes location of heater, humidifier, and HVAC units. B) Sampling scheme for exposure within environmental chamber and the extraction mode: extraction performed inside chamber (left) and extraction performed outside chamber (right).

Dedicated HVAC and heater units allow temperature adjustments between 0-43 degrees Celsius. Supplemental humidifiers and de-humidifiers can further control relative humidity conditions. SPME extractions of exposed samples was monitored via two extraction modes for all explosive classes evaluated in the chamber. The first extraction mode evaluated the target odorant while explosive remained in the extreme targeted environmental condition. This mode aimed to understand how the chemical odor profile remained during an “active operational” environmental effect. The second mode of SPME extraction monitored the targeted VOCs after removal of sample from chamber conditions. This second mode was aimed to understand the effect of a drastic change of environmental conditions on target odorant, mimicking a change of odor profile from an active operational condition to regular storage and handling in a well-ventilated standard condition.

Composition 4

Headspace analysis of C4 samples (5 g) was conducted via SPME-GC-MS. Sample amounts were added to 40 mL clear glass vials and allowed to equilibrate to environmental conditions listed in Table 9. Samples were placed in an environmental chamber and left open (no lid) to equilibrate in the chamber for 30 min. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1 h or (2) retained inside the chamber for headspace extraction under extreme environmental conditions for 1 h. Analytes were extracted via polydimethylsiloxane (PDMS) SPME fiber (Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 10.

Table 10: Composition 4 (C-4) GC-MS parameters

Oven temperature program	40 °C, hold 4 min. 10 °C/min to 300 °C, hold 2 min. Total 32.0 min
Inlet split ratio	Splitless
Inlet temperature	265 °C

Double based smokeless powder

Headspace analysis of DB SP samples (10 g) were conducted via SPME-GC-MS. Sample amounts were added to 40 mL clear glass vials and allowed to equilibrate to environmental conditions listed in Table 9. Samples were placed in an environmental chamber and left open (no lid) to equilibrate in the chamber for 30 min. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1 h or (2) retained inside the chamber for headspace extraction at extreme environmental conditions for 1 h. Analytes were extracted via divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 11.

Table 11: Double base smokeless powder GC-MS parameters

Oven temperature program	40 °C, hold 1 min. 15 °C/min to 200 °C, hold 1 min. 15 °C/min to 240 °C, hold 6.5 min. 25 °C/min to 270 °C 5 °C/min to 280 °C, hold 4 min. Total 29.03 min.
Inlet split ratio	Split 1:5
Inlet temperature	280 °C

Ammonium nitrate powder

Headspace analysis of AN powder samples (10 g) were conducted via SPME-GC-MS. Sample amounts were added to 40 mL clear glass vials and allowed to equilibrate to environmental conditions listed in Table 9. Samples were placed in an environmental chamber and left open (no lid) to equilibrate in the chamber for 1 h. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1 h or (2) retained inside the chamber for headspace extraction under extreme environmental conditions for 3 h. Analytes were extracted via divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber

(Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 12.

Table 12: Ammonium nitrate powder GC-MS parameters

Oven temperature program	40 °C, hold 1 min. 20 °C/min to 240 °C, hold 3 min. Total 14.0 min
Inlet split ratio	Splitless
Inlet temperature	250 °C

Ammonium nitrate prill

Headspace analysis of AN prill samples (7 g) were conducted via SPME-GC-MS. Sample amounts were added to 8 oz clear glass wide-mouth canning jars and allowed to equilibrate to environmental conditions listed in Table 9. Samples were placed in an environmental chamber and left open (no lid) to equilibrate in the chamber for 1 h. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1 h or (2) retained inside the chamber for headspace extraction under extreme environmental conditions for 3 h. Analytes were extracted via divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 13.

Table 13: Ammonium nitrate prill GC-MS parameters

Oven temperature program	40 °C, hold 1 min. 20 °C/min to 240 °C, hold 3 min. Total 18.0 min
Inlet split ratio	Splitless
Inlet temperature	250 °C

TNT

Headspace analysis of TNT flake samples (10 g) were conducted via SPME-GC-MS. Sample amounts were added to 40 mL clear glass vials and allowed to equilibrate to environmental conditions listed in Table 9. Samples were carried to the environmental chamber and left open (no lid) to equilibrate in the chamber for 30 min. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1 h or (2) retained inside the chamber for headspace extraction under extreme environmental conditions for 1 h. Analytes were extracted via polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fiber (Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 14.

Table 14: 2,4,6-trinitrotoluene (TNT) GC-MS parameters

Oven temperature program	40 °C, hold 2 min. 20 °C/min to 200 °C, hold 2.5 min. 20 °C/min to 250 °C, hold 5 min. Total 20.0 min
Inlet split ration	Splitless
Inlet temperature	250 °C

PETN

Headspace analysis of PETN samples (2-3 inch segments of 25 grain detonation cord) were conducted via SPME-GC-MS. Sample amounts were added to 40 mL clear glass vials and allowed to equilibrate to environmental conditions listed in Table 9. Samples were placed in an environmental chamber and left open (no lid) to equilibrate in the chamber for 30 min. After this time, vials were closed and (1) removed from the chamber for headspace extraction under ambient conditions by SPME for 1.5 h or (2) retained inside the chamber for headspace extraction at extreme environmental conditions for 1.5 h. Analytes were extracted via polydimethylsiloxane (PDMS) SPME fiber (Supelco, Millipore Sigma) and thermally desorbed by GC-MS. Other GC-MS parameters are listed in Table 15.

Table 15: Pentaerythritol tetranitrate (PETN) GC-MS parameters

Oven temperature program	70 °C, hold 1 min. 30 °C/min to 120 °C, 10 °C/min to 170 °C, 60 °C/min to 280 °C, hold 5.5 min. Total 15.0 min
Inlet split ration	Splitless
Inlet temperature	200 °C

Statistical Analysis

Matched paired t-test was performed to determine significant difference between extraction modes for each target VOC from each explosive tested. Target VOC concentrations from different climatic conditions (temperature and humidity) were analyzed with Analysis of Variance (ANOVA) and Tukey's multiple comparison test (normally distributed) or by the Kruskal-Wallis test with Dunn's multiple comparison test (if not normally) distributed using JMP statistical analysis software.

Results

Target VOC Concentrations

Target VOC concentrations present in the headspace were measured and monitored as a function of operational climate conditions. Quantitation of target VOC concentrations extracted by the SPME fiber was performed via external calibration using a standard mix of signature VOCs within each explosive class. Average concentrations (mg/L) are listed in Table 16 (outside chamber) and Table 17 (inside chamber). Experimental environmental chamber conditions and the total VOC concentrations for each explosive class are listed in Table 18 (Hot/Humid, Hot/Dry) and Table 19 (Cold/Humid, Cold/Dry).

Table 16: Average target VOC concentrations (Outside Environmental Chamber)

Explosive	VOC	Hot/Humid	Hot/Dry	Cold/Humid	Cold/Dry
Composition 4 (C4)	Cyclohexanol	3.46±0.27	4.16±0.50	5.58±0.72	5.99±0.55
	Cyclohexanone	19.28±2.38	23.43±2.89	33.83±7.74	35.95±5.79
	2-ethyl-1-hexanol	51.47±4.98	51.07±15.4	76.56±6.91	79.93±6.81
	DMNB	452.67±33.2	301.96 ±93.9	301.62±26.2	317.85±24.4
	C15	8.25±0.10	8.25±0.15	7.90±0.08	7.96 ± 0.07
Ammonium nitrate powder	2-ethyl-1-hexanol	37.13±5.42	13.43±4.04	4.89±1.14	4.65±0.79
Ammonium nitrate prill	2-ethyl-1-hexanol	50.17±3.09	34.19±5.87	39.92±2.04	32.43±1.39
	Hexanal	2.90±0.93	3.07±1.20	6.74±1.86	5.89±2.27
	DPA	11.07±0.80	9.87±0.56	9.47±0.22	9.12±0.19

Double base smokeless powder	DBP	4.59±0.31	4.19±0.29	4.27±0.16	4.38±0.21
2,4,6-trinitrotoluene (TNT)	Hexanal	10.00±0.07	9.88±0.005	10.17±0.05	9.88±0.003
	2-ethyl-1-hexanol	9.71±1.71	4.31±0.07	15.84±0.93	7.94±0.48
	DNB	73.46±14.3	71.86±13.0	82.51±20.0	61.23±12.8
	TNT	40.21±10.3	36.75±11.3	27.60±11.8	20.00±4.50
Pentaerythritol tetranitrate (PETN)	TXIB	47.14±6.16	59.99±9.39	10.45±3.39	3.78±0.84

Average mean concentrations with report standard deviation for target VOCs. Target volatiles: 2-ethyl-1-hexanol (2E1H); 2,3-dimethyl-2,3-dinitrobutane (DMNB); pentadecane (C15); diphenylamine (DPA); dibutyl phthalate (DBP); 2,4,6-trinitrotoluene (TNT), 2,2,4-Trimethyl-1,3-pentadienol diisobutyrate (TXIB).

Table 17: Average target VOC concentrations (Inside Environmental Chamber)

Explosive	VOC	Hot/Humid	Hot/Dry	Cold/Humid	Cold/Dry
Composition 4 (C4)	Cyclohexanol	5.51±0.15	3.52±0.28	5.51±0.61	5.03±0.45
	Cyclohexanone	30.50±4.00	35.61±5.76	33.27±6.52	27.92±5.86
	2-ethyl-1-hexanol	38.63±3.98	56.81±5.45	76.16±6.50	66.80±5.13
	DMNB	580.30±47.4	433.23±63.8	264.38±19.8	252.99±17.4
	Pentadecane	9.00±0.18	9.07±0.22	7.87±0.06	7.80±0.05
Ammonium nitrate powder	2-ethyl-1-hexanol	11.10±1.63	6.99±0.82	8.00±1.00	3.98±0.65
Ammonium nitrate prill	2-ethyl-1-hexanol	38.25±9.60	28.37±2.15	20.49±26.4	7.71±0.62
	Hexanal	4.21±1.96	2.31±0.87	2.97±1.36	2.97±1.37
Double base smokeless powder	DPA	15.51±6.37	17.29±5.93	9.08±0.12	8.98±0.05
	DBP	3.70±2.09	4.94±2.92	2.43±1.63	0.98±0.64

2,4,6-trinitrotoluene (TNT)	Hexanal	9.86±0.0006	10.43±0.22	10.02±0.06	10.06±0.48
	2-ethyl-1-hexanol	6.67±0.82	6.91±1.03	7.31±0.43	5.12±0.32
	DNB	454.60±55.1	422.25±57.1	9.22±1.60	9.93±1.82
	TNT	1034.89±239.4	988.32±231.6	NA	NA
Pentaerythritol tetranitrate (PETN)	TXIB	118.74±14.9	3.10±0.61	1.89±0.46	1.36±0.30

N/A = Not applicable. Average mean concentrations with report standard deviation for target VOCs. Target volatiles: 2-ethyl-1-hexanol (2E1H); 2,3-dimethyl-2,3-dinitrobutane (DMNB); pentadecane (C15); diphenylamine (DPA); dibutyl phthalate (DBP); 2,4,6-trinitrotoluene (TNT), 2,2,4-Trimethyl-1,3-pentadienol diisobutyrate (TXIB).

Table 18: Experimental extreme environmental conditions and total odor concentration (mg/L) for Hot/Humid and Hot/Dry conditions

Explosives	Hot/Humid		Total VOC Accumulation (ppm) (Outside/Inside)		Hot/Dry		Total VOC Accumulation (ppm) (Outside/Inside)	
	(Temp °C/RH%)				(Temp °C/RH%)			
C4	39.8	70.0	535.14	663.97	39.5	40.6	338.9	538.27
TNT	39.2	69.4	133.4	1506.56	39.5	40.2	122.8	1427.93
AN prill	39.8	69.6	53.08	42.47	39.9	40.0	37.26	30.69
AN powder	40.2	71.0	11.10	37.13	40.1	39.8	7.00	13.43
SP	40.1	69.0	15.66	19.22	40.1	39.9	14.07	22.24
PETN	39.9	70.4	47.14	118.73	40.8	40.1	60.00	3.10

Temperature is reported in (°C). Relative Humidity (%RH) is reported in (%). Total VOC accumulation is reported in the average concentration (mg/L) from external calibration of SPME-GC-MS method.

Table 19: Experimental extreme environmental conditions and total odor concentration (mg/L) for Hot/Humid and Hot/Dry conditions

Explosives	Cold/Humid (Temp °C/RH%)		Total VOC Accumulation (ppm) (Outside/Inside)		Cold/Dry (Temp °C/RH%)		Total VOC Accumulation (ppm) (Outside/Inside)	
C4	0.3	89.7	425.5	387.2	0.5	50.0	447.7	360.6
TNT	0.6	89.7	136.1	26.56	0.5	50.6	99.07	25.13
AN prill	0.5	89.9	46.67	23.47	0.2	49.8	38.32	8.94
AN powder	0.7	89.8	8.01	4.24	0.1	51.2	3.99	4.24
SP	0.3	91.0	13.75	9.97	0.2	49.8	13.50	9.97
PETN	0.3	89.6	10.45	1.89	0.4	49.5	3.78	1.36

Temperature is reported in (°C). Relative Humidity (%RH) is reported in (%). Total VOC accumulation is reported in the average concentration (mg/L) from external calibration of SPME-GC-MS method.

Composition 4

A total of ten (10) replicate samples of Composition 4 explosive were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameter for this explosive was a SPME fiber chemistry of polydimethylsiloxane (PDMS) with an extraction time of 1 h. Based on previous literature reports targeted signature VOCs for C-4 included: cyclohexanol, cyclohexanone, 2-ethyl-1-hexanol (2E1H), pentadecane (C15), and 2,3-dimethyl-dinitrobutane (DMNB) (K. G. Furton & Myers, 2001; Sanchez, Huertas, Prada, & Furton, 2016). Figure 16 shows the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure. DMNB was the most abundant VOC for all conditions and extraction modes. Cyclohexanol was the least abundant VOC across all conditions and extraction modes. There was a significant difference between all volatile concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction mode as follows: cyclohexanol – all conditions; cyclohexanone – Hot/Humid, Hot/Dry, Cold/Dry; 2-ethyl-1-hexanol – Hot/Humid, Cold/Dry; DMNB – all conditions; C15 – Hot/Humid, Hot/Dry, Cold/Dry. Concentrations of cyclohexanol and DMNB were significantly different between extraction modes as expected. 2-ethyl-1-hexanol, C15, and cyclohexanone had concentrations with significant differences among several environmental conditions. Concentrations that were not significantly different between extraction modes are attributed to variation in environmental chamber conditions and location within the chamber. This variability led to fluctuations among the average concentration value for VOCs influenced by environmental factors.

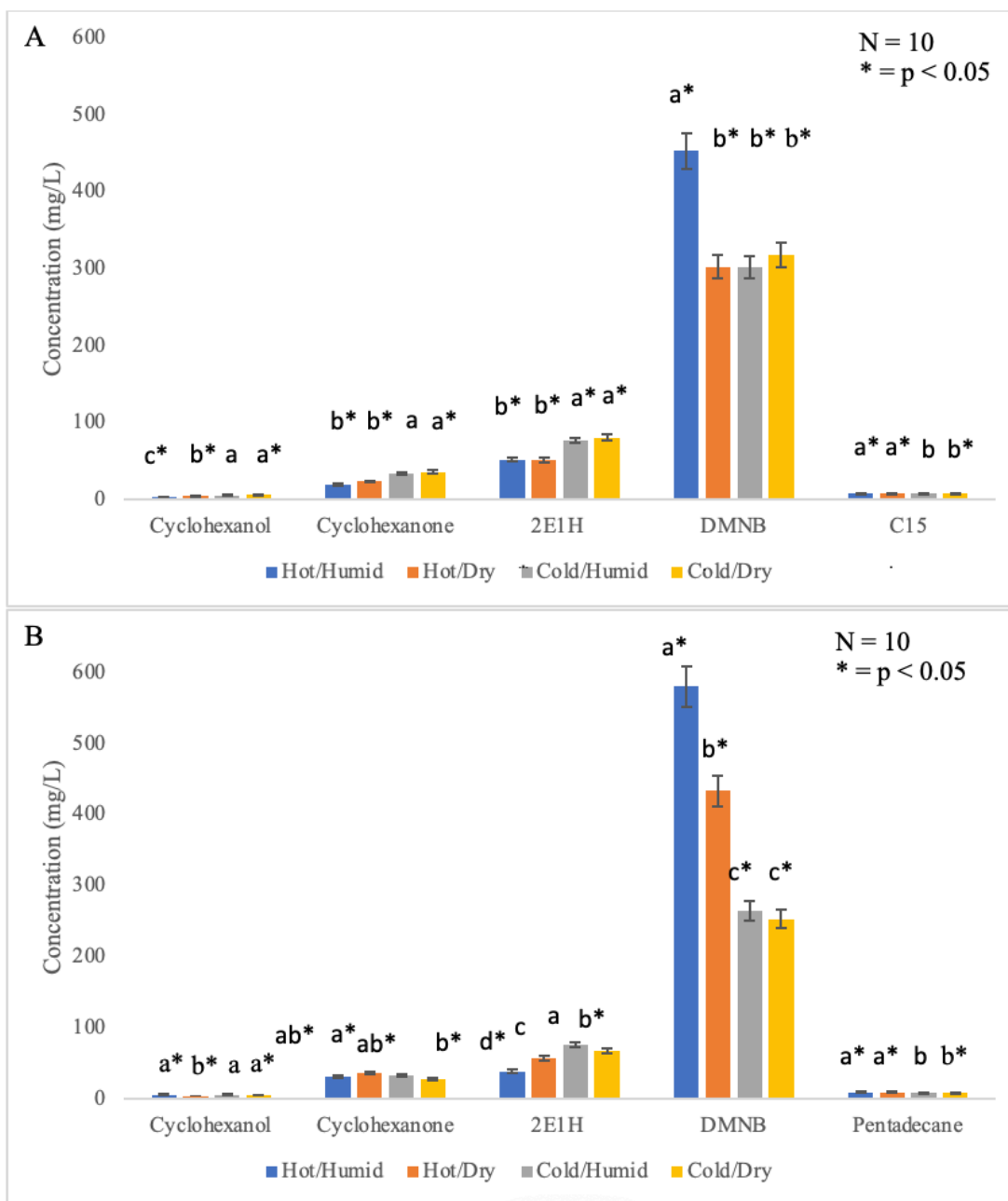


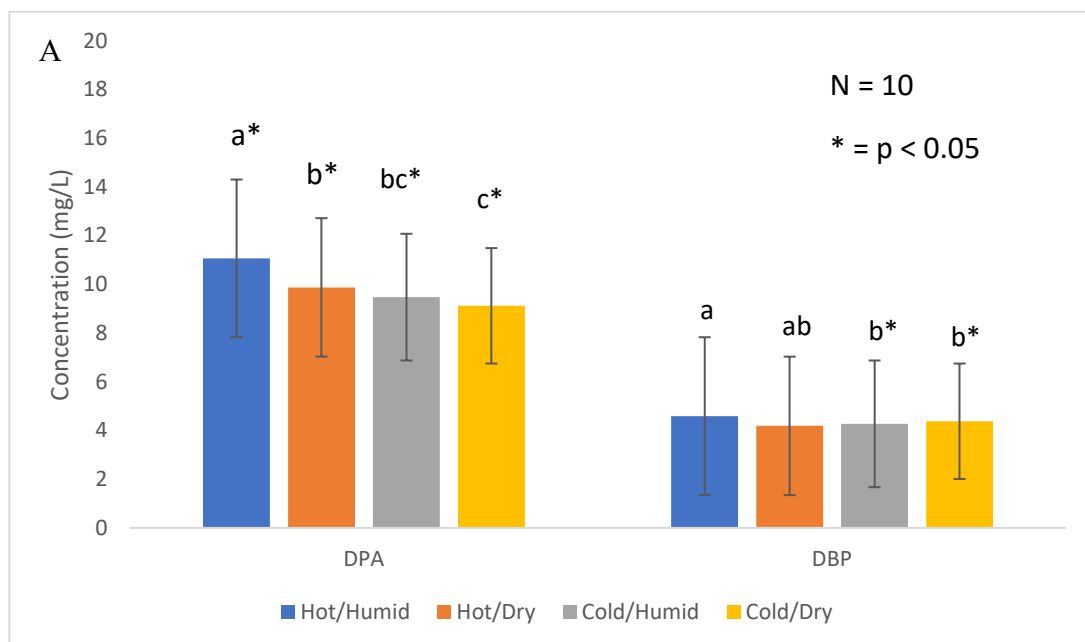
Figure 16: Composition 4 average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. 2-ethyl-1-hexanol (2E1H); 2,3-dimethyl-2,3-dinitrobutane (DMNB).

Double base smokeless powder

A total of ten (10) replicate samples of smokeless powder explosive were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameters for

this explosive was a SPME fiber chemistry of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with an extraction time of 1 hour. Based on previous literature reports, targeted signature VOCs for double-based smokeless powder included: diphenylamine and dibutyl phthalate (Joshi, Rigsby, & Almirall, 2011). Figure 17 depicts the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure.

Diphenylamine was the most abundant VOC for all conditions and extraction modes. Dibutyl phthalate was the least abundant VOC across all conditions and extraction modes. There was a significant difference between all volatile concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction mode as follows: DPA – all conditions; DBP – Cold/Humid, Cold/Dry.



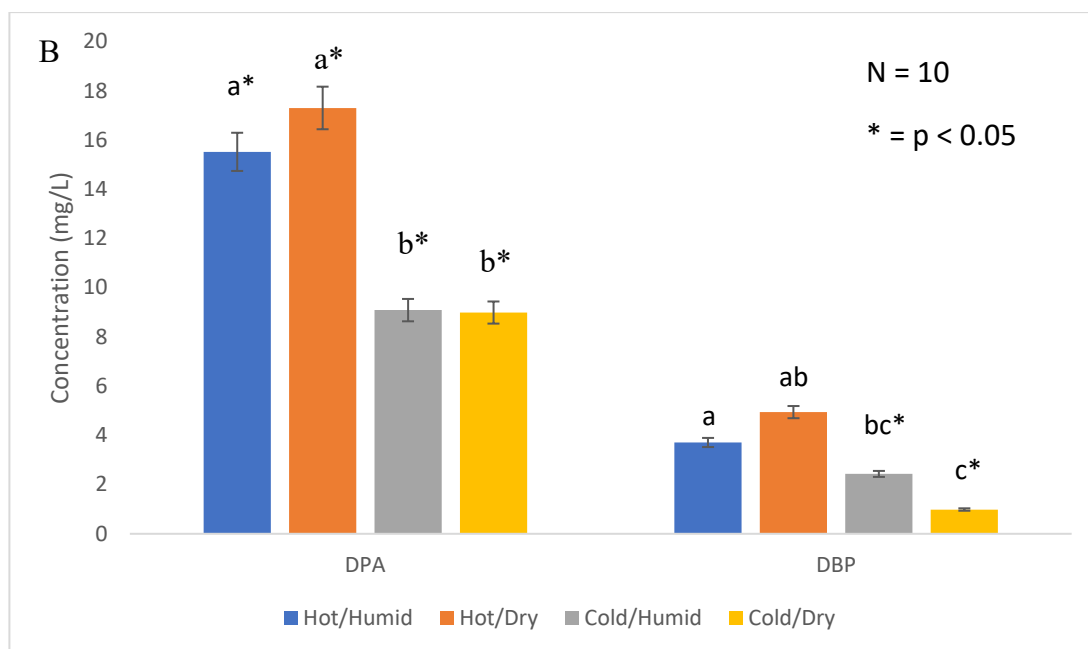


Figure 17: Smokeless powder average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. Diphenylamine (DPA) and dibutyl phthalate (DBP).

Ammonium nitrate powder

A total of ten (10) replicate samples of smokeless powder explosive were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameters for this explosive was a SPME fiber chemistry of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with an extraction time of 3 hours. Based on previous literature reports targeted signature VOCs for AN powder was 2-ethyl-1-hexanol (L. E. DeGreeff & Peranich, 2021). Figure 18 concluded the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure. 2-ethyl-1-hexanol was concluded to have the highest abundance in the hot/humid condition and the least abundance in the cold/dry condition. There was a significant difference between all volatile target VOC concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction modes for Hot/humid, Hot/Dry, Cold/Humid.

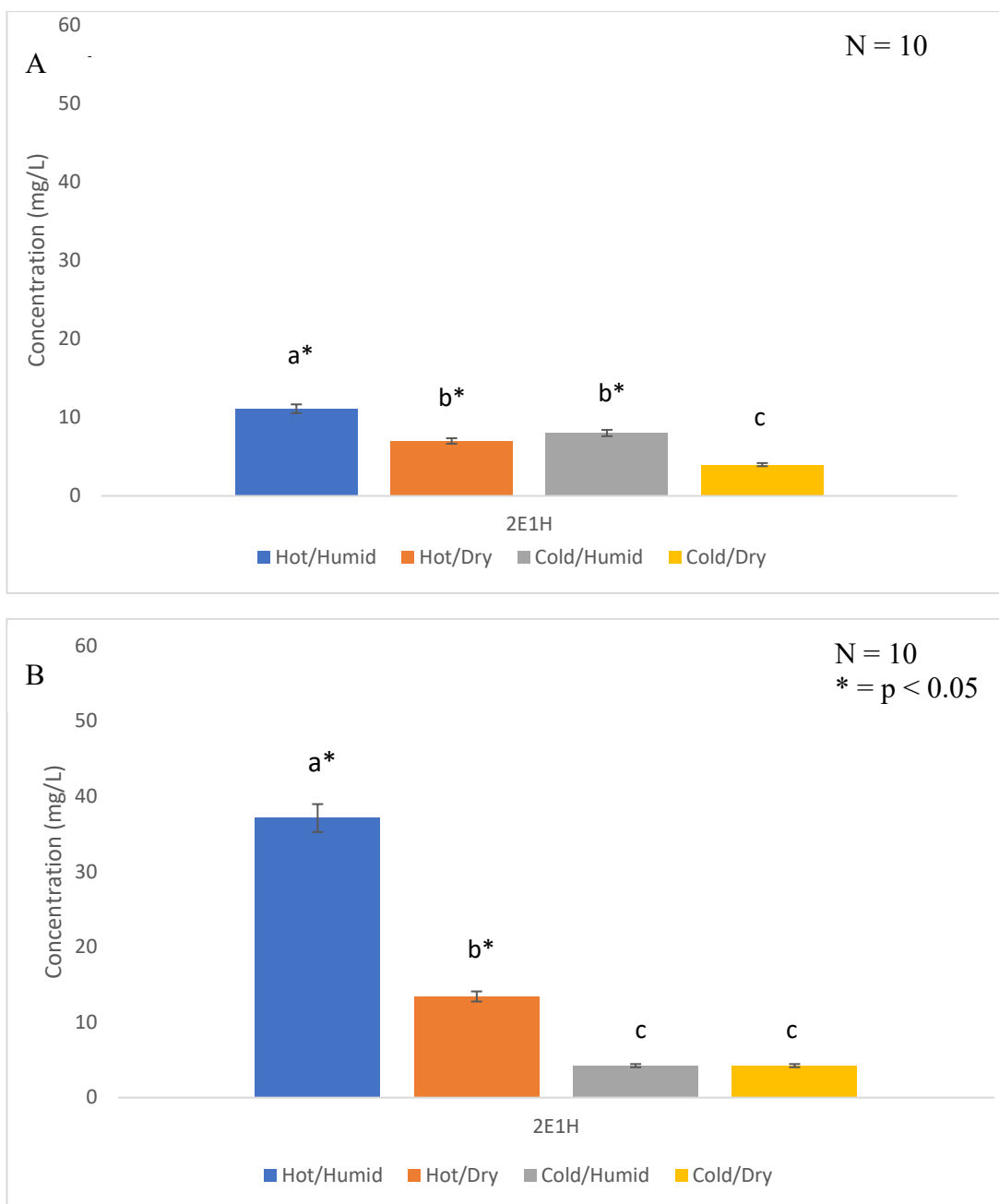
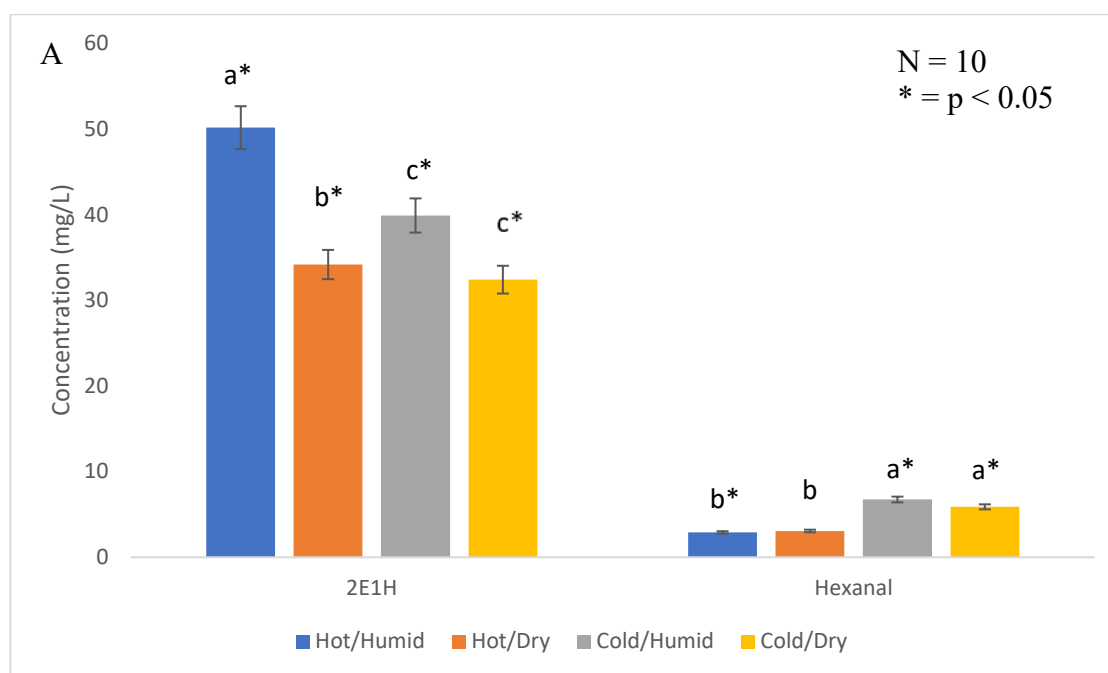


Figure 18: Ammonium nitrate powder average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. 2-ethyl-1-hexanol (2E1H).

Ammonium nitrate prill

A total of ten (10) replicate samples of ammonium nitrate powder explosive were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameters for this explosive was a SPME fiber chemistry of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with an extraction time of 3 hours. Based on previous literature reports targeted signature VOCs for AN prill was 2-ethyl-1-hexanol and hexanal (L. E. DeGreeff & Peranich, 2021). Figure 19 concluded the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure. 2-ethyl-1-hexanol was concluded to have the highest abundance in all conditions and hexanal had the least abundance in all conditions. There was a significant difference between all volatile target VOC concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction modes for as follows: 2-ethyl-1-hexanol – all conditions; hexanal - Cold/Humid, Cold/Dry.



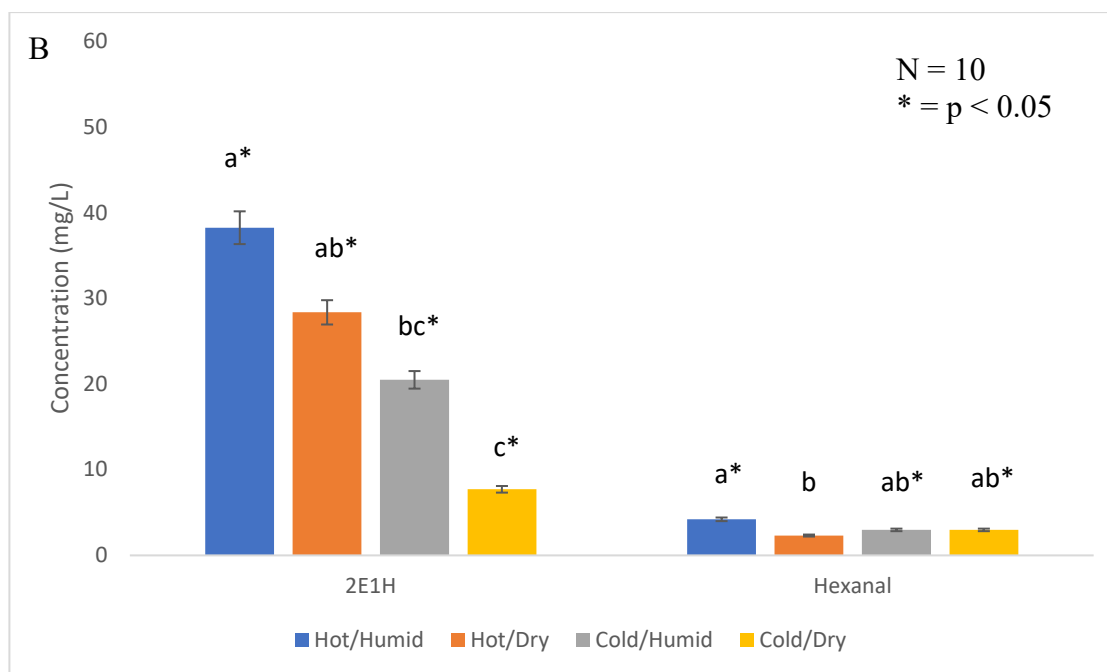


Figure 19: Ammonium nitrate prill average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. 2-ethyl-1-hexanol (2E1H).

TNT

A total of ten (10) replicate samples of TNT flake samples were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameters for this explosive was a SPME fiber chemistry of polydimethylsiloxane/divinylbenzene (PDMS/DVB) with an extraction time of 1 hour. Based on previous literature reports targeted signature VOCs for TNT was hexanal, 2-ethyl-1-hexanol, 1,3-dinitrobenzene (DNB), and 2,4,6-trinitrotoluene (TNT) (Lorenzo et al., 2003; Psillakis, Naxakis, & Kalogerakis, 2000). Figure 20 depicts the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure. DNB was the most abundant volatile concluded for all conditions in the outside chamber extraction mode. DNB was the most abundant for all conditions in the inside chamber extraction mode; however, in cold conditions, hexanal is the most abundant volatile. 2-ethyl-1-hexanol was the least abundant volatile in all conditions but cold/humid extracted outside the chamber. The TNT volatile was lost in both cold conditions when extracted inside the chamber. There was a significant difference between all volatile concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction modes for as follows: 2-ethyl-1-hexanol – all conditions; hexanal – Hot/Humid, Hot/Dry. Cold/Humid; DNB – all conditions; TNT – all conditions.

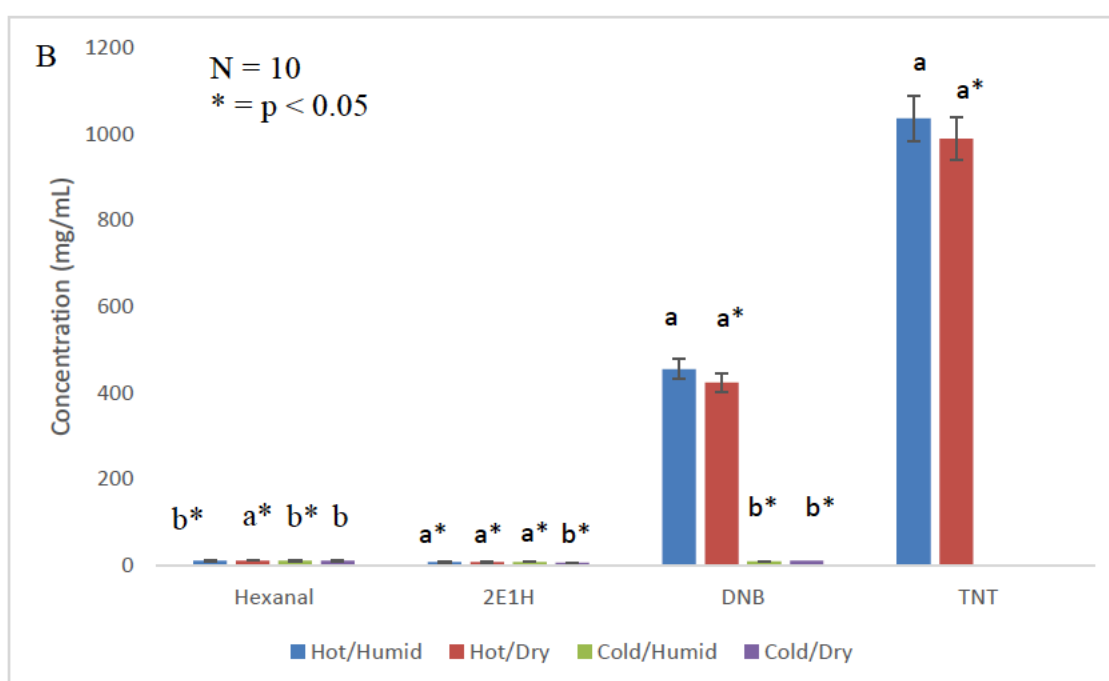
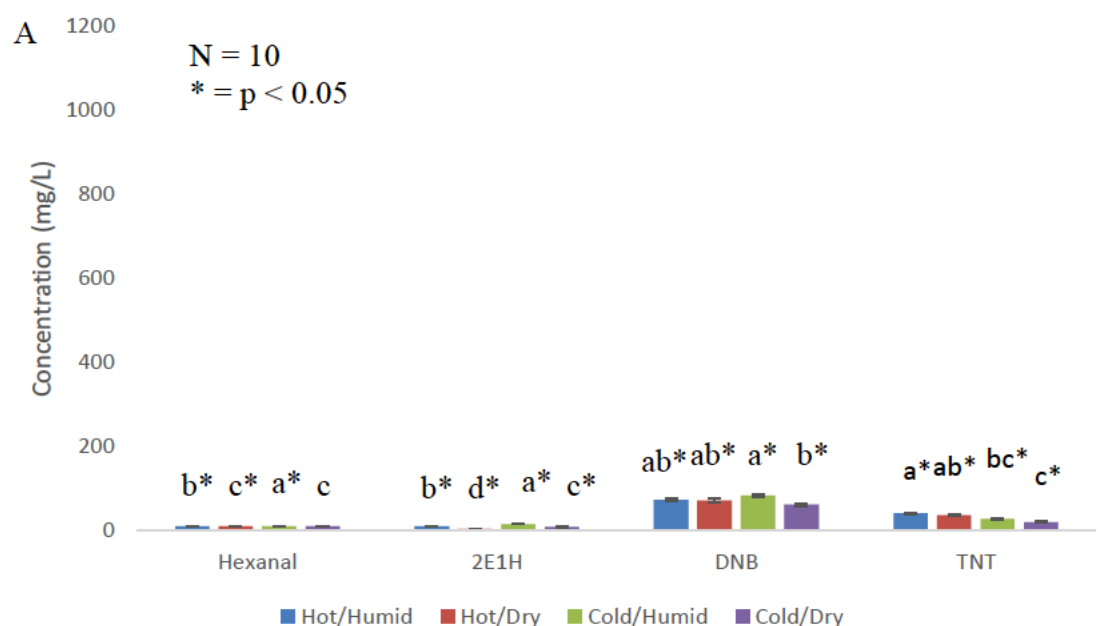


Figure 20: TNT average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. 2-ethyl-1-hexanol (2E1H).

PETN

A total of ten (10) replicate samples of pre-cut PETN (2-3 inch segments, 25 grain) were tested in the environmental chamber at the four targeted conditions. The optimal extraction parameters for this explosive was a SPME fiber chemistry of polydimethylsiloxane (PDMS) with an extraction time of 1.5 hour. Based on previous literature reports targeted signature VOCs for PETN was 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB) (Dsikowitzky et al., 2014). Figure 21 depicts the abundances of VOC concentrations among all environmental conditions for A) extraction performed outside chamber after exposure and B) extraction performed inside chamber after exposure. TXIB was concluded to have the highest abundance in the hot/dry condition for extractions performed outside the chamber and the least abundant in cold/dry conditions. TXIB concentrations were the highest abundance in the hot/dry condition. There was a significant difference between all volatile target VOC concentrations across all conditions. There was a significant difference between target VOC concentrations among extraction modes for as follows: TXIB- all conditions.

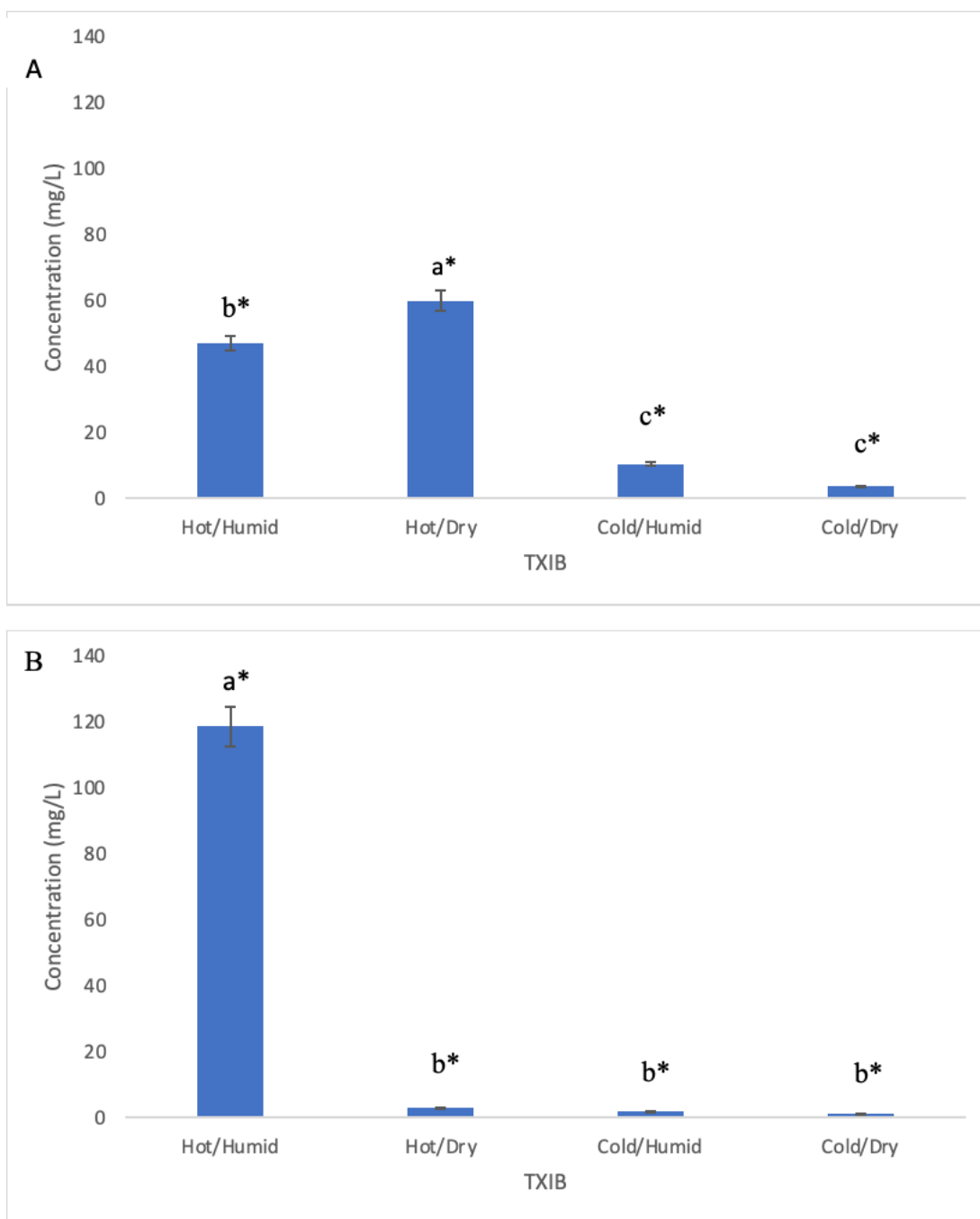


Figure 21: PETN average concentrations for target VOCs. A) Extractions performed outside environmental chamber. B) Extractions performed inside environmental chamber. Error bars indicate standard error. Asterisks indicate statistical differences ($p < 0.05$) in mean concentrations among environmental conditions. 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB).

Discussion

SPME fiber extraction efficiency

The stability of the SPME fiber coating over time is an important parameter to consider in the analytical technique. Determination of endurance and reusability of the fiber was evaluated in previous studies and was subjected to 130 successive thermal desorption cycles for analysis (Souza Silva & Pawliszyn, 2012). The amount of analyte extracted by SPME is proportional to the concentration of target VOCs in the sample matrix (Souza Silva & Pawliszyn, 2012). Reusability of the SPME fiber shows a decrease in amount extracted after the 90th extraction; however, amounts remain reproducible between the 90th and 130th extraction (Souza Silva & Pawliszyn, 2012). SPME fundamentals applies to headspace analysis of explosives and introduces variability between samples in the same environmental condition. Target VOCs demonstrated variability among calculated concentrations due to repeated use of SPME fibers for all environmental conditions and extraction modes. Variability within replicate samples for each environmental condition demonstrated fluctuations among VOC concentrations but were detected above 80% peak area quality as concluded. Furthermore, it is important to understand that sample location within the chamber and airflow fluctuations during sampling might also cause slight deviations in reported values for each replicate sampling. Odor transport and movement is not taken into consideration in the experimental design, however, might play a vital role in odor concentrations detected. It is known that odor plumes fluctuate differently as a function of environmental conditions, with hot air causing molecules to rise and colder air causing molecules to stay closer to the ground. Hence, variation of odor volatile concentrations is an intrinsic response of odor plume development as a result of molecular diffusion and turbulence. While the environmental chamber provides an option for temperature and humidity fluctuations, it is important to understand that other factors such as wind, solar radiation and shade are not introduced to explosive samples, thereby highlighting a limitation to simulating actual operational settings.

SPME fiber selection and optimization

Fiber selection is taken into consideration as the first step of method development as the sorbent material defines the detection capabilities of target VOCs (Marrubini et al., 2020). Selection of fiber is based on personal experience (Gaurav, Malik, & Rai, 2008, 2009; Monteleone, Naccarato, Sindona, & Tagarelli, 2013; Szultka-Mlynska, Pomastowski, & Buszewski, 2018) or literature data (L. E. DeGreeff & Peranich, 2021; Gallegos, Aviles-Rosa, Hall, & Prada-Tiedemann, 2022; Psillakis et al., 2000; Sanchez et al., 2016). The selected fiber chemistry has a significant impact on the extraction of analytes as extraction efficiency is dependent upon the properties of the fiber coating and selectivity of target VOCs versus other matrix components (Gaurav, Kaur, Kumar, Malik, & Rai, 2007).

There are seven different SPME fibers coatings commercially available: PDMS, PDMS/DVB, PA, CAR/PDMS, CW/TPR, CW/DVB, and DVB/CAR/PDMS (stableflex). Among these fibers, PDMS (Halasz et al., 2002), CW/DVB (Kenneth G Furton, Wu, & Almirall, 2000), CW/TPR

(Kenneth G Furton et al., 2000; Monteil-Rivera, Beaulieu, Deschamps, Paquet, & Hawari, 2004), PDMS/DVB (Haag, 1996; Wu, Almirall, & Furton, 1999; Zhang, Yang, & Pawliszyn, 1994), PA (Fanny Monteil-Rivera et al., 2004), DVB/CAR/PDMS (Garzon-Serrano, Sierra, Rodriguez-Bejarano, & Sinuco, 2020). PA SPME fibers gave the lowest GC/MS response and large errors for all explosive classes. The decomposition of the PA fiber under increased pressure is concluded by Rivera et al. (Fanny Monteil-Rivera et al., 2004). PDMS/DVB and PDMS are more selective to nitroaromatic compounds, while smokeless powder and ammonium nitrate VOCs are more selective towards DVB/CAR/PDMS based on laboratory exposure and extraction methods. In this study, preliminary studies were conducted at ambient temperature and humidity to determine the appropriate SPME fiber to be used for environmental chamber studies. A total of three replicates were conducted per fiber type to determine SPME fiber optimization. Optimization of SPME fibers ensured detection of VOCs within the environmental chamber to reduce variability among environmental conditions.

Environmental Chamber

Temperature and humidity conditions targeted operational conditions for canine detection teams in extreme environments. Evaluation of these effects on explosive VOCs examined the influence of vapor pressure and odor availability for select compounds in the headspace. Extractions performed outside of the environmental chamber produced results with cold conditions as having an elevated concentration versus extractions conducted inside the environmental chamber. The difference in exposure only in the chamber affected target VOCs to acclimate to the ambient conditions during the extraction, allowing for samples exposed to cold conditions an opportunity to equilibrate, increasing the abundance of VOCs in the headspace. Samples exposed and extracted within the chamber are subjected to the operational condition for the duration of sampling and do not have an acclimation period. Studies were conducted inside versus outside to demonstrate the differences of extraction modes after exposure to extreme environmental conditions. Optimization of extraction time was determined from ambient conditions in the laboratory upon evaluation of SPME fiber selection. Temperature and humidity conditions within $\pm 5\%$ of the target value were accepted for environmental chamber studies. Variations among the ten replicates ($n=10$) per condition can be attributed to heating and cooling system cycling during the exposure and extraction process.

Composition 4

The extraction mode determined common trends among the environmental conditions. Temperature and humidity induced various effects on volatiles based on vapor pressure and variations within the environmental chamber. Target VOC concentrations increased in the presence of hot conditions within the chamber but decreased when removed for the outside chamber extraction mode. Comparatively, concentrations increased when removed from cold conditions to ambient temperatures. Rapid changes to environmental conditions during the extraction procedures suggests an influence on the volatility of the bulk material (vapor pressure of target odorants) across replicate samplings. All VOCs present in the headspace for C-4

decreased in concentration when extracted inside the chamber, thus suggesting a direct relationship with temperature and humidity correlated to volatile concentrations.

Smokeless powder

Significant differences in DBP concentrations were attributed to a decrease in target VOC concentrations exposed to cold conditions during the extraction process due to a lower vapor pressure compared to diphenylamine. Low vapor pressure compounds are influenced by warmer temperatures and produce more volatiles in the headspace during extraction. Less volatiles were produced in cold conditions. There was a significant difference in volatiles generated in the outside chamber extraction mode as they were given a period of time to equilibrate in ambient temperatures during the extraction compared to constant exposure to the cold conditions inside the chamber.

Ammonium nitrate powder

Acclimation of AN powder samples within the chamber in the cold/dry condition was influenced by the optimized extraction time (3 h), which resulted in low target VOC concentrations in the cold/dry condition. Average target VOC concentrations were not significantly different. The effect on vapor pressure in the inside chamber mode resulted in decreased volatility compared to the outside chamber mode. Constant exposure to below freezing conditions restricted the production of volatiles, thus affecting overall concentrations of ammonium nitrate.

Ammonium nitrate prill

AN prill concentration differences are attributed to low vapor pressure and the use of 8 oz mason jars as the sampling container. The larger jar was used to increase the surface area of the prill for improved detection of signature VOCs. Optimization of vial size (8 oz) and extraction time (3 h) determined detection of VOCs over 80% quality for qualitative data. The increased surface area for AN prill provided capabilities for detection but is not comparable to samples in smaller vial sizes.

TNT

The cold/dry condition did not have a significant effect on hexanal concentration and is likely attributed to exposure to colder conditions slowed the rate of odor vaporization from the solid explosive. Replicate samples from cold/humid and cold/dry conditions were analyzed to ensure the loss of TNT VOCs in the headspace when extracted inside the environmental chamber.

PETN

The hot/dry condition with an increased abundance of TXIB is a result of optimal environmental parameters for optimal odor vaporization from the bulk material. PETN was cut into segmented pieces but exposure to energetic material was accessible only from the cut ends of the detonation cord. Limited availability of the active material resulted in low TXIB concentrations for extractions within the environmental chamber. Active constituents of PETN odor profile did not include energetic material due to thermal instability of the material within the GC-MS. Previous literature studies have documented only active component of PETN with no reported VOCs of additional plasticizers as TXIB.

Limits and future challenges

The disadvantages and limitations of commercially available coatings in SPME fibers results from poor interactions with polar compounds and/or incompatibility with complex samples (Sajid et al., 2019). Further limitations of explosive headspace analysis of target VOCs that are semi-volatile or non-volatile and do not partition well into the vapor phase contribute to the difficulty of odor detection and characterization (Godage & Gionfriddo, 2019). Matrix composition and physiochemical properties of the target VOCs and bulk material strongly affect the headspace analysis (De Giovanni & Marchetti, 2020) as volatile compounds are extracted faster than semi-volatile. This results in a higher concentration in the headspace, contributing to faster mass transport rates in headspace analysis (De Giovanni & Marchetti, 2020). Challenges to improving extraction efficiency for the headspace analysis of explosives are bulk material age and storage. Bulk material that is stored for an extended time frame versus fresh will alter the odor profile of the material and influences the dominant VOCs present in the sample. This effect can be seen within the environmental chamber with target VOCs not being detected in the sample due to temperature, humidity, or age of the sample.

Future studies should consider the longevity of volatiles to correlate to the effectiveness of canine training aids in extreme environments. As the odor profile changes temporally, canine training aids are crucial to provide a developed regime for these environmental and temporal changes. Additionally, insight to sample location within the chamber is to be evaluated to ensure optimal target VOC detection. Location of samples in relation to heating or cooling system cycling has a potential effect on exposure of explosives due to odor movement and transport effects influenced by flux within the chamber.

Conclusion

Headspace analysis via SPME-GC-MS was implemented to provide chemical characterization of odor behavior in extreme environments to double base smokeless powder, ammonium nitrate (powder and prill form), composition 4, and TNT to provide insight into environmental factors on target explosive VOCs. Environmental parameters influenced the concentration of target VOCs throughout the study for each extraction mode. Quantitation of target VOC concentrations fell within the linear dynamic range > 0.98 for all VOCs analyzed.

Explosives extracted outside the chamber in ambient conditions resulted in decreased concentrations of explosives exposed in hot conditions and increased concentrations for explosives exposed in cold conditions. Conversely, concentrations of explosives exposed and extracted inside the environmental chamber resulted in expected odor behavior principles influenced by physiochemical properties and environmental factors. Explosive concentrations increased in hot conditions and decreased in cold conditions. Smokeless powder, ammonium nitrate (powder and prill), composition 4, and TNT were affected at all conditions for each target VOC in the headspace.

Chemical analyses of explosive bulk material demonstrated C4 had a significantly higher total VOC accumulation compared to all other explosives across all conditions (Table 18 and Table 19). The higher abundance of VOCs was attributed to the presence of C4 taggant which was detected in low concentrations as expected. TNT was most susceptible to environmental conditions given fluctuations between extractions modes (inside chamber vs outside chamber). TNT total VOC accumulation fluctuated based on extraction mode and environmental conditions. This trend indicates TNT is not rugged and canine training is to reflect this pattern for select explosives with sensitivity to environmental factors. AN prill and powder were subject to limitations from the surface area of bulk material and low vapor pressure. Both forms required additional extraction time and produced rugged results with total VOC accumulation across environmental conditions. Double based smokeless powder total VOC accumulation across extraction mode and environmental conditions were stable and resistant to change. PETN was subject to a decrease in total VOC accumulation across environmental conditions for the plasticizer detected as the primary VOC. PETN requires further studies to identify additional odor signatures for optimal odor profile identification. These results highlight the impact factor of extreme environmental conditions and odor behavior to the measured odor signature of key explosive classes.

These results highlight the impact factor of extreme environmental conditions and odor behavior to the measured odor signature of key explosive classes. The need for enhanced characterization of odor behavior in the presence of operational conditions is vital to promote the field of biological detectors. Understanding how the odor profile changes under extreme environmental conditions can help promote better canine training regimens and help understand odor quality from regularly used explosive training aids. Furthermore, this study implements instrumental analysis with dynamic headspace sampling and the effects of changes to the training environment for EDDs when exposed to explosives in extreme environmental conditions.

Task 4: Characterizing canine detection sensitivity and odor interactive effects in a simulated operational environment

Abstract

Task 2 demonstrated that detection canines can show poorer detection sensitivity under extreme environmental conditions when the concentration of the odor is controlled. This highlights that canine factors are driving this reduced sensitivity (rather than environmental changes on the odor), but this may not reflect real-world effects when odor availability is also changing based on environmental conditions (Task 3). The objective of this test was to evaluate changes in detection sensitivity limits from standard conditions when the odor availability was allowed to also change based on those conditions. Eight dogs from Task 2 participated in Task 4. Dogs were trained to search a series of three olfactometers with dilution capabilities of 80% to 3% and identify the olfactometer presenting the target odor. Dogs' thresholds were measured using a descending staircase 3-down 1-up procedure. All dogs received one threshold assessment per condition for four different energetic materials. Results were similar to Task 2 such that dogs showed best detection in standard conditions, and heterogeneous decrements were otherwise observed across the different environmental conditions for different odors. C-4 showed the greatest degree of disruption for the high temperature conditions (both high and low humidity). Additional measures collected confirmed Task 2's results that higher mean subcutaneous temperature was again associated with poorer detection limits and that longer latencies to start search were associated with poorer detection limits. In addition, we were able to measure dogs' time in navigating from one box to the next. This inter box interval (or speed of movement) was associated with poorer detection limits such that slower movement was associated with poorer detection limits. Results were overall similar to Task 2, suggesting that decrements in detection sensitivity in hot conditions are likely driven by canine factors and that increased odor availability in these conditions do not mitigate or improve detection limits. This does suggest, however, that canine acclimatization may be an important tool to mitigate detection decrements.

Methods and Materials

Participants

Eight dogs (6M, 2F; 4 Labrador retrievers, 4 German Short-Haired Pointers), were former candidates of a government detection program, and were available as participants in this project. The dogs had various amounts of prior training with explosives.

Animal welfare considerations

This project was conducted at Texas Tech University and was overseen by the Institutional Animal Care and Use Committee (Protocol #21051-07). In addition to their participation in this project all the dogs had additional enrichment activities every day and received food enrichment in their kennels. Throughout all testing, all dogs always had access to water.

Experimental Set up

The dogs were randomly split into two groups throughout the duration of this experiment (A and B). These groups worked with the target odorants in the same order but to limit order effects they worked the environmental conditions in a pseudorandomized order (see Table 23)

Research was conducted in a 3.6 x 3.4 m environmental chamber. The room was equipped with a heater, AC unit, dehumidifiers (hOmeLabs and ALORAIR), humidifiers (AILINKE), a fan and a mister system (homenote Misting Cooling System). The misters hung from the ceiling to help the room reach the required humidity conditions. The room also contained a SensorPush HT.w wireless thermometer/hygrometer sensor which measured the humidity and temperature in the room. Three Bluetooth enabled olfactometer boxes were arranged as a triangle in the room and presented all odors for canines (Figure 22).

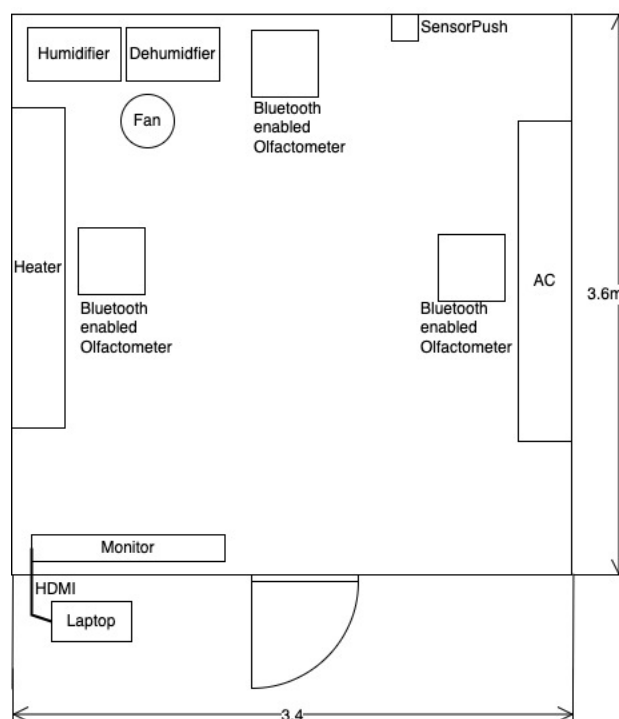


Figure 22: Environmental condition chamber room set up (overhead view). The humidifier and dehumidifier number varied based on conditions requirements. The Bluetooth enabled olfactometer boxes (3) or BLE olfactometers are pictured.

Odorants

Four odorants were used in this project; C-4, double-based smokeless powder, ammonium nitrate prill (agricultural grade), and TNT (see Table 20). There were no distractor odorants, all other valves of the olfactometer (described below) were connected to empty borosilicate glass vials matching the type used to contain the target odorants.

Table 20: Explosive material used as target odorants in Experiment 2

Odor	Mass per vial (g)	Brand of energetic material	Vial diameter (cm)
Double-base smokeless powder (SP)	1.039g	Hogdon H335	2.54
Ammonium nitrate (AN)	20.422g	Omni Explosives	5.08
Trinitrotoluene (TNT)	20.6	Omni Explosives	5.08
C-4	4.23g	Omni Explosives	2.54

Data collection equipment

Three Bluetooth enabled olfactometers were used in this project, which were a modification of our prior automated line up equipment (see Aviles-Rosa et al., 2021). The odor generation design was identical, but instead of having a horizontal panel of odor ports, the odor port was integrated into the olfactometer box itself. Additionally, the programming was modified to allow Bluetooth control of the olfactometers.

Each olfactometer was an independent replicate containing the same odor delivery system and odorants. Each olfactometer had six vials connected. One vial contained the target odorant (C-4, TNT, AN, SP) while the remaining five control vials were vials cleaned and prepared in a manner identical to the target vial.

Odor was generated from a 4L/minute oil-less air pump. Air from the pump first passed through a charcoal filter to scrub the air of odorants. The air was then split to an odor line and a dilution line via a T-connector. The odor line was regulated by a 0-1 lpm rotameter flowmeter and the dilution line was regulated by a 0-4 lpm rotameter flowmeter. After the regulating flowmeter, the odor line passed to a manifold of one-way valves. Upon activation of the valve, air passed into a vial containing the odorant (or a clean distractor vial). This displaced headspace of the vial through a check valve into a PTFE mixing manifold. The PTFE manifold mixed with the dilution line flow to present a controlled odor dilution to the odor port for the dog to sniff.

The olfactometer was controlled by a custom program (<https://github.com/njhall1/CanineOlfactometer>). Odor dilutions were controlled by manual manipulation of the flowmeters controlling the odor line and dilution line. The flowmeters allowed a practical dilution from 80 to 3% (see Table 21). Each olfactometer contained an infrared sensor beam pair that measured all canine entries into the odor ports.

This study utilized an alternative forced choice test design, in which the target odor was always present in one of the three olfactometers. The other two olfactometers were pushing air from one of the five control vials.

Table 21: Odor concentration changes for the 3 down 1 up staircase threshold procedure

Concentration (% odor line to clean air line)	Step number	Odor line flow rate (cc/min)	Clean line flow rate (L/min)
80%	1	1000	0.25
50%	2	1000	1.0
25%	3	750	2.25
12%	4	360	2.65
3%	5	90	2.91

For threshold testing, a series of dilutions was designed 80% then 50%, 25%, 12% and 3%. The flow rate, or the amount of odor being pushed into the port was consistent for each concentration, except for 80%. To get to the 80% concentration, the flow rate had to be lower.

Physiological Measures

During threshold testing, all dogs wore a wGT3X-BT Actigraph™ and a Polar® H10 Heart Rate monitor. The wGT3X-BT device was attached to the dogs' collar at the start of every session and monitored accelerometry data. The Polar® connected via Bluetooth to the Acrigraph™ device. The Polar® was placed around the abdomen of the dog just posterior to their front legs, beneath the harness (see Figure 4). Alligator clips were used to secure the sensor to the body and keep the rubber heart rate reader flush with the rib cage of the dog. Aquasonic Clear Ultrasound Transmission Gel was rubbed along the rubber part of the Polar® monitor band to improve conduction.

Temperature sensor

Each dog was equipped with a subcutaneous microchip over the shoulder that scanned their internal body temperature. At the start of every testing session, approximately every 5 minutes during the session, at the end of the session and 5-minutes post, an experimenter scanned the 134.2 kHz ISO microchip with a HomeAgain™ Universal Worldscan™ Reader Plus (by MERCK animal health) to get the body temperature of the dog.

Environmental conditions

There were 5 specific temperature and humidity conditions used in this experiment (see Table 22). Experimenters allowed the standard, low-temperature high humidity, high temperature low humidity and high temperature high humidity to vary by 3°C and 5% relative humidity (RH) from the stated condition to allow for natural fluctuations as airflow changed when the door of the environmental chamber was opened, or the AC unit began a thaw cycle etc. The low temperature low humidity condition was allowed to vary by 3°C and 10% RH because this condition was difficult to reach and maintain at the time of year this study was conducted.

Dogs were tested for one threshold session under each condition for each target odor. All dogs were first tested with each odor under standard conditions. Groups A and B (each four dogs randomly selected) followed a pseudo-randomized test order for the environmental conditions (see Table 23 for order). Each odor was tested sequentially to reduce the potential of olfactometer contamination by switching odors multiple times per day.

Table 22: Temperature and humidity conditions for each of the 5 conditions the dogs worked in

Condition	Temperature (°C)	Relative humidity (% RH)
Standard (STD)	21 ± 3	50 ± 5
High temperature high humidity (HTHH)	40 ± 3	70 ± 5
high temperature low humidity (HTLH)	40 ± 3	40 ± 5
Low temperature high humidity (LTHH)	0 ± 3	90 ± 5
Low temperature low humidity (LTLH)	0 ± 3	50 ± 10

Table 23: Indicates the orders each group of dogs worked through the conditions for each target odorant. Blocking of testing order was done to help limit order effects. STD=standard, HTHH= high temperature high humidity, HTLH=high temperature low humidity, LTHH= Low temperature high humidity, LTLH= low temperature low humidity.

Groups (n=4)	Odor	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5
Group A	C-4	STD	LTHH	HTHH	HTLH	LTLH
Group B	C-4	STD	HTLH	LTLH	LTHH	HTHH
Group A	SP	STD	HTLH	LTHH	LTLH	HTHH
Group B	SP	STD	LTHH	HTLH	HTHH	LTLH
Group A	AN	STD	HTHH	LTLH	LTHH	HTLH
Group B	AN	STD	LTHH	HTHH	LTLH	HTLH
Group A	TNT	STD	LTHH	HTHH	HTLH	LTLH
Group B	TNT	STD	HTHH	HTLH	LTLH	LTHH

Canine odor detection testing and training

All canines had been previously trained to detect explosives in an olfactometer during a previous study (see Task 2). In the previous experiment, the dogs used an air dilution olfactometer with a go/no-go paradigm. Dogs transitioned to a 3 alternative forced choice test prior to starting threshold testing. Canines were trained on the procedure for each target odor until dogs reached 85% accuracy at the 80% concentration.

All training and testing was conducted under double blind conditions such that the handler was never aware of the location of the olfactometer presenting the target odor. Canine response (i.e. alerts), were scored automatically using the infrared beam pairs. Dogs were trained to hold their nose within the port containing the target odor (breaking the IR beam pair) for 4 consecutive seconds. If dogs made a correct response, a “correct” tone played indicating to provide a reinforcer to the dog. If the dog made a false alert or failed to respond to the correct port, an “incorrect” tone played. At the end of the trial, there was a 20s inter-trial interval to purge the olfactometers of any residual odor.

Canine odor threshold testing

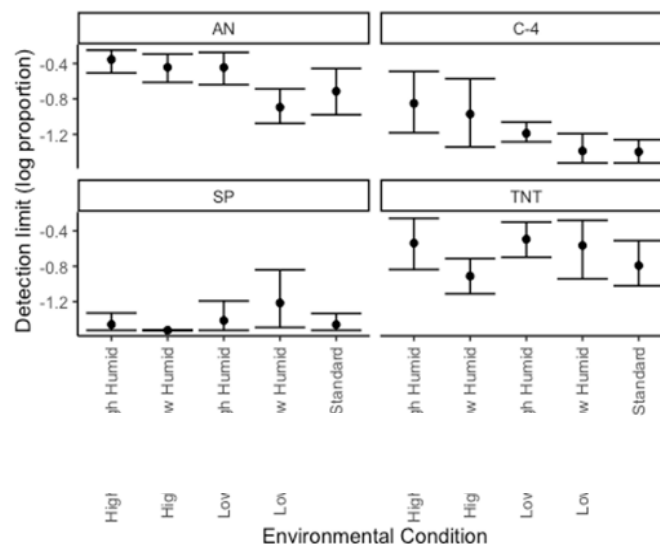
The threshold testing was conducted as an alternative forced choice task with a 3-down 1-up descending staircase procedure. In an alternative forced choice task, the target odorant was present in one of the three olfactometers in every trial. In the three down, one up threshold test procedure, after three consecutive correct trials the odor concentration was decreased by one step. If the dog made an incorrect response, the odor concentration was increased by one step. A reversal point was defined as anytime the direction of concentration change reversed directions. Threshold testing continued until dogs completed seven reversals. Testing also terminated if dogs made three consecutive correct responses at the lowest concentration step (3%). Testing also terminated if dogs met a welfare criterion by not searching for five consecutive trials or laid down and would not search the ports when prompted.

Data Analysis

Threshold was calculated as the geometric mean of all seven reversal points. Threshold was log transformed for all analyses. If dogs' detection exceeded the maximum dilution capabilities, the lowest dilution point was used as a reversal point. If dogs exceeded the dilution capability prior to reaching all reversals, the remaining reversals were treated as missing. Thus, if a dog scored perfectly, going from 80% to 3%, threshold was imputed as 3% (maximum dilution).

Results and Discussion

The thresholds for each odor in each condition can be seen in Table 24 and Figure 23. The detection limits (log scale) for each odor varied by condition and odor identity, as was seen in Task 2. For smokeless powder, dogs' detection limits were lower than the dilutions



available by the Bluetooth olfactometers. This was expected based on the detection limits observed in Task 2. Overall, dogs' estimated detection limits were slightly lower (improved)

Figure 23: Detection limit (log scale) for each odorant in each environmental condition. The figure indicates that the detection limit for smokeless powder (SP) was not reached.

than that observed in Task 2, which is expected in changing from a go/ no go task to a forced choice procedure and as a result of further training.

To evaluate the effect of environmental conditions on detection limits, a linear mixed effect regression model fit in which log threshold was predicted by breed, environmental condition, odor and an interaction between both factors, with a random effect of individual dog. This model produced a residual plot with some boundary effects, due to the limitations of the highest and lowest concentrations produced by the olfactometers (maximum concentration was 80% and minimum was 3%). Otherwise, the residual plot showed no patterns and so the model was deemed fit for interpretation. The anova (Fox & Weisberg, 2018b) analysis of this model indicated that there was a significant effect between odor ($X^2=176.3$ DF=3, $P<0.001$) and conditions ($X^2= 14.71$, DF=4, $P<0.01$) and their interaction ($X^2= 31.04$, DF= 12, $P<0.01$) on detection limit. Breed, however, had no impact ($X^2= 0.46$, DF=1, $P=0.50$).

Post hoc tests were conducted comparing each environmental condition to standard conditions for each target odor (see Table 25). Post hoc tests showed that detection limits for C-4 were higher (poorer) under high temperature high humidity (est=0.54, $t=3.29$, $p=0.005$) and high temperature low humidity conditions (est=0.43, $t=2.57$, $p=0.02$). Low temperature low humidity and low temperature high humidity conditions did not differ from standard conditions ($p>0.05$). Smokeless powder thresholds did not differ between environmental conditions (all $p>0.05$), but this may reflect that dogs' thresholds was below the limits of dilution of the olfactometer.

For AN, poorest detection limits occurred under the hot temperature and low temperature high humidity conditions, however none of these differences from standard reached a traditional statistical criterion. For smokeless powder, dogs' detection limits were below that of the olfactometer. Thus, dogs exceeded the dilution capacity for all environmental conditions. For TNT, dogs showed detection difficulty in all conditions and no environmental condition differed from chance.

Table 24: Detection limit by odor in each condition.

Odor		Standard	High Temp High Humid	High Temp Low Humid	Low Temp High Humid	Low Temp Low Humid
AN	Mean	-0.71	-0.36	-0.44	-0.45	-0.89
	95% CI	-0.96, -0.47	-0.60, -0.11	-0.69, -0.20	-0.69, -0.20	-1.14, -0.65
C-4	Mean	-1.40	-0.85	-0.97	-1.19	-1.39

	95% CI	-1.64, -1.15	-1.09, -0.61	-1.22, -0.73	-1.43, -0.94	-1.63, -1.14
SP	Mean	-1.46	-1.46	-1.52	-1.41	-1.21
	95% CI	-1.70, -1.2	-1.70, -1.21	-1.77, -1.28	-1.66, -1.17	-1.46, -0.97
TNT	Mean	-0.79	-0.54	-0.91	-0.49	-0.57
	95% CI	-1.04, -0.545	-0.785, -0.292	-1.16, -0.66	-0.741, -0.248	-0.811, -0.318

Table 25: Detection limit comparisons for each odorant in each condition vs. standard.

Conditions	High Temp High Humid		High Temp Low Humid		Low Temp High Humid		Low Temp Low Humid	
Odor:	t-ratio	P	t-ratio	P	t-ratio	P	t-ratio	P
AN	2.16	0.13	1.63	0.14	1.62	0.14	-1.09	0.28
C-4	3.29	0.005	2.57	0.02	1.26	0.28	0.06	0.95
SP	0.01	0.99	-0.38	0.99	0.28	0.99	1.48	0.57
TNT	1.53	0.23	-0.72	0.47	1.79	0.23	1.36	0.23

Factors affecting detection limit

Canine subcutaneous temperature and detection limit

Canine subcutaneous temperature across the session is shown in Figure 24. Only the first 25 min are shown due to the variable session length to complete reversals, this captured most data from all dogs. Similar to that observed before in Task 2, temperatures were highest in the high temperature and high humidity condition. A simple mixed effect model evaluated the impact of environmental condition on the temperature recorded (with a random effect of dog). Condition had a significant impact on subcutaneous temperature recorded ($\chi^2=1082$, $df=4$, $p<0.0001$). Post hoc tests indicate that all temperature conditions differed except the low temperature low humidity and low temperature high humidity conditions. Overall, temperature was highest in the high temperature and high humidity conditions and even higher than that observed at the same temperature but lower humidity ($t=3.19$, $p=0.013$).

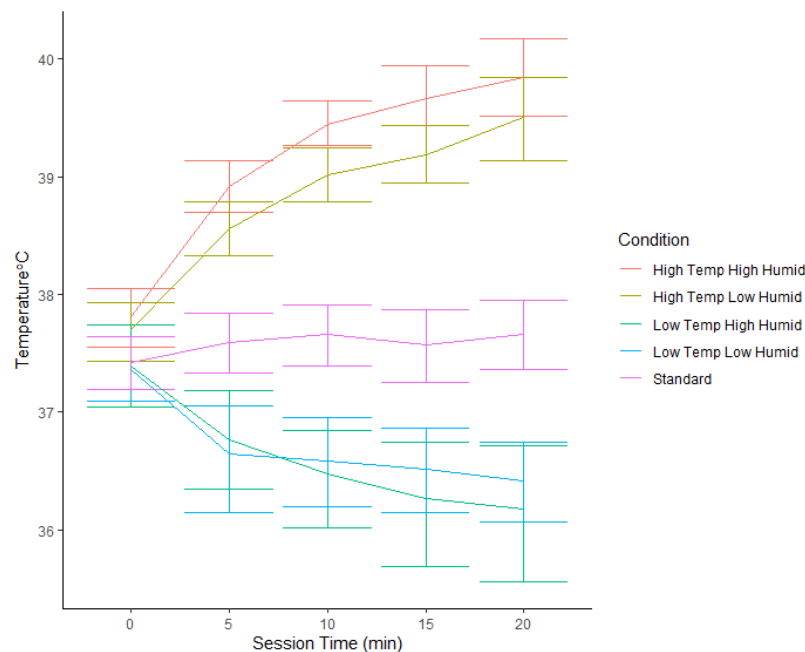


Figure 24: Canine internal temperature change throughout a session, broken down by condition. The maximum session time was 40m, however most dogs finished the detection task within 20 min (which is shown). Error bars show the 95% boot strap estimated confidence intervals.

Next, we evaluated the impact of mean subcutaneous temperature within a session on measured thresholds. First, we removed the low temperature conditions due to the biologically implausible values which suggested that subcutaneous temperature readings were unlikely useful in cold temperatures. Next, we conducted a mixed effect model to evaluate whether threshold was predicted by mean subcutaneous temperature (for hot and standard conditions), odor and their interaction. There was no significant interaction between odor and mean temperature on threshold ($\chi^2=2.07$, $df=3$, $p=0.56$). Threshold was however impacted by odor ($\chi^2=111$, $df=3$, $p<0.0001$) and mean temperature ($\chi^2=6.59$, $df=1$, $p=0.01$). As mean temperature in a session

increased by a degree C, threshold increased (became poorer) on average by 0.18 on the log scale. Thus, dogs with higher overall mean temperatures during a session was associated with poorer detection thresholds (see Figure 25).

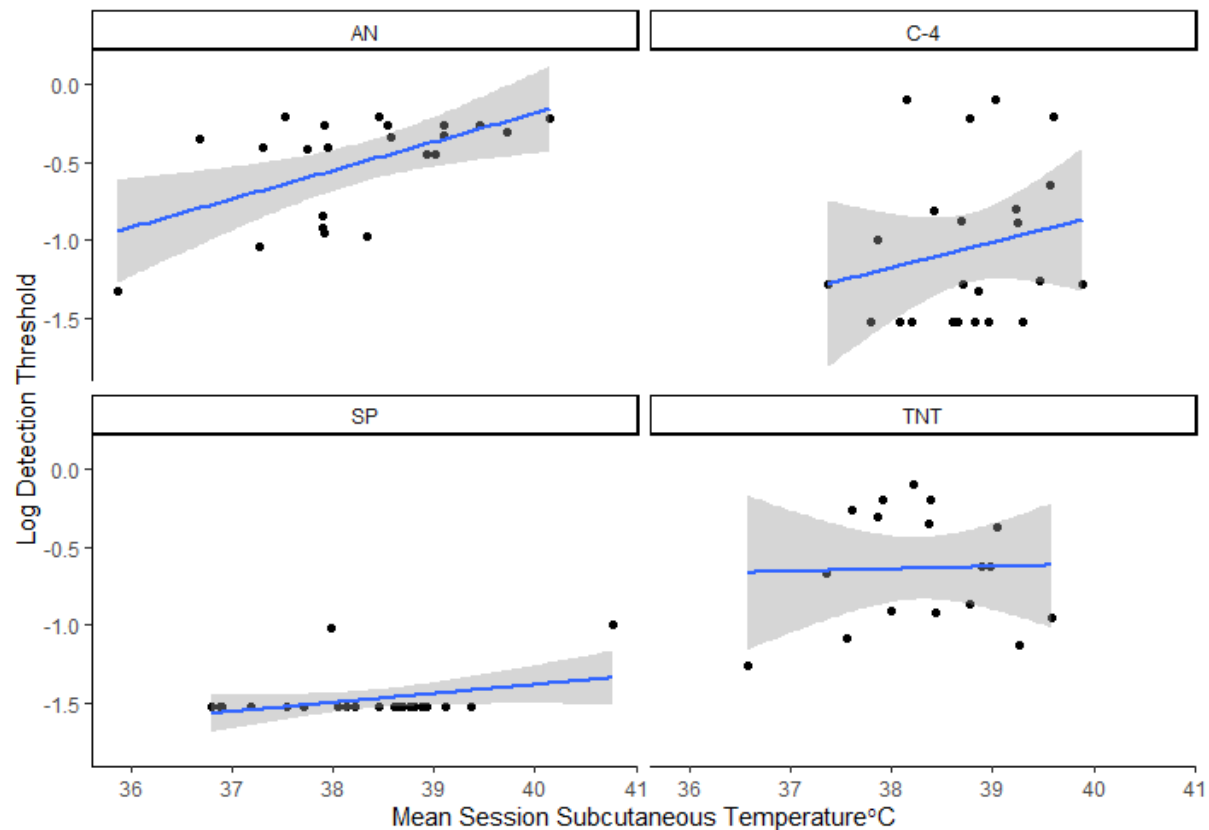


Figure 25: Effect of Subcutaneous temperature on Detection Limits. Line shows the best fit regression for each odor.

Heart rate and detection limit

Another physiological measure gathered during the study was canine heart rate (HR). Similar to Task 2, substantial sensor drift was observed during the session with a high frequency of inadequate reads during animal movement. Thus, HR data was not leveraged for further analysis.

Latency and Inter-Olfactometer-Interval

Two additional measures were examined for their relationship to detection threshold. First latency was measured as the time from the start of the trial until the dog inserted its nose into an odor port. Latency to start a trial did differ by the environmental conditions ($\chi^2=10.19$, $p=0.04$). Post hoc test indicate, however, that the only pairwise difference between conditions was that longer latencies were observed in the low temperature high humidity condition compared to standard ($t=2.81$, $p=0.04$).

Although there was minimal difference in latency between conditions, a mixed effect model was fit in which mean session latency, odor, and their interaction predicted log threshold. Results indicate there was no interaction between odor and latency ($\chi^2=2.06$, $p=0.56$), but there were main effects of latency ($\chi^2=27.09$, $p<0.0001$) and odor ($\chi^2=157.49$, $p<0.0001$). For every 1 s mean increase in latency there was a 0.06 increase in log threshold detection limit (poorer detection; see Figure 26).

Secondly, mean inter-box-interval was calculated for each session and trial. The inter-box-interval reflected the time the dogs took between nose pokes, reflecting time to get from one box to the next. The inter-box-interval did not vary in a statistically significant manner between the environmental conditions ($\chi^2=6.57$, $p=0.16$). Interestingly, however, the mean session inter box interval was related to overall threshold detection. A mixed effect model predicting threshold by the mean session inter box interval, odor, and their interaction, indicated that mean inter box interval predicted threshold ($\chi^2=11.06$, $df=1$, $p=0.001$). Odor also predicted threshold as expected ($\chi^2=143.25$, $df=3$, $p<0.0001$) and there was no interaction between inter box interval and odor ($\chi^2=5.26$, $p=0.15$). An average increase in the inter box interval of 1s led to an average increase in log threshold of .22, indicating the taking longer to go from one box to the next was associated with poorer thresholds (see Figure 27).

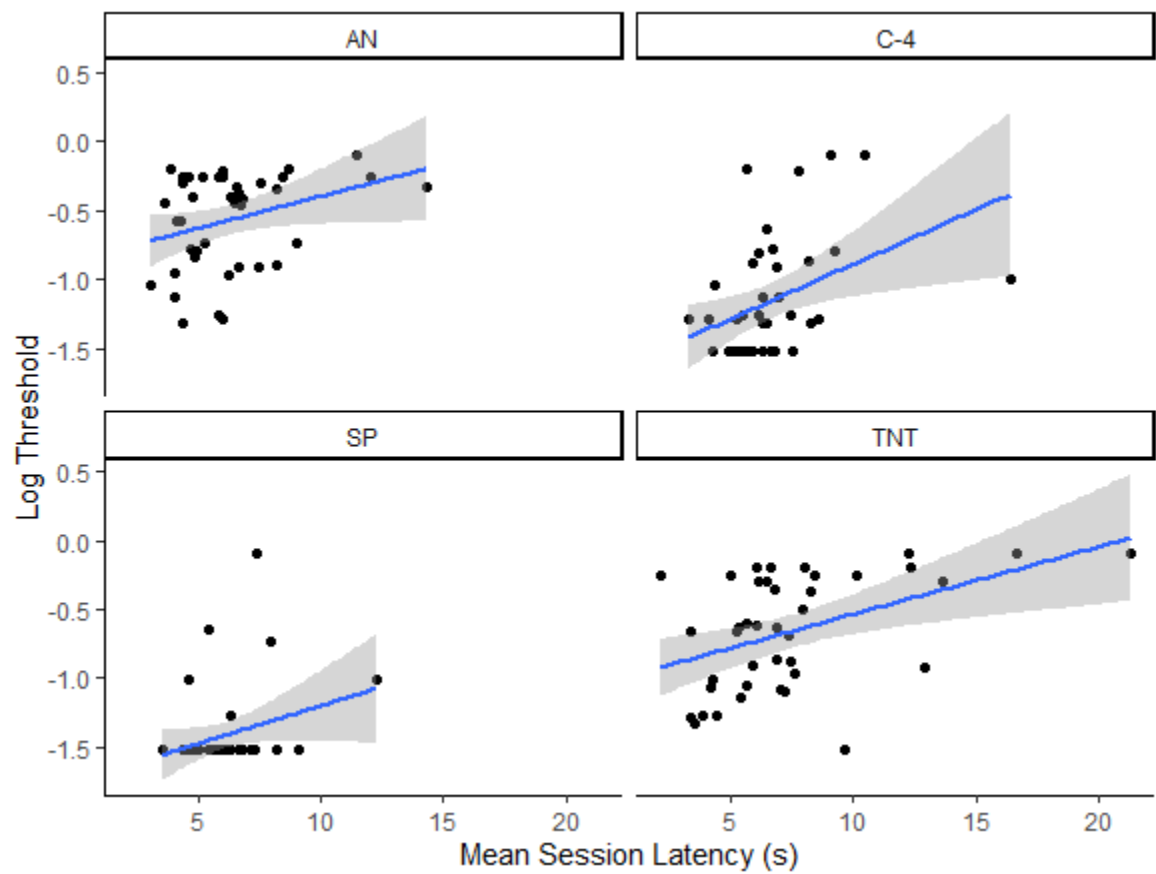


Figure 26: Effect of mean session latency on log detection threshold.

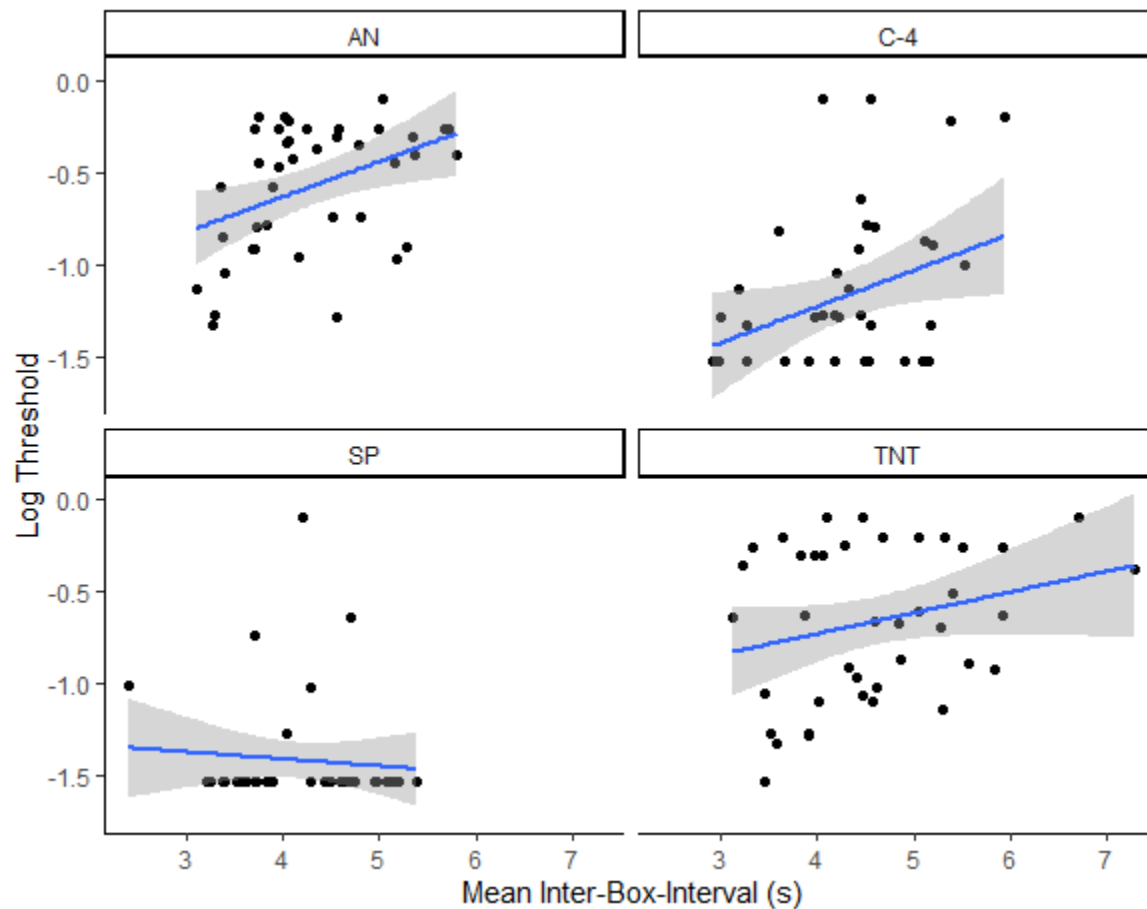


Figure 27: Effect of the interval between olfactometer boxes on log detection threshold.

Discussion and Conclusions

The results of Task 4 indicate that dogs showed a significant decrement in detection limits for C-4 under high temperature and high humidity and high temperature low humidity conditions. Detection limits changed such that under standard conditions dogs could detect <3% dilution of C-4; however, in the high temperature conditions dogs failed to detect ~14% dilution (nearly a 10-fold change). Results were highly similar in direction and magnitude as with C-4 for the target odor of AN, but the comparisons did not reach the statistical criterion. Due to the limited sample size and inability to conduct long term testing that may cause acclimatization, the test may have been too underpowered to reach the statistical criterion.

Smokeless powder thresholds were unfortunately below the dilution range capable of the olfactometer. Dogs could detect smokeless powder at the lowest dilution (3%) across all

conditions. If an olfactometer with a greater dynamic range was leveraged, it's likely the environmental effects observed in Task 2 would have been replicated. TNT, however, showed poor detection sensitivity across all environmental conditions, which therefore made it difficult to detect decrements.

In addition to environmental condition alone, there were several additional physiological and behavioral measures that served as predictors for poor threshold detection limits. First was mean subcutaneous temperature. Higher mean subcutaneous temperature was associated with poorer detection limits. This suggests that canine subcutaneous temperature maybe a simple and useful metric to predict or warn handlers of poorer detection limits. Additionally, latency to start search also predicted detection threshold where dogs that took longer to initiate search showed poorer detection limits. Similarly, the duration it took dogs to move from one box to the next box (inter-box-interval) was also associated with detection limits. When dogs took longer to navigate from one box to the next, detection threshold limits were higher (poorer). Together, this suggests that when dogs start moving slower and take longer to start a search, this could suggest a simple inductive behavior of poorer detection.

One consideration is whether slower movement from one box to the next may reflect more detailed search due to a more challenging task. However, given that similarly, decreases in latency (time from given the command to search and searching) also lead to poorer performance suggest that the result likely reflects overall decreased activity/speed rather, decreases in speed due to more detailed search. Thus, although further testing would be important, this does suggest that declines in search speed or delays to initiate search maybe a useful marker of reduced canine sensitivity and performance.

There are important limitations to the present study. First the dilution range of the olfactometer used was inadequate to provide the range of dilutions necessary to reach threshold for smokeless powder and under standard conditions, for C-4. Further, some of the environmental conditions, particularly the cold conditions, were not as extreme as what many detection dogs may need to operate in. The low temperature conditions of 0°C was chosen based on the physical limits of the environmental chamber. However, this is not an extreme cold by which many detection dogs may need to work. Thus, further research is necessary is subzero conditions. This could be accomplished with future tests that evaluate more dogs under a greater range of natural environmental conditions and would be a useful next step.

Overall, the results from this study suggest that decrements in detection performance appear to be largely, or at least importantly, impacted by biological/physiological effects on the canine rather than grossly changing odor availability. This is exemplified by performance decrements occurring in high temperature conditions, especially in high temperature and high humidity conditions where odor availability was at its greatest. This highlights the potential that canine acclimatization and conditioning could have on mitigating potential performance decrements.

Supplemental Task: Instrumental Validation of explosive odor delivery method: Olfactometer dilution evaluation

Abstract

Explosive Detection Dogs (EDDs) possess an innate superior olfactory system and can detect a multitude of pure or complex odors within seconds of exposure time. EDDs are utilized by government and law enforcement agencies for a range of detection applications for routine field operations. Evaluation of current target odors is needed to develop and advance odor delivery systems used in canine training regimes. Composition 4 (C-4); 2,4,6-trinitrotoluene (TNT), ammonium nitrate (AN) prill, and double base smokeless powder were used as the target bulk material to validate and confirm the target volatile organic compounds (VOCs) emitted from explosives at set odor dilutions (80%, 50%, and 25%) using an in house olfactometer as the odor delivery method. Instrumental analysis included solid phase microextraction fiber (SPME) chemistry for target odors as a function of peak area response via gas chromatography-mass spectrometry (GC-MS). Target odors were externally calibrated to determine target odor concentrations (mg L⁻¹) with a linear dynamic range > 0.98. Studies were conducted measuring the output line of the olfactometer device to monitor target explosive odor volatile concentration at the corresponding odor dilution. Explosive odor presence was detected for Composition 4, TNT, and ammonium nitrate prill; providing a robust delivery system for three explosive classes tested. Smokeless powder was not detected at any dilution percentage. Odor dilution showed a direct linear relationship with odor response, increased odor availability resulted in an increased odor response. This study evaluates the presence of target explosive odors using an olfactometer. Successful validation of the odor delivery device resulted in C-4 and TNT identified and validated at all odor dilutions and ammonium nitrate prill was validated at 80% and 50%. Smokeless powder was not detected at any dilution factor. Presence of explosive odor provides a need for monitoring of indirect headspace sampling for canine detection training to validate the use of odor delivery systems in canine detection training.

Chemicals and explosive samples

Composition 4 (C4), ammonium nitrate (AN) prill, and 2,4,6-trinitrotoluene (TNT) were purchased from Omni Distribution, Inc (Marion, AR, USA). Double based smokeless powder #335 was purchased from Hodgdon Powder Co., Inc (Shawnee, KS, USA). Ammonium nitrate (AN) powder was purchased from Sigma-Aldrich (St. Louis, MO, USA). Calibration chemical standards used included diphenylamine (DPA), 2,3-dimethyl, 2-3-dinitrobutane (DMNB), and hexanal from Sigma-Aldrich (St. Louis, MO, USA). 2-ethyl, 1-hexanol from Thermo Fisher Scientific (Waltham, MA, USA). Analytical grade solvents used for calibration included acetone, methylene chloride, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Olfactometer Design

Construction, electronic components, and dimensions are denoted (Aviles-Rosa et al., 2021). Briefly, an electromagnetic air pump functioned as the air source to compress ambient room air. The compressed air was passed through an activated charcoal filter to remove environmental contaminant volatiles. The filtered air was then split and distributed into two rotameters, regulating two independent flow paths. Line one (dilution flow path) was used as for continuous airflow, directly connected to the odor mixing PTFE (Teflon) manifold and delivered to the odor port. Line two (odor line) and carried filtered air to a six-channel solenoid valve manifold (Figure 28A). A 1/8" OD PTFE tube connected each valve of the manifold to a glass VOC vial with septa lid (PTFE, silicone, PTFE septa). Activation of a solenoid valve passed clean air from the selected valve to the jar connected. A second 1/8" PTFE tube inserted into the septa carried the headspace of the sample into the PTFE manifold. This tube was connected to a stainless-steel check valve to prevent reverse flow contamination between the manifold and sample jar. The mixing manifold produced dilutions at 80%, 50% and 25% for select explosive odorants to the odor port. Line one and two settings for each dilution are shown in Table 26. Odor valves were activated and controlled via Bluetooth. To ensure consistent airflow, one valve remained active for the duration of the sampling period. The valve was connected to a clean blank vial or a vial containing bulk explosive sample.

A



B



Figure 28: A) Olfactometer six-channel solenoid valve manifold. B) Solid phase microextraction (SPME) fiber olfactometer sampling procedure.

Table 26: Olfactometer Dilution Ratios

Dilution (%)	Odor Line (mL min ⁻¹)	Dilution Line (L min ⁻¹)
80	1000	0.25
50	1000	1.0
25	750	2.25

Odor line- continuous airflow directly connected to mixing manifold and output line for dilution percentage. Dilution line – airflow is routed to mixing manifold to create dilution percentage.

Solid-Phase Microextraction (SPME) Sampling Procedure

Identification of target odorants for smokeless powder, AN prill, C4, and TNT from the odor port of the olfactometer were analyzed via SPME-GC/MS. Olfactometer odor dilutions were determined by the ratio of line one and line two settings calculated in Equation 1:

$$\text{Dilution factor (\%)} = \frac{L_D}{L_O + L_D} * 100 \quad (1)$$

where L_D represents the dilution flow path line (cc min⁻¹), and L_O represents the odor line (cc min⁻¹). L_O expressions are measured in L min⁻¹ and converted to cc min⁻¹ for dilution factor calculation. All samples were analyzed with a SPME fiber injected into the odor port to extract for 1 min. SPME fiber was wrapped in parafilm to ensure complete transfer from the odor port to the injected fiber (Figure 28B). The fiber was retracted, removed, and the blank valve was activated for 1 min to purge the line of residual odor. Six extraction replicates (n = 6) were analyzed to obtain average peak area responses for target volatiles and evaluated for concentrations of volatiles by external standard calibration. All chromatographic separations utilized an Agilent 7890A GC Series GC system, equipped with front and rear split/splitless injection ports, fitted with an HP5 30 m, 0.25 mm i.d., 0.25 mm capillary column. Helium was the carrier gas at a flow rate of 1 mL min⁻¹ at an average velocity of 37 cm s⁻¹. The mass spectrometer (MS) operated in electron ionization (EI) full scan mode from 45 to 550 amu, with a 3-min solvent delay. Target VOCs were identified using the National Institute of Standards and Technology (NIST) (2017) mass spectral reference library and verified with external standard

chemical calibration. Criteria for identified compounds were samples with detected peak areas greater than a match quality of 80%. Blank background subtraction was performed to exclude compounds as products of the column or sampling process. Optimized GC-MS methods are listed in Table 27, Table 28, Table 29, Table 30.

Table 27: C4 GC-MS parameters

Oven temperature program	40 °C, hold 4 min. 10 °C/min to 300 °C, hold 2 min. Total 32.0 min
Inlet split ration	Splitless
Inlet temperature	265 °C

Table 28: TNT GC-MS parameters

Oven temperature program	40 °C, hold 2 min. 20 °C/min to 200 °C, hold 2.5 min. 20 °C/min to 250 °C, hold 5 min. Total 20.0 min
Inlet split ration	Splitless
Inlet temperature	250 °C

Table 29: Ammonium nitrate prill GC-MS parameters

Oven temperature program	40 °C, hold 1 min. 20 °C/min to 240 °C, hold 3 min. Total 18.0 min
Inlet split ration	Splitless
Inlet temperature	250 °C

Table 30: Double based smokeless powder GC-MS parameters

Oven temperature program	40 °C, hold 1 min. 15 °C/min to 200 °C, hold 1 min. 15 °C/min to 240 °C, hold 6.5 min. 25 °C/min to 270 °C 5 °C/min to 280 °C, hold 4 min. Total 29.03 min.
Inlet split ration	Split 1:5
Inlet temperature	280 °C

Target VOC External Calibration

Composition 4 (C-4)

The target VOC detected via dynamic airflow sampling for C-4 is 2,3-dimethyl-2,3-dinitrobutane (DMNB). Calibration curves were performed using direct liquid injections of 5, 10, 20, 40, 80, and 100 mg L⁻¹ (Figure 29). 2,3-dimethyl-2,3-dinitrobutane (DMNB) standard solutions were prepared in analytical grade methanol. The response factor for all concentrations yielded an r^2 value of 0.9924 for DMNB. The average response factor for DMNB was evaluated to interpolate the concentration of target VOCs in the odor port expressed in milligrams/liter (mg L⁻¹). The calculated limit of detection (LOD) and limit of quantitation (LOQ) for DMNB were 0.67 and 2.03 mg L⁻¹, respectively.

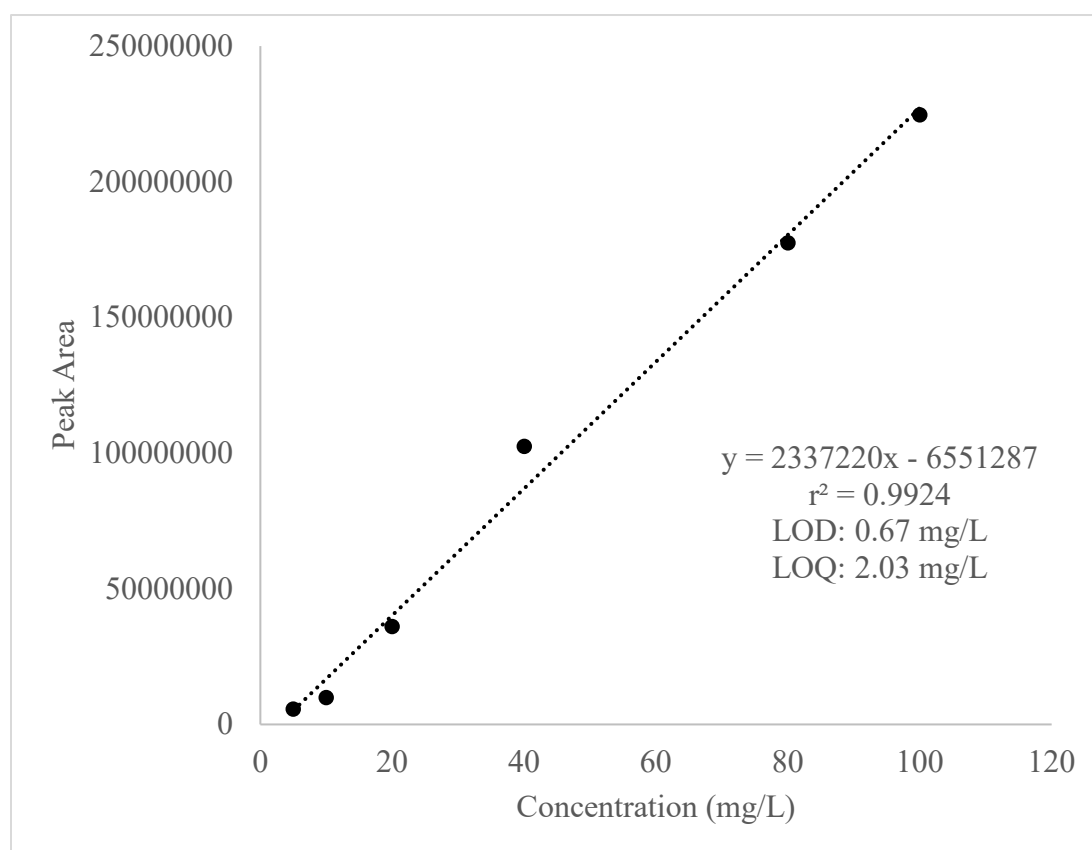


Figure 29: Composition 4 (C-4) target VOC calibration curve 5-100 mg L⁻¹. External calibration for 2,3-dimethyl, 2,3-dinitrobutane. r^2 – coefficient of determination, LOD – limit of detection, LOQ – limit of quantitation.

2,4,6-trinitrotoluene (TNT)

The target VOC detected via olfactometry for TNT is 2-ethyl, 1-hexanol (2E1H). Calibration curves were performed using direct liquid injections of 5, 10, 20, 40, 80, and 100 mg L⁻¹ (Figure 30). 2-ethyl, 1-hexanol standard solutions were prepared in analytical grade acetonitrile. The response factor for all concentrations yielded an r^2 value of 0.9905 for 2E1H. The average response factor for 2E1H was evaluated to interpolate the concentration of target VOCs in the odor port expressed in milligrams/liter (mg L⁻¹). The calculated LOD and LOQ for 2E1H were 0.27 and 0.83 mg L⁻¹, respectively.

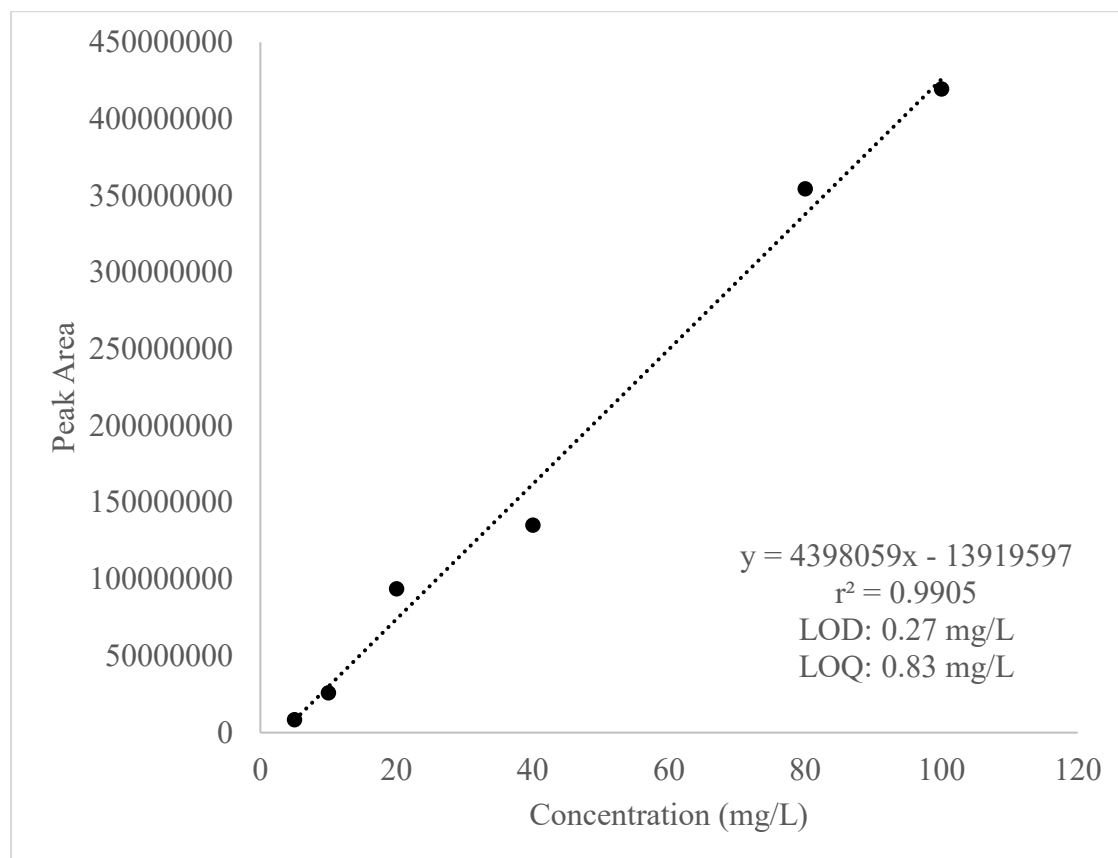


Figure 30: 2,4,6-trinitrotoluene (TNT) target VOC calibration curve 5-100 mg/L. External calibration for 2-ethyl, 1-hexanol (2E1H). r^2 – coefficient of determination, LOD – limit of detection, LOQ – limit of quantitation.

Ammonium nitrate prill

The target VOC detected via olfactometry for ammonium nitrate prill is 2-ethyl, 1-hexanol (2E1H). Calibration curves were performed using direct liquid injections of 5, 10, 20, 40, 80, and 100 mg L⁻¹g (Figure 31). 2-ethyl, 1-hexanol standard solutions were prepared in analytical grade acetone. The response factor for all concentrations yielded an r^2 value of 0.9902 for 2E1H. The average response factor for 2E1H was evaluated to interpolate the concentration of target VOCs in the odor port expressed in milligrams/liter (mg L⁻¹). The calculated LOD and LOQ for 2E1H were 0.62 and 1.87 mg L⁻¹, respectively.

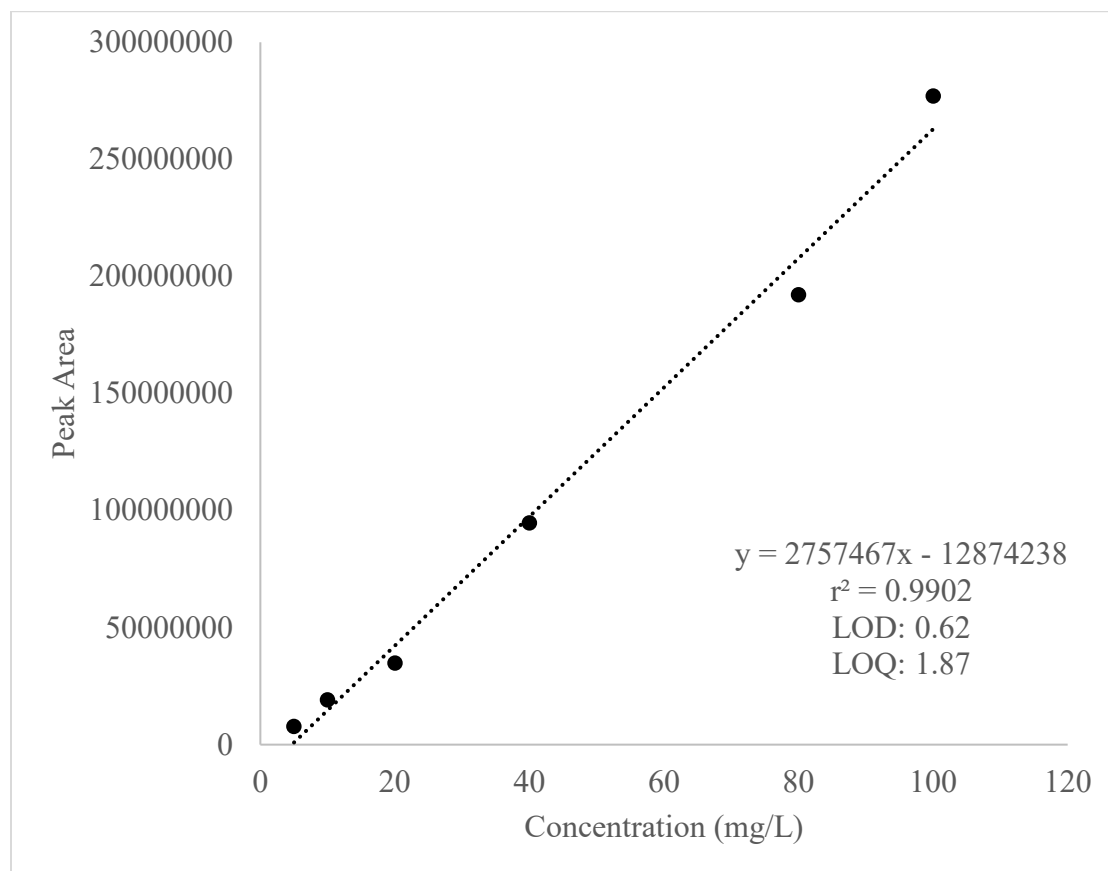


Figure 31: Ammonium nitrate prill (AN prill) target VOC calibration curve 5-100 mg/L. External calibration for 2-ethyl, 1-hexanol (2E1H). r^2 – coefficient of determination, LOD – limit of detection, LOQ – limit of quantitation.

Double base smokeless powder

The target VOC detected via olfactometry for ammonium nitrate prill is diphenylamine (DPA). Calibration curves were performed using direct liquid injections of 5, 10, 20, 40, 80, and 100 mg L⁻¹ (Figure 32). Diphenylamine standard solutions were prepared in analytical grade methylene chloride. The response factor for all concentrations yielded an r^2 value of 0.9902 for 2E1H. The average response factor for 2E1H was evaluated to interpolate the concentration of target VOCs in the odor port expressed in milligrams/liter (mg L⁻¹). The calculated LOD and LOQ for DPA were 0.44 and 1.33 mg L⁻¹, respectively.

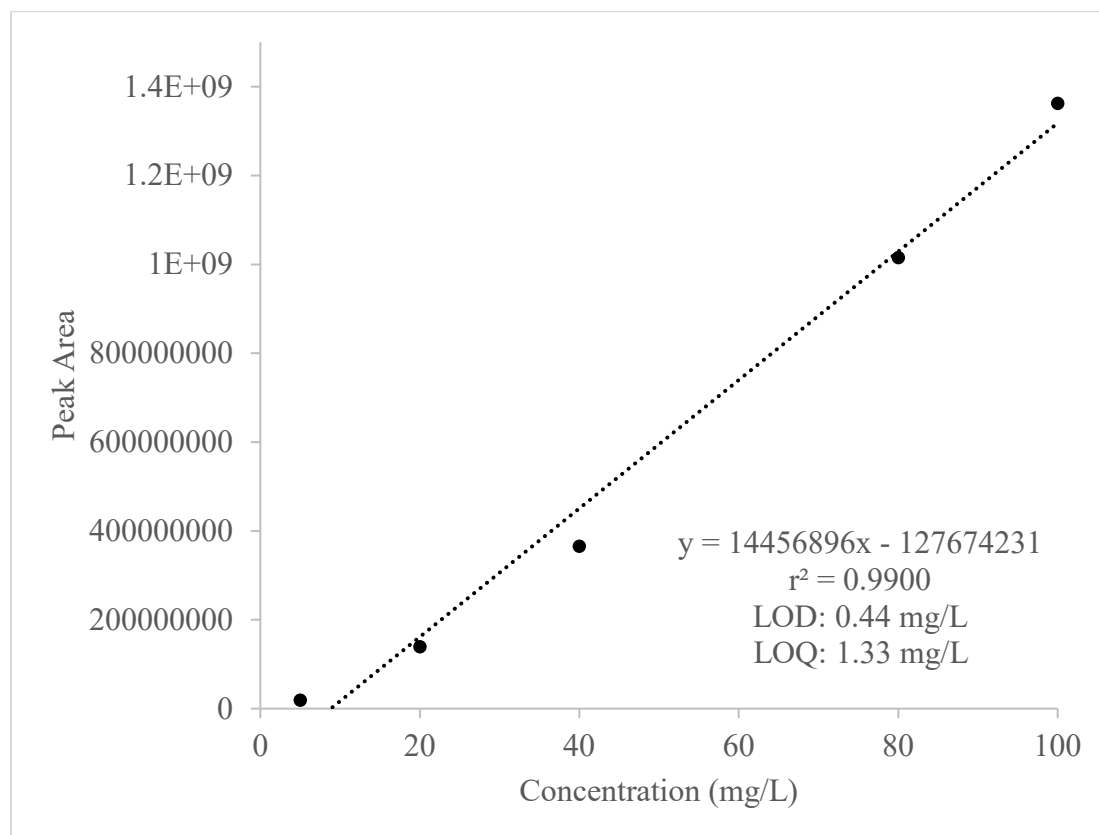


Figure 32: Double-based smokeless powder target VOC calibration curve 5-100 mg/L. External calibration for diphenylamine (DPA). r^2 – coefficient of determination, LOD – limit of detection, LOQ – limit of quantitation.

Statistical Analysis

Target VOC concentrations from intraday sampling were analyzed with Analysis of Variance (ANOVA) and Tukey's multiple comparison test (normally distributed) or by the Kruskal-Wallis test with Dunn's multiple comparison test (if not normally) distributed using JMP statistical analysis software.

Composition 4

The optimal extraction fiber for this explosive was a SPME fiber chemistry of polydimethylsiloxane (PDMS). Intraday and interday mean concentrations are shown in Figure 33. Interday concentrations of DMNB for 80%, 50%, and 25% dilutions were 41.96, 32.24, and 5.40 mg L⁻¹, respectively. The coefficient of variation (CV) at each dilution percentage was 32%, 4.5%, and 25%, respectively. Figure 34 shows average peak area of DMNB for each odor dilution factor.

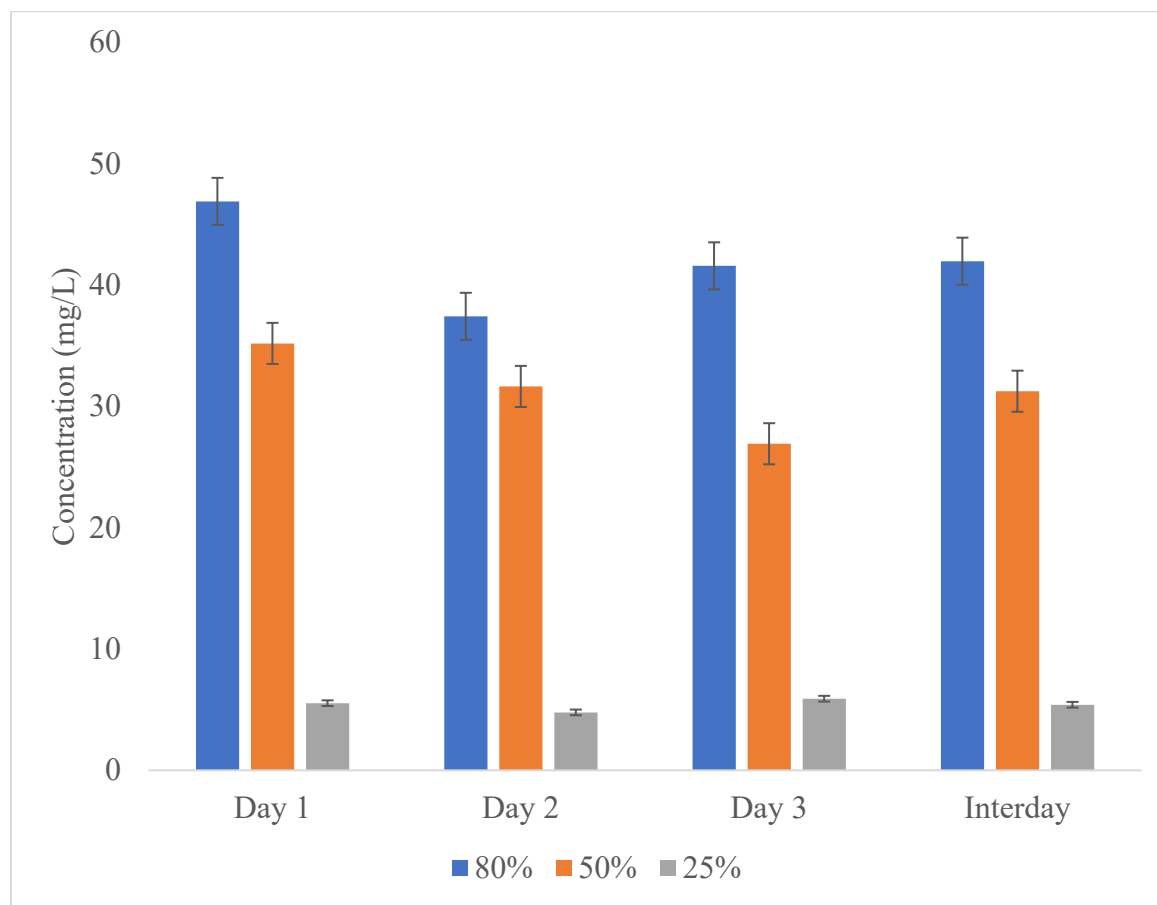


Figure 33: C-4 Odor Dilution - Inter- and intra-day mean concentrations for 2,3-dimethyl-2,3-dinitrobutane (DMNB) at 80%, 50%, and 25% (N = 6). Replicates were sampled three consecutive days. No significant differences for average concentration reported between all days (intraday). Ambient laboratory conditions were not statistically different among consecutive sampling

days. All dilutions (80%, 50%, 25%) were not affected by ambient sampling conditions due to relative temperature and humidity each sampling day.

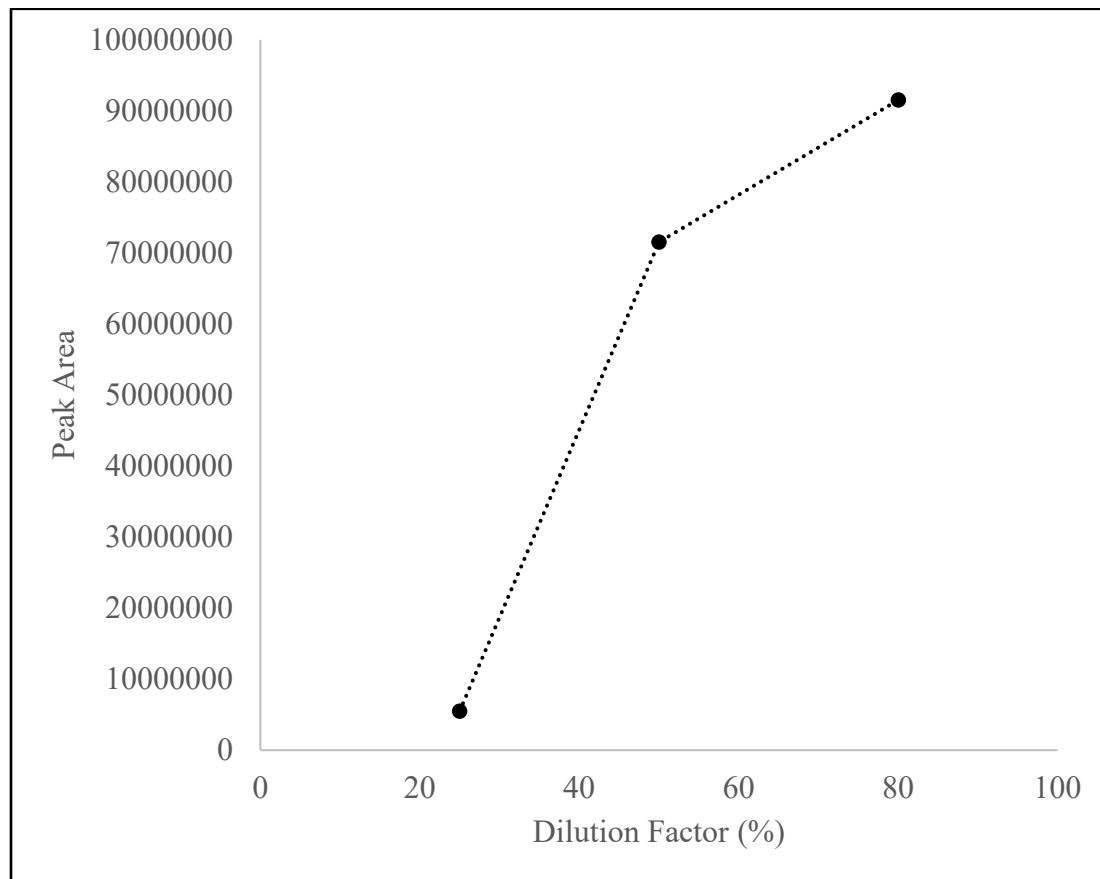


Figure 34: Composition 4 (C-4) Olfactometer Odor Dilution Response for DMNB. C-4 mean peak areas for 2,3-dimethyl-2,3-dinitrobutane (DMNB) at 80%, 50%, and 25% dilution percentages.

TNT

The optimal extraction fiber for this explosive was a SPME fiber chemistry of polydimethylsiloxane/divinylbenzene (PDMS/DVB). Intraday and interday mean concentrations are shown in Figure 35. Interday concentrations of DMNB for 80%, 50%, and 25% dilutions were 6.53, 5.94, and 4.05 mg L⁻¹, respectively. The CV at each dilution percentage was 11%, 16%, and 2.7%. Figure 36 shows average peak area of 2E1H for each odor dilution factor.

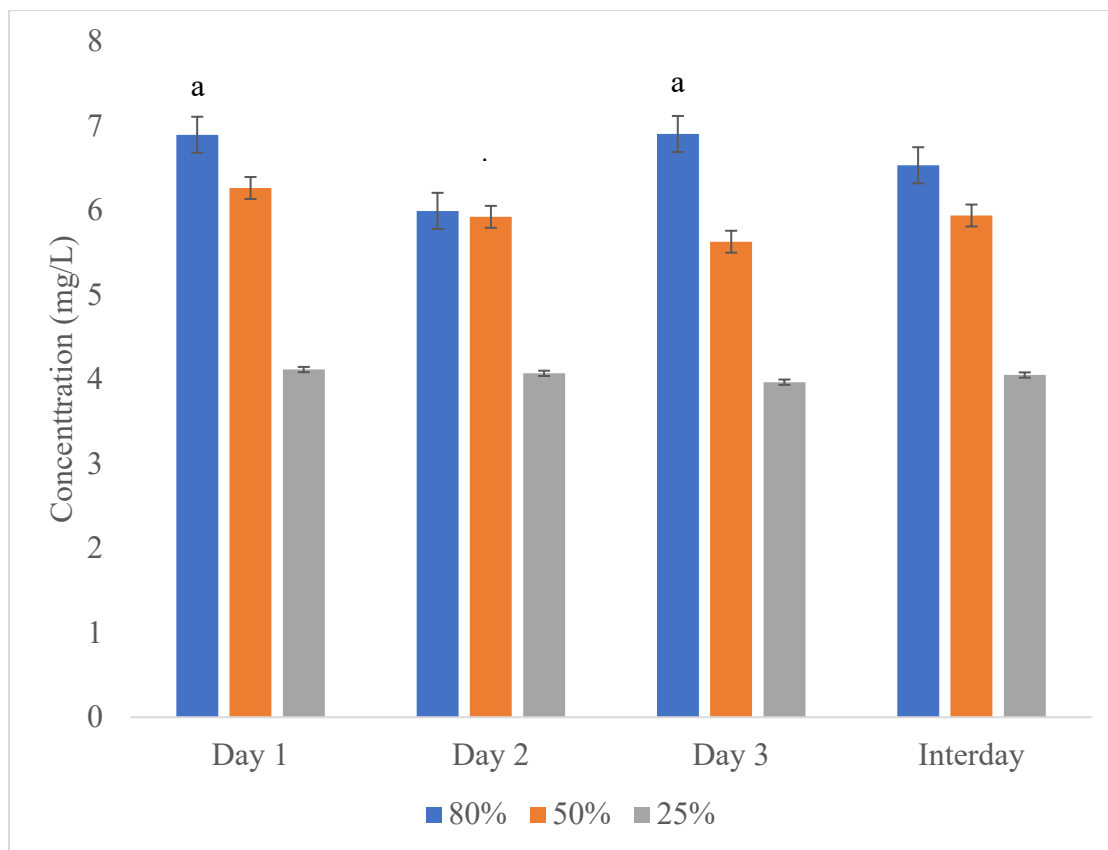


Figure 35: TNT Odor Dilution. Inter- and intra-day mean concentrations for 2-ethyl,1-hexanol (2E1H) at 80%, 50%, and 25%. ($N = 6$). Replicates were sampled on three consecutive days. Non-matched letters indicate significant differences between average concentrations on separate sampling days. Average concentration differences are attributed to variance among ambient laboratory conditions between sampling days. Lower dilution (50%, 25%) were not affected by ambient sampling conditions due to relative temperature and humidity each sampling day.

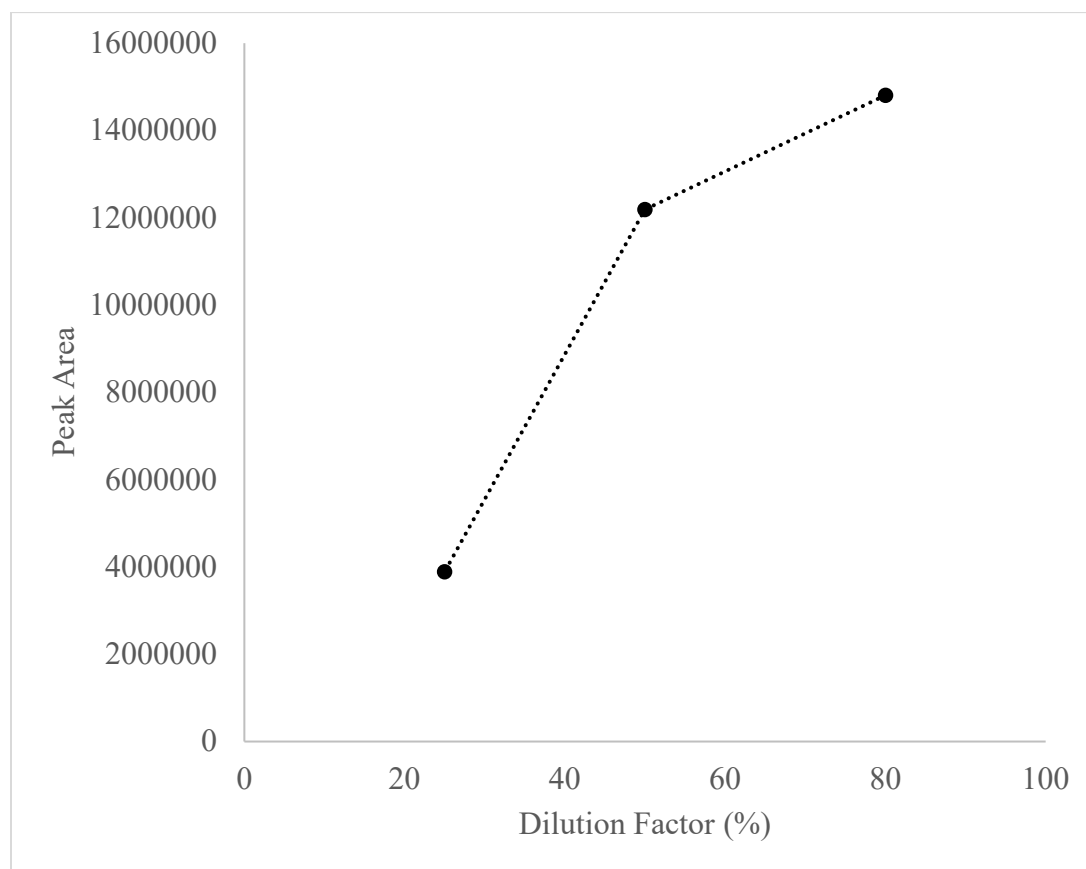


Figure 36: TNT Olfactometer Odor Dilution Response for 2-ethyl,1-hexanol. 2,4,6-trinitrotoluene (TNT) mean peak areas for 2-ethyl,1-hexanol (2E1H) at 80%, 50%, and 25% dilution percentages.

Ammonium nitrate prill

The optimal extraction fiber for this explosive was a SPME fiber chemistry of divinylbenzene/carboxen/polydimethylsiloxane/divinylbenzene (DVB/CAR/PDMS). Intraday and interday mean concentrations are shown in Figure 37. Interday concentrations of DMNB for 80%, 50%, and 25% dilutions were 5.07, 4.94, and 0 mg L⁻¹, respectively. 2E1H was not detected across intraday sampling periods at the 25% dilution percentage. The CV at each dilution factor was 19% and 4.7%, respectively. Figure 38 shows average peak area of 2E1H for each odor dilution factor.

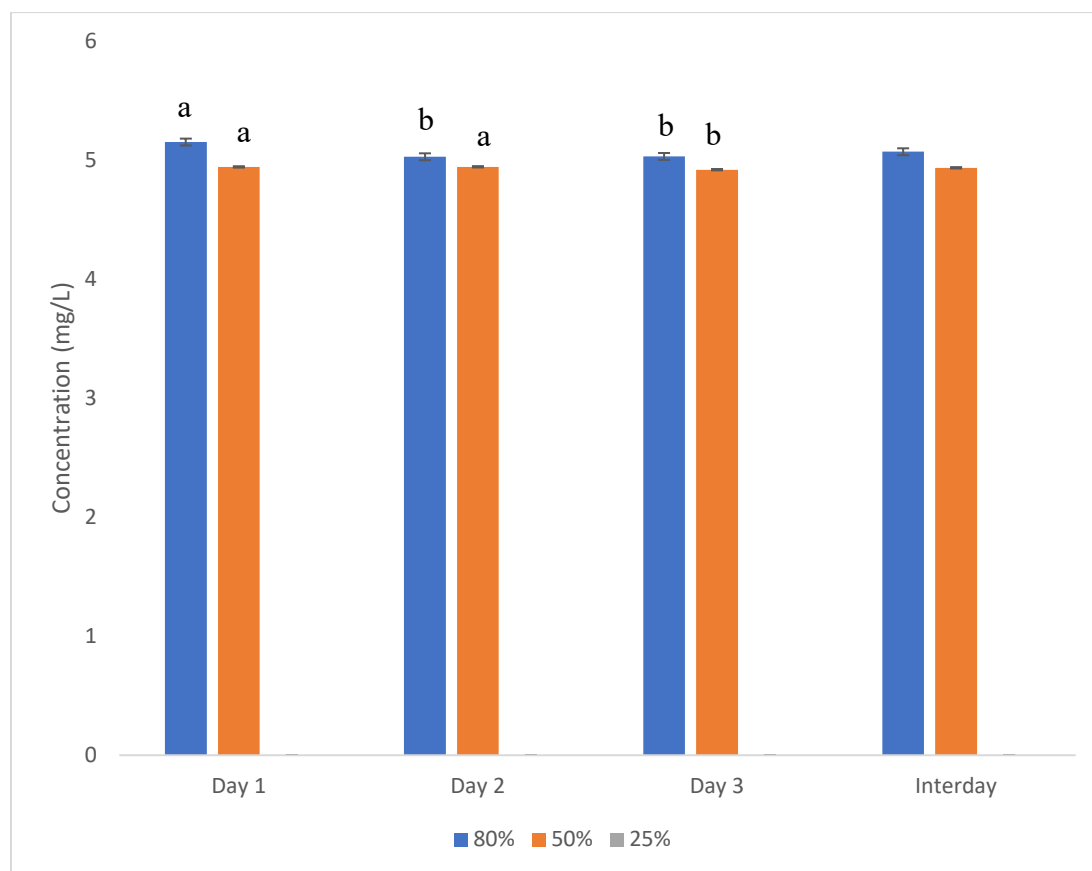


Figure 37: AN Prill Odor Dilution. Inter- and intra-day mean concentrations for 2-ethyl,1-hexanol (2E1H) at 80%, 50%, and 25% (N = 6). Replicates were sampled on three consecutive days. Non-matched letters indicate significant differences between average concentrations on separate sampling days. Average concentration differences are attributed to variance among ambient laboratory conditions between sampling days. Loss of target VOC concentration at 25% dilution.

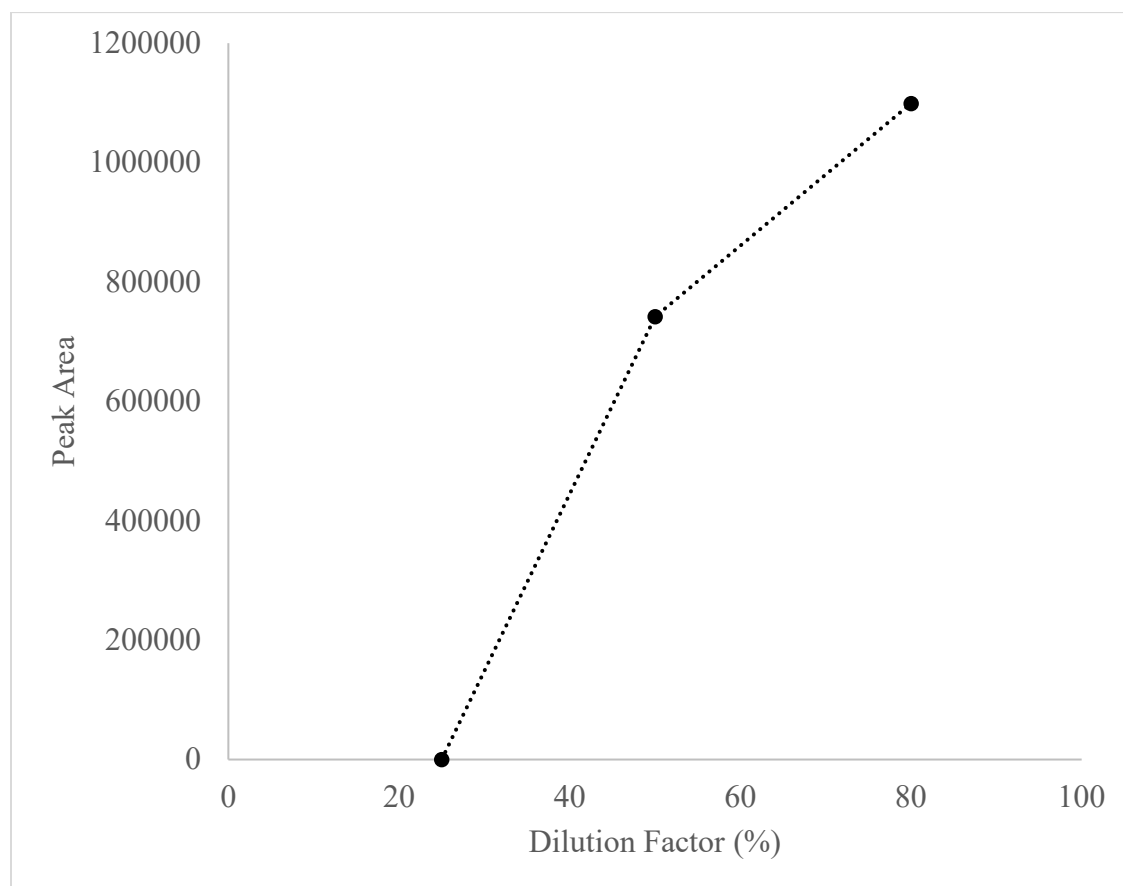


Figure 38: AN prill Olfactometer Odor Dilution Response for 2-ethyl,1-hexanol. Ammonium nitrate (AN) prill mean peak areas for 2-ethyl,1-hexanol (2E1H) at 80%, 50%, and 25% dilution percentages.

Double base smokeless powder

The optimal extraction fiber for this explosive was a SPME fiber chemistry of divinylbenzene/carboxen/polydimethylsiloxane/divinylbenzene (DVB/CAR/PDMS). Target VOCs from direct headspace sampling were not detected among dilution percentages for smokeless powder.

Discussion

The main objective of this study was to instrumentally validate the olfactometer device using a range of explosive classes at distinctive odor dilutions to instrumentally monitor effective odor delivery as measured by odor profile response. Explosives were selected based on the need for instrumental validation for current olfactometer studies in tandem with canine detection. Given the increased use of olfactometers in odor delivery system technology and canine detection research, it is crucial to monitor and verify odor quality and quantity via an analytical instrumental approach. Validation between instrumental analyses and odor delivery devices

bridges the gap, providing optimized methodology to approach the standardization of canine detection research. SPME fiber sampling procedures were optimized to improve detection of target VOCs reported in the literature of selected explosives.

Composition 4

No significant differences for VOC mean concentrations were observed between intraday sampling of 2,3-dimethyl-2,3-dinitrobutane (DMNB) for all odor dilutions. Optimization of the sampling procedure included the use of parafilm to prevent the loss of target VOC at the 80% dilution factor (Figure 39). Optimization concluded the use of parafilm to seal the odor port and SPME fiber junction to contain maximum extraction of VOCs from the odor port. DMNB average peak area decreased as odor dilution factor decreased, indicative of odor availability decreasing as a function of dilution factor. The stability of DMNB concentrations in C-4 odor dilutions are attributed to the use as an explosive taggant predominantly used in the United States (Lai, Leung, Magee, & Almirall, 2010). Confirmation of the presence of DMNB in diluted odor concentrations validates the use for improved detection of plastic explosives (Kranz et al., 2014). Reported headspace VOCs for Composition 4 include: cyclohexanol, cyclohexanone, 2-ethyl, 1-hexanol, 2,3-dimethyl-2,3-dinitrobutane, and pentadecane (Sanchez et al., 2016). Loss of cyclohexanol, cyclohexanone, 2-ethyl, 1-hexanol, and pentadecane in the headspace analysis correlates to extraction time of the olfactometer (1 min) and the overall abundance in the headspace of bulk explosive material. The detection of DMNB instrumentally validates the use of a taggant for high order explosives and detection via dynamic airflow sampling.

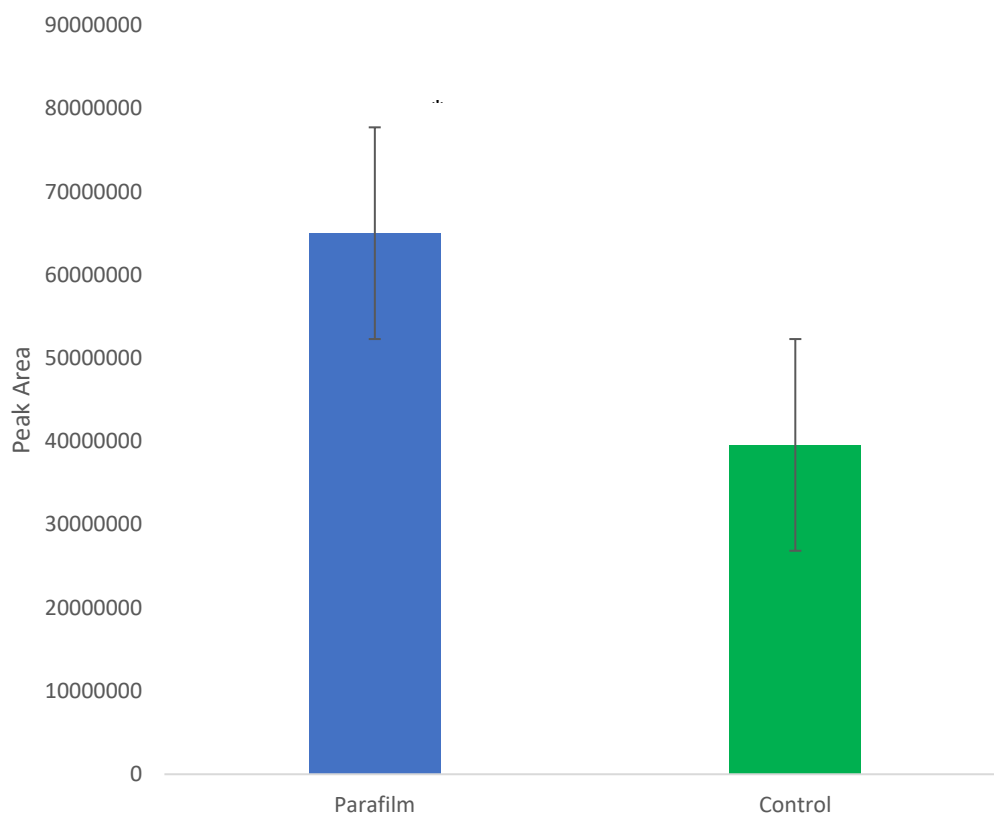


Figure 39: Parafilm optimization for sampling procedure. DMNB = 2,3-dimethyl-2,3-dinitrobutane. Samples ($N = 3$) were exposed to odor port of olfactometer and analyzed via SPME-GC-MS. Asterisk indicates significant difference of peak area for the use of parafilm to increase odor collection target VOCs.

TNT

Significant differences were observed for mean concentrations between intraday sampling of 2-ethyl,1-hexanol at 80% odor dilution. Odor dilutions at 50% and 25% did not show significant differences. Odor availability of 2-ethyl,1-hexanol was minimally influenced by the ambient relative humidity conditions (A. G. Simon et al., 2019) between intraday sampling: Day 1 (41%), Day 2 (20%), and Day 3 (52%), respectively. Environmental factors at ambient conditions are to be noted to gain insight to general trends among calculated concentrations for inter- and intraday sampling. Ambient conditions for 50% and 25% odor dilutions were performed under similar ambient conditions. 2-ethyl,1-hexanol average peak area decreased as odor dilution factor decreased. 2-ethyl,1-hexanol is a common additive to explosives and is termed a contaminant due to the high abundance within many types of different explosives (Harper, Almirall, & Furton, 2005). Confirmation of 2-ethyl,1-hexanol is an indication of potential explosive detection but would require additional VOCs to positively confirm the detection of explosive. TNT direct headspace analysis reports confirmatory VOCs as 1,3-dinitrobenzene, 2,6-dinitrotoluene, or 2,4,6-trinitrotoluene (Psillakis et al., 2000). Loss of TNT specific volatiles via odor dilution

requires further development in odor delivery methodology to identify optimal odor volatile markers at low dilution thresholds for this explosive type.

Ammonium nitrate prill

Significant differences were observed for mean concentrations between intraday sampling of 2-ethyl,1-hexanol at 80% and 50% odor dilution. Detection of 2-ethyl,1-hexanol was not found at 25% odor dilution. Ammonium nitrate prill requires an increased surface area for detection of volatiles (Katilie, Simon, & DeGreeff, 2019). Odor availability is influenced by surface area for the prill form due to its porous nature. To increase detection at 25% odor dilution, optimization of the sample vials in the olfactometer and duration of exposure are to be evaluated.

Double-based smokeless powder

Smokeless powder odor volatiles were not detected in any samples at all odor dilutions. Lack of detection is attributed to potential SPME fiber chemistry, as recent studies have reported polyacrylate SPME fiber is the optimized fiber used in dynamic airflow sampling (Gallegos et al., 2022) compared to direct headspace analysis. Gallegos et al., 2022 reported a 3-hour extraction period with a volatile trapping system to capture diphenylamine concentrations. SPME fiber chemistry for analysis was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and was extracted for 1 min. The inability to detect diphenylamine contributes to odor availability and extraction time for validation of smokeless powders. Further insight is needed to develop a standardized method for the detection of smokeless powder target VOCs.

Limitations and future challenges

Limitations to the study included odor port purge time. Initial experiments were conducted with a 30 sec purge of the odor port. This did not allow sufficient time for target VOCs to equilibrate in the system; therefore, depleting total abundance of VOCs. Further evaluation deemed an increase to the purge time with a blank vial purging clean air to compensate for depletion of VOCs in the headspace. Initial data demonstrated a decrease in peak area throughout consecutive sampling. Replicate sampling depleted the headspace, which increases the coefficient of variance among intraday samples. An optimized purge time is to be investigated to allow for analysis of replicate sampling without loss of analyte peak area. Future studies could include fiber chemistry optimization for explosives compared to direct headspace analysis to confirm appropriate SPME fiber. As olfactometers improve detection capabilities, further studies are needed to validate the reliability of these instruments.

Conclusion

Headspace analysis via SPME-GC-MS was implemented to provide an instrumental validation of odor delivery via the olfactometer device for four targeted explosive classes. These targeted explosive samples included: Composition 4 (C-4), 2,4,6-trinitrotoluene (TNT), ammonium nitrate prill, and double-based smokeless powder. Dynamic airflow sampling yielded the successful detection of target VOCs: 2,3-dimethyl-2,3-dinitrobutane and 2-ethyl,1-hexanol across odor dilutions of 80%, 50%, and 25%. There was a decrease in peak area for all detected VOCs as odor dilution factor decreased, thus suggesting an efficient and linear odor delivery as a function of odor amount. TNT and AN prill intraday samples were statistically different in mean concentrations at 80% odor dilution. These results indicate indirect odor sampling undergoes a decrease in concentration of target VOCs for inter- and intra-day and is crucial for monitoring and evaluation for optimal canine training regimes for olfactometry. The development and improvement of olfactometry-based methods validates the reliability of biological sensors. This study evaluates indirect dynamic headspace sampling of explosives via olfactometer and the successful use of SPME-GC/MS methods for confirmation of odor presence.

Task 5: Acclimatization/mitigation training under extreme environments

Abstract

Task 2 and 4 demonstrated that detection canines have decrements in detection sensitivity limits when operating in challenging environmental conditions. Task 4 further found that decrements still occur in hot and humid conditions, even when Task 3 indicates odor is most available for the canine to detect. This Task therefore explored whether canines can receive a brief acclimatization protocol over 10 and 20 days to mitigate detection sensitivity decrements. The eight dogs that participated in the previous tasks were randomly assigned to experimental or control treatments. In the experimental treatment dogs received a 5 min exercise conditioning phase, followed by an approximately 20 min threshold test. The environmental conditions started at standard conditions but increased daily by 5 °C and 5% RH until reaching the high temperature high humidity conditions. The control dogs received a petting session (instead of 5 min exercise) followed by a threshold test always at standard temperature and humidity conditions. Both groups therefore received identical amounts of odor/detection training/testing. All threshold training/testing was run as a descending staircase three alternative forced choice test with an automated olfactometer. Following 10 (Test 1) and 20 days (Test 2) of acclimatization training, control and experimental dogs received a standard threshold assessment at the High Temperature and High Humidity conditions. On the first day of training, where both groups were at standard conditions, control and experimental dogs showed similar detection sensitivity limits. On the test days at High Temperature and Humidity, experimental dogs had lower (better) detection sensitivity than control dogs across Test 1 and Test 2. Additionally, experimental dogs showed reduced latency to initiate trials and moved from one olfactometer box to the next more rapidly than control dogs, which were both shown to be predictors of detection sensitivity in Task 4. In contrast, mean subcutaneous temperature was not different between experimental and control dogs, suggesting the effects observed may be more behavioral (de-sensitization to the conditions) than physiological (improved thermoregulation), but additional work is needed to isolate these effects.

Introduction

Results from Task 4 demonstrated that canines showed a decrement in detection sensitivity when operating under high temperature and high humidity conditions. This decrement could have important impacts on detection canine operational performance. However, it remains unclear whether this decrement could be mitigated. The objective of this task is to evaluate whether dogs can undergo a brief acclimatization training to reduce deficits in detection sensitivity in the high temperature and high humidity condition.

Methods and Materials

Participants

The same 8 dogs (4 Labrador Retrievers, 4 German Short haired pointers) that participated in Tasks 2 and 4 participated in this experiment. The dogs were again randomly split into two groups throughout the duration of this experiment (Experimental and Control). These groups worked with the same target odorant (C-4) but were trained under different environmental acclimatization treatment plans.

Animal welfare considerations

This project was conducted at Texas Tech University and was overseen by the Institutional Animal Care and Use Committee (Protocol #21051-07). All additional enrichment and care procedures were identical to Experiment Task 4. Identical criterion for early discontinuation of a session (e.g., failure to respond during trials) used in Tasks 2 and 4 were maintained.

Odorants

C-4 (Omni Distribution) was selected as the target odor because dogs showed a specific deficit for this target in Task 4. All other vials in the olfactometer were empty and acted as controls, identical to the procedure in Task 4.

Data collection equipment

Olfactometers used were identical to those used in Task 4.

Environmental conditions

The same environmental chamber was used as in Task 4. Only two of the environmental conditions were utilized, the standard conditions (22°C and 50% relative humidity), and high temperature, high humidity (40°C and 70% relative humidity). In both conditions there was a 3°C and 5% relative humidity window on either side to allow changes caused by the chamber door opening and closing.

Table 31: Details the environmental conditions in the acclimatization plan by group

Day	Experimental group (n=4) condition	Control group (n=4) condition
1	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
2	$76^{\circ} \pm 3^{\circ}; 53\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
3	$82^{\circ} \pm 3^{\circ}; 56\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
4	$88^{\circ} \pm 3^{\circ}; 59\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
5	$94^{\circ} \pm 3^{\circ}; 62\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
6	$100^{\circ} \pm 3^{\circ}; 65\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$
7-22	$104^{\circ} \pm 3^{\circ}; 70\% \pm 5\%$	$70^{\circ} \pm 3^{\circ}; 50\% \pm 5\%$ (excluding testing days 10 and 22)

Canine acclimatization training and testing

The eight dogs were randomly split into two groups: control and experimental. Both groups consisted of 4 dogs. Dogs in the experimental group received 5-minute exposure with play-based exercise within the environmental condition followed by a threshold detection session following an identical threshold procedure to that of Task 4 with the exception of completing four reversals instead of seven. The environmental conditions for the experimental group, slowly escalated over the course of six days to reach the high temperature and high humidity conditions (see Table 31). In contrast, the control group received a 5-minute pet session in the environmental chamber (instead of exercise) followed by a threshold detection session. Critically, environmental conditions remained at standard conditions during the training phase for control dogs and both experimental and control dogs received equal amounts of threshold training.

This exercise treatment consisted of either a toy toss (Kong™) or food toss (the food used as their reward during odor training and testing) that was determined to likely be physically fatiguing in increasingly warmer conditions. Prior to the start of the experiment, experimenters ran a preference test to determine which reward the dog would prefer to work for in this initial 5 minutes of exercise. In this test, the experimenter tossed a toy and a piece of the dog's normal kibble out in front of the restrained dog. The experimenter then released the dog and whichever reward the dog engaged with first was recorded. This procedure was repeated five times, and whichever reward the dog chose more was the reward that was used in acclimatization training.

Three of the four dogs initially used the Kong™ as their reward. However, after the dogs reached the high temperature and high humidity condition requirements (Day 7) only 1 dog still engaged with the toy. The other two dogs were switched to a food toss, which they continued to engage with the rest of the study.

Canine testing

On day 11 (after 10 days of acclimatization training) and on day 22 (after twenty days of acclimatization training) both groups underwent threshold testing. This testing consisted of the same threshold test done during acclimatization, in the absence of the exercise/pet 5 minute prior at the high temperature and high humidity condition (40°C and 70% RH).

Results and Discussion

Detection limit changes and acclimatization plan

The primary goal of this study was to determine if the acclimatization plan improved the detection limits for C-4 under high temperature and humidity conditions for the treatment (n=4) group compared to the control group (n=4). Figure 40A shows that there were minimal differences in detection limit at Day 0 under standard conditions.

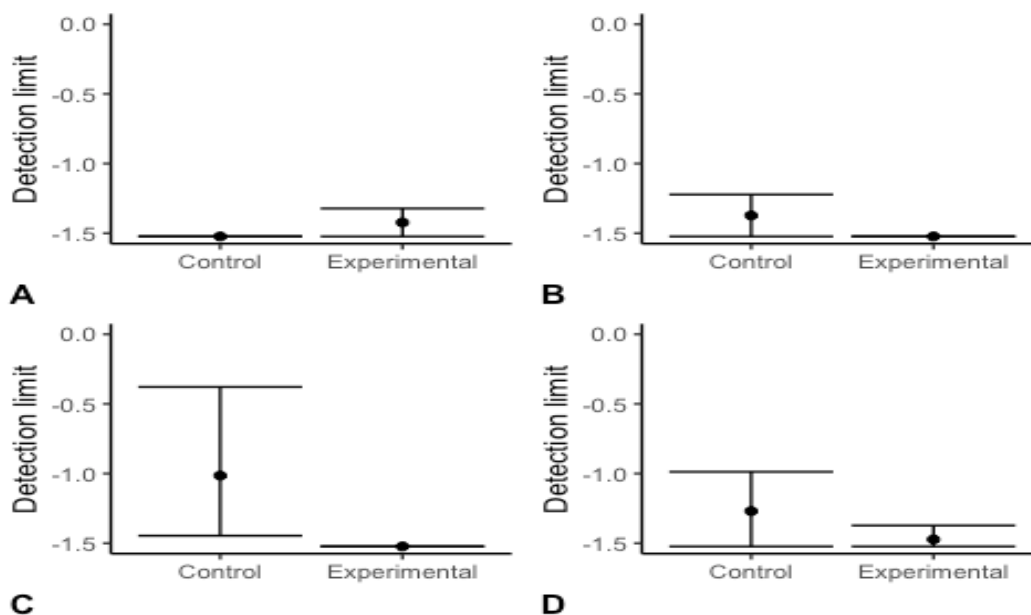


Figure 40: Detection limits for each of the two treatment groups on a baseline standard condition. A) initial test day, B) average of Test 1 and 2, C) Test 1, and D) Test 2 at 20 days.

In fact, detection limits were slightly poorer for the experimental group compared to the control. Figure 40B, however, shows that Experimental dogs outperformed control dogs under the high temperature and high humidity conditions averaged across Test 1 and Test 2 at 10 and 20 days of acclimatization. Figure 40C shows that Experimental dogs showed optimal performance following just 10 days of acclimatization training, outperforming control dogs. A similar result is seen for Test 2, although with less variability in the control dogs. A linear mixed effect model predicting detection threshold by treatment across Test 1 and Test 2 indicate that treatment did have a significant impact on canine detection thresholds ($\chi^2=4.5$, $df=1$, $p=0.03$). This indicates that acclimatization training did lead to lower (better) detection thresholds under the high temperature high humidity condition.

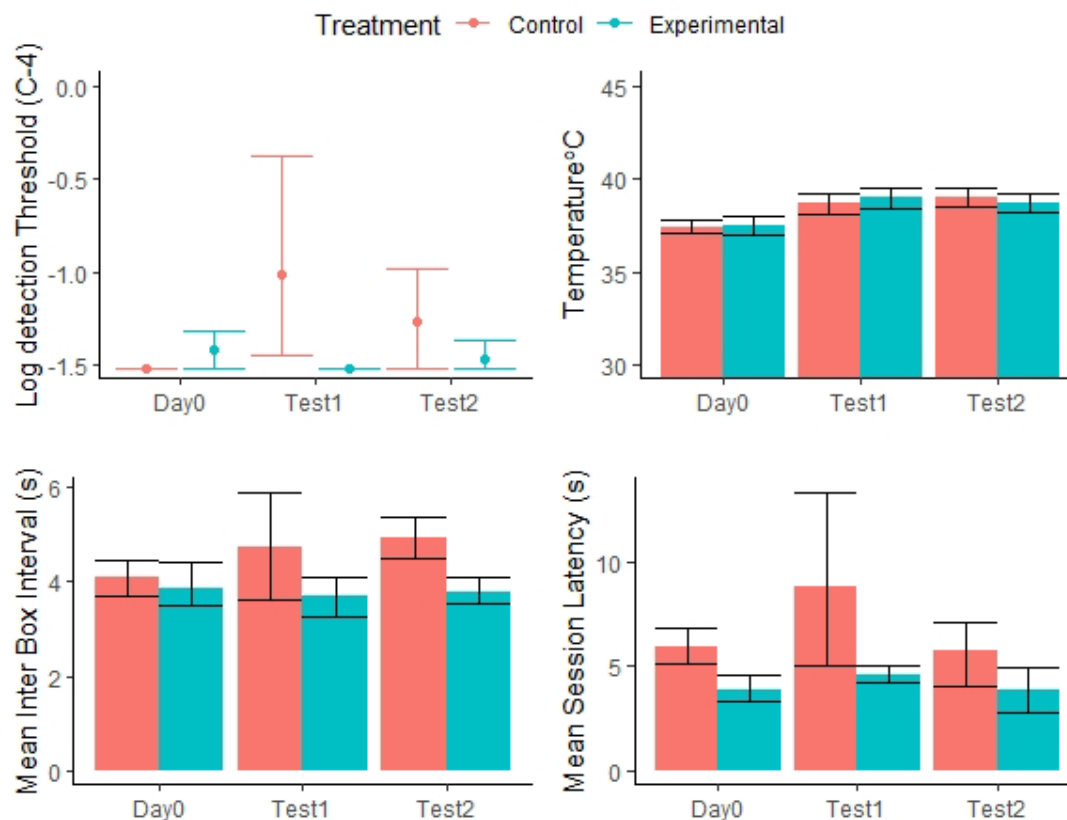


Figure 41. Effect of acclimatization on detection threshold, subcutaneous temperature, inter box interval and latency. Day0 is the baseline at standard conditions. Test 1 and Test 2 is under High Temperature and High Humidity conditions following 10 and 20 days of acclimatization, respectively.

Figure 41 shows the effect of acclimatization training on (Control vs. Experimental) again for threshold and for the three measures that were previously found to be associated with detection thresholds in Task 4 (mean subcutaneous temperature, mean inter box interval, and mean session latency). A linear mixed effect model indicates that mean temperature was not different by treatment across the two Test days at high temperature and high humidity ($\chi^2<.1$ $df=1$, $p=0.98$).

In contrast, there was a significant difference between treatments on the mean inter-box-interval, such that experimental dogs walk on average 1 s faster from one box to the next than control dogs during the test days ($\chi^2=9.66$, $df=1$, $p=0.002$). In addition, latency almost met the criterion for traditional statistical significance, and showed that experimental dogs initiated trials approximately 3 s faster than did controls during the high temperature high humidity tests ($\chi^2=3.35$, $df=1$, $p=0.07$).

Conclusions

The results of the acclimatization treatment showed that when challenged with the high temperature high humidity condition, acclimatized dogs showed lower (better) detection limits, shorter latency to start search (~3s) and maneuvered from one olfactometer box to the next about 1s faster.

Interestingly, subcutaneous temperature was not different between the treatment and control group. This suggests that although behavioral performance improved (e.g., inter-box interval) and detection limits improved, there was not likely directly caused by improved physiological responses to heat stress (e.g., subcutaneous temperature).

The acclimatization protocol leveraged for this study combined brief exercise with de-sensitization and exposure training to the environmental condition. Although this combined program (exercise + de-sensitization) led to improved performance, it's not clear if both components are necessary. Further, it is possible that both components have important effects. However, given that the exercise conditioning was minimal (~5 minutes) it is possible that a more extensive fitness training program (Farr et al., 2020; Stigall et al., 2022) could lead to greater benefits. Given that the brief exercise did not improve (reduce) mean subcutaneous temperature, this suggests further improvement could be made with more robust physical conditioning.

One important limitation for this study is the limited sample size. Due to the group design, there were only four dogs per treatment. Nonetheless, clear differences in performance were observed during the test phase, even though there were no differences in baseline. This suggests that the sample size was not a substantial limitation.

A second limitation is that following acclimatization, dogs' detection limits to C-4 were below the dilution capabilities of our olfactometer system. This limited the ability to measure differences between groups because threshold reached a floor. Future work could utilize multi-stage dilution to generate higher dilutions which would be more sensitive to detecting detection threshold changes.

Lastly, our analysis was focused on the decrements caused by high temperatures on C-4 detection limits. However, our prior work in Task 2 and 4 demonstrated that canines can also show reduced performance in cold temperatures, particularly cold and humid conditions. Thus, additional work is necessary to explore de-sensitization and/or physical conditioning effects on performance decrements in cold conditions.

Overall, results from the acclimatization treatment show that brief exercise and de-sensitization to working in High Temperature and High Humid conditions mitigated detection threshold decrements for C4 in those environmental conditions. Performance improvements were also observed for mean inter-box interval (search speed). Further, performance improvements were observed after 10 days of conditioning, and we did not observe greater performance returns following 20 days of acclimatization training compared to ten. Future research is needed to investigate separate effects of physical conditioning and de-sensitization to the environmental conditions to identify optimal and sufficient combinations to mitigate performance decrements.

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