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Headspace analysis of ammonium nitrate variants and the effects of differing vapor profiles on canine detection

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ABSTRACT

Canines continue to be one of the most frequently deployed tool in the detection of explosives, and particularly homemade explosives (HMEs), in part, due to the ease in training to new HME materials as threats arise. The majority of HMEs encountered contain ammonium nitrate (AN), and previous research has measured the release of ammonia from AN, and found that the ammonia vapor concentration varies with form, purity, and environment, but this is has not been correlated to canine detection proficiency. In this research, the headspace analysis of AN variants was carried out using solid phase microextraction (SPME) with gas chromatography/mass spectrometry (GC/MS). Ammonia vapor from the AN was extracted using on-fiber derivatization, while the presence of other volatiles in the headspace of these variants were also characterized by a traditional SPME extraction. These results were correlated to canine testing, where canines previously trained in odor detection were provided laboratory-grade AN for odor imprinting, after which they were to locate other AN variants in a series of simple detection tasks. Headspace analysis showed variations in both the amount of ammonia as well as other volatile compounds in the headspace of the various AN samples, as well as changes in the vapor profiles due to changing environmental conditions. Canine data indicated that the differences in the headspace profiles of the samples may confound detection when canines were trained on laboratory-grade AN alone, while increased ammonia vapor availability from certain samples may have improved detection by this group of canines.

1. Introduction

Homemade explosives (HMEs) include a range of non-industrially-produced explosives easily synthesized from improvised or commercially available materials [1]. Due to the commercial availability of a wide range of starting materials and the availability of bomb-making instructions on the internet, the threat from homemade explosives is continually evolving, making identification and detection of HMEs challenging [2]. Though many devices exist for the detection of HMEs, canines continue to be the most effective tool compared to other field-portable explosive detection systems, in part, due to their ability to quickly learn to detect new explosive threats. In 2013, the U.S. Military Working Dog Program (341st Training Squadron) maintained 2500 trained dogs to detect explosives, find drugs, and protect troops [3], and in 2019 there were more than 1000 canines deployed by the Transportation Security Administration (TSA), accumulating more than 300,000 work hours in 2019 alone [4]. Today, trained canines are an essential tool to the military, law enforcement and homeland security. Although canines play a vital role in protection from improvised explosive devices (IEDs) and HME detonations, there remains a dearth of information regarding canine olfaction capabilities in this area. A greater understanding of these capabilities could contribute to improve training and detection proficiency.

Many HMEs are composed of simple binary mixtures of fuels and oxidizers. Of these binary mixtures, the most frequently utilized oxidizer is Ammonium Nitrate (AN). Of the IEDs seen in Afghanistan by 2012, 86% contained HMEs, and 83% of these were AN-based [5], and in 2018 AN was listed as a highest priority threat in the Consensus Study Report, *Reducing the Threat of Improvised Device Attacks* [6]. AN is sold as a fertilizer in the form of small, compressed pellets, or prills; however, as an explosive, AN is most effective ground and is often used in this form. In an attempt to thwart the use of AN for terrorism, it is frequently sold in the form of calcium AN or CAN [1]. CAN is also found in instant cold packs, as the hydration of AN is an endothermic process and, when crushed, the AN prills mix with water setting off the endothermic reaction and causing the water in the pack to freeze.

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To aid in the deployment of successful non-contact, vapor detection by biological (i.e. canine) or electronic sensors, it is imperative to understand the vaporous constituents emitted from the target of interest. To date, there is minimal research on AN and other oxidizers as it relates to HMEs, particularly in comparison to the body of literature available for traditional explosive materials, such as trinitrotoluene (TNT) [7]. It is known that AN is highly hydroscopic, deliquescing in humid air above 62% RH (25 °C). As a salt, AN does not have an appreciable vapor pressure on its own, and instead dissociates under ambient temperature and humidity into its precursors, ammonia and nitric acid. The vapor pressure, owing to these vaporous products, is thought to be similar to that of TNT, at 1.47×10^{-8} atm at 25 °C [8]. The vapor pressure may be affected by the presence of contaminants, the form in which AN is found (i.e. prill, ground, or crystalline), and environmental conditions (i.e. temperature and humidity) [9].

A. Lubrano et al. (2016) and C. Katilie et al. (2019) developed techniques for the headspace analysis of AN using static and dynamic sampling approaches, respectively. Lubrano et al. utilized solid phase microextraction (SPME) with derivatization and gas chromatography / mass spectrometry (GC/MS) to quantitate the concentration of ammonia vapor from 2 g preparations of AN and fuel oil (ANFO) and AN with aluminum powder (AN/Al), measuring and average of 6 and 21 ppb of ammonia, respectively [10]. Katilie et al. applied the same derivatization reaction to dynamic sampling of AN vapor onto a programmable temperature vaporization GC inlet with separation and detection by GC/MS [11]. The method was then used to quantify ammonia from bulk AN, AN with aluminum, and AN with petroleum jelly. The work noted differences in ammonia concentration depending on the source of AN, with 9 g of CAN yielding approximately 1268 ng/L of ammonia and laboratory-grade AN yielding 5.21 ng/L. Furthermore, when the same source of AN was compared in prill or ground forms, the ground material produced three times as much ammonia as the prill material (3.45 ng/L and 9.97 ng/L, respectively) [11].

Such differences in ammonia concentration, as well as the presence of extraneous or unintended vaporous compounds, not previously explored in the literature, may have an effect on canine detection. The detection of AN by canine is highly dependent on its physiochemical properties, as well as the presence of volatile contaminants; however, few studies have been conducted relating this to canine detection of HMEs [7]. Lazarowski et al. (2015) tested canines previously trained on a single variant of AN on similar compounds including other ammonium- and nitrate-containing salts (ammonium sulfate and sodium nitrate), other AN forms (fertilizer-grade AN), and AN mixtures (AN in soil and AN with aluminum powder). The canines in this study did detect these variants at a rate greater than chance; however, the detection rates were low, ranging from 58% on the related salts to 73% on the AN in soil. In comparison, the performance for the trained AN sample was above 80% [12]. These results point to detection challenges due to differences in the vapor signatures of the AN materials, although no headspace measurements were made. Another study by Hall & Wynne (2018) went further, exploring how training to AN alone or as part of a mixture influences generalization to novel AN mixtures. They found that canines trained using only pure AN were significantly less likely to locate the novel AN mixtures. Training using the mixtures, while initially more challenging, led to greater success in locating AN in novel mixtures [13].

Unlike other explosives materials, there has been minimal headspace analysis studies on AN. Furthermore, previous studies using canines in the detection of AN were not correlated to headspace characterization of AN materials. In this study, we conducted headspace analyses of pure AN (laboratory-grade) and related AN variants (ground, prill, CAN etc.) and hypothesized that differences and similarities between the AN variants would affect the canines in their ability to detect AN and to generalize to or discriminate between related variants.

2. Materials and methods

2.1. Instrumental analysis

2.1.1. Materials

Six AN variants were tested and are included in Table 1. For analysis, 20 mL screw-thread headspace vials (Restek Co.) were filled about 1/3 full to equal approximately 6 cm^3 of AN (total mass varied with density of each AN type). The samples were prepared in triplicate and allowed to equilibrate for 24 h prior to sampling.

2.1.2. Headspace analysis

The headspaces of all AN samples were investigated using SPME-GC/MS. Two separate SPME methods were used in this analysis, one for the detection of ammonia (from the dissociation of AN), and one for the detection of all other volatiles coming from impurities in the AN variants. Detection of ammonia vapor by standard SPME-GC/MS methods is problematic due to poor trapping/retention and separation on typical stationary phases. For this reason, an on-fiber derivatization technique, developed by Brown et al. [14], was used. Two mL of the derivatizing agent, butylchloroformate (98%, Sigma-Aldrich), was pipetted neat into 20 mL headspace vials, and allowed to equilibrate for at least two hours. An 85 μm, polyacrylate SPME fiber (Supelco, Millipore Sigma) was exposed to the butylchloroformate for 1 min. The SPME fiber was then immediately removed and exposed within the AN sample vial for 1 h. The derivatized ammonia was detected as butyl carbamate, and relative amounts of butyl carbamate from each sample were compared. For extraction of the other volatiles, a polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR) SPME fiber (Supelco, Millipore Sigma) was exposed directly to the headspace of the AN samples for 4 h. For both extraction methods, analytes were thermally desorbed from the SPME fibers in the GC (Agilent 6890A gas chromatograph with a 5973 mass selective detector) inlet at 260 °C with a flow rate of 2 mL/min. Both analyses utilized a Rtx-5MS GC column (15 m × 0.25 mm ID × 0.25 μm thickness; Restek Co.). Other GC/MS parameters are listed in Table 2. All compounds in the headspace were assigned based on mass spectra matches to the NIST mass spectral library.

All headspace measurements were made at room temperature (22 °C ± 1, 32% RH ± 5%). In addition, the headspaces of several AN variants were compared at varying temperatures and humidities using an environmental test chamber. The test chamber is capable of providing a temperature range of −34 to 85 °C and a relative humidity range of 10 – 95%. It houses an exhaust apparatus that purges the air at 300 CFM. Temperatures and humidities were chosen to mimic outdoor conditions in the mid-Atlantic region, and included 6 °C at 20% RH, 20 °C at 20% RH, 26 °C at 40% RH, and 32 °C at 60% RH. For these analyses, AN samples were again first placed in the 20 mL headspace vials, approximately 1/3 full, under ambient temperatures. The samples were then carried to the environmental chamber and left open (no lid) in the chamber, allowing the AN to interact with the environment for one hour. After this time, the vials were closed and allowed to equilibrate for an additional 1 h. The vials were then removed from the chamber for extraction at ambient temperatures by SPME. Extraction and analysis protocols were the same as previously described. Three AN variants, representing three AN forms, were tested including laboratory-grade

Table 1
AN type and form (C – crystal, P – prill, G - Ground used in study.

AN type (form)	Source/manufacturer
Laboratory-grade (C)	Sigma-Aldrich
Industrial-grade (P)	GSF Chemical
Industrial-grade (G)	GSF Chemical
Fertilizer (P)	Garden Naturals
Fertilizer (G)	Garden Naturals
Instant cold pack (P)	Dynarex
Calcium AN (CAN) (P)	Yara (YaraBela CAN 27)

Table 2
GC/MS analysis parameters for two SPME extraction methods.

	Ammonia extraction parameters	Volatiles extraction parameters
Oven temperature program	1. 40 °C, hold 0 min. 2. 40 °C/min to 240 °C 3. Hold 2 min	1. 40 °C, hold 1 min. 2. 40 °C/min to 240 °C 3. Hold 3 min
Inlet split ratio	Splitless	10:1
MS scan range	*m/z* 33–220	*m/z* 30–300

(crystalline), industrial-grade (ground), and industrial-grade (prill). Fresh samples, prepared in triplicate, were used for each SPME extraction method (i.e. ammonia extraction or VOC extraction). Blank vial samples were also taken at each temperature/humidity combination.

2.2. Canine testing

Note that all canine training and testing protocols were performed in compliance with relevant laws and institutional guidelines and approved by the Florida International University Institutional Animal Care and Use Committee and the Navy Bureau of Medicine. All canines involved in the study participated with knowledge and consent of their owner.

2.2.1. Training and testing materials

All canine training and testing materials were from the same lots as used for the headspace analysis experiments. For all testing and training, approximately 500 mg of each AN variant was placed in small "breather" tins, so the tins were about half-full. The tins were 2 oz., round, rust-resistant, screw top, steel tins (purchased from PaperMart) with solid lids. For testing and training exercises, lids were prepared with five small holes drilled in the tin lids to allow odor to escape. During testing, the breather tins were placed into larger one quart cans and allowed to equilibrate for a minimum of 30 min. When not in use, odor tins were topped with the solid lids and stored in either glass jars (16 oz. canning jars with unlined lids, Fillmore Container) or metalized Mylar barrier bags (7.5" x 11.5" x 3.5", ESP Packaging). Types of AN were packaged separate to prevent the possibility of cross-contamination.

Testing also included non-target odors, also known as distractor odors. Distractor odors included household items, including crayons, bar soap, bandages, deodorant, unused tea bag, batteries, soil/grass, shredded paper, or shampoo, purchased from local retail stores and selected at random, as well as the nitrile gloves of the same brand used to prepare the training and testing materials. Distractor materials were placed in identical tins as the AN material, but were stored separately to prevent cross-contamination.

Finally, blank tins were also used in the training and testing. These consisted of fresh tins with identical breather lids. Again, all blank materials were stored separately from the testing odors to prevent cross-contamination.

2.2.2. Canine participants and canine training

All canine participants were members of the National Association of Canine Scent Work®, LLC (NACSW™); a.k.a. K9 Nose Work®. K9 Nose Work is a social group that trains non-working (pet) canines in search and scenting activities using scents from essential oils (birch, anise, and clove). The group offers classes and competitions in scent detection that mimic training and testing scenarios for actual working dogs [15]. The canines that participated in this research were a range of breeds including traditional and non-traditional working dog breeds, and were 2–13 years old with at least one year of experience in odor detection. A full list of participant breed, age, and experience can be found in the supplemental data (Appendix A).

The canines used in this study had no prior experience with AN nor any other explosive-related odor. For this study, the canines were trained to locate a single AN type, laboratory-grade AN, and then were tested on the other variants listed in Table 1.

Upon receipt of the training odor(s), canine handlers were instructed to train "as usual", meaning in the same manner in which they train with K9 Nose Work odors. Details of the recommended training methods are included in the supplemental materials (Appendix B), though each participant trained on their own, as such the number and length of training sessions and exact training method varied.

2.2.3. Test set-up

After having been trained on laboratory-grade AN, canines were tested on their recognition of (generalization to) other types of AN. Canine recognition of AN odor was tested using a series of odor recognition tests. An odor recognition test (ORT) is defined as "a test of the dog's ability to alert to a trained odor" [16]. It is a standardized method used to demonstrate the canine's ability to recognize a desired odor. In these trials, the ORTs consisted of a line of five one-quart cans held in place by a rigid PVC "ladder", as can be seen in Fig. 1. Each can in the set contained a breather tin with either a target (1 per line-up), a distractor (1 per line-up), or a blank (3 per line-up). Negative tests were also used and consisted of one distractor and four blanks in identical breather tins and cans. Canines were tested on novel AN variants as well as fresh laboratory-grade AN, identical to which they had previously been trained. Canines had two chances to locate the trained odor (laboratory-grade), and any canine that was not able to locate the trained odor on either occasion was not included in the data. Additionally, the data from any canine showing excessive false alerts (defined as more than two false alerts to distractor odors or more than four total [distractor + blank] false alert rate) was also removed from the study.

The location of each target within an ORT was chosen by a random number generator for each canine. The test coordinators were responsible for placing the testing materials for each ORT and were privy to the location of the target odors. In order to maintain search motivation, the handler vocalized the canine alert, and received either a "yes" or a "no" from the test coordinators. The handler rewarded "yes" responses (with either food or toy) before continuing. The test coordinators were otherwise removed from the testing scenario and handlers, and did not interact with the handlers or test assessors to maintain a double-blind testing scenario. Test areas were inspected and cleaned after each trial. Canines and handlers waiting to be tested were prevented from observing other canines during testing. To minimize "learning" of the novel odors, no canine saw any of the novel odors more than one time.

All testing was observed by two impartial test assessors, both

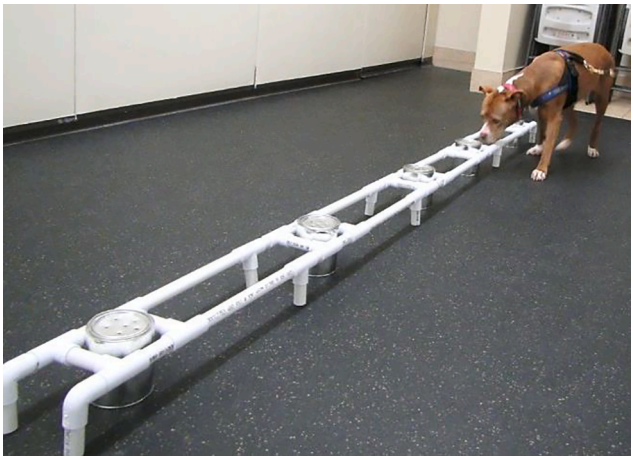

Fig. 1. Canine completing odor recognition test. Five cans were placed in a line and held in place by the PVC "ladder". Breather tins containing the target, a distractor, or a blank were placed inside of a 1 qt can with a perforated lid.

experienced in reading canine behavior during olfaction exercises. All trials were double-blind, meaning neither the handler nor the test assessors knew the identity or location of the target odors. The assessors were physically separated from the test coordinator and were tasked to independently note canine alert or non-alert prior to the handler's vocalization. Test assessors noted alerts based on clear behavioral indications, including on the rare occasion when it was missed by the handler. Video was used to address any disparities between test assessors in such instances. Other, more subtle, changes in canine behavior were not considered, as changes in behavior are not uncommon when approaching novel including distractor odors. Following each testing day, the alerts were tabulated by a test coordinator, to determine if the alerts were true positives (i.e. alert to AN) or false positives (i.e. an alert on a distractor or a blank).

2.2.4. Statistical analysis

The chi-square test for independence was used to compare the distribution of discrete responses for independent comparison groups, in this case comparison in detection of the trained AN variant versus the novel variants. The null hypothesis states that there was no difference in outcomes between the chance of detecting a trained versus untrained AN odor. The alternative hypothesis states that there was a difference in the distribution of responses to the variables among comparison groups.

3. Results

3.1. Headspace analysis

Ammonia available in the headspace of the six AN variants is compared in Fig. 2, given as the averaged (n = 3) peak area of derivatized ammonia (i.e. butyl carbamate). The amount of ammonia present was correlated to AN purity, with the more pure substances releasing less ammonia vapor. Of the selected AN materials, the laboratory-grade AN would be considered the most pure, followed by the industrial-grade material, and then the fertilizer-grade, CAN, and ice pack materials. The laboratory-grade material yielded the least amount of available ammonia compared to the other samples, followed by the industrial-grade, which yielded low quantities of ammonia as well. CAN, AN mixed with 19% calcium magnesium carbonate, produced the greatest amount of ammonia in the headspace. The abundance of ammonia from CAN is likely owing to the presence of the calcium magnesium carbonate, which is weakly basic and thus favors the consumption of nitric acid and, presumably, freeing more ammonia vapor. Grinding the fertilizer

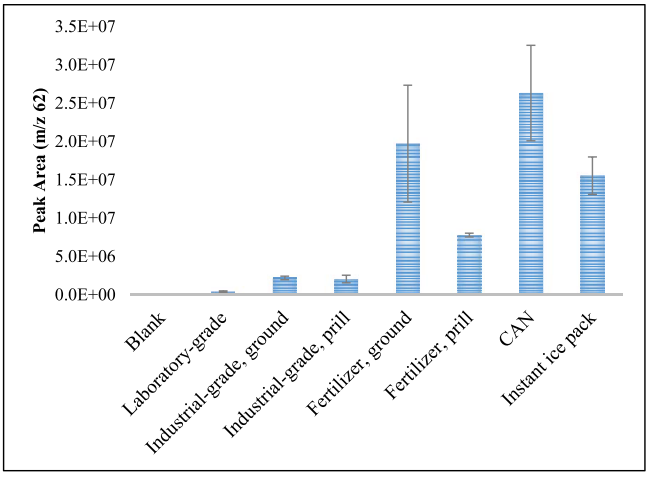

Fig. 2. Ammonia vapor measured from the headspace of AN variants. The magnitude of ammonia vapor present is given as the peak area of the main ion, *m/z* 62, of butyl carbamate, which is the product of ammonia derivatization. Note. Error bars equal one standard deviation.

AN liberated significantly more ammonia than was measured in the same prill sample, though the same trend was not identified in the industrial-grade material, as these were statistically similar (*t*-test, 95% confidence).

Other volatiles extracted from the headspace are summarized in Table 3. As AN is a salt, composed solely of ammonia and nitric acid, all other volatiles are imparted through manufacturing or packaging/storage. Besides ammonia, no one volatile was detected in all samples, though oxime-methoxy phenyl, a volatile compound found in the headspace of a variety of commercial products, was found in all samples but the CAN. AN from the instant ice pack had significantly more vaporous contaminants than any other sample. Most of these are pyridines, which can be produced through reactions between acetylaldehyde or formaldehyde with ammonia [17].

Previous research by Steinkamp et al. (2010) demonstrated that increasing humidity *or* temperature increases the dissociation of AN, thus increasing the concentration of ammonia [9]. The effect of temperature and humidity on several of the AN variants is shown in Figs. 3 and 4. As expected, there was an overall increase in ammonia in the headspace corresponding to an increase in temperature and humidity, with a significant increase in ammonia vapor moving from the 20 °C / 20 RH data point to the 26 °C / 40 RH (Fig. 3). Interestingly, in the industrial-grade samples, the maximum ammonia was collected at 26 °C / 40 RH, with 32 °C / 60 RH being appreciably lower, though this was not the case for the laboratory-grade material. It appears that the environmental conditions affect the different AN forms differently. Without further study, it is difficult to say if this divergence was owing to differences in how the AN form interacts with change in humidity, temperature increase, or a combination of both. Overall, the changes in ammonia availability could be significant for vapor detection as greater ammonia availability increases the chance of detection, while cooler and drier temperatures, which suppress ammonia formation, could prohibit detection should the ammonia concentration fall below the minimum threshold for olfactory detection by canine.

Examining the effects of temperature and humidity on the presence of other VOCs from the headspace of the AN samples, Table 4 compares the most prominent VOCs and their relative abundance in a heat map for

Table 3
Volatiles detected in the headspace (HS) of AN samples, excluding ammonia. All compounds were identified by comparison to the NIST mass spectral library. Note. All volatiles also found in the blank vial were removed from this data.

AN variant/ HS component	Lab	Indust, ground	Indust, prill	Fert, ground	Fert, prill	CAN	Ice pack
Acetic acid	X	X	X			X	
Propanoic acid	X						
Oxime-methoxy phenyl	X	X	X	X	X		X
1-butanol				X	X		X
Acetamide						X	
2-ethyl-1-hexanol						X	
Acetone							X
Pentanal							X
Hexanal							X
Pyridine							X
2-methyl pyridine							X
4-methyl pyridine							X
2,6-dimethyl pyridine							X
2,4-dimethyl pyridine							X
2,3-dimethyl pyridine							X

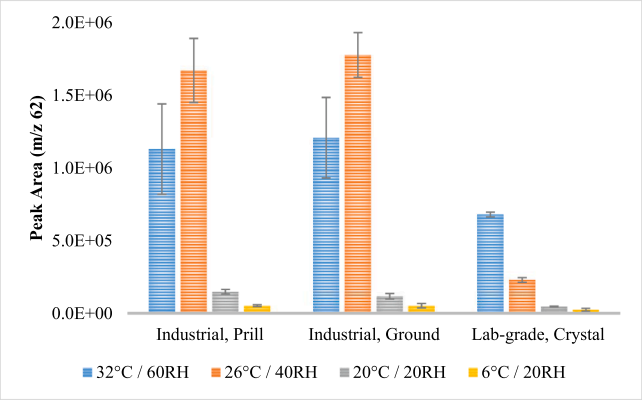

Fig. 3. Ammonia vapor measured from the headspace of AN samples at varying temperatures and humidities. The magnitude of ammonia vapor present is given as the peak area of the main ion, *m/z* 62, of butyl carbamate (derivatized ammonia). Note. Error bars equal one standard deviation.

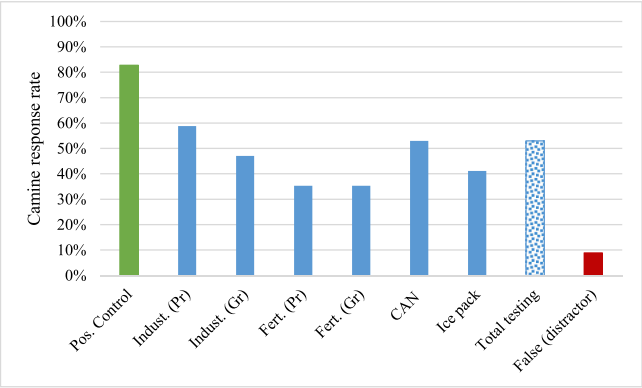

Fig. 4. Canine response rate to the novel AN variants, as well as the positive control (laboratory-grade AN) and false alerts to distractor odors. The alert rates for all variants were significantly higher than the false alert rate and significantly lower than the positive control (χ^2, 95% confidence, N = 17).

each variant at the differing environmental conditions (full chromatograms given in Appendix A). The first result to note is the general increase in VOCs in the industrial-grade AN samples even at moderate temperatures, compared to the results from the samples discussed above (Table 2). For the samples in Table 2, each AN material was removed from its respective bulk storage vessel and placed into a vial, which was then quickly lidded, allowed to equilibrate for one hour and then sampled. In contrast, after being placed in the vials, the samples referenced in Table 4 were left unlidded to interact with the environment for one hour prior to equilibration and extraction. Even at ambient temperatures, this exposure to the ambient environment seemed to increase the availability of VOCs from the AN, particularly the industrial-grade samples.

Both industrial-grade samples, ground and prill, yielded similar volatiles in similar quantities at each temperature / humidity point. In these samples, there was a significant increase in hydrocarbons, particularly alkanes (C10-21) and organic acids (acetic and butanoic), as well as lesser quantities of branched aromatics, branched cyclics, and aldehydes that were more difficult to distinguish (see Appendix A, Figure A1) as these samples were heated above room temperature. The colder temperature suppressed many of these components (see Appendix A, Figure A1), leaving only predominantly lower amounts of the alkanes (Table 4) to be detected. When heated above room temperature, the laboratory-grade sample yielded significant amounts of acetic acid in addition to 2-(2-methoxyethoxy)ethanol and 2,4,4-trimethyl-3-(3-methylbutyl) cyclohex-2-enone, both likely artifacts of the manufacturing process. When cooled, acetic acid was the only predominant volatile detectable in this sample besides ammonia. The significant increase or decrease in volatiles from impurities or manufacturing alters the scent picture appreciably and could confound detection. For this reason, the environmental conditions should be taken into account during detection tasks.

3.2. Canine testing

A total of 17 canines participated in in this test, and none were removed from the study due to unsuccessful detection of the positive controls (laboratory-grade AN) or due to excessive false alerts (defined as more than two false alerts to distractor odors or more than five total [distractor + blank] false alert rate). It should be noted that, due to circumstances beyond control of the test coordinators, some canines were only tested on a single positive control. This is noted in the supplemental data and was taken into consideration when calculating true alert and false alert rates (Appendix A).

A summary of the results are included in Fig. 4, though all canine responses are included in the Appendix A. The detection rate for the positive control (laboratory-grade AN) was 83% (for comparison, working dog requirements generally range from 90 to 95% proficiency [16 18]) with a 9% false alert rate to distractor odors and a total false alert rate of 8%. Overall, the canines located the novel AN variants significantly less than the trained odor at a rate of 53% (χ^2, 95% confidence, N = 17). The alert rate for all testing odors ranged from 35% to 59%, significantly lower than that of the positive control but significantly greater than chance (false alert rate) (χ^2, 95% confidence, N = 17). The majority of the canines (71%) alerted to less than four of the six novel odors, with no single canine responding to all six testing odors (Fig. 5). Largely, the type and number of variants to which the canines generalized appeared to be dependent on the individual canine olfaction process, though several trends can be noted. Canines were more likely to detect the industrial-grade prill AN (59%), the AN with the most similar odor profile to the laboratory-grade material. Then canine response decreased as similarity to the trained material also decreased, industrial-grade ground (47%) followed by fertilizer prill or ground (35%). Conversely, canines more readily detected the CAN (53%) and the ice pack AN (41%) than the fertilizer-grade materials, which were the most *dissimilar* to the trained materials. Those materials, however, did have the highest odor availability (see Fig. 2 and Table 3). These results indicate that both total odor availability and similarity to the trained material play roles in generalization.

4. Discussion

Canines were trained to laboratory-grade AN and asked to detect other less pure AN variants. This is a common practice in operational canine training, where canines may be trained on the purest form of a substance, but are expected to detect less pure forms in the field. After initial training, all canines did show some generalization to the AN variants as all were detected at a rate significantly higher than chance. This was, however, significantly lower than the detection rates of the trained odor. These results agree with the previous study carried out by Larazowski et al. (2015) who found that canines trained to a single AN source displayed "weak generalization" to AN from different manufacturers [12]. These results also agree with the study by Hall & Wynne (2018) that determined that canines trained only to pure AN failed to successfully generalize to mixtures containing the substance [13].

The participants were asked to complete a survey providing average training time per week during the study (data included in Appendix A). No correlation was found between a canine's chance of detecting the novel AN variants and time spent training.

No particular variant was found to be more "similar" or more "distinct" to the training odor by this group of canines. Odor

Table 4
Relative abundance, given as a heat map, of VOCs detected in the headspace of ammonium nitrate samples, including laboratory-grade and industrial-grade ground and prill. Relative abundance was determined based on averaged peak areas of three replicate samples, with the highest abundance indicated in dark red, followed by red, orange, yellow and then green. Note that the identification of the compounds is based solely on comparison to the NIST mass spectral library and not based on retention time comparison to standard compounds, as such, the exact identity of some compounds may be different than what is listed below.

	Laboratory-grade				Prill				Ground			
Compound ID	32°C / 60RH	26°C / 40RH	20°C / 20RH	6°C / 20RH	32°C / 60RH	26°C / 40RH	20°C / 20RH	6°C / 20RH	32°C / 60RH	26°C / 40RH	20°C / 20RH	6°C / 20RH
Acetic acid												
Pentanoic acid												
2-(2-methoxyethoxy)ethanol												
Decane												
Undecane												
1-ethyl-3,5-dimethyl benzene												
Dodecane												
2,6,10-trimethyl dodecane												
Tridecane												
2,4,4-trimethyl-3-(3-methylbutylcyclohex)-2-enone												
Tetradecane												
2,6-di-tert-butyl-p-benzoquinone												
Pentadecane												
Hexadecane												
Heptadecane												
Octadecane												
2,6,10-trimethyl tetradecane												
Nonadecane												
Eicosane												
Docosane												

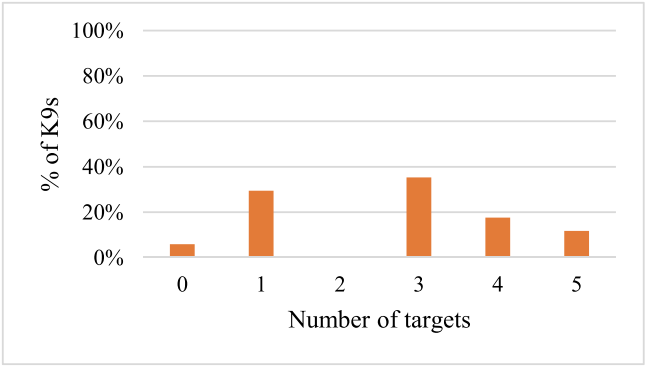

Fig. 5. Percentage of canines that responded to a given number of the AN variants (targets).

"similarity", based on canine detection rate, seemed to vary based on interpretation of individual canines (individual canine data is given in Appendix A). Such individuality across canines was also noted by Lazarowski et al., who observed that some canines display a higher tendency to generalize to related odors than others [12].

5. Conclusion

Headspace analysis by SPME-GC/MS was used to characterize and compare the headspace profiles of AN samples differing in form, manufacturer, and purity, as well as effects of environmental conditions. The results indicated differences in both the quantity of ammonia vapor and the presence of contaminating volatiles between the AN samples tested. Furthermore, exposure to increasing environmental temperature and humidity increased the presence of ammonia, as well as confounding volatiles in the headspace, although these results were dependent on the type of AN tested. In addition, these data were correlated to canine detection proficiency, where, for this group of canines, generalization from the trained material (laboratory-grade AN) to other AN variants was explored using a series of simple detection tasks. The outcomes of the canine testing indeed were correlated to the headspace profiles with both quantity of ammonia and differences between extraneous volatile compounds in the headspace affecting detection rate with this set of canine participants.

CRediT authorship contribution statement

Lauryn E. DeGreeff: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Project administration, Funding acquisition. **Kimberly Peranich:** Conceptualization, Methodology, Investigation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2021.100342.

References

[1] Defense Intelligence Agency, Homemade Explosives, 3rd Edition, July 2014.
[2] Department of Homeland Security, Science and Technology, Homemade explosives identification, detection, and mitigation program, Department of Homeland Security, https://www.dhs.gov/science-and-technology/heidm-program (Accessed 08 September 2020).
[3] D. K. Sypesteyn, Canines in Combat: Military Working Dogs, San Antonio Magazine, https://www.sanantoniomag.com/canines-in-combat-military-working-dogs/, November 2013.
[4] Transportation Security Administration, TSA Canine Training Center: Facts Sheet, https://www.tsa.gov/sites/default/files/resources/caninetrainingcenter_factsheet_0.pdf, 2019 (Accessed 3 September 2020).
[5] Remarks by LTG M.G. Babero, delivered at RUSI Land Warfare Conference; London, UK, https://jieddo.mil/content/docs/20120608_LTG Barbero Remarks_RUSI_As Prepared.pdf, 8 June 2012 (Accessed 4 October 2017).
[6] Committee on Reducing the Threat of Improvised Explosive Device Attacks by Restricting Access to Chemical Explosive Precursors, Reducing the Threat of Improvised Explosive Device Attacks by Restricting Access to Explosive Precursor Chemicals, Washington, DC: National Academies Press, 2018.
[7] A. G. Simon, L. E. DeGreeff, M. Maughan, J. Gadberry, Canine detection of explosives: Shifting focus from traditional to homemade explosives, Naval Research Laboratory, NRL/MR/6181–1-9794, Washington, DC, 2018.
[8] R.G. Ewing, M.J. Waltman, D.A. Atkinson, J.W. Grate, P.J. Hotchkiss, The vapor pressure of explosives, Trends Anal. Chem. 42 (2013).
[9] F. Steinkamp, B. Giordano, G. Collins, S.L. Rose-Pehrsson, Volatile emissions of ammonium nitrate under flowing conditions, Propellants Explos. Pyrotech. 35 (2010) 1–7.
[10] A.L. Lubrano, B. Andrews, M. Hammond, G.E. Collins, S. Rose-Pehrsson, Analysis of ammnium nitrate headsapce by on-fiber solid phase microextraction derivatization with gas chromatography mass spectrometry, J. Chrom. A 1429 (2016) 8–12.
[11] C.J. Katilie, A.G. Simon, L.E. DeGreeff, Quantitative analyis of vaporous ammonia by online derivatization with gas chromatography – mass spectrometry with applications to ammonium nitret-based explosives, Talanta 193 (2018) 87–92.
[12] L. Lazarowski, M. Foster, M. Gruen, B. Sherman, N. Fish, R. Milgram, D. Dorman, Olfactory discrimination and generalziation of ammonium nitrate and structurally related odorants in Laborador retrivers, Anim. Cognit. 18, no. 6 (2015) 1255–1265.
[13] N.J. Hall, C.D.L. Wynne, Odor mixture training enhances dogs' olfactory detection of Home-Made Explsovie precursors, Heliyon 4 (2018), e00947.
[14] H. Brown, K.P. Kirkbride, P.E. Pigou, G.S. Walker, New developments in SPME Part 2: Analyis of ammonium nitrate-based explosives, J. Forensic Sci. 49 (2) (2004) 1–7.
[15] National Association of Canine Scent Work, https://www.nacsw.net, 2017.
[16] Scientific Working Group for Dogs and Orthogonal Devices Guidlines, SC2 General Guidelines from SWGDOG Approved Guidelines, SWGDOG.org, 2012.
[17] N.G. Grigor'eva, N.A. Filippova, M.R. Agliullin, B.I. Kutepov, N. Narender, Crystalline and amporphous aluminosilicates with different pore structures for the synthesis of pyridines, J. Chem. Res. 41, no. 5 (2017) 253–261.
[18] Communications with Charles County Sheriff's Office, Special Operation Divsion, La Plata, MD, 31 Oct. 2017.