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Canine olfactory detection of trained explosive and narcotic odors in mixtures using a Mixed Odor Delivery Device

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ABSTRACT

Like using a substandard calibrant to test and calibrate an instrumental detector, when detection canines are regularly exposed to less than optimal training material, their detection proficiency is diminished, risking the lives of their handlers and civilians they are intended to protect. This research examined canine detection proficiency to odor mixtures and the use of mixture training to improve said proficiency. Trained detection canines were tested on their ability to correctly locate their trained target odors, explosives or narcotics, in various mixtures from a series of blanks and distractor odors. After making base measurements, canines were trained on the target odor in mixtures using the Mixed Odor Delivery Device (MODD), which was previously developed to safely contain separated explosive components and deliver the mixed odor to a canine detector for training purposes. Headspace measurements, made using solid phase microextraction with gas chromatography/mass spectrometry (SPME-GC/MS), were also taken of mixture components in and out of the MODD to confirm that odor mixtures were accurately portrayed to the canines during MODD training. Following mixture training, canines were retested on the same mixtures. Results of the headspace analysis showed that the MODD did not alter the delivery of the odorants from the mixture components. As such, canines showed an improved proficiency in detection of target mixtures following mixture training, increasing the detection rate from 63% to 72% for pseudo cocaine mixtures and from 19% to 100% for explosive mixtures.

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1. Introduction

While numerous devices exist for the detection of contraband, canines are currently the most effective tool for non-contact detection of narcotics and explosives, as well as many other targets of interest. Canine detection has demonstrated superior sensitivity and selectivity over most field-deployable detectors and sensors across a broad range of target analytes, and, unlike instrumental detectors, canines are easily trained to new target odors [1,2]. With proper and consistent training, canine detectors are capable of locating a wider range of targets with lower false alert rates than most or all currently deployable detectors [3]. However, due to safety and legal restrictions, consistent access to new and potentially dangerous explosives and narcotic mixtures to support detection of field-relevant targets is often limited. For instance, homemade explosives (HMEs), specifically fuel-oxidizer mixtures assembled from commercially available materials, pose a dangerous threat both at home and overseas. These binary mixtures are simply made by mixing an oxidizer, such as ammonium nitrate (AN) or potassium chlorate ($KClO_3$), with a number of possible fuels. Because of the danger associated with these homemade materials, explosive detection canines are often trained on the oxidizer alone, and expected to detect it in the presence of any fuel [2]. This can be challenging due to the inherently low, or negligible, vapor pressures of oxidizer salts and the potential high vapor pressures of fuel components.

Like HMEs, street narcotics are often mixtures of a pure drug, or multiple drugs, and cutting agents. The presence of these impurities complicate the odor profile and may impede detection or prevent the canine from giving a final alert, which is a major liability for the department and has important legal implications. However, similar to HMEs, safety and security restrictions prevent many canine handlers from training on street drugs or physically mixing their training aids with common impurities to replicate street mixtures. This potentially has a negative impact on canine proficiency.

In this regard, a HME or narcotics detection canine will come across their trained target odorants in mixtures from many sources

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in the course of their working life, including fuels and adulterants physically mixed with the target, as well as other odorants from the surrounding environment. Component odorants in a mixture may be perceived elementally (i.e. individually) or configurally (i.e. experienced as a new single odor). Binary or other simple mixtures are more likely to be perceived elementally, while more complex mixtures are more likely to be perceived configurally [4]. As such, when a trained target is in a mixture, the canine may either discriminate the target from the other odorants in the mixture, and thus correctly locate the target, or the mixture may be perceived configurally as a new odor, preventing the detection of the target. In addition, overshadowing, where a single odorant in a mixture overwhelms the odor profile, may prevent detection of other odorants in a mixture [5,6]. In the case of trained detection canines, Waggoner et al. showed that detection canines require a very high excess, approximately three orders of magnitude, of an extraneous odor to reduce the detection to a trained target odor [7]. If one considers explosive mixtures, such as AN and diesel fuel, the vapor pressure of diesel fuel (approximately 14.1 mmHg at 25 °C) exceeds that of the AN (1.11×10^{-5} mmHg at 25 °C [8]) by much more than three orders of magnitude [9]. As such, it is not surprising that previous research with explosive detector canines has shown that canines trained on a HME oxidizer alone do not necessarily detect the HME mixtures with high proficiency [10–12].

The way mixtures are perceived can also be changed through learning. Similar to optimizing the selectivity of a sensor, selective attention, which is the ability to concentrate on a single odor while ignoring other confounding odors, can be achieved through training [13]. Selective attention can enhance elemental perception when mixtures contain the trained odor [14]. For instance, researchers have shown that mixture training has the potential to heighten selective attention and thus improve detection of explosive mixtures [11,12]. Hall and Wynne explored how experience and training with an individual explosive component effects canines' abilities to detect the component in novel mixtures. In this study, canines (n = 6) were trained either solely to AN or to AN in various mixtures. After training, all canines were presented with novel AN mixtures. Their results showed that the canines trained on mixtures out-performed those trained solely on AN [11]. Becker et al. conducted a similar experiment training canines (n = 5) to discriminate chamomile in herb mixtures after having been trained on chamomile alone or in mixtures, yielding the same results [15]. Furthermore, in a study by Lazarowski et al., canines were trained to detect potassium chlorate ($KClO_3$) alone, after which all canines correctly discriminated pure $KClO_3$ from other distractor odors. The canines (n = 16) were then tested on their ability to detect $KClO_3$ in four different mixtures, with a 69% correct response rate. Only two of the 16 canines successfully detected all four mixtures. Canines were subsequently trained on the same $KClO_3$ mixtures using a novel mixture training device, and in further testing, the canines correctly identified the four mixtures 81% of the time [10].

With the success of the novel mixture training device used by Lazarowski et al. [10], the Mixed Odor Delivery Device (MODD) (Fig. 1) was designed to alleviate difficulties in training with hazardous mixed explosives and improving mixture detection proficiency. The MODD can safely separate up to four explosive components in removable vials. The vials are placed securely in a lower compartment, while an upper component, where the canine sniffs, fits on top and is secured by an o-ring and latches on either side. The design allows vapors from the separated vials to diffuse to the base of a neck centered between the vials where the vapors meet and mix and then continue around a restrictor plug above the neck. The analyte vapor escapes from around the neck and restrictor plug to the MODD outlet as a plume of mixed vapor (Fig. 1, Center). The outlet is a bowl-shaped area around the restrictor plug in the upper compartment where the canine places its snout for sampling. The canine samples an odor mixture instead of separated component odors at this outlet. The MODD safely contains HME components and allows for accurate presentation of the vapor mixture without the physical mixing of explosives components, while also offering transportability and ruggedness for field use, and adaptability for the varied components one might encounter in the field [16].

In DeGreeff et al. [17], experiments were carried out across multiple investigative strategies, including computational modeling of vapor diffusion, analytical headspace vapor measurements, and canine testing, to evaluate and characterize the vapor distribution in the MODD. The laboratory experiments, in agreement with the vapor diffusion models, showed that separated HME component vapors mix and distribute evenly around the MODD outlet. Further headspace measurements and canine trials using ammonium nitrate-based HME mixtures revealed that the MODD accurately portrays the vapor profile of mixed materials from separated components; however, the effects of mixture training with the MODD were not explicitly tested.

Furthermore, previous research using AN-based explosives showed that physical mixing of AN with a fuel did not alter the odor profiles of individual components, meaning AN and diesel fuel, for example, have the same odor whether separated or physically combined. This indicates that there is no chemical reaction associated with mixing that alters the odor [17]. However, this has not

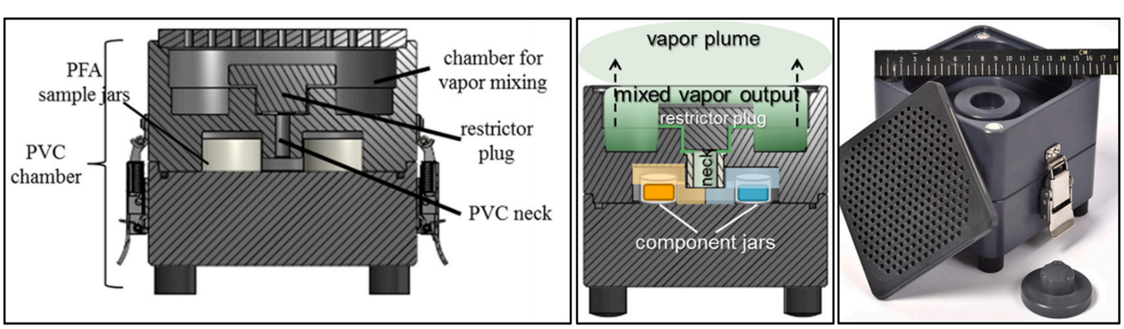

Fig. 1. The Mixed Odor Delivery Device (MODD): (Left) Diagram of MODD, sliced vertically; (Center) Diagram of vapor flow path through MODD [reprinted with permission from Elsevier; 17]; (Right) Photograph of MODD.

been tested for potassium chlorate ($KClO_3$), another oxidizer common to HMEs. Though the MODD was designed as a training device for mixed explosives, it also has utility in training narcotics detection canines for detection of adulterated materials.

In this study, two populations of trained detection canines were used to explore operational implications and training strategies in the detection of narcotics and explosives mixtures. Previous studies, such as that by Waggoner et al., did consider detection of operationally-relevant mixtures, but did not explore effects of training or prior experience on mixture detection [7]. Previous experience, through training or operational deployment, can have a significant effect on how well a target is discriminated from confounding odorants [18]. Lazarowski et al. did investigate the effect of mixture training on detection proficiency, but did not consider novel mixtures, testing the canines only on the same mixtures to which they were trained [10]. This approach makes it unclear whether mixture training enhanced selective attention to the trained oxidizer, or if the canines learned each mixture configurally. Hall et al. did explicitly test detection proficiency to novel mixtures containing a common component [11]; however, the odor delivery and testing was carried out using laboratory equipment and laboratory testing dogs trained exclusive for the research, leaving it still unknown if and how the approach translates to operational training and detection. The research discussed herein addresses these variables using operationally-relevant testing scenarios. Additionally, headspace analysis using solid phase microextraction with gas chromatography/mass spectrometry (SPME-GC/MS) was utilized to assess the volatile compounds delivered from the MODD outlet and available for detection by the canines during testing. These headspace measurements are imperative to ensure that the odorants in interest are detectable from the MODD and that the MODD does not alter the volatile profiles in such a way that would inhibit detection.

2. Materials and methods

2.1. *Experiment 1 Detection of cocaine odorants in mixtures before and after mixture training in a controlled setting.*

2.1.1. *Headspace analysis*

All narcotic samples were handled with appropriate safety precautions in a hood with proper personal protective equipment including nitrile gloves and a clean laboratory coat, and were stored in accordance with DEA guidelines. The odor profiles of actual cocaine samples were analyzed and compared to the same materials mixed with adulterants. 20 mL volatile organic compound analysis (VOA) vials of freebase cocaine (100 mg) and cocaine HCl (100 mg), previously seized by U.S. Customs and Border Patrol and legally obtained by the researcher, were prepared and allowed to equilibrate for 24 h. Confiscated samples were analyzed by CBP for purity prior to delivery to NRL. The headspace components were sampled with a solid phase microextraction (SPME) fiber (divinylbenzene/carboxen/polydimethylsiloxane [DVB/CAR/PDMS]; Millipore Sigma) inserted into the vial for a 1 h extraction time. This and all SPME extraction times were chosen based on previous extraction time optimization. Additionally, both types of cocaine (freebase – 300 mg, HCl – 1.8 g) were mixed with two adulterants (erythritol and caffeine – 3 g each, purchased from PureBulk) in individual VOA vials and extracted in the same manner. All samples were prepared in triplicate.

For comparison, 600 mg freebase cocaine and 2.6 g cocaine HCl were placed separately in the MODD for 2 h. Odorants emanating from the MODD outlet were then sampled by SPME for 2 h. Additionally, vials with one cocaine sample (freebase – 600 mg or HCl – 2.6 g) were then placed in the MODD with either erythritol (2 vials, 3 g each) or caffeine (2 vials, 3 g each). Note that while the amount of cocaine and adulterant was higher when used in the MODD, the ratio of cocaine to adulterants remained the same. Again, all samples were prepared in triplicate.

The MODD was cleaned by wiping thoroughly with isopropanol wipes, rinsing with running water, and the leaving it to dry for at least 18 h prior to use. This method of cleaning was shown to remove odorants form the PVC material composing the MODD to below the limit of detection of canines in a previous study [19].

The compounds on the fibers were thermally desorbed at 260 °C and analyzed by gas chromatography/mass spectrometry (GC/MS; Agilent 6890 GC/5975 MSD) using a 30 m × 0.25 ID × 1.00 µm df Rtx-Volatiles GC column (Restek Co.) with a flow rate of 2.0 mL/min and a split ratio of 10:1. Ultra high purity helium (Airgas) was used as the carrier gas. The GC oven program started at 40 °C for 1 min, then increased to 240 °C at 40 °C/min, and finally held for 3 additional minutes. The MS transfer line temperature was 250 °C and the scan range was m/z 30–300. Electron ionization was used at 70 eV. The compounds in the headspace were identified by comparison to the NIST Mass Spectral library and a methyl benzoate reference standard (50 ppm methyl benzoate [Aldrich] in methanol [99.8%, Sigma-Aldrich]). The ratios of the odorants from the pure and mixed material in vials and in the MODD were compared.

2.1.2. *Canine testing materials*

While the odor delivery from actual narcotics could be carried out in the laboratory, canine subjects canine subjects were not trained or tested with actual narcotics due to safety concerns. In lieu of actual cocaine, cocaine mimics were used for canine testing. Two types of mimics were used in training and testing, both were made by distributing methyl benzoate on a substrate. Methyl benzoate has previously been identified as the main odorant used in the detection of canine by trained canines [20] and was identified as the main volatile component in the headspace analysis described above. Cocaine training aids that were provided to the study participants consisted of Controlled Odor Mimic Permeation Systems (COMPS) with methyl benzoate (MB, cocaine odorant). The COMPS devices were also used for some of the distractor odors, as shown in Table 1. All COMPS were made by spiking 5 µL of the pure compound on gauze and enclosing it in a permeable bag (3″ × 4″, 6 MIL low density polyethylene, Uline). Further discussion and the method for creating COMPS can be found in Simon et al. [21]. Separately, a pseudo cocaine was used only for testing and consisted of methyl benzoate (SigmaAldrich) on a cellulose substrate (Sigmacell, SigmaAldrich). The cellulose powder gave the pseudo cocaine the look of pure cocaine and facilitated the mixing of pseudo cocaine with the powdered adulterants. Pseudo MDMA and methamphetamine were also made in the same fashion using piperonal and benzaldehyde (SigmaAldrich), respectively, as odorants on a cellulose substrate. A list of all target, control, and distractor odors used in Experiment 1 are included in Table 1 (all materials not already defined, were purchased from local supermarkets).

The pseudo cocaine alone or in a mixture was placed in small tins (2 oz. rust-resistant screw lid tin cans) purchased from Papermart. When not in use, a solid lid was placed on the tins, which were then stored in plastic storage containers with air tight lids. For testing the solid lid was removed and replaced with a perforated lid, and, to ensure no contamination of the testing area by spilled powder, the tin was then placed into a cotton bag (6″ × 4″, Grainger) before being

Table 1
Materials used in canine Trials 1 and 2, to include the positive and negative controls, adulterants (materials mixed with pseudo cocaine), and distractors. Training materials are listed in the far right column. All canines were trained with the methyl benzoate COMPS and blank COMPS. The Experimental Group also trained with adulterants in the MODD with the methyl benzoate COMPS, as noted by a (*).

Material type	Trial 1	Trial 2	Training materials (**Experimental Group only*)
Positive controls	methyl benzoate COMPS	methyl benzoate COMPS	methyl benzoate COMPS
	1 g pseudo cocaine	1 g pseudo cocaine	
Adulterants (components mixed with 1 g pseudo cocaine)	1 g pseudo MDMA	1 g pseudo MDMA	*piperonal (MDMA) COMPS
	1 g pseudo methamphetamine	1 g pseudo methamphetamine	*plaster of paris
	3 g powdered milk	3 g caffeine	*powdered milk
	3 g meat tenderizer	3 g erythritol	*citric acid
	3 g corn starch	3 g corn starch	*chalk
	3 g creatine	3 g chalk (crushed)	
	1 g pseudo MDMA + 3 g powdered milk	1 g pseudo MDMA + 3 g chalk	
	1 g pseudo methamphetamine + 3 g corn starch	1 g pseudo methamphetamine + 3 g corn starch	
Distractors(no mixtures)	1 g pseudo MDMA	1 g pseudo MDMA	
	1 g pseudo methamphetamine	1 g pseudo methamphetamine	
	3 g powdered milk	3 g caffeine	
	3 g meat tenderizer	3 g erythritol	
	3 g corn starch	3 g corn starch	
	3 g creatine	3 g chalk (crushed)	
	cuminaldehyde COMPS	cuminaldehyde COMPS	
	furfuryl COMPS	furfuryl COMPS	
	carvone COMPS	carvone COMPS	
	eucalyptol COMPS	eucalyptol COMPS	
	eugenol COMPS	eugenol COMPS	
	cinnamaldehyde COMPS	cinnamaldehyde COMPS	
Negative controls	blank COMPS	blank COMPS	blank COMPS
	1 g cellulose powder	1 g cellulose powder	

put in the box. All positive controls, distractor odors, and blanks (clean cellulose) were also placed in similar tins and cotton bags.

All testing materials were prepared the day prior to the testing and stored under cool, indoor, ambient conditions. Care was taken to minimize any chance of cross-contamination. Blanks and distractors were prepared prior to the pseudo cocaine and COMPS. Blanks, distractors, positive controls, and adulterants were all double contained and stored and transported separately. Gloves and counter surface coverings were changed after handling each individual odor.

2.1.3. Canine selection and training

Canines were selected from the National Association of Canine Scent Work (NACSW). The NACSW organization trains pet dogs to locate specific odors (essential oils of anise, clove, and birch) for competition. Competition and training replicate operational detection dog scenarios, such as interior and exterior building, luggage, and vehicle searches. Twenty NACSW members participated in this testing held at the Richmond Dog Obedience Center (Richmond, VA). The canine participants were chosen by the NACSW trainer and held either the Elite (highest) or III (second highest) level of detection proficiency as given by the NACSW through successful completion of competitive trials of increasing difficulty, or were chosen by the trainer for having equivalent detection proficiency. No participants had previously trained or encountered cocaine or cocaine training materials. The age, breed, and years of experience of each canine participant is included in the Supplemental information (Table S1).

Canine handlers were provided with training aids that mimic the odor of cocaine (MB COMPS), as described above, and were given six weeks to train their dogs to locate that odor. The method and frequency of training was chosen by the handler. Recommended training methods were given to the handlers and are included in the Supplement information. Confirmation that individual canines had successfully been trained to the target odor was provided through use of three positive controls containing the odor of cocaine during the testing. Canines that did not correctly respond to at least two of the three positive controls were not included in the data.

After the first trial, the participants were divided into two groups at random. The Control Group continued to train with the cocaine training aids (MB COMPS) only, while the Experimental Group trained on the cocaine training aids, but were also given a set of MODDs along with a number vials containing various cutting agents (according to Table 1) with the instructions to use the MODD to create mixtures of their choosing (Supplemental information). MODDs were cleaned in the same manner as used in the laboratory analysis. Both groups were given an additional six weeks to train as described, and then were tested again using some of the same and some novel pseudo cocaine mixtures. Training records for all canine participants can also be seen in the Supplemental information (Table S2).

2.1.4. Canine tests

Tests consisted of a series of rows or line-ups of five new white cardboard boxes (9″ × 6.5″ × 2.75″ purchased from Uline). Each row contained a single target material or control, a distractor material, and three blanks. There were eleven rows in total, with one of the eleven having no target or control odor, and only a distractor odor and four blank boxes. The identity and location of the targets, blanks, and distractors were randomized for each canine. Both Trials 1 and 2 included a MB COMPS and two pseudo cocaine (alone) as positive controls, as well as blank COMPS and blank cellulose. The materials used in canine testing are given Table 1.

The testing was conducted double-blind, meaning neither the participants nor the assessors knew the location or identity of the materials used during testing. The test assessors recorded canine

Table 2
Potassium chlorate/fuel mixtures used in Task 1 – component ratios and total mass used.

Fuel	$KClO_3$: fuel ratio	Mass of $KClO_3$	Mass of fuel
Petroleum jelly	9:1	2.7 g	0.3 g
Sugar	1:1	1.5 g	1.5 g
Automatic transmission fluid	9:1	2.7 g	0.3 g
Vegetable oil	9:1	2.7 g	0.3 g

behavior in addition to the handler observed final response. Test coordinators were also present to clean between participants, as necessary, change the order of the line-ups and positioning of the targets, and confirm or deny the presence of a target odor after the handler called the "alert". The test coordinators remained separated from and did not interact with the handlers or test assessors during testing to maintain the double-blind testing scenario. Handlers were allowed to work on or off-leash and use the reward type of their choice (i.e. food, toy, or praise). False alerts were also noted by the assessor.

2.1.5. Data analysis

Chi-square test for independence was used to compare the distribution of discrete responses between groups (e.g. Control vs. Experimental or Trial 1 vs Trial 2). The null hypothesis assumes no difference in responses between groups. The alternative hypothesis states that there was a difference in response distribution between groups. In all cases the confidence level used was 95% and the N value for each group being compared is located on the corresponding figure legends.

2.2. Experiment 2 Detection of homemade explosive mixtures before and after mixture training in an operational setting.

2.2.1. Headspace analysis

Previous work by DeGreeff et al. examined ammonium nitrate mixtures in the MODD [17]. As such, this work completes the headspace analysis of mixed and unmixed potassium chlorate-based explosives. Potassium chlorate ($KClO_3$) was purchased from a fireworks-supply company (Skylighter). The fuels used were commercially available, purchased from local stores (Washington, DC), and included petroleum jelly (Vaseline brand), vegetable oil (Crisco brand), transmission fluid, and sugar. The odor of each fuel and $KClO_3$ were each sampled separately in addition to $KClO_3$-fuel mixtures. Replicates of five mixtures were made in ratios according to Table 2, totaling 3 g of material for each. When testing individual components, the same masses were tested as were used in the mixtures.

For sampling, mixtures or individual components were placed in a custom headspace sampling chamber designed by NRL [22] and allowed to equilibrate for 18 h prior to headspace extraction. Headspace sampling was carried out using a previously established method for chlorine detection [23]. In brief, the headspace of the $KClO_3$ was extracted by SPME (DVB/CAR/PDMS; Millipore Sigma) and analyzed by GC/MS. However, the chlorine from $KClO_3$ would not be detectable at trace levels using traditional SPME-GC/MS methods. For this reason, the SPME fiber was exposed to the derivatizing agent, vaporous propylene oxide (≥ 99.5%, Aldrich), in a 20 mL VOA vial for 15 s. The fiber was then removed and immediately exposed to the headspace of the $KClO_3$ samples, also in 20 mL VOA vials, for an additional 18 h. The propylene oxide derivatizes the chlorine directly on the SPME fiber resulting in chloro-2-propanol, detectable by GC/MS. The other non-chlorine containing products could be detected by this method, as well, without effects of the derivatizing agent. The same GC/MS instrument and GC column were used as above. The instrumental parameters were also the same, though the GC oven program was started at 35 °C instead of 40 °C. Additional details of the analysis method can be found in Crespo Cajigas et al. [23]. The relative amounts of headspace products (i.e. odorants) were measured and compared between unmixed and mixed materials.

2.2.2. Canine testing materials

Experiment 2 was carried out in the same manner as Experiment 1, except in an operational setting using homemade explosive –related materials. For security reasons, the actual testing supplies used will not be named in this publication. The testing and training materials are listed in Table 3. All testing and training materials were purchased locally from commercial retailors (Colorado Springs, CO). Mixtures were made the day of testing and held in drawstring cotton bags (8″ × 10″, Grainger) inside of clean, 64 oz glass jars. Distractors

Table 3
Materials used in canine Trials 1 and 2, to include the positive and negative controls, additives (materials mixed with targets), and distractors. Training materials are listed in the far right column. All canines were trained in the same manner for the duration of the study.

Material type	Trial 1	Trial 2	Training materials
Positive controls	Target X (T-X)	Target X (T-X)	Target X (T-X)
	Target Y (T-Y)	Target Y (T-Y)	Target Y (T-Y)
Target mixtures (additive + target)	Additive A (A-A) + T-X	Additive A (A-A) + T-X	Additive D (A-D)
	Additive B (A-B) + T-X	Additive B (A-B) + T-X	Additive E (A-E)
	Additive B (A-B) + T-Y	Additive B (A-B) + T-Y	Additive F (A-F)
	Additive C (A-C) + T-Y	Additive C (A-C) + T-Y	Additive G (A-G)
Distractors (no mixtures)	Additive A (A-A)	Additive A (A-A)	
	Additive B (A-B)	Additive B (A-B)	
	Additive C (A-C)	Additive C (A-C)	
	Additive D (A-D)	Additive D (A-D)	
	Latex gloves	Latex gloves	
	Permanent markers	Permanent markers	
	Food products	Food products	
	Electrical components	Electrical components	
	Oil	Oil	
	Human-scented objects	Human-scented objects	
Negative controls	Empty cotton bags in jar	Empty cotton bags in jar	Empty PFA vials

were also placed inside of cotton bags and placed within the jars, as well. Care was taken to prevent cross-contamination. Gloves were changed regularly between handling materials, distractors and controls. Mixtures were prepared separately and physical distance was maintained between materials when not in use on testing days. For training with the MODD, aliquots of target and additive materials were kept in 22 mL PFA vials (Savillex) and stored in such a way to prevent cross-contamination.

2.2.3. Canine selection and training

All canine participants were certified explosive detection dogs with previous training on both Targets X and Y (T-X and T-Y). While the canines had not explicitly been trained on mixtures of T-X and T-Y with other materials, such mixtures may have been encountered in operational use. Canines were of traditional working dog breeds, including German Shepherds and Belgian Malinois, male and female, ranging in age from three to seven years.

After Trial 1, handlers were given an additional three weeks of training time. All canines trained in the same manner using T-X and T-Y placed in MODDs alone or in combination with the Additives listed in Table 3. Note that the additives used in training were different than those used in Trials 1 and 2. Instructions for the use of the MODDs during training were the same as given for Experiment 1 and are included in the Supplemental information. At the completion of the MODD training, canines were re-tested on the same mixtures as previously seen in Trial 1.

2.2.4. Canine tests

Tests consisted of a series of rows of four glass jars (canning-type jars purchased locally [Colorado Springs, CO]). Each row contained a Target/Additive mixture or positive control, a distractor material, and two blanks. There were eight rows total, with two of the eight having no target odor, and instead three blank jars and a distractor. The materials used in canine testing are given Table 3.

The testing was conducted double-blind, meaning neither the participants nor the assessors knew the location or identity of the materials used during testing. The test assessors recorded canine behavior in addition to the handler observed final response. Test coordinators were also present to change out the mason glass jars to new ones between dogs, change the order of the line-ups and positioning of the targets, and confirm or deny the presence of a target odor after the handler called the "alert". The test coordinators remained separated from and did not interact with the handlers or test assessors during testing to maintain the double-blind testing scenario. The test scenarios were set up using two phones that were placed on speaker, one phone in the test room and the other phone with the test coordinator at a different location so the test coordinators could hear the handler call the alert as well as the location of the alert. Handlers were allowed to work on or off-leash. Any canine that did not correctly locate both of the positive controls was excluded from the data. False alerts were also noted by the assessor and any canine that gave more than two false alerts (25%) on distractors was also excluded.

3. Results and discussion

3.1. Experiment 1 Detection of cocaine odorants in mixtures before and after mixture training in a controlled setting.

3.1.1. Headspace analysis

The odor profiles of freebase cocaine and cocaine HCl alone (top) and in the MODD (lower) are presented in the chromatograms below (Fig. 2). Note the major odorant associated with cocaine, methyl benzoate, in both samples was in greater abundance in the freebase cocaine than the cocaine HCl. Other products in the headspace are also noted. Methyl benzoate is a degradant of cocaine and was previously identified as the main odorant involved in canine detection of cocaine. The research showed that, though methyl benzoate may not be the only component in the headspace of cocaine, it is present in cocaine samples of varying purity levels and is the active odorant involved in detection by canine [20,24]. Comparing the upper and lower chromatograms, it can be seen that the components

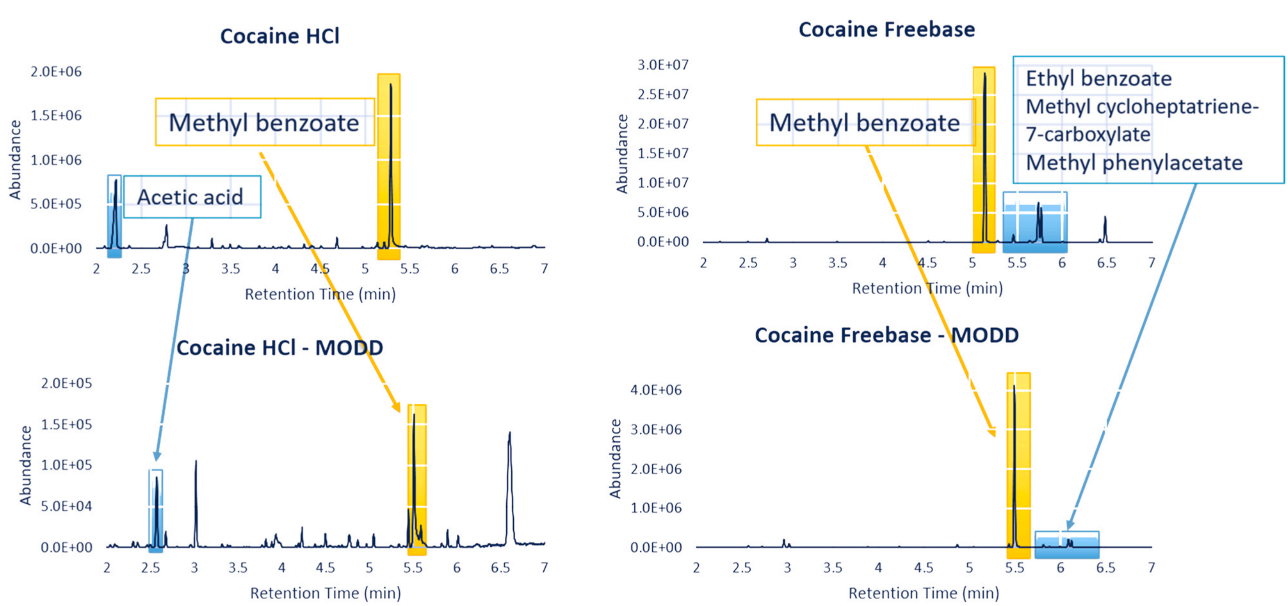

Fig. 2. GC/MS chromatograms collected from the headspace of cocaine HCl (left) and cocaine freebase (right) samples alone (top) and in the MODD (bottom). Major components of the odor profiles of the cocaine are noted on the chromatograms.

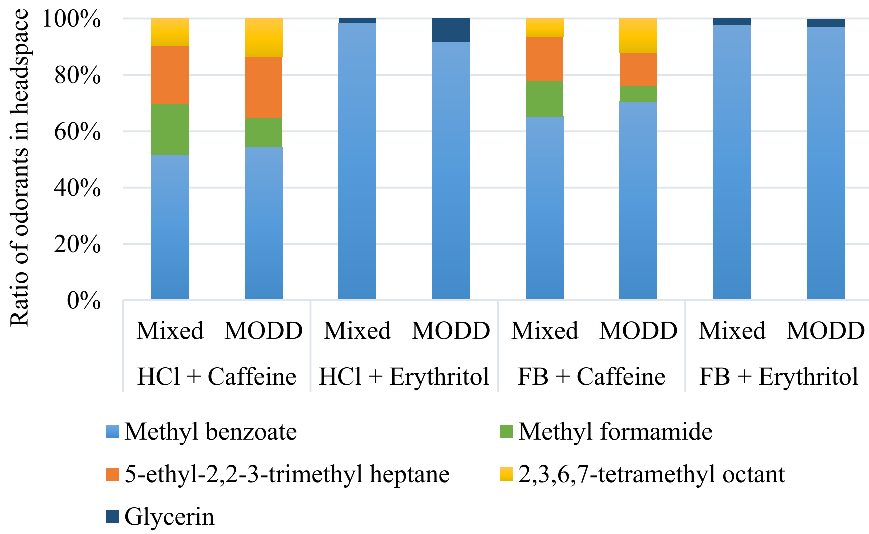

Fig. 3. Comparison of odorants detected in the headspace of cocaine HCl (HCl) or cocaine freebase (FB) physically mixed with adulterants (caffeine or erythritol) and as separate components in the MODD. The data presented as ratio of odorants measured in the headspace from averaged measurements (N = 3).

of the odor profiles of both cocaine samples are present emanating from the MODD as well, implying that the main odorant of cocaine and other less odorants are detectable from the MODD.

Having evaluated cocaine alone in the MODD, it is necessary to evaluate cocaine with adulterants. It was hypothesized that ratio of odorants achieved by physically mixing cocaine with these adulterants will remain the same as those produced when placed separately in the MODD. Fig. 3 shows the ratio of the adulterant odorants to methyl benzoate from the cocaine samples. As seen in previous research by DeGreeff et al. [17], there was little difference between the actual mixed samples and those in the MODD, indicating the MODD provides an accurate odor mixture for these components.

3.1.2. Canine testing

Seventeen canines took part in the first trial, but two were not included in the data for missing two or more of the positive controls; none were eliminated for excessive false alerts. Of the 15 subjects included in the data, the total positive response rate to the positive controls (MB COMPS + pseudo cocaine) was 91%. The positive response rate to all mixtures was 67%, statistically different than the response to the positive controls, and statistically greater than the false response rate (4%). The data is summarized in Fig. 4. The distribution of the total number of mixtures to which canines alerted are given in Fig. 5, dark blue plot. Individual canine data is given in the Supplemental information.

The lower detection rate to the mixtures could be due to effects of overshadowing or configural mixture perception, or due to physical depletion of target odorant in the headspace. The canines had the highest response rate to pseudo cocaine mixed with creatine (87%) and the lowest response rate to the mixtures containing corn starch (27% and 33%). As corn starch does not have a strong odor in comparison to the pseudo cocaine, it is unlikely that this low detection rate is due to overshadowing, and is more likely that the corn starch, being a naturally adsorbent material actually adsorbs

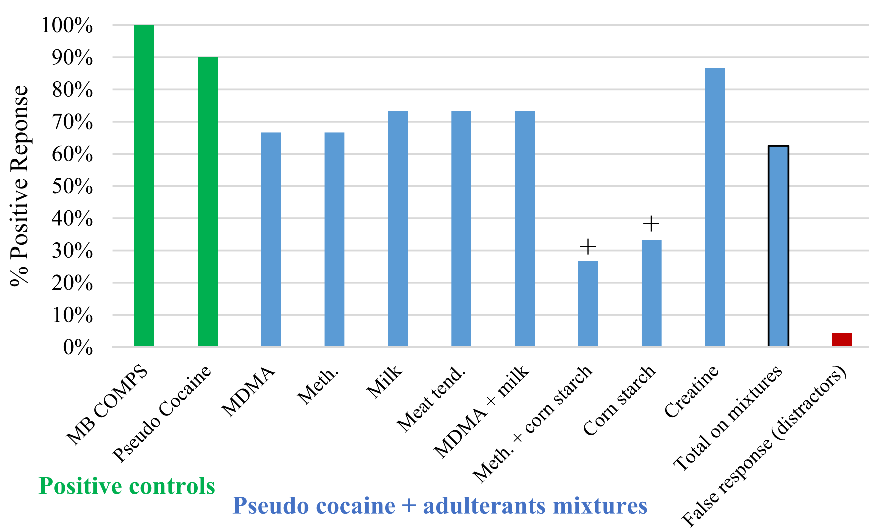

Fig. 4. Percentage of canines that responded to positive controls, pseudo cocaine mixtures, or distractors (false response) in Trial 1. All data were statistically different than chance. (+) indicates mixtures with response rates statistically different than the response to the positive controls. N = 15.

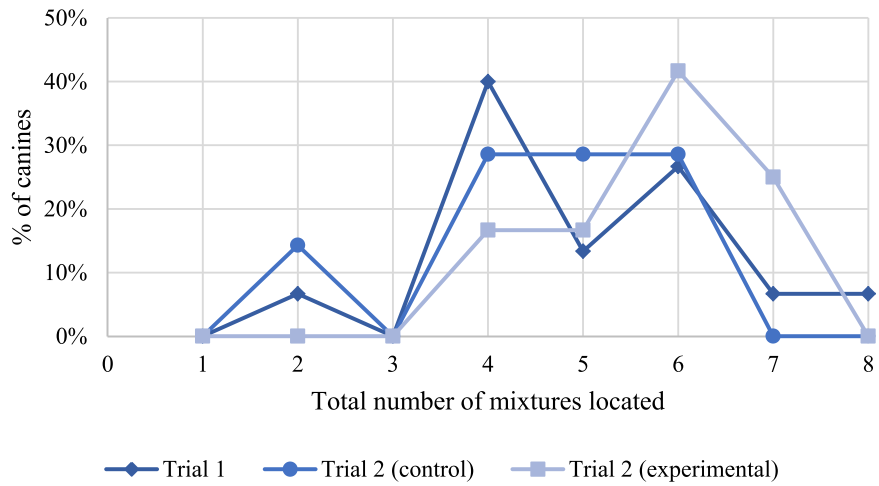

Fig. 5. Portion of dogs that responded to a given number of the eight pseudo cocaine mixtures in the first and second trial. Data from the second trial are divided between the control and experimental groups. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

vaporous methyl benzoate, depleting the abundance of odor available for detection [25].

Nineteen canines participated in the second trial, and no canine data were removed for lack of detection of the positive controls or excessive false alerts. Fig. 6 compares the performance of the canines in Trial 1 and Trial 2 for both the Control Group, trained only on MB COMPS, and the Experimental Group, trained on MB COMPS and mixtures in the MODD. In Trial 2, the total positive response rate to the positive controls was 86%, with the Control Group correctly responding 81% of the time, and the Experimental Group 89% of the time. Comparing the positive response rate to all mixtures, there was some improvement from the MODD-trained group, though this difference was not statistically significant. However, examining Fig. 5, there was a notable shift in the number of mixtures each canine detected in Trial 2 from the Experimental Group (light blue line), shifting from a peak at four mixtures in Trial 1 to six mixtures in Trial 2, and indicating some improvement in the detection of methyl benzoate in the mixtures. The data do show a notable improvement in the detection of pseudo cocaine when mixed with corn starch in Trial 2 for both groups, indicating an improvement simply due to training time, and not mixture training. If indeed the corn starch adsorbs odorant molecules, lowering the odor concentration available for detection, then increased training time effectively lowered the

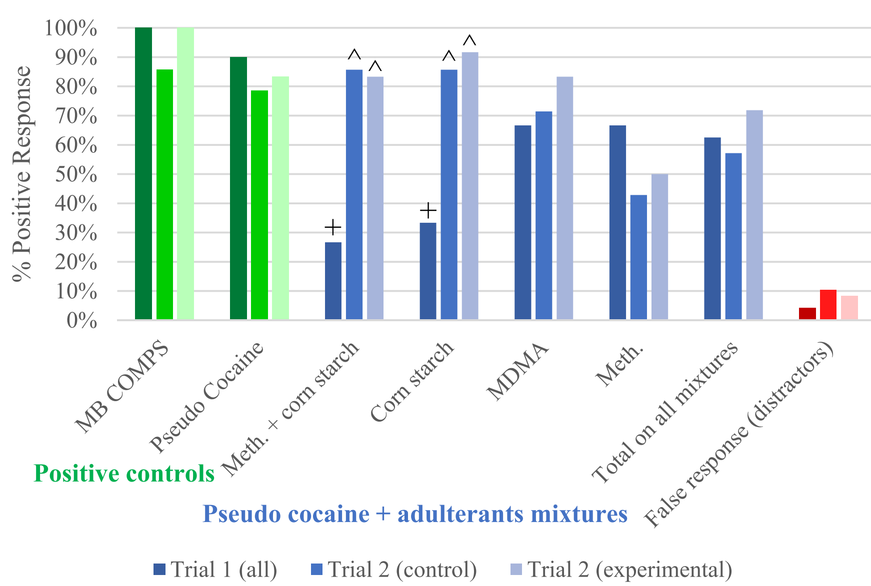

Fig. 6. Comparing results from Trial 1 and Trial 2, control and experimental groups. Figure gives the percentage of canines that responded to positive controls, pseudo cocaine mixtures, or distractors (false response). (^) indicates Trial 2 data that is significantly different than Trial 1 data. (+) indicates mixtures with response rates statistically different than the response to the positive controls. All data was statistically different than chance. Trial 1: N = 15; Trial 2 (control): N = 7; Trial 2 (experimental): N = 12.

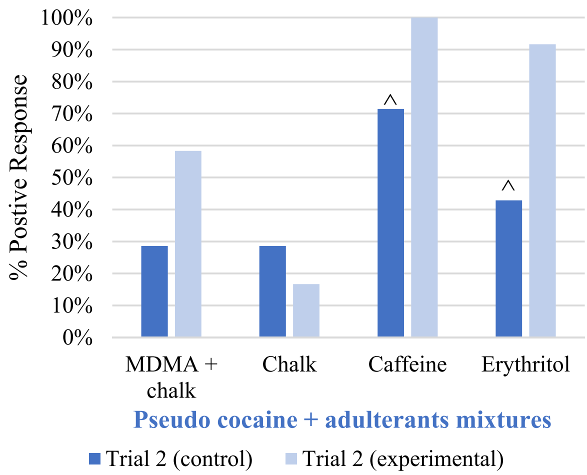

Fig. 7. Percentage of canines that responded to novel pseudo cocaine mixtures in Trial 2, comparing the control and experimental groups. (^) indicates that the Control Group data were statistical different than the Experimental Group. Trial 2 (control): N = 7; Trial 2 (experimental): N = 12.

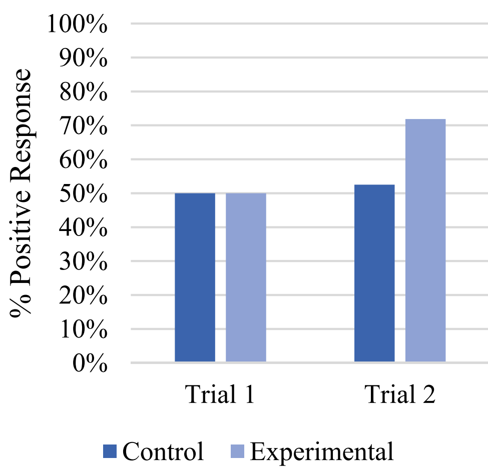

Fig. 8. Percentage of positive responses to all mixtures presented in Trials 1 and 2 from canines that locate five or less pseudo cocaine mixtures in Trial 1, comparing the Control Group (N = 5) and the Experimental Group (N = 4).

canines' detection threshold allowing for the improved detection proficiency.

Fig. 7 compares the canine response to solely the novel mixtures (those not seen in Trial 1) in the Control and Experimental Groups. There was improved detection of three out of the four mixtures for the Experimental Group, two of which were statistically different than the Control Group, indicating an improved ability to detect mixtures due to the mixture training. The canines were not provided caffeine or erythritol for mixture training, and thus, these mixtures were entirely novel to the canines.

As we saw in Fig. 4, some canines initially had a greater propensity for finding their target odorant in mixtures. This is akin to some humans having a greater natural ability to pick out individual ingredients or aromas from foods or fragrances. As these canines were more readily proficient, we turn our focus to improving selective attention in the less proficient subjects. Nine canines correctly responded to five or less mixtures in Trial 1. Results from solely these subjects these subjects are compared in (Fig. 8). Here, one can see that the initial positive response rate to all mixtures was 50% for both groups, but after continued training the correct response rate increased to 72% for the Experimental Group and only 53% in the Control Group in Trial 2. In agreement with Hall et al. [11], it can be concluded that, in this experiment, mixture training did improve detection of novel mixtures compared to training on the target odorant alone.

3.2. Experiment 2 Detection of homemade explosive mixtures before and after mixture training in an operational setting.

3.2.1. Headspace analysis

The results of the headspace analysis of mixed vs unmixed components of $KClO_3$-based explosives are given in two ways. First, chromatograms representing the headspace constituents of each fuel are given in Fig. 9. The headspace of fuels can be very complex, as such several major odorants in each fuel are noted on the graphs. The compounds labeled in red were used for the calculation results shown in Fig. 10. In Fig. 9A and C the compounds in red were chosen because they have previously been associated with the odor of that fuel. In Fig. 9B and D the red compounds were chosen to be used because they reproducibly occurred in replicate samples and were easily identified in the chromatogram. The main odorant associated with $KClO_3$ is chlorine. The ratio of chorine to the main odorant of each fuel was compared for the mixed and unmixed pairs (Fig. 10). The size of the bars (given as odorant ratios) were statistically similar (*t*-test, 95% confidence) for all $KClO_3$/fuel combinations, indicating that the physical mixing of $KClO_3$ HME components did not significantly alter the odor signatures of the individual components, thus supporting the use of the MODD for $KClO_3$-based HME training. Similar conclusions were previously drawn based on headspace analysis of ammonium nitrate-based mixtures in the MODD [17].

3.2.2. Canine testing

Eight canines participated in Trial 1 and the results are listed in Table 4. All of the eight canines correctly responded to the two positive controls (100% positive response), T-X and T-Y, and there was only one false response to Distractor A-B. This subject, OP001, was also the only canine that alerted to all mixtures. One other subject located both mixtures containing A-B. There were no other positive responses to the mixtures, and total positive response rate to all mixtures was 19%.

Seven dogs participated in Trial 2 (OP001 and OP002 were not able to participate), all of which correctly responded to both positive controls, and there were no false responses (Table 5). There was a notable improvement in the detection of the target mixtures, with all dogs alerting to all four mixtures (100% positive response to mixtures and positive controls). This data is similar to observation by Lazarowski et al. [10], where mixture training indeed made a dramatic difference in detection of HME mixtures.

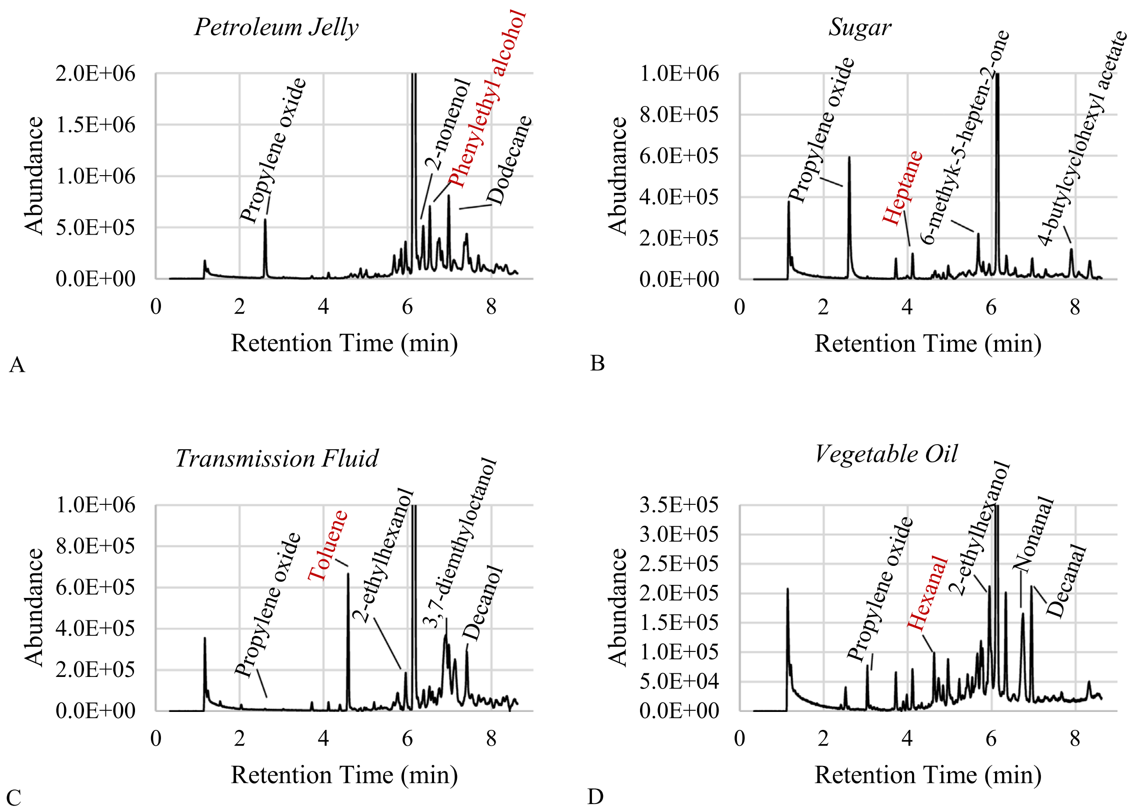

Fig. 9. Chromatograms representing the headspace constituents of for HME fuels (A) petroleum jelly, (B) sugar, (C) transmission fluid, and (D) vegetable oil. Some of the odorants in the chromatograms have been labeled. The compounds labeled in red were used in the calculations for Fig. 10. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

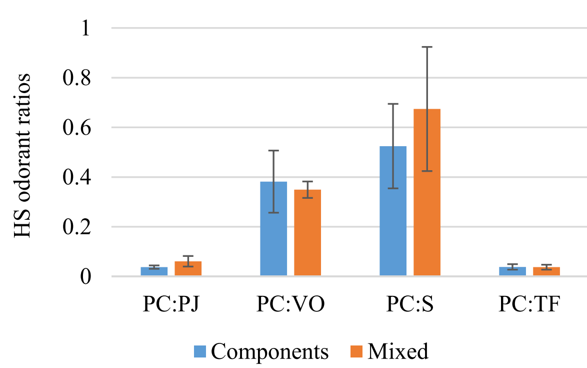

Fig. 10. Comparison of odorants detected in the headspace (HS) in potassium chlorate (PC) mixed with fuels (PJ = petroleum jelly, VO = vegetable oil, S = sugar, TF = transmission fluid) and as separate components. Note. Data presented as ratio of odorants measured in the headspace and error bars equal one standard deviation of the mean, N = 5.

Table 4
Trial 1 canine responses to positive controls (T-X and T-Y), mixtures (as described in Table 3), and distractors. 1 = positive response; 0 = no response; n.d. = no data.

Dog ID	T-X	T-Y	T-X/A-A	T-X/A-B	T-Y/A-B	T-Y/A-C	False response
OP001	1	1	1	1	1	1	1 (A-B)
OP002	1	1	0	1	1	0	0
OP003	1	1	0	0	0	0	0
OP004	1	1	0	0	0	0	0
OP005	1	1	0	0	0	0	0
OP006	1	1	0	0	0	0	0
OP007	1	1	0	0	0	0	0
OP008	1	1	0	0	0	0	0
OP009	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total (of 8)	**8**	**8**	**1**	**2**	**2**	**1**	**1/64**

Table 5
Trial 2 canine responses to positive controls (T-X and T-Y), mixtures (as described in Table 3), and distractors. 1 = positive response; 0 = no response; n.d. = no data.

Dog ID	T-X	T-Y	T-X/A-A	T-X/A-B	T-Y/A-B	T-Y/A-C	False response
OP001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OP002	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
OP003	1	1	1	1	1	1	0
OP004	1	1	1	1	1	1	0
OP005	1	1	1	1	1	1	0
OP006	1	1	1	1	1	1	0
OP007	1	1	1	1	1	1	0
OP008	1	1	1	1	1	1	0
OP009	1	1	1	1	1	1	0
Total (of 7)	**7**	**7**	**7**	**7**	**7**	**7**	**0/56**

4. Conclusion

The MODD was previously proven valuable for training canines to detect HME mixtures, with analytical data to support its use for ammonium nitrate-based explosives mixtures [17]. Previous research on the MODD showed that individual components of ammonium nitrate (AN)-based explosives in the MODD produced an odor profile equivalent to that from the same components physically mixed in the same vessel. The research also showed that canines, previously trained to located mixed AN-based explosives would successful locate those materials in the MODD [17]. In this research, headspace analysis demonstrated that the MODD can be utilized to train canines to detect potassium chlorate-based explosive mixtures as the odor profiles of the mixed explosives and unmixed components in the MODD are the same.

This work also described the first evaluation of narcotics mixtures with the MODD. Since “street” narcotics are rarely pure substances, it was hypothesized that the MODD could be beneficial to improve canine detection proficiency for these real-life mixtures. Canine olfactory trials supported this hypothesis, demonstrating that training with the MODD indeed improved detection of novel mixtures. Laboratory analysis of cocaine mixtures in the MODD corroborated the canine trial outcomes.

Canines trained for detection of either explosives or pseudo cocaine were tested on their ability to locate their detection targets in a variety of mixtures. In agreement with previous literature, the detection of the targets when in a mixture was statistically lower than detection of the targets alone. However, after mixture training using the MODD, there was a notable improvement in detection of target mixtures, particularly in canines that did not show an early propensity for locating the mixtures. The results of this study were intended to support canine trainers and handlers in fostering the best and most effective tools to do their job, and to safeguard canine teams on the front lines.

CRediT authorship contribution statement

Lauryn E. DeGreeff conceived of the research, carried out the laboratory work, and was the primary author of the manuscript. **Kimberly Peranich** and **Lauryn E. DeGreeff** both conceived, planned and organized the canine testing. **Kimberly Peranich** also assisted in manuscript editing.

Conflict of interest statement

Kimberly Peranich has no conflicts of interest to report. Dr. Lauryn DeGreeff, when part of the Naval Research Laboratory, was involved in patenting and licensing the Mixed Odor Delivery Device, but currently has no financial interest in the device or other conflicts of interest to report.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.111059.

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