Identification of dominant odor chemicals emanating from explosives for use in developing optimal training aid combinations and mimics for canine detection

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Abstract

Despite the recent surge in the publication of novel instrumental sensors for explosives detection, canines are still widely regarded as one of the most effective real-time field method of explosives detection. In the work presented, headspace analysis is performed by solid phase microextraction (SPME)/gas chromatography–mass spectrometry (GC–MS), and gas chromatography–electron capture detection (GC-ECD), and used to identify dominant explosive odor chemicals seen at room temperature. The activity of the odor chemicals detected was determined through field trials using certified law enforcement explosives detection canines. A chemical is considered an active explosive odor when a trained and certified explosives detection canine alerts to a sample containing that target chemical (with the required controls in place). A sample to which the canine does not alert may be considered an inactive odor, but it should be noted that an inactive odor might still have the potential to enhance an active odor's effect. The results presented indicate that TNT and cast explosives share a common odor signature, and the same may be said for plasticized explosives such as Composition 4 (C-4) and Detasheet. Conversely, smokeless powders may be demonstrated not to share common odors. The implications of these results on the optimal selection of canine training aids are discussed.
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1. Introduction

The use of canines as a method of detection of explosives is well established worldwide and those applying this technology range from police forces and military to humanitarian agencies in the developing world. Until recently, most data regarding optimal training protocols and the reliability of canine detection has been anecdotal, leading to successful challenges regarding the admissibility of evidence obtained with the assistance of canines and hampering the improvement of performance of canines as biological explosive detectors [1]. Challenges facing the field of canine detection include the limited ability to evaluate their performance with standardized calibration standards. Unlike instrumental methods, it is currently difficult to determine detection levels,

perform a calibration of the canines' ability or produce scientifically valid quality control checks. In addition, there are increasingly strict requirements being applied to the admissibility of the application of detector dogs in locating items of forensic interest, highlighting the need for better a scientific understanding of the process of canine detection. This current research is targeted towards the identification of active odors for canine detection of items of forensic interest and the development of what we are calling odor mimics, or training aids that contain the odor chemicals that mimic the real substances. There are presently several theories about what is responsible for the canines' high selectivity and specificity to explosives including (i) that canines alert to the parent explosives regardless of their volatility; (ii) that canines alert to more volatile, non-explosive chemicals that are present in explosives, and which are characteristic to explosives; or (iii) both parent explosives as well as characteristic volatiles are used to accurately locate explosives. To date, there are no

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definitive peer-reviewed studies to support any of these theories. The results presented here are part of an ongoing research program aimed to improve the scientific validity of canine detection, through better understanding of the chemistry of odors emanating from forensic specimens. By identifying the key odors of items of forensic interest, in this case explosives, levels of detection and linearity ranges may be determined, and better documentation of training and deployment will serve to benefit reliability studies. In addition, identification of active odor signature chemicals aids in the selection of the fewest number of target substances needed for optimal training and facilitates the development of reliable, cost-effective non hazardous odor mimics which can be used to enhance the capabilities of detector dogs. A previous Talanta article reviewed the use of dogs as chemical detectors, and the scientific foundation and reliability of explosive detector dogs, including a comparison with analytical instrumental techniques [2]. Recent reviews of electronic noses have highlighted the current limitations of instrumental methods with Yinon concluding that electronic noses for detecting explosives have a long way to go before being field operational [3] and Gopel concluding that, for most applications, the performance of electronic noses containing sensor arrays are insufficient compared to established analytical instruments such as GC/MS [4]. A recent extensive review of instrumentation for trace detection of high explosives concluded that there is still no instrument available that simultaneously solves the problems of speed, sensitivity and selectivity required for the real time detection of explosives [5]. Overall, detector dogs still represent the fastest, most versatile, reliable real-time explosive detection device available. Instrumental methods, while they continue to improve, generally suffer from a lack of efficient sampling systems, selectivity problems in the presence of interfering odor chemicals and limited mobility/tracking ability.

2. Explosive detection technologies

There are a variety of technologies currently available and others under development. Fig. 1 illustrates some trace explosive technologies including separation techniques ranging from high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) commonly with fluorescence or electrochemical detection and gas chromatography (GC) combined with mass spectrometry (GC/MS) electron capture (GC/ECD) or luminescence detection. In addition, techniques based on mass spectrometry and ion mobility spectrometry (IMS) continue to improve [4]. Currently, the most widely deployed explosives screening technology deployed at airports is ion mobility spectrometers which rely primarily of the on detection of particles contaminated on the outside of baggage or paper tickets. Recently, a new IMS inlet has been developed which allows for the detection of odor chemicals using solid phase microextraction (SPME) sampling [6,7]. Microsensors have the potential for selective

GC detectors and also as remote sensors when combined in arrays often referred to as "electronic noses". Promising microsensors include surface acoustic wave (SAW) detectors normally coated with different semi-selective polymeric layers and microelectromechanical systems (MEMS) including microcantilever sensors. Recently, a handheld sensor based on piezoresistive microcantilevers named "SniffEx" has been demonstrated to detect PETN and RDX at levels below 10 parts per trillion within a few seconds of exposure [8]. The hope is that, in the future, hundreds of such microcantilevers, coated with suitable coatings, may be able to achieve sufficient selectivity to provide a cost-effective platform for detecting explosives in the presence of potentially interfering compounds in real environments. Other electronic nose technologies under development include the use of fiber optics and sensor beads, polymeric thin films, nanocluster metal-insulator-metal ensembles (MIME), and fluorescent polymers using amplifying chromophore quenching methods [3]. To date, there has been limited testing of these devices with noisy chemical backgrounds under operational conditions, however the handheld "FIDO" system based upon quenching chromophore amplifying fluorescent polymers (AFP) was recently field tested against certified explosive detection canines for the detection of TNT based explosives, and was reported to share similar detection capabilities with canines [9].

Optical techniques under investigation include transmission and reflectance spectophotometry including infrared (IR) detection of decomposition products including the well established EGIS system, UV–vis absorption methods including cavity ring down spectroscopy (CRDS), Raman scattering including using localized surface plasmon resonance (LSPR) and optoacoustic (OA) spectroscopy [5]. Standoff technologies under development include laser, light detection and ranging (LIDAR), differential absorption LIDAR (DIAL) and differential reflection LIDAR (DIRL) for imaging. Nonlinear optical techniques offer the potential for increased signal-to-noise ratios in sensing modes including coherent anti-Stokes Raman scattering (CARS), optical phase configuration, and coherent control of the specific states of molecules and optimize their luminescence [10]. A recent report on standoff explosive detection techniques conducted by the National Academy of Sciences concluded that it is important to use multiple orthogonal detection methods (methods that measure the properties of explosives that are not closely related) as no single technique solves the explosive detection problem [10]. Studies conducted include free-running and remote explosive scent tracing (REST) in which the odor is collected on a sorbent in the field and presented to the animal at a different location [11]. Biological explosive detectors, including detector dogs can be considered orthogonal detectors to sensors under development as they generally rely on different detection modalities. In addition to canines, other animal and plant species have been proposed as alternative methods of biological explosives detectors. A research project in Tanzania, under the support
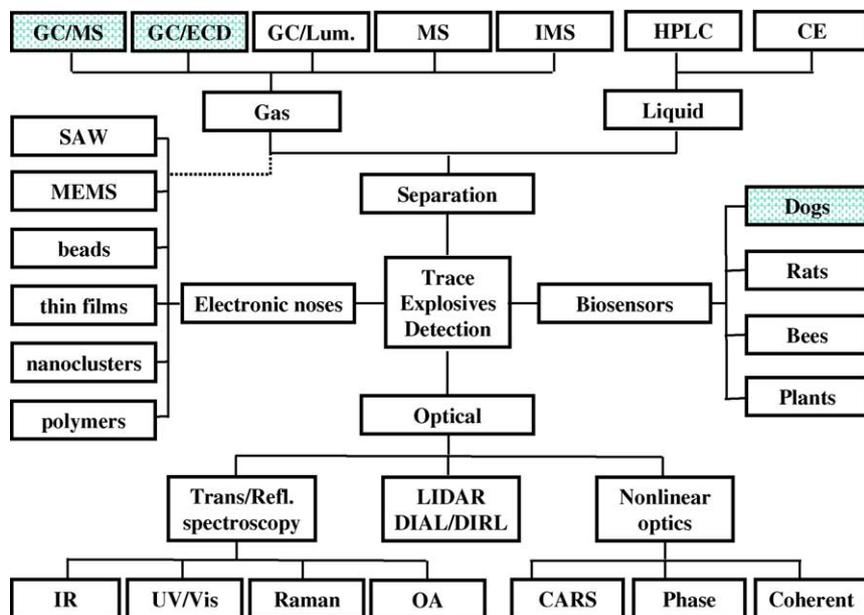
Fig. 1. Chart showing some of the technologies presently available and under development for the detection of trace explosives.

of the Belgian research organization APOPO, trains African Giant Pouched rats to detect landmines. Reports indicate that rats may be capable of detecting similarly low levels of explosive odors compared to dogs with advantages including their small size and low cost but with more challenging training and retrieval aspects [12]. Bees are also being studied as a biological explosive detection system. It has been demonstrated that bees are capable of detecting explosive odors at concentrations below those of most instruments and comparable to dogs [13]. The bees can be imaged or traced to the source or, more commonly, used to survey areas by examining chemical residues brought back to the hive. Advantages include that they can be trained quickly and will not set off any mines. Limitations include that bees do not fly at night, in heavy rain or in cold weather (below 40 °F). Danish scientists at Aresa Biodetection have developed a genetically modified (GM) cress crop which, when sown over a suspected mine field, will change its leaves from green to red upon detection of buried explosives [14]. The GM thale cress crop is altered to change color should its roots contact NO_2 in the soil. Since this method relies upon seepage of NO_2 from leaking mines, it has the potential to miss the more recent models that are specially sealed to obscure detection.

3. Explosives detection canines

Whilst the sensitivity of some of the emerging instrumental technologies is on par with, if not beyond that of the canine, the dogs still hold an advantage over the instruments on selectivity. Canines are renowned for their ability to individualize the scent picture, ignoring the 'background matrix' to focus upon a single odor. It is this selectivity, combined with mobility and independent thinking that still ranks the canines as the current best method for real-time detection of explosives. The research described in this paper is part of an overall project aimed to test and improve the reliability and detection limits of these biological explosive detectors. The authors have previously published preliminary results on the identification of active odor signature chemicals of forensic specimens including explosives employing SPME with GC/MS and GC/ECD [15–18] and in this paper, a more detailed comparison of these methods and additional field experiments of alerts to target chemicals by deployed law enforcement explosive detector dog teams is reported. Unlike narcotic detection canines, which are expected to face a predictable line-up of 5 or 6 drug odors, the explosive detection canine is expected to face dozens of different explosive products during its service. Narcotics detection canines are typically trained on cocaine (HCl and base), heroin and marijuana. In addition, they may be trained on additional drugs depending on the training agency and the locations where they are deployed including methamphetamine, MDMA, hashish, opium, mescaline, L.S.D. [19]. While there are six principle chemical categories of explosives including aliphatic nitrates ($C–NO_2$), aromatic nitrates ($Ar–C–NO_2$), nitramines ($C–N–NO_2$), nitrate esters ($C–O–NO_2$) peroxides ($C–O–O–C$) and acid salts (NH_4^+, NO_3^-), there are dozens of individual explosive chemicals which must be detected. Some common explosives and their properties are shown in Table 1. Inorganic explosives include ammonium nitrate, ammonium perchlorate and potassium nitrate (in black powder) and are mixed with organic com-

Table 1
Some common explosives and properties (data from [9] and [17])

Explosive class	Explosive		Molecular weight (amu)	Formula	Vapour pressure at 25 °C (Torr)
Acid salt		Ammonium nitrate	80.04	NH_4NO_3	5.0×10^{-6}
Aliphatic nitro		Nitromethane	61.04	CH_3NO_2	2.8×10^1
	DMNB	2,3-Dimethyl-dinitrobutane	176.17	$C_6H_{12}N_2O_4$	2.1×10^{-3}
Aromatic nitro	o-MNT	2-Nitrotoluene	137.14	$C_7H_7NO_2$	1.5×10^{-1}
	p-MNT	4-Nitrotoluene	137.14	$C_7H_7NO_2$	4.1×10^{-2}
	DNT	2,4-Dinitrotoluene	182.14	$C_7H_6N_2O_4$	2.1×10^{-4a}
	TNT	2,4,6-Trinitrotoluene	227.13	$C_7H_5N_3O_6$	3.0×10^{-6}
	Picric acid	2,4,6-Trinitrophenol	229.11	$C_6H_3N_3O_7$	5.8×10^{-9}
Nitrate ester	EGDN	Ethylene glycol dinitrate	152.06	$C_2H_4N_2O_4$	2.8×10^{-2}
	NG	Trinitroglycerin	227.09	$C_4H_5N_3O_9$	2.4×10^{-5}
	PETN	Pentaerythritol tetranitrate	314.14	$C_5H_8N_4O_{12}$	3.8×10^{-10}
	NC	Nitrocellulose	327.21	$[C_8H_{13}N_3O_{11}]_n$	N/A
Nitramin	Tetryl	Tetranitro-N-methylamine	287.15	$C_7H_5N_5O_8$	5.7×10^{-9}
	RDX	Trinitro-triazacyclohexane	222.12	$C_3H_6N_6O_6$	1.4×10^{-9}
	HMX	Tetranitro-tetrazacyclooctane	296.16	$C_4H_8N_8O_8$	1.6×10^{-13a}
	CL20	Hexanitro-hexaazaisowurzitane	438.19	$C_6H_6N_{12}O_{12}$	N/A
Peroxide	TATP	Triacetone triperoxide	138.08	$C_3H_6O_6$	3.7×10^{-1a}
	HMTD	Hexamethylene triperoxide diamine	208.17	$C_6H_{12}N_2O_6$	N/A

N/A not available.

[a] Extrapolated values.

pounds in common explosive mixtures. The extremely low vapor pressures for many of the common explosives complicates the detection of these compounds directly. For this reason, the compound 2,3-dimethyldinitrobutane (DMNB) is one of four chemicals now added as a marker to plastic and sheet explosives. DMNB was chosen due to its high vapor pressure, high permeability through textiles and uniqueness, with no known industrial applications [20] as seen in Table 2, the vapor pressure for DMNB is more than 1 million times greater than any of the nitramine explosives. These explosive mixtures also generally contain minor components with relatively high vapor pressures which themselves may be used to locate the explosives and can contribute to odor contamination from one training aid to another unless proper care is taken when storing samples in close proximity [21]. Depending on the training agency and deployment locations, explosive detection canines are currently trained on a wide variety of samples ranging from half a dozen samples to upwards of twenty. Using at least one representative sample from each explosive chemical class would require an acid salt such as ammonium nitrate, an aromatic nitro such as TNT, a nitrate ester such as PETN a nitramine such as RDX, an aliphatic nitro such as DMNB, a peroxide such as TATP and representative black and smokeless powders. Unfortunately, there is currently little scientific information available to aid in the optimal selection of training aids. Due to the challenges in handling and storing of a wide variety of explosives, non hazardous training aids are commercially available but with limited types available and limited testing of their effectiveness under field conditions in double-blind studies.

The difficulty in choosing an optimal number of training aids lies in the multiple explosives within each category, and is then confounded by the wealth of explosive products that employ various combinations of the explosives, as highlighted in Table 2. One of the most important decisions a canine trainer has to make is in choosing which explosives to use as odor targets. Many trainers and canine programs choose to focus on one main explosive from each principle category. However, there is scant peer reviewed data to demonstrate the utility of this practice. The difficulty in selecting the optimal number and combinations of training aids can be highlighted by looking at the choice of a smokeless powder training aid. While bombs made from black and smokeless powder are generally relatively small, these devices are the ones most commonly used in criminal bombings in the U.S. and are readily available with millions of individuals purchasing these powders for sport use each year and hundreds of different formulations available from different manufacturers [22]. Finding one smokeless powder that adequately represents the hundreds of possibilities seems unlikely, and the results presented here support this. A previous study reported results consistent with the theory of stimulus generalization, indicating that odor generalization is a function of the similarity of the vapor chemistry between trained and untrained target substances and the extent of training across multiple variants of the substances. Conclusions from this study include the importance of identifying the variants of explosives that will yield optimal effectiveness [23]. The results presented here are amongst the first scientific studies aimed at identifying the optimal number of explosive variants.

Table 2
Common military and commercial explosive contents (from [26,27])

Explosive	Components
Amatol	Ammonium nitrate + TNT
Ammonal	Ammonium nitrate + TNT + Al
ANFO (Amex or Amite)	Ammonium nitrate + fuel oil (diesel)
Black powder	Potassium nitrate + C + S
Composition A-3	RDX + wax
Composition B	RDX + TNT
Composition C-2	RDX + TNT + DNT + NC + MNT
Composition C-3	RDX + TNT + DNT + tetryl + NC
Composition C-4	RDX + plasticisers
Composition D	Ammonium picrate
Cyclotol	RDX + TNT
Detasheet (Flex-X)	RDX + plasticisers
DBX	TNT + RDX + ammonium nitrate + Al
Demex 200	RDX
Detonation cord (commercial)	PETN
Detonation cord (military)	RDX or HMX
Dynamite (ammonia)	NG + NC + sodium nitrate
Dynamite (gelatine)	NG + NC + ammonium nitrate
Dynamite (military)	TNT
HBX-1	RDX + TNT + Al
Helhoffnite	NB + nitric acid
HTA	HMX + TNT + Al
Nitropel	TNT
Non-el cord	HMX
PE-4	RDX + plasticiser
Pentolite	PETN + TNT
Picratol	TNT + ammonium picrate
Primasheet 1000	PETN + plasticisers
Primasheet 2000	RDX + plasticisers
PTX-1	RDX + TNT + tetryl
PTX-2	RDX + TNT + PETN
Red diamond	NG + EGDN + sodium nitrate + ammonium nitrate
SEMTEX A	PETN + plasticisers
SEMTEX H	RDX + PETN + plasticisers
Smokeless powder (single based)	NC
Smokeless powder (double based)	NC + NG
Smokeless powder (triple based)	NC + NG + nitroguanidine
Tetrytol	TNT + tetryl
Time fuse	Potassium nitrate + C + S
Torpex	TNT + RDX + Al
Tritonal	TNT + Al
Water gel/slurry (aquaspex)	NG
Water gel/slurry (hydromex)	Ammonium nitrate + TNT
Water gel/slurry (powermex)	Ammonium nitrate + sodium nitrate + EGMN
Water gel/slurry (tovex)	Ammonium nitrate + sodium nitrate + MMAN

4. Methodology

The odor of a wide selection of explosive samples was determined through headspace analysis. The use of solid phase microextraction permits rapid, solvent free extraction of the headspace. SPME was combined with gas chromatography–mass spectrometry and gas chromatography-electron capture detector to produce a comprehensive screening method that was optimized for general volatiles and specific explosives respectively.

The general volatiles SPME-GC–MS method used a 70 um StableFlex^{TM} Carbowax$^{®}$/Divinylbenzene (CW/DVB) SPME fiber from Supelco (Bellefonte, PA) to sample the headspace at ambient temperature with injections into a Supelco$^{®}$ SPME liner at 220 °C. The SPME exposure time was optimized to 30 min for the high explosives and 15 h for the low explosive powders. The GC–MS used was the Agilent 6890-5973 combination running Chemstation software. The column used was an HP5 30 m, 0.25 mm i.d., 25 um film thickness column also obtained from Agilent. The injection port was held at 220 °C, with a 5 min SPME desorption. The oven program was a 40 °C hold for 5 min followed by a 10 °C/min ramp to 280 °C, with a 1 min hold at 280 °C. The carrier gas was helium at 1.0 mL/min. The MS was operated in electron ionization (EI) full scan mode from 50 to 500 amu, with a 1 min solvent delay.

The explosive specific SPME-GC-ECD method used a 100 um polydimethylsiloxane (PDMS) SPME fiber from Supelco with an optimized exposure time of 5 min with injections into a 1 mm Restek$^{®}$ deactivated liner at 250 °C. The GC used was an Agilent 5890 with ECD. The column employed was a 6.0 m 0.53 mm i.d., 1.5 um film thickness Restek (Bellefonte, PA) Rtx$^{®}$-TNT column, a specialist column for explosives analysis. The injection port was held at 250 °C with a 1 s desorption. The oven program began with a 1 min hold at 80 °C, followed by 10 °C/min ramp to 180 °C. This was followed by a 30 °C/min ramp to 300 °C and finished with a 3 min hold at 300°. The carrier was Helium at 15 mL/min with a Nitrogen 60 mL/min makeup in the ECD. The detector was held at 330 °C with anode purge.

Explosive standards were obtained from Cerilliant (Round Rock, TX) including EPA method 8330 components (nitrobenzene, 1,3-dinitrobenzene, 1,3,5-trinitrobenzene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, RDX, HMX and tetryl) and EDGN, trinitroglycerin and PETN. Explosive training aid samples were sampled from local and state law enforcement agencies, including Miami Dade Police Department Bomb Squad, Florida International University Police Department K9 Unit, Palm Beach County Sheriff's Office Bomb Squad and K9 Division, and Florida Highway Patrol K9 Division. NESTT Training Aids were purchased from Ray Allen Manufacturing Company (Colorado Springs, CO). A range of samples of smokeless powders was obtained from various manufacturers including Vihtavouri Lapua, IMR, Accurate Arms and Hodgdon Powder companies. Trinitroglycerin was in the form of a package of trinitroglycerin tablets (25 × 0.4 mg dose). Samples ranging from 0.25 to 2.50 g of explosive were placed inside 10 mL glass vials from Supelco and capped with Silica/PTFE septa. The headspace of the sample was

then extracted using the SPME fiber. The fiber was inserted through the septum and exposed approximately 1.0 cm above the sample within the closed vial for the allotted time, prior to immediate GC analysis.

5. Results and discussion

5.1. High explosive chromatographic analysis

The analysis of the high explosives was separated into cast explosives and polymer bonded (plastic) explosives. The cast explosives are commonly based around TNT and other aromatic nitrates whereas the plastic explosives generally involve the nitramine or nitrate ester explosives such as RDX and PETN. Three samples of cast explosive used as canine training aids were sampled from local law enforcement agencies. The odor headspace, shown in Fig. 2, analyzed by SPME-GC–MS, was shown to contain the parent explosive 2,4,6-trinitrotoluene in addition to 2,4-dinitrotoluene as major component in two of the samples. 2,4-Dinitrotoluene was not detected in one sample. The cast primer was analyzed for content and shown to be Tetratol, a combination of TNT and tetryl, but no tetryl was observed in the headspace.

Six samples of polymer bonded explosive sampled from local and state agencies were also prepared for analysis; Flex-X Detasheet, Composition 4 (C-4), PETN booster and TNT booster charges, no parent explosives, such as the RDX in the C-4 or PETN in the booster, were observed in the headspaces; however, 2-ethyl-1-hexanol (a common additive to plastic explosives) was detected in five of the six samples often as the most abundant headspace chemical as seen in for representative samples shown in Fig. 3. The detection marker 2,3-dinitrodimethylbutane was also observed in four of the six samples. Although labeled as TNT based booster, no TNT or other aromatic nitrates were detected in the headspace of the sample. Recent analysis of further samples of C-4, C-4 caulking, and Flex-X has further confirmed the commonality of 2-ethyl-1-hexanol and DMNB in the odor headspace. The polymer bonded explosives were also analyzed using the SPME-GC-ECD method to ensure that the parent explosives, if present in the headspace, were not being lost due to the GC–MS conditions. SPME exposure was shortened to 5 s to prevent excessive overloading of the more sensitive detector. Similar headspace signatures were seen employing SPME-GC-EDC. No parent explosives (PETN and RDX, respectively) were observed in the headspace of the PETN booster or C-4 samples.
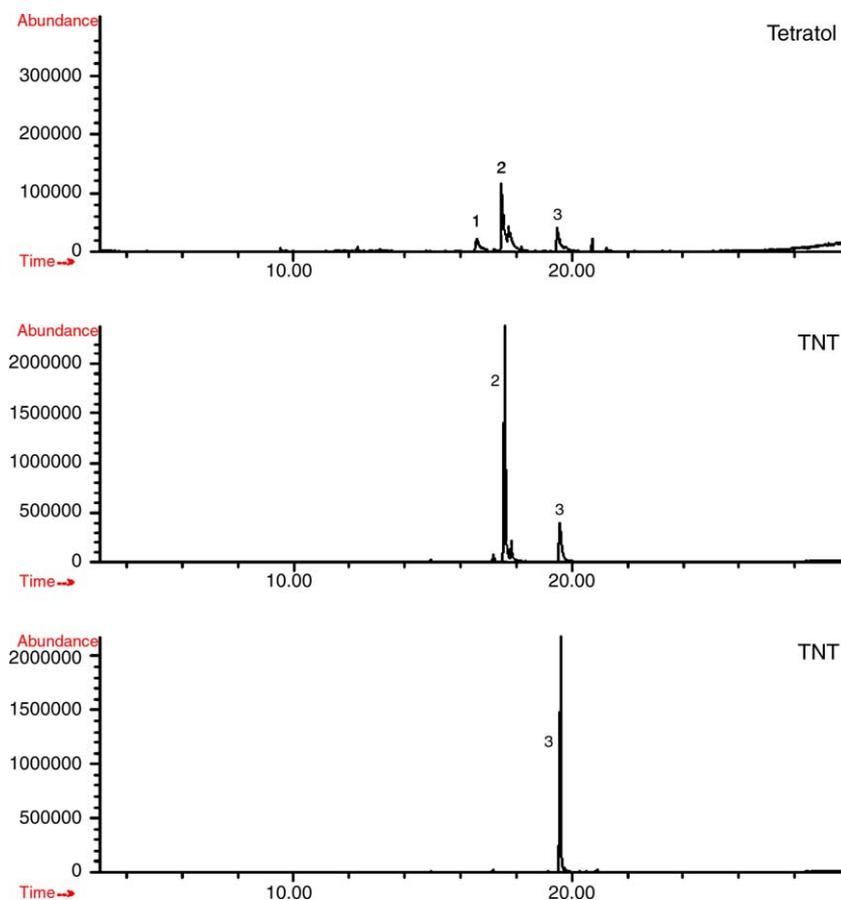

Fig. 2. Headspace SPME-GC–MS of selected cast explosives (1. 1,3-dinitrobenzene; 2. 2,4-dinitrotoluene; 3. 2,4,6-trinitrotoluene).
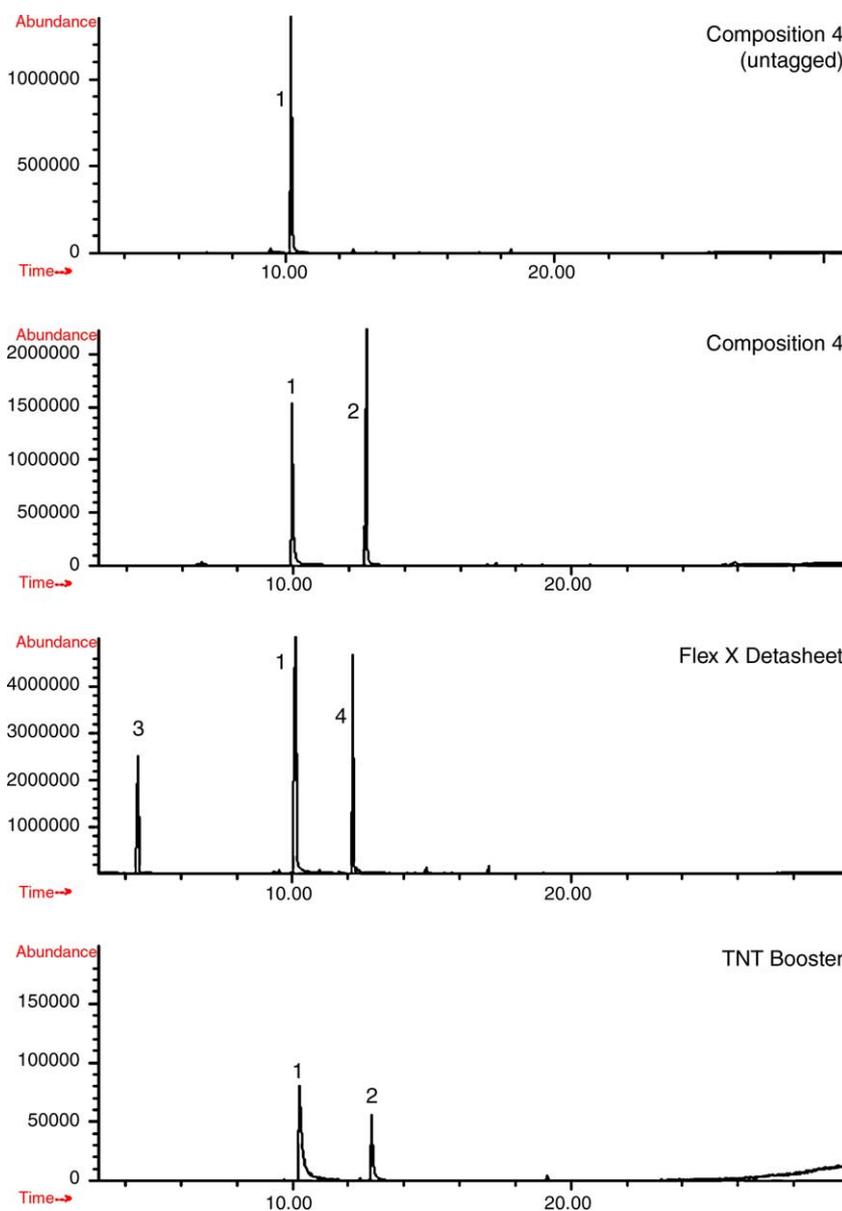
Fig. 3. Headspace SMPE-GC–MS of Composition 4 (C-4) explosives and other plasticised explosives (1. 2-ethyl-1-hexanol; 2. 2,3-dimethyl-dinitrobutane; 3. 1-butanol acetic acid ester; 4. 2-ethyl-1-hexanol acetic acid.

5.2. Low explosive chromatographic analysis

The single-based smokeless powders adopt nitrocellulose as the explosive propellant, whereas the double-based powders contain trinitroglycerin in addition to the nitrocellulose. Additives added to the composition are selected to improve stability, burn properties and shelf life to optimize safety and product performance (including phthalates, diphenylamine, ethyl centralite and methyl centralite, and many other volatile organic compounds). Different manufacturers may choose different additives, leading to the potential individuality of odor headspaces. Analysis of the powders from the various manufacturers highlighted significant differences in observed odor chemicals. Review of

material safety data sheets (MSDS), available from each manufacturer, indicated a range of additives and stabilizers in addition to the nitrocellulose/trinitroglycerin expected, including diphenylamine and nitrated derivatives, ethyl centralite and 2,4-dinitrotoluene. Results reveal the common occurrence of diphenylamine in most single-based powders, in addition to the presence of either 2,4-dinitrotoluene or ethyl centralite in high abundance in the headspace. The range of chemicals detected was in good agreement with a qualitative analysis recently published [24]. Trinitroglycerin and dinitroglycerin were observed in the headspace of double-based powders from all companies, as observed in Figs. 4 and 5 analyzed by SPME-GC–MS and SPME-GC-ECD respectively. Additionally, dinitrotoluenes were
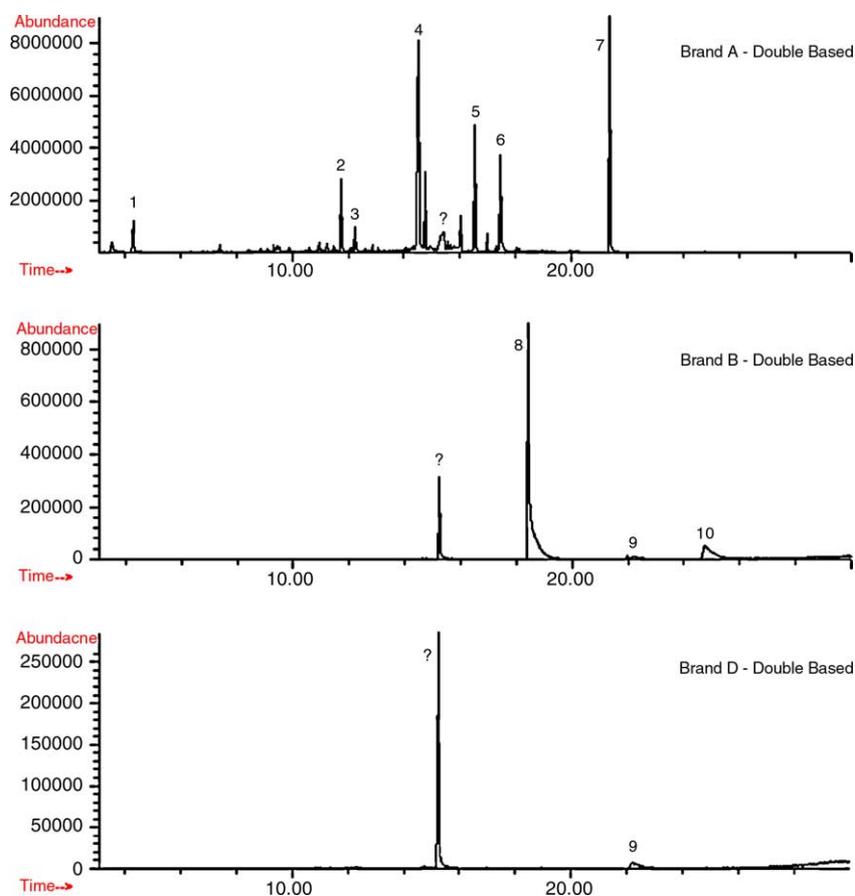
Fig. 4. Headspace SPME-GC–MS of selected double based smokeless powders (1. 1-butanol acetic acid ester; 2. 2-nitrophenol; 3. 2-nitrotoluene; 4. *N,N*-dimethylaniline; 5. 2,6-dinitrotoluene; 6. 2,4-dinitrotoluene; 7. ethyl centralite; 8. diphenylamine; 9. 2-nitrodiphenylamine; 10. 4-nitrodiphenylamine; ?. unidentified).

observed in the one brand of double-based powder, and nitrodiphenylamines were observed in a separate brand of double-based samples. The combination of SPME-GC–MS and SPME-GC-ECD provided complementary information with ethyl centralite and diphenylamine seen as major components using MS detection whereas ECD highlighted the presence of glycerins and nitrotoluenes as seen on Figs. 4 and 5.

5.3. Non-explosive training aid chromatographic analysis

Non explosive training aids are marketed for use in areas where the use of live explosives is not practical and to potentially provide more consistency in the odor chemicals used in training. The leading line of non-explosive training aids is the non-hazardous explosives for security training and testing (NESTT) range from Van Aken International (Rancho Cucamonga, CA). Purified explosives are diluted to 4–8% by silica granules or petrolatum jelly. The complete range of NESTT aids (including the blank distracters) were obtained and analyzed by SPME-GC–MS and SPME-GC-ECD. The headspace analysis of the TNT and RDX training aids are shown in Fig. 6 for the petrolatum based aids and Fig. 7 for

the silica based aids. The petrolatum jelly aids showed a large unresolved complex hydrocarbon mixture, with only 2,4,6-trinitrotoluene observed in the TNT aid by mass spectrometry, in addition to dinitrotoluenes, and 1,3-dinitrobenzene were observed in the ECD spectra and no target odor compounds or RDX observed above the petrolatum based RDX training aid. The silica based aid revealed similar results, with only the TNT aid providing related odor chemicals in the headspaces. The RDX aid did produce a small peak by ECD analysis with indications of dusting of the silica matrix.

5.4. High explosives field testing with canines

Having confirmed the multiple presences of certain suspected odor chemicals, field trials were arranged with local law enforcement agencies that were operating trained and certified explosives detection canines. The odor chemicals were prepared as acetonitrile solutions. The acetonitrile was obtained from Fisher Scientific (address) and the odor chemicals ordered from Sigma Aldrich (address). Solutions at 1000 and 100,000 ppm (mg/L) were prepared. Aliquots (100 uL) of the solutions could then be used to apply 0.1 and 10 mg, respectively, of the odor chemical to test substrates. The
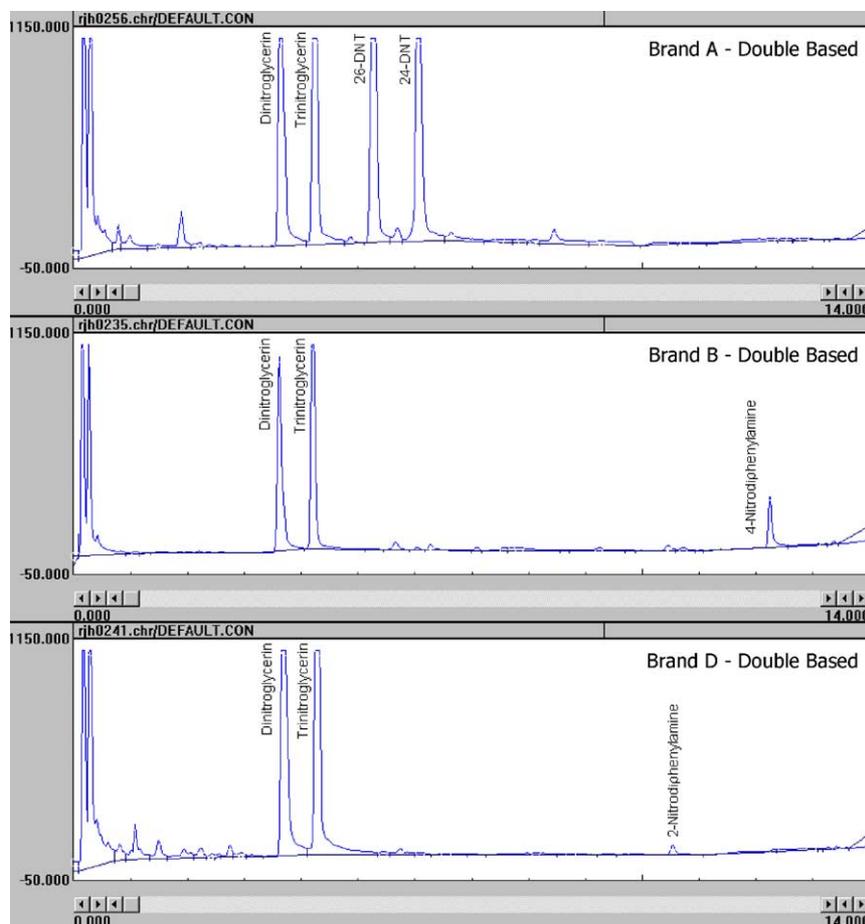
Fig. 5. Headspace SPME-GC-ECD of selected double based smokeless powders.

100 uL of acetonitrile was shown to evaporate within 90 min leaving a residue of the odor compound. Typical odor delivery substrates were 125 mm filter paper, or 5.1 cm × 5.1 cm gauze sponge heat sealed within a 2 mL low-density polyethylene (LDPE) bag. Negative controls were applied, including the acetonitrile solvent and uncontaminated filter papers and gauze.

The odor aids were presented to the canines in metal electrical boxes that had been previously cleaned with soap, then rinsed with water and baked at 200 °C overnight. The odor chemicals detected were then presented to the canines in an "odor line-up" of hide boxes. The electrical boxes were placed 1.5 m apart along a floor surface and presented to the canines. The detection canines would then walk the boxes, with the handlers ensuring that the canines detailed each box with a sniff directly above it. The handlers were not informed of the content of the electrical boxes, and there was no specific marking to indicate the contents. One box contained the suspected odor chemical, other boxes contained distracters or negative controls including the acetonitrile solvent. A positive control was presented separately, also in a hide box. Twenty-five certified police detection canines participated in this study, although actual numbers at each field test ranged from 8

to 14 on any given day. Each canine was assigned a three digit identification to assure anonymity in reporting the results.

A representative field trial of odor chemicals from cast explosives is summarized in Table 3. 2,4-Dinitrobenze yielded alerts from half the dogs tested and commonly seen in the headspace of the cast explosives sampled. 1,3-Dinitrobenzene also elicited positive responses from half the dogs tested, but is less commonly seen in the samples tested by headspace SPME. The less volatile parent explosive, 2,4,6-trinitrotoluene, also elicited responses from the canines but for less than 50% of dogs tested. Field experiments with the most abundant odor chemicals found above the headspace of plasticized explosives are summarized in Table 4 with limited response observed for cyclohexanone, but strong response with 2-ethyl-1-hexanol, reaching a maximum of 70% when spiked amount are increased 10 μL on cotton gauze sealed within LDPE plastic. The 0.5 μL aid appeared to be below the level of detection for most canines; whereas the 25 μL aid may have resulted in saturation of the odor, making it difficult for the canines to trace the aid to source. A study into the controlled permeation of 2-ethyl-1-hexanol from prototype training aids with different polymer chemistry and thicknesses is under-
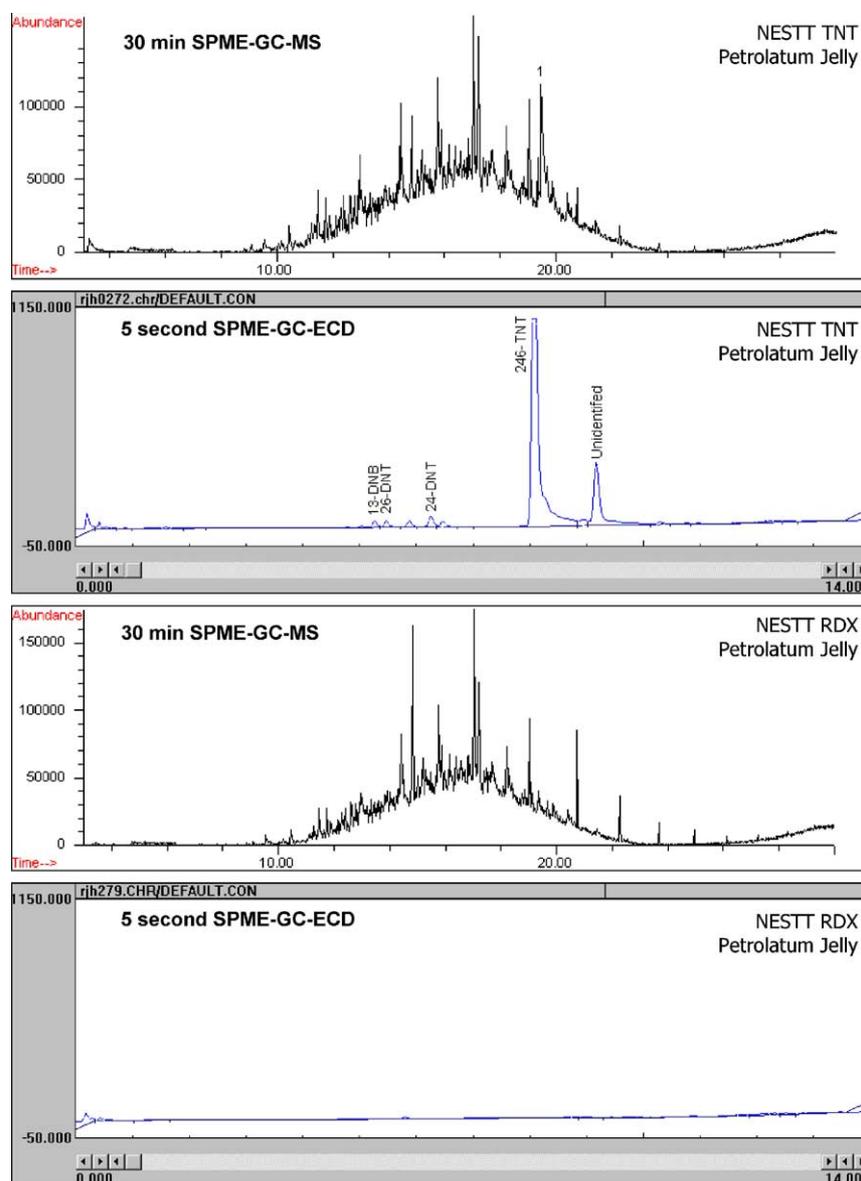
Fig. 6. Headspace SPME-GC–MS and headspace SMPE-GC-ECD of NESTT petrolatum jelly aids (1. 2,4,6-trinitrotoluene).

way to improve the quantitation of odor chemical delivered in the field. A previous study, using canines trained under behavioral laboratory conditions with dilution olfactometry, indicated that cyclohexanone and 2-ethyl-1-hexanol may be

odor signature chemicals for C-4 explosive [25]. In a separate field test with different odor chemicals, no alerts were observed for diphenylamine or DMNB but all nine dogs tested showed interest or alerted when exposed to 5 μL of 2-ethyl-

Table 3
Results from field testing of cast explosive odour chemicals

| Hide contents	No alert	Interest	Alert	% alert
Empty scratch box	106, 112, 113, 114, 115, 116	–	–	–
Scratch box containing cotton in open vial	106, 112, 113, 114, 115, 116	–	–	–
Scratch box containing 100 ul acetonitrile on cotton in open vial	106, 112, 113, 114, 115, 116	–	–	–
Scratch box containing 1,3-dinitrobenzene (100 uL at 1000 ppm acetonitrile) on cotton in open vial	–	106, 113, 114	112, 115, 116	50.0
Scratch box containing 2,4-dinitrotoluene (100 uL at 1000 ppm acetonitrile) on cotton in open vial	–	106, 112, 114	113, 115, 116	50.0%
Scratch box containing 2,4,6-trinitrotoluene (100 uL at 1000 ppm acetonitrile) on cotton in open vial	113, 114	106, 112	115, 116	33.3
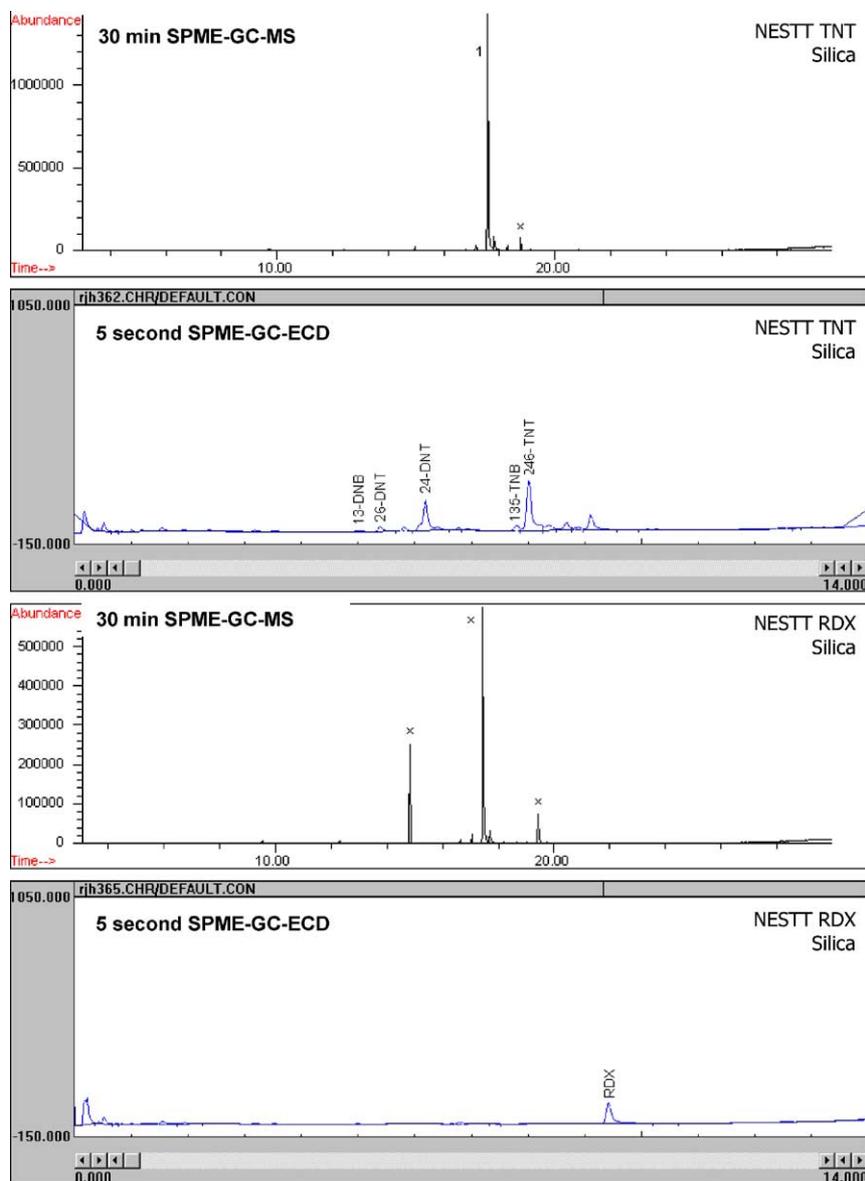
Fig. 7. Headspace SPME-GC–MS of NESTT silica aids (1. 2,4-dinitrotoluene; x = siloxanes).

1-hexanol on cotton gauze in an open vial as summarized in Table 5.

5.5. Low explosives field testing with canines

No individual low explosive headspace compounds tested in this study elicited responses in a majority of the canines tested. The canines did not alert to the diphenylamine observed in the odor headspace of the smokeless powders, although one dog did show interest without alerting as shown in Table 5. In another field experiment, summarized in Table 6, the canines tested showed no interest in nitrodiphenylamine, with 2 of 12 dogs alerting to ethyl centralite and 3 of 12 dogs alerting to trinitroglycerin tablets. Testing is currently underway using other headspace compounds, different concentrations and combination of chemicals. Both the ethyl centralite and trinitroglycerin also observed some interest, promoting further work on these odors. The wide variability of observed odor chemical signatures for smokeless powders and the variability in the smokeless powders used in the training of the canines tested may preclude the identification of active odor chemical(s) for low explosives. Additional work is underway to classify smokeless powders into various groups based on similarities in headspace odor chemicals.

5.6. Non-explosive training aid field testing with canines

The complete range of NESTT aids (including the blank distracters) were obtained and placed in the same metal hide boxes used in the field trials above. Again, negative controls (the blank distracter) and positive controls (real explosive) were made available separately. The dogs had difficulty in

Table 4
Results of field testing of plasticized explosive odor chemicals

Hide contents	No alert	Interest	Alert	% alert
Empty electrical box	108, 109, 110, 112, 113, 115, 116, 119, 127, 132	–	–	–
Electrical box containing gauze in 2 mL LDPE	108, 109, 110, 112, 113, 115, 116, 119, 127, 132	–	–	–
Electrical box containing cyclohexanone (0.5 uL) on gauze in 2 mL LDPE	103, 108, 109, 111, 113, 115, 119, 126, 127, 128	–	–	–
Electrical box containing 2-ethyl-1-hexanol (0.5 uL) on gauze in 2 mL LDPE	108, 109, 111, 113, 115, 119, 126, 127, 128	–	103	10
Electrical box containing cyclohexanone (10 uL) on gauze in 2 mL LDPE	108, 109, 110, 112, 113, 116, 119, 127, 132	115	–	–
Electrical box containing 2-ethyl-1-hexanol (10 uL) on gauze in 2 mL LDPE	–	108, 109, 127	110, 112, 113, 115, 116, 119, 132	70.0
Electrical box containing cyclohexanone (25 uL) on gauze in 2 mL LDPE	107, 109, 113, 118, 124, 125, 127	116, 119, 126, 129	106	8.3
Electrical box containing 2-ethyl-1-hexanol (25 uL) on gauze in 2 mL LDPE	107, 124, 125, 126, 129	109, 113, 116, 118, 127	106, 119	16.7

Table 5
Results from field testing of several explosive odour chemicals

Hide content	No alert	Interest	Alert	% alert
Empty quart can	101, 103, 106, 110, 113, 116, 117, 118, 119	–	–	–
Quart can containing cotton in open vial	101, 103, 106, 110, 113, 116, 117, 118, 119	–	–	–
Quart can containing 50 ul acetonitrile on cotton in open vial	101, 103, 106, 110, 113, 116, 117, 118, 119	–	–	–
Quart can containing diphenylamine (50 uL at 10%, w/v, acetonitrile) on cotton in open vial	101, 103, 106, 110, 113, 116, 117, 118	119	–	–
Quart can containing 2-ethyl-1-hexanol (50 uL at 10%, w/v, acetonitrile) on cotton in open vial	–	117	110, 101, 103, 116, 106, 113, 119, 118	88.9
Quart can containing 2,3-dimethyldinitrobutane (50 uL at 10%, w/v, acetonitrile) on cotton in open vial	101, 103, 106, 110, 113, 116, 117, 118, 119	–	–	–
Quart can containing TNT aid	–	–	110, 101, 103, 116, 106, 113, 119, 117, 118	100.0

locating the NESTT aids in several separate tests. The only positive result observed was 1 dog in 10 alerting to the NESTT TNT (silica base), although there was 1 interest in the NESTT RDX (petrolatum base), shown in Table 7. Previously [16], we observed more positive results with NESTT aids with the majority of the dogs tested alerting using TNT and RDX NESTT (silica base) aids although different dogs and field conditions were employed. These results demonstrate a lack of consistency in the results when using NESTT aids and that the mode of delivery may play an important role in the available odor of these aids. The results are not surprising, however, when comparing the odor signatures seen for the NESTT aids and live explosive samples. For example, the C4 RDX samples tested showed 2-ethyl-1-hexanol as the

Table 6
Results from field testing of smokeless powder odour chemicals

Hide contents	No alert	Interest	Alert	% alert
Empty electrical box	101, 108, 109, 110, 112, 115, 117, 119, 126, 127, 128, 130	–	–	–
Electrical box containing 50 ul acetonitrile on gauze in 2 mL LDPE	101, 108, 109, 110, 112, 115, 117, 119, 126, 127, 128, 130	–	–	–
Electrical box containing 2-nitrodiphenylamine (50 uL at 10%, w/v, acetonitrile) on gauze in 2 mL LDPE	101, 108, 109, 110, 112, 115, 117, 119, 126, 127, 128, 130	–	–	–
Electrical box containing ethyl centralite (50 uL at 10%, w/v, acetonitrile) on gauze in 2 mL LDPE	101, 109, 110, 115, 119, 126, 127, 128, 130	108	117, 112	16.7
Electrical box containing trinitroglycerin tablets 25 × 0.4 mg in 2 mL LDPE	108, 109, 112, 119, 126, 127	117, 101, 115	110, 128, 130	25.0

Table 7
Results from field testing of NESTT petrolatum explosive training aids

Hide content	No alert	Interest	Alert	% alert
Empty quart can	101, 102, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	–	–	–
Quart can containing NESTT chlorate 3 g petrolatum jelly in open vial	101, 102, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	–	–	–
Quart can containing NESTT nitrate 3 g petrolatum jelly in open vial	101, 102, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	–	–	–
Quart can containing NESTT PETN 3 g petrolatum jelly in open vial	101, 102, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	–	–	–
Quart can containing NESTT RDX 3 g petrolatum jelly in open vial	101, 102, 103, 106, 109, 108, 110, 111, 113, 116, 117	112	–	–
Quart can containing NESTT TNT 3 g petrolatum jelly in open vial	101, 102, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	–	–	–
Quart can containing detonating cord aid	–	102	101, 103, 106, 109, 108, 110, 111, 112, 113, 116, 117	91.7%
Empty electrical box	102,109, 110, 113, 115, 116, 118, 127, 128	–	–	–
Electrical box containing NESTT distractor 20 g petrolatum jelly in open tin	102,109, 110, 113, 115, 116, 118, 127, 128	–	–	–
Electrical box containing NESTT PETN 20 g petrolatum jelly in open tin	102,109, 110, 113, 115, 116, 118, 127, 128	–	–	–
Electrical box containing NESTT RDX 20 g petrolatum jelly in open tin	102,109, 110, 113, 115, 116, 118, 127, 128	–	–	–
Electrical box containing NESTT TNT 20 g petrolatum jelly in open tin	102,109, 110, 113, 115, 116, 118, 127, 128	–	–	–

Table 8
Summary of explosive odor chemicals

Category of explosive	Common odor chemicals[a]	Uncommon odor chemcals[b]	Primary canino odor chemical[c]
Cast explosive	2,4-Dinitrotoluene; 2,4,6-trinitotoluene	1,3-Dinitrobenzene	2,4-Dinitrotoluene
Polymer bonded explosives	2-Ethyl-1-hexanol; 2,3-dimethyldinitrobutane	Cyclohexanone; 1-butanol; 2-ethyl-1-hexanol acetate; 1-butanol acetate	2-Ethyl-1-hexanol
Smokeless powders (single based)	2,4-Dinitrotoluene; 2,6-dintrotoluene diphenylamine	Ethyl centralite	2,4-Dinitrotoluene
Smokeless powders (double based)	Ethyl Centralite; dinitroglycerin; trinitroglycerin	2-Nitrodiphenylamine; 4-nitrodiphenylamine; 2,4-dintrotoluene	–
NESTT TNT silica	1,3-DNB; 2,6-DNT; 2,4-dinitrotoluene; 1,3,5-TNB; 2,4,6-trinitotoluene	–	–
NESTT RDX silica	RDX	–	–
NESTT TNT petrolatum	1,3-DNB; 2,6-DNT; 2,4-dinitrotoluene; 2,4,6-trinitotoluene	–	–
NESTT RDX petrolatum	–	–	–

[a] Observed in more than 75% of samples.
[b] Observed in less than 75% of samples.
[c] Canine response greater than 50% alert of canines tested.

dominant odor chemical while the only chemical seen in the NEST RDX aid was RDX and only in the headspace of the silica aid. It is probable that canines trained to alert to the NESTT aid could also locate C-4 samples containing RDX using a different odor chemical which is present in a significantly lower quantity and may have limited availability depending on the packaging of the explosive. Table 8 summarizes some of the most abundant odor chemicals identified in the headspace of various categories of explosives

training aids and the primary odor chemical identified in this study.

6. Conclusions

These results raise concerns regarding the scientific soundness of the choice of explosive samples used in training explosives detection canines. These results indicate that dogs

may not need to be trained on as many plasticized explosives as typically employed in canine training programs (some have comparable headspace odor signatures such as those observed for C4, Flex X Detasheet and TNT booster tested in this study). In addition, dogs trained on certain smokeless powders may not need to train on cast explosives such as Tetratol and TNT as they both contain abundant amounts of one of the active odor signature chemicals, 2,4-DNT. Conversely, significant odor differences have been highlighted between smokeless powder brands and types suggesting that dogs should be trained on multiple smokeless powders with additional research needed to identify the optimal number and types grouped according to dominant odor chemicals present. While major headspace odor components such as diphenylamine and ethyl centralite in smokeless powders were not identified as active odor signature chemicals used by the dogs tested, they can still be useful in the calibration of instrumental techniques to improve detection.

This study has identified several key odor chemicals which illicit positive responses from deployed explosives detection canines with 2,4-dinitrotoluene and 2-ethyl-1-hexanol identified as important odor chemicals for canine detection of cast and polymer based explosives, respectively. These results support the hypothesis that most dogs use the most abundant chemicals available in the headspace to locate concealed explosives. For example, the detector dogs tested alerted to 2-ethyl-1-hexanol found in the headspace of the plasticized explosives and explosive mimics tested but not the RDX observed in the headspace of the silica based RDX NESTT aid. The results also suggest that dogs may use single dominant odor chemicals as a primary mechanism with the dogs tested alerting to 2-ethyl-1-hexanol but not to the added marker DMNB although it was present in the majority of the canine training aids tested. These results also suggest that training dogs on the pure marker chemical DMNB and other single chemicals in low quantities may be advantageous in enhancing their performance.

Reliable non-hazardous training aids are needed for use in areas where live explosive aids are not practical and to provide more consistency in the odor chemicals released in training and in order to improve canine detection performance. In the present studies, NESTT aids yielded inconsistent results with most of the deployed bomb dogs tested in this double-blind study with most dogs not alerting to these materials under field operational conditions. These results also show that NESTT aids have potentially undesirable matrix effects with a large hydrocarbon background observed for the petrolatum based aids and dusting with the silica based aids. Preliminary field tests with explosive odor mimics which provide controlled polymer permeation of active odor chemicals are promising with consistent alerts observed for target chemicals tested including 2-ethyl-1hexanol. Fieldwork is ongoing with additional certified canines in order to identify additional odor chemicals of high and low explosives, including additional smokeless powders, under various environmental conditions and to provide additional explosive odor mimics as improved non hazardous training aids.

Acknowledgements

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