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Lessons from the development of a canine training aid mimic for the detection of fentanyl: Canines versus instruments

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ABSTRACT

Fentanyl, a potent synthetic opioid, presents an increasing challenge to public health and law enforcement due to its high overdose potential and widespread illicit distribution. Canine detection remains one of the most effective field-based methods for narcotics identification; however, direct training with fentanyl poses significant health risks to dogs. This study evaluated N-phenylpropanamide (NPPA) – a known degradant consistently detected in the headspace of both reference-grade and illicit fentanyl – as a potential surrogate odorant for canine training aids. Three NPPA-based mimic training aids were developed using varying amounts of NPPA and different concealment methods – metal tins, Controlled Odor Mimic Permeation Systems (COMPS), and Training Aid Delivery Devices (TADDs) – to regulate NPPA odor availability to canines. Nine drug-detection canines with no prior fentanyl exposure were divided into two groups: one group of three dogs were trained on reference-grade fentanyl and tested on NPPA-based mimics, while the other group of six dogs were trained on NPPA-based mimics and tested on reference-grade fentanyl. The objective was to assess whether canines could generalize across the two materials. Although NPPA was analytically confirmed in both reference-grade fentanyl and NPPA-based mimics, canine behavioral trials did not support NPPA as the sole odorant capable of eliciting a reliable response for fentanyl. These findings suggest that NPPA alone does not adequately represent the fentanyl odor profile relevant to canine detection. This study underscores the importance of combining canine behavioral validation with headspace profiling for the development of safe and effective training aids. Future research should focus on identifying compound mixtures that more accurately reflect the fentanyl odor profile signature to enhance canine detection reliability.

1. Introduction

Drug detection canines are specialized canines trained to detect illicit substances, but their operational performance depends on effective training, which ideally requires repeated exposure to true material, the drugs, in this case. The illicit substances (such as heroin, cocaine, fentanyl etc.) are hazardous, have controlled access and/or often have limited availability for canine training purposes; thus, there is a need for alternative materials which can mimic the odor of the true material without the hazards associated with it. These substitute materials are referred to as alternative training aids. The Organization of Scientific Area Committees (OSAC) [1] describes alternative training aids as training materials that do not use actual substances typically encountered by canines during field searches. These are of different types including: 1) sorption aids (obtained through ab/dsorption of gas-phase compounds from target material), 2) mimic aids (formulated using chemicals chosen to replicate the real odor of a target material), and 3) dilution aids (developed by adding or embedding trace amounts of liquid- or solid-phase target material in a substrate).

Developing a mimic aid for canine training involves understanding and replicating the odor profile of the target material. The odor profile is often complex as it is comprised of multiple odorants (volatile organic compounds [VOCs]) that interact with the olfactory receptors resulting in the perception of an odor. These odorants can result from the degradation of the target material, chemicals associated with its manufacturing process, and/or contaminants introduced during usage and storage of the target material. The first step in mimic development is characterization of the odorant profile of the target material through

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headspace analysis. A common method for this is solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC/MS), resulting in a list of potential odorant(s) of interest that can be used in mimic development.

Prior studies characterized the headspace of drugs including methamphetamine [2], cocaine [3,4] and N-Methyl-3,4-methylenedioxyamphetamine (MDMA) [4,5], resulting in the development of effective mimic training aids for each. In 2001 a study by Vu concluded that among several compounds identified in the headspace of methamphetamine, 1-phenyl-2-propanone and benzaldehyde were likely suited for formulation of a mimic. The study then showed that training detection canines on benzaldehyde enabled them to locate illicit methamphetamine, indicating that canines can be trained to detect specific odorants associated with drugs [2]. In 2002, Furton *et al.* used headspace analysis and canine detection trials to reveal methyl benzoate as the active odorant responsible for eliciting canine response to cocaine [3]. Similarly, piperonal, detected from the headspace of MDMA, was shown to be an effective mimic [5]. In the above instances, a single active odorant elicited a canine response; nonetheless, this is not always true for all substances. For example, in the case of heroin, even though acetic acid was identified as the dominant odorant, acetic acid alone did not elicit a canine response; rather, 90% of the canines showed interest or alerted to a 3:1 mixture of acetic acid and acetylsalicylic acid as the odor profile of heroin [6]. Hence, one odorant or combination of odorants must be tested with canines to identify the most effective mimic.

One particularly dangerous drug, fentanyl, has highlighted the need for development of a mimic for safer training protocols. Several incidents have underscored this necessity, one such being the 2016 federal drug raid in Florida, where three police canines exhibited overdose symptoms after exposure to fentanyl, such as lethargy and refusal to drink [7]. More recently, during a drug bust in Everett, Washington, in 2023 resulting in 500,000 fentanyl pills seized by law enforcement, a drug detection canine became unresponsive after sniffing fentanyl and required three doses of Narcan along with emergency veterinary care [8]. These cases, in addition to several others, have emphasized the potential risks of using true material during training and the critical role of safer alternatives. In addition to being non-hazardous and safer substitutes for canine training purposes, alternative training aids offer the advantage of being usable in various scenarios (e.g., schools, airports etc.) without requiring extensive safety protocols. Furthermore, some alternative training aids provide a consistent odor profile, in contrast to true materials that may degrade over time, leading to changes in their odor profiles. Thus, alternative training aids offer several advantages over true materials.

With the growing concern for public health and the increasing interest in fentanyl-related scientific literature, recent studies have analyzed the headspace of reference-grade and illicit fentanyl, thus, laying the foundation for developing a fentanyl mimic for canine training. A study conducted by Vaughan *et al.* [9] to determine the VOCs in the headspace (using SPME/GC-MS) of pure reference-grade fentanyl concluded the presence of benzaldehyde, styrene, aniline, heptane, N-phenethyl-4-piperidone (NPP), N-phenylpropanamide (NPPA) and 1-phenethyl-4-propionyloxypiperidine. A parallel study by the above-mentioned research group on headspace analysis of fentalogs and illicit fentanyl found NPP and NPPA in 75% of the samples [10]. A comprehensive investigation into the passive degradation of fentanyl under varying environmental conditions, including temperature, humidity, oxygen levels, and storage, identified NPPA as the predominant degradation product in the vapor phase [11]. Thus, the consistent presence of NPPA emphasized its suitability as an ideal surrogate compound for non-contact fentanyl detection. As a result, Fulton *et al* [12]. developed a field-deployable ion mobility spectrometry (IMS) method targeting NPPA for non-contact detection of fentanyl. The handheld IMS, with a limit of detection as low as 5 ng for NPPA, demonstrated successful detection of NPPA in pure, diluted, and even confiscated fentanyl samples. These findings underscore NPPA's effectiveness as an odorant marker, enabling rapid and safe presumptive detection of fentanyl. Furthermore, its consistent detectability and specificity suggested potential for NPPA to serve as a safe and reliable mimic in the development of training aids for detection canines.

Therefore, the current study explores an effort in creating a fentanyl mimic training aid with NPPA and outlines lessons learned for future development. During this study, three NPPA-based mimics were developed with varying levels of odor availability, manipulated using containment systems including a permeable bag which allowed control and consistent release of odorants or Controlled Odor Mimic Permeation Systems (COMPS) [13,14] and the Training Aid Delivery Device (TADD®) [15]. The presence of NPPA in the mimics was confirmed through SPME/GC-MS headspace analysis and their effectiveness as a fentanyl training aid was tested using drug detection canines that had no prior formal training in the detection of fentanyl.

2. Materials and methods

2.1. Overview

This study evaluated the potential of N-phenylpropanamide (NPPA) as a surrogate odorant for fentanyl by assessing its effectiveness as a canine training aid through controlled canine testing. NPPA-based mimics were prepared in different dilutions (elaborated in 3.4) and distributed to canine handlers for training and testing. Headspace analysis through solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME/GC-MS) was conducted to confirm the presence of NPPA in all three dilutions. An initial baseline testing was conducted with nine canines to confirm their non-recognition of reference-grade fentanyl and NPPA-based mimics, as these were novel odors to them. The canines were then divided into two groups: the control group ($n = 3$), which was trained on reference-grade fentanyl, and the experimental group ($n = 6$), which was trained on NPPA-based mimics. Following training, testing was conducted in a setup identical to baseline testing to evaluate whether reference-grade fentanyl-trained canines could detect NPPA-based mimics and whether NPPA-based mimic-trained canines could detect reference-grade fentanyl. Thus, establishing (or not) the effectiveness of NPPA as a canine-relevant odorant for fentanyl detection through canine testing.

2.2. Materials

Reference-grade fentanyl [N-(1-(2-phenethyl)-4-piperidinyl-N-phenyl-propanamide] was purchased from Cayman Chemical. 98% NPPA, a degradation product of fentanyl, was purchased from Tokyo Chemical Industry (TCI). 99.8% acetonitrile was purchased from Sigma-Aldrich. Alumina adsorption 80–200 mesh was purchased from Fischer Scientific. All chemicals were used as received without further purification. 2"x3" 1 MIL low-density polyethylene (LPDE), 2"x3" 2 MIL LDPE bags, 2 oz screw top metal tins, and 2"x2" gauze pads were purchased from ULINE. 32 oz canning jars were purchased from a local store.

2.3. Safety precautions for handling hazardous materials

All fentanyl was purchased and received according to DEA requirements. In compliance with internally developed Standard Operating Procedures, any handling or manipulation of fentanyl powder was performed in the presence of two trained personnel. Both personnel wore proper personal protective equipment (PPE), and any transfer or manipulation of the powder was conducted in a ventilated fume hood. The second personnel, not handling the fentanyl, maintained a safe distance but within eyesight of the primary personnel, with Naloxone available in case of emergency.

2.4. Training aid preparation

The Controlled Odor Mimic Permeation System (COMPS) training aids were prepared to represent differing levels of odorant (NPPA) availability. The COMPS comprises a LDPE permeable plastic bag that securely contains the odor source, enabling the consistent release of volatiles [13,14]. In this study, the lower odor level COMPS, or Mimic 1, contained 20 mg of NPPA diluted in 80 mg of alumina powder heat sealed in a 2"x3" 2 MIL LDPE permeable plastic bag. The higher odor level COMPS, or Mimic 2, included 60 mg of NPPA neat, sealed in a 2"x3" 1 MIL LDPE permeable plastic bag. The third training aid, or Mimic 3, was prepared without COMPS by placing 60 mg of NPPA neat in a 2 oz screw top metal tin with 15 holes each approximately 4 mm. Table 1 summarizes the formulations of the three training aid mimics. The COMPS training aids were placed inside Training Aid Delivery Device (TADDs) before headspace analysis and conduction of canine trials. TADDs consist of glass containers that hold the odor source and use a membrane to allow controlled, passive release of volatiles while preventing exposure of the canine and handler to the actual material [15].

2.5. Headspace analysis

The availability of NPPA in the headspace of Mimics 1, 2, and 3 were compared to 5 mg of reference-grade fentanyl. The NPPA-based mimics were placed in separate 32 oz canning jars for headspace collection. The headspace of each mimic was extracted following the parameters previously devised by Vaughan *et al.* [11]. To summarize this procedure, all training aids mimics were equilibrated at room temperature (approximately 21 ± 3 °C) for 24 h. The mimics were then heated to 35 °C for 30 min. The divinylbenzene/carboxen/ polydimethylsiloxane SPME fiber was inserted into the septum of the jar and exposed to the headspace of one mimic for 4 hrs.

To assess the headspace of reference-grade fentanyl and NPPA-based training aid mimics, a 7890 A/5975 C Agilent GC-MS with a 30 m x 0.25 mm ID x 0.25 µm, Rtx-5MS column was utilized. The collection of analytes adsorbed to the SPME fiber was thermally desorbed in the GC inlet at 260 °C for 3 mins with a 10:1 split ratio and a 2 mL/min flow rate. The GC column was temperature programmed, which was held at 40 °C for 30 s, ramped to 250 °C at 30 °C/min, and held at 250 °C for 30 s. The MS transfer line was set to 250 °C, with the MS Quad and MS Source held at 150 °C and 230 °C, respectively. The mass scan range was *m/z* 40–300. NPPA was preliminarily identified using the NIST Mass Spectral Library and confirmed by preparing a standard solution of 50 ppm of NPPA in acetonitrile.

2.6. Canine trial

Regarding the validity of NPPA as the odorant of fentanyl, a canine trial was conducted with a total of nine drug detection canines (details in Table 2) provided by government and law enforcement agencies. All canine trial protocols were reviewed and approved by the Florida International University Animal Care and Use Committee (IACUC-21–015-CR02). All canines had only recently completed the olfactory detection training with cocaine and other drugs immediately prior to this study.

Table 1
Formulations of the three NPPA-based mimic training aids prepared for headspace analysis and canine trials.

Mimic name	Formulation	Containment
Mimic 1	20 mg of NPPA diluted in 80 mg of alumina powder	2"x3" 2 MIL LDPE permeable plastic bag
Mimic 2	60 mg of NPPA neat	2"x3" 1 MIL LDPE permeable plastic bag
Mimic 3	60 mg of NPPA neat	2 oz screw top metal tin with holes

Table 2
Summary of canine identification details and their purpose of participation in this study.

Canine ID#	Breed	Age	Years of experience	Purpose of participation in this study
C1	Belgium Malinois	3 years	1 year	Canines trained on reference-grade fentanyl and tested for detection of NPPA-based mimics.
C2	Dutch Shepherd	2 years 6 months	1 year	
C3	German Shepherd	3 years	< 1 year	
C4	Belgium Malinois	4 years	3 years	Canines trained on NPPA-based mimics and tested for detection of reference-grade fentanyl.
C5	Dutch Shepherd	5 years	3 years	
C6	Dutch Shepherd	2 years 6 months	1 year	
C7	Belgium Malinois	8 years	7 years	
C8	German Shepherd	2 years	1 year	
C9	Dutch Shepherd	3 years	1 year	

The canines had no formal training in fentanyl detection. None of the participants had prior operational search experience and thus had not encountered fentanyl in the field prior to the study. Canines C1-C9 were divided into two groups: control and experimental. The control group (C1–C3) was trained on reference-grade fentanyl and tested for detection of Mimics 1 and 2. The experimental group (C4–C9) was sequentially trained on Mimic 3 and Mimic 2 before being tested for detection of reference-grade fentanyl. Both the control and experimental group canines were tested before and after their training, thereby establishing the baseline testing (elaborated in section 3.6.1), training, and post-training testing (hereafter referred to as testing – elaborated in section 3.6.2) sessions of the trial. Fig. 1 outlines the experimental design of the trial.

During the trial, each canine response was recorded and classified as a true positive when the canine correctly alerted to a target odor, a false positive when the canine incorrectly alerted to a blank or distractor, a true negative when the canine correctly ignored a blank or distractor, and a false negative when the canine failed to alert to a target odor. Performance metrics, including true positive rate (Eq. 1) and false positive rate (Eq. 2), were calculated for each sample type where,

$$\text{True positive rate} = \frac{\text{Number of true positive alerts}}{\text{Total number of possible true positive alerts}} \text{x } 100 \quad (1)$$

$$\text{False positive rate} = \frac{\text{Number of false positive alerts}}{\text{Total number of possible false positive alerts}} \text{x } 100 \quad (2)$$

2.6.1. Baseline testing

Baseline testing was conducted to establish the canines' initial detection capabilities prior to training. This step was essential to establishing non-recognition of the new target material including reference-grade fentanyl and NPPA-based Mimics 1 and 2. Additionally, cocaine, which the canines had previously been trained on, was used as a positive control to verify their baseline detection performance. The tests were conducted as double-blind by not informing the handlers or the evaluators of the location of the target odors. A total of six setups of six-arm carousel were used, where the odor sources were placed in TADDs and stored in shaker cans positioned at the ends of the carousel arms. The odor sources used in the trial included positive control (cocaine) and target materials (reference-grade fentanyl and NPPA-based Mimics 1 and 2), blanks (empty TADDs) and non-target odors (i.e., distractors). Non-target odors are utilized in addition to blanks to ensure that the

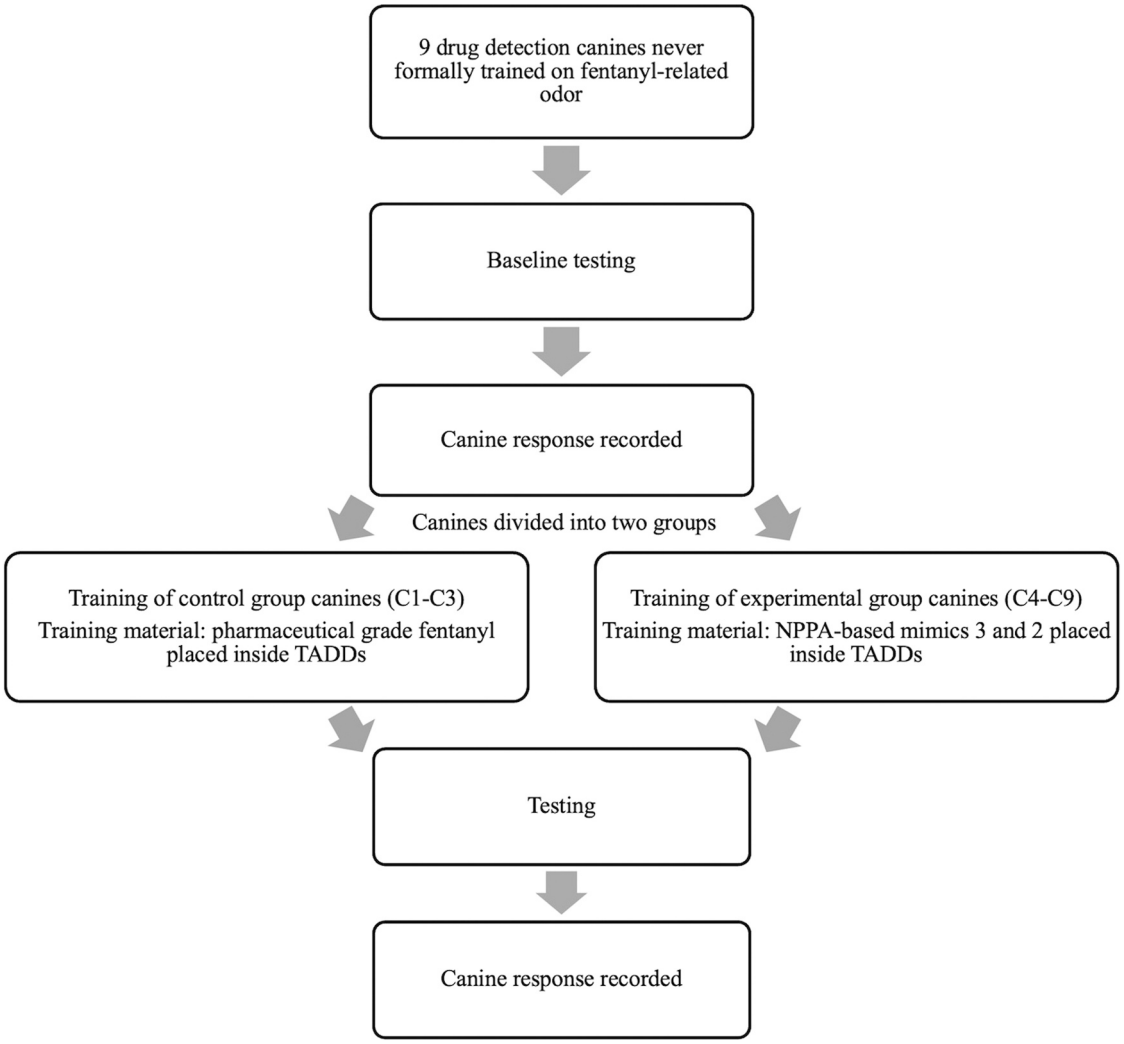

Fig. 1. Flowchart representing steps involved in the canine trial conducted with nine drug detection canines never previously trained on fentanyl-related odor.

canine is responding to recognized odors, not simply any odor other than a blank. Non-target odors included dog food, β-hydroxybutanoic acid, blank mimic (alumina without NPPA), o-toluic acid, nitrile gloves, dog treats, and other local commercial substances chosen by the agency handlers.

The handlers were directed to let the canine conduct their searches independently. Only the individual who organized the trial was aware of the lineup contents and informed the handler whether the canine's alert was accurate. To maintain the double-blind, the test organizer was located away from the sightline of the handler or canine. If the alert was correct, the canine received a reward; if incorrect, the handlers were instructed to continue searching. Table 3 details the odor source placements in the six carousel setups utilized during baseline testing.

2.6.2. *Final testing*

The final testing, also referred to as testing, was conducted after canines C1-C3 were successfully trained on reference-grade fentanyl, and canines C4-C9 were successfully trained on Mimics 3, 2 and 1 at the

Table 3
Odor source placement matrix for six setups of six-arm carousel used during baseline testing of nine drug detection canines.

Carousel arm	1	2	3	4	5	6
Wheel 1	Blank (Empty TADD)	Dog food (Distractor 1)	Blank (Empty TADD)	Positive control (cocaine)	O-toluic acid (Distractor 4)	Blank (Empty TADD)
Wheel 2	Distractor 11	Reference-grade fentanyl 1	Blank (Empty TADD)	Dog treats (Distractor 6)	Blank (Empty TADD)	Alumina powder (Distractor 3)
Wheel 3	Blank (Empty TADD)	β-hydroxybutanoic acid (Distractor 2)	Blank (Empty TADD)	Distractor 7	Blank (Empty TADD)	Distractor 9
Wheel 4	Distractor 8	Alumina powder (Distractor 3)	NPPA-based Mimic 1	Blank (Empty TADD)	Nitrile gloves (Distractor 5)	Blank (Empty TADD)
Wheel 5	Blank (Empty TADD)	Blank (Empty TADD)	Distractor 10	Distractor 11	Blank (Empty TADD)	Reference-grade fentanyl 2
Wheel 6	NPPA-based Mimic 2	Dog food (Distractor 1)	Blank (Empty TADD)	Blank (Empty TADD)	Distractor 8	Blank (Empty TADD)

end of two weeks of training. The training protocols and methods were not dictated by the researchers of this study; instead, they were determined by the handlers, who decided when the canines were considered 'successfully trained'. Double-blind testing was conducted using the same protocol as baseline trials; however, the placement of odor sources was randomized across different arm positions and carousel setups. Table 4 details the odor source placements in the six carousel setups utilized during testing.

3. Results

3.1. Headspace

The availability of NPPA based on the odor profiles of reference-grade fentanyl (5 mg) and Mimics 1, 2, and 3 was compared in Fig. 2. The odor levels of NPPA availability present within Mimics 2 and 3 were significantly higher (1-tailed *t*-test, 95% confidence) than the detectable levels present within Mimic 1.

3.2. Canine trial

The training concluded after two weeks, by the end of which canine handlers reported successful training of the control group canines (C1-C3) on reference-grade fentanyl and the experimental group canines (C4-C9) on NPPA-based mimics. The results from baseline testing and testing of canines are summarized in Fig. 3. Results for each canine response during the trial are detailed in Tables 1–2 of Supplementary Material. In the control group, the true positive response rate to their training material – reference-grade fentanyl – increased from 16.67% to 66.67% after training. Similarly, in the experimental group, the true positive response rate to their training material – NPPA-based mimics – also increased, from 0% to 58.34%, indicating improvements in both groups, though detection rates of the trained targets were low for both groups. By comparison, for the positive control, cocaine, the true positive rate increased from 50%–66.67–100% in the testing sessions. It should be noted that there was no statistical difference in detection of distractor odors during the testing. This is because, given the small canine sample size (n = 6), the associated margin of error is substantially larger than the observed difference, indicating that this variation is most likely due to random chance rather than a change in performance.

Contrastingly, when evaluating performance on the testing material (i.e., the material to which the canines were not trained), the true positive rate for the control group increased from 0% to 33.34% when tested on NPPA-based mimics, while the experimental group showed a smaller increase from 0% to 16.67% when tested on reference-grade fentanyl – based on a comparison of baseline and post-training test results. Although the improvement in detecting the test material was less pronounced than the increase observed for the training materials, it still indicates some generalization between the reference-grade fentanyl and NPPA-based mimics, and vice versa. The false positive rate was significantly low throughout the trial, ranging between 0% and 10.41% across all the baseline testing and testing sessions of both canine groups.

4. Discussion

Evidence from prior studies including headspace analysis of fentanyl [9; 10], investigations into its passive degradation [11], and instrumental detection of fentanyl via ion mobility spectrometry (IMS) [12, 16], all consistently highlighted NPPA as a promising candidate for developing a fentanyl mimic canine training aid. Additionally, NPPA is chemically distinct as it has not been detected in the headspace of other forensically relevant matrices, allowing canine discrimination of NPPA from background/environmental odor and other non-relevant odor sources. NPPA being detected in both reference-grade and illicit fentanyl samples [9; 10], supported its potential to enable canines to generalize detection across different fentanyl formulations. This further added value to the hypothesis of NPPA being an ideal candidate for a fentanyl mimic training aid. Thus, this study aimed to evaluate the efficacy of NPPA as a target odorant in the development of a non-hazardous fentanyl canine training aid through canine testing.

The NPPA-based mimics were developed with varying levels of odor availability by adjusting the amount of NPPA (20 mg and 60 mg) and packaging it either in COMPS (Mimic 1 and 2), to lower odor availability, or tin containers (Mimic 3). Odor availability, which refers to the accessible odorant in the vapor phase that a canine can detect, is influenced not only by the quantity of the odorant but also by the packaging material, surface area, and environmental interactions such as temperature and airflow [17]. Although all three mimics contained measurable NPPA, Mimic 1, containing 20 mg of NPPA in a COMPS, which was most representative of NPPA levels found in reference-grade fentanyl samples, likely had the lowest vapor-phase concentration due to both the smaller amount of material and the restrictive permeation properties of the COMPS. In contrast, Mimic 3 (60 mg NPPA in a tin) likely offered higher odor availability which was supported through headspace analysis results.

Anecdotal evidence from handlers that participated in this study indicated challenges in training canines to detect NPPA, particularly with Mimic 1, which had the lowest odor availability. This difficulty suggested that canines may naturally have a relatively high odor detection threshold for NPPA. While quantifying this threshold was beyond the scope of the study, the hypothesis was supported by handler observed canine behavior. Odor detection canine threshold is defined as the lowest vapor-phase concentration at which canines can reliably detect the odor [18]. This threshold can vary depending on the odorants, canine training, and environmental factors. Consequently, during training on NPPA-based mimics, canines initially required exposure to Mimic 3 (with higher odor availability) before gradually detecting lower concentrations in Mimics 2 and 1 through continued training. The potentially higher odor detection threshold of NPPA in canines limits its suitability as a mimic training aid for fentanyl detection. This would imply that canines would need a more concentrated presence of NPPA in vapor-phase to consistently detect fentanyl, exhibiting diminished

Table 4
Odor source placement matrix for six setups of six-arm carousel used during testing of nine drug detection canines.

Carousel arm	1	2	3	4	5	6
Wheel 1	NPPA-based Mimic 1	Dog treats (Distractor 6)	Blank (Empty TADD)	Blank (Empty TADD)	β-hydroxybutanoic acid (Distractor 2)	Blank (Empty TADD)
Wheel 2	Distractor 7	Dog food (Distractor 1)	Blank (Empty TADD)	Nitrile gloves (Distractor 5)	Blank (Empty TADD)	Reference-grade fentanyl 1
Wheel 3	Blank (Empty TADD)	Alumina powder (Distractor 3)	Blank (Empty TADD)	Distractor 11	Blank (Empty TADD)	Distractor 10
Wheel 4	Alumina powder (Distractor 3)	Positive control	O-toluic acid (Distractor 4)	Blank (Empty TADD)	Dog treats (Distractor 6)	Blank (Empty TADD)
Wheel 5	Blank (Empty TADD)	Blank (Empty TADD)	Distractor 9	NPPA-based Mimic 2	Blank (Empty TADD)	Distractor 8
Wheel 6	Blank (Empty TADD)	β-hydroxybutanoic acid (Distractor 2)	Reference-grade fentanyl 2	Blank (Empty TADD)	Distractor 11	Blank (Empty TADD)

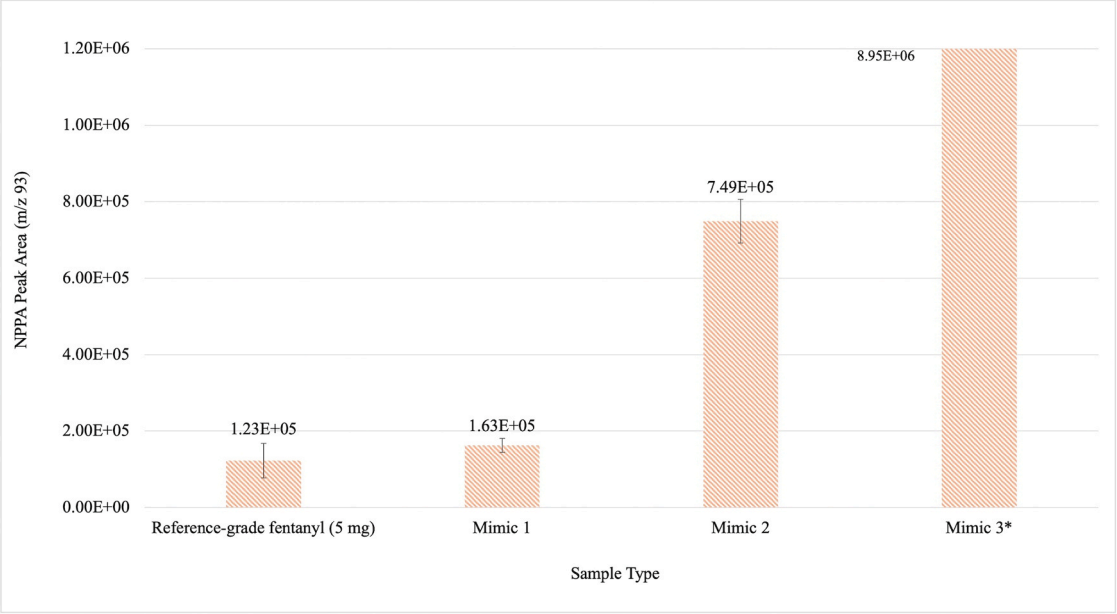

Fig. 2. Bar graph depicting the availability of NPPA in the headspace of reference-grade fentanyl (5 mg) and Mimics 1, 2, and 3. The standard error bars pertained to one standard deviation (n = 3). *The peak area of Mimic 3 exceeded the limit on the bar graph.

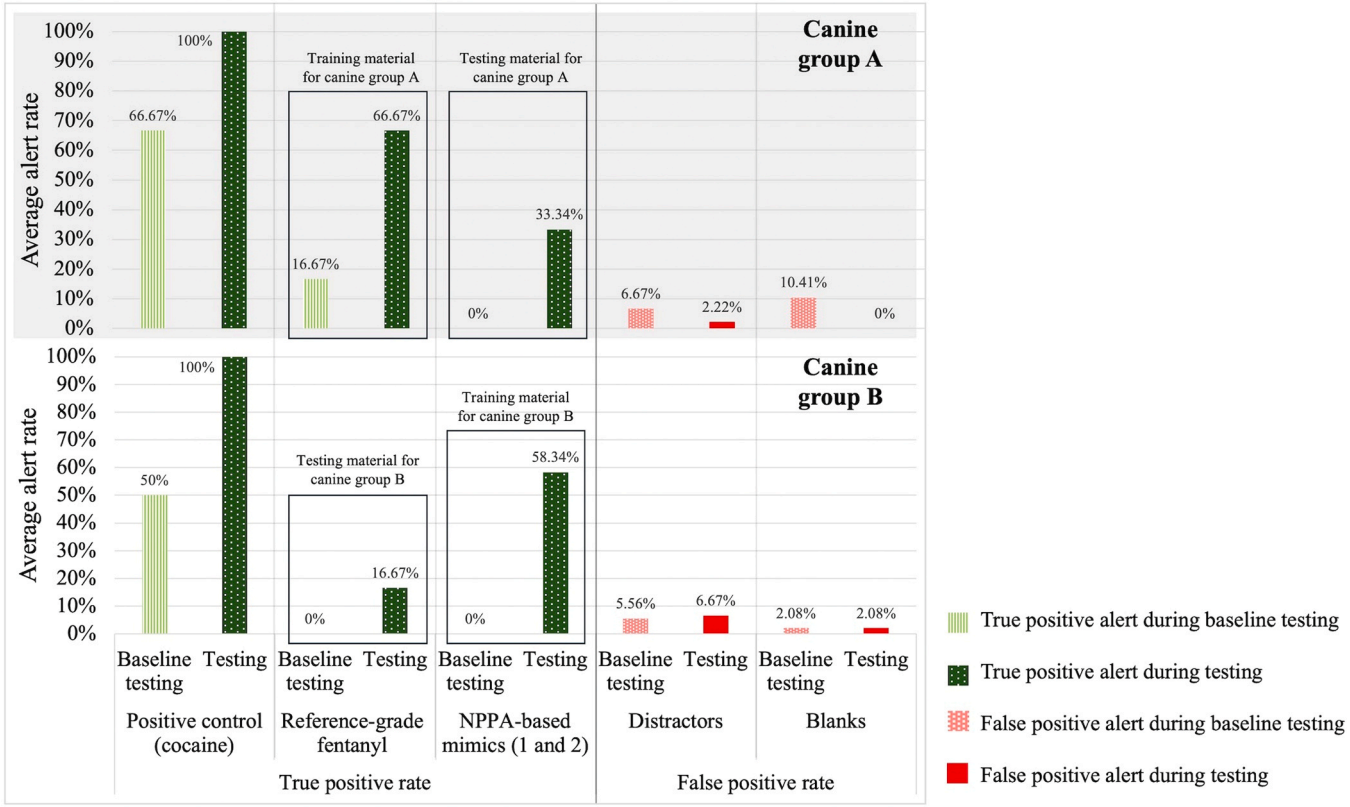

Fig. 3. True positive rate and false positive rate recorded during baseline and testing for A) Control group canines (C1-C3) trained on reference-grade fentanyl B) Experimental group canines (C4-C9) trained on NPPA-based mimics, when exposed to positive control (cocaine); target odors (reference-grade fentanyl, NPPA-based mimics 1 and 2); distractors and blanks.

sensitivity as a detector in practical search scenarios where the target material may typically occur at very low concentrations.

The results of the canine trial yielded several important insights. First, following training, both canine groups – the control group (C1-C3) trained on reference-grade fentanyl and the experimental group (C4-C9) trained on NPPA-based mimics demonstrated improved detection of all target material (cocaine, reference-grade fentanyl and NPPA-based mimics) regardless of what they were trained on. Improved alert rate for their respective training material was an anticipated outcome, as the canines utilized in this study had only recently completed their final

detection training and had not previously had experience with the scent wheels. Training and repeated reinforcement are well-documented to enhance canine detection capabilities. The primary objective of the study, however, was to determine whether generalization was possible: specifically, whether control group canines trained on reference-grade fentanyl could detect NPPA-based mimics, and whether experimental group canines trained on NPPA-based mimics could detect reference-grade fentanyl. While both groups did show the ability to generalize their respective test materials, the observed true positive alert rates (33.34% in the control group and 16.67% in the experimental group canines) were not sufficiently high to support the conclusion that NPPA-based mimics can function effectively as standalone mimic training aids for fentanyl detection. Finally, when analyzing true positive alert rates for individual canines (depicted in the Tables 3 and 4 of the supplementary material), regardless of what the canines were trained, some canines could either successfully detect reference-grade fentanyl or NPPA-based mimics but failed to detect both. This was evident in control group canine C2, which was trained on reference-grade fentanyl but responded only to NPPA-based mimics during testing. A similar divergence was observed in canine C6 from the experimental group, which was trained on NPPA-based mimics but responded only to reference-grade fentanyl during testing. A possible explanation for this pattern may be that the perceived odor of reference-grade fentanyl and NPPA-based mimics are not interchangeable to the canine. As a result, some canines may have simply responded to the presence of a novel odor which is not uncommon [19]. A limitation to this study in hindsight, was the failure to include Mimic 3 (highest concentration mimic) in the generalization trial. This higher concentration could have instigated responses from canines that did not respond to Mimic 2 due to detection threshold limitations. To conclude, NPPA may be one of the key odorants that, in combination with other compounds present in the headspace of fentanyl, contribute to the canine detection response, but it is not sufficient on its own.

Findings from studies like the present one consistently emphasize the crucial importance of employing orthogonal study designs when developing canine training aids. Specifically, combining headspace analysis, which allows for objective instrumental detection of odorants or volatile organic compounds (VOCs), with observational studies that assess actual canine behavioral responses, is vital. This dual approach ensures a comprehensive evaluation from both a chemical and biological perspective. It is important to recognize that an odorant identified as a promising candidate through instrumental analysis alone does not necessarily translate into effective training material for detection canines. The canine olfaction is complex and may not be fully captured by instrumental methods. It is well established that instrumental analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), do not match the sensitivity and selectivity of canines [19]. These instruments may not capture the full complexity of the headspace profile, often leaving out trace-level odorants that could be critical to canine detection. Furthermore, it is possible that a non-salient or chemically minor odorant in the headspace, which may be undetected in instrumental analysis, may be responsible for eliciting a canine alert to a target material. Thus, integrating canine behavioral assessments with chemical profiling provides a more reliable foundation for selecting and validating odorants that can be used in the development of mimic training aids. Orthogonal studies bridge the gap between laboratory findings and real-world canine applications, ensuring that training aids are truly representative of what the canines are expected to detect in the field.

Future studies that focus on developing a non-hazardous fentanyl mimic training aid for canines must consider a combination of other compounds previously detected in the headspace of fentanyl. These include benzaldehyde, styrene, aniline, heptane, NPP, NPPA, and 1-phenethyl-4-propionyloxypiperidine [9]. However, there is a possibility that none of these compounds elicit a canine response and that a currently unknown or undetected compound in the fentanyl headspace or a byproduct produced during the synthesis of fentanyl is the key odorant for canine detection. Therefore, future studies could focus on using alternative advanced analytical instruments for headspace analysis, as well as additional canine testing.

5. Conclusion

The primary objective of this study was to evaluate whether NPPA (N-phenylpropanamide) serves as a key odorant capable of eliciting a reliable and specific olfactory response in detection canines trained to identify reference-grade fentanyl. This hypothesis was based on previous instrumental studies, which consistently identified NPPA as a major component in the headspace of reference-grade fentanyl. Based on these findings, behavioral assessment with detection dogs were undertaken to assess whether NPPA alone could serve as an effective surrogate in training materials. However, results from the canine trials indicated that dogs trained on reference-grade fentanyl did not consistently alert to NPPA-based mimic materials and vice versa, despite the analytically verified presence of NPPA in the headspace of both. This suggests that the presence of NPPA alone may not be sufficient to drive the desired behavioral response in canines. One possible explanation lies in the relatively high odor detection threshold of NPPA for canines. Alternatively, the odor of NPPA may not be recognizable to the canines without the presence of other possible volatiles. These findings underscore the importance of complementing analytical headspace data with behavioral validation studies when developing and validating canine training aids.

CRediT authorship contribution statement

Lauryn E. DeGreeff: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Kenneth G. Furton:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Jeff Bosnich:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Leann Forte:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kayla A. Hogan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Rushali Dargan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation.

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

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2026.112911.

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