#### Check for updates

#### **OPEN ACCESS**

EDITED BY Jennifer Lynn Essler, State University of New York at Cobleskill, United States

REVIEWED BY Adee Schoon, Animal Detection Consultancy, Netherlands Shari L. Forbes, University of Windsor, Canada

\*CORRESPONDENCE Alison G. Simon, agsforensics@gmail.com

<sup>†</sup>These authors have contributed equally to this work

RECEIVED 19 April 2023 ACCEPTED 01 August 2023 PUBLISHED 17 August 2023

#### CITATION

Simon AG, Lazarowski L, Krichbaum S, Singletary M, Angle C, Waggoner P, Van Arsdale K and Barrow J (2023), A method for validating a non-hazardous canine training aid. *Front. Anal. Sci.* 3:1208709. doi: 10.3389/frans.2023.1208709

#### COPYRIGHT

© 2023 Simon, Lazarowski, Krichbaum, Singletary, Angle, Waggoner, Van Arsdale and Barrow. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is

reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

# A method for validating a non-hazardous canine training aid

Alison G. Simon<sup>1\*†</sup>, Lucia Lazarowski<sup>2†</sup>, Sarah Krichbaum<sup>2†</sup>, Melissa Singletary<sup>2†</sup>, Craig Angle<sup>2†</sup>, Paul Waggoner<sup>2†</sup>, Kelly Van Arsdale<sup>3†</sup> and Jason Barrow<sup>1,3†</sup>

<sup>1</sup>Center for Health Sciences, School of Forensic Sciences, Oklahoma State University, Tulsa, OK, United States, <sup>2</sup>Canine Performance Sciences, Auburn University College of Veterinary Medicine, Auburn, AL, United States, <sup>3</sup>Federal Bureau of Investigation Laboratory Division, Quantico, VA, United States

Detection dogs are trained to locate a variety of substances to provide security and protection for the public and the environment, but access to substances for training is often limited. Various training aids have been created to deliver target odors as safer or more accessible alternatives to using the actual substance material, many of which are commercially available. However, the methods used to create and validate the effectiveness of these training aids are rarely reported or available to consumers, leading to uncertainty regarding their use. There has been a recent drive in the detection canine community to create standards by which to measure the manufacture and utility of canine training aids, but little progress has been made in determining how a reliable canine training aid should be developed and which analytical measurements should be utilized. While the interest in and need for an independently evaluated training aid is clear, developers typically do not release the necessary information, whether for proprietary or other reasons. Transparent analysis and procedures would allow for direct examination of training aids using objective measures, which in turn would allow canine teams to select the best tool to achieve their mission. To this end, the current manuscript provides a stepwise method for the development and validation of a novel canine training aid, using triacetone triperoxide as an example target. This method can be applied to the creation of training aids of many different target odors, such as explosives, narcotics, chemical warfare agents, or biological diseases and viruses.

#### KEYWORDS

triacetone triperoxide, detection canine, training aid, validation, working dog

# **1** Introduction

Canines are trained to detect a range of substances for the protection of both the public and the environment. While access to training materials is often limited due to security restrictions and other constraints, several training aids have been designed to provide safe and accessible training aid alternatives to the true material. The methods of manufacture, testing, and validation of these training aids are rarely provided or made available to consumers, which leads to uncertainty regarding their use. This lack of transparency within the canine training aid industry is a concern largely due to a lack of standards to guide developers and researchers. The National Institute of Standards and Technology's (NIST's) Organization of Scientific Area Committees (OSAC) Dogs and Sensors Subcommittee has identified several research areas that need to be explored, including the "Development of Reliable Surrogate Aids" (OSAC Research Needs Assessment Form 2021). Such materials

Training aid	Bench science data reported	Bench science methods provided	Canine data reported	Canine training methods provided	Canine testing methods provided	Third-party validated
TrueScent™	No	No	Conclusions only	Vague	Vague	No
ScentLogix™	No	No	Yes	No	No	No
SPOT*	Qualitative comparisons	Chemical instrument stated	No	No	No	No
Precision Explosives	No	No	No	No	No	No
TOIDS	No	No	No	No	No	No
Microtrace Marker	No	No	No	No	No	No
Getxent*	No	No	No	No	No	No
SOKKS*	No	No	No (anecdote given)	No	No	No

TABLE 1 Summary of information available on the websites of seven different pseudo or non-pseudo alternative training aids available commercially for TATP. Table includes only information available on the website of each training aid. \*Training aid not specific to TATP only.

would allow for controlled odor delivery to help train or calibrate canine sensors. Despite the identification of this gap first by the OSAC forerunner SWGDOG (the Scientific Working Group for Dog and Orthogonal detector Guidelines) in 2007 (approved in 2010), little progress has been made.

In a recent review of the types of canine training aids, three categories were identified, with four individual types of non-pseudo alternatives (Simon et al., 2022a). The categories were based on the method of manufacture: 1) true material, or the actual target substance, 2) pseudo-odors, or training aids where the true material had no part in the manufacture, and 3) non-pseudo alternatives, or training aids where the true material did have a part in the manufacture but is not present in bulk. The types of nonpseudo alternatives included dilution, encapsulation, ad/absorption, and extraction. Such alternative aids can be of great use to the community. However, they should be selected with caution because while some commercially available training aids provide chemical analysis of the odor to consumers, many do not. Further, the information provided is not consistent across training aids or manufacturers. The authors concluded that any manufacturer of canine training aids should provide transparent information regarding chemical, biological, or physical validation in tandem with canine trial results, all of which should be evaluated by an independent third-party.

An example of a target odor for which many alternative training aids exist is the explosive triacetone triperoxide (TATP), due to the restrictions and safety hazards associated with accessing and handling the true material. At least eight pseudo or non-pseudo alternative training aids are available commercially for TATP, listed in **Table 1** along with a summary of the type of information available on the website of each training aid at the time of writing. This summary of information is not meant to be a reflection of the quality of these training aids, which has yet to be evaluated. Rather, it notes the analytical work available regarding each aid. Only one of the seven aids provides any bench science information, and two provide information related to canine testing. While some state that research occurred, they do not provide the methods or data. None of the aids claim to have been validated by a third-party. Analytical validation of a training aid should evaluate similarity to the true material. There are several types of chemical, biological, or physical data that provide information related to a training aid's efficacy. For example, the qualitative and quantitative comparison of the headspace present above the alternative training aid and the true material should be determined. It is also important to determine the odor associated with the matrix of the training aid, or the background odor. This information is essential in establishing quality assurance and quality control measures that allow first for reproducibility and reliability in manufacture. Second, it allows for the establishment of end user recommendations, such as shelf life (Simon et al., 2022a; Consensus Analytical Methods for K9 Training Aid Verification, 2021). A qualitative comparison of the headspace profiles of most of the training aids in Table 1 was published by Simon 2022b.

Canine validation of a training aid should demonstrate that a trained detection dog responds equivalently to a training aid and the actual material it is designed to represent. Depending on the intended use of the training aid, validation efforts could include initially training dogs to detect the odor using only the training aid, and then testing the dogs' ability to detect and respond to the true material. Conversely, dogs already trained to detect the true material could be tested for detection of the training aid. To conclude that a training aid is perceived by dogs in the same way as the actual material, response rates (i.e., correct indications of the target) should be statistically equivalent between the two. For example, Moser et al. (2020) compared the effectiveness of two types of training aids for live insect detection. Two groups of dogs were trained on either dead insects or an extraction-based training aid, and tested for detection of the live insects. Dogs trained with the scent extract training aid detected the live insects with 100% sensitivity and specificity, demonstrating strong support for the effectiveness of the training aid. Live insect detection by the dead specimen trained dogs was less straightforward, requiring further examination into its reliability as a training aid.

Regarding studies evaluating detection canine capabilities, critical controls should be included to eliminate extraneous variables influencing dogs' performance. However, wide variability in testing protocols such as experimental design, methodological controls, and data analysis have resulted in difficulty interpreting and comparing



results directly (Johnen et al., 2013). Recently, published literature has identified best practices for designing methods for detection canine testing. For example, Porritt et al. (2015) reported a validated odor discrimination procedure for detection dogs that consisted of a short, double-blind, and reliable odor discrimination test for detection canine accreditation. Lazarowski et al. (2020) further detailed methodological aspects of testing detection canines that must be considered in rigorous experimental designs. For example, sensory and behavioral considerations should be taken into account when selecting canine subjects. Ideally, canines included in training aid validation studies should be representative of operational detection canines for which the training aids are intended. The experimental design is also an important consideration involving the type of detection task (which again should include those representative of operational detection canines), minimization of potential bias from handlers or experimenters by use of single- or double-blind testing, and the inclusion of positive and negative odor controls. Finally, odor sample characterization, delivery, and possible sources of contamination should be considered.

Complementary analytical and canine testing provides the most rigorous evaluation of alternative training aids in comparison to the true material. For example, in the Moser et al. (2020) study, canine responses and volatile odor component (VOC) analysis for the various training aids were compared. The chemical similarity between the training aids and the live insects supported the canine detection rates, suggesting that non-pseudo alternatives may be a viable option for the detection of certain difficult-tomaintain pests. Importantly, the interplay of analytical and canine testing can lead to problem-solving efforts that increase training aid proficiency and create a robust final product. Used in tandem, this information can also inform end user guidelines for use of the alternative training aids and even the true material.

Third party validation of both analytical and canine testing is an essential final measure in canine training aid development. The test design, evaluation, and analysis should be objective and conducted by an unbiased party separate from the manufacturer or original training aid development sponsor, with no conflict of interest. Objective reviewers are essential for providing results that evaluate the utility of the aid and in developing training aids based on unbiased and rigorous analyses that can be used reliably across canine cohorts.

Despite the evolving consensus for reliable methods of testing general detection canine capabilities, there has been little similar movement for canine training aid materials. While such published literature and the OSAC subcommittee identify the need for standards of canine training aids, no peer-reviewed or ASTM (i.e., the American Society for Testing and Materials which provides technical standards for a variety of industries, including OSAC) method for their development and validation currently exists. The current manuscript therefore offers a stepwise method for providing a validated alternative canine training aid using the explosive TATP as an example.

# 2 Method and results

Because standards do not currently exist for the validation of a canine training aid, a recommended method is outlined below (Figure 1). This stepwise program describes the testing of a canine training aid from its inception to the validated product called a Polymer Odor Capture and Release training aid (POCR<sup>TM</sup>) through integrated canine and chemical methods. Each step will be discussed, using the TATP POCR<sup>TM</sup> as an example. The authors acknowledge that TATP has a relatively simple headspace in comparison to many canine targets. The process may therefore need to be adapted for more complex targets. However, it is still a useful guideline in developing a validated training aid.

# 2.1 Step 1: intelligence-driven target identification

Selection of a target should be based on gathered intelligence from the end-user community or related government intelligence. Such considerations may include: any target that a canine trainer requests, whether a target is required for certification, how often the target is encountered operationally, how difficult it is to obtain true samples of the target material for training, and how easily the true material can be manipulated for training in operationally relevant scenarios. Another consideration is the variations of the target that may be available. For example, many canines train on pure samples obtained from laboratory- or industrial-grade sources. However, clandestine versions may have different headspace components (Lazarowski et al., 2021).

In the TATP POCR<sup>™</sup> example, TATP was selected as a target because it is a hazardous and highly sensitive primary improvised explosive that is difficult for canine teams to access regularly. It is, however, an increasing threat to the populace and military personnel alike. Since the 1990s TATP has become an increasingly popular explosive used by terrorists and criminals. Both internationally and domestically, it has changed from a relatively obscure novelty explosive to a common primary explosive filler for improvised detonators. It has even been used as a main charge in several bombings, such as the Paris (2015), Brussels (2016), and Manchester (2017) attacks. Because of the increased use of TATP as a weapon, it is provided to teams in controlled settings by bomb technicians or other such trained personnel to allow controlled access for canine training. Due to this reliance on specialized personnel to provide access, opportunities to train with TATP are constrained and infrequent. Further, the heat and impact sensitivity of TATP often prevent it from being used in training scenarios that provide canines with realistic operational experience. Restrictions on training environments can lead to context-specificity which can reduce detection of targets in different, realistic settings (Porritt et al., 2015). Thus, any number of local, state, federal, or private explosives detection canine teams would have use for a non-hazardous TATP training aid that can be used in various operational settings. Finally, testing has shown that canines trained on pure, laboratorygrade TATP are able to locate clandestine versions at a rate above 90% (Lazarowski et al., 2021). Thus, there was evidence that using pure TATP vapor as the target would allow for success in the field.

#### 2.2 Step 2: initial analytical chemistry

Initial development of a TATP POCR<sup>™</sup> was performed by the National Institute of Standards and Technology (NIST). Tests were performed to evaluate the release of TATP vapor from various substrates before the polymer polydimethylsiloxane (PDMS) was selected as the most efficient for providing a steady release of vapor pressure over time (MacCrehan et al., 2012).

Once PDMS was chosen as the matrix, initial chemical analyses were designed to identify certain properties of the PDMS that were deemed essential prior to presenting the training aid to canines. First, the amount of TATP absorbed into the polymer was determined using an extraction method described in detail in Simon et al., 2022a. It was determined that this initial iteration of the training aid contained 0.10% TATP by weight.

Second, quantitative and qualitative headspace analysis was performed using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) (MacCrehan et al., 2012; Simon et al., 2021a). This analysis provided two important pieces of information: 1) the headspace profile of the training aid was qualitatively similar to the true material, meaning that both odor sources contained TATP with no notable contaminants and 2) the blank POCRs<sup>TM</sup> (i.e., a training aid with no TATP odor) contained minimal background odors.

Finally, it was determined that no physical or toxicological hazard existed in the TATP POCR<sup>TM</sup>. This was done by consulting a Subject Matter Expert (SME) in explosives at the Federal Bureau of Investigation, who determined that the amount of TATP vapor absorbed by the polymer was not hazardous. Similarly, a safe method of presenting true TATP was designed by the SME for future canine testing in comparison studies (Steps 3-6 below). This method included using a metal cage over a single-compartment concrete cinder block on top of a wooden rolling cart. The TATP could then be placed safely in this configuration during testing, so that the canines could not access the explosive and it could be moved for testing randomization (see Simon et al., 2021a or Lazarowski et al., 2021 for further details).

#### 2.3 Step 3: initial canine testing

Canine validation of a novel training aid should occur after the chemical studies have ensured that the headspace of the training aid is analytically the same as the true material and that the blank training aid has no significant odor profile. The experimental design used should be dictated by the intended use of the training aid and end-user requirements.

Canine testing should begin with an internal, standardized examination of the effectiveness of the training aid using a controlled population of dogs in order to minimize potentially extraneous variables such as breed, age, and training history.

To determine whether a training aid can be effectively used to initially train dogs to recognize an odor leading to detection of the true material in the field without explicit prior training, dogs should first be trained with the training aid until meeting a pre-defined performance criterion and determine a baseline level of performance. The predefined performance criterion may differ based on the target, material, or end-user requirements. Such steps are critical to ensure the dog is prepared to advance to testing to eliminate potential training issues that may interfere with interpretation of results. Once proficiency with the training aid is demonstrated, subsequent testing should examine a dog's detection of the true material.

Methods used for evaluating canines' generalization to the true material should include a standardized, controlled odor discrimination test to determine dogs' ability to detect the true material and discriminate it from relevant distractors. Odor discrimination testing typically occurs in a line-up or circular configuration in which the target odor is placed in a randomly selected position, with the remaining positions containing nontarget odors (i.e., distractors), and allows for straightforward quantification of sensitivity (correct detection of the target odor) and specificity (discrimination against non-target odors). This method is advantageous as it can be performed in a setting controlling for environmental variables while ensuring discrete exposures to target and non-target odors necessary for calculating accuracy metrics. Distractors are critical to ensure that the dog is responding to target odor and not another cue (e.g., odor of the container or packaging material) or rule (e.g., responding to novelty) For validation of a training aid, it is essential to include as a distractor the container or vehicle used to deliver the odor to ensure that the dog has not learned to respond to the background odor rather than the target odor (ideally incorporated

earlier in training). For example, canines were trained to detect the bovine viral diarrhea virus (BVDV) using the POCR<sup>TM</sup> technology charged with BVDV (Singletary et al., 2022) and distractors which included blank POCR<sup>TM</sup> (i.e., PDMS with no viral culture) to ensure that the dogs were responding to the viral odor and not the background odor of the PDMS. In addition, any generalization test should include novel distractors to firmly conclude that any response to the tested target (i.e., the true material) is not merely a response to novelty. Detailed discussion of such factors that impact canine detection research is included in Lazarowski et al. (2020).

In addition to controlled odor discrimination testing, operational testing of dogs' ability to locate and alert to a target odor in a real-world environment can provide a valuable evaluation of the training aid's effectiveness and provide a more realistic assessment compared to the artificial nature of odor discrimination testing. For example, for training aids intended to facilitate generalization to detection of true material in an operational environment after never having experienced the true material in training (i.e., in the case of hazardous or restricted agents not available in training), operational testing is a key determinant of the value of the training aid. The environment used for such testing should match that of the operational environment where the canine will work and where the true material may be encountered. However, drawbacks of operational search testing such as inability to control environmental factors or exposures to targets and non-targets may limit the robustness of the results for the purpose of validation (see Lazarowski et al., 2020). Advantages and disadvantages of both methods should be considered in relation to the intended purpose of the training aid and when possible, used as complimentary assessments to provide a well-rounded evaluation of the training aid.

Initial canine testing of the TATP POCR<sup>TM</sup> was previously reported in Simon et al., 2022a, showing positive alert rates to raw TATP above 90% for dogs trained only on the TATP POCR<sup>TM</sup>. At this stage of testing, refinement of the training aid has not yet occurred, and further troubleshooting may be needed before a final product suitable for the end user is developed. Initial canine testing may therefore include further intermediate steps prior to the final validation. In the current example, blank PDMS POCRs<sup>TM</sup> accounted for two-thirds of the false alerts. However, trainers considered that the results were adequate to proceed to refinement of the training aid, upon which the false alert rates could be addressed. Results of this step indicate that canines can generalize to the target odor, but future refinement is necessary to optimize the odor delivery system.

#### 2.4 Step 4: refinement

Following initial internal canine and analytical testing demonstrating perceptual and chemical similarity between the training aid and the true material (Steps 2 and 3), additional chemical analyses or development of the training aid will likely be necessary. This step resembles troubleshooting and may be more informal in data collection. Several different arrangements of the training aid are tested to determine the format of odor delivery that will be most efficient. Refinement may therefore be considered method optimization, where any variable change and the effect is recorded but the design and execution are less formal than initial testing and later validations.

#### 2.4.1 Alpha testing

Any change made to the training aid itself or the delivery arrangement requires additional canine testing to be conducted. This process may go through several iterations until the canines achieve a proficiency of detection of the training aid using predetermined criteria. However, it is important to minimize the number of exposures to the test material in order to conclude dogs' ability to spontaneously generalize from the training aid to the true material and rule out learning due to experience. This can be achieved by training dogs on an intermittent reinforcement schedule and not reinforcing responses to the probed targets or restricting analysis to the first few exposures.

The following example is provided to highlight how alpha testing may occur. Due to the design of troubleshooting trials, however, such results are not typically published. Following initial canine testing, manipulations of the TATP POCR<sup>™</sup> were done to observe canine reactions to variable levels of odor concentration. In these exploratory tests, canines were able to locate the TATP POCRs<sup>™</sup>, but it was presumed that the POCRs<sup>™</sup> released odor at a lower concentration than the true material (approximately 1/25th the level of trained odor), as the dogs were more successful (80% alert rate compared to 43% alert rate) after learning the new POCR<sup>™</sup> with higher odor concentration (unpublished data). Alert rates were above the pre-defined performance criterion, so the study progressed to Step 5. Therefore, the concentration of TATP in the POCRs<sup>™</sup> was subsequently increased from 0.10% mass fraction of the final TATP POCR<sup>™</sup> arrangement, 0.54% mass fraction (Simon et al., 2022a).

#### 2.4.2 Chemical data collection

Chemical analysis during the refinement step is first used in alignment with the canine alpha testing, as in the previous example where TATP concentration in the POCR<sup>TM</sup> was increased. Chemical refinement further explores the training aid once a final arrangement is determined using the above process. This involves characterizing the training aid using quantitative and qualitative techniques to provide information for the end users. The aspects of this characterization may be different for each training aid target depending on their operational use. For the TATP POCR<sup>TM</sup>, it was important to show reproducibility, to measure dissipation and diffusion, and to determine how the matrix may affect TATP.

Quantitative comparisons of the TATP POCR<sup>™</sup> headspace *versus* TATP were performed using both SPME-GC-MS and a whole air injection method called a cooled injection system (CIS)-GC-MS. These analyses showed that the amounts of TATP released from both sources was comparable, even across different batches of the TATP POCRs<sup>™</sup> (Simon et al., 2022a). Other analyses performed using direct analysis in real time-mass spectrometry (DART-MS) determined the reproducibility between TATP POCRs<sup>™</sup> (Simon et al., 2021a).

Another important characterization for the TATP POCR<sup>TM</sup> was to determine the dissipation and diffusion rates of TATP from the TATP POCR<sup>TM</sup> compared to the true material. First, the dissipation rate of TATP from the POCR<sup>TM</sup> was determined using gravimetric analysis (Simon et al., 2021a; Simon et al., 2021b).

Next, thermogravimetric analysis (TGA) was used to explore how TATP volatilized from each odor source. In this experiment, the

effect of the polymer on TATP vaporization was studied, showing that while the enthalpy of volatilization and vapor pressure were altered for TATP from the POCR<sup>TM</sup>, these effects allowed for similar headspace concentrations to be produced (Simon et al., 2021b). These experiments were pivotal in showing how the matrix of the training aid influences the volatilization of the target odor, a variable that is not often considered in the creation of non-hazardous training aids.

The TATP POCR<sup>™</sup> and TATP vapor diffusion was studied using direct analysis in real time-mass spectrometry (DART-MS). These experiments allowed for the comparison of TATP vapor behavior given several different parameters that may be encountered operationally. Primarily, it was shown that TATP from the POCR<sup>™</sup> has a diffusion rate of 7.78 m/min up to 0.5 m (Simon et al., 2022a). It further compared the behavior of TATP from the POCR<sup>™</sup> and the true material given the following variables: distance from the detector, heights of the odor source, concealment, and purposeful air disturbances (Simon et al., 2021a).

The final analytical step prior to further canine testing was to determine proper containment systems for maintaining training aid integrity (Simon et al., 2021a). For security reasons, the final configuration of the containment system will not be disclosed.

It is important to note that canine sensitivity to many targets is better than chemical instrumentation and that the selectivity of canine sensors may differ from that of instrumentation. It is therefore important to consider the advantages and limitations of each method applied. A variety of methods may be used to verify findings, as in the TATP POCR<sup>™</sup> example. Further, chemical results should be considered in tandem with canine trials, especially during the refinement period. Together, the information produced in Step 4 provide a training aid tested by chemical and canine analyses to verify that the odor of the true material and the training aid are perceived indistinguishably. It will also provide a guideline for basic use and storage based on data.

# 2.5 Step 5: external canine cohort testing and chemical monitoring

#### 2.5.1 Initial external validation

External testing should be conducted with operational detection canines representative of the intended end-user to determine the generalizability of the results and provide ecological validity. Because operationally deployed canines may have experience with true material, testing can utilize a cross-over design in which dogs are tested either for generalization from the true material to the training aid or *vice versa*, depending on their prior experience. Inclusion of dogs with varying levels of experience (i.e., "green dogs" at the start of detection training and certified detection dogs) can be important for determining the effectiveness of the training aid across a spectrum of canine experience. Canines should meet a predetermined criteria with their testing odor to suggest the canines and aid's efficacy prior to moving forward with beta testing (see Simon et al., 2022a for TATP POCR<sup>TM</sup> results).

#### 2.5.2 Internal beta testing

Once the training aid is sufficiently characterized to provide a measure of quality assurance and information regarding odor

quality, production, and movement, training aids should next undergo internal beta testing. The purpose of this step is to allow the end user the opportunity to utilize the training aid in real-world training environments and provide feedback on its utility and potential issues. Initial external validation is similar to internal beta testing in that they both use operational detection canines rather than controlled internal cohorts. However, the key difference is that in initial external validation, researchers design and perform tests. Beta testing is objective with minimal researcher influence in testing or training protocols. This step should also be used for chemical monitoring to ensure that operational use does not negatively impact the function of the training aid. For example, the level of odor should maintain consistency with the expected levels and contamination/cross-contamination should be minimal.

In the beta testing for the TATP POCR<sup>TM</sup>, kits were distributed to canine teams within the sponsoring organization for use in training and the kits were replaced with fresh materials every 3 months. Kits included the TATP POCRs<sup>TM</sup>, blank POCRs<sup>TM</sup>, gloves, alcohol swipes, an anemometer, and extra storage containers. These teams were provided with an informational video to answer basic questions about the POCR<sup>TM</sup> and suggest best practices regarding its use, but their training frequency and specific methods were not dictated by the research and development team. Teams were asked to provide training records for analysis (see Supplementary Material for an example data collection sheet).

Two different groups of canine teams were included in the internal beta tests. All canines were experienced in detecting TATP, but training methods differed across teams. For example, some handlers worked their dogs off leash, while some solely on-leash. Despite the many differences, the overall results were as follows: 88% positive alert rate with three false alerts to the blank POCR<sup>™</sup>. Most of the false alerts occurred during an isolated training session on a single POCR<sup>™</sup> kit. Because of this, the kit was returned to the laboratory and tested for contamination. While some visual contamination was observed (i.e., dirt), no odor contamination was detected above the TATP POCRs<sup>™</sup> or the blank POCRs<sup>™</sup>. The amount of TATP detected from the TATP POCRs<sup>™</sup> in the kit was consistent with the expected quantity. Further conversations with the handlers determined that the issue was probably due to either airflow in the training space or a training challenge that was later corrected.

#### 2.6 Step 6: program refinement

This final step prior to external testing was important in determining what information should be provided to the end user to make the TATP POCR<sup>TM</sup> as effective and user-friendly as possible, and to ensure training aid kit integrity. Based on handler feedback, adjustments to the kit were made. For example, due to handler preference based on feedback provided in a handler survey, nitrile gloves replaced the polyethylene gloves originally provided. This change was later supported by research of odor permeation through gloves (Gauthier et al., 2022). Lifetime and "set" or "soak" times were extracted from the chemical analysis and provided to users of the TATP POCR<sup>TM</sup>. The efficacy of the packaging techniques in maintaining aid integrity was tested and maximized. Such information can provide end users with guidelines for best practice when utilizing the TATP POCRs<sup>TM</sup>.

The training aids continue to undergo evaluation based on handler feedback and questions. For example, effects of certain environmental conditions (e.g., wind and humidity effects and possible background absorption) are currently being evaluated. The continuous evaluation of the POCRs<sup>TM</sup> allows more information to be provided, which further creates a dialogue between researchers and end users that continues to optimize the product for effective use.

# 2.7 Step 7: external beta testing and chemical monitoring

Following successful internal beta testing and program refinement, the final step of testing should include external beta testing with independent parties and no involvement from the research or development team other than provision of materials and recommendations for use. TATP POCRs<sup>TM</sup> were distributed to other federal entities for canine and chemical testing without the influence of the sponsoring agency. This step can also be described as third-party testing as neither the sponsor nor the research team involved in the prior development and testing were involved in the training or testing for this phase aside from providing the TATP POCR<sup>TM</sup> materials.

Canine testing involved a group of canines naïve to TATP as well as a group with prior TATP experience, and utilized a crossover design to test for generalization between TATP and TATP POCR<sup>™</sup>. Testing was performed using an odor recognition set up and the handlers were blind to the presence and location of targets and distractors. Chemical testing involved headspace analysis using SPME-GC-MS to identify the presence of TATP and PDMS-related compounds, conducted by the organization's chemist who was not affiliated with the sponsor or research team. While the TATP POCR<sup>™</sup> was reportedly successful in these settings and continues to be used by external agencies, the entities cannot be named for security purposes. The authors recognize that the anonymous nature of the third-party testing is a limitation of the study as it prevents complete transparency. However, sufficient data has been provided here and in previous publications to support the claim of success in these environments.

# **3** Conclusion

There is a growing demand in the canine training aid industry for effective non-hazardous training aids to be developed using transparent and systematic methods. The current publication provides the first attempt to document a process for creating such an aid. Using TATP as an example, a seven-step process that incorporates chemical and canine analyses in tandem has been demonstrated. The authors acknowledge that each of these steps may not apply to all target odors. However, the integrity of the general process can and should still be maintained. By providing this information in a transparent manner, the authors hope to set a standard by which canine training aid information may be presented.

### Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

### Author contributions

AS: Conceptualization, Methodology, Investigation, Data curation, Writing, Project administration. LL: Methodology, Investigation, Data curation, Writing. SK: Methodology, Investigation, Data curation, Writing. MS: Methodology, Project administration. Data curation, Writing. CA: Methodology, Project administration. PW: Methodology, Project administration. KV: Methodology, Project administration. JB: Methodology, Project administration. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

### Funding

This research was funded by contracts with the FBI Chemical and Biological Advanced Detection Technologies program within the Emerging Threats and Technologies Unit of the Weapons of Mass Destruction Directorate.

# Acknowledgments

The authors would like to thank all the canine teams who participated in the testing and provided feedback during beta tests.

# Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

# Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

# Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frans.2023.1208709/ full#supplementary-material

# References

Consensus Analytical Methods for K9 Training Aid Verification (2021). *Consensus analytical methods for K9 training aid verification*. Lexington: hosted by Massachusetts Institute of Technology Lincoln Laboratory.

Gauthier, Q. T., Riley, P., and Simon, A. G. (2022). Permeation of human scent through laboratory examination gloves. J. Forensic Sci. 67, 2308–2320. doi:10.1111/1556-4029.15123

Lazarowski, L., Krichbaum, S., DeGreeff, L. E., Simon, A., Singletary, M., Angle, C., et al. (2020). Methodological considerations in canine olfactory detection research. *Front. Veterinary Sci.* 7 (7), 408–417. doi:10.3389/fvets.2020.00408

Lazarowski, L., Simon, A., Krichbaum, S., Angle, C., Singletary, M., Waggoner, P., et al. (2021). Generalization across acetone peroxide homemade explosives by detection dogs. *Front. Anal. Sci.* 1. doi:10.3389/frans.2021.797520

MacCrehan, W., Moore, S., and Schantz, M. (2012). Reproducible vapor-time profiles using solid phase microextraction with an externally sampled internal standard. *J. Chromatogr. A* 1244 (1244), 28–36. doi:10.1016/j.chroma.2012.04.068

OSAC Research Needs Assessment Form (2021). Development of reliable surrogate aids. Available at: https://www.nist.gov/system/files/documents/2021/03/12/Dogs% 20%26%20Sensors%20SC\_R%26D%20Need\_Development%20of%20Surrogate% 20Aids.pdf (Accessed March 10, 2022).

Porritt, F., Shapiro, M., Waggoner, P., Mitchell, E., Thomson, T., Nicklin, S., et al. (2015). Performance decline by search dogs in repetitive tasks, and mitigation strategies. *Appl. Animal Behav. Sci.* 166 (166), 112–122. doi:10.1016/j.applanim.2015.02.013

Simon, A. (2022b). Analysis of non-hazardous canine training aids for triacetone triperoxide (TATP). Forensic Chem. 30 (30), 100440. doi:10.1016/j.forc.2022.100440

Simon, A., Lazarowski, L., Barrow, J., Van Arsdale, K., Singletary, M., Angle, C., et al. (2022a). Validation of a polymer odor capture-and-release (POCR) aid for training canines to detect triacetone triperoxide (TATP). *Forensic Chem.* 31, 100454. doi:10. 1016/j.forc.2022.100454

Simon, A., Van Arsdale, K., Barrow, J., and Wagner, J. (2021a). Real-time monitoring of TATP released from PDMS-based canine training aids versus bulk TATP using DART-MS. *Forensic Chem.* 23 (23), 100315–15. doi:10.1016/j.forc.2021.100315

Simon, A., Van Arsdale, K., Wagner, J., and Barrow, J. (2021b). Effects of polydimethylsiloxane (PDMS) on triacetone triperoxide (TATP) volatilization. *Forensic Chem.* (28), 1–8.

Singletary, M., Krichbaum, S., Passler, T., Lazarowski, L., Fischer, T., Silvis, S., et al. (2022). A novel method for training the interdiction of restricted and hazardous biological materials by detection dogs. *Front. Med.* 9 (9), 847620. doi:10.3389/fmed. 2022.847620