Effectiveness of Canine Odor Detection Containment Aids in Resisting Contamination from a Storage Environment

by

Rhiannon LaFortune

A Manuscript
Submitted to the Faculty
in fulfillment to the requirement for Experimental Research Capstone
Degree of Laboratory Science Technology- Biology Emphasis

Northeast Wisconsin Technical College
May 2022

Abstract

In the field of odor detection, many tools exist to store target materials and deliver scent, but with such an abundance of choice comes a bombardment of misconceptions as to which option is best. Subjectivity fuels fallacies in the market, but as objectivity and research are beginning to enter the field, better practices for handling and storing training tools may become commonplace. Contamination of target odors remains a threat to the success of detection canines. In comparing two commonly used, commercially available containers, this study addresses the ingress of contaminants into the widely used mason jar compared to the Training Aid Delivery Device (TADD). This study aimed to determine if the TADD would outperform the mason jars in preserving the target material within and investigate the influence of time. Contaminant levels were analyzed at three different time points to establish a relationship between time and quantity, but discovered levels remained too low to be conclusive. Regardless, the findings of this study suggest that the mason jar did not effectively resist contamination stemming from neighboring explosive compounds during storage. At the same time, the TADD showed an ability to withstand the pollution of the materials stored within. Environmentally sourced contamination consistent with explosives leached into the mason jars after only four weeks in a storage magazine that housed a range of explosives detection training materials. The results suggest that rudimentary devices, such as mason jars, may be an inferior method of containing training materials and could result in signature odors being tainted.

Introduction

Dogs' noses, once exploited only for hunting, herding, and protection, are now utilized as biosensors for a vast range of applications (13). With high trainability and great sensitivity, canines' role in scent detection have diversified greatly since the early days of dog and men (4,7,8,9,12). Canine olfaction is now utilized in an extensive number of fields from conservation to healthcare, humanitarian aid, security, military, and even in sport. The most recognizable role of the modern working dog is unquestionably in law enforcement (2). From cadaver dogs sniffing out remains to apprehension K9s trailing those evading arrest, canines have extensive roles in working alongside police officers. They may assist in search and rescue, locating evidence at crime scenes, or alerting for the presence of narcotics as well as explosives (2,7,9). As the work of police canines becomes more visible to the public, the dogs are often viewed as a positive and proactive tool. Members of the public train their dogs to emulate the tasks police dogs perform in the line of duty (2,8,11,12).

Odor detection training begins with imprinting a specific odor on the dog through various operant and classical conditioning techniques (8,12). The desired odor is introduced and paired with positive reinforcement, incentivizing the canine to seek it out. The canine quickly learns that a reward comes when the target odor is successfully found. Through repetition and reiteration, numerous odor profiles can be easily introduced and mastered (8,12). In operational settings, volatile organic compounds (VOCs) emanate from hidden scent sources, flowing and filling the area to be searched (5,6). This target odor is then sought out and localized by a dog who indicates the presence of the odor to the handler (8,12).

As interest and applications of detection work using canines grows, the variation in training methodology expands as well (8,11,12). With this expansion, the lack of standardization leads to inconsistencies in handling and storage protocols, which potentially taints training aids and increases alerting error (3,6). Odor detection presents many challenges, and it is vital to understand the influence odor profile characteristics have on the detection dog's success (5,6) and how contamination can alter the goal.

Improper storage of training supplies can create a blending of odors via off-gassing (5,6). Off-gassing is the release of volatile organic compounds (VOCs) that are absorbed in a material. This is desired during searches and training but not ideal during storage (6). The inability to stop VOCs from releasing and mixing with those of other training materials may corrupt all training aids' odor profiles (13) present in storage potentially creating novel odors. To limit blending, industry-recommended best practices suggest a system of using primary,

secondary, and tertiary containment methods (11). This storage method minimizes headspace volume and prevents the permeation of contamination into the training aids, keeping odors ultimately to themselves (5,6,11).

Canines have an exceptional ability to process unique odor signatures and distinguish target odors from background smells, but this ability is tainted when training aids used during maintenance training are polluted (5,6,8,11,12). Often the contaminant scents have a much higher particle count and may be a more accessible odor profile for the dog to detect. Though canines' olfactory threshold has been estimated as being within the parts-per-billion to parts-per-trillion range for various chemical odors (1,4), if introduced to an overpowering odor in a high concentration, the dogs can misunderstand the intended odor profile (11). This becomes an issue with explosive odor detection because many of the target compounds have low volatility and the target odor can be easily overshadowed. If the target odor is compromised, unknowingly to the handler, the dog is now being trained and maintained and will consequently alert on an entirely different odor than the goal. Proper handling practices and storage will minimize this mixing of smells.

The consequences resulting from the presence of contaminants should be researched and addressed, but first experimentation should be done to discover which tools minimize or eliminate the polluting of odor profiles where the scent stems, within the training aids. A comparison of two commercially available containers will be performed and analyzed for the presence of contaminates stemming from storage to bring awareness to the importance of using best practices when working with training materials (6,8,12,14). The objective of this experiment is to investigate whether mason jars will show less resistance to the ingress of environmental contaminants stemming from storage compared to the Training Aid Delivery Device (TADD) and whether a positive correlation between time and contaminant level exists.

Materials and Methodology

Training Aid Delivery Device Sample Preparation
Three Training Aid Delivery Devices (TADDs), products of SciK9.com, were removed from their packaging and opened. All containers were washed with isopropyl alcohol, rinsed with deionized water, and dried using a lint-free wipe. Into each container, a single blank 50mm diameter Whatman filter paper was inserted. The gasket was set in place at the rim of the glass jar and secured by screwing on the membrane holder and lid. A tamper-proof sticker with an identification number was affixed where the glass and plastic membrane holder met.

Mason Jar Sample Preparation
Three mason jars were washed with isopropyl alcohol, rinsed with deionized water, and dried with a lint-free wipe. A single 50mm diameter Whatman filter paper was inserted into each of the containers. The snap lid was centered on the jar, and the screw band was applied and secured firmly. A tamper-proof sticker with identification number was secured at the transition point from the glass lip and metal lid.

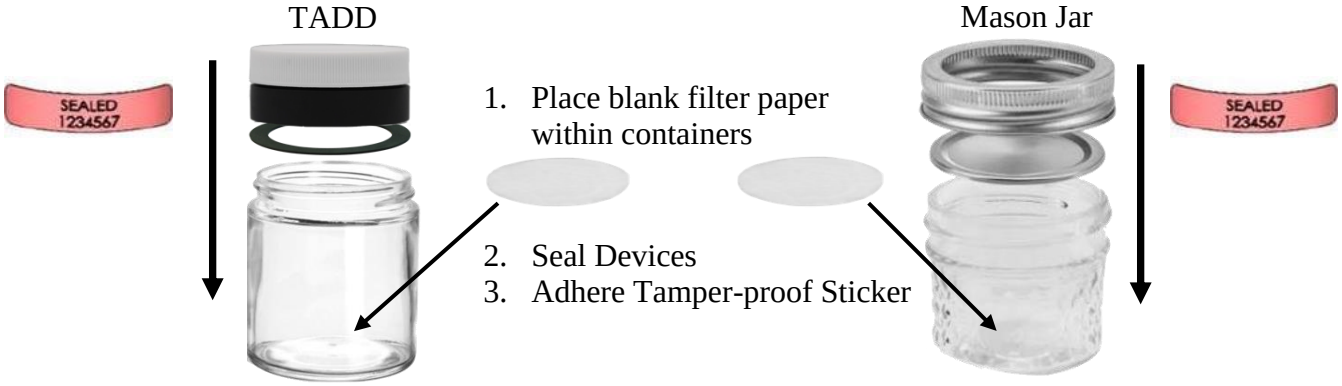

Figure 1. Visualization of container assembly, TADD (left) and Mason Jar (right).

Preparation of Containers for Delivery to Storage Magazine

Individually, each container was placed into an odor-resistant metalized envelope. The envelope was sealed by interlocking the mechanism along the edge. This was then inserted into a second metalized envelope and sealed. A strip of tape was placed across the lip of the envelope to prevent opening further. This tape was signed and dated by the researcher.

Transit of Containers to Storage Magazine

The envelopes securing the containers were placed in a backpack and driven by the researcher to the Appleton Police Department. The supplies were carried into the office of the EOD officer and removed from the pack. Individually, the tape was cut, and envelopes opened to reveal the containers. All containers were placed into the storage magazine by the officer and left to cohabitate with active training aids and tools held within. Containers were left to mingle for four weeks before the initial collection time.

Preliminary Investigation

Shimadzu GC-2010 Plus Model Gas Chromatograph Limit of Detection

Into a 10.00mL graduated cylinder, 1.00mL of limonene was pipetted and diluted with 9.00mL of acetone. This solution was mixed by pulling a portion of the sample into a pipette and forcefully shooting it back out until the solution was believed to be homogeneous. Approximately 1mL of this solution was pipetted into a gas chromatography (GC) vial. The vial was labeled, and all but 5.00mL of the solution was discarded from the graduated cylinder. An additional 5.00mL of acetone was added to the cylinder and again the solution was thoroughly mixed with a portion then pipetted into a GC vial. This dilution procedure was repeated for a total of sixteen trials with the intention of reducing the limonene concentration to a great extent.

Investigation of Negative Control

Three pieces of 50mm diameter Whatman filter paper were cut into quarters and submerged in approximately 5.00mL of acetone. Each remained submerged for five minutes with intermittent agitation before approximately 1mL of each solution was pipetted into labeled GC vials and was run on the gas chromatograph.

Investigation of RDX Odor Print from within TADD

The tamperproof seal was broken on the TADD and the charged Odor Print removed. This Odor Print was cut into quarters. Each quarter was placed into a 25mL beaker and submerged in 5.00mL of acetone for five minutes. Approximately 1.00mL of the solution was pipetted into labeled GC vials once five minutes had elapsed.

First Timepoint

Retrieval of Containers

The first time point was reached four weeks after the initial drop-off date. One TADD and one mason jar were removed from the storage magazine by the officer. Each container was handed over to the researcher and placed into a metalized envelope. This envelope was sealed and then placed into a second metalized envelope. The outer envelope was taped closed, signed by the researcher, and dated. A photograph was taken to document the exchange. These envelopes were then placed into a backpack, where they remained until being returned into the lab the following morning.

Preparation of Samples for Gas Chromatography

The seals were broken on the outer envelopes and the containers were removed from the packaging. The identification numbers found on the tamper-proof seals were recorded and the containers were opened. Four small beakers were cleaned for the dissection of each container. This was done using a series of deionized water and acetone rinses. The filter paper within both the TADD and the mason jar were cut into quarters and placed into individual 25mL beakers. An addition of 5.00mL of acetone was pipetted into all beakers to submerge each quarter of filter paper. To minimize evaporation, a watch glass was placed atop each of the beakers. Once five

minutes had passed, approximately 1mL of solution from each beaker was pipetted into a labeled GC vial. This resulted in eight GC vials total to be run on the gas chromatograph.

Blinding of Samples before Gas Chromatography
The researcher had labeled all vials before adding solution into them. To blind both the researcher and advisor, a code was created. The advisor assigned random numbers to each of the vials without knowing the researcher's labeling system and covered the original label with a piece of colored tape. All vials were randomly placed into the autosampler before GC was run. The placement was recorded for later analysis.

Second Timepoint

Retrieval of Containers
An identical procedure was followed for this time point as was utilized in the first time point, with a collection date three weeks after the previous timepoint, seven weeks from the initial drop off.

Preparation of Samples for Gas Chromatography
An identical procedure was followed for this time point as was utilized in the first time point.

Blinding of Samples before Gas Chromatography
An identical procedure was followed for this time point as was utilized in the first time point.

Final Timepoint

Retrieval of Containers
An identical procedure was followed for this time point as was utilized in the first time point, with a collection date two weeks after the previous timepoint, nine weeks from the initial drop off.

Preparation of Samples for Gas Chromatography
An identical procedure was followed for this time point as was utilized in previous time points.

Blinding of Samples before Gas Chromatography
An identical procedure was followed for this time point as was utilized in previous time points.

All procedures above were performed wearing nitrile gloves. While on the campus of Northeast Wisconsin Technical College, all experiments were conducted within the lab's fume hood to minimize contamination stemming from researchers. A chain of custody was maintained during pickup and transit all contents were sealed within the metalized envelopes with tape, initialed with dates and times of exchange being noted on the packaging. Photos were also taken to document exchanges.

This procedure aimed to minimize any influence the researchers had on the contents within each device with the goal of exploring contamination stemming solely from storage.

Data Analysis Strategy
Once all GC data was collected, graphs were compared. Peaks that were identified in the control were disregarded and all other peaks noted. The retention times of unique peaks were recorded and compared alongside the area below each of the peaks. Corresponding peaks from separate readings were analyzed using a t-test to determine whether a significant difference exists.

Results

Samples contained within the TADDs tested at all time points revealed readings that matched the negative control, indicating the integrity of the sample was preserved. All samples contained within the mason jars were tested and found to have the expected control peaks as well as an additional mystery peak that appears consistently throughout the timepoints.

The gas chromatograph's limit of detection needed to be investigated. After an extensive series of dilutions, a true limit of detection for the instrument was never established, yet the dilutions ceased once the known peak for limonene became difficult to discern from the surrounding baseline peaks, observed in the graphs below.

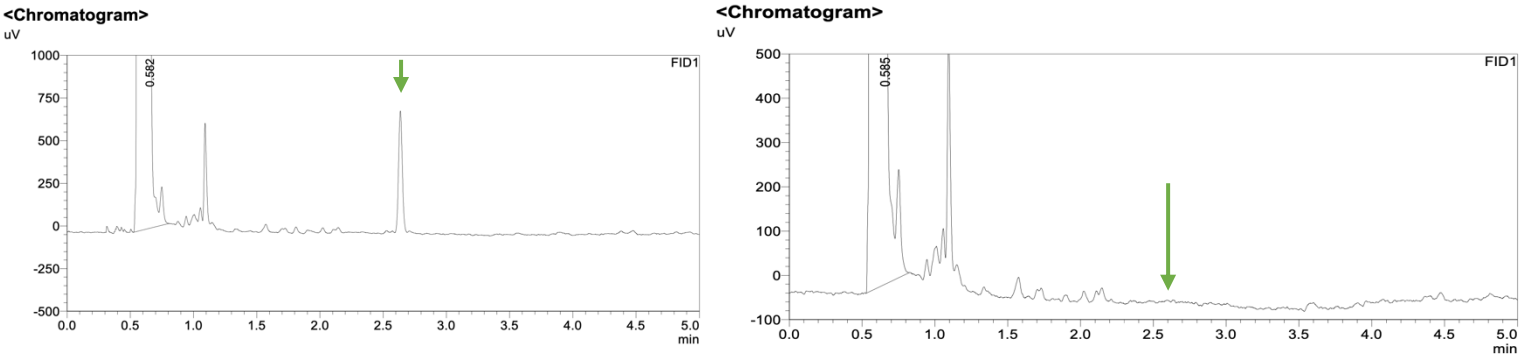

Figure 2. Gas chromatography readings discovering the detection limits of gas chromatography using limonene in acetone.

Note. Easily viewed peak of limonene in acetone at approximately 2.65min for reference (left) and the lowest concentration of limonene in acetone explored (right) with reference arrow indicating where limonene peak presents.

The series of dilutions ended as the limonene peak faded into the baseline peaks. This equated to 0.000153% by volume. It was determined that the gas chromatograph would meet the needs of the study. It is a highly sensitive machine while being a reliable tool for separating volatile compounds.

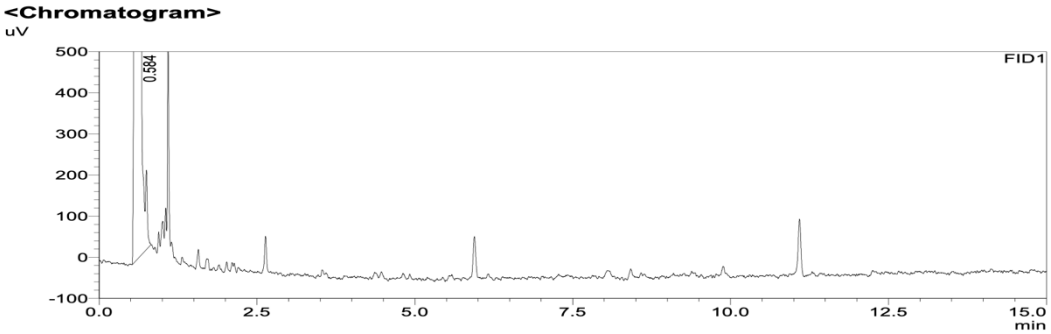

Figure 3. Gas chromatography reading of acetone.

Note. The above graph is representative of all the gas chromatography readings taken using blank filter paper and acetone. A total of twelve readings were run to increase reliability.

Filter paper soaked in acetone served as a negative control in this experiment. Readings were done to establish which peaks would stem from the solvent or fresh filter paper for later elimination from gas chromatograph readings. Significant peaks are visualized between 0.5min and 2.5min, while lesser, more distinctive peaks were observed at the retention times of approximately 5.9min and 11.2min.

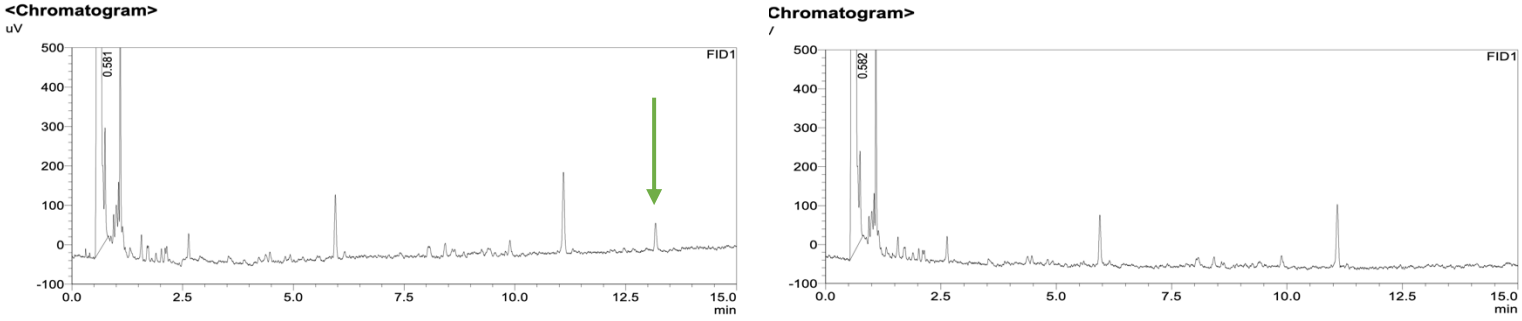

Figure 4. Gas chromatography readings from sample set pulled at the first timepoint, mason jar (left) and TADD (right).

Note. Disregarding the peaks observed from the control, a peak of interest appears at approximately 13.2min in the sample stored within the mason jar. No peaks observed in sample held within the TADD.

The above are representative graphs for both sample sets. A total of four readings were performed on individual samples resulting in eight total readings. These findings suggest there is an extra substance found within the mason jar that was not within the TADD.

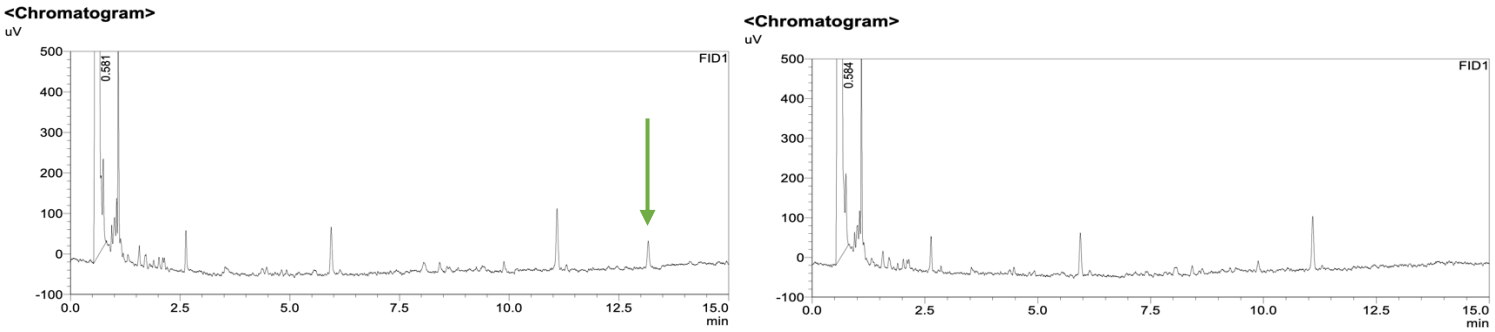

Figure 5. Gas chromatography readings from sample set pulled at the second timepoint, mason jar (left) and TADD (right).

Note. A peak is again observed at approximately 13.2min from the sample within the mason jar. No unexpected peaks are observed in the sample stored within the TADD.

The above graphs are representative for both sample sets pulled at the second timepoint. A total of four readings were performed on individual samples resulting in a total of eight readings.

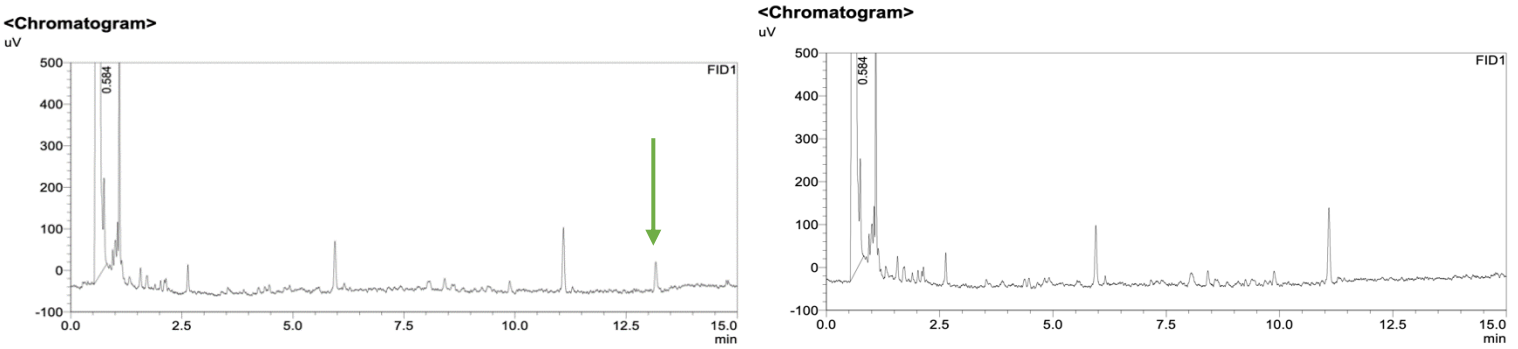

Figure 6. Gas chromatography readings from sample set pulled at the third timepoint, mason jar (left) and TADD (right).

Note. A peak is observed at approximately 13.2min again only in the samples stored within the mason jar. The TADD reveals only the peaks seen in the control sample.

The graphs previous are representative of both sample sets pulled at the final time point. A total of four readings were performed on individual samples, resulting in eight charts total.

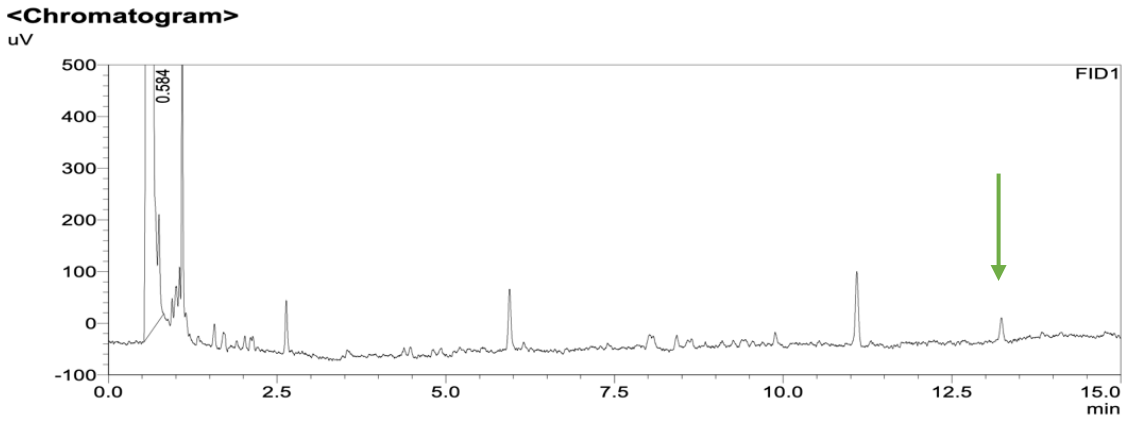

Figure 7. Gas chromatography reading of an RDX-charged Odor Print in acetone.

Note. Excluding the known peaks from our negative control, a single peak at approximately 13.2min was observed stemming from the RDX charged Odor Print.

This sample, like all others, was run in quadruplicate for validity. The above graph is representative of all the readings taken from the RDX Odor Print. RDX is a nitramine explosive compound that is often utilized as a propellant, gunpowder or high explosive depending on the initiation type. It is often a target material used in training and is a compound found in other explosives, such as composition C4 and detonating cord.

A comparison of the results reveals peaks at identical retention times witnessed in all sample sets stored within the mason jars and the RDX Odor Print. This suggests an ingress of explosive odor contamination from the storage environment into the mason jars, tainting the blank filter within.

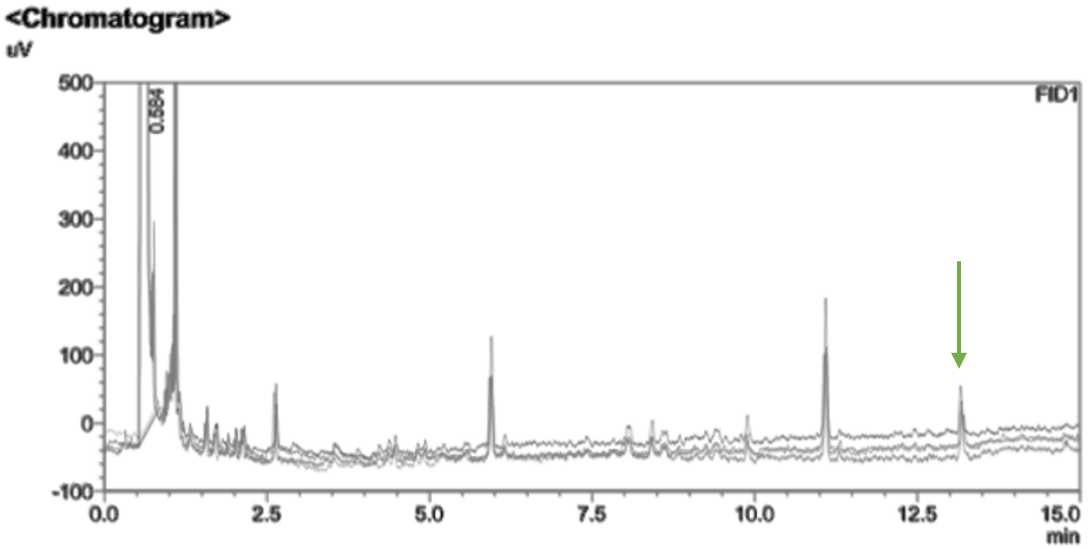

Figure 8. Overlay of Gas Chromatograph readings of RDX sample and representative graphs of samples stored within mason jars from all time points.

Conclusions

The findings of this experiment align with the hypothesis that the TADDs would outperform the mason jars in their ability to resist environmental contamination. The TADD maintained the integrity of the blank filter paper more effectively than the mason jars. Unfortunately, the relationship between time and the level of contaminants could not be analyzed. Due to the contaminant levels being so low, the area below the peaks of interest could not be calculated and therefore not integrated, and this led to the factor of time being disregarded.

For this study, the amount of contaminant within the sample set pales in comparison to the significance of its presence. The fact that any substance penetrated a sealed vessel is momentous. The unexpected discovery of matching peaks from our samples and the known RDX Odor Print is notable. The identical peaks suggest that contaminants are penetrating the mason jars even in *unrealistically* ideal storage conditions. After only four weeks in the storage magazine, the containers remained unmoved and unopened, untouched, likely contamination from the environment managed to ingress.

With the data collected, it is possible to theorize a contaminant originated from within the mason jars. Yet, more likely, the matching peaks of the sample and RDX suggests the source stems from the environment. There are numerous open bricks of composition C4, coils of detonation cord, and other active training aids present within the storage container that could be the source. One cannot say with absolute certainty what the compound was that had been observed in the sample sets but identifying trace contaminants could be explored in the future.

Strong evidence was discovered that contaminants are capable of rapidly penetrating mason jars in ideal conditions, but more research needs to be done. Introducing more controls into the experiment to isolate when contaminants are infiltrating and where from would be beneficial. If this research were to be continued, the parameters of the storage protocols should be loosened as well. This study could be furthered by observing how both containers perform under grittier, more realistic conditions. Devices should undergo handling that matches training aids used in practical application, broadening the scope of the research to contamination pathways stemming from both use and storage for an extended period. If the duration of the study was extended, contamination levels may be measured and the influence of time on contaminant levels better explored.

This study faced many limitations. The sample size was incredibly small, with the use of only six containers for the duration of the experiment, which reduced the study's overall power. The limited time frame and unrealistic conditions contribute as well to questionable validity. Addressing that, in real-world applications training aids and storage containers are exposed to a vast array of contamination pathways that would intensify the probability of tainted target odors. Observing such pollution within the limited conditions of the study is eye-opening.

The findings suggest a harsh reality that very likely the target materials stored inappropriately within basic devices, such as mason jars, are not remaining pure. With further study, the conclusions of this experiment could be strengthened, bringing greater awareness to contamination in the odor detection community, shedding light on better tools and methodology that resist the detriment of our training aids and increase the success rates of our canines.

References

1. Aviles-Rosa, Edgar O, et al. “Case Study: An Evaluation of Detection Dog Generalization to a Large Quantity of an Unknown Explosive in the Field.” *Animals: an Open Access Journal from MDPI*, U.S. National Library of Medicine, 8 May 2021, https://pubmed.ncbi.nlm.nih.gov/34066869/.

2. Chapman, Samuel G. *Police Dogs in North America*. C.C. Thomas, 1990.

3. Concha, Astrid, et al. “Using Sniffing Behavior to Differentiate True Negative from False Negative Responses in Trained Scent-Detection Dogs.” *Chemical Senses*, Oxford University Press, 11 Sept. 2014, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4201303/.

4. Concha, Astrid R., et al. “Canine Olfactory Thresholds to Amyl Acetate in a Biomedical Detection Scenario.” *Frontiers*, Frontiers, 1 Jan. 1AD, https://www.frontiersin.org/articles/10.3389/fvets.2018.00345/full.

5. DeGreeff, Lauryn. “The Chemistry of Odor and Odor Detection.” *Iwdba.org*, U.S. Naval Research Laboratory, https://www.iwdba.org/wp-content/uploads/2021/05/17_iwdc_odor-2.pdf.

6. DeGreeff, Lauryn. “The Chemistry of Odor: How Understanding Odor Can Foster a Better Detector.” *Iwdba.org*, U.S. Naval Research Laboratory, https://www.iwdba.org/wp-content/uploads/2019/12/l-degreff_presentation.pdf.

7. Farr, Brian D, et al. “Expert Perspectives on the Performance of Explosive Detection Canines: Operational Requirements.” *Animals: an Open Access Journal from MDPI*, MDPI, 1 July 2021, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8300389/.

8. Gerritsen, Resi, and Ruud Haak. *K9 Explosive and Mine Detection: A Manual for Training and Operations*. Dog Training Press, 2017.

9. Greatbatch, Ian, et al. “Quantifying Search Dog Effectiveness in a Terrestrial ...” *Wilderness & Environmental Medicine*, May 2005, https://www.researchgate.net/publication/277080838_Quantifying_Search_Dog_Effectiveness_in_a_Terrestrial_Search_and_Rescue_Environment.

10. Guest, Claire, et al. “Feasibility of Integrating Canine Olfaction with Chemical and Microbial Profiling of Urine to Detect Lethal Prostate Cancer.” *PLOS ONE*, Public Library of Science, 17 Feb. 2021, https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0245530.

11.“K9s Talking Scents.” *011 Dr. Michele Maughan and Jenna Gadberry.... Keep That Odor to Itself*, 29 Aug. 2019, https://talkingscents.libsyn.com/dr-michele-maughan-and-jenna-gadberry-keep-that-odor-to-itself.

12. Mistafa, Ron. *K9 Explosive Detection A Manual for Trainers*. Detselig Enterprises Ltd., 1998.

13. Morey, Darcy F. *Dogs Domestication and the Development of a Social Bond*. Cambridge University Press, 2010.

14. Simon, Alison, et al. “A Review of the Types of Training Aids Used for Canine Detection Training.” *Frontiers in Veterinary Science*, Frontiers Media S.A., 5 June 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7301692/.