A Preliminary Study of the Odorants of Interest in Native Crude Oils to Oil Detection Canines

Stephanie R. Vaughan[a], Vidia Gokool[b], Michelle Karpinsky[b], Paul Bunker[c], Ed Owens[d], Steven G. Tuttle[e], and Lauryn E. DeGreeff[b,f*]

[a] National Research Council Postdoctoral Fellow at the Naval Research Laboratory, Washington, DC, United States
[b] Florida International University, International Forensic Research Institute, Miami, FL, United States
[c] Chiron K9, San Antonio, TX, United States
[d] Owens Coastal Consultants, Bainbridge Island, WA, United States
[e] Naval Research Laboratory, Code 6185, Washington, DC, United States
[f] Formerly of the Naval Research Laboratory, Code 6181, Washington, DC, United States
* ldegreef@fiu.edu

Abstract

Detection technologies for subsurface oil during shoreline spill responses rely heavily on labor-intensive and time-consuming techniques since it is obscured by sediment. Canines have been successful at detecting small deposits of oil over widespread areas of shoreline and can cover a greater area in less time than other techniques with high accuracy. The headspace of crude oil is composed of a complex mixture of volatile organic compounds (VOCs) that yield a unique odor profile allowing for canine detection; however, the volatile components that canines utilize for olfactory detection is unknown. The goal of this research was to determine the components used by oil detection canines for olfactory detection. The odor profiles of several crude oils were characterized by solid phase microextraction (SPME) with gas chromatography and mass spectrometry (GC-MS). To elucidate which VOCs are responsible for detection, canine testing probes were created from fractions of the oil headspace. To do this, fractions of the chromatogram were eluted and collected onto sorbent materials. The sorbent materials containing the oil vapor fractions, as well as positive and negative controls, were used as probes in canine testing exercises. Three fractions from fresh crude oils were presented and the canines gave a positive response rate of 67% to two out of three fractions and 100% response to the remaining fraction. These results indicate that canines are capable of detecting crude oil from any fraction of the odor profile and speak to the canines' ability to generalize across many types of crude oils even when source or weathering may affect the headspace profile of the oil. Future testing of canines will further elucidate the effects of sample source on canine detection and odor profile composition.

1 Introduction

The ongoing collection, transportation, and processing of crude oil throughout the world sustains a risk of environmental contamination by crude oil. In the case of an oil spill, depending on the severity and duration, significant damage to an ecosystem and the economy can occur. To systematically survey the damage to shorelines proximate to the Exxon Valdez Oil Spill in 1989, Shoreline Cleanup and Assessment Technique (SCAT) was conceived. SCAT is a method for surveying an affected shoreline after a spill has occurred (National Oceanic and Atmospheric

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Administration, 2021). “It is a logical, standardized, science-based approach and methodology to oil-on shoreline response decision making” (American Petroleum Institute, 2016).

Since its advent, SCAT techniques have been deployed to many oil spills. The most noteworthy oil spill to occur within the last 20 years was the Deepwater Horizon (DWH) oil spill, where, in 2010, a blowout explosion aboard the oil rig killed 11 people and caused hundreds of millions of gallons of crude oil to pour into the Gulf of Mexico. The silver lining of the horrible accident was the notable breakthrough in the understanding of both the physical and chemical properties of crude oil. BP provided approximately $500 million in funding for independent research on the impacts of oil spills (Ward & Overton, 2020) (Ward, Reddy, & Overton, 2020). SCAT includes both aerial and ground observations to locate and delineate a spill, and incorporates both surface and subsurface level surveys.

During the DWH spill, initial ground surveys of more than 7,000 km of coastline were required, and over a span of three years, more than 31,000 km of shoreline was surveyed. A post-Deepwater Horizon study determined that the detection technology for shoreline spill response relied on SCAT techniques that were labor-intensive, had slow survey speeds, and were able to encompass only limited or partial areal coverage by spot sampling. Within this broader issue, a report on “Current Practice and Prospective Developing Technologies for the Detection and Delineation of Subsurface Oil in Sediment Shorelines” found two key issues with the surveys of the DWH spill. The first issue was much of the effort was put forth in survey areas where there was only a low potential for shoreline oiling, 75% of shorelines had no observed oil. The second issue was the time and effort required to detect and delineate subsurface oil. Over the course of 2 years, more than 180,000 pits, trenches, and auger holes were used to search for delineated buried oil. These difficulties could be obviated by a professional canine oil detection team (K9-SCAT) providing low-risk, high confidence support capacity to survey teams (American Petroleum Institute, 2016) (Owens & Bunker, 2022).

Detection canines are used in a variety of government agencies and industries for their keen olfactory capacity as well as for their reliability, versatility, and speed. Canines have been deployed for the detection and location of drugs, explosives, humans, and human remains. They exhibit high selectivity and sensitivity towards locating odors, with a sense of smell 10,000-100,000 times greater than that of an average human and have been shown to be able to detect volatile organic compounds (VOCs) down to one part per trillion, which is more sensitive than some analytical instruments (Furton, Caraballo, Cerreta, & Holness, 2015) (DeGreeff, Singletary, & Lazarowski, 2022). Recent experiences with canines on oil spill response surveys have shown that K9-SCAT can help to locate surface and subsurface oil much more quickly than traditional methods, clearing large areas in a very short amount of time, and also searching difficult terrain which cannot be easily accessed on human foot (American Petroleum Institute, 2016) (Owens & Bunker, Canine detection teams to support oil spill response surveys, 2022).

In a field study conducted by Owens et al. (2017), trained oil detection canines were deployed following a residual leak of crude oil from a sunken ship. The K9-SCAT team was deployed 9 months after the residual leak and was imprinted on the “fresh” samples harvested from the source. The canine team detected visible and non-visible oil deposits at the surface and subsurface level along the shoreline of Chedabucto Bay, Nova Scotia (Owens et al., 2017). The positive identification of surface and subsurface weathered oil deposits suggest a persistence of volatile organic compounds (VOCs) that are indicative of a petroleum product vapor profile.

Additional proof-of-concept studies have been conducted, such as the K9-SCAT team’s recovery of subsurface oil in North Saskatchewan River and Prince William Sound, Alaska. At

North Saskatchewan River, canines were employed along with traditional SCAT efforts to search for oil along the lower reaches of the shoreline. A total of 718 km was surveyed using four K9-SCAT teams over 202 field days. Out of the 8,689 canine alerts made in that time period, 7,748 were verified. The canines detected subsurface, sub-subsurface, and sub-water/in sediment oil, which would not have been located by a human without the use of strenuous effort or an excess of resources (Owens & Bunker, 2022).

Although canines have successfully been deployed to detect hidden or obscured crude oil, currently, the advances and developments in the field have been based on trial and error using the basics of canine detection strategies learned from other applications (mine detection, unexploded ordnance detection, etc.). This research endeavors to elevate the technology to a science-based approach in which we can apply the knowledge gained on the discrimination capabilities of a canine to many real-world situations and to significantly improve training procedures. The authors utilize headspace analysis using solid-phase microextraction with gas chromatography– mass spectrometry (SPME-GC-MS) to characterize the VOCs from a Gulf Coast and Alaskan crude oil. SPME is a common method for the extraction of VOCs from the headspace of a sample. The SPME fiber consists of a thin, polymer-coated rod which, when not in use, is covered by a stainless-steel sheath for projection. The polymer-coated fiber is placed in the sample headspace for a pre-determined amount of time, after which it is removed and analytes are thermally desorbed in the heated inlet of a GC-MS, where they are separated and identified. Portions of the resulting chromatogram were then collected onto a sorbent media that were used to probe canine detection capabilities and determine which portion, or portions, of the odor profile constitute the odor of interest for canine detection.

2 Methods

2.1 Crude Oil Samples

Hoover Offshore Oil Pipeline System (HOOPS) and Alaskan North Slope (ANS) crude oils were obtained and stored in a flammables cabinet until ready for use. Odor profiles of HOOPS and ANS were generated by placing 10 mL of each crude oil in a 20 mL VOA sampling vial with septa. All samples were prepared in triplicate.

In addition to fresh crude oil, a preliminary study of the effects of weathering on the odor profile of crude oil was carried out. For this purpose, weathering was simulated using a Q-Sun Xe-3 Xenon test chamber (Q-Lab). Triplicate samples of approximately 1 g of fresh HOOPS crude oil was placed in a Pyrex petri dish, which was then irradiated for 4, 12, 24, or 168 hours at an irradiance of 0.68 W/m^2 at 340 nm. This irradiance was selected as it is approximately equivalent to the summer sun in the Southern U.S. at noon. Following irradiation, the petri dish was placed in a Teflon jar containing two septa-covered ports for sampling and analysis, as described below.

2.2 Headspace Analysis

Headspace analysis was carried out using solid phase microextraction (SPME) with gas chromatography– mass spectrometry (GC-MS). SPME is a non-exhaustive extraction method where a polymer-coated SPME fiber was placed into the headspace of a small amount of crude oil contained in a vial with a septum lid. VOCs from the crude oil were adsorbed to the polymer coating for a given amount of time before the fiber was removed. The fiber was then thermally desorbed into a heated injection port in the GC where the analytes are deposited onto the GC column for separation and detection in the MS.

Four types of SPME fibers were considered: Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), CAR/PDMS, PDMS/DVB, and 100 µm PDMS (Millipore Sigma). The 100 µm PDMS fiber yielded the greatest abundance of VOCs from HOOPS crude oil as compared to the other fibers and was thus chosen for analysis. Additional SPME parameters were optimized using aliquots of HOOPS oil, including extraction temperature, sample equilibration time, and SPME extraction time. The optimized parameters are given in Table 1.

Table 1. Optimized equilibration and SPME parameters.

Final SPME Parameters	
Extraction Temperature	35 °C
Equilibration Time	20 min
Extraction Time	10 min
SPME Fiber	100 µm PDMS

Following extraction of the VOCs onto the SPME fiber, the fiber was removed from the vial containing the crude oil sample and the analytes were thermally desorbed in the inlet of the GC (Agilent 7890A) at 260 °C with a 10:1 split ratio and a flow rate of 40 mL/min. The analytes were then separated on a Rtx-Volatiles (Restek Co.) starting with the GC oven at 40 °C, increasing at 8 °C/min to 80 °C, followed by 5 °C/min to 200 °C, and finishing with a 0.5 min hold. The transfer line to the MS (Agilent 5975C) was set to 250 °C with a scan range of *m/z* 30 - 300. The MS used EI ionization at 70 eV.

2.3 Data Handling and Statistical Approach of Headspace Data

To statistically compare the similarity between odor profiles, a chemometric approach was designed for analysis of the complex chromatograms resulting from the ANS and HOOPS crude oils. The intra-sample and inter-sample variations in the triplicate analyses of both crude oil headspaces were quantified using Spearman's rank correlation (Equation 1). Spearman's rank correlation coefficients related the similarity of total ion chromatogram (TIC) features between samples.

Spearman's rank correlation coefficient (ρ), is a value between -1 to +1. Spearman's rank correlation coefficient is calculated using the difference between the ranked values (d) and the number of values being compared (n). The larger the magnitude of ρ, the stronger the correlation, the closer the value is to 0, the weaker the correlation observed.

$$\rho = \frac{6\Sigma d^2}{n(n^2-1)} \quad (1)$$

Additionally, the Spearman's rank correlation of the TIC features required the coordination of aligned peaks across the dataset (i.e., chromatogram data). A proprietary program developed at Florida International University was used to perform the peak alignment procedure across the submitted dataset. The peak matching software was created to perform retention time-based peak matching across multiple data files and correct for run-to-run retention time shifts. Due to the untargeted nature of the performed SPME-GC-MS method, this program

acts as a data preparation step for Spearman's rank correlation by identifying peaks of interest and their frequency of appearance across the submitted sample set.

2.4 Preparation of Canine Testing Materials

In order to determine which compounds detection canines utilize in the detection of crude oil, testing probes were prepared from sections, or fractions, of the crude oil chromatograms. Similar to the approach used by Simon (2017) and Hudson (2009), a fractionation technique was used to collect portions of the odor profile as they exit the GC (Simon, Mills, & Furton, 2017) (Hudson, 2009). For this purpose, when a SPME sample was thermally desorbed in the inlet, the compounds were separated by the GC in the same manner as described above; however, instead of the compounds going to the MS detector, the column instead vented into the environment (Figure 1). Fractions of the chromatogram, as seen in the example in Figure 1, were collected onto a polymer sorbent, which were then presented to the canines for testing. Three fractions were collected approximately representing the highly volatile (HVOC), volatile (VOC), and semi-volatile compounds (SVOC) in the headspace (Table 2).

Table 2. Fractions of odor profile collected for canine testing. HVOC = highly volatile, VOC = volatile, and SVOC = semi-volatile. *Note that designations are approximate.

Fraction #	Portion of odor profile*	Retention time collected (min)
1	HVOC	0-7.4
2	VOC	7.4-12.6
3	SVOC	12.6-30

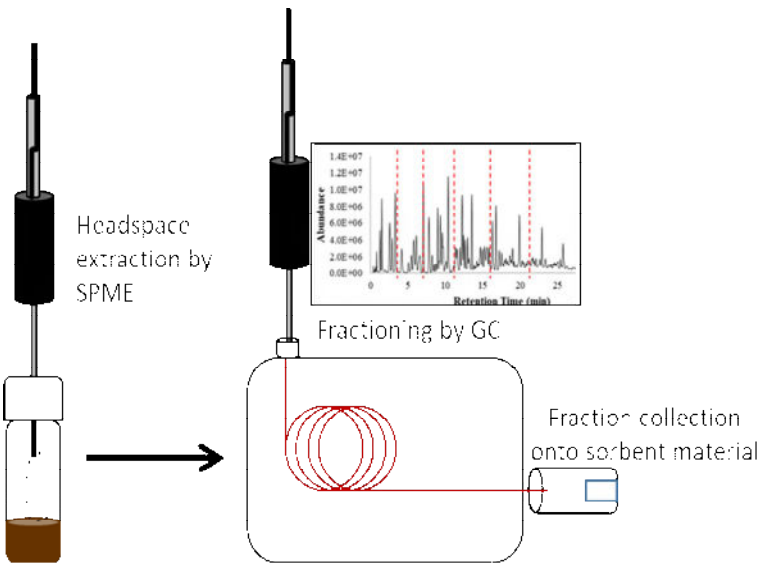

Figure 1. Schematic of experimental setup for fractionation and collection of odor signature.

The sorbents used in this trial were GetXent tubes. These tubes consist of a polymer technology that is impregnated with odorant molecules, by the user, which then continuously releases them into the environment for detection by canines (GetXent, 2020). A single, unused, GetXent tube was placed into a vial lidded with a septum. The vial was then inverted and the

septum was pierced by the end of the GC column, allowing the chosen fraction of the chromatogram to be collected (see Table 2). Solely the HOOPS oil was used for this preliminary canine testing. The volatiles from the headspace of the crude oil were extracted for 10 minutes, prior to separation and collection on the GC. The presence of the crude oil headspace analytes was confirmed on the tubes by SPME-GC/MS using the above extraction and chromatographic method.

In addition to fractions from the crude oils, positive and negative controls and distractor odors were collected onto the GetXent tubes, as listed in Table 3. Positive controls consisted of a collection of the entire chromatogram resulting from the headspace of HOOPS crude oil or simply a tube left to soak in the headspace of the crude oil for 5 hours, without use of the SPME-GC method. Several different negative controls were made – blank GetXent tubes, controls made from blank GC runs, and controls made from blank SPME-GC runs. Finally, distractors are used to confirm that when a canine alerts to a testing odor, it is indeed due to recognition of the training odor and not due to a behavioral change in response to any novel odor. Distractor odors were prepared from common household items by placing the odor in a vial for 24 hours, followed by the above mentioned SPME-GC method. The total chromatograms of these items were collected onto the GetXent tubes. Each individual tube was packaged separately in heat-sealed mylar, "odor-proof" bags (ESP Packaging) with precautions taken to avoid any cross-contamination of the odors. The tubes were prepared within 72 hours of shipping and were stored in the freezer when not in use. Tubes were shipped on dry ice. Negative controls and distractors were shipped separately from the positive control and testing probes. Upon receipt, all materials were stored in a freezer until use, no more than 48 hours after they were received. Prior to use the tubes were thawed in training laboratory ambient temperature (70 °F) for at least one hour.

Table 3. Positive and negative controls and distractor odors prepared for canine testing on GetXent tubes using the SPME-GC fractionation technique.

Sample on GetXent tube	*Type*
Nitrile glove	Distractor
Glade wax melt	Distractor
Milkbone dog treat	Distractor
Rubber bands	Distractor
Peanut butter	Distractor
Oxiclean	Distractor
SPME-GC blank	Negative control
GC blank	Negative control
Blank tubes	Negative control
HOOPS positive control – full chromatogram	Positive control
HOOPS positive control – soak	Positive control

2.5 Canine Testing

The trials were observational in nature and were not considered to be outside the normal realm of working dog training guidance. As such, the trials did not require review and approval by an Institutional Animal Care and Use Committee (IACUC) (NRC 2011; NIH 2019). The activities were conducted by professional handlers trained and certified in the care and ethical use of canines. Training methods for these ODCs, handler responsibilities, and handler certification are described by Bunker (2017) and ICSALDA (2019).

Testing materials were sent to Chiron K9 for on-site testing. The canines were tested utilizing stainless steel odor stands (Figure 2) offering 6 potential locations of target odor. Each stand held a sample within its VOA vial. Testing was conducted under normal room temperature and humidity. All testing materials were assigned random numbers corresponding to position on the stands allowing the canine testing to be double-blind, meaning neither the canine handler nor the test assessor knew the correct identity of the testing materials. The assistant observed through mirrored glass from the control room. Two canines were used for testing and have previously been trained to detect crude oil of differing origins and condition (fresh and weathered). The canines utilized have had significant operational success, with one being deployed on a spill, and both having training and research experience on crude oil. The handler reported a response and the assistant recorded the data on a form designed for the trials. The completed data forms were digitized and transmitted to the Naval Research Laboratory as the Principal Investigators.

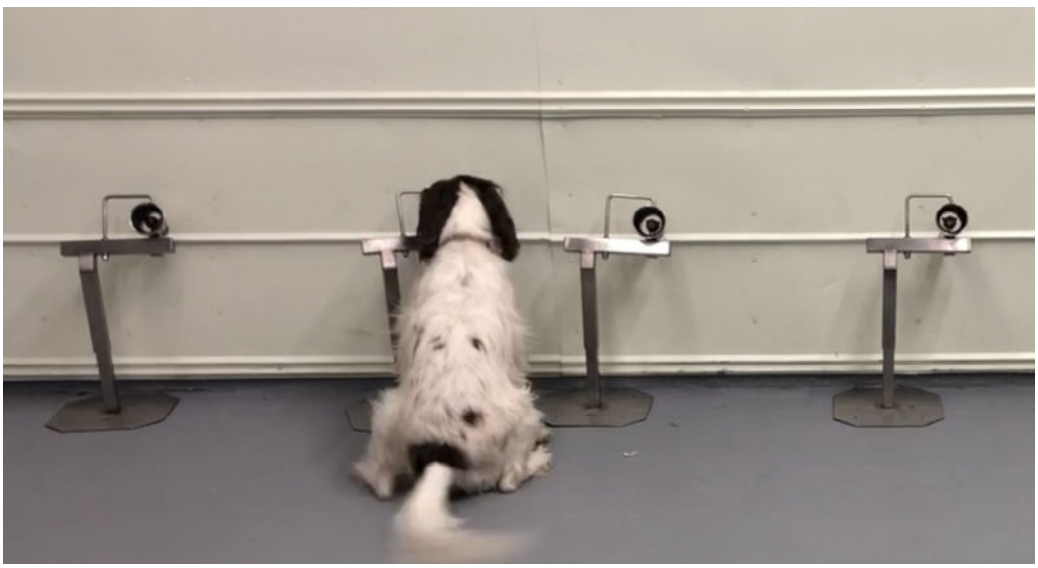

Figure 2. Example odor stands at Chiron K9.

In a preliminary test, canines were presented with three positive controls, in addition to several blanks and distractors. Positive controls of HOOPS included one tube made with a full chromatogram of HOOPS crude oil, a single tube comprised of a total of two subsequent GC runs of HOOPS, and one 5-hour soak made directly from the headspace of HOOPS (not using the GC). In addition, blank materials and negative controls prepared from blank GC runs were sent. Duplicates of each positive and negative control were provided for testing.

After the canines were shown to be proficient at detecting the odor of the crude oil from the GetXent tubes prepared using SPME-GC, trials were run with the chromatographic fractions, in addition to positive controls, negative controls, and distractors, as seen in Table 4. This test took place on two separate occasions, each time with freshly-prepared GetXent tubes.

Table 4. Testing probes, controls, and distractor odors prepared by the SPME-GC fractionation technique and used in canine testing.

Sample on GetXent tube	*Type*	*Number of replicates*
Nitrile glove	Distractor	1
Glade wax melt	Distractor	1
Milkbone dog treat	Distractor	1
Rubber bands	Distractor	1
Peanut butter	Distractor	1
Oxiclean	Distractor	1
SPME-GC blank	Negative control	2
GC blank	Negative control	1
Blank tubes	Negative control	4
HOOPS fraction 1	Testing probe	1
HOOPS fraction 2	Testing probe	1
HOOPS fraction 3	Testing probe	1
HOOPS positive control – full chromatogram	Positive control	2

3 Results and Discussion

3.1 Headspace Analysis of Fresh Crude Oils

The headspace analysis of HOOPS and ANS was carried out by SPME-GC-MS (Figure 3). The headspace of ANS and HOOPS crude oils contained 63 and 62 distinct peaks found in all triplicates of each oil, respectively, which corresponded to predominately alkanes, cyclic alkanes, and aromatic compounds. In Figure 4, the VOCs extracted from the headspaces of HOOPS and ANS were classified by respective functional groups. Here, it can be seen that the odor profiles of HOOPS and ANS slightly differed, with HOOPS being composed mainly of branched and straight alkanes, followed by aromatics, cyclic alkanes, and then terpenes and polycyclic aromatics. Comparatively, ANS could be differentiated by an increased abundance of cyclic alkanes and reduced quantity of branched alkanes compared to HOOPS (Figure 4).

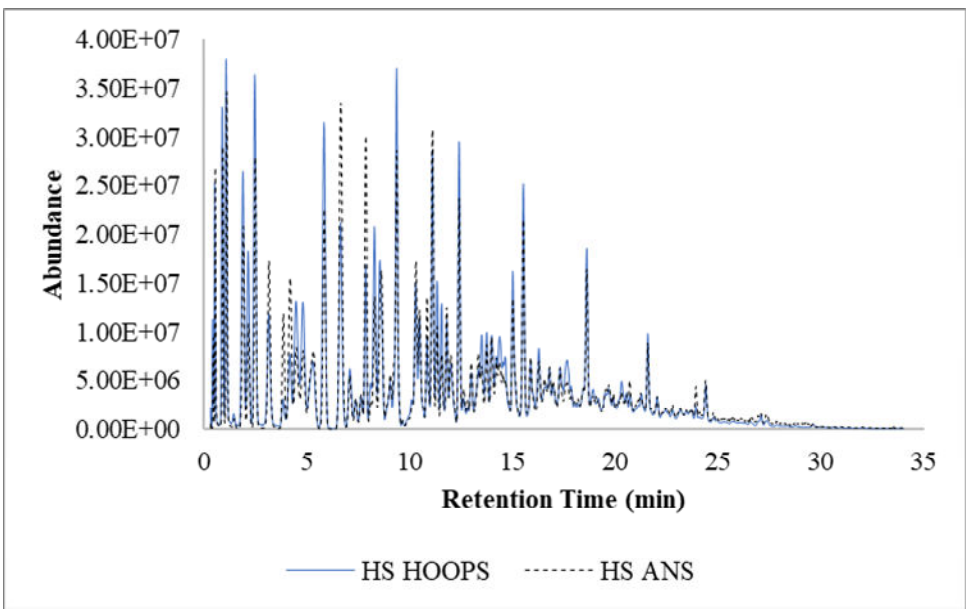

Figure 3. Overlaid chromatograms of HOOPS and ANS crude oil for comparison of extracted headspaces.

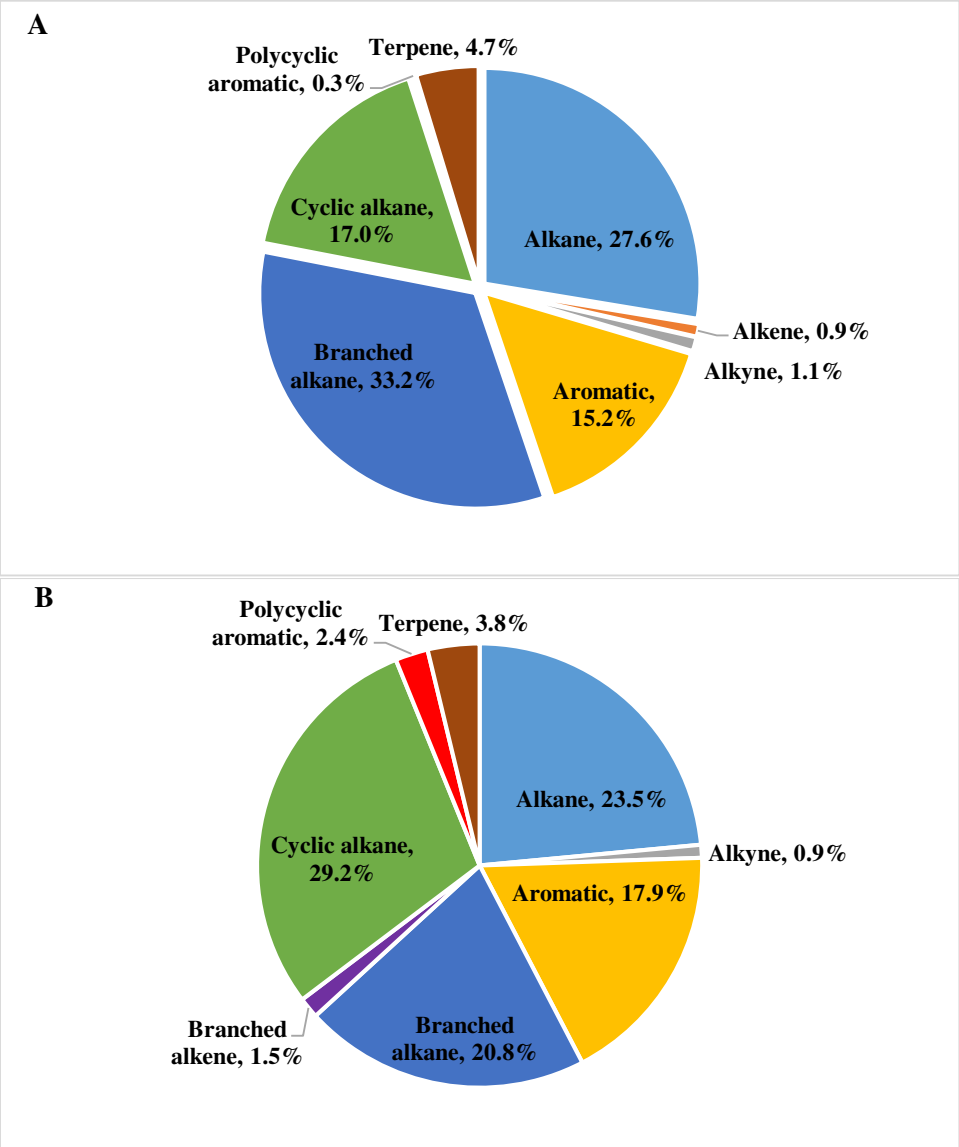

Figure 4. Compound class distribution of VOCs extracted from the headspace of HOOPS (A) and ANS (B).

Of the 63 peaks found in triplicates of ANS and 62 peaks found in triplicates of HOOPS, 55 of those peaks were found to be retention-time aligned and present in both sets of samples. Furthermore, 26 of these common peaks were present in statistically different quantities, based on peak area (t-test, 95% confidence). Spearman's rank correlation was performed using the peak areas of 55 retention time-matched peaks. The values displayed in Table 5 are the Spearman's rank correlation coefficients (Spearman's rho or ρ); a higher value denotes a stronger correlation in the information being compared. The Spearman's rank correlation of the ANS and HOOPS samples revealed higher Spearman's rank correlation coefficients for comparisons made between samples originating from the same source versus different sources. The higher value indicates a stronger correlation between same source samples and the positivity of the value indicates that the correlation includes similar trends in peak area values.

Table 5. Spearman's rank correlation values (ρ) from retention time-matched peaks in the chromatograms of triplicate samples of ANS and HOOPS crude oils. Blue indicates a comparison between samples that originate from the same source (ANS vs ANS; HOOPS vs. HOOPS).

	ANS_01.D	*ANS_02.D*	*ANS_03.D*	*HOOPS_01.D*	*HOOPS_02.D*	*HOOPS_03.D*
ANS_01.D	1.000	0.923	0.945	0.752	0.793	0.786
ANS_02.D		1.000	0.979	0.688	0.727	0.726
ANS_03.D			1.000	0.701	0.746	0.738
HOOPS_01.D				1.000	0.967	0.974
HOOPS_02.D					1.000	0.992
HOOPS_03.D						1.000

3.2 Headspace Analysis of Weathered Crude Oil

A preliminary study on the effects of weathering on the VOC composition of HOOPS crude oil was carried out. Figure 6 visibly compares the change in appearance of the oil from fresh to 12 hours of irradiation, showing the crude oil becoming increasingly concentrated and viscous during the weathering process. Figure 7 shows the chromatograms produced from the headspace of fresh and weathered crude oil. After only four hours of irradiation a significant change in the odor signature can be seen, with the total abundance of VOCs decreasing substantially and the majority of the high volatility compounds (HVOCs), eluting before 11 minutes, no longer being detected. There was little change from 4 to 12 hours of weathering. After 24 hours, there was a greater reduction in total abundance and the few compounds eluting before 18 minutes could be detected. Finally, after 168 hours of irradiance, there were only a small number of the semi-volatile compounds (SVOCs) remaining on the back end of the chromatogram.

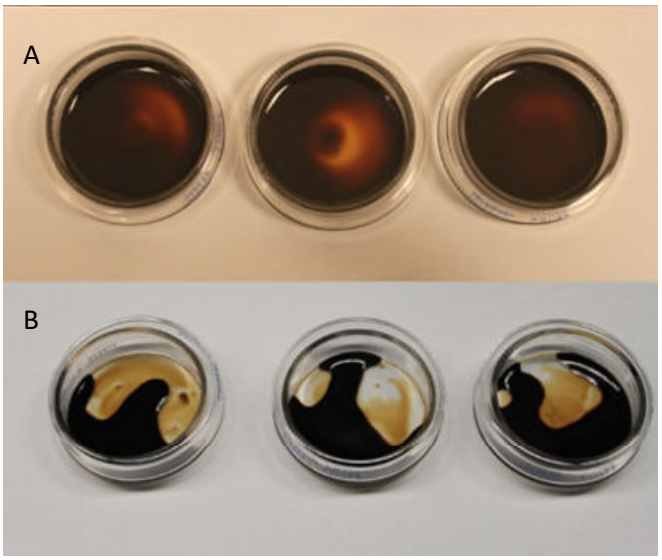

Figure 6. HOOPS crude oil prior to weathering (A), and HOOPS after 12 hours of irradiation (B).

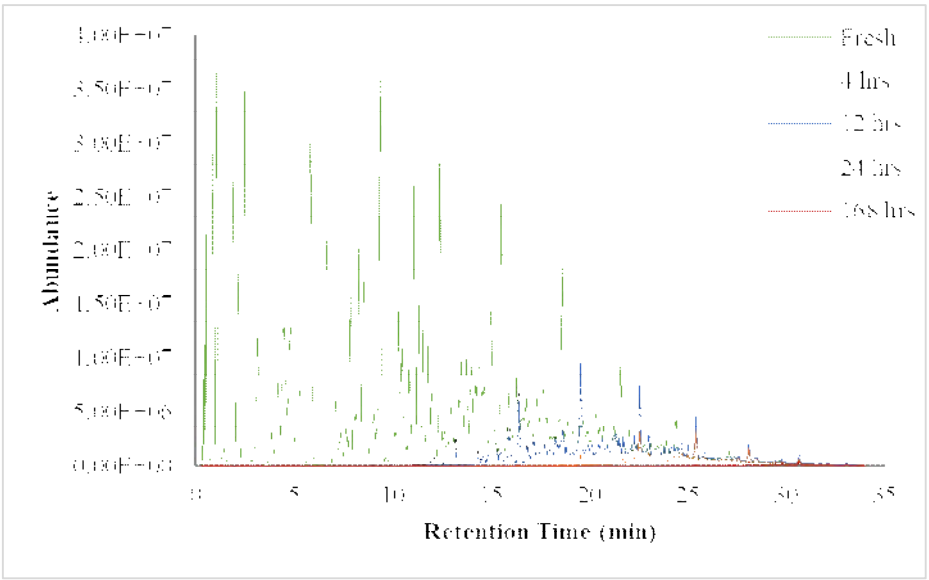

Figure 7. Overlaid chromatograms of fresh and weathered HOOPS crude oil for comparison of extracted headspaces.

Further analysis of the headspace data revealed significant differences in the weathered samples compared to the fresh (Figure 8). After weathering for only 4 hours, polycyclic aromatic compounds, not detected in the fresh oil, were identified and increased with each irradiation time. After one week of weathering (168 hours), the headspace was predominantly composed of straight-chain alkanes (62%), whereas fresh HOOPS was mostly composed of a mix of branched alkanes (33%) and straight-chain alkanes (28%). It was also found that as weathering time increased, the reproducibility of sample headspace decreased. For example, the relative standard deviation (RSD) for triplicates of weathered for 168 hours at peak 22.5 min (dodecane) was 173%, while the RSD for this same peak of fresh was 16%.

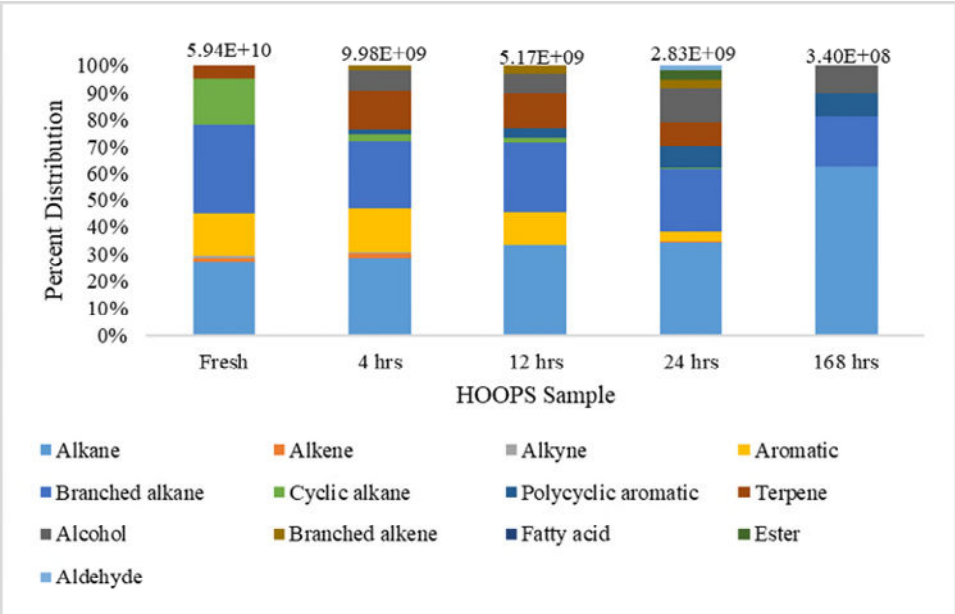

Figure 8. Compound class distribution of VOCs extracted from the headspace of simulated weathered HOOPS crude oil in comparison to fresh HOOPS crude oil. Values above bars represent total abundance in area counts.

3.3 Canine Testing

Results from this preliminary testing session are given in Table 6. Two canines previously trained to detect crude oil were tested. Each canine had three separate occasions to respond to each item, and duplicates of each item were provided, equating to 12 chances for detection. Both canines alerted to all positive controls, with the exception of one canine, which missed one of the HOOPS GC x 1. There were no false alerts to the blank materials or to additional distractor odors that were included in the odor stands and were selected by the test provider (separate from the handler and assessor). Due to the one miss and because the fractions will have less total odor than the total chromatogram, it was determined that fractions needed to be made with double collections of crude oil headspace.

Table 6. Canine response to positive and negative controls of HOOPS oil on GetXent tubes.

Sample on GetXent tube	Canine response (out of 12)
HOOPS GC x1	11
HOOPS GC x2	12
HOOPS headspace (no GC)	12
Blank tubes	0
GC blank	0

Following preliminary canine testing and confirmation that the canines indeed recognized the odor of crude oil deposited on GetXent tubes by the SPME-GC method, the canines were tested on three crude oil fractions (Fig. 9), in addition to the positive and negative controls and distractors listed above. Each presentation of a crude oil fraction was repeated a second time with fresh, previously unseen, materials. Results from the canine testing are given in Table 7. There were no false alerts to distractors or blanks from either canine on either occasion. Each canine alerted to each fraction in at least two of the three trials, indicating that they are capable of using parts of the odor signature for detection. This is likely because the canines have been taught to

generalize across many types and conditions of crude oil. The canines alerted to Fraction 3 in all trials. From the preliminary weathering data presented above, this fraction remains the most consistent during weathering, and is thus, apparently, the most recognizable portion of the odor profile. Future testing will be done with canines trained only on fresh crude oil to determine if they also use all portions of odor profile, or if they utilize the more abundant HVOC or VOC portion.

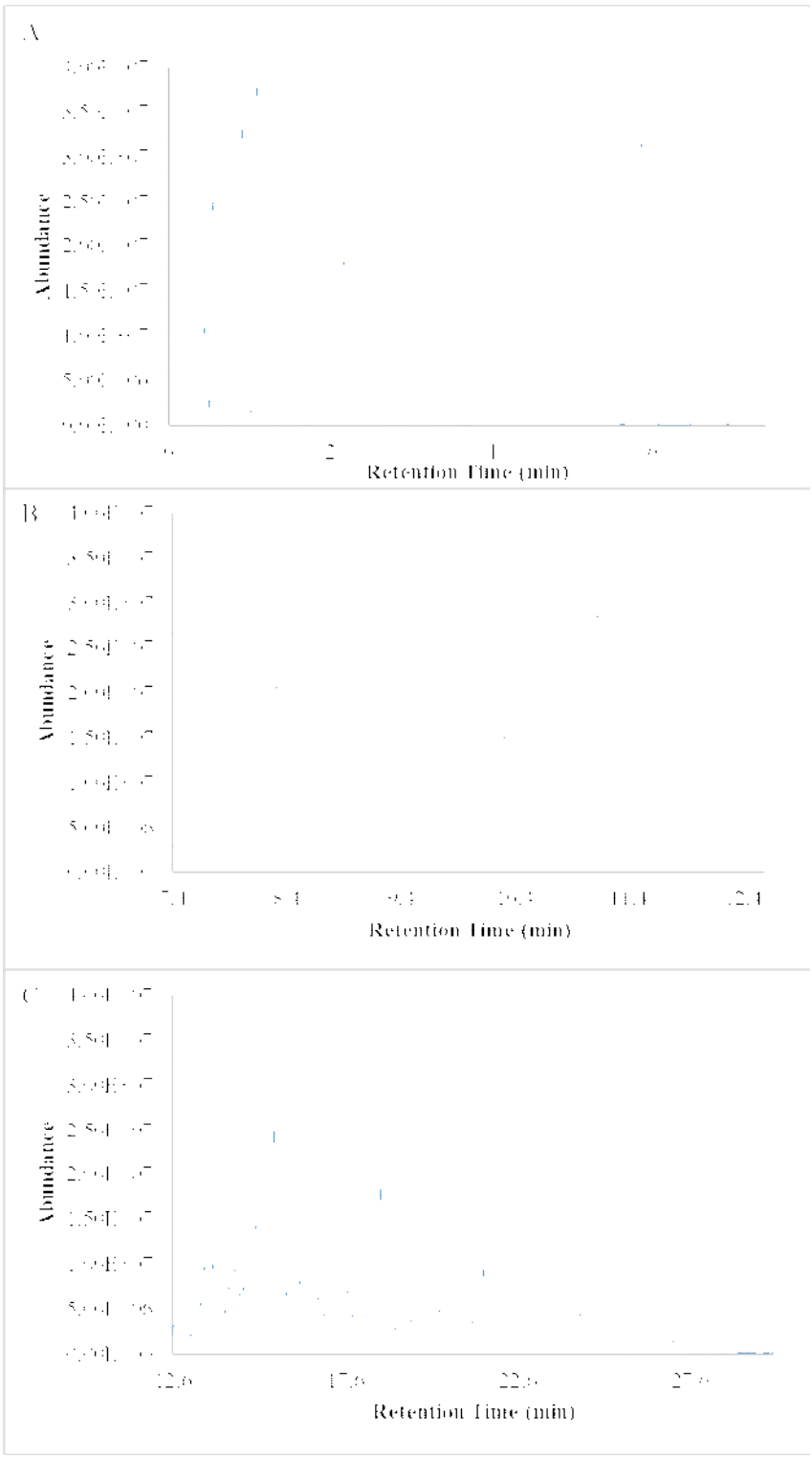

Figure 9. Chromatograms of fresh HOOPS fractions. Fraction 1: 0 – 7.4 min (A), fraction 2: 7.4 – 12.6 min (B), and fraction 3: 12.6 – 30 min (C).

Table 7. Canine responses to fresh HOOPS training aids. "A" denotes a positive alert, while "N" denotes no alert. (HVOC = highly volatile organic compound; VOC = volatile organic compound; SVOC = semi-volatile organic compound_

	Fraction 1 (HVOCs)		Fraction 2 (VOCs)		Fraction 3 (SVOCs)	
	Canine 1	Canine 2	Canine 1	Canine 2	Canine 1	Canine 2
Fresh Trial 1	A	A	N	N	A	A
Fresh Trial 2	A	A	A	A	A	A
Fresh Trial 3	N	N	A	A	A	A
Total Alerts	4		4		6	
Alert Response %	67%		67%		100%	

4 Conclusions and Future Work

In the preliminary year of this research project, a SPME-GC-MS method was developed and optimized using fresh HOOPS crude oil, allowing for the characterization of the odor profiles of HOOPS and ANS crude oils. A comparison of the odor profiles of the two oils showed overlapping compositional makeup and many retention time-matched peaks in common; nevertheless, there were enough differences to correctly categorize samples as HOOPS or ANS using Spearman's rank correlation coefficients for individual sample comparisons. In terms of canine detection, these results could, in the future, assist the training of specific oil detection canines. In this way the canines *only* respond to a particular oil as the result of a spill incident and ignore all other presentations of oil (natural and historical), within an incident response location such as a beach.

Additionally, weathered HOOPS oil was compared to fresh, and a notable shift in the odor profile in as little of 4 hours of irradiation, characterized by a loss of the early-eluting HVOCs and the appearance of polycyclic aromatic compounds with increasing irradiation time.

Canine testing was carried out to determine the portion of the odor profile was used in oil detection. Testing probes were prepared by absorbing the VOCs emanating from the oil onto polymer tubes. Double-blind testing showed that the canines were capable of detecting the odor profiles from these tubes and differentiating the odor profiles of crude oil from that of non-target distractor odors. This achievement could lead to the development of a training aid that does not need specific storage, transportation, or handling protocols like actual oil samples currently require. In this way, teams can travel to incident locations with a single, non-hazardous, training aid and conduct calibration and maintenance training on site without the requirement to use spilled oil samples. The training aid could also be transported onto an aircraft or within the postal system without restrictions.

It was shown that the canines are capable of distinguishing crude oil from any fraction of the odor profile. This capability allows the canines to readily generalize between oils of different types and conditions. However, the canines most readily detected the heavier SVOC fraction of the fresh oil, likely because this fraction was the most consistent between types of oil and its higher boiling point makes it resilient to a degree of weathering.

In its application, the devised approach will inform a greater body of work regarding the volatile components of crude oil, how these components change when subjected to weathering, and how separately sourced crude oil samples vary throughout this process. These findings will garner a fundamental understanding of crude oil composition and weathering, informing crude oil detection procedures.

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