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Author(s):	Michael E. Sigman, Ph.D., Mary Williams, M.S.
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Degraded Ignitable Liquids Database: An Applied Study of Weathering and Bacterial Degradation on the Chromatographic Patterns of ASTM E 1618 Ignitable Liquid Classes

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Author/ Principal Investigator

Michael E. Sigman, Ph.D.
Department of Chemistry and National Center for Forensic Science
University of Central Florida
PO Box 162367
Orlando, FL 32816-2367
407-823-6469

Co-Authors

Mary Williams, M.S.
National Center for Forensic Science
University of Central Florida
PO Box 162367
Orlando, FL 32816-236

Abstract

A study has been completed on the effects of weathering and biological degradation on ignitable liquids from each of the ASTM E1618 designated classes. Fifty ignitable liquids in the Ignitable Liquids Reference Collection (ILRC) database were weathered by evaporation of 0, 0.25, 0.5, 0.75, 0.9 and 0.95 fractions of the original volume. The same liquids were biologically degraded on potting soil for 0, 7, 14 and 21 days. Each of the weathered and degraded samples was added as a new record in the ILRC database. The ILRC database was modified to link all associated records from weathering and degradation of the same ignitable liquid. The data from all of the samples was reviewed by the ILRC Committee and evaluated with regard to the potential impacts of weathering and biological degradation on the interpretation of fire debris data. A "Best Practices" statement was prepared by the ILRC Committee and placed on the ILRC database website. The "Best Practices" statement will serve to inform forensic practice and policy in laboratories conducting fire debris analysis. The evaporation rates were also modeled as a function of the fraction weathered and found to follow first order kinetic profiles. The natural logarithm of the fitted rate constants were found to exhibit varying degrees of linearity as a function of retention index. The kinetic analysis constitutes a preliminary investigation that may provide direction for future attempts to implement a digital weathering algorithm that would aid fire debris analysts in predicting, *a priori,* the chromatographic profiles of weathered ignitable liquids.

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Executive Summary

The Problem

Identification of ignitable liquid residues in fire debris is complicated by several factors. Background contamination from volatile organics formed as a result of the pyrolysis and partial combustion of household furnishings and building materials can obscure the chromatographic pattern of an ignitable liquid. When pyrolysis leads to the formation of a chemical component of the ignitable liquid, the resulting chromatogram will have distorted peak ratios relative to the original liquid. Partial evaporation (weathering) of the ignitable liquid can also lead to significant distortion of the chromatographic profile. Weathering follows an understood and generally predictable pattern with the loss of the “front end” of the chromatogram where the most volatile components normally elute. Although the pattern of weathering may be understood, the loss of ignitable liquid components can complicate the assignment of the ignitable liquid into an ASTM E1618 designated class based on the weathered residue. An extensive study of weathering across all ASTM classes has not previously been reported. The analyst’s options for coping with the weathering action of fire are (1) to retreat to the laboratory and engage in many hours of in-house weathering of ignitable liquids for comparison to the fire debris samples or (2) to search databases for examples of weathered ignitable liquids that match the casework results. Neither of these options is attractive because the first option is laborious and time consuming, while the second option is not realistic due to a lack of accessible data on weathered liquids. Not only does additional weathering data need to be available, but the data also needs to be tabulated in a way that facilitates finding meaningful results. Finally, the presence of microbes in fire debris samples can alter the composition of the ignitable liquid residue, resulting in a chromatographic profile that may not be readily placed into the appropriate ASTM class of the non-degraded liquid. A significant study of the effects of microbial degradation on the chromatographic patterns of representative liquids from all ASTM classes, when exposed to the same microbial population, had not been reported, but extensive work covering all classes has been performed by Goodpaster.

Purpose of the Research

The purpose of this research was to address all of the problems identified in the preceding paragraph which are associated with the effects of weathering and biological degradation pertaining to the process of ignitable liquid residue identification and class assignment in fire debris. Example liquids from each of the ASTM E1618 designated classes were selected from the Ignitable Liquids Reference Collection (ILRC) - and each liquid was weathered and biologically degraded. Records for four new liquids were also added to the ILRC database, along with their weathering and biological degradation studies. The resulting data records were added to the ILRC database and linked to the record of the original sample (before weathering and biodegradation). The ILRC Committee collaborated with the team from the National Center for Forensic Science (NCFS) to evaluate the effects of weathering and biological degradation and formulate a "Best Practices" statement to inform fire debris analysis policy and practice to mitigate the impact of these effects on data interpretation. Additional work completed under this research program included an investigation of the influence of weathering and biological degradation on the correct ASTM classification rates for a simple linear discriminant analysis method that had previously been reported by NCFS under peer review. And finally, a preliminary investigation was conducted to address digital modeling of the weathering process across all ASTM classes of ignitable liquid. The results from the study point to a potential approach to allow the analyst to predict weathering patterns for ignitable liquids and possibly avoid the time consuming task of weathering several ignitable liquids in search of a weathered chromatographic pattern that matches a casework sample.

Research Design

A total of 50 ignitable liquids (46 from the ILRC and 4 new samples) were weathered and biologically degraded in this study. The samples were chosen to be representative of the classes designated in ASTM E1618-11. The represented classes, class abbreviations and number of liquids from each class were: aromatic (AR, 5); gasoline (GAS, 8); petroleum distillates (PD) representatives of the sub-classes light petroleum distillates (LPD, 3), medium petroleum distillates (MPD, 6) and heavy petroleum distillates (HPD, 3); isoparaffinic liquids (ISO, 5), naphthenic paraffinic liquids (NP, 4), normal alkanes (NA, 4), oxygenated liquids (OXY, 5), and miscellaneous liquids (MISC, 7). Each liquid was weathered by removal of 0 (unweathered), 0.25, 0.5, 0.75, 0.9 and 0.95 fractions of the original volume. In some cases it was not possible to reach the highest fractions of weathering in reasonable periods of time due to the extremely high boiling points of the heavier components. Each liquid was also biologically degraded by placing a small amount of liquid on potting soil and allowing degradation to continue for 0, 7, 14 and 21 days.

The online interface to the ILRC database was modified to provide the user a link to related samples. This allows the user to view the total ion chromatograms for all weathered and/or biologically degraded samples, along with the original sample. As described earlier in the *Executive Summary*, weathering and biological degradation challenge the fire debris analyst by increasing the possibility of assigning the ignitable liquid residue into an ASTM E1618 designated class that does not correspond to the correct class assignment for the original liquid. Perhaps more problematic are the possibilities that weathering and biological degradation would lead to failure to identify the presence of an ignitable liquid residue. Two approaches were taken to assess the possibility of incorrect classification and the ability to identify the presence of ignitable liquid residue.

The first way that these issues were addressed was by continued ILRC Committee review of the data from weathered and biologically degraded samples. The committee examined the records to look for signs of preferential loss of specific types of chemical compounds (i.e., normal alkanes, branched alkanes, cyclic alkanes, aromatic compounds, polycyclic aromatic compounds, etc.). The loss of lower boiling components could cause a MISC class liquid, perhaps corresponding to a blend of liquids, to resemble a single class corresponding to the higher boiling component. The ILRC Committee members reviewed each data record before the record was released to the public side of the database. The committee also met to collectively discuss the comprehensive set of data produced during this work and formulate a recommended "Best Practice" to inform policy and practice within each agency that evaluates fire debris evidence.

The effects of weathering and biological degradation on correct classification were also addressed by examining the influence of these processes on the true positive rate and false positive rate of discrimination among the ASTM classes using linear discriminant analysis (LDA). It has previously been demonstrated that LDA can provide true positive rates > 80% for the detection of the presence of ignitable liquid residue in fire debris; however, the false positive rate increased as the contribution of substrate pyrolysis product in the model increased. In this work, an LDA model was built for assigning unweathered and non-degraded liquids into the 8 ASTM classes. The true positive rates and false positive rates for assignment of liquids into each class, versus all other classes, were determined for the weathered and degraded liquids.

Data for the weathered samples produced in this work also provided an opportunity to expand on previous research on discrimination models for samples containing ignitable liquid residues. The training data for the chemometric methods in the previous work was prepared by computationally mixing ignitable liquid and substrate pyrolysis data to generate representative fire debris data. The weathered sample data generated in this work was added to the computationally generated models to produce more representative fire debris data wherein the ignitable liquid component was weathered.

Laboratory weathering of ignitable liquids is a time consuming process, but necessary for verification of casework results in some cases. In order to examine the possibility of saving the analyst time by providing estimates of weathered chromatographic profiles through a process of digitally weathering, kinetic profiles were developed for multiple peaks in the weathered samples produced in this research. Peak intensities were fit to a kinetic first order decay as a function of the fraction that the sample was weathered. Use of the fraction weathered provides a convenient substitute for time that would allow the analyst to address the change in chromatographic profile in terms of the extent of evaporation. Chromatographic peaks from each weathered liquid were located based on the derivative of the chromatographic profile using a Savitzky-Golay algorithm. The fitted rate constants, k, had units of f^{-1}, where f is the fraction weathered. Rate constants with a high coefficient of expectation (r^2) were retained for further analysis. The natural logarithm of the rate constants, ln(k), for peaks throughout the chromatographic profile were examined as a function of the retention index for the peak.

Findings and Conclusions

The results from this research led to a significant expansion and upgrade of the ILRC Database and provided the ILRC Committee with data on the weathering and biological degradation of ignitable liquids from each of the ASTM E1618 designated classes of ignitable liquid. The data was studied by the committee members and a “Best Practices” statement was published on the ILRC website (http://ilrc.ucf.edu/). The Best Practices statement warns analysts of the possibility that weathering will lead to a change in ASTM class for an ignitable liquid and complicate the assignment of ignitable liquid residue. ASTM E1618 does not provide a stepwise process by which the analyst first decides if a sample is positive or negative for ignitable liquid with subsequent assignment of the residue to a class if the sample is determined to be positive. Rather, the process of determining that a sample is positive for ignitable liquid residue depends on determining that a pattern exists in the gas chromatography – mass spectrometry data that is consistent with an ASTM class. If this condition is met, the assigned class information may be passed to the investigator along with possible examples of commercial products that fall into the specified ASTM class. It is at this point that an incorrect class assignment could potentially lead investigators down the wrong path. The Best Practices statement coupled with enhancements in the ILRC Database, and with the increased educational value of the database, provide a useful product for the forensic fire debris analysis community.

The effects of weathering and biological degradation can be misleading to the analyst when it comes to class assignment for ignitable liquid residue in fire debris. Likewise, the processes of weathering and biological degradation can decrease the ability of automated (chemometric based discriminant) techniques to properly assign liquids into ASTM classes. While weathering and biological degradation both led to decreases in the true positive rate for classification, the effects were more significant for biological degradation. Weathering decreased the true positive rate, but did not significantly increase false positive rates; however, biological degradation led to a significant increase in false positive rate for assignment to the OXY ignitable liquid class. This may be partially attributed to the production of aldehydes that were recovered from some samples when desorption temperature was increased for headspace sampling.

Weathering rates for compounds across the chromatographic profile were found to follow first order rate laws when the degree of weathering was used to express the time component of the weathering. The ln(k) versus retention index plots were linear for many of the ignitable liquids, although there was significant variance in the fitted rates for some liquids. The slope of the ln(k) versus retention index were in different ranges for each class.

The slope of the plot and the intercept are required to allow effective modeling of the weathering of an ignitable liquid. The observed variation in the slopes and intercepts of the plots complicate the prediction of which values to use to give optimal modeling of the weathered chromatographic profile of a liquid that was not involved in the study. Nonetheless, the preliminary results from this study are encouraging and point to the need for more work in this area.

Implications for Policy and Practice

The results from this research have been made available on the ILRC database web site (http://ilrc.ucf.edu/) throughout the course of the research. The work has also been presented in a number of forums throughout the course of the research. The ILRC Committee has participated in the research throughout the process and has utilized the results to formulate a "Best Practices" statement, which is posted on the web site. Consequently, the results have had an impact on fire debris analysis practices and policy within individually laboratories as the data and best practices recommendations have been accessed by the forensic fire debris analysis community for casework and educational purposes.

The following recommendations are provide by the ILRC committee within the Best Practices Guide. Measures should be taken to prevent microbial growth since major degradation occurs within 7 days. Currently, this is done by freezing or refrigerating the sample prior to extraction. A threshold on the abundance in a total ion chromatogram should be set to determine whether the sample is positive for ignitable liquid residue. The threshold will be dependent on the instrumentation and laboratory procedures. Below the threshold there was not sufficient mass spectral data to confirm individual peak identification.

Analysts are reminded that target compounds are identified by a mass spectral and - retention time matching to a standard.

I. Introduction

Statement of the problem

The identification and classification of ignitable liquid residue from fire debris can be complicated by weathering (evaporation) and biological degradation of the liquid. While evaporation affects the chromatographic profile, primarily through loss of the “front end” of the chromatographic profile by evaporation of the most volatile components, biological degradation tends to selectively remove certain types of hydrocarbons. ASTM E 1618-14, “The Standard Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry”, emphasizes the need for laboratories to consult libraries of gas chromatography – mass spectrometry (GC-MS) data for ignitable liquid references, including a set of weathered and biologically degraded samples [1]. Libraries are intended to assist the analyst in identifying reference materials for analysis within their laboratory in comparison to casework samples. The National Center for Forensic Science (NCFS) at the University of Central Florida and the Ignitable Liquids Reference Collection (ILRC) Database Committee of the Scientific/Technical Working Group for Fire and Explosions (S/TWGFEX) have collaboratively produced the ILRC database [2] and a Substrate database[3], both of which are freely available via the internet. These databases are consulted daily by fire debris analysts throughout the U.S. and other countries from around the world. Although the ILRC previously contained in excess of 600 records for ignitable liquids encompassing the

ASTM E 1618 classes, the collection had relatively few weathered and no biologically degraded samples. The research conducted under this award provided:

1) Hundreds of new weathered and biologically degraded records for the database;
2) An assessment of the possibility of misclassification due to degradation;
3) Studies to further the fire debris community's understanding of the processes that lead to the degradation of ignitable liquids in fire debris;
4) A best practices guideline for the interpretation of weathered and degraded samples;
5) Preliminary investigations into the potential use of digital weathering to assist fire debris analysts in the identification of highly evaporated ignitable liquids.

Literature review

Ignitable liquid residues recovered from fire debris are altered during a fire and possibly after the fire. A study by Borusiewicz, Zieba-Palus and Zadora investigated how many factors including the duration of the fire influence the ignitable liquids residue recovered at a fire scene [4]. During a fire, ignitable liquids evaporate (weather) as temperatures in the fire reach the boiling points of the compounds in the ignitable liquid. The original composition of the ignitable liquid changes due to evaporation of the more volatile compounds compared to the less volatile compunds resulting in a composition with a greater concentration of the less volatile compounds [5]. Comparisons between the ignitable liquid residue and a reference ignitable liquid becomes challenging when the composition has been changed. Hirz [6] and Vergeer et. al [7]discuss the possible difficulties in identifying gasoline once it has been weathered. A majority of the studies are concerned with overcoming the problem by finding characteristic compounds in the ignitable liquids that are not affected by the weathering. Coulombe utilized the low volatile aromatic disulfides to provide positive evidence of the presence of gasoline [8]. A series of C_0 to C_2 naphthalenes were used by Sandercock and DuPasquier to discriminate 96 samples of gasoline [9]. Barnes, Dolan, Kuk, and Siegel examined various compound ratios in gasoline to determine if an un-evaporated gasoline sample could have originated from the same source as an evaporated gasoline residue extracted from fire debris [10]. Smaller bicyclic sesquiterpanes (decahydronaphthalenes) were used as biomarkers by Wang et al. to identify the source of spilled petroleum products such as jet fuels, diesel fuels and oils [11, 12]. Zadora, Borusiewicz and Zieba-Palus, were able to distinguish weathered kerosene from diesel fuel by statistically comparing the relative content of n-alkanes ($C_{11}H_{24}$ – $C_{15}H_{32}$) [13]. A chemometric approach to associate an evaporated ignitable liquid residue with an un-evaporated ignitable liquid was investigated by Baerncopf, McGuffin, and Smith [14]. Bruno, Lovestead, and Huber tried to predict the weathering process by using an advanced distillation curve method which indicates the volume fraction of liquid at a series of temperatures [15]. Bruno and Allen used the advanced distillation curve method to simulate weathering patterns of ignitable liquids by examining the composition of specific cuts of the distillate [16]. Simulation of weathered gasoline chromatographic data by Hirz and Rizzi reported accurate results up to 30% evaporation [17].

Modeling the evaporation of hydrocarbon mixtures is important in understanding and predicting the environmental fate and transport of these materials [18]. Models have been presented which model evaporation based on physical properties, such as vapor pressure and boiling point [19-22]. McGuffin has recently pointed out the advantage of using a chromatographic retention index as a surrogate of the physical properties [18]. The retention index allows the modeling of evaporation rates and circumvents the need for identification of the evaporating component [18]. Forensic scientists also have need to model the evaporation of commercial ignitable liquids. It is often the case that ignitable residues recovered from fire debris appear to be highly weathered based on the chromatographic profile. The appearance of weathering may be the result of actual weathering or displacement of more volatile components from the charcoal adsorbents often used in fire debris sampling [23]. Irrespective of the origin of the chromatographic distortion, the fire debris analyst would like to identify potential sources of the ignitable liquid residue in the sample. One option is to obtain commercially available liquids and evaporate them in the laboratory to produce a pattern that resembles the recovered pattern. This is laborious and time consuming and, though perhaps necessary, it would be advantageous to be able to model the evaporation of candidate liquids before undertaking the physical evaporation in the laboratory. Modeling for the fire debris analyst must accommodate high degrees of evaporation (frequently over 90% evaporated) and account for the variability in exposure time and extreme temperatures (often 800 °C or higher) to which the ignitable liquid is exposed in a fire. This work is a first examination into possible methods to model extensive evaporation in a way that is useful to the fire debris analyst.

Ignitable liquid residues recovered from soils or other organic matter may potentially be degraded by microorganisms. One of the first studies conducted by Mann and Gresham demonstrated microbial degradation of gasoline after reviewing microbiological literature [24]. Seven microorganisms were identified in soil by Kirkbride et al. in the examination of the effects microorganisms have on ignitable liquids [25]. They found that individual strains within each species of microorganism preferred a particular hydrocarbon type. One species metabolizes aliphatic hydrocarbons and the other species metabolizes aromatic hydrocarbons [25]. Chalmers, Cassista, Hrynchuk, and Sandercock studied the microbial degradation of three classes of ignitable liquids; gasoline, medium petroleum distillates, and heavy petroleum distillates [26]. Their results indicated the majority of degradation occurred between 7 and 14 days and the normal alkanes and mono-substituted aromatics were depleted prior to the poly-substituted aromatics and substituted alkanes. A semi-quantitative study on microbial degradation was performed by Turner and Goodpaster on several classes of ignitable liquids [27]. They found that normal alkanes in the range of C_9-C_{16} and mono-substituted benzenes were preferentially consumed in all samples. Effects of microbial degradation of gasoline due to soil types and seasonal conditions were studied by Turner et al. where they discovered a reduction in hydrocarbon depletion during Summer and more activity in residential soil [28-30]. Turner and Goodpaster also studied the microbial degradation of a de-aromatized distillate, naphthenic paraffinic product, and a miscellaneous product with results indicating the possibility to misclassify an ignitable liquid due to microbial degradation when viewing the chromatographic patterns [31]. Most studies only considered soil as a substrate for microbial degradation; however, Hutches found similar results when the ignitable liquids residue was recovered from building materials [32]. A study on preventing microbial degradation by Turner and Goodpaster other than the recommended freezing or refrigeration methods indicated that triclosan was able to preserve gasoline residues for at least 30 days [33]. A statistical approach to comparing the effects of weathering to microbial degradation showed that lower boiling point compounds are most susceptible to weathering, but a different set of compounds are subject to microbial degradation [34].

Hypothesis or Rationale for the Research

Currently, there are a few studies on the weathering of ignitable liquids. Most of these studies attempt to associate a weathered sample to the original unweathered sample for ignitable liquid classification. Recently, there have been more studies on the microbial degradation of ignitable liquids. These have typically discussed the preferential loss of particular compound types which may lead to misclassifications. The research thus far has been performed mostly on gasoline with a small number of examples from other ASTM E 1618 classes. The rationale for this research was to provide numerous examples of weathering and microbial degradation over many classes. Different results between the two mechanisms of degradation (i.e. weathering and microbial) are demonstrated by incorporating the examples into the ILRC database. Statistical classification by linear discriminant analysis and committee review determined if there is a possibility of misclassification. These techniques ascertained which classes were most susceptible and whether the misclassifications are predictable. This research provides best practices for recognizing degradation and recommendations on determining the presence and classification of degraded ignitable liquids. Finally, the current method of visual pattern recognition requires that the analyst have access to the chromatographic profiles of weathered (often highly weathered) ignitable liquids. Preparing highly weathered samples in the laboratory is time consuming. This work examines the potential for modeling weathering as a function of evaporative loss and the possible application of the models to new liquids not examined in the modeling process.

II. Methods

Forty-six ignitable liquids from the Ignitable Liquids Reference Collection repository were used for the degradation of ignitable liquids project, plus four new ignitable liquids, see Appendix A. Each ignitable liquid was re-analyzed as an un-altered (neat) liquid, where 20 µl was diluted with 1 ml of carbon disulfide.

Biological Degradation Methods

Biological degradation of the ignitable liquids was performed by Indiana University Purdue University Indianapolis (IUPUI). Twenty microliters of ignitable liquid was deposited into a quart size metal paint can (Best Containers) with 100 grams of Hyponex® brand potting soil purchased from Kmart. Once sealed, the liquid and soil were mixed. The remaining ignitable liquid was extracted from the soil following ASTM E1412-12, Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with Activated Charcoal [35]. After a specified time period (0, 7, 14, or 21 days), a whole activated charcoal strip (Albrayco) attached to paperclip and nylon string was suspended into the headspace of the can. The can was place into an oven at 65 °C for 16 hours. Once the can was removed from the oven, it was allowed to cool to room temperature and then the charcoal strip was placed inside a 4 ml vial with a screw cap. The activated charcoal strip was sent to NCFS for analysis. Half of the activated charcoal strip was placed into a 2 ml auto-sampler vial with 500 µl of carbon disulfide (Fisher Scientific). For quality assurance purposes, each sample was prepared in duplicate. A secondary sample was extracted in pentane and analyzed by IUPUI. The ignitable liquid patterns of the total ion chromatograms were compared to one another by visual pattern recognition.

Oxygenated liquids studies followed the same methods with variation of the time period (days) that the liquid remained on the soil and the extraction temperature/time, see Table 1.

Table 1: ignitable liquid samples with time periods of biological degradation and the extraction time and temperature.

Experiment	IL Classes	Ignitable Liquid(s)	Time period(s)	Extraction
1	AR, NA, OXY, OXY, AR	SRN 69, 149, 218, 220, 231	0, 7, 14, 21 days	85°C for 4 hours
2	NA	SRN 149	0 days	85°C for 1 hr, 2 hrs, overnight
3	OXY	SRN 220	0, 2, and 7 days	85°C for 4 hours

The quantitative study on biological degradation of ignitable liquids utilized a hydrocarbon mixture comprised of 14 hydrocarbons representing normal alkanes, branched alkanes, cycloalkanes, single ring aromatics, double ring aromatics, and oxygenated compounds, see Table 2. The concentration of the hydrocarbon mixture was equal molar. Twenty microliters of the hydrocarbon mixture was deposited onto 90 grams of potting soil for 0, 2, 7, and 14 days. Extraction was performed by passive headspace as described above at a temperature of 85°C for 4 hours. An internal standard calibration method was used with n-dodecane as the internal standard.

Table 2: hydrocarbons in the equal molar mixture used for a quantitative study of biological degradation.

Compound
toluene
2-methylheptane
1,3-dimethylcyclohexane
2,2,5-trimethylhexane
ethylcyclohexane
p-xylene
2-heptanone
2-butoxyethanol
n-nonane
o-ethyltoluene
1,2,4-trimethylbenzene
n-undecane
2-methylnaphthalene
n-tetradecane

Gasoline studies followed the same methods. The volume of gasoline applied to the soil, the time periods (days) that the liquid remained on the soil and the extraction temperature/time were all varied, see Table 3.

Table 3: gasoline samples with volumes, time periods, and extraction parameters.

Exp	Ignitable Liquid(s)	Volume (μl)	Time period(s)	Temperature and time
1	SRN 116	20 and 80	0, 1, 2, 7 days	65°C for 16 hours
2	SRN 116	80	0 and 7 days	85°C for 4 hours

Evaporation Degradation (weathering) Methods

All ignitable liquids were evaporated and analyzed at NCFS. A graduated microbial was filled with 10 ml of ignitable liquid. The vial was placed into a dry bath in which the height of vial and temperature of dry bath were adjusted according to the percent volume evaporated. Nitrogen flowed gently above the ignitable liquid and a vacuum pump used to remove the ignitable liquid vapor from the vial into a trap. This was to prevent the vapor from condensing back into the vial. The nitrogen needle and vacuum tubing were cleaned between samples and replaced when needed. Evaporation percentages were 25%, 50%, 75%, 90% and 95%, corresponding to volume reductions of 2.5 ml, 5.0 ml, 7.5 ml, 9.0 ml, and 9.5 ml, respectively. During the evaporation process, twenty microliters of ignitable liquid was collected at each of the volume reductions. One milliliter of carbon disulfide was added to an autosampler vial containing the evaporated ignitable liquid. For quality assurance purposes, all samples were evaporated and analyzed in duplicate. The ignitable liquid patterns of the total ion chromatograms were compared to one another by visual pattern recognition.

Instrument Methods

All samples analyzed by NCFS followed the current instrumental method for the Ignitable Liquids Reference Collection (ILRC) database [2]. Gas chromatography-mass spectrometry analysis was performed utilizing an autosampler on an Agilent 6890 gas chromatograph interfaced to a 5973 mass spectrometer. One microliter of the CS2-diluted ignitable liquid was injected into a 250°C injection port. The compounds were separated by a 100 % dimethylpolysiloxane (HP-1) capillary column with a film thickness of 0.50µm, a nominal diameter of 200 µm, and 25 m length. Helium gas was maintained at a constant flow rate of 0.8 ml/min with an average velocity of 36 cm/sec. The injection was split in a 50:1 ratio. The initial oven temperature of 50°C was held for 3 minutes, followed by a temperature ramp of 10°C/min to a final temperature of 280°C, which was held for 4 minutes. The mass spectrometer transfer line temperature was 280°C with a source temperature of 230°C and a quadrupole temperature of 150°C. Mass spectra were scanned between 30 and 350 mass to charge ratio at an acquisition rate of 2-3 scans/second. The detector was turned off at 1.54 minutes until 2.00 minutes during solvent elution.

Samples analyzed by IUPUI were performed on an Agilent gas chromatograph-mass spectrometer (GC-MS). One microliter of the pentane diluted ignitable liquid was injected into a 250°C injection port. The compounds were separated by a 5 % phenyl methyl siloxane (HP-5) capillary column with a film thickness of 0.25µm, a nominal diameter of 250 µm, and 34.7 m length. Helium gas was maintained at a constant flow rate of 1.0 ml/min with an average velocity of 22 cm/sec. The injection was split in a 20:1 ratio. The initial oven temperature of 40°C was held for 3 minutes, followed by a temperature ramp of 10°C/min to a final temperature of 280°C, which was held for 3 minutes. The mass spectrometer transfer line temperature was 280°C with a source temperature of 230°C and a quadrupole temperature of 150°C. Mass spectra were scanned between 40 and 300 mass to charge ratio at an acquisition rate of 2-3 scans/second. The detector was turned on at 2.75 minutes after solvent elution.

Database

Data from each sample was added to the ILRC. After quality assurance review of the data was complete, the following data and information was entered into the ILRC database by NCFS:

- Brand Name; extent of degradation
- Original Classification
- Product Use
- Date of Run
- Total ion chromatogram image
- Data set
- Ion Profiles
- Material Safety Data Sheet

The ILRC committee reviewed the data and provided the following information:

- Classification (confirmed)
- Component class (if applicable)
- Predominant ion profile
- Hydrocarbon range
- Identified major peaks
- Supplemental information concerning the degradation of the liquid

NCFS amended the record with the information supplied by the ILRC committee and release the record to the public section of the database.

Chemometric techniques

The dataset contained 1040 total ion spectra (TIS) of which 574 were undegraded samples (neat liquids), 266 were weathered and 200 were biologically degraded. Total ion spectra are comprised of the sum of ion intensities for each mass-to-charge (m/z) ion over the chromatographic time range [36]. The ion range was 30 m/z to 200 m/z with 32 m/z and 76 m/z omitted since they represented the baseline contribution. The 574 non-degraded TIS were combined into a dataset with 169 variables (m/z) to be used for classification modeling. Pre-processing techniques included normalization in which the intensities of the TIS summed to one and mean centering.

Linear discriminant analysis (LDA) was chosen to classify the samples into the 10 ASTM E1618 classes (with sub-classes for the petroleum distillates): aromatic (AR), gasoline (GAS) heavy petroleum distillate (HPD), isoparaffinic product (ISO), light petroleum distillate (LPD), miscellaneous (MISC), medium petroleum distillate (MPD), normal alkane product (NA), naphthenic paraffinic product (NP), and oxygenated solvents (OXY). Principal component analysis (PCA) is a data reduction technique commonly used prior to LDA to meet the requirements that the number of variables is smaller than the number of samples. PCA was performed and ninety percent of the variance, in this case 13 principal components were retained for LDA. The scores from the 13 principal components became the new variables used to create the LDA model. The model was evaluated using a k-fold or repeated cross-validation method where the dataset of 574 TIS were divided into 10 subsets in which one of the subsets was designated as the test set and the others were combined to form the training set. Each subset was designated the test set once. The training sets were used to create a model and the test sets were used to evaluate the models ability to predict class membership. This process was repeated ten times. After cross-validation, the model was used to predict the classes for three data sets: 1) undegraded samples (n=46), weathered samples (n=266) and the biologically degraded samples (n=200).

Digital Weathering

Ignitable liquids from each of the ASTM E1618 classes were evaporated under a flow of nitrogen, as described above to obtain prescribed fractions f_i $(i=1, 2 \ldots 6)$ of the liquid removed, where the values of f_i correspond to 0.0, 0.25, 0.50, 0.75, 0.90 and 0.95. Evaporations were done at room temperature initially and the temperature of the evaporation pot was increased as necessary to obtain the desired percent evaporation.

The six evaporation fractions from each ignitable liquid were analyzed by automated fitting of the evaporation rates for multiple peaks in the liquid. The automated fitting routine was written in-house and performed in the R Statistical Computing environment [37]. Peaks were selected in the unevaporated sample TIC for each liquid based on a signal-to-noise ratio that would allow for multiple peaks to be selected throughout the chromatographic profile. Each sample TIC was normalized to an integrated area of one and then scaled by the fraction evaporated. This normalization and scaling procedure implicitly assumes that all compounds in an ignitable liquid sample have the same GC-MS response factor and contribute to the volume of the solution in proportion to their integrated intensities. The calibration data in Table 4 show the response factor (Slope=$[A_a/A_{IS}]/[C_a/C_{IS}]$, unitless response factor for internal standard calibration) for a set of aromatic, normal and branched hydrocarbons and two oxygenated solvents [38]. With the exception of n-nonane, the response factors for this variety of compounds range from 0.45 – 1.99, roughly a factor of four.

Table 4: Calibration data, including the response factor (Slope) for 14 analytes representing the range of components found in most ignitable liquids.

Compound	Slope	Slope Std Error	Intercept	Intercept Std Error	R^2 value
toluene	0.7508	0.00494	-0.00115	0.00029	0.9997
p-xylene	0.8882	0.01065	-0.00121	0.00062	0.999
2-ethyltoluene	1.3005	0.01625	-0.00205	0.00094	0.9989
1,2,4-trimethylbenzene	1.3116	0.01706	-0.00230	0.001	0.9988
2-methylnaphthalene	1.839	0.01847	-0.00144	0.00089	0.9989
n-tetradecane	1.9895	0.01612	-0.00371	0.00094	0.9995
n-nonane	0.0807	0.00105	-3.729×10^{-6}	0.00061	0.9988
n-undecane	1.243	0.01535	-0.00144	0.00089	0.9989
2-methylheptane	0.5738	0.00396	-0.00049	0.00023	0.9997
1,3-dimethylcyclohexane	0.6504	0.00484	-0.00108	0.00028	0.9996
2,2,5-trimethylhexane	0.7442	0.00398	-0.0003	0.00012	0.9998
ethylcyclohexane	0.8080	0.00816	-0.00083	0.00047	0.9993
2-heptanone	0.5316	0.00692	-0.00062	0.00040	0.9988
2-butoxyethanol	0.4479	0.00849	-0.00148	0.00049	0.9975

Peak positions were identified by calculating the first derivative of the TIC using a Savitzky-Golay routine (pracma R package) [37]. The rates of loss of the selected peaks were followed throughout the evaporation and modeled as first order processes using the linearized form of the decay, given in Equation 1. In Equation 1, I_0 is the intensity of a peak in the TIC for the 0.0 fraction evaporated sample and I is intensity of the peak in the TIC at other fractions evaporated.

$$ln\left(\frac{I_0}{I}\right) = kt \qquad (1)$$

The fraction evaporated was utilized as the time intervals for the kinetic fitting. The changing profile for a gasoline, petroleum distillate and naphthenic paraffinic samples are shown as a function of the fraction evaporated in Figures 1 – 3. The peaks that were fitted for kinetic decay are marked with a filled circle at the top of the peak in the 0.0 fraction evaporated TIC in each figure. The approach of using the evaporated fraction as the time interval was taken because the forensic application is interested in the change in the TIC profile as a function of the fraction evaporated. In a fire, there is no control over the temperature and time profiles experienced by the sample. The most volatile samples elute early in the chromatographic profile and are lost at faster rates than the less volatile samples, which elute later. This can be clearly seen in Figure 1. A minimum of three points are required to estimate the single fitting variable, k, in Equation 1. Multiple rate constants were calculated for each peak by fitting the intensity at f_1 through f_i *(i=3, 4, 5 and 6)*. The best fitted rate constants were selected by (1) requiring the intercept of the plot from Equation 1 to not be statistically different from 0 at the $\alpha = 0.05$ level, (2) by requiring that the fitted rate constant be positive (indicating a decay process) and statistically differ from 0 at the $\alpha = 0.05$ level and (3) requiring that the coefficient of determination (r^2) be greater than or equal to 0.9. The analysis and rate selection resulted in a total of 1899 rate constants from 50 different ignitable liquids in eight ASTM E1618 classes.

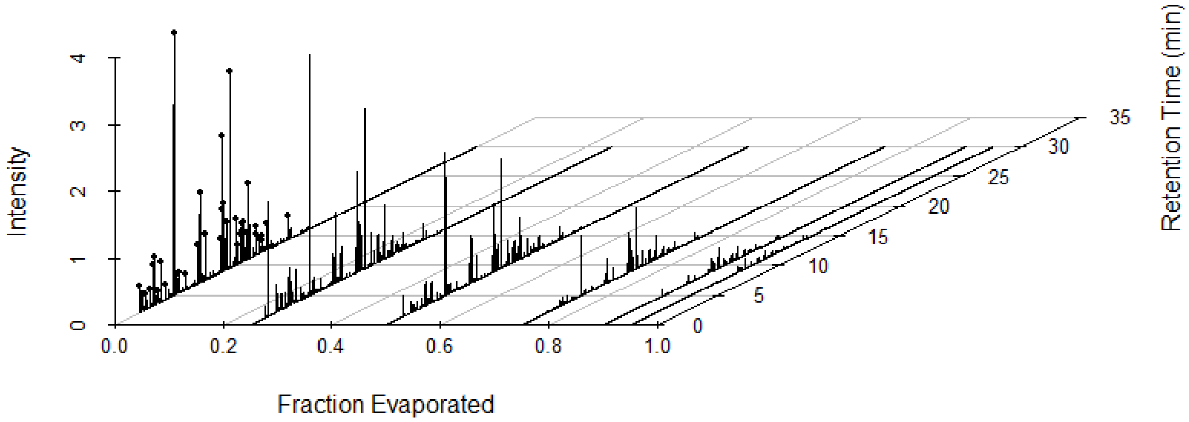


Figure 1 Changing profile for a gasoline sample is shown as a function of the fraction evaporated.

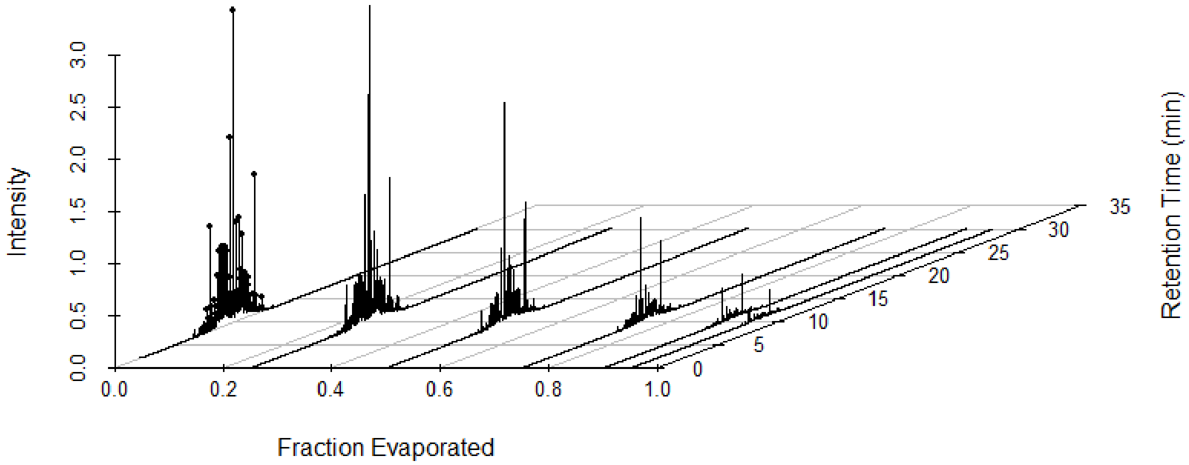


Figure 2 Changing profile for a petroleum distillate sample is shown as a function of the fraction evaporated.

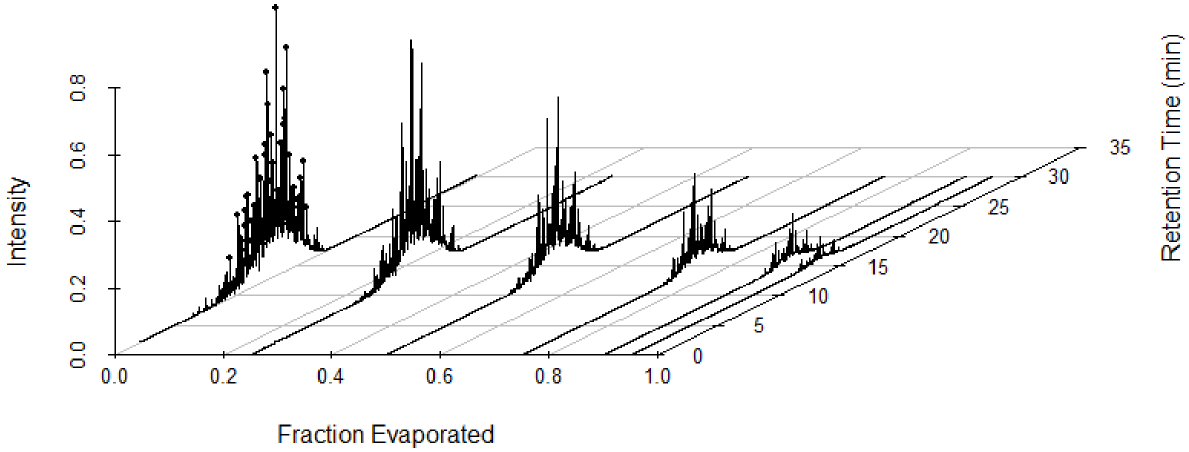


Figure 3. Changing profile for a naphthenic paraffinic sample is shown as a function of the fraction evaporated.

III. Results

Modification of Ignitable Liquids Reference Collection Database

Design Modifications

The Ignitable Liquids Reference Collection database was re-designed by NCFS and the ILRC committee of S/TWGFEX to include the following new features (see also Figure 4):

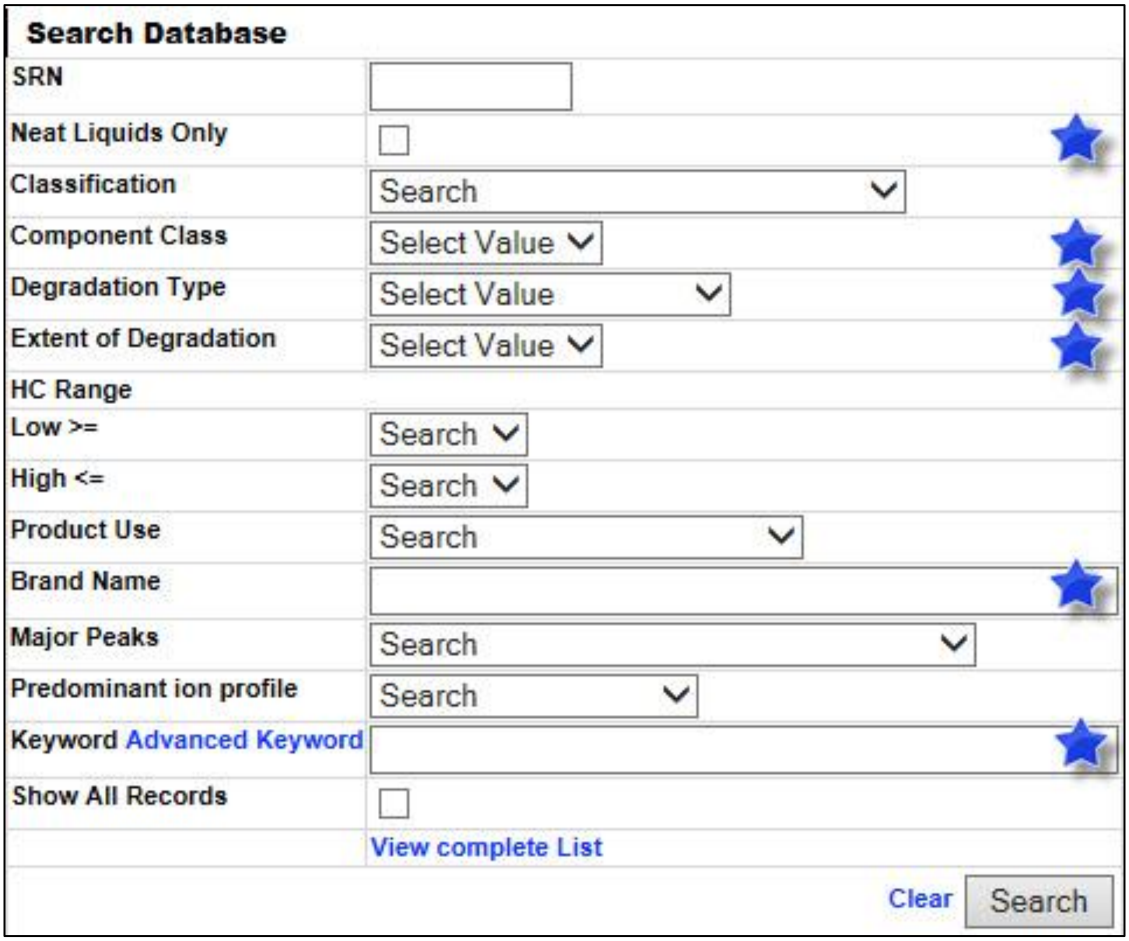
Search

- ★ View only neat liquids
- ★ Component Class: a list of the classes identified within an ignitable liquid classified as either a miscellaneous category or oxygenated solvent.
- ★ Degradation Type: Biological or Weathered
    - ▪ Extent of Degradation: 0, 7, 14, or 21 days

        - 25%, 50%, 75%, 90%, 95%, or 99%

★ Brand Name: As user types in text a list is provided.

★ Keyword: As user types in text a list is provided.



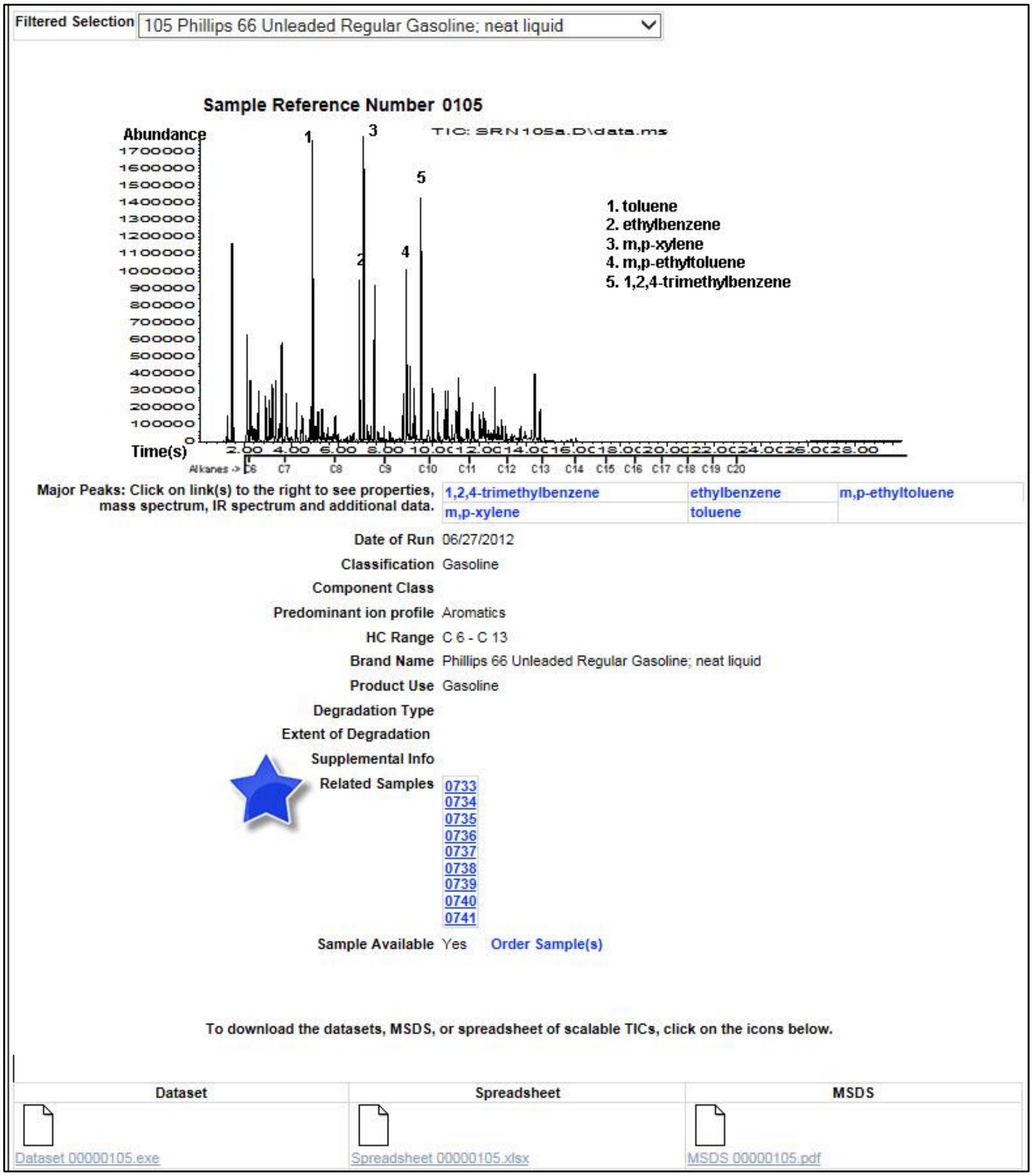
Search Database

SRN		
Neat Liquids Only	☐	★
Classification	Search	
Component Class	Select Value	★
Degradation Type	Select Value	★
Extent of Degradation	Select Value	★
HC Range		
Low >=	Search	
High <=	Search	
Product Use	Search	
Brand Name		★
Major Peaks	Search	
Predominant ion profile	Search	
Keyword Advanced Keyword		★
Show All Records	☐	
	View complete List	
	Clear Search	

Figure 4. Screen shot of modified ILRC database search parameter input.

<u>Sample Detail and Download Page</u>: All records related to a degraded ignitable liquid are linked to one another (see also Figure 5).

- ★ Related Samples: Produces a page with all related record total ion chromatograms. The user can filter the page according to the type of degradation.



Filtered Selection: 105 Phillips 66 Unleaded Regular Gasoline; neat liquid

Major Peaks: Click on link(s) to the right to see properties, mass spectrum, IR spectrum and additional data.

1,2,4-trimethylbenzene	ethylbenzene	m,p-ethyltoluene
m,p-xylene	toluene	

Date of Run	06/27/2012
Classification	Gasoline
Component Class	
Predominant ion profile	Aromatics
HC Range	C 6 - C 13
Brand Name	Phillips 66 Unleaded Regular Gasoline; neat liquid
Product Use	Gasoline
Degradation Type	
Extent of Degradation	
Supplemental Info	
Related Samples	0733 0734 0735 0736 0737 0738 0739 0740 0741
Sample Available	Yes Order Sample(s)

To download the datasets, MSDS, or spreadsheet of scalable TICs, click on the icons below.

Dataset	Spreadsheet	MSDS
Dataset 00000105.exe	Spreadsheet 00000105.xlsx	MSDS 00000105.pdf

Figure 5. Screen shot of Sample Detail and Download page.

New Records

Fifty ignitable liquids were diluted and analyzed as neat liquids (parent record). Forty-six were updated records with four new records for the new ignitable liquids. Each ignitable liquid was biologically degraded at 0, 7, 14, and 21 days (200 records). Evaporation occurred at 25%, 50%, 75%, 90%, and 95% when possible; however, some liquids containing higher molecular weight compounds did not evaporate at the higher percentages (236 records). The total number of samples prepared, analyzed, reviewed, and added to the database as a new or updated record was 486.

Biological Degradation Studies

Oxygenated Solvents

Initial results from the biological degradation of the five oxygenated solvents exhibited limited recovery of the oxygenated compounds, results are in Table 5. Especially disconcerting were the results from the Day 0 samples. Two possible reasons for the results were examined: 1) the soil was retaining the oxygenated compounds, or 2) the passive headspace extraction parameters were not optimal. A study was undertaken to determine why a majority of the oxygenated compounds were not present in the biologically degraded samples. Experiment 1 degraded all five of the oxygenated solvent ignitable liquids for the same time periods of 0, 7, 14, and 21 days; however the extraction parameters for oxygenated solvents were changed to 85°C for 4 hours, results are in Table 6. The IUPUI instrument method was modified from a solvent delay to turning off the source (1.42 – 1.51 minutes) only during solvent elution. This was done in order to detect some of the oxygenated compounds that eluted during the solvent delay in the original method. More oxygenated compounds were detected in Day 0 samples with an extraction temperature of 85°C for 4 hours compared to 65°C for 16 hours indicating the extraction parameters were affecting the ability of the oxygenated compounds to remain adsorbed onto the activated charcoal strip. The few that were not recovered at Day 0 have low boiling points and were not highly concentrated. No oxygenated compounds were recovered at Days 7, 14, and 21.

Table 5: Results from original biological degradation of oxygenated solvents. The extraction temperature was 65°C and the time was 16 hours. SD indicates the compound elutes during the solvent delay.

	0 days		**7 days**		**14 days**		**21 days**	
	IUPUI	NCFS	IUPUI	NCFS	IUPUI	NCFS	IUPUI	NCFS
SRN 69								
acetone	SD	No	SD	No	SD	No	SD	No
2-butoxyethanol	No	No	No	No	No	No	No	No
SRN 149								
Ethanol	No	No	No	No	No	No	No	No
Ethyl acetate	No	No	No	No	No	No	No	No
Methyl isobutyl ketone	Yes	Yes	No	No	No	No	No	No
SRN 218								
2-butanone	SD	No	SD	No	SD	No	SD	No
1-methoxy-2-propylacetate	No	No	No	No	No	No	No	No
SRN 220								
2-butanone	SD	No	SD	No	SD	No	SD	No
butylacetate	No	No	No	No	No	No	No	No
Ethyl-3-ethoxypropionate	Yes	Yes	No	No	No	No	No	No
SRN 231								
isopropanol	No	No	No	No	No	No	No	No
2-butanone	Yes	No	No	No	No	No	No	No

Table 6: Results from second biological degradation of oxygenated solvents. The extraction temperature was 85°C and the time was 4 hours. SD indicates the compound elutes during the solvent delay.

	0 days		7 days		14 days		21 days	
	IUPUI	NCFS	IUPUI	NCFS	IUPUI	NCFS	IUPUI	NCFS
SRN 69								
acetone	Yes	No	No	No	No	No	No	No
2-butoxyethanol	No	No	No	No	No	No	No	No
SRN 149								
Ethanol	Yes	No	No	No	No	No	No	No
Ethyl acetate	No	No	No	No	No	No	No	No
Methyl isobutyl ketone	Yes	Yes	No	No	No	No	No	No
SRN 218								
2-butanone	Yes	No	No	No	No	No	No	No
1-methoxy-2-propylacetate	No	No	No	No	No	No	No	No
SRN 220								
2-butanone	Yes	Yes	No	No	No	No	No	No
butylacetate	Yes	No	No	No	No	No	No	No
Ethyl-3-ethoxypropionate	Yes	Yes	No	No	No	No	No	No
SRN 231								
isopropanol	No	No	No	No	No	No	No	No
2-butanone	Yes	Yes	No	No	No	No	No	No

Experiment 2 was conducted by two committee members using SRN 149 on soils native to their location. The same volume of liquid and weight of soil were applied; however the liquids remained on the soil only for Day 0, the extraction temperature was 85°C, and the samples were heated for 1 hour, 2 hours, and overnight. Another extraction technique was utilized where the headspace vapors were collected and directly injected into the GC-MS following ASTM E1388 Standard Practice of Sampling Headspace Vapors from Fire Debris Samples [39]. These results also indicate that the extraction parameters were affecting the recovery of the oxygenated compounds.

Table 7: Results from experiment 2 biological degradation of SRN 149. The time period in which the liquid remained on the soil was shortened. Also, another extraction method was employed (headspace vapor). SD indicates the compound elutes during the solvent delay.

	1 hour	2 hours	overnight	Headspace Vapor
Alabama				
Ethanol	Yes	Yes	No	No
Ethyl acetate	SD	SD	SD	SD
Methyl Isobutyl Ketone	Yes	Yes	Yes	No
Georgia				
Ethanol	Yes	Yes	No	Yes
Ethyl acetate	No	No	No	No
Methyl Isobutyl Ketone	Yes	Yes	Yes	Yes

Experiment 3 was designed to determine whether there was any interaction between the soil and the oxygenated compounds thus causing their limited recovery. The oxygenated solvent SRN 220 containing toluene, butyl acetate, m or p-xylene, and ethyl-3-ethoxpropionate remained on the soil for periods of 0, 2, and 7 days and was extracted at temperatures of 85°C for 4 hours. Half of the samples deposited the liquid on untreated soil (as in previous experiments) and the other half of the samples deposited the liquid on sterilized soil. All oxygenated compounds were recovered from the sterilized soil suggesting no interaction with the soil, see Table 8. One of the oxygenated compounds from the untreated soil was recovered on Day 2, but not Day 7.

Table 8: Results from experiment 3 biological degradation of SRN 220 on untreated and sterilized soil.

	0 days	**2 days**	**7 days**
Untreated soil			
toluene	Yes	Yes	No
butyl acetate	No	No	No
m/p-xylene	Yes	Yes	No
ethyl-3-ethoxypropionate	Yes	No	No

Sterilized soil			
toluene	Yes	Yes	Yes
butyl acetate	Yes	Yes	Yes
m/p-xylene	Yes	Yes	Yes
ethyl-3-ethoxypropionate	Yes	Yes	Yes

The study on biological degradation of oxygenated solvents indicates there are difficulties in extracting low boiling point oxygenated compounds by passive headspace adsorption onto activated charcoal. More studies are required to optimize the extraction of these compounds. The rate of biological degradation is rapid for oxygenated compounds, possibly within 2 days.

Gasoline Studies

Even though biological degradation results of the five gasoline samples were similar to one another; the committee requested an additional study because the results were not similar to those published by Mann [24]. The additional study consisted of depositing a larger volume of gasoline (80 µl) onto the 100 grams of soil and leaving the gasoline on the soil for shorter periods of time (0, 1, 2, and 7 days). These conditions were analogous to the Mann experiments. The same experiments were repeated using the 20 µl of gasoline to ensure any differences in the results were due to the volume of gasoline deposited onto the soil and not the condition of the soil or the time period. Ten months had elapsed between the original biodegradation experiments (September 2012) and the experiments for the study (July 2013). Possible changes in the bacterial populations in the soil were not monitored in this work.

Total ion chromatograms for the original 2012 experiments and those from the study in 2013 are shown in Figures 6 – 8. A list of the major peaks identified in the samples is in Table 9. All samples except for the Day 7 samples contained the same major peaks; however, the peaks have different relative ratios. In the Day 7 samples, the branched alkane, 2,3,3-trimethylpentane which co-eluted with toluene was identified. This was possible because toluene had been completely consumed and was no longer present. Even though the relative ratios of the major peaks were not the same for all the samples, the general tendency was complete degradation of toluene, a significant reduction of ethylbenzene compared to the xylenes, and less degradation of the branched alkanes compared to the aromatics. There were notable differences between the 20 µl samples. The increase in volume did not increase the rate of consumption whereby no aromatics except 1,3,5-trimethylbenzene remained as observed in the Mann studies. It appears as though the 0 day from 2013 was slightly weathered compared to the 2012 sample. The relative ratios between the 7 day samples also indicate possible weathering; however there are peaks such as 2,2-dimethyl-3-pentanone in the 2012 sample which are not in the 2013 sample. In 2013, the soil was observed to be much drier than in 2012.

Table 9: List of major peaks identified in gasoline samples. Identification of 2, 2-dimethyl-3-pentanone was a tentative identification with NIST mass spectral library and not confirmed with a standard.

Number	Compound	Retention Time (min.)
1	2,2,4-trimethylpentane	3.59
2	2,3,4-trimethylpentane	4.81
3	toluene	4.88
4	2,3,3-trimethylpentane	4.90
5	2,2-dimethyl-3-pentanone	5.46

6	ethylbenzene	6.88
7	m- or p- xylene	7.05
8	o-xylene	7.53
9	m-ethyltoluene	8.89
10	1,3,5-trimethylbenzene	9.04
11	1,2,4-trimethylbenzene	9.51

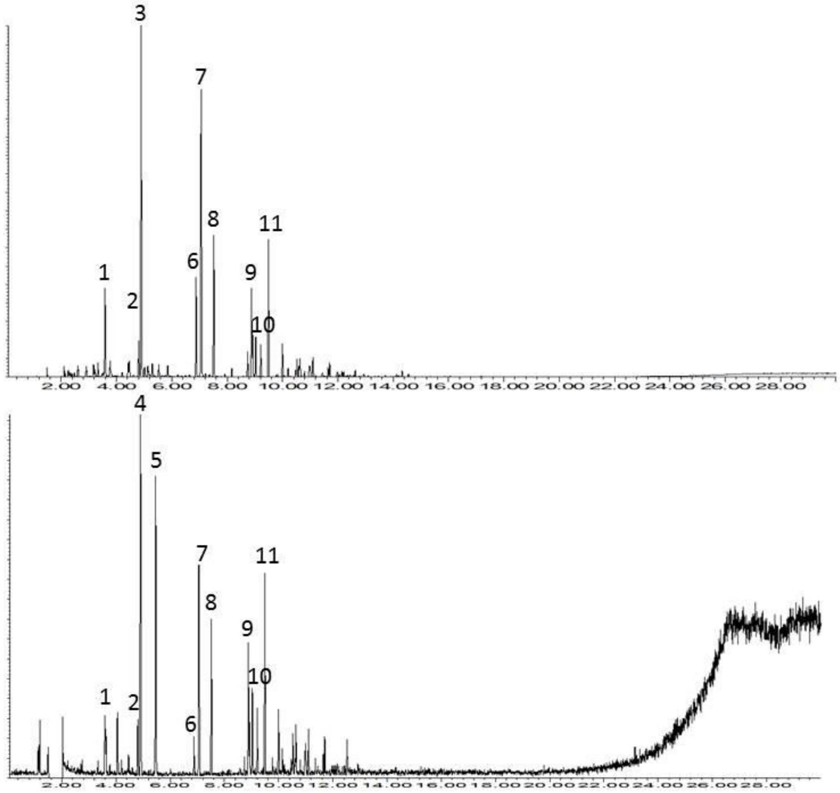


Figure 6: Total ion chromatograms of 20µl of SRN 116 gasoline on 100 grams of soil biologically degraded for 0 and 7 days from September 2012.

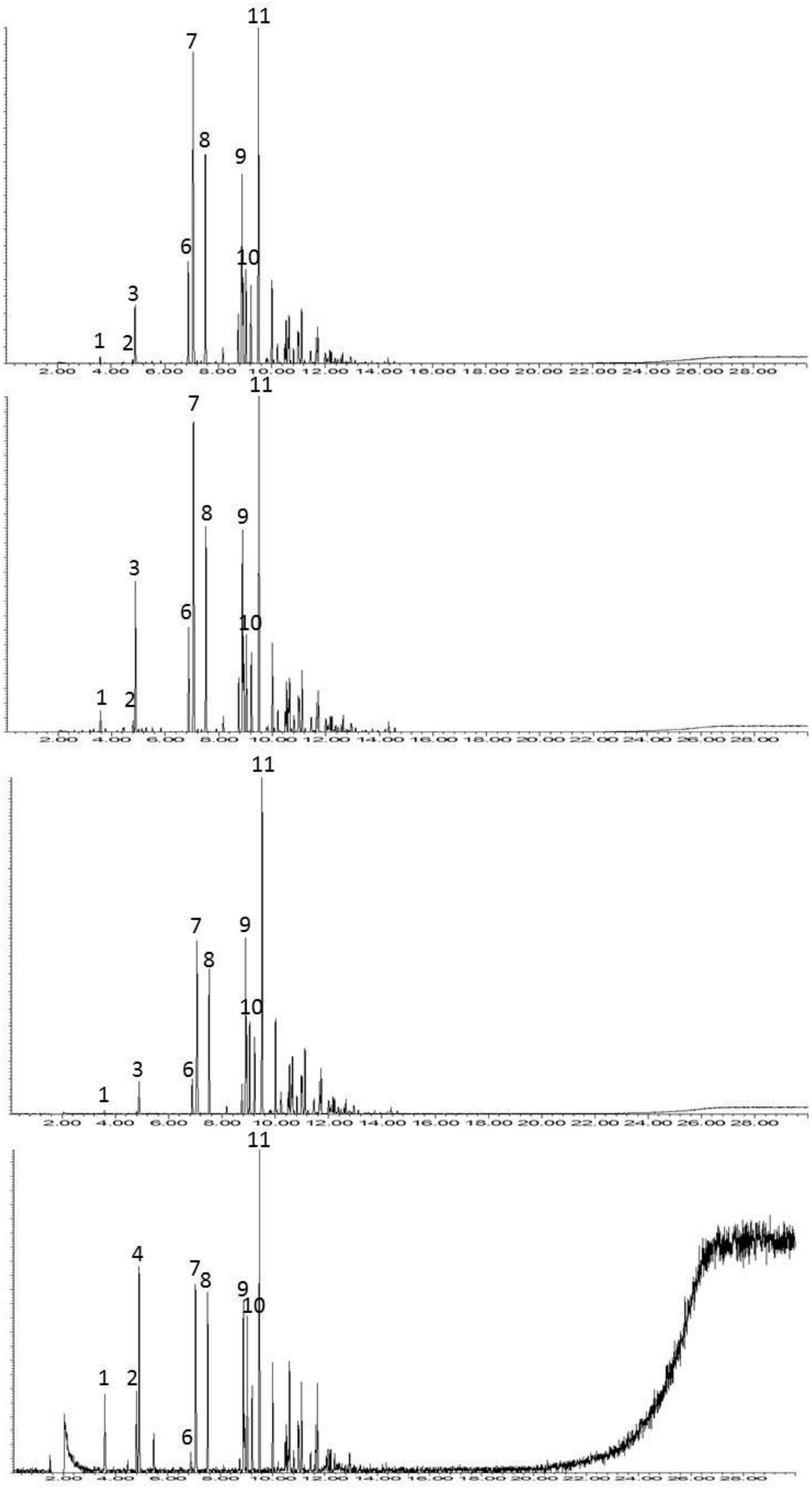


Figure 7: Total ion chromatograms of 20µl of SRN 116 gasoline on 100 grams of soil biologically degraded for 0, 1, 2, and 7 days from July 2013.

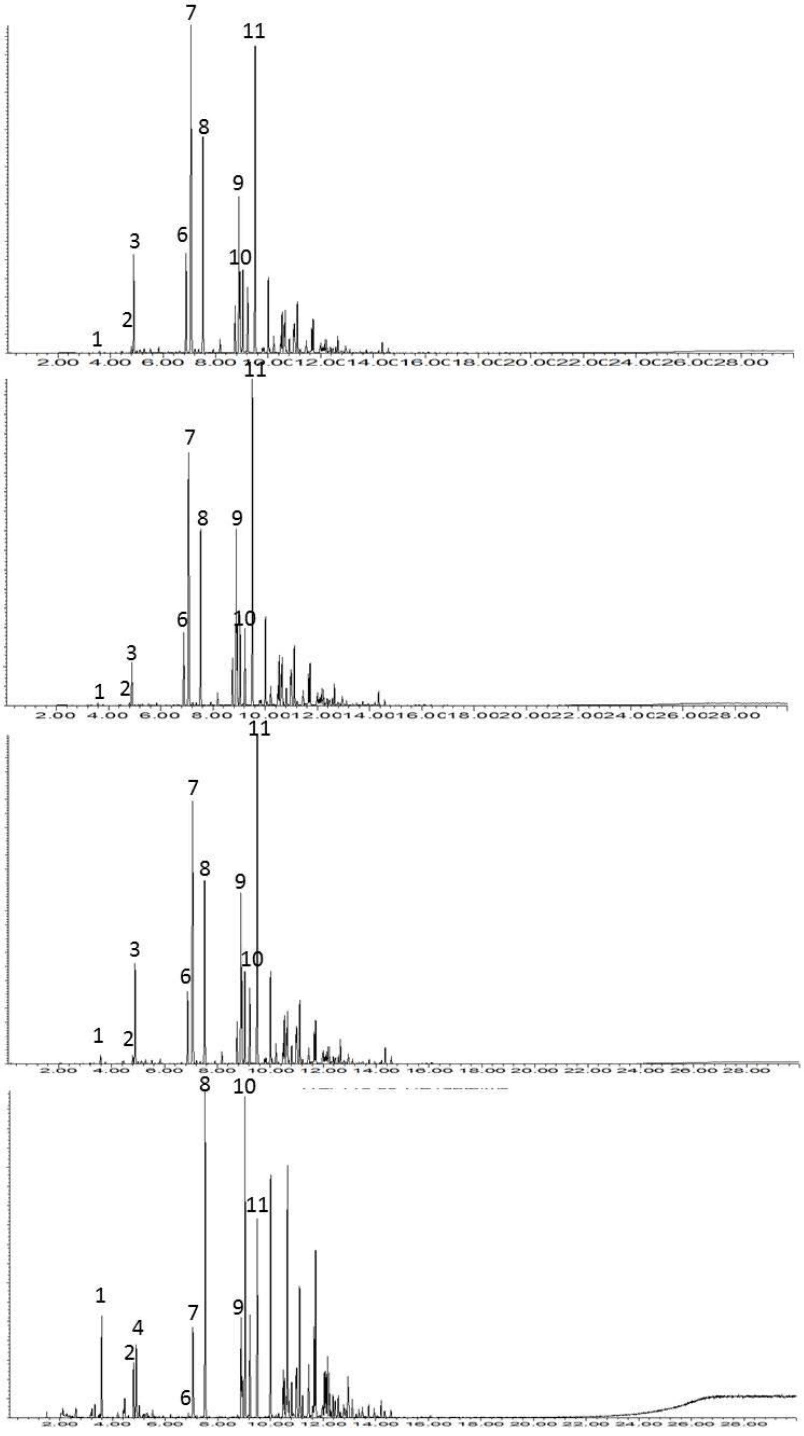


Figure 8: Total ion chromatograms of 80µl of SRN 116 gasoline on 100 grams of soil biologically degraded for 0, 1, 2, and 7 days from July 2013.

The other difference between the NCFS experiments and the Mann experiments was the extraction time and temperature. Therefore, two samples were created with 80 µl of SRN 116 gasoline on 100 grams of the same soil for 0 and 7 days. One was extracted at 65 °C for 16 hours and the other at 85 °C for 4 hours. Total ion chromatograms are shown in figure 9. Day 0 results are comparable to one another although the C2 alkylbenzenes are more abundant for the 65°C for 16 hours sample. Day 7 results are also similar to one another with slight variations in the relative abundances; however all of the major compounds are the same. Day 0 samples contain mostly aromatics, but the Day 7 samples are mostly branched alkanes with some heavier aromatics. The extraction temperature and time did not significantly affect the results. The presence of the branched alkanes and 1,3,5 –trimethylbenzene in Day 7 coincide with Mann's results yet the NCFS samples still have identifiable aromatics such as m,p-xylene, o-xylene, m-ethyltoluene, and 1,2,4-trimethylbenzene.

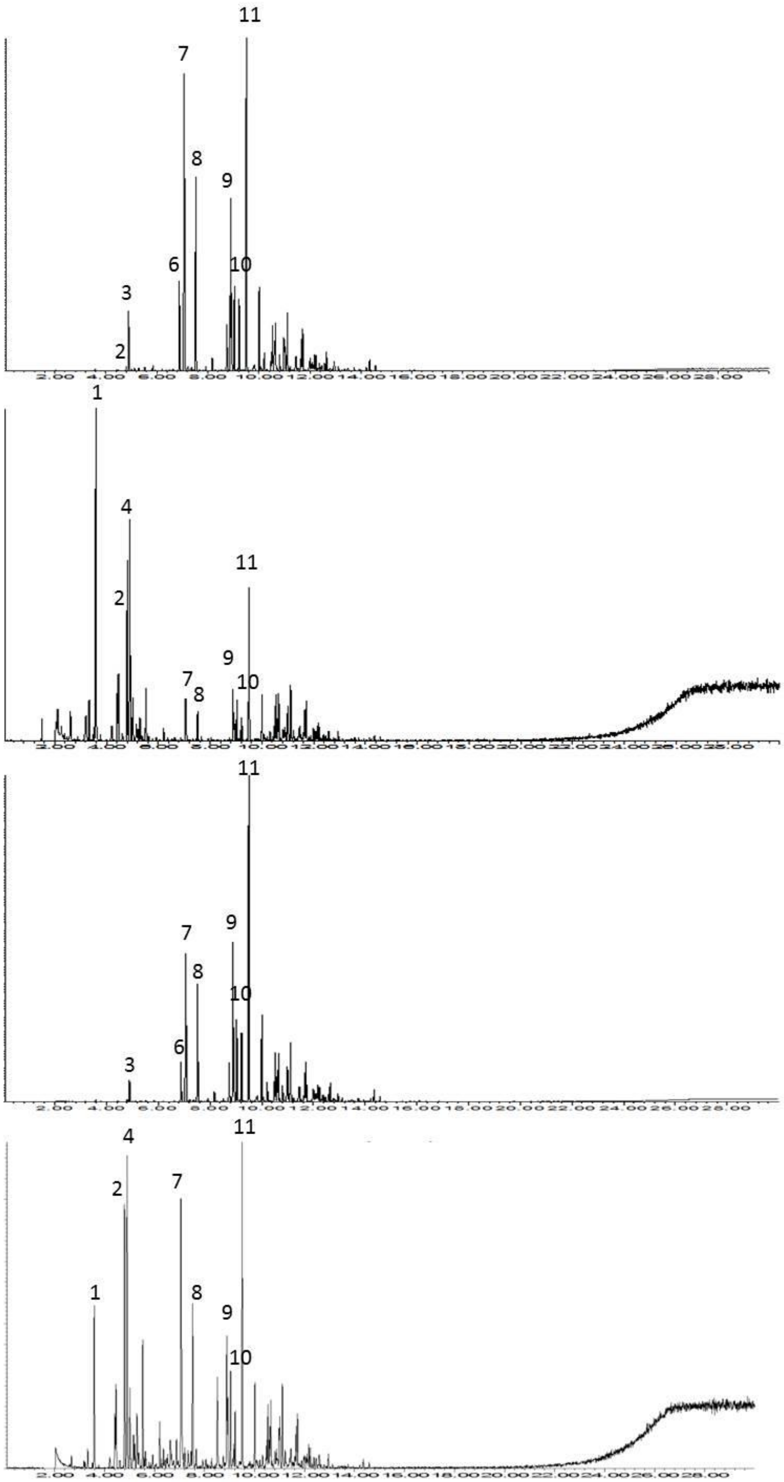


Figure 9: Total ion chromatograms of 80µl of SRN 116 gasoline on 100 grams of soil biologically degraded for 0 and 7 days. The first two TICS were extracted at 65°C for 16 hours and the last two were extracted at 85° for 4 hours.

Quantitative Study

A quantitative study on the biological degradation of ignitable liquids was performed using a simple hydrocarbon mixture of compounds typically identified in ignitable liquids and encompassing a range of compound types (i.e. aromatics, normal alkanes, branched alkanes, etc.). See Table 4 for a list of the compounds in the mixture. When the compounds were biologically degraded individually, the formation of by-products was not observed. Relative recovery of each compound as related to Day 0 is demonstrated in Figure 10. All compounds are recovered at Day 0 with the exception of 2-butoxyethanol. By Day 2, the other oxygenated compound (2-heptanone) is no longer present and there is a marked reduction in the abundance of normal alkanes and toluene. The aromatics and 2-methylheptane (minimally branched) are absent or significantly reduced in abundance by Day 7 with the polynuclear aromatic, a tri-substituted branched alkane and 2 cycloalkanes remaining. There were only trace amounts of a few identifiable compounds from the mixture at Day 14. Relative recovery of two compounds 1,3-dimethylcyclohexane and 2,2,5-trimethylhexane are greater than one. The reason is believed to be related to the heterogeneity of the soil (i.e. bacteria population and the number of absorption sites). The total moles of recovered hydrocarbon is plotted over the biological degradation periods in Figure 11 revealing a progressive loss over time. The total moles recovered (x 10^6) were 13.89, 9.43, and 3.67 for Days 0, 2, and 7 respectively. The calculated overall decay rate constant was 0.22 days^{-1}, corresponding to a half-life of 3.5 days. Figure 12 represents a first order graph where the rate constant indicates approximately 0.2 moles per day were lost due to biological degradation.

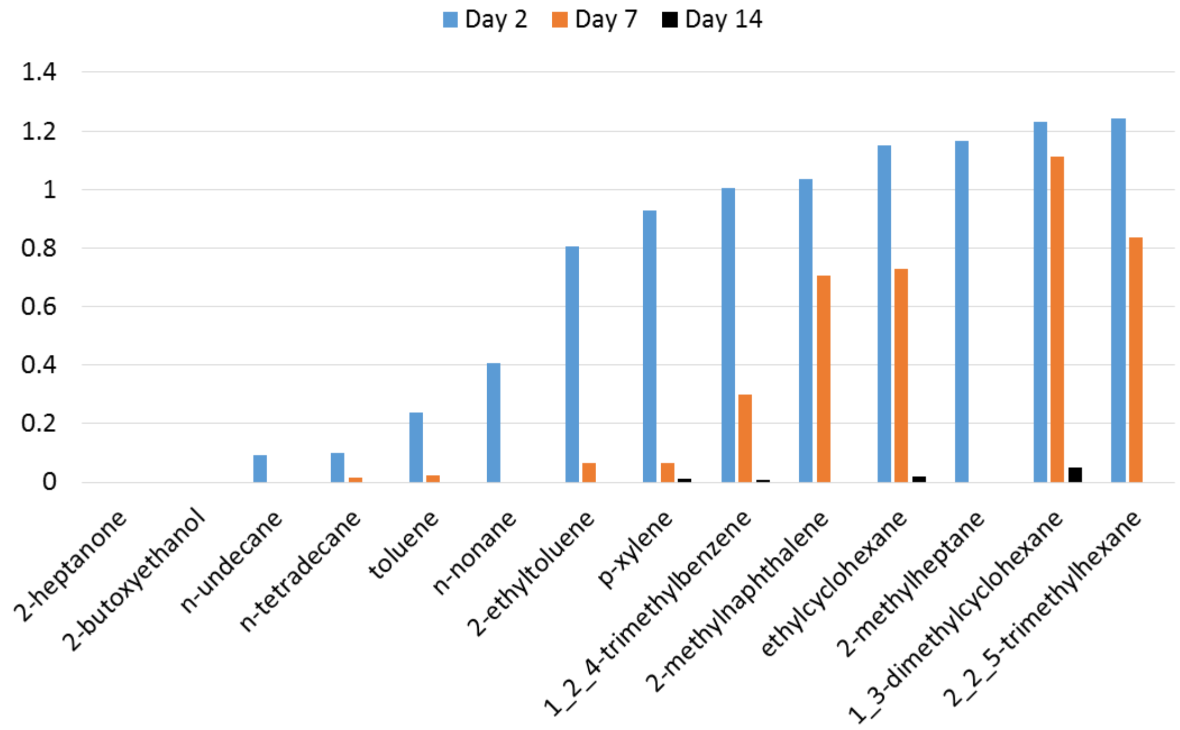


Figure 10. Recovery of hydrocarbons relative to Day 0 in order of earliest to degrade to the latest to degrade.

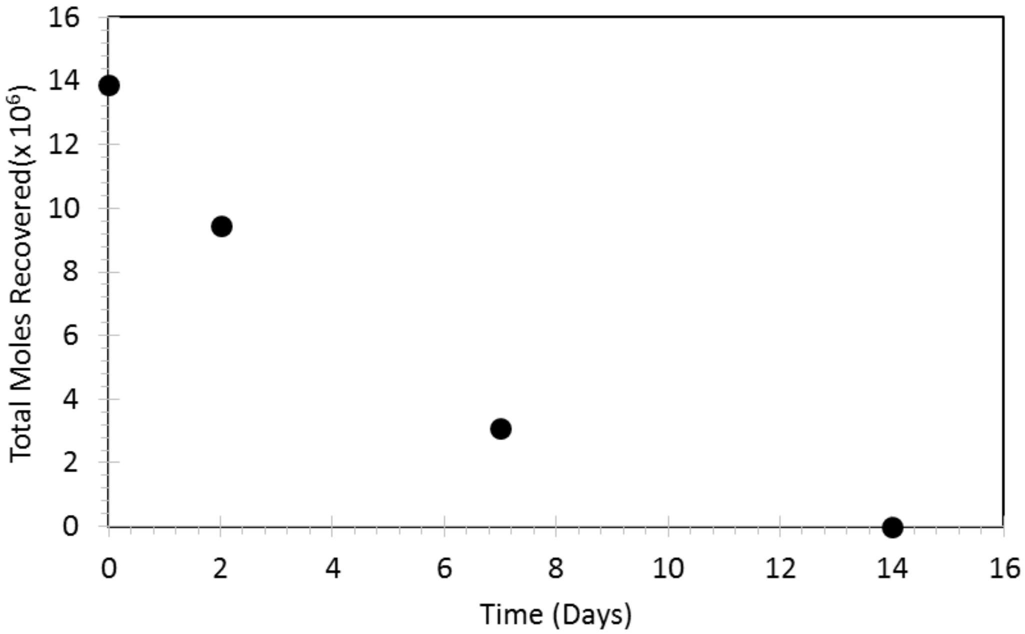


Figure 11. Recovery of hydrocarbons relative to Day 0 in order of earliest to degrade to the latest to degrade.

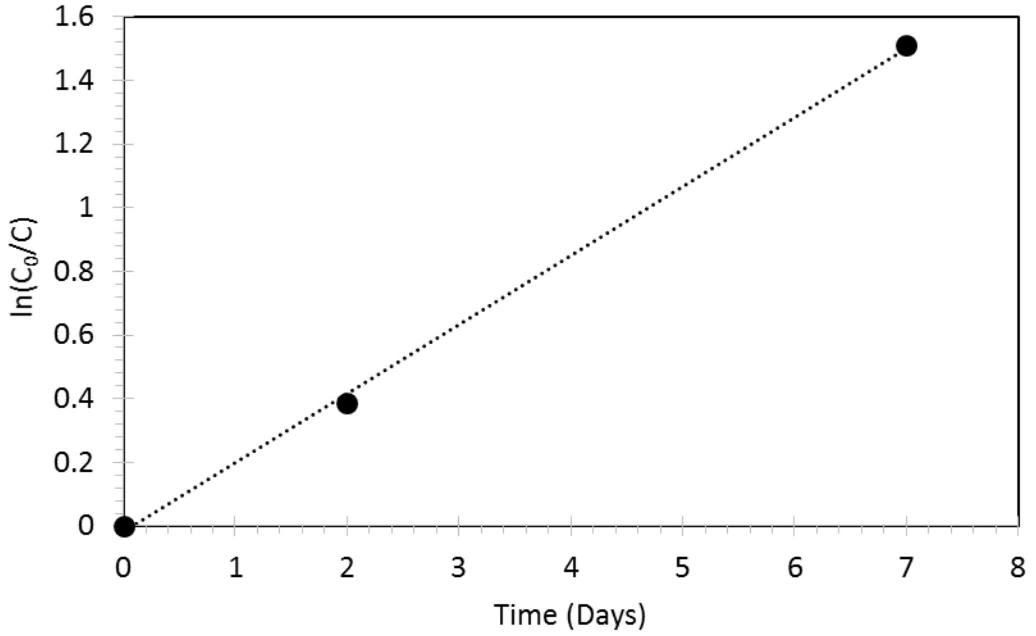


Figure 12. Total moles recovered over the biological degradation periods if 0, 2, 7, and 14 days.

Analysis of the possibility of misclassification

Linear Discriminant Analysis

Results for the cross validation, non-degraded samples, weathered samples, and biologically degraded samples are compiled into individual confusion matrices. The classes assigned to the sample in the database are located in the columns of the confusion matrices. The model predicted classes are located in the rows. Assignment of the classifications as True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) is demonstrated for a simple three class system in Table 10. True positives refer to samples correctly classified (i.e., class **a** assigned to **A**) and these numbers lie along the diagonal of the confusion matrix for each class. False positives for a specific class refer to samples that were incorrectly predicted to belong to that class (i.e. samples of class **b** and **c** that were assigned to **A**). True negatives refer to samples that do not belong to a specified class and were classified into one of the other classes (i.e. class **b** and **c** samples that were assigned, correctly or incorrectly, to **B** and **C**). False negatives refer to samples from a specific class that were incorrectly assigned to a different class (i.e., class **a** samples assigned to **B** and **C**). These relationships are shown for a sample of class **a** in Table 10. The true positive rate (TPR) or sensitivity is TP/ (TP+ΣFN). The false positive rate (FPR) or specificity is ΣFP/ (ΣTN+ΣFP). The accuracy of the model is (TP + ΣTN)/(total population). In these formulas, the summation over multiple confusion table entries is symbolized by Σ. For a given class (**a** in Table 10), only the TP will correspond to a single table entry, all others will require summing multiple entries.

Table10. : Confusion matrix showing TP, TN, FP and FN entries for a sample of known class a (see text for further explanation.

		True Class		
		a	**b**	**c**
Predicted Class	**A**	TP	FP	FP
	B	FN	TN	TN
	C	FN	TN	TN

The TPR, FPR and accuracy, along with the confusion matrices for the model cross validation, and application of the LDA model to un-weathered, weathered and biologically degraded ignitable liquids can be seen in Tables 11 – 14, respectively. The TPR, FPR and accuracy values are calculated for each class of ignitable liquid by treating the data as a dichotomous classification (i.e., the specified class against all others), as described above. The TPR and FPR values are plotted in ROC space in Figure 13. The plot depicts LDA model performance for the cross validation data and for class predictions for un-weathered, weathered and biologically degraded ignitable liquids. Perfect prediction for membership in a class would be reflected by a point with a TPR = 1 and a FPR = 0. As performance of the classifier decreases, points move away from the upper left-hand corner of the ROC graph. The graph for the cross validation and un-weathered sample class predictions reflect similar performance of the model. Although TPR values are low for some classes, the FPR values stay very low for all classes. Classification of the weathered samples results in a clear separation between those classes with a TPR ≥ 0.8 (AR, HPD, ISO, LPD and NA) and those with a TPR <0.5 (GAS, MISC, MPD, NP and OXY). All of the weathered samples are classified with a FPR ≤0.11. These results indicate that weathering will reduce the TPR for classification of some classes by LDA, but does not significantly increase the FPR. The reduction in TPR is due to classification of weathered samples into the wrong ASTM class. Most GAS samples began to misclassify as AR when weathered at 75% or 90%. All 95% weathered GAS misclassified as AR and the 99% weathered GAS misclassified as HPD. MISC samples that are blends (combination of 2 classes) classified into the class with the higher carbon range. For example, SRN 16 is a blend of an AR and an HPD, which loses the more volatile AR component upon weathering. Some distillates classified into the distillated class with the higher carbon range (i.e. LPD to MPD and MPD to HPD). A significant number of weathered NP samples misclassified as HPD. NP samples that exhibited this behavior were initially misclassified as MPD. Some weathered OXY samples misclassified as MISC after the oxygenated compounds evaporated completely. There is a decrease in the percentage of correct classification as the samples are weathered.

Table 11: Cross validation results for the LDA classification model.

		Database Assigned Class												
		AR	**GAS**	**HPD**	**ISO**	**LPD**	**MISC**	**MPD**	**NA**	**NP**	**OXY**	**TPR**	**FPR**	**Accuracy**
Predicted Class	**AR**	**24**	4	0	0	0	11	0	0	0	3	0.86	0.03	0.96
	GAS	0	**22**	0	0	0	4	0	0	0	2	0.76	0.01	0.98
	HPD	0	0	**58**	0	1	18	0	0	2	2	0.88	0.05	0.95
	ISO	0	0	0	**36**	0	14	0	0	0	0	0.95	0.03	0.97
	LPD	0	0	0	0	**23**	5	1	0	0	6	0.82	0.02	0.97
	MISC	4	1	2	1	1	**60**	13	0	0	18	0.41	0.09	0.78
	MPD	0	0	1	0	3	20	**96**	0	4	2	0.86	0.06	0.92
	NA	0	0	3	0	0	1	0	**18**	0	0	1.00	0.01	0.99
	NP	0	1	2	0	0	5	2	0	**8**	0	0.57	0.02	0.97
	OXY	0	1	0	1	0	8	0	0	0	**62**	0.65	0.02	0.93

Table 12: LDA results for analysis of un-weathered samples

		Database Assigned Class												
		AR	GAS	HPD	ISO	LPD	MISC	MPD	NA	NP	OXY	TPR	FPR	Accuracy
Predicted Class	**AR**	5	0	0	0	0	0	0	0	0	0	1.00	0.00	1.00
	GAS	0	4	0	0	0	2	0	0	0	0	1.00	0.05	0.95
	HPD	0	0	2	0	0	0	0	0	0	0	0.67	0.00	0.98
	ISO	0	0	0	4	0	1	0	0	0	0	0.80	0.02	0.95
	LPD	0	0	0	0	3	0	0	0	0	0	1.00	0.00	1.00
	MISC	0	0	1	0	0	3	2	0	0	0	0.43	0.08	0.84
	MPD	0	0	0	0	0	1	4	0	3	0	0.67	0.10	0.86
	NA	0	0	0	1	0	0	0	4	0	0	1.00	0.02	0.98
	NP	0	0	0	0	0	0	0	0	1	0	0.25	0.00	0.93
	OXY	0	0	0	0	0	0	0	0	0	3	0.60	0.00	1.00

Table 13: LDA classification results for weathered ignitable liquids.

		Database Assigned Class												
		AR	GAS	HPD	ISO	LPD	MISC	MPD	NA	NP	OXY	TPR	FPR	Accuracy
Predicted Class	**AR**	21	21	0	0	0	5	0	0	0	0	0.88	0.11	0.89
	GAS	0	21	0	0	0	1	0	0	0	0	0.41	0.00	0.88
	HPD	0	2	17	0	0	4	8	0	13	0	1.00	0.11	0.90
	ISO	0	0	0	23	0	6	0	0	0	0	0.92	0.02	0.97
	LPD	0	0	0	0	12	0	0	0	0	0	0.80	0.00	0.99
	MISC	0	0	0	0	0	10	9	0	0	13	0.29	0.09	0.83
	MPD	0	0	0	0	3	8	14	0	3	0	0.44	0.06	0.88
	NA	0	0	0	2	0	0	0	20	0	0	1.00	0.01	0.99
	NP	2	0	0	0	0	0	0	0	7	0	0.30	0.01	0.93
	OXY	1	7	0	0	0	0	1	0	0	12	0.48	0.04	0.92

For the biologically degraded samples, the TPR drops to less than 0.5 for all classes except ISO and OXY; however, the FPR remains very low for all classes, except OXY (FPR = 0.54). The class least affected by the biological degradation was ISO with a TPR of 0.750. OXY has the highest TPR at 0.88; however OXY has the highest FPR at 0.54 signifying that typically if a biologically degraded sample misclassifies it classify as OXY. All GAS and HPD samples remaining on the soil 7 – 21 days were misclassified as OXY with the exception of one HPD at 7 days misclassifying as NP. MPD and NP classified correctly in days 0 and 7 then misclassifies as OXY. The remaining classes began to misclassify somewhere between 7 or 14 days on the soil. The correct classification rate decreases rapidly with the number of days the ignitable liquid residue remains on the soil.

Table 14: LDA results for classification of biologically degraded ignitable liquids.

		Database Assigned Class												
		AR	**GAS**	**HPD**	**ISO**	**LPD**	**MISC**	**MPD**	**NA**	**NP**	**OXY**	**TPR**	**FPR**	**Accuracy**
Predicted Class	**AR**	10	2	0	0	0	1	0	0	0	0	0.50	0.02	0.94
	GAS	0	5	0	0	0	0	1	0	0	0	0.18	0.01	0.88
	HPD	0	0	3	0	0	2	0	0	0	0	0.25	0.01	0.95
	ISO	0	0	0	15	0	2	0	1	0	0	0.75	0.02	0.96
	LPD	0	0	0	0	4	1	0	0	0	0	0.33	0.01	0.96
	MISC	0	0	0	1	0	4	2	0	0	3	0.14	0.03	0.85
	MPD	0	0	0	0	0	1	8	0	1	0	0.33	0.01	0.91
	NA	0	0	0	4	0	0	0	4	0	0	0.25	0.02	0.92
	NP	0	0	1	0	0	1	0	0	7	0	0.44	0.01	0.95
	OXY	10	21	8	0	8	16	13	11	8	21	0.88	0.54	0.51

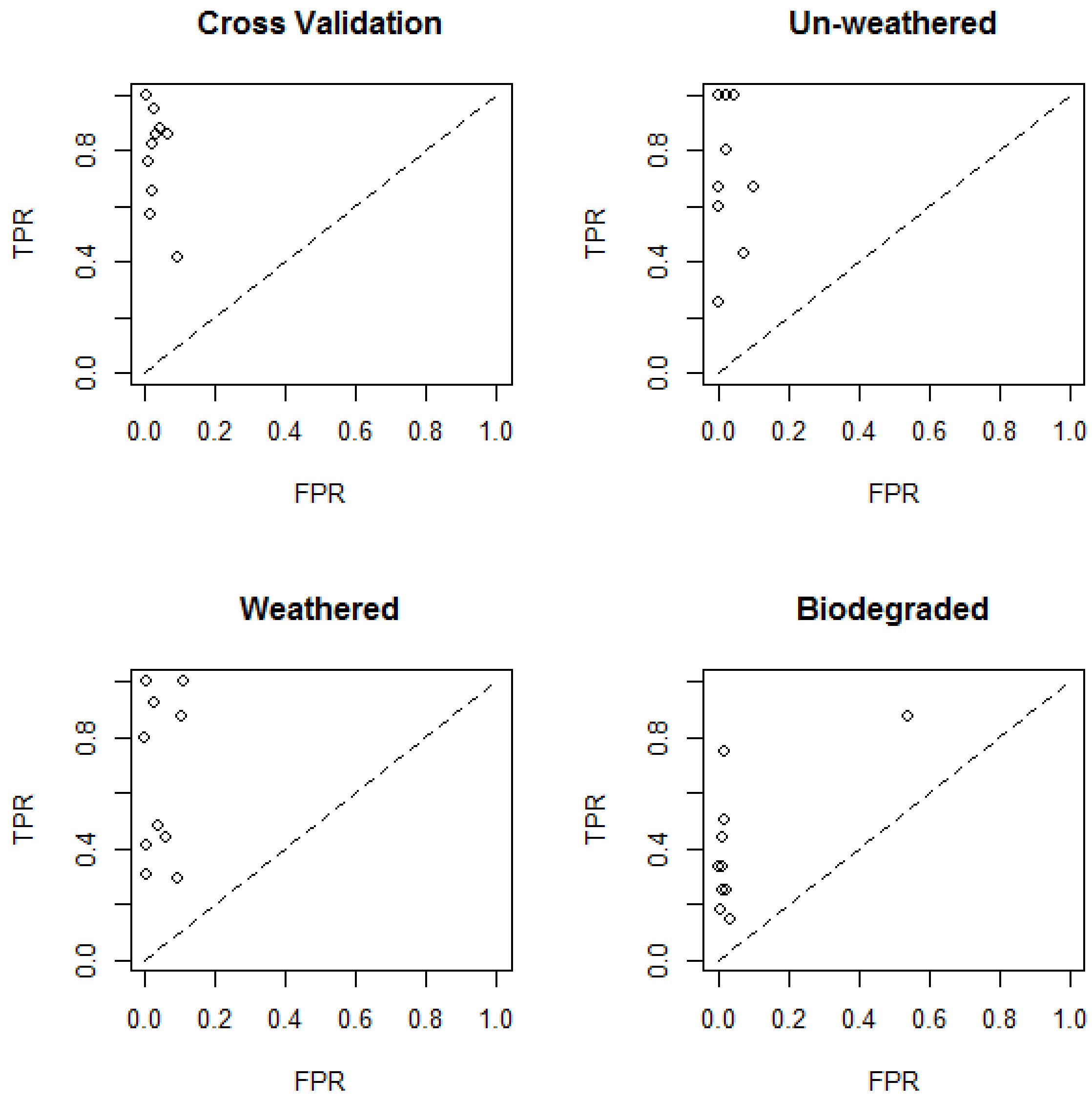


Figure 13. LDA model results plotted in ROC space for ignitable liquid class predictions.

Additional Uses of the Data

Data from this research also provided a valuable tool for increasing the reality of computationally generated fire debris data for training chemometric methods. The more realistic models were modeled well by support vector machine, LDA, quadratic discriminant analysis (QDA) and k-nearest neighbors (kNN) methods. These modeling studies were not included in the scope of this research; however, the results were leveraged to improve modeling results under award 2009-DN-BX-K227. Results from this work have been submitted for publication in Forensic Science International [40].

Best Practices for the Interpretation and Use of the Weathered and Biologically Degraded Samples in the Ignitable Liquids Database

This guide is accessible on the Ignitable Liquids Reference Collection Database.

See Appendix B.

Digital Weathering

Examples of weathered (evaporated) TICs from the gasoline, petroleum distillate and naphthenic paraffinic liquids are shown in Figures 1, 2 and 3 respectively. The kinetics of evaporation was analyzed without identification of chemical compounds. The faster evaporation rates for compounds eluting early in the chromatographic profile can be seen in each Figure 1 – 3. The same effect can be observed in each of the data sets (not shown). The variable temperature retention index was calculated for each peak based on the retention time for a set of normal hydrocarbons using Equation 2, which is based on Kovats' index [41].

$$R = \left\lfloor \frac{t_a - t_n}{t_{n+1} - t_n} * (C_{n+1} - C_n) + C_n \right\rfloor * 100 \tag{2}$$

In Equation 2, t_a is the retention time of the analyte, t_n and t_{n+1} are the retention times of the normal hydrocarbon standards that bracket t_a. The quantities C_n and C_{n+1} are the number of carbons in the two standards with retention times that bracket t_a. The term (C_{n+1} - C_n) is introduced to account for sequential standards that differ by more than one carbon. The natural logarithm of the rate constant, ln(k), has previously been shown to be a linear function of the retention index, R, for samples undergoing less evaporative loss that typically observed in this work [18]. In the following paragraphs, the relationships between ln(k) and R is examined for each of the ASTM E1618 classes.

Gasolines

A plot of ln(k) versus retention index for all of the gasolines examined in this study is shown in Figure 14. Note that the plots of ln(k) vs R for all of the ASTM classes will be shown on the same scale to allow direct comparison of the rate and retention index ranges. The graph in Figure 14 depicts relationship of varying linearity between ln(k) and the retention index for gasolines. A similar set of rates are observed across seven gasoline samples. The numerical values for the slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the data sets in Figure 14 are given in Table 15. Previous work has shown that a linear relationship may be observed across multiple compound types [18]. The lowest r^2 value is observed for the SRN 116 gasoline sample. The low coefficient of expectation is the result of a very low slope and spread of the data around the best fit line, Figure 15(a). Panels 15(b) and 15(c) demonstrate the normal distribution of the standardized residuals and a Q-Q plot for the standardized residuals that shows some deviation at the high and low ends. The overall appearance of non-linearity observed in

Figure 14 is the result of the samples having the same retention index range and somewhat differing slopes; however, no single sample depicts well-defined curvature (the reader is referred to Figure 15).

Table 15. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the gasoline data sets.

IL	Slope	Std. E. Slope	r^2
MURPHYE85	-5.32E-03	3.65E-04	7.83E-01
SHELL	-6.46E-04	2.18E-04	1.01E-01
SRN105	-4.19E-03	1.60E-04	8.60E-01
SRN116	-2.84E-04	9.68E-05	9.31E-02
SRN258	-1.74E-03	1.39E-04	6.30E-01
SRN259	-1.81E-03	2.54E-04	5.91E-01
Texaco	-3.07E-03	2.42E-04	7.85E-01

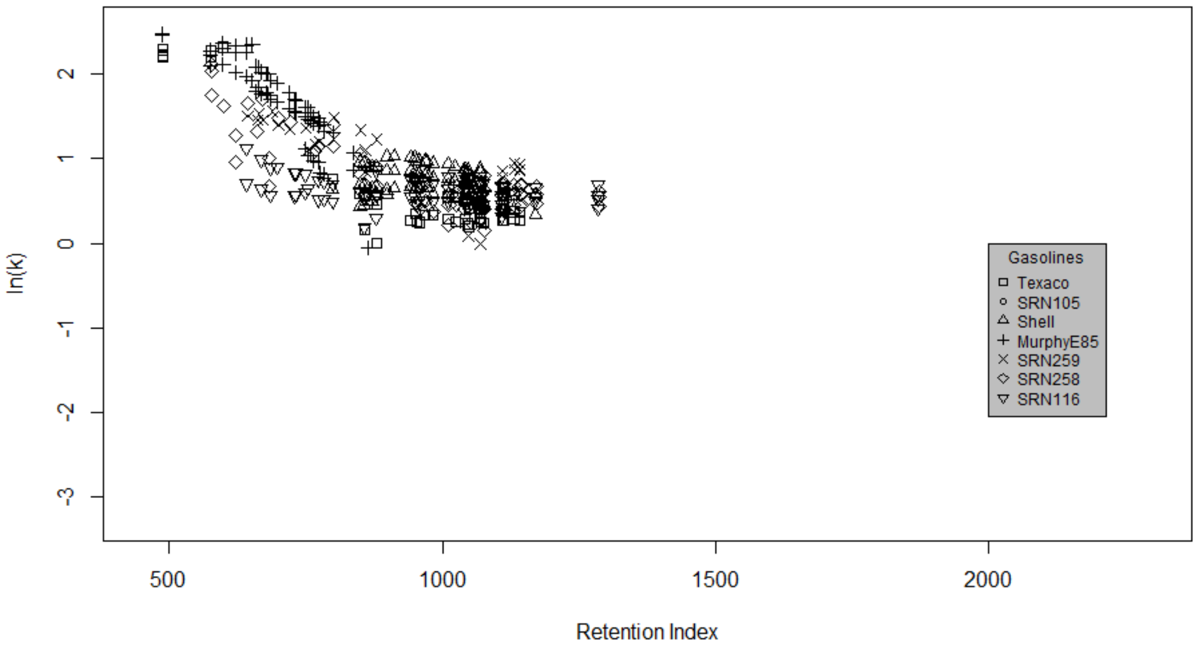


Figure 14. Plot of ln(k) versus retention index for gasoline samples examined in this study.

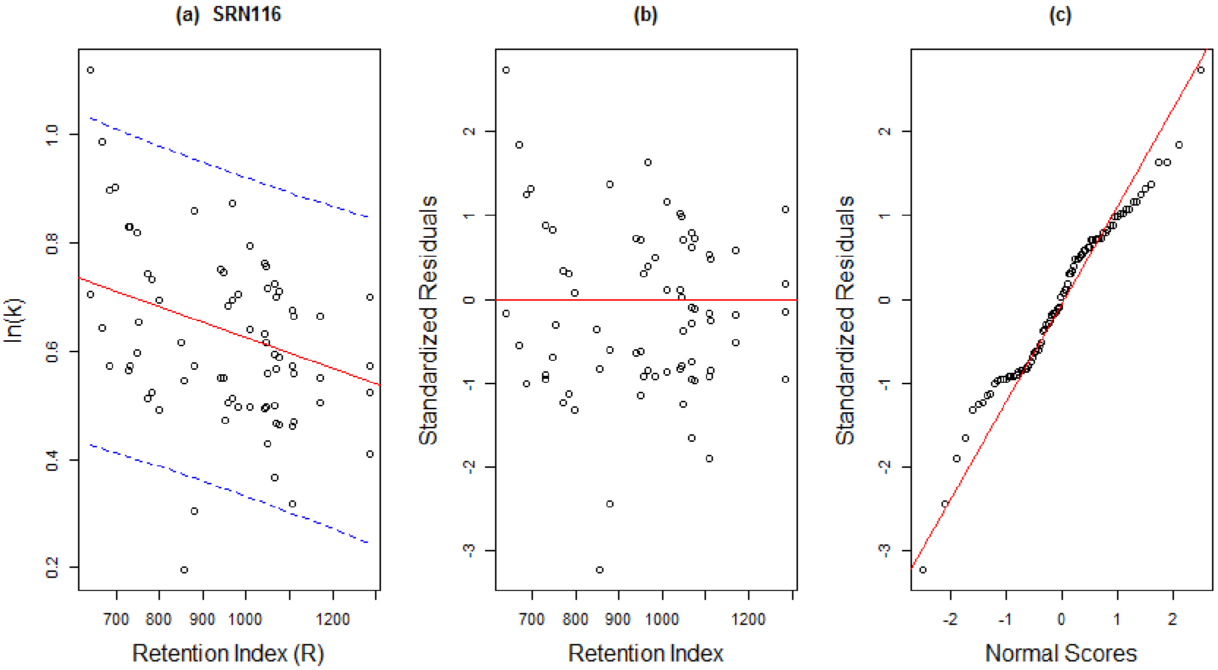


Figure 15. (a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Naphthenic Paraffinic Liquids

The ln(k) versus retention index plot of four naphthenic paraffinic ignitable liquids is shown in Figure 16. The graph demonstrates a set of linear plots with nearly identical slopes for four naphthenic paraffinic liquids. Naphthenic paraffinic liquids contain branched and cyclic aliphatic hydrocarbons, whereas gasolines contain branched and cyclic aliphatic compounds and aromatic compounds. The three samples that overlap most strongly on the graph (SRN 53, SRN 140 and SRN 185) are in the "heavy" sub-classification and have carbon ranges of C_{9-16}, C_{9-15} and C_{9-15}, respectively, whereas SRN 201 is in the "medium" sub-classification and has a carbon range of C_{10-13}. The slopes of the regression lines through the naphthenic paraffinic samples are similar (mean = -9.11E-3, SDev = 3.51E-4, n = 4). The slopes, standard error in the slope and the coefficient of expectation for the best fit line through each of the data sets in Figure 16 are given in Table 16. The coefficients of expectation for the best fit linear regressions of SRN 185 and SRN 201 are the lowest of the four liquids. The best fit line and 95% prediction limits for SRN 201 is shown in Figure 17(a). The standardized residuals and the QQ-plot for the standardized residuals are shown in Figure 17(b) and 17(c) respectively.

Table 16. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the naphthenic paraffinic data sets.

IL	Slope	Std. E. Slope	r^2
SRN140	-8.96E-03	3.72E-04	9.37E-01
SRN185	-8.68E-03	4.89E-04	8.85E-01
SRN201	-9.44E-03	7.07E-04	7.74E-01
SRN53	-9.34E-03	3.98E-04	9.32E-01

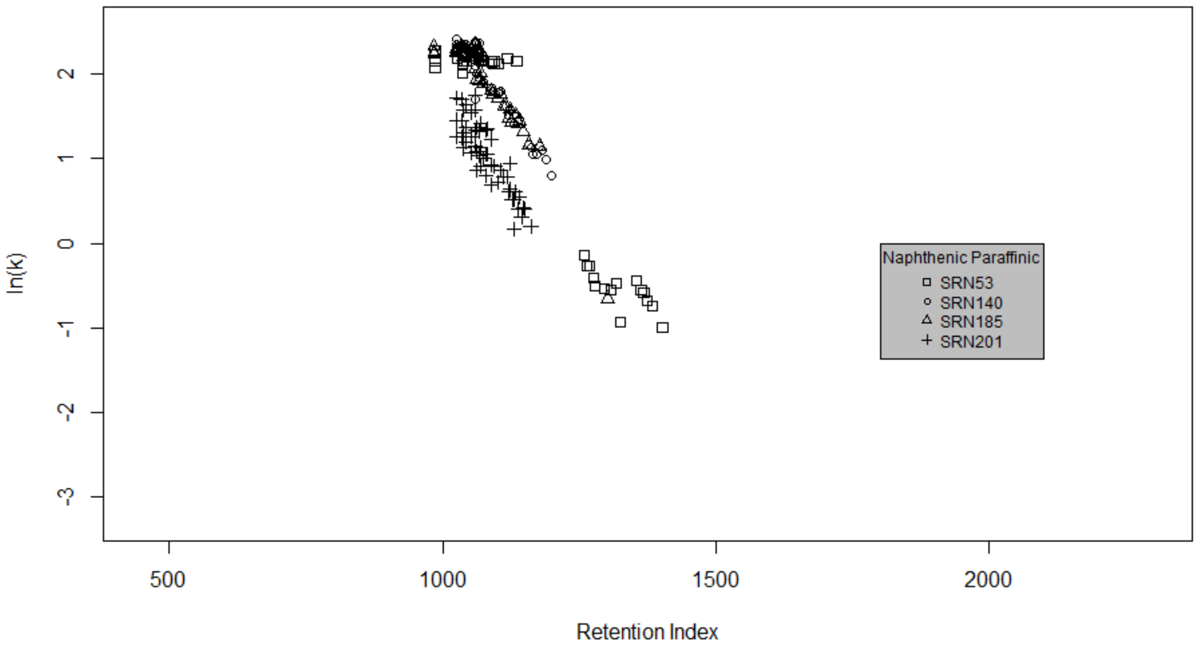


Figure 16. Plot of ln(k) versus retention index for naphthenic paraffinic samples examined in this study.

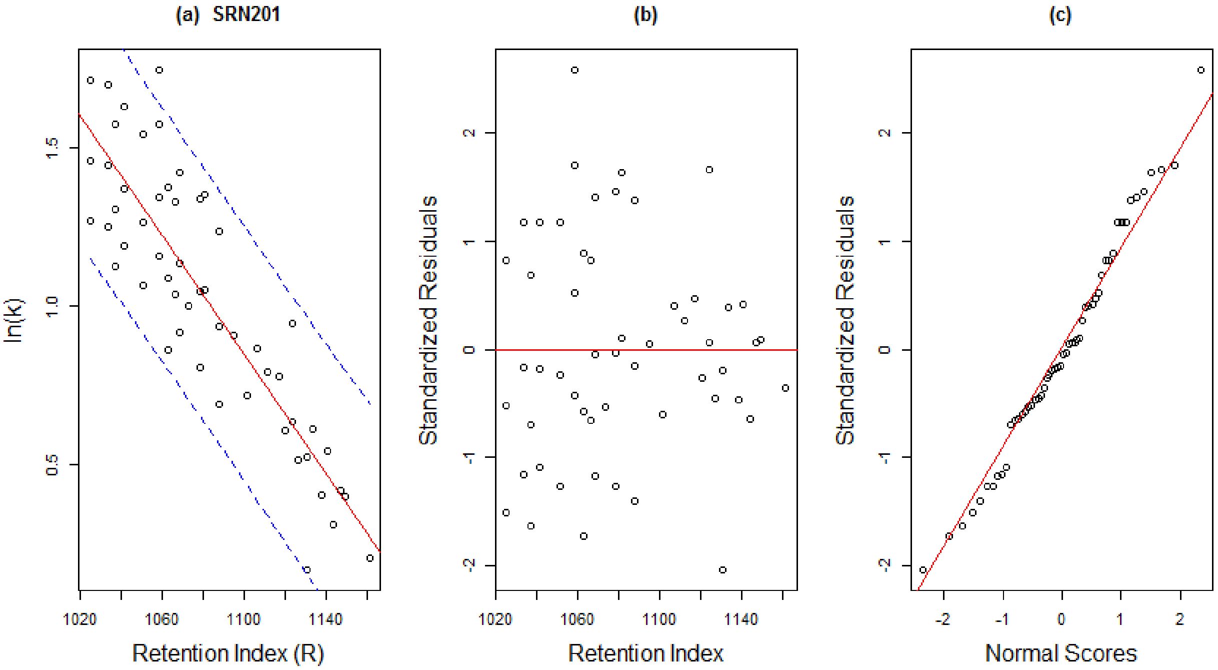


Figure 17. (a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Isoparaffinic Liquids

The ln(k) versus retention index plot of four isoparaffinic ignitable liquids is shown in Figure 18. The graph demonstrates a set of linear relationships with nearly identical slopes for the liquids. Isoparaffinic liquids contain only branched hydrocarbons, making a single liquid more homogeneous in composition than a gasoline; however, gasolines are more homogeneous as a class of liquids. Heterogeneity across the class of isoparaffinic liquids is reflected in the sets of parallel lines in Figure 18, which correspond to samples of varying carbon range. The samples that overlap most strongly on the graph have similar carbon ranges, for example SRN 87 (C_{7-10}) and SRN 120(C_{6-8}). A second example is SRN 12 (C_{8-12})

and SRN 119 (C_{9-12}). The carbon range for SRN 201 (C_{11-16}) extends to 16 carbons on the upper end and does not overlap with any of the other four isoparaffinic liquids in Figure 18. Although the slopes are relatively similar (mean = -7.30E-3, SDev = 1.79E-3, n = 5) for the best fit linear regression lines through the data in Figure 18, the intercepts for the three sets of parallel lines are very different. Since both the slope and intercept are required to model the evaporation of a given liquid, a general application of these results to model evaporation of an isoparaffinic liquid not in the data set would be subject to significant error. The slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the data sets in Figure 18 are given in Table 17.

Table 17. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the isoparaffinic data sets.

IL	Slope	Std. E. Slope	r^2
SRN87	-9.14E-03	5.98E-04	8.66E-01
SRN120	-5.86E-03	1.09E-03	6.17E-01
SRN12	-8.93E-03	2.95E-04	9.40E-01
SRN119	-5.15E-03	5.36E-04	6.36E-01
SRN89	-7.43E-03	2.62E-04	8.91E-01

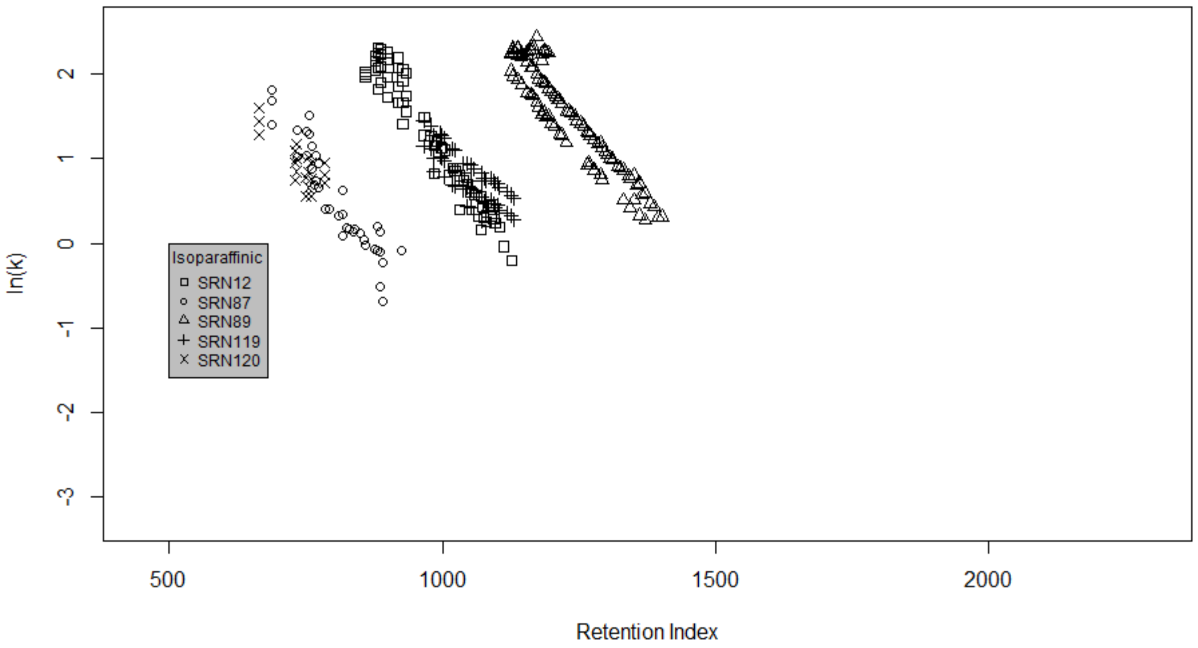


Figure 18.Plot of ln(k) versus retention index for isoparaffinic samples examined in this study.

Petroleum Distillate Liquids

The ln(k) versus retention index plots of 14 petroleum distillates are shown in Figures 19 - 21. The data is divided among three plots to reduce clutter resulting from overlap of the large number of liquids in this class. The three graphs also demonstrate sets of linear relationships of varying quality, as reflected in the coefficient of determination values in Table 18. The chemical composition of petroleum distillates includes linear and branched alkanes, cycloalkanes and may or may not contain aromatic components; however, aromatics will generally be a smaller component. In Figures 19 – 21, we also see the data aggregating into several sets of parallel lines; however, the variation in the slopes (mean = -

7.29E-3, SDev = 3.9E-3, n= 14) is larger than observed for the previously discussed classes. Two ignitable liquids, SRN 14 and SRN 20, have especially low coefficients of expectation (r^2 0.1 – 0.12), which is the result of the very low slope and relatively large scatter in the data. The plots of ln(k) against retention index for these two samples can be seen in Figures 19 and 20, respectively. Figure 22a shows plot of ln(k) versus retention index for SRN 14, along with the regression line and the 95% prediction intervals. Figure 22(b) shows the standardized residuals plot and the QQ-plot is shown in Figure 22(c). Figure 22(a) more clearly indicates the low slope of the best fit line, while panels (b) and (c) demonstrate the normal distribution of the standardized residuals. Figure 23 shows an analogous set of plots for SRN 64, which has a much higher r^2 of 0.772. The linearity of the plot in Figure 23(a) is readily apparent, as is the normality of the residuals. Sample SRN 4 has two outlier points at retention index values above 1,100 (see figure 21), which significantly influence the linear fit.

Table 18. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the petroleum distillate data sets.

IL	Slope	Std. E. Slope	r^2
SRN14	-1.40E-04	2.39E-04	1.21E-02
SRN21	-9.08E-03	6.01E-04	8.64E-01
SRN35	-8.17E-03	5.47E-04	7.94E-01
SRN64	-8.26E-03	4.81E-04	7.72E-01
SRN91	-8.55E-03	3.43E-04	9.20E-01
SRN4	-1.13E-02	8.75E-04	7.99E-01

SRN8	-6.22E-03	6.08E-04	8.97E-01
SRN20	4.54E-04	1.82E-04	1.02E-01
SRN33	-8.27E-03	3.52E-04	9.37E-01
SRN46	-9.54E-03	6.21E-04	9.40E-01
SRN50	-3.85E-03	2.19E-04	9.01E-01
SRN156	-5.46E-03	1.22E-04	9.71E-01
SRN160	-1.17E-02	5.34E-04	9.25E-01
SRN164	-1.20E-02	5.05E-04	9.22E-01

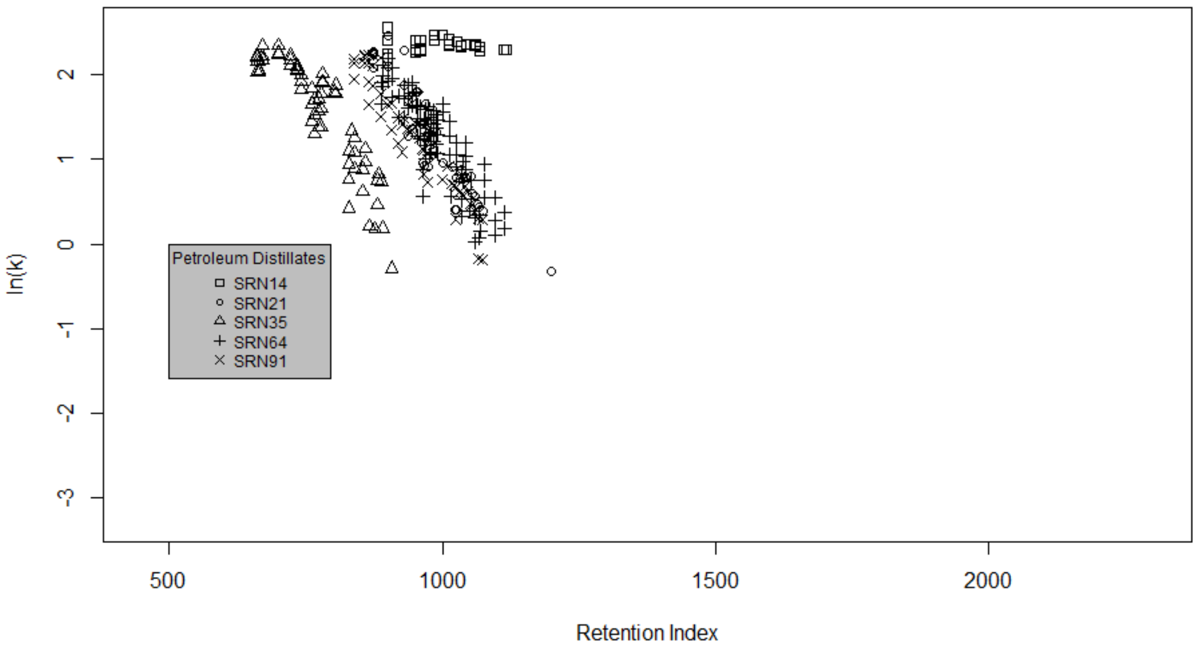


Figure 19.Plot of ln(k) versus retention index for petroleum distillate samples examined in this study.

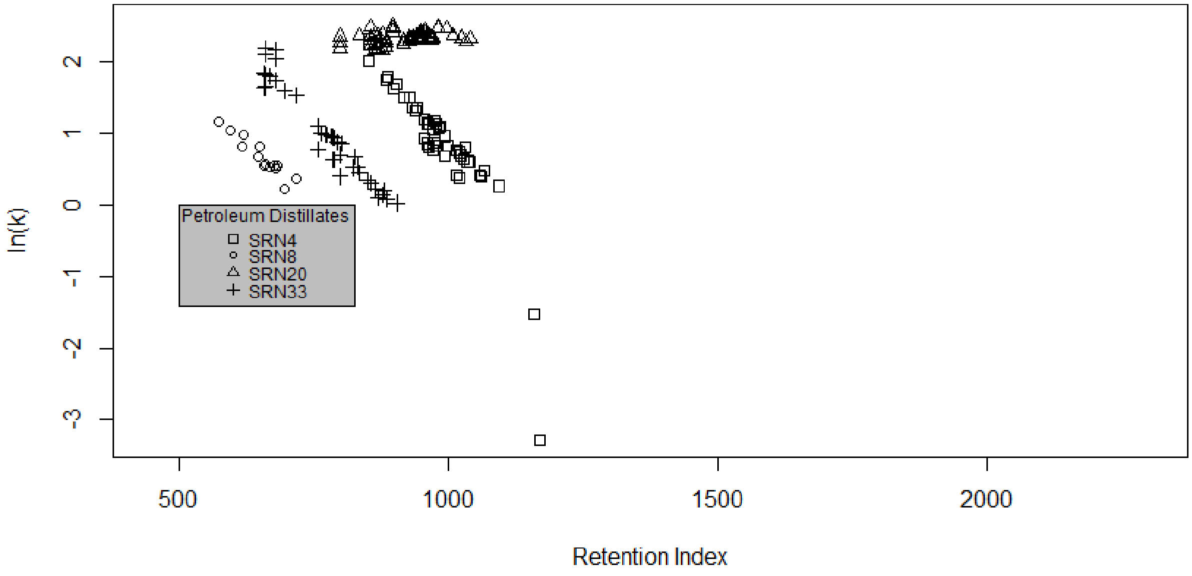


Figure 20.Plot of ln(k) versus retention index for petroleum distillate samples examined in this study.

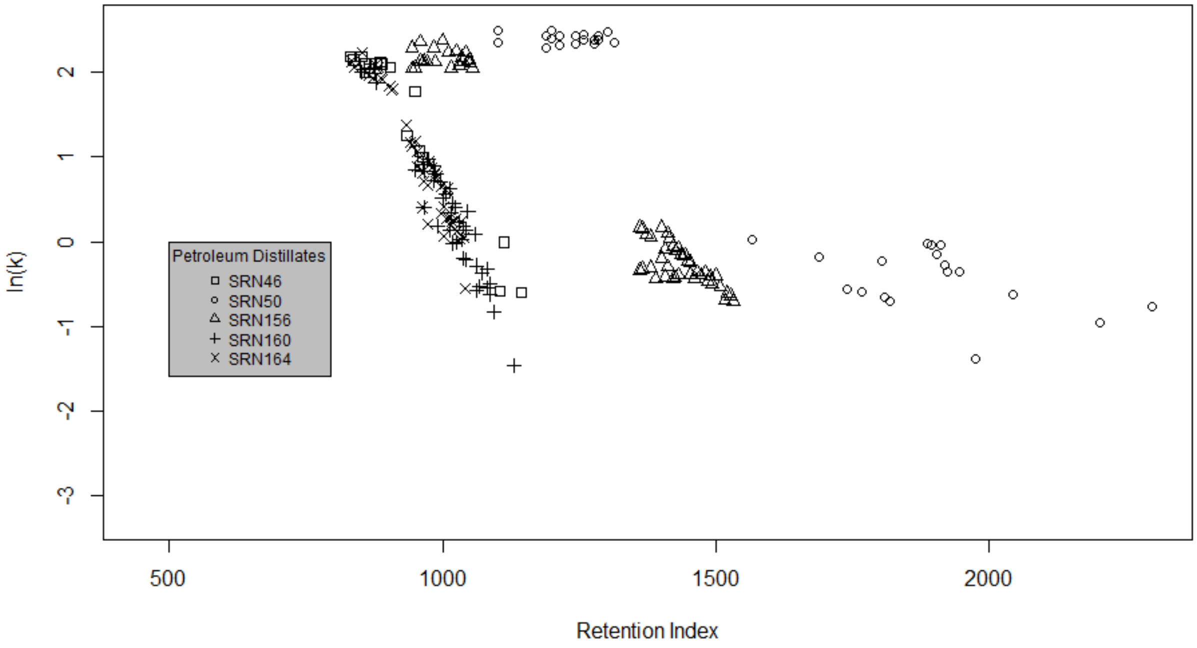


Figure 21. Plot of ln(k) versus retention index for petroleum distillate samples examined in this study.

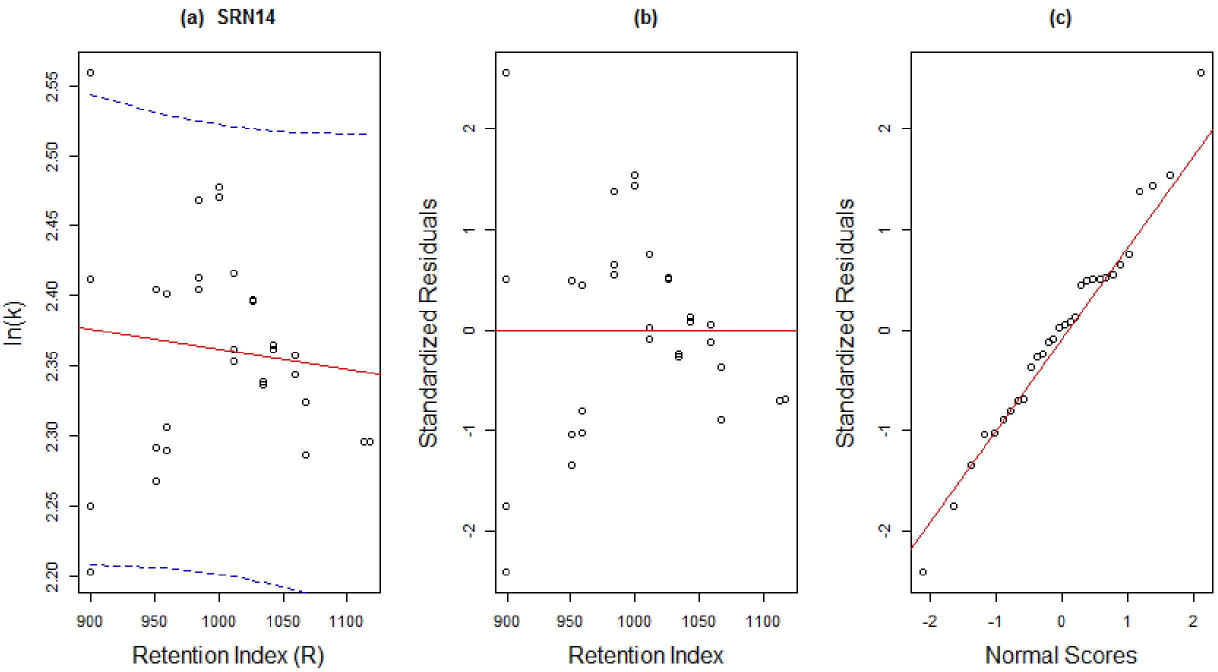


Figure 22. (a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

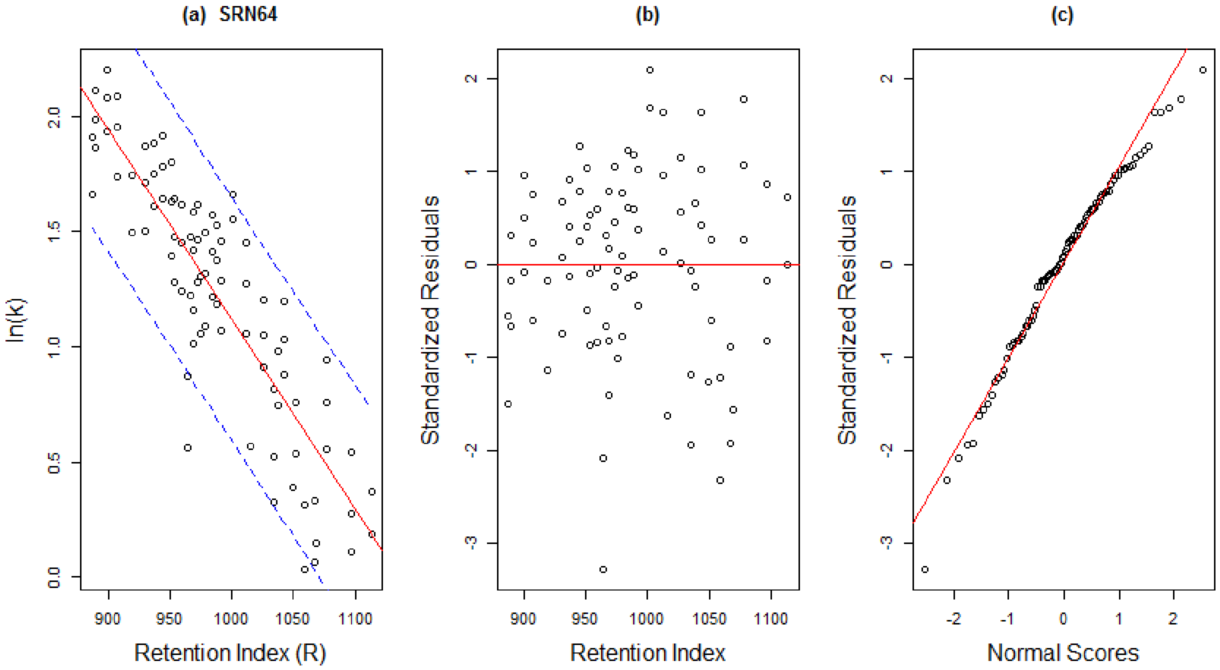


Figure 23.(a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Normal Alkane Solvents

Solvents in the normal alkane class typically have a very limited number of components since composition is restricted to the normal alkanes. Figure 24 shows a plot of ln(k) against the retention index for four normal alkanes. The slope, standard error in the slope and coefficient of expectation is given in Table 19 for only one of the liquids, SRN 176. The other three liquids had an insufficient number of high quality first order decay rate constants to allow for prediction of a best-fit line through the data. The coefficient of determination for SRN 176 is extremely low, as is the slope. This result is due to a very limited range in the retention index and calculated rates, coupled with a large uncertainty in the rates, relative to the range.

Figure 25 shows the best fit line through the data, the standardized residuals and the QQ-plot for the standardized residuals.

Table 19. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the normal alkane data sets.

IL	Slope	Std. E. Slope	r^2
SRN176	-4.48E-04	7.54E-04	6.60E-02
SRN192	-	-	-
SRN236	-	-	-
SRN77	-	-	-

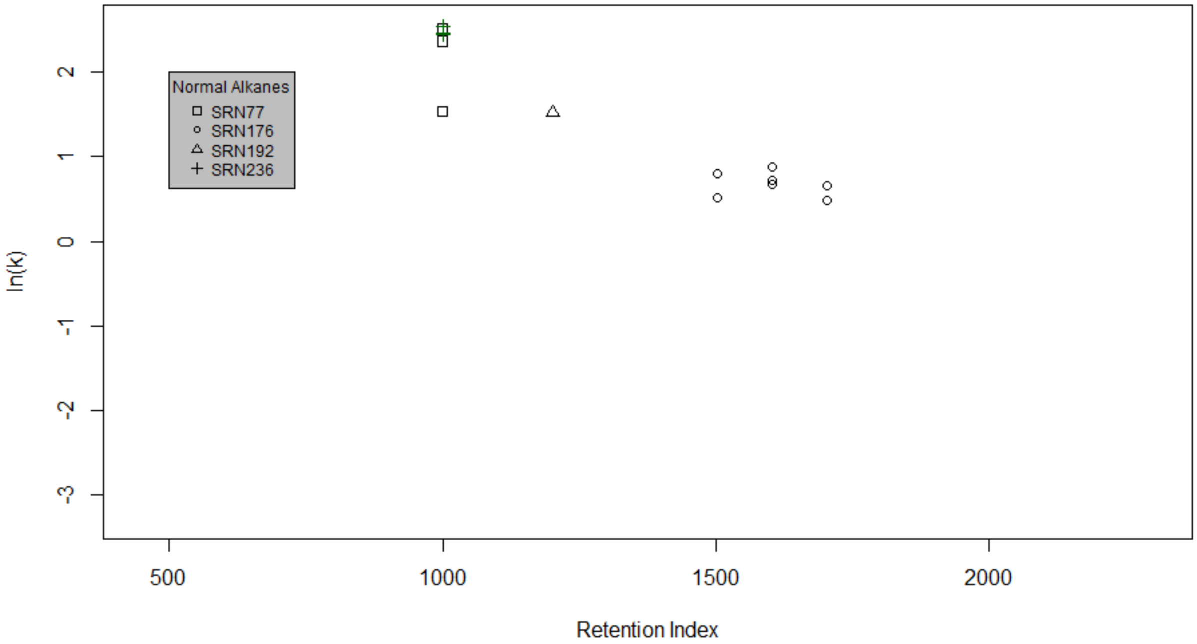


Figure 24. Plot of ln(k) versus retention index for normal alkane samples examined in this study.

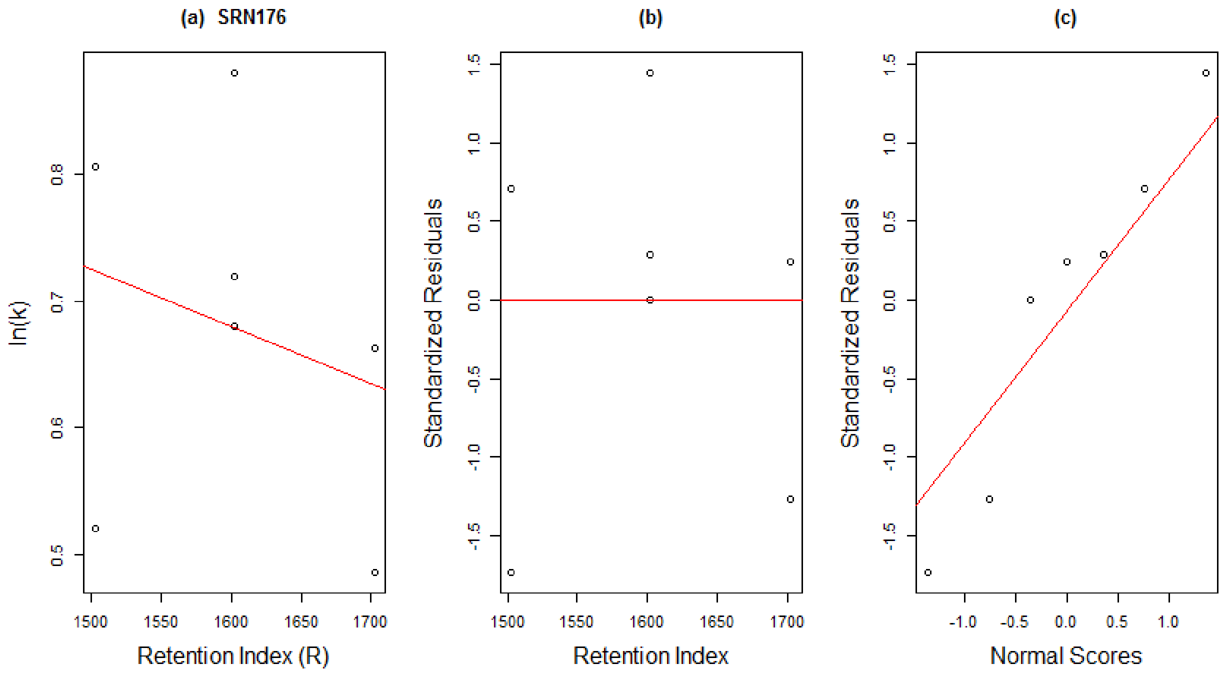


Figure 25(a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Aromatic Solvents

The aromatic solvents contain abundant amounts of monocyclic aromatics and may contain polynuclear aromatic compounds as well. The number of individual compounds in an aromatic solvent may, in some cases, be rather limited. A plot of ln(k) against the retention index is shown for four aromatic solvents in Figure 26. The plot shows fairly linear relationships for the data and similar slopes (mean = -1.03E-2, SDev = 2.02E-3, n = 3). The slope, standard error in the slope and coefficient of expectation is given in Table 20 for three of the four liquids. One of the liquids, SRN 59, had an insufficient number of high quality first order decay rate constants to allow for prediction of a best-fit line through the data. Figure 27a shows plot of ln(k) versus retention index for SRN 284, along with the regression line and the 95% prediction intervals. Figure 27(b) shows the standardized residuals plot and the QQ-plot is shown in Figure 27(c).

Table 20. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the aromatic solvent data sets.

IL	Slope	Std. E. Slope	r^2
SRN284	-8.00E-03	1.14E-03	5.99E-01
SRN5	-1.14E-02	5.42E-04	9.22E-01
SRN59	-	-	-
SRN73	-1.16E-02	4.35E-04	9.53E-01

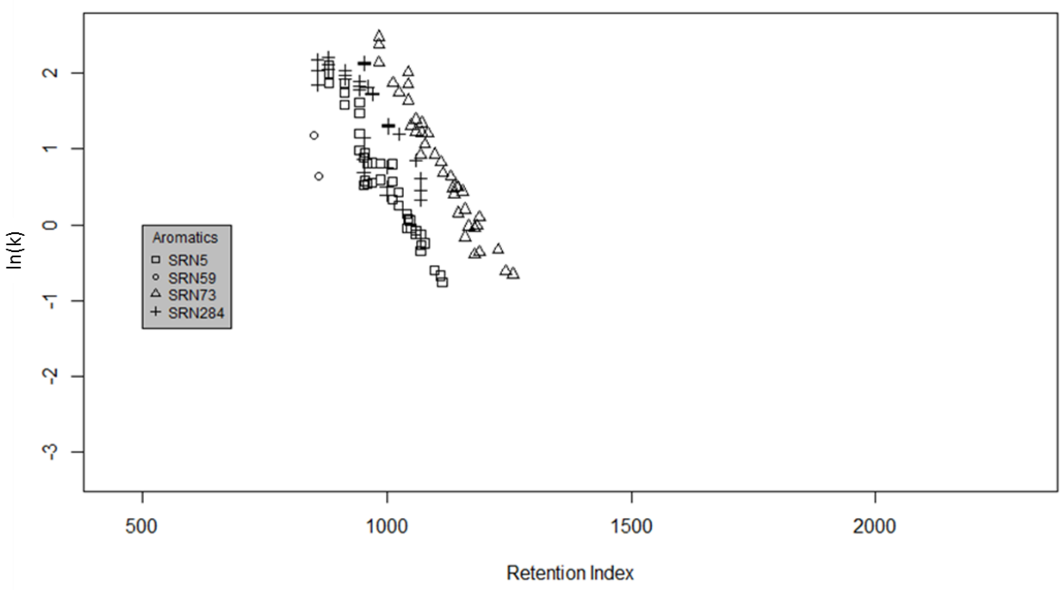


Figure 26. Plot of ln(k) versus retention index for aromatic samples examined in this study.

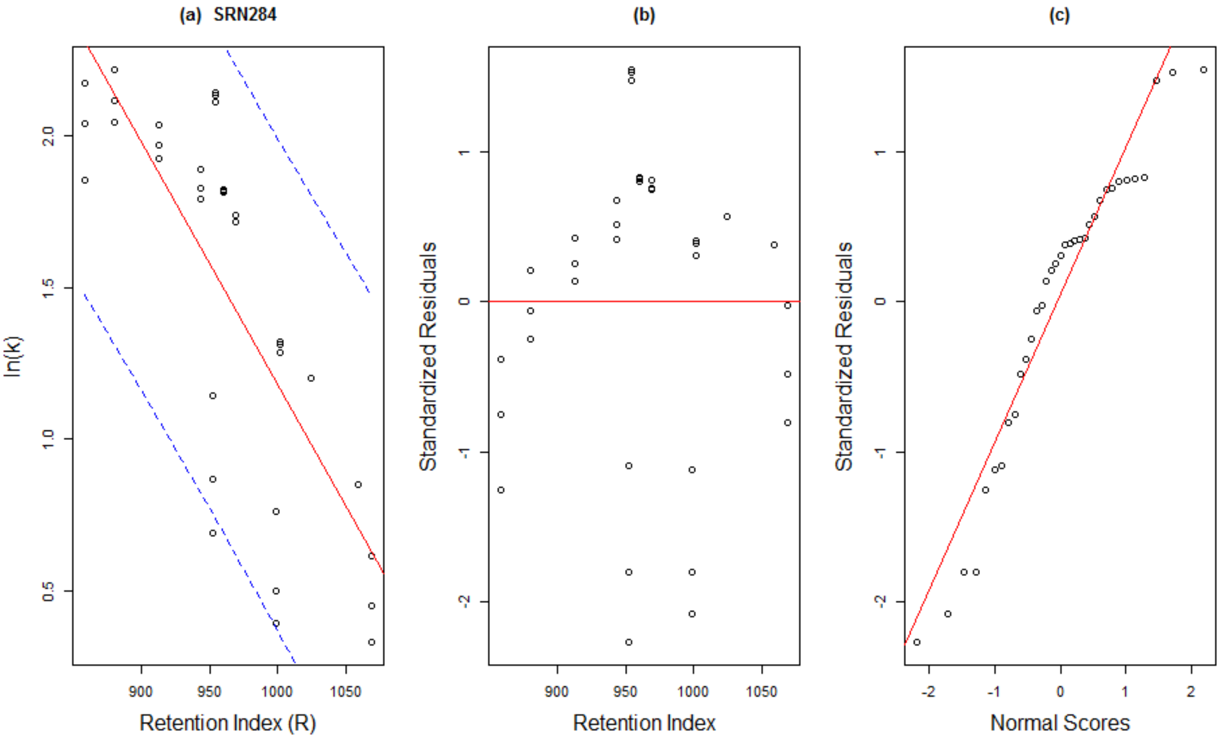


Figure 27(a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Oxygenated Solvents

Oxygenated solvents can be very heterogeneous, so long as they contain major oxygenated components. Figure 28 shows a plot of ln(k) against the retention index for five oxygenated solvents. The slope, standard error in the slope and coefficient of expectation is given in Table 21 for only one of the liquids, SRN 231. The other four liquids had an insufficient number of high quality first order decay rate constants to allow for prediction of a best-fit line through the data. The coefficient of determination for SRN 231, 0.691, corresponds to 69% of the variance in ln(k) accounted for by variance in the retention index . Figure 29 shows the best fit line through the data, the standardized residuals and the QQ-plot for the standardized residuals. The QQ-plot shows significant deviation of the residuals from normality.

Table 21. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the oxygenated solvent data sets.

IL	Slope	Std. E. Slope	r^2
SRN149	-	-	-
SRN218	-	-	-
SRN220	-	-	-
SRN231	-4.68E-03	6.53E-04	6.91E-01
SRN69	-	-	-

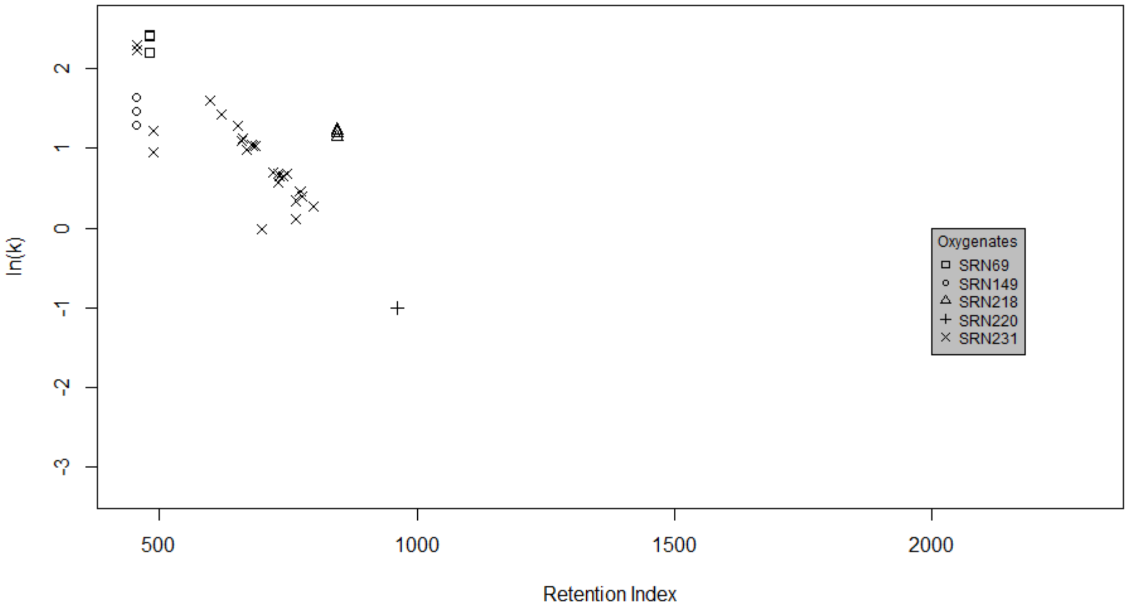


Figure 28. Plot of ln(k) versus retention index for oxygenated liquid samples examined in this study.

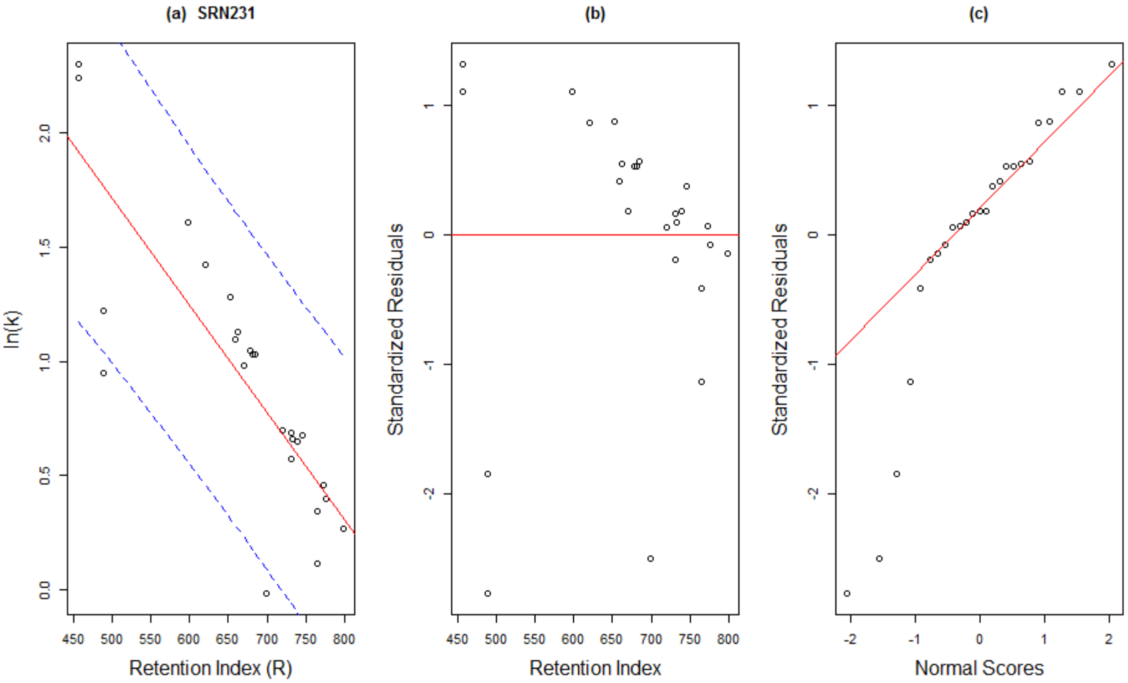


Figure 29 (a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Miscellaneous Solvents

The miscellaneous solvents comprise a category of solvents that serves as a collection of samples that do not fit into the other ASTM E1618 classes. Liquids in this category are often mixtures of liquids from two or more of the other classes. Figure 30 shows a plot of ln(k) versus the retention index for seven ignitable liquids from the miscellaneous category. Most of the plots appear linear, although SRN 42 is obviously curved (concave down). The slopes, standard error in the slope and coefficient of expectation for the best fit line through six of the seven miscellaneous ignitable liquid data sets are shown in Table 22. The data for SRN 16 had an insufficient number of high quality first order decay rate constants to allow for prediction of a best-fit line through the data. Figure 31 shows the best fit line through the data for SRN 42, the standardized residuals and the QQ-plot for the standardized residuals. The curvature of the data is obvious from the best fit line and the deviation of the QQ-plot.

Table 22. Slopes, standard error in the slope and coefficient of expectation for the best fit line through each of the miscellaneous ignitable liquid data sets.

IL	Slope	Std. E. Slope	r^2
SRN10	-9.59E-03	1.24E-03	7.79E-01
SRN131	-1.07E-02	8.68E-04	8.22E-01
SRN146	-1.49E-02	7.77E-04	9.68E-01
SRN16	-	-	-
SRN182	-5.52E-03	1.81E-04	9.97E-01
SRN39	-1.32E-02	1.18E-03	8.02E-01
SRN42	-7.72E-03	7.82E-04	8.16E-01

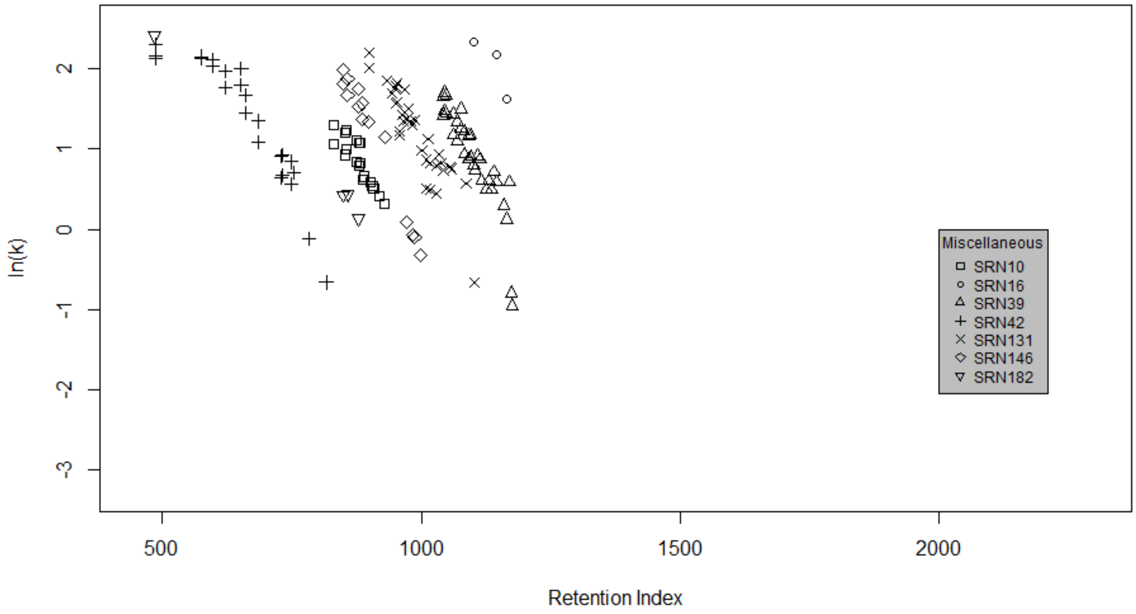


Figure 30. Plot of ln(k) versus retention index for oxygenated liquid samples examined in this study.

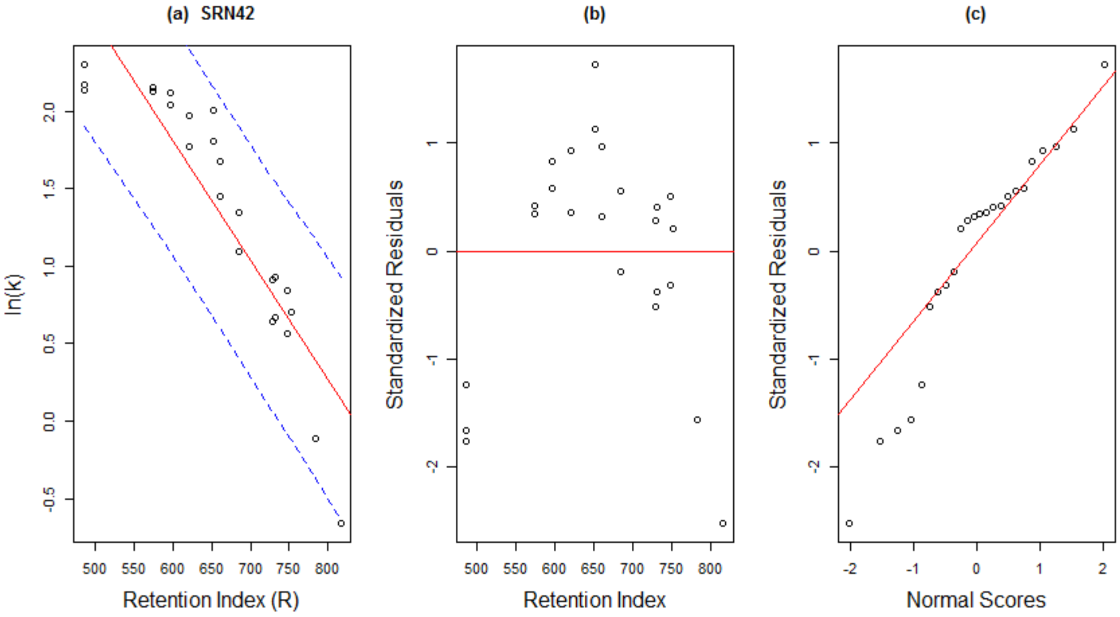


Figure 31 (a) ln(k) vs. R with best fit line and 95% prediction levels; (b) standardized residuals; (c) QQ-plot of standardized residuals.

Comparison of ASTM E1618 Classes

Although the ln(k) versus the retention index plots for the ignitable liquids examined in this work show higher scatter than previously reported [18]; however, in this work the evaporation proceeds through greater than 90% volume reduction. The ranges in the slope of the ln(k) versus retention index are shown as box plots in Figure 32 for each of the ASTM classes. The ranges in the slope of the ln(k) versus retention index **for $r^2 \geq 0.9$** are shown as box plots in Figure 33 for each of the ASTM classes. The rates in Figure 33 are data for which 90% of the variation in ln(k) is explained by variation in the retention index. Each slope represents the rate of change in the natural logarithm of the rate of evaporative loss across the chromatographic profile for an individual ignitable liquid. The range of slopes within an ASTM class represents the range of variability of evaporation rates within a class. From Figure 32, the range in rates is seen to be largest for the miscellaneous and petroleum distillate classes and smallest for the naphthenic paraffinic class. Only single values were available in the normal alkane and oxygenated liquids classes (single line in the plot). When the data is restricted to the most linear plots, Figure 33, the picture is not changed significantly; however, the gasoline, normal alkane and oxygenated solvent classes are no longer populated, a single value was remaining for the ISO class and only two values were remaining for the AR, MISC and NP classes (box with no whiskers in Figure 33).

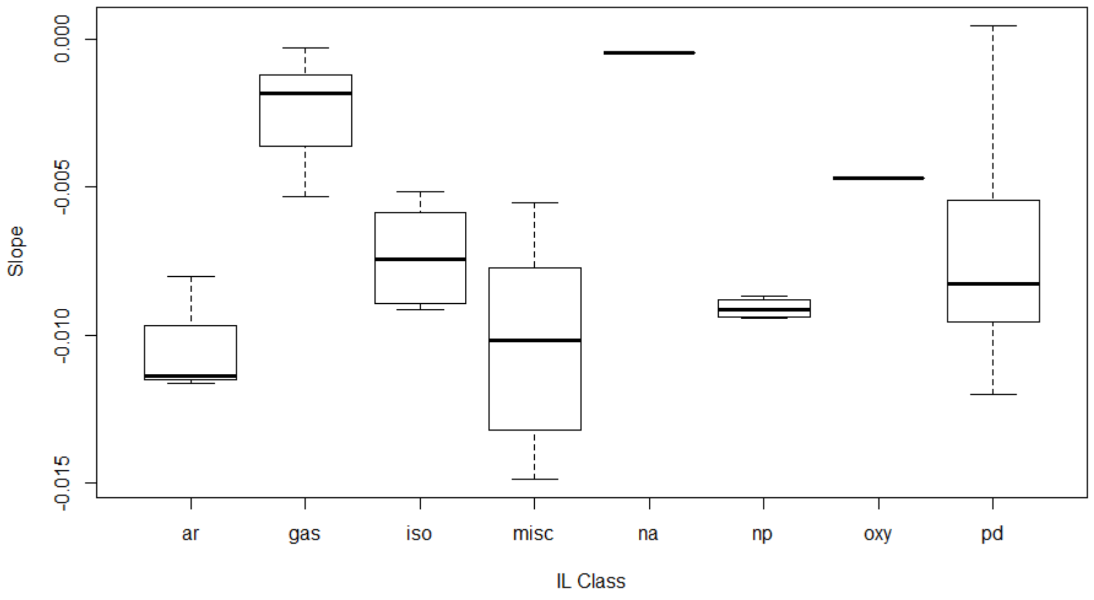


Figure 32 The range in the slope of the ln(k) versus retention index is shown as a box plot for each of the ASTM classes.

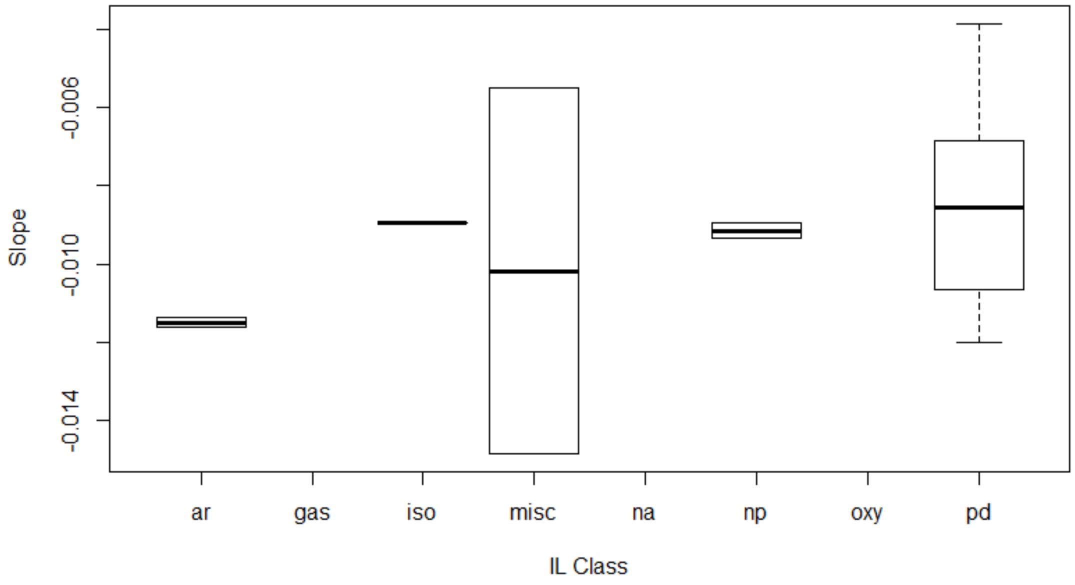


Figure 33 The range in the slope of the ln(k) versus retention index for $r^2 \geq 0.9$ are shown as a box plot for each of the ASTM classes.

IV. Conclusions

Discussion of Findings

The findings from this research have provided fire debris analysts hundreds of examples of weathered and biologically degraded ignitable liquid samples encompassing all ASTM E1618 classes. These examples are contained within the Ignitable Liquids Reference Collection Database, which is searchable and links all related records to one another. The ILRC committee has produced a guide of best practices when interpreting ignitable liquids that have been altered by evaporation or microbes. Possible misclassifications have been determined by both the traditional interpretations outlined in ASTM E1618 and a chemometric method called linear discriminant analysis.

Results from the weathered ignitable liquids were predictable and correspond with findings in the literature; however more ASTM E 1618 classes were represented in this work. A sequential loss of low boiling point hydrocarbons leaves more representation of the higher boiling point hydrocarbons. This is clearly seen when comparing the total ion chromatograms of an ignitable liquid at various evaporation points. Typically, it is not difficult to relate a weathered sample to the undegraded sample. The ignitable liquids that can potentially be misclassified are those with a broader range of hydrocarbons where two different compound types reside in different carbon ranges. Upon evaporation, the compound type residing in the lower carbon range (low boiling point compounds) is lost leaving behind the second group of compounds of a different type. Gasoline is an example, where the majority of the alkanes are low boiling point compounds. Once they are lost due to evaporation, the remaining liquid consists mostly of aromatics. Other classes having a potential to misclassify upon weathering are some miscellaneous and oxygenated solvents. Miscellaneous category ignitable liquids that have characteristics of multiple classes will misclassify when the compounds of the low boiling point compounds have evaporated. When the low boiling point oxygenated compounds evaporate, the ignitable liquids may resemble another class, the class depending on the remaining hydrocarbons.

Data from the weathering of the ignitable liquids has been used in a recent publication to increase performance of a statistical analysis method for classification. The method uses the total ion spectra calculated from the GC-MS data and performs in a class-conditional feature space. New samples are classified based on their similarity to samples contained within a known collection [42].

Unlike those from weathering, the results from microbial degradation were not as predictable and did not always correspond with results from the literature. Degradation by microbes is not consistent and produces variability in the results. Variability was demonstrated in the gasoline study where the same gasoline sample, soil, and sample preparation methods were used; however the results were slightly different. Suggested reasons were a change in the condition of the soil and possibly a change in the microbe population over time. There are numerous species of microbes which may have preferences for particular types of hydrocarbons, which is substantiated by the different rate of consumption of different compound types. There are studies indicating that microbes are opportunistic and will adapt to consume any hydrocarbon available possibly leading to the variability in results. It has been demonstrated that microbes can consume ignitable liquid residues within 7 days which leads not to misclassification, but to an inability to identify the presence of an ignitable liquid. A rate of 0.2 moles of hydrocarbons per day was observed to be consumed by microbes at soil loading used in this work. The first hydrocarbons to be consumed were oxygenated, followed by normal alkanes, aromatics, and then branched and cycloalkanes.

First order evaporative losses of the chemical components of commercial ignitable liquids were observed across all eight ASTM E1618-defined classes of ignitable liquids. Linear ($r^2 \geq 0.9$) relationships were observed between ln(k) and the retention index for liquids in all eight ASTM classes. The ln(k) versus retention index plots appeared nearly parallel for some classes, leading to variable intercepts. Accurate prediction of the evaporation profile of an ignitable liquid can only be modeled if the correct parameters (slope and intercept) can be determined from an analogous liquid.

Implications for Policy and Practice

The results from this research have been made available on the ILRC database web site (http://ilrc.ucf.edu/) throughout the course of the research. The work has also been presented in a number of forums throughout the course of the research. The ILRC Committee has participated in the research throughout the process and has utilized the results to formulate a "Best Practices" statement, which is posted on the web site. Consequently, the results have had an impact on fire debris analysis practices and policy within individually laboratories as the data and best practices recommendations have been accessed by the forensic fire debris analysis community for casework and educational purposes.

The following recommendations are provided by the ILRC committee within the Best Practices Guide. Measures should be taken to prevent microbial growth since major degradation occurs within 7 days. Currently, this is done by freezing or refrigeration the sample prior to extraction. A threshold on the abundance in a total ion chromatogram should be set to determine whether the sample is of sufficient quality to warrant further evaluation as positive for ignitable liquid residue. The threshold will be dependent on the instrumentation and laboratory procedures. Below the threshold there was not sufficient mass spectral data to confirm individual peak identification. Analysts are reminded that target compounds are identified by a mass spectral match to a standard and the retention time.

Implications for Further Research

A more complete understanding of the microbial population found with in organic fire debris would be advantageous in order to determine mechanisms for destroying the microbes or reducing their consumption of the hydrocarbons in the ignitable liquid residues.

While the ln(k) versus retention index were relatively linear with similar slope, more work is required to determine a useable method that allows the analyst to digitally compute a good representation of a weathered total ion chromatogram. A calculation of this type would save enormous time and effort by allowing the analyst to predict the correct ignitable liquid and degree of weathering that would best match the casework sample before going into the laboratory.

Weathering and biological degradation can lead to incorrect class assignments. Computational models, as they currently exist, are not immune to these mistakes. More robust classification methods, experimental and computational, should be pursued with the goals of improving the true positive classification rates (TPR) while keeping the false positive rates low.

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VI. Dissemination of Research Findings

Publications and Thesis

Lopatka, Martin; Sigman Michael; Sjerps, Marjan J; Williams, Mary R;, Vivo-Truyols, Gabrielle; *Class-conditional feature modeling for ignitable liquid classification with substantial substrate contribution in fire debris analysis, Forensic Science International*, **2015**, (252) 177-186.

Kindell JH. Quantitative Assessment of the Effects of Microbial Degradation of a Simple Hydrocarbon Mixture: University of Central Florida; 2015 (Forensic Science M.S. Thesis)

Dee A. Turner, Mary Williams, Michael A. Sigman, John V. Goodpaster, "A Comprehensive Study of the Alteration of Ignitable Liquids by Weathering and Microbial Degradation", manuscript in preparation, **2016**.

Jessica H. Kindell, Mary R. Williams, Michael Sigman, "Biological Degradation of a Hydrocarbon Mixture on Soil: Implications for Fire Debris Analysis" manuscript in preparation, **2016**.

Databases

The Ignitable Liquids Reference Collection (ILRC) Database (http://ilrc.ucf.edu/) was re-designed and updated.

Patents

None

Presentations

Williams, Mary R.; Sigman, Michael; Turner, Dee Ann; Goodpaster, John; *Expansion of the ILRC Database: Addition of Weathered and Biologically Degraded Liquids.* American Academy of Forensic Sciences Conference. Washington, DC. February 19-22, 2013. Oral Presentation.

Turner, Dee Ann; Goodpaster, John; Williams, Mary R.; Sigman, Michael; *A Comprehensive Study of Weathering and Microbial Degradation of Ignitable Liquids*. American Academy of Forensic Sciences Conference. Washington, DC. February 19-22, 2013. Oral Presentation.

Williams, Mary R.; Sigman, Michael; *Degradation of Ignitable Liquids*. Florida Annual Meeting and Exposition. Palm Harbor, FL May 8-10, 2014. Oral Presentation.

Kindell, Jessica; Williams, Mary R. Sigman, Michael; *Weathering and Microbial Degradation of Ignitable Liquids.* American Academy of Forensic Sciences Conference. Orlando, FL. February 16-21, 2015. Poster Presentation.

Appendices

Appendix A: Ignitable Liquids

SRN	Product Description	ASTM Class
59	Goof Off The Ultimate Remover	Aromatic
116	Exxon Unleaded Premium Gasoline	Gasoline
21	Parks 100% Mineral Spirit Paint Thinner	MPD
119	Isopar H	Isoparaffinic
77	Exxon Norpar 12	Normal Alkane
53	Ortho Bug B Gon Multi-Purpose Insect Killer Conc.	Naphthenic Paraffinic
131	Ace Pure Gum Turpentine	Miscellaneous
69	Whitaker # 51 Lacquer Thinner	Oxygenate
5	ShellSol A100	Aromatic
105	Phillips 66 Unleaded Regular Gasoline	Gasoline
35	Zippo Premium Lighter Fluid	LPD
87	Isopar E	Isoparaffinic
176	V&O Lanterns Candle and Lamp Oil	Normal Alkane
140	Lamplight Farms Citronella Torch Fuel	Naphthenic Paraffinic
16	STP Octane Booster	Miscellaneous
220	PPG DT 895 Reducer	Oxygenate
52	Ortho Malathion 50 Plus Insect Spray	Aromatic

258	Chevron Regular Unleaded Gasoline	Gasoline
14	STP Fuel Injector/ Carburetor Cleaner	HPD
12	Shellsol Odorless Mineral Spirits	Isoparaffinic
192	Northern Lights Lamp Fuel	Normal Alkane
185	Pennzoil Marine Fuel System Cleaner	Naphthenic Paraffinic
146	Sunnyside Brush Cleaner	Miscellaneous
218	PPG DT 870 Reducer	Oxygenate
73	Whitaker Aro-Sol 15 (Aromatic 150)	Aromatic
259	Chevron Plus Unleaded Gasoline	Gasoline
43	Chevron Techron Concentrate	MPD
89	Isopar M	Isoparaffinic
236	Aura Lamp Oil	Normal Alkane
201	Summer Lights Citronella Outdoor Lamp Oil	Naphthenic Paraffinic
10	Cypar 9	Miscellaneous
231	E-Z Water Wash Brush Cleaner	Oxygenate
284	Exxon Aromatic 100	Aromatic
940	Texaco Unleaded Regular Gasoline	Gasoline
8	Shell Rubber Solvent 332	LPD
46	Pro-Gard Fuel Injector Plus Intake Valve Cleaner	MPD
50	Chevron Low Sulfur Diesel Fuel 2	HPD
120	Isopar C	Isoparaffinic

39	Pennzoil Roadside Rescue Emergency Fuel Additive	Miscellaneous
149	Sunnyside Denatured Alcohol Solvent	Oxygenate
1001	Meijer E 85 Gasoline	Gasoline
1011	Shell Regular Unleaded Gasoline	Gasoline
33	Ronsonol Lighter Fuel	LPD
4	Shellsol D43	MPD
20	Penske Fuel Injector/ Carburetor Cleaner	HPD
64	Whitaker Paint Thinner Mineral Spirits	MPD
91	E-Z Paint Thinner	MPD
42	Chevron Aviation Gasoline 100LL	Miscellaneous
1075	Murphy USA Unleaded Regular Gasoline	Gasoline
182	Prestone Heavy Duty Brake Parts Cleaner	Miscellaneous

Appendix B: Best Practices for the Interpretation and Use of the Weathered and Biologically Degraded Samples in the Ignitable Liquids Database

The Ignitable Liquids Reference Committee of the Technical and Scientific Working Group for Fire and Explosions has prepared the following guide so users of the database are aware of the considerations and limitations of the weathered and biologically degraded samples contained within the ignitable liquids database. The guide describes sample preparation and defines the database limitations. This Best Practice Guide is continually under revision and we will accept comment and input from any user for consideration by the committee and potential inclusion in future revisions of the document.

CAUTION: Care must be exercised in relating the contents of this database to extractions from debris collected in fire scenes. The samples in this database were prepared under controlled conditions.

METHODS

Weathered samples were prepared using the following methodology:

A graduated microbial was filled with 10 mL of ignitable liquid (parent). The vial was placed into a dry bath in which the height of the vial and temperature of the dry bath were adjusted according to the percent volume evaporated. Nitrogen flowed gently above the ignitable liquid and a vacuum pump was used to remove the ignitable liquid vapor from the vial into a trap. The nitrogen needle and vacuum tubing were cleaned between samples and replaced when needed. Evaporation percentages (v/v) were 25, 50, 75, 90 and 95%, corresponding to volume reductions of 2.5, 5.0, 7.5, 9.0, and 9.5 mL, respectively. One milliliter of carbon disulfide was added to an autosampler vial containing 20 µL of the weathered ignitable liquid. The weathered liquid was collected as the volume of the parent ignitable liquid was reduced by evaporation.

Biologically degraded samples were prepared using the following methodology: Twenty microliters of ignitable liquid was deposited into a quart sized metal paint can with 100 grams of Hyponex® brand potting soil purchased from Kmart. The same bag of Hyponex® was used throughout the experiment (approximately 1.5 years) and stored in a closed plastic container. Once the quart can was sealed, the liquid and soil were mixed. After the specified time period (0, 7, 14, or 21 days), an activated carbon strip (standard full size 10 mm x 22 mm) attached to a paperclip and nylon string was suspended into the headspace of the can. The can was placed into an oven at 65° C for 16 hours. After heating, the can was removed from the oven and allowed to cool to room temperature. The activated carbon strip was cut in half, and one half was placed into an autosampler vial with 500 µL of carbon disulfide. The other half was retained for use in other research investigations.

OBSERVATIONS AND DISCUSSION

Generally, weathering (evaporation) resulted in a sequential loss of the lower molecular weight compounds. Within each volume reduction, lower boiling point components were lost before the heavier components. In some instances, weathering of the samples caused the ignitable liquid to change ASTM classification when the early components were lost. For example, SRN 775, STP Octane Booster, is classified as "miscellaneous" because of the significant abundance of earlier eluting aromatics in the product; however, after 50% weathering had occurred the sub-component class changed to "heavy petroleum distillate," as the early abundant aromatics were lost.

While most of the weathered samples (especially those lightly weathered) are directly relatable to the parent (un-weathered) samples, this is not consistently true for the biologically degraded samples. Degradation by microbes was inconsistent and highly variable. Replicate analysis of the same ignitable liquid placed in the same batch of potting soil and sampled after the same amount of time was performed. While the only difference was the elapsed time between the preparations and the moisture of the potting soil, variations in the resulting data were seen. The major degradation observed in the ILRC study typically occurred between 0 to 7 days. Some peaks present in biodegraded samples may not be from the liquid but from the soil itself or metabolites from the microbial action. In additional testing, samples heated to 85° C for 4 hours contained aldehydes; suspected by-products of the microbial digestion of the ignitable liquids. While some ignitable liquids may be suggested in a biodegraded sample, the changes may not allow a conclusive classification.

Microorganisms may have preferences for the types of chemicals they use as carbon sources. In addition, bacteria may be opportunistic in the selection of compounds preferred for consumption and may evolve to change their preferred food source. This transition is dependent on the chemicals available and the microbe's ability to utilize inducible enzymes. As one preferred source becomes depleted, the microbes adapt and are increasingly able to consume a separate source. For example, some microbes may initially prefer to consume alkanes. As the alkanes are consumed and are no longer present, the microbes adapt and consume other classes of compounds (e.g. alkene or aromatic compounds); though the mechanism to do so requires more energy and a different approach [3]. The committee feels that this factor has contributed to some of the variations we have seen. The same would also relate to differences between the data generated and available in this database, and what is observed in real world fire debris samples.

RECOMMENDATIONS / LABORATORY CONSIDERATIONS

Based on an examination of experimental data from the biologically degraded ignitable liquid studies, the Ignitable Liquid Reference Committee (ILRC) recommends setting a threshold limit for accepting data as positive. This threshold will be different and dependent on each laboratory's procedures and instrumentation. Based on the instrument used to collect data at the National Center for Forensic Science, the threshold selected by the ILRC members was 15,000 to 20,000 counts in the total ion chromatogram. It was determined that below this threshold, there was not sufficient mass spectral data available to confirm individual peak identification. Even though some extracted ion profiles appeared to be recognizable, the committee was unable to verify individual components. Patterns with very few components often required the higher threshold while patterns with many components and a complex pattern could often be determined using the lower threshold. Regardless of the threshold, the committee has developed a criterion of making a negative determination if there is not sufficient conclusive evidence of the presence of an ignitable liquid.

Caution must be exercised when reviewing Total Ion Chromatogram data that is at a very low level.   Extracted ion profiles (EIP), by filtering away some of the less desirable compounds from the TIC, will sometimes produce what appears to be an EIP pattern comparable to a reference standard. However, comparison of only the retention time patterns of peaks from EIP from the debris sample to a reference standard is not enough on which to base a positive determination. The mass spectra of target compounds must be examined. If, due to the levels of their concentration or presence of co-elutants, the mass spectra are not sufficiently clear, the analyst should opt for a negative determination.

The ability to make a positive determination on a biologically degraded ignitable liquid is dependent on the compounds remaining from the parent liquid, the lower threshold for the instrument, and the comparison of the unknown against an ignitable liquid standard degraded under controlled conditions. The degree and pattern of degradation for ignitable liquids is affected by:

The abundance of microbes in the sample

The types of microbes in the sample [9]

Ability of the microbes to adapt to different food sources [3]

The amount of ignitable liquid in the sample

The time the microbes and ignitable liquids are in contact

The temperature of the sample over time [2]

The moisture content of the soil or other organic matrices [3]

The soil type [4] [5]

The records in this database do not represent all possible results for microbial degradation. The committee urges the users of this database to obtain a comparison soil (if possible) and spike it with the suspected ignitable liquid to approximate what was found in the debris sample. Analysts should note the date of the incident and take measures to retard microbial growth prior to sample extraction. Major degradation in this study occurred most often by day 7. There are references and suggested readings for the user at the end of this guide. Some of the variations seen may also relate to individual laboratory protocols. Any positive determination of the presence and identification of an ignitable liquid requires a combination of the total ion chromatogram, the appropriate ion profiles, and the mass spectra of key compounds. Chromatographic patterns are not sufficient by themselves to confirm that an ignitable liquid is still identifiable after being deteriorated or degraded. Confirmation of components and target compounds by extracted ion chromatograms, mass spectra and library matches must be used to confirm the ignitable liquid.

CAUTION – DO NOT make any identification of a target compound by retention time alone.

Prior to extraction, the method commonly used to retard microbial degradation of ignitable liquids in fire debris is to refrigerate or freeze them. During extraction, microbes are affected by heat and may be completely destroyed depending on the time and temperature utilized. However, the tolerance for heat is not the same for all microbes [2]. The passive headspace extraction method for fire debris usually progresses with the sealed containers being heated for 12 to 16 hours at 60 to 85° C. This may be sufficient to kill microbes present in the debris as some studies indicate that they are destroyed in a logarithmic process where the time is a more significant factor than temperature in their destruction [2]. Further studies on this subject may be warranted.

When performing a search in the database for a particular product, the initial screen may have a link for "Related Samples". This "Related Samples" link takes the user to a screen where the neat liquid and all available biologically degraded and weathered total ion chromatograms for that sample are shown. The list can be further sorted to show only the weathered or biologically degraded samples as compared to the original product. This allows the user to see the progression of the loss of components from the parent product to the most affected sample. Click here to hyperlink an example in the database.

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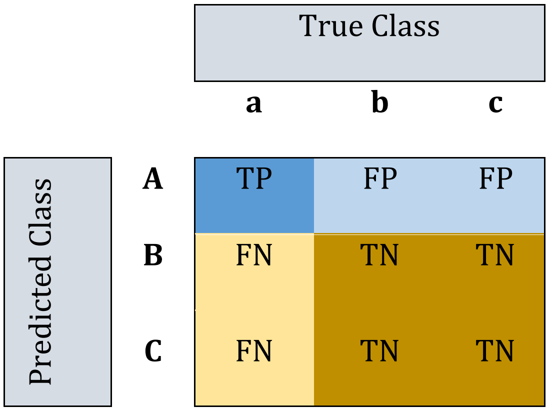
Appendix C. Example calculation of TPR, FPR and Accuracy For AR from Table 11

Use the following table and color scheme to identify the cells that will be used in the equations for calculating TPR, FPR and accuracy for the Aromatics (AR).



Predicted Class		True Class		
		a	**b**	**c**
	A	TP	FP	FP
	B	FN	TN	TN
	C	FN	TN	TN

The true positive rate (TPR) or sensitivity is TP/ (TP+ΣFN). The false positive rate (FPR) or specificity is ΣFP/ (ΣTN+ΣFP). The accuracy of the model is (TP + ΣTN)/(total population), as described in the text.

The following table gives the assigned and predicted classes (Table 11) and has been shaded to indicate the TP, FP, FN and TN cells.

		Database Assigned Class									
		AR	GAS	HPD	ISO	LPD	MISC	MPD	NA	NP	OXY
Predicted Class	AR	**24**	4	0	0	0	11	0	0	0	3
	GAS	0	**22**	0	0	0	4	0	0	0	2
	HPD	0	0	**58**	0	1	18	0	0	2	2
	ISO	0	0	0	**36**	0	14	0	0	0	0
	LPD	0	0	0	0	**23**	5	1	0	0	6
	MISC	4	1	2	1	1	**60**	13	0	0	18
	MPD	0	0	1	0	3	20	**96**	0	4	2
	NA	0	0	3	0	0	1	0	**18**	0	0
	NP	0	1	2	0	0	5	2	0	**8**	0
	OXY	0	1	0	1	0	8	0	0	0	**62**

The following table gives the calculated sums for each of the shaded areas and reduces the larger table into a 2x2 confusion matrix.

		True Class	
		ar	$\overline{ar}$
Predicted Class	**AR**	24	18
	$\overline{AR}$	4	506

The true positive rate (TPR) or sensitivity is 24/ (24+4)=0.86. The false positive rate (FPR) or specificity is 18/ (506+4)=0.03. The accuracy of the model is (24 + 506)/(24 + 4 +18 +506) = 0.96.