

A Comprehensive Study of the Alteration of Ignitable Liquids by Weathering and Microbial Degradation

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ABSTRACT

The differing effects of weathering and microbial degradation are described here in a comprehensive study that involved 50 different ignitable liquids from the Ignitable Liquids Database and Reference Collection. Examples of ILRs from each of the main classes established by the American Society of Testing and Materials (ASTM) are presented. Weathering was accomplished via evaporation whereas microbial degradation was carried out on soil at room temperature for periods of up to 21 days. Major trends included the rapid degradation of long n-alkanes and monosubstituted alkyl benzenes (e.g., toluene, ethylbenzene, and propylbenzene). Surprisingly, some longer branched alkanes (e.g., trimethyloctanes) were also susceptible to microbial attack. Although all ignitable liquids examined suffered at least to some extent from microbial degradation, gasoline, petroleum distillates, and oxygenates were the most susceptible. Isoparaffinic and naphthenic paraffinic products were the most resistant to microbial degradation.

KEY WORDS: Forensic science, ignitable liquids, microbial degradation, weathering, soil, biodegradation

The investigation of a suspicious fire relies upon a successful collaboration of criminal investigators, fire investigators, technical experts, and forensic chemists. One of the key findings in such an investigation is that debris gathered from the fire scene contains residues of an ignitable liquid. As most ignitable liquid residues (ILRs) originate from readily available petroleum products, it has been a long-standing practice to classify ILRs per their chemical composition and boiling point range [1].

However, the chaotic nature of a fire can confound this effort. This is manifested as low levels of residue, naturally present volatiles (e.g., terpenes), background contaminants (e.g., tar, asphalt) and pyrolysis products from polymeric substrates (e.g., rubber, plastic, carpet padding) [2,3]. The nature of the ILR itself can also be perturbed via two main processes. The first, weathering, describes the degradation of an ignitable liquid due to evaporation or partial burning. This results in a predictable loss of low boiling compounds relative to high boiling compounds. The second, microbial degradation, can occur in fire debris samples that contain soil, rotting vegetation or other substrates that may support large populations of bacteria. This results in preferential loss of readily metabolized compounds such as straight chain alkanes and mono-substituted aromatics [4]. Prior research into the forensic implications of microbial degradation began over 20 years ago with the seminal work of Mann and Kirkbride [5,6]. Since then, the effect of microbial degradation on a wide variety of ignitable liquids has been reported [7,8]. The extent to which degradation is reproducible and results in changes different than those seen in weathering has also been described for gasoline [9]. Abiotic factors such as soil type and season have also been studied [10,11]. Fortunately, preserving ILRs in fire debris evidence can be accomplished by either freezing evidence upon arrival to the laboratory or by adding chemical preservatives in the field [12].

For the results from a fire debris analysis to be successfully interpreted, a comprehensive database is needed that contains samples of all classes of ignitable liquids that

have been subjected to both evaporation (weathering) and microbial degradation. To date, a direct comparison of weathering and degradation has been reported only for gasoline [9]. A database will be a valuable tool for fire debris analysts in the identification and classification of ignitable liquid residues.

The purpose of the work presented here was to observe any changes in an ignitable liquid due to weathering or microbial degradation. Multiple examples from each ASTM E1618-11 classification were obtained from the Ignitable Liquids Reference Collection (ILRC) at the University of Central Florida (<http://ilrc.ucf.edu/>). The resultant data were added to the ILRC database and related back to the original non-degraded ignitable liquid. The ILRC database was developed by the National Center for Forensic Science (NCFS) at the University of Central Florida and the Scientific Working Group for Fire and Explosions (SWGEX) to aid forensic chemists in interpretation of the ILR in fire debris.

Materials and Methods

Materials

The ignitable liquids were obtained from the ILRC and are listed in Table 1. The substrate used was Hyponex[®] brand potting soil obtained from K-Mart. Activated charcoal strips were obtained from Albrayco Technologies. Quart-sized paint cans were obtained from Best Containers. Carbon disulfide was purchased from Fisher Scientific.

Weathering Experiments

All weathering experiments were conducted at NCFS. The ignitable liquids were evaporated (weathered) by volume to remove approximately 25, 50, 75, 90 and 95 percent of the original volume. Weathering was performed in 10 mL conical vials with 0.1 graduations. The vial was filled to the 10 mL mark and evaporated to volume under a continuous stream of nitrogen to assist in evaporation. Heat was applied via a dry bath to aid in the evaporation of some ignitable liquids. A volume of 20 microliters (0.02 mL) was added to 1 mL of CS₂ for GC-MS analysis for all samples.

Microbial Degradation Experiments

All microbial degradation experiments were conducted at IUPUI. Each ignitable liquid (20µL) was spiked onto approximately 100g of potting soil inside a quart-sized paint can. Four identical cans were prepared for each ignitable liquid. One can was immediately extracted as described below and the remaining three were sealed and aged. After storage at room temperature for 7, 14, and 21 days, the sealed cans were re-opened and a whole carbon strip was suspended on a paper clip into the headspace of the can. The can was then resealed and baked at 65°C for 16 hours. Upon cooling the can to room temperature, the carbon strip was removed and cut in half. One half of the strip was placed into a vial with 500 µL of carbon disulfide for analysis.

Instrumental Analysis

Gas chromatography-mass spectrometry analysis was performed using an Agilent 6890 gas chromatograph and 5973 mass spectrometer utilizing an auto-sampler. One microliter of the 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 linear 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 250°C with a source temperature of 230°C and a quadrupole temperature of 150°C. Mass spectra were acquired between 30 and 350 mass to charge ratio at an acquisition rate of 2-3 scans/second. The detector was turned off between 1.54 and 2.00 minutes during solvent elution.

Results and Discussion

The effects of weathering and microbial degradation are distinct and can be separately observed in the chromatograms of the ignitable liquids presented here. In this work, several ignitable liquids in each ASTM class were weathered up to 95% (v/v) via evaporation under nitrogen. In addition, un-evaporated samples were subjected to microbial degradation on potting soil for up to 21 days. The results for one liquid from each ASTM class will be presented and discussed. The chromatograms for all other liquids listed in Table 1 can be found in the Ignitable Liquids Database and Reference Collection (ILRC).

Gasoline is the most common ignitable liquid encountered in casework. This liquid contains a significant contribution of aromatic compounds but also contains normal, branched and cyclic alkanes with a minor contribution of naphthalenes. The set of chromatograms on the left side of Figure 1 show the effects of weathering while the set of chromatograms on the right show the effects of microbial degradation. As can be seen in Figure 1, weathering of gasoline results in the loss of the branched alkanes that elute between 0 and 4 minutes. There is also nearly total loss of toluene ($t_r = 4.9$ min) and the C₂-alkylbenzenes (between 6.9 – 7.5 min) once

the liquid is highly (90%) weathered. Conversely, weathering results in a relative increase in abundance of heavier components, beginning with the C₃-alkylbenzenes (between 8 – 10 min).

In contrast, microbial degradation causes the selective loss of certain compounds based upon chemical structure, not boiling point. The compounds that are degraded most rapidly are the mono-substituted alkylbenzenes: toluene ($t_r = 4.9$ min), ethyl benzene ($t_r = 6.9$ min) and propyl benzene ($t_r = 8.9$ min). After 7 days, the loss of ethyl- and propyl benzene clearly perturb the peak height ratios of the C₂- and C₃-alkylbenzenes. The biodegradation of SRN 116 also demonstrates these changes, as found in the ILRC.

For most gasolines, samples aged between 7 and 21 days were degraded to the point where the alkylbenzenes could not positively be identified by their mass spectra. By 21 days, the peaks for all alkyl benzenes essentially disappeared from the chromatogram. Note that the C₃-alkylbenzenes must be present in a fire debris sample to classify an ignitable liquid residue as gasoline according the ASTM E1618-11. In addition, the peak height ratios of these compounds must be consistent with that of a gasoline standard.

Figure 2 compares the effects of weathering and microbial degradation in a heavy petroleum distillate (HPD). Weathering of an HPD results in the systematic loss of low boiling compounds regardless of chemical structure (i.e., alkyl benzenes, branched alkanes and n-alkanes). As the extent of weathering increases, more and more of the higher boiling compounds are lost, increasing the relative intensity of the less volatile compounds left behind. The result of this phenomenon is a clear difference between the range of retention times observed in a pristine HPD (9.5 – 22.5 min) and the range of retention times seen in a 90% weathered sample (20.7 – 27.2 min).

Before discussing the biodegradation of HPD, there is an important abiotic factor to consider. These ILRs contain a significant amount of high boiling n-alkanes (e.g., n-C₁₉ and

up), however, the recovery of these compounds from a soil substrate is relatively poor. A comparison of the unevaporated HPD to that of an HPD recovered from soil at 0 days illustrates this point in that n-alkanes as large as n-C₂₃ are readily identified in the liquid but nothing above n-C₁₉ is recovered from the soil. This poor recovery is related to the low vapor pressure of the large n-alkanes but also their affinity for soil organic matter, which can serve as an efficient absorbent.

Taking this into account, there are clear biotic effects on a HPD, most notably the n-alkanes are rapidly degraded, eventually resulting in an unresolved alkane envelope comprised largely of branched alkanes. This creates the possibility that a petroleum distillate whose n-alkanes had degraded could be misclassified as either an isoparaffinic or naphthenic-paraffinic liquid. Determining factors would be the extent of n-alkane degradation and the presence of cyclic alkanes. In this sample, the chromatogram takes on such an appearance after 7 days. Overall and in general, HPD samples degrade relatively rapidly (i.e., in less than 7 days), yielding a weak chromatographic profile that may present challenges for the determination of ILR in the sample.

There also appears to be some additional, albeit subtle, effects on the degraded HPD samples. The first is a systematic shift in the retention time of the unresolved envelope as degradation progresses (e.g., from ~15 min at 0 days to ~18 min after 21 days). This would typically be interpreted as a weathering effect, but as the samples were sealed in air tight cans, evaporative loss was not possible. As will be discussed below, there may be a relationship between the molecular weight and vapor pressure of a compound and the extent to which it is degraded by microbes.

A clearer trend is a greater relative loss of high boiling n-alkanes (e.g., n-C₁₅, n-C₁₇, and n-C₁₉) relative to low boiling n-alkanes (e.g., n-C₁₁ and n-C₁₃). There is evidence that soil

bacteria preferentially degrade alkanes that fall within a specific size range [4]. Alkanes that fall between 6 and 15 carbon atoms are neither too hydrophilic nor too hydrophobic, hence they can more readily diffuse through cell membranes [4]. However, our experiments show that the alkanes in HPDs can be well above this “goldilocks” zone – and it is these alkanes that appear to be degrading faster in this work. The biodegradation of SRN 020 exhibits similar changes, as found in the ILRC. Ultimately, additional experiments are warranted to ensure that this behavior can be reproduced in degradation experiments that proceed to a lesser degree.

The effects of weathering and microbial degradation on an aromatic liquid are shown in Figure 3. This aromatic liquid is comprised predominantly of alkyl benzenes with smaller contributions from naphthalene compounds. As the aromatic liquid is weathered, the trimethylbenzenes, which are the lowest boiling components in the sample, are the first to be affected followed by the higher boiling alkylbenzenes. As these components are lost, a relative increase in abundance of the higher boiling alkylbenzenes and naphthalenes is observed.

In contrast, as the liquid biodegrades, there is an overall degradation of all compounds at relatively similar rates. For example, although the relative amount of C₃-alkylbenzenes is reduced over time, these compounds remain identifiable after 21 days on soil. Similarly, the C₄-alkylbenzenes remain identifiable and their peak height ratios are largely unchanged. Two notable exceptions are naphthalene and 2-methylnaphthalene, which are rapidly diminished relative to the other aromatic compounds. This implies a potential selectivity of soil microbes for polyaromatic compounds such as the naphthalenes, however this requires further investigation. Interested readers can also examine SRN 284 in the ILRC.

Figure 4 compares the effects of weathering and microbial degradation on a miscellaneous liquid which contains a mixture of an aromatic and a heavy petroleum distillate. The changes in this liquid with weathering are solely based upon the boiling point of the various

components. For example, at 25% weathered the C₂-alkylbenzenes are lost, with some loss of the C₃-alkylbenzenes and a slight increase in relative abundance of the alkane portion. Total loss of the C₃-alkylbenzenes is observed at 50% weathered while the alkane portion remains largely unweathered. However, since the aromatics no longer remain, this liquid would be classified as an HPD. At 75% weathered, a significant decrease in n-C₁₁ and n-C₁₂ is observed and at 90% weathered, a clear shift in the chromatographic profile of the n-alkanes is observed.

A quite different trend is noted in the microbial degradation of this miscellaneous liquid. After exposure to the soil for 14 days, the C₂-alkylbenzenes are completely lost. In addition, the n-alkanes also suffer from bacterial action and are largely consumed after 7 days. The C₃-alkylbenzenes, being the most resistant to degradation, remain in the sample after 21 days.

The effects of weathering and microbial degradation on an isoparaffinic product are compared in Figure 5. As weathering increases, a loss of the lower boiling compounds is observed, while the relative abundance of the less volatile compounds increases. For example, the trimethylpentanes that are most abundant in the 0% weathered sample become less abundant while the trimethylhexanes, trimethylheptanes, and trimethyloctanes increase in abundance. However, in microbial degradation of this isoparaffinic product, the trimethylpentanes remain largely unaffected, while the trimethylhexanes, trimethylheptanes, and trimethyloctanes are almost completely degraded over the course of 21 days. While bacteria preferentially degrade n-alkanes and aromatics, when presented with a liquid comprised of only branched alkanes, they will degrade the long chain, lesser branched alkanes such as the trimethyloctanes found in the isoparaffinic liquid shown in Figure 5.

Figure 6 demonstrates the effects of weathering and microbial degradation of a normal alkane product. Although n-C₁₂ remains the most abundant alkane in all the weathered

chromatograms, there is a significant change in the relative amounts of low boiling n-alkanes (i.e., n-C₁₀ and n-C₁₁) versus high boiling n-alkanes (i.e., n-C₁₃ and n-C₁₄).

In microbial degradation, the chromatograms are initially skewed towards lower boiling n-alkanes, with n-C₁₁ being the most abundant. This is expected based upon the relatively low recovery of high boiling compounds from the soil matrix (as discussed above with HPDs). As the degradation process proceeds, n-C₁₀ and n-C₁₁ are lost at a slightly faster rate than n-C₁₂, resulting in a small change in the relative peak heights. The overall pattern, however, is preserved. It is important to note that a liquid composed solely of n-alkanes represents a much higher concentration of these compounds as opposed to other liquids with an n-alkane component. This allows this liquid to be readily identified, even after 21 days of degradation. In addition, this liquid consisted of a relatively narrow carbon number range, hence any preferences of the bacteria based upon molecular weight are not readily visible.

The naphthenic-paraffinic product shown in Figure 7 is not as significantly affected by weathering and microbial degradation. This liquid is comprised largely of branched alkanes and naphthenic compounds such as 2-methyl-trans-decalin. This liquid does contain a small contribution of n-alkanes, however. In weathering, the n-C₁₁ and 2-methyl-trans-decalin as well as the earlier eluting compounds are lost due to evaporation while the less volatile compounds, including n-C₁₂, show an increase in relative abundance.

In contrast, both n-C₁₁ and n-C₁₂ are the first to be degraded by the bacteria, while the relative abundance of 2-methyl-trans-decalin increases. Note that the prominent peak at ~14 min in the most degraded sample is safrole – a component of the root bark of the sassafras tree. This is an artifact from the potting soil substrate, which in some cases contained wood fragments. This anomaly was found in only one sample and it did not interfere with the identification of any other peaks in the chromatogram.

Lastly, Figure 8 demonstrates the effects of weathering and microbial degradation on an oxygenated liquid. This liquid contains 2 oxygenated species, isopropanol (IPA) and butanone, which are the first two compounds to elute. This liquid also contains aromatic and aliphatic compounds. When this liquid was subjected to weathering, a loss of compounds based on boiling point was observed, where IPA and butanone decreased quickly followed by heptane and methylcyclohexane. At 95% weathered, toluene and very small amounts of heptane and methylcyclohexane remained.

When this liquid was placed on a soil substrate, there was a dramatic and immediate decrease in recovery of IPA and butanone (0 days). This is attributed to adsorption and/or absorption of these small, water soluble components into the soil matrix. Thus, these compounds are not able to serve as visual references for the loss of the remaining compounds in the mixture. This essentially results in the degradation experiment being carried out on a three-component mixture (i.e., heptane, methylcyclohexane and toluene). Being an n-alkane, heptane is rapidly degraded in the soil. Similarly, toluene is a mono-substituted benzene that is degraded relatively easily, although it is still identifiable after 21 days due to its high initial concentration. Finally, methylcyclohexane is the most resistant to degradation and remains identifiable after 21 days.

Conclusions

Microbial degradation is based on the ability of bacteria to metabolize the compounds in ignitable liquids. In contrast, weathering results in the loss of all lower boiling compounds without bias, other than well-known changes in boiling point with molecular structure. In general, bacteria prefer to utilize n-alkanes and lesser substituted alkylbenzenes. Among the alkylbenzenes, toluene is degraded first, followed by the C₂-alkylbenzenes, then the C₃-alkylbenzenes, and finally the C₄-alkylbenzenes. Longer, lesser branched alkanes (e.g.,

trimethyloctanes) are also susceptible to microbial attack. All ignitable liquids examined suffered at least to some extent from microbial degradation, although gasoline, petroleum distillates, and oxygenates suffered the most, while the isoparaffinic and naphthenic paraffinic products were affected the least.

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Table 1: Ignitable liquids used for this study, along with the Sample Reference Number (SRN) and ASTM classification (according to [13]).

SRN*	Liquid	Class
1940	Texaco Regular Unleaded Gasoline	Gasoline
1075	Murphy USA Regular Gasoline	Gasoline
1011	Shell Gasoline	Gasoline
1001	Meijer E-85 Gasoline	Oxygenated
004	Shellsol D43	Medium Petroleum Distillate
005	ShellSol A100	Aromatic
008	Shell Rubber Solvent 332	Light Petroleum Distillate
010	Cypar 9	Miscellaneous (MPD+Aromatic)
012	Shellsol Odorless Mineral Spirits	Medium Isoparaffinic
014	STP Fuel Injector/Carburetor Cleaner	Heavy Petroleum Distillate
016	STP Octane Booster	Miscellaneous (HPD + aromatic)
020	Penske Fuel Injector/Carburetor Cleaner	Heavy Petroleum Distillate
021	Mineral Spirits/Paint Thinner	Medium Petroleum Distillate
033	Ronsonol Lighter Fluid	Light Petroleum Distillate
035	Zippo Premium Lighter Fluid	Light Petroleum Distillate
039	Pennzoil Roadside Rescue Emergency Fuel Additive	Miscellaneous (Isopar+Aromatic)
042	Chevron Aviation Gasoline 100 LL	Miscellaneous (Isopar+toluene)
043	Chevron Techron Concentrate	Heavy Petroleum Distillate
046	Pro-Gard Fuel Injector PLUS Intake Valve Cleaner	Medium Petroleum Distillate
050	Chevron Low Sulfur Diesel Fuel 2	Heavy Petroleum Distillate
052	Ortho Malathion 50 Plus Insect Spray Conc.	Aromatic

053	Multipurpose Insect Killer	Naphthenic Paraffinic
059	Adhesive Remover	Aromatic
064	Whitaker Paint Thinner/Mineral Spirits	Medium Petroleum Distillate
069	Whitaker #51 Lacquer Thinner	Oxygenated
073	Whitaker Ar-Sol 15 (Aromatic 150)	Aromatic
077	Norpar 12	Normal Alkane
087	Isopar E	Light Isoparaffinic
089	Isopar M	Medium Isoparaffinic
091	E-Z Paint Thinner	Medium Petroleum Distillate
105	Phillips 66 Unleaded Regular Gasoline	Gasoline
116	Gasoline	Gasoline
119	Isopar H	Medium Isoparaffinic
120	Isopar C	Isoparaffinic
131	Gum Turpentine	Miscellaneous
140	Lamplight Farms Citronella Torch Fuel	Naphthenic Paraffinic
146	Sunnyside Brush Cleaner	Miscellaneous (MPD+Aromatic)
149	Sunnyside Denatured Alcohol Solvent	Oxygenated
176	V & O Lanterns Candle and Lamp Oil	Normal Alkane
182	Prestone Heavy Duty Brake & Parts Cleaner	Miscellaneous (LPD + aromatic)
185	Pennzoil Marine Fuel System Cleaner	Naphthenic Paraffinic
192	Northern Lights Lamp Fuel	Normal Alkane
201	Summer Lights Citronella Outdoor Lamp Oil	Naphthenic Paraffinic
218	PPG DT870 Reducer	Oxygenated
220	PPG DT 895 Reducer	Oxygenated
231	E-Z Water Wash Brush Cleaner	Oxygenated

236	Aura Lamp Oil	Normal Alkane
258	Chevron Regular Unleaded Gasoline	Gasoline
259	Chevron Plus Unleaded Gasoline	Gasoline
284	Exxon Aromatic 100	Aromatic
* Sample Reference Number from the ILRC.		

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FIGURE LEGENDS

Figure 1: The effects of weathering versus microbial degradation of gasoline.

Figure 2: The effects of weathering versus microbial degradation of a heavy petroleum distillate.

Figure 3: The effects of weathering versus microbial degradation of an aromatic product.

Figure 4: The effects of weathering versus microbial degradation of a miscellaneous liquid.

Figure 5: The effects of weathering versus microbial degradation of an isoparaffinic product.

Figure 6: The effects of weathering versus microbial degradation of a normal alkane product.

Figure 7: The effects of weathering versus microbial degradation of a naphthenic-paraffinic product.

Figure 8: The effects of weathering versus microbial degradation of an oxygenated product.