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 microvial 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^{o} 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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