Detection of gasoline from internal tissues for use in determining victim status at the time of a fire

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Certificate of Authorship/Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are appropriately referenced in the thesis.

Kevin Pahor

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Abstract

In Ontario, fire investigators from the Office of the Fire Marshal (OFM) are responsible for determining the origin and cause of suspicious fires. As part of the investigation, fire debris samples are collected from the scene and analyzed by the Centre of Forensic Sciences. The standard practice is to collect items that are porous, highly absorbent or adsorbent with high surface areas as they allow for better retention of the ignitable liquids. The evidence typically collected includes carpets, cardboards, soils, cloths and other items that have not been impinged by flame such as beneath baseboards. These samples are analyzed for the presence of ignitable liquid residues which may be evidence that an accelerant was used at the fire. When a body is recovered from a fire it can provide another source from which to collect samples for analysis. These samples can be especially helpful in instances where the fire generated an intense heat which may cause a loss of ignitable liquid residues from the fire debris. The tissue samples have a greater likelihood of still containing residues as the organs and body fluids can act as a shield protecting the residues from volatilization.

The purpose of this study is to validate whether a victim was alive or deceased at the time a fire was intentionally set by detecting presence or absence of gasoline residues within their lungs and heart blood post fire. It was hypothesized that only when a victim was alive and performing respiration would sufficient gasoline vapours enter the airways and bloodstream for detection postmortem. Contamination becomes a significant issue when these samples are collected at autopsy and this study aimed to determine the accuracy with which a gasoline signature can be interpreted following the collection and analysis of lung tissue and heart blood.

Pig (*Sus domesticus*) carcasses were chosen as acceptable analogues for humans in this study. The experiments involved anaesthetizing a pig (with Animal Ethics Approval), exposing the pig to gasoline vapours for 10 minutes, and then euthanizing it. The carcass was clothed with a cotton t-shirt and placed in a house where additional gasoline was poured onto it. The house also contained two additional clothed pig carcasses which did not inhale gasoline vapours; one with gasoline poured directly onto it and the other with no gasoline exposure (negative control). Thermocouples were placed under each carcass and in the centre of each room at ceiling and floor level to record the temperature. The house was set ablaze and monitored by a volunteer fire service. After the fire had reached flashover and was suppressed, the carcasses were collected and their lungs and heart blood excised at a necropsy. The lungs and heart blood were then placed into glass mason jars following the OFM protocol. The headspace from each sample was analyzed by thermal desorption-gas chromatography-mass spectroscopy to determine the presence or absence of a gasoline signature. Two full scale house fires were conducted in order to obtain three replicates.

The results showed that only the lungs and heart blood from the pig that inhaled gasoline contained gasoline residues. This indicates that it is possible to determine a victim's status at the time of the fire based on the detection of gasoline in the lungs and/or heart blood. It was also concluded that contamination of samples during an autopsy can be minimized by changing gloves before handling the internal tissues. The thermal data showed that the bodies act as an insulator and protects the underside as the temperatures under the carcasses did not exceed 30°C while the room reached over 900°C at the first full scale house fire.

These results will impact the forensic community by demonstrating the importance of analyzing a deceased victim's internal tissues for ignitable liquid residues post fire as they may provide evidence of an intentionally set fire as well as providing information about the victim's status when a fire was started. These findings will have a direct impact to the OFM as additional evidence can be obtained by completing internal tissue analysis. This will intern impact the Centre of Forensic Science (CFS) as it confirms the importance of analyzing internal tissues in order to provide results to fire investigators. Finally these findings should be used to implement new protocols at the Coroner's Office so contamination can be minimized during fire autopsies and accurate samples are collected and sent to the CFS for analysis.

Key Words: forensic science, fire investigations, fire chemistry, ignitable liquid residues, accelerants, gas chromatography-mass spectrometry (GC-MS)

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Chapter 1- Introduction

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1.1 Fire Investigations

A fire investigation is the complex process of determining the origin, cause, and development of a fire or explosion (NFPA 921, 2011). A single fire investigation could involve an array of technical skills and knowledge in order to reach a final conclusion with respect to where the fire started, how it started and how it progressed. The knowledge needed could include; electrical, heat and air conditioning knowledge, metallurgy, fire prevention techniques, and forensic chemistry (Hine, 2004). Should the investigation require expertise beyond the knowledge of the investigator then a skilled professional may be hired to assist with the investigation (Hine, 2004).

Fires involving over five hundred thousand dollars damage, a historical building, personal injury, fatality or an explosion in Ontario, will be investigated by the Ontario Office of the Fire Marshal (OFM). The investigators employed by the OFM are trained in a variety of disciplines so that they are able to respond to the multitude of different scenes encountered in the field. The scenes can range from an investigation of a single room fire or car fire to a multiday excavation of a collapsed house or industrial explosion. Should an investigator can request assistance from the engineering group that is employed by the OFM. The engineer can attend the scene to assess any technical aspects or advise the investigator on the type of evidence they would need to conduct an engineering examination.

1.1.1 Scene Examination

The majority of a fire investigation will take place at the scene which should remain secured until the investigator arrives and begins the examination. In order to ensure that all investigations are carried out in a consistent manner a systematic approach is taken by employing the scientific method (NFPA 921, 2011). This involves conducting a thorough examination and documentation of the scene, along with proper collection of evidence.

When the investigator arrives at the scene they will meet with the first responders and witnesses to obtain a better understanding of the events before they enter. By interviewing the firefighters, police and any other bystanders, the investigator can

determine the best place to begin. A detailed account from the first responders will also inform the investigator of any actions that were completed after the fire which may have disturbed the scene. This can involve the investigator walking through the scene with the firefighters to determine how they interacted with the fire scene. Once the initial interviews are completed the scene documentation can begin.

The investigator will document each scene with written notes, photography, sketches, and evidence collection. Typically this will begin from the outside and work inward (Lentini, 2006). It is important to describe all aspects of the scene so that it can be recreated in court for a jury. Photographs and a detailed description will be taken for each side of the structure involved. It is important to note and photograph any burn or smoke patterns.

When the entire exterior has been documented the investigator will then move inside to conduct the internal examination. By examining the burn and smoke patterns, the investigator may be able to determine where the fire originated. The patterns are followed from the areas of least damage to the areas of most damage (Hine, 2004; DeHaan, 2007). Following the patterns from the area of least to most damage can lead the investigator to the origin of the fire as it is assumed that the origin will have the greatest amount of damage. The reason for this assumption is that the fire burned the longest in this area and would therefore cause the most amount of damage (Hine, 2004; DeHaan, 2007). This may not always be the case as fuel load and ventilation can cause areas other than the origin to have greater amounts of damage (Hine, 2004; DeHaan, 2007; Lentini 2006). The size of the origin will be dependent on the size of the scene being investigated. For example a large industrial fire may have originated from the southwest corner of the property while a small kitchen fire could have originated from the stove. When the origin is located the investigator can then begin to determine the cause of the fire.

Examining the origin is crucial for determining the cause of a fire as this is where the first fuels were likely ignited. The first fuels of the origin will be covered by debris that has resulted from the fire, therefore a systematic removal of this debris must be completed (Hine, 2004; Lentini, 2006). This will involve removing the debris by hand

and setting aside any relevant materials for further examination or reconstruction. In some cases the debris may be sieved in order to discover small items. Once all the debris is removed the area can be washed with water in order to allow a better observation of the burn patterns. At this point furniture and other large items can be placed back in their original positions to complete the recreation. By examining the fire patterns on the furniture and structure in the reconstructed origin the investigator can determine how the fire traveled throughout the structure (DeHaan, 2007). This subsequently concludes the scene examination and allows for a classification of the cause.

1.1.2 Cause Classification

When an investigation is complete the investigator will classify the fire into one of four categories; accidental, natural, incendiary, or undetermined (NFPA 921, 2011). A classification can only be assigned if there is enough evidence to support the hypothesis formed using the scientific method.

Accidental fires are classified as fires that are caused without the intentional human act to ignite or spread fire into an area where fire should not occur (NFPA 921, 2011). Examples of this type of fire would include electrical malfunctions, gas leaks, or other nonhuman causes. Some intentionally set fires could still be classified as accidental (NFPA 921, 2011). An example would be a campfire that was intentionally set but accidentally spread by a gust of wind.

Natural fires are those fires that are set without any human interactions (NFPA 921, 2001). This classification includes fires that result from natural phenomena such as tornadoes, volcanic explosion, and lightning.

Incendiary fires are intentionally ignited under circumstances where the person igniting the fire knows that it should not be ignited (NFPA 921, 2011). The intent of the person needs to be proven in order for a fire to be classified under this category.

Undetermined is the classification given to those fires where the cause cannot be proven with an acceptable level of certainty (NFPA 921, 2011). This classification can be changed at a later date if further evidence is discovered that will allow for an acceptable category to be assigned.

1.1.3 Evidence Collection

In a fire investigation, evidence is collected for the purpose of supporting a cause classification. Evidence can be anything that will aid in supporting a cause theory which can include but is not limited to; furniture, appliances, wiring, smoking materials, and fire debris. As with other crime scene investigation any evidence collected must be well documented to establish a chain of custody. This involves photographing the item in situ, documenting its location, seizing the item and packing it with the appropriate labelling.

The most common reason for the collection of evidence is when the investigator suspects that an accelerant may have been utilised to promote the growth and spread of the fire. The best evidence to collect is from the origin of the fire as accelerants will most likely have been used to start the fire. Most common accelerants, including gasoline, are hydrophobic and will not be degraded when the fire is suppressed by water (Pert, Baron, & Birkett, 2006). The water instead causes the accelerants to be sealed in porous materials where they are protected from rapid degradation and can be kept intact for up to 3 months (Pert et al., 2006). The items that will have the highest probability of containing remnants of accelerant use are those that are porous, highly absorbent or adsorbent and have a high surface area to aid in the retention of the ignitable liquid residues (Pert et al., 2006 & Stauffer, Dolan, & Newman, 2008). With this knowledge; soil, cloth, paper/cardboard, and carpets have become the preferred items for collection (Pert et al., 2006).

It is imperative when the items are seized that they be packaged effectively to ensure the integrity of the evidence and limit any contamination risks. All items that are collected for the purpose of detecting accelerant use must be packaged in a way that ensures they are protected from contaminant exposure and evaporation or degradation (Stauffer et al., 2008). If the item is not packaged effectively it can easily be contaminated during transportation by gasoline vapours from a vehicle or other volatiles which could lead to false positives. Conversely if the sample is not packaged in an air tight container, any ignitable liquid residues (ILRs) present in the sample may evaporate and lead to a false negative. In order to prevent this from happening there are several different containers that are utilized specifically for collecting items that will be analyzed

for accelerant use. These containers include; metal paint cans, glass mason jars, and polymer/nylon bags (Williams & Sigman, 2007; Stauffer et al., 2008; DeHaan 2007).

Studies have been conducted to test the reliability of each of these containers. All demonstrated some amount of leakage with the fastest leak rate occurring in the mason jars and the slowest leak rate in properly heat sealed polymer bags (Williams & Sigman, 2007). If the bags were not heat sealed or heat sealing was not performed correctly, the leak rate increased considerably (Williams & Sigman, 2007). The mason jars were shown not to contain any significant contaminants and if any were observed they could be removed by washing the jars with hot water (Stauffer et al., 2008). In Ontario the guidelines created by the Centre of Forensic Sciences (CFS) state that items should be packaged in glass mason jars, and items that are too large for mason jars should be packaged in a nylon bag (Chemistry Section Head, 2009). The CFS also recommends that if the items have a noticeable petroleum odour they should be packaged in a mason jar and subsequently placed in a nylon bag for extra protection against residue loss (Chemistry Section Head, 2009). The OFM investigators should adhere to these guidelines for all their evidence collections. Once the items are properly packaged they are transported to a laboratory for the detection of ignitable liquid residues. In Ontario this analysis is completed at the CFS.

It is important to distinguish the words "accelerant" and "ignitable liquid" as they can often be used synonymously (Stauffer et al., 2008) even though in the fields of fire investigations and fire chemistry they have individual meanings and should not be used interchangeably. An accelerant by definition is any substance that is used to accelerate the combustion of materials that do not readily burn, e.g., furniture (Hine, 2004). For the accelerant to be effective it needs to have a flashpoint close to or below room temperature (Hine, 2004). The most common accelerants are liquids, with gasoline being one of the most predominant due to its easy accessibility (Hine, 2004; Per et al., 2006). Gasoline may also be referred to as an ignitable liquid as its properties make it readily ignitable when exposed to an ignition source that contains sufficient energy to commence combustion.

An ignitable liquid is one that will readily ignite when exposed to an ignition source, while a fire accelerant is a material that is used to increase the rate of combustion for materials that do not readily burn (Hine, 2004). The reason these words may be used synonymously is due to the fact that ignitable liquids can be used as fire accelerants. It is important to note however that ignitable liquids could be present at a scene without having been used as an accelerant. For example, gasoline may be detected in debris from a garage as it is common for gasoline to be present in a garage under normal circumstances. In this scenario, the investigator must determine whether or not it was utilized as an accelerant. If an ignitable liquid were detected in an area where it normally should not be present, such as a bedroom, then this would suggest a higher likelihood that it was used as an accelerant. Regardless, the investigator must still make the determination as the laboratory can only report if an ignitable liquid was present in a sample and its identity.

1.2 Ignitable Liquid Residue Detection

The detection of ignitable liquid residues is of vital importance to a fire investigation as their detection could be the difference between classifying a fire as an accidental fire or an incendiary one. In order for ILR detection to occur, samples must be collected from the scene and sent to a competent forensic laboratory for analysis. The investigator must determine which samples will have the highest likelihood of containing an ILR at the time of collection.

1.2.1 Sample Selection

There are several techniques an investigator can utilize to determine the best evidence to collect for ILR detection. The investigator will know which materials can retain ILR but they do not know if these materials were exposed to accelerants. Collecting evidence from the origin will increase the likelihood that the materials came into contact with accelerants. This is due to the fact that when accelerants are used in a fire their primary use is to start the fire and their remnants would be found at the origin.

The main method for determining the optimal location for sample collection is a thorough physical examination. An investigator can identify patterns left by the fire to determine if accelerants were used. A trailer or pour pattern is one pattern that may be

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indicative of accelerant use. This pattern is created when an accelerant is intentionally poured so that the fire will spread from one location to another (Hine, 2004). These patterns can be hard to distinguish when they are covered with fire debris but once the debris is removed and the area cleaned with water the patterns may become apparent (Hine, 2004).

Since pattern recognition can be difficult depending on the amount of damage caused by the fire, other techniques have emerged to determine if accelerants were used and to locate their remnants. Accelerant Detection Canines (ADC) can be used to pinpoint a precise location where an ILR may be present. An ADC is specifically trained to detect and indicate the presence of an ILR at a fire scene (Furton & Harper, 2004). These canines are trained to distinguish the scent emitted by fire debris and an ILR to prevent false positives from occurring (Furton & Harper, 2004). A study by Kurz et al. (1994) evaluated the detection limits of ADC by exposing the dogs to spiked samples of wood and carpeting ranging from 10 to 0.01 μ L of accelerant. They found that the dogs were capable of alerting on samples containing 0.01 μ L of gasoline and kerosene but that they would also on occasion falsely alert on background scents from the debris (Kurz et al., 1994). The final test of the study was conducted at heavily damaged fire scenes where partially evaporated gasoline was spotted at concentrations of 0.02 to 0.1 μ L (Kurz et al., 1994). The dogs had an excellent positive alert record at 0.1 μ L but were not as successful at concentrations lower than 0.1 μ L (Kurz et al., 1994).

One of the most recently employed techniques for detecting ILRs at a scene is an electronic hydrocarbon detector, more commonly known as a "sniffer". These sniffers draw in a vapour sample through a small vacuum and analysis is conducted with a chemical detector system designed to detect hydrocarbons and organic vapours (Furton & Harper, 2004). The detector system will vary depending on the company manufacturing the sniffer. Studies have been conducted to evaluate the usefulness of these sniffers and other accelerant detectors and compare them to ADC. A study comparing a sniffer, portable combustible gas leak detector, and a portable arson sampler was conducted by testing all techniques on both burned and unburned materials spiked with various accelerants (Conner, Chin, & Furton, 2006). The portable arson sampler is a device

manufactured by Portable Arson Samplers (Tooele, UT), that draws sample headspaces at the scene through adsorbent tubes that can be analyzed at a laboratory (Conner et al., 2006). The sniffer gave several false positives and was not able to locate small concentrations of accelerants as accurately as an ADC (Conner et al., 2006). However the study concluded that the sniffer could be a useful tool for fire investigators as it can locate and alert correctly on accelerants at the appropriate thresholds and may be effective for confirming ADC alerts (Conner et al., 2006). The gas leak detector was not useful for fire investigations as it was not sufficiently selective or sensitive while the portable arson sampler was shown to effectively collect volatile compounds from burned debris at the scene (Conner et al., 2006). The portable arson sampler was determined to be very useful for fire investigations as it can allow for volatiles to be collected at the scene which would eliminate the need for collecting and transporting fire debris. This would be especially useful for large items that an ADC alerts on that could otherwise not be collected.

The constant advancement in technology has led to an increased development of fire investigation equipment. However these new technologies still have yet to demonstrate the same effectiveness as an ADC. Nowland, Stuart, Basara, and Sandercock (2007) tested a commercial solid absorbent which is intended to assist investigators in sample location by changing colour in the presence of a hydrocarbon. The solid absorbent was only able to absorb ignitable liquids from 9 out 18 test panels that were burnt and of those samples, the indicator dye only changed colour on 3 samples. In comparison an ADC was able to alert on 16 panels (Nowland, Stuart, Basara, & Sandercock., 2007). The absorbent could not absorb any gasoline in six panels that were tested even though a GC-MS test confirmed that gasoline was still present on the panels after burning (Nowland et al., 2007).

1.2.2 Extraction of Ignitable Liquid Residues

In order for an ignitable liquid residue to be identified it must first be extracted from the fire debris. There are a variety of techniques that can be utilized for this extraction, the choice of which will depend on a number of factors. These factors could include equipment availability, cost, time, and whether the method is destructive or non-

destructive. The extraction techniques commonly used today can be classified into three categories; solvent extraction, headspace extraction, and adsorption (Stauffer et al., 2008).

The solvent extraction technique removes the ILRs from the debris with the use of a solvent. For the extraction of petroleum based residues the solvent chosen will need to be nonpolar, evaporate easily, safe to handle and should be inexpensive (Stauffer et al., 2008). The most common solvents used are n-pentane, n-hexane, and carbon disulfide (Stauffer et al., 2008). If heavier oxygenated compounds are suspected then the most commonly used solvent is diethyl ether (Stauffer et al., 2008). The extraction is completed by washing the sample with the solvent, filtering the solution to remove unwanted compounds, and concentrating the remaining solution by evaporation (Stauffer et al., 2008). Solvent extraction is comparatively fast and easy to complete, allows for the extraction of heavier compounds and can be used for small nonporous samples (Stauffer et al., 2008). However the technique may be destructive to the sample and the solvent can create interfering products by reacting with the sample substrate (Stauffer et al., 2008).

Headspace extraction is accomplished by collecting compounds present in the headspace above the sample rather than those that are trapped within the sample. It is the simplest, most expedient, and most convenient method of extraction as it requires little equipment other than a syringe and oven (Stauffer et al., 2008). The first step involves heating the sample to generate a sufficient headspace. The headspace is withdrawn using a syringe which is then fitted into a gas chromatograph for injection. The two major advantages of this technique are that it is non-destructive which leaves the evidence intact for further processing and that it is extremely rapid (Stauffer et al., 2008). The drawbacks are that it lacks a good recovery of heavier compounds and it can sometimes exhibit low sensitivity (Stauffer et al., 2008).

Adsorption extraction is a headspace extraction which utilizes a material known as an adsorbent that has a high affinity for ILRs (Stauffer et al., 2008). The adsorbent traps the ILRs and concentrates the compounds until they are removed for analysis. There are two main methods of adsorption extraction: passive and dynamic (Stauffer et al., 2008).

In a passive adsorption method the adsorbent is placed directly into the container and exposed to the headspace. The adsorbent will adsorb the residues until equilibrium is reached between the concentration of residues on the adsorbent and the concentration in the headspace (Stauffer et al., 2008). The adsorbent that is primarily used for this method is an activated charcoal strip (Lentini, 2006; Stauffer et al., 2008). The charcoal strip will be secured to a safety pin or paperclip and placed as high as possible in the container to absorb the ILRs (Lentini, 2006; Stauffer et al., 2008). This process can be completed at room temperature, when the sample is heated, or under both temperatures with two separate charcoal strips. When the exposure period is complete, the charcoal strip is removed and the residues are isolated from the strip by solvent extraction (Stauffer et al., 2008). A portion of the solvent is then analyzed by gas chromatography (GC). This is the most commonly utilized technique due to the fact that it can be applied to all ILRs and the carbon strips can be archived by cutting the strip into pieces for later analysis (Pert et al., 2006).

Another form of passive headspace adsorption involves the use of a solid phase micro-extraction (SPME) fibre. The greatest benefit to this technique is that the fibre is much smaller than the charcoal and can be directly desorbed into the GC thermally, ensuring maximum sensitivity (Stauffer et al., 2008). Other benefits include the fact that it is less labour intensive, faster than other techniques, and the fibre is protected inside the SPME cartridge until it is needed (Stauffer et al., 2008).

Dynamic headspace adsorption is conducted by drawing the headspace of a sample through a tube filled with the adsorbent. Typically, 30 to 60 mL of the headspace will be drawn through the tube, and will be thermally desorbed and analyzed with a GC (Stauffer et al., 2008). Once the ILRs are trapped inside the tube the end caps are replaced until the tube is inserted into the heating chamber of a thermal desorption unit. Inside the chamber, the tube can be heated between 50 to 350° C for a specified amount of time in order to weaken the bonds between the residues and the adsorbent (Stauffer et al., 2008). A flow gas, usually helium, will flow through the tube and carry any of the ignitable liquid residues present into the cold trap. The cold trap ranges from -100 to 30° C which causes the compounds to rapidly cool and concentrate (Stauffer et al., 2008).

The trap is then swiftly heated to a temperature between 200 and 400° C to instantaneously vaporize the compounds (Stauffer et al., 2008). Once vaporized the sample is forced into the injection port of the GC by the carrier gas. This method is quick, has low labour intensity, and will have maximum sensitivity as the residues are desorbed directly into the GC.

Tenax TA and Carbotrap 300 are two adsorbents that have been routinely used for the dynamic headspace extraction of ILRs (Borusiewicz & Zieba-Palus, 2007, Stauffer et al., 2008). These two adsorbents were analyzed to determine their effectiveness by adsorbing a test mixture consisting of 18 different compounds and then desorbing the tubes for analysis via gas chromatography-mass spectrometry (GC-MS) (Borusiewicz & Zieba-Palus, 2007). The study found Tenax TA to be more effective for the adsorption of nonpolar, high boiling compounds but less effective than the Carbotrap 300 for polar and volatile compounds (Borusiewicz & Zieba-Palus, 2007). The study also determined that Tenax TA desorption was so effective that the tubes could be reused immediately after the desorption while the Carbotrap tubes needed additional conditioning before reuse (Borusiewicz & Zieba-Palus, 2007). The study concluded that the average thermal desorption efficiency for all the compounds was 95% for Carbotrap 300 and 99.5% for Tenax TA (Borusiewicz & Zieba-Palus, 2007).

1.2.3 Detection and Interpretation

Once the samples are injected into the gas chromatograph, the analytes are separated and detection is completed using a mass spectrometer (Hine, 2004; Stauffer et al., 2008; Touron, Malaquin, Gardebas, & Nicolai, 2000). The chromatograms generated for the samples are compared to chromatograms generated from standards analyzed on the same instrument to determine the identity of any ignitable liquids present.

With advancements in technology the method of using GC-MS may no longer be the most common method. A study conducted by de Vos, Froneman, Rohwer, & Sutherland (2002) analyzed gasoline using gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS). Various materials were tested from case work that had been identified by the use of an accelerant detecting canine and subsequently collected (de Vos, Froneman, Rohwer, & Sutherland, 2002). The authors found that the use of MS/MS for detection enhanced a single MS ion profile and had an improved elimination of pyrolysis interference (de Vos et al., 2002).

One difficulty in interpreting results for an ignitable liquid residue test is distinguishing the ILRs from the background noise. The chromatogram comparison begins with the total ion chromatogram and continues with isolated ion chromatogram comparisons. The chromatograms are compared to determine if a similar peak pattern is observed between the standard and sample chromatograms. Based on how closely the pattern in the sample matches the pattern observed in the standard, the analyst will conclude the ignitable liquid residue is either present, absent, or they will reanalyze the samples. This comparison is completed in accordance with the American Society for Testing and Materials standard test method, ASTM E1618-06¢1 *Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry* (2006).

1.3 Human Remains

When a fire investigation involves a fatality the investigator will have to locate, identify and recover the human remains. The recovery of remains is not only important for the investigation but also for the family of the deceased. Recovering a body for burial will aid the family in attaining closure with the loss of a loved one (Olson, 2009). Any remains recovered will aid the investigation as they will be sent to the Coroner's office or to the local pathologist so that a cause and manner of death can be determined. It is important to maximize the amount of human remains recovered as this could have a significant impact on the cause and manner determination (Olson, 2009).

1.3.1 Cause and Manner of Death Determination

When remains are recovered from a fire scene, a cause and manner of death investigation must occur. An autopsy will be completed to identify signs of trauma or irregularities that may be present on the body which could have contributed to the victim's death. Along with the standard autopsy procedure, additional observations and tests are completed for victims of a fire. The first is an observation of the inside of the trachea which is completed by dissection. This procedure is completed to determine whether soot is present inside the trachea. A large amount of soot inside the trachea could indicate that the victim was still alive while the fire was burning as they were able to inhale smoke and soot which was subsequently trapped in the trachea.

The second additional test is the analysis of blood for carbon monoxide levels (HbCO). If the HbCO level is above the lethal concentration, the pathologist can conclude that the victim died as a result of carbon monoxide poisoning assuming no other significant findings. Additional causes of death for a fire victim can include; heat, burning, lack of oxygen, or intoxication by gases other than carbon monoxide (Rodge & Olving, 1995). Even though these two procedures carry a significant weight in the cause of death determination, the results can vary from victim to victim even when exposed to the same circumstances.

A study by Rodge and Olving (1995) compared the autopsy findings in victims of different fires with respect to HbCO levels and the amount of soot in the respiratory tract. In 49 cases where an inflammable fluid was used, 15 victims had lethal levels of HbCO, 15 had negative HbCO levels, and in the remaining 19, HbCO was detected but not at a lethal concentration (Rodge & Olving, 1995). The study also found that in four cases of homicide with an accelerant the level of soot varied from no soot to substantial amounts of soot (Rodge & Olving, 1995). The authors concluded that a large variation in HbCO levels and amount of soot present in the respiratory tract can be observed in fire victims (Rodge & Olving, 1995). This study illustrates the difficulty in determining the cause of death for fire victims even involving the same types of fire.

Determining if a victim was alive at the time of a fire is also difficult. It is commonly believed that if soot is found in the respiratory tract, the victim was alive during the fire. If one follows this assumption then the absence of soot should indicate that the victim was not breathing or deceased during the time of a fire. This is was not found to be the case in an examination of 169 fire fatalities (Gormsen, Jeppesen, & Lund, 1984). Gormsen, Jeppesen, and Lund (1984) found seven cases where there was evidence that a victim was alive when the fire started but no soot was found in the respiratory tract and no HbCO was found in the blood. This finding was supported in the study conducted by Rodge & Olving (1995).

Fire investigators may attend any autopsy conducted on the victims involved in the fire as the cause and manner of death will impact their investigation. If the investigator suspects that accelerants may have been used at the fire they can request that the pathologist remove the lungs and send them for analysis to test for any ILRs. The pathologist will remove the lungs and package them in a 1 L glass mason jar which is then refrigerated until analysis. A major problem with this analysis is the possibility of contamination if the pathologist handles clothing and other debris prior to handling the lung tissue and does not change their gloves in between. (Dr. M. Pollanen, Chief Forensic Pathologist, personal communication, November 19, 2010). This may result in false identifications of ILRs in the lung tissue and an incorrect determination of the cause and manner of death.

1.4 Current Knowledge

There is a lack of data in the literature with regards to the detection of ignitable liquid residues from post-mortem tissue samples after a fire as the majority of research is focused on improving or developing detection techniques. Only a few studies have been conducted using post-mortem blood samples from a fire victim in an attempt to detect ignitable liquid residues (Schuberth, 1994; Schuberth, 1997; Morinaga et al., 1996). There are even less data on the use of other tissues for fire debris analysis.

Analysis of tissue samples is important for fire investigations because a positive identification of an accelerant can rule out an accidental fire, thus changing the dynamic of the investigation and potential for criminal prosecutions. Tissue samples are particularly valuable because the organs and body fluids can act as an evaporation shield and protect residues retained in the body (Schuberth, 1997). This was demonstrated in two studies by Schuberth who was able to detect low boiling residues of gasoline and engine starting fluid from post-mortem blood samples of fire victims (Schuberth, 1994; Schuberth, 1997). Although the author concluded gasoline was present in the blood samples his evidence for the conclusion would not be accepted by the standards set today for an identification of gasoline. His identification was predominantly based on the detection of methyl-tert-butyl ether (MTBE) which is no longer a prevalent additive in gasoline today.

A study by Morinaga et al. (1996) examined the blood of 47 fire scene victims for the presence of 24 petroleum related compounds by using headspace capillary GC-MS. By detecting these compounds in differing ratios, the authors concluded that the victims had inhaled gases which could be characterized as either gasoline, kerosene, automobile exhaust, or construction fire gases (Morinaga, Kashimura, Hara, Hieda, & Kageura, 1996). The characterization completed in this study is based on a relatively low number of compounds and would not be considered a positive identification according to the current ASTM standards.

Takayasu, Ohshima, Kondo, and Sato (2001) analyzed the intratracheal gas of 20 fire victims and found that for at least 48 hours after the fire, the intratracheal gas can provide valuable information on the volatile hydrocarbons that the victim was exposed to before death. However, similar to the previous studies, this study did not identify ignitable liquid residues in accordance with the current ASTM standard.

Other studies have identified volatile organic compounds in tissue samples but did not use them to determine whether an ILR was present. One such study identified 33 different volatile organic compounds (VOCs) from post-mortem blood samples from fire victims and concluded that these could lead to the identification of gasoline or kerosene residues in blood (Houeto, Borron, Marliere, Baud, & Levillain, 2001). Another study detected multiple VOCs in various tissues which including brain, skin, lung, and muscle (Gottzein, Musshoff, & Madea, 2009). The authors found variations in the type of VOCs present in each type of tissue (Gottzein et al., 2009). It was suggested that different parts of the body were burned more than others and that different chemical affinities to different tissues would cause certain volatiles to be found only in specific tissues (Gottzein et al., 2009).

A study conducted in Japan found that the analysis of blood samples using headspace and solvent extraction with GC-MS allowed for the detection of ILRs and could differentiate gasoline and kerosene (Kimura, Nagata, Hara, & Kageura, 1988). The study analyzed human samples from a blood bank that were spiked with gasoline and heated blood samples from rats and rabbits that were exposed to gasoline and kerosene vapours respectively before they were pithed (Kimura et al., 1988). Studies have also investigated the distribution and concentration of various ILRs in human and animal tissues that had exposure to the ignitable liquids other than in a fire (Martinez & Ballesteros, 2005; Zahlsen, Eide, A.M. Nilsen, & Nilsen 1992; Zahlsen, Eide, A.M. Nilsen, & Nilsen, & Nilsen, 1993). One study which focused on gasoline poisoning determined that, after ingestion, residues could be found in the stomach and respiratory tract but the highest concentrations were present in the blood (Martinez & Ballesteros, 2005). Additionally, recreational gasoline sniffing resulted in the highest gasoline concentrations detected in the liver, lungs, and brain (Martinez & Ballesteros, 2005).

Two studies conducted by Zahlsen et al. examined the absorption, distribution, and accumulation of petroleum related hydrocarbons after repeated exposure in rats (Zahlsen et al., 1992; Zahlsen et al., 1993). They found that, 12 hours after exposure, various concentrations of the hydrocarbons could be found in the blood, brain, liver, kidneys, and fat tissue (Zahlsen et al., 1992; Zahlsen et al., 1992; Zahlsen et al., 1993).

As part of an Honours thesis by Pahor in 2010, a study was conducted to determine if a gasoline signature could be identified from porcine skin post arson. The results suggested that it is possible to identify gasoline from porcine tissue after it has been exposed to gasoline and high intensity burning (Pahor, 2010).

The combustion of human and animal tissues can also produce a variety of nalkanes, n-aldehydes, alkenes, and light aromatics (DeHaan, Brein,, & Large, 2004). De Haan et al. (2004) found that burning both porcine and human fat tissues released a high concentration of volatiles which could be mistaken for ignitable liquid residues.

Importantly, none of these studies focused on determining the status of the victim at the time of the fire. This information is important as it can be used to determine if the victim was alive during the fire (suggesting suicide or homicide) or that the victim was already deceased and the fire was an attempt to destroy evidence. This makes the current study significant as this would provide novel data that could allow investigators to gain a new significant piece of evidence for fire related homicides.

1.5 Thesis Objectives

1.5.1 Long Term Objective

The overall goal of this study is to determine whether there can be reliable evidence that a victim was alive during a fire which involved the use of gasoline. This study will attempt to detect a gasoline signature in lung tissue or heart blood samples post fire using automated thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). The results of this study will aid in determining which tissues are the most appropriate to collect at an autopsy which is valuable information for the organizations investigating fires involving human remains.

1.5.2 Short term objectives

- To determine if a positive result for gasoline can be found from lung and heart blood samples collected from a deceased victim who was exposed to gasoline post-mortem.
- 2. Develop a standard operating procedure for collecting samples of the lungs and heart blood that will avoid contaminating the sample with gasoline volatiles present on the outer tissues and surrounding material.
- Conduct full scale experiments in the field to determine if the gasoline signature persists in the lungs and/or heart blood post fire.

Chapter 2: Materials and Methods

2.1 Objective 1

Domestic adult pigs (*Sus domesticus*) weighing approximately 30 kg were used to determine if a positive gasoline signature could be detected from the lung tissue or heart blood when exposure to gasoline occurred postmortem. This was completed to test the validity of this method as obtaining a positive signature for gasoline from a deceased victim would indicate that this method could not be used to determine whether the victim was alive at the time of a fire.

2.1.1 Non-burning Trial

A deceased pig carcass was placed on its side in a body bag made of nylon and had 250 mL of gasoline (Regular 89 octane, Petro-Canada) poured on top of it starting from the head and moving to the tail. Immediately after the gasoline was poured the nylon body bag was sealed by tying a 'goose neck' on the open end and securing it with duct tape. The carcass was left in the nylon body bag for a 30 minute exposure period (Figure 2.1). Once the exposure period was complete, the bag was opened and the carcass was removed. Excess gasoline was wiped off the carcass using paper towels and a necropsy was completed to remove the lungs and heart blood for analysis.

Three replicates were completed for this objective along with one reference pig carcass. The reference carcass was a deceased pig that was placed inside a nylon body bag and sealed for 30 minutes without any gasoline exposure.

This trial was completed in the Decomposition Chemistry Laboratory at the University of Ontario Institute of Technology (UOIT).



Figure 2.1: Deceased pig carcass in a sealed nylon body bag exposed to gasoline postmortem

2.1.2 Burning Trial

For the second part of objective one deceased pig carcasses were exposed to gasoline and then burned. This trial was carried out at the UOIT Decomposition Facility.

The deceased carcass was clothed in a 100% cotton t-shirt and then placed on top of steel sheets inside a dirt pit (Figure 2.2). The carcass had 250 mL of gasoline poured onto it from head to tail and was immediately ignited with a road flare. The carcass was allowed to self-extinguish and was cooled with water from a water filled fire extinguisher. Once the carcass was cool it was placed inside a nylon body bag that was 'goose necked' with duct tape. The carcasses were then transported back to the laboratory where the necropsy was completed to remove the lungs and heart blood.

This trial was carried out with three separate pig carcasses along with one reference carcass. The reference carcass in this trial was a deceased pig that was clothed with a

100% cotton t-shirt and singed with a road flare. The carcass was then packaged in the same manner as the test subjects and underwent a necropsy.



Figure 2.2: Deceased pig carcass exposed to gasoline post-mortem prior to burning in a pit

2.2 Objective 2

In order to ensure that contamination of the lungs and heart blood samples did not occur, a standard operating procedure for the necropsies was designed. This procedure involved the collection and analysis of the gloves used during the necropsy to determine if gasoline was being transferred and samples were subsequently being contaminated.

The first step in the necropsy was to remove the carcass from the nylon bag. This was carried out wearing nitrile laboratory gloves (Kimberly-Clark, On., Canada). Once the carcass was removed from the nylon bag it was placed onto clean plastic bags and held on its back. With a new scalpel blade the chest cavity was cut open to expose the internal organs. At this point the gloves were removed and collected in a 1 L glass mason jar. These gloves were labelled as the "before lung collection" gloves. While wearing a new set of gloves both lungs were removed from the carcass and placed inside a 1 L glass mason jar. The gloves were then packaged in a separate glass mason jar and were

classified as the "after lung collection" gloves. Finally with a new pair of gloves the heart blood was collected by cutting the arteries and draining the blood into a 125 mL glass mason jar. This technique was utilized to collect the heart blood as it was clotted to a point that would not allow effective collection using a needle and syringe which was attempted. The final pair of gloves were also collected in a mason jar and were labelled the "after heart blood collection" gloves. Each sample, including the gloves, was then analyzed to determine if a gasoline signature could be detected, thus confirming contamination (Figure 2.3).



Figure 2.3: A complete sample set from one necropsy (From left to right), Before lung collection gloves, Lungs, After lung collection gloves, After heart blood collection gloves, and heart blood (in front)

2.3 Objective 3

In order to replicate an actual fire involving human remains, pig carcasses were placed inside a house that was ignited and allowed to reach flashover. This full scale experiment was completed on two separate occasions with two different houses.

2.3.1 Test subjects

At both of the house fires, three different test subjects were utilized. Test subject 1 involved the live inhalation of gasoline which was completed in a manner that met with the approval of the UOIT Animal Care Committee. The live inhalation of gasoline was completed under the supervision of a veterinarian. In each instance, the pig was anaesthetized by the veterinarian using Ketamine HCL and Acepromazine with doses of 2 mg/kg and 0.22 mg/kg respectively. Once the veterinarian confirmed that the drugs had taken effect, the pig was exposed to gasoline. The exposure was completed by holding a surgical mask close to the pigs face for approximately 10 minutes containing a 2000 ppm concentration of gasoline. The concentration and exposure time were chosen as they have been shown to only cause mild anesthesia and not death based on the weights of the pigs (Martinez & Ballesteros, 2005). The mask was prepared by pouring 2.7 mL of gasoline onto it and placing it in a 1 L mason jar to produce a concentration of ~ 2000 ppm. This concentration was chosen as it was determined to be a concentration that would not harm the pig based on its weight of 35 kg. After the 10 minute inhalation period the pig was euthanized by the veterinarian using Euthansol at a dosage of 0.3 mL/kg until cardiac arrest was reached. Once this was completed the pig carcass was clothed in a 100% cotton t-shirt and placed inside the house. Prior to ignition of the first house fire 250 mL of gasoline was poured onto the carcass from head to tail to mimic a forensic scenario. This was not repeated at the second house fire to further confirm that any gasoline detected in the lung or heart blood samples only resulted from the gasoline inhaled prior to death.

Test subject number 2 had no live inhalation of gasoline and was clothed in a 100% cotton t-shirt postmortem. When this carcass was placed inside the house 250 mL of gasoline was poured onto it starting from the head and moving to the tail prior to the house being ignited. Test subject number 3 was also clothed in a 100% cotton t-shirt and had no exposure to gasoline while alive or deceased. Both of these pigs were euthanized by electrocution on the farm.

2.4 House Fire 1

The first house fire was conducted in Strathroy, Ontario on June 25, 2011. The structure was a two-story farm house with brick siding (Figure 2.4).



Figure 2.4: Farm house used for fire 1

For this fire, each test subject was represented with one pig carcass for a total of three carcasses. Each of these carcasses was placed on the main level in a separate area, directly on the concrete floor (Figure 2.5). A pile of wood and debris was utilized as the origin point in the main room and 250 mL of gasoline was poured onto the debris prior to ignition (Figure 2.6). Test subject 1 (F1A) was placed in the corner to the left of the origin, test subject 2 (F1B) was placed in the corner to the right of the origin, and test subject 3 (F1C) was placed across from the origin in another room. Once all the carcasses were in place and the gasoline was poured on test subjects 1 and 2, the origin was ignited by the fire chief with a flare to start the fire.

Thermocouples were used to monitor the temperatures in the house during the fire. These wires were run throughout the house in the centre of each of the rooms containing carcasses. They were placed on the ceiling and on the floor. There was also a wire placed under each of the pig carcasses to monitor the temperature underneath the carcass during the fire.

Once the fire had reached flashover, as determined by a visual observation and the fire reaching temperatures over 600°C, the fire was suppressed with water by the local fire department. Once the scene was safe to enter, local firefighters entered the house and removed the carcasses. The carcasses were then allowed to cool outside for several minutes before they were packaged in nylon bags for transport. Once sealed in the nylon bags the carcasses were transported back to the laboratory at UOIT where the necropsies were completed as outlined in section 2.2.

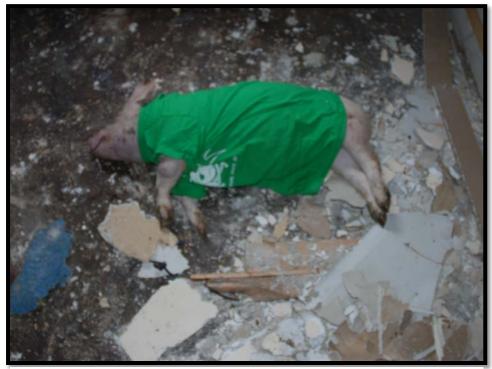


Figure 2.5: Test subject 2 inside the farm house used for fire 1 prior to gasoline exposure



Figure 2.6: Origin point of fire 1 and test subject 2 in the main room prior to ignition

2.5 House Fire 2

The second house fire was conducted on December 3rd 2011 in Midhurst, Ontario. The structure was a two-storey farm house with brick and stone siding and a tin roof (Figure 2.7).



Figure 2.7: Farm house used for fire 2

For this fire, each test subject was represented by two pig carcasses for a total of six carcasses. Each of these carcasses was placed on the main level with the replicates being placed side by side but separate from the other test subjects. All the carcasses were placed in the room of origin on top of cardboard boxes on the floor (Figure 2.8). The cardboard boxes were used to generate fire debris underneath the carcasses for use in another study which was testing a new method for ILR detection in debris. A pile of wooden skids and a couch were utilized as the origin point and 250 mL of gasoline was poured onto it prior to ignition. The two carcasses used as test subject 1 (F2A and F2B) were placed underneath the window directly beside the wooden skids. The carcasses used as test subject 2 (F2C and F2D) were placed in the alcove to the right of the window. The

two pigs used as test subject 3 (F2E and F2F) were placed to the right of the alcove. In this fire, the pigs used as test subject 1 were both exposed to gasoline prior to death as outlined in section 2.3.1, but were not exposed to gasoline post-mortem. They were both clothed in 100% cotton t-shirts. Test subjects number 2 had no live inhalation of gasoline and were clothed in a 100% cotton t-shirt postmortem. When these carcasses were placed inside the house 250 mL of gasoline was poured onto each of them starting from the head and moving to the tail prior to the house being ignited. Test subjects number 3 were also clothed in a 100% cotton t-shirt and had no exposure to gasoline while alive or deceased. All of these pigs were euthanized by electrocution on the farm except for the live inhalation test subjects which were euthanized by injection. Once the gasoline was poured on the appropriate test subjects and on the origin the fire was ignited with a flare.

Thermocouples were used to monitor the temperatures in the house during the fire. Wires were run throughout the house in the centre of each of the rooms containing pig carcasses. The wires were placed on the ceiling and on the floor. There was also a wire placed under each of the pig carcasses to monitor the temperature underneath the carcass during the fire.

For this trial the fire did not reach flashover due to safety concerns and was suppressed with water before flashover temperatures were reached. Once safe to enter, local firefighters entered the house and removed the carcasses. The carcasses were then allowed to cool outside for several minutes before they were packaged in nylon bags for transport. Once sealed in the nylon bags, the carcasses were transported back to the laboratory at UOIT where the necropsies were completed as outlined in Section 2.2.



Figure 2.8: Test subjects for fire 2 (from left to right), test subject 1, test subject 2, test subject 2 replicate, test subject 3

2.6 Laboratory Sampling

For each sample, 50 mL of headspace was withdrawn from the mason jar through a stainless steel sampling tube packed with Tenax TA 60/80 mesh (PerkinElmer, Norwalk, CT), using a clean 60mL plastic syringe (Becton Dickinson and Company, New Jersey, Ref 309653). Headspace was withdrawn separately from each sample at room temperature.

This was accomplished by puncturing a hole in the metal lid of the mason jar so that the headspace could be drawn out. The sampling tube was fitted with disposable pipette tubes on both ends once the caps were removed and the tip on the sampling end (double lines) was inserted through the hole in the lid. The other end was fitted into the plastic syringe and 50 mL of headspace was withdrawn. The hole was then sealed with a piece of scotch tape.

2.6.1 Standards

Four standards including; a blank packed tube, a blank unpacked tube, a gasoline standard, and an ASTM Test Mixture, were analysed along with the samples.

The gasoline standard was prepared by placing 1 μ L of gasoline in a 1 L mason jar. This was completed by spotting a KimwipeTM with 1 μ L of regular 87 gasoline and placing it inside the jar. A 50 mL headspace sample was extracted using the sampling procedure previously described.

The ASTM standard was prepared by placing 10 μ L of the ASTM Test Mixture in a 1 L mason jar. This was completed by spotting a KimwipeTM once with a glass capillary tube dipped in an ATSM standard solution provided by the CFS. The jar was heated in an oven at approximately 130°C for 20 min. Following heating, 10 mL of headspace was extracted using the sampling procedure.

2.7 Instrumentation

Samples were analyzed using a Unity 2 Thermal Desorber (Markes International Ltd, Llantrisant, United Kingdom) coupled to a GC-MS unit which incorporated a 450-GC and 240-MS (Varian Inc, Walnut Creek, CA). After the headspace was drawn into the tubes they underwent a two-stage thermal desorption process. Sample components were desorbed from the tube packing at 300°C for 25 minutes, onto a cold trap packed with Tenax TA, held at -30°C. The cold trap was then flash heated to 300°C, to transfer the components to the analytical column through a fused silica transfer line held at 200°C. Chromatographic separations were achieved using a CP-Sil 5 CB MS column, 5% phenyl/ 95% dimethylpolysiloxane column, 30 m x 0.25 mm i.d. x 0.25 µm film thickness (Varian Inc, Walnut Creek, CA). The GC oven temperature was started at 50°C, held for eight minutes and then increased at 10°C per minute to 240°C and held for six minutes. Helium was used as the carrier gas at a flow rate of 1.7 mL/minute constant flow. The mass spectrometer was operated in Electron Ionization full scan mode, 40 to 350 amu, at a trap temperature of 220°C. The total run time for each sample was 33 minutes.

2.7.1 Optimization and Equipment preparation

Prior to sample collection the instrumentation needed to be optimized to ensure that the peaks related to gasoline would resolve effectively. This was accomplished by analyzing a gasoline standard and adjusting the split ratios to ensure that the correct amount of sample was reaching the column. Optimization was completed at the beginning of the study and any time maintenance had been completed on the instruments.

Glass mason jars used during this study were cleaned and analyzed prior to use to ensure no contaminants were present that could affect the samples. This was accomplished by rinsing the jars and lids with hot water and allowing them to air dry. A headspace sample was then collected and analyzed following the above outlined procedure. The only difference in the sampling procedure involved moving the lid slightly to the side to maintain the integrity of the container rather than puncturing a hole for sample collection.

The nylon bags used to transport the carcasses were also analyzed to ensure they would not contaminate the samples. This analysis was completed at the CFS by their technicians.

2.8 Limit of Detection for Gasoline

The limit of detection for gasoline was determined for the instrumentation used in this study by analyzing decreasing concentrations of gasoline standards. The concentrations analysed were; $1\mu L/1L$ (1 ppm), $0.5\mu L/1L$ (0.5 ppm), and $0.1\mu L/1L$ (0.1 ppm).

2.9 Data Analysis

Interpreting the findings to make an identification of gasoline in the samples is accomplished by comparing the sample chromatogram with the gasoline standard chromatogram. This interpretation is completed in accordance with ASTM E1618-06¢1 *Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris* Samples by Gas Chromatography-Mass Spectrometry (2006). The overall pattern of the sample and gasoline standard are compared using the total ion count chromatograms. At this point the sample chromatogram is compared to the gasoline standard to determine if gasoline is present in the sample. Following this, isolated ions from both chromatograms are compared with each other. The ions observed represent each of the following classes of compounds, Alkanes, Alkenes, Cycloalkanes, Aromatics, and Naphthalenes (Table 1). These compounds were chosen as they are specifically used to characterize gasoline according to ATSM E1618-06 ϵ 1 (2006) and are used by the CFS for their case work.

Compound Type	Mass-to- Charge Ratio (m/z)	
Alkanes	43+57+71	
Alkenes	55+69+85	
Cycloalkanes	82+83	
Aromatics	91+105+119	
Naphthalenes	128+142+156	

 Table 1: Major ions present in Mass Spectra of Gasoline

Each of these classes is individually observed by isolating the ions from both the sample and the standard and comparing to ensure the compounds of gasoline are present in the sample. Any similarities are noted and a decision is made according to the number of similarities and the quality of the similarities based on their peak height, peak width and general pattern.

In order to make an identification for gasoline the entire chromatographic pattern of the gasoline standard must be displayed in the samples at a similar sensitivity. This is particularly important for the aromatic ions as the compounds that form the later peaks are some of the first to degrade and would no longer be present in a sample that does not contain an entire gasoline pattern. Therefore it is important to ensure these peaks are present by observing an expanded view of the aromatic chromatogram. A complete pattern of peaks would indicate the presence of gasoline in a sample and be classified as a "positive" result whereas an incomplete pattern of peaks would indicate the presence of a partial gasoline profile and would be classified as "partial". The absence of the peaks would indicate that gasoline was not present or that the concentrations were too low for detection and this would be classified as "negative".

Chapter 3: Results

3.1 Limit of Detection and Retention

The limit of detection for the GC-MS utilized in this study was determined to be 1μ L of gasoline in a 1 L mason jar or 1 ppm. The samples F1A lungs and F1A heart blood were reanalyzed 10 months after they were collected with no significant changes observed in the chromatograms which demonstrates the ability of the samples to retain the ILRs during storage.

3.2 Objective 1- Exposure of Gasoline to Deceased Pig Carcasses

There were three positive identifications (Entire pattern of peaks for gasoline present in sample) and two partial identifications (More than 3 peaks of the gasoline standard present in the sample but not an entire pattern) of gasoline from samples collected for the non-burning trial of objective 1. All three positive results were found in the heart blood collected from each of the three replicates. Representative total ion chromatograms and aromatic region chromatograms for the heart blood samples can be seen in Figures 3.1a and 3.1b, respectively. Two of the lung samples contained partial profiles for gasoline (Figure 3.2a and Figure 3.2b) and the third was negative for the presence of gasoline. The heart blood and lung samples from the reference pig were negative (Figure 3.3). The samples collected from the burning trial of objective 1 all exhibited negative results for gasoline. A summary of the results for objective 1 can be seen in Table 2.

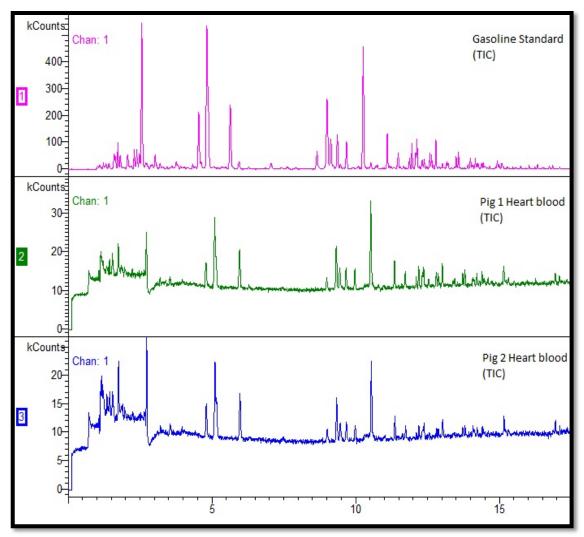


Figure 3.1a: Total ion chromatograms (TIC) for deceased non-burning gasoline exposure (from top to bottom); gasoline standard, Pig 1 heart blood sample, Pig 2 heart blood sample

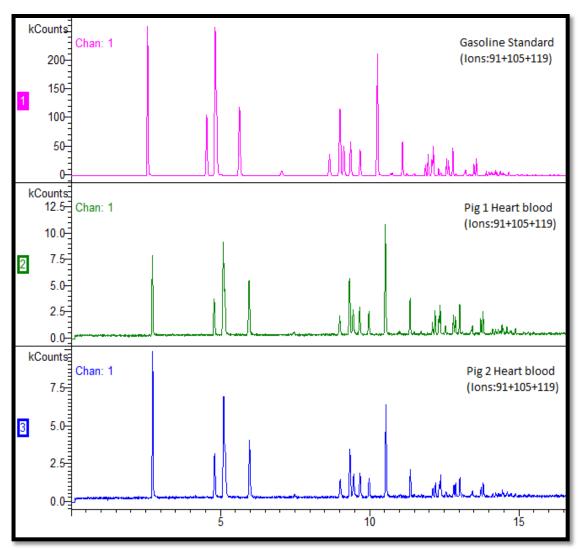


Figure 3.1b: Aromatic region chromatograms for deceased non-burning gasoline exposure (from top to bottom); gasoline standard, Pig 1 heart blood sample, Pig 2 heart blood sample

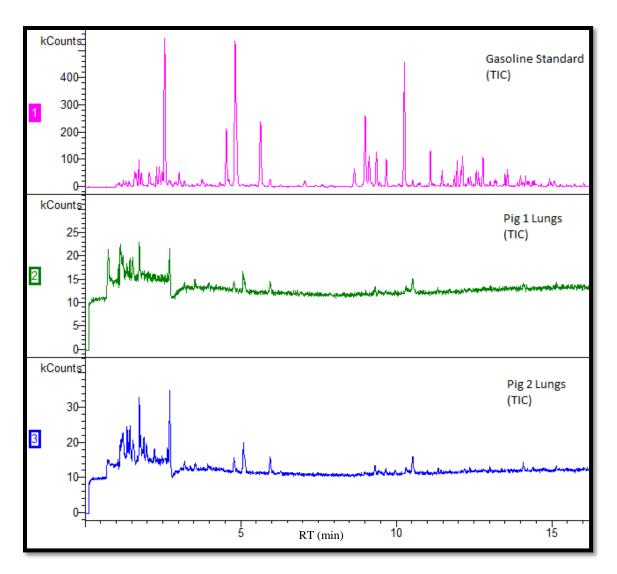


Figure 3.2a: Total ion chromatograms (TIC) for the deceased non-burning gasoline exposure (from top to bottom); gasoline standard, Pig 1 lung sample, Pig 2 lung sample

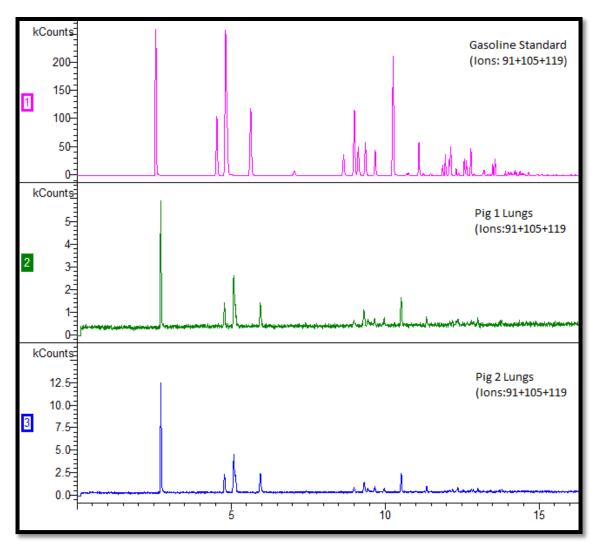


Figure 3.2b: Aromatic region chromatograms for the deceased non-burning gasoline exposure (from top to bottom); gasoline standard, Pig 1 lung sample, Pig 2 lung sample

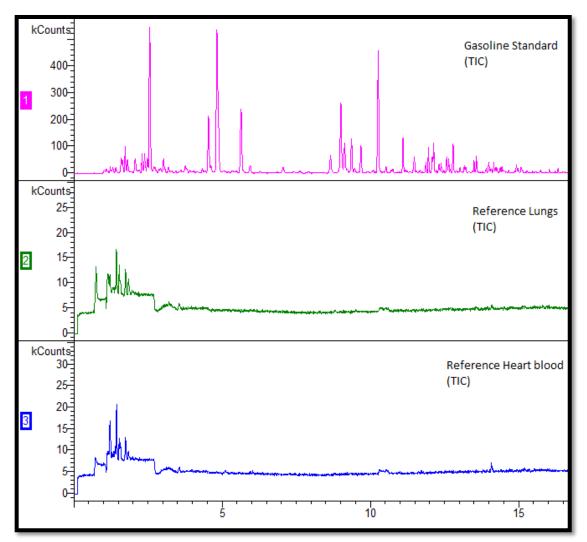


Figure 3.3: Total ion chromatograms (TIC) for the deceased non-burning gasoline exposure (from top to bottom); gasoline standard, Non-burnt reference pig lung sample, Non-burnt reference pig heart blood sample

Non-burnt Carcasses		Burnt Carcasses	
Sample #	Identification of gasoline	Sample #	Identification of gasoline
Pig 1 Lungs	Partial	Pig 4 Lungs	Negative
Pig 1 Heart blood	Positive	Pig 4 Heart blood	Negative
Pig 2 Lungs	Partial	Pig 5 Lungs	Negative
Pig 2 Heart blood	Positive	Pig 5 Heart blood	Negative
Pig 3 Lungs	Negative	Pig 6 Lungs	Negative
Pig 3 Heart blood	Positive	Pig 6 Heart blood	Negative
Reference Lungs	Negative	Reference Lungs	Negative
Reference Heart blood	Negative	Reference Heart blood	Negative

Table 2: Identification of gasoline from lung and heart blood samples collected from the deceased carcasses exposed to gasoline

3.3 Objective 2- Detection of Contamination on Gloves Used During the Necropsies

All gloves used during the necropsies for the non-burnt carcasses demonstrated positive identifications of gasoline except for the "After lung collection gloves" for pig 3. Representative chromatograms for one set of gloves that demonstrated positive identifications for gasoline can be seen in Figure 3.4a and Figure 3.4b for all other positive chromatograms see Appendix. The gloves used for the necropsy of the non-burnt reference carcass all exhibited negative results for the identification of gasoline. The gloves used for the necropsy of the burnt carcasses demonstrated two positive identifications and one partial identification for gasoline. All three results were observed in the "Before lung collection" gloves used for each of the replicates. The gloves used for the necropsy of the burnt reference carcass all exhibited negative results for the results for the identification.

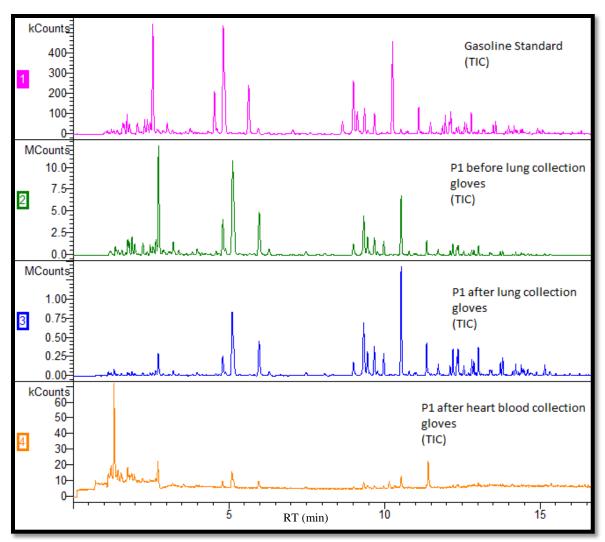


Figure 3.4a: Total ion chromatograms (TIC) for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P1 before lung collection, P1 after lung collection, P1 after heart blood collection

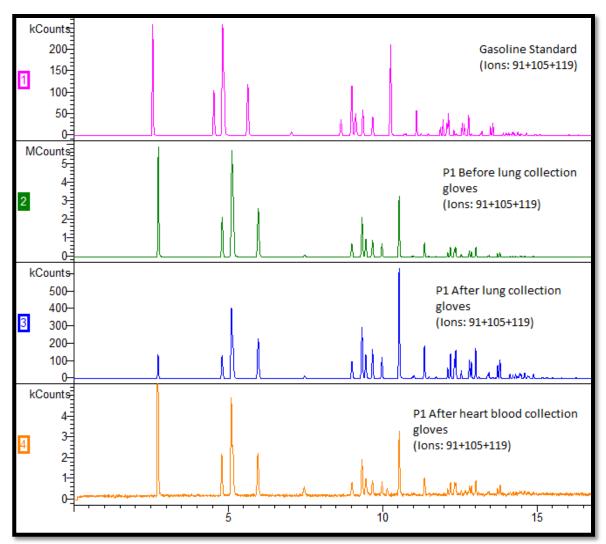


Figure 3.4b: Aromatic region chromatograms for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P1 before lung collection, P1 after lung collection, P1 after heart blood collection

Table 3: Identification of gasoline from gloves used during the necropsies of the deceased carcasses exposed to gasoline

Non-burnt Carcasses		Burnt Carcasses	
Sample #	Identification of gasoline	Sample #	Identification of gasoline
P1 Before lung collection gloves	Positive	P4 Before lung collection gloves	Positive
P1 After lung collection gloves	Positive	P4 After lung collection gloves	Negative
P1 After heart blood collection gloves	Positive	P4 After heart blood collection gloves	Negative
P2 Before lung collection gloves	Positive	P5 Before lung collection gloves	Partial
P2 After lung collection gloves	Positive	P5 After lung collection gloves	Negative
P2 After heart blood collection gloves	Positive	P5 After heart blood collection gloves	Negative
P3 Before lung collection gloves	Positive	P6 Before lung collection gloves	Positive
P3 After lung collection gloves	Negative	P6 After lung collection gloves	Negative
P3 After heart blood collection gloves	Positive	P6 After heart blood collection gloves	Negative
Reference Before lung collection gloves	Negative	Reference Before lung collection gloves	Negative
Reference After lung collection gloves	Negative	Reference After lung collection gloves	Negative
Reference After heart blood collection gloves	Negative	Reference After heart blood collection gloves	Negative

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3.4 Objective 3- Full Scale House Burns

The samples collected during the full scale house burns produced six positive identifications for gasoline. All positive identifications were obtained from the lung and heart blood samples collected from the pig carcasses that experienced live inhalation of gasoline prior to euthanasia (F1A, F2A, and F2B). Representative chromatograms for the positive identifications can be seen in Figures 3.5a through 3.6b. All other lung and heart blood samples along with the gloves used during the necropsies for this objective were negative for the presence of gasoline. Table 4 and 5 summarize the results for the full scale house burns.

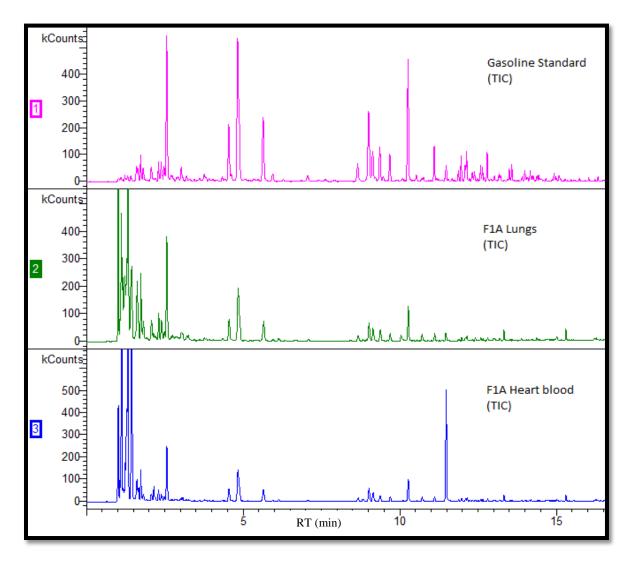


Figure 3.5a: Total ion chromatograms (TIC) for the full scale house burns (from top to bottom); gasoline standard, lung sample from live inhalation test subject 1 in fire 1, heart blood sample from live inhalation test subject 1 in fire 1

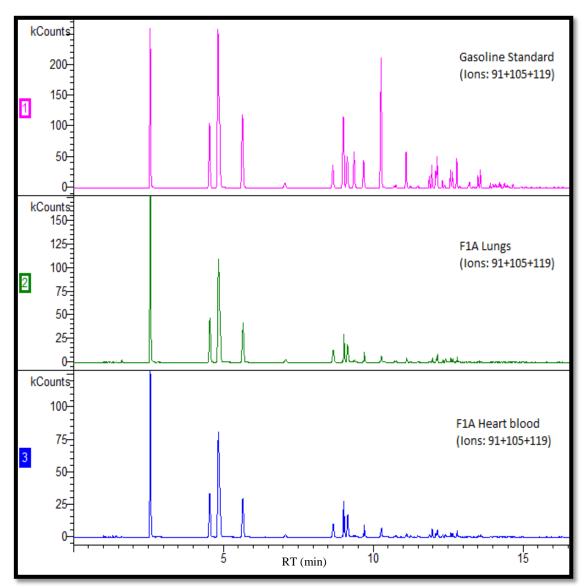


Figure 3.5b: Aromatic region chromatograms for the full scale house burns (from top to bottom); gasoline standard , lung sample from live inhalation test subject 1 in fire 1, heart blood sample from live inhalation test subject 1 in fire 1

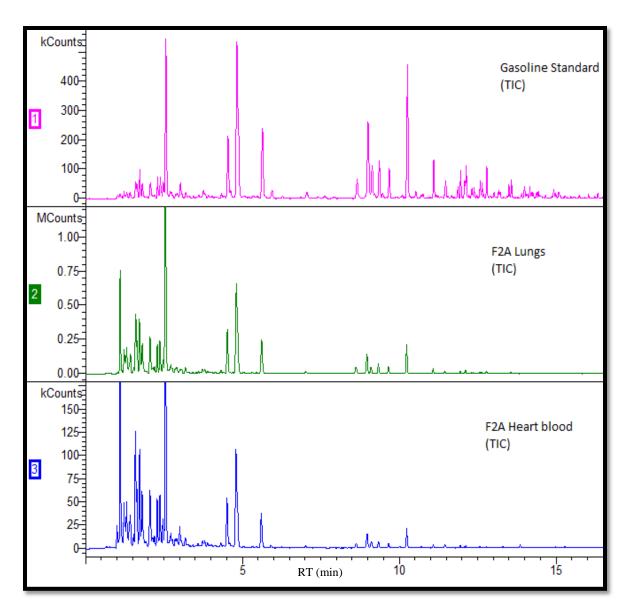


Figure 3.6a: Total ion chromatograms (TIC) for the full scale house burns (from top to bottom); gasoline standard, lung sample from live inhalation test subject 1 in fire 2, heart blood sample from live inhalation test subject 1 in fire 2

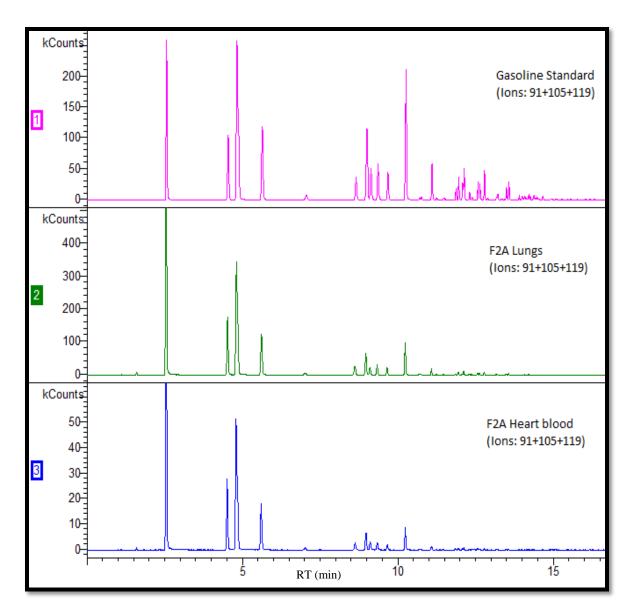


Figure 3.6b: Aromatic region chromatograms for the full scale house burns (from top to bottom); gasoline standard, lung sample from live inhalation test subject 1 in fire 2, heart blood sample from live inhalation test subject 1 in fire 2

Sample #	Identification of gasoline
F1A Lungs	Positive
F1A Heart blood	Positive
F1A Before lung collection gloves	Negative
F1A After lung collection gloves	Negative
F1A After heart blood collection gloves	Negative
F1B Lungs	Negative
F1B Heart blood	Negative
F1B Before lung collection gloves	Negative
F1B After lung collection gloves	Negative
F1B After heart blood collection gloves	Negative
F1C Lungs	Negative
F1C Heart blood	Negative
F1C Before lung collection gloves	Negative
F1C After lung collection gloves	Negative
F1C After heart blood collection gloves	Negative

 Table 4: Identification of gasoline in samples collected from the first full-scale house fire

Sample # (Replicate 1)	Identification of gasoline	Sample # (Replicate 2)	Identification of gasoline
F2A Lungs	Positive	F2B Lungs	Positive
F2A Heart blood	Positive	F2B Heart blood	Positive
F2A Before lung collection gloves	Negative	F2B Before lung collection gloves	Negative
F2A After lung collection gloves	Negative	F2B After lung collection gloves	Negative
F2A After heart blood collection gloves	Negative	F2B After heart blood collection gloves	Negative
F2C Lungs	Negative	F2D Lungs	Negative
F2C Heart blood	Negative	F2D Heart blood	Negative
F2C Before lung collection gloves	Negative	F2D Before lung collection gloves	Negative
F2C After lung collection gloves	Negative	F2D After lung collection gloves	Negative
F2C After heart blood collection gloves	Negative	F2D After heart blood collection gloves	Negative
F2E Lungs	Negative	F2F Lungs	Negative
F2E Heart blood	Negative	F2F Heart blood	Negative
F2E Before lung collection gloves	Negative	F2F Before lung collection gloves	Negative
F2E After lung collection gloves	Negative	F2F After lung collection gloves	Negative
F2E After heart blood collection gloves	Negative	F2F After heart blood collection gloves	Negative

 Table 5: Identification of gasoline in samples collected from the second full-scale house fire

3.4.1 Thermal Data From Full-Scale House Burns

The maximum ceiling temperature reached for the first full-scale house burn was 906°C and the maximum floor temperature was 445 °C. The maximum temperature underneath the pig carcass in the same room was 28°C. The maximum ceiling temperature reached for the second full-scale house burn was 529°C and the maximum floor temperature was 83 °C. The maximum temperature underneath the pig carcass in the same room was 26°C. The temperatures at the second house fire were lower as the fire was suppressed before flashover. An illustration of the thermal data from the first and second full-scale house burns can be seen in Figure 3.7 and Figure 3.8 respectively. The discrepancy between the x-axis of these two figures is due to the fact that the second fire took longer to start due to the larger room size, colder temperatures, and smaller fuel load. This resulted in more time passing before maximum temperatures were reached.

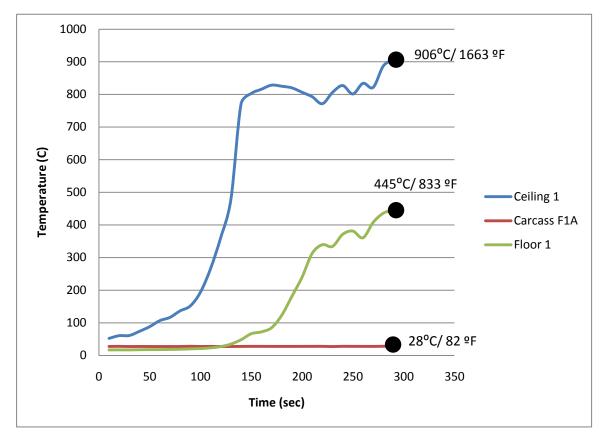


Figure 3.7: Thermal data collected from ceiling, floor, and underneath subject F1A during the first full-scale house burn

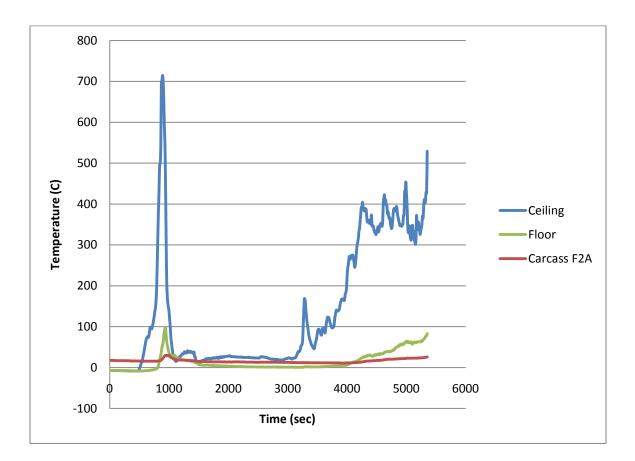


Figure 3.8: Thermal data collected from ceiling, floor, and underneath subject F2A during the second full-scale house burn

Chapter 4: Discussion

The current study provides novel information to fire investigators as it is the first study to determine a victim's status at the time of a fire by examining internal tissues for ignitable liquid residues. Several previous studies (Schuberth, 1994; Schuberth, 1997 & Morinaga et al., 1996) have analyzed post-mortem blood samples and were able to detect ILRs. However, the detection of ILRs in those studies was completed with a relatively low number of compounds which would not be considered positive identifications based on the currently accepted ASTM protocols. The identifications in the current study were completed in accordance with ASTM E1618-06¢1 *Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry* (2006) which is the currently accepted method. This requires a larger number of compounds to be identified in the sample before a positive identification of gasoline can occur. The current study also utilized the detection of ILRs to determine the victim's status at the time of the fire which has not previously been attempted.

A study by Takayasu et al. (2001) analyzed the intratracheal gas of fire victims and determined that these samples could provide useful information on the antemortem hydrocarbon exposure. Although these results are useful for intratracheal gas samples the identifications of ILRs were once again not completed in accordance with the current ASTM standards. The authors in this study used less compounds to make their identification then is required by the current ASTM. The current study chose to sample the victim's heart blood and lungs instead of the intratracheal gas as these samples are routinely collected by pathologists in Canada and can be sent for analysis at the CFS.

In 1988, Kimura et al. analyzed spiked human blood samples from a blood bank and collected blood samples from rats and rabbits that were exposed to gasoline and kerosene vapours respectively. The researchers were able to detect ILRs from these samples using solvent extraction coupled with GC-MS analysis. The current study expanded on these findings by collecting samples from pig carcasses that had undergone live inhalation of gasoline vapours prior to death and were subsequently burned postmortem in a full scale house fire. The samples in the current study would better reflect samples that would be analyzed in case work as they have been exposed to the extreme temperatures of a real fire. Two additional studies were able to identify volatile organic compounds from internal tissue samples taken from fire victims post-mortem (Houeto et al., 2001 & Gottzein et al., 2009). The first identified 33 different VOCs from post-mortem blood samples but did not make any inferences with respect to ILR detection. The second study showed that multiple VOCs could be detected from various tissues which included, brain, skin, lung, and muscle. In contrast to the current study these findings were not used to detect ILRs or to determine the victim's status at the time of the fire.

4.1 Objective 1 & 2 Non-Burnt Samples

The first test was the exposure of three deceased pig carcasses to 250 mL of gasoline. The lungs and heart blood were collected and analyzed after the exposure of all three carcasses for a total of six samples. Of these six samples, the three heart blood samples displayed positive identifications for gasoline and two of the lung samples displayed partial identifications for gasoline. These positive results were unexpected as the pigs were deceased when exposed to the gasoline and therefore their internal tissues should not have had signatures of gasoline. These positive results can be explained by the inferences from the observations made during sample collection and the results collected from objective 2.

During the necropsies it was noted that there was gasoline present on the carcasses. This was indicated by a strong gasoline odour surrounding the carcass, the visualization of wet tissue on the carcass and the fact that the carcass still felt damp when handled. This residual gasoline remained on the carcass as the carcass was not ignited and the gasoline was not consumed by any flames. Based on the amount of gasoline present on the carcass during the necropsy it is feasible that the internal tissues were contaminated during the necropsy. Contamination of internal tissues from the handling of outer tissue areas of a body has been suspected by the Ontario Coroner's Office (Dr. M. Pollanen, Chief Forensic Pathologist, personal communication, November 19, 2010) and was confirmed in this trial.

The suspected contamination issue in this trial was supported by the results from the analysis of the gloves used during the necropsy. All the gloves used during the necropsies for the three carcasses that were exposed to gasoline without burning

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demonstrated positive identifications for gasoline except for the P3 after lung collection gloves. These results directly correlate with the results for the tissue samples. The only negative results recorded were the lung sample collected from Pig 3and the gloves used to collect this sample. These findings indicate that the gasoline was being transferred from the gloves into the tissue samples. Other sources of contamination would not have been a factor as the reference carcasses (which were not exposed to gasoline) showed negative results for gasoline in both the heart blood and lung tissue as well as all the gloves used during the necropsy. There have been several studies that support this finding (Almirall, Wang, Lothridge, & Furton 2000, Coulson & Morgan-Smith, 2000, & Darrer, Jacquemet-Papilloud, & Delemont, 2008).

A study by Almirall et al. (2000) demonstrated that gasoline can persist on skin for up to 90 minutes when it is not burned. The authors spiked volunteers' hands with 10 μ L of gasoline and subsequently sampled the hands using SPME at several time intervals after exposure including; 30 min, 45 min, 75 min and 90 min (Almirall et al., 2000). The authors found that some gasoline components could be detected after 90 minutes but an identification as per ASTM guidelines could only be accomplished up to 45 minutes after exposure with such a low volume deposited (Almirall et al. 2000). Based on these results the skins of the carcasses in the current study would have contained transferable levels of gasoline as they were exposed to a much larger volume of gasoline and the necropsies took place 30 minutes after exposure which was in the identifiable time frame found by Almirall et al (2000).

The second study conducted by Coulson and Morgan-Smith (2000) supports the finding of gasoline transfer between materials. The authors found that gasoline could be transferred to clothing and shoes simply by pouring gasoline around a room. The author found that as much as 30 mL could be transferred to a suspect's shoes when pouring gasoline around a room (Coulson & Morgan-Smith, 2000). In the current study there was direct contact with the gasoline soaked carcass which would have transferred the gasoline to the gloves and then subsequently to the internal tissues when they were extracted.

The third study demonstrated that gasoline can be transferred from skin to several different types of gloves (Darrer et al., 2008). The authors spiked volunteers hands with

 $50 \ \mu\text{L}$ of gasoline and had the subjects rub their hands together and then put on a pair of gloves for 20 minutes. After the 20 minutes the gloves were removed, placed in a nylon bag and subsequently analyzed for the presence of gasoline using gas chromatography. Three different types of gloves were utilized including, polyethylene gloves with no talcum powder, latex gloves with no talcum powder, and polyvinylchloride (PVC) gloves containing talcum powder. Gasoline was detected on all of the gloves with the polyvinylchloride gloves retaining the most gasoline. The authors tested the persistence of the gasoline with the PVC gloves by spiking volunteers' hands with 500 and 1000 μ L of gasoline and found that trace amounts could be detected up to 2 hours and 4 hours after exposure respectively (Darrer et al., 2008). This study illustrates that small quantities of gasoline can be transferred to gloves. The current study utilized much larger quantities of gasoline which would likely be easier to transfer to the gloves and subsequently to the tissue samples.

4.2 Objective 1 & 2 Burnt Samples

The results obtained for the burning trial in objective 1 were a better representation of a deceased victim being exposed to gasoline during a fire as ignition of the gasoline took place. In this trial all the heart blood and lung samples collected from these carcasses yielded negative gasoline identifications. Since the pigs were deceased they did not inhale the gasoline vapours and therefore were not expected to have gasoline present in their heart blood or lungs. In contrast to the non-burning trial, contamination was not an issue in this trial because the fire consumed most of the gasoline reducing the amount left on the carcass that could be transferred into the samples when the necropsy occurred.

Two positive identifications and one partial identification for gasoline were detected from the glove samples used during the necropsies. The three results occurred for the "Before lung collection" gloves which were the first gloves to be used during the necropsy. These gloves were used for the initial handling of the carcass from the nylon bag which involved the removal of the t-shirt. There was still a large portion of the t-shirt remaining after the fire as the fire did not reach a high enough intensity, due to the lack of fuel load. The t-shirt and the tissue under the t-shirt likely contained gasoline as the

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gasoline was poured directly onto this area and there was little impingement by the fire in these areas. When handling these areas during the beginning stages of the necropsy the gasoline could have been transferred to the gloves, resulting in the positive and partial identifications. This is the only plausible explanation for these gloves having positive identifications for gasoline as the samples collected from the reference carcass were all negative.

This burning trial illustrates how properly changing gloves during an autopsy, or in this case a necropsy, can prevent samples from being contaminated. By handling the external tissues and clothing of the carcass the gloves became contaminated with gasoline. Had the gloves not been changed prior to collecting the heart blood and lung samples they likely would have transferred gasoline onto these tissues as was seen in the non-burning trial. With these findings it is recommended that during any fire autopsy new gloves should be used prior to handling any internal tissues.

An additional way to avoid contamination issues when conducting an autopsy would be to collect the blood directly using a needle and syringe. This would eliminate any contamination issues as the blood is being directly drawn from the heart into the syringe. As this procedure for blood sampling is routinely completed for toxicology tests it would be relatively straight forward to collect a second sample for ILR detection. This was attempted during this study but was not successfully accomplished due to the level of clotting which occurred in the heart blood.

4.3 Objective 3 Full Scale House Burns

The only positive identifications for gasoline obtained during this objective came from the tissue samples collected from the test subjects that had experienced live inhalation of gasoline prior to death. Gasoline was identified in both the lung and heart blood samples in all of the live inhalation test subjects (Test subject 1). This indicates that ILRs will only be detected in the lungs and heart blood post fire if the victim was alive and inhaling gasoline vapours prior to death. This finding was observed in three replicates during two different scenarios. The first was a fire that reached flashover where the carcasses had severe burning and the second was a fire that was suppressed prior to flashover where the carcasses only suffered moderate burns. The results for the tissue

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samples collected from the deceased carcasses exposed to gasoline post-mortem (Test subject 2) also support this finding as these samples were all negative for the presence of gasoline demonstrating that a gasoline signature cannot be detected in victims who are already deceased when gasoline exposure occurs.

Gasoline was identified in the blood samples from the live inhalation test subjects because the hydrocarbons are portioning from the lung tissue and entering the blood. It is known that after the hydrocarbons are absorbed through inhalation they will be stored in adipose tissue and then be released into the blood (Cox, Hwang, Himel, & Edlich, 1996). This process was described in a case report of a recreational gasoline sniffer (Cox et al., 1996).

An additional factor that may have contributed to the tissues' ability to retain the ILRs of gasoline was the use of nylon bags to transport the carcasses from the fires to the laboratory. By utilizing body sized nylon bags the carcasses were sealed in air tight bags that do not permit gas exchange and would subsequently trap any volatiles with the carcass. The nylon bags also prevent any external volatiles from entering the carcasses during the transportation which ensures the tissues are not contaminated with gasoline volatiles from the vehicle transporting the remains. Using nylon bags to package bodies is not a routine practice in Ontario so it is possible that bodies of fire victims may be contaminated during transportation as they are not sealed in air tight bags. Additional research is needed to determine if the packaging can affect the results obtained from the analysis of the internal tissues. It is known that improperly packaged fire debris can result in a loss of volatiles from the samples and expose the samples to external contaminates (Williams & Sigman, 2007).

The positive results obtained for objective 3 cannot be attributed to pyrolysis or background interference (Stauffer et al., 2008 & Hine, 2004) as three reference carcasses (Test subject 3) were placed under the same conditions as the live inhalation test subjects and no gasoline was detected in the lungs or heart blood of these carcasses. Contamination during the necropsy can also be ruled out due to the fact that all the gloves used during the necropsies for this objective were negative for the presence of gasoline. This finding demonstrates that the gasoline signature being detected in the lungs and heart blood is a direct result of the gasoline being inhaled by the victim prior to death.

The findings in this study support those found in other studies. Gasoline was identified in post-mortem blood samples in two different studies conducted by Schuberth and a third conducted by Morinaga et al. (Schuberth, 1994; Schuberth, 1997; Morinaga et al., 1996). In these studies the authors analyzed blood samples obtained from fire victims and concluded that gasoline was present in the samples based on a relatively low number of compounds. The current study validates these prior findings by utilizing the currently accepted ASTM for fire debris analysis to identify gasoline in the samples.

In the study conducted by Schuberth in 1997, the tissue samples were described as being particularly valuable because the organs and body fluids can act as evaporation shields and protect the residues retained in the body from thermal degradation. This statement was not only confirmed by detecting gasoline in lung and heart blood samples collected during the current study but was also supported by the thermal data. The thermal data showed that the body can act as an insulator and protect not only the internal organs but also anything underneath the body. Although the room reached flashover in the first house fire and temperatures over 900°C, the temperature underneath the carcass never reached a temperature greater than 28°C. This is a significant finding because it indicates that materials underneath a victim will be exposed to far less heat than other areas and will therefore have a higher likelihood of retaining ILRs. This finding was illustrated in an honours thesis conducted by the author (Pahor, 2010) where the only positive identifications for gasoline in porcine tissues collected from a full scale house fire were observed in the skin collected from the underside of the carcass. Hence it is important that bodies not be moved until the fire investigator can arrive on scene and collect samples because the samples with the highest likelihood of containing ILRs will be found underneath the body.

Chapter 5: Conclusions

The current study attempted to determine a victim's status at the time of a fire by detecting a gasoline signature from lung tissue and heart blood post-fire using thermal desorption and gas-chromatography mass-spectrometry detection. This objective was accomplished by exposing domestic pigs (*Sus domesticus*) to gasoline vapours, euthanizing them and then placing them in a full scale house fire. An additional objective of the study was to develop a standard operating procedure to reduce the chance of internal tissue samples being contaminated by ILRs during an autopsy.

The results from this study demonstrate that it is possible to determine a victim's status at the time of a fire by detecting gasoline from the lung tissue or heart blood. Six positive results for the identification of gasoline were obtained from the full scale house fires conducted. The positive findings all resulted from the live inhalation test subjects (Test subject #1). These findings suggest that internal tissues, specifically the lungs and heart blood, should be routinely analyzed for the presence of ILRs in order to aid in making a determination on the victim's status at the time of the fire. Presently, only lung tissue is analyzed by the CFS and this is only completed if the fire investigator attends the autopsy and requests that the lung tissue be collected and sent for analysis.

One reason for internal tissues not being routinely analyzed is that pathologists do not change their gloves during an autopsy and it is speculated that this can result in contamination of the internal tissues with ILRs. This study proved that this is a possibility since lung and heart blood samples were contaminated by gloves during the necropsy of the pig carcasses. The current study also showed that if the gloves are changed prior to handling the internal tissues, contamination can be minimized. This procedure should be followed by pathologists conducting fire autopsies to minimize the risk of contaminating samples. An alternative method for the collection of heart blood samples was recommended which could further reduce the risk of contamination. If a sample of the heart blood was collected using a needle and syringe then there would be minimal chance for the sample to be contaminated as the blood would enter directly from the heart and into the syringe in a closed system.

An additional finding from this study is that a body can act as an insulator and protect the underside from thermal degradation. This was illustrated by the thermal data

collected from the two full scale house fires. This is an important finding for fire investigators as it provides the best location to collect samples for ILR detection. The area under a body would be protected from high intensity heat and would therefore protect any ILRs present from thermal degradation. With this knowledge the standard practice should recommend that the body is not moved until the fire investigator arrives on scene and can collect samples from underneath the body.

The findings in the current study should be used to implement a new protocol for the collection of lungs and heart blood for the detection of ILRs. This would allow for the analysis to be completed without the concern of the sample being contaminated and would result in more valuable evidence for a fire investigation. This study has successfully demonstrated that the information gained from analyzing internal tissue samples can be valuable for determining a victim's status at the time of a fire.

5.1 Future Considerations

Further research into the detection of gasoline from internal tissues is necessary, as every fire is unique and will affect the bodies differently. Future studies should include: varying the length of the fire, changing fuel loads, completing the experiment in various structures including vehicles, and varying the exposure time and concentration of gasoline. Furthermore, additional steps should be taken to eliminate contamination risks during an autopsy which would involve testing different methods for collecting the internal tissues.

Once sufficient data is collected for the detection of gasoline from internal tissues other accelerants should also be tested. Although gasoline is the main accelerant used in intentionally set fires, it is not the only one encountered by fire investigators. Other accelerants could include lighter fluid, kerosene, and diesel fuel.

Finally, other tissues should be tested to determine if they could provide useful data for ILR detection. These tissues could include brain, liver, and intratracheal gases should also be collected.

Chapter 6: References

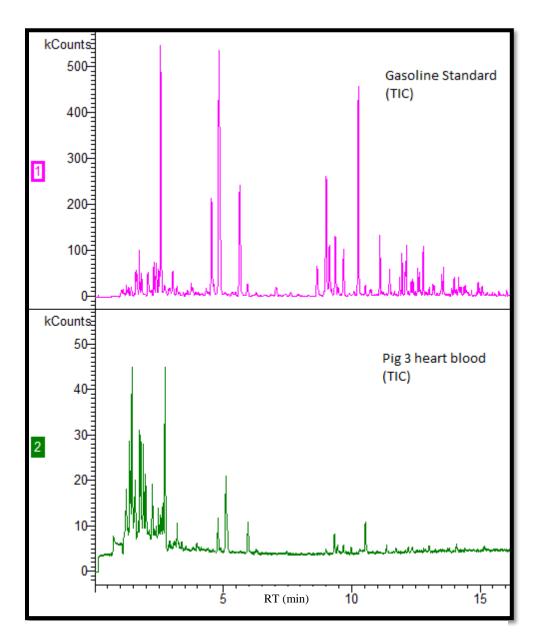
- Almirall, J., Wang, J., Lothridge, K., & Furton, K. (2000). The detection and analysis of ignitable liquid residues extracted from human skin using SPME/GC. *Journal of Forensic Sciences*, 45(2), 453-461.
- ASTM International. ASTM E1618-06€1 Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography-Mass Spectrometry. West Conshohocken, PA: ASTM International 2006.
- Borusiewicz, R., & Zieba-Palus, J. (2007). Comparison of the effectiveness of Tenax TA and Carbotrap 300 in concentration of flammable liquids compounds. *Journal of Forensic Sciences*, 52, 70-74.
- Chemistry Section Head. (2009). Fire Debris Packaging Guide. Unpublished guide, Chemistry Section, Centre of Forensic Sciences and Northern Regional Laboratory, Ontario Canada.
- Conner, L., Chin, S., & Furton, K., (2006). Evaluation of field sampling techniques including electronic noses and a dynamic headspace sampler for use in fire investigations. *Sensors and Actuators B:Chemical*, 116, 121-129
- Coulson, S.A., & Morgan-Smith, R.K. (2000). The transfer of petrol on to clothing and shoes while pouring petrol around a room. *Forensic Science International*, 112, 135-141.
- Cox, M.J., Hwang, J., Himel, H., & Edlich, R. (1996). Severe burn injury from recreational gasoline use. *Journal of Emergency Medicine*, 14, 39-43.
- Darrer, M., Jacquemet-Papilloud, J., & Delemont, O. (2008). Gasoline on hands: Preliminary study on collection and persistence. *Forensic Science International*, 175, 171-178.
- DeHaan, JD., Brein, DJ., & Large, R. (2004). Volatile organic compounds from the combustion of human and animal tissue. Science & Justice: Journal of the Forensic Science Society, 44, 223-236.
- DeHaan, JD., & Nurbakhsh, S. (2001). Sustained combustion of an animal carcass and its implications for the consumption of human bodies in fires. *Journal of Forensic Sciences*, 46(5), 1076-1081.
- DeHaan, JD. (2007). Structure fires and their investigations. In *Kirk's Fire Investigation Sixth Edition*. (pp. 197-257). Upper Saddle River, NJ, USA: Pearson Education.

- de Vos, B., Froneman, M., Rohwer, E., & Sutherland, D. (2002). Detection of petrol (gasoline) in fire debris by gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS). *Journal of Forensic Sciences*, 47(4), 1-21.
- Furton, K., & Harper, R. (2004). Fire scene investigation: an introduction for chemists. In
 J. Almirall, & K. Furton (Eds.), *Analysis and Interpretation of Fire Scene Evidence*. (pp. 75-95). Boca Raton, FL, USA: Taylor & Francis Group
- Gormsen, H., Jeppesen, N., & Lund, A. (1984). The cause of death in fire victims. *Forensic Science International*, 24(2), 107-111.
- Gottzein, A., Musshoff, F., & Madea, B. (2009). Qualitative screening for volatile organic compounds in human blood using solid-phase microextraction and gas chromatography-mass spectrometry, *Journal of Mass Spectrometry*, 45, 391-397.
- Griffith, R., Jayachandran, K., Shetty, K., Whitstine, W., & Furton, K. (2007).Differentiation of toxic molds via headspace SPME-GC/MS and canine detection. *Sensors*, 7, 1496-1508.
- Hine, G. (2004). Fire scene investigation: an introduction for chemists. In J. Almirall, &
 K. Furton (Eds.), *Analysis and Interpretation of Fire Scene Evidence*. (pp. 35-70).
 Boca Raton, FL, USA: Taylor & Francis Group
- Hieda, Y., Tsujino, Y., Xue, Y., Takayama, K., Fujihara, J., Kimura, K., & Dekio, S.
 (2004). Skin analysis following dermal exposure to kerosene in rats: the effect of post-mortem exposure and fire. *International Journal of Legal Medicine*, 118, 41-46.
- Houeto, P., Borron, S., Marliere, F., Baud, F., & Levillain, P. (2001). Development of a method for measuring volatile organic compounds in the blood of fire victims using 'purge and trap' gas chromatography. *Indoor and Built Environment*, 10, 62-69.
- Kimura, K., Nagata, T., Hara, K., & Kageura, M. (1988), Gasoline and kerosene components in blood – A Forensic Analysis. *Human Toxicology*, 7, 299-305.
- Kurz, M.E., Billard, M., Rettig, M., Augustiniak., J., Lange, J., Larsen, M., Warrick, R., Mohns, T., Bora, R., Broadus, K., Hartke, G., Glover, B., Tankersley, D., & Marcouiller, J. (1994). Evaluation of canines for accelerant detection at fire scenes. *Journal of Forensic Science*, 39 (6) 1528-1536.

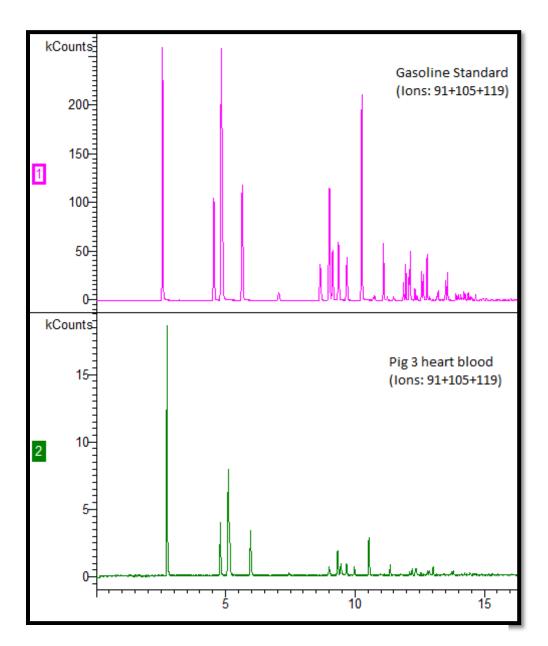
- Lentini, J. (2006). Analysis of Ignitable Liquid Residues. In *Scientific Protocols for Fire Investigation*. (pp. 140-143). Boca Raton, FL, USA: Taylor & Francis Group
- Martinez, M., & Ballesteros, S. (2005). Investigation of fatalities due to acute gasoline poisoning. *Journal of Analytical Toxicology*, 29, 643-651.
- Morinaga, M., Kashimura, S., Hara, K., Hieda, Y., & Kageura, M. (1996). The utility of volatile hydrocarbon analysis in cases of carbon monoxide poisoning. *International Journal of Legal Medicine*, 109, 75-79.
- National Fire Protection Association. NFPA 921: Guide for Fire & Explosion Investigations. Orlando, 2011 Edition.
- Nowlan, M., Stuart, A., Basara, G., & Sandercock, M. (2007). Use of solid absorbent and an accelerant detecting canine for the detection of ignitable liquids burned in a structure fire. *Journal of Forensic Sciences*, 52 (3), 643-648.
- Olson, G. (2009). Recovery of human remains in a fatal fire setting using archaeological methods (Unpublished Master's thesis). Mercyhurst College, Erie, PA, USA.
- Pahor, K. (2010). Detection of gasoline from porcine tissue post arson by headspace analysis using thermal desorption and gas chromatography mass spectrometry detection (Unpublished Honours thesis). University of Ontario Institute of Technology, Oshawa, ON, Canada.
- Pert, A., Baron, M., & Birkett, J. (2006). Review of analytical techniques for arson residues. *Journal of Forensic Sciences*, 51, 1033-1047.
- Rogde, S., & Olving, J. (1996). Characteristics of fire victims in different sorts of fires. *Forensic Science International*, 77, 93-99
- Schuberth, J. (1994). Post-Mortem test for low-boiling arson residues of gasoline by gas chromatography-ion-trap mass spectrometry. *Journal of Chromatography B*, 662, 113-117.
- Schuberth, J. (1997). Gas residues of engine starting fluid in postmortem sample from an arsonist. *Journal of Forensic Science*, 42(1), 144-147.
- Stauffer, E., Dolan, J., & Newman, R. (2008). *Fire Debris Analysis*. (pp. 188, 379-426). Burlington, MA, USA: Academic Press.

- Takayasu, T., Ohshima, T., Kondo, T., & Sato, Y. (2001). Intratracheal gas analysis for volatile substances by gas chromatography/mass spectrometry-application to forensic autopsies. *Journal of Forensic Sciences*, 46(1), 98-104
- Touron, P., Malaquin, P., Gardebas, D., & Nicolai, J. (2000). Semi-automatic analysis of fire debris. *Forensic Science International*, 110, 7-18.
- Williams, M., & Sigman, M. (2007). Performance Testing of Commercial Containers for Collection and Storage of Fire Debris Evidence. *Journal of Forensic Sciences*, 52, 579-585.
- Zahlsen, K., Eide, I., Nilsen, A. M., & Nilsen, O. G. (1992). Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures. *Basic & Clinical Pharmacology & Toxicology*, 71, 144-149.
- Zahlsen, K., Eide, I., Nilsen, A. M., & Nilsen, O. G. (1993). Inhalation kinetics of C8 to C10 1-alkenes and iso-alkanes in the rat after repeated exposures. *Basic & Clinical Pharmacology & Toxicology*, 73, 163-168.

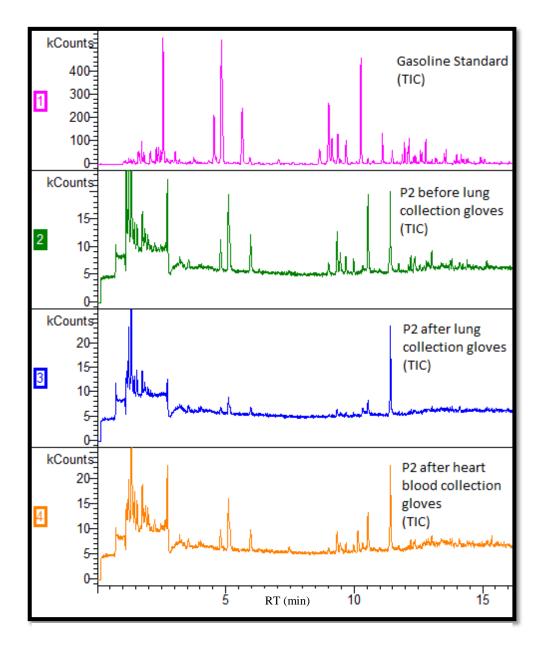
Chapter 7- Appendix



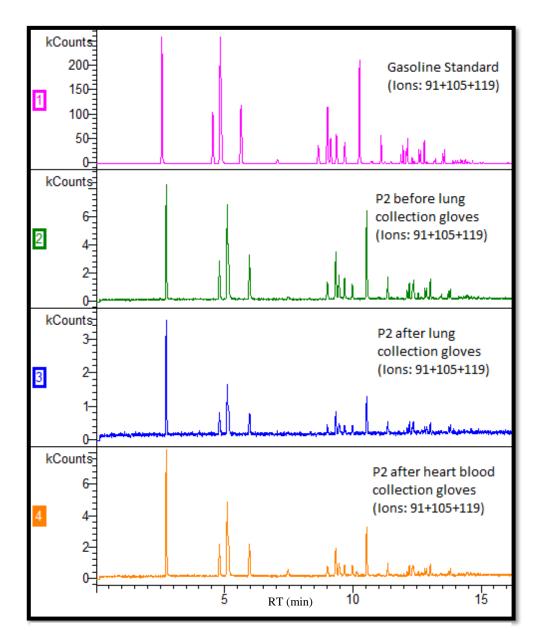
Appendix 7.1: Total ion chromatograms (TIC) for the deceased exposure to gasoline ((from top to bottom); gasoline standard, Pig 3 heart blood sample



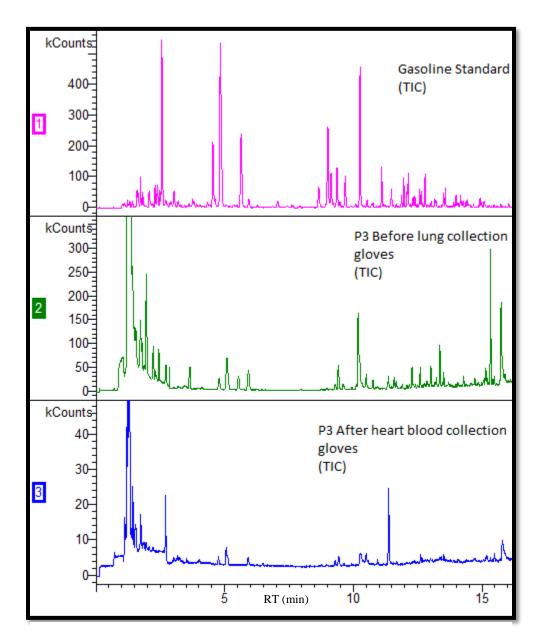
Appendix 7.2: Aromatic region chromatograms for the deceased exposure to gasoline (from top to bottom); gasoline standard, Pig 3 heart blood sample



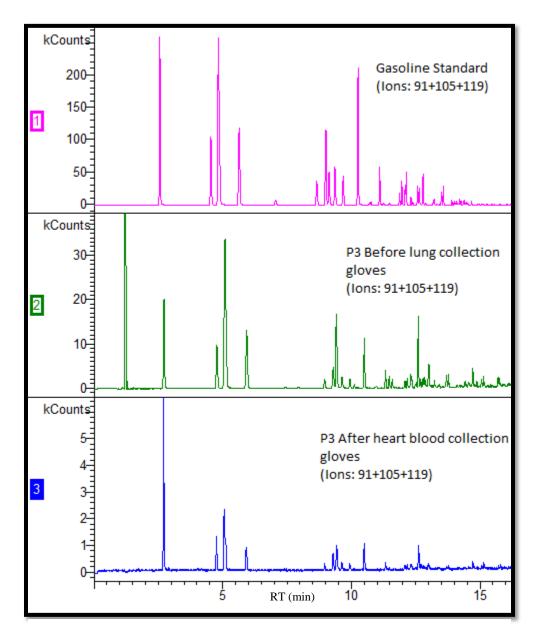
Appendix 7.3: Total ion chromatograms (TIC) for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P2 before lung collection, P2 after lung collection, P2 after heart blood collection



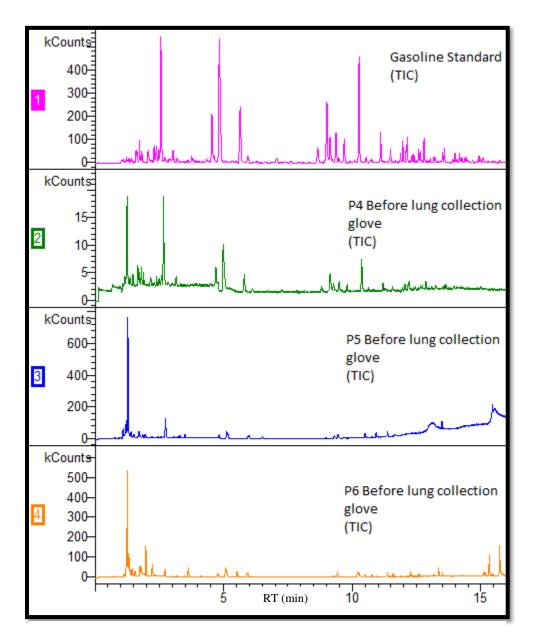
Appendix 7.4: Aromatic region chromatograms for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P2 before lung collection, P2 after lung collection, P2 after heart blood collection



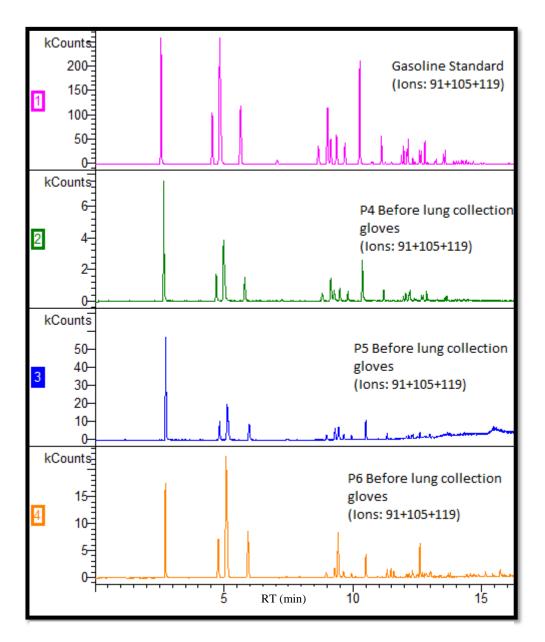
Appendix 7.5: Total ion chromatograms (TIC) for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P3 before lung collection, P3 after heart blood collection



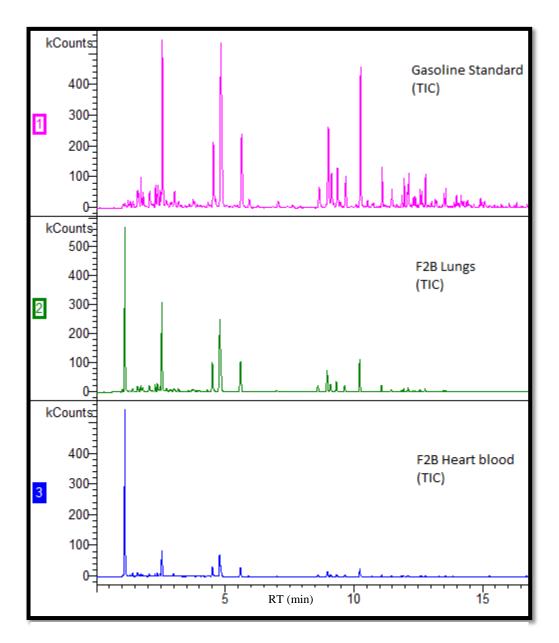
Appendix 7.6: Aromatic region chromatograms for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P3 before lung collection, P3 after heart blood collection



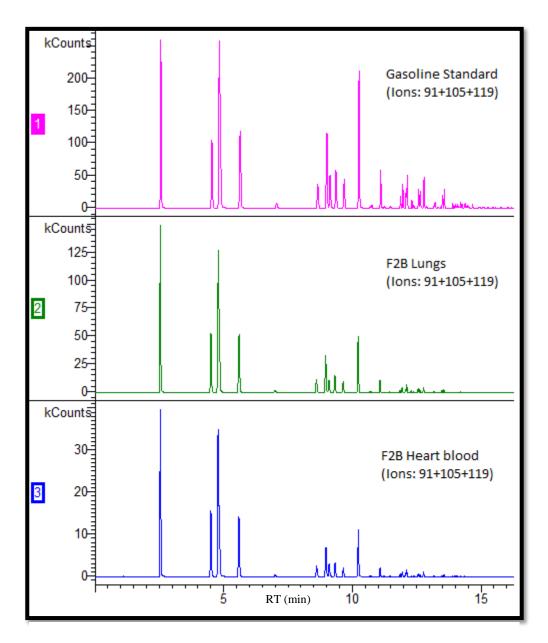
Appendix 7.7: Total ion chromatograms (TIC) for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P4 before lung collection, P5 before lung collection, P6 before lung collection



Appendix 7.8: Aromatic region chromatograms for a gasoline standard (top) and the gloves used in the necropsy of the deceased carcasses exposed to gasoline (from second to bottom); P4 before lung collection, P5 before lung collection, P6 before lung collection



Appendix 7.9: Total ion chromatograms (TIC) for the full scale house burns (from top to bottom); gasoline standard, lung sample from live inhalation test subject 1 replicate in fire 2, heart blood sample from live inhalation test subject 1 replicate in fire 2



Appendix 7.10: Aromatic region chromatograms for the full scale house burns (from top to bottom); gasoline standard, lung sample from live inhalation test subject 1 replicate in fire 2, heart blood sample from live inhalation test subject 1 replicate in fire 2