

# Analytical Methods for the Determination and Characterization of Jet Propellant 8 (JP-8)

By

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(Under the Direction of Michael G. Bartlett)

## Abstract

Jet propellant 8 (JP-8) is a complex mix of hydrocarbons used by NATO (North Atlantic Treaty Organization) countries in military aircrafts and vehicles. The exposure of civilians as well as servicemen is of great importance. JP-8 contains many components that have shown to be related to many ill effects including: benzene (leukemia), naphthalene (cataract, laryngeal carcinoma, neoplasms), and long chain hydrocarbons have been shown to increase the carcinogenicity of other compounds present in JP-8 related to the incidence of lung cancer). The analysis of this fuel is a very arduous task because its composition varies from batch to batch and contains thousands of components. Therefore accurate identification of the compounds present in this fuel along with an accurate, sensitive and precise analytical method are needed to improve the efforts to develop a PB/PK model for this fuel. A PB/PK model will be effective in describing and predicting plasma and tissue concentrations of JP-8 and many of its components in rodents and humans. Chapter 1 reviews methods currently in the literature for the analysis of JP-8 hydrocarbons and petroleum. The identification of compounds present in JP-8, including the best sampling methods for the three phases of JP-8 are presented in Chapter 2. The validation of an analytical method for a representative mix of JP-8 in blood and liver matrices is included in Chapter 3.

INDEX WORDS: Jet propellant 8, JP-8, hydrocarbon, petroleum, Volatile organic hydrocarbons, VOC, Polyaromatic hydrocarbons, PAH, Mass spectrometry, Gas Chromatography, Solid phase Microextraction, GC/MS, SPME, ion trap, BTEX

ANALYTICAL METHODS FOR THE DETERMINATION AND CHARACTERIZATION  
OF JET PROPELLANT 8 (JP-8)

by

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## DEDICATION

I would like to dedicate this dissertation to the memory of Margaret Green, Joseph Green, and Louise Gregg, because of their perseverance, strength and fortitude I am able to reach this great milestone in my life. I stand on your shoulders.

To mamma, daddy (Michael and Loretta Gregg), thank you for always believing in me and sharing your wisdom. Thank you for your sacrifice, your love, and your unconditional support. I am what I am because of you. You felt every triumph and disappointment. You too have earned doctorate degrees in this process. I love you.

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## Chapter 1

### Introduction and Literature Review

Jet Propellant 8 (JP-8) is a fuel used by North Atlantic Treaty Organization (NATO) countries in their military aircrafts and vehicle. The study of JP-8 is fairly new; therefore there are still a number of unanswered questions about the fuels toxicity and phase composition. It has become increasingly important to study this fuel, because military personnel are continually being exposed. Exposure to military personnel is so great that the Department of Defense (DOD) has declared JP-8 the main source of all reported chemical exposures to its workers. Therefore they have become interested in the potential effects that accompany contact with this fuel.

There are three phases of JP-8 each of which has the potential to cause different toxicological effect. Neat JP-8 is the liquid fuel containing all of the components of kerosene and additional performance additives. The vapor is the volatile fraction of JP-8. The aerosol is composed of small droplets and is believed to represent the higher molecular weight fraction of JP-8. Occupational exposure to JP-8 occurs through a number of routes including inhalation of the vapor and/or aerosol and dermal absorption of the aerosol. It is important to understand the difference between the aerosol and the vapor phases of JP-8 in order to know how to accurately incorporate these two routes of exposure into physiological models of human internal dosimetry.

To understand the difference in these phases as well as to correlate the compound concentration with resulting exposure effect a sensitive and selective analytical method must be developed. Analysis of JP-8 is very complex. The composition of JP-8 varies greatly due to the refining process or due to where the fuel originated. No methods are currently available that show validation data to include precision, accuracy, stability or recovery.

In the studies to follow we have examined the three phases of JP-8 and determined the best sampling method for the vapor and aerosol phase of the fuel. In chapter 2, a compilation of literary analytical methods used to analyze components within JP-8 in matrices to include: liver, sweat, blood, plasma, fish tissue, water, oil and soil have been reviewed. This paper also discusses extraction methods, detectors, and other separation techniques commonly used in JP-8 and hydrocarbon analysis, in general. Lastly, the effects of exposure and the future of JP-8 and petroleum analysis with respect to human health are discussed.

In chapter 3, the three phases of JP-8 are examined. Each phase is generated and analyzed using a GC/MS system and a previously developed method. In the first part of these experiments, neat JP-8 is compared to 37 standards of components believed to be present in JP-8. The vapor and aerosol phases are generated and compared. The three phases are shown to be unique; therefore exposure effect for each phase of the fuel can be different. For the next part of the experiments, we set out to determine the best way to sample the vapor phase of the fuel. Three sorbent tubes were examined: charcoal tube, custom tube and a Tenax tube. Each tube was exposed to the vapor phase of this fuel and desorbed using an organic solvent. Based on statistical comparison and recovery, the Tenax tube was determined to be most representative of the vapor phase of the fuel. Lastly we examined the aerosol phase of the fuel exposed to a glass fiber filter. The filter showed adequate adsorption of the fuel when compared to the aerosol standard.

As a result of the characterization performed in chapter 3, we were able to choose twelve compounds that were representative of the JP-8 fingerprint. These

twelve components were combined into a mixture and analyzed. In chapter 4, a method is developed and validated to examine the JP-8 mixture in rat liver and whole blood. Using a solid phase microextraction (SPME), a gas chromatograph and an ion trap mass spectrometer a method that was accurate, precise and stable was validated and can be used confidently in the analysis of JP-8. During method development optimization of the following parameters were achieved: extraction time, extraction temperature, incubation time, desorption time, injection temperature, and salt addition. Animals were exposed by IV administration of JP-8. Timed blood samples were taken and the terminal liver samples were analyzed so that toxicokinetic parameters and tissue distribution could be determined.

## Chapter 2

### A Review of Analytical Methods for the Identification and Quantification of Hydrocarbons Found in Jet Propellant 8 and Related Petroleum Based Fuels

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**Abstract:**

Jet Propellant 8 (JP-8) is a complex mixture of compounds that varies from batch to batch. Quantification of various compound classes of JP-8 including: BTEX, PAH's and VOC's have been accomplished. Very few papers have tackled total JP-8 quantification because of its complexity. The components in JP-8 tend to co-elute and present at low concentrations often nondetectable. JP-8 is the major source of chemical exposure for Department of Defense personnel and a potential hazard for civilians and marine animals. Some components of JP-8 have been identified as possible human carcinogens and have been studied extensively. Analytical methods developed to analyze components of this fuel are essential to measure the extent of exposure, as well as the short-term and long-term exposure in rodents, humans and marine life. To date, JP-8 has been examined in urine, blood, contaminated water and fish tissue. This paper reviews methods currently utilized in the literature for the analysis of JP-8 and its components. This paper also discusses extraction methods and detectors commonly used in JP-8 and hydrocarbon analysis, in general. Finally, the effects of exposure and the future of JP-8 and petroleum analysis with respect to human health are discussed.

**Introduction**

Jet Propellant-8 (JP-8) is a kerosene-based fuel used universally by North Atlantic Treaty Organization (NATO) countries. Over 20 years it has replaced JP-4 as the main fuel of the military, because it is less volatile and has a higher flash point (Bogdan, Boulares et al. 2001). These characteristics make this fuel less likely to produce catastrophic events in crashes or warfare. JP-8 is composed of 33-61% n-

alkanes and isoalkanes, 12-22% aromatics (benzene, substituted benzenes), 10-45% polycyclic aromatic hydrocarbons (naphthalene, substituted naphthalene and cycloalkanes), 0.5-5% olefins, and 0-0.3% sulfur-containing heterocyclics (The United States National Academy of Sciences, 2003). Because JP-8 is manufactured to a performance standard, the composition of this fuel varies from batch to batch. JP-8 is responsible for the majority of all chemical exposures reported for U.S. Department of Defense personnel (ATSDR 1998). As a result, exposure to JP-8 has been studied extensively, and a wide variety of health effects have been noted.

The composition of JP-8 is determined based on operational requirements including heat content, fluidity, corrosion, stability and cost. Over the years many fuels have been made, yet not all were used due to various performance failures (Figure 1.1). JP-8 has surpassed its predecessors in terms of price/gallon, alleviation of coking (blocking of fuel injectors by solid build up), temperature capability and safety. This is the fuel upon which all future fuels will be based. JP8+100 consists of JP-8 and several additives used to prevent corrosion, metal deterioration and gum formation. The addition of these additives prevents fuel degradation and improves engine-cooling capabilities (Maurice, Lander et al. 2001).

The United States alone uses 609,550 barrels of jet fuel per year (without wartime consideration) (Maurice, Lander et al. 2001). Exposure to JP-8 is very common among military personnel, but uncommon in those not directly associated with military activity. The most common route for JP-8 to enter the environment is through accidental release. When JP-8 is released it can evaporate into the air, as well as leach into the soil and ground water (McDougal and Robinson 2002). Individuals become

exposed by living near accident sites, touching or eating soil-containing JP-8, or by drinking JP-8 contaminated water. The effects of short-term exposure include lightheadedness, immunotoxicity, and skin irritation (ATSDR, 1998). The immune system in rodents has been shown to be the most sensitive to the toxic effects of JP-8. These effects may include decreases in immune organ weights and loss of immune function time, depending on the route and length of exposure (Harris, Sakiestewa et al. 1997a; Harris, Sakiestewa et al. 1997b; Harris, Sakiestewa et al. 1997c). Long-term effects may include decreased neurological function, memory impairment, and immunotoxicity (Harris, Sakiestewa et al. 2000). Some components of this fuel have also shown carcinogenicity (ATSDR, 1997; NTP 1992; NTP 2000). Various toxicological effects from exposure to this fuel have been demonstrated.

Benzene, one of the components reduced when JP-4 was replaced by JP-8, has been shown to be carcinogenic. Leukemia, a cancer of the bone marrow, lymph nodes, and spleen, has been linked to benzene exposure in humans. Long-term exposure to benzene can affect bone marrow and blood production. Short-term exposure to high levels of benzene may cause drowsiness, dizziness and death. The suggested exposure limit for benzene is 1 ppm in air per 8 hours (OSHA). Short-term exposure limit is 5 ppm. NIOSH sets the exposure limit to 0.1ppm (10 hr time weighted average) and 1ppm for short term exposure (ATSDR 1997).

Naphthalene, another component of JP-8, has been linked to cataract formation in workers following short-term exposure. Workers who have been exposed to naphthalene vapors have developed laryngial carcinomas and neoplasms (NTP, 1992). Several substituted naphthalene's have been linked to cancer when applied to the skin

of mice. However, no conclusive studies have been able to associate a carcinogenic effect in humans solely from exposure. Long-term exposure to naphthalene has been associated with retinal hemorrhaging and chronic inflammation of the lungs. Short-term exposure has been linked to cataract formation and neurological damage. OSHA has set an exposure limit for naphthalene. Long-term exposure should not exceed 10 ppm and short-term exposure should not exceed 15 ppm. The US EPA has designated naphthalene as group C, a possible human carcinogen (NTP, 2000).

The long-chain hydrocarbons decane through hexadecane have been shown to increase the carcinogenicity of other compounds present in JP-8 that have been connected with lung cancer (Zielinski 1987). The health effects of these compounds are determined by the concentration and duration of exposure. Analyzing components in this fuel separately and together would allow improved understanding of their affects and possible side effects that military workers and personnel may incur.

A number of analytical techniques have been employed to examine JP-8 as a neat fuel and for specific compound classes within the fuel. JP-8 is an extremely complex mixture of thousands of components. To analyze this fuel is a very arduous task. However, studies have characterized the breath of exposed and non-exposed individuals to observe overall JP-8 exposure. JP-8 vapor as well as aerosol has also been studied.

Analysis of JP-8 and its components has been attempted using gas chromatography using mass spectrometry, flame ionization, electron capture and photoionization detection. Gas chromatography is the predominant mode for the determination of this mixture. GC analysis is performed due to the physical properties

of JP-8's components, including volatility. There is no single method available to quantify all components of JP-8. Its composition varies from batch to batch therefore making characterization difficult and batch dependent. This paper will review analytical methods used to examine the three major classes of compounds that comprise JP-8: aliphatic hydrocarbons, alicyclic hydrocarbons, aromatics individually and within JP-8 itself.

Hydrocarbons are the largest class of compounds in JP-8. They are the principle components of the crude petroleum from which JP-8 is refined. There are over 200 aliphatic and aromatic hydrocarbons that have been identified in this fuel. In petroleum there are 3 major classes of hydrocarbons: parafins, naphthalenes and aromatics. Hydrocarbons are naturally occurring chemicals processed mainly into gas/fuel. They can also be processed into plastics and solvents (Zielinski 1987). These 3 major classes of compounds account for over 90% of JP-8's total mass.

Four detectors have been used in the analysis of hydrocarbons: flame ionization (FID), photoionization (PID), electron capture detector (ECD) and mass spectrometry (MS). These detectors are usually paired with a gas chromatograph. GC's are used for hydrocarbon analysis because of versatility in detectors, column substrate materials, high resolving power, stability and reliability for volatilization of broad classes of hydrocarbons (Grob 1995). High performance liquid chromatography (HPLC) has been used in the analysis of polyaromatic hydrocarbons (PAH).

### **Extraction Techniques**

JP-8 and the classes of compounds therein have been analyzed in matrices including: blood, urine and breath. Before analysis of these compounds can be

achieved using an analytical instrument, they must be separated from the matrix. There are four main methods used for extraction of these compounds. These include: solid phase microextraction (SPME), static headspace, purge and trap and liquid liquid extraction (LLE). Each of these methods has advantages as well as disadvantages. Determining which method is optimal for a particular experiment is dependent on the specific circumstances. The best conditions for the performance of each extraction method and a brief description are included in this section.

### **Solid Phase Microextraction:**

SPME was developed in the late 1980's by Janusz Pawliszyn (Pawliszyn and Liu 1987). This solvent free technique was developed to aid in rapid sample preparation. The introduction of this sample preparation technique gave an alternative to preceding sample preparation techniques including: LLE, purge and trap and solid phase extraction.

This technique includes the exposure of a fiber following direct placement in the sample (direct extraction) or into the headspace of a vial that contains the sample (headspace). The fiber is coated with a nonpolar or polar coating depending on the physical properties of the target compounds. Analytes must have a high affinity for the fiber coating. A strong affinity for the coating is important because the sample matrix and the fiber are competing for analyte binding. The analytes absorbed to the fiber, are thermally desorbed in the GC inlet (most common) (Pawliszyn 1990; Pawliszyn 1997; Wercinski 1999).

SPME is suited for samples that contain volatile or semivolatile organic compounds. The concentration of these compounds must be kept relatively low when

coming into contact with the fiber. Concentrations that are too high may have adverse effects on the fiber due to their solvating properties. Headspace SPME is most commonly used for JP-8 extraction, because exposing the fiber to the headspace of the sample allows for protection of the fiber from interferences from high molecular weight and non-volatile components in the sample matrix. Direct extraction is rarely used with a biological matrix because proteins and other blood or urine products may bind to the fiber, decreasing the fiber's capacity for analyte absorption. As a result the method sensitivity for analytes decreases.

As stated earlier, SPME has some significant advantages over other techniques. These include: being solvent free, simple, selective and fast. Disadvantages include: carryover, the fragile nature of the fiber, and limited fiber coating selections. SPME is a well established technique and is employed by many laboratories for the analysis of volatile and semivolatile compounds, however many papers studying JP-8 choose to use LLE because of shipping considerations. However, for studies where shipping of samples is not involved SPME is becoming the method of choice.

SPME is an all-inclusive process, which includes sampling, extraction, concentration and sample injection. Smaller amounts of sample can be used along with matrices that would normally require extensive cleaning and purification. Headspace sampling is the most popular form for SPME because the fiber does not come into contact with the sample, therefore, lowering equilibration time and increasing the life of the fiber. Two methods employ headspace SPME to analyze compounds including benzene, toluene, ethylbenzene and xylene (Alegretti, Thiesen et al. 2004). The alkanes hexane through tridecane, benzene, toluene, ethylbenzene, m- and o-xylene,

cumene, propylbenzene, 2- and 3-ethyltoluene, mesitylene, pseudocumene and 1,2,3-trimethylbenzene were analyzed by Liu and co-workers (Liu, Hara et al. 2000). Both methods used standard GC conditions for analysis. Alegretti and co-workers examined the first set of listed compounds in human whole blood using a 10mm x 100µm polydimethylsiloxane (PDMS) fiber. Heating, adsorption and desorption conditions were optimized. Into a 10ml vial was placed a 10mm magnetic stirrer, 1g NaCl, and a 2 ml blood sample. Vials were sealed using polytetrafluoroethylene (PTFE) silicon septa. Samples were heated at 40°C with stirring for 20 minutes. The fiber was exposed to the headspace for 2 minutes and injected into the GC injector with a set temperature of 200 °C for 3 minutes. A DB-624 megabore column (30m x .53mm i.d. x 3µm film thickness) was used to analyze these compounds. It is important to know the temperature at which the analyte compounds will partition into the gas phase, adsorb to the fiber and desorb to the GC column. The time allotted to each step of the adsorption/desorption process is equally important. Too short a time could result in insufficient equilibration or adsorption to the fiber or desorption to the column. Too long a time could result in fiber saturation for adsorption or decreased peak areas for certain compounds. This method did not use an internal standard, observed no carryover and did not have any co-elution of analytes.

Liu and co-workers examined hydrocarbons and some aromatic compounds in male human whole blood also using a PDMS fiber (100µm). This paper also optimized heating temperature, adsorption and desorption time. Into a 12 ml vial, 0.2g of blood along with 0.2µg or 1µg/g of each compound (C<sub>7</sub>-C<sub>13</sub>, benzene, toluene, ethylbenzene, m- and o-xylene, cumene, propylbenzene, 2-ethyltoluene, 3-ethyltoluene, mesitylene,

pseudocumene and 1,2,3-trimethylbenzene) and 0.8 ml of VHC-free water were mixed. This vial was sealed with a Teflon coated silicone rubber septum. The sample was cooled at -5 °C for 30 minutes. Unlike the other study, cryogenic trapping was used to optimize low level compounds before fiber exposure. Liquid CO<sub>2</sub> was used as the coolant. The fiber was exposed for 30 minutes and introduced to the GC injector for 3 minutes. The injector temperature was set to 250 °C. A XTI-5 capillary column (30m x .25mm i.d. x .25µm film thickness) was used in the analysis of these compounds. There was no mention of stirring or salt addition. Deuterated toluene was used as the internal standard.

These methods have many differences yet both examined whole blood and used SPME for compounds in JP-8. The first method did not use an internal standard or cryocooling, while the second method did not use stirring/agitation or salt. The choice of sample preparation technique may be a result of the compounds being analyzed, the use of different detectors, instrumentation or sensitivity, availability and accuracy. Both methods provided sufficient sensitivity for analysis of a subset of components of JP-8 in whole blood. However, one could speculate that the combination of stirring, sample salting, and using cryocooling would provide improved sensitivity for these components.

### **Liquid Liquid Extraction:**

Liquid Liquid Extraction is a conventional technique used in the separation of compound mixtures. LLE works by transferring an analyte from one solvent to another according to its solubility. LLE consists of two phases, an aqueous phase and an organic phase. Hydrophilic compounds will have an affinity for the aqueous phase and hydrophobic compounds will have affinity for the organic phase. Therefore, the analyte

will partition into the solvent that offers the greatest solubility. The two solvents should be immiscible to allow for facile isolation. Liquid Liquid extraction is the choice of many researchers, because of shipping considerations.

To begin separation, the sample is placed into an extraction vial and the immiscible solvent is added. The mixture is shaken and the two phases are allowed to separate. Both phases can be drawn off and the amount of analyte in the appropriate phase measured. LLE should be used if 1) separation by distillation is ineffective or difficult, 2) boiling points of mixtures are close (characteristic of JP-8), 3) flexibility in operating conditions is desired, and 4) more than two components are present (also characteristic of JP-8). Disadvantages to using LLE include: emulsion formation, analytes may strongly adsorb to particulates, the analytes may bind to high molecular weight compounds, and the analytes may have mutual solubility in the two phases (Snyder 1997). Advantages of LLE include that solvents are usually readily available and inexpensive.

Liu and co-workers (Liu and Pleil 1999) employed LLE for the determination of bovine plasma and phosphate buffered saline (surrogate blood). They employed a HP-5890 GC and an HP-5971A MSD with a RTX-1 fused silica (30m x .25mm x 1 $\mu$ m) column. A 10m x .32mm i.d. guard column was also used. This group examined two liquid liquid extraction solvents pentane and dichloromethane. Analysis of JP-8 was done to choose targets that would be monitored following liquid-liquid extraction. Based on the results, benzene, toluene, nonane, decane, undecane, dodecane, tridecane, tetradecane and pentadecane were chosen.

When LLE was done with these solvents, varying effects were seen. Fluctuations in peak area were more prevalent with the use of pentane as an extracting solvent. Pentane had a higher efficiency for the extraction of aliphatic alkanes with a higher carbon number, where as dichloromethane had a greater affinity for the aromatics. After the extraction was performed using both solvents the authors found that when using pentane, there was a concentration dependence on the recovery efficiency from PBS solutions. Additionally at the same concentration the relative efficiency was greater for aliphatic alkanes vs. aromatics. When using DCM, lower efficiency was seen for long chain aliphatics, higher efficiency for short chain aliphatics and aromatics. This study showed that both solvents were efficient in extracting components from JP-8. LLE could accommodate a broad range of compounds (benzene –pentadecane) from a blood surrogate. The following parameters were taken into consideration when using these solvents for LLE of JP8 and its components: Pentane, should be used as a solvent with compounds of similar volatility. When using DCM, to get the same amount of sensitivity seen when using pentane, solvent reduction and purification must be performed. With DCM contamination with various hydrocarbons was often seen.

This same group later realized that the sensitivity they were seeing in the PBS (blood surrogate) solution could not be duplicated. They attributed this to possible binding/ interaction of lipids or proteins in the plasma with the sample. Therefore to improve partitioning they added salt. They began diluting the plasma with a sodium chloride saturated PBS solution before extraction. Adding salt has been shown to increase the distribution ratio for selected analytes. In this case, there was a 2-3 fold

improvement in recovery. Benzene was the exception, its percent recovery decreased from 35.5 +/- 7.4 to 24.2 +/- 16.4. After optimizing their procedure they concluded that the extraction of VOC's from plasma with the sodium chloride saturated PBS solution with 20 minutes of hand shaking was the most favorable procedure for the extraction of the VOC's in JP-8. They applied this method to human whole blood and found that analyte recovery varied from 14.6-63.9. Toluene and benzene were not detected from the blood samples.

### **Static Headspace:**

Static headspace is a technique that uses a volume of headspace vapor to define the nature and concentration of volatile compounds in the original sample. Gas chromatography coupled with headspace sampling was first introduced in 1958 by Bovijn at the Amsterdam symposium (Bovijn, Pirotte et al. 1958).

For headspace analysis a sample including analyte and matrix are placed in to a vial and sealed. The vial is then heated to increase the concentration of volatiles in the headspace. After the analyte concentration in the headspace reaches equilibrium, a small amount of the vial's headspace is introduced into the GC system. In static headspace, the sample is transferred under equilibrium conditions. Advantages of this method include: its long history of use in the literature, and its ease of use. Disadvantages include: sampling, lack of concentrating the sample prior to analysis, which ultimately limits method sensitivity. The matrix can also affect the method sensitivity if the analyte has a high affinity for the matrix (Kolb and Ettre 1997).

Static Headspace is a popular form of sampling for JP-8 because most of the components are volatile and readily partition into the headspace. In addition, when

headspace is used it is usually not necessary to perform any other actions for sample preparation. Therefore this technique is desired because of its simplicity and short sample preparation time. A number of studies have used this technique to examine biological samples. Schroers and co-workers (Schroers, Jermann et al. 1998) used headspace analysis with three detectors in series. A type 4160 GC with a type 250 headspace autosampler was used. For separation Schroers used a DB-5 fused silica column (60m x .32mm x .25 $\mu$ m). The headspace autosampler was equipped with a device made in the lab to incubate the sample at 65°C for 6 hours to prevent coagulation that could be caused by over heating the sample. For this experiment the syringe temperature was 80 °C, the injection volume was 2.5 ml and the GC injection temperature was 130 °C. Using this method BTEX was successfully extracted and analyzed. Other papers to use static headspace analysis include Perbellini and co-workers (Perbellini, Pasini et al. 2002). This paper examined benzene, toluene, ethylbenzene, and m-xylene in blood and urine. Hattori and co-workers (Hattori, Iwai et al. 1998) examined xylenes in whole blood using static headspace for extraction. Safarova and co-workers (Safarova, Sapelnikova et al. 2004) examined VOC's in wastewater.

### **Purge and Trap:**

This technique is a dynamic headspace sampling technique that has been used for years to extract volatile organic hydrocarbons from a matrix prior to injection into a GC system. It was first used in the early 1960's to extract VOC's from body fluids. Now this sampling method is being used widely for various environmental applications.

For Purge and Trap extraction an inert gas, usually helium is passed through a liquid sample that is introduced to a purge vessel. The flow of the gas is constant and is in contact with the sample for a fixed period of time. Gas bubbles agitate the sample increasing the rate of transfer of volatile compounds to the headspace. This process is referred to as purging the sample. Volatile compounds in the headspace are then passed through an adsorbent material. This adsorbent may be Tenax, silica gel, coconut charcoal, graphitized carbon or carbon molecular sieves which are used to trap the analytes. The choice of adsorbent is dependent on the chemical properties of the compounds of interest. When the purge and trap sequence is complete, the adsorbent trap is heated and flushed with the GC carrier gas. Analytes are released from the trap quickly and are introduced directly into the GC system. When compared to static headspace, purge and trap extraction holds two important advantages. These include the ability to reduce matrix effects and its ability to increase sensitivity by concentrating the analytes prior to analysis. The major disadvantages in purge and trap analysis are the low sample throughput and increased mechanical complexity of the system.

Purge and trap analysis has been done for numerous aqueous sample methods and a few solid or matrix laden analyses. In methods using purge and trap analysis for blood samples, carryover was seen that interfered with quantitation of sample compounds (Zielinski 1987). Ashley and co-workers (Ashley, Bonin et al. 1992) examined volatile organic compounds including: benzene, ethylbenzene, m/p-xylene, o-xylene and toluene in blood using this extraction method, but found trouble when constructing calibration curves due to background that could not be reduced. As a

result, the calibration curves had to be constructed in water. The VOC's were seen at relatively low concentration in these blood samples (< 4 ppb). For these experiments an HP-5890 GC and VG analytical 70E high resolution mass spectrometer was used with a LSC 2000 purge and trap concentrator attached to an ALS 2016 autosampler. Separation was performed on a J&W DB-624 column (30m x 1.8 $\mu$ m)

Methods examining compounds from JP-8 in water include Wang and co-workers (Wang and Chen 2001) who looked at VOC's using several small sorbent beds in series vs. a single sorbent bed, which allowed them to forgo using cryogen for cooling. While examining water samples heptane, octane, decane, toluene and styrene were observed. This method would work well for semivolatile or less volatile compounds because they did not need to be cryofocussed for GC analysis. A HP-6890 GC with FID and ECD was used in these experiments. The separation was performed using a DB-1 column (60m x .32mm x 1 $\mu$ m). Hino and co-workers (Hino, Nakanishi et al. 1996), Ashley and co-workers (Ashley, Bonin et al. 1992), and Rosell and co-workers (Rosell, Lacorte et al. 2003) also used purge and trap analysis to examine VOC's in water.

Helium was used as the carrier gas for most methods. Parameters such as flow rate, purge time and bake time were optimized for individual methods to ensure that a sufficient amount of sample was extracted from the matrix, introduced to the GC and cleaned from the system to minimize carryover. Most purge and trap methods, unless specifically looking at materials that had unknown hydrocarbon content, examined benzenes and xylenes. Purge and Trap has a history for the determination of these compounds and is often seen as the gold standard for VOC sampling. However, its

place is being challenged by newer methods such as SPME which offer comparable sensitivity and improved ruggedness.

## **Detectors**

Several detectors have been used in various applications for the determination of JP-8. The analysis of JP-8 and its components requires high sensitivity, specificity, stability and dynamic range. The detectors discussed in this section include: Electron capture (ECD), Flame ionization (FID), Photoionization (PID), and Mass Spectrometry (MS). To date all of these detectors have been coupled with a gas chromatograph for the determination of JP-8.

### **Electron Capture Detector**

The electron capture detector was introduced in the 1960's by J.E. Lovelock (Lovelock 1968). It is highly sensitive for electronegative compounds. This detector has the capability of detecting concentrations in the picogram to femtogram range. These characteristics make this detector suitable for many compounds in environmental analysis.

A sample passes from the GC column into the radiation source ( $\text{Ni}^{63}$  or tritium) where it is ionized. The radiation source produces beta particles, which collide with the carrier gas (argon or nitrogen) generating free electrons. These free electrons move to an anode generating a current. The sample molecules capture a portion of these electrons, as a result, fewer of them reach the anode causing a decrease in the current that is proportional to the concentration of the analyte. Advantages of this detector include its high sensitivity and its selectivity for compounds with a high electronegativity.

The disadvantages of this detector are its limited dynamic range (Scott 1996) and the fact that it is only applicable to certain classes of compounds.

The electron capture detector is not often used for the analysis of JP-8 or its components since these compounds are not expected to provide much response for this detector. Few papers used this detector, but some compared this detector to the flame ionization detector or the mass spectrometer. These studies found that none of the compounds (JP-8 components) being measured provided a detectable response Ramey and co-workers (Ramey and Flannigan 1982), Scheoers and co-workers (Schroers, Jermann et al. 1998).

### **Flame Ionization Detector**

McWilliam and co-workers (McWilliam, 1958) introduced the flame ionization detector in 1958. It is one of the most commonly used GC detectors. It is mainly used for the detection of organic compounds. This detector has limits in the low picogram to femtogram range.

The heart of this detector is the hydrogen-air diffusion flame. When organic compounds are introduced into the flame they become ionized. The ions are then collected and an increase in current is seen at an electrode that is proportional to the amount of carbon in the flame. The signal is amplified by an electrometer, because of this amplification lower concentrations can be observed. Advantages of this detector include its wide linear dynamic range and its high sensitivity for most all-organic compounds. This detector decomposes the sample in the flame; therefore, destroying it so the sample cannot be analyzed any further. FID also does not provide any structural information (Scott 1996).

The flame ionization detector is widely used in the analysis of JP-8 and its components because of its ability to determine all of the components with high sensitivity. Ketola and co-workers (Ketola, Virkki et al. 1997) examined several JP-8 components in water. They combined FID with static headspace and compared it to purge and trap GC/MS and membrane inlet mass spectrometry. A HP-5890 Series II GC was used in these experiments. A LSC 2000 purge and trap autosampler was also used. A DB-1 column (30m x .32mm x 1 $\mu$ m) was used for separation. In the detection of BTEX, the FID showed detection limits 10x higher than the other methods, and its dynamic range was wider. Alegretti and co-workers (Alegretti, Thiesen et al. 2004) also examined BTEX. In blood the dynamic range was observed between 1-100 $\mu$ g/ml and the limits of detection were determined to be between 0.5-1 $\mu$ g/ml. Schroers and co-workers (Schroers, Jermann et al. 1998) used headspace analysis with three detectors. They compared the flame ionization detector, electron capture detector and the photoionization detector using static headspace sampling. The photoionization detector proved to have the lowest detection limits from .026 ng/ml - .067 ng/ml for benzene, toluene, m,p,o-xylene, and ethylbenzene. The flame ionization detector had detection limits of 1.520 - .370 ng/ml for the same set of VOC's. The electron capture detector could not detect these components at this level. Other papers using FID included Wang and co-workers (Wang and Chen 2001), Hino and co-workers (Hino, Nakanishi et al. 1996), Hattori and co-workers (Hattori, Iwai et al. 1998) and Ramey and co-workers (Ramey and Flannigan 1982).

The FID is an excellent detector to use for JP-8 analysis. All but one of the cited references uses this detector for the analysis of BTEX. Though it does not have other

capabilities like the mass spectrometer, the flame ionization detector can be used when structural information or confirmation of identity is not needed.

### **Photoionization**

This detector was introduced in 1958 by Lovelock (Lovelock 1958) and is used in the detection of aromatic hydrocarbons. Detection limits are in the picogram range for these compounds.

Following separation by the GC, the sample is ionized using UV light. The UV light source is usually an argon lamp. An electrode then detects analyte ions that are generated following photon impact. The generated current is proportional to the analyte concentration. This detector has a wide dynamic range and is relatively inexpensive. The major disadvantage of this detector is its sensitivity to water in the sample (Grob 1995).

Photoionization detectors are not as widely used as mass spectrometers or FIDs, but are quite sensitive. Schroers and co-workers (Schroers, Jermann et al. 1998) compared this detector to the electron capture detector as well as the flame ionization detector. This comparison was made using blood samples and static headspace as the extraction technique. Various VOC's were used including: benzene, toluene, m/p-xylene, o-xylene and ethylbenzene. For these compounds photoionization showed the lowest detection limits 26,45,67,32 and 33 ng/L respectively. These values were 10-100x lower than the FID detector. While the PID is not often used in the laboratory setting, it is used widely in field sampling. Photoionization is more selective for aromatic compounds and alkenes than the FID but is less sensitive to alkanes, perhaps explaining its decrease in prevalence in the literature for JP-8 analysis.

## **Mass spectrometry**

Mass spectrometry (MS) was first introduced by J.J. Thompson in 1912 when he constructed his mass spectrograph (Thompson 1913). In MS, ions generated from the analytes are separated according to their mass-to-charge ratio. Mass spectrometry is the leading detector for JP-8 and its components. This detector has outstanding sensitivity, stability and dynamic range. Mass spectrometry has been widely used in the identification, quantification, and structure elucidation. Mass spectrometry has traditionally been coupled with a separation technique like high performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE).

The sample is introduced by the inlet system into the source where it is ionized. The ions are then passed through the mass analyzer where they are separated according to their mass-to-charge ratio. The detector (MS) counts these ions as an electrical signal generated by impact on the detector. A mass spectrum is produced by plotting the arrival time of an ion versus the abundance of these ions.

The production of ions is critical to successful MS detection. The source is where ion production occurs. There are various types of sources used today; however the source most often used in the analysis of JP-8 and its components is electron ionization (EI).

### **Electron Ionization:**

A.J. Dempster introduced EI in 1918 (Dempster 1918). This ionization source is good for the analysis of organic compounds, but produces extensive fragmentation where the molecular ion is not always observed. To produce ions from the sample, electrons are made by heating a wire filament using an electric current. The electrons

are then accelerated toward an anode and during this process interact with the gaseous analyte molecules, ionizing them via a Franck-Condon vertical transition (de Hoffman and Stroobant 2002).

After the ions leave the source, they travel to the mass analyzer to be separated. Many analyzers exist including magnetic sector, quadrupole, Fourier transform ion cyclotron resonance, time-of-flight (TOF), and the ion trap. Of these, the quadrupole and time-of-flight mass analyzers have been most widely used in the analysis of JP-8 and its components.

### **Quadrupole:**

The quadrupole mass filter was introduced in 1955 by W. Paul and H. S. Steinwedel (Paul and Steinwedel 1953; Paul, Reinhard et al. 1958). This analyzer consists of four parallel rods. Separation of the ions occurs by placing both Rf and DC potentials on the four rods in a manner where alternating rods are held at positive or negative potential values. Depending on the potentials applied only ions of one mass-to-charge value will adopt a stable path as they move through the quadrupole. These stable ions will be attracted toward an oppositely charged rod. This potential is then moved to the adjacent rod causing ions to travel through the quadrupole in a spiraling trajectory following the potential around the rod assembly. By scanning the amplitude of the Rf signal and the applied DC potential, ions of varying mass-to-charge values can successfully traverse the quadrupole allowing for the detection of specific ions. The quadrupole provides good reproducibility, sensitivity and linearity at relatively low cost, but provides limited mass resolution.

**Time-of-Flight:**

W. Stephens introduced the time-of-flight mass analyzer in 1946 (Stephens 1946). This analyzer measures the transit time it takes for an ion from the source to the detector. By having a constant acceleration and with fixed distance between the source and the detector, the transit time is directly related to the mass-to-charge ratio of the ion. The time-of-flight mass analyzer has the highest scan rate and ion transmission of any mass analyzer. In addition, the poor resolution that plagued the earlier versions of this mass analyzer have largely been resolved through the use of reflectrons and delayed extraction techniques. The time-of-flight mass analyzer is a natural fit for gas chromatography where the narrow peak widths require fast detector response in order to generate a mass spectrum for the identification of unknowns. In addition, the high resolution that is now available on time-of-flight instruments allows empirical formula data to be acquired enhancing the ability of this type of instrument to identify unknown compounds. To date, gas chromatography using time-of-flight instruments has been primarily qualitative. It remains to be seen if the newer time-of-flight instruments can overcome limitations in linearity that have left quantitation primarily to the quadrupole mass analyzer.

**MS analysis**

Mass spectrometry is one of the most popular detectors in JP-8 analysis. When coupled with the gas chromatograph it is capable of identification via interpretation of fragmentation patterns or comparison to libraries of EI mass spectra. In the area of environmental analysis it is useful because of its ability to aid in the identification of compounds, which is not possible with many other detectors. While the use of MS has

many advantages it is still relatively expensive and sophisticated relative to other GC detectors.

Mass spectrometry is frequently used for the analysis and quantitation of JP-8 and its components, because agencies or labs that monitor contamination of water and other matrices need to know what is in the sample as well as how much, so that better treatment methods can be employed or developed to reduce levels of hazardous compounds such as benzene and naphthalene. Huybrechts and co-workers (Huybrechts, Dewulf et al. 2000) examined volatile organic hydrocarbons in marine water from the Netherlands and Belgium. Twenty-seven compounds were examined including: cyclohexane, benzene, toluene, ethylbenzene, m/p-xylene, and o-xylene had detection limits of 1.25, 22.05, 4.99, 2.31, 2.83, and 1.93 ng/ml respectively. These limits were achieved by using a Carlo Erba QMD 1000 GC-MS system employing selected-ion monitoring with a quadrupole mass analyzer. The separation was performed on a Restek Rtx 502.2 column (60m x .32mm x 1.8 $\mu$ m). Other methods examining water samples include Ketola and co-workers (Ketola, Virkki et al. 1997) who compared static headspace, purge and trap, and membrane inlet mass spectrometry, Safarova and co-workers (Safarova, Sapelnikova et al. 2004) examined compounds including benzene, toluene, m-, p-, o-xylene, propylbenzene, mesitylene, pseudocumene, and naphthalene using GC/MS. Rosell and co-workers (Rosell, Lacorte et al. 2003) examined BTEX and gasoline products in groundwater also using GC/MS.

Blood is a complicated matrix to perform analysis of hydrocarbons. Consideration must be taken to ensure that the compounds that are being studied have

no interaction with proteins or lipids in the matrix. Care must also be taken in the choice of extraction method.

Methods examining blood most often use SPME, headspace, or LLE. Methods examining components of JP-8 in this matrix include Liu and co-workers (Liu, Hara et al. 2000) using male human whole blood C<sub>6</sub>-C<sub>13</sub>, benzene and xylenes were observed. The limit of quantitation was reported as .01 µg/g whole blood, detection limits were not reported. Ashley and co-workers (Ashley, Bonin et al. 1992) also examined compounds in blood. Detection limits for benzene, ethylbenzene, m/p-xylene, o-xylene and toluene were .032, .008, .010, .024, and .088 ppb respectively. Other papers observing components of JP-8 in blood include Perbelli and co-workers (Perbellini, Pasini et al. 2002).

Few papers have examined JP-8 in other tissues or matrices. Human liver, sweat and urine were studied by Schulnegger (Schlunegger 1972). Schulnegger was looking at the distribution patterns of n-alkanes in these matrices. Using GC/MS, in the liver C<sub>12</sub>-C<sub>33</sub> was observed, in urine C<sub>12</sub>-C<sub>30</sub> was observed and in sweat C<sub>12</sub>-C<sub>35</sub> was observed. Schnulnegger observed that in the liver, every n-alkane present was accompanied by a small amount of the mono-unsaturated hydrocarbon. In urine he also observed that the most pronounced alkanes were hexadecane and octadecane. Perbelli and co-workers (Perbellini, Pasini et al. 2002) also examined urine.

Methods to examine the fuel components in marine animals are very important. Spills or accidental release of oil from refineries or oil tankers have happened more recently and methods have been developed to analyze fish contamination. Ingestion of contaminated fish is also a medium for human exposure. The following papers

developed methods to examine the extent of exposure as well as effects of exposure to petroleum from aquatic species: Isigigur and co-workers (fish muscle tissue) (Isigigur, Heras et al. 1996), Klein and Jenkins (flag fish, rainbow trout and gold shiners) (Klein and Jenkins 1983), Ogata and co-workers (eels and short-necked clams) (Ogata, Miyake et al. 1979), Bridie and co-workers (goldfish) (Bridie, Wolff et al. 1979), Donkins and co-workers (water and mussels) (Donkin and Evans 1984) and Reddy and Quinn (sea water sample after oil spill) (Reddy and Quinn 1999).

### **JP-8 exposure**

A number of papers have been published examining health effects of JP-8 exposure. A GC/MS method was developed to study the 3 forms of JP-8 for potential exposure (neat JP-8 (liquid from the fuel), vapor and aerosol) (Dietzel, Campbell et al. 2005).

This method validated a 34 component surrogate hydrocarbon mix using GC/MS. This method was later used to analyze aerosol and vapor samples from an exposure chamber. This method is the first method to address quantification of JP-8 in different forms. This method reported good accuracy and precision data for a difficult mixture of compounds. This method could be applied to future inhalation chamber studies and be used to understand the difference in component concentrations between various aerosol and vapor generation systems.

Identifying the components in JP-8 is an important aspect in determining its toxicological effects. In another study, 37 compounds were identified from JP-8 neat fuel, vapor and aerosol (Gregg, Campbell et al. in press). These compounds were identified using retention time matches with authentic standards and by comparison to

El mass spectra from databases. This method also examined different collection and extraction methods for the examination of aerosol and vapor samples. Vapor samples were tested on a number of sorbents and then extracted using solvents. Tenax based sorbents followed by extraction using methyl t-butyl ether was found to provide hydrocarbon distributions nearly identical to direct injection of vapor samples.

### **Analysis of compound classes**

Gas chromatography is the predominant mode of analysis for JP-8 as well as most volatile and semivolatile compounds. The first published article examining volatile compounds used an automatic burette for detection and 4 foot columns for separation (James and Martin 1952). Overtime this method was modified and improved into the modern GC systems we employ today.

Thus far, all methods in this review have used GC for separation. The GC has been coupled with various detectors and extraction techniques to make the analysis of JP-8 both sensitive and accurate. HPLC is also a very popular separation tool, but has not been used extensively in the analysis of JP-8 since GC has far more theoretical plates and therefore provides better separations. However, HPLC separation has been used to analyze some of the aromatic components of petroleum. HPLC-UV-DAD has been used in the identification of several aromatics from petroleum (Pasadakis, Gaganis et al. 2001), HPLC-UV-RI has been used to separate crude oils (Akhlaq 1993), normal phase HPLC was used in the separation of coal liquids (Padlo, Subramanian et al. 1996), and normal phase HPLC has been used in the determination of aromatics in 7 crude oils (Sarowha, Sharma et al. 1996). However, since the bulk of petroleum samples are hydrocarbons with little or no absorbance in the ultraviolet portion of the

electromagnetic spectrum of light, GC will continue to dominate the analysis of this class of samples.

Several GC methods exist for the analysis of JP-8 and petroleum products these include: capillary GC/MS with SPME and HS to characterize VOC and petroleum products in ground water samples (Wang, Li et al. 2002). Detection limits for SPME were 100 times lower than those for HS. Using this method the final detection limits for BTEX and alkylbenzenes were .004-.01 µg/ml and .001 µg/ml for PAHs. The transfer of supercritical fluid chromatography flow to a GC to achieve a two dimensional separation apparatus alleviates co-elution for better analysis (Pal, Juhasz et al. 1998). Using this method, the desired effect was seen for aromatics, but not for alkanes because of their quantity and abundance. Headspace SPME was used to analyze VOC's in soil samples. Two soil samples contaminated with diesel fuel were examined. Detection limits achieved with this method range between .05-.23 ng/g. Quantification limits ranged from 0.16-0.78 ng/g. When compared against traditional headspace, HSSPME yielded greater responses and better precision and accuracy (Llompart, Li et al. 1999).

### **Two-Dimensional Gas Chromatography (GCxGC)**

Two-dimensional gas chromatography (GCxGC) was first described by Liu and Phillips in the early 1990's (Liu and Phillips 1991). GCxGC has become a great source of separation for many complex mixtures including petroleum and jet fuel. When compared to traditional GC, which yields many unresolved and overlapping peaks which are not quantifiable, GCxGC allows for the following: large peak capacity, ordered peak distribution, separation of thousands of peaks per chromatogram, an order of

magnitude increase in sensitivity, and an order of magnitude increase in analysis speed (Phillips, Gaines et al. 1999). GCxGC has also been combined with detectors including mass spectrometers and flame ionization detectors to gain more complete separation and analysis.

GCxGC is a relatively new technique. In GCxGC analysis, the sample is partially separated using a primary column and completely separated using a secondary column. These columns have different stationary phase polarities increasing the possibility of resolution. Components not separated using the primary column may be separated by the secondary column (more polar). Once the sample leaves the primary column it is modulated and injected into the secondary column. There are different modulation techniques that are discussed below. The signal from the analysis is plotted according to retention times on the columns. A contour plot is generated which separates compounds according to their chromatographic characteristics.

GCxGC is the newest and most promising technique in the analysis of volatile and semivolatile compounds. GCxGC uses traditional GC with the addition of a thermal modulator (one technique for sample modulation) used to connect the two columns being used. GCxGC uses two columns. Typically the first column is of longer length than the second column, these columns separate compounds according to volatility and polarity. As a result, the compounds being analyzed can be grouped by these characteristics. The thermal modulator interface is the most important aspect in the function of GCxGC. It connects the columns as well as transfers the analytes from the first column to the second. There are four important steps in the operation of GCxGC. These include: Accumulation, where the analytes fill the modulator tube after they are

passed through the first column. Secondly, cut, where a section of sample is heated and detached from the sample remaining at oven temperature. After the analytes are cut, they are focused. The analytes are retained at oven temperature before the final step, launch, where the chemical pulse of analyte is formed and pushed onto the second column (Phillips, Gaines et al. 1999).

Frysiner and co-workers (Frysiner and Gaines 1999) examined diesel fuel using a HP GCD Plus system that included a HP 6890 GC and a HP 5972 series quadrupole mass spectrometer. For sample modulation a thermal modulator was used. The primary column was a 13 m x .100 mm x 3.5  $\mu\text{m}$  dimethylpolysiloxane column. The secondary column was a 2 m x .100 mm x .10  $\mu\text{m}$  methylpolysiloxane column. The oven temperature ramped from 30-250  $^{\circ}\text{C}$  at a rate of 0.5  $^{\circ}\text{C}/\text{min}$  over 440 minutes. The thermal modulator was set to 100  $^{\circ}\text{C}$  over the oven temperature, and rotated every 14.41s. During the analysis they encountered several problems including: the slow scan speed of MS, to correct this problem they slowed the GCxGC separation. They also observed discontinuities in the chromatogram, to solve this problem they removed the mismatched data. It was determined that the use of a mass spectrometer with 2-dimensional GC allows for greater identification capabilities for minor components and for improved characterization of peaks.

Frysiner and co-workers (Frysiner and Gaines 1999) published another study examining BTEX and gasoline as a reference standard using a HP 6890GC and an FID. Thermal modulation was also used for this study. The primary column used was a 3.80 m x .100 mm with 3.5  $\mu\text{m}$  dimethylpolysiloxane. The secondary column was a 2.0 m x .100 mm with .10  $\mu\text{m}$  methylpolysiloxane. The column temperatures were maintained

by two ovens. The first column oven was held at 0 °C for 10 minutes and ramped to 180 °C at a rate of 3 °C/min. The second column oven was held at 25 °C for 10 minutes and ramped to 226 °C at a rate of 3.36 °C/min. The modulator was again set to 100 °C above the first column oven and rotated every 4s. Calibration solutions were made using methylene chloride. The range of concentrations correlated to the expected amount in gasoline. R<sup>2</sup> values ranged from .9887-.9999. RSD% ranged from 0.6-14. This method yielded complete separation of BTEX and aromatics in gasoline.

Since its introduction 14 years ago there have been alternative techniques employed to take advantage of GCxGC. Other methods have been developed to transfer the analyte from column one to column two. Flow switching is a method where a solenoid valve located outside of the GC oven is used to direct analyte to a second column or columns. See Figure 2. The columns were connected to the solenoid valve using stainless steel tubing and T unions. The first column was connected to the center T union using a fused silica capillary, which was also used to connect the outside unions. The first column flow travels through union C to the fill line. Some of the auxiliary gas flow is used to guide the first column effluent to the fill loop the rest travels to union D. The flow from the first column is then joined at the D union with the auxiliary gas flow and injected in the second column. As can be seen in Figure 1.2, the two outside unions are responsible for 1) Filling with sample from the first column and 2) Flushing sample from the first column. The position of the solenoid valve determines the function of each line. The positions should be alternated at regular intervals to allow pulses from the first column to enter the second column (Bueno and Seeley 2004).

Bueno and co-workers used this hardware modification to examine diesel fuel and volatile organic compounds. A Perkin-Elmer Autosystem XL GC with dual FID detectors was used for these analyses. The primary column used for the determination of VOC's was a 5.0 m x 250  $\mu$ m DB-624 capillary column, while the secondary columns were 1) A 5.2 m x 250  $\mu$ m DB- Wax column and 2) A 5.2 m x 250  $\mu$ m DB-210 column. The oven program was held at 40 °C for 1.0 minute and ramped to 75 °C at 14.0 K/min, ramped to 120 °C at 10.0 K/min and lastly ramped to 160 °C at 6.5 K/min and held for 1.0 minute. The split between the two secondary columns were measured to be even within 2%. A 41 component mix was examined that included: n-alkanes C<sub>5</sub>-C<sub>14</sub>, alkyl aromatics, alcohols, acetates and ketones. Chromatograms produced using flow switching are similar to other methods of sample modulation. Slight peak tailing was observed, however this was not expected to compromise resolution or quantitation. For diesel fuel analysis, a 27.0 m x 250  $\mu$ m HP-5 capillary column was used as the primary column. The secondary columns used were a 1.4m x 250 $\mu$ m deactivated fused silica column and a 5.7 m x 250  $\mu$ m DB-wax column. The oven temperature was held at 50 °C for 1.5 minutes, ramped to 240 °C at 12.5 K/min and held for 6.0 minutes. A ratio of 1:4 was seen between the two secondary columns. Diesel analysis tested the ability of the flow switching system to handle high temperatures. The response of the switching system was favorable and similar to that of other sample modulation techniques and samples analyzed at lower temperatures.

Micyus and co-workers (Micyus, McCurry et al. 2005) used GCxGC with flow switching to analyze aromatic compounds in gas. Using an Agilent Technologies 6890N GC with dual FID detectors they were able to successfully analyze compounds

including: BTEX, naphthalene, 1 and 2-methylnaphthalene, and ethyltoluene. A standard of gasoline was also analyzed for comparison using an ASTM method with GC/MS. For the GCxGC analysis the first column was a 15.0 m x 250  $\mu$ m DB-1 capillary column the flow of this column was maintained at 1.0 ml/min. After the injected sample entered the flow switching device it was divided between two secondary columns each connected to a different FID detector. The first secondary column was a 5.0 m x 250  $\mu$ m DB-Wax column and the second column was a 5.0 m x 250  $\mu$ m DB-1701 column. After examining the data it was decided that only the data from the DB-wax column would be used because narrower peaks were observed. All subsequent analysis would be done using this column alone. The oven was held at 35°C for 3 minutes and ramped to 250°C at 8°C/min. The ASTM standard gasoline analysis used an Agilent Technologies 6890/5973 inert GC/MS. The sample was injected onto a 60.0m x 250 $\mu$ m HP-1 capillary column. The oven temperature began at 60 °C and was ramped to 120 °C at a rate of 3 °C/min; a second oven ramp of 10 °C/min to 250 °C was also used. All the analytes injected onto the column were analyzed by the mass spectrometer. When the GCxGC method was compared to the standard gasoline method, the results were similar. For benzene, the volume percentage was observed to be  $0.79 \pm 0.11$  for the GCxGC method and  $0.81 \pm 0.18$  for the standard GC/MS method. For the total of analyzed aromatic compounds, the volume percentages were observed to be  $22.4 \pm 0.1$  for the GCxGC method and  $21.81 \pm 2.3$  for the standard GC/MS method. As shown by this study, GCxGC was comparable to the traditional GC/MS methods when examining aromatic compounds.

Differential flow modulation is another form of sample modulation introduced by Seeley and co-workers (Seeley, Kramp et al. 2000). This method uses a six port diaphragm valve placed between the detector and the GC oven to collect the sample leaving the primary column and inject onto the secondary column. After leaving the primary column the sample is collected in a sample loop and its contents are passed to the secondary column. The valve switches between collect and injection mode to allow for proper transfer of analytes. To test the effectiveness of this technique, Seeley et al. used a Perkin-Elmer Autosystem XL with an FID. The primary column used was a 10 m x .25 mm o.d. DB-624 column. The two secondary columns used were a 5.0 m x .25 mm o.d. DB-Wax column and a 5.0 m x .25 o.d. DB-210 column. The oven temperature was held at 45 °C for 0.3 minutes, ramped to 95 °C at a rate of 36 °C/min, then ramped to 180 °C at a rate of 32 °C/min and lastly held at 180 °C for 1 minute. A 21 component mix was examined that included straight chain primary alcohols, ketones and n-alkanes. Minimal tailing and good resolution was observed. Maximum separation was seen for the n-alkanes and the primary alcohols using the combination of the columns and conditions. The differential flow sample modulation technique is a simple alternative to other more complex techniques and allows for suitable analysis of VOC's.

#### **Future of JP-8 analysis:**

A number of techniques for extraction and detection of JP-8 and its components have been discussed. The choice of extraction method and detection depends on the compounds being examined. However, the analysis of JP-8 requires sensitive and robust instrumentation because many compounds present in this fuel have low

concentrations. Since the field of JP-8 analysis is growing, the newer methods should be validated to improve transferability.

Classic extraction techniques such as LLE and P&T have proven themselves efficient in the analysis process. However, it appears that SPME will soon surpass these techniques. SPME is a solvent free technique, the SPME apparatus allows for agitation, concentration and heating of a sample. Agitation and heat help analytes to partition into headspace faster as well as keep the headspace concentration constant. These aspects are important when dealing with volatile components. Fiber exposure as well as desorption time can be controlled by the SPME setup. All these parameters aid in a more accurate extraction and analysis of JP-8.

GC/MS will continue to lead in the quantification of this fuel. Mass spectrometry is currently the most widely used detector for jet fuel analysis. Mass spectrometry allows for definitive identification with the use of spectral libraries, provides structural information and is able to detect compounds at low concentrations. Combining GC/MS with various mass analyzers or even other detectors like the ion trap or the time of flight, PID or FID will further increase sensitivity and the speed of analysis. Two-dimensional GC separations also are becoming more prevalent in fuel analysis. To date, these studies have primarily relied on quadrupole mass analyzers but have noted that the scan speed of this analyzer is a limitation. It appears that the TOF mass analyzer would be a more appropriate detector for these types of analyses.

### **Conclusion:**

There are many significant challenges that still exist with regard to the analysis of JP-8. JP-8 contains thousands of components, all of which are not detectable because

of concentration or due to evaporation. It is also difficult to have a standard method for JP-8 analysis when its composition varies from batch to batch. Despite these difficulties in analysis this field is moving forward. The methods mentioned in this paper have helped to fill a void that will allow the scientific community to more accurately recognize components of JP-8 in environmental and biological samples. Toxicity can be studied further and the effects of exposure can be more accurately determined. Improvements upon these methods will allow application to total JP-8, more accurate results and the detection of compounds present at lower concentrations. These methods give an important and needed outlet for those examining exposure risks and other health effects from JP-8 and its components.

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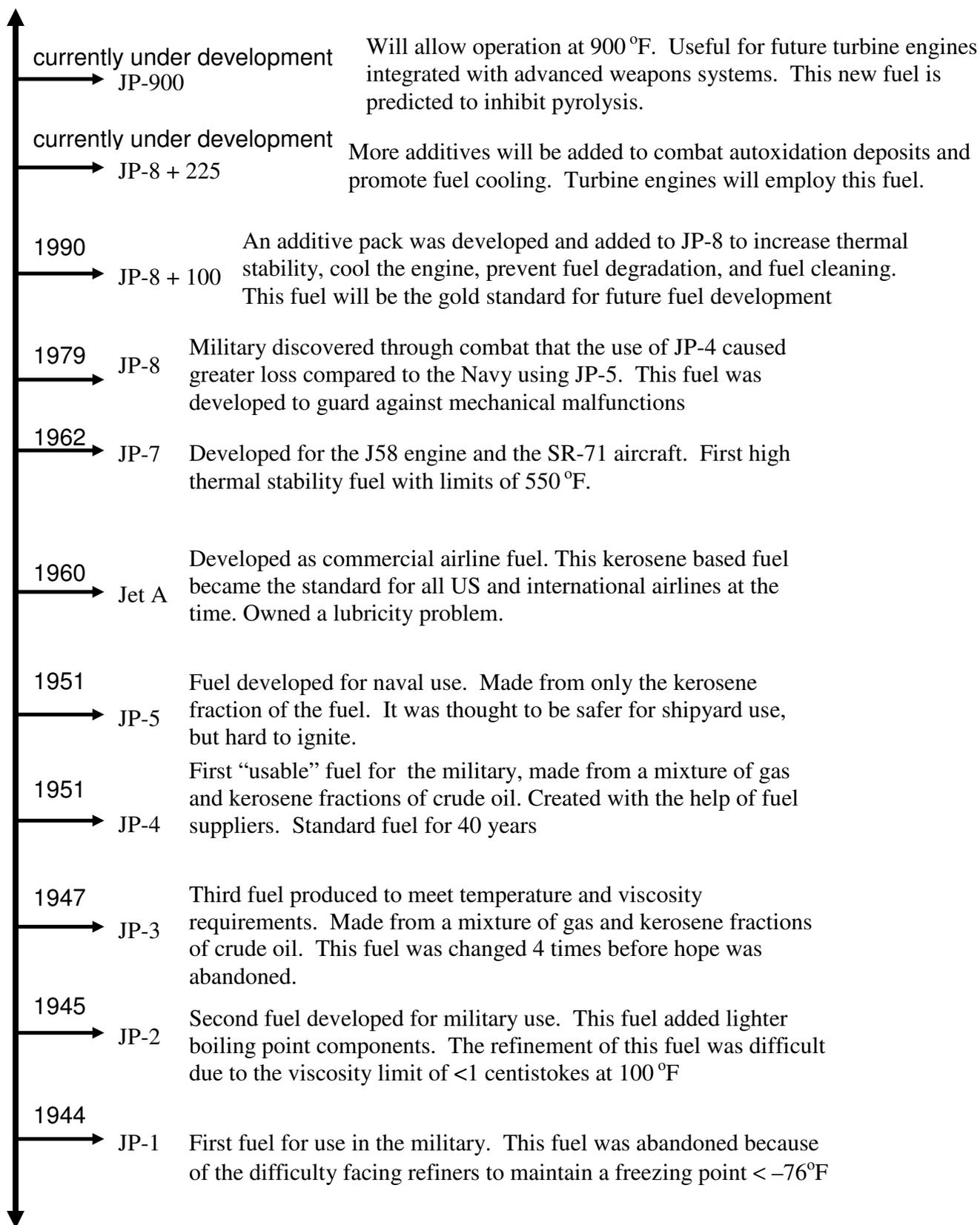
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**Table 1.1 Acronyms used in this review paper**

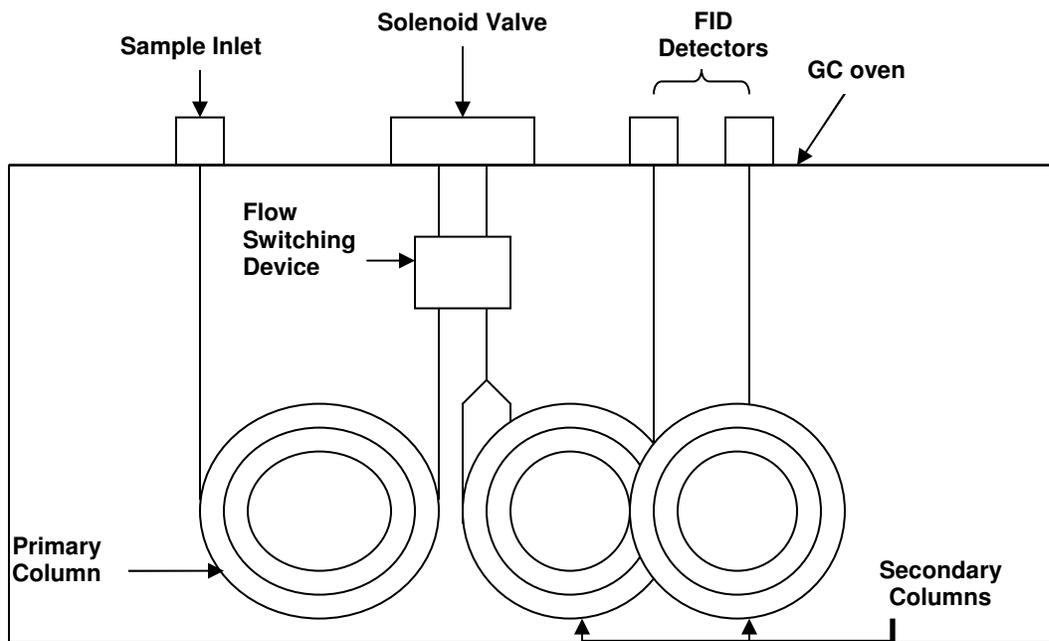
BTEX	Benzene, toluene, ethylbenzene and xylene
DCM	Dichloromethane
ECD	Electrochemical detector
EI	Electron ionization
ELSD	Evaporative light scattering detector
FID	Flame ionization detector
GC	Gas chromatography
GC/MS	Gas chromatography with mass spectrometry
HPLC	High performance liquid chromatography
HPLC-UV-DAD	High performance liquid chromatography with ultraviolet and diode array detection
HPLC-UV-RI	High performance liquid chromatography with ultraviolet and refractive index detection
HS	Headspace
HSSPME	Headspace solid phase microextraction
JP-8	Jet Propellant 8
LLE	Liquid liquid extraction
MIMS	Membrane inlet mass spectrometry
MS	Mass spectrometry
NP-HPLC	Normal phase high performance liquid chromatography
NTP	National toxicology program
OSHA	Occupational safety and health administration
P&T	Purge and trap
PAH	Polyaromatic hydrocarbons
PBS	Phosphate-buffered saline
PDMS	Polydimethylsiloxane
PID	Photoionization detector
PPM	Part per million
SFC	Super critical fluid chromatography
SIM	Selected ion monitoring
SPME	Solid phase microextraction
TOF	Time-of-flight
UV	Ultraviolet detection
VOC	Volatile organic compounds



**Figure 1.1:** Jet fuel time line: JP-8 has survived many years and will continue to survive with the addition of additives and other minor modifications

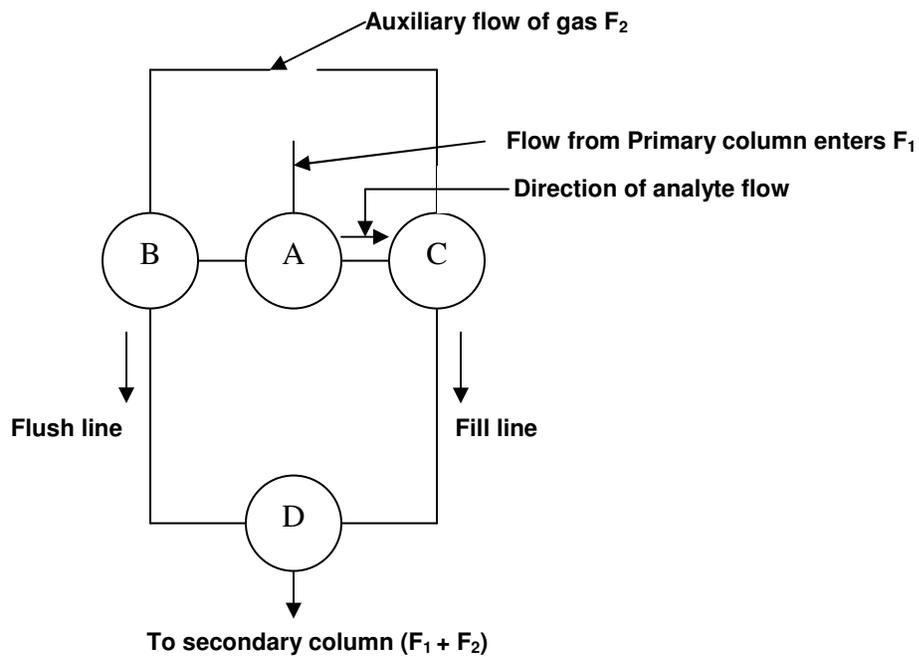
**Table 1.2:** Analytical Methods for the Analysis of JP-8 and Petroleum Components

Author	Matrix	Analyte	Extraction Method	Separation	Detection
Schlunegger et al.	Liver, urine, sweat	Alkanes	N/A	GC	MS
Reddy et al.	Water	Petroleum	LLE	GC	MS
Ogata et al.	Eels and clams	Petroleum	N/A	GC	MS
Klein et al.	Fish Tissue	JP-8	Purge and trap	GC	FID
Bridie et al.	Gold fish	Petrochemicals	N/A	GC or Total Organic Carbon Analyzer	N/A
S. Liu et al.	Plasma/PBS solution	JP-8	LLE	GC	MS
Tu et al.	Breath, blood, urine	JP-8	Thermal desorption	GC	FID
Pleil et al.	Breath	JP-8	Thermal desorption	GC	MS
Alegretti et al.	Blood	BTEX	SPME	GC	FID
Liu et al.	Blood	Alkanes/BTEX	SPME	GC	MS
Ashley et al.	Human blood	VOC	Purge and trap	GC	MS
Tellez et al.	Water	Alkanes	Purge and trap	GC	MS
Wang et al.	Water	VOC	Purge and trap	GC	FID/ECD
Hino et al.	Water	VOC	Purge and trap	GC	FID
Hattori et al.	Whole blood	Xylenes	HS	GC	FID
Schroers et al.	Blood	VOC	HS	GC	PID/FID/ECD
Ramey et al.	Blood	VOC	HS	GC	FID/ECD
Alegretti et al.	Blood	BTEX	SPME	GC	FID
Ketola et al.	Water	VOC	HS/P&T/MIMS	GC	FID/MS
Sarowha et al.	Oil	Aromatics	N/A	HPLC	UV
Padlo et al.	Coal liquids	Aromatics	N/A	NP-HPLC	UV-DAD/ELSD
Akhlaq	Crude oil	Aromatics	N/A	HPLC/GC	UV-RI/FID/TCD
Pasadakis et al.	Petroleum	Aromatics	N/A	HPLC	UV-DAD
Pal et al.	Petroleum	Aromatics	N/A	SFC-GC	MS/FID/UV-DAD
Llompart et al.	Soil	Volatile and semivolatiles	SPME	GC	MS
Wang et al.	Water	Hydrocarbons	SPME/HS	GC	FID/MS
Perbelli et al.	Blood/urine	BTEX	HS	GC	MS
Sakata et al.	Solvent mix	VOC	HS	GC	MS
Huybrechts et al.	Water	VOC	P&T	GC	MS
Frysiner et al.	N/A	BTEX and Gasoline	N/A	GCxGC	FID
Frysiner et al.	N/A	Diesel Fuel	N/A	GCxGC	MS
Bueno et al.	N/A	VOC and Diesel	N/A	GCxGC	FID
Micyus et al.	N/A	Aromatics	N/A	GCxGC	FID
Seeley et al.	N/A	Alkanes, ketones, alcohols	N/A	GCxGC	FID



Zoom

↓



**Figure 1.2:** This figure depicts the schematic of flow switching

## Chapter 3

### Methods for the Characterization of Jet Propellant-8: Vapor and Aerosol

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Gregg, S.D., Campbell, J.C., Fisher, J.V., and Bartlett, M.G. Accepted by *Biomedical Chromatography*. Reprinted here with permission of publisher, 11/08/2006

**Abstract:**

Jet Propellant-8 (JP-8) has been responsible for the majority of reported chemical exposures by the U.S. Department of Defense. Concerns related to human exposure to JP-8 are relatively new; therefore, there is a lack of literature data. Additionally, health effects related to the composition of the exposure have only recently been considered. Two major questions exist 1) What is the compositional difference between the aerosol and vapor portions of JP-8 under controlled conditions and 2) What is the most representative method to sample JP-8 aerosol and vapor. Thirty-seven standards, representing greater than 40% of the mass of JP-8, were used for characterization of the neat fuel, vapor and aerosol portions. JP-8 vapor samples at a concentration of  $1600 \text{ mg m}^{-3}$  were prepared in Tedlar bags. A portion of the vapor samples was adsorbed on charcoal, Tenax and custom mixed phase sorbents. These samples were then extracted using organic solvent and analyzed using gas chromatography/mass spectrometry. The vapor samples extracted from the sorbent tubes were directly compared to a vapor bag. The samples collected using Tenax sorbent tubes were found to be most representative of the composition of the vapor bags. In another set of experiments, aerosolized JP-8 was generated using a collision nebulizer. Aerosol samples were collected and the chemical composition was characterized. The entire aerosol distribution was collected on a glass filter, extracted into solvent, and analyzed by GC-MS. Finally, the composition of the vapor and aerosol was compared. The vapor was found to represent the lower molecular weight components of JP-8, while the aerosol was composed of higher molecular weight

components. Therefore, the vapor and aerosol should be treated as two discrete forms of exposure to JP-8.

**Key Words:** Gas Chromatograph-mass spectrometry; JP-8 hydrocarbon fuel; Aerosol composition; Vapor composition

**Introduction:**

Jet Propellant-8 (JP-8) is a kerosene-based fuel used universally by North Atlantic Treaty Organization (NATO) countries. Over 20 years it has replaced JP-4 as the main fuel of the military, because it is less volatile and has a higher flash point [1]. These characteristics make this fuel less likely to produce catastrophic events in crashes or during warfare. JP-8 is colorless and contains additives specific to its military use. JP-8 is formulated based on performance and therefore its composition varies from batch to batch. In general, the composition of JP-8 is 33-61% hydrocarbons, 12-22% aromatics (benzene, substituted benzenes, naphthalene, and substituted naphthalene), 10-45% alicyclic hydrocarbons (cycloalkanes), 0.5-5% olefins, and 0-0.3% sulfur-containing heterocyclics [2]. JP-8 is responsible for the majority of all chemical exposures reported for the U.S. Department of Defense personnel [3]. As a result, exposure to JP-8 has been studied with a wide variety of health effects having been noted. However, the composition and subsequent effects of the vapor and aerosol portions of JP-8 have not been considered in any of these studies.

The United States alone uses 609,550 barrels of jet fuel per year (without wartime consideration) [4]. Exposure to JP-8 is very common among military personnel, but uncommon in those not directly associated with military activity. Tu et al. conducted a study observing human exposure to JP-8 [5]. They examined breath, blood, and urine

samples from military workers in 6 areas including: fuel cell workers, crew chiefs, mechanics, fuel specialists, and incidental workers. Eighteen students from Johns Hopkins were also examined for this study in the same manner. These subjects came into contact with the vapor and aerosol portion of this fuel. Breath samples were taken before and after work, blood and urine samples were taken after work only. A neurocognitive test was given along with a questionnaire. In this study, they observed that the Johns Hopkins group showed significantly lower concentrations of aliphatic and aromatic hydrocarbons versus the military group. Little difference was seen pre and post work. This finding was expected because there was no direct contact with the aerosolized fuel. In the military group, fuel specialists showed the greatest exposure to JP-8. This study also found that smokers showed a significantly greater exposure to JP-8. In another study, Scandinavian fuel workers exposed to the aviation fuel Jet-A, which is similar to JP-8 but lacks the performance enhancing additives, reported fatigue, headache, dizziness, nausea, anxiety, vegetative hyperactivity, and attention span deficiency [6-8]. In another study, JP-8 exposed workers were shown to display subtle deficits in their ability to maintain balance [9].

In addition to the human studies mentioned above, there are many animal studies that have looked at the effect of exposure to vapor [10-12] and aerosolized JP-8 [13-19]. These studies find few if any effects from exposure to the vapor but note adverse effects from exposure to the aerosolized fuel at occupational levels. The findings of both the human and animal studies suggest that there is significantly greater risk from exposure to the aerosolized fuel than simply the vapor.

In collaboration with the Air Force Office for Scientific Research, our laboratory is involved in the development of a physiologically based pharmacokinetic model (PB/PK) for JP-8 that will be effective in describing and predicting plasma and tissue concentrations of JP-8 and many of its components in rodents and humans. A PB/PK model has been developed for decane (a component present in JP-8) [20]. The next and most important step in further model development is to characterize the vapor and aerosolized fuel, the two major forms of exposure.

JP-8 samples can be divided into three classifications. Neat JP-8 is the liquid fuel containing all of the components of kerosene and additional performance additives. The vapor represents the volatile fraction of JP-8. The aerosol is composed of small droplets and is believed to represent the higher molecular weight fraction of JP-8. Several methods have been developed to measure components of JP-8 from these types of samples [21]. Occupational exposure to JP-8 occurs through a number of routes including inhalation of the vapor and/or aerosol and dermal absorption of the aerosol and/or neat JP-8 [22, 23]. It is important to understand the difference between the aerosol and the vapor portions of JP-8 in order to know how to accurately incorporate these two routes of exposure into physiological models of human internal dosimetry.

A major issue in this type of analysis is the collection of vapor samples. The National Institute for Occupational Safety and Health (NIOSH) approves monitoring of vapor samples by their collection on an adsorbent, such as activated charcoal or Tenax [24]. These collected samples are then analyzed by thermal desorption or direct injection following liquid extraction. There are many types of adsorbents that can be

used for complex samples such as JP-8, but it is important to compare these extracted samples to unabsorbed vapor samples because part of the sample distribution may be lost during the collection and extraction process. Therefore, it is important to understand the sample collection process and its potential influence on the sample composition.

Neat JP-8, vapor, and aerosol samples were analyzed to determine the differences in their composition. The samples were characterized with respect to 37 standards that represent >40% of the weight of the fuel [25]. Several different adsorbents are used to trap the vapor samples. Statistical comparisons were made between the different adsorbents to find the one that had the least difference from unabsorbed vapor samples. This information can be used to aid in the construction of a more realistic physiologically based pharmacokinetic model to predict occupational and environmental exposure to JP-8.

### **Experimental Section:**

Experiments were carried out using an Agilent Technologies 6890N gas chromatograph directly connected to a model 5973 mass selective detector. This system was controlled using Chemstation version G1701DAD.01.00 software. A Petrocol DH 150 column with dimensions 150m x .25mm I.D. x 1.0 $\mu$ m film was used for this separation. The initial oven temperature program was as follows: hold at 90 $^{\circ}$ C for 30 min followed by a ramp to 210 $^{\circ}$ C over 303 min. For liquid samples, 3  $\mu$ L was introduced into the GC for analysis. Helium (National Specialty Gas, Durham, N.C., USA, Ultra Carrier Grade) was used as the carrier gas. The instrument was run in split mode with a ratio of 3:1. The total flow of gas was 6.7 mL min $^{-1}$ . All analysis was done

using electron ionization (EI) in the positive ion mode with a filament voltage of 70 eV. Mass spectrometric conditions were as follows: the source temperature was set to 230 °C, and the instrument was operated in full scan mode low mass 35.0 to high mass 400.0 at a scan rate of 5.87scans second<sup>-1</sup> [26]. All chemicals used were analytical grade.

Five samples of neat JP-8 (Wright-Patterson Air Force Base, Dayton, OH, USA) were run for characterization. A concentration of 2µL mL<sup>-1</sup> (1.6 mg mL<sup>-1</sup>) of JP-8 in chloroform (EMD Chemicals, Gibbstown, NJ, USA) was placed into a vial for analysis by the GC-MS system. Analysis of the neat fuel was needed to compare all subsequent analysis done with the vapor and aerosol portions. After the completion of the GC-MS sequence, samples were characterized using retention time matches with 37 authentic standards and comparison to the Wiley EI library (version G1035).

A generated vapor bag, charcoal tubes (SKC Inc., Eighty Four, PA, USA), Tenax tubes (SKC Inc., Eighty Four, PA, USA) and custom tubes (Supelco, Bellefonte, PA, USA) were used to analyze the vapor phase of JP-8. To make the vapor bags, 4L of air were pumped into a 5L Tedlar bag (SKC Inc., Eighty Four, PA, USA). After the bag was completely filled with air, 8 µL of neat JP-8 was injected into a septum located on the bag. The bag was then heated for 5 min using a heat gun at 197°C, allowed to cool and then reheated. The final concentration of the bag was approximately 1600 mg m<sup>-3</sup>. After the bag returned to ambient temperature, 1 mL of vapor was extracted from the bag using a gas tight syringe and directly injected into the GC-MS system for analysis. This process was repeated using five different bags to allow for statistical comparisons.

Experiments were also conducted to look at the effects of heating the bag versus allowing the vapor phase to arrive at equilibrium without any heating.

Using the above procedure, vapor bags were made to examine components adsorbed to Tenax, charcoal, and custom made mixed phase charcoal sorbent tubes. Each collection tube was attached to the vapor bag. A low flow rate vacuum pump (SKC Inc., Eighty Four, PA, USA, Model # 210-2002) attached with Tygon tubing was used to pull approximately 500 ml of vapor from the tedlar bag through each sorbent tube at a flow rate of  $100 \text{ cm}^3 \text{ min}^{-1}$  for 5 min. The main chamber of each sorbent tube was removed and extracted for 1h using 1 ml chloroform for charcoal adsorbents or methyl tertiary butyl ether- MTBE (Aldrich, Milwaukee, WI, USA) for Tenax adsorbents. No mechanical agitation or heating was used to assist the extraction process.

Two milliliters of chloroform was used to extract the vapor from the custom made tubes because the packing for the tube was twice the mass of the other tubes, one milliliter was therefore, not sufficient for extraction. When adjusted for dilution, the custom sorbent tubes did not show a reduction in concentration when compared to the other sorbent tubes. After desorption, 100 $\mu\text{L}$  of the extracting solvent was placed into sample vials containing an insert for small sample volumes and 3 $\mu\text{L}$  was injected into the GC-MS system. This entire process was repeated 5 times for each type of sorbent tube ( $n=5$ ). Schematics of the various sorbent tubes used for the sample collections are shown in Fig. 2.1. The sorbent tubes contained additional chamber(s) to determine sample breakthrough. No breakthrough from the first sorbent chamber was noted for any of the tubes in this study.

The aerosolized fuel was generated using a collision nebulizer (BGI, Inc Waltham, MA, USA) (Shown in Fig. 2.2) [27, 28]. 50 ml of JP-8 was placed into the nebulizer jar. Compressed air was used at a flow rate of  $12\text{L min}^{-1}$  to generate an aerosol. The aerosolized fuel was pulled through a glass fiber filter followed in series by a sorbent tube using the low flow rate vacuum pump set to  $200\text{ mL min}^{-1}$ . The sampling time was 15 min. The JP-8 was extracted by placing the glass fiber filter in 5 mL of chloroform for 1h. The sorbent tubes were extracted as described above.  $100\mu\text{L}$  of the solvent was transferred into vial inserts and analyzed using the GC-MS system. The process was repeated 5 times ( $n=5$ ).

Recovery studies were performed for all of the sorbent tubes being used. Concentrations of 2, 25, and  $100\mu\text{g mL}^{-1}$  of JP-8 in chloroform were examined. The main chamber of each tube was removed and spiked with  $10\ \mu\text{L}$  of the working standard and desorbed in 1mL chloroform (charcoal tube), 2 mL chloroform (custom charcoal tube), and 1mL MTBE (Tenax tube) for 1h.  $10\ \mu\text{L}$  of the working standard were also spiked into blank solvent for comparison. Chloroform and MTBE were used as stated above.  $3\ \mu\text{L}$  of each sample was injected into the GC-MS system for analysis. The recoveries were run in replicates of five ( $n=5$ ) for each sorbent tube and blank. To determine the recovery, the area of each compound identified was compared to the area of the spiked solvent blank. The results of the recovery study were used to determine the tube that provided the highest recovery and that best represented the vapor composition of JP-8.

Statistical comparisons were made using one-way analysis of variance (ANOVA) on the peak area ratios between two sets of data to determine if differences observed

between the groups could be explained by the variance in the data. If the F score showed that the differences between the two groups was not explained by the variance in the data, a Tukey's pairwise comparison was conducted to determine which component(s) contributed to the observed differences. All of the statistical tests were performed at the 99% level of confidence using SAS/STAT (SAS Institute Inc., Cary, NC).

### **Results and Discussion:**

Upon analysis of a solution of  $1.6 \text{ mg mL}^{-1}$  of neat JP-8 in chloroform, 34 of the 37 standards were identified above the limit of detection (S/N of 3/1) of  $1 \text{ ug mL}^{-1}$  for the individual components. The three compounds that were below this threshold were octadecane, 3,4,5- trimethylheptane, and indene. The compounds 3,4,5- trimethylheptane and indene were not observed in any experiments involving the diluted fuel, vapor or aerosolized JP-8. The other 35 components were identified in the vapor, the aerosol or both. Table 2.1 lists the 37 standard compounds as well as their respective retention times, molecular weights and vapor pressures.

The vapor and aerosolized JP-8 concentrations selected for these studies were  $1600 \text{ mg m}^{-3}$ . Acute neurological effects have been observed in humans exposed to as little as  $250 \text{ mg m}^{-3}$  of aircraft fuel (not necessarily JP-8) [6-8]. Dopamine levels have been shown to decrease leading to behavior modification in rats when exposed to JP-8 vapor at  $1000 \text{ mg m}^{-3}$  [11, 12]. Lung toxicity has been observed in rats exposed to aerosolized JP-8 above  $520 \text{ mg m}^{-3}$  and in mice to exposures above  $2800 \text{ mg m}^{-3}$  [18, 29]. Therefore, the concentration chosen for these studies was comparable to those

used in many animal studies and was at a level that would be expected to cause acute toxicity in humans.

One of the main goals for this part of the study was to identify the compositional differences between the aerosol and vapor portions of JP-8. In the aerosol generation system that was used in the study only 5-10% of the fuel was present as aerosol droplets. Therefore, it was difficult to directly compare the vapor and aerosol samples because of differences in their concentrations. Therefore, all of the peak area data was normalized to undecane. Undecane provided the largest peak area for almost all of the JP-8 samples (vapor, aerosol, neat fuel).

In studies involving aerosol and vapor sampling it is important to assure that the vapor samples are not significantly impacted by the presence of aerosol and vice versa. We first studied the adsorption of JP-8 vapor on the glass fiber filter. Using a vapor bag, we pulled 500 ml of 1600 mg/m<sup>3</sup> JP-8 vapor sample through the glass fiber filter (n=3). After solvent extraction, we did not observe any JP-8 components on the glass fiber filter. We were also concerned about the aerosol samples contributing to the signals obtained from the vapor traps. We therefore, compared the results of the vapor samples generated from the vapor bags to those generated from the aerosolized fuel and found no statistical difference in the samples. Therefore, over the 15 minutes that we collect samples we do not observe any evidence that the aerosol particle samples are impacting the vapor samples.

**Aerosol:** 29 of the 37 standard compounds were identified in this portion of JP-8. It was hypothesized that the aerosol samples would not contain some of the lower molecular weight components (i.e. heptane, methylcyclohexane), but would contain

most of the heavier compounds (i.e. octadecane). This expectation was confirmed. The aerosol was missing the following lower molecular weight components seen in the vapor as well as the neat fuel: mesitylene, methylcyclohexane, and 1,3-dimethylcyclohexane. The aerosol also contained a detectable amount of the high molecular weight component octadecane. The aerosol also contained 2-methylnonane, another higher molecular weight component not observed in the vapor.

The aerosol was first compared to the neat fuel. It was unclear whether there would be much difference between the aerosol and the neat fuel. However, it was hypothesized that these small droplets with high surface area would preferentially evaporate the more volatile components of JP-8 causing differences in composition. When compared using the statistical approach described above, several components present in both samples showed a significant difference in their means. The components octane (21%), nonane (55%), phenylcyclohexane (84%), and ethylbenzene (25%), were all significantly lower in the aerosol sample relative to the neat fuel. It was also noted that heptane was absent from the aerosol sample but was observed in the neat fuel. All other components that were present in both the neat fuel and aerosol were statistically indistinguishable when compared. In general, the components that were reduced have higher vapor pressures and lower molecular weights relative to other components of JP-8. However, there was a notable exception. Phenylcyclohexane was much higher in molecular weight and, while it does not have a reported vapor pressure, the fact that it elutes 70 min after the other compounds that were reduced in concentration demonstrated that its vapor pressure was likely much lower. It was unclear why this particular compound was so different. The following

compounds showed enrichment in the aerosol relative to the neat fuel, 1,4 diethylbenzene (463%), 1-methylnaphthalene (164%), 2-methylnaphthalene (194%), naphthalene (183%), 1,2,3,4 tetrahydronaphthalene (156%), dodecane (113%), tridecane (124%), tetradecane(140%), pentadecane (141%), hexadecane(181%), and heptadecane(177%). The higher levels of the above compounds in the aerosol suggest that these compounds may be involved in the greater toxicity noted from exposure to the aerosolized fuel relative to the vapor.

**Vapor:** Studies of the vapor involved two basic questions. First what, if any, were the compositional differences between the neat fuel and the vapor? Second, what is the most appropriate method to collect a vapor sample of JP-8? To address this first question we prepared a vapor bag as described previously. Of the 37 standards, 28 were identified in the vapor bag sample (See Table 2.2). The vapor bag did not contain a detectable amount of octadecane. In addition, six components that were observed in neat JP-8 were not identified in the vapor bag, these included heptane, 1,2-dimethylnaphthalene, 2-methylnonane, 2,3,5-trimethylnaphthalene, heptadecane and cumene. From the statistical comparison of the neat fuel to the vapor bag it was determined that phenylcyclohexane (70%), 1,4 diethylbenzene (411%), 2-methyldecane (350%), propylbenzene (355%), pseudocumene (151%), 1,2,3,4 tetrahydronaphthalene (124%), naphthalene (190%), propylcyclohexane (144%), octane (135%), nonane (121%), decane (121%), dodecane (89%), tridecane (80%), tetradecane (74%), pentadecane (83%), and hexadecane (13%) were the components present in both samples that showed significant difference when comparing the normalized peak areas.

These compounds represent a wide range of molecular weights and volatility. Heptane and cumene had low abundance in the neat fuel and their absence was likely a reflection of the lower overall concentration of the vapor sample relative to the neat fuel. However, the higher molecular weight compounds were believed to demonstrate discrimination against these species from the evaporation process. These findings were consistent with the earlier findings from ExxonMobile that several higher molecular weight components of JP-8 were decreased in vapor samples [29].

We also investigated two different methods to generate JP-8 vapor bags. In one method the vapor bags were allowed to reach equilibrium by heating followed by allowing the vapor bag to return to room temperature. This bag was then sampled using a gas tight syringe. Secondly, to insure that we were not forcing less volatile compounds into the vapor by heating the vapor bag, we allowed another set of vapor bags to arrive at equilibrium at ambient temperature for 24 hours. Using the statistical approach described above, the two methods for creating vapor bags were found to be equivalent. Therefore, we used the heated vapor bags for all experiments because these could be prepared in approximately an hour.

A final set of experiments were done to determine the most representative method to sample JP-8 vapor. Three different sorbent tubes were evaluated for their ability to retain and then allow the components to be extracted using solvents. The three types of sorbent materials were charcoal, Tenax and a customized mixed phase. Each of the sorbents was compared to determine which of the tubes most resembled samples taken directly from the vapor bag.

The first sorbent tube characterized was the charcoal tube. Of the 28 components present in the vapor bag, the charcoal tube contained 21. The charcoal tube was missing several of the higher end components including: pentadecane, hexadecane, heptadecane, octadecane, 1-methylnonane, 2-methylnonane, 1,2-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene. The charcoal tube was also missing the following lower molecular weight components: heptane, xylene, mesitylene, and 1,2,3-trimethylbenzene. Of these only heptane was not found in the vapor bag. When the 21 detected compounds were compared statistically with the vapor bag, tridecane (31%), naphthalene (19%), ethylbenzene (85%), 1,4 diethylbenzene (58%), phenylcyclohexane (980%), and nonane (31%) were different. The charcoal tube had significant differences when compared to the vapor bag. Higher molecular weight compounds were lower in abundance or were missing indicating that these compounds may not be efficiently desorbed from the charcoal tubes.

The second vapor trap to be analyzed was the custom sorbent tube. Of the 28 components present in the vapor bag, the custom tube contained 24, about 85.7% of the components. Like the charcoal tube, several of the higher molecular weight components were absent. These included: pentadecane, hexadecane, heptadecane, octadecane, 1-methylnonane, 2-methylnonane, 1,2-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene. These components were also not identified in the charcoal tube. Heptane, xylene, and mesitylene were observed in the custom tube, but not in the charcoal tube. Therefore, the custom tube appears to provide better representation of the vapor samples for the lower molecular weight components versus the charcoal sorbent tubes.

Next the custom tube was statistically compared to the vapor bag. When compared to the vapor bag, octane (213%) and methylcyclohexane (309%) showed a significant difference in their mean areas. These two components were 2-3 times higher in concentration in vapor samples collected on the custom sorbent tubes relative to the vapor bag. These are two of the most volatile components in JP-8. These findings, along with the generally higher relative levels of the lower molecular weight compounds, indicate that the custom sorbent tubes may actually be over representing these components.

The last sorbent tube to be analyzed was the Tenax tube. Of the 28 components present in the vapor bag, 26 were identified in the Tenax tube. Like the previous tubes, several higher end components were absent during analysis: hexadecane, heptadecane, octadecane, 1,2-dimethylnaphthalene, and 2,3,5-trimethylnaphthalene. Lower end components absent included: heptane and 1,3-dimethylcyclohexane. 3 components present in the Tenax tube were not seen in the vapor bag. When compared to the vapor bag, only phenylcyclohexane (38%) was found to show a statistically significant difference in mean area. A side-by-side view of the sorbent tubes and the vapor bag can be seen in Table 2.2.

To determine which sorbent tube most accurately represented the vapor bag standard, we used two factors 1) the intensities of components that were observed and 2) the number of identified components present in the vapor bag versus the various sorbent tubes. Using these criteria we concluded that the Tenax tube provided the best representation of the vapor bag compared to the carbon based sorbent tubes. Fig. 2.3 shows the superimposed gas chromatograms showing vapor, aerosol, neat fuel and a

comparison of the Tenax sorbent tube versus the vapor bag used for collection. Fig. 2.4 shows the amount of each compound present in JP-8.

**Aerosol versus Vapor:** Since exposure to aerosolized JP-8 appears to cause greater health effects in human, we felt that it was important to compare the vapor and the aerosol portions of JP-8. There were four compounds that were observed in the aerosol that were not observed in the vapor samples. These were cumene, 2-methylnonane, heptadecane and octadecane. In addition seven compounds were found to be statistically lower in the vapor bag relative to the aerosol, 1-methylnaphthalene (42%), 2-methylnaphthalene (53%), dodecane (79%), tridecane (64%), tetradecane (53%), pentadecane (59%), and hexadecane (7%). Overall the compounds that are lowered or missing were higher molecular weight components with the exception of cumene. Cumene was a low abundance component of JP-8 and was likely observed in the aerosol because of higher overall concentrations for these samples. The aerosol samples were missing three compounds relative to the vapor samples. These were mesitylene, methylcyclohexane and 1,3-dimethylcyclohexane. In addition, octane (16%), nonane (45%), pseudocumene (68%), propylbenzene (27%), propylcyclohexane (53%), and 2-methyldecane (36%) were observed in statistically lower abundances in aerosol samples. In this case all of the compounds that were missing or decreased in abundance were the lower molecular weight compounds.

The aerosol should be viewed as a liquid suspension. This reasoning may explain why some compounds were observed in the aerosol portion and not the vapor portion of JP-8. As a result of being a suspension, heavier compounds tended to be present in higher concentrations for longer periods of time. In the vapor portion, these

heavier components were present for shorter periods of time making the relative abundance of lighter weight compounds more pronounced.

## **Recovery**

Recovery studies were performed for each of the sorbent tubes used to analyze JP-8 vapor. Data obtained from the charcoal tube revealed that recovery ranged from 19-118% with 2-ethyltoluene, pseudocumene, naphthalene, ethylbenzene, and propylbenzene yielding recovery percentages of <60%. The Tenax tube recovery ranged from 72-112%. All components had recovery percentages of >90% with the exception of naphthalene and 1-methylnaphthalene (seen in the lowest concentration). The custom tube recovery ranged from 40-107%. This tube also showed slightly lower recovery percentages for the aromatic compounds <72%. The Tenax tubes showed the greatest recovery for all compounds. Results can be seen in Table 2.3.

## **Conclusions**

Both JP-8 vapor and aerosol samples were analyzed and compared with neat fuel to determine if these sample types were unique. GC-MS analysis followed by statistical analysis of the data showed that these two types of samples were unique. The aerosol contained a greater abundance of higher molecular weight compounds, while the vapor was found to contain a greater abundance of lower molecular weight compounds. Comparison of three different types of sorbent tubes revealed that vapor samples trapped and extracted from Tenax were most representative of the vapor and therefore, this sorbent is recommended for sampling JP-8 atmospheres. The glass fiber filters were sufficient in the collection of the aerosol samples.

The knowledge of the composition of JP-8's three major forms will aid in the design of more realistic exposure models for this fuel. Due to these differences, the vapor and the aerosol should be treated as discrete exposure routes in future models of toxicity. Finally, the increased abundances of several higher molecular weight components in the aerosol samples present a possible explanation for increased health effects noted for exposure to aerosolized JP-8 when compared to vapor exposures.

### **Acknowledgements**

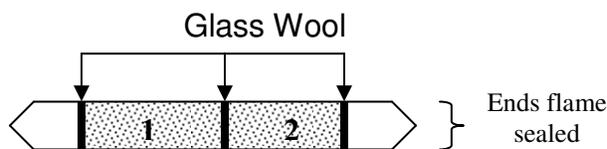
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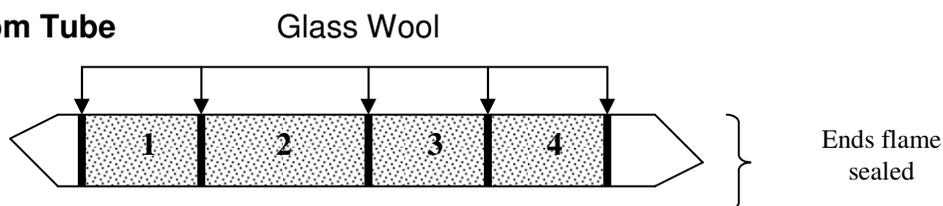
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### A. Tenax Tube



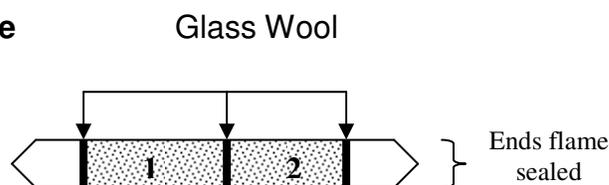
- 1: 100 mg 60/80 mesh Tenax TA
- 2: 50 mg 60/80 mesh Tenax TACarboxen-1018

### B. Custom Tube



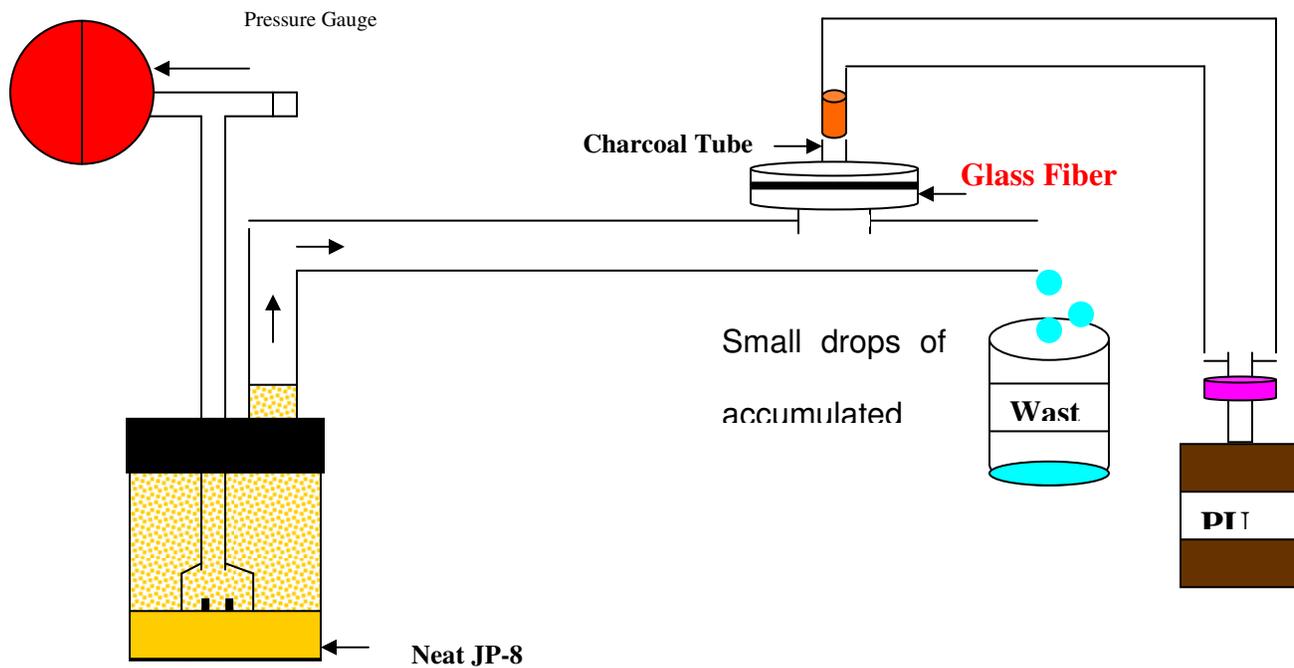
- 1: 150 mg 60/80 mesh Carbopack X
- 2: 150 mg 60/80 mesh Carboxen-1018
- 3: 150 mg 60/80 mesh Carboxen-1021
- 4: 150 mg 60/80 mesh Carboxen-1021

### C. Charcoal Tube



- 1: 100 mg Anasorb CSC
- 2: 50 mg Anasorb CSC

**Figure 2.1:** Depiction of the vapor traps used to characterize the vapor phase of JP-8. Traps A and C have two distinctive chambers. Chamber 1 is the sorbent layer, this is the chamber analyzed in this study. Chamber 2 is the backup sorbent layer. This chamber served to detect any breakthrough that may occur during sample collection. Little breakthrough occurred using our sampling method because of the low concentration and small sampling window. Tube B was a tube custom made. This tube consists of 2 sorbent layers and 2 backup sorbent layers.



**Figure 2.2:** Schematic showing the apparatus used to generate aerosol samples. Using compressed air at a flow of 30 psig, JP-8 was nebulized and traveled through a stainless steel tube. JP-8 was then pulled through the glass fiber filter and through the adsorbent tube for analysis.

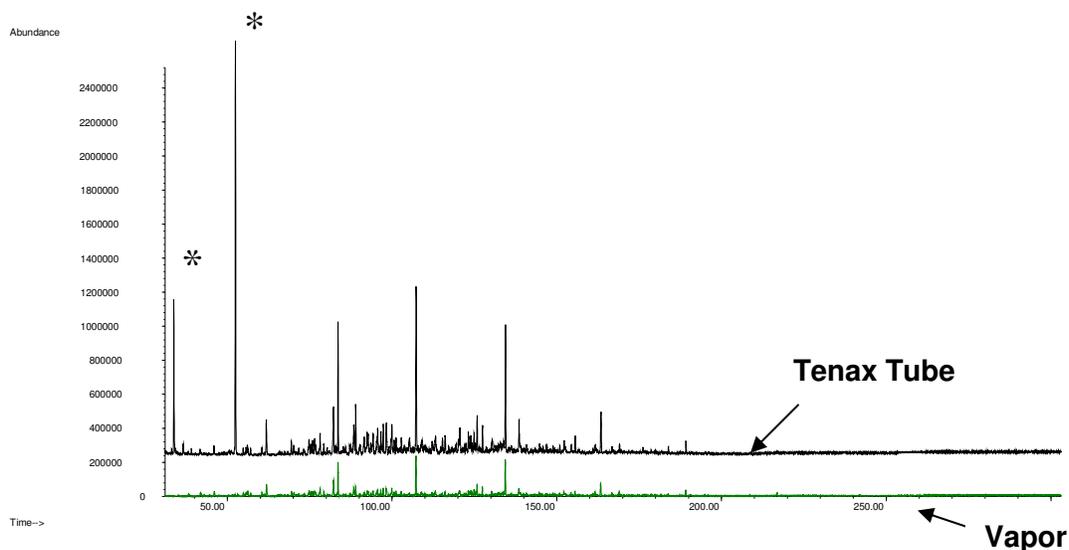
**Table 2.1:** Identity of components and gas chromatographic

retention times from JP-8.

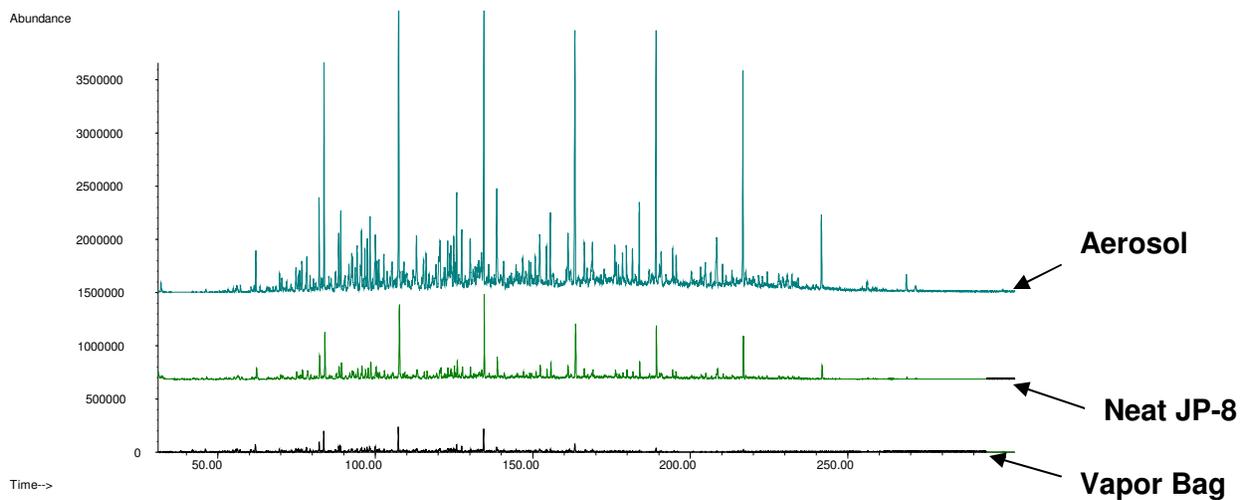
Compounds	Retention Time (min)	Molecular Weight (g)	Vapor Pressure (mmHg)
Heptane	35.44	100.20	40 @ 20 °C
Octane	46.50	114.23	11 @ 20 °C
Nonane	62.39	128.26	10 @ 20 °C
Decane	84.00	142.28	1 @ 16.5 °C
Undecane	107.70	156.31	1 @ 33 °C
Dodecane	134.64	170.34	1 @ 47.8 °C
Tridecane	163.61	184.36	1 @ 59.4 °C
Tetradecane	189.31	198.39	1 @ 76.4 °C
Pentadecane	216.91	212.42	1 @ 92 °C
Hexadecane	241.90	226.44	1 @ 105.3 °C
Heptadecane	268.81	240.47	1 @ 115 °C
Octadecane	299.44	254.50	1 @ 119 °C
Xylene	60.84	106.17	8.6 @ 25 °C
2-Ethyltoluene	79.56	120.19	8 @ 20 °C
Mesitylene	76.97	120.19	2.49 @ 25 °C
Pseudocumene	82.41	120.19	7 @ 44.4 °C
1,2,3 Trimethylbenzene	88.51	120.19	N/A
Naphthalene	130.22	128.17	.05 @ 20 °C
1-methylnaphthalene	166.47	142.2	N/A
2-methylnaphthalene	161.32	142.2	1 @ 61 °C
1,2-Dimethylnaphthalene	204.94	156.23	N/A
Ethylbenzene	55.21	106.17	10 @ 20 °C
Propylbenzene	73.54	120.19	2.5 @ 20 °C
Methylcyclohexane	38.95	98.19	37 @ 20 °C
1,3 Dimethylcyclohexane	44.95	112.21	N/A
Propylcyclohexane	70.63	126.24	8.7 @ 37.7 °C
1,4-Diethylbenzene	95.47	134.22	N/A
2-Methylnonane	76.95	142.28	N/A
2-methyldecane	98.57	156.31	N/A
3-methyldecane	100.32	156.31	N/A
4-methyldecane	97.73	156.31	N/A
3,4,5-trimethylheptane*	74.44	142.28	N/A
2,3,5-trimethylnaphthalene	232.81	170.25	N/A
1,2,3,4 THN	125.20	132.20	.18 @ 20 °C
Indene*	93.06	116.16	N/A
Phenylcyclohexane	169.56	160.26	N/A
Cumene	66.90	120.19	8 @ 20 °C

**Table 2.2:** Comparison of components observed and absent from the various vapor traps used to characterize the vapor phase of JP-8. Components in bold were not seen in the analysis.

Tenax	Charcoal	Custom	Vapor Bag
<b>Heptane</b>	<b>Heptane</b>	Heptane	<b>Heptane</b>
Octane	Octane	Octane	Octane
Decane	Decane	Decane	Decane
Undecane	Undecane	Undecane	Undecane
Dodecane	Dodecane	Dodecane	Dodecane
Tridecane	Tridecane	Tridecane	Tridecane
Tetradecane	Tetradecane	Tetradecane	Tetradecane
Pentadecane	<b>Pentadecane</b>	<b>Pentadecane</b>	Pentadecane
<b>Hexadecane</b>	<b>Hexadecane</b>	<b>Hexadecane</b>	Hexadecane
<b>Heptadecane</b>	<b>Heptadecane</b>	<b>Heptadecane</b>	<b>Heptadecane</b>
<b>Octadecane</b>	<b>Octadecane</b>	<b>Octadecane</b>	<b>Octadecane</b>
Xylene	<b>Xylene</b>	Xylene	Xylene
2-Ethyltoluene	2-Ethyltoluene	2-Ethyltoluene	2-Ethyltoluene
Mesitylene	<b>Mesitylene</b>	Mesitylene	Mesitylene
Pseudocumene	Pseudocumene	Pseudocumene	Pseudocumene
1,2,3 TMB	<b>1,2,3 TMB</b>	1,2,3 TMB	1,2,3 TMB
Naphthalene	Naphthalene	Naphthalene	Naphthalene
1-methylnaphthalene	<b>1-methylnaphthalene</b>	<b>1-methylnaphthalene</b>	1-methylnaphthalene
2-methylnaphthalene	<b>2-methylnaphthalene</b>	<b>2-methylnaphthalene</b>	2-methylnaphthalene
<b>1,2-DMN</b>	<b>1,2-DMN</b>	<b>1,2-DMN</b>	<b>1,2-DMN</b>
Ethylbenzene	Ethylbenzene	Ethylbenzene	Ethylbenzene
Propylbenzene	Propylbenzene	Propylbenzene	Propylbenzene
Methylcyclohexane	Methylcyclohexane	Methylcyclohexane	Methylcyclohexane
<b>1,3Dimethylcyclohex</b>	1,3Dimethylcyclohex	1,3Dimethylcyclohex	1,3Dimethylcyclohex
Propylcyclohexane	Propylcyclohexane	Propylcyclohexane	Propylcyclohexane
1,4-Diethylbenzene	1,4-Diethylbenzene	1,4-Diethylbenzene	1,4-Diethylbenzene
2-Methylnonane	2-Methylnonane	2-Methylnonane	<b>2-Methylnonane</b>
2-methyldecane	2-methyldecane	2-methyldecane	2-methyldecane
3-methyldecane	3-methyldecane	3-methyldecane	3-methyldecane
4-methyldecane	4-methyldecane	4-methyldecane	4-methyldecane
<b>2,3,5-trimethylnap</b>	<b>2,3,5-trimethylnap</b>	<b>2,3,5-trimethylnap</b>	<b>2,3,5-trimethylnap</b>
1,2,3,4 tetrahydron	1,2,3,4 tetrahydron	1,2,3,4 tetrahydron	1,2,3,4 tetrahydron
Phenylcyclohexane	Phenylcyclohexane	Phenylcyclohexane	Phenylcyclohexane
Cumene	Cumene	Cumene	<b>Cumene</b>
Nonane	Nonane	Nonane	Nonane



B. Superimposed total ion chromatograms from the three phases of JP-8. The aerosol was the most abundant followed by the neat JP-8 and lastly by the vapor bag.



**Figure 2.3**

A. Total ion chromatogram demonstrating that the Tenax tube is lacking the higher end components. After about 225 minutes no additional peaks were observed. In the Tenax tube extraneous peaks (\*) are seen due to impurities in the extracting solvent used (MTBE), these were acetic acid and 2 pentanone. The Tenax tube was determined to be most like the vapor standard.

**Table 2.3:** Recovery results shown for charcoal, custom and Tenax sorbent tubes. ND=Not Detected

Compound	Charoocal Tube			Custom Tube			Tenax tube		
	2 µg/ml	25 µg/ml	100 µg/ml	2 µg/ml	25 µg/ml	100 µg/ml	2 µg/ml	25 µg/ml	100 µg/ml
Heptane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Octane	86%	94%	105%	80%	87%	87%	105%	95%	102%
Nonane	107%	96%	100%	82%	91%	89%	104%	101%	102%
Decane	110%	92%	97%	84%	92%	89%	101%	99%	102%
Undecane	111%	90%	95%	84%	93%	91%	96%	100%	103%
Dodecane	110%	85%	92%	86%	94%	91%	96%	100%	102%
Tridecane	107%	79%	87%	85%	93%	90%	99%	101%	102%
Tetradecane	106%	71%	80%	85%	93%	89%	99%	101%	102%
Pentadecane	ND	ND	ND	ND	ND	ND	94%	101%	102%
Hexadecane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptadecane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Octadecane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Xylene	ND	39%	32%	40%	45%	48%	107%	97%	100%
2-Ethyltoluene	79%	34%	44%	66%	81%	74%	100%	100%	105%
Mesitylene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pseudocumene	81%	43%	49%	72%	71%	58%	95%	97%	100%
1,2,3 Trimethylbenzene	ND	ND	ND	77%	86%	86%	91%	98%	101%
Naphthalene	98%	57%	66%	ND	71%	71%	85%	100%	100%
1-methylnaphthalene	ND	ND	ND	ND	ND	ND	72%	98%	98%
2-methylnaphthalene	ND	ND	ND	ND	ND	ND	97%	97%	100%
1,2-Dimethylnaphthalene	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethylbenzene	103%	19%	32%	ND	ND	ND	ND	ND	ND
Propylbenzene	ND	55%	66%	ND	68%	71%	95%	97%	102%
Methylcyclohexane	ND	103%	108%	67%	100%	94%	91%	98%	109%
1,3 Dimethylcyclohexane	ND	98%	108%	ND	ND	ND	ND	ND	ND
Propylcyclohexane	110%	101%	103%	99%	104%	98%	101%	99%	101%
1,4-Diethylbenzene	ND	74%	84%	78%	96%	92%	90%	99%	100%
2-Methylnonane	118%	85%	85%	85%	95%	92%	101%	98%	101%
2-methyldecane	107%	74%	79%	91%	93%	91%	112%	100%	102%
3-methyldecane	105%	99%	103%	95%	104%	98%	103%	102%	103%
4-methyldecane	116%	101%	102%	107%	105%	98%	99%	101%	101%
1,2,3,4 tetrahydronaphthalene	116%	52%	61%	ND	84%	85%	92%	94%	98%
Phenylcyclohexane	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cumene	ND	ND	ND	ND	ND	ND	ND	ND	ND

## Chapter 4

Determination of Twelve Major Components of Jet Propellant 8 from Rat Blood and Liver by Solid Phase Microextraction Gas Chromatography Mass Spectrometry

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## **Abstract**

JP-8 is a complex mixture of hydrocarbons and various performance enhancing additives that is used as the universal fuel for North Atlantic Treaty Organization military forces. The complex nature of this mixture makes the analysis of JP-8 a challenge. In order to address health concerns resulting from exposure to this mixture, the development of sensitive and selective methods are needed. In this study we have developed and validated a method for assessing twelve major components of JP-8 from rat liver and whole blood. These twelve components represent a sampling of the major classes that compose JP-8. The method was validated according to precision, accuracy, recovery, and stability. The linear range for blood was 2-200 ng/ml and liver homogenate was 1-100 ng/ml. Precision (% RSD) and accuracy (% Bias) were less than 20%. The recovery ranged from 5%-98% in blood and 4%-98% for liver and was highly correlated to the boiling point of the component. This method was then applied to an animal study involving JP-8 exposure allowing an assessment of partition coefficients, bioavailability and basic toxicokinetic parameters for these components.

## **Introduction**

Jet Propellant-8 (JP-8) is a kerosene-based fuel used universally by North Atlantic Treaty Organization (NATO) countries. JP-8 is responsible for the majority of all chemical exposures reported for U.S. Department of Defense personnel.<sup>1</sup> This fuel is of major interest because exposure to JP-8 has resulted

in a wide variety of health effects. Like other chemicals the effect of exposure is dependent on the length of exposure, concentration of the exposure, route of exposure (dermal, oral, inhalation) and physical form of the exposure (liquid, vapor or aerosol).

Since JP-8 has been named the principle fuel for NATO countries, limited studies on the effects of this fuel on humans<sup>2,3</sup> and marine life<sup>4-6</sup> have been conducted. Most of these studies are descriptive in nature but there is significant evidence to link JP-8 exposure to effects including: carcinogenicity,<sup>7-9</sup> neuropathy,<sup>10</sup> death,<sup>11</sup> and immunotoxicity.<sup>12-15</sup> However, there are no studies that have evaluated the concentrations of more than a few individual components during an exposure.

JP-8 is a complex mixture of compounds consisting mainly of linear and branched alkanes and alkenes along with various aromatic hydrocarbons. JP-8 is manufactured to performance specifications and therefore, there is significant variation in the composition of the individual components from batch to batch. Each individual component of JP-8 is present in very low concentrations which presents a challenge for many methods. To date, sample preparation techniques including: purge and trap,<sup>16-19</sup> solid phase microextraction (SPME),<sup>3, 20, 21</sup> liquid-liquid extraction (LLE),<sup>22, 23</sup> and headspace<sup>24-26</sup> have been used. Detectors have included: mass spectrometry,<sup>27-29</sup> flame ionization,<sup>30-32</sup> and photoionization detection.<sup>33</sup> Gas chromatography (GC) has been used almost exclusively as the separation technique for the analysis of JP-8 due to the volatility of the individual components. To date, there are no methods providing validation data

demonstrating sensitivity, linearity, precision, accuracy and recovery to determine the concentrations of individual components from a biological sample. In order to correlate the concentrations of individual components of JP-8 to observed health effects, it is necessary to have a well defined analytical method that measures components that represent the diversity of JP-8. Based on previous studies, we have identified 12 representative components of JP-8.<sup>36</sup> These compounds include: nonane, decane, undecane, dodecane, tridecane, tetradecane, toluene, o-xylene, ethylbenzene, naphthalene, 2-methylnaphthalene, and pseudocumene. These components were chosen based on their overall abundance in JP-8 (approximately 20% of the mass) and previous health effects data.

In this study, we have used solid phase microextraction (SPME) with gas chromatography employing an ion trap mass spectrometer for detection. The methods were validated in accordance with current US Food and Drug Administration guidance<sup>37</sup>. We chose to analyze these compounds in rat whole blood to determine toxicokinetic parameters. The liver was selected because of its role in cancer and because it provides information related to the general tissue distribution of highly perfused organs.

## **Experimental**

Samples were analyzed using a Varian CP-3800 Gas Chromatograph (GC) with a 2000 series Ion Trap Mass Spectrometer (MS) equipped with a Combi Pal autosampler (Varian Inc., Palo Alto, CA). The mass spectrometer was operated in full scan mode from  $m/z$  45 to 260. Helium was used as the GC carrier gas and electron ionization at 70 eV was used to create ions.

For efficient separation, a HP-1 (50m x 200 $\mu$ m x .5 $\mu$ m) GC column was used. The flow rate was held constant at 1ml/min with a split ratio of 20. The oven temperature program was identical for both matrices. The initial oven temperature was 35 $^{\circ}$ C, which was held for 8 minutes, then increased to 230  $^{\circ}$ C at a rate of 4  $^{\circ}$ C/min and held for 7 minutes. The oven was then increased to 250  $^{\circ}$ C at a rate of 20  $^{\circ}$ C/min and was held for another 3 minutes. The total run time for a sample was 67.75 minutes.

Solid phase microextraction was used for extraction and introduction of the samples to the GC column. Blood and liver samples were incubated for 30 minutes at a temperature of 65 $^{\circ}$ C, extracted for 15 minutes and desorbed for 1 minute at a temperature of 250  $^{\circ}$ C. Before extraction or injection the sample was agitated during incubation to keep the concentration of analytes in the headspace constant. After each sample the SPME fiber was heated for 15 minutes at 260  $^{\circ}$ C to reduce carryover.

## **Sample Preparation**

Calibration curves were constructed using a 1mg/ml stock solution. Each of the twelve components was placed into a 20ml vial and diluted to the initial

concentration using chloroform ((99.9% purity, ECD tested, Arcos Organics, Morris Plains, NJ). Serial dilutions were made to the following concentrations: 1, 5, 10, 15, 35, 50 and 100 $\mu$ g/ml. The quality control points were made (n=5) at 1, 3, 25 and 100 $\mu$ g/ml.

Blood and liver were the matrices used in these experiments. For blood: 3 ml of a 3% NaCl solution, 1 ml of blood and 1 $\mu$ l of each standard was added to a 10 ml autosampler vial. For liver: 3 ml of a 3% NaCl solution, 500  $\mu$ l of liver homogenate and 1 $\mu$ l of the standard was added to a 10 ml vial. The vials were closed and vortexed for 20 seconds and set in the autosampler for analysis. Two blanks were run before each batch of samples to insure that there was not a detectable background level for any analyte before beginning.

### **Validation Procedure**

The twelve components of JP-8 that were quantitated are shown in Table 1 along with their manufacturer and GC retention time. The conditions that were considered for a successful validation were as follows: accuracy (%Bias) of better than 20% and precision (%RSD) of less than 20%<sup>37</sup>. For each validation batch an individual calibration curve was constructed for each component. Using the calibration curves, four quality control (QC) points were prepared in replicates of 5 and then analyzed. Calibration curves and QC samples were generated and analyzed three times to determine inter- and intraday precision and accuracy. The effect of weighting the calibration curves was also evaluated. Unweighted, 1/x, 1/x<sup>2</sup>, 1/y, and 1/y<sup>2</sup> weighting were compared to find the equation for the line

with the lowest sum of the squares of residual error. Using these criteria  $1/x^2$  weighting was chosen for all experiments.

### **Stability Studies**

Because these compounds are very complex and volatile, every precaution was taken for these compounds to ensure they remain stable during analysis. We examined samples over a period of forty hours to determine if there was any loss of signal due to the time a sample spends in the autosampler prior to analysis. We also examined freeze/thaw stability for blood and liver samples to ensure samples could be stored for later determination. Each sample was prepared as mentioned above and analyzed over three freeze/thaw cycles. The area response for each cycle was compared and the %RSD was calculated.

Blood and liver samples were found to be stable.

### **Recovery Studies**

Recovery is normally not determined for SPME studies because it is difficult to separate the concentrating effects of the technique from its extraction efficiency. We choose to look at the relative recovery of the samples from water solutions versus biological samples to evaluate the role of the matrix. Samples were prepared at three concentrations (1, 50, 200 ng/ml). For each concentration 10 samples were prepared, five in water and five in the biological matrix. Recovery was assessed by comparing the response for each component from water versus the biological.

## **Animal Studies**

Male Sprague-Dawley rats weighing 225-275g were housed individually in a temperature controlled environment. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of Georgia. After their arrival, animals were allowed to acclimate for one week. All animals had a carotid cannula inserted to allow for serial blood samples. A jugular cannula was inserted for the IV experiments. Animals were rested for 24h following surgery prior to dosing.

JP-8 was given as an IV bolus at concentrations of 100 and 400mg/kg, orally at 400 mg/kg and by inhalation at 400 mg/m<sup>3</sup>. Oral and IV doses were given using alkamulus 620 as the vehicle. Blood samples from each rat (n=6) per dose were taken at 10, 30, 60, 120, and 240 minutes. The animals were euthanized after the 4h exposure by cervical dislocation and the livers were extracted and homogenized following 2:1 w/w addition of deionized water.

## **Results and Discussion**

### **Method Development**

The final procedure for this method was determined after extensive method optimization. Optimization included: fiber selection, fiber extraction and desorption time, fiber cleaning, and sample salt addition. The 100 µm polydimethylsiloxane (PDMS) fiber and the Carboxen SPME fibers have both been used in the past for hydrocarbon analysis. These two fibers have different extraction mechanisms. The PDMS fiber acts as an *absorbent* type fiber where the components of JP-8 are extracted by partitioning into a “liquid-like” fiber

coating. The ability of the coating to retain and release the analyte is dependent primarily on the thickness of the coating and the size of the analyte.<sup>38</sup>

The carboxen fiber acts as an *adsorbent* fiber where JP-8 components are isolated by trapping molecules on the high surface areas created by the porous material.<sup>38</sup> Both fibers showed good sensitivity for the components of JP-8.

However, the carboxen fiber showed carryover for several of the compounds in the mixture. Despite efforts to regenerate clean fiber surfaces, we were unable to eliminate this carryover. The PDMS absorbent fiber extracted each component without any measurable carryover and since the two fibers had similar general performance, the PDMS fiber was selected for this method.

### **Extraction time**

The extraction time is an important aspect of SPME method development. This parameter is the amount of time the fiber is exposed to the sample. This time must be sufficient so that the fiber and the sample concentration can reach equilibrium. Optimization of this factor improves sensitivity, recovery, precision and accuracy. Optimization of the extraction time for the twelve component mixture was done examining times ranging from 1-25 minutes for both matrices. After these samples were run, the responses they were plotted and observed for the time that yielded the best overall response for the mixture of compounds. Response increased over the first ten minutes for all of the components. The more volatile components (toluene, ethylbenzene and xylene) required fifteen minutes to arrive at equilibrium with the SPME fiber. Therefore, an extraction time of fifteen minutes was used for all experiments.

### **Extraction temperature**

Extraction temperature affects the equilibrium between the analytes on the fiber and those in the headspace. Extraction temperatures for both matrices were examined from: 30-65°C. For liver samples, the components response maximized at or just below 65°C. For blood, an identical pattern was observed; therefore, the extraction temperature for these samples was also 65 °C.

### **Incubation time**

Incubation time is the amount of time required to establish the analyte equilibrium between the sample and the headspace. The incubation time was examined from 15-60 minutes based on values used in the literature for hydrocarbon determination. The response for the aromatic components (benzenes and naphthalenes) showed no significant differences over this range of times. However, the alkanes required at least 30 minutes to attain equilibrium. As a result, 30 minutes was chosen as the incubation time.

### **Desorption time**

Desorption time is the amount of time needed to sufficiently extract the analyte from the fiber into the GC inlet. This parameter was examined over times ranging from 1-25 minutes. The response showed no significant change over the range for both matrices. Therefore, 1 minute was chosen as the optimal desorption time.

### **Injection temperature**

Injection temperature must be sufficient to desorb analytes from the fiber to the GC system. This parameter was examined over a range of 200-265 °C.

For blood the peak response was observed at 250 °C, and for liver the peak response was observed at 225 °C. The difference in analyte response for the liver between 225 °C and 250 °C was <1%. As a result, 250 °C was selected to ensure that the contents on the fiber were being sufficiently desorbed and to reduce any potential carryover.

### **Salt Addition**

Salt addition to the sample enhances the extraction from the aqueous matrix.<sup>34, 39</sup> Previous studies of JP-8 using SPME reported that NaCl was the optimal salt to use.<sup>40, 41</sup> However, there was no discussion of the optimization of the concentration of NaCl. We examined salt concentrations ranging from 0-35%. We observed that lower salt concentrations enhanced the recovery of the alkanes, while higher salt concentrations enhanced the recovery of the aromatics (especially the BTEX compounds). We also observed that the precision of measurements became poorer with increases in the salt concentrations. Since a 3% solution showed the highest response for the alkanes and an acceptable response for the aromatic compounds, this concentration was used for all other studies.

### **Carryover**

Fiber cleanup time was also examined to eliminate carryover that may be present from the analysis of consecutive samples. We employed the use of a fiber baking station that was able to heat the fiber after each injection to remove any residual sample. To determine the optimum time required for the fiber regeneration we examined a temperature of 260°C at the following times: 3,5,10

and 15 minutes. It was determined by the above experiment that 14 minutes was sufficient for fiber cleanup.

## **Validation**

The quantitation of the twelve components was conducted using an abundant ion that was representative of the compound of interest and did not have significant interference from other components of JP-8. The ions that were monitored for each component are shown in Table 3.1. The validation of this method began with the evaluation of precision and accuracy for each component from each matrix over a range of concentrations. In blood, the accuracies ranged from 1-7% over the three day validation period (See Table 3.2). Precision for these compounds ranged between 1-16% over the three day validation period as seen in Table 3.3. In liver, as seen in Table 3.2, accuracy for the compound mixture ranged between 1-12% over the validation period. Precision for the mixture ranged between 3-20% over the validation period as seen in Table 3.3. These values for precision and accuracy were acceptable for conducting basic toxicokinetic studies and for the determination of blood to liver partition coefficients (see Table 3.4).

While recovery studies are normally performed in bioanalytical method validation the values are not normally reported for SPME studies. From the few studies that do report recovery numbers they are typically only a few percent. We examined the recovery for the 12 components from both matrices. In blood, recoveries ranged between 5-84% and for liver the recoveries ranged between 4-98%. It is interesting to note that the recoveries for both matrices are highly

correlated with the boiling points of the compounds (See Figure 3.1). The components with lower boiling points exhibited higher recovery, while compounds with higher boiling points exhibited lower recovery. It was not clear to us initially that this would be the case, because it was possible that the more hydrophobic components may have preferentially partitioned into the hydrophobic fiber coating. However, this data demonstrates that the availability of the compound in the headspace is the major factor determining analyte recovery.

Using these optimized parameters, we were able to determine each of the twelve components over the concentration range from 2-200 ng/ml from blood and from 1-100 ng/ml from liver homogenate (representing 3-300 ng/g from liver tissue). Figure 3.2 shows the gas chromatogram of the blank and a 1 ng/ml spiked liver homogenate sample. The liver homogenate samples have a very low background even when taking the two-fold dilution with water from the homogenizing into account. Figure 3.3 shows the gas chromatogram of the blank and a 2 ng/ml spiked blood sample. The background from the blood samples was higher but we did not observe any interference with the twelve components that were measured.

Examining the stability of compounds during analysis is very important and often not addressed. There are several issues to address when discussing stability. Since the analysis of JP-8 requires a long run time to adequately separate the many structurally related compounds, samples may sit in the autosampler for hours or days. We have evaluated blood and liver homogenate

samples for up to 40 hours and do not observe any significant loss of sensitivity. Another type of stability test is the freeze-thaw stability. It is often necessary to freeze samples prior to analysis. However, calibration curves are normally prepared prior to analysis. Therefore, it is important to demonstrate that samples that have been frozen and then thawed provide equivalent responses to those that have not been frozen. The twelve compounds were examined over three freeze-thaw cycles. Samples were frozen at -80°C and then thawed. Over a range of concentrations covering the calibration curve we did not observe a significant decrease in the responses for any of the components following freezing and thawing up to three times.

### **Toxicokinetic Study of JP-8**

Following IV administration of JP-8 there is a distribution phase where the components partition into various tissue compartments. This process appears to take approximately 60 minutes for all twelve components. Following this rapid distribution phase, we observe a much slower elimination phase. Table 3.4 shows the liver/blood partition coefficients. The concentration for the pseudocumene and the alkanes undecane through tetradecane show higher levels in the liver indicating that this tissue may act as a deep compartment.

### **Conclusion**

The development of a validated method to analyze twelve major components from JP-8 has been demonstrated. This method has been applied to the study of IV and oral doses in the rat. The components rapidly distribute to tissues and then are slowly eliminated. The ability of these compounds to

accumulate in the liver may be related to the higher incidence of cancer observed in this tissue.

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**Table 3.1:** Shows the components present in the mix and gives their purity, retention time, and the ions used for analysis.

<b>Compound</b>	<b>Purity</b>	<b>Supplier</b>	<b>Retention Time (Minutes)</b>	<b>Ions Observed</b>
n-Nonane	99%	Sigma	23.9	97+85
n-Decane	99%	Sigma	27.7	98+85
n-Undecane	99%	Sigma	32.7	98+85
n- Dodecane	99%	Sigma	36.6	97+85
n-Tridecane	99%	Sigma	40.2	97+85
n-Tetradecane	99%	Sigma	43.6	97+85
Toluene	99.8%	Aldrich	16.0	91+92
o-Xylene	99.5%	Fluka	21.2	91+106
Ethylbenzene	99.8%	Arcos	22.8	91+106
Naphthalene	NK	Supelco	35.4	128+102
2-Methylnaphthalene	97%	Aldrich	39.6	141+115
Pseudocumene	NK	Sigma	27.0	120+105

**Table 3. 2:** Shows blood and liver validation accuracy interday results for days 1-3.

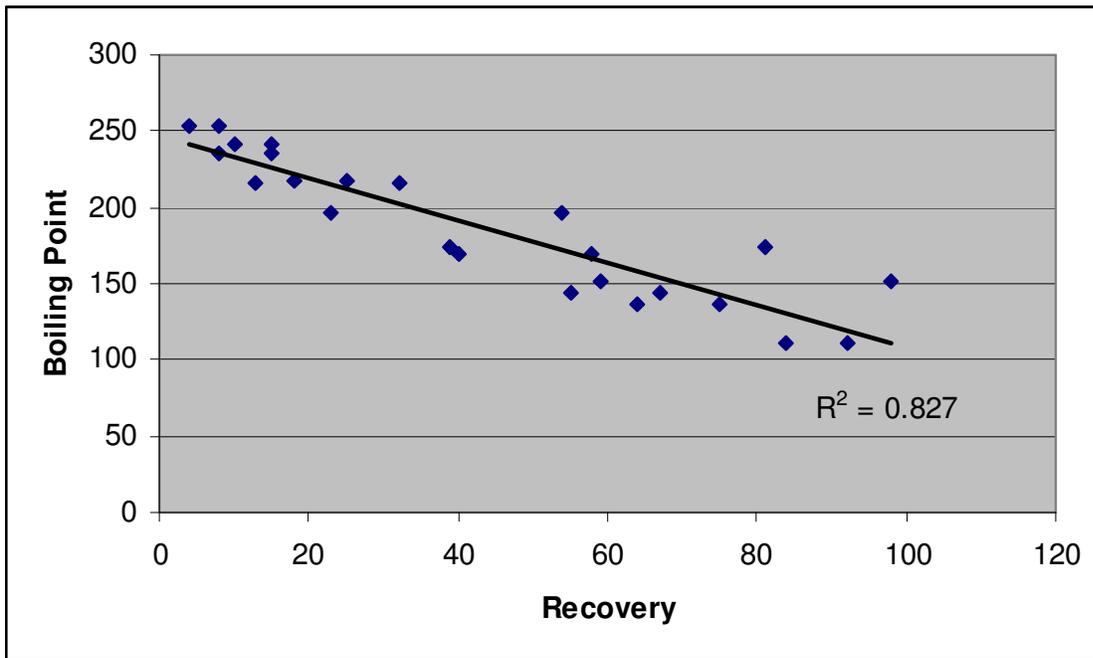
<b>Blood</b>	<b>Nonane</b>	<b>Decane</b>	<b>Undecane</b>	<b>Dodecane</b>	<b>Tridecane</b>	<b>Tetradecane</b>	<b>Toluene</b>	<b>Xylene</b>	<b>Ethylbenzene</b>	<b>Naphthalene</b>	<b>2MN</b>	<b>Pseudocumene</b>
<b>2ng/ml</b>	4.904374	2.512305	2.637650	2.101174	1.605809	1.893437	3.278281	2.733621	1.865503	2.006021	1.978235	2.721987
<b>6ng/ml</b>	4.711229	3.932547	5.478214	3.979281	3.298188	4.359106	4.114975	6.884102	5.240515	3.412522	1.432235	2.994925
<b>50ng/ml</b>	3.901783	4.584636	5.577560	4.191136	3.548132	4.353446	4.209262	5.551215	3.414101	5.905705	6.483232	5.140444
<b>200ng/ml</b>	6.915702	5.202084	3.319016	5.215380	4.553722	5.572761	3.076380	2.988112	4.816176	4.669585	3.220519	5.782653
<b>Liver</b>												
<b>1ng/500µl</b>	2.828154	4.917878	1.438569	8.564682	9.395535	10.186941	5.929405	4.681809	5.136564	3.764575	5.189981	3.170534
<b>3ng/500µl</b>	3.553308	4.409638	7.242523	7.746545	10.065726	8.848554	5.119369	5.965682	5.737787	7.974094	9.199537	6.145353
<b>25ng/500µl</b>	3.117088	8.291400	6.120101	5.066829	4.114335	3.617593	3.107593	1.692693	2.933846	2.517943	2.805547	2.441404
<b>100ng/500µl</b>	7.466932	11.304120	11.447274	10.938320	11.872563	9.672364	3.639185	7.466932	5.140270	3.612277	4.802318	3.659337

**Table 3.3:** Shows liver and blood validation precision interday results (n=15).

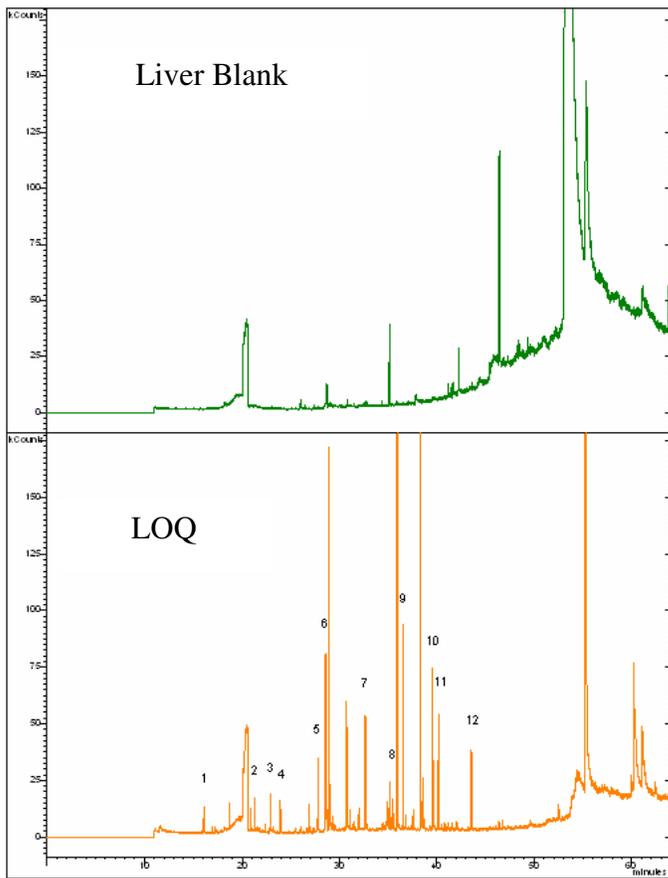
<b>Blood</b>	<b>Nonane</b>	<b>Decane</b>	<b>Undecane</b>	<b>Dodecane</b>	<b>Tridecane</b>	<b>Tetradecane</b>	<b>Toluene</b>	<b>Xylene</b>	<b>Ethylbenzene</b>	<b>Naphthalene</b>	<b>2MN</b>	<b>Pseudocumene</b>
<b>2ng/ml</b>	6.240609	3.309876	3.4024314	2.8854346	2.2090509	2.369515561	4.357413	3.460368	2.571999045	2.696814835	2.9107	3.485175178
<b>6ng/ml</b>	5.686591	4.71398	5.8411489	5.0143312	4.9011091	5.672325449	5.587458	6.61334	6.806093169	4.511500723	2.47179	3.937623211
<b>50ng/ml</b>	7.484839	6.55849	5.7240204	4.2319234	4.5417286	5.190204942	7.220421	6.685726	5.769072307	5.017931579	5.38737	5.103925598
<b>200ng/ml</b>	15.47768	10.63536	10.916754	11.762113	12.158771	13.18316903	10.74858	10.53679	10.68206912	5.930704349	4.1868	8.469286554
<b>Liver</b>												
<b>1ng/500µl</b>	6.456702	9.168956	4.8637026	11.148008	16.144551	14.80516952	6.621233	5.749715	6.452914378	4.613602883	8.5679	3.57824594
<b>3ng/500µl</b>	3.035764	11.94778	8.486271	12.384583	15.175957	10.08466329	6.412097	7.220525	7.231393744	8.874658467	8.76125	7.802453126
<b>25ng/500µl</b>	8.220207	11.30412	13.525802	12.772749	12.243554	13.51407955	4.841546	7.552048	5.042009962	6.597697619	6.58374	6.266575846
<b>100ng/500µl</b>	9.992737	13.54376	12.789244	14.237766	17.948018	19.09247962	5.216105	7.655446	8.442702589	5.461580155	6.91291	6.41978521

**Table 3.4:** lists the partition coefficients for liver:blood at 4h.

Compounds	Partition Coefficients (average)
Nonane	ND
Decane	ND
Undecane	1.401469058
Dodecane	5.84364206
Tridecane	25.30867779
Tetradecane	12.88939137
Toluene	ND
Xylene	ND
Ethylbenzene	ND
Naphthalene	ND
2-methylnaphthalene	ND
Pseudocumene	1.339432628



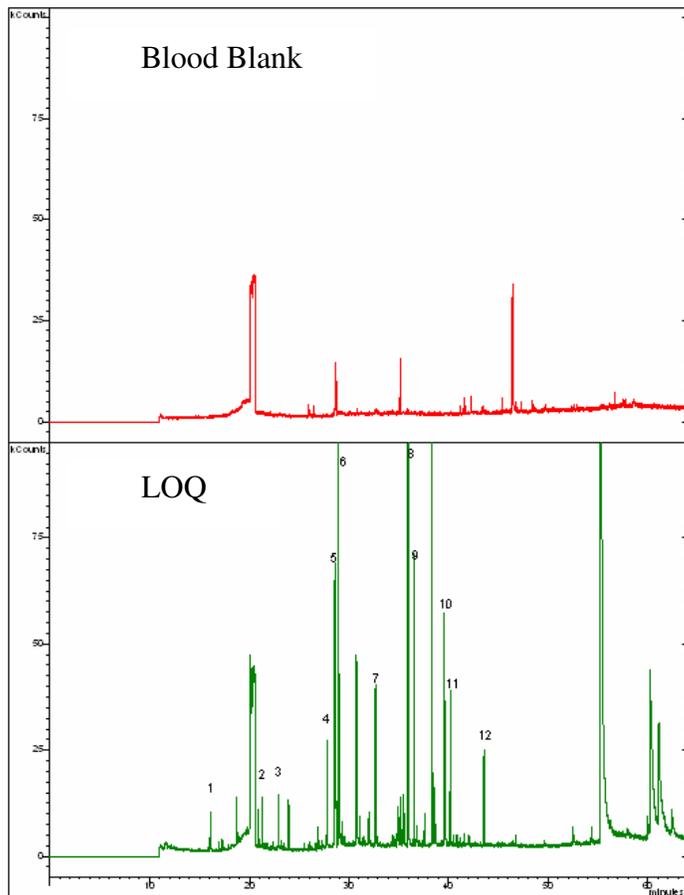
**Figure 3.1:** Recovery correlation; boiling point vs. recovery for blood and liver



Legend

1	Nonane
2	Decane
3	Undecane
4	Dodecane
5	Tridecane
6	Tetradecane
7	Toluene
8	o-Xylene
9	Ethylbenzene
10	Naphthalene
11	2-Methylnaphthalene
12	Pseudocumene

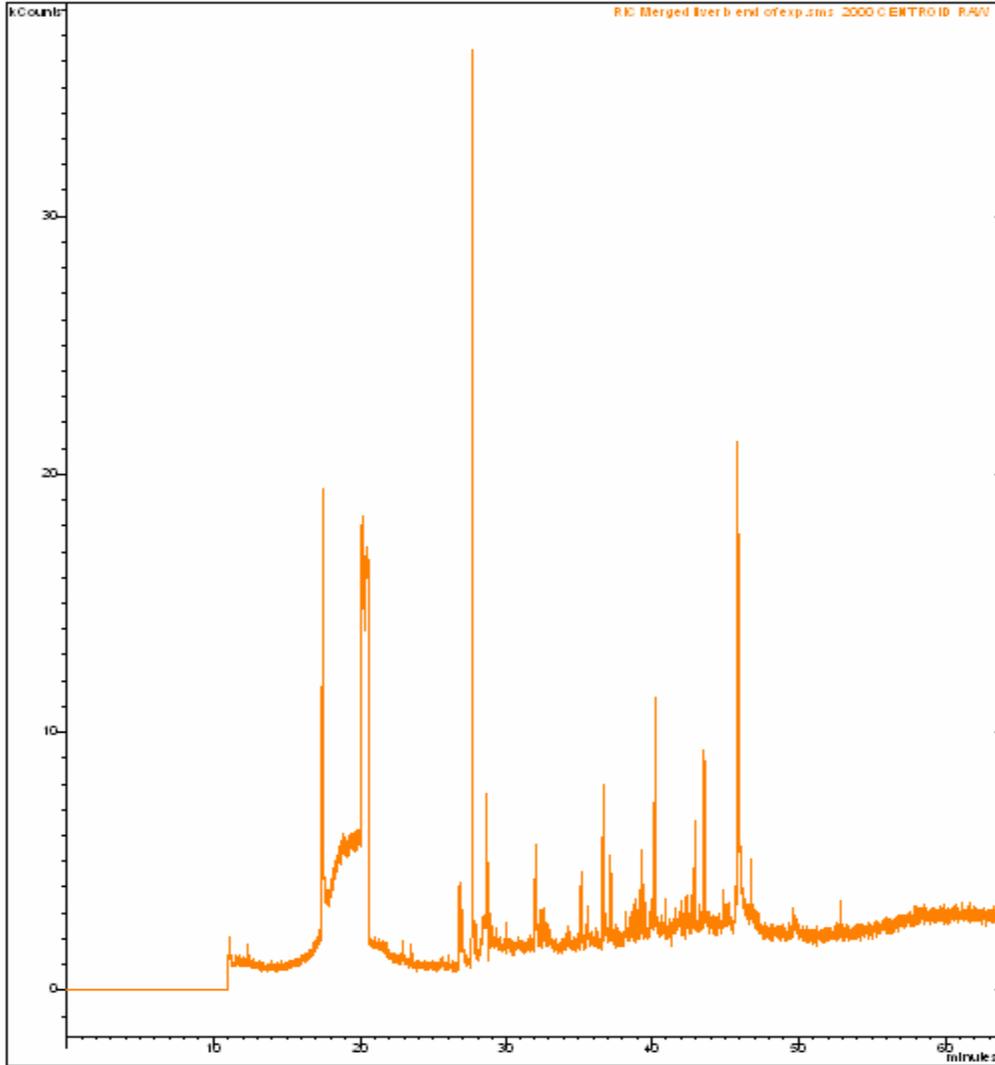
**Figure 3.2:** Shows chromatograms of a liver blank stacked above the LOQ for the liver



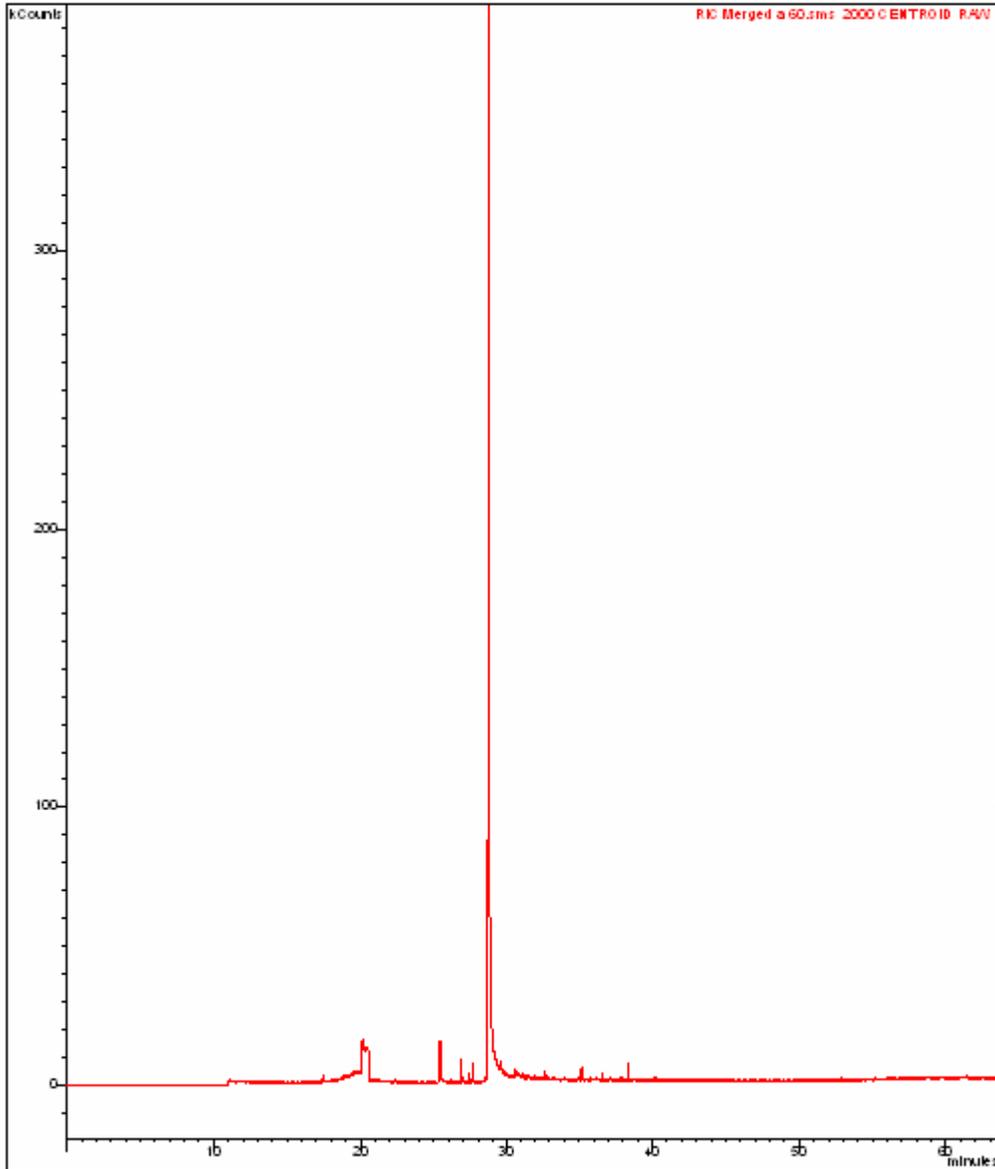
Legend

1	Nonane
2	Decane
3	Undecane
4	Dodecane
5	Tridecane
6	Tetradecane
7	Toluene
8	o-Xylene
9	Ethylbenzene
10	Naphthalene
11	2-Methylnaphthalene
12	Psuedocumene

**Figure 3.3:** Shows chromatograms of a blood blank stacked above the LOQ for the blood



**Figure 3.4:** Shows a chromatogram of the terminal (4h) liver sample after exposure.



**Figure 3.5:** Shows a chromatogram of the 60 minute blood sample during exposure.

## Conclusions

Jet Propellant 8 (JP-8) and its related components are of great interest to the military community. Military personnel are continually exposed to this fuel as it is used to power military aircrafts and vehicles in wartime and not. There are several reasons for the difficulty in analyzing JP-8. These reasons include: The volatility of its compound make up and batch variance. The phases of JP-8 are also of interest (neat, vapor and aerosol). It is widely believed that exposure to different phases of JP-8 will have an effect on what happens after exposure, as it pertains to toxicity.

Neat JP-8, JP-8 vapor and aerosol were generated and characterized. Identification of the compounds present in these phases of the fuel can allow for accurate accountability for toxicity. Knowing the most accurate way to sample these phases is of great importance. For the vapor phase of the fuel three tubes were tested and it was observed that the Tenax tube was the most representative of the vapor, in terms of presence and abundance of compounds. The two remaining tubes were not effective in picking up many of the higher end components and other components in abundance similar to JP-8 vapor. The glass fiber filter was used to analyze JP-8 aerosol and was efficient in doing so. Of the phases the aerosol showed the largest abundance of the identified components, therefore; it can be assumed that it has the greatest effect when one becomes exposed, followed by the neat fuel and lastly the vapor phase of JP-8.

Analytical methods were developed for a JP-8 surrogate mix using SPME, GC and iontrap technology. SPME is a method of sample extraction that is growing in popularity. More scientists are beginning to apply this technique because of its simplicity and ease of use. SPME combines sampling, extraction, concentration and sample injection in one step. SPME will likely grow into the main sample extraction technique for JP-8 surpassing its predecessors. This sample extraction technique combined with the GC ion trap successfully analyzed representative components of JP-8 in biological tissues at levels below those seen in previous methods.

In brief, methods have been developed to identify components present in JP-8 and to analyze a representative component mixture of JP-8. These methods are straightforward, effective, and efficient in analyzing JP-8. Through these studies the Tenax tube has emerged as the supreme sampling method for analysis of JP-8 vapor and the glass fiber filter has proven to be excellent for aerosol analysis. The use of SPME has simplified the analysis therefore cutting experiment time and eliminating the stigma of complicated fuel analysis. Use of these methods will aid in the development of a PBPK model, which will effectively describe and predict plasma and tissue concentrations of JP-8.