EFFECTS-DRIVEN FRACTIONATION OF HEAVY FUEL OIL TO ISOLATE COMPOUNDS TOXIC TO TROUT EMBRYOS

by

Jason Michael Bornstein

A thesis submitted to the Department of Chemistry

In conformity with the requirements for

the degree of Master of Science

Queen's University

Kingston, Ontario, Canada

(July, 2012)

Abstract

Heavy Fuel Oil (HFO) is a petroleum product and emerging contaminant used as fuel by cargo ships, cruise liners, and oil tankers. As a high-frequency, low volume commodity shipped by pipeline, train, truck, and ship, it is at high risk for small-scale spills in terrestrial, aquatic, and marine environments. There are few reports characterizing HFOs and quantifying the contaminants therein, but previous studies have shown that the most toxic classes of compounds in petroleum products are polycyclic aromatic hydrocarbons (PAHs). This project seeks to address that by analyzing HFO 7102, the specific HFO spilled in Wabamun Lake, Alberta in August 2005.

Through an Effects-Driven Fractionation and Analysis, HFO 7102 was successively fractionated by physical and chemical means. First, a low-temperature vacuum distillation separated the oil into three fractions by volatility. The most toxic of these (lowest median toxic concentration, or LC_{50}), F3, underwent a series of solvent extractions to remove asphaltenes and waxes. The remaining PAH-rich extract (F3-1) was further separated using open column chromatography into non-polar, mid-polar, and polar fractions with groupings approximately by number of aromatic rings. At each stage, fractions and sub-fractions were characterized by GC-MS for compositional analysis and bioassays were conducted with rainbow trout embryos. In this fashion, toxicity thresholds were developed for all fractions and the components of HFO 7102 associated with toxicity were identified and quantified.

The F3 fraction was six times more toxic than the whole oil. While the wax fraction (F3-2) was shown to be non-toxic, the remaining PAH-rich extract (F3-1) accounted for all of the toxicity in F3. Future work may be done to determine the relative toxicity of the last fractions generated and identify a range of PAH responsible for fish toxicity. It is expected that the F3-1-2

fraction will be most toxic, as it contains nearly all of the three-ring and most of the four-ring PAH. These size classes of PAH have been associated with chronic toxicity to fish embryos in studies of crude oil. Further separations may be attempted to identify a more specific range of toxic compounds, such as by degree of alkylation.

Co-Authorship

1. Jason Bornstein wrote all chapters in this thesis. Dr. R. Stephen Brown edited and co-authored all chapters. Dr. Stephen Brown is Mr. Bornstein's research supervisor and a co-investigator on this project. His advice and guidance were provided throughout.
2. Dr. Peter Hodson co-authored chapter three and edited chapters one, two and three. Dr. Hodson is a co-investigator on this project, and a fish toxicologist/environmental biologist at Queen's University. All biological work reported in this thesis was performed in his laboratory.
3. Miss Julie Adams co-authored the work reported in chapter three. She is a Master's student in the biology department at Queen's University. She exposed the fish to HFO fractions and performed chronic toxicity testing.
4. Dr. Bruce Hollebone co-authored chapter two. He is an environmental chemist at the Environment Canada labs in Ottawa, Ontario, and supervised the low-temperature vacuum distillation work performed there.
5. Dr. Tom King co-authored chapter three. He is an environmental scientist with the Department of Fisheries and Oceans and supervised the GC-MS analysis of HFO fractions, performed at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia.

Acknowledgements

Wow. Here I am. It's hard to believe that I made it, and even harder to believe that it took as long as it did. A "Master of Chemistry" as it were, having "fended off the attack of the scientists". There are so many people I want to thank and it's safe to say that I would not have completed this document without their love, friendship, assistance, guidance, and companionship over the years. I have accepted the fact that this document will probably interest almost none of them, and much of it will go over their heads. However, it is my sincerest hope that they read this section and know that they have made a difference in my life; not just in the last three years, but for the last twenty-five.

First, I have to thank my parents, Neil and Carolyn. They helped me understand the value of academics and hard work, to stand tall in the face of adversity, and to always be the best that I can be. My sister and brother, Jen and Adam, even though you both went to Western I still love you. We have had our differences over the years, but you have always supported my endeavours. To my aunts, uncles, cousins and grandparents: your love and support means everything to me. Even though I always dreaded the "when are you graduating?" question, it would have taken much longer without my extensive support network.

My time in Kingston was almost equally split between my undergraduate and graduate degrees, so I feel the need to thank the people who got me through the first half: the Queen's Engineering program, particularly the class of Engineering Chemistry 2009, as well as my good friends and housemates, including Robert Baraniecki, Jason Munn, Lucas Vanderpluym, Rob Ingratta, Craig Norval, Scott Coulter, Holden Sumner, Zach Schiller, and Jane Purificati. We had plenty of great times together, and I will always look back on those years fondly.

From the Chemistry Department, I am forever indebted to members of the Brown Group: Pat Cashin, Gillian Mackey, Moru Zhang, and Jessamyn Little, as well as our summer students Hilary Chung and Makenna Schwab. You made coming to work every day an absolute pleasure. To Makenna: you really helped me get back on track at a time when I was floundering and I am extremely grateful. Also, thank you to Dr. Ray Bowers for his assistance with column chromatography; I learned so much from you and I wish I could have picked your brain a little more. I also owe a great deal of thanks to my colleagues from other groups in the department: Julian Kwok, Jen Adams, Alex Dunlop-Briere, Klaus Bescherer-Nachtmann, Lili Mats, and John Saunders. I really value your friendship and wish you all the success in the world in your future careers in chemistry.

Next, I would like to thank our sister group in the Biology Department, past and present members of the Hodson Group, particularly Jon Martin, Julie Adams, Shirin Fallahtafti, Colleen Greer, and Troy Arthur. Whether it was lab meetings or SETAC conferences, we always had good laughs and good fun. Also, Julie: thanks for killing all of those fish in the name of science.

From my time in Ottawa, summer 2010, I would like to thank my colleagues and collaborators at Environment Canada ES&T: Mike Landriault, Bruce Hollebone, Zhendi Wang, Ben Fieldhouse, and Pat Lambert. I'm sorry that I broke so much glassware! Most importantly from that summer, I must express my extreme gratitude to Aunty Jan & Uncle Arlen – for their hospitality and for their sound advice and positive reinforcement when I needed it most. Many times that summer I felt like an absolute disaster, only to come home to a warm meal and loving hugs at the best place to stay in Ottawa!

Of course, I am most grateful for the guidance, support and leadership of my supervisor Dr. Stephen Brown. When you were in the country you always had good tips for my experiments and excellent advice. Special thanks to Dr. Peter Hodson for being my pseudo-supervisor. You

seemed to be around whenever Stephen wasn't, and you always supported and encouraged my work. Also, thanks for taking me to all of those conferences! Thank you to Dr. Philip Jessop, who showed me the ropes of research as my undergraduate thesis supervisor and served on my examination committee. A big thanks goes out to our collaborators in the Department of Fisheries and Oceans, Tom King and his team at BIO, for providing the GC-MS analysis of my oil and fractions.

Finally, I must express my thanks to two wonderful individuals and role models who were taken too soon and are not able to share in my success. Aunt Karen: you were like a second mother to me. I will always remember our time together, and hope to emulate your selfless nature, generosity, sense of fun, and love of life. Louis Gyori: you are one of the greatest men I have ever met. You helped me become a strong person, teacher and leader and taught me so many things about life. It was an incredible honor to work alongside you for so long at Camp. I hope to live my life and do everything I do with a little "Can-Aqua spice".

Now, without further ado: "Oil Kills Fish".

(You don't have to read any more if you don't want to.)

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AET – Atmospheric Equivalent Temperature

ANSC – Alaska North Slope Crude

ASTM – American Society for Testing and Materials

BIO – Bedford Institute of Oceanography

BSD – Blue sac disease

BTEX – Benzene, toluene, ethylbenzene, xylenes

CCME – Canadian Council of Ministers of the Environment

CEWAF – Chemically-Enhanced Water-Accommodated Fraction

CYP1A – Cytochrome P4501A1 protein

DCM – Dichloromethane

EC50 – Median effective concentration

EDFA – Effects-Driven Fractionation and Analysis

EROD – Ethoxyresorufin-*O*-deethylase

ES&T – Emergencies Science & Technology Division (Environment Canada)

GC-FID – Gas Chromatography-Flame Ionization Detector

GC-MS – Gas Chromatography-Mass Spectrometry

HE-CEWAF – High-Energy Chemically-Enhanced Water-Accommodated Fraction

HFO – Heavy Fuel Oil

HPLC – High Performance Liquid Chromatography

IBP – Incipient Boiling Point

K_{ow} – Octanol-water partition coefficient

LC50 – Median lethal concentration

PAH – Polycyclic Aromatic Hydrocarbon

SARA – Saturates, Aromatics, Resins, Asphaltenes

SIM – Selective Ion Mode

TLC – Thin Layer Chromatography

UV – Ultraviolet

WO – Whole Oil

Chapter 1

Introduction to Heavy Fuel Oils and the Problems they Pose

1.1 Oil Spills in Aquatic Environments

The long and notorious history of oil spills has been well-documented. For as long as people have been extracting oil from the ground and drilling it from the sea floor, some of the oil has found its way into bodies of water, soils and sediments. Worldwide oil production, estimated at 82 million barrels per day in 2009, is projected to increase to between 108 and 115 million barrels per day by 2035 [1]. While small leaks and seeps may be unavoidable, full-scale spills due to cargo ship crashes are amongst the biggest anthropogenic disasters on Earth. In March 1989, the *Exxon Valdez* ran ashore in Prince William Sound, Alaska, and lost approximately forty-one million litres of Alaskan North Slope crude (ANSC) oil [2]. The 2010 Deepwater Horizon blowout resulted in an estimated 779 million litres of South Louisiana Sweet crude oil being released into the Gulf of Mexico, making it the largest ever to originate in U.S.-controlled waters [3]. The composition and effects of these and other crude oil spills have been studied extensively [4-6], but there is also a need to characterize and study refined oils.

Heavy Fuel Oils (HFOs) are a refined product of crude oils, sometimes classified as Bunker C or Fuel Oil No. 6. As a refined product, the environment is not susceptible to natural seeps or large-scale releases at the source of drilling; the primary sources of HFO are spills during transportation by pipeline, train, transport truck, or cargo ship. As such, these spills tend to be of smaller magnitude but, since their composition and toxic effects are not well-known, may result in similar or even greater damage to aquatic environments than crude oil spills. Some recent large volume HFO spills include the oil tanker *Prestige*, which sank and spilled about sixty million litres of HFO into the Bay of Biscay near Spain in 2002 [7]. In 2006, during the Israel-Lebanon conflict, the Jiyeh Power Station near Beirut was bombed, releasing an estimated thirty-eight million litres of HFO into the Mediterranean Sea [8]. Closer to home, a recent heavy fuel oil spill

happened in Wabamun Lake, Alberta in August 2005. Nearly 800,000 litres of HFO 7102 was spilled when a train derailed north of the lake, and an estimated 150,000 litres made its way to the shore, spreading into the sediment, spawning shoals, and water column [9]. Strong winds spread the oil all across the lake, affecting the populations of a great many species of fish, plants and animals. It is this particular oil, HFO 7102, which was studied in this project.

1.2 Impacts of Oil Spills

Oil spills in aquatic environments have a wide range of negative effects on the environment and its occupants. The toxic effects of oil are well-documented and have been observed and studied in birds [10], humans [11], plants [12, 13], shorelines and sediments [14, 15], fish [7, 9, 16-26], and other marine organisms [27, 28]. It is clear that even small amounts of oil can have adverse effects on a wide variety of species that live in and around aquatic environments.

Due to the toxic nature of oil, spills are often swiftly followed by clean-up attempts. One of the most common methods of dealing with spills in aquatic environments is adding dispersant so that the oil will break up. This is an effective method of getting oil slicks off the water surface where it can be harmful to birds, mammals, shorelines, and is highly noticeable by the public. However, it distributes the oil into the water column, where it can be harmful to fish, cephalopods, crustaceans, and even benthic organisms.

In the case of Wabamun Lake, the spilled oil was observed with a wide variety of behaviours (sinking, floating, neutral buoyancy) and in many aggregate forms (‘logs’, sheets, lumps and tar balls). These density variances were primarily attributed to differences in the uptake of sediment [29]. Shortly after the spill, strong winds and breaking waves caused the oil to

spread throughout the lake, including 52.5% of the total shoreline [30]. Part of the efforts to clean-up the shoreline involved removing oiled vegetation, resulting in increased erosion and sediment release [31]. The components of the spill that could not be cleaned up along the shore persisted as submerged oil and tar balls.

In the days and weeks following the spill, Alberta Environment took water and sediment samples for analysis. They found that, in most cases, the concentrations of various contaminants were within acceptable limits in the open water. Closer to the shoreline, elevated concentrations were observed, possibly due to recovery operations along the shoreline. Sediments from open water similarly had contaminant concentrations close to background concentrations from previous studies [32]. This shows that the spilled oil collected near the shore, which likely posed a problem for many freshwater fish that are known to spawn in shoreline vegetation or near shore gravel/cobble substrates.

1.3 Heavy Fuel Oils vs. Crude Oils

Over a twenty-five year period at the end of the twentieth century, nearly forty percent of the four-hundred plus ship-source oil spills involved medium or heavy fuel oils, either as cargo or fuel for the ships [33]. Although both the annual number of spills and volume of oil spilled has decreased fairly consistently since 1970 (less than four percent of the total oil spilled since 1970 happened from 2000-2009, compared to fifty-six percent in the 1970s), the proportion of heavy oil spills has increased to about sixty percent [34].

Crude oils are unrefined; they exist in the same state as when they were extracted from the ground. As such, crude oils differ widely in composition and other characteristics based on where they are extracted and the decomposed organic matter that contributed to their formation.

They can enter the environment by natural "seeps" through the ocean floor or by anthropogenic means, such as leaks during extraction, transportation, refining, or usage. It is estimated that natural and anthropogenic means of crude oil entering the environment are approximately equal, although it is much harder to estimate the volume leaked through seeps [35].

Heavy Fuel Oils (HFOs), on the other hand, are refined oils. They are petroleum products used as fuel by large ships such as cruise ships and oil tankers. They are dense (specific gravity usually 0.92-1.02 g/cm^3), viscous (kinematic viscosity of 5,000 to 30,000 mPas at 15°C) and have high pour points (i.e. they are too viscous to flow at room temperature) [33]. HFOs are composed of the "bottom of the barrel" compounds; i.e., whatever is left over when all other compounds are removed in the refining process. They often contain viscous and tarry residues of the refining process as well as complex mixtures of heavy aliphatic and aromatic compounds, bitumens, and asphaltenes [33]. As a product of the refining process, 100% of HFOs leaked to the environment are from anthropogenic sources, such as leaks during transportation or high volume spills.

Another important difference between crude oils and HFOs is the way they behave in aquatic environments. Crude oils generally have low densities, and often float to the surface [36]. Floating oils form sheens that are easily spotted by observers, and easily tracked as they spread. Over time, they will weather by processes including loss by evaporation, dissolution and emulsion formation, and loss by degradation through chemical and biochemical processes. During the first weeks after an oil spill, weathering is dominated by evaporation and dissolution, which favour loss of low-molecular weight compounds. This causes density to increase but not usually by enough to cause the oil to sink [37]. HFOs, due to their high density, may sink in aquatic environments. This poses two problems: spills are not very visible and their movement cannot be tracked easily. In addition, they do not usually experience significant losses due to

evaporation because most low-molecular weight compounds have already been removed in the refining process.

Previous work by our group has linked chronic toxicity to fish embryos with the PAHs in crude oils [4, 5, 6]. An analysis of MESA, a medium crude oil, and two heavy fuel oils (HFO 6303 and HFO 7102) shows that HFOs are significantly more toxic than some crude oils (Figure 1.1). The toxicity of HFO 7102 is about 7.5 times more toxic than MESA, with HFO 6303 a further 7 times more toxic than HFO 7102 (Julie Adams, personal communication). This shows that heavy fuel oils are significantly more toxic than medium crude oils, and different heavy fuel oils may have significantly different toxic effects. The higher relative toxicity of HFOs may be due to higher concentrations of such PAHs as phenanthrene, chrysene, and napthalene [6].

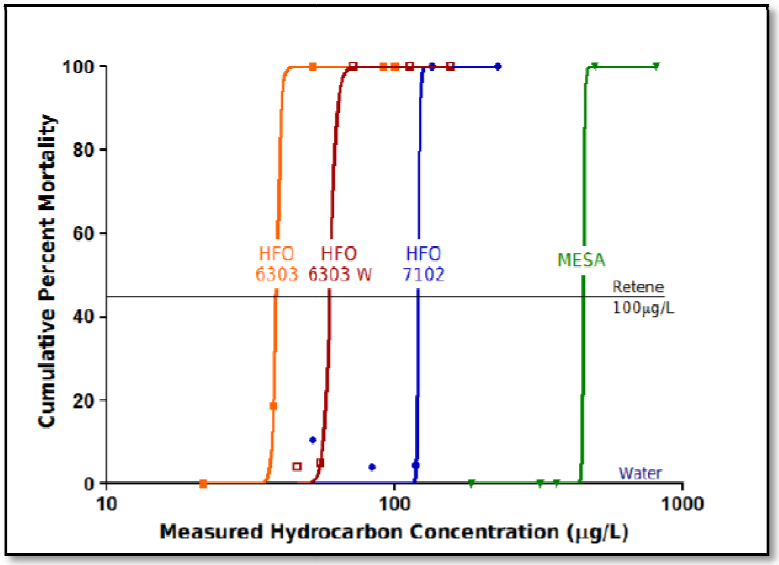

Figure 1.1: Cumulative Percent Mortality for HFO 6303, weathered HFO 6303, HFO 7102 and MESA (a medium crude oil). Graph reproduced with permission from Julie Adams, Dept. of Biology, Queen's University.

The purpose of this work is to explain some of the reasons why HFOs are significantly more toxic than crudes. This will be accomplished through a multi-stage fractionation using a

scheme that isolates different chemical groups into different fractions for group-specific toxicity testing. This will determine the relative proportions of the most toxic and least toxic fractions. An emphasis will be made on isolation of different groups of PAHs to provide further evidence that these are responsible for most of the toxicity of the oil. After the oil and its fractions are chemically analyzed, the results will be discussed to explain the relatively high toxicity of this HFO in terms of its chemical composition. The Effects-Driven Fraction and Analysis (EDFA) developed here will establish a framework for testing other HFOs where relative toxicity could be predicted from chemical composition.

1.4 Chemical Composition & Properties of Heavy Fuel Oils

Heavy fuel oils, being a refined petroleum product, contain a myriad of hydrocarbons, as well as some nitrogen, oxygen and sulfur-containing compounds. They also contain trace levels of metals, such as vanadium, nickel, iron and copper [38, 39]. The oil components are broadly grouped into four categories: saturates, aromatics, resins and asphaltenes (SARA) (Figure 1.2), which may be separated chemically based on solubility using a variety of solvents and techniques. The primary techniques used are gravity-driven chromatographic separation, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) [40]. Each of these categories contains hundreds or even thousands of individual compounds.

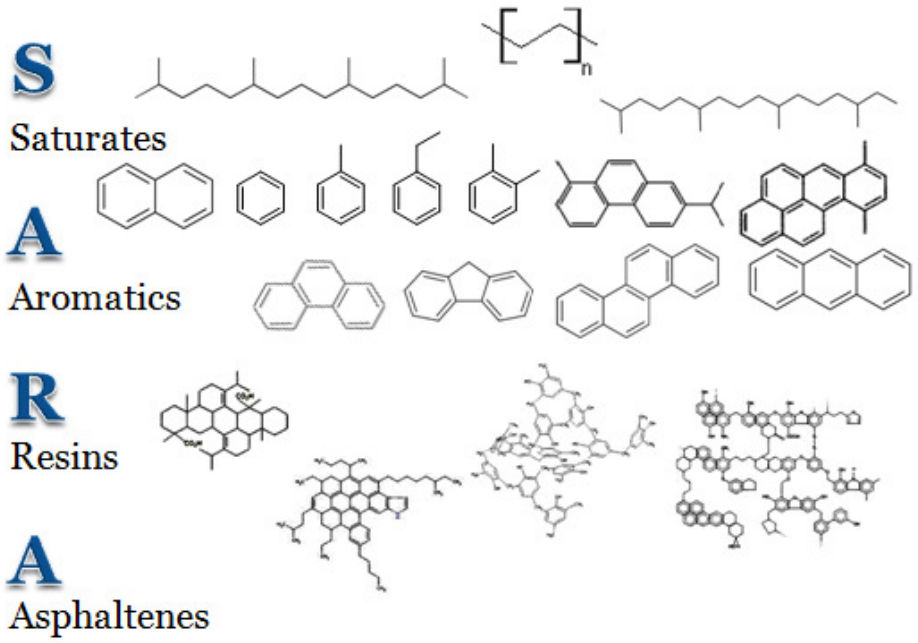

Figure 1.2: Structures of some compounds belonging to the classes Saturates, Aromatics, Resins and Asphaltenes

1.4.1 Saturates

Within the saturates class, there are multiple sub-classes. Straight chain alkanes, also called n-alkanes, generally range from five to forty carbons in length (C5-C40). HFOs contain mostly longer-chain n-alkanes (above C17) as the lower molecular weight compounds are removed in the distillation process [41]. Some short chain n-alkanes may be added later to improve flow properties [42]. Iso-alkanes are branched chain hydrocarbons. Two of the most commonly analyzed iso-alkanes are pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) [43]. Cycloalkanes contain saturated alkyl rings (for example, cyclohexane), and their class includes alkylated derivatives. Again, due to distillation, low molecular weight cycloalkanes would be present only in very small concentrations. Finally, hopanes, steranes and terpanes consist of multiple cyclic rings with long alkyl side chains. Due to

their persistence, these compounds, such as 17α(H),21β(H)-Hopane, are commonly used as chemical markers for identifying and differentiating oils, as well as determining their weathering status [44, 45].

1.4.2 Aromatics

Aromatic hydrocarbons contain only carbon and hydrogen, and one or more fused benzene rings. Compounds with multiple aromatic rings are commonly referred to as polycyclic aromatic hydrocarbons (PAHs). These compounds are widely studied due to their toxic effects. It is expected that the refining process removes most one-ring aromatics and perhaps some of the two-ring aromatics as well. A large portion of the remaining PAHs are alkylated (i.e. have alkyl side-chains) [36]. In addition to aromatic hydrocarbons, this class includes aromatic compounds containing heteroatoms (e.g. nitrogen, oxygen and sulfur).

1.4.3 Resins

Resins are large, polar molecules. Like other classes, there are a wide variety of different compounds with similar structures. The structure of resins is not well understood. It is accepted that resins are the pre-cursors for the formation of asphaltenes. Resins are generally classified as heptane soluble and pentane insoluble. They may also be isolated from crude and refined oils through silica gel chromatography [46]. HFOs generally contain a substantial amount of resins.

1.4.4 Asphaltenes

Asphaltenes are the class containing the most polar and highest molecular weight compounds. Mass spectrometry and fluorescence polarization have shown that asphaltenes are large molecules with a molecular weight of about 500-1000 amu and about four to ten aromatic rings, and may be bound to metals such as nickel and vanadium [47]. Asphaltenes may be

separated chemically, as they precipitate in solutions of n-alkanes such as n-pentane or n-heptane [48]. HFOs contain a substantial amount of asphaltenes.

1.4.5 Unique Properties of Heavy Fuel Oils

As a refined petroleum product, fuel oils are very different in composition from light, medium and even heavy crude oils and bitumens. Fuel oils are distilled from crude oils, and may be classified into one of the following categories: number 1 fuel oil (kerosene), number 2 fuel oil (home heating oil, similar to diesel), number 4 fuel oil (commercial heating oil), number five fuel oil (a residual industrial heating oil), or number six fuel oil (a high viscosity residual fuel oil). Fuels formerly classified as number 3 fuel oils have been merged with the number 2 fuel oil class. Fuel oils are numbered in the order they distill; higher numbered oils contain higher boiling point components, and are generally more viscous and denser than lower numbered oils. Another classification system for oils is the Bunker oil categorization. Bunker A is the same as number 2 fuel oil, Bunker B comprises number 4 and number 5 fuel oils, and Bunker C is the same as number 6 fuel oil. HFOs are a Bunker C, or number 6 fuel oil, which is the heaviest class of fuel oils. After distillation, HFOs may be blended with low molecular weight hydrocarbons to improve pouring and flow properties [42].

The compounds removed by distillation during the production of HFOs are the same compounds that are typically observed to volatilize when crude oil is weathered: saturated hydrocarbons, mono-aromatics such as BTEX (benzene, toluene, ethylbenzene and xylenes) and (to a lesser extent) naphthalenes [37]. Thus, HFOs experience much lower weathering losses due to evaporation compared with crude oils. Another result of the distillation process is that the concentration of sulfur is greatly increased; sulfur in various forms occurs in all crude oils, but is concentrated in heavier fractions [38, 42]. This means that HFOs are likely to have higher

concentrations of such compounds as high molecular weight thiols and dibenzothiophene than crude oils. Additionally, if stored for an extended period of time, HFOs may oxidize, resulting in the presence of various by-products [42]. As with all oils, there will be slight (and widely varying) amounts of heavy metals such as vanadium, iron, magnesium, nickel and others. These metals are not removed in the distillation process and accumulate in HFOs relative to the original crude oils.

1.5 Potentially Toxic Components of Heavy Fuel Oils

It is critically important to identify the toxic components of heavy fuel oils. By establishing a relationship between the concentrations of specific compounds (or groups of compounds) and their effects, a better effort can be made to assess and remediate contaminated sites. Furthermore, chemical and biological fingerprints could be used to identify the source of pollution in a site with unknown history, and to relate it to observed effects.

In addition to toxicity tests on crude and heavy fuel oils, previous studies have shown many things about the relative toxicity of different classes of compounds. For example, Turcotte *et al.* showed that alkyl-PAH are substantially more toxic than unsubstituted PAH (specifically alkyl-phenanthrene vs. phenanthrene). They also showed that increasing octanol-water partition coefficient, K_{ow}, is linked to a proportional increase in toxicity [49]. Similarly, Barron *et al.* suggest that alkyl-phenanthrenes are a primary cause of toxicity of crude oil in the early life stages of fish [50]. HFO 7102 may or may not have higher alkyl-PAH concentrations than other oils, so those compounds (specifically alkyl-phenanthrene) will be important to monitor. Fallahtafti *et al.* studied various hydroxylated alkyl-phenanthrenes, finding that some were more toxic than their alkyl-derivatives and some were less toxic [51]. These hydroxylated compounds

would likely be present in higher concentrations in oxidized oils, or as metabolized products of alkyl-phenanthrenes. Basu *et al.* showed that, while the toxicity of complex mixtures of PAHs with similar properties may be predicted, it is substantially more difficult to predict the toxicity of complex mixtures of PAHs with different properties [18]. This would indicate that PAHs and other toxicants that have been previously tested for toxicity individually may have positive or negative effects when mixed with other PAHs in HFOs.

1.6 Chemical Analysis of Heavy Fuel Oils

The purpose of this project is to correlate the composition of heavy fuel oils to their toxic effects. Chemical analysis can be done by Gas Chromatography coupled to either Mass Spectrometry (GC-MS) or Flame Ionization Detection (GC-FID), or by High Performance Liquid Chromatography (HPLC), all of which are common methods for petroleum and hydrocarbon analysis [26, 52-61].

1.6.1 GC-MS and GC-FID Analysis

GC-MS and GC-FID are both excellent at analyzing saturated hydrocarbons [44]. Typical analysis requires the identification and quantitation of n-alkanes (C8 to C40), hopanes and steranes [62]. The distribution of n-alkanes is a strong indicator of whether the source is biogenic or petrogenic. For biogenic sources, n-alkanes with an odd carbon number are substantially more prevalent than n-alkanes with an even carbon number. In cases of petrogenic contamination, concentration decreases with increasing carbon number. The source may be determined by calculating the carbon performance index (CPI) as shown below (Equation 1.1). Typical oil samples have a CPI of approximately one, while samples with a strong biogenic influence will have a CPI of at least two, and possibly much higher [62]. In addition, the ratio of pristane to

phytane will be quite high for samples with a strong biogenic influence, as pristane is derived predominantly from plant sources.

$$CPI = \frac{nC_{21}+nC_{23}+nC_{25}+nC_{27}+nC_{29}+nC_{31}+nC_{33}}{nC_{22}+nC_{24}+nC_{26}+nC_{28}+nC_{30}+nC_{32}} \qquad (1.1)$$

It is also essential to analyze and quantify PAH because of their strong link to toxicity [49, 50]. Different classes of crude and heavy fuel oils have very different PAH profiles, and GC-MS analysis is excellent at "fingerprinting" oils to determine the source of pollution [56]. A number of un-substituted and alkyl-substituted PAH (one to four alkyl carbons; C0 to C4), including the United States Environmental Protection Agency (US EPA) 16 priority pollutant PAH, are routinely analyzed and quantified in petroleum analysis (Table 1.1). PAH with more than four alkyl carbons surely exist, but are not quantified in this method.

The PAH profile of crude oils and HFO can be compared with combustion (or pyrogenic) sources of PAH. First, petrogenic sources generally have much higher concentrations of alkyl-PAH than unsubstituted PAH. Conversely, a PAH signature from a combustion source shows that unsubstituted PAH have much higher concentrations than their alkylated homologues [44]. The second trend is that the alkylated concentration profile is bell-shaped for a given PAH (i.e. for C0-C4 phenanthrenes, the concentration of C0 and C4 isomers will be significantly lower than the isomers of the intermediate carbon number alkyl-phenanthrenes). PAH signatures from a pyrogenic source favour low degrees of alkyl substitution, such that C0>C1>C2>C3>C4 [44]. Additionally, when oil is weathered, the compounds with lower degrees of alkylation are degraded more readily, resulting in a significant decrease in the proportion of C0 and C1 isomers [37].

Table 1.1: List of PAHs quantified by GC-MS and their abbreviations

Compound	Abbrev.	# of Rings	Compound	Abbrev.	# of Rings
Naphthalenes			**Naphthobenzothiophenes**		
C0 naphthalene	C0-NAPH	2	C0 napthobenzothiophene	C0-NBT	4
C1 naphthalenes	C1-NAPH	2	C1 napthobenzothiophenes	C1-NBT	4
C2 naphthalenes	C2-NAPH	2	C2 napthobenzothiophenes	C2-NBT	4
C3 naphthalenes	C3-NAPH	2	C3 napthobenzothiophenes	C3-NBT	4
C4 naphthalenes	C4-NAPH	2	C4 napthobenzothiophenes	C4-NBT	4
Dibenzothiophenes			**Chrysenes**		
C0 dibenzothiophene	C0-DBT	3	C0 chrysene	C0-CHRY	4
C1 dibenzothiophenes	C1-DBT	3	C1 chrysenes	C1-CHRY	4
C2 dibenzothiophenes	C2-DBT	3	C2 chrysenes	C2-CHRY	4
C3 dibenzothiophenes	C3-DBT	3	C3 chrysenes	C3-CHRY	4
C4 dibenzothiophenes	C4-DBT	3	C4 chrysenes	C4-CHRY	4
Fluorenes			**Other Priority PAH**		
C0 fluorene	C0-FLUOR	3	Acenaphthene	Ace	3
C1 fluorenes	C1-FLUOR	3	Acenaphthylene	Acl	3
C2 fluorenes	C2-FLUOR	3	Anthracene	Anth	3
C3 fluorenes	C3-FLUOR	3	Fluoranthene	Fl	4
Phenanthrenes			Benz[*a*]anthracene	BaA	4
C0 phenanthrene	C0-PHEN	3	Benzo[*b*]fluoranthene	BbF	4
C1 phenanthrenes	C1-PHEN	3	Benzo[*k*]fluoranthene	BkF	4
C2 phenanthrenes	C2-PHEN	3	Benzo[*e*]pyrene	BeP	5
C3 phenanthrenes	C3-PHEN	3	Benzo[*a*]pyrene	BaP	5
C4 phenanthrenes	C4-PHEN	3	Perylene	Per	5
Pyrenes			Indeno[*1,2,3-cd*]pyrene	IP	6
C0 pyrene	C0-PYR	4	Dibenz[*a,h*]anthracene	DBA	5
C1 pyrenes	C1-PYR	4	Benzo[*ghi*]perylene	BP	6
C2 pyrenes	C2-PYR	4			
C3 pyrenes	C3-PYR	4			
C4 pyrenes	C4-PYR	4			

Most of the environmentally relevant PAH are reasonably volatile and thermally stable, thus gas chromatography is a viable form of analysis. Generally, the GC column used will have either a methyl or phenyl substituted polysiloxane stationary phase [63]. While GC-MS and GC-

FID have similar sensitivity, the co-eluting peaks of complex petroleum samples usually result in reduced selectivity of the GC-FID method. Thus, GC-MS methods operating in selective ion mode (SIM) are most commonly used for PAH identification and quantitation in petroleum samples. A suite of per-deuterated PAHs are included as internal standards; they behave similarly to the target PAHs during sample preparation and analysis but can be distinguished in the detector. Including them is a reliable indicator of percent recovery of PAH during solvent extraction and other pre-treatment of oil samples [63].

1.6.2 HPLC Analysis

HPLC is a good tool for characterizing heavy fuel oil and oil fractions. It may be used to isolate specific PAH from complex mixtures for further analysis, through the use of HPLC-fluorescence, HPLC coupled to an ultraviolet absorbance (UV) detector, or HPLC-MS. Normal-phase HPLC is quite adept at separating PAH mixtures by number of aromatic rings [58]. Reverse-phase HPLC may then be used to separate alkyl-PAH from un-substituted PAH [61]. The chromatogram of an oil sample may also be compared to that of a PAH mixture to establish the presence or absence of various compounds and to quantify their approximate concentrations. However, UV detectors lack the sensitivity and selectivity to perform highly accurate quantitative analysis. In general, much better resolution could be achieved with gas chromatography, and mass spectrometry detectors have much higher selectivity than ultraviolet detectors [64]. While normal- and reverse-phase HPLC have been shown to be excellent at separating mixtures of aromatic compounds by number of rings and degree of alkylation respectively, more accurate quantification and characterization of aromatic compounds in a given sample may be achieved through GC-MS.

1.7 Identifying Toxic Compounds: Effects-Driven Fractionation and Analysis

This project utilizes Effects-Driven Fractionation and Analysis (EDFA) to determine the most toxic compounds and groups of compounds in heavy fuel oil (Figure 1.3). The HFO is separated through physical and chemical means and the resulting fractions are analyzed and tested for toxicity to the early life stages of rainbow trout (*Oncorhynchus mykiss*). The fractions that prove most toxic are further fractionated, and their sub-fractions tested for toxicity, and so on. Each fraction is chemically analyzed to identify individual toxicants. This iterative process concentrates the toxicity while reducing the chemical complexity at each stage.

The EDFA approach is quite advantageous for several reasons. It is a good technique to identify toxic and non-toxic sub-fractions of complex chemical mixtures. It may identify toxic groups of compounds whose pollution history is not well-known. In combination with toxicity tests, the end point (i.e. disease or death) may be accurately assessed in an unbiased and unambiguous fashion.

However, there is no set method for fractionating all complex mixtures. Due to variations in composition, each EDFA on a new mixture (or new type of oil) will need to be re-optimized and these modifications may be time-consuming, laborious, and expensive. Additionally, a large amount of starting material is required so that enough material remains in the smallest fractions for toxicity tests.

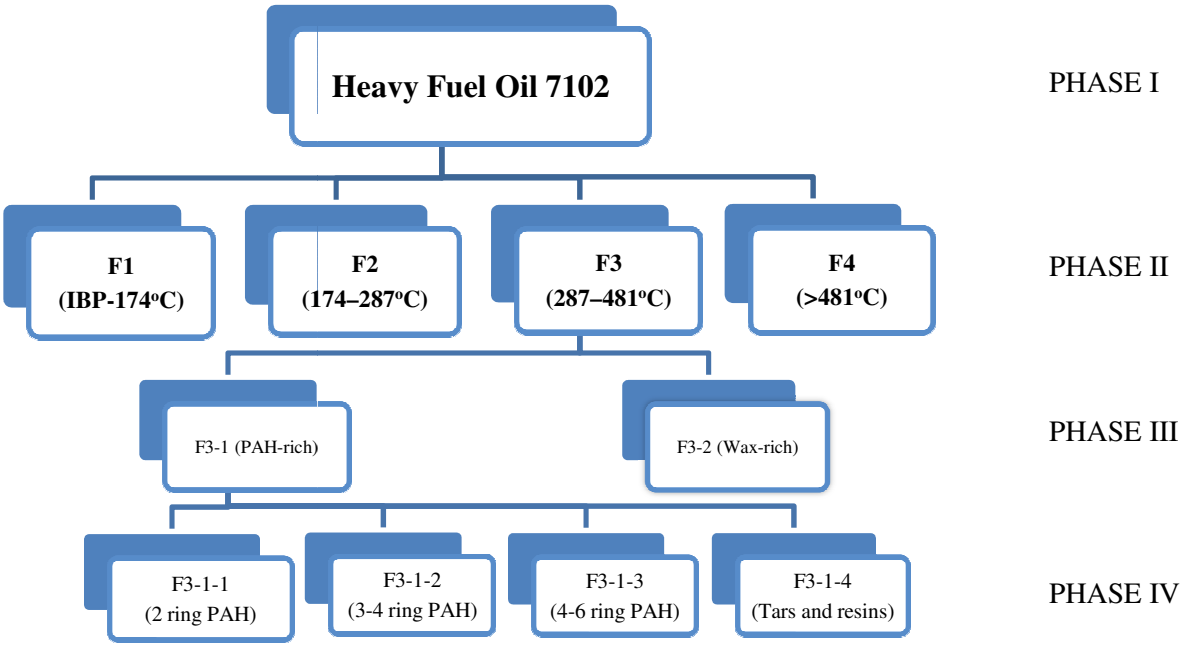

Figure 1.3: Effects-Driven Fractionation Flow Chart for HFO 7102

The primary aim of fractionation is to reduce the chemical complexity of the oil. With each successive fractionation step, chemical analyses are performed to verify desired separations and to identify the toxicants and the non-toxic components. Initial fractionation must be done on a large scale so that a sufficient amount of material carries through for further separations. While earlier separations may be based on volatility (Phase II) or solubility (Phase III), latter-stage fractionations rely on chromatography (Phase IV).

1.7.1 Phase II Separation: Low-Temperature Vacuum Distillation

A simple preliminary separation for HFOs is by volatility. A technique for low-temperature vacuum distillation was developed by the American Society for Testing and Materials (ASTM) [65] and optimized by Zhendi Wang's lab at Environment Canada, Ottawa. A large amount of oil may be heated and divided at pre-determined temperature cut-off points

(according to the Canadian Council of Ministers of the Environment [66]); the oil can be separated into four fractions (labelled F1, F2, F3, F4). Because a vacuum is used, the oil boils at a lower temperature than normal, which helps to reduce or eliminate some adverse effects of distillation (e.g. cracking, strain on glassware).

1.7.2 Phase III Separation: Asphaltene and Wax Precipitation

In an effort to concentrate known toxicants, other groups of compounds may be removed based on a difference in solubility. Buenrostro-Gonzalez *et al.* have optimized the procedure for asphaltene precipitation: dissolving an oil (or oil fraction) in n-pentane at room temperature over a 24 hour period and collecting the precipitate by vacuum filtration [48]. Another group of compounds shown to be relatively non-toxic and that can be removed by precipitation is waxes (long chain alkanes) [4, 5]. The method for their separation was developed by Burger *et al.* [67] and optimized through previous work by our group [5]. The oil fraction may be dissolved in acetone and cooled in the freezer. Over a five hour period, the waxes precipitate and can be collected by vacuum filtration.

1.7.3 Phase IV Separation: Chromatography

The final phase of separation can be achieved through chromatography. Open column chromatography, with a polar stationary phase (such as alumina), can be used to separate components into non-polar, slightly-polar, and polar fractions [68]. Normal phase HPLC, with a stationary phase such as silica or aminopropylsililated silica has also been used to separate PAH mixtures by number of aromatic rings [68]. After trying both of these methods, it was determined that open column chromatography was more practical for higher volume separations (up to 11 g), while HPLC is best for more precise analysis or small volume separations (less than 200 mg).

With either method, a PAH-rich oil fraction may be separated into multiple sub-fractions with different polarities and number of aromatic rings.

1.7.4 Fish Exposure and Toxicity Testing

Toxicity tests for this work were done by colleagues in the Department of Biology and School of Environmental Studies at Queen's University. Because it is likely for spilled oil to contaminate aquatic environments, toxicity tests of petroleum products have been performed on a variety of fish species, including rainbow trout [4], whitefish [9], zebrafish [69], and Japanese medaka [51]. Sprague *et al.* developed standard methods of toxicity testing in the 1960s and 1970s [24, 70, 71]. The term "toxicity test" can be replaced with the term "bioassays", meaning "the measurement of the potency of any stimulus, physical, chemical, or biological, physiological or psychological, by means of the reactions that it produces in living matter" [72]. In other words, the fish are used like an analytical instrument. They are exposed to a complex mixture whose components are not exactly known in their entirety (such as heavy fuel oil), and the effects are monitored to establish the presence and relative concentration or potency of the toxic components. In addition to mortality, there are several sub-lethal signs of toxicity that can be monitored. Cytochrome P4501A (CYP1A) enzymes are known to be induced by PAH [73], and can be assayed as ethoxyresorufin-O-deethylase (EROD) activity in liver tissue of juvenile fish [74]. Any measured increase in EROD activity is an indicator of exposure to PAH, and may be linked to toxic effects caused by reactive oxygen species [75, 76]. An important syndrome of sub-lethal toxicity to fish embryos is blue sac disease (BSD), a non-contagious disease that includes such signs as "yolk sac edema; subcutaneous hemorrhages of the yolk sac, ocular and cranial tissues; craniofacial malformations; and fin rot and erosion" [75].

There are many methods of determining the effects of exposing fish to petroleum products. There are *in-situ* observations of fish in their natural habitat after addition of oil or an actual spill, exposure after partitioning of hydrocarbons to water from oil-coated substrates, and immersion of fish in solutions of chemically dispersed oil. Both oil-coated gravel and chemically dispersed oil exposures have been used by our group in the past [4, 6]. The first method involves gravel coated with various amounts of oil stored in a column, with water pumped through to a bowl of fish. The amount of oil that dissolves in the water can be measured and fish toxicity can be observed. For the second method, the oil is first mixed with water and dispersant to create a chemically-enhanced water accommodated fraction (CEWAF), and the fish are exposed to the CEWAF by daily addition to a fresh medium, as outlined by Singer *et al.* [77]. Median lethal concentrations (LC_{50}) and median effective concentrations (EC_{50}) may be determined for the oil or oil fraction.

1.8 Objectives

The objective of this project was to fractionate and identify compounds and groups of compounds in HFO 7102 based on their toxicity to rainbow trout embryos. By corollary, compounds and groups of compounds that are non-toxic to rainbow trout embryos at all concentrations tested would also be identified. Better understanding of the composition of HFO 7102 and the most toxic fractions will provide a more accurate assessment of the potential environmental and ecological impacts of a spill. Additionally, it is our hope that the EDFA approach developed may be considered the framework for future HFO compositional analysis and bioassay studies. The separations conducted, toxicity tests performed, and chemical analyses carried out will explain the nature of heavy fuel oils as a toxic petroleum product. The most toxic

components will be isolated and grouped, and the fractions will be quantified by GC-MS. Specifically, the three- to five-ring PAHs and alkyl-PAHs will be followed due to the fact that they were previously identified as a component of major concern. Other HFOs may be analyzed in a similar manner to identify compositional differences and predict the effects that those differences will have on aquatic populations. The results of this work will be especially useful in the implementation of ecological risk assessments, natural resource damage assessments, establishing cause and effect for liability, and environmental forensics.

1.9 Organization of this Thesis

This thesis is divided into four chapters including this introduction to illustrate the fractionation approach conducted as well as the ensuing toxicity tests and chemical analysis. Chapter 2 describes all three phases of separation in the EDFA (low-temperature vacuum distillation, asphaltene and wax precipitation, and column chromatography) of HFO 7102. Chapter 3 explores the analysis of each fraction generated: the bioassays and their results (as conducted by Julie Adams, Department of Biology, Queen's University) and the analytical methods used to characterize the fractions. Finally, Chapter 4 summarizes the results with an overview of the advantages and limitations of the EDFA, as well as potential applications for the method developed and the resulting information about HFO 7102.

1.10 References

1. U.S. Energy Information Administration. *Annual Energy Outlook 2011;* Technical Report;, April 2011.

2. Wolfe, D. A.; Hameedi, M. J.; Galt, J. A.; Watabayashi, G.; Short, J.; O'Claire, C.; Rice, S.;

Michel, J.; Payne, J. R.; Braddock, J.; Hanna, S.; Sale, D. The Fate of the Oil Spilled from the Exxon Valdez. *Environmental Science & Technology* **1994,** *28* (13), 561-568.

3. McNutt, M.; Camilli, R.; Guthrie, G.; Hsieh, P.; Labson, V.; Lehr, B.; Maclay, D.; Ratzel, A.; Sogge, M. *Assessment of Flow Rate Estimates for the Deepwater Horizon/Macondo Well Oil Spill;* Flow Rate Technical Group report to the National Incident Command, Interagency Solutions Group, March 10, 2011.

4. Khan, C. *Identification of Compounds in Crude Oil that are Chronically Toxic to the Early Life Stages of Fish;* MSc Thesis; Queen's University, Biology Department: Kingston, ON, 2007.

5. Saravanabhavan, G. *An Effect-Driven Fractionation Approach for the Isolation and Characterization of CYP1A Inducing Components of Crude Oils;* PhD Thesis; Queen's University: Kingston, ON, 2007.

6. Martin, J. D. *Comparative Toxicology and Bioavailability of Heavy Fuel Oils to Fish Using Different Exposure Scenarios;* M.Sc. Thesis ; Queen's University: Kingston, ON, 2011.

7. Bilbao, E.; Raingeard, D.; Diaz de Cerio, O.; Ortiz-Zarragoitia, M.; Ruiz, P.; Izagirre, U.; Orbea, A.; Marigomez, I.; Cajaraville, M.; Cancio, I. Effects of exposure to Prestige-like heavy fuel oil and to perfluorooctane sulfonate on conventional biomarkers and target gene transcription in the thicklip grey mullet Chelon labrosus. *Aquatic Toxicology* **2010,** *98,* 282-296.

8. Shaban, A.; Ghoneim, E.; Hamzé, M.; El-Baz, F. A Post-Conflict Assessment to Interpret the Distribution of Oil Spill Off-Shore Lebanon Using Remote Sensing. *Lebanese Science Journal* **2007,** *8* (2), 75-85.

9. Debruyn, A. M.; Wernick, B. G.; Stefura, C.; McDonald, B. G.; Rudolph, B.-L.; Patterson, L.; Chapman, P. M. In Situ Experimental Assessment of Lake Whitefish Development Following a Freshwater Oil Spill. *Environmental Science & Technology* **2007,** *41* (20), 6983-6989.

10. Alonso-Alvarez, C.; Pérez, C.; Velando, A. Effects of acute exposure to heavy fuel oil from the Prestige spill on a seabird. *Aquatic Technology* **2007,** *84,* 103-110.

11. Baird, S. J.; Bailey, E. A.; Vorhees, D. J. Evaluating Human Risk from Exposure to Alkylated PAHs in an Aquatic System. *Human and Ecological Risk Assessment* **2007,** *13* (2), 322-338.

12. Baek, K.-H.; Kim, H.-S.; Oh, H.-M.; Yoon, B.-D.; Kim, J.; Lee, I.-S. Effects of Crude Oil, Oil Components, and Bioremediation on Plant Growth. *Journal of Environmental Science and*

Health, Part A **2004,** *A39* (9), 2465-2472.

13. Kazlauskiene, N.; Svecevicius, G.; Vosyliene, M.; Marciulioniene, D. Comparative Study on Sensitivity of Higher Plants and Fish to Heavy Fuel Oil. *Environmental Toxicology* **2004,** *19,* 449-451.

14. Phillips, B. M.; Anderson, B. S.; Hunt, J. W.; Clark, S. L.; Voorhees, J. P.; Tjeerdema, R. S.; Casteline, J.; Stewart, M. Evaluation of phase II toxicity identification evaluation methods for freshwater whole sediment and interstitial water. *Chemosphere* **2009,** *74,* 648-653.

15. Venosa, A. D.; Suidan, M. T.; Wrenn, B. A.; Strohmeier, K. L.; Haines, J. R.; Eberhart, B. L.; King, D.; Holder, E. Bioremediation of an Experimental Oil Spill on the Shoreline of Delaware Bay. *Environmental Science & Technology* **1996,** *30* (5), 1764-1775.

16. Anderson, B. S.; Arenella-Parkerson, D.; Phillips, B. M.; Tjeerdema, R. S.; Crane, D. Preliminary investigation of the effects of dispersed Prudhoe Bay Crude Oil on developing topsmelt embryos, Atherinops affinis. *Environmental Pollution* **2009,** *157,* 1058-1061.

17. Barron, M. G.; Heintz, R.; Rice, S. D. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor antagonists in fish. *Marine Environmental Research* **2004,** *58,* 95-100.

18. Basu, N.; Billiard, S.; Fragoso, N.; Omoike, A.; Tabash, S.; Brown, S.; Hodson, P. Ethoxyresorufin-o-deethylase induction in trout exposed to mixtures of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry* **2001,** *20* (6), 1244-1251.

19. Duarte, R. M.; Honda, R. T.; Val, A. L. Acute effects of chemically dispersed crude oil on gill ion regulation, plasma ion levels and haematological parameters in tambaqui (Colossoma macropomum). *Aquatic Toxicology* **2010,** *97,* 134-141.

20. Hatlen, K.; Sloan, C. A.; Burrows, D. G.; Collier, T. K.; Scholz, N. L.; Incardona, J. P. Natural sunlight and residual fuel oils are an acutely lethal combination for fish embryos. *Aquatic Toxicology* **2010,** *99,* 56-64.

21. Jung, J. H.; Yim, U. H.; Han, G. M.; Shim, W. J. Biochemical changes in rockfish, Sebastes schlegeli, exposed to dispersed crude oil. *Comparative Biochemistry and Physiology, Part C* **2009,** *150,* 218-223.

22. Olsvik, P. A.; Hansen, B. H.; Nordtug, T.; Moren, M.; Holen, E.; Lie, K. K. Transcriptional evidence for low contribution of oil droplets to acute toxicity from dispersed oil in first feeding Atlantic cod (Gadus morhua) larvae. *Comparative Biochemistry and Physiology, Part*

C **2011,** *154,* 333-345.

23. Short, J. W. Long-Term Effects of Crude Oil on Developing Fish: Lessons from the Exxon Valdez Oil Spill. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* **2003,** *25* (6), 509-517.

24. Sprague, J. B. Measurement of Pollutant Toxicity to Fish I. Bioassay Methods for Acute Toxicity. *Water Research* **1969,** *3,* 793-821.

25. Van Scoy, A. R.; Lin, C. Y.; Anderson, B. S.; Philips, B. M.; Martin, M. J.; McCall, J.; Todd, C. R.; Crane, D.; Sowby, M. L.; Viant, M. R.; Tjeerdema, R. S. Metabolic responses produced by crude versus dispersed oil in Chinook salmon pre-smolts via NMR-based metabolomics. *Ecotoxicology and Environmental Safety* **2010,** *73,* 710-717.

26. Yu, Y.; Wade, T. L.; Fang, J.; McDonald, S.; Brooks, J. M. Gas Chromatographic-Mass Spectrometric Analysis of Polycyclic Aromatic Hydrocarbon Metabolites in Antarctic Fish (Notothenia gibberifrons) Injected with Diesel Fuel Arctic. *Archives of Environmental Contamination and Toxicology* **1995,** *29,* 241-246.

27. Saco-Alvarez, L.; Bellas, J.; Nieto, O.; Bayona, J. M.; Albaiges, J.; Beiras, R. Toxicity and phototoxicity of water-accommodated fraction obtained from Prestige fuel oil and Marine fuel oil evaluated by marine bioassays. *Science of the Total Environment* **2008,** *394,* 275-282.

28. Singer, M. M.; Smalheer, D. L.; Tjeerdema, R. S. Effects of Spiked Exposure to an Oil Dispersant on the Early Life Stages of Four Marine Species. *Environmental Toxicology & Chemistry* **1991,** *10* (10), 1367-1374.

29. Fingas, M.; Hollebone, B.; Fieldhouse, B. *The Density Behaviour of Heavy Oils in Freshwater: The Example of the Lake Wabamun Spill;* Technical Report; Emergencies Science and Technology Division, Environment Canada, 2006.

30. Birtwell, I. *Comments on the effects of oil spillage on fish and their habitat – Lake Wabamun, Alberta;* Report submitted to Public Prosecution Service of Canada, Alberta Regional Office; Fisheries and Oceans Canada, 2008.

31. Evans, D. *An evaluation of the harmful alteration, disruption and destruction (HADD) of fish habitat following the Bunker 'C' and pole treating oil release on Wabamun Lake, Alberta;* Report submitted to Public Prosecution Service of Canada, Alberta Regional Office; Fisheries and Oceans Canada, Prairies Area Operations, Central and Arctic Region, 2008.

32. Anderson, A.-M. *Wabamun Lake Oil Spill August 2005: Data Report for Water and Sediment Quality in the Pelagic Area of the Lake;* Alberta Environment, April 2006.

33. Ansell, D.; Dicks, B.; Guenette, C.; Moller, T.; Santner, R.; White, I. A Review of the Problems Posed by Spills of Heavy Fuel Oils. *International Oil Spill Conference*, Tampa, FL, 2001; pp 591-596.

34. International Tanker Owners Pollution Federation. *Oil Tanker Spill Statistics 2011;* Technical Report;, 2011.

35. Kvenvolden, K. A.; Cooper, C. K. Natural seepage of crude oil into the marine environment. *Geo-Marine Letters* **2003,** *23* (3-4), 140-146.

36. Wang, Z.; Hollebone, B.; Fingas, M.; Fieldhouse, B.; Sigouin, L.; Landriault, M.; Smith, P.; Noonan, J.; Thouin, G.; Weaver, J. W. *Characteristics of Spilled Oils, Fuels, and Petroleum Products: 1. Composition and Properties of Selected Oils;* Environmental Protection Agency, 2003.

37. Wang, Z.; Fingas, M.; Blenkinsopp, S.; Sergy, G.; Landriault, M.; Sigouin, L.; Foght, J.; Semple, K.; Westlake, D. Comparison of oil composition changes due to biodegradation and physical weathering in different oils. *Journal of Chromatography A* **1998,** *809,* 89-107.

38. Speight, J. G. *Handbook of Petroleum Analysis;* John Wiley & Sons: Toronto, 2001.

39. Environment Canada, ES&T. *Bunker C Fuel Oil;* Technical Report;, 1999.

40. Fan, T.; Buckley, J. S. Rapid and Accurate SARA Analysis of Medium Gravity Crude Oils. *Energy & Fuels* **2002,** *16,* 1571-1575.

41. Baird, C.; Cann, M. *Environmental Chemistry,* 4th ed.; W.H. Freeman and Company: New York, 2008.

42. American Bureau of Shipping. *Notes on Heavy Fuel Oil;* Technical Report;, 2001.

43. Wang, Z.; Fingas, M. Oil and Petroleum Product Fingerprinting Analysis by Gas Chromatographic Techniques. In *Chromatographic Analysis of the Environment, 3rd Ed.;* Taylor & Francis Group: Boca Raton, FL, 2006.

44. Wang, Z.; Fingas, M.; Li, K. Fractionation of a Light Crude Oil and Identification and Quantiation of Aliphatic, Aromatic, and Biomarker Compounds by GC-FID and GC-MS, Part I. *Journal of Chromatographic Science* **1994,** *32,* 361-382.

45. Prince, R. C.; Elmendorf, D. L.; Lute, J. R.; Hsu, C. S.; Halth, C. E.; Senius, J. D.; Dechert, G. J.; Douglas, G. S.; Butler, E. L. 17α(H),21β(H)-Hopane as a Conserved Internal Marker for Estimating the Biodegradation of Crude Oil. *Environmental Science & Technology* **1994,** *28* (1), 142-145.

46. Kazakova, L. P.; Gundyrev, A. A.; Litvin, N. I.; Shubina, E. S.; Mukhin, M. L. Composition and Structure of Resins in Lube Distillates from West Siberian Crudes. *Chemistry and Technology of Fuels and Oils* **1994,** *30* (1-2), 91-96.

47. Groenzin, H.; Mullins, O. C. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy & Fuels* **2000,** *14,* 677-684.

48. Buenrostro-Gonzalez, E.; Lira-Galeana, C.; Gil-Villegas, A.; Wu, J. Asphaltene Precipitation in Crude Oils: Theory and Experiments. *American Institute of Chemical Engineers Journal* **2004,** *50* (10), 2552-2570.

49. Turcotte, D.; Akhtar, P.; Bowerman, M.; Kiparissis, Y.; Brown, R. S.; Hodson, P. V. Measuring the toxicity of alkyl-phenanthrenes to early life stages of medaka (Oryzias lapites) using partition-controlled delivery. *Environmental Science & Technology* **2011,** *30* (2), 487-495.

50. Barron, M. G.; Carls, M. G.; Heintz, R.; Rice, S. D. Evaluation of Fish Early Life-Stage Toxicity Models of Chronic Embryonic Exposures to Complex Polycyclic Aromatic Hydrocarbon Mixtures. *Toxicological Sciences* **2004,** *78,* 60-67.

51. Fallahtafti, S.; Rantanen, T.; Brown, R. S.; Snieckus, V.; Hodson, P. V. Toxicity of hydroxylated alkyl-phenanthrenes to the early life stages of Japanese medaka (Oryzias latipes). *Aquatic Toxicology* **2012,** *106-107,* 56-64.

52. Anyakora, C.; Ogbeche, A.; Palmer, P.; Coker, H.; Ukpo, G.; Ogah, C. GC/MS analysis of polynuclear aromatic hydrocarbons in sediment samples from the Niger Delta region. *Chemosphere* **2005,** *60,* 990-997.

53. Hites, R. Gas Chromatography Mass Spectrometry. In *Handbook of Instrumental Techniques for Analytical Chemistry;* Prentice Hall: Upper Saddle River, NJ, 1997.

54. Lao, R.; Thomas, R.; Oja, H.; Dubois, L. Application of a Gas Chromatograph-Mass Spectrometer-Data Processor Combination to the Analysis of the Polycyclic Aromatic Hydrocarbon Content of Airborne Pollutants. *Analytical Chemistry* **1973,** *45* (6), 908-915.

55. Sporstol, S.; Gjos, N.; Lichtenthaler, R. G.; Gustavsen, K. O.; Urdal, K.; Oreld, F.; Skel, J. Source Identification of Aromatic Hydrocarbons in Sediments Using GC/MS. *Environmental Science & Technology* **1983,** *17* (5), 282-286.

56. Wang, Z.; Fingas, M.; Sergy, G. Chemical Characterization of Crude Oil Residues from an Arctic Beach by GC/MS and GC/FID. *Environmental Science & Technology* **1995,** *29* (10), 2622-2631.

57. Borgund, A.; Erstad, K.; Barth, T. Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils. *Journal of Chromatography A* **2007,** *1149,* 189-196.

58. Kaminski, M.; Kartanowicz, R.; Gilgenast, E.; Namiesnik, J. High-Performance Liquid Chromatography in Group-Type Separation and Technical or Process Analytics of Petroleum Products. *Critical Reviews in Analytical Chemistry* **2005,** *35* (3), 193-216.

59. Naikwadi, K.; Charbonneau, G.; Karasek, F. Separation and identification of organic compounds in air particulate extracts by high-performance liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography* **1987,** *398,* 227-237.

60. Palmentier, J.-P. F.; Britten, A. J.; Charbonneau, G. M.; Karasek, F. W. Determination of polycyclic aromatic hydrocarbons in lubricating oil base stocks using high-performance liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography* **1989,** *469,* 241-251.

61. Saravanabhavan, G.; Helferty, A.; Hodson, P. V.; Brown, R. S. A multi-dimensional high performance liquid chromatographic method for fingerprinting polycyclic aromatic hydrocarbons and their alkyl-homologs in the heavy gas oil fraction of Alaskan North Slope crude. *Journal of Chromatography A* **2007,** *1156,* 124-133.

62. Alimi, H.; Ertel, T.; Schug, B. Fingerprinting of Hydrocarbon Fuel Contaminants: Literature Review. *Environmental Forensics* **2003,** *4,* 25-38.

63. Poster, D. L.; Schantz, M. M.; Sander, L. C.; Wise, S. A. Analysis of Polycyclic Aromatic Hydrocarbonds (PAHs) in Environmental Samples: a Critical Review of Gas Chromatographic (GC) Methods. *Analytical and Bioanalytical Chemistry* **2006,** *386,* 859-881.

64. Lawrence, J. F. Advantages and Limitations of HPLC in Environmental Analysis. *Chromatographia* **1987,** *24,* 25-50.

65. ASTM International. *Standard Test Method for Distillation of Petroleum Products at Reduced Pressure;*, 2006.

66. Canadian Council of Ministers of the Environment. *Canada-Wide Standards for Petroleum Hydrocarbons (PHC) in Soil;* Technical Report; Winnipeg, April 30-May 1, 2001.

67. Burger, E.; Perkins, T.; Striegler, J. Studies of Wax Deposition in the Trans Alaska Pipeline. *Journal of Petroleum Technology* **1981,** *33* (6), 1075-1086.

68. Brack, W.; Kind, T.; Hollert, H.; Schrader, S.; Moder, M. Sequential fractionation procedure for the identification of potentially cytochrome P4501A-inducing compounds. *Journal of Chromatography A* **2003,** *986,* 55-66.

69. Scott, J. A.; Incardona, J. P.; Pelkki, K.; Shepardson, S.; Hodson, P. V. AhR2-mediated, CYP1A-independent cardiovascular toxicity in zebrafish (Danio rerio) embryos exposed to retene. *Aquatic Toxicology* **2011,** *101* (1), 165-174.

70. Sprague, J. B. Measurement of Pollutant Toxicity to Fish II. Utilizing and Applying Bioassay Results. *Water Research* **1970,** *4,* 3-32.

71. Sprague, J. B. Measurement of Pollutant Toxicity to Fish III. Sublethal Effects and "Safe" Concentrations. *Water Research* **1971,** *5,* 245-266.

72. Finney, D. J. *Probit Analysis,* 3rd ed.; Cambridge University Press: New York, 1971.

73. Stegeman, J. J.; Lech, J. J. Cytochrome P-450 Monooxygenase Systems in Aquatic Species: Carcinogen Metabolism and Biomarkers for Carcinogen and Pollutant Exposure. *Environmental Health Perspectives* **1991,** *90,* 101-109.

74. Whyte, J. J.; Jung, R. E.; Schmitt, C. J.; Tillitt, D. E. Ethoxyresorufin-O-deethylase (EROD) Activity in Fish as a Biomarker of Chemical Exposure. *Critical Reviews in Toxicology* **2000,** *30* (4), 347-570.

75. Billiard, S. M.; Querbach, K.; Hodson, P. V. Toxicity of Retene to Early Life Stages of Two Freshwater Fish Species. *Environmental Toxicology and Chemistry* **1999,** *18* (9), 2070-2077.

76. Brinkworth, L. C.; Hodson, P. V.; Tabash, S.; Lee, P. CYP1A induction and blue sac disease in early developmental stages of rainbow trout (Oncorhynchus mykiss) exposed to retene. *Journal of Toxicologicy and Environmental Health, Part A* **2003,** *66,* 627-646.

77. Singer, M. M.; Aurand, D. V.; Coelho, G. M.; Bragin, G. E.; Clark, J. R.; Sowby, M.; Tjeerdema, R. S. Making, Measuring, and using Water-Accommodated Fractions of Petroleum for Toxicity Testing. *International Oil Spill Conference*, Tampa, FL, 2001; pp 1269-1274.

Chapter 2

Fractionation of HFO 7102

2.1 Low-Temperature Vacuum Distillation of HFO 7102

The first phase of heavy fuel oil fractionation is low-temperature vacuum distillation. This was carried out at the Environment Canada Emergencies Science & Technology facilities in Ottawa, Ontario. The goal of the distillation is to split the oil into four fractions by boiling point, with the temperature cut-offs corresponding to the n-alkanes listed in Table 2.1, according to the Canadian Council of Ministers of the Environment (CCME) standards [1]. In general, this means that F1 should contain short-chain alkanes and BTEX-type aromatic compounds. The second fraction, F2, should contain some longer-chain alkanes and small (2 ring) PAH, F3 should contain long-chain alkanes and 3-6 ring PAH, while F4 should contain primarily asphaltenes and resins with some large (5-6+ ring) PAH [2]. These cuts provide adequate separation of compounds such that toxicity testing will consider a more manageable group (or groups) of compounds.

Table 2.1: Boiling Points and Corresponding n-alkanes of Four Oil Fractions. Fractions are separated by low-temperature vacuum distillation and separation points are according to CCME standards.

Fraction	Boiling Range (°C)	Corresponding n-alkanes
F1	Incipient Boiling Point (IBP) – 174	n-C_6 to n-C_{10}
F2	174 – 287	>n-C_{10} to n-C_{16}
F3	287 – 481	>n-C_{16} to n-C_{34}
F4	>481	>n-C_{34}

2.1.1 Materials and Methods

The low temperature vacuum distillation apparatus (Figure 2.1), consisted of a twelve litre round bottom flask containing the whole oil, a distillation column, a two-way, five-to-one distillation head (meaning that distillate either goes down to the initial flask or, one out of six

drops passes through to the receiving flask), two condensers and a pre-weighed one litre round bottom receiving flask. The system was under vacuum, protected by a cold trap (dry ice and acetone). The reflux condenser used cooling water, the distillation condenser used ethylene glycol, and each bath's temperature was controlled. The twelve litre round bottom flask sat in a heating mantle and was covered with another mantle. A temperature probe measured the liquid temperature in the large flask and a high-temperature thermometer was used to measure the vapour temperature in the distillation head. The pressure of the system was monitored and controlled by an MKS Pressure Transducer and Valve Controller.

The temperature cut-off points were according to vapour temperature, but must be considered at one atmosphere of pressure. Thus, vapour temperature and system pressure were used to calculate the atmospheric equivalent temperature (AET) according to the following equations [3].

$$AET = \frac{748.1A}{[1/(T+273.1)]+0.3861A-0.00051606} - 273.1 + t \qquad (2.1)$$

Where T is the observed vapour pressure in °C, and A is calculated in Equation 2.2 as follows:

$$A = \frac{5.994295-0.972546\log_{10}P}{2663.129-95.76\log_{10}P} \qquad (2.2)$$

Where P is the operating pressure in torr (provided P≥2 torr, confirmed during the procedure). These equations only work for a Watson K-factor of 12, and as such a temperature correction factor, *t*, must also be considered and is calculated in Equation 2.3 as follows:

$$t = -1.4[K-12]\left[\log_{10}\left(\frac{P_a}{P_o}\right)\right] \qquad (2.3)$$

Where P_a is atmospheric pressure, P_o is the observed pressure and K is the Watson K-factor, as determined by Equation 2.4:

$$K = \frac{\sqrt[3]{1.8(B+273.1)}}{D} \qquad (2.4)$$

Where B is the mean average boiling point (in °C) and D is the relative density at 15.6°C.

Values of B and D were obtained from previous research on this oil (596.2°C and 0.972 respectively) [4], giving a K-factor of 11.94. Thus, all temperature corrections to AET were minimal (less than 0.2°C).

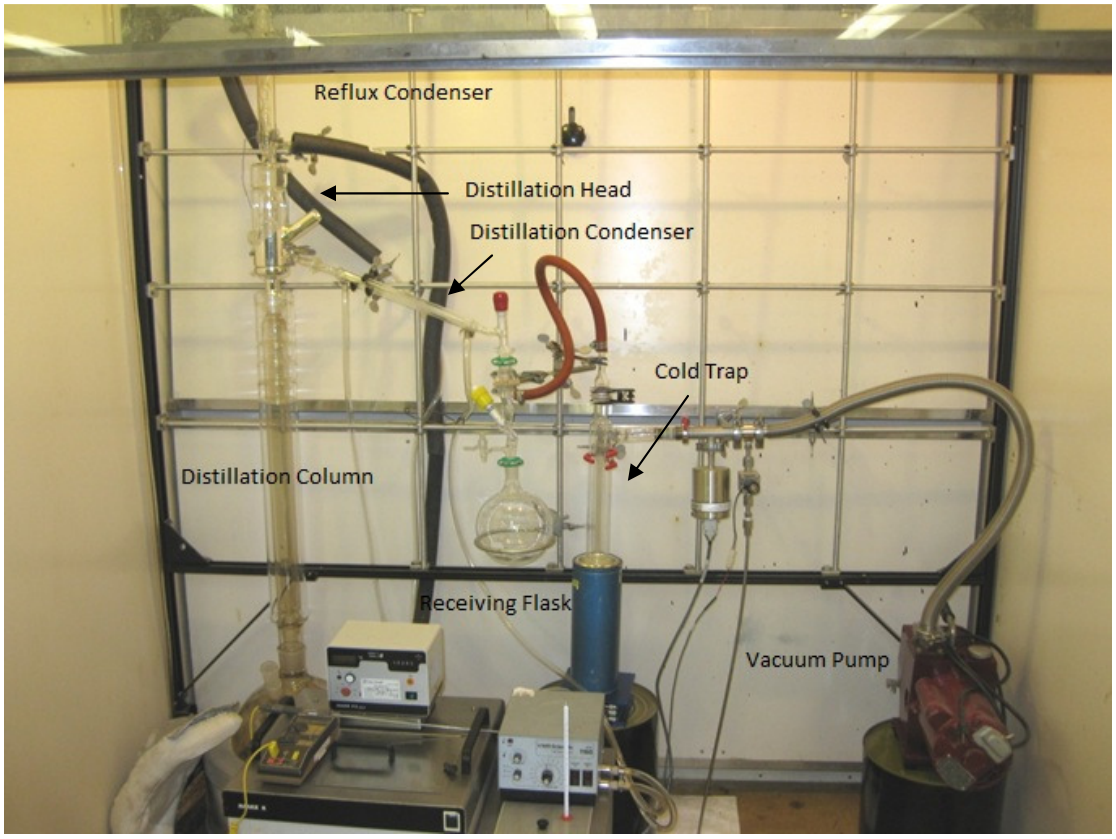

Figure 2.1: Low-Temperature Vacuum Distillation Apparatus: photograph and schematic.

A measured amount of HFO 7102 (3692 g) was loaded into the lower round bottom flask. The mantles were heated and pressure was lowered gradually (initially atmospheric, by the end 3 torr). All process variables were monitored regularly, and the process was not run overnight. As the oil boiled, the liquid and vapour temperatures were noted and AET calculated. Once a steady reflux developed, the distillation head was switched so that condensate flowed to the receiving flask. When the appropriate cut-off temperature (AET) was reached, the valves were shut and the receiving flask was removed and weighed. The cold trap was emptied at this time and added to

the fraction in the receiving flask to complete a mass balance. Fractions F1, F2 and F3 were collected in separate receiving flasks and F4 was the remaining oil in the original flask.

The cooling water in the reflux condenser was maintained near freezing point to facilitate quick condensation of the hot vapours. The distillation condenser fluid was kept slightly higher (30-40°C), particularly during the later stages, so that the more viscous and wax-containing fractions did not solidify in the condenser, but passed through to the receiving flask.

2.1.2 Results and Discussion

Over a period of seven days, HFO 7102 separated into three fractions; because the incipient boiling point was higher than 174°C, there was no F1. This is not entirely unexpected, because heavy fuel oils contain the heavier compounds of crude oil (as discussed in Chapter 1). The distribution of the remaining fractions by weight is shown in Table 2.2. Small losses occurred, perhaps due to oil sticking to various pieces of equipment, or being caught in the cold trap.

Table 2.2: Results from the fractionation of HFO 7102. Weight distribution of each fraction is shown.

Fraction	Mass (g)	Percent of Whole Oil
F1	0	0.0%
F2	250.3	6.8%
F3	937.1	25.4%
F4	2426.4	65.7%
TOTAL	*3613.8*	*97.9%*

Approximately 7% of the oil fractionated into F2, while about one-quarter separated into F3 and nearly two-thirds remained in F4. HFO 7102 is a very heavy oil. In contrast, Scotian Light (a light crude oil studied previously by our group) had 46.6% of its mass in F1 and was only 0.4%

F4 by weight [5]. A slightly higher (close to 100%) mass recovery should be attainable, but any material in the cold trap was inadvertently disposed rather than added to the fraction collected. The three fractions generated were analyzed chemically and the results are discussed in detail in Chapter 3. Toxicity testing to the early life stages of rainbow trout identified F3 as the most toxic fraction (see Chapter 3), so this was fractionated further.

2.2 Solvent Extractions of F3

The second level of the EDFA was a series of two chemical separations, each causing a group of compounds to precipitate from the F3 fraction. The first was an asphaltene precipitation accomplished by dissolving F3 in n-pentane. The second was a wax precipitation, achieved by dissolving F3 in acetone at low temperatures. By removing groups of similar compounds and performing toxicity tests on sub-fractions, certain groups of compounds were targeted for further fractionation and toxicity testing. The groups of compounds removed by precipitation were shown to be non-toxic at all concentrations tested.

2.2.1 Asphaltene Precipitation

In principle, F3 may contain some asphaltenes [6, 7], though the majority would be expected to be in F4 [2, 9]. To confirm the relative amount in F3, the asphaltenes were removed and their contribution to the overall composition noted.

2.2.1.1 Materials and Methods

The procedure was a modified version of the one outlined by Buenrostro-Gonzalez et al. [8]. The F3 oil fraction was first heated to 75°C for 30 minutes and sonicated to ensure that it was well-mixed. About 13-15 g of hot F3 was dissolved in n-pentane (Reagent Grade 98%, Caledon

Laboratories, Georgetown, Ont.) at a ratio of 50 mL: 1 g, the optimal n-alkane and solvent-to-oil ratio for the process [8]. The mixture was sonicated for 10 minutes and left for 24 hours at room temperature and pressure. The solution was filtered by vacuum filtration with a 0.45 μm Teflon membrane. The flask and membrane were rinsed with small amounts of n-pentane. To adequately measure the asphaltenes, they were dissolved in dichloromethane, transferred to a pre-weighed glass vial, and dried by nitrogen blow-down (for small amounts) or rotary evaporation (for larger solvent volumes). The amount of asphaltenes was determined gravimetrically.

2.2.1.2 Results and Discussion

This procedure was conducted three times (see Table 2.3 below). In each case, the amount of asphaltenes was 0.032% or less of the initial mass of F3. This confirmed the prediction that the asphaltenes should be primarily in the F4 fraction, based on their very high boiling points [9]. Given that the asphaltenes content in F3 was so miniscule, it was impractical to repeat this procedure to generate sufficient asphaltenes for toxicity testing. Thus, all further fractionation of F3 skipped the asphaltene precipitation step.

Table 2.3: Asphaltene Precipitation Results

Trial Number	Initial Mass F3 (g)	Volume n-Pentane (mL)	Mass Asphaltenes (g)	Asphaltene Content (%)
1	14.2	710	0.0026	0.018
2	13.7	685	0.0044	0.032
3	14.8	740	0.0047	0.032

2.2.2 Wax Precipitation

The process of wax precipitation, initially developed by Burger et al. [10], involves dissolving the oil or oil fraction in a polar solvent at low temperatures. At these conditions, any

wax content in the oil will precipitate out over time, eventually reaching equilibrium. Considering the work of Burger and optimization carried out during previous research by our group [5], the optimal solvent for this procedure is acetone. The solvent ratio and extraction temperature were optimized for maximum wax precipitation and minimal PAH contamination in the wax fraction (F3-2).

Wax precipitation furthered the goal of isolating toxic compounds and groups of compounds in HFO 7102. By removing as much wax as possible in its purest form, the F3 fraction is divided into a PAH-rich fraction (F3-1) and a wax fraction (F3-2). Toxicity testing of these fractions directed the next phase of fractionation.

2.2.2.1 Materials and Methods

F3 (usually stored at -20°C) was first heated to about 70°C in a hot water bath for thirty minutes, then agitated for thirty seconds to ensure that all components were properly mixed. Approximately one gram of F3 was weighed precisely in a 50 mL Erlenmeyer flask, and the appropriate amount of acetone was added. All solvents used were HPLC grade and were obtained from Fisher Scientific (Fair Lawn, New Jersey). Flasks were sealed with rubber septa and sonicated for five minutes at about 35°C, then placed in an explosion-proof freezer (-20°C) for at least five hours.

After five hours, the mixtures were filtered by vacuum filtration using a 0.2 μm nylon filter. The Erlenmeyer flasks were rinsed at least twice with -20°C acetone, with the washings added to the filter, and the solids on the filter were washed twice more. When the solids on the filter were fairly dry, they were transferred to a 250 mL round bottom flask and the filter was rinsed with hexanes and dichloromethane. Usually, the solids from four filters were combined at

this stage. The solvent was removed by rotary evaporation and the wax-rich residue (F3-2) was collected in a pre-weighed glass vial.

The solvent in the filtrate was removed by nitrogen blowdown and rotary evaporation, leaving the PAH-rich extract (F3-1), which was collected in a pre-weighed glass vial. A mass balance was conducted to determine percent recovery and mass distribution of the two sub-fractions. A schematic of this process is shown in Figure 2.2 below.

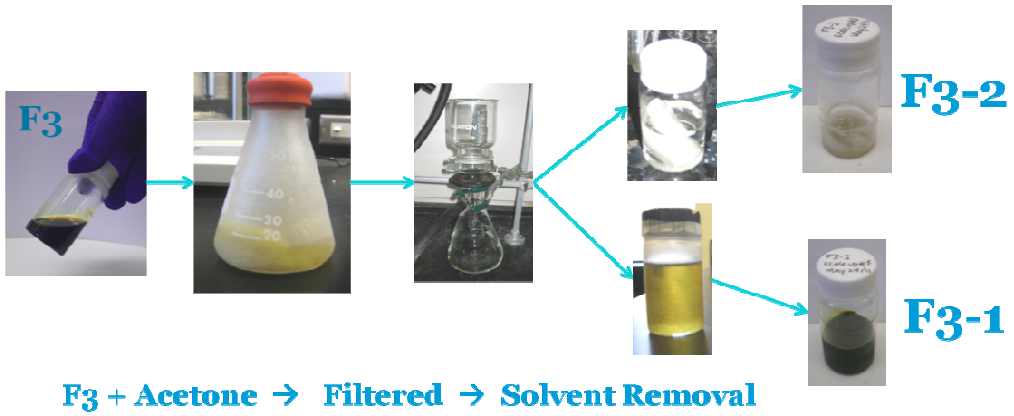

Figure 2.2: Wax Precipitation procedure. Fraction F3 was dissolved in acetone and stored in the freezer (-20°C) for five hours. Vacuum filtration separated the wax rich residue (F3-2) from the PAH-rich extract (F3-1).

2.2.2.2 Determining Optimal Solvent Ratio

Three different ratios of acetone to F3 were tested: 5 mL:1 g, 10:1 and 20:1. The goal was to identify the solvent ratio that removed as much of the wax as possible from F3, while concurrently having the least amount of PAH contamination in F3-2. With smaller amounts of solvent, solubility will decrease, meaning that trials run at 5:1 should have the largest residue by mass. The question was whether or not those solids that precipitated contained entirely waxes, or were contaminated with PAH.

2.2.2.3 Determining Optimal Extraction Temperature

In addition to trials in the freezer (-20°C), extractions were also conducted at -40°C using an acetone/dry ice bath. Similarly, the goal was to remove as much wax as possible from F3 with minimal PAH contamination in F3-2. At lower temperatures, solubility decreases. Thus, there should be a larger residue (by mass) when trials are conducted at -40°C. The question was whether or not this F3-2 contained entirely waxes or also some PAH.

2.2.2.4 Quantitative Analysis for Optimization

In addition to gravimetric analysis for weight distribution, additional analysis was done by High Performance Liquid Chromatography coupled to an ultraviolet-visible detector (HPLC-UV-Vis). The HPLC column was a semi-preparative Zorbax silica column with dimensions 9.4 mm ID x 250 mm (Agilent Technologies). The apparatus was a Varian ProStar 215 with a Varian ProStar 320 UV-Vis detector set to 254 nm. The mobile phase was a 49:1 mixture of hexanes to dichloromethane (all solvents HPLC grade, Fisher Scientific, Fair Lawn, New Jersey) with column regeneration by dichloromethane backflush. Samples were injected at a concentration of 650 µg for a 20µL injection loop. Analysis was performed using Varian Star Chromatography Workstation version 5.31 software.

2.2.2.5 Results and Discussion

The first variable to be tested was solvent ratio. In previous work by our group studying crude oils, changing the solvent ratio from 40:1 to 5:1 caused the residue to vary from about 25% to over 45% by mass. [5] However, a much smaller difference was observed with HFO 7102. As shown in Table 2.4 below, the wax-rich fraction F3-2 was fairly consistent at 15-16%. Although

average values trended in the predicted direction, the variability within each set of trials was such that there was no significant difference in wax precipitated regardless of solvent ratio.

Table 2.4: Solvent Ratio Optimization Results. Experiments conducted at -20°C (n=4). Average values are shown with uncertainties equal to one standard deviation.

Solvent Ratio	Average Mass % F3-1	Average Mass % F3-2	Average Mass Recovery %
5:1	74.4 ± 4.5	16.7 ± 2.6	91.1 ± 1.8
10:1	77.6 ± 4.5	15.1 ± 2.5	92.3 ± 1.5
20:1	80.9 ± 1.8	15.0 ± 2.0	95.9 ± 3.4

Given that the mass separation was relatively independent of solvent ratio, another important property to assess is PAH content in the wax-rich fraction. HPLC analysis of each trial revealed (as expected) that trials done with a solvent ratio of 5:1 had the highest amount of PAH contamination, as determined by UV absorbance. Each two-fold increase in solvent ratio corresponds to roughly a three-fold increase in PAH content (see Table 2.5). To minimize PAH loss and to use a fairly minimal amount of solvent, all further wax precipitations were conducted at a solvent ratio of 10:1, where the total PAH content in the wax was less than 5% of that in the whole fraction F3.

Table 2.5: Solvent Ratio Comparison for PAH Content (n=1)

Solvent Ratio	F3-2 Area Under Curve (Arbitrary Units)	Percentage of Area Under Curve for F3
5:1	28,646,782	8.8%
10:1	9,366,137	2.9%
20:1	2,725,603	0.8%

The next variable tested was extraction temperature. An acetone/dry ice bath was maintained at -40°C, and acetone/F3 mixtures were placed in the bath (instead of the freezer) for a minimum of five hours. While the mass composition of F3-2 was relatively similar to that

obtained at -20°C (Table 2.6), it was observed that this F3-2 was much more contaminated with PAH due to its darker colour. This was confirmed by HPLC-UV analysis (Table 2.7) – indicating more than 10% of the PAH in this wax fraction. To avoid PAH loss from the extract, -20°C was selected as the optimal extraction temperature. The lower temperature did not provide a significant increase in mass of F3-2 wax precipitated, and the difficulty of working at lower temperatures relative to the convenience of using the freezer confirmed the decision. This last point is especially relevant because it allowed samples to be left in the freezer overnight, whereas an acetone/dry ice bath needs to be constantly monitored to ensure that it remains the correct temperature.

Table 2.6: Extraction Temperature Optimization Results. Experiments conducted at a solvent ratio of 20:1 (n=4). Uncertainties shown are one standard deviation.

Temperature	Average Mass % F3-1	Average Mass % F3-2	Average Mass Recovery %
-20°C	77.6 ± 4.5	15.1 ± 2.6	92.3 ± 4.1
-40°C	83.8 ± 2.3	14.2 ± 1.2	97.9 ± 2.6

Table 2.7: Extraction Temperature Comparison for PAH Content (n=1)

Extraction Temperature	F3-2 Area Under Curve (Arbitrary Units)	Percentage of Area Under Curve for F3
-20°C	9,366,137	2.9%
-40°C	45,552,520	13.9%

2.2.3 Wax Precipitation Scale-up

To produce enough F3 sub-fractions for toxicity testing, the procedure described earlier was modified slightly. First, instead of extracting about one gram of F3, about two to three grams were used. Experiments were performed in quadruplicate: the solid residue and liquid filtrate

were collected from four flasks and dried (average results shown in Table 2.8, results from each run shown in Appendix A). Occasionally, a mass recovery greater than one hundred percent was observed. This was due to condensation (water) contamination in the F3-1 (PAH-rich) fraction. The small amounts of water proved difficult to remove by rotary evaporation. A decision was made not to pursue other methods (i.e. distillation) to maintain the integrity of the oil fraction (i.e. to avoid cracking).

Table 2.8: Wax Precipitation Scale-up Average Recoveries (n=35). Uncertainties shown are one standard deviation. All data from each scale-up replicate are available in Appendix A.

	F3-1 %	F3-2 %	Total Recovery %
Average	81.5 ± 4.0%	16.1 ± 2.6%	97.5 ± 3.3%

2.3 Column Chromatography for Separation of F3-1

High performance liquid chromatography (HPLC) should be an effective technique for analyzing and separating petroleum products, crude oils, and their sub-fractions [11-15]. However, this method proved ineffective for fractionation of F3-1 for two reasons. First, due to the size of the semi-prep column, only a small amount of F3-1 could be injected at one time (about 200 mg). Second, difficulties with the apparatus caused almost eighty percent of the injected oil fraction to be lost. Thus, other techniques were explored and it was decided to use preparative-scale column chromatography, as described below.

2.3.1 Materials and Methods

The first step was to choose a suitable mobile phase, or eluent. The solvent (or solvent mixture) must adequately separate marker PAHs (in this case phenanthrene and chrysene) with a

suitable retention time, as determined by thin-layer chromatography (TLC). Many solvent mixtures were tried, including hexanes, hexanes/DCM, hexanes/toluene, cyclohexane, and cyclohexane/ethyl acetate in various ratios. The only potential eluent that satisfied all requirements was 98% Hexanes: 2% DCM.

A glass column (75 mm ID) was packed with a silica gel stationary phase (Ultra Pure, SiliCycle, Quebec, QC), approximately fifty times the mass of F3-1 to be added. The stationary phase was added using the wet method, i.e., a slurry of silica gel and the eluent was prepared and poured into the column. A known amount of F3-1 (close to 10g) was dissolved in a minimal amount of eluent and added to the column with a Pasteur pipette. This layer was covered with cotton and mobile phase was added.

The mobile phase was collected manually in glass containers of various sizes. Close to separation points, glass tubes were used so that a precise cut-point was achieved with the small volumes. Eluate from these tubes was analyzed using TLC and compared to standards of phenanthrene and chrysene. Three sub-fractions were generated: F3-1-1 (initial eluate), F3-1-2 (eluate beginning with the presence of phenanthrene) and F3-1-3 (eluate beginning with the presence of chrysene). When no more compounds came off the column, it was flushed with acetone, generating a fourth fraction, F3-1-4.

Glass containers were grouped into the target fractions and their contents were combined. Each container was rinsed at least twice with DCM into a round bottom flask and solvents were removed by rotary evaporation. Each of the four fractions was collected in a pre-weighed glass vial.

2.3.2 Results and Discussion

It was readily apparent that the two major problems with HPLC would not be an issue with column chromatography. A large amount of F3-1 could easily be added to the column and nearly all of it could be recovered in one of the four sub-fractions. Additionally, the tubes of eluate were relatively easy to group into the first three sub-fractions based on colour and fluorescence. F3-1-1 was clear and did not fluoresce, F3-1-2 was a pale yellow, with a blue fluorescent tinge, and F3-1-3 was bright yellow, with a bluish green fluorescence. The fourth fraction, F3-1-4 from the acetone flush, was a dark brown with a dark green fluorescence.

After some initial work on a smaller column (45 mm ID) with one-tenth the F3-1 loading, the scaled-up method (75 mm ID) described above was performed multiple times to collect a sufficient amount of each fraction for bioassays and chemical analysis. The average mass distributions and percent recovery are listed in Table 2.9. All data on experimental repetitions are available in Appendix A.

Table 2.9: Average Mass Distribution and Percent Recovery of F3-1 by Column Chromatography (n=4). Uncertainties are equal to one standard deviation. All data on experimental repetitions are available in Appendix A.

	F3-1-1 %	F3-1-2 %	F3-1-3 %	F3-1-4 %	% Recovery
Average	48.1 ± 1.6%	22.4% ± 6.2%	10.1 ± 1.0%	5.7 ± 1.5%	86.4 ± 2.4%

2.4 Relative Contribution of Each Fraction to Whole Oil

With all fractionation complete, it is worth discussing the relative contribution of each fraction and sub-fraction to the weight of the whole oil (WO), as well as to look at the total losses from each phase combined (Table 2.10). At 81.5% of its parent fraction F3, the PAH-rich extract F3-1 comprised 20.7% of the weight of the WO. This is quite substantial compared to medium

and light crude oils previously studied by our group, of which F3-1 contributed only 5 to 10% of the weight of the WO [5]. The wax-rich F3-2 fraction was only 4.1% of the weight of the WO. Amongst Phase IV sub-fractions, F3-1-1 had the largest contribution at about 10% of the WO. F3-1-2 was next most at 4.6%, followed by F3-1-3 at 2.1% and F3-1-4 at 1.2%. The composition of these fractions will be discussed in Chapter 3. Toxicity studies for Phase II and Phase III are discussed in Chapter 3 as well. The total losses from all three phases of separation amounted to 5.5% of the WO.

Table 2.10: Contribution of Each Fraction to the Weight of the Whole Oil (WO). n=1 for Phase II, n=35 for Phase III, n=4 for Phase IV

Phase	Fraction	Parent Fraction	% of Parent Fraction	% of WO
II	F2	WO	6.8	6.8
II	F3	WO	25.4	25.4
II	F4	WO	65.7	65.7
II	Lost	WO	2.1	2.1
III	F3-1	F3	81.5 ± 4.0	20.7 ± 1.0
III	F3-2	F3	16.1 ± 2.6	4.1 ± 0.7
III	Lost	F3	2.5 ± 3.3	0.6 ± 0.8
IV	F3-1-1	F3-1	48.1 ± 1.6	10.0 ± 0.6
IV	F3-1-2	F3-1	22.4 ± 6.2	4.6 ± 1.3
IV	F3-1-3	F3-1	10.1 ± 1.0	2.1 ± 0.2
IV	F3-1-4	F3-1	5.7 ± 1.5	1.2 ± 0.3
IV	Lost	F3-1	13.6 ± 2.4	2.8 ± 0.5
Total Loss				5.5 ± 1.3

2.5 References

1. Canadian Council of Ministers of the Environment. *Canada-Wide Standards for Petroleum Hydrocarbons (PHC) in Soil;* Winnipeg, April 30-May 1, 2001.

2. Wang, Z.; Hollebone, B.; Fingas, M.; Fieldhouse, B.; Sigouin, L.; Landriault, M.; Smith, P.; Noonan, J.; Thouin, G.; Weaver, J. W. *Characteristics of Spilled Oils, Fuels, and Petroleum Products: 1. Composition and Properties of Selected Oils;* Environmental Protection Agency, 2003.

3. ASTM International. *Standard Test Method for Distillation of Petroleum Products at Reduced Pressure;*, 2006.

4. Environment Canada, ES&T. *Bunker C Fuel Oil;*, 1999.

5. Saravanabhavan, G. *An Effect-Driven Fractionation Approach for the Isolation and Characterization of CYP1A Inducing Components of Crude Oils;* PhD Thesis; Queen's University: Kingston, ON, 2007.

6. Altgelt, K.; Boduszynski, M. *Composition and Analysis of Heavy Petroleum Fractions;* Marcel Dekker: New York, 1994.

7. Thanh, N. X.; Hsieh, M.; Philp, R. P. Waxes and Asphaltenes in Crude Oil. *Organic Geochemistry* **1999,** *30,* 119-132.

8. Buenrostro-Gonzalez, E.; Lira-Galeana, C.; Gil-Villegas, A.; Wu, J. Asphaltene Precipitation in Crude Oils: Theory and Experiments. *American Institute of Chemical Engineers Journal* **2004,** *50* (10), 2552-2570.

9. Groenzin, H.; Mullins, O. C. Molecular Size and Structure of Asphaltenes from Various Sources. *Energy & Fuels* **2000,** *14,* 677-684.

10. Burger, E.; Perkins, T.; Striegler, J. Studies of Wax Deposition in the Trans Alaska Pipeline. *Journal of Petroleum Technology* **1981,** *33* (6), 1075-1086.

11. Saravanabhavan, G.; Helferty, A.; Hodson, P. V.; Brown, R. S. A multi-dimensional high performance liquid chromatographic method for fingerprinting polycyclic aromatic hydrocarbons and their alkyl-homologs in the heavy gas oil fraction of Alaskan North Slope crude. *Journal of Chromatography A* **2007,** *1156,* 124-133.

12. Borgund, A.; Erstad, K.; Barth, T. Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils. *Journal of Chromatography A* **2007,** *1149,* 189-196.

13. Kaminski, M.; Kartanowicz, R.; Gilgenast, E.; Namiesnik, J. High-Performance Liquid Chromatography in Group-Type Separation and Technical or Process Analytics of Petroleum

Products. *Critical Reviews in Analytical Chemistry* **2005,** *35* (3), 193-216.

14. Naikwadi, K.; Charbonneau, G.; Karasek, F. Separation and identification of organic compounds in air particulate extracts by high-performance liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography* **1987,** *398,* 227-237.

15. Palmentier, J.-P. F.; Britten, A. J.; Charbonneau, G. M.; Karasek, F. W. Determination of polycyclic aromatic hydrocarbons in lubricating oil base stocks using high-performance liquid chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography* **1989,** *469,* 241-251.

Chapter 3

Chemical Analysis and Toxicity of HFO 7102 and its Sub-Fractions

3.1 Introduction

Heavy fuel oil 7102 is an emerging contaminant and its composition and toxicity are not well characterized. In August 2005, an estimated 150,000 litres of HFO 7102 was spilled into Wabamun Lake, Alberta, causing damage to the aquatic life, sediments, and shoreline plants and grasses [1]. In an effort to better understand the toxic effects of this oil, it was separated by the effects-driven fractionation described in Chapter 2. Each fraction and sub-fraction generated was subjected to a detailed chemical analysis and bioassays with trout embryos to identify toxic components and their relative concentrations.

The types of compounds in oil known to cause most chronic toxicity to fish are polycyclic aromatic hydrocarbons (PAH) [2, 3]. Two techniques proven to be useful in PAH detection and quantitation are HPLC and GC-MS [4, 5]. Normal phase HPLC can separate PAH mixtures by number of aromatic rings [4]. However, UV detectors lack the sensitivity and selectivity to provide adequate quantitation of target analytes. Gas chromatography can achieve significantly better resolution, and mass spectrometry detectors are much more selective than ultraviolet detectors [6]. Thus, GC-MS methods operating in selective ion mode are most commonly used to measure oils and other complex mixtures of PAHs. A suite of per-deuterated PAH compounds are included as internal standards; they behave similarly to the target PAHs during sample preparation and analysis, but are distinguished by the detector. Thus, their inclusion is a reliable method of determining percent recovery of PAH during solvent extraction of oil samples [7]. Therefore, samples will be analyzed by GC-MS.

To determine the toxicity of each fraction, animals should be exposed to well-characterized oil samples. These bioassays are commonly done with a standard test species, such as rainbow trout (*Oncorhynchus mykiss*) [2], zebrafish (*Danio rerio*) [8], or Japanese medaka

(*Oryzias latipes*) [9]. Singer *et al.* developed standard procedures for making solutions of chemically-dispersed oil [10, 11]. After fish are exposed to dispersed oil or oil fractions, toxicity can be measured in many forms. For the purposes of this work, the only end-point considered is death. Fluorescence of water samples can be used to determine measured hydrocarbon concentration and statistical software can be used to calculate median lethal concentrations (LC_{50}). LC_{50} values are inversely proportional to toxicity; a six-fold decrease in LC_{50} represents a six-fold increase in toxicity.

3.2 Composition and Analysis of HFO 7102 and its Fractions

An in-depth chemical analysis was performed on HFO 7102 and all fractions and sub-fractions generated. While fractionation was directed by the results of the bioassays described in Section 3.3.1, a detailed analysis provides necessary data on specific compounds and groups of compounds that may be responsible for toxicity. In addition, analysis of non-toxic fractions provides information on compounds and groups of compounds that may be considered relatively benign at tested concentrations.

3.2.1 Analysis of Phase I/Phase II (HFO 7102, F2, F3, F4)

3.2.1.1 GC-MS Analysis of Phase I/II

The whole oil and its fractions (F2, F3, and F4) were characterized by gas chromatography-mass spectrometry (GC-MS) at the Bedford Institute of Oceanography (BIO) in Dartmouth, Nova Scotia. Important data were gathered on concentrations of many n-alkanes and PAH and alkyl-PAH, as well as distribution of PAH by number of aromatic rings. The results of

the GC-MS data are given in Table 3.1 below, and original chromatograms are available in Appendix B.

Table 3.1: GC-MS Data for HFO 7102 and its Fractions: F2, F3, and F4

Compound	Whole Oil ($\mu g \cdot g^{-1}$)	Fraction 2 ($\mu g \cdot g^{-1}$)	Fraction 3 ($\mu g \cdot g^{-1}$)	Fraction 4 ($\mu g \cdot g^{-1}$)
Alkanes				
n-decane **(C10)**	72	478	357	91
Undecane **(C11)**	103	671	474	120
Dodecane **(C12)**	130	929	534	121
Tridecane **(C13)**	178	1,274	601	137
Tetradecane **(C14)**	244	1,749	781	160
Pentadecane **(C15)**	346	1,837	1,287	152
Hexadecane **(C16)**	410	1,619	1,903	148
Heptadecane **(C17)**	532	2,857	2,802	175
2,6,10,14-tetramethylpentadecane **(Pristane)**	234	2,002	892	36
Octadecane **(C18)**	669	4,501	3,369	178
2,6,10,14-tetramethylhexadecane **(Phytane)**	345	3,782	1,514	57
Nonadecane **(C19)**	757	5,929	3,971	194
Eicosane **(C20)**	779	5,844	4,401	169
Heneicosane **(C21)**	1,035	5,335	5,516	186
Docosane **(C22)**	1,396	4,147	7,123	208
Tricosane **(C23)**	1,542	2,928	7,562	232
Tetracosane **(C24)**	1,670	2,164	8,175	266
Pentacosane **(C25)**	1,649	1,338	7,322	315
Hexacosane **(C26)**	2,050	757	7,167	402
Heptacosane **(C27)**	2,019	417	5,873	516
Octacosane **(C28)**	2,424	378	4,968	927
n-nonacosane **(C29)**	2,197	186	3,235	1,241
Triacontane **(C30)**	629	44	1,528	482
n-heneicontane **(C31)**	651	26	869	535
Dotriacontane **(C32)**	452	14	508	467
Tritriacontane **(C33)**	418		340	507
Tetratriacontane **(C34)**	290		177	468
n-pentatriacontane **(C35)**	255		99	449
17β(H), 21α (H)-hopane	18		18	20
∑ Alkanes ($\mu g \cdot g^{-1}$)	**23,495**	**51,208**	**83,366**	**8,961**
C17/Pristane ratio	**1.11**	**1.28**	**1.11**	**1.02**
C18/Phytane ratio	**0.18**	**0.14**	**0.18**	**0.25**

PAHs				
Naphthalenes (NAPH)				
naphthalene **(C0-NAPH)**	335	2,225	25	
methylnaphthalenes **(C1-NAPH)**	764	5,272	86	14
dimethylnaphthalenes **(C2-NAPH)**	1,056	5,694	1,025	23
trimethylnaphthalenes **(C3-NAPH)**	981	2,802	2,607	27
tetramethylnaphthalenes **(C4-NAPH)**	565	1,706	1,726	18
Total Naphthalenes	**3,701**	**17,699**	**5,470**	**81**
Dibenzothiophenes (DBT)				
dibenzothiophenes **(C0-DBT)**	105	322	380	
methyldibenzothiophenes **(C1-DBT)**	268	1,175	1,186	12
dimethyldibenzothiophenes **(C2-DBT)**	523	2,253	2,334	27
trimethyldibenzothiophenes **(C3-DBT)**	471	1,761	2,110	27
tetramethyldibenzothiophenes **(C4-DBT)**	295	891	1,284	22
Total Dibenzothiophenes	**1,662**	**6,401**	**7,293**	**88**
Fluorenes (FLUOR)				
fluorene **(C0-FLUOR)**	92	213	288	
methylfluorenes **(C1-FLUOR)**	249	971	1,119	11
dimethylfluorenes **(C2-FLUOR)**	492	1,832	2,014	18
trimethylfluorenes **(C3-FLUOR)**	521	2,102	2,211	31
Total Fluorenes	**1,353**	**5,118**	**5,631**	**59**
Phenanthrenes (PHEN)				
phenanthrene **(C0-PHEN)**	373	1,400	1,727	5
methylphenanthrenes **(C1-PHEN)**	1,247	4,377	5,699	23
dimethylphenanthrenes **(C2-PHEN)**	1,866	5,273	7,927	38
trimethylphenanthrenes **(C3-PHEN)**	1,602	3,963	6,969	41
tetramethylphenanthrenes **(C4-PHEN)**	932	1,731	4,627	48
Total Phenanthrenes	**6,021**	**16,743**	**26,948**	**155**
Pyrenes (PYR)				
pyrene **(C0-PYR)**	172	333	564	13
methylpyrenes **(C1-PYR)**	749	1,182	3,182	44
dimethylpyrenes **(C2-PYR)**	1,441	997	4,996	116
trimethylpyrenes **(C3-PYR)**	1,232	571	5,132	232
tetramethylpyrenes **(C4-PYR)**	1,074	401	3,445	436
Total Pyrenes	**4,669**	**3,484**	**17,320**	**841**
Naphthobenzothiophenes (NBT)				
naphthobenzothiophenes **(C0-NBT)**	124	102	475	13
methylnaphthobenzothiophenes **(C1-NBT)**	543	331	1,892	146
dimethylnapthobenzothiophenes **(C2-NBT)**	1,969	645	5,099	1,038
trimethylnaphthobenzothiophenes **(C3-NBT)**	507	143	988	398
tetramethylnaphthobenzothiophenes **(C4-NBT)**	244	51	383	231
Total Naphthobenzothiophenes	**3,386**	**1,272**	**8,836**	**1,826**

Chrysenes (CHRY)				
chrysene **(C0-CHRY)**	244	117	1,016	35
methylchrysenes **(C1-CHRY)**	904	319	3,069	389
dimethylchrysenes **(C2-CHRY)**	1,735	491	5,818	1,047
trimethylchrysenes **(C3-CHRY)**	941	142	1,487	791
tetramethylchrysenes **(C4-CHRY)**	691	61	792	661
Total Chrysenes	**4,515**	**1,131**	**12,181**	**2,923**
∑ Methylated PAHs ($\mu g \cdot g^{-1}$)	**23,861**	**47,135**	**79,205**	**5,909**
Unsubstituted PAH				
Acenaphthene **(Ace)**	59	214	115	5
Acenaphthylene **(Acl)**		51	10	
Anthracene **(Anth)**	41	143	191	
Fluoranthene **(Fl)**	43	86	164	8
benz[a]anthracene **(BaA)**	148	90	478	35
benzo[b]fluoranthene **(BbF)**	86	19	142	90
benzo[k]fluoranthene **(BkF)**	11	6	14	
benzo[e]pyrene **(BeP)**	106	16	149	120
benzo[a]pyrene **(BaP)**	71	10	82	87
Perylene **(Per)**	23		24	23
indeno[1,2,3-cd]pyrene **(IP)**	19		14	25
dibenz[a,h]anthracene **(DBA)**	49	23	38	62
benzo[ghi]perylene **(BP)**	50	13	27	81
∑ Unsubstituted PAHs ($\mu g \cdot g^{-1}$)	**2,150**	**5,384**	**5,923**	**602**
Total Analytes ($\mu g \cdot g^{-1}$)	**49,506**	**103,727**	**168,494**	**15,472**
Mass % of analytes	**5.0%**	**10.4%**	**16.8%**	**1.5%**
Mass % of parent fraction	**n/a**	**6.8%**	**25.4%**	**65.7%**

While the GC-MS method used targets many important compounds, there are a great deal more compounds known to be present in heavy fuel oils which are not measured. For example, only two branched alkanes are measured (pristane and phytane). There are likely many other branched alkanes present in HFO 7102. There are potentially also PAHs with higher degrees of alkylation (e.g. C5 or C6). Additionally, the 13 PAHs listed at the end all have alkylated analogues which are not measured in this method. Finally, there are not any asphaltenes or resins considered in this method. HFOs are known to be very high in that class of compounds (66% of

the weight of the oil is in F4, but only 1.5% of the weight of F4 is accounted for). This explains why only 5% of the weight of the whole oil is accounted for by the target analytes.

As HFO 7102 is a complex mixture of many chemicals closely related in structures and properties, it is not prudent to expect clean cuts between fractions. It is clear that the PAH distribution is quite varied. For example, C3-NAPH and C4-NAPH are present in approximately equal concentrations in F2 and F3. The same is true of all fluorenes (C0-C4) and dibenzothiophenes (C0-C4). As it is, there is at least a detectable amount of all PAH and alkyl-PAH in each fraction with very few exceptions (There is no C0-NAPH, C0-FLUOR, C0-DBT, Acl, Anth or BkF present in F4, and no Per or IP in F2). In addition, the longest alkanes (C33-C35 and hopane) are not present in F2, while all other alkanes are present in all three fractions. The distributions of alkanes and PAHs are shown in Figure 3.1 and Figure 3.2 respectively, below.

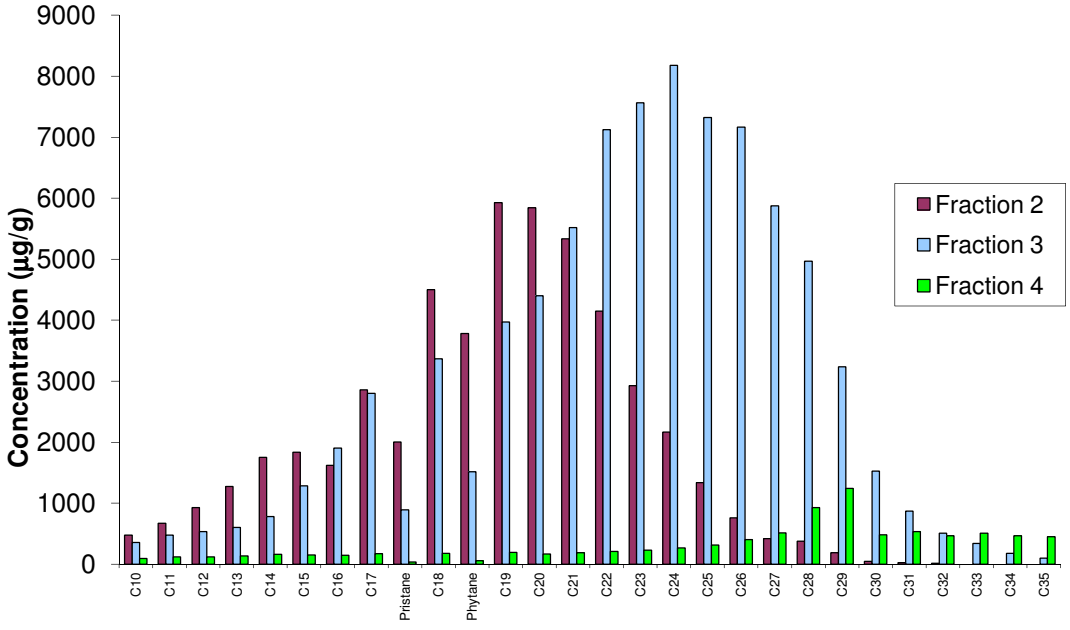

Figure 3.1: Distribution of alkanes in F2, F3 and F4

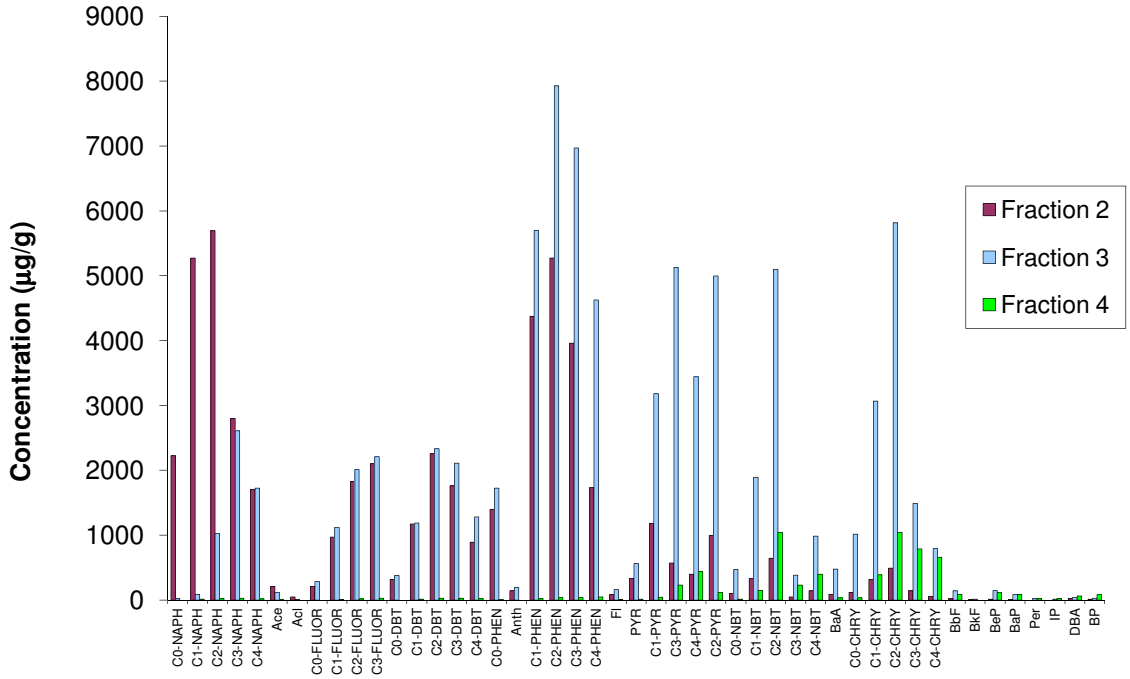

Figure 3.2: Distribution of PAH and alkyl-PAH in F2, F3 and F4

Other trends to note: total alkanes and alkylated PAH are significantly concentrated in F3 while the total concentration of unsubstituted PAH is only about 10% higher in F3 than F2, though the larger unsubstituted PAH are primarily in F3. F4 has hardly any alkane and PAH analytes, with by far the lowest concentrations of total alkanes, unsubstituted, and alkylated PAH. In fact, the analytes tested account for only 1.5% by mass of F4 (compared to 10.4% of F2 and 16.8% of F3). The rest of the fractions are likely composed of other organic compounds, including a significant amount of asphaltenes and resins in F4.

The composition of fractions based on number of aromatic rings in PAH and alkyl-PAH are listed in Table 3.2. Out of all PAH quantified in the whole oil, about half are four-ring compounds (PYR, NBT, CHRY, BaA, BbF, BkF), while the remainder are about equally divided between two-ring (NAPH, FLUOR, DBT, Ace, Acl) and three-ring (PHEN, Anth, Fl)

compounds, with a very small amount of five or six ring compounds (BeP, BaP, Per, IP, DBA, BP). About half of the quantified PAH and alkyl-PAH in F2 are two-ring compounds, with about one-third three-ring compounds and just over ten percent four-ring compounds (almost negligible five to six-ring compounds). The quantified PAH distribution in F3 is about half four-ring compounds, about one-third three-ring compounds and about one-fifth two-ring compounds, with almost negligible five to six-ring compounds. Lastly, the total quantified PAH in F4 are almost ninety percent four-ring compounds, with approximately equal proportions of the remaining two, three, five and six-ring compounds.

Table 3.2: Distribution of PAH and Alkylated PAH in HFO 7102 and its Fractions by Number of Aromatic Rings

	Whole Oil		**F2**		**F3**		**F4**	
Number of Aromatic Rings	Total (µg/g)	Total (%)	Total (µg/g)	Total (%)	Total (µg/g)	Total (%)	Total (µg/g)	Total (%)
Σ 2 Ring PAH	6,775	26.0	29,482	56.1	18,519	21.8	234	3.6
Σ 3 Ring PAH	6,106	23.5	16,973	32.3	27,303	32.1	163	2.5
Σ 4 Ring PAH	12,815	49.3	6,002	11.4	38,972	45.8	5,715	87.8
Σ 5-6 Ring PAH	317	1.2	63	0.1	334	0.4	398	6.1
Sum	**26,012**		**52,519**		**85,128**		**6,511**	

As presented is Section 3.3.1, F3 is the most toxic fraction by at least six fold, so it is safe to say that the most toxic components of HFO 7102 are preferentially concentrated in F3. The primary candidates are three- and four-ringed PAH, which are approximately 1.6 and 6.5 times more concentrated in F3 than in F2 respectively. F4 is significantly less toxic than the whole oil, so either its components are of low relative toxicity, or they are not bioavailable enough to be significantly toxic. The concentrations of PAH and alkyl-PAH are quite low in this fraction, with only four-ringed PAH contributing appreciably, and even then at lower concentrations than in F2. Specifically, high concentrations of phenanthrenes and pyrenes were observed in F3, along with

significant contributions from C2-NBT, C1-CHRY and C2-CHRY. Two-ringed PAH are not likely to contribute significantly, since they are more concentrated in F2 than F3 (by about 1.6 times) yet toxicity is more contained in F3. Five- and six-ringed compounds are also unlikely to contribute to overall toxicity based on their low composition; only 0.1% of the PAH in F2 and 0.4% of the PAH in F3 have five or six rings. While it is unlikely that alkanes would contribute to overall toxicity, it should be noted that the total concentration of alkanes is about 1.6 times higher in F3 than in F2. The next phase of fractionation attempts to remove as many of the long-chain alkanes as possible (F3-2) while leaving the PAH in F3-1. This will lead to a less speculative conclusion regarding waxes and other long-chain alkanes.

3.2.1.2 Volatile Components

Fractions F2 and F3 were tested for volatile components. A measured amount of each was subjected to rotary evaporation and periodically weighed over about a seven hour period. Due to the extreme amount of rotary evaporation involved in the solvent extraction processes, it is important to know whether a significant portion of the fractions will be lost. Each fraction was tested twice, and the average of the two trials is shown in Figure 3.3 below. The loss of F2 was fairly consistent, and amounted to about 1.5% of the total mass over the seven hour period. F3 had a lower rate of loss after the first ninety minutes and may have even been approaching a plateau. In total, less than 1% of the mass was lost in the seven hour period. For both fractions this loss is very small, and allows us to draw one conclusion and one corollary. The conclusion is that mass loss from evaporation is not significant, and the total loss should not greatly impact the toxicity testing. The corollary is that, since any volatile components will likely not contribute to chronic toxicity [2], the only possible effect of evaporation on toxicity testing will perhaps be a

slight increase in toxicity due to the concentrations of larger PAH and alkyl-PAH increasing slightly with the mass loss.

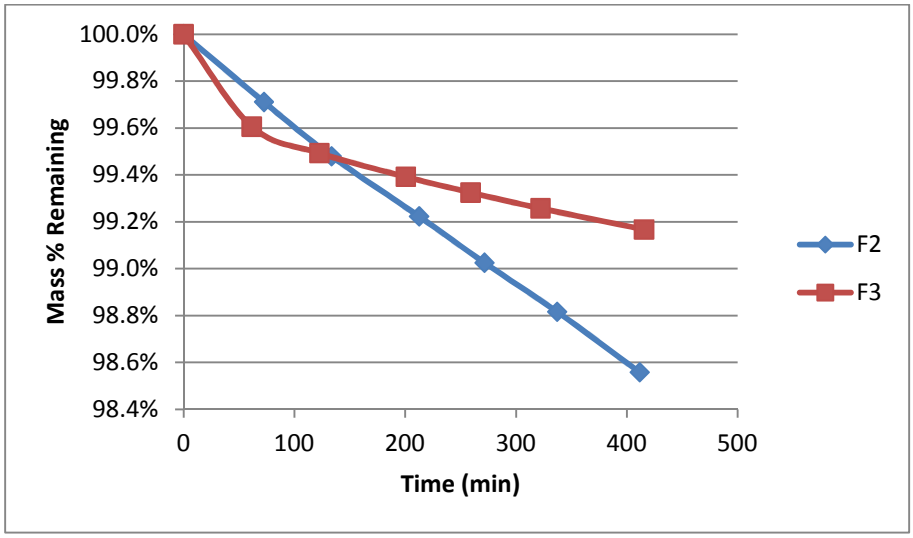

Figure 3.3: Results from Rotary Evaporation of F2 and F3

The higher total loss in F2 is likely due to a higher proportion of volatile components. As shown above, F2 has significantly higher concentrations of naphthalenes than F3. The slopes of the two lines are similar for about the first two hours. This is likely a good representation of the expected rate of loss for volatile components in heavy fuel oil fractions. It is predicted that the F2 mass loss trend would eventually level out similarly to that of F3. It is unknown when this might happen, but the time frame of this experiment exceeded the length of any rotary evaporation that these fractions would otherwise be exposed to during the course of other experiments in Phase II analysis or Phase III and Phase IV separations.

3.2.2 Composition and Analysis of Phase III Sub-Fractions (F3-1, F3-2)

Phase III sub-fractions were analyzed by GC-MS at BIO. The purpose of the Phase III separation was to remove the non-polar waxes from the bulk fraction as F3-2, leaving a PAH-

rich, wax-free fraction (F3-1). The analysis of target n-alkanes, unsubstituted PAH, and alkyl-PAH is shown in Table 3.3, along with data from the parent fraction, F3. Original chromatograms are available in Appendix B.

Table 3.3: GC-MS Data for F3 and its Sub-Fractions: F3-1 and F3-2

Compound	F3 ($\mu g \cdot g^{-1}$)	F3-1 ($\mu g \cdot mL^{-1}$)	F3-2 ($\mu g \cdot mL^{-1}$)
Alkanes			
n-decane **(C10)**	357	212	
undecane **(C11)**	474	538	
dodecane **(C12)**	534	817	
tridecane **(C13)**	601	1,015	22
tetradecane **(C14)**	781	1,259	40
pentadecane **(C15)**	1,287	1,715	93
hexadecane **(C16)**	1,903	2,149	279
heptadecane **(C17)**	2,802	3,023	1,239
2,6,10,14-tetramethylpentadecane **(Pristane)**	892	1,023	77
octadecane **(C18)**	3,369	2,702	4,054
2,6,10,14-tetramethylhexadecane **(Phytane)**	1,514	1,504	174
nonadecane **(C19)**	3,971	2,289	8,533
eicosane **(C20)**	4,401	1,398	12,594
heneicosane **(C21)**	5,516	1,050	17,225
docosane **(C22)**	7,123	765	22,322
tricosane **(C23)**	7,562	507	24,470
tetracosane **(C24)**	8,175	191	27,645
pentacosane **(C25)**	7,322	168	26,742
hexacosane **(C26)**	7,167		27,215
heptacosane **(C27)**	5,873		25,715
octacosane **(C28)**	4,968		25,121
n-nonacosane **(C29)**	3,235		19,799
triacontane **(C30)**	1,528		10,866
n-heneicontane **(C31)**	869		7,794
dotriacontane **(C32)**	508		4,736
tritriacontane **(C33)**	340		2,610
tetratriacontane **(C34)**	177		1,222
n-pentatriacontane **(C35)**	99		716
17β(H), 21α(H)-hopane	18	25	
∑ Alkanes ($\mu g \cdot mL^{-1}$)	**83,366**	**22,351**	**271,303**
C17/Pristane ratio	**1.11**	**2.95**	**16.2**

C18/Phytane ratio	0.18	1.80	23.3
PAHs			
Naphthalenes (NAPH)			
naphthalene **(C0-NAPH)**	25	32	
methylnaphthalene **(C1-NAPH)**	86	139	
dimethylnaphthalene **(C2-NAPH)**	1,025	1,847	
trimethylnaphthalene **(C3-NAPH)**	2,607	3,783	11
tetramethylnaphthalene **(C4-NAPH)**	1,726	2,389	
Total Naphthalenes	**5,470**	**8,190**	**11**
Fluorenes (FLUOR)			
fluorene **(C0-FLUOR)**	288	428	
methylfluorene **(C1-FLUOR)**	1,119	1,126	
dimethylfluorene **(C2-FLUOR)**	2,014	1,718	
trimethylfluorene **(C3-FLUOR)**	2,211	2,006	
Total Fluorenes	**5,631**	**5,277**	**0**
Dibenzothiophenes (DBT)			
dibenzothiophene **(C0-DBT)**	380	488	
methyldibenzothiophene **(C1-DBT)**	1,186	1,152	
dimethyldibenzothiophene **(C2-DBT)**	2,334	2,308	
trimethyldibenzothiophene **(C3-DBT)**	2,110	2,082	
tetramethyldibenzothiophene **(C4-DBT)**	1,284	1,128	16
Total Dibenzothiophenes	**7,293**	**7,157**	**16**
Phenanthrenes (PHEN)			
phenanthrene **(C0-PHEN)**	1,727	1,617	
methylphenanthrene **(C1-PHEN)**	5,699	5,193	16
dimethylphenanthrene **(C2-PHEN)**	7,927	7,157	25
trimethylphenanthrene **(C3-PHEN)**	6,969	6,300	24
tetramethylphenanthrene **(C4-PHEN)**	4,627	4,133	
Total Phenanthrenes	**26,948**	**24,400**	**66**
Pyrenes (PYR)			
pyrene **(C0-PYR)**	564	487	
methylpyrene **(C1-PYR)**	3,182	2,906	
dimethylpyrene **(C2-PYR)**	4,996	4,756	
trimethylpyrene **(C3-PYR)**	5,132	4,285	
tetramethylpyrene **(C4-PYR)**	3,445	3,151	29
Total Pyrenes	**17,320**	**15,585**	**29**
Naphthobenzothiophenes (NBT)			
naphthobenzothiophene **(C0-NBT)**	475	547	
methylnaphthobenzothiophene **(C1-NBT)**	1,892	2,199	
dimethylnapthobenzothiophene **(C2-NBT)**	5,099	8,436	60
trimethylnaphthobenzothiophene **(C3-NBT)**	988	1,317	
tetramethylnaphthobenzothiophene **(C4-NBT)**	383	485	

Total Naphthobenzothiophenes	**8,836**	**12,985**	**60**
Chrysenes (CHRY)			
chrysene **(C0-CHRY)**	1,016	1,115	
methylchrysene **(C1-CHRY)**	3,069	3,332	
dimethylchrysene **(C2-CHRY)**	5,818	9,672	
trimethylchrysene **(C3-CHRY)**	1,487	1,675	
tetramethylchrysene **(C4-CHRY)**	792	1,021	
Total Chrysenes	**12,181**	**16,814**	**0**
∑ Methylated PAHs (μg·mL^{-1})	**79,205**	**85,696**	**181**
Unsubstituted PAH			
acenaphthene **(Ace)**	115	195	
acenaphthylene **(Acl)**	10		
anthracene **(Anth)**	191	191	
fluoranthene **(Fl)**	164	166	
benz[a]anthracene **(BaA)**	478	571	
benzo[b]fluoranthene **(BbF)**	142	146	
benzo[k]fluoranthene **(BkF)**	14	18	
benzo[e]pyrene **(BeP)**	149	150	
benzo[a]pyrene **(BaP)**	82	98	
perylene **(Per)**	24	28	
indeno[1,2,3-cd]pyrene **(IP)**	14	49	
dibenz[a,h]anthracene **(DBA)**	38	46	
benzo[ghi]perylene **(BP)**	27	30	
∑ Unsubstituted PAHs (μg·mL^{-1})	**5,923**	**6,399**	**0**
Total Analytes	**168,494**	**114,446**	**271,485**
Mass % of analytes	**16.85%**	**11.44%**	**27.15%**
Mass % of parent fraction	**n/a**	**81.5%**	**16.1%**

Clearly, the non-polar F3-2 fraction contained the vast majority of the n-alkanes observed. C10-C12 n-alkanes were observed exclusively in F3-1, indicating some solubility in acetone for these low molecular weight alkanes. The C13-C25 n-alkanes were increasingly partitioned into F3-2, with greater than 90% of each C-23 or larger n-alkane in F3-2. The largest n-alkanes observed, C-26 to C-35, were below detection limits in F3-1. The branched alkanes analyzed (pristane, phytane and 17β(H), 21α(H)-hopane) were contained almost exclusively in

F3-1 (98.5%, 97.8%, and 100% by weight, respectively). The quantified branched alkanes remained dissolved in acetone, rather than precipitate with the wax-rich residue, likely due to their lower melting points.

The unsubstituted and alkylated PAH were almost entirely present in F3-1. Only very small amounts of C3-NAPH, C4-DBT, C1-PHEN, C2-PHEN, C3-PHEN, C4-PYR, and C2-NBT were observed in F3-2, with the highest concentration being 60 ppm C2-NBT. All unsubstituted (C0) PAH were below their respective detection limits in F3-2. Greater than 99.96% of PAH detected were in F3-1. When considering the extremely low concentrations in F3-2, it was expected that that fraction would be non-toxic to trout embryos at all concentrations tested and that all F3 toxicity would be accounted for and concentrated in the F3-1 fraction (see Section 3.3.1). The distribution of alkanes and PAH are shown in Figure 3.4 and Figure 3.5 respectively.

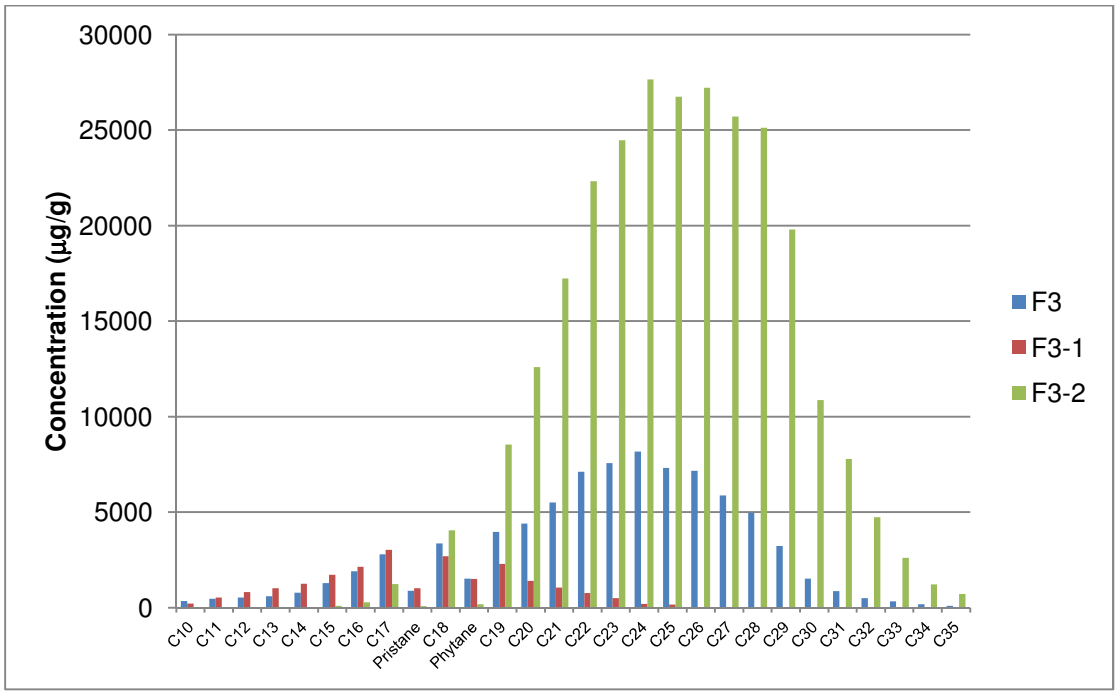

Figure 3.4: Distribution of Alkanes in F3, F3-1 and F3-2

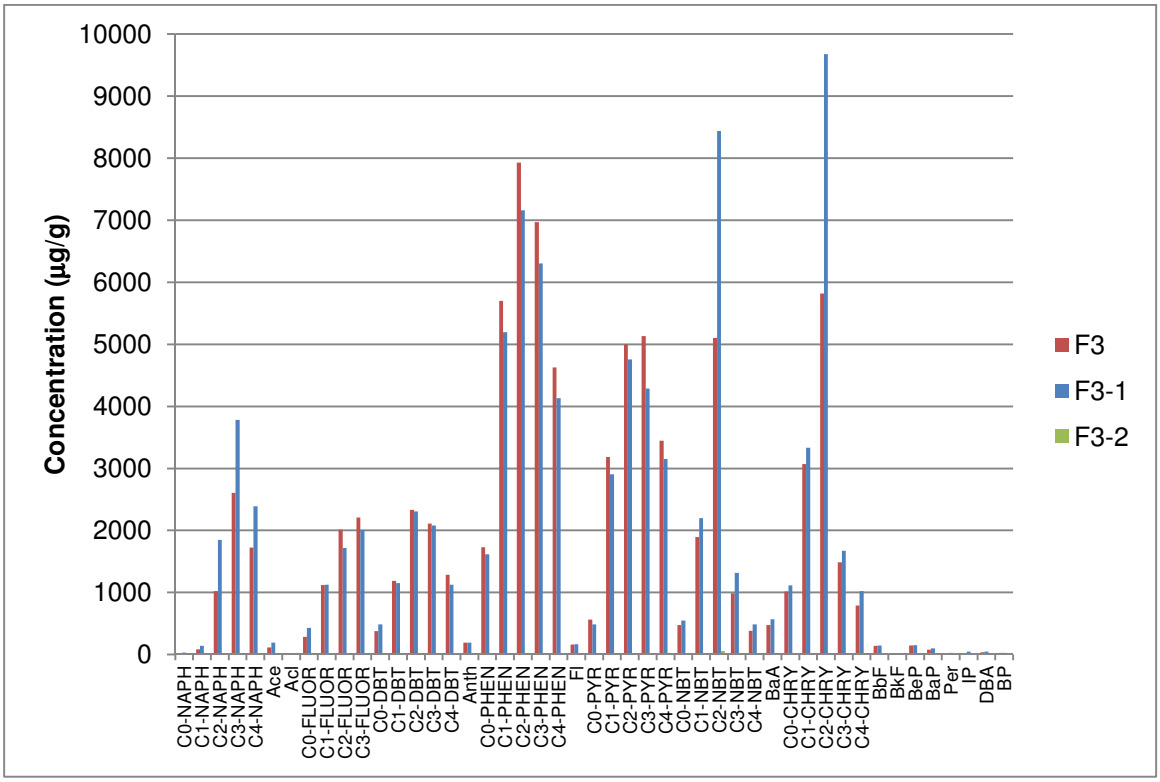

Figure 3.5: Distribution of PAH and alkyl-PAH in F3, F3-1 and F3-2

3.2.3 Composition and Analysis of Phase IV Sub-Fractions (F3-1-1, F3-1-2, F3-1-3 F3-1-4)

Phase IV sub-fractions were analyzed by GC-MS at BIO. The open column chromatography attempted to separate fractions by number of aromatic rings, and partly by polarity. Analysis was used to determine the proportions of each group of compounds in each fraction (Table 3.4). Original chromatograms are shown in Appendix B.

Table 3.4: GC-MS Data for F3-1 and its Sub-Fractions: F3-1-1, F3-1-2, F3-1-3, and F3-1-4

Compound	F3-1 (µg·mL^{-1})	F3-1-1 (µg·mL^{-1})	F3-1-2 (µg·mL^{-1})	F3-1-3 (µg·g^{-1})	F3-1-4 (µg·g^{-1})
Alkanes					
n-decane **(C10)**	212	147			
undecane **(C11)**	538	1,069			
dodecane **(C12)**	817	2,278			
tridecane **(C13)**	1,015	3,079			6.2
tetradecane **(C14)**	1,259	3,911			16
pentadecane **(C15)**	1,715	5,337			27
hexadecane **(C16)**	2,149	5,361	12	1.2	52
heptadecane **(C17)**	3,023	7,051		1.4	78
2,6,10,14-tetramethylpentadecane **(Pristane)**	1,023	2,682			26
octadecane **(C18)**	2,702	6,499		1.4	81
2,6,10,14-tetramethylhexadecane **(Phytane)**	1,504	3,690			37
nonadecane **(C19)**	2,289	5,632		1.1	76
eicosane **(C20)**	1,398	4,102	150	1.1	61
heneicosane **(C21)**	1,050	3,215	234		45
docosane **(C22)**	765	2,496	353		31
tricosane **(C23)**	507	2,102	416		21
tetracosane **(C24)**	191	1,365	451		13
pentacosane **(C25)**	168	265	408		7.7
hexacosane **(C26)**		145	403		4.0
heptacosane **(C27)**		348	356		9.0
octacosane **(C28)**		162	340		4.8
n-nonacosane **(C29)**		103	241		4.0
triacontane **(C30)**		86	151		2.8
n-heneicontane **(C31)**		64	119		2.8
dotriacontane **(C32)**		34			2.2
tritriacontane **(C33)**					1.6
tetratriacontane **(C34)**					
n-pentatriacontane **(C35)**					
17β(H), 21α(H)-hopane	25				
∑ Alkanes (µg·mL^{-1}) (µg·g^{-1})*	**22,351**	**61,224**	**3,632**	**6.3**	**609**
C17/Pristane ratio	**2.95**	**2.63**			**2.96**
C18/Phytane ratio	**1.80**	**1.76**			**2.20**
PAHs					
Naphthalenes (NAPH)					
naphthalene **(C0-NAPH)**	32	43			
methylnaphthalene **(C1-NAPH)**	139	170	73		
dimethylnaphthalene **(C2-NAPH)**	1,847	1,922	2,022		26

trimethylnaphthalene **(C3-NAPH)**	3,783	3,738	5,885	2.0	63
tetramethylnaphthalene **(C4-NAPH)**	2,389	2,289	4,134	1.7	37
Total Naphthalenes	**8,190**	**8,162**	**12,114**	**3.7**	**126**
Fluorenes (FLUOR)					
fluorene **(C0-FLUOR)**	428		1,975		
methylfluorene **(C1-FLUOR)**	1,126	136	5,615	11	7.1
dimethylfluorene **(C2-FLUOR)**	1,718	169	8,358	124	16
trimethylfluorene **(C3-FLUOR)**	2,006	167	7,781	435	10
Total Fluorenes	**5,277**	**472**	**23,729**	**570**	**33**
Dibenzothiophenes (DBT)					
dibenzothiophene **(C0-DBT)**	488	261	1,410		9.4
methyldibenzothiophene **(C1-DBT)**	1,152	594	3,725	1.3	20
dimethyldibenzothiophene **(C2-DBT)**	2,308	833	8,993	4.0	29
trimethyldibenzothiophene **(C3-DBT)**	2,082	561	8,587	12	23
tetramethyldibenzothiophene **(C4-DBT)**	1,128	286	4,498	15	16
Total Dibenzothiophenes	**7,157**	**2,536**	**27,214**	**32**	**98**
Phenanthrenes (PHEN)					
phenanthrene **(C0-PHEN)**	1,617	26	6,979	16	6.7
methylphenanthrene **(C1-PHEN)**	5,193		23,167	57	20
dimethylphenanthrene **(C2-PHEN)**	7,157		31,433	194	39
trimethylphenanthrene **(C3-PHEN)**	6,300	32	27,372	900	45
tetramethylphenanthrene **(C4-PHEN)**	4,133	73	15,524	1,179	80
Total Phenanthrenes	**24,400**	**131**	**104,476**	**2,345**	**191**
Pyrenes (PYR)					
pyrene **(C0-PYR)**	487		2,291		3.0
methylpyrene **(C1-PYR)**	2,906		9,666	870	15
dimethylpyrene **(C2-PYR)**	4,756		12,356	2,271	28
trimethylpyrene **(C3-PYR)**	4,285		6,427	2,548	30
tetramethylpyrene **(C4-PYR)**	3,151	55	3,850	2,618	135
Total Pyrenes	**15,585**	**55**	**34,589**	**8,306**	**211**
Naphthobenzothiophenes (NBT)					
naphthobenzothiophene **(C0-NBT)**	547		2,057	27	3.3
methylnaphthobenzothiophene **(C1-NBT)**	2,199		7,004	729	35
dimethylnapthobenzothiophene **(C2-NBT)**	8,436	31	23,668	2,764	74
trimethylnaphthobenzothiophene **(C3-NBT)**	1,317		2,898	554	16
tetramethylnaphthobenzothiophene **(C4-NBT)**	485		1,196	214	7.1
Total Naphthobenzothiophenes	**12,985**	**31**	**36,824**	**4,287**	**135**
Chrysenes (CHRY)					
chrysene **(C0-CHRY)**	1,115		159	854	6.2
methylchrysene **(C1-CHRY)**	3,332		1,050	2,412	29
dimethylchrysene **(C2-CHRY)**	9,672		3,679	8,843	168

trimethylchrysene (**C3-CHRY**)	1,675		754	1,379	24
tetramethylchrysene (**C4-CHRY**)	1,021		424	849	54
Total Chrysenes	**16,814**	**0**	**6,066**	**14,337**	**282**
∑ Methylated PAHs (µg·mL^{-1}) (µg·g^{-1})*	**85,696**	**11,057**	**230,140**	**28,985**	**1,047**
Unsubstituted PAH					
acenaphthene (**Ace**)	195	136	271		2.8
acenaphthylene (**Acl**)					
anthracene (**Anth**)	191		1068	7.5	
fluoranthene (**Fl**)	166		685	23	
benz[a]anthracene (**BaA**)	571		168	438	3.1
benzo[b]fluoranthene (**BbF**)	146			111	2.0
benzo[k]fluoranthene (**BkF**)	18			13	
benzo[e]pyrene (**BeP**)	150			133	5.3
benzo[a]pyrene (**BaP**)	98			93	
perylene (**Per**)	28			33	
indeno[1,2,3-cd]pyrene (**IP**)	49			4.0	
dibenz[a,h]anthracene (**DBA**)	46			12	
benzo[ghi]perylene (**BP**)	30			17	
∑ Unsubstituted PAHs (µg·mL^{-1}) (µg·g^{-1})*	**6,399**	**466**	**17,064**	**1,780**	**42**
Total Analytes	**114,446**	**72,747**	**250,836**	**30,771**	**1,698**
Mass % of analytes	**11.4%**	**7.3%**	**25.1%**	**3.1%**	**0.2%**
Mass % of parent fraction	**n/a**	**48.1%**	**22.4%**	**10.1%**	**5.7%**

For the most part, successive fractions were expected to contain compounds of increasing molecular weight and number of aromatic rings. The final fraction, F3-1-4, contained all compounds remaining on the column at the end of the experiment, and accounted for only 5.7% of F3-1 by weight. Distribution of n-alkanes and PAHs are shown in Figure 3.6 and Figure 3.7 respectively.

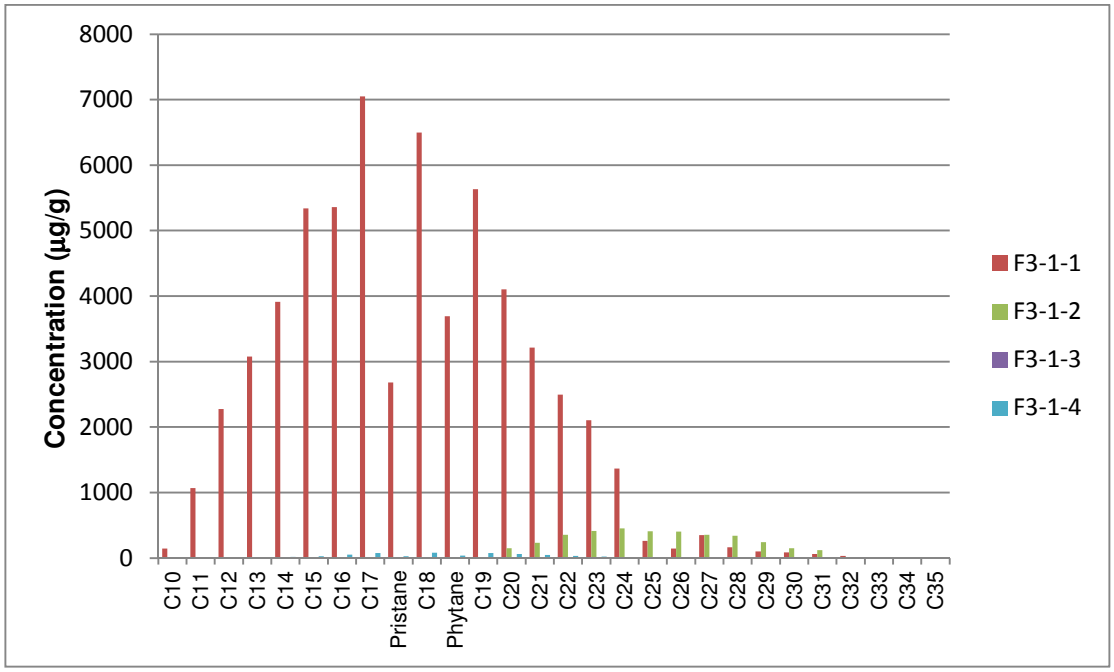

Figure 3.6: Distribution of Alkanes in Phase IV Sub-Fractions

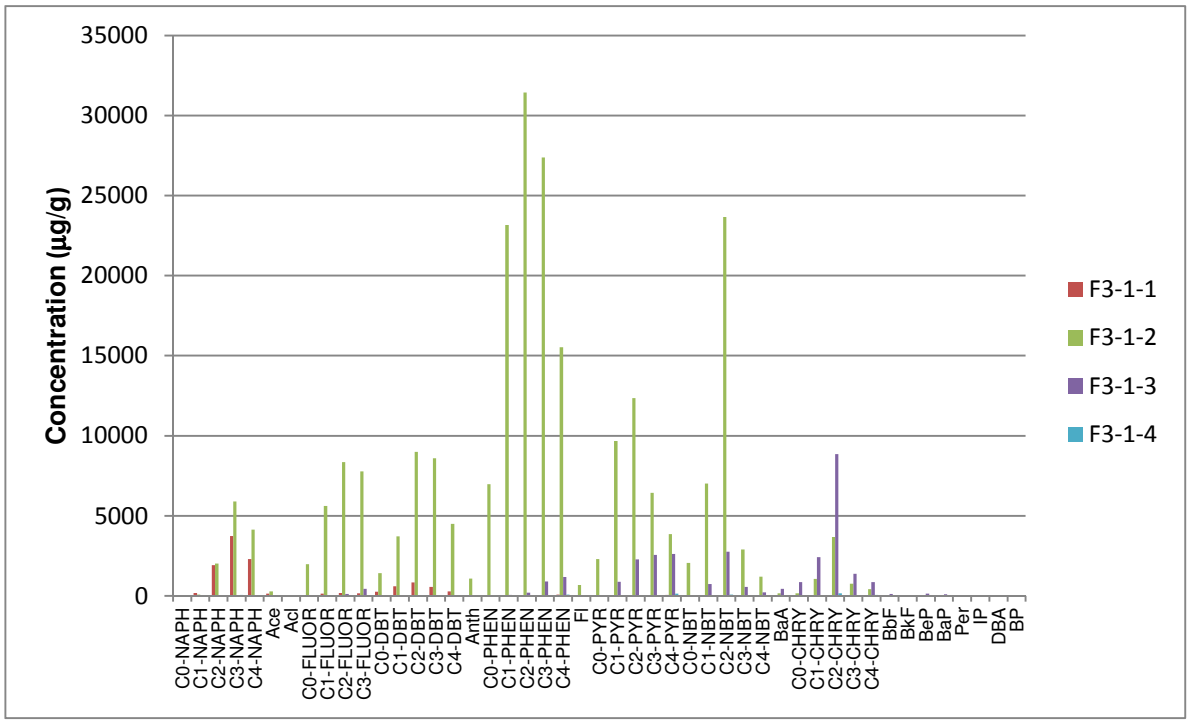

Figure 3.7: Distribution of PAHs and alkyl-PAHs in Phase IV Sub-Fractions

The n-alkanes mostly eluted in the first fraction, F3-1-1, which accounted for nearly all of the C10-C24 n-alkanes, and about half of the remaining C25-C35 n-alkanes. This is consistent with the lack of aromatic rings in these compounds to interact with the more polar silica stationary phase. F3-1-2 contained nearly all of the remaining C20-C31 n-alkanes, while F3-1-3 contained only trace amounts (about 6 ppm) of n-alkanes. F3-1-4, which contained a hodgepodge of compounds in small concentrations, accounted for the last of the n-alkanes, mostly in the C14-C24 range. Curiously, n-alkanes C-26 and larger were not detected in the original F3-1 sample but appeared in small quantities in its sub-fractions – up to a few hundred ppm. This could be due to slight variations in the wax precipitation process. Ultimately, these compounds are unlikely to contribute significantly to toxicity, as explored with fraction F3-2 in Section 3.3.1.

The aromatic compounds tell a much more interesting story. As expected, F3-1-1 contained mostly C0-C4 NAPH and C0-C4-DBT, along with lesser amounts of C1-C3 FLUOR and Ace. These 2-3 ring compounds are unlikely to contribute significantly to toxicity. This fraction accounted for nearly 50% of F3-1 by weight, but only 9% of the total PAH (along with 97% of the total n-alkanes). F3-1-2 contained nearly all of the unsubstituted and alkylated PAH – 86% of the total PAH, only lacking C0-NAPH and the 4-6 ring unsubstituted PAH (BbF, BkF, BeP, BaP, Per, IP, DBA and BP). F3-1-3 was primarily composed of 3-4 ring PAH: C0-C4 PHEN, C1-C4 PYR, C0-C4 NBT, and C0-C4 CHRY, as well as the unsubstituted 4-6 ring PAH missing in F3-1-2. Although F3-1-3 was mainly comprised of 4 ring PAHs (Table 3.5), it contained less than 5% of the total PAH measured in Phase IV sub-fractions. The pattern of PAH in F3-1-1 to F3-1-3 is also consistent with compounds interacting with the stationary silica phase based on number of aromatic rings, with overall molecular weight and non-polarity playing a secondary role in elution. Finally, F3-1-4 again contained small concentrations of most

compounds, with only C1-NAPH measuring below detection limits among methylated PAHs. However, these concentrations are almost negligible; this fraction accounts for less than 0.1% of the total PAH in Phase IV sub-fractions.

Table 3.5: Distribution of PAH and Alkylated PAH in Phase IV Sub-Fractions

	F3-1-1		**F3-1-2**		**F3-1-3**		**F3-1-4**	
Number of Rings	Total (µg/mL)	Total (%)	Total (µg/mL)	Total (%)	Total (µg/g)	Total (%)	Total (µg/g)	Total (%)
Σ 2 Ring PAH (Totals)	11,306	98.1%	63,328	25.6%	605	2.0%	260	23.9%
Σ 3 Ring PAH (Totals)	131	1.1%	106,229	43.0%	2,375	7.7%	191	17.5%
Σ 4 Ring PAH (Totals)	87	0.8%	77,647	31.4%	27,493	89.4%	633	58.1%
Σ 5-6 Ring PAH (Totals)	0	-	0	-	291	0.9%	5.3	0.5%
Sum	**11,523**		**247,203**		**30,765**		**1,089**	

Clearly, the majority of PAH, regardless of number of rings, were fractionated into the second fraction, F3-1-2. This fraction, which accounts for about 22% of F3-1 and 4.6% of the whole oil, is therefore expected to contain the majority of the toxicity amongst the Phase IV sub-fractions.

There is one more issue that can be discussed after analyzing the GC-MS results. A mass balance of Phase IV sub-fractions showed that the column chromatography process had an 86.4% recovery. Of the nearly 14% lost, (whether by sticking to the column, the silica, or lost in washing, transfer, etc.) which compounds are unaccounted for? When a weighted sum of Phase IV sub-fraction concentrations is compared to F3-1 concentrations (Table 3.6) a proper mass balance can be made.

Table 3.6: Recovery of Target Analytes in Phase IV Sub-Fractions

Compound	F3-1 (µg•mL^{-1})	Phase IV Sum (µg•mL^{-1})	% in sub-fractions	Compound	F3-1 (µg•mL^{-1})	Phase IV Sum (µg•mL^{-1})	% in sub-fractions
Alkanes				**PAHs**			
C10	212	71	33%	**Naphthalenes (NAPH)**			
C11	538	515	96%	**C0-NAPH**	32	21	65%
C12	817	1097	134%	**C1-NAPH**	139	98	70%
C13	1015	1483	146%	**C2-NAPH**	1847	1380	75%
C14	1259	1884	150%	**C3-NAPH**	3783	3122	83%
C15	1715	2571	150%	**C4-NAPH**	2389	2031	85%
C16	2149	2586	120%	**Total NAPH**	**8,190**	**6,652**	**81%**
C17	3023	3399	112%	**Fluorenes (FLUOR)**			
Pristane	1023	1293	126%	**C0-FLUOR**	428	443	103%
C18	2702	3134	116%	**C1-FLUOR**	1126	1325	118%
Phytane	1504	1779	118%	**C2-FLUOR**	1718	1968	115%
C19	2289	2715	119%	**C3-FLUOR**	2006	1869	93%
C20	1398	2012	144%	**Total FLUOR**	**5,277**	**5,604**	**106%**
C21	1050	1603	153%	**Dibenzothiophenes (DBT)**			
C22	765	1282	168%	**C0-DBT**	488	442	91%
C23	507	1107	218%	**C1-DBT**	1152	1122	97%
C24	191	759	398%	**C2-DBT**	2308	2418	105%
C25	168	219	131%	**C3-DBT**	2082	2197	106%
C26		160		**C4-DBT**	1128	1148	102%
C27		248		**Total DBT**	**7,157**	**7,328**	**102%**
C28		155		**Phenantrenes (PHEN)**			
C29		104		**C0-PHEN**	1617	1579	98%
C30		75		**C1-PHEN**	5193	5199	100%
C31		58		**C2-PHEN**	7157	7066	99%
C32		16		**C3-PHEN**	6300	6243	99%
C33		0.1		**C4-PHEN**	4133	3638	88%
C34				**Total PHEN**	**24,400**	**23,724**	**97%**
C35							
Hopane	25		0%				
∑ Alkanes	**22,351**	**30,322**	**136%**				

Compound	F3-1 (µg•mL^{-1})	Phase IV Sum (µg•mL^{-1})	% in sub-fractions	Compound	F3-1 (µg•mL^{-1})	Phase IV Sum (µg•mL^{-1})	% in sub-fractions
PAHs				**Unsubstituted PAH**			
Pyrenes (PYR)				**Ace**	195	126	65%
C0-PYR	487	514	106%	**Acl**			
C1-PYR	2906	2255	78%	**Anth**	191	240	126%
C2-PYR	4756	3000	63%	**Fl**	166	156	94%
C3-PYR	4285	1700	40%	**BaA**	571	82	14%
C4-PYR	3151	1162	37%	**BbF**	146	11	8%
Total PYR	**15,585**	**8,630**	**55%**	**BkF**	18	1.3	7%
Naphthobenzothiophenes (NBT)				**BeP**	150	14	9%
C0-NBT	547	464	85%	**BaP**	98	9.4	10%
C1-NBT	2199	1645	75%	**Per**	28	3.3	12%
C2-NBT	8436	5603	66%	**IP**	49	0.4	1%
C3-NBT	1317	707	54%	**DBA**	46	1.2	3%
C4-NBT	485	290	60%	**BP**	30	1.7	6%
Total NBT	**12,985**	**8,709**	**67%**	**∑ Unsubstituted PAHs**	**6,399**	**4,231**	**66%**
Chrysenes (CHRY)							
C0-CHRY	1115	122	11%	**Total PAH**	**92,095**	**64,121**	**70%**
C1-CHRY	3332	481	14%				
C2-CHRY	9672	1729	18%				
C3-CHRY	1675	310	19%				
C4-CHRY	1021	184	18%				
Total CHRY	**16,814**	**2,826**	**17%**				
∑ Methylated PAHs	**85,696**	**59,890**	**70%**				

Amongst n-alkanes, C12-C33 were recovered at a significantly higher percentage than initially measured in F3-1 (including C26-C33, which were not observed at all). As discussed earlier, this may be due to variations in the wax precipitation process. That is, the F3-1 sample

submitted for GC-MS analysis had much more wax removed than the F3-1 used for column chromatography. If all F3-1 generated was pooled, a more certain conclusion could be drawn; however, this was not possible due to the ongoing nature of this work and the need for intermittent sample deliveries. The total alkanes were recovered at 136%, with a total concentration about 8000 ppm higher (0.8% by weight) than the parent fraction. This discrepancy is well within the variance of the wax precipitation process (± 2.6%). Another possible explanation is matrix effects (lower perceived values in the parent fraction due to the presence of other compounds). There may also be some amount of instrument variability, but this is probably not significant since all alkane concentrations are much higher than 100%, while most PAHs are recovered close to 100%.

Within a reasonable error, the following PAHs were fully recovered: C0-C4 FLUOR, C0-C4 DBT, C0-C4 PHEN, C0-PYR, Anth, and Fl (88%-126%). Meanwhile, C0-C4 NAPH, C1-C4 PYR, C0-C4 NBT and Ace experienced significant losses, with recoveries between 37% and 85%. The most substantial losses were observed in the largest PAH: C0-C4 CHRY, BaA, BbF, BkF, BeP, BaP, Per, IP, DBA and BP. These 4-6 ring compounds were recovered at only 1% to 19%.

Overall, n-alkanes were observed at a 136% recovery, and total PAHs at 70% recovery. The biggest losses were in NAPH (perhaps due to evaporation) and the 4-6 ring compounds. While the losses are somewhat upsetting empirically, future toxicity work will show whether the remaining compounds can account for all F3-1 toxicity, or if some toxic equivalents have also been lost.

3.3 Bioassays of HFO 7102 and its Fractions

Due to previous work in this field [3, 8, 9, 12-17], it is expected that the majority of compounds toxic to the early life stages of fish are present in F3 (i.e. 3-5 ring PAH and alkyl-PAH). The majority of compounds in F2 may be acutely toxic by injection, but are not much of a threat in terms of chronic toxicity at concentrations normally encountered in aquatic environments [2]. Meanwhile, F4 contains such large and bulky compounds that uptake is relatively low. Since these compounds should have low bioavailability, it is expected that F4 will have low relative toxicity [2]. Bioassays were performed by Julie Adams, under the supervision of Dr. Peter Hodson, Queen's Biology Department.

3.3.1 Fish Exposures

Various concentrations of High Energy Chemically Enhanced Water Accommodated Fraction (HE-CEWAF) of each fraction were added to bowls of rainbow trout embryos (*Oncorhynchus mykiss*). Ratios of oil, water, and dispersant in HE-CEWAF were based on those developed by Singer *et al.* [10, 11]. The exposure period was twenty-four days, with daily static renewal of the HE-CEWAF. Water samples were taken daily, and dead fish were removed. Fluorescence was used to convert nominal loading values to measured hydrocarbon concentrations. At the conclusion of the tests, cumulative mortality data were used to determine median lethal concentrations (LC_{50}) for each fraction and sub-fraction.

In Phase II, the whole oil (WO) was compared to its fractions (F2, F3 and F4) (Figure 3.8). The most toxic fraction was F3, approximately six times more toxic than the WO, which had an overlapping toxicity curve with F2. The least toxic fraction was F4, which was at least seven times less toxic than the WO. At concentrations higher than those shown, 100%

mortality was observed, but that toxicity was attributed to the amount of dispersant required to mix the required amount of F4 into solution and those data points were removed (Julie Adams, personal communication). The difference in toxic effects observed amongst the three fractions indicates that the low-temperature vacuum distillation was successful in creating fractions with different toxicological properties, and is a valid first step in the EDFA approach. Since toxicity was concentrated in F3, that fraction was targeted for further separations.

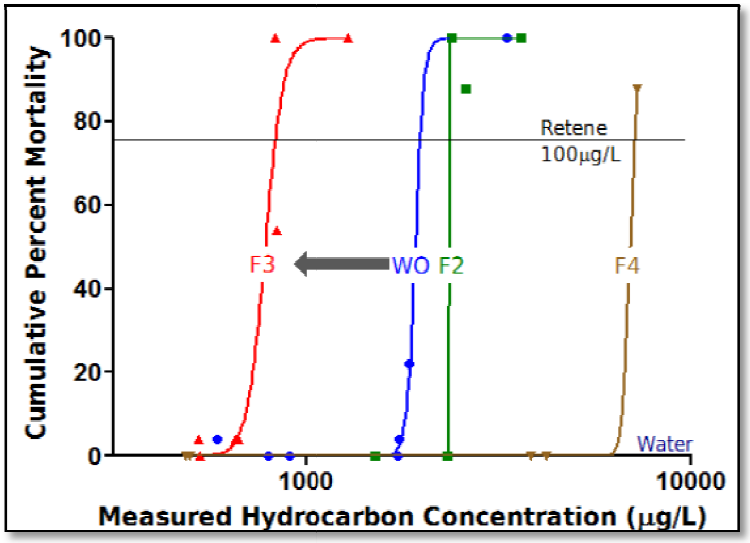

Figure 3.8: Cumulative Mortality of HFO 7102 (WO) and its fractions. Reproduced with permission from Julie Adams.

After wax precipitation was conducted, the resulting Phase III sub-fractions – the PAH-rich extract (F3-1) and wax-rich residue (F3-2) – were each exposed to rainbow trout embryos (Figure 3.9). The wax-rich fraction F3-2 did not show any toxicity at all, while the toxicity curves for F3 and F3-1 overlapped (Julie Adams, personal communication). This indicates that the wax precipitation process was successful in separating the F3 fraction, generating a toxic and non-toxic sub-fraction. There was a slight concentration of toxic components in F3-1.

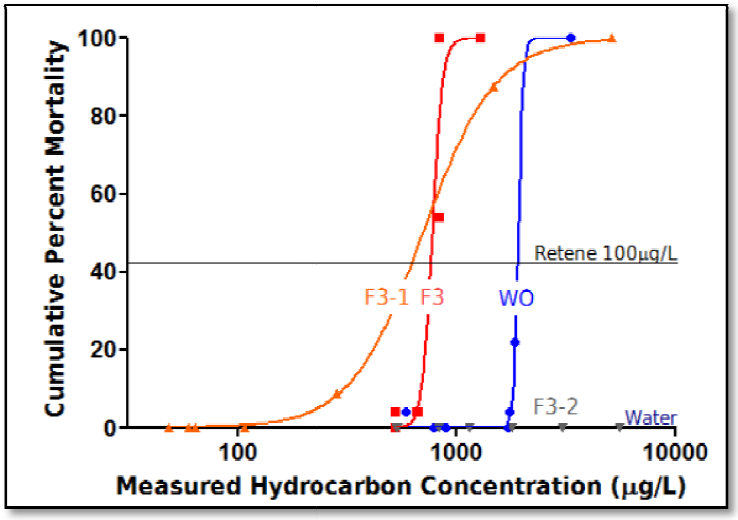

Figure 3.9: Cumulative Mortality of F3 and its sub-fractions. Reproduced with permission from Julie Adams.

Due to time constraints, Phase IV exposures have not yet been completed. Our group intends to analyze these fractions in the near future.

3.4 Discussion of Relative Toxicity of HFO 7102 Sub-Fractions

Despite accounting for almost two-thirds of the weight of HFO 7102, F4 proved non-toxic at all concentrations tested. The other non-toxic fraction, F3-2, contained nearly all waxes with minimal PAH contamination and accounted for only about 4% of the weight of HFO 7102. As expected, the toxicity of HFO 7102 sub-fractions increased as PAHs were concentrated. F3 was about six times more toxic than the whole oil and F2. In terms of relative composition, F3 is about one quarter of the whole oil. Therefore, if all toxicity was isolated in F3 it should be four times more toxic than WO, consistent with the result. Four-ring PAH are 6.5 times more concentrated in F3 than in F2, suggesting these compounds could be important in fuel oil toxicity.

In the future, bioassays of Phase IV sub-fractions will give further insight into the relative toxicity of various components of HFO 7102.

3.5 References

1. Debruyn, A. M.; Wernick, B. G.; Stefura, C.; McDonald, B. G.; Rudolph, B.-L.; Patterson, L.; Chapman, P. M. In Situ Experimental Assessment of Lake Whitefish Development Following a Freshwater Oil Spill. *Environmental Science & Technology* **2007,** *41* (20), 6983-6989.

2. Khan, C. *Identification of Compounds in Crude Oil that are Chronically Toxic to the Early Life Stages of Fish;* MSc Thesis; Queen's University, Biology Department: Kingston, ON, 2007.

3. Saravanabhavan, G. *An Effect-Driven Fractionation Approach for the Isolation and Characterization of CYP1A Inducing Components of Crude Oils;* PhD Thesis; Queen's University: Kingston, ON, 2007.

4. Kaminski, M.; Kartanowicz, R.; Gilgenast, E.; Namiesnik, J. High-Performance Liquid Chromatography in Group-Type Separation and Technical or Process Analytics of Petroleum Products. *Critical Reviews in Analytical Chemistry* **2005,** *35* (3), 193-216.

5. Wang, Z.; Fingas, M.; Li, K. Fractionation of a Light Crude Oil and Identification and Quantiation of Aliphatic, Aromatic, and Biomarker Compounds by GC-FID and GC-MS, Part I. *Journal of Chromatographic Science* **1994,** *32,* 361-382.

6. Lawrence, J. F. Advantages and Limitations of HPLC in Environmental Analysis. *Chromatographia* **1987,** *24,* 25-50.

7. Poster, D. L.; Schantz, M. M.; Sander, L. C.; Wise, S. A. Analysis of Polycyclic Aromatic Hydrocarbonds (PAHs) in Environmental Samples: a Critical Review of Gas Chromatographic (GC) Methods. *Analytical and Bioanalytical Chemistry* **2006,** *386,* 859-881.

8. Scott, J. A.; Incardona, J. P.; Pelkki, K.; Shepardson, S.; Hodson, P. V. AhR2-mediated, CYP1A-independent cardiovascular toxicity in zebrafish (Danio rerio) embryos exposed to retene. *Aquatic Toxicology* **2011,** *101* (1), 165-174.

9. Fallahtafti, S.; Rantanen, T.; Brown, R. S.; Snieckus, V.; Hodson, P. V. Toxicity of hydroxylated alkyl-phenanthrenes to the early life stages of Japanese medaka (Oryzias latipes). *Aquatic Toxicology* **2012,** *106-107,* 56-64.

10. Singer, M. M.; Smalheer, D. L.; Tjeerdema, R. S. Effects of Spiked Exposure to an Oil Dispersant on the Early Life Stages of Four Marine Species. *Environmental Toxicology & Chemistry* **1991,** *10* (10), 1367-1374.

11. Singer, M. M.; Aurand, D. V.; Coelho, G. M.; Bragin, G. E.; Clark, J. R.; Sowby, M.; Tjeerdema, R. S. Making, Measuring, and using Water-Accommodated Fractions of Petroleum for Toxicity Testing. *International Oil Spill Conference*, Tampa, FL, 2001; pp 1269-1274.

12. Saravanabhavan, G.; Helferty, A.; Hodson, P. V.; Brown, R. S. A multi-dimensional high performance liquid chromatographic method for fingerprinting polycyclic aromatic hydrocarbons and their alkyl-homologs in the heavy gas oil fraction of Alaskan North Slope crude. *Journal of Chromatography A* **2007,** *1156,* 124-133.

13. Barron, M. G.; Heintz, R.; Rice, S. D. Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor antagonists in fish. *Marine Environmental Research* **2004,** *58,* 95-100.

14. Basu, N.; Billiard, S.; Fragoso, N.; Omoike, A.; Tabash, S.; Brown, S.; Hodson, P. Ethoxyresorufin-o-deethylase induction in trout exposed to mixtures of polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry* **2001,** *20* (6), 1244-1251.

15. Billiard, S. M.; Querbach, K.; Hodson, P. V. Toxicity of Retene to Early Life Stages of Two Freshwater Fish Species. *Environmental Toxicology and Chemistry* **1999,** *18* (9), 2070-2077.

16. Brinkworth, L. C.; Hodson, P. V.; Tabash, S.; Lee, P. CYP1A induction and blue sac disease in early developmental stages of rainbow trout (Oncorhynchus mykiss) exposed to retene. *Journal of Toxicologicy and Environmental Health, Part A* **2003,** *66,* 627-646.

17. Turcotte, D.; Akhtar, P.; Bowerman, M.; Kiparissis, Y.; Brown, R. S.; Hodson, P. V. Measuring the toxicity of alkyl-phenanthrenes to early life stages of medaka (Oryzias lapites) using partition-controlled delivery. *Environmental Science & Technology* **2011,** *30* (2), 487-495.

Chapter 4

Discussion and Conclusion

4.1 Overall Project Objectives

The goal of this project was to separate and analyze HFO 7102 to characterize the oil, identify and quantify its toxic and non-toxic components, and to provide commentary on the relative risks of HFO spills to crude oil spills. The goal of fractionation was to concentrate 90% of the toxicity of HFO 7102 into 10% of its weight or less. The Effects-Driven Fractionation approach provided a standard for future HFO analysis, and bioassays with rainbow trout embryos clearly identified toxic and non-toxic fractions, while providing room for future analysis. Thorough GC-MS analysis characterized each fraction, making it possible to discuss the relative amounts of various toxicants present in HFO 7102.

In previous work by our group, the toxicity of crude oils was explored relative to HFOs [1, 2]. Conclusions indicated that HFOs are, in general, substantially more toxic than crudes. To explore this relationship more thoroughly, and understand the toxicity of HFOs better, this project sought to properly fractionate and characterize HFO 7102, and correlate composition with toxicity through bioassays with trout embryos. A specific emphasis of the separation process involved isolation of PAHs and alkyl-PAHs, since that group of compounds was previously shown to have a major impact on chronic toxicity to the early life stages of fish and are responsible for most of the toxicity of the whole oil [1, 2].

4.1.1 Fractionation Methods

A three-phase effects-driven fractionation approach was taken to separate HFO 7102 by groups of similar compounds (Table 4.1). The first step was a low-temperature vacuum distillation to divide the oil into three fractions by volatility. The components were distributed in a 7:25:66 (F2:F3:F4) fashion from lightest to heaviest, indicating a very high relative composition

of asphaltenes and resins in the whole oil. The second fractionation step removed non-polar compounds from F3 by solubility in cold acetone. Approximately 16.1% of F3 collected as a wax-rich residue (F3-2), and 81.5% remained in the PAH-rich extract (F3-1). The final fractionation procedure separated F3-1 into four sub-fractions, approximately by number of aromatic rings, by column chromatography in an open-bore silica-packed column. A 48:22:10:5.7 (F3-1-1:F3-1-2:F3-1-3:F3-1-4) fractionation profile was observed, indicating a high proportion of 2-3 ring PAH and other non-polar components due to 70% of F3-1 eluting in the first two fractions. Although these procedures did not have the highest degree of reproducibility, they proved capable of providing the type of separations required to tease apart fractions with varying degrees of toxicity.

Table 4.1: Relative Contribution of Each Fraction to the Weight of the Whole Oil (WO). n=1 for Phase II, n=35 for Phase III and n=4 for Phase IV.

Phase	Fraction	Parent Fraction	% of Parent Fraction	% of WO
II	F2	WO	6.8	6.8
II	F3	WO	25.4	25.4
II	F4	WO	65.7	65.7
II	Lost	WO	2.1	2.1
III	F3-1	F3	81.5 ± 4.0	20.7 ± 1.0
III	F3-2	F3	16.1 ± 2.6	4.1 ± 0.7
III	Lost	F3	2.5 ± 3.3	0.6 ± 0.8
IV	F3-1-1	F3-1	48.1 ± 1.6	10.0 ± 0.6
IV	F3-1-2	F3-1	22.4 ± 6.2	4.6 ± 1.3
IV	F3-1-3	F3-1	10.1 ± 1.0	2.1 ± 0.2
IV	F3-1-4	F3-1	5.7 ± 1.5	1.2 ± 0.3
IV	Lost	F3-1	13.6 ± 2.4	2.8 ± 0.5
Total Loss				5.5 ± 1.3

4.1.2 Toxicity of Sub-Fractions

Rainbow trout embryos were exposed to various concentrations of dispersed oil solutions for each fraction generated. HFO 7102 was about 7.5 times more toxic than MESA, a medium crude oil, and about 7 times less toxic than another heavy fuel oil, HFO 6303. The more toxic of these oils are known to contain higher concentrations of PAH and alkyl-PAH, particularly phenanthrene and chrysene [1]. The most toxic fraction of HFO 7102 was F3, at about a six-fold toxicity increase over the whole oil. Results from the other fractions showed that F2 had similar toxicity to the whole oil, while only dispersant toxicity was observed in fish exposed to F4. The wax-rich residue F3-2 proved non-toxic at all concentrations tested, while the PAH-rich fraction F3-1 accounted for all of the toxicity in F3. Bioassays on Phase IV sub-fractions (F3-1-1 through F3-1-4) will be performed in future work. Considering that F3-1-2 contains the majority of three and four ringed PAHs, it is expected that this fraction will be the most toxic.

4.2 Major Research Findings

In accordance with project objectives, great strides were made in the compositional analysis and toxicological characterization of Heavy Fuel Oil 7102. The oil was found to be composed of less than seven percent “light compounds” and nearly two-thirds asphaltenes and resins, leaving only one-quarter in the most toxic fraction, F3. This fraction contains about 81.5% polar materials as a PAH-rich extract (F3-1) and about 16% waxes (F3-2), which means that the most toxic fraction of those bioassayed (F3-1) represents about 21% of the total composition of HFO 7102. This is a much larger proportion than that observed in medium and light crude oils previously studied by our group (ANSC 9.4% of total mass in F3-1, SCOT 5.7% of total mass in F3-1) [2]. This substantial difference is likely a major reason why HFOs are so much more toxic

than crudes: they contain much higher concentrations of PAHs and alkyl-PAHs, and the fraction containing these groups of compounds forms a much higher proportion of the whole oil.

The most toxic groups of compounds are likely three and four-ringed PAHs, according to bioassays of fractions generated and GC-MS analysis of those fractions. The F3 fraction contains significantly more three and four-ringed compounds than other fractions, which likely contribute to that fraction's substantial relative toxicity. The toxicity of long-chain alkanes can be discounted at all concentrations tested due to the fact that none of the fish exposed to the wax precipitate F3-2 showed signs of toxicity. Previous work by our group showed that light and medium crude oils are much richer in waxes, with F3 being as high as 40-60% wax [2]. Since these compounds are non-toxic at all concentrations tested, it is logical that an oil with a higher composition of waxes would be less toxic than HFO 7102, which had a much lower wax composition (F3-2 only 4.1% of the weight of the whole oil).

The column chromatography methods used generated four further fractions. F3-1-1 contained primarily saturates and two-ring PAHs. F3-1-2 contained nearly all of the three and four-ringed PAHs. F3-1-3 contained the remaining four-ringed PAHs, while F3-1-4 contained a hodgepodge of compounds which remained on the column after elution. This is in accordance with the expectation that compounds with fewer aromatic rings and lower molecular weight would elute first. The most toxic compounds are likely concentrated in F3-1-2 (4.6% of the weight of HFO 7102). If future toxicity tests agree with this likelihood, the vast majority of the toxicity of HFO 7102 will be concentrated in less than 5% of its weight, achieving the project goal of concentrating toxicity into 10% of the weight or less! This separation will make it possible to further study HFO 7102 and target more specific compounds for future analysis.

4.3 General Applications of EDFA

The methods described in this document were carefully developed and optimized. The three-step fractionation technique (low-temperature vacuum distillation, wax precipitation, and open column chromatography) may be considered a standard method for future analysis and separation of heavy fuel oils, and may serve as a guideline for other oils. As expected, the most toxic fractions were those containing the highest concentration of PAHs and alkyl-PAHs. Future bioassays of Phase IV sub-fractions will contribute to the identification of the most toxic PAH by number of aromatic rings. The relative composition of these compounds can be used as a measure of the effects on fish exposed to heavy fuel oil and will serve to guide clean-up efforts of spilled HFO 7102. Some of the potential impacts of dispersed oil on fish populations can be estimated if a known volume of oil is spilled in a relatively contained area, including contributions to ecological risk assessments, natural resource damage assessments, establishing cause and effect for liability, and environmental forensics.

4.4 Future Work

Although this part of the project has finished, there is still more work to be done in the field of HFO analysis, including work with HFO 7102. To begin, the Phase IV sub-fraction exposures to rainbow trout embryos for bioassay must be completed. In addition to identifying specific groups of PAH that are more toxic than others, this bioassay would direct the next phase of separation. If, as expected, the toxicity is concentrated in F3-1-2, it will mean that three and four-ring PAHs are the most likely causes of toxicity. F3-1-1 is representative of remaining saturates and two-ring PAHs, while F3-1-3 contains about half of the chrysenes and some other

four to six-ring PAHs. F3-1-4 contains a wide variety of compounds in small concentrations and accounts for less than six percent of the weight of F3-1, and thus is unlikely to reveal much information about toxic compounds, if it proves toxic at all.

Chromatography with a non-polar stationary phase (e.g. reverse-phase HPLC) could be used to further fractionate a PAH mixture by degree of alkylation. Another series of exposures with these sub-fractions could also be conducted to determine whether un-substituted, mono-alkylated, di-alkylated, tri-alkylated, or tetra-alkylated PAH are the most toxic to fish. For these bioassays to be completed, a significant amount of Phase IV sub-fractions would have to be generated (by column chromatography) such that there would be sufficient material in the resulting sub-fractions. This could be a somewhat expensive and time-consuming process, but the results would likely be quite valuable.

GC-MS analysis of Phase IV sub-fractions revealed that the fractional divisions are not entirely representative of the number of aromatic rings. The second fraction, F3-1-2, clearly began after the elution of phenanthrenes, but included nearly as many chrysenes as F3-1-3 (which was supposed to begin with chrysene elution). Further refining of this process, including greater care as cut-points are approached, and a higher resolution analysis than TLC, may lead to a point where the first three fractions more closely resemble a saturates & two-ring PAH/three-ring PAH/four or more ring PAH split. The analysis has shown that some overlap is to be expected, but the model has been set and F3-1 definitely elutes smaller (fewer ringed) PAH earlier than larger (many ringed) PAH compounds.

As more information is learned about HFO 7102, and heavy fuel oils in general, a better effort can be made to estimate the effects on aquatic life due to heavy fuel oil spills and to remediate said spills. It is important to distinguish between the effects of crude oil spills and

heavy fuel oil spills due to differences in composition, density, viscosity, octanol-water partition coefficient, and other behavioural properties. Ultimately, this work is a stepping stone for impact assessments of heavy fuel oil spills, and may lead to variations in the refining process to eliminate (or reduce the concentration of) certain chemical species which have been shown to cause significant damage to the early life stages of fish.

4.5 References

1. Martin, J. D. *Comparative Toxicology and Bioavailability of Heavy Fuel Oils to Fish Using Different Exposure Scenarios;* M.Sc. Thesis ; Queen's University: Kingston, ON, 2011.

2. Saravanabhavan, G. *An Effect-Driven Fractionation Approach for the Isolation and Characterization of CYP1A Inducing Components of Crude Oils;* PhD Thesis; Queen's University: Kingston, ON, 2007.

Appendix A

Scale-up Replicate Data

Table A.1: Scale-up Replicate data for Wax Precipitation of F3 (detailed in Chapter 2). Uncertainties are calculated as one standard deviation.

Run #	Total F3 (g)	Mass F3-1 (g)	F3-1%	Mass F3-2 (g)	F3-2 %	Total Recovery (g)	Total Recovery %
1	8.7336	3.2831	37.59%	1.5662	17.93%	4.8493	55.52%[1]
2	10.2011	7.7118	75.60%	2.0238	19.84%	9.7356	95.44%
3	10.3577	8.1796	78.97%	1.6392	15.83%	9.8188	94.80%
4	7.0894	5.6899	80.26%	1.3391	18.89%	7.0290	99.15%
5	10.7000	8.3666	78.19%	1.9195	17.94%	10.2861	96.13%
6	9.0258	7.3302	81.21%	1.3044	14.45%	8.6346	95.67%
7	8.8661	7.1706	80.88%	1.3773	15.53%	8.5479	96.41%
8	9.2608	7.3215	79.06%	1.5815	17.08%	8.9030	96.14%
9	9.5691	7.4403	77.75%	1.6303	17.04%	9.0706	94.79%
10	10.3318	n/a	n/a	n/a	n/a	n/a	n/a[2]
11	9.2287	8.6868	94.13%	0.6731	7.29%	9.3599	101.42%
12	8.7977	7.5760	86.11%	0.9127	10.37%	8.4887	96.49%
13	10.3100	8.5100	82.54%	1.4118	13.69%	9.9218	96.23%
14	10.3229	8.6784	84.07%	1.3887	13.45%	10.0671	97.52%
15	11.0615	9.0971	82.24%	1.7513	15.83%	10.8484	98.07%
16	10.7491	8.6376	80.36%	1.7694	16.46%	10.4070	96.82%
17	10.4613	8.5447	81.68%	1.6044	15.34%	10.1491	97.02%
18	10.3950	7.8869	75.87%	2.1432	20.62%	10.0301	96.49%
19	9.4804	8.1385	85.85%	1.5863	16.73%	9.7248	102.58%
20	10.0262	7.6721	76.52%	1.9390	19.34%	9.6111	95.86%
21	10.5564	8.6845	82.27%	1.6179	15.33%	10.3024	97.59%
22	10.6049	8.5462	80.59%	1.7582	16.58%	10.3044	97.17%
23	9.5928	7.9796	83.18%	1.5775	16.44%	9.5571	99.63%
24	9.8267	7.9616	81.02%	1.8276	18.60%	9.7892	99.62%
25	10.4828	8.3157	79.33%	1.7897	17.07%	10.1054	96.40%
26	10.6070	8.7146	82.16%	1.9058	17.97%	10.6204	100.13%
27	11.2812	9.2191	81.72%	1.7900	15.87%	11.0091	97.59%
28	10.0894	8.2386	81.66%	1.4702	14.57%	9.7088	96.23%

[1] Some F3-1 was spilled in Run #1. Data for F3-1 and total recovery omitted in Average calculations.
[2] Trial 10 aborted.

29	10.5894	8.5502	80.74%	1.8677	17.64%	10.4179	98.38%
30	10.5768	8.3248	78.71%	1.8777	17.75%	10.2025	96.46%
31	13.6212	12.1520	89.21%	2.1341	15.67%	14.2861	104.88%
32	13.5645	11.9822	88.33%	2.3112	17.04%	14.2934	105.37%
33	13.1773	11.0031	83.50%	2.3385	17.75%	13.3416	101.25%
34	13.0001	10.9074	83.90%	1.8550	14.27%	12.7624	98.17%
35	9.5037	7.4331	78.21%	1.2881	13.55%	8.7212	91.77%
36	12.0479	9.1395	75.86%	1.4577	12.10%	10.5972	87.96%
Average	**n/a**	**n/a**	**81.5 ± 4.0%**	**n/a**	**16.1 ± 2.6%**	**n/a**	**97.5 ± 3.3%**

Table A.2: Replicate Scale-up data for Column Chromatography of F3-1 (detailed in Chapter 2). Uncertainties are calculated as one standard deviation.

Run #	F3-1 (g)	F3-1-1 (g)	F3-1-1 %	F3-1-2 (g)	F3-1-2 %	F3-1-3 (g)	F3-1-3 %	F3-1-4 (g)	F3-1-4 %	% Recovery
1	10.4912	5.1452	49.04%	1.8937	18.05%	1.1719	11.17%	0.8127	7.75%	86.01%
2	10.5650	5.1826	49.05%	2.8281	26.77%	0.9813	9.29%	0.5963	5.64%	90.76%
3	10.5512	n/a	n/a	n/a	n/a	n/a	n/a	0.4529	4.29%	n/a[3]
4	10.9491	5.0709	46.31%	n/a	n/a	1.0855	9.91%	0.5520	5.04%	n/a[4]
Average	**n/a**	**n/a**	**48.14 ± 1.58%**	**n/a**	**22.41%**[5]	**n/a**	**10.12 ± 0.96%**	**n/a**	**5.68 ± 1.48%**	**86.35 ± 2.37%**[6]

[3] In Scale-up #3, the same column was used as Scale-up #2 without re-packing. The column was clearly not fully regenerated, and the F3-1 loading eluted more quickly and with less repeatability than expected. Due to a "blurring" of the fractions, only F3-1-4 could be used, as it is eluted afterwards with a different solvent.

[4] In Scale-up #4, all of F3-1-2 was lost due to experimenter error.

[5] Since there were only two usable replicates for F3-1-2, standard deviation (and thus uncertainty) could not be calculated.

[6] Average recovery was calculated as the sum of average recoveries for each individual fraction, and uncertainty is the square root of the sum of the squared uncertainties of each individual fraction.

Appendix B

GC-MS Chromatograms of HFO 7102 and its Fractions

B.1 Phase I Chromatogram

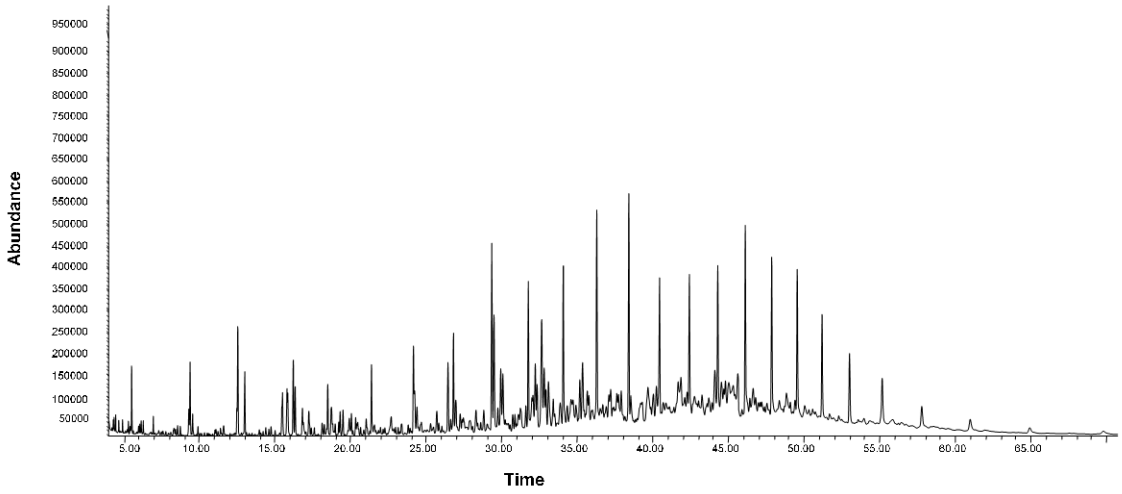

Figure B.1: GC-MS Chromatogram for HFO 7102 (Whole Oil)

B.2 Phase II Chromatograms

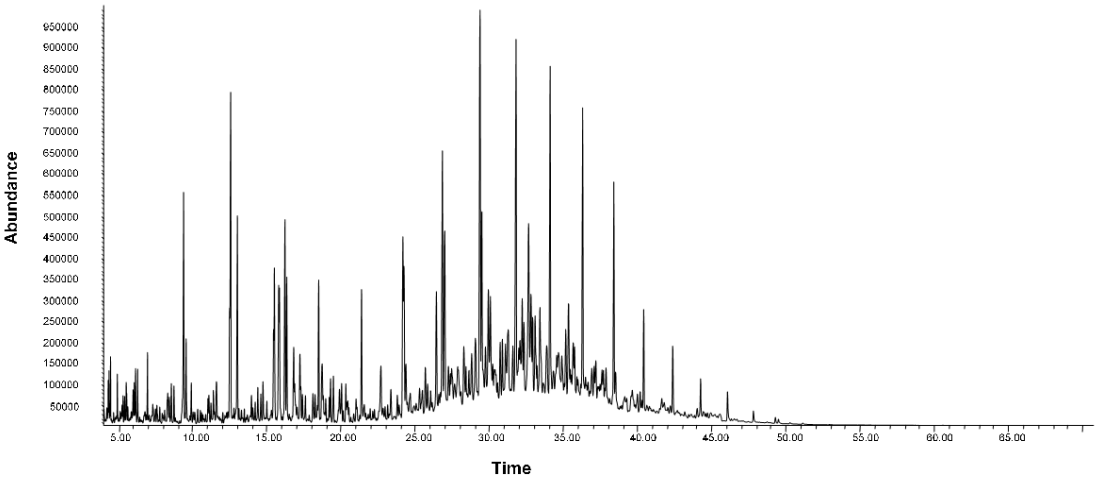

Figure B.2: GC-MS Chromatogram for F2

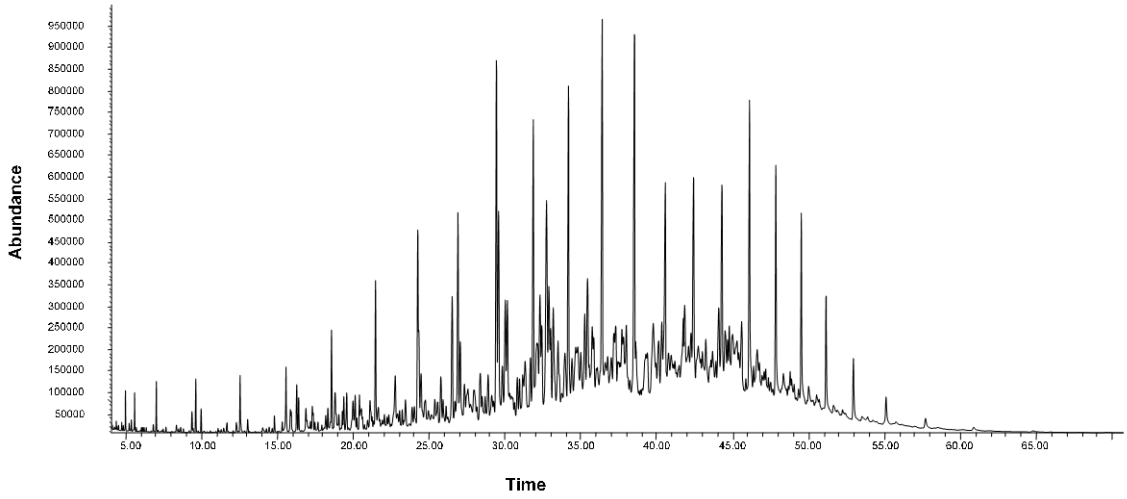

Figure B.3: GC-MS Chromatogram for F3

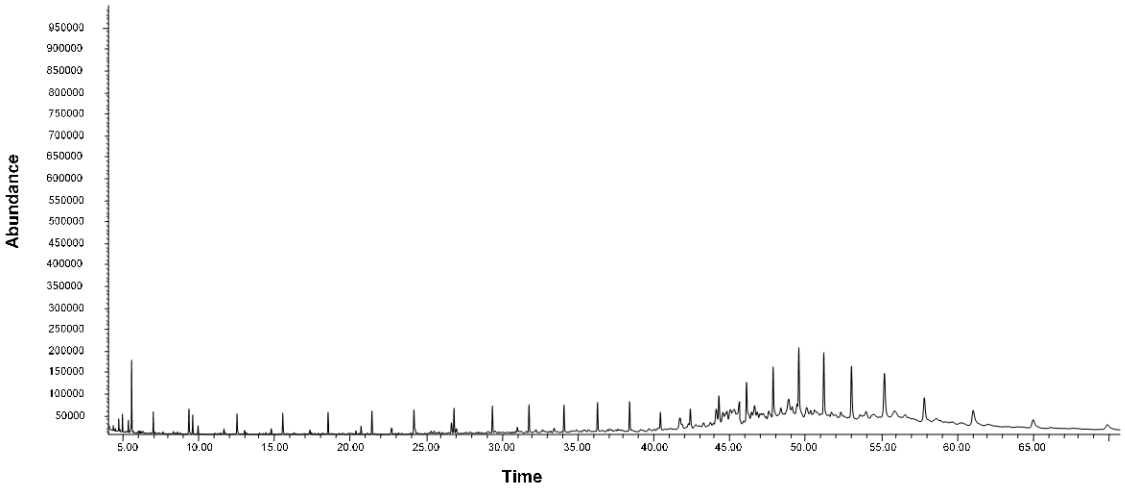

Figure B.4: GC-MS Chromatogram for F4

B.3 Phase III Chromatograms

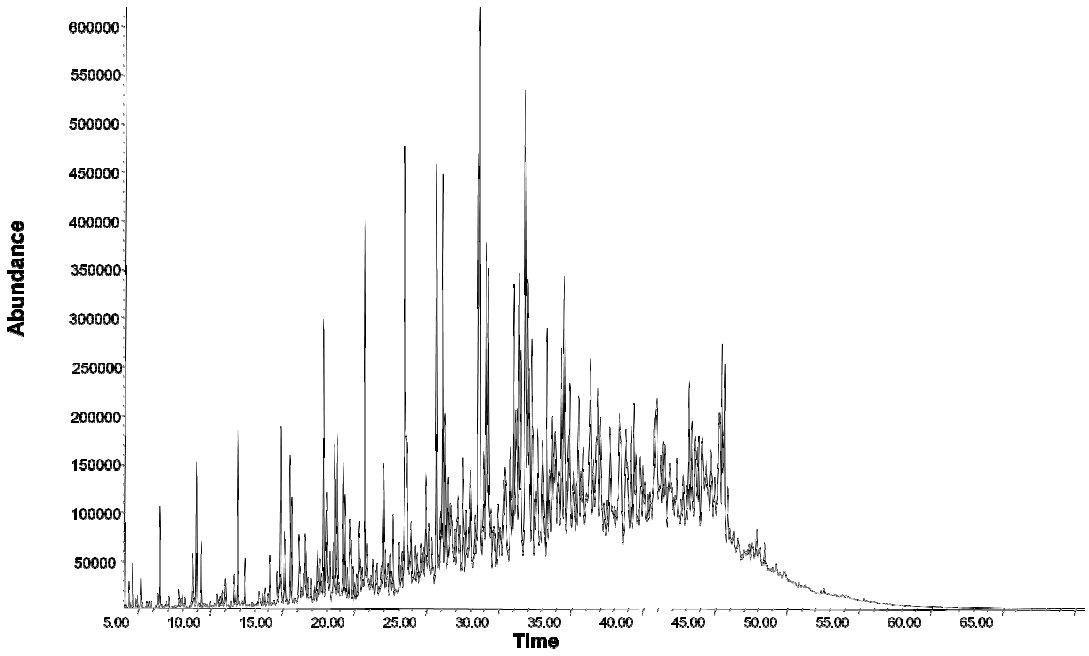

Figure B.5: GC-MS Chromatogram for F3-1

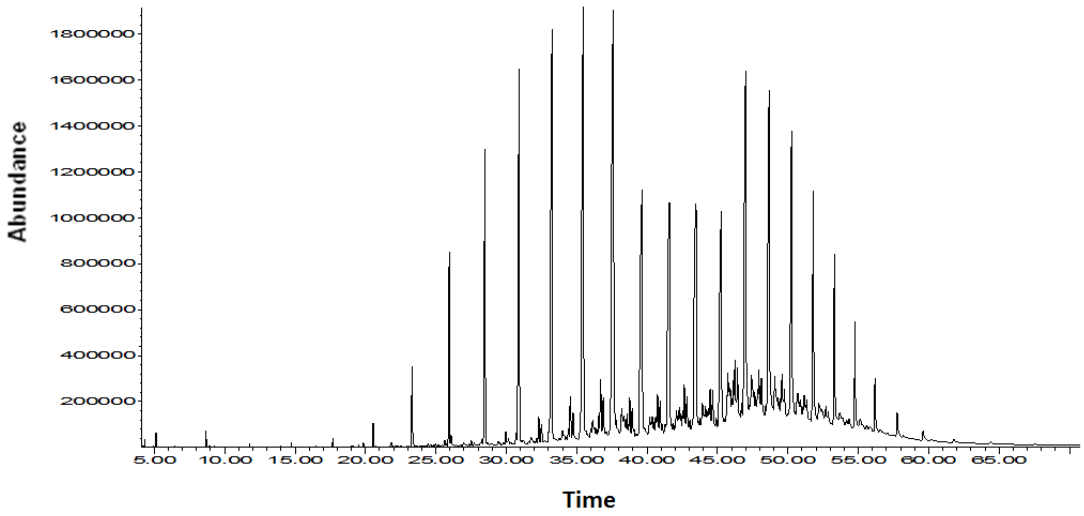

Figure B.6: GC-MS Chromatogram for F3-2

B.4 Phase IV Chromatograms

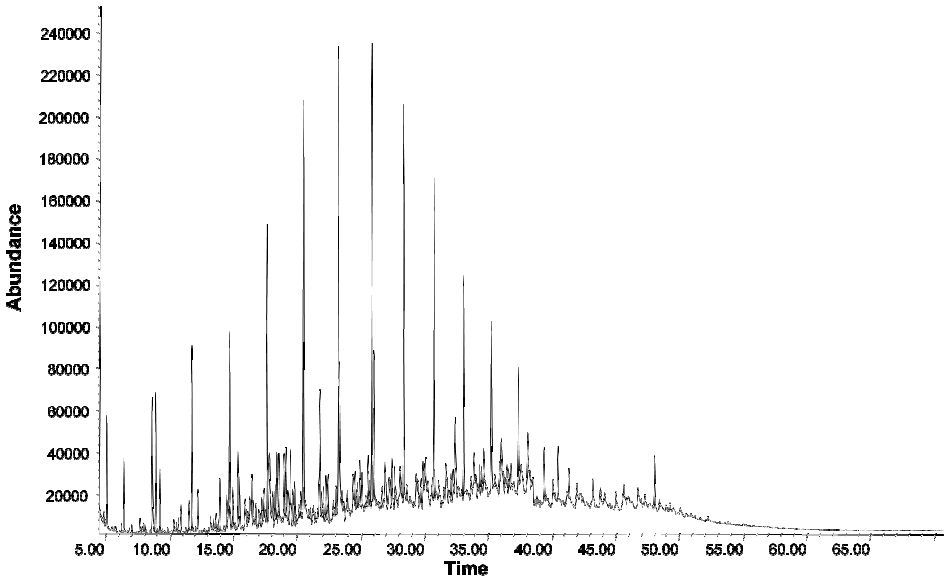

Figure B.7: GC-MS Chromatogram for F3-1-1

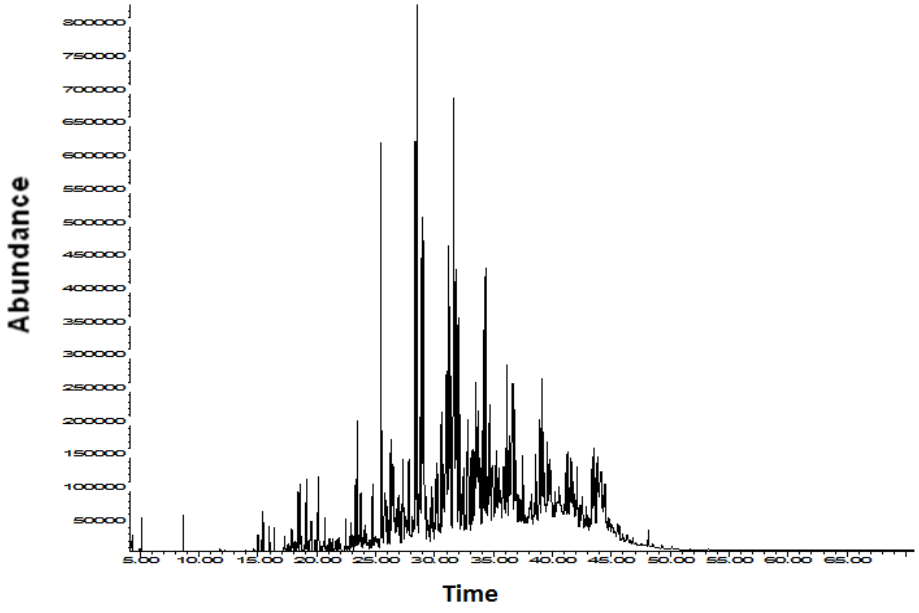

Figure B.8: GC-MS Chromatogram for F3-1-2

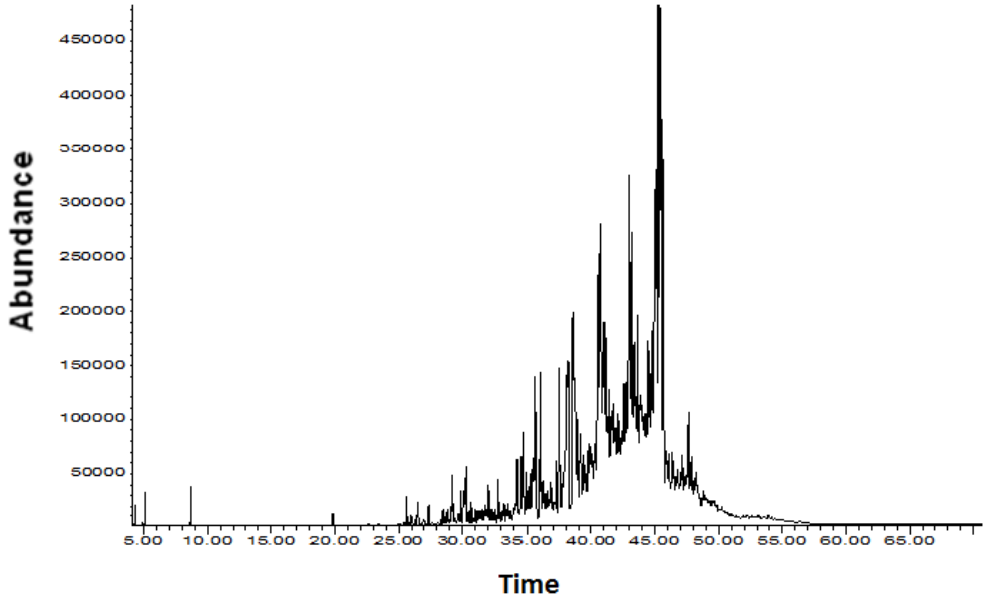

Figure B.9: GC-MS Chromatogram for F3-1-3

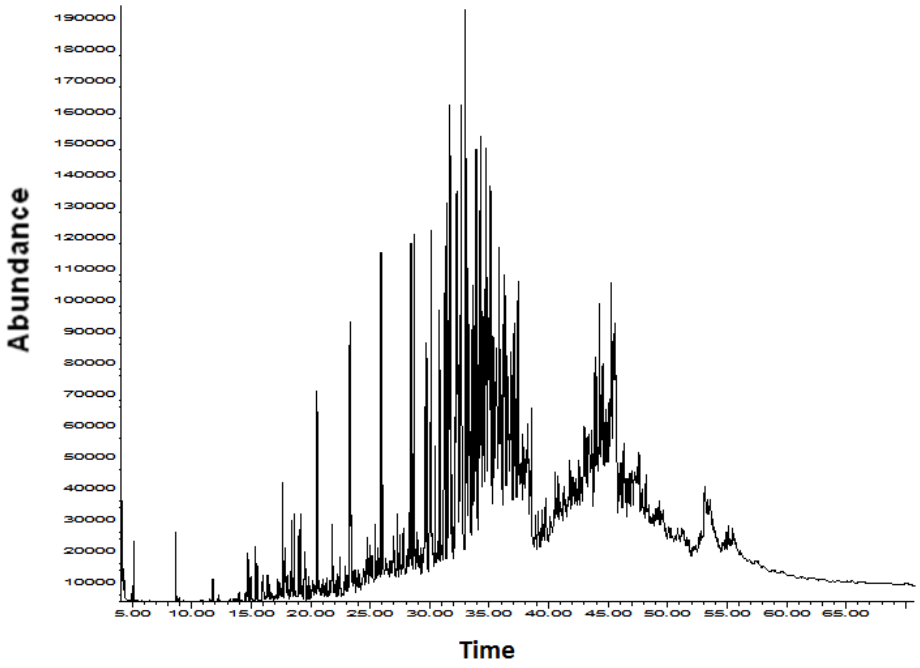

Figure B.10: GC-MS Chromatogram for F3-1-4