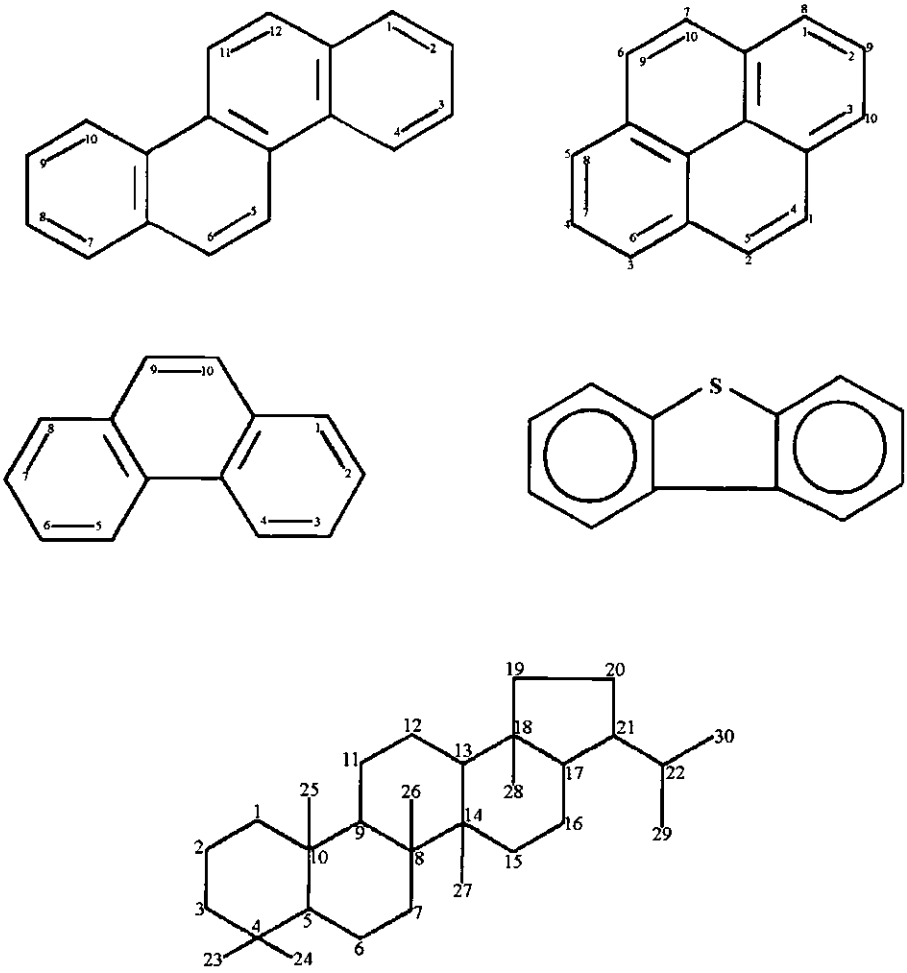
OCS Study
MMS 2000-086

Coastal Marine Institute

Studying and Verifying the Use of Chemical Biomarkers for Identifying and Quantitating Oil Residues in the Environment



MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

LSU Cooperative Agreement
Coastal Marine Institute
Louisiana State University

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ABSTRACT

Analytical chemistry and instrumentation provides environmental scientists with the ability to identify and track the fate of spilled oil residues in the marine environment. Compounds commonly used for the identification of spilled oil to a source are called biomarkers. Biomarker compounds are universal in crude oils and petroleum products and are generally more resistant to environmental weathering than most other oil constituents. The distribution of biomarker compounds is unique for each oil and different sources of petroleum exhibit different oil fingerprints. Self-normalizing fingerprint indexes (SFI) calculated from the oil fingerprints provide a stable and useful tool for determining a match or nonmatch for different oil residues present in some environmental samples. A combination high-resolution gas chromatography and mass spectrometry, visual comparison and self-normalizing fingerprint indexes (SFI) were utilized to establish eight petroleum biomarkers for oil spill identification and assessment.

The eight petroleum biomarkers chosen for detection and analysis were determined through a literature search and previous research. SFI calculated from GC/MS analysis of tarballs and an oil degradation study validated the use of the eight biomarkers chosen. Of the eight SFI, four remain stable over an extended period of time and laboratory simulated weathering. Visual comparison of biomarker fingerprints played an important role in distinguishing gross, and in some cases subtle, differences between unknown environmental samples. Double SFI scatterplots were also utilized as a screening tool for source match/nonmatch determinations.

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INTRODUCTION

Research Objective

Analytical chemistry and instrumentation provides environmental scientists with the ability to identify and track the fate of spilled oil residues in the marine environment. Since the 1960's many advances have been made in analytical chemistry techniques and instrumentation; however, despite these advances, more accurate methods are necessary for identifying and quantifying oil contamination in the environment. Biomarkers, for the purpose of this study, are petroleum components that remain relatively unchanged in oil residues even after natural environmental weathering processes. Since these biomarker compounds are relatively persistent, they are commonly used for the identification of a spilled oil to its source. The objective of this research is to refine the use of biomarkers as tools for oil spill identification and assessment, and to verify the analytical approach utilized by the Louisiana State University, Institute for Environmental Studies, Response and Chemical Assessment Team (LSU IES/RCAT) with both laboratory and field evaluation studies. High-resolution gas chromatography and mass spectrometry (GC/MS) and source-fingerprinting techniques will be employed to fulfill the research objective. Visual comparison, self-normalizing fingerprint indexes (SFI) calculated from GC/MS analysis and biomarker normalization methods will provide further validation for the importance of analyzing biomarkers in oil and oil residues found in the marine environment.

Background Information

Oil spills into marine environments have been recognized as major environmental insults for more than 30 years. The 1968 *Torrey Canyon* spill accentuated the large volumes of oil and potential environmental threat posed by the then newly introduced "supertanker." In 1969, a production well blow-out off the California coastal city of Santa Barbara caused another major spill in the marine environment further highlighting environmental concerns generated by oil pollution. Since the 1960s, there have been numerous spills around the world, including the infamous *Amoco Cadiz* and *Exxon Valdez* spills, as well as major well blow-outs in the North Sea, Bay of Campeche, and the Persian Gulf. In addition to these incidents, intentional oil fires and marine oil spills which occurred during the 1991 Persian Gulf War have also contributed to environmental contamination and resulted in considerable media attention. Media attention often leads to public outrage and in some cases, such as in the United States following the *Exxon Valdez*, laws are created and passed to control and give liability to the responsible parties (i.e. The Oil Pollution Act of 1990).

High profile oil spills are not the only source of oil pollution in marine and other environments. Chronic oil discharges occur from a variety of small spills, natural seeps of oil from rivers and oceans, non-point source run-off, operational discharges from tankers, and development associated with petroleum exploration. As a result, there is approximately 5 million tons of oil released into the oceans each year. Approximately 6% of the 5 million tons (300,000) are from tanker accidents such as the *Exxon Valdez*, while the remaining 4.7 million tons are from natural, chronic and acute events (Henry 1995).

Crude oil is a complex mixture of organic compounds derived from the partial decomposition of animals and plants that were once alive, but have been long since dead. The process of oil formation occurs slowly and produces "simple" organic molecules (petroleum) from more complex organic structures (organic biomass). Petroleum-type hydrocarbons may exist as a gas (natural gas), as a liquid (crude oil), as a solid (tar, bitumen), or any combination. All crude oils are composed primarily of carbon (80-87%) and hydrogen (10-15%), and to a lesser degree, sulfur (0-10%), nitrogen (0-1%) and oxygen (0-0.5%). Metals are a minor constituent in petroleum and contribute little to the toxicity of oil. The physical and chemical properties of crude oil vary with regions of production and zones within these regions. Since crude oils vary, no single definition or composition is valid for all oils.

In general, all crude oils tend to be composed of the same individual compounds but the relative abundance of these compounds may vary significantly between different oils. These differences are important from the refinery processing and environmental chemistry perspective because they are useful for predicting how an oil will behave if spilled in the marine environment. Common oil constituents can be classified into four general groups: (1) individual saturated hydrocarbons (the normal alkanes and isoprenoids); (2) polycyclic aromatic hydrocarbons (PAHs) including the dominant alkylated homologues in oil; (3) sulfur heterocyclic aromatic hydrocarbons and related alkylated homologues; and (4) oil biomarkers (primarily the steranes and triterpanes) which are polycyclic aliphatics. Even though aromatic hydrocarbons (groups 2, 3, and 4) represent less than 5% of the bulk composition of most oils, they are essential for assessing biological effects, aid in determining exposure pathways, source characterization, and for monitoring the extent of oil weathering and degradation (Sauer and Boehm 1991).

Once oil is discharged into the marine environment, it undergoes various physical and chemical interactions such as spreading, drifting, dispersion, evaporation, dissolution, emulsification, photochemical degradation, and biodegradation. These effects on bulk oil composition are collectively called weathering and result in changes in the aromatic hydrocarbon profile of an oil. The two primary weathering processes affecting oil in the marine environment are evaporation and biodegradation. The chemical effects of weathering can be broken down into four simplified stages. Samples are classified as either relatively unweathered or slightly, moderately, or heavily weathered. Relatively unweathered oils exhibit no apparent change in the aromatic hydrocarbon profile. Loss of compounds more volatile than naphthalene may have occurred, but any weathering is minor. Slightly weathered oils exhibit no major changes in the relative order or abundance of aromatic homologues. The alkylated naphthalenes are the most abundant constituents, but may be slightly reduced; and the normal alkanes are generally still present. The total naphthalenes are significantly depleted in moderately weathered oils; and the total alkylated dibenzothiophenes and phenanthrenes dominate the histogram plot. Furthermore, moderately weathered oils have a highly degraded normal alkane fraction. Dibenzothiophenes and phenanthrenes are significantly depleted from heavily weathered oil, and dominant constituents are alkylated napthobenzothiophenes, pyrenes and chrysenes.

Tarballs are examples of moderately to heavily weathered oil. Light petroleum products and light crude oils, such as many South Louisiana Crude production oils, spread rapidly and are often removed from the ocean surface by dispersion during high sea state conditions. On the

other hand, the very heavy crude oils, refined heavy bunker oils, and other petroleum products with high pour points are slow to spread, exposing little surface area for the natural degradation process. Thick oils are generally only degraded at the surface interface resulting in the encapsulation of "fresher" oil. As a result, heavier oils are the most persistent in the environment and are often encountered as stranded tarballs along the debris line of the beach face. The formation of "mousse", a stable water-in-oil emulsification, may enhance the process of tarball formation and extend environmental persistence of the lighter crude oils. Mousse formation is common and is a prime factor for the formation of tarballs from otherwise easily dispersible light crude oils.

Factors that influence weathering include weather conditions, the environment in which the oil was spilled, and the type of oil. Once oil is stranded on a beach or shoreline, weathering is modified by the microenvironment in which the oil is entrapped and the rate of natural biodegradation at any specific location is dependent on a variety of factors. The amount of oil, mixing energy, microfauna, and the availability of oxygen are a few of the key factors of biodegradation. The limiting factor is often the availability of oil to the microbial community. Other physical/chemical properties that affect the fate of individual constituents in crude oil include vapor pressure, water solubility, the organic carbon partitioning coefficient (K_{oc}) and octanol/water partitioning coefficients (K_{ow}). Different oils spilled under similar conditions may undergo entirely different weathering changes. The ability to examine a sample of residual oil (such as a tarball) and speculate on the composition of the unweathered oil from which it was derived is valuable information. Environmental forensic investigations to determine a potential source of an unknown or mystery spill must exploit both morphological, as well as, chemical compositional differences.

Biomarkers

As previously stated, petroleum spilled into the marine environment undergoes varying degrees of environmental weathering that affect its composition. As weathering proceeds, certain groups of oil constituents are lost in a predictable sequence. The first compounds to be depleted are the n-alkanes and isoprenoids, followed by the lighter PAHs, then the remaining PAHs and their alkyl homologues. As a result, unique identification of sources of oil spills may be difficult due to the loss of these oil constituents and their distribution patterns relative to the amount of weathering.

Biomarkers, as defined for this research, are petroleum components that remain detectable and relatively unchanged in oil residues even after natural environmental weathering processes. They are also typically resistant to biodegradation and are therefore, useful as chemical markers. Biomarker fingerprinting by GC/MS may be necessary for environmental samples in which identification of spilled oil is difficult due to weathering of target compounds. Two classes of biomarkers commonly referred to in the literature are the triterpane and sterane compounds (Figure 1). The triterpane and sterane distributions are unique for each type of oil, and, because of their relatively refractory nature, these compounds help to identify a particular oil, even oils with similar geographical origins, when other target analytes are lost. Biomarkers suffer little interference from weathering effects because of their high resistance to degradation, which can be attributed to their high molecular weights. As an oil becomes more degraded, the

concentration of biomarker compounds should increase relative to the more easily degraded constituents (Wang and Fingas 1995b). These compounds are also useful in distinguishing a spilled oil from other oils and sources that may be present within the same sample matrix. As a result, biomarkers provide chemical fingerprinting information about the source, degree of weathering, characteristics, and fate of the spilled oil.

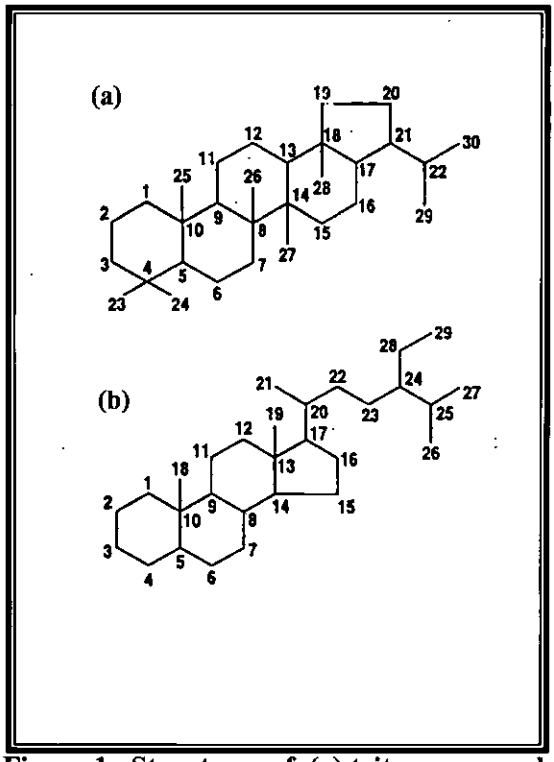


Figure 1. Structures of (a) triterpanes and (b) steranes, both common biomarker compounds.

While the hopanes and steranes are useful biomarker compounds, other biomarkers from one of the four groups of oil constituents previously listed are essential due to the fact that refinery processes remove the triterpanes and steranes. Previous research in the IES/RCAT laboratory, as well as other studies reported in the literature, suggest that the compounds and ratios listed in Table 1 are most promising as biomarkers. The "n" represents normal alkane (m/e 85), and "C" relates to the number of carbons attached to the parent molecule. For example, C-3 dibenzothiophene indicates that the unalkylated sulfur heterocyclic parent structure has three carbons attached as either a propyl group or a combination of methyl and ethyl groups. The structures of a few of these compounds are displayed in Figure 2.

Table 1. Target Petroleum Biomarkers.

IES/RCAT Biomarkers	Abbreviations
nC-17/pristine	nC-17/Pris
nC-18/phytane	nC-18/Phy
(Table 1 Continued)	

IES/RCAT Biomarkers	Abbreviations
C-3 Dibenzothiophene	C-3 DBT
C-3 Phenanthrene	C-3 Phen
C-1 Pyrene	C-1 Pyr
C-1 Chrysene	C-1 Chr
17α (H), 21β(H) -30 Norhopane/17α (H), 21β(H) - Hopane	Nor/Hop
Sum of C-3 Dibenzothiophene divided by the sum of the C-3 Phenanthrenes	C-3 DBT area/C-3 Phen area

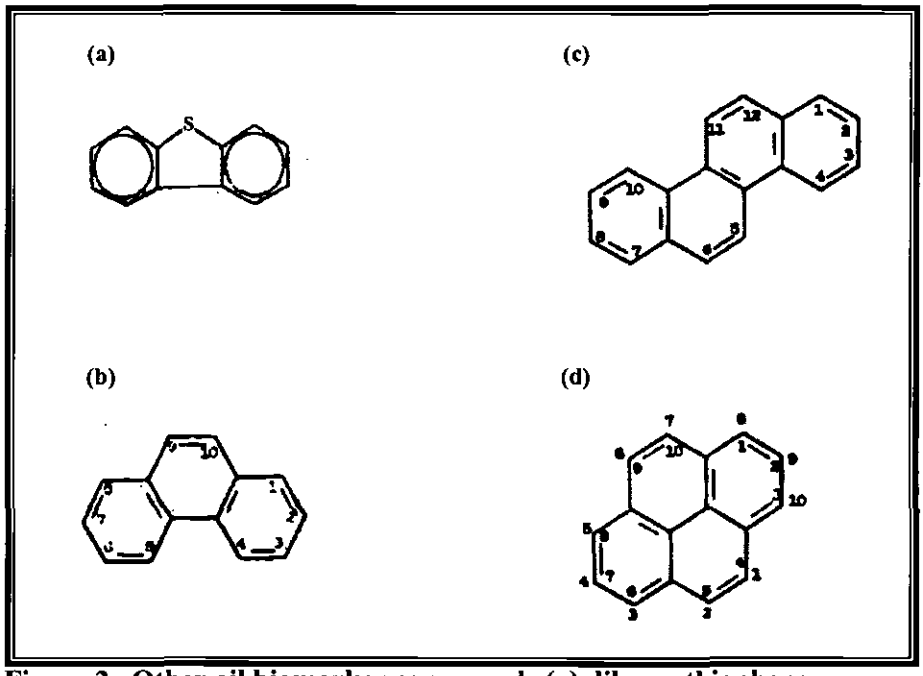


Figure 2. Other oil biomarker compounds (a) dibenzothiophene; (b) phenanthrene; (c) chrysene; and (d) pyrene.

Analytical Chemistry and Oil Characterization

The foremost questions asked about spilled oil are its source and quantity in various compartments of the environment, and the risk and consequences associated with various levels of petroleum within these compartments. Spilled oil is not evenly or uniformly dispersed throughout the environment because many chemical compounds that make up petroleum are not water-soluble. Consequently, spilled oil is dispersed into aquatic environments in a very heterogeneous fashion. The physical and chemical processes that constitute environmental weathering continually alter its composition and distribution into different facets of the environment. The heterogeneous distribution of oil, with a continually changing composition, causes considerable uncertainty in assessing the impacts of oil spills and chronic petroleum releases. These factors also pose a problem in the evaluation and selection of various technologies that are utilized to mitigate the incidence.

Furthermore, the complex mixture and alteration of spilled oil in the environment creates a problem for most analytical detection techniques. This is due to the fact that most analytical methods provide little or no discrimination between sources and the calculated concentrations are often accepted as real. Other situations that complicate the ability to assess trace level oil pollution are matrix effects and multiple pollution sources. Analytical approaches to oil spill assessment include U.S. Environmental Protection Agency (EPA) methods for volatile aromatic compounds and polycyclic aromatic hydrocarbons (PAHs). Unfortunately, these methods were originally developed to assess industrial and hazardous waste (Wang and Fingas 1995a), not oil pollution. When studying oil pollution in aquatic environments, these analytical approaches lack chemical specificity for oil compounds and are unable to differentiate sources of contamination and quantify oil residues in complex environmental samples. To further complicate matters, many of the petroleum compounds of interest have no commercially available standards and identification is often based on the extensive qualitative mass spectrometer analyses during method development. Therefore, the risks and consequences associated with various levels of petroleum in all parts of the environment are very difficult to accurately assess and the effectiveness of mitigating technologies are not easily ascertained. In an effort to better identify and quantify spilled oil, document its weathered residues, and assess the physical/chemical transformations caused by weathering, the IES/RCAT laboratory in 1990 developed an advanced GC/MS methodology and self-normalizing fingerprint indexes (SFI) for source-fingerprint analysis of moderate to heavily contaminated samples associated with oil spills.

Analytical methods for identifying oil in a marine environment should accomplish the following tasks: detect the presence of oil; provide compound specific quantification of target petroleum compounds; and provide data applicable to source-fingerprinting. Other criteria to consider when developing an analytical method that targets both common oil constituents and analytes for baseline oil pollution include the ability of the method to differentiate between petroleum and other natural and anthropogenic sources of hydrocarbon pollution (i.e. terrestrial plant waxes and combustion by-products).

Information about the bulk physical and chemical properties of oil provides limited data to assess damages to the environment. Standard EPA methodologies are inadequate for assessing petroleum pollution since they lack key target compounds characteristic of oil. Therefore, a more chemically selective approach is required to determine the concentration of constituents in spilled oil in environmental samples. While no standardized methodology currently exists at a national level, there is fundamental acceptance of GC/MS for the analysis of petroleum. This is due to the fact that GC/MS provides highly selective source-fingerprinting information along with compound specific quantitative results for target aromatic and aliphatic hydrocarbons. The target analytes may be a single compound or isomers quantified as a group. Target compounds must be useful for monitoring oil weathering and biodegradation; assessing response activities, as well as fate and effects of spilled oil; and aid in the determination of baseline oil pollution. Furthermore, legally defensible analytical procedures and data should be incorporated into the selection of target oil constituents. Oil constituents selected for analysis in the IES/RCAT laboratory are displayed in Table 2. The hopanes and steranes ions are primarily used for source-fingerprinting and generally are not quantified.

Table 2. List of Target Oil Constituents Analyzed for by GC/MS-SIM in the IES/RCAT Laboratory.

Compound	Quantification Ion	Abbreviation
Alkanes	85	
Naphthalene	128	NAPH
C-1 Naphthalenes	142	C-1 NAPH
C-2 Naphthalenes	156	C-2 NAPH
C-3 Naphthalenes	170	C-3 NAPH
C-4 Naphthalenes	184	C-4 NAPH
Fluorene	166	FLU
C-1 Fluorenes	180	C-1 FLU
C-2 Fluorenes	194	C-2 FLU
C-3 Fluorenes	208	C-3 FLU
Dibenzothiophene	184	DBT
C-1 Dibenzothiophenes	198	C-1 DBT
C-2 Dibenzothiophenes	212	C-2 DBT
C-3 Dibenzothiophenes	226	C-3 DBT
Phenanthrene	178	PHEN
C-1 Phenanthrenes	192	C-1 PHEN
C-2 Phenanthrenes	206	C-2 PHEN
C-3 Phenanthrenes	220	C-3 PHEN
C-4 Phenanthrenes	234	C-4 PHEN
Fluoranthrene	202	FLANT
Pyrene	202	PYR
C-1 Pyrenes	216	C-1 PYR
C-2 Pyrenes	230	C-2 PYR
C-3 Pyrenes	244	C-3 PYR
C-4 Pyrenes	258	C-4 PYR
Chrysene	228	CHRY
C-1 Chrysenes	242	C-1 CHRY
C-2 Chrysenes	256	C-2 CHRY
C-3 Chrysenes	270	C-3 CHRY
C-4 Chrysenes	284	C-4 CHRY
Benzo (b) Fluoranthene	252	B(b)F
Benzo (k) Fluoranthene	252	B(k)F
Benzo (e) Pyrene	252	B(e)P
Benzo (a) Pyrene	252	B(a)P
Perylene	252	PERYL
Indeno (g,h,i) Pyrene	276	INDPYR
Dibenzo (a,h) Anthracene	278	DIBENZ
Benzo (1,2,3-cd) Perylene	276	BENZP
Hopanes (191 family)	191	
Steranes (217 family)	217	

Identification, or source-fingerprinting as it is commonly called, is based around the use of high-resolution GC/MS analysis of selected components in oil and associated residues such as those listed in Table 2. Source-fingerprinting is an environmental forensics technique that utilizes analytical chemistry to compare samples of spilled oil to a suspected source to assess if the oil is a positive match. Therefore, source determinations, such as a damaged tanker or

deliberate ocean dumping, can be derived. The results of oil fingerprinting are similar to other forensic analyses (i.e. blood typing) in that oil-fingerprinting techniques provide stronger evidence to prove that a sample is a nonmatch to a suspect source rather than prove, without question, a positive match. Since oil is a very complex mixture of compounds that cannot be completely resolved by gas chromatography, a highly selective detector such as a mass spectrometer used in conjunction with a high-resolution chemical separation system, the GC, discrimination of specific target compounds from the bulk oil can be achieved. The target polycyclic aromatic hydrocarbons (PAHs) listed in Table 2 represent less than 5% of the bulk oil composition by weight, and many of the target analytes are present at the low part per million (ppm) levels in whole or bulk oil. This list of PAHs is highly useful in differentiating crude petroleum from byproducts of fuel combustion. This is possible because incomplete combustion of fuels produces PAHs that are characterized by 3, 4, and 5 ring aromatic compounds with few substituted alkyl homologues. For example, fluoranthene, chrysene and benzopyrenes are more common in combustion-sourced pollution than oil pollution.

Quantification of oil and oily residues can be obtained from several different methods. Oil and oil residue concentrations can be directly measured as "total petroleum hydrocarbons" using appropriate analytical and instrumental (gas chromatograph coupled with a flame ionization detector, or GC/FID) methods. However, due to the heterogeneous distribution of oil in aqueous systems and the non-selective properties of the detector, this method requires extensive replication before statistically valid results can be achieved. Alternately, oil quantities can be inferred by examining the distribution of selected hydrocarbon components remaining in oily residues or tarballs found in the environment. This method is based on the fact that not all components in oil are readily degraded by natural weathering processes. Therefore, if the quantities of hydrocarbons that remain are compared to the quantity of undegraded components, the percent of residual oil remaining after environmental weathering can be estimated. Comparing selected hydrocarbon components to the undegraded components is known as biomarker normalization. Biomarker normalization shows great promise as a tool for both identifying spilled oil and quantifying environmental residues. It is not a perfect tool and can, in contaminated systems, overestimate the actual percent degradation. One objective of this research is to extend and further validate the use of biomarker normalization by incorporating certain calculated fingerprint indexes that are not subject to day-to-day variances in an analytical laboratory.

Self-Normalizing Fingerprint Indexes

Since biomarker compounds are more resistant to environmental weathering processes, compared to most other oil compounds, they can be utilized as conserved reference compounds against which the loss of less stable oil components can be quantitatively estimated by calculating certain ratios. These ratios may be useful in differentiating unknown spill samples from a suspected source. Furthermore, the distributions of oil biomarkers is unique for different types and blends of petroleum products and represent an oil-specific fingerprint to which distinct oil samples can be correlated. Match/nonmatch determinations can be achieved qualitatively through visual comparison of ion chromatogram patterns, and quantitatively from calculating the ratio of one biomarker to another. Ratios of certain biomarkers are referred to as self-normalizing fingerprint indexes (SFI) throughout this research. Figures 3 – 8 display

the peaks chosen from the biomarker ion chromatograms for calculating the SFI. SFI are calculated by using the ratio of different peaks within the same isomer having similar retention times and identical mass to charge ratios. Choosing isomer groups that have similar water solubilites, vapor pressures, and parent masses will result in potentially useful SFI and contribute to the reduction of instrumental variance effects. As instrument conditions change because of matrix effects, column degradation, sensitivity, or tune degradation, both integers used to calculate the index (assuming they are similar in molecular weight, chemistry and quantitation ion) will be affected by the same relative degree of change; therefore, the index or ratio of the two integers, should remain constant. After a corrected base line value and peak heights have been determined, the SFI are calculated by dividing peak a by peak b (labeled in each figure).

SFI are a quantitative technique capable of reducing the potential for false-positive results and is not subject to most day-to-day laboratory variances. Goals of the SFI approach are to reduce investigator bias through the use of improved quantitative fingerprint techniques; and allow investigators to distinguish subtle differences in actual spill samples that can be easily missed by standard qualitative approaches.

In 1978 Overton and colleagues verified the application of alkyl aromatic ratios in the investigation of the fire and oil spill at the West Hackberry, Louisiana, Strategic Petroleum Reserve Complex (Overton et al. 1981). They found that the ratio of sulfur containing alkyl-dibenzothiophenes to alkyl-phenanthrenes was distinctly different between the spill oil, an Arabian light, and the South Louisiana crude oil (domestically produced and the principal source of chronic or periodic release in the study area). Match/nonmatch determinations were derived primarily from qualitative (i.e. visual) comparisons of the chromatographic profiles of specific aromatic hydrocarbons and petroleum biomarker compounds such as the steranes and hopanes, as well as indexes derived from specific compound ratios and simple plots.

Visual comparisons of chromatographic fingerprints provide very significant information as opposed to comparison of a few indexes. Visual comparison of a series of analyzed samples and their corresponding ion fingerprints can easily distinguish one type of oil from another based on the pattern or fingerprint and the ratios of the different constituents within the ion being analyzed. Visual match/nonmatch determinations are often criticized as being highly subjective and potentially biased by the interpreter. However, refined analytical skills, knowledge of oil chemistry, and experience reduce bias and provide a very efficient means for distinguishing qualitative differences between the fingerprints being compared and can provide further direction of analysis.

Project Goals

The overall objective of this research was to develop more accurate methods to identify and quantify spilled oil in the environment. Biomarker normalization and self-normalizing fingerprint indexes show great promise as a tool for both identifying spilled oil and quantifying environmental residues. Research performed by IES/RCAT will refine the use of biomarkers commonly analyzed in our laboratory as tools for oil spill identification and assessment; verify

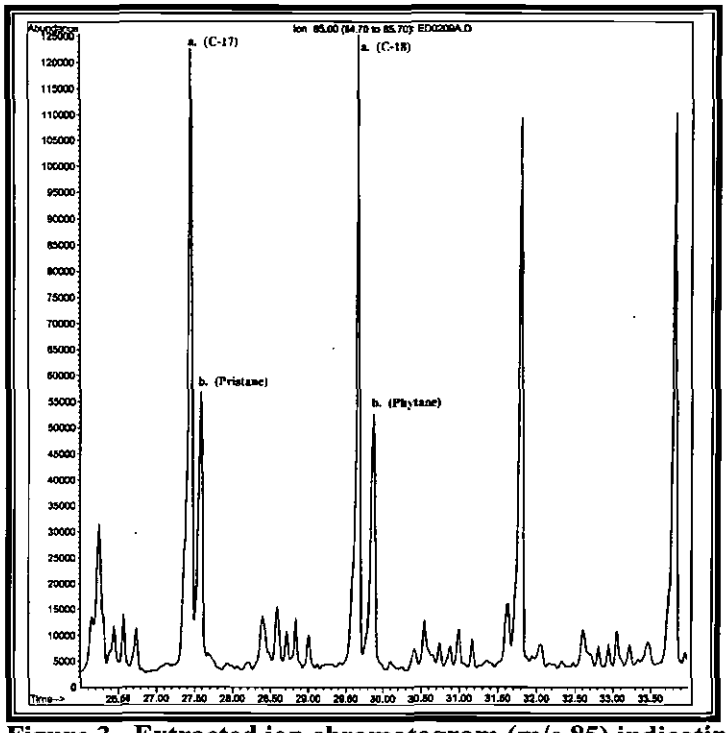


Figure 3. Extracted ion chromatogram (m/e 85) indicating C-17/Pristane and C-18/Phytane SFI.

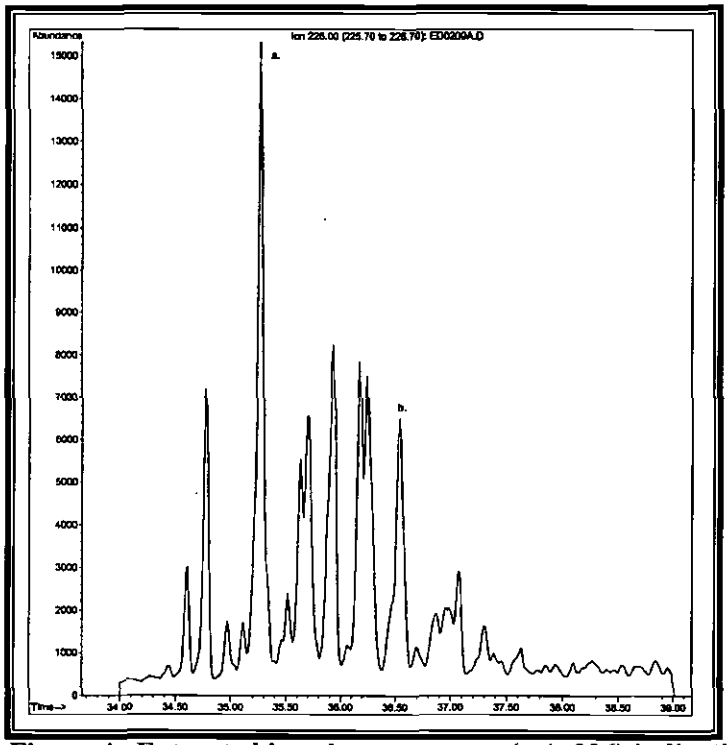


Figure 4. Extracted ion chromatogram (m/e 226) indicating C-3 DBT (a/b) SFI.

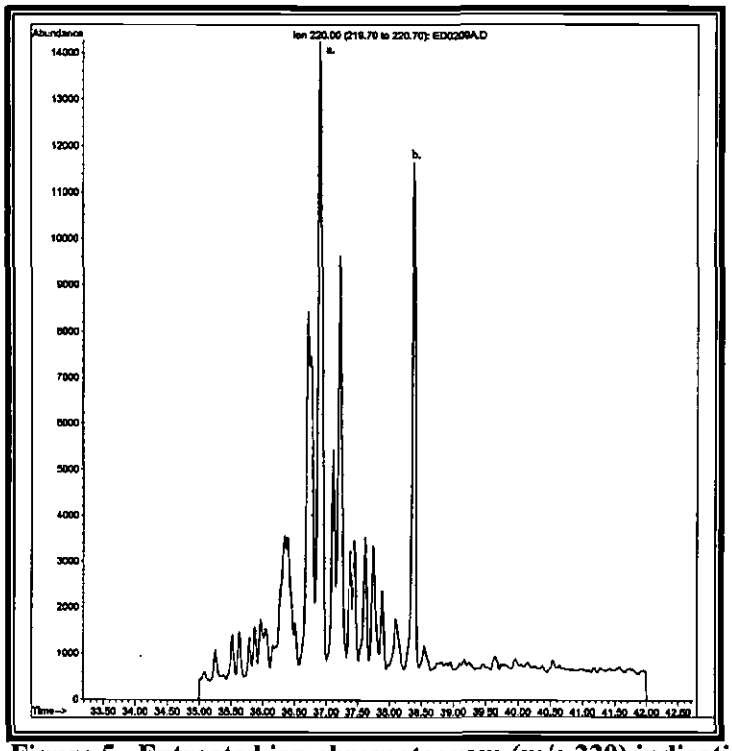


Figure 5. Extracted ion chromatogram (m/e 220) indicating C-3 Phen (a/b) SFI.

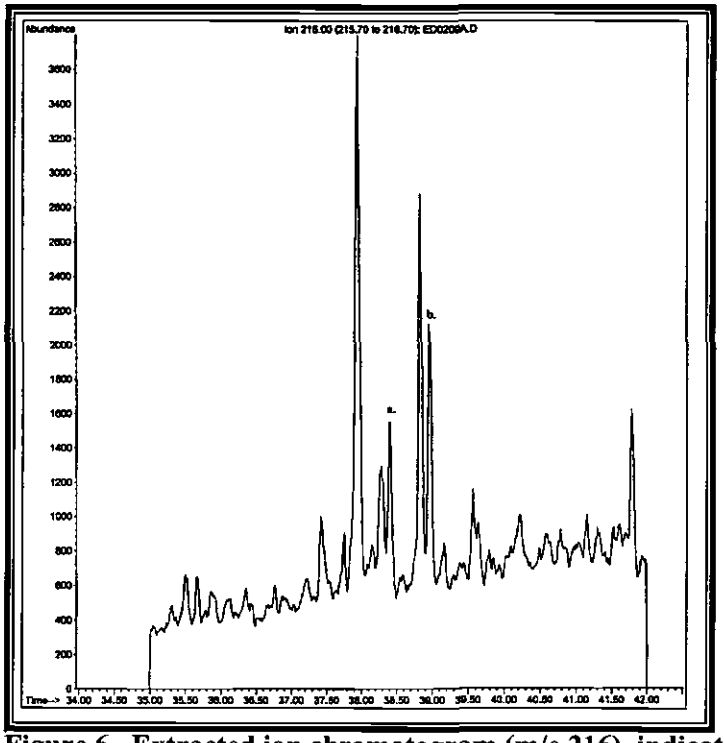


Figure 6. Extracted ion chromatogram (m/e 216) indicating C-1 Pyr (a/b) SFI.

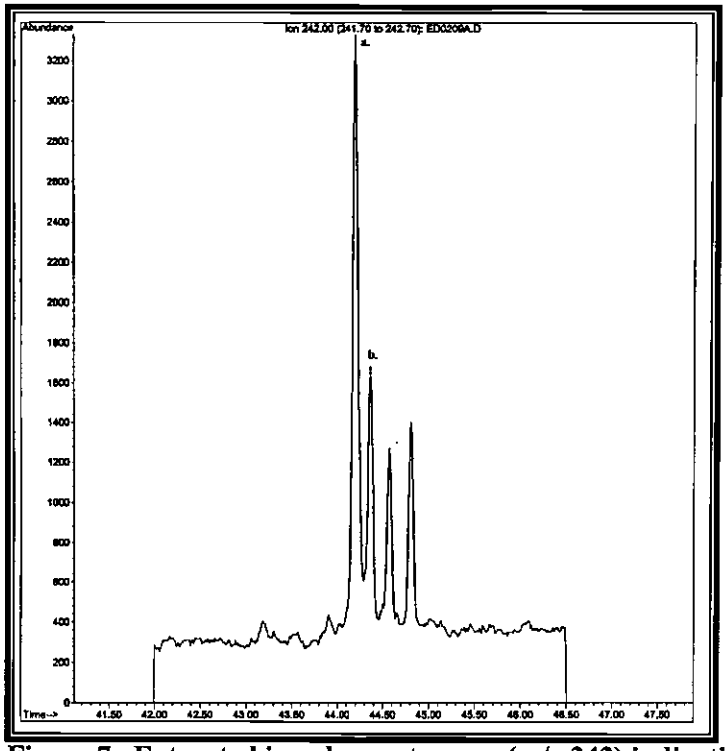


Figure 7. Extracted ion chromatogram (m/e 242) indicating C-1 Chr (a/b) SFI.

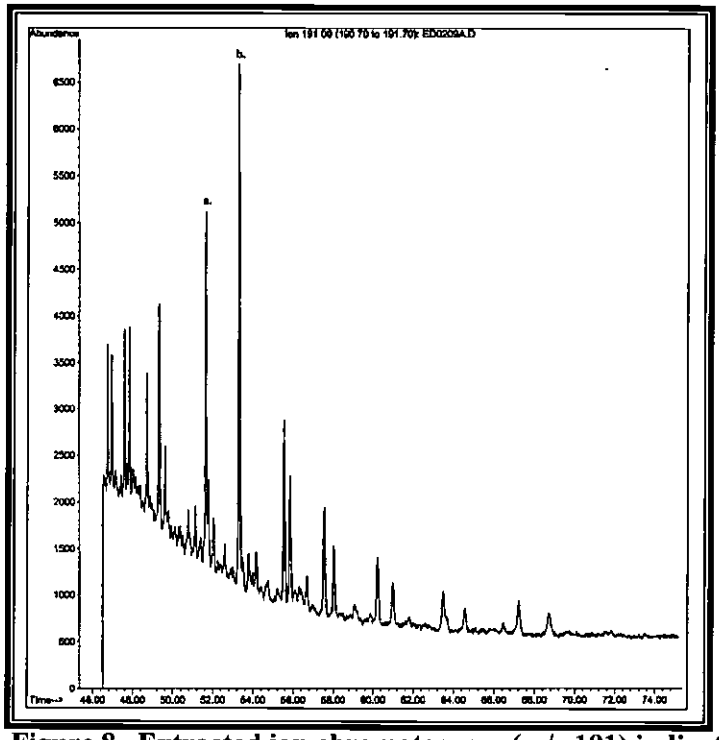


Figure 8. Extracted ion chromatogram (m/e 191) indicating Norhopane (a)/Hopane (b) SFI.

this method as compared to the use of other conventional ratio and TPH technologies; and apply this technique to a tarball and oil residue survey along Louisiana's coastline. IES/RCAT will utilize its cache of several collected and preserved reference oils commonly transported in U.S. waters including coastal Louisiana and a few internationally documented sites. The research originally was broken down into eight different research goals, or "tasks" that were divided into one of four categories: preliminary stages (i.e. literature search and instrument methodology); water degradation of one oil in nine natural waters; bioaccumulation of biomarker compounds in clams and mussels; and classification and identification of tarballs using SFI.

Preliminary Stages

The literature search established the foundation for oil biomarkers, past and present, which can be compared to the oil biomarkers utilized by IES/RCAT. A comprehensive literature review of the environmental, petroleum industry, and geological journals was conducted. Information gained from the literature survey included the identification of various biomarkers and profiles of biomarkers previously determined in petroleum reservoirs from around the world. Key words utilized during the literature search are given in Table 3.

Table 3. List of Keywords Utilized in the Literature Search.

Assessment	Oil
Biodegradation	PAHs
Biomarker	Persistence
Crude Oil	Petroleum
Fate	Pollution
Fingerprinting	Salt Marsh
Fuel Oil	Spill
Hydrocarbon	Weathering
Ocean	

Two previously compiled bibliographies provided additional references and were beneficial in the initial literature search. The citations for these two bibliographies are:

> Light, Melvin and Joseph Lanier. 1978. Biological effects of oil pollution: a comprehensive bibliography with abstracts. *US Coast Guard Research and Development Center Report No. CG-D-75-78*: 647 pp.
>
> GESAMP (IMO, FAO, UNESCO, WMO, IAEA, UN, UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution). 1993. Impact of oil and related chemicals on the marine environment. *Rep Stud. GESAMP (50)*: 180 pp.

The literature survey resulted in 180 pertinent articles out of over 300 titles acquired and reviewed. The 180 relevant articles appear in the Appendix titled *Biomarkers of Petroleum Products in the Marine Environment: A Bibliography.*

The instrument methodology involved the development of an improved GC/MS analytical method that included newly identified biomarker families in addition to those compounds currently used for oil spill response. Accomplishment of this stage involved determinations from the literature review and data generated from all the other experiments.

Laboratory-Simulated Weathering and Degradation Study

The water degradation experiment was initially intended to assess compositional changes in six selected oils due to weathering with respect to biomarker normalization and compositional changes within the laboratory. In evaluating the effectiveness of this task in conjunction with past experience with oils weathered in the environment, it was realized that the major altering factor is not the oil compositions that can be identified, but the different types of changes due to environmental factors. One significant factor for oil released on water is the ability of the natural microbial communities within the water to affect the oil composition. Therefore, the water degradation experimental design was altered to evaluate the changes in the composition of one oil, a South Louisiana Crude, after exposure to nine natural waters collected across the country. Water samples were collected from the coast of Alaska, Hawaii, Florida, Louisiana, California, and from inland waters of the Mississippi River. By evaluating the degradation changes that occur to the oil when exposed to 28-days of laboratory simulated weathering, in addition to a simulated nutrient influx and no nutrient inputs, environmental alterations of an oil can be projected, and ultimately affect the emphasis of this research.

In order to determine whether significantly different bacterial communities were present in the various waters, nutrient substrate uptake was analyzed with Biolog™ microplates. The 96-well Biolog™ microplate contained a variety of carbon and nitrogen sources, as well as a redox dye that records increases in metabolic rate (Bochner 1989). After the microplate is inoculated with the bacterial isolate it is incubated, usually for 24 hours. The patterns of positive and negative responses are input into a computer through a microplate density reader and the database scanned for the best statistical match of the substrate utilization profile. Although initially utilized for identification of bacterial isolates, the Biolog™ system, with some modifications to the procedure, has been demonstrated to be a reproducible measurement of substrate utilization by whole bacterial communities and very useful in distinguishing among different communities (Garland and Mills, 1991; Haack et al., 1995; Wunsche et al. 1995). The results of the Biolog™ microplates were used for characterizing the nutrient substrate utilization patterns of the microbial communities in each of the water samples.

The final objective of the water degradation experiment was to examine the changes in SFI for one type of oil pre- and post- weathering. Completion of this task relied upon the SFI generated from pre- and post-weathered Eugene Island Crude (EIC) reference oil utilized in the 28-day laboratory simulated weathering step of the water degradation experiment. Statistical analysis was performed to determine the stability of the oil biomarkers present in the EIC after the 28-days of weathering, along with nutrient or no nutrient additions, and exposure to natural microorganism populations in the different waters tested.

Bioaccumulation

The objective of the bioaccumulation experiment was to examine biomarker up-take in bivalves exposed to oil of known biomarker composition from samples collected recently from Prince William Sound, Alaska and impacted by the T/V *Exxon Valdez*. Theoretically, biomarkers are not significantly altered by microbial degradation; hence, there is a possibility that these lipid soluble materials will accumulate in marine organisms and could be used as an indicator of possible exposure during oil spills. The results of this task will establish a time frame in which biomarker compounds may be detected in bivalves since the samples were collected ten years after the T/V *Exxon Valdez* incident.

Classification and Identification of Tarballs

The methods of biomarker normalization developed throughout this research were used to analyze "unknown" environmental samples collected from three areas previously sampled that accumulated tarballs as documented in the 1993 report titled *Characterization of Chronic Sources and Impacts of Tar Along the Louisiana Coast* by Henry et al. The results of the classification and identification of the 1998 tarballs further extended the use of the SFI as biomarker normalization tools to identify and estimate relative environmental oil residues. A semi-quantitative approach for identifying different oil sources based on pattern matches of the GC/MS fingerprints and calculated self-normalizing fingerprint indexes were the final results of this task.

An initial step in the classification and identification of tarballs included the analysis of the eight biomarker compounds chosen in fourteen unweathered reference oils (two of which were daily reference oils). The reference oil analysis was performed prior to the analysis of the tarballs collected in 1998. The reference oils were obtained from the IES/RCAT oil library and were categorized into three groups: crude oils; fuel oils; and other oils (i.e. motor oil). Detailed GC/MS analysis was performed on these samples and data analysis included the calculation of the SFI. The results of this task included an attempt to correlate the oils in each of the categories, and/or support the use of the SFI for the biomarkers chosen to aid in source determination since the quantities of biomarkers are different for each type of oil. The SFI data from the reference oils was also utilized to calculate target source areas on double SFI scatterplots containing samples collected during 1992 and 1998, and 1998 alone.

A total of 88 new tarballs were collected in 1998. All 88 of the tarballs were characterized morphologically, and 70 tarballs were extracted and analyzed by GC/MS. After the tarballs were analyzed, the SFI were calculated and a comparison between the SFI from three sites previously sampled in 1992 to the SFI calculated from the 1998 tarballs from the same three sites was performed. Double SFI scatterplots were produced for the 1992 and 1998 tarballs, and the 1998 tarballs only. The overall objective of the classification and identification of tarballs was to determine whether or not the SFI and double index scatterplots used by IES/RCAT are suitable for oil and/or source identification purposes.

METHODOLOGY

General Laboratory Methodology

Established good laboratory procedures were utilized throughout the course of the research. All of the sample preparation, experimentation, extraction, and analytical analyses were conducted at the Institute for Environmental Studies (IES) at Louisiana State University. Samples collected in the field were brought back to the IES/RCAT laboratory to be logged in and given an identification number. The samples were properly stored until time of preparation and extraction.

Quality assurance and quality control was assured through a five-point calibration curve for target analytes and internal standards that demonstrated the linear range of the analysis. After linearity of the five-point calibration curve was established, a reference oil (North Slope Crude) and a continuing calibration standard containing naphthalene-d8, anthracene-d10, chrysene-d12, and perylene-d12 was injected every day prior to any sample analysis to verify instrumental performance.

GC/MS Methodology

Two different Hewlett-Packard 5890 Series GCs coupled with a HP 5971Series Mass Selective Detector were used for all instrumental analyses. Both instruments were equipped with 30 meter (m) by 0.250 millimeter (mm) capillary columns with a DB-5 stationary phase and a 0.25 micron inner diameter. The GCs were operated in temperature program mode with an initial column temperature of 55°C held for 3 minutes and then increased to 280°C at a rate of 5°C /minute. A final temperature of 300°C was achieved at a rate of 0.5°C/min and held for 15 minutes. The injection temperature was set to 270°C and only high-temperature, low thermal bleed septa were used. The GC/MS interface was maintained at 280°C. Helium gas was used as the mobile phase. The column head pressure was 13 psi and the flow rate was 1.31 milliliters (mL)/min.

The MS was operated in Selected Ion Monitoring (SIM) mode to maximize the detection of the target crude oil compounds. The instrument was operated such that the selected ions for each acquisition window were scanned at a rate greater that 1.5 scans/sec. At the start of an analysis period, the MS was tuned to perfluorotributylamine (PFTBA). Nineteen groups with differing scan start times were established and Table 4 provides detailed information for these 19 groups.

Sample introduction into the instruments for the water degradation experiment and the tarball analysis were achieved by manual injection. Sample introduction for the bioaccumulation study was by a HP 6890 Series Auto Injector. One microliter (μL) of each sample extract was injected. Both injectors were operated in splitless mode. HP Chemstation software was used for control, data acquisition and data analysis.

Table 4. SIM Ion Grouping for Target Crude Oil Compounds.

Group ID	Scan Start Time (minutes)	Ions in Group
1	04.50	82;85;128;136;142;152;156;166;172;180
2	19.20	85;142;152;154;156;164;166;170;172;180;184
3	25.00	85;165;166;170;176;178; 179;180;184; 188
4	29.00	85;165;178;180;184;188;192;194;198;212
5	30.00	85;178;182;194;208;230;500
6	32.00	85;179;191;192;194;198;208;212;226;500
7	33.00	85;100;192;198;206;208;212; 245;260;500
8	34.00	85;101;198;202;206;208;212;226;260
9	35.00	85;202;205;206;208;212;216;220;226;245
10	37.00	85;205;206;216;220;226;234;244;245;500
11	39.00	85;205;216;220;228;230;234;240;244;248
12	42.00	85;228;230;234;240;242;244;248;258;500
13	44.00	85;230;240;242;244;248;256;258;262;270;276
14	46.50	85;191;252;256;258;262;270;276;284
15	47.00	85;191;217;252;256;262;270;276;284
16	48.00	85;191;217;252;256;264;270;276;284
17	49.00	85;191;217;252;264;270;276;284;500
18	52.00	85;191;217;252;253;270;276;278;284
19	54.00	85;191;200;217;276;278;284;500

Calculation of SFI

All SFI were calculated from peak height except the C-3 DBT area/C-3 Phen area SFI that was determined by the division of the area sums (area under each homologues series). Peak height was chosen over an area integration method for most of the indexes since the peaks used in the calculation are generally not baseline resolved. All peak height determinations were calculated manually, corrected for baseline value, and entered into a Microsoft Excel spreadsheet. Refer to Figures 3-8 for the peaks chosen from the biomarker ion chromatograms used in the calculation of the SFI adapted by IES/RCAT. After a corrected baseline value and peak heights have been determined, the SFI were calculated by dividing peak a by peak b.

Visual Matching

Comparison of extracted ion chromatographic profiles for the indexes listed in Table 1 were conducted to determine if any of the samples appear to be related. This process compares the relative composition and extent of weathering for each sample analyzed, providing a detailed interpretation of the alkylated aromatic hydrocarbon series, sterane, and biomarker distribution patterns.

The visual method of qualitatively comparing each sample to every other sample is generally referred to as the "standard method" and is labor intensive. By this process all the GC/MS data is printed to create a hard copy, sorted by extracted ions and stacked in a pile. Each sample in a single ion group is compared to the other samples with the same ion to determine if each is a match or nonmatch. Nonmatch samples form a new pile until every chromatogram has been

compared. The result is generally dozens of small piles of the one ion being compared with each pile signifying a different source or exhibiting significantly different weathering patterns. The piles are then given an alphanumeric identification.

Laboratory-Simulated Weathering and Degradation Study

The oil used in this experiment was Eugene Island Crude, a type of South Louisiana crude oil. Two, one-liter water samples collected from areas outside of Louisiana included: Coronodo Beach, California; Jax Beach, Florida; and Kilua Beach, Hawaii. Water samples collected within Louisiana included: Holly Beach; water from Lake Charles; Mississippi river water collected in Port Allen; Price Lake in Rockefeller Refuge; Sabine Lake; and LSU University Lakes. Two control waters were also used. One was just deionized water and the other was a 32 part per thousand (ppT) Instant Ocean solution. Properties such as salinity, pH, and temperature were measured in the field. The water samples were brought back to the IES/RCAT laboratory where they were logged in, subsampled and stored at 1°C (34°F) until the experiment began.

Two, 250mL Erlenmeyer flasks were filled with 100mL of water from each site. The flasks were placed on a digital scale (one at a time) and the scale was then tared to zero. A disposable 9-inch soda glass pipette was used to add 0.50g of Eugene Island Crude oil to the water. The first flask containing the water and the oil was removed and covered with sterile gauze secured by a rubber band. After the oil was added to the water in the second flask, it was removed and 1mL of nutrient mixture was added. The nutrient mixture contained 5g of potassium phosphate (mono), 2.5g of potassium phosphate (dibasic), 2.5g of magnesium sulfate, 5.0g of ammonium nitrate, and 2.5g of yeast extract in 100 mL of deionized water. The second flask was also covered with sterile gauze secured by a rubber band. These steps were repeated twice for a total of three samples without nutrients and three samples with nutrients for most of the water samples.

The samples were placed on orbital shakers in two different rooms with two different temperatures. The water samples with cooler temperatures at collection were placed on the orbital shaker in the room with an average temp of 23.6°C (74.5°F), and the waters with warmer temperatures at collection were placed in a room with an average temperature of 27.7°C (81.9°F). The rotations per minute (rpm) of the two orbital shakers was set to 100 ± 10 rpm. The flasks remained on the orbital shaker for 28-days. On the 14th day of the experiment, an additional 1mL of nutrients was added to all the flasks originally containing nutrients. This was the only time the orbital shakers were stopped during the course of the experiment.

After 28-days, all the flasks were removed from the shakers to be extracted. Four samples, selected at random from all of the flasks, were poured into 500mL separatory funnels. The 250mL flasks were rinsed with 10mL of dichloromethane (DCM), which was then poured into the corresponding separatory funnel. This step was repeated two more times for a total of three rinses and 30mL of DCM. Each separatory funnel was inverted and vented several times and allowed to stand for 10 to 30 minutes depending on emulsification. Extent and color of the emulsification for each sample was recorded. A glass funnel with glass wool and anhydrous sodium sulfate (Na_2SO_4) was placed on top of a 40mL VOA vial and set under each separatory

funnel. The Na_2SO_4 was moistened with DCM before removal of the DCM fraction of the extraction in the separatory funnel. DCM was used to rinse the Na_2SO_4 after removal of the organic DCM fraction and to reach a final volume of 40mL for each extract.

After the first set of four extractions was completed, all the equipment utilized was washed and solvent rinsed prior to the next four extractions. All extracts were capped and stored at 1°C (34°F) until GC/MS analysis.

In addition to this degradation experiment, Biolog™ microplate analyses were performed to determine whether significantly different bacterial communities were present in the various waters. For this study, 2mL subsamples of the water samples collected from the various sites were frozen at –70°C until analysis. Prior to analysis, 1mL aliquots of the samples were inoculated onto plates containing BUGM agar (Biolog) supplemented with defibrinated sheeps blood to promote abundant growth. The plates were incubated at 37°C (~124°F) for 72 hours. Cotton swabs were used to transfer bacteria into test tubes containing a sterile saline solution, with salinity adjusted with NaCl to match the salinity of the samples. The saline solution was inoculated onto the Biolog™ microplates using a multipipettor. The plates were incubated for 3 days at 37°C (~124°F), in a modification of a technique used for soil samples (Garland and Mills 1991). The yeast extract solution was then inoculated directly onto the Biolog™ plates.

Finally, pre- and post-weathered Eugene Island Crude oil samples were analyzed. The pre-weathered oil sample utilized was prepared by adding 0.25 grams of EIC to 10 mL of hexane, and was manually injected everyday prior to analysis of the extracts. The post-weathered EIC samples were obtained from the extracted waters after 28-days of weathering on the orbital shaker. SFI were calculated after HP Chemstation data integration. The stability of the biomarker SFI was determined through statistical analysis of each individual SFI.

Bioaccumulation

Because biomarkers are not significantly altered by microbial degradation, there is the possibility that these lipid soluble materials will accumulate in marine organisms and could be used as an indicator of possible exposure during oil spills. Selected marine bivalves recently collected and still exposed to the T/V *Exxon Valdez* oil were analyzed for composition, uptake and alteration of hydrocarbon components including the biomarker compounds. Biomarkers normally elute in the biogenic lipids fraction (F1) during size exclusion chromatographic fractionation. F1 fractions from 6 mussel tissues and 6 clam tissues from different collection sites in Prince William Sound (PWS), along with a blank and rotary evaporator blank for each tissue type, were analyzed by the GC/MS-SIM method given previously in the methodology.

An IES/RCAT scientist collected the tissue samples during the National Oceanographic and Atmospheric Administration's (NOAA) summer monitoring cruise of PWS in 1999. The samples arrive at the IES/RCAT laboratory frozen and in ice chests. They are logged in, given a LSU ID#, and stored in a freezer until they are removed to be cleaned. The external shell and tissue of all bivalves in each sample were rinsed with deionized water to remove any particles or extraneous material. The animals were removed from their shells and placed into a

precleaned jar. A Teckmar Tissuemizer™ was used to homogenize each sample. All homogenized samples were stored in a freezer until extraction.

Approximately 5g of the homogenized tissue was removed from the sample and placed into a cleaned, 50mL beaker and spiked with surrogate standards. Between 15 to 25g of Na_2SO_4 was added to the tissues depending upon the amount of water within the tissues or until a paste consistency was obtained. The extraction solvent, DCM, was added in 35mL aliquots to the sample. The samples were then placed in a sonicator for 15 minutes. The solvent extract was then filtered through additional Na_2SO_4 and glass wool into a round bottom flask. The extraction procedure was carried out a total of three times for each sample.

To concentrate the solvent extract, the sample was rotary evaporated to approximately 8mL final volume. The concentrated extract was split into 4mL for lipid analysis and 4mL for PAH analysis. The samples were then fractionated using alumina/silica gel solid phase extraction. The first fraction, or the F1 fraction, is usually composed of lipid compounds in hexane. A final F1 fraction volume of 4mL was stored in a vial until concentrated under a gentle stream of nitrogen to a final volume of 0.1mL. A 1mL Hamilton Gas tight syringe was used to transfer the 0.1mL sample into a 2mL autosampler vial containing 100 µL wide opening inserts. Ten microliters of a 100 µg/mL internal standard were added to each of the samples. The vials were capped with aluminum/PTFE lined crimp caps.

An HP 6890 Auto Injector was used to inject 1µL onto the GC column for this task. After analysis, HP Chemstation software was utilized to integrate the compounds listed in Table 2 on page 7. After integration, the peak areas for each of the samples were transferred into a spreadsheet where the concentrations, in parts per billion, were calculated.

Classification and Identification of Tarballs

Fourteen reference oil samples were prepared by adding 0.25 grams (g) of oil into 10mL of hexane. Oils selected from the IES/RCAT oil library included: Arabian Light Crude; Basrah Crude; Bunker C; Diesel No. 2 Fuel Oil; Eugene Island Crude; Hondo; IFO 380; Louisiana Blend; Mega Borg Light Crude; North Slope Crude; South Louisiana Crude; SS Block 126 Condensate; Strategic Petroleum Reserve Crude; and Valvoline SAE 30. The North Slope Crude oil was used to check daily instrument responses. The Eugene Island Crude oil was used during the water degradation experiment. One microliter of the oil in hexane was manually injected into the GC/MS. SFI were calculated as stated previously and entered into a spreadsheet. The SFI for all the oils were divided into three types of oil: crude; fuel; and other. Statistical analysis was performed to determine, if any, correlation within the three categories.

Tarballs for the 1998 classification and identification field study were collected from three sites in the southwestern portion of the Louisiana Gulf Coast that were sampled previously in 1992. Nine original sampling locations were designated in 1992 and are given in the report titled: *Characterization of Chronic Sources and Impacts of Tar along the Louisiana Coast* by C.B. Henry, P.O. Roberts, and E.B. Overton (1993). Only three of the nine original station locations were revisited in 1998 (Figure 9). The three stations revisited were: Martin's Beach; Holly Beach; and Rutherford Beach.

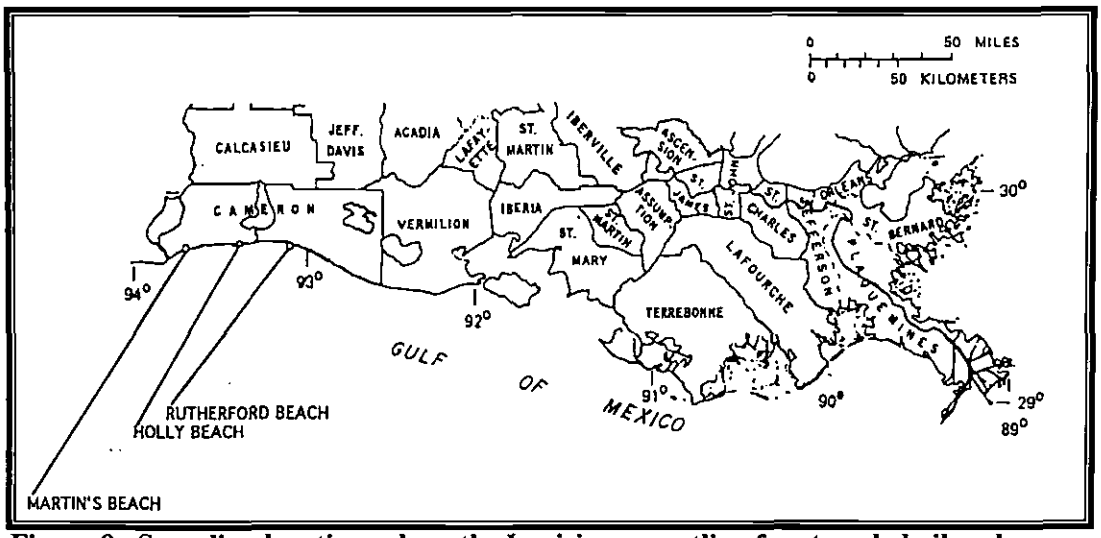


Figure 9. Sampling locations along the Louisiana coastline for stranded oil and tar survey, 1998.

Each sampling location was subdivided during sampling into backshore and foreshore regions. The backshore region is defined as the area behind the high tide debris line and includes the storm berm area. The foreshore area included the high tide debris line down to the water's edge. The differentiation between the foreshore and backshore was intended to distinguish between recently deposited oil (i.e. from the last high tide) and oil deposited in the upper beach from past storm activities. Tarball collection was performed by systematically walking each sampling location, collecting all tarballs which were greater than a few millimeters in size, and wrapping each individually in aluminum foil. All samples were stored at ambient temperature while in the field.

For the 1998 survey, a total of 52 tar balls were collected from Martins Beach (47 from the foreshore, one from midshore, four from backshore); 10 tarballs were collected from Holly Beach (eight from the foreshore, two from the backshore); and 26 tarballs were collected from Rutherford beach (25 from the foreshore, one from the midshore). The cumulative tarball total was 88, of which 70 were sampled and analyzed. Eighteen samples were not extracted due to the fact that they were smaller than the cutoff weight established during the morphological characterization step of analysis; or were pieces of asphalt too hard to sample.

Once the tarballs were brought back to the lab, they were weighed, and visually and physically characterized. Morphological characterizations included: outside/inside color, extraneous materials, pliability, and any comments regarding the outside/inside texture. The color categories were black, black-red, brown, grey, green, and white. Extraneous materials were visually estimated as a percentage of organic, sand, and shell. Materials considered as organic matter were seaweed and other plant material, and occasionally worms. Barnacles on the outside surface were considered when determining the percent range for shell composition. Pliability was also determined and ranked from 0 to 5 by the extent the tarball would bend when manual pressure was applied. A value of 0 represents solid tarballs without any pliability, while 5 indicated stranded tar that is almost fluid at ambient laboratory room temperature. A pliability ranking of 3 is representative of tar pieces that can bend without

breaking. The pliability characteristic can be related to some degree to the residual oil's pour-point and provide insight to the extent of weathering.

Each tarball was cut in half (after being weighed) and any additional comments were noted in regards to the appearance of the interior portion of the sample. Common interior descriptions were glassy, slightly glassy, shiny, slightly shiny, and dull. These descriptions were used in conjunction with a description of the texture of the tarball interior which included hard, relatively soft, soft, waxy, grainy/gritty, and rocky. All of these observations were qualitative only and decided on by the researcher.

A 0.20 to 0.30g core of each tarball was sampled and placed into individual 40mL VOA vials. The average weight of the samples was 0.22g. Two grams of Na_2SO_4 were added to all the vials. Ten milliliters of hexane were then added to each vial. The samples were sonicated in a cold bath for 15 to 20 minutes. After sonication, the vials were refrigerated at 1°C (34°F) until GC/MS analysis. After GC/MS analysis, the samples were integrated and the SFI were calculated as stated previously in the methodology and entered into a spreadsheet.

Visual classification of the tarballs was based on a source classification scheme established by Henry et al. in the 1993 report *Characterization of Chronic Sources and Impacts of Tar along the Louisiana Coast*. The tarballs for this report were classified into one of nine categories: relatively unweathered, high aromatic; relatively unweathered, high paraffin; relatively unweathered bimodal wax; weathered, high aromatic; weathered, high paraffin; weathered, bimodal unresolved complex mixture (UCM); weathered, bimodal wax; weathered, bimodal UCM, and wax (trimodal); and unclassifiable.

A slightly modified version of the same source classification was utilized to separate the tarballs collected during the 1998 survey (i.e. high was not used as a descriptive of the type of oil and only eight categories, instead of nine, were established for the 1998 tarballs). The categories for the 1998 tarballs were classified into one of eight categories: relatively unweathered, aromatic; relatively unweathered, paraffin; relatively unweathered, wax/bimodal wax; weathered, aromatic; weathered, paraffin; weathered, wax/bimodal wax; weathered, UCM; and unclassifiable.

SFI from the tarballs collected in 1992 were then compared to the SFI calculated for the tarballs collected in 1998. Data for 27 of the 1992 tarballs was obtained from Appendix II in Henry et al.'s 1993 report. Data for the 1998 samples totaled 69 tarballs. Double index scatterplots were then generated with the SFI data from both years. SFI data from twelve of the fourteen reference oils (excluding North Slope Crude and Eugene Island Crude) were also plotted to determine, if possible, source of the tarballs and/or if any of the tarballs from both years matched each other. Calculating ±20% of the x-axis SFI and y-axis SFI for each reference oil established target match zones.

The 1998 tarballs were plotted alone on another set of double SFI scatterplots with the reference oil target match zones. The tarballs included in the target zones were visually compared to the reference oil and/or other tarballs in the zone. Match/nonmatch determinations

were then made. Data evaluation also included an extensive visual matching process for the eight biomarker fingerprints.

RESULTS AND DISCUSSION

Laboratory-Simulated Weathering and Degradation Study

The original goal of the water degradation study was to assess compositional changes in six selected oils due to weathering with respect to biomarker normalization and compositional changes within the laboratory. However, after some consideration, it was decided that studying the ability of microbial communities to naturally degrade oils would be more prudent. The major altering factor, in regards to oil degradation in the marine environment, is the effect caused by the natural microbial communities within the water. This task evaluated the changes in composition of one oil, Eugene Island Crude (EIC), after exposure to nine natural waters collected across the country and two control waters, after 28-days of weathering.

Two analytical endpoints, percent change in total target aromatic hydrocarbons (TTAH) and the stability of the SFI, were chosen to assess the biodegradation and biomarker persistence of the one oil (EIC) in the nine water samples and the two control waters. The change in TTAH is reflective of bulk oil loss; while the SFI represent specific classes of biodegradation-resistive hydrocarbons, or biomarkers.

Table 5 gives the percent reduction in total target aromatic hydrocarbons after the 28-day weathering period for the nine water sites and Figure 10 shows the sum of TTAH in the samples with and without nutrients analyzed after 28-days of weathering. Abiotic factors that may affect the degradation of the EIC were controlled to some extent; the oil was the same and temperatures remained relatively constant in both experimental locations. One important abiotic factor not capable of being replicated in the laboratory study was the effect of tides. Oxygen and nutrients, often the limiting factors in real world situations, were provided to the microbial communities present in the different waters (i.e. the flasks were covered with sterile gauze and nutrients were provided to half the flasks). Biodegradation potentials of each water with additional nutrients were readily apparent in the percent reduction of TTAH.

Table 5. Percent of TTAH Reduction for the Nine Water Samples After 28-Day Weathering Experiment.

	% Reduction, No Nutrients	% Reduction, With Nutrients
Fresh Water Sites		
Lake Charles, LA	37	87
Port Allen, LA (Mississippi River water)	15	68
Rockefeller Refuge, LA	43	88
Sabine Lake, LA	19	49
LSU University Lakes, LA	39	93

(Table 5 Continued)

	% Reduction, No Nutrients	% Reduction, With Nutrients
Salt Water Sites		
Coronodo Beach, CA	17	76
Holly Beach, LA	30	90
Jax Beach, FL	0	74
Kilua Beach, HI	0	35

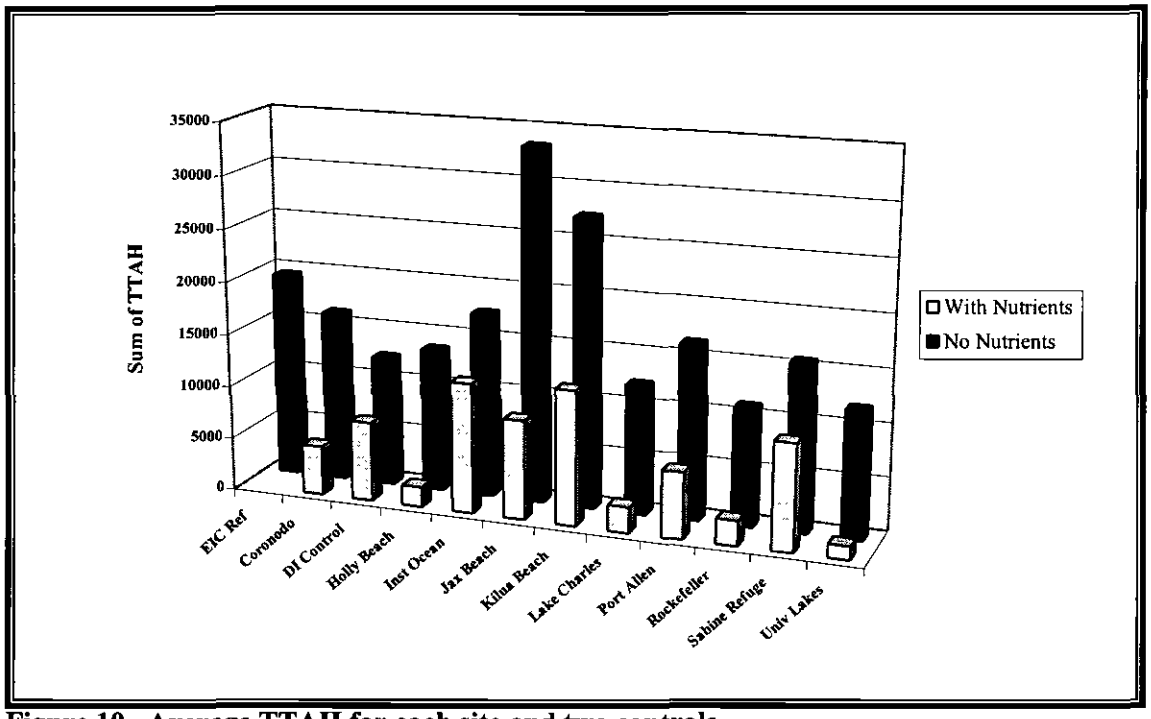


Figure 10. Average TTAH for each site and two controls.

Biolog™ microplate analyses were also performed for the nine water sites in order to provide a preliminary characterization of the microbial communities present in the waters and their ability to utilized a variety of carbon sources. The results of the Biolog™ microplate analyses indicate that the bacteria in the different water samples had widely varying metabolic capabilities. For purposes of comparison among samples, carbon sources were grouped according to compound type and then the response to compounds within the class averaged. Full responses (indicated by high color development in the well) was given a rating of 100, low response a rating of 50, and no response a rating of 0. Compound classes included (1) polymers, (2) carbohydrates, (3) esters, (4) carboxylic acids, (5) brominated chemicals, (6) amides, (7) amino acids, (8) aromatic chemicals, (9) amines, (10) alcohols, and (11) phosphorylated chemicals. The total number of sources used was also calculated. Figure 11 provides the total sources, as calculated from the Biolog™ results, used by the indigenous microorganisms from each water. The carbon source of interest for correlation to the laboratory weathering is the aromatic chemicals. Figure 12 displays the percent aromatic chemicals utilized by the indigenous microorganisms present in each of the water samples.

The results of the Biolog™ microplates do not correlate well with the TTAH endpoint. The results of the 28-day weathering experiment demonstrate that LSU University Lakes water with the additional nutrients had the highest percent change, 93%, in total target aromatic hydrocarbons. This is interpreted as the water from University Lakes and the indigenous microbial community present utilized the oil more efficiently than the other waters. The lakes are subjected to chronic, urban runoff that results in a constant flux of nutrients and oxygen demands. The results of the Biolog™ microplates show the opposite of this conclusion. LSU University Lakes had a low response in both total source utilization and aromatic chemical utilization. This could be due to several factors. The aromatic chemicals provided as a carbon source in the Biolog™ microplates consisted of urocanic acid, inosine, uridine and thymidine. The carbon source in the 28-day weathering experiment was a crude oil that was analyzed for 40 different petroleum hydrocarbons. The nutrient substrates also differed. Biolog™ utilized BUGM agar supplemented with defibrinated sheep's blood; the 28-day weathering study utilized a nutrient mixture containing potassium mono-phosphate, potassium dibasic phosphate, magnesium sulfate, ammonium nitrate and yeast extract. Larger samples of water should have been taken for the Biolog™ analyses to provide a more sufficient sample to be cultured. Only 2 mL of water from each collection site was saved for the microplate analysis.

The differences in nutrient substrate and carbon sources available in the natural environment and in the experimental flasks could have affected the combined metabolic utilization of the microbial community present in each of the waters tested. As a result, dominant species may have been different for the Biolog™ and the 28-day weathering experiment. Furthermore, Smalla et al. (1998) found that the relative abundance of populations present in the inocula changes during incubation of the Biolog™ plates, resulting in a dominance of species that are best able to compete with the substrate concentrations in the microplates and under the conditions of incubation. Both experiments provide information on the microbial populations as a whole and how they work together to degrade sources of nutrients provided for them.

It appears as though the temperature of the two experimental locations may have had an effect on the degradation potential of the nine water sites. The temperature of each water was recorded at the time of collection, along with other physical characteristics such as salinity and pH. When the waters were brought back to the laboratory, they were stored in a refrigerator until sample preparation, at with time the samples were removed. Two different experimental locations were established to correspond to the approximate collection temperature of the water samples. One room had an average temperature of 23.3°C (73.9°F) and the other room had an average temperature of 27.4°C (81.4°F). The percent reduction in TTAH for the samples in the room with the warmer average temperature, with one exception, was almost one to three orders of magnitude higher than the percent reduction in TTAH for the samples in the room with the cooler temperature. The one exception was the water sample from Sabine Lake. Average ambient temperature of the two experimental locations appears to only affect the percent TTAH reduction for the no nutrients. Table 6 contains the physical characteristic data and the percent reduction of TTAH for the nine water samples and is sorted based on the average temperature of the two rooms.

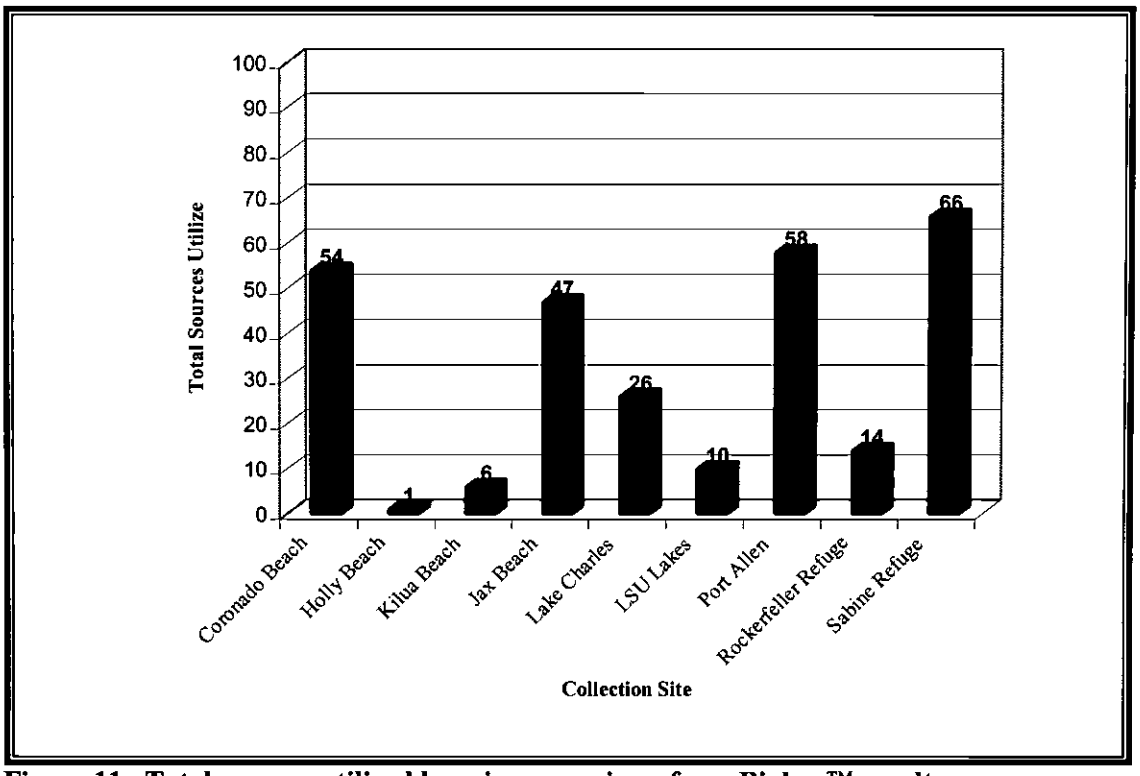


Figure 11. Total sources utilized by microorganisms from Biolog ™ results.

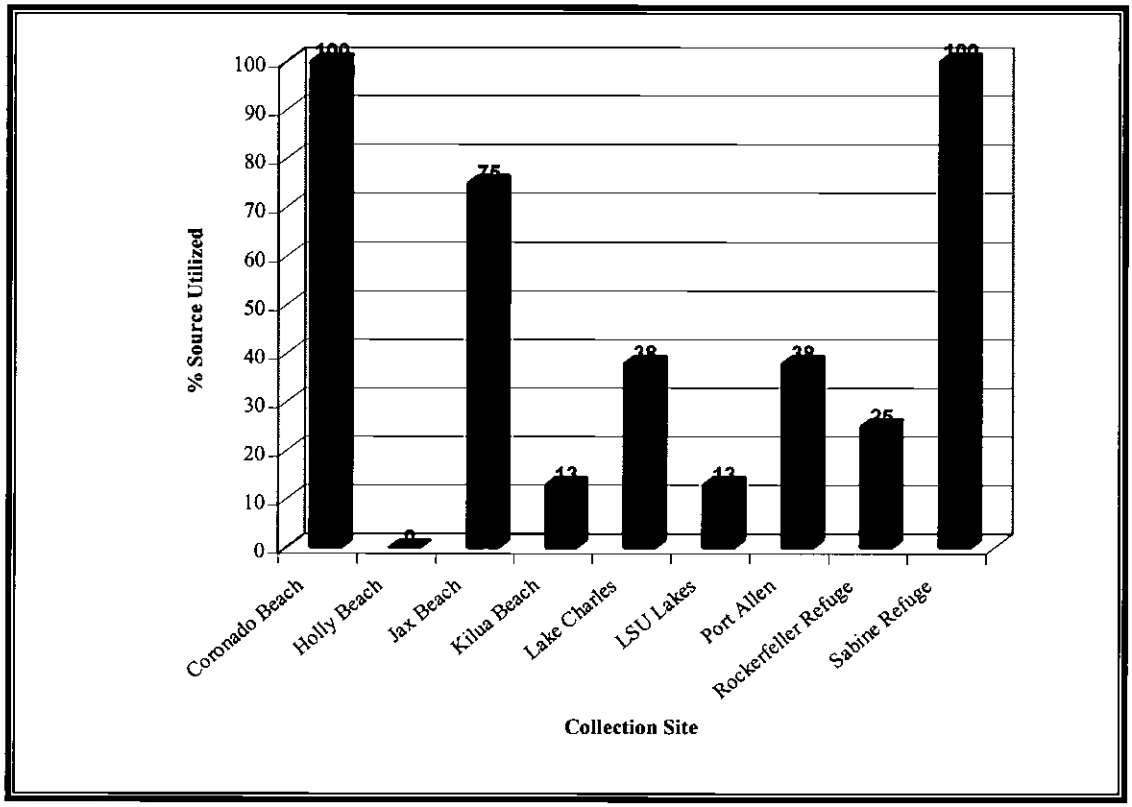


Figure 12. Percent aromatic chemicals utilized by microorganisms from Biolog™ results.

Table 6. Physical Characteristic Data and % Reduction in TTAH for the Water Samples Based on the Experimental Location Average Temperature.

Water Sample	Temp at Collection (°C)	Experimental Location*	%Reduction, No Nutrients	% Reduction, With Nutrients
Holly Beach	27.8	1	30	90
Lake Charles	30.0	1	37	87
Rockefeller Refuge	28.3	1	43	88
Sabine Lake	28.3	1	19	49
University Lakes	28.9	1	39	93
Coronodo	16.1	2	17	76
Jax Beach	23.3	2	0	74
Port Allen	25.0	2	15	68
Kilua	None Given	2	0	35

*Average temperature of location 1 = 27.4°C; average temperature of location 2 = 23.3°C

SFI were calculated for the EIC oil in the nine different waters and two control waters, with and without nutrients, after the 28-day weathering experiment. The different collection sites were divided into no nutrients and with nutrients, and statistical analyses were performed (averages, standard deviations, and the percent relative standard deviation were calculated). Table 7 provides the SFI data and statistical results for all the collection sites without nutrients after 28-days of weathering; and Table 8 provides the SFI data and statistical results for the collection sites with nutrients after 28-days of weathering. Table 9 displays the daily SFI data for the EIC reference oil.

Table 7. SFI Data and Statistical Results for All Collection Sites, Without Nutrients.

Site	nC-17/ Pris	nC-18/ Phy	C-3 DBT	C-3 Phen	C-3DBT/ C-3Phen	C-1 Pyr	C-1 Chr	Nor/ Hop
Coronodo Beach, CA	2.30	1.90	1.90	4.90	1.10	0.71	2.30	0.91
Coronodo Beach, CA	2.50	2.00	2.10	5.00	1.90	0.71	2.30	0.87
Coronodo Beach, CA	2.30	2.00	2.10	4.60	1.90	0.73	2.30	0.92
DI Control	2.60	2.10	2.10	4.30	1.90	0.74	2.20	0.94
DI Control	2.70	1.80	2.00	4.70	1.90	0.75	2.20	0.93
DI Control	2.50	2.00	2.10	5.00	1.90	0.72	2.40	0.97
Holly Beach, LA	2.50	2.00	1.90	4.30	1.80	0.79	2.00	0.91
Instant Ocean	2.40	1.90	1.90	4.70	1.70	0.74	2.20	0.94
Jax Beach, FL	2.40	2.00	2.00	4.80	1.80	0.79	2.30	0.86
Kilau Beach, HI	2.50	2.10	2.00	4.60	1.70	0.80	2.20	1.00
Lake Charles, LA	2.40	2.00	2.00	4.60	2.00	0.75	2.30	0.85
Port Allen, LA	2.00	1.60	2.00	4.50	1.90	0.78	2.40	0.92
Port Allen , LA	2.10	1.60	2.00	4.60	1.80	0.77	2.30	0.85
Port Allen , LA	2.20	1.70	2.00	4.90	1.80	0.78	2.30	0.92
Rockefeller Refuge, LA	2.60	2.10	2.00	4.90	1.90	0.76	2.30	0.86
Sabine Lake, LA	2.70	2.00	2.10	4.90	1.80	0.83	2.20	0.91
University Lakes, LA	2.40	1.80	1.90	4.50	1.80	0.74	2.30	0.82

(Table 7 Continued)

	nC-17/ Pris	nC-18/ Phy	C-3 DBT	C-3 Phen	C-3DBT/ C-3Phen	C-1 Pyr	C-1 Chr	Nor/ Hop
Average	2.42	1.92	2.01	4.69	1.80	0.76	2.26	0.90
Standard Deviation	0.19	0.16	0.07	0.22	0.20	0.03	0.09	0.05
Percent Relative Standard Deviation	8.04	8.50	3.73	4.73	10.94	4.43	4.11	5.22

Table 8. SFI Data and Statistical Results for All The Collection Sites, With Nutrients.

Site	nC-17/ Pris	nC-18/ Phy	C-3 DBT	C-3 Phen	C-3DBT/ C-3Phen	C-1 Pyr	C-1 Chr	Nor/ Hop
Coronodo Beach, CA	0.00	0.00	2.20	4.80	1.70	0.69	2.20	0.94
Coronodo Beach, CA	0.00	0.00	2.10	5.10	1.80	0.73	2.20	0.92
Coronodo Beach, CA	0.00	0.00	2.20	5.70	1.80	0.00	2.20	0.90
DI Control	0.61	0.00	2.20	4.90	1.80	0.76	2.50	0.89
DI Control	1.80	0.00	2.00	5.10	1.80	0.72	2.30	0.84
DI Control	0.71	0.00	2.20	4.90	1.90	0.74	2.40	0.88
Holly Beach, LA	0.00	0.00	2.10	7.30	2.20	0.00	4.50	0.89
Instant Ocean	0.06	0.04	2.10	5.00	1.80	0.77	2.20	0.91
Jax Beach, FL	0.00	0.00	1.50	6.40	2.00	0.00	3.20	0.90
Jax Beach, FL	0.52	0.20	2.50	4.80	1.90	0.70	2.20	0.91
Jax Beach, FL	0.00	0.00	2.00	6.10	2.00	0.85	4.30	0.92
Kilua Bch, HI	0.11	0.01	2.10	5.20	1.90	0.80	2.40	0.92
Kilua Bch, HI	0.21	0.13	2.20	5.50	1.80	0.84	2.30	1.00
Kilua Bch, HI	0.03	0.03	2.00	4.30	1.80	0.74	2.30	0.87
Lake Charles, LA	0.90	0.15	1.40	8.10	1.70	0.45	3.20	0.86
Port Allen, LA	0.00	0.00	2.00	5.30	1.60	0.65	2.20	0.92
Port Allen, LA	0.00	0.00	2.10	4.40	1.70	0.76	2.40	1.00
Port Allen, LA	0.00	0.00	2.20	6.10	1.60	0.84	2.56	0.89
Rockefeller Refuge, LA	0.00	0.00	1.80	7.80	1.80	0.54	3.20	1.00
Sabine Lake, LA	0.61	0.17	1.90	5.30	1.90	0.73	2.40	0.90
University Lakes, LA	0.00	0.00	2.30	6.70	1.50	0.00	2.50	0.88
University Lakes, LA	0.37	0.00	2.40	6.20	1.70	0.00	4.40	0.95
University Lakes, LA	0.00	0.00	1.90	4.30	1.00	0.00	13.80	0.90
Average	0.26	0.03	2.06	5.62	1.77	0.54	3.21	0.91
Standard Deviation	0.44	0.06	0.25	1.07	0.22	0.34	2.42	0.04
Percent Relative Standard Deviation	170.22	198.96	12.13	18.95	12.69	62.93	75.43	4.64

Table 9. SFI Data and Statistical Results for Daily EIC Reference Oil.

EIC Ref Oil	nC-17/ Pris	nC-18/ Phy	C-3 DBT	C-3 Phen	C-3DBT/ C-3Phen	C-1 Pyr	C-1 Chr	Nor/ Hop
Rep 1	2.30	2.00	1.80	4.20	1.80	0.75	2.20	0.90
Rep 2	2.40	1.90	1.80	4.10	1.70	0.77	2.00	1.00
Rep 3	2.20	1.90	1.80	4.50	1.80	0.73	3.80	0.97

(Table 9 Continued)

EIC Ref Oil	nC-17/ Pris	nC-18/ Phy	C-3 DBT	C-3 Phen	C-3DBT/ C-3Phen	C-1 Pyr	C-1 Chr	Nor/ Hop
Rep 4	2.30	1.90	1.90	6.50	1.70	0.85	2.30	0.94
Rep 5	2.40	2.00	1.90	4.30	1.70	0.84	2.20	0.91
Rep 6	2.40	1.80	1.90	4.30	1.70	0.78	2.30	0.94
Rep 7	2.30	1.90	2.00	7.00	1.70	0.76	2.20	0.91
Rep 8	2.40	1.90	2.00	4.80	1.80	0.75	2.10	0.93
Rep 9	2.50	2.00	1.90	4.10	1.70	0.79	2.30	0.97
Rep 10	2.30	1.90	2.00	4.60	1.90	0.81	2.10	0.93
Average	2.35	1.92	1.90	4.84	1.75	0.78	2.35	0.94
Standard Deviation	0.08	0.06	0.08	1.04	0.07	0.04	0.52	0.03
Percent Relative Standard Deviation	3.62	3.29	4.30	21.43	4.04	5.07	22.09	3.36

The SFI for the EIC after 28-days of weathering and no nutrients in the nine waters and two controls were acceptable based on the percent relative standard deviation (%RSD), which ranged from 4% to 11%. Acceptable data can be defined in many ways and is usually governed by the criteria established in different scientific fields. For the Environmental Protection Agency, acceptable data is anything less than or equal to 25% RSD. The SFI for the daily EIC analysis had similar results to the weathered EIC. Percent relative standard deviations for the daily EIC SFI ranged from 3% to 22%.

The nC-17/Pris and nC-18/Phy SFI for all the waters with nutrients would not be acceptable to use for accurate determinations of source based on their %RSD, 170% and 199% respectively. However, they should not be entirely overlooked due to the fact that they would provide information in regards to the extent of weathering. The C-1 Pyr and C-1 Chr SFI were also unacceptable as shown by their %RSD (63% and 75% respectively).

When each water sampling site was analyzed individually, most of the waters with the additional nutrients had nC-17/pris and nC-18/phy SFI that were either not within acceptable data limits (%RSD of 25 or less) or no peaks were present in the ion chromatogram to calculate the SFI (this would result in an SFI of 0.00). There were three sites out of nine and two controls that contained unacceptable relative standard deviations (>25% RSD) for SFI other than the nC-17/Pris and nC-18/Phy. Coronodo beach, no nutrients, had an unacceptable C-3 DBT area/C-3 Phen area SFI (RSD=28%) and an unacceptable C-1 Pyr SFI for the samples with nutrients (RSD=87%). Jax Beach had unacceptable data for C-1 Pyr and C-1 Chr SFI for samples containing nutrients (RSD=88% and 33% respectively). The third site with unacceptable data was University Lakes with nutrients; %RSD for the C-3 DBT area/C-3 Phen area SFI was 26% and the C-1 Chr SFI was 88%. All of the other individual sites, with and without nutrients had SFI ranging from 0 to 25% RSD. Despite some of the individual site data, the water degradation study provides the best evidence of the stability of the biomarker compounds analyzed (refer again to Tables 7, 8, and 9).

The SFI that would provide the best information in regards to proving that a sample of spilled oil is a match/nonmatch to a suspect source would be C-3 DBT; C-3 Phen; C-3 DBT area/C-3 Phen area; and Nor/Hop (refer to Figures 4, 5, and 8 respectively for fingerprint). These compounds and their calculated SFI will distinguish one oil type from another, even oils of

similar geographical origin, when other target biomarkers and analytes are lost. An investigator using these four SFI will have the ability to distinguish subtle differences in actual spill samples that can be easily missed by standard qualitative approaches (i.e. visual matching). The C-3 DBT, C-3 Phen, C-3 DBT area/C-3 Phen area, and Nor/Hop SFI, when used in conjunction with double SFI scatterplots with a 20% target zone for the reference oil and visual comparisons, will further assure the investigator in determining match/nonmatch of the unknown sample to the reference oil and other unknown samples. The remaining SFI, (nC-17/Pris; nC-18/Phy; C-1 Pyr; and C-1 Chr) appear to be greatly affected by weathering (%RSD were greater than 25 for the EIC after 28-days of weathering). Regardless of the fact that these four SFI were not within acceptable statistical ranges, visual examination of the corresponding fingerprint will still aid the investigator in match/nonmatch determinations.

Bioaccumulation

Up-take of biomarkers and, in general PAHs, were examined in bivalves exposed to oil from the T/V *Exxon Valdez*. The samples were collected in 1999 from Prince William Sound, Alaska, 10 years after the T/V *Exxon Valdez* spill. Since biomarkers are not usually altered by microbial degradation, there is a possibility that these lipid soluble materials will accumulate in marine organisms and could be used as an indicator of possible exposure during oil spills.

Since biomarkers normally elute with biogenic lipids during chromatographic fractionation (F1 fraction), random samples of seven F1 mussel tissues and six F1 clam tissues were analyzed by GC/MS. Figure 13 is a histogram of the mussel tissue analysis. One mussel sample (LSU ID# N9201-22) included in the F1 fraction analysis was taken from a dock that undergoes chronic pollution from boats. This sample had the highest concentration of both total target aromatic hydrocarbons (TTAH; synonymous with total polycyclic aromatic hydrocarbons or PAHs) and biomarker compounds; however, a baseline concentration of biomarker compounds has not been established for biological samples from the beginning of the T/V *Exxon Valdez* spill. Figure 14 is a histogram of the clam tissue analysis. Both histograms have the TTAH and biomarker concentrations given in parts per billion (ppb).

The F1 analysis provides important information about the uptake and utilization of petroleum hydrocarbons by filter feeding organisms after a duration of time that would be difficult to perform in a laboratory based experiment. Overall, the biomarker concentrations for both tissue types were low. The PAH and biomarker concentrations in the F1 fractions from the clam tissues were lower than the mussel. Detection limits of the GC/MS utilized for the analysis was 0.01 ppb. The low levels of both PAHs and biomarkers can be viewed in one of two ways: the mussel and clam tissues almost have non-detectable quantities of petroleum hydrocarbons; or biomarker compounds are not at detectable concentrations in biological samples 10 years after exposure to oil. The concentrations of PAHs in the tissue samples from Prince William Sound have been relatively low the past few years.

Two factors that may explain the low levels are the metabolic capabilities of the mussels and clams, and the age of the population sampled in 1999. Generally, biomarkers are resistant to biodegradation, which implies bacterial degradation and not enzymatic transformation. The life span of the mussel and clam organisms themselves is another factor. The population

sampled in 1999 may have been either newly established populations, therefore exposed to lower concentrations of oil to begin with; or the population was well established and was able to readily metabolize the petroleum compounds. Unfortunately, biomarker analysis or SFI were not calculated for any of the samples from previous years, and as a result, a baseline biomarker concentration was not established. In regards to the literature review concerning biological tissue samples, the focus was on bioavailability of PAHs compounds in the marine environment and not biotransformation.

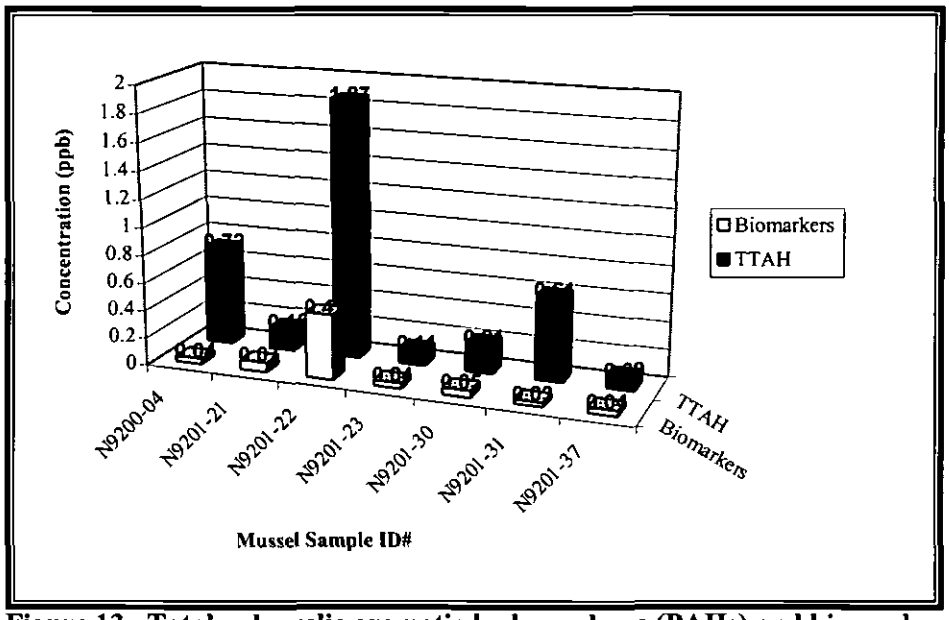


Figure 13. Total polycyclic aromatic hydrocarbons (PAHs) and biomarker concentrations in F1 fractions from 1999 PWS mussel tissues.

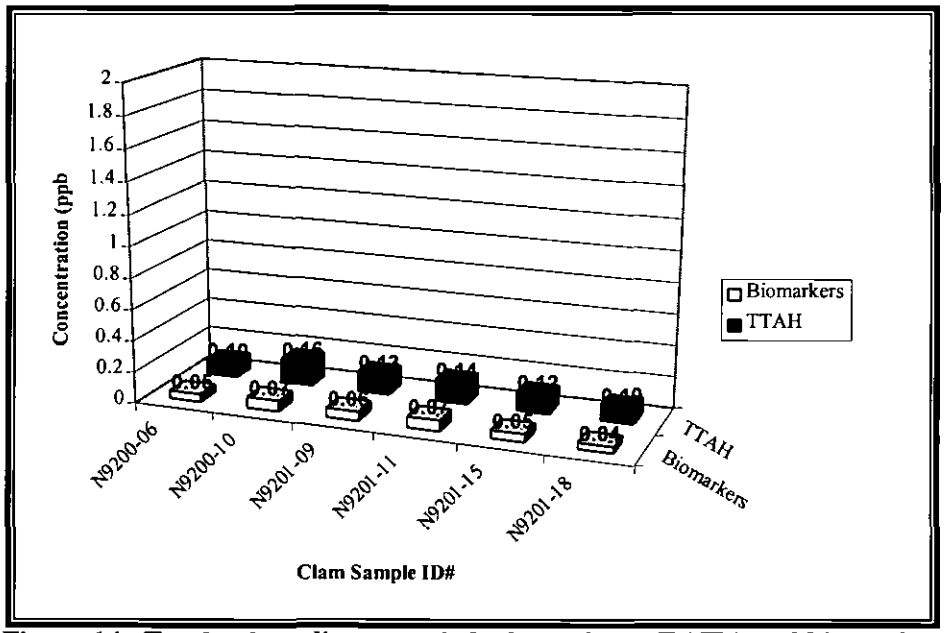


Figure 14. Total polycyclic aromatic hydrocarbons (PAHs) and biomarker concentrations in F1 fractions from 1999 PWS clam tissues.

Classification and Identification of Tarballs

Fourteen reference oils, including the two reference oils utilized throughout the research (NSC and EIC), from the oil library at IES/RCAT were analyzed and SFI were calculated. The oils were categorized into three groups: crude oils; fuel oils; and other. There were a total of nine crude; three fuel; and two other. The ratios generated from these reference oils are given in Table 10.

Table 10. SFI Profiles for Fourteen Reference Oils.

	C17/ Pris	C18/ Phy	C3 DBT	C3 Phen	C3 DBT/ C3 Phen	C1 Pyr	C1 Chr	Nor/ Hop
Crude Oils								
Arabian Light	6.34	3.18	1.27	3.06	3.28	0.35	2.22	1.36
Basrah	1.72	2.59	1.61	2.64	1.64	0.70	2.15	0.88
Eugene Island	2.35	1.92	1.90	4.84	1.75	0.78	2.35	0.94
Hondo	1.79	0.95	1.14	1.34	1.90	0.55	1.09	0.75
LA Blend	1.75	1.85	2.79	5.88	0.83	0.93	2.28	0.67
Mega Borg Light	1.97	1.77	2.40	26.77	0.38	1.04	0.79	0.74
North Slope	2.23	2.39	2.20	1.18	0.80	0.57	2.02	0.65
So. Louisiana	1.62	2.34	2.99	4.75	0.55	0.70	2.46	0.53
SPR	2.80	2.20	1.83	1.58	1.30	0.49	1.73	0.77
Fuel Oils								
Bunker C	3.35	2.64	1.67	10.44	0.84	0.98	1.71	0.92
Diesel No. 2	3.03	2.72	9.30	21.38	0.46	1.39	2.66	0.00
IFO 380	2.58	2.37	1.39	7.10	0.72	0.99	1.63	0.79
Other Oils								
SS Block Condensate	2.32	3.60	3.89	1.95	0.37	1.06	1.89	0.68
Valvoline	1.79	1.12	1.95	3.22	2.82	0.00	0.00	1.19

The SFI profiles given in Table 10 display the varying characteristics of biomarker compounds present in different types of oils. Even though no correlation can be made within the three categories, the SFI are representative of each individual oil type analyzed; therefore, supporting the SFI approach to source match/nonmatch determinations. Sources of an unknown sample can be eliminated based on the SFI profiles given for these fourteen oils since the biomarker distributions are relatively unique for each reference oil.

SFI are capable of providing evidence to prove that a sample of spilled oil is a match/nonmatch to a suspect source. The SFI profiles given in Table 10 provide a baseline for relatively unweathered or recently spilled oil. As time and weathering progress, certain SFI will remain more constant when dealing with environmental forensic determinations, as demonstrated by the weathering experiment. For example, the nC-17/Pris and nC-18/Phy ratios are both in the n-alkane fraction, which is generally the first group of oil compounds to be affected by weathering. Therefore, if the oil has been in the environment for more than a week or so, instrumental response of these compounds may be low, which would in turn make calculating these two SFI difficult. On the other hand, SFI such as the C-3 DBT, C-3 Phen, C-3 DBT area/C-3 Phen area, and Nor/Hop had %RSD less than 11% after 28-days of weathering with no additional nutrients. Therefore, these four SFI would be the best option for match/nonmatch

determinations for crude oils with similar physical properties. Another important factor to consider is the extent of oil refinement. Some fuel oils, such as diesel in Table 10, are devoid of the triterpane biomarker compounds due to the refinery process. Furthermore, certain oils contain differing levels of sulfur would be reflected in the C-3 DBT index. Oils with higher levels of sulfur will have lower C-3 DBT SFI; oils with lower levels of sulfur will have higher C-3 DBT SFI. In conclusion to the reference oil analysis, just as relative abundances of biomarker compounds in individual oils are unique, so are the SFI, making them a very valuable tool for the characterization of oil.

Biomarkers as a tool were applied to tarball samples collected in 1992 and 1998. SFI data from three of nine sites surveyed in 1992 were obtained from Appendix II of *Characterization of Chronic Sources and Impacts of Tar Along the Louisiana Coast* (Henry et al. 1993). SFI were also calculated for the 1998 tarballs. The SFI calculated for the tarballs collected from both tarball surveys were separated by beach and plotted on double SFI scatterplots. The beaches were Martin's, Rutherford, and Holly. SFI data from 1992 included 17 tarballs collected from Martin's beach, three tarballs from Rutherford, and seven tarballs from Holly (a total of 27). These same three sites from 1992 were revisited in 1998. SFI data from 1998 included 40 tarballs from Martin's beach, 21 tarballs from Rutherford, and eight from Holly (a total of 69). Two different double SFI plots were utilized. One plot consisted of the C3 Phen SFI vs. the C3 DBT area/C3 Phen area. The other plot was Nor/Hop vs. C3 DBT area/C3 Phen area. Twelve out of the fourteen reference oils (excluding NSC and EIC) were also plotted on the graphs. A 20% relative standard deviation of each SFI utilized in the double index plots was calculated for each reference oil and considered as that reference oil's positive match target area. Acceptance of scientific data is usually governed by EPA standards, in which data is considered valid when the relative standard deviation is 25% or less. The 20% variance used to establish the reference oil target zones is based on EPA standards, but a slightly lower range so that the chance for false positive matches is decreased.

No matches were visually confirmed between any of the 1992 and 1998 tarballs. Two tarballs from the 1992 survey, one from Martin's Beach and the other from Rutherford Beach, when plotted on the Nor/Hop vs. C3 DBT area/C3 Phen area, fell into the target zone for the Bunker C and South Louisiana Crude reference oils. Two 1998 samples (again one from Martin's and the other from Rutherford) were also plotted on the Nor/Hop vs. C3 DBT area/C3 Phen area, fell into the South Louisiana Crude and SS Block Condensate target zones. The 1992 and 1998 samples within the South Louisiana Crude range did not match upon visual examination.

Another tool for characterizing oil types based on visual scrutinization was established by Henry et al.'s 1993 report. Tarballs collected and analyzed in 1992 for this report were classified into one of nine categories: relatively unweathered, high aromatic; relatively unweathered, high paraffin; relatively unweathered bimodal wax; weathered, high aromatic; weathered, high paraffin; weathered, bimodal unresolved complex mixture (UCM); weathered, bimodal wax; weathered, bimodal UCM, and wax (trimodal); and unclassifiable. The most common classification identified for the 1992 tarballs was the relatively unweathered, high paraffin which represented 32% of the samples analyzed. A total of 26% of the samples analyzed contained the bimodal wax component that is generally believed to be related to crude oil tanker washing or sludge discharges resulting from cleaning waxy residues from the sides of

storage tanks or cargo holds (Butler et al. 1973). Cleaning bunker fuel tanks and fuel lines may also result in a similar wax signature.

A slightly modified version of the same source classification was utilized to separate the tarballs collected during the 1998 survey (i.e. high was not used as a descriptive of the type of oil and only eight categories, instead of nine, were established for the 1998 tarballs). The 1998 tarballs were classified into one of eight categories: relatively unweathered, aromatic; relatively unweathered, paraffin; relatively unweathered, wax/bimodal wax; weathered, aromatic; weathered, paraffin; weathered, wax/bimodal wax; weathered, UCM; and unclassifiable. The most common classification identified for the 1998 tarballs was weathered, UCM which represented approximately 57% of the samples analyzed. These tarballs are indicative of weathered crude oils and the normal alkane distribution suggest that the oils were a mid to heavy range. A total of 17% of the samples analyzed were categorized into the unclassifiable category. Samples in the unclassifiable category were oils that did not fit any of the other classifications and generally were so heavily weathered, or not oils at all, that they could not be classified with any confidence.

Outside of the comparison between the 1992 and 1998 samples, the data for just the tarballs collected in the 1998 survey was processed and interpreted at several levels. Morphological characterizations were determined for all 88 tarballs collected. GC/MS analysis was performed for 70 of the 88 tarballs. The data generated from the GC/MS analysis were compared based on eight weathering stages and six extracted ion chromatograms/fingerprints (m/e 85, 191, 216, 220, 226, and 242). The SFI were calculated from the ion chromatograms and were plotted on double SFI scatterplots to determine if any of the samples containing oil appeared to be related, or if any of the samples fell within the reference oil target match zones previously plotted.

Morphological Appearance

The weight distribution of the tarballs is a function of the amount of oil and the amount of adsorbed material. As indicated by the morphological characterizations, numerous samples contained sand and other extraneous debris. The percentage of weight contributed from debris (not oil) could possibly account for 50% of the weight of some samples. It is practically impossible to remove these included materials; therefore, the weight values presented are over estimations of the true values. The total weight of the tarballs collected was 3054.15g. Weights ranged from 0.15g to 296.01g. A cutoff weight of 0.70g was established for sampling and analyzing due to the fact that tarballs weighing less than the cutoff were too small to sample.

A wide gradation of colors was observed and each tarball was classified as black, brown, grey, green, and dark red. Of the 88 tarballs collected, 84 (~95%) were black, followed by grey (two tarballs or ~2%) on the outside surface as shown in Figure 15. The remaining colors were less than 2% of the total. The majority of the inside color of the tarballs was black (82 tarballs, or ~93%) followed by brown (2 tarballs, or ~2%). The distribution of inside color for tarballs collected in the 1998 survey is displayed in Figure 16. Two samples were too small for the inside color to be described.

Color often suggests an oil type. The dark green tarballs appeared to be globs of heavy grease or lube oil. The pitch-black tarballs appear to be sourced either from a high pour-point bunker oils or other refined products derived from heavy petroleum residuum. Brown tarballs often suggest oil that has been oxidized and moussed during the weathering process; often these represent spilled crude oils.

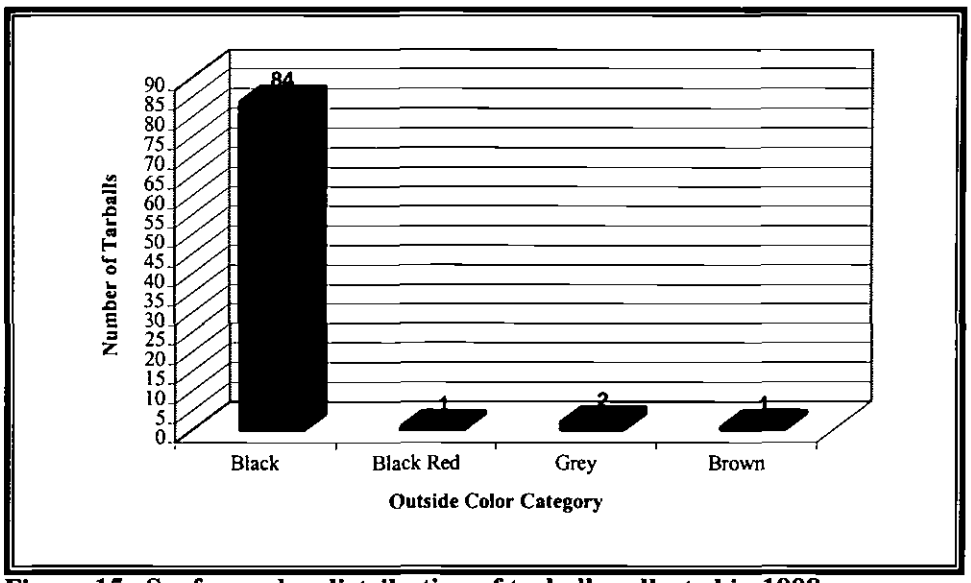


Figure 15. Surface color distribution of tarballs collected in 1998 survey.

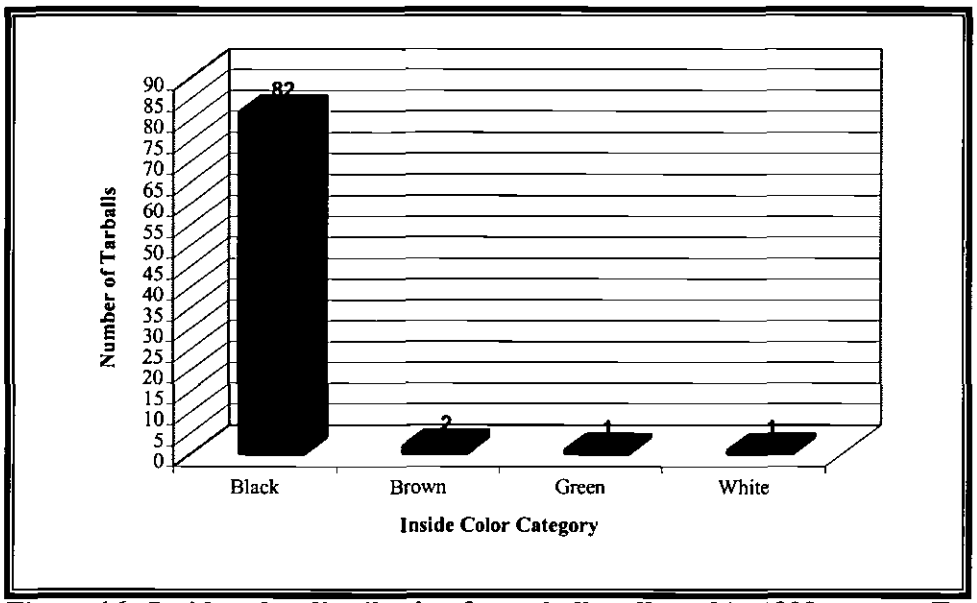


Figure 16. Inside color distribution for tarballs collected in 1998 survey. Two were too small to be characterized for inside color.

Many of the samples collected contained extraneous matter including organic debris (i.e. seaweed), sand, and shell fragments. These observations are not unusual. The sticky nature of oil floating on the water's surface and repeated strandings often result in the accumulation of

organic debris and beach substrate. The potential for extraneous material to become incorporated in the tarball is a function of physical weathering processes as well as the physical/chemical composition of the spilled oil. Very high pour-point oils do not easily spread and are limited to surface encrustations only. Figures 17, 18, and 19 present the relative contribution of organic matter, sand and shell fragments to the entire tarball population. Organic debris from pelagic materials and beach erosion is a very ubiquitous feature in the marine environment and was the predominant extraneous material encountered in all the 88 collected tarballs. Sand was more common than shell for most of the tarballs collected. This is consistent with the distribution of beach substrates; sandy beaches are more numerous than shell beaches along the northern Gulf of Mexico.

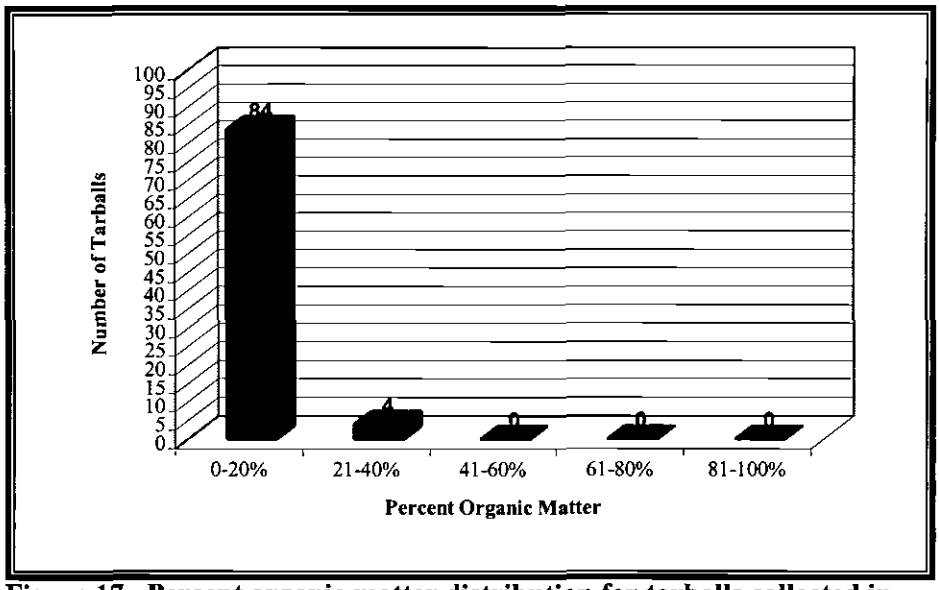


Figure 17. Percent organic matter distribution for tarballs collected in 1998 survey.

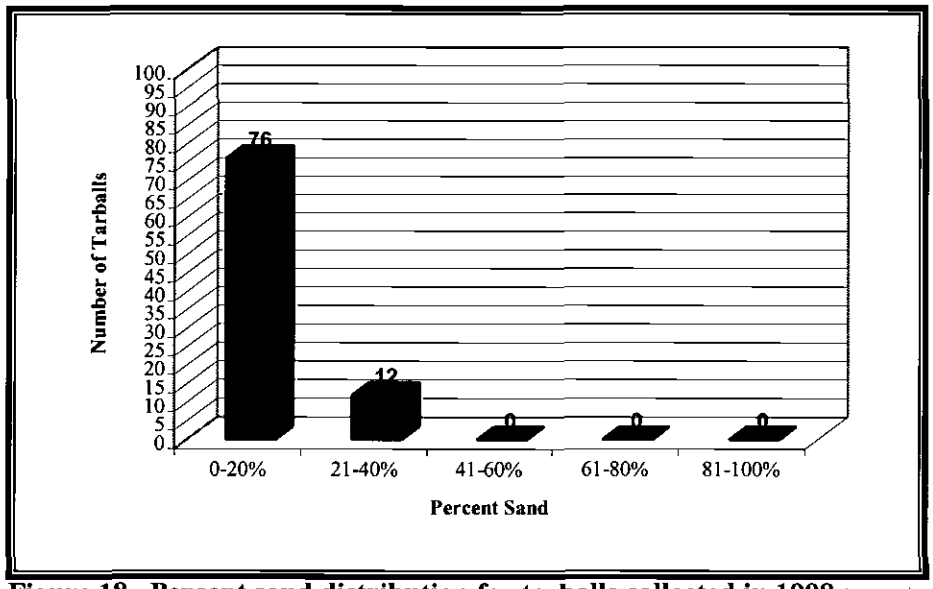


Figure 18. Percent sand distribution for tarballs collected in 1998 survey.

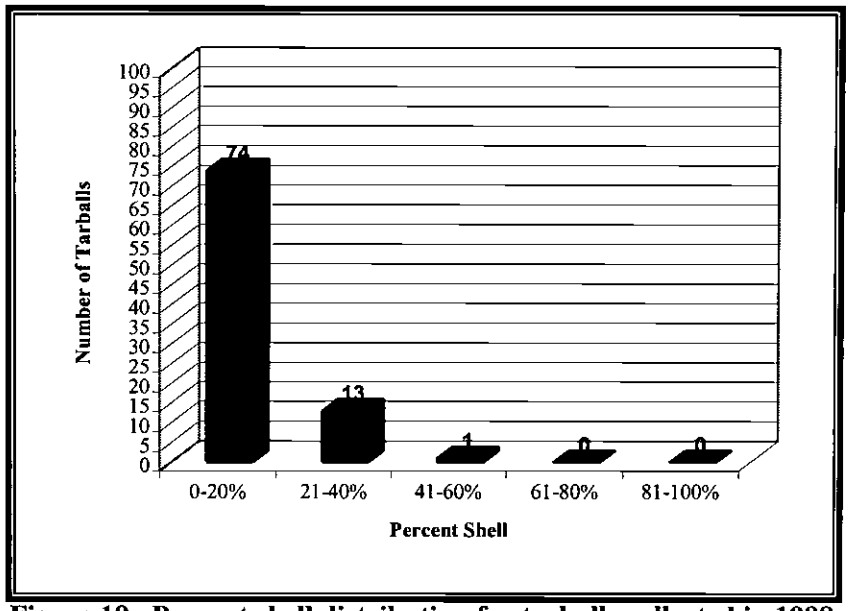


Figure 19. Percent shell distribution for tarballs collected in 1998 survey.

Each tarball was also characterized for its pliability. Pliability is generally associated with the physical/chemical composition of the spilled oil and the extent of weathering which has occurred. Highly weathered tarballs tend to become nonpliable, often brittle or very difficult to break. These pliability characterizations were qualitative only. Each sample was rated from 0 to 5. A rating score of 0 is nonpliable and nonbreakable using normal hand pressure. A score of 5 represents stranded tar that was almost fluid. Figure 20 displays the pliability score for the tarballs collected in 1998. The majority of the tarballs sampled for this research had a score of 1, which represents tarballs that are difficult to bend with normal hand pressure. Less than 7% of the tarballs collected were nonpliable (score of 0) and none were scored as very fluid (pliability score of 5).

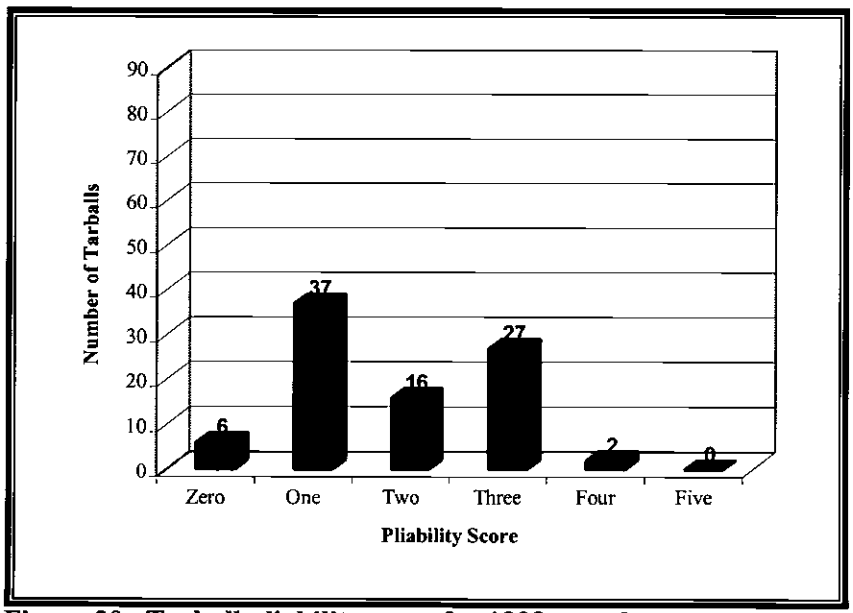


Figure 20. Tarball pliability score for 1998 samples.

Analytical Chemistry Results

Of the 88 tarballs collected during the 1998 survey, 70 were sampled and analyzed by GC/MS. Eighteen samples were not extracted due to the fact that they were smaller than the cutoff weight established during the morphological characterization step of analysis; or were pieces of asphalt too hard to sample. The first stage of data processing for the GC/MS analyses for each of the 70 samples was classification into one of eight classifications adapted from *Characterization of Chronic Sources and Impacts of Tar Along the Louisiana Coast* (Henry et al. 1993). Some of the earlier classification schemes were based primarily on alkanes (Boehm et al. 1981), and more recently, polycyclic aromatic hydrocarbons (PAHs) classification have been published (Sauer et al. 1993). Any weathering classifications are most useful when they describe a specific oil type. The classification scheme used for this project consisted of eight categories, which provided basic chemical information related to compositional change in the aromatic hydrocarbon fingerprint. The eight classifications are as follows:

1) Relatively unweathered, aromatic. Oils classified into this category were identified by a nC-18/phytane SFI ratio of greater than 1 and were enriched with target aromatic hydrocarbons. Oils in this classification are often representative of slightly weathered, refined, blended fuel oils.

2) Relatively unweathered, paraffin. Oils classified into this category were identified by a nC-18/phytane SFI ratio of grater than 1 and were enriched with normal paraffins between nC-15 and nC-33 often with the most abundant normal paraffin being nC-19. Oils in this classification generally have high pour-points and many may be representative of heavy fuel oils.

3) Relatively unweathered, wax/bimodal wax. These tarballs are characterized by a nC-18/phytane SFI ratio greater than 1 and, in some instances, a bimodal distribution of normal alkanes with a pronounce wax component from nC-21 extending to nC-37. These oils are believed to be representative of crude oil and heavy fuel oil tank washings or sludge discharges.

4) Weathered, aromatic. Oils classified into this category were identified by a nC-18/phytane SFI ratio of less than 1 and were enriched with target aromatic hydrocarbons. Oils in this classification are often representative of weathered refined fuel oils and some highly weathered crude oils.

5) Weathered, paraffin. Oils classified into this category were identified by a nC-18/phytane SFI ratio of less than 1 and were enriched with normal paraffins between nC-15 and nC-33. Oils in this classification have high pour-points and many may be derived from heavy, high pour-point fuel oils.

6) Weathered, wax/bimodal wax. These tarballs are characterized by a nC-18/phytane ratio less than 1 and in some instances, a bimodal distribution of normal alkanes with a pronounced nC-21 extending to nC-37 wax component. These oils are

believed to be representative of crude oil and fuel oil tank washings and sludge discharges as noted from the unweathered bimodal wax.

7) Weathered, unresolved complex mixture (UCM). These tarballs are characterized by a range of nC-18/phytane SFI ratios and contain some normal alkanes and aromatic hydrocarbons, but could not be distinguished as weathered aromatic oils. All tarballs in this classification exhibited a large UCM in both the TIC and m/e 85. Oils in this classification are often highly weathered crude oils.

8) Unclassifiable. Oils that did not fit any of the above classifications. Generally, these oils were so heavily weathered, or not oils at all, that they could not be classified with any confidence.

Table 11 provides a summary of the GC/MS tarball classifications for the samples analyzed in the 1998 collection. The most common classification identified was weathered UCM, which represented ~57% of the samples analyzed. Figures 21, 22, 23, 24, 25, 26, and 27 show chromatographic ion fingerprints of oils typically classified as one of the eight categories, except Unclassified.

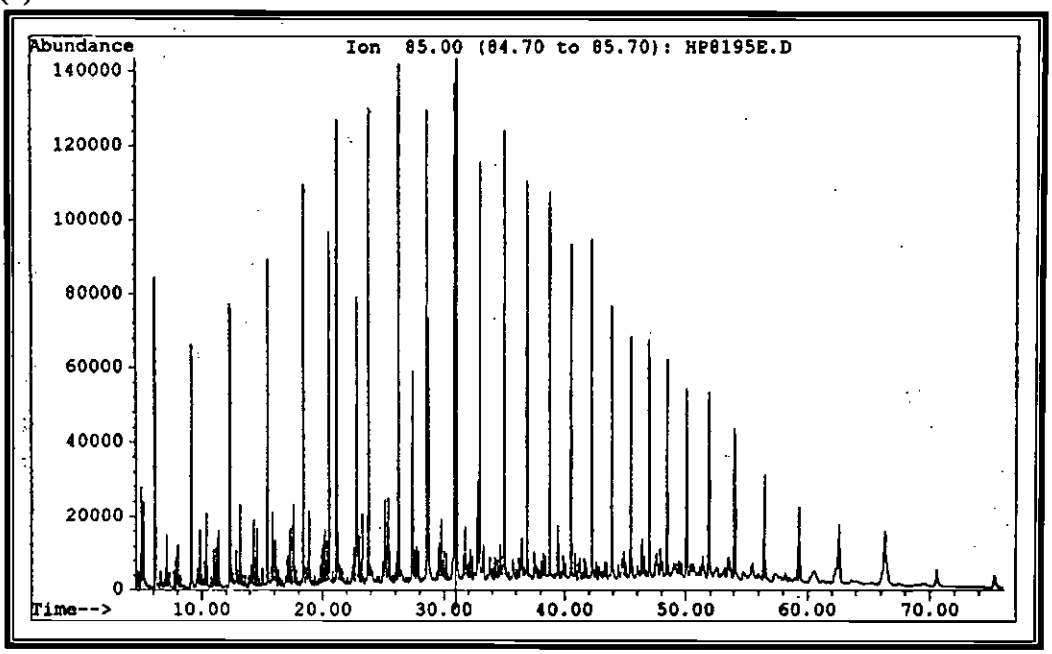
Table 11. Distribution of All Tarballs into Classifications.

	Number of Samples*	% of Total
Relatively Unweathered		
Aromatic	1	~1.4
Paraffin	1	~1.4
Wax/Bimodal Wax	2	~2.9
Relatively Weathered		
Aromatic	9	~12.9
Paraffin	2	~2.9
Wax/Bimodal Wax	3	~4.3
UCM	40	~57.1
Unclassifiable	12	~17.1

Visual Matching

An exhaustive matrix comparison process that utilized all the chromatographic data and provided separation of the tarballs by source was the next step of the data interpretation. This comparison involved visual scrutinization of each ion fingerprint for all biomarker ions chosen for analysis. After analysis, a hard copy of all the ion fingerprints for each tarball was printed out and the biomarker ions were separated from the rest of the ions and stacked into separate piles. Each tarball fingerprint in each pile was visually compared to each other to determine if any matched or were nonmatches. Matched samples went into one pile and nonmatch samples formed a new pile until every chromatogram had been compared. Each source was assigned an alphabetical identification such as Source A and Source B. The comparisons were completed for each tarball analyzed. Through this manual process and previous experience, certain ion components within the samples were shown to be quite unique and descriptive.

(a)



(b)

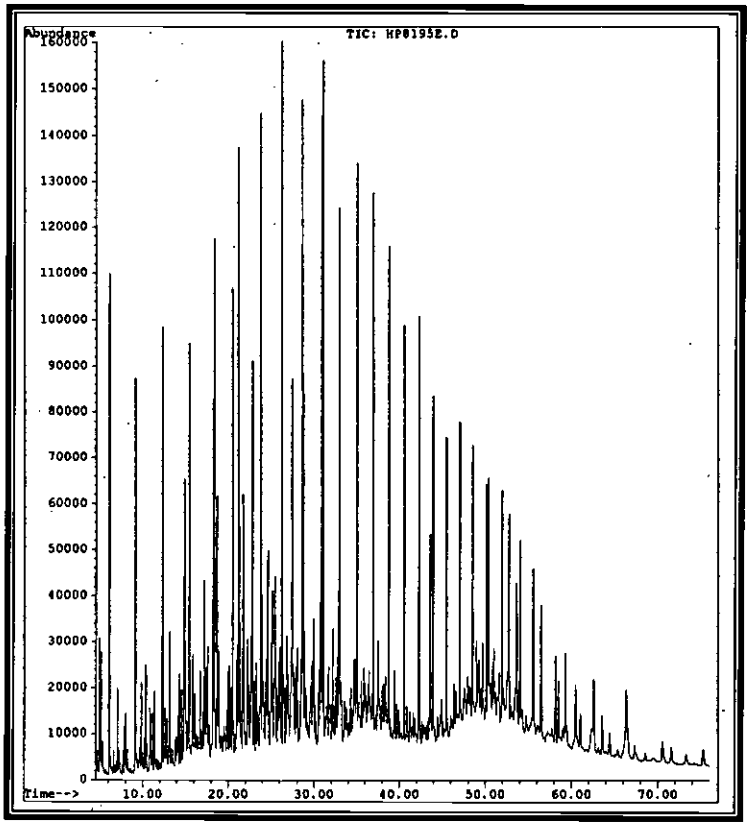
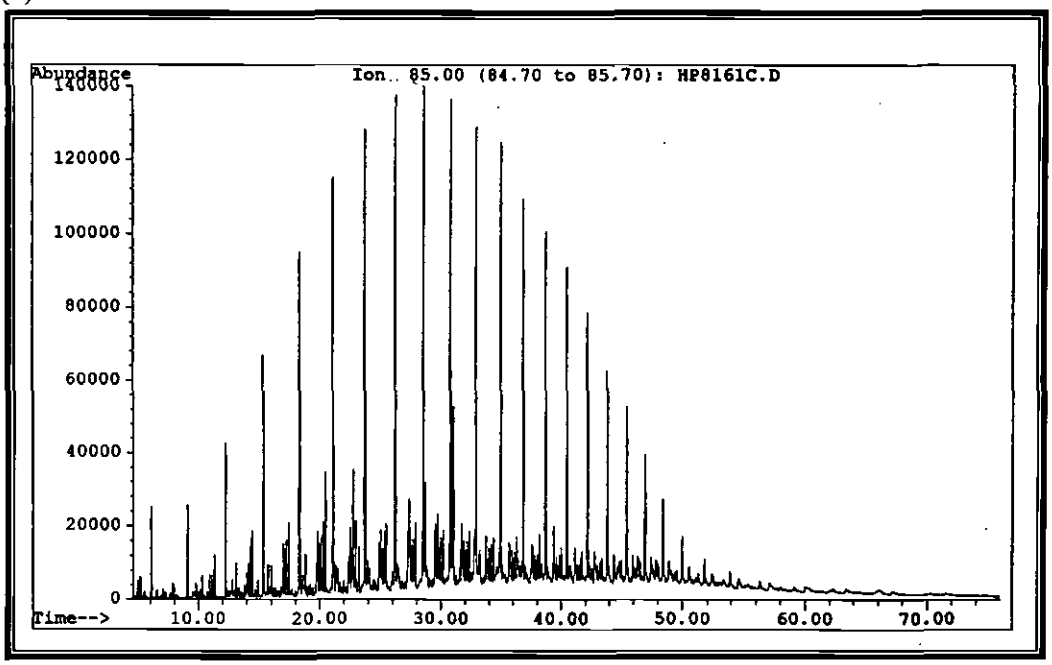


Figure 21. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a relatively unweathered, aromatic oil.

(a)



(b)

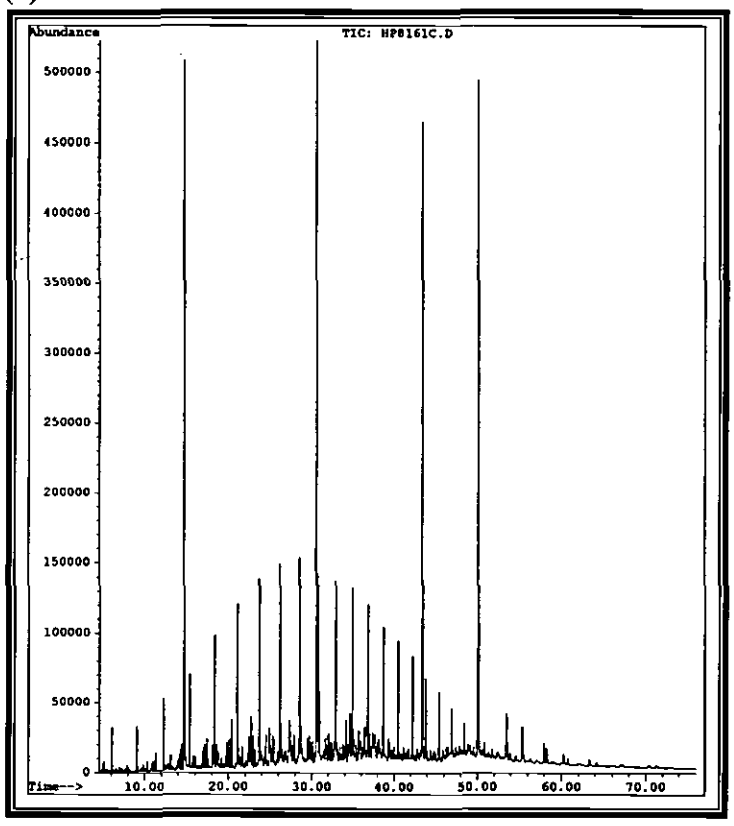
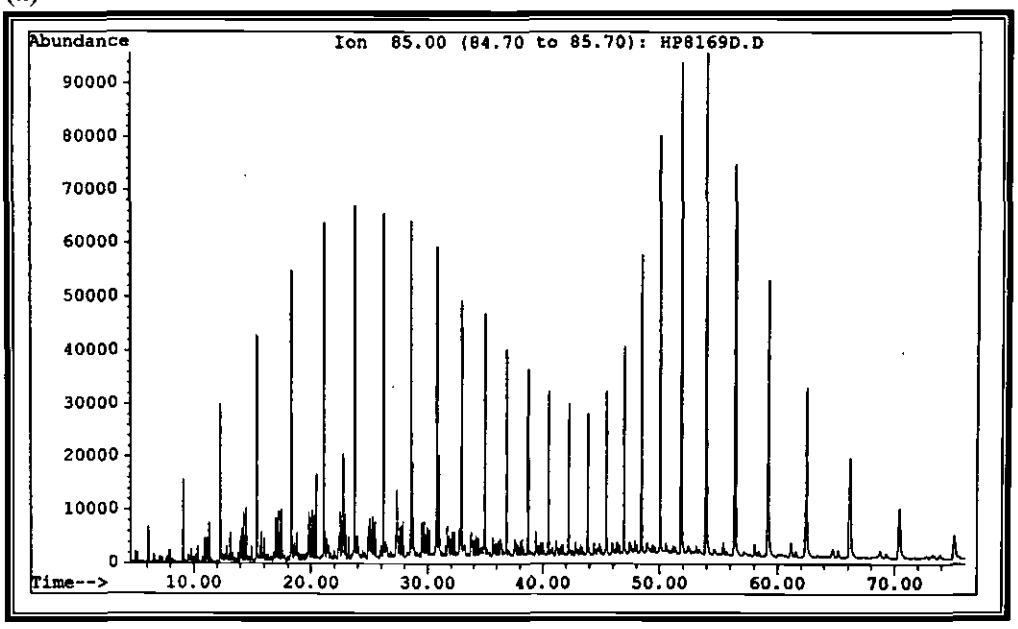


Figure 22. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a relatively unweathered, paraffinic oil.

(a)



(b)

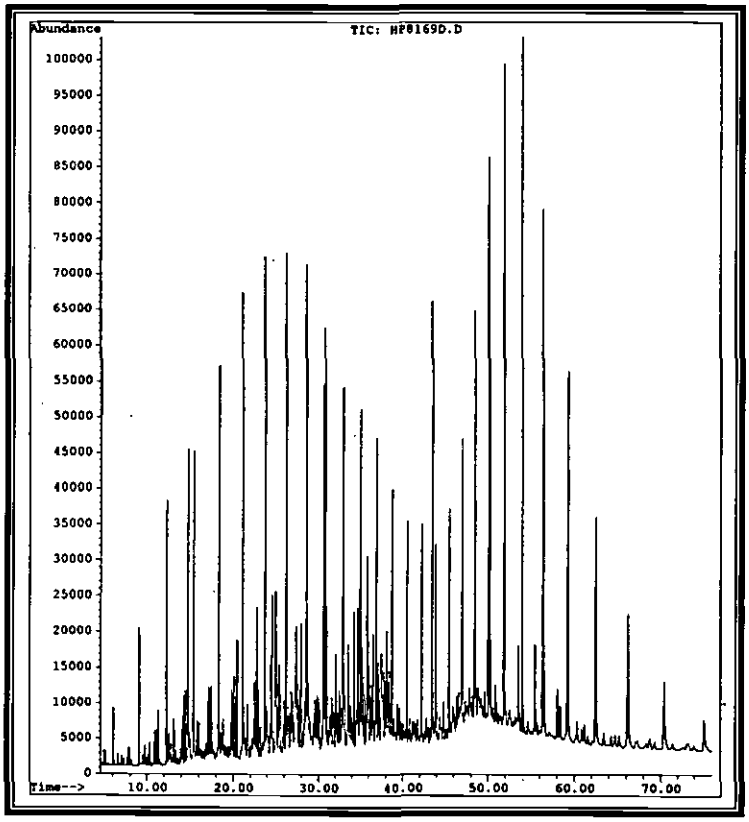
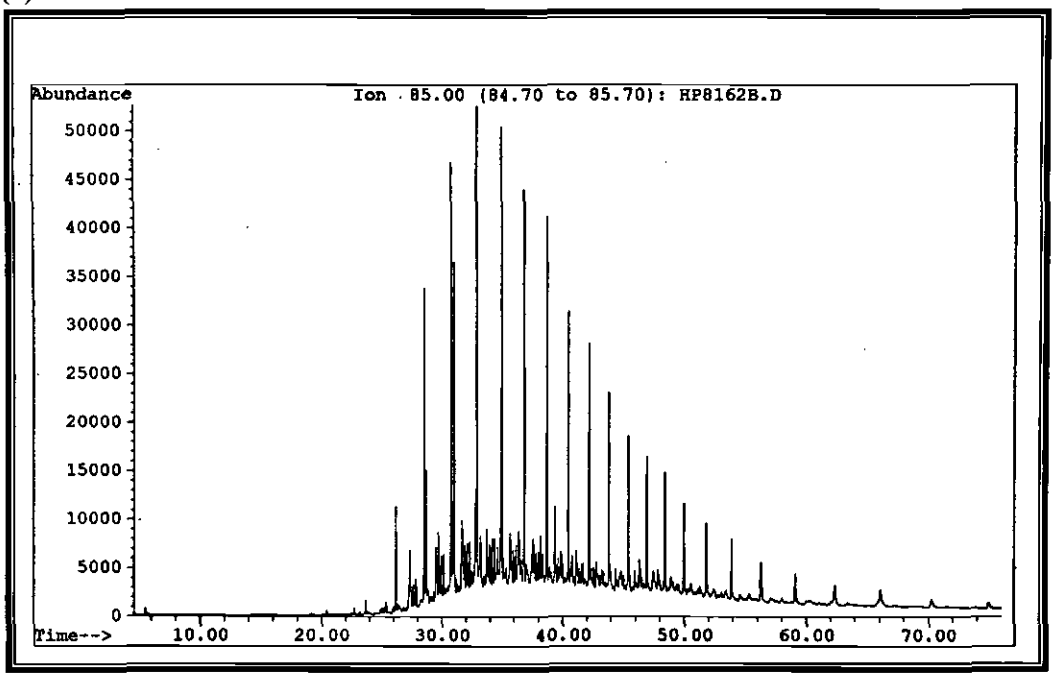


Figure 23. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a relatively unweathered, bimodal wax.

(a)



(b)

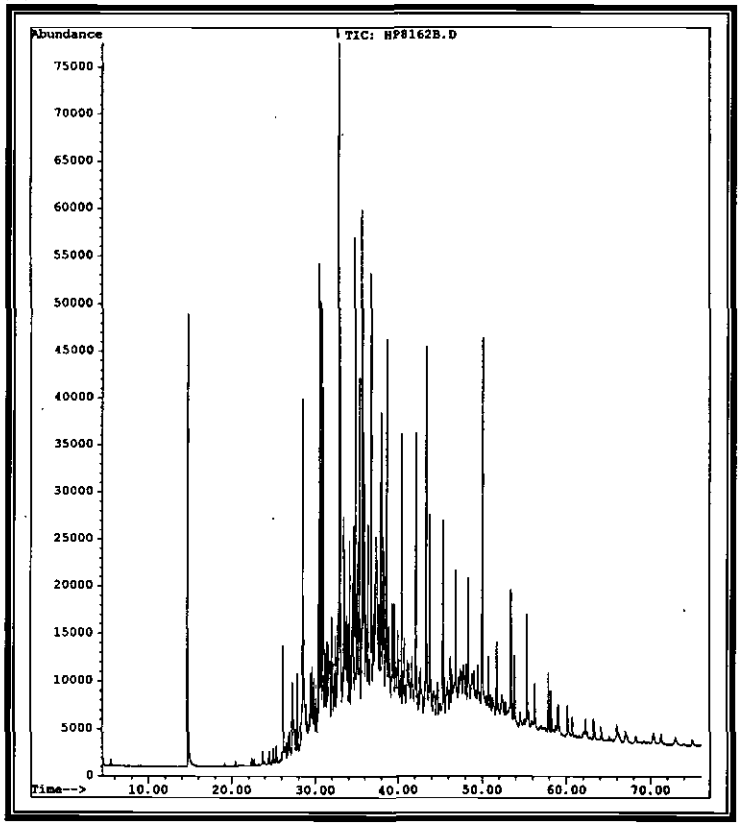
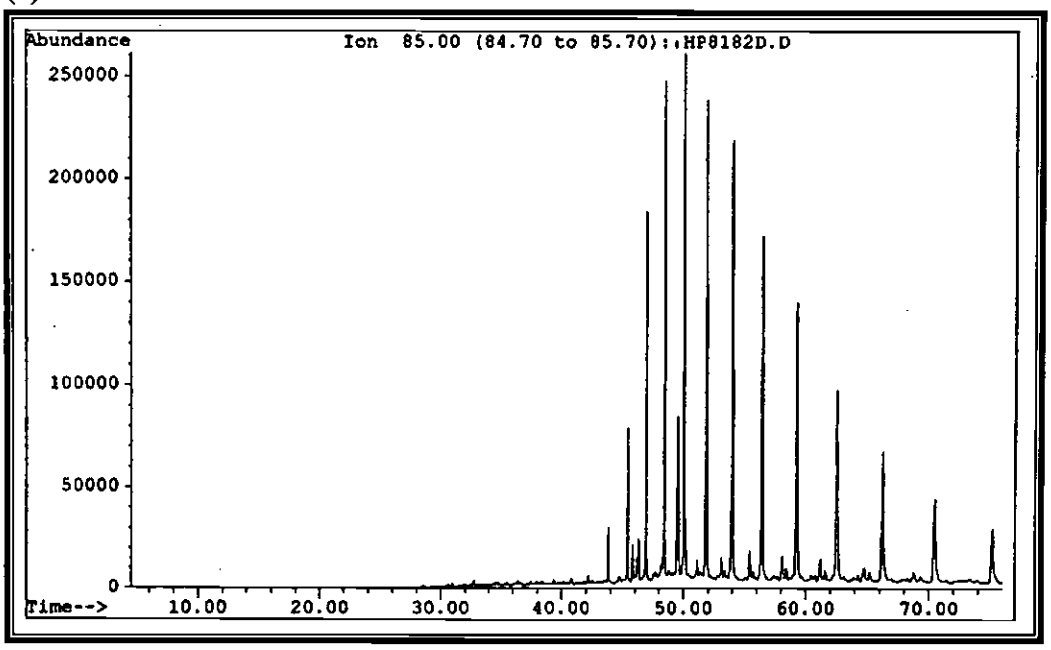


Figure 24. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a weathered, aromatic oil.

(a)



(b)

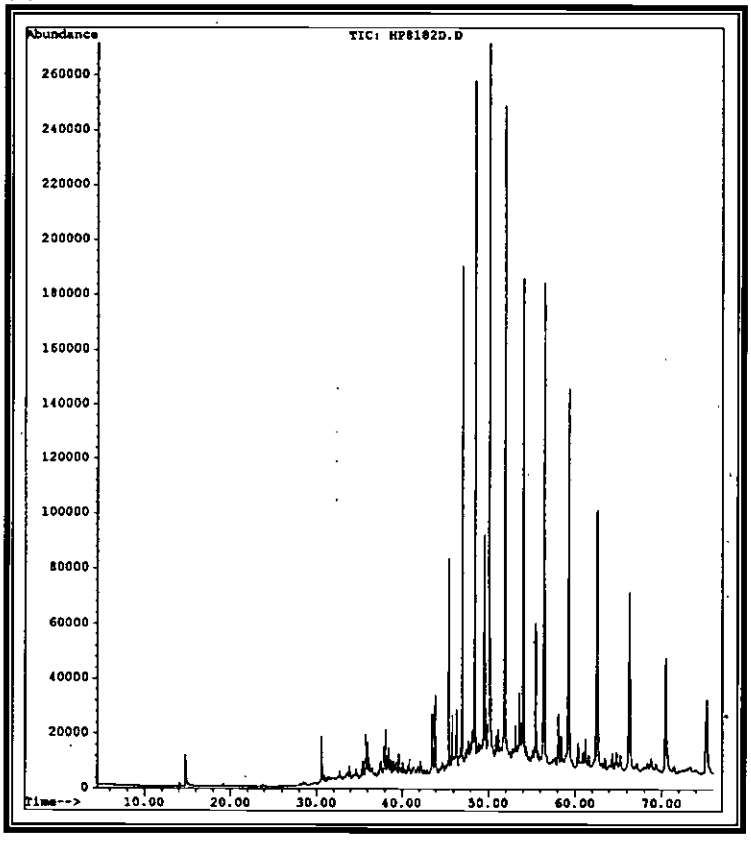
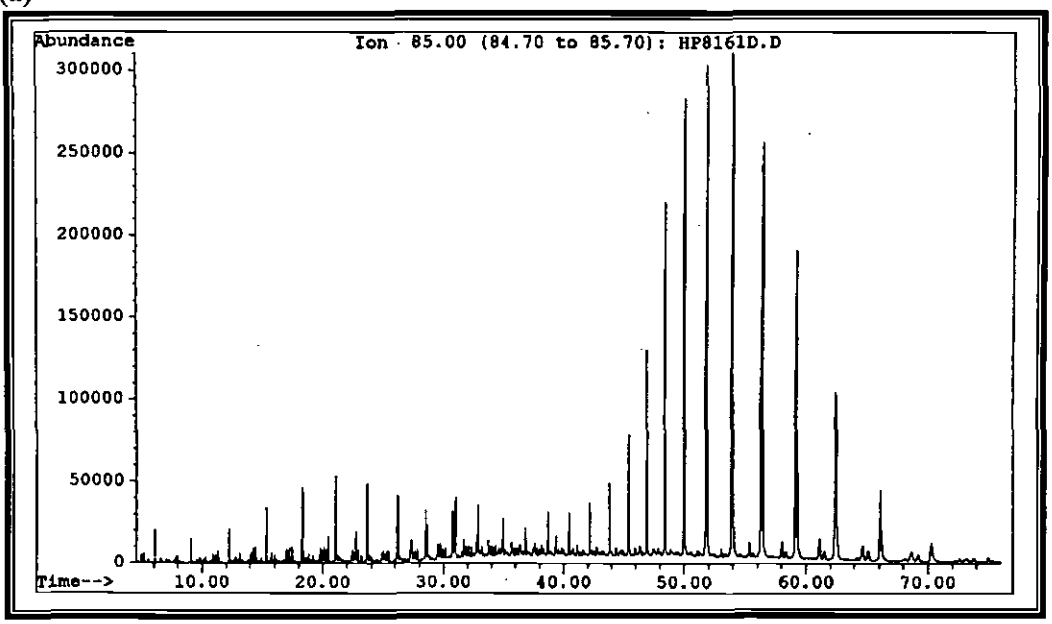


Figure 25. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a weathered, paraffinic oil.

(a)



(b)

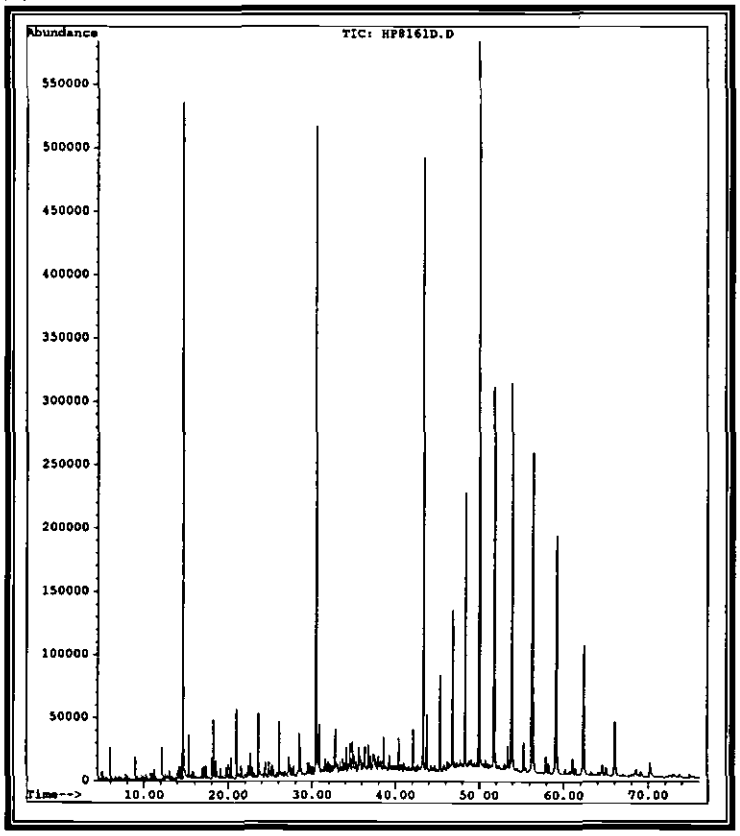
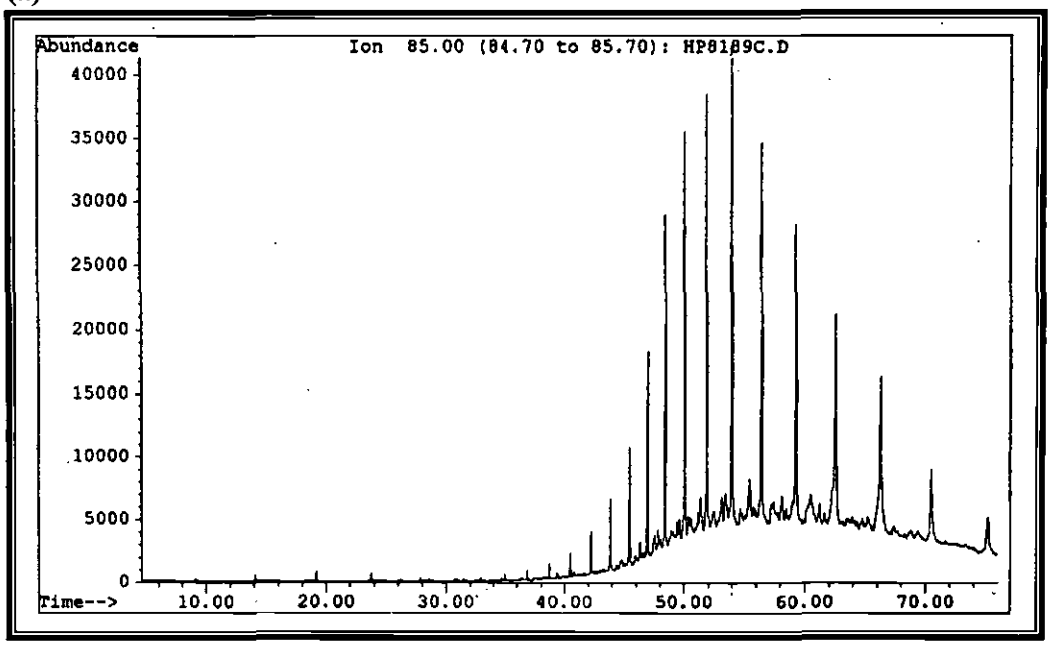


Figure 26. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a weathered, bimodal wax.

(a)



(b)

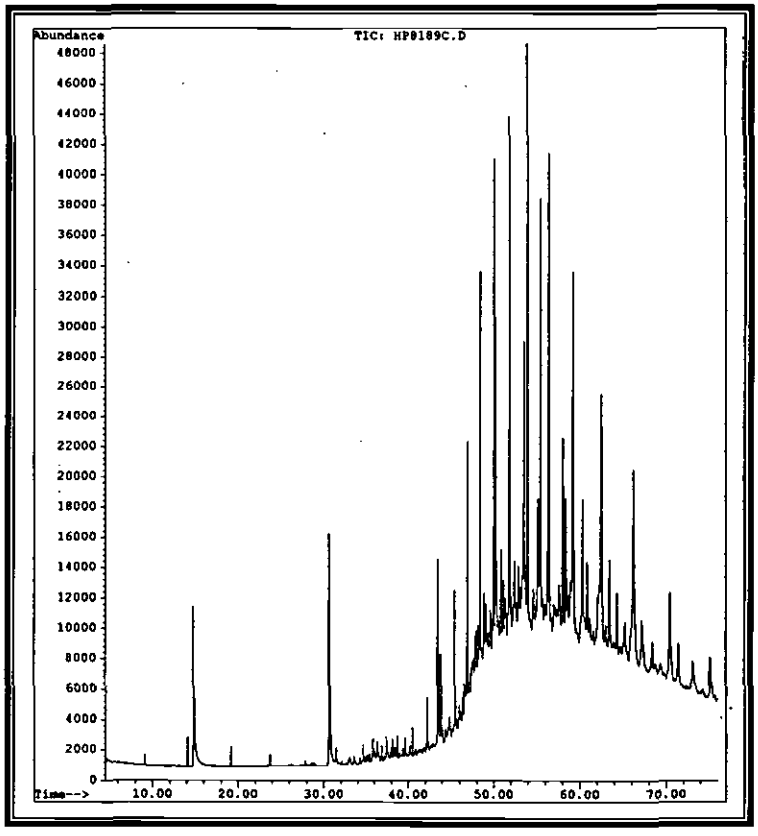


Figure 27. Normal alkane distribution (a) and Total Ion Chromatogram (b) for a weathered, UCM.

Source-fingerprinting of the 70 tarballs analyzed identified 66 different sources. Four matches were found through visual comparison and double SFI scatterplots. The matches included one tarball matching Valvoline; one tarball matching Hondo Crude; one tarball matching SPR crude; and two tarballs that matched each other but did not match one of the reference oils. An interesting observation is the wide distribution of sources from the three sampling locations. Apparently, the tarballs collected represent oil contamination from small petroleum and/or bilge waste discharge.

SFI and Scatterplots

The initial group of SFI scatterplots included all eight of the biomarkers chosen by IES/RCAT. As a result of the visual comparison, a selection of three of the SFI were chosen and consisted of two SFI per plot (i.e. C-3 Phen vs. C-3 DBT area/C-3 Phen area; and norhopane/hopane vs. the C-3 DBT area/ C-3 Phen area). These indexes exhibited resistance to weathering during the water degradation experiment and displayed chromatographic uniqueness. Figures 28 and 29 are two examples of the double SFI plots.

All the samples analyzed were plotted as a scatterplot in an attempt to further confirm sources. The reference oils prepared during the initial stages of the characterization and identification of tarballs were also plotted and target match zone were calculated based on a 20% standard deviation of the SFI chosen. A total of 29 possible matches, either to one of the reference oils or to another tarball, were determined from the two different double SFI scatterplots. However, only four of the matches were confirmed by visual comparison. Since the initial visual comparison indicated an abnormal distribution of several non-related sources (66 different sources), it was decided that principle component analysis would not provide the discrimination necessary for statistical source-fingerprint results; therefore, scatterplots were created instead. Henry (1995) established that the use of principle component analysis did not provide the degree of source resolution anticipated in a tarball population containing 67 different sources in a sample of 133. Had each source not been differentiated by visual comparison, identification of which tarballs were related statistically could not be determined to any degree of confidence. Factors that contributed to the loss in discrimination included the limited value range of the index values used and the extremely high number of different sources in the study population. Principle component analysis may have been useful if the study population demonstrated a more normal distribution.

The double SFI scatter plots that were generated did not provide a high degree of identification, but were considered a useful screening tool. The resulting scatterplots were often more confusing than enlightening, yielding "inconclusive" results for all double SFI plots attempted. The major factor influencing the interpretation of the scatterplots was the various stages of weathering and sources for the tarballs sampled. Therefore, it has been concluded that double SFI scatterplots are more efficient and defensible when one source oil and a few unknown samples have been collected for analysis.

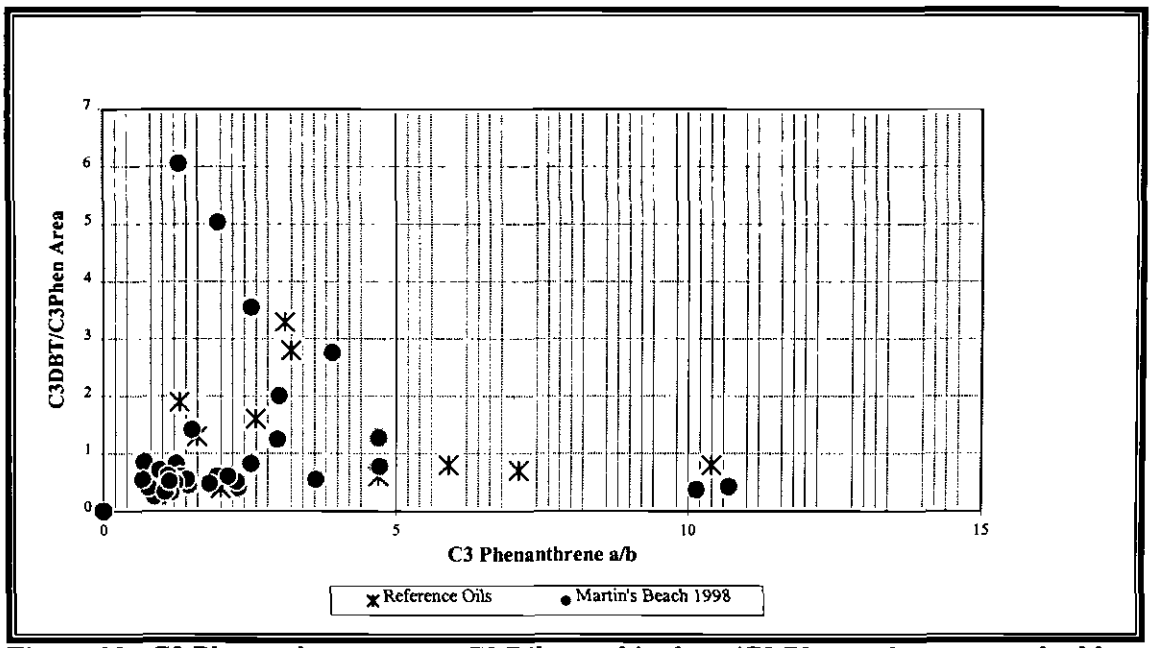


Figure 28. C3 Phenanthrene versus C3 Dibenzothiophene/C3 Phenanthrene area double SFI scatterplot for tarballs from Martin's beach.

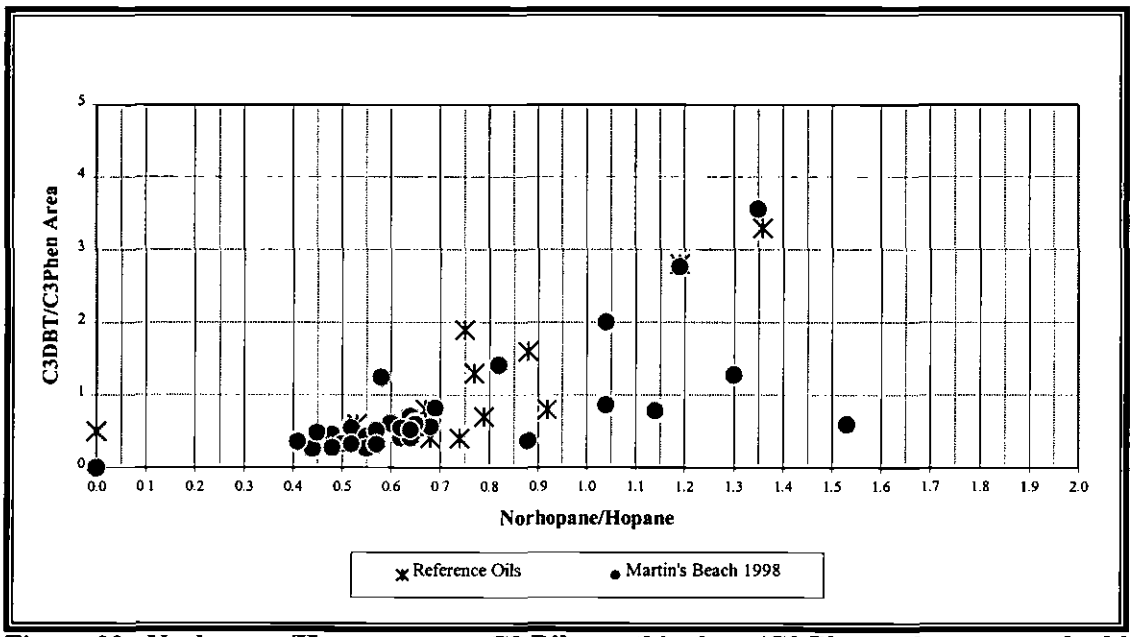


Figure 29. Norhopane/Hopane versus C3 Dibenzothiophene/C3 Phenanthrene area double SFI scatterplot for tarballs from Martin's beach.

The SFI values were validated for instrumental variability from the percent relative standard deviation calculated from the daily North Slope Crude (NSC) reference oil. The %RSD for the daily NSC ranged between 3.63 and 9.17%. Individual %RSD were as follows: nC-17/pristane, 3.63%; nC-18/phytane, 9.17%; C-3 DBT a/b, 6.08%; C-3 Phen a/b, 4.21%; C-1 Pyr a/b, 7.59%; C-1 Chr a/b, 5.13%; Nor/Hop, 5.33%; and C-3 DBT area/C-3 Phen area, 3.83%. One goal of this research was to choose SFI that were unaffected by changes in

instrumental operation. Each index, except for the C-3 DBT area/C-3 Phen area, was hand calculated on the basis of peak height rather than area to reduce integration error due to peaks not being baseline resolved. The C-3 DBT area/C-3 Phen area SFI is based on total area represented by each isomer group.

Of the 70 tarballs analyzed by detailed GC/MS, ~57% had fingerprints indicative of weathered crude oils. The normal alkane distribution observed from the GC/MS analysis indicate that the majority of the weathered crude oils were a mid to heavy range. Light oils spilled in the marine environment spread very thinly on the water's surface and disperse by natural processes, such as storms, and usually do not form tarballs. However, some of the tarballs were most likely formed due to the formation of stable water-in-oil emulsifications, or mousses. Mousse formation is common for many crude oils and is a prime factor for the formation of tarballs from otherwise easily dispersible light crude oils. Very heavy crude oils are prone to form tarballs due to the fact that they are slow to spread and expose little surface area for natural degradation processes.

CONCLUSIONS AND RECOMMENDATIONS

Biomarker compounds, by definition, are universal in crude oils and petroleum products. These compounds are generally more resistant to environmental weathering than most of the other oil constituents. The distribution of biomarker compounds is unique for each oil and different sources of petroleum exhibit different oil fingerprints. SFI calculated from these fingerprints provide a stable and useful tool for determining a match or nonmatch for different oil residues present in some environmental samples. The presence and detection of biomarker compounds is an indicator of some sort of oil exposure or contamination. Analytical chemistry, biomarker normalization determined by SFI, and visual comparisons of oil biomarker fingerprints, provide environmental scientists with valuable information regarding the identification of spilled oil and quantification of oil residues.

As far as general methodology is concerned, GC/MS operated in selected ion monitoring mode provides detailed compositional and comprehensive information on saturated and aromatic hydrocarbons, without requiring involved sample clean-up procedures prior to sample extraction and analysis. High-resolution GC/MS analysis and source-fingerprinting techniques were employed throughout all experimental stages to validate and extend the use of biomarkers as tools for oil spill identification and assessment. Eight (8) petroleum biomarkers were chosen for detection and analysis through a literature search and from previous research. Self-normalizing fingerprint indexes, or SFI, calculated from the GC/MS analysis of a water degradation experiment; a bioaccumulation study; and the classification and identification of tarballs assisted in the validation of the eight biomarkers chosen by LSU IES/RCAT.

Visual comparison played an important role in distinguishing gross, and in some cases subtle, differences in the biomarker fingerprints. This method is often the first step in GC/MS data interpretation of spilled oil. Visual comparison of a small number of samples is very efficient for match/nonmatch determinations. A suggested method (Figure 30) for data interpretations for match/nonmatch determinations is as follows: print out hard copies of biomarker ions and

the TIC; separate each copy into separate biomarker ions; compare other copies of each biomarker ion to one another to determine a visual match/nonmatch; calculate the SFI and compare each value with the others in the group; plot the reference oil with the 20% target match zone on one of the double index scatterplots from above and then plot the samples; and finally, reconfirm matches with a visual comparison.

SFI reflect the relative rates at which selected oil biomarkers are dissipated during the combined physical, chemical, and biological processes that constitute oil weathering. The effects of weathering on the value of biomarker indexes are generally greatest in the period immediately following spillage or leakage. For SFI to be valid throughout the weathering processes, they must consist of components that are truly conserved during oil weathering and not display significantly different physical properties (Whittaker and Pollard 1997). The SFI that meet this criterion and provide the best tool for oil spill identification, both visually and statistically, are C-3 DBT, C-3 Phen, C-3 DBT area/C-3 Phen area, and Norhopane/Hopane. These compounds, as demonstrated by the water degradation experiment, remain stable even after weathering and will distinguish one oil source from another, even oils of similar geographical origin, when other biomarkers are lost (i.e. nC-17/Pris and nC-18/Phy).

Generally, the simpler the chemical structure, the easier it is to degrade, which is the case for nC-17/Pristane and nC-18/Phytane. The latter two SFI are straight-chain hydrocarbons that are metabolized by microorganisms through successive oxidation and cleavage of terminal methyl groups of a hydrocarbon molecule. The C-3 DBT, C-3 Phen, C-3 DBT area/C-3 Phen area, and Norhopane/Hopane are branched or cyclic compounds that experience slower biotransformation rates due to the presence of secondary, tertiary, and quaternary carbon atoms within the basic hydrocarbon structure, rendering them more difficult to metabolize (Whittaker and Pollard 1997).

Even though four of the eight SFI are statistically valid, it is recommended that GC/MS analysis and visual examination include all eight because they provide very valuable information about the extent of weathering and biodegradation from their respective fingerprints. Visual examinations of chromatographic fingerprints provide very significant qualitative information, as opposed to the comparison of a few quantitative indexes, and can easily distinguish one type of oil or weathering stage from another based on the pattern of the fingerprint alone.

One significant aspect of oil released on water is the ability of natural microbial communities within the water to affect the oil composition; or in other words, their biodegradation potential. The key elements of biodegradation include the amount of oil, the mixing energy, the types of microfauna, and the availability of oxygen. The water degradation experiment assessed the effects of microorganism populations present in different water sources in two ways: Biolog™ microplate analyses and percent reduction of total target aromatic hydrocarbons (TTAH). The organisms in the different water samples had widely varying metabolic capabilities. Unfortunately, the results from the Biolog™ analyses and the %TTAH calculated from the GC/MS data did not correlate. This is due to several factors: differing carbon sources; differing nutrient substrates; and differing dominate microbial communities. However, the experiment provided the best data in regards to the stability of the SFI chosen by IES/RCAT.

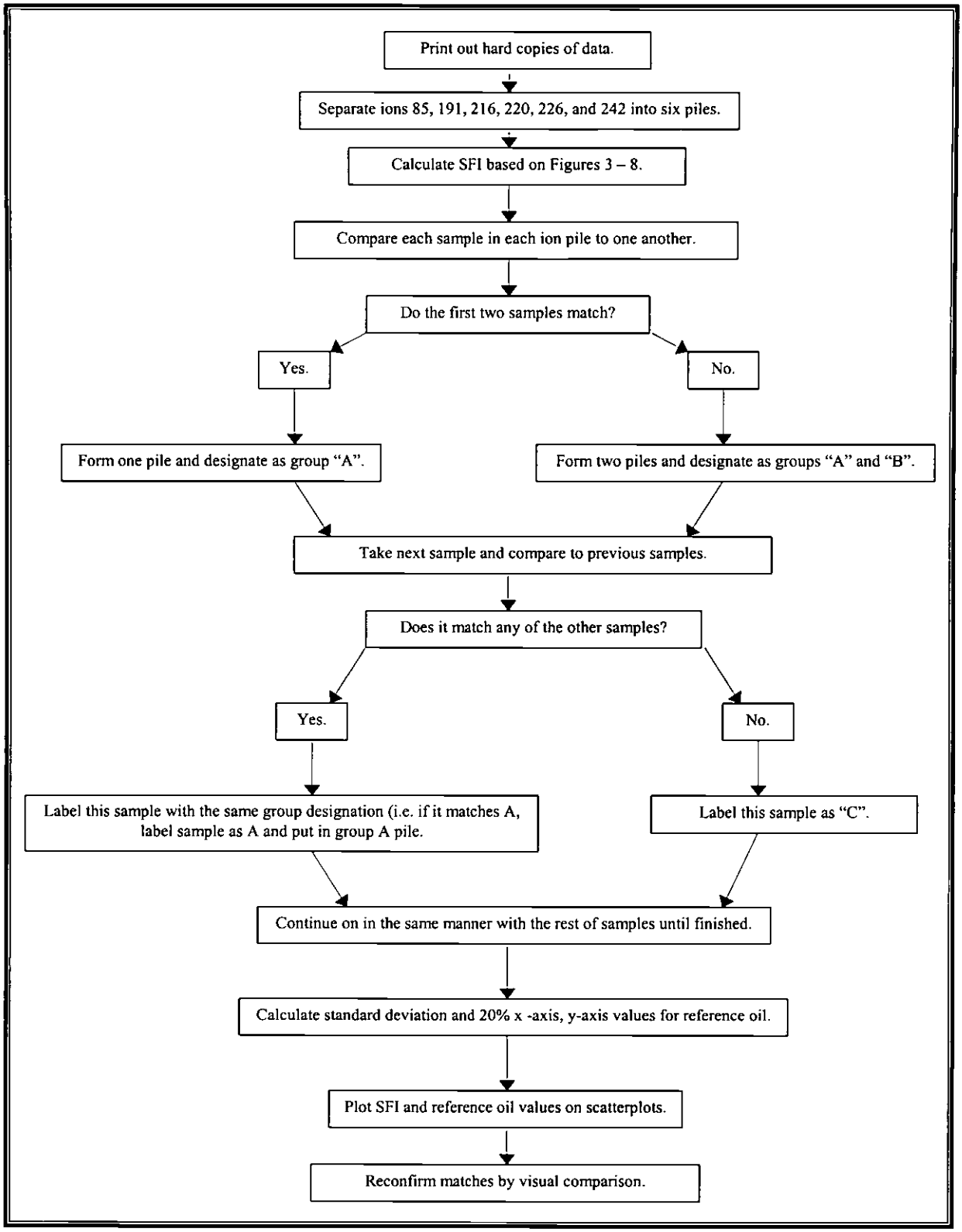


Figure 30. Suggested method for match/nonmatch determinations of unknown environmental oil samples to a reference oil based on visual comparison and SFI data.

The uptake of biomarkers and, in general PAHs, was examined in bivalves exposed to oil from the T/V *Exxon Valdez*. Theoretically, biomarkers are not significantly altered by microbial degradation, and since biomarkers are lipid soluble materials, they will accumulate in marine organisms and could be used as an indicator of possible exposure during oil spills. Selected marine bivalves were collected during the 1999 NOAA Shoreline Monitoring Program of Prince William Sound, Alaska and analyzed for PAH content and biomarker concentrations. The analysis provided important information about the uptake and utilization of petroleum hydrocarbons by filter feeding organisms after a duration of time (10 years) that would be difficult to perform in a laboratory based experiment. Overall, the biomarker and PAH concentrations for both mussel and clam tissues were low, and have been relatively low the past few years. Unfortunately, a baseline concentration of biomarker compounds has not been established for biological samples from the beginning of the T/V *Exxon Valdez* incident.

When spilled oil is weathered to the state of a tarball, the oil is generally considered less acutely toxic than the fresh crude oil or the refined petroleum product from which it was derived. This is due to the loss of the more water soluble mono- and di-aromatic hydrocarbons, such as the benzenes and naphthalenes, by evaporation and dissolution processes. However, tarballs are important environmental samples to analyze due to the fact that oil remaining in the tarball is enriched with the possible carcinogenic and chronically toxic 3,4, and 5 ring aromatic hydrocarbons such as chrysene and benzo(a)pyrene.

The classification and identification of tarball samples collected in 1998 suggests that the majority of the tarballs collected were from mid- to heavy-range crude oil sources. Of the 70 tarballs extracted and analyzed, four matches were found through visual comparison and double SFI scatterplots. The double SFI scatterplots were beneficial as a preliminary identification step of an unknown sample to a source oil. However, the various stages of weathering and abnormal distribution of several non-related sources limited the scatterplots ability to discriminate, within defensible limits, a match or nonmatch to a source. Since the distribution of sources was extensive, it is believed that the tarballs collected represent oil contamination from small petroleum and/or bilge waste discharge.

In conclusion, the analysis of biomarker compounds can be further advanced through the use of tandem mass spectrometry. Triple-stage quadrupole (TSQ) mass spectrometers are currently available and several researchers have been developing new GC-MS/MS techniques for the analysis of complex mixtures, such as oil and biomarker compounds above the nC-40 region (Philip and Oung 1988).

The water degradation experiment could be further advanced with a longer-term oil weathering study. The duration of the study would be anywhere from one to two months. Several oils could be tested in several types of natural waters and extracted and sub-sampled for microbial analysis at select intervals throughout the experiment (i.e. days 0, 7, 14, 28, etc). Instrumental analysis would be by GC/MS or GC-MS/MS and data analysis would include calculation of SFI. This would provide fingerprints of the various stages of weathering for each of the oils tested, which in turn, would be a valuable tool for oil spill responders. A more detailed study of the microbial affects on oil could coincide with the longer-term weathering study and characterize the fluctuation of dominant species in the microbial population throughout the

study. This would include Biolog™ characterization of the microbial communities present in the waters and their ability to utilize a variety of carbon sources. The dominant communities identified from the Biolog™ analyses, using its carbon and nutrient sources, could then be compared to the dominant species identified from the sub-samples taken throughout the water degradation study. This analysis would clarify the differing results between the carbon source utilization from the Biolog™ analyses and the GC/MS % reduction of petroleum hydrocarbons.

The important bioaccumulation factors that need further exploring are how bivalves metabolize petroleum hydrocarbons throughout their life cycle. Generally, biomarker compounds are resistant to biodegradation, which implies bacterial degradation and not biological transformation by the indigenous enzymes in the marine bivalves. A laboratory- simulated oil spill that would expose populations of mussels and clams to an oil would help establish concentrations of the biomarker compounds throughout one to several life cycles of the animals. Both F1, biogenic fractions, and F2, PAH fractions, of the extracted tissues would be analyzed by GC/MS or GC-MS/MS. Results from an experiment such as this would aid environmental scientists and oil spill responders in determining recent and conceivably previous oil exposure to biological specimens.

Developing more quantitative schemes for classifying and identifying tarballs would be difficult due to the varying stages of weathering encountered throughout sampled populations. Double SFI scatterplots and principle component analysis is one way of obtaining quantitative schemes; however, they are most effective when there is only one source in question with several unknown samples. Further experimentation, such as the recommended water degradation study, could enhance the ability of scientists to develop more quantitative schemes for classifying and identifying tarballs. At this point, classification of tarballs into different oil compositions based on qualitative methods (i.e. morphological descriptions) and visual scrutinization of biomarker fingerprints remain the best tools for oil spill responders and environmental scientists.

LITERATURE CITED

Ahsan, A. and D.A. Karlsen. 1997. Petroleum biodegradation in the tertiary reservoirs of the North Sea. *Marine and Petroleum Geology*. 14(1):55-64.

Bochner, B.R. 1989. Sleuthing out bacterial identities. *Nature*. 339;157-158.

Boehm, P.D., D.L. Fiest, and A. Elskus. 1981. Comparative weathering patterns of hydrocarbons from the *Amoco Cadiz* oil spill observed at a variety of coastal environments. In *Amoco Cadiz*, Fates and Effects of the Oil Spill Proceedings of the 1981 International Symposium, Breat (France). pp. 159-173.

Butler, J.N., B.F. Morris, and J. Sass. 1973. Pelagic tar from Bermuda and the Sargasso Sea. Bermuda Biological Station Special Publ. No. 10.

Garland, J.L. and A.L. Mills. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology*. 57:2351-2359.

Haack, S.K., H. Garchow, M.J. Klug, and L.J. Forney. 1995. Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. *Applied and Environmental Microbiology*. 61:1458-1468.

Henry, C.B, P.O. Roberts, and E.B. Overton. 1993. Characterization of Chronic Sources and Impacts of Tar Along the Louisiana Coast. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Regional Office, New Orleans, LA. OCS Study MMS 93-0046. 64 pp.

Henry, C.B. 1995. Advancement of Forensic Petroleum Fingerprinting and Application of GC/MS to Investigate Sources of Oil Pollution in the Marine Environment. MS thesis, Louisiana State University.

Overton, E.B, J. McFall, S.W. Mascarella, C.F. Steele, S.A. Antoine, I.R. Politzer, and J.L. Laseter. 1981. Identification of petroleum residue sources after a fire and oil spill. In Proceedings of the 1981 Oil Spill Conference. pp. 541-546.

Philip, R.P. and J.N. Oung. 1988. Biomarkers: Occurrence, Utility, and Detection. *Analytical Chemistry*. 60(15):887A-896A.

Sauer, T.C., J.S. Brown, P.D. Boehm, D.V. Aurand, J. Michel, and M.O. Hayes. 1993. Hydrocarbon source identification and weathering characterization of intertidal and subtidal sediments along the Saudi Arabian coast after the Gulf War oil spill. *Marine Pollution Bulletin*. 27:117-134.

Sauer, T. and P. Boehm. 1991. The use of defensible analytical chemical measurements for oil spill natural resource damage assessment. In Proceedings of the 1991 Oil Spill Conference. pp. 363-369.

Smalla, K., U. Wachtendorf, H. Heuer, W-T Liu, and L. Forney. 1998. Analysis of BIOLOG GN Substrate Utilization Patterns by Microbial Communities. *Applied and Environmental Microbiology*. 64: 1220-1225.

Wang, Z. and M. Fingas. 1995a. Chemical analysis methods for crude oil. In Proceedings of the 1995 Oil Spill Conference. pp. 1004-1006.

Wang, Z. and M. Fingas. 1995b. Using biomarker compounds to track the source of spilled oil and to monitor the oil weathering process. *LC/GC*. 13(2):951-958.

Whittaker, M. and S.J.T. Pollard. 1997. A performance assessment of source correlation and weathering indices for petroleum hydrocarbons in the environment. *Environmental Toxicology and Chemistry*. 16(6):1149-1158.

Wunsche, L., L. Bruggemann, and W. Babel. 1995. Determination of substrate utilization patterns of soil microbial communities: An approach to assess population changes after hydrocarbon pollution. *FEMS Microbiology Ecology*. 17:295-306.

APPENDIX

Biomarkers of Petroleum Products in the Marine Environment: A Bibliography.

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Abdullah, A. R., W. C. Woon, et al. (1996). "Distribution of Oil and Grease and Petrolem Hydrocarbons in the Straits of Johor, Peninsular Malaysia." Bulletin of Environmental Contamination and Toxicology **57**: 155-162.

Aboul-Kassim, T. A. T. and B. R. T. Simoneit (1995). "Petroleum Hydrocarbon Fingerprinting and Sediment Transport Assessed by Molecular Biomarker and Multivariate Statistical Analyses in the Eastern Harbour of Alexandria, Egypt." Marine Pollution Bulletin **30**(1): 63-73.

Aboul-Kassim, T. A. T. and B. R. T. Simoneit (1996). "Lipid Geochemistry of Surficial Sediments from the Coastal Environment of Egypt I. Aliphatic Hydrocarbons - Characterization and Sources." Marine Chemistry **54**: 135-158.

Ahsan, A., D. A. Karisen, et al. (1997). "Petroleum Biodegradation in the Tertiary Reservoirs of the North Sea." Marine and Petroleum Geology **14**(1): 55-64.

Alajbeg, A., V. Britvic, et al. (1990). "Geochemical Study of the Oils and Source Rocks in the Pannonian Basin (Yugoslavia)." Organic Geochemistry **16**(1-3): 339-352.

Albaiges, J. and P. Albrecht (1979). "Fingerprinting Marine Pollutant Hydrocarbons by Computerized Gas Chromatography-Mass Spectrometry." International Journal of Environmental Analytical Chemistry **6**: 171-190.

Alexander, R., R. I. Kagi, et al. (1994). "The Effect of Maturity on the Relative Abundances of Cadalene and Isocadalene in Sediments from the Gippsland Basin, Australia." Organic Geochemistry **21**(2): 115-120.

Al-Hadhrami, M. N., H. M. Lappin-Scott, et al. (1996). "Effects of the Addition of Organic Carbon Sources on Bacterial Respiration and n-Alkane Biodegradation of Omani Crude Oil." Marine Pollution Bulletin **32**(4): 351-357.

Ali, L. N., R. Fauzi, et al. (1995). "The Dissolution and Photodegradation of Kuwaiti Crude Oil in Seawater. Part 1: Quantitative Dissolution and Analysis of the Seawater-Soluble Fraction." Marine Envirionmental Research **40**(1): 1-17.

Ali, L. N., R. F. Mantoura, et al. (1995). "The Dissolution and Photodegradation of Kuwaiti Crude Oil in Seawater. Part 2: A Laboratory Photodegradation Apparatus and Photodegradation Kinetics of a Model Seawater Soluble Hydrocarbon (Phenanthrene)." Marine Environmental Research **40(4)**: 319-335.

Andersen, B. (1990). "Fingerprinting Petroleum Problems." Energy and Technology(July-August): 90.

Anderson, B. (1994). Elimination of Chemicals Urged. The Advocate. Baton Rouge, LA: 2B.

Anderson, C. B. and B. D. Starer (1990). "Contingency Planning Crucial to Oil-Spill Response." Oil and Gas Journal **OGJ Special**(March): 41-42,44,46.

Armanios, C., R. Alexander, et al. (1994). "Fractionation of Sedimentary Higher-Plant Derived Pentacyclic Triterpanes Using Molecular Sieves." Organic Geochemistry **21**(5): 531-543.

Armstrong, D. W., Y. Tang, et al. (1991). "Resolution of Enantiometric Hydrocarbon Biomarkers of Geochemical Importance." Analytical Chemistry **63**: 2858-2861.

Atlas, R. M., P. D. Boehm, et al. (1981). "Chemical and Biological Weathering of Oil, from the Amoco Cadiz Spillage, within the Littoral Zone." Estuarine, Coastal and Shelf Science **12**: 589-608.

Baker, J. M. (1970). The Effects of a Single Oil Spillage. Ecological Effects of Oil Pollution on Littoral Communities. E. B. Cowell: 16-20.

Baker, J. M. (1971). Successive Spillages. Ecological Effects of Oil Pollution on Littoral Communities. E. B. Cowell: 21-32.

Baker, J. M., L. M. Guzman, et al. (1993). Long-Term Fate and Effects of Untreated Thick Oil Deposits on Salt Marshes. 1993 Oil Spill Conference.

Beiger, T., J. Hellou, et al. (1996). "Petroleum Biomarkers as Traces of Lubricating Oil Contamination." Marine Pollution Bulletin **32**(3): 270-274.

Benner, B. A., Jr., N. P. Bryner, et al. (1990). "Polycyclic Aromatic Hydrocarbon Emissions from the Combustion of Crude Oil on Water." Environmental Science & Technology **24**(9): 1418-1427.

Bernard, D., H. Pascaline, et al. (1996). "Distribution and Origin of Hydrocarbons in Sediments from Lagoons with Fringing Mangrove Communities." Marine Pollution Bulletin **32**(10): 734-739.

Blumer, M. and S. Jeremy (1972). "Oil Pollution: Persistence and Degradation of Spilled Fuel Oil." Science **176**: 1120-1122.

Boehm, P. D. (1989). **Exxon Valdez Chemistry Program**: Recommended Procedure for the Chemical Analysis of Oil, Mousse, and Tarball Samples and the Use of Data in Determinations of Source and Weathering Extent.

Boehm, P. D., H. J. Costa, et al. (1994). Assessment of the Changes in Composition and Concentration of Spilled San Joaquin Valley Crude Oil and Bioavailability of Spilled Oil Residues in Suisan Bay Sediments.

Boehm, P. D., G. S. Douglas, et al. (1995). Advanced Chemical Fingerprinting for Oil Spill Identifications and Natural Resource Damage Assessments. 1995 International Oil Spill Conference, Long Beach, CA.

Boehm, P. D. and J. W. Farrington (1984). "Aspects of the Polycyclic Aromatic Hydrocarbon Geochemistry of Recent Sediments in the Georges Bank Region." Environmental Science & Technology **18**(11): 840-845.

Boehm, P. D., D. L. Fiest, et al. (1981). Comparative Weathering Patterns of Hydrocarbons From the *Amoco Cadiz* Oil Spill Observed at a Variety of Coastal Environments. International Bolume on the *Amoco Cadiz* : Fate and Effects of the Oil Spill, Brest, France, Brest, France.

Boehm, P. D., J. C. Foster, et al. Monitoring Chemical Fate of Spilled Oil: 19-107.

Boehm, P. D., P. J. Mankiewicz, et al. (1996). "Characterization of Mussel Beds With Residual Oil and the Risk to Foraging Wildlife 4 Years After the *Exxon Valdez* Oil Spill." Environmental Toxicology and Chemistry **15**(8): 1289-1303.

Boehm, P. D. and J. G. Quinn (1976). "The Effect of Dissolved Organic Matter in Sea Water on the Uptake of Mixed Individual Hydrocarbons and Number 2 Fuel Oil by a Marine Filter-feeding Bivalve (*Mercenaria mercenaria*)." Estuarine and Coastal Marine Science **4**: 93-105.

Bogan, B. W. and R. T. Lemar (1996). "Polycyclic Aromatic Hydrocarbon-Degrading Capabilities of *Phanerochaete laevis* HHB-1625 and Its Extracellular Ligninolytic Enzymes." Applied and Environmental Microbiology **62**(5): 1597-1603.

Boon, J. J., F. W. Meer, et al. "Organic Geochemical Analysis of Core Samples from Site 362, Walvis Ridge, DSPS Leg 40.": 627-637.

Borzelli, G., A. Ciappa, et al. (1996). "A New Perspective on Oil Slick from Space by NOAA Satellites." International Journal of Remote Sensing **17**(7): 1279-1292.

Brakstad, F. and O. Grahl-Nelson (1988). "Identification of Weathered Oils." Marine Pollution Bulletin **19**(7): 319-324.

Broman, D., A. Colmsjo, et al. (1987). "'Fingerprinting' Petroleum Hydrocarbons in Bottom Sediment, Plankton, and Sediment Trap Collected Seston." Marine Pollution Bulletin **18**(7): 380-388.

Brown, J. S. and P. D. Boehm The Use of Double Ratio Plots of Polynuclear Aromatic Hydrocarbon (PAH) Alkyl Homologues for Petroleum Source Identifiction.

Bruheim, P., H. Bredholt, et al. (1997). "Bacterial Degradation of Emulsified Crude Oil and the Effect of Various Surfactants." Canadian Journal of Microbiology **43**: 17-22.

Burns, W. A., P. J. Mankiewicz, et al. (1997). "A Principal-Component and Least-Squares Method for Allocating Polycyclic Aromatic Hydrocarbons in Sediment to Multiple Sources." Environmental Toxicology and Chemistry **16**(6): 1119-1131.

Chosson, P., C. Lanau, et al. (1991). "Biodegradation of Refractory Hydrocarbon Biomarkers from Petroleum Under Laboratory Conditions." Nature **351**(June 20): 640-642.

Colombo, J. C., E. Pelletier, et al. (1989). "Determination of Hydrocarbon Sources Using *n*-Alkane and Polyaromatic Hydrocarbon Distribution Indexes. Case Study: Rio de La Plata Estuary, Argentina." Environmental Science & Technology **23**(7): 888-894.

Conde, J. E., E. Pena, et al. (1996). "Sources of Tar Balls and Oil Slicks on the Coasts of the Canary Islands." International Journal of Environmental and Analytical Chemistry **62**: 77-84.

Connan, J. (1984). Biodegradation of Crude Oils in Reservoirs. Advances in Petroleum Geochemistry. J. Brooks and D. Welte. London, Academic Press (Harcourt Brace Jovanovich). **1:** 299-335.

Curiale, J. A. and B. W. Bromley (1996). "Migration Induced Compositional Changes in Oils and Condensates of a Single Field." Organic Geochemistry **24**(12): 1097-1113.

Curl, H. J. and K. O'Donnell (1977). Chemical and Physical Properties of Refined Petroleum Products, United States Department of Commerce.

Dahl, D. (1993). A New Weapon in Environmental Cases "Chemical Fingerprinting" Traces an Oil Spill's Origins. Lawyers Weekly USA: B3.

Dahl, J., J. M. Moldowan, et al. (1992). "A New Class of Natural Products Revealed by 3B-Alkyl Steranes in Petroleum." Nature **355**(January): 154-157.

D'Elia, C. F., J. G. Sanders, et al. (1989). "Analytical Chemistry for Environmental Sciences." Environmental Science and Technology **23**(7): 768-774.

Dingchuang, Q., S. Jiyang, et al. (1996). "Novel Extended Side-Chain-Unsaturated Released from the Kerogen Macromolecules under Artificial Conditions." Organic Geochemistry **24**(89): 815-823.

Disnar, J. R. and M. Harouna (1994). "Biological Origin of Tetracyclic Diterpanes, n-alkanes and Other Biomarkers Found in Lower Carboniferous Gondwana Coals (Niger)." Organic Geochemistry **21**(2): 143-152.

Dixon, I. (1996). "Photoacoustic Monitoring of Oil Pollutants." Marine Pollution Bulletin **32**(6): 454-455.

Douglas, G. S., A. E. Bence, et al. (1996). "Environmental Stability of Selected Petroleum Hydorcarbon source and Weathering Ratios." Environmental Science and Technology **30**: 2332-2339.

Douglas, G. S., R. C. Prince, et al. (1994). The Use of Internal Chemical Indicators in Petroleum and Refined Products to Evaluate the Extent of Biodegradation. Hydrocarbon Bioremediation. R. E. Hinchee, B. C. Alleman, R. E. Hoppel and R. N. Miller. Ann Arbor, MI, Lewis Publishers: 219-235.

Douglas, G. S. and A. D. Uhler (1993). "Optimizing EPA methods for Petroleum-Contaminated Site Assessments." Environmental Testing and Analysis(May/June): 1-6.

Draper, W. M., J. S. Dhaliwal, et al. (1996). "Determination of Diesel Fuel and Motor Oil in Water and Wastes by a Modified Diesel-Range Organics Total Petroleum Hydrocarbon Method." Journal of AOAC International **79**(2): 509-519, 629-632.

Ehrhardt, M. G. and K. A. Burns (1990). "Petroleum-Derived Dissolved Organic Compounds Concentrated from Inshore Waters in Bermuda." Journal of Experimental Marine Biology and Ecology **138**: 35-47.

Ellis, L., R. K. Singh, et al. (1996). "Formation of Isohexyl Alkylaromatic Hydrocarbons from Aromatization-Rearrangement of Terpenoids in the Sedimentary Enviornment: A New Class of Biomarker." Geochimica et Cosmochimica Acta **60**(23): 4747-4763.

Essaid, H. I., B. A. Bekins, et al. (1995). "Simulation of Aerobic and Anaerobic Biodegradation Processes at a Crude Oil Spill Site." Water Resources Research **31**(12): 3309-3327.

Farrington, J. (1992). "Biomarker and Molecular Paleontology Working Group Report." Marine Chemistry **39**: 51-65.

Fayad, N. M. and E. Overton (1995). "A Unique Biodegradation Pattern of the Oil Spilled During the 1991 Gulf War." Marine Pollution Bulletin **30**(4): 239-246.

Fazeelat, T., R. Alexander, et al. (1994). "Extended 8,14-Secohopanes in Some Seep Oils From Pakistan." Organic Geochemistry **21**(3/4): 257-264.

Fingas, M. (1995). The Evaporation Oil Spills. Proceedings of the Eighteenth Artic and Marine Oilspill Program (AMOP) Technical Seminar, Edmonton, Alberta, Canada, Environment Canada.

Fingas, M. (1996). The Evaporation of Oil Spills: Variation with Temperature and Correlation with Distillation Data. Proceedings of the Nineteenth Artic and Marine Oilspill Program (AMOP) Technical Seminar, Calgary, Alberta, Canada, Environment Canada.

Frank, U., D. Stainken, et al. (1979). Methods for the Source Identification and Quantification of Oil Pollution. 1979 Oil Spill Conference.

Gerlach, C. L. (1996). "New Instrument Brings PAH Analysis to the Field." Environmental Science and Technology (Featured Article) **30**(6): 252A-254A.

Glegg, G. A. and S. J. Rowland (1996). "The *Braer* Oil Spill - Hydrocarbon Concentrations in Intertidal Organisms." Marine Pollution Bulletin **32**(6): 486-492.

Goodwin, N. S., P. J. D. Park, et al. (1981). "Crude Oil Biodegradation under Simulated and Natural Conditions." Advances in Organic Chemistry: 650-658.

Grantham, P. J. and L. L. Wakefield (1988). "Variations in the Sterane Carbon Number Distributions of Marine Source Rock Derived Crude Oils through Geological Time." Organic Geochemistry **12**(1): 61-73.

Grigson, S. and G. Baron (1993). The European Approach to the Source Identification of Oil Spills: A Study of its Specificity and Reliability. 1993 International Oil Spill Conference, Tampa, FL, American Petroleum Institute.

Harkey, G. A., P. L. V. Hoof, et al. (1995). "Bioavailability of Polycyclic Aromatic Hydrocarbons from a Historically Contaminated Sediment Core." Environmental Toxicology and Chemistry **14**(9): 1551-1560.

Hartley, J. P. (1996). "Environmental Monitoring of Offshore Oil and Gas Drilling Discharges-A Caution on the Use of Barium as a Tracer." Marine Pollution Bulletin **32**(10): 727-733.

Hayes, M. O., J. Michel, et al. (1993). "Distribution of Oil From the Gulf War Spill Within Intertidal Habitats-One Year Later." Oil Spill Conference: 373-381.

Hellebust, J. A., B. Hanna, et al. (1975). Experimental Crude Oil Spills on a Small Subarctic Lake in the Mackenzie Valley, N.W.T.: Effects on Phytoplankton, Periphyton, and Attached Aquatic Vegetation. Proceedings of the 1975 Oil Spill Conference (Prevention, Behavior, Control, Cleanup).

Henry, C. B., Jr. and E. B. Overton (1993). Chemical Composition and Source Fingerprinting of Depositonal Oil from the Kuwait Oil Fires. 1993 International Oil Spill Conference, Tampa, FL, American Petroleum Institute.

Henry, C. B., Jr. and E. B. Overton (1993). Source-Fingerprinting and Compound Specific Quantitative Analysis of Oil Contaminated Soils and Sediments, Institute for Environmental Studies Louisiana State University.

Henry, C. B., Jr., E. B. Overton, et al. (1990). Standard Method: Source-Fingerprint Analysis of Moderate to Heavily Contaminated Samples Associated with the Exxon Valdez.

Henry, C. B., P. O. Robert, et al. (1993). Characterization of Chronic Sources and Impacts of Tar along the Louisiana Coast, Institute for Environmental Studies, Louisiana State University.

Horstad, I., S. R. Larter, et al. (1989). "Degradation and Maturity Controls on Oil Field Petroleum Column Heterogeneity in the Gullfaks Field, Norwegian North Sea." Advances in Organic Geochemistry **16**(1-3): 497-510.

Hostettler, F. D. and K. A. Kvenvolden (1994). "Geochemical Changes in Crude Oil Spilled from the Exxon Valdez Supertanker into Prince William Sound, Alaska." Organic Geochemistry **21**(8/9): 927-936.

Hostettler, F. D., J. B. Rapp, et al. (1992). "Use of Geochemical Biomarkers in Bottom Sediment to Track Oil from a Spill, San Francisco Bay, California." Marine Pollution Bulletin **24**(1): 15-20.

Hund, K. and W. Traunspurger (1994). "Ecotox-Evaluation Strategy for Soil Bioremediation Exemplified for a PAH-Contaminated Site." Chemosphere **29**(2): 371-390.

Hwang, R. J., A. S. Ahmed, et al. (1994). "Oil Composition Variation and Reservoir Continuity: Unity Field, Sudan." Organic Geochemistry **21**(2): 171-188.

Jacob, J. (1996). "The Significance of Polycyclic Aromatic Hydrocarbons as Environmental Carcinogens." Pure and Applied Chemistry **68**(2): 301-308.

Jacquot, F., M. Guiliano, et al. (1996). "*In Vitro* Photooxidation of Crude Oil Maltenic Fractions: Evolution of Fossil Biomarkers and Polycyclic Aromatic Hydrocarbons." Chemosphere **33**(4): 671-681.

Kanga, S. A., J. S. Bonner, et al. (1997). "Solubilization of Naphthalene and Methyl-Substituted Naphthalenes from Crude Oil Using Biosurfantants." Environmental Science and Technology **31**: 556-561.

Kayal, S. and D. W. Connell (1995). "Polycyclic Aromatic Hydrocarbons in Biota from the Brisbane River Estuary, Australia." Estuarine, Coastal, and Shelf Science **40**(5): 475-493.

Kennicutt, M. C., II (1988). "The Effect of Biodegradation on Crude Oil Bulk and Molecular Composition." Oil & Chemical Pollution **4**: 89-112.

Khalili, N. R., P. A. Scheff, et al. (1995). "PAH Source Fingerprints for Coke Ovens, Diesel and Gasoline Engines, Highway Tunnels, and Wood Combustion Emissions." Atmospheric Environment **29**(4): 533-542.

Killops, S. D., M. S. Massoud, et al. (1991). "Biomarker Characterisation of an Oil and its Possible Source Rock from Offshore Korea Bay Basin." Applied Geochemistry **6**: 143-157.

Kornacki, A. S., J. W. Kendrick, et al. (1994). "Impact of Oil and Gas Vents and Slicks on Petroleum Exploration in the Deepwater Gulf of Mexico." Geo-Marine Letters **14**(2/3): 160-169.

Kropp, K. G., J. A. Goncalves, et al. (1994). "Bacterial Transformations of Benzothiophene and Methylbenzothiophenes." Environmental Science and Technology **28**: 1348-1358.

Kucklick, J. R., S. K. Siversten, et al. (1997). "Factors Influencing Polycyclic Aromatic Hydrocarbon Distributions in South Carolina Estuarine Sediments." Journal of Experimental Marine Biology and Ecology **213**: 13-29.

Kvenvolden, K. A., P. R. Carlson, et al. (1993). "Possible Connection Between Two Alaskan Catastrophes Occuring 25 yr Apart (1964 and 1989)." Geology **21**: 813-816.

Kvenvolden, K. A., F. D. Hostettler, et al. (1993). "Hydrocarbons in Oil Residues on Beaches of Islands of prince William Sound, Alaska." Marine Pollution Bulletin **26**(1): 24-29.

Lavine, B. K., H. Mayfield, et al. (1995). "Source Identification of Underground Fuel Spills by Pattern Recognition Analysis of High-Speed Gas Chromatograms." Analytical Chemistry **67**: 3846-3852.

Law, R. J. and J. L. Biscaya (1994). "Polycyclic Aromatic Hydrocarbons (PAH)-Problems and Progress in Sampling, Analysis and Interpretation." Marine Pollution Bulletin **29**(4-5): 235-241.

Li, M., S. R. Larter, et al. (1995). "Biomarkers or Not Biomarkers? A New Hypothesis for the Origin of Pristane Involving Derivation from Methyltrimethyltridecylchomans (MTTCs) Formed during Diagenesis from Chlorophyll and Alkylphenols." Organic Geochemistry **23**(2): 159-167.

Licht, D., B. K. Ahring, et al. (1996). "Effects of Electron Acceptors, Reducing Agents, and Toxic Metabolites on Anaerobic Degradation of Heterocyclic Compounds." Biodegradation **7**: 83-90.

Mackenzie, A. S. (1984). Applications of Biological Markers in Petroleum Geochemistry. Advances in Petroleum Geochemistry. J. Brooks and D. Welte. London, Academic Press (Harcourt Brace Jovanovich). **1:** 115-214.

Marshall, K. C. (1980). "Microorganisms and Interfaces." BioScience **30**(4): 246-249.

Martin, G. (1991). "Marked for Life." Discover: 28.

McCaffrey, M. A., B. R. T. Simoneit, et al. (1994). "Functionalized Biological Precursors of Tricyclic Terpanes: Information from Sulfur-bound Biomarkers in a Permian Tasmanite." Organic Geochemistry **21**(5): 481-487.

McGee, B. L., C. E. Schlekat, et al. (1995). "Sediment Contamination and Biological Effects in a Chesapeake Bay Marina." Ecotoxicolgy **4**: 39-59.

Mello, M. R., P. C. Gaglianone, et al. (1988). "Geochemical and Biological Marker Assessment of Depositional Environments using Brazilian Offshore Oils." Marine and Petroleum Geology **5**(August): 205-223.

Morel, G. (1996). "Method Development and Quality Assurance for the Analysis of Hydrocarbons in Environmental Samples." International Journal of Environmental Analytical Chemistry **63**: 269-288.

Neff, J. M. and W. A. Burns (1996). "Estimation of Polycyclic Aromatic Hydrocarbon Concentrations in the Water Column Based on Tissue Residues in Mussels and Salmon: An Equilibrium Partitioning Approach." Environmental Toxicology and Chemistry **15**(12): 2240-2253.

Nichols, P. D., D. S. Nichols, et al. (1993). "Recent Developments with Marine Oil Products in Australia." Chemistry in Australia(July): 336-340.

Overton, E. B., J. McFalls, et al. (1981). Identification of Petroleum Residue Sources After A Fire And Oil Spill. 1981 Oil Spill Conference, Atlanta, GA.

Owens, E. H. (1984). "Variablility in Estimates of Oil Contamination in the Intertidal Zone of a Gravel Beach." Marine Pollution Bulletin **15**(11): 412-416.

Page, D. S., P. D. Boehm, et al. (1993). "Identification of Hydrocarbon Sources in the Benthic Sediments of Prince William Sound and the Gulf of Alaska Following the *Exxon Valdez* Oil Spill." Aquatic, Plant and Terrestrial(April 26-28): 1-42.

Page, D. S., P. D. Boehm, et al. (1996). "The Natural Petroleum Hydrocarbon Background in Subtidal Sediments of Prince William Sound, Alaska, USA." Environmental Toxicology and Chemistry **15**(8): 1266-1281.

Parsons, K. C. (1996). "Recovering from Oil Spills: The Role of Proactive Science in Mitigating Adverse Effects." Colonial Waterbirds **19**(1): 149-153.

Peters, K. E. and J. M. Moldowan (1991). "Effects of Source, Thermal Maturity, and Biodegradation on the Distribution and Isomerization of Homohopanes in Petroleum." Organic Geochemistry **17**(1): 47-61.

Peters, K. E., G. L. Scheuerman, et al. (1992). "Effects of Refinery Processes on Biological Markers." Energy & Fuels **6**: 560-577.

Philip, R. P., J. N. Oung, et al. (1990). "The Determination of Biomarker Distributions by Tandem Mass Spectrometry." Advances in Organic Geochemistry (Organic Geochemistry) **16**(4-6): 1211-1220.

Philp, R. P. and J.-N. Oung (1988). "Biomarkers." Anaytical Chemistry **60**(15): 887A-896A.

Pieri, N., F. Jaquot, et al. (1996). "GC-MS Identification of Biomarkers in Road Asphalts and in Their Parent Crude Oils. Relationships Between Crude Oil Maturity and Asphalt Reactivity Towards Weathering." Organic Geochemistry **25**(1/2): 51-68.

Porter, P. S., R. C. Ward, et al. (1988). "The Detection Limit." Environmental Science & Technology **22**(8): 856-861.

Prahl, F. G., L. A. Pinto, et al. (1996). "Phytane from Chemolytic Analysis of Modern Marine Sediments: A Product of Desulfurization or Not?" Geochimica et Cosmochimica Acta **60**(6): 1065-1073.

Pu, F., R. P. Philp, et al. (1990). "Geochemical Characteristics of Aromatic Hydrocarbons of Crude Oils and Source Rocks from Different Sedimentary Environments." Organic Geochemistry **16**(1-3): 427-435.

Quinn, M. F., K. Marron, et al. (1990). "Modelling of the Ageing of Crude Oils." Oil and Chemical Pollution **7**: 119-128.

Radke, M., P. Garrigues, et al. (1990). "Methylated Dicyclic and Tricyclic Aromatic Hydrocarbons in Crude Oils from the Handil Field, Indonesia." Organic Geochemistry **15**(1): 17-34.

Readman, J. W., J. Bartocci, et al. (1996). "Recovery of the Coastal Marine Environment in the Gulf Following the 1991 War-Related Oil Spills." Marine Pollution Bulletin **32**(6): 493-498.

Readman, J. W., S. W. Fowler, et al. (1992). "Oil and Combustion-Product Contamination of the Gulf Marine Environment following the War." Nature **358**(August): 662-665.

Readman, J. W., I. Tolosa, et al. (1996). "Discrete Bands of Petroleum Hydrocarbons and Molecular Organic Markers Identified Within Massive Coral Skeletons." Marine Pollution Bulletin **32**(5): 437-443.

Reed, W. E. (1977). "Molecular Compositions of Weathered Petroleum and Comparison with its Possible Source." **41**: 237-247.

Reilley, K. A., M. K. Banks, et al. (1996). "Organic Chemicals in the Enviornment." Journal of Environmental Quality **25**(2): 212-219.

Requejo, A. G., R. Sassen, et al. (1996). "Polynuclear Aromatic Hydrocarbons (PAH) as Indicators of the Source of Maturity of Marine Crude Oils." Organic Geochemistry **24**(10/11): 1017-1033.

Richnow, H. H., R. Seifert, et al. (1995). "Rapid Screening of PAH-Residues in Bioremediated Soils." Chemosphere **31**(8): 3991-3999.

Riediger, C. L., M. G. Fowler, et al. (1990). "Triassic Oils and Potential Mesozoic Source Rocks, Peace River Arch Area, Western Canada Basin." Organic Geochemistry **16**(1-3): 295-305.

Roberts, P. O., C. B. Henry, et al. (1995). Fast Source-Fingerprinting Analysis for Oil Spill Response. 1995 International Oil Spill Conference, Long Beach, CA, American Petroleum Institute.

Roques, D. E., E. B. Overton, et al. (1994). "Using Gas Chromatography/Mass Spectroscopy Fingerprint Analyses to Document Process and Prograss of Oil Degradation." Journal of Environmental Quality **23**(4): 851-855.

Rullkotter, J., C. Cornford, et al. Organic Geochemistry of Sediments Cored During Deep Sea Drilling Project Legs 56 and 57, Japan Trench: Organic Petrography and Extractable Hydrocarbons.

Saliot, A., C. Andrie, et al. (1986). Hydrocarbons in the Mediterranean Sea: Their Occurrence and Fate in the Sediment and in the Water Column, as Dissolved and Associated with Small and Large Size Particulates. Fate of Hydrocarbons in the Environment: An Analytical Approach. J. Albaiges and R. W. Frei. New York, New York, Gordon and Breach Science: 255-276.

Saliot, A., J. Laureillard, et al. (1991). "Evolutionary Trends in the Lipid Biomarker Approach for Investigating the Biogeochemistry of Organic Matter in the Marine Environment." Marine Chemistry **36**: 223-248.

Salmeen, I. T., B. R. Kim, et al. (1995). "Case of Lognormally Distributed TPH in Contaminated Soil." Journal of Environmental Engineering **121**(9): 664-667.

Sauer, T. and P. Boehm (1991). The Use of Defensible Analytical Chemical Measurements for Oil Spill Natural Resource Damage Assessment. 1991 Oil Spill Conference.

Sauer, T. C. and A. D. Uhler (1994). "Pollutant Source Identification and Allocation: Advances in Hydrocarbon Fingerprinting." Remediation(Winter): 25-50.

Saxton, W. L., R. T. Newton, et al. (1993). "Polycyclic Aromatic Hydrocarbons in Seafood from the Gulf of Alaska Following a Major Crude Oil Spill." Bulletin of Environmental Contamination and Toxicology **51**(4): 515-522.

Schoell, M. (1984). Stable Isotopes in Petroleum Research. Advances in Petroleum Geochemistry. J. Brooks and D. Welte. London, Academic Press (Harcourt Brace Jovanovich). **1:** 215-245.

Seifert, W. K. and J. M. Moldowan (1979). "The Effect of Biodegradation on Steranes and Terpanes in Crude Oils." Geochimica et Cosmochimica Acta. **43**: 111-126.

Seifert, W. K., J. M. Moldowan, et al. (1984). "Source Correlation of Biodegraded Oils." Organic Geochemistry **6**: 633-643.

Seifert, W. K. and J. M. Moldwan (1978). "Applications of Steranes, Terpanes and Monoaromatics to the Maturation, Migration and Source of Crude Oils." Geochimica et Cosmochimica Acta **42**: 77-95.

Shen, J. (1984). "Minimization of Interferences from Weathering Effects and Use of Biomarkers in Identification of Spilled Crude Oils by Gas Chromatography/Mass Spectrometry." Analytical Chemistry **56**: 214-217.

Simcik, M. F., S. J. Eisenreich, et al. (1996). "Atmospheric Loading of Polycyclic Aromatic Hydrocarbons to Lake Michigan as Recorded in the Sediments." Environmental Science and Technology **30**: 3039-3046.

Simoneit, B. R. T. "Organic Geochemistry of Albian Sediment from Hess Rise, Deep Sea Drilling Project Hole 466.": 939-942.

Simoneit, B. R. T. (1979). "Organic Geochemistry of the Shales from the Northwestern Proto-Atlantic, DSDP Leg 43.": 643-649.

Simpson, C. D., W. R. Cullen, et al. (1995). "Methodology for the Determination of Priority Pollutant Polycyclic Aromatic Hydrocarbons in Marine Sediments." Chemosphere **31**(9): 4143-4155.

Sosrowidjojo, I. B., A. P. Murray, et al. (1996). "Bicadinanes and Related Compounds as Maturity Indicators for Oils and Sediments." Organic Geochimstry **24**(1): 43-55.

Sporstol, S., N. Gjos, et al. (1983). "Source Identification of Aromatic Hydrocarbons in Sediments Using GC/MS." Environmental Science & Technology **17**(5): 282-286.

Sugai, S. F., J. E. Lindstrom, et al. (1997). "Environmental Influences on the Microbial Degradation of *Exxon Valdez* Oil on the Sorelines of Prince William Sound, Alaska." Environmental Science and Technology **31**: 1564-1572.

Sugiura, K., M. Ishihara, et al. (1997). "Physicochemical Properties and Biodegradability of Crude Oil." Environmental Science and Technology **31**: 45-51.

Swartz, R. C., S. P. Ferraro, et al. (1997). "Photoactivation and Toxicity of Mixtures of Polycyclic Aromatic Hydrocarbon Compounds in Marine Sediment." Environmental Toxicology and Chemistry **16**(10): 2151-2157.

Tanacredl, J. T. and R. R. Cardenas (1991). "Biodepuration of Polynuclear Aromatic Hydrocarbons from a Bivalve Mollusc, *Mercenaria mercenaria L.*" Environmental Science and Tehnology **25**: 1453-1461.

Tegelaar, E. W., R. M. Matthezing, et al. (1989). "Possible Origin of n-alkanes in High-wax Crude Oils." Nature **342**(November): 529-531.

Thompson, K. F. M. and M. C. K. II (1992). "Correlations of Gulf Coast Petroleums on the Basis of Branched Acyclic Alkanes." Organic Geochemistry **18**(1): 103-119.

Traxler, R. W. and L. S. Bhattacharya (1977). Effect of a Chemical Dispersant on Microbial Utilization of Petroleum Hydrocarbons. Chemical Dispersants for the Control of Oil Spills. L. T. McCarthy, G. P. Lindblom and H. F. Walter. Williamsburg, VA, American Society for Testing and Materials: 180-187.

Urdal, K., N. B. Vogt, et al. (1986). "Classification of Weathered Crude Oils Using Multimethod Chemical Analysis, Statistical Methods and SIMCA Pattern Recognition." Marine Pollution Bulletin **17**(8): 366-373.

van Graas, G. W. (1990). "Biomarker Maturity Parameters for High Maturities: Calibration of the Working Range up to the Oil/Condensate Threshold." Organic Geochemistry **16**(4-6): 1025-1032.

Vandermeulen, J. H. and J. G. Singh (1994). "*Arrow* Oil Spill, 1970-90: Persistence of 20-yr Weathered Bunker C Fuel Oil." Canadian Journal of Fisheries and Aquatic Sciences **51**: 845-855.

Venosa, A. D., M. T. Suidan, et al. (1996). "Bioremediation of An Experimental Oil Spill on the Shoreline of Delaware Bay." Environmental Science and Technology **30**: 1764-1775.

Volkman, J. K., R. Alexander, et al. (1983). "Demethylated Hopanes in Crude Oils and Their Applications in Petroleum Geochemistry." Geochimica et Cosmochimica Acta **47**: 785-794.

Volkman, J. K., D. G. Holdsworth, et al. (1992). "Identification of Natural, Anthropogenic and Petroleum Hydrocarbons in Aquatic Sediments." Science of the Total Environment **112**: 203-219.

Wang, Z. (1995). "Use of Methyldibenzothiophenes as Markers for Differentiation and Source Identification of Crude and Weathered Oils." Environmental Science and Technology **29**: 2842-2849.

Wang, Z. and M. Fingas (1995). "Using Biomarker Compounds to Track the Source of Spilled Oil and Monitor the Weathering Process." LC-GC **13**(12): 951-958.

Wang, Z., M. Fingas, et al. Fractionaton of ASMB Oil and Identification and Quantitation of Aliphatic, Aromatic and Biomarker Compounds by GC/FID and GC/MS. Sixteenth Arctic and Marine Oil Spill Program Technical Seminar.

Wang, Z., M. Fingas, et al. (1994). "Fractionation of a Light Crude Oil and Identification and Quantitation of Aliphatic, Aromatic, and Biomarker Compounds by GC-FID and GC-MS, Part II." Journal of Chromatographic Science **32**: 367-382.

Wang, Z., M. Fingas, et al. (1994). "Study of 22-Year-Old *Arrow* Oil Samples Using Biomarker Compounds by GC/MS." Enviornmental Science and Technology **28**: 1733-1746.

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

Wang, Z. and M. F. Fingas (1995). "Chemical Analysis Methods for Crude Oil.": 1004-1006.

Warner, J. S. (1976). "Determination of Aliphatic and Aromatic Hydrocarbons in Marine Organisms." Analytical Chemistry **48**(3): 578-583.

West, N., R. Alexander, et al. (1990). "The Use of Silicate for Rapid Isolation of Branched and Cyclic Alkane Fractions of Petroleum." Organic Geochemistry **15**(5): 499-501.

Whittaker, M. and S. J. T. Pollard (1997). "A Performance Assessment of Source Correlation and Weathering Indices for Petroleum Hydrocarbons in the Environment." Environmental Toxicology and Chemistry **16**(6): 1149-1158.

Widdows, J., C. Nasci, et al. (1997). "Effects of Pollution on the Scope for Growth of Mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy." Marine Environmental Research **43**(1/2): 69-79.

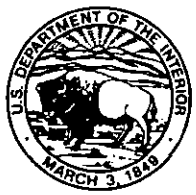
Wolfe, D. A., M. J. Hameedi, et al. (1994). "The Fate of the Oil Spilled from the Exxon Valdez: The Mass Balance Is the Most Complete and Accurate of Any Major Oil Spill." Environmental Science and Technology **28**(13): 561A-568A.

Yawanarajah, S. R. and M. A. Kruge (1994). "Lacustrine Shales and Oil Shales From Stellarton Basin, Nova Scotia, Canada: Organofacies Variations and Use of Polyaromatic Hydrocarbons as Maturity Indicators." Organic Geochemistry **21**(2): 153-170.

Yunker, M. B., R. W. Macdonald, et al. (1995). "Terrestial and Marine Biomarkers in a Seasonally Ice-Covered Arctic Estuary-Integration of Multivariate and Biomarker Approaches." Marine Chemistry **49**: 1-50.

Zeng, E. Y. and C. L. Vista (1997). "Organic Pollutants in the Coastal Environmental Off San Diego, California. 1. Source Identification and Assessment By Compositional Indices of Polycyclic Aromatic Hydrocarbons." Environmental Toxicology and Chemistry **16**(2): 179-188.

Zhou, S., R. G. Ackman, et al. (1996). "Very long-chain Aliphatic Hydrocarbons in Lipids of Mussels (*Mytilus edulis*) Suspended in the Water Column near Petroleum Operations off Sable Island, Nova Scotia, Canada." Marine Biology **126**: 499-507.



The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.