Petroleum in the Marine Environment

Published on May 1, 1980 on http://pubs.acs.org | doi: 10.1021/ba-1980-0185.fw001

Petroleum in the Marine Environment

Leonidas Petrakis, EDITOR
Gulf Research & Development Company

Fred T. Weiss, EDITOR
Shell Development Company

A symposium jointly sponsored by the Divisions of Petroleum and Analytical Chemistry at the 176th Meeting of the American Chemical Society, Miami Beach, Florida, September 13–14, 1978.

ADVANCES IN CHEMISTRY SERIES **185**

AMERICAN CHEMICAL SOCIETY
WASHINGTON, D. C. 1980

Library of Congress CIP Data

Petroleum in the marine environment.
(Advances in chemistry series; 185 ISSN 0065–2393)

Includes bibliographies and index.

1. Oil pollution of the sea—Congresses. 2. Petroleum—Analysis—Congresses.
I. Petrakis, Leonidas, 1935– . II. Weiss, Frederick T., 1916– . III. American Chemical Society. Division of Petroleum Chemistry. IV. Series.

QD1.A355 no. 185 [QH545.05] 540'.8s [574.5'2636]
ISBN 0–8412–0475–6 79–25524
ADCSAJ 185 1-371 1980

PRINTED IN THE UNITED STATES OF AMERICA

Advances in Chemistry Series

M. Joan Comstock, *Series Editor*

FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all. Papers are reviewed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Volumes in the ADVANCES IN CHEMISTRY SERIES maintain the integrity of the symposia on which they are based; however, verbatim reproductions of previously published papers are not accepted. Papers may include reports of research as well as reviews since symposia may embrace both types of presentation.

PREFACE

This volume addresses an important scientific issue at the interface of the dual concern for the quality of the environment and the adequacy of energy and its proper use: namely, the analytical methodology for monitoring the fate and effects of petroleum as it enters the aquatic environments.

It is well accepted that for at least the near future, there will be continued primary reliance on fossil fuels for our energy needs. This continued reliance on fossil fuels as a major energy source entails, among others, the transportation of increasingly greater quantities of petroleum over great distances; the issue of oil possibly entering the oceans from the offshore drilling in the Atlantic and Pacific coasts; possible oil spills, which in the past have received spectacular coverage in the popular press; and petroleum hydrocarbons as they may accumulate in marine fauna and flora. Proper analytical methodology can be effective in evaluating the significance of these issues as well as consideration of the natural entry of hydrocarbons in the aquatic environments (natural seepage and airborne materials).

When the scientific community—chemists, marine ecologists, microbiologists, toxicologists, and many others—turned its attention to the problem of petroleum in the aquatic environments, it became apparent very quickly that, popular expectations not withstanding, the analytical chemistry involved is both critical and complex. One cannot discuss the fate and effects of petroleum by simply monitoring one or even a few compounds. Petroleum is too complex a mixture for such simplistic approaches. Too, the great diversity of conditions under which petroleum might be found in the marine and aquatic environments; the many physical, photochemical, and microbiological changes that it might undergo under these diverse conditions; its highly complex initial composition; the variable propensity for the alteration of its constituent chemical classes; and the minute quantities that one might be dealing with after extraction or separation; all combine to attest to the great complexity of the analytical chemical issues involved.

This volume is a collection of topics that were discussed at a Symposium sponsored by the Petroleum and Analytical Chemistry Divisions of the American Chemical Society at its 176th National Meeting. An attempt has been made to balance the chapters between methodology

and important paradigms, after an initial assessment of the broad issues involved. The authors are a cross section of important contributors from academic, industrial, and governmental laboratories.

It is hoped, of course, that this volume will be timely and useful. It is deliberately addressed to a broad readership including active workers of various disciplines dealing with aspects of this problem; persons contemplating entering the field and who may be in need of a cogent up-to-date review; administrative or legal personnel who may be dealing with questions of appropriate methodology in proposed work or forensic problems; persons interested in a general overview of the subject; and professors and students who may find the volume a good source of supplementary material in appropriate courses.

The editors express their great appreciation to the authors who prepared their manuscripts with diligence and enthusiasm in order to make the publication of this volume timely and useful. Sincere thanks are also due to the Officers of the Petroleum and Analytical Divisions of the American Chemical Society, the ACS Books Department, and all the others—too numerous to mention by name—who have contributed materially to the preparation of this volume.

Gulf Research & Development Company
Pittsburgh, Pennsylvania

LEONIDAS PETRAKIS

Shell Development Company
Houston, Texas

FRED T. WEISS

December 14, 1978

An Overview of the Biogeochemistry of Fossil Fuel Hydrocarbons in the Marine Environment

JOHN W. FARRINGTON

Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Analyses of hydrocarbons in the aquatic environment are reviewed within the context of biogeochemical research. Intercalibration of analyses of hydrocarbons in surface sediments shows discrepancy among data reported by different laboratories by as much as a factor of 30. Chronic release of fossil fuel compounds to the marine environment from fossil fuel combustion, sewage sludge, and harbor dredge spoils is discussed. Examples cited and discussed include studies of New York Bight surface sediments and mussels and oysters from the U.S. East and Gulf Coasts using glass capillary gas chromatography–mass spectrometer–computer systems analyses of aromatic hydrocarbons. Recommendations for future studies are presented and range from "bench chemistry" solubility studies to studies of the global transport of hydrocarbons by aeolian and fluvial processes.

Biogeochemical studies of fossil fuel hydrocarbons provide information on inputs, routes, and rates of transfers and reaction, and reservoirs of accumulation of these compounds in aquatic environments. Such research is necessary in conjunction with research on the lethal and sublethal effects of fossil fuel compounds on aquatic ecosystems. They tell us where and for how long organisms will be in contact with these compounds and what the form of the compound will be (e.g., dissolved, colloidal, particulate, adsorbed on sediment).

Throughout this chapter I will refer primarily to studies of marine ecosystems. Generally the results are applicable to riverine and lacustrine

0-8412-0475-6/80/33/001$05.50/0

ecosystems, although each type has unique properties and warrants separate, focused studies. To date, marine ecosystems have received most of the attention because of the variety and magnitude of fossil fuel inputs. Freshwater ecosystems should receive increasing attention in the future, as will be demonstrated later in this chapter. I will also limit the chapter primarily to hydrocarbons with more than 15 carbon atoms.

Early biogeochemical studies of fossil fuel compounds in the marine environment involved observing the disappearance of slicks from the surface of bodies of water. Surveys of the distribution and frequency of "tar balls" on the East Coast of the United States in the 1950s documented one fate of oil spilled into the ocean (*1*). Another chapter in this volume deals with present efforts to understand the biogeochemistry of "tar balls" in a more broadly defined but scientifically rigorous manner. Other chapters in this volume deal with analytical techniques to detect trace quantities of fossil fuel compounds in environmental samples. This is a results of progress from an *out-of-sight–out-of-mind,* or *the oil slick disappeared–all is well,* philosophy to a fuller realization of the complexity of the biogeochemistry of fossil fuel compounds in aquatic environments.

A stylized representation of the fate of oil inputs taken from a variety of studies since 1969 (*1, 2*) is presented in Figure 1. Although we know that most of the pathways shown in Figure 1 are important, we still do not have a quantitative measure of the relative importance of each for different types of inputs. However, substantial progress has been made towards this goal as reported in a number of recent reviews and symposia (*2–10*). For example, we know that oil spills are only a small part of the total oil input to the marine environment. A perusal of Tables I and II shows that chronic releases such as municipal and industrial effluents and river discharges account for a fairly large portion of the inputs. We also know that under certain circumstances spilled oil can continue to contaminate intertidal sediments for at least five years (*11, 12*).

The continuing research challenge can be stated as follows: petroleum is a complex mixture of tens of thousands or more chemicals. This complex chemical soup is discharged into the aquatic environment, itself a complex chemical soup, then acted on by a variety of physical, chemical, biological, and geological processes such as wind, waves, heat, light, oxygen, microbial degradation, metabolism by fish, and adsorption onto particulate matter. During and after all this interaction the geochemist wants to know, "Where did all the petrochemicals go and why?" The various biological and ecological disciplines want to know what the effects were over time spans of hours to a decade or longer, for levels of biological organization from cells to ecosystems. Throughout the research aimed at answering these questions there has been a challenging need

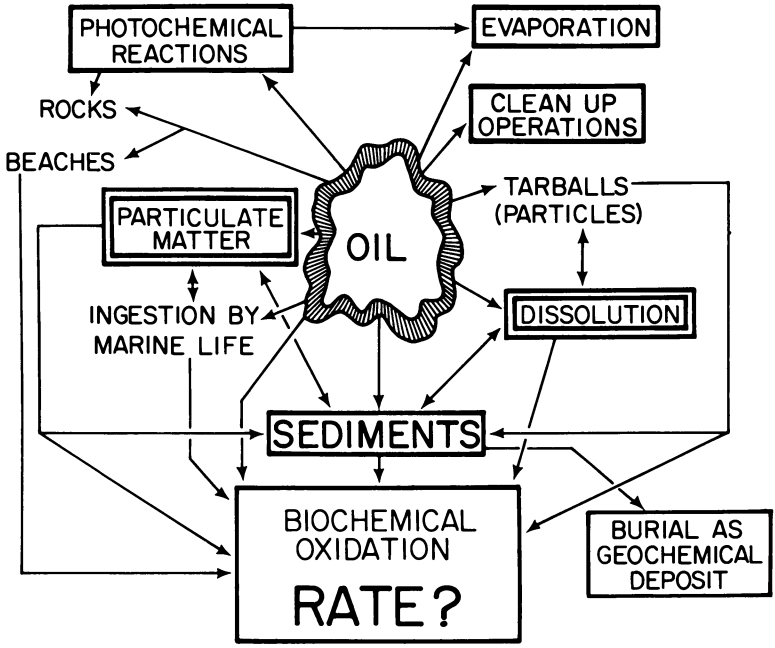


Figure 1. Stylized outline of fate of oil inputs to the marine environment

Table I. Estimates for Petroleum Hydrocarbon Input to the Oceans (from Ref. 1)

Source	*NAS Workshop (1973) (mta)*[a]
Marine transportation	2.133
Offshore oil production	0.08
Coastal oil refineries	0.2
Industrial waste	0.3
Municipal waste	0.3
Urban runoff	0.3
River runoff[b]	1.6
Subtotal	4.913
Natural seeps	0.6
Atmospheric rainout[c]	0.6
Total	6.113

[a] Millions of tons per annum.
[b] PHC input from recreational boating assumed to be incorporated in the river runoff value.
[c] Based upon assumed 10% return from the atmosphere.

Table II. Estimated Inputs of Petroleum Hydrocarbons in the Ocean During the Early 1980s[a]

	Estimated Magnitude and Relative Confidence in Estimate (mta)[b]		
Input Source	*High*	*Modest*	*Low*
Natural seeps	—	0.6	—
Offshore production	0.2	—	—
Transportation	0.8	—	—
Coastal refineries	0.02	—	—
Atmosphere	—	—	0.6
Municipal and industrial	0.45	—	—
Urban runoff	—	0.3	—
River runoff	—	1.6	—
Total	1.47	2.5	0.6
Grand total	—	4.57	—

[a] Input values are directly subject to global output values that may experience major shifts because of political, financial, economic, or exploration/production considerations.
[b] Millions of tons per annum.

for obtaining measurements of fossil fuel compounds, more often than not at 10^{-6}–10^{-9} g/g sample concentration levels.

Another challenge to analysts has been the application of analytical methods to the forensic approach of matching mystery oil spills with the "spillee." This topic will be addressed by another chapter in this volume.

Analytical Methodology

Prior to the late 1960s measurements of total "grease and oil" were the only routine analyses that would qualify as attempting to measure fossil fuels in environmental samples—mainly sewage effluents or industrial effluents. The measurement techniques varied but typically involved organic solvent extraction of grease, oil, and fats or lipids followed by either gravimetric determinations after evaporation of the solvent or IR spectrometry (*13*). During the late 1960s and early 1970s it was recognized that more discriminating and sensitive analytical methodology had to be applied and/or developed to provide measurements of petroleum compounds in environmental samples (*1, 3*). It was no longer sufficient to be able to measure total oil and grease in samples in which the fossil fuel compounds were present at such high concentrations that they overwhelmed the concentrations of natural lipids. Measurements of fossil fuel compounds at concentrations less than natural lipids and even less than biogenic hydrocarbons were needed. Furthermore, the dynamics of the changing compositions of the fossil fuel compound mix as a result of microbial degradation, evaporation, and water/particle partitioning could

be better understood if more sophisticated analytical methods were employed. This resulted in the application of a variety of column, thin-layer, and high-pressure liquid chromatography (HPLC) techniques to separate hydrocarbons from naturally occurring lipids and use of UV-fluorescence, UV, IR, glass capillary gas chromatography, gas chromatography–mass spectrometry–computer system analyses of the separated hydrocarbons.

One approach to such analyses is illustrated by the flowchart of one of the analytical schemes we have used in our laboratory for the analysis of hydrocarbons in sediments (Figure 2). There are, of course, many

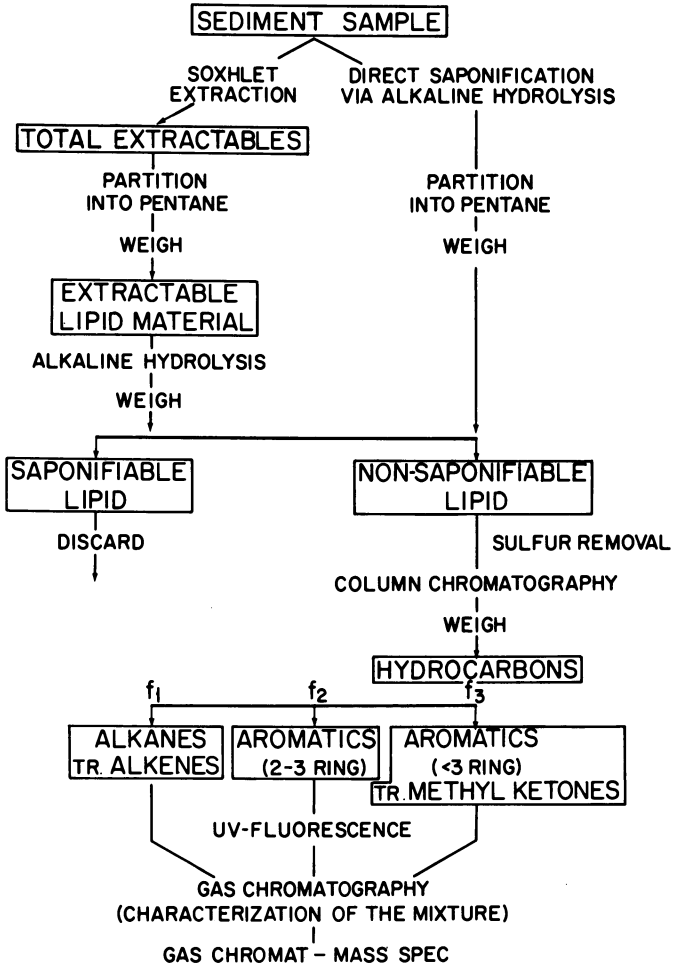


Figure 2. Flow scheme for one type of hydrocarbon analysis for sediment samples

other schemes that have been applied depending on sample types and information desired. For example, if it is desirable to have a rapid means of measuring the spread of the aromatic components of a No. 2 fuel oil in subsurface waters away from a spill, then it may be satisfactory to employ a towed UV-fluorescence instrument package that measures the fluorescence of aromatic hydrocarbons in the water (*14*). However, once the extent of the spread of the spill is mapped, it may be desirable to sample and analyze for specific aromatic compounds. This analysis would require the application of techniques such as HPLC, gas chromatography (GC), gas chromatography–mass spectrometry–computer systems. In the best of all possible situations and with unlimited funds, it would be possible for all biologists studying effects of fossil fuel compounds and geochemists studying the geochemistry of fossil fuel compounds to have available analytical facilities equipped with IR, UV, UV-fluorescence, HPLC, reporting glass capillary GC systems, glass capillary GC–mass spectrometry–computer systems and the scientists to operate such systems and interpret the analytical data. Such a utopian situation is wishful thinking. The basic responsibility of the analysts involved in these studies is to choose the appropriate analytical technique commensurate with the needs of sensitivity, specificity, and accuracy for each situation. The basic responsibility of the person or group requesting analyses is to define the analytical needs adequately.

This may seem to be an obvious set of requirements. However, I have been involved the past two years in providing reviews of two programs of analyses of hydrocarbons where there was no carefully thought-out rationale for the types of measurements being made. As a result, data were inadequate, data interpretation was in a state of confusion, and in one case data interpretation by a requesting agency was wrong.

Intercalibration and Quality Control. The large increase in the number of laboratories measuring or attempting to measure fossil fuel compounds in environmental samples raises a very serious question that has been posed many times previously, "How comparable are analyses from different laboratories?" The validity of data on incorporation of hydrocarbons into surface sediments at the concentration level of 1 mg/g is of prime importance to fate-and-effect studies in coastal and continental shelf areas. It is not difficult to see how this could also be of importance to legal considerations. The need for intercalibration seemed obvious and a few efforts to establish this practice were completed (*15*).

The largest scale program that I am aware of for measuring hydrocarbons in environmental samples is the U.S. Department of the Interior, Bureau of Land Management Outer Continental Shelf Environmental

Studies Program. Under the aegis of this program thousands of hydrocarbon measurements by more than 15 laboratories have been undertaken. Given this large number of analyses, we should have a good data base from which to establish present fossil fuel hydrocarbon concentrations in OCS ecosystems and to arrive at some conclusions regarding biogeochemistry of these compounds. This would require a pooling of data from several laboratories, which depends on the comparability of data from different laboratories.

A recently published intercalibration experiment (*16*) involved hydrocarbon analyses of surface sediment samples from two locations. In many cases the eight participating laboratories did not agree on what the major hydrocarbons were in a sample. In addition, concentrations for individual and total hydrocarbons differed by as much as a factor of 10 for data from some laboratories. The sediment samples used in this intercalibration contained low concentrations of hydrocarbons. Perhaps if the concentrations had been higher there would have been better agreement among laboratories. This hypothesis was tested by distributing samples of Santa Barbara Basin surface sediment spiked with South Louisiana crude oil. Again, the participating laboratories were instructed to use the methods of analysis they usually applied to such samples. This involved some scheme along the lines indicated in Figure 2.

A summary of the data on some *n*-alkanes, pristane, and phytane is presented in Table III. Aromatic hydrocarbon data are given in Table IV. The ranges of concentrations for individual hydrocarbons are a factor of 9 to 33 (Tables III, and IV). This means that comparison

Table III. Summary of Selected Alkane Data from BLM Intercalibration Program with Santa Barbara Sediment Spiked with South Louisiana Crude Oil (μg/g dry weight)

	Carbon Number of Alkanes							
	15	*16*	*17*	*18*	*19*	*20*	*Pristane*	*Phytane*
Range:								
high	46	43	21.0	17.7	26.5	26.5	14.6	8.0
low	1.6	1.5	1.6	1.5	1.2	0.8	1.2	0.9
range factor	29	29	13.1	11.8	22	33	12	9
Range with highest and lowest values removed								
Range:								
high	32.2	30.2	18.9	15.2	16.7	13.3	14.1	6.8
low	4.6	4.1	3.4	2.9	2.8	2.0	3.0	1.3
range factor	7.0	7.4	5.6	5.2	6.0	6.7	4.7	5.2
Number of laboratories reporting	10	10	9	8	8	7	7	6

Table IV. Summary of Selected Aromatic Hydrocarbon Data from BLM Intercalibration Program with Santa Barbara Sediment Spiked with South Louisiana Crude Oil (μg/g dry weight)

	1-Methyl Naphthalene	*2-Methyl Naphthalene*	*DDE*
Range:			
high	9.7	15	37
low	0.32	0.52	1.7
range factor	30	29	22
Number of laboratories reporting	5	5	4

of hydrocarbon concentration values that have been generated by more than one laboratory can be a factor of 10 or more apart even when concentrations are on the order of 1 to 50 μg/g dry weight in sediment. This must be kept in mind when using such data in assessing inputs, rates, and effects of fossil fuel compounds in aquatic environments.

Obviously, analyses should be in better agreement. One of the most serious problems was a lack of common reporting format. Some laboratories corrected their data for losses during workup; other laboratories did not, nor did they report recoveries for standard compounds carried through the analysis procedures. A positive note can be struck: we are now aware of the problem. Also, it was encouraging that four of the laboratories identified DDE as one of the major compounds in the gas chromatograms of the aromatic hydrocarbon fractions.

Interpretation of Hydrocarbon Analyses. Although obtaining accurate hydrocarbon measurements in samples is often a difficult task, it is equally as difficult to determine the sources of hydrocarbons from the data. The following criteria for differentiating petroleum hydrocarbons from biogenic hydrocarbons that have been suggested and applied over the past several years were outlined in (*1*) and are taken from there.

Differentiation of Petroleum Hydrocarbons from Biogenic Hydrocarbons. Petroleum and biogenic hydrocarbons can be distinguished as follows, thus providing useful means for detecting petroleum. Note that not all differences apply to all organisms, nor to all crude oils and refined products.

1. Petroleum contains a much more complex mixture of hydrocarbons, with much greater ranges of molecular structure and weight.
2. Petroleum contains several homologous series with adjacent members usually present in nearly the same concentrations.

The approximate unity ratio for even- and odd-numbered alkanes is an example, as are the homologous series of C_{12}–C_{22} isoprenoid alkanes. As previously mentioned, marine organisms have a strong predominance of odd-numbered C_{15} through C_{21} alkanes.

3. Petroleum contains more kinds of cycloalkanes and aromatic hydrocarbons. Also, the numerous alkyl-substituted ring compounds have not been reported in organisms. Examples are the series of mono-, di-, tri-, and tetramethyl benzenes and the mono-, di-, tri-, and tetramethyl naphthalenes.
4. Petroleum contains numerous naphthenoaromatic hydrocarbons that have not been reported in organisms. Petroleums also contain numerous hetero-compounds containing S, N, and O, metals, and the heavy asphaltic compounds.

One criterion added since then is the ^{14}C activity of isolated hydrocarbons. If the bulk of the hydrocarbons are fossil then there should be little ^{14}C activity (*17*). These criteria have enjoyed some success but only differentiate biogenic and fossil fuel compounds.

Once having established that the bulk of a hydrocarbon mixture of specific hydrocarbons is of fossil fuel and not of biogenic origin, the next question arises: Are these compounds from oil or from some other fossil compound source?

Hydrocarbons in New York Bight Surface Sediment. As an example of the problems involved let us consider the case of hydrocarbons in surface sediments of the New York Bight. Our previous work on alkanes and cycloalkanes in that area has been published (*18*). By applying the previously discussed criteria we were able to conclude that the bulk of the alkanes and cycloalkanes were of fossil fuel origin. The measurements of polynuclear aromatic hydrocarbons (PNAs) in one of our samples from this area indicated that the bulk of those hydrocarbons were of pyrolytic origin (*19*).

Since that time we have analyzed samples at stations indicated in Figure 3 as OC-26 (R/V OCEANUS Cruise, June 1977). Aromatic hydrocarbon distributions in Station H and Station M were similar to the distributions found for urban air particulate matter (*20, 21*) and for surface sediments in other areas of the Northeast (*19, 22*). The glass capillary gas chromatograms of the aromatic fraction are given in Figure 4. The listing of the peak identity numbers is given in Table V. Note the Peaks or groups of Peaks 2, 3, 4, and 6 that are present in the Station H gas chromatogram. The distribution of these alkylated naphthalenes is not found in air particulate matter analyzed to date and is similar to distributions of these compounds found in crude oil and fuel oil. For

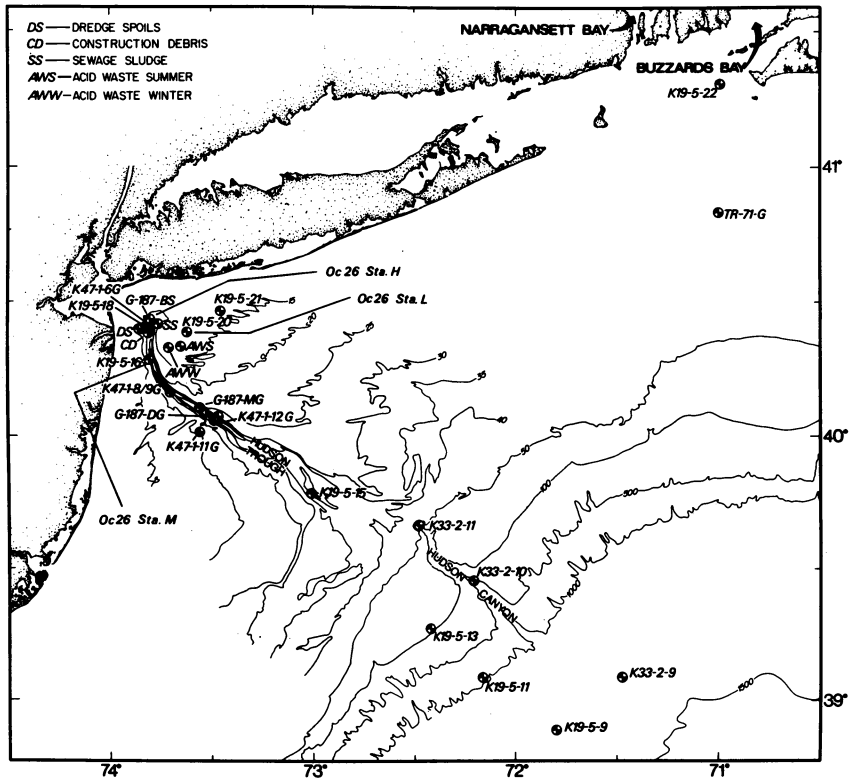


Figure 3. Station locations for studies of hydrocarbons in sediments

example, see Figure 5 for the distribution of aromatic hydrocarbons in Santa Barbara Basin surface sediment spiked with South Louisiana crude oil. Does this mean that small amounts of aromatic hydrocarbons are being contributed to the sediments by petroleum spilled or released to the environment in some manner? The presence of the naphthalenes suggests that this is the case.

However, we must consider other sources as well. It is not uncommon when sieving surface sediments from the coastal areas of the Northeast United States to pick out coal from the coarse fraction (*23*). This made us wonder if some of the contributions of aromatic hydrocarbons and other hydrocarbons in sediment samples might originate in coal particles. We extracted some coal obtained from a dealer and analyzed it using our usual procedure for sediments (*18*). The total aromatic fraction was 200 μg/g of coal. The qualitative distribution of the aromatic hydrocarbon fraction is given in the gas chromatogram of Figure 5. The similarity of the gas chromatogram profile of coal aromatics

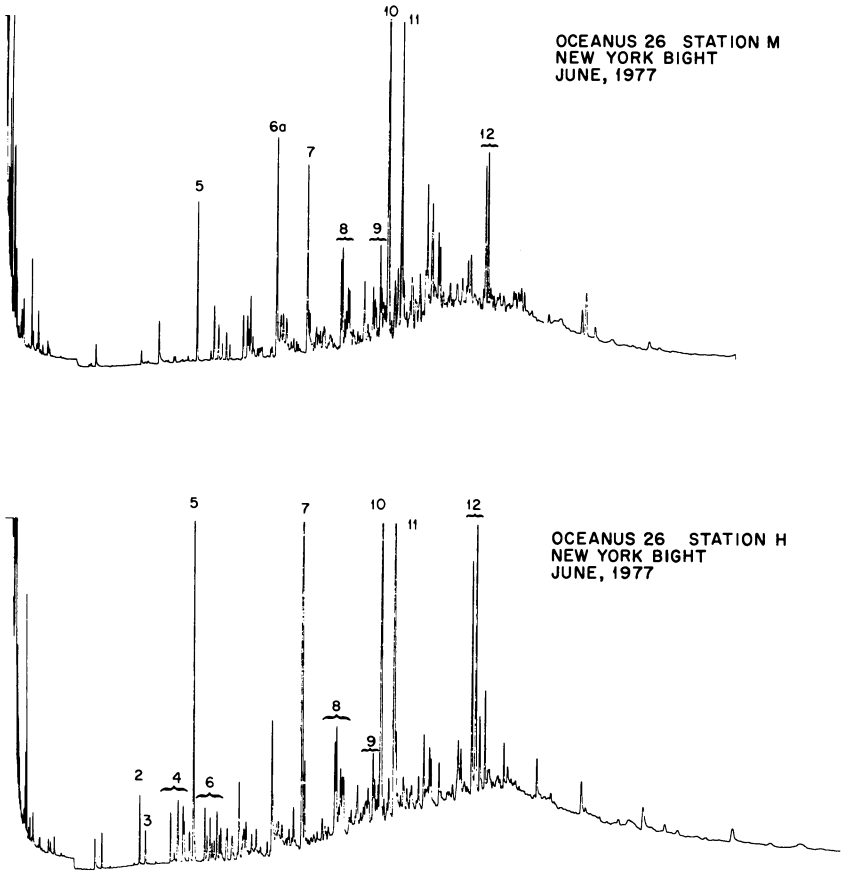


Figure 4. Glass capillary gas chromatograms of aromatic hydrocarbons in New York Bight surface sediments. Analysis conditions: 20 m × 0.32-mm i.d. Jaeggi SE-54 column installed in a Carlo Erba Model 2150 gas chromatograph equipped with split/splitless injection; helium carrier gas at 0.55 kg/cm²; injection at room temperature, program 80°–240°C at 3°/minute; injector and detector at 250°C. Numbered peaks are identified in Table V.

and petroleum aromatics was striking and indicates that distinguishing the two sources when they are each contributing low concentrations will be a challenge. Perhaps this can be accomplished with minor component analyses. Obviously, analyses of several different types of coal with more detailed qualitative and quantitative analyses of individual compounds are necessary. My point here is to raise this issue of coal in sediments as a source of aromatic hydrocarbons, especially at low concentration levels.

Table V. Compound List for Figures 4 and 5

Peak(s) Number	*Compound(s)*
1	naphthalene
2	methylnaphthalene
3	methylnaphthalene
4	C_2-substituted naphthalenes
5	hexamethylbenzene (internal standard)
6	C_3-substituted naphthalenes
7	phenanthrene
8	methylphenanthrenes
9	C_2-substituted phenanthrenes
10	fluoranthene
11	pyrene
12	aromatic compounds with $C_{18}H_{12}$ formula, e.g., chrysene

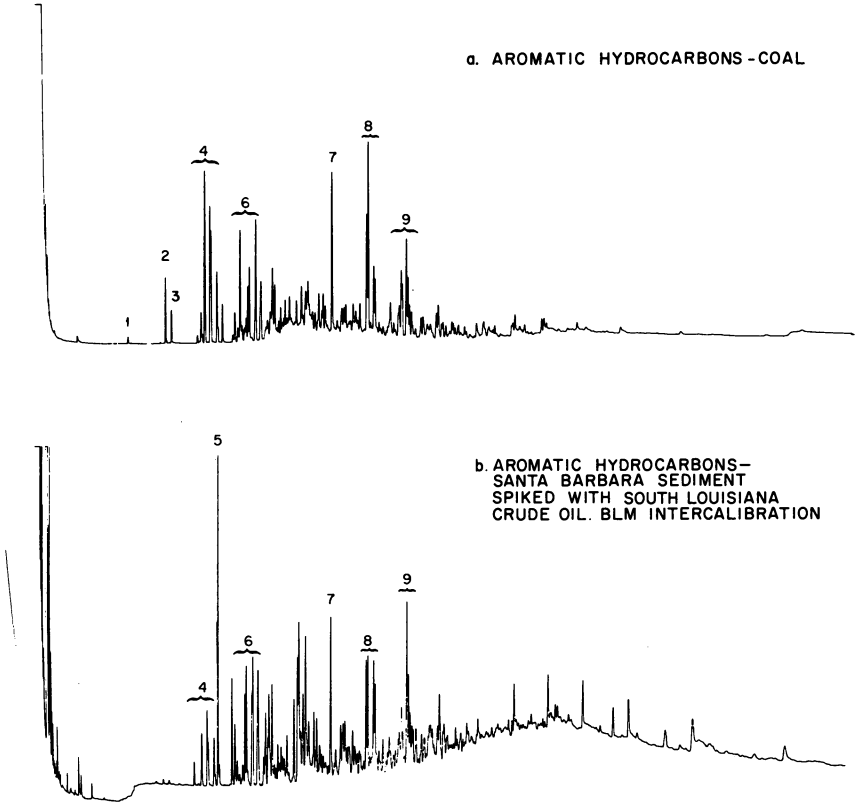


Figure 5. Glass capillary gas chromatogram of aromatic hydrocarbons in coal and crude oil spiked sediment. (See also *the legend in Figure 4.)*

The data from the New York Bight surface sediment raise a very important consideration. The input to these sediments appears to be primarily dumping of sewage sludge and harbor dredge spoils. We have estimated that 4×10^3 tons of hydrocarbons are dumped each year into the New York Bight area (*18*). This rate of pollutant hydrocarbon input to the New York Bight is at least 2% of the 1973 global OCS discharge rate for production and drilling. Surface sediments in this area are moving distances of meters to kilometers on time scales from days to years—it is an area with a dynamic benthic boundary layer (*24*). As we have pointed out previously (*18*), these inputs have to be considered when interpreting the analytical data for sediments from the Mid-Atlantic and George's Bank OCS areas near the New York Bight. Since there are other dump sites in continental margin areas, this reasoning generally applies to other OCS areas.

Historical Record of Fossil Fuel Hydrocarbons in Aquatic Sediments. There are now at least four areas in the world where an historical record of anthropogenic hydrocarbon inputs is recorded in aquatic sediments (*21, 25–29*). The data from these areas strongly suggest that fossil fuel combustion is the main source of the compounds, based on knowledge of alkyl homologue distribution for parent aromatic hydrocarbons in uncombusted fossil fuels and combustion products. Fossil fuel hydrocarbon concentrations in surface sediments are an order of magnitude to a factor of 50 to 60 above the concentrations in sediments deposited in the early 1800s to 1850s.

The consequence of this in terms of the health of benthic animals and benthic ecosystems is not known and is an important consideration. An even more important consideration is the public health aspect. The increases in PAH content of aquatic sediments reflect an increase in PAHs in the environment as a whole. Many of the PAHs are mutagens or carcinogens (*19*). With the advent of increased coal combustion as a source of energy, we can expect an increased discharge of PAHs to the environment. Technological improvements to decrease effluent emission rates will be needed. The extent to which we will need to spend money on these technological improvements to protect the public health will depend in part on our knowledge of how these compounds move through the environment (i.e., their biogeochemistry). I recently heard that we do not need to know much more about aromatic hydrocarbons in petroleum, because we will soon run out of oil to spill or discharge to the environment. My answer is that as long as we are combusting fossil fuels, an understanding of the biogeochemistry of fossil fuel compounds in aquatic ecosystems is of fundamental importance to public health and aquatic food resource protection.

We must also remember that coal mining and transportation processes of the future may require increasing use of freshwater resources to mine and transport the coal. The effluents discharged and the receiving ecosystems—mainly riverine and lacustrine—will require careful study to ensure adequate food resource and public health protection.

Long-Term Fate of Fuel Oil Aromatic Hydrocarbons in Marsh Sediments. A study of the fate of No. 2 fuel oil aromatic hydrocarbons in marsh sediments at one location in Buzzards Bay, Massachusetts, over a 6½-year period of time has recently been reported (*11*). An important finding of this study was that phenanthrene and substituted phenanthrenes and higher-molecular-weight alkylated naphthalenes were removed from the sediments by water washing and/or degradative activities at a slower rate than were lower-molecular-weight naphthalene and 2–3-carbon alkyl naphthalenes. Gas chromatograms illustrating this are given in Figure 6 with peak identities given in Table VI. A study of another marsh in the same area of Buzzards Bay subjected to a second No. 2 fuel oil spill showed similar results for the first 2½ years, which was up to the time the paper (Ref. *11*) was written.

At the end of the 6½-year period the fuel oil phenanthrenes were still present at concentrations at least an order of magnitude above background levels in the marsh sediments. As was noted in that paper (*11*), most experiments or measurements of the uptake and depuration of aromatic hydrocarbons have been conducted using naphthalene and C_1–C_2-substituted naphthalenes. A few results (*30, 31*) indicate that heavier molecular weight compounds such as phenanthrenes have a longer residence time than lower-molecular-weight compounds once they are incorporated into animal tissue. The measurements in the marsh show that organisms living in the marsh were exposed to heavier molecular weight fuel oil aromatics for at least 6½ years. Experiments by others (*31, 32*) show that organisms living in conjunction with oiled sediment take up small amounts of oil during short-term exposure of a few weeks. When the two data sets are combined, it seems clear that investigation of the toxicology, biochemistry, and geochemistry of aromatic compounds over time periods of years will be necessary to understand the long-term fate and effects of fossil fuel compounds in aquatic ecosystems whether they are subjected to acute spills or chronic inputs. This conclusion is the same as that reached by those studying the long-term fate of a Bunker C fuel oil spill (*12*).

Up to this point I have been talking mainly about hydrocarbons in sediments. One of the advantages of sediment analyses, as already noted, is that within some limits they can give an indication of the history of the inputs of fossil fuel compounds to aquatic ecosystems and assist in

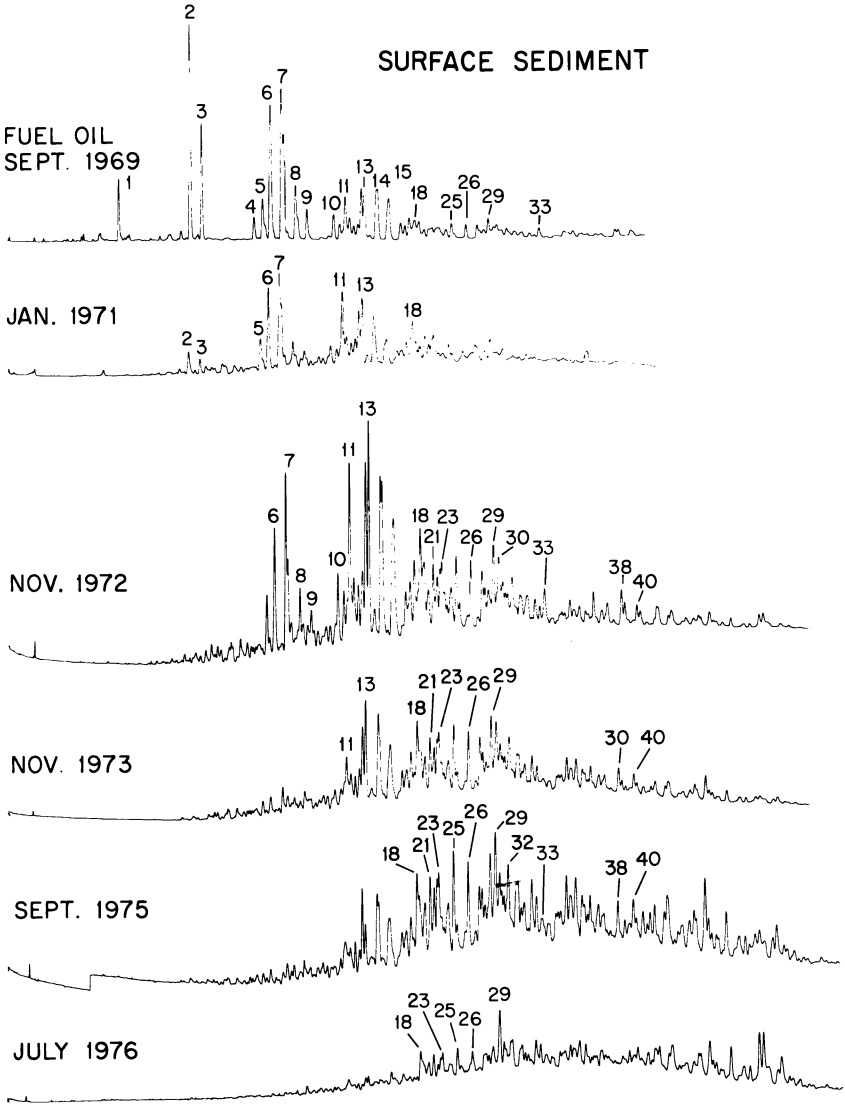


Figure 6. Glass capillary gas chromatograms of aromatic hydrocarbons in marsh sediments from an oil spill area (Ref. 11). Numbered peaks are identified in Table VI.

Table VI. Compound List for Figure 6 [from Ref. (11)]

Peak Number	*Compound*	*Identification*[a]
1	naphthalene	GC and MSA
2	2-methylnaphthalene	GC and MSA
3	1-methylnaphthalene	GC and MSA
4–9	C_2-naphthalenes	MSA
10–17	C_3-naphthalenes	MSA
18	C_4-naphthalene	MSA
19	tetrahydrophenanthrene	MS
20–29	C_4-naphthalene	MS
30	C_4-naphthalene + C_1 fluorene	MS
31	C_4-naphthalene	MS
32	dibenzothiophene	MSA
33	phenanthrene	GC and MSA
35	C_2-fluorene	MS
36	methyldibenzothiophene	MSA
37	methyldibenzothiophene	MSA
38	methylphenanthrene	MSA
39	methylphenanthrene	MS
40	methylphenanthrene	MS
41	methylphenanthrene	GC and MS

[a] GC—gas chromatography retention indices; MS—mass spectral interpretation; MSA—mass spectra match with API reference spectra.

defining the geographical extent of the contamination or pollution. The major drawback of sediment analyses is the following: compounds extracted chemically from sediments are not necessarily available for incorporation into benthic organisms. Having defined areas of contamination of sediments, we need to analyze benthic animals with various life histories and feeding strategies to understand the relationship between pollutants in sediments and pollutants in benthic animals. This is the focus of one research project in our laboratories at the present time.

Mussel Watch. Another strategy that complements sediment analyses is the use of sentinel organisms in assessing the severity and extent of fossil fuel compound pollution in the marine environment. The rationale behind this approach has been presented elsewhere (*33*). In the U.S. EPA-funded "Mussel Watch" Program, mussels on the Mid-Atlantic, Northeast, and West coasts and oysters on the south and Gulf Coasts are analyzed for a collective of pollutants. The sampling locations are given in Figure 7. A preliminary data set for a few polynuclear aromatic hydrocarbons is given in Tables VII and VIII. Previous analyses of mussels in West Coast areas had reported benzopyrene concentrations in mussels (*33, 34*). We have not yet analyzed for the five- and six-ring aromatics. However, the data in Tables VII and VIII are very interesting. There are higher concentrations of hydrocarbons in the samples near

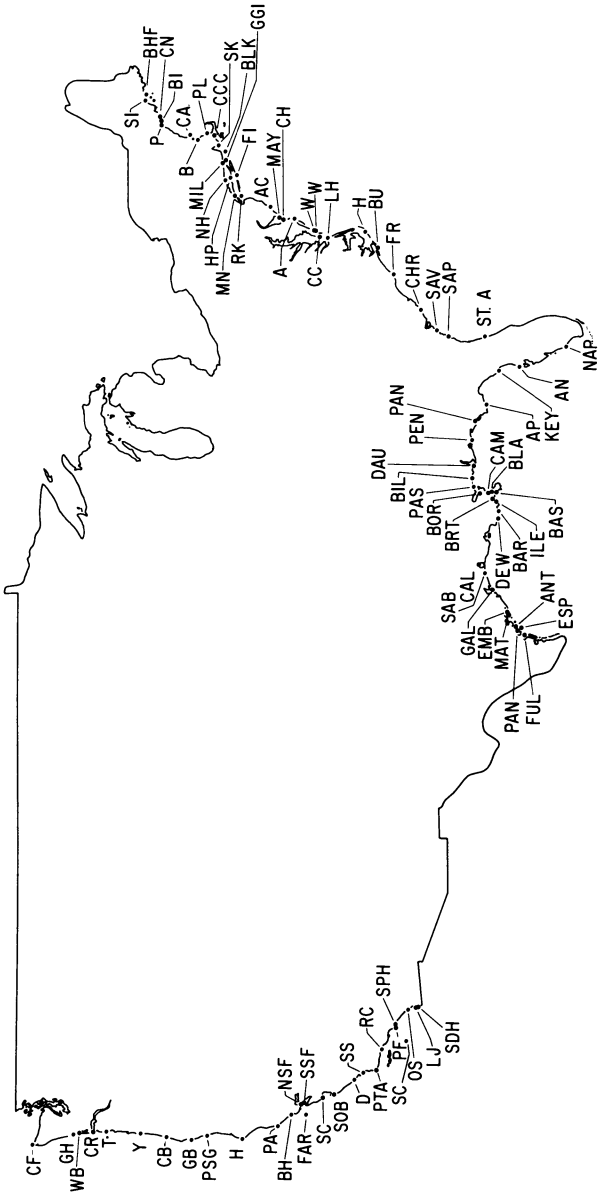


Figure 7. U.S. EPA "Mussel Watch" stations

Boston and Biloxi. This indicates higher inputs of fossil fuel hydrocarbons to these locations. A second interesting feature of the data is the relative ratios of dimethylphenanthrene and methylphenanthrene to phenanthrene. In the Boston and Biloxi samples there is a definite increase in methyl and dimethylphenanthrenes, relative to phenanthrene, in comparison to the other samples.

Does this mean that these samples are mainly subjected to fuel oil or crude oil aromatic hydrocarbon inputs while the others are mainly subject to combustion product aromatics? The ratios of alkylated phenanthrenes to the parent phenanthrene would seem to indicate that this is the case. However, we do not know enough about the partitioning of

Table VII. Polynuclear Aromatic Hydrocarbons in East Coast Mussels (10^{-9} g/g dry weight[a])

Compound	*Narragansett, Rhode Island*	*Blue Hill Falls, Maine*	*Boston, Massachusetts*	*Fire Island, New York*
Naphthalene	9.8	8.2	4.2	2.6
Methylnaphthalenes	9.1	8.2	25	7.2
Dimethylnaphthalenes	12	4.8	184	2.7
Phenanthrene	23	7.9	87	13
Methylphenanthrenes	29	5.7	577	11
Dimethylphenanthrenes	59	—[b]	1445	—
Fluoranthene	81	5.2	239	20
Pyrene	59	3.4	327	12

[a] Error estimated ± 15%.
[b] None detected above 0.1 × 10^{-9} g/g dry weight.

Table VIII. Polynuclear Aromatic Hydrocarbons in Oysters (10^{-9} g/g dry weight[a])

Compound	*Wachapreague Inlet, Virginia*	*St. Augustine, Florida*	*Pass Christian, Mississippi*	*Biloxi, Mississippi*
Naphthalene	1.8	2.0	—	8.6
Methylnaphthalenes	2.4	4.8	—	18
Dimethylnaphthalenes	—[b]	11.0	9.0	85
Phenanthrene	8.4	29	33	60
Methylphenanthrenes	8.9	45	26	170
Dimethylphenanthrenes	—	49	40	294
Fluoranthene	4.6	61	26	60
Pyrene	3.5	39	18	90

[a] Error estimated at ± 15%.
[b] None detected above 0.1 × 10^{-9} g/g dry weight.

aromatic hydrocarbons into and out of the mussels or oysters to answer this question at present. We also do not have a firm enough grasp of the biogeochemistry of the aromatic hydrocarbons in water, particulate matter, and sediment. These Mussel Watch data are preliminary and we need further study.

These data and other data on mussels sampled elsewhere on the U.S. West Coast (*33, 34, 35*) clearly indicate that there is a low concentration of 10^{-9} g/g dry weight of fossil fuel or fossil fuel combustion product aromatics in one species of biota sampled in several locations of the coastal zone of the United States. In two cases to date, samples have shown elevated levels of aromatic hydrocarbons. These samples were taken near Boston and Biloxi, areas thought to be contaminated due to chronic release of these compounds to the marine environment.

Concluding Remarks and Recommendations

The analytical methodology currently available for fossil fuel hydrocarbon analyses is sensitive and selective to the point that individual aromatic hydrocarbons can be measured in sediment and tissue samples at 10^{-9} g/g dry weight concentration levels. We should all be concerned that intercalibration exercises reveal wide differences, often a factor of 10 or more in values reported by different laboratories. We cannot ignore this and we should strive to calibrate and refine the methodology to the point where we and others can be more confident of the data comparisons from area to area and from one laboratory study to another. A factor of 10 in concentration of toxic compounds can easily be the difference between a healthy and a severely impacted ecosystem.

A second aspect of analytical methodology that concerns me is the lack of suitable standards. In my laboratory and certainly in many other laboratories the application of HPLC, glass capillary GC, and glass capillary GC–mass spectrometry–computer systems allows us to separate relatively easily hundreds of individual aromatic compounds, e.g., 9 to 12 isomers of C-3 phenanthrenes. However, there are no commercial sources for standards to verify our identifications or calibrate the quantification of these compounds. Synthesis of all isomers is clearly a monumental task. In the interim, perhaps the analytical chemists interested in this problem should be encouraged to develop systematic rules for interpreting glass capillary GC and HPLC retention indices, subtle mass spectral differences, and UV-fluorescence spectra.

A third aspect of analytical methodology in need of increased attention is analysis for reaction products of fossil fuel compounds and metabolites resulting from chemical or photochemical reactions in the environment and from metabolism in organisms. Coupled with this is

the need for more attention to the heteroatom compounds such as toluidines in petroleum, as they have been shown to be relatively more toxic than some of the aromatic hydrocarbons (*36*).

A fourth problem is being able to get at the vast data base on hydrocarbon biogeochemistry collected by the BLM Program and other programs funded by DOE, EPA, and industry over the past several years. It is easy to show that there are data management problems; these are the usual ones such as how to integrate computer files and reformat data. However, my concern is more fundamental. Environmental scientists, including those interested in biogeochemistry of the hydrocarbons in aquatic ecosystems, need time to sit and think about what all of these data are telling us and where to go from here.

With the increase in environmental exposure levels for man and biota as combustion of fossil fuels increases, questions on the fate and effects of compounds such as aromatic hydrocarbons in aquatic ecosystems gain in importance. We have learned something of the biogeochemistry of these compounds in aquatic ecosystems, as indicated by my previous remarks. However, the investigations cited were limited in spatial and temporal scope compared with the long-term global nature of the fate-and-effect questions. For example, we have yet to get at the question of the relative importance of aeolian vs. fluvial transport of combustion product aromatic compounds to coastal and open ocean ecosystems. Perhaps the best way to illustrate the very fundamental nature of some of the research that needs to be addressed is to mention the question of solubility of aromatic hydrocarbons such as naphthalenes, phenanthrenes, and pyrenes. We often invoke "solubilization" or water washing of sediments to remove aromatics from sediments or in controlling partitioning between organisms and their aquatic habitat. Yet our knowledge of the solubility of aromatic hydrocarbons in waters of different salinities, pH, and organic matter content is rudimentary (*37, 38, 39*).

Fossil fuel compound biogeochemistry research needs range from bench chemistry solubility studies to studies of global transport phenomena for the atmosphere and oceans.

Acknowledgments

Collaborative research with John Teal and Ronald A. Hites stimulated many thoughts incorporated into this chapter. The laboratory and field assistance of Bruce W. Tripp and Alan C. Davis was also quite helpful. Nelson Frew deserves a special acknowledgment for the GC-mass spectrometry measurements.

The support given by the U.S. Department of Energy, Contract No. EE-77-5-02-4256, and the U.S. Bureau of Land Management via Interagency Agreement AA-550-IA-7-20 as well as Grants 804215 and R-803-

902020 from the U.S. Environmental Protection Agency is gratefully acknowledged. Contribution No. 4215 from Woods Hole Oceanographic Institution.

Literature Cited

1. "Petroleum in the Marine Environment"; National Academy of Sciences: Washington, DC, 1975.
2. Farrington, J. W. In *"Estuarine Pollution Control and Assessment," U. S. Environ. Prot. Agency Conf., Proc.; 1975;* Vol. II, pp. 385–400.
3. "Marine Pollution Monitoring (Petroleum)," *Nat. Bur. Stand. (U.S.)* **1974,** *Spec. Publ.* No. 409.
4. Farrington, J. W.; Meyers, P. A. *Environ. Chem.* **1975,** *1,* 109–136.
5. McIntyre, A. D.; Whittle, K. J., Eds. "Petroleum Hydrocarbons in the Marine Environment"; Proceedings from ICES Workshop, Aberdeen, Scotland, September, 1975. Rapports et Proces-Verbaux Des Reunions Counseil International Pour l'Exploration de la Mer, **1977,** *171.*
6. "Sources, Effects, and Sinks of Hydrocarbons in the Aquatic Environment"; American Institute of Biological Sciences: Washington, DC, 1976.
7. Duce, R. A., Parker, P. L., Eds. "Pollutant Transfer to the Marine Environment. Deliberations and Recommendations of the NSF/IDOE Pollutant Transfer Workshop, 1974"; National Science Foundation: Washington, DC, 1974.
8. Wolfe, D. A., Ed. "Fates and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms"; Pergamon: New York, 1977; pp. 78–94.
9. *Proc.—Oil Spill Conf. (Prev., Behav., Control, Cleanup).* American Petroleum Institute: Washington, DC, 1977.
10. "The Impact of Oil on the Marine Environment. GESAMP, Workshop Group Report, 1976," Food and Agricultural Organization of the United Nations: 1976.
11. Teal, J. M.; Burns, K. A.; Farrington, J. W. *J. Fish. Res. Board Can.* **1978,** 35, 510–520.
12. Vandermeulen, J. H.; Gordon, D. C., Jr. *J. Fish. Res. Board Can.* **1976,** *33,* 2002–2010.
13. "Manual for Chemical Analysis of Water and Wastes," U. S. EPA Office of Technology Transfer **1974.**
14. Environmental Devices Corporation; Towed Underwater Fluorometer System, ENDECO, Marion, MA.
15. Farrington, J. W.; Teal, J. M.; Medeiros, G. C.; Burns, K. A.; Robinson, E. A., Jr.; Quinn, J. G.; Wade, T. L. *Anal. Chem.* **1976,** *48,* 1711–1715.
16. Hilbert, L. R.; May, W. E.; Wise, S. A.; Chesler, S. N.; Hertz, H. S. *Anal. Chem.* **1978,** *50,* 458–463.
17. Zafiriou, O. C. *Estuarine Coastal Mar. Sci.* **1973,** *1,* 84–87.
18. Farrington, J. W.; Tripp, B. W. *Geochim. Cosmochim. Acta* **1977,** *41,* 1627–1641.
19. LaFlamme, R. E.; Hites, R. A. *Geochim. Cosmochim. Acta* **1978,** *42,* 289–303.
20. Lee, M.; Prado, G. P.; Howard, J. B.; Hites, R. A. *Biomed. Mass Spectrom.* **1977,** *4,* 182–186.
21. Giger, W.; Schaffner, C. *Anal. Chem.* **1978,** *50,* 243–249.
22. Youngblood, W. W.; Blumer, M. *Geochim. Cosmochim. Acta* **1975,** *39,* 1303–1314.
23. Goldberg, E. D.; Gamble, E.; Griffin, J. J.; Koide, M. *Estuarine Coastal Mar. Sci.* **1977,** *5,* 549–561.
24. Stubblefield, W. L.; Parmenter, R. W.; Swift, D. J. P. *Estuarine Coastal Mar. Sci.* **1977,** *5,* 549–561.

25. Hites, R. A.; LaFlamme, R. E.; Farrington, J. W. *Science* **1977**, *198*, 829–831.
26. Müller, G.; Grimmer, G.; Böhnke, H. *Naturwissenschaften* **1977**, *64*, 427–431.
27. Wakeham, S. G.; Carpenter, R. W. *Limnol. Oceanogr.* **1976**, *21*, 711–723.
28. Wakeham, S. G., Ph.D. Thesis, University of Washington, Seattle, WA, 1976.
29. Farrington, J. W.; Frew, N. M.; Gschwend, P. M.; Tripp, B. W. *Estuarine Coastal Mar. Sci.* **1977**, *5*, 793–808.
30. Sanborn, H. R.; Mallins, D. C. *Proc. Soc. Exp. Biol. Med.* **1977**, *154*, 151–155.
31. Roesijadi, G.; Anderson, J. W.; Blaylock, J. W. *J. Fish. Res. Board Can.* **1978**, *35*, 608–614.
32. Gordon, D. C., Jr.; Dale, J.; Keizer, P. D. *J. Fish. Res. Board Can.* **1978**, *35*, 591–603.
33. Goldberg, E. D. *Mar. Pollut. Bull.* **1975**, *6*, 111.
34. Dunn, B. P. *Environ. Sci. Technol.* **1976**, *10*, 1018–1021.
35. Dunn, B. P.; Young, D. R. *Mar. Pollut. Bull.* **1976**, *7*, 231–234.
36. Winters, K.; O'Donnell, R.; Batterton, J. C.; Van Baalen, C. *Mar. Biol.* **1976**, *36*, 269–276.
37. May, W.; Wasik, S. P.; Freeman, D. H. *Anal. Chem.* **1978**, *50*, 175–179.
38. Sutton, C.; Calder, J. *J. Chem. Eng. Data* **1975**, *20*, 320.
39. Boehm, P. D.; Quinn, J. G. *Estuarine Coastal Mar. Sci.* **1976**, *4*, 93–105.

RECEIVED October 31, 1978.

Analytical Chemistry of Petroleum

An Overview of Practices in Petroleum Industry Laboratories with Emphasis on Biodegradation

L. PETRAKIS,[1] D. M. JEWELL, and W. F. BENUSA

Gulf Research and Development Company, Pittsburgh, PA 15230

This chapter presents an overview of analytical practices not generally well known outside the petroleum industry laboratory. The methodology surveyed has been developed primarily for processing purposes, but more recently it has been applied to oil pollution problems. Mass spectrometry, chromatography, and ^{1}H and ^{13}C NMR are emphasized. The synergism of the techniques is illustrated through the structural profiles of the "group types" of a South Louisiana and a Kuwait crude prior to biodegradation. Physical properties are given. In addition, crudes have been separated into asphaltenes, resins, mono-, di- plus triaromatics, polyaromatics, n-paraffins, cycloparaffins, and i-methyl paraffins. These fractions are "fingerprinted" with a variety of analytical tools. The "baseline" profiles are then compared with similar profiles of the South Louisiana crude after biodegradation with mixed bacterial cultures from Chesapeake Bay. Special consideration is given to the novel use of ^{13}C NMR.

Problems at the energy–environment interface have received much attention in the recent past. Prominent in this context has been the problem of the fate of petroleum as it enters the marine environment, whether through natural seepage, during exploration and production activities, during transportation activities (including normal operations and accidental spills), or, finally, through its ultimate disposal. Key questions raised include the determination of the effects of oil on the marine fauna and flora and the overall fate of oil in the ecosystem (*1–8*).

[1] Author to whom correspondence should be addressed.

0-8412-0475-6/80/33/023$07.75/0

The fate and effects of petroleum depend on the prevailing environmental conditions in any particular oil–water system as well as on the chemical nature of the petroleum involved.

Clearly, the questions raised constitute an extremely complex set, given the many variables that are important in this problem. One technical problem that enters in all considerations of oil and oil pollution is the problem of the physical and chemical characterization of the oil itself as well as how it may be altered in a given environment and under a given set of climatological conditions. In oil spill identification, in biodegradation studies, and in the monitoring of oil as it moves through the hierarchy of organisms, the means for the qualitative and quantitative identification of oil are indeed important considerations. How important a consideration is further indicated by a brief look at the chemical complexity of petroleum.

Petroleum is a highly complex mixture of mostly hydrocarbons, but with significant representation from other elements including oxygen, sulfur, and smaller amounts of nitrogen, nickel, and vanadium. Molecular weights observed easily reach the several thousand mark, and, therefore, the number of chemical compounds present is very great. Thus, any chemical and physical characterization precludes the monitoring of individual components. It also precludes any general statement about the behavior of the "oil," for not only can various oils be significantly different, but the very large number of chemical species present guarantees a variability in response to environmental and other factors. It is not surprising then that the question of chemical analytical methodology has received much attention, especially in the laboratories of petroleum companies. Much of this methodology has not been generally known outside of the industry, however. As a result, and in view of the considerable and diverse interest that has arisen in oil, both in its environmental implications and in conjunction with the "energy crisis," the highly developed and available analytical methodology has not been used to its greatest advantage.

This chapter attempts to bring the potential of the available analytical methodology to the attention of those interested in the broad aspects of petroleum pollution. Much of this methodology has been used by the authors in conjunction with biodegradation studies (*9–14*) as well as in petroleum processing studies (*15*). Presented here is simply an indication of what a proper approach to the problem of analysis of petroleum and its environmental effects might be. We do this by following closely two different oils and by establishing a "baseline" study that would be required, in any event, in the consideration of the fate and effects of oil in the ecosystem. In addition, the same analytical methodology is used in following the biodegradation of a South Louisiana crude.

Properties of Crude Oils

Due to the complexity and variety of crude oils, certain common properties are routinely determined for purposes of intercomparison. The petroleum industry frequently utilizes a method developed by DOE (U.S. Bureau of Mines) (*16, 17*) that determines elemental distribution, density, viscosity, and distillation characteristics. Variations of this method are attributable to the different experimental techniques employed and to the particular needs of an investigator. For the purpose of our discussion in this chapter, two different crude oils having the properties shown in Table I were selected (*18*). We chose to work with these particular oils because they are the subject of a very extensive API-sponsored investigation. Domestic crude oil produced in the South Louisiana area usually contains less sulfur, nitrogen, and metals than crude oils from the Middle East (Kuwait). The higher API gravity of the South Louisiana crude oil strongly implies that it has an overall lower mid-boiling point, is less aromatic, and contains less asphaltic-type components than does the Kuwait crude oil. Refinery experience with each crude verifies these facts. Although the quantity of naphtha distilling up to 204°C is a valuable parameter for refining purposes, it is not the sole determinant of material lost to the environment when crude oils are mixed with water and subjected to heat, oxidation, and/or biological action. The analyses obtained normally include nickel and vanadium, which are found in small quantities but play a very important role in refining through their deleterious effects on catalyst life and performance. Also, the same analyses have been used in oil spill identification and other problems of the oil in the environment.

Table I. Typical Properties of Crude Oils (*18*)

	South Louisiana	*Kuwait*
API—gravity	34.5	31.4
Sulfur (wt %)	0.25	2.44
Nitrogen (wt %)	0.07	0.14
Nickel (ppm)	2.2	7.7
Vanadium (ppm)	1.9	28.0
Naphtha (< 204°C—wt %)	18.6	22.7

American Petroleum Institute

Chromatographic Separation Methods

Due to the complexity of crude oils and their products, analyses may provide different compositional profiles of the same oil. This leads to confusion and makes meaningful comparisons difficult. There is a need

for some standardization in terminology and approaches for determining significant, useful average structural (molecular) parameters for oils on a microsize sample (20–500 mg). The most common usage of compositional information by environmentalists and microbiologists is comparative in nature. That is, they are looking for significant changes from some baseline features rather than individual compound identifications, although the latter may be highly desirable or required in selected situations. This requires consistency, accuracy, and repeatability in data acquisition over a large sample size range.

What is really needed is "compositional mapping" (*19*). The more accurate and useful points one can place on the "map," the more useful it becomes and the more valuable are the conclusions drawn therefrom. When only one or two techniques are used to delineate the composition of petroleum (e.g., GLC, IR, NMR, etc.), data are suspect and may lead to false-positive conclusions. The characterization of any petroleum residual is relative and never complete, but its degree is directly related to the extent that discrete classes of compounds can be isolated and analyzed by chemical and spectroscopic methods. With the philosophy that "compositional mapping" does offer the soundest analytical approach to study petroleum in any context (environmental, biological, geochemical, refining, etc.), we suggest a standardized technique as outlined in Figure 1.

This scheme is illustrated for studies in which petroleum–water mixtures exist, as would be the case in oil spills, biological degradation studies, and similar situations. Intimate mixing of petroleum in aqueous systems that are then subjected to heat, oxidation, light, and bacterial action always induces some irreversible changes in composition. Increased solubility of oxidized products and losses by evaporation are the most obvious changes. For this reason, exhaustive benzene extraction is used to recover the petroleum; pure, unaltered petroleums are completely miscible in benzene. Drying the extract and removing the solvent under a stream of nitrogen at 70°C provides a residual oil containing only those components boiling above 230°C. Experience has shown that aliphatic hydrocarbons containing less than 12 carbon atoms (dodecane) have a vapor pressure sufficiently high to be lost in this concentration step; these same hydrocarbons are usually lost by combination of evaporation and solubilization in real life problems (e.g., oil spills). This indicates that the naphtha content (*see* Table I) is not an accurate predictor for these losses.

The residue (Figure 1) is rapidly and sequentially separated into a discrete number of compound types by techniques that have been described in detail elsewhere (*19, 20*). These are:

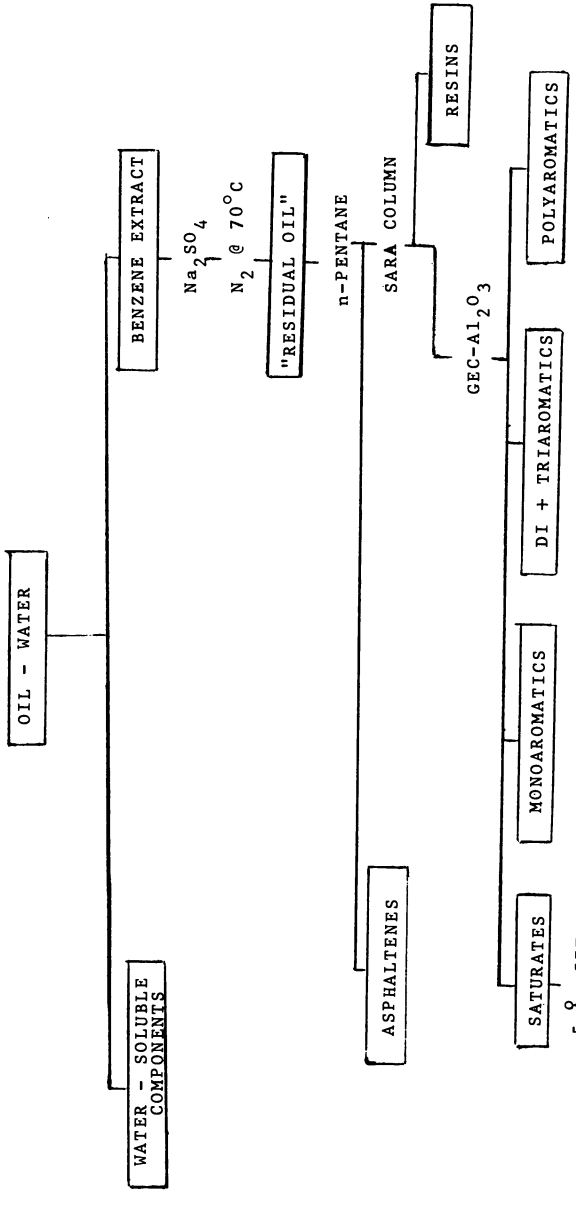


Figure 1. Standardized separation scheme for petroleum crudes

1. *Asphaltenes*: high-molecular-weight polar molecules that are insoluble in *n*-pentane but are soluble in benzene. This class contains most of the metalloorganics such as porphyrins.
2. *Resins*: pentane-soluble nonhydrocarbons such as pyridines, phenols, carbazoles, amides. This class is isolated by means of their chemical reaction with ion exchange resins or transition metal salts.
3. *Saturates*: molecules containing only carbon and hydrogen and no unsaturation.
4. *Aromatics*: hydrocarbons containing at least one benzenoid ring; ethers and thioethers may also be present.

The total aromatics can be subdivided according to their number of rings and type of condensation by adsorption and charge transfer chromatography (*21, 22*). The total saturates can be subdivided by selective reactions with 5-Å molecular sieves and urea adduction into straight-chain, methyl-branched chain, and cyclic hydrocarbons. Isoprenoids do not react with urea and as a result they are isolated in the cyclic paraffin fractions. Nearly all these separations can be performed quantitatively on 500 mg of residue by modern medium-to-high-pressure liquid chromatographic techniques using multiple detectors. The use of these instruments minimizes human error and greatly improves the reproducibility and accuracy of the data. Most of the existing separation techniques (e.g., ASTM methods) do not use these instrumental techniques (*18*).

Table II is an example of the application of this scheme to the crude oils shown in Table I. Each crude oil was analyzed by this scheme.

When separating the untreated crude, volatiles are lost during the concentration of aromatics and saturates and are subsequently grouped with the resins (since the latter are determined by difference). Kuwait

Table II. Molecular Types in Crude Oils (Chromatographic Determination—Wt %)

	Kuwait	*South Louisiana*
Total saturates	28.0	40.8
n-paraffins	8.5	6.8
non-*n*-paraffins	19.5	34.0
(isoparaffins)	(1.7)	(0.8)
(cycloparaffins)	(17.8)	(33.2)
Total aromatics	34.0	22.0
monoaromatics	7.6	8.5
di- + triaromatics	26.1	12.0
polyaromatics	0.3	1.5
Resins and volatiles	36.5	36.9
Asphaltenes	1.6	0.3

crude oils always contain more polar compounds, even in the low-molecular-weight portions, which increases losses due to evaporation. The structural differences among all fractions can be seen by GLC, NMR, and mass spectroscopic techniques and are discussed below.

Mass Spectrometry

Mass spectrometric analyses of crudes and petroleum fractions have proved to be of great value in the understanding of the compositional problems in petroleum and in petroleum processing. The approach taken in these kinds of analyses is the so-called group-type analysis. (*Note*: A "class" of compounds here means broad chemical categories such as aromatics, saturates, etc., while "group types" refer to subdivisions within a class such as alkanes, cycloalkanes, etc.) The very large number of individual compounds present in a petroleum fraction makes the detection and quantitative determination of each individual component impossible. Therefore, the determination of chemical group types is essentially the only practical analytical procedure applicable to such materials. These group-type analyses utilize combinations of mass peaks in the mass spectrum of a fraction that are characteristic of specific compound types.

There are two very useful and commonly used mass spectrometric group-type analyses employed to provide quantitative information in some 25 molecular types. One is the so-called Robinson–Cook (*23*) method for the analysis of aromatic fractions, while the other is the saturate group-type analysis (ASTM) (*24*). Table III lists the results that have been obtained using these two spectrometric analyses for Kuwait and South Louisiana crude prior to any degrading or weathering but after removal of the light ends ("topping"). The results are quoted in weight percent of unweathered material ("residue"). The lower portion of the table lists 7 broad classes of aromatic compounds according to the number of rings. In the actual analysis there are 19 classes altogether, because the mass spectral features allow the further breakdown into subclasses. For example, the monoaromatics are further broken down into alkyl benzenes, naphthene benzenes, and dinaphthene benzenes; the tetraaromatics into pyrenes and chrysenes, etc.

Direct comparison of subgroup concentrations (e.g., monoaromatics) as determined by chromatographic (Table II) or mass spectrometric (Table III) techniques cannot be made. Comparison of subgroups should only be made within the same technique. Since the mass spectrometric analysis is based on fragmentation patterns, a true distinction of every precursor (parent molecule) in a mixture cannot be made. Noncondensed di- and triaromatics (*21*) and many sulfur compounds (*25*) in petroleum yield fragments reported as "monoaromatics"; this value is frequently higher when determined in this manner.

Table III. Mass Spectrometric Analysis of Pretreated Crude Oils (Wt %)

	Kuwait Crude	*South Louisiana Crude*
Saturate Group-Type Analysis		
alkanes	9.8	9.8
1-ring cycloalkanes	3.7	9.4
2-ring cycloalkanes	2.9	6.8
3-ring cycloalkanes	2.4	4.6
4-ring cycloalkanes	2.5	5.0
5-ring cycloalkanes	0.0	4.1
6-ring cycloalkanes	0.1	2.1
	21.4	41.8
Aromatic Group-Type Analysis		
monoaromatics	14.0	16.6
diaromatics	9.4	12.2
triaromatics	3.7	3.9
tetraaromatics	2.0	1.5
pentaaromatics	0.5	0.5
aromatic sulfur species	7.8	1.4
unidentified aromatics	3.2	1.9
	40.6	38.0

Nuclear Magnetic Resonance

Proton Spectra—"Average Molecule" Parameters. Proton NMR has, of course, found considerable and important use in the characterization of petroleum constituents and fractions. In the case of isolated individual components, NMR can be a powerful tool in the elucidation of structure. However, this approach is obviously limited when we are dealing with immensely complex, multicomponent mixtures such as petroleum fractions.

A different, simpler, and more useful approach has been discussed (*26*) in terms of "average molecule" parameters. The ^1H NMR chemical shifts from aromatic and nonaromatic protons are very different. In addition, the intensities of these signals quantitatively reflect the relative number of protons in these different environments. The integrated intensities due to the distinct aromatic and aliphatic signals can be used to derive a series of parameters describing the ensemble profile of the sample.

Table IV gives some of the parameters that have been derived from the manipulation of the integrated intensities of the various chemically shifted signals of the aromatic fractions and subfractions of Kuwait and South Louisiana crudes. Profiles that emerge from these baseline studies are the following:

(a) In comparing the monoaromatics, di- plus triaromatics, and total aromatics of South Louisiana crude, the total fraction has an aromaticity that is intermediate between that of the monoaromatics and that of the di- + triaromatics fraction. This situation is not unexpected because the di- + triaromatics tend to have a greater fraction of aromatic carbons even as the molecular weight of the molecules increases. The monoaromatic fraction has a greater number of alkyl substituents (4.0 per molecule) than the di- + triaromatic fraction (3.6 per molecule). In the former fraction, the alkyl substituents are also longer (4.4 carbons/substituent) than on the di- + triaromatic fraction (3.3 per molecule). In addition, the naphthenic material in the monoaromatic fraction is greater than in the di- + triaromatics fraction. An apparent discrepancy appears in the Aromatic Rings/Molecule column, with the di- + triaromatic fraction showing a value of 1.6 when clearly one would expect something greater than 2.0. The explanation for this is that there is a difference in what NMR and separations (scheme of Figure 1) consider as mono- or diaromatics. For example, biphenyls would give NMR signals quite typical of monoaromatic species, but they would be concentrated during separation with the di- + triaromatic fractions. This clearly indicates that care must be taken in making cross-comparisons between results of various techniques, while comparisons between baseline materials and degraded materials may be much more significant when the same technique is applied.

(b) Comparing the monoaromatic fractions of the two crudes, we find profiles that are essentially identical except for the number of alkyl substituents, which is somewhat greater in the Kuwait crude. However, comparing the di- + triaromatic fractions of the two crudes, we find some important differences. The aromaticity of the South Louisiana crude is higher (41% vs. 34%). Also, the number of alkyl substituents of the South Louisiana crude is lower than the Kuwait crude (4.0 vs. 4.2 per molecule, respectively). Moreover, these alkyl substituents are smaller, 3.3 vs. 3.8 carbons per alkyl substituent, for the South Louisaina and Kuwait crudes, respectively.

The significance of these indices of the molecular profiles of crudes is that they should be very useful in establishing average biodegradation rates for different molecular species. Also, they would be quite useful in establishing semiquantitatively the propensity of different microbiological flora to attack various molecular species. For example, if alkyl chains are removed preferentially, then the overall aromaticity would increase. Also, one would expect a decreasing number and length of the alkyl substituents. If, on the other hand, smaller chains were removed first, aromaticity would increase and the carbons per alkyl substituent would also increase.

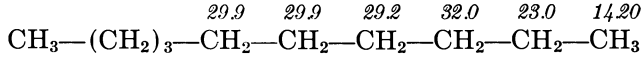
Table IV. Some "Average Molecule" Parameters for

	Aromatic Carbon (%)	*Saturate Carbon (%)*
S. LA crude—total aromatics	38	62
S. LA—"monoaromatics"	26	74
Kuwait crude—"monoaromatics"	24	76
S. LA—"di- + triaromatics"	41	59
Kuwait crude—"di- + triaromatics"	34	66

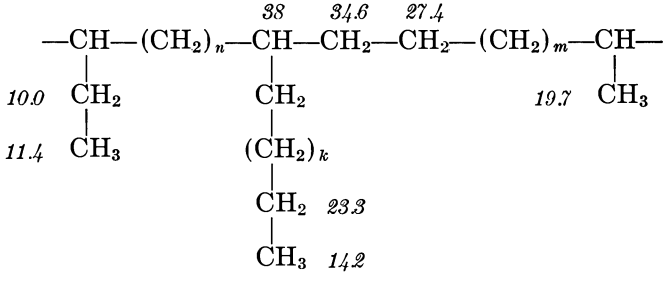
Such subtle average profile changes could also establish specific points of microbial attack and help design microbial cleanup schemes for oil spills.

Carbon-13 NMR. The recent widespread availability of ^{13}C spectrometers has allowed their utilization in elucidation of structural problems in petroleum fractions. The advantages of using ^{13}C NMR over the more traditional ^{1}H NMR stem primarily from the fact that ^{13}C NMR resonance signals are spread out over a wider range and therefore allow a better characterization of the molecular sepcies present. Nevertheless, even in ^{13}C NMR, the number of independent resonances is quite limited, given the very great number of independent constituents of petroleum. Thus, again, we are led to using ^{13}C NMR spectra either to determine a group profile as with ^{1}H NMR or to fingerprint fractions as has been done with GLC fingerprints. The recent availability of extensive ^{13}C chemical shift data (*27–30*) has made it also possible to make some specific assignments of various chemical species present, but still one has to rely primarily on comparisons of spectral fingerprints.

The chemical shifts of the ^{13}C NMR spectra of hydrocarbons are well studied and tabulated (*27–30*). The spectra of hydrocarbons of interest here can be understood from the following values of observed chemical shifts:



and



Aromatic Fractions of Crudes (^{1}H NMR Results)

Aromatic Rings/Molecule	*Alkyl Substituents/Molecule*	*Carbons/Alkyl Substituent*	*Naphthenic Rings/Molecule*
1.4	3.5	3.6	0.8
1.0	4.0	4.4	1.1
1.0	4.2	4.4	1.1
1.6	3.6	3.3	0.8
1.5	3.9	3.8	0.8

The indicated values are chemical shifts in ppm from tetramethylsilane (TMS), which is a commonly accepted standard.

Figure 2a shows the ^{13}C NMR spectra of the following fractions of Kuwait crude: total saturates, normal paraffins, and nonnormal paraffins. Figure 2b shows the corresponding spectra of isoparaffins and cycloparaffins. These fractions have been obtained from the scheme outlined in Figure 1. The spectra demonstrate the need for such detailed separation schemes, for the spectra of the total saturates are too detailed to allow any significant conclusions as to contributions from the various molecular types present.

The spectra of the normal paraffins are the simplest; they show the five peaks expected at the five frequencies associated with terminal methyls (14 ppm), methylenes α to terminal methyls ($\sim$ 23 ppm), methylenes β to terminal methyls (32 ppm), γ methylenes ($\sim$ 29.2 ppm), and the remaining methylenes in the middle of the long chain grouped at approximately 29.9 ppm. From the relative intensities of these terminal methyls and the methylenes, the average carbon number of the normal paraffins is calculated to be about 14 carbons (Table V). It turns out that the GLC fingerprint of the normal paraffins shows a peaking at about 15 carbons (vide infra).

The spectra of the nonnormal paraffins are extremely rich. The resonances expected of terminal methyls and of other groups associated with long alkyl chains are evident in the spectra. In addition, signals are observed that are associated with branching, such as methyls attached to long chains ($\sim$ 19.7 ppm). From the intensities of these methyls and the

Table V. ^{13}C NMR Structural Parameters of Crudes

	Average Carbon Number	*Methyl Branches (%)*	*Ethyl Branches (%)*
Kuwait *n*-paraffins	14.0	—	—
Kuwait cycloparaffins	19.6	6	$\sim$ 1
Kuwait isoparaffins	19.9	2.7	$\sim$ 1

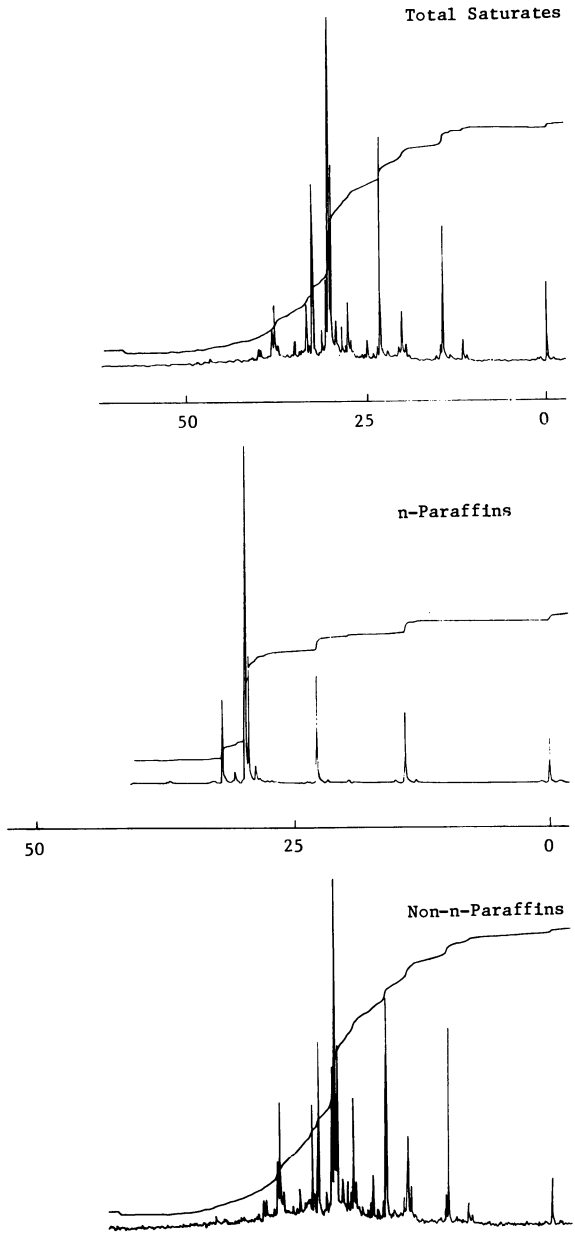


Figure 2a. ^{13}C *NMR of saturate fractions from Kuwait crude oil (total saturated,* n-*paraffin, nonnormal paraffins)*

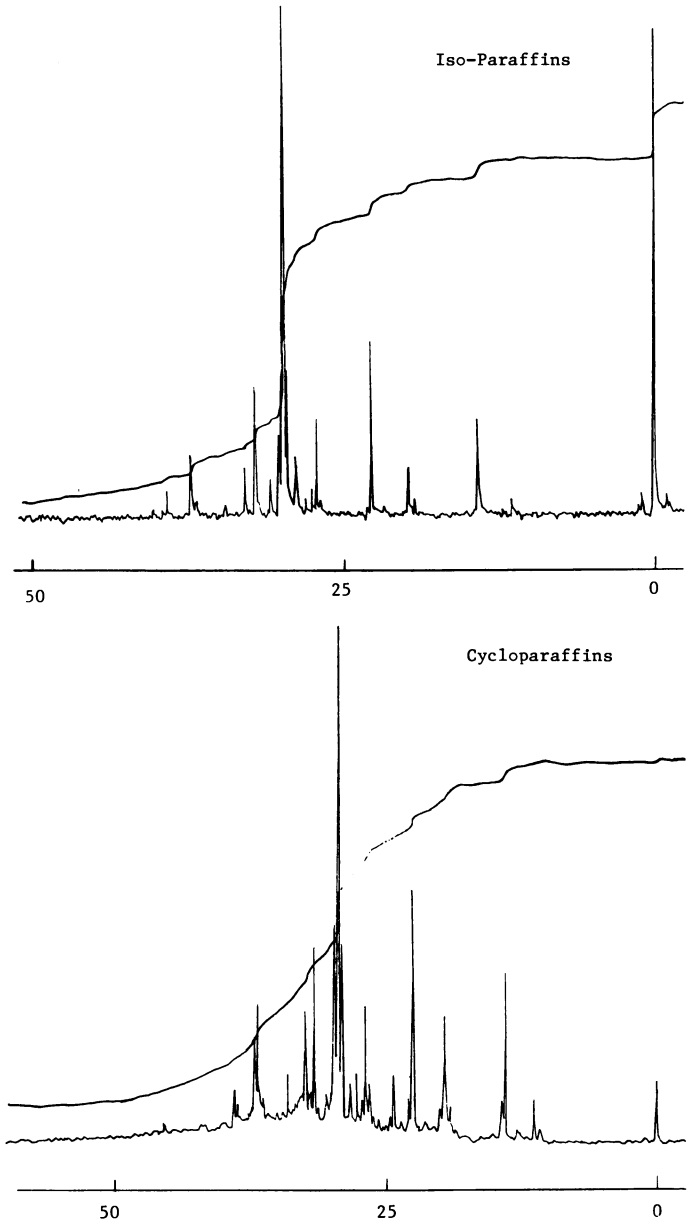


Figure 2b. ^{13}C *NMR of saturate fractions from Kuwait crude oil*

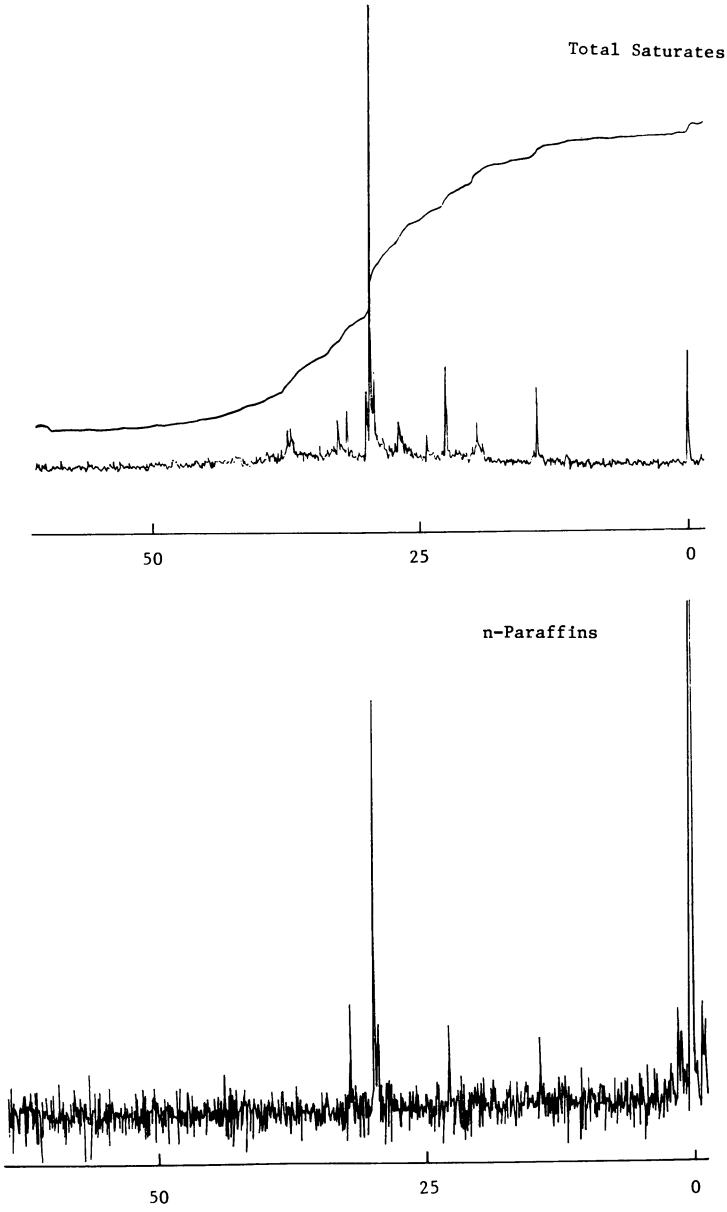


Figure 3. ^{13}C *NMR of total saturates and* n-*paraffins from South Louisiana crude oil*

methylenes, the average carbon number of the nonnormal paraffin is 16 carbons. The ratio of methyl to ethyl to higher branches is 6:2:6.

The spectra of the isoparaffins and cycloparaffins are extremely rich also, showing all the expected features. The isoparaffins have a signal in the 11.4-ppm region typical of the CH^3 directly attached to the alkyl chain, while that is missing from the cycloparaffin spectra. Figure 3 shows the ^{13}C NMR spectra of the total saturates and the normal paraffins of South Louisiana crude. Again, when compared with the spectra of the corresponding Kuwait fraction (Figure 2a), the same expected resonances are observed, although the relative intensities may be somewhat different.

The significance of these spectra is that they allow one to follow unequivocally the biodegradation path. If, for example, the methyls directly attached on the chains are first removed, then the 19.6-ppm signal would disappear selectively. Removal of the ethyl groups would be indicated by the removal of the methyl at 11.4 ppm and 10 ppm. Given the great power of the technique, it is surprising that this tool has not been used much more extensively to study the biodegradation paths of the various hydrocarbon chains.

Figure 4a shows the ^{13}C spectra of the total aromatics and di- + triaromatics subfractions of Kuwait crude oils. Figure 4b shows the ^{13}C spectra of the monoaromatics and di- + triaromatics from South Louisiana crude. These have been recorded with a commercially available CFT-20 Varian Associates ^{13}C NMR spectrometer. $CDCl_3$ solutions of each fraction have been utilized along with tetramethylsilane (TMS) for calibration purposes. The numbers under the individual peaks indicate the chemical shifts of nonaromatic carbons in the range of 14.11 ppm to about 37.16 ppm.

South Louisiana monoaromatics show a very large peak at 27 ppm, which is completely missing from the di- plus triaromatics. It is also interesting to compare the terminal methyls intensity (Table VI) in these four fractions. Clearly, Kuwait fractions have a greater portion of their intensity as methyls (5%–6% vs. 2%–3% for the South Louisiana crude).

Table VI. ^{13}C Parameters of Mono and Di- + Triaromatic Fractions of Kuwait and South Louisiana Crudes

	Aromatic Aliphatic	*Terminal Methyl Total Intensity*
Kuwait monoaromatics	0.11	0.06
Kuwait di- + triaromatics	0.24	0.05
S. LA monoaromatics	0.09	0.03
S. LA di- + triaromatics	0.40	0.02

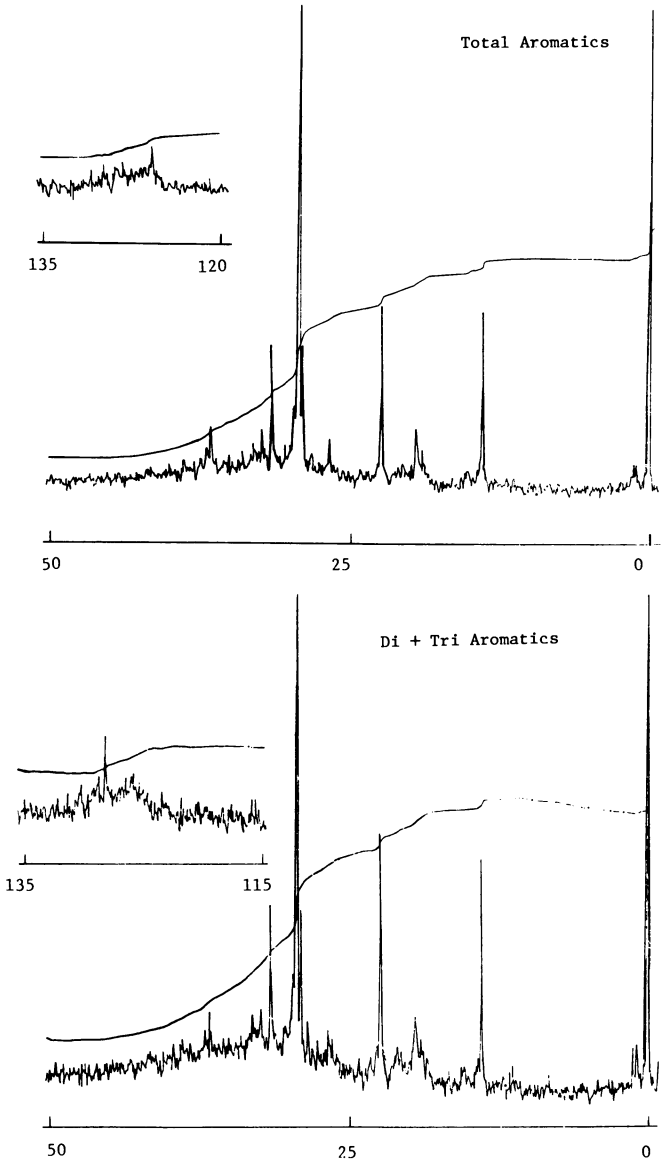


Figure 4a. ^{13}C *NMR of aromatic fractions from Kuwait crude oil*

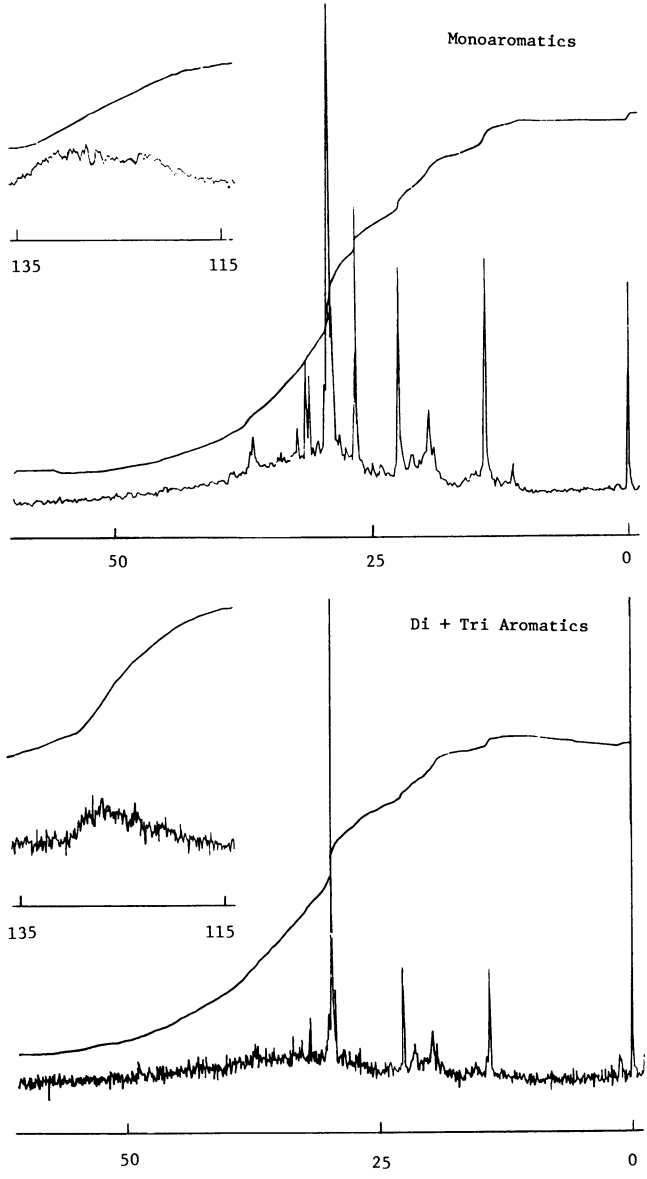


Figure 4b. ^{13}C *NMR of aromatic fractions from South Louisiana crude oil*

Gas Liquid Chromatography

Gas liquid chromatography (GLC) has been used for a number of years (*31–34*) to obtain fingerprints of oil spilled in the aquatic environment. In addition, the ASTM in 1974 published a tentative GLC method (*35*) for the identification of waterborne oils and in 1970 published a tentative GLC method (*36*) for determining the boiling range of petroleum fractions. Primary intent of these methods (excepting the 1970 ASTM method) has been to assist in identifying the source of oil in the aquatic environment. Several investigators using GLC have also followed the degradation of spilled oil as a function of time. Because of the complexity of petroleum, the exact identification of each component in the GLC chromatogram is not possible. Normal paraffins can usually be identified, as can the isoprenoids, pristane, and phytane. For oil identification purposes, the overall features of the chromatogram are used as a fingerprint to be compared with the corresponding fingerprint of suspected sources.

We propose here that fingerprinting be carried out after separation of the oil into fractions. Blumer and Sass (*37*) followed the degradation of a fuel oil spill by GLC fingerprinting of the aromatic and saturate fractions. We suggest that much more useful information can be obtained by separating the saturate fraction into normal paraffins, isoparaffins, and cycloparaffins. To illustrate this, a Kuwait crude was fractionated to the scheme of Figure 1, and the GLC fingerprints were obtained on various saturate fractions (Figures 5a and 5b). These chromatograms show that all components having boiling points less than C_{12} normal paraffins were removed in the pretreatment process mentioned previously. The numbers on the chromatogram correspond to the carbon numbers of the normal paraffin, that is, 15 or C_{15} refers to $C_{15}H_{32}$ (pentadecane).

The chromatogram (Figure 5a) for the total saturate fraction is typical of a moderately paraffinic crude. It contains a broad, unresolved envelope that underlies the partially resolved peaks of normal paraffins, isoparaffins, cycloparaffins, and isoprenoids. The middle chromatogram in Figure 5a is the homologous series of normal paraffins after the nonnormal paraffins, including the isoprenoids, are removed. The lower chromatogram shows what is referred to as the nonnormal paraffin fraction. It contains the unresolved envelope on which the isoparaffins, cycloparaffins, and isoprenoids are superimposed. The peaks are generally not well resolved because of the large number of possible isomers that can be present.

The nonnormal fraction can be separated further into an isoparaffin and cycloparaffin (naphthene) fraction. The nonnormal paraffin chromatogram is shown again in Figure 5b for comparison purposes. The

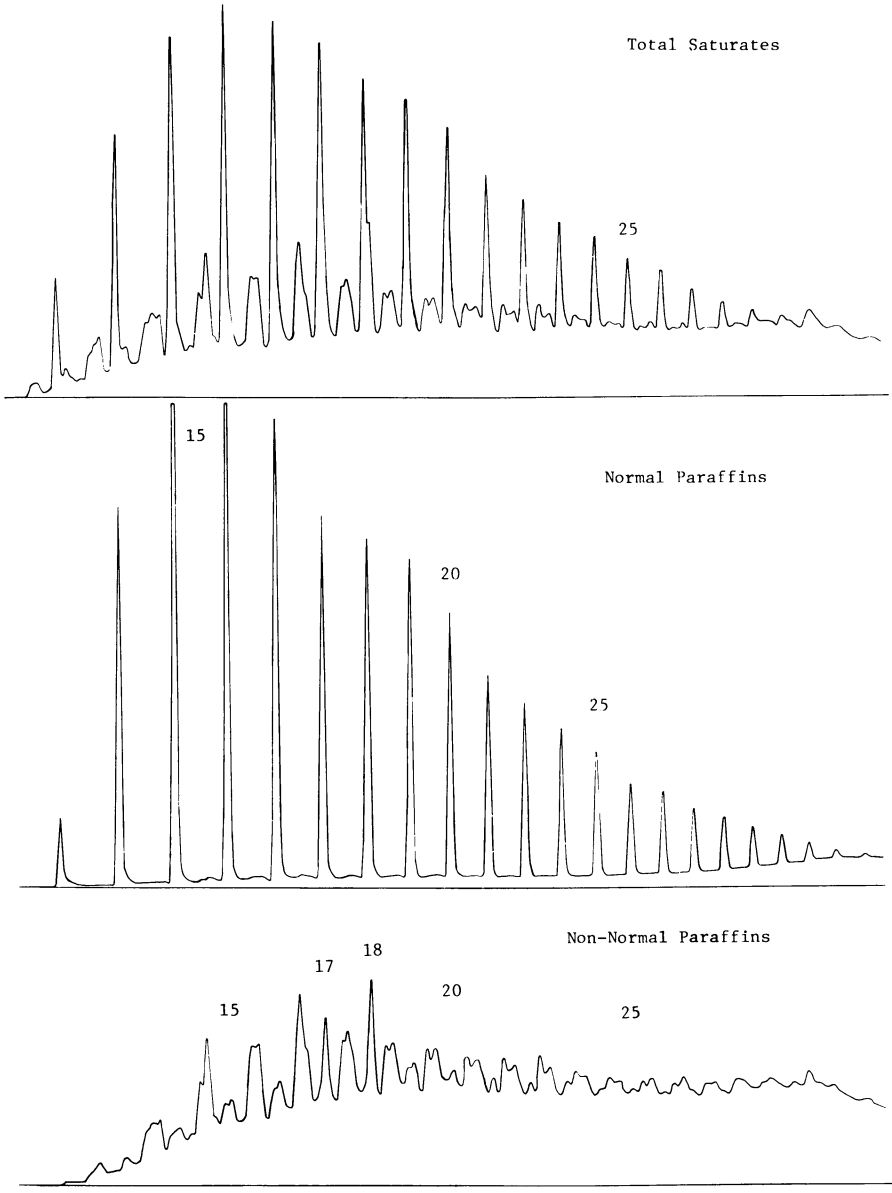


Figure 5a. GLC fingerprints of Kuwait crude saturates (total saturates, normal and nonnormal paraffins)

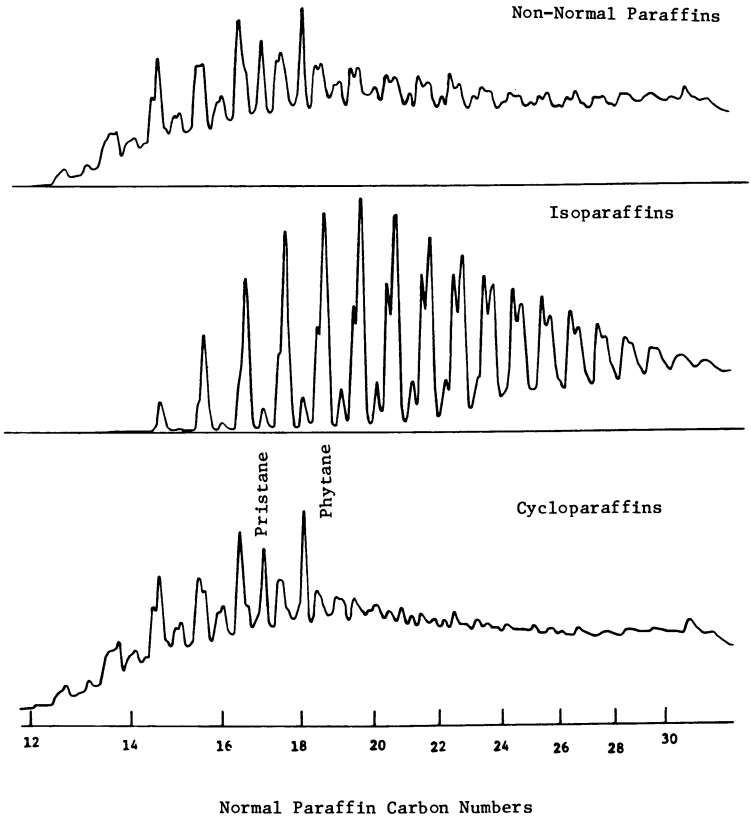


Figure 5b. GLC fingerprints of Kuwait crude saturates (nonnormal, iso- and cycloparaffins)

middle chromatogram is the fingerprint of the isoparaffins after the cycloparaffins have been removed. The cycloparaffin fraction is shown in the lower chromatogram, and it contains the broad, unresolved envelope with partially resolved peaks. The ratio of total cycloparaffins to isoparaffins is about 10:1 (Table II). The isoparaffin fraction, being only a small portion of the total saturate fraction, is nearly indistinguishable in the total saturate fingerprint. Only by separating the saturate into its fractions can the degradation of the isoparaffins be followed. The fingerprints indicate that the isoparaffins have a hydrocarbon distribution with a higher boiling range than do the cycloparaffins or normal paraffins.

The isoprenoids are not isolated with urea but are concentrated in the cycloparaffin fraction. They can be isolated with 7-Å molecular sieves (38). Pristane and phytane are indeed enhanced in the cycloparaffin fraction as shown in Figure 5b. The retention time of pristane was verified by spiking. Both of these isoprenoids are considered to be

present since the peaks for these two components are much greater than would be predicted by the peak heights for the hydrocarbons occurring at the normal paraffin markers on either side (C_{16}) and (C_{19}). Pristane occurs as a doublet with heptadecane (C_{17}), but it is masked in the fingerprint of the total saturate fraction by the relatively large amount of heptadecane. It is present in the fingerprint of the nonnormal fraction at the C_{17} marker. Phytane appears in the nonnormal fraction at the C_{18} marker and also in the total saturate fingerprint as a doublet with octadecane (C_{18}).

Different crudes have different GLC fingerprints, which are the basis for their use as an identification technique. The fractions also most likely will have different fingerprints. The isoparaffin fractions of Kuwait crude and South Louisiana crude are shown in Figure 6. The Kuwait isoparaffin fingerprint has a series of doublets between normal paraffin markers 17 and 21, whereas the South Louisiana isoparaffin fingerprint does not have any apparent doublets in this region of the chromatogram.

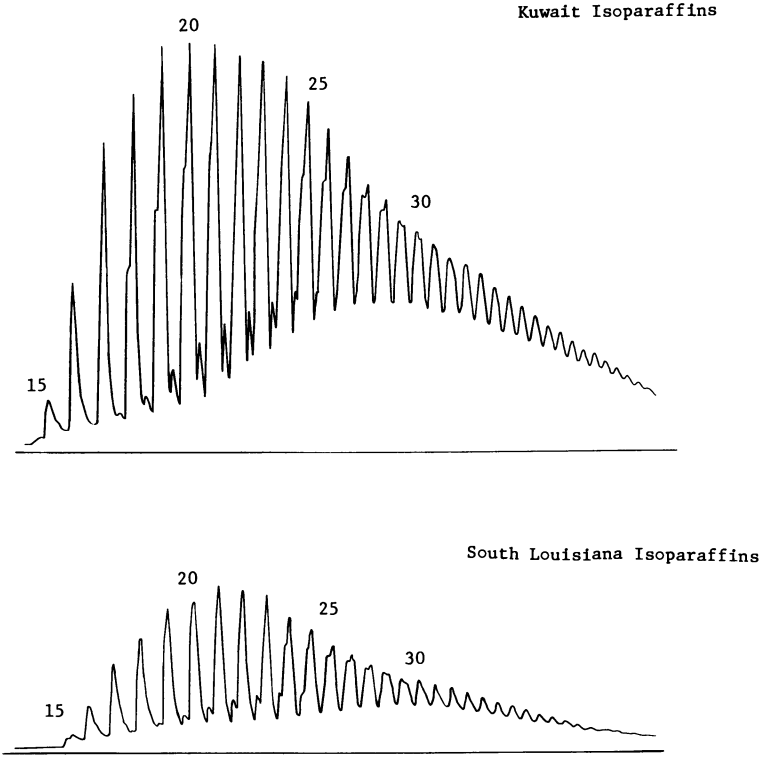


Figure 6. GLC fingerprint of the isoparaffins from Kuwait and South Louisiana crude

Biodegradation

To better understand the chemical changes occurring during the biodegradation of petroleum, a study with a domestic crude oil from South Louisiana (*18*) was conducted in which the oil–water system was inoculated with a mixed bacterial culture from Chesapeake Bay (*9–14*).

The use of the combination of techniques discussed previously produces a greater insight into the fate of the various chemical classes of petroleum that occurred during biodegradation than the information obtained when single techniques are used or when only individual components are monitored.

Table VII summarizes chromatographic separations of the pretreated, weathered, and biodegraded South Louisiana crude. Pretreatment (flowing N_2 at 70°C for 24 hr) simply removes the volatile components (up to and comparable to C_{12} saturates): it is a necessary step in the analysis; it simulates, to a degree, the natural volatilization that takes place in exposed crude oils; and it establishes a baseline against which weathering and biodegradation can be measured. The amount of the virgin crude volatilized under these conditions varies, depending on the nature of the crude (38% loss in this case). The nonvolatile components (residue) are the components of interest in the study of structural changes attributable to factors other than volatility.

Table VII. Distribution of Molecular Types of a South Louisiana Crude Comparing Biodegraded, Weathered, and Original Material

	Pretreated Original		*Sterile Weathering*		*4-Week Biodegradation*	
	Wt (mg)	*Wt %*	*Wt (mg)*	*Wt %*	*Wt (mg)*	*Wt %*
Total saturates	36.0	58.0	37.0	43.0	14.0	39.0
n-paraffins	6.0	9.7			—	—
non-*n*-paraffins	30.0	48.3			14.0	39.0
(isoparaffins)	(0.9)	(1.5)				—
(cycloparaffins)	(29.1)	(46.8)			(14.0)	(39.0)
Total aromatics	17.5	28.2	32.0	37.2	9.7	27.0
monoaromatics	6.8	11.0			2.7	7.5
di- + triaromatics	9.5	15.3			7.0	19.5
polyaromatics	1.2	1.9			—	—
Resins	8.0	12.9	16.0	18.6	12.0	33.4
Asphaltenes	0.5	0.9	1.0	1.2	0.2	0.6
Total residue (per 100 mg of virgin crude)	62.5	100.0	86.0	100.0	35.9	100.0

The combined processes of volatilization plus oxidation in a sterile environment are referred to as weathering. It is interesting to compare pretreatment and weathering results (Table VII). While the amount of saturates in the residue is the same (36 mg vs. 37 mg), on a percentage basis saturates contribute a larger amount in the pretreated material. The reason is that during pretreatment there remain fewer aromatics (17.5 mg vs. 32 mg) and resins (8 mg vs. 16 mg) than during weathering. The amount of asphaltenes remains essentially unchanged and at a very low level. Oxidation, with resulting increase of resins, can take place even in a sterile environment, and it may result in a further decrease of residue through the enhanced solubilization of the oxidized material. When actual weathering effects are assessed on a crude oil, the data in the pretreated columns represent the best baseline information for comparative purposes.

Microbial action (Table VII) can lead to further decrease in the residue as well as to important changes in the distribution of the various chemical classes. In the example on hand, there is a 75% loss of the original material (35.9 mg of residue) corresponding to a removal of crude four times greater than on mere weathering. This further loss is clearly due to microbial action.

The four-week bacterial action results in a significant increase of oxygenated chemical species. The resins now constitute some 33% of the residue compared with 13% in the baseline material. This increase would be expected to be primarily at the expense of the condensed aromatics, which have a greater propensity to undergo oxidation compared with monoaromatics. Indeed, the distribution of aromatic types shows that upon biodegradation, the polyaromatics have been removed completely. The other aromatics have been reduced, but on a percentage basis the di- plus triaromatics are a larger fraction of the residue, indicating a preferential loss of the monoaromatics, either by volatilization (lower molecular weights) or a greater solubilization of the corresponding oxygenated species.

Significant changes are evident in the saturate fraction. Not only are the saturates affected to a greater degree than any of the other fractions, but the distribution of the types of saturates is also greatly affected. Specifically, all the normal paraffins are removed, as well as the isoparaffins, leaving only the cycloparaffins after the bacterial action of four weeks.

These conclusions, drawn from the chromatographic separations of the various chemical classes, are supported and are further refined by ^{13}C NMR, GLC, ^{1}H NMR, and mass spectrometric measurements.

The complete removal of all but the cycloparaffins is further demonstrated by the GLC tracings of the pretreated, weathered, and

four-week biodegraded saturate fraction (Figure 7). The total loss of the sharp peaks in the calibrated chromatogram of the biodegraded sample is clear evidence of the total absence of normal and isoparaffins. This group of GLC tracings gives further support to preferential removal of more normal and isoparaffins by weathering compared with the pretreatment.

Significant corroborative information as to the structural changes taking place can be gained from the ^{13}C NMR spectra. Figure 8 shows the spectra of the cycloparaffins of the baseline or pretreated material, as

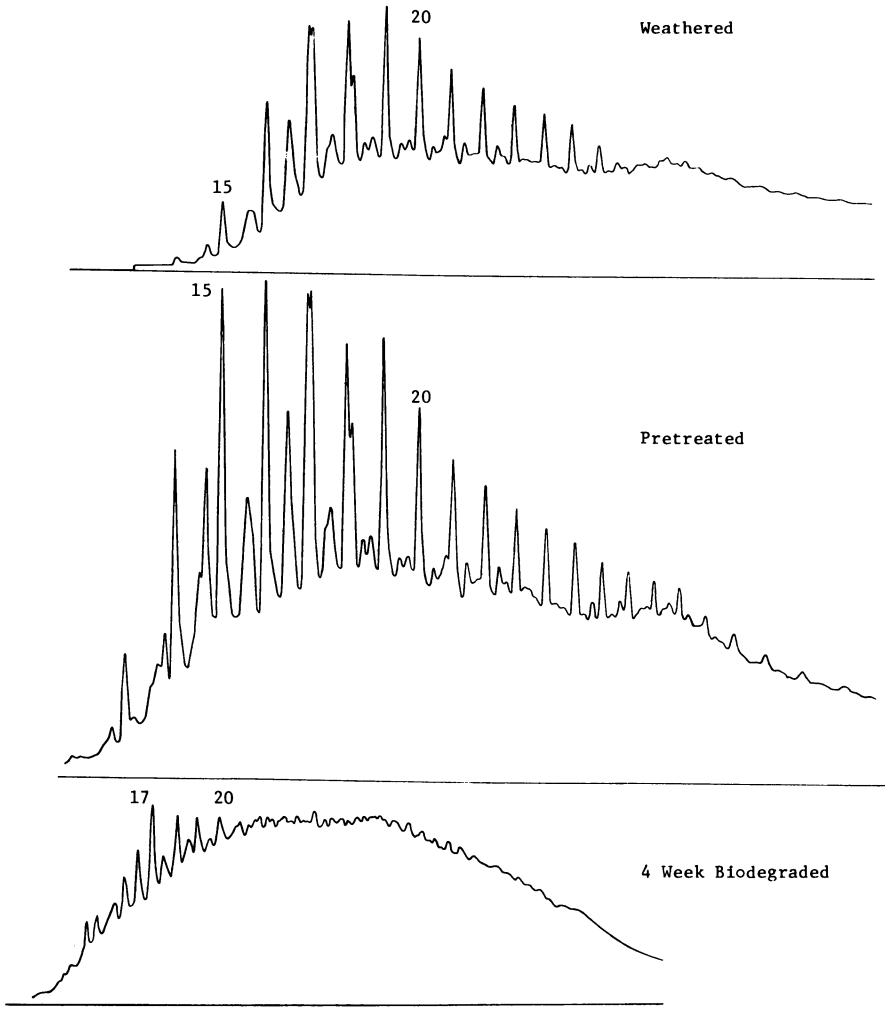


Figure 7. GC fingerprints of saturates

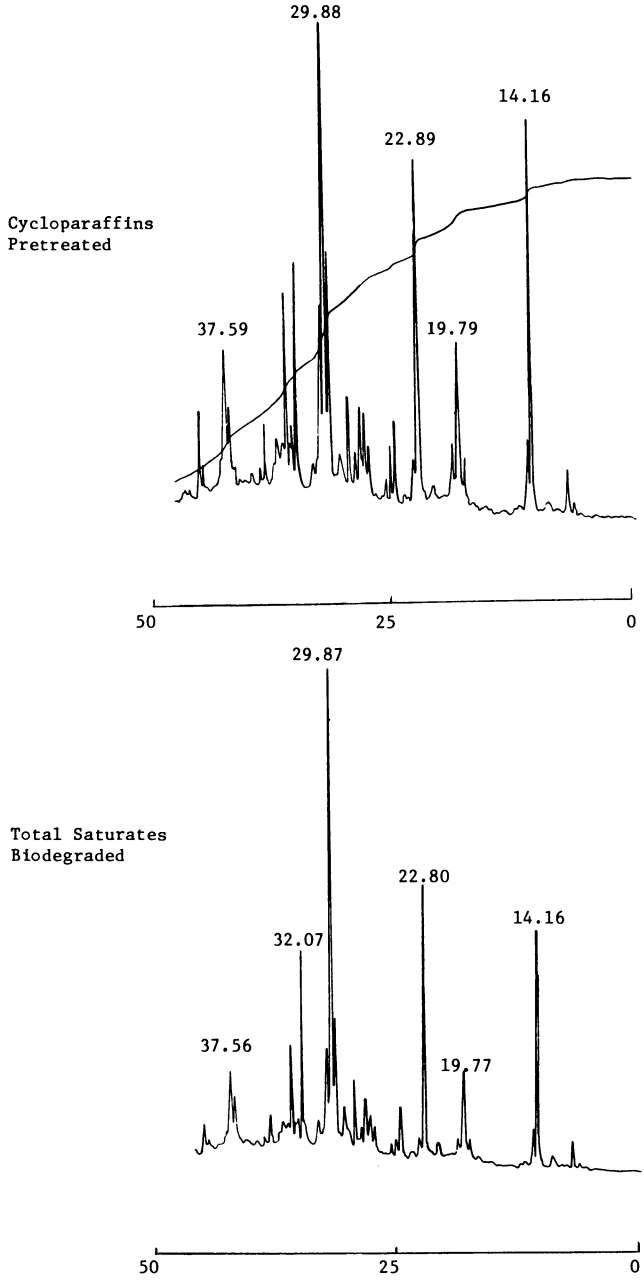


Figure 8. *^{13}C NMR spectra of aliphatic fractions*

well as the total saturates of the biodegraded material. Clearly, the two spectra are identical in both chemical shifts and relative intensities.

The carbons of the main skeletons of the saturated hydrocarbons are present (14, 23, 29.2, 29.9, and 32 ppm). In addition, signals are observed that are associated with branching of the saturated hydrocarbons, such as methyls attached to long chains ($\sim$ 19.7 ppm) and ethyl groups at 11.4 ppm. It is most interesting that these small side chains are evident in the biodegraded material. This observation, coupled with the total removal of normal paraffins, leads to the conclusion that bacteria strongly prefer not to attack cyclic hydrocarbons with branched chains.

Table VIII lists the ratio of the intensities of methylene and terminal methyl groups as an indication of average chain length. The pretreated material, which still contains normal paraffins and even few isoparaffins, has an average chain of 13 carbons compared with 9 carbons for the chains of cycloparaffins. The biodegraded fraction has an average chain length of 9 carbons, further supporting the contention that the biodegraded material is exclusively cycloparaffin. The removal of normal paraffins would reduce the chain length since the very small chains are already lost during weathering.

The ^{13}C NMR spectra of aromatic fractions (Figure 9) and average parameter calculations therefrom (Table IX) indicate that biological reactions primarily occurred on the condensed aromatic rings and the

Table VIII. ^{13}C NMR-Derived Profiles of Total Saturates of South Louisiana Crude

	Intensity (CH_2):Intensity (CH_3)	*Average Chain Length (No. of Carbons)*
Total saturate fraction	7.6	13
Cycloparaffins of virgin crude	4.3	9
Total saturates of 4-week biodegraded	3.8	9

Table IX. NMR-Derived Structural Profile of the Aromatic Fraction of Virgin and 4-Week Biodegraded South Louisiana Crude

	Virgin Crude	*4-Week Biodegraded*
Aromatic carbon	0.38	0.32
Saturate carbon	0.62	0.68
Aromatic rings/molecules	1.4	1.1
Alkyl substituents/molecule	3.5	3.4
Number of carbons/alkyl substituent	3.6	3.9
Naphthenic rings/molecule	1.5	0.8

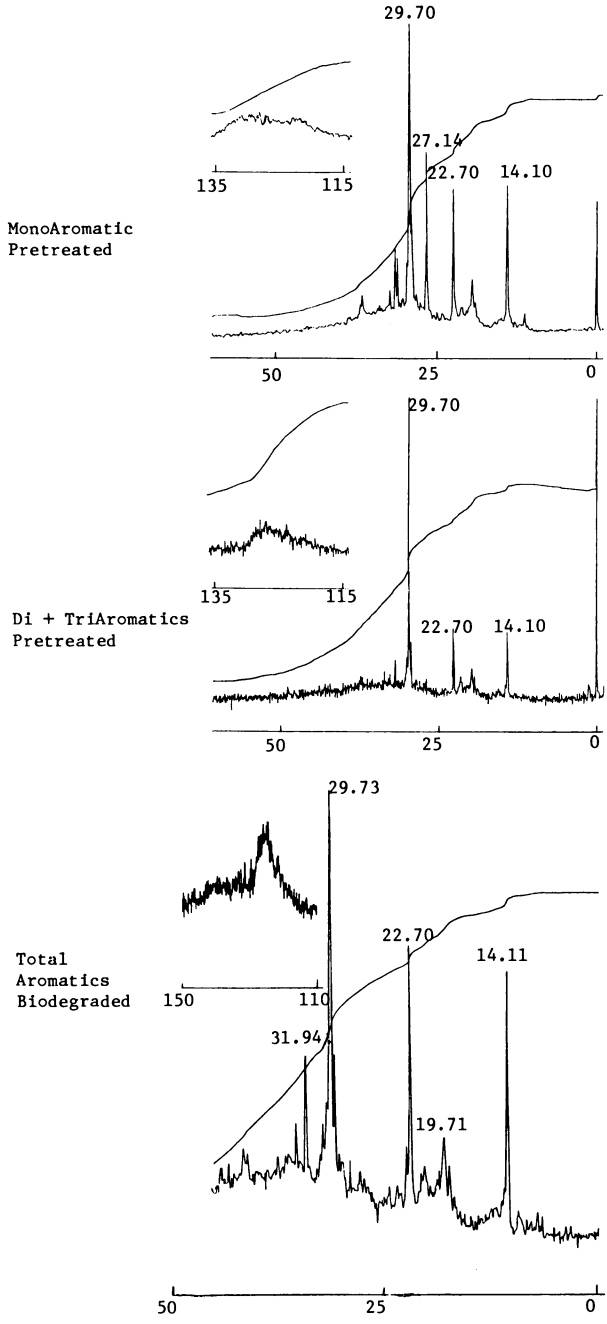


Figure 9. ^{13}C *NMR spectra of aromatic fractions*

longer chain substituents on monoaromatic rings, leaving noncondensed aromatic structures in the residue. The remaining high-molecular-weight aromatic hydrocarbons consist of benzenoid molecules connected by alkyl chains or cycloparaffin rings, which are not fused with the aromatic ring (hydroaromatics).

This is consistent with the separation data in Table VII, which show the decrease in monoaromatics and loss of polyaromatics.

It is very interesting to compare the saturate region (10–40 ppm) for both Figures 8 and 9. It is clear that except for the carbon directly attached to the aromatic nucleus, the signals are identical. The ring part of the molecule, whether it is saturated or not, plays little role in determining the chemical shift. However, the presence of a ring, saturated or unsaturated, inhibits the attack of the molecule by bacteria. Finally, we point out the prominent peak at 27.14 ppm, which is present in the monoaromatics of the pretreated material but lacking completely from the biodegraded material. Such a signal is due most likely to a methine or methylene alpha to an aromatic ring.

Table X lists similar information obtained from the mass spectrometric analyses of weathered South Louisiana and Kuwait crudes.

Table X. Mass Spectrometric Analyses of Weathered Crude Oils (Wt %) [a]

	Kuwait Crude	*South Louisiana Crude*
Saturate Group-Type Analysis		
alkanes	5.9	11.2
1-ring cycloalkanes	4.2	11.7
2-ring cycloalkanes	3.0	9.6
3-ring cycloalkanes	2.3	6.9
4-ring cycloalkanes	2.3	7.3
5-ring cycloalkanes	1.3	5.25
6-ring cycloalkanes	0.6	3.1
	19.6 (32.9)	55.0 (57.8)
Aromatic Group-Type Analysis		
monoaromatics	13.9	8.6
diaromatics	9.4	7.4
triaromatics	3.8	3.0
tetraaromatics	1.7	1.3
pentaaromatics	0.5	0.2
aromatic sulfur species	6.7	1.0
unidentified aromatics	2.6	0.9
	38.6 (46.8)	22.4 (28.8)

[a] % referenced to unweathered material.

Comparison of studies in Tables III and X shows that the mass spectrometric analyses demonstrate the differences in behavior of the various chemical classes due to weathering. For example, the alkanes of the Kuwait crude are 5.9% after five weeks of weathering, while in the pretreated material they are 9.8%. Actually, on an absolute weight basis, the amounts present may be considerably different because of the continued disappearance of some classes of compounds upon weathering.

Similar information is provided by the mass spectrometric analyses of the aromatic fractions. For example, for the South Louisiana crude the monoaromatics are 8.6% by weight after five weeks of weathering compared with 16.6% of the starting material. Similarly, the diaromatics show a dimunition, while the Kuwait crude shows relatively little change.

The significance of this kind of approach is shown in Figure 10, which depicts the mass spectrometric results of the alkanes remaining in a South Louisiana crude exposed to bacteria from two different sites of Chesapeake Bay. The remaining alkanes after exposure to microorganisms for various time intervals are referred to as the alkanes that remain after weathering. Thus, Figure 10 clearly illustrates not only that the microorganisms consume the alkanes to an extent greater than simple weathering but also that the rates and extent of biodegradation are dependent on the microorganisms present (*9–14*).

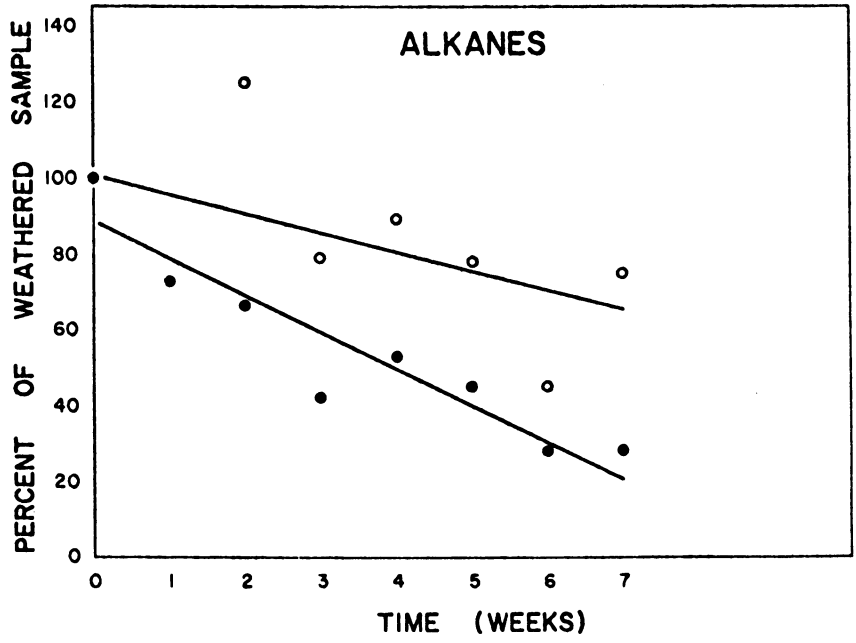


Figure 10. Alkane degradation by sediment bacteria from (○) Eastern Bay and (●) Colgate Creek

Conclusion

We have shown that modern analytical methodology presents us with the opportunity to develop detailed structural profiles of the highly complex, multicomponent mixtures of petroleum and petroleum fractions. These structural profiles can be extremely valuable in studies of the fate of oil in the marine environment as well as in the development of oil spill cleanup schemes. This is possible because the various chemical classes do not behave in the same manner in a given set of environmental conditions, nor do they respond identically to various bacterial flora. The detailed structural profiles involve the use of separation as well as characterizational tools particularly suitable to the study of petroleum. Novel use is suggested for ^{13}C NMR, which holds much promise, especially for delineating such questions as point of microbial attack in biodegradation of petroleum. Emphasis is placed on the synergism of the various analytical tools and on the need to compare biodegraded material with set baseline data (starting material). The need for group-type analyses is also emphasized. These analytical schemes are illustrated with the profiles of two different petroleum crudes.

Literature Cited

1. "Proc. 1977 Conference on Prevention and Control of Oil Pollution," API/EPA/USCG, New Orleans, LA, March 1977.
2. "Proc. 1975 Conference on Prevention and Control of Oil Pollution," API/EPA/USCG, San Francisco, CA, March 1975.
3. "Proc. Symp. on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment," Am. Inst. Biol. Sci., American University, Washington, DC, August 1976.
4. Malins, D. C., Ed. "Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms"; Academic: New York, 1977; Vols. I and II.
5. "Petroleum in the Marine Environment"; National Academy of Sciences: Washington, DC, 1975.
6. Stevenson, J. C. *J. Fish. Res. Board Can.* **1978**, *35*(5), 499–796.
7. Wolfe, D. A., Ed. "Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems"; Pergamon: New York, 1977.
8. Betz, A. P. *Anal. Chem.* **1976**, *48*, 454A.
9. Walker, J. D.; Colwell, R. R.; Petrakis, L. *Appl. Microbiol.* **1975**, *30*, 1036.
10. Ibid., p. 79.
11. Walker, J. D.; Colwell, R. R.; Petrakis, L. *Can. J. Microbiol.* **1976**, *22*, 423.
12. Ibid., p. 598.
13. Walker, J. D.; Colwell, R. R.; Petrakis, L. *J. Water Pollut. Control Fed.* **1975**, *47*, 2058.
14. Walker, J. D.; Colwell, R. R.; Petrakis, L. "Proc. 1975 Conference on Prevention and Control of Oil Pollution," March 1975, p. 601.
15. Jewell, D. M.; Ruberto, R. G.; Albaugh, E. W.; Query, R. C. *I&EC Fund.* **1976**, *15*(3), 206.
16. DOE, U. S. Bureau of Mines, "Characterization of the Heavy Ends of Petroleum," Semi-annual Report No. 5, API Research Project 60, **1968**, p. 7.
17. Smith, H. M.; Hale, J. H. *U. S. Bur. Mines Rep. Invest.* **1966**, 6846.

18. Pancirov, R. J. API Report No. AID IBA 74, February 5, 1974, American Petroleum Institute: Washington, DC.
19. Jewell, D. M.; Albaugh, E. W.; Davis, B. E.; Ruberto, R. G. *I&EC Fund.* **1974,** *13*(3), 278.
20. Jewell, D. M.; Weber, J. A.; Bunger, J. W.; Plancher, H.; Latham, D. R. *Anal. Chem.* **1972,** *44*(8), 1391.
21. Jewell, D. M.; Ruberto, R. G.; Davis, B. E. *Anal. Chem.* **1972,** *44*(14), 2318.
22. Jewell, D. M.; Ruberto, R. G.; Davis, B. E. *Anal. Chem.* **1975,** *47*(12), 2048.
23. Robinson, C. J.; Cook, G. L. *Anal. Chem.* **1969,** *41,* 1548.
24. ASTM "Annual Book of Standards," American Society for Testing and Materials: Philadelphia, PA, 1978; Part 24, Method D2786.
25. Jewell, D. M.; Ruberto, R. G.; Swansiger, J. T. *Am. Chem. Soc., Div. Petr. Chem., Prepr.* **1975,** *20*(1), 19.
26. Clutter, D. R.; Petrakis, L.; Stenger, R. L.; Jensen, R. K. *Anal. Chem.* **1972,** *44,* 1395.
27. Lindeman, L. P.; Adams, J. R. *Anal. Chem.* **1971,** *43,* 1245.
28. Grant, D. M.; Paul, E. G. *J. Am. Chem. Soc.* **1964,** *86,* 2984.
29. Jensen, R. K.; Petrakis, L. *J. Magn. Reson.* **1972,** *6,* 105.
30. Levy, G. C.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience: New York, 1972.
31. Kneider, R. E. "Joint Conference on Prevention and Control of Oil Spills," API/EPA/USCG, Washington, DC, June 1971.
32. Institute of Petroleum Standardization Committee. *J. Inst. Pet. London* **1970,** *56,* 107–117.
33. Brunnock, J. V. *J. Inst. Pet. London* **1968,** *54,* 310–325.
34. Ramsdale, S. J.; Wilkinson, R. E. *J. Inst. Pet. London* **1968,** *54,* 326–332.
35. ASTM "Annual Book of Standards," American Society for Testing and Materials: Philadelphia, PA, 1978; Part 31, Method D3328.
36. ASTM "Annual Book of Standards," American Society for Testing and Materials: Philadelphia, PA, 1978; Part 24, Method D2887.
37. Blumer, M.; Sass, J. Woods Hole Oceanographic Institution, April 1972, WHOI-72-19.
38. Curran, R.; Eglinton, G.; MacLean, I., Douglas, A. G.; Dungworth, G. *Tetrahedron Lett.* **1968,** *14,* 1669.

RECEIVED October 12, 1978.

3

Oil Spill Identification and Remote Sensing[1]

ALAN P. BENTZ

United States Coast Guard Research and Development Center,
Avery Point, Groton, CT 06340

This chapter is comprised of two distinct parts. The first is a review of the state of the art in oil spill identification techniques. It includes significant developments that have firmly established oil fingerprinting as a valid approach to identification of oil spill sources. These include: increased understanding of weathering phenomena, refinement of analytical techniques and pattern recognition techniques for oil comparison, adoption by ASTM of new standard methods for spill identification, establishment of a Central Oil Identification Laboratory (COIL) and satellite Field Oil Identification Laboratories (FOIL) by the U.S. Coast Guard, and four successful tests of the analytical methodology in court. The second part of this chapter is a brief account of development of planned and operational remote sensing systems as applied to oil spill detection, identification, and mapping.

Since the author's review on "Oil Spill Identification" (*1, 2*), a number of significant occurrences have firmly established oil fingerprinting as a valid approach to the identification of spill sources. These include increased understanding of the effects of weathering on oils, continued refinement of various analytical techniques, continued refinement of computer pattern recognition techniques for comparison of oils, the adoption by ASTM of new methods of analysis of waterborne oils (*3, 4, 5*), the establishment of a Central Oil Identification Laboratory (COIL)

[1] The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Commandant or the Coast Guard at large.

by the U.S. Coast Guard with satellite Field Oil Identification Laboratories (FOIL) throughout the country, and, most significantly, four successful tests of the analytical methodology as evidence in courts; three in federal courts and one in a New York State court. This, coupled with airborne surveillance by the Coast Guard, has provided sufficient deterrence, for example, to stop significant spills in the Florida Straits since July 1977 (*6*). Kleineberg (*7*) recently reviewed the operational oil identification techniques employed by the U.S. Coast Guard to identify the sources of spilled oil.

Interest in the impact of spills has been given additional impetus by the *Argo Merchant* disaster in December 1976 and by the more recent (March 1977) grounding of the *Amoco Cadiz* off the coast of France, which resulted in the largest oil spill ever recorded (over 200,000 tons of light crude oil).

The problem of oil spills is here to stay. Spill incidents in and around U.S. waters have been averaging between 10 and 11 thousand per year in recent years (*8*), with 80% of the spills being less than 800 gal (*9*). About 350,000 tons of oil are being spilled accidentally each year. For the year 1975, the Coast Guard estimated that four times that amount was dumped deliberately by oil tankers in the normal course of operations—cleaning cargo tanks or emptying ballast water (*10*). Sleeter (*11*) cites National Academy of Sciences figures for the same year as showing six times as much oil per year from cleaning operations as from spills (tanker and nontanker accidents). Oostdam and Anderlini (*12*) point out that although spectacular oil spill accidents are well documented, very little has been published concerning the level of pollution along the coasts of the world's major oil-producing countries in the Middle East, where the specter of a major oil spill in the semienclosed gulf between Iran and Arabia becomes a frightening possibility.

To assess the full short- and long-term ecological impacts of major oil spills, an immediate and coordinated scientific response is required. To delineate the requirements for such a response, the Montauk Workshop on Oil Spill Studies: Strategies and Techniques was convened in February 1976 (*13*) and dealt with such topics as information management, chemistry, and the effects on plankton, benthos, fish, birds, marine mammals, and sea turtles. The Conference on Assessment of the Ecological Impact of Oil Spills (held by the American Institute of Biological Sciences and cosponsored by the American Petroleum Institute and seven government agencies at Keystone, Colorado, June 1978) addressed the ecological studies of numerous recent major oil spills. The quality of information improved with each spill as experience was gained and the degree of coordination was increased. Immediate, well-integrated response in the case of the *Amoco Cadiz* resulted in better sampling and a

clearer picture of the oil distribution. The scope of this paper does not permit further discussion of fate and effects of oil spills other than to cite a key bibliography (*14*) soon to appear that has over 1200 references, each with abstracts.

New responses for cleanup are required; one of these is the use of chemical dispersants. Obviously, if dispersants come into common use, the question must be answered whether any given analytical technique can still identify the source of the spill. At least two studies are underway in this area: one by the Coast Guard and the other by the University of Rhode Island, under a Department of Energy contract.

Legal Aspects

Oil spills have a large economic impact on fisheries and resort and recreational areas, with the result that legal settlements are frequently large. Ultimately, the oil spill analyst must also be a forensic chemist: this author addressed the oil spill chemist's philosophy and technical approach in a presentation to the American Bar Association's Session on Marine and Tanker Problems (*15*).

Sleeter (*11*) examined the evidentiary aspects of the techniques used for oil fingerprinting and cited one case where the fingerprinting techniques resulted in a pretrial liability settlement. He concluded that a significant amount of experience has been gained in fingerprinting and that it meets the FRYE test of having "gained general acceptance in the particular field in which it belongs." He thus concludes that "the case for the admissability of fingerprint identification seems strong," since the court in *Stifel* had noted "neither newness nor lack of absolute certainty in a test suffices to render it inadmissable in court . . . (and) . . . every useful new development must have its first day in court."

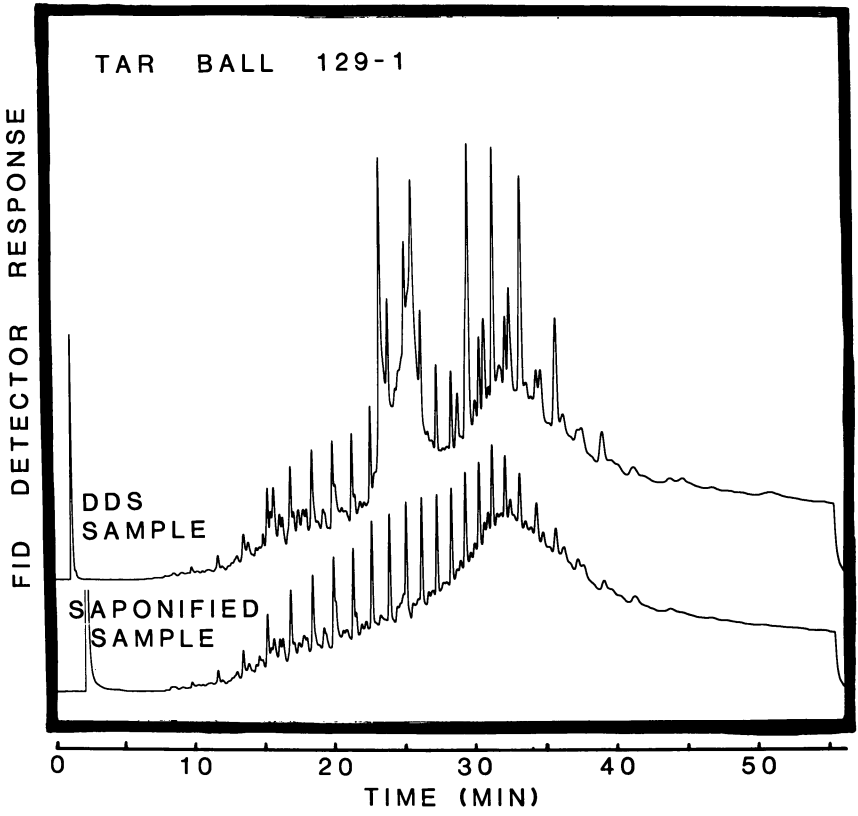
Since Sleeter's review, several cases have been tried using chemical fingerprinting to determine the source of an oil spill. The three cases tried in 1977 emphasized the importance of documentation, beginning with the taking of the sample (long before the chemist becomes involved) and requiring an unbroken chain of custody until the sample is disposed of.

At the time of a spill investigation, it is hard to realize that the case may not appear in court for three to five years, at which time the fallibility of the human memory becomes agonizingly apparent. The result is that everything must be fully documented. In the first federal court test of chemical oil fingerprinting conducted in 1977 in Houston, the judge made it clear that the on-scene investigation was not thorough and made equally clear that the scientific testimony was persuasive. Judge Finis E. Cowan stated, ". . . if anything, the physical facts lean in favor of the defendant.

This court is basing its ruling entirely and one hundred percent on the (scientific) testimony . . . which the court finds to be credible and believable and scientifically reliable."

Several civil court cases have been decided on the basis of a single analytical method. This is certainly warranted when the results are unequivocal. The first citable case is that of *U.S.* vs. *Slade, Inc.* (*16*), in which the unreported case of Judge Cowan's is referenced. Both cases used gas chromatography with flame ionization and flame photometric (sulfur) detection as the only analytical method.

The merit of the multimethod approach lies in the added confidence given the results from a concurrence of several methods. The multimethod approach is most desirable when contaminants picked up by the spill may



U.S. Coast Guard

Figure 1. Gas chromatograms of a contaminated Cape Cod tar ball before (upper curve) and after (lower curve) cleanup by a saponification technique

obscure the results by one or more methods. The presence of contaminants that may come from biologically generated glycerides, bilge cleaners, etc., will be apparent from the nonpetroleum aspects of the fingerprint by any given method. Some specific methods used, however, may be insensitive to contaminants and thus allow for designation of a match despite the presence of contamination in one of the two samples compared.

Aside from having backup methods available in the event of interference, it is possible in many instances to clean up the sample to obtain the true petroleum fingerprint. One such method is that of Frame et al. (*17*), in which saponification is used for sample cleanup. An example of the use of saponification is shown in Figure 1, which shows gas chromatograms of a tar ball from Cape Cod before and after cleanup. These analyses were conducted on a 3-m packed column with 10% OV-101. The temperature was programmed from 75°–325°C at 8°C/min and held isothermally at the maximum temperature for 32 min. The helium flow rate was 30 mL/min. After the saponification treatment, it is clear that a normal petroleum-type chromatogram that can be compared with suspected sources is obtained. In this instance, the tar ball was shown not to originate from either the *Argo Merchant* or the *Grand Zenith,* as first thought, thus releasing the shipowners from liability.

A major legal aspect is the adoption of standard methods for oil fingerprinting such as the ASTM consensus standards, because it facilitates acceptability of the findings as evidence in court cases.

Recent Developments in Spill Fingerprinting Methodology

Overview. The Coast Guard recently published a definitive manual on spill identification (*18*) that included sections on oil sampling, sample handling and transmittal, simulated weathering of oils, identification by gas chromatography (GC), fluorescence spectroscopy (FL), low-temperature luminescence (LTL), infrared spectroscopy (IR), thin-layer chromatography (TLC), and high-pressure liquid chromatography (HPLC). The basic Coast Guard oil identification system is comprised of GC, FL, IR, and TLC methods. Sections also included are a field manual for infrared oil spill identification, a field classification of oils by infrared spectroscopy, and one on safety aspects of the laboratory procedures.

The ASTM has already published a "preamble" to oil identification (*19*) and methods on preservation of samples (*20*), elemental analysis (*21*), and infrared analysis (*22*), in addition to the GC and FL methods (*4, 5*) and sample preparation methods (*3*) previously mentioned. Widespread adoption of these carefully developed methods and their under-

lying philosophy in the analytical approach used should lead to more uniform practices.

The Coast Guard has selected two complementary methods for use by its Central Oil Identification Laboratory (COIL), which is an operational unit devoted full time to oil spill fingerprinting, namely LTL and HPLC. Other methods that have been used are atomic absorption spectroscopy (AA), emission spectroscopy (ES), X-ray fluorescence (XRF), gas chromatography–mass spectrometry (GC–MS), mass spectrometry (MS), and limited wet methods of analysis (*23*).

Following is a look at the most recent developments in oil spill identification by several of these oil fingerprinting techniques, including sampling and a discussion of an interlaboratory study on the effects of weathering on the capability to match oils.

Sampling. Representative samples of spills and suspect oils are a must to obtain definitive results in oil matching. Teflon strips and Lipopore screens (*24*) continue to show the most promise for sampling slicks for laboratory analysis. The Lipopore screen, manufactured by Hydroil Corporation, of Plymouth, New Hampshire, is undergoing extensive field testing by the operational Coast Guard. The Lipopore screen is used to admit oil, with the exclusion of water, into a cannister lowered to the water surface by means of a monofilament line. The cannister is replaced into its shipping jar for transmittal back to the laboratory.

Weathering. Most of the analytical methods selected for oil spill fingerprinting work very well on "neat" (unweathered) oils. When oil is spilled on water, it immediately starts to change composition as a function of the effects of water solubility, evaporation, temperature, sunlight, wind and wave action, bacterial action, etc. Therefore, most of the development of instrumental methodology requires taking weathering into account, on the premise that the weathering changes of a given oil are smaller in magnitude than differences between two different oils of the same type. To a large extent this is true, although it may not be in the very light petroleum products.

The Coast Guard, perceiving a need to test its fingerprinting methods and those of ASTM, conducted a large-scale interlaboratory (*25*) weathering study to see the effects of weathering on four different oil types (No. 2, No. 4, and No. 6 fuel oils, and a Louisiana crude) weathered over four days, sampling daily. Sixteen laboratories, most of them active on ASTM task groups, participated.

The interlaboratory study showed overall that the GC procedure gave the best results, followed closely by IR and then FL. When the IR spectra were interpreted by those expert in oil spill matching, the IR results were significantly better than GC or FL, indicating that IR is the method most influenced by "expert" interpretation.

The major conclusion of the interlaboratory study confirms our growing belief in the necessity for a simple, standard method for accelerated or simulated weathering in the laboratory of suspect samples prior to comparing them with spill samples. All of the methods were better able to match weathered samples with other weathered samples of the same oil than they were to the unweathered oil.

Numerous workers have studied simulated weathering. Brown et al. (*26*) and Frankenfeld (*27*) conducted two-year studies under Coast Guard contract on the weathering of oil and devised some effective rapid simulation methods that could be the basis for future standardized methods. Ahmadjian et al. (*28*) studied simulated weathering by infrared spectroscopy. Flanigan et al. (*29*) investigated the effects of several methods of laboratory weathering on the results of various analytical methods. They also attempted to separate the relative importance of variables such as dissolution, evaporation, photooxidation, etc. as to their effects. Dissolution and evaporation were the variables that most affected GC and IR; photooxidation most affected fluorescence.

Anderson et al. (*30*) have been developing a technique for weathering neat samples of suspect oils (i.e., with total absence of water) in a thin film under long-wavelength ultraviolet radiation with air passed over the surface. This technique permits convenient accelerated weathering of small samples—from approximately 1 mL in a small petri dish to 30 mg on an infrared salt plate. Under these conditions, the ultraviolet becomes an important factor in weathering, particularly in the generation of carbonyl.

Gruenfeld and Frederick (*31*) developed an evaporative method that can be used for samples as small as 70 mg. The sample, contained in a vial, is suspended in a 40°C water bath for 15 min in the presence of a filtered airstream. Gas chromatograms of the "weathered" sample showed that it gave virtually the same trace as a 50-mL sample distilled according to the ASTM sample preparation technique. Even more remarkable is the fact that they obtained the same trace from 0.5–30 mg of oil that had been dispersed in water and was extracted and evaporated, that is, they were able to match a sample of South Louisiana crude oil from the water column with a neat sample of oil that had been "weathered."

Zürcher and Thüer (*32*), in a recent paper, examined the effects of suspended solids (kaolinite) on the weathering of a No. 2 fuel oil by GC and IR. They found that there was fractionation of the oil with alkylated benzenes and naphthalenes enriched in the water phase while aliphatic hydrocarbons (above MW 250) were adsorbed. They also found that with increased turbulence, oil droplets were agglomerated with suspended minerals. They observed the same fractionation pattern for a groundwater oil spill, despite the fact that the oil had been biochemically altered.

Infrared. The use of infrared for oil fingerprinting is well documented (*32, 33, 34, 35*). The technique has a well-demonstrated capability and is one of the four primary Coast Guard methods (*18*) that is in operational use by COIL. In addition, it has been tested in the field for over a year in Philadelphia and will shortly begin testing in San Francisco, California, and New York, using relatively inexpensive dual-beam grating instruments.

The biggest breakthrough in the medium-priced instrument field is the advent of commercially available spectrophotometers with digitizing capability. At least two of these spectrometers have software that permits curve ratioing or subtraction and other valuable routines. Digitizing in these instruments assumes a flat baseline, as was anticipated at our first pattern recognition seminar in 1975 (*36*). The advantages of using the horizontal baseline were subsequently demonstrated by Mattson et al. (*37, 38*). At this same seminar, it was decided to standardize on a horizontal baseline drawn from 1975 ± 20 cm^{-1} for oil fingerprinting. Any deviation from this line was considered to be real and meaningful. The cell window material selected was KBr with AgBr or AgCl acceptable for field use.

Commercial instruments, for the most part, read frequencies only at peak maxima that exceed specified thresholds. Unfortunately, on one instrument we found that only half of the standard 18 wavenumbers we use in pattern recognition were recorded. Furthermore, weathering shifts peak positions, which results in different wavenumbers being printed out for nominally the "same" peak, meaning that the pattern recognition was comparing absorbances at slightly different wavenumbers. To correct these problems and to get the data at the desired wavenumbers, it was necessary to interface the commercial instruments with our own designed microprocessor systems, which digitized the absorbance at prespecified wavenumbers, regardless of whether or not there were peak maxima.

In our work, although we use the 18 peaks listed in Table I, some others are digitized because they give valuable information about the sample. Three such peaks are: the 1708 cm^{-1}, which gives a quick estimate of the degree of oxidative weathering; the 1600 cm^{-1}, which gives an idea of the aromaticity; and the 1375 cm^{-1}, which, because of its little variance over the entire population of oils [Mattson et al. (*37, 38*)], is a ready index of thickness.

Mattson also found that the 1375-cm^{-1} peak had the largest analytical error of those peaks measured. Part of the reason is that the peak is so strong that its absorbance approaches 1 in a 0.05-mm cell and 2 in a 0.1-mm cell. According to Hannah (*36*), thick cells in optical null instruments give nonlinear absorbance. They are linear only up to absorbances of 0.8 to 1.0. Ratio recording systems, such as that used by Mattson, are

linear up to 1.3 absorbance units. This suggests that the thickness of 0.05 mm, as used by Brown and the Coast Guard, is best for oil fingerprinting in optical null instruments.

The availability of digitized data has led to a number of pattern recognition schemes (*26, 37–41*). Of these, one of the most useful is Brown's log-ratio method (*26*), which is adaptable to GC and other data as well as IR data. Ahmadjian et al. (*28*) used this method to study simulated weathering. We applied the log-ratio method first to single-beam infrared data (*42*); then, using dual-beam infrared data, we studied the quantitative effects of weathering using a microprocessor of our own design to evaluate all 20 oils used in the previously described interlaboratory weathering study (*43*).

In one of our most recent applications of the Brown log-ratio method, we compensated for weathering by applying weighting factors to the data for each of the peaks, weighting most strongly those peaks that changed the least with weathering (*44*). The peaks we used for matching oils are shown in Table I, along with the empirically assigned weighting factors first selected and a second set labeled vector weighting factors. Both sets of weighting factors improved the results of the log-ratio method and gave clean matches of all but No. 2 fuel oils. We expect that simulated weathering of suspects will be required for optimal results with light oils.

The vector, referred to in Table I, alludes to a promising new pattern recognition technique developed by Killeen et al. (*45*). It uses the data from a suspect and a partially weathered sample of the suspect to estab-

Table I. Weighting Factors for 18 Peaks Used for Comparison

Wavenumber	*Empirical*	*Vector*
1304	0.8	0.9
1165	0.5	0.3
1145	0.3	0.3
1070	0.3	0.3
1032	0.5	0.3
955	0.6	0.9
918	0.7	0.9
890	1.0	0.9
871	0.9	0.9
849	1.0	0.9
832	1.0	0.9
810	0.4	0.4
790	0.5	0.9
782	0.4	0.4
766	0.5	0.9
744	0.7	0.4
722	0.6	0.4
700	0.4	0.4

lish the direction of change for each data point, actually generating an 18-dimensional hyperplane. The spill is then compared with the closest fit to that plane, the closest fit being called the "computer-simulated weathering" data for the suspect. An example of a computer plot for a spilled No. 4 fuel oil is shown in Figure 2, along with the plot of the suspect, which has a significantly different trace. However, the computer-simulated plot for the suspect can be seen to be a virtual overlay to the spill, including a crossover of both at about 980 cm^{-1}. When two different No. 4 fuel oils were compared by the vector method, the simulated weathering curve from one actually diverged from the other, that is, the method does not make all oils look more alike. An illustration with a No. 6 fuel oil can be seen in Ref. *24*.

In a second part of the report on the interlaboratory weathering study (*25*), different pattern recognition techniques were evaluated using the Mann–Whitney *U*-statistic (*46*) . For each of the four oil types 15 pairwise comparisons were made quantitatively, and the results were serially ranked according to the closeness of match. An example is given below for only 5 pairwise comparisons. After ranking the oils sequentially, their identities are tabulated as to whether or not they are the same (*S*) or different (*D*) from the known oils that were analyzed in the interlaboratory study. Ideally, all of the weathered oils from a common source should be grouped together, as shown in Table II. In the actual case, this is not true. The *U*-statistic is determined by counting how

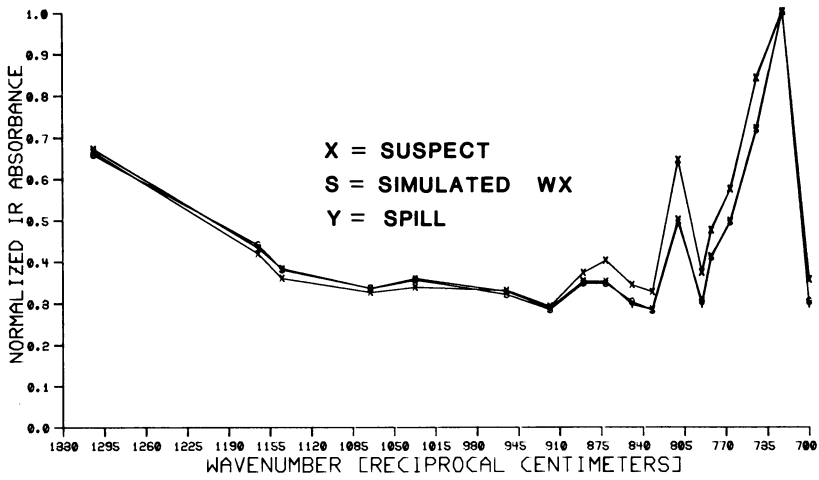


Figure 2. Computer printout of connected data points from a spilled No. 4 fuel oil and a suspect. It also shows the computer-simulated weathering data for the suspect as a near overlay for the spill.

Table II. Illustration of Mann–Whitney *U*-Statistic

	Rank					
	1	*2*	*3*	*4*	*5*	*Score*
Ideal	*S*	*S*	*S*	*D*	*D*	0
Actual	*S*	*S*	*D*	*S*	*D*	1

many times an *S* is separated by a *D* from the other *S*'s; that is, each time an *S* must pass a *D* to join the other *S*'s, the *U*-statistic is incremented by one. In the actual case illustrated, the *S*-ranked No. 4 must pass one *D* at rank 3 and the *U*-value is therefore 1.

With 15 comparisons involving six pairs of *S*'s and nine pairs of *D*'s unweathered oils, the maximum *U*-value would be 54; the value for random ranking would be 27. The better pattern recognition schemes gave values of 0–5; that is, the lower the number generated, the better the method was in making oil comparisons. (*Note*: The random chance of getting a numerical value of less than 6 is 0.4%.)

The evaluation involved four basic methods and variants of them. They included: the standard visual overlay method, or a modification of it that involved some linear measurements in the fingerprint region of the infrared spectrum; the Brown log-ratio method (*26*); the vector method devised by Killeen et al. (*44, 45*); and the standard ASTM method (*22*). The overlay method was the best method when used by someone experienced in interpreting weathered oils.

The quantitative methods required digitization of 18 peaks (*see* Table I), either manually or electronically. The log-ratio method was evaluated using an average percent difference value for the 18 peaks, or alternatively the Brown S^2 statistic (*26*), which seemed a better measure of the validity of the method. The log-ratio method was also evaluated using a variety of weighting factors for each peak (*see* previous discussion). The vector method and log-ratio methods were both significantly improved by artificially weathering suspects prior to comparison with spill samples. Further work is being conducted in this area. The primary conclusion is that for quantitative comparisons, an artificial weathering of suspects is needed for optimum results.

Grizzle and Coleman (*34, 35*) recently reported an extensive study on crude oils. Although its results are internally consistent, some practices are not recommended for routine oil spill fingerprinting. They are:

1. *The Use of Distillation as an Artificial Weathering with a 10-mL Sample.* There is nothing inherently wrong with this approach. It closely parallels the standard ASTM D3326 procedure. The problem arises from the fact that in real world situations actual samples may be 1 mL or less.

2. *The Use of Sodium Chloride Cells.* This is perfectly acceptable when comparing to other NaCl spectra. However, Brown (*26*) showed that KBr and AgCl gave better results and could be compared with each other but not with NaCl. The consensus (*36*) was that KBr is the recommended window material.
3. *The Use of 0.1-mm Cells.* For routine use, we recommend the 0.05-mm sealed-demountable cells because some of their absorbances in 0.1-mm cells are in the nonlinear range for optical null instruments and the standard deviations rise significantly. Furthermore, a single sealed cell changes with continued use, particularly when some intractable spill samples may have traces of residual water. We have demonstrated that triplicate analyses of a No. 2 fuel oil show not even line width variations in a properly used sealed-demountable cell.
4. *The Use of a Straight Percent Difference Calculation.* The method used by Grizzle and Coleman again was satisfactory for their purposes. It lacks two properties of the Brown log-ratio method, which make the latter much better suited for routine oil comparisons. First, the Brown equation cancels out thickness dependence. This not only includes variations within a given cell but also, and more importantly, permits comparison with any oil in the data file analyzed at any time in any cell. The second, and still more important, distinction for anyone comparing oils in data files is the fact that the Brown method, by using logs, makes it immaterial how the oils are compared, X:Y or Y:X. This is not true of the Grizzle–Coleman equation, which compares absorbances A at the ith frequency for two oils, X and Y:

$$\left(\frac{A_i^X}{A_i^Y} - 1.0\right) 100 = \% \text{ difference}$$

From this equation, it can be seen that the ratio and its reciprocal would give different answers, conceivably indicating a match when compared one way and a nonmatch when compared the other way.

Despite the refinements yet to be made with the use of infrared fingerprinting of oils, there is no doubt that it is one of the better methods.

Gas Chromatography. Gas chromatography is one of the "workhorse" methods used for oil identification. The newly approved ATSM Method D3328-78 (*4*) incorporates simultaneous flame photometric detection along with flame ionization detection. Such dual detection, using a high-resolution SCOT column is the routine method employed by COIL for Coast Guard analyses.

Since the last review by this author (*1, 2*), an early paper in this field came to light in which Johnson and Fulmer (*47*) used three detectors simultaneously—FID, and FPD for both sulfur and phosphorus. The phosphorus FPD was considered especially useful for detecting the presence of contaminants of biological origin, such as vegetable oils.

The thermionic nitrogen–phosphorus detector (NPD) was employed by Lee et al. (*48*), using an SE-52 SCOT column for fingerprinting used engine oils for forensic purposes. Nitromethane extracts containing the polynuclear hydrocarbon fraction yielded peak patterns with either an FID or NPD, which were characteristic for a particular engine.

Meanwhile, Flanigan and Frame (*49*) successfully applied a commercially available NPD with an independently heated rubidium bead to fingerprint petroleum and synthetic oils. They found that FID, used simultaneously with the NPD, was useful to assess NPD response to the large excess of nonnitrogen-containing hydrocarbons coeluting with the nitrogenous compounds. They also demonstrated that the NPD-active nitrogen compounds could be concentrated and isolated from the majority of interfering hydrocarbons by the use of an alumina chromatographic column. The hydrocarbons were first eluted with a nonpolar solvent, followed by a more polar solvent to remove the sample of interest.

Frame et al. (*50*) have published an atlas of over 70 dual FID/NPD chromatograms of a wide variety of oils separated on a 15.2-m Dexsil 300 SCOT column. They note that the NPD fingerprints of light oils may be lost after weathering, but that those of spilled heavy crudes and residual oils are very stable. For example, they were able to match the NPD peak pattern of an open ocean oil slick sample to the *Argo Merchant* cargo after the slick had been on the water for three weeks.

Kawahara (*51*) found that he could identify spilled asphalts by making the pentafluorobenzyl thioethers and ethers from traces of mercaptans and phenols in asphalts, and separating them by gas chromatography, using an electron capture detector to obtain the distinctive chromatograms.

Rasmussen (*52*) used a 30.4-m Dexsil 300 SCOT column (compared with the 15.2-m used by COIL) to obtain high-resolution separations. He then numbered the minor peaks between the *n*-alkane peaks and expressed the areas of each of these as a percentage of the preceding *n*-alkane. For each alkane peak, strings of numbers were generated that could be compared between oils for identification, particularly those above C_{14}.

Another use of SCOT SE-52 columns was reported by Overton et al. (*53*), in which a glass capillary 35 m long was coated with SE-52 to obtain sufficiently high resolution to perceive crude oil pollution among sediment

hydrocarbons. The authors feel that glass capillaries offer an answer to the difficult problem of distinguishing petroleum-type hydrocarbons in the marine environment from hydrocarbons of recent biogenic origin.

MacLeod et al. (*54, 55*) have found the high resolution achieved by glass capillaries to be extremely useful in identifying the source of oil pollution in marine biota. Two-meter capillaries with rapid temperature programming were excellent for screening, since they could analyze C_{10}–C_{34} hydrocarbons in 7 min vs. 40 min for a 2-m packed column of comparable resolution. High-resolution separations were achieved with a 20-m capillary column. The saturated and aromatic hydrocarbon fractions were separated on a silica gel column first and analyzed separately by capillary GC. Horizontal scale expansion showed 15–40 discrete hydrocarbon components in the intervals between adjacent *n*-alkane peaks; these were found useful in determining the identity of the source oil.

Figure 3 shows the separation achieved of the saturated hydrocarbons from the stomach contents of cod near the *Argo Merchant* oil slick. These were virtually identical to saturated hydrocarbons in the *Argo Merchant* cargo. These samples were obtained from homogenized tissue that was digested with alkali, solvent extracted, and chromatographed on silica gel. The GC analysis was carried out on a 20-m glass capillary of 0.25-mm i.d., coated with 0.5-μm film of SE-30. A 0.2-μL sample was injected (splitless for 12 sec, then split 10:1 with 14 psig He carrier). The temperature was isothermal at 40°C for 5 min, then programmed to 270°C at 4°C/min. The findings showed that the stomach contents of cod collected at a particular location undeniably had been contaminated with *Argo Merchant* oil. There were indications that windowpane flounder may have ingested some of the oil, based on the saturated hydrocarbon profile, but the aromatics were too low for comparison.

MacLeod et al. (*56*) made use of their capillary technique to make weathering estimations for spilled oil from the barge *Bouchard No. 65* (Buzzard's Bay, Massachusetts, January 1977). This was accomplished by obtaining peak areas for the *n*-alkanes, C_9–C_{22}, plus pristane and phytane in the aliphatic fraction and for 21 arenes among the aromatics. The alkanes were normalized to C_{17}; the arenes to phenanthrene. From these numbers, the percentage loss of each was computed, and the overall oil losses were estimated by taking a weighted average of the alkene and arene loss estimates. The arenes exhibited greater percentage losses than the alkanes (as much as 89% despite the frigid conditions of the spill). The losses generally correlated with exposure to the atmosphere.

In a study reported at the 1977 Pittsburgh Conference (*57*), we examined quantitative changes in gas chromatograms for different oil types weathered over four days. All peak heights were normalized to C_{20} on the supposition that the C_{20} peak would be stable. However, even C_{20}

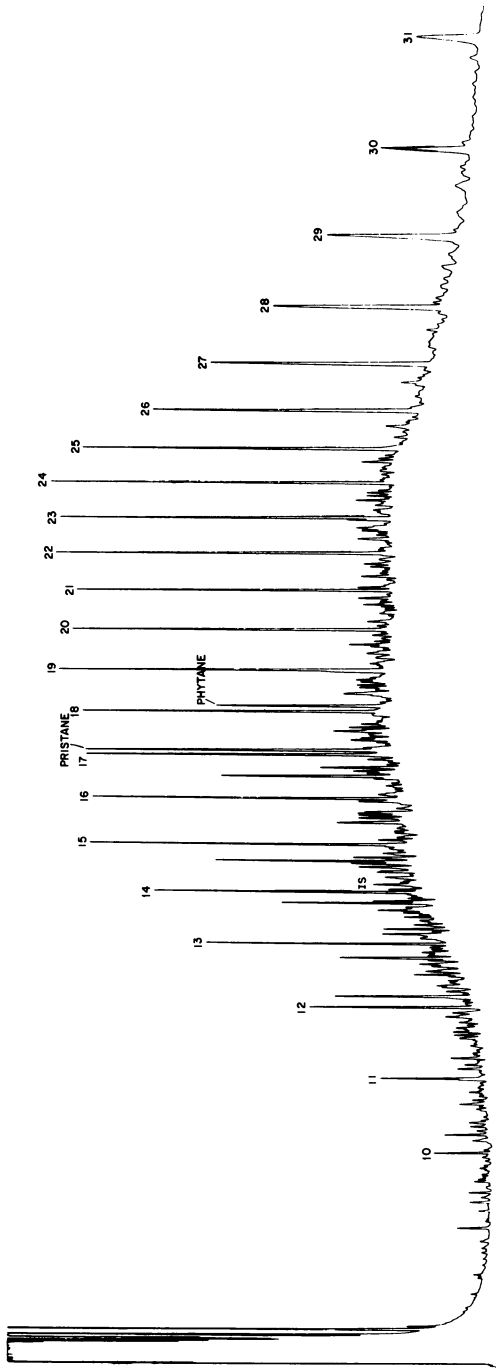


Figure 3. Glass capillary gas chromatogram (high resolution) of saturated hydrocarbons from stomach contents of cod near the Argo Merchant *oil slick, which is virtually identical to saturated hydrocarbons in* Argo Merchant *cargo oil. Numerals denote* n*-alkane chain length* (55).

showed some loss in No. 2 fuel oil. The peak heights were found generally to diminish logarithmically. A semilog plot of normalized peak heights vs. days of weathering gave a straight line of steep slope for C_{12} and lesser slopes for C_{13} and C_{14}. From C_{15} upward, the lines were nearly horizontal, with some slight discontinuities between Day 0 and Day 1 weathering. The lines were given by the expression

$$Z = Z_0 e^{mt}$$

where Z = peak height, Z_0 = intercept, m = slope, and t = weathering time (days).

This equation suggested that once the slope and intercept are known for a given oil type, a peak height measurement could be used to estimate the weathering time in days. This calculation was made for a No. 2 fuel oil as shown in Table III. These data seem to indicate that under certain circumstances, some rough estimates of weathering time might be possible, but the problem would require further study for this assumption to be made generally applicable.

Other conclusions of the quantitative study were that sample preparation is very important and samples being compared should be analyzed under identical conditions—preferably on the same day. Best matching was achieved if suspects were subjected to accelerated weathering before analysis. The Brown log-ratio (*26*) was found applicable and was used to demonstrate that a good range of hydrocarbons to use (one which is relatively insensitive to weathering) is C_{16}–C_{22}, including pristane and phytane peaks.

Fluorescence. The fluorescence spectroscopic (FL) method approved by ASTM (*5*) is similar in most respects to that used by the Coast Guard. Unless the samples are collected soon after the spill occurs, it is not recommended for volatile fuels such as gasoline, kerosene, and No. 1 fuel oils because of their rapid changes with weathering. Some No. 2 fuel oils and light crude oils may be identifiable with only up to one or two days of weathering.

Table III. Calculated Weathering Time from C_{12} and C_{14} Peak Heights

Actual Days	C_{12}	C_{14}
1	0.91	1.42
2	1.78	2.52
3	2.99	3.50
4	3.97	4.02

Fluorescence applied to oil identification has been an active field, with 17 papers presented on the subject at the last three Pittsburgh Conferences. A number of interesting developments for fluorescence and low-temperature luminescence (LTL) are described by Eastwood et al. (*58*). These include synchronous scanning, difference spectrofluorometry, synchronous difference spectroscopy, derivative spectroscopy, and total luminescence (or contour) spectroscopy and combinations of these techniques. In a recent presentation, Eastwood and Hendrick (*59*) reported an extension of their low-temperature luminescence studies to include polarized excitation and emission spectroscopy, and time-resolved phosphorescence. Preliminary studies of polarization effects indicate that differences exist in low-temperature polarized luminescence spectra of oils, which may aid in oil identification. In the time-resolved phosphorescence spectra of oils, the most significant difference observed was enhancement of the vanadyl porphyrin signal at approximately 700 nm for short delay times (20 μsec).

Fortier and Eastwood (*60*) reported on the use of low-temperature luminescence to achieve more structure than room temperature fluorescence spectra for fuel oils, thereby enhancing oil-matching capabilities. Eastwood et al. (*61*) conducted FL and LTL studies on weathered oils. They tested corrected emission spectra at room temperature and at 77 K (20 ppm oil in methylcyclohexane) using excitations at 254, 290, and 330 nm; synchronous scanning, with a 25-nm wavelength offset between excitation and emission to obtain additional structure; and difference spectra (dual-beam) for direct comparison of changes due to weathering. Matching capabilities usually appeared to be satisfactory for up to one week of weathering for heavy oils and up to two days of weathering for light oils.

Another recent paper showing the advantage of synchronous scanning over conventional fluorescence emission, as applied to geochemistry, is that of Wakeham (*62*). He found that greater information on the aromatic content of oils could be obtained by separating the aromatic hydrocarbon fractions by liquid–solid column chromatography prior to fluorescence analysis. He was able to distinguish indigenous and petroleum hydrocarbons found in sediments of Lake Washington.

With the advent of computerized systems and readily available hardware, total luminescence is gaining adherents. Brownrigg and Hornig (*63*) and Hornig and Giering (*64*) have reported on the low-temperature total luminescence applied to weather oils. Warner et al. (*65*) at the University of Washington applied sophisticated pattern recognition techniques to resolve a model mixture of nine petroleum-type polynuclear aromatic compounds from the complex total luminescence emission–excitation matrix (EEM).

From the same University of Washington group, Johnson et al. (*66*) reported rapid scanning fluorescence spectroscopy based on a "novel" scheme for polychromatic irradiation of the sample cuvette and the use of a silicon-intensified target (SIT) vidicon detector to measure all regions of the EEM simultaneously. The computer-controlled instrument, patented in 1977 by Callis and Davidson (*67*) is capable of acquiring an EEM spanning up to 240 nm in emission and excitation wavelengths at a spatial resolution of 1 nm per point in a time as short as 16.7 msec. The instrument shows promise for improving multicomponent capabilities of fluorescence assays, at some cost in sensitivity. One major disadvantage is that for absolute spectroscopy each of the resolution elements must be calibrated individually, but only a few matrix locations may suffice for certain types of analysis.

Independently, Jadamec et al. (*68*) developed a similar system for continuous real-time acquisition of fluorescence emission spectra of eluting petroleum oil fractions separated by high-pressure liquid chromatography. Using an optical multichannel analyzer detector, which has a spectral response over the range of 190 to 900 nm, the spectral window between 290 to 400 nm was examined (0.2 nm per channel) with a complete frame scan of the detector target every 31 msec. This system too prints out quasi-three-dimensional displays in numerous formats. Its value lies in the fact that the short scan times permit assessment of eluting peak homogeneity and identification of the compounds present in pure fractions. The prototype instrument designed by this group will soon be modified to permit simultaneous analysis by ultraviolet absorption spectra for those compounds that do not fluoresce.

The University of Washington group, Christian et al. (*69*), recently introduced a unique micro-flow-through cell for fluorescence determination of effluents from liquid chromatography columns. A laminar flow design reduces scatter and minimizes dead volume. The sample volume can be maintained at less than 100 nL with a wide range of flow rates.

Miscellaneous Methods for Oil Identification. High-pressure liquid chromatography has already been mentioned in conjunction with rapid fluorescence detection. It fully characterizes the polar fractions containing the polynuclear aromatic hydrocarbons and could be of use in those cases where their overall fluorescence fingerprint cannot distinguish between two oils.

Saner et al. (*70*) report on the use of reverse-phase liquid chromatography for the identification of oils using dual ultraviolet absorption detection (210 and 254 nm). During these studies, Saner et al. (*71*) showed that temperatures elevated to 60°C diminished resolution for 5-μm-diameter particles (both C_8 and C_{18}) but increased resolution for 10-μm-diameter C_{18} reverse-phase columns.

In the most recent edition of *Chromatography* by Heftman (*72*), 11 references from the early 1970s are cited in which gel permeation chromatography (GPC) is used for either the examination of high-molecular-weight petroleum fractions or the "identification of crude oils, etc., by 'fingerprint' comparison."

Saner and Fitzgerald (*73*) reported a method for thin-layer chromatographic identification of oils that was the basis for the current Coast Guard TLC method employed by COIL and FOIL.

Hendrick and Eastwood (*74*) recently reexamined the use of vanadium and nickel concentrations and V:Ni ratios as a means of identifying weathered oils, because of the reputed stability of the ratios. They used a DC argon plasma emission echelle spectrometer equipped with a microprocessor. A statistically designed experiment (two-level factorial) showed that there was no enhancement effect of sodium in the concentration ranges of interest. These workers showed that the plasma emission method was applicable to heavy crude and fuel oils even after several days of weathering with as little as a half a gram of sample when the oil has a concentration of at least 20 ppm of V and Ni. The sample is analyzed merely by diluting with spectroquality xylene (no ashing or sample pretreatment required). For lighter oils where metal ion concentrations are lower, and where larger samples of weathered oils are not available, the method is less useful. This was a preliminary evaluation and not all instrument parameters were fully optimized.

Finally, an interesting new approach using computerized gas chromatography–mass spectrometry (GC–MS) is being developed by Flory et al. (*75*) and Koons et al. (*76*). The technique involves analysis of selected fractions with their fingerprints plotted by computer. Specific ions indicative of indigenous biological markers have been monitored. Two series of biological markers chosen were the C_{15+} alkane series and their corresponding isoprenoids, and the steroid- and triterpenoid-type hydrocarbons (the C_{27+} cyclics). For the two groups, *m/e* 191 (five-ring naphthenes or triterpanes) and 217, four-ring naphthenes (steranes), respectively, were monitored and the computer enhanced the data by subtracting background to produce a reconstructed gas chromatogram from each. The mass chromatograms thus plotted serve as specific indicators for the isoprenoid and cyclic compounds, provide resolution of compounds not completely separated by the GC column, and greatly increase the dynamic range. These workers believe that this technique will substantially improve the potential for classifying oils over other currently used methods.

Remote Sensing

The growing worldwide concern over pollution of our finite planet has led to increased international interest in remote sensing. An example

of this is current interest of the European Economic Community in satellite surveillance of the Mediterranean for pollution (*77*). Applications abound as more sophisticated hardware becomes available. Twelve international symposia have been held on remote sensing of the environment with voluminous proceedings attesting to the extent of ongoing research.

Although the term "remote sensing" includes sensing from satellites, the Coast Guard originally perceived airborne surveillance and local area surveillance as the two most practical approaches to satisfy its needs. Local area surveillance, as the name implies, involves monitoring harbors and waterways that are more or less confined. Airborne surveillance is the approach used for wide area coverage of open sea.

The Federal Water Pollution Control Act (Public Law 80-845) prohibits "discharges of oil into or upon the navigable waters of the United States." The International Convention for the Prevention of Pollution at Sea by Oil (1954), which was ratified by the U.S. Senate as the Oil Pollution Control Act in 1961, prohibited tankers from discharging oily mixtures within at least 50 miles of land. Today, the prohibited discharge zone extends to 200 nautical miles. This means that the surveillance of the 4500 miles of contiguous U.S. coastline must provide regular coverage of 900,000 square miles of ocean (*78*). The amount of ocean to be covered necessitates much more emphasis on airborne surveillance. The EPA has similar responsibilities under the law; Melfi et al. (*79*) recently reviewed the EPA needs in remote sensing.

The literature on the subject of remote sensing began to grow significantly in the late 1960s for all applications. Every conceivable technique seems to have been tried for feasibility for oil identification. Some of these include infrared reflectance sensors [in which the signals at two wavelengths in the 3-μm region are ratioed (*80*)], an active fluorosensor system (*81*), a laser backscatter sensor called a Dichromatic Lidar Polarimeter [LIDAR for light detection and ranging—see also Rause et al. (*82*)] that uses 632-nm red and 442-nm blue lasers and measures horizontally and vertically polarized backscattered light, laser Raman (*83*), and vapor sensors based on (a) a coated piezoelectric crystal, (b) a semiconductor gas sensor (Taguchi Gas Sensor or TGS), (c) a vapor absorption/resistor sensor, and (d) a differential evaporation sensor based on differential evaporation rates on water and oil-coated temperature probes.

Several of the sensors mentioned above were evaluated by the U.S. Coast Guard [White and Arecchi (*84*)] for use in local area pollution surveillance systems in various ports and estuarine areas. They were tested for their ability to give early alerts to spills, which would permit prompt containment and cleanup efforts and thereby minimize

environmental damage. All those tested had limited capabilities in one aspect or another for local pollution surveillance. Three promising sensors were put through field tests for six months in New York Harbor. White (*85*) encountered operational difficulties involving thin-film sensitivity, threshold levels in the presence of background oil films, and alarm time delays. He felt these types of sensors would be more effective monitoring specific problem areas such as moored tankers and storm drain outfalls. One of the most difficult problems with point sensors is determining the placement location. Bacon (*86*) addressed this from the oceanographic point of view in Providence Harbor, Rhode Island. Spill movement models were used to estimate movement patterns, which were transferred to a map grid to estimate the number of spills that might occur in a given grid space. For precise location of sensors, a very detailed study would be required of currents on a small scale.

Preliminary studies for airborne systems were reported by Meeks et al. (*87*), who studied microwave radiometric detection of oil slicks. A couple of years later, Fantasia and Ingrao (*88*) and Ingrao et al. (*89*) described the development of an experimental airborne remote sensing system for oil spills, based on laser-stimulated oil fluorescence. At the Tenth International Symposium on Remote Sensing, in 1975, several techniques were reported, including a passive infrared (*90*), radar observation of spills (*91*), passive luminescence with a Fraunhofer line discriminator (FLD) (*92*), and active luminescence (*93*) and fluorescence (*94*).

Two groups are pursuing the use of active fluorescence. They are the Canada Centre for Remote Sensing and NASA Wallops Flight Center. Both have studied extensively spectral and temporal identification of oils (*95, 96, 97*). Time-resolved spectra are possible since the red portion takes longer to decay than the blue portion of the spectrum; by gating, a time-delayed spectrum can be obtained with a temporal resolution of 5–10 nsec. The Canadian group has reported on a laboratory study of time-resolved fluorosensors for remote characterization of oils (*98*). Both the Canadian and NASA groups have decided, for the present, to concentrate on active fluorescence using a single narrow laser pulse with a spectrometer receiver tuned to multichannels to obtain a real-time total fluorescence spectrum. The Canadians use a 16-channel receiver in their operational active fluorosensor. NASA uses a 40-channel receiver with a spectral resolution of 11.25 nm/channel in the measured range of 350–800 nm, using a laser tuned to 337 nm. Both groups are awaiting spills of opportunity for real world testing.

Radar observations of spills have already been mentioned (*91*). A synthetic aperture radar on board *Seasat A* will be used with a 16-ft vertically polarized antenna to look at natural seeps over Santa Barbara

Channel and Baffin Bay (*99*) after studying icebergs off Newfoundland.

The Coast Guard's Airborne Surveillance System (AOSS) has been very successful, so much so that it is now entering its third generation. In 1972 Catoe (*100*) presented a state-of-the-art survey that contained elements of the AOSS predecessors. The current system being used is AOSS II (*78, 101, 102*). Edgerton et al. (*103, 104*) described the prototype of the AOSS system, which is a multisensor system that provides real-time, day or night, all-weather detection, mapping, and documentation of oil spills at sea. The prototype system was installed on a Coast Guard HU-16 Albatross aircraft and tested off the California coast with natural oil seeps, routine shipping, and targets of opportunity. The system consisted of a side-looking radar, a passive microwave imager, a multispectral low-light-level TV, a multichannel line scanner, a position reference system, and a real-time processor/display console.

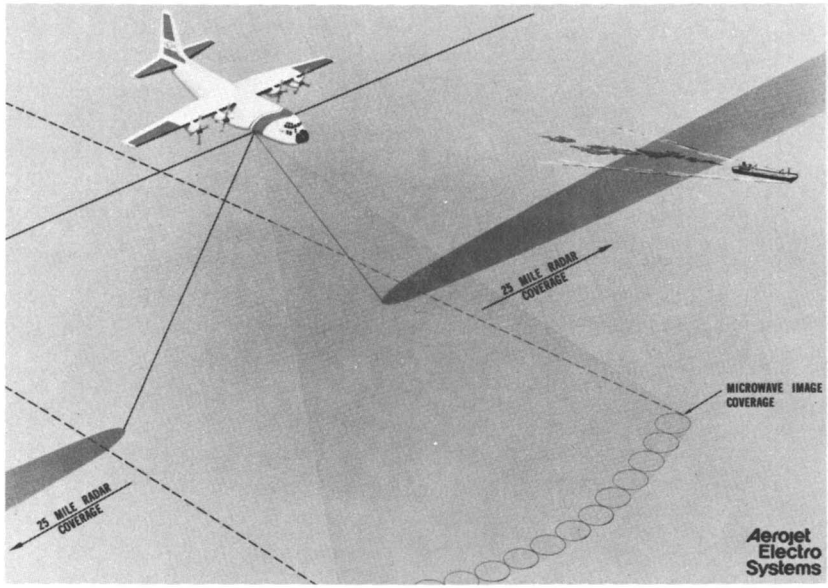
The AOSS I flight evaluations were reported by Mauer and Edgerton (*105*), and the entire system reviewed by Woolever et al. (*101*). The planned phasing out of the Coast Guard's Albatross fleet, coupled with required improvements in the AOSS I system, led to installation of an improved system, designated AOSS II in the U.S. Coast Guard HC-130B aircraft (*106*).

The major subsystems of the AOSS II include the following improvements:

1. Double-sided SLAR antenna installation for left/right detection and mapping capability.
2. Increased microwave scanner rotation speed to permit lower-altitude operations and higher resolution.
3. Installation of an improved aerial reconnaissance camera (KS-72) in lieu of the low-light-level television.
4. Use of IR line scanner to provide printout of surface water temperature along the flight track.
5. Expanded hard-copy recording capability, computerized target latitude/longitude readout, and image smoothing at the operator's display console.

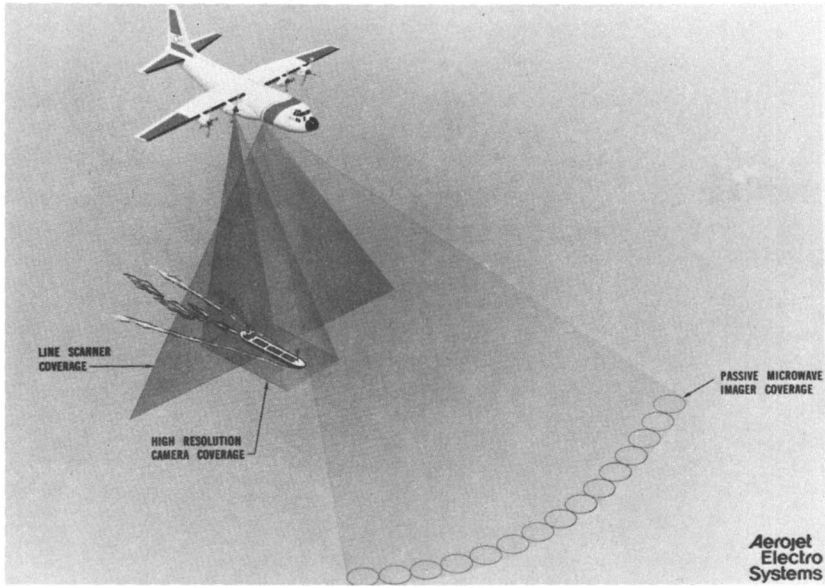
AOSS II became operational in April 1977 and has increased the surveillance productivity over that of the standard HC-130B aircraft fourfold. According to White et al. (*102*), "Never before in Coast Guard history has a single aircraft imaged as much territory in as short a time."

The AOSS II integrated multisensor system provides effective surveillance around the clock and under all but extreme weather conditions. Figure 4 illustrates how the passive microwave imager and side-looking radar system provide long-range surveillance—up to 25 miles on either side of the aircraft. Figure 5 shows how the system employs the reconnaissance camera and multichannel line scanner for high-resolution documentation of oily discharges and suspected violators.



U.S. Coast Guard

Figure 4. AOSS II long-range ship/discharge detection and mapping mode (106)



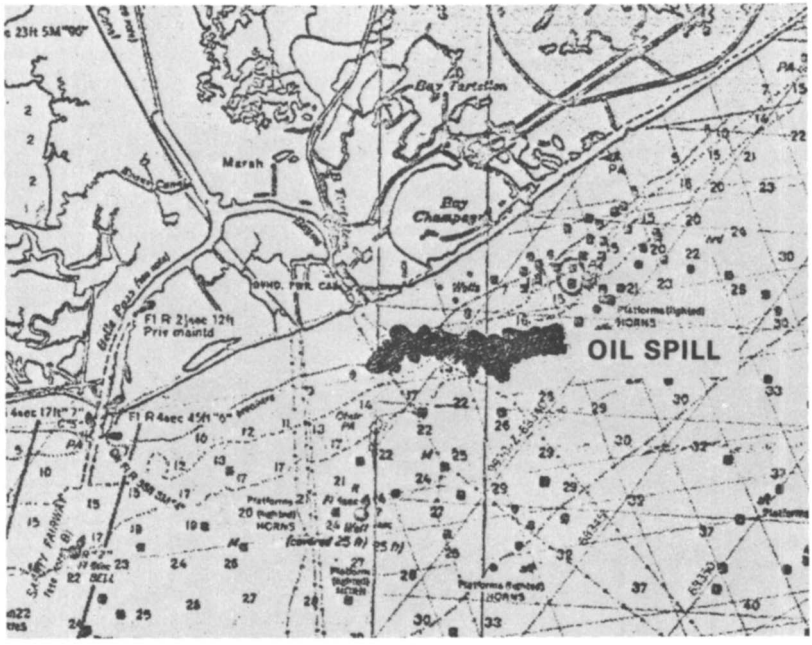
U.S. Coast Guard

Figure 5. AOSS II short-range inspection/identification and mapping mode (106)

The side-looking radar mapping system utilizes a unique 8-ft vertically polarized antenna for oil detection and mapping. A standard 16-ft antenna complements the 8-ft antenna to provide a 50-nautical mile swath width, and digital filters are employed for automated target detection. This system produces a near-real-time radar map on film that can be sent to a ground station while the aircraft continues its patrol. Figure 6 shows the outline of a spill superimposed on a chart as it is received on the ground. It shows the location and extent of the slick, so that ground response teams can take appropriate action.

The inertial navigation system and airborne data annotation system of AOSS II provide position references that are superimposed on all data products. The four sensors and position reference system are integrated by means of a software-controlled operator console that requires only one operator. Color and black-and-white TV monitors display information from the sensors. Under low-contrast conditions, false color enhancement is used to facilitate interpretation.

The third generation, called AIREYE (from the acronym ARI, which stands for Airborne Remote Instrumentation) is on the drawing boards to be installed in Falcon 20G jet aircraft starting in mid-1979. This will



U.S. Coast Guard

Figure 6. Outline of spill superimposed on chart as it is received on the ground

replace the HU-16E Albatross and will be designated the HU-25A. It will serve as the Coast Guard's Medium Range Surveillance (MRS) aircraft. The AIREYE system is based on the AOSS II system but will be smaller in size (2.25 m^3 vs. 3.75 m^3) and weight (one-third the weight of the AOSS II package). There will be improvements in all components of the system. The components will consist of side-looking radar (SLAR), an infrared/ultraviolet line scanner (IR/UV), an aerial reconnaissance camera (KS-87B), an airborne data annotation system (AN/ASQ-154), and an active gated television (AGTV) and a control display and record console (CDRC). Figure 7 shows the location of the AIREYE sensors on the aircraft.

The AIREYE SLAR will have a 200-kW transmitter vs. 45 kW in AOSS. It will also have a dry film silver radar processor instead of a wet chemical processor. It will be the primary oil pollution detection sensor, detecting slicks by reduction of ocean clutter from 2–25 miles on both sides of the aircraft and providing a near-real-time annotated map.

The IR/UV line scanner is the same as AOSS, except the UV sensitivity will be increased by a factor of 10. The IR/UV will scan a line missed by the SLAR. It can differentiate between oil and false targets and is effective in calm water when SLAR is degraded. The IR portion provides a day or night capability.

The KS-87B camera is in the Department of Defense inventory. It is a pulse-operated frame camera with a high-speed focal plane shutter, integral automatic exposure control and data recording, and forward

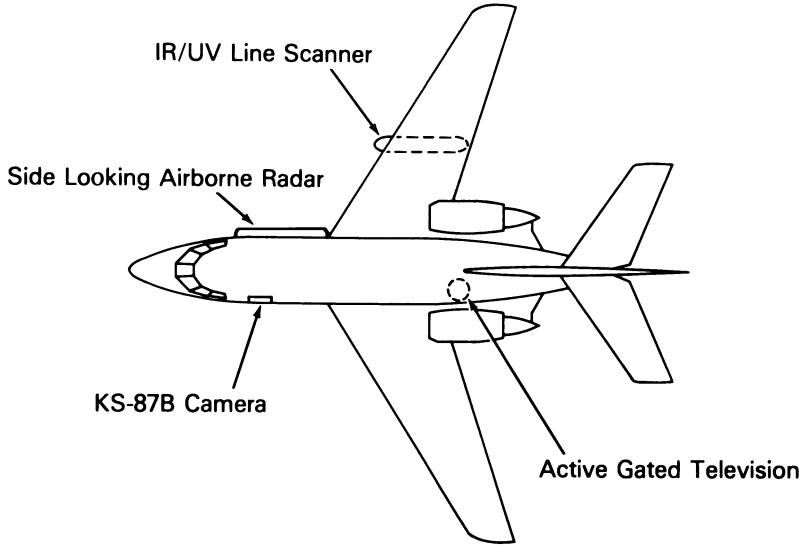


Figure 7. AIREYE sensor locations on the HU-25A (78)

motion compensated. It provides documentary evidence (ship identification) and can be operated by the pilot as well as the sensor operator.

The data system (AN/ASQ-154) is also in the Department of Defense inventory.

The AGTV is a capability the earlier AOSS II system did not have and will provide the ability to identify discharging vessels at night. It has a pulsed laser illuminator that will permit reading painted letters at night on the bow or stern of vessels and provide absolute identification for forensic purposes. This is possible because of an annotated videotape record that has a playback and freeze-frame capability to ensure the evidence is readable while the aircraft is still on scene.

The CDRC will have only one black-and-white multipurpose display in the console as a result of space limitations. However, it will provide a limited map display capability.

In its operational form, AIREYE will detect oil pollution at sea, identify violators, and provide permanent records suitable for prosecution of polluters.

Summary

The chapter presents some of the significant recent developments and indicates the direction of future developments in oil spill fingerprinting and remote sensing. Widespread adoption of uniform practices in oil identification will benefit all who are concerned with the problem of identifying sources of oil pollution. Airborne remote sensing is, at present, the only practical way to maintain pollution surveillance over wide areas of open sea.

Acknowledgments

I am indebted to a number of people for source material, particularly in the area of remote sensing. J. White and Lloyd Breslau (78) gave valuable assistance and provided the remote sensing graphics on AOSS II and AIREYE. I am also indebted to Denny Farmer and R. D. Lassiter for helpful discussions. George Frame gave me useful input in the gas chromatographic section as well as providing Figure 2. I wish to thank William MacLeod, Jr., for Figure 3. DeLyle Eastwood was especially helpful in the areas of fluorescence and plasma emission spectroscopy. I also want to thank J. R. Jadamec for his suggestions and key references regarding fluorescence and fluorosensors. G. A. Flanigan provided me with some key references.

Literature Cited

1. Bentz, A. P. "Oil Spill Identification," *Anal. Chem.* **1976,** *48*(6), 454A.
2. Bentz, A. P. "Oil Spill Identification Bibliography," National Technical Information Service: March 1976; Accession No. ADA029126.
3. ASTM Method D3326-78, "Preparation of Sample for Identification of Waterborne Oils." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
4. ASTM Method D3328-78, "Method of Test for Comparison of Waterborne Petroleum Oils by Gas Chromatography." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
5. ASTM Method D3650-78, "New Standard Method of Test for Comparison of Waterborne Petroleum Oils by Fluorescence Analysis." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
6. Wilkins, Bill, Lt. Comdr., Chief Maritime Environmental Protection Branch, Seventh Coast Guard District, Miami, Florida, personal communication Feb 1978.
7. Kleineberg, G. "Capabilities of Analytical Techniques for Identification of Unknown Oil Samples Employed by the U.S. Coast Guard," Symposium on Oil Analysis, Erding, Germany, July 1978.
8. Commandant, U.S. Coast Guard, "Polluting Incidents in and Around U.S. Waters," Comdtinst M16450.2 (old CG-487).
9. Harwood, M. "The Rising Tide of Oil Spills," *New York Times Magazine,* p. 74, April 9, 1978; quoting K. E. Biglane, Director of EPA's Division of Oil and Special Materials Control, New York.
10. "Tanker Pollution," *Ocean Reporter* April–May 1978, p. 9.
11. Sleeter, Thomas. "Methods for Identifying the Source of Spilled Oil," *Harvard Environmental Law Review* **1977,** *2,* 514.
12. Oostdam, B. L.; Anderlini, V. "Oil Spills and Tar Pollution Along the Coast of Kuwait," Marine Pollution Program, Earth and Science Division, Kuwait Institute for Scientific Research: Kuwait, 1978.
13. "Oil Spill Studies: Strategies and Techniques," *API Publ.* **1976,** No. 4286.
14. Light, M.; Lanier, J. J. "The Biological Effects of Oil Pollution, A Bibliography with Abstracts and Index," NTIS Accession No. ADA064196.
15. Bentz, A. P. "Chemical Identification of Oil Spill Sources," *The Forum* **1978,** *XIII*(2), 425.
16. U.S. v. SLADE, Inc., 447 F. Supp. 638 (1978).
17. Frame, G. M.; Flanigan, G. A.; Chamberlain, C. P. "Cleanup Procedure for Contaminated Oils Prior to Fingerprinting by Gas Chromatography and IR Spectroscopy," Pittsburgh Conference, Cleveland, 1978, paper 554.
18. "Oil Spill Identification System," Chemistry Branch, U.S. Coast Guard Final Report, 1977; NTIS Accession No. ADA044750.
19. ASTM Method D-3415-75T, "Practice for Identification of Waterborne Oils." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
20. ASTM Method D-3325-78, "Preservation of Waterborne Oil Samples." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
21. ASTM Method D-3327-74T, "Analysis for Selected Elements in Waterborne Oils." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.
22. ASTM Method D-3414-75T, "Method for Infrared Analysis of Waterborne Oils." In "Annual Book of ASTM Standards"; American Society for Testing and Materials; part 31.

23. Dick, J. G.; Pant, B. C. Concordia University, Montreal, Quebec, personal communication, Mar 1978.
24. Bentz, A. P. "Who Spilled the Oil?" In "The Analytical Approach," *Anal. Chem.* **1978**, *50*(7), 655A.
25. Bentz, A. P.; Chamberlain, C. P.; Killeen, T. J., unpublished data.
26. Brown, C. W.; Lynch, P. F.; Ahmadjian, M. "Identification of Oil Slicks by Infrared Spectroscopy," Final Report, U.S. Coast Guard Contract DOT-CG-81-74-1099, August 1976; NTIS Accession No. ADA040975.
27. Frankenfeld, J. W. "Weathering of Oil at Sea," Final Report, U.S. Coast Guard Contract DOT-CG-23035-A, September 1973; NTIS Accession No. AD787789.
28. Ahmadjian, M.; Baer, C. D.; Lynch, P. F.; Brown, C. W. "Infrared Spectra of Petroleum Weathered Naturally and Under Simulated Conditions," *Environ. Sci. Technol.* **1976**, *10*(8), 777.
29. Flanigan, G. A.; Copperthite, R. D.; Buchanan, S. "Laboratory Weathering of Petroleum," Pittsburgh Conference, 1978, paper 556.
30. Anderson, C. P.; Killeen, T. J.; Taft, J. B.; Bentz, A. P. "Artificial Oil Weathering Techniques," presented at the Pittsburgh Conference, 1979, paper 527.
31. Gruenfeld, M.; Frederick, R. "The Ultrasonic Dispersion, Source Identification, and Quantitative Analysis of Petroleum Oils in Water," *Rapp. P.-V. Reun. Cons. Int. Explor. Mer* **1977**, *171*, 33–38.
32. Zürcher, F.; Thüer, M. "Rapid Weathering Processes of Fuel Oil in Natural Waters: Analyses and Interpretations," *Environ. Sci. Tech.* **1978**, *12*(7), 838.
33. Brown, C. W.; Lynch, P. F.; Ahmadjian, M. "Applications of Infrared Spectroscopy in Petroleum Analysis and Oil Spill Identification," *Appl. Spectrosc. Rev.* **1975**, *9*, 223.
34. Grizzle, P. L.; Coleman, J. H. "Infrared Analysis Techniques for Oil Identification," Bartlesville Energy Research Center, ERDA, April 1977, Report No. BERC/RI-77-4.
35. Grizzle, P. L.; Coleman, J. H. *Am. Chem. Soc., Div. Pet. Chem., Prepr.* **1977**, *22*(3), 878.
36. U.S. Coast Guard R&D Center, "Proceedings of the Infrared Pattern Recognition Seminar," May 1975.
37. Mattson, J. S. "Classification of Oils by Application of Pattern Recognition Techniques to Infrared Spectra," Final Report, U.S. Coast Guard Contract DOT-CT-81-75-1364, March 1 976; NTIS Accession No. ADA039387.
38. Mattson, J. S.; Mattson, C. S.; Spencer, M. J.; Starks, S. A. "Multivariate Statistical Approach to the Fingerprinting of Oils by Infrared Spectrometry," *Anal. Chem.* **1977**, *49*(2), 297.
39. U.S. Coast Guard, University of Connecticut, IEEE, "Proceedings, Workshop on Pattern Recognition Applied to Oil Identification," Coronado, CA, November 1976; IEEE Catalog No. 76 CH 1247-6 C.
40. Curtis, Morton L. "Use of Pattern Recognition Techniques for Typing and Identification of Oil Spills," Final Report, April 1977 U.S. Coast Guard Contract DOT-CG-81-75-1383; NTIS Accession No. ADA043802.
41. Jurs, P. C. "Pattern Recognition Techniques Applied to the Identification of Oil Spills," Final Report, EPA Contract 68-01-1739.
42. Mar, T. L.; Westervelt, V. M.; Bentz, A. P. "A Preliminary Look at Microprocessing Infrared Data for Oil Identification," Pittsburgh Conference, 1976, paper 323.
43. Gronlund, W. R.; Chamberlain, C. P.; Bentz, A. P. "Quantitative Effects of Weathering on Infrared Spectra of Oils," Pittsburgh Conference, 1977, paper 334.

44. Anderson, C. P.; Killeen, T. J.; Bentz, A. P. "Weighting Factors for Infrared Data Points in Pattern Recognition for Oil Identification," Pittsburgh Conference, 1978, paper 397.
45. Killeen, T. J.; Eastwood, D.; Hendrick, M. S. "Oil Matching Using a Simple Vector Model," Pittsburgh Conference, 1978, paper 398.
46. Noether, G. E. "Introduction to Statistics, a Nonparametric Approach," Houghton Mifflin: Boston, 1976; p. 167.
47. Johnson, W. D.; Fuller, F. D. "Identification and Characterization of Oil Pollutants in Water Via Simultaneous Gas Chromatography Employing Flame Ionization and Flame Photometric Detectors for Sulfur and Phosphorus," Proceedings 13th Conference, International Association Great Lakes Research, 1970; pp. 128–136.
48. Lee, M. L.; Bartle, K. D.; Novotny, M. V. "Profiles of the Polynuclear Aromatic Fraction from Engine Oils Obtained by Capillary-Column Gas–Liquid Chromatography and Nitrogen-Selective Detection," *Anal. Chem.* **1975**, *47*(3), 540.
49. Flanigan, G. A.; Frame, G. M. "Oil Spill 'Fingerprint' with Gas Chromatography," *Res./Dev.* **1977**, 28.
50. Frame, G. M.; Carmody, D. C.; Flanigan, G. A. Feb 1978 "An Atlas of Gas Chromatograms of Oils Using Dual Flame-Ionization and Nitrogen–Phosphorus Detectors," Coast Guard Report No. CG-D-19-78, NTIS Accession No. ADA054966.
51. Kawahara, F. K. "Trace Organic Components as Fingerprints in Gas Chromatographic Identification of Spilled Asphalts," *Environ. Sci. Technol.* **1976**, *10*(8), 761.
52. Rasmussen, D. V. "Characterization of Oil Spills by Capillary Column Gas Chromatography," *Anal. Chem.* **1976**, *48*, 1562.
53. Overton, E. B.; Bracken, J.; Laseter, J. L. "Application of Glass Capillary Columns to Monitor Petroleum-Type Hydrocarbons in Marine Sediments," *J. Chromatogr. Sci.* **1977**, *15*, 169.
54. MacLeod, W. D., Jr.; Thomas, L. C.; Uyeda, M. Y.; Jenkins, R. C. "Evidence of *Argo Merchant* Cargo Oil in Marine Biota by Glass Capillary GC Analysis," Proceedings of the "Conference on Assessment of Ecological Impacts of Oil Spills," Keystone, CO, June 1978.
55. MacLeod, W. D., Jr.; Uyeda, M. Y.; Thomas, L. C.; Brown, D. W. "Hydrocarbon Patterns in Some Marine Biota and Sediments Following the *Argo Merchant* Spill," Proceedings of Symposium, "In the Wake of the *Argo Merchant*," January 1978. University of Rhode Island Press: Kingston, RI, in press.
56. MacLeod, W. D., Jr.; Uyeda, M. Y.; Friedman, A. J.; Prohaska, P. G. "Weathering Estimations for Spilled Oil from *Bouchard* No. 65," Proceedings of the "Conference on Assessment of Ecological Impacts of Oil Spills," Keystone, CO, June 1978.
57. Flanigan, G. A.; Bentz, A. P. "Quantitative Effects of Weathering on GC Identification of Oils," Pittsburgh Conference, 1977, paper 333.
58. Eastwood, D.; Fortier, S. H.; Hendrick, M. S. "Oil Identification—Recent Developments in Fluorescence and Low-Temperature Luminescence," *Am. Lab.* **1978**, 45.
59. Eastwood, D.; Hendrick, M. S. "Further Investigations in Fluorescence and Low-Temperature Luminescence for Oil Identification," Pittsburgh Conference, 1978, paper 551.
60. Fortier, S. H.; Eastwood, D. "Identification of Fuel Oils by Low-Temperature Luminescence Spectrometry," *Anal. Chem.* **1978**, *50*(2), 334.
61. Eastwood, D.; Hendrick, M. S.; Fortier, S. H. "Fluorescence and Low-Temperature Luminescence Studies on Weathered Oils," unpublished data.

62. Wakeham, S. G. "Synchronous Fluorescence Spectroscopy and Its Application to Indigenous and Petroleum-Derived Hydrocarbons in Lacustrine Sediments," *Environ. Sci. Technol.* **1977**, *11*(3), 272.
63. Broownrigg, J. T.; Hornig, A. W. "Improved Identification of Oils by Low-Temperature Total Luminescence," Pittsburgh Conference, 1977, paper 396.
64. Hornig, A. W.; Giering, L. P. "Total Luminescence Study of the Effects of Artificial Weathering on Oils," Pittsburgh Conference, 1978, paper 552.
65. Warner, I. M.; Callis, J. B.; Davidson, E. R.; Christian, G. D. "Oil Characterization by Multicomponent Fluorescence Analysis," U.S. Coast Guard, University of Connecticut, IEEE, "Proceedings, Workshop on Pattern Recognition Applied to Oil Identification," Coronado, CA, November 1976; IEEE Catalog No. 76 CH 1247-6 C.
66. Johnson, L. W.; Callis, J.; Christian, G. D. "Rapid Scanning Fluorescence Spectroscopy," *Anal. Chem.* **1977**, *49*(8), 747A.
67. Callis, J. B.; Davidson, E. R. U.S. Patent 4 031 396, June 1977.
68. Jadamec, J. R.; Saner, W. A.; Talmi, Y. "Optical Multichannel Analyzer for Characterization of Fluorescent Liquid Chromatographic Petroleum Fractions," *Anal. Chem.* **1977**, *49*(9), 1316.
69. Christian, G. D.; Johnson, D. W.; Hershberger, L. W.; Callis, J. B. "Recent Advances in Video Fluorometry," Pittsburgh Conference, 1978, paper 22.
70. Saner, W. A.; Fitzgerald, G. E.; Welsh, J. P. "Liquid Chromatographic Identification of Oils by Separation of the Methanol Extractable Fraction," *Anal. Chem.* **1976**, *48*, 1747.
71. Saner, W. A.; Jadamec, J. R.; Sager, R. W. "Change in Resolution of Reverse-Phase Liquid Chromatographic Columns with Temperature," *Anal. Chem.* **1978**, *50*(6), 749.
72. Heftman, L. "Chromatography. A Laboratory Handbook of Chromatographic and Electrophoretic Methods," 3rd ed.; Van Nostrand Reinhold: New York, 1975.
73. Saner, W. A.; Fitzgerald, G. E., II. "Thin-Layer Chromatographic Technique for Identification of Waterborne Petroleum Oils," *Environ. Sci. Technol.* **1976**, *10*, 893.
74. Hendrick, M. S.; Eastwood, D. "Trace Metal Analysis (V/Ni) by Argon Plasma Optical Emission Spectrometry for Weathered Oil Identification," Pittsburgh Conference, 1978, paper 404.
75. Flory, D. A.; Rubenstein, A. E.; Lichtenstein, H. A.; Koons, C. B.; Rogers, M. A.; Mercer, J. N. "Sophisticated Equipment Fingerprints Crude Oils," *Oil Gas J.* **1978**, *76*(8), 102.
76. Koons, C. B.; Rogers, M. A.; Mercer, J. N.; Flory, D. A.; Rubenstein, A. E.; Lichtenstein, H. A. "Pattern Recognition of Output from GC–MS–COM for Crude Oil Classification," U.S. Coast Guard, University of Connecticut, IEEE, "Proceedings, Workshop on Pattern Recognition Applied to Oil Identification," Coronado, CA, November 1976; IEEE Catalog No. 76 CH 1247-6 C.
77. Geiss, F. Joint Research Center of the EEC, personal communication.
78. White, J. R.; Breslau, L. R. "Remote Sensing for Oil Pollution Control Along Coastal Waters of the United States," Proceedings of the Twelfth International Symposium on Remote Sensing of Environment, Manila, Philippines, April 1978.
79. Melfi, S. H.; Koutsandreas, J. D.; Moran, J. "Tracking Pollutants from a Distance," *Environ. Sci. Technol.* **1977**, *11*(1), 36.
80. Wright, D. E.; Wright, J. A. "A New Infrared Instrument for Monitoring Oil Films on Water," *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1974**, *409*, 93.

81. Schwemmer, G. K.; Kim, H. H. "Mapping and Identification of Oil on Water by the Use of an Airborne Laser System," *Natl. Bur. Stand. (U.S.) Spec. Publ.* **1974**, *409*, 95.
82. Rause, J. W., Jr.; Hulse, W. C.; Blanchard, A. J.; Malek, H. "Development of a LIDAR Polarimeter Sensor for Remote Detection and Monitoring of Oil and Other Hazardous Materials on Water," Final Report, U.S. Coast Guard Contract DOT-CG-34017-A, April 1975.
83. Ahmadjian, M.; Brown, C. W. "Feasibility of Remote Detection of Water Pollutants and Oil Slicks by Laser-Excited Raman Spectroscopy," *Environ. Sci .Technol.* **1973**, *7*, 452.
84. White, G. P.; Arecchi, A. V. "Local Area Pollution Surveillance Systems: A Summary of the Coast Guard's Research and Development Activities," Proc. Pollution Control Conference, 1975, San Francisco, CA, API.
85. White, J. R. " Preliminary Test of a Government-Owned Local Area Oil-On-Water Surveillance System," Final Report, U.S. Coast Guard R&D Center Report No. CG-D-121-76, June 1976; NTIS Accession No. ADA040541.
86. Bacon, J. C. "An Oceanographic Technique for Determination of Placement of Oil-On-Water Sensors and Its Application to Providence, Rhode Island, Harbor," Final Report, U.S. Coast Guard R&D Center, Groton, Connecticut, March 1976.
87. Meeks, D. C.; Williams, D. P.; Wilcox, R. M.; Edgerton, A. T. "Microwave Radiometric Detection of Oil Slicks," Final Report No. 1335-2, Coast Guard Contract DOT-CG-93228A, March 1971.
88. Fantasia, J. F.; Ingrao, H. C. "The Development of an Experimental Airborne Laser Oil Spill Remote Sensing System," Proceedings Pollution Control Conference, 1973, Washington, DC, API.
89. Ingrao, H. C.; Cartwright, M. F.; Yaffee, M. "Airborne Laser Remote Sensor for Oil Detection and Classification," Interim Report, Coast Guard Contract DOT-TSC-74-4, August 1975.
90. Matsui, M.; Tsutsumi, S. "Detection and Analysis for Water Surface Covered with Oil Film," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, Ann Arbor, October 1975; Vol. I, p. 223.
91. Van Kuilenburg, J. "Radar Observations of Controlled Oil Spills," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, Ann Arbor, October 1975; Vol. I, p. 243.
92. Watson, R. D.; Hemphill, W. R.; Bigelow, R. C. "Remote Sensing of Luminescing Environmental Pollutants Using a Fraunhofer Line Discriminator (FLD)," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, October 1975; Vol. I, p. 203.
93. Gross, H. G. "Los Angeles Harbor Field Investigation of Oil and Background Luminescence Signatures," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, Ann Arbor, October 1975; Vol. I, p. 253.
94. Kung, R. T. V.; Itzkan, I. "A New Concept for the Remote Measurement of Oil Fluorescence Conversion Efficiency," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, Ann Arbor, October 1975; Vol. I, p. 231.
95. O'Neil, Robert A. Canada Center for Remote Sensing, personal communication.
96. O'Neil, R. A.; Davis, A. R.; Gross, H. G.; Kruus, J. "A Remote Sensing Laser Fluorometer," Proceedings of the Hydrographic LIDAR Conference, NASA, Wallops Island, VA, September 1974.

97. Hoge, Frank. NASA Wallops Flight Center, personal communication.
98. Rayner, D. M.; Szabo, A. G. "Time-Resolved Laser Fluorosensors: a Laboratory Study of Their Potential in the Remote Characterization of Oil," *Appl. Opt.* **1978**, *17*, 1624.
99. Freezer, D., Lt. Comdr.; Vollmers, R. R. U.S. Coast Guard Headquarters (DOE-2), personal communication.
100. Catoe, Clarence E. "The Applicability of Remote Sensing Techniques for Oil Slick Detection," Fourth Annual Offshore Technology Conference, Houston, May 1972.
101. Woolever, G. F.; Kidd, L. A.; Welsh, J. P.; McIntosh, J. A.; Farmer, L. D. "Utilization of Remote Sensing Techniques for U.S. Coast Guard Missions," Proceedings of the Tenth International Symposium on Remote Sensing of the Environment, October 1975; Vol I, p. 3.
102. White, J. R.; Freezer, D. R.; Vollmers, R. R. "U.S. Coast Guard Utilization of Remote Sensing Techniques for Ocean Surveillance," Proceedings OCEAN '77 Conference, October 1977; Los Angeles, CA.
103. Edgerton, A. T.; Bommarito, J. J.; Schwantje, J. J.; Meeks, D. C. "Development of a Prototype Oil Surveillance System," Final Report, U.S. Coast Guard Contract DOT-CG-22170A (CG-D-90-75), May 1975.
104. Ketchel, P. J.; Edgerton, A. T. Oil Spill Conference, 1973, Washington, DC, API.
105. Maurer, A.; Edgerton, A. T. "Flight Evaluation of U.S. Coast Guard Airborne Oil Surveillance System," Proceedings Pollution Control Conference, 1975, San Francisco, CA, API.
106. Meeks, D. C.; Bommarito, J. J.; Schwantje, R. S.; Edgerton, A. T. "Transfer, Installation and Flight Testing of the Modified Airborne Oil Surveillance System (AOSS II) in an HC-130B Aircraft," Final Report, U.S. Coast Guard CG-D-60-77, August 1977; NTIS Accession No. A 052-434.

RECEIVED October 31, 1978.

Recent Advances in the Determination of Aromatic Hydrocarbons in Zooplankton and Macrofauna

J. S. WARNER, R. M. RIGGIN, and T. M. ENGEL

Battelle–Columbus Laboratories, 505 King Avenue, Columbus, OH 43201

The following methods were applied to the determination of aromatic hydrocarbons in marine organisms: (1) high-pressure liquid chromatography using a fluorescence detector, (2) glass-capillary-column gas chromatography combined with mass spectrometry and a computerized search of selected ions in full-mass-range mass spectra, (3) gel permeation chromatographic cleanup, and (4) purging and trapping of volatiles. The methods overcome three major analytical problems, namely, interference from biogenic hydrocarbons, inadequate detection limits using small samples, and losses of volatile aromatic hydrocarbons. By using the purge-and-trap method, light aromatic hydrocarbons, benzene to dimethylnaphthalene, can be determined at levels down to 0.02 μg/g using only 100 mg of sample.

This paper addresses three major analytical problems in the determination of aromatic petroleum hydrocarbons in marine organisms, namely, interference from biogenic hydrocarbons, inadequate detection limits using small samples, and losses of volatile aromatic hydrocarbons.

Our efforts have been concentrated on the determination of aromatic hydrocarbons rather than saturated hydrocarbons primarily because biological studies (*1*) have indicated that the aromatic hydrocarbons are the most significant petroleum components from the standpoint of potential environmental hazard.

The methods most commonly employed for the analysis of petroleum hydrocarbons in marine organisms (*2–5*) involve extraction with or without saponification, fractionation by column chromatography on silica gel

0-9412-0475-6/80/33/087$05.00/0

and/or alumina to give a saturate fraction and an "aromatic" fraction, and gas chromatographic analysis of each fraction using a flame ionization detector. Unfortunately, the "aromatic" fraction contains nonaromatic polyunsaturated hydrocarbons, the biogenic olefins, in addition to any aromatic petroleum hydrocarbons. The levels of biogenic olefins vary widely depending upon the species and its food supply. In many cases the biogenic olefins do not interfere in the determination of individual aromatic hydrocarbons (e.g., naphthalenes and phenanthrenes) when the aromatics are present at levels of 0.1–10 μg/g wet weight. However, levels that high are found usually only in animals that are highly contaminated by oil, as in a fresh spill situation or in laboratory exposure studies at high levels. The determination of aromatic hydrocarbons in most field samples, especially for baseline studies, is quite a different matter. The levels of individual aromatic hydrocarbons in such samples are frequently below 0.01 μg/g wet weight. At that level it is generally not possible to determine whether or not an aromatic hydrocarbon is present by the usual gas chromatography (GC) method, because there are too many interfering olefins present at higher levels. The problem is usually worse in the analysis of zooplankton than of macrofauna because the levels of biogenic components are higher. In an effort to overcome the interference problem, methods to detect aromatic hydrocarbons selectively (e.g., selected ion mass spectrometry or fluorescence analysis) and methods to separate aromatic hydrocarbons from biogenic components have been studied in our laboratory.

The problem of inadequate detection limits is associated with the interference problem as indicated above but is also dependent upon the amount of sample available, concentration methods used, and the inherent sensitivity of the detection system used. When the usual extraction–GC analysis procedure is used, a 10- to 100-g sample is used but only about one percent of the extract is injected into the GC. The resulting gas chromatogram thus represents only 100 mg to 1 g of sample. If all the sample could be injected into the gas chromatograph, the advantages of either decreasing the sample size by a factor of 100 or of lowering the detection limit by a factor of 100 could be achieved, at least in theory. In practice, the detection limit cannot be effectively lowered in this manner in most cases, because the amounts of interfering components and column overloading would increase proportionately. However, the advantage of decreasing the sample size without a loss in sensitivity can be achieved. This advantage is highly desirable for studies involving small animals or specific organs in which less than a gram of sample is available. We have developed a purge-and-trap method, which involves the GC analysis of the total sample and thus is suitable for sample sizes of a few hundred milligrams. The method also has the advantage of being suitable for the

determination of benzene, toluene, and xylenes, which cannot be determined by solvent extraction methods because of losses during solvent concentration.

GC–MS Options

One approach to selectively detecting aromatic hydrocarbons in the presence of interfering biogenic olefins is the use of combined gas chromatography–mass spectroscopy (GC–MS). The mass spectra of aromatic hydrocarbons, which are characterized by large molecular ions, are entirely different from mass spectra of biogenic olefins, which are characterized by a pattern of low-molecular-weight fragment ions.

With the computerized GC–MS systems now routinely used, various operational options are available. They are: (1) full-range monitoring with a study of the spectra from each GC peak, (2) full-range monitoring with selected ion scanning to detect preselected aromatic hydrocarbons, and (3) selected ion monitoring of preselected aromatic hydrocarbons during the run. The usefulness of three different options will be considered.

The most generally used option is to monitor the full mass range by repetitive scanning during the run from approximately m/e 40 to 450 or higher. In this manner the complete mass spectrum of each individual GC peak can be obtained. For the GC–MS work a Finnigan Model 9500 gas chromatograph interfaced with a Finnigan Model 3200 quadrupole/mass spectrometer and System Industries Model 150 data system was used. The mass spectrometer was operated in the electron impact mode at 70 eV and data were collected using the IFSS mode with an integration time of 5 msec. To achieve as much resolution of individual components as possible, an SE-30 glass capillary column GC system having approximately 75,000 theoretical plates was used.

The gas chromatogram of an "aromatic" fraction from a representative macrofauna sample is shown in Figure 1. The corresponding total ion chromatogram from a GC–MS run of the same sample is shown in Figure 2. A manual study of the mass spectra from the 40 most intense peaks, the first GC–MS option, revealed that none of the peaks were aromatic hydrocarbons. Instead, they all gave a high degree of fragmentation indicative of biogenic olefins. A representative mass spectrum of a biogenic olefin is shown in Figure 3. Note that ions at m/e 128, 142, 156, 170, 178, 192, 206, and 202, indicative of naphthalenes, phenanthrenes, anthracenes, fluoranthenes, and pyrenes, are completely absent. This indicates that aromatic hydrocarbons, which give relatively intense molecular ions, should be detectable in the presence of biogenic olefins by a GC–MS approach in which the full mass range is monitored by repetitive scanning

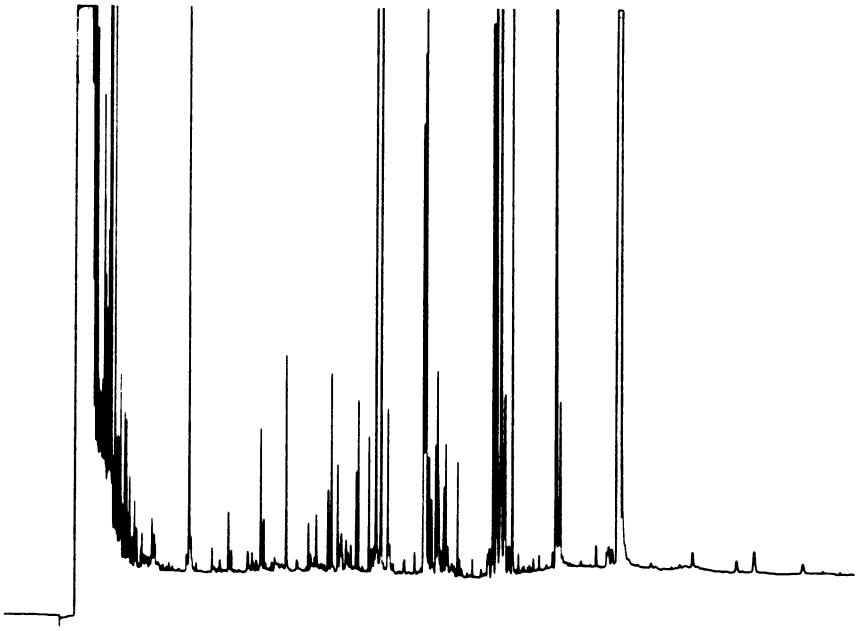


Figure 1. Gas chromatogram of "aromatic" fraction

as usual but which is coupled with a computer program that extracts selected ions, such as those listed above, from each full-mass-range mass spectra. In many cases two or more characteristic ions (e.g., 155 and 156 for dimethylnaphthalenes) are summed for each mass spectrum. The plots of the selected ion summations (SIS) vs. spectrum number are referred to as SIS plots. This second GC–MS option was found to be very effective. For the GC–MS run shown in Figure 2, the mass spectra were scanned for the ions indicative of aromatic hydrocarbons commonly

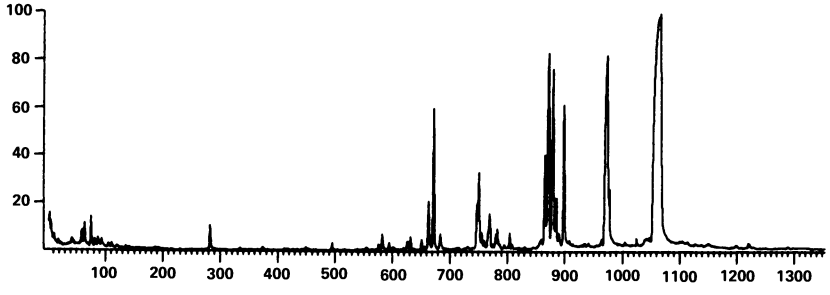


Figure 2. Total ion chromatogram of "aromatic" fraction

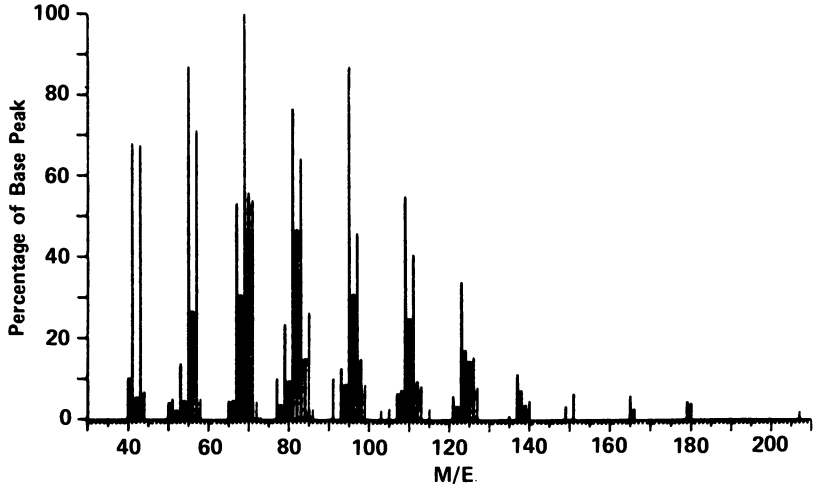


Figure 3. Mass spectrum of representative biogenic olefin, Spectrum No. 769 from GC–MS run shown in Figure 2

found in petroleum. The SIS plot for *m/e* 128 (naphthalene), *m/e* 155 and 156 (dimethylnaphthalenes), *m/e* 177 and 178 (phenanthrene), and *m/e* 101 and 202 (fluoranthene and pyrene), given in Figure 4, clearly show that small amounts of the aromatic hydrocarbons were present. The complete mass spectra for the individual peaks identified by the SIS plots frequently confirmed the presence of the aromatic hydrocarbons. For example, even though no peak is detectable in the total ion chromatogram at Spectrum No. 185, which is the peak for naphthalene identified by the SIS plot for *m/e* 128, the complete mass spectrum shown in Figure 5, clearly confirms the presence of naphthalene. The retention times of the SIS peaks were consistent with those expected for the identified hydrocarbons. The amounts of the selected aromatic hydrocarbons found were estimated on the basis of selected ion responses of standard solutions to be 0.001–0.005 g/g wet weight. Since these components were not detected at all by GC analysis or by the usual interpretation of full-range mass spectra from the GC–MS run, the advantage of SIS is clearly demonstrated.

A third GC–MS option is selected ion monitoring in which the mass spectrometer is set to monitor only certain preselected ions during the GC–MS run. With this option the sensitivity of the method is increased by a factor of 10–50, but the ability to obtain confirmatory information by referring to full-range mass spectra is lost. Since the sensitivity of the SIS option is generally adequate, selected ion monitoring is not recommended.

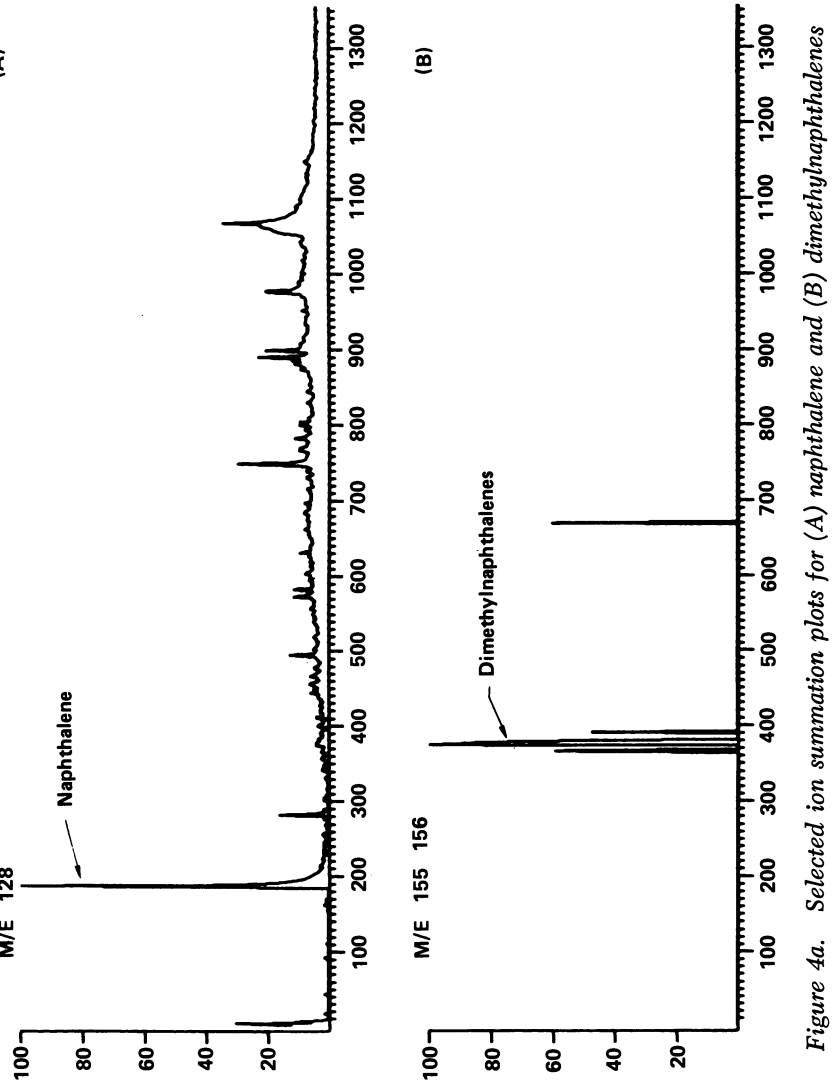


Figure 4a. Selected ion summation plots for (A) naphthalene and (B) dimethylnaphthalenes

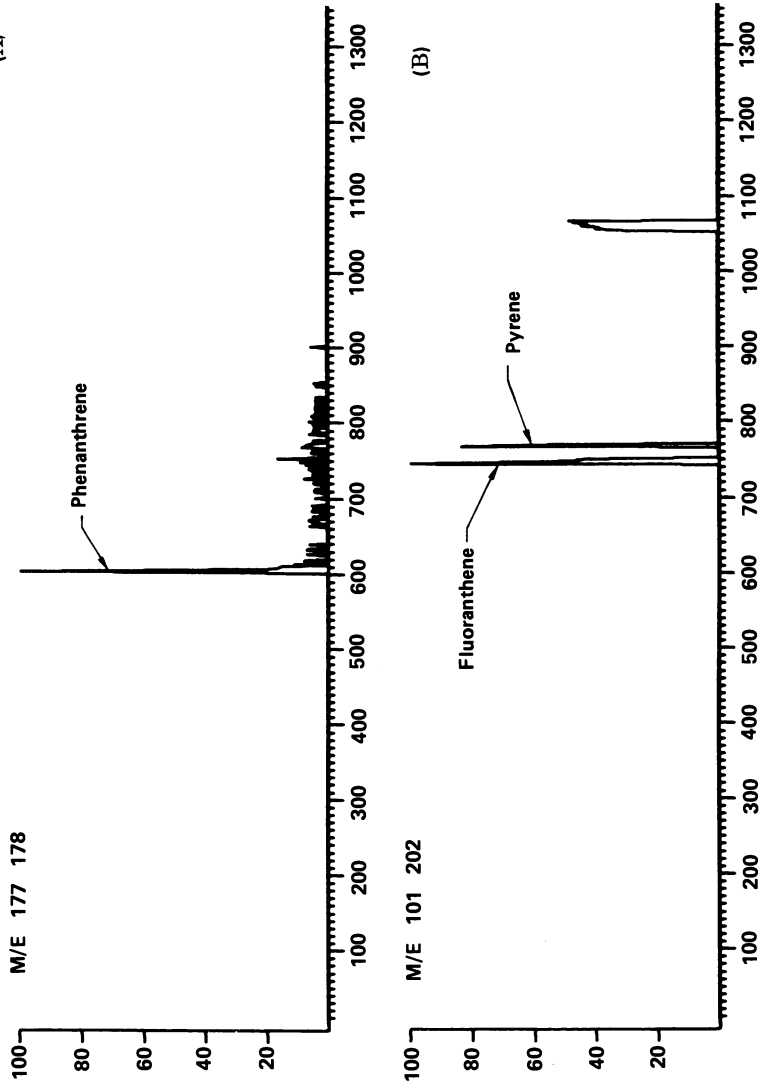


Figure 4b. Selected ion summation plots for (A) phenanthrene and (B) fluoroanthene and pyrene

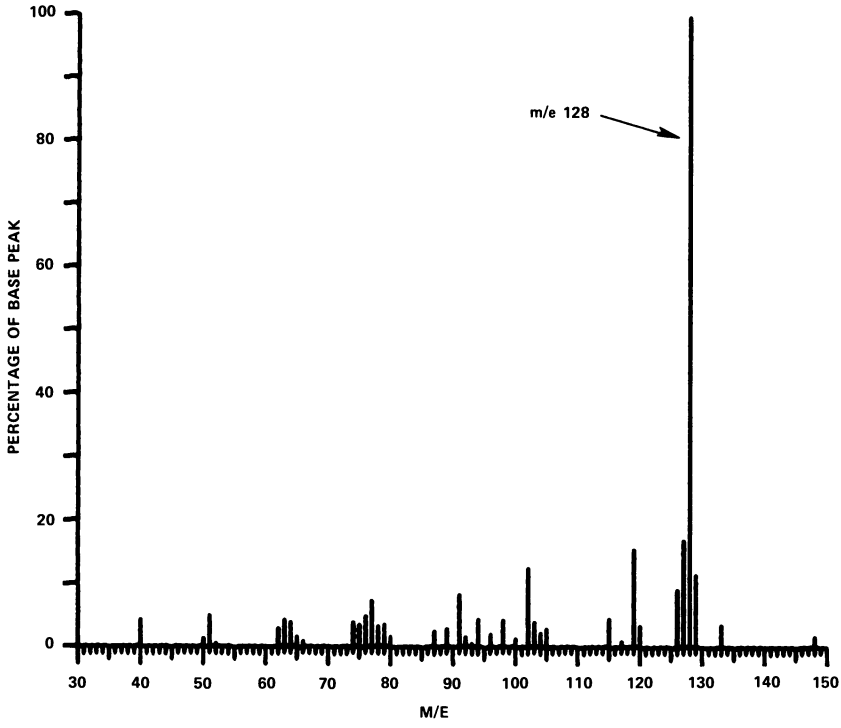


Figure 5. Mass Spectrum No. 185 from GC–MS run shown in Figure 2.

HPLC Using Fluorescence Detection

Naphthalenes, phenanthrenes, and other polycyclic aromatic hydrocarbons are very strongly fluorescing compounds and can be readily distinguished from biogenic olefins on this basis. High-performance liquid chromatography (HPLC) using a fluorescence detector is particularly well suited to this work, as has been described by various workers (*6, 7, 8*). HPLC analysis of the "aromatic" fraction from macrofauna and zooplankton samples is no more involved or costly than GC analysis and the detection limits are as low or even lower. However, in the presence of biogenic olefins, HPLC can detect trace amounts of aromatic hydrocarbons that would be completely overlooked by GC. The gas chromatogram shown in Figure 6 was obtained from the "aromatic" fraction of a fish sample. Although a very complex pattern of biogenic olefins was obtained, the gas chromatogram gave no information concerning the presence of aromatic hydrocarbons. The same fraction run by HPLC using a reverse-phase ODS column, an elution gradient of 50% acetonitrile in water to 100% acetonitrile at 1% per minute and a fluorescence detector set for excitation at 250 nm and emission detection

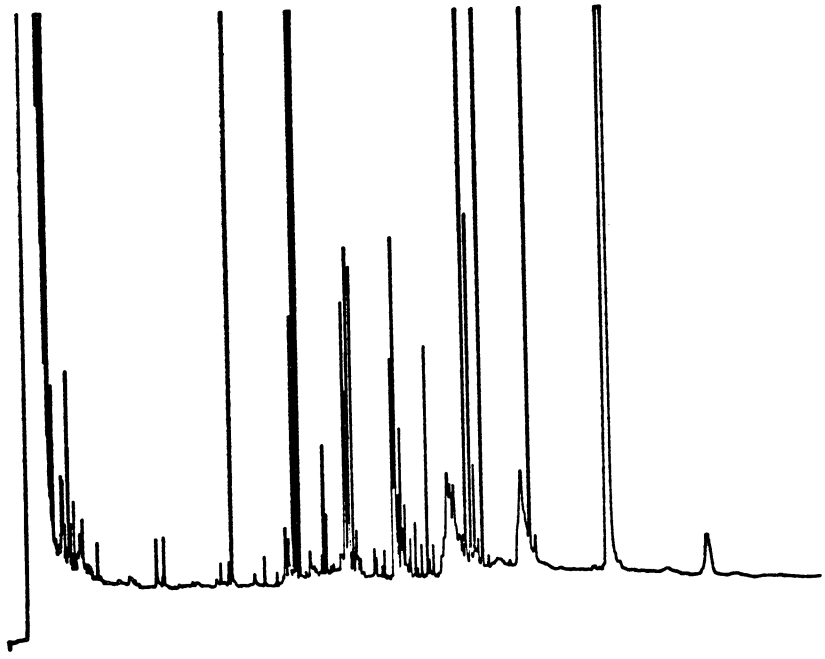


Figure 6. Gas chromatogram of "aromatic" fraction from a fish sample

at above 370 nm, gave the chromatogram shown in Figure 7. In this case, the aromatic hydrocarbons, phenanthrene, anthracene, and fluoranthene, were detected without interference from biogenic olefins. Hence for the determination of polycyclic aromatic hydrocarbons, especially in samples containing low levels of such compounds as in baseline studies, HPLC is far superior to GC. The chief disadvantages of HPLC are that fluores-

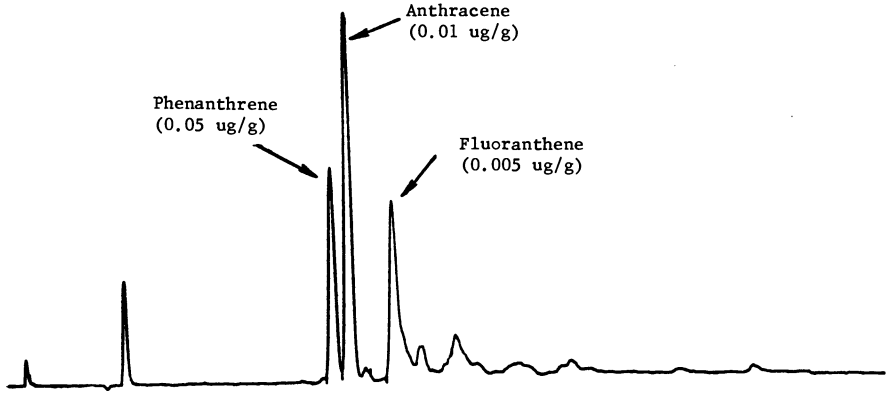


Figure 7. HPLC chromatogram of "aromatic" fraction from a fish sample

cence varies widely from compound to compound and partial quenching by interferences may occur. Therefore, the amounts of unidentified compounds present can only be roughly approximated. Nevertheless, such an approximation of compounds that are very probably polycyclic aromatic hydrocarbons provides much more valuable information concerning possible petroleum pollution than GC quantitation of compounds that are most likely nonaromatic and nonpetroleum hydrocarbons.

GPC Removal of Interfering Biogenic Components

The biogenic components that interfere with the GC analysis of aromatic hydrocarbons are primarily long-chain components. Such compounds are much larger molecules than the compact aromatic hydrocarbons. Because of this major difference, gel permeation chromatography (GPC), which separates on the basis of molecular size, can be used very effectively to separate the two classes of compounds (9). The GPC materials that can be used for the separation include modified dextrans, for example, Sephadex LH-20, and styrene–divinylbenzene copolymers, for example, BioBeads, Styragel, or μ-Styragel.

One system we used for this work was comprised of four 30-cm 100-Å μ-Styragel columns (from Waters Associates) connected in series with a Rheodyne Model 7105 injector valve and a Varian 4200 syringe pump that maintained a constant flow of either 1 or 2 mL/min. At 1 mL/min the overall resolution efficiency was about 15,000 theoretical plates and the run time was about 50 min. By doubling the flow rate, the run time was cut in half with only a slight loss of column efficiency. The pressure required for a flow rate of 2 mL/min was usually about 1200 psi. Tetrahydrofuran (UV grade from Burdick and Jackson Laboratories) was used as the eluting solvent. The amount of sample solution injected into the system varied from 10 μL to 500 μL.

The resolution achievable by the system is indicated by the separation of a standard hydrocarbon mixture shown in Figure 8. A refractive index detector was used. In addition to showing the resolution of *n*-paraffins achievable, Figure 8 also indicates that aromatic hydrocarbons, even 4-ring and 5-ring compounds, can be readily separated from straight-chain hydrocarbons.

For the removal of biogenic olefins from the aromatic fraction of a macrofauna or zooplankton extract, we discarded the GPC fraction that eluted prior to *n*-dodecane. The later eluting fraction, which would contain any aromatic hydrocarbons present (including any of the alkyl homologues commonly found in petroleum), was collected and analyzed by gas chromatography. A gas chromatogram of the aromatic fraction

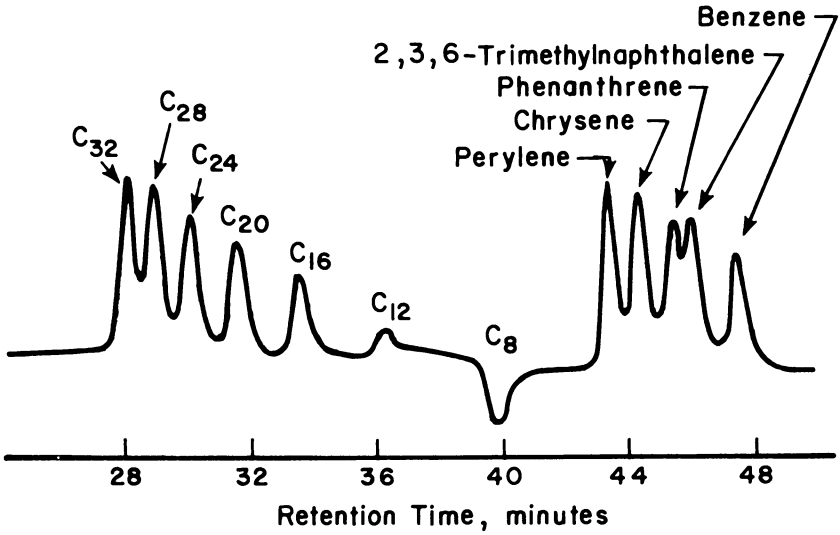


Figure 8. GPC separation of selected hydrocarbons using 100-Å μ-Styragel

from a zooplankton sample prior to cleanup by this GPC method is shown in Figure 9. Large amounts of biogenic olefins were present, making it impossible to determine whether any aromatic hydrocarbons were present. The gas chromatogram of the same fraction after GPC cleanup is shown in Figure 10. A trace of contaminants was found but no aromatic hydrocarbons.

By applying this GPC cleanup procedure to dozens of zooplankton samples, we have been able to determine by GC analysis alone that very few of the samples contained any aromatic hydrocarbons at levels of 0.01 μg/g wet weight or higher. Without the GPC cleanup, GC analysis would have been of no value in determining whether or not the samples contained aromatic hydrocarbons at such a low level.

GPC cleanup can also be used to advantage in place of silica gel chromatography in a gravity-flow chromatographic cleanup procedure. We have used BioBeads S-X8 with 50:50 acetone–methylene chloride as an eluting solvent. For this work a 9-mm i.d. × 400-mm glass column was packed with 10 g of BioBeads S-X8 that had been preswelled for at least 4 hr in 50:50 acetone–methylene chloride. The column was packed and used in the same manner as described previously (*4*) for silica gel chromatographic cleanup and fractionation. Prior to use the column was washed with 40 mL of eluting solvent (50:50 acetone–methylene chloride) to remove possible contamination. One milliliter of macrofauna or zooplankton extract in 50:50 acetone–methylene chloride and containing no

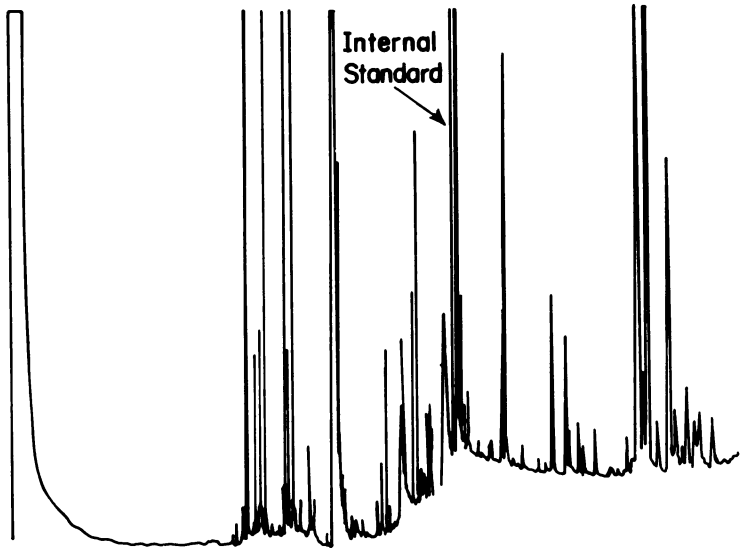


Figure 9. Gas chromatogram of "aromatic" fraction from a zooplankton sample before GPC cleanup

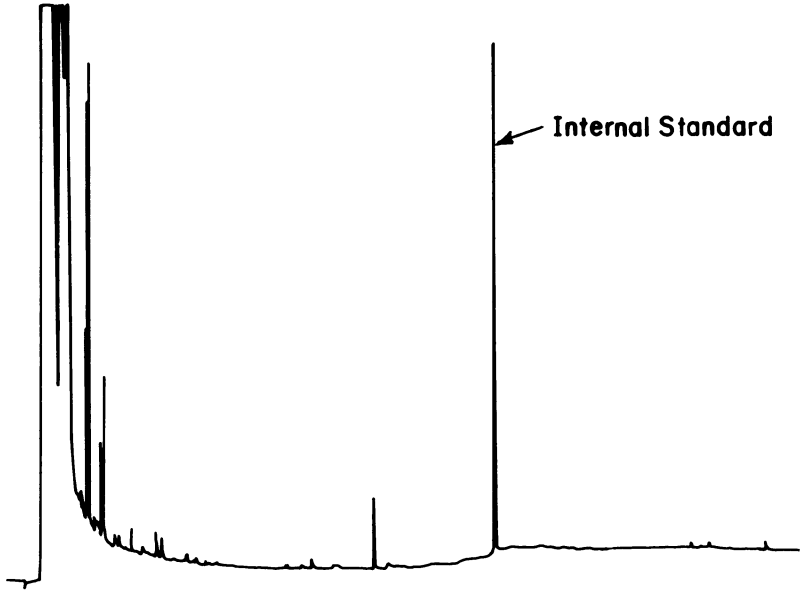


Figure 10. Gas chromatogram of "aromatic" fraction from a zooplankton sample after GPC cleanup

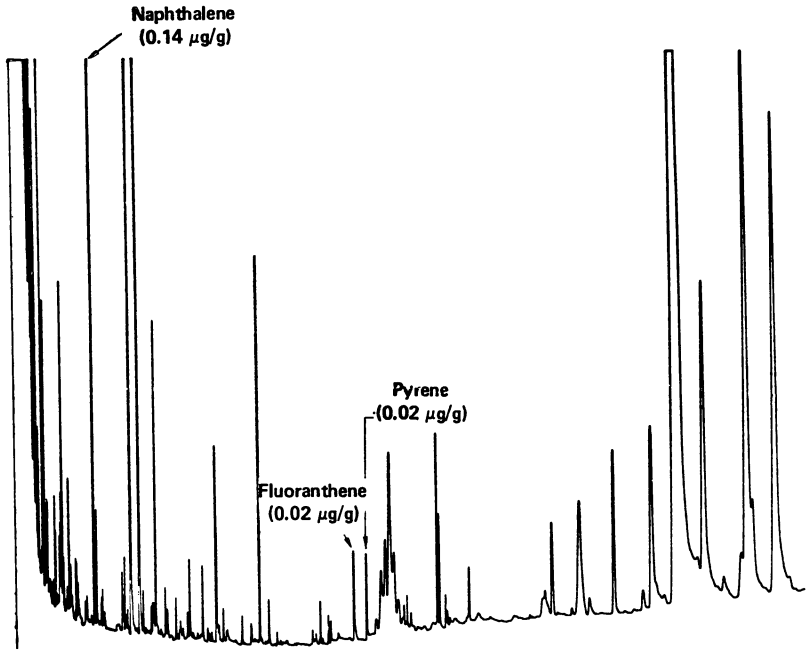


Figure 11. Gas chromatogram of tissue extract cleaned up by GPC using Biobeads S-X8

more than 100 mg of lipid material was added to the column and eluted with the same solvent. The elution rate was adjusted to 5 mL/hr. The first fraction eluted, 14 mL, contained the large molecules, including triglycerides, fatty acids, and long-chain biogenic olefins, and was discarded. The second fraction, 16 mL, contained the smaller cyclic compounds including aromatic hydrocarbons and was concentrated for GC analysis. Figure 11 shows a gas chromatogram of such a fraction obtained from mussels. Several aromatic hydrocarbons, namely, naphthalene, fluoranthene, and pyrene, are clearly discernible. The fraction also contains steroidal compounds and heterocyclic nitrogen compounds as was determined by GC–MS analysis. Further cleanup of the extract by silica gel chromatography either before or after the GPC cleanup would give a fraction containing only the aromatic hydrocarbons and could be analyzed satisfactorily by GC alone.

Benzenes and Naphthalenes by Purge-and-Trap Method

The most volatile aromatic hydrocarbons, benzene, toluene, and xylene, cannot be determined in tissue samples by extraction methods because they are largely or entirely lost during the solvent concentration

step. A purge-and-trap method was therefore developed in which the volatile hydrocarbons were swept out during the caustic digestion of tissue, trapped on Tenax-GC, and subsequently thermally desorbed onto a GC column for analysis.

The sample was contained in a 50-mL round-bottomed flask that was heated with a heating mantle and that contained a Teflon-covered magnetic stirring bar. The flask had a 0.25-inch o.d. side arm on which was attached with Swagelok fittings a 0.125-inch o.d. Teflon tube used as an inlet for a helium purge. The flask was fitted with a 15-cm condenser that was connected in series with a scrubber containing 80% sulfuric acid to remove amines, a 10-inch × 0.25-inch o.d. stainless steel U-tube containing 2.2 g of Tenax-GC, and a flowmeter. The Tenax-GC was extracted with methanol in a Soxhlet extractor and conditioned overnight at 250°C in a stream of helium prior to use. The Tenax trap was fitted in an aluminum block that was designed specially to permit temperature control at 20°–50° ± 1°C by water circulating from a constant temperature bath, and also to permit rapid controlled heating to 200°–250°C ± 5° and to permit rapid water cooling to room temperature. The trap was connected to a heated valving system (two 8-port switching valves, Carle No. 2012 and 2013) that permitted flushing and venting and also backflushing of desorbed components to a GC column. The GC system used was a Varian Model 2840 equipped with cryogenic capabilities and a flame ionization detector and fitted with a 30-m × 0.2-mm i.d. glass capillary column coated with SF-96. The helium carrier gas flow was pressure controlled at 10 psi, which gave a flow of about 2 mL/min.

One of the most critical features of the system, in terms of achieving satisfactory GC resolution, was the manner in which the column was connected at the injector end. It was essential that the desorbed components be condensed as a sharp plug at the head of the GC column and that the section of the column containing the plug be readily heated when the column oven was heated. This required a sharp temperature transition from a heated transfer line to a cooled column. This was achieved by using a separate temperature-controlled oven for the stainless steel transfer line and the stainless steel 1/16-inch Swagelok fitting used to connect the glass capillary column. The Swagelok fitting was insulated from the column oven to make sure that it remained heated while the column oven was cooled to − 70°C or lower. If the Swagelok fitting was not insulated sufficiently and became cold during the desorption step, the sample would condense at that point and would be subsequently only slowly released to give broad, poorly resolved peaks.

In making an analysis, 0.02–1.0 g of homogenized tissue was added along with 200 ng of hexylcyclohexane in 2 μL of CS_2, added as an internal standard, and was refluxed and stirred with 20 mL of 1*N* aqueous

sodium hydroxide while purging with helium at 50 mL/min to carry volatile components to the Tenax trap maintained at 30°C. At the end of a 1-hr digestion-and-purging period, the Tenax trap was flushed with helium at 50 mL/min for 15 min to remove any water vapor.

The trap was then heated rapidly to 220°C and backflushed with helium at 40 psi for 30 min, which swept the desorbed components onto the GC column with a flow rate of approximately 10 mL/min. The GC column oven was maintained at − 70°C during the desorption. The switching valves, transfer lines, and Swagelok fittings were maintained at 180°–200°C. At the end of the desorption the valves were turned to permit the Tenax trap to be reconditioned at 220°C, with 50 mL/min of helium for 30 min, and to permit a GC run. The GC column oven was temperature programmed at 16°C/min for 5 min and then 4°C/min to 192°C.

A gas chromatogram obtained from the analysis of 200 mg of clams exposed to a South Louisiana crude oil is shown in Figure 12. The naphthalenes are particularly discernible. Although the method works well for benzene, toluene, and xylenes, it is even better for the naphthalenes, because there are fewer interferences in that region of the chromatogram. Essentially quantitative recovery of dimethylnaphthalenes is obtained. The method was applied very successfully to the analysis of over 100 samples. It was particularly useful in the analysis of fish fry, fish eggs, and liver samples using 25–100 mg of material. The

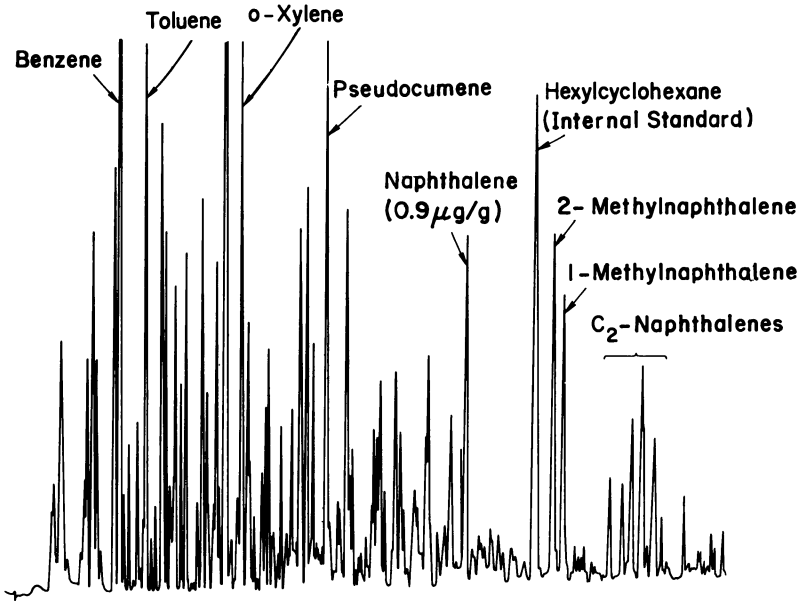


Figure 12. Gas chromatogram of volatiles from 0.2 g of clams exposed to South Louisiana crude oil

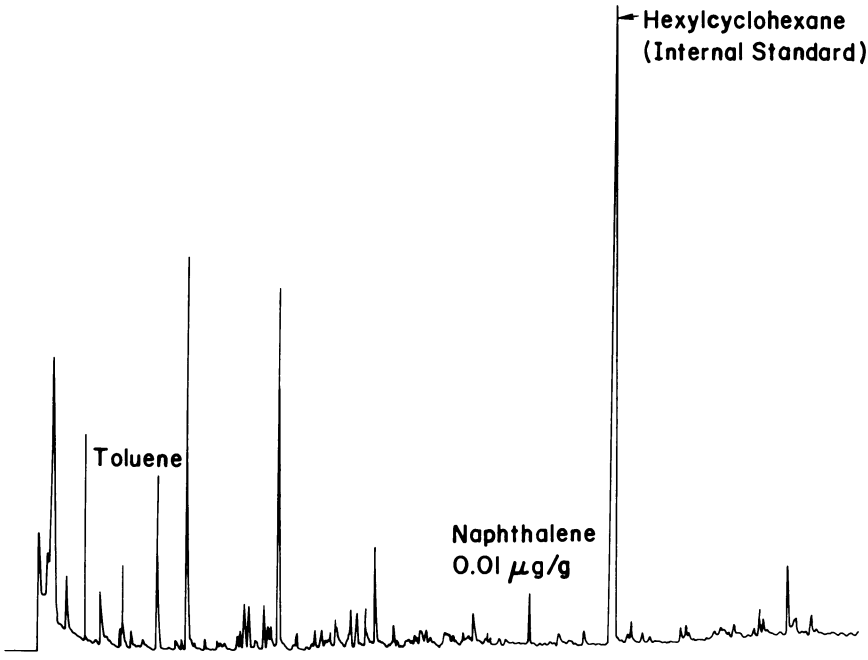


Figure 13. Gas chromatogram of volatiles from 1.0 g of control clams

limit of detection for the method is about 0.002 μg/g wet weight using a 1.0-g sample. A gas chromatogram from the analysis of 1.0 g of control clams in which naphthalene was found at a level of 0.01 μg/g is shown in Figure 13.

Conclusions

Interference by biogenic olefins, insufficient amounts of sample, and losses of volatile components generally make it impossible to determine low levels (< 0.05 μg/g wet weight) of aromatic petroleum hydrocarbons in macrofauna and zooplankton samples if the analytical method involves simply saponification, solvent extraction, silica gel fractionation, and GC analysis using a flame ionization detector. The interference problem can be overcome, however, by GC–MS analysis with specificity provided by selected ion scanning, by HPLC analysis with specificity provided by a fluorescence detector that selectively detects the polycyclic aromatic hydrocarbons, or by using gel permeation chromatography to remove the interferences prior to GC analysis. The GPC cleanup can be used alone or in conjunction with silica gel fractionation depending upon the degree of cleanup required. A purge-and-trap technique can be very

useful for determining low levels of light aromatic hydrocarbons, benzene to dimethylnaphthalenes, in small samples (25 mg–1 g) of macrofauna or zooplankton.

Acknowledgments

This work was supported in part by the Bureau of Land Management, the Department of Energy, the American Petroleum Institute, and the National Oceanic and Atmospheric Administration.

Literature Cited

1. Anderson, J. W.; Neff, J. M.; Cox, B. A.; Tatem, H. E.; Hightower, G. M. *Mar. Biol.* **1974**, *27*, 75.
2. MacLeod, W. D.; Brown, D. W.; Jenkins, R. G.; Ramos, L. S.; Henry, V. D. *Nat. Oceanic Atmos. Admin.* Technical Memorandum ERL MESA-8, November, 1976.
3. Farrington, J. W.; Madeiros, G. C. *Proc. Conf. Prev. Control Oil Pollut. March*, **1975**, 115.
4. Warner, J. S. *Anal. Chem.* **1976**, *48*, 578.
5. Lawler, G. C.; Loong, W. A.; Laseter, J. L. *Environ. Sci. Technol.* **1977**, *11*, 47.
6. Cretney, W. S.; Wong, C. S. *Nat. Bur. Stand. (U.S.), Spec. Publ.* **1974**, *409*, 175.
7. Fox, M. A.; Staley, S. W. *Anal. Chem.* **1976**, *48*, 992.
8. Wise, S. A.; Chesler, S. N.; Hertz, H. S.; Hilpert, L. R.; May, W. E. *Anal. Chem.* **1977**, *49*, 2306.
9. Giger, W.; Schaffner, C. *Anal. Chem.* **1978**, *50*, 243.

RECEIVED October 31, 1978.

5

High Resolution Gas Chromatography: An Overview

STUART P. CRAM and FRANK J. YANG

Walnut Creek Division, Varian Associates, 2700 Mitchell Dr., Walnut Creek, CA 94598

An overview of capillary gas chromatography is presented. Selected environmental applications, such as PCB's in water, PAH's in airborne particulate matter, and TCDD's at the part-per-trillion level illustrate the separation and analysis of complex mixtures. The chromatographic performance, characteristics, and trade-offs of packed and capillary columns are described in terms of permeability and efficiency, sample capacity, choice of stationary phase, high temperature capabilities, quantitative accuracy, and the development of GC separation methods.

Although the concept of capillary column chromatography $(GC)^2$ was first described by Golay (*1*) in 1957, the technique has now been developed to the point where it is easy to use and amenable to routine use in analytical laboratories. Since 1957, a great deal of technology has been developed to bring GC instruments, techniques, and thin-film glass capillary columns up to the point where they are highly efficient, more reproducible than packed column separations, chemically inert, and thermally stable. Special techniques, injectors, detectors, accessories, and instrumentation are now available for doing $(GC)^2$, and the literature contains a number of papers demonstrating the capabilities of the technique (*2*). Further, the need now exists, more than ever, for analytical techniques in trace organic analysis that are capable of separating increasingly complex samples and analyzing them at trace levels.

0-8412-0475-6/80/33/105$05.00/0

Applications

To illustrate a few of the problems in trace organic analysis being studied today by $(GC)^2$, the following selected examples indicate the diversity and power of the technique in approaching difficult separation and analysis problems.

Giger and co-workers (*3*) at the Swiss Federal Institute for Water Resources and Water Pollution Control, Dubendorff, traced the origin and the environmental pathways of polycyclic aromatic hydrocarbons (PAH) by $(GC)^2$. Figure 1 shows the capillary separation with dual detection (flame ionization and electron capture) of the aromatic hydrocarbons extracted from rain water. The major peaks in flame ionization detector (FID) trace are three-to-six ring unsubstituted PAH's. The electron capture detector (ECD) chromatogram shows the additional information content obtained using specific GC detectors to characterize trace samples. The FID chromatogram shows less than 20 major components of interest, and yet the same separation analyzed by ECD shows regions of the chromatogram that could be assumed to be "clean" from the FID analysis. Part of the reason for the higher degree of complexity of the ECD chromatogram is that the ECD has a detection limit for most chlorinated compounds approximately 100 times lower than that of the FID. By using both detectors in parallel it was found that PAH's and PCB's are subject to similar transport and transformation mechanisms in the environment. They both have low solubilities in water and a pro-

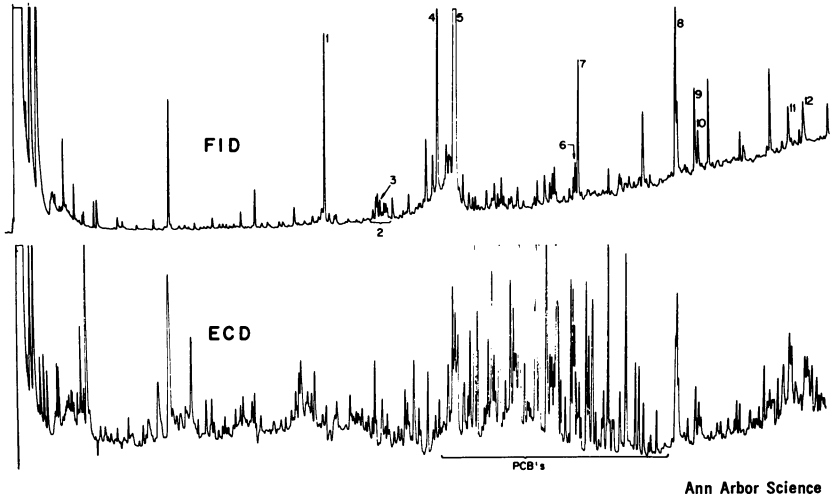


Figure 1. Capillary GC separations of the aromatic hydrocarbon fraction extracted from rain water. Column was 30 m × 0.35 mm OV-101 and temperature programmed from 60° to 240°C/min. Carrier gas was H_2 at 5 mL/min. (3)

nounced tendency to absorb on particulate matter. Therefore, the two classes of compounds are found in much higher concentrations in sediments.

Sewage effluents have also been characterized by $(GC)^2$. The chromatograms in Figure 2 compare the primary and secondary aliphatic

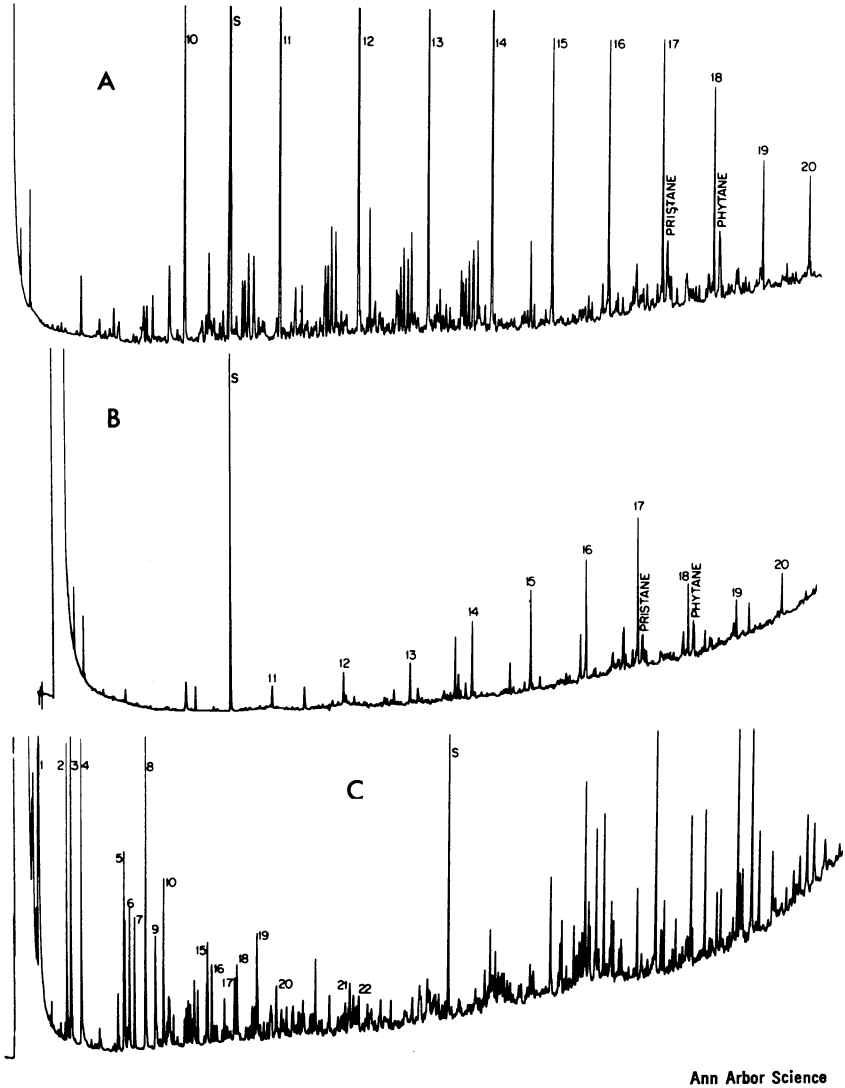


Figure 2. Capillary GC separation of the saturated hydrocarbon fractions from the (A) primary and (B) secondary effluent, and the (C) aromatic hydrocarbons in the secondary effluent. Column was 50 m × 0.36 mm OV-101 and temperature programmed from 30° to 240°C at 2.8°C/min. Carrier gas was H_2 at 4 mL/min (3).

hydrocarbon effluents and the secondary aromatic fractions. It can be seen from the n-C_{17}:pristane and n-C_{18}:phytane ratios of the primary and secondary effluents that almost no biological degradation is taking place in the n-C_{14} to n-C_{20} range. Therefore, other mechanisms such as gaseous stripping and adsorption during aeration and floculation may be responsible for the loss of the lower alkanes. The aromatic secondary effluent contains mainly alkylated benzenes, which are not removed as efficiently as the lower boiling alkanes. This may be explained by their higher solubility in water. Other studies of polar fractions from the same sewage effluents have shown that materials such as α-terpineol, a widely used synthetic flavor, is eliminated with 99% efficiency by rapid biodegradation (*3*).

Lee et al. (*4*) reported capillary GC separations and identification by mass spectroscopy (MS) and nuclear magnetic resonance (NMR) of more than 100 polycyclics, including trace alkylated compounds, in airborne particulate matter. Figure 3 shows the separation of more than 120 compounds in less than 90 min on an 11-m column. This chromatogram shows the importance of good sample cleanup and pre-GC sample fractionation methods. In this case, Sephadex LH-20 columns were used. Of particular significance are the GC results obtained with high-molecular-weight compounds at moderately high column temperatures. Figure 3 shows the elution of compounds ranging from three-ring PAH's (MW = 178) such as phenanthrene and anthracene (Peaks 2 and 3, respectively) to the seven-ring coronene and dibenzopyrene (Peaks 120–122). These separations and analyses represent an attractive tool for routine screening for a number of PAH pollutants, analyzing the combustion products of automobile exhaust, and modeling combustion processes.

Another example is the high sensitivity and specificity required for the separation and determination of 2,3,7,8-tetrachlorodioxin (TCDD) in natural products and the environment. Buser (*5*) screened grass and soil samples from Switzerland that were presumably uncontaminated. Figure 4 shows portions of the capillary GC–MS chromatograms, which indicate that if TCDD were present in the Swiss samples, the concentration was less than 3–5 ppt. The partial chromatograms inserted show the signal obtained for samples "spiked" with 20 ppt TCDD before extraction. Interfering peaks can be observed eluting within seconds of the TCDD peak. Conventional packed columns would not have separated all of the interferences at the ppt level, and a GC–MS method specific for the 2,3,7,8-TCDD isomer would be routinely feasible only if high resolution mass spectrometry were used.

The use of capillary GC is not limited to the separation of complex of multicomponent mixtures. Through the development of very high separation efficiencies, many relatively simple samples (< 20 components)

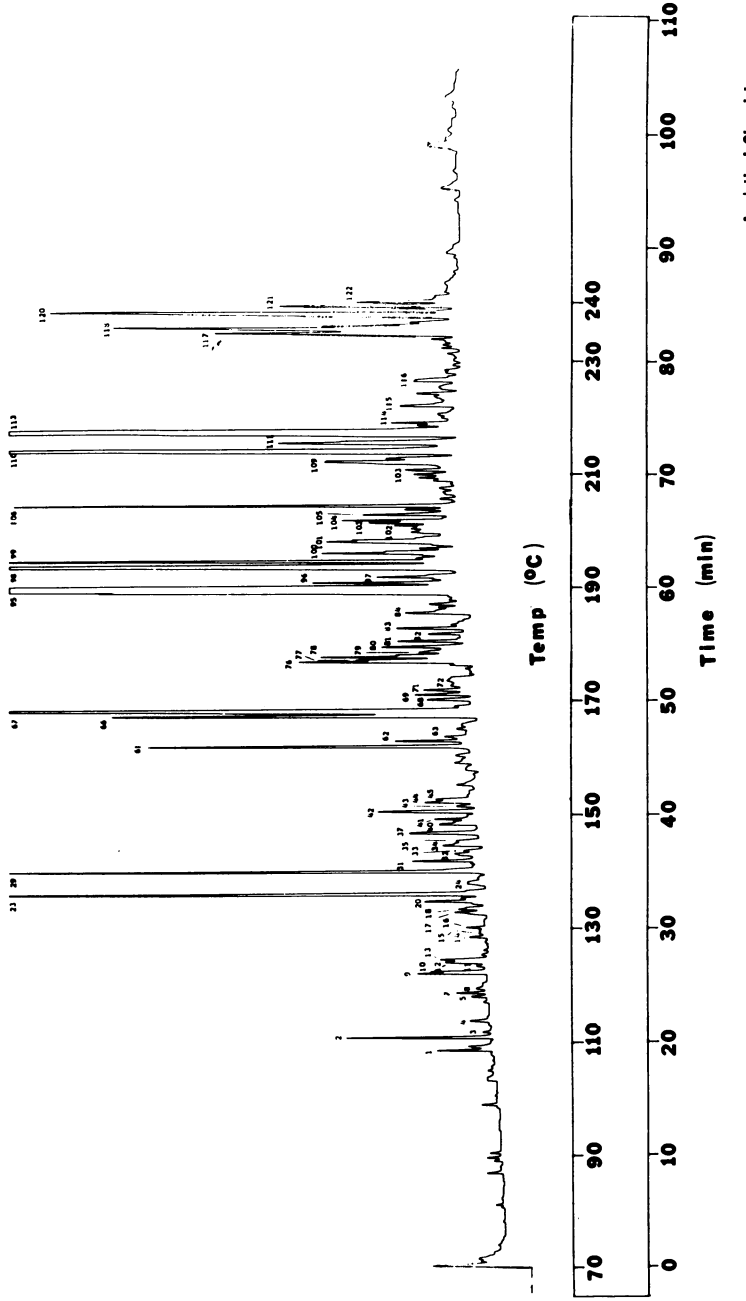


Figure 3. Capillary GC separation of the total PAH fraction of an airborne particulate sample. Column was 11 m × 0.26 mm SE-52, and temperature programmed from 70° to 240°C at 2°/min (4).

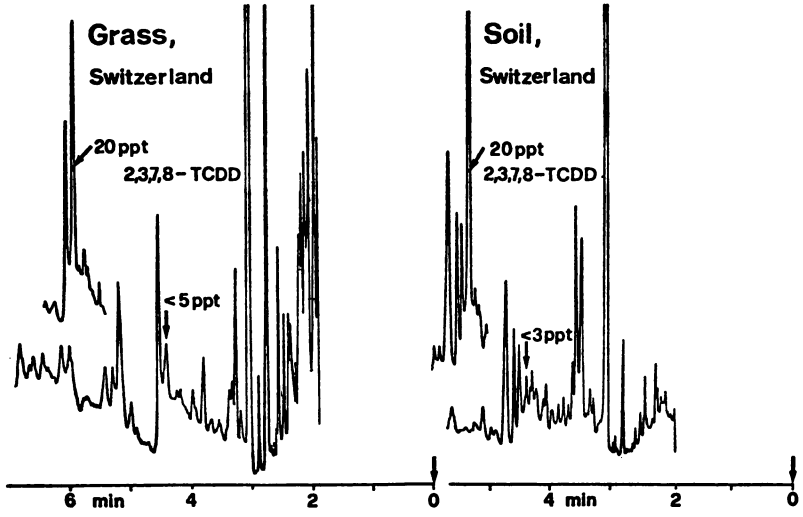


Figure 4. Mass chromatogram (m/e = 320) of grass and soil samples from Switzerland, analyzed for part-per-trillion (ppt) levels of TCDD. Inserted partial chromatograms show samples spiked with 20 ppt TCDD. Column was 25 m OV-17, at 220°C. (5)

may be separated on short columns (2–10 m) with short analysis times. Alternatively, the separation of isomers may require much longer columns and/or more chemically selective stationary phases. Examples are the separation of optically active enantiomers on optically active stationary phases (*6*) and geometrical isomers (*7*). The latter is shown in Figure 5 where the cis- and trans-isomers and the dimethylcyclododecanes resulting from the methylene insertion reaction of methylcyclododecane are separated. Thus, $(GC)^2$ can be a useful analytical technique to the synthetic or mechanistic organic chemist when a relatively small number of components are to be separated. Potential areas of application include solvent analysis, pesticide extracts, and drug assays.

Comparison of Packed and Capillary Column Separations

A good comparison of packed and glass capillary column separations was demonstrated by Grob (*8*). The two chromatograms shown in Figure 6 are of the same lake water extract, run on the same stationary phase (OV-1). Packed and wall coated open tubular (WCOT) columns and methods are compared in Table I.

The difference between the two methods is highlighted by noting the ratio of the capillary and packed column parameters. For example, though the capillary column is 12 times longer, it has 1% of the sta-

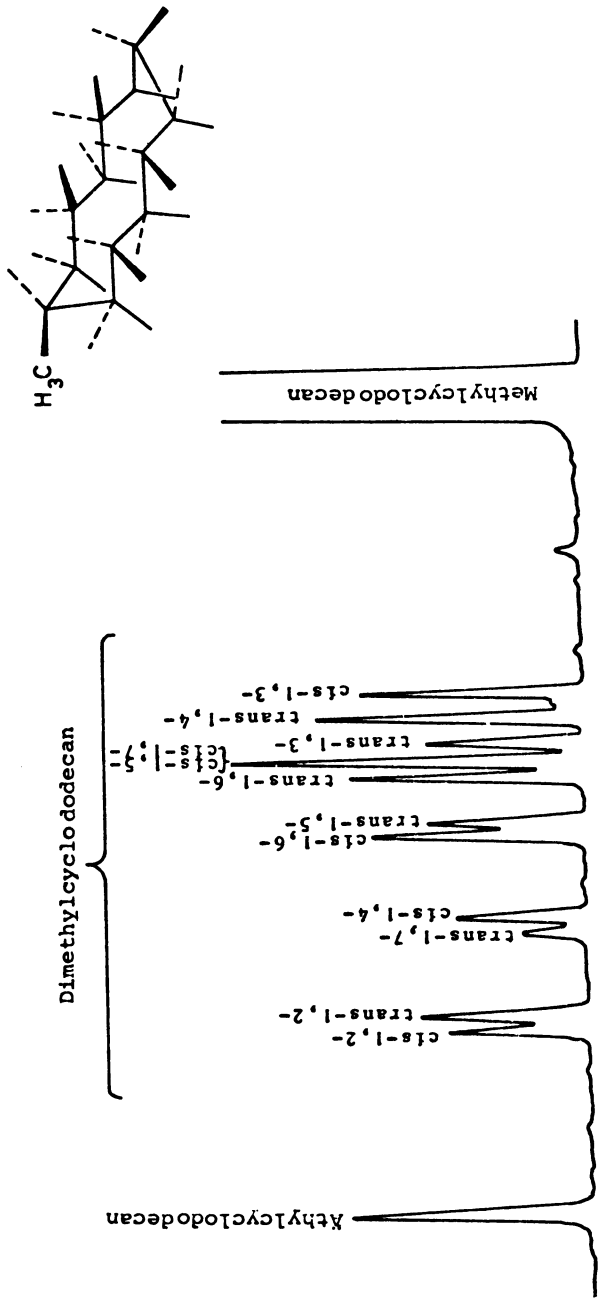


Figure 5. Capillary GC separation of the methylene insertion reaction products of methylcyclododecane. Column was 100 m × 0.25 mm DC-200, and run isothermally at 120°C with H_2 as a carrier gas. (7)

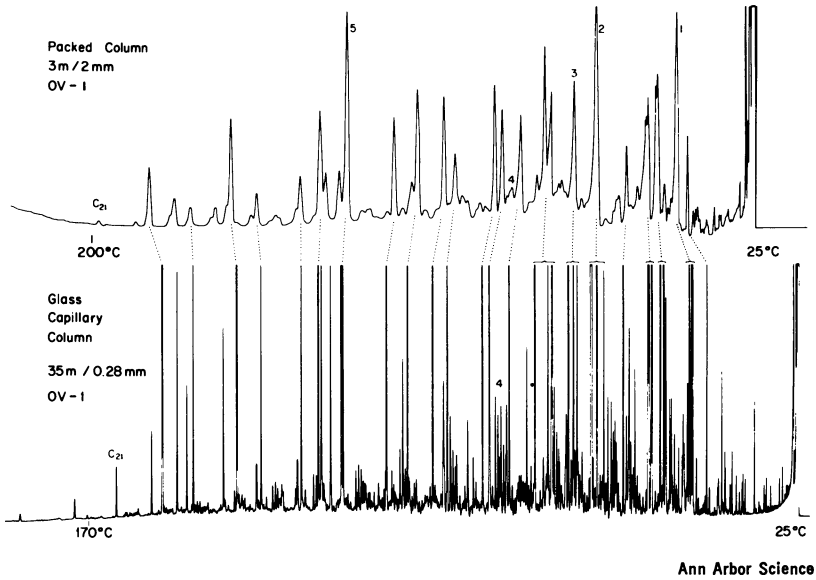


Figure 6. Comparison of packed and capillary column separations of a river water extract. Dotted lines indicate corresponding sample components. The numbered peaks (Peaks 1–3) were shown by GC–MS to be composed of at least three sample components; Peak 5 contains two equivalent major substances. (8)

Table I. Comparison of Packed and Capillary Columns and Methods[a]

	Packed Column	*Capillary Column*	*Ratio of Capillary: Packed*
Length	3 m	35 m	12
Inside diameter	2 mm	0.28 mm	0.1
Stationary phase	3% OV-1 on 80/100 mesh Gas Chrom	OV-1 $d_f = 1 \times 10^{-5}$ cm	—
Stationary phase load	120 mg	1.5 mg	0.01
Temperature program	50–200°C @ 20°C/min	25–170°C @ 3.5°C/min	—
Carrier gas flow rate	10–22 mL/min, He	2.2 mL/min, H_2	0.1
Sample size	0.15 μL	0.024 μL (0.6 μL split 25:1)	0.2
Analysis time	90 min	45 min	0.5
Number of peaks	118	450	4

[a] Data from Figure 6.

tionary phase of the packed column and, therefore, the maximum sample loading on the column is proportionally smaller. The capillary column is capable of higher separation efficiency (more than four times more peaks were found in one-half the time). With the packed column 118 peaks were eluted through special column preparation techniques and flow and temperature programming. The capillary column separation can be considered to be more "routine."

Packed column GC will remain a useful technique, especially for: the analysis of light hydrocarbons, low-boiling-point compounds, and fixed gases; samples known to contain less than 10 components; separations requiring large throughputs for preparative work; quantitation of very high concentration components in samples; and analytical work with low sensitivity detectors such as IR and NMR.

Capillary columns are potentially useful in methods research and quality control applications because they are: effective, especially with complex samples, as in environmental analyses; easy to use in developing separation methods; and more reproducible in terms of performance than packed columns. This last point follows from the fact that capillary columns are available that are individually tested and guaranteed on the basis of performance specifications; guaranteed packed columns are not available normally.

The baseline stability of the capillary separation in Figure 6 indicates nearly complete sample resolution, good experimental technique, and good thermal stability. The capillary peak width is narrow and the peak symmetry for the capillary peaks is virtually triangular (i.e., the peaks start in the noise, go straight up, and straight back into the noise without tailing). These characteristics are the mark of good capillary GC separations and analyses. Long capillary columns usually are not required for the complex samples, and thus the analysis time of the capillary column separation is one-half that of the packed column separation for this analysis.

A number of differences exist between packed and capillary columns that are responsible for their distinction in terms of performance:

(a) The permeability and efficiency of capillary columns is higher. Therefore, the pressure drop is less and, consequently, much longer columns can be used. A common misnomer is that capillary columns are more efficient than packed columns. In total they are, but per unit length they are comparable. A good packed column is nominally on the order of 2000–3000 plates/m. In comparison, thin-film, narrow-bore ($\leq$ 0.3 mn i.d.), wall-coated, open tubular columns are nominally 3000 plates/m. Because of the pressure drops involved and other experimental considerations, packed and capillary column lengths are usually limited to 3 and 100 m, respectively. Therefore, the total number of theoretical plates

available for separation will be less than 6000–10,000 plates for packed columns and less than 300,000 plates for capillaries. The separation efficiency of the capillary columns is principally determined by the column inside diameter, stationary phase film thickness, and uniformity of the stationary phase coating.

The relationship between capillary column inside diameter, column efficiency, expressed as the number of theoretical plates per meter (N/L), and film thickness is illustrated in Figure 7. These curves represent the theoretical limits of column efficiency for a given column inside diameter and stationary phase coating. The very thin-film, narrow-bore columns will be the most efficient and their efficiency changes markedly as the film thickness increases. The efficiency of a 0.25-mm i.d. column with a stationary phase film thickness of less than 1 μm is shown to be limited by mass transfer in the mobile phase (designated by C_g from the van Deemter equation), whereas mass transfer in the stationary phase (C_L) becomes predominate in thick-film columns. The point at which $C_g = C_L$ is shown for narrow- and wide-bore capillary columns in Figure 7.

Narrow bore columns usually range in film thickness from 0.1–1.0 μm. Columns between 0.1 and 0.5 μm demonstrate very high separation efficiencies, give minimum retention times for a given temperature, and

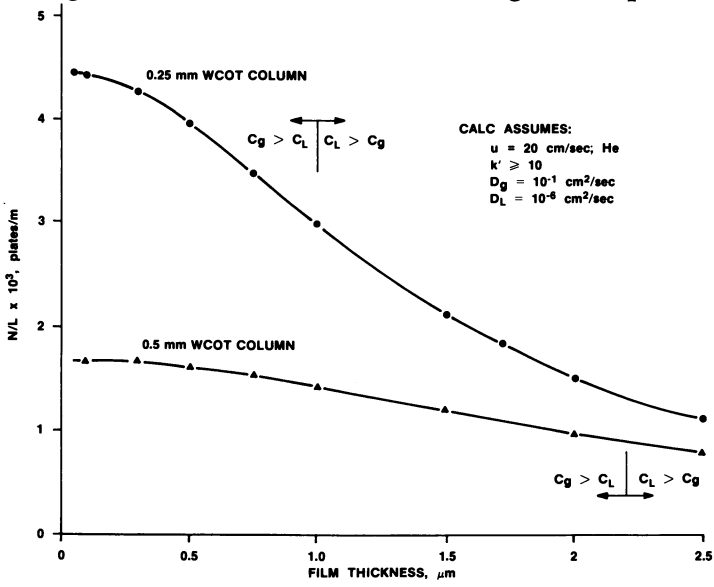


Figure 7. Effect of the stationary phase film thickness on the number of theoretical plates per meter (N/L, *column efficiency) as a function of the film thickness of the stationary phase for capillary columns of different inside diameters.*

have the lowest sample capacity. These columns are suggested for the analysis of highly complex samples ($\leq$ 100 components), for minimizing retention times, and for those samples containing high-boiling-point materials. Those columns with a film thickness greater than 0.5 μm have higher sample capacities, tend to demonstrate fewer surface or interfacial adsorption effects, and show longer retention times. They can be used for general purpose separations such as steroids and pesticides.

Figure 7 also shows that stationary phase films up to 2.0 μm can be used with the large bore columns, nominally 0.5 mm i.d. The advantages of wide-bore over narrow-bore columns are that they: allow the use of direct injection techniques; require minimum instrument modifications; allow the analysis of larger sample sizes (e.g., GC–MS); make it easier to use nondestructive detectors (e.g., TCD); cover a larger range of sample concentrations; are chemically inert and exhibit low absorption; use higher column flow rates (e.g., 5–15 mL/min); are not as susceptible to dead volume effects; and demonstrate excellent quantitation. The disadvantages are basically that: it is difficult to elute high-boiling-point compounds; lower column efficiencies are realized ($\sim 10^3$ plates/m); and longer analysis times are required.

An application of these columns was demonstrated by the separation of black pepper oil extract fractions for GC–IR/MS/NMR identification on columns as large as 0.9 mm i.d. (*10*). A partial chromatogram of one of the fractions is shown in Figure 8.

(b) The sample capacity of capillary columns is principally determined by the thickness of the stationary phase on the wall of the column. For high column capacity, wider bore columns with thicker films of stationary phase are used at the expense of some separation efficiency. For the highest separation efficiencies very thin-film, narrow-bore columns (e.g., 0.25 mm i.d.) are used. For the highest resolution, sample sizes of less than 10–50 ng/component are recommended. Special capillary injectors and injection techniques are used, which allow microliter injections and yet reduce the amount of sample reaching the column to a few nanograms.

Generally, the smallest amount of sample injected will give the best separation performance on thin-film columns. Thin films of stationary phase are easily destroyed or their polarity altered by overheating or by injecting "dirty samples" that contain nonvolatile and/or particulate residues. However, many workers point out that it is easier and faster to break off the first few coils of the column and reinstall it rather than to do extensive sample cleanup. For best separation efficiency, good retention time reproducibility, quantitative accuracy and precision, and to

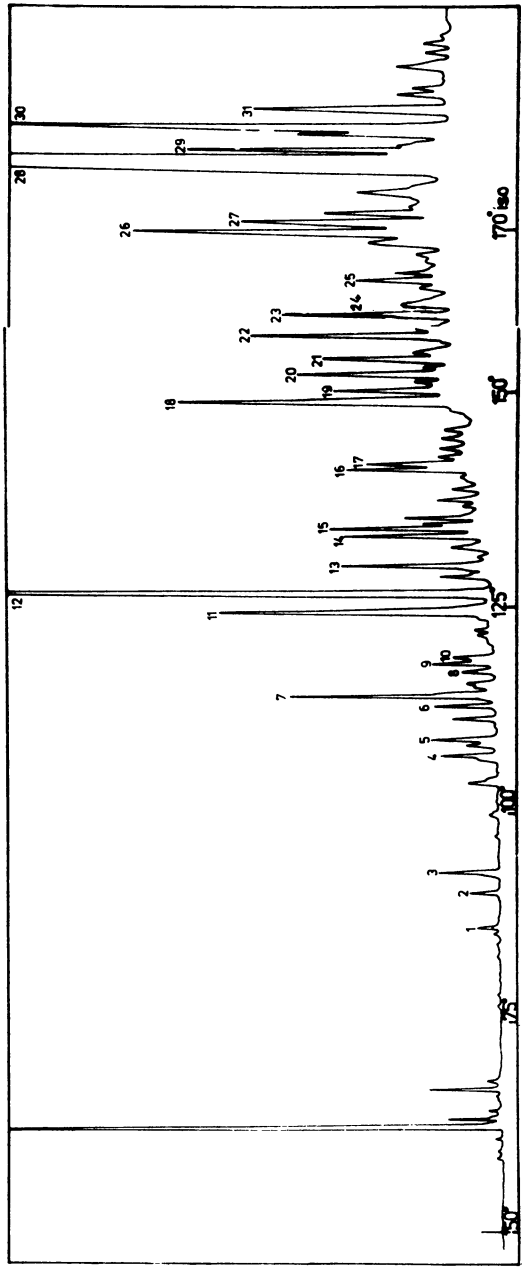


Figure 8. High capacity capillary column separation of a black pepper oil extract. Column was 70 m × 0.9 mm i.d. with Carbowax 20M, and was temperature programmed from 50° to 170°C. (9)

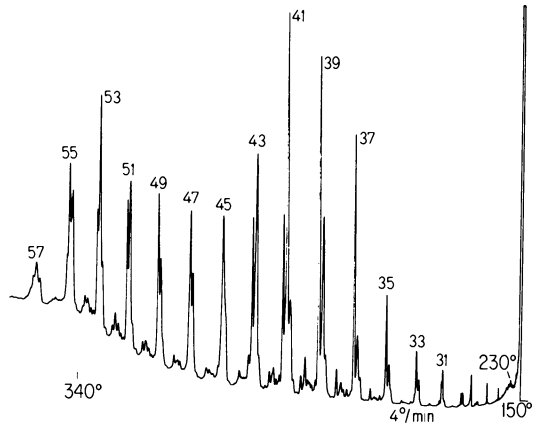
ensure a long column lifetime, sample cleanup is highly recommended. With care, the lifetime of a good capillary column should be comparable to that of a packed column.

(c) The choice of stationary phases is less critical for capillary than for packed columns for most applications because the total column efficiency is high. Therefore, fewer different stationary phases will be available and the need for special phases or columns will be limited to separations such as isomers. A good polar (e.g., Carbowax 20M), nonpolar (e.g., SE-30), and intermediate polarity column (e.g., FFAP or OV-17) will resolve the majority of separation problems and can be selected for the application on the basis of matching the polarity of the stationary phase to that of the sample or sample components of interest.

(d) High temperature separations on glass capillary columns were first reported in 1978 by Schomburg et al. (*10*) on a POLY S column temperature programmed to 390°C. This column was used for the separation of coal tar constituents. Subsequently, Maskarinec (*11*) analyzed PAH's as large as eight fused rings on Dexsil-400 columns at temperatures as high as 375°C. Triglycerides in whole butter up to tristearin/triolein (C_{57}) were eluted on a SE-52 column after temperature programming to 340°C as shown in Figure 9. However, the upper temperature limits of capillary GC separations are dependent upon the type of stationary phase, the method of coating, the type of column material used, the method of preparation of the surface, the presence or absence of deactivation agents, and other column parameters.

(e) The quantitative accuracy of GC analyses should be improved by $(GC)^2$ because of the high resolution of glass capillary columns. This should reduce the number of overlapping or fused peaks, which have been generally treated as single components in the past. The shape of the capillary peak is much sharper and more symmetric than packed column chromatograms, and the baselines are more stable, exhibiting less baseline bleed. Therefore, data systems can identify peak integration limits more easily than with packed columns and there is less ambiguity about where to assign the baseline if good capillary GC practice is followed. This often entails more sample cleanup than packed column GC.

(f) Another important distinction is that the time required to develop a capillary GC method is usually less than for packed columns. Less experimental work is required to find the best liquid phase, the proper amount of stationary phase, type of solid support, temperature program profiles, and all of the other parameters that must be chosen for packed column methods. In $(GC)^2$ the important considerations are choosing



Huthig Publications

Figure 9. Analysis of triglycerides in whole butter on a 20 m × 0.30 mm SE-52 column with a stationary phase film thickness of 0.05 μm and the on-column injection technique. (12)

the right sampling technique and pre-GC sample preparation methods, particularly if the sample is "dirty" or contains high concentration of fats, lipids, particulate, or other nonvolatile materials.

Future Column Technique Developments

Even higher resolution separations may be feasible in the future via techniques such as "two-dimensional gas chromatography" and "coupled column techniques." These techniques generally involve the use of two or more columns in series. The first may be a packed or nonpolar capillary column and is coupled to a capillary column with intermediate trapping and column switching by microvolume techniques. These techniques have been demonstrated and discussed by Schomburg (*13*), Grob (*14*), Bertsch (*15*), Kaiser (*16*), and others, and require advanced instrument capabilities. An example of the two-dimensional capillary separation of diesel oil is shown in Figure 10. The sample was first run on the 70-m, OV-1 column (A) and the *n*-C_{12} to *n*-C_{16} alkanes pneumatically switched to the 30-m, Carbowax 600 column (B). The branched alkanes in the sample still elute between the same alkanes on the polar column (B) but yield a higher resolution fingerprint. In addition, this section is now seen to contain some isomeric methylnaphthalenes. This technique has promising implications for confirmational identification by shifting retention times. This is seen in Figure 10 where the methylnaphthalenes are shifted from the *n*-C_{16} to the *n*-C_{13}–*n*-C_{15} region.

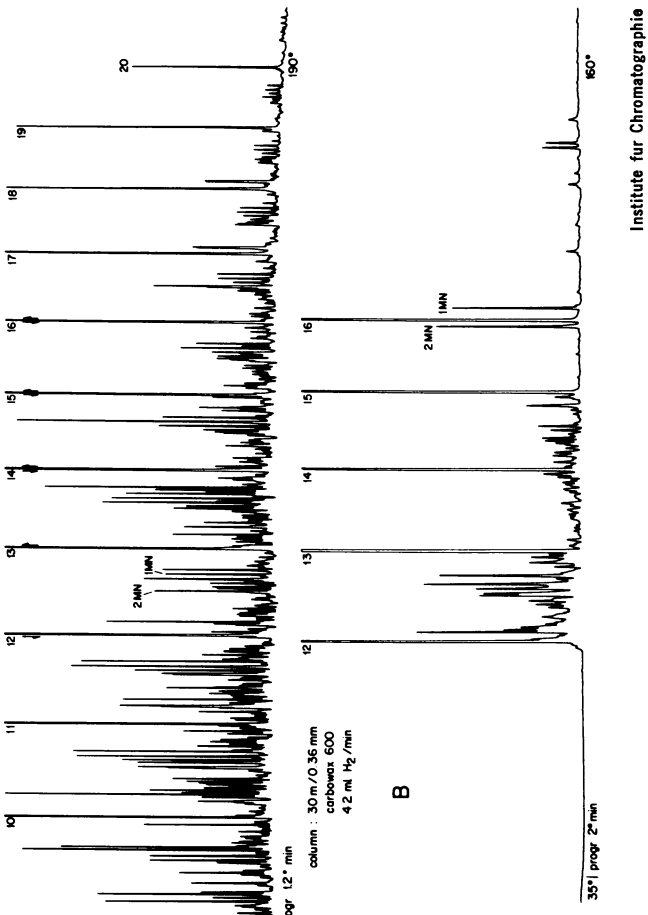


Figure 10. Analysis of diesel oil by "two-dimensional chromatography." Separation on Column A is with a 70 m × 0.27 mm OV-1 column, and the n-C_{18}–C_{16} *region is switched to Column B for further separation on a 30 m × 0.26 mm Carbowax 600 column.* (14)

Summary

New separation capabilities are now available to the analytical scientist for high resolution separations of multicomponent mixtures. Examples of important and complex problems in trace organic analysis being studied with $(GC)^2$ have been described in this chapter. $(GC)^2$ systems have been shown to be efficient, chemically inert at the nanogram–picogram level, and can be operated at lower injector and column temperatures than packed column systems. Therefore, the chance of thermal, catalytic, adsorptive, and other types of rearrangements and sample degradation should be decreased. This has been demonstrated by extending pesticide (*17*) and steroid (*18*) analyses down to the subpicogram range and by separating free fatty acids (*19*) and free sterols (*20*) without derivatization. Because lower column temperatures are required for elution on capillary columns, the analysis of compounds with lower volatility, higher molecular weights and highly polar compounds is now possible. This is exemplified by the elution of subnanogram quantities of seven-ring PAH's such as coronene (*4*), decabromobiphenyls, and polychlorinated dibenzo-*p*-dioxins (*5*).

Literature Cited

1. Golay, M. J. E. "Gas Chromatography"; Coates, V. J., Noebels, H. J., Fagerson, I. S., Eds.; Academic: New York, 1958; p. 1.
2. Cram, S. P.; Risby, T. *Anal. Chem.* **1978**, *50*(5), 213R.
3. Giger, W.; Reinhard, M.; Schaffner, C.; Zurcher, F. "Identification and Analysis of Organic Pollutants in Water"; Keith, L. H., Ed.; Ann Arbor Science: Ann Arbor, Michigan, 1976; p. 433.
4. Lee, M. L.; Novotny, M.; Bartle, K. D. *Anal. Chem.* **1976**, *48*, 1566.
5. Buser, H.-R., presented at the 2nd International Symposium on Glass Capillary Chromatography; Hindelang, Germany; 1977.
6. Koenig, W. A.; Stoelting, K.; Kruse, K. *Chromatographia* **1977**, *10*, 444.
7. Schomburg, G.; Husmann, H.; "Proceedings of the First International Symposium on Glass Capillary Chromatography Including Micropacked Columns"; Kaiser, R. E., Ed.; Inst. fur Chromatographie: Bad Durkheim, Germany, 1975; p. 61.
8. Grob, K.; Grob, G. "Identification and Analysis of Organic Pollutants in Water"; Keith, L. H., Ed.; Ann Arbor Science: Ann Arbor, MI, 1976; p. 75.
9. Debrauwere, J., Doctoral Dissertation, Rijksuniversiteit, Gent, Belgium, 1975.
10. Schomburg, G.; Dielmann, R.; Borwitzky, H.; Hussmann, H. *J. Chromatogr.* **1978**, *167*, 337.
11. Maskarinec, M. P. Ohio Valley Chromatography Symposium, 1979.
12. Grob, K.; Grob, G. *HRC & CC, J. High Resol. Chromatogr. Chromatogr. Commun.* **1979**, 3(2), 109.
13. Schomburg, G.; Husmann, H.; Weeke, F. *J. Chromatogr.* **1975**, *112*, 205.
14. Grob, K. "Proceedings of the First International Symposium on Glass Capillary Chromatography Including Glass Micropacked Columns"; Kaiser, R. E., Ed.; Institute fur Chromatographie: Bad Durkheim, Germany, 1975; p. 1.

15. Anderson, E. L.; Bertsch, W. "High Resolution Gas Chromatography"; Cram, S. P., Ed.; 1979, in press.
16. Kaiser, R. E. *Chromatographia* **1974**, *7*, 688.
17. Cramers, C. A.; Vermeer, E. A.; Franken, J. J. "Proceedings of the Second International Symposium on Glass Capillary Chromatography"; Kaiser, R. E., Ed.; Institute fur Chromatographie: Bad Durkheim, Germany, 1977; p. 95.
18. Horning, E. C.; Horning, M. G.; Carroll, D. I.; Dzidic, I.; Stillwell, R. N. "Advances in Chromatography 1973"; Zlatkis, A., Ed.; University of Houston: Houston, Texas, 1973; p. 1.
19. Maskarinec, M. P.; Alexander, G.; Novotny, M. *J. Chromatogr.* **1976**, *126*, 559.
20. Reed, W. E.; Stuermer, D. H. "High Resolution Gas Chromatography"; Cram, S. P., Ed., 1979; in press.

RECEIVED October 31, 1978.

6

Methods of Analysis for Polynuclear Aromatic Hydrocarbons in Environmental Samples

R. J. PANCIROV, T. D. SEARL, and R. A. BROWN[1]

Exxon Research and Engineering Co., Linden, NJ 07036

Levels of polynuclear aromatic hydrocarbons (PNAs) reported in this work for marine organisms, foodstuffs, sediments, and wastewater streams were found to be in the low ppb range. Data obtained on sediments and shellfish indicate that these PNAs are not petroleum derived but arise from a higher temperature combustion source. This conclusion is based on the alkyl-substituted PNAs measured relative to the parent PNAs. Analytical methods applicable to PNA analysis are almost as varied as the number of laboratories doing this type of work. This chapter describes the Exxon methods as they apply to various environmental samples.

For many years industry, academia, and the federal government have been involved in the development of methods for the analysis of polynuclear aromatic hydrocarbons (PNAs) in the environment. This concern for the measurement of PNAs has arisen because some of these materials are known carcinogens. The presence of these compounds in the environment is well established. In particular, these compounds have been found in sediments (*1*) and marine organisms (*2, 3*). They can enter the ocean by many routes, including petroleum spills, runoff from roads, sewage, effluents from industrial processes, and fallout from the atmosphere. To obtain baseline data on this subject, several years ago API initiated a project in which a continuing effort was made to measure PNAs in petroleum, fish, and foodstuffs.

The question of the origin, distribution, and fate of PNAs in the environment has been of keen interest for many years. Data obtained

[1] Deceased.

0-8412-0475-6/80/33/123$05.00/0

from the API program tend to support earlier conclusions drawn by Blumer and Youngblood (*4*) and Hites (*5*) that PNAs present in the marine environment are most likely derived from high-temperature combustion sources (i.e., forest fires or other sources) rather than from petroleum. This conclusion is based on the distribution of alkyl-substituted PNAs measured relative to the parent PNAs.

Analytical methods applicable to PNA analysis are almost as varied as the number of laboratories doing this type of work. An attempt will be made here to summarize the more pertinent techniques and to review their applicability to various types of environmental samples.

Analytical Methods

Analysis of PNAs is a complex and challenging task for the analytical chemist, because even in relatively simple samples such as air particulates the PNAs must be isolated from a mixture of materials. A much more difficult problem is the determination of PNAs in crude oil or petroleum products. In this case the parent PNAs are present as minor constituents in a matrix of materials very similar to the PNAs themselves. Another extremely difficult sample type to analyze for PNA content is foodstuffs. Difficulty arises here because of the very low PNA content (ppb) and the complex organic nature of the materials.

Methods used to handle the more difficult sample types generally include the following steps: preparation of a saturate hydrocarbon (*n*-pentane, cyclohexane, isooctane) solution of the sample; liquid/liquid extraction of the prepared solution with dimethylsulfoxide or dimethylformamide; preparation of a PNA concentrate using column chromatography; qualitative and/or quantitative measurements based on gas chromatography (GC), high-pressure liquid chromatography (HPLC), ultraviolet (UV) spectrophotometry, mass spectrometry (MS), or a combination of these techniques. In these procedures extensive sample handling occurs. This leads to incomplete and variable recoveries of the PNAs being measured. For this reason, internal standards are frequently used. These standards may be PNAs known to be absent from the sample or, preferably, isotopically labeled compounds that are the same as the PNAs being measured. ^{14}C labeled benzo[*a*]pyrene (BaP) and benzanthracene (BaA) are ideally suited for this task.

A number of laboratories have developed and put to practice a variety of methods for environmental problems. This includes Grimmer and co-workers (*6, 7, 8*), Exxon Research and Engineering (*9*), and Giger and Blumer (*1*). The GC–MS technique is extensively used as demonstrated by Lao et al. (*10*), Strup et al. (*11*), and Hites and Biemann (*12*).

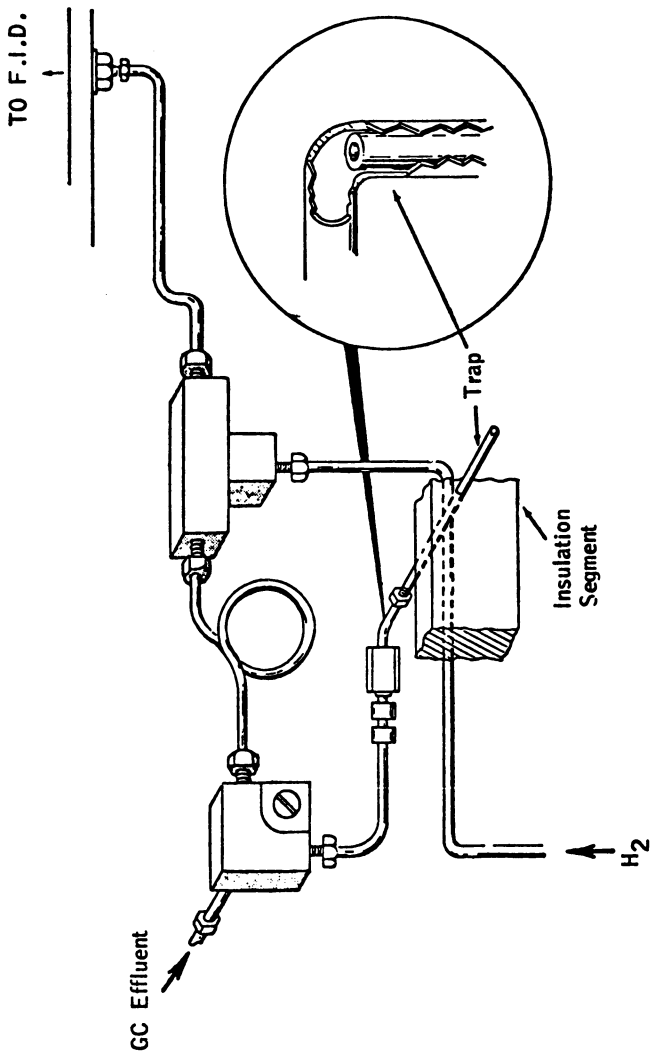


Figure 1. Gas chromatograph splitter and trapping mechanism

Experimental

The data reported here were obtained using the Exxon GC–UV procedure (*9*) as the final steps of the analytical method. To identify and quantify the PNA of interest, an aliquot of a PNA concentrate of the sample is injected into a gas chromatograph and each GC peak is trapped and its UV spectrum measured. These spectra provide a quantitative measure of individual PNAs in the aliquot. Quantitation on a total sample basis is derived from the observed recovery of ^{14}C BaA and ^{14}C BaP in their respectively trapped GC peaks. These activities compared with the amounts originally added are used to calculate the microgram quantities of each PNA present in the original sample. The ^{14}C BaA factor is used to calculate BaA, chrysene, and triphenylene, which all elute in the same peak. BaP and benzo[*e*]pyrene (BeP) are calculated using the ^{14}C BaP factor. All the remaining PNAs are calculated using whichever ^{14}C factor is the lower.

^{14}C counting is performed on an Intertechnique SL-30 Liquid Scintillation Counter. Aliquots of samples of recovered ^{14}C BaA and ^{14}C BaP are assayed for ^{14}C. Counting efficiency is determined by recounting after the addition of an aliquot of a standardized ^{14}C toluene solution.

The GC work is performed on a modified Perkin Elmer 900 gas chromatograph equipped with a flame ionization detector (FID). Figure 1 shows the modified effluent splitter of the 900. The GC effluent is split 15% to the FID detector, 85% to the trap. Stainless steel tubes (1/8″ × 7″) are used to trap individual peaks as recorded by the FID detector. The traps are washed with cyclohexane and a UV spectrum measured for each trap of interest.

The GC conditions are listed in Table I.

Table I. GC Conditions for GC–UV Procedure

Column	*1/8″ × 10′, 2% SE 30 on Chromosorb G*
Injection port temperature	300°C
Detector temperature	340°C
He flow	40 mL/min
Temperature program	175°–300°C at 4°/min

Samples of wastewater, sediments, marine tissues, foodstuffs, petroleum, and coal liquids have been analyzed using this method. Though the GC–UV finishing step was the same in each case, sample workup prior to GC injection varied considerably depending on sample types.

Sample Methods

Wastewater. This method is described by Searl, et al. (*13*) and may be summarized as follows:

One- to 4-L samples are collected by manually compositing the effluent over a 1–3-day period. The composites are kept in ice during the collection period and at less than 4°C until analyzed. ^{14}C labeled

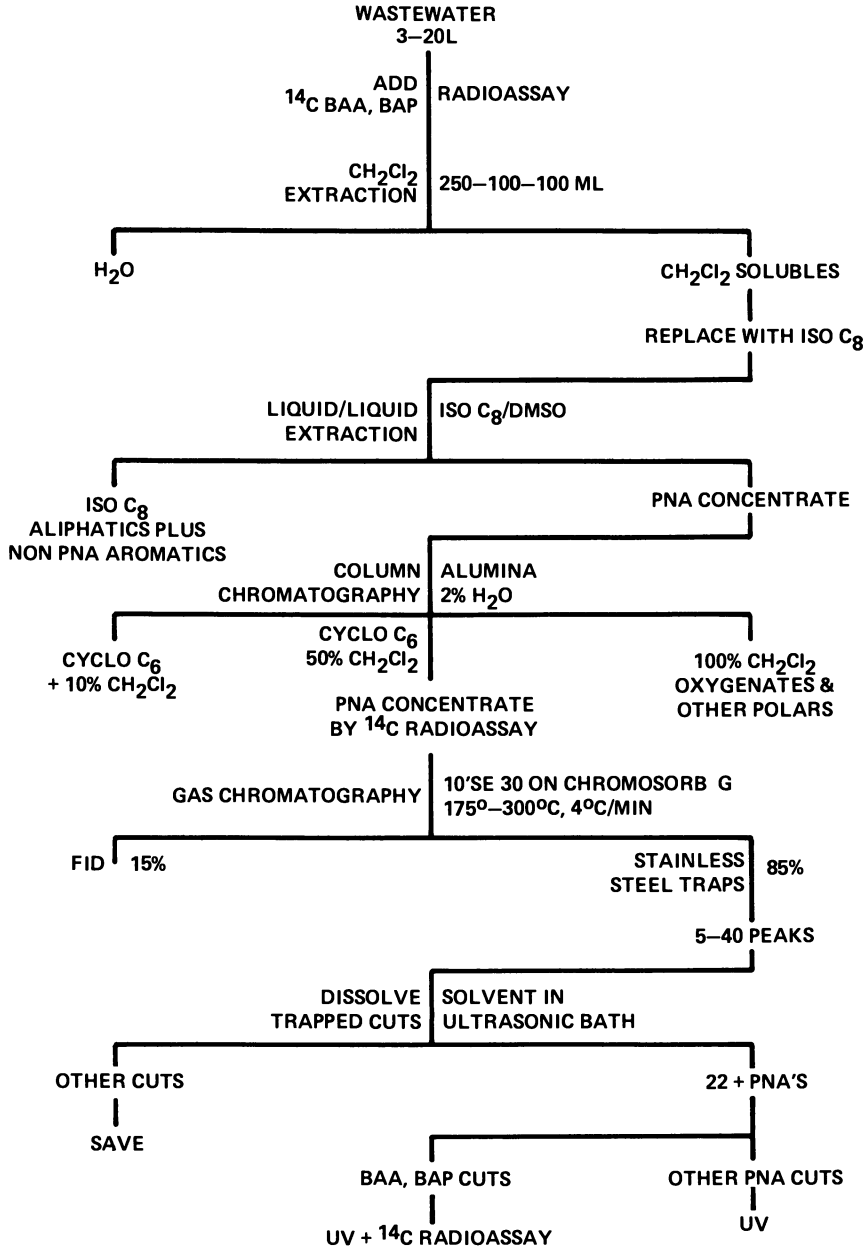


Figure 2. GC–UV method for PNA in wastewater

BaA and BaP is added and the entire sample is extracted with methylene chloride (250-, 100-, and 100-mL portions). The extracts are combined, 10 mL of isooctane added, and the solution evaporated to about 10 mL. When the solution approaches 10 mL, a second 10-mL volume of isooctane is added. This is repeated three times. The isooctane solution is made up to 75 mL and extracted with three 100-mL portions of dimethyl sulfoxide (DMSO) that contains 20% phosphoric acid. The DMSO extracts are each washed with three 30-mL portions of fresh isooctane, combined and diluted with 500 mL of water. This solution is extracted with three 80-mL portions of isooctane. The isooctane extracts are combined, rinsed once with water, and evaporated to 25 mL. At this point, a 1-mL portion of the solution is removed for intermediate radioassay and a UV scan. Usually the concentrate is sufficiently free from interferences and is evaporated down to about 0.05 mL and an aliquot injected into the GC. If further purification is indicated, the solution is chromatographed on a 1.1-cm $\times$ 90-cm column containing 75 g of Woelm Neutral Alumina deactivated with 2% water. Before deactivation, the alumina is dried in an oven for 1 hr at 150°C. The solvent elution schedule is as follows: 25 mL cyclohexane prewash, 25-mL sample in cyclohexane, 100 mL cyclohexane, 100 mL cyclohexane–methylene chloride (9:1), 100 mL cyclohexane–methylene chloride (1:1), 100 mL methylene chloride. After the first 125 mL has eluted, 10 cuts are then radioassayed and combined into a single PNA fraction. This fraction is evaporated to about 0.05 mL in preparation for the GC–UV finishing step. Figure 2 is a diagram of the wastewater procedure.

Sediments (*14*). Accurately weigh about 200 g of sample and place in a clean evaporating dish. Break up and spread out the sample and place the dish in a clean desiccator, with $CaSO_4$ as desiccant, and keep in the dark for three days. Remove the sample from the desiccator and weigh. Split the sample in the following manner: approximately 100 g for PNA extraction and determination, approximately 30 g for extractable organics and $C_{14}+$ analysis, and approximately 30 g for dry weight determination. Take the 100-g sample and split two ways for separate Soxhlet extractions. To 2 L of "distilled in glass" methylene chloride add ^{14}C labeled BaA and BaP ($\sim$ 32,000 DPM each). Split the 2-L spiked solution in half and Soxhlet extract each 50-g sample for 5 hr. After extraction, evaporate down to 25 mL while replacing with isooctane, DMSO extract, isooctane solution; chromatograph on alumina as outlined under method for marine tissues/foodstuffs. Heat one of the 30-g samples for 12 hr at 100°C in a clean oven to obtain dry weight of sediment. Use this calculated factor to obtain PNA content on a dry weight basis.

Marine Tissues/Foodstuffs (*15*). Approximately 450 g of sample is dried and placed into a 2-L flask containing 300 mL ethanol, 15 g of KOH pellets, and boiling chips. The sample is spiked with ^{14}C BaA and BaP, approximately 60,000 DPM of each, and the mixture is refluxed for 2 hr using a Friedrich condenser. After refluxing, the warm mixture is transferred to a 2-L separatory funnel. The boiling flask is washed successively with two 125-mL portions of distilled H_2O and two 100-mL portions of ethanol. Finally, the flask is washed with 150 mL of isooctane. All the wash solutions are added to the 2-L separatory funnel.

Shake funnel for 3 min and allow layers to separate, making sure residual solids settle. Draw off lower aqueous layer into a second separatory funnel. Extract this aqueous solution with 100 mL of isooctane. Allow these layers to separate and again draw off the lower aqueous layer into a third funnel. Extract this solution with 100 mL of isooctane. Discard the remaining aqueous layer and any residual solids. Wash each of the three isooctane extracts four times with 250 mL of warm (50°C) distilled H_2O, discarding the aqueous layer after each wash. After washing, combine the isooctane extracts.

Reduce the isooctane extract, on a steam bath under nitrogen, to 20 mL. A 1-mL sample may be removed at this time for an intermediate radioassay. Add an equivalent amount of isooctane to the sample and place in a 500-mL separatory funnel. Extract with five 100-g portions of DMSO/H_3PO_4 (10:1), combining the extracts in a 2-L separatory funnel. Add 720 mL of H_2O and back extract with three 100-mL portions of isooctane. Place each portion of isooctane into a separatory funnel and wash three times with 250 mL of distilled H_2O. Combine and reduce the isooctane extracts to 10 mL.

The isooctane solution is eluted through a long, narrow (6 mm × 90 cm) column of 2% partially deactivated alumina. The solvent elution procedure is as follows: 5 mL prewash of cyclohexane, 10 mL sample, 30 mL 5% methylene chloride in cyclohexane, 30 mL 10% methylene chloride in cyclohexane, 30 mL 50% methylene chloride in cyclohexane, and 30 mL of methylene chloride. Ten-milliliter cuts are collected, counted, and combined according to the radioassay data. The combined cuts are reduced to approximately 0.05 mL on a steam bath under nitrogen after adding 5 μL of *n*-hexadecane to prevent going to dryness. An aliquot (10 μL) of this concentrate is used for the GC–UV finishing step.

Petroleum (*16*). Where applicable, samples are distilled and the 200°C+ fraction retained, spiked with ^{14}C BaA and BaP, and analyzed for PNA content. Samples are first separated on Attapulgus clay to remove the very polar heteroatom portion of the sample. The Attapulgus clay was purchased from the National Bureau of Standards to have adsorptive characteristics as specified in ASTM D 2007. The column, 760 mm × 22 mm, is fitted with a 500-mL reservoir and a Teflon stopcock; 170 g of clay is placed in the column and prewet with 50 mL of *n*-pentane. A 15- to 20-g sample, dissolved in 100 mL of pentane, is placed on the column. The sample is then successively eluted with 600 mL of *n*-pentane and 500 mL of acetone. The entire separation is carried out in a blanket of nitrogen. Each fraction is carefully evaporated on a steam bath under nitrogen until a constant weight is obtained.

The *n*-pentane clay fraction is chromatographed on silica gel to obtain a saturate and aromatic fraction. The silica gel used is from Davison Chemical Company, Baltimore, Maryland, and is grade H 100–200 mesh. The column dimensions are the same as for the clay separation. One hundred eighty grams of silica gel are placed in the column and prewet with 50 mL of *n*-pentane. Ten grams of sample obtained from the *n*-pentane wash of the clay separation are dissolved in 10 mL of *n*-pentane and placed on the prewet column. This is followed by 600 mL of *n*-pentane and 500 mL of acetone. Each fraction is evaporated on a steam bath under nitrogen until a constant weight is obtained.

The acetone wash (aromatic fraction) from the gel separation is then separated on 2% deactivated Woelm Neutral Alumina (Nutritional Biochemicals, Cleveland, Ohio). The column is 890 mm × 6.5 mm and is fitted with a 50-mL reservoir and tapered end. Fifteen grams of the alumina are placed in the column. The column is prewet with 25 mL of cyclohexane and pressurized to 15 lb of nitrogen. One-fifth gram of the acetone fraction from the silica gel separation is dissolved in 10 mL of cyclohexane and placed on the column. When the cyclohexane has entered the column, the following washes are added: 15 mL cyclohexane; 30 mL 10% benzene in cyclohexane; 30 mL 20% benzene in cyclohexane; 30 mL benzene; and 30 mL 55% benzene in methanol.

Fractions are collected in the following sequence: The first 15 mL is collected and each fraction after is collected in 10-mL portions. The 10-mL fractions are combined according to the radioassay and prepared for GC–UV.

The procedure above for the alumina column separation was used at the time of analyzing the petroleum samples. A preferred procedure now would be to employ cyclohexane and methylene chloride–cyclohexane as described for wastewater samples.

Coal Liquids (*17*). A procedure similar to that applied to petroleum liquids was used for coal liquids. In particular, the separation steps on clay, silica gel, and alumina were identical. The methods differ in that the coal liquids were obtained from the feed coals and liquefaction products by first refluxing the material in a stirred Erlenmeyer flask for 8 hr with benzene. After removing the benzene from the extract by vacuum stripping, the remaining oily liquid was refluxed in a stirred Erlenmeyer flask with cyclohexane. The cyclohexane-soluble portion of the oil was analyzed for PNAs following the petroleum liquids separation scheme.

Results and Discussion

Wastewater. Three wastewater samples were analyzed for PNA content using the procedure outlined in Figure 2 (*13*). These were a refinery dual-media filter effluent, a final refinery effluent, and a municipal sanitary wastewater plant effluent. Figure 3 presents the chromatograms of the PNA concentrates from the dual-media filter (DMF) effluent and the final refinery effluent. The high attenuation (1024 × 10) on the DMF chromatogram and the large peaks indicate PNA levels in the 10–100-ppb range. Also, the earlier peaks are more pronounced than the later, indicating higher proportions of lower-molecular-weight compounds. The peaks observed in the final refinery effluent samples are much less pronounced than for the DMF sample, indicating a high percentage of PNA removal during water treatment.

The results obtained for 18 PNAs found in the refinery dual-media filter and final filter are presented in Table II. Only traces of PNAs were found in the final filters from the refinery. These data illustrate that the refinery-activated sludge unit removed 95% of the 5-ring PNAs and 99% of the 4-ring PNAs.

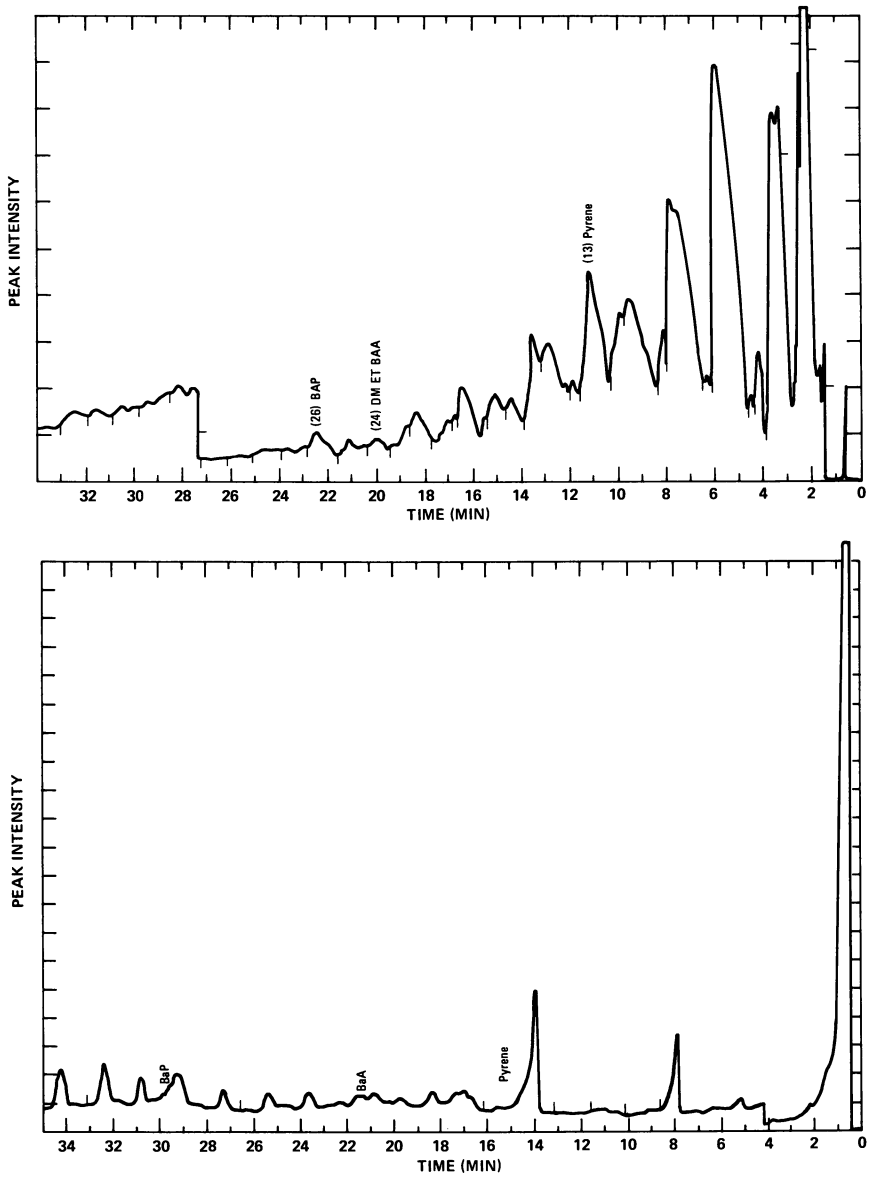


Figure 3. (Top) GC chromatogram of PNA concentrate from dual-media filter; (bottom) *GC chromatogram of PNA concentrate from final refinery effluent.*

Table II. Polynuclear Aromatic Compounds in Wastewater Effluents

Compound	*Refinery Effluents*			
	Dual-Media Filter[a]	*Final Refinery Effluent*[a]	*% PNA Removal*	*Municipal Effluent*[a]
Fluoranthene	17	ND (0.2)[b]	99+	ND (0.03)
Pyrene	106	0.07	99.9	0.10
Benzanthracene	31	ND (0.03)	99.9	0.00
Chrysene	16	ND (0.03)	99.8	0.02
Triphenylene	11	ND (0.03)	99.7	0.03
Methyl benzanthracene	20	0.10	99.5	ND (0.03)
Dm/Et benzanthracene	7	0.07	99	ND (0.03)
Benzo[*ghi*]fluoranthene	6	ND (0.4)		ND (0.15)
Benzo[*b*]fluoranthene	ND (3)	ND (0.1)		ND (0.06)
Benzo[*j*]fluoranthene	ND (6)	ND (0.2)		ND (0.10)
Benzo[*k*]fluoranthene	ND (5)	ND (0.2)		ND (0.07)
Perylene	2.6	0.14	95	ND (0.05)
Benzo[*a*]pyrene	20	0.57	97	0.03
Benzo[*e*]pyrene	11	0.65	94	ND (0.06)
Methyl benzo[*a*]pyrene	11	0.27	97	ND (0.10)
Methyl benzo[*e*]pyrene	7	0.38	95	ND (0.07)
Benzo[*ghi*]perylene	6	0.36	94	ND (0.1)
Coronene	ND (0.1)	ND (0.01)		—
Indenopyrene		ND (0.02)		

[a] Data recorded as μg/L.
[b] ND—not detected, detection limit in parentheses.

The refinery effluent is in fact comparable to the municipal sanitary wastewater plant effluent. These data are presented in the last column of Table II. As can be seen, PNA levels in the municipal effluent are in close agreement with those from the refining effluent.

Figure 4 is the chromatogram obtained from the PNA concentrate of the municipal sanitary wastewater. Here again the chromatogram is very similar to the final refinery effluent.

Figure 5 is the UV spectrum of the BaA peak in the DMF refinery sample. The GC peak is a mixture of three PNAs, BaA, chrysene, and triphenylene, and without the UV spectrum the peak could not be correctly assigned. The spectrum shows peaks at 269 nm and 259 nm, attributable to chrysene and triphenylene, as well as the BaA peak at 289 nm. This spectrum also provides a good example of the baseline technique used for measuring UV absorbance.

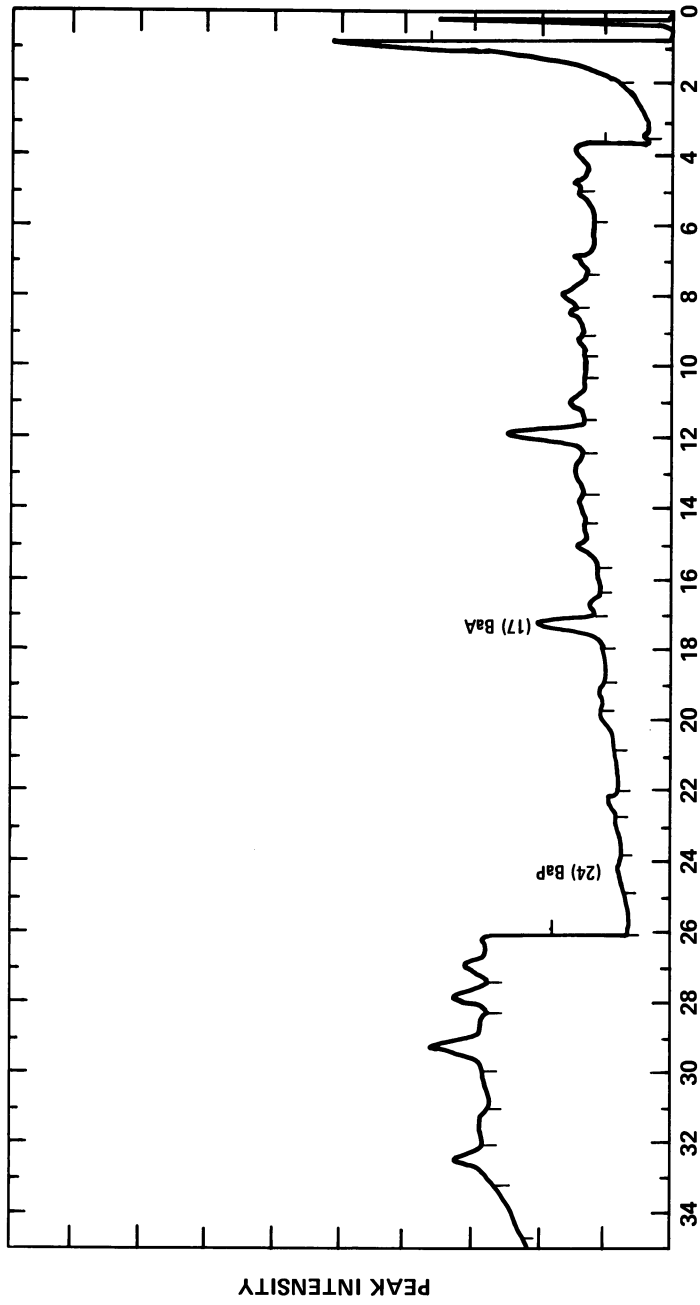


Figure 4. GC chromatogram of a municipal sanitary wastewater plant

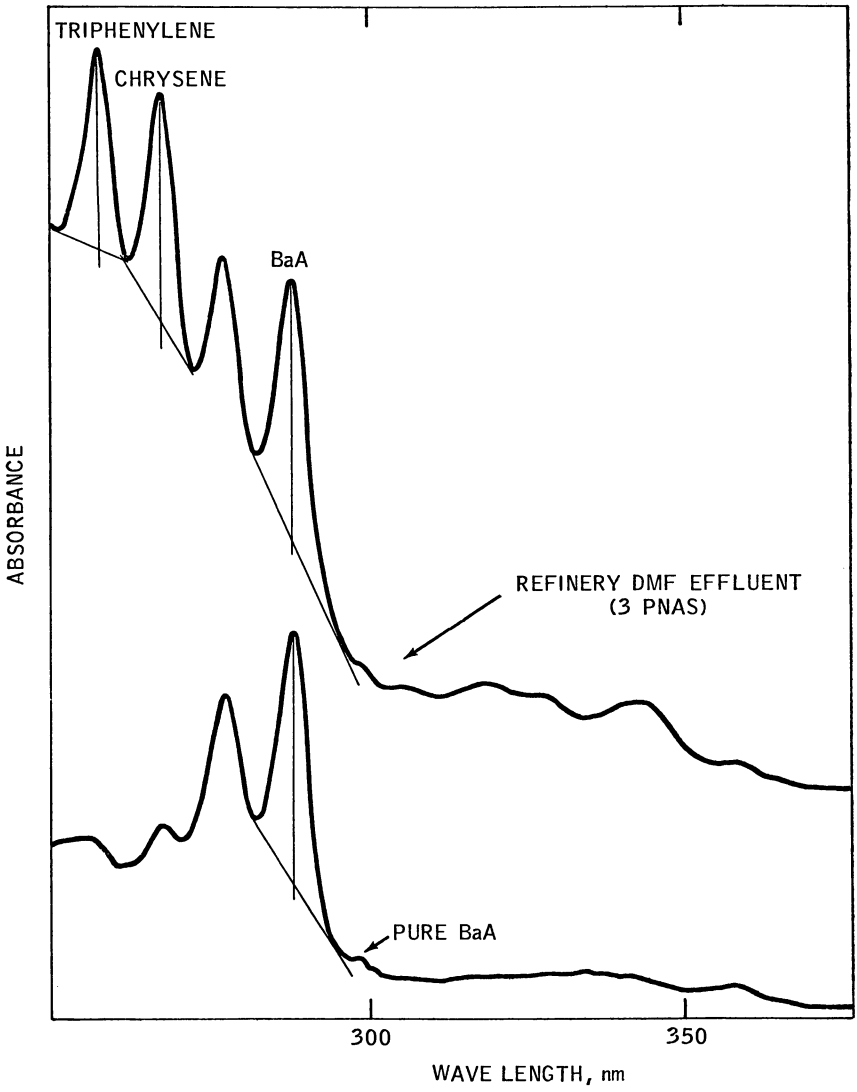


Figure 5. UV spectrum of BaA peak in dual-media filter effluent

To check the efficiency of the direct extraction of PNAs from wastewater with methylene chloride, a water sample was spiked with ^{14}C BaA and ^{14}C BaP extracted with methylene chloride and both the radioactivity and UV absorption measured on successive extracts. Figure 6, a plot of the UV absorbance and ^{14}C radioactivity vs. number of extractions, illustrates very clearly that 98% of the PNAs are recovered by three extractions.

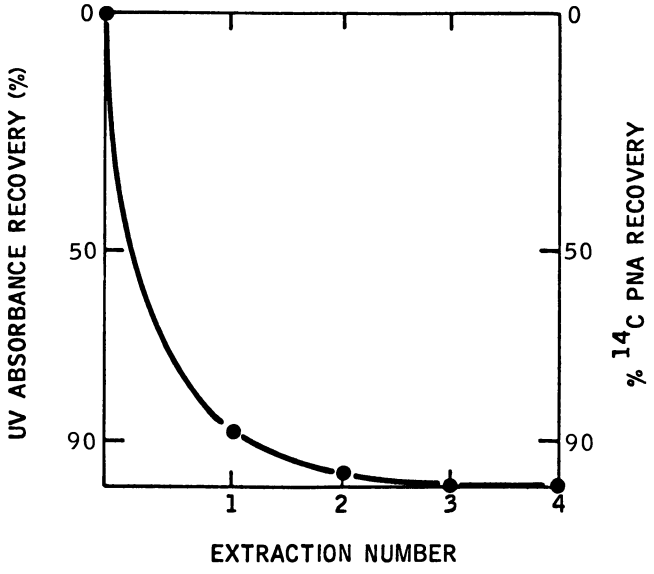


Figure 6. Extraction efficiencies of PNA hydrocarbons from wastewater with methylene chloride

Sediments. Figure 7 is a chromatogram of a sediment taken from Colin Scott Lake, Ontario, Canada (*14*). Here, as in the case of the wastewater chromatogram, is an excellent illustration of the high degree of selectivity the method has for obtaining a PNA concentrate. Figures 8 and 9 are the UV spectra measured on the BaA and BaP GC peaks. As mentioned earlier, BaA elutes along with chrysene and triphenylene and these three components can be readily determined from the UV trace. BaP and benzo[*e*]pyrene also elute in the same peak, but again their concentrations can be calculated from the UV spectrum.

Data obtained from three sediment samples are listed in Table III. These data are in agreement with other investigators (*18, 19*). Their work points out that PNAs are widely distributed in soils and marine sediments at very low concentrations. One of the significant observations from this earlier work was the distribution of the alkyl-substituted PNAs measured relative to their parents. In all cases the parent PNA was of much higher concentration than its methyl-substituted PNA. This type of behavior was observed in our data as seen by the pyrene and methyl pyrene levels listed in Table III. This type of distribution suggests a high-temperature combustion source as the origin of the PNAs rather than a petroleum source. Blumer and Youngblood (*4*) made the point that PNAs may have been present on earth, from forest fires and other natural combustion sources, for a long while, actually over geologic time.

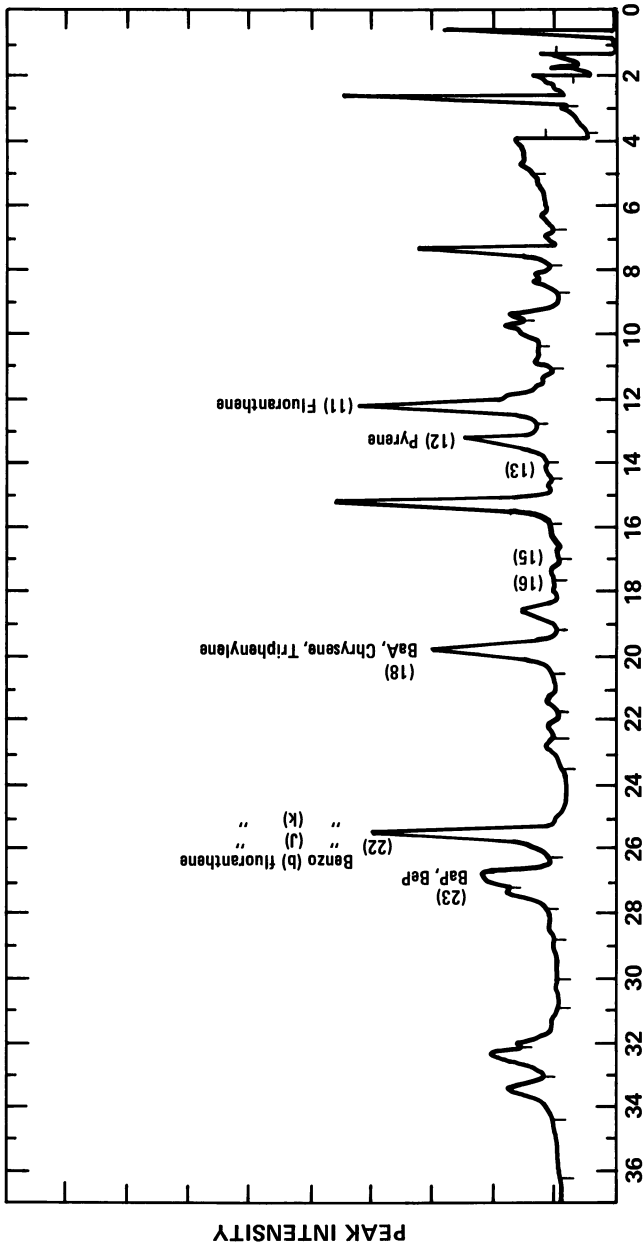


Figure 7. GC chromatogram of Colin Scott Lake sediment

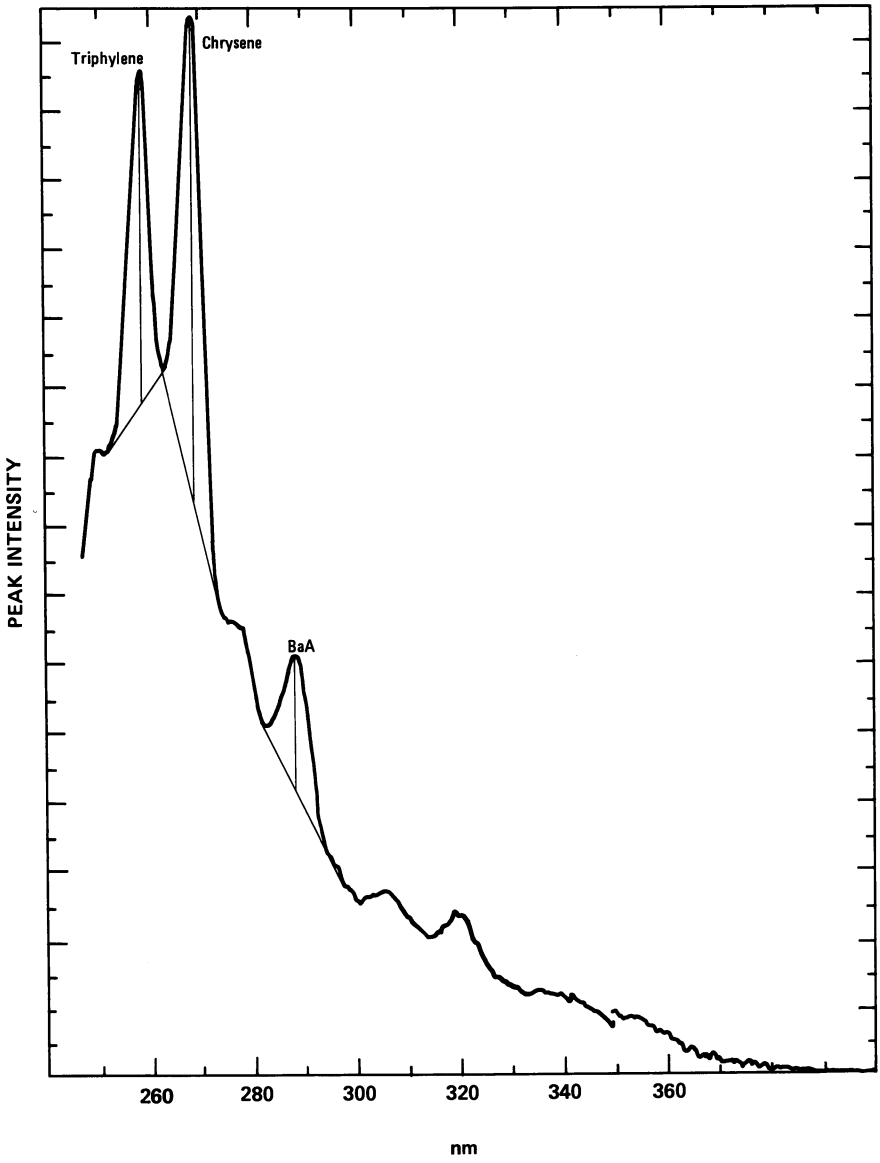


Figure 8. UV spectrum of BaA GC peak from Colin Scott Lake sediment

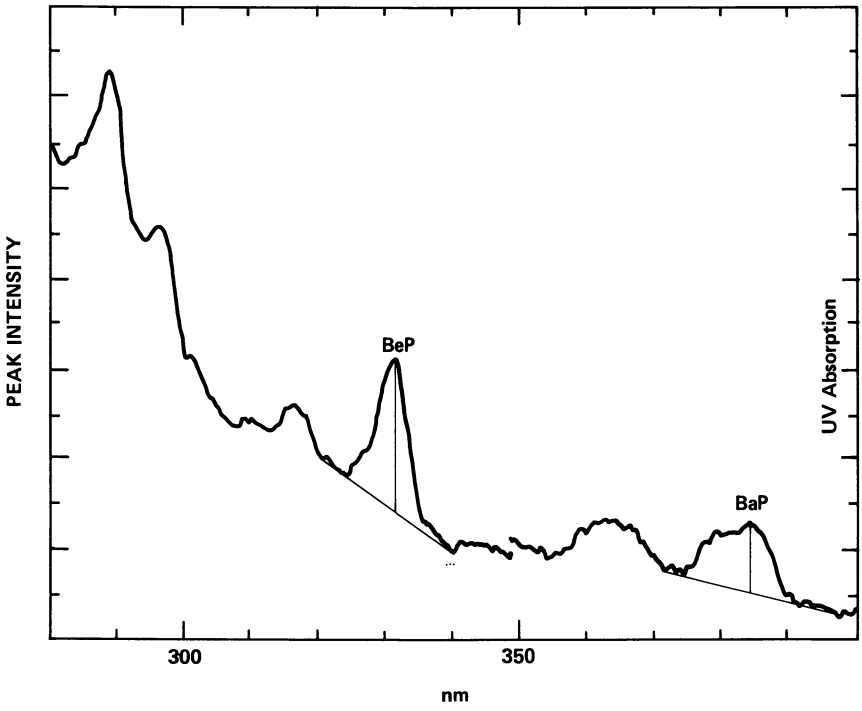


Figure 9. UV spectrum of BaP GC peak from Colin Scott Lake sediment

Table III. Polynuclear Aromatic Hydrocarbons in Sediments[a]

	West Falmouth Downslope[b]	*West Falmouth Upslope*[b]	*Scott Lake*
Fluoranthene	—	13.8	32
Pyrene	< 0.24	15.6	19
Methyl pyrene	< 0.30	4.3	< 1.7
Benzanthracene	1.1	1.2	6
Chrysene	1.3	6.0	19
Triphenylene	0.28	2.1	8
Benzo[*a*]pyrene	2.6	5.5	11
Benzo[*e*]pyrene	1.3	6.6	22
Perylene	1.0	< 5.3	17

[a] All data recorded as ppb.
[b] Corrected for loss on drying.

Marine Tissues (Edible Portion). A number of marine tissues, both fin and shellfish, have been analyzed for PNA content (*15*). These samples were obtained from both contaminated and noncontaminated areas. Table IV lists the data obtained on fin fish samples and Table V contains data from shellfish. These data indicate that it is rare to observe the presence of PNAs in fin fish. Only in the case of a menhaden caught in Raritan Bay (a contaminated area) and a flounder caught south of Long Island did we detect any PNAs: 1.5 ppb of BaP for the menhaden and 2 and 0.5 ppb for pyrene and methyl pyrene in the flounder.

For shellfish the opposite is true. It is rare to find a shellfish that does not contain PNAs. This is not surprising, because it is known that marine sediment contains measurable quantities of PNAs. Shellfish are harvested from sediment areas and consequently are continuously exposed to PNAs. Even under these conditions, however, it should be noted that PNA levels in shellfish are in the low ppb level. In shellfish as in sediments, the ratio between pyrene and methyl pyrene is an indicator of the source of these compounds. This ratio in shellfish favors the parent PNA over the methyl-substituted PNA, again indicating that the source of the PNAs is not petroleum but a higher-temperature combustion process.

Table IV. Polynuclear Aromatic Hydrocarbons in Fin Fish (*20*)[a]

	Menhaden (Raritan Bay)	*Flounder (40°27′N, 73°06′W)*	*Codfish (39°57′N, 73°35′W)*	*Lake Trout (Canada)*
Pyrene	< 0.3	2	0.5	< 0.3
Methylpyrene	< 0.6	< 0.5	< 2	< 0.6
Benzanthracene	< 0.3	< 1	< 2	< 0.5
Chrysene	< 0.5	< 0.3	< 1.8	< 1
Triphenylene	< 0.5	< 0.2	< 3.5	< 1
Benzo[*a*]pyrene	1.5	< 11.4	<1.0	< 0.5
Benzo[*e*]pyrene	< 1	< 8.3	< 4.4	< 1
Perylene	< 1	< 6.9	< 0.2	< 1

[a] All data recorded as ppb, wet weight.

Foodstuffs. The presence of PNAs in common foodstuffs is well documented (*20*). Highest concentrations are observed for charcoal-broiled, barbecued, and smoked meat and fish. Benzo[*a*]pyrene has been observed at a concentration of 12 ppb in such food. Results for vegetables and miscellaneous other foodstuffs reflect a fairly wide range, varying from less than 1 ppb to as much as 20–50 ppb for benzo[*a*]pyrene. In order to provide additional insight regarding PNAs in foodstuffs, the GC–UV method is being applied to a number of vegetables and miscellaneous other foodstuffs. Our measurements generally show low PNA contents for these foodstuffs.

Table V. Polynuclear Aromatic

	Oyster (Chincoteague, Virginia)	*Oyster (Long Island Sound)*
Pyrene	0.5	58
Methyl pyrene	< 0.1	11
Benzanthracene	0.02	8
Chrysene	0.3	7.0
Triphenylene	0.3	15.1
Benzo[*a*]pyrene	0.2	2.4
Benzo[*e*]pyrene	0.5	5.1
Perylene	0.7	1.1

[a] All data recorded as ppb, wet weight.

Table VI. Polynuclear Aromatic Hydrocarbons in Petroleum Materials[a]

	South Louisiana Crude	*Kuwait Crude*	*No. 2 Heating Oil*	*Bunker C*
Pyrene	4.3	4.5	41	23
Fluoranthene	6.2	2.9	37	240
Benzanthracene	3.1	2.3	1.2	90
Chrysene	23	6.9	2.2	196
Triphenylene	13	2.8	1.4	31
Benzo[*a*]pyrene	1.2	2.8	0.6	44
Benzo[*e*]pyrene	3.3	0.5	< 0.1	10
Perylene	37	—	—	22

[a] All data recorded as ppm.

Table VII. Polynuclear Aromatic

	Kentucky Homestead Feed Coal	*Kentucky Liquefaction Product*
Pyrene	0.8	2040
Fluoranthene	1.2	0.5
Benzanthracene	5.8	0.2
Chrysene	6.6	1.1
Triphenylene	0.4	0.3
Benzo[*a*]pyrene	6.2	1.3
Benzo[*e*]pyrene	3.5	5.0

[a] All data recorded as ppm.

Hydrocarbons in Shellfish[a]

Clam (Virginia)	*Clam (Long Island Sound)*	*Crab (Chesapeake)*	*Crab (Raritan)*
1.0	12	< 0.2	6
< 0.2	2.5	< 0.2	1.6
0.3	1	< 1.5	2
< 0.1	1.6	< 1.2	2
< 0.1	3.3	< 1	1
0.3	0.2	< 0.5	2.9
< 0.4	3.7	< 1 (< 0.9)	—
< 0.6	1.1	< 1 (< 0.9)	1.4

Petroleum. Four petroleum samples were analyzed for PNA content (*16*) as shown in Table VI. These samples cover a range of materials including: a South Louisiana crude (low sulfur), a Kuwait crude (high sulfur), a distillate product (No. 2 fuel oil), and a heavy fuel oil (Bunker C). Table VI lists the data collected from these samples. These PNA levels are in the ppm range. Bunker C is shown to be highest in PNA content. Relatively low concentrations occur in crude oil, and No. 2 fuel oil was lowest of all in 4- and 5-ring compounds.

Coal Liquids. Coal liquids from three feed coals and three liquefaction products from the Synthoil process were analyzed for PNA content (*17*). Data obtained from these coal liquids are presented in Table VII. Here, as in the case of the petroleum materials, the concentrations are in the ppm level. In general, liquefaction appears to decrease the level of 5-ring PNAs but significantly, at least in the case of Kentucky Homestead and Clearfield, Pennsylvania, coal, to increase the concentration of pyrene.

Hydrocarbons in Coal Liquids[a]

Clearfield, Pennsylvania, Manor Feed Coal	*Clearfield Liquefaction Product*	*West Virginia Ireland Mine Feed Coal*	*West Virginia Liquefaction Product*
0.6	2280	1.9	1.5
ND[b]	8.1	4.3	0.4
1.3	0.5	9.3	0.4
12.9	2.7	9.9	1.8
2.6	1.1	1.2	0.1
1.6	1.6	19.2	2.9
1.6	1.1	9.3	2.9

[b] Not detected.

Literature Cited

1. Giger, W.; Blumer, M. *Anal. Chem.* **1974**, *46*(12), 1663–1671.
2. Zechmeister, L.; Koe, B. K. *Arch. Biochem. Biophys.* **1952**, *35*, 1–11.
3. ZoBell, C. E. *Proc. Jt. Conf. Prev. Control Oil Spills* **1971**, *pp.* 451.
4. Blumer, M.; Youngblood, W. W. *Science* **1975**, *188*, 53–55.
5. Hites, R. A. *Symp. Sources, Effects, and Sinks of Hydrocarbons in the Aquatic Environment, Washington, DC, 1976.*
6. Grimmer, G.; Böhnke, H. *Z. Anal. Chem.* **1972**, *261*, 310–314.
7. Grimmer, G. *Erdöl Kohl* **1972**, *25*, 339–342.
8. Grimmer, G.; Böhnke, H. *Chromatographia* **1976**, 9(1), 30–40.
9. Brown, R. A.; Searl, T. D.; King, W. H., Jr.; Dietz, W. A.; Kelliher, J. M. CRC-APRAC Project CAPE-12-68, Final Report, U.S. Document No. PB-219-025, 1971.
10. Lao, R. C.; Thomas, R. S.; Monkmann, J. L. *J. Chromatogr.* **1975**, *112*, 681–700.
11. Strup, P. E.; Giammar, R. D.; Stanford, T. B.; Jones, P. W. "Carcinogenesis" in "Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis 55"; Freudenthal, R. I.; Jones, P. W., Eds.; Raven: New York, 1976, Vol. 1.
12. Hites, R. A.; Biemann, W. G. "Analytical Methods in Oceanography," *Adv. Chem. Ser.* **1975**, *147*, 188–201.
13. Searl, T. D.; Robbins, W. K.; Brown, R. A. *ASTM Symp. on the Measurement of Organic Pollutants in Water and Wastewater, Denver, 1978.*
14. Brown, R. A.; Starnes, P. K., submitted for publication in *Marine Pollution Journal.*
15. Pancirov, R. J.; Brown, R. A. *J. Environ. Sci. & Technol.* **1977**, *11*, 989–992.
16. Pancirov, R. J.; Brown, R. A. *Jt. Conf. Prevention Control Oil Pollution, San Francisco,* 1975, pp. 103–113.
17. Aczel, T.; Williams, R. B.; Pancirov, R. J.; Karchmer, J. H. ERDA Contract No. E (46-17-800), Report No. MERC-8007-1.
18. Hites, R. A.; LaFlamme, R. E. *Science* **1977**, *198*, 829–831.
19. Hilpert, L. R.; May, W. E.; Wise, S. A.; Chester, S. N.; Hertz ,H. S. *Anal. Chem.* **1978**, *50*, 458–463.
20. Pancirov, R. J.; Searl, T. D.; Brown, R. A. *Prepr., Div. Petr. Chem., Am. Chem. Soc.* **1978**, *23*(3), 868.

RECEIVED October 31, 1978.

7

The Solubility Behavior of Polycyclic Aromatic Hydrocarbons in Aqueous Systems

WILLIE E. MAY

National Measurement Laboratory, National Bureau of Standards, Washington, DC 20234

A dynamic coupled-column liquid chromatographic technique was used to obtain aqueous solubility data on 11 aromatic hydrocarbons. The aqueous solubility at 25°C was determined for each compound. The precision of replicate solubility measurements was better than ±3%. The variation of the solubility of each compound with temperature is expressed in the form of either a quadratic or cubic equation based on a least-squares fit of the solubility to temperature. These equations can be used to interpolate the solubility to within ±2% of the experimentally measured values between 5° and 30°C. Enthalpies of solution (ΔH_s) were then calculated from the values obtained and Setschenow constants were calculated from the effect of salinity on solubility. This system was also used to investigate the partitioning of PAHs between aqueous solutions and some sediment samples.

The chief concerns of this chapter are to present a review of the analytical methodologies that have been used to measure the aqueous solubility of polycyclic aromatic hydrocarbons (PAHs) and to describe a new approach to the determination of the aqueous solubility behavior of PAHs.

An understanding of the solubility behavior of PAHs in aqueous systems is important in several fields. In water pollution control, such information is helpful in devising abatment processes (*1*), in modeling natural water systems (*2*), in designing toxicity experiments, and in developing analytical techniques. In petroleum research, aqueous solubilities are useful in understanding how hydrocarbons might migrate and accumulate to form oil fields (*3*). In biology, a knowledge of how

hydrocarbons behave in water is important for understanding the effects of hydration on the configuration of biopolymers (*4*). And in chemistry, solubility data are needed for testing models concerned with the behavior of these compounds in aqueous solution (*5*). For example, Hites (*6*) has suggested the natural mechanism that modifies PAH homologue distribution on sediments, following initial deposition, is the differential water solubility of the various alkyl homologues.

The aqueous solubility is a fundamental parameter in assessing the extent and rate of the dissolution of PAHs and their persistence in the aquatic environment. The extent to which aquatic biota are exposed to a toxicant such as a PAH is largely controlled by the aqueous solubility of the toxicant. These solubilities are also of thermodynamic interest since they give information on the nature of these highly nonideal solutions.

Data on the aqueous solubility of PAHs in the presence of a third component, such as an electrolyte, are also very important. The practical implications are that the presence of this third component may substantially change the solubility, an example being the salting-out effect of sodium chloride and other salts present in seawater (*7*).

Methods for Measurement of the Aqueous Solubility of Aromatic Hydrocarbons

The aqueous solubilities of benzene, the alkylbenzenes, and the naphthalenes have been measured by several investigators (*7–22*). All these investigators prepared saturated solutions by adding an excess quantity of the solute to water and mechanically stirring the mixture for at least 24 hr. These solutions were usually allowed to settle prior to filtration, extraction, and quantitative analysis by ultraviolet (UV) spectroscopy.

This method worked well for the fairly soluble compounds being studied. Interlaboratory precision was good. Therefore, this basic methodology, with minor modifications, such as the use of gas chromatography (GC) or fluorescence spectroscopy for analysis of the aqueous solutions has been generally adopted for the determination of the aqueous solubilities of PAHs (*23–32*), even though the solubilities of some PAHs differ from that of benzene by a factor of more than 10^6. In the remainder of this section some of the modifications that have been made on this basic method and some new approaches to the measurement of the aqueous solubility of aromatic hydrocarbons will be briefly reviewed.

Measurement of Solubility by Nephelometry. In 1942 Davis and co-workers (*24*) determined the aqueous solubility of 30 PAHs at 29°C. The solubilities varied from 2–1600 μg/kg. In this procedure, the test

substance was first dissolved in a water-miscible solvent such as ethanol or acetone. Dilutions of increasing amounts of this solution with relatively large volumes of water gave a series of turbid suspensions. The turbidity was measured nephelometrically, and the relative intensity of scattered light was plotted against the concentration of the test substance. Extrapolation of this standard curve to the relative intensity of a reagent blank gave the experimental solubility.

The precision of replicate analyses was reported to be ±10%. The major source of uncertainty was the narrow time frame between saturation of the solution and coalescence of the dispersed crystals to an extent that would alter the nephelometric behavior of the solution. This approach was also nonselective. It was impossible to discriminate against nonanalyte signals arising from crystalline impurities, dust particles, and the like.

Measurement of Solubility by UV Spectroscopy. In 1972 Wauchope and Getzen (29) studied the temperature dependence of the aqueous solubility of some PAHs between 25° and 75°C. Saturated solutions were prepared by adding 20 g of each solid to a 250-mL glass-stoppered flask containing distilled water. The flasks were suspended in an open water bath and shaken gently for one to three weeks between measurements. Temperature control was maintained to within ±0.5°C. Samples of the solutions for measurement were withdrawn with pipets through glass wool plugs and emptied into volumetric flasks containing measured amounts of cyclohexane for extraction of the aqueous solutions. The volume of cyclohexane was chosen so that the measured signal fell in the range between 0.5 and 1.5 absorbance units. Although the temperature of the equilibrated aqueous solutions varied from 25° to 75°C, this technique allowed the analyses and the Beer–Lambert coefficient determinations to be made at room temperature. This method was more selective and gave better precision (±5%) than the nephelometric method because quantitative analysis was performed at the λ maximum for each individual compound.

Measurement of Solubility by GC. Although his method was not applicable for PAHs, McAuliffe (8) was the first investigator to use chromatography as an analytical tool in the determination of aqueous hydrocarbon solubilities. He measured the solubility of some light alkanes, olefins, and aromatics by making direct 50-μL injections of mechanically prepared saturated solutions into a gas chromatograph. This chromatographic step allowed him to eliminate (via separation in time) nonanalyte signals contributed by dissolved impurities associated with the analyte. For example, he found that the gas chromatogram of cyclopentane

revealed an associated 0.2% impurity when injected neat. The size of this impurity peak increased to 25% of the size of the cyclopentane peak after equilibration with water.

Sutton and Calder (9) have also measured the solubilities of several alkylbenzenes in distilled water and in seawater by a method based on GC. Saturated solutions were prepared by equilibrating water with aromatic vapor in an all-glass apparatus consisting of a 1-L Erlenmeyer flask with an insert tube. The insert tube was used to store the compound. It was capped with a ground-glass stopper. The liquid hydrocarbon did not come into contact with the water except through a perforation in the insert, which allowed hydrocarbon vapors to enter the headspace above the water in the flask. The flask was placed in a constant-temperature shaking bath controlled at 25.0 ± 0.1°C. The water was equilibrated for 48 hr prior to analysis. The solubilities were determined by solvent extraction of the saturated solutions with subsequent analyses of the extracts by GC.

The solubilities reported by Sutton and Calder were 5%–20% higher than those determined by McAuliffe. This is not surprising since the McAuliffe method is susceptible to serious losses due to adsorption of the hydrocarbons on the walls of the syringe. (*See* Accommodation section.) Sutton and Calder injected concentrated organic extracts rather than dilute aqueous solutions. This reduced the chances for adsorptive losses during injection and increased the sensitivity of the method. However, neither McAuliffe's nor Sutton and Calder's method is sufficiently sensitive for determination of the aqueous solubility of PAHs.

Determination of Solubility by Headspace Analysis. Some researchers have determined the aqueous solubility of several benzenes and naphthalenes using a headspace analysis method. In 1971, McAuliffe (33) reported hydrocarbon solubilities determined by the following method. A 50-mL glass hypodermic syringe was filled with equal volumes of an aqueous hydrocarbon solution and helium. The mixture was vigorously agitated for 20 min on a wrist-action shaker to establish equilibrium between the phases. The gas phase was then flushed through a sample loop of a gas-sampling valve and injected into a gas chromatograph. A fresh supply of helium was added to the syringe and equilibrated with the aqueous solution and treated as before. A plot of log peak area in the gas phase vs. equilibration number (gas volume) produced a straight line. The partition coefficient (C_l/C_g) between the liquid and gas phase then can be calculated from the slope of the plot and the liquid and gas volumes. The solubility is the product of the partition coefficient and the saturated vapor pressure.

Wasik and Brown (34) later modified McAuliffe's static headspace method. They constructed a dynamic headspace analysis unit where

hydrocarbons were introduced into the apparatus as vapors to avoid the danger of emulsion formation (*35*), which can occur when liquid hydrocarbons are mixed with water. They allowed the gas mixtures to circulate through the liquid phase for at least half an hour to ensure that equilibrium was attained. After the first equilibration, a small portion of the headspace was sampled via a gas-sampling valve and measured by gas chromatography, while the remainder was vented to waste. Fresh helium then was added, equilibrated with the aqueous solution, and analyzed as before. The partition coefficients were calculated as in McAuliffe's method.

Determination of solubility by headspace analysis offers several advantages over spectrophotometric techniques. First, because of the selectivity of chromatographic analysis, compound purity is not a critical factor; second, absolute calibration of the gas chromatographic detector is not necessary if the response is linearly related with concentration over the range necessary for the measurements; and finally, this method does not require the preparation of saturated solutions, since a partition coefficient, not a solubility, is actually measured. However, headspace methodology would probably not be applicable for determining PAH solubilities for three reasons. First, there is little data in the literature on the vapor pressures of PAHs. Second, the aqueous solubilities of most PAHs are too low to be measured by this procedure. Finally, adsorptive losses of PAHs to glass surfaces from the vapor phase would cause errors.

Measurement of Solubility by Fluorescence Spectroscopy. Recently, Schwarz (*31*) has measured the temperature dependence of the solubilities of several PAHs in aqueous solutions by a fluorescence method. Saturated solutions were prepared and measured in situ in modified fluorescence cells. Each cell contained 5 mL of an aqueous solvent with an excess amount of the PAH to be studied. The cells were rotated for at least 24 hr at each temperature before the solution was allowed to settle and be measured. The fluorescent intensity of these saturated aqueous PAH solutions was found to be proportional to concentration between 8° and 30°C. The fluorescence measurements were put on an absolute scale by UV spectroscopic measurements at 25°C.

This fluorescence method has two major advantages over the methods previously described for measuring the solubilities of PAHs in aqueous solutions. First, no transfers of the saturated solutions are made, so systematic errors arising from adsorption on the transfer tools are eliminated. Fluorescence is also inherently more selective for PAHs than for UV and other nonluminescence spectroscopic techniques. Nonanalyte signals may be reduced through both selective excitation and selective monitoring of fluorescence (emission). This latter advantage is partially negated, however, since the absolute measurement is made by UV

spectroscopy. One of the disadvantages of the method lies in the fact that the solutions cannot be filtered prior to analysis to remove suspended microcrystals and other particulate matter.

Estimations of Solubility by HPLC. Locke (*36*) has used a reverse-phase HPLC method to estimate the aqueous solubilities of some PAHs. He made the following postulates: selectivity in reverse-phase HPLC is governed by the solubilities of similar solutes in the mobile phase; group selectivity is determined by both the stationary and mobile phases; selectivity toward individual components within a group is set by the eluant. The eluant used in reverse-phase HPLC is a blend of water and an organic solvent that is miscible with water. Acetonitrile or methanol is most often used as the organic component. PAHs (up to five condensed rings) are fairly soluble in both acetonitrile and methanol. The aqueous solubilities of PAHs are low and vary between 1–2000 μg/kg. Selectivity then is based on the differences in the aqueous solubility of the individual PAHs. Locke suggested that a linear relationship should exist between log retention volume and log solubility of unsubstituted PAHs. He used the solubilities of benzene and fluoranthene to define the slope of the line, and he determined the solubilities of other aromatic hydrocarbons from this relationship.

Locke's method provides a rapid and simple means for estimating the aqueous solubilities of PAHs. Individual solubility values can be estimated to within 200%. However, these extrapolated values differ too greatly from measured solubilities for this procedure to be categorized as a valid method of determining the aqueous solubility of PAHs.

Summary. Methods for determining the aqueous solubilities of PAHs are subject to errors associated with the preparation, extraction, and quantitative analysis of saturated solutions. There is no one method that has addressed the problems associated with each of these processes. Systematic errors associated with quantitative analyses of saturated solutions should be reduced in methods where selective analytical measurement techniques are used. Chromatographic methods allow separation of nonanalyte signals-in-time from those of the analyte. Fluorescence spectroscopy allows greater selectivity than UV spectroscopy, though less than gas or liquid chromatography.

The efficient extraction of saturated aqueous solutions of PAHs with organic solvents is usually not a problem. Problems are associated, however, with the transfer of aliquots of the saturated solution to extraction vessels. Adsorptive losses of PAHs on the surfaces of transfer tools (pipets, beakers, etc.) are possible. These errors can be eliminated by rinsing the transfer tools with the extracting solvent or by employing methods that do not involve transfer steps.

The source of systematic error that remains and that is present in all methods used to measure PAH aqueous solubilities is associated with

the preparation of saturated solutions. In all methods that have been reported, saturated solutions were prepared by adding excess quantities of the PAH to water and mechanically mixing the solution for at least 24 hr.

Peake and Hodgson (*37*) have shown that when hydrocarbons are dissolved by mechanical means, the resulting solutions are often supersaturated. They called this phenomenon accommodation. They showed that accommodated hydrocarbons are not in equilibrium with the water and that their concentrations are a function of hydrocarbon supply, settling time, and mode of introduction.

Wasik and Brown (*34*) have shown that accommodation is prevented when saturated solutions are prepared by equilibration of hydrocarbon vapors, rather than liquids or crystalline hydrocarbons with water. The methods that employ this approach (*33, 34*), however, do not have the sensitivity or dynamic range necessary to measure PAH aqueous solubilities accurately.

Although there are values for the aqueous solubility of many PAHs in the literature, they have been reported at only one temperature. The agreement between values determined by different methods is sometimes poor. Furthermore, there have been few determinations of the aqueous solubility of PAHs in seawater. Because of the increasing need for information about these systems, a study was undertaken to investigate the solubility behavior of PAHs in aqueous systems.

To begin this investigation, it was necessary to develop a method capable of accurately measuring PAH solubilities. In this method, saturated solutions are prepared by an equilibrium process and extracted almost instantaneously. Quantitative analyses of the extract are done by reverse-phase HPLC. Preparation, extraction, and analysis of the saturated solutions all occur within the same system.

In this investigation, a dynamic coupled-column HPLC method was used to measure the aqueous solubility of some aromatic hydrocarbons, the effect of temperature on solubility, the effect of salinity on solubility, and the partitioning of some PAHs between water and sediments. This methodology is also being used in the development of an aqueous PAH standard reference material (SRM).

Development of Dynamic Coupled-Column Liquid Chromatography—An Accurate Method for the Determinations of the Aqueous Solubility of PAHs

The dynamic coupled-column liquid chromatographic (DCCLC) technique that will be discussed in this section circumvents all the problems stated above. In addition to a description of this technique, this section includes a critical evaluation of the method in terms of the

saturated solution preparation, transfer, extraction, and quantitative analysis processes. The section concludes with a discussion of the precision and potential accuracy of this method.

Methodology. The DCCLC method for determining aqueous solubility is based on generating saturated solutions by flowing water through a column packed with glass beads coated with the compound to be measured (generator column). The concentration of the desired compound in the effluent from the generator column is measured by a modification of the coupled-column liquid chromatographic procedure developed by May et al. (38). A flow diagram of the system is shown in Figure 1.

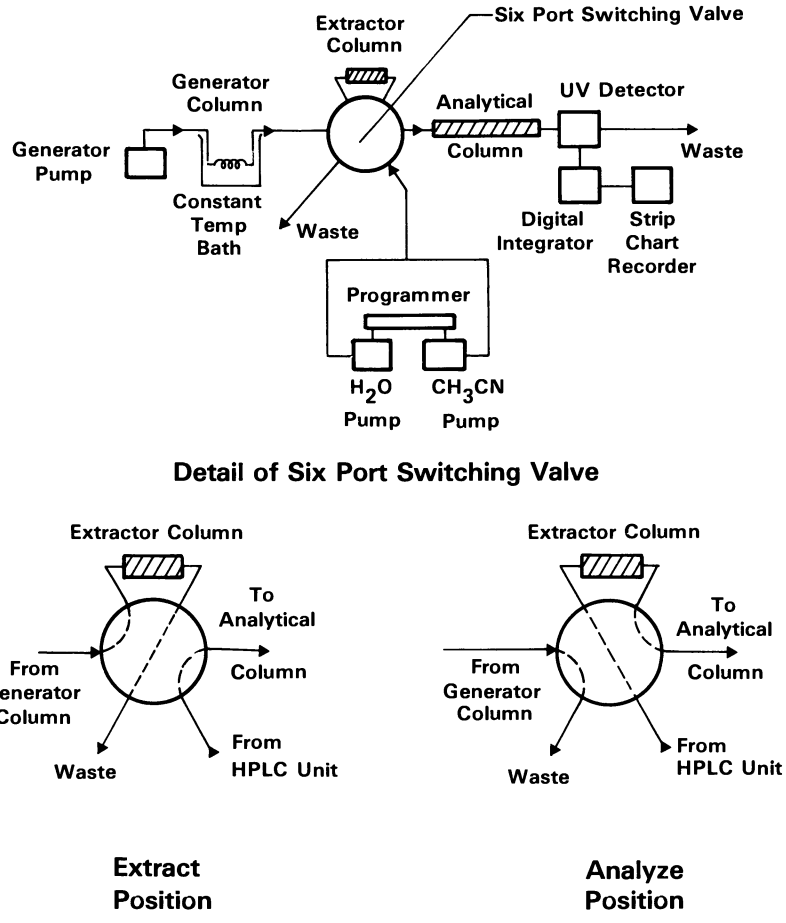


Figure 1. DCCLC flow diagram

REAGENTS. The water used in this study was distilled from a potassium permanganate–sodium hydroxide solution and passed through an XAD-2 column (*38*). The acetonitrile and sodium chloride were spectro and reagent grades, respectively. All of the PAHs used were obtained commercially and were reported to contain less than 3% impurities by their respective manufacturers. Gas and liquid chromatographic analyses of these materials supported these claims.

PREPARATION OF SATURATED SOLUTIONS USING "GENERATOR COLUMNS." Columns for the generation of saturated solutions of PAHs were prepared by packing 60-cm × 0.6-cm stainless steel columns with 60–80 mesh glass beads coated with 1% (w/w) of the compound of interest. The beads were coated by adding 20 g of the beads to 200 mL of a 0.1% methylene chloride solution of the PAH of interest and stripping the solvent with a rotary evaporator.

Columns for the generation of saturated solutions of liquid aromatic hydrocarbons such as benzene were prepared by pumping 50 mL of the liquid through a 60-cm × 0.6-cm stainless steel column with uncoated glass beads. Excess amounts of benzene were purged from these columns with 50 mL of water.

Saturated solutions were generated by pumping distilled water or saline solutions through these columns at flow rates ranging between 0.1 and 5 mL/min. These columns were thermostated by means of a water bath controlled to ±0.05°C.

EXTRACTION OF GENERATED SOLUTIONS. Extraction of the PAHs from the generated solutions was accomplished by flowing a measured volume of the solution through a 6-cm × 0.6-cm stainless steel "extractor column" packed with a 37–50-μm superficially porous support with a bonded C18 stationary phase (Bondapak C18, Waters Associates, Milford, MA) and fitted on both ends with 2-μm frits. This column has been found to provide greater than 98% extraction efficiency for less than 25-mL volumes of aqueous PAH solutions (*39*). Generated solutions of benzene were not passed through the extractor column but were injected directly into the chromatographic system via a loop sample injector. Reasons for this modification are discussed later in the section and in Appendix A-1.

CHROMATOGRAPHIC TRANSFER AND ANALYSIS OF EXTRACT. After extraction, flow from the generator column was diverted to waste, while an acetonitrile–water solvent blend from a HPLC gradient pumping system (Waters Associates, Milford, MA) was simultaneously routed through the extractor column to elute the adsorbed compounds. The eluate then passed through a 30-cm × 0.6-cm microparticulate analytical column (μBondapak C18, Waters Associates) for separation of individual PAHs from nonanalyte interferences such as those caused by crystalline impurities. Isocratic elution conditions were usually employed, resulting

in some band broadening in the transfer of extracted components from the extractor to the analytical column. The band broadening resulting from this transfer process was eliminated when necessary by employing a linear solvent gradient from water to acetonitrile. This caused elution focusing of the components at the head of the analytical column. A detailed discussion of this phenomenon is given elsewhere (39).

The integrated UV detector signal produced by each of the aromatic hydrocarbons was determined to be proportional to its concentration. Individual response factors were found for each compound by first replacing the extractor column with a calibrated sample loop and then injecting acetonitrile solutions with known concentrations of the individual hydrocarbons. Details concerning the loop calibration technique and preparation of the acetonitrile solutions are given in Appendices A-3 and A-4.

Critical Evaluation of the DCCLC Method. The development of an accurate method for determining the aqueous solubility of PAHs is contingent upon the ability of that method to prepare, maintain, transfer, and analyze saturated solutions. In this section some of the problems associated with PAH solubility methodology in general are discussed along with explanations of how these problems are circumvented by the DCCLC approach.

Production of Saturated Solutions Via an Equilibrium Process. Stable saturated solutions were eluted from generator columns after an initial aqueous purge volume of between 100 and 500 mL. After this initial conditioning, equilibrium was obtained and the PAH concentration at constant temperature became independent of flow rate between 0.1 and 5 mL/min (*see* Table I). Equilibrium could be reestablished after a change in temperature or salinity by passing 10 mL of water through the column under the new conditions. A purge volume of approximately 50 mL was necessary to recondition a generator column after a shelf storage period of three months.

Table I. Phenanthrene Solubility Dependence on the Aqueous Flow Rate Through a Generator Column

Flow Rate (mL/min)	*Concentration[a] (μg/kg)*
5.0	865 ± 7
0.4	868 ± 4
0.1	866 ± 3
temperature	22.0°C

[a] The concentrations reported represent the averages of five measurements at each flow rate. The uncertainties represent the standard deviation of the mean at these flow rates.

Table II. A Demonstration of the Equilibrium Nature of the Solution-Generating Process with Generator Columns[a]

Generator Column A		*Generator Column B*		*Generator Column A + B in Series*	
Temp. (°C)	*Conc. (μg/kg)*	*Temp. (°C)*	*Conc. (μg/kg)*	*Temp. (°C)*	*Conc. (μg/kg)*
24.3	42.7	25.3	45.5 ± 0.2	25.3	45.4 ± 0.1
		12.8	21.3 ± 0.2	12.8	20.7 ± 0.1
		6.6	14.0 ± 0.1	6.6	14.2 ± 0.2

[a] The flow rate through these anthracene generator columns was 5.0 mL/min.

The reversible nature of this process was demonstrated by the following experiment. Distilled water was pumped through two independently thermostated anthracene generator columns that were connected in series. The temperature of the first, Column A, was maintained at 24.3°C. Concentration measurements were made on the effluent from the second column in the series, Column B, at temperatures of 25.3°, 12.8°, and 6.6°C. The concentration of the solution that eluted from Column B of the series, at each temperature, was identical within experimental error to the concentration that had been obtained from Column B alone. The data from this experiment are presented in Table II.

Normally saturated PAH solutions were generated by pumping distilled water through generator columns. The results of this experiment show that identical results were obtained when the opposite situation was imposed; that is, the PAH concentration in the feed solution was greater than that expected in the effluent. This could only happen through an equilibrium process.

ACCOMMODATION. It is well established that hydrocarbons can exist in water in colloidal, micellar, or particulate form in appreciable quantities. This was first noted by Peake and Hodgson (*37*), who found that filtration reduced the apparent solubility or accommodation measured for hydrocarbon solutions prepared by mechanical means. They also found that the degree of accommodation (difference between the measured and equilibrium amounts of hydrocarbons in the water) was a function of hydrocarbon supply, settling time, filtration pore size, and the mode by which crystalline hydrocarbons were introduced into the solutions.

The saturated solutions produced by generator columns are independent of hydrocarbon supply. Phenanthrene solutions of identical concentrations were generated by a generator column prepared by the procedure described earlier (coated glass beads) and one prepared by packing a 60-cm × 0.6-cm column with crystals of phenanthrene (*see* Table III). The concentrations of the solutions produced by generator

Table III. Solubility Dependence on the Supply of Phenanthrene on a Generator Column[a]

Phenanthrene Concentration in Generator Column Effluent	
1% Phenanthrene on Glass Beads Column (60 cm × 0.6 cm) (μg/kg)	*Crystalline Phenanthrene Column (60 cm × 0.6 cm) (μg/kg)*
959	938
947	935
958	943
955 ± 7[b]	939 ± 4[b]

[a] Aqueous flow rate, 5 mL/min; temperature, 24.3°C.
[b] Average values.

columns are also stable through large volumes of aqueous purge, where the supply of hydrocarbon on the column is being steadily depleted. This depletion, however, represents only a small percentage of the total amount of compound on the column.

Differential settling times are not a problem because the saturated solutions are generated and measured almost instantaneously, all within the same system.

Filtration of the saturated solutions does not alter the concentration of the solutions generated by generator columns because the solutions are actually filtered during, and not after, preparation. The filters (snubbers) that are fitted on both ends of these columns become saturated with the PAH of interest during the initial column-conditioning period and are therefore an integral part of the solution-generating system.

NONANALYTE SIGNALS. One of the problems that reduces the accuracy of methods that use spectroscopic techniques for the measurement of solubility is the presence of dissolved impurities that contribute to the spectroscopic signal. The most direct method for eliminating this problem is by the use of ultrapure chemicals. Ultrapure PAHs, however, are difficult to obtain. Thus selective analytical techniques must be used to minimize such interferences.

The method discussed in this report employs a reverse-phase liquid chromatographic procedure that separates the signals produced by nonanalyte constituents, in time, from the signal produced by the analyte. The use of this technique can also relax the purity requirements for the individual PAHs. The following experiment was performed to illustrate this point. A generator column coated with a 1% blend of a 90% 2-methylanthracene, 7% phenanthrene, and 3% of several other PAHs was prepared, conditioned, and purged with 4500 mL of water at 25.4°C. The results of this experiment are presented numerically in Table IV and graphically in Figure 2. It should be noted that impurities are eluted

Table IV. Effect of an Added Impurity, Phenanthrene, on the Concentration of 2-Methylanthracene Solution Generated at 25.5°C[a]

Volume Pumped Through Generator Column (mL)	*Phenanthrene Conc. (μg/kg)*	*2-Methylanthracene Conc. (μg/kg)*
0	< 1215	21.6
75	1215	21.6
225	1219	22.3
375	1198	21.4
525	1194	22.1
675	1196	22.8
825	1193	21.8
975	1183	21.9
4500	161	21.7

[a] Measured 2-methylanthracene concentration is 21.8 ± 0.4 μg/kg. Concentration calculated from a least-squares fit of solubility and temperature obtained using a generator column packed with 99% pure 2-methylanthracene is 21.9 ± 0.2 μg/kg.

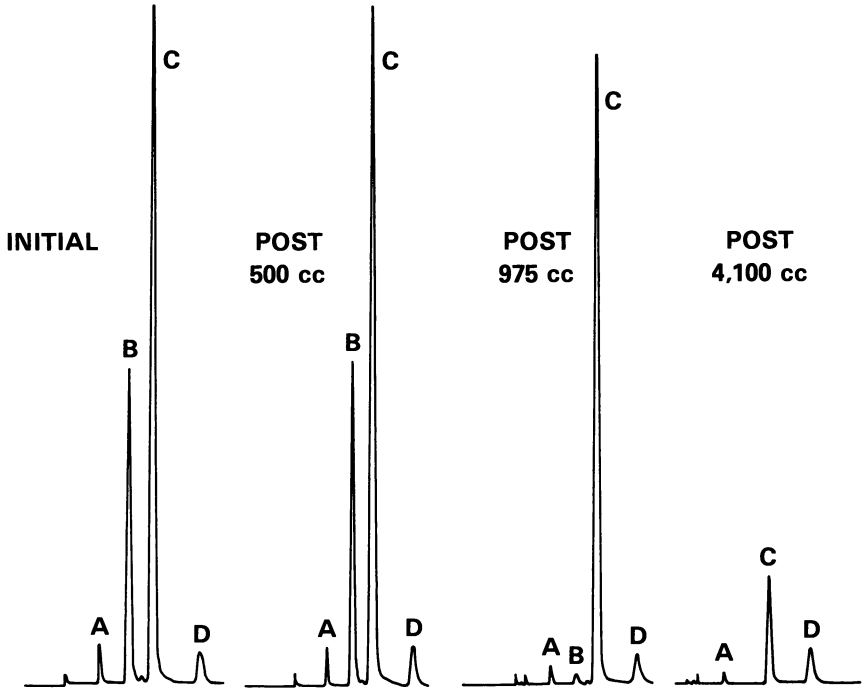


Figure 2. Effect of impurities on analyte solubility measurement; (A) unidentified impurity, (B) unidentified impurity, (C) phenanthrene, and (D) 2-methylanthracene.

Table V. Surface Adsorption Characteristics of PAHs Aqueous Solutions[a]

	Percent Loss from Solution					
	Phenanthrene		*Chrysene*		*Benz[a]pyrene*	
	1 hr	*13 hr*	*1 hr*	*13 hr*	*1 hr*	*13 hr*
Glass	8		46	71	53	82
Silanized glass	5	73	64	81	73	93
Platinum	35	87	66	85	57	93
Aluminum	5	76	50	89	67	95

[a] The concentration of each PAH was 1 μg/kg. Surface-to-volume ratios are equal to approximately 1 cm^2/cm^3 for all materials studied. All measurements are made by the coupled-column LC technique described by May et al. (*38*).

from the column along with the analyte. After initial conditioning, the concentration of the 2-methylanthracene is stable, while the concentrations of the impurities continue to diminish. The measured concentration of 2-methylanthracene produced by the impurity-containing column at 25.4°C was 21.8 ± 0.4 μg/kg. The concentration of the solution produced by a generator column packed with glass beads coated with only 2-methylanthracene and run under identical conditions was 22.6 ± 0.5 μg/kg.

ADSORPTION. The most elusive analytical interference associated with the measurement of PAH aqueous solubilities is that of adsorptive losses of these compounds from solution to the surfaces of containers and transfer tools. PAH adsorption causes reductions in the measured analyte signal, whereas the other interferences discussed up to this point cause positive errors.

The magnitude of the adsorption effect is variable and a function of the manner in which the solutions are handled. The adsorptive properties of three PAHs on four different surfaces are shown in Table V.

Table VI. Losses of Various PAHs from Aqueous

Experiment	*Complexing Agent*	*Experimental Conditions*
1	0.1% caffeine	stir 4 hr, then analyze
	none	stir 4 hr, then analyze
2	0.1% caffeine	stir 16–20 hr, then analyze
	none	stir 16–20 hr, then analyze
3	0.1% caffeine	stir 40 hr, then analyze
	none	stir 40 hr, then analyze

[a] 0.5 μg of each PAH present in 500 mL of distilled water (1 μg/kg per compound).

These results show that losses of PAHs from static solutions to surfaces occur in short periods of time. The use of caffeine as a PAH complexing agent (*32*) reduces these losses but does not eliminate them (*see* Table VI). Stirring the solutions causes only small reductions in these losses. The PAH losses shown in Table VI for stirred solutions are less than those reported in Table V for static solutions.

The DCCLC system was also designed to circumvent problems due to adsorption. After preparation and filtration, saturated solutions are transferred and extracted by a process that minimizes PAH contact with surfaces. The volume between the generator column and the extractor column is approximately 6 μL. The time required for transfer of the saturated solutions at a flow rate of 5 mL/min is less than 75 msec. Furthermore, the walls of the transfer lines are presaturated with the compound being studied during the column-conditioning process. This further reduces the possibility of adsorptive losses of the PAHs during the brief time that the saturated solutions remain in these lines.

Direct injection of the generated saturated solutions into the chromatographic system via a sample loop was initially attempted. PAH adsorptive effects, however, restricted the use of this more simple and rapid measurement approach to only relatively soluble aromatic hydrocarbons such as benzene (solubility 1800 ppm at 25°C). The results presented in Tables V and VI indicate that the magnitude of the adsorption problem is inversely related to aqueous solubility. Therefore, substitution of a stainless steel sample loop for the extraction column would be expected to cause positive systematic errors. The results presented in Table VII indicate that the size of these errors would be a function of both the solubility of the hydrocarbon and the volume of saturated solution passed through the loop prior to injection into the chromatographic system. The data presented in Table VII also show

Solutions Containing Caffeine as a Complexing Agent[a]

Percent Loss

Pyrene	*Chrysene*	*3,4-Benz[a]pyrene*	*1,2,5,6-Dibenzanthracene*
10	10	10	44
29	61	68	78
25	29	32	61
74	72	86	85
12	22	25	61
96	93	96	97

Table VII. Adsorption of Some Aromatic Hydrocarbons from Aqueous Solution to Stainless Steel Sample Loop at 25°C[a]

Volume of Saturated Solution Passed through Loop (mL)	*Apparent Concentration (mg/kg)*
Benzene	
0.05	1795
1.00	1800
10	2273
25	2728
Naphthalene	
0.05	32.0
0.20	32.2
2.0	35.0
5.0	36.4
10	37.8
Phenanthrene	
0.05	0.97
4.0	1.36
9.2	1.61
11.0	1.70

[a] The concentrations of these generated solutions as determined by either solvent extraction followed by liquid chromatographic analysis (benzene) or DCCLC were: benzene, 180 ± 50 (mg/kg); naphthalene, 31.8 ± 1; phenanthrene, 0.95 ± .02. Sample loop volume, 23.1 μL; generator column flow rate, 0.4 mL/min in all cases.

that adsorptive errors may be eliminated from the aqueous solubility determination of benzene by limiting the volume of saturated solution passed through the loop to 50 μL prior to injection (one stroke of the pump used to pass water through the generator column delivers 50 μL). The magnitude of the errors caused by adsorption on the walls of the sample loop was much greater for naphthalene (*see* Figure B-2 in the Appendix) and the less soluble PAHs, and the errors were not eliminated by limiting the volume of saturated solution passed through the loop to 50 μL prior to injection. Because of these adsorptive errors, saturated solutions of all the compounds studied in this investigation, with the exception of benzene, were extracted via an extraction column and transferred to the chromatographic system via the coupled-column process that was described in this section.

THE POTENTIAL ACCURACY OF THE DCCLC METHOD. The preparation of saturated solutions by generator columns has been shown to be

the result of an equilibrium process. The extractor column provides quantitative extraction of naphthalene and the PAHs from aqueous solutions. Problems associated with the stability of aqueous PAH solutions are avoided because the solutions are instantaneously analyzed after they are generated. Nonanalyte signals are separated from those of the analyte by chromatographic means. The potential accuracy of the DCCLC method then is limited by the liquid chromatographic analysis of the generated PAH solutions.

The accuracy of the liquid chromatographic analysis of the generated solutions is limited by the uncertainties involved with the calibration of the sample loop, with the preparation of standard acetonitrile solutions of the PAHs, and with the volumetric measurement of the amount of saturated solution sampled for a given analysis. The random errors associated with each of these processes have been estimated to be less than ±1.2%, ±0.1%, and ±1.0%, respectively. A detailed explanation of how each of these estimates was made is presented elsewhere (39). Quadratic addition of these random errors yields a minimum uncertainty of 1.6% for the quantitative analysis of the generated saturated solutions and hence a potential accuracy of greater than 98% for the method.

REPRODUCIBILITY. Both the short- and long-term precision with which saturated solutions can be generated and measured are better than ±3%. The results presented in Table VIII demonstrate this fact.

Table VIII. Precision with Which Anthracene Solutions May Be Generated and Measured at 25.4°C

Volume of Distilled Water Eluted Through Column (mL)	*Concentration Measured (μg/kg)*
1	45.2
190	47.0
230	46.6
500	46.1
775	46.9
910	44.6
1175	45.5

Average measured concentration (6/21/77)	46.0 ± 0.9
Concentration calculated from calibration curve (12/20/76)	45.7
Concentration measured by solvent extraction followed by GC analysis (6/21/77)	45.8 ± 1.0

The Application of DCCLC to the Determination of the Aqueous Solubility and Other Related Parameters of Some Aromatic Hydrocarbons

Aqueous solubility data for the 12 aromatic hydrocarbons studied in this investigation are reported in this section. The solubilities determined spanned a range of 10^6. The solubilities measured at 25°C are compared with values reported by other investigators and are correlated with molecular parameters such as carbon number, molar volume, and molecular length.

The variations of solubility with both temperature and salinity are reported. Enthalpies of solution (ΔH_s) are calculated from the temperature dependence of the solubility, and the effect of salinity on solubility, expressed in terms of the Setschenow constant, is reported for each compound.

Aqueous Solubilities at 25°C. Table IX compares the solubilities determined by DCCLC with some values reported by other investigators. Of the 12 values reported, there are only two cases of gross disagreement with the consensus literature value. Those are the values for anthracene and triphenylene.

Table IX. The Aqueous Solubilities of Some Aromatic

		Solubilities (mg/kg)	
		This Work	
Compound	*Molecular Weight*	*25°C*	*29°C*
Benzene	78.1	1791 ± 10	
Naphthalene	128.2	31.69 ± 0.23	
Fluorene	166.2	1.685 ± 0.005	
Anthracene	178.2	0.0446 ± 0.0002	0.0570 ± 0.003
Phenanthrene	178.2	1.002 ± 0.011	1.220 ± 0.013
2-Methylanthracene	192.3	0.0213 ± 0.003	
1-Methylphenanthrene	192.3	0.269 ± 0.003	
Fluoranthene	202.3	0.206 ± 0.002	0.264 ± 0.002
Pyrene	202.3	0.132 ± 0.001	0.162 ± 0.001
Benzanthracene	228.3	0.0094 ± 0.0001	0.0122 ± 0.0001
Chrysene	228.3	0.0018 ± 0.00002	0.0022 ± 0.00003
Triphenylene	228.3	0.0066 ± 0.0001	

[a] Solubilities determined by the nepholometric method described in Ref. *24*.
[b] Solubilities determined by method described in Ref. *26*.

The solubilities reported for anthracene are clustered about two values. A possible reason for this phenomenon is that most commercial preparations of anthracene contain at least 2% phenanthrene. Though the two compounds are structural isomers, phenanthrene is approximately 20 times more water-soluble than anthracene. The presence of phenanthrene in solution would contribute a positive systematic error to methods that employ nonspecific analytical measurement techniques, such as UV spectroscopy (*29*) and nephelometry (*24*). The value reported by Schwarz (*31*), who employed a more selective analytical technique (fluorescence), agrees with that determined by DCCLC.

The aqueous solubility of triphenylene as determined by DCCLC is in gross disagreement with all other literature values. The reason for this discrepancy is unknown. DCCLC values obtained through use of triphenylene prepared by different commercial manufacturers were identical. Neither variations in the length of, nor triphenylene supply on, the generator column had any effect on the solubility determined.

Variation of Solubility with Temperature. Solubility data for benzene, naphthalene, and 10 other PAHs are presented in Table X in the form of either a quadratic or a cubic least-squares fit of the solubility to temperature for each compound. These fits have no theoretical signifi-

Hydrocarbons as Determined by Several Investigators

	Solubilities (mg/kg)			
Davis et al.[a] *29°C (1942)*	*MacKay*[b] *and Shui 25°C (1977)*	*Schwarz*[c] *25°C (1977)*	*Wauchope*[d] *and Getzen (1972)*	*Others*
				1780 (*34*), 1796 (*22*), 1755 (*13*), 1780 (*12*)
	31.7 ± 0.2	30.3 ± 0.3	31.2	34.4 (*11*)
	1.98 ± 0.04		1.90	
0.075 ± 0.005	0.073 ± 0.005	0.041 ± 0.0003	0.075	0.075 (*28, 30*)
1.600 ± 0.050	1.290 ± 0.070	1.151 ± 0.015	1.180	0.994 (*32*)
0.240 ± 0.020	0.260 ± 0.020		0.265	0.240 (*32*)
0.165 ± 0.007	0.135 ± 0.005	0.129 ± 0.002	0.148	
0.011 ± 0.001	0.014 ± 0.0002			0.010 (*28*)
0.0015 ± 0.0004	0.002 ± 0.0002			0.006 (*28*)
0.038 ± 0.005	0.043 ± 0.001			0.043 (*28*)

[c] Solubilities determined by fluorimetric method described in Ref. *31*.
[d] Solubilities determined by UV method described in Ref. *29*.

Table X. Variation of Aqueous Solubility with Temperature

Compound	Solubility Dependence on Temperature ($\mu g/kg$)	Correlation Coefficient[a]
1. Benzene	$(0.0247t^3 - 0.6834t^2 + 0.3166t + 1833) \times 10^3$	0.9443
2. Naphthalene	$(0.0189t^2 + 0.2499t + 13.66) \times 10^3$	0.9987
3. Fluorene	$0.0185t^3 + 0.4543t^2 + 22.76t + 543$	0.9999
4. Phenanthrene	$0.0025t^3 + 0.8059t^2 + 5.431t + 324$	0.9992
5. 1-Methyl-phenanthrene	$0.0080t^3 - 0.1301t^2 + 6.802t + 55.4$	0.9994
6. Pyrene	$-0.0011t^3 + 0.2007t^2 - 1.051t + 50.2$	0.9997
7. Fluoranthene	$0.0072t^3 - 0.1047t^2 + 4.322t + 50.4$	0.9988
8. Anthracene	$0.0013t^3 - 0.0097t^2 + 0.8861t + 8.21$	0.9998
9. 2-Methyl-anthracene	$0.0011t^3 - 0.0306t^2 + 0.8180t + 2.79$	0.9988
10. Benzan-thracene	$0.0003t^3 - 0.0031t^2 + 0.1897t + 1.74$	0.9991
11. Thiphenylene	$-0.002t^3 + 0.0250t^2 - 0.4250t + 4.89$	0.9990
12. Chrysene	$0.0024t^2 + 0.0144t + 0.609$	0.9982

[a] These equations and correlation coefficients were obtained by fitting solubility vs. temperature data to a polynomial regression (2° or 3°) program supplied with a Hewlett Packard 9830A Calculator.

cance but can be used to interpolate the solubility as a function of temperature to within ±2% of the experimentally measured values between 5° and 30°C. The experimental values from which these equations were derived are given in Appendix B.

The data presented in Table B-1 and Figure B-1 of Appendix B show that the DCCLC method has both sufficient precision and accuracy to detect a minimum in solubility profile of benzene in the region between 17.5° and 18.0°C, although the solubility never varies by more than 2.5% between 5° and 25°C. This effect has been previously observed and reported by several other investigators (*10, 11, 16, 34*).

Calculation of ΔH_s. The solubility data presented in the preceding section can be used to calculate ΔH_s from Equation 1, where T is the

$$\frac{d(\ln S)}{d\left(\frac{1}{T}\right)} = \frac{-\Delta H_s}{R} \tag{1}$$

absolute temperature, R is the ideal gas constant, and S is the molar solubility at T. Values of ΔH_s at 298 K were calculated from Equation 2, a least-squares fit of the integrated form of Equation 1. The results of

$$\ln S = \frac{-\Delta H_s}{RT} + b \qquad \text{where b is a constant} \tag{2}$$

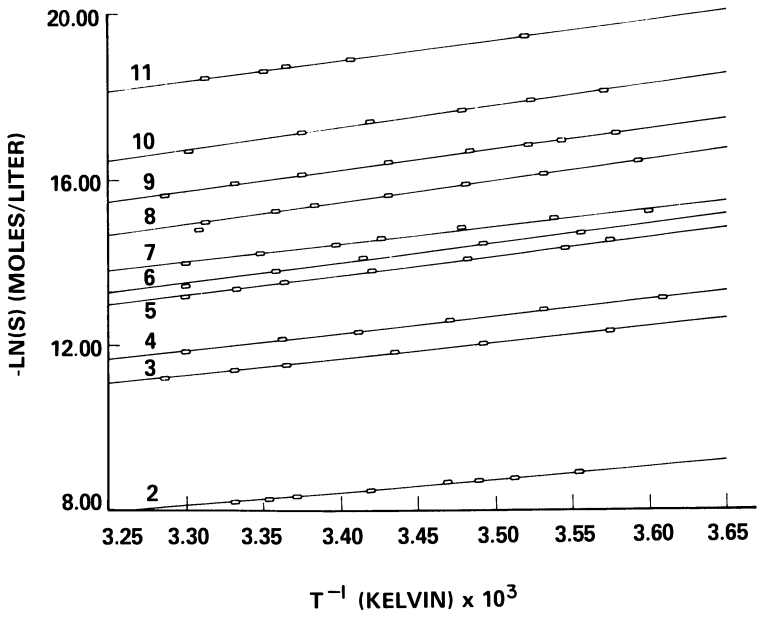


Figure 3. Dependencies of the solubility of some PAHs on temperature. (The numbers refer to the corresponding compounds listed in Table X.)

these least-squares fits are presented graphically in Figure 3. Values of ΔH_s for 11 of the compounds studied in this investigation are found in Table XI along with values for ΔH_s determined by other investigators.

Table XI. Enthalpies of Solution of Some Aromatic Compounds Between 5° and 30°C

	Enthalpy of Solution (kcal/mol)		
Compound	*This Work*	*Schwarz*[a]	*Wauchope*[b] *and Getzen*
Naphthalene	6.30 ± 0.12	5.29 ± 0.10	6.10 ± 0.28
Fluorene	7.88 ± 0.15		6.99 ± 0.16
Anthracene	10.46 ± 0.10	8.32 ± 0.38	10.4 ± 0.8
Phenanthrene	8.32 ± 0.14	8.68 ± 0.23	7.7 ± 0.9
1-Methylphenanthrene	9.34 ± 0.18		
2-Methylanthracene	10.10 ± 0.17		
Fluoranthene	9.52 ± 0.38		
Pyrene	8.47 ± 0.36	11.4 ± 0.2	7.3 ± 0.2
Benzanthracene	10.71 ± 0.25		
Chrysene	9.86 ± 0.24		
Triphenylene	10.71 ± 0.39		

[a] Reported in Ref. *31*.
[b] Calculated from solubility data reported in Ref. *29* in the 0°–30°C range.

A single value of ΔH_s cannot be reported for benzene because the enthalpy of solution varies with temperature.

Variation of Solubility with Salinity. Determination of the solubility of PAHs in water is of both thermodynamic interest and practical relevance in assessing the environmental fate and effects of oil present in rivers, lakes, groundwater, and oceans. Although the aqueous solubilities of a large number of specific PAHs have been measured and correlated, few data exist on the solubility in the presence of a third component such as an electrolyte. The practical implications are that the presence of this third compound may substantially change the solubility; an example of this is the salting-out effect of sodium chloride and other salts present in seawater.

Setschenow (*40*) derived an empirical relationship for the magnitude of the salting-out effect, that is, the dependence of solubility on the salt concentration of saline solutions:

$$\log \frac{S_o}{S_s} = K_s C_s$$

where S_o and S_s are the concentrations of the solute in fresh and salt water, respectively, K_s is the Setschenow constant for the sodium chloride solution, and C_s is the molar salt concentration. The solubility of

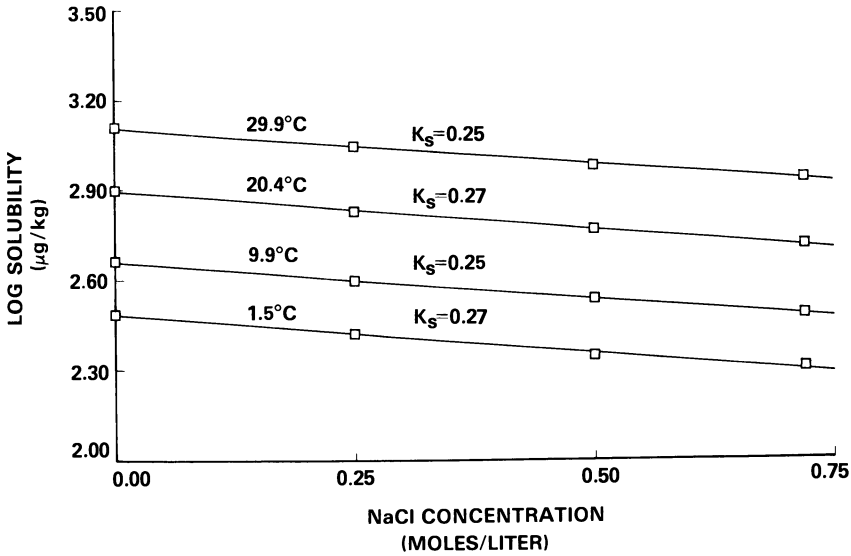


Figure 4. Solubility of phenanthrene as a function of NaCl concentration

Table XII. Setschenow Constants for Some Aromatic Hydrocarbons at 25°C

Compound	K_s *(L/mol)*
Benzene	0.175 ± 0.006
Naphthalene	0.213 ± 0.001
Fluorene	0.267 ± 0.005
Anthracene	0.238 ± 0.004
Phenanthrene	0.275 ± 0.010
2-Methylanthracene	0.336 ± 0.006
1-Methylphenanthrene	0.211 ± 0.018
Pyrene	0.286 ± 0.003
Fluoranthene	0.339 ± 0.010
Chrysene	0.336 ± 0.010
Triphenylene	0.216 ± 0.010
Benzanthracene	0.354 ± 0.002

phenanthrene as a function of ionic strength and temperature is illustrated graphically in Figure 4. The value of the Setschenow constant is shown to be independent of temperature over the range studied. The Setschenow constants calculated in the same fashion for the compounds studied in this investigation are presented in Table XII.

The theory developed by McDevit and Long (*35*) for the salting out of liquid hydrocarbons from aqueous solutions predicts an increase in K_s with increasing liquid molar volume for nonelectrolyte solutes. This relationship seems also to be valid for this series of crystalline compounds, with triphenylene again giving anamolous results. (Molar volumes are presented in Table XIII.)

Table XIII. Correlations of Solubility with Molecular Parameters

Compound	*Carbon No.*	*Molar Volume*[a] *(mL)*	*Molecular Length*[b] *(Å)*	*−ln (S)*
1. Benzene	6	189	5.5	3.77
2. Naphthalene	10	125	8.0	8.30
3. Fluorene	13	153	—	11.5
4. Phenanthrene	14	159	9.5	12.1
5. Anthracene	14	160	10.5	15.2
6. Pyrene	16	172	9.5	14.2
7. Fluoranthene	16	175	9.4	13.8
8. Benzanthracene	18	194	11.8	17.0
9. Triphenylene	18	190	9.5	17.4
10. Chrysene	18	194	11.8	18.7

[a] Reported by Davis and Gottlieb in Ref. *52*.
[b] Reported by Klevens in Ref. *25*.

Correlations of Solubility with Molecular Parameters. The aqueous solubility of aromatic hydrocarbons has been shown by Klevens (*25*) to be related to carbon number, molar volume, and molecular length. These parameters along with the molar solubilities (expressed as $-\ln S$) of the compounds studied are presented in Table XIII. Figures 5 through 7 demonstrate the relationship between each of these parameters and solubility. These figures show that there are several compounds whose anomalous behavior makes accurate extrapolations of solubility from these relationships impossible. For example, anthracene and phenanthrene are structural isomers. They, therefore, have identical carbon numbers and very similar molar volumes. However, their aqueous solubilities differ by more than a factor of 20. Phenanthrene, fluoranthene, pyrene, and triphenylene all have very similar molecular lengths; but their respective aqueous molar solubilities at 25°C are 5.6×10^{-6}, 1.0×10^{-6}, 6.8×10^{-7}, and 2.8×10^{-8}.

Tsonopoulos and Pransnitz (*27*) have reported that the hydrocarbon infinite dilution coefficient, γ^{∞}, is the appropriate quantity for correlating the aqueous solubilities of hydrocarbons. They, along with Leinonen et al. (*23*) and Pierotti et al. (*28*) have successfully correlated γ^{∞} with carbon number, molar volume, and degree of branching. Recently MacKay and Shiu (*26*) have correlated the hydrocarbon infinite dilution coefficients of 32 aromatic hydrocarbons (using the supercooled standard state) with carbon number. From this relationship, they derived a

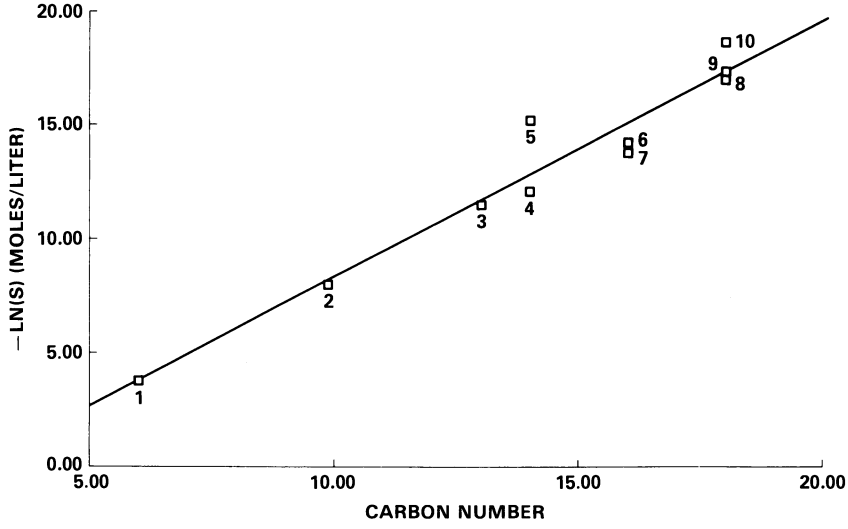


Figure 5. Variation of solubility with carbon number

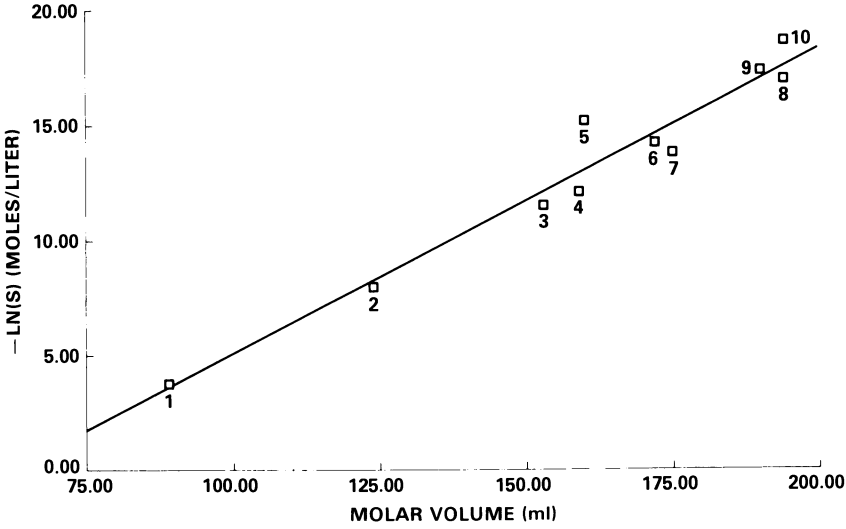


Figure 6. Variation of solubility with molar volume

parabolic equation from which individual solubilities could be calculated. A comparison of the correlated and experimental solubility values showed that solubilities could be estimated only to within a factor of 3.

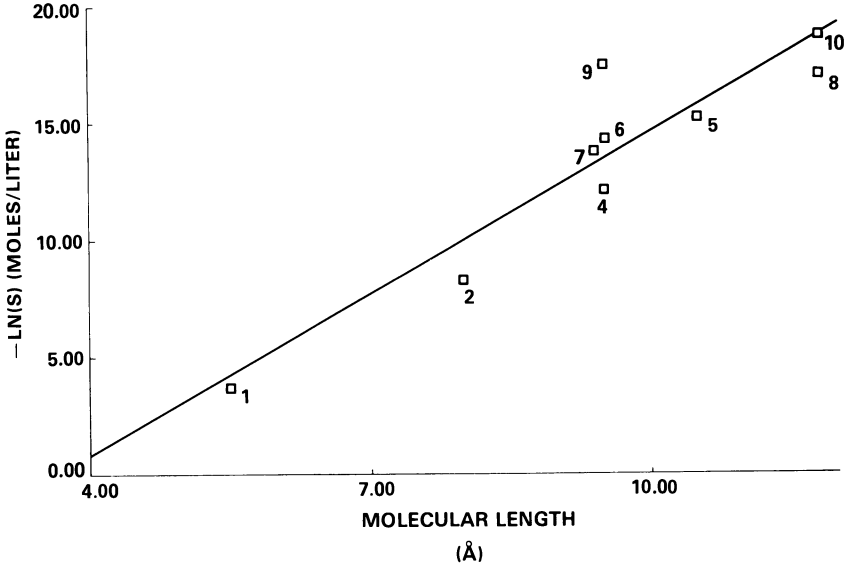


Figure 7. Variation of solubility with molecular length

Summary. The DCCLC technique is a very rapid, precise, and accurate method for determining aqueous solubilities and related parameters for PAHs. The agreement with solubilities and calculated solubility parameters, such as ΔH_s, for which values have been previously reported in the literature helps to confirm the validity of this approach. In all cases replicate solubility measurements of the generated PAH solutions at constant temperature were better than $\pm 3\%$ and in most cases better than $\pm 1\%$. Quantitative analyses of hexane extracts of the generated solutions by both GC and HPLC have agreed with the DCCLC values to within $\pm 3\%$ in all cases.

The lack of an appropriate physical parameter for accurately extrapolating PAH solubilities further enhances the utility of the DCCLC approach. PAH aqueous solubilities can be accurately measured in about the same time it would take to make an estimation via correlation with a physical parameter.

PAH Sorption on Sediments

Background. Recent studies conducted in this laboratory and elsewhere have shown that sediments taken from areas thought to be pristine and those taken from areas exposed to low levels of petroleum both contain PAH mixtures of similar distribution, with the alkyl homologues of PAHs predominating over the unsubstituted species. Different possible sources of the PAHs in these and other marine sediments have been suggested. These include biosynthesis; petroleum spillage; and combustion from mobile and stationary sources, including refuse burning, forest fires, and industrial activity.

There have been suggestions that at least some PAHs originate from biosynthesis of algae (*6*), plants (*41*), or various bacteria (*42*). Hites and Hase (*43*) have shown, however, that bacteria accumulate but do not biosynthesize PAHs.

The PAHs found on marine sediments may also originate from petroleum. In petroleum, the three- and four-carbon alkyl homologues predominate. Petroleum-derived PAH mixtures are usually very deficient in the unsubstituted parent PAH.

Combustion-produced PAHs transported by air are another likely source of these compounds in marine environments. Low-temperature combustion (such as 1100 K, as in a cigarette) will yield a soot with an abundance of the alkyl-substituted PAHs. Blumer and Youngblood (*44*) have concluded that PAHs found in several recent sediments are likely to have originated from forest fire particulates. Hites (*6*), however, refutes this conclusion and offers instead the belief that PAH mixtures are initially deposited by anthropogenic sources and then undergo an

in situ modification. Hites postulates that the differential water solubility of the higher alkyl homologues vs. the unsubstituted species is the mechanism responsible for the PAH distributions found on recent sediments. He proposes the following model: After airborne particulate deposition on soil or in water, the lowest homologues continuously fractionate into the water phase to an extent proportional to their solubility (increasing alkylation lowers aqueous solubility). The remaining species, which accumulate on the particulate matter and on sediment, are, therefore, devoid of the lowest homologues, thus increasing the relative abundance of the higher homologues.

Measurement of PAH Sorption by DCCLC. The DCCLC system is capable also of providing a means of characterizing the sorption characteristics of PAHs on solid adsorbents. Since the concentrations of generated PAH solutions are known functions of temperature, PAH sorption data for the adsorbent were determined by placing a column packed with the material to be characterized between the generator column and the switching valve (*see* Figure 1). A plot of the concentration measured at the exit of the adsorbent column vs. time (or volume) provided data pertaining to the kinetics of the sorption process (interpretation of such data is beyond the scope of this work). Equilibrium was defined as that point at which the PAH concentrations at the adsorbent column inlet and outlet were the same. At that point the equilibrium uptake was measured by removing the adsorbent from the column and extracting it with diethyl ether. An aliquot of that extract was then analyzed by liquid chromatography.

Results. Table XIV presents some results obtained when using this method for measuring the equilibrium partition coefficient between distilled water and several silica gels. The sorption of phenanthrene is shown to be a function of surface area for these materials. Tables XV through XVIII present the results obtained for the partitioning of a PAH

Table XIV. The Partitioning of Phenanthrene[a] Between Silica Gel and Distilled Water as a Function of Nitrogen Surface Area[b]

N_2 Surface Area[c] (m^2/g)	Concentration Sorbed on Silica (mg/kg)	Partition Coefficient, K $\left(\frac{\text{concn. sediment}}{\text{concn. water}}\right)$
2	8 ± 2	8
22	50 ± 4	52
200	95 ± 7	99
400	130 ± 3	135

[a] Phenanthrene aqueous concentration is 0.96 mg/kg.
[b] All measurements were made at room temperature.
[c] Surface areas used are those reported by the manufacturer.

Table XV. The Partitioning of Some PAHs Between Zipax[a] and Water at Room Temperature

	Concentration in Water (μg/kg)	*Concentration on Sediment at Equilibrium (μg/kg)*	*Partition Coefficient, K* $\left(\frac{\text{concn. sediment}}{\text{concn. water}}\right)$
Phenanthrene	710	1790	2.5
1-Methylphenanthrene	114	816	7.2
Anthracene	15.9	25.7	1.6
2-Methylanthracene	8.1	94.0	12

[a] Zipax is a superficially porous adsorbent marketed by the E. I. du Pont de Nemours Co., Wilmington, Delaware.

mixture of phenanthrene, 1-methylphenanthrene, anthracene, and 2-methylanthracene on two silica gels and two Alaskan sediment samples. The absolute values of the partition coefficients (K) vary, but the preferential sorption of the methylated PAHs is evident in all four experiments. These partition coefficients are not simply inverse functions of solubility. The partition coefficient for anthracene is less than that for 1-methylphenanthrene, although 1-methylphenanthrene is five times more soluble.

These trends in PAH sorption in aqueous systems are not predicted by chromatographic retention on liquid–solid (adsorption) or normal bonded phase-partition chromatographic systems. Table XIX compares the relative chromatographic retention of these four compounds on silica, alumina, and μBondapak NH_2 using hexane as a mobile phase, and on μBondapak C_{18} using a water–acetonitrile mobile phase. There is little difference shown in the chromatographic retention of these three condensed ring PAHs on the two classical adsorbents, silica and alumina, or μBondapak NH_2, a new "bonded phase" chromatographic material. However, the trends in the retention of these three condensed ring PAHs on μBondapak C_{18} (a bond phase usually used in the reverse-phase

Table XVI. The Partitioning of Some PAHs Between Porasil (250)[a] and Water at Room Temperature

	Concentration in Water (μg/kg)	*Concentration on Sediment at Equilibrium (μg/kg)*	*Partition Coefficient, K* $\left(\frac{\text{concn. sediment}}{\text{concn. water}}\right)$
Phenanthrene	809	2.93×10^4	36
1-Methylphenanthrene	92.4	1.00×10^3	108
Anthracene	12.0	5.85×10^2	49
2-Methylanthracene	9.18	1.46×10^3	159

[a] Porasil (250) is a totally porous silica material manufactured by Waters Associates, Milford, Massachusetts.

Table XVII. The Partitioning of Some PAHs Between Water and Alaskan Sediment "A" at Room Temperature

	Concentration in Water (μg/kg)	*Concentration on Sediment at Equilibrium (μg/kg)*	*Partition Coefficient, K* $\left(\frac{\text{concn. sediment}}{\text{concn. water}}\right)$
Phenanthrene	710	7.3×10^3	10
1-Methylphenanthrene	114	2.3×10^3	20
Anthracene	15.9	3.0×10^2	19
2-Methylanthracene	8.1	2.9×10^2	36

Table XVIII. The Partitioning of Some PAHs Between Water and Alaskan Sediment "B" at Room Temperature

	Concentration in Water (μg/kg)	*Concentration on Sediment at Equilibrium (μg/kg)*	*Partition Coefficient, K* $\left(\frac{\text{concn. sediment}}{\text{concn. water}}\right)$
Phenanthrene	874	3930	4.5
1-Methylphenanthrene	94.8	1185	13
Anthracene	11.7	127	11
2-Methylanthracene	9.80	246	25

Table XIX. Comparison of Log Retention Indices (*I*)[a] of Phenanthrene, Anthracene, and Methylated Homologues on Several Liquid Chromatographic Packing Materials

	Silica (45)	*Alumina (64)*	*μBondapak NH_2 (48)*	*μBondapak C_{18} (48)*
Phenanthrene	3.00	3.00	3.00	3.00
1-Methylphenanthrene	3.26	3.18	2.98	3.43
Anthracene	2.97	3.00	2.95	3.02
2-Methylanthracene	—	—	2.92	3.63

[a] The retention indices were calculated as previously described by Popl et al. (*45*), with the hydrocarbon standards being assigned the following retention indices: benzene, 10; naphthalene, 100; phenanthrene, 1000; benzanthracene, 10,000; and benzo[*b*]chrysene, 100,000. The retention index of an aromatic hydrocarbon was then calculated in a manner analogous to the calculation of Kováts indices for gas chromatography.

mode) closely resembles the trends of the partition coefficients measured for the sorption of these compounds from water onto silica gels and sediments.

Summary. The results of this brief study indicate that there should be much more work directed toward the understanding of the mechanisms of the sorption (partitioning, adsorption, etc.) of slightly soluble components from water onto particulate matter. The DCCLC technique is an excellent tool for conducting such studies.

Development of an Aqueous PAH Standard Reference Material

Introduction. The National Bureau of Standards (NBS) currently issues over 900 Standard Reference Materials (SRMs), with various groups being represented, such as clinical laboratory standards, trace element standards, nuclear materials, glass viscosity standards, rubber materials, color standards, and coating thickness standards. We are now endeavoring to add an additional group to this list, namely, trace organic chemical SRM.

The first SRM from this new group of materials will be an aqueous PAH SRM. There are several problems associated with the preparation, storage, and handling of aqueous solutions of PAH that previously prevented the development of this SRM. Now, through the use of the DCCLC method, we have been able to circumvent these problems. The use of this technique for the preparation and certification of an aqueous PAH SRM will be discussed.

Background. In the fall of 1975 NBS and the Environmental Protection Agency (EPA) jointly sponsored a series of workshops entitled "Standards and Reference Materials for Environmental Analysis Associated with Energy Development." The objective of these workshops was to obtain input for NBS on the methodology and certified standards necessary for the accurate analysis of environmental samples associated with the production of alternate fuels.

At the conclusion of these workshops, a number of SRMs were recommended by the participants for NBS consideration. One of the SRMs recommended was a PAH in a water matrix. Although many PAHs have demonstrated mutagenic properties, this SRM was given a low priority because of the presumed difficulties associated with the preparation and stabilization of such a material.

Problems Associated with the Preparation and Stabilization of a PAH–Water SRM. Preparation of aqueous PAH solutions of known concentration by gravimetric procedures is difficult because of the extremely low aqueous solubilities of PAH. As shown in Table IX many PAHs have aqueous solubilities of less than 500 μg/kg (ppb). Preparation of aqueous solutions of known concentration by serial dilutions of a more concentrated organic solution is both hazardous and wasteful. After small aliquots are taken, large volumes of organic solvent containing toxic and expensive chemicals remain to be disposed of.

Preservation of stable aqueous solutions of PAH is hampered by adsorptive losses of the PAH to the surfaces of containers and transfer tools. The magnitude of the adsorptive effect is variable and is a function of the manner in which the solutions are handled. The adsorptive properties of three PAHs on four different surfaces were shown in Table V. These results show that losses of PAH from static solutions to surfaces

occur in short periods of time. Stirring such solutions only slightly reduces such losses.

Preparation of Standard Aqueous Solutions of Individual PAHs. With the development of the DCCLC technique, accurate preparation of standard aqueous PAH solutions is possible. The aqueous solubility of a compound is a well-defined thermodynamic quantity. Generator columns loaded with single PAHs produce saturated solutions. The use of generated solutions circumvents the problems that are usually associated with maintaining aqueous PAH solutions, since the solutions need not be generated until they are needed.

Preparation of Aqueous Solutions Containing Several PAHs Using Generator Columns. Although they do not generate saturated solutions, generator columns coated with three or more compounds are of more practical value. The initial attempt to prepare such a column met with only limited success. A column was prepared that was composed of 50 mg each of phenanthrene, 1-methylphenanthrene, anthracene, and 2-methylanthracene coated on 20 g of glass beads. The results of this experiment are reported in Table XX. After 6 L of water had been pumped through this generator column (G-1), equilibrium had not been achieved.

The same four compounds were used in a second attempt to prepare a generator column that would be capable of generating a stable solution of these four components. The amounts of each compound comprising the 1% coating on the glass beads were made to be proportional to their aqueous solubility. The results reported in Table XXI demonstrate the feasibility of this approach. After initial conditioning, over 6 L of solution was generated with the maximum change being less than 10% over that range.

Table XX. Effluent Stability of Generator Column G-1

Water Purge Volume (mL)	*Phenanthrene Concn. (μg/kg)*	*1-Methylphenanthrene Concn. (μg/kg)*	*Anthracene Concn. (μg/kg)*	*2-Methylanthracene Concn. (μg/kg)*
100	610	167	22.1	14.6
500	541	155	26.0	15.9
860	520	148	26.6	15.1
1330	506	150	29.1	15.7
4500	405	157	34.9	15.5
5350	388	162	35.4	15.8
550	380	164	36.1	15.9
6400	377	170	35.2	17.7
$\bar{x}$ (1300–6499 mL)	411 ± 53	161 ± 7.6	34.1 ± 2.9	16.1 ± 8.9
Maximum range between high and low value	29%	13%	19%	12%

Table XXI. Effluent Stability of Generator Column G-2

Water Purge Volume (mL)	*Phenanthrene Concn. (μg/kg)*	*1-Methylphenanthrene Concn. (μg/kg)*	*Anthracene Concn. (μg/kg)*	*2-Methylanthracene Concn. (μg/kg)*
25	913	96.3	12.1	12.2
550	897	73.6	20.1	9.6
2250	890	71.8	18.4	11.1
3700	906	69.9	16.6	10.2
5200	898	70.8	16.4	10.0
6320	888	74.4	16.0	8.94
7100	903	71.0	17.0	10.3
8100	876	78.1	16.5	9.24
$\bar{x}$ (2250–8100 mL)	894 ± 11	72.7 ± 3.1	16.8 ± 0.9	10.0 ± 0.8
Maximum range between high and low value	1.8%	8.1%	5.8%	19.5%

A third generator column (G-3) loaded with three compounds was prepared by the same technique used to prepare G-2. The column was purged with 5 L of water at 25°C, after which a state of equilibrium was reached. Although the concentrations generated by this column were not equal to the equilibrium saturation concentrations for the individual components, they did not change by more than ±5% while more than 6 L of water was purged through the column (*see* Table XXII). The concentrations of the individual compounds in the effluent of this column over this interval at 25°C were: anthracene, 62.1 ± 1.3 μg/kg; 2-methylanthracene, 13.3 ± 0.2; and benzanthracene, 10.8 ± 0.3. Figure 8 shows that the relationship between ln solubility and temperature is

Table XXII. Effluent Stability of Generator Column G-3

Water Purge Volume	*Anthracene*	*2-Methylanthracene*	*1,2-Benzanthracene*
200	63.6	13.3	15.8
2000	58.9	11.9	14.0
5000	62.0	13.3	10.9
6000	64.1	12.9	11.0
7200	62.8	13.4	10.7
8100	62.2	13.4	11.0
9300	61.7	13.2	10.7
9990	60.6	13.1	10.4
11,500	61.0	13.4	11.1
$\bar{x}$ (5000–11,500 mL)	62.1 ± 1.3	13.3 ± 0.2	
Minimum range between high and low value	5.5%	3.0%	10.8 ± 0.3 6.3%

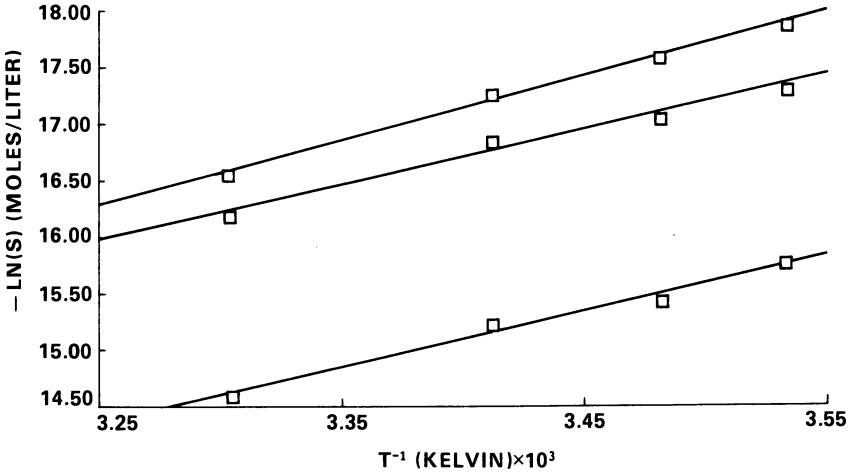


*Figure 8. Concentration dependence of components of a ternary solution on temperature; (*top plot*) anthracene, (*center plot*) 2-methylanthracene, (*bottom plot*) benzanthracene.*

linear for each of the three compounds over the 0°–30°C temperature range. The linear relationship indicates that this solution-generating process is thermodynamically well behaved and allows one to predict the concentration of each component in the ternary solution, given the temperature.

Preparation of Dilute Organic Standard PAH Solutions. Small volumes of dilute organic solutions may also be accurately prepared by extracting the generated aqueous solutions with the desired volume of an immiscible organic solvent, such as hexane. Since PAH hexane–water distribution coefficients are very large, the concentration of these dilute solutions can be calculated if the volume ratios are known. For example, 5 mL of a 10-(μg/kg) ppb solution of chrysene in hexane could not be prepared gravimetrically. Such a solution would have to be prepared by serial dilutions of a more concentrated solution. This process is both wasteful and hazardous. After an aliquot is taken from the concentrated solution, a large volume of toxic solution will remain to be discarded. However, such a solution can be prepared by extracting 25 mL of a saturated aqueous solution ($\sim$ 2 ppb) of chrysene with 5 mL of hexane. This is only a hypothetical example, but it does demonstrate the utility of using generator columns to prepare organic PAH solutions of known concentrations indirectly.

Evaluation of DCCLC as a Method for the Preparation and Certification of an Aqueous PAH SRM. There are several factors that make the DCCLC approach ideal for the preparation and analysis of very dilute aqueous solutions of individual PAHs. Many of the factors have been discussed earlier in other contexts and will be only referred to here.

1. Saturated solutions are prepared by an equilibrium process. This process has been shown to be reversible and PAH concentrations are independent of the rate of flow through the generator columns between 0.1 and 5.0 mL/min.
2. The use of generator columns to produce aqueous PAH solutions circumvents the problems that are usually associated with storing such solutions, since the solutions need not be generated until they are needed.
3. The concentration of the generated aqueous solutions are a function of temperature and may be expressed in terms of least-squares fits of the concentration as a function to within $\pm 2\%$ of the experimentally determined values between 5° and 30°C (*see* Appendix B).
4. The short- and long-term precision with which aqueous PAH solutions can be made appear to be $\leq 2\%$ (*see* Table VIII).
5. Shelf storage of generator columns does not present a problem. They have been stable for longer than 1½ years and through more than 100 L of aqueous purge.
6. DCCLC is a rapid and accurate method for analyzing the generated dilute aqueous PAH solutions. Analytical errors due to adsorption are minimized in DCCLC because the solution is extracted and concentrated, on line, in less than 500 msec after generation. It has been estimated that this analytical method has an uncertainty of less than 2%.

Conclusion. The DCCLC technique has been shown to be an ideal method for the preparation and certification of an aqueous PAH SRM. Tentative plans are to issue individually certified anthracene, benzanthracene, and 3,4-benz[*a*]pyrene generator columns by early 1979.

Acknowledgment

The author is grateful to the Office of Air and Water Measurement, National Bureau of Standards, for partial support of this work. This work is from a dissertation submitted to the Graduate School, University of Maryland (December 1977), by Willie E. May, in partial fulfillment of the requirements for a Ph.D. degree in chemistry. Identification of any commercial product does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Literature Cited

1. Tsonopoulos, C.; Prausnitz, J. *Ind. Eng. Chem., Fundam.* **1971**, *10*, 503.
2. LeFeuvre, A. "Water and Water Pollution Handbook"; Ciaccio, L. L., Ed.; Marcel Dekker, Inc.: New York, 1971; Vol. I, p. 263.
3. Peak, E.; Hodgson, G. *J. Am. Oil Chem. Soc.* **1966**, *215*.
4. Ben-Naim, A. *J. Chem. Phys.* **1972**, *57*, 5257.
5. Ben-Naim, A. "Water and Aqueous Solutions"; Horne, R. A., Ed.; Wiley: New York, 1972; p. 425.

6. Hites, R. *Proc. Source, Effects and Sinks of Hydrocarbons in the Aquatic Environment, American University, Washington, DC, 1976.*
7. MacKay, D.; Shiu, W. *Can. J. Chem. Eng.* **1975,** *53,* 239.
8. McAuliffe, C. *J. Phys. Chem.* **1966,** *70,* 1274.
9. Sutton, C.; Calder, J. A. *J. Chem. Eng. Data* **1975,** *20,* 320.
10. Arnold, D.; Plank, C.; Erickson, E. *Chem. Eng. Data Ser.* **1958,** *3,* 253.
11. Bohon, R.; Claussen, W. *J. Am. Chem. Soc.* **1951,** *73,* 1571.
12. Vermillion, H. Ph.D. Thesis, Duke University, Durham, NC, 1939.
13. Stearns, R.; Oppenheimer, H.; Simon, E.; Harkins, W. *J. Chem. Phys.* **1947,** *14,* 496.
14. Hill, A. *J. Am. Chem. Soc.* **1922,** *44,* 1163.
15. Franks, F.; Gent, M.; Johnson, H. *J. Chem. Soc.* **1973,** *2716.*
16. Hayashi, M.; Sasaki, T. *Bull. Chem. Soc. Jpn.* **1956,** *29,* 857.
17. Booth, H.; Everson, H. *Ind. Eng. Chem.* **1948,** *40,* 1491.
18. Morrison, T.; Billet, F. *J. Chem. Soc.* **1952,** 3819.
19. Horiba, S. *Mem. Coll. Eng., Kyoto Imp. Univ. 1* **1914,** *49.*
20. Fühner, H. *Chem. Ber.* **1924,** *57,* 510.
21. Andrews, L.; Keefer, R. *J. Am. Chem. Soc.* **1950,** *72,* 5034.
22. MacKay, D.; Shiu, W.; Wolkoff, A. "Gas Chromatographic Determination of Low Concentrations of Hydrocarbons in Water, in Water Quality Parameters," *ASTM Spec. Tech. Publ.* **1975,** *573.*
23. Leinonen, P.; MacKay, D.; Phillips, C. *Can. J. Chem. Eng.* **1971,** *49,* 288.
24. Davis, W.; Krohl, M.; Clower, G. *J. Am. Chem. Soc.* **1942,** *64,* 108.
25. Klevens, H. *J. Phys. Colloid Chem.* **1950,** *54,* 283.
26. MacKay, D.; Shiu, W. *J. Chem. Eng. Data,* in press.
27. Tosonopoulos, C.; Prausnitz, J. *Ind. Eng. Chem., Fundam.* **1971,** *10,* 593.
28. Pierotti, G.; Deal, C.; Derr, E. *Ind. Eng. Chem.* **1959,** *51,* 95.
29. Wauchope, R.; Getzen, F. *J. Chem. Eng. Data* **1972,** *17,* 38.
30. Weimer, R.; Prausnitz, J. *J. Chem. Phys.* **1965,** *42,* 3643.
31. Schwarz, F. *J. Chem. Eng. Data* **1977,** 22, 273.
32. Eisenbrand, J.; Baumann, K. *Z. Lebensm. Unters. Forsch.* **1970,** *144,* 312.
33. McAuliffe, C. *Chem. Technol.* **1971,** *1,* 46.
34. Brown, R.; Wasik, S. *J. Res. Nat. Bur. Stand., Sect. A* **1974,** *78,* 453.
35. McDevit, W.; Long, F. *J. Am. Chem. Soc.* **1952,** *74,* 1773.
36. Locke, D. *J. Chromatogr. Sci.* **1974,** *12,* 433.
37. Peake, H.; Hodgson, G. *J. Am. Oil Chem. Soc.* **1967,** *44,* 696.
38. May, W.; Chesler, S.; Cram, S.; Gump, B.; Hertz, H.; Enagonio, D.; Dyszel, S. *J. Chromatogr. Sci.* **1975,** *13,* 535.
39. May, W. Ph.D. Dissertation, University of Maryland, College Park, MD, 1977, pp. 34–40.
40. Setschenow, J. *Z. Phys. Chem.* **1889,** *4,* 117.
41. Hancock, J.; Applegate, H.; Dodd, J. *Atmos. Environ.* **1970,** *4,* 363.
42. Niaussat, P.; Auger, C.; Mallet, L. *C. R. Helod. Seances Acad. Sci., Ser. D.* **1970,** *270,* 1042.
43. Hase, A.; Hites, R. "Identification and Analysis of Organic Pollutants in Water"; Ann Arbor Science Pub.: Ann Arbor, MI, 1976.
44. Blumer, M.; Youngblood, W. *Science* **1975,** *188,* 53.
45. Popl, M.; Dolansky, V.; Mostecky, J. *J. Chromatogr.* **1976,** *117,* 117.
46. Ibid; **1974,** *91,* 649.
47. Popl, M.; Dolansky, V.; Coupek, J. *J. Chromatogr.* **1977,** *130,* 195.
48. Wise, S.; Chesler, S.; Hertz, H.; Hilpert, L.; May, W., unpublished data.
49. Instruction Manual # 4930 for CAHN Electrobalance Model "4700."
50. U.S. Government Printing Office, 1941, NBS Circular C434.
51. "The International System of Units (SI)," *Nat. Bur. Stand. (U.S.), Spec. Publ. 330.*
52. Davis, H.; Gottlieb, S. *Fuel* **1962,** *8,* 37.

Appendix A: Processes That Affect the Accuracy of DCCLC

This section presents a brief discussion of the major processes that affect the accuracy of the DCCLC technique. They are:

A-1 Extractor Column Extraction Efficiency;
A-2 Measurement of Sample Volume;
A-3 Calibration of the Sample Loop;
A-4 Preparation of Standard Solutions.

This appendix also includes:

A-5 Calculation of Response Factors;
A-6 Calculation of Concentration.

A-1. Extractor Column Extraction Efficiency. Extraction of the PAHs from the generated solutions was accomplished by pumping volumes varying between 5.0–25.0 mL through the extractor column. Over this range, extraction efficiencies are quantitative for 11 of the 12 compounds studied in this investigation. Benzene was not extracted efficiently by the extractor column. The solubility of benzene, therefore, was determined by direct injection of 23.2 μL of the generated solutions via a sample loop.

The extraction efficiencies for 5-, 10-, and 25-mL volumes of benzene, naphthalene, fluorene, and phenanthrene are given below.

	Extraction Efficiency (%)		
Compound	*5 mL*	*10 mL*	*25 mL*
Benzene	60.0 ± 5.0	4.3 ± 0.9	3.1 ± 1.2
Naphthalene	101.4 ± 2.2	98.8 ± 1.0	98.5 ± 0.5
Fluorene	99.5 ± 1.0	98.4 ± 1.1	98.6 ± 1.2
Phenanthrene	99.5 ± 0.6	98.1 ± 1.5	98.7 ± 0.9

It has been shown elsewhere (39) that retention on, and hence the extraction efficiency of, C_{18} packed columns is a function of carbon number. Extraction of the eight larger PAHs studied in this investigation are, therefore, also quantitative.

A-2. Measurement of Sample Volume (*see* Figure 1). The volume of saturated solution extracted was determined and by placing the sample valve in the "extract" position, a designated volume of the effluent from the extractor column was collected in a class "A" 5-, 10-, or 25-mL volumetric flask. After the volumetric flask had been filled, the sample valve was manually switched to the "analyze position," thus diverting the saturated solution to waste.

The uncertainty associated with this process was dependent on human reflexes. One stroke of the generator pump (Milton Roy Controlled Volume Mini-Pump) is 0.05 mL. If we assume that the volumetric

flasks could be reproducibly filled to within one stroke of the generator pump, then the maximum uncertainty associated with the measurement of sample volume is ±1.0% (0.05/5.0 mL). This is a very liberal estimate and is equivalent to a very slow reaction time of 0.6 sec at the maximum flow rate used, 5 mL/min. (The density of water varies from 0.9960 g/mL at 30°C to 1.0000 g/mL at 5°C. This represents a maximum change in mass/volume of 0.4%. In this investigation, it is assumed that 1 L of water has a mass of 1 kg.)

A-3. Calibration of the Sample Loop. The volume of the sample loop was determined by an indirect gravimetric procedure. The loop was initially filled with mercury. The mercury was swept from the loop into a tarred weighing dish with approximately 1 mL of pentane. Since mercury and pentane are not miscible, the bulk of the pentane was decanted and the remainder was allowed to evaporate. This process was repeated four times, yielding the following results:

Mass of Mercury Displaced from Loop

	0.3097 g
	0.3167 g
	0.3128 g
	0.3178 g
$\bar{x}$	0.3143 ± 1.2%

The density of mercury at room temperature is 13.538 g/mL. The volume of the loop was then determined to be 23.2 ± 0.3 μL. Since determination by mass is a definitive method, the errors associated with this determination are random and associated with the ability to reproducibly fill the loop. The accuracy is then the precision with which the mass measurements can be made and is equal to 1.2%.

A-4. Preparation of Standard Solutions. Standard solutions of the aromatic hydrocarbons studied in this investigation were prepared by the direct method. Solutes were weighed on a Cach Electrobalance, dissolved in acetonitrile, and made up to a 500-mL volume in a class "A" volumetric flask.

The uncertainty involved in weighing the solutes was small. The balance has a certified accuracy of 5×10^{-4} or 0.05% (*49*). Class "A" 500-mL volumetric flasks have tolerances of less than 0.05% (*50*). The uncertainty associated with the preparation of these solutions is estimated to be less than 0.1%.

A-5. Calculation of Response Factors. Detector response factors for the 12 compounds studied in this investigation were determined by injecting 23.2 μL of standard solutions of the compounds into the liquid chromatographic system. These response factors are presented below.

Compound	*Response Factor*[a] $\left(\frac{area\ units}{ng}\right)$
Benzene	20.14 ± 0.22
Naphthalene	184.0 ± 1.18
Fluorene	1071 ± 10
Anthracene	7123 ± 66
Phenanthrene	2890 ± 14
2-Methylanthracene	10647 ± 95
1-Methylphenanthrene	3042 ± 32
Fluoranthene	659.7 ± 8
Pyrene	463.0 ± 3
Benzanthracene	1479 ± 10
Chrysene	2063 ± 15
Triphenylene	3081 ± 10

[a] Calculated from areas obtained when using Perkin Elmer System I electronic integrator with a chromatographic flow rate of 2.77 mL/min.

$$\text{Response factor} = \frac{\text{Area}}{(23.2\ \mu\text{L})\ (\text{conc. std.})\ (\text{purity of PAH})}$$

A-6. Calculation of Concentration. The concentration of the aromatic hydrocarbons in the aqueous solutions was calculated from the following equation:

$$\text{Concentration } (\mu\text{g/kg or ppb}) = \frac{\text{Area}}{(\text{R.F.})\ (\text{sample volume})}$$

where Area is the area under the chromatographic peak as determined by the electronic integrator, R.F. is the response factor (area/ng), and sample volume is the size of aqueous sample analyzed in mL (g).

Appendix B: Variation of Solubility with Temperature

The equations relating aqueous solubility to the temperature that were presented in the Accommodations section were derived from the data presented in this section. Experimental values of the solubilities between 5° and 30°C are given in Tables B-I through B-XII along with the standard deviations associated with each measurement. Each table is accompanied by a plot of this data (Figures B-1 to B-12).

The SI unit for concentration is moles per cubic meter (mol/m^3). Mole (mol) is the base SI unit for the amount of substance. Cubic meter (m^3) is a derived unit for volume. The use of regularly formed multiples such as cubic centimeter and cubic decimeter is also allowed. The special name liter (*51*) has been approved for use instead of cubic decimeter, but its use is restricted to the measurement of liquids and gases.

The PAH solubilities reported in the tables are expressed in both the SI unit mol/L and metric units mg/kg or μg/kg. The concentration axes for the least-squares fits of the solubility to temperature are labeled as either parts per million (ppm) or parts per billion (ppb). Since the density of water between 4° and 30°C is essentially constant and equal to 1.000, 1 L of water has a mass of 1 kg. Thus μg/kg equals ppb and mg/kg equals ppm.

Table B-I. Benzene

t (°C)	Concentration Measured (mol/L × 10^{-2})	Concentration Measured (mg/kg)	Concentration[a] Calculated (mg/kg)
0.2	2.35	1836 ± 12	1833
6.2	2.31	1804 ± 2	1815
11.0	2.30	1799 ± 13	1787
14.0	2.27	1770 ± 13	1771
16.9	2.26	1762 ± 10	1762
18.6	2.26	1767 ± 5	1761
25.0	2.29	1791 ± 10	1799
25.8	2.33	1819 ± 21	1810

[a] Solubility = $0.0247t^3 - 0.6838t^2 + 0.3166t + 1833$ (mg/kg).

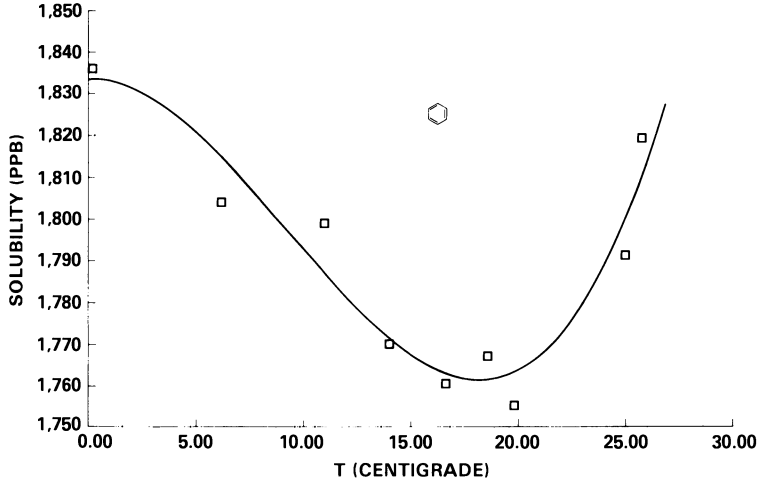


Figure B-1. Temperature dependence of the aqueous soluibility of benzene

Table B-II. Naphthalene

t (°C)	Concentration Measured (mol/L × 10^{-4})	Concentration Measured (mg/kg)	Concentration[a] Calculated (mg/kg)
27.0	2.66	34.15 ± 0.47	34.16
25.0	2.49	31.91 ± 0.30	31.69
23.4	2.30	29.47 ± 0.21	29.83
19.3	2.01	25.79 ± 0.31	25.51
15.1	1.68	21.48 ± 0.11	21.73
13.4	1.59	20.37 ± 0.05	20.39
11.5	1.50	19.23 ± 0.20	19.02
8.2	1.32	16.91 ± 0.26	16.97

[a] Solubility = $0.0189t^2 + 0.2499t + 13.66$ (mg/kg).

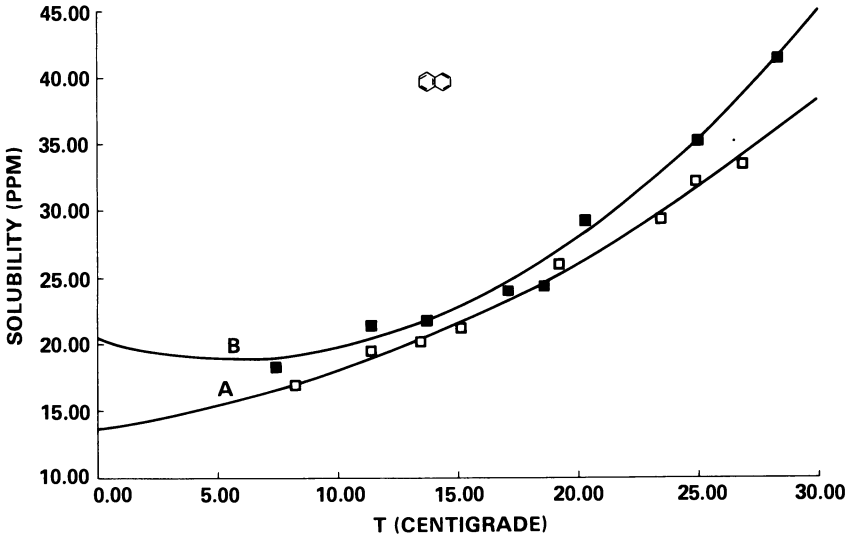


Figure B-2. Temperature dependence of the aqueous solubility of naphthalene

Table B-III. Fluorene

t (°C)	Concentration Measured (mol/L × 10^{-6})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
31.1	13.5	2248 ± 5.0	2246
27.0	11.1	1845 ± 4.9	1853
24.0	9.72	1616 ± 6.0	1607
18.0	7.24	1203 ± 1.1	1208
13.2	5.82	967.3 ± 9.7	965.4
6.6	4.32	718.4 ± 0.7	178.6

[a] Solubility = $0.0185t^3 + 0.4543t^2 + 22.76t + 543.3$ (μg/kg).

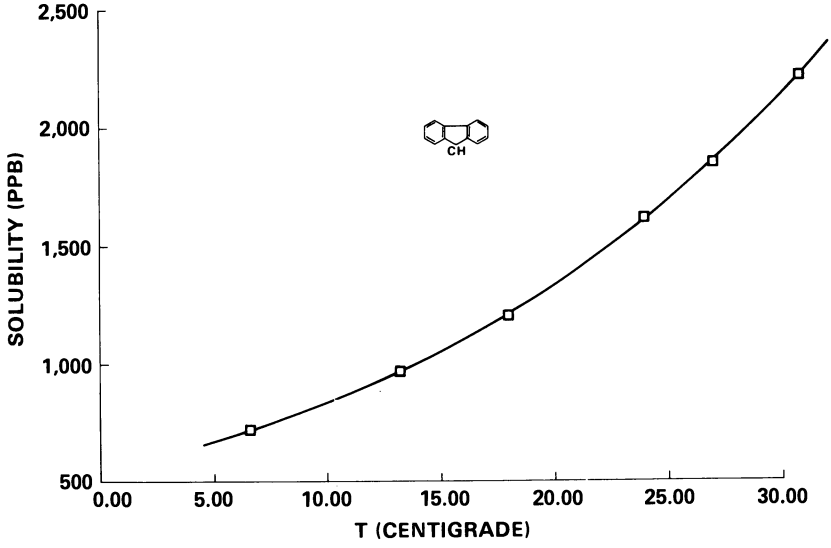


Figure B-3. Temperature dependence of the aqueous solubility of fluorene

Table B-IV. Anthracene

t (°C)	Concentration Measured ($mol/L \times 10^{-7}$)	Concentration Measured ($\mu g/kg$)	Concentration[a] Calculated ($\mu g/kg$)
28.7	3.13	55.7 ± 0.7	55.8
24.6	2.44	43.4 ± 0.1	43.1
22.4	2.09	37.2 ± 1.1	37.5
18.3	1.63	29.1 ± 0.6	29.0
14.1	1.25	22.2 ± 0.1	22.3
10.0	0.98	17.5 ± 0.3	17.4
5.2	0.71	12.7 ± 0.4	12.7

[a] Solubility $= 0.0013t^3 - 0.0097t^2 + 0.8886t + 8.21$ ($\mu g/kg$).

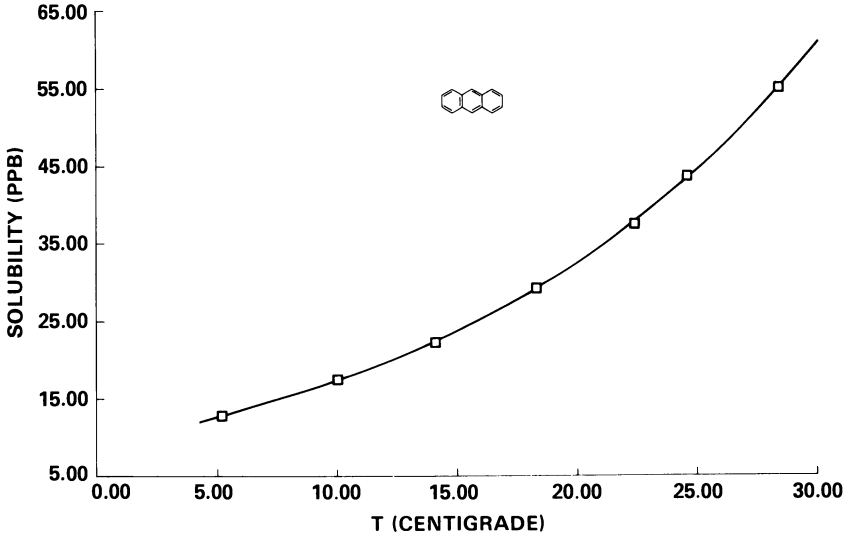


Figure B-4. Temperature dependence of the aqueous solubility of anthracene

Table B-V. Phenanthrene

t (°C)	Concentration Measured (mol/L × 10^{-6})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
29.9	7.16	1227 ± 11	1274
24.3	5.36	955 ± 1	967
21.0	4.58	816 ± 8	816
20.0	4.42	787 ± 2	775
15.0	3.37	601 ± 7	595
12.5	2.87	512 ± 1	523
10.0	2.63	468 ± 2	461
8.5	2.37	423 ± 4	430
4.0	1.97	361 ± 1	359

[a] Solubility = $0.0025t^3 + 0.8059t^2 + 5.413t + 324$ (μg/kg).

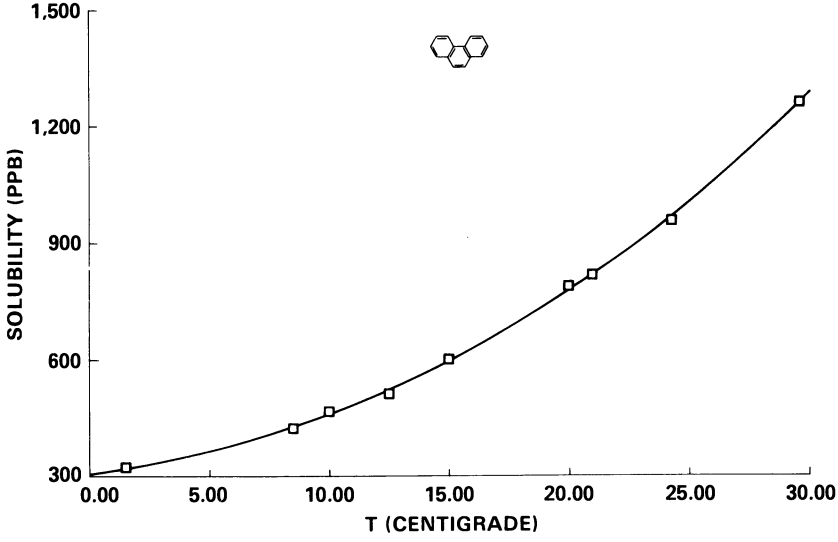


Figure B-5. Temperature dependence of the aqueous solubility of phenanthrene

Table B-VI. 2-Methylanthracene

t (°C)	Concentration Measured (mol/L × 10^{-7})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
31.1	1.67	32.1 ± 0.3	32.0
27.0	1.26	24.2 ± 0.1	24.4
23.1	0.994	19.1 ± 0.6	19.0
18.3	0.754	14.5 ± 0.1	14.3
13.9	0.575	11.1 ± 0.3	11.2
10.8	0.490	9.43 ± 0.37	9.45
9.1	0.441	8.48 ± 0.09	8.52
6.3	0.367	7.06 ± 0.18	7.00

[a] Solubility = $0.0011t^3 - 0.0306t^2 + 0.8180t + 2.79$ (μg/kg).

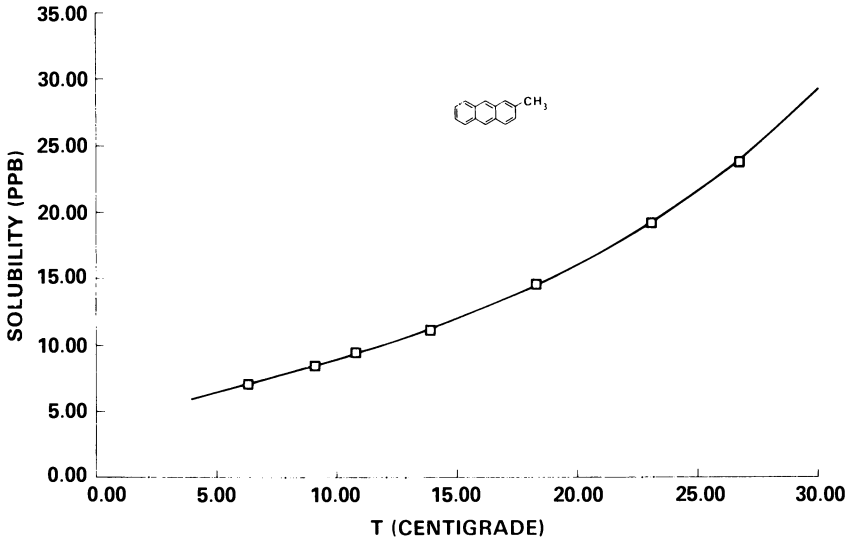


Figure B-6. Temperature dependence of the aqueous solubility of 2-methylanthracene

Table B-VII. 1-Methylphenanthrene

t (°C)	Concentration Measured (mol/L × 10^{-6})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
29.9	1.85	355 ± 2	357
26.9	1.58	304 ± 1	302
24.1	1.32	255 ± 5	257
19.2	1.01	193 ± 1	195
14.0	0.765	147 ± 1	147
8.9	0.594	114 ± 4	111
6.6	0.495	95.2 ± 0.2	97.3

[a] Solubility = $0.0074t^3 - 0.0858t^2 + 5.785t + 62.9$ (μg/kg).

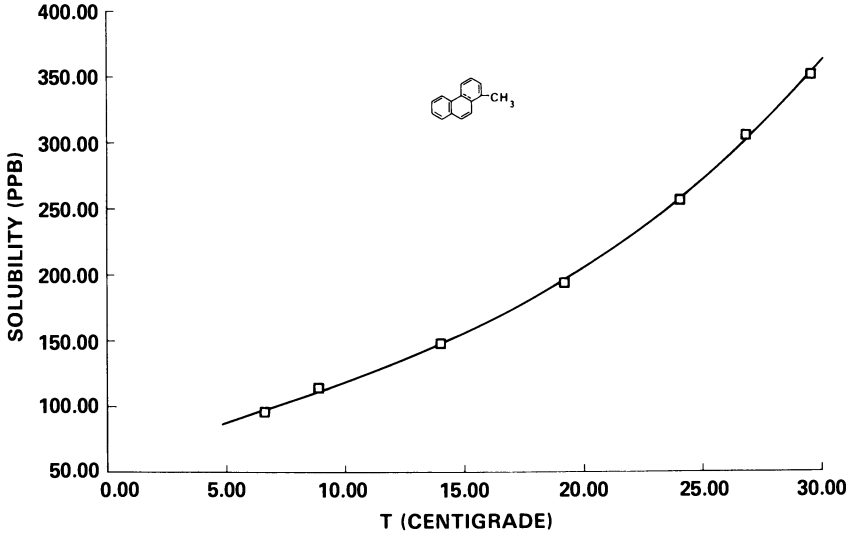


Figure B-7. Temperature dependence of the aqueous solubility of 1-methylphenanthrene

Table B-VIII. Fluoranthene

t (°C)	Concentration Measured ($mol/L \times 10^{-7}$)	Concentration Measured ($\mu g/kg$)	Concentration[a] Calculated ($\mu g/kg$)
29.9	13.8	279.3 ± 5.9	280
24.6	10.0	202.7 ± 0.2	201
19.7	7.33	148.3 ± 0.1	150
13.2	5.29	107.0 ± 0.4	106
8.1	4.05	82.0 ± 2.1	82.3

[a] Solubility $= 0.0072t^3 - 0.1047t^2 + 4.322t + 50.4$ ($\mu g/kg$).

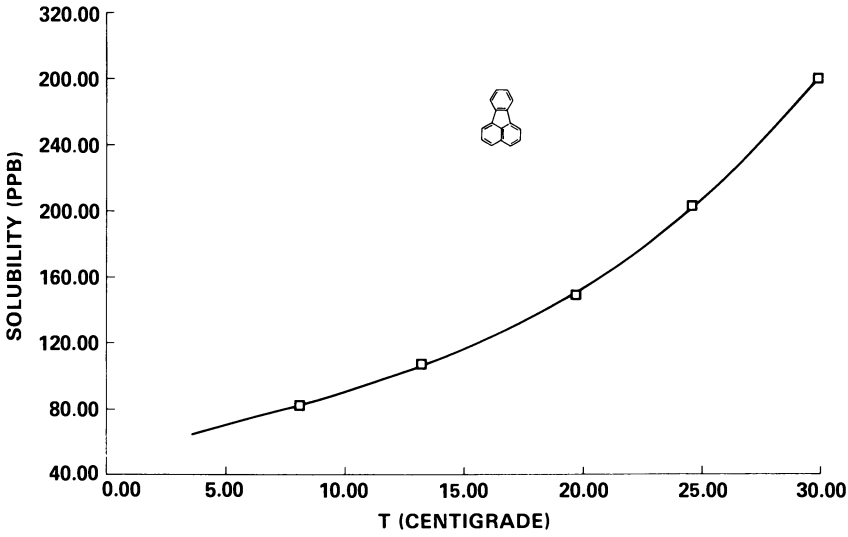


Figure B-8. Temperature dependence of the aqueous solubility of fluoranthene

Table B-IX. Pyrene

t (°C)	Concentration Measured (mol/L × 10^{-7})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
29.9	8.39	170 ± 1	170
25.5	6.73	136 ± 2	136
21.2	5.37	109 ± 1	108
18.7	4.61	93.3 ± 1.0	93.7
14.3	3.56	72.0 ± 1.0	73.1
9.5	2.89	58.5 ± 0.6	57.4
4.7	2.43	49.2 ± 0.1	49.5

[a] Solubility = $-0.0011t^3 + 0.2007t^2 - 1.051t + 50.2$ (μg/kg).

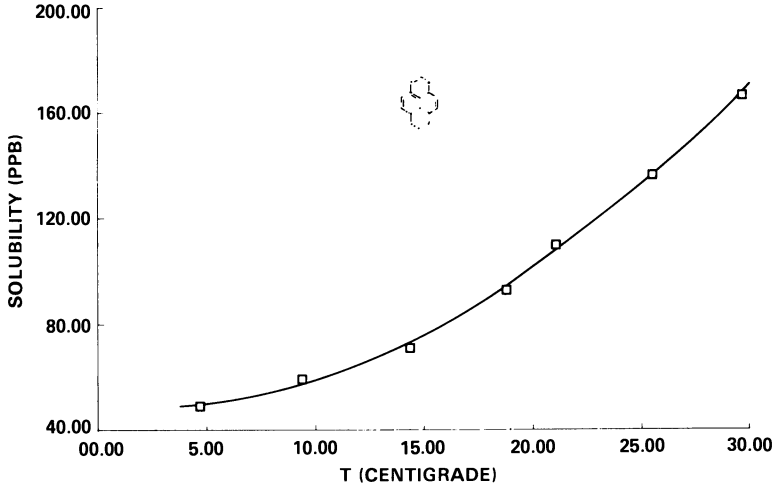


Figure B-9. Temperature dependence of the aqueous solubility of pyrene

Table B-X. Benzanthracene

t (°C)	Concentration Measured (mol/L × 10^{-8})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
29.7	5.58	12.7 ± 0.2	12.8
23.1	3.67	8.37 ± 0.03	8.28
19.3	2.77	6.33 ± 0.02	6.47
14.3	2.10	4.79 ± 0.02	4.72
10.7	1.66	3.78 ± 0.05	3.79
6.9	1.31	2.99 ± 0.10	3.00

[a] Solubility = $0.0003t^3 - 0.0031t^2 + 0.1897t + 1.74$ (μg/kg).

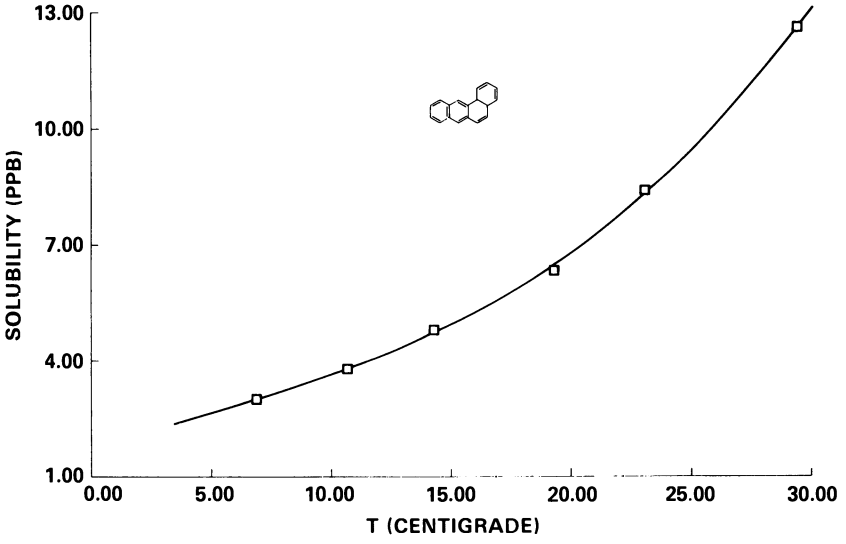


Figure B-10. Temperature dependence of the aqueous solubility of benzanthracene

Table B-XI. Chrysene

t (°C)	Concentration Measured (mol/L × 10^{-9})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
28.7	9.68	2.21 ± 0.02	2.23
25.3	8.28	1.89 ± 0.03	1.84
24.0	7.36	1.68 ± 0.03	1.71
20.4	6.13	1.40 ± 0.02	1.38
11.0	3.50	0.80 ± 0.02	0.82
6.5	3.10	0.71 ± 0.02	0.69

[a] Solubility = $0.0024t^2 - 0.0144t + 0.69$ (μg/kg).

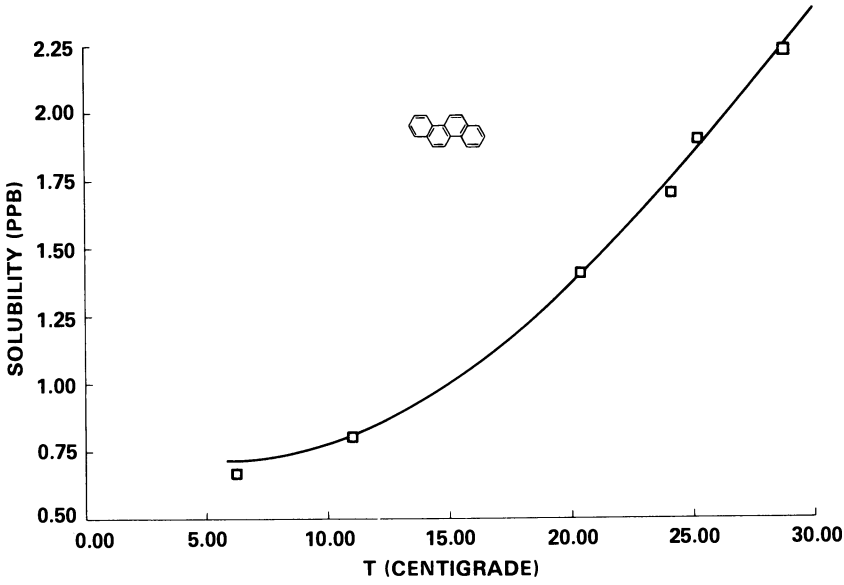


Figure B-11. Temperature dependence of the aqueous solubility of chrysene

Table B-XII. Triphenylene

t (°C)	Concentration Measured (mol/L × 10^{-8})	Concentration Measured (μg/kg)	Concentration[a] Calculated (μg/kg)
28.2	3.55	8.11 ± 0.11	8.10
27.3	3.35	7.65 ± 0.09	7.67
20.5	2.14	4.89 ± 0.05	4.88
14.8	1.49	3.39 ± 0.06	3.40
12.0	1.33	3.03 ± 0.02	3.03
8.0	1.18	2.99 ± 0.08	2.98

[a] Solubility = $-0.0002t^3 + 0.0250t^2 - 0.4250t + 4.89$ (μg/kg).

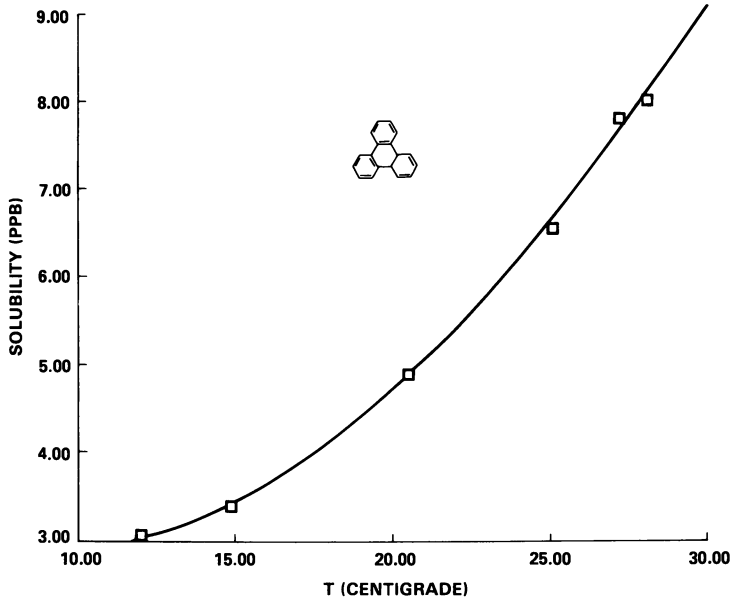


Figure B-12. Temperature dependence of the aqueous solubility of triphenylene

RECEIVED October 12, 1978.

8

The Multiple Gas-Phase Equilibration Method and Its Application to Environmental Studies

CLAYTON D. McAULIFFE

Chevron Oil Field Research Company, La Habra, CA 90631

Equilibrating a gas two or more times with an aqueous sample (waters, sediment slurries, biological fluids) permits calculation of distribution coefficients and measurements of volatile organic compounds, such as hydrocarbons and halocarbons at sub-μg/L concentrations. Classes of volatile organic compounds have different distribution coefficients, which aids in their separation and identification. The multiple gas-phase equilibration method has been used to measure the solubilities of pure hydrocarbons in waters of various salinities and of volatile hydrocarbons in oils and in water from the Cook Inlet, Gulf of Mexico, and Santa Barbara Channel. It was first to detect small amounts of chloroform and other contaminants in New Orleans drinking water; it measured the loss of C_1–C_{10} hydrocarbons from oil slicks on the ocean surface and the apparent absence of dissolved hydrocarbons under the slicks in less than 8 hr. It has simultaneously measured up to 8 anesthetic gases in blood and plasma.

Volatile compounds such as low-molecular-weight hydrocarbons and halogenated hydrocarbons dissolved in aqueous media can be determined by analyzing a gas phase that has contacted the water phase. Two principal methods have been used: gas stripping (*1–7*) and gas equilibration (*8–11*). These methods were developed for geochemical and oceanographic investigations. More recently, the methods have been applied to a variety of environmental problems.

Both methods have principal application to measuring lower-molecular-weight compounds as well as those that have favorable distribution coefficients (a relatively high vapor pressure compared with aqueous solubility).

0-8412-0475-6/80/33/193$06.50/0

In principle, gas stripping and gas equilibration are similar in that both depend upon volatile compounds diffusing from the water into a gas phase. Gas stripping involves bubbling a nonreactive gas through the aqueous phase to remove volatile compounds and subsequently trapping these compounds on a solid adsorbent or in a cold trap. The compounds are then desorbed or released from the cold trap to a suitable analytical instrument, often a gas chromatograph. Gas equilibration involves mixing a given volume of nonreactive gas with the aqueous sample, often in equal volumes. This establishes equilibrium of the organic compounds between water and gas phases. The gas is subsequently analyzed, usually by gas chromatography (GC).

However, there are also differences between the two methods. Gas stripping is a partial equilibrium method, whereas gas equilibration provides for true equilibrium of the volatile compounds.

Each method has advantages and disadvantages. The principal advantage of the gas equilibration method is that all volatile hydrocarbons, from the lowest molecular weight to the highest, will be present in the gas phase in proportion to the compound's vapor pressure and solubility in the aqueous phase (its distribution coefficient). Thus both low- and high-molecular-weight volatile hydrocarbons can be determined accurately.

The multiple gas-phase aspect (repeating equilibration with a second volume of pure gas) provides for the separation of a given class of organic compounds (hydrocarbons) from others. The measured distribution coefficient also assists in the identification of compounds found by GC. These and other aspects of the gas equilibration method will be presented in greater detail later.

Because gas stripping is a nonequilibrium method, lower-molecular-weight organic compounds (for a given class of hydrocarbons, for example) are stripped most rapidly. Therefore the amount removed is proportional to vapor pressure and inversely proportional to solubility (i.e., proportional to distribution coefficient). This results in complete removal of low-molecular-weight organic compounds of a given class but a decreasing percentage of the higher-molecular-weight ones under a given set of stripping conditions. Different classes of compounds also strip differently (for hydrocarbons, alkanes > cycloalkanes > aromatics). As an example, cyclohexane strips faster than benzene despite their having similar molecular weight. Thus careful calibration of the stripping conditions is needed, and/or the use of several representative internal standards (*1*). If the stripping method analyzes a mixture of classes of organic compounds of different molecular weights in a single gas chromatographic run, the analysis lacks information provided by distribution coefficients obtainable by the gas equilibration method.

The use of an adsorbent or cold trap introduces another step into the gas-stripping method. If stripping is carried out long enough to quantitatively remove the most difficult to strip organic compounds from the aqueous phase, the easiest to strip start to be lost from the solid adsorbent, or cold trap. For example, with Tenex solid adsorbent it is difficult to obtain high percentage removal of trimethylbenzenes from water and still retain benzene (*12*).

A principal advantage of the gas-stripping method and particularly the gas-cycling method (*2, 3*) is high sensitivity. Gas stripping removes volatile organic materials from 10 mL to 2.0 L of aqueous samples, whereas gas equilibration typically introduces the organic compounds from 1- to 50-mL samples (*8, 10, 11*). The higher sensitivity, however, is often negated by contaminants in the stripping gas, air contamination, water interference, adsorption losses in recovering volatile organics from the solid adsorbent, and production of artifacts by heating organic polymer adsorbents. These problems have been discussed and largely overcome by Grob and co-workers (*1, 2, 3*), but their technique requires very careful manipulation.

Gas stripping requires careful calibration throughout the stripping and desorption phases, even for drinking water samples. The problems become much more difficult if applied to other aqueous media of varying ionic strengths (brackish water, seawater, and subsurface brines); natural water samples containing inorganic mineral matter, bacteria, phytoplankton, and zooplankton; sediment samples; and biological samples such as body fluids and tissues.

The gas-equilibration method is not subject to the disadvantages above. A small amount of gas is used. This avoids errors introduced by contaminants that become significant only when adsorbed from the large volume of gas used for stripping. No adsorbent is used for gas equilibration, water does not interfere, and the technique is simple. Because distribution coefficients are measured, gas equilibration can accurately determine volatile organics in all types of aqueous samples, without prior standardization or use of internal standards.

In the following sections the gas equilibration method is described in greater detail, followed by its application to several environmental problems.

Multiple Gas-Phase Equilibration

Organic contaminants in water can be easily determined at sub-μg/L concentrations if they partition favorably from water to a gas phase. The method (*10*) is based on gas chromatographic analyses of the gas phases for two or more equilibrations with an aqueous sample.

Laboratory Measurements. Typically a 50-mL glass hypodermic syringe (with Luer–Lok fitting) is flushed several times with portions of the aqueous sample and capped with a stainless steel–Teflon gas-tight valve. Twenty-five mL of water is retained (care taken to exclude air), and 25 mL of hydrocarbon-free, nonreactive gas (helium, air, nitrogen, etc.) is added. The valve is closed, and the syringe is vigorously shaken (for example, on a paint shaker) for 3–5 min to establish equilibrium between phases. Longer shaking with a wrist-action shaker or some other means of establishing equilibrium is suitable also. From 20 to 23 mL of the gas phase then is flowed through the sample loop of a gas chromatograph, and a measured volume (usually 1–10 mL) of the gas is introduced for analysis.

With the syringe tip upward, the remaining gas is carefully and completely discharged by moving the solution to the syringe tip, and 25 mL of fresh gas is added. Equilibration is repeated as many times as required for the specific application. If some water should be accidentally lost, it is necessary only to proportionately reduce the equilibrating gas volume. Temperature must be kept constant during successive equilibrations.

Two or more successive gas-phase equilibrations give all the necessary data to calculate the concentrations in the aqueous phase. The mathematics of multiple-phase equilibration are presented in Reference *10*. A plot of the log of the compound's concentration in each gas phase equilibration vs. the number of equilibrations produces a straight line (Figure 1). The negative slope of this line is the log of the distribution coefficient plus 1. The intercept is the product of the initial concentration of the unknown compound, its distribution coefficient, and a constant related to sample size, the instrument, and its sensitivity.

From the semilog line, any two adjacent gas-phase concentrations can be read. Dividing the greater by the lesser and subtracting 1 gives the distribution coefficient (k). The intercept of the line divided by k gives the compound concentration in the original sample.

Although the results will be slightly less accurate, the data need not be plotted. For most environmental samples, two equilibrations give sufficient accuracy. The concentration in the aqueous phase is calculated by dividing the concentration in the first gas equilibration by that in the second and then multiplying the first concentration by this value to give the intercept. The intercept divided by k gives the compound concentration of the original sample.

Figure 1 shows the analysis of three different salinity waters all made up to contain 42 ppm toluene. The calculated values were: tap water (very low salinity), 42.2; 3.5% NaCl, 41.8 ppm; and 20% NaCl, 42.2

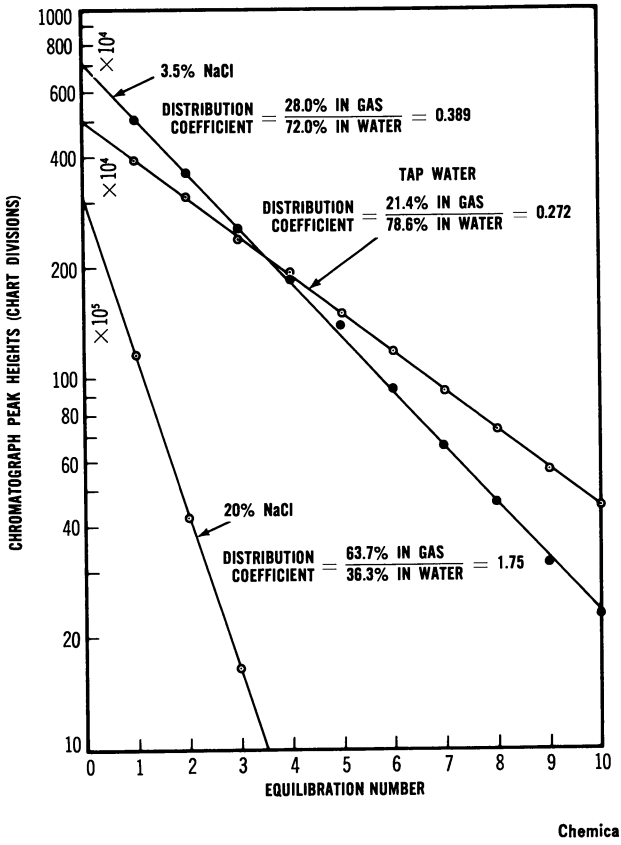


Figure 1. Partitioning of toluene between equal volumes of three salinity waters and helium at 25°C for successive equilibrations (10)

ppm. The mean was 42.06 ± 0.23 (0.55%) ppm. These salinities cover the range normally encountered and demonstrate the accuracy of the method in solutions of varying ionic strength (markedly changed distribution coefficients). The solubility of toluene in distilled water (fresh tap) is 530 ppm (*13, 14*). The measured solubilities calculated from the distribution coefficients shown on Figure 1 for 3% and 20% NaCl solutions are 370 and 82 ppm, respectively. These values check the values for toluene-saturated solutions measured by Price (*14*).

If samples are always of the same composition (e.g., fresh water or seawater) from a given location, the k's need be measured on only a few samples; thereafter, a single equilibration suffices. The concentration of each compound in the first equilibration gas-phase times $(1 + k)$ divided by k gives the concentration in the original sample.

Partitioning of Organic Compounds. Groups of compounds, such as hydrocarbons and chlorinated hydrocarbons, have different *k*'s. For example, each class of hydrocarbons (alkanes, alkenes, cycloalkanes, and aromatics) have different *k*'s. The alkanes partition 95+% into the gas when equal volumes of gas and water are equilibrated. For this reason two, or at most three, equilibrations will transfer all the alkanes present into the gas phase. Thus, it is more accurate and convenient merely to sum the concentrations found in the successive gas phases rather than back-extrapolate. If 5% accuracy is adequate for analysis of alkanes, a single equilibration is sufficient. Two or three equilibrations are also adequate for alkenes and cycloalkanes.

Aromatic hydrocarbons, however, partition less favorably to the gas phase, approximately 20%. Therefore, many equilibrations would be required to obtain an analysis by summing successive gas phases (Figure 1, fresh and seawater). Some chlorinated hydrocarbons behave in a similar manner.

Because different classes of hydrocarbons partition differently, successive equilibrations remove alkanes, alkenes, and cycloalkanes, leaving only aromatic hydrocarbons in solution. Variations in the way different classes of hydrocarbons partition are shown graphically in Figure 2. The original solution was formulated in tap water to give approximately the same peak heights in the first equilibration for benzene, cyclohexane, and *n*-hexane. The results also show a small peak for cyclopentane, an impurity in the cyclohexane.

The second equilibration resulted in a small *n*-hexane peak, a small cyclohexane peak, and a toluene peak reduced by approximately 20%. A small benzene peak exists as an impurity from the toluene sample. The third equilibration shows no *n*-hexane peak, a very small cyclohexane peak, a small benzene peak, and toluene as the major hydrocarbon remaining.

Table I. Partitioning of Representative Hydrocarbons Between

	Hydrocarbons			
	Normal Alkanes			
Gas–Water Ratio	*Methane*	*Ethane*	*Pentane*	*Decane*
1:10	72.9	67.0	83.7	98.2
1:5	84.3	80.3	91.1	99.1
1:1	96.4	95.3	98.1	99.8
5:1	99.3	99.0	99.6	100
10:1	99.6	99.5	99.8	100

Hydrocarbons and chlorinated hydrocarbons can be separated qualitatively from other organic compounds having high water solubilities, such as alcohols, acids, aldehydes, and ethers. Because the latter compounds have high water solubilities relative to their vapor pressures, the distributions greatly favor the water phase, and little is found in the gas phase. If the water phase should contain a sufficiently high concentration of an organic compound of high water solubility, successive gas equilibrations would show a very slow decrease in concentration in the gas phase. For example, with equal volumes of gas and water, only 3% of diethyl ether is released to the gas phase for each equilibration.

The distribution can be changed, however, by using different gas–water ratios. Table I shows the partitioning of various classes of hydrocarbons for selected gas–water ratios. Increasing the gas–water ratio partitions a higher percentage of the individual hydrocarbons to the gas phase. However, the concentration per unit volume of gas decreases with increase in gas–water ratios for *n*-alkanes, alkenes, and cycloalkanes. Aromatic hydrocarbons, by coincidence, partition to give approximately the same concentration per unit volume of gas over gas–water ratios from 1:10 to 10:1.

Table I also demonstrates the difference in partitioning for different classes of hydrocarbons shown in Figure 2. Although the classes partition approximately the same, they all show an increase with increase in molecular weight. This happens because the water solubilities decrease more rapidly than the vapor pressures.

Method Sensitivity and Accuracy. When we use the described procedure and introduce a 5-mL gas sample into the chromatograph, the method is capable of detecting alkane, alkene, and cycloalkane hydrocarbons down to 1–3 parts in 10^{12} parts of water by weight (ppt). Aromatic hydrocarbons, because of their lower partitioning into the gas phase, can be detected at 4–12 ppt. Reasonable accuracy requires concentrations

Different Volumes of Gas and Water (Percent in Gas Phase)

Hydrocarbons							
Alkenes			*Cycloalkanes*		*Aromatics*		
Propene	*1-Hexene*	*1-Octene*	*Cyclopentane*	*Methylcyclohexane*	*Benzene*	*Toluene*	*Ethylbenzene*
46.2	62.0	78.9	27.4	60.8	2.2	2.6	3.3
63.2	76.6	88.2	43.0	75.6	4.3	5.1	6.3
89.6	94.2	97.4	79.0	93.9	18.5	21.2	25.3
97.7	98.8	99.5	95.0	98.7	53.2	57.4	62.8
98.9	99.4	99.7	97.4	99.4	69.4	72.9	77.2

20–30 times higher. For trimethylbenzenes the peaks broaden with the gas chromatograph operated isothermally, so sensitivity becomes less.

The method has high precision if attention is paid to gas–water volumes and temperatures are constant (normal air conditioning appears satisfactory). Using these conditions McAuliffe (*10*) measured four replicates of the same solution of *n*-hexane and obtained a standard deviation of ±0.7%, and ±0.8% for three replicates of water containing cyclohexane. These values, along with the ±0.5% for toluene in the three different-salinity waters reported above, give a measure of method precision. If the GC-detector calibration is this accurate (it probably is not), the precision above is also the method accuracy. Wasik and Brown (*15*) have modified the technique to permit automation and constant temperature conditions.

As will be discussed later, most of the volatile (and soluble) hydrocarbons in crude oils and light refined products have less than 10 carbon atoms in the molecule. These hydrocarbons, especially the aromatics, are

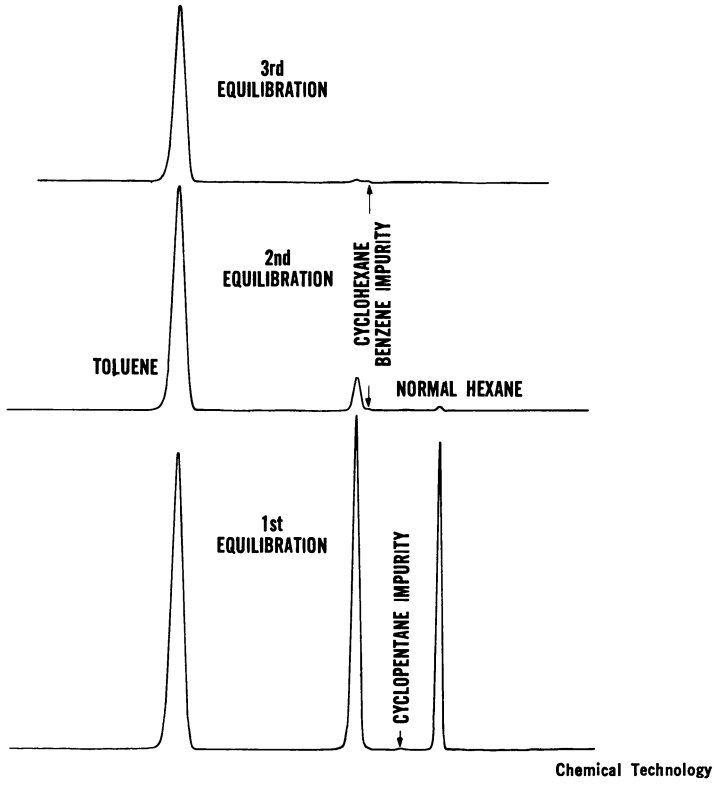


Figure 2. Partitioning of three different classes of hydrocarbons between equal volumes of tap water and helium at 25°C for successive equilibrations (10)

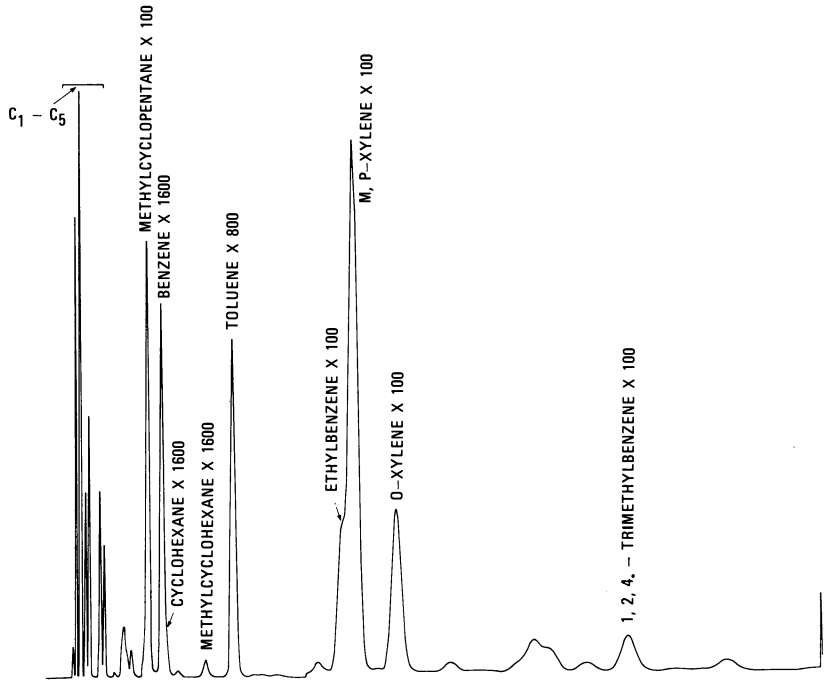


Figure 3a. Hydrocarbons dissolved in 2% NaCl solution from an excess of a South Louisiana crude oil. Chromatograph conditions: 4 m × 6.4 mm stainless steel column packed with 15% SE-30 and operated isothermally at 140°C.

considered the most likely to cause immediate toxicity to aqueous plants and animals. Thus analysis by isothermal GC is satisfactory.

If needed, increased sensitivity can be obtained for trimethylbenzenes, and the range can be extended to include the naphthalenes if the equilibrated gas is analyzed by temperature-programmed gas chromatography. Figure 3a shows an isothermal run of hydrocarbons dissolved in seawater from an excess of a Murban crude oil. Figure 3b shows a temperature-programmed run on a similar water sample.

Applications of the Method

The multiple gas-phase equilibration method has been used to measure hydrocarbons and halogenated hydrocarbons in several studies, many related to environmental problems. Some of these are reported in this section as representative of the method's use.

Waters. As mentioned previously, most hydrocarbons dissolved in water associated with crude oils or light refined products, such as gasoline and diesel fuels, have less than 10 carbon atoms in the molecule. This is

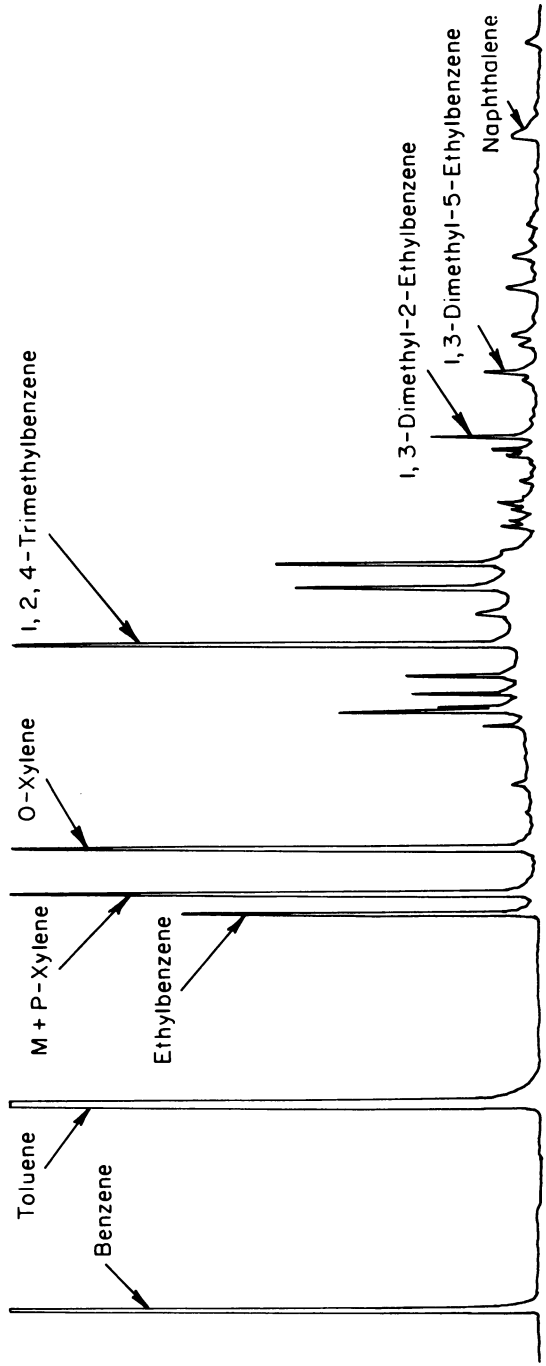


Figure 3b. Hydrocarbons from a water-soluble fraction of a biological bioassay solution (satured hydrocarbons lost) prepared from a South Louisiana crude oil. Chromatograph conditions: Stainless column (91 m × 0.25 mm) wall coated with low viscosity DC-200. Gas sample containing hydrocarbons collected from sample loop on head of column at −100°C, quickly raised to 20°C, and temperature programmed to 140°C to 6°C/min. (12)

a reflection of the decrease in solubility within each class of hydrocarbons (alkanes, alkenes, cycloalkanes, and aromatics) with an increase in molecular weight, and of the general trend for the concentrations of individual hydrocarbons in crude oils to decrease with increase in molecular weight. Figure 4 summarizes some of the solubility data for pure alkane and aromatic hydrocarbons in distilled water. Some of the data were obtained using the gas equilibration method (9). For normal alkanes the solubility decreases 6–7 orders of magnitude for a change in carbon number from 1 to 12. For aromatics the solubility decreases similarly for carbon-number increases from 6 to 20 carbon atoms in the molecule.

The data in Figure 4 also demonstrate that for a given carbon number, aromatics are very much more soluble than alkanes. For example, hexane, cyclohexane, and benzene, each with 6 carbon atoms in the molecule, have respective solubilities of 9.6, 60, and 1750 mg/L (ppm). The respective values for the seven-carbon-atom hydrocarbons (heptane, methylcyclohexane, and toluene) are 2.5, 15, and 530 mg/L (*13, 14*).

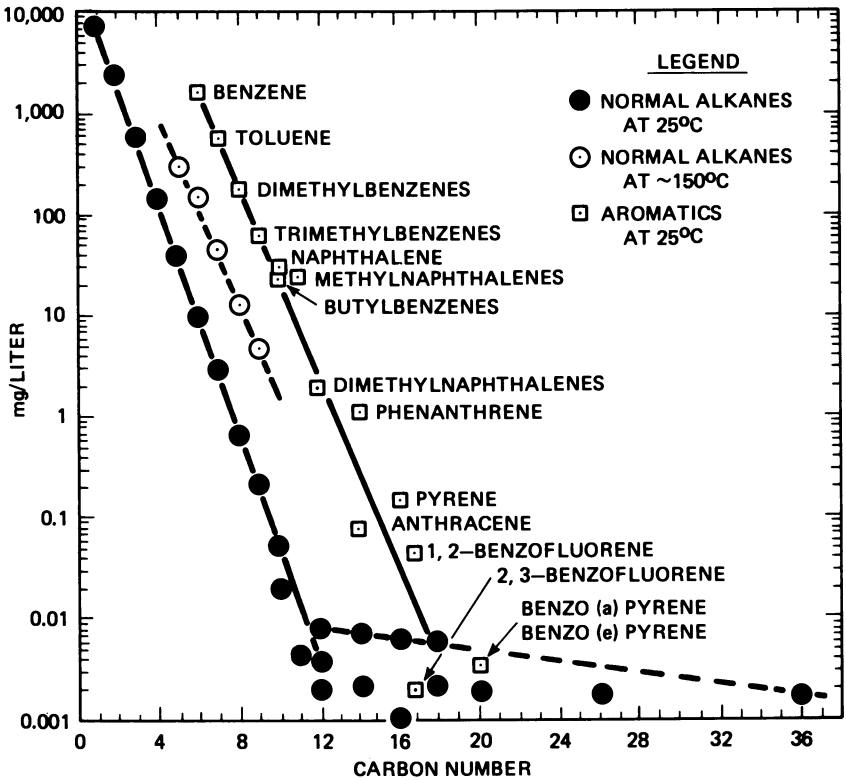


Figure 4. Solubilities of normal alkane and aromatic hydrocarbons in water (9, 14, 29–36)

Table II. Hydrocarbons Dissolved in Crude Oil (water salinity)

Hydrocarbons	*Murban*[b] *(sea)*	*La Rosa*[b] *(sea)*
Methane	—	0.26
Ethane	0.23	2.01
Propane	2.15	3.63
Isobutane	0.80	0.76
n-Butane	2.88	1.88
Isopentane	1.03	0.60
n-Pentane	1.34	0.60
Hexanes + cyclopentane	0.85	0.50
n-Hexane	0.50	0.15
Methylclclopentane	0.35	0.27
Benzene	6.08	3.30
Cyclohexane	0.41	0.19
n-Heptane	0.33	0.10
Methylcyclopentane	0.23	0.16
Toluene	6.16	2.80
Ethylbenzene	0.82	0.27
m-, *p*-Xylene	1.94	0.84
o-Xylene	1.01	0.35
Trimethylbenzenes	0.75	0.30
C_1–C_5 Alkanes	8.43	9.48
$C_6 + C_7$ Saturates[c]	2.67	1.37
Total saturates	11.10	11.11
Benzene + toluene	12.24	6.10
Dimethylbenzenes (C_8)	3.77	1.46
Trimethylbenzenes (C_9)	0.75	.30
Naphthalenes (Table III)	—	—
Biphenyls, fluorenes, and phenanthrenes (Table III)	—	—
Total aromatics	16.76	7.86
Total hydrocarbons	27.87	19.97

[a] All analysis performed at Chevron Oil Field Research Company. All data reported as mg/L.
[b] Data from Ref. *17*.
[c] Data from Ref. *16*.

Thus, benzene is 185 times more soluble than hexane; and toluene, 210 times more than heptane. The aromatic:*n*-alkane ratio increases with carbon number (Figure 4), so that dimethylnaphthalenes are over 600 times more soluble than *n*-C_{12}.

The usual method of measuring hydrocarbon solubility in water is to ensure equilibrium saturation with an excess of hydrocarbon. If the vapor pressure of an organic compound is known, its solubility can be determined without knowing whether the water is truly saturated. The

Water Equilibrated with Oil Samples[a]

Crude Oil (water salinity)			
South Louisiana[e] *(2% NaCl)*	*Kuwait*[c] *(2% NaCl)*	*Cook Inlet*[d] *(sea)*	*Prudhoe Bay*[d] *(sea)*
—	—	0.81	—
0.54	0.23	3.63	0.01
3.01	3.30	10.70	0.79
1.69	0.90	1.90	0.33
2.36	3.66	5.02	1.50
0.70	0.98	1.02	0.40
0.49	1.31	1.11	0.56
0.38	0.59	0.96	0.50
0.09	0.29	0.21	0.13
0.23	0.19	0.63	0.32
6.75	3.36	8.10	9.30
[g]	[g]	0.80	0.24
0.06	0.09	0.16	0.11
0.22	0.08	0.42	0.21
4.13	3.62	4.10	6.58
[f]	[f]	0.35	0.49
1.56	1.58	1.06	1.58
0.40	0.67	0.45	0.52
0.76	0.73	0.29	0.34
8.79	10.38	24.19	3.59
0.98	1.24	3.18	1.51
9.77	11.62	27.37	5.10
10.88	6.98	12.20	15.88
1.96	2.25	1.86	2.59
0.76	0.73	0.29	0.34
0.30	0.07	—	—
0.01	0.01	—	—
13.60	9.96	14.35	18.81
23.37	21.72	41.72	23.90

[d] Data from Ref. *18*.
[e] Included with benzene.
[f] Included with *m*-, *p*-xylene.
[g] Saturated (alkane plus cycloalkanes).

water can be very much undersaturated. All that is required for the gas equilibration method is that sufficient compound be present for accurate analysis. The measured distribution coefficient and the vapor pressure of the pure compound are used to calculate its solubility.

If water is equilibrated with crude oils, each hydrocarbon dissolves in the water phase in proportion to its solubility and its mole fraction concentration in the crude oil. Table II summarizes the measured concentrations of individual C_1-to-C_{10} hydrocarbons in water equilibrated

with representative crude oils (*16, 17, 18*). The crude oils had most of the gas removed. Thus, the concentrations of methane-through-pentanes in water were quite low, particularly the lower-molecular-weight hydrocarbons such as methane and ethane, compared with their concentrations in "live" oil (without major gas separation). Note the high relative concentrations of benzene and toluene.

Table III presents the solubilities in water of the higher-molecular-weight hydrocarbons ($> C_9$) from two crude oils (*16*).

The analyses shown in Tables II and III were of the prepared water-soluble stock solution used by these investigators in different dilutions to test the toxicities of these compounds to marine organisms. Tables II and III show that the amount of hydrocarbons with more than 9 carbon atoms dissolved in water from an excess of crude oil is minor, compared with that of hydrocarbons with less than 10 carbon atoms. This latter fraction is 98.7% of total dissolved hydrocarbons for the South Louisiana crude oil and 99.6% for Kuwait (assuming that there is no contribution from hydrocarbons higher in molecular weight than phenanthrenes).

These data indicate that the gas-equilibration method is well adopted to analyze C_1–C_{10} hydrocarbons in aqueous media.

The technique was used on the University of Alaska's research vessel *Acona* to study low-molecular-weight hydrocarbons in Cook Inlet waters (*19*). Only methane was found, in approximate equilibrium with methane in the atmosphere. In the northern Cook Inlet, methane concentrations appeared to be somewhat higher, suggesting possible seepage from subsurface reservoirs.

The method has also been used to measure low-molecular-weight hydrocarbons in seawater, in the oil seep area of the Santa Barbara Channel (*20*) and other Pacific Ocean waters (*8*).

Use of the method has been made to monitor hydrocarbons and chlorinated hydrocarbons in drinking and effluent waters. New Orleans' drinking water was daily analyzed for 18 months starting in April 1970 by gas equilibration. Contaminants were present throughout this period, chloroform being present in the highest concentration (*21*). Benzene and toluene also were consistently present, along with unidentified nonhydrocarbon compounds. Dow Chemical Company (*22*) and the Ethyl Corporation (*23*) use the technique to monitor chlorinated hydrocarbons in effluent streams, and Jefferson Parish, New Orleans, has used it to monitor drinking water.

G. R. Umbreit (*24*) has used the gas-equilibration technique to measure several halogenated hydrocarbons, employing both flame ionization detection (FID) and electron capture (EC). Halogenated hydrocarbons included dichloromethane, chloroform, carbon tetrachloride, trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, 1,2,2-trichlo-

Table III. Higher Molecular Weight Aromatic Hydrocarbons Dissolved in 2% NaCl Solution Equilibrated with Two Crude Oils (*16*)[a]

Hydrocarbon	*South Louisiana Crude Oil*	*Kuwait Crude Oil*
Naphthalene	0.12	0.02
1-Methylnaphthalene	0.06	0.02
2-Methylnaphthalene	0.05	0.008
Dimethylnaphthalenes	0.06	0.02
Trimethylnaphthalenes	0.008	0.003
Biphenyl	0.001	0.001
Methylbiphenyls	0.001	0.001
Dimethylbiphenyls	0.001	0.001
Fluorene	0.001	0.001
Methylfluorenes	0.001	0.001
Dimethylfluorenes	0.001	0.001
Dibenzothiophene	0.001	0.001
Phenanthrene	0.001	0.001
Methylphenanthrenes	0.002	0.001
Dimethylphenanthrenes	0.001	0.001
Totals	0.298	0.071

[a] All data reported as mg/L.

Marine Biology

roethane, 1,2-dichloroethane, chlorobenzene, *o*-, *m*-, and *p*-dichlorobenzenes, 1,2,4-trichlorobenzene, bromoform, dichlorobromomethane, dibromochloromethane. For some halogenated compounds (two-carbon chloro-compounds and chlorinated aromatics), FID is a better GC detector than EC. For others, electron capture is much more sensitive. Umbreit found, for example, that overloading of the EC detector occurred with only 1 mL of the gas phase equilibrated with water containing 20 ppb carbon tetrachloride. Hence it was necssary to use only 2 ppb as a calibration standard. Thus, for carbon tetrachloride and similar compounds such as tetrachloroethylene, the method will measure concentrations in water in the sub-ppt range.

Oils. Water can be equilibrated with an excess of oil, and the water subsequently analyzed for dissolved hydrocarbons. Individual hydrocarbons at equilibrium in water have concentrations dependent on their mole fractions in the oil and their solubilities in water. Therefore, analysis of the water permits calculation of the amount of each soluble hydrocarbon in the oil phase.

As the oil changes the composition (for example, when spilled on water), the changes can be monitored by equilibrating an excess of the oil with water. This was done for four research oil spills made on the

open ocean (*17*), two with a 39.0° API gravity Murban crude oil (Abu Dhabi) and two with a 23.9° API gravity La Rosa crude oil (Venezuela). These were selected to bracket the densities and viscosities of many crude oils produced in offshore waters or transported by tankers in world trade.

Samples of oil were collected in a time sequence from each oil slick. Collections were made principally with a small skimmer developed by JBF Scientific Corporation and towed by a boom. Some oil samples were collected by repeatedly dipping a galvanized steel bucket into the slick and pouring into a separatory funnel. Excess water was drained from the funnel.

Oil samples were analyzed for remaining low-molecular-weight hydrocarbons by equilibrating 10–20 mL of the original or surface-collected oil with 70 mL of seawater collected outside the spill area. The oil and water were hand-shaken gently and periodically for 24 hr or more to establish equilibrium, Mercuric chloride added to water samples at time of collection prevented possible biodegradation of hydrocarbons during the oil–water equilibration. This water was analyzed by the gas-equilibration technique.

Table IV shows the dissolved hydrocarbons found in water equilibrated with the original La Rosa crude oil and in samples collected with time following the La Rosa spill. These data are representative of the four spills. The italicized values are the percentages remaining in the surface-collected oil samples.

Seawater equilibrated with unweathered La Rosa crude oil (from the spill tank) contained hydrocarbons dissolved in proportion to their individual solubilities and amounts in the oil. The C_2–C_4 alkane concentrations are high, but benzene and toluene concentrations stand out compared with C_6 and C_7 alkane and cycloalkane hydrocarbons. This hydrocarbon signature is typical for water equilibrated with crude oils reported previously in Table II.

Table IV shows hydrocarbons weathering from the surface oil rapidly and in accordance with their vapor pressures. Figure 5 is a plot of the losses of benzene, toluene, dimethylbenzenes, and trimethylbenzenes from the surface samples collected with time. The length of the bar represents the time the skimmer was towed through the slick. Percentage loss lines have been drawn through the midpoint of the towing time.

The loss of these aromatic hydrocarbons may not be representative of the weathering of all the discharged oil because of varying slick thickness and nonuniform sample collection. However, the curves show the maximum time for the oil to become depleted of these constituents, because the oil samples were collected from the thickest parts of the slicks.

Table IV. Hydrocarbons Dissolved in Seawater Equilibrated with Oil Samples and Percentage Remaining in Surface Slick (La Rosa crude oil)[a]

	Time After Spill[b] (Source of Oil)							
Hydrocarbon	*Time 0 (tank)*	*18–73 min (skimmer)*		*3.3–5.2 hr (skimmer)*		*5.3–6.5 hr (skimmer)*	
Methane	260						
Ethane	2011	8.0	*0.4*[c]				
Propane	3630	2.0	*0.1*				
Isobutane	760	12.5	*1.6*				
n-Butane	1880	6.9	*0.4*				
Isopentane	600	11.7	*2.0*				
n-Pentane	600	7.4	*1.2*				
Hexanes	500	5.8	*1.2*				
n-Hexane	150	3.2	*2.1*				
Methylcyclopentane	275	8.5	*3.1*				
Benzene	3300	80	*2.4*	0.63	*0.02*		
Cyclohexane	190	4.0	*2.1*				
n-Heptane	105	5.9	*5.6*				
Methylcyclohexane	165	13.7	*8.3*	0.17	*0.10*		
Toluene	2800	300	*11*	2.60	*0.09*	0.4	*0.01*
Ethylbenzene	275	110	*40*	3.20	*1.2*	0.9	*0.3*
m-, *p*-Xylene	840	315	*38*	10.1	*1.2*	3.4	*0.4*
o-Xylene	350	150	*43*	7.1	*2.0*	2.4	*0.7*
Trimethylbenzenes	300	170	*57*	23.8	*7.9*	12.8	*4.3*
Total saturates[d]	11,100	90		0.2			
Total aromatics[d]	7860	1125		47.0		20	
Total hydrocarbons[d]	19,000	1210		47		20	

[a] All data reported as μg/L (ppb).
[b] Samples collected 22 and 24 hr after spill contained no detectable C_2–C_{10} hydrocarbons.
[c] Italicized value is percentage of hydrocarbon remaining in surface-collected oil.
[d] Numbers are rounded to prevent implied high accuracy.

The rates of loss of these low-molecular-weight hydrocarbons from surface oil slicks determine the maximum time soluble hydrocarbons can be contributed to underlying waters. These rates also determine whether the volatiles will remain in oil sufficiently long to be transported to shorelines or, possibly, to bottom sediments.

The loss of volatile hydrocarbons shown by analysis of the surface oil samples is by evaporation and solution. Water samples were collected under the oil slicks to determine the concentrations of dissolved constituents. A total of 68 water samples were collected with time, 5 and 10 ft under the oil slicks. Each was analyzed for C_2–C_{10} low-molecular-weight hydrocarbons using the gas equilibration method.

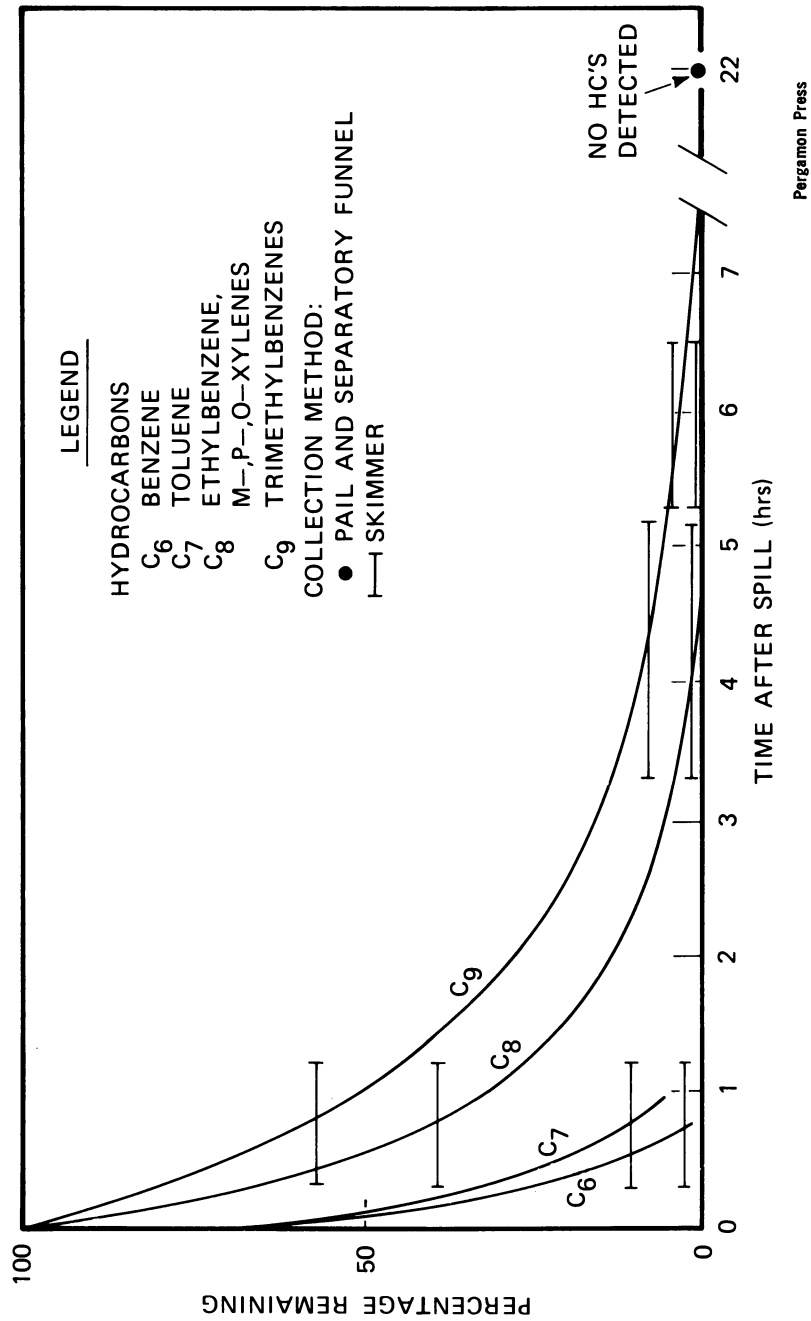


Figure 5. Percentage of aromatic hydrocarbons remaining in an oil slick on the ocean surface—La Rosa crude oil (Venezuela) (17)

Of the 68 samples, C_2-to-C_{10} hydrocarbons were found only in five. These were the first samples collected, 15–20 min after each spill (the time required for the research vessel to sample after discharging the oil). Low-molecular-weight hydrocarbons were not found in water samples collected 30 min or later after the spills.

Table V shows the concentrations of individual low-molecular-weight hydrocarbons in the water samples collected. The highest total concentration was 60 μg/L (ppb) in the 5-ft water sample collected after the first Murban spill (39° API gravity crude). Total hydrocarbon concentrations in the other four samples ranged from 2 to 16 ppb. The difference is probably due to higher winds at the time of the first Murban spill.

Table V. Low-Molecular-Weight Hydrocarbons in Water Samples under Oil Slicks[a]

	Oil Spill				
	Murban 1	*La Rosa 2*	*Murban 2*	*Murban 2*	*Murban 2*
Time after spill (min)	20	19	15	15	18
Depth (m)	1.5	1.5	1.5	3	1.5
Hydrocarbon	*Concentrations in μg/L (ppb)*				
Ethane	[a]				
Propane					
Isobutane	0.01	0.02			
n-Butane	0.02	0.04			
Isopentane	0.01	0.05			
n-Pentane	0.04	0.04			
Hexanes	0.07				
n-Hexane	0.24				
Methylcyclopentane	0.20	0.10	0.05	0.04	
Benzene	1.58	0.50	0.51	0.25	0.12
Cyclohexane	[b]	[b]	0.68		
n-Heptane	2.30	0.54			
Methylcyclohexane	7.60	0.80	0.30		
Toluene	6.20	0.61	1.22	0.61	0.41
Ethylbenzene	5.30	0.55	1.66	0.34	0.50
m-, *p*-Xylene	11.4	1.43	3.57	0.71	1.07
o-Xylene	12.2	0.79	3.95	0.39	0.79
Trimethylbenzenes	12.9	0.23	3.87		
Total saturates	10.5	1.59	1.03	0.04	
Total aromatics	49.6	4.11	14.8	2.30	2.89
Total hydrocarbons	60	5.7	16	2.3	2.9

[a] No value, not detected.
[b] Present but not resolved by GC integrator from benzene.

The relative concentrations of the individual low-molecular-weight hydrocarbons in Table V indicate that these were residual in dispersed oil droplets, so were not in true solution at the time of collection. As for evaporation (discussed above), hydrocarbons (each class, i.e., alkane, cycloalkane, and aromatic) would dissolve into water in inverse proportion to their molecular weights. The smaller the molecule, the higher was the amount found in solution for a given concentration in the oil phase.

In Table V the lowest-molecular-weight hydrocarbons are not present in the highest concentrations. Instead, concentrations increase with increase in molecular weight; thus, the conclusion that truly dissolved hydrocarbons were minor constituents under the oil slicks was made. The measured hydrocarbons shown in Table V have relative concentrations expected for partly weathered oil and resemble the evaporative loss of volatile hydrocarbons from surface slicks (Figure 5).

A similar distribution of C_2–C_{10} hydrocarbons was observed in water samples collected from a chemically dispersed emulsion plume of 34° API gravity crude oil in a Gulf of Mexico spill (*25*).

These results show that for oil moving on a water surface with wave action, evaporation greatly predominates. Hydrocarbons that dissolve appear to dilute or evaporate quickly.

Sediments. Sediments are unlikely to contain appreciable dissolved hydrocarbons. Oil would have to pass through the water column, probably as small droplets, and the soluble hydrocarbons would be quickly removed. Nevertheless, a modified equilibration method has been devised for sediments, because mineral matter prevents satisfactory operation of a glass syringe.

Sediment samples collected following the 1970 Chevron Gulf Coast spill were analyzed by this method (*25*). Known amounts of sediment, hydrocarbon-free water (obtained from the Gulf of Mexico because of the presence of the above-mentioned compounds in New Orleans' water) and hydrocarbon-free gas in appropriately sized glass bottles with metal screwcaps were shaken to establish equilibrium. After shaking, a sharpened stainless steel point was inserted through the metal cap. Hydrocarbon-free water added through one opening in the point displaced gas through a second opening, into a gas chromatograph sample loop.

Carlisle et al. (*26*) used a similar technique to measure C_1–C_7 hydrocarbons in sediment samples by mixing sediment, water, and gas in a sealed mixer (such as Waring). They also report evidence of submarine seeps in samples collected in the Gulf of Mexico. Hunt (*27*), using the technique of Carlisle, documented the apparent generation of small amounts of butane-through-heptane hydrocarbons in deep-sea drilling project cores, from burial depths of 28–800 m.

If multiple equilibration of the sample is required, rather than adding water to displace the gas, a small metal diaphragm pump can be used to circulate and mix the gas in the sample loop for injection into the chromatograph. The remaining gas can be replaced by hydrocarbon-free gas before the next equilibration.

If C_1–C_{10} hydrocarbons are present in a sediment sample, a second or third equilibration will document the partitioning of aromatic or other hydrocarbons with the solid surfaces present. The distribution coefficients probably are different for sediment slurries than for waters.

Biological Samples. Multiple gas equilibration has been used to measure up to 8 foreign inert gases in blood, plasma, and dextrose solutions (*11*). The method is used to measure distributions of ventilation–perfusion ratios based upon the simultaneous preliminary clearance of several inert gases (*28*). It is well suited to monitoring anesthetics and trace blood levels of anesthetic agents.

Table VI summarizes the solubilities of 9 gases in blood and 5% dextrose solution measured by multiple gas-phase equilibration. From 24° to 37°C, the solubility of the gases in dextrose solutions decreased in solubility. On this basis, the slightly higher temperature of 38°C for dog blood compared with 37°C for human blood would predict slightly lower solubilities for these gases in dog blood. However, all gases were more soluble in dog blood. Mean homoglobin, hematocrit, and plasma proteins were very similar in human beings and dogs and appear unlikely to explain these systematic differences in solubility. However, the largest differences for human and dog blood occurred for sulfur hexafluoride and halothane, which also have the highest oil–water partition coefficients of the 9 gases. This suggests that blood lipid levels may be important in determining the solubilities in blood. Figure 6 is a plot of the solubility of halothane vs. the lipid content of each blood sample, the mean of which is shown in Table VI.

The mean total lipids for the samples in Table VI were 384 mg/100 mL for human blood and 588 mg/100 mL for dog blood. Least-squares fits of sulfur hexafluoride and halothane solubilities vs. total blood lipids content for all blood samples gave correlation coefficients of $R = 0.82$ for halothane and 0.70 for SF_6. For the cholesterol fraction, the R's were 0.90 and 0.76, respectively. For the phospholipid component, the R's were 0.63 and 0.62, respectively, while for triglycerides and fatty acids, R was less than 0.40 for both gases.

For all gases except diethylether and acetone, Wagner et al. (*11*) used equal volumes of helium and liquid for gas equilibration. For the highly soluble ether and acetone, they took advantage of the change in distribution coefficients shown in Table I and introduced a measured

Table VI. Solubilities of Gases in Human Blood, 37°C, n = 9

Gas	
Sulfur hexafluoride	0.0084 ± 0.0013
Methane	0.0533 ± 0.0059
Ethane	0.133 ± 0.011
Cyclopropane	0.762 ± 0.056
Acetylene	1.17 ± 0.07
Fluroxene	2.03 ± 0.16
Halothane	3.28 ± 0.25
Diethyl ether	16.4 ± 1.24
Acetone	449 ± 32

[a] All data reported as (mg gas/L)/mm Hg.

amount of fluid (0.25–0.5 mL) from a 1-mL graduated syringe into the 50-mL volume of helium in a 50-mL glass syringe. With this high gas-to-liquid ratio, the highly soluble organic compounds partitioned sufficiently to the gas phase for accurate analysis.

As far as is known, the multiple gas-phase equilibration method has not been applied to tissue samples. However, it should be possible to place tissue, a suitable liquid (water or sodium hydroxide solution), and a gas in a sealed homogenizer and analyze the gas phase after adequate mixing. As with sediment samples, the first gas phase can be displaced with fresh gas and a second equilibration performed.

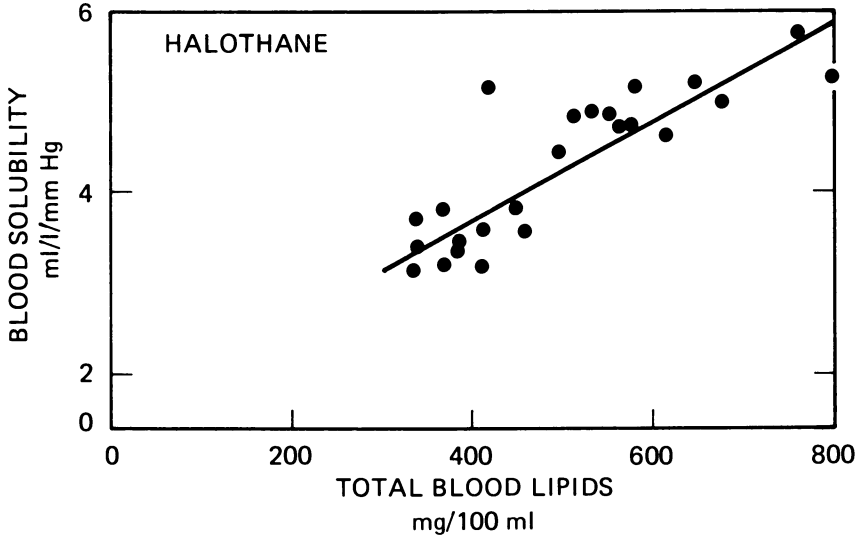


Figure 6. Relationship between solubility of halothane and total blood lipids. Dog and human data were pooled (correlation coefficient, R = 0.82) (11).

Blood and Dextrose Solutions (*11*)[a]

Dog Blood, 38°C, n = *21*	*5% Dextrose, 37°C*, n = *6*
0.0125 ± 0.0035	0.0040 ± 0.0005
0.0575 ± 0.0038	0.0396 ± 0.0026
0.168 ± 0.024	0.0464 ± 0.0011
0.970 ± 0.079	0.296 ± 0.009
1.30 ± 0.04	1.14 ± 0.05
2.28 ± 0.11	1.18 ± 0.04
4.99 ± 0.33	1.52 ± 0.09
16.7 ± 0.67	19.6 ± 1.94
478 ± 30	367 ± 28

The presence of minerals (as in sediment or some natural water samples), highly variable salinities (fresh water to subsurface brines), and the many substances in biological samples (as for blood, just discussed) introduces complexities in the determination of volatile organic compounds. Because these vary from sample to sample, the multiple gas-phase equilibration method determines distribution coefficients and permits accurate analyses.

Summary

Gas equilibration involves agitating aqueous samples containing volatile organic compounds with a gas phase, typically in a 50-mL glass syringe, until the dissolved compounds are in equilibrium in both phases. The gas phase is analyzed usually by GC. Multiple equilibration (replacing the gas phase two or more times) determines the distribution coefficients of all volatile compounds and permits calculation of their concentrations in the aqueous phase. The aqueous phase can be waters of widely varying salinities (fresh to subsurface brines), sediment slurries, and biological samples (blood and other body fluids). The concentrations of low-molecular-weight hydrocarbons in oils can be determined by first equilibrating water with oil and then analyzing the water.

The method is one of true equilibrium and permits the measurement of a wide range of molecular weight compounds from a single analysis (hydrocarbons from methane through methylnaphthalenes; methylene chloride through trichlorobenzenes).

Sensitivities for multiple gas equilibration are 1–3 ppt by weight for alkanes, alkenes, and cycloalkanes, and 4–12 ppt for aromatics. Quantitative use requires concentrations 10–30 times higher. Representative standard deviations achieved include ±0.5% for toluene, ±0.7% for *n*-hexane, and ±0.8% for cyclohexane.

The various classes of volatile organic compounds have different distribution coefficients, which aid in their separation and identification. Two equilibrations transfer all alkanes and cycloalkanes into the gas phase, leaving aromatics in the water. Alcohols, acids, aldehydes, and ethers partition little to the gas phase and generally do not interfere in hydrocarbon analyses. If present in amounts that interfere, they can be identified as nonhydrocarbons by their distribution coefficients. They can be analyzed if desired by greatly increasing the gas-to-water ratio.

The gas equilibration method has been used to measure the solubility of various hydrocarbons and halogenated hydrocarbons in varying salinity waters. It is well adapted to measure the C_1–C_{12} volatile hydrocarbons that are considered toxic to marine organisms. It has been used to measure traces of low-molecular-weight hydrocarbons in Cook Inlet waters, the Santa Barbara Channel, and other Pacific Ocean water. It was used to identify and monitor chloroform and other contaminants in New Orleans drinking water. It has been used to monitor effluent streams for halogenated hydrocarbons from manufacturing plants.

The technique was used to show the loss of C_1–C_{10} hydrocarbons in 4–8 hr from four research oil slicks in the ocean surface. Similarly, it showed the loss of this fraction from oil droplets in the water column under the oil slicks in 20–30 min. The hydrocarbon composition measured by gas equilibration showed the hydrocarbons in water under the slick to be residual in the oil droplets with little in true solution in seawater. The same was true for chemically dispersed oil in the Gulf of Mexico. Apparently evaporation quickly removed these hydrocarbons.

Gas equilibration has been used to look for low-molecular-weight hydrocarbons in sediment samples in an offshore oil spill area. Naturally occurring hydrocarbons have been measured in Gulf of Mexico and deep-sea sediments.

Multiple gas equilibration has been used to measure anesthetic gases in blood, plasma, and dextrose solutions. Halothane and sulfur hexafluoride solubilities were correlated with total blood lipids. The technique is routinely used in ventilation–perfusion measurements.

Literature Cited

1. Grob, K. "Organic Substances in Potable Water and in Its Precursor. Part I. Methods for Their Determination by Gas–Liquid Chromatography," *J. Chromatogr.* **1973,** *84,* 255–273.
2. Grob, K.; Grob, K., Jr.; Grob, G. "Organic Substances in Potable Water and Its Precursor. Part III. The Closed-Loop Stripping Procedure Compared with Rapid Liquid Extraction," *J. Chromatogr.* **1975,** *106,* 299–315.
3. Grob, K.; Zürcher, F. "Stripping of Trace Organic Substances from Water. Equipment and Procedure," *J. Chromatogr.* **1976,** *117,* 285–294.

4. McAuliffe, C. D. "Geochemical Method of Prospecting for Petroleum," U. S. Patent 3 345 137, 1967.
5. Novák, J.; Zluleckeý, J.; Kubelka, V.; Mostecký, J. "Analysis of Organic Constituents Present in Drinking Water," *J. Chromatogr.* **1973,** *76,* 45–50.
6. Swinnerton, J. W.; Linnenbom, V. J. "Gaseous Hydrocarbons in Sea Water: Determination," *Science* **1976,** *156,* 1119–1120.
7. Swinnerton, J. W.; Linnenbom, V. J. "Determination of C_1 to C_4 Hydrocarbons in Sea Water by Gas Chromatography," *J. Gas Chromatogr.* **1967,** *5,* 570–573.
8. Koons, C. B. "Distribution of Volatile Hydrocarbons in Some Pacific Ocean Waters," *Proc.—Oil Spill Sonf. (Prev., Behav., Control, Cleanup),* Washington, DC **1977,** 589–591.
9. McAuliffe, C. D. "Solubility in Water of Normal C_9 and C_{10} Alkane Hydrocarbons," *Science* **1969,** *158,* 478–479.
10. McAuliffe, C. D. "GC Determination of Solutes by Multiple-Phase Equilibration," *Chem. Technol.* **1971,** *1,* 46–51.
11. Wagner, P. D.; Naumann, P. F.; Laravuso, R. B. "Simultaneous Measurement of Eight Foreign Gases in Blood by Gas Chromatography," *J. Appl. Physiol.* **1974,** *36,* 600–605.
12. Warner, J. C. Battelle, Columbus, Ohio, personal communication, 1978.
13. McAuliffe, C. D. "Solubility in Water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons," *J. Phys. Chem.* **1966,** *10,* 1267–1275.
14. Price, L. C. "Aqueous Solubility of Petroleum as Applied to Its Origin and Primary Migration," *Bull. Am. Assoc. Pet. Geol.* **1976,** *60,* 213–244.
15. Wasik, S. P.; Brown, R. L. "Determination of Hydrocarbon Solubility in Sea Water and the Analysis of Hydrocarbons in Water-Extracts," *Proc. Jt. Conf. Prev. Control Oil Spills,* Washington, DC, **1973,** 223–227.
16. Anderson, J. W.; Neff, J. M.; Cox, B. A.; Tatem, H. E.; Hightower, G. M. "Characteristics of Dispersion and Water-Soluble Extracts of Crude and Refined Oils and Their Toxicity to Esturine Crustaceans and Fish," *Mar. Biol.* **1974,** *27,* 75–88.
17. McAuliffe, C. D. "Evaporation and Solution of C_2 to C_{10} Hydrocarbons from Crude Oils on the Sea Surface," in "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms"; Wolfe, D. A., Ed.; Pergamon: New York, 1977, p. 263–372.
18. Rice, S. D.; Short, J. W.; Brodersen, C. C.; Mechlenburg, T. A.; Moles, D. A.; Misch, C. J.; Cheatham, D. L.; Karinen, J. F. "Acute Toxicity and Uptake–Depuration Studies with Cook Inlet Crude Oil, Prudhoe Bay Crude Oil, No. 2 Fuel Oil and Several Subarctic Marine Organisms," Northwest Fisheries Center Auke Bay Laboratory Report, May 1976.
19. Kinney, P. J.; Schell, D. M.; Button, D. K. University of Alaska, 1970, "Analytical Detection of Hydrocarbon Pollution in Alaska's Cook Inlet," Institute of Marine Science Report R-69-16.
20. Koons, C. B.; Brandon, D. E. "Hydrocarbons in Water and Sediment Samples from Coal Oil Point Area, Offshore California," in "Proceedings 1975 Offshore Technology Conference, Volume III," **1975,** 3, 513–521.
21. McAuliffe, C. D.; Smalley, A. E.; Groover, R. D.; Welsh, W. M.; Pickle, W. S.; Jones, G. E. "Chevron Main Pass Block 41 Oil Spill: Chemical and Biological Investigations," *Proc.—Conf. Prev. Control Oil Pollut.,* Washington, DC **1975,** 555–566.
22. Brown, E., Dow Chemical U.S.A., personal communication.
23. Mungall, T. G., Ethyl Corporation, personal communication, 1978.
24. Umbreit, G. R., Greenwood Laboratories, personal communication, 1978.
25. McAuliffe, C. D., unpublished data.

26. Carlisle, C. T.; Bayliss, G. S.; VanDelinder, D. G. "Distribution of Light Hydrocarbons in Seafloor Sediments: Correlations between Geochemistry, Seismic Structure, and Possible Reservoired Oil and Gas," in "Proceedings 1975 Offshore Technology Conference, Volume III, 65–70.
27. Hunt, J. M. "Origins of Gasoline Range Alkanes in the Deep Sea," *Nature* **1975**, *254*, 411–413.
28. Wagner, P. D.; Saltzman, H. A.; West, J. B. "Measurement of Continuous Distributions of Ventilation-Perfusion Ratios: Theory," *J. Appl. Physiol.* **1974**, *36*, 588–599.
29. Baker, E. G. "A Geochemical Evaluation of Petroleum Migration and Accumulation," in "Fundamental Aspects of Petroleum Geochemistry"; Elsevier: New York, 1967; 299–329.
30. Eganhouse, R. P.; Calder, J. A. "The Solubility of Medium Molecular Weight Aromatic Hydrocarbons and the Effects of Hydrocarbon Co-Solutes and Salinity," *Geochim. Cosmochim. Acta* **1976**, *40*, 555–561.
31. MacKey, D.; Shiu, W. Y. "Aqueous Solubility of Poly-Nuclear Aromatic Hydrocarbons," *J. Chem. Eng. Data* **1977**, *22*, 399–402.
32. McAuliffe, C. D. "Solubility in Water of Paraffin, Cyclo-Paraffin, Olefin, Acetylene, Cyclo-Olefin, and Aromatic Hydrocarbons," *J. Phys. Chem.* **1966**, *70*, 1267–1275.
33. Sutton, C.; Calder, J. A. "Solubility of Higher-Molecular-Weight *n*-Paraffins in Distilled Water and Seawater," *Environ. Sci. Technol.* **1974**, *8*, 654–657.
34. Sutton, C.; Calder, J. A. "Solubility of Alkylbenzenes in Distilled Water and Seawater at 25.0°C," *J. Chem. Eng. Data* **1975**, *20*, 320–322.
35. Schwartz, F. P.; Wasik, S. P. "Fluorescence Measurement of Benzene, Naphthalene, Anthracene, Pyrene, Fluoranthene, and Benzo(e)pyrene in Water," *Anal. Chem.* **1976**, *48*, 524–527.
36. Wauchope, R. D.; Getzen, F. W. "Temperature Dependence of Solubilities in Water and Heats of Fusion of Solid Aromatic Hydrocarbons," *J. Chem. Eng. Data* **1972**, *17*, 38–41.

RECEIVED October 12, 1978.

9

The Role of Nonhydrocarbons in the Analysis of Virgin and Biodegraded Petroleum

D. M. JEWELL

Gulf Research & Development Company, P.O. Drawer 2038, Pittsburgh, PA 15230

Nonhydrocarbons vary from trace amounts to as much as 75% of certain crude oils. Biological reactions with petroleum not only alter the nonhydrocarbons naturally present but always form new oxygen-containing species from the basic hydrocarbon framework. Selective isolation and characterization techniques that have been used successfully in this field are demonstrated. Experimental evidence for the presence of amides, esters, and carboxyl groups on "neutral nitrogen compounds" is presented. The isolation of structural isomers of both oxygen (ketones) and nitrogen (diaza-) compounds having a molecular weight of 180 is discussed. The fact that structural isomers involving CH, CHO, CHS, and CHN are all present at many nominal molecular weights implies that no single analytical technique should be considered sufficient evidence for any particular class of compounds.

Nonhydrocarbons have always been the enigma of petroleum chemists and engineers; for different reasons, they both ask, "What do you do with them?" The emphasis of each usually has been limited to certain hydrocarbon species. While it is correct to emphasize the major components in petroleum, it is misleading to limit analysis to them. This is because of the implication that the trace nonhydrocarbons in many (but not all) crudes can be ignored to the same degree that high percentages ($> 50\%$) of nonhydrocarbons are ignored in severely degraded crudes.

The presence of any heteroatom (N, O, S, V, Ni) in a molecule is the basis of defining nonhydrocarbons. The heteroatom frequently has a drastic influence on the reactivity of the molecule; in general, it decreases

0-8412-0475-6/80/33/219$05.00/0

the molecule's ease of catalytic reduction but increases its ease of oxidation. This latter point has significance to biodegradation. Although bacteria can affect all types of molecules in petroleum, depending on the selectivity of organisms present in any particular system, the new products are always nonhydrocarbons quite different from those present in the virgin crude. To determine the structure of nonhydrocarbons from either virgin or altered petroleum, numerous selective, microisolation, and characterization techniques are required. The purpose of this chapter is to illustrate by numerous examples that the nonhydrocarbon constituents present a unique challenge to the chemist and play a significant role in the comprehensive analysis of any virgin or degraded petroleum.

Background

The ideal approach to studying nonhydrocarbons is no different than that used on any natural product problem: isolate, purify, and characterize by spectroscopic and/or chemical reaction techniques. While the bulk concentration of nonhydrocarbons is desirable, one finds that this is impossible because of the wide range of reactivity that they exhibit for different adsorbents or reagents. The standard approach used to isolate nonhydrocarbons has been clay-type adsorbents (ASTM methods), primarily because they were simple, cheap, and had a history of process applications in actual refining operations (e.g., lube oils). Adsorbents such as clays, alumina, silica gels, and Florisil generally have the advantage of retaining polar compounds without altering the structure of either the nonadsorbed hydrocarbons or polar constituents; they have the general disadvantage of low capacity and nonselectivity.

Some of the earliest chemical studies of nonhydrocarbons, which resulted in the isolation of numerous strong acids and bases, were conducted at the University of Texas under the leadership of Lochte and Littman (*1*). This work used the classical extraction of petroleum with mineral acids and caustic followed by chemical identification (boiling point, refractive index, derivatives, etc.). Since only strong Brönsted acids and bases can be isolated by aqueous solvents, their identifications were limited to low-molecular-weight species; aqueous solvents also do not isolate weak acids or bases (low pK values).

The Bureau of Mines together with the American Petroleum Institute made concentrated efforts to study both nitrogen and sulfur compounds in domestic crudes in the 1950–1964 period (*2, 3, 4, 5*). The sulfur studies (API Report of Project 48), while limited to low-molecular-weight compounds, were the first comprehensive attempt to integrate liquid–solid and gas–liquid chromatography with selective chemical desulfurizations and mass spectrometry; more than 100 individual sulfur compounds were

identified in this effort. The nitrogen studies (API Report of Project 52) were among the first efforts to recognize that nonbasic functions were major constituents and that nonaqueous reagents were necessary approaches for the isolation and the analysis of these types. They thus were able to identify the carbazoles as a major compound type and various amides/lactams as probable types.

A fundamental study of surface reactions of many organic structures and various adsorbents (e.g., aluminas, silicas) was done by L. R. Snyder in the 1958–1968 period. This provided an excellent evaluation of the relative elution of both hydrocarbons and nonhydrocarbons in numerous chromatographic systems. These studies were a necessary ingredient in the LEAC technique that he utilized on petroleum products. These studies indicated that carbazoles and benzocarbazoles are major nonhydrocarbon types in petroleum (*6*). With the advent of many selective isolation techniques for nonhydrocarbons since 1965 and high-pressure liquid chromatography technology, the correlations that he developed should now find numerous applications.

The work of Seiffert in recent years was specifically oriented toward the role that oxygen compounds play in affecting the surface activity of petroleum (*7, 8*). His studies were innovative in using classical chemical reactions on a microscale, and they illustrated the immense complexity of one particular class of compounds, carboxylic acids. These studies provided the first firm evidence for the steroid ring system in nonhydrocarbons, which is quite valuable to the geochemist.

The studies of Copelin (*9*) provided the first unequivocal isolation of the quinolone-type compounds. Although quinolones were isolated as a base (via HCl salt), these compounds are excellent examples of amphoterism; they can be titrated (nonaqueous) as either acid or base and isolated with either anion or cation exchange resins.

The early studies of Hartung, Jewell et al. (*10, 11, 12, 13*) identified numerous nitrogen, oxygen, and sulfur compounds or compound types that are present in either virgin petroleum or petroleum products and suggested several novel approaches for their isolation. These and subsequent studies have demonstrated: the immense complexity of any structural type; the presence of isomers within any structural type and between different types; the necessity of selective isolation and characterization techniques for any particular nonhydrocarbon type to make unequivocal structure assignments; and the vast amount of work needed to be done, especially in the high-molecular-weight portion (residuals) of petroleum. These points are particularly relevant to biodegraded petroleum because, to the author's knowledge, no systematic study of the nonhydrocarbons in a biodegraded petroleum has ever been made; and the compounds remaining from severe biodegradation will be high

molecular weight and multifunctional, with oxygen as the prevalent heteroatom. The following sections will illustrate some of our recent observations on nonhydrocarbons, and oxygen functions in particular, that we believe are relevant to this symposium.

Recent Studies of Nonhydrocarbons

Amides and Esters in Petroleum. Since the amide and lactam structures are very common in natural products, their presence in virgin petroleum is not unexpected. The isolation of quinolones, which are also present in natural products, was the first solid evidence for this functional group. Since carbonyl groups are frequently found in concentrates of nitrogen compounds (*13*), the amide group may be suspected, as well as esters and ketones. The specific approaches for isolating and charcterizing amides involved: bulk concentration on basic alumina followed by further separation on cation exchange resin [Dowex 50 W]; hydrolysis with KOH; gas–liquid chromatography of isolated free carboxylic acid; and elemental and spectroscopic [infrared (IR), ultraviolet (UV), and mass] analysis of all steps and fractions. Figure 1 shows representative IR spectra of isolated species; Figure 2 shows the UV spectra of chromatographically pure carboxylic acids. Cuts 1–4 are alkylated benzoic acids, while Cuts 5 and 6 represent phenylacetic and hydrocinamic acids (all corroborated by mass spectrometry). On the basis of the identified carboxylic acids, we can conclude that amides are definitely present in virgin crude oils. To date, those identified appear to be alkylated homologues of two types—the benzamide type in which the amide moiety is attached directly to the aromatic ring, and phenylacetamide and hydrocinamide in which the aromatic ring is separated from the amide group by methylene groups. No evidence has been obtained for secondary or tertiary amides. The primary amides isolated in these studies have been from both virgin middle distillates as well as their hydrogenated products, which indicate the resistance of these amides to commercial catalytic treatment.

The early publications by Russian investigators (*15*) disclosed the belief that the neutral nitrogen compounds of crude oils are essentially tertiary amides. This deduction was based solely on two observations: the nonbasic nitrogen compounds are reduced quantitatively to "bases" with $LiAlH_4$; and these "bases" are potentiometrically titrated at the same half-wave potential region as quinolines or aromatic amines. This technique for classifying nitrogen types was further expanded by the Bureau of Mines investigations in 1965 (*5*). No attempts were made by these groups to isolate the individual neutral compounds, to study exhaustively their reactions with $LiAlH_4$, or to study the new "bases" in

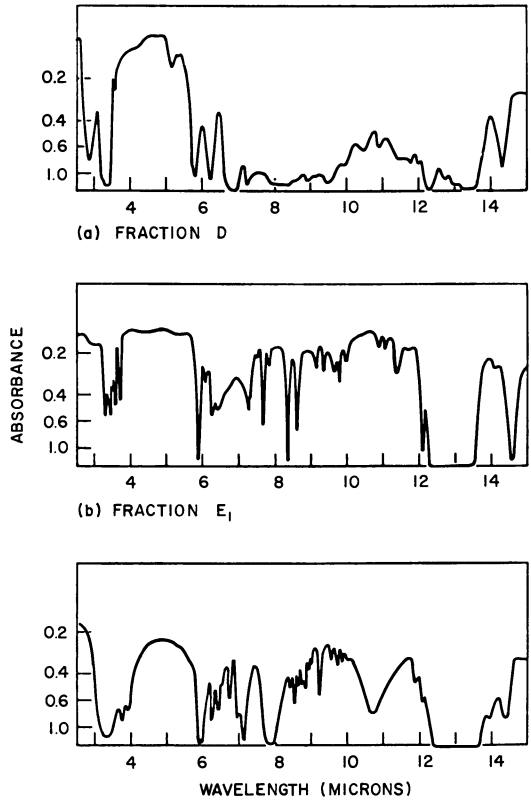


Figure 1. IR spectra of: (a) Fraction D, material isolated by cation exchange; (b) Fraction E_1, nonhydrolyzable portion of Fraction D; (c) Fraction E_2, acidic artifacts formed by hydrolysis of Fraction D.

detail. Although studies from our laboratory have confirmed the presence of benzamides (as stated earlier), results have also indicated that the precursors of new "bases" need not be limited to the amide structure. This is particularly true for the high-molecular-weight components.

An early study (*13*) was made of the basic nitrogen compounds present in a virgin Kuwait vacuum gas oil. The nonbasic nitrogen has also been studied by various approaches including that shown in Figure 3. Two separate approaches provide similar concentrates of new "bases," which were separated and studied by the same techniques as in (*13*). These studies revealed the predominance of quinolines, benzoquinolines, and cycloalkylbenzoquinolines, which are the same major structures found in "natural bases." It is apparent that the mixture of "bases" is quite complex, although the heterocyclic system is predominantly a "pyridine." No evidence for free amino groups (Hinsberg test) was found.

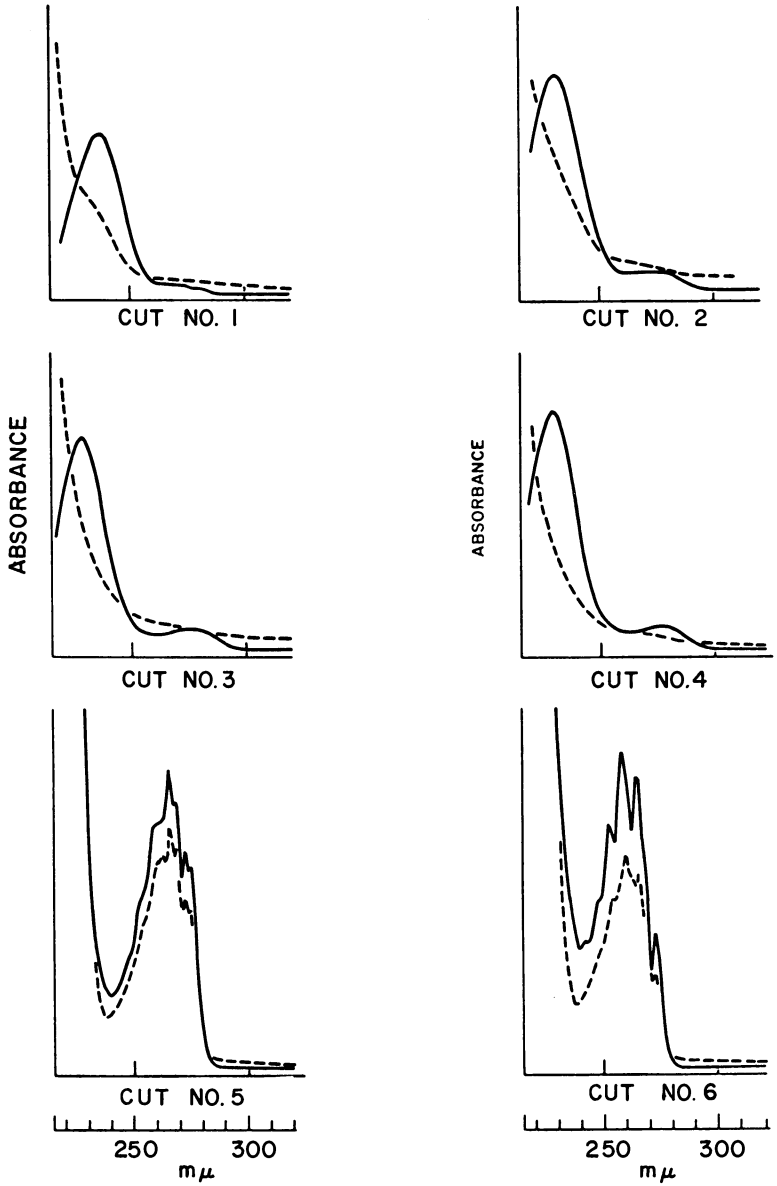


Figure 2. UV spectra of the gas chromatographically separated carboxylic acids (Fraction E_2) (——) in methanol and (– – –) in methanol HCl

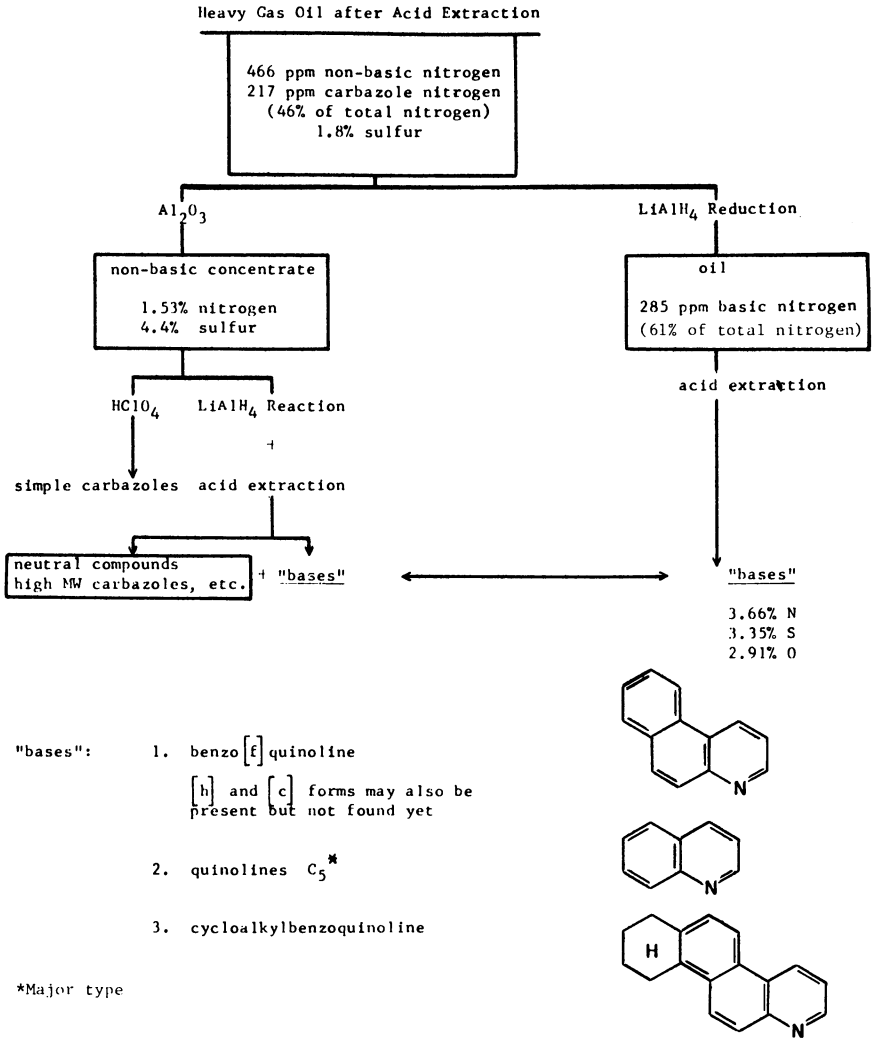


Figure 3. Reduction of neutral compounds in Kuwait gas oil with $LiAlH_4$

Figure 3 indicates that all the noncarbazolic nitrogen compounds are reduced with $LiAlH_4$. Therefore, chemical and spectroscopic evidence has been obtained to support the claim that the new "bases" are tertiary aromatic amines. However, these observations raise several questions: How do various carbonyl-functional groups react with $LiAlH_4$? What is the influence of a heterocyclic nitrogen on (1)? What is the influence of various carbonyl groups on the basicity of heterocyclic nitrogen (pyridine rings)? Are amides the true precursors of "bases?" What are some precursors other than amides?

The general behavior of most common carbonyl-containing compounds towards $LiAlH_4$ is known. A comprehensive study of the reduction of carbonyl-containing heterocyclics is not available for all systems. However, Treibs, in his porphyrin chemistry studies, has evaluated these reactions on the pyrrole systems (*16*). Table I summarizes these studies. An examination of this work discloses the complete reduction of the carbonyl group to the methylene stage, in most examples, rather than stopping at the carbinol stage. The presence of an adjacent electrophillic heterocyclic system apparently weakens the C=O metal bonds to enhance C—O cleavage. From these studies, one could speculate on the presence of such functional groups (R′ and R″) in petroleum; the products of these reductions are still nonbasic through the pyrrole function. If the ring were six-membered, "basic" pyridine or quinolines would result.

We have extended these studies slightly as shown in Figure 4 to include compounds specifically related to this problem. Each compound was chosen to demonstrate a specific point. All reactions were done in a fivefold excess of $LiAlH_4$ in refluxing diethyl ether for 72 hr to ensure complete reaction and simulate the conditions used for petroleum concentrates (Figure 3).

Reactions 1 and 2 (*see* Figure 4) demonstrate the quantitative reduction of common secondary amides without any bond cleavage or cyclization as possible side reactions.

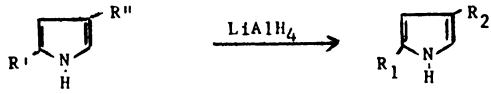
2-Hydroxyquinoline (α-quinolone) is the most likely amide precursor of quinolines known to be present in petroleum. Although they are weak bases (and weak acids), it is conceivable that higher homologues would remain in oils after aqueous acid extraction and appear to be "neutral" compounds. Performing Reaction 2 (*see* Figure 4) for long reaction times (100 hr) did not reveal any appreciable reductions to quinoline.

Reaction 4 (*see* Figure 4) illustrates the usual reduction of a carboxyl group; it is unlikely that further reduction would occur by replacing the benzene ring with pyridine or quinoline (*see* Table I, Reactions 2 and 8).

Reactions 5 and 8 (*see* Figure 4) demonstrate the difficulty in achieving complete reduction of the carbonyl group when two or more carbonyl groups are present. These products are very unstable in air, preventing accurate structural analyses.

Finally, Reactions 9, 10, and 11 (*see* Figure 4) illustrate the reduction of carboxyl and ester groups on a quinoline system. These groups are reduced in high yields to the basic quinoline; some C—C cleavage is also observed, as found earlier (*17*). In our opinion, Compounds 9 and 10 represent the most likely precursors of "quinolines" present in the neutral fractions of the heavy gas oils and residuals. The basicity of the heterocyclic N is sharply reduced by the α-substituted acid or ester, as demon-

Table I. Complete Reduction of Carbonyl Groups on Pyrroles



Reaction Number	R'	R''	R_1	R_2
1.	H	$-\overset{O}{\overset{\|}{C}}-CH_3$	H	$-CH_2CH_3$
2.	H	$-CH_2CH_2\overset{O}{\overset{\|}{C}}-OH$	H	$-CH_2CH_2CH_2OH$
3.	H	$-\overset{O}{\overset{\|}{C}}-OCH_3$	H	CH_3
4.	$-\overset{O}{\overset{\|}{C}}-OC_2H_5$	$-C_2H_5$	$-CH_3$	C_2H_5
5.	$-\overset{O}{\overset{\|}{C}}-OH$	$\overset{O}{\overset{\|}{C}}-CH_3$	$-CH_3$	$-CH_2CH_3$
6.	$-\overset{O}{\overset{\|}{C}}-OH$	$-\overset{O}{\overset{\|}{C}}H$	$-CH_3$	$-CH_3$
7.	$-\overset{O}{\overset{\|}{C}}-OH$	$-\overset{O}{\overset{\|}{C}}-OH$	$-CH_3$	$-CH_3$
8.	$-\overset{O}{\overset{\|}{C}}-OC_2H_5$	$-CH_2CH_2\overset{O}{\overset{\|}{C}}-OH$	$-\overset{O}{\overset{\|}{C}}-OC_2H_5$	$-CH_2CH_2CH_2OH$
9.	$-\overset{O}{\overset{\|}{C}}-OC_2H_5$	$-\overset{O}{\overset{\|}{C}}-C_3H_7$	$-\overset{O}{\overset{\|}{C}}-OC_2H_5$	$-CH_2-C_3H_7$
10.	$-\overset{O}{\overset{\|}{C}}CH_3$	$-\overset{O}{\overset{\|}{C}}CH_3$	$-C_2H_5$	$-C_2H_5$
11.	H	$-\overset{O}{\overset{\|}{C}}-C_6H_5$	H	$-CH_2C_6H_5$
12.	$-\overset{O}{\overset{\|}{C}}CH_2\overset{O}{\overset{\|}{C}}CH_3$	H	$-\overset{O}{\overset{\|}{C}}CH_2CH_2CH_3$	H
13.	$-\overset{O}{\overset{\|}{C}}H$	H	$-CH_3$	H

strated by their low solubliity in dilute mineral acids. This neutralizing effect diminishes rapidly as the substituents move away from the nitrogen; Compound 11 is a very strong base.

The reactions illustrated in Figure 4 serve as a guide in the search for better techniques for isolating and studying the nonbasic nitrogen compounds in heavy oils or residuals. Further reactions with model compounds may be worthwhile in an effort to deduce the structures of isolated compounds; a microsaponification reaction is one example. The

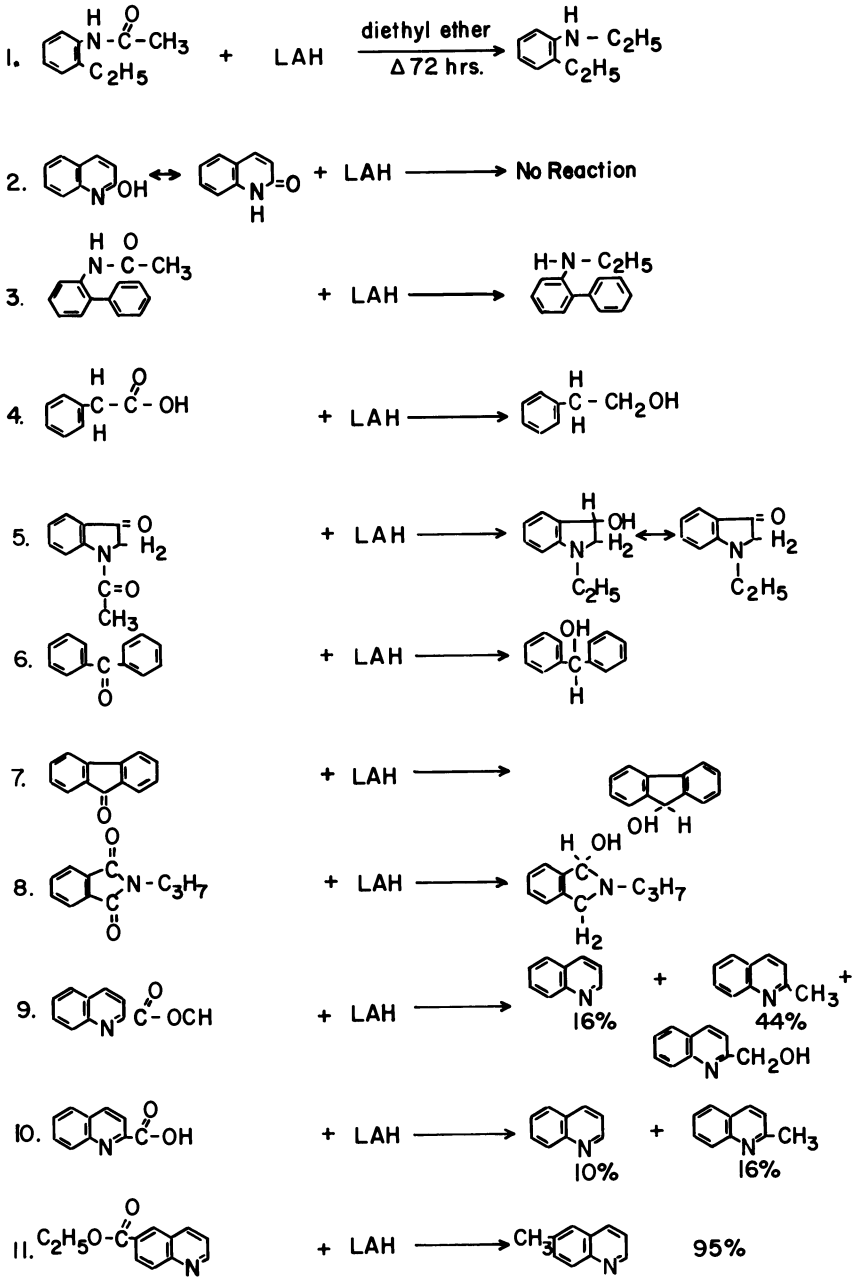


Figure 4. Reaction of model compounds with $LiAlH_4$

α-esters of quinoline are also severely hydrolyzed upon percolation through basic Al_2O_3, indicating that a neutral adsorbent is necessary for isolating these compounds. Some of our studies in this area utilized acidic alumina and preparative thin-layer chromatography to isolate several fractions of polyfunctional esters or amides. Figure 5 is a composite of several UV spectra of these fractions, together with that of 2-methylcarboxyquinoline. The compounds are highly alkylated (MW 300–500) and yield very weak absorption patterns. Figure 6 illustrates the IR spectra of these fractions. Again, many similarities are obvious, with the strong carbonyl band near 5.8 μm in each. Finally, when these thin-layer-chromatography zones are reduced with $LiAlH_4$, we observe complete reduction to the methylene stage. In one fraction, hexyl- and heptylalcohol were isolated. Another fraction yielded *n*-propanol upon saponification. The primary products from $LiAlH_4$ reaction were found to be quinolines and benzoquinolines similar to those naturally present in the strong-base fractions. Collectively, these data are excellent evidence for the presence of heterocyclic esters in petroleum.

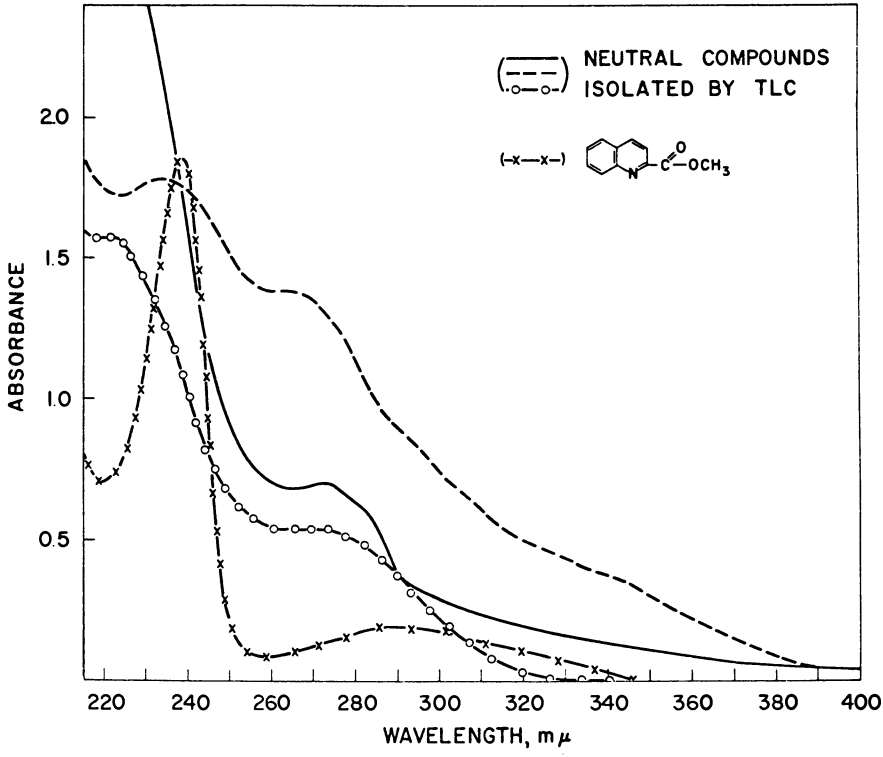


Figure 5. UV spectra of isolated nonbasic compounds and 2-methylcarboxyquinoline in CH_3OH

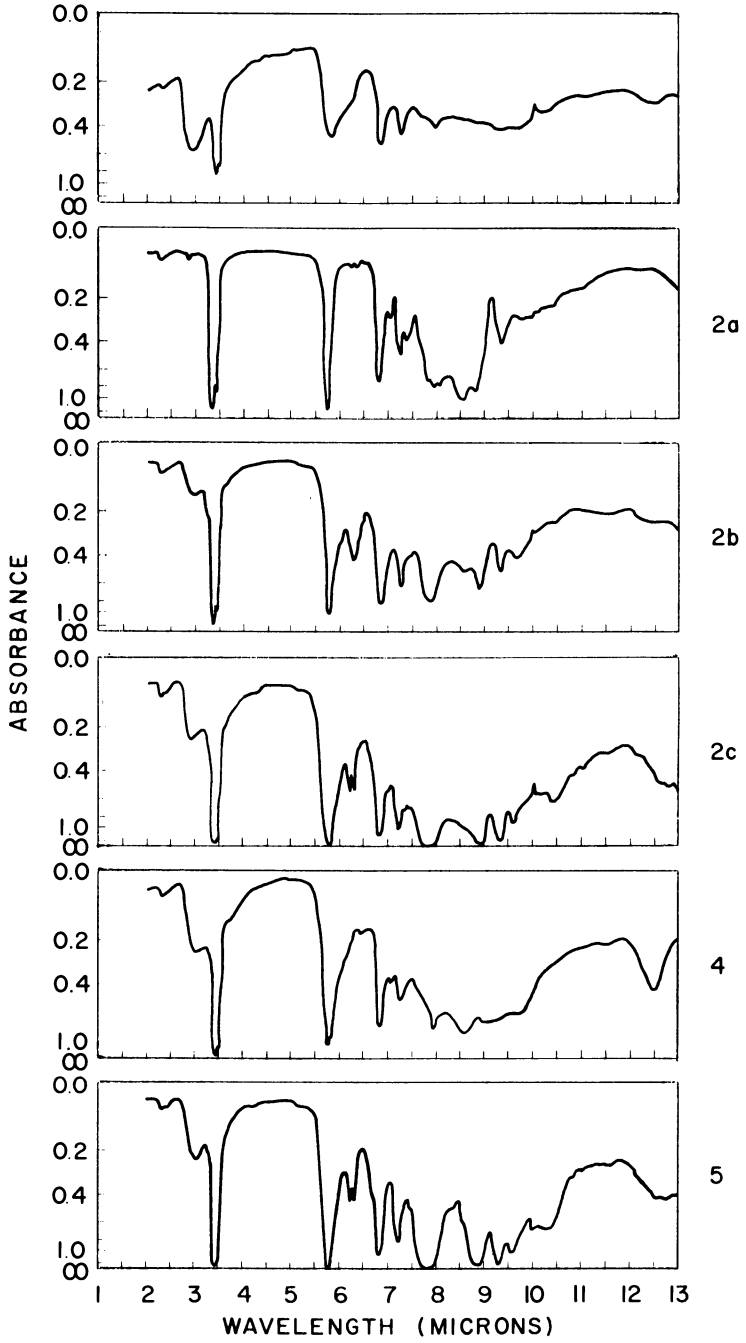


Figure 6. IR spectra of isolated nonbasic compounds

Isomers of Nitrogen and Oxygen Compounds in Petroleum. The study of nonhydrocarbons should and will continue to reveal unique structures within the same crude, as the studies from this laboratory demonstrate (*11, 12, 13*). The presence of isomers is a further complication to consider. Diaza compounds are present as basic and nonbasic forms in Kuwait crude oil; these are phenazines and phenanthrolines, respectively. The phenazines were isolated by means of their perchlorate salts. Application of this procedure (*14*) to numerous petroleum distillates resulted in the isolation of a salt of a new aromatic ketone, perinaphthenone. This compound was well studied years ago in the natural products laboratory of Fieser (*18*) and this ring system should be expected in petroleum. Figure 7 shows the UV and IR spectra of both

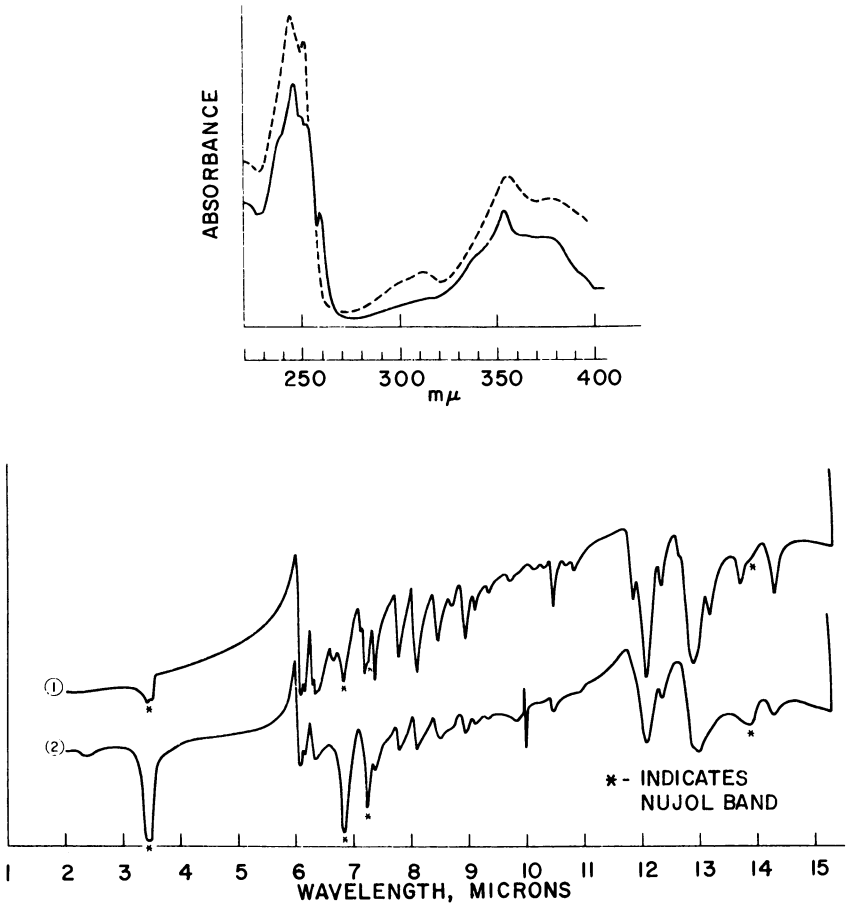
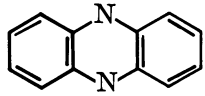
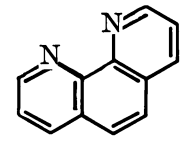


Figure 7. (Top) *UV spectra (——) of isolated oxygen compound and (– – –) of pure perinaphthenone in methanol;* (bottom) *IR spectra of (1) isolated oxygen compound and (2) of pure perinaphthenone in Nujol mull.*

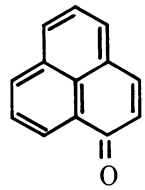
isolated and pure ketone (MS, mixed mp, and NMR are also identical). Besides the novelty of this structure to petroleum, it should be noted that this compound is a structural isomer of the 9-fluorenone previously isolated from petroleum (*19*) and the diaza compounds previously mentioned. The parent structures of each have a molecular weight of 180. These four known isomers have the following structures:



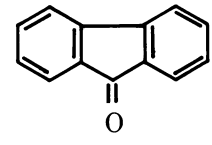
phenazine



phenanthroline



perinaphthenone



fluorenone

In our opinion, the isolation of significantly different structures at such a low molecular weight is confirming evidence for the true complexity of petroleum, the need for predictive characterization techniques, and the need for extreme caution in interpreting data from any one particular analytical method (e.g., GLC, MS).

Sulfur Compounds. Sulfur compounds are the largest class of nonhydrocarbons in petroleum. When the elemental sulfur content reaches 5–6 wt %, as is the case for certain residuals and tar sands bitumen, this implies that over 50% of the molecules are actually nonhydrocarbons. Considering these facts, structural studies have been successful only on the low-molecular-weight components containing only C, H, and S. The most intensive of these studies was conducted by the API Project 48 from which more than 100 compounds were identified (*2*). The highest-molecular-weight sulfur compounds positively identified from petroleum were tetramethyl dibenzothiophenes. These types of compounds, which contain only C, H, S, are present in "aromatic" portions of residuals and, as shown earlier for Kuwait crudes, may account for only 50% of the total sulfur (*20, 22*). In the case of certain tar sands bitumen (Alberta), these simple sulfur structures may be even less than 50%. This implies that a vast amount of work remains to be done in understanding how sulfur is present with nitrogen and oxygen compounds in resins and asphaltenes.

Although the thiophene system is certainly one important form of sulfur in petroleum, our studies have indicated that it is grossly misleading to assume that these are the only structures to consider (*21*).

The single most important property of sulfur compounds is their susceptibility to oxidants. They are particularly reactive to peroxide or hydroperoxides, a fact commonly used in analysis (*22*). Since biodegradation may well generate these oxidants, the sulfur functions could then be converted to sulfoxides–sulfones and eventually to sulfonic acids. These new functional groups would affect the distribution of sulfur between the water and hydrocarbon phases, as well as the distribution among the aromatic resin and asphaltene fractions. To our knowledge, a systematic study of the role that sulfur (and naturally occurring nitrogen or oxygen) compounds play in any biodegradation of petroleum has not been made.

Nonhydrocarbons in Biological Processes. A variety of biological reactions were done on South Louisiana and Kuwait crude oils in the past five years (*23, 24*). By following the variation of compound classes with reaction time, these studies indicated the gross changes that occur. A significant amount of information on the structural selectivity for biological attack on the hydrocarbon structures also was obtained (*25*). In general, petroleum must be converted to nonhydrocarbons (resins and/asphaltenes) before eventually becoming water-soluble acids. To date, specific studies on these nonhydrocarbon intermediates in the degradation process have not been made but are badly needed. These should include the role played by naturally occurring nitrogen, oxygen, and sulfur compounds in controlling their competition with hydrocarbons for attack by the various species such as light, O_2, H_2O, and bacteria.

Conclusions

The foregoing discussion has attempted to emphasize that nonhydrocarbons are or can be major constituents of petroleum and their concentration always increases, at the expense of hydrocarbons, with biodegradation. A reasonable amount of structural information exists in the literature for nonhydrocarbons in several virgin crude oils. Essentially no information has been published on biodegraded crudes. An effort is badly needed to determine both weathering and biological effects on naturally present nonhydrocarbons and hydrocarbons (independently) and the synergism, if any, among compound type during biodegradation.

Literature Cited

1. Lochte, H. L.; Littmann, E. R. "The Petroleum Acids and Bases"; Chemical Publishing Company: 1955.
2. Rall, H. T., et al. *U.S. Bur. Mines, Bull.* **1972**, *659*.
3. Ball, J. S.; Rall, H. T. *Proc. Am. Pet. Inst. Sect. 3* **1962**, *42*, 128.

4. Helm, R. V.; Latham, D. R.; Ferrin, C. R.; Ball, J. S. *Anal. Chem.* **1960,** *32,* 1765.
5. Okuno, I.; Latham, D. R.; Haines, W. E. *Anal. Chem.* **1965,** *37,* 54.
6. Snyder, L. R.; Buell, B. E. *Anal. Chem.* **1968,** *40,* 1295.
7. Seiffert, W. K. *Anal. Chem.* **1969,** *41,* 554.
8. Ibid., p. 562.
9. Copelin, E. C. *Anal. Chem.* **1964,** *36,* 2274.
10. Hartung, G. K.; Jewell, D. M.; Larson, O. A.; Flinn, R. A. *J. Chem. Eng. Data* **1961,** *6,* 477.
11. Hartung, G. K.; Jewell, D. M. *Anal. Chim. Acta.* **1962,** *26,* 514.
12. Ibid., *27,* 219.
13. Jewell, D. M.; Hartung, G. K. *J. Chem. Eng. Data* **1964,** *9,* 297.
14. Yevich, J. P.; Jewell, D. M., unpublished data.
15. Bezinger, N. N.; Abdurakhmanov, M. A.; Galpern, G. D. *Pet. Chem. USSR (Engl. Transl.)* **1962,** *1,* 13.
16. Treibs, A.; Derra-Scherer, H. *Justus Liebigs Ann. Chem.* **1954,** *589,* 188.
17. Micovic, V. M.; Michailovic, M. L. *Recl. Trav. Chim. Pays-Bas* **1952,** *71,* 970.
18. Fieser, L. F.; Newton, L. W. *J. Am. Chem. Soc.* **1942,** *64,* 917.
19. Latham, D. R.; Ferrin, C. R.; Ball, J. S. *Prepr., Div. Pet. Chem., Am. Chem. Soc.* **1961,** *6*(3), B67.
20. Jewell, D. M.; Ruberto, R. G.; Albaugh, E. W.; Query, R. C. *Ind. Eng. Chem., Fundam.* **1976,** *15*(3), 206.
21. Jewell, D. M.; Ruberto, R. G.; Swansinger, J. T. *Prepr., Div. Pet. Chem., Am. Chem. Soc.* **1975,** *20*(1), 19.
22. Drushel, H. V.; Sommers, A. L. *Anal. Chem.* **1967,** *39,* 1819.
23. Walker, J. D.; Colwell, R. R.; Petrakis, L. *Appl. Microbiol.* **1975,** *30,* 79.
24. Ibid., p. 1036.
25. Petrakis, L.; Jewell, D. M.; Walker, J. D., presented at the 174th National Meeting of the American Chemical Society, Chicago, IL, September 1977.

RECEIVED October 31, 1978.

10

Application of Trace Analytical Techniques to a Study of Hydrocarbon Composition Upon Dispersion of Petroleum in a Flowing Seawater System

ROGER M. BEAN, J. W. BLAYLOCK, and ROBERT G. RILEY

Battelle, Pacific Northwest Laboratory, Battelle Boulevard, Richland, WA 99352

A combination of analytical techniques has been used to determine the concentrations of hydrocarbons in very dilute dispersions of crude oil in flowing seawater. The volatile aromatic components were determined directly from seawater samples using a helium equilibration gas chromatographic technique. Less volatile hydrocarbons were sampled by pumping the seawater through columns of macroreticular resin using positive displacement pumps and analyzed by capillary gas chromatography. The results show that the lower-molecular-weight aromatic hydrocarbons are present in the seawater dispersions in much higher concentrations than would be predicted from their concentration in the crude oil. These studies demonstrate that detailed compositional analysis of systems used for oil toxicity studies is a prerequisite for interpretation of observed biological effects.

The scientific studies that followed a spill of fuel oil off West Falmouth, Massachusetts, in 1969 clearly showed that petroleum and its refined products could act as a chemical poison to a wide variety of commercially important sea life (*1*). One of the first attempts to systematically quantify the toxicity of oil to marine animals was conducted at Battelle, Pacific Northwest Laboratories (*2*), and it was followed by other investigations in which a number of test conditions were used. These early studies quickly showed that the experimental method used

0-8412-0475-6/80/33/235$05.00/0

to determine the toxicity of oils could have a profound effect upon the result obtained; Vanderhorst (*3*), for instance, pointed out that the reported LD50 to shrimp of No. 2 fuel oil has varied from 1.5 to 50 mg/L. Thus, there was an obvious need to understand the physical and chemical properties of oil-in-water dispersions so that an understanding of the dosimetry in toxicity measurements could be applied to an interpretation of the results.

Early studies of the hydrocarbon composition of seawater-saturated petroleum products (*4, 5, 6, 7*) demonstrated that there are large numbers of hydrocarbon constituents present in the dispersions. These studies also showed that the contribution of many of the individual hydrocarbon components in the water to the total hydrocarbon found was substantially different from their contribution to the original oil. In addition, investigations (*5, 8, 9*) have pointed to a complex physical situation in the dispersions in which the hydrocarbon constituents can exist in true solution as discrete oil particles or in an "accommodated" state (*10*) as hydrated molecular aggregates. It has been suggested that many of the hydrocarbon components can exist in equilibrium among the phases (*9, 11*).

The analytical chemistry applied to the study of oil-in-water systems used for toxicity testing has pursued two major goals that tend to be mutually exclusive. First, there is a need for rapid and relatively inexpensive analytical methods that can be repeated frequently enough to permit the biological investigator to determine the variability of exposure throughout the duration of the test. Second, there is a need for detailed compositional data so that the toxic effects can be placed in context with actual environmental situations. By far the most commonly employed method to accomplish the first goal is that of "total oil" determination by infrared (IR) absorbance (*12, 13*). This method, which involves extracting the oil with carbon tetrachloride and measuring the IR absorbance at 2927 cm^{-1}, has been applied to the analysis of environmental samples (*14*) as well as to samples derived from toxicity studies (*2, 3, 4, 6, 9*). Detailed compositional studies using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) have generally been applied to solvent extracts of oil–water suspensions (*4, 5, 6, 7*). A rapid method of determining the light hydrocarbon solutes in solvent extracts of toxicity test water has been developed (*15*) as a compromise between the desire for rapidity and analytical detail. However, the method can only be applied to studies in which oil concentrations are acutely toxic, that is, when concentrations of individual aromatic hydrocarbon components are in the mg/L range.

Marine biology studies at Battelle's Marine Research Laboratory at Sequim, Washington, have been directed toward understanding long-term effects of petroleum hydrocarbons on marine ecosystems and asso-

ciated organisms. To this end, studies have considered the toxicological and ecological effects of petroleum in sediments and water in concentrations considerably below those that can cause acute toxic (lethal) effects (*9*). As a part of these long-term studies, experiments are being conducted over periods of many months in flowing seawater systems (*16*) containing total hydrocarbon concentrations on the order of 1 mg/L or less. At these concentrations, many of the individual petroleum components of interest are in concentrations of less than 1 μg/L. These concentration levels of hydrocarbons present problems to the analytical chemist in representative sampling and with method sensitivity. The methodologies described herein were adopted in an attempt to circumvent these analytical problems and to develop a reasonably accurate determination of the important component composition of these very dilute oil-in-water suspensions. The methods also may be useful in environmental oil pollution research involving studies of hydrocarbon concentrations in natural waters.

Experimental

Solvents and Reagents. All solvents used in this study were Burdick and Jackson "Distilled in Glass." The diethyl ether used was ethanol-free, available from B & J on special order. The XAD-2 resins used for sampling the seawater suspensions were purchased from Supelco, Inc., and cleaned by soxhlet extraction according to the method of Junk et al. (*17*).

Seawater Suspensions of Prudhoe Bay Crude Oil. Continuous-flow suspensions of Prudhoe Bay Crude (PBC) were generated in an experimental apparatus described previously (*16*). The initial suspensions were diluted to the desired concentrations with additional seawater and were then sent directly to exposure tanks. In some cases, the initial oil suspension was filtered through 100-μm polypropylene filters prior to use. The filtered suspensions were necessary to avoid buildup of oil films on the exposure apparatus in studies where simulated tidal cycles were employed.

Analysis of Seawater Suspensions for Volatile Hydrocarbons by Helium Extraction Technique. The helium extraction–gas chromatographic method for determination of volatile aromatic hydrocarbons in water has been previously described (*11*). Briefly, the method involves equilibrating the aqueous sample with an equal volume of helium in a large syringe and injecting the helium extract into the gas chromatograph via a sample loop. Two sequential equilibrations and analyses are sufficient to calculate the original concentration of hydrocarbons in the sample according to the method described by McAuliffe (*18*).

Analysis of Seawater Suspensions for Hydrocarbons by XAD-2 Extraction Technique. Stainless steel tubing 0.95 cm × 22.8 cm was packed with about 10 mL of cleaned XAD-2 resin, using silanized glass wool to plug either end. The tubes were sealed using Swagelok end plugs and stored at 4°C prior to use. The seawater suspension of PBC was sampled by forcing it through the XAD-2 column at a rate of 40 mL/min using a positive displacement pump (Lab Pump, Fluid Meter-

ing, Inc.). Only stainless steel, ceramic, and Teflon were in contact with the sample during pumping. Samples were obtained in triplicate by employing three sampling devices simultaneously. Normally, 10 L was sampled; however, for the lowest concentration, 20 L was taken. After sampling, the columns were resealed and transported to Battelle's Richland laboratories where they were again stored at 4°C prior to analysis.

The hydrocarbons were extracted by passing 100 mL diethyl ether through the columns over a period of about 15 min. The ether extracts were dried with anhydrous sodium sulfate overnight, and then they were evaporated in a conical tube under a stream of dry nitrogen to 2 mL without external heating. One mL hexane was added, and the sample evaporated to 1 mL. The extracted hydrocarbons were separated into saturate and aromatic types using a modification of a procedure described by Warner (*19*) and employed in these laboratories for sediment analysis (*20*). Fifteen grams of Davison silica gel (100–200 mesh) in a Chromaflex column were washed with 30 mL methylene chloride, then 40 mL hexane. The 1-mL sample was first eluted with 40 mL hexane to obtain the saturate fraction, and then with 86 mL 20% methylene chloride in hexane to obtain the aromatic fraction. Each fraction was evaporated to 1 mL; then 1 mL of internal standard was added and the sample reevaporated to 1 mL and analyzed by GC. A 20-μg/mL solution of 2,6,10-trimethyldodecane in hexane was used for the saturate internal standard and a solution of 20 μg/mL hexamethyl–benzene was used for the aromatics analysis.

The isolated fractions were analyzed for hydrocarbon constituents using a Hewlett-Packard 5840A gas chromatograph fitted with a conventional capillary column injection port. A 30-m $\times$ 0.25-mm OV101 column (J & W Scientific) having $N_{eff} > 90{,}000$ at 130°C, was used for the separation, at a split ratio of 10:1. Detection was by flame ionization. Temperature programming was from 60°C (4-min hold) to 250°C at 4°C/min and held for 15 min.

For one set of samples (the "unfiltered suspension"), a Varian 2800 chromatograph equipped with a 30-m SE-30 column ($N_{eff} = 60{,}000$) was used for the analysis. Initial temperature was 70°C and programming at 4°C/min was initiated upon sample injection. These conditions precluded analysis for the benzene aromatic types.

Saturated hydrocarbons were quantitated for *n*-alkanes with 8–25 carbons (C_8 to C_{25}) as well as pristane and phytane, using analytical standards. Analysis of the aromatic fraction was more complex because of the great diversity of aromatic types and isomers. A chromatogram of the aromatic fraction from a filtered seawater suspension of PBC is shown in Figure 1. Determination of the total quantity of any one aromatic structural type was made by applying a response factor derived from the average of several standard hydrocarbons of the indicated structural type to each of the peaks in the region of interest and summing the values obtained.

Analysis of PBC Oil for Water-Soluble Hydrocarbons. A solution of 40 mg PBC in 1 mL hexane was separated into saturate and aromatic fractions as described above. The saturate fraction was diluted to 50 mL with hexane and the aromatic fraction was diluted to 100 mL with 20%

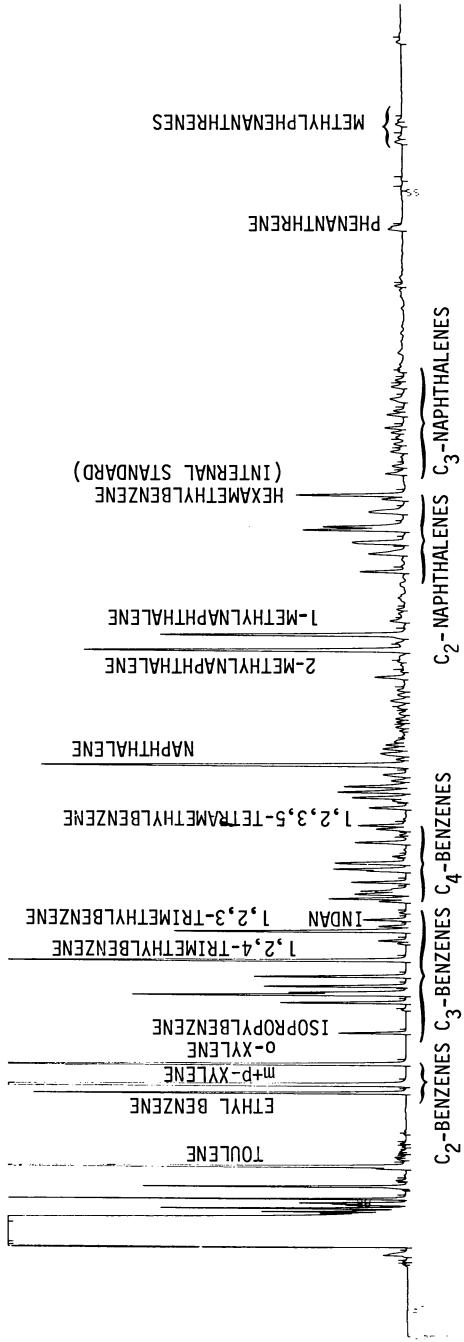


Figure 1. Capillary gas chromatogram of aromatic hydrocarbons extracted from a seawater suspension of Prudhoe Bay crude oil

methylene chloride in hexane. One mL of each fraction was taken, an equal volume of internal standard was added, and the GC analysis described above was performed.

Analysis of Seawater Suspensions for Total Oil by IR. The stainless steel XAD-2 sampling tubes described above were extracted with carbon tetrachloride until the extract contained no IR absorbance at 2927 cm^{-1}. The columns were rinsed with methanol, then distilled water, and used to sample the seawater–oil suspension as described above. After sampling, the columns were extracted with 25 mL carbon tetrachloride and the IR absorbance at 2927 cm^{-1} was measured. Oil quantitation was accomplished by referring to a calibration curve prepared from known concentrations of Prudhoe Bay Crude oil in carbon tetrachloride.

Results and Discussion

Examination of the aromatics fraction of PBC using capillary GC–MS has enabled us to assign regions of the chromatogram (Figure 1) having peaks corresponding to components with similar hydrocarbon structural type. Although the structural types indicated in the figure predominate in the regions shown, GC–MS analysis indicated the presence of other structural types in most of the regions. For instance, the C_3-naphthalene region contains peaks having mass spectra consistent with methyl biphenyls, fluorene, and methyl acenaphthenes. Also, there is overlap of component types among the C_3-, C_4-, and C_5-benzene regions. Other regions, such as those containing the dimethylfluorenes and trimethylnaphthalenes, were not included in the quantitative scheme. These simplifications were introduced to facilitate the routine use of the analytical method and to make sample intercomparison a less complex process.

The results obtained from replicate analysis of three different oil-in-water suspensions are shown in Table I. The data reported for benzene through the C_3-benzenes were generated using the helium extraction technique, while the higher-molecular-weight hydrocarbon types were determined with the XAD-2 extraction method. The higher relative standard deviations experienced with the helium extraction procedure reflect the small sample size employed (50 mL). In contrast to other sampling techniques (9), good precision was achieved with the XAD-2 technique. The standard deviations for each component type through C_3-naphthalenes were generally less than 5% of the reported value.

Although the XAD-2 extraction method gives satisfactory precision at the hydrocarbon levels studied, the accuracy of the technique is dependent on the extraction efficiency of the XAD-2 resin and the recoveries of hydrocarbon experienced during the workup procedure (the helium method does not have this shortcoming since it is a direct analysis). Therefore, several experiments were performed to obtain estimates of the recovery efficiency of the resin.

Table I. Component Analysis of Three Different Seawater Suspensions of Prudhoe Bay Crude Oil (Values in μg/L ± standard deviation)

Component	*Unfiltered Suspension*[a]	*Higher-Concentration Filtered Suspension*[a]	*Lower-Concentration Filtered Suspension*[b]
Total oil by IR	141 ± 14	320 ± 50	80
Saturates (C_8–C_{25})	2.93 ± 0.13	14.90 ± 2.67	3.87 ± 0.62
Benzene[c]	36.1 ± 5.2	389.1 ± 92.6	66.9 ± 1.2
Toluene[c]	55.5 ± 4.5	319.7 ± 35.0	74.6 ± 15.5
C_2-Benzenes[c]	44.7 ± 10.3	214.1 ± 19.3	47.2 ± 4.2
C_3-Benzenes[c]	9.2 ± 1.0	55.8 ± 7.5	16.3 ± 3.5
C_4-Benzenes[d]	Not determined	11.25 ± 0.23	1.80 ± 0.06
Naphthalene[d]	1.60 ± 0.16	6.90 ± 0.24	1.21 ± 0.09
Methylnaphthalenes[d]	2.40 ± 0.09	11.43 ± 0.29	2.00 ± 0.10
C_2-Naphthalenes[d]	1.39 ± 0.06	8.46 ± 0.17	1.54 ± 0.05
C_3-Naphthalenes	0.57 ± 0.04	3.88 ± 0.69	0.98 ± 0.02
Phenanthrene[d]	0.10 ± 0.01	0.24 ± 0.05	0.07 ± 0.01
Methylphenanthrenes[d]	0.18 ± 0.02	0.67 ± 0.06	0.11 ± 0.00
Aromatics (total)	151.7	1021.5	212.7

[a] Triplicate determinations.
[b] Duplicate determinations for helium extraction results, triplicate for column extraction results.
[c] Helium extraction method.
[d] XAD-2 method, uncorrected for procedural losses.

Total oil recoveries were estimated by sampling an unfiltered seawater suspension of oil through two XAD resin columns placed in series. The quantity of oil trapped in each column was then determined by IR absorbance. Three such experiments performed simultaneously showed that 92.4 ± 1.7% of the total oil recovered from both columns was collected on the first column. The average oil concentration in the seawater suspension was determined to be 116 ± 3 μg/L, based on the total oil recovered by both first and second columns.

Although recovery of total oil as measured by IR analysis appears to be satisfactory, the extraction efficiency of the resin columns with respect to individual components is still in question, since alkylbenzenes are relatively soluble in water (*21*) and thus might be expected to adsorb poorly on the XAD resin. Therefore, a direct comparison was made between the quantities of toluene, C_2-benzenes, and C_3-benzenes measured by both helium extraction and by XAD-2 adsorption. The results of this intermethod comparison are presented in Table II.

Procedural recoveries of toluene, *o*-xylene, and 1,3,5-trimethylbenzene during the workup procedure were determind to be 15.2 ± 0.9, 51.4 ± 1.0, and 54.7 ± 0.8%, respectively (Table III). These values were used to determine the corrected values for the XAD-2 technique reported in

Table II. Estimate of Recovery Efficiency of Lower-Molecular-Weight Hydrocarbons by Adsorption on XAD-2 Resins

	Hydrocarbon Type	*Concentration by XAD-2 Extraction*[a] *(μg/L)*	*Concentration by Helium Extraction (μg/L)*	*Calculated XAD-2 Efficiency (%)*
A. High-concentration filtered suspension	toluene	34.3	319.7	10.7
	C_2-benzenes	58.7	214.1	27.4
	C_3-benzenes	46.4	55.8	83.2
B. Low-concentration filtered suspension	toluene	5.6	74.6	7.6
	C_2-benzenes	10.5	47.2	22.2
	C_3-benzenes	8.8	16.3	54.0

[a] Corrected for procedural losses (*see* text).

Table II. An inspection of Table II reveals that neither toluene nor the C_2-benzenes are sufficiently retained on the XAD column to provide a useful analytical result.

The adsorption efficiencies increase with molecular weight in a manner that might be expected from the relative solubilities of the different compound types in water. McAuliffe (*21*) reported the solubilities of toluene, *o*-xylene, and 1,2,4-trimethylbenzene in water to be 515, 175,

Table III. Recovery of Hydrocarbons Added to the Top of XAD Column (triplicate determinations)

n-*Alkanes*	*Percent Recovery ± Standard Deviation*	*Aromatics*	*Percent Recovery ± Standard Deviation*
C_8	38.6 ± 3.2	toluene	15.22 ± 0.9
C_9	55.7 ± 4.3	*o*-xylene	51.4 ± 1.0
C_{10}	64.9 ± 5.6	1,3,5-trimethylbenzene	54.7 ± 0.8
C_{11}	61.3 ± 3.4	C_4-benzenes[a]	59.5 ± 1.6
C_{12}	61.3 ± 3.4	naphthalene	59.2 ± 1.5
C_{13}	60.6 ± 4.1	methylnaphthalenes	61.2 ± 3.1
C_{14}	61.5 ± 4.0	dimethylnaphthalenes[b]	63.2 ± 3.4
C_{15}	63.2 ± 3.7	2,3-6-trimethylnaphthalene	66.4 ± 3.6
C_{16}	64.0 ± 4.2	phenanthrene	68.3 ± 3.0
C_{17}	68.8 ± 4.1	methylphenanthrenes[c]	73.3 ± 1.7
C_{18}	74.8 ± 4.2	pyrene	81.0 ± 1.8
C_{19}	82.1 ± 4.4		
C_{20}	89.1 ± 5.5		

[a] Five C_4-benzene isomers: 1,2,4,5- and 1,2,3,5-tetramethylbenzene, 1,2-dimethyl-4-ethylbenzene, 1,3-dimethyl-5-ethylbenzene, and 1,2-diethylbenzene.
[b] 1,3-, 2,3-, and 2,6-dimethylnaphthalene.
[c] 1- and 2-methylphrenanthrene.

Table IV. Observed Recoveries of Hydrocarbon Standards from Silica Gel Chromatography–Gas Chromatography Procedure (triplicate determinations)

Component	*Percent Recovery ± Standard Deviation*
n-Alkanes	
C_{12}	90.94 ± 1.10
C_{13}	91.57 ± 0.93
C_{14}	91.29 ± 1.97
C_{15}	91.44 ± 1.42
C_{16}	91.85 ± 2.09
C_{17}	91.91 ± 3.16
C_{18}	93.41 ± 3.75
C_{19}	95.90 ± 4.30
C_{20}	97.27 ± 6.42
C_{22}	97.09 ± 4.55
C_{24}	95.51 ± 5.02
Naphthalenes	
2-methylnaphthalene	95.56 ± 4.91
1-methylnaphthalene	91.21 ± 0.50
2,6-dimethylnaphthalene	90.71 ± 3.75
1,3-dimethylnaphthalene	92.51 ± 0.15
Phenanthrene	90.82 ± 0.14

and 57 μg/g, respectively. The observed recoveries parallel the component solubilities in an inverse manner. Thus, there is sufficient agreement between the C_3-benzene results to indicate that adsorption efficiencies of the higher-molecular-weight (and less soluble) components on the XAD column are satisfactory. The low adsorption efficiencies of C_2-benzenes experienced in these studies contrast with results reported by Junk et al. (*17*), who reported 81% recovery of ethyl benzene using very similar XAD-2 methodology. The complexity of the system under study may be a contributing factor to the lower efficiencies experienced in this case.

The recovery data given in Table III show that the lower-molecular-weight hydrocarbons are lost in significant amounts, as would be expected because of their relatively high volatility. However, the recovery of higher-molecular-weight saturates as well as the recovery of naphthalene and phenanthrene hydrocarbons in these studies was somewhat lower than expected when compared with other analytical procedures involving these compounds (*19*). Thus a study of the sources of losses was made. Table IV gives the recoveries of 20-μg quantities of saturate and aromatic hydrocarbons from the silica gel procedure, including final evaporation. The data show that the silica gel procedure is not the major source of hydrocarbon loss. Extraction of XAD columns with 150 mL instead of

100 mL of ether did not increase the recoveries of naphthalenes and phenanthrenes, but resulted in reduced yields of lower-molecular-weight components. Recoveries were substantially the same whether the XAD columns used for the studies were wet with water prior to the experiment or not. Thus it is probable that the major portion of the losses are associated with either the ether drying or the ether evaporation step. It should be pointed out that the recovery experiments are not exactly analogous to what is experienced in the analytical procedure itself, since in the latter case the hydrocarbons are probably more distributed throughout the length of the column.

The studies described above give evidence that the XAD-2 method provides a useful determination of the hydrocarbon components in dilute seawater–oil suspensions. The quantity of "total oil" reported in Table I is in sharp contrast to the total hydrocarbons found in the water by the combined helium extraction/XAD extraction techniques. The discrepancy between total oil by IR and hydrocarbons found in water by component analysis was previously reported (*5, 11*) and can be explained by the low contribution to the IR absorbance at 2927 cm^{-1} of the soluble aromatic constituents relative to the saturate hydrocarbons. The difference between IR analytical result and component analysis by GC becomes much greater in the filtered systems, where the total hydrocarbons found are three times that reported by the IR method. It is clear that the IR analytical technique is only useful in systems where there is a preponderance of particulate, bituminous petroleum or where it is used as a monitoring tool. It provides no information about actual levels of hydrocarbons in systems where there is a preponderance of water-soluble aromatic compounds.

The analysis of Prudhoe Bay Crude Oil for the hydrocarbon components under study is presented in Table V, together with the percentage of the total hydrocarbons found represented by each of the hydrocarbon types. For comparison, the contribution of component types to the total hydrocarbon is listed for both a filtered and unfiltered seawater suspension. The comparison is somewhat biased because benzene was not determined in the crude oil, being poorly separated from the hexane solvent, and because C_4-benzenes were not determined in the unfiltered sample. However, it can be readily seen from the results that while aromatic hydrocarbon types are present in the crude oil in roughly equal concentrations, the preponderance of the total hydrocarbons in the seawater suspension is composed of the low-molecular-weight aromatic hydrocarbons. In both unfiltered and filtered systems, 90% of the water-soluble aromatic hydrocarbons found are composed of benzene, toluene, ethyl benzene, and the xylenes. This is in contrast to their concentration in the whole crude oil, which is at most a few percent and where their contributions to the hydrocarbons analyzed for is probably less than 30%.

Table V. Comparison of Hydrocarbon Composition of Whole Prudhoe Bay Crude Oil with That in Exposure Apparatus

	Prudhoe Bay Crude Oil		*Unfiltered Suspension*	*High-Concentration Filtered Suspension*
Component	*mg/g Oil*	*(% of total hydrocarbons found)*	*(% of total hydrocarbons found)*	*(% of total hydrocarbons found)*
Saturates (C_8–C_{25})	68.36	52.53	1.89	1.53
Benzene	[a]	[a]	23.34	37.51
Toluene	9.30	7.15	35.88	30.81
C_2-Benzenes	12.39	9.52	28.90	20.64
C_3-Benzenes	12.03	9.25	5.95	5.38
C_4-Benzenes	7.48	5.75	[a]	1.08
Naphthalene	1.48	1.14	1.03	0.67
Methylnaphthalene	5.78	4.44	1.55	1.10
C_2-Naphthalene	7.33	5.63	0.90	0.82
C_3-Naphthalene	4.59	3.52	0.37	0.37
Phenanthrene	0.58	0.45	0.06	0.02
Methylphenanthrenes	0.78	0.60	0.12	0.06
Aromatics (total)	61.74	47.45	98.10	98.46

[a] Not determined (*see* text).

Since a number of investigators are employing continuous-flow oil delivery systems for determining effects of petroleum hydrocarbons on marine biota (*9, 22, 23*), it is important that the differences between hydrocarbon composition in these systems and hydrocarbon composition in the crude oils be measured and understood so that valid predictions of the effects of petroleum hydrocarbons in marine environments can be made.

It is also important to place the results from continuous-flow apparatus in context with those that might obtain in an actual oil spill. McAuliffe (*24*) has reported that dissolved hydrocarbon concentrations under slicks of spilled petroleum are very low, less than 1 μg/L. This being the case, additional means of studying crude oil toxicity to marine life must be developed to supplement the soluble oil studies underway.

Acknowledgments

This work was performed under Contract EY-76-C-06-1830 with the U.S. Department of Energy and under contract IAG D6-E681 with the Environmental Protection Agency.

The authors would like to thank Phil Ryan for the GC–MS analysis and Berta L. Thomas for her technical assistance.

Trade names are supplied throughout this paper to assist the reader in replicating the experiment but use does not imply endorsement by Battelle Memorial Institute.

Literature Cited

1. Blumer, M.; Sanders, H. L.; Grassle, J. F.; Hampson, G. R. *Environment* **1971**, *18*, 2–12.
2. "Effects of Oil and Chemically Dispersed Oil on Selected Marine Biota—A Laboratory Study"; Vaughan, B. E., Ed. Battelle, Pacific Northwest Laboratories: Richland, WA, 1973.
3. Vanderhorst, J. R.; Gibson, C. I.; Moore, L. J. *Mar. Pollut. Bull.* **1976**, *7*, 106–107.
4. Boylan, D. B.; Tripp, B. W. *Nature* **1971**, *230*, 44–47.
5. Bean, R. M.; Vanderhorst, J. R.; Wilkinson, P. "Interdisciplinary Study of the Toxicity of Petroleum to Marine Organisms," Battelle, Pacific Northwest Laboratories: Richland, WA, 1974.
6. Anderson, J. W.; Neff, J. M.; Cox, B. A.; Tatem, H. E.; Hightower, G. M. *Mar. Biol.* **1974**, *27*, 75–88.
7. Lewis, B. W.; Walker, A. L.; Bieri, R. H. *NASA Technical Memo.* **1974**, NASA TM X-72009.
8. Boehm, P. D.; Quinn, J. G. *Mar. Pollut. Bull.* **1974**, *5*, 101–105.
9. Vanderhorst, J. R.; Bean, R. M.; Moore, L. J.; Wilkinson, P.; Gibson, C. I.; Blaylock, J. W. *Proc. Oil Spill Conf. (Prev., Behav., Control, Cleanup)* **1977**, pp. 557–561.
10. Peake, E.; Hodgson, G. W. *J. Am. Oil Chem. Soc.* **1966**, *43*, 215–222.
11. Bean, R. M.; Blaylock, J. W. In "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms"; Wolfe, D. A., Ed.; Pergamon: 1977; pp. 397–403.
12. Simard, R. G.; Hasegawa, I.; Bandaruk, W.; Headington, C. *Anal. Chem.* **1951**, *23*, 1384–1387.
13. Gruenfeld, M. *Environ. Sci. Tech.* **1973**, *7*, 636–639.
14. Brown, R. A.; Searl, T. D.; Elliot, J. J.; Philips, B. G.; Brandon, D. E.; Monaghan, P. H. *Proc. Oil Spill Conf.* **1973**, pp. 505–519.
15. Bean, R. M. "Proceedings, Marine Pollution Monitoring (Petroleum)," *Natl. Bur. Stand. (U.S.), Spec. Publ.* **1974**, *409*, 127–130.
16. Vanderhorst, J. R.; Gibson, C. I.; Moore, L. J.; Wilkinson, P. *Bull. Environ. Contam. Toxicol.* **1977**, *17*, 557–584.
17. Junk, G. A.; Richard, J. J.; Grieser, M. D.; Witiak, D.; Witiak, J. L.; Arguello, M. O.; Vick, R.; Svec, H. J.; Fritz, J. S.; Calder, G. V. *J. Chromatogr.* **1974**, *99*, 745–762.
18. McAuliffe, C. *Chem. Technol.* **1971**, *1*, 46–51.
19. Warner, J. S. *Anal. Chem.* **1976**, *48*, 578–583.
20. Riley, R. G.; Bean, R. M. "Application of Liquid and Gas Chromatographic Techniques to a Study of the Persistence of Petroleum in Marine Sediments," in *Proceedings, 9th Materials Research Symposium, National Bureau of Standards, Gaithersburg, MD, April 10–13, 1978*, in press.
21. McAuliffe, C. *J. Phys. Chem.* **1966**, *70*, 1267–1271.
22. Hyland, J. L.; Rogerson, P. F.; Gardner, G. R. *Proc. Oil Spill Conf. (Prev., Behav., Control, Cleanup)* **1977**, pp. 547–550.
23. Roubal, W. I.; Bovee, D. H.; Collier, T. K.; Stranahan, S. I. *Proc. Oil Spill Conf. (Prev., Behav., Control, Cleanup)* **1977**, pp. 551–555.
24. McAuliffe, C. D. In "Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems"; Wolfe, D. A., Ed.; Pergamon: 1977; pp. 363–372.

RECEIVED October 31, 1978.

A Scheme for Analysis of Oily Waters

IHOR LYSYJ, RONALD RUSHWORTH, and ROBERT MELVOLD

Rockwell International, Environmental Monitoring & Services Center, Newbury Park, CA

EDWARD C. RUSSELL

U.S. Army MERADCOM, Fort Belvoir, VA

A scheme is described for gross and detailed chemical characterization of oily waters. Total, suspended, and dissolved organic content and hydrocarbon levels of the sample are determined. Volatile and water-soluble fractions are characterized in greater detail. Lower aliphatic and aromatic hydrocarbons are separated from the water by nitrogen sparging and are collected in an activated carbon absorption column. They are then extracted into carbon disulfide and analyzed gas chromatographically. The water-soluble fraction is extracted into chloroform or adsorbed on Amberlite XAD type of resin. Class characterization of this fraction is performed using the HPLC procedure. GC–MS–C is used for detailed analysis. The methodology was used for studying the effectiveness of bilge and ballast water treatments.

One of the principal analytical tools in control of oil pollution is the standard method for analysis of oil and grease in water and wastewater. This method, as promulgated in a number of official publications, involves extraction of the oil into a nonpolar solvent, followed by the gravimetric or spectrometric measurement of the separated oil. The most commonly used variations of this method are as postulated in the *Standard Methods for the Examination of Water and Wastewater,* Methods 502A and 502B (*1*). Freon 113 is used as a solvent, and the oil content is determined either gravimetrically (Method 502A) or spectrometrically (Method 502B). Such methods have proved quite satisfactory for the estimation of the nonvolatile hydrocarbon content of oily wastewaters, and produce fairly accurate results when applied to the analysis of water

0-8412-0473-6/80/33-185-247$05.00/0

contaminated by refined nonvolatile oils such as lubricants, greases, and heavy fuel oils. Product oils consist largely of hydrocarbons, which are effectively extracted into nonpolar solvents.

Standard methods, however, have a number of shortcomings when they are applied to the analysis of ballast and bilge waters. Ballast waters are normally contaminated by crude oils, while bilge waters contain used lubricating oils and light fuel oils. Crude oils are of very complex organic composition and contain, in addition to hydrocarbons, many substituted and heterocyclic compounds. Substituted compounds include nitrogen, sulfur, and oxygen moieties. Some crude oils are known to contain substantial amounts of resins and asphaltenes, which are made up of a variety of organic compounds including esters, phenols, carboxylic acids, ketones, pyridines, quinolines, carbazoles, and other substituted compounds. Many of these are only partially soluble in nonpolar solvents such as Freon 113 and consequently cannot be effectively extracted from the water. Out of five crude oils (Wilmington, California; Prudhoe Bay, Alaska; Recluse, Montana; Swan Hills, Alberta; and Gach Sārān, Iran) that were tested in the laboratory, only one (Recluse, Montana) appeared to be largely soluble in Freon 113. Nonsoluble residues were observed in all other cases. It has been our experience that the analysis for oil in wastes contaminated by the crude oils suffers from three principal shortcomings:

1. Not all of the crude oil components are soluble in nonpolar solvents.
2. The volatile fraction may be lost during evaporation (in a gravimetric procedure).
3. The water-soluble fraction is discarded.

The losses could be quite substantial and produce inaccurate results when standard extraction methods are used for the analysis of wastewaters containing crude oils. In our experience, typical recoveries for crude oils were in the 50%–60% range when Standard Extraction Method 502B (*1*) was used. This was in sharp contrast to crude oil determination by the Total Organic Carbon (TOC) method (modified for analysis of oil in water) where TOC recoveries were close to 100%.

Methodology

The composition of crude and refined oils is quite complex and covers a broad spectrum of organic compounds. It is not uncommon to find in it compounds ranging from very volatile, low-molecular-weight hydrocarbons to nonvolatile tarry residues composed of very complex polynuclear aromatic hydrocarbons. Some of the crude oil components (hydrocarbons) are essentially not soluble in water, while others (phe-

nols, quinolines) are highly soluble. When crude oils are mixed with water, partition of specific compounds takes place between aqueous and oil phases. More polar compounds tend to dissolve in the water phase, while nonpolar compounds remain in the surface oily film.

No single analytical technique (such as standard methods for oil analysis or modified TOC determination) can provide for meaningful chemical definition of crude oil contaminants, such as are found, for example, in ballast waters of an oil tanker. To deal with this problem, we have developed over a number of years an analytical scheme that characterizes in gross and detailed terms chemical composition of oily wastewater with special attention to water-soluble (primarily aromatic) content of such waters (Figure 1). The gross characterization includes determination of total, dissolved, and suspended organic matter, and an estimation of aliphatic hydrocarbon content. Detailed chemical analysis includes gas chromatographic determination of volatile organics and characterization of dissolved organics by high pressure liquid chromatography (HPLC) and gas chromatography–mass spectroscopy (GC–MS).

The gross chemical determinations are performed on neat oily water samples, while detailed chemical analysis requires separation of organic matter from the water matrix prior to analysis. Volatile organics are stripped by nitrogen purging, adsorbed in activated carbon tubes, and analyzed gas chromatographically after desorption into carbon disulfide. The dissolved fraction is separated from the suspended organic matter by Millipore filtration and is either extracted into chloroform or adsorbed on Amberlite XAD-7 column. Both separation methods have advantages and disadvantages. The chloroform extraction method is generally satisfactory for compounds of intermediate polarity, while Amberlite XAD-7 was proved to be superior for the separation of highly polar phenolic compounds (pyrogallol, resorcinol, creosol, hydroquinone, phenol).

The analytical scheme described here was used in a study of the effectiveness of small-scale bilge water treatment (Fort Eustis, Virginia), and in a study of the effectiveness of large-scale ballast water treatment in Port Valdez, Alaska. In our experience this scheme provided a good starting point for a comprehensive assessment of chemical composition of a given type of oily waste. The scheme was usually modified somewhat in subsequent investigations to be fully responsive to the specific characteristics of a chemical composition of a given source of oily waste.

Experimental

Principal analytical operations performed include:

1. Determination of total, dissolved, and suspended organic content by the TOC analysis

nols, quinolines) are highly soluble. When crude oils are mixed with water, partition of specific compounds takes place between aqueous and oil phases. More polar compounds tend to dissolve in the water phase, while nonpolar compounds remain in the surface oily film.

No single analytical technique (such as standard methods for oil analysis or modified TOC determination) can provide for meaningful chemical definition of crude oil contaminants, such as are found, for example, in ballast waters of an oil tanker. To deal with this problem, we have developed over a number of years an analytical scheme that characterizes in gross and detailed terms chemical composition of oily wastewater with special attention to water-soluble (primarily aromatic) content of such waters (Figure 1). The gross characterization includes determination of total, dissolved, and suspended organic matter, and an estimation of aliphatic hydrocarbon content. Detailed chemical analysis includes gas chromatographic determination of volatile organics and characterization of dissolved organics by high pressure liquid chromatography (HPLC) and gas chromatography–mass spectroscopy (GC–MS).

The gross chemical determinations are performed on neat oily water samples, while detailed chemical analysis requires separation of organic matter from the water matrix prior to analysis. Volatile organics are stripped by nitrogen purging, adsorbed in activated carbon tubes, and analyzed gas chromatographically after desorption into carbon disulfide. The dissolved fraction is separated from the suspended organic matter by Millipore filtration and is either extracted into chloroform or adsorbed on Amberlite XAD-7 column. Both separation methods have advantages and disadvantages. The chloroform extraction method is generally satisfactory for compounds of intermediate polarity, while Amberlite XAD-7 was proved to be superior for the separation of highly polar phenolic compounds (pyrogallol, resorcinol, creosol, hydroquinone, phenol).

The analytical scheme described here was used in a study of the effectiveness of small-scale bilge water treatment (Fort Eustis, Virginia), and in a study of the effectiveness of large-scale ballast water treatment in Port Valdez, Alaska. In our experience this scheme provided a good starting point for a comprehensive assessment of chemical composition of a given type of oily waste. The scheme was usually modified somewhat in subsequent investigations to be fully responsive to the specific characteristics of a chemical composition of a given source of oily waste.

Experimental

Principal analytical operations performed include:

1. Determination of total, dissolved, and suspended organic content by the TOC analysis

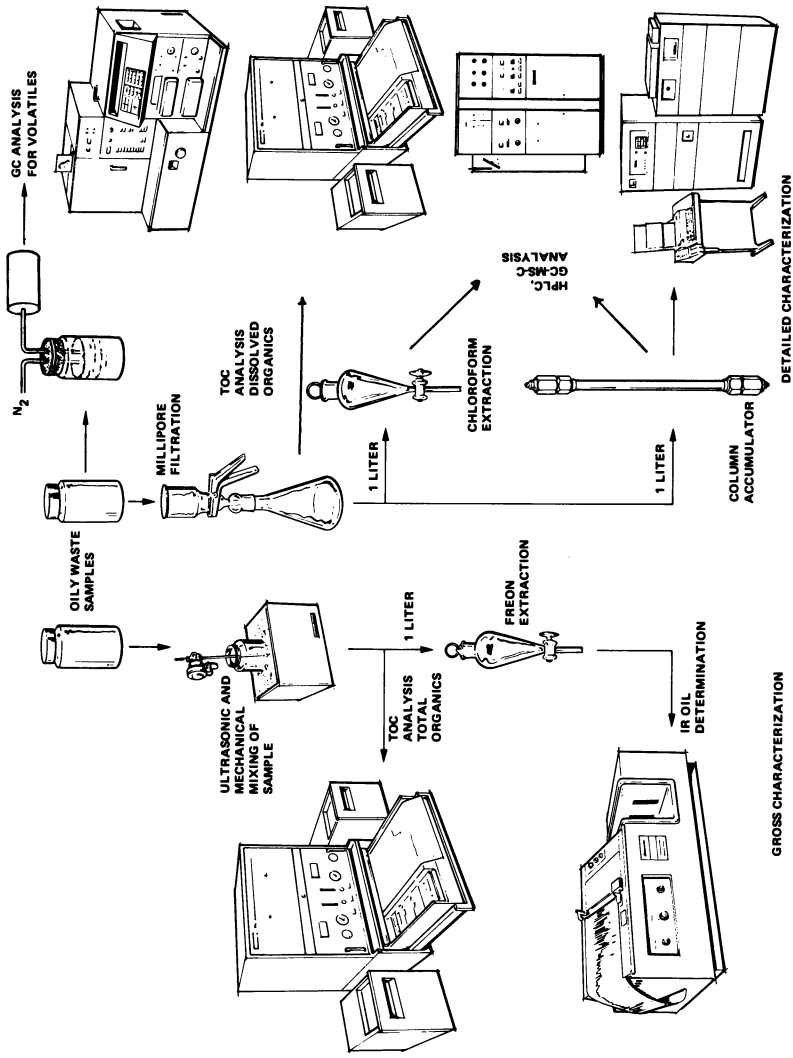


Figure 1. Protocol for oily wastewater analysis

2. Estimation of aliphatic hydrocarbon content by Freon 113 extraction–IR detection method
3. Determination of volatile organics by gas sparging and GC analysis
4. Characterization of dissolved fraction by IR and GC–MS
5. Class characterization of aromatic fraction by HPLC.

Each of the methods was evaluated initially in the laboratory using known synthetic compositions and then field-tested using actual oily wastewater samples.

Total Organic Carbon (TOC). A standard method for TOC analysis (*1*) was modified by a special procedure for preparation of oily wastewater samples prior to the analysis. Two methods of oily water pretreatment were tested and used. In one method oily water samples were dispersed by mechanical stirring and ultrasonic vibration in an ultrasonic bath. The other method involved ultrasonic dispersion of oil in water by means of an ultrasonic probe (*2*). The best results in terms of emulsion stability and reproducibility were obtained using the ultrasonic probe method (Table I).

Erlenmeyer flasks were used in the laboratory work for sample preparation, while 4-oz cylindrical vials (VWR Catalog No. 66015-224) were used for sampling in the field. An ultrasonic vibrator probe was immersed a few centimeters below the surface of the sample in the cylindrical vial and the power to the probe was gradually increased from 0 to 300 W. A Brunsonic Model 1510 probe manufactured by B. Braun, Mesungen AG, was used in this work. The shape of the sample container was found to be rather important to the oil-in-water dispersion effectiveness. In narrow cylindrical vessels, oily film forms in the relatively small surface area and adheres to glass walls in close proximity to the ultrasonic probe tip. The use of cylindrical vials for samples and ultrasonic probes for dispersion facilitates effective dispersion of oil in water in 1–5 min and leads to formation of stable emulsions (10–30 min). Reproducible aliquots of such emulsions can be injected in either a Beckman or Dohrmann TOC analyzer for the determination of total organic carbon. The Dohrmann TOC analyzer provides data on both the volatile and nonvolatile organic content of the sample. The acidified and sparged waste

Table I. Accuracy of Modified TOC Method in Analysis of Prudhoe Bay, Alaska, Crude Oil

Oil in Water (mg/L)	*Present (mgC/L)*	*Found (mgC/L)*	*% Recovery*
21.4	18.4	19.7	107
38.5	33.1	32.5	98
54.9	47.2	43.8	93
78.2	67.3	62.0	92
113.0	97.1	93.7	97
3479.0	2950.0	3100.0	105
			99

samples are used for the Backman TOC analysis. The results obtained in this manner correspond to the nonvolatile organic content of the sample.

The dissolved organic carbon is determined similarly after the sample is filtered through a 0.45-μm Millipore filter. The suspended organic carbon is determined as a difference between total and dissolved organic carbon.

Both methods are calibrated using potassium acid phthalate as a standard.

Estimation of Hydrocarbon Concentration. Standard Method 502B (*1*) is used for the estimation of the aliphatic hydrocarbon content of the sample. A 1-L sample is transferred quantitatively into a separatory funnel where hydrocarbons are extracted into a 100-mL volume of Freon 113. The concentration of hydrocarbons is estimated by measuring IR absorption of a main peak in the 3200–2700 cm^{-1} spectral region using a low-resolution IR spectrometer. The instrument is calibrated using a specified standard solution (isooctane, hexadecane, and benzene).

The effectiveness of this procedure was evaluated using samples of Prudhoe Bay (Alaska) and other types of crude oil. Known amounts of crude oils were introduced into distilled water, dispersed, and then analyzed closely following Standard Method 502B (*1*). The results of laboratory experiments are shown in Table II. As can be seen from this table, recoveries of selected crude oils ranged between 50%–60% using this method.

The use of this method is further complicated by different magnitudes of IR response in the spectral region to aromatic and aliphatic hydrocarbons. The method becomes of limited value when oily waters containing large proportions of aromatic hydrocarbons are analyzed. The treated ballast waters, for example, were found to contain very high proportions of aromatic hydrocarbons, and this fact negated the usefulness of this procedure. It is believed that the Freon 113 extraction–IR detection method should be used only for the determination of aliphatic hydrocarbon content of oily wastes that contain largely aliphatic hydrocarbons.

Table II. Accuracy of Crude Oil Determination by the Freon Extraction–IR Method of Analysis

	Oil in Water (mg/L)		
Crude Oil	*Present*	*Found*	*% Recovery*
Recluse, Montana	19.7	11.3	57
Swan Hills, Alberta	20.1	11.0	55
Gach Saran, Iran	20.0	10.0	50
Wilmington, California	21.3	12.1	57
Prudhoe Bay, Alaska	18.9	11.5	61
	43.9	22.2	51
	84.0	38.6	48
	2027.0	1130.0	56
	2027.0	1170.0	58
Average recovery			55

Characterization of Volatile Fraction. Volatile organic compounds found in oily wastewaters consist primarily of lower-molecular-weight aliphatic and aromatic hydrocarbons. Because of its relatively high vapor pressure, this fraction is quite often lost during analysis of oily wastes. For this reason a separate procedural step was incorporated into the overall scheme for analysis of the volatile fraction. An unfiltered sample of oily waste is used in this determination. The volatile fraction is separated from water by means of nitrogen sparging and collected in an activated carbon absorption column. The collected compounds are desorbed into carbon disulfide and analyzed by GC.

The head of the sparging assembly consists of a rubber stopper or a jar lid into which a gas dispersion tube and adsorption train are inserted (Figure 2). The collection assembly consists of a drying tube (6-mm-i.d. × 13-cm-long glass tube filled with 10–20 mesh Drierite) and a charcoal tube (NIOSH-approved for determining organic vapors in air, obtainable from Supelco or MSA). A 2-L round-bottomed flask was used as a sample vessel in the laboratory study of accuracy of the method. In the field work, however, glass jars used for sampling were equipped with a sparg-

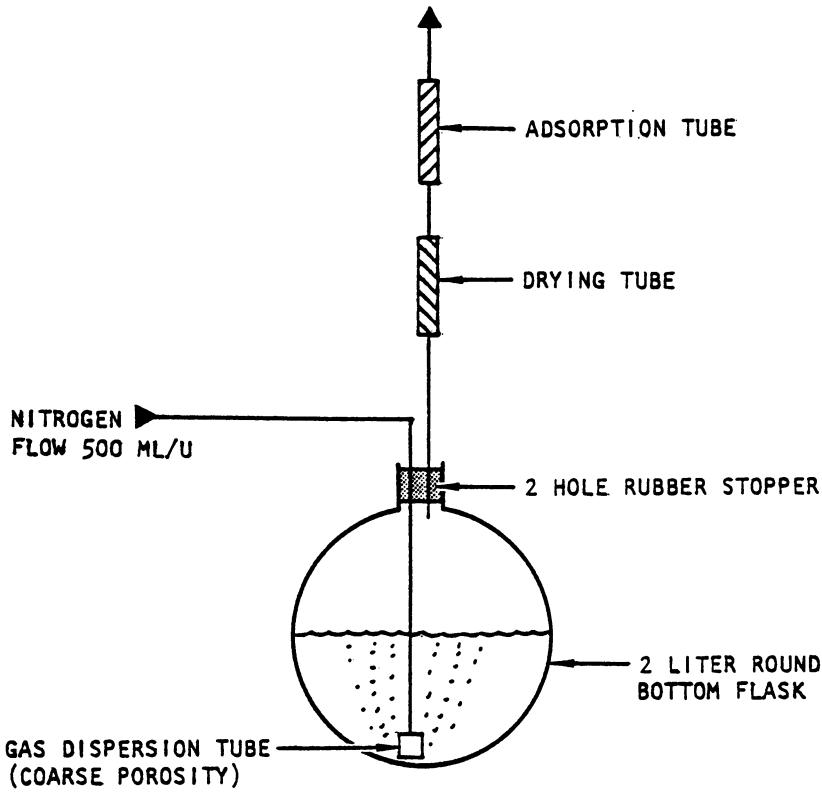


Figure 2. Diagram of apparatus used for recovery of volatile hydrocarbons

ing head, and volatile organics were sparged directly from the sampling container.

Nitrogen is purged through the sample at 500 mL/min for a period of 30 min. The charcoal tube is in two sections to ensure complete trapping. The total capacity of the charcoal section is approximately 15 mg of hydrocarbons (this would correspond to a 1-L sample containing 15 ppm hydrocarbons).

Activated carbon cartridges are opened after the sparging operation and the front and back portions of the carbon are transferred into vials equipped with Mininert valve seal. One milliliter of carbon disulfide is introduced through the septum by means of a syringe (to prevent losses of volatiles due to heat of adsorption). The vials are allowed to stand at room temperature for 30 min with periodic shaking. A 1-μL aliquot is withdrawn and injected into the gas chromatograph.

The analysis of separated volatile fraction can be carried out using a number of gas chromatographic columns and conditions including those described in the Standard Test Method ANSI/ASTM D 2267-68 (3). The following conditions were used by us in the laboratory studies: GC column, 6% OV 101 on 100–120 mesh Chromosorb G-HP, 5 ft $\times$ $\frac{1}{8}$ in. stainless steel; column temperature, 1.5 min hold at 60°C, temperature programmed to 105°C at 10°C/min; detector, hydrogen flame ionization.

The recoveries achieved with this procedure are shown in Table III.

During the field studies a simpler isothermal Gow-Mac gas chromatograph equipped with a hydrogen flame ionization detector was used. A 12-ft $\times$ $\frac{1}{8}$-in. column packed with 5% OV-17 substrate on Chromosorb W-HP was used for separation at 80°C and 20 mL/min flow of carrier gas.

Characterization of Dissolved Fraction. The dissolved organic fraction is separated from the suspended organic matter by Millipore filtration using a 0.45-μm filter, Type HA. The TOC of the filtrate is determined first. Thereafter, the filtrate that contained the dissolved organic matter is separated from the water by chloroform extraction or by means of adsorption on Amberlite XAD-type resin.

Chloroform extraction was used as a principal method for separation of dissolved nonvolatile organic matter in our earlier studies. More recently, we have been using Amberlite XAD-7 resins for such separations. Our laboratory studies indicated significant superiority of this technique for the separation of highly polar organic compounds. Recoveries obtained for phenols were typically 70%–80%.

Table III. Recovery of Volatile Hydrocarbons from Water Solution

	Concentration (μL/L, vpm)			
		Found		
Compound	*Present*	*Total*	*First Section*	*Backup Section*
Hexane	1.0	1.07	1.07	0
Benzene	1.0	1.00	1.00	0
Toluene	1.0	1.01	1.01	0
Xylene	1.0	1.00	1.00	0

Special pretreatment of commercially available Amberlite XAD-7 resin was necessary to overcome the problem of high blank values. Fines were removed by slurrying the resin in methanol and decanting. Then the impurities present in the resin were extracted by refluxing the resin in the Soxhlet apparatus for 8 hr each with methanol, acetonitrole, and diethyl ether. The purified resin was introduced as a slurry into a glass column 140 mm long × 8½ mm i.d. Accumulator columns were always stored filled with methanol.

The accumulator column separation method was similar to a method described by Junk (*4*). The apparatus consisted of a 1-L reservoir bulb connected to a 140-mm Amberlite XAD-7 column equipped with a stopcock. The separation of organics from the water sample was accomplished by rinsing the column with 25 mL of methanol (under gravity flow) and then introducing the water sample into a 1-L reservoir bulb and letting the sample pass through the column under the gravity flow. The retained organics were then desorbed into 25 mL of methanol. The chloroform separation procedure involved extraction of approximately 1-L samples into three 50-mL volumes of chloroform, which were then combined and evaporated at room temperature with a flow of nitrogen to 2-mL volume.

This concentrate was then analyzed for functionality by IR spectrometry. General profiling and specific compound identification was accomplished by computerized GC–MS and characterization of the aromatic subfraction is done by HPLC. The IR analysis was performed in a conventional manner on a Perkin–Elmer high-resolution IR spectrometer Model 283. Computerized GC–MS work was performed on the Olfax II instrument (Universal Monitors Corporation). The Cornell University computer facility was used for spectra matching.

Conditions for GC separation in the computerized GC–MS procedure were as follows:

Column: 6 ft × ¼ in. glass tube filled with 3% OV-17 on Chromosorb

Temperature Program From 70°C to 280°C at 8°/min

Carrier: Nitrogen, flow rate 20 mL/min

Combustion Gases: Oxygen inlet pressure, 50 psig
Hydrogen inlet pressure, 25 psig

The HPLC analysis for the aromatic fraction is described in greater detail in the following paragraphs.

Characterization of Aromatic Subfraction. Aromatic compounds, including hydrocarbons, are generally more water-soluble than aliphatic hydrocarbons. They are also believed to be more detrimental to aquatic life than aliphatic hydrocarbons because of higher orders of toxicity. For this reason this subfraction deserves close examination.

Crude and refined oils are known to contain the following aromatic compounds: aromatic and polynuclear aromatic hydrocarbons (PAHs), phenols and cresols, heterocyclics (such as pyridine, quinoline), benzoic acid, esters and ethers. Many are quite water-soluble (Table IV) and are expected to be found in the dissolved fraction.

A quantitative class analysis was devised for the characterization of this environmentally important component of oily wastes. This analysis is performed using a HPLC procedure.

Table IV. Solubility of Aromatic Compounds in Water (*1*)

Compound	*Concentration (mg/L)*
Benzene	1780/20°C
Toluene	515/20°C
Xylene	198/25°C
Naphthalene	30
Phenol	82,000/15°C
Resorcinol	2,290,000/30°C
Pyrogallol	625,000/25°C
Creosol	28,000/30°C
Benzoic acid	2,900
Pyridine	65,000/20°C
Quinoline	60,000

Under the conditions specified in Table V, aromatic compounds elute in order of polarity: phenolic materials in a 1–4 min retention time window, nitrogen heterocyclics in 4–6 min, alkylbenzenes in 6–14 min, and PAH after 14 min (Figure 3). Detection and analysis of the elutants are performed using ultraviolet (UV) detection at a fixed absorption band of 254 nm. It was found that while the absorption characteristics of different

Table V. Conditions for HPLC Analysis for Class Characterization of Aromatic Compounds

Instrument	Spectra Physics HPLC—Model 3500B
Column	Partisil PXS/10/25 ODS-2, 25 cm, Whatman Corp.
Mobile phase	30% water, 70% methanol
Flow rate	1.32 mL/min, pressure 1000 psi
Sensitivity	AUFS[a]—0.16
Recorder speed	0.5 in./min
Sample size	10 μL

[a] Absorption units full scale.

Table VI. HPLC Separation and Calibration Data for Phenolic

Class	*Compound*
Phenolic compounds	pyrogallol isomers
	hydroquinone isomers
	resorcinol
	phenol
Nitrogen heterocyclics	pyridine
	quinoline
Aromatic hydrocarbons	benzene
	toluene
	xylene

[a] Conditions of analysis as described in Table V.

aromatic compounds vary, there is a similarity in the intensity of UV responses within each of the specified groupings: alkylbenzenes, phenolic compounds, and heterocyclics (Table VI). Because of this, it is possible

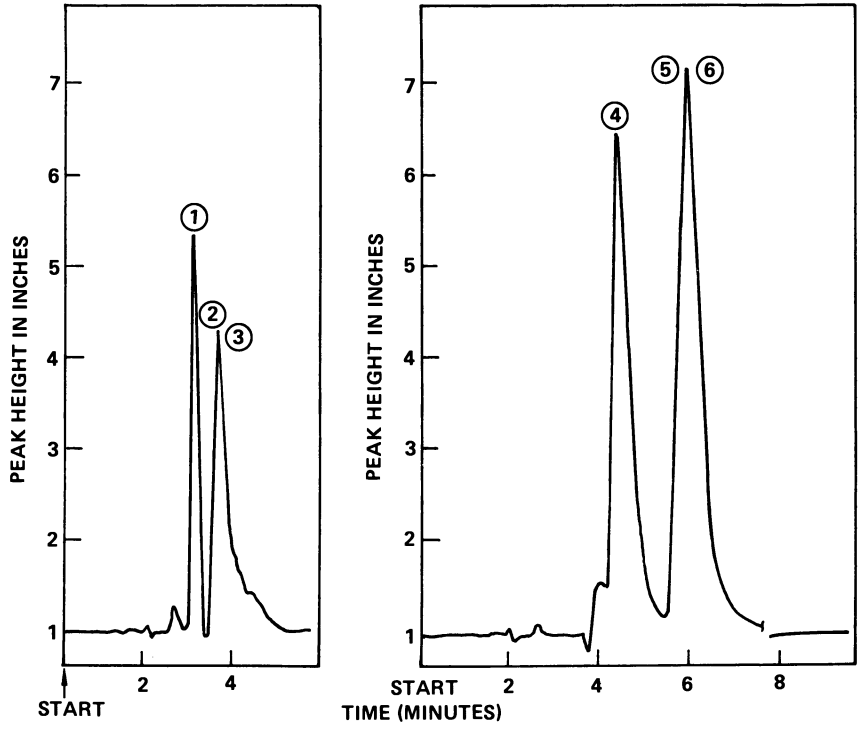


Figure 3. HPLC calibration of phenolic and heterocyclic compounds. Peaks: 1, phenol; 2, 3, O-P-creosol; 4, pyridine; 5, 6, 3-picoline and quinoline.

Compounds, Nitrogen Heterocyclics, and Aromatic Hydrocarbons[a]

Retention Time (min)	*Response μV-sec*[b] *(mg/L)*	*Class Factors μV-sec*[b] *(mg/L)*
1.67; 2.21; 2:58; 2.34; 2.98; 2.45	4099	2738
	2021	
	2012	
3.29	2818	
4.48	15,723	13,637
6.64	11,550	
6.40	1261	1291
9.39	1385	
14.55	1226	

[b] Hewlett Packard Integrator Model 3370A was used.

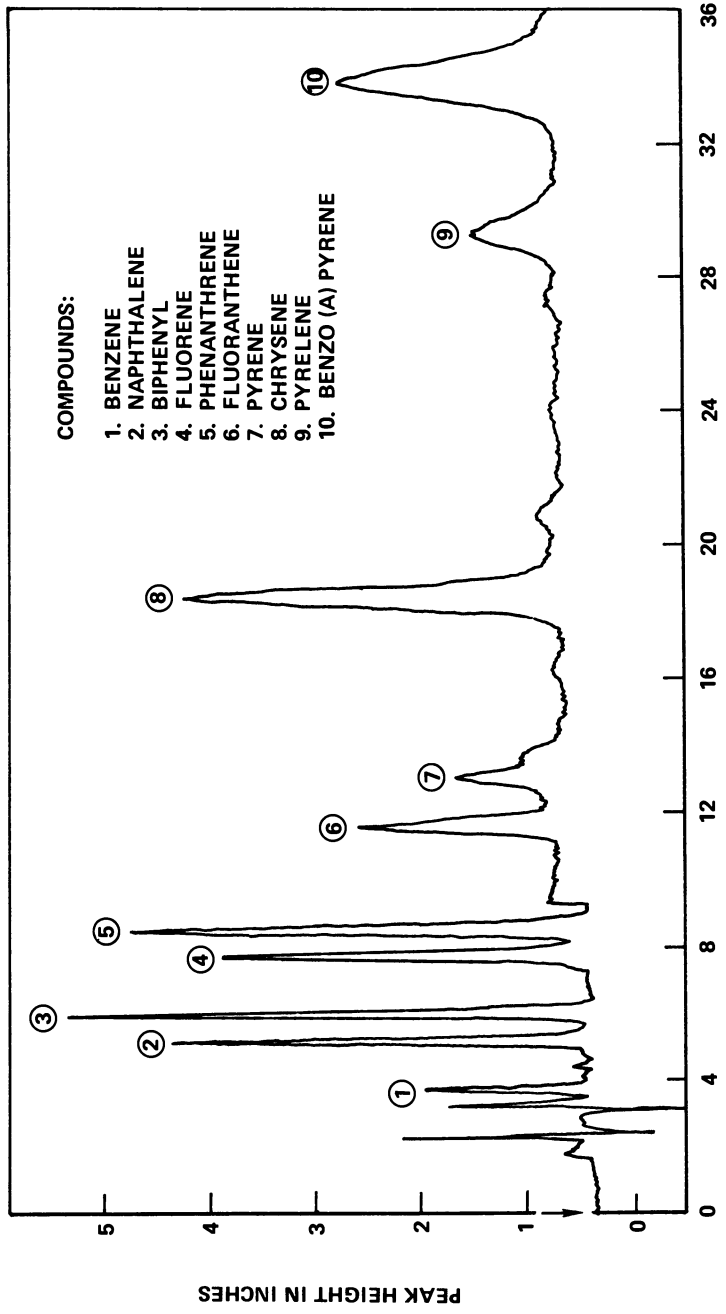


Figure 4. Calibration of PAHs

to establish an average UV response factor for each class of interest. The calculations are then performed by integrating the total area in each time window (0–4, 4–6, and 6–14 min, which correspond to phenolics, heterocyclics, and aromatic compounds, respectively) and proportioning such areas to the respective group response factors.

The accuracy of this procedure was tested by preparing and analyzing a standard known mixture of aromatic compounds, including pyrogallol, resorcinol, hydroquinone, phenol, pyridine, quinoline, toluene, and xylene. The recoveries were found to be: phenols, 113%; heterocyclics, 105%; and aromatic hydrocarbons, 75%. This HPLC procedure provides for estimation of the makeup of the principal organic types found in dissolved, nonvolatile fractions of oily wastewater. The analysis for PAH is performed under similar conditions, but 15% water and 85% methanol are used as the moving phase. This modification permits a speedup of the elution sequence and facilitates analysis for two- to six-ring PAH compounds. An example of an HPLC analysis for PAH is shown in Figure 4.

Applications

The analytical scheme described here was used with some modifications in a field study of the effectiveness of small-scale bilge water treatment (*5*) and in a study of the effectiveness of large-scale ballast treatment (*6*).

The bilge water treatment operation is located at the U.S. Army Transporation Center at Fort Eustis, Virginia. The treatment involves primary gravity separation in three tanks of approximately 250-barrel capacity, followed by a secondary treatment using an experimental three-stage coalescence device. In the normal mode of operation, bilge water can be stored for two weeks or more in gravity separation tanks prior to secondary treatment and subsequent discharge into the harbor.

The ballast treatment facility studied is located at the terminal of the Alaska pipeline in Port Valdez, Alaska. The treatment involves primary separation by gravity in 430,000-barrel-capacity tanks, followed by a secondary treatment consisting of a combination of chemically aided flocculation and dissolved air flotation processes, with final pH adjustment and an effluent impound basin. The total residence time of ballast water in the treatment facility is usually less than 48 hr.

These two oily waste treatment facilities are different in three major aspects: the nature of oily waste treated, plant capacity, and retention time of waste during treatment. Not surprisingly, the effectiveness of the treatment and the chemical composition of the treated effluent were found to be quite dissimilar.

The bilge waste treatment study in Fort Eustis, Virginia, involved sampling and analysis of untreated bilge water aboard a number of different kinds of watercraft and of the treated effluent. The ballast water

treatment study in Port Valdez, Alaska, involved sampling and analysis of effluents from a primary gravity separator and of the treated final effluent.

Results

The results of the bilge waste treatment study indicated that suspended organics ranged between 10 and 300 ppm and dissolved organics between 10 and 150 ppm in the untreated bilge water collected aboard operating watercraft. All samples were collected below the bilge surface and no attempt was made to estimate the amount of the oil contained in a surface film. The treated effluent contained no detectable amount of suspended matter but rather high levels of dissolved organic matter (700–2000 ppm).

Up to 70% of the dissolved organics in untreated bilge wastewater were chloroform-extractable, while less than 10% of the dissolved organics in treated bilge wastewater were extractable into chloroform.

The water-soluble organics of the treated bilge effluent were separated into chloroform-extractable and ethanol-soluble fractions. This was done by extracting organic matter first into chloroform and then dissolving the residue (after evaporation of water) into ethanol. The ethanol fraction was found to correspond to more than 90% of the organic matter present in the treated bilge effluent, while chloroform-extractable compounds comprised less than 10% of dissolved organics. The IR-spectrometric examination of the ethanol fraction revealed that it is composed primarily of aliphatic polyhydroxy alcohols, large portions of which might be of a glycolic type. Such compounds are known to be associated with biological processes and are most likely formed as a result of biological degradation of petroleum matter during long periods of contact between the oil and the water in primary separation tanks.

The GC–MS profiling of organic compounds present in the chloroform-extractable fraction of both treated and untreated bilge water disclosed that the aromatic compounds constituted the great bulk of organic matter present.

HPLC analysis of the aromatic subfraction of the water-soluble and the chloroform-extractable fractions of untreated bilge water produced the following average proportions: hydroxylated aromatics, 50%; aromatic hydrocarbons, 46%; and heterocyclics, 4% (Figures 5 and 6). In the treated effluent, the aromatic subfraction was found to be: hydoxylated aromatics, 79%; aromatic hydrocarbons, 15%; and heterocyclics, 5% (Figure 7).

The PAHs were found only in trace concentration (low ppb concentration range) and this concentration was further reduced in the treated effluent (Figure 8).

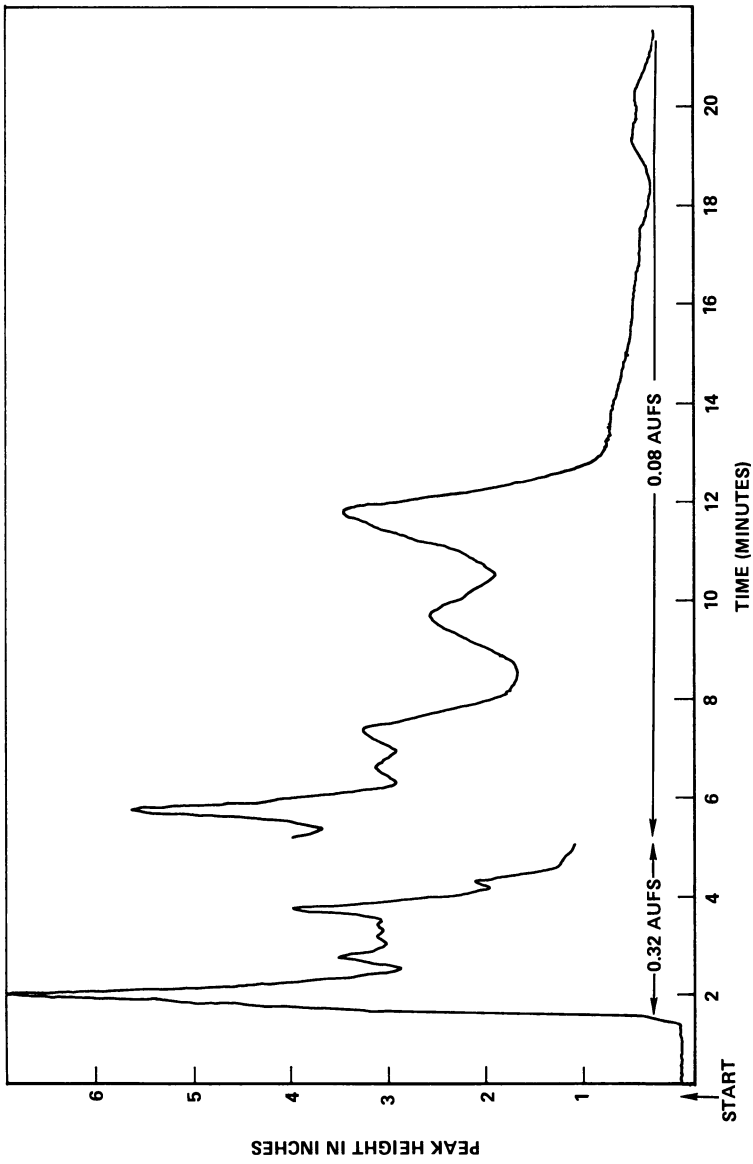


Figure 5. HPLC chromatogram of untreated bilge waste from landing craft utility; AUFS = absorption unit full scale.

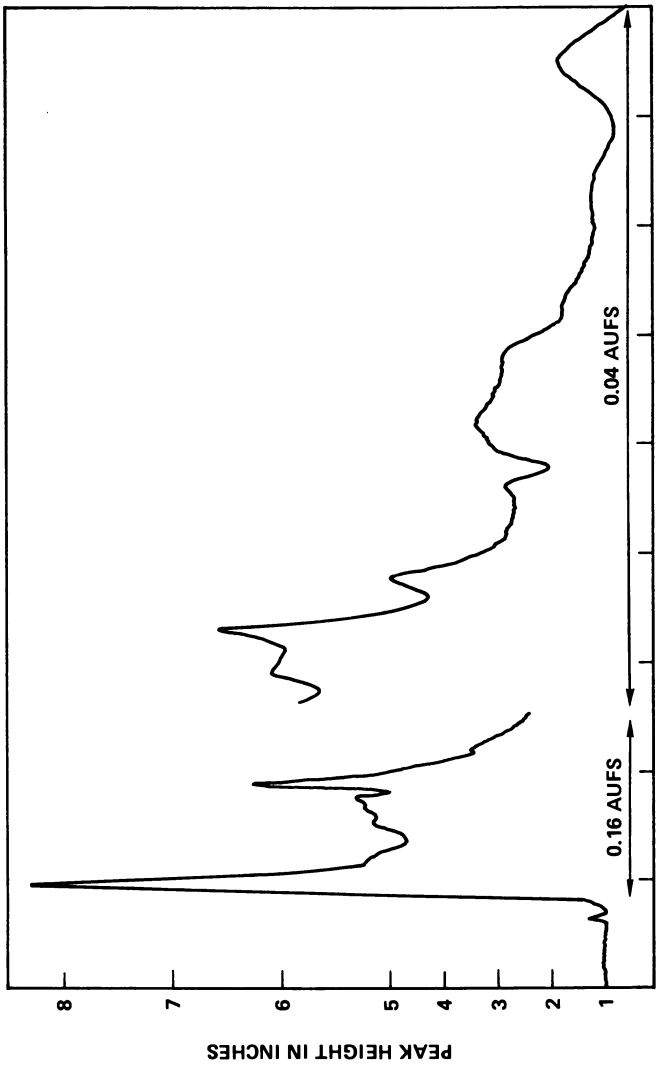


Figure 6. HPLC chromatogram of untreated bilge waste from transport ship; AUFS = absorption unit full scale.

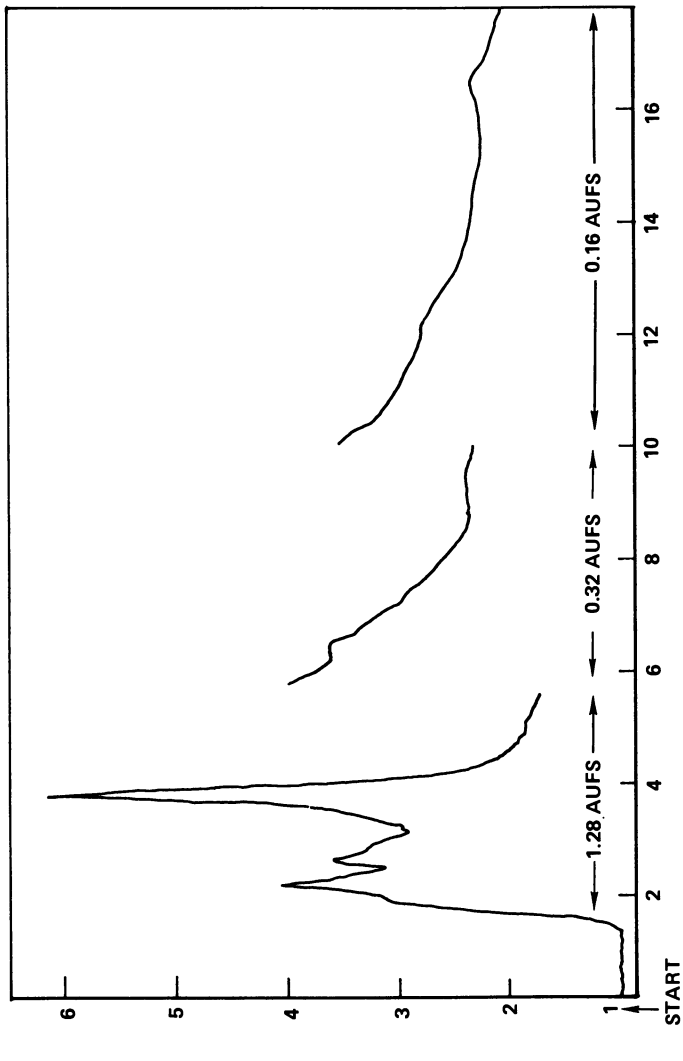


Figure 7. HPLC chromatogram of treated effluent from U.S. Army oily waste treatment facility in Fort Eustis, VA; AUFS = absorption unit full scale.

In a study of large-scale ballast treatment operations in Port Valdez, Alaska (Summer 1978), it was found that the effluent from the gravity separator and the final effluent contained volatile organic matter composed primarily of aromatic hydrocarbons (benzene, toluene, and xylenes), dissolved organic materials of the petroleum nonhydrocarbon

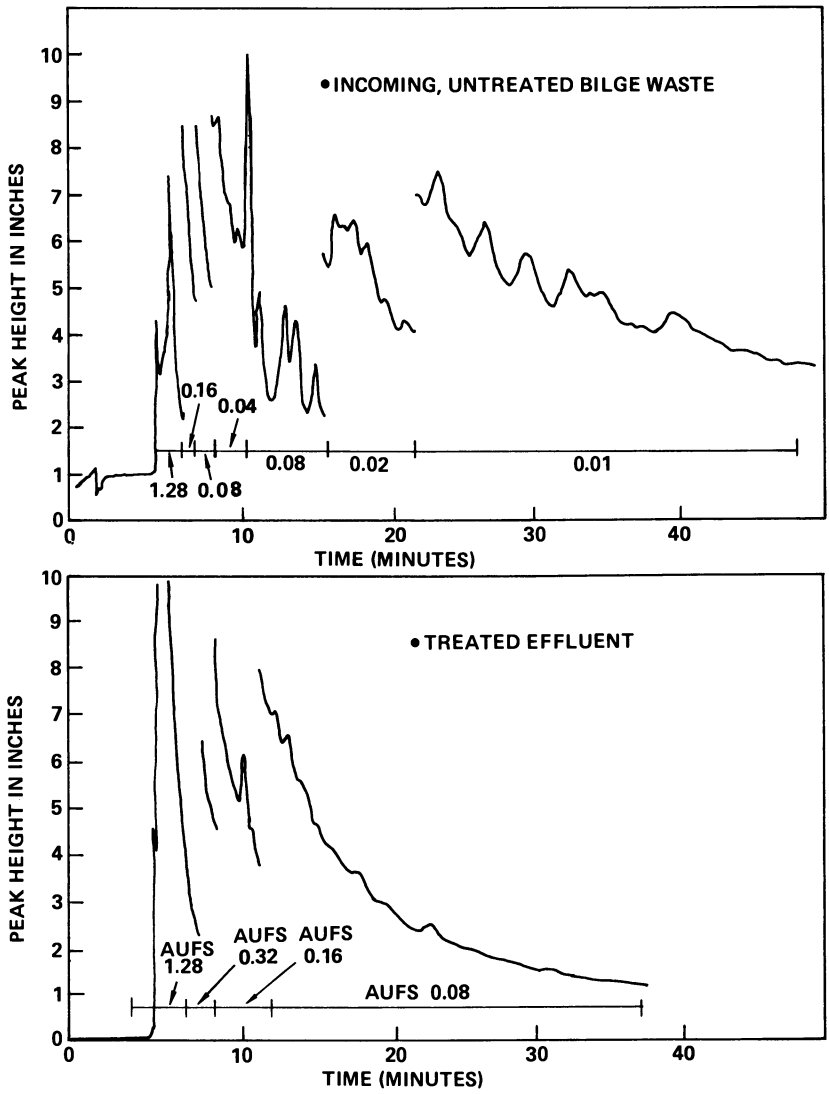


Figure 8. PAHs in bilge wastewater; AUFS = absorption unit full scale.

type, and suspended organic matter traditionally defined as water-insoluble "oil." The breakdown between these three principal types of organics in treated effluent was found to be: aromatic hydrocarbons, 48%; dissolved nonvolatile organics, 36%; and suspended organic matter, 16%. A typical composition of final effluent is shown in Table VII.

The average total organic load of the effluent from the primary separator was approximately 20 mgC/L, and the approximately average load of organics in the final treated effluent was found to be 10 mgC/L. It was estimated from plant operating records that incoming ballast contained an average of 6000 ppm of crude oil. From this it appears that the bulk of oil removal from the process stream takes place in the gravity separator.

Effectiveness studies of the two different treatment facilities for oily waste disclosed gross differences in the quality of the treated effluent and in the nature of organic matter present. The small-scale ballast treatment plant with long waste retention times in a gravity separator, followed by coalescence treatment, produced an effluent essentially free of suspended organic matter (the "oil") but containing very high levels of dissolved organic matter. The bulk of dissolved organics were of the glycolic type and were probably biologically derived. The effluent also contained smaller quantities of dissolved, chloroform-extractable organic matter, which was found to be largely aromatic in nature.

The large-scale, high-throughput, low-residence-time plant for treatment of ballast waters produced treated effluent containing small amounts of volatile, dissolved, and suspended organic matter. The principal components in the treated effluent were found to be volatile aromatic hydrocarbons and water-soluble organics. There was no indication of any significant contribution of biological nature to organics in treated effluent from this plant.

Table VII. Typical Chemical Composition of Treated Effluent Ballast Treatment Plant, Port Valdez, Alaska

Compound	*Concentration (mgC/L)*	*Percent*
Volatile organics	5.1	48
benzene	2.3	21
toluene	1.9	18
xylenes	1.0	9
Dissolved nonvolatile organics	3.7	36
phenols	0.6	6
Suspended organics	1.9	16

Literature Cited

1. "Standard Methods for the Examination of Water and Wastewater," 14th ed.; American Public Health Association: Washington, DC, 1975.
2. Gruenfeld, M., *Int. Counc. Explor. Sea, Workshop on Petroleum Hydrocarbons, Aberdeen, Scotland,* 1975.
3. "Standard Test Method for Aromatics in Light Naphthas and Aviation Gasolines by Gas Chromatography," ANSI/ASTM; American Society for Testing and Materials: Philadelphia, 1977; p. 2267–2268.
4. Junk, G. A., et al., "Resin Sorption Method for Monitoring Selected Contaminants in Water," *Identif. Anal. Org. Pollut. Water [Chem. Congr. North Am. Cont.], 1st;* Ann Arbor Science Publishers, Inc.: Ann Arbor, 1976.
5. Lysyj, I.; Russell, E. C. "Effectiveness of Bilge Water Treatment: A Field Study," submitted for publication in *Environ. Int.*
6. Lysyj, I.; Rushworth, R.; Melvold, R.; Farlow, J. "Effectiveness of a Large-Scale Ballast Treatment Process," presented at the *1979 Oil Spill Conference,* Los Angeles, CA.
7. Verschueren, K. "Handbook of Environmental Data on Organic Chemicals"; Van Nostrand Reinhold: New York, 1977.

RECEIVED October 12, 1978.

12

Hydrocarbons in the Sediments of the Bermuda Region: Lagoonal to Abyssal Depths

THOMAS D. SLEETER, JAMES N. BUTLER, and JACK E. BARBASH

Division of Applied Sciences, Harvard University, 29 Oxford St., Cambridge, MA 02138

Surficial sediment was sampled at 20 stations (50 cores) from the Bermuda platform including one deep-water sediment sample from 1400 m. Forty-eight-hour soxhlet extraction (methanol + benzene) was followed by column chromatography (alumina/silica). The aliphatic (pentane-extractable) hydrocarbons from the column chromatography are reported. Along the shoreline, subtidal sediment contained residues from beached pelagic tar. Outside the protective boiler reef total aliphatic hydrocarbon concentration was less than inside the reef (3–10 μg/g dry weight outside; 10–65 μg/g inside). Samples from 1400-m depth showed very low concentrations (< 1.0 μg/g). The chromatograms from the shipping channels showed a fresh petroleum source in concentrations ranging from 8 to 31 μg/g. Harbors yielded chromatograms typical of chronic petroleum contamination, with concentrations from 30 to 110 μg/g. Several biogenic compounds (including C_{15} and C_{17} normal alkanes) were noted in addition to the petroleum-derived hydrocarbons mentioned above. These are most probably derived from marine algae.

Pelagic tar lumps have been quantitatively surveyed in the ocean since 1969. Highest concentrations have been found in the Sargasso Sea (9.4 mg/m² ocean surface) (*1*) and the Mediterranean Sea (9.7 mg/m²) (*2*). Estimates of the standing stock of pelagic tar in the Atlantic Ocean are from 23,000 to 86,000 metric tons, of which 65%–75% is found in the Sargasso Sea (*3, 4*).

0-8412-0475-6/80/33/267$05.50/0

The principal source of pelagic tar lumps appears to be routine tank cleaning and deballasting by crude oil tankers (*4, 5,6*). Tar has been reported from all oceans, with high concentrations in major shipping lanes (*6, 7, 8, 9*).

On the other hand, the ultimate fate of pelagic tar lumps and other petroleum residues in the ocean is largely unknown. Although tar is found on island beaches, coastal stranding in the open ocean cannot be a major mechanism for removal. For a fraction of the petroleum residues, evaporative weathering (*10, 11*) and microbial degradation (*13*) appear to be significant mechanisms of removal. Evaporation is normally limited to the more volatile fractions (below $C_{16\text{-}18}$ for paraffins).

The mean residence time of a pelagic tar lump has been estimated to be of the order of several months to a year (*1, 3, 4*) because the standing stock of tar lumps (5×10^5 metric tons) is approximately equal to the estimated yearly input after allowance for evaporation (*6*).

The biota associated with tar lumps also provide clues to their age. Organismal growth on tar lumps confirms their age is at least on the order of several months (*8, 9, 13, 14*). Growth of organisms on tar lumps may be an important mechanism for removal from surface waters; attachment of calcareous organisms (barnacles and tubeworms) change the specific gravity of tar lumps, causing them to sink.

Tar lumps collected in neuston nets may, however, be only a fraction of the total dispersed in the upper layers of the ocean. Particles flaking off pelagic tar lumps from weathering and organismal action become suspended in the water column. Seawater samples collected in the Sargasso Sea between 1 and 100-m depth were filtered (Whatman GFC #3 glass fiber and Millipore 0.45 μm) and pentane extracts were analyzed by gas chromatography (GC) (*15*). The pentane extracts were extremely similar to the paraffinic inclusions found in pelagic tar lumps. The total mass of these particles in the top 100 m of the water column off Bermuda was estimated to be four times the standing crop of pelagic tar lumps at the surface (*15*).

This chapter presents data on the extent to which petroleum residues find their way into the sediments of the Bermuda platform. Bermuda was chosen since its mid-Atlantic location provides a range of sedimentary environments from shallow lagoonal to abyssal without the complications of contamination by continental shelf runoff and coastal pollution. We present results of the analyses of the aliphatic fraction of 50 surface sediments (from the upper 1–3 cm) and two species of benthic algae.

Methods and Materials

Fifty sediment samples were collected around Bermuda from various subtidal environments including a transect from the high-tide line to 40-m depth along the South Shore (Figure 1). Divers using scuba

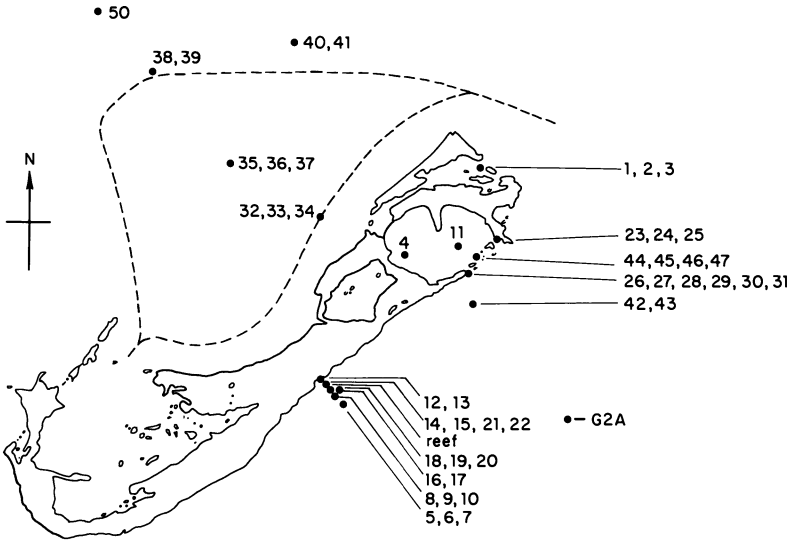


Figure 1. Locations of core samples

obtained samples in Plexiglas core tubes (6-cm diameter) capped with foil-lined rubber stoppers. The sediment core was immediately returned to the laboratory and prepared for extraction. One deep-water sediment sample (1400 m) was obtained with a Van Veen grab.

After interstitial water had drained from the cores approximately 50 g of wet surface sediment (top 1–3 cm) was placed in a preextracted cellulose thimble for soxhlet extraction. Care was taken not to use any sediment that had contacted the corer. All possible precautions were taken against contamination during sampling and handling of the samples.

The methods for hydrocarbon extraction and analysis are similar to those previously described (*16, 17, 18*). Sediment samples were soxhlet-extracted in methanol:benzene (1:1) for 48 hr. Copper wool was placed beneath the thimble to facilitate drainage and removal of elemental sulfur. Lipids were partitioned into a pentane–benzene layer (3 combined washes) followed by a wash of saturated NaCl solution, dried overnight with Na_2SO_4, and vacuum-evaporated to dryness. The extract was redissolved in pentane and fractionated by column chromatography on alumina over silica gel (both 5% deactivated with H_2O). Aliphatics were eluted with three column volumes of pentane, followed by three column volumes of benzene to elute aromatics. Only analyses of the pentane fractions are reported here.

The pentane fraction was analyzed on a Hewlett-Packard 5700A gas chromatograph equipped with a packed, medium resolution 3-m, SP-2100 on Supelcoport column (100–120 mesh) that was temperature pro-

grammed from 75° to 290°C at 8°/min with nitrogen carrier gas flow at 30 mL/min. Major peaks in the chromatogram were identified and calibrated by comparison of retention indices of known *n*-alkanes from external standards. Resolved peaks were quantitated using a Hewlett-Packard 3380S integrator. Procedural blanks were run with each set of extractions and showed a normal baseline with no resolved peaks.

Results

Sampling station locations are shown in Figure 1 and the results of gas chromatographic analysis of the pentane fraction given in Table I. Figure 2 summarizes the average amount of aliphatic hydrocarbons obtained in the various environments sampled. Standard deviations and other details are summarized in Table II.

As predicted, the enclosed harbors show highest concentrations of hydrocarbons: St. George's Harbor (107 μg/g dry sediment ± 35%) and Castle Harbor (45.9 μg/g ± 60%). Castle Harbor is used almost solely by recreational vessels while St. George's Harbor is used by larger commercial vessels such as ocean liners and cargo ships.

At two locations in the North Lagoon and the two stations at the mouth of Castle Harbor, South Shore, the concentrations are the same (8.3–9.1 μg/g). The chromatograms are in Figures 3 and 4, Cores 25, 28 and 29.

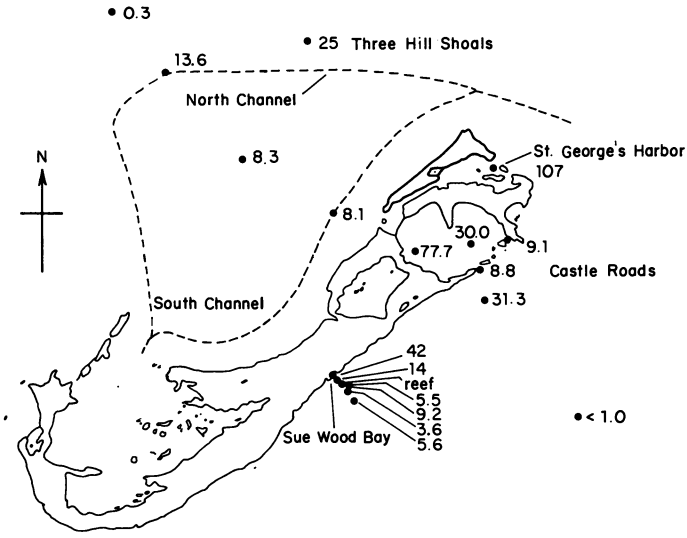


Figure 2. Total pentane-extractable hydrocarbons (μg/g dry sediment)

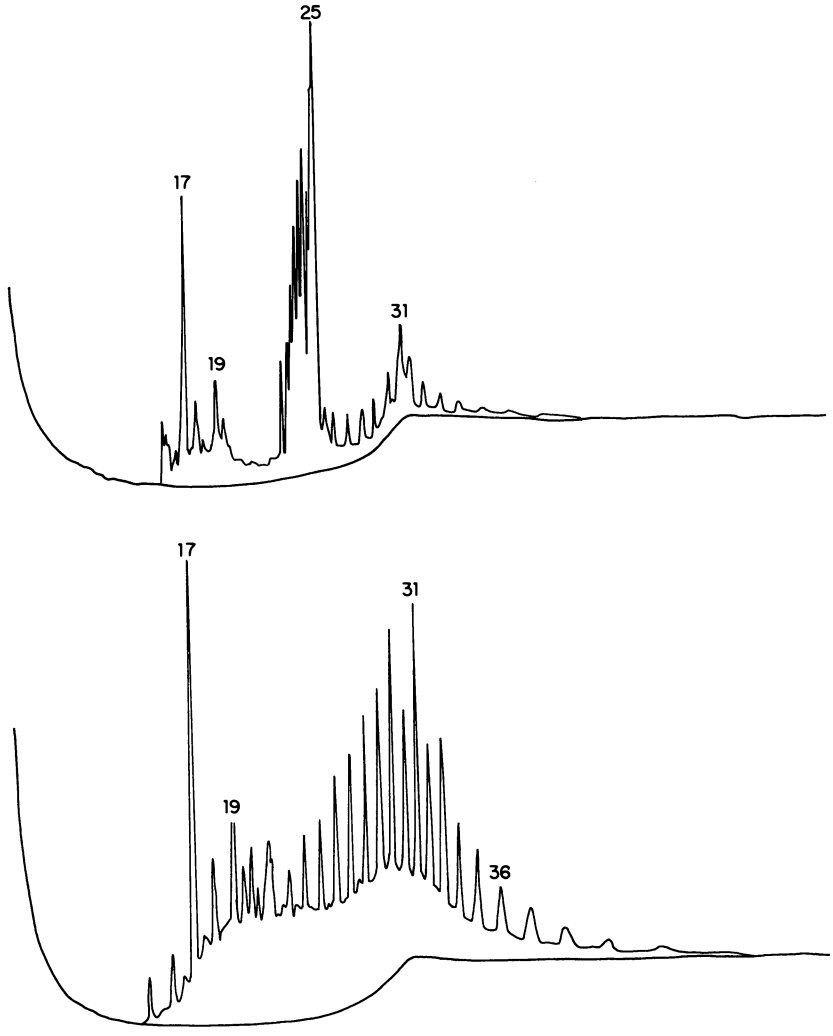


Figure 3. Gas chromatograms of core samples: (top) *28–77 Castle Roads, South Shore; 15.9 μg/g total, 5.5 μg/g C.U.M.;* (bottom) *29–77 Castle Roads, South Shore; 5.44 μg/g total, 3.87 μg/g C.U.M.*

The North Lagoon is scattered with patch reefs and all ships must enter Hamilton Harbor through the North or South Channels (Figure 2). Analyses of the cores from the North Channel and nearby Three Hill Shoals showed higher concentrations than the more remote parts of the North Lagoon: 13.6 μg/g and 25 μg/g, respectively. The chromatograms for these samples are shown in Figure 5, Cores 39 and 42. At the outer edge of the North Lagoon (40-m depth) concentrations are much lower (0.27 μg/g).

Table I. Results of Hydrocarbon Analyses

Sample	*Total HC (μg/gr)*	*Total n-Alkanes*	*Other Resolved Peaks*	*C.U.M. (μg/gr)*
1–77	61.34	1.08	0.04	60.23
2–77	122.86	4.89	26.18	91.80
2–77	149.35	2.17	32.06	115.12
3–77	96.18	——24.38——		71.80
4A–77				74.67
4A–77	77.71	7.27	9.19	61.25
4B–77				
4C–77		15.41	6.49	?
4D–77	51.94	4.14	7.81	39.99
5–77				4.65
5–77	3.98	0.32	0.03	3.63
6–77	5.87	0.35	0.14	5.38
6–77	6.49	0.67	0.12	5.69
7–77				5.77
7–77	5.79	0.42	0.15	5.22
8–77				3.16
8–77	2.00	——0.16——		1.84
8–77	3.18	0.49	0.03	2.66
9–77	4.52	——0.48——		4.04
9–77	3.80	——0.47——		3.34
9–77	7.04	0.16	0.05	6.83
9–77				8.43
10–77	4.37	0.30	0.41	3.66
11A–77	28.13	1.76	0.31	26.06
11B–77	31.86	1.10	0.38	30.38
12–77	65.77	17.20	2.77	45.79
13–77	17.41	2.78	0.11	14.52
14–77	15.46	——5.04——		10.43
14–77	15.74	1.12	0.31	14.31
15–77	13.93	——3.30——		10.63
15–77	16.02	1.27	0.76	13.99
16–77				5.18
16–77	8.17	1.88	0.50	5.79
17–77	3.06	0.14	0.04	2.89
18–77	10.64	0.45	0.19	10.0
19–77				9.53
19–77	10.61	0.85	0.29	9.48
20–77	6.42	0.45	0.05	5.92
21–77	12.55	2.55	5.20	4.80
22–77	9.99	——0.90——		9.09
22–77	10.82	0.76	0.18	9.88

and CPIs for the Aliphatic Fraction

CPI Total	*CPI* ≤ 20	*CPI* ≥ 21	C_{17}*:Pristane*	C_{18}*:Phytane*	*Pristane: Phytane*
1.37	1.99	1.22	N.D.	1.0	N.D.
1.68	1.79	1.64	1.14	0.74	3.04
1.49	2.85	1.32	N.D.	0.96	N.D.
0.88	1.58	0.82	N.D.	1.28	N.D.
0.66	2.78	0.64	N.D.	1.0	N.D.
0.71	2.69	0.67	N.D.	1.0	N.D.
1.22	5.6	0.76	2.64	1.60	6.46
2.50	5.45	1.44	2.93	1.36	2.86
2.58	8.9	0.91	6.12	1.48	3.0
1.41	3.41	0.82	2.90	1.57	2.71
0.72	0.70	0.73	2.67	2.00	0.22
1.03	3.19	0.71	1.87	1.4	2.70
1.43	3.99	0.82	N.D.	1.0	N.D.
1.36	2.81	1.23	N.D.	0.80	N.D.
1.86	2.36	1.78	N.D.		N.D.
1.20	7.76	0.88	7.30	1.67	3.36
5.90	27.81	1.07	8.81	1.80	14.33
1.42	2.95	1.20	1.0	1.47	7.11
1.53	5.99	0.97	2.69	1.28	6.97
0.89	1.72	0.83	0.59	1.67	8.12
0.94	1.72	0.74	N.D.	1.33	N.D.
1.77	4.66	1.18	2.88	1.50	5.55
2.10	5.40	1.18	1.08	2.33	16.74
4.01	25.58	0.75	30.13	1.25	2.13
1.65	11.67	1.36	2.5	1.0	67.55
1.22	3.06	0.99	0.82	1.50	10.67

(continued)

Table I.

Sample	*Total HC (μg/gr)*	*Total n-Alkanes*	*Other Resolved Peaks*	*C.U.M. (μg/gr)*
23–77				7.98
23–77	11.03	0.59	0.20	10.24
24–77	2.81	0.17	0.16	2.48
25–77	13.35	4.27	2.09	6.99
27–77	1.66	0.43	0.12	1.11
28–77				3.58
28–77	15.93	4.64	5.94	5.35
28–77				3.51
29–77	5.44	1.46	0.11	3.87
30–77	4.31	1.21	0.30	2.80
31–77				
32–77	7.09	0.59	0.24	6.25
33–77	6.88	——1.18——		5.71
33–77	10.33	0.92	0.73	8.67
34–77	2.04	0.11	0.05	1.88
35–77	11.81	——2.95——		8.86
35–77	7.19	1.92	0.90	4.37
36–77				7.36
36–77	9.92	1.72	0.86	7.34
37–77	6.39	——1.22——		5.17
37–77	6.04	0.84	0.44	4.76
38–77		5.65	1.59	
39–77	13.64	4.40	0.13	9.11
40–77	25.47	6.49	0.85	18.14
41–77	25.41	——10.21——		15.20
41–77	24.41	8.67	2.45	13.30
42–77	31.32	8.24	1.20	21.88
43–77				
45–77	16.61	5.88	0.84	9.89
47–77				
48–77	20.85	5.12	0.32	15.41
49–77				4.13
49–77				0.22
49–77	0.71	0.17	0.01	0.53
50–77	0.27	0.20	0.02	0.05
51–77	0.65	0.20	0.01	0.44
G2A				
G2A				
Udotea fans	182.50	——78.42——		104.08
Udotea roots	81.63	——2.39——		79.25
Penicillus	205.56	——38.34——		167.22

Continued

CPI Total	*CPI* ≤ 20	*CPI* ≥ 21	C_{17}*:Pristane*	C_{18}*:Phytane*	*Pristane: Phytane*
1.10	1.86	0.94	1.24	1.29	3.43
1.08	2.14	0.84	1.22	1.0	2.25
1.01	1.52	0.99	N.D.	1.0	N.D.
2.70	7.42	1.27	3.55	1.33	7.55
1.94	4.25	1.59	2.68	1.75	2.55
1.28	2.22	1.19	0.70	1.71	8.47
1.07	2.18	0.95	3.58	1.88	2.22
2.76	5.44	1.93	N.D.	2.0	N.D.
1.99	4.57	1.39	N.D.	1.71	N.D.
0.64	0.32	0.80	0.80	0.80	1.79
2.70	7.65	1.60	N.D.	3.50	N.D.
2.78	3.97	2.02	N.D.	2.0	N.D.
5.11	11.31	3.06	N.D.	N.D.	N.D.
1.15	0.81	1.22	1.11	1.66	1.11
1.02	0.64	1.04	1.0	1.60	0.96
1.14	1.99	1.00	2.25	1.54	2.39
1.02	1.39	0.93	2.21	1.85	2.18
1.16	1.69	1.02	1.24	1.60	2.24
1.01	1.77	0.86	1.59	2.33	2.95
1.10	1.63	0.99	1.08	1.33	1.54
2.05	1.96	2.11	N.D.	N.D.	N.D.
1.71	2.50	1.58	N.D.	N.D.	N.D.
1.62	2.17	1.43	N.D.	N.D.	N.D.

Table II. Summary of

Core Numbers	*Total Pentane-Extractable Hydrocarbons*		
	X	*S*	
St. George's Harbor			
1, 2, 3	107	38	[4]
Castle Harbor			
4A	78		[1]
Castle Roads			
27, 28, 29, 30, 45	8.8	7.0	[5]
29, 30 (trough)	4.9	0.8	[2]
27, 28, 45 (crest)	11	8.4	[3]
42	31.3		[1]
Nonsuch Island			
23, 24, 25	9.1	5.5	[3]
Three Hill Shoals			
40, 41	25	0.6	[3]
South Channel			
32, 33	8.1	1.9	[3]
Patch Reefs			
35, 36, 37	8.3	2.5	[5]
North Channel			
38, 39	13.6		[1]
Sue Wood Bay, Devonshire Transect			
12, 13	42	34	[2]
21, 22, 14, 15	14	2.4	[7]
5, 6, 7	5.5	1.1	[4]
18, 19, 20	9.2	2.4	[3]
8, 9, 10	3.6	1.0	[5]
16, 17	5.6	3.6	[2]

* X, mean value; S, standard deviation; [ ] = number of analyses.

The South Shore (Sue Wood Bay, Devonshire) transect consists of 6 stations (17 cores) from the subtidal zone (2-m depth) to 40-m depth (2 km from shore). A long patch reef, which runs the length of the South Shore, separates the transect between the second and third station (*see* Figure 2). The two stations on the inside of the reef (Figure 6) show higher concentrations (14–42 μg/g) than those stations on the outside of the reef (3.6–9.2 μg/g) (Figure 4, Core 8). One deep-water sediment sample (G2A, 1400 m) was lower still (1.0 μg/g).

Benthic green algae (*Udotea* sp. and *Penicillus* sp.) collected from St. George's Harbor showed large amounts of total aliphatics: 206 μg/g

Core Sample Analyses[a]

C.U.M.			*Resolved Peaks*			*Total n-Alkanes*		
X	*S*		*X*	*S*		*X*	*S*	
85	24	[4]	23	15	[4]	2.7	2.0	[3]
68	9.5	[2]	16		[1]	7.3		[1]
4.3	2.8	[7]	4.2	4.3	[5]	2.7	2.4	[5]
3.3	0.76	[2]	1.5	0.04	[2]	1.3	0.18	[2]
5.0	3.7	[5]	6.0	6.0	[3]	3.7	2.9	[3]
21.8		[1]	9.4		[1]	8.2		[1]
7.0	3.1	[4]	2.4	3.5	[3]	1.7	2.3	[3]
16	2.4	[3]	9.6	2.0	[3]	7.6	1.5	[2]
6.9	1.6	[3]	1.2	0.41	[3]	0.76	0.23	[2]
6.3	1.8	[6]	2.2	0.85	[5]	1.5	0.57	[3]
9.1		[1]	5.9	1.9	[2]	5.0	0.88	[2]
30	22	[2]	11	12	[2]	10	10	[2]
12	2.1	[7]	2.0	1.7	[7]	1.4	0.78	[4]
5.1	0.81	[6]	0.55	0.18	[4]	0.44	0.16	[4]
8.7	1.9	[4]	0.76	0.34	[3]	0.74	0.25	[3]
4.2	2.2	[8]	0.42	0.21	[6]	0.32	0.17	[3]
4.6	1.5	[3]	1.3	1.6	[2]	1.0	1.2	[2]

dry weight for *Penicillus* sp., 182 μg/g for *Udotea* fans, and 97 μg/g for *Udotea* roots (261 μg/g total). In the *Udotea* extracts (Figure 7) large peaks occurred at C_{17}, C_{23}, C_{25}, and a double peak appeared at the retention time expected for normal C_{19}. *Penicillus* showed dominant peaks at C_{17}, C_{21}, C_{23}, and a very large double peak at C_{19}. Both algae produced chromatograms with an unresolved envelope and a series of normal alkanes similar to those in the sediment extracts. For example, the chromatogram of the holdfast (roots) of the benthic algae *Udotea* sp. (Figure 7) is identical to sediment extracts from a nearby location in St. George's Harbor.

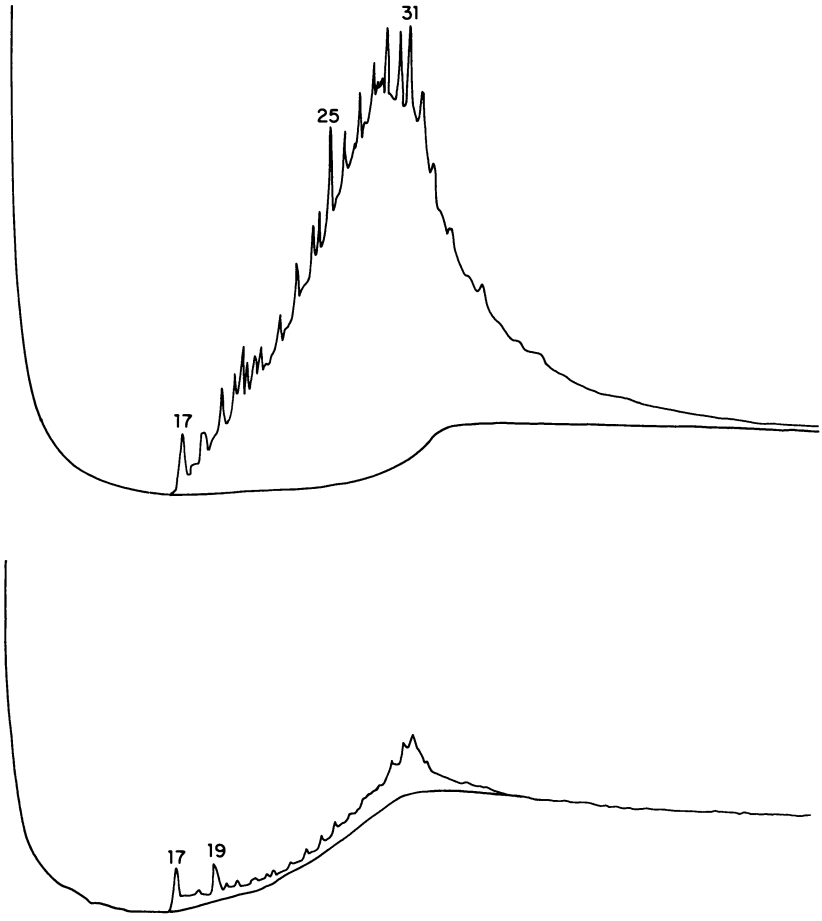


Figure 4. Gas chromatogram of core samples: (top) *25–77 Nonsuch Island, North Beach Channel; 13.35 μg/g total, 6.99 μg/g C.U.M.;* (bottom) *8–77 1 km off Devonshire Bay; 3.18 μg/g total, 2.66 μg/g C.U.M.*

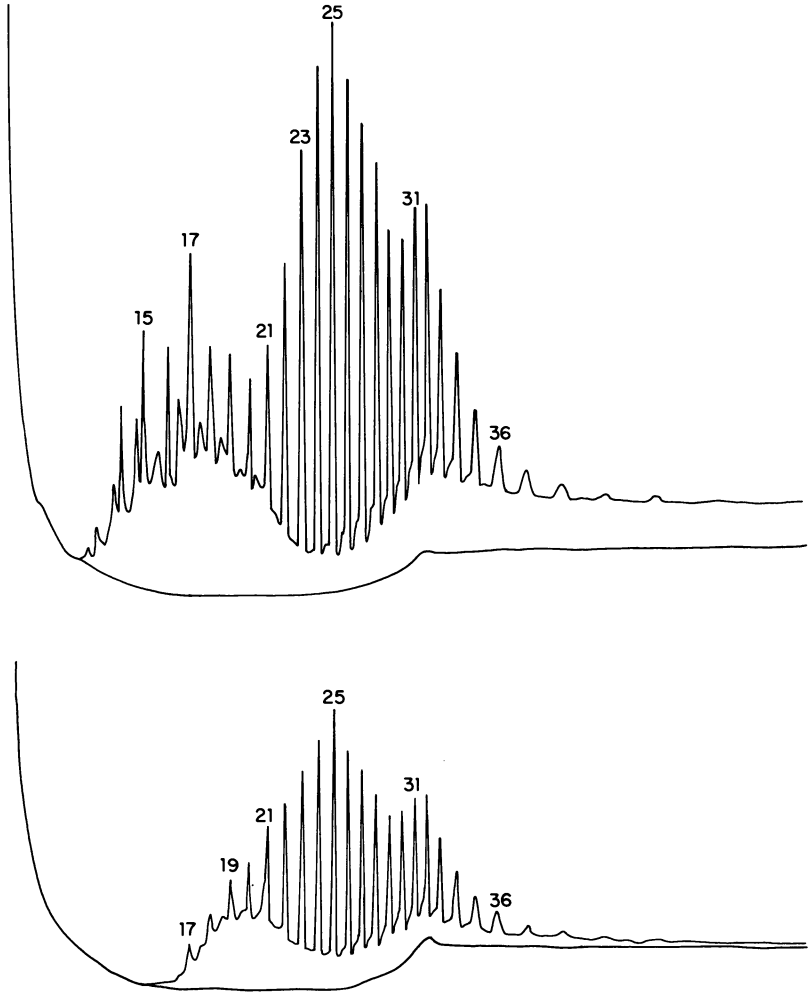


Figure 5. Gas chromatogram of core samples: (top) *42–77 Three Hill Shoals; 31.3 μg/g total, 21.8 μg/g C.U.M.;* (bottom) *39–77 North Channel; 13.6 μg/g total, 9.1 μg/g C.U.M.*

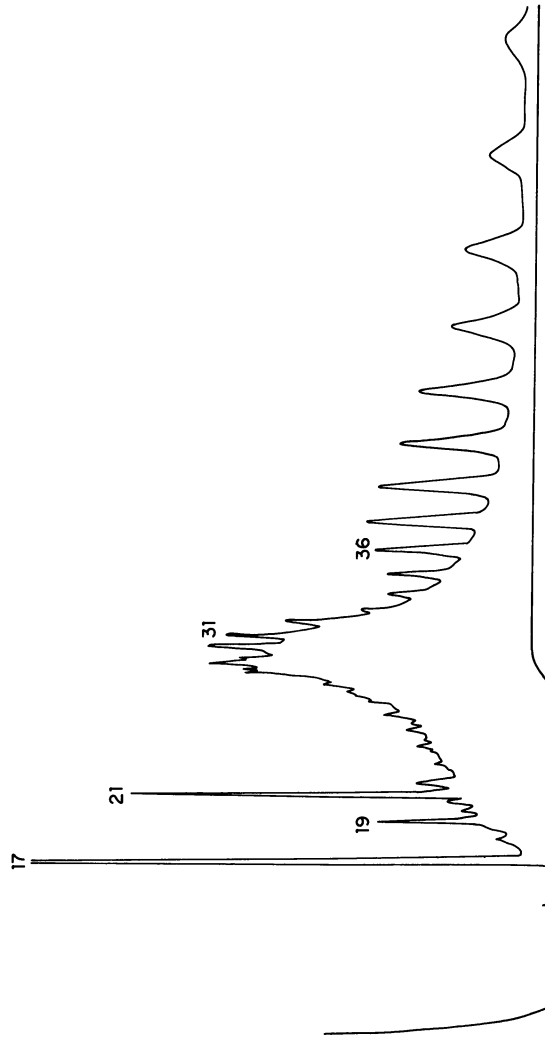


Figure 6. Gas chromatogram of core sample 12–77 Sue Wood Bay, Devonshire; 65.7 μg/g total, 45.7 μg/g C.U.M.

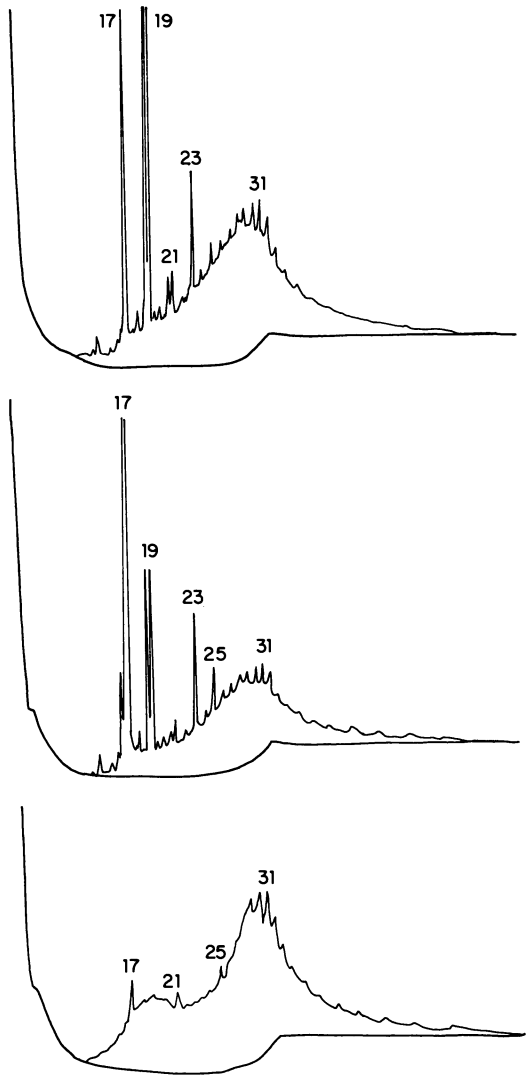


Figure 7. Gas chromatograms: (top) Penicillus *sp. St. George's Harbor; 205. μg/g total, 167. μg/g C.U.M.;* (center) Udotea *sp. fans, St. George's Harbor; 182. μg/g total, 104. μg/g C.U.M.;* (bottom) Udotea *sp. roots, St. George's Harbor; 81.6 μg/g total, 79.2 μg/g C.U.M.*

Biogenic Hydrocarbons in the Marine Environment

Knowledge of the *n*-alkane distributions in marine organisms, as well as in terrestrial plants, is of considerable importance in any study of sedimentary hydrocarbons. *n*-Alkanes in organisms often exhibit a characteristic predominance of odd-numbered homologues over even-numbered ones. This feature has been observed in organisms grown in laboratory cultures, as well as in those taken from the natural environment (*18, 19, 20*). Odd-carbon preference may be expressed by the ratio between the total weight of odd-carbon homologues and the weight of the even-carbon homologues. This ratio is known as the Carbon Preference Index (CPI), and is calculated as follows:

$$\mathrm{CPI}_{x \to y} = \left(\frac{\sum_{n=y}^{n=x} \mathrm{HC}_{\mathrm{odd}}}{\sum_{n=y}^{n=x} \mathrm{HC}_{\mathrm{even}}} \right) \frac{D}{N}$$

where D = number of even-numbered homologues between x and y, inclusive; and N = number of odd-numbered homologues between x and y, inclusive.

Odd–even ratios are frequently observed to vary from one portion of a given *n*-alkane distribution to another. For example, see the chromatogram of Core 12–77 from South Shore transect (Figure 6). The CPI ratio for *n* not greater than 20 is 7.76 and contains a large bias produced by prominent C_{17} and C_{19} biogenic peaks. This is typical of most of the samples listed in Table I. In contrast, the CPI for *n* not less than 20 is within 20% of 1 in most cases.

Marine algae are characterized by predominant *n*-alkanes at C_{15}, C_{17}, C_{19}, and C_{21}. Clark (*18*) found that the major classes of benthic algae fall into two groups based on this difference. In brown algae (Phaeophyceae, both Fucales and Laminariales), *n*-C_{15} predominates (constitutes up to 98% of the total *n*-alkane content). In red and green algae (Rhodophyceae and Chlorophyceae, respectively), *n*-C_{17} predominates (representing between 36% and 95% of the total *n*-alkanes). This finding was later confirmed by Youngblood and Blumer (*19*).

In marine phytoplankton the odd-carbon homologues between *n*-C_{15} and *n*-C_{21}, inclusive, tend to dominate the *n*-alkanes. Recent analyses (*15*) of *Sargassum natans* and *Sargassum fluitans* have shown biogenic

components at C_{15}, C_{17}, and to a lesser extent C_{19}, but if there is an odd-carbon predominance at higher-molecular-weight alkanes, it is obscured by the ubiquitous petroleum pollution of the Sargasso Sea.

Hydrocarbons in Marine Sediments

Normal Alkanes. Of all alkanes found in sediments, *n*-alkanes are by far the most abundant (*21*). Studies of *n*-alkane distributions in uncontaminated recent sediments by Cooper and Bray (*22, 23*) have indicated that they exhibit a very strong odd-carbon predominance, with CPIs ranging from 2.4 to 5.5. These high CPI values indicate that planktonic or benthic algae are not the sole contributors of sedimentary *n*-alkanes. Clark and Blumer (*24*) suggest that organisms indigenous to the sediments or terrestrial plant detritus may be providing a large fraction of the *n*-alkanes observed in sediments with such a pronounced odd-carbon predominance. In addition to these high CPI values, unpolluted recent sediments often show relatively large *n*-alkane concentrations within the range from *n*-C_{23} to *n*-C_{31} (*25*). Our samples often showed substantial concentrations of C_{23}–C_{31} alkanes but little odd-carbon predominance (Table I and Figures 3–6).

Stevens, Bray, and Evans (*26*) were the first to point out the distinct difference between the distributions of *n*-alkanes from *n*-C_{22} to *n*-C_{34} in recent sediments, crude oils, and ancient sediments. Recent sediments show far less odd-carbon preference, and most crude oils show practically none at all.

Isoprenoids. While both pristane and phytane (as well as other isoprenoids) are quite common in ancient sedimentary rocks (*28, 29*), only pristane has been found in uncontaminated recent sediments. Blumer and Snyder (*30*) have suggested that organisms rich in pristane, such as copepods, are a major source of sedimentary pristane.

Phytane in sediments would normally be interpreted as a result of petroleum contamination, but it might also be a natural product of post-depositional reactions involving the catalytic hydrogenation of phytadienes (*20, 30*). The latter group of compounds has been detected in zooplankton (*31*) and may comprise a large proportion of the olefinic fraction in benthic algae (*32*). Sedimentary phytane could also be a product of clay-catalyzed hydrogenation of sedimentary phytol (*30*). Because of the presence of phytadiene in sediments, Lytle and Lytle (*20*) caution against the use of phytane as an indicator for petroleum contamination in recent sediments. However, the work of Blumer and Snyder (*30*) suggests that the natural reduction of phytadiene to phytane takes such a long time that natural phytane is not likely to be found in

recent sediments. In our samples, phytane was nearly always present in amounts comparable with n-C_{18} but often quite a bit smaller than pristane or C_{17}.

Differentiating Between Petroleum and Biogenic Hydrocarbons

As we have seen, biogenic hydrocarbons in the sediments under study tend to be few in number and their chromatograms are simple in structure. Petroleum, on the other hand, contains many thousands of components, of which only the normal paraffins and isoprenoids are easily quantified by low-resolution GC. The remaining compounds appear as a complex unresolved mixture. This distinction has been accepted by many workers (*6, 33*) as the basis for interpreting gas chromatograms. Methods such as fluorescence spectroscopy depend on the relative scarcity of polycyclic aromatic hydrocarbons in biogenic material and the relative abundance of these compounds in petroleum; but it does not give an analysis on a compound-by-compound basis. High-resolution GC, of course, permits more detailed separation and accurate quantification of many petroleum components. The most detailed work (*27, 34, 35*) has employed a combination of column chromatography, GC, and mass spectrometry to separate homologous series of substituted polycyclic aromatic hydrocarbons in an attempt to distinguish between the input of these compounds from pyrogenic sources (forest fires, industrial combustion) and petroleum pollution.

We have used only medium-resolution GC thus far in our studies and have adopted the following criteria for distinguishing biogenic and petroleum hydrocarbons:

1. Presence of an envelope (complex unresolved mixture) well above the baseline produced by a blank sample. This is an indication of a very large number of components, typical of petroleum (*6, 16, 17*). Although the quantitative amount depends on column resolution as well as the sample composition, resolution of the various chromatograms reported here is essentially the same, and thus a comparison of the values within the series can be quantitatively meaningful.
2. Presence of large individual components, typically at the retention times for normal C_{15}, C_{17}, C_{19}, etc. This is indicative of the class of biogenic hydrocarbons typically found in marine algae, as discussed above (*6, 15*). (*See also* Chapter 1 of this volume.)
3. CPI showing odd/even preference substantially greater than one. This is an indication of more complex biogenic sources, but it is easily obscured by petroleum contamina-

tion, which tends to reduce CPI to approximately 1.0. The CPI index ranges must be used with care and are never a substitute for trained visual examination.

4. The ratio of the isoprenoids pristane (C_{19}) and phytane (C_{20}). When these compounds are resolved from the normal C_{17} and C_{18} peaks with which they are closely associated in the chromatogram, their ratio (if near one) is diagnostic for petroleum, or (if pristane >> phytane) for biogenic sources. Pristane is common in plankton (copepods) and various marine animals; phytane has only rarely been reported except as a component of petroleum (*30*). In Table I all the C_{18}:phytane ratios are between 0.8 and 2.3, as would be expected for a petroleum source. Pristane:phytane ratios are (with one exception) substantially greater than one, indicating a planktonic source as well.

Discussion

Chromatograms of hydrocarbons from the living portions of the algae *Udotea* sp. and *Penicillus* sp. show clear peaks at the retention times for C_{17}, C_{19}, C_{21}, and C_{23} (C_{17} being 73% of all resolved peaks in *Udotea* fans), including multiple peaks that probably represent phytadienes (*20*). The doublets at C_{19} and C_{17} respectively in *Penicillus* account for 50% and 18% of the resolved peaks in that chromatogram. These are almost certainly biogenic. In addition there is a large unresolved envelope and a series of normal paraffins (C_{23}–C_{31}) with CPI approximately one. These features are almost certainly the result of petroleum contamination of St. George's Harbor, where the algae were collected. The "root" (holdfast) of *Udotea,* in contrast to the fan parts, shows almost no normal paraffins and little of the biogenic compounds present in the fans. Indeed, the chromatogram of the roots is identical to that obtained from the nearby sediments. It appears to be a partially degraded petroleum residue, the normal paraffins possibly having been removed by an endemic population of hydrocarbon-utilizing microorganisms (Sleeter, unpublished data).

Hydrocarbons in sediments from Castle Harbor, which have chromatograms similar to that labeled "Nonsuch" (25–27, Figure 4), also appear to be primarily petroleum residues, although less degraded than those from St. George's Harbor. In contrast, chromatograms of sediments from Castle Roads, just south of the outlet of Castle Harbor, showed great variability. One sample (29–77, Figure 3) appears to be a combination of an unresolved envelope, waxy paraffin mixture (C_{22}–C_{40} with CPI $\sim$ 1.0), and biogenic (C_{17}, C_{19}, C_{29}, C_{31}) hydrocarbons. It probably represents inputs from degraded pelagic tar as well as features from the local algae (C_{17}, C_{19}) and terrestrial plants (C_{29}, C_{31}). Another chromatogram

(28–77, Figure 3) is quite different. It shows little that could be identified as petroleum, and its most dominant feature is a group of at least nine peaks in the range of retention times corresponding to C_{22}–C_{25}, as well as the usual C_{17} and C_{19} biogenic peaks. This central group of closely spaced peaks (of which at least some are probably phytadienes) may have been the same as those noted by Lytle and Lytle (*20*) in the sediments of the Florida Shelf and by Gearing (*37*) in the Northern Gulf of Mexico.

At Castle Roads on the South Shore, a brief study was made of the partitioning of hydrocarbons between crests and troughs of the sand ripples in the subtidal region. The total hydrocarbons as well as the unresolved envelope were lower in the troughs (Samples 29, 30) than in the crests (Samples 27, 28, 45). Furthermore, the variability was much less in the troughs than on the crests (3% vs. 85% for the resolved peaks' standard deviation).

Further to the southwest, at Sue Wood Bay, Devonshire, we made a detailed transect from subtidal to 40-m depth (Table II). The shallow sediment (Figure 6) shows features to be expected from an area well known for its heavy pollution by pelagic tar. Both the unresolved envelope and the high-molecular-weight paraffins (C_{28}–C_{45}) are characteristic of crude oil sludge and of many pelagic tar lumps. Superimposed on this, however, are the usual biogenic hydrocarbons at C_{15}, C_{17}, C_{19}, and C_{21}. The other stations inside the reef were similar. The deeper area, outside the reef, shows far less hydrocarbon content (8–77, Figure 4) but the features are similar: an unresolved envelope with normal paraffins, plus biogenic peaks at C_{17} and C_{19}.

It appears that some pelagic tar stranded on the south shore beaches is degraded sufficiently to form sand-size grains (these can be observed with a low-power magnifier as black particles among the predominantly pink and white algal and coralline sands). These grains, although less dense than the calcareous sands, move with the sands and are transported subtidally to deeper waters. At the outlet of Castle Harbor, we have seen (Core 42–77) that this transport can extend for 1 km and to depths of at least 25 m. Where the boiler reefs impede transport of sediment (e.g., Sue Wood Bay), the amount of these tar particles that can be transported beyond the reef is much smaller, and the reef acts as a clear line of demarcation between a high-hydrocarbon and low-hydrocarbon sediment region.

The two ship channels in the North Lagoon have substantially different hydrocarbon contents: the North Channel is higher than the South Channel, even though traffic is greater in the South Channel. This difference may be the result of disturbance in the fine-grained (*36*) sediments of the shallower near-shore region, which disperse the hydrocarbon pollu-

tion from the ships. In addition, during the summer (when these samples were taken) there is a general offshore movement of water, because evaporation in the North Lagoon (*36*) produces hypersaline water of density greater than the surrounding Sargasso Sea. These offshore currents would tend to transport sediments from the South Channel toward the North Channel. Note that the samples from Three Hill Shoals (just north of the North Channel, Figure 5) have large unresolved envelopes and high-molecular-weight paraffins. Note also that the lowest-molecular-weight peaks are about C_{13}, indicating that the petroleum was not extensively weathered (as is pelagic tar) and probably derived from ship bilges or ballast (*38*).

Further offshore, either to the north (50–77) or the south (G2A), the hydrocarbon content of the sediment becomes very much lower than the near-shore areas, less than 1 μg/g total. The chromatograms for these deep (to 1400 m) samples consisted of a small unresolved envelope and many small paraffin peaks (but significantly greater than the blank for that set of samples), and appear to be primarily of petroleum origin. Additional analysis of deeper sediments will help elucidate the possible sources and modes of transport of hydrocarbons in this region.

Acknowledgments

This work was supported by the National Science Foundation under grants OCE 76-19901 and OCE 77-18662, and by a grant from the Zemurray Foundation. The authors thank the personnel of the Bermuda Biological Station, particularly David Martin, John Holland, and Tony Riker (who volunteered his time) for their good-humored and patient assistance with the field work. This is Contribution No. 758 from the Bermuda Biological Station for Research.

Literature Cited

1. Morris, B. F. *Science* **1971,** *173,* 430–432.
2. Morris, B. F.; Butler, J. N.; Zsolnay, A. *Environ. Conserv.* **1975,** *2,* 275–281.
3. McAuliffe, C. D. *Mar. Sci. Commun.* **1976,** *2,* 13–42.
4. Butler, J. N.; Morris, B. F.; Sass, J. *"Pelagic Tar from Bermuda and the Sargasso Sea,"* Bermuda Biological Station, 1973, No. 10.
5. Blumer, M.; Ehrhardt, M.; Jones, J. H. *Deep-Sea Res.* **1973,** *20,* 239–259.
6. "Petroleum in the Marine Environment"; National Academy of Sciences: 1975.
7. "Marine Pollution Monitoring (Petroleum)," Junghans, R. C., Ed. National Bureau of Standards, **1974,** No. 409.
8. Sleeter, T. D.; Morris, B. F.; Butler, J. N. *Deep-Sea Res.* **1974,** *21,* 773–775.
9. Ibid. **1976,** *23,* 467–474.

10. Butler, J. N. *Mar. Chem.* **1975**, *3*, 9–21.
11. Butler, J. N. In "Marine Pollutant Transfer"; Windom, H. L., Duce, R. A., Eds.; Lexington Books: Lexington, MA, 1976; Chapter 9, pp. 201–211.
12. Bartha, R.; Atlas, R. M. *Adv. Appl. Microbiol.* **1977**, *22*, 225–266.
13. Heyerdahl, T. *Biol. Conserv.* **1971**, *3*, 164–167.
14. Ehrhardt, M.; Derenbach, J. *Meteor Forshungsberichte* **1978**, *19*.
15. Morris, B. F.; Butler, J. N.; Sleeter, T. D.; Cadwallader, J. In "Marine Pollutant Transfer"; Windom, H. L., Duce, R. A., Eds.; Lexington Books: Lexington, MA, 1976; Chapter 10, pp. 213–234.
16. Blumer, M.; Robertson, J. C.; Gordon, J. E.; Sass, J. *Biochemistry* **1969**, *8*, 4067–4074.
17. Farrington, J. W.; Giam, C. S.; Harvey, G. R.; Parker, P.; Teal, J. M. In "Marine Pollution Monitoring: Strategies for a National Program"; Goldberg, E. D., Ed.; Workshop, University of Southern California and the Allan Hancock Foundation, 1972; pp. 152–176.
18. Clark, R. C., Jr. M.S. Thesis, MIT, Cambridge, MA, 1966.
19. Youngblood, W. W.; Blumer, M. *Mar. Biol.* **1973**, *21*, 163–172.
20. Lytle, J. S.; Lytle, T. F. In "Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems"; Wolfe, D. A., Ed.; Pergamon: Elmsford, NY, 1977; Chapter 41, pp. 404–412.
21. Meinschein, W. G. In "Organic Geochemistry"; Eglinton, G., Murphy, M. T. J., Eds.; Springer: New York, 1969; pp. 330–356.
22. Bray, E. E.; Evans, E. D. *Geochim. Cosmochim. Acta* **1961**, *22*, 2–9.
23. Cooper, J. E.; Bray, E. E. *Geochim. Cosmochim. Acta* **1963**, *37*, 1127–1133.
24. Clark, R. C.; Blumer, M. *Limnol. Oceanogr.* **1967**, *12*, 79–87.
25. Giger, W.; Reinhard, M.; Schaffner, C.; Stumm, W. *Environ. Sci. Technol.* **1974**, *8*(5), 454–455.
26. Stevens, N. P.; Bray, E. E.; Evans, E. D. *Am. Assoc. Pet. Geol. Bull.* **1956**, *40*, 975–983.
27. Farrington, J. W.; Tripp, B. W. *Geochim. Cosmochim. Acta* **1977**, *41*, 1627–1641.
28. Meinschein, W. G., Barghoorn, E. S.; Schopf, J. W. *Science* **1964**, *145*, 262–264.
29. Eglinton, G.; Scott, P. M.; Belsky, T.; Burlingame, A. L.; Calvin, M. *Science* **1964**, *145*, 263–264.
30. Blumer, M.; Snyder, W. D. *Science* **1965**, *150*, 1588–1589.
31. Blumer, M.; Thomas, D. W. *Science* **1965**, *147*, 1148–1149.
32. Lytle, J. S.; Lytle, T. F., unpublished data.
33. Zafiriou, O. C. *Anal. Chem.* **1973**, *45*, 952–956.
34. Youngblood, W.; Blumer, M. *Geochim. Cosmochim, Acta* **1975**, *39*, 1303–1314.
35. Hites, R. A.; LaFlamme, R. E. *Geochim. Cosmochim. Acta* **1977**, *42*, 289–303.
36. Morris, B. F.; Barnes, J.; Brown, I. F.; Markham, J. *"The Bermuda Marine Environment,"* Bermuda Biological Station, 1977, No. 15.
37. Gearing, P.; Gearing, J. N.; Lytle, T. F.; Lytle, J. S. *Geochim. Cosmochim. Acta* **1976**, *40*, 1005–1017.
38. Sleeter, T. D.; Butler, J. N. *Environ. Conserv.* **1978**, *5*, 1–4.

RECEIVED October 31, 1978.

13

Polycyclic Aromatic Hydrocarbons in Marine/Aquatic Sediments: Their Ubiquity

RONALD A. HITES[1], R. E. LAFLAMME, and J. G. WINDSOR, JR.

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139

Fifty soils and sediments from many areas of the globe have been analyzed for polycyclic aromatic hydrocarbons (PAH) using gas chromatographic mass spectrometry. The results of these analyses indicate that PAH are ubiquitous. The source of most of the PAH in these samples is combustion. The concentrations of PAH are highest in areas of high anthropogenic activity. PAH concentrations relative to distance from anthropogenic point sources also indicate that the major source of PAH in these sediments is attributable to human activity.

Polycyclic aromatic hydrocarbons (PAH) occur widely in the lithosphere. They have been reported in various soils (*1, 2*), in marine sediments (*1–3*), and in urban limnic sediments both in the United States (*4, 5*) and in Europe (*6, 7, 8*). The concentrations of PAH range from less than 100 ppb for abyssal plain sediments to more than 100,000 ppb for sediments from highly urbanized areas. Much of the past work on the organic geochemistry of PAH has centered on understanding their sources, and there now seems to be a consensus (*1, 2, 3*) that most (but not all) PAH in the sedimentary environment are attributable to combustion processes. The goal of this chapter is to test this consensus. We use two experimental approaches for this study.

The first approach is an analysis of soils and sediments from many different locations. Since one facet of source determination is to distinguish between anthropogenic and natural contributions, it is desirable

[1] Current address: School of Public and Environmental Affairs and Department of Chemistry, Indiana University, Bloomington, IN 47405.

0-8412-0475-6/80/33/289$05.75/0

to analyze samples close to and far from human activity. Thus, we report on the analyses of approximately 50 samples from all over the globe. Included in these samples are sites in the northeast and continental United States, deep ocean sediments, soil samples from South Pacific islands and atolls, and sediments from the Southern Hemisphere. These samples include most types of depositional environments: soils (both forested and prairie) and sediments (oxic and anoxic; fresh water, marine, and estuarine; deep ocean and near shore). In each case, the quantitative and qualitative composition of the PAH assemblages have been measured. These data provide valuable information on the global distribution of PAH. By correlating PAH distributions and concentrations with sample locations, an assessment of the combustion source in these samples, either anthropogenic or natural, can be made.

The second experimental approach to source recognition is the analysis of the PAH mixtures in samples along a transect from a point source. Since anthropogenic burning of fossil fuels is known to produce PAH, a logical point source would be a large city or group of cities. Thus, samples taken from the New York Bight, near New York City, and on a transect through the Hudson Channel and Canyon out to the Abyssal Plain are analyzed. The PAH concentrations in these samples give information on the direct environmental impact of an anthropogenic point source as well as an indication of the impact on areas located at varying distances from it. These data can also be used for understanding transport mechanisms of PAH. A similar treatment has been used for samples taken on a transect from Boston through Massachusetts Bay to the Gulf of Maine and is reported elsewhere (*9*).

Experimental

Sample Preparation and PAH Isolation. All samples were treated so that the portion extracted represented the entire sample. In the soil and sediment samples, large twigs and stones were removed either by sifting or by manually removing them with tweezers. Extremely wet sediments were slurried to obtain a representative grain-size distribution. The samples (5–300 g, depending on availability) were soxhlet-extracted (without drying) with methanol for 24 hr followed by methanol–benzene (2:3) for an additional 24 hr. The benzene and methanol were removed under vacuum. The remaining water was extracted with five volumes of hexane followed by one volume of methylene chloride. The extracts were combined, evaporated, dissolved in cyclohexane–nitromethane (1:1), and extracted with five volumes of nitromethane (*10*). The nitromethane was removed at 40°C under vacuum. The extract was then subjected to column chromatography on 2.0 g of silicic acid with elution by 300 mL of hexane. The hexane was removed and the eluate dissolved in methylene chloride for subsequent analysis.

Sample Analysis. The PAH extracts were first analyzed by gas chromatography with a flame ionization detector (GC–FID). Since most samples were too dilute in PAH to make identification and/or quantitation by GC–FID possible, gas chromatographic mass spectrometry (GC–MS) was performed to obtain the necessary sensitivity and selectivity. The chromatographic columns for both GC–FID and GC–MS were 6-ft glass (2 mm i.d., 0.25 in. o.d.) or 6-ft stainless steel (0.085 in. i.d., 0.125 in. o.d.) packed with 3% OV-17 on 80–100 mesh Supelcoport. The two-column materials gave the same results. An FID trace or total ionization plot was obtained by temperature programming the gas chromatograph from 70° to 310°C at 8°C/min. The GC–MS system was a Hewlett-Packard 5982A quadrupole mass spectrometer with an H.P. 5933A data system. The mass spectrometer was used in three modes: totol ion monitoring, selected ion monitoring, and direct probe distillation. In the total-ion-monitoring mode, the mass spectrometer was continuously scanned over a wide mass range, usually m/e 50–350, in approximately 3–4 sec. Each scan was recorded by the computer and stored on a disc where data were retrieved for inspection and interpretation. The total ion voltage accumulated during each scan was also recorded and stored. A plot of this total voltage (ionization) vs. time corresponds to the output of an FID gas chromatograph.

In the selected-ion-monitoring (SIM) mode, the mass spectrometer scanned only certain preselected masses, and the computer stored the intensity of each mass. A plot of this intensity vs. time is a mass chromatogram. In the total ionization mode, 300 mass units were scanned in approximately 3 sec, which means the mass spectrometer spent less than 10 msec on each mass per scan. In the SIM mode, the operator can choose a dwell time of up to 500 msec. Our experiments utilized a 250-msec dwell time. Thus, SIM allows greater sensitivity; however, because of the greater dwell time, not all masses can be scanned during a gas chromatographic run. The operator can choose up to four masses to be scanned at any one time. Five sets of four masses can be chosen and instructions as to which of these sets are to be scanned at any given time can be given manually or automatically. This allows up to 20 masses to be monitored during a chromatographic run. This type of monitoring is especially suited to PAH, since their mass spectra are dominated by their molecular ions and they have very few fragment ions. Mass chromatograms of each of the masses scanned during a run are plotted, and the area under each curve is calculated with the aid of the computer. These areas can then be compared with the responses obtained from standard PAH of the same molecular weight and degree of alkyl substitution.

The third mode is direct probe distillation; in this method, the mixtures of PAH are introduced directly into the ion source of the mass spectrometer via a glass capillary tube on a probe that passes through a vacuum lock. The heat of the ion source distills the PAH from the capillary tube into the ionization chamber. Scanning a very limited mass range (m/e 150–350) in 4 sec results in a dwell time of 20 msec per mass. The increased dwell time and the increased sensitivity accompanying direct introduction into the ion source allows one to detect molecular ions of PAH too weak to be detected using the total ionization mode. By plotting mass chromatograms of parent PAH and their alkyl deriva-

Table I. Percentage

Matrix	*Phenanthrene*	*2-Methylphenanthrene*
Standard solution only	10	21
Preextracted soil with spike	57	NA[a]
Unmodified soil with spike	110	110

[a] NA = not analyzed.

tives and computing the area under these curves, the relative quantity of alkylated PAH can be obtained. This mode has the disadvantage that samples abundant in PAH and devoid of other organic species are necessary to avoid interferences. Most samples taken in areas remote from urban activity, having complex organic matrices, do not meet these requirements.

Evaluation of Methodology. Several blank analyses were performed during the course of the investigation. These included system blanks, which involved extraction of empty thimbles and simulation of the entire analytical procedure as well as individual solvent blanks, which were evaporations of known volumes of solvent, dissolution in methylene chloride, and analysis using GC–MS in the SIM mode. The median blank concentration for an individual PAH was 0.2 ppb based on a sample size of 200 g. All PAH concentrations reported here have been corrected for the appropriate blank.

Recovery studies were also performed to determine the efficiency of the analytical methodology. Several methods were used. Recoveries of selected PAH were measured using only a standard solution with no environmental matrix. Recoveries were also determined using spiked preextracted soil and spiked "uncleaned" soil. In the case of the "uncleaned" soil, a comparison between a spiked and a nonspiked sample was used to calculate recovered PAH concentrations. The results are summarized in Table I. It is interesting to note the low recoveries in samples with no additional organic matrix to act as a carrier (lines 1 and 2 of Table I). This is particularly striking in the case of perylene. The

Table II. Reproducibility Studies: Replicate PAH

Soil	*Experiment*	*PAH Concentration[a] (ppb)* $C_{14}H_{10}$	$C_{15}H_{12}$
Maine	1	78	38
Maine	2	48	24
Springfield	1	37	25
Springfield	2	33	20
Springfield	3	30	21

[a] See footnotes a to i in Table III.

Recoveries of Added PAH

Pyrene	*1-Methylpyrene*	*Chrysene*	*Perylene*
55	66	81	2
110	NA[a]	140	0
110	100	110	98

spiking experiment (line 3 of Table I) indicates nearly quantitative recovery of all PAH measured. Given these data, we estimate recoveries of 70%–100% for the range of PAH being analyzed. The results of the analyses reported here have not been adjusted for recovery efficiency in view of the high numbers obtained in these experiments.

Reproducibility is always an important question in the analysis of environmental samples. Homogeneity of the sample, precision of measurement of the GC injection volumes, precision of measurement using the computer for area calculations, and variability in procedural blanks are all factors contributing to a lack of reproducibility. An estimation of the reproducibility can be obtained by examination of the data presented in Table II. The first two lines represent two analyses of a Maine soil taken from the same sample jar. The variability is probably a result of lack of homogeneity of the sample or differences in the activity of the chromatographic materials. The last three lines of Table II present the results from two aliquots of the same sample. The first set of data is from the workup and quantitation of one aliquot by one of us (J.G.W.), while the second set of data is from the workup and quantitation of a second aliquot by another author (R.E.L.). The third set of data is concentrations obtained by J.G.W. on an aliquot worked up by R.E.L. This experiment represents the maximum number of variables that could affect reproducibility. Despite this, the data in Table II indicate good agreement. With all variables considered, we estimate the average reproducibility of the methodology is better than ±30%.

Abundances (ppb on dry weight basis) for Two Soils

PAH Concentration[a] (ppb)				
$C_{16}H_{14}$	$C_{16}H_{10}$	$C_{17}H_{12}$	$C_{18}H_{12}$	$C_{20}H_{12}$
18	160	52	54	NA[b]
12	120	36	60	98
30	100	37	63	110
14	84	27	55	98
22	89	32	49	96

[b] NA = not analyzed.

Sample Sites

PAH data on some of the following sample sites have been published previously (*2*). They are included here for completeness and for comparison.

1. Charles River sediment, Boston, Massachusetts: Anoxic sediment previously described by Hites and Biemann (*4*).
2. Maine soil, North Anson, Maine: Soil from a wooded area 100 yd from a paved rural road, two miles off Routes 8 and 201A, north of the center of North Anson. This is similar to the location described by Youngblood and Blumer (*1*). The sample was collected by shovel at 10–20 cm below the surface.
3. Gulf of Maine: Obtained from J. Farrington, Woods Hole Oceanographic Institute (WHOI). This sample was the 2–3-cm section of a core labeled Oceanus 1/1, which was collected in November 1975 under 214 m of water at approximately 43° N, 70° W (*11*).

4–6. Buzzards Bay sediment, Massachusetts: Obtained from J. Farrington (WHOI). The core sample was obtained from Buzzards Bay, Massachusetts (Station P, 41°29.0′ N, 70°52.5′ W, 17 m water) from an area where measurements of ^{210}Pb (*12*), ^{137}Cs, and ^{239}Pu and ^{240}Pu (*13*) provide a means of estimating the sedimentation rate. An earlier study (*11*) of alkanes, cycloalkanes, and phenanthrenes in another sediment core from this location showed an interesting trend of decreasing concentrations between the upper 2 cm and 54–58 cm, which pointed towards fossil fuel combustion as the principal source of hydrocarbons in these surface sediments. The core was collected in August 1975 with a 21-cm-diameter, 1-m-long sphincter corer (*14*). Three sections of the core were used for this study: Sample 4, the top 4 cm; Sample 5, 20–24 cm; and Sample 6, 38–42 cm. There was no sulfide present in the top 8 cm. An oxic, bioturbation zone of about 4 cm is indicated by the ^{210}Pb depth profile and benthic ecology studies (*12, 15*).

7. Urban soil, Stoneham, Massachusetts: Soil from a wooded area in the Middlesex Fells Reservation. The area is more than 500 yd from any road and approximately 5 mi from the Charles River. There is little direct anthropogenic influence other than airborne transport. The sample was collected by shovel at 10–20 cm below the surface.
8. Springfield soil, East Longmeadow, Massachusetts: Soil from a lightly wooded area with little surface vegetation in a small town 100 mi west of Boston. The

area is populated, but runoff from paved roads should not be possible. The sample was collected by shovel at 5–20 cm below the surface.

9. Cape Cod Bay, Massachusetts: Obtained from J. Farrington (WHOI). This sample was the top 2 cm of a sediment taken as a grab sample, labeled AST7-2 at 41°50′ N, 70°15′ W.
10. Outer Channel, Gulf of Maine: Obtained from J. Farrington (WHOI). This sample was the top 2 cm of box core 11, section 14, obtained on the R/V Knorr Cruise 69-1 at approximately 41° N, 64° W.
11. Pettaquamscutt River, Rhode Island: These samples are from Core 7408-2913 as reported by Goldberg et al. (*16*). They were received in 2-cm sections from the surface to a depth of 50 cm. The core was taken under 17 m of water.
12. New York Bight, New York: Obtained from J. Farrington (WHOI). It is a grab sample taken on February 5, 1975, at a water depth of 28 m at 40°25.7′ N, 73°48.1′ W on the R/V Knorr Cruise 47-1.
13. Hudson Channel, New York: Obtained from J. Farrington (WHOI). It is a combination of grabs 8 and 9 taken on the R/V Knorr Cruise 47-1 at 40°10.0′ N, 73°42.7′ W on February 5, 1975, under 60 m of water.
14. Hudson Canyon, New York: Obtained from J. Farrington (WHOI). It is the top 2 cm of a core obtained on the R/V Knorr Cruise 33-2 on October 6, 1973, at 29°27.8′ N, 72°13.0′ W under 986 m of water.
15. Continental slope, East Coast, U.S.: Obtained from J. Farrington (WHOI). It is an anchor dredge sample obtained in the R/V Knorr Cruise 19-5 at 38°53.5′ N, 71°47.8′ W on April 14, 1971, under 1830 m of water.
16. Abyssal plain, East Coast, U.S.: Obtained from J. Farrington (WHOI). The sample is from a core obtained on the R/V Knorr Cruise 33-2, September to October, 1973, at 32°25′ N, 70°13′ W under 5465 m of water.
17. Deep sea, East Coast, U.S.: Obtained from J. Farrington (WHOI). The sample is the top 8 cm of section 1 of a core taken on the R/V Knorr Cruise K19-4 at 30°01′ N, 60°00′ W on March 29, 1971, under 5250 m of water.
18. South Carolina soil, Berkely County, South Carolina: Soil from a marshy area 50 ft off an unpaved road numbered 220A in the Wambaw-Hunting Unit of the Francis Marion National Forest. The sample was collected by a cleaned shovel at a depth of 10–20 cm.

19, 20. Nebraska soils, Knox County, Nebraska: Collected by a cleaned shovel. Sample 19 was obtained 10 mi west of Crofton by digging into a recently eroded stream bank so that the depth of sample from surface

was 1.5 m. Soil was exposed at the surface during the early 1900s. Sample 20 was obtained at a depth of 2–10 cm 14 mi northwest of Bloomfield in a grassy meadow where there has been no farming.

21–25. Wyoming soils, Wyoming: Obtained from C. McAuliffe (Chevron Research). These samples are from remote wooded areas. Sample 21 was collected in a mountain meadow near the inlet of Grave Lake. It is a moist clay with some vegetation. Sample 22 was taken along a path to Onion Meadows. It is the top pine needle layer (2 cm thick) from under pine trees with no other surface vegetation. Sample 23 is the next 5 cm under sample 22. It is a mossy, pine needle layer. Sample 24 is the next 5–7 cm of soil under Sample 23. It is a soil with no pine needles and only a few roots. Sample 25 is a mossy soil with some pine needles taken from a different site than that at which Samples 22, 23, and 24 were taken. Samples were taken in August 1977.

26, 27. Mono Lake sediment, Lee Vining, California: Obtained using a small hand-operated dredge (Benthos Inc.). Sample 26 was taken about 1 mi offshore under 8–10 m of water. Sample 27 was obtained next to a cove near Niget Island under 5–8 m of water. Mono Lake is a strongly alkaline desert lake in eastern California that has a combination of high organic productivity with almost no scavengers (*17*).

28. Mono soil, Lee Vining, California: Obtained with a cleaned shovel at a depth of 10–20 cm half a mile from Route 395. The soil was taken from an area that may have been uncovered in the last decade by the shrinking of the lake.

29. Yosemite soil, Yosemite National Park, California: Obtained with a cleaned shovel at a depth of 10–20 cm. The site was located in a wooded area (various species of pines) behind Summit Meadow (elevation 8000 ft) approximately 500–1000 yd from a paved road.

30. Nevada soil, Nevada: Obtained with a cleaned shovel at a depth of 10–20 cm. The site was located 40 mi east of Lee Vining, California, 1 mile off Nevada Route 31, and 50 m from a unpaved road. This is a sandy, desert environment.

31. Alaska K-30: Obtained from H. Hertz of the National Bureau of Standards (NBS). This is an intertidal surface sediment taken in the vicinity of the Katalla River near the site of a suspected oil seep.

32. Alaska H-24: Obtained from H. Hertz (NBS). This was an intertidal sediment taken near Hitchinbrook Island, an area considered free from anthropogenic or oil contamination.

33–35. Polynesian atolls: Obtained by J. Trichet (University of Orleans, France). These samples are from lagoons of the Mururoa and Hao atolls. They are described by Niaussat et al. (*18*) and were received as freeze-dried samples. Sample 33 is from the Mururoa atoll. Samples 34 and 35 are from the Hao atoll at depths of 0 cm (still living algae) and 1 cm, respectively.

36. Oahu, Hawaiian Islands: Obtained from R. Duce (University of Rhode Island, School of Oceanography). The sample is a soil from the face of a vertical bank. Six to 8 cm of soil were removed from the bank at a depth of 5 cm from the horizonal surface. The soil was collected from a 5–15-cm depth from the surface. The site was on the windward coast of Oahu.

37. Enewetok Island, South Pacific: Obtained from R. Duce (U.R.I.). The sample is a soil collected on the north end of the island in a populated area. The soil is from a depth of 4–9 cm.

38. Wake Island, South Pacific: Obtained from R. Duce (U.R.I.). The sample is a soil collected near the east coast of the island with no buildings or roads upwind. The soil is from a depth of 4–9 cm below the surface.

39–41. Samoan soils, American Samoa, South Pacific: Obtained from R. Duce (U.R.I.). Sample 39 was taken at Duce Station, which is on Oh Point. This site is in the trade winds. The soil was dark brown loam. Sample 40 was taken near the NOAA station on Samoa 50 ft down a windward slope. The soil was a red clay. Sample 41 was taken from the banks of a road "cut" half a mile from the NOAA station. The soil was a light brown loam.

42. Walvis Bay, Africa: Obtained from J. Farrington (WHOI). Sample is the top 4 cm of Core 21, Station 14, from the Namibian Shelf of Africa in an upwelling area at 22°11.5′ W, 13°51.4′ S.

43. Cariaco Trench: Obtained from J. Farrington (WHOI). This was the top 10 cm of an anaerobic organic-rich ooze from the Cariaco Trench at 10°42.5′ N; 65°10.5′ W. This was a core sample.

44. Flood plain, Amazon River, Brazil: Obtained from J. Edmond, Department of Earth and Planetary Sciences (MIT). The sample is a sediment of the 1976 flood plain deposit taken on the downstream tip of Isle Santa Helena No. 1, which is 15 km upstream of Leticia, Colombia. It was very recently deposited sediment, less than one year old, when sampled.

45. Station 7, Amazon River: Obtained from J. Edmond (MIT). The sample was collected with a dredge from

Station 7 (02°0.3′ S, 55°23.1′ W) of the Amazon River between Santarem and Obidos. The sandy nature is characteristic of the Amazon's main channel.

46. Coari River, Amazon River system: Obtained from J. Edmond (MIT). The sample was collected with a dredge from Station 5 (0.4°04.1′ S, 63°09.1′ W), which is 1 km from Rio Solimoes.
47. Rio Ica, Amazon River system: Obtained from J. Edmond (MIT). The sample was collected with a dredge approximately 12 mi upstream from one of the least inhabited large tributaries in the Amazon River Basin. Sample was obtained June 30, 1976, at 03°14.2′ S, 68°04.1′ W.
48. Obidos 16, Amazon River: Obtained from J. Edmond (MIT). The sediment was taken from Station 16 which is 500 m from shore in a 60-m-deep, scoured hole near the city of Obidos. This sample was taken with a dredge.
49. Station 1, Amazon River system: Obtained from J. Edmond (MIT). The sediment was taken in the Tocantins River with a bed load sampler at 01°42.7′ S, 49°08.7′ W.
50. Rio Negro, Amazon River system: Obtained from J. Edmond (MIT). The sediment (mixed silt, clay, and sand) was taken upstream of Manaus at 03°07′ S, 60°06′ W. The sample was obtained with a grab sampler.

Results and Discussion

Complex mixtures of PAH were found in almost all samples. Table III summarizes the results of the analysis of samples from New England (Samples 1–11), the continental United States (Samples 18–30), the north and south Pacific Ocean (Samples 31–41) and Africa and South America (Samples 42–50). Concentrations are given for PAH with seven molecular weights representing over 25 alkylated and nonalkylated compounds. The quantities were determined using the SIM mode of the mass spectrometer with external standards, as described previously.

The data in Table III indicate that the presence of PAH in the environment is worldwide. Only two samples [one from a coarse, sandy, desert like environment (Nevada soil, Sample 30) and the other the freeze-dried remains of a still living algal layer from the lagoon of a South Pacific atoll (Hao 232, Sample 34)] were found to contain no PAH above blank levels. The concentrations of PAH in these global samples vary drastically, however. The total concentration of nonalkylated three-to-five-ring PAH ranges from less than 1 ppb (Samoan soils and an

Amazon sediment) to nearly 100 ppm (Charles River sediment); this represents a dynamic range of five orders of magnitude. The geometric mean of these 50 total PAH levels is 110 ppb.

Careful examination of these data shows that the highest concentrations of PAH are in the northeastern United States. This section of the country is densely populated and highly industrialized. No other sample has more than 1 ppm (1000 ppb) total PAH. (Enewetok soil is an exception that will be discussed below.) The concentrations within the Northeast samples are by no means uniform, however. The highest value is from the Charles River Basin. This sample site has been described previously (*4*). The salt water intrusion, low flow, and occasional input of sanitary sewage have resulted in a highly anoxic sediment. The location of the river in the middle of the densely populated Boston–Cambridge urban area results in a high input of street runoff, storm sewer overflow, and direct deposition of urban air particulate matter. These factors combine to yield a sediment with the highest reported concentration of PAH. Certainly this sample reflects a very high anthropogenic input.

The next highest concentration of PAH (approximately one order of magnitude less) is from the Pettaquamscutt River in Rhode Island. This is a small river located about 20 mi south of a large urban area (Providence, RI). There are some housing developments at isolated locations along its banks, but the overall area is rural. The nearest macadamized roadways run parallel to the river more than half a mile away. Some street runoff is possible, but this should not be significant in view of the half mile of wooded terrain separating the roadways from the river. Given these conditions, the high quantity of PAH is somewhat surprising. We present data elsewhere (*19*) on the analysis of a dated core from the Pettaquamscutt that indicates the source of the PAH at this location is anthropogenic. The mechanism by which these anthropogenic PAH enter the sediment of the Pettaquamscutt is unclear, but the lack of direct point sources (such as exist for the Charles River) suggests atmospheric transport and deposition.

Examination of data from three northeastern U.S. soils, taken from areas where the contribution from street runoff is low, indicates that PAH concentrations are highest closest to an urban center (urban soil, Sample 7) but are still present in samples removed from large metropolitan areas (Maine soil, Sample 2, and Springfield soil, Sample 8). As in the Pettaquamscutt sediment, the lack of direct point sources at these locations suggests atmospheric transport and deposition as the mechanism by which the PAH enter these soils.

Analyses of sediments in Cape Cod Bay, Buzzards Bay, and the Gulf of Maine indicate PAH concentrations ranging from 1300 ppb to 540 ppb, with the lowest value from the sediment most distant from

Table III. PAH Concentrations

	Sample	$C_{14}H_{10}$[b]	$C_{15}H_{12}$[c]	$C_{16}H_{14}$[d]
1.	Charles River	5000	NA[m]	NA
2.	Maine soil	63	31	15
3.	Gulf of Maine	44	46	46
4.	Buzzards Bay 1	53	53	50
5.	Buzzards Bay 2	42	31	29
6.	Buzzards Bay 3	8	NA	NA
7.	Urban soil	120	68	39
8.	Springfield soil	33	22	22
9.	Cape Cod Bay	140	86	48
10.	Outer channel	ND[n]	ND	ND
11.	Pettaquamscutt[k]	940	580	280
12.	New York Bight	740	500	370
13.	Hudson Channel	62	51	41
14.	Hudson Canyon	74	62	63
15.	Continental slope	7	7	8
16.	Abyssal Plain	ND	NA	NA
17.	Deep sea	ND	6.9	7.7
18.	South Carolina soil	78	48	90
19.	Nebraska 1	4.4	NA	NA
20.	Nebraska 2	8.0	NA	NA
21.	Wyoming soil 1	4.8	5.5	NA
22.	Wyoming soil 2	30	NA	NA
23.	Wyoming soil 3	23	NA	NA
24.	Wyoming soil 4	16	12	9.4
25.	Wyoming soil 5[l]	NA	NA	NA
26.	Mono Lake 1	91	NA	NA
27.	Mono Lake 2	110	NA	NA
28.	Mono soil	9.5	NA	NA
29.	Yosemite soil	7.0	6.3	9.6
30.	Nevada soil	ND	ND	ND
31.	Alaska K-30	67	120	58
32.	Alaska H-24	2.5	4.5	2.7
33.	Mururoa 300	90	32	10
34.	Hao 232	ND	ND	ND
35.	Hao 233	35	25	12
36.	Oahu soil	3.6	8.6	5.8
37.	Enewetok soil	130	280	600
38.	Wake soil	31	10	3.7
39.	Samoa soil 1	0.4	0.4	0,2

in Worldwide Samples (ppb) [a]

$C_{16}H_{10}$ [e]	$C_{16}H_{10}$ [f]	$C_{17}H_{12}$ [g]	$C_{18}H_{12}$ [h]	$C_{20}H_{12}$ [i]	*Total* [j]
15,000	13,000	NA	21,000	33,000	87,000
63	80	44	57	98	360
120	100	53	79	200	540
130	120	110	160	340	800
140	120	NA	200	380	870
11	7	NA	12	25	60
180	170	140	280	380	1100
48	43	32	56	100	270
200	180	160	260	520	1300
ND	7.9	11	19	39	66
1600	1600	880	1500	2800	8400
1200	1300	740	890	1700	5800
110	100	100	150	320	740
130	100	82	120	390	810
11	10	8	10	33	70
4.1	4.4	NA	13	34	56
4.3	2.2	3.0	3.5	2.3	12
15	26	14	22	13	150
5.1	5.1	NA	31	NA	—
8.4	8.5	NA	17	37	74
2.1	1.4	NA	2.2	7.6	18
35	25	NA	29	95	210
14	9.7	NA	20	43	110
3.8	3.6	NA	9.6	NA	—
NA	NA	NA	NA	NA	—
13	24	NA	29	NA	—
74	65	NA	94	57	400
8.7	9.2	NA	26	9.7	63
1.4	1.1	1.3	1.6	0.9	12
ND	ND	ND	ND	ND	0
4	8	28	23	11	110
0.6	0.6	1.1	1.4	0.6	5.7
7.4	2.7	7.2	5.7	1.7	110
ND	ND	ND	ND	ND	0
4.3	1.2	8.8	5.2	3.0	49
13	16	12	25	30	88
260	410	520	560	600	2000
49	54	20	75	140	350
0.7	0.8	0.5	3.7	3.0	8.6

(continued)

Table III.

	Sample	$C_{14}H_{10}$ [b]	$C_{15}H_{12}$ [c]	$C_{16}H_{14}$ [d]
40.	Samoa soil 2	0.3	0.3	0.2
41.	Samoa soil 3	ND	ND	0.8
42.	Walvis Bay	7.6	26	23
43.	Cariaco Trench	18	29	15
44.	Flood plain	2.1	5.3	6.5
45.	Station 7	ND	0.3	0.4
46.	Coari River	9.3	19	20
47.	Rio Ica	5.5	7.2	5.4
48.	Obidos 16	6.4	13	13
49.	Station 1	3.2	9.7	12
50.	Rio Negro	1.2	2.0	1.6

[a] Dry weight basis.
[b] Phenanthrene and anthracene.
[c] Methylphenanthrenes and methylanthracenes.
[d] Dimethyl- and ethylphenanthrenes and anthracenes.
[e] Fluoranthene.
[f] Pyrene.
[g] Methylfluoranthenes and methylpyrenes.
[h] Chrysene, triphenylene, and benzanthracene.

Boston. This observation suggests a correlation of PAH concentrations with anthropogenic activity. This argument will be presented in more detail later.

The quantities of total PAH differ, but does the relative distribution of the individual species differ? Figure 1 graphically compares the distribution of PAH in several samples. The PAH concentrations have been normalized to the most abundant species in each sample. All mixtures are nearly identical; the nonalkylated PAH are the most abundant compounds. This type of pattern is most compatible with combustion as the source of these PAH assemblages (*1, 2, 3*).

From these data, it appears that in areas where PAH concentrations are high, such as the northeastern United States, one can associate the PAH mixtures found with both anthropogenic activity and combustion. There is a possibility that natural combustion, such as forest fires, could contribute to the more remote areas (Maine soil, for example). The contribution of PAH to the offshore sediments (Samples 3, 4, 9) by forest fires should be minimal compared with the anthropogenic contribution because of the proximity of these sites to urban areas and their distance from large forested areas.

Can the PAH found in the remainder of the samples listed in Table III also be associated with anthropogenic combustion? The total concentrations of PAH from the samples outside the Northeast are considerably lower: the highest is 400 ppb for a Mono Lake sediment. These

Continued

$C_{16}H_{10}$[e]	$C_{16}H_{10}$[f]	$C_{17}H_{12}$[g]	$C_{18}H_{12}$[h]	$C_{20}H_{12}$[i]	*Total*[j]
0.2	0.1	NA	0.2	0.1	0.9
ND	0.2	0.6	0.4	ND	0.6
9.8	15	8	9.7	25	67
8.9	16	27	13	50	110
1.2	1.5	3.0	5.9	6.4	17
0.2	0.1	0.1	0.2	0.4	0.9
3.4	3.7	5.4	4.0	6.8	27
2.9	2.6	4.2	13	5.8	30
5.5	5.9	17	16	32	66
4.9	4.1	6.8	7.0	8.6	28
0.8	0.6	0.6	0.9	1.8	5.3

[e] Benzofluoranthenes and benzopyrenes but not perylene.
[j] The total of the five nonalkylated species listed in the table.
[k] Surface layer 0–4 cm of core listed in sample description.
[l] Interference from many naturally occurring compounds prohibits quantitation of PAH species.
[m] NA = not analyzed.
[n] ND = not present above blank level.

samples were all selected because they are remote from human activities. Table III indicates that the more remote the location, the lower is the PAH concentration. For example, the average PAH concentration of the Amazon River system is less than 25 ppb. The Samoan soils have total PAH abundances of less than 4 ppb. These samples have PAH patterns which reflect a common source. Figure 2 compares some of these global samples. Some variations occur between the PAH distributions of these samples and those normally associated with combustion. These variations occur mainly in the phenanthrene/anthracene series (molecular weights 178, 192, 206). The problems that arise from analyzing very low-level samples, including contributions from blanks, sample contamination, and recoveries, are the probable source of these variations. The combustion pattern for the rest of the PAH is still evident. Note the abundance of the alkylated fluoranthenes and pyrenes (molecular weight 216) compared with the nonalkylated species (molecular weight 202).

The Mono Lake (Sample 27) and South Pacific atoll (Sample 35) all show extremely high amounts of the phenanthrene series compared with the other PAH. We believe this variation is the result of other than experimental complications. Studies on Mono Lake sediments by Henderson et al. (*17*) indicate that algae are the predominant source for the organic content of the sediments. The same might be expected to be true of the atoll samples (see sample site descriptions). It is therefore possible that an alteration of the sterol content of the algae into phenan-

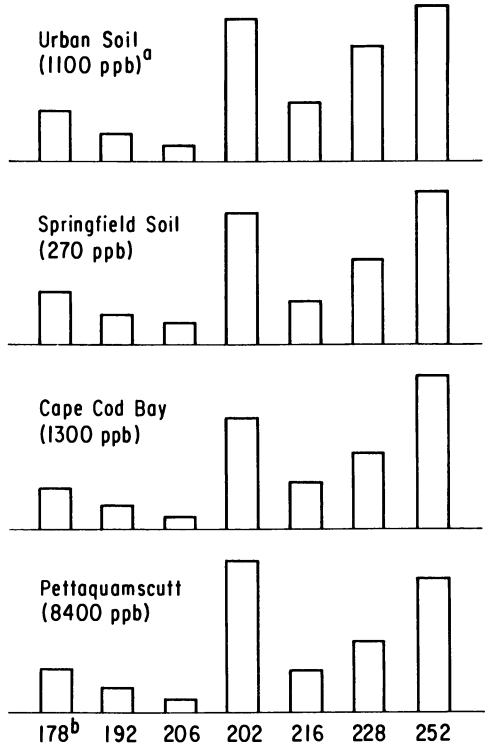


Figure 1. Relative distribution of PAH in samples from New England; values are normalized to most abundant species. [a] *Concentration of total PAH (dry weight basis);* see *footnote j in Table III.* [b] *Molecular weight of PAH species: 178=$C_{14}H_{10}$, phenanthrene and anthracene; 192=$C_{15}H_{12}$, methylphenanthrenes and methylanthracenes; 206 = $C_{16}H_{14}$, dimethyl and ethyl phenanthrenes and anthracenes; 202 = $C_{16}H_{10}$, fluoranthene and pyrene; 216 = $C_{17}H_{12}$, methyl fluoranthenes and pyrenes; 228 = $C_{18}H_{12}$, chrysene, triphenylene, and benzanthracene; 252 = $C_{20}H_{12}$, benzofluoranthenes and benzopyrenes but not perylene.*

threne and alkyl phenanthrenes occurs in these sediments (*2, 21*). The possibility of PAH biosynthesis by the algae is unlikely because no PAH were found in the still living algal layer.

Two other samples have PAH distributions that do not correspond to the pattern seen in the other sediments and soils. They are the Alaska K-30 sediment and the Enewetok soil. Both have patterns with relatively high abundances of alkylated PAH (*see* Figure 3). The sample site description for the Alaskan sediment indicates that the sample was taken near an oil seep. It is not surprising that the PAH pattern should reflect the high degree of alkylation found in petroleum (*1, 22*). Figure 3 graphically demonstrates this pattern for a crude oil as well as for a shale

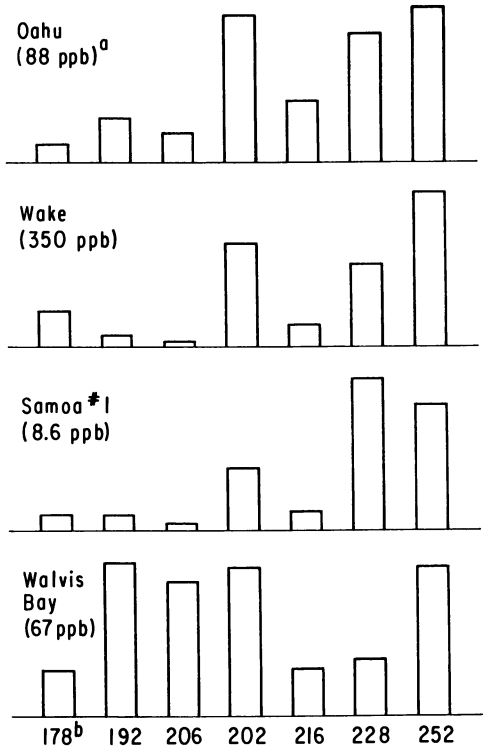


Figure 2. Relative distribution of PAH in samples from remote locations; values are normalized to most abundant species. [a,b] *For explanations of concentration and molecular weights,* see *Figure 1, caption.*

oil and coal. The Enewetok soil has an absolute abundance that is remarkably high (greater than 2000 ppb). This must indicate local contamination. The gas chromatogram of the PAH extract (Figure 4) indicates three major components. These components have been identified by MS as DDE and two isomers of DDT. They have been quantified by the GC–FID response to be 67, 24, and 120 ppm, respectively. The presence of these insecticides provides a clue about the source of the high quantity of PAH found in the soil. DDT can be purchased commercially for spraying as a 40% DDT, 60% petroleum mixture (23), and the phenanthrene series (Figure 3) of the Enewetok soil sample reflects a petroleum contribution. Because this section of Enewetok Island is populated, one can assume that the application of such DDT–petroleum mixtures was used to control insects. The high abundance of the 202, 228, and 252 molecular weight PAH may indicate that volatilization of the lower-molecular-weight PAH species occurred during or after spraying of the pesticide formulation.

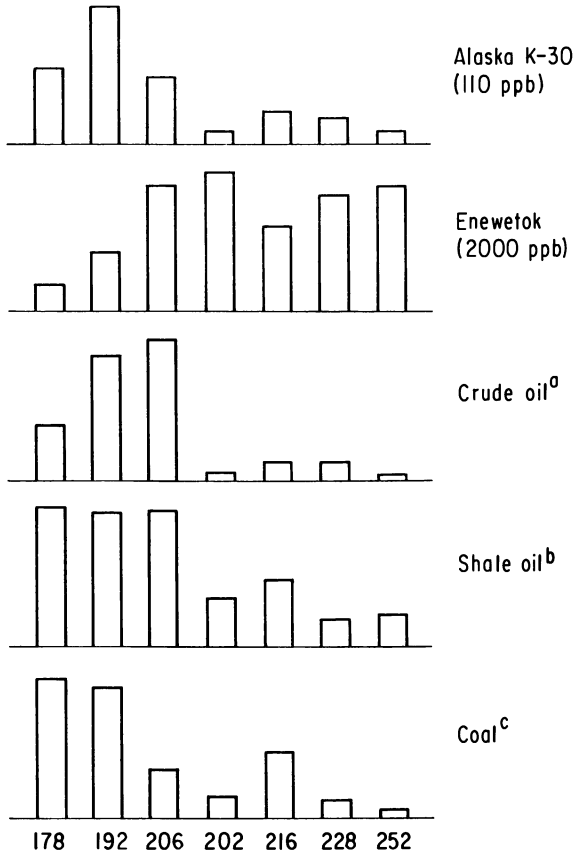


Figure 3. Relative distribution of PAH in two environmental samples and three fossil fuels; values are normalized to most abundant species. [a] Amna crude oil: Spiked in a sediment and carried through PAH isolation procedure. [b] Shale oil: Partitioned using cyclohexane and nitromethane followed by silicic acid chromatography for PAH isolation. [c] Pittsburg seam bituminous coal: Extracted with methylene chloride and carried through PAH isolation procedure.

The samples from South Carolina (Sample 18) and Yosemite National Park (Sample 29) demonstrate a peculiar abundance pattern in the phenanthrene/anthracene series. The nonsubstituted and disubstituted species are both abundant relative to the monosubstituted phenanthrenes/anthracenes. Information from the mass spectral analysis of other components in these soil extracts indicates the source for these particular PAH is neither combustion nor fossil fuels but rather aromatization of certain natural products (2).

One can also assess human input of PAH into the environment by looking at PAH concentrations in an area of high anthropogenic activity and following the concentrations as one moves away from the point source.

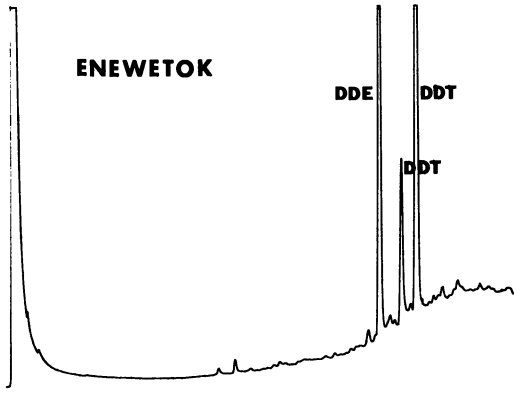


Figure 4. Gas chromatogram of extract from Enewetok soil. The larger DDT peak is the p,p' *isomer.*

The New York Bight is an area affected by shipping, dumping of industrial wastes and sludge, industrial and sewage effluents in rivers flowing into the Bight, land runoff, and atmospheric deposition from the metropolitan New York area. Mueller et al. (*24*) have estimated flow inputs and organic carbon loads into the New York Bight. Air and urban runoff accounted for 25% of the organic carbon, while waste water and barge dumping accounted for more than 50%. Obviously, the New York Bight is an area of high anthropogenic activity.

The transect from the Bight to Bermuda is interesting (Figure 5) because the Hudson Channel and Canyon system deliver sediments to the abyssal plain by means of the slumping of sediments down the channel or by the flow of water down the canyon (*25*). The aliphatic hydrocarbons in sediments along this transect have been studied (*26*). These authors concluded that the New York Bight surface sediments contain pollutant hydrocarbons. They attributed these hydrocarbons to fossil fuel contamination and suggested that the most likely source is the dumping of sewage sludge and harbor dredge spoils. They also concluded that there is clear evidence for the transport of land-derived *n*-alkanes to the continental shelf and slope and to abyssal plain surface sediments. The question of the predominant mode of transport is unresolved, however.

The analysis of PAH in a surface sediment from the New York Bight is presented in Table IV and Figure 6. The high PAH abundance and the distribution pattern indicate a high degree of contamination with combustion-generated PAH. The combustion PAH pattern is maintained in sediments as one moves along the transect from the Bight to the deep sea (*see* Figure 6). The deep-sea sample has some indication that noncombustion PAH (i.e., fossil fuel) might be contributing to the total sedimentary PAH. (Alkylated phenanthrenes and anthracenes are more

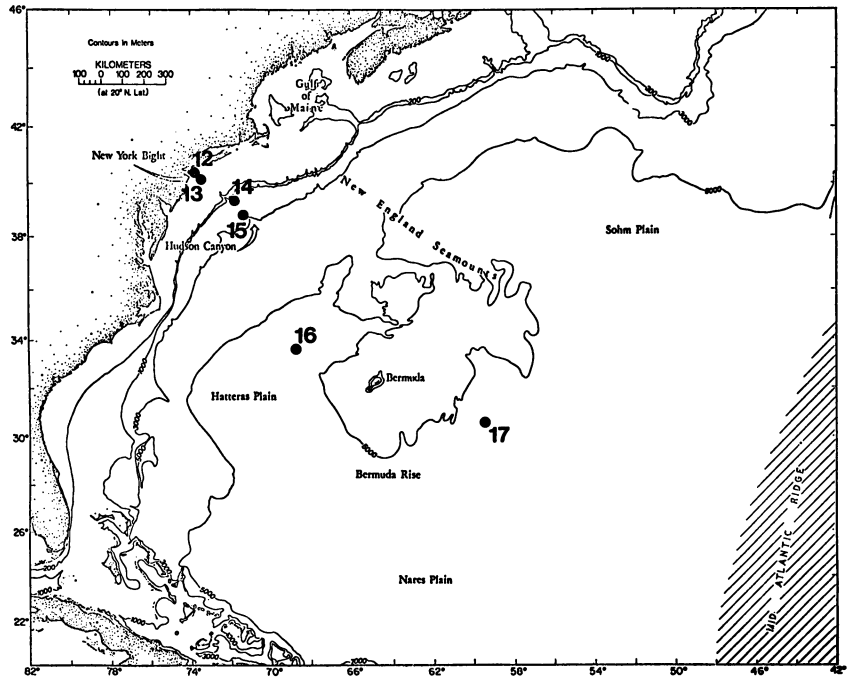


Figure 5. Sample sites on transect from New York Bight to the ocean southeast of Bermuda. Numbers refer to sample numbers in site descriptions.

abundant than the nonalkylated compounds.) However, the ratio of molecular weights 202 to 216 is greater than 1, thus indicating combustion-produced PAH are still a significant portion of the total PAH found in the deep-sea sediment. The rapid falloff from the Bight to the deep ocean sediment (5800 to 12 ppb; *see* Table IV) indicates that metropolitan New York serves as a point source for combustion-generated PAH and some of these PAH are transported long distances. The mode of transport is still unknown.

The anomalously high PAH concentration for the Hudson Canyon sample agrees with the similarly high alkane values obtained by Farrington and Tripp (*26*) for this site. They relate this anomaly to the accumulation of sediment transported in the channel and across the shelf break to this location (*25*). Sediment movement therefore can be an important mode of transport in some areas of the transect. Whether this mode is solely responsible for PAH found in the Abyssal Plain sediments is unknown.

A similar analysis of sediments along a transect from Boston to the basins in the Gulf of Maine (*9*) leads the authors to propose a dual mode of transport of combustion-generated PAH. We suggest a deposition of

Table IV. PAH Concentrations in Sediments Along a Transect from the New York Bight to the Deep Ocean Southeast of Bermuda

Sample	*Cruise*[a]	*Total PAH (ppb)*[b]
12 New York Bight	K47-1-G6	5800
13 Hudson Channel	K47-1-G8/9	740
14 Hudson Canyon	K33-2-10	810
15 Continental slope	K19-5-9	70
16 Abyssal Plain	K33-2-6	56
17 Deep sea	K19-4-9	12

[a] Sample numbers as assigned by J. Farrington (WHOI). They can be used to compare these data with those reported on other hydrocarbons by Farrington and Tripp (*26*) and Farrington et al. (*11*).
[b] Parts per billion (dry weight basis); *see* Table III.

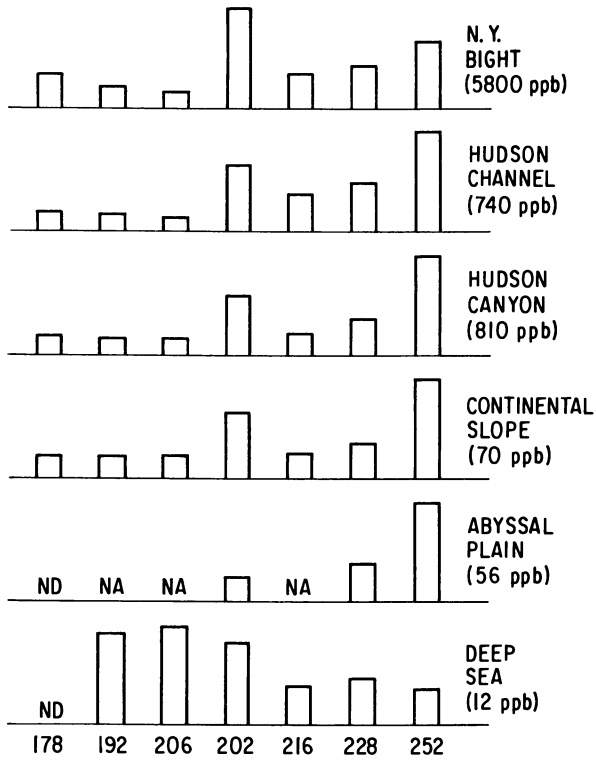


Figure 6. Relative distribution of PAH in samples taken along a transect from New York Bight. ND = Not present above blank levels; NA = not analyzed. (See Figure 5 for site locations.)

large (>10 μm) urban particulate matter close to the point source and sediment transport to the basins where accumulation occurs. The second mode of transport is the airborne movement of the smaller (<1 μm) particles long distances to the open ocean sediments. Long-range atmospheric transport of certain anthropogenic compounds is a well-known phenomenon (*27*). However, there has been little information concerning the long-range transport of aromatic hydrocarbons. Two recent studies (*28, 29*) report finding low levels of aromatic hydrocarbons in air samples distant from anthropogenic activity, indicating that long-range atmospheric transport of PAH is possible.

Additional support for the airborne transport of PAH is suggested by the results on the global distribution of PAH reported here. Thus, airborne transport of combustion-generated PAH from the New York City area to the sediments along the transect studied (Figure 5) is quite likely, especially since aeolian transport of land-derived material in this region has been documented (*30*).

Conclusions

The data and discussion presented above indicate that PAH are ubiquitous in the sedimentary environment. In most instances, they are present as complex mixtures of alkylated and nonalkylated species whose relative abundances suggest a common source: combustion. Nearby and at some distance from areas of high anthropogenic activity, these combustion-generated PAH can be attributed to human beings. There are low levels of PAH in remote areas of the globe, which may represent either long-range transport of anthropogenically derived PAH or a background level due to natural combustion such as forest fires.

Acknowledgment

Work was supported by the National Science Foundation (Grant OCE-77-20252).

Literature Cited

1. Youngblood, W. W.; Blumer, M. *Geochim. Cosmochim. Acta* **1975**, *39*, 1303.
2. Laflamme, R. E.; Hites, R. A. *Geochim. Cosmochim. Acta* **1978**, *42*, 289.
3. Hites, R. A.; Laflamme, R. E.; Farrington, J. W. *Science* **1977**, *198*, 829.
4. Hites, R. A.; Biemann, W. G. In "Analytical Methods in Oceanography," *Adv. Chem. Ser.* **1975**, *147*, 188.
5. Wakeham, S. G. *Environ. Sci. Technol.* **1977**, *11*, 272.
6. Grimmer, G.; Bohnke, H. *Cancer Lett. (Amsterdam)* **1975**, *1*, 75.
7. Muller, G.; Grimmer, G.; Bohnke, H. *Naturwissen.* **1977**, *64*, 427.
8. Giger, W.; Schaffner, C. *Anal. Chem.* **1978**, *50*, 243.

9. Windsor, J. G., Jr.; Hites, R. A. *Geochim. Cosmochim. Acta* **1979,** *43,* 27.
10. Hoffman, D.; Wynder, E. *Anal. Chem.* **1960,** *32,* 295.
11. Farrington, J. W.; Frew, N. W.; Gschwend, P. M.; Tripp, B. W. *Est. Coast Mar. Sci.* **1977,** *5,* 793.
12. Farrington, J. W.; Henricks, S. M.; Anderson, R. *Geochim. Cosmochim. Acta* **1977,** *41,* 289.
13. Bowen, V. T., U.S. Energy Res. Dev. Admin. Health Safety Lab Rpt., HASL-291.
14. Burke, J. C. *Limnol. Oceanog.* **1978,** *13,* 714.
15. Rhoads, D. *Oceanog. Mar. Biol.* **1974,** *12,* 263.
16. Goldberg, E. D.; Gamble, E.; Griffin, J. J.; Koide, M. *Est. Coast. Mar. Sci.* **1977,** *5,* 549.
17. Henderson, W.; Reed, W. E.; Steel, G.; Calvin, M. *Nature* **1971,** *231,* 308.
18. Niaussat, P. M.; Trichet, J.; Heros, M.; Luong, N. T.; Ehrhardt, J. P. *C. R. Helod. Seances Acad. Sci., Ser. D* **1975,** *281,* 1031.
19. Hites, R. A.; Laflamme, R. E.; Windsor, J. G., Jr.; Farrington, J. W., unpublished data.
20. Douglas, A. G.; Mair, G. J. *Science* **1965,** *147,* 499.
21. Gaskell, S. J.; Eglinton, G. *Adv. Org. Geochem.* **1974,** *1973,* 963.
22. Speers, G. C.; Whitehead, E. V. In "Organic Geochemistry"; Eglinton, G., Murphy, M. T., Eds.; Springer-Verlag: New York, 1969; pp. 638–675.
23. *"Pesticide Handbook—Entoma";* Frear, D. E. H., Ed.; College Science: State College, PA, 1969.
24. Mueller, J. A.; Anderson, A. R.; Teris, J. S. *J. Water. Pollut. Control Fed.* **1976,** *48,* 2309.
25. Keller, H.; Lambert, D.; Rowe, G.; Staresinic, N. *Science* **1973,** *180,* 181.
26. Farrington, J. W.; Tripp, B. W. *Geochim. Cosmochim. Acta* **1977,** *41,* 1627.
27. Windom, H. L.; Duce, R. A. "Marine Pollutant Transfer"; Lexington Books: Lexington, MA, 1976.
28. Ketseridis, G.; Hahn, J.; Jaenicke, R.; Junge, C. *Atmos. Environ.* **1976,** *10,* 603.
29. Cautreels, W.; Cauwenberghe, K. V.; Guzman, L. A. *Sci. Total Environ.* **1977,** *8,* 79.
30. Folger, D. *Deep-Sea Res.* **1970,** *17,* 337.

RECEIVED October 31, 1978.

14

Ambient-Temperature Extraction of Hydrocarbons From Marine Sediment—Comparison with Boiling-Solvent Extractions

DONALD W. BROWN, L. SCOTT RAMOS, MARIANNE Y. UYEDA, ANDREW J. FRIEDMAN, and WILLIAM D. MACLEOD, JR.[1]

NOAA National Analytical Facility, Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Boulevard East, Seattle, WA 98112

Effective measurement of hydrocarbons in marine sediments requires standardized extraction procedures that are efficient and reproducible. Most hydrocarbon extractions from sediment depend on solvent reflux, which poses difficulties for routine processing of large numbers of samples. As an alternative, an ambient-temperature solvent extraction was devised for a ball-mill tumbler and compared with three boiling-solvent extractions. Aliphatic and aromatic hydrocarbons from a moderately contaminated sediment were quantitated using glass capillary gas chromatography. After dewatering the sediment with methanol, the tumbler extraction with 2:1 dichloromethane:methanol gave hydrocarbon yields comparable to the boiling-solvent extractions. The tumbler extraction did not require benzene, hood space, expensive glassware, boiling solvents, running water, or freeze-drying.

Concern over oil pollution has led to considerable interest in measuring amounts of hydrocarbons in the marine environment (*1, 2*). A number of researchers have developed specialized procedures for analyzing hydrocarbons in marine sediments (*3–14*). Unfortunately, because

[1] Author to whom reprint requests should be sent.

the various procedures have not been adequately assessed (*3, 4*), interpretations of analytical data from these analyses have been difficult, especially for individual aliphatic and aromatic hydrocarbons. To help overcome such deficiencies, it is desirable to compare important analytical methods for hydrocarbons in marine sediment using a representative set of individual hydrocarbons. Clearly, the extraction techniques compared should be reproducible, accurate, and efficient within meaningful limits. In addition, these techniques must be safe and convenient for processing large numbers of samples.

Soxhlet extraction with benzene and methanol (*3, 5, 9, 10, 14*) is generally considered the most efficient technique for hydrocarbon recovery. However, it is not convenient for processing large numbers of samples (*6*). Recently, alternative techniques have been reported that may be as efficient as soxhlet extraction. For example, Farrington and Tripp (*5*) showed that sediment refluxed for 3 hr with benzene and methanol afforded about the same gross weight of hydrocarbons as by the soxhlet technique. Similarly, Rohrback and Reed (*3*) reported that by shaking sediment with various solvents, they extracted almost the same weight of hydrocarbons as by soxhlet extraction with the same solvents. However, MacLeod et al. (*6*) often found that shaking produced stable emulsions. To avoid emulsions, Warner suggested extracting sediments with diethyl ether–water using a ball-mill tumbler (*8*). This approach reduced emulsions and was convenient for mass sample processing (*6, 7*), but Carpenter and Bates found that this technique generally extracted only about one-third the amount of hydrocarbons extracted by the conventional soxhlet technique (*9*).

We have derived an extraction method that retains the convenience of the ball-mill tumbler for large numbers of extractions, and have investigated various solvent systems for improved recoveries. Preliminary results indicate that a tumbler extraction using methanol and dichloromethane recovered hydrocarbons from an intertidal sediment as efficiently as the soxhlet technique (*12*). This report describes improvements in our tumbler procedure and compares it with three boiling-solvent procedures (*10, 13, 15*).

Materials and Methods

Materials, reagents, apparatus, and their cleaning procedures have been published previously (*6, 12*). All solvent ratios were on a v/v basis. Sediment dry weights were determined on 10–20-g samples (*6*).

Methanol Purification. To check methanol purity, 200 mL was diluted with an equal volume of contaminant-free dichloromethane and extracted twice with 200-mL portions of distilled water. After the resulting dichloromethane phase was concentrated to 1 mL, it was

analyzed for contaminants by glass capillary gas chromatography (GC). When contaminants exceeded 0.1 ng/μL in the dichloromethane concentrate (i.e., 0.5 μg/L methanol), 1500 mL of the methanol was purified by diluting it with 500 mL of water and extracting it twice with 50-mL aliquots of contaminant-free hexane. Then the aqueous methanol was redistilled at 65°–67°C through an efficient fractionation column and checked by GC analysis.

Sediment Extraction Procedures. BALL-MILL TUMBLER EXTRACTION. Figure 1 shows the extraction scheme. A 100-g sample of wet sediment was weighed into a 1-L bottle, and 50 mL of methanol were added. Aliquots of recovery standards (*n*-decylcyclohexane and 1,3,5-triisopropylbenzene) were added to each sample, except for the reagent blank. An aliquot containing the compounds to be quantitated was added to a second blank to estimate typical losses due to handling. The bottles containing the samples and methanol were gently swirled by hand to dewater the sediment. The methanol was decanted into a 600-mL beaker, and the methanol-dewatering step was repeated with another 50 mL of methanol. Then 100 mL of 2:1 dichloromethane:methanol was added to the sediment, and the bottles were sealed with all-Teflon screwcaps and rolled on a ball-mill tumbler for 16 hr (overnight) at about 75 rpm. The extract was decanted into the 600-mL beaker containing the methanolic extracts. Then the sample and bottle were washed with about 5 mL of dichloromethane (dispensed from a clean Teflon wash bottle), and the washings were decanted into the 600-mL beaker. The dichloromethane–methanol sediment extraction step was repeated twice, first for 6 hr, then for 16 hr.

All extracts were combined and filtered through a 65-mm-i.d., coarse fritted-glass filter funnel into a 1-L separatory funnel. The beaker and filter were washed twice with 25–50 mL of dichloromethane. The total filtrate was gently swirled for 2 min with 500 mL of distilled water to remove methanol from the dichloromethane phase. After the phases separated, the dichloromethane (lower) phase was drained into a 500-mL Erlenmeyer flask. The aqueous (upper) phase was then back-extracted with 20 mL of dichloromethane and the dichloromethane phases were combined. The aqueous phase was discarded. The aqueous wash–dichloromethane back-extraction steps were repeated on the combined dichloromethane extracts prior to cleanup (below).

DIRECT REFLUX EXTRACTION. Wet sediments (50 g) were refluxed in 250 mL of 0.5*N* KOH in methanol and 35 mL of distilled water for 2 hr (*13*). After cooling, the mixture was poured through a 65-mm-i.d., coarse fritted-glass filter funnel into a 1-L separatory funnel. The extraction flask and the filter were rinsed with 20 mL of methanol and three times with 35 mL of dichloromethane. Distilled water (150 mL) was added to the extract and the mixture was shaken. After the phases separated, the dichloromethane layer was drained into a 500-mL Erlenmeyer flask, and the aqueous phase was back-extracted with 50 mL of dichloromethane. The combined dichloromethane extracts then passed to the cleanup step (below).

SOXHLET EXTRACTION WITH BENZENE–METHANOL. The procedure of Clark and Finley (*10*) was employed, using two 24-hr extractions of a 100-g sample of wet sediment, each with 250 mL of 1:1 benzene:methanol.

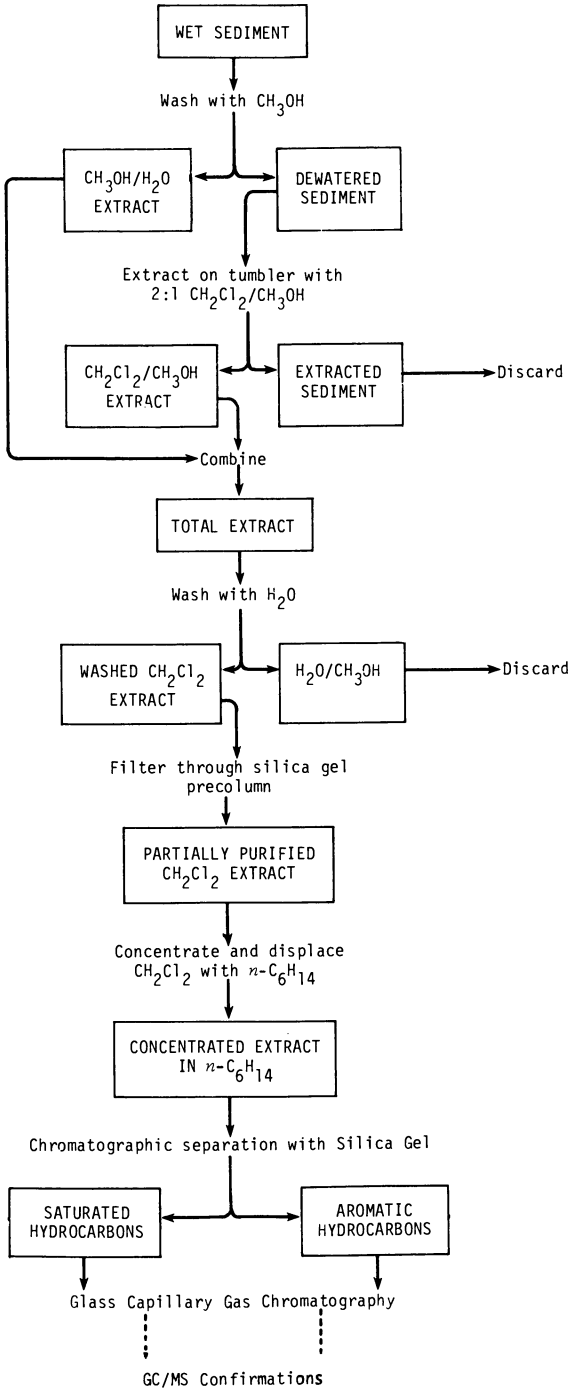


Figure 1. Ball-mill tumbler extraction scheme

The combined extracts were washed with water, dried over Na_2SO_4, and evaporated just to dryness with a rotary evaporator. The residue was dissolved in dichloromethane for extract cleanup (below).

SOXHLET EXTRACTION WITH DICHLOROMETHANE–METHANOL. Wet sediments (100 g) were extracted by a similar soxhlet procedure using 2:1 methanol:dichloromethane (*15*). The extracts were combined, washed, and concentrated as done in the tumbler procedure prior to cleanup.

Extract Cleanup. The sediment extract in dichloromethane was filtered through a 19-mm-i.d. chromatography column containing 20 mL of activated silica gel previously prepared in dichloromethane and covered with a 1-cm layer of sand (*6*). Then the column was eluted with one bed volume of dichloromethane. The combined eluates were collected in a 500-mL Erlenmeyer flask equipped with a 24/40 outer joint. Residual methanol, water, particulates, and gel-forming polar materials (which could plug the silica gel chromatography column used later) were removed by this step. A Snyder distillation column equipped with a 24/40 inner joint was attached to the flask, and the assembly was placed in a heated (ca. 60°C) water bath. The dichloromethane extract and eluate were concentrated to ca. 15 mL and transferred to a 25-mL Kontes concentrator tube. After a Teflon boiling chip was added, and a Kontes micro-Snyder column (modified with indentations) was attached, the extract was further concentrated on a modified Kontes tube heater to about 1 mL. After adding 2 mL of hexane, the extract was reconcentrated to about 1 mL for fractionation into hydrocarbon classes.

Fractionation into Hydrocarbon Classes. All extracts were chromatographed on Davison grade 923 silica gel, as reported earlier (*6, 7*). Two fractions, containing saturated and unsaturated hydrocarbons, respectively, were collected in separate 25-mL Kontes concentrator tubes. These fractions were concentrated to 1 mL on a modified Kontes tube heater. After adding 2 mL of hexane, the extract was reconcentrated to 1 mL and transferred to GC sample vials. After adding 4 μg of hexamethylbenzene (GC internal standard) in hexane, the vials were sealed for GC analysis.

Gas Chromatographic Analysis. The vial contents were automatically sampled and analyzed by GC (*6, 7*) using high-resolution glass capillary columns (*see* Figure 2 for column parameters and operating conditions). Major alkanes ranging from decane (n-$C_{10}H_{22}$) through hentriacontane (n-$C_{31}H_{64}$), plus pristane and phytane, were quantitated in the saturated hydrocarbon fraction. The arenes listed in Table I were quantitated in the unsaturated hydrocarbon fraction.

Results and Discussion

Ball-Mill Tumbler Extraction. To avoid unnecessary cost, inconvenience, and hazards involved in extracting large numbers of sediment samples with boiling solvents (*6*), we have investigated alternative procedures. Ideally, a suitable procedure should extract efficiently and reproducibly and be simple, safe, and convenient. In particular, we needed a method that used minimal benchtop or hood space; could

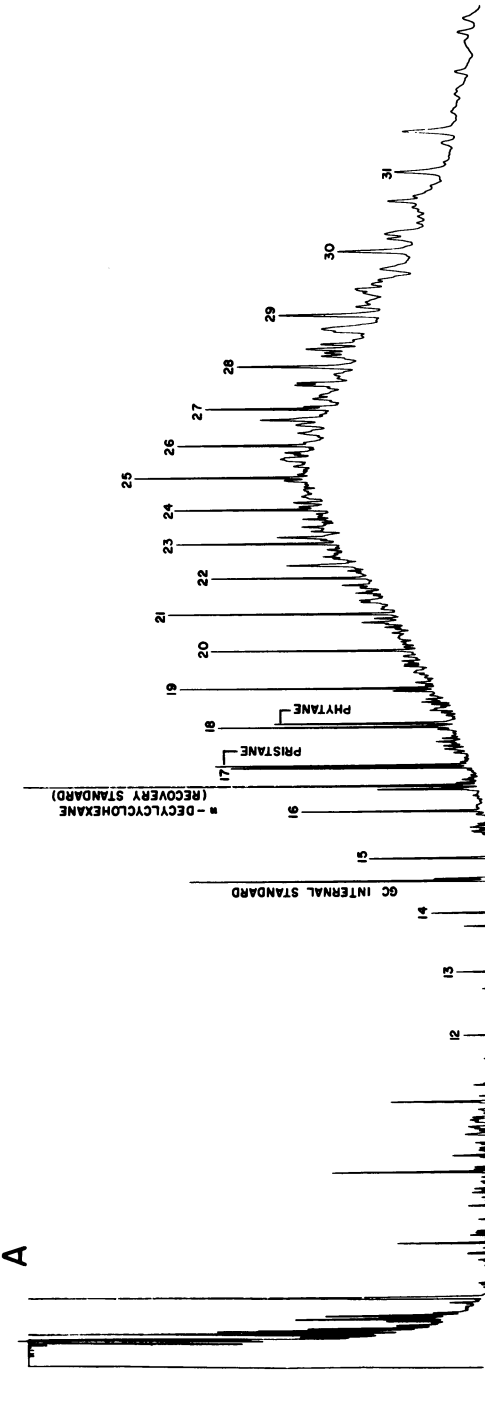


Figure 2A. Gas chromatograms of the saturated hydrocarbons from an extract of lower Duwamish River sediment, West Waterway, Seattle, Washington, Hewlett-Packard 5840A GC with flame ionization detector. J & W Scientific 30 m × 0.25-mm SE-54 WCOT column obtained from Supelco. 2μL splitless injection, vaporization at 280°C, split 10:1 after 18 sec with 22 psig helium carrier gas. Column temperature 40°C for 5 min, then programmed to 270°C at 4°/min. Numbered peaks refer to n*-alkanes of corresponding chain length.*

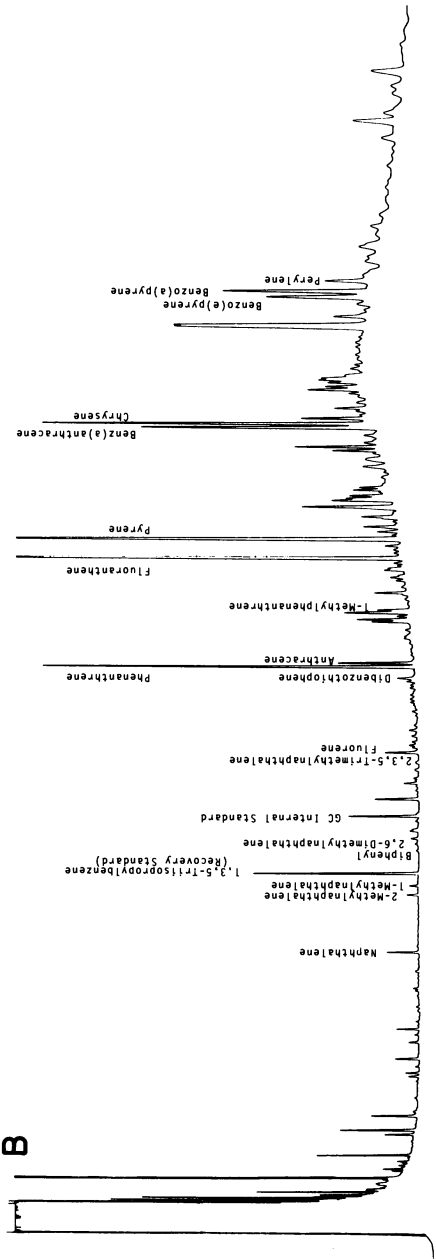


Figure 2B. Gas chromatograms of the unsaturated hydrocarbons from an extract of lower Duwamish River sediment, West Waterway, Seattle, Washington. Same conditions as for Figure 2A.

Table I. Concentrations of Aromatic Hydrocarbons Extraction

	CH_2Cl_2–CH_3OH Tumble	
Aromatic Hydrocarbon	$\bar{x}$ (n = 11) (ng/g)	RSD (%)
2-Methylnaphthalene	10	33
1-Methylnaphthalene	6	33
Biphenyl	2	39
2,6-Dimethylnaphthalene	8	26
2,3,5-Trimethylnaphthalene	6	58
Fluorene	30	28
Dibenzothiophene[b]	28	32
Phenanthrene	330	28
Anthracene	57	26
1-Methylphenanthrene	22	24
Fluoranthene	570	23
Pyrene	760	21
Benzanthracene	440	23
Chrysene	270	20
Benzo[*e*]pyrene	150	26
Benzo[*a*]pyrene	170	33
Perylene	36	36

[a] Data reported as ng/g dry weight; $\bar{x}$ = mean, n = number of analyses, RSD = relative standard deviation of the mean (100 SD/$\bar{x}$).

function at ambient temperature; avoided freeze-drying; and avoided benzene, a suspected carcinogen. After more than a year's experience with solvent–slurry extractions of sediments using a ball-mill tumbler (*6, 7*), we devised a procedure using methanol and dichloromethane that largely met these criteria.

In developing this tumbling extraction procedure, a number of solvent systems were investigated, including:

Diethyl ether–acidified water
Dichloromethane–acidified water
Dichloromethane:diethyl ether (2:1)–acidified water
Diethyl ether:methanol (2:1)
Dichloromethane:methanol (2:1), (1:1), and (1:2)

Comparison of these tumbler solvent systems showed that if methanol was one of the solvents, hydrocarbon extraction efficiencies were 2–3 times better than without it, regardless of the less polar cosolvent used. Hence, methanol was chosen as a cosolvent for use with dichloromethane, which is safer than diethyl ether or benzene.

To optimize our tumbler method, we tested various ratios of dichloromethane to methanol at various solvent-to-sediment ratios. Replicate

Found in Homogenized Duwamish River Sediment by Four Methods[a]

		Soxhlet Extraction			
CH_3OH–KOH Reflux		*Benzene–CH_3OH*		*CH_2Cl_2–CH_3OH*	
$\bar{x}$ (n = 4) (ng/g)	*RSD* (%)	$\bar{x}$ (n = 4) (ng/g)	*RSD* (%)	$\bar{x}$ (n = 4) (ng/g)	*RSD* (%)
7	25	14	28	11	59
4	16	8	25	7	67
1	28	9	24	2	95
5	21	9	27	6	118
< 1	—	7	29	4	76
14	8	50	50	35	51
20	2	50	57	51	63
180	2	610	44	370	47
34	7	120	50	65	49
16	8	56	38	33	48
320	3	840	40	560	41
280	5	1100	38	550	46
170	3	870	71	620	31
200	5	530	48	370	30
150	5	230	31	310	38
180	4	410	45	300	43
56	5	97	22	160	66

[b] A sulfur-substituted aromatic hydrocarbon.

extractions were performed on 100-g samples of a homogenized, moderately contaminated intertidal sediment (fine-to-medium-grained sand) from the West Waterway of the lower Duwamish River, Seattle, Washington. Generally, when dichloromethane:methanol ratios were between 2:1 and 1:2, extraction efficiencies approximated those achieved by benzene–methanol soxhlet extraction. Since a higher ratio of dichloromethane to methanol simplifies subsequent extract workup, a 2:1 ratio was chosen. Further study showed that when less than 100 mL of 2:1 dichloromethane–methanol were used to extract a 100-g sample of sediment, extraction efficiencies decreased.

Samples that had a substantial aqueous phase floating on the dichloromethane–methanol during extraction also gave lower hydrocarbon yields. Apparently, very wet samples reduced the methanol concentration in the solvent mixture to the point of ineffectiveness. To minimize this effect, sediments were dewatered by swirling briefly with methanol twice before extracting with 2:1 dichloromethane–methanol. The methanol from the dewatering steps was combined with the subsequent dichloromethane–methanol extracts before further contact with water; this was necessary to avoid erratic hydrocarbon recoveries.

Comparison of Extraction Methods. Preliminary analytical results (*12*) indicated that our ambient-temperature tumbler sediment extraction was about as efficient for hydrocarbon recoveries as soxhlet extraction. To test the tumbler extraction performance more completely, we have compared it with an alkaline methanol reflux extraction (*13*); a 1:1 benzene:methanol soxhlet extraction (*10*); and a 2:1 dichloromethane: methanol (azeotrope 7.6:1) soxhlet extraction (*15*), using replicate analyses of the homogenized harbor sediment.

Tables I and II list individual aromatic and aliphatic hydrocarbon yields, respectively and relative standard deviations (RSDs) for the four methods. Within the experimental uncertainties (i.e., the RSDs), the tumbler method compared favorably with the soxhlet extractions in the C_{13}–C_{26} alkanes (Table II), while the soxhlet extractions were generally more efficient in the C_{27}–C_{31} range. Soxhlet extraction with dichloromethane–methanol was least reproducible (e.g., 7 of 21 RSDs > 33%). Usually, direct reflux extraction was least efficient but most reproducible

Table II. Concentrations of Aliphatic Hydrocarbons Extraction

	CH_2Cl_2–CH_3OH Tumble	
n-*Alkane*	$\bar{x}$ (n = *14*) *(ng/g)*	*RSD (%)*
C_{13}	6	23
C_{14}	11	25
C_{15}	18	19
C_{16}	23	26
C_{17}	36	16
Pristane[b]	51	18
C_{18}	44	15
Phytane[b]	39	16
C_{19}	54	15
C_{20}	40	14
C_{21}	28	24
C_{22}	29	14
C_{23}	39	15
C_{24}	36	15
C_{25}	52	15
C_{26}	43	23
C_{27}	51	32
C_{28}	54	33
C_{29}	72	30
C_{30}	98	35
C_{31}	96	55

[a] Data reported as ng/g dry weight; $\bar{x}$ = mean, n = number of analyses; RSD = relative standard deviation of the mean (100 SD/$\bar{x}$).

for both alkanes and aromatics. Recoveries for the polynuclear aromatics below fluorene in Table I were generally highest by benzene–methanol soxhlet extraction. Again dichloromethane–methanol soxhlet extraction was least reproducible (highest RSDs).

Variability of Aromatic Hydrocarbons. Figure 2B shows that the unsubstituted aromatics are generally more abundant than their alkyl-substituted homologues. Recent research of LaFlamme and Hites (*16*) and Youngblood and Blumer (*17*) indicates that this pattern is characteristic of combustion byproducts as opposed to spilled fossil fuels. If these aromatics had such an origin and were deposited with various types of airborne particulates, they could give uneven results for this sediment, even though it was homogenized in a mixer for 3 hr. This is consistent with the greater variability observed for the aromatic data compared with the alkane data (e.g., 28 out of 68 RSDs > 33% in Table I vs. 10 out of 84 RSDs > 33% in Table II).

Found in Homogenized Duwamish River Sediment by Four Methods[a]

		Soxhlet Extraction			
CH_3OH–KOH Reflux		*Benzene–CH_3OH*		*CH_2Cl_2–CH_3OH*	
$\bar{x}$ (n = 5) (ng/g)	RSD (%)	$\bar{x}$ (n = 5) (ng/g)	RSD (%)	$\bar{x}$ (n = 4) (ng/g)	RSD (%)
4	17	6	14	5	32
8	16	11	15	9	29
15	12	18	16	15	29
20	10	23	16	20	24
29	7	29	24	29	15
40	8	40	42	37	15
28	9	39	17	33	22
33	7	33	29	32	15
31	5	41	33	32	7
30	9	38	20	28	4
35	12	39	15	36	4
22	8	30	15	30	7
27	7	34	12	40	20
31	5	35	13	46	20
37	9	44	8	74	51
34	11	47	15	69	48
59	15	62	23	150	63
45	11	110	15	170	57
42	11	100	17	160	46
36	15	98	20	180	120
36	10	144	23	130	87

[b] A branched alkane.

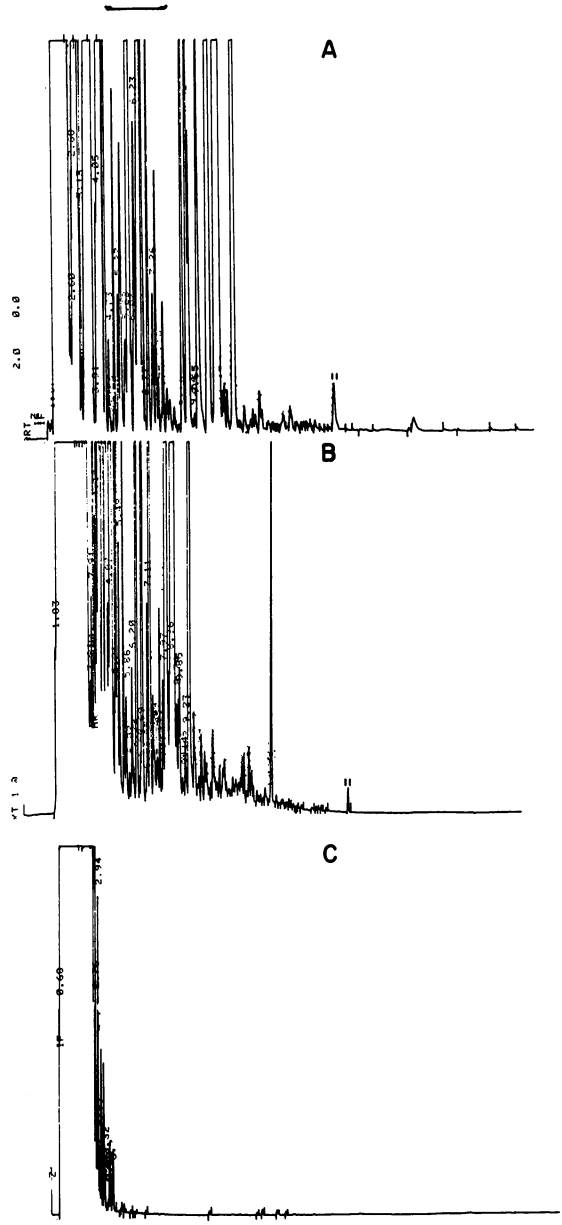


Figure 3. Gas chromatograms of 200-fold concentrated extracts of (A) high-quality, commercially available methanol, (B) the same methanol after fractional redistillation, and (C) the same methanol purified by dilution with water, extraction with hexane, and fractional redistillation. GC parameters and conditions same as in Figure 2.

In the extreme, the overall recoveries of aromatics in 5 of 28 analyses exceeded the overall mean of their respective subsets ($n \geq 5$) by factors of 3, 3, 5, and 10, while the corresponding alkane recoveries did not. These exceptional step increases in only the aromatics may be due to the presence of one or more aromatic-rich particles (e.g., soot). This possibility is not excluded since the sediment came from an active harbor in an urban industrial area. Dixon's statistical method of outlier analysis (*18*) was used to exclude such extreme results from Tables I and II.

Impurities in Methanol. We found that methanol was an important cosolvent for extracting hydrocarbons efficiently from the wet sediment. Even the purest of more than 10 top grades of commercial methanol had impurities that interfered with hydrocarbon analyses (Figure 3A). Attempts to purify such methanol by fractional redistillation removed only contaminants boiling higher than n-$C_{12}H_{26}$ (Figure 3B). To reduce contaminants less volatile than n-C_8H_{18} to acceptable levels (< 0.5 μg/L), methanol was diluted with water, extracted with hexane, and redistilled (Figure 3C).

Summary

We developed a hydrocarbon extraction procedure for sediments that uses methanol and dichloromethane with a ball-mill tumbler. Within the limits of experimental error (1σ), this procedure is generally as efficient as soxhlet extraction with benzene–methanol or dichloromethane–methanol and more efficient than direct reflux with alkaline methanol. The new procedure, which is convenient for processing large numbers of samples, uses less expensive equipment and less toxic solvents than previously employed soxhlet techniques. Ambient-temperature extraction eliminates the problems inherent with boiling solvents and cooling water. Moreover, dewatering with methanol makes freeze drying or air drying unnecessary.

Acknowledgments

This study was supported by the Bureau of Land Management through the Outer Continental Shelf Environmental Assessment Program (OCSEAP) of the National Oceanic and Atmospheric Administration (NOAA). We thank J. A. Calder and J. J. Kineman of OCSEAP for 50 kg of homogenized sediment; D. D. Gennero, P. G. Prohaska, D. D. Dungan, and J. S. Finley for laboratory assistance; R. G. Jenkins and T. I. Sherman for data preparation; and F. J. Ossiander for statistical evaluation. Reference to a company or a product does not imply endorsement by the U.S. Department of Commerce to the exclusion of others that may be suitable.

Literature Cited

1. Clark, R. C., Jr.; Brown, D. W. "Petroleum: Properties and Analyses in Biotic and Abiotic Systems," in "Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, Nature and Fate of Petroleum"; Malins, D. C., Ed.; Academic: New York, 1977; Vol. I, pp. 1–89.
2. Farrington, J. W.; Teal, J. M.; Parker, P. L. "Petroleum Hydrocarbons," in "Strategies for Marine Pollution Monitoring"; Goldberg, E. D., Ed.; John Wiley: New York, 1976; pp. 3–34.
3. Rohrback, B. G.; Reed, W. E. "Evaluation of Extraction Techniques for Hydrocarbons in Marine Sediments," Institute of Geophysics and Planetary Physics, University of California, Los Angeles, CA, 1975, No. 1537.
4. Hilpert, L. R.; May, W. E.; Wise, S. A.; Chesler, S. N.; Hertz, H. S. "Interlaboratory Comparison of Determinations of Trace Level Petroleum Hydrocarbons in Marine Sediments," *Anal. Chem.* **1978,** *50,* 458.
5. Farrington, J. W.; Tripp, B. W. in "Marine Chemistry in the Coastal Environment," *ACS Symp. Ser.* **1975,** *18,* 267–284.
6. MacLeod, W. D.; Brown, D. W.; Jenkins, R. G.; Ramos, L. S.; Henry, V. D. "A Pilot Study on the Design of a Petroleum Hydrocarbon Baseline Investigation for Northern Puget Sound and Strait of Juan de Fuca," NOAA Tech. Memo. ERL MESA-8, National Oceanic and Atmospheric Administration, Boulder, CO, 1977.
7. MacLeod, W. D.; Brown, D. W.; Jenkins, R. G.; Ramos, L. S. "Intertidal Sediment Hydrocarbon Levels at Two Sites on the Strait of Juan de Fuca," In "Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems"; Wilfe, D. A., Ed.; Pergamon: New York, 1977; pp. 385–396.
8. Warner, J. S., Battelle Memorial Institute, Columbus, OH, personal communication, 1976.
9. Carpenter, R.; Bates, T., Department of Oceanography, University of Washington, Seattle, WA, personal communication, 1977.
10. Clark, R. C., Jr.; Finley, J. S. "Techniques for Analysis of Paraffin Hydrocarbons and for Interpretation of Data to Assess Oil Spill Effects in Aquatic Organisms," *Proc. Jt. Conf. Prev. Control Oil Spills* **1973,** 161–172.
11. Chesler, S. N.; Gump, B. H.; Hertz, H. S.; May, W. E.; Dyszel, S. M.; Enaganio, D. P. "Trace Hydrocarbon Analysis: The National Bureau of Standards Prince William Sound/Northeastern Gulf of Alaska Baseline Study," *Nat. Bur. Stand. (U.S.), Tech. Note* **1976,** 889.
12. Brown, D. W.; Ramos, L. S.; Friedman, A. J.; MacLeod, W. D. "Analysis of Trace Levels of Petroleum Hydrocarbons in Marine Sediments Using a Solvent Slurry Extraction Procedure," In "Proceedings 9th Materials Symposium, Trace Organic Analysis: A New Frontier in Analytical Chemistry"; National Bureau of Standards: Washington, DC, 1979.
13. Quinn, James G., Graduate School of Oceanography, University of Rhode Island, Kingston, RI, personal communication, 1978.
14. Shaw, D. G., Institute of Marine Science, University of Alaska, College, AK, personal communication, 1977.
15. Calder, J. A., Florida State University, personal communication, 1977.
16. LaFlamme, R. E.; Hites, R. A. "The Global Distribution of Polycyclic Aromatic Hydrocarbons in Recent Sediments," *Geochim. Cosmochim. Acta* **1978,** *42,* 289.
17. Youngblood, W. W.; Blumer, M. "Polycyclic Aromatic Hydrocarbons in the Environment: Homologous Series in Soils and Recent Marine Sediments," *Geochim. Cosmochim. Acta* **1975,** *39,* 1303.
18. Dixon, W. J. "Processing Data for Outliers," *Biometrics* **1953,** *9,* 74.

RECEIVED October 31, 1978.

Distribution of Aromatic Hydrocarbons in Sediments from Selected Atlantic, Gulf of Mexico, and Pacific Outer Continental Shelf Areas

E. B. OVERTON and J. L. LASETER[1]

Center for Bio-organic Studies, University of New Orleans, New Orleans, LA 70122

Approximately one hundred near-surface sediments from selected Atlantic, Gulf of Mexico, and Southern California OCS areas were analyzed. Hydrocarbon components were extracted by reflux with hexane and benzene. The aromatic components were isolated by silica gel absorption chromatography and were analyzed by high-resolution gas chromatography and GC–MS methods. Total aromatics ranged from 9.0 to 1080 ng/g dry weight of sediment. Aliphatics were present from 3 to 5 times higher in concentration than aromatics. Pristane:phytane ratios were generally 1:6 in samples that were rich in the aromatic components. In terms of relative abundance, two-ring PAHs were more abundant than three rings, which were more abundant than four rings. Alkyl substitution was common in the two- and three-ring PAHs. Little alkyl substitution was observed in the four or more ring systems. Correlations of aromatic types with geographic distribution and contemporary petroleum sources are discussed.

The existence and sources of polynuclear aromatic hydrocarbons (PAHs) in the environment is of great interest for several reasons. One of the most important reasons is that several PAHs are known carcinogens to human beings and may be accumulating in the environment.

[1] Author to whom correspondence should be addressed.

0-8412-0475-6/80/33/327$05.00/0

Furthermore, many PAHs are not thought to be produced biogenically or diagenetically; so their presence in the environment indicates contamination from natural events, such as forest fires and oil seeps, and a variety of anthropogenic inputs, such as automobile exhaust, industrial pollution, and oil spills. A number of reports have recently appeared in the literature on the evaluation of the sources of PAHs in the environment (*1–6*). These authors generally relate the distribution of alkyl substitution on PAHs in sediments from urban areas to their potential sources. They rely on the fact that low-temperature combustion results in highly alkylated PAHs, while high-temperature sources yield primarily nonalkylated species. Alkyl homologue distribution plots thus are used to indicate source temperatures of PAHs and, by inference, the actual source materials. These assumptions, however, are predicted on the tenuous hypothesis that the alkyl homologue distributions are not subjected to environmental modifications, such as differential water solubilities or preferential biodegradation. One firmly established fact concerning these types of compounds, though, is that elevated levels of a series of different ring-size PAHs show close correlation to anthropogenic activity.

At present there are no reports in the open literature that describe the distribution of PAHs in a large number of samples from the outer continental shelf (OCS) areas of the United States. It is important to study sediments from these areas, because they are generally remote from common centers of urban pollution and therefore may not be grossly affected by anthropogenic sources of PAHs. Furthermore, several of the OCS regions investigated are potential oil and gas production areas and it is certainly desirable to develop baseline data on their PAH content prior to exploration. This presentation reports on the analysis and distribution of PAHs and their alkyl homologues in 92 surface sediment samples from selected Atlantic, Gulf of Mexico, and Pacific OCS areas. It represents an effort to present these important results in the open literature for evaluation by marine scientists. A brief discussion of the potential sources of PAHs in recent sediments, based on contemporary understanding of these sources, is also presented.

Experimental

All sediment samples were analyzed according to the analytical scheme shown in Figure 1. This procedure was designed to analyze aliphatic and aromatic hydrocarbons in the low ng/g (ppb) range in many sediment samples and sediment types. The samples were freeze-dried in an oven-type lyophilizer. Approximately 100 g, dry weight, was taken for analysis from each of the sediment samples. The dried sediments were reflux-extracted overnight using first 200 mL of *n*-hexane followed by another overnight reflux extraction using 200 mL of benzene. The extracts were combined and reduced in volume to approximately

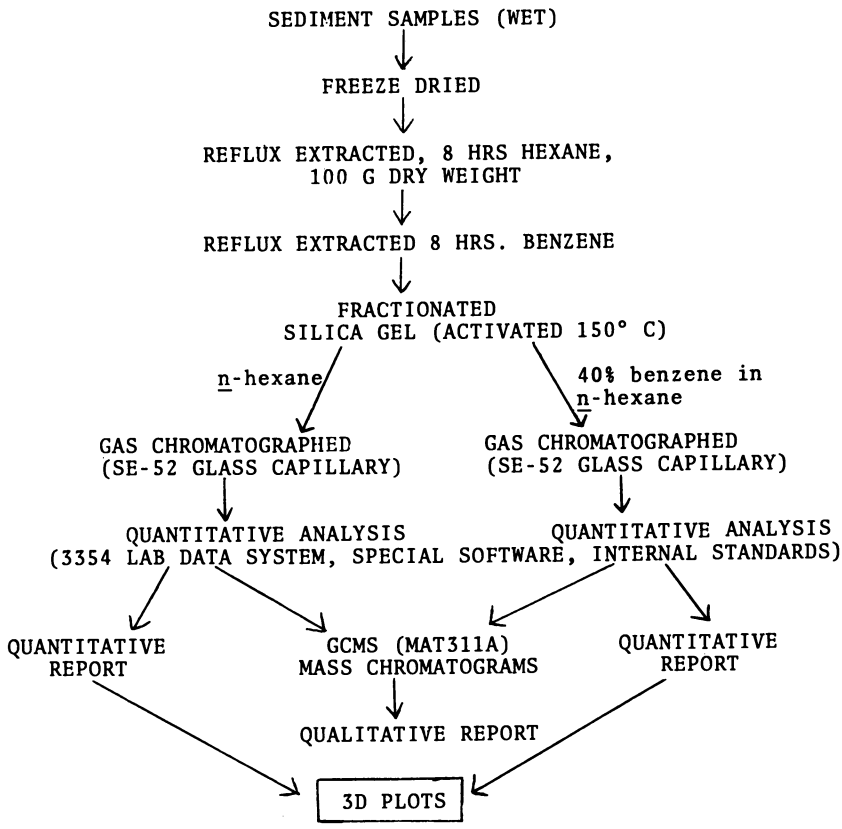


Figure 1. Schematic of the analytical scheme used for analysis of OCS sediment samples by the University of New Orleans

100 μL. These extracts were then fractionated on silica gel columns (Davison grade 923, 100–200 mesh, activated overnight at 150°C) using three bed volumes of *n*-hexane followed by three bed volumes of 40% benzene in *n*-hexane. Each fraction was reduced in volume on a rotary evaporator to below 5 mL and stored in a freezer until analyzed by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) techniques.

The *n*-hexane and 40% benzene in *n*-hexane fractions of all samples were quantitatively analyzed using high-resolution glass capillary–gas chromatography (HR (GC)^2). Figure 2 shows the computer reconstructed HR (GC)^2 of the 40% benzene in *n*-hexane fraction of a sediment sample collected off the southern Texas coast. Typical columns were 25 m × 0.3 mm i.d. and were statically coated with SE-52 liquid phase according to the barium carbonate procedure of Grob (7). Helium was the carrier gas and had a linear velocity of 38 cm/sec (3 mL/min). Samples were injected using our modification of the splitless procedure described by Grob (8). Eluting peaks were detected by flame ionization detection and the data digitized and stored by a HP 3354A dual-disc

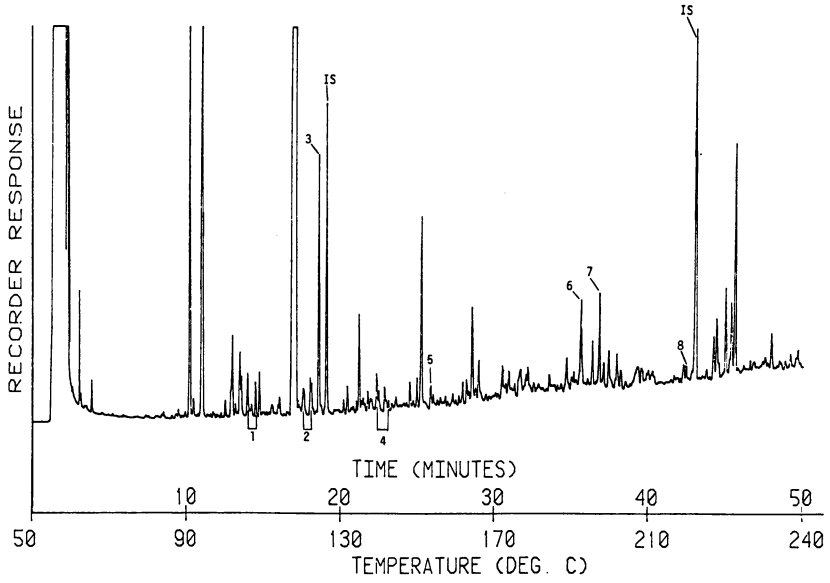


Figure 2. A 50-min portion of a typical HR (GC)² chromatogram of a 40% benzene in hexane fraction of a Gulf of Mexico sediment sample collected off the south Texas coast. 1, C_1-naphthalene; 2, C_2-naphthalene; 3, biphenyl; 4, C_3-naphthalenes; 5, C_5-naphthalene; 6, fluoranthene; 7, pyrene; and 8, chrysene. IS represents internal standards (hexamethyl benzene and 3-methyl tricosane). Chromatographic conditions are described in the text.

Laboratory Data System. All quantitative calculations were based on the internal standard. The internal standards were added prior to the final concentration step necessary for GC injection. Special software to integrate resolved GC peaks accurately, eluting on top of complex unresolved mixtures, was used for most quantitative calculations (9). The three-dimensional plots of the distribution of PAH in sediments from the various locations were generated from digital chromatographic data using a Textronix 4662 XY plotter and software developed in-house. A more detailed discussion of the chromatographic procedures used for these analyses can be found in the literature (*10*). Generally, spiking experiments revealed that this procedure gives recoveries for the high-molecular-weight hydrocarbons of approximately 60% and has a precision of ±5% (sediment spiked with a standard mixture of 19 petroleum-type hydrocarbons at the 8.65-ppm level, $n = 4$).

Chromatographically resolved components were characterized using a Varian MAT 311A high-resolution gas chromatograph–mass spectrometer–computer system. Eluting peaks were introduced directly into the ion source through a Henneburgh-type coupling (*11*). The ionization potential was 70 eV, source temperature was 250°C, and the mass spectrometer was operated at a resolution of 1000 ($M/\Delta m$ at 10% valley). All mass spectrometric data were acquired and processed using a Varian

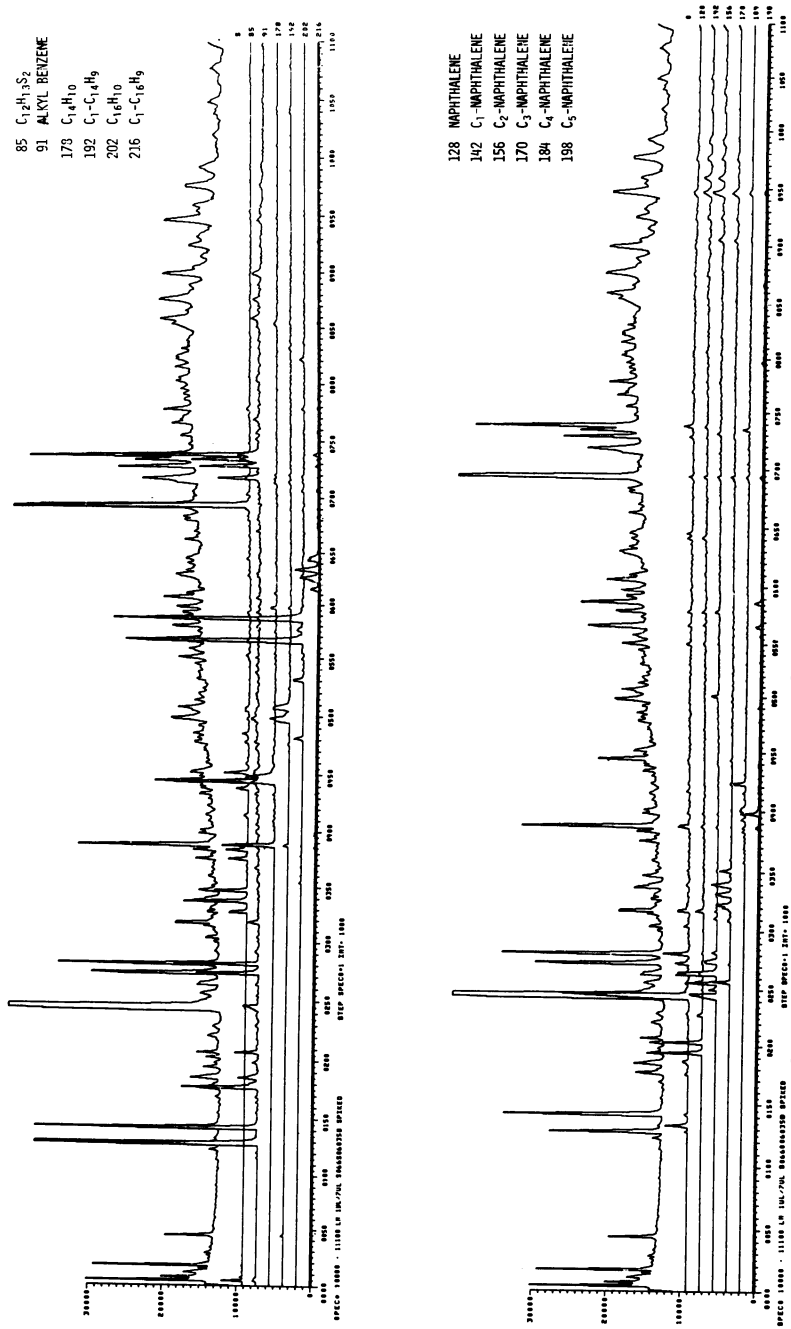


Figure 3. Total summation chromatogram and selected mass chromatograms of a 40% benzene–n-hexane fraction of a Gulf of Mexico sediment sample

Spectro System 100 Data System. The scanning rate was 2.5 sec per decade with a 3.8-sec cycle time. Mass ranges from 40 to 600 amu were scanned. Data display included total ion summation plots (total ion chromatograms), mass chromatograms of selected masses, and background-subtracted mass spectra. Figure 3 shows typical mass chromatograms from a northern Gulf of Mexico sediment sample that indicate the potential presence of a series of PAHs from naphthalene ($m/e = 128$) to pyrene ($m/e = 202$) and several alkylated homologues of these PAHs. All identifications of specific compounds were based on comparison of the background-subtracted mass spectra of these sample components with the mass spectra of known compounds.

Samples were collected as part of the quality assurance effort associated with the benchmark analytical program of the U.S. Department of Interior, Bureau of Land Management, Outer Continental Shelf Environmental Studies Program. Figure 4 shows the general location for sample collection under this program and the approximate boundaries for the OCS. Specific sample locations and other physical parameters associated with collection of these samples can be found in quality control reports to the Bureau of Land Management for the years 1977–1978. The sample locations within an area were generally separated by distances on the order of kilometers. No replicate sample analyses are reported in this document. The North Atlantic samples came from off the New England coast south of Cape Cod George's Bank). The Middle Atlantic samples were collected from locations off the Virginia coast, and the South Atlantic samples came from the area off the coast of Georgia. MAFLA (Mississippi, Alabama, Florida) samples came from the northern Gulf of Mexico off the Mississippi, Alabama, and Florida coasts, while south Texas sam-

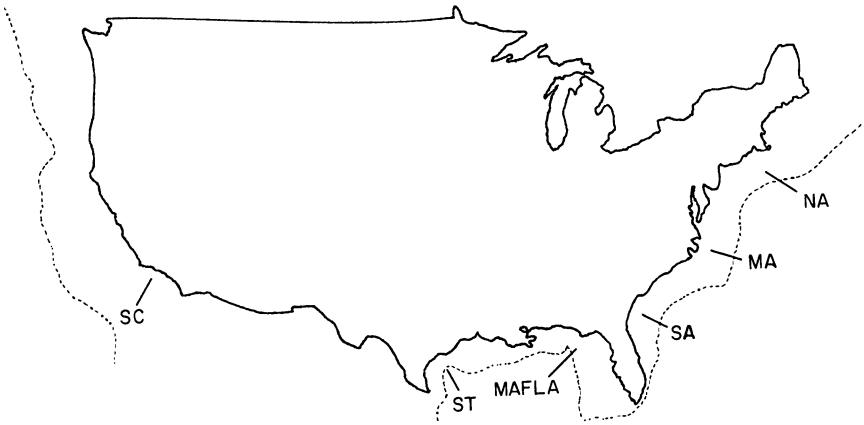


Figure 4. Diagram showing general areas of sample collection for the BLM benchmark program. NA, North Atlantic; MA, mid-Atlantic; SA, South Atlantic; MAFLA, Mississippi, Alabama, Florida, Gulf of Mexico; ST, south Texas, Gulf of Mexico; SC, southern California Pacific; and (– – –) indicates approximate boundry for the OCS.

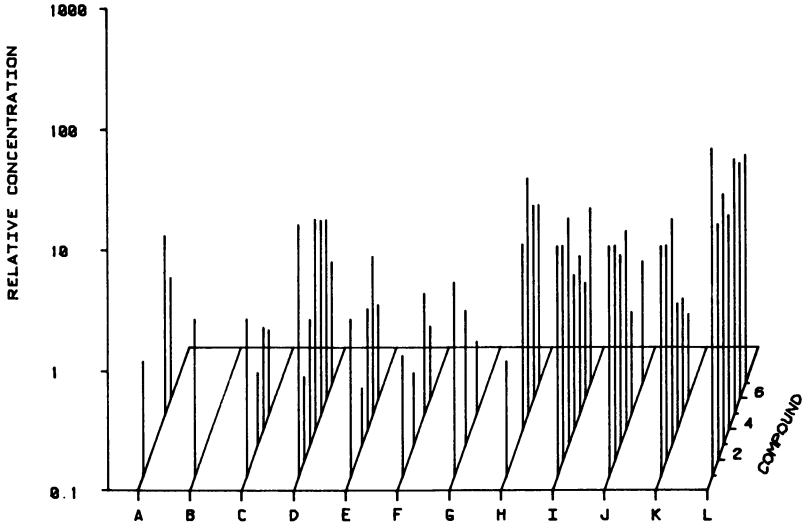
ples came from the general locations off the southwestern Texas coast. The Pacific OCS samples were collected from the area of the Los Angeles Bight. All samples were surface sediments collected with either cores or grabs. Great care was taken to ensure that samples were then collected, stored, and processed so as to exclude hydrocarbon contamination.

Discussion and Results

All samples reported in this presentation were obtained from pristine OCS areas that were not in close proximity to populated urban centers. Furthermore, the analytical techniques were of a general nature and were designed to analyze both aliphatic and aromatic hydrocarbons in the molecular weight range above 125 (high-molecular-weight hydrocarbons) in diverse sediment types. Detection limits for this procedure are in the low ng/g (ppb) range. Figure 2 shows a typical computer-reconstructed HR $(GC)^2$ of the 40% benzene in *n*-hexane fraction from a Gulf of Mexico sediment collected off the south Texas coast. Quantitative data on the PA components were derived from this and similar chromatograms. Figure 3 illustrates the total summation chromatogram (reconstructed gas chromatogram) and several mass chromatograms for this gulf sediment sample. Mass chromatograms were used to aid in the identity of PAHs and their alkyl homologues. The lower six mass chromatographs in Figure 3 indicate the presence of a small amount of naphthalene ($m/e = 128$) and the presence of C_1, C_2, and C_3 alkyl homologues of naphthalene. Apparently there were no C_4 or C_5 alkyl homologues of naphthalene indicated in this sample. The existence of specific components can only be inferred from mass chromatograms; their presence must be confirmed from background-subtracted mass spectra of the peaks eluting at the indicated scan number (retention time). The upper portion of Figure 3 also shows mass chromatograms that were used to indicate the possible presence of dihexyldisulfide and alkane components ($m/e = 85$), alkyl benzene homologues ($m/e = 91$), phenanthrene or anthracene ($m/e = 178$), C_1-phenanthrene isomers ($m/e = 192$), fluoranthene and pyrene ($m/e = 202$), and C_1-pyrene isomers ($m/e = 216$). A careful examination of the data displayed in these upper traces, for example, indicates the presence of fluoranthene and pyrene eluting at scan numbers 0565 and 0590, respectively. The possible presence of the C_1 alkyl homologues of these substances, eluting between scan numbers 0600 and 0650, is indicated from the mass chromatograms. However, because of coeluting substances and low concentrations, the identity of these C_1 homologs could not be confirmed from background-subtracted mass spectra and their quantities could not be accurately determined from GC. These C_1

alkyl homologues, if present in this sample, are in concentrations below the detection limits of the analytical procedure used for analysis of these samples.

The data displayed in Figures 5–11, using a three-dimensional plotting format, show the concentration (vertical axis, log scale) in ng/g of selected PAHs and their alkylated homologues (axis into page, numbered) for a number of samples (horizontal axis, lettered) from selected OCS areas of the Atlantic, Gulf of Mexico, and Pacific. The selected PAHs and alkyl homologues represent essentially all the aromatic hydrocarbons found in samples from the given areas. Not shown in these data are the concentrations of numerous biogenic olefins and other compounds found in the aromatic fraction or the distribution of aliphatic hydrocarbons for each sample. The aliphatic fraction for these samples contained normal, branched, cyclic, and isoprenoid hydrocarbons. The Carbon Preference Index (CPI) for n-C_{20}/n-C_{31}, the pristane/phytane, n-C_{17}/pristane, and



CPI	13	--	--	4.2	9.9	54	--	3.0	3.6	--	3.9	3.8
Pr/Ph.	--	--	.4	--	.9	--	--	6.3	4.	5.9	5.3	2.9
n-C_{17}/Pr.	--	--	1.4	--	.33	--	--	.15	5.	.59	2.5	.25
n-C_{18}/Ph.	--	--	.21	.4	.125	--	--	.43	10.	1.7	5	2.5

Figure 5. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the North Atlantic OCS areas. 1, Biphenyl; 2, C_2-naphthalenes; 3, C_3-naphthalenes; 4, C_1-phenanthrenenes; 5, fluoranthene; 6, pyrene; 7, $C_{20}H_{12}$; CPI, Carbon Preference Index (n-C_{20} to n-C_{31}); Pr., pristane; Ph., phytane.

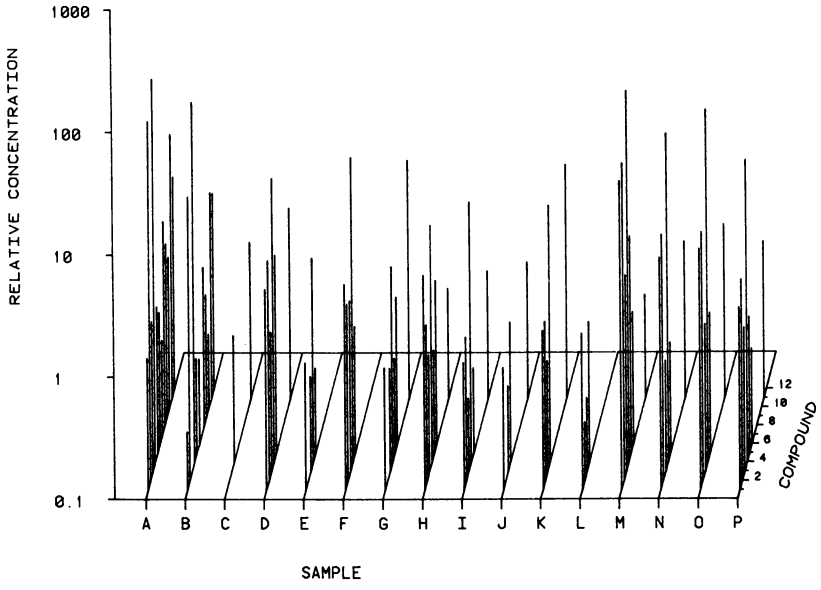


Figure 6. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the mid-Atlantic OCS areas. 1, Naphthalene; 2, C_6-alkylbenzene; 3, C_1-naphthalenes; 4, biphenyl; 5, C_2-naphthalenes; 6, C_3-naphthalenes; 7, fluorene; 8, phenanthrene/anthracene; 9, C_1-phenanthrenes; 10, C_2-phenanthrenes; 11, fluoranthene/pyrene; 12, $C_{20}H_{12}$

n-C_{18}/phytane ratios have been tabulated for a selected group of samples collected from the northern Atlantic, Gulf of Mexico, and Pacific OCS areas and can be seen in Figures 5, 9, and 10. Careful examination of these aliphatic data fails to reveal a general correlation between these parameters and the nature or abundance of PAHs in sediment samples. The only general statement concerning aliphatic and aromatic components in these samples is that samples containing elevated levels of aromatic hydrocarbons also contained elevated levels of aliphatic substituents.

An examination of Figures 5 through 11 can lead to several generalizations about the presence and distribution of PAHs in the marine sediments of the OCS area. First, numerous aromatic components were observed in most sediment samples taken from OCS pristine areas. This is significant because very few PAHs are thought to be produced biogenically and, therefore, other sources must account for the presence of these compounds. Also, within a given region and between regions there appeared to be a significant qualitative and quantitative variation in the PAH distribution. This observation is important because it emphasizes

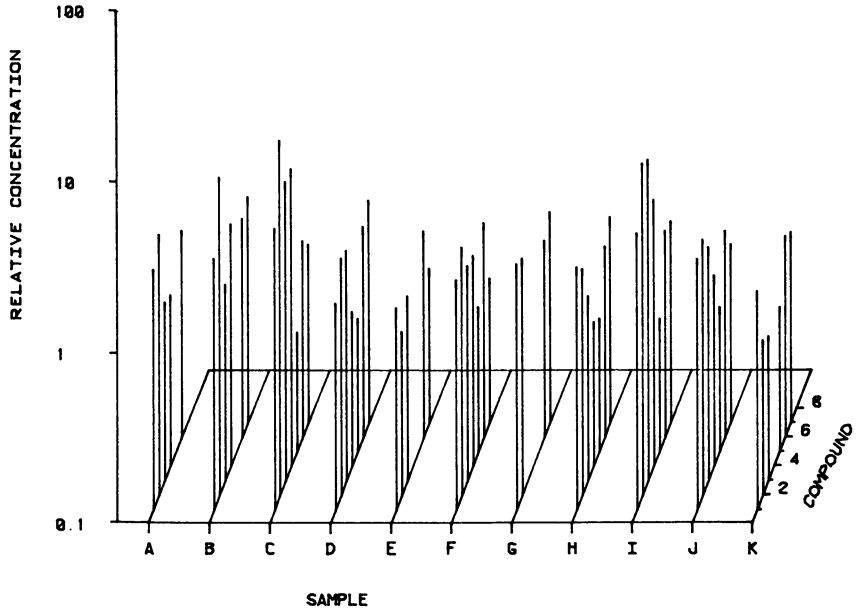


Figure 7. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the South Atlantic OCS area. 1, Naphthalenes; 2, C_1-naphthalenes; 3, biphenyl; 4, C_2-naphthalenes; 5, C_3-naphthalenes; 6, phenanthrene; 7, fluoranthene; 8, pyrene.

the need for extensive sample collection, analysis, and examination of the data before conclusions can be drawn about the distribution and sources of PAHs in environmentally remote areas of the OCS. The concentration observed for the total PAH components were, in general, similar to those reported by Laflamme and Hites (5) for nonurban areas.

Second, as can be seen most clearly in Figures 10 and 11, surface sediments from off the southern California coast were somewhat richer in PAHs and their alkylated homologues than samples from other regions. This is not surprising, considering the close proximity of these samples to known natural oil seep areas and abundant anthropogenic activity. Also, mid-Atlantic sediments were rich in a variety of PAHs. This may reflect ocean dumping and urban runoff associated with the New York area.

Third, a general inspection of the data reveals that these sediment samples contained PAHs with two to five aromatic rings. Most samples, however, did not contain PAHs with more than four rings. In fact, fluoranthene and pyrene were present in a large majority of the samples. Also, there is very little alkyl substitution above the C_3 homologue. This

is in contrast to recent findings of Hites and Biemann (*3*) of abundant alkyl substitution, in some cases up to 15 carbon atoms in the alkyl group, in samples from urban areas.

Fourth, in samples containing abundant two- and three-ring aromatic compounds, the alkyl homologues of each species were more abundant than the unsubstituted species. These data are consistent with a low-temperature source, such as petroleum, for these components.

Fifth, in the four-or-more-ring aromatic compounds, the nonalkylated species were more abundant than the alkylated species. This observation indicates a high-temperature source for these substances. Other explanations for the data, however, such as preferential solubilities and transports or environmental modifications, must be considered before the sources of the larger PAHs can be unequivocally determined.

Sixth, there appears to be no general correlation between the distribution and concentration of PAHs and the various aliphatic hydrocarbon ratios and parameters generally used to indicate petroleum contamination of samples. These ratios may be used in detecting relatively gross levels of petroleum contamination; however, they are of little value in determining trace petroleum hydrocarbon contamination in sediment samples.

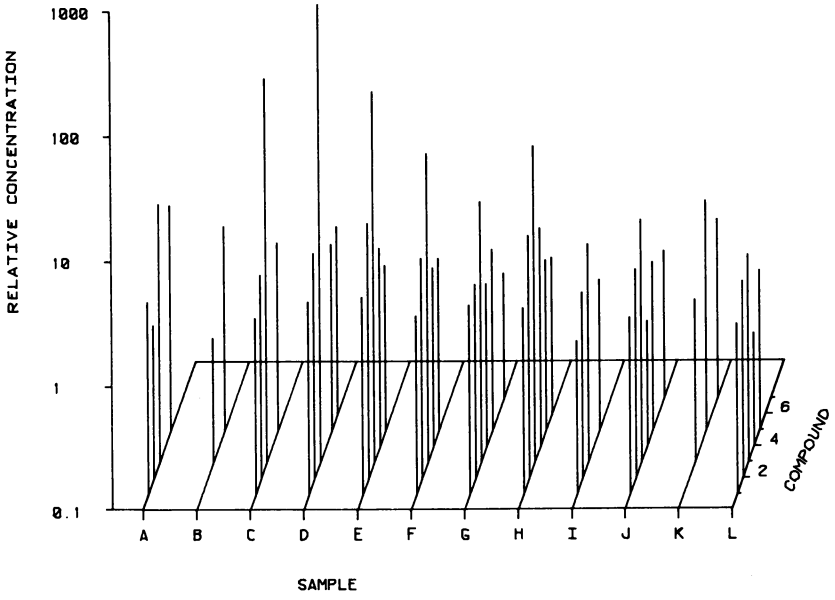
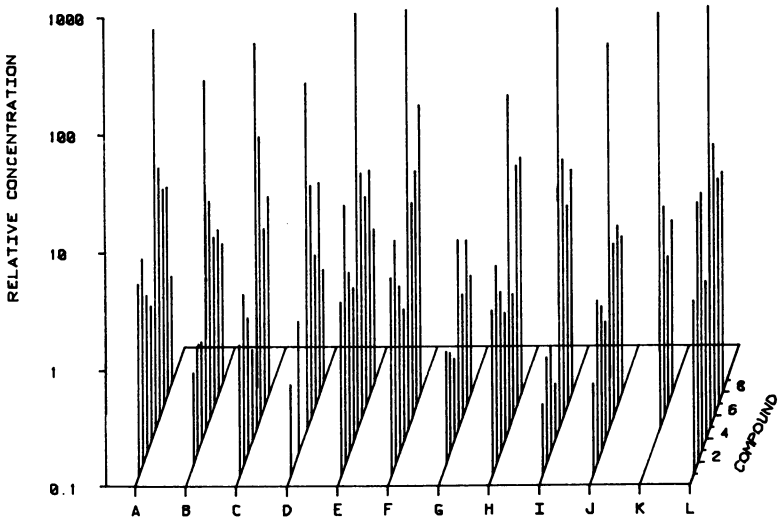


Figure 8. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the MAFLA OCS area. 1, Naphthalene; 2, C_1-naphthalenes; 3, biphenyl; 4, C_2-naphthalenes; 5, phenanthrene; 6, fluoranthene; 7, pyrene.

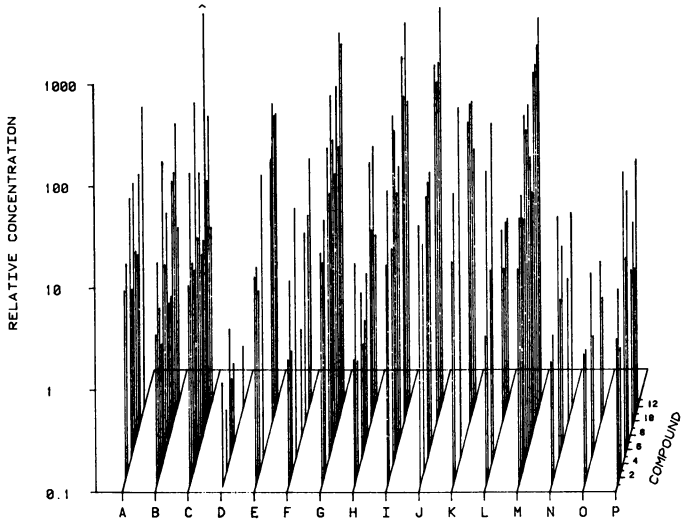
Seventh, based on contemporary thought, the PAHs in these samples appear to have dual origins. Other factors not now obvious, however, may eventually provide an explanation for this seemingly contradictory data.

Eighth, the data presented in Figures 5–11 represent a comparative study of the concentration of selected PAHs in a large number of sediment samples. All samples were analyzed using the same analytical procedure. Much care should be exercised when comparing these data with other data generated through different analytical procedures. For example, wet chemical extraction and treatment of samples can be used to enhance the analysis of selected compound types. General extraction procedures do not benefit from this selected enhancement. However, selected enhancement of specific compound types generally precludes the analysis of other types of compounds that may eventually prove to be environmentally significant. Another example of the differences in ana-



CPI	8.2	4.0	1.9	5.2	3.2	5.8	--	5.1	5.5	6.3	1.1	3.8
Pr./Ph.	.55	.8	1.1	--	.82	1.2	1.02	.75	1.7	.95	1.03	.20
C_{17}/Pr.	1.5	1.7	2.0	2.5	1.2	1.06	1.6	1.5	1.1	2.2	1.4	0.2
C_{18}/Ph.	.35	--	.68	--	.45	.22	--	1.03	--	.95	--	1.6

Figure 9. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the South Texas OCS area. 1, C_1-naphthalenes; 2, C_2-naphthalenes; 3, C_3-naphthalenes; 4, C_5-naphthalenes; 5, biphenyl; 6, C_1-biphenyl; 7, fluoranthene; 8, pyrene; 9, chrysene; CPI, Carbon Preference Index (n-C_{20} to n-C_{31}); Pr., pristane; Ph., phytane.



CPI	5.7	4.9	3.7	--	2.7	6.7	1.9	8.0	3.1	2.6	2.8	.92	1.1	2.4	3.3	2.3
Pr./Ph.	3.5	2.5	2.5	--	1.26	1.5	--	1.6	1.7	2.7	1.5	1.2	1.5	1.3	.69	1.25
C_{17}/Pr.	1.0	1.45	2.5	1.3	1.1	.43	1.3	.35	.65	.41	.99	1.9	.6	.99	.77	1.1
C_{18}/Ph.	2.0	1.5	1.4	--	.9	1.2	--	2.9	.68	.35	.71	1.3	.39	1.05	.55	1.2

Figure 10. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the southern California OCS area. 1, Naphthalene; 2, C_6-alkylbenzene; 3, C_1-naphthalenes; 4, C_6-alkenylbenzene; 5, biphenyl; 6, C_2-naphthalenes; 7, C_3-naphthalenes; 8, C_2-biphenyls; 9, C_3-biphenyls; 10, fluoranthene; 11, pyrene; 12, unknown olefin; 13, DDT; CPI, Carbon Preference Index (n-C_{20} to n-C_{31}); Pr., pristane; Ph., phytane.

lytical procedures that can significantly affect the data is the use of mass chromatograms and background-subtracted mass spectra as compared to the use of selected ion monitoring techniques. The former is more reliable and specific, but less sensitive, than the latter. Much additional work is needed to improve the precision, accuracy, and detection limits for trace hydrocarbon analysis of these sediment samples.

Conclusions

The sources of PAHs in the environment are the subject of much speculation. Of major importance is the ability, using analysis of these types of compounds, to detect contamination from hydrocarbons of petroleum origin. The results accumulated by our laboratory and others indicate that no single parameter can easily be used to describe low levels of petroleum contamination. Furthermore, the ability to differen-

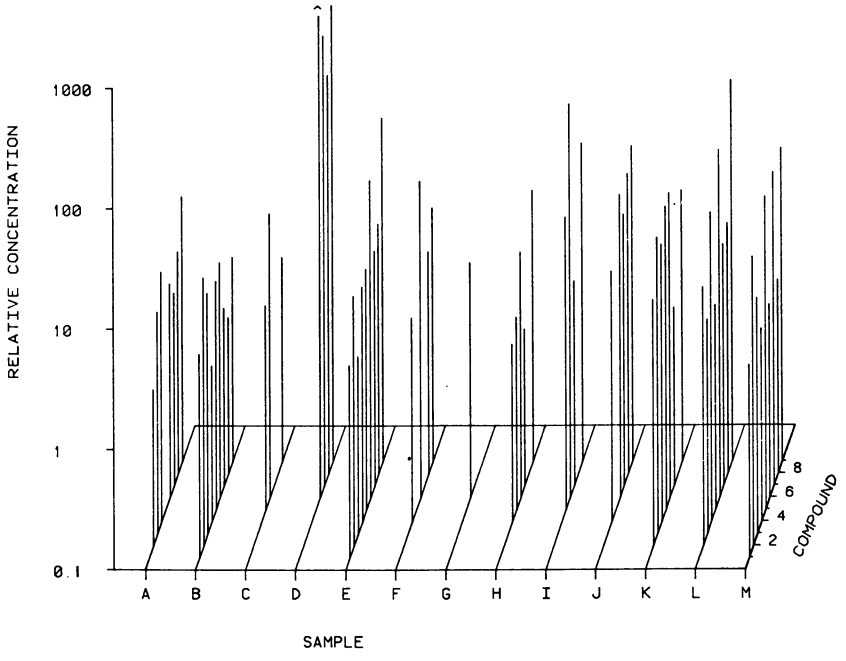


Figure 11. Three-dimensional diagram showing the distribution of selected aromatic hydrocarbons in samples from the southern California OCS area. 1, C_1-Naphthalenes; 2, C_2-naphthalenes; 3, C_3-naphthalenes; 4, C_1-phenanthrenes; 5, fluoranthene; 6, pyrene; 7, C_1-tetrahydronaphthalenes; 8, C_1-benzo[c]anthracenes; 9, DDE.

tiate petroleum hydrocarbons from others of contemporary origin will depend on the analysis of the many chemical parameters and should not rely on the presence and concentration of certain common aromatic components.

Much care should be exercised when comparing hydrocarbon data obtained by using different extraction and analytical techniques. Procedures designed to analyze high-molecular-weight hydrocarbons in large numbers of samples, such as those we have used, are inherently less specific than methods designed to analyze solely PAHs.

Petroleum and low-temperature combustion processes yield primarily highly alkylated PAHs, while high-temperature combustion sources result in many nonalkylated PAHs. If this is generally the case, we must conclude, from an examination of the data in Figures 5–11, that the PAHs in these samples generally have dual sources or that they have undergone substantial biochemical and/or physical modifications. If petroleum is a

contributing factor to the PAH distribution, it represents a very modest input and one that has been considerably altered from the PAH distributions in recent source crude oils.

Acknowledgments

The authors wish to thank the Bureau of Land Management for partial support of this work under contract AA550-CT6-19; Chuck Steele for technical support; and Chris Raschke, Nancy Foster, and Diane Trembley for help in preparing the figures and manuscript.

Literature Cited

1. Blumer, M.; Youngblood, W. A. *Science* **1975**, *188*, 53–55.
2. Hase, A.; Hites, R. A. *Geochim. Cosmochim. Acta* **1976**, *40*, 1141–1143.
3. Hites, R. A.; Biemann, W. G. In "Analytical Methods in Oceanography," *Adv. Chem. Ser.* **1975**, *147*, 188–201.
4. Hites, R. A.; LaFlamme, R. F.; Farrington, J. W. *Science* **1977**, *198*, 829–831.
5. LaFlamme, R. E.; Hites, R. A. *Geochim. Cosmochim. Acta* **1978**, *42*, 289–303.
6. Muller, G.; Grimmer, G.; Boehnke, H. *Naturwessenschaften* **1977**, *64*, 427–431.
7. Grob, K.; Grob, G. *J. Chromatogr. Sci.* **1976**, *125*, 471–485.
8. Grob, K.; Grob, G. *J. Chromatogr. Sci.* **1969**, *7*, 584–586.
9. Overton, E. B.; Steele, C. F.; Laseter, J. L. "Abstracts of Papers," 27th Pittsburgh Conference in Analytical Chemistry and Applied Spectroscopy, March 1978.
10. Overton, E. B.; Bracken, J.; Laseter, J. L. *J. Chromatogr. Sci.* **1977**, *15*, 169–173.
11. Henneberg, D.; Henricks, U.; Schomburg, G. *J. Chromatogr.* **1975**, *112*, 343–352.

RECEIVED October 31, 1978.

16

A Comparison of Methods for the Analysis of Hydrocarbons in Marine Sediments

JAMES L. LAKE, CRANDALL W. DIMOCK, and CURTIS B. NORWOOD

Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, RI 02882

The ability of several methods to extract polycyclic aromatic hydrocarbons (PAHs) and other petroleum hydrocarbons from marine sediments was examined. Comparisons of soxhlet and methylene chloride reflux methods gave extraction efficiencies that showed no statistical difference in the return of PAHs; however, the return when using ball-mill tumbling was significantly lower. The relative content of individual parent PAH compounds, parent compound distributions (PCDs), and alkyl homologue distributions (AHDs) of PAHs was calculated using capillary column GC–MS. The similarities of the distributions showed that any of the three methods could have been used to calculate these distributions. An examination of several extraction methods commonly used for the extraction of petroleum hydrocarbon material from sediment samples showed differences in results that depended on the extraction method employed. In addition, the methods varied in their ability to extract resolved versus unresolved material and to return aliphatic and aromatic hydrocarbon compounds.

Analytical determinations of the quantities and types of pollutant hydrocarbons entering the marine environment are essential for an understanding of the fate and effects of these compounds in marine systems. Since substantial amounts of these hydrocarbon compounds are deposited in marine sediments, research studies have examined the content of polycyclic aromatic hydrocarbons (PAHs) and other petroleum hydrocarbons in marine sediment samples.

0-8412-0475-6/80/33/343$05.00/0

Several studies have examined the total PAH content, the relative content of individual parent PAH compounds, that is, parent compound distributions (PCDs), and the alkyl homologue distributions (AHDs) of PAHs from extracts of marine sediments (*1–9*). The analytical methods used in these studies were generally complex and lengthy, and consisted of an extraction, an isolation of the PAH material (by either complex formation or chromatographic separation), and an analysis. Based on comparisons of PCDs and AHDs of the sediment samples with those of samples from known origins (e.g., petroleum, combustion products), these studies discussed the origins of PAH compounds found in sediments.

Researchers have utilized numerous techniques to determine the content and composition of petroleum hydrocarbons in marine sediments. Unfortunately, no standard technique for the analysis of hydrocarbons from sediments exists, and the variety of techniques utilized has made the comparison of results from different studies nearly impossible. Recent publications have examined extraction and analytical methods (*10, 11, 12*), while other studies have intercalibrated on a standard reference sediment (*13*). In a study using different solvents for extraction, it was concluded that benzene was more efficient than hexane or chloroform in extracting hydrocarbon material from sediment (*10*). It was found, however, that the percentages of each class of hydrocarbons (alkane, cyclic alkanes, and aromatics) extracted by the solvents were relatively similar. The efficiency of soxhlet extraction, followed by alkaline hydrolysis, was compared with that of acid wash prior to soxhlet extraction (*11*). For the methods examined, no substantial differences in extraction efficiencies were found. An interlaboratory comparison of trace level (ppb) hydrocarbons present in two marine sediment samples was discussed (*13*). Eight procedures using different extraction methods and different methods of analysis resulted in considerable differences in estimates of the concentrations of hydrocarbons and in determinations of the most abundant aliphatic and aromatic compounds present. While the latter study showed that considerable variation among laboratories resulted from different extraction and analytical methodologies, other factors inherent to the sediment samples (e.g., total organic carbon, geophysical parameters, acute vs. chronic contamination) may also affect extraction efficiency.

This chapter reports the results of experimentation designed to examine the extraction and characterization of petroleum hydrocarbons, including PAHs, from marine sediments. The efficiencies of several methods for the extraction of PAHs from marine sediments were compared, and the ability of these extraction methods [followed by gas chromatographic–mass spectrometric (GC–MS) analysis] to reproduce consistently AHDs and PCDs from contaminated sediments was exam-

ined. The research involving petroleum-contaminated samples included a two-laboratory intercalibration, an experiment that compared the ability of several sediment extraction methods to recover different classes of petroleum hydrocarbons from sediments, and a study that examined the effectiveness of methods for extracting aliphatic and aromatic hydrocarbons from recently contaminated sediments.

Methods

The sediment samples used in the present study were obtained from Narragansett Bay. The sediment sample used for the PAH extraction study and in Experiments 1 and 4 was obtained adjacent to a pier in Narragansett, Rhode Island. The intercalibration sediment (ICS) was obtained near the north end of Jamestown Island (*14*). Recently contaminated sediments were produced by dispersing sediment obtained near the south end of Jamestown Island in seawater, dosing it with No. 2 fuel oil in a continuous flow oil-dosing system (*15*), and collecting it after it had settled to the bottom of the tanks.

To ensure uniformity, sediments were thoroughly mixed before use. The ICS was wet-sieved through a 1.0-mm sieve, followed by resuspension in distilled water and mixing on a ball mill for 2 hr. The intercalibration sediment was then filtered to remove most of the water. Other sediments were thoroughly mixed by hand. Replicate analysis of aliquots of these sediments showed subsampling variability was small (Table II).

Brief descriptions of the three methods used for extracting PAH material from sediment samples are listed in Methods section (Methods I–III). Method II was adapted from a method used by another laboratory (*16*). Aliquots of the extracts obtained from these methods were analyzed by splitless, injection-glass-capillary-column GC–MS techniques. Concentrations of PAH compounds were calculated by integrating the extracted ion current profiles (EICPs) corresponding to the molecular ions of the compounds of interest. Examples of EICPs for the $Z = -22$ homologue series are shown in Figure 1. Z numbers are determined from the molecular formula C_nH_{2n+z}. Specific peaks in the EICPs were not integrated if their spectra did not correspond to those of the compounds of interest or if they did not have characteristic retention times. The integrated values were normalized and displayed in a semilogarithmic format as PCDs and AHDs. PCDs represent the relative concentrations of the parent compounds of interest, that is, PAHs with molecular weights of 178, 202, 228, 252, 276, and 278. These distributions were obtained by correcting the raw data for instrument response with the aid of response factors calculated from known PAH standards. In the few cases where a standard was not available, raw data were corrected by the use of another

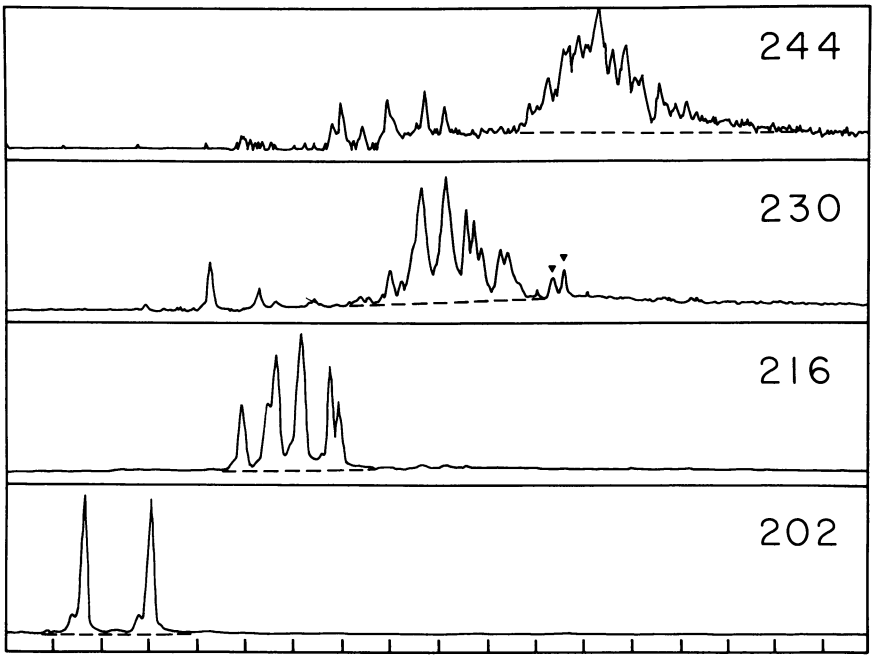


Figure 1. Extracted ion current profiles (EICPs) of the molecular ions for the Z = −22 AHD for a marine sediment. Arrowheads indicate the $(M + 2)^+$ ions of compounds with MW = 228.

PAH standard with the same molecular weight. AHDs show the concentration of the parent compound in relation to its C_1–C_3 alkyl homologues. AHDs were not corrected for instrument response because of a lack of necessary alkylated standards.

Both PCDs and AHDs are helpful in graphically demonstrating the similarities and/or differences among samples. Figure 2 shows the total ion current profiles obtained from samples of coal tar from a dock and sediment from the Charles River, Boston. The numbers above the peaks refer to tentative identifications listed in Table I. The differences in the PAH content of the samples are graphically demonstrated by the shapes of the PCDs and by the lengths of the Z = −22 AHDs (Figure 3). The sediment exhibits a much larger proportion of higher-molecular-weight PAHs. Similar distributions have been found in other samples, and these characteristic distributions have been used to provide evidence as to the origins of PAH compounds in marine environments (*8*).

The methods used for the extraction of petroleum hydrocarbon compounds are listed in Methods section (Methods III–VII). In an attempt to demonstrate the efficiency of Method III relative to Method V,

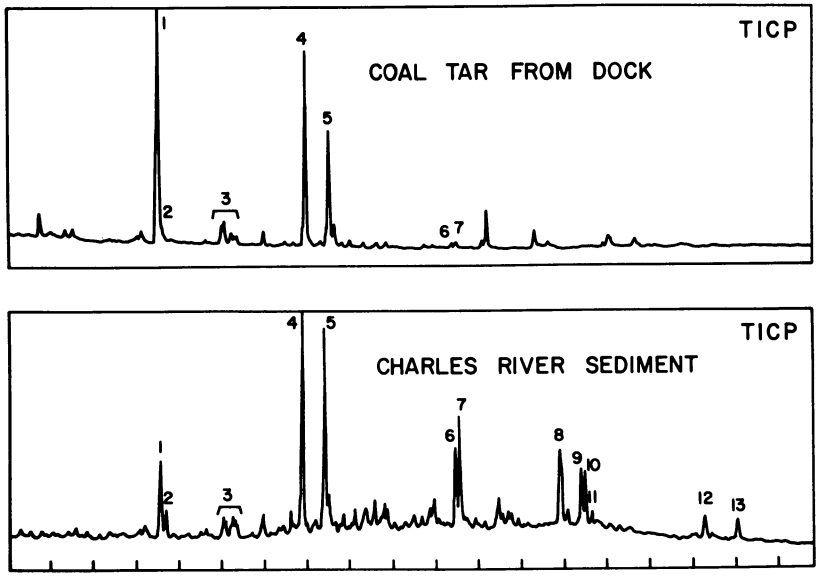


Figure 2. A comparison of total ion current profiles (TICPs) of a dock tar and sediment. Numbers refer to compound identifications presented in Table I.

Table I. PAH Compounds in Figure 2

Compound Number	*Molecular Weight*	*Z Number*	*Tentative Compound Identifications*
1	178	−18	phenanthrene
2	178	−18	anthracene
3	192	−18	C_1-phenanthrenes + C_1-anthracenes
4	202	−22	fluoranthene
5	202	−22	pyrene
6	228	−24	benzanthracene
7	228	−24	chrysene
8	252	−28	benzofluoranthene
9	252	−28	benzo[*e*]pyrene
10	252	−28	benzo[*a*]pyrene
11	252	−28	perylene
12	276	−32	6-ring PAH
13	276	−32	6-ring PAH

successive sample extractions were applied to some sediments and the resulting extracts were analyzed. Results were compared to determine whether any extractable hydrocarbons remained following the initial extraction.

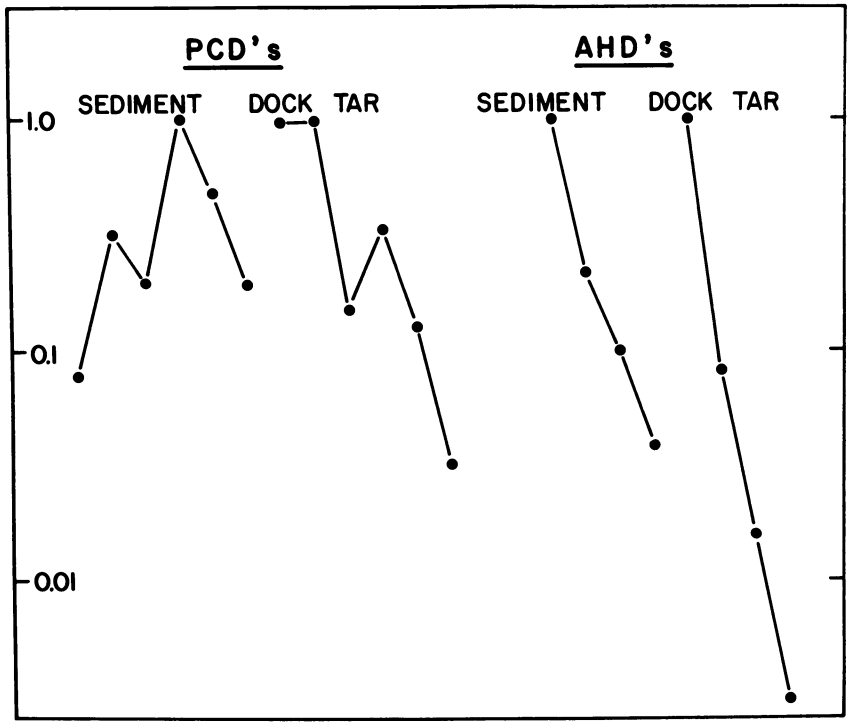


Figure 3. A comparison of PAH PCDs and Z = −22 AHDs of a sediment and a dock tar

The organic fractions resulting from all extractions were purified by passage through a column of silica gel, sodium sulfate, and copper powder. Extracts from Methods II, III, IV, and VI were reduced in volume on a Kuderna–Danish apparatus, solvent-exchanged with hexane, and reduced in volume to 1 mL under a stream of nitrogen. The extracts from Methods I, V, and VII were handled similarly but were evaporated to dryness (to remove benzene, toluene, and methanol) before being redissolved in hexane.

The sample extract was then separated on a 45-cm × 1-cm-o.d. column of silica gel (100–200 mesh, deactivated with 5% water) into F-1 (aliphatic) and F-2 (aromatic) fractions. Final analysis was accomplished by gas chromatography (GC) or GC–MS.

The GC analyses were performed on several glass capillary columns with various liquid phases (OV-101, SE-52, and SE-54) in a Hewlett-Packard 5840-A GC with splitless injection. Except for the intercalibration study, quantitation of chromatograms was based upon external standards. Areas from chromatograms were manually integrated using a digitizing planimeter.

The GC–MS analyses were performed on similar glass capillary columns in a Shimazdu Model GC-4CM GC connected to a Finnegan 1015 mass spectrometer equipped with a Systems Industries data system with Riber 400 D-8 software. The mass spectrometer was operated in the EI (electron impact) mode at 70 eV.

Reagent blanks and standard samples were periodically processed to ensure continued satisfactory performance of the methods.

Methods for the Extraction of Hydrocarbons from Sediment

Method I. (Soxhlet): Set up soxhlet with 10–200 g wet sediment in the extraction thimble, and 125 mL methanol and 125 mL benzene in the attached boiling flask. Reflux 24 hr. Stop with extraction chamber filled with solvent and exchange extract in boiling flask for 100 mL benzene. Reflux 24 hr. Combine extracts with 25 mL water and 25 mL pentane, shake vigorously, and separate aqueous and organic phases. Wash organic phase with 25 mL water. Extract combined aqueous phases with approximately 10 mL pentane. Combine organic phases.

Method II. (Ball-mill tumbler): Dewater 10–200 g wet sediment with a 2-fold excess of methanol (based upon the calculated dry weight). Repeat. Extract sample by rotating in a 3-fold excess of CH_2Cl_2 at 5 rpm for 18 hr. Repeat for 7 and 64 hr, respectively. Methanol and CH_2Cl_2 fractions are combined and filtered and then partitioned into aqueous and organic phases by adding an equal volume of water. Extract aqueous phase with 20 mL CH_2Cl_2 and combine organic phases. Repeat partitioning steps.

Method III. (Methylene chloride reflux): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 3-fold excess of CH_2Cl_2 and a 1.5-fold excess of distilled water. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Separate organic and aqueous phases. Wash sediment twice with 25 mL CH_2Cl_2 and 25 mL H_2O. Separate aqueous and CH_2Cl_2 phases and combine all CH_2Cl_2 fractions.

Method IV. (Shaking with CH_2Cl_2): Weigh 25–50 g of wet sediment into a centrifuge bottle. Based upon the calculated dry weight, add a 2-fold excess of CH_2Cl_2 and extract the sediment by shaking for 5 min. Centrifuge. Draw off supernatant and repeat twice. Separate CH_2Cl_2 and aqueous phases in a separatory funnel. Retain and combine all CH_2Cl_2 phases.

Method V. (Methanolic saponification/benzene): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 5-fold excess of 0.5*N* methanolic NaOH and a 2.5-fold excess of benzene. Add a volume of distilled water equal to 10%–20% of the volume of the

aqueous phase. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Separate organic and aqueous phases. Extract the aqueous phase with 50% of its volume of CH_2Cl_2. Wash sediment twice with approximately 25 mL CH_2Cl_2 and 25 mL H_2O. Combine each washing with the aqueous phase, extract by shaking, and separate the CH_2Cl_2. Combine all CH_2Cl_2 fractions.

Method VI. (Saponification): Weigh 10–200 g of wet sediment into a flask. Based upon the calculated dry weight, add a 4-fold excess of 0.5*N* NaOH. Reflux mixture for 2 hr while stirring with a magnetic stirrer. Centrifuge. Extract the aqueous phase with 50% of its volume of CH_2Cl_2. Wash sediment twice with approximately 25 mL CH_2Cl_2 and 25 mL water. Combine each washing with the aqueous phase, extract by shaking, and separate the CH_2Cl_2. Combine all CH_2Cl_2 fractions.

Method VII. (Methanolic saponification/toluene): Follow Method V, but substitute a 5-fold excess of a mixture consisting of 70% 0.5*N* methanolic KOH and 30% toluene, for the methanolic NaOH and benzene (*21*).

Results

PAH Investigation. The steps in the PAH procedures following extraction were examined by the addition of a standard containing alkylated and nonalkylated PAH compounds. A comparison of the quan-

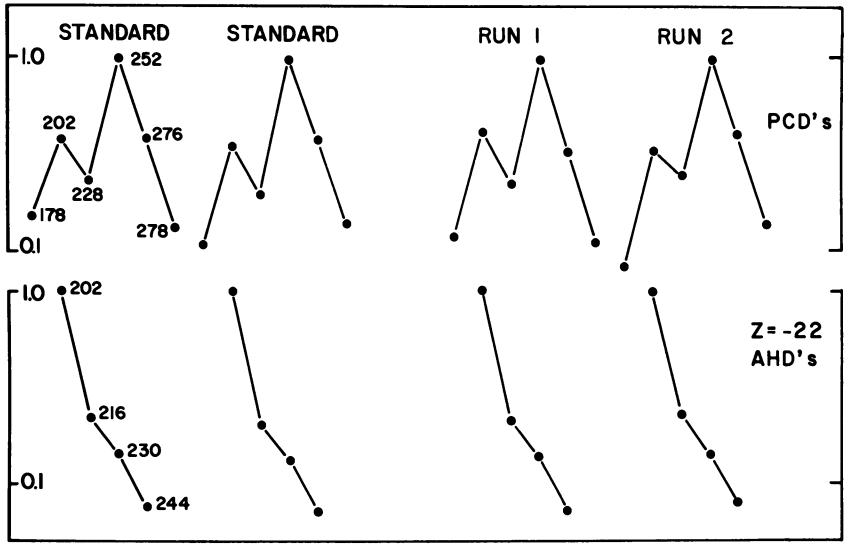


Figure 4. Comparison of the PCDs and the Z = −22 AHDs of a PAH standard before (standards) and after (Runs 1 and 2) being carried through the volume reduction and column chromatography portions of the sample preparation procedure

tity of nonalkylated PAH in the standard with the amount returned after it was carried through the volume reduction and chromatographic steps of the procedure showed that there was an average difference of 5%. Comparisons of the PCDs and AHDs from the standard and from the results of this experiment (Figure 4, Runs 1 and 2) showed that this portion of the procedure caused little alteration of these PAH distributions. Comparisons of phenanthrene/anthracene ratios obtained from the standard with those from Runs 1 and 2 showed no significant differences. These data indicate that no significant photooxidation of susceptible PAHs had occurred.

The results of the three PAH extraction methods are shown in Figure 5. The soxhlet extraction (I) and CH_2Cl_2 reflux (III) were about equal in their ability to extract PAH compounds. The ball-mill tumbler (II) was only 72% as efficient as the other methods. A two-way, crossed-factor analysis of variance on the log-transformed data was used to test whether or not there were significant differences in the amount of parent compounds returned by these three methods. An F-test indicated signifi-

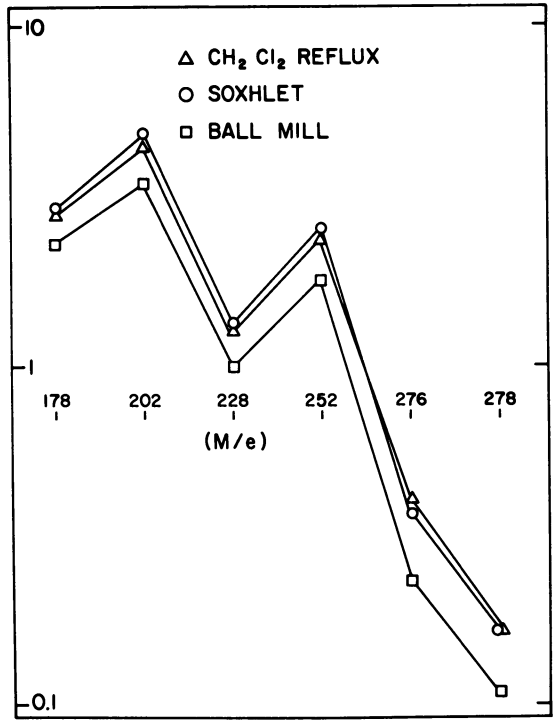


Figure 5. Recovery [log (ppm dry weight)] of nonalkylated PAH compounds for methylene chloride reflux, soxhlet, and ball-mill tumbler extraction methods

cant differences ($\alpha = 0.05$). The Student–Newman–Keuls test (*17*) showed that Method III and Method I returns were not significantly different from one another ($\alpha = 0.05$), while the return from Method II was significantly lower than the other methods ($\alpha = 0.05$). All PCDs and $Z = -22$ AHDs (Figures 6 and 7) from the three methods were similar, as were other AHDs at $Z = -18$, $Z = -24$, and $Z = -28$. Therefore, any of these three extraction methods could have been used to characterize the PAH compounds in this sediment sample.

The high resolution inherent in a capillary column analysis proved valuable when characterizing the PAH assemblages in the samples, but the low-temperature tolerance of the glass capillary column was found to preclude the collection of data on PAHs with molecular weights greater than 300.

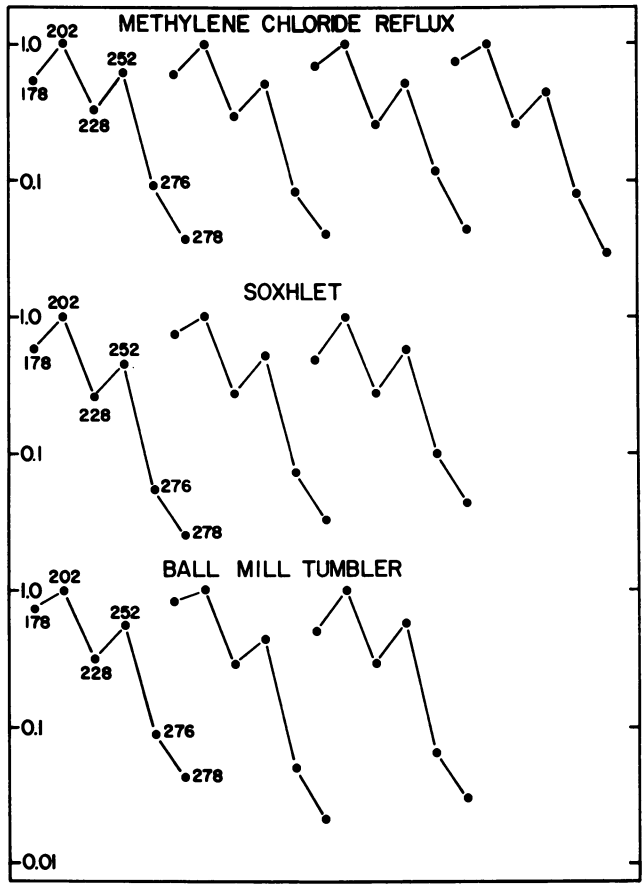


Figure 6. Comparison of PAH PCDs for replicate extractions by methylene chloride reflux, soxhlet, and ball-mill tumbler methods

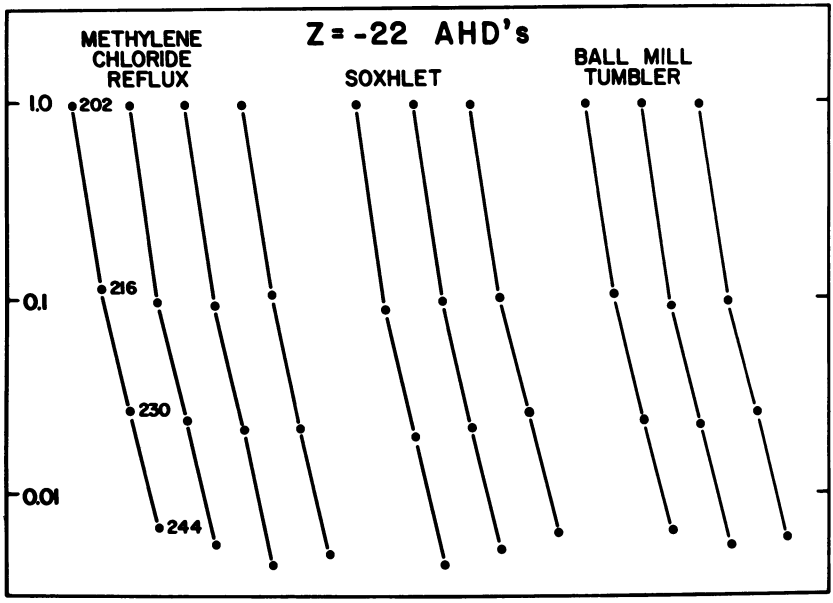


Figure 7. Comparison of PAH Z = −22 AHDs for methylene chloride reflux, soxhlet, and ball-mill tumbler extraction methods

The collection of full-mass-range spectral data is advantageuos because it allows the molecular ions of the compound of interest to be distinguished from fragment ions of other compounds, so that only the peaks of interest are included in the EICP area calculations. However, this mode of data acquisition is not as sensitive as select ion monitoring.

Hydrocarbon Investigations. The results of Experiments 1–4, which compared the ability of five commonly used methods to extract petroleum hydrocarbons from sediments, are listed in Tables II–V.

Experiment 1 (Table II) was an intercalibration between this laboratory (EPA Environmental Research Laboratory—Narragansett) and the laboratory of James Quinn (University of Rhode Island, Graduate School of Oceanography) using aliquots of the ICS and the pier sediment samples. The amounts of hydrocarbon material extracted from both sediment samples were very close when similar extraction methods (V and VII) were used.

Experiment 2 (Table III) compared the extraction efficiencies of three methods for the recovery of hydrocarbon material from ICS samples. Method IV, the most rapid and least vigorous (shaking with CH_2Cl_2), was only about 17% as efficient as Method V (reflux and saponification with MeOH, NaOH, and benzene, followed by extraction with CH_2Cl_2). Method VI (NaOH reflux followed by CH_2Cl_2 extraction) was only about 31% as efficient as Method V.

Table II. Experiment 1: Return of Total Hydrocarbons from Marine Sediments for Interlaboratory Calibration

Analytical Laboratory	*Method*	*Standard*	*Sediment*	*Concentration (μg/g dry wt)*	*Average Concentration*
ERL-N	V	[a]	ICS	215 210	213
URI/GSO	VII	[a]	ICS	213 236 193	214
ERL-N	III followed by V	[b]	pier sediment	33	
ERL-N	V	[b]	pier sediment	33	
URI/GSO	VII	[a]	pier sediment	33.8 32.9	33.4

[a] Quantitation based on Docosane as an internal standard.
[b] Quantitation based on No. 2 fuel oil as an external standard.

Experiment 3 (Table IV) compared the abilities of Methods V and III (CH_2Cl_2 reflux) to extract sediments recently contaminated by No. 2 fuel oil. Method V (the more vigorous extraction) returned more hydrocarbon material from the sediment samples than did Method III. The difference in return, however, was primarily in the form of unresolved complex material (*11*) that was believed to have resulted from the long-term chronic contamination of these sediments while they were in Narragansett Bay. Both methods returned approximately equal amounts of resolved material.

Experiment 4 (Table V) examined sediments obtained near a pier in Narragansett Bay. Two extraction methods were used alone and in sequence to examine their ability to remove hydrocarbons from the sediment samples. The results of Method III followed by Method V on

Table III. Experiment 2: Comparison of Extraction Efficiencies of Methods IV, VI, and V for an ICS Sediment Sample[a]

Method	*F-1*	*F-2*	*Total*	*Average*
IV	38	2	40	36
	30	2	32	
VI	62	2	64	64
	63	2	65	
V	189	16	205	206
	197	11	208	

[a] Quantitation based on No. 2 fuel oil as an external standard.

Table IV. Experiment 3: Comparison of Extraction Efficiencies of Methods III and V for a Narragansett Bay Sediment Exposed to No. 2 Fuel Oil in an Oil-Dosing System[a]

	Concentration (μg/g dry weight)								
	F-1			*F-2*			*Total*		
Method	R	U	*Total*	R	U	*Total*	R	U	*Total*
III	38	111	149	9	37	46	47	148	195
	37	105	142	10	39	49	47	144	191
V	37	172	209	15	47	62	52	219	271
	36	161	197	12	47	59	48	208	256

[a] Quantitation based on No. 2 fuel oil as an external standard. R = Resolved peak areas on chromatograms. *U* = Unresolved peak areas on chromatograms.

Table V. Experiment 4: Comparison of Extraction Efficiencies of Methods III and V[a]

		Concentration (μ/g dry weight)		
Part[b]	*Method*	*F-1*	*F-2*	*Total*
A	III	4	19	23
	V	8	2	10
	Total	12	21	33
B	V	10	23	33

[a] Quantitation based on No. 2 fuel oil as an external standard.
[b] Part A: Sample extracted by Method III followed by Method V. Part B: Sample extracted only by Method V.

the same sample showed that more hydrocarbon material was returned by the more vigorous Method V. Most of this additional material, however, was returned as unresolved complex material and was found primarily within the F-1 (aliphatic) fraction. The second extraction with Method V returned approximately 200% additional F-1 material, but only 11% additional F-2 (aromatic) material. Method III therefore appeared to be adequate for extracting F-2 (aromatics) from these contaminated sediments but inefficient for the extraction of F-1 (aliphatic) material.

Reagent blanks processed periodically yielded no material that would have interfered with the analyses above.

Discussion

PAH Investigations. Studies examining the input of PAHs to marine systems have examined the PAH assemblages present in samples of petroleum (*6*), airborne particulates (*18, 19*), water particulates (*19*), combustion products (*18*), and coal tar (*8*). The AHDs and the PCDs

of PAHs found in marine sediment have been used to indicate the sources and origins of these compounds in marine benthic environments (*8*). A variety of extraction and analytical techniques has been utilized to characterize PAH assemblages in samples; however, many of the techniques involved complex and lengthy extraction and isolation procedures.

The present study compared the ability of three techniques to extract PAH compounds and their alkylated homologues from marine sediments. Specifically, this study was designed to determine if the results of a shorter and simpler extraction (CH_2Cl_2 reflux) were comparable with those from longer yet more commonly used extraction methods (exhaustive soxhlet and ball-mill tumbling).

Prior to conducting the extraction efficiency experiments, an examination of the volume reduction and column chromatographic portions of the procedure was undertaken to determine if selective photooxidation or other processes were altering the characteristic PCDs and AHDs. Previous studies examining PAH methodology have demonstrated that radiation from room light can destroy some PAH molecules (*20*). It was found that the light degraded anthracene, napthacene, benzanthracene, dibenz[*a,c*]anthracene, pyrene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*ghi*]perylene, and coronene on silica gel plates, but did not degrade phenanthrene, chrysene, and triphenylene. In the present study two aliquots of a PAH standard were taken through the latter portions of the procedure. The PCDs and $Z = -22$ AHDs of the standard are shown with those of the replicate runs in Figure 4. The quantities of nonalkylated PAH material returned from this portion of the procedure were only slightly different than those of the standard. The PCDs were very similar and the AHDs were nearly identical. In addition, calculation of the phenanthrene/anthracene ratios showed that the average ratio for the initial standard was not significantly different from that for Runs 1 and 2. These findings indicated that no significant losses or alterations of PAHs occurred during the volume reduction and column chromatographic portions of the procedure.

The results of the three extraction experiments for PAH compounds are shown in Figures 5, 6, and 7. The concentrations of nonalkylated parent PAH compounds at m/e 178, 202, 228, 252, 276, and 278 were determined by GC–MS analysis. These concentrations (corrected for instrument response) are shown in Figure 5. The results showed that the soxhlet extraction and the CH_2Cl_2 reflux were approximately equal in their ability to extract these PAH compounds, while the ball-mill tumbler extraction method was only about 72% as efficient as the other two methods. PCDs obtained from the different extraction methods are shown in Figure 6. This figure demonstrates the consistency of each method for extracting nonalkylated PAH compounds and shows the close similarity

of PCDs obtained from these different extraction methods. The AHDs were calculated for the $Z = -22$ homologue series from extracts obtained from the three extraction methods. The close agreement of the C_0 through C_3 AHDs is graphically demonstrated in Figure 7. Other AHDs at $Z = -18$, $Z = -24$, and $Z = -28$ calculated for these three extraction methods were also very similar.

A capillary column inlet system and collection of full-range mass spectral data offer several advantages over other inlet systems (e.g., packed column GC, direct probe) and/or limited-scan-range data acquisition modes (e.g., select ion monitoring). For example, with packed column, solid probe, or batch inlet systems, it would be exceedingly difficult to resolve certain isomers (e.g., phenanthrene and anthracene) whose relative abundances may prove valuable in evaluations of the origins, sources, and pathways of PAH compounds in the marine environment. In addition, the capillary GC inlet allows calculation of retention time data that can be used in conjunction with other data to include or delete peaks from EICP integrations (described in next paragraph). Unfortunately glass capillary columns have a limited temperature range. AHD data up to the C_3 homologue of MW = 252 compounds can be obtained, but column life is shortened by the high temperature needed to elute these compounds. As glass capillary column technology improves, the temperature limit may increase and thus allow the examination of higher-molecular-weight PAHs.

The collection of full-scan-range data from samples allows the spectra of peaks to be checked. From an examination of the spectra, peaks can be included or deleted from EICP area calculations. An example of this is shown in Figure 1, where EICPs for *m/e* 202, 216, 230, and 244 are presented. The C_2 homologue is quantified by integrating the area under the *m/e* 230 EICP. An examination of the retention times and the spectra of the major peaks in the EICP indicated that the two peaks to the right of the main group resulted from the M + 2 ions of the benzanthracene and chrysene compounds (MW = 228). These peaks, therefore, were not included in the calculation of the C_2 alkyl homologue. A disadvantage of collecting full-scan-range data from GC–MS is that of decreased sensitivity. The loss in sensitivity usually precluded the collection of data for AHDs above the C_3 alkyl homologue. While this is a distinct disadvantage, an examination of data collected by the present study and from other research shows that the shape of the AHD is usually defined by the first four points. Therefore, depending on the research, calculation of the alkyl homologues above C_3 may not be necessary.

Hydrocarbon Investigations. Since no standard method exists for the analysis of hydrocarbon material from marine sediment, the extraction and analytical procedures used to determine the level of hydrocarbons in

samples of marine sediments have varied substantially. This study compared the ability of five methods commonly used for petroleum hydrocarbon analysis (Methods section, Methods III through VII) to extract aliphatic and aromatic hydrocarbons from sediment samples.

The results from the intercalibration study (Experiment 1) between the EPA Environmental Research Laboratory—Narragansett and the laboratory of James Quinn at the U.R.I. Graduate School of Oceanography agreed closely (Table II). This agreement also indicates that subsampling variability of the intercalibration sediments was quite low. The amount of material recovered from sediments by three extraction methods (Table III) was compared in Experiment 2. A considerable increase in the amount of hydrocarbon material returned from sediment samples was observed as the method of extraction became more vigorous.

The results of Experiment 3 are shown in Table IV. This experiment compared the ability of different methods (III and V) to extract recently added petroleum hydrocarbons from marine sediment. The more vigorous extraction (Method V) increased the yield, but the additional material returned was unresolved. It appeared that the extractions were equally effective in returning the resolved hydrocarbons. In the unresolved portions of chromatograms, it was not possible to differentiate between recently added hydrocarbons and those from long-term inputs. This differentiation may be important for determining the extent of contamination from oil spills where extensive areas are impacted and control or prespill sediment samples (to determine background hydrocarbon levels) are not available.

In Experiment 4 the same sediment sample was extracted by Methods III and V successively. This experiment (Table V) showed that the saponification (Method V) returned additional material from the sediment; however, most of this material was unresolved and in the F-1 fraction. The amount of additional F-2 material returned by the second extraction was only about 11%. This experiment showed that a method may be suitable for one class of petroleum hydrocarbons but not be as effective for recovering other classes.

Conclusions

A comparison of three extraction methods used for recovery of PAH material from sediments showed that the CH_2Cl_2 reflux and the soxhlet extraction were approximately equal in their ability to extract PAH material; however, ball-mill tumbling was only approximately 72% as efficient. The similarities of the AHDs and PCDs obtained from the three extraction methods indicate that any of these methods could have been utilized to characterize the PAH assemblages in the sediment; but these findings

could not necessarily be generalized to include all PAH contamination levels and all sediment types. Full-mass-range data acquisition and a glass capillary GC inlet to the mass spectrometer were advantageous for the calculation of AHDs and PCDs, but these adaptations limited the sensitivity of the method and the range of compounds that could be examined.

An examination of several extraction methods commonly used for the extraction of petroleum hydrocarbon material from sediment samples showed differences in results depending upon the extraction method employed. In addition, the methods varied in their ability to extract resolved versus unresolved material and to return different classes of compounds (aliphatics and aromatics) .

The factors considered in this paper and other variations in the extraction and analytical scheme (e.g., a method's ability to return lower-molecular-weight compounds, sediment-dependent differences in extractability, differences in analytical instrumentation) indicate that close agreement in results from laboratories utilizing different extraction methodologies should not be expected. While these variations may be acceptable to those who understand that the extraction of hydrocarbon material from a complex matrix (such as marine sediment) is still the subject of research studies, these variations may complicate the assessment of ecological damage resulting from inputs of hydrocarbon contaminants to the marine environment. These considerations, the results of the present study, the variation in results from other intercalibration studies (*13*), and the difficulties encountered when results from different studies are compared, suggest that a standard method of extraction and analysis of hydrocarbon material from sediments should be established. An increased amount of research effort should be directed toward this problem.

Acknowledgments

The authors wish to thank Robert Bowen and David Mizenko for their research assistance and Peter Rogerson for his careful review of the manuscript. The support of Grant No. R805477 from the Environmental Protection Agency is also greatly appreciated.

Literature Cited

1. Blumer, M.; Youngblood, W. W. *Science* **1975**, *188*, 53–55.
2. Giger, W.; Blumer, M. *Anal. Chem.* **1974**, *46*, 1663–1671.
3. Hites, R. A. In "Sources, Effects, and Sinks of Hydrocarbon in the Aquatic Environment"; Weiss, F. T., Ed.; American Institute Biological Sciences: 1976; pp. 325–333.

4. Hase, A.; Hites, R. A. In "Identification and Analysis of Organic Pollutants in Water"; Keith, D. H., Ed.; Ann Arbor Science Publishers; 1976; pp. 205–214.
5. Hites, R. A.; Beimann, W. G. In "Analytical Methods in Oceanography," *Adv. Chem. Ser.* **1975,** *147,* 188–201.
6. Youngblood, W. W.; Blumer, M. *Geochim. Cosmochim. Acta* **1975,** *39,* 1303–1314.
7. LaFlamme, R. E.; Hites, R. A. *Geochim. Cosmochim. Acta* **1978,** *42,* 289–303.
8. Lake, J. L.; Norwood, C. B.; Dimock, C. W.; Bowen, R. D., presented at Symposium on Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment, Pensacola Beach, FL, August 14–18, 1978.
9. Hites, R. A.; Laflamme, R. E.; Farrington, J. W. *Science* **1977,** *198,* 829–831.
10. Walker, J. D.; Colwell, R R..; Hamming, M. C.; Ford, H. T. *Bull. Environ. Contam. Toxicol.* **1975,** *13,* 215–218.
11. Farrington, J. W.; Tripp, B. W. In "Marine Chemistry in the Coastal Environment," *ACS Symp. Ser.* **1975,** *18,* 267–284.
12. Hargrave, B. T.; Phillips, G. A. *Environ. Pollut.* **1975,** *8,* 193–215.
13. Hilpert, L. R.; May, W. E.; Wise, S. A.; Chesler, S. N.; Hertz, H. S. *Anal. Chem.* **1978,** *50,* 458–463.
14. Wade, T. L.; Quinn, J. G. "Marine Environmental Research," in press.
15. Hyland, J. L.; Rogerson, P. F.; Gardner, G. R. *Proc. Oil Spill Conf. (Prev., Behav., Control, Cleanup)* API/EPA/USCG, **1977,** 547–550.
16. MacLeod, W. D.; Brown, W. D.; Jenkins, R. G.; Ramos, S. L.; Henry, V. D. *NOAA Technical Memorandum ERL MESA-8,* November **1976.**
17. Snedecor, G. W.; Cochran, W. G., "Statistical Methods"; Iowa State Univ. Press: Ames, IA, 1976.
18. Lee, M. L.; Prado, G. P.; Howard, J. B.; Hites, R. A. *Biomed. Mass Spectrom.* **1977,** *4,* 182–186.
19. Giger, W.; Schaffner, C. *Anal. Chem.* **1978,** *50,* 243–249.
20. Inscoe, M. N. *Anal. Chem.* **1964,** *36,* 2505–2506.
21. Van Vleet, E. S.; Quinn, J. G. *J. Fish. Res. Board Can.* **1978,** *35,* 536–543.

RECEIVED October 12, 1978.

INDEX

C

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Z