United States
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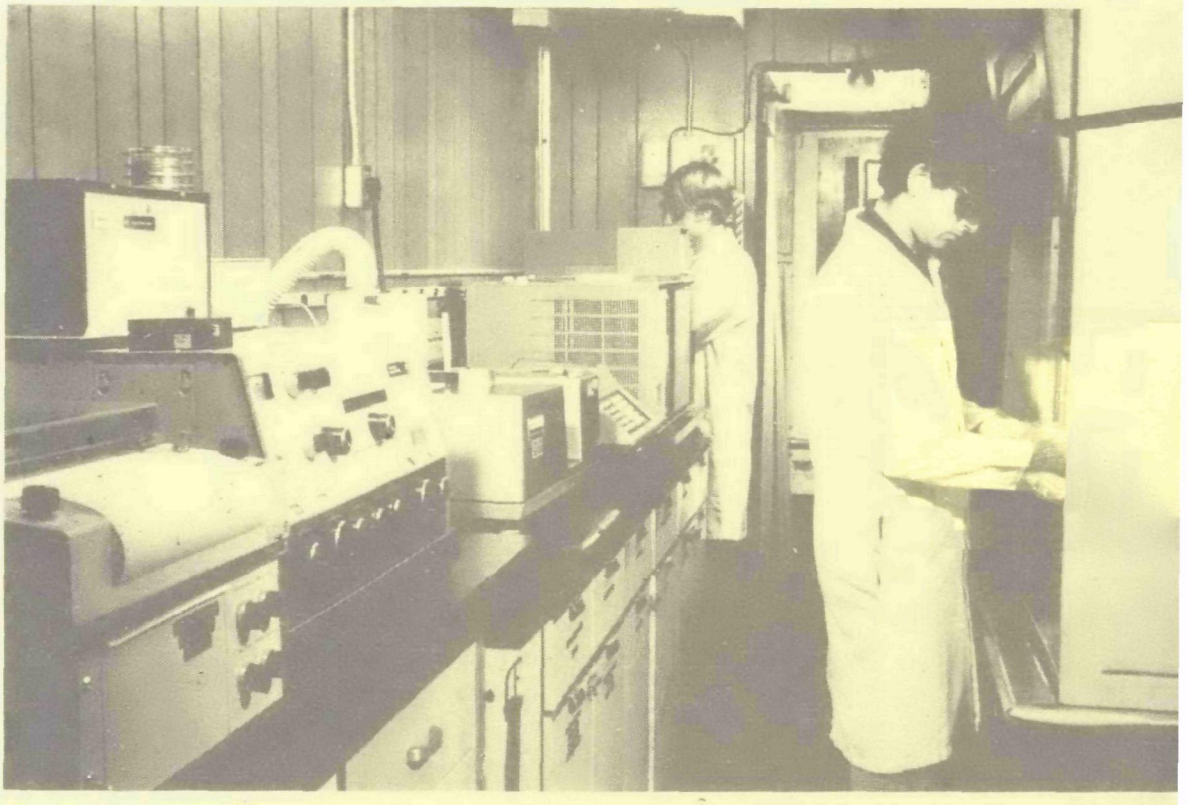
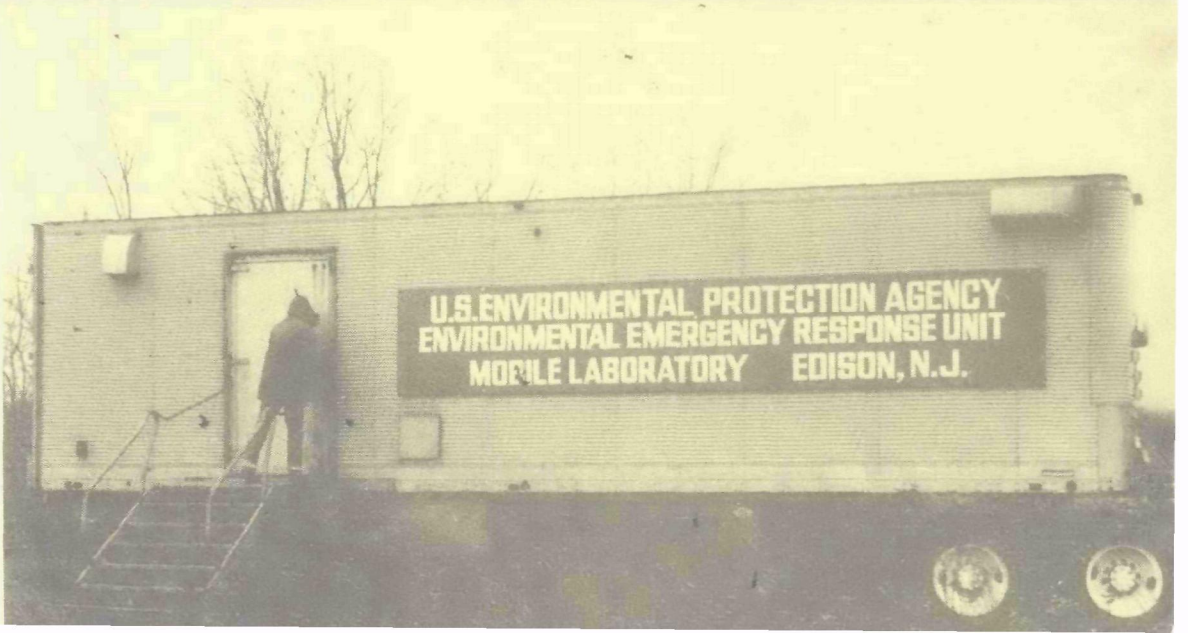
Cincinnati OH 45268

Research and Development

EPA

Publications on the Analysis of Spilled Hazardous and Toxic Chemicals and Petroleum Oils





PREFACE

THIS COLLECTION OF OIL AND HAZARDOUS CHEMICAL POLLUTION RELATED REPORTS DESCRIBES NEWLY DEVELOPED ANALYTICAL TECHNIQUES AND ENVIRONMENTAL STUDIES OF THE OIL AND HAZARDOUS MATERIALS SPILLS BRANCH AT EDISON, NEW JERSEY. THIS MATERIAL IS REPRINTED FROM JOURNALS, NEWSLETTERS, AND CONFERENCE PROCEEDINGS, WHICH ARE REFERENCED.

COVER ILLUSTRATION FEATURES A HIGHLY INSTRUMENTED MOBILE EMERGENCY RESPONSE LABORATORY. FOR FURTHER INFORMATION SEE THE ARTICLE ON PAGE 23.

PUBLICATIONS ON THE ANALYSIS OF SPILLED HAZARDOUS AND TOXIC CHEMICALS AND PETROLEUM OILS

OIL AND HAZARDOUS MATERIALS SPILLS BRANCH
INDUSTRIAL ENVIRONMENTAL RESEARCH LABORATORY
U. S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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I. HAZARDOUS CHEMICALS

A. FLUORESCENCE ANALYSES

Quantitation of Hazardous Materials

The use of synchronous excitation fluorescence spectroscopy for the analysis of hazardous materials (40 CFR, Part 116) has been described in AQC Newsletters Nos. 31 and 32. We are now extending this technique to non-fluorescent materials by using derivatization techniques. Fluorescamine (available from Fisher Scientific Company as Roche Fluran) was used for in-situ quantitations of butylamine and ethylenediamine in water. The derivatization reaction proceeded rapidly in aqueous media at room temperature. Preliminary findings indicate nearly 100% recoveries and detection limits in the ppb range. (U. Frank, Coml. 201-321-6626, FTS 340-6626/J. Munn/N. Pangaro, Coml. 201-321-6628, FTS 340-6628)

Comparison of Synchronous and Single Wavelength Excitation Fluorescence Spectroscopy

Synchronous excitation and conventional single wavelength excitation fluorescence spectroscopy were compared for in-situ water analyses of aromatic compounds that contaminated a town's water reservoir. Fluorescence analyses were performed on site, on board our Mobile Spills Laboratory. Single wavelength excitation yielded spectra that were totally obscured by Rayleigh-Tyndall scatter as a consequence of colloidal particulates in the water samples. Synchronous excitation (described in AQC Newsletters Nos. 31 and 32) yielded spectra that were free of scatter interferences and that contained only one sharp peak and a complex envelope. The peak's fluorescence intensity served to monitor the extent of the reservoir's contamination, and also served to determine the feasibility of water detoxification. A total of 250 in-situ water measurements were performed by this method in four days. (U. Frank, Coml. 201-321-6626, FTS 340-6626)

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Cincinnati, Ohio 45268

Quantification of Mixed Hazardous Materials

Synchronous excitation (SE) fluorescence spectroscopy for the in situ quantification of Hazardous Materials in water samples containing only the individual toxicants, was previously described in AQC Newsletters 31, 32 and 34. A current effort in our laboratory addresses the in situ quantification of specific toxicants in water samples containing mixtures of toxicants. Preliminary findings indicate that well resolved fluorescence spectra of individual toxicants in mixtures are obtained by the SE procedure, when these toxicants differ in their number of aromatic rings. For example, phenol and naphthenic acid yield two well resolved fluorescence peaks which are readily quantified. Conversely, aniline, phenol and toluene are all single ring compounds, which as a mixture yield only one fluorescence peak. Quantification of each toxicant in this mixture was accomplished by suppressing the fluorescence of phenol and aniline through pH adjustment. Observation of the behavior of the three materials demonstrated that all three toxicants fluoresced at pH 7, aniline and toluene fluoresced at pH 11, while phenol and toluene fluoresced at pH 2. Quantification of the individual toxicants was successfully accomplished by performing these pH adjustments, and by using three simultaneous equations. (U. Frank, Coml. 201-321-6626, FTS 340-6626, P. Ward/J.L. Callender, Coml. 201-321-6628, FTS 340-6628)

* See page 2.

Rapid Quantification of Hazardous Materials in Sediments by Synchronous Excitation Fluorescence Spectroscopy - The use of synchronous excitation fluorescence spectroscopy for the in situ quantification of hazardous materials (40 CFR 116) in water was previously described in AQC Newsletters 31-34 and 36. We have now extended this technique to the quantification of hazardous materials in sediments. Phenol, resorcinol, and styrene were spiked into fluvial sediments at concentrations of 10 mg/Kg. Analyses were performed by adding 2-propanol to the spiked sediments and vigorously agitating for 15 minutes. The propanol extract was then filtered and analyzed by the synchronous excitation technique. Recoveries in the range 80-100% were obtained. (U. Frank, FTS 340-6626, Coml. 201-321-6626/D. Remeta, FTS 340-6628, Coml. 201-321-6628)

Purification of Isopropanol for Fluorescence Analyses - While quantifying hazardous materials in sediments by Synchronous Excitation (SE) fluorescence spectroscopy, an interfering impurity was encountered in the solvent isopropanol. Use of the SE technique was previously described in AQC Newsletters 31-34 and 36. The impurity yielded a single peak at 290 nm, when scanning the excitation monochromator through 270 nm. This impurity was found in both Burdick and Jackson distilled-in-glass isopropanol and in Fisher certified 99 Mol % pure isopropanol. This impurity was also apparent when using the Single Wavelength Excitation technique. Attempts to remove the contaminant by distillation were unsuccessful. This problem was satisfactorily resolved by percolating the solvent through a 17 mm (i.d.) chromatographic column containing a 425 mm height of granular activated carbon and using a solvent flow of 6-10 ml/min. Subsequent fluorescence analyses revealed no further interference. (S. Frysinger/D. Remeta/N. Pangaro, FTS 340-6628, Coml. 201-321-6628)

* See page 2.

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Use of Synchronous Excitation Fluorescence Spectroscopy for In Situ Quantifications of Hazardous Materials in Water

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INTRODUCTION

Spilled hazardous materials often cause substantial economic damage and also threaten human life. Consequently, their rapid quantification in environmental samples is highly desirable. Most analytical methods address the quantification of toxicants in complex aqueous matrices such as industrial and municipal effluents. These methods require tedious and time consuming procedures involving solvent extraction, evaporation and concentration steps and sometimes chromatographic cleanup steps.

Hazardous materials are often spilled into bodies of water that yield less complex sample matrices and can therefore be more rapidly and directly analyzed. Few available methods, however, specifically address the quantification of toxicants in spill samples. The following discussion therefore describes an unusually rapid, sensitive and novel method for the quantification of fluorescent hazardous materials directly in water. This method avoids time consuming extraction, concentration and cleanup steps, and can be used to rapidly and accurately quantify fluorescent hazardous materials in aqueous samples.

Ultraviolet fluorescence spectroscopy is a useful analytical technique for the measurement of many hazardous materials. It is exceptionally sensitive and often more specific than other spectroscopic methods such as ultraviolet, visible, and infrared absorption techniques. Kullbom, *et al*[1], used fluorescence spectroscopy to analyze for phenols and lignins in water. Other investigators[2-6] used it to measure petroleum and non-petroleum hydrocarbons in water. Some of the latter investigators[2,3,5,6] extracted the toxicants from water with solvents prior to fluorescence measurement. Kullbom, *et al*[1] and Frank[4], measured toxicants directly in water. All of these investigators used conventional single wavelength excitation (SWE) techniques.

SWE fluorescence spectroscopy is not satisfactory for *in-situ* measurements of hazardous materials in water. Spectra derived from use of the SWE technique are distorted by Rayleigh-Tyndall and Raman scatter radiations, especially when these spectra are obtained from samples containing only mg/l of toxicants. Consequently, the authors describe, in this paper, the use of "Synchronous Excitation" (SE) fluorescence spectroscopy for rapid *in-situ* quantifications of hazardous materials in water. While other investigators[7,8] have demonstrated that the SE fluorescence technique yields substantially improved spectral resolution when applied to spill source identifications of petroleum oil pollutants, the authors demonstrate that this technique also yields spectra free of Rayleigh-Tyndall and Raman scatter when used for *in-situ* quantifications of hazardous materials in water.

Differences between SWE procedures and the SE technique and the advantages of the latter for *in-situ* quantifications of hazardous materials in water are discussed within the context of a three dimensional coordinate system. This system was previously described by Frank[9], and Frank and Gruenfeld[10]. Three interdependent variables that are inherent to fluorescence spectroscopy are used as axes: (1) excitation wavelength (x), (2) emission wavelength (y) and (3) fluorescence intensity (z). Within this context, the fluorescence properties of hazardous materials are viewed as "total fluorescence spectra" and the SE and SWE fluorescence approaches are readily compared.

A three dimensional total spectrum of a compound that resembles a mountainous region is shown in Figure 1. The two mountain peaks (solid lines) are in fact the fluorescence maxima of this compound. SWE spectra are two dimensional slices through the three dimensional spectrum, parallel to the (y) axis. According to Lloyd[7], such spectra provide only limited useful information. SE fluorescence spectra, however, are slices oriented at a 45° angle to the (x) and (y) axes and often yield more useful information.

The present study of SE fluorescence spectroscopy for *in-situ* quantifications of hazardous materials in water addresses representative materials that fluoresce and that are designated as hazardous by 40 CFR Part 116. These materials were also shown by a recent EPA study[11] to be the most frequently spilled toxicants. In fact, 80% of the organic hazardous materials shown by this EPA study to be frequently spilled, fluoresce strongly and can be quantified directly in water by the SE procedure. This discussion of these analyses also addresses spectral interferences, concentration quenching phenomena and optimum wavelength intervals set between excitation and emission monochromators. Measurements were performed using synthetically prepared samples consisting of hazardous materials in waters collected from areas of heavy marine traffic and high spill potential.

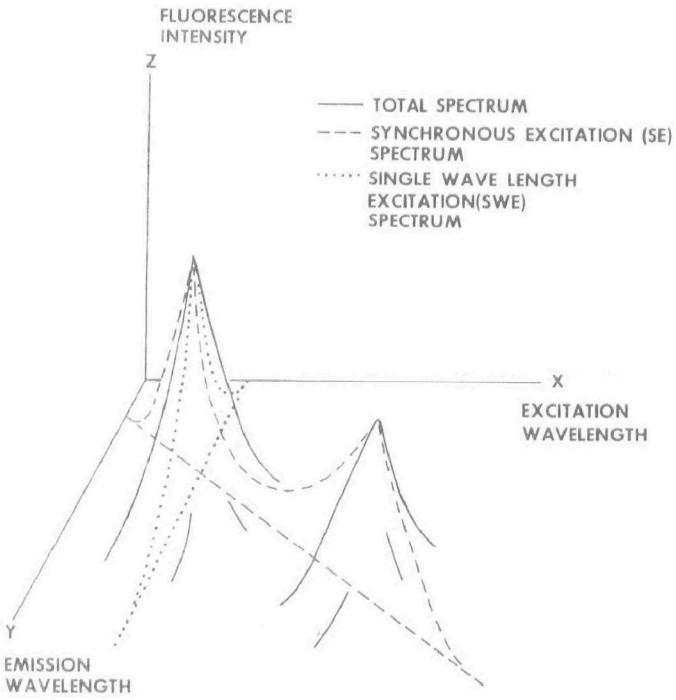

Figure 1: Comparison of SE, SWE and Total Fluorescence Spectra Within the Context of the Three Dimensional Coordinate System

Experimental

Apparatus: A Perkin-Elmer model MPF-3 Fluorescence Spectrometer, and 10 mm pathlength quartz cell were used.

Materials: High purity reagents were used.

Procedure: Accurate aqueous stock solutions were prepared. Water soluble liquids were dispensed directly by using appropriate syringes; solids were weighed; water insoluble materials were dissolved in 2-propanol, prior to water addition. Further dilution of the stock solutions were performed with water to yield final concentrations that were below each material's solubility limit. SE fluorescence spectra were obtained by simultaneously scanning the instrument's excitation and emission monochromators. The excitation monochromator was set ahead of the emission monochromator by an "optimum" wavelength interval. Wavelength intervals in the range 20-100 nm were evaluated for each toxicant.

Some SWE spectra of the above solutions were also obtained for comparison purposes. These solutions were excited at single wavelengths, which yielded maximum emission intensities for each compound. The emission spectra were scanned in the range 220-500 nm.

Results and Discussion

The ability of SE fluorescence spectroscopy to avoid Rayleigh-Tyndall and Raman scatter distortions of spectral profiles is demonstrated within the context of the three dimensional coordinate system. The combined effect of these scattering phenomena can be viewed as resembling a three dimensional "interference wedge" that bisects total fluorescence spectra (Figure 2). Although this interference wedge causes detrimental spectral distortions when using SWE techniques, it can be avoided by using the SE procedures (Figure 3). Rayleigh-Tyndall (reflected) scatter radiation is especially troublesome when measuring natural waters that contain suspended particulates. Spectra obtained with the SE and SWE procedures are contrasted in Figure 4; a synthetically prepared sample of phenol in brackish marsh water containing suspended particulates was measured. This example illustrates the obvious advantage of the SE technique, i.e., its elimination of scatter interferences.

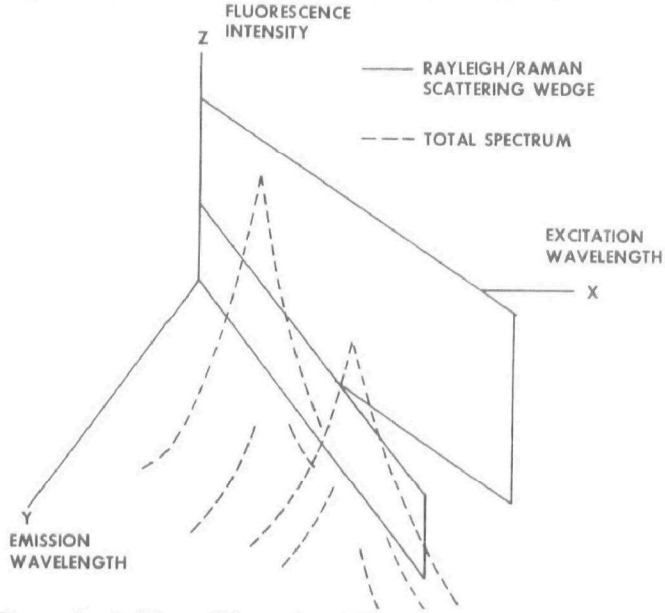

Figure 2: A Three Dimensional Presentation of a Rayleigh-Tyndall/Raman Radiation "Scattering Wedge", Superimposed on a Total Fluorescence Spectrum of a Hazardous Material

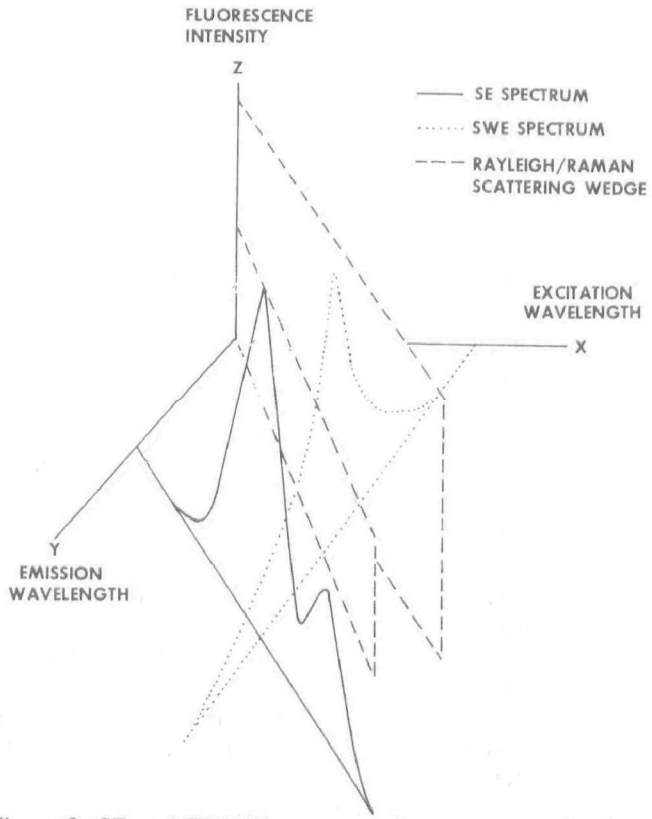

Figure 3: SE and SWE Fluorescence Spectra, and a Rayleigh-Tyndall/Raman "Scattering Wedge" (This Demonstrates that the Scattering Wedge is Readily Avoided by Use of the SE Technique)

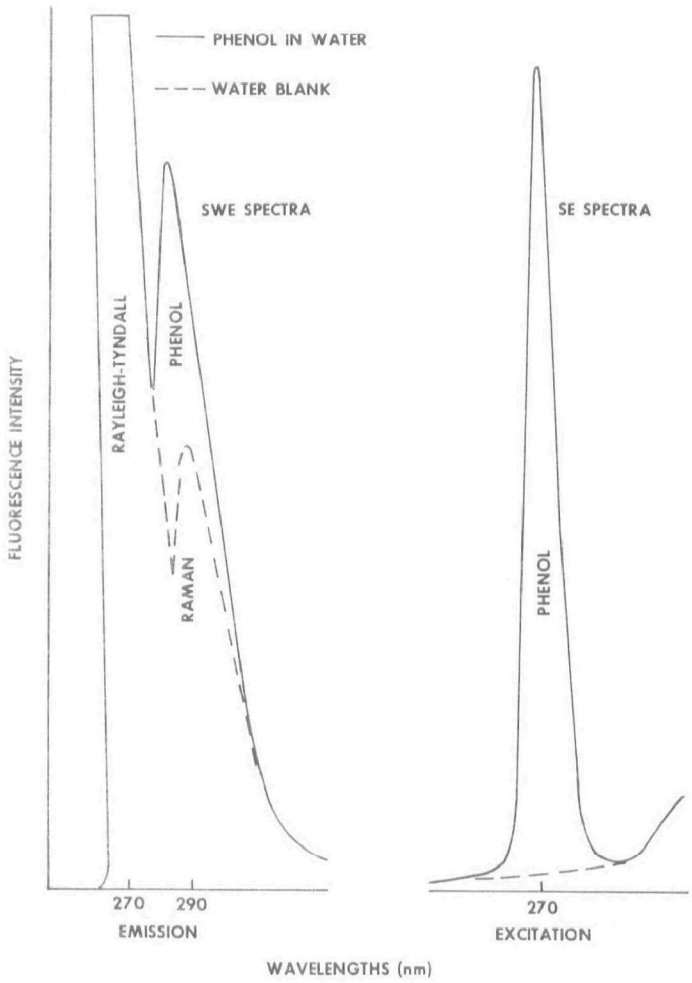

Figure 4: SE and SWE Spectra of Phenol "Spiked" into Water Obtained from a Marsh in Sayreville, New Jersey (The Water Contains Suspended Colloidal Particulates. Phenol Concentration is 1mcl/l)

Raman scatter interferences emanating from water are less pronounced than Rayleigh-Tyndall scatter. They become apparent, however, when using high instrument sensitivities for measuring low toxicant concentrations. The SWE technique fails to avoid this scatter problem, while the SE procedure avoids it readily. Elimination of scatter interferences by the SE procedure also minimizes the need for blank or background sample analyses. SWE techniques require blank sample measurements whenever instrument parameters are changed resulting in a large number of additional and time consuming analyses.

Elimination of scatter interferences by using the SE procedure also permits increased sensitivity. While actual detection limits obtained with the SE procedure are determined by a fluorescence instrument's design, SWE techniques detection limits are severely influenced by scatter interferences. Detection limits of several hazardous materials in distilled water, determined by the SE technique, are listed in Table I.

The effect of indigenous fluorescent species in natural waters, on analysis results is illustrated in Figure 5. The SE fluorescence spectrum of aniline in Raritan River water is shown in Figure 5a while the SE fluorescence spectrum of aniline in Arthur Kill River water is shown in Figure 5b. These rivers represent heavily trafficked and polluted bodies of water in New Jersey. The SE fluorescence spectrum of aniline in distilled water is shown in Figure 5c. The spectrum of the latter figure was used as standard for quantifying aniline in Figure 5a and 5b; recoveries were 96% and 94%, respectively. In effect, the aniline in distilled water solution was used as the standard for quantifying the aniline content of synthetically prepared samples containing this hazardous material in two polluted rivers waters.

Table I: Detection Limits for Several Hazardous Materials in Water

Hazardous Material	Detection Limit (ppm)
Aniline	0.005
O-Cresol	0.1
Dodecyl Benzene Sulfonic Acid	0.1
Naphthalene	0.02
Naphthenic Acids	0.1
Phenol	0.01
Quinoline	0.005
Resorcinol	0.02
Styrene	0.005
Toluene	0.1
Xylene	0.1
Xylenol	0.1

The fluorescence intensity versus concentration plot of aniline in water is illustrated in Figure 6. Linearity extends to slightly above 20 mg/l. Measurements of solutions having concentrations below this value should be free of concentration quenching phenomena. The deleterious effects of quenching become progressively worse at higher concentrations. Fluorescence intensities may actually decrease with increasing concentration when aniline in water concentrations exceed 200 mg/l. Deviations from linearity, therefore must be considered, if grossly erroneous hazardous material quantifications are to be avoided. Excessively high concentrations yield spectral shifts to longer wavelengths, in addition to non-linear plotted results. These properties are useful for establishing optimum concentrations for the quantification of hazardous materials.

The effect of various wavelength interval adjustments between emission and excitation monochromators, when using synthetically prepared samples of o-cresol in water is illustrated in Figure 7. Polluted water from a stream adjoining an oil refinery was used in these samples. The different wavelength intervals clearly determined the degree to which indigenous fluorescent materials in this water distorted the spectrum of o-cresol. An evaluation of the "optimum wavelength interval" is necessary for each type of sample analysis of a hazardous material in water. The optimum wavelength interval in Figure 7 is 40 nm. This interval sacrifices

a small amount of sensitivity in exchange for adequate spectral resolution from indigenous fluorescent species. Optimum wavelength intervals between emission and excitation monochromators were determined for several hazardous materials in a number of polluted waters (Table II).

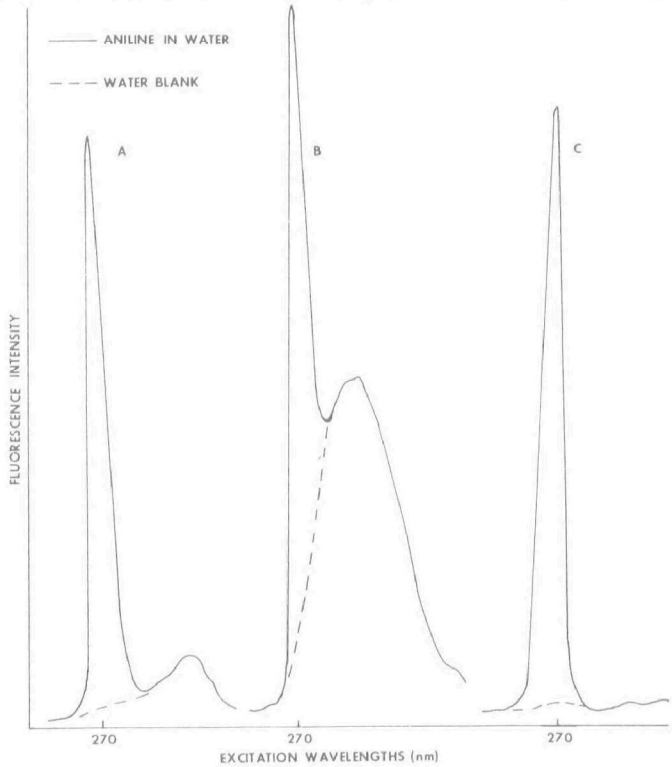

Figure 5: SE Spectra of Aniline "Spiked" into River Waters (A) and (B) and Distilled Water (C). Aniline in Water Concentration is 1 mcl/l. The Waters used to Obtain (A) and (B) were Collected from the Raritan and Arthur Kill Rivers in New Jersey, Respectively. Both Rivers are Subject to Heavy Marine Traffic and Chronic Pollution from Petroleum and Industrial Chemicals

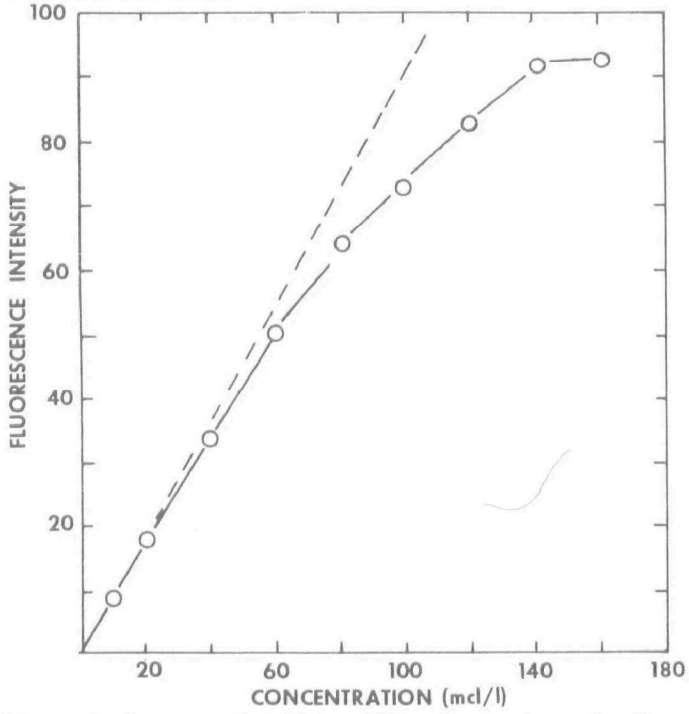

Figure 6: Concentration Versus Fluorescence Intensity Plot of Aniline in Distilled Water

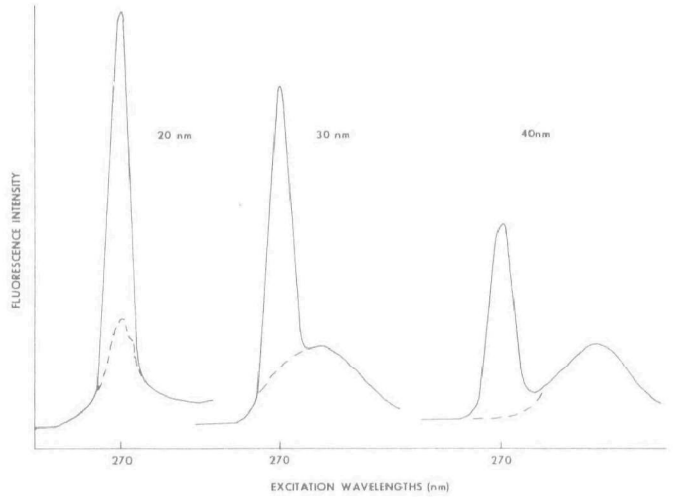

Figure 7: SE Spectra Obtained at Three Different Wavelength Intervals Between the Emission and Excitation Monochromators. The Spectra Derive from 1 mcl/l of o-cresol Spiked into Water Collected from the Woodbridge Creek in Sewaren, New Jersey. Dashed lines Represent Water Blanks.

Table II: Optimum Wavelength Intervals for Quantification of Several Hazardous Materials in Water

Hazardous Material	Optimum Wavelength Interval (nm)
Aniline	20
Benzene	35
Benzoic Acid	15
O-Cresol	40
Dodecyl Benzene Sulfonic Acid	50
Ethyl Benzene	20
Naphthalene	45
Naphthenic Acids	60
Phenol	25
Quinoline	60
Resorcinol	60
Strichnine	40
Styrene	60
Toluene	15
Xylene	15
Xylenol	30

SUMMARY

Use of SE fluorescence spectroscopy was evaluated for *in-situ* quantifications of hazardous materials in water. This method is discussed and contrasted with SWE methods within the context of a three dimensional coordinate system. The relative impact of Rayleigh-Tyndall and Raman scatter interferences on quantifications by SE and SWE methods are contrasted and the advantage of the SE technique is illustrated. *In-situ* quantifications of several hazardous materials in representative polluted waters were performed, good recovery capabilities were demonstrated and analysis parameters are provided. The SE fluorescence pro-

cedure is shown to facilitate accurate quantifications of fluorescent hazardous materials directly in water, thereby avoiding the tedious and time consuming solvent extraction steps that are normally associated with quantifications of organic toxicants in water.

ACKNOWLEDGMENTS

The Authors wish to express grateful appreciation to Mr. Nick Pangaro, Ms. Pamela Ward, Ms. Jocelyn Callender and Mr. John Munn, for the data acquisition that has made these findings possible.

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I. HAZARDOUS CHEMICALS

B. OTHER ANALYSES

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #20, January 1974.

STABILITY OF STORED CHROMATOGRAPHIC AND SPECTRAL DATA

A severe fading problem of chromatograms and spectra recorded in red ink has been encountered. This appears to be inherent to several commercial red inks and manifests itself only after prolonged storage of such data output. These chromatograms and spectra were used in our laboratory for display purposes, and were generally found to fade beyond recognition within 16 months. Similar data recorded in black ink exhibited no image degradation. The commercial sources of the red inks involved cannot be positively identified because such records were not maintained. A cursory test was developed that is thought to have predictive value about the image stability of inks. Normal thickness lines of several red, black, and blue inks were exposed to the fumes of full strength hydrochloric acid, for 30 seconds. Two of the four red inks exhibited severe fading, the one blue ink tested exhibited some fading, while the two black inks tested did not fade. It is believed that these test results correlate well with observation of stored spectra, especially because the two red inks that faded in this test are thought to be the ones that faded during data storage. These observations underscore the need for care in the choice of inks.

(F. Behm/M. Gruenfeld, FTS 201-548-3543, Coml. 201-548-3347)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #25, April 1975.

Determination of EDTA by NMR

The possible discharge of sodium ethylenediaminetetraacetate in some industrial waste effluents is being evaluated by the Region II Edison Laboratory. This compound is present as the tetrasodium salt (Na_4EDTA) in some sommercial formulations of boiler cleaners, and is used as a chelating agent. As a first step in determining the concentration of Na_4EDTA in waste effluents, distilled water samples were prepared from a commercial formulation and analyzed by ASTM Method D 3113-72T. Erroneously low results were obtained, however, and these were ascribed to the presence of interfering additives. The samples were then analyzed with a 60 MHz Nuclear Magnetic Resonance (NMR) Spectrometer and the results agreed very closely with the declared formulation. The accuracy of the NMR determination was also confirmed by using a pure reference standard. Na_4EDTA exhibited two sharp singlets at 3.1 and 2.6 ppm, relative to sodium 3-trimethylsilyl-propane sulfonate, and integration of these peaks yielded the expected ratio 2:1. The 3.1 ppm peak was used for quantitating the EDTA salt, and methanol was selected as the internal standard.

The NMR detection limit of Na_4EDTA in the distilled water samples of the formulation was 0.5% (w/v). We are now testing a number of concentration procedures in anticipation of actual industrial wastewater samples. (U. Frank/F. Iaconianni, FTS 340-6626, Coml. 201-321-6626)

* See page 2.

Kepone Analyses

At the request of the special EPA Kepone Task Force, established by the Office of the Administrator, Washington, D.C., our laboratory conducted analyses to measure the removal of Kepone Pesticide from water that had been contaminated in the washdown and dismantling of the Life Sciences Products Plant in Hopewell, Virginia. Our "Mobile Hazardous Spills Treatment System", which utilizes three activated carbon columns about 7 ft high x 7 ft in diameter, was used for the on-site removal of 99.996% of Kepone from the washdown water. Samples deriving from clean up operations of 22 railroad tank cars containing 220,000 gallons of contaminated water were concurrently analyzed by extracting one liter portions with one 40 ml and two 30 ml aliquots of benzene in glass stoppered graduated cylinders. A minimum of 1 part per billion (µg/l) of Kepone was detected by electron capture gas chromatography without concentrating the extracts. Column influent concentrations ranged from 100 to 3 ppm (mg/l); treated effluent concentrations ranged from 6 to 0.2 µg/l of Kepone. This data was verified by two other laboratories that analyzed replicate samples and used diethyl ether as the extraction solvent. Procedures and results of these interlaboratory analyses are available on request. (U. Frank, FTS 342-7510, Coml. 201-548-3347, J. Lafornara, FTS 342-7523)

Mobile Laboratory

Several ongoing extramural efforts at Edison involve the development of large scale hazardous material clean up systems that are needed to provide flexibile emergency response capability in the event of accidental spills of hazardous materials. Optimum utilization of these systems necessarily requires minimum analytical turn around time in order to monitor treatment effectiveness. At the present time, samples are usually mailed or otherwise transported to laboratories that are distant from the spill site, and this often causes intolerable delays. Consequently, we constructed a mobile laboratory to support the treatment systems in the field, during emergency response situations. The laboratory was constructed in a 35 ft semi-trailer, equipped with heating and air conditioning systems. Laboratory construction was accomplished largely through in-house efforts, using laboratory furniture and accessories that were available within our facility. The mobile laboratory is fully equipped for solvent storage, and contains a fume hood, explosion proof refrigerator, computerized gas chromatograph, and an infrared spectrometer. An atomic absorption spectrometer will soon be added. Other associated equipment includes a hazardous spill detection field kit, pH meter, centrifuge, and a sanitary facility, distilled water supply, and a solvent disposal facility. This mobile laboratory was built in about four months, at a cost of approximately $5,000. This figure includes both salaries and hardware, but does not include the cost of analytical instruments. Testing of the laboratory is now in progress, prior to actual use. (U. Frank, FTS 342-7510, Coml. 201-548-3347, R. Frederick, FTS 342-7524, M. Urban, FTS 342-7517)

* See page 2.

MOBILE LABORATORY

Our mobile laboratory (described in AQC Newsletter No. 29, April 1976) just completed 6 weeks of field operation in Haverford, Pennsylvania. The laboratory was used to monitor the effectiveness of pentachlorophenol (PCP) removal operations from ground water, using a mobile activated carbon treatment system. Concentrations of PCP in water exceeded 20 mg/l (ppm) before treatment, and were less than 1 mcg/l (ppb), after treatment. Approximately 150 sample analyses were performed in the mobile laboratory, on site, using gas chromatography. Concurrent oil analyses were also performed using infrared spectroscopy. A method by Chau, A.S.Y. and Coburn, J.A., JAOAC, 57 (2), 389 (1974), was used for the PCP analyses. (M. Urban/R, Losche, FTS 340-6628, Coml. 210-321-6628.)

* See page 2.

Analysis of Hazardous Materials in Water by NMR

A preliminary evaluation of the use of NMR for the analysis of ppm levels of hazardous materials in water has been performed. Fifty milligram portions of activated carbon retained in a funnel over glass wool were used to adsorb ppm levels of cyclohexane and xylene from one liter of water. Following this "filtration" step, the carbon was rinsed with 2 ml of deuterium oxide and transferred to an NMR tube containing 1.0 ml carbon tetrachloride. The presence of powdered carbon in the NMR tube caused little spectral interference; only slight peak broadening was observed. Recoveries were in the range 85 - 90 percent. Evaluation of this method for other hazardous materials listed in 40 CFR Part 116 is now underway. (U. Frank, Coml. 201-321-6626, FTS 340-6626)

Interference by Sodium Sulfate Impurities

While analyzing water samples for PCBs at sub-part per billion levels (0.05) by GC-ECD, interferences were encountered that were traced to sodium sulfate. Sodium sulfate was used for dehydrating the extraction solvent prior to concentration. Impurities in sodium sulfate yielded extraneous peaks with retention times that overlapped the PCB range. Repeated rinsings of sodium sulfate with 15 percent methylene chloride in hexane failed to eliminate the interferences. This problem was successfully resolved by the method of Garrison, (AQC Newsletter No. 14, July 1972), by maintaining sodium sulfate at 600°C for two hours prior to its use for dehydration. (R. Losche/M. Urban, Coml. 201-321-6628, FTS 340-6628/M. Gruenfeld, Coml. 201-321-6625, FTS 340-6625)

* See page 2.

Evaluation of an Emission Spectroscope

The evaluation of a low cost emission spectroscope capable of rapid identifications of metals is described. This unit, a Vreeland Spectroscope, Model 7 (Spectrex Company, Redwood City, California) costs approximately $3,000 and incorporates a novel method for rapid spectral interpretation. Matching of spectral lines is accomplished by comparison of sample spectra with spectral reference lines that are incorporated into a movable reference film in the instrument. Spectral interpretation and instrument use are simple and require little experience. Our anticipated use of this instrument is aboard the Mobile Spills Laboratory described in AQC Newsletters 29 and 32. Instrument dimensions are approximately 16" H X 18" W X 24" L, and it weighs approximately 35 lbs. Synthetic water and sediment samples containing mixtures of the following metals were analyzed: V, Cr, Ni, Co, Ti, Pb, Ba, and Hg. The instrument yielded reliable identifications at high metal concentrations (0.5%), but was less useful at low concentrations (below 0.1%). These samples required preconcentration prior to measurement. The emission spectroscope will be used in conjunction with the Atomic Absorption Spectrometer while responding to spills of hazardous substances containing toxic metals. It will be used for monitoring source samples or grossly contaminated environmental samples to identify their metal content. Quantification of metals identified in this manner will be accomplished by Atomic Absorption Spectroscopy. (R. Losche, Coml. 201-321-6628, FTS 340-6628/M. Gruenfeld, Coml. 201-321-6625, FTS 340-6625)

* See page 2.

Removal of Sulfur from Sediment Extracts - Estuarine sediments were recently analyzed for PCB content according to EPA's manual, "Analysis of Pesticide Residues in Human and Environmental Samples". Removal of the sulfur in the hexane extracts was accomplished by modifying a more complex column procedure detailed in the EPA report, "Correlation of Oils and Oil Products by Gas Chromatography", (EPA-600/2-77-163). Each extract concentrate (2 ml) was eluted through a short stem disposable Pasteur pipette (9 in. length) packed with copper. This was prepared by placing a glass wool plug at the base of the pipette, and adding 2-2.5 cm of copper metal (electrolytic dust). This was activated with one column volume of 1 N HCL followed by a rinse of two column volumes of hexane. Samples were then passed through the columns to remove sulfur. The bright copper turned black as the sulfur was removed, and the solvent flow was accelerated as necessary, by using positive pressure from a pipette bulb. (D. Stainken, Coml. 201-321-6628, FTS 340-6628).

Sediment Hydrocarbon Quantification - While quantifying volatiles and extractable hydrocarbons in estuarine sediments, a statistical relationship was found between the silt-clay fraction of the sediments and percent volatiles. Generally, the percent volatiles of the sediments ranged from 0.85 to 11.39, and increased as the silt-clay fractions of the sediments increased. Percent volatiles were determined by heating predried sediments in a muffle furnace at 500-800^{o}C for 24 hours. Modifying a procedure by Giger et al, (1974, Environ. Sci. & Technol. 8, 454), extractable hydrocarbons in dried sediments were measured after tumbling the ground sediments in flasks containing Freon® 113 solvent. The extracts were quantified by IR spectroscopy following silica gel treatment. This procedure is described by Gruenfeld and Frederick (Publication announced in AQC Newsletter 36). A statistical relationship was also found between the percent volatiles and micrograms of extractable hydrocarbons. Values of extracted hydrocarbons ranged from 2.2 to 1098 µg/g of dry sediment. Generally, the amount of hydrocarbons extracted increased as the amount of volatiles increased. GC analysis of selected samples exhibited a large unresolved complex mixture with few well resolved peaks. The maximum peak in many of the chromatograms had a retention time that matched the n-C_{28} alkane. An n-C_{28} sewage derived hydrocarbon has been reported from English sediments, and it is possible that this n-C_{28} compound derives from sewage. Analysis of these samples by synchronous excitation fluorescence spectroscopy (described in AQC Newsletters 31-34 and 36) indicated the frequent presence of 1-6 ring polynuclear aromatic hydrocarbons (PAH). Sediments with large amounts of volatiles, and silt-clay fractions, are therefore expected to contain large amounts of extractable hydrocarbons, possibly necessitating several extractions. The hydrocarbons may be PAH or other complex mixtures. (D. Stainken, Coml. 201-321-6628, FTS 340-6628)

* See page 2.

Interaction of m-Cresol with Plastic Tubing - While using pilot scale water decontamination equipment to evaluate the efficiency of activated carbons for removing organic hazardous materials from water, we examined the interaction of one of the materials, m-cresol, with two kinds of plastic tubing. m-cresol solutions in water, at 100 ppm, were exposed to clear industrial grade PVC tubing, and to bev-a-line VHT tubing (Thermoplastic Scientifics, Inc., 57 Stirling Road, Warren, New Jersey 07060). Contact between the m-cresol solution and the tubing was accomplished by: (1) maintaining an unbroken solution flow at approximately 15 ml/min. through 9 foot lengths of each tubing; (2) maintaining the solution flow, containing air gaps, through 19 feet of the clear PVC tubing, and (3) performing a static test whereby portions of solution were sealed for two hours in one foot lengths of both types of tubing. These tests yield progressively longer contact times between the m-cresol solution and the plastic materials. The bev-a-line tubing was of special interest because it is less rigid than Teflon® tubing, and according to product literature it strongly resists chemical interaction. Our test findings confirm this for m-cresol. While m-cresol was progressively lost to the clear industrial grade PVC tubing in amounts proportional to increasing contact time, no measurable loss to the bev-a-line VHT tubing occurred. Test results were as follows: test (1) yielded no measurable loss of m-cresol to either tubing materials; test (2) yielded a lowering of m-cresol from approximately 100 ppm to approximately 93 ppm by the clear PVC tubing; while test (3) yielded a lowering of m-cresol from approximately 100 ppm to approximately 60 ppm by the clear PVC tubing, but no lowering by the bev-a-line tubing. (M. Royer/S. Frysinger/M. Gruenfeld/G. Fraser, Coml. 201-321-6627, FTS 340-6627)

Analysis of Polynuclear Aromatic Hydrocarbons in Estuarine - Water - Raritan Bay bottom waters, sediments, and clams were surveyed to determine the quantities of polynuclear aromatic compounds (PNA's) present. The estuarine bottom water samples were recently analyzed for PNA content using synchronous excitation (SE) fluorescence spectroscopy (Quality Assurance Newsletter, Volume 1, Number 1, April 1978). Use of the SE technique yielded spectra of PNA mixtures that were resolved according to the number of aromatic rings. Our procedure involved the extraction of one liter water samples with two 50 ml portions of cyclohexane (glass distilled). Emulsions were alleviated by adding five ml of concentracted HCl prior to extraction. One to three grams of anhydrous sodium sulfate (precleaned by baking three hours at 450°C) was added to each extract. SE spectra were then obtained using a 25 nm interval with the excitation monochromator leading the emission monochromator. SE spectra of PNA standards at concentrations of one mg/l in cyclohexane were similarly obtained for comparison purposes. Confirmation of the samples' PNA content was obtained by HPLC analyses of sample extract concentrates. Appropriate HPLC fractions were collected and analyzed by SE and single wavelength excitation fluorescence spectroscopy. Results indicated that PNA compounds in the water were in the low ppb range. The identity of some of the compounds detected included isomers of methyl naphthalenes, pyrene, and benz-a-pyrene. The SE fluorescence method is currently being used to analyze sediments and bivalve tissue extracts for their PNA content. (D. Stainken, Coml. 201-321-6628, FTS 340-6628/U. Frank, Coml. 201-321-6626, FTS 340-6626)

* See page 2.

REPRINTED FROM: Chemical Marketing Reporter, April 3, 1978, pp 61 & 64.

Mobile Lab Johnny-on-the-Spot In EPA's Toxic Spill Operations

Two summers ago, residents of Haverford,Pa., noticed that "oil slicks" were forming in a nearby stream called Naylors Run. The pollution seemed most evident at a number of places where storm drains and springs emptied into the Delaware River tributary. Alarmed, they reported the problem to the regional office of the Environmental Protection Agency.

Normally,EPA would have obtained samples of the stream's water and sent them to a large laboratory equipped to carry out the sophisticated analyses necessary to detect chemicals which may be toxic in concentrations as low as a few parts per million.

That time-consuming process was avoided, however, by bringing EPA's mobile laboratory into action. An addition of only a few years to the agency's physical assets, the laboratory consists of a close-packed array of delicate detection and measuring instruments housed in the back of a semi-trailer truck.

LAB ARRIVES QUICKLY

It took little time for the laboratory, manned by two chemists, to be driven to Haverford from its base with EPA's Oil and Hazardous Materials Spills Branch in Edison, N.J. Once on the scene, where strong chemical odors hung above the stream and dead and dying fish had begun to dot the surface, the team set to work quickly. A combination of field investigation and laboratory analysis pinpointed the source of the pollution at a wood-treating operation where a 5% solution of pentachlorophenol (PCP) suspended in fuel oil was used as a preservative. Tests by a Perkin-Elmer infrared spectrophotometer and a gas chromatograph confirmed that the Naylors Run oil slicks contained large amounts of PCP.

EPA investigators later discovered that the wood-preservative plant site was saturated with the chemical. Wastes from the operation had been injected by the company into a twenty-five-foot deep well for approximately thirty years, and when the well could hold no more, PCP-containing oil had seeped into an underground culvert, leaked into storm drains and emptied into Naylors Run. The location of the pollution made the agency particularly apprehensive, because the stream ultimately flows through the suburbs of Philadelphia and into Tinicum National Wildlife Refuge, the last freshwater tidal marsh in Pennsylvania.

PCP, a fungicide and pesticide, is mixed with fuel oil before being applied to wood as a preservative. It is commonly used on farm buildings and other outdoor structures, much like creosote. It has also been linked to many human health problems, including lung, liver and kidney damage. A year ago, PCP was blamed for the death of a number of dairy cows in Michigan, but subsequent testing was unable to prove that the chemical, present in large quantities in the dead animals, was the cause.

At Naylor's run, the EPA laboratory team determined that the concentration of PCP in the oil was greater than 10,000 parts per million (ppm) and in the stream water was 0.5 ppm. Concentrations of 0.2 ppm are lethal to fish.

POLLUTION ATTACKED

The laboratory was accompanied to Haverford by another arm of EPA's mobile strike force - a hazardous spill treatment system consisting of trailer-mounted activated carbon columns and prefiltration, flocculation and sedimentation tanks.

Through well-drilling and trenching to collect oil-contaminated groundwater, skimming of the oil in tanks and the use of the activated carbon columns, the Oil and Hazardous Materials Spills Branch and EPA's Region III response team cut the PCP concentrations in the water to below 1 part per billion (ppb). It took three months to process 210,000 gallons from the stream. The laboratory chemists monitored the efficiency of the clean-up effort throughout with their instruments.

The two mobile units have been used in a number of detection and clean-up operations.

"Once we become involved in a toxic chemical spill," says Michael Gruenfeld, director of the Oil and Hazardous Materials Spills Branch, "because of the urgency of the situation it is not always logistically feasible to request analytical support from permanent laboratory facilities. If we were to depend on a centralized laboratory, or other laboratory, it would mean taking samples, transporting them to the laboratory and waiting for answers. By the time we received the analysis, the priorities for the spill clean-up might have changed.

"Additionally", he explains, "laboratories normally do not do this specific type of

work, nor would they want to interrupt their normal flow to handle it. Therefore, we often send experts into the field to do these dedicated analyses."

The panelled inside of the mobile laboratory bristles with the machines of precise chemical analysis. The mainstays are the Perkin-Elmer atomic absorption spectrophotometer, infrared and flourescense spectrophotometers and a gas chromatograph.

The atomic absorption instrument can analyze metallic components of a sample in concentrations as low as ppb. The technology of the EPA machine, which uses a heated graphite atomizer to reduce the samples, is three times more delicate a detection device than the earlier generation of atomic absorption spectrophotometers, which worked on what is called the "flame mode."

"The fluorescense instrument," Mr. Gruenfeld says, "has an extremely high sensitivity for materials that fluoresce under ultraviolet light. It is valuable for aqueous analyses at a site and possesses the advantage over gas chromatograph analysis in not needing a solvent in sample preparation. A sample can be used directly from the source. It also has the ability to measure the presence and levels of fluorescing toxicants, even if they remain unknown. The identity of contaminating material can then be adduced from corresponding evidence. In some cases, the instrument has been able to measure reductions in levels of unknown aromatic toxicants, following water treatment, while other labs were still trying to identify the contaminants."

The infrared spectrophotometer analyzes petroleum oils that are used as vehicles for toxicants. The device can also identify unknown materials through "fingerprint" spectra. The classified spectra are stored in the Edison library of spectra, and the mobile lab has at times called into the library for telefacsimile transfer of a spectrum's distinguishing characteristics.

The gas chromatograph separates complex mixtures into their components. The lab can also carry out total organic carbon analysis (TOC), a process in which the total organic content of water is rapidly measured without extraction.

For the most part, the rolling lab has dealt with bodies of water than have been contaminated and that threaten drinking water supplies or aquatic life.

For the purposes of broad distinction, OHM divides spills and their subsequent clean-up problems into three categories.

"The different environments where water-threatening or contaminating spills occur can be divided into spills on soils, into stream or into large unconfined bodies of water," says Frank Freestone, head of the hazardous materials spill group at Edison. "Water bodies present different problems depending on their size. A small stream or pond, for instance, in an impoundable, or damable, body of water. A spill could be isolated from the remainder of the stream, permitting clean-up to proceed relatively easily.

"Large water courses are unconfinable. The treatment approach must be different for such large bodies of water.

"Our function has been to develop large-scale equipment to return the environment as closely as possible to its prespill state," Mr. Freestone adds. "Our analytical work is in support of this primary engineering responsibility. We have to provide answers as to how well the equipment words, and also, research in how to improve the engineering."

VANDALS CAUSE SPILL

The hazardous materials detectives got another chance to test their sleuthing machinery last June. Vandals in Kernersville, N.C. crept onto the grounds of a chemical wastes disposal company there one night and opened the valves of six storage tanks. Over 30,000 gallons of mixed wastes gushed into the town's reservoir.

It didn't take long for the townspeople to realize something was wrong. Vapors drove a thousand residents from their homes and dead fish began to float to the surface. Mistakenly, local authorities believed an oil spill had occurred. When they treated the water accordingly, there was no effect.

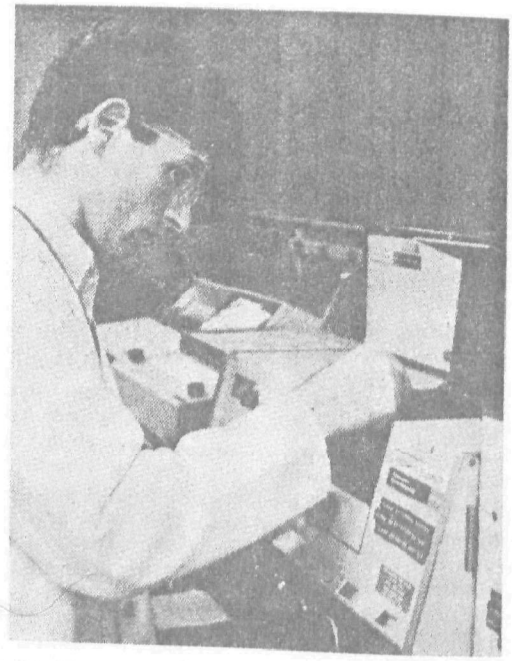
At that point, the EPA mobile laboratory was called in. Fish were dying in increasing numbers, fleeing the spreading chemicals to the mouths of the inlets of the reservoir. Within seventy-two hours, the toxic chemicals overtook the fish and killed 20,000 of them, about 90 percent of the aquatic life of the reservoir. Town officials reacted by closing down the water source.

For the EPA chemists, the problem was a tough one. The toxicants were unknown and thoroughly mixed, meaning proper identification would be a lengthy process. The scientists ran samples of the polluted water through a pilot-scale carbon adsorption system. That, tied in with a bioassay system, revealed that the water going into the carbon columns was toxic, but the water coming out was not. The discovery suggested to the EPA team the most likely way to clean up the reservoir - carbon adsorption.

"The fluorescense device was very valuable in this situation," Mr. Gruenfeld says. "By running samples with the fluorescense spectrometer, we were able to isolate distinctive profiles that were not present in samples taken before the spill occurred.

"It was intial use of the mobile treatment system that first pointed to the need for a mobile laboratory when it was noted that although oil spills are visible enough to locate and isolate, chemical spills most often are not. With the critical aid of the laboratory, we are able to deal with an acute public safety hazard, such as chlorine, as well as with hazards of a chronic nature like PCB's, or low-level pesticide releases, on a nationwide 24-hour-response basis. In effect, the mobile laboratory is an extension of the base laboratory, bringing to the field all the sophisticated technology that is required, in more immediate and timely way."

TREATMENT SYSTEM: EPA's trailer-mounted hazardous materials treatment system is equipped to clean-up chemical spills with activated carbon columns and prefiltration, flocculation and sedimentation tanks.



INSIDE THE MOBILE LAB, an adjustment is made in the operation of a Perkin-Elmer fluorescence spectrophotometer.

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Development and Use of a Mobile Chemical Laboratory for Hazardous Material Spill Response Activities

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INTRODUCTION

As part of a continuing effort to provide prompt response to spills of oil and hazardous materials, EPA's Oil and Hazardous Materials Spills Branch at Edison, New Jersey, developed a Mobile Spills Laboratory to perform chemical analyses in remote field locations. The laboratory was specifically designed to provide on-site analytical services during hazardous material spill response and cleanup operations. It was constructed to aid in the cleanup of spill impacted areas by eliminating the delay associated with the transport of samples to distant laboratories for analysis. Immediate laboratory data is often urgently needed to make decisions regarding spill cleanup efforts and to monitor cleanup efficiency.

The laboratory was constructed within a 35-foot van semi-trailer, obtained as an excess government property item (Figure 1); the van is equipped with heating and air conditioning systems. Laboratory construction was accomplished largely through the efforts of Branch personnel using available laboratory furniture and accessories; the interior construction required four months and cost approximately $5,000. This figure includes salaries and hardware but excludes analytical instruments and supplies.

Capabilities

In order to provide optimum laboratory services during any potential spill situation and to assure analysis of virtually all organic and inorganic hazardous substances (pesticides, PCB's, heavy metals, etc.), the Mobile Spills Laboratory was equipped with a spectrum of sophisticated analyt-

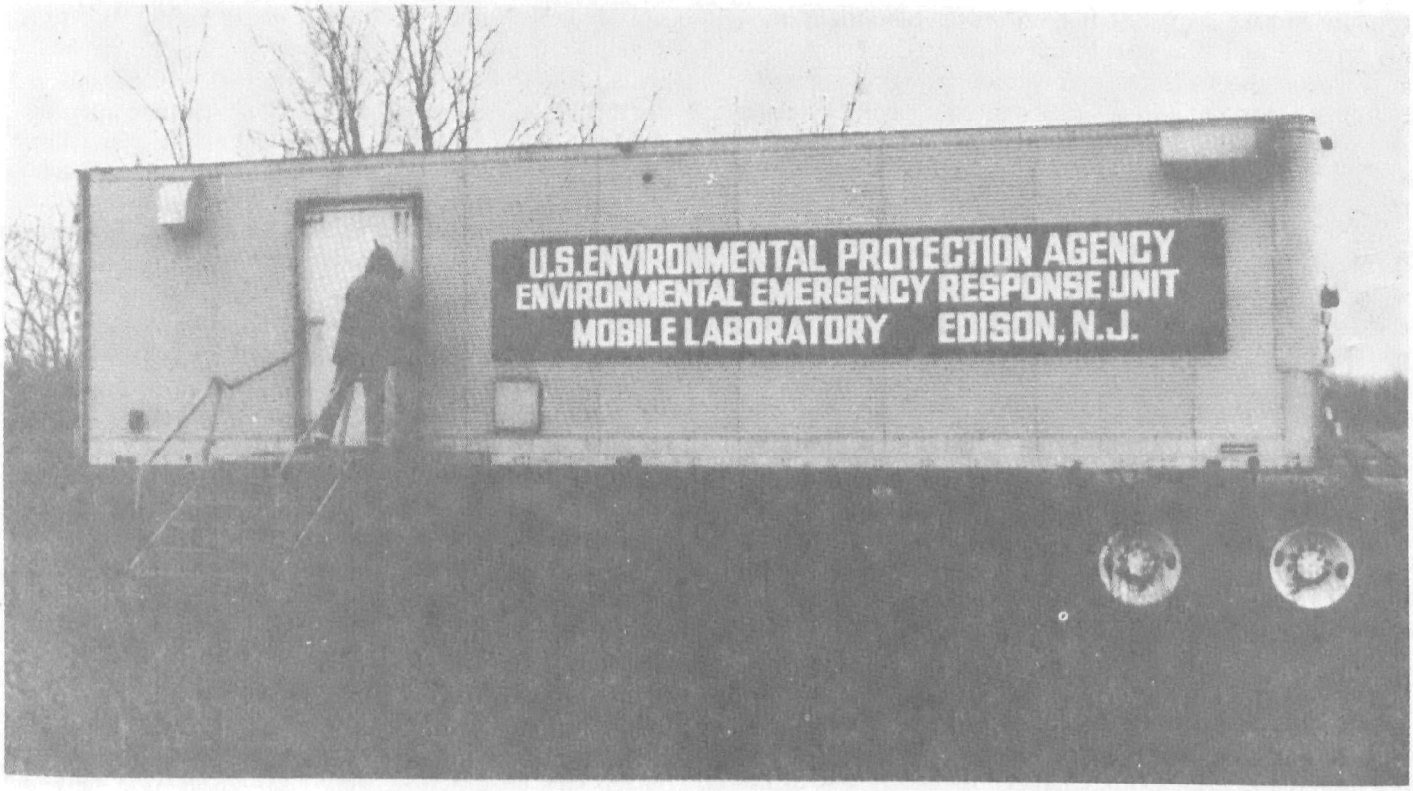

Figure 1: Exterior View of the EPA Mobile Spills Laboratory Operating in a Remote Field Location

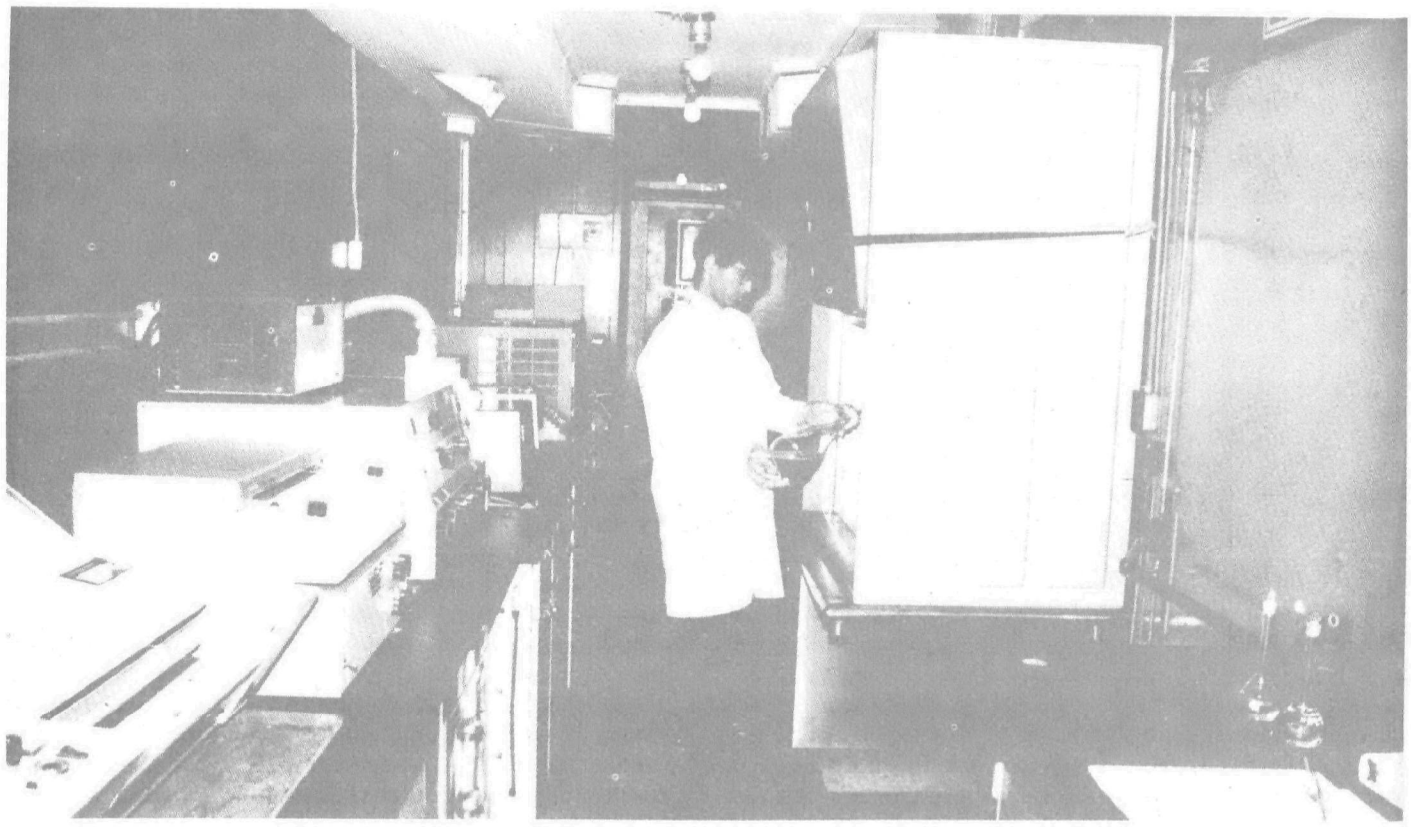

Figure 2: Interior View of the Mobile Spills Laboratory. Instruments and Equipment Include a Fume Hood, Telefacsimilie, Fluorescence and Infrared Spectrophotometers, Gas Chromatograph, and an Atomic Absorption Spectrometer

ical instruments (Figures 2, 3 & 4) including (1) two gas chromatographs, one computerized and one portable, both with flame ionization and electron capture detectors, (2) a computerized atomic absorption spectrophotometer with a graphite furnace accessory and automatic sampling system, (3) infrared and fluorescence spectrophotometers and (4) an emission spectrograph. Safety considerations necessitated the inclusion of a fume hood, vented solvent locker, explosion-proof refrigerator, running water (100 gal.), safety shower, eyewash, fire blanket, fire extinguishers and a modification of the existing air conditioning and heating systems, to allow a once through pass of air.

Other dedicated equipment include a telefacsimile, which permits transmission of hard copy data (spectra, chromatograms, methods of chemical analysis, etc.) via telephone lines, a pH meter, balances, hot plates, magnetic stirrers, desiccator, steambath and all necessary glassware, solvents, reagents, and supporting equipment needed for fully independent operation in remote field locations.

The laboratory was also equipped to carry a pilot scale water treatment system consisting of four three-inch I.D. by four foot deep pyrex columns. These can be charged with activated carbon, ion exchange resins, or other mass transfer agents to conduct treatment feasibility studies, before initiating full scale cleanup operations.

Case Histories

The following summary describes the use of the Mobile Spills Laboratory during several repsonse actions to hazardous material spill incidents. Included is an overview of the type of incident encountered and an outline of analytical support provided. Additional detailed descriptions of the incidents are provided by other authors[1,3].

Haverford, Pennsylvania

During November 1976, at the request of the U.S. EPA's Region III Environmental Emergency Branch, the Mobile Spills Laboratory was sent to Haverford, Pennsylvania. On-site analytical assistance was required in cleanup operations involving groundwater contaminated with pentachlorophenol (PCP) and oil. The contaminants had been leaching into a stream called Naylor's Run, killing higher aquatic life forms. The pollution originated from a wood preserving company which, prior to 1963, disposed of its wastes by means of shallow well injection on its property. Through the years, the waste, which consisted of PCP in fuel oil, had found its way into storm drainage pipes and ultimately into Naylor's Run.

Cleanup efforts consisted of drilling several wells on the company's property from which oil and contaminated water could be removed. An intercepting trench was also dug at the point where the contaminant had been entering Naylor's Run, permitting its collection and subsequent removal for treatment. Following the removal of the oil and water mixture from the trench and wells, the oil was gravity separated and incinerated. The water was purified by carbon adsorption, by the Mobile Physical Chemical Treatment System (MPCTS)[4]. The Mobile Spills Laboratory continuously conducted numerous sample analyses for PCP and oil to monitor the effectiveness of each stage of treatment by the MPCTS. Electron capture-gas chromatrography and

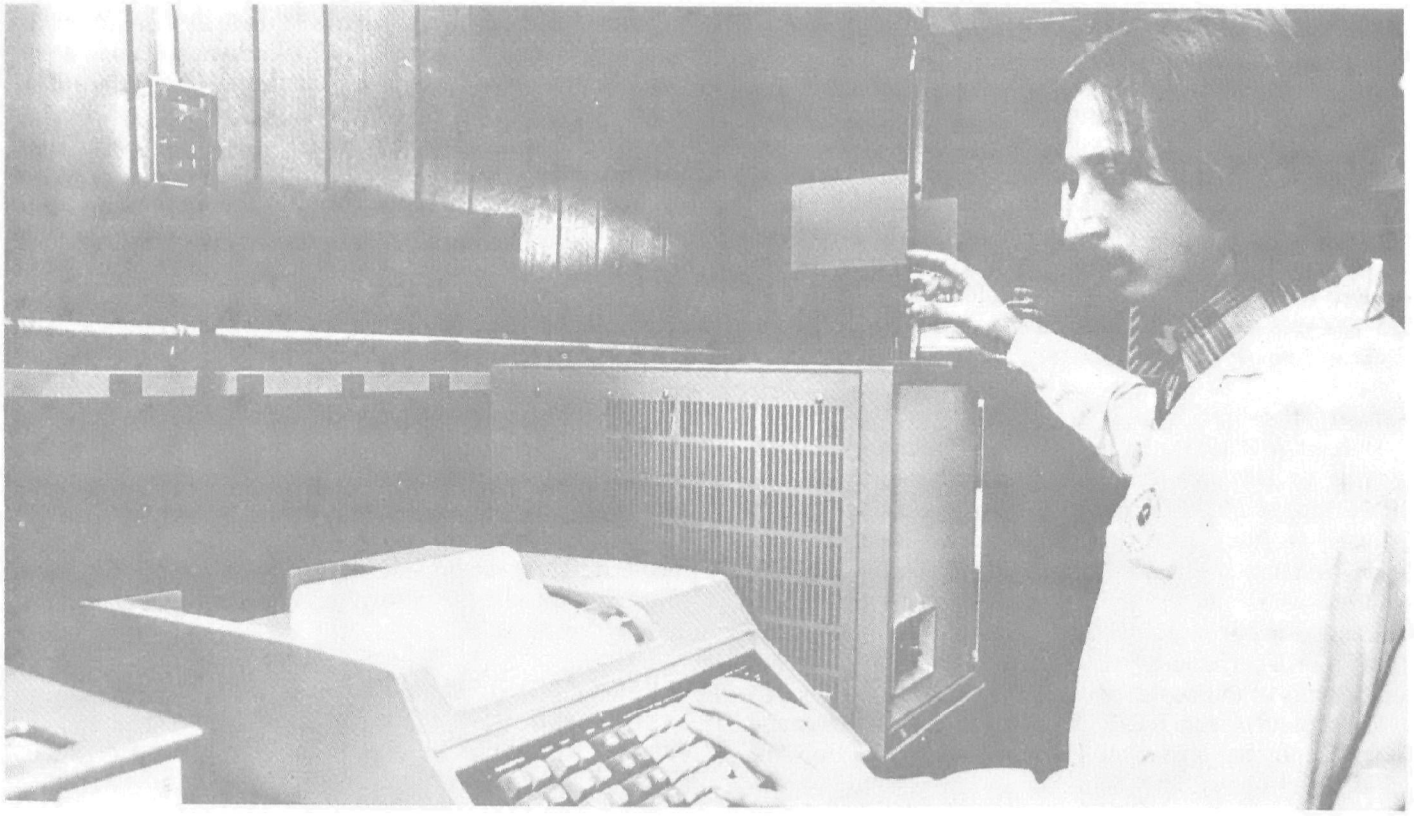

Figure 3: The Use of a Computerized Gas Chromatograph Aboard the Mobile Spills Laboratory for the Analysis of Spilled Organic Hazardous Substances

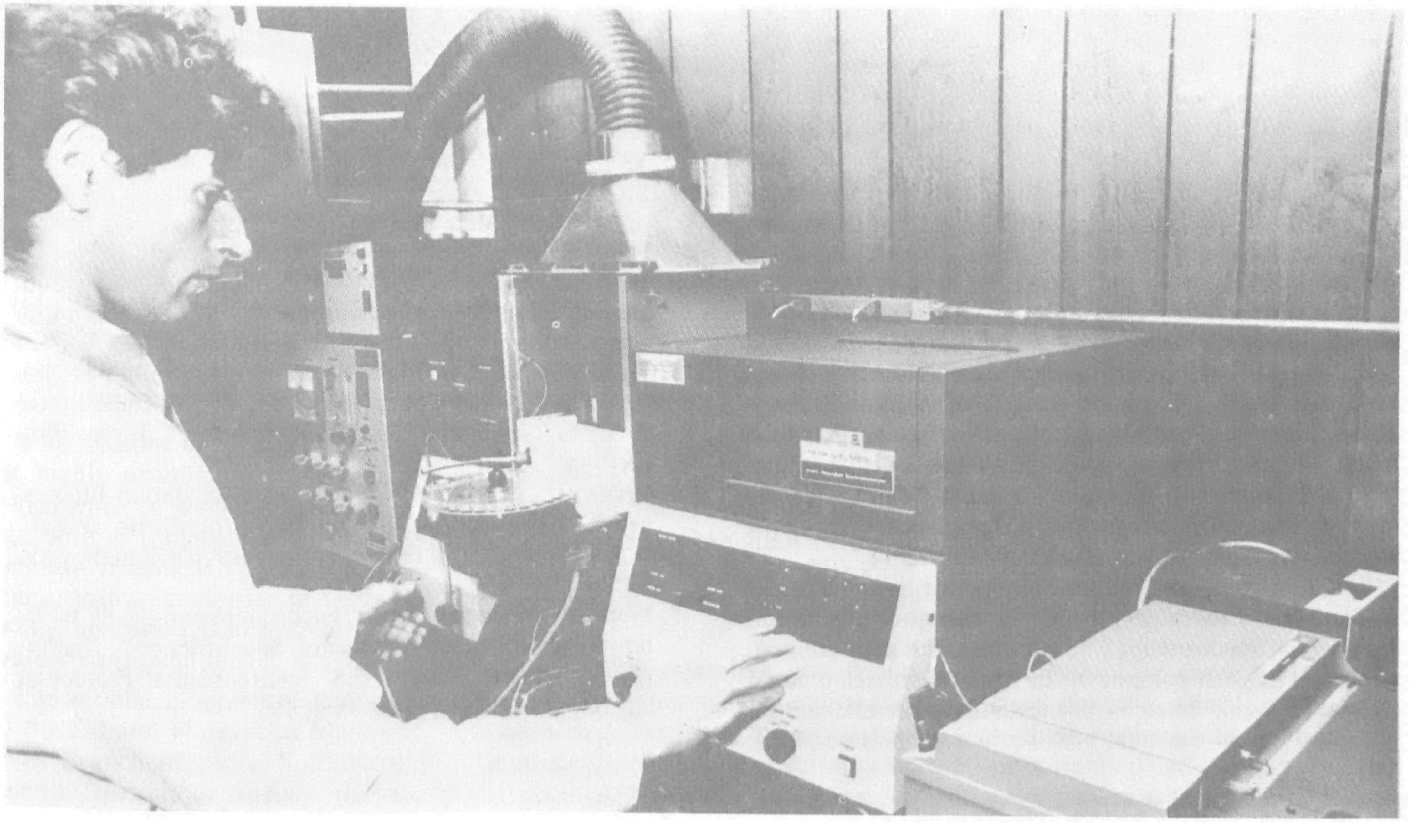

Figure 4: The Use of a Computerized Atomic Absorption Spectrometer Aboard the Mobile Spills Laboratory for the Analysis of Toxic Metals

infrared spectroscopy were used for the PCP and oil analyses.

After treatment, the water was held in five 3,000 gal. storage tanks for analysis prior to discharge. PCP concentrations in the effluent were consistently below 1 ppb. Analysis required less than one hour thereby permitting storage of the treated water until its purity was determined while continuing processing. In addition to support provided during this cleanup, the laboratory was utilized daily to monitor PCP levels in two sections of Naylor's Run. More than 125 analyses were performed on-site over a period of five weeks in support of these efforts.

Dittmer, Missouri

During April 1977, the Mobile Spills Laboratory was dispatched to Dittmer, Missouri, at the request of the U.S. EPA's Region VII Emergency Response Section. On-site chemical analyses were required to support cleanup operations involving an oil and mixed chemical spill. The spill occurred when rainwater caused the contents of a waste chemical disposal pit to overflow and contaminate a nearby stream. PCB's (Aroclor 1260) were identified as major components of the waste.

Cleanup efforts included diversion of the stream around the contaminated area, excavation of the material from the pit, disposal of this material in an approved landfill, treatment of the stream water by the MPCTS, removal of contaminated vegetation and debris from the streambed and installation of a field-improvised activated carbon water treatment device for continued long term stream water decontamination. During the commitment of the Mobile Spills Laboratory to this spill, numerous chemical analyses were performed, using electron capture-gas chromatography. This work supported each segment of the cleanup effort. Measurements included the concentration of PCB's in the contaminated streambed, at the perimeter of the pit during excavation and in the stream water before and after treatment by both the MPCTS and the field improvised treatment device. Approximately 75 PCB analyses were performed during the laboratory's one-month involvement in this spill.

Kernersville, North Carolina

In June 1977, an oil and mixed chemical spill contaminated the water supply reservoir of Kernersville, North Carolina. After the pollutants entered the reservoir, a total fishkill resulted. The Mobile Spills Laboratory, together with an EPA Region IV Mobile Bioassay Unit, were deployed to the reservoir to monitor pilot plant studies of the ability of activated carbon to detoxify this water.

Numerous chemical analyses were performed aboard the Mobile Spills Laboratory, using Synchronous Excitation Fluorescence Spectroscopy[5-7]. Although the absolute concentrations of each component in the toxic mixture could not be readily calculated by this technique, a semi-quantitative fingerprint of the total mixture in water was obtained. Reductions in concentration of the toxic mixture following activated carbon treatment were measured; these data correlated well with the bioassay data. During a period of 12 days, more than 250 analyses were performed.

SUMMARY

The Mobile Spills Laboratory has proven itself to be an excellent means for providing reliable analytical services in remote field locations during hazardous material emergency response actions. It can be readied for transport in a few hours to any part of the United States, and operates with total independence of other support. Telephone communications are maintained with a fixed laboratory facility in Edison, New Jersey. Data output, calculations, reports, and published and specially modified methods of analysis are transmitted back and forth by telefacsimile. Addition of a gas chromatograph-mass spectrometer to the complement of instruments aboard the Mobile Spills Laboratory is now planned. This will provide the laboratory with the on-site capability to identify virtually any unknown organic hazardous substance.

ACKNOWLEDGMENTS

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EPA'S MOBILE LAB AND TREATMENT SYSTEM RESPONDS TO HAZARDOUS SPILLS *

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Industrial Environmental Research Laboratory-Cincinnati Oil and Hazardous Materials Spills Branch

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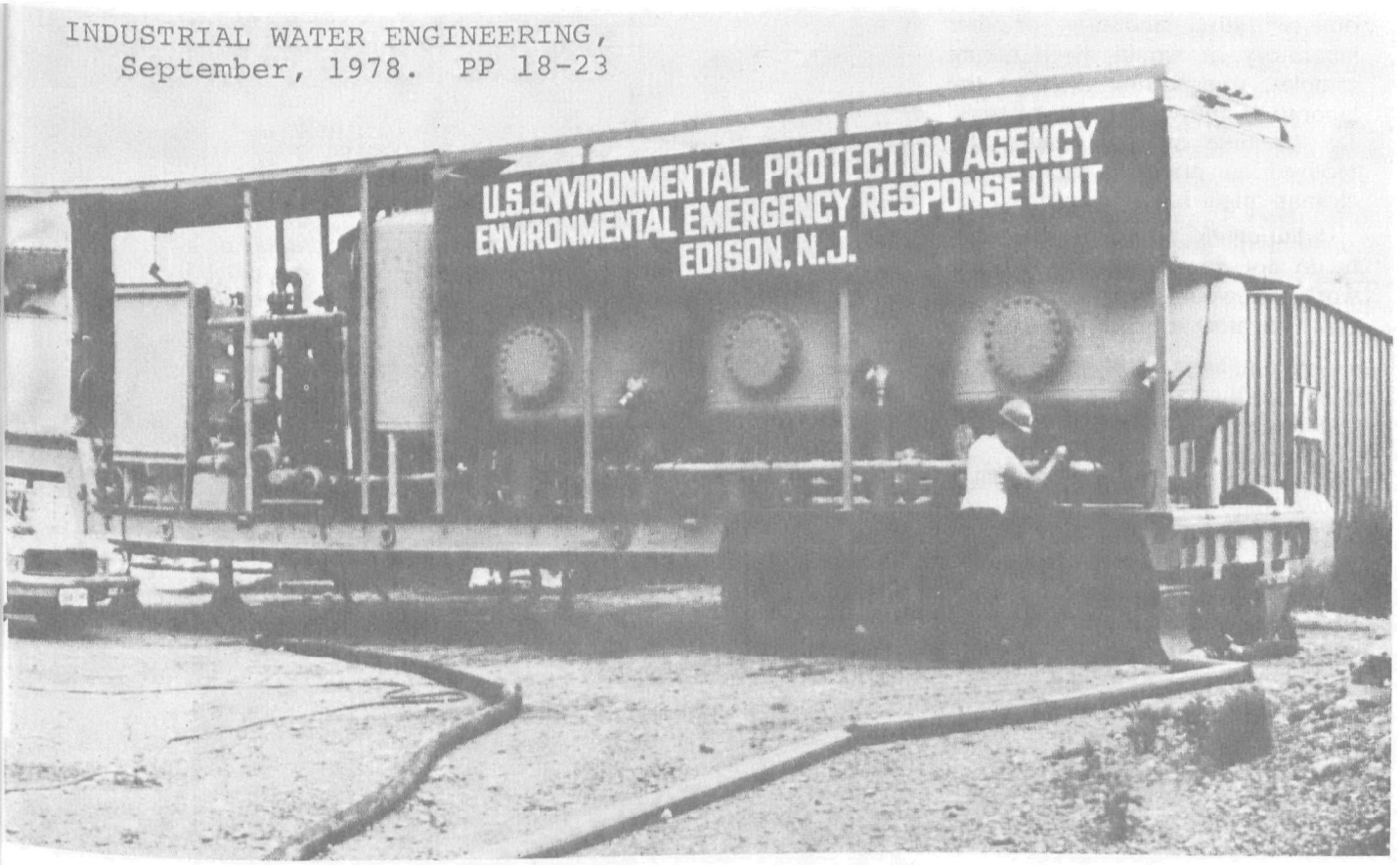
September, 1978. PP 18-23



A view of the Mobile Physical-Chemical Treatment System. The unit, together with a mobile laboratory containing analytical instruments, responds to hazardous materials spills for urgent identification and cleanup of toxic water contaminants.

A laboratory-on-wheels, containing highly sophisticated analytical instruments, is working in conjunction with a trailer-mounted mobile chemical treatment system in emergency cleanup responses to spills of hazardous materials into streams and soil across the nation.

The mobile laboratory, managed by the Oil and Hazardous Materials Spills Branch of the U.S. Environmental Protection Agency (EPA), Edison, New Jersey, and manned by two chemists, is contained within a 35-foot-long semi-trailer[1]. It is readily transported for service throughout the United States, together with the treatment unit consisting of trailer-mounted activated carbon columns, plus prefiltration, flocculation and sedimentation tanks[2].

The mobile treatment unit so far has treated about a dozen major contamination incidents, the latest four making use of the mobile laboratory. It was successful in removing between 90 and 99.9% of the toxic substances. In most cases, the spills have threatened drinking water supplies and aquatic life with such toxic chemicals as dinitrobutylphenol, PCB, toxaphene, chlordane, Kepone, pentachlorophenol and other aromatic and chlorinated organics.

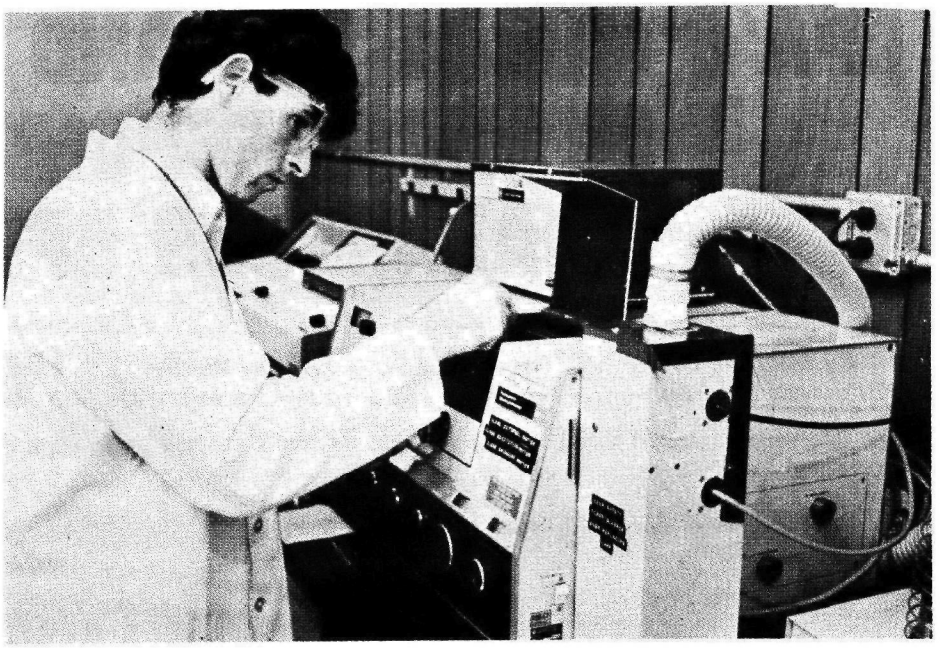
The rapid detection of spills and their accurate identification, as well as their marking and tracking, are all important if effective response to emergencies is to be made. A key to the ability of the emergency response system to work rapidly and accurately lies in the precision analytical in-

*Mention of trade names or commercial products does not constitute an endorsement or recommendation by the U.S. Government.

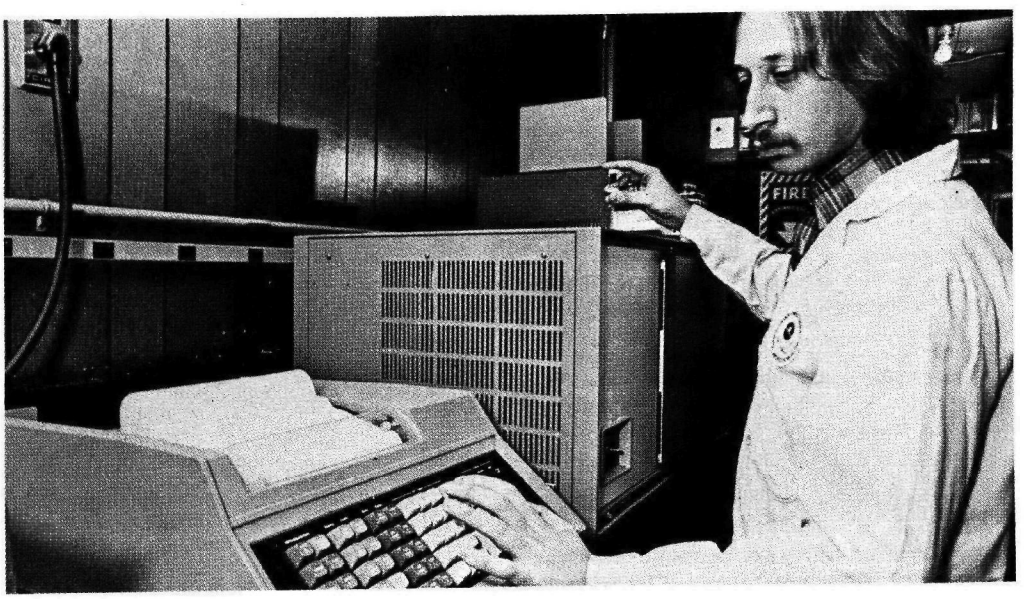
struments contained in the mobile laboratory. Among these are atomic absorption, infrared and fluorescence spectrophotometers by the Perkin-Elmer Corporation, as well as a gas chromatograph) by the Hewlett Packard Corporation, and an emision spectroscope by the Spectrex Corporation.

Once the Branch becomes involved in a toxic chemical spill, because of the urgency of the situation it is not always logistically feasible to request analytical support from permanent laboratory facilities. If it is to depend on a centralized laboratory, or other laboratory, it would mean taking samples, transporting them to the laboratory and waiting for answers. By the time analysis results, are received the priorities for the spill cleanup might have changed.

Additionally, laboratories normally do not do this specific type of work, nor would they want to interrupt their normal work flow to handle it. Therefore, we often send specially trained personnel into the field to accomplish these dedicated analyses.



An adjustment is made in the operation of a fluorescence spectrophotometer in the mobile laboratory where analysis for contaminants in a spill situation is taking place.



A sample is analyzed with a computerized gas chromatograph.

The atomic absorption instrument in use in the laboratory is provided with an automatic sampling system and a heated graphite atomizer (HGA). The HGA is used instead of the more common, and earlier, flame mode. It permits analysis of metallic components to approximately threefold lower limits (down to ppb's) than the flame mode. It does this by combusting, ashing and vaporizing samples at high temperatures in an electric furnace. Measurements for the purpose of quantification are usually accomplished after using the emission spectroscope to identify the metals that are present.

The fluorescence instrument has an extremely high sensitivity for materials that fluoresce under ultraviolet light. It is valuable for water analyses, and possesses the advantage over gas chromatograph analyses in not needing a solvent, in sample preparation, to improve sensitivity; a sample can be used directly from the source. it also has the ability to measure the presence and levels of fluorescing toxicants, even if they remain unknown. The identity of contaminating materials can then be adduced from corresponding evidence. In some cases, the fluorescence instrument has been able to measure reductions in levels of unknown aromatic toxicants, following water treatment, even before identification of the contaminants was accomplished.

The infrared instrument is used for analysis of petroleum oils that are used

as venicles for toxicants, and for identification, through "fingerprint" spectra, of unknown materials. In some instances, it has even provided identification following transmission of the spectra via a telefacsimile carried in the mobile laboratory.

We anticipate also equipping the mobile laboratory with a mass spectrometer, and communicating information to experts throughout the country by means of the telefacsimile.

In addition to gas chromatography, a method used to separate complex mixtures for the purpose of quantification and identification, the laboratory also engages in total organic carbon analysis (TOC) whereby the total organic content of water is rapidly measured, without extraction.

The EPA has published a list of 271 chemicals considered hazardous to the environment. The emergency response system is concerned with even more than these.

The laboratory is concerned with hazardous chemicals under a wide variety of circumstances. These include gases, volatile components, liquids and solids involved in situations or incidents that pose public health or environmental threats, sometimes beyond spill situations.

Predominantly, the laboratory has dealt with bodies of water that have been contaminated and that threatened drinking water supplies, or aquatic life. Chemicals that are being transported by truck, or rail, and are spilled through accident, also come under the branch's emergency purview, if the spill is sufficiently large to present problems of acute toxicity.

Spills are divided into a number of categories, depending upon what is spilled and where it takes place. These each demand different cleanup treatment.

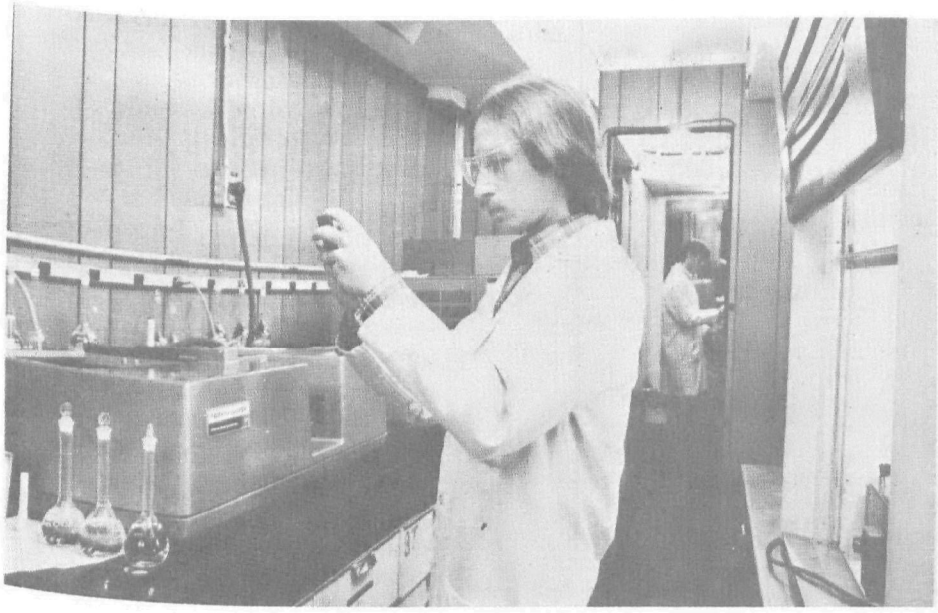
Spills can be grouped into those on soils, into streams, or into large unconfined bodies of water. A small stream, or a pond, is considered to be an impoundable, or damable, body of water in which a spill can be isolated from the remainder of the stream, permitting cleanup to proceed relatively easily. Large water courses (such as the Ohio River, or Raritan Bay, off New Jersey, where there have been hazardous spills), all categorized as unconfinable, require different treatment approaches.

The Edison Branch views its main function as the development of equipment for returning the environment as closely as possible to its pre-spill state. The chemical analysis work supports this primary engineering responsibility, i.e., it provides answers as to how well the equipment works, and how to improve the engineering.

Some successful applications of the emergency of the emergency response unit have been:

- Oswego, New York, where leakage from three lagoons holding waste chemicals of over a million gallons was discovered to be draining into a tributary of Lake Ontario, threatening to pollute that body of water. The EPA mobile treatment unit arrived on April 1, 1977, and, after analyses by the mobile laboratory, started to filter the waste leakage. By

The sample tray of an atomic absorption spectrophometer equipped with an automated sampler and heated graphite atomizer is filled by a chemist in an effort to measure quantities of metallic elements to be found in the spill of a toxic substance.



The sample holder of an infrared spectrophotometer is filled by a chemist prior to spill-site analysis of petroleum oils containing toxicants.

TOXIC COMPOUNDS REMOVED FROM WATER USING THE MOBILE TREATMENT UNIT

	LOCATION OF INCIDENT	QUANTITY OF WATER TREATED — GAL.	INFLUENT CONC, ppb	EFFLUENT CONC, ppb	% REMOVAL
DNBP	Clarksburg, NJ	2,000,000	8	< .002	99.9
PCB	Seattle, WA	600,000	400 ppb	< .075	99.9
		12,000,000	1 ppb	< .075	92.5
Toxaphene	The Plains, VA	250,000	36	1	97.2
Chlordane	Strongstown, PA	100,000	13	.35	97.3
		3,000	1,430	.43	99.9
Heptachlor	Strongstown, PA	100,000	6.1	.06	99.0
		3,000	80	.1	99.9
Aldrin	Strongstown, PA	100,000	8.5	.19	97.8
		3,000	60.5	.15	99.8
Dieldrin	Strongstown, PA	100,000	11	< .01	99.9
		3,000	60.5	< .01	99.9
Kepone	Hopewell, VA	225,000	4,000	< 1	99.9
Pentachorophenol	Haverford, PA	215,000	20,000	< 1	99.9
Methylene Chloride	Oswego, NY	1,000,000	190	51	73.2
Carbon Tetrachloride	Oswego, NY	1,000,000	1.1	< .1	90.9
Benzene	Oswego, NY	1,000,000	1	< .1	90
Toluene	Oswego, NY	1,000,000	120	.3	99.8
Xylenes	Oswego, NY	1,000,000	140	< .1	99.9
Trichloroethane	Oswego, NY	1,000,000	12	< .1	99.2
Trichloroethylene	Oswego, NY	1,000,000	21	.3	98.6
Phenol	Oswego, NY	1,000,000	140	< .1	99.9
Cresol	Oswego, NY	1,000,000	230	8.1	96.5
Dimethylphenol	Oswego, NY	1,000,000	1,220	5.4	99.6
Trimethylphenol	Oswego, NY	1,000,000	130	10	92.3
Butylphenol	Oswego, NY	1,000,000	300	15	95
Dimethylaniline	Oswego, NY	1,000,000	380	23	94.0
PCB	Ditmer, MO	180,000	19	< .1	99.5

November 1977, it had treated over 1 million gallons of waste and contaminated runoff waters. The wastes, consisting of mixed chemicals and oils containing PCB's, had been dumped into the overflowing lagoons by a waste disposal company that had had its incinerating facilities shut down for air and water pollution violations. At the height of operations, during the month of November 1977, from 50,000 to 60,000 gallons of water were processed.

- Haverford, Pennsylvania, where, in the summer of 1976, residents of the town reported to the EPA regional office that a tributary of the Delaware River, Naylors Run, was being polluted by toxic "oil slicks" at a number of places where storm drains and springs emptied into the stream[4]. There was a strong odor along the stream's course, and fish were dying. The source was discovered to be a wood treating operation where a 5% solution of pentachlorophenol (PCP) in fuel oil was used as a preservative. Laboratory analyses by infrared spectrophotometer, and by gas chromatograph, confirmed that PCP was present in large amounts in the Naylors Run oil slicks. Further investigation showed that the wood-preservative plant site was saturated with the chemical and that waste from the operation had been injected into a 25-foot-deep well for some 30 years. Seepage from the well entered an underground culvert, leaked into storm drains and emptied into Naylors Run. This stream passes through Philadelphia suburbs and enters Tinicum National Wildlife Refuge, the last freshwater tidal marsh in Pennsylvania.

PCP, a fungicide and pesticide, is added to fuel oil before being applied to wood as a preservative. It has been linked to many human health problems, including lung, liver and kidney damage. The concentration in the oil was greater than 10,000 parts per million (ppm), and in the stream water, 0.5 ppm. Concentrations of 0.2 ppm are lethal to fish.

Through well drilling and trenching operations for oil-contaminated groundwater collection, skimming of the oil in tanks, and use of the activated carbon columns of the mobile treatment unit, the Oil and Hazardous Materials Spills Branch assisted EPA Region III response personnel to reduce PCP concentrations in the water to below 1 part per billion (ppb). Oil-in-water concentrations were also reduced from 100 ppm to less than 1 ppm. Altogether, 215,000 gallons were processed during a three-month operation. In all stages of the cleanup, infrared spectroscopy and gas chromatography were used to monitor removal efficiency.

- Kernersville, North Carolina, where, in June, 1977, vandals opened the valves on six storage tanks at a chemical wastes disposal company and emptied 30,000 gallons of mixed wastes into the reservoir that supplied drinking water. Vapors from the wastes were so strong that 1,000 people in the area of the spill had to be evacuated. Dead fish were observed in the reservoir and were reported by town officials. It was believed at first that an oil spill had occurred and the water was treated accordingly. When this did not clear up the problems, the

EPA mobile laboratory and a pilot scale water treatment unit were called in. Fish, meanwhile, continued to die, fleeing the chemicals to the mouths of freshwater inlets to the reservoir. Within 72 hours after the spill, the toxic chemicals caught up with the fish, killing about 20,000 of them, some 90% of the aquatic population. At this point, town officials closed down the reservoir.

Because the toxicants were unknown and the materials were grossly mixed, identification could not immediately be made. A mobile bioassay system was brought in to determine the acute toxicity in the influent to, and effluent from the pilot scale water treatment unit. It was discovered that the influent was toxic, but the effluent not, suggesting cleanup of the reservoir system by carbon adsorption methods.

The fluorescence spectrophotometer was very valuable in this situation. By running samples with this instrument, we were able to isolate distinctive profiles that were not present in samples taken before the spill occurred. The reservoir materials yielded a profile for the presence or absence of materials, that worked hand-in-glove with the bioassay toxicity tests, providing excellent correlation for monitoring contamination levels.

The greatest benefit of the use of the fluorescence instrument was this relative indication of toxicant concentrations through correlation with the behavior of bioassay organisms.

Other significant operations in which the mobile treatment unit has engaged[2] include: Kepone contamination at Hopewell, Va., in January 1976; spillage of dinitrobutylphenol (DNBP) into a tributary of the Millstone River, a public water supply for Allentown, N.J., at Clarksburg, N.J., in August 1974; spillage of 265 gallons of PCB from an electrical transformer into the Duwamish Waterway, Seattle, in September 1974; spillage of a pesticide containing chlordane, heptachlor, aldrin and dieldrin, to a concentration of 39 ppb, into Carney Run, at Strongstown, Pa., in July, 1975.

It was initial use of the mobile treatment unit that first pointed to the need for a mobile laboratory, when it was noted that although oil spills are visible enough to locate and isolate, chemical spills are not. The waters or soils have to be analyzed to find out the areas of toxic concentrations. With the critical aid of the laboratory, we are able to deal with an acute public safety hazard, such as chlorine, as well as with hazards of a chronic nature, such as PCB, or low-level pesticide releases on a nationwide 24-hour-response basis. In effect, the mobile laboratory is an extension of our base laboratory at Edison, bringing to the field all the sophisticated technology that is required, but, in more immediate and timely terms.

The attached table lists some of the hazardous chemicals, their amounts, and their removal efficiencies achieved at a number of sites.

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Frank J. Freestone obtained his Bachelor of Mechanical Engineering Degree from Rutgers University in 1967, and his Masters Degree in Ocean Engineering from the University of Miami in 1969. He has been with the Environmental Protection Agency since 1971 and is presently serving as Chief of the Hazardous Materials Spills Staff of the Oil and Hazardous Materials Spills Branch. Mr. Freestone formerly served as a commissioned officer in the U.S. Army, including one year's service in Vietnam.

Ira Wilder obtained his Bachelor of Chemical Engineering Degree in 1954 from the City College of New York. He has been with the Environmental Protection Agency since 1970 and is presently serving as Chief of the Oil and Hazardous Materials Spills Branch of the Industrial Environmental Research Laboratory, Edison, New Jersey. The Branch is responsible for the initiation, planning and management of research and development programs relative to the prevention, control and treatment of Oil and hazardous material spills. Mr. Wilder formerly served with the Naval Applied Science Laboratory where he was responsible for the management of R&D programs relative to liquid fuel fire protection for the Navy.

II. PETROLEUM OILS

A. FLUORESCENCE ANALYSES

Analysis for Crankcase Oil in Water by Fluorescence Spectrophotometry - Potential environmental damage from repeated applications of spent crankcase oil onto unpaved roadways, for dust control, is under investigation at Edison. Our laboratory recently examined a suspected "run-off" water sample, collected in the vicinity of such a road, for traces of crankcase oil. After the usual extraction and concentration steps, analysis was attempted by gas chromatography (GC), using a flame ionization detector. An unresolved chromatogram was obtained, in which the presence of crankcase oil could not be established. This difficulty was attributed to excessive interfering contaminants in the sample extract, that masked any GC profile resulting from traces of crankcase oil. This analysis was successfully performed, however, by using a modified version of the fluorescence spectrophotometric method previously reported by Thruston and Knight (Env. Science and Tech., 5, 64-69, 1971) We excited the sample extract at 290 mμ instead of the recommended 340 mμ, because we had previously established that this change considerably improved differentiation between the spectrum of a known crankcase oil and the spectra of ten other common oils that were examined (crudes and crude oil fractions). The entire sample extract was dissolved in the minimum cyclohexane volume needed to fill a 1 cm rectangular cell. A Perkin Elmer MPF-3 fluorescence spectrophotometer was used for this analysis. The water sample was found to contain traces of crankcase oil. The pollutant was identified through the shape of its emission spectrum, and the wavelength of its maximum emission (329 mμ). It matched in these spectral properties a known crankcase oil, but differed considerably from all the other oils examined. Fluorescence spectrophotometry therefore shows unique promise as a method for characterizing oils in situations that are not amenable to analysis by gas chromatography. (U. Frank)

* See page 2.

PASSIVE TAGGING OF OILS BY FLUORESCENCE SPECTROPHOTOMETRY

Fluorescence spectrophotometry is currently under evaluation as a method for passive tagging of oils. The potential usefulness of this technique was previously reported by Thruston and Knight (Env. Science and Tech., 5, 64-69, 1971), but difficulties were encountered while applying some of their recommendations. Misleading emission spectra were obtained from dilute solutions of crude and processed oils in cyclohexane (0.5 mg/liter) when these were excited at the recommended wavelength of 340 mμ. All the resulting spectra were essentially identical, consisting of a pronounced maximum at 380 mμ surrounded by a variable background envelope. These spectra were more fully resolved by examining several solutions containing different oil concentrations, at various excitation wavelengths in the range 290 mμ - 400 mμ. Two distinct emission spectra resulted; the maximum that was previously noted at 380 mμ, was due to the cyclohexane solvent Raman C-H stretch band, while the background envelope was due to oil. These conclusions were confirmed by separately exciting at 340 mμ pure cyclohexane and the oils as tnin solvent-free films. A decrease in dissolved oil concentration increased interference by the Raman band. Several other solvents were also excited at 340 mμ and yielded interferring Raman bands: hexane, pentane and isooctane were tested. In this study 290 mμ was selected as the excitation wavelength of choice. It yielded improved spectral separation, with the emission maxima of the oils occurring between 330 mμ - 360 mμ and the Raman band of cyclohexane occurring at 320 mμ. *(U. Frank, 201-548-3347)*

* See page 2.

PASSIVE TAGGING OILS BY FLUORESCENCE SPECTROMETRY

A question has been raised by several investigators about the stability of fluorescing components in oils exposed to environmental conditions, especially sunlight. A preliminary evaluation of the reliability of fluorescence spectra for passive tagging oils was carried out by performing a limited weathering study. Six oils (No. 2, No. 4 and No. 6 Fuel Oils; and South Louisiana, W. Texas Sour, and Bachaquero Crude oils) were placed as surface slicks on ocean water, contained in wide mouth jars, and exposed on a building roof for 30 days. Similarly, four other oils (one light Arabian and four Persian Gulf Crude oils) were subjected to outdoor weathering for 50 to 300 hours in 500 gallon tanks provided with constantly circulating salt water drawn from Casco Bay, Maine. After the indicated weathering, the oils were recovered from the water surface and solutions were prepared having concentrations of 1 and 10 mg/l oil in solvent, using 99 mol % pure cyclohexane. Solutions of the corresponding unweathered oils were prepared in a similar manner. Solvent selection was in accordance with AQC Newsletter #18, "Solvent Impurities and Fluorescence Spectrometry". Analyses were performed by exciting the solutions at 290, 320, 340 and 360 nm and scanning each emission spectrum in the range of 220 to 600 nm. The resulting spectral envelopes, which are used for passive tagging oils, were found to retain their characteristic shapes despite extensive weathering, although their intensities were slightly reduced.

(U. Frank, FTS 201-548-3510, Coml. 202-548-3347)

* See page 2.

AN IMPROVED SOLVENT FOR FLUORESCENCE ANALYSES OF OILS

Increased resolution in the fluorescence spectra of oils is obtained by using dipropylene glycol instead of cyclohexane, as the solvent matrix. The effect of this solvent on the fluorescence spectra of five oils (No. 2 and No. 6 Fuel Oils, South Louisiana, W. Texas Sour and Bachaquero Crude Oils) was investigated. Because of some solubility problems, solutions were initially prepared in cyclohexane, from which 1 ml aliquots were diluted to 25 ml with dipropylene glycol. The final concentrations were 10 mg oil per liter of solvent. Similar solutions in cyclohexane, were also prepared. Emission spectra were obtained in the range 220-600 nm, by exciting at 290 nm. The spectral envelopes of the oils in dipropylene glycol showed distinct and sharp bands, while the envelopes of the oils in cyclohexane displayed only slight deflections as shoulders. The price of dipropylene glycol offers an additional advantage. While cyclohexane that is suitable for fluorescence analysis costs $47.00 per gallon (3kg), an equal quantity of dipropylene glycol costs about $7.25. (U. Frank, FTS 340-6626, Coml. 201-340-6626).

RECLAMING A WASTE SOLVENT

Due to current restrictions on the availability of cyclohexane, which is a required solvent for most of our fluorometric analyses of oils, it was necessary to reclaim the waste solvent. Because of time constraints only a minimum effort could be expended, and therefore a simple, one step distillation procedure was tested. An all glass apparatus was used, consisting of a 2 liter pot in a heating mantle, a reflux condenser, and a collection flask. Cyclohexane (99 Mol%Pure) that was contaminated with up to 5% oil was distilled at a rate of 2 liters per hour. The reclaimed product regained its initial purity to fluorescence analysis. (U. Frank, FTS 340-6626, Coml. 201-321-6626)

* See page 2.

Effect of Fluorescence Quenching on Oil Identification

Questions have been raised by several investigators about the effects of quenching on fluorescence spectra when used for passively tagging oils. Quenching as defined for the fluorescence analysis of oil, occurs at high concentrations and is characterized by supression of the oil's emission radiation. The exact concentrations at which quenching becomes apparent was determined for four oils. Solutions having known concentrations of the oils in 99 Mol % pure cyclohexane were prepared. Emission spectra were obtained in the range 220-600 nm, by exciting at 290 and 340 nm. Graphs were then constructed by plotting fluorescence intensities versus concentrations. The points at which the plots began to deviate from linearity marked the "onset of quenching." Those points corresponded to the following concentrations:

OIL	CONCENTRATION OF "ONSET OF QUENCHING" mg/l	
	Ex 290 nm	Ex 340 nm
#2 Fuel Oil	30	No Emission
#6 Fuel Oil	6	7
Bachaquero Crude	6	18
Iran - Gach Crude	16	25

By inspection of the spectral envelopes it was found that above the onset of quenching the shapes of the envelopes varied with concentration, while below the onset (region of linarity) the shapes remained constant. In addition, at concentrations above 100 mg/l the envelopes were sometimes distorted beyond recognition. It is therefore vital to prepare oil solutions with concentrations below the onset of quenching when passively tagging oils by the fluorescence technique. (U. Frank, FTS 201-548-3510, Coml. 201-548-3347)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #24, January 1975.

Identification of Petroleum Oils by Fluorescence Spectroscopy

A simple and rapid method for fingerprinting petroleum oils by taking advantage of the three dimensional character of their fluorescence spectra has been developed. Our approach involves excitation of the oils at 15 wavelengths, in the range of 220-500 nanometers (nm), at 20 nm intervals. The emission monocromator is rapidly scanned at each excitation wavelength to obtain the emission maximum. These maxima are then plotted versus the excitation wavelengths to derive "silhouette profiles", which are used to fingerprint the oils. We tested our method by matching weathered with unweathered portions of nine petroleum oils, and discriminating among them. Each oil yielded a unique and different profile that remained substantially unchanged despite weathering. (U. Frank, FTS 340-6626, Coml. 201-321-6626)

* See page 2.

Synchronous Excitation Fluorescence Spectroscopy

A preliminary evaluation of the utility of synchronous excitation fluorescence spectroscopy for the quantitative determination of petroleum oil and phenol has demonstrated that by the use of this technique Rayleigh-Tyndall and Raman scatter interferences are totally eliminated. These scatter interferences, which are usually encountered in emission spectra obtained by conventional constant wavelength excitation techniques, were previously described in AQC Newsletter No. 13. Since the scatter interferences are avoided by synchronous excitation, this technique offers two advantages over constant wavelength excitation: (1) shorter analysis time because solvent blank determinations and corrections are not necessary, and (2) increased sensitivity because spectral interferences are avoided. In our study we compared synchronous excitation spectra to emission spectra of oil and phenol solutions, and of appropriate solvent blanks. Synchronous excitation spectra were obtained by simultaneously scanning the excitation and emission monochromators with the emission monochromator leading by a 20 nm interval. Emission spectra were obtained at 290 nm for oil and 270 nm for phenol. (U. Frank, FTS 342-7510, Coml 201-548-3347/L. Pernell, FTS 342-7517, Coml 201-548-3347)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER # 32, January 1977.

Synchronous Excitation Fluorescence Spectroscopy

Use of synchronous excitation fluorescence spectroscopy for the quantification of petroleum oils and phenol was previously described in AQC Newsletter No. 31, October 1976. Use of this technique has now been extended to aniline, benzene, benzolic acid, cresol, ethyl benzene, naphthalene, phenol, toluene, and xylene. These materials are part of more than 300 toxicants that may be designated "hazardous" by anticipated legislation (40 CFR Part 116). Detection limits obtained by the synchronous excitation technique varied between 0.5 mcg/l (ppb) - 0.1 mg/l (ppm), using 100 ml cyclohexane for extracting 1 liter water. A preliminary evaluation of interference by commonly present materials in brackish lake and marsh waters, was also performed. No interference was found. (U. Frank, FTS 340-6626, Coml. 201-321-6626, M. Gruenfeld, FTS 340-6625, Coml. 201-321-6625).

* See page 2.

Synchronous Excitation Fluorescence Spectroscopy

Synchronous excitation fluorescence spectroscopy (AQC Newsletters 31 and 32) was used for the measurement of residual aromatics in water. In-situ analyses of benzene, methyl substituted benzenes, and dimethyl aniline in water samples from an industrial waste holding pond were performed in order to monitor the effectiveness of a mobile carbon column treatment unit in an emergency response situation (AQC Newsletter No. 29). One of the two peaks in the fluorescence spectrum was attributed to benzene and methyl substituted benzenes. The other peak was attributed to dimethyl aniline. These characterizations were confirmed with standard compounds, and by GC/MS. (U. Frank, FTS 340-6626, Coml. 201-321-6626/J. Munn, FTS 340-6628, Coml. 201-321-6628)

Adsorption of Fluorescing Oil Components Onto Silica Gel

Silica gel and alumina adsorption techniques that are used for the separation of hydrocarbons from other organics, prior to petroleum oil quantitation by IR (AQC Newsletter No. 26), should not be used prior to fluorescence measurements. Major fractions of fluorescing oil components adsorb onto silica gel, causing substantial spectral distortions and losses of emission intensities. (U. Frank, FTS 340-6626, Coml. 201-321-6626)

Substitute Deozonator for Fluorescence Spectrometers

Following breakdown of the deozonator of our fluorescence spectrometer, a hot air blower (2,000 watt, 400°C-540°C) was modified to serve as a substitute. The air blower was connected to the outlet hose of the fluorescence instrument's lamp housing, using a cardboard adaptor. This arrangement was used satisfactorily for several days, without a preceptable odor of ozone. We believe that a simple hot air blower (approximate cost $65.00) is adequate for the commercial deozonator (approximate cost $500.00). (R. Frederick, FTS 340-6627, Coml. 201-321-6627)

* See page 2.

Oil Quantitation by Fluorescence Spectroscopy

Hydrocarbon solvent contamination of sample jars precludes the quantitation of petroleum oils by infrared spectroscopy. Recently, jars that were rinsed with n-hexane prior to water sample collection were forwarded to our laboratory. Residual hexane in the water prevented use of our usual IR procedure, but the analyses were performed successfully by the synchronous excitation fluorescence method that was outlined in AQC Newsletter No. 31, October 1976. (U. Frank, Coml. 201-321-6626, FTS 340-6626/ J. Susman, Coml. 201-321-6628, FTS 340-6628)

* See page 2.

(*) EPA QUALITY ASSURANCE NEWSLETTER, Volume 1 No.2, July 1978.

Rapid Quantification of Petroleum Oils in Sediments - Wet sediment extraction combined with Synchronous Excitation (SE) fluorescence spectroscopy are currently under evaluation as a rapid method for estimating petroleum oil concentrations in sediments. This method circumvents time comsuming procedures, such as soxhlet extraction, sediment drying and saponification steps. Wet sediments are simply extracted with a solvent mixture consisting of 60 percent isopropanol and 40 percent cyclohexane. The method was tested with several estuarine sediments spiked with No. 2 and No. 6 fuel oils, and Kuwait and South Louisiana Crude oils, at concentrations of approximately 10 mg/Kg oil in wet sediment. Five grams of the spiked sediments were placed into 50 ml, glass-stoppered graduated cylinders and diluted to 50 ml with the solvent mixture. The cylinders were then agitated for 15 minutes using a mechanical rotary mixer and 10 minutes were allowed for settling. Analysis of the supernatants was performed by the SE fluorescence technique as described in EPA's Quality Assurance Newsletter, Vol. 1, No. 1, April 1978, using 25 nm wavelength intervals. Recoveries obtained were in the range of 100-106 percent. Use of this procedure for the quantification of weathered oils in sediments is now under evaluation. (U. Frank, Coml. 201-321-6626, FTS 340-6626)

* See page 2.

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IDENTIFICATION OF PETROLEUM OILS BY FLUORESCENCE SPECTROSCOPY

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ABSTRACT

A simple and rapid method for the identification of weathered petroleum oils (passive tagging) by fluorescence spectroscopy is described. The approach used takes advantage of the three-dimensional character of the oil fluorescence spectra. Oil identification methods of other investigators that use fluorescence spectroscopy are also reviewed within the context of the three-dimensional system. Our method involves excitation of the oils at 15 wavelengths, between 220-500 nanometers (nm), at 20-nm intervals. The emission monochromator is rapidly scanned at each excitation wavelength to obtain an emission spectrum. The maximum emission intensities are then plotted versus the excitation wavelengths to derive silhouette profiles. These are used as fingerprints for passive tagging petroleum oils. The influence of weathering, quenching, and solvent effects on our method are also examined.

INTRODUCTION

The discharge of petroleum products into the marine environment has caused extensive environmental damage in the past. The current increasing transportation and offshore production of petroleum is, therefore, of vital concern to the U.S. Environmental Protection Agency. Effective legislation, with adequate analytical support for enforcement, should reduce this damage, and methodology for the identification of the source of discharged oil is needed.

Fluorescence spectroscopy is a rapid and promising tool for the source identification of weathered oil (passive tagging). While in the past few years many laboratories have used this tool for passive tagging both crude and refined petroleum products, few innovations in the technique have come about. Although three parameters are inherent to the fluorescence technique, only two of them have been commonly used. This can be explained more clearly within the context of a three-dimensional system. Because fluorescence spectroscopy entails three parameters (excitation wavelength, emission wavelength, and fluorescence intensity), the total spectrum of an oil can be presented as a topographical map of a mountainous region. Figure 1 depicts the total fluorescence spectrum of an oil with three fluorescence maxima; the excitation wavelength, emission wavelength, and fluorescence intensity represent the *x*, *y*, and *z* axes, respectively.

Most publications on the identification of oils by fluorescence spectroscopy do not mention the three-dimensional character of oil fluorescence spectra. Their authors limit themselves by using only two of the three parameters. Within the context of the three-dimensional system, the approach of these investigators can be described as taking "cuts" through figure 1 in planes that are parallel to the *y* axis and at specific points along the *x* axis. Figure 2 illustrates this; each cut is an emission spectrum at one excitation wavelength.

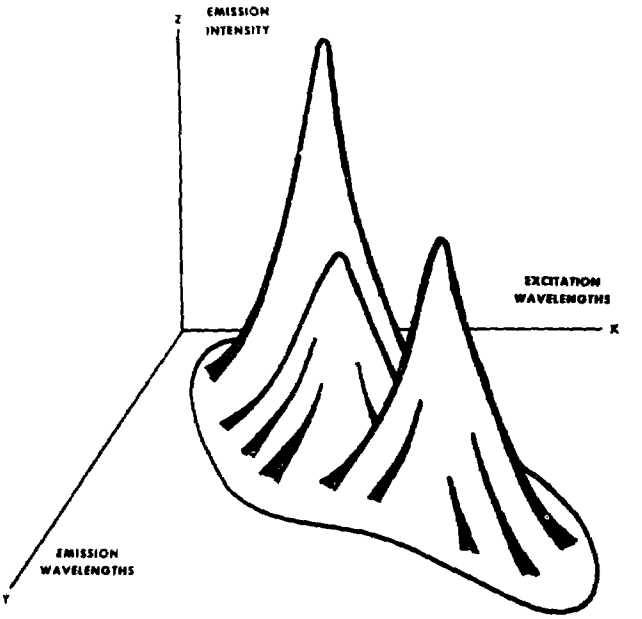

Figure 1. Three-dimensional presentation of the total fluorescence spectrum of an oil

Thus, Thruston and Knight [1] utilize 340 nanometers (nm) as the excitation wavelength for oil solutions in cyclohexane at three concentration levels. They then ratio the 386-nm and 440-nm emission maxima to one another. A similar approach is taken by Coakley [2] who excites each oil at a discrete wavelength. He selects for each oil the excitation wavelength that yields the maximum emission for that oil. His wavelengths are usually in the range 290-320 nm. Jadamec [3], in a more recent method, uses 254 nm as the excitation wavelength. He generates emission spectra for passive tagging 8 oil spill samples.

All of these methods utilize the same basic approach. The oils are excited at specific wavelengths and their emission spectra are obtained. The methods differ only in their wavelength of excitation.

Other fluorescence methods are available or have been proposed for passive tagging oils, but their utility for this purpose has not been established. Lloyd [4,5] describes a method whereby cross-sectional cuts are taken at 45° angular planes to the *x* and *y* axes. This is accomplished by simultaneously scanning the excitation and emission monochromators of the fluorescence instrument. Another method by Freegarde et al. [6] proposes the analysis of oils by constructing contour maps. While this approach somewhat simulates a three-dimensional system, it is extremely time consuming and requires computer services for data manipulation.

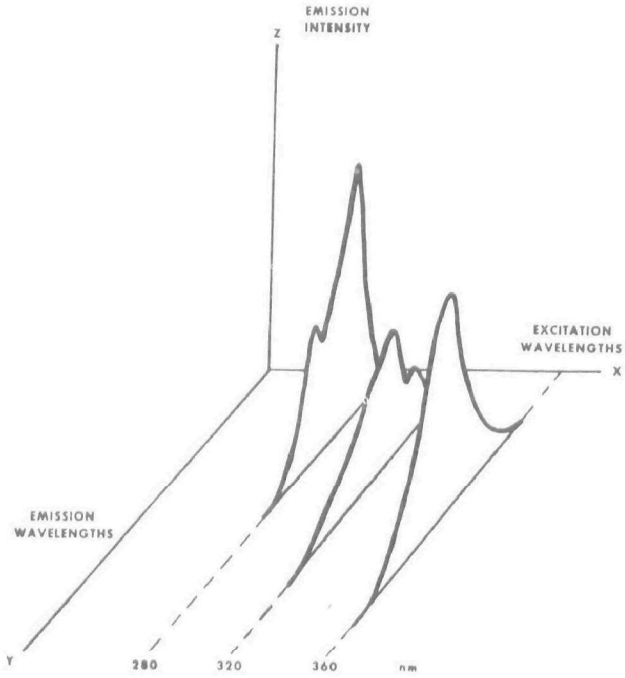

Figure 2. Three-dimensional presentation of the emission spectra of the oil in figure 1 excited at three wavelengths

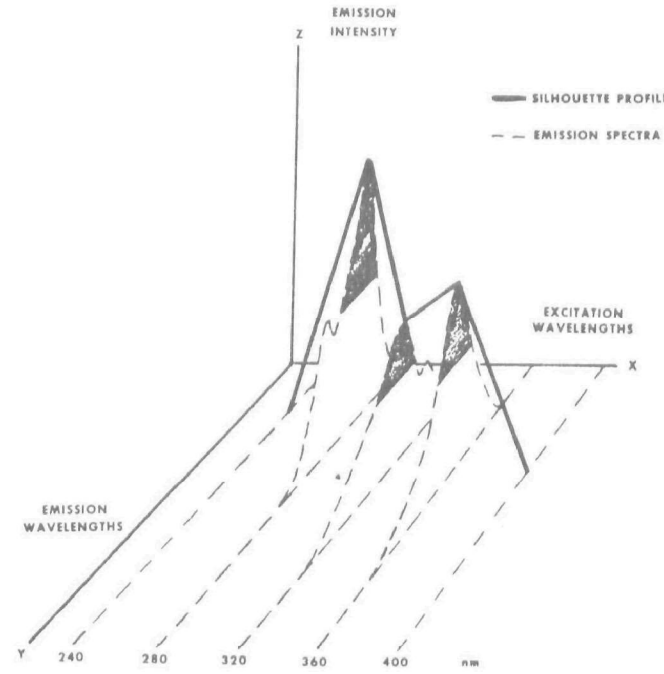

Figure 3. Three-dimensional presentation of the derivation of the silhouette profile from the oil in figure 1

Our method circumvents these limitations; sample analysis and data handling are rapid and commensurate with the other commonly used fluorescence techniques [1,2,3,]. We use all three fluorescence parameters, and thereby exploit the advantages that derive from the three-dimensional system that is illustrated in figure 1. We obtain in our method a silhouette profile of an oil's total three-dimensional spectrum as it is observed in a plane that parallels the x axis. Figure 3 illustrates how a simplified silhouette profile of an oil is obtained from the three-dimensional spectrum that is illustrated in figure 1. In actual practice, we excite each oil at 20-nm wavelength intervals between 220 nm and 500 nm. The emission monochromator is rapidly scanned at each excitation wavelength, and the maximum emission intensity of each scan is recorded. These maximum intensities are then plotted manually versus the excitation wavelengths, and the silhouette profiles of the oils are obtained by connecting the points with straight lines.

We evaluated the ability of the method to identify weathered oils, i.e., to correlate weathered oils with unweathered portions of the same oils (passive tagging). Pertinent environmental factors such as photodecomposition, evaporation, dissolution, and biodegradation were considered separately and in combination. Quenching and solvent effects that may influence the accuracy of our method were also examined.

Weathering effects

Previous investigators indicate that weathering may drastically degrade petroleum oils. Thruston and Knight [1], Coakley [2], and Freegarde et al. [6] surmised that significant changes in the intensity and shape of the emission spectrum can occur when oils are exposed to sunlight. Two studies were therefore conducted in order to establish our method's ability to cope with weathering effects. The first study dealt primarily with photodecomposition effects, and the second study examined the combined effects of water, radiation, heat, and bacteria.

Quenching effects

Thruston and Knight [1] found that the intensity and shape of oil fluorescence spectra substantially depend on the concentrations of the solutions that are measured. Fluorescence quenching is a phenomenon that occurs at high solution concentrations and is characterized by the formation of excimers. These are combinations of excited molecules. Excimer formation and their effect on fluorescence spectra was first noted by Forster and Kasper [7], who demonstrated that while a polynuclear aromatic compound (PNA) yielded a fluorescence emission maximum at 390 nm, a hundredfold increase in the concentration of this compound shifted its fluorescence maximum to 480 nm. Other PNAs, exhibit similar behavior. Because other investigators [8,9] have demonstrated that petroleum oil fluorescence is primarily attributable to PNAs, we maintain that the distortion of high concentration oil fluorescence spectra is due to excimer formation.

Excimer formation is considered important to our method because its effects can combine with or can be confused with the effects of weathering. Also, losses of volatiles by spilled oils invariably result in increased concentrations of PNAs in the weathered residues; the PNAs are higher boiling materials that do not readily volatilize. As a consequence, excimer formation can become quite prominent and can cause ambiguities during passive tagging analyses. Therefore, the oil solution concentrations at which substantial quenching appears must be known before our method can be successfully used.

Solvent effects

Cyclohexane, our solvent of choice, presents two problems: (1) interference by Raman scatter, and (2) interfering emissions by solvent impurities. Raman scatter is caused by the interaction of excitation radiation with the bonds of the cyclohexane molecules. It reveals itself as an emission band that appears 0.3 microns^{-1} from the Rayleigh band in cyclohexane [10]. Also, impurities in cyclohexane yield spectra that can coincide with those of oils. The effects of these interferences on our method are also examined in this paper.

Experimental

Apparatus. A Perkin-Elmer model MPF-3 Fluorescence Spectrophotometer,[1] with a constant temperature cell bath (P.E. Part No. 220-1419) was used. The cell (10 mm pathlength, quartz; P.E. Part No. 990-2711) was maintained at 20° ± 0.5°C.

[1] Mention of trade names or commercial products does not constitute endorsement by the U.S. Government.

Procedure. The silhouette profile of a typical oil was obtained in the following manner:

A stock solution with an accurately known concentration approximating 1000 mg/l oil in cyclohexane was prepared. An aliquot of this solution was then diluted to obtain a final concentration of 5 mg/l. This was measured in a cell which was placed into the constant temperature cell holder for a five-minute equilibration period. The analysis was performed at a constant temperature of 20°C.

In order to prevent the recorder pen from going off scale during a run, the excitation wavelength that yielded the maximum emission resonse was determined. This was performed by manually scanning the excitation and emission monochromators until a maximum pen deflection was obtained. Next, the instrument settings were adjusted to yield ca. 90% recorder scale deflection at this excitation wavelength.

The oil was then excited at 15 wavelengths, 20 nm apart, in the range 220 to 500 nm (i.e. 220, 240, 260, ···, 480, 500 nm), to obtain the maximum emission at each wavelength. This was accomplished by setting the excitation monochromator at each excitation wavelength and rapidly scanning the emission monochromator over the entire fluorescence spectral region of the oil. Fifteen compressed emission spectra were thereby obtained. A cyclohexane "blank" was similarly analyzed by exciting at the 15 wavelengths to determine its contributions to the oil spectra. None were found at the instrument settings used.

The silhouette profiles were prepared by manually plotting the maximum emission intensity of each spectrum versus its excitation wavelength and then connecting these points with straight lines. Two criteria were used to compare one profile with another: (1) shape, and (2) the excitation wavelength of the maximum emission intensity.

Weathering effects. Photodecomposition effects were examined by placing four oils (No. 2 and No. 6 fuel oil; South Louisiana and Bachaquero crude oil) as surface slicks in wide-mouth, quart-size jars, containing ocean water. The jars were exposed to the elements on a building roof during the entire month of August.

The other environmental effects were examined by subjecting five other crude oils to weathering conditions which simulate typical oil spill incidents. The weathering was performed by the Department of Environmental Protection, State of Maine, at the facilities of the TRIGOM laboratory, South Portland, Maine, as part of a U.S. Environmental Protection Agency (EPA) sponsored grant. The oils were weathered by spilling one pint of oil on salt water in 500-gallon fiberglass tanks. Seawater drawn from Casco Bay, Maine, was constantly circulated through the tanks whose geometry was such that the outlets were located at the bottom and the inlets at the top. All weathering was performed under ambient outdoor conditions, during the summer months, and for periods of 7-14 days. The weathered oils were skimmed from the water surface, dissolved in cyclohexane, and centrifuged in order to separate the oil from particulate matter and residual water. The solutions were then decanted, and the solvent stripped off with the aid of an air stream at room temperature. Silhouette profiles of the weathered and unweathered oils were then prepared and compared.

Quenching effects The concentration at which quenching phenomena became apparent was determined for four representative crude and refined oils. A No. 2 fuel oil (a low-viscosity distillate), a No. 6 fuel oil (a high-viscosity residual), a South Louisiana crude oil (a low-viscosity crude), and a Bachaquero crude oil (a medium-viscosity crude) were examined. Solutions having accurately-known concentrations of these oils in cyclohexane were prepared. Emission spectra in the range 220-600 nm were obtained by exciting at 290 nm and 340 nm, and fluorescence intensity versus concentration plots were prepared. The maximum concentration within the linear region of each plot was designated at the onset of quenching (figure 4).

Solvent effects. The emission spectra of ten oils were examined in order to demonstrate the effects of solvent Raman scatter on the fluorescence spectra of the oils. Solutions having concentrations of 1 mg/l and 10 mg/l oil in cyclohexane were excited at 290 and 340 nm. A cyclohexane blank measurement was obtained concurrently with each oil measurement using the same instrument settings. The emission spectra of two solvents (Fisher Scientific Co., Spectro-analyzed and 99 Mol % Pure cyclohexanes) were also checked for the presence of fluorescing impurities.

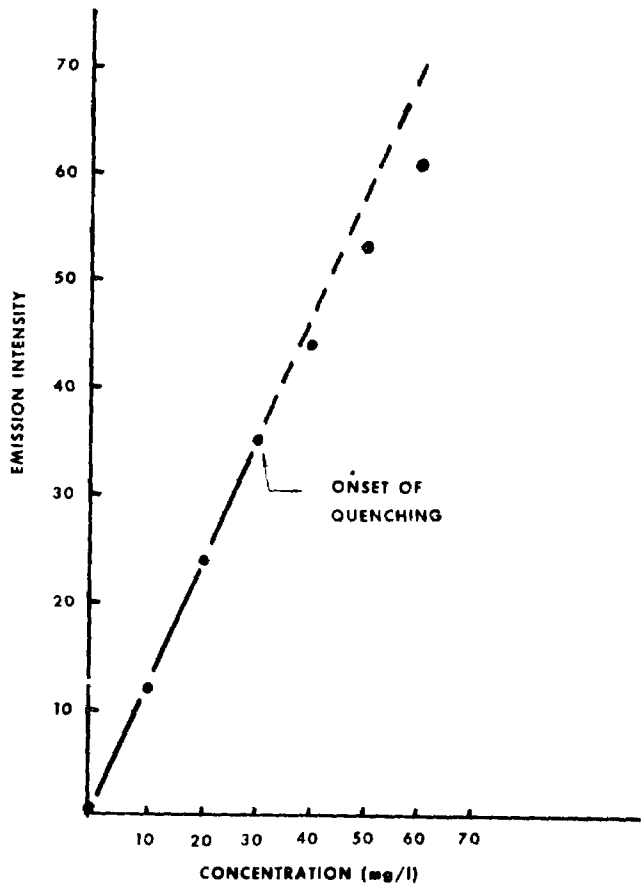

Figure 4. Emission intensity versus concentration plot of No. 2 fuel oil in cyclohexane demonstrating the onset of quenching. The maximum concentration within the linear region is 30 mg/liter

Results and discussion

Weathering effects. The criteria that are used to judge the efficacy of a method for passive tagging oils are: (1) whether the method can correctly correlate weathered and unweathered portions of the same oils, and (2) whether it can distinguish between oils. Our first weathering study, i.e., the study of photodecomposition effects, demonstrates that the profiles of four oils remain relatively unchanged despite weathering (figure 5) and that the method distinguishes between the oils (figure 6). Four discrete silhouette shapes and excitation wavelength maxima were obtained, yet the profiles of the weathered and unweathered portions of each oil were quite similar. We therefore conclude that our method not only discriminates between oils but also that exposure of the oils to sunlight did not adversely affect our method.

The results of our second weathering study, i.e., the study of other environmental effects, also support the contention that our method is adequate for passive tagging oils. The silhouette profiles of the weathered and unweathered portions of five crude oils are shown in figure 7. Each weathered oil is again uniquely matched with its unweathered counterpart.

Quenching effects. The optimum solution concentration for fluorescence measurement was found to be 5 mg/l oil in cyclohexane. Table 1 illustrates our reasons for selecting this concentration by listing the concentrations of the oils at which the onset of quenching occurs (figure 4). Oil solutions that exceeded this

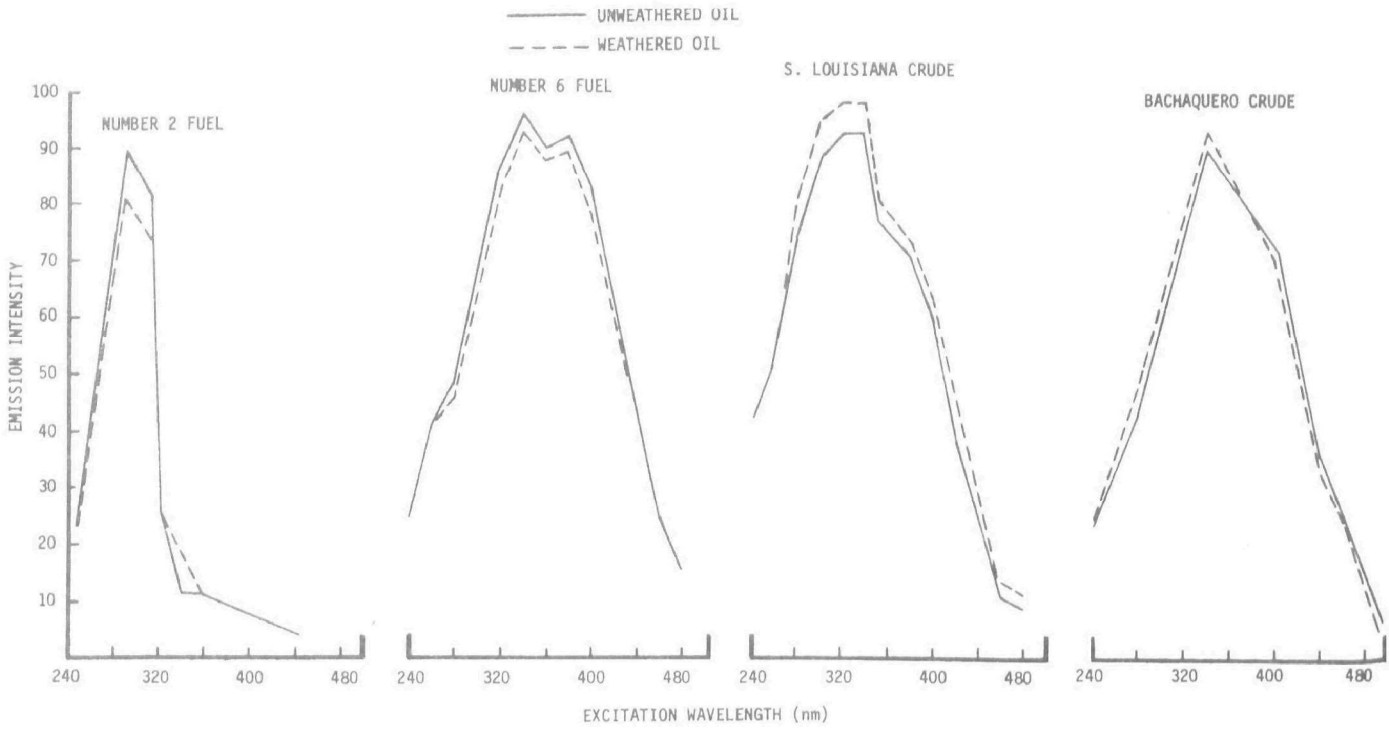

Figure 5. Silhouette profiles of weathered and unweathered portions of four crude and processed oils

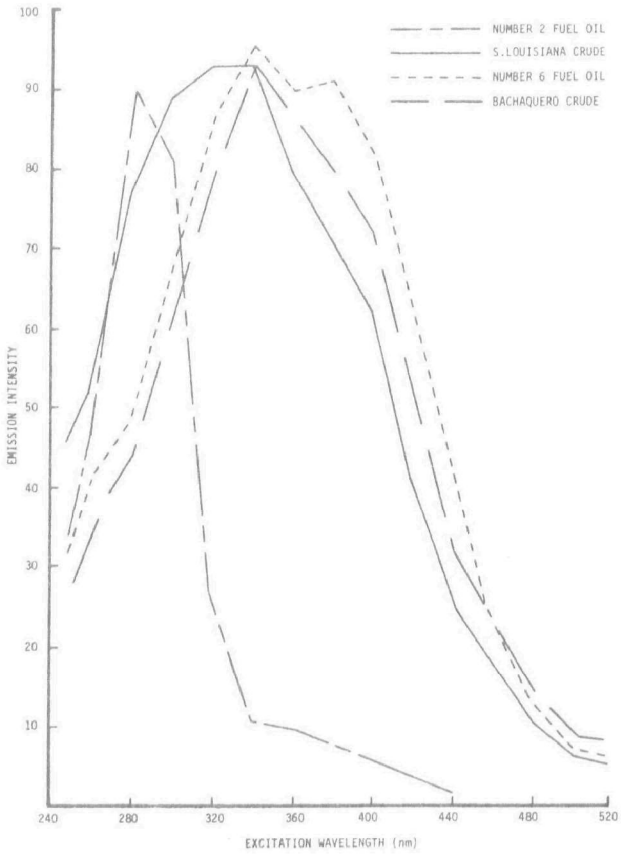

Figure 6. Overlay of the silhouette profiles of the unweathered oils in figure 5

concentration yielded fluorescence spectral envelopes that changed with solution concentration. The spectral envelopes of less concentrated solutions remained constant despite concentration changes. We, therefore, suggest that it is vital to use solution concentrations that are within the linear portion of figure 4 to avoid spectral distortion by quenching effects.

Solvent effects. Raman scatter can be quite prominent in cyclohexane at concentrations below 10 mg/l oil in solvent. Its impact depends on the excitation wavelength that is used and on the fluorescence efficiency (emission intensity) of the oil. Typical Raman band interference with a spectrum of oil is illustrated in figure 8. Two excitation wavelengths are used, and they produce different effects. This figure demonstrates the importance of measuring neat solvent prior to oil analysis. Fortunately, most petroleum oils have such a high fluorescence efficiency that this solvent Raman band overlap does not handicap our ability to achieve oil identification.

The presence of fluorescing impurities in the solvent is a factor that must also be taken into account. Two grades of cyclohexane were examined: Spectroanalyzed and 99 Mol % Pure cyclohexane (Fisher Scientific Company). Impurities in the spectroanalyzed grade solvent yielded substantial interfering fluorescence envelopes, but the 99 Mol % Pure solvent was essentially free of these interferences. We successfully purified the spectroanalyzed cyclohexane, however, by simple distillation [11].

Summary

We have described the three-dimensional character of oil fluorescence spectra and reviewed within this context the pertinent work of previous investigators. We have also presented a straightforward method for identifying the source of discharged petroleum oil, i.e., correlating a weathered oil correctly with an unweathered portion of the same oil (passive tagging). Our method utilizes the three-dimensional aspect of fluorescence spectra, and requires only simple data manipulation. In order to validate our method for passive tagging oils, we tested its ability to match weathered with unweathered portions of nine petroleum oils and to discriminate among them. Each oil yielded a unique and different profile in these tests, and the profiles remained substantially unchanged despite weathering. We also demonstrate that some common phenomena such as fluorescence quenching, Raman scatter, and solvent impurities do not handicap our method.

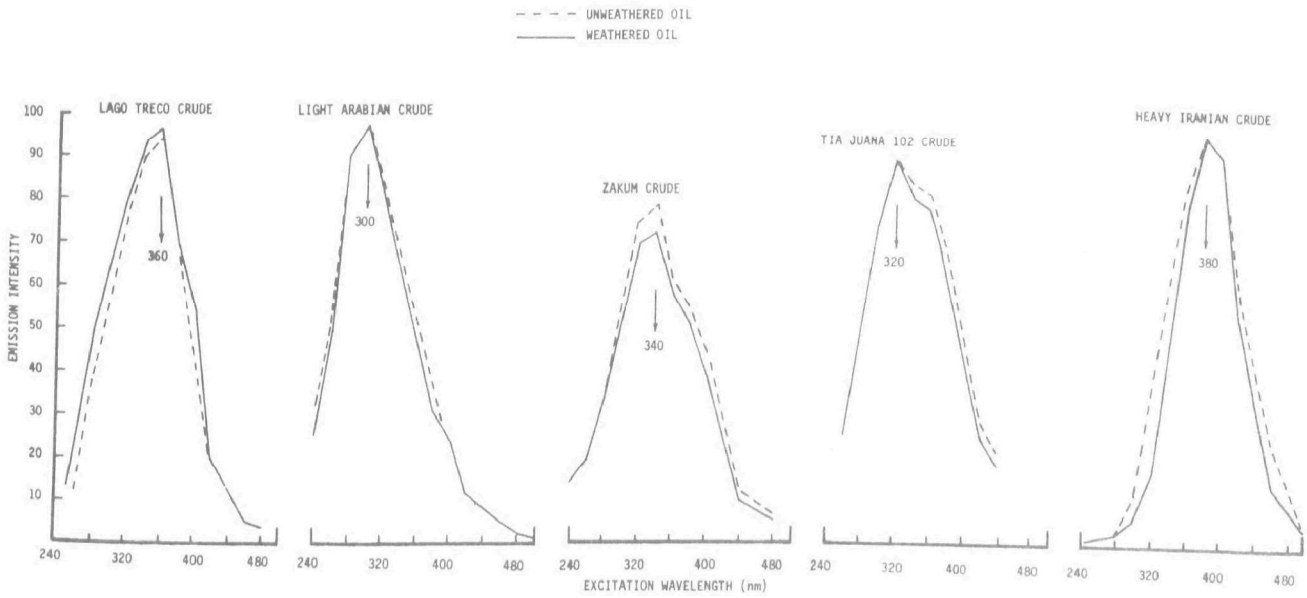

Figure 7. Silhouette profiles of weathered and unweathered protions of five crude oils

Table 1. Concentrations of oil in cyclohexane at which the onset of quenching appears for 4 crude and refined oils

OIL	CONCENTRATION (mg/l)	
	Ex 290 nm	Ex 340 nm
Number 2 Fuel Oil	30	No Emission
Number 6 Fuel Oil	6	7
Bachaquero Crude	6	18
Iran - Gach Crude	16	25

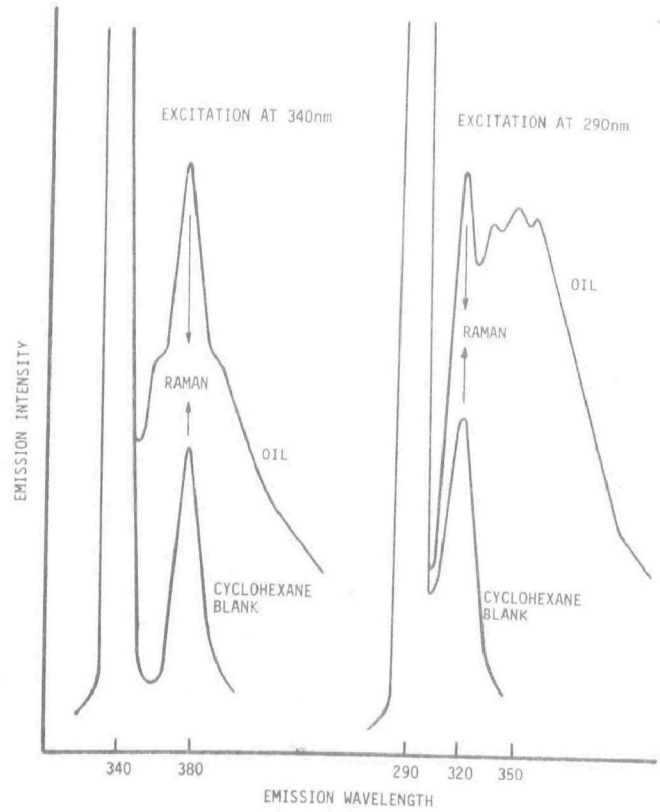

Figure 8. Raman band interference with the emission spectra of a La Rosa crude oil. The concentration 1 mg/l oil in 99 Mol % Pure cyclohexane is used

ACKNOWLEDGMENTS

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NO. 400

DETERMINATION OF PETROLEUM OILS IN SEDIMENTS BY FLUORESCENCE SPECTROSCOPY AND NMR

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The use of several fluorescence methods and one NMR procedure were evaluated for monitoring the presence of petroleum oils in sediments. Analyses were performed on sediments from a mangrove swamp in Puerto Rico that was impacted by a major oil spill in 1973, and on sediments from a proximate area that had no known history of petroleum oil pollution. The latter sediments served as control samples.

The following fluorescence techniques, that are believed to be most commonly used for oil spill source identification, were examined: (a) Single Wavelength Excitation[1,2,3,4]; (b) Synchronous Excitation[5]; and (c) Derived Silhouette Profiles[6]. These techniques are compared and contrasted within the context of a three dimensional system, using the three interdependent variables that are inherent to fluorescence spectroscopy, i.e., excitation wavelength (x), emission wavelength (y), and fluorescence intensity (z). Within this context, the fluorescence characteristics of petroleum oils are presented as "total fluorescence spectra" and the spectral information that is obtained by each technique is discussed as an appropriate portion of such spectra. Figure 1 shows a hypothetical three dimensional total oil spectrum (illustrated as mountains by solid lines), and the spectral information that is available. This figure also highlights the rather limited information used by techniques (a) and (b). Technique (a) measures only the slice of the total spectrum that parallels the y axis; technique (b) measures only the slice of the total spectrum that lies at a 45° angle to the x and y axes. Technique (c) differs, however. This method measures the total spectrum's silhouette profile as projected onto the plane bordered by the x and z axes. Unlike methods (a) and (b), silhouette profiles of method (c) incorporate the essence of three dimensional fluorescence spectra, within a two dimensional format. A comprehensive discussion of method (c) is available in Reference 6. In addition to its greater informational content, this method is rapid and easily used. It was therefore selected for the mangrove sediment analyses.

Use of NMR spectroscopy for confirming the presence of petroleums in the mangrove sediments was also evaluated. The NMR procedure is based on the absorption of aromatic compounds in the 6.5-8.0 ppm spectral region, relative to tetramethylsilane at 0 ppm; substantially elevated levels of aromatics in sediments are thought to indicate the presence of petroleums. These absorptions were observed in the spill impacted sediments but not in the control sediments. Figure 2 illustrates a composite section of the NMR spectra of the contaminated sediment (upper), and the control sediment (lower). The fluorescence and the NMR data agree on the presence of petroleums in the spill impacted sediments.

FIGURE 1

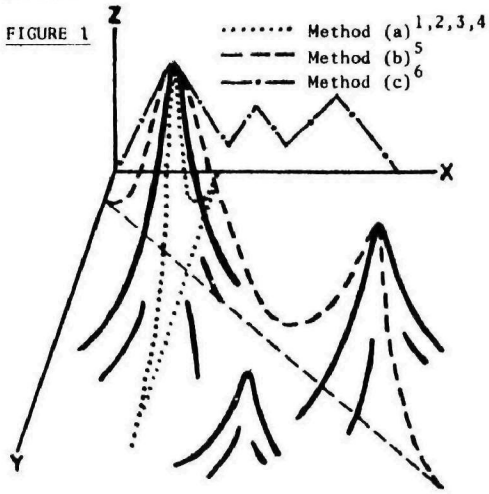
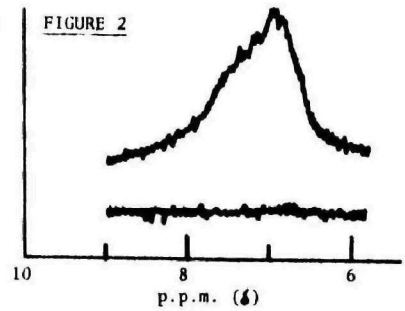


FIGURE 2



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II. PETROLEUM OILS

B. OTHER ANALYSES

Extraction of Oil from Water for Quantitative Analysis by IR - A preliminary study was made to compare the extraction efficiency of two solvents that are commonly used to separate oil from water. This study also examined the changes in extraction efficiency that result from additions of acid and salt. Trichlorotrifluoroethane (Freon 113) and carbon tetrachloride (CCl_4) were the solvents of interest, and both are usable for the quantitative analysis of oils by infrared spectrophotometry. Carbon tetrachloride exposure to laboratory personnel can be extremely hazardous. This material is highly toxic when inhaled (10 ppm TLV), or when absorbed through the skin, and should be handled with gloves in well ventilated laboratory hoods. Freon 113 is much safer (1,000 ppm TLV) and is better, particularly in situations where adequate ventilation may be lacking, such as in mobile laboratory and field use. The solvents were found to be about equally efficient for extracting a broad range of oils from water; i.e., light and heavy, processed and unprocessed oils. Freon 113 is therefore generally recommended as the solvent of choice. Tests were performed with 1 liter emulsions prepared from tap water and No. 2 and No. 6 fuel oils, which are light and heavy processed oils, and South Louisiana and Bachaquero crudes, which are light and moderately heavy crude oils. Addition of acid and salt dramatically improved extraction efficiency. When acid and salt were not present, complete separation of the oils was not achieved even after fifteen 25 ml solvent extractions; however, complete separation was achieved with only four extractions when 5 gms sodium chloride and 5 ml 50% sulfuric acid were added to the 1 liter synthetic samples. Additional salt yielded no further improvement. During the latter determinations, more than 90% of each emulsified oil was separated in the first extract. Quantitative analyses were performed by measuring the oil absorbance band intensity at 2,930 cm^{-1} in the infrared spectral region, using 10 mm and 100 mm path length potassium bromide cells and near infrared silica cells. (M. Gruenfeld)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #15, October 1972.

PREPARATION OF HEAVY OILS FOR INFRARED ANALYSIS

Although Freon is adequate for extracting dispersed oil from water, it is not usable for preparing IR standard solutions of heavy oils. Bachaquero Crude and No. 6 Fuel oils do not readily dissolve in Freon. Satisfactory "simulated" standard solutions were prepared by mixing 2.0 ml carbon tetrachloride containing known amounts of oil with 98 ml of Freon in a 100 ml volumetric flask. The preferred amounts of oil for measurements in 10 mm and 100 mm cells are 1 - 40 mg and 0.2 - 2.5 mg, respectively. The $E^{1\%}_{1cm}$ values (absorbances in 10 mm cells normalized to 1% w/v dissolved oil) of No. 2 Fuel and South Louisiana Crude oils in 98% Freon - 2% carbon tetrachloride are virtually identical to these values in Freon. All the $E^{1\%}_{1cm}$ values of the oils in the solvents are listed below. This parameter may merit inclusion in passive tagging profiles; e.g. Bachaquero Crude oil is clearly distinguishable from the other oils by virtue of its diverging $E^{1\%}_{1cm}$ value.

	Carbon Tetrachloride	Freon 113	2% - 98% Solvent Mix
No. 2 Fuel Oil	23.3	21.5	21.5
South Louisiana Crude Oil	25.3	22.5	23.1
Bachaquero Crude Oil	17.8	--	16.5
No. 6 Fuel Oil	26.2	--	23.7

(M. Gruenfeld, 201-548-3347)

* See page 2.

STORAGE AND TRANSPORT OF OIL CONTAINING SAMPLES IN PLASTIC BOTTLES

HALAR plastic bottles manufactureed by Vanguard Plastics, Inc. (104-118 Wagaraw Road, Hawthorne, New Jersey 07506) were tested for storing and transporting oil containing samples for laboratory analysis. Preliminary findings indicate that these bottles are generally usable for this purpose. Hexane, chloroform, carbon tetrachloride, and Freon 113, used as solvents for quantitative analysis of oil by gravimetric and infrared spectroscopic methods, did not extract any materials from the plastic that interfere with: (a) infrared analysis of oil at concentrations exceeding 2 mg/liter water (slight interference was found at lower levels), or (b) gravimetric analysis of oil. Exposing the plastic bottles to methanol and acetone yielded similar results. The HALAR bottles prevented volatile oil components from escaping and thereby assured sample integrity as effectively as glass stoppered Pyrex bottles. They also cleansed free of oil, for reuse, as easily as Pyrex containers. A slight "memory" effect for polar solvents was noted, however. Traces of methanol and acetone were not as readily removed as from Pyrex. Following exposure to polar solvents the plastic bottles should be carefully cleansed before reuse. These findings are based on the very limited number of plastic bottles that were available for testing and that were repeatedly exposed to the solvents. The reported findings and conclusions should therefore be considered preliminary. *(B. F. Dudenbostel/M. Gruenfeld, 201-548-3347)*

QUANTITATIVE ANALYSIS OF OIL BY IR

A preliminary evaluation of infrared spectrophotometry for quantitating petroleum oils has been completed. No. 2 and No. 6 fuel oils and South Louisiana and Bachaquero Crude oils were examined. Solutions having known concentrations of these light and heavy, processed and unprocessed oils in carbon tetrachloride, Freon 113, and mixtures of these solvents were prepared. Trichlorotrifluoroethane and Freon-TF are synonymous with Freon-113 which is obtainable from E. I. DuPont de Nemours, Co. Oil absorbances were measured at 2930 cm^{-1} in 10 mm path lengths silica cells at ordinate expansions 1 and 5. Absorbance versus concentration plots derived from the measurements were linear and passed through the origin (Lambert-Beer Law). Concentrations of 0.2 - 2.5 mg/100 ml and 1 - 40 mg/100 ml were preferred for measurements at ordinate expansion 1 in 100 mm and 10 mm cells, respectively. The detection limit of these oils by IR at ordinate expansion 5 using 100 mm cells was estimated to be 0.05 mg/100 ml. This represents 0.05 mg/liter oil in water when using the extraction method described in the Analytical Quality Control Laboratory Newsletter #12, January 1972. A final volume of 100 ml was selected to facilitate direct correlation with the previous method.

(M. Gruenfeld, 201-548-3347)

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #16, January 1973.

A TLC Method to Facilitate the Quantitation of Oil by IR

Approximately 200 water samples containing dispersed JP-4 jet fuel were recently analyzed by the IR method outlined in AQC Newsletter Number 15. The initial sample extracts differed widely in petroleum content, and often required repeated dilutions for measurement. Estimation of these dilutions by trial and error IR measurements, proved to be very time consuming and cumbersome. Therefore, a thin layer chromatographic method was developed to reduce analysis time and simplify sample extract handling. TLC plates coated with cellulose (Chromagram Sheet 6064, Eastman Kodak Company) were simply spotted (optimum spot diameters ca. 5 mm) with equal volumes of the sample extracts. Similarly, a set of standards with known concentrations of JP-4 was spotted onto the same plate. Since no chromatographic development was needed the entire plate surface was utilized, thereby accommodating up to 80 samples. By comparing the fluorescence intensity of the sample spots with the standard spots, it was possible to estimate needed dilutions for IR measurements. A UV box containing long and short wavelength light sources was used (Chromato-Vue, Ultra Violet Products, Inc., San Gabriel, California). Spot fading due to evaporation did not occur for several hours, even though JP-4 jet fuel is quite volatile. The same procedure was also attempted with a number 2 fuel oil and proved successful. It is therefore thought that this TLC method can also be readily extended to yield semi-quantitative analyses of water dispersed oils which would be simpler and less costly than instrumental methods such as IR, but less accurate.

(U. Frank, 201-548-3347)

* See page 2.

Ultrasonification for Preparing Stable Oil in Water Dispersions

The use of ultrasonification to prepare stable concentrated oil in water dispersions is currently being investigated. Resulting stock solutions can be readily diluted further to yield any desired concentration of oil in water, and these dispersions are useful for evaluating analytical methods, environmental cleanup procedures, and for performing bioassay evaluations of oil toxicity. Most previous methods for preparing oil in water dispersions involved addition of known quantities of oil to water, followed by vigorous agitation. Such procedures are considered unsatisfactory because rapid oil separation can occur, yielding a system that no longer represents a dispersion. Ultrasonification can yield stable dispersions and is therefore more satisfactory. In an effort to determine whether ultrasonification causes changes in oil that interfere with its analysis, a low viscosity South Louisiana crude oil (4.8 centistokes at 100°F) was examined by gas chromatography, and by infrared, ultraviolet, and fluorescence spectrophotometry. These measurements were made after ultrasonification at 23°C - 36°C in tap water, solvent extraction, and recovery after solvent stripping. Comparison was made to the chromatogram and spectra of a portion of this oil not exposed to ultrasonification, but otherwise subjected to the same treatment. No significant spectral or chromatographic changes were evident. Stability of oil in water dispersions, appears to diminsh with increasing oil viscosity. The South Louisiana crude oil yielded 1% dispersions that remained stable for more than four days. But a more viscous Bachaquero crude oil (1,070 centistokes at 100°F) yielded less stable dispersions. A model W185 Sonifier Cell Disruptor, sold by Heat Systems-Ultrasonics, Inc., Plainview, L.I., N.Y., was used.

(M. Gruenfeld/F. Behm, 201-548-3347)

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #17, April 1973.

Plastic Bottles for Storing and Transporting Oil Containing Samples

In AQC Newsletter No. 15, HALAR plastic bottles were evaluated for storing and transporting oil containing samples. Vanguard Plastics, Inc., was cited as the source of supply. We have since been informed that Vanguard only manufactured a small number of these bottles for research purposes. They do not now plan to market HALAR bottles unless orders are placed for large, single-size lots. Another company, the Nalge Company (75 Panorama Creek Drive, Rochester, New York 14602), may retail HALAR bottles if the market warrants. Information from Nalge can be obtained by contacting Mr. Stephen Hooper *(716-586-8800)*.

(B. F. Dudenbostel/M. Gruenfeld, 201-548-3347)

* See page 2.

USE OF GAS CHROMATOGRAPHIC PEAK HEIGHT RATIOS FOR PASSIVE TAGGING OF PETROLEUM OILS

A novel GC data handling system is under investigation as a simple method to utilize both the quantitative and qualitative aspects of gas chromatograms for passive tagging of petroleum oils, both weathered and unweathered. Samples are compared by using only the "heart fraction" of the gas chromatograms, eliminating volatile components and heavy-end components from consideration to reduce the possible effect of environmental change, such as weathering. A well-resolved GC peak, present in all samples under consideration, is chosen as the reference and the other peak heights in the "heart fraction" are ratioed to this reference peak height. By comparison of the data obtained in this fashion on all samples under consideration, it appears possible to "match" an environmental sample with possible source mathematically to those obtained from the environmental sample and the closest match determined. This approach has been shown to give almost identical results on an authentic No. 2 fuel oil and a sample of the same oil that had been naturally weathered in the Sandy Hook area for one month. The method was also successful in identifying the source gasoline sample (from among several possibilities) in two contamination cases handled for the New Jersey State Department of Environmental Protection. *(B.F. Dudenbostel, FTS 201-548-3419, Coml. 201-548-3347)*

STORAGE AND TRANSPORT OF OILS IN SOLVENTS FOR QUANTITATIVE ANALYSIS BY IR

Dispersed oil in water samples is susceptible to evaporative and microbial degradation losses. Prolonged transport and storage is therefore not recommended. Prompt solvent extraction, and subsequent transport and storage in the extraction solvent is a promising alternate approach. A preliminary evaluation of the stability of oils in some common solvents was therefore performed. A No. 2 fuel oil and a South Louisiana Crude Oil, which are non-viscous, moderately volatile oils, were used. Ten mg/100 ml solutions were prepared in carbon tetrachloride (CCl_4) and Freon 113, while 2 mg/100 ml solutions were prepared in a 2% CCl_4 - 98% Freon 113 solvent mixture. The solvents were selected to correlate this work with oil quantitation procedures in the Analytical Quality Control Laboratory Newsletters, Nos. 12 and 15. All solutions were stored in stoppered 100 ml volumetric flasks for eight days, at normal room temperature and lighting conditions. Solution stabilities were monitored by measuring the 2930 cm^{-1} absorbance band of oil. All the solutions proved to be stable; less than 1% change in absorbance occurred during the test period. This preliminary study suggests that dispersed oils should be promptly extracted from water, prior to prolonged transport or storage.

(M. Gruenfeld/J. Puglis, FTS 201-548-3543, Coml. 201-548-3347)

* See page 2.

SOLVENT EXTRACTION OF OIL FROM WATER

Difficult emulsion problems were encountered while analyzing 200 water samples for oil, by the IR method described in the AQCL Newsletter #15, October 1972. These samples originated from the overboard discharges of a refined cargo tanker during its ballast run. It was determined by visual inspection that many of the samples contained more than 2 ml of oil as a surface layer. This exceeded the upper concentration limit of the method and often yielded difficult-to-break emulsions. Use of the IR method was possible only after additions of 100 ml portions of concentrated hydrochloric acid to the sample - carbon tetrachloride mixtures in the separatory funnels. This caused the emulsions to separate readily into two distinct layers.

(U. Frank, FTS 201-548-3510, Coml. 201-548-3347)

GLASSWARE CLEANING FOR THE QUANTITATION OF OIL IN WATER

Carbon tetrachloride is frequently used for cleaning glassware to remove trace organic impurities. This solvent is expensive and its excessive handling is hazardous. A comparison of the ability to remove oil from glassware was therefore made between carbon tetrachloride and a powdered detergent (Tide Laundry Detergent). A small quantity of detergent and warm water was added to a separatory funnel coated with oil. Light and heavy crude and processed oils were used. The funnel was thoroughly shaken, and then rinsed repeatedly with water. After the elimination of suds no interference was encountered at 2930 cm^{-1}, which is the infrared region used for quantitating oil. The detergent appears essentially equal to carbon tetrachloride for removing the oils. It is believed that other detergents will yield similar results.

(J. Puglish/M. Gruenfeld, FTS 201-548-3543, Coml. 201-548-3347)

CALCULATION OF ABSORBANCE FROM IR ORDINATE EXPANSION MEASUREMENTS

An equation was developed in order to transpose into absorbance units the 5X ordinate expansion readings obtained with a Perkin-Elmer Model 457A (now sold as Model 467) infrared spectrophotometer. This instrument expands any 20% portion of the transmittance range to cover the entire chart ordinate. The equation can also be applied to similar data output of other IR instruments with ordinate expansion features that operate in the same manner. Transposition of expansion factors other than 5X, into absorbance units, can be accomplished by substituting this new expansion factor for 5 in the numerator and denominator of the equation.

$$\text{Absorbance} = \log_{10} \frac{5B}{5B+D-C}$$

B = % T of the unexpanded baseline

C = % T of the expanded baseline

D = % T of the expanded peak maximum.

(M. Gruenfeld, FTS 201-548-3543 - Coml. 201-548-3347)

SULFUR INTERFERENCE IN U.V. ANALYSIS

The U.V. absorption spectrum of elemental sulfur has been found to resemble the absorption spectra of some petroleum oils. As a consequence, leaching of elemental sulfur from the environment (sediments, et al), by spilled oils, can result in erroneous analyses. This possibility should be considered whenever quantitating oil in water by a U.V. absorption technique (Harva, O., Somersalo, A., Suomen Kem, 31 (B): 384-7 (1958)), and whenever using a U.V. method for "passive tagging" oils (Levy, E. M., Water Research, 6, 57-69 (1972); also - Shields, D. C., "Forensic Aspects of Oil Pollution", presented at the 1973 CIC-CCIW Symposium on Water Quality Parameters, Author's address - R.C.M.P., Chemistry Section, Crime Detection Laboratory, Vancouver, B.C., Canada). These conclusions derive from our limited measurements of South Louisiana and Bachaquero Crude, and No. 2 and No. 6 Fuel Oils. These are viscous and non-viscous crude and processed oils that are considered to be representative of many petroleum oils. Solutions of the oils and sulfur, in cyclohexane, were prepared for U.V. measurement. The spectrum of sulfur was found to exhibit absorption maxima at approximately 225 mμ and 260 mμ. The oil spectra also exhibit absorption maxima or shoulders at these approximate wavelengths. The spectral profiles of South Louisiana Crude and No. 6 Fuel Oils most closely resemble that of elemental sulfur, but the spectral profile of No. 2 Fuel Oil differs from it significantly. *(J. Lafornara/M. Gruenfeld, FTS 201-548,3543, Coml. 201-548-3347)*

REMOVAL OF CHARRED OIL DEPOSITS FROM GLASSWARE

During our laboratory's recent participation in an ASTM collaborative study of methods for "passive tagging" oils, difficulties arose in cleaning charred oil deposits from distillation equipment, and from GC injector sleeves. These items were constantly reused and, therefore, required frequent cleaning. Scrubbing with steel wool and detergent, and soaking in cold Chromerge solution (sulfuric acid and sodium dichromate) proved ineffective. Hot Chromerge solution was effective, but is considered too dangerous for routine use. Complete removal of all visible traces of oil deposits was achieved by heating the glassware for 10 minutes at 500°C, in a muffle furnace. It is thought that this technique can be used to remove other carbonaceous deposits from glassware that can withstand this temperature.

(H. Jeleniewski/U. Frank, FTS 201-548-3510, Coml. 201-548-3347)

* See page 2.

SOLVENT FOR OIL ANALYSIS

Recurring questions have been raised about the identity and commercial source of trichlorotrifluorethane, a solvent that is often recommended for the quantitative analysis of water dispersed oils. While this term describes two isomers, the isomer that is actually the recommended solvent is 1,1,2-trichloro-1,2,2-trifluoroethane. It is available from E.I. duPont De Nemours and Company as a refrigerant (Freon-113) and as a cleaning agent (Freon TF). It is also available from a number of chemical supply companies, in several grades of purity. (M. Gruenfeld, FTS 340-6625, Coml. 201-321-6625.)

EVALUATION OF A PORTABLE IR SPECTROPHOTOMETER

A preliminary evaluation of a compact portable infrared spectrophotometer that is suitable for the quantitative analysis of oils in the field, is described. This unit, a Miran I Fixed Filter Infrared Analyzer (Wilks Scientific Corporation, South Norwalk, Connecticut) is a single beam device with a fixed 3.4 micron filter. It accommodates a 10 mm path length sample cell, incorporates ordinate expansion capability 5x and 20x, and can operate off a 12-volt automobile battery. Battery operation was used during most of this study, but AC operation was found to yield identical readings. Solutions of four oils (South Louisiana and Bachaquero Crude and No. 2 and No. 6 Fuel Oils) with known concentrations in carbon tetrachloride were measured in 10 mm path length cells without ordinate scale expansion, and by using expansion settings 5x and 20x. Absorbance versus concentration curves were generated, and these prove to be nonlinear. Miran "absorbances" do not match absorbance readings of a double beam IR instrument. In accordance with a recommendation by Wilks, appropriate correction factors were determined as the difference between linearity and the curve of one oil. These correction factors, when applied to the curves of the oils, translated them into linear plots that passed through the origin. This demonstrates the utility of the instrument for single point analysis of oils. Similar curves were also derived by using ordinate scale expansions, but no attempt was made to correct these to linear plots. At ordinate expansion 20x, points where quite scattered and did not yield a smooth curve. The concentration range 0.1 - 150 mg/100 ml oil in carbon tetrachloride was examined in this study, and the lower concentration figures is considered to be the reasonable detection limit of the instrument at 20x expansion. This represents an oil in water concentration of 0.1 ppm when 1 liter water samples are extracted with 100 ml carbon tetrachloride. (M. Gruenfeld and U. Frank, FTS 340-6625, Coml. 201-321-6625.)

* See page 2.

IDENTIFICATION OF MILLIGRAM QUANTITIES OF PETROLEUM OILS

Several new methods for fingerprinting petroleum oils have been published in the 1974 Annual Book of ASTM Standards, Part 31, Water. They include a procedure for sample preparation and laboratory weathering (D3326-74T), and for gas chromatographic correlation (D3328-74T). Fairly large volumes of oil (50 ml) are required by D3326, and this amount may be difficult to collect at the scene of a spill. This is also an excessive amount of oil to extract from the water column for those analyses requiring fingerprinting of water dispersed oils. A procedure has therefore been developed for laboratory weathering and gas chromatographic correlation of small amounts of oil, i.e., 0.5-30 mg. Our procedure yields chromatograms that closely resemble those of D3328, Method A, (packed column GC); work with SCOT columns (Method B) will soon be undertaken. Carbon tetrachloride or 1,1,2 - trichloro - 1,2,2, - trifluorethane (Freon 113, et al.) solutions of the spilled oils are quantitatively analyzed by IR. Solution volumes of 100 ml are then prepared, which contain either 0.5 or 30 mg oil. A steam table and a filtered air stream are used to strip the solutions to a final 1-2 ml volume in 150 ml beakers, and these concentrates are transferred to 10 x 30 nm vials. The vials that contain the 30 mg oil portions are suspended in a 40°C water bath, and a filtered air stream is used to remove the final trace of solvent. This condition is then maintained for an additional 10 minutes. The vials that contain 0.5 mg portions of oil are held at room temperature, the airflow is stopped just before total solvent removal, and final solvent evaporation is achieved spontaneously. This condition is then maintained for an additional 10 minutes. Distilled solvent is used for the lower concentration determinations. Small amounts of CCl_4 (10-20 µl) are then added to each vial prior to GC injection. Laboratory weathering of the reference oils is achieved by suspending vials containing 70 mg of each reference oil in a 40° C water bath, for 15 minutes, in the presence of a filtered air stream. Portions of these reference oils are then injected onto the GC. (Michael Gruenfeld/R. Frederick, FTS 201-548-3543, Coml. 201-548-3347)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #26, July 1975.

Separation of Petroleum and Non-Petroleum Oils

The direct addition of silica gell to freon has been recommended (Newsletter #22, p. 7) for separating petroleum oils from animal oils and vegetable oils. Silica gel removes the non-hydrocarbons from the solution while leaving the hydrocarbons unaffected. This technique avoids column chromatographic and solvent stripping steps and is therefore very straighforward. We examined this method as part of a project to develop a procedure for quantitating 1 ppb levels of petroleum oils in water. The interaction of silica gel with petroleum and non-petroleum oils in carbon tetrachloride was monitored. The consequence of silica gel deactivation was also examined. This information was then plotted versus the degree of oil removal, and the influence of different stirring times. The resulting graph suggests optimum operating conditions, and is available on request. (M. Gruenfeld, FTS 340-6625, Coml. 201-321-6625.)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #29, April 1976.

Replicate Oil Chromatograms

Replicate high resolution gas chromatograms of four weathered and unweathered crude oils were obtained in order to evaluate a pattern recognition computer program for fingerprinting oils. This program was previously described in AQC Newsletter No. 25, April 1975, Page 8. Ten replicate runs of each oil (total of 80 chromatograms) were prepared, using a 50 ft OV-101 SCOT column. The injection and quality assurance techniques used were in accordance with Technical Report 72-55 of the Woods Hole Oceanographic Institution (1972) by Zafiriou, O., Blumer, M., and Myers, J. Photocopies of the chromatograms are available on loan, for duplication, together with information regarding oil identity and weathering history. (M. Gruenfeld, FTS 342-7543, Coml. 201-548-3347, M. Urban, FTS 342-7517)

* See page 2.

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #30, July 1976.

Determination of Oil in Sediment by NMR

Nuclear magnetic resonance (NMR) spectrometry was found to be a rapid and useful technique for determining the presence of petroleum contamination in aquatic sediment samples. Determinations were performed in support of a study conducted by Region II to establish whether a major oil spill, that occurred in 1973, caused long term irreversible damage and plant mortalities in a mangrove swamp in Puerto Rico. Column chromatographic "clean-up" procedures and gas chromatography (GLC-FID) were initially used to analyze carbon tetrachloride extracts of the sediments, but failed to provide conclusive results because of interferences from possible biogenic organics. Extracts of sediments from both the spill site and a similar uncontaminated location (control) were therefore examined with our 60MHz NMR spectrometer. The extracts from the spill site were found to exhibit absorptions in the range 6.5 - 8.0 ppm (ring aromatic protons) and 0.5 - 2.0 ppm (paraffinic protons) relative to tetramethylsilane (TMS); the control extracts exhibited no aromatic absorptions. The low-field aromatic absorptions found only in sediments from the spill site were ascribed to the presence of petroleum oil suspected of originating from the past spill incident. (U. Frank, FTS 342-7510, Coml. 201-548-3347, M. Gruenfeld, FTS 342-7543, Coml. 201-548-3347)

* See page 2.

(*)ANALYTICAL QUALITY CONTROL NEWSLETTER #36, January 1978.

Differentiation of a Synthetic Lubricating Oil from Several Petroleum Derived Automotive Lubricating Oils

In response to recent requests for distinguishing between synthetic and petroleum-derived automotive lubricating oils, our laboratory analyzed a number of oils by several techniques. One synthetic oil and several petroleum-derived oils were tested. The synthetic oil proved to be distinguishable from the other oils through its high content of carbonyl compounds, mainly esters. The petroleum oils yielded broad, unresolved GC profiles, one moderately broad HPLC peak, substantial absorption in the 1 ppm region (relative to TMS) of the NMR, and absorbance in the 2930 cm^{-1}, 1450 cm^{-1}, and 1360 cm^{-1} spectral regions of the IR. The synthetic oil produced large, well resolved peaks by GC, a second sharp peak by HPLC and additional adsorptions at approximately 3.8 ppm and 1740 cm^{-1} by NMR and IR, respectively. The GC profile of the synthetic oil was clearly atypical of petroleum oils, while its additional absorptions at approximately 3.8 ppm by NMR and 1740 cm^{-1} by IR were indicative of carbonyl compounds. These unique spectral and chromatographic features of the synthetic oil were also used to determine our ability to detect adulterations of the petroleum oils with the synthetic oil. Detection limits were established at approximately 10%. (R. Frederick, FTS 340-6627, Coml. 201-321-6627/N. Pangaro.)

* See page 2.

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IDENTIFICATION OF OIL POLLUTANTS: A REVIEW OF SOME RECENT METHODS

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ABSTRACT

Some recent studies of methods for identifying weathered oils through chemical fingerprints (passive tagging) are reported. These studies were performed by Esso Research and Engineering Company, Phillips Scientific Corporation, Woods Hole Oceanographic Institution, and Baird Atomic Corporation under U.S. Environmental Protection Agency (EPA) sponsored grants and contracts. A broad range of analytical instruments and techniques were used, e.g., adsorption chromatography, molecular emission and absorption spectrophotometry, atomic absorption spectrophotometry, gas chromatography, computerized mass spectrometry, et. al. Some of the oil parameters evaluated as potential fingerprint indices are vanadium, nickel, sulfur and nitrogen content, gas chromatographic profile appearance, carbon and sulfur isotope ratios, API gravity, and pour point. Several promising methods for passive tagging oils are suggested by these studies.

INTRODUCTION

Discharges and spills of crude, residual, and distillate oils are occurring with ever increasing frequency in coastal waters. They cause extensive damage to marine life, coastal life, recreational beaches, and to their dependent industries, and are therefore of special concern to the U.S. Environmental Protection Agency (EPA). Establishing the true source of the pollutant, where several suspect sources exist, and proving identity between the weathered residue and an unweathered authentic often requires extensive analytical instrumental procedures. Many methods are now used, but there is a lack of agreement among laboratories about the advantage or necessity of any single technique or combination of techniques. This variety of methods is illustrated in a recent publication[1] that reviews some current procedures for correlating weathered and unweathered oils (passive tagging). Some techniques such as infrared spectrophotometry require only simple inexpensive instrumentation that is readily available and usable by most laboratory personnel. But other techniques, such as neutron activation analysis, require such expensive instruments and highly specialized personnel that they are available in very few laboratories.

The Edison Water Quality Research Laboratory, a Laboratory of EPA's National Environmental Research Center in Cincinnati (NERC), investigated numerous methods for passive tagging of oils that occur as slicks and shoreline residues. Several analytical systems that integrate simple techniques such as gas chromatography with more sophisticated ones such as mass spectrometry were developed through grants and contracts. These techniques were evaluated singly and in combination as a basis for recommending reasonable analytical approaches to oil identification problems. Funding was provided to the Esso Research and Engineering Company[2], the Phillips Scientific Corporation[3], the Woods Hole Oceanographic Institution[4], and the Baird Atomic Corporation[5]. Resulting analytical methods and oil parameters measured are summarized (Table 1).

The Esso System

Esso examines a variety of crude and processed oils (Table 2) by mass spectrometry and gas chromatography to determine high molecular weight paraffins, naphthenes (cyclic paraffins), and polynuclear aromatics. Emission spectroscopy is used to determine bulk vanadium-nickel content; X-ray spectroscopy and Kjeldahl analysis are used to determine bulk sulfur-nitrogen content. Adsorption chromatography with several different columns is used to isolate paraffins, naphthenes, and aromatics from the oil matrix and to separate the aromatics from the aliphatics. A schematic presentation of these analysis steps is provided (Figure 1).

Portions of the authentic and weathered[2] samples are first separated for determination of bulk nickel-vanadium and sulfur-nitrogen content. Separate portions are distilled

[1]The Baird Atomic study was funded through the EPA Analytical Quality Control Laboratory, NERC, Cincinnati.

[2]Weathering of oils is simulated by using a continuously recirculating salt water system with controlled temperature, agitation, light and wind conditions.

Table 1: Summary of Techniques, Fingerprint Indices, and Investigators

Oil Parameter	Method of Determination	Investigator
Vanadium & Nickel Content	Emission Spectrometry	1
	Atomic Absorption Spectrophotometry	2
Nitrogen Content	Kjeldahl Method	1
	Dumas Method & Gas Chromatography	2
Sulfur Content	X-Ray Spectroscopy	1
	ASTM Method[a]	2
Total Profile	Gas Chromatography (Flame Ionization):	
	Packed Column	2
	Open Tubular Column	3
N-Paraffins	Gas Chromatography/Adsorption Chromatography	1
	Gas Chromatography/Urea Adduction	2
Isotopic Composition		
Carbon & Sulfur in:		
Total High Boiling Residue	Isotope Mass Spectrometry	2
Carbon in:		
Saturate Fraction	Isotope Mass Spectrometry	2
Aromatic Fraction	Isotope Mass Spectrometry	2
Asphaltic Fraction	Isotope Mass Spectrometry	2
Polynuclear Aromatics	Gas Chromatography	1
	Mass Spectrometry (Low Voltage)	1
	Ultraviolet Absorption Spectrophotometry	1
	Fluorescence and Phophorescence Spectrophotometry (77°K)	4
Naphthenes	Mass Spectrometry (High Voltage)	1
Saturates	Gravimetric/Adsorption Chromatography	2
Polynuclear Aromatics	Gravimetric/Adsorption Chromatography	2
Asphaltics	Gravimetric/Adsorption Chromatography	2
API Gravity	ASTM Method[a]	2
Pour Point	ASTM Method[a]	2

1-Esso Research and Engineering Company
2-Phillips Scientific Corporation
3-Woods Hole Oceanographic Institute
4-Baird Atomic Corporation

[a]ASTM Method cited in text.

to remove components boiling below 400° F; these volatiles are discarded. Accurately weighed portions of the higher boiling residues are dispersed in 50 cc n-pentane, insolubles are separated by centrifugation and discarded. Each solution is then fed to a clay column and successively eluted with n-pentane, benzene-acetone, and acetone. The n-pentane eluate preferentially contains the paraffins, naphthenes, and aromatics, and the acetone-benzene and acetone eluates contain the more polar sample fractions. These polar materials are thought to be more water soluble and therefore unreliable indices; they are not used in the analysis scheme.

The n-pentane eluate is stripped of solvent, and yields a concentrate rich in paraffins, naphthenes and aromatics which is fed to a silica gel column. The aliphatic components are selectively eluted with n-pentane, and the aromatic components with acetone. The two solvents are then stripped and the residues weighed. These material balance data may be useful for oil characterization (Table 3). The saturate fraction is subsequently analyzed by gas chromatography for n-paraffins and by computerized high voltage mass spectrometry for various naphthene types. The aromatic fraction is analyzed by low voltage computerized mass spectrometry

Table 2: Oil Samples Examined by Esso

Sample No.	Sample Type		Oil Field	Location
1	Crude oil		Tia Juana	Venezuela
2	Crude oil		Lago	Venezuela
3	Crude oil		Grande Isle	Indonesia
4	Crude oil		Nigeria	Nigeria
5	Crude oil		Zuitina	Libya
6	No. 2 Heating oil			
7	No. 4 Fuel oil	Refined and		
8	No. 5 Fuel oil	formulated from Venezuelan stock		

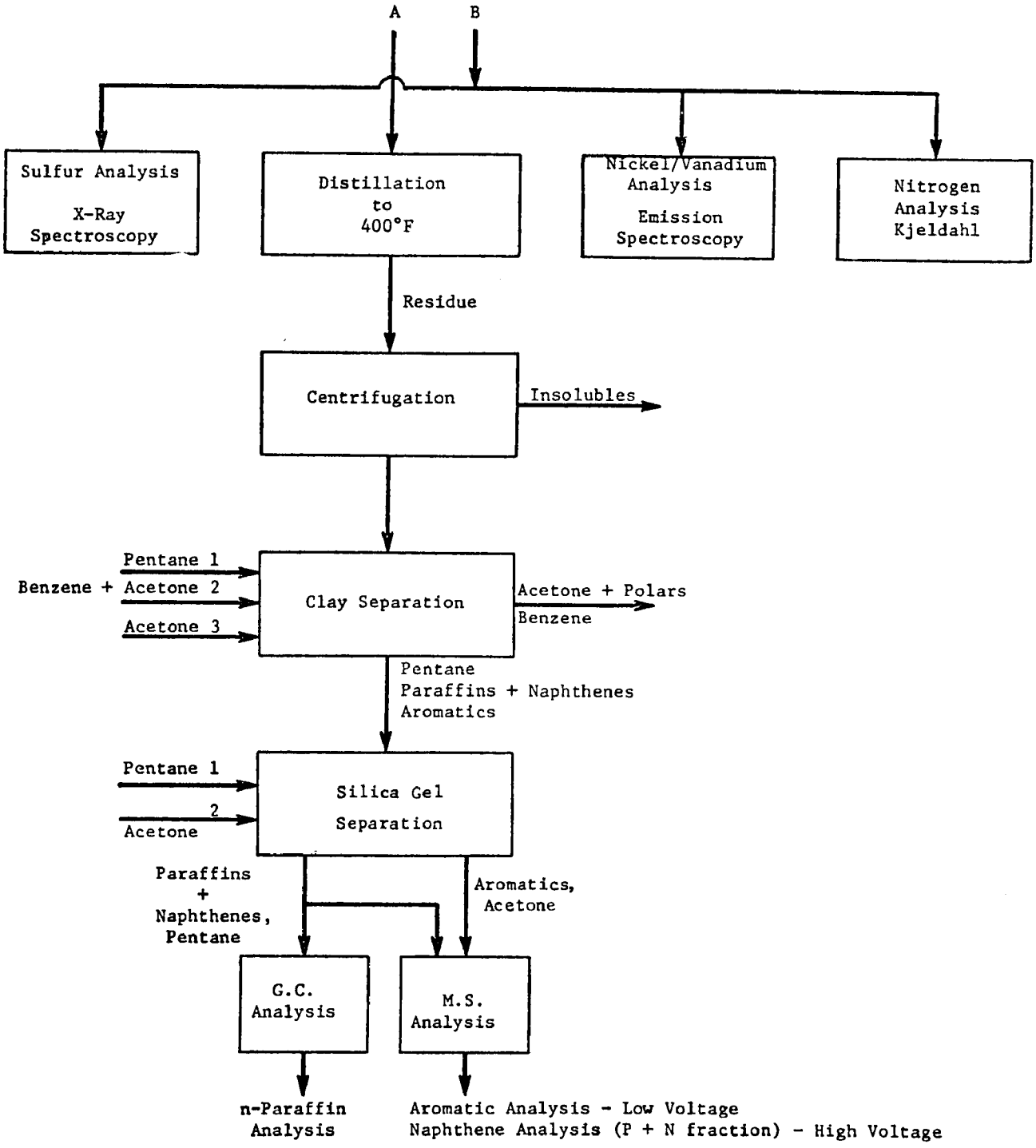


Figure 1: Analysis Schematic of the Esso System.

Table 3: Typical Material Balance Data from Esso Sample Separation Processing[a]

Oil Type	Paraffins + Naphthenes	Aromatics	Weight % of Sample Polars	Pentane Insolubles	Σ Fractions
Tia Juana Medium Crude Oil	46.0	21.6	19.3	9.0	95.9
Lago Crude Oil	39.8	21.8	24.7	15.5	101.8
Grande Isle Mix Crude Oil	73.0	16.1	12.3	1.2	102.6
Nigerian Crude Oil	61.2	20.7	10.6	1.0	93.5
Zuitina Crude Oil	66.1	12.1	15.2	3.1	96.5
No. 2 Heating Oil	49.7	46.6	0.0	0.2	96.5
No. 4 Heating Oil	63.2	26.2	8.6	1.0	99.0
No. 5 Heating Oil	64.2	20.4	12.1	5.8	102.5
Replicate Tia Juana	48.2	19.5	18.4	10.9	97.0
Medium Crude Oils	47.7	20.8	20.4	9.4	97.9
	47.4	18.6	21.5	10.9	98.4

[a]Data based on unweathered samples.

for various polynuclear aromatic types. Twenty-six promising fingerprint indices derived by these techniques (Table 4) are subjected to Discriminant Function Analysis to identify the best discriminators for the test oils (Table 5). This statistical treatment is needed to isolate five indices having the lowest probability of mismatching two oils (Table 5). Estimation of confidence levels for oil classification could also have been performed with the Bonferroni "t" Statistics.

In this study, Esso uses only four fingerprint indices and, with high statistical confidence, distinguishes among any possible pairs of the oils, even after the oils have experienced extensive laboratory weathering. These indices are generally unaffected by simulated weathering, but their applicability to other oils has not been established. Other indices may be required for individual situations.

Another Esso Approach

In a supplemental study Esso evaluates a combined gas chromatographic-ultraviolet spectrophotometric procedure for passive tagging oils (Figure 2). Individual polynuclear aromatics (PNA's) separated by gas chromatography are collected and measured by ultraviolet absorption spectrophotometry. After 1-gram portions of the weathered and unweathered oils are extracted with caustic and dissolved in cyclohexane, triphenyl benzene is added as internal standard. Each solution is fed to a deactivated alumina column (2%H O) that is successively eluted with cyclohexane, cyclohexane/benzene, benzene, and benzene/methanol. The cyclohexane/benzene fraction, which is enriched in three-ring and heavier PNA's, is stripped of solvent and the residue dispersed in toluene. A gas chromatogram of the PNA's is then obtained (Figure 3). Selected components appearing as gas chromatographic peaks are trapped and their ultraviolet absorption spectra obtained. These spectra together with appropriate calibration coefficients are used to quantitate the individual polynuclear aromatics in the oils (Table 6). Esso uses these quantitative results and visual examination of the chromatograms for passive tagging oils. Preliminary data indicate that this approach is promising.

The Phillips Study

Phillips surveys a multitude of parameters that may be useful for passive tagging oils. Main emphasis is on identifying promising "fingerprint" indices; extensive controlled weathering experiments are not performed. Seventy-seven crude oils representing world-wide production (Appendix A) are stripped of components having boiling points below 600°F and analyzed by mass spectrometry for carbon and sulfur isotope compositions, by atomic absorption spectrophotometry for bulk vanadium-nickel content and by gas chromatography for general profile appearance and odd-even n-paraffin predominance. Total sulfur, nitrogen, saturates, aromatics, and asphaltics, as well as API gravity and pour point are also determined. A schematic presentation of these analysis steps is provided (Figure 4).

The oils are first freed of insolubles by centrifugation at 20,000 RPM for 90 minutes. The volatiles are then stripped by heating to 214°F at 0.15 mm Hg. This is equivalent to approximately 600°F atmospheric. The 600°F + residuals are analyzed for total nitrogen (Dumas method followed by gas chromatography); sulfur content (ASTM method D 1552-64); API gravity and pour point (ASTM methods D287-67 and D97-66, respectively); total nickel and vanadium (incineration in the presence of sulfur and subsequent analysis by atomic absorption spectrophotometry); carbon and sulfur isotope ratios (mass spectrometry); and gas chromatographic profile appearance. A novel classification scheme is used for coding gas chromatographic information in a form amenable to computer input: a straight line is drawn between the C_{20} and C_{30} peak tips, and the locations of the intermediate nine n-paraffin peaks are noted as "above" or "under" the line (Figure 5). Designating the gas chromatographic profile as Type A (above) or Type U (under) is based on the location of the majority of peaks. The number of peaks in the minority is designated by a digit following the type letter. For example, Type U-2 indicates that seven peaks are under the line and two above (Figure 5). A Type B profile has a broad envelope with n-paraffin peaks poorly defined, which prevents the construction of the characterization line between peaks (Figure 6). A summary of all these "fingerprint" indices is presented (Table 7).

Phillips examines still more potential "fingerprint" indices. The 600°F + residuals are separated by adsorption chromatography over silica into three fractions: saturates, aromatics, and asphaltics (Figure 4). Pentane, dichloromethane, and methanol-dichloromethane, respectively, are

Table 4: Some Promising Esso Finger

$\frac{V}{Ni}$	$\frac{\Sigma\text{–3 Ring Naphthenes}}{\Sigma \quad (P+N)}$
$\frac{S}{N}$	$\frac{\Sigma\text{–4 Ring Naphthenes}}{\Sigma \quad (P+N)}$
$\frac{C_{20}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\text{–5 Ring Naphthenes}}{\Sigma \quad (P+N)}$
$\frac{C_{21}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-6}}{\Sigma\, C_nH_{2n-18}}$
$\frac{C_{24}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-6} + C_nH_{2n-18}}{\Sigma\, C_nH_{2n-14}}$
$\frac{C_{25}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\text{–1 Ring + 2 Ring Naphthenes}}{\Sigma\text{–5 Ring + 6 Ring Naphthenes}}$
$\frac{C_{26}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-6}}{\Sigma\text{ Aromatics}}$ (Benzenes)
$\frac{C_{27}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-6}}{\Sigma\text{ Aromatics}}$ c = 20, c = 36
$\frac{C_{30}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-10}}{\Sigma\text{ Aromatics}}$ (Indenes)
$\frac{C_{31}}{\Sigma\text{nParaffin}}$ c = 20, c = 40	$\frac{\Sigma\, C_nH_{2n-10}}{\Sigma\text{ Aromatics}}$ c = 20, c = 36
$\frac{\Sigma\, C_{20} + C_{21} + C_{22}}{\Sigma\, C_{30} + C_{31} + C_{32}}$	$\frac{\Sigma\, C_nH_{2n-14}}{\Sigma\text{ Aromatics}}$ (Acenopthenes)
$\frac{\Sigma\, C_{20} + C_{21} + C_{22} + C_{30} + C_{31} + C_{32}}{\Sigma\ C_{24} + C_{25} + C_{26} + C_{27} + C_{28}}$	$\frac{\Sigma\, C_nH_{2n-16}}{\Sigma\text{ Aromatics}}$ (Acenopthalenes)
$\frac{\Sigma\text{nParaffins}}{\Sigma\ (P+N)}$	$\frac{\Sigma\, C_nH_{2n-18}}{\Sigma\text{ Aromatics}}$ (Phenanthrenes)

used for elution. Percent composition of each fraction in the 600°F + residuals, and the carbon isotope ratios in these fractions are measured as additional indices. The n-paraffins in the saturates fraction are further separated by urea adduction and measured by gas chromatography for relative abundance according to carbon number distribution. Odd-numbered to even-numbered n-paraffins are calculated as a function of carbon number. A summary of these additional "fingerprint" indices is presented (Table 8). A limited assessment is also made of measurement repeatability and weathering effects.

The Woods Hole Oceanographic Institution System

Woods Hole Oceanographic Institution (WHOI) uses gas chromatography with support-coated open tubular (SCOT) columns for passive tagging oils. The oils are merely dis-

Table 5: Most Promising Esso Fingerprint Indices

$$\frac{V}{Ni}$$

$$\frac{\Sigma nParaffins}{\Sigma (P + N)}$$

$$\frac{\Sigma\text{-5 Ring Naphthenes}}{\Sigma (P + N)}$$

$$\frac{C_{20}}{\Sigma nParaffin_{c=40}^{c=20}}$$

$$\frac{\Sigma\text{-1 Ring+2 Ring Naphthenes}}{\Sigma\text{-5 Ring+6 Ring Naphthenes}}$$

solved in carbon disulfide (ca 5-10% W/V) and injected; exact concentrations need not be known. The gas chromatograph injector is equipped with a glass liner that is removable for cleaning. Column specifications are 50 feet × 0.02 inch packed with nonpolar liquid silicone OV-101 and rated at 25,000 effective plates. Oil chromatograms are compared visually, and certain features are abstracted and tabulated when analyzing many samples and for statistical purposes. These features are measured as vertical distances between superimposed baselines and appropriate peak heights (Figure 7, Table 9).

The WHOI method requires frequent evaluation of system performance by injecting a standard oil[3] that yields a repeatable known chromatogram. The extent of weathering undergone by oils is estimated from the onset of the gas chromatographic signal: if below n-C_{14} then the tabulated indices are considered unaffected by weathering, but if at or above n-C_{15} then weathering alterations are implied. Tabulated indices of oils that exhibit considerable weathering should not be compared directly with the indices of unweathered oils. Instead, each unweathered suspect is artificially weathered in a rotary evaporator by dropping pressure slowly to a high vacuum while heating to 60°C or warmer. Small portions are withdrawn periodically for GC analysis and the procedure continued until the oil resembles the environmental sample. Similarity is judged by the shape of low-boiling — range signals, or by comparing background ratios at C_{15} to any signal parameter at C_{17} or C_{18}. The remaining indices (Table 9) are then tabulated and compared to those of the weathered sample. SCOT column lifetimes generally exceed 200 oil analyses (injections), and tabulated

[3]A Number 2 Fuel Oil "stabilized" by passage through an alumina and silica gel column.

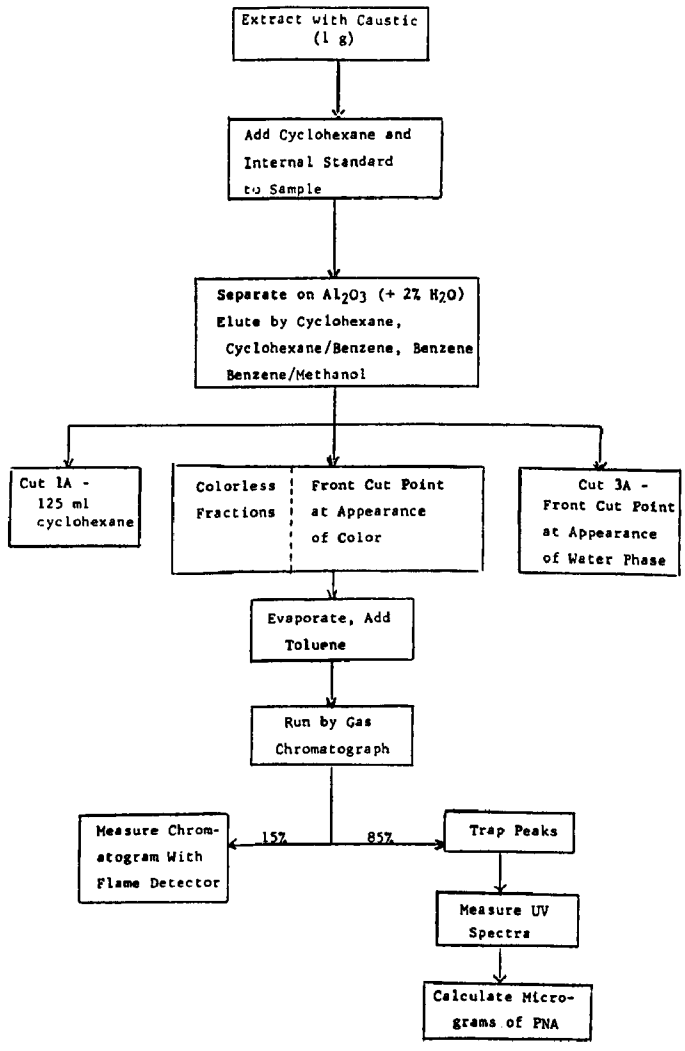


Figure 2: Analysis Schematic of Another Esso Approach.

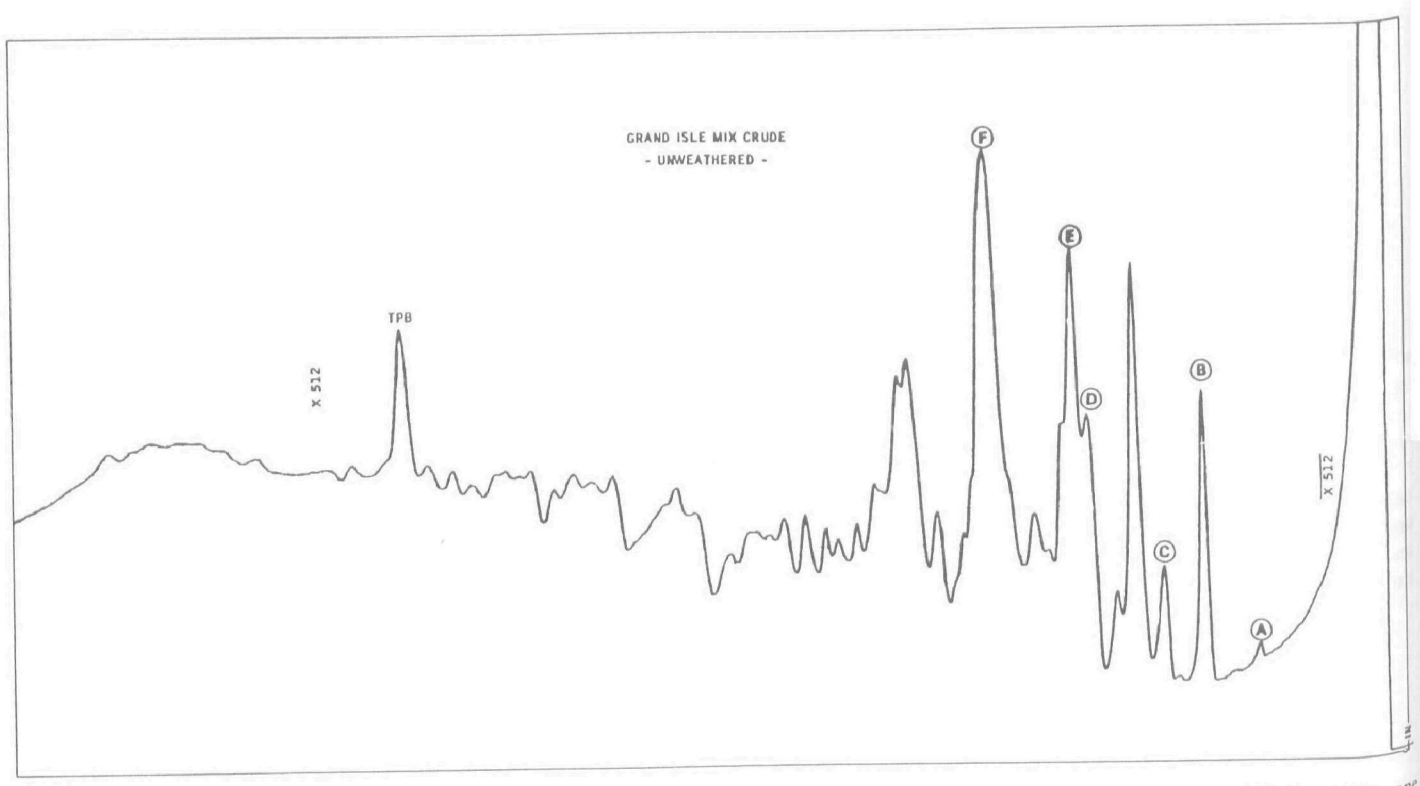
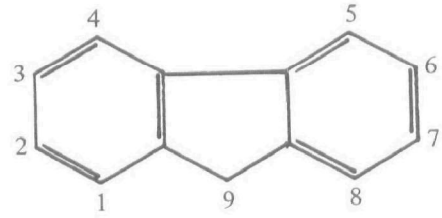


Figure 3: Gas Chromatogram by Esso of Polynuclear Aromatics in a Crude Oil. Marked Peaks Are Identified in Table 6. Triphenyl Benzene (TPB) Is Used as Internal Standard.

Table 6: GC Components Trapped by Esso for UV Analysis

Peak A[a] fluorene

Peak B methyl fluorene

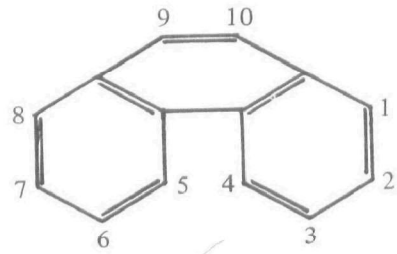


Peak C phenanthrene

Peak D 2-methyl phenanthrene

Peak E 1-methyl phenanthrene

Peak F dimethyl phenanthrene



[a]GC peaks are identified in Figure 3

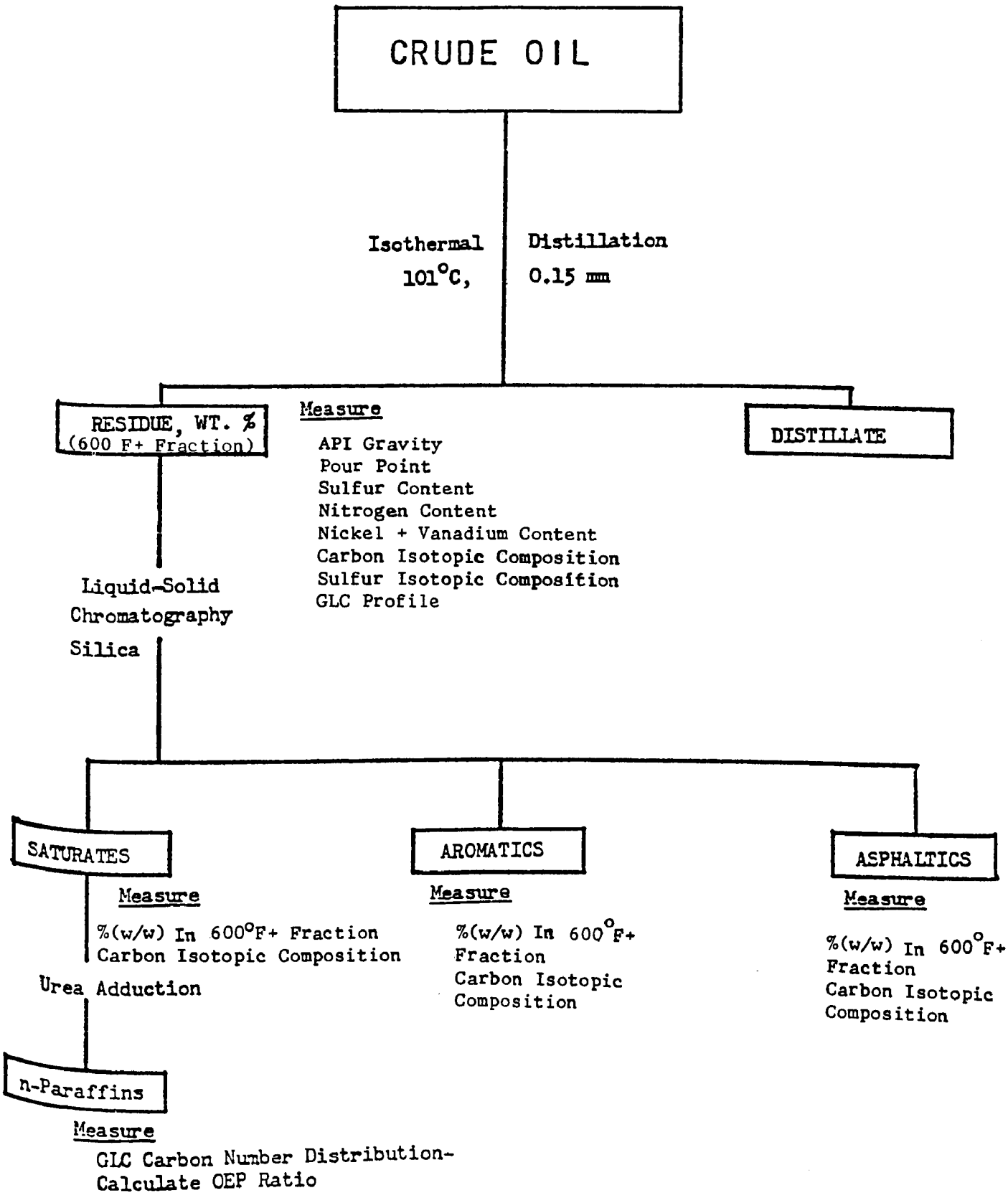


Figure 4: Analysis Schematic of the Phillips Study.

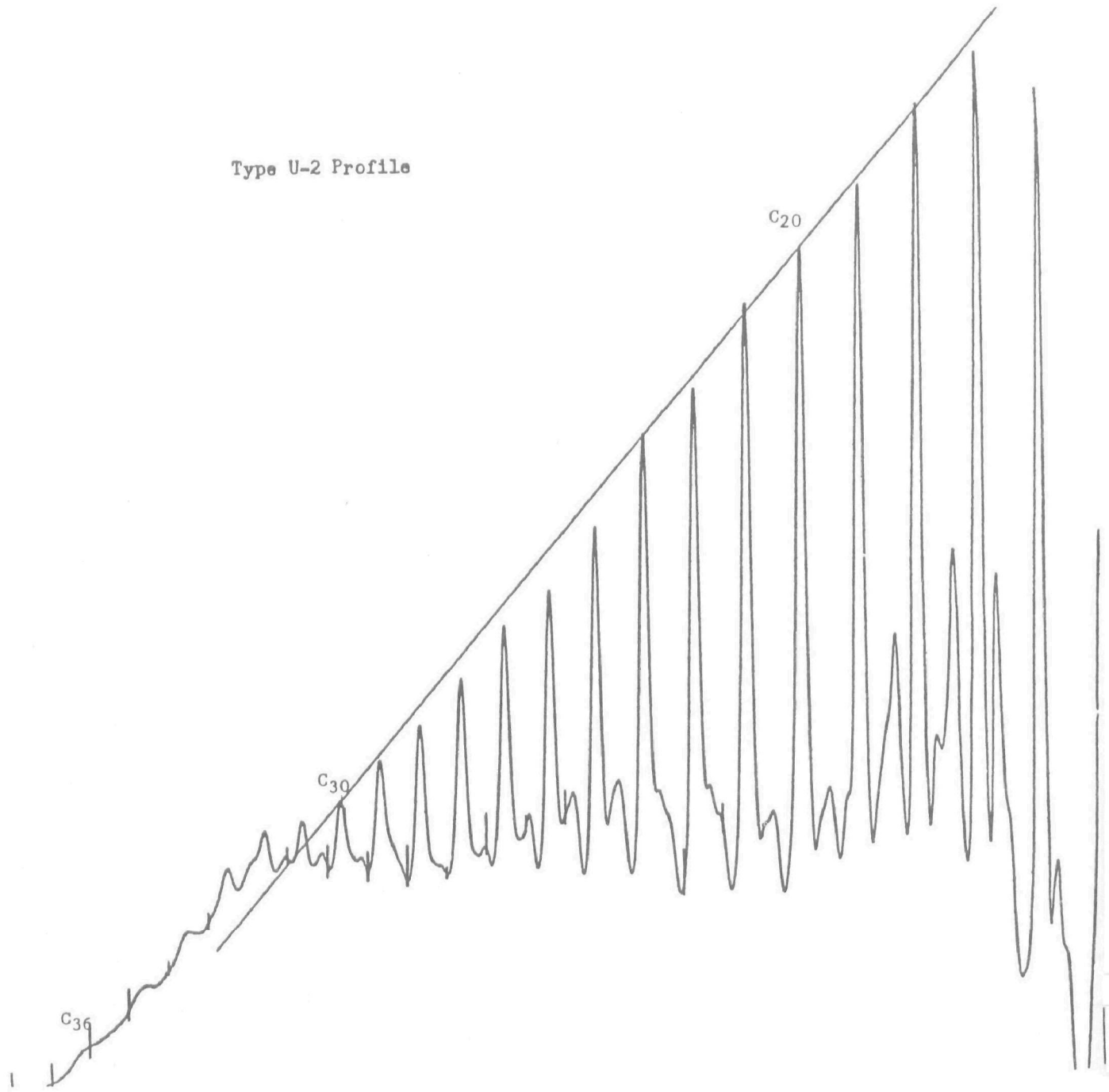


Figure 5: Coding of Gas Chromatogram Profile by Phillips — Type U-2 System.

indices of individual oils remain quite stable from column to column.

WHOI reports on a preliminary evaluation of their system with oils exposed by the Edison EPA Laboratory to various weathering conditions for different time intervals. Common spill-control chemicals were also added to some of the oils. Unweathered portions of 16 out of 17 oils used for this test accompanied 35 simulated spill samples. All the samples were number coded and identified only as potential sources or simulated spills. Test results are reported as "definite correlation", "probable correlation", or "no definite or probable correlation". Correct "definite correlation" is achieved in 74% of the cases, and only one "probable correlation" is incorrect (Table 10). An evaluation of the system's ability to distinguish among similar unweathered oils and oil products is also reported; in the majority of cases the oils were uniquely identifiable (Table 11). It is thought that since short term weathering does not significantly affect the correlation ability of the method, these results obtained with unweathered oils also apply to many weathered oils.

The Baird Atomic Study

Baird-Atomic provides a preliminary assessment of molecular emission (fluorescence and phosphorescence) at low temperatures as a method for identifying oils. Their ef-

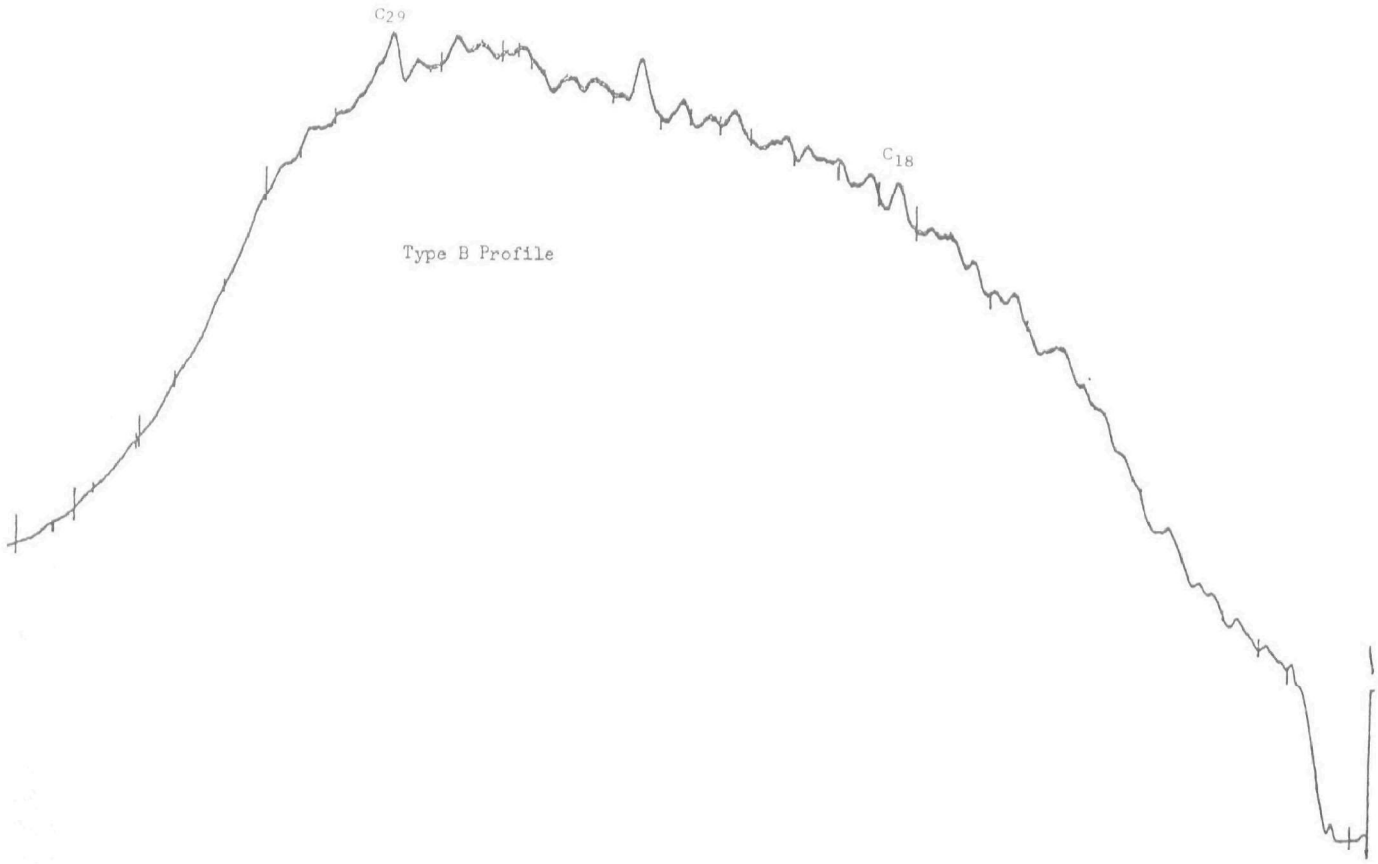


Figure 6: Coding of Gas Chromatogram Profile by Phillips — Type B System.

fort is a probe to evaluate the utility of the technique for discriminating among oils and to determine whether an advantage derives from using cryogenic temperatures. This study deals mostly with unweathered heavy crude and processed oils (Table 12); only two weathered oils were tested and no substantial effort was made to correlate them with the appropriate unweathered oils. Emphasis is on assessing the utility of low temperature molecular emission for distinguishing among different oils and not to demonstrate the utility of this technique for correlating appropriate weathered and unweathered oils; i.e., passive tagging.

The oils are dissolved in methylcyclohexane and cooled to 77°K. Methylcyclohexane is a good solvent that forms a clear glass at the cryogenic temperature. The use of other solvents and different oil concentrations is described; 10 ppm oil in methylcyclohexane yields the most satisfactory results. 290 mμ and 340 mμ are the diagnostic excitation wavelengths used to compare the oils. In every case oil emission spectra resulting from excitation at 340 mμ are more intense than those resulting from excitation at 290 mμ, but excitation at this latter wavelength yields broader emission spectra; all the oils yield characteristic emissions in the 380-400 mμ region when excited at 340 mμ. Dramatically improved spectral resolution is achieved by cooling to 77°K (Figures 8a and 8b). These emission spectra at cryogenic temperature are generally a mixture of fluorescence and phosphorescence, the latter occurring at the longer wavelengths. Phosphorescence and fluorescence spectra of several oils are compared; they differ sufficiently to distinguish the oils. This study also attempts to identify major luminescing compound types in the oils. Porphyrins, compounds similar to 4-methylpyrene, et. al., are identified. Several solvents in addition to methylcyclohexane are also evaluated; they include pentane, hexane, heptane, and octane. Initial difficulties with spectral resolution were traced to oxygen-quenching that was more evident in these normal paraffin solvents. The difficulty was resolved by degassing the solutions with dry nitrogen.

The Baird Atomic study demonstrates the potential utility of low temperature molecular emission for passive tagging oils. Cooling to cryogenic temperature enhances spectral resolution and the technique can differentiate among very similar oils; e.g., several number 6 fuel oils (Figure 9).

Table 7: Initial Phillips Fingerprint Indices

API Gravity
Pour Point
Carbon Isotopic Composition
Sulfur Isotopic Composition
Nickel/Vanadium Content (Ratio)
Sulfur/Nitrogen Content (Ratio)
GC Profile

Table 8: Additional Phillips Fingerprint Indices

% (w/w) Saturates
% (w/w) Aromatics
% (w/w) Asphaltics
Odd-Even N-Paraffin Predominance
Carbon Isotopic Composition (Saturates)
Carbon Isotopic Composition (Aromatics)
Carbon Isotopic Composition (Asphaltics)

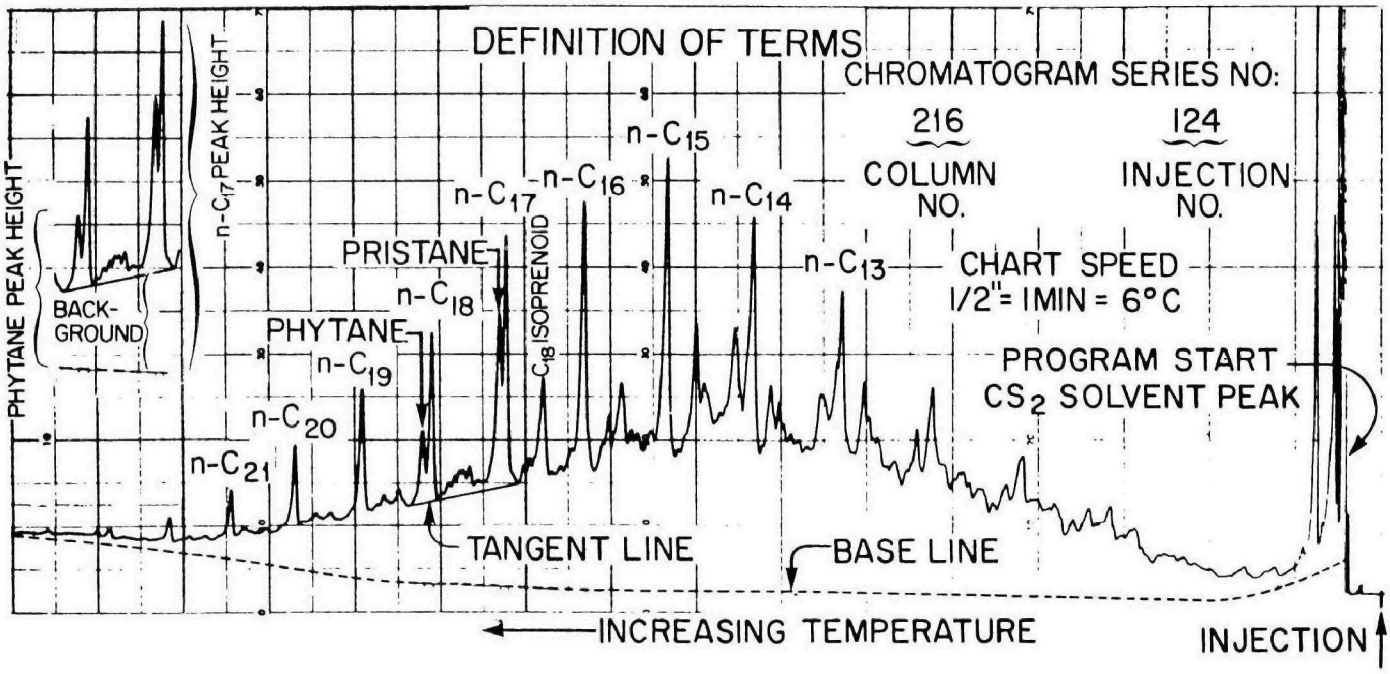


Figure 7: Gas Chromatogram by Woods Hole Oceanographic Institution with Terms Defined.

Table 9: Woods Hole Oceanographic Institute Fingerprint Indices[a]

Pristane/Phytane	C_{17}/C_{18}
C_{17}/ Pristane	C_{17}/Background
C_{18}/Phytane	C_{17}/C_{17}-Pristane Valley

a - Ratios measured as in Figure 7.

This study does not evaluate the susceptibility of low temperature fluorescence spectra to changes that result from weathering.

Discussion

These EPA sponsored studies are an effort to develop passive tagging methods having broad scope of application, and to appraise the usefulness of many analytical techniques for this purpose. Methods are sought that permit identifying weathered oils by comparing them with libraries of unweathered oil indices, but that can also be readily simplified for comparing a weathered oil with only a few unweathered oils. Although comparison with a few unweathered oils is now the common procedure, libraries of standard oil indices may come into use around harbors and other high density oil storage and handling locations.

Of these studies, only those by Esso and WHOI yield ready-to-use passive tagging methods. The others mostly evaluate promising oil parameters and analytical techniques, and determine their utility for pairing and discriminating among unweathered oils. Phillips and Baird Atomic do not perform extensive weathering tests and, therefore, do not confirm the reliability of their parameters and techniques for passive tagging oils. A considerable difference also exists in the usability of the instrumental techniques in these studies by "average" analytical laboratories.

The *Esso System* yields a ready-to-use passive tagging method that can be applied, in whole or in part, according to analysis needs and available instrumentation. For example, if

Table 10: Woods Hole Oceanographic Institute Test Results of EPA Weathered Samples

Prepared by EPA		Correlated by Woods Hole	Result
17 Source Oils:	35 Simulated Spills:	26 Unique Correlations	All Correct
8 Crudes	Exposures:	6 "Probable" Correlations (A Second Source Possible)	1 Error[a]
5 Fuels	1, 3, 30 Days		
4 Re-refined	Freshwater, Saltwater, Beach Sand	3 No Definite or Probable Correlations Found	No Correlation Exists[b]
	With or Without 1 of 4 Spill Control Chemicals Added.		

[a]Error attributable to presence of oil-based spill control chemical.
[b]The source of these samples was not supplied to Woods Hole by EPA.

Table 11: Ability by Woods Hole Oceanographic Institute to Distinguish Among 30 Oils and Oil Products from Greater New York Harbor[a]

Product Type	No. of Samples	Assignment
Gasoline	12	Not Analyzed
#2 Fuel Oil	14	7 Unique
		3 Pairs of Indistinguishable Oils
		1 Trio of Indistinguishable Oils
#4 Fuel Oil	4	All Unique
#6 Fuel Oil	8	All Unique
Miscellaneous:		
Kerosene	1	Unique
Marine Diesel	1	Unique
Nigerian Crude	1	Unique
Asphalt	1	Unique
Overall:		77% Unique 20% Indistinguishable *Pairs* 3% Indistinguishable *Triplets*

[a]Each oil was analyzed and compared with the 29 others to determine distinguishability.

pairing a weathered oil to one of only a few unweathered oils is required, then nickel-vanadium, sulfur-nitrogen, and GC analyses may suffice; but, where comparison with many similar unweathered oils is to be made, the entire Esso Method should be used. This assumes, of course, that a computerized mass spectrometer is available. Therefore, the extent of method utilization depends on problem complexity and available instrumentation.

The Esso Method is somewhat inconvenient because Adsorption chromatography is used repeatedly and because new selections among the 26 fingerprint indices (Table 4) may have to be made for different analyses. Additionally, computerized mass spectrometry is not available in most laboratories, thereby limiting the usability of the Esso Method. The method should permit unique correlations with extensive libraries of oil indices, however. It also benefits from the extensive weathering studies that were performed in its development by Esso.

Another Esso approach evaluates a combined GC-UV procedure that may provide additional fingerprint indices for passive tagging oils. Weathered and unweathered portions of the same oils are used in order to establish method integrity in the presence of weathering. If successful, this technique should find wide acceptance because gas chromatographs and ultraviolet spectrophotometers are generally available in analytical laboratories. It could also be combined to advantage with those portions of the Esso Method that require more commonly available instruments. The chromatograms of polynuclear aromatics differ considerably from the aliphatic GC profiles of the Esso Method. These aromatic profiles are easily obtained and may facilitate passive tagging oils, even without further UV analysis.

Use of adsorption chromatography and GC fraction collection make this procedure somewhat inconvenient. Losses of oil components can also occur during these steps, thereby yielding erroneous results. But, Esso's work at the time of this writing is still too preliminary for meaningful conclusions. Usable chromatograms of polynuclear aromatics could perhaps also have been obtained in the previous Esso study if the aromatic fraction, following silica gel separation (Figure 1), had been analyzed by GC.

The *Phillips Study* evaluates the suitability of many parameters and techniques for distinguishing and correctly pairing unweathered crude oils that represent worldwide production. Emphasis is directed toward identifying those parameters that distinguish oils and that may retain their integrity in the presence of weathering. No in-depth evaluation of weathering effects is done, however, and therefore, no ready-to-use passive tagging method results.

The potential fingerprint indices examined in this study include those that require only minimal equipment (API gravity and pour point) and those that require unusually expensive instruments (carbon and sulfur isotope ratios). The suitability of some commonly used passive tagging indices (sulfur, nitrogen, nickel, and vanadium content) for distinguishing among a large number of crude oils is also determined. Phillips aims to evaluate a sufficiently broad range of parameters to yield a comprehensive method, such as the Esso Method.

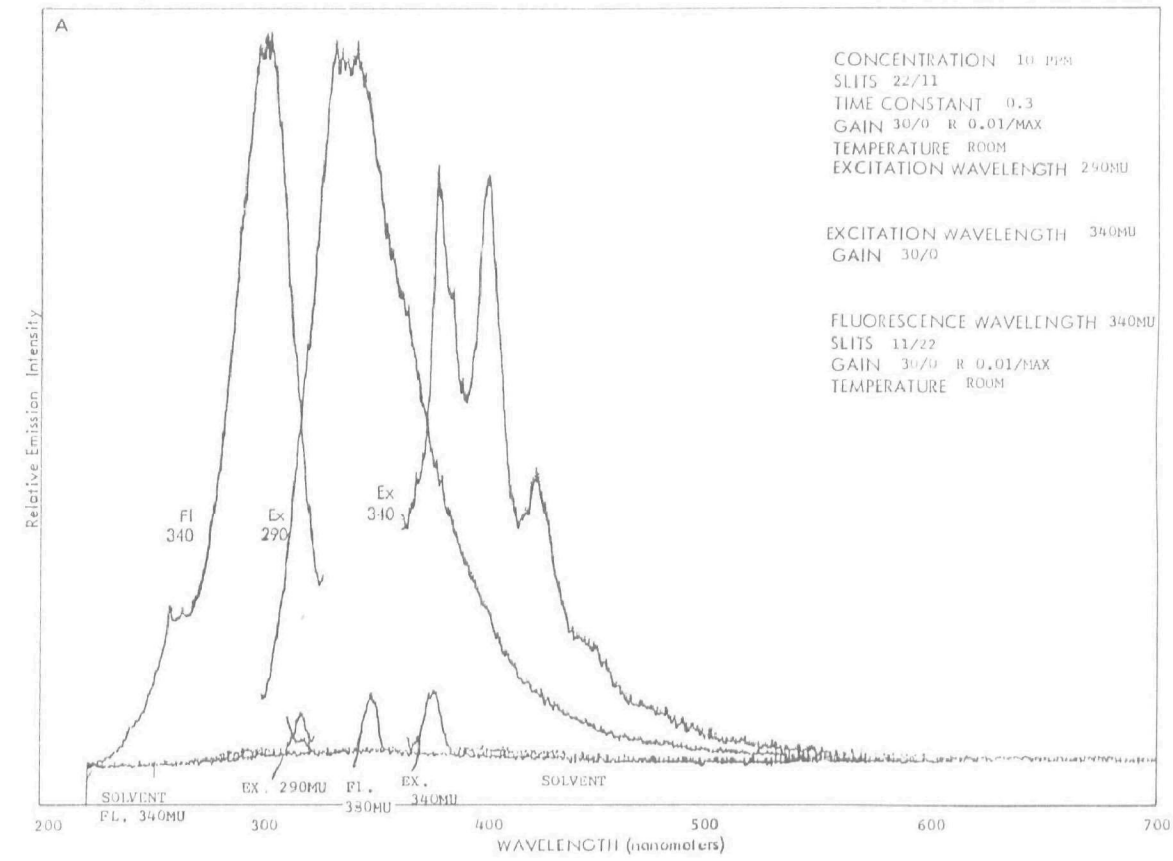
Phillips' general scheme (Figure 4) suggests a comprehensive passive tagging method. Identification of parameters that are unaffected by weathering and extension of the study to processed oils (distillates and residuals) must necessarily precede acceptance of this outline as a method. Use of adsorption chromatography and urea adduction are thought to be somewhat inconvenient.

The *WHOI System* is a ready-to-use passive tagging method requiring minimum sample pretreatment and using only one instrumental technique. WHOI provides explicit

Table 12: Oil Types[a] Tested by Baird-Atomic

Five Crude Oils
Two Weathered Crudes
Seven Number 6 Fuel Oils
Two Asphalts

a - Individual oils not identified.



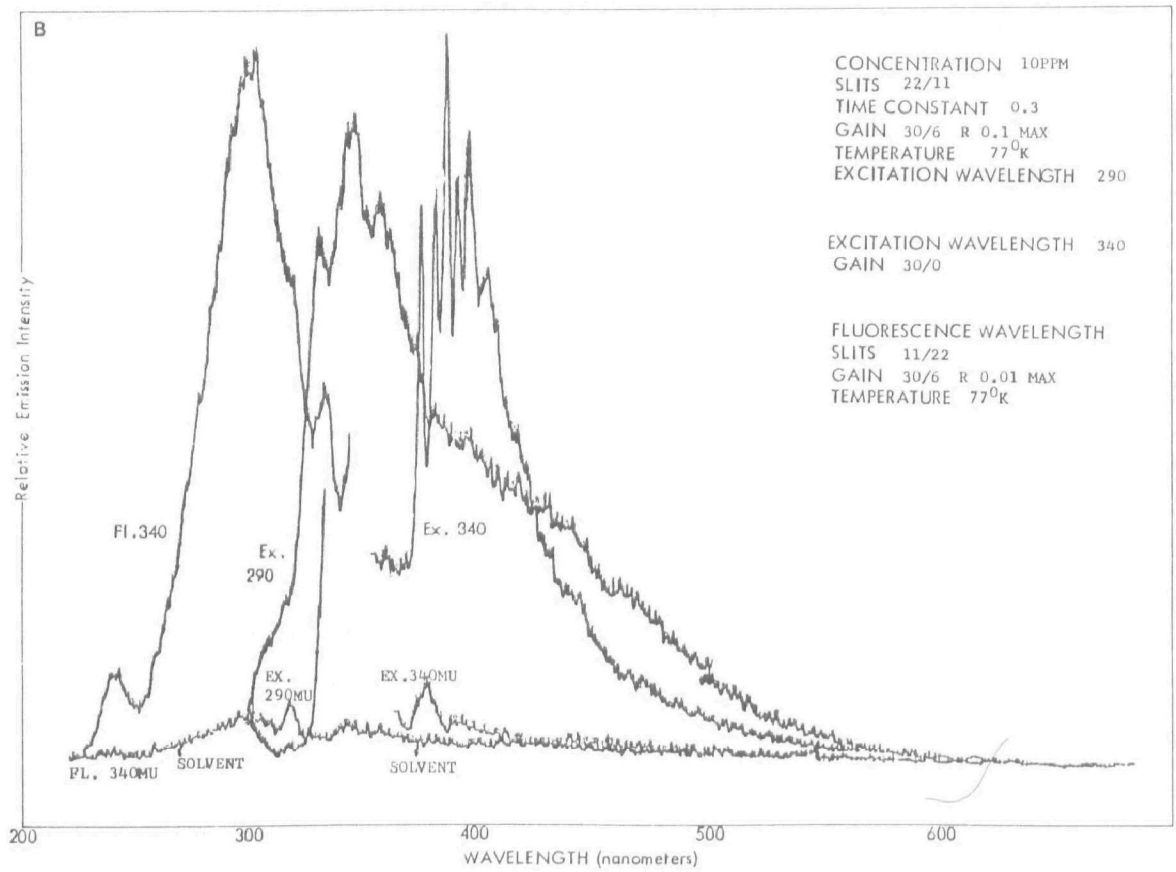


Figure 8: Fluorescence Excitation and Emission Spectra by Baird Atomic of a Crude Oil (a) at Room Temperature, and (b) at 77°K. Concentrations Are 10 ppm in Methylcyclohexane.

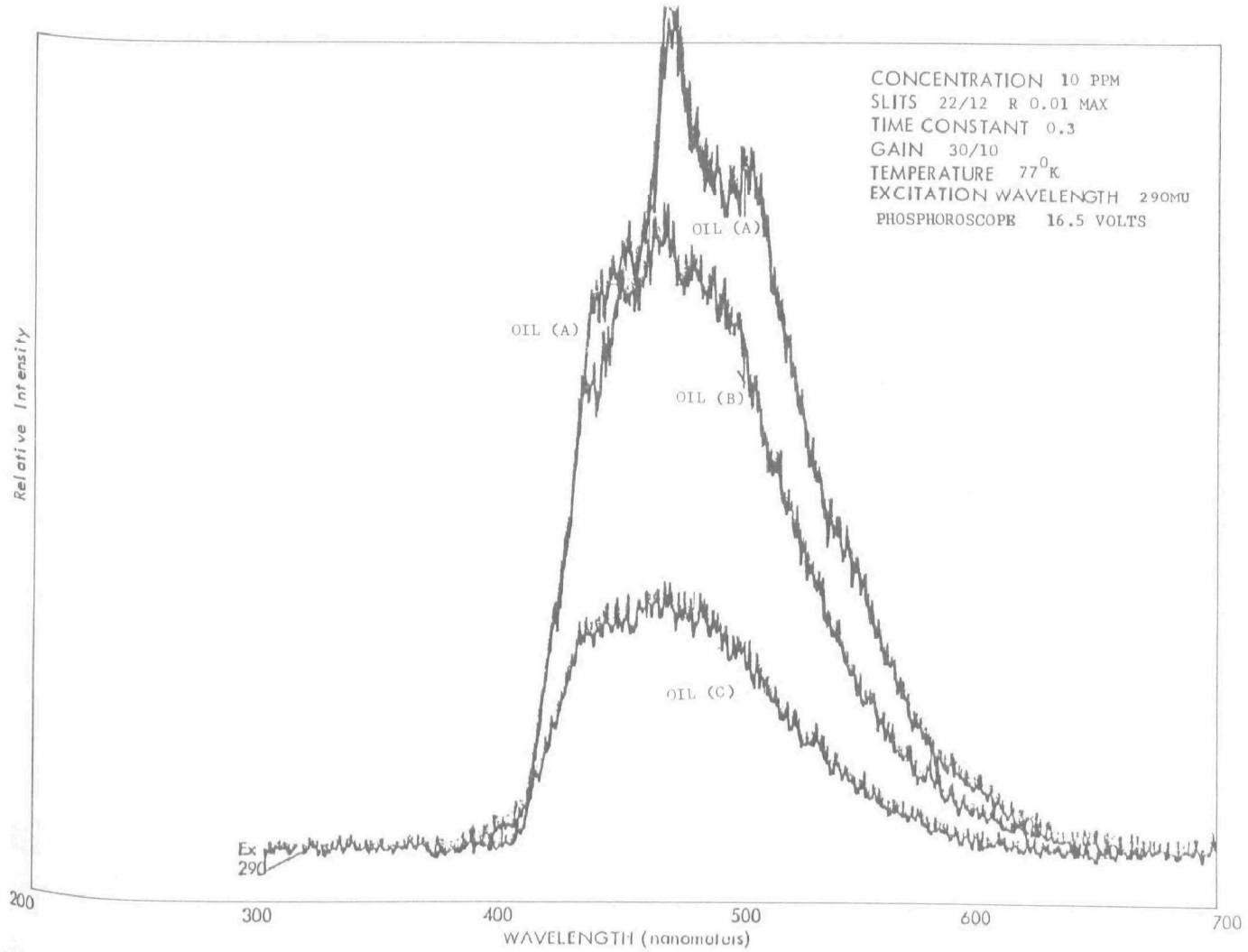


Figure 9: Phosphorescence Spectra of Three Different Number 6 Fuel Oils at 77°K by Baird Atomic. Concentrations Are 10 ppm in Methylcyclohexane.

instructions for separating oils from water, sand, and animal tissue matrices. The oils are then injected directly into the GC instrument in carbon disulfide solution. Unweathered suspect oils are also merely dissolved in carbon disulfide and injected. No preweathering, high speed centrifugation, adsorption chromatographic, or multi-instrumental techniques, such as those used by Esso and Phillips, are used by WHOI. The method requires some modification of most GC instruments, however. These are now generally available instruments in most analytical laboratories.

The WHOI Method was evaluated by using weathered and unweathered portions of oils supplied by EPA's Edison Laboratory. Most, but not all of the weathered oils were uniquely correlated; several correlations were only "probable". The WHOI Method, therefore, seems usable for comparisons to small numbers of standard oils and possibly for correlations with small libraries of oil indices. But, it may not achieve the "definite" correct correlations with extensive libraries of oil indices that should be achievable by the Esso Method, and by a method that may derive from the Phillips study. Use of additional fingerprint indices, such as nickel, vanadium, sulfur, and nitrogen content, in conjunction with the WHOI Method, should yield further improvement.

The requirement for support-coated open tubular (SCOT) columns, capillary injectors, glass liners, etc., may diminish the acceptability of this method. SCOT columns are unusually expensive GC columns (ca. $160) and have useful lifetimes that may not greatly exceed 200 injections. Excellent resolution and reproducibility of fingerprint indices are achieved. however; the usual packed GC columns yield inferior results. The use of capillary injectors and glass liners will require modification of many GC instruments.

The *Baird Atomic Study* is an evaluation of low temperature molecular emission as a technique for distinguishing among oils. Efforts are also made to identify individual fluorescing and phosphorescing components in oil and to maximize spectral differences among oils. An enhanced ability to distinguish between oils and to yield more detailed spectra, by cooling to 77°K, is demonstrated. But, Baird Atomic does not demonstrate the usability of their technique for correctly pairing weathered and unweathered oils; i.e., passive tagging.

Although this study does not yield a passive tagging method, it does demonstrate that low-temperature molecular emission is promising for this purpose. Equipment cost and operating complexity is commensurate with other commonly used analytical instruments. The technique should, therefore, find ready acceptance. From this point of view, the Baird Atomic technique, like the WHOI Method, appears more attractive for normal use than the Esso Method or Phillips

approach in their entirety. But, the effect of weathering on the low temperature molecular emission of oils has not yet been determined.

CONCLUSION

These EPA-sponsored studies were performed to evaluate many existing and suggested techniques for passive tagging oils. The gas chromatographic Method of WHOI and the fluorescence spectroscopic approach of Baird Atomic are readily usable in many "normal" laboratory facilities; the Esso Method and Phillips study encompass techniques that range beyond the capabilities of most laboratories. Consideration is given in these studies to "simple" methods that permit correct pairing of weathered and unweathered oils when dealing with a small selection of oils, and to more complex methods that may permit identification of totally unknown weathered oils by using extensive libraries of appropriate "fingerprint" indices. Evaluation of so many analytical techniques by these studies should facilitate the selection by other personnel of the most appropriate passive tagging procedures that suit individual situations and available facilities. These descriptions of the Esso and Phillips projects are preliminary and subject to change; final reports are not yet available to EPA at this time. Most tables and figures presented were extracted in whole or in part from investigators' reports.

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4. Woods Hole Oceanographic Institution (EPA Project Number 15080 HEC).

5. Baird Atomic Corporation (EPA Project Number 16020 GBW).

APPENDIX A

Crude Oils Tested By Phillips

Crude Oil	*Crude Oil Sample Number*	*County/Region*
Abu Dhabi	20	Offshore
Abu Dhabi	59	
Abu Dhabi	60	
Alaska	1	Cook Inlet
Alaska	2	Cook Inlet
Alaska	3	Cook Inlet
Alaska	4	Gulf of Alaska Shore
Alaska	5	North Slope
Alaska	6	North Slope
Algeria	21	
Algeria	22	
Algeria	23	
Algeria	24	
Argentina	70	
California	7	Off Shore St. Barbara
California	8	Los Angeles Co.
California	9	St. Barbara Co.
California	75	St. Barbara
California	76	St. Barbara
Canada	47	Alberta
Colombia	71	
Cuba	72	
Cuba	73	
Cuba	74	
Florida	51	Collier
Florida	52	Collier
Florida	53	Hendry
Florida	54	Lee
Florida	55	Hendry-Lee
Gabon	57	
Gabon	58	
Indonesia	25	Brunet
Indonesia	61	
Iran	26	
Iran	28	Offshore
Iran	29	Offshore
Iran	30	Offshore
Israel	62	
Israel	63	
Kuwait	64	
Kuwait Neu	31	
Kuwait Neu	27	Khasji
Libya	32	
Libya	33	Cyrenaica
Libya	34	
Louisiana	13	Timbalier Off Shore
Louisiana	14	Jackson Parish
Louisiana	77	Claiborne Parish
Louisiana	78	Offshore
Louisiana	79	Offshore
Louisiana	80	Offshore
Louisiana	81	Offshore
Mississippi	15	Wayne
Mississippi	16	Wayne
Mississippi	17	Clark
Mississippi	18	Jones
Mississippi	19	Jasper
Nigeria	35	
Nigeria	36	
Norway	37	North Sea
Norway	56	North Sea
Norway	82	
Qatar	65	
Qatar	66	
Saudi Arabia	67	
Saudi Arabia	68	
Saudi Arabia	69	
Texas	10	Yoakum & Gaines Co.
Texas	11	Nueces Co.
Texas	12	Brazoria Co.
United Arab Republic	41	
United Arab Republic	42	
United Arab Republic	43	
Venezuela	44	Lake Maracaibo
Venezuela	45	
Venezuela	46	Lake Maracaibo
Venezuela	50	Anzoategui

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Extraction of Dispersed Oils from Water for Quantitative Analysis by Infrared Spectrophotometry

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■ Some parameters that optimize the extraction of dispersed oils from water for quantitative analysis by infrared spectrophotometry (ir) are examined, and an improved extraction procedure is recommended. Trichlorotrifluoroethane, an essentially nonpoisonous solvent (1000 ppm TLV), is compared with carbon tetrachloride, which is highly poisonous to operating laboratory personnel (10 ppm TLV). Both solvents are usable for extracting dispersed oils from water, and for their quantitative analysis by ir, but trichlorotrifluoroethane is preferred. Changes in extraction efficiency following small additions of sulfuric acid and sodium chloride are examined. Great improvement results in extraction efficiency, but no further improvement derives from addition of more salt. Absorbance measurements are at 2930 cm^{-1} in 10-mm path length cells.

Many biological processes necessary for the survival of aquatic organisms may be adversely affected by the presence of extremely low levels—0.1 mg/l.—of petroleum oils in water. Jacobson (1972), for example, has shown that kerosine extracts in water, in the part-per-billion range, upset the chemotactic response of *Nassarius obsoletus* to oyster and scallop tissue. Identification of the particular oil and its quantitative determination in the water column are essential properly to monitor and assess potential biological damage resulting from oil spill incidents.

The development of a method that can be used to rapidly and completely extract dispersed oils from water for quantitative analysis by infrared spectrophotometry, and one that can be safely used in a mobile laboratory operating at the scene of a spill incident is described in this paper. Methods currently available for the quantitative analysis of petroleums dispersed in water are broadly identified as gravimetric and spectroscopic procedures.

Table I. Fraction of Oil Removed by Individual Extracts from Synthetic Dispersions Containing 5 Ml of 50% H_2SO_4 and 5 Grams of NaCl

No. of 25-ml extractions	Percent recovered[a]							
	South Louisiana Crude Oil		No. 2 Fuel Oil		Bachaquero Crude Oil		No. 6 Fuel Oil	
	Freon 113	CCl_4	Freon 113	CCl_4	Freon 113	CCl_4	Freon 113	CCl_4
1	92.6	94.4	97.2	97.8	90.0	95.4	91.1	92.2
2	99.3	99.7	99.5	100	98.7	99.5	98.7	98.6
3	99.8	100	100		99.7	99.9	100	100
4	100				100	100		

[a] Determined as $A/A_t \times 100 + B$: A, infrared absorbance at 2930 cm^{-1} due to the extract of interest; A_t, sum of absorbances at 2930 cm^{-1} of all the extracts (ΣA); B, total percent oil recovered by the previous extracts.

The gravimetric methods produce losses of the more volatile petroleum fractions making their use questionable for measuring light oils and distillates. Spectroscopic methods are inherently more sensitive and accurate, as indicated by Harva and Somersalo (1958). Infrared and ultraviolet procedures therefore seem to hold greater promise for yielding sensitive and accurate techniques.

The extraction of petroleum pollutants from water is a necessary part of quantitative analysis by either gravimetric or spectroscopic methods. Parameters that influence these extractions are evaluated in this study, and an optimum extraction scheme is presented. These parameters include the degree to which additions of acid and salt influence extraction efficiency and the utility of trichlorotrifluoroethane (Freon 113) and carbon tetrachloride (CCl_4) for such extractions; the latter solvent is highly toxic when inhaled (10 ppm TLV) or when absorbed through the skin (Sax, 1968).

The literature contains reports on the extraction of oils from water with carbon tetrachloride and trichlorotrifluoroethane, and acid and salt have previously been used to increase extraction efficiency. The American Petroleum Institute's spectroscopic procedure (1958) utilizes carbon tetrachloride as the extracting solvent, after addition of sulfuric acid and salt. Carbon tetrachloride is also used in a spectroscopic procedure developed by the Beckman Instrument Co. (1968) without, however, the addition of acid or salt. Freon 113 is used as the extracting solvent in a gravimetric procedure reported by the American Public Health Association (1971), following addition of sulfuric acid, but not salt. These methods suggest that there is a lack of uniformity and general agreement about the advantages of using acid and salt. They also do not examine the extent to which additions of these materials influence extraction efficiency, or the possibility of using Freon 113 for spectroscopic analyses. Freon is safer than carbon tetrachloride from the analyst's viewpoint.

In the present study four oils were used to compare the efficiencies of the two solvents and the influence of acid and salt: No. 2 Fuel Oil, which is a low-viscosity distillate oil (2.4 cSt at 100°F); No. 6 Fuel Oil, which is a high-viscosity residual oil (2300 cSt at 100°F); South Louisiana Crude Oil which has a low viscosity (4.8 cSt at 100°F); and Bachaquero Crude Oil which has a moderately high viscosity (1070 cSt at 100°F). Consecutive extractions of each oil from synthetic dispersions in water were carried out with each solvent; the quantity of oil in the individual extracts was monitored by measuring the oil absorbance band intensity at 2930 cm^{-1} in the ir spectral region (Figure 1). This band is not unique to oils, but derives from the CH_2 group that is common to many organics. Freon 113 and carbon tetrachloride yield minimal absorbance in the 2930 cm^{-1} region and are amenable for such analyses.

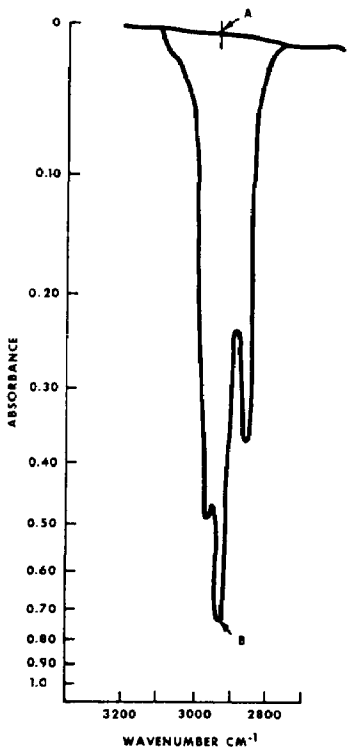

Figure 1. Infrared absorbance band of No. 2 Fuel Oil dissolved in Freon 113 (0.034% W/V), using 10-mm path length silica cells; Freon is in the reference beam

Absorbance at 2930 cm^{-1} is determined as the difference between points A and B

The changes in extraction efficiency that accompanied additions of sulfuric acid and sodium chloride were established by monitoring resulting changes in the quantity of oil separated by the individual extracts. The degree to which the dispersed oils were separated by each extract, following addition of acid and salt, is also estimated (Table 1). Since the tabulated results are derived solely from our synthetic oil-water dispersions however, they should not be extrapolated to other types of dispersions without further study.

Experimental

Apparatus. Perkin Elmer Model 457A and Beckman IR-33 infrared grating spectrophotometers were used for the determinations. Absorbance of the solutions was measured in 10-mm path length glass-stoppered rectangular silica cells (Beckman Instruments, Inc., Catalog No. 580015).

Reagents. Extractions were performed with Freon TF (Freon 113) solvent (E. I. Du Pont De Nemours and Com-

pany, Inc.), and carbon tetrachloride, spectroanalyzed (Fisher Scientific Co., Catalog no. C-199).

Procedure. Synthetic 1-liter oil-water samples containing identical concentrations of emulsified oil were prepared by shaking 5-ml portions of each test oil with 1½ liters of tap water for 1 min in 2-liter separatory funnels. The mixtures with No. 2 Fuel Oil, No. 6 Fuel Oil, and South Louisiana Crude Oil were allowed to separate undisturbed for 15 min, while the mixture with Bachaquero Crude Oil was allowed to separate for 1 hr. The homogeneous oil-water emulsions obtained as bottom layers were used to prepare the duplicate synthetic 1-liter samples. This was accomplished by mixing two 500-ml portions of each emulsion with an equal amount of tap water, in 2-liter separatory funnels. Subsequent extraction of the oils from the synthetic samples was also carried out in these funnels.

The relative extraction efficiencies of Freon 113 and carbon tetrachloride and the influence of acid and salt were examined by extracting the 1-liter synthetic samples with successive 25-ml portions of each solvent; acid and salt were added to some of the samples (Figures 2-4). The quantity of oil in each extract was monitored by measuring the oil absorbance band intensity at 2930 cm^{-1} in 10-mm path length silica cells (Figure 1). Extraction efficiencies and the effects of sulfuric acid and sodium chloride were determined by plotting the absorbance of each extract as a function of the number of 25-ml extractions (Figures 2-4). Removal of oil from water was considered complete only after attaining zero absorbance. That the absence of an infrared absorption band indicates "complete" extraction of oil was also shown by submitting residual water, emulsified with No. 2 Fuel Oil and extracted with Freon 113, to analysis by the gravimetric procedure of the American Public Health Association (1971). No oil was found in this water. The fraction of total emulsified oil separated by each extract was estimated by determin-

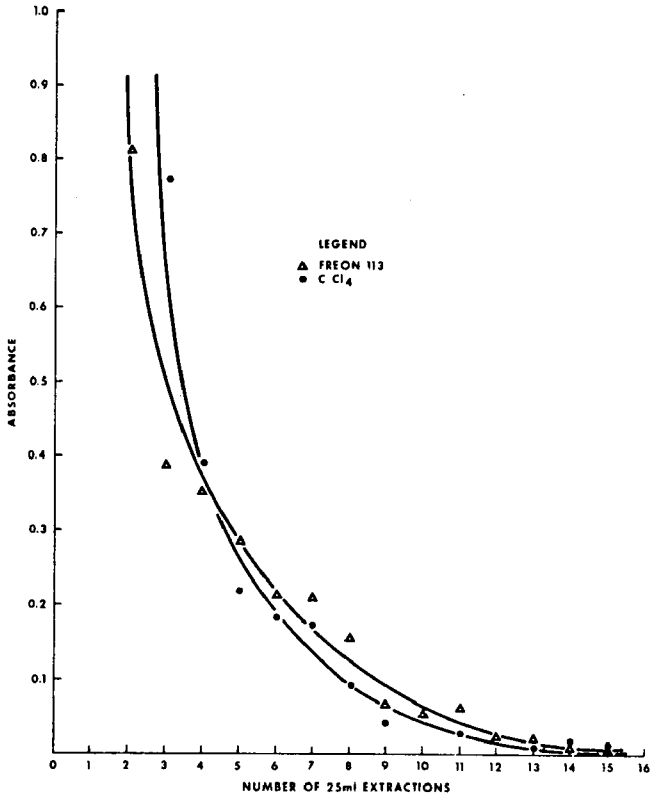

Figure 2. Number 2 Fuel Oil extracted from 1-liter duplicate synthetic oil-water samples containing no added acid or salt

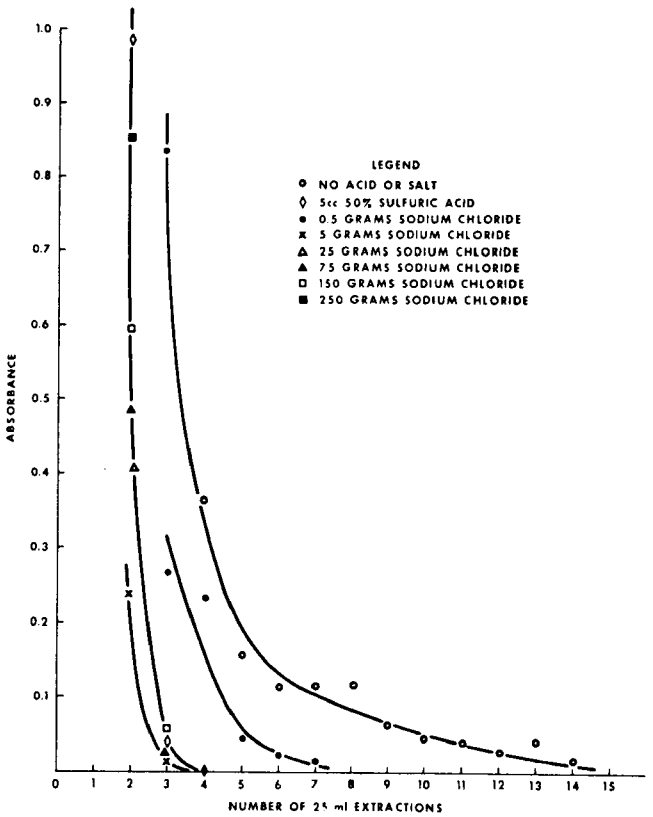

Figure 3. Number 2 Fuel Oil extracted from similar 1-liter synthetic oil-water samples containing acid or salt

Acidified sample is extracted with Freon 113; untreated sample and samples containing the indicated amounts of salt are extracted in CCl_4

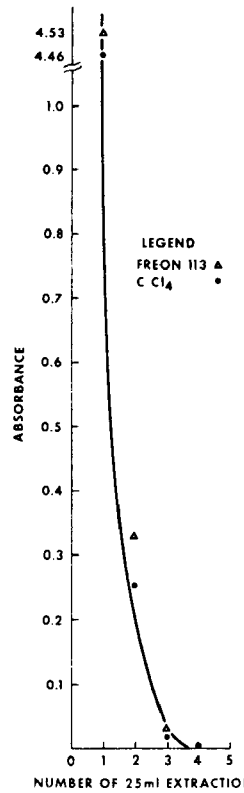

Figure 4. South Louisiana Crude Oil extracted from 1-liter duplicate synthetic oil-water samples containing 5 grams of sodium chloride and 5 ml 50% sulfuric acid

Absorbance intensities greater than 1.0 are calculated values

ing the ratio of its absorbance to the sum of the absorbances of all the extracts (Table I).

Results and Discussion

Freon 113 and carbon tetrachloride were found to be about equally effective for extracting the dispersed oils from water. Virtually the same number of extractions with each solvent effected removal of the oils (Figures 2 and 4). Additions of sulfuric acid and sodium chloride dramatically improved extraction efficiency. In the absence of these materials, the complete separation of No. 2 Fuel Oil was not possible even after 15 separate 25-ml extractions with carbon tetrachloride and Freon 113. However, complete separation of oil was achieved with only four extractions when 5 grams of sodium chloride and 5 ml of 50% sulfuric acid were added to the 1-liter synthetic samples. The addition of more than 5 grams of salt yielded no further improvement (Figures 2 and 3). Four 25-ml extractions with either solvent achieved complete separation of all the test oils when these quantities of acid and salt were added to the synthetic 1-liter samples (Figure 4). In these latter determinations, more than 90% of each emulsified oil was removed by the first extract (Table I).

Freon 113 is recommended as the solvent of choice for extracting dispersed oils from water, because it is virtually as efficient for these extractions and as usable for the infrared determination of oil as carbon tetrachloride, but is much less poisonous to laboratory personnel. It is especially preferable to carbon tetrachloride in situations where adequate ventilation may be lacking, such as in some mobile laboratory and field use.

The recommended procedure for extracting dispersed oils from water is the addition of 5 ml of 50% sulfuric acid and 5 grams of sodium chloride to 1-liter samples. Extraction should be carried out with four 25-ml portions of Freon 113 in 2-liter separatory funnels. Checks for acidity (below pH 3) and completeness of extraction should be performed. Initial dilution is to 100 ml. Seawater samples are an exception because they already contain adequate salt and can therefore probably be analyzed without addition of sodium chloride. Such samples were not examined in the present study, however. A "blank" determination of the reagents and water should be performed to prevent interference with the oil measurement at 2930 cm^{-1} by extraneous solvent extractable organics.

Acknowledgment

Special thanks are given to Joseph Lafornara for providing valuable background information, and to Henry Jeleniewski, Michael Killeen, Susan Rattner, and Peter Furth for their assistance.

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REVIEW OF METHODS & STANDARDS USED FOR OIL IN BRINE ANALYSIS BY CONTRACTING LABORATORIES ON THE LOUISIANA COAST

by

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INTRODUCTION

The increased demand for energy is expanding the exploration and development of offshore petroleum production. Wastes generated by offshore drilling operations and discharged into the marine environment are therefore an increasing menace and are of vital concern to the Environmental Protection Agency (EPA). Production platforms on the Outer Continental Shelf offshore Louisiana alone produce ca. 410,000 barrels of waste water (brine) per day[1]; by 1983 this figure will have risen to and estimated 1.54 million barrels per day[2]. These brines contain oils, toxic metals, a variety of salts, solids and organic chemicals. To curb the indiscriminant discharge of these pollutants into our environment, the Federal Water, Pollution Control Act Amendments of 1972 require that anyone discharging wastes into the nation's waters from a point source must acquire a permit. Once a discharger has applied for a permit, EPA and State pollution control officials decide on effluent limits and conditions after soliciting and considering public comments. In addition to meeting effluent limitations, dischargers are also required to monitor and analyze their discharge continuously and report the results to the State Agency and EPA. The explicit numerical limits for petroleum derived oils are listed in Table I[3].

Petroleum values reported by the offshore oil industry are usually determined by contracting laboratories. To establish the merit of these values the EPA recently conducted a "Field Verification" study. I participated in this study and reviewed analytical methods used by contracting laboratories on the Louisiana coast. In this presentation I will discuss and evaluate these methods and examine the necessary standards.

DISCUSSION

The methods used by the Louisiana laboratories for offshore brine analyses basically entail extraction of the oil and determination by one of four techniques, i.e., infrared, gravimetric, ultraviolet fluorescence and colorimetric techniques. However, the accuracy of these techniques varies widely. Factors such as brine characteristics, physical and chemical properties of oil and standards used, all influence their accuracy. The extent to which each technique is affected is primarily governed by the oil constituents which are measured. Ideally, the determinative technique measures all, and only, the oil constituents. However, due to the complex chemical composition inherent to petroleum oil, each technique measures only some specific oil constituents. In the case of the infrared, fluorescence and colorimetric techniques the constituents measured are then used as a "handle" for determining the total oil.

Table II lists the determinative techniques, the respective constituents measured and the primary factors influencing the accuracy of each technique.

Standards play a critical role in all but the gravimetric technique. It appears, therefore, that this technique is more universally applicable than the others. Its accuracy however is not on par with the fluorescence and infrared techniques. A recent collaborative study conducted by the American Society for Testing and Materials (ASTM) shows that the gravimetric results were consistently much lower. This is illustrated in Table III, which shows the results obtained by our laboratory using both the infrared and gravimetric techniques.

As a consequence of measuring only a fraction of the total oil constituents and using it as a "handle", the infrared, fluorescence and colorimetric techniques need standard oils of comparable composition to the sample oil for accurate results. Any imbalance in the ratios of components being measured to the total composition in the standard and sample oil results in either erroneously low or high results.

The standard of choice is an oil identical to the oil being measured. However, the availability of such source oil standards is not always assured, necessitating the use of a substitute. In the case of the Louisiana laboratories, an oil of different identity than the sample oil, was often used. This recourse critically affects the accuracy of results obtained with the fluorescence and colorimetric techniques. Both techniques use only minor oil constituents as a "handle" for total oil determinations. Slight variations in the composition of these constituents in the oil will therefore significantly affect the accuracy.

Conversely, the infrared technique uses the major constituents of oil as a "handle". Its accuracy in comparison is therefore only slightly impaired by compositional variations of oil. The infrared technique also allows the use of a synthetic or arbitrary standard consisting

of a mixture of isooctane, hexadecane (cetane) and benzene. The merit of this standard is based on the assumption that the infrared absorptivities of oils from different sources are very close and are similar to the synthetic standard absorptivity. Gruenfeld[4] and others[5,6] have shown that the absorptivity of a large variety of oils are in fact very similar and close to the synthetic standard.

An alternate approach suitable for the infrared technique and routinely used in our laboratory entails the derivation of standard oil from the sample itself. The standard oil is prepared by stripping the solvent from the sample extract. Although some volatility losses are incurred during solvent stripping they do not significantly alter the ratio of components measured by infrared to the total oil composition. The availability of an adequate oil standard is thereby always assured.

CONCLUSIONS

This study has shown that the concentration of oil in brine is defined by the determinative technique used. Only minor and specific component classes of oils are measured by the fluorescence and colorimetric techniques. Accurate quantitation of oil in brine samples, to assure compliance with Federal effluent limitations, is therefore only attained by using standards of identical compositions as the sample oils. In situations where such standards are unavailable, the infrared technique was found to provide more accurate results because a major class of oil components (paraffinic hydrocarbons) is measured. The gravimetric technique does not require standards and is applicable in situations where the loss of volatile oil components does not affect the accuracy

of results. The technique is not applicable to measurement of oils that contain significant amounts of petroleum compounds that volatilize at temperatures below 70°C.

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TABLE I. Effluent Limitations for Point Sources

Petroleum Derived Oil - mg/l

	Maximum for any one day	Average of daily values for thirty consecutive days shall not exceed
	Compliance by July 1, 1977	
Near Offshore produced water	72	48
Far Offshore produced water	72	48
	Compliance by July 1, 1983	
Near Offshore produced water	No discharge	
Far Offshore produced water	52	30

TABLE II. Determinative Techniques Used by the Louisiana Laboratories

Determinative Technique	Main Constituents Measured	Factors Affecting Measurements
Infrared	Paraffinic Hydrocarbons	Standards Used
Gravimetric	Non-volatile, Residual Oil Constituents	Volatility of Oil
Ultraviolet - Fluorescence	Polynuclear Aromatic Compounds	Standards Used
Colorimetric	Dark Colored Oil Constituents	Color of Oil, Standards Used

TABLE III. IERL Results of ASTM Collaborative Study

Mg/l of Petroleum Hydrocarbons

Sample No.	Infrared	Gravimetric	Avg. of 28 Labs Infrared
1	261	215	261
2	272	221	263
3	130	70.8	131
4	118	82.5	110
5	47.8	26.3	49
6	26.8	24.2	21

Michael Gruenfeld[1]

Quantitative Analysis of Petroleum Oil Pollutants by Infrared Spectrophotometry

REFERENCE: Gruenfeld, Michael, "**Quantitative Analysis of Petroleum Oil Pollutants by Infrared Spectrophotometry,**" *Water Quality Parameters, ASTM STP 573*, American Society for Testing and Materials, 1975, pp. 290-308.

ABSTRACT: The accuracy and sensitivity of infrared spectrophotometry are evaluated for the quantitative analysis of water dispersed oils, by single point analysis. Absorbance versus concentration (Beer-Bouguer Law) plots are prepared for viscous and nonviscous crude and processed oils in Freon 113, carbon tetrachloride, and in a mixture of these solvents. Absorbances at 2930/cm are measured in 10 and 100-mm path length cells, with and without ordinate scale expansion. Solution concentrations in the range 0.5 to 40 mg/100 ml oil in solvent yield linear plots that pass through the origin. The concentration 0.05 mg/100 ml oil in solvent yields a recognizable absorption band at approximately 2930/cm when measured in 100-mm path length cells with ordinate scale expansion ×5. This is considered the practical detection limit of these oils by the infrared (IR) technique. Stability of oil absorptivities following solution storage, and use of IR absorptivities for oil identification are also examined briefly.

KEY WORDS: water quality, oils, infrared spectrophotometers, spectrophotometry, environmental tests, water pollution

Improved methods for the quantitative analysis of water dispersed petroleum oils are under investigation by the Industrial Waste Treatment Research Laboratory of the National Environmental Research Center (NERC), Cincinnati. Such methods are needed to evaluate the performance of oil-water separator devices, to measure the dispersed oil concentration in water below surface floating oil slicks, to monitor oil concentrations in effluent waters of various industrial plants and watercraft, and to support studies of the effect of water dispersed petroleum oils on the biosphere. That low concentrations of water dispersed oils adversely affect living organisms is well known: for example, Jacobson [*1*][2] has shown that kerosene extracts in water, in the parts-per-billion range,

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[2] The italic numbers in brackets refer to the list of references appended to this paper.

upset the chemotactic response of *Nassarius Obsoletus* to oyster and scallop tissue.

Most methods that are currently used for the quantitative analysis of water dispersed oils can be classified as gravimetric or spectroscopic procedures. The determinative step of gravimetric methods is simple weighing, while the determinative step of spectroscopic methods is the measurement of radiation absorption by dissolved oils. Solvent extraction is commonly used by gravimetric and spectroscopic methods to isolate and concentrate water dispersed oils for quantitative measurement. Solvent evaporation (stripping), which precedes weighing in gravimetric methods, has a drawback because the more volatile petroleum fractions are lost. A gravimetric method by the American Public Health Association [*2*] illustrates yet another drawback: questionable sensitivity and accuracy are achieved by weighing minute oil residues in "large" (approximately 125 ml) distilling flasks. Harva and Somersalo [*3*] conclude that the spectroscopic methods are more accurate and sensitive—a justifiable conclusion.

Infrared spectrophotometry (IR) is the most commonly used spectroscopic technique for the quantitative analysis of water dispersed oils. In an American Petroleum Institute procedure [*4*] dispersed oils are extracted from water with carbon tetrachloride; a mechanical shaking apparatus and a 5-liter extraction flask are used. The absorption band maxima of oil at 2850 and 2930/cm are measured, using 10-mm path length quartz cells. Oil concentrations are determined by comparing the sum of these absorbances to the sum of absorbances derived from a prepared carbon tetrachloride solution containing an accurately known concentration of the oil. The latter solution is the standard solution, and the determination is performed by single point analysis. These terms are discussed later. In this method a blend of hydrocarbons (37.5 percent isooctane, 37.5 percent cetane, and 25 percent benzene) that is thought to approximate the IR absorptivity of an average petroleum oil is used to prepare the standard solution when a portion of the dispersed oil is not available.

In an IR method by Beckman Instruments, Inc. [*5*], 100-ml of dispersed oil in water samples are extracted with 2-ml carbon tetrachloride, using hand-held separatory funnels. The absorption band maximum of oil at 2930/cm is measured as in the previous method, using 10-mm path length near IR silica cells. The 2850/cm band is not used. Reference solutions are prepared as oil in water dispersions having known oil content, and each of these dispersions is extracted with 2-ml carbon tetrachloride. The extracts are then measured at 2930/cm, and a concentration versus absorbance plot is derived. This plot is used to quantitate dispersed oil in water samples.

The two preceding methods utilize the same extraction solvent, but they differ in most other respects. The method by the American Petroleum Institute uses a large oil extraction flask and a mechanical shaker; salt

and acid are added; extraction is performed with successive 100-ml portions of carbon tetrachloride; two IR absorption band maxima are measured; and comparison is made to only a single standard solution. The Beckman Instrument method uses a small hand-held separatory funnel; one 2-ml portion of carbon tetrachloride is used to extract a 100-ml sample volume; acid or salt are not added; one IR absorption band maximum is measured; and a concentration versus absorbance plot derived from several oil-in-water reference solutions is used for sample determination. These methods thus differ in the type of apparatus used, sample and solvent volumes, addition of salt and acid, and the method of IR measurement and data handling. Gruenfeld [*6*] recently investigated some of these parameters in detail. He examined the influence of salt and acid, and compared the extraction efficiencies of carbon tetrachloride and Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane). Freon 113, like carbon tetrachloride, can be used for IR measurement of oil at 2930/cm. Gruenfeld [*6*] extracts 1-liter dispersed oil in water samples with four consecutive 25-ml portions of solvent, using 2-liter hand-held separatory funnels. Use of Freon 113 is of special interest because it is far less poisonous to exposed personnel than carbon tetrachloride. According to Sax [*7*], carbon tetrachloride is highly toxic (10 ppm TLV) when inhaled or absorbed through the skin, while Freon 113 is much safer (1000 ppm TLV).

The present paper describes a study of the accuracy and sensitivity of the IR technique, when used for the quantitative determination of petroleum oils by single point analysis. Carbon tetrachloride, Freon 113, and a mixture of these solvents are used. The detection limit of oils by IR, the stability of oil absorptivities during prolonged solution storage, and the utility of these absorptivities for oil identification are also examined. Four oils were used: No. 2 Fuel Oil, which is a low viscosity distillate oil (2.4 cSt at 100°F); Number 6 Fuel Oil, which is a high viscosity residual oil (2300 cSt at 100°F); South Louisiana Crude Oil, which has a low viscosity (4.8 cSt at 100°F); and Bachaquero Crude Oil, which has a moderately high viscosity (1070 cSt at 100°F). IR measurements were made in 10 and 100-mm path length silica cells, at approximately 2930/cm. The oils, solvents, final solution volumes, and cells were selected in order to correlate this work with the previously reported investigation by Gruenfeld [*6*].

Atwood et al [*8*] reported a somewhat similar, though more limited study of IR for the quantitative analysis of oils. A blend of hydrocarbons (isooctane, hexadecane, and benzene) was used to represent "typical" oils, and carbon tetrachloride solutions of this blend were measured in 10 and 100-mm path length quartz cells. No actual oils, or solvents other than carbon tetrachloride were used. An effort was not made to establish whether concentration versus absorbance (Beer-Bouguer Law) plots of oils

are linear and pass through the origin; that is, whether oils can be quantitatively determined by single point analysis.

Experimental

Apparatus

A Perkin Elmer Model 457A infrared grating spectrophotometer[3] was used for the determinations. Solution absorbances were measured in 10-mm (Beckman Instruments, Inc., Catalog No. 580015) and 100-mm (Fisher Scientific Co., Catalog No. 14-385-930F) path length cells. These are rectangular silica and cylindrical Supracil cells, respectively. Cell holders obtained from the Perkin Elmer Corporation (Catalog No. 186-0091) were used for the 10-mm path length cells, while holders obtained from International Crystal Laboratories (Catalog No. R 100-22 with Teflon gaskets as spacers) were used for the 100-mm path length cells.

Reagents

The oil solutions were prepared in spectroanalyzed carbon tetrachloride (Fisher Scientific Co., Catalog No. C-199), Freon 113 solvent (E. I. DuPont De Nemours and Company, Inc.), and in a 98 percent Freon 113/2 percent carbon tetrachloride (by volume) solvent mixture. Freon 113 is a DuPont refrigerant. It is 1,1,2-trichloro-1,2,2-trifluoroethane, of specified purity. This isomer of trichlorotrifluoroethane is available from several manufacturers, under a variety of trade names.

Procedure

Oil solution concentrations were adjusted to yield absorbances that were within the ordinate scale range of the IR chart paper. Measurements were made with matched 10 and 100-mm path length cells, without ordinate scale expansion, and with ordinate scale expansion ×5. Measurements without scale expansion required oil concentrations of 2 to 40 mg/100 ml and 0.5 to 4.0 mg/100 ml for measurements in the 10 and 100-mm path length cells, respectively. Measurements with ordinate scale expansion ×5 required concentrations of 0.5 to 4.0 mg/100 ml and 0.05 to 0.4 mg/100 ml for measurements in the 10 and 100-mm path length cells, respectively. All the solutions contained accurately known oil concentrations. The reference cell was filled with solvent from the same reagent bottle that was used to prepare the oil solution. Absorbances derived from measurements without scale expansion were read directly from a nonlinear absorbance type chart paper. These absorbances were measured as vertical distances between the 2930/cm absorption band maximum of oil and a baseline

[3]Mention of trade names or commercial products does not constitute endorsement by the U.S. Government.

drawn tangent to absorption minima adjoining the band maximum (Fig. 1). Absorbances that were determined by using ordinate scale expansion ×5 required use of a linear transmittance type chart paper, and calculation by special equation (Fig. 2). The solvents used were Freon 113, carbon tetrachloride, and 98 percent Freon 113/2 percent carbon tetrachloride (by volume). Absorbance versus concentration (Beer-Bouguer Law) plots of the oils in the solvents were derived.

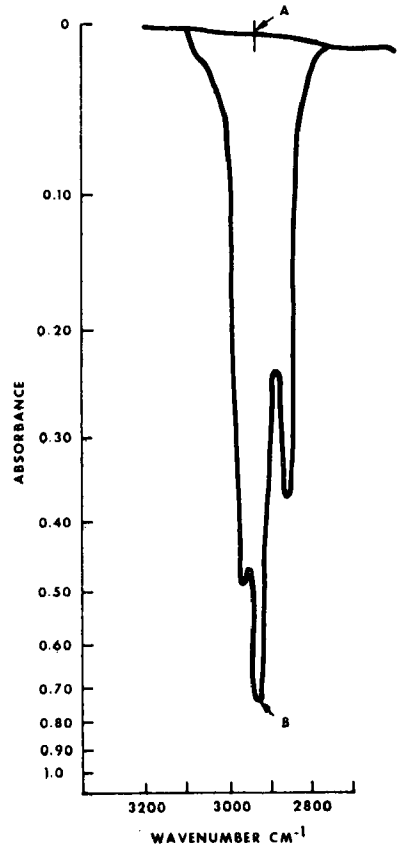
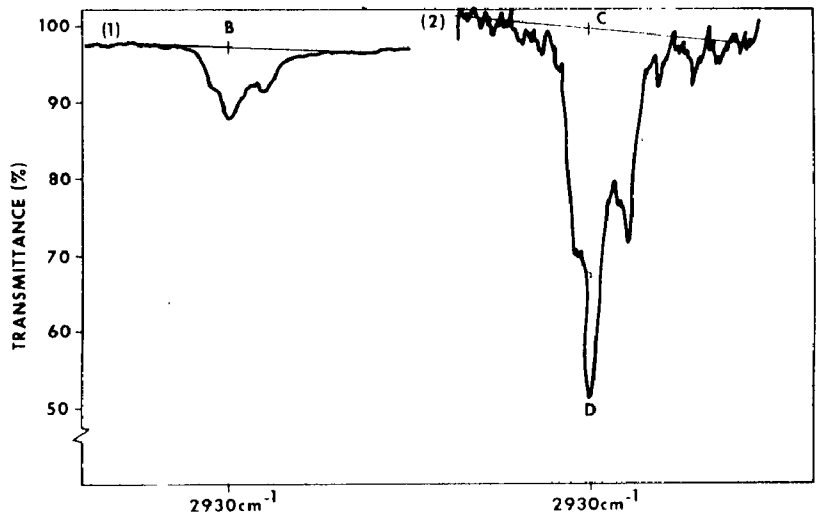


FIG. 1—*Infrared absorption band of No. 2 Fuel Oil dissolved in Freon 113 (0.034% w/v), using 10-mm path length silica cells; Freon 113 is in the reference beam. Absorbance at 2930/cm is determined as the difference between Points A and B.*

The utility of IR absorptivities as a parameter for identifying weathered oils was also briefly investigated. Diverging slopes of Beer-Bouguer Law plots illustrate differences in oil absorptivities. $E_{10mm}^{1\%}$ values (absorbances measured in 10-mm path length cells, normalized to 1 percent weight/ volume dissolved oil) of the oils were calculated and evaluated for stability, following loss of volatile oil components as a consequence of



$$\text{Absorbance} = \log_{10} \frac{5B}{5B + D - C}$$

B = % T of the unexpanded baseline
C = % T of the expanded baseline
D = % T of the expanded peak maximum

FIG. 2—*Infrared absorption band of No. 2 Fuel Oil dissolved in Freon 113: (1) without ordinate scale expansion, and (2) with ordinate scale expansion ×5.*

solvent distillation (stripping). An oil solution was stripped free of solvent, following IR measurement in 10-mm path length cells. The solvent was discarded and the oil residue weighed. The residue was then redissolved, diluted to the previous volume (100-ml), and the solution measured again by IR. The new $E_{10mm}^{1\%}$ absorptivity was compared to the original value. The solvent stripping procedure of the American Public Health Association [*2*] was used.

A brief investigation was also made of the stability of oil absorptivities following prolonged solution storage under normal room light and temperature conditions. Oil solutions were prepared in carbon tetrachloride, Freon 113, and in the 98 percent/2 percent solvent mixture. South Louisiana Crude and No. 2 Fuel Oils were used; solution concentrations were 2 and 10 mg/100 ml. Following the initial absorbance measurements, the solutions were stored for eight days on an exposed laboratory shelf, in clear-glass tightly sealed 100-ml volumetric flasks. The absorbance measurements were then repeated and compared with the original values.

Results and Discussions

The major purpose of this study was to assess the utility of IR for the

quantitative determination of petroleum oils, by single point analysis. Solutions of four representative oils in two solvents, and in a mixture of these solvents, were used to prepare the appropriate absorbance versus concentration (Beer-Bouguer Law) plots. Quantitative determination by single point analysis requires linear plots that pass through the origin. Use of single point analysis usually offers a considerable time saving because only one standard solution is used for each analysis, rather than a Beer-Bouguer Law plot derived from several solutions. The term standard solution designates a solution containing an accurately known concentration of oil with the same identity as the dispersed oil in the water sample. The following equation is used for single point analysis

$$C_x = C_s \cdot \frac{A_x}{A_s}$$

where

C_x = the unknown oil concentration of the sample extract used for IR measurement;

A_x and A_s = the absorbances of the sample extract and standard solution, respectively; and

C_s = the standard solution concentration used for IR measurement.

The measurements were made in cells with identical path lengths, and using identical ordinate scale expansion settings. The four oils selected for study were thought to be fairly representative of viscous and nonviscous crude and processed petroleum oils. The choice of solvents was determined by the previously cited publications, and by consideration of oil solubility, IR absorptivity, and solvent toxicity.

Carbon tetrachloride is used in the previously described IR methods by the American Petroleum Institute [*4*], Beckman Instruments, Inc. [*5*], and Atwood et al [*8*]. As previously indicated, this solvent is highly toxic. Freon 113 is used in a gravimetric procedure by the American Public Health Association [*2*], and is much safer than carbon tetrachloride. Freon 113 is evaluated in the present study for IR measurement of oils. Gruenfeld [*6*] compared the ability of these solvents to extract dispersed oils from water. He found them equally effective for extracting small quantities (less than 200 ppm) of water dispersed oils. Although Freon 113 is adequate for extracting low concentrations of water dispersed oils, it does not readily dissolve (cut) undispersed viscous oils such as No. 6 Fuel and Bachaquero Crude oils. Freon 113 is therefore not recommended for preparing IR standard solutions of viscous oils. This problem is solved by first dissolving a sufficient amount of viscous oil in carbon tetrachloride to yield an accurately known concentration approximating 20 mg/ml. This solution (2.0-ml) is then diluted to 100-ml with Freon 113, to yield a final 98 percent Freon 113/2 percent carbon tetrachloride mixture that contains

approximately 40-mg oil. A fresh portion of the 98 percent/2percent solvent mixture is used for further dilutions. These standard solutions will be discussed further.

The oils used in the present study were dissolved directly in the solvents, to yield solutions having accurately known concentrations. These solutions simulate solvent extracts of actual dispersed oil in water samples. Beer-Bouguer Law plots were prepared and evaluated for compliance with the criteria for single point analysis; that is, plots that are linear and pass through the origin. Solutions of the four oils in the two solvents and in the solvent mixture were measured by IR. A range of concentrations was examined in 10 and 100-mm path length cells, with and without ordinate scale expansion. Linear plots that pass through the origin were obtained for the four oils in carbon tetrachloride (Fig. 3) and in 98 percent Freon

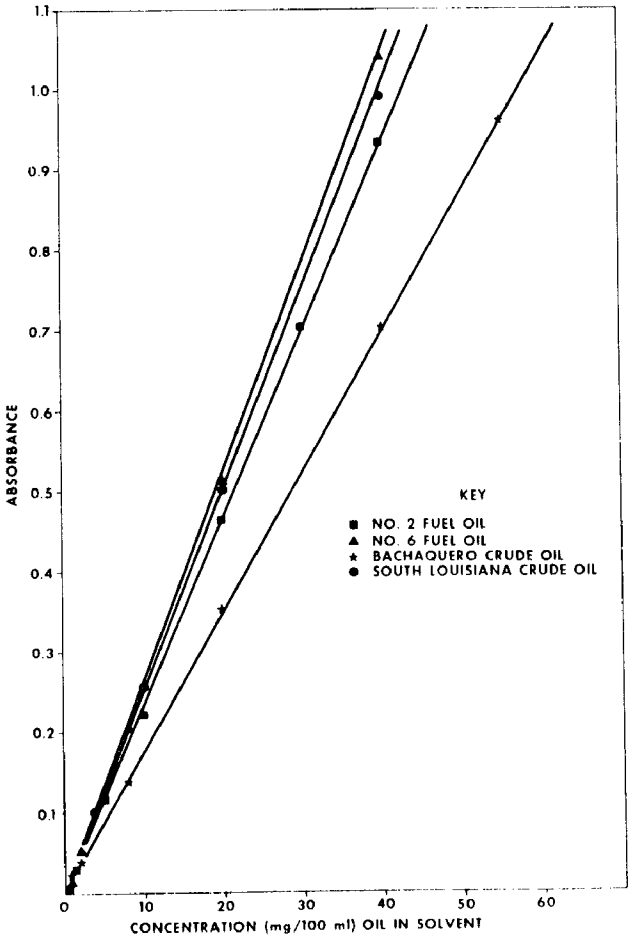


FIG. 3—*Oil solutions in carbon tetrachloride measured in 10-mm path length cells without ordinate scale expansion.*

113/2 percent carbon tetrachloride (Fig. 4), and for the two less viscous oils in Freon 113 (Fig. 5). Number 6 Fuel and Bachaquero Crude oils were not readily soluble in Freon 113. Therefore, they were dissolved initially in carbon tetrachloride, and then diluted further with Freon 113, by the previously described procedure, to yield a final 98 percent Freon 113/2 percent carbon tetrachloride solvent mixture. The potential absorptivities of viscous oils in Freon 113 are thought to match their absorptivities in the solvent mixture, because Beer-Bouguer Law plots of the nonviscous oils in Freon 113 yield slopes that match the slopes of these oils in 98 percent Freon 113/2 percent carbon tetrachloride (Figs. 4, 5, and 6). Preparation of standard solutions in 98 percent Freon 113/2

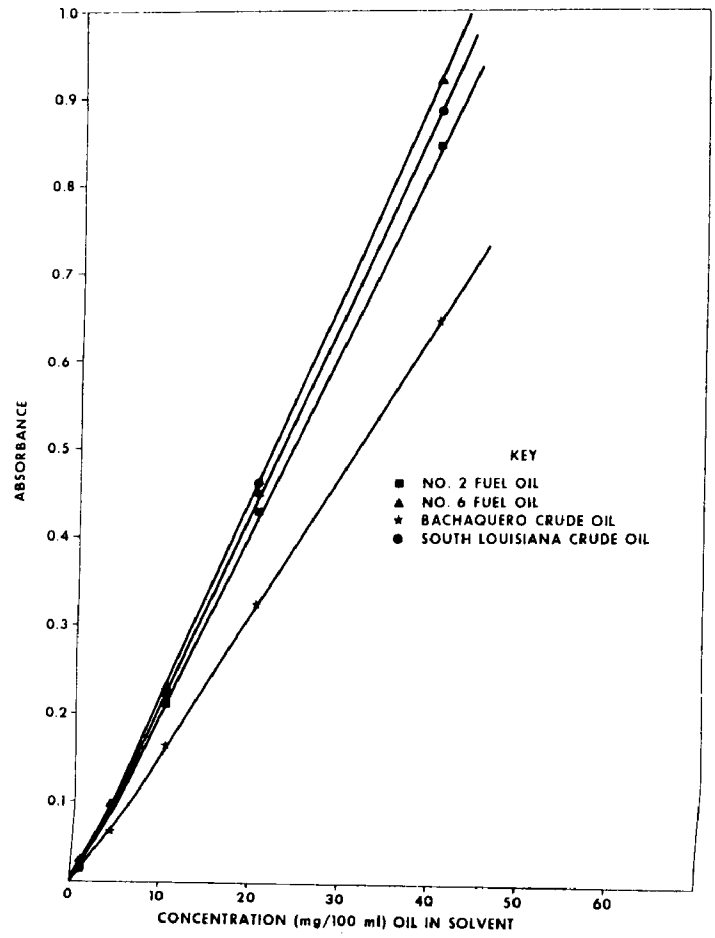


FIG. 4—*Oil solutions in 98 percent Freon 113/2 percent carbon tetrachloride measured in 10-mm path length cells without ordinate scale expansion.*

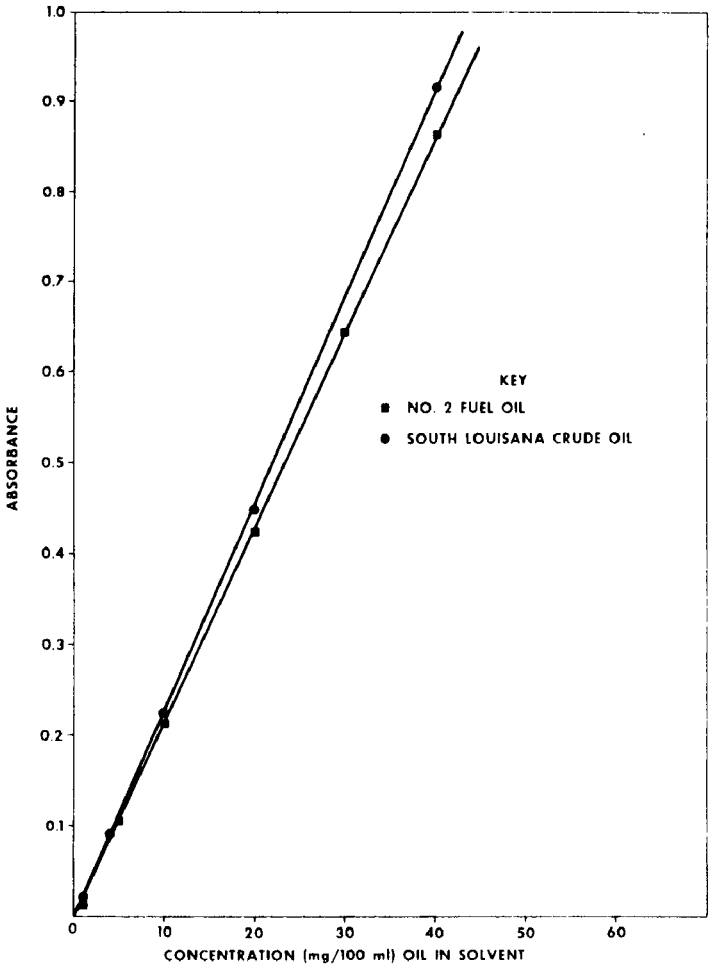


FIG. 5—*Oil solutions in Freon 113 measured in 10-mm path length cells without ordinate scale expansion.*

percent carbon tetrachloride is recommended whenever Freon 113 is used as the extraction solvent for quantitating dispersed oil in water samples.

Use of IR for the quantitation of water dispersed oils at concentrations below 1 ppm, by single point analysis, was also examined. Appropriate Beer-Bouguer Law plots were derived from measurements in 100-mm path length cells without ordinate scale expansion, using solutions of the four oils in carbon tetrachloride (Fig. 7), in 98 percent Freon 113/2 percent carbon tetrachloride (Fig. 8), and solutions of the two less viscous oils in Freon 113 (Fig. 9). While some of the plots exhibit deviations from linearity above 0.7 absorbance, all the plots are linear below this value and pass through the origin. In some cases, portions of an oil used for

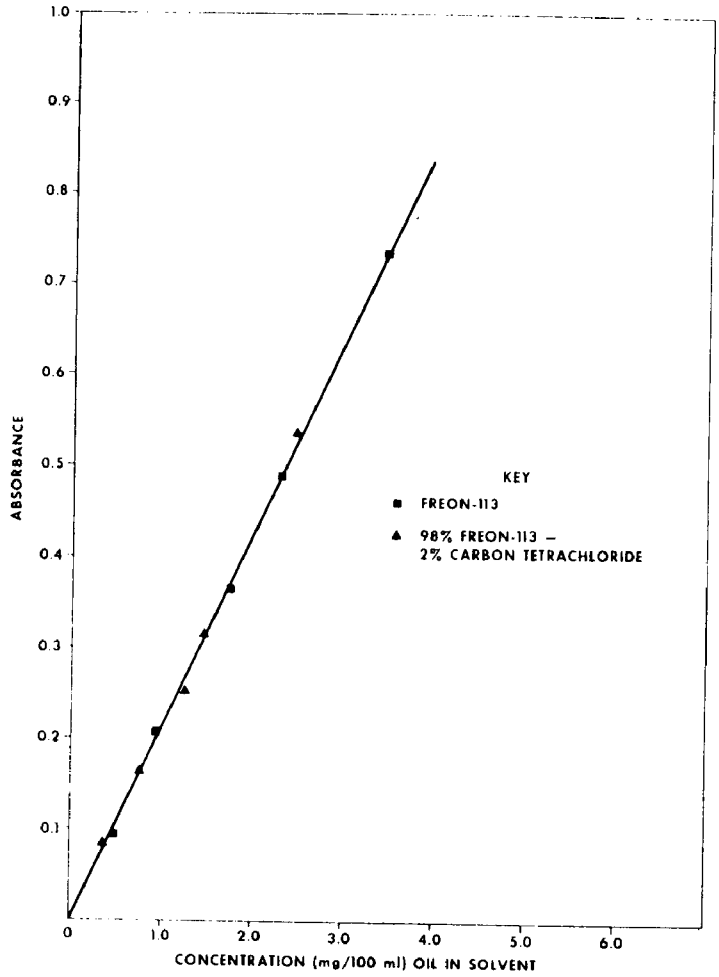


FIG. 6—*Solutions of No. 2 Fuel Oil measured in 100-mm path length cells without ordinate scale expansion.*

measurements in 10 and 100-mm path length cells were inadvertently taken from different drums. This caused the slopes of some plots deriving from measurements in 10-mm path length cells (Figs. 3, 4, and 5) to differ from slopes of plots deriving from measurement of the same oils in 100-mm path length cells (Figs. 7, 8, and 9). Use of Freon 113 or 98 percent Freon 113/2 percent carbon tetrachloride in 100-mm path length cells results in a sluggish response of the IR instrument recorder pen; Freon 113 has a higher absorptivity at 2930/cm than carbon tetrachloride. The IR instrument gain setting should be increased sufficiently for these measurements to yield a properly shaped absorption band at approximately 2930/cm (Fig. 1). Linear Beer-Bouguer Law plots that pass

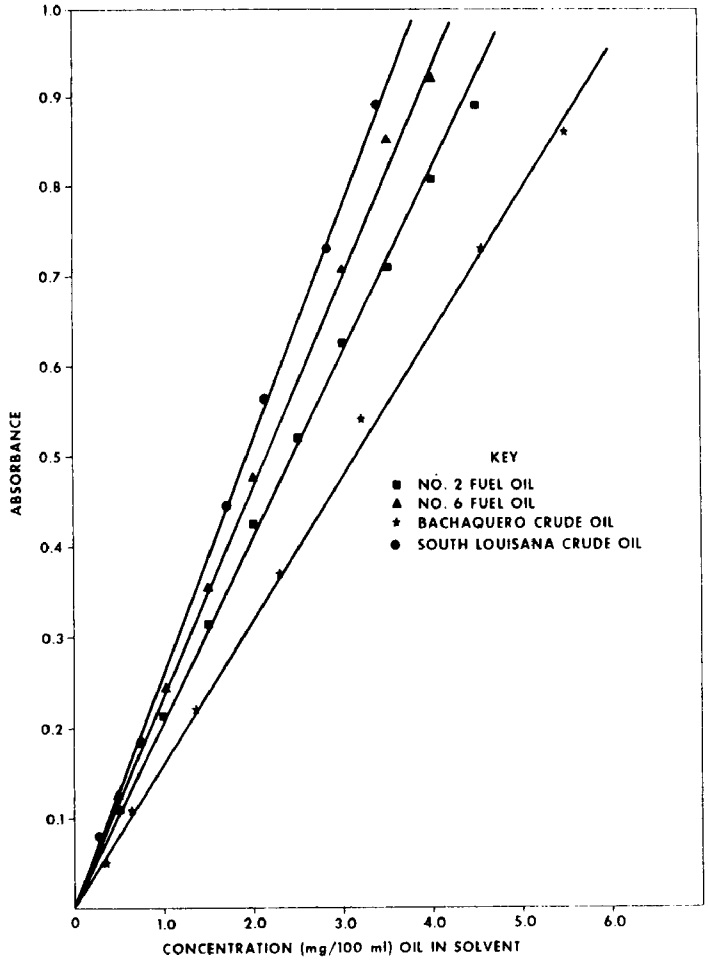


FIG. 7—*Oil solutions in carbon tetrachloride measured in 100-mm path length cells without ordinate scale expansion.*

through the origin were also obtained for measurements of the four oils in carbon tetrachloride, using 10-mm path length cells with ordinate scale expansion ×5 (Fig. 10). But, considerable deviation from linearity resulted from the measurement of Bachaquero Crude oil in 100-mm path length cells with ordinate scale expansion ×5 (Fig. 11).

Accurate quantitative determination of water dispersed oils by single point analysis can be accomplished in the concentration range 2 to 40 mg/liter (ppm) oil in water, by using 10-mm path length cells without ordinate scale expansion (Figs. 3 to 5). This concentration range assumes use of the extraction procedure by Gruenfeld [*6*], whereby 1-liter oil in water samples are extracted with four 25-ml portions of solvent. Improved

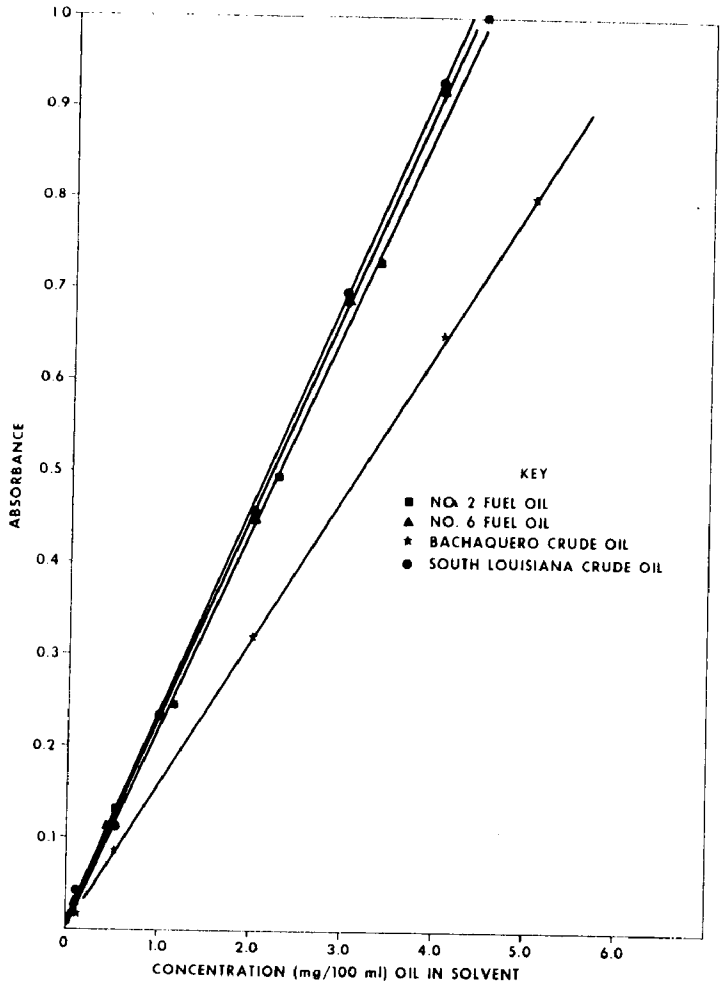


FIG. 8—*Oil solutions in 98 percent Freon 113/2 percent carbon tetrachloride measured in 100-mm path length cells without ordinate scale expansion. A higher than normal IR instrument gain setting was used.*

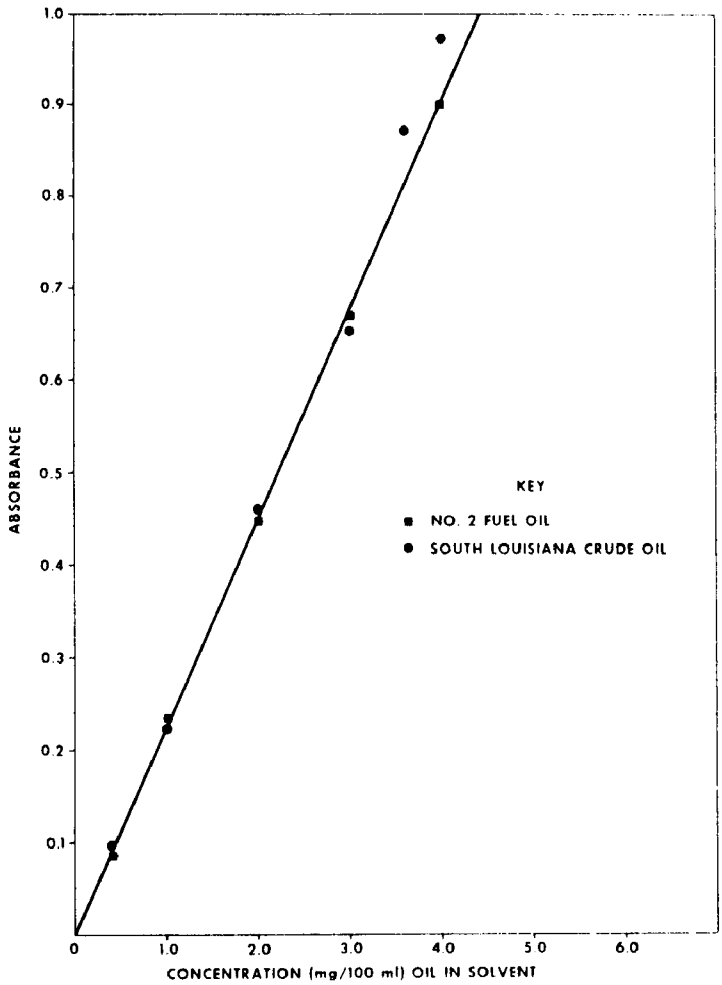


FIG. 9—*Oil solutions in Freon 113 measured in 100-mm path length cells without ordinate scale expansion. A higher than normal IR instrument gain setting was used.*

sensitivity can be achieved by using less solvent. Accurate quantitation of oils in the concentration range 0.5 to 3 ppm oil in water can be achieved by single point analysis when using 100-mm path length cells without ordinate scale expansion (Figs. 7 to 9), or 10-mm path length cells with ordinate scale expansion ×5 (Fig. 10). The measured absorbances in 100-mm path length cells should not exceed 0.7. Use of 100-mm path length cells with ordinate scale expansion ×5 yields nonlinear Beer-Bouguer Law plots (Fig. 11). While accurate quantitation of oils by single point analysis is not possible under these conditions, useful information is gained about the detection limit of the IR technique. These measurements

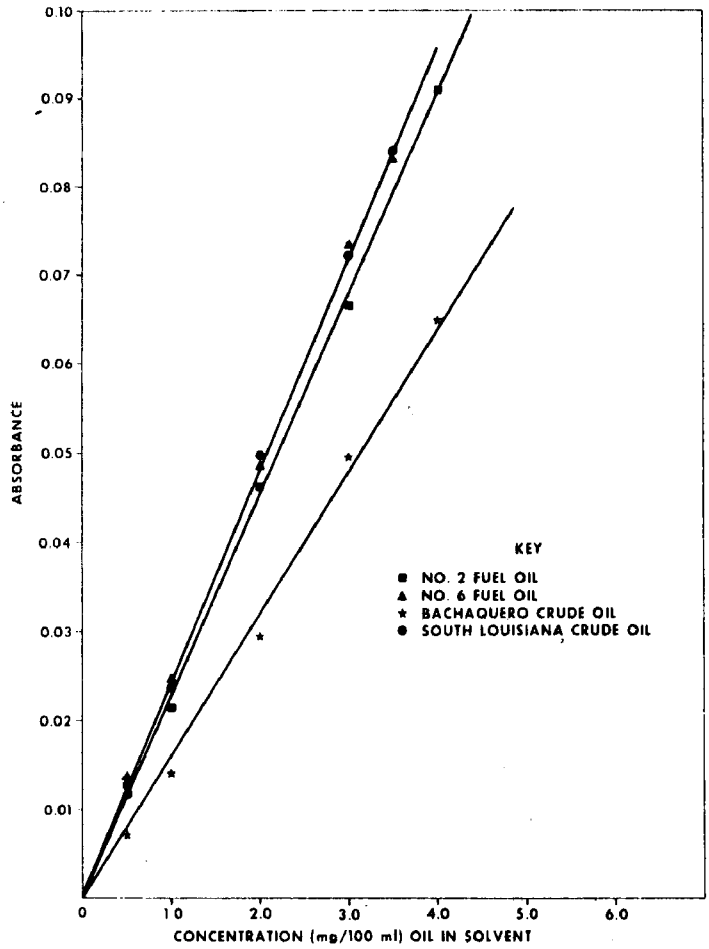


FIG. 10—*Oil solutions in carbon tetrachloride measured in 10-mm path length cells with ordinate scale expansion×5.*

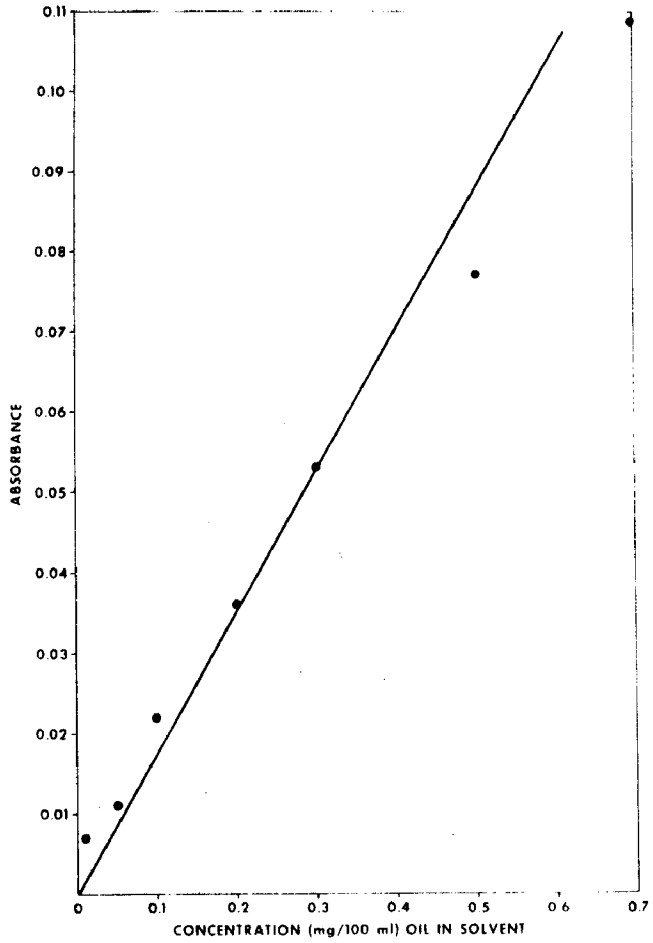


FIG. 11—*Carbon tetrachloride solutions of Bachaquero Crude Oil measured in 100-mm path length cells with ordinate scale expansion* ×5.

yield a recognizable absorption band at approximately 2930/cm (Fig. 1) for an oil solution containing 0.05 mg/100 ml oil in solvent. This is equivalent to 0.05 ppm dispersed oil in water when the extraction procedure by Gruenfeld [*6*] is used. This detection limit of the IR technique can be improved further by reducing the volume of extraction solvent.

Consideration of the Beer-Bouguer Law plots demonstrates that the lines diverge and, therefore, that the oils have different absorptivities. These absorptivities appear to remain reasonably stable, despite loss of the more volatile oil components (Table 1). They are solvent dependent, however, as demonstrated by the diverging Beer-Bouguer Law plots that

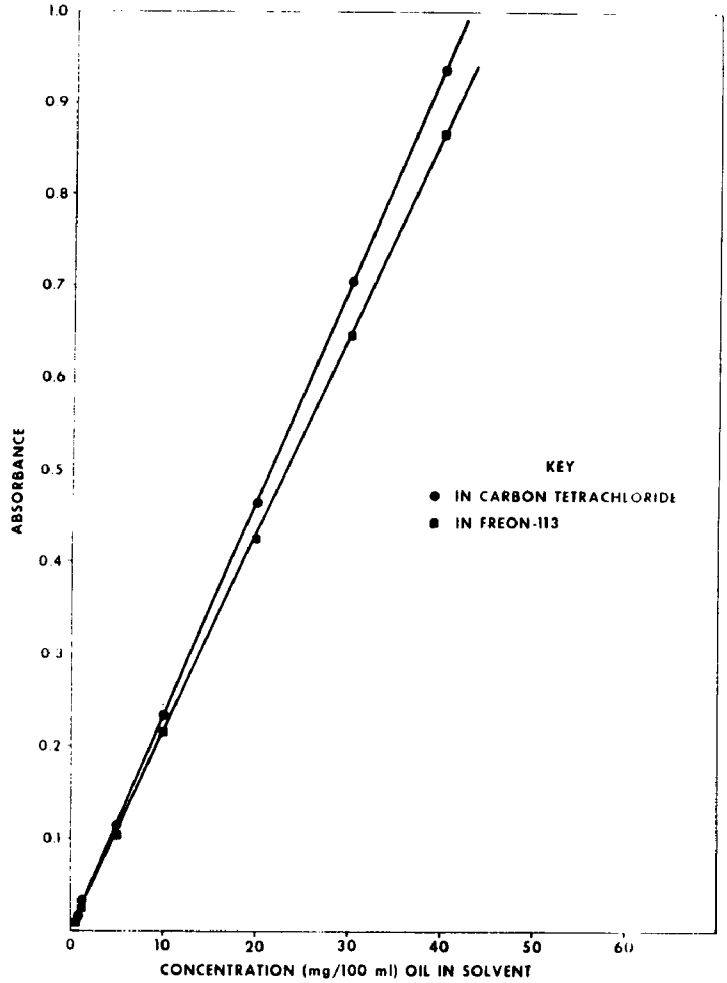


FIG. 12—*Solutions of No. 2 Fuel Oil measured in 10-mm path length cells without ordinate scale expansion.*

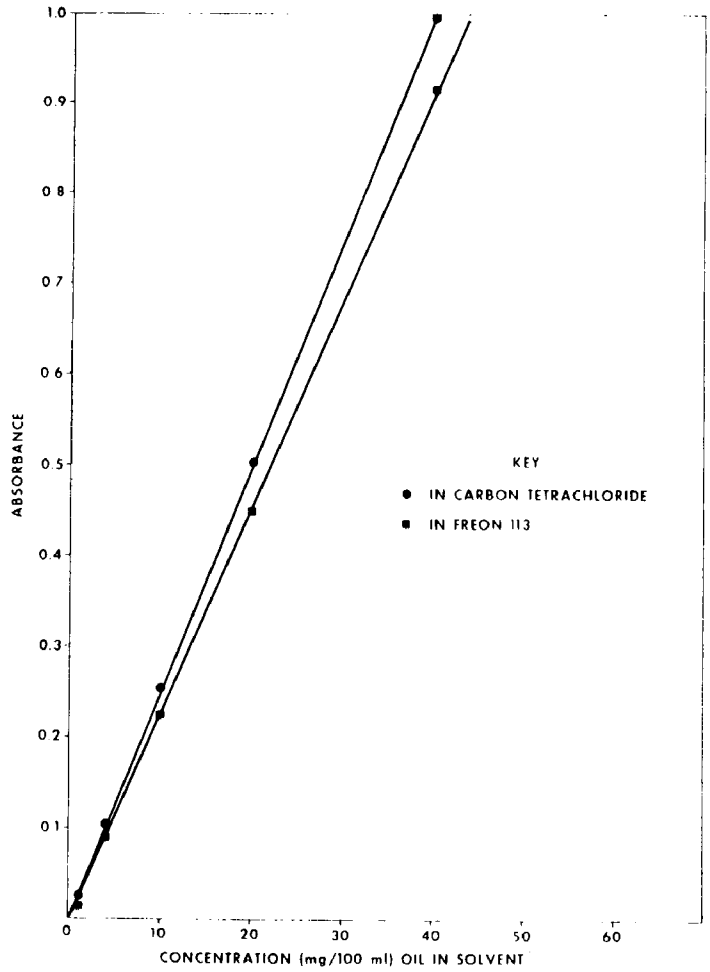


FIG. 13—*Solutions of South Louisiana Crude Oil measured in 10-mm path length cells without ordinate scale expansion.*

are obtained from the same oil in different solvents (Figs. 12 and 13): No. 2 Fuel and South Louisiana Crude oils yield absorptivities in Freon 113 that differ from their absorptivities in carbon tetrachloride. These differences are also apparent when comparing Figs. 3 and 4, and others. IR absorptivities are thus a promising parameter for "passive tagging" oils because they differ from oil to oil, and yet remain reasonably stable. Their solvent dependence may also be useful for distinguishing among similar oils. The term "passive tagging" describes a procedure whereby a weathered oil residue, that is, an environmental pollutant, is correlated (matched) with an unweathered portion of the same oil.

The stability of solution absorbances, and therefore oil absorptivities,

TABLE 1—*Effect of solvent distillation on oil absorptivity. (No. 2 Fuel and South Louisiana Crude Oils are in Freon 113 solution; No. 6 Fuel and Bachaquero Crude Oils are in 98% Freon 113/2% carbon tetrachloride solution).*

Oil	Before Distillation		After Distillation[a]		
	Oil in Solvent (mg/100 ml)	Absorptivity[b] ($E_{10\ mm}^{1\%}$)	% Loss (by weight)	Absorptivity ($E_{10\ mm}^{1\%}$)	% Change
No. 2 Fuel	104	21.4	11.5	21.1	1.4
	14.5		23.0	19.6	8.4
South Louisiana Crude	101	22.4	3.0	21.6	3.6
	10.5		14.3	21.7	3.1
No. 6 Fuel	10.6	23.8	0.0	23.8	0.0
Bachaquero Crude	103	16.1	4.4	15.3	5.0

[a] Solvent distilled (stripped) from 100-ml solutions having known oil content, using the procedure of the APHA [2]. The weighed residues are rediluted to 100-ml and the solution absorbances measured at 2930/cm.

[b] $E_{10\ mm}^{1\%}$ values are absorbances at 2930/cm, measured in 10-mm path length cells, normalized to 1% (weight/volume) dissolved oil.

following prolonged solution storage was also examined. Oil solutions in carbon tetrachloride, Freon 113, and 98 percent Freon 113/2 percent carbon tetrachloride were stored under normal room light and temperature conditions, in clear glass flasks for eight days. The absorbances following storage matched those prior to storage, within 1 percent. Prompt solvent extraction of dispersed oil in water samples is therefore recommended. Oil transport and storage in carbon tetrachloride or Freon 113 solution should prevent biological degradation and evaporative losses that can occur in the water matrix.

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Petroleum hydrocarbons from effluents: detection in marine environment

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The marine environment has become the primary disposal ground for an increasing quantity of petroleum wastes. Mushrooming demands for petroleum products and the lack of economic incentive to recycle waste oil will increase the concentrations of detrimental petroleum hydrocarbons in the marine environment.

Although a continuous, low-level discharge of waste petroleum hydrocarbons into the marine environment may not be as dramatic as a major oil spill, the consequences could be more devastating over an extended period. As noted by Blumer,[1] earlier interpretations of the environmental effects of oil must now be re-evaluated in the light of recent evidence of its effects on marine organisms [2-5] and its environmental persistence, which resembles that of DDT, PCBs, and other synthetic materials.[6-9]

Of the 4.73 mil m^3 (1.25 bil gal) of new oils purchased annually by the automotive industry,[10] an estimated 68 percent will leave automobile engines as waste. Of this 3.22 mil m^3 (850 mil gal), it is estimated [11] that 0.7 to 1.5 mil m^3 (200 to 400 mil gal)are recycled annually, with only 0.4 mil m^3 (100 mil gal) actually being refined. In the New York metropolitan area, of the estimated 0.3 mil m^3 (94 mil gal) of automotive and industrial waste petroleum recorded annually only 40 percent is being reprocessed.[12] The sources of waste petroleum in municipal wastewater systems and their receiving waters range from individuals who change the oil in their automobiles and indiscriminately dump the wasted crankcase oil into a nearby sewer to large establishments that sporadically discard accumulated stocks of waste oils. On many occasions, especially during periods of plant by-pass, large "oil slicks" pass through water pollution control facilities undetected and untreated.[13] A major portion of this waste petroleum will eventually be discharged to a receiving body of water because most hydrocarbons are more resistant to degradative processes than other compounds commonly found in wastewater,[14] and because skimming and setting operations fail to remove a major portion of petroleum hydrocarbons which are finely dispersed.[15]

The current energy dilemma makes it imperative to consider this waste not simply as a pollutant but also as a potentially re-usable source of energy. This paper will attempt to determine:

1. Whether waste automotive petroleum hydrocarbons are present in the treated effluents discharged by water pollution control facilities into Jamaica Bay,
2. Whether there is a sufficient quantity of petroleum-derived hydrocarbons present in the Jamaica Bay waters to warrant immediate attention,
3. Whether a significant portion of this waste petroleum persists in the surface waters of the Bay, and causes a chronic exposure of this ecosystem to petroleum-derived hydrocarbons.
4. Whether petroleum hydrocarbons are being incorporated in biological tissue of a Bay marine organism, *Mya arenaria*.

ANALYTICAL APPROACH

Adlard [16] showed that a variety of analytical techniques should be used in a multi-parameter approach to oil analysis. Because of the complex chemistry of the oil, each oil sample lends itself to differentiation from others. This passive-tagging approach establishes specific qualitative parameters for oil samples, in the form of "profiles" or "fingerprints" to be compared to a "standard profile." Thus, positive correlations for environmental samples are either established or not established with standards depending upon those portions of the petrochemical waste that exhibit themselves in fingerprints and remain stable under environmental conditions.

Jamaica Bay, one of the few viable wetland ecosystems in the New York Metropolitan area,

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The author conducted the chemical analyses for this report at a facility of the U.S. Environmental Protection Agency, Industrial Environmental Research Laboratory-Ci, Edison, New Jersey. Use of these laboratory facilities is provided in order to encourage graduate level environmental research.

was chosen as the study site because its unique hydrological characteristics afford a long residence time for treated or untreated effluents (Figure 1). The sampling scheme involved collections of weekly (September 7, 1973 through November 5, 1973) final effluent samples from the four major water pollution control facilities emptying into the Bay, surface water samples of the Bay (0.61 m (2 ft) below surface by Kemmerer water sampler), and marine organism samples. *Mya arenaria* were collected from an area considered to be of high pollution potential (HPP) (Mya II) and an area of low pollution potential (LPP) (Mya III).

METHODOLOGY

Water samples were collected in 980 ml wide-mouth, glass Mason jars with Teflon-lined caps. Five ml of 1:1 sulfuric acid and 20 ml of carbon tetrachloride (CCl_4) were added to all water samples to retard bacterial action and for extraction purposes. All samples were refrigerated after collection. Glassware was detergent washed and oven heated at 120°C to ensure removal of possible contaminants.[17]

The quantity of total extractable hydrocarbons in water sample extracts was determined by use on an infra-red (IR) method tentatively accepted for oil in water analysis by the EPA's Industrial Waste Treatment Research Laboratory, Edison, N. J., with some slight modifications that were necessary because of sample concentration factors.[18] Carbon tetrachloride extracts were jet-air evaporated, concentrated, and residues weighed and brought to volume in hexanes for ultraviolet (UV)-fluorescence and gas chromatographic (GC) analysis. Organism extraction required shucked clams to be homogenized in a blender with 50 ml of *n*-hexane, mixed with three times wet tissue weight of anhydrous Na_2So_4, and refrigerated for 24 h. The tissue extract mix was Soxhlet extracted in *n*-hexane for 6 h. These extracts were divided into subfractions by hexane/benzene/methanol elution through a 3 percent water deactivated silica gel/alumina column at a flow rate of 120 ml/s. The three analytical methods used for sample analyses were gas chromatography, UV-fluorescence spectroscopy and GC-mass spectroscopy.

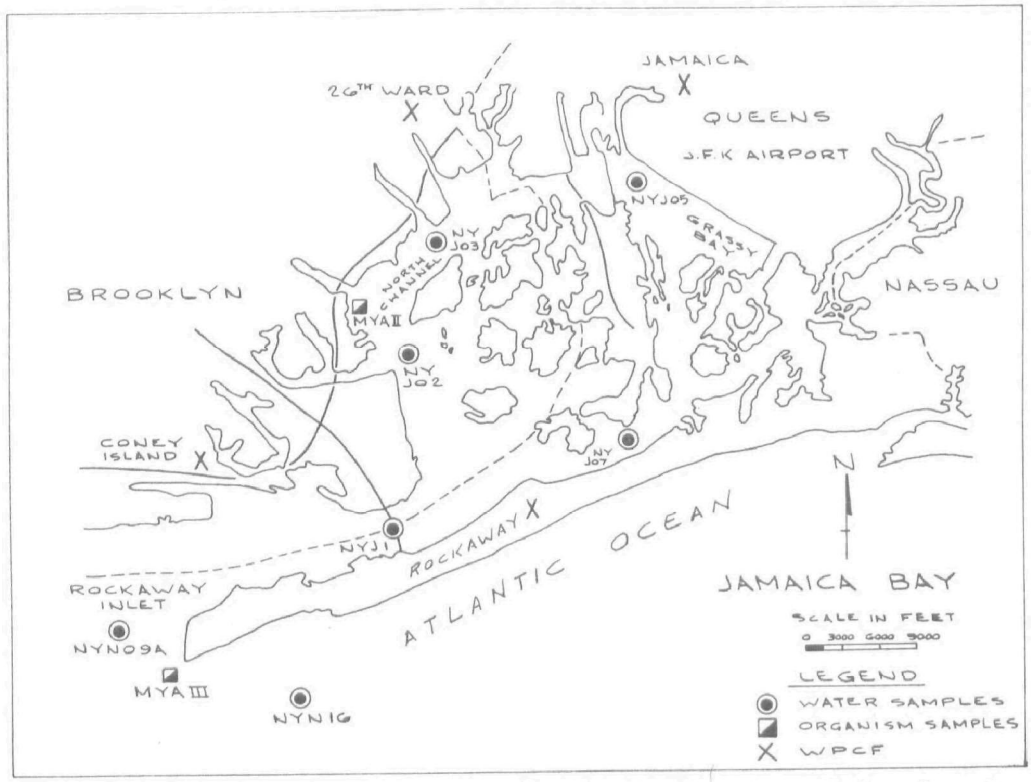

FIGURE 1. Tri-phase sampling scheme for Jamaica Bay, New York. Organism samples were designated Mya II and Mya III representative of high pollution potential (HPP) and low pollution potential (LPP), respectively.

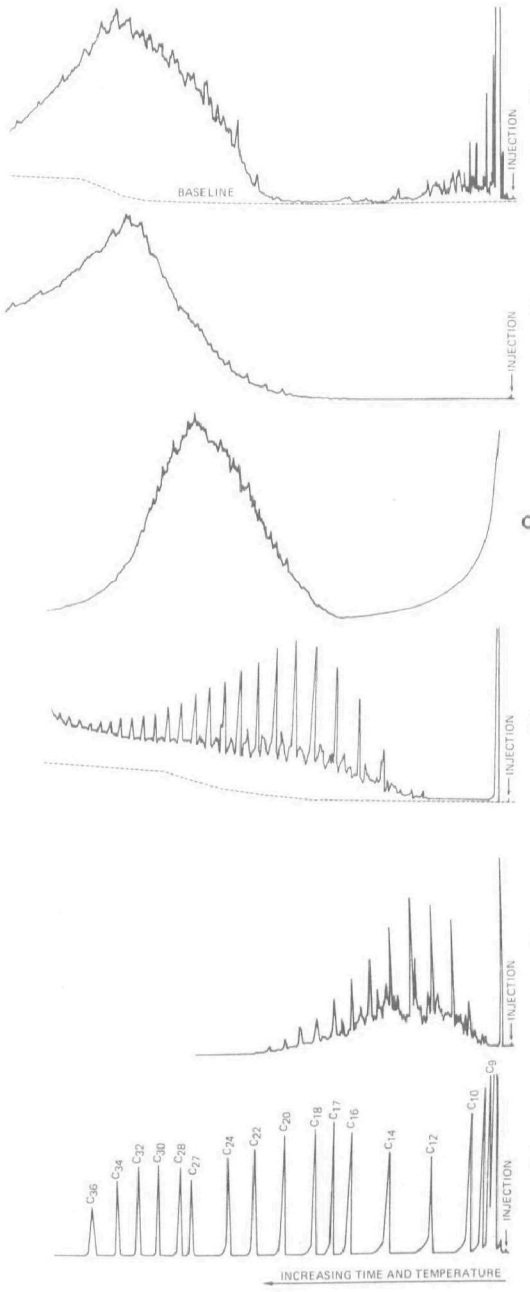

FIGURE 2. Gas chromatographic profiles of "standard" petroleum entities: (A) waste automobile crankcase oil, (B) 10W/30 virgin motor oil, (C) transmission fluid. (D) Arabian light crude oil. (E) No. 2 fuel oil, (F) standard C_6-C_{36} hydrocarbon "spike" used for retention time and relative peak height correlations.

Gas chromatographic method. Hydrogen-flame gas chromatography with flame ionization detector and a 15.24 m × 0.51 mm (50 ft × 0.02 in.) ID, stainless steel, support-coated, open-tubular column (SCOT OV-101; non-polar silicone oil*) operated with nitrogen carrier gas with a flow rate at the column outlet of 240 ml/s. Samples were temperature programmed from 75° to 300°C at 6°C/min with isothermal operation at 300°C for 12 min.

Waste crankcase oil GC profiles exhibited a large envelope area above the baseline attributable to polynuclear and polycyclic aromatic ring compounds; a "light-end" of hydrocarbons with carbon numbers under C_{12}; C_{17}-pristane/C_{18}-phytane peak-pairs; various peaks atop the envelope portion indicative of branched paraffinic, olefinic, and hetero-compounds over a wide range of boiling points. The chromatograms generated by refined petroleum products differed from those of crude or fuel oils (Figure 2). GC-retention times and relative peak heights were used to identify petroleum hydrocarbons in environmental samples. A C_6 through C_{36} n-paraffin hydrocarbon "spike" (Figure 2F) was added to samples after noting the original samples' profiles and retention times for resolved peaks. Increases in peak heights of previously noted sample components indicated its presence in the sample extract. Organism sample sub-fractions were analyzed by retention times generated in the GC-MS system.

UV-fluorescence spectroscopic method. Recent investigators [19] have exhibited the ability of fluorescence spectroscopy to detect trace quantities of petroleum-derived hydrocarbons in oceanic waters. Goldberg and Devonald [20] have been able to differentiate between a lubricating oil and a crude or fuel oil using fluorescence spectroscopic techniques. All petroleum products fluoresce when excited by UV light because of the presence of aromatic hydrocarbons with multi-ring configurations such as fused ring polynuclear aromatics.[21] A UV-fluorescence spectrophotometer with two independent monochromators (150 watt xenon arc light source), and a constant temperature cell bath that maintained a 10 mm path length, quartz cell at 20° ± 0.5°C were used for all fluorescence analyses. Each standard oil was excited at 290 mμ while scanning the emission spectrum from 240 to 540 mμ. Thurston and Knight [22] had used 340 mμ as the excitation wave length for the characterization of petro-

*Perkin-Elmer Corp.

leum entities; however, it has been recently demonstrated [23] that Raman scattering at that frequency obscures the fluorescence profiles, making sample differentiation difficult.

A relatively new fluorescence technique [24] was also used to excite each sample at successive excitation wavelengths from 240 mμ to 440 mμ (at 20-mμ intervals) while scanning for the maximum fluorescence emission (Figure 3). Each maximum peak was used as a point to be plotted graphically; this process generated a "fluorescence maxima profile" (FMP) for each sample. Correlation was determined by visually comparing the maxima profile plots of known oil standards to the maxima profile plots of environmental samples. When these maxima profiles "fit" (Figure 4) in addition to exhibiting the other qualitative characteristics established by standards, detection was established for the particular sample under investigation.

When the profile for a sample met the qualitative criteria established by standard oils, it was recorded in a "correlates" column. When profiles generated were not totally consistent with the qualitative criteria for a correlation, it was recorded in a "slight-correlation" column. Slight correlations were felt to be the result of mixtures of petrochemical entities. It should be emphasized that the correlation criteria are essentially qualitative in that source identification, (such as gas stations or individuals dumping crankcase oil into a sewer), of the detected waste petrochemical cannot be directly established by this technique. This method was originally intended to match spilled weathered petroleum products with already-known stock oils. When a positive correlation exists in all fluorescence categories, it is an extremely strong indication that a waste automotive petroleum product is present; however, the correlation arrays are based solely upon generated profiles and fluorescence analysis of standard oils.

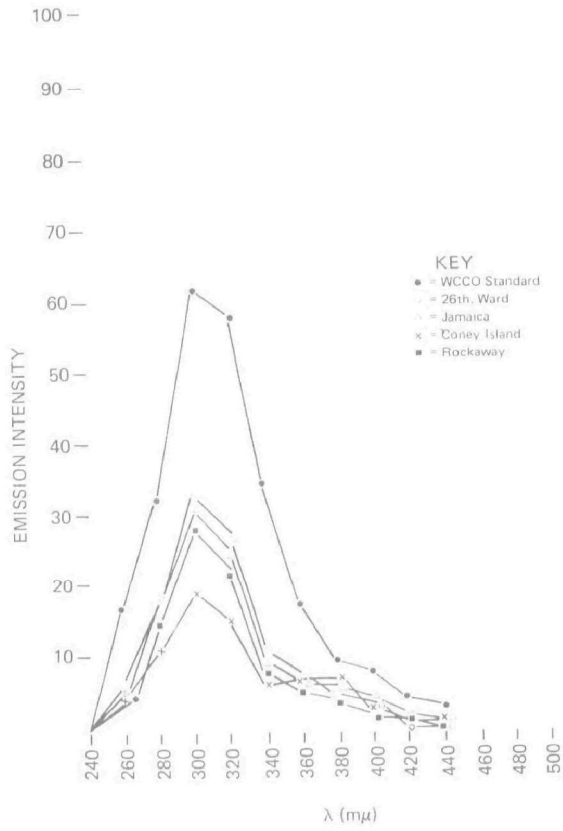

FIGURE 4. Fluorescence Maxima profile (FMP) "fit" for waste crankcase oil with pollution control facility final effluent extract samples collected on September 7, 1973.

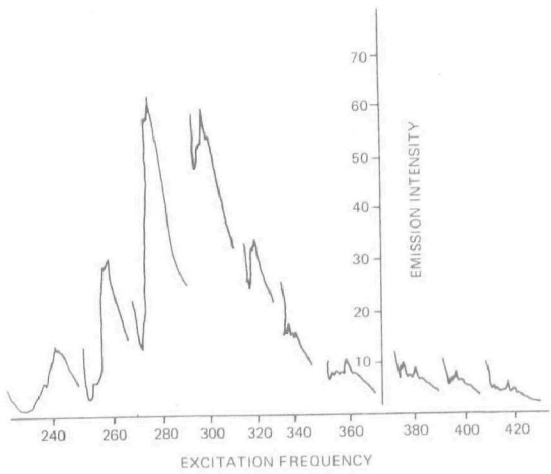

FIGURE 3. Fluorescence emission maxima peaks for the standard waste automobile crankcase oil.

GC-mass spectroscopic method. The final analytical phase of this project used a computerized GC-mass spectrometer combination with 1.52 m × 2 mm ID glass, packed with 3 percent OV-1 on a Chromsorb W column. Samples were temperature programmed from 100° to 280°C at 6°C/min with isothermal operations at 280°C for approximately 10 min. Helium carrier gas at 90 ml/s was measured at the column outlet. Only organism sub-

fractions were analyzed for specific petroleum-derived aromatic compounds by this method.

RESULTS

When a waste petroleum product is discarded and enters the environment, it is subjected to a variety of weathering phenomena. Volatiles are lost as a result of evaporation and bacteria sequentially degrade different hydrocarbons.[25] To see if detection parameters established for standard oils would be appreciably affected by weathering, standard oils were weathered in filtered sea water for a period of 32 days. Profiles generated indicated no significant changes in fluorescence detection criteria even though some profiles exhibited decreases in intensity because of concentration factors.[26] Waste crankcase oil chromatograms did lose "light-ends"; however, weathering had little effect on other detection parameters such as the unresolved envelope portion.

Treated wastewater samples. Thirty-nine treated effluent samples were analyzed for total CCl_4 extractable hydrocarbons (Table I). Regular and spiked (C_6 through C_{36} n-paraffin mix) GC runs were conducted and qualitative characteristics and retention time data of resolved peaks were noted. In all cases, chromatograms indicated an unresolved envelope portion above 200°C. Spiked samples exhibited increases in peak heights for a major portion of previously resolved peaks. Those peaks that occurred between standard peaks were tentatively labeled as being a series of isomeric or branched chain compounds.[27]

C_{17}-pristane, C_{18}-phytane hydrocarbon peak pairs, indicative of petroleum contamination, were observed in some effluent chromatograms, but not in all cases.

UV-fluorescence analysis and correlation results for treated effluent extracts are shown in Table II. Figure 5 exhibits fluorescence maximum profiles for wastewater effluents and their relation to standard waste crankcase oil and No. 2 fuel oil fluorescence maxima profiles.

Surface water samples. By using a similar solvent extraction method, average background levels of petroleum hydrocarbons in oceanic waters have been established at approximately 2 μg/l; greater than average, or excessively high levels of petroleum hydrocarbons for oceanic waters, ranged between 10 and 20 μg/l. Table III reveals that the values obtained for the Jamaica Bay surface waters are significantly above normal background oceanic levels of hydrocarbon concentrations. Non-petroleum, biologically derived compounds are extracted with CCl_4 and are not all lost during the jet-evaporation step. Extensive care and consideration was taken to prevent contamination from sample containers, extraction operations, solvents or from the research vessel during collection. The January 8 Jamaica Bay surface water extracts (Figure 6) were analyzed by gas chromatography using the same sample injection quantities and attenuation conditions in order to observe a gradient response of increased petroleum hydrocarbons as one moves in and through the Bay.

Table IV exhibits fluorescence results on surface water samples and reveals the presence of petroleum fractions in those samples taken from the Bay proper. Again, the degree of weathering to which the oil had been subjected probably dictated the intensity of the fluorescence response, yet in no instance did profiles correlate with other types of petroleum entities such as fuel or crude oils.

Organism samples. Mya II (HPP) and Mya III (LPP) extracts were analyzed by GC, UV-fluorescence and GC-MS methods outlined previously. Results of analyses clearly demonstrate the presence of petroleum-derived hydrocarbons from *Mya arenaria* tissue extracts considered to be of high pollution or contamination potential. The aromatic portion of the Mya II

TABLE I. IR Quantification of Total Extractable Hydrocarbons from Treated Effluents.

Water Pollution Facility	Date 9/10	9/15	9/17	9/24	9/29	10/1	10/8	10/15	10/22	10/29	11/5
Coney Island plant	1.50	16.4	7.1	2.0	3.5	15.6	3.0	39.8†	10.5	416	8.6
26th Ward plant	29.7	20.0	34.9	28.9†	22.9	12.3	19.2	19.1	9.3	—	—
Jamaica plant	—	10.7	12.0†	7.2	5.3	4.7	9.6	4.6	9.4	14.2	18.8
Rockaway plant	—	—	4.9	1.3	4.7	0.5	10.0†	13.8	8.5	7.7	3.2

* All values in mg/l.
† = Gas chromatographic analysis.

TABLE II. Effluent Sample Correlation Data.

Date 1973	Sample	Fluorescence Maxima Profile Fit		Maxima Region Fit		332 mμ Peak Fit	
		Correlates With WCCO	Slight Correlation	Correlates With WCCO	Slight Correlation	Correlates With WCCO	Slight Correlation
7 Sept.	26th Ward	+		+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
10 Sept.	CIP	+		+		+	
	26th Ward	+		+		+	
15 Sept.	26th Ward		+	+		+	
	CIP		+	+		+	
	Jamaica	+		+		+	
17 Sept.	26th Ward		+	+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
24 Sept.	26th Ward	+		+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
29 Sept.	26th Ward	+		+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
1 Oct.	26th Ward		+	+		+	
	CIP		+	+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
8 Oct.	26th Ward		+	+		+	
	CIP		+	+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
15 Oct.	26th Ward	+		+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
22 Oct.	26th Ward	+		+		+	
	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
29 Oct.	CIP		+	+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	
5 Nov.	CIP	+		+		+	
	Jamaica	+		+		+	
	Rockaway	+		+		+	

extract (Figure 7A) was shown to contain a wide range of aromatic compounds with boiling ranges above 250°C. Mass spectral analysis of this aromatic portion indicated the presence of substituted akyl-benzenes, a group of aromatic hydrocarbons found in extremely small amounts or not at all in marine organisms.[28, 29] Chromatograms of the Mya II-saturated portion revealed the presence of isomeric compounds or other members of the homologous series. The Mya III aromatic portion (Figure 7B) was considerably less complex than the Mya II aromatic fraction; this suggested that there was less exposure of the organisms to petroleum hydrocarbons outside the Bay than within. Brown and Huffman [30] showed this when they observed lower concentrations of aromatic hydrocarbons in

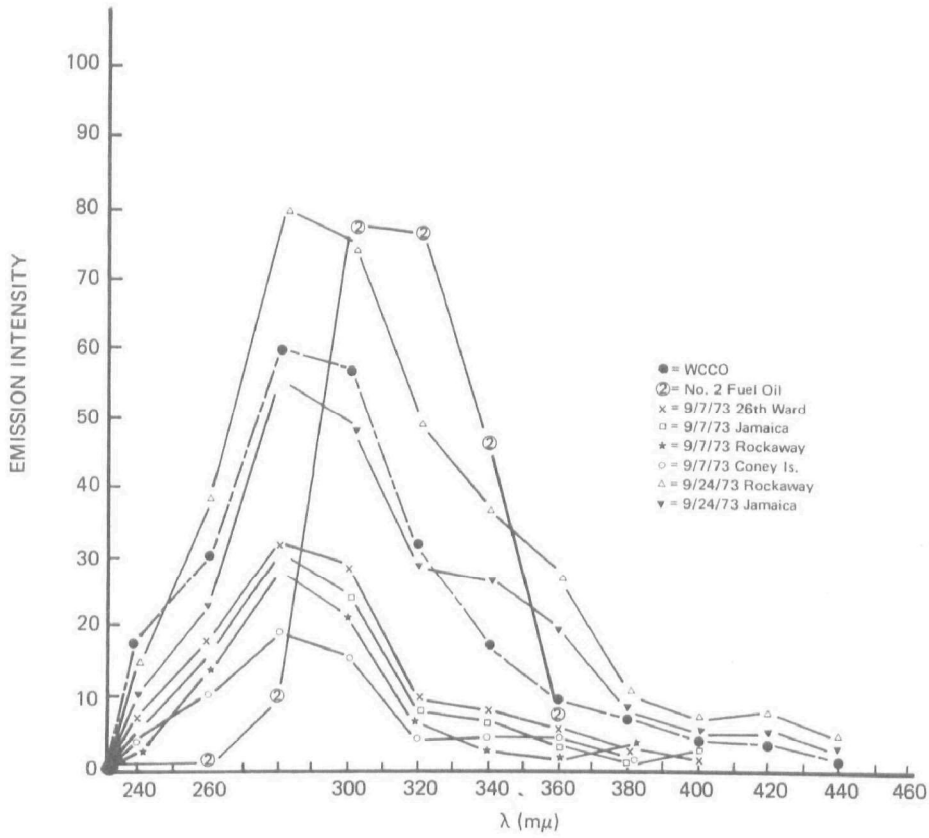

FIGURE 5. UV-Fluorescence Maxima profiles for pollution control facility extracts of September 1973 in correlation with standard crankcase oil and No. 2 fuel oil.

ocean waters than would be found during petroleum pollution incidents.

The results of the fluorescence analysis on Mya II extracts, (Table V) indicate that samples collected from the Bay proper correlated excellently with standard crankcase oil waste; however, the Mya III extract did not correlate successfully with these standards. When the Mya III extract was excited at 290 mμ, an additional peak of greater intensity occurred at 370 mμ. This peak could be attributable to contamination from other petroleum pollutants such as residual fuel oil, which peaks between 350 and 400 mμ.[31] Further investigation is needed to determine whether or not similar findings are obtained from non-petroleum materials in such samples.

DISCUSSION

Chromatograms generated by waste automotive lubricating oil and refined petroleum have been shown to be characteristic and differentiable from chromatograms of other petroleum entities. Once a waste oil enters the environment, it seems that weathering phenomena such as evaporation and bacterial degradation will have little effect upon the less soluable aromatic and higher molecular weight components; thus, detection parameters are

TABLE III. Jamaica Bay Surface Water Total Extractable Hydrocarbons.*

Sample Site	7 November 1973	8 January 1974
NYN16	0.94	1.13
NYN09A	1.20	0.88
NYJ01	2.10	2.16
NYJ02	1.17	2.20
NYJ03	3.10	5.10
NYJ05	0.50	1.50
NYJ07	1.08	1.40

* All values in mg/l.

preserved. The wide-boiling range, variety of substituents separated, and the unresolved envelope portions of chromatograms indicate the presence of crankcase oil, although they are not conclusive. These chromatographic data may be considered as preliminary findings for crankcase oil in wastewater and will require a more quantitative investigation.

Though the specific sources of the detected waste petroleum could not be established, the accumulated evidence from all analyses strongly indicates a crankcase oil origin. Gas chromatograms of pollution control plant extracts did show a wide range of hydrocarbon compounds above C_{20}, a characteristic of lube oils.[29] They did exhibit unresolved envelope portions with some samples revealing the C_{17}-pristane/C_{18}-phytane peak-pairs. Gas chromatograms generated by organism extracts strongly exhibited the presence of aromatic compounds in body tissue. Tentative GC-MS identification of organism subfractions indicated the presence of alkyl-substituted benzene structures, which are highly toxic substances indicative of petroleum contamination. UV-fluorescence analysis furnished dramatic evidence for the presence of crankcase oil in environmental samples and greatly strengthened the other analytical results obtained. Emission spectra of environmental samples consistently demonstrated the presence of polynuclear aromatics (PNA), compounds which could only

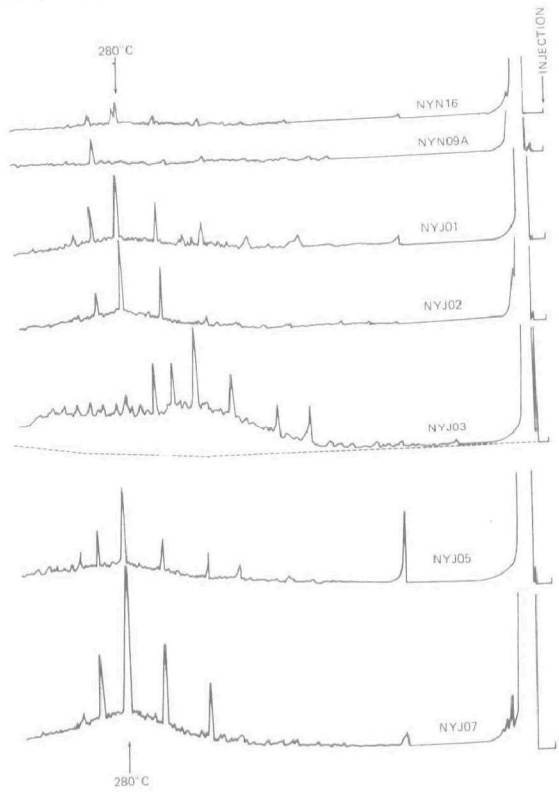

FIGURE 6. Gas chromatographic profiles generated by Jamiaca Bay surface water samples collected on January 8, 1974.

TABLE IV. Fluorescence Correlation Data for Jamaica Bay Surface Water Samples.

Date	Source	Fluorescence Maxima Profile Fit		Maxima Region Fit		332 mμ Peak Fit	
		Correlates With WCCO	Slight Correlation	Correlates With WCCO	Slight Correlation	Correlates With WCCO	Slight Correlation
7 November 1973	NYN16		+	+		+	
	NYN09A		+	+		+	
	NYJ01		+	+		+	
	NYJ02		+	+		+	
	NYJ03	+		+		+	
	NYJ05	+		+		+	
	NYJ07		+	+		+	
9 January 1974	NYN16		+	+		+	
	NYN09A		+	+		+	
	NYJ01		+	+		+	
	NYJ02	+		+		+	
	NYJ03	+		+		+	
	NYJ05	+		+		+	
	NYJ07	+		+		+	

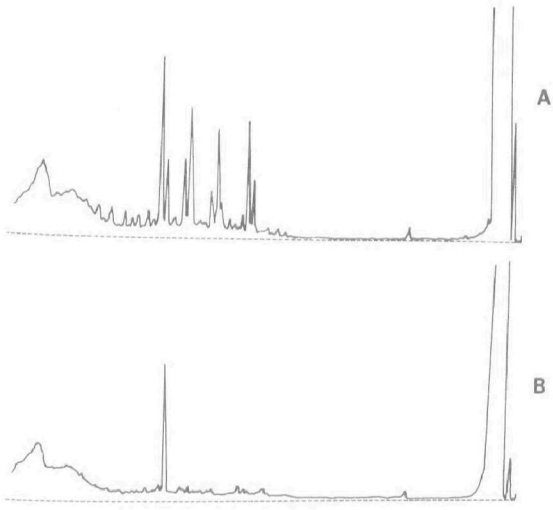

FIGURE 7. Gas chromatograms of (A) Mya II aromatic sub-fraction, and (B) Mya III aromatic sub-fraction.

be attributable to petroleum pollution.[32] Due to crankcase oil's unique fluorescence and characteristic profiles, its presence in environmental samples was tentatively established, even though the fluorescence techniques used for this project were originally to be used for the comparative identification of unknown oils to standard oils.

The results presented give a strong indication that hydrocarbons that are discharged with treated wastewater into Jamaica Bay and that ultimately accumulate in biological tissue are of a waste automotive petroleum origin. Whether this is from intentional or accidental dumping into sewers, cannot be determined here. Because of the unique characteristics of the study area and the fact that the only fresh water input comes from pollution control plants, there is a good indication that a major portion of detected hydrocarbons originate from combined wastewater effluents. Further investigations are necessary to determine the contributions of hydrocarbons from atmospheric washings, recreational boating, and biotic sources.

CONCLUSION

The results of this project indicate the existence of an unusually large hydrocarbon burden in Jamaica Bay. Continued addition of these hydrocarbons can only lead to a further deterioration of this ecosystem. Tidal-wetland areas provide food and shelter for a variety of indigenous and migratory wildlife, and thus provide critical support to marine food chains reaching all the way to man. It seems that problems of aquatic pollution in Jamaica Bay will be magnified in the future even though portions of the Bay may be included in the Gateway National Park. Added pollution from new housing, continued land-fill operations, and off-shore oil drilling will continue to pollute, perhaps irreversibly, this coastal area. Increased efforts should be aimed at restoring these waters so that they may support a much greater variety of marine life. The idea of fostering a shell-fishery in Jamaica Bay is a good one.

The analytical results of this study suggest that appreciable quantities of hydrocarbons attributable to waste automotive petroleum products are present in treated wastewater effluents entering Jamaica Bay. The discharge of petroleum hydrocarbons in the effluent is chronic. Significant quantities of detectable hydrocarbons remain in solution in the surface waters of the Bay, while aromatic hydrocarbons from waste petroleum are found in tissue extracts of marine benthic organisms collected in the Bay. The establishment of a national waste oil recycling program would not only reduce the pollution already developing in the Bay, but would be an exemplary step towards the most efficient use of U. S. energy sources.

TABLE V. Fluorescence Data for MYA Extracts.

		Fluorescence Max Profile		Max Region Fit		Excitation 290 mμ	
Date	Source	Correlation	Slight Correlation	Correlation	Slight Correlation	Correlation	Slight Correlation
12/3/73	Mya II†	+		+		+	
12/3/73	Mya III‡		+		+		+

† Collected at Diamond Point, Jamaica Bay.
‡ Collected at Rockaway point, Atlantic Ocean.

ACKNOWLEDGMENTS

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A REVIEW OF SOME COMMONLY USED PARAMETERS FOR THE DETERMINATION OF OIL POLLUTION

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ABSTRACT

A state-of-the-art review is provided describing specific parameters of petroleum oils that are used by various investigators to demonstrate the presence of oil pollution in water, sediments, and biological tissues. Several representative publications are discussed with regard to the techniques used for distinguishing between petroleum hydrocarbons and organics that are of recent biological origin. The techniques include chromatographic procedures using alumina and silica gel for separating hydrocarbons from other organics, followed by instrumental methods such as gas chromatography, fluorescence spectroscopy, ultraviolet absorption spectroscopy, ***et al****. The various oil parameters that are used to demonstrate the presence of petroleum oils are discussed, and the most effective ones are recommended. In addition, a recent study is also described in which several of the parameters were used to demonstrate the presence of oil pollution in sediments from a mangrove swamp in Puerto Rico.*

INTRODUCTION

Petroleum oils that are spilled or otherwise discharged into the aqueous environment migrate in all directions. Spilled oil floats on water and is carried onto shorelines. Oil also migrates downward through the water column and contacts fish, benthic sediments, animals and plants. Several kinds of chemical analyses are commonly performed to monitor the presence of oil pollution. "Fingerprinting" methods are used to locate the source of oil discharge. Methods of quantitation are used to measure dispersed oil levels in water, and oil levels that are incorporated by benthic sediments and tissues of animals and plants. While most fingerprinting methods require gram quantities of oil and are therefore restricted to oil rich environmental samples such as surface slicks and shoreline residues, the quantitation methods are used to measure ppm (mg/kg) or smaller amounts of oil. These methods are emphasized in the following discussion. Descriptions of highly satisfactory fingerprinting methods are available, however.[2]

Although hydrocarbons are the major components of petroleum oils, they are also produced by living marine organisms. Methods for measuring petroleums in sediments, plants and animals, and methods for measuring sub-ppm levels of oils in water consequently select characteristic petroleum oil parameters that differentiate between recent biologically produced hydrocarbons and petroleum derived hydrocarbons. The following discussion reviews some of these parameters and illustrates their use for confirming the presence of petroleum oil in samples.

Unresolved complex mixture

Petroleum is an extremely complex mixture of thousands of different hydrocarbons and related compounds. Hydrocarbons, according to Anderson, *et al.*[1], are the most numerous and abundant organic compounds comprising crude oils and refined petroleum products. Comprehensive discussions of petroleum oil composition are available in the preceding reference, and in reports by Zafiriou, *et al.*,[20] Bieri, *et al.*,[3] and the U.S. Department of Commerce.[19]

When injected into a gas chromatograph (GC), petroleum oils exhibit an inverted "cup and saucer effect" (Figure 1). This description of a rather characteristic feature of GC profiles of petroleum oils was obtained from the Department of Commerce report. This report also describes this configuration as a "smear of unresolved hydrocarbons", Zafiriou, *et al.*[20] call it an "unresolved envelope", while Farrington and Medeiros[11] describe it as due to an "unresolved complex mixture (UCM)." The presence of this configuration results from the inability of gas chromatographic methods to separate all petroleum oil components from one another. According to Blumer and Sass,[4] this unresolved envelope is characteristic of the homologous and isomeric hydrocarbons in fossil fuels. According to Clark and Finley,[8, 9] the presence of this large unresolved envelope below discrete n-paraffin peaks (discussed below) strongly suggests the presence of petroleum oils in environmental samples.

Environmental samples that contain only recently biologically produced hydrocarbons exhibit less complex chromatograms. According to Anderson, *et al.*[1], living organisms use rather specific biosynthetic pathways which favor the production of hydrocarbons in preferred size ranges. Such chromatograms exhibit a few exceptionally large peaks and often show some degree of baseline resolution.

n-alkane homologs

Crude oils and most refined petroleum products contain a homologous series of n-alkanes. These appear as discrete peaks above the unresolved envelope of the GC profiles of oils (Figure 2). According to Zafiriou, *et al.*[20] n-alkanes from C_1-C_{60} are present in petroleum oils with adjacent members occurring in similar quantities. Zafiriou contrasts this with the distribution of n-alkanes in petroleum-free plants, organisms, recent sediments, and shales, all of which yield GC profiles showing only a limited range of normal

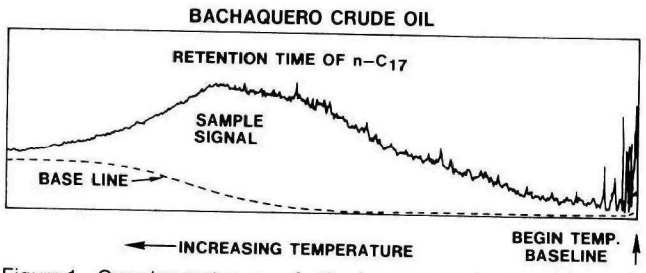

Figure 1. Gas chromatogram of a Bachaquero crude oil exhibiting the presence of an unresolved complex mixture

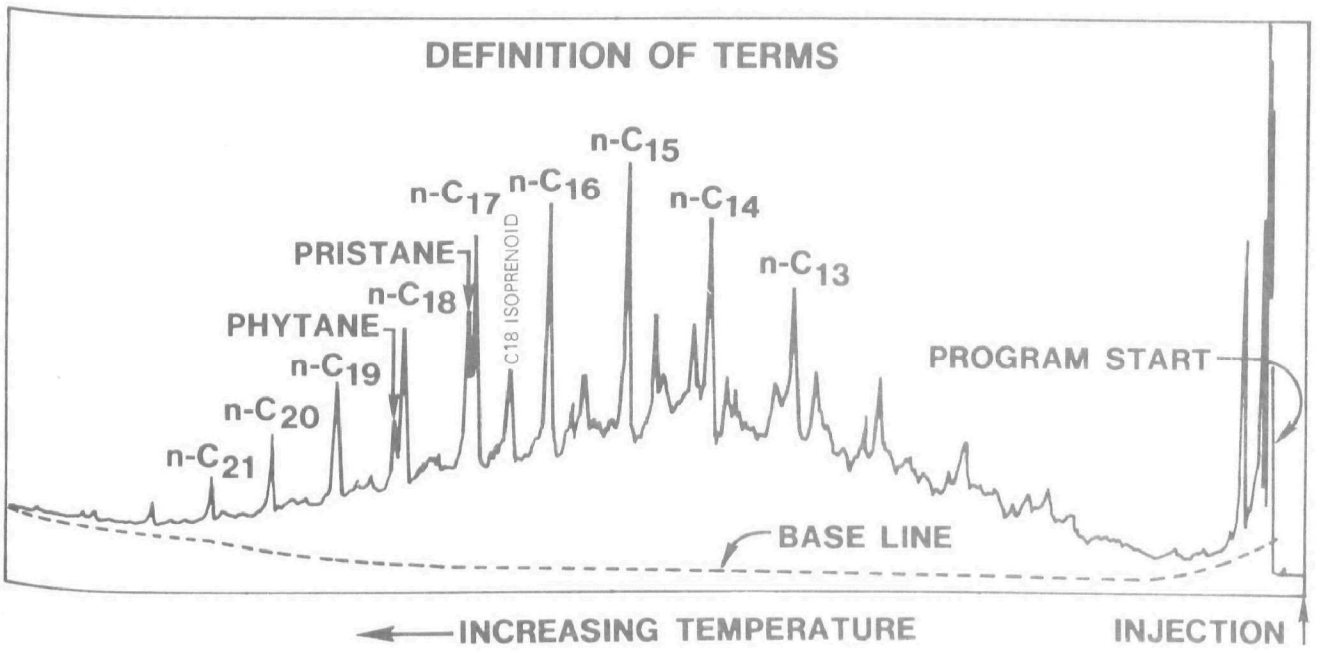

Figure 2. Gas chromatogram of a No. 2 fuel oil exhibiting the presence of n-alkane homologs, and the isoprenoids pristane and phytane

alkanes (mostly C_{15}-C_{35}), and a strong predominance of odd-numbered over even-numbered compounds. The exceptionally broad distribution of n-alkanes (C_1-C_{60}) in crude oils and most refined products is also emphasized by Anderson, *et al.*[1] These authors find a distinct predominance of odd carbon number versus even carbon number n-alkanes in petroleum-free environmental samples (i.e., samples containing only recent biologically produced hydrocarbons). While odd-to-even n-alkane carbon number ratios in petroleum oils are approximately 1.0, GC profiles of environmental samples containing only recently produced hydrocarbons often show a strong predominance of one or two n-alkanes over all others. Use of GC profiles of environmental samples to demonstrate petroleum incorporation is also suggested by Clark and Finley.[9] According to these authors, the first indication of petroleum uptake is often seen by the presence of a large unresolved envelope below the n-alkane peaks, and by the presence of n-alkane peaks in the range C_{12}-C_{22} which have the same order of magnitude. The n-alkanes are quite susceptible to microbial catabolism, and may not appear in GC profiles of substantially weathered oils. Such chromatograms should retain their characteristic inverted cup and saucer appearance, however.

Isoprenoids

Gas chromatograms of petroleum oils often contain major peaks that appear in addition to those of n-alkanes. Two isoprenoids, pristane (2, 6, 10, 14-tetramethylpentadecane) and phytane (2, 6, 10, 14-tetramethylhexadecane) are of particular interest in evaluating the presence of petroleums in samples. When relatively non-polar stationary phases are used (e.g., Apiezon L, OV-1, OV-101, or SE-30), pristane and phytane yield GC peaks that adjoin and are usually only partially separated from the C_{17} and C_{18} n-alkane peaks (Figure 2). This characteristic four peak configuration is common to many oils and indicates the likely incorporation of petroleums by marine environmental samples. Pristane is commonly produced by marine organisms, however, and consequently does not confirm petroleum incorporation. But, according to Anderson, *et al.*,[1] and Ehrhardt and Heinemann,[10] phytane has not been found as a natural component of marine organisms. Its presence in samples therefore suggests oil incorporation.

Fluorescing components

Aromatic hydrocarbons are abundant in most crude and refined petroleum products, and their presence in marine samples is often thought to indicate petroleum incorporation. For example, Hargrave and Phillips[15] use the presence of triaromatic and greater substituted aromatic compounds as a useful indication of petroleum pollution. The U.S. Department of Commerce[19] report emphasizes that aromatic hydrocarbons in marine waters are currently believed to be from petroleum sources. Similarly, Brown and Huffman[7] cite evidence suggesting that marine organisms do not produce aromatic hydrocarbon mixtures. Conversely, Blumer and Youngblood[6] describe forest fires and prairie fires as a likely source of many polynuclear aromatic hydrocarbons in marine sediments. Similarly, Anderson, *et al.*[1] indicate that although aromatic hydrocarbons have not been isolated from plankton, they may be produced by some marine organisms. Despite these possibilities, elevated levels of polynuclear aromatic hydrocarbons in marine samples are widely thought to indicate the presence of petroleums.

Several publications describe methods for measuring aromatic hydrocarbons in marine samples. Fluorescence spectroscopy is the most highly recommended technique. According to Gordon, *et al.*[14] fluorescence methods are rapid, sensitive, and simple. While these authors believe fluorescing materials to be aromatics, they describe the uncertainty of actual compound identity as a major drawback. Gordon and Keizer[13] describe several fluorescence methods for measuring aromatics in water, while Zitko,[21] and Hargrave and Phillips[15] provide some useful fluorescence data describing petroleum incorporation by animals and sediments. Several fluorescence spectra of oils are illustrated in Figure 3.

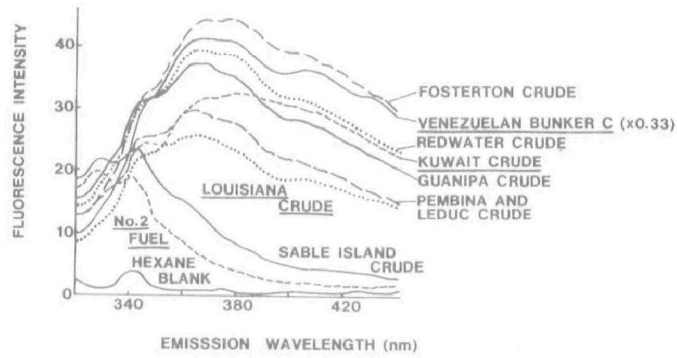

Figure 3. Fluorescence spectra of several petroleum oils[13]

Naphthalenes and substituted naphthalenes

The toxicity of higher boiling aromatics such as napthalenes, to fish, is well established according to Anderson, *et al.*[1] In fact, these authors feel that the toxicity of naphthalenes to fish may even exceed that of lower boiling aromatics such as benzene, toluene, and xylene. Naphthalenes are more soluble in water than most other high boiling aromatic hydrocarbons, and consequently have a greater tendency to migrate from petroleum oils into marine waters, sediments, and animals. Discussions of these properties and of the unique uptake and depuration characteristics of naphthalenes, and their known toxicity to marine organisms are presented by Rossi, *et al.*, [16] and also in several other papers by Anderson that are cited by these authors.

Naphthalene, methyl naphthalene, and dimethyl naphthalene, according to the preceding authors, are the major high-boiling aromatic hydrocarbons that transfer from oils into the water column and consequently contact marine organisms. In addition, napthalenes are readily absorbed by the organisms, but only slowly depurated. As a consequence of their enhanced availability, high toxicity, rapid uptake and slow depuration, napthalenes are often viewed with special interest during oil pollution studies. These aromatics are apparently not produced by marine organisms, and consequently serve to indicate petroleum incorporation. Naphthalenes are readily measurable, using the ultraviolet spectroscopic method in Neff and Anderson.[18]

Use of parameters

The preceding discussion addressed several readily measurable and commonly used parameters for monitoring and confirming the presence of petroleum oils in waters, sediments and animals of the marine environment. Oil pollution is indicated whenever samples from oil spill or discharge impacted areas are found to contain petroleums, while samples from proximate non-impacted areas do not contain oil. Several parameters are suggested for monitoring the presence of petroleum oils in marine environmental samples:

1. gas chromatograms exhibiting the presence of an inverted "cup and saucer effect"-unresolved complex mixture
2. gas chromatograms exhibiting the presence of n-alkane homologs covering a broad range of molecular weights, and with adjoining n-alkane peaks of essentially equal size
3. gas chromatograms exhibiting the presence of the isoprenoid phytane, most often as a partially resolved peak adjoining the n-C_{18} alkane peak
4. the occurrence of substantial fluorescence spectra, following excitation at specified wavelengths; aromatic components are thereby indicated
5. the presence of napthalene, methyl naphthalene, and dimethyl naphthalene; measurement can be accomplished by ultraviolet absorption spectroscopy

Measurement of these parameters is usually accomplished after chromatographic separation of hydrocarbons from other organics, using silica gel, alumina, or florisil chromatographic techniques. The occurrence of any one of these parameters may suffice to establish the presence of petroleum oils. Of course if more than one is found, then petroleum is shown to be present with a higher degree of certainty.

Sample analysis

Application of these parameters is illustrated by discussing a recent study in which some of the parameters were used to establish the presence of oil pollution in aquatic sediments from a mangrove swamp in Puerto Rico. The area was impacted by a major oil spill in 1973. General ecological aspects of this project are discussed in greater detail by Nadeau and Bergquist.[17]

Gas chromatography (GLC), fluorescence spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy were used to analyze extracts of sediments from the spill site, and from an area having no known history of petroleum oil pollution. Extractions were performed by triturating 20 g portions of air dried sediment with four 50 ml portions of carbon tetrachloride. Separation of hydrocarbons from other organics was accomplished as in Blumer, *et al.*,[5] using silica gel and alumina packed columns. The eluates were evaporated to dryness, and the residues analyzed by the following procedures.

GLC. The analysis was performed with a 50 ft OV-101 support-coated open tubular (SCOT) column, using a flame ionization detector (FID) and a temperature program of 75°-275°C at 6°/minute. Program activation was initiated to coincide with solvent peak elution.

NMR. Spectra were obtained using a 60 Megahertz (MHz) instrument. All peak field positions were referred to tetramethylsilane (TMS) at 0 ppm, using the delta scale.

Fluorescence. Measurements were made according to Frank,[12] using 15 different excitation wavelengths between 220-500 nanometers (nm) at 20 nm intervals. This procedure differs somewhat from that illustrated in Figure 3.

Analysis results are summarized in Figures 4-6. GC profiles of the contaminated sediments exhibit the typical inverted "cup and saucer effect". This unresolved complex pattern does not appear in GC profiles of the clean sediments, however (Figure 4). The n-alkane homologs and the

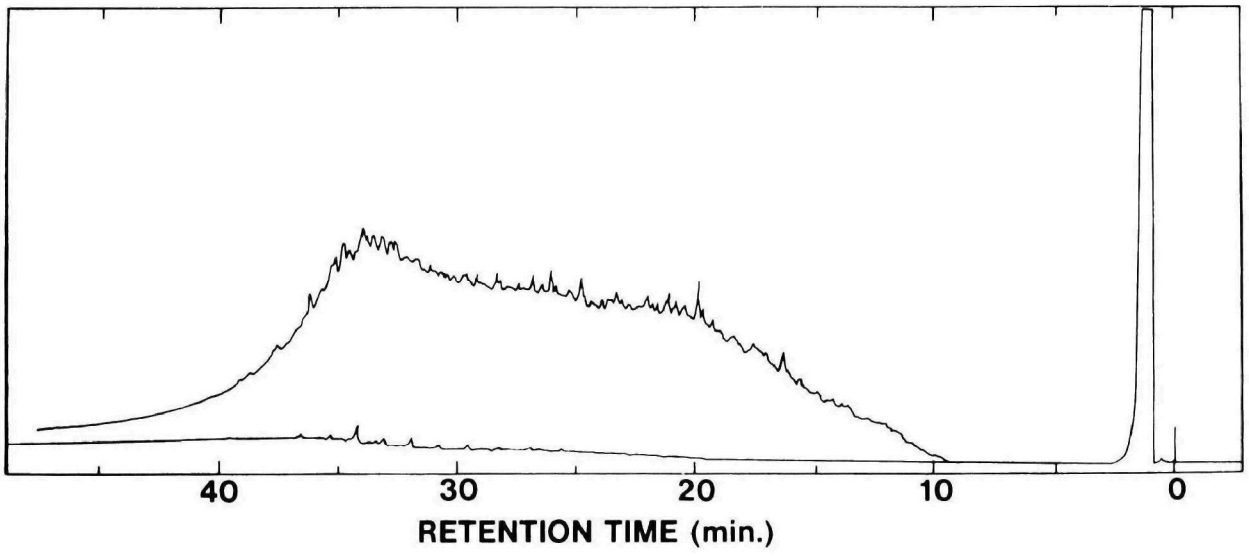

Figure 4. Gas chromatograms of sediment sample extracts: (upper line) sediment from an oil spill impacted area; (lower line) sediment from a non-impacted area

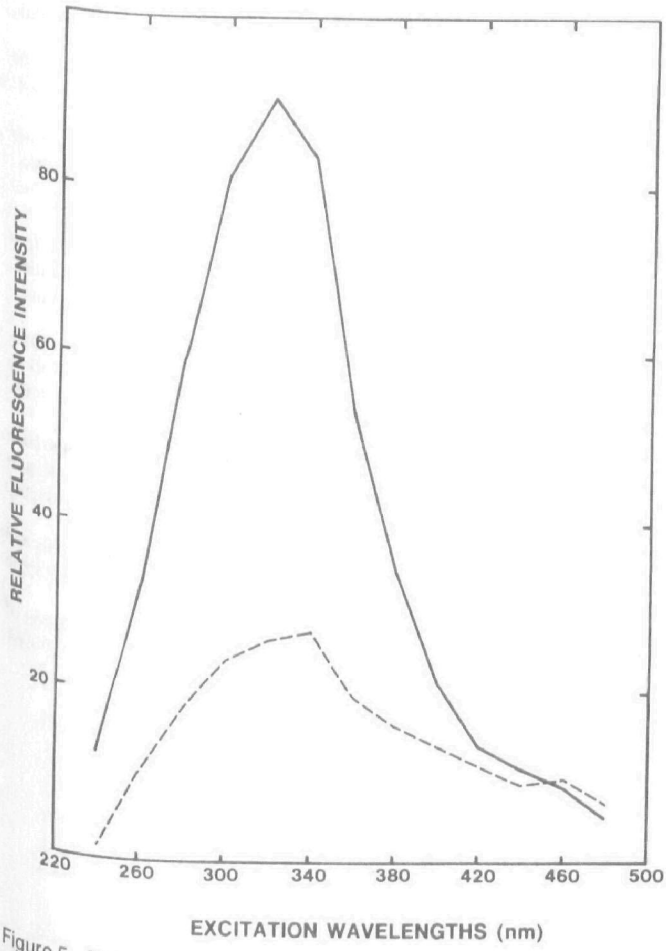

Figure 5. Fluorescence spectra of sediment sample extracts: (upper) sediment from an oil spill impacted area; (lower) sediment from a non-impacted area

isoprenoid phytane peaks are not evident. This is probably a consequence of extensive microbial degradation that has occurred during the preceding three years. Substantial fluorescence profiles resembling those of known oils were obtained from the contaminated sediments, but only weak profiles differing substantially from those of known oils were obtained from the uncontaminated sediments (Figure 5). NMR was used in addition to fluorescence spectroscopy, to confirm the presence of aromatics in the contaminated sediments. Absorption peaks in the range 6.5-8.0 ppm resulted from the contaminated sediments, but not from the uncontaminated sediments (Figure 6). This absorption region is typical of aromatic compounds, and confirms the presence of petroleum oil in the sediment samples that were presumed to be contaminated. This conclusion is also supported by the gas chromatographic and fluorescence data.

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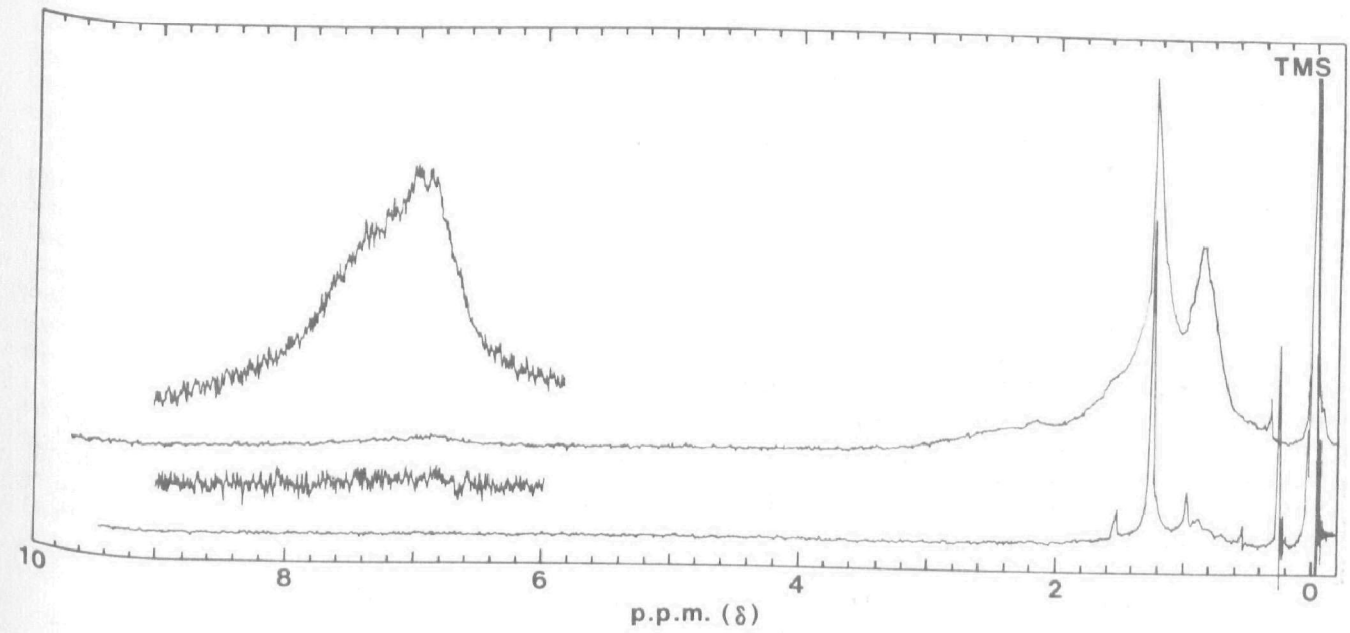

Figure 6. NMR spectra of sediment sample extracts: (upper) sediment from an oil spill impacted area; (lower) sediment from a non-impacted area; peaks between 0.5-2.0 ppm and 6.5-8.0 ppm are in absorption regions characteristic of aliphatic and aromatic hydrocarbons, respectively; the aromatic absorption region is emphasized by increasing instrument sensitivity

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THE ULTRASONIC DISPERSION, SOURCE IDENTIFICATION, AND QUANTITATIVE ANALYSIS OF PETROLEUM OILS IN WATER

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We describe three current projects involving the development of methods for the quantification and source identification of water dispersed oils, and for the preparation of stable oil-in-water dispersions. Adaptations of existing solvent extraction, IR quantification, and GC identification methods are discussed, and some newly developed techniques are presented. The techniques include: 1) a method for inducing comparable volatility losses in milligram and sub-milligram amounts of oils, and thereby enhancing their source identification; 2) a rapid adsorption method for separating hydrocarbons from other organics, and thereby achieving more selective petroleum oil quantification; 3) a method for using an ultrasonic device to prepare stable oil-in-water dispersions, having a known oil content.

INTRODUCTION

Petroleum oils enter the water column of the aqueous environment through many pathways. Offshore drilling operations, ships' cleaning operations, damaged tankers and storage tanks, industrial and municipal outfalls, and natural seeps all contribute their share. Discharged petroleum oils thereby constitute a major pollution problem, that is under intensive evaluation by the U.S. Environmental Protection Agency (EPA), other government agencies, various industrial organizations, and by segments of the academic community.

Most standardized laboratory methods that are currently used to monitor oil pollution are procedures for identifying the point of discharge of visible oil slicks and shoreline residues, and methods for quantifying the total extractable organics in water. Few corresponding methods exist for identifying the source of sub-milligram quantities of water dispersed oils, and for estimating the petroleum oil content of waters that also contain substantial amounts of other extractable organics.

Our paper addresses these needs. We describe two current projects to develop methods for the quantification and source identification of water dispersed petroleum oils, and a procedure for preparing stable oil-in-water dispersions, having known oil content. These projects are still underway, and our conclusions are therefore tentative. We hope to publish final results in the near future.

OIL IDENTIFICATION

Several oil identification methods were recently published by the American Society for Testing and Materials (ASTM). They include a gas chromatographic (GC) procedure (ASTM D3328-74T), and a method for using nickel, vanadium, sulfur, and nitrogen components of oil for the identification of oil (ASTM D3327-74T). Other recently published, and soon to be published ASTM methods are also relevant. They include a sample preservation technique (ASTM D3325-74T), a sample preparation method (ASTM D3326-74T), an infrared (IR) procedure (provisional ASTM designation D3414-75T), and a data handling technique (provisional ASTM designation D3415-75T). When properly combined, these methods can be used to identify the source of discharge of oil pollutants, i.e., to "fingerprint" oils.

While these ASTM methods emphasize the identification (fingerprinting) of oil pollutants that occur as visible surface slicks and shoreline residues, our present interest as described in this paper, is the development of a method for fingerprinting much smaller quantities of oils, i.e., oils that are extracted from the water column. We decided to utilise and integrate existing procedures wherever possible. Consequently, ASTM D3328 was selected for matching oils by GC. Similarly, existing procedures were used for solvent extraction (Gruenfeld, 1973) and IR quantification (Gruenfeld, 1975). The need for quantification prior to identification is discussed below.

Optimum intercomparison of oils is enhanced by stripping volatile components from weathered and unweathered oils, to yield residues with similar volatility losses. ASTM D3326 achieves this by distillation: 50 ml portions of slicks, shoreline residues, and unweathered oils are heated to yield 280°C+ distillation residues that are then matched by GC, IR, and the elemental

analysis methods. But, the requirement for 50 ml quantities of oil greatly exceeds the amount of oil that is usually available from the water column; i.e., water samples normally yield only microlitre (mg) quantities of dispersed oil. Therefore, we developed a procedure for treating 0·5 and 30 mg quantities of oil to yield residues with GC profiles that closely resemble the residue profiles of ASTM D3326.

It should be noted that other methods are available for GC identification of water dispersed oils (CONCAWE, 1972; Dell'Acqua et al., 1975). But these procedures do not attempt to optimize intercomparison of oils by yielding residues with similar volatility losses, nor do they benefit from the incorporation of a standardized ASTM method.

OIL QUANTIFICATION

Many methods are available for estimating the amount of oil in water. Some measure extractable aromatics by UV absorption or fluorescence techniques (Zsolnay, 1973; Levy, 1971; Gordon and Keizer, 1974). Others measure extractable hydrocarbons by IR, following Florisil or silica gel adsorption chromatography (CONCAWE, 1972; Brown et al., 1974; Yu and Coleman, 1975), while still others measure total extractable organics by IR and gravimetric procedures (EPA, 1974; Gruenfeld, 1975). All of these methods can estimate µg/ml levels of oil in water, but only the UV absorption and fluorescence methods, and one IR procedure (Brown et al., 1974) estimate 1—10 µg/litre levels. This IR method is somewhat cumbersome, however, because exceedingly large water samples are needed (usually 20 litre), while the UV absorption and fluorescence methods suffer from substantial variability in response from oil to oil, thereby handicapping selection of meaningful reference standards.

Our current interest as described in this paper, is the development of a method for estimating the dispersed petroleum oil content in water columns near surface slicks, ships' ballast water discharges, offshore waste disposal sites, and offshore platforms *inter alia*. The method should be capable of distinguishing petroleum oils from other extractable organics, and be appropriate for estimating oil in water concentrations below 10 µg/litre. We are evaluating, as the method of choice, a rapid silica gel adsorption technique (Longbottom, 1974), followed by IR measurement of hydrocarbons. The work is still incomplete, however, and consequently discussion is limited to evaluation of the silica gel adsorption step.

OIL DISPERSION

Adequate methods are needed for preparing stable oil-in-water dispersions that can be further diluted with water. Use of stable dispersions having known oil content can enhance bioassay tests of oil toxicity, and performance evaluations of oil quantification methodology. Most dispersions are now prepared by vigorously shaking or mechanically mixing oils with water, but separation of oils can quickly ensue. Consequently, we evaluated the use of a commercially available ultrasonification device for preparing stable dispersions. We then examined the resulting dispersions for stability and miscibility with water. We also tested the ultrasonically dispersed oils for spectral and chromatographic alterations that may interfere with their quantification and identification.

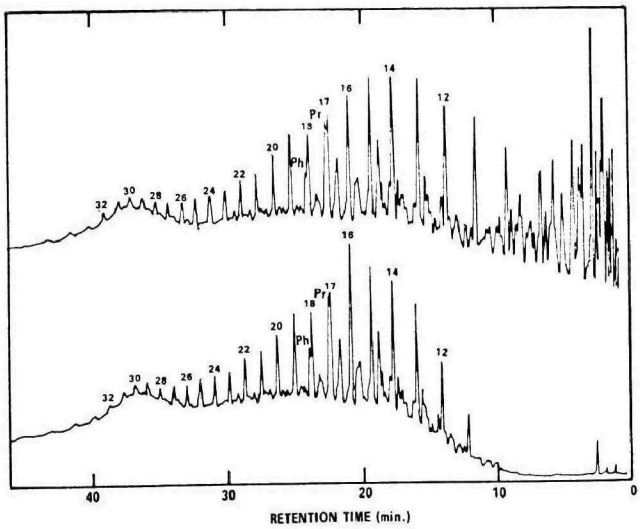

Figure 21. South Louisiana Crude oil. Untreated neat oil (upper chromatogram). After ASTM D3326 distillation (lower chromatogram). Numbers refer to *n*-alkanes. Pr and Ph refer to pristane and phytane, respectively.

EXPERIMENTAL

APPARATUS

Gas chromatographic determinations were performed with a Perkin-Elmer Model 900 GC instrument[1], equipped with a flame ionization detector. A 3 m by 3 mm O.D. (0·6 mm wall) stainless steel column was used, packed with 10 % OV-101 on 60/80 chromosorb W (AW-DMCS treated). Helium at 40 ml/min was the carrier gas. Temperature programme: initial-50°C for 2 min., final-325°C, programme rate-8°C/min., injector-300°C.

Infrared spectroscopic determinations were performed with a Perkin-Elmer Model 457A IR instrument. Absorbances were measured in 10 mm rectangular silica

[1] Mention of trade names or commercial products does not constitute endorsement by the U.S. Government.

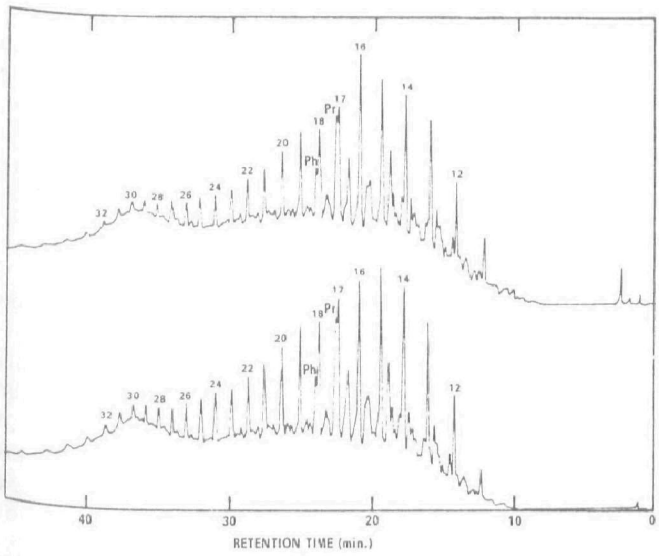

Figure 22. South Lousiana Crude oil. After ASTM D3326 distillation (upper chromatogram). After the 70 mg neat oil treatment procedure (lower chromatogram). See legend of Figure 21 for key to numbers and letters.

cells (Beckman Instruments Incorporated, catalogue number 580015), using Perkin-Elmer cell holders (catalogue number 186-0091).

Ultrasonic dispersions were prepared with a Branson Sonic Power Company (Danbury, Connecticut, USA) model W185 Sonifier Cell Disruptor, using a ½ inch disruptor horn with plain conical tip.

REAGENTS

Spectroanalyzed carbon tetrachloride (Fisher Scientific Company, Catalogue Number C-199), and Freon 113 solvent (E. I. Du Pont De Nemours and Company, Inc.) were used. Freon 113 is a Du Pont designation for 1,1,2-trichloro- 1,2,2-trifluoroethane, and this reagent is also available from other manufacturers under various trade names.

Separation of hydrocarbons from other organics was accomplished with Davidson Type 923 silica gel (100—200 mesh).

PROCEDURE

Oil identification

Simulated oil in water samples were prepared by ultrasonically dispersing a South Louisiana Crude oil in water. Sample extraction was achieved with four, 25 ml portions of Freon 113, following addition of acid and salt (Gruenfeld, 1973). Quantification of oils was accomplished by IR spectroscopy (Gruenfeld, 1975), followed by further dilution to yield final 100 ml Freon solutions containing 0·5 or 30 mg oil. Simulation of the ASTM D3326 distillation was accomplished by stripping the solutions to final 1—2 ml volumes, in 150 ml beakers, with the aid of a steam table and a filtered air stream and the concentrates were transferred to 10 by 30 mm glass vials. The vials containing 30 mg portions of oil were suspended in a 40°C water bath, and a filtered air stream was used to remove final solvent traces. This condition was maintained for 10 additional minutes. The vials containing 0·5 mg portions of oil were maintained at room temperature, the air flow was turned off just before total solvent removal and final solvent evaporation occurred spontaneously. This condition was maintained for 10 additional minutes. Distilled solvent was used for the lower concentration determinations. Small amounts of CCl_4 (10—20 µl) were then added to each vial for GC injection.

In order to enhance intercomparisons of neat oils with dispersed oils, the neat oils were treated as follows: vials containing 70 mg portions of neat (undispersed) oils were suspended in a 40°C water bath for 15 minutes, in the presence of a filtered air stream.

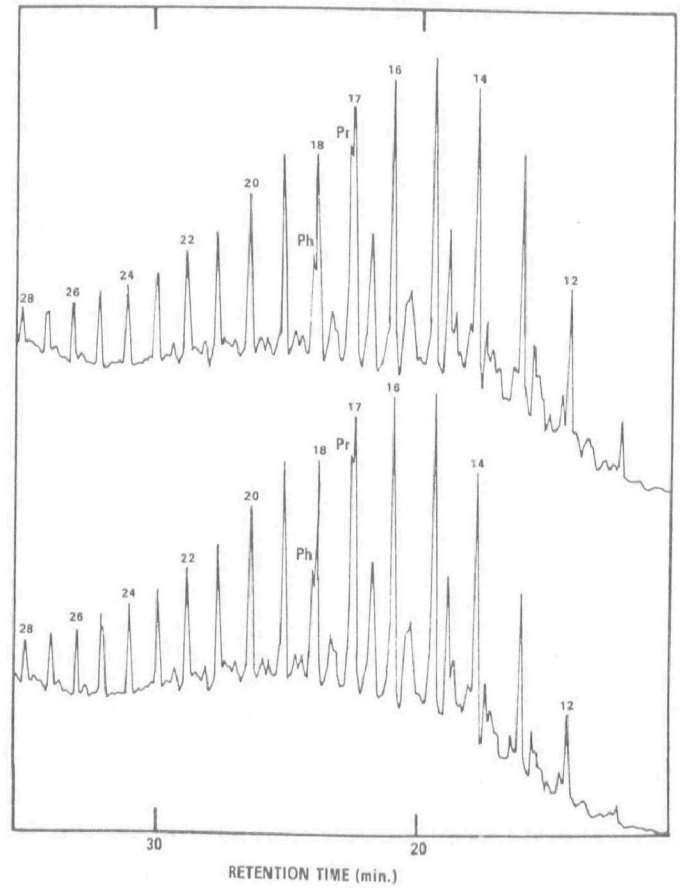

Figure 23. South Lousiana Crude oil. After the 70 mg neat oil treatment procedure (upper chromatogram). After the 30 mg dispersed oil treatment procedure (lower chromatogram). See legend of Figure 21 for key to numbers and letters.

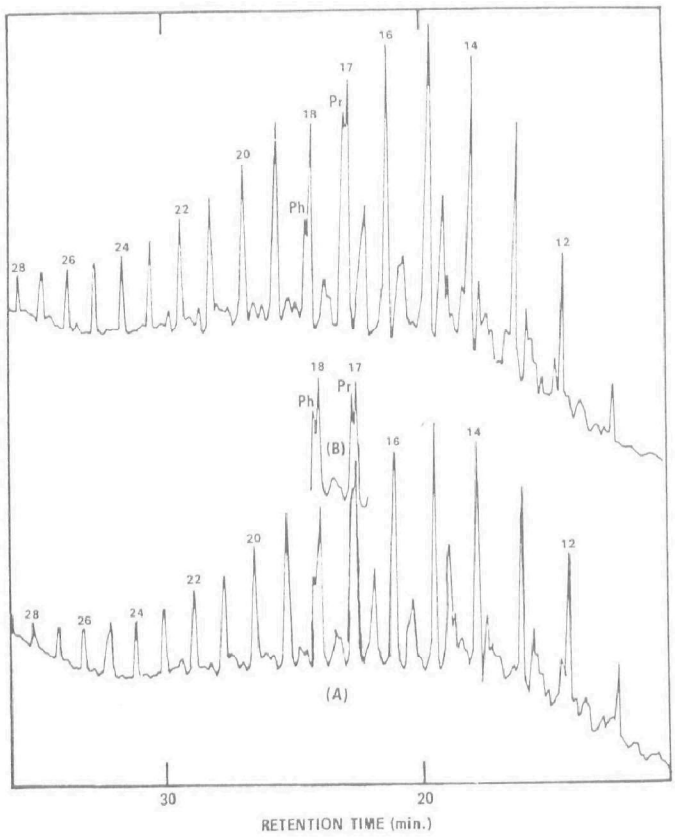

Figure 24. South Louisiana Crude oil. After the 70 mg neat oil treatment procedure (upper chromatogram). After the 0·5 mg dispersed oil treatment procedure (lower chromatogram): (A) – shows slight column degeneration, (B) – new column. See legend of Figure 21 for key to numbers and letters.

Portions of the residues were then injected onto the GC column.

Oil quantification

While modifications of existing procedures for oil extraction (Gruenfeld, 1973) and quantification (Gruenfeld, 1975) to improve sensitivity are planned, our present discussion deals only with a silica gel adsorption technique for separating petroleum oils from animal and vegetable oils, directly in CCl_4 solution. The latter oils contain many components that typify the water dispersed non-hydrocarbon organics that separate together with petroleum oils during solvent extraction. Carbon tetrachloride solutions of South Louisiana Crude and Number 2 fuel oils, and vegetable, olive, and cod liver oils were prepared in 100 ml volumetric flasks, at the following concentrations: petroleum oils - 20 mg/100 ml, non-petroleum oils - 100 mg/100 ml. These solutions were vigorously stirred for 5 and 10 minute intervals with a magnetic stirrer, after additions of 3 g activated and partly deactivated silica gel. Activation was achieved by maintaining 100 g portions of silica gel at 150°C for two hours. The degree of adsorption of the non-petroleum oils by silica gel, and the impact of silica gel adsorption on the petroleum oils were monitored by IR (Gruenfeld, 1975). The degree of oil removal from solution was compared graphically with the degree of silical gel deactivation and solution stirring time.

Oil dispersion

Ultrasonic dispersion was achieved by inserting the instrument probe into 50 ml graduate cylinders containing 50 ml water and accurately weighed amounts of oil. Maximum dispersing energy was applied for two minutes, while suspending the cyclinders in an ice bath. Dispersions of a South Louisiana Crude oil (4·8 cSt at 38°C) and a Bachaquero Crude oil (1070 cSt at 38°C) were tested for stability and miscibility with water. Portions of the South Louisana oil were also recovered after dispersion, and examined for ultrasonically induced chromatographic and spectral changes. Gas chromatography, and IR, UV, and fluorescence spectroscopy were used.

RESULTS AND DISCUSSION

OIL IDENTIFICATION

As previously explained, our immediate goal was to develop a procedure for enhancing comparisons of undispersed oils, with 0·5 and 30 mg quantities of water dispersed oils. We hoped to induce losses of volatiles in minute oil residues, that approximated losses induced by ASTM D3326.

The ASTM method strips off almost all components below the C_{11} normal alkane (Fig. 21). Our procedure for treating neat oils yields equivalent losses (Fig. 22). Similarly, our procedure for treating 0·5 and 30 mg portions of dispersed oils yields chromatograms that nearly match the chromatogram of the neat oil (Figs. 23 and 24). Therefore, within the constraints of our limited tests (one oil was used) it appears that our method can successfully correlate water dispersed oils with neat oils, for the purpose of source identification (fingerprinting). However, evaluation of the method with more oils is needed, especially following short term weathering in the water column. This work is now underway and will be reported in the near future. Meanwhile, our method offers a convenient technique whereby reproducible and intercomparable chromatograms of milligram and sub-milligram amounts of oil can be obtained.

OIL QUANTIFICATION

As part of our work to develop an appropriate oil quantification method, we evaluated a rather unique

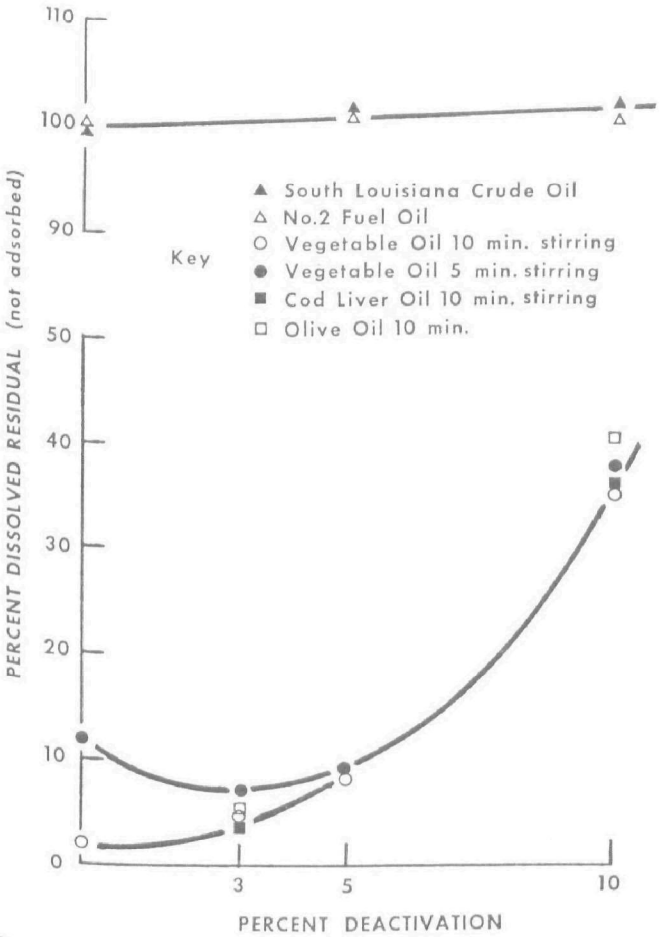

Figure 25. Adsorption of oils by silica gel in CCl_4 solution. IR measurements are at 2930 cm^{-1}, using 10 mm path length cells.

adsorption technique for separating petroleum oils from animal and vegetable oils. Separation was achieved by merely adding small amounts of silica gel to CCl_4 solutions of oils, and then stirring briefly. The non-petroleum oils were removed by silica gel, while the petroleum oils remained in solution. Consequently, we expect that this procedure will successfully separate most water dispersed petroleum oils from the non-hydrocarbon organics that are also extracted from the water column. It should be noted that Florisil can also be used in a similar manner (CONCAWE, 1972), but that silical gel is more effective (Longbottom, 1975).

In order to optimize the adsorption procedure, we evaluated the interaction of silica gel deactivation and solution stirring time, with oil separation efficiency (Fig. 25). Maximum separation of the petroleum oils from the non-petroleum oils was achieved with fully activated silica gel, and a 10 minute stirring period (stirring rate was not monitored). These conditions did not cause a visible loss of the petroleum oils. Increased silica gel deactivation, and reduced stirring time adversely affected the method. The method appears quite practical for separating petroleum oils from non-hydrocarbon organics, prior to IR measurement. But, fully activated silica gel, and ample magnetic stirring (10–15 min.) are recommended.

OIL DISPERSION

Preparation of stable oil-in-water dispersions by ultrasonification offers some attractive possibilities. The concentrates are stable, infinitely miscible with water, and have a known oil content. They are useful for critical method evaluations, and can be added to water columns for various biological and toxicity tests. Our method was used recently to advantage in a study of the mode of accumulation of Number 2 fuel oil by the soft shell clam *Mya arenaria* (Stainken, 1975). Dispersion stability appears to diminish with increasing oil viscosity. The South Louisiana oil yielded 1 % (10 000 µg/ml) dispersions that remained stable beyond four days. But, the Bachaquero oil yielded less concentrated dispersions, that were also less stable.

Since ultrasonification causes considerable heating resulting in oil vapourization losses, we incorporated an ice bath in the method to prevent these losses. Following ultrasonification of 6 and 30 mg portions of the two oils, recoveries in the range, 96–100 %, were obtained. In addition, ultrasonically treated and untreated portions of the South Louisiana oil were compared by gas chromatography, and IR, UV absorption and fluorescence spectroscopy. No significant chromatographic or spectral changes were observed.

CONCLUSION

Current work to develop methods for the quantification and source identification of water dispersed petroleum oils, and for the preparation of stable oil-in-water dispersions, is discussed. Tentative procedures are presented for: 1) separating hydrocarbons from non-hydrocarbon organics for the purpose of oil quantification; 2) inducing comparable volatility losses in milligram quantities of oils to enhance their source identification; 3) preparing stable oil-in-water dispersions with known oil content, that are water miscible. Preliminary conclusions are provided regarding the successful application of the methods, but these conclusions are subject to future tests with more oils, and to integration of our procedures with already existing methods. Final results will be published in the near future.

ACKNOWLEDGEMENTS

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My subject is - The Ultrasonic Dispersion, Source Identification, and Quantitative Analysis of Petroleum Oils In Water*

I would like to discuss three projects that are now underway in our laboratory. We are developing methods for identifying and quantitating small amounts of petroleum oils in water, and a procedure for preparing stable oil in water dispersions containing known amounts of oil. The identification method will correlate milligram and sub-milligram amounts of water dispersed oils with undispersed oils, for the purpose of source identification; the quantitation method will estimate part-per-billion amounts of petroleum oils in water, in the presence of non-hydrocarbons; and the oil in water dispersion method is needed by biologists for oil toxicity evaluations and by chemists for evaluation of oil quantitation methodology. Our work on these projects is still in progress and our conclusions are therefore tentative. In fact, the present discussion and paper deal only with the rather limited segments of each project that have actually been completed.

Source identification of waterborne oils is accomplished in three steps. The oils are first separated from water. The weathered and neat oils to be compared are then submitted to distillation or to some other vaporization procedure, which induces comparable volatility losses in the oils. The non-volatiles are then compared by techniques such as gas chromatography, infrared, and fluorescence spectroscopy. We used a gas chromatographic procedure of the American Society for Testing and Materials (ASTM) to compare the oils. But, we developed a procedure for inducing volatility losses in milligram and sub-milligram amounts of oils, that simulated volatility losses resulting from an ASTM distillation method that requires approximately 40 gm quantities of oil. My present discussion of oil identification is restricted to our vaporization procedure.

*Presented by Michael Gruenfeld, September 8, 1975, at the ICES Workshop on Petroleum Hydrocarbons, in Aberdeen, Scotland.
This is the oral presentation of the preceeding paper.

Oil quantitation is the second subject of my discussion. We are developing an IR spectroscopic method for estimating part-per-billion amounts of petroleum oils in water, even in the presence of non-hydrocarbons. We are evaluating as part of this method a rapid silica gel adsorption technique for separating petroleums from non-hydrocarbons, directly in carbon tetrachloride solution. My present discussion of oil quantitation deals only with this adsorption procedure.

Preparation of stable oil in water dispersions having a known oil content is my third subject for discussion. A commercial ultrasonification device is used to prepare oil in water concentrates that readily mix further with water. We also checked whether ultrasonification causes spectral or chromatographic changes in oils.

My discussion therefore emphasizes the procedures and results of the oil vaporization, silica gel adsorption, and ultrasonic dispersion techniques.

We evaluated vaporization techniques by preparing oil in water dispersions and then extracting them with Freon 113. Dilutions were then made to yield solutions containing 0.5 or 30 mg oil. (Slide 1) These were transferred to 150 ml beakers and stripped to 1-2 ml, with a steam table and a filtered air stream. (Slide 2) The concentrates were then transferred to small glass vials (10 x 30 mm). Vials containing 30 mg oil were suspended in water at 40^{o}C. The filtered air stream was used to remove final solvent traces, and this condition was maintained for 10 additional minutes. Vials containing 0.5 mg oil were maintained at room temperature, and the air was turned off just before total solvent removal. Final evaporation then occurred spontaneously. These vials remained open at

room temperature for 10 additional minutes. Finally, small amounts of carbon tetrachloride (10 - 20 ul) were added to each vial for gas chromatographic injection.

The neat reference oils were treated somewhat differently. Seventy milligram oil was added directly to the vials, which were then suspended in water for 15 minutes, at 40°C, while exposed to the air stream. The oils were then injected onto the gas chromatographic instrument without further dilution.

(Slide 3) Our vaporization techniques, as I previously mentioned, induced losses in small amounts of oil that approximate losses induced by an ASTM distillation procedure for large amounts of oil. This slide illustrates the results of the ASTM procedure. The upper chromatogram is South Louisiana Crude oil before distillation; the lower chromatogram results from use of the ASTM distillation. The numbers refer to n-alkanes, while pristane and phytane are abbreviated as Pr and Ph. The ASTM procedure causes major loss of components below the C_{11} n-alkane. (Slide 4) Our procedure for inducing vaporization losses in 70 mg neat oils yields similar results. The upper chromatogram is South Louisiana oil after ASTM distillation; the lower chromatogram is the neat oil after our procedure. (Slide 5) The lower chromatogram here results from our treatment of 30 mg oil that was extracted from water. The upper chromatogram is the 70 mg portion of neat oil. (Slide 6) The upper chromatogram here is again the 70 mg portion of neat oil; the lower chromatogram results from our treatment of 0.5 mg oil that was extracted from water. The lower chromatogram shows evidence of gas chromatographic column degeneration, but adequate resolution of pristane and phytane from the normal alkanes was achieved with a new column. I should emphasize that although oils that actually weathered in the water

column have not yet been evaluated, our techniques are usable for identifying small amounts of oil that occur as slicks and shoreline residues.

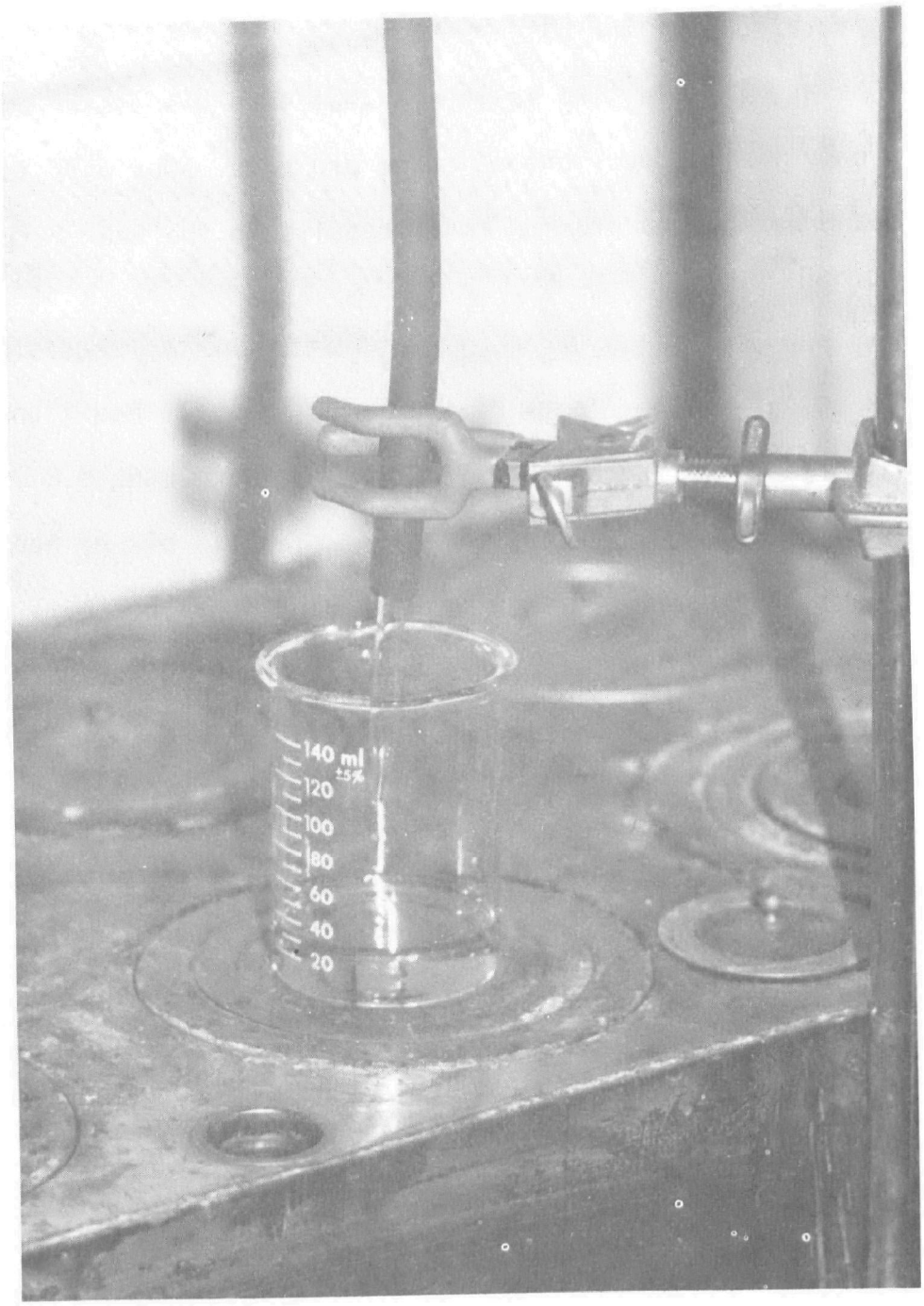
Next I will discuss the silica gel adsorption technique that was evaluated in the oil quantitation project. Silica gel was tested for separating petroleum oils from animal and vegetable oils directly in carbon tetrachloride solution. We contrasted the sorption capacity of silica gel with its solution contact time, and degree of activation. Carbon tetrachloride solutions of two petroleum oils at 20 mg/100 ml, and vegetable, olive and cod liver oils at 100 mg/100 ml were prepared in 100 ml volumetric flasks. 3.0 gram portions of activated and partly deactivated silica gel were added to the flasks. (Slide 7) The solutions were vigorously stirred for five and ten minute intervals with a magnetic stirrer. (Slide 8) A plot was prepared contrasting oil removal, with silica gel deactivation and solution stirring time. Maximum separation of petroleum from non-petroleum oils resulted from fully activated silica gel and ten minute stirring. No loss of petroleum oils occurred. Decreased activation, and reduced stirring yielded adverse results.

I will now briefly describe the preparation of oil in water dispersions. (Slide 9) The probe of an ultrasonic instrument was inserted into a 50 ml graduated cyclinder containing water and an accurately weighed amount of oil. Maximum dispersion energy was applied for two minutes, while keeping the cylinder in an ice bath. Cooling prevented oil vapor losses during ultrasonification, which causes substantial heating. Dispersion stability diminished with increasing oil viscosity. A non-viscous crude oil yielded stable 1% (10,000 PPM) dispersions that were stable for days; but a more viscous oil yielded less stable and less concentrated dispersions. This technique permitted oil recoveries in the range 96 - 100%. A check for

ultrasonically induced changes showed no substansive chromatographic or spectral alterations of the oils.

In closing, I would like to repeat that this discussion and paper deal with only limited portions of three projects. Our work is still underway, and we hope to provide final results in the near future.

- THANK YOU -



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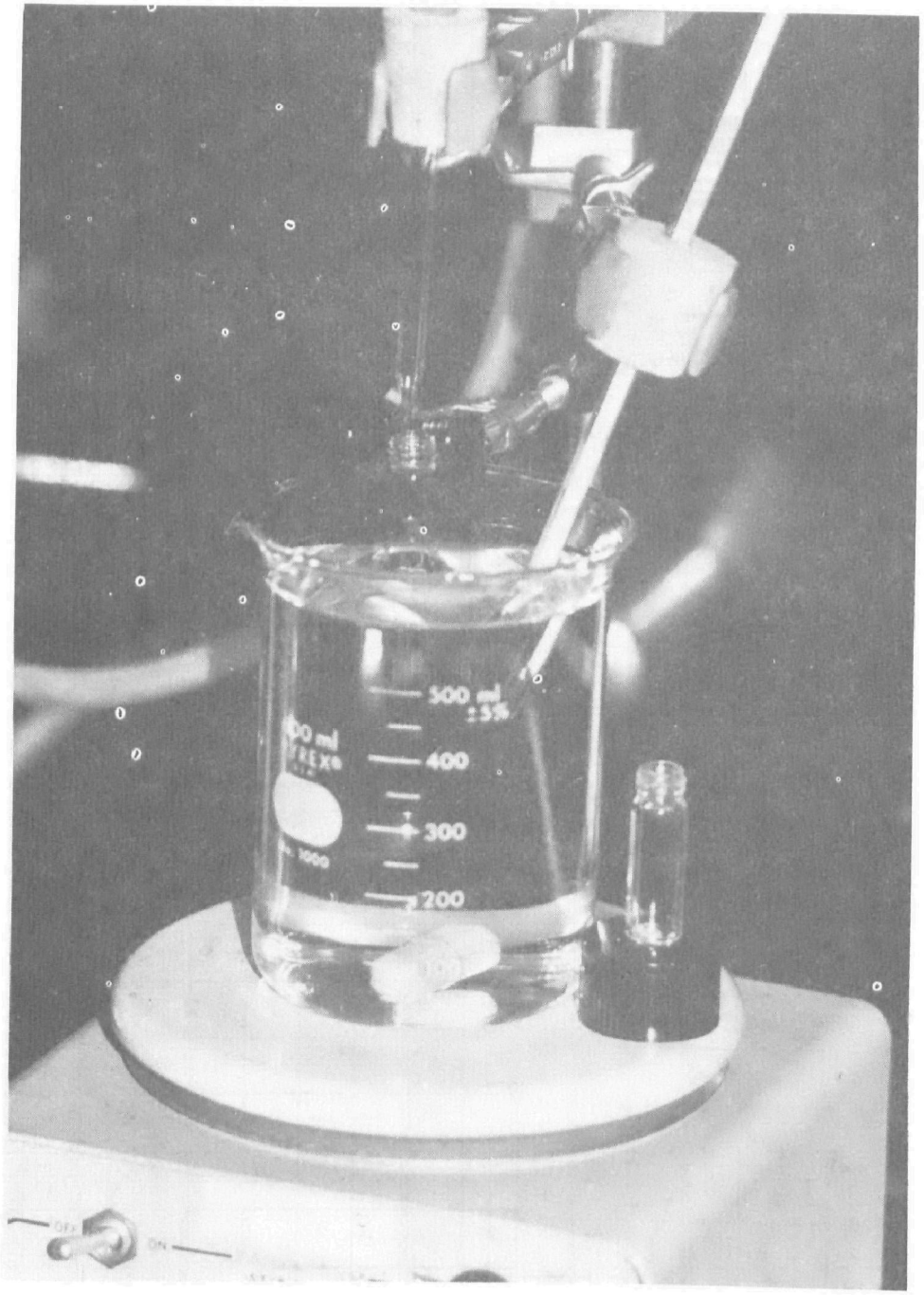

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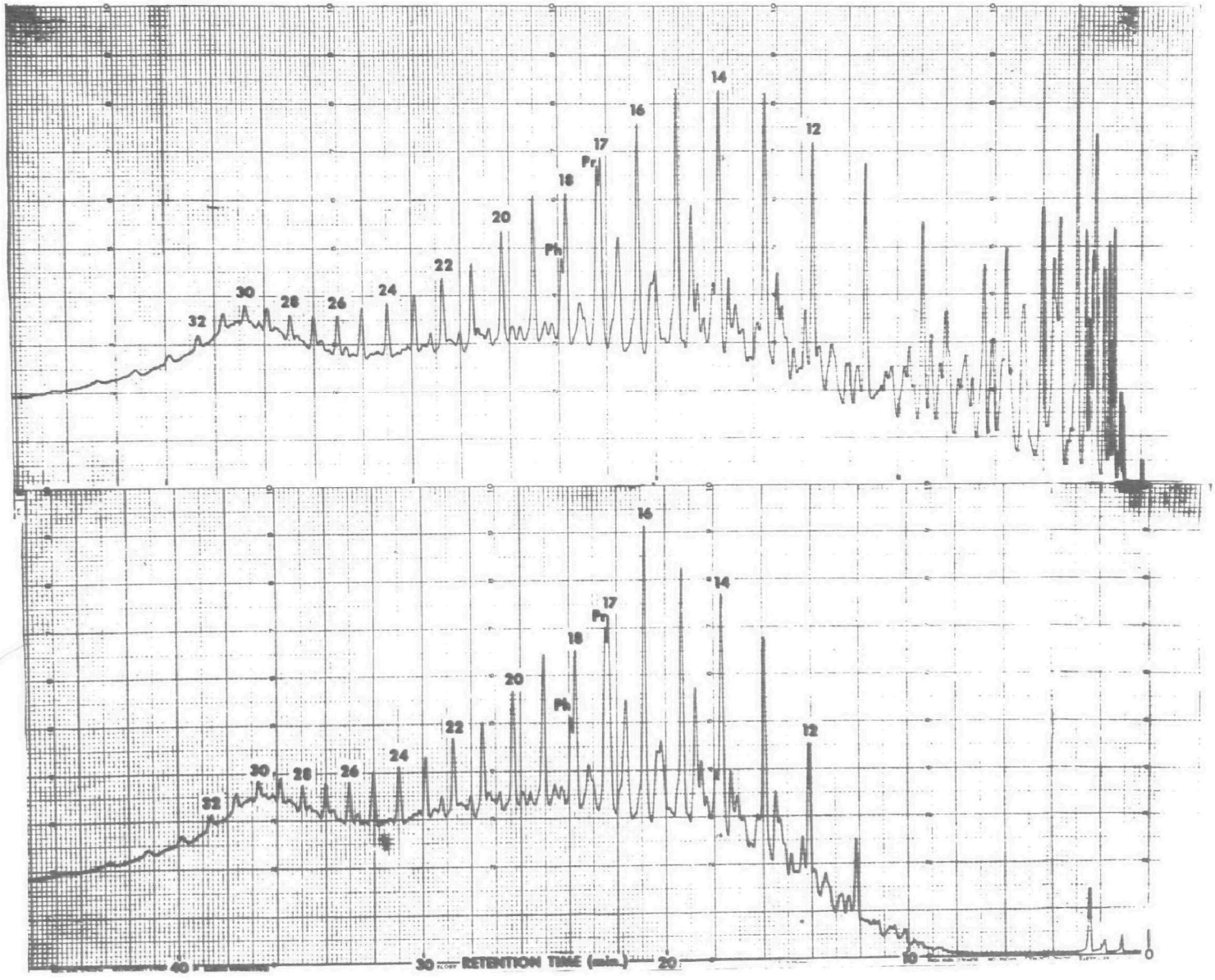

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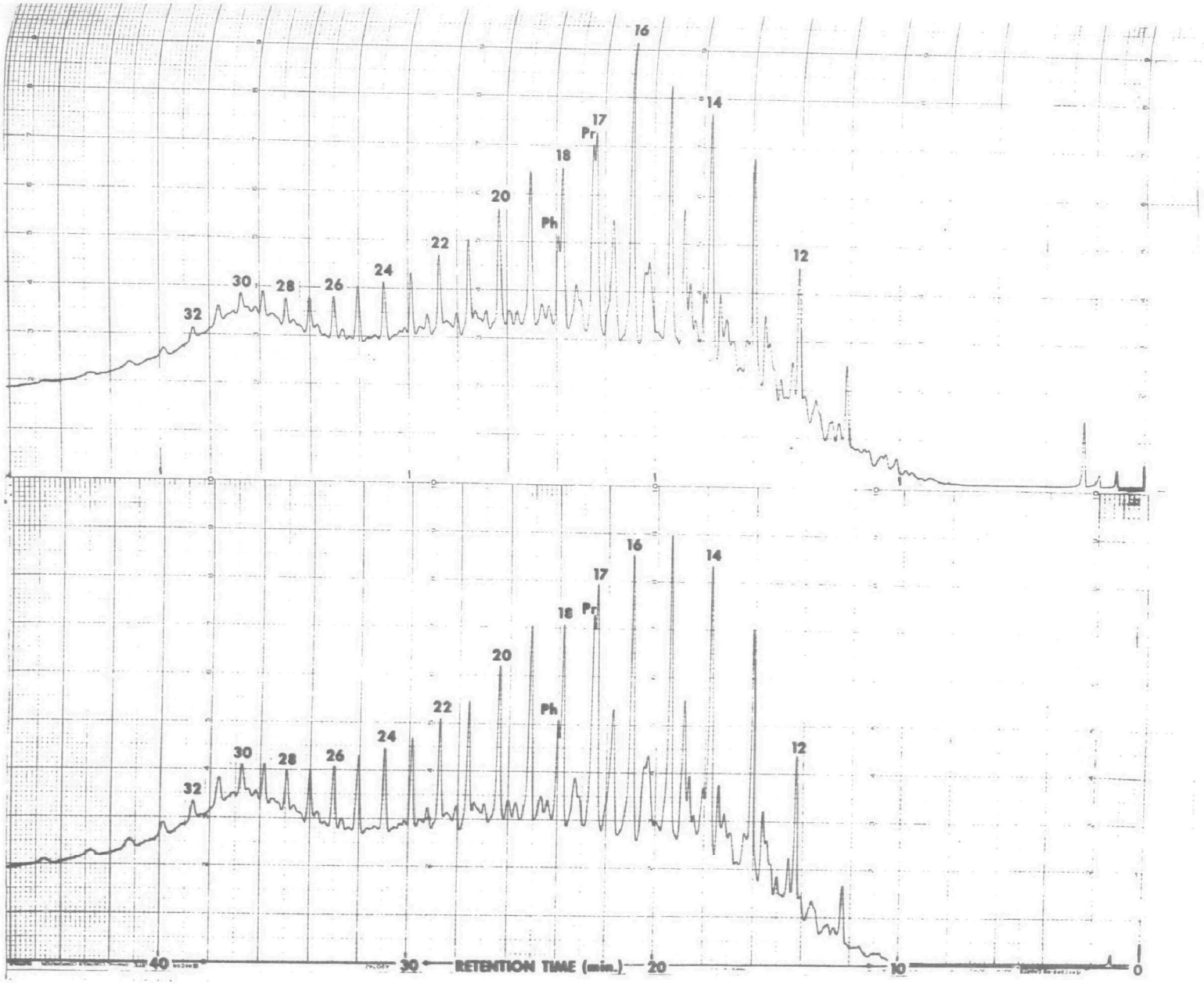
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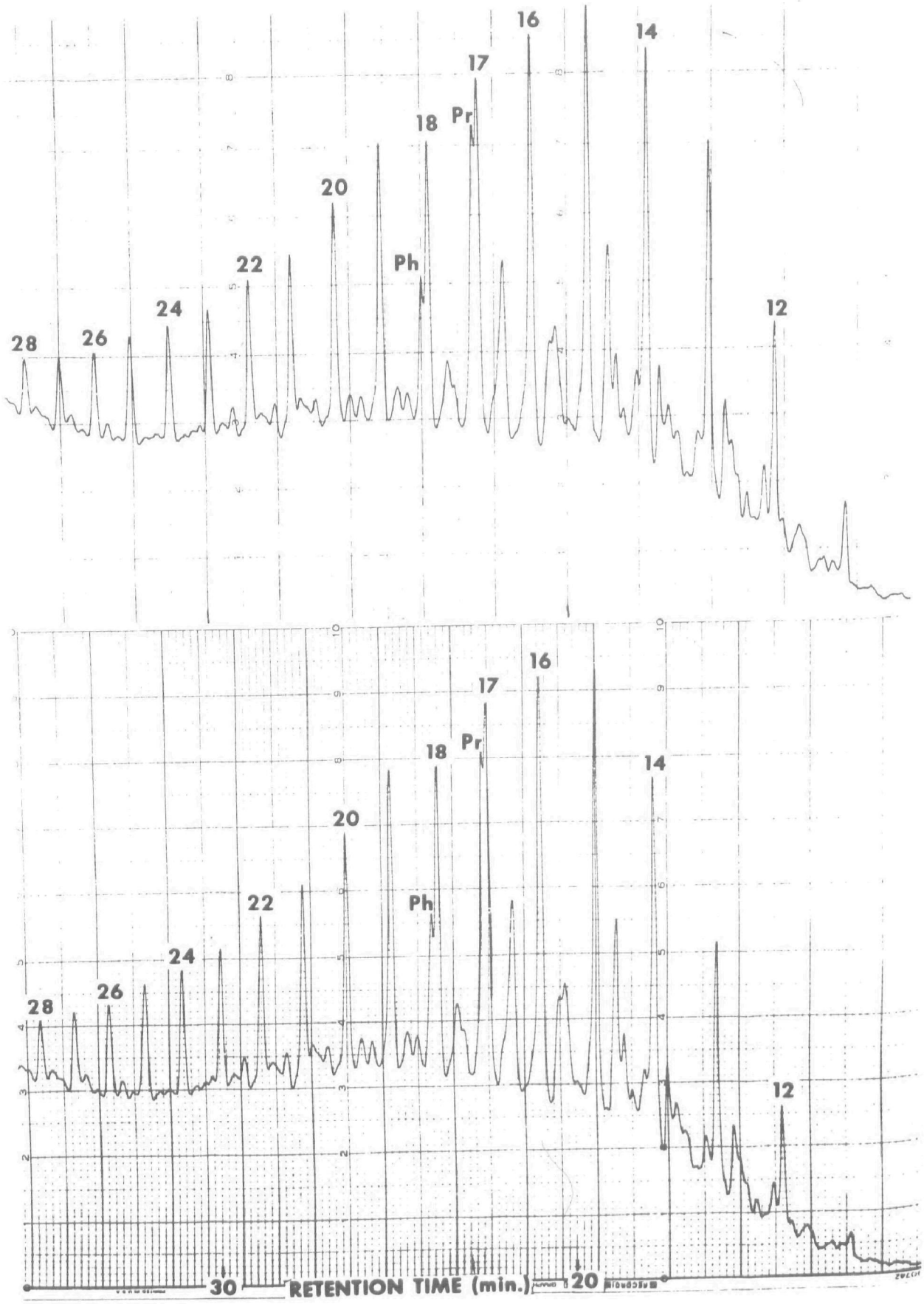

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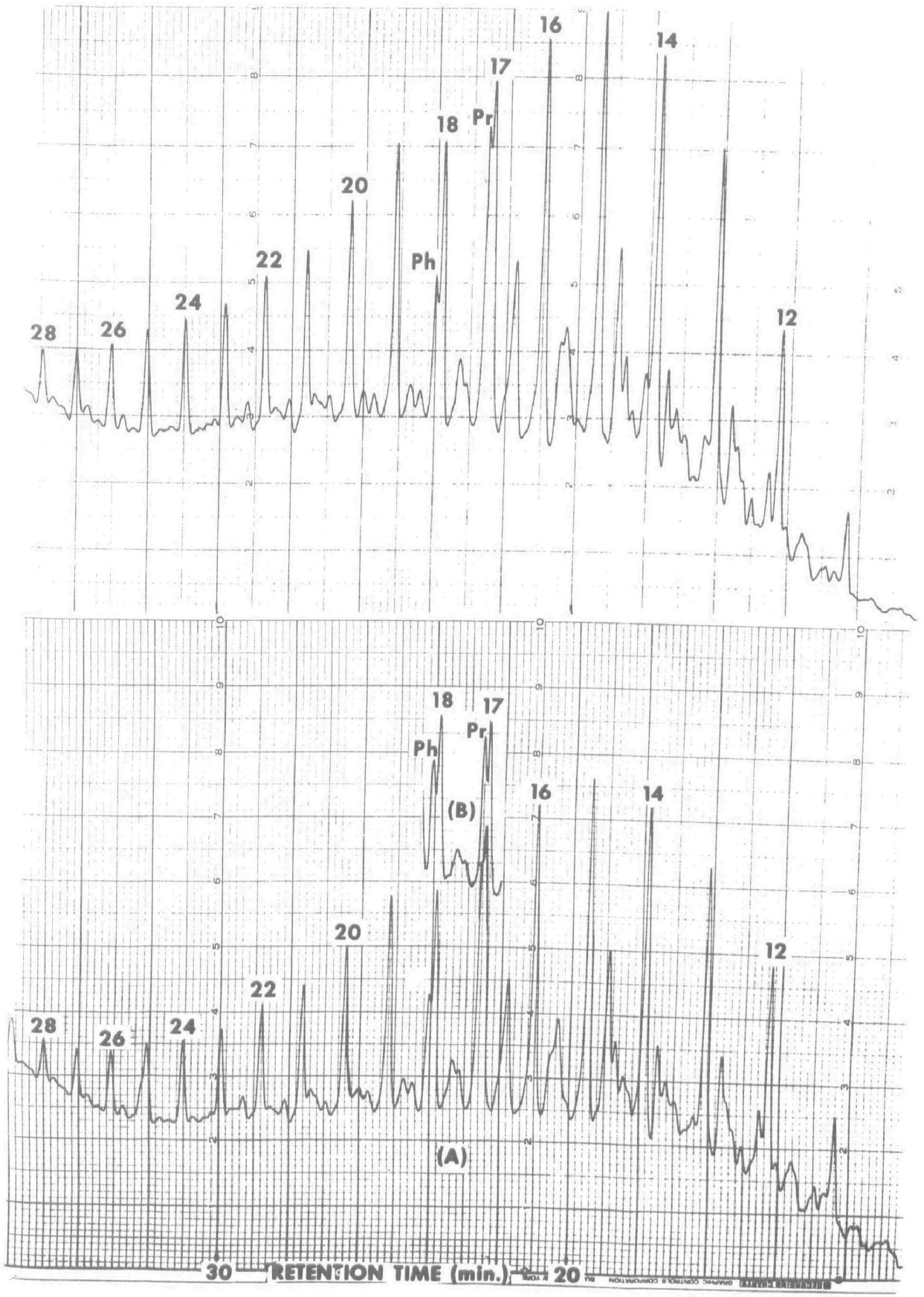

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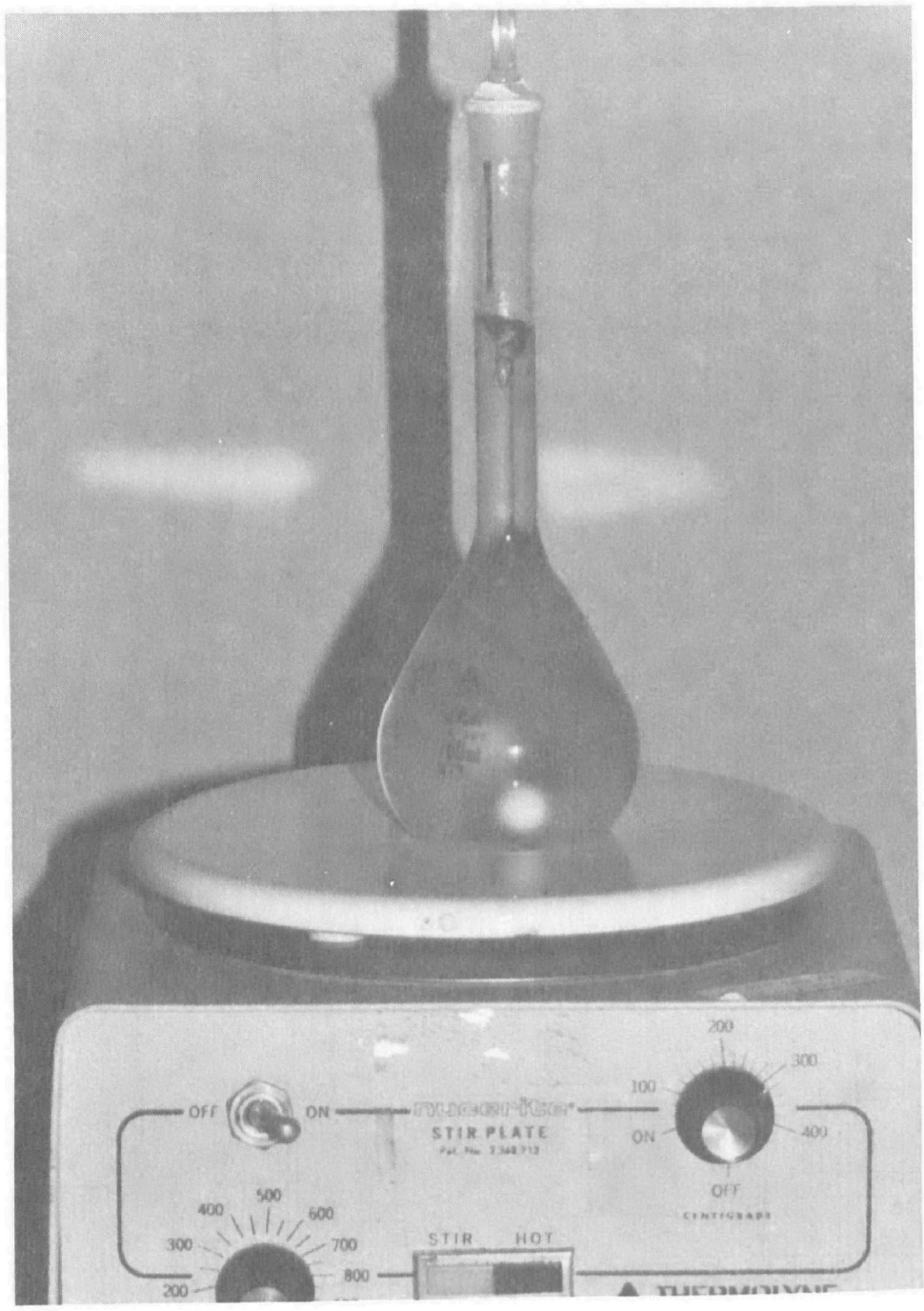
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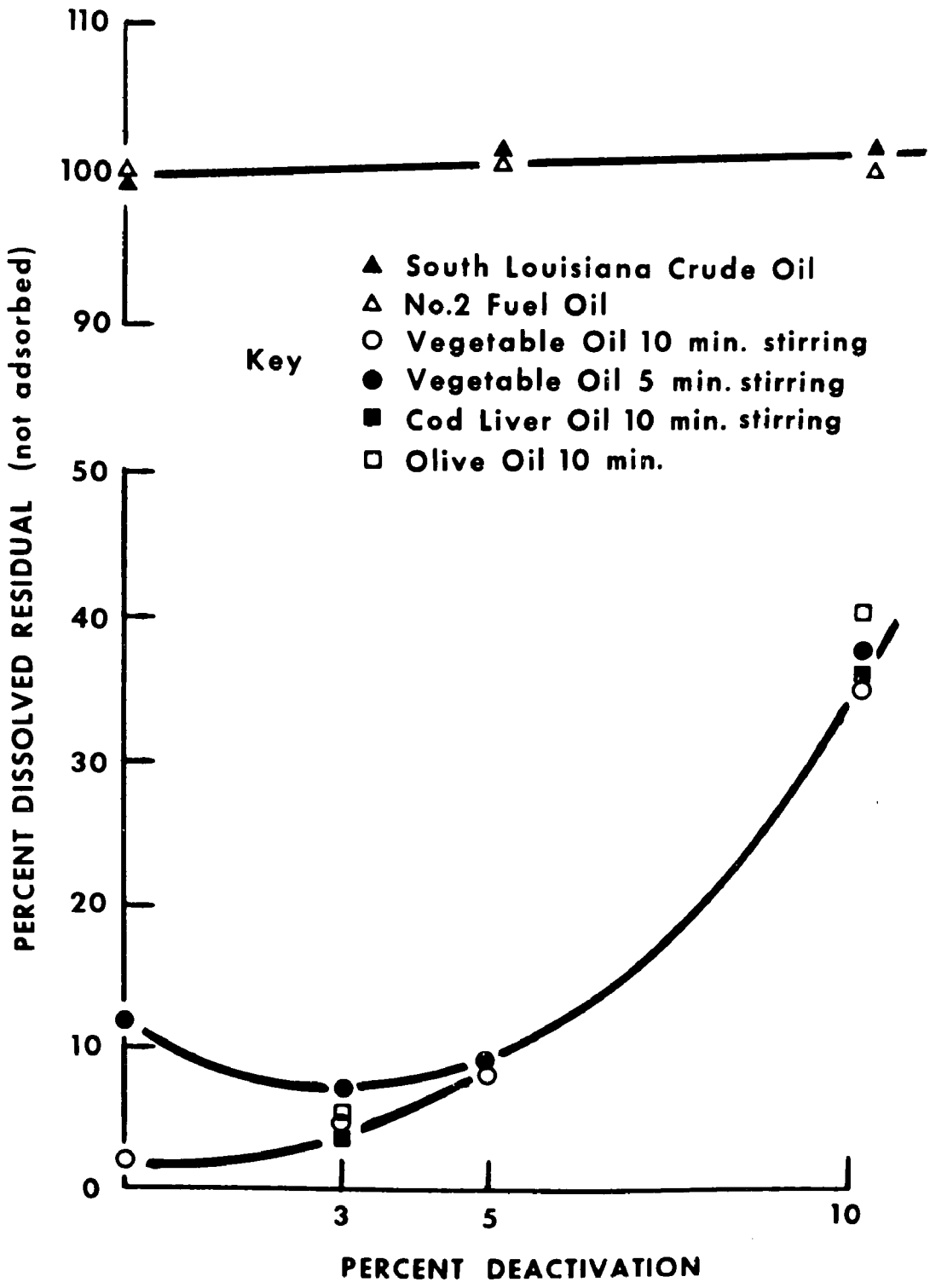
Slide 7


110
100
90
50
40
30
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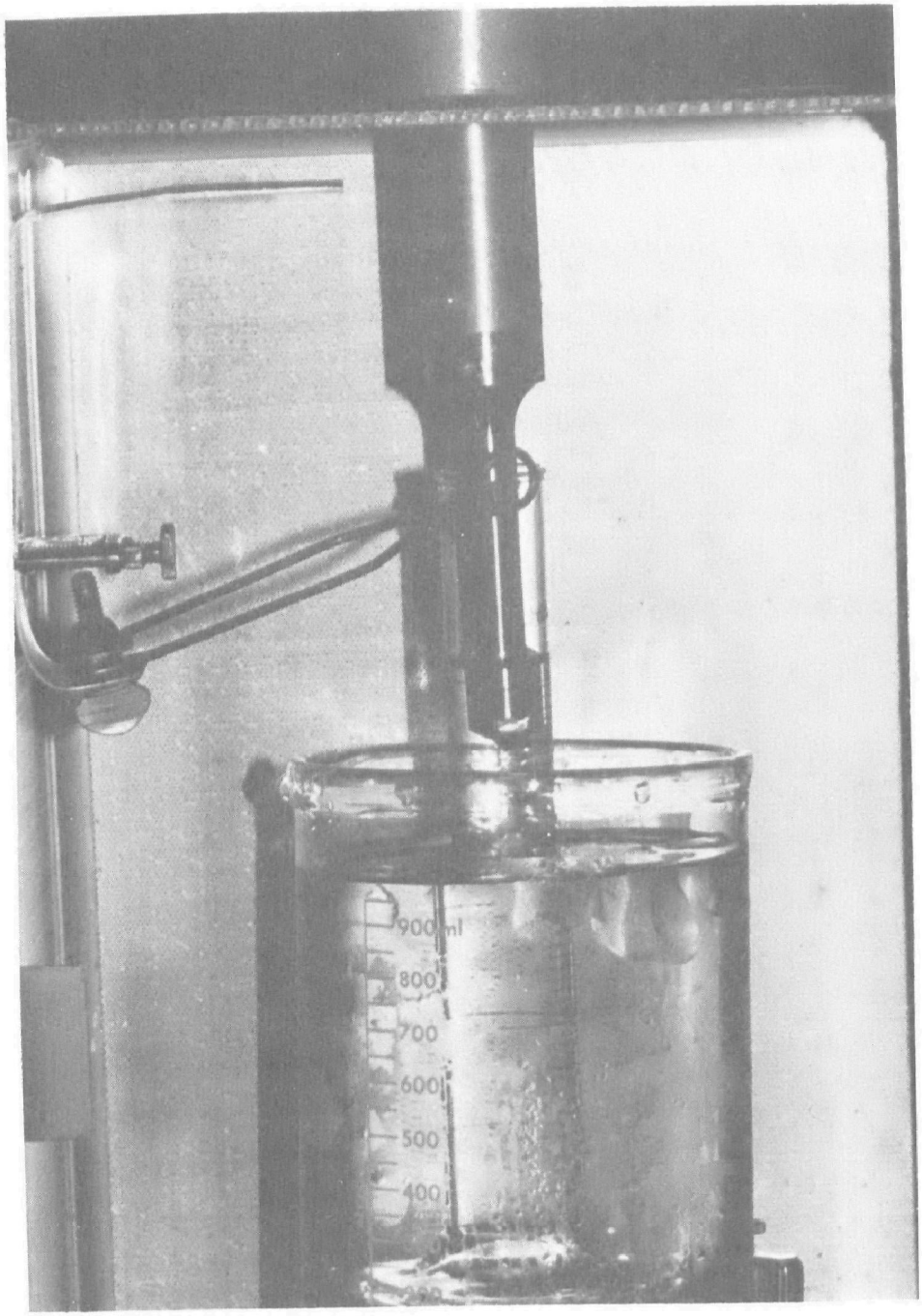
5
10
PERCENT DISSOLVED RESIDUAL (not adsorbed)
PERCENT DEACTIVATION
Key
South Louisiana Crude Oil
No.2 Fuel Oil
Vegetable Oil 10 min. stirring
Vegetable Oil 5 min. stirring
Cod Liver Oil 10 min. stirring
Olive Oil 10 min.

Slide 8



Slide 9

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II. PETROLEUM OILS

C. ENVIRONMENTAL STUDIES

(*) ANALYTICAL QUALITY CONTROL NEWSLETTER #13, April 1972.

Comparison of Hydrocarbons in Marine Organisms from Unpolluted Water with Petroleum Oils - Passive tagging and quantitative analysis of petroleum oil pollutants in fish and other pelagic marine organisms are under investigation. Rapid and accurate methods are needed for analyzing these pollutants of low concentrations in pelagic organisms. Previous investigations examined mainly benthic organisms such as shellfish. As part of the investigation a summary table has been prepared comparing the hydrocarbons found in crude petroleum oils with those oils normally present in organisms taken from "unpolluted" waters. A table showing comparisons and the references from which the information was obtained are available from the Edison Laboratory. (B. F. Dudenbostel)

* See page 2.'

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PRELIMINARY OBSERVATIONS ON THE MODE OF ACCUMULATION OF #2 FUEL OIL BY THE SOFT SHELL CLAM, *MYA ARENARIA*

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ABSTRACT

Chemical analysis has shown that various components of oils can accumulate within marine invertebrates. Several mechanisms by which this may occur have been conjectured. This paper offers experimental verification of a mechanism by which a commercially important bivalve, Mya arenaria, can accumulate oil within its tissues. The paper also documents the behavioral response of Mya arenenaria and deleterious ecological side effects resulting from oil accumulation.

Young Mya (25-35 mm) were exposed to #2 fuel oil and an oil soluble dye (Oil Red 0) which were ultrasonically emulsified in water. The concentrations tested were 50 ppm, 100 ppm and 150 ppm. Exposures were done in both natural and artificial seawater at 4° C and 22° C. Exposure periods ranged from 3 hours to 4 days.

Macroscopic observations were performed to determine the effects of the dyed oil contacting the gill surfaces and the means by which the oil was either ingested or ejected. Definite patterns of response to the dyed oil were established. Essentially, the clams treat oil micelles and globules as food or detritus particles. The smallest oil micelles are passed by ciliary currents directly to the stomach. Larger globules are bound by mucus secreted by the gill ctenidia. Gas chromatography and mass spectrometry confirmed the binding of oil-mucus. The oil-mucus is ingested or rejected by means of the clam ciliary pathways. Implications of the oil-mucus mechanism and the ejection of this mucus into the environment are discussed.

INTRODUCTION

Oil spillage continues to be a serious problem in coastal and estuarine waters. Many spills reported from barges, tankers, and industrial installations involved fuel oils [8,2,32,22,20]. Spilled oil may be altered by many factors including evaporation and photo-oxidation. The water soluble portions of the oil, mostly aromatics [5,21], will dissolve and be dispersed. Many of the petroleum hydrocarbons may be dispersed throughout the water column by formation of emulsions, either by turbulent wave action [13,12] or chemical dispersion. Morris [23] documented the occurrence of oil emulsions in the eastern Mediterranean. Some of the oil may be adsorbed to particulate matter within the water column or mixed in the sediment. Boehm and Quinn [4], experimenting with #2 Fuel Oil, have demonstrated the existence of accomodated (particulate or droplet), solubilized (colloidal micelles), and soluble hydrocarbon components in fuel oil-seawater mixtures.

The solubilized petroleum hydrocarbons dispersed throughout the water column and sediments may be ingested by benthic marine filter feeders. These animals filter the water to obtain planktonic food, maintain respiration, and excrete wastes. Chemical analyses have shown that various components of oils can accumulate within marine bivalves [11,6,7,33,10].

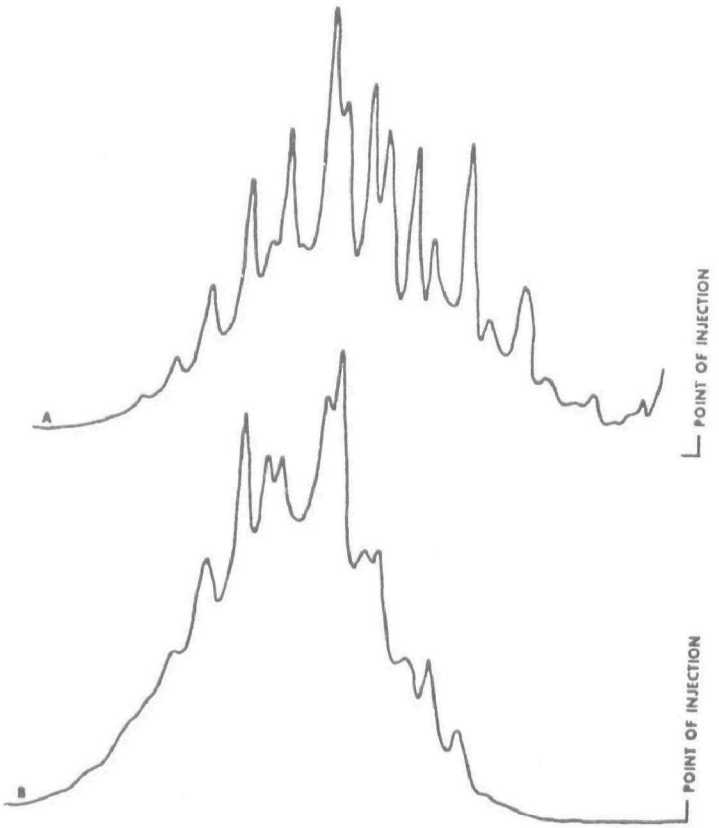

Figure 1. Gas Chromatograms: (a) 8 ppm #2 fuel oil in hexane, attenuation 1 X 128; (b) Carbon tetrachloride extract of clam mucus, attenuation 10 X 1024

Number two fuel oil was chosen for study because it is commonly shipped in coastal waters, used in coastal industrial installations, and has already been involved in a well-documented spill [2]. The #2 fuel oil was supplied by the United States Environmental Protection Agency, Industrial Waste Treatment Research Laboratory in Edison, New Jersey. Two temperatures, 4°C and 22°C, were used to represent the winter and summer temperature regime found in the New York region. The bulk of the study was performed at 4°C assuming that the winter with its accompanying wind and

storms would be the most likely time for spills to occur. Oil was added as an emulsion assuming that initially much of the spilled oil is naturally emulsified through turbulent wave action. Subsequent treatment by oil dispersants would increase emulsion formation.

Mya arenaria were chosen to study because they are a commercially valuable bivalve commonly found in the littoral and sublittoral zones on the American eastern Atlantic coast from the coast of Labrador to the region of Cape Hatteras, North Carolina, and on the Pacific coast from Monterey, California, to Alaska. *Mya* occur on the European coast from Norway to the Bay of Biscay, France, and along the western Pacific coast from the Kamchatka Peninsula to the southern regions of the Japanese Islands [16]. Young *Mya* with a mean length of 25 mm [25,27] were utilized because young bivalves tend to have higher respiratory and filtration rates than older bivalves [28,29,19,9,35]. Young clams would therefore probably accumulate oil present in the water column faster than older clams. They would also be prone to recurrent exposure to oil during their maturation process with the concurrent possibility of continuous low-level accumulation of petroleum hydrocarbons within their tissues. A continuing re-exposure to oil can occur in cases where the oil is mixed into the sediments and slowly released again. Farrington and Quinn [11], Zafiriou [36], Blumer et al. [2], and Scarratt and Zitko [31] have reported the occurrence of petroleum hydrocarbons from marine sediments. Blumer et al. [2] demonstrated that oil-contaminated sediments can continue to release relatively undegraded oil for extended periods of time.

Numerous studies have reported the occurrence of petroleum-derived hydrocarbons in bivalved molluscs, and several mechanisms by which this may occur have been conjectured. This paper offers experimental verification of a mechanism by which a commercially important bivalve, *Mya arenaria*, can accumulate oil within its tissues. The paper also shows the behavioral response of *Mya arenaria* and the possible deleterious ecological side effects that result from oil accumulation.

Methods and Materials

The mode of accumulation of #2 fuel oil and behavioral response of *Mya* was followed by using an oil soluble dye, Oil Red 0. Adapting a procedure developed by Gruenfeld and Behm [14], #2 fuel oil and Oil Red 0 (5 mg) were emulsified in 100 ml of chilled tap water by ultrasonification to produce a stable 20,000 ppm dyed oil in water emulsion. This was diluted to the desired concentrations for each test. After exposure to the dyed oil, each clam was removed from the water and its left valve was removed. Dyed oil accumulation was then followed by observations using a binocular dissecting scope. A color photographic record was maintained of selected specimens.

A preliminary series of four experiments was run at different exposure periods (3-24 h) and concentrations at 22°C to determine procedures and patterns. In the first experiments, lab acclimated clams were placed in specimen dishes with natural filtered seawater (26%) or artificial Rila seawater mix (26%). The dyed oil emulsion was added either by syringe when the clam was siphoning or by adding the desired concentration to the water. In subsequent experiments, clams were placed in 1,600 ml of Rila artificial seawater mix (26%), and enough dyed oil emulsion was added to achieve the test concentration. The salinity chosen for the tests was the salinity of the water at the time the clams were collected. In those experiments in which clams were kept in 1,600 ml seawater, aeration was provided through a 1 ml pipet with a moderate airflow.

In the experiments performed at 4°C, clams were kept in 1,600 ml of artificial seawater (26%) and exposed to concentrations of 50 ppm, 100 ppm, and 150 ppm dyed oil emulsion for periods of 1, 2, 3, and 4 days.

The dye was determined to be totally insoluble in mucus by taking human mucus and mixing the raw dye in. The dye and mucus remained insoluble. A drop of #2 fuel oil instantly solubilized both. After mixing and standing for several minutes in seawater, the mucus-oil-dye combination had the same appearance as seen in the clams in the experiments.

Results

The dyed oil emulsions were very stable in the water column and did not crack readily. Whenever the dyed oil emulsion cracked, small red oil globules would appear on the water surface. Even when some of the dyed oil emulsion accumulated on the surface, there was still a visible red tint in the water column. This may have been due to the solubilized (colloidal micelles) and soluble hydrocarbon components in the dyed fuel oil emulsion-seawater mixture [4]. Because some of the dyed emulsion did crack with time, actual exposure concentrations are relative only to time 0 when the emulsion was added. The Oil Red 0 (C.I. 26125) is a neutral dis-azo dye which does not contain water solubilizing groups and hence is insoluble in aqueous media but is soluble in oils, fats, waxes, etc. [15]. The Oil Red 0 dye was totally insoluble in aerated and nonaerated control mixtures of dye and artificial seawater and dye and natural seawater. Earlier toxicity and ancillary experiments had confirmed that the patterns of mucus secretion and behavior response were elicited by the emulsified fuel oil. The dye had no effect on the clam.

In preliminary experiment I at 22°C, seven clams (25-35 mm) were used. Tests were run for 3 hours. All clams behaved similarly. The dyed oil emulsion present in the water while the clam was filtering resulted in periodic "coughing" and periodic rejection of dye-oil in a mucus binding. This periodic coughing is a rapid contraction of the adductor muscles which forces water out the inhalant siphon. It is a mechanism commonly used by bivalves to rid themselves of accumulated detritus and pseudo feces.

Within 2 to 3 hours, two clams had dyed oil-mucus visible in the stomach. One of these plus two others now had dyed oil-mucus in the digestive diverticula and around the style. Most commonly, the dyed oil was found bound in mucus near the palps. The behavior of the dyed oil on the gills and palps was closely followed. It was noted that the oil droplets and globules were passed to the edge of the gills, bound in mucus as a food or detritus particle, and passed towards the palps. Smaller oil droplets passed directly to the palps. The oil usually followed the ciliary pathways outlined by Kellog [18].

The results from preliminary experiments II and III are listed in tables 1 and 2 respectively. In preliminary experiment IV at 22°C, clams were exposed to 50 ppm and 100 ppm of dyed #2 fuel oil emulsion for 24 hours. Large clams were used (40-50 mm), four per concentration. At 50 ppm exposure, one clam accumulated dyed oil and oil-dye-mucus in the stomach and diverticula. All had much oil-dye-mucus on the mantle, gills, and some on the palps. At 100 ppm exposure, three died. One accumulated oil in the stomach and diverticula, with oil-dye-mucus on gills and mantle.

The first accumulation experiment at 4°C was a 24-hour exposure of clams (20-25mm) to the dyed-oil emulsion at concentrations of 50 ppm and 100 ppm. Fourteen clams were used in the test at 50 ppm exposure. Four clams accumulated dyed oil in the stomach and diverticula. Two clams had no oil visible and ten had dyed oil in mucus on the gills, palps, mantle, and foot. Some dyed oil-mucus exited through the pedal aperture. Seven clams were exposed to the 100 ppm concentration. Five clams accumulated dyed oil in the stomach and digestive diverticula. All clams had oil-dye-mucus on gills, mantle, and palps.

The results from accumulation experiment II at 4°C are listed in table 3. In these experiments, clams were exposed to the dyed oil emulsion for 1, 2, 3, and 4 days. Figure 2 illustrates the results of accumulation experiment II at 4°C. Experimental problems precluded the measurement of the incorporation of the 150 ppm concentration of the dyed oil emulsion on day one.

Chemical confirmation of the oil being bound or adsorbed to mucus is shown in figure 1. The data was obtained from a different series of experiments in which *Mya* were exposed to sublethal concentrations of #2 fuel oil. Complete results from these experiments will be published at a later date. The mucus extracted was secreted by clams exposed to an initial concentration of 100-ppm emulsified #2 fuel oil. Four weeks later some of the mucus was collected. It had a gray-green flocculent appearance and formed flocculent clumps on the surface and in the water column. A sample of water and mucus was taken from the water column (there was no surface

Table 1. *Preliminary experiment II*–22°C, natural filtered seawater, clams (20-25 mm) were placed in specimen dishes and dyed oil added

Group 1–66 ppm, exposure 5 hours (3 clams)
Much of the dyed oil was visibly bundled in mucus, appearing as semisolid viscous globs on the surface. No oil was readily visible in the gut. Most oil was bundled in mucus near pedal and palp area or following general ciliary rejection pathways.

Group 2–83 ppm, exposure 5 hours (5 clams)
All clams had packaged the oil-dye within mucus. This oil-dye-mucus was always found on and around the palps. A small amount was sometimes found on the gills.

Group 3–83 ppm, exposure 5 hours (kept anaerobic 1½ hours before test by placing on wet towel to induce filtering when placed in oil and water) (7 clams)
Oil-dye-mucus strings were near, on, and around the palps in 5 clams. None had oil visible in the visceral mass; some oil-dye-mucus was being shunted out the pedal aperture and siphon in several clams.

Group 4–83 ppm, exposure 8 hours (kept anaerobic 6 hours before test to induce filtering when placed in oil and water) (8 clams)
Three clams showed no trace of oil. One clam's digestive diverticula were tinged red. Five clams had oil-dye-mucus on, near, and around palps and siphon and very small amounts in mantle rejection tracts.

Group 5–83 ppm, exposed 9 hours (kept anaerobic 6 hours before test) (8 clams)
Two clams showed no trace of oil. The other 6 clams had oil-dye bound in mucus near the palps and/or siphon. A few had some in the mantle rejection tracts.

Group 6–Kept anaerobic 1½ hours prior to test, placed in seawater, and fed 2,000 ppm oil-dye emulsion from syringe whenever pumping for 8 hours (5 clams)
Four clams had accumulated oil in stomach. No oil was visible in the digestive diverticula. Several clams had some oil-dye bound in mucus near the palps or siphon.

Groups 7 & 8–Kept anaerobic 6 hours prior to test, then placed in seawater and fed dye-oil emulsion by syringe for 9 hours. Four clams were fed a 100 ppm emulsion. All accumulated oil in the stomach and in mucus around palps and on mantle. Four clams were fed a 2,500 ppm emulsion. Three accumulated oil in stomach; all accumulated oil in mucus on palps, near siphon, and some on gills and mantle.

slick) and filtered through no. 1 Whatman paper. Infrared analysis of the water at the time of sampling indicated a concentration of approximately 0.466 ppm oil in the water column. Solvents were glass distilled and checked for impurities. All glassware was prewashed with solvents. Approximately 40 ml of mucus-water sample was filtered and then washed with artificial seawater. The filter was then extracted in an erlenmeyer flask with 50 ml of carbon tetrachloride with constant shaking for ten minutes. The sample was then filtered through prewashed sodium sulfate and concentrated to a volume of 0.15 ml under nitrogen. During this procedure, all carbon tetrachloride evaporated. Gas chromatographic analysis was done using a Perkin Elmer Model 900 gas chromatograph equipped with a flame ionization detector and a six-foot stainless steel column packed with 8% Dexsil on 80/100 mesh of Chromosorb W. Standard operating conditions were as follows: carrier gas N_2, 2 cc/min; detector H_2, 20 cc/min, air 40 cc/min; injector temperature 200°C, detector temperature 300°C, manifold temperature 300°C; column temperature 70°C with two-minute hold, programmed at an increase of 8°C per minute to 300°C; injection sample 0.1 micro liter. The mucus sample gas chromatographic tracing was quantitated gravimetrically by comparison to the peak area of 16 micrograms of octacosane dissolved in hexane. The calculated quantity of oil derived from the mucus was 833 micrograms. The hydrocarbon content of the water was 4.66 ppm or 0.466 micrograms per milliliter. Therefore, the most the hydrocarbon background of the water

Table 2. *Preliminary experiment III*–22°C, clams (25-34 mm) placed in 1,600 ml Rila seawater mix (26%), 12-hour exposure

conc.: 25 ppm (6 clams), 50 ppm (6 clams), 100 ppm (5 clams)–all in #2 fuel + dye
50 ppm (5 clams) in So. Lo. crude oil

25 ppm (#2 Fuel):	Five clams had oil-dye and or dye-mucus accumulated in the stomach and diverticula. All had oil-dye wrapped in mucus near and on palps, and some on mantle and gills.
50 ppm (#2 Fuel):	All clams had oil-dye and or oil-dye-mucus accumulated in the stomach and diverticula. In some the style tip was slightly tinted. All had oil-dye-mucus on, near, and around palps, some near foot and on mantle, gills, and pedal aperature.
100 ppm (#2 Fuel):	Two clams accumulated oil-dye in stomach and diverticula, but no oil was visible in three. All had large amounts of oil-dye-mucus on gills, palps, mantle, siphon, and pedal aperture.
50 ppm (So. Lo. Crude):	All 5 clams accumulated dyed oil and dyed oil-mucus in the stomach and digestive diverticula. Only 2 clams had small amounts of oil-dye-mucus visible on gills, mantle, and pedal aperture.

could have contributed by adhering to the filter was 18.64 micrograms, and the remainder was from the oil bound in mucus. Mass spectrometric analysis of the extracted sample was provided by the U.S. Environmental Protection Agency, Industrial Waste Treatment Research Laboratory, Edison, New Jersey. Analysis demonstrated that the sample was predominantly dimethylnaphthalenes and trimethylnaphthalenes with paraffins, mainly in the C-14 and C-15 regions.

Discussion

When dyed oil initially contacts the gill surfaces, the beating of the gill frontal cilia often disintegrates the film or globules. The resultant small oil globules and micelles then pass along ciliary tracts to the palps directly, or enwrap in mucus secreted by the gills. The clams treat the oil globules and micelles as food or detritus particles. The mucus covered particles will then either fall onto rejection paths of the mantle or enter the esophagus and stomach. When the dyed oil is bound in mucus it follows the ciliary pathways as outlined by Kellog [18]. The movement is similar to the general patterns described for bivalves by Morton [24] and Jørgensen [17]. If the mucus strings accumulate on the gills and palps and fall onto the mantle, they may be ejected out the siphon or pedal aperture. As oil concentration and exposure time increases, production of mucus binding the oil increases. This tends to accumulate in the anterior portion of the clam around the palps causing the clams to increasingly shunt the bulk of the rejecta out the pedal aperture.

Bernard [1] described particle sorting and labial palp function in the Pacific oyster. Suspended particles, both organic and inorganic, which impinge on the gill ctenidium are entrapped in mucus and transported by the action of the frontal cilia. Stimulation of two or more adjacent ctenidial filaments results in a copius secretion. The ctenidial surfaces are normally covered with a thin watery fluid not subject to ciliary movements. A definite band of mucus overlays the frontal cilia. Tactile stimulation of the ctenidia results in the abundant production of "rejection" mucus forming a sheet over the ctenidium. The mucus masses are then carried to the free margins of the ctenidia. Overstimulation of the filaments by particles will cause the release of rejectory mucus which is removed from the ctenidium by muscular action or its inability to enter the food grooves. Much of the mucus is carried to the palps. In the normal state the inner surfaces of the palpi are always pressed together. The complicated ciliation of the palps brings any large mucus masses to the free edges

Table 3. ***Accumulation experiment II*****–4°C, clams (20-25 mm) exposed 1, 2, 3, and 4 days to #2 fuel oil-dye emulsion.**

Conc.: 50 ppm, 100 ppm, and 150 ppm in 1600 ml Rila s.w. (26%)

	Day 1
100 ppm:	(6 clams)–Four clams accumulated dyed oil in stomach and digestive diverticula. One had no oil visible. All others had varying degrees of oil-dye-mucus on palps, gills, mantle, and in mantle rejection tracts near siphon and pedal aperture.
	Day 2
50 ppm:	(6 clams)–Three clams accumulated dyed oil in the stomach and digestive diverticula. All clams had varying amounts of oil-dye-mucus on gills, palps, mantle, and foot, and near siphon and pedal aperture, and in mantle rejection tracts. There were many visual observations of oil following ciliary pathways.
100 ppm:	(6 clams)–Two clams had dyed oil and oil-dye-mucus in stomach and diverticula. All had varying amounts of oil-dye-mucus on gills, palps, mantle, and foot. Oil globules in all clams followed standard ciliary pathways, [18].
	Day 3
50 ppm:	(6 clams)–Four clams accumulated dyed oil in the stomach and diverticula. All had varying amounts of oil-dye-mucus on gills, palps, mantle, and foot, and out siphon and pedal aperture. There were many visual observations of oil being bound in mucus and passing along ciliary paths.
100 ppm:	(6 clams)–Four clams accumulated dyed oil in stomach and diverticula. Oil-dye-mucus observations were the same as for 50 ppm, except more was present.
150 ppm:	(6 clams)–Two clams accumulated dyed oil and oil-dye-mucus in stomach and diverticula. All had varying amounts of oil-dye-mucus on gills, palps, etc., as did 100 ppm and 50 ppm, except much more was present for 150 ppm. Many observations were made of oil-dye being passed along ciliary tracts, wrapped in mucus, and passing to palps and into stomach or along rejection tracts.
	Day 4
50 ppm:	(6 clams)–All accumulated dyed oil and dye-oil-mucus in either the stomach, digestive diverticula, or both. All had varying amounts of oil-dye-mucus on gills, palps, mantle, etc., as outlined. There were many visual observations of oil following ciliary tracts and being enwrapped in mucus.
100 ppm:	(6 clams)–Four clams accumulated dyed oil and dye-oil-mucus in stomach. All had much oil-dye-mucus passing out the pedal aperture, near palps and siphon, and some was on gills, mantle, and foot. There were many observations of oil being ensnared in mucus and passing along ciliary paths.
150 ppm:	(6 clams)–Four clams accumulated some dyed oil and oil-dye-mucus in stomach, digestive diverticula, or both. All were relatively unresponsive and slow. Much oil-dye-mucus was in each clam on gills, mantle, and near siphon. It was also passing out the pedal aperture, and it was near the siphon and on visceral mass and foot. There were many observations of oil following ciliary paths and being ensnared in mucus.

of the palps for rejection. The mechanism is presumably the cause of oil-dye-mucus commonly occurring in large masses on, near, and around the palps of *Mya*. When sufficient rejecta accumulate in the palp region, the clam contracts the tip of the siphons and then rapidly contracts the anterior adductor muscle forcing water and rejecta out the pedal aperture. There also is a slow ciliary beat on the mantle expelling rejecta out the pedal aperture. This pattern of rejection may not be a primary pathway in the field. When the clam is burrowed in the sand mud substrate, it is extremely difficult to

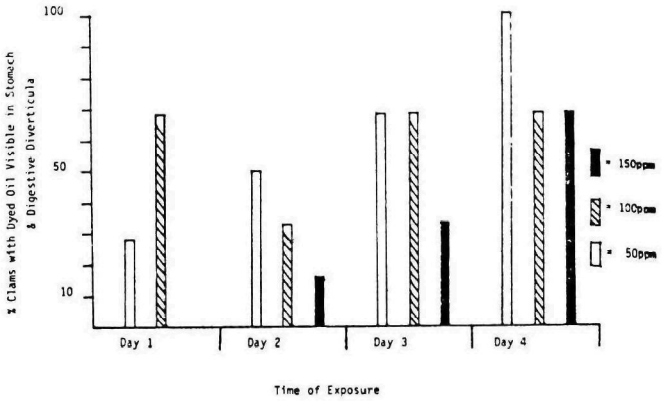

Figure 2. Accumulation experiment II. Clams exposed to dyed #2 fuel oil emulsion at 4°C

expel rejecta out the pedal aperture. The bulk of the rejecta accumulates within the mantle cavity. If much rejecta accumulates, the clam has to do more work to expel it out the inhalant siphon. In the interim, the oil-dye-mucus may possibly release some of the oil again. In essence, this would constitute an internal re-exposure to oil. Other detrimental effects are the increased energy demand on the clam for mucus secretion, ciliary beating, and muscular contractions and the synergistic effects of narcotization by the oil and clogging of gills, palps and digestive tract. Concentrations of #2 fuel oil above 100 ppm over a 24-hour or longer exposure cause an increasingly greater degree of general narc zation with increasing oil concentration.

Figure 3 illustrates the general ciliary pathways which dyed #2 fuel oil globules and micelles follow in *Mya*, as they are bound in mucus. The oil enters through the inhalant siphon into the pallial cavity. Here the oil follows pathways depicted where the micelles pass to the edge of gill ctenidia to the marginal groove. Ciliary currents then sweep the material along the marginal groove towards the palps. Some of the rejecta passes slowly along mantle rejection tracts towards the inhalant siphon and accumulates at the base of the inhalant siphon. Occasionally, the clam contracts expelling the dye-oil-mucus out the inhalant siphon.

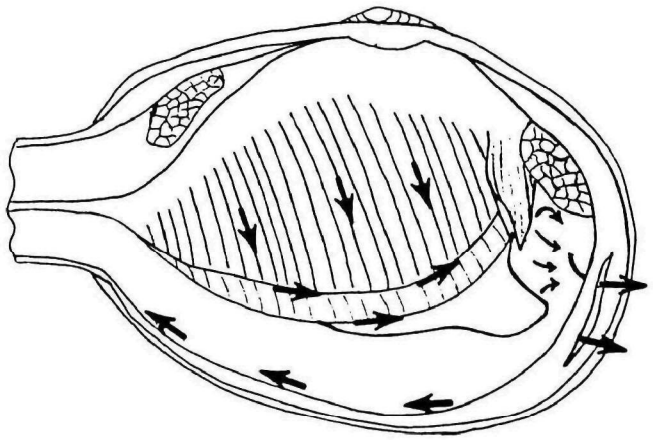

Figure 3. General ciliary pathways which dyed #2 fuel oil droplets follow as they are bound in mucus

Whether a clam will accumulate the dyed oil as dyed oil-mucus in the stomach and diverticula seems to be related to prior feeding, oil concentrations, length of exposure, and temperature. Figure 2 illustrates the results of accumulation experiment II at 4°C. At all oil concentrations, 50 ppm, 100 ppm, and 150 ppm, there was a general trend for an increasing number of clams to visibly accumulate and concentrate low concentrations of oil from the water column, since test concentrations in the water column were relative

only to time zero at the beginning of the test. The higher temperature, 22°C, seemed to accelerate the accumulation of dye-oil-mucus, general narcotization, and death. The lower temperature, 4°C, tended to enhance the stability of the oil emulsion and slow the narcotization of the clam.

If the clam's gut is full of detritus and silt there is little visual accumulation. The most rapid accumulation of oil seems to be correlated with a partially empty gut. When the dyed oil is in the stomach, it may be in mucus fragments or as minute droplets. Occasionally, the style tip may be tinted red. Next, the digestive diverticula gradually become tinted from the stomach outward toward the limit of the digestive diverticula where the tissue becomes undifferentiated gonad.

The ability of the clam to accumulate oil, concentrate it in mucus, and release it, may have deleterious ecological side effects. While the clam is accumulating oil within its own tissues, it may also bind and concentrate any oil entering its pallial cavity. Some of this is accumulated and some ejected from the clam as concentrated oil-mucus. This may occur at high-oil concentrations even while the clam is dying. At low-oil concentrations, the effect would be more insidious. Filter-feeding bivalves must filter large volumes of water to sustain their nourishment and respiration. Even very low concentrations of oil in the water column can be concentrated by the clam in its tissues and mucus. Essentially, the clams serve as bioconcentrators, accumulating oil in their tissues and rejecting some in the form of concentrated oil-mucus. This situation is greatly aggravated in the case of coastal spills when dispersants and sinking agents are used. The effect of sinking oil or dispersing it is primarily cosmetic and increases the potential for oil accumulation by filter and detritus feeders.

The ability of the clam to accumulate and concentrate oil has other side effects. Eventually, the effects of the accumulated oil can cause death. The oil in the decaying clam flesh is deposited into the sediments at depths of 2-14 centimeters. Several studies including Blumer et al. [2], Blumer and Sass [3], and Farrington and Quinn [11] have shown that oil may exist in sediments in an undegraded state for extended periods of time with the potential for release into the environment again. The release of oil-mucus rejecta by the clam also has the potential for further contamination. The mucus strings may be worked into the sediment or utilized as food by a variety of detritus feeders and benthic scavengers, resulting in their contamination. Scavengers such as green crabs and brown shrimp are common prey for fish. The clams themselves are a common food for many food and game fish such as striped bass [26], black drum, and flounder. Trevallion et al. [34] reported that the siphons of clam were a major source of food for plaice. Many crabs, including those used for human consumption and those utilized by marine predators such as striped bass, also feed on clams [30]. By these means, low concentrations of oil may rapidly be disseminated through the marine sediments and food chains.

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THE ACCUMULATION AND DEPURATION OF NO. 2 FUEL OIL BY THE SOFT SHELL CLAM, Mya arenaria L.

by
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Abstract

Young soft shell clams, Mya arenaria, were exposed to subacute concentrations of No. 2 fuel oil-in-water emulsions under simulated winter (4°C) spill conditions. A pattern of accumulation and discharge of petroleum constituents, an experimental depuration time (biological half-life, TB_{50}), and a potential transport mechanism of aromatic compounds from the fuel oil to the clams were experimentally determined. The clams were exposed to single dose concentrations of 10, 50, and 100 ppm of No. 2 fuel oil-in-water emulsion for 28 days. Clams accumulated the greatest amount of hydrocarbons within one week after the initial exposure. The accumulated hydrocarbons decreased each week as the hydrocarbon content of the water decreased. Mass spectrometric analysis determined that the principle compounds accumulated and retained after three weeks of oil exposure were monomethyl, dimethyl, and trimethylnaphthalene isomers. Depletion of oil from the water column and accumulation and discharge of fuel oil constituents appeared to involve a mucus-oil complex formation by the clam.

The depuration period was determined when the clams were transferred to an uncontaminated system for 14 days subsequent to the 28 day oil exposure. Accumulated hydrocarbons were rapidly, although incompletely discharged. At the end of the depuration period, mass spectrometric analysis revealed that many of the hydrocarbons present in the clams were dimethyl and trimethylnaphthalene isomers. The biological half-lifes (TB_{50}) calculated were: 10ppm (50 days); 50ppm (11 days); 100ppm (13.5 days).

Key Word Index: Mya arenaria, oil accumulation, depuration, retention, No. 2 fuel oil, aromatic molecules, oil emulsion.

Introduction

Chemical analyses have shown that various components of petroleum oils are accumulated within marine invertebrates. Blumer et al (1970), Ehrhardt (1972), Clark and Finley (1973) and Farrington and Quinn (1973) have reported the occurrence of petroleum oils in bivalve molluscs sampled from the environment. These molluscs were sampled from areas which had either been exposed to oil spills or from areas considered industrially contaminated. Laboratory studies of the accumulation of petroleum oils and oil fractions by mussels or oysters were investigated by Lee et al (1972), Blaylock et al (1973) and Stegeman and Teal (1973). Their work indicated that various fractions of petroleum oils can be accumulated and retained by mussels and oysters.

Oil spillage continues to be a serious problem in coastal and estuarine waters. Field studies of spills and their effects on Mya arenaria were documented by Thomas (1973) and Dow and Hurst (1975), and the occurrence of oil in Mya arenaria subsequent to environmental contamination was reported by Zitko (1971) and Scarratt and Zitko (1972).

Soft shell clams, Mya arenaria, occur frequently in areas receiving acute and chronic oil exposures and are often harvested for human consumption. This study was therefore performed to determine experimentally, a pattern of accumulation and discharge of petroleum constituents, a depuration time (biological half-life, TB_{50}), and a potential transport mechanism of aromatic constituents of the oil in soft shell clams exposed to No. 2 fuel oil-in-water emulsions. Few studies of oil accumulation and discharge have been performed at winter temperature conditions and experimentation was therefore performed at 4°C to simulate the winter state during which time spills and discharges were more likely to occur resulting from winter storms.

A number two fuel oil was chosen for study because it is commonly shipped in coastal waters, used in coastal industrial installations, and has already been involved in a well documented spill (Blumer, et al, 1970). The oils were added in an emulsified form to simulate a potential naturally occurring condition. Forrester (1971), Gordon et al (1973) and Kanter (1974) have reported the formation of oil emulsions in sea water by various mechanisms. In the event of an oil spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action. Emulsions tend to be relatively stable and a mechanism for the accumulation of emulsified oil by soft shell clams has been reported by Stainken (1975).

Materials and Methods

The No. 2 fuel oil was supplied by the U.S. Environmental Protection Agency, Industrial Environmental Research Laboratory-Ci, Edison, N.J. The specific gravity of the oil was 2.40 centistokes. The oil was composed of 14% aromatics and 86% nonaromatics according to ASTM method No. D2549-68. Oil-in-water emulsions were ultrasonically prepared according to a procedure developed by Gruenfeld and Frederick (1977).

Clams for the experiments were collected from Sequine Point, Staten Island, N.Y. Young clams with a mean shell length of 25 mm (Newcombe, 1936; Pfitzenmeyer, 1965) were utilized because young bivalves tend to have greater respiration and filtration rates than those of older bivalves (Prosser and Brown, 1961; Walne, 1972).

An exposure period of 28 days to No. 2 fuel oil emulsions having concentrations of 10, 50, and 100ppm was utilized. Four 20 gallon covered aquaria containing 60 liters of filtered sea water per aquaria (Salinity = 20‰) were employed. The sea water was collected from Sandy Hook Bay and filtered through a coarse plankton net to remove macro debris. One aquaria served as a control. Each of the remaining aquaria received a sufficient volume of a stock oil emulsion to attain a final concentration of either 10, 50 or 100ppm of oil emulsion per aquaria. The time at which the stock emulsions were added to the aquaria was termed Time 0. The water was continuously aerated and the temperature was maintained at 4°C. The clams were acclimated to the experimental conditions for a duration of 6 days before the stock emulsions were added. Sampling for hydrocarbon contents of water and clams was performed every 7 days after Time 0. The controls were sampled at Time 0 and every 7 days subsequent to Time 0.

Chemical confirmation of the oil being bound or adsorbed to mucus was provided by extracting mucus secreted by the clams exposed to 100 ppm emulsified No. 2 oil. The mucus was collected after the four weeks oil exposure. It had a gray-green flocculent appearance and formed flocculent clumps on the surface and in the water column. The methodology used for hydrocarbon analysis has been described by Stainken (1975).

The depuration of the clams was determined for 14 days after the 28 day oil exposure. Each experimental group of clams was removed from their aquaria and rapidly placed in clean 20 gallon aquaria containing 60 liters of fresh coarse filtered Sandy Hook Bay sea water maintained at the same temperature and salinity. As clams were transferred, the valves were wiped clean to remove deposited pseudofaeces, etc. Samples for chemical analysis were obtained on day 7 and 14.

The hydrocarbon contents of the Sandy Hook Bay sea water and the experimental aquaria water were determined using a Freon extraction technique (Gruenfeld, 1973; Zeller, 1974). Each week, 400 ml samples were removed from the center of each tank and at the same depth. Prior to the experiments, the hydrocarbon content of the sea water was analyzed to ensure external contamination did not occur.

The extraction and column chromatograph method used for chemical analysis of tissue was a modification of that employed by Blaylock, *et al* (1973). All solvents were of distilled spectral grade (Burdick & Jackson, Muskegon, Mich.). All glassware was prerinsed with hexane. Once a week, 5 clams (5-8 gm tissue weight) from each experimental exposure were placed in a round bottom flask (500 ml) having a standard taper ground glass neck, and mixed with 95% ethanol (150 ml) and KOH (10 gm). Clams were shucked and the water from the mantle cavities were drained before being placed in the flasks. The shells were not extracted. The mixtures were then refluxed at approximately 65-70°C on a heating mantle and under a Friedrich reflux condenser for 1½ hours. After cooling to ambient temperatures, the interior of each condenser was washed with 5 ml hexane. The washing was collected in the receiver flask.

The digested material was transferred to a Teflon stoppered separatory funnel (1 liter) using distilled water (80 ml) and two portions (50 ml each) of hexane. The mixture was then shaken by hand for one minute. The two phases were then drained into separate flasks and the aqueous phase was returned to the separatory funnel using a hexane (50 ml) wash. The extraction and separation was repeated for a total of three times.

The combined extracts were washed (minimum 3 times) with aliquots (500 ml each) of distilled water and transferred to an Erlenmeyer flask (300 ml) using additional hexane. Water was removed from the extract by adding 2-3 gm of anhydrous Na_2SO_4. The extracts were then concentrated to approximately 40 ml under a stream of filtered nitrogen in a warm water bath (50°C) and transferred to a conical flask (50 ml) where concentration was continued to a final volume of 2 ml.

The concentrated extract was placed on a glass column (25 cm length x 1.0 cm I.D.). The column was packed with 6.5 cm of 5% water deactivated silica gel (100-200 mesh, Grade 923, Davison Chemical, Baltimore, Md.). Another 6.5 cm of 5% water deactivated alumina (Neutral, 90-20 mesh, Brockman Activity 1: Fisher Chemical Co.) was layered over the silica. Both the silica gel and alumina were activated at 110°C for 24 hours and then deactivated prior to column packing. Sand was layered over the alumina (0.5 cm). When packed, the column was rinsed with one column volume of hexane.

The residual concentrate was charged onto the column and then elutriated with 50 ml hexane. Subsequent analysis demonstrated that all petroleum hydrocarbons in the sample had passed through. The column was rinsed with 50 ml of benzene but few hydrocarbons were found in this fraction and it was later discarded. The eluate was concentrated for gas chromatographic analysis to a volume of 0.1 ml under nitrogen.

The identification of sample hydrocarbons was achieved by comparison with gas chromatograms of hydrocarbon standards and of No. 2 fuel oil in hexane. A Perkin-Elmer (Model 900) gas chromatograph was employed, equipped with a flame ionization detector and a 6 ft stainless steel column packed with 8% Dexsil on 80/100 mesh of Chromosorb W. Standard operating conditions were as follows: carrier gas: N_2, 2 cc/min; detector: H_2, 20 cc/min; air: 40 cc/min; injector temperature: 200°C; detector temperature: 300°C; manifold temperature: 300°C; column temperature: 70°C with a two minute hold, and programmed at an increase of 8°C per minute to 300°C; injection sample: 0.1 microliter. Further identifications of compounds were accomplished by mass spectrometric analysis provided by the U.S. EPA (IERL-Ci), Edison, N.J.

Hydrocarbons were quantified by cutting and weighing the chromatograms. The total areas under the peaks were obtained by subtraction of the baseline area of the first week control measurement. It was assumed that the area under the first week control measurement would be most representative of background levels and therefore any material above this baseline were considered accumulated petroleum hydrocarbons. The sample peak areas were compared to that of an internal standard (octacosane, C_{28}) having a known concentration (2 µg/gm clam tissue).

Hydrocarbon quantification results were analyzed for regression. The regression analysis used the No. 1987A/ST3 package from the 700 series Standard Statistical Program, Wang Laboratories, on a taped program. The significance of the regressions were further analyzed by a t-test (Zar, 1974).

Results

Water quality appeared excellent throughout the 28 day period. The dissolved oxygen and ph remained optimum in all tanks and bacterial contamination was not apparent. Control animals were reactive to tactile stimuli and appeared to be feeding. Very little mortality occurred at all oil concentrations. The actual mortalities were: Controls - 0.0%, 10ppm - 0.5%, 50ppm - 2.0%, 100ppm - 3.0%.

During the 14 day depuration period, the 10ppm clams seemed to recover their tactile irritability, in comparison to the controls. The 50 and 100ppm clams were sluggish and after shucking many smelled foul. Their visceral mass was less firm than controls and their color had paled from the normal gold-brown. There was an increase in mortality in the clams previously exposed to the concentration of 50 and 100ppm. The actual mortality at the end of the depuration priod was: Control 0.0%, 10ppm - 0.0%, 50ppm - 18.3% and 100ppm - 13.6%.

The sea water before all tests and the controls during the tests did not have detectable hydrocarbon contents. Within the oiled tanks, some of the oil emulsion had broken within two hours after addition. Much of the oil emulsion and water soluble fraction remained in in the water column as indicated in Table 1. One week later, the detectable hydrocarbon concentrations in the water column of the oiled tanks were similar. Much of the oil was bound in mucus which adhered to the glass cooling coils and tank walls or formed floating organic flocculent conglomerates. The IR spectra also showed the emergence of an apparent aromatic band at 3030 cm^{-1}. The hydrocarbon peaks at 2930 cm^{-1} often masks the aromatics. Apparently, the water soluble aromatics of the fuel oil were becoming unmasked as more of of the non-aromatic hydrocarbons became dissipated from the water column. The Week 2 data were similar to those of Week 1 and a peak emerged at ca. 3000 cm^{-1}.

Table 1. Hydrocarbon concentration (ppm) in the water column during the 28 day exposure period

Aquaria	Time 0*	Week 1	Week 2	Week 3	Week 4
Control	0	0	0	0	0
10 ppm	4.5	1.31	0.56	0.37	0
50 ppm	43.7	1.04	0.71	0.37	0.29
100 ppm	60.7	1.52	0.78	0.32	0.47

*The Time 0 sample measurement was made two hours after addition of the emulsified oil.

By Week 3, the measurable hydrocarbon content had dropped further and in the 10 and 50ppm tank samples the possible aromatic peak became less apparent. The 100ppm tank sample still had a strong peak at 3030 cm^{-1}. At the end of the fourth week, the hydrocarbon content in the tanks had fallen to almost trace concentrations and peaks were becoming indistinquishable.

The tanks used in the oil exposure experiments were drained after the fourth week. There was an extremely strong odor of No. 2 fuel oil, especially in the 100ppm tank. As the mucus was wiped off the sides and cooling oils, the odor became readily apparent in all the oiled tanks. This indicates that much of the oil was bound by the mucus which had settled on the cooling coils and sides of the tanks. The mucus-oil complex formation may have aided in the dissipation of hydrocarbons from the water column. The mechanism of the binding of oil in mucus by the clams has been documented (Stainken, 1975). Mass spectrometric analysis demonstrated that the mucus contained predominantly dimethyl and trimethylnaphthalenes with paraffins, mainly in the C-14 and C-15 regions. Figure 1 illustrates the gas chromatograms of No. 2 fuel oil and an extract of hydrocarbons from a mucus sample. A close correlation of peak heights is readily noticeable. The chromatogram pattern of the mucus is similar to No. 2 fuel oil but some of the lower boiling compounds are missing.

During the depuration period, measurable amounts of hydrocarbons in the aquaria water were not detected. There was an emergence of a peak at 3000 cm^{-1} during the second week in the experimental tanks.

Gas chromatograms for the control clams are contained in Fig. 2. The basic pattern for the normal control clams remained unchanged during the experiment. The chromatogram for Week 1 was chosen as a baseline for the exposed animals to compare the amount of No. 2 fuel oil hydrocarbons accumulated. The chromatograms for the clams exposed to 10ppm No. 2 fuel oil are illustrated in Fig. 3. The chromatograms A-C show a characteristic well defined pattern of peaks. Mass spectrometric analysis of the third weeks sample (chromatogram C) revealed that the bulk of the chromatogram consisted of monomethyl, dimethyl and trimethylnaphthalenes. By the fourth week, the resolution of the peaks had diminished and the chromatograms of the clams placed in fresh sea water would superficially indicate there were few hydrocarbons present. However, mass spectrometric analysis revealed that the sample (Chromatogram F, Fig. 3) contained dimethyl and trimethylnaphthalenes at the end of the depuration period.

Figure 4 depicts the chromatograms from the clams exposed to 50ppm of No. 2 fuel oil emulsion. The same pattern of peaks as those of the 10ppm is present. The peaks have much higher resolution and are still apparent even after the depuration period. A higher attenuation (10x64) had to be used because the samples contained a much higher hydrocarbon concentration. Mass spectrometric analysis of the Week 3 sample revealed that most of the sample was comprised of monomethyl, dimethyl and trimethylnaphthalenes. Mass spectrometric analysis of the clam sample at the end of depuration (Chromatogram F, Fig. 4) showed that the sample was composed of alkyl benzenes, monomethyl, dimethyl and trimethylnaphthalenes. The chromatogram of Week 1 control was drawn under chromatograms E and F, Fig. 4, to illustrate the quantity of petroleum hydrocarbons retained by the clams.

Chromatograms of clams exposed to a concentration of 100ppm No. 2 fuel oil are illustrated in Fig. 5. The same recurrent pattern is readily visible and the peaks have sharper resolution. Mass spectrometric analysis of the week 3 sample showed it to consist of naphthalene, monomethylnaphthalene, a mixture of dimethyl and trimethylnaphthalene isomers, phenantherene, and C_{12} and C_{14} paraffins. Mass spectrometric analysis of the clam sample after the two week depuration (Chromatogram F, Fig. 5) confirmed that the sample consisted of naphthalene, a mixture of dimethyl and trimethylnaphthalene isomers and no paraffins.

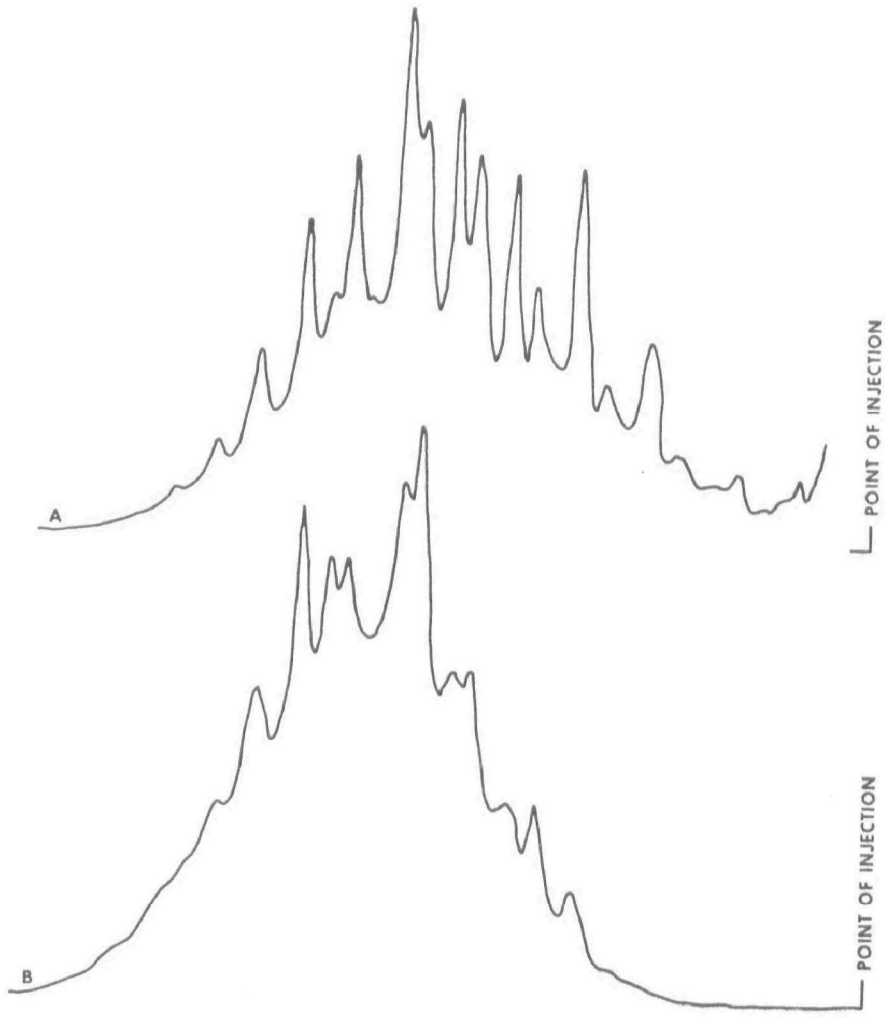

Fig. 1. Gas chromatograms.

(A) 8ppm #2 FUEL OIL IN HEXANE, ATTENUATION 1×128

(B) CARBON TETRACHLORIDE EXTRACT OF CLAM MUCUS, ATTENUATION 10×1024

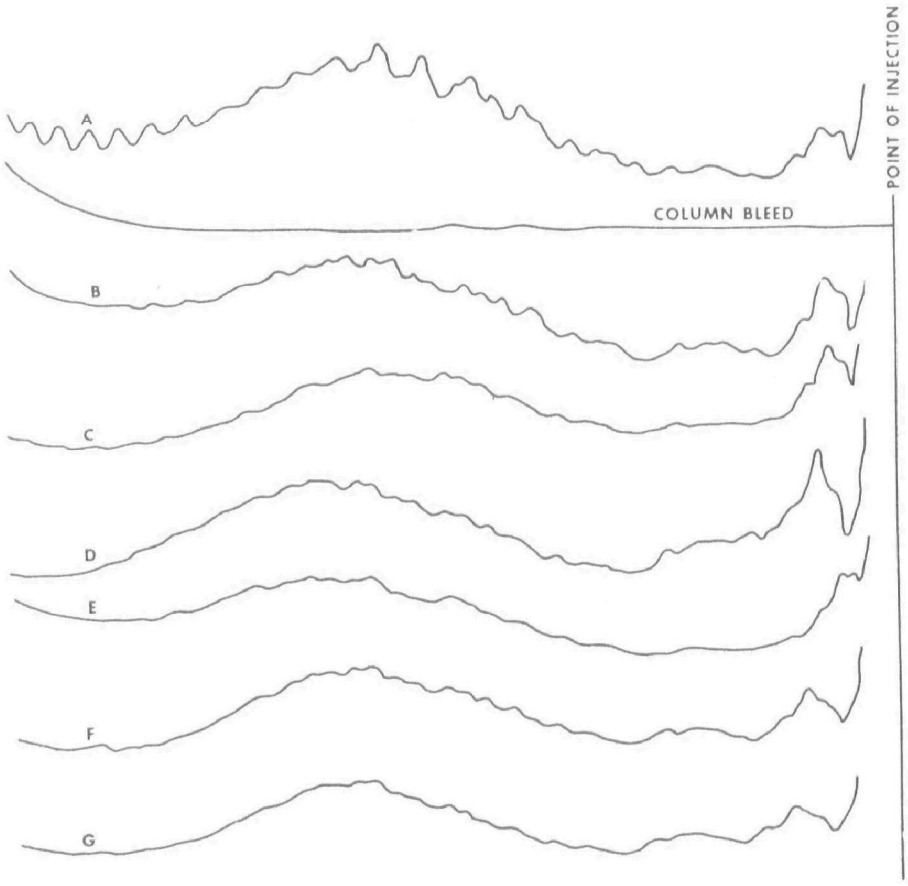

Fig. 2. Control animal gas chromatograms.

(A) TIME 0, WITH COLUMN BLEED DRAWN BELOW; (B) WEEK 1; (C) WEEK 2; (D) WEEK 3; (E) WEEK 4; (F) CLAMS IN FRESH SEA WATER, WEEK 1; (G) CLAMS IN FRESH SEA WATER, WEEK 2

ALL CHROMATOGRAMS AT 1×128 ATTENUATION

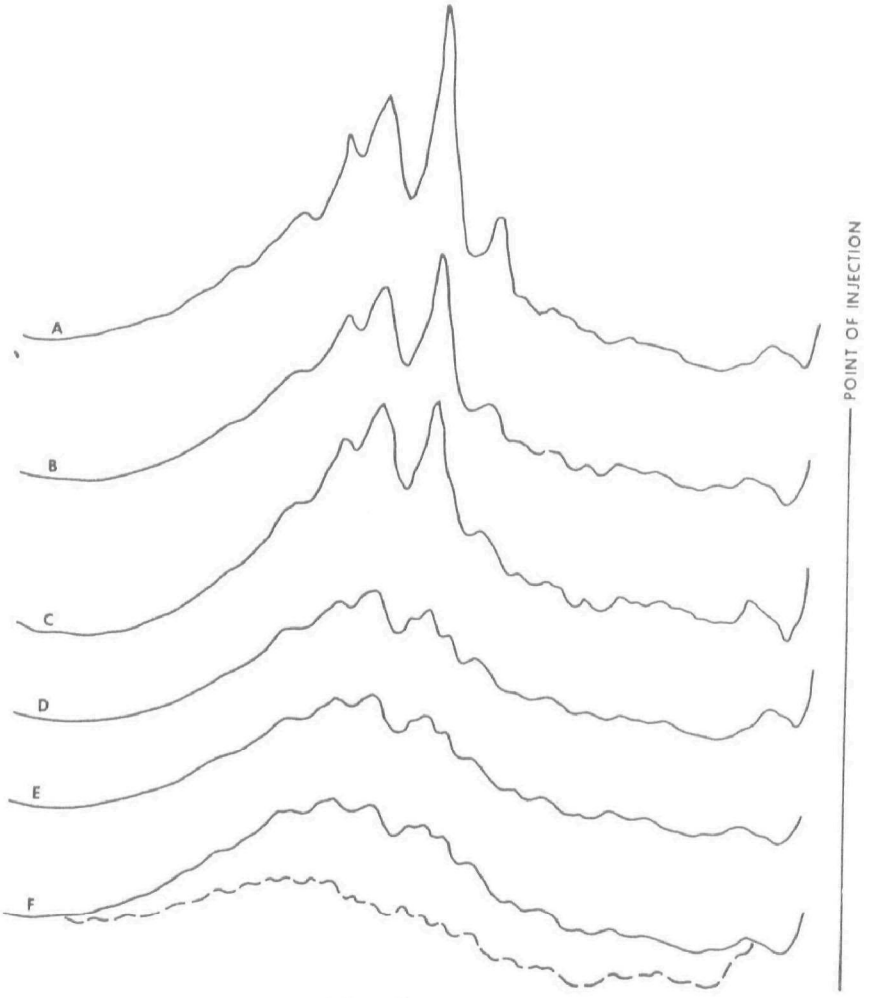

Fig. 3.

Gas chromatograms clams exposed to 10 ppm #2 Fuel Oil.
(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER, WEEK 1;
(F) CLAMS PLACED IN FRESH SEA WATER, WEEK 2, WITH CONTROL WEEK 1 DRAWN AS BASE LINE
ALL CHROMATOGRAMS AT 1×128 ATTENUATION

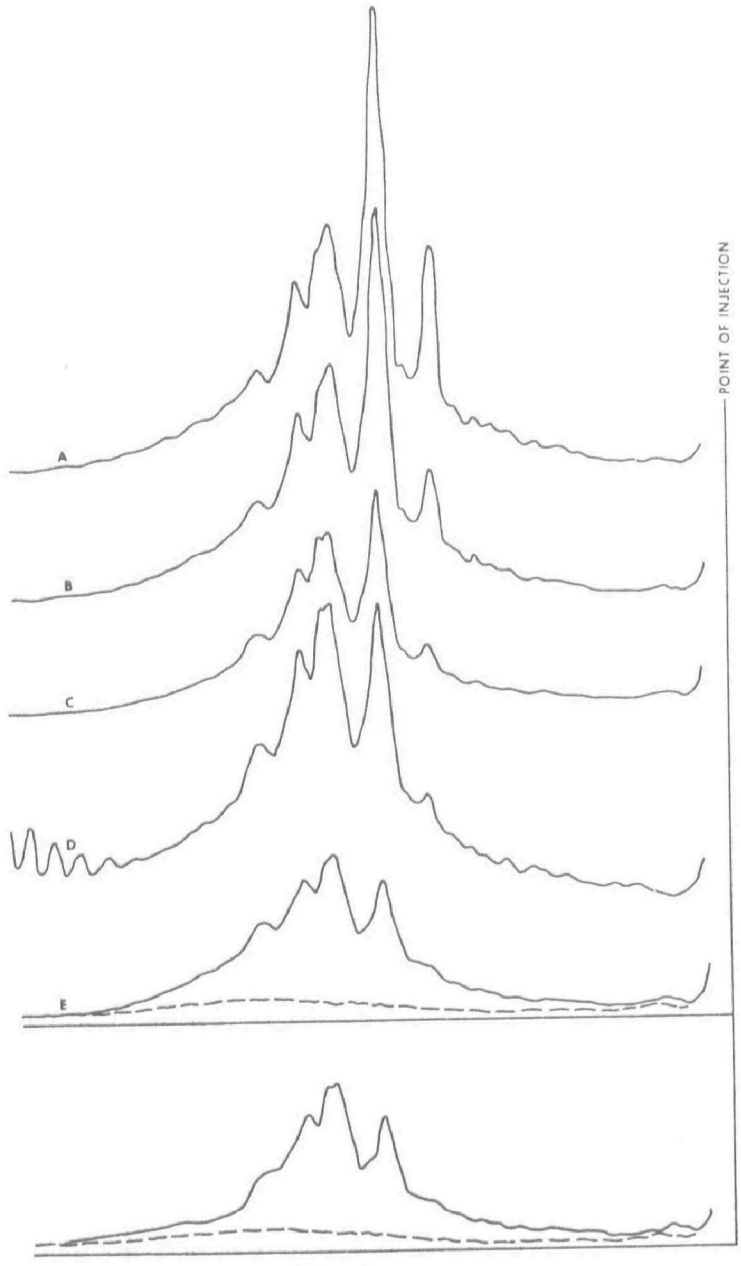

Fig. 4.

Gas chromatograms clams exposed to 50ppm #2 Fuel Oil.

(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER, WEEK 1, WITH CONTROL WEEK 1 AS BASELINE; (F) CLAMS IN FRESH SEA WATER, WEEK 2, WITH CONTROL WEEK 1 AS BASELINE

ALL CHROMATOGRAMS AT 10×64 ATTENUATION

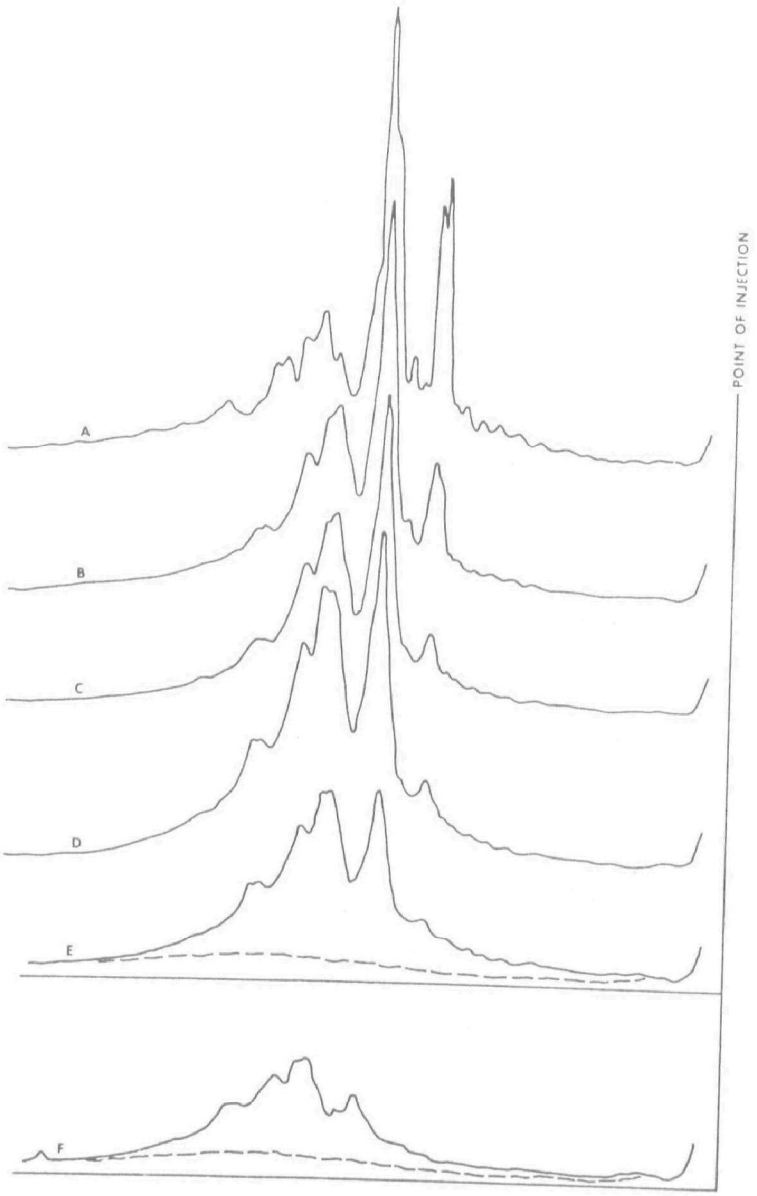

Fig. 5.

Gas chromatograms clams exposed to 100 ppm #2 Fuel Oil.

(A) WEEK 1; (B) WEEK 2; (C) WEEK 3; (D) WEEK 4; (E) CLAMS PLACED IN FRESH SEA WATER, WEEK 1, WITH CONTROL WEEK 1 AS BASELINE (F) CLAMS IN FRESH SEA WATER, WEEK 2, CONTROL WEEK 1 AS BASELINE

ALL CHROMATOGRAMS AT 10×64 ATTENUATION

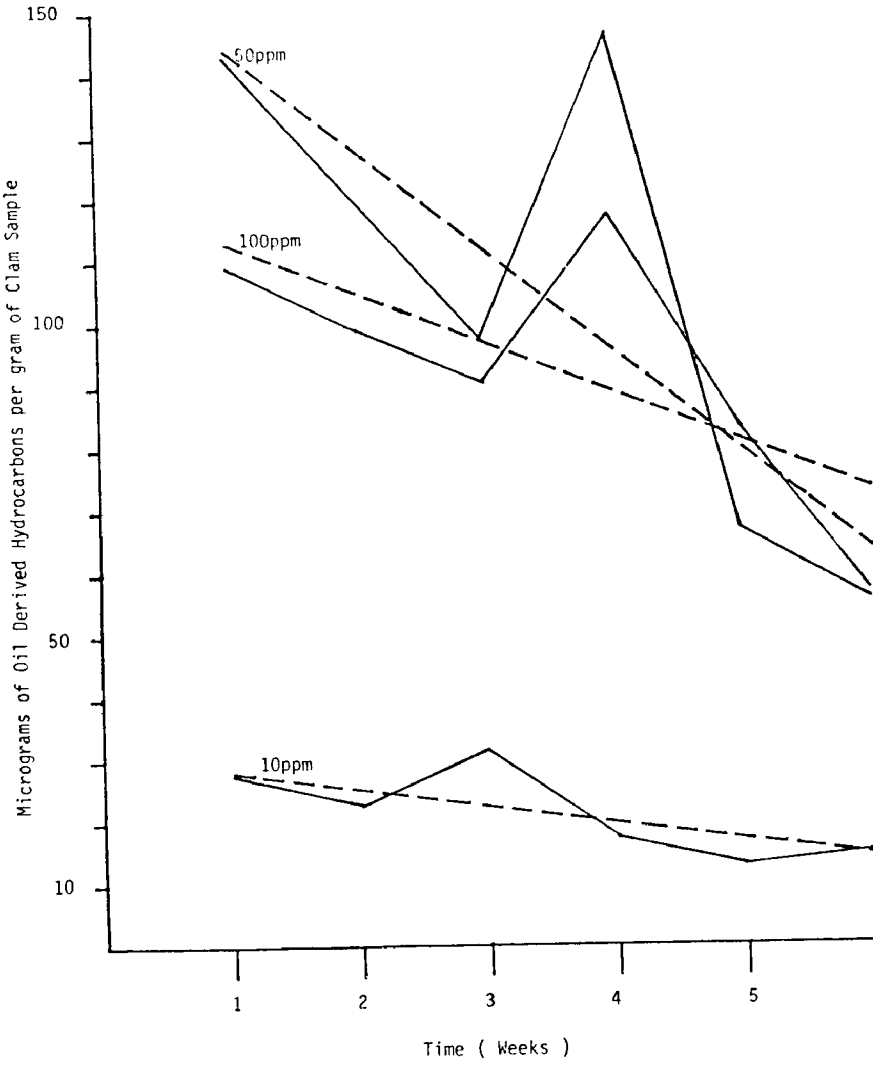

Fig. 6.

Accumulation and depuration of petroleum derived hydrocarbons.

Weeks 1-4 were the oil exposure period.
Weeks 5 & 6 were the depuration period.
(Dotted line is calculated regression.)

The results of quantification of the gas chromatograms are listed in Table 2 and illustrated in Fig. 6. Results are expressed as micrograms of petroleum hydrocarbons/gram clam sample. After one weeks exposure to oil, the values diminish with time. The sudden upsurges in Week 3 at 10ppm and in Week 4, of the 50 and 100ppm are unexplained. The calculated regressions are the broken lines in Fig. 6. A gradual loss of petroleum hydrocarbons with time is demonstrated. Analysis of the regressions indicated that the 50 and 100ppm regressions were not significantly different (.05P) although they are both significantly different from the 10ppm regression.

Experimental concentration factors were calculated for the first week of exposure from the data in Table 1 and 2. The hydrocarbon concentration of the water column, Week 1, was subtracted from the Time 0 value. This net hydrocarbon concentration in the water column was then divided into the hydrocarbon value accumulated by the clam after the first week of oil exposure. The computed concentration factors were: 10ppm - 8.5, 50ppm - 3.3, 100ppm - 1.8.

Table 2. Quantification of petroleum hydrocarbons (micrograms per gram of clam tissue).

	10 ppm	50 ppm	100 ppm
Week 1	27.28	143.15	109.27
Week 2	22.92	120.50	99.23
Week 3	31.65	97.03	90.73
Week 4	17.27	146.80	117.52
Week 5 (depuration)	12.94	66.80	83.56
Week 6 (depuration)	14.95	55.34	57.05

The biological half lifes (TB_{50}) or residence times were calculated using a regression analysis of the depuration period and plotting on semilog paper. The fourth weeks data were calculated as Time 0 for the depuration period. The calculated TB_{50} were: 10ppm - 50 days; 50ppm - 11 days; 100ppm - 13.5 days. A theoretical time period for the clams to completely depurify accumulated petroleum hydrocarbons can be estimated if the regressions in Fig. 6 are extrapolated. The extrapolation results in the following depurification periods: 10ppm - 61 days; 50ppm - 93 days; 100ppm - 109 days.

Discussion

The concentrations of oil in the water column began to decrease several hours after the addition of the oil emulsion. Several factors were probably responsible for this. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. Large masses of mucus were ejected from the clams and were accumulated on the cooling coils. Subsequent chemical analysis revealed a large content of oil in the mucus. It is presumed that as the clams filtered water, they were exposed primarily to naphthalene derived compounds. Earlier investigators reported that fuel oil can form droplets and micelles in sea water, and that the major water soluble components were the aromatic compounds, particularly the naphthalenes (Boylan & Tripp, 1971; Boehm & Quinn, 1974). The accumulation of large amounts of oil and aromatic molecules in the mucus may be a potential mechanism of concentrating and disseminating petroleum hydrocarbons in the environment. The mechanism for the formation of an oil-mucus complex was discussed by Stainken (1975).

After Time zero, the oil rapidly disappeared from the water column. The amount of dissolved compounds attained a peak measured in the first week, and then decreased to an equilibrium value for several weeks ranging from 0.5-0.29ppm. Analysis of mucus and clam tissue indicated that naphthalenes and methyl substituted naphthalenes were the apparent predominant components in the water. This is further substantiated by other reports (Frankenfeld, 1973; Anderson, et al., 1974) which indicate that the water soluble fractions of oils often contain aromatic compounds, particularly naphthalenes.

The peak accumulation of petroleum derived hydrocarbons by the clams was reached one week after exposure, followed by a gradual loss in accumulated hydrocarbons. The bulk of the material accumulated were naphthalenes and methyl substituted naphthalene isomers. Other aromatics were detected and accumulated but the naphthalenes were the most persistent. The results of this study are comparable to those of Vaughan (1973). He reported that oysters exposed to No. 2 fuel oil often accumulated methyl and dimethyl naphthalenes. Other studies also indicate that aromatic hydrocarbons may be accumulated by bivalves (Ehrhardt, 1972; DiSalvo, et al, 1973).

Paraffins or aliphatics were not detected in clam tissue in this study, except in the higher oil exposures. The aromatic compounds constituted a much greater percentage of the total oil extracted from the clams. The extracted concentrations of aromatics from the clams were also much greater than the original diluted concentrations when added at Time 0 as part of the fuel oil emulsion. Similar results of Stegeman and Teal (1973) demonstrate that a high aromatic content in the organism need not be the result of a high aromatic content in the contaminating oil. This phenomena is probably due to the greater solubility of the aromatic compounds (Anderson, et al., 1974; Boehm and Quinn, 1974).

The greater uptake of hydrocarbons by clams exposed to 50ppm may be due to a dose dependent narcosis. Galtsoff, et al., (1935) reported that water soluble substances of crude oil could produce anaesthetic effects on the ciliated epithelium of the gills. The 100ppm oil concentration may have reduced the filtration activity of the clams below that of the clams exposed to 50ppm. The reduced filtration rate may account for the animals exposed to the higher concentration (100ppm) accumulating less oil than the animals epxosed to the lower concentration (50ppm).

Depuration of accumulated petroleum derived hydrocarbons actually began as the hydrocarbons decreased in the water column. When the clams were removed to fresh sea water, depuration proceeded rapidly for the first week. The calculated half-time for depuration ranged from 11-50 days. If the calculated regression of the accumulation of petroleum hydrocarbons is extrapolated (Fig. 6), 61-109 days are necessary for the clams to completely depurify the accumulated petroleum hydrocarbons. Although extrapolation is not necessarily accurate, the retention of hydrocarbons at the end of the depuration period indicated that after oil exposure, depuration will proceed rapidly to a low concentration after which depuration proceeds slowly. Other investigators have reported similar findings in other bivalve species (Lee, et al., 1972; Stegeman & Teal, 1973; Vaughan, 1973). However, the actual tissue concentrations are related to the concentration of the initial oil exposure. The retention of oil by Mya arenaria in this study is further substantiated by the observations of Thomas (1973) in which clams were observed to eject oil for at least one month after an oil spill.

Oil retention can have several deleterious effects. Long term low level petroleum hydrocarbon contamination may interfere or weaken the ability of the clam to withstand further environmental stresses such as those of temperature, salinity, spawning, disease and insult from other contaminants. Futhermore, oil retained in the sediment and clams may also be passed on to predators, including man.

Summary

1. Clams accumulate large amounts of petroleum derived hydrocarbons after initial exposure to petroleum (No. 2 fuel oil). The amount and the type of petroleum hydrocarbon accumulated are related to the initial dose, time following initial exposure and the relative "solubilities" of the various hydrocarbon components. Naphthalene and methyl substituted naphthalene isomers (mono, di, tri) are the most common compounds concentrated by the clams. Occasionally, some alkyl benzenes, phenanthrenes and paraffins in the C12 and C14 region are accumulated.

Depuration by soft-shell clams of accumulated petroleum hydrocarbons may actually begin as soon as hydrocarbon levels drop in the water column. When clams are placed in fresh hydrocarbon free sea water, depuration proceeds rapidly within the first week after which the rate slows. Dimethyl and trimethylnapthalenes were still present in the clams after two weeks in fresh sea water.

2. Chemical analysis of clam mucus extract demonstrated that clams can concentrate and ingest or release to the environment extremely large amounts of petroleum hydrocarbons by means of their mucus - oil binding mechanism. Analysis of petroleum compounds present in the mucus revealed them to be methyl substituted naphthalenes and paraffins, all compounds similar to those which the clams accumulate. Types of chemical compounds accumulated or bound in the mucus are probably related to differential solubilities of petroleum constituents.

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The Effect of a No. 2 Fuel Oil and a South Louisiana Crude Oil on the Behavior of the Soft Shell Clam, *Mya arenaria* L.

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Introduction

The toxic effects other than lethality of oils has often been treated as a secondary problem in bioassays. According to STIRLING (1975), there is a need to enlarge the concept of the routine bioassay test to include quantitative measurements of the effects of pollutants on behavior, physiology and metabolism. The inherent interspecific and intraspecific physiological differences of test species and various test conditions make direct comparison of bioassay results difficult. VAUGHAN (1973) and BEAN, et al. (1974) discussed these problems in detail.

Few studies have characterized the behavioral effects of oils on bivalves or examined the effects of temperature simultaneously. WILSON (1974) noted that bivalve molluscs have been avoided for toxicity tests because it is difficult to establish a simple criteria of effect. This study was therefore performed to determine the behavioral effects of a No. 2 fuel oil and a South Louisiana crude oil in bioassay tests conducted at winter temperatures. The winter temperatures were chosen because spills are more likely to occur during the inclement winter weather.

The oils were added in an emulsified form to simulate a potential naturally occurring condition. FORRESTER (1971), FOSTER, et al. (1971), GORDON, et al. (1973) and KANTER (1974) have recorded the formation of oil emulsions in sea water by various mechanisms. In the event of an oil spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action. Many of these emulsions tend to be relatively stable and a mechansim for the accumulation of emulsified oil by bivalves has been reported (STAINKEN, 1975).

Materials and Method

Behavioral observations were recorded during bioassay tests. The tactile responses of the clams were examined by lightly tapping the shell with a glass stirring rod. Tests were conducted according to the "Standard Dispersant Effectiveness and Toxicity Tests" published by the U.S. Environmental Protection Agency (McCARTHY, 1973). An experimental concentration at which 50% of the experimental animals survived (LC_{50}) was determined during a 96 hr. exposure period. The method employed requires the use of a standard toxicant. Benzene was used initially but in subsequent tests phenol was utilized because benzene emulsions were not stable.

Oil-in-water emulsions were ultrasonically prepared according to a procedure developed by GRUENFELD and BEHM (1973). Twenty thousand parts per million of Southern Louisiana Crude oil and No. 2 fuel oil were prepared and dilutions were made to achieve desired concentrations. The No. 2 fuel oil and Southern Louisiana were supplied by the U.S. Environmental Protection Agency, Industrial Waste Treatment Research Laboratory, Edison, N.J. The fuel oil was composed of 14% aromatics and 86% non-aromatics. Benzene was also sonified to yield a stock emulsion of 10,000 ppm. A 10,000 ppm stock solution of phenol was prepared.

All toxicants except benzene were used at $4^{o}C$ and $14^{o}C$. Benzene was tested at $14^{o}C$. The oils were added at concentrations from 50-800 ppm. The test concentrations of the oils were later increased to 1,600 ppm.

Clams for the experiments were collected from the Princes Bay - Sequine Point Area of Staten Island, N.Y. from December, 1973 - February, 1974. Clams were collected at water temperatures and salinity closely approximating test conditions. Young clams with a mean shell length of 25 mm or less were utilized. Prior to each experiment, clams were acclimated 24-48 hours in 1600 ml of artificial sea water (salinity - 26‰). Fifteen clams (15-24 mm) were used at each oil concentration. Five clams were placed in each aerated container. Five clams per container were also used for standard toxicant testing (benzene or phenol) at each concentration.

Results

Behavioural observations were made in each LC_{50} test. A noticeable reaction was not observed in clams exposed to benzene. The clams merely contracted upon tactile stimulation.

Clams exhibited an identical response pattern to oil exposure in all tests. Initially, at low oil concentrations (50 ppm) mucus was given off by the clam out the pedal opening and siphon. Higher oil concentrations resulted in proportionally higher mucus secretion. As more mucus was secreted, the clams increasingly shunted more out the pedal opening. All effects appeared to be both time and dose related, although crude oil effects were never as severe as the effect of fuel oil. Both oils depressed muscular contraction. A decrease in irritability and contractibility of the siphon was noticeable at 50 ppm. At concentrations greater than 100 ppm, the pedal opening musculature rapidly became totally relaxed and did not contract . The adductor muscles simultaneously lost the ability to contract rapidly. At concentrations greater than 400 ppm, the adductors began to relax in less than 15-20 sec and the animal consequently 'gaped'. It appeared that the anterior adductor was affected first, then the posterior.

The general relaxation of the musculature (adductors, siphon, pedal aperture) occurred at all oil concentrations. The intensity varied with time and dose.

The large amounts of mucus secreted at the 400 ppm concentrations appeared to be clogging the cavity between the valves by the end of the tests. The clams also appeared too weak to expel the mucus.

The response pattern of mucus secretion and muscular narcotization occurred in all tests with oil and seemed to be enhanced at 14°C compared to responses at 4°C.

The response of clams to phenol was not completely similar to that of oil. Mucus secretions were never as heavy in response to phenol, but phenol appeared to narcotize the musculature quicker. Phenol exposed clams reacted differently than oil exposed clams. The muscles remained at one length, turgid and lost their irritability. Adductor muscles remained half or sometimes fully contracted and the pedal aperture remained partially open. The muscles of the phenol exposed group rapidly became turgid at death. The tactile response of control clams remained normal during the experiments and mucus secretion was not evident.

The static bioassay data derived from the tests were inconclusive. The LC_{50} values were computed on semilog paper. Results of the tests are in Table I. Tests numbers 1A and 1B were run in natural filtered sea water to determine whether testing in natural sea water or artificial sea water would have an effect. An effect was not found and all other tests were conducted in artificial sea water. Scattered mortality was observed in the majority of tests but in most cases there was insufficient mortality after 96 hours to calculate a LC_{50}. Mortality was not found in the controls. Death was defined as a total lack of muscle response.

At 14°C, two 96 hour LC_{50} values for No. 2 fuel were obtained. These were 475 ppm (Test IIA) and 535 ppm (Test IIIA). Comparison by a t test revealed no significant difference and a mean LC_{50} value of 505 ppm was calculated. Some of the tests were continued 3 days beyond the 96 hour period, to see if there was a time effect. At 14°C, test IIIA and IIIP were continued. An LC_{50} (7 day) was found for No. 2 fuel oil to be less than 100 ppm, compared to test IIIA LC_{50} (96 hour) of 535 ppm. The LC_{50} (7 day) of phenol in test IIIB dropped to 535 ppm.

At 4°C, test IVA, IVP, VIA and VIP were also continued for 3 days (total 7 days exposure). In test IVA, a LC_{50} could not be found. In test IVP, the 7 day semilog plot had the shape of a backwards 'S' and two values were derived, 80 and 225 ppm phenol. In test VIP, the LC_{50} (7 day) for phenol was 450 ppm. It is probable that more mortality would have been encountered if the tests were continued beyond seven days.

TABLE I. Calculated toxicity (LC_{50}) during a 96 hour exposure period.

14°C

(IA) So. Louisiana Crude	(IB) Benzene
conc: 50,100,200,400,800 ppm	10,20,30,40,50 ppm
LC_{50} = none	LC_{50} = none
(IIA) #2 Fuel Oil	(IIB) Benzene
conc: 50,100,200,400,800 ppm	50,60,70,80,90,100 ppm
LC_{50} = 475 ppm	LC_{50} = none
(IIIA) #2 Fuel Oil	(IIIP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = 535 ppm	LC_{50} = 565 ppm

4°C

(IVA) So. Louisiana Crude	(IVP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = none	LC_{50} = 365 ppm
(VA) #2 Fuel Oil	(VP) Phenol
conc: 50,100,200,400,800 ppm	10,20,30,40,50 ppm
LC_{50} = none	LC_{50} = none
(VIA) #2 Fuel Oil	(VIP) Phenol
conc: 100,200,400,800,1600 ppm	50,100,200,400,800 ppm
LC_{50} = none	LC_{50} = 535 ppm

The Southern Louisiana Crude oil appeared to have a few acute toxic effects within the test parameters.

In the tests with No. 2 fuel oil, there seemed to be a temperature effect. At 4°C, a LC_{50} was not found in any tests (except VIA, 7 days), while at 14°C a mean LC_{50} of 505 ppm was found in the two tests with No. 2 fuel oil. A temperature effect was not apparent in clams exposed to phenol.

Discussion

The behaviour effects found for M. arenaria were repeatable for both crude and refined oil. The increasingly greater concentrations of oil elicited greater mucus secretion and decreased tactile response. SWEDMARK, et al. (1973) reported the effect of crude oil and oil emulsions on bivalves and found a similar decrease in tactile response. The general behavior sequence they reported was: increased activity; successively imppaired activity; immobilization and death. The observations of this report are similar to their observations.

The copious production of mucus by M. arenaria had several effects. It imposed a steady drain on the energy reserves of the clams. The continual production of mucus clogged the gills and mantle cavity and would disrupt normal feeding mechanisms. The clogging mucus must be expelled by the clam by increased contraction of the adductors and mantle musculature which puts an additional strain on the metabolic rate. The increased metabolic demands for mucus production and excretion and the disruption of normal physiological and biochemical processes occurred at much lower concentrations of oil exposure than the LC_{50} indicates.

A problem encountered in comparing the results of this study was the lack of definition of death in bivalves in published reports. Terms such as "moribund" are found in the literature but the criteria of death was not defined. The definition of death in this report was the total lack of muscular response, though this was often difficult to determine. Stimulation of the musculature by pinching or light rapping with a stirring rod often elicited muscular twitches though the clam appeared "dead".

The bioassay LC_{50} values obtained for M. arenaria are greater in concentration (ppm) than those reported for other species exposed to No. 2 fuel oil (VAUGHAN, 1973; ANDERSON, et al., 1974). However, the values are lesser than those reported for many species exposed to crude oils. According to the ranking system of SPRAGUE and CARSON (1970), the No. 2 fuel tested in this report can be classified as moderately toxic to M. arenaria. An additional factor noted in the bioassay test was the time of exposure. Increasing the period of exposure from 96 hours to seven days decreased the LC_{50} values obtained. Similar increases in mortality of molluscs during longer exposure periods were reported by SPRAGUE and CARSON (1970), KANTER et al., (1971) and KASYMOV and ALIEV (1973).

Future bioassay work with molluscs, particularly bivalves, should be determined over a 7 day exposure period. However, behavioral observations appear to have advantages as a toxic criterion over lethality, and shorter exposure periods can be employed.

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A Descriptive Evaluation of the Effects of No. 2 Fuel Oil on the Tissues of the Soft Shell Clam, *Mya arenaria* L.

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Introduction

Bivalve molluscs have been found to accumulate petroleum and petrochemical derivatives. BLUMER, et al (1970) described hydrocarbon uptake by oysters and scallops following a fuel oil spill. ZITKO (1971), LEE, et al. (1972), STEGEMAN and TEAL (1973), VAUGHAN (1973), and NEFF and ANDERSON (1975) have reported the occurrence and uptake of petroleum hydrocarbons by bivalve molluscs. A mechanism by which soft shell clams may accumulate oil was described by STAINKEN (1975).

The effects of petroleum oils and derivatives on bivalves are varied. Some of the effects reported have described alterations in oxygen consumption, carbon budgets, larval development, behavior, filtration rates, mortality and biochemical effects. The histological effects of petrochemicals are also varied. Deleterious effects of oil on bivalve tissue structure have been reported by LAROCHE (1972), CLARK, et al. (1974) and GARDNER, et al. (1975). Histological aberrations in bivalves have been reported by BARRY, et al. (1971), JEFFRIES (1972), and BARRY and YEVICH (1975). The aberrations were believed to be due to pollution effects. In contrast, VAUGHAN (1973) found few effects of oils on bivalves.

Reports on the effects of petroleum oils are often conflicting. Some of the studies were field studies and exposure concentrations were unknown. The reported effects of petroleum oil exposure have ranged from extensive to relatively none. Bivalves in the environment are frequently exposed to single spill or chronic discharges. This study was therefore performed to experimentally determine the effects of subacute concentrations of No. 2 fuel oil on the soft shell clam.

A No. 2 fuel oil was chosen for study because it is commonly shipped in coastal waters, used in coastal industrial installations, and has already been involved in a well documented spill (BLUMER, et al. 1970). A winter temperature (4°C) was chosen because spills are more likely to occur during the inclement winter weather. In the event of a spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action. The clams were therefore exposed 28 days to oil initially added in an emulsified form to simulate a potential naturally occurring condition of chronic exposure.

Materials and Method

The No. 2 fuel oil was supplied by the U. S. Environmental Protection Agency, Industrial Waste Treatment Laboratory, Edison, N. J. The specific gravity of the oil was 2.40 centistokes. The oil was composed of 14% aromatics and 86% nonaromatics according to ASTM method No. D2549-68. Oil-in-water emulsions were ultrasonically prepared according to a procedure developed by GRUENFELD and BEHM (1973).

Clams for the experiments were collected from Sequine Point, Staten Island, N. Y. Young clams with a mean shell length of 25 mm were utilized because young bivalves tend to have greater filtration rates than those of older bivalves. The clams were acclimated to the experimental conditions for a duration of 6 days before the emulsions were added.

An exposure period of 28 days to No. 2 fuel oil emulsions having concentrations of 10, 50 and 100 ppm was utilized. Four 20 gallon aquaria containing 60 liters of filtered sea water/aquaria (salinity = 20‰) were employed. The sea water was collected from Sandy Hook Bay and filtered through a coarse plankton net. One aquaria served as a control and each of the remaining aquaria received either 10, 50 or 100 ppm of oil emulsion. The water was continuously aerated and the temperature was maintained at 4°C. Sampling for hydrocarbon content of water and clams was performed every 7 days. The hydrocarbon content of the water was determined by the method of GRUENFELD (1972). Complete results from these experiments will be published at a later date.

At the beginning of the experiment, just before addition of the emulsions (Time 0), five clams were fixed in Davidson's fixative (SHAW and BATTLE, 1957). At the end of the 28 day exposure period, ten clams from each concentration and the control tank were removed for histological examination. The clams sampled represented 10% of the experimental population. Five of the ten clams randomly removed from each concentration were fixed in Davidson's fixative and five were fixed in cold 10% acetate buffered neutral formalin, pH 7. The two fixatives were employed to determine whether alterations in tissue structure were fixation artifacts. The results indicated that artifacts did not occur.

All fixation was done in a refrigerator. After 24 hours, clams fixed in Davidson's were transferred to cold 70% ethanol. All fixed material was stored in a refrigerator until further processing. Prior to embedding, the formalin fixed animals were washed 1½ hours in running tap water. All tissues were dehydrated and brought to paraffin utilizing an Autotechnicon. Final infiltration was accomplished employing a vacuum infiltrator for 15 minutes at 13-15 inches Hg. The paraffin embedded clams were oriented to cut beginning at the anterior pedal opening. Serial cross sections were cut at 6 and 7 microns to the level of the

heart and kidney. Sections were mounted according to the procedure of LUNA (1968). The sections were stained with Harris-Lillie hematoxylin (Fisher Scientific) and 0.5% Eosin Y in 95% ethanol.

Several cross sections of the visceral mass, stomach and pallium of each clam from each experimental group were stained for mucosubstances and necrotic tissue. Mucosubstances were stained using the aldehyde fuchsin-alcian blue method of LUNA (1968) and azure A/eosin B was employed for necrotic tissues (GRIMSTONE and SKAER, 1972).

One section of the visceral mass and stomach from each clam from each experimental group was stained by a modification of McManus's method for glycogen, the Periodic Acid - Schiff reaction or PAS (LUNA, 1968). The modifications were made from procedures described by LUNA (1968) and LILLIE (1965). To check the specificity of the PAS reaction for glycogen, sections were treated in an amylase solution (Diastase, Sigma chemical Co.) at a concentration of 0.1 g/100 ml in distilled water, pH 6.8 for one hour.

Results

Throughout the oil exposure period, all clams in the control and oil exposed groups seemed to remain in good condition. After the addition of the oil emulsions, the concentrations of oil in the water column decreased rapidly. The actual hydrocarbon concentrations are listed in Table 1.

Table 1. Hydrocarbon concentration (ppm) in the water column during the 28 day exposure period.

	Tank #	Time 0*	Week 1	Week 2	Week 3	Week 4
Control	1	0	0	0	0	0
10 ppm	2	4.5	1.31	0.56	0.37	0
50 ppm	3	43.72	1.04	0.71	0.37	0.29
100 ppm	4	60.71	1.52	0.78	0.32	0.46

* The Time 0 sample measurement was made two hours after addition of the emulsified oil.

Several factors were probably responsible for the gradual depletion of oil from the water. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. Large masses of mucus were

ejected from the clams and were accumulated on the cooling coils. Subsequent chemical analysis revealed a large content of oil in the mucus. After 28 days, a sample of the mucus from the 100 ppm aquaria was found to contain 833 micrograms of hydrocarbons. Mass spectrometric analysis demonstrated that these hydrocarbons were mostly dimethyl and trimethyl naphthalenes and paraffins in the C-14 and C-15 regions (STAINKEN, 1975).

Harris's Hematoxylin was used to examine the general morphology of clams. Radical tissue aberrations were not observed. A gradation of tissue effects was apparent. The clams exposed to 100 ppm exhibited the largest number of anomalies from the controls. The pallial muscle appeared edematous in four clams, similar to that described by PAULEY and SPARKS (1965, 1966). There were more leukocytes in the pallial blood sinuses of 100 ppm exposed clams than controls, with occasional leukocyte nests as described by PAULEY and CHENG (1968) occurring in the pallial blood sinuses. The leukocytes often formed a band underlying the mantle epithelium similar to that illustrated by DES VOIGNE and SPARKS (1968). In some clams, the anterior adductor muscle was midly edematous and infiltrated with leukocytes. The area between the mantle membrane and the cell layer next to the shell was also edematous and contained many leukocytes in some clams. In one clam, they formed a plug in the hemocoel of part of the foot. In seven clams, the style sac, intestine and diverticula appeared very vacuolar and the diverticula appeared much reduced in size. There was a small loss of chromatophilic material at the top of the gill filaments in several clams exposed to 100 ppm oil.

At 50 ppm, the effects were less marked, except in the diverticula and intestine. The diverticula of seven clams were shrunken in size. The diverticula epithelium appeared almost cuboidal instead of the normal columnar epithelium. Portions of the intestinal mucosa appeared to be sloughing into the lumen which was not prevalent in the controls. The intestine, style sac and diverticula were abnormally vacuolar in appearance. In one clam, a few diverticula near the gonad appeared necrotic and undergoing resorbtion. The pallial muscle was edematous in one clam. Several clams had more leukocytes and leukocyte nests in the blood sinuses below the inner pallial epithelium than the controls. Only one clam had more than normal leukocytes and leukocyte nests between the mantle epithelium and the cell layer next to the shell. In one clam, a portion of the gill had a pavement of leukocytes along the lining of the blood sinuses next to the central water tube.

The 10 ppm clams showed fewer effects of oil exposure than the 100 or 50 ppm clams. The diverticula were reduced in size and the diverticula, stomach and intestine were vacuolated in appearance. In two clams, a moderate number of leukocytes were observable in the pallial blood sinuses.

Cross sections of the clam visceral mass and pallium were stained with azure A/eosin B to demonstrate necrotic tissues. Major histological differences were not found between controls and oil exposed clams. The general effect of holding the clams for four weeks was an increase in vacuolization of the digestive diverticular and intestinal cells. The vacuolization was present in all groups. The diverticular cells of the Time 0 clams were distinct and contained few vacuoles. After four weeks, the diverticular cells of all clams appeared to contain many vacuoles and the cell membranes were often indistinct. However, this effect was exacerbated in the oil exposed clams compared to controls (Figure 1-3).

Figure 1. Section of the digestive diverticula. 100x. Stained Azure A/eoson B. Time 0.

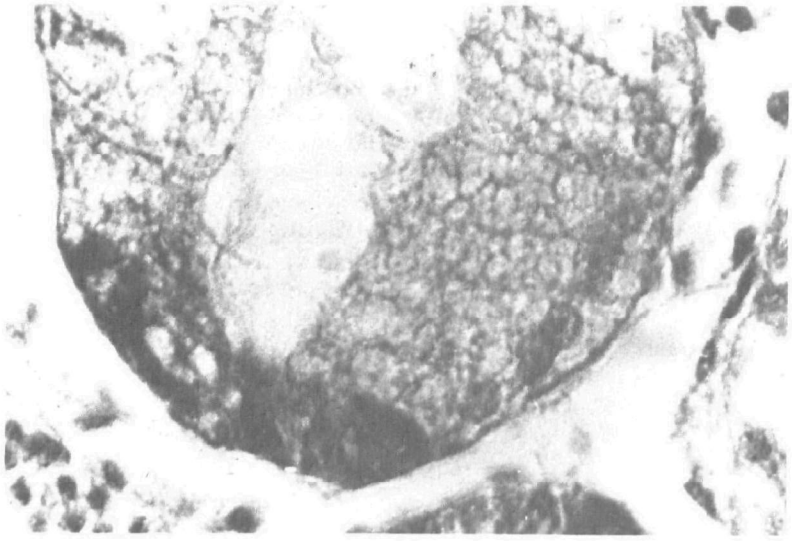

Figure 2. Section of the digestive diverticula. 100x. Stained Azure A/eosin B. Control.

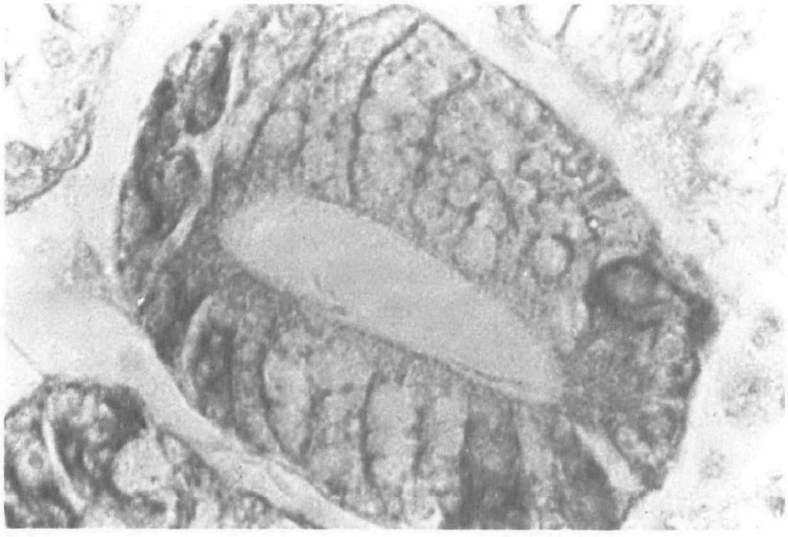

Figure 3. Section of the digestive diverticula. 100x. Stained Azure A/eosin B. 100 ppm exposed.

An aldehyde Fuchsin - Alcian Blue 8GX stain was used to stain mucosubstances. Histological differences were not found between exposed and control groups along the intestines and the periphery of the visceral mass. There was a decrease in mucoid cells and staining intensities from two of the 100 ppm clams. The same effect occurred in one 50 ppm clam.

Clam sections were stained for glycogen according to the PAS technique. There was a gradation of effects of oil exposure in the digestive diverticular and intestinal cells. The 100 ppm clams had the least amount of PAS positive material (glycogen). Six of the 100 ppm clams had observable differences from the control clams. Generally, the diverticular cells decreased in size and contained much less PAS positive material. The diverticular cells of all 100 ppm clams appeared very vacuolar with few glycogen deposits. Several cells almost appeared amylase treated. The intestinal cells and basement membranes also contained less PAS positive material. Most of the diverticular cells appeared to be devoid of cytoplasm. The stomach mucosa of two of the 100 ppm clams contained less PAS positive material than did the controls. The gill filament tips and margins appeared to have a decrease in PAS positive material.

Generally, a pattern of cellular glycogen depletion was observed in 50 ppm clams similar to that described for 100 ppm clams. The diverticular cells were reduced in size, vacuolar in appearance and contained less PAS positive material than did the controls. The cells also appeared to be depleted of cytoplasm. The intestinal mucosa appeared to be sloughed into the lumen. The stomach mucosa of the 50 ppm clams also showed a depletion of PAS positive material as compared to controls.

Most digestive cells in the 10 ppm clams appeared normal. However, several clams diverticular and intestinal cells were depleted of PAS positive material and were more vacuolar in appearance than were the controls.

In the control clams, the bulk of the diverticular cells were full, round, and most of the cytoplasm was red. In the 10 ppm, 50 ppm and 100 ppm clams, the diverticular cells were more vacuolar and contained less cytoplasm than did those of the control group.

Discussion

Petroleum hydrocarbons are generally assumed to be carcinogenic, particularly the polycyclic aromatics. Reviews of the general carcinogenic effects of oil have been published by HEIDELBERGER (1970) and ZOBELL (1971). CLARK, et al. (1974) reported alterations in tissue structure of oysters and mussels exposed to outboard motor effluent. LAROCHE (1972), BARRY and YEVICH (1975) have reported a high incidence of gonadal tumors in soft shell clams exposed to oils. Hyperplastic germ cell

tumors were also present in the gills. BARRY, et al. (1971) reported a high incidence of hyperplasia of the gills and kidneys in soft shell clams from areas believed polluted. In contrast, VAUGHAN (1973) did not find evidence of histopathological change in oysters exposed to No. 2 fuel oil. There were some nonpathological changes evident in the epithelial layer of the inner mantle lobe, and it was suggested that oil restricted feeding activity.

The results of this study with Mya arenaria revealed that radical tissue changes did not occur after exposure to No. 2 fuel oil. It is possible, however, that either the very low concentration of oil present in the water column was not suffiecient to alter tissue structure (i.e. neoplasms), or the exposure time was not long enough. The hydrocarbon concentrations in the water column of each tank measured during the last 3 weeks of exposure varied from 1.52 to 0.29 ppm.

The general effects of subacute oil exposure can be characterized as a depletion of glycogen and generalized leukocytosis particularly evident in the blood sinuses of the pallium and mantle membrane. There was also an increase in vacuolization of the diverticula, stomach and intestines. The histological effects in Mya arenaria appeared to be dose dependent. The clams exposed to the initial 100 ppm oil emulsion had more frequent and noticeable histological differences from the controls. The depletion of glycogen and vacuolization may have been due to a suppression of feeding and consequent use of body reserved coupled with an altered respiratory rate. The increased vacuolization of oil-exposed clams may also represent inclusion and intracellular compartmentalization of hydrocarbons. The leukocytosis of the mantle blood sinuses beneath the inner epithelium probably represents an inflammation reaction with a migration of leukocytes into the affected areas.

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Effects of Uptake and Discharge of Petroleum Hydrocarbons on the Respiration of the Soft-Shell Clam, *Mya arenaria*[1]

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STAINKEN, D. M. 1978. Effects of uptake and discharge of petroleum hydrocarbons on the respiration of the soft-shell clam, *Mya arenaria*. J. Fish. Res. Board Can. 35: 637–642.

A winter (4°C) spill condition was simulated in which young soft-shell clams, *Mya arenaria*, were exposed to subacute concentrations of No. 2 fuel oil-in-water emulsions for 28 d. Clams were exposed at the beginning of the experiment to single dose concentrations of 10, 50, and 100 ppm. Hydrocarbons were rapidly accumulated by clams within 1 wk after exposure. The accumulated hydrocarbons then decreased each week as the hydrocarbon content of the water decreased. Methyl substituted naphthalene isomers were the principal compounds accumulated and retained by the clams after 3 wk of oil exposure. A dose–response relationship was observed in the respiratory rates as measured by oxygen consumption (QO_2). Significant differences ($P = .05$) in respiratory rates were found in clams exposed to low concentrations of oil. The lowest concentrations of oil caused a doubling of the respiratory rates and greater oil concentrations caused a depression in rate. The respiratory rates of the clams exposed to low oil concentrations decreased as the hydrocarbon content of the water and clam tissues decreased, but remained significantly altered from the controls. Clams were transferred to an uncontaminated system for 14 d subsequent to the 28-d oil exposure to determine effects of depuration on the respiratory rate. During the depuration period, many of the hydrocarbons present in clam tissue were again found to be methyl substituted naphthalene isomers. During this period, the respiratory rates of the clams initially exposed to 10 ppm fuel oil emulsion remained significantly altered above the controls. The respiratory rates of all groups of oil-exposed clams remained altered from the controls, but the magnitude of difference tended to decline toward the controls. A dose–response narcosis may have been evident during this period.

Key words: Mya arenaria, respiration, No. 2 fuel oil, petroleum emulsion, petroleum accumulation, depuration

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Nous avons simulé une condition hivernale (4°C) de déversement dans laquelle de jeunes myes communes (*Mya arenaria*) furent exposées à des concentrations subaiguës d'émulsions de fuel-oil nº 2 dans de l'eau durant 28 jours. Au début de l'expérience, les myes ont été exposées à des concentrations, par doses uniques, de 10, 50 et 100 ppm. Les hydrocarbures s'accumulent rapidement dans les myes en dedans d'une sem après exposition. Les hydrocarbures accumulés diminuent ensuite à chaque sem à mesure que la teneur de l'eau en hydrocarbures diminue. Les isomères naphtalène à méthyl substitué sont les principaux composés qui s'accumulent dans les coques et qui sont retenus après 3 sem d'exposition au pétrole. Nous avons observé une relation dosage–réponse dans les taux respiratoires tels que mesurés par la consommation d'oxygène (QO_2). Nous avons observé des différences significatives ($P = .05$) dans les taux respiratoires des myes exposées à de faibles concentrations de pétrole. Les plus basses concentrations entraînent une augmentation du double des taux respiratoires et les hautes concentrations produisent un abaissement des taux. Les taux respiratoires des myes exposées à de faibles concentrations de pétrole diminuent à mesure que la teneur en hydrocarbures dans l'eau et dans le tissu des myes diminue, mais demeurent nettement différents de ceux des témoins. Les myes furent ensuite transférées dans un système non contaminé durant 14 jours après l'exposition au pétrole

de 28 jours dans le but de déterminer les effets de l'épuration sur le taux respiratoire. Durant la période d'épuration, on constate que plusieurs des hydrocarbures présents dans le tissu des myes sont de nouveau des isomères naphtalène à méthyl substitué. Durant cette période, les taux respiratoires des myes initialement exposées à 10 ppm d'émulsion de fuel-oil demeurèrent nettement supérieurs à ceux des témoins. Les taux respiratoires de tous les groupes de myes exposées au pétrole se maintiennent à un taux différent de ceux des témoins, mais la différence a tendance à diminuer et les taux à se rapprocher de ceux des témoins. Il se peut qu'il y ait eu une réponse de narcose liée à la dose durant cette période.

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BIVALVE molluscs in the environment are frequently exposed to single spills or chronic discharges of petroleum oils. Chronic subdetectable oil pollution may occur from seeps, undetected spills, leaks, marine vessels, and urban and industrial sewage. Pollution incidents in temperate and subarctic estuarine and coastal marine waters will probably increase as further exploration, development, and transportation of oil increases. Lee et al. (1972), Stegeman and Teal (1973), and Neff and Anderson (1975) have reported the occurrence and uptake of petroleum hydrocarbons by bivalve molluscs. Various effects of petroleum oils and derivatives on bivalves have been described. Some of the effects reported were alterations of behavior (Galtsoff et al. 1935), and tissue structure (Barry and Yevich 1975; Stainken 1976a).

Few studies have characterized the effects of oils on the respiration of bivalves or related physiological responses to concentrations of contaminants in bivalve tissues. Gilfillian (1973, 1975) and Gilfillan et al. (1976) reported alterations in the carbon budgets of mussels and soft-shell clams exposed to crude and fuel oils and noted that O_2 consumption was altered. Avolizi and Nuwayhid (1974) found that exposure to crude oils decreased the O_2 consumption of mussels. In contrast, Fong (1976) described an increase in respiration rates of soft-shell clams exposed to crude oil. It is difficult to compare directly some studies because of the varying test parameters (i.e. salinities, temperatures, methods of oil addition, period of exposure, animal size, species, etc.). Therefore, this study was performed to determine the effect(s) of uptake and discharge of petroleum hydrocarbons on the O_2 consumption of the soft-shell clam, *Mya arenaria*. Effects on O_2 consumption were determined during a 28-d exposure to subacute concentrations of No. 2 fuel oil emulsions and a subsequent depuration period of 12 d. The effect on the clams was then related to the body burden of petroleum hydrocarbons and specific fractions.

Mya arenaria was chosen for study because these clams occur frequently in areas receiving acute and chronic oil exposures. Oil spills and their effects on *M. arenaria* have been documented by Thomas (1973), and the occurrence of oil in *M. arenaria* has been reported by Scarratt and Zitko (1972). Mechanisms by which *M. arenaria* may accumulate oil have been described by Stainken (1975) and Fong (1976). A No. 2 fuel oil was chosen for study because it is commonly used in coastal industrial installations and shipped in coastal waters. Experimentation was performed at 4°C because spills are more likely to occur during inclement winter weather.

The oils were added in an emulsified form to simulate a potential naturally occurring condition. Forrester (1971) and Gordon et al. (1973) reported the formation of oil emulsions in seawater. In the event of an oil spill during the colder months, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent wave action.

Materials and Methods

The No. 2 fuel oil was supplied by the U.S. Environmental Protection Agency, Industrial Environmental Research Laboratory, Edison, N.J., (specific gravity, 2.40 centistokes; composition, 14% aromatics and 86% nonaromatics according to ASTM method No. D2549-68). Oil-in-water emulsions were prepared according to Gruenfeld and Frederick (1977).

Clams with a mean shell length of 25 mm (Newcombe 1936; Pfitzenmeyer 1965) were collected from Sequine Point, Staten Island, N.Y. Young clams were used because they tend to have greater respiration and filtration rates than those of older bivalves (Read 1962; Walne 1972). The clams were acclimat to the experimental conditions for 6 d before the emulsions were added.

An exposure period of 28 d to No. 2 fuel oil emulsions having concentrations of 10, 50, and 100 ppm was utilized. Four, 20-gal covered aquaria containing 60 L of filtered seawater per aquarium (salinity = 20‰) were used. The seawater was collected from Sandy Hook Bay and filtered through a coarse plankton net to remove macrodebris. One aquarium served as a control. Each of the remaining aquaria received a sufficient volume of a stock oil emulsion to attain an initial concentration of either 10, 50, or 100 ppm of emulsified oil. The time of addition was termed Time 0. The water was continuously aerated and the temperature maintained at 4°C. The photoperiod was approximately 16L:8D. Previous studies (D. M. Stainken unpublished data) have shown that *M. arenaria* establishes normal O_2 consumption patterns within these experimental parameters.

The hydrocarbon contents of the Sandy Hook Bay seawater and the experimental aquaria water were determined using a Freon extraction technique (Gruenfeld 1973). Before the experiments, the hydrocarbon content of the seawater was analyzed to ensure no external contamination. Each week, a 400-mL sample was removed from the same depth at the center of each tank. Concomittant studies of hydrocarbon accumulation and depuration of the clams (Stainken 1976b), and the effects (Stainken 1976a), were

performed. The method used for hydrocarbon analyses was described by Stainken (1976b).

Beginning at Time 0, the O_2 consumption of 18 clams from each of the groups, control and oil-exposed, was measured every 4 d for 28 d. At the end of this period, each experimental group of clams was rapidly removed to clean 20-gal aquarium containing seawater as described above. As clams were transferred, the valves were wiped clean to remove deposited pseudofeces, etc. The O_2 consumption of 18 clams from each of the groups, control and oil-exposed, was then measured every 4 d for 12 d. Samples for hydrocarbon analyses were obtained on days 7 and 14.

The procedure used for O_2 consumption measurements was modified from that of Kennedy and Mihursky (1972). A Scholander respirometer (The Mark Co., Brockton, Mass.) was used. Each respirometer vessel received 8 mL of natural seawater collected with the clams. This seawater had been stored at 4°C in the dark. The water in the respirometer vessels was prefiltered through a 1.4- and 0.8- filter (Millipore Corp., Bedford, Mass.) and aerated 1 h at 4°C prior to use. To absorb CO_2, 0.2 mL of 10% KOH was placed in a plexiglass boat with a piece of No. 4 Whatman paper. The valves of the clams were gently wiped dry before the animals were placed in the respirometer vessels. Animals were equilibrated 1½ h prior to measuring O_2 uptake. O_2 consumption was than measured for 1 h. Measurements were made in the morning or afternoon at the same time for each experimental group. Procedural blanks were run with each experiment. Shell length was measured at the end of each experiment and dry tissue weight (minus shell) was determined by drying the tissue to constant weight at 75–80°C for 48 h. The O_2 consumption data was then calculated as μL O_2/mg dry weight per hour or QO_2. This method has been used by Kennedy and Mihursky (1972) and Widdows (1973); it decreases much of the variability inherent in molluscan oxygen consumption rates.

Respirometric results were analyzed by linear regression. For regression analysis the procedures of Ostle (1963) and Draper and Smith (1966) were used. An analysis of variance and "in level" comparisons of the mean QO_2 measurements of oil-exposed and depurated clams were performed according to Campbell (1974). Results were considered significant at the $P = 0.05$ level.

Results

The dissolved O_2 and pH remained optimal in all aquaria throughout the 28-d exposure period; bacterial contamination was not apparent. Control animals were reactive to tactile stimuli and appeared to be feeding. Very little mortality occurred at any of the oil concentrations employed. The actual mortalities in the oiled phase of the experiment were controls 0.0%, 10 ppm 0.5%, 50 ppm 2.0%, 100 ppm 3.0%.

In the 10-ppm aquaria, the oil odor disappeared by the 3rd wk and the clams appeared to react more slowly than the controls to tactile stimuli. The 50-ppm aquaria still had a faint odor of oil by the 3rd wk and the clams reacted much more slowly to tactile stimuli. In the 100-pm aquaria, there was still a faint odor of oil detectable after 3 wk, and the clams appeared narcotized and were relatively unreactive.

During the 14-d depuration period, the 10-ppm clams appeared to recover their tactile sensitivity to a level similar to that of the controls, but the 50- and 100-ppm clams continued to react sluggishly. Mortality increased during the depuration period among the 50- and 100-ppm clams. The actual mortality in the depuration was controls 0, 10 ppm 0.1%, 50 ppm 18.26%, 100 ppm 13.63%. The seawater before all tests and in the control tank during the tests did not have detectable hydrocarbon contents (0.2 ppm). Within the oiled tanks, some of the oil emulsion had broken within 2 h after addition. As indicated in Table 1, much of the oil emulsion and water-soluble fraction remained in the water column after 2 h.

TABLE 1. Hydrocarbon concentration (ppm) in the water column during the 28-d exposure period. The Time 0 sample measurement was made 2 h after addition of the emulsified oil.

Aquaria	Time 0	Wk 1	Wk 2	Wk 3	Wk 4
Control	0	0	0	0	0
10 ppm	6.0	0.8	0.4	0.3	0.3
50 ppm	31.5	1.2	0.7	0.6	0
100 ppm	55.3	1.5	1.5	0.4	0.3

One week later, the detectable hydrocarbon concentrations in the water column of the oiled aquaria were similar. Much of the oil appeared bound to mucus which adhered to the glass cooling coils and tank walls or formed floating organic flocculent conglomerates. The IR spectra also showed the emergence of an apparent aromatic band at 3030 cm^{-1}. The week 2 data were similar to those of week 1 and a peak emerged at ca 3000 cm^{-1}.

By week 3, the measurable hydrocarbon content had dropped further, and in the 10- and 50-ppm tank samples the possible aromatic peak became less apparent. The 100-ppm tank sample still had a strong peak at 3030 cm^{-1}. At the end of the 4th wk, the hydrocarbon content in the tanks had fallen to almost trace concentrations and peaks were becoming indistinguishable.

The mucus–oil complex formation may have aided in the dissipation of hydrocarbons from the water column. The mechanism of the binding of oil in mucus by *M. arenaria* has been documented earlier (Stainken 1975). Mass spectrometric analysis demonstrated that the mucus contained predominantly dimethylnaphthalenes and trimethylnaphthalenes with paraffins, mainly in the C_{14} and C_{15} regions.

During the depuration period, measurable amounts of hydrocarbons in the aquaria water were not detected. There was an emergence of a peak at 3000 cm^{-1} during the 2nd week in the experimental tanks.

Figure 1 illustrates the calculated regression of QO_2 of clams exposed to oil over the 28-d period, and Table 2 contains the correlation coefficient, slope, y-intercept, upper and lower confidence intervals for the regression lines of Fig. 1. Table 3 contains the mean QO_2 and standard errors for the depuration period.

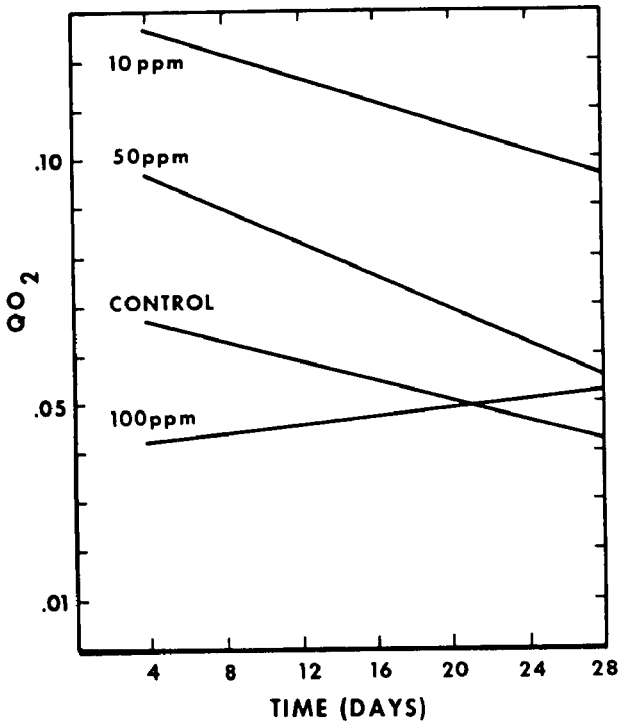

FIG. 1. Calculated regression of QO_2 of clams exposed to No. 2 fuel oil for 28 d.

The normal QO_2 rates of *M. arenaria* acclimated to 4°C ranged between 0.06 and 0.04 QO_2 units. This agrees with the results of Kennedy and Mihursky (1972) who found that clams acclimated and measured at 1°C had a QO_2 range from 0.07 to 0.40. Although these rates are not directly comparable due to temperature, latitude, and other unknown variables, it does indicate that O_2 consumption by *M. arenaria* is relatively low.

TABLE 3. Mean QO_2 and standard error (SE) or clams during the depuration period.

	4 d	8 d	12 d
Control			
QO_2	.041	.099	.046
SE	.008	.016	.008
10 ppm			
QO_2	.065	.154	.087
SE	.010	.011	.010
50 ppm			
QO_2	.023	.040	.038
SE	.007	.011	.008
100 ppm			
QO_2	.043	.120	.058
SE	.007	.021	.010

The 10-ppm regression of QO_2 in Fig. 1 appears to have translated to greater rates than those of the controls. After the initial exposure to oil and elevation of QO_2, there is a continual decline toward the control values. After 28 d, the 10-ppm QO_2 remained almost double the control values. During the depuration period, O_2 consumption by the 10-ppm-exposed clams remained elevated compared to the controls.

The 50-ppm regression of QO_2 in Fig. 1 was also elevated above that of the controls. Following an initial elevation, the 50-ppm rates gradually declined toward the levels of the controls. After 28 d of oil exposure, however, the 50-ppm rates remained elevated. During the depuration period, the rates were depressed slightly below those of the controls. This could be due to several factors including the more rapid loss of hydrocarbons possibly affecting cellular respiration, or generally depressed body condition subsequent to oil exposure.

The calculated regression of QO_2 of the 100-ppm

TABLE 2. Correlation coefficient (r), slope (a_1), y intercept (a_0), upper confidence intervals (UCI), and lower confidence intervals (LCI) for the regression lines of the control, 10 ppm, 50 ppm, and 100 ppm QO_2 rates of Fig. 1. *For each day measurement, $n = 18$.

Control	10 ppm	50 ppm	100 ppm
$a_0 = 0.072$	$a_0 = 0.132$	$a_0 = 0.103$	$a_0 = 0.039$
$a_1 = -0.001$	$a_1 = -0.001$	$a_1 = -0.002$	$a_1 = 0.001$
$r = -0.573$	$r = -0.712$	$r = -0.683$	$r = 0.482$

	95% confidence intervals*							
	Control		10 ppm		50 ppm		100 ppm	
Day	UCI	LCI	UCI	LCI	UCI	LCI	UCI	LCI
4	.092	.044	.147	.107	.125	.067	.067	.016
8	.082	.044	.138	.106	.112	.066	.065	.025
12	.074	.044	.130	.104	.100	.064	.065	.033
16	.069	.041	.124	.100	.092	.059	.067	.037
20	.066	.036	.119	.093	.087	.051	.072	.040
24	.065	.027	.117	.085	.085	.039	.079	.039
28	.066	.018	.116	.076	.084	.026	.087	.036

clams rotated around that of the controls (Fig. 1). Rates were depressed until the 16th d when the rates approximated those of the controls, followed by a continual elevation in rate until the end of the experiment. During the depuration period, oil consumption was slightly greater than the controls.

A single factor analysis of variance calculated for the mean QO_2 of the 28-d exposure period and the 12-d depuration period showed significance ($P = 0.001$) for the exposure period. Further "in level" analysis showed that differences existed between the control and 10-ppm rates ($P = 0.01$) and between those of the control and 50 ppm ($P = 0.05$). There was no significant difference between controls and 100 ppm. Comparisons of the depuration results were not significant ($P = 0.05$) for the rates of the controls and of those clams previously exposed to 50- and 100-ppm concentrations. However, the difference between the 10 ppm and controls was still significant ($P = 0.05$).

The total mean QO_2s for oil exposure and depuration periods were calculated. Generally the oil-exposed clams had greater O_2 consumption during exposure and depuration periods than the controls. The incidence of respiration was also increased by oil. The mean percent of clams respiring during the oil exposure period was controls 65%, 10 ppm 99.2%, 50 ppm 88%, and 100 ppm 75.4%. During depuration, the mean percent of clams respiring was controls 77%, 10 ppm 90.7%, 50 ppm 46.3%, and 100 ppm 72.2%.

Discussion

The concentrations of oil in the water column began to decrease several hours after the addition of the oil emulsion. Several factors were probably responsible for this. Much of the oil was apparently removed from the water column by the mucociliary feeding and ejection mechanisms of the clams. The mechanisms for the formation of an oil–mucus complex and its chemical analysis were described earlier (Stainken 1975). A large content of oil, especially di- and trimethylnaphthalenes, was found in the mucus. Boehm and Quinn (1974) found that fuel oil could form droplets and micelles in seawater and that the major water soluble components were aromatic compounds, particularly naphthalenes.

After Time 0, the emulsified oil rapidly disappeared from the water column. The amount of dissolved compounds attained a peak measured in the 1st wk, and then decreased to equilibrium values (ranging from 1.5 to 0.3 ppm) for several weeks. An analysis of mucus and tissue from clams concomittantly exposed and sampled indicated that naphthalenes and methyl-substituted naphthalenes were probably predominant components in the water (Stainken 1976b). Anderson et al. (1974) have also reported that the water soluble fractions of oils often contains naphthalenes.

After 1-wk exposure, the peak accumulation of petroleum derived hydrocarbons was reached, followed by a gradual loss in accumulated hydrocarbons. Most of the materials accumulated were naphthalene and methyl substituted naphthalene isomers. Details of these chemical analyses were reported earlier (Stainken 1976b). Although other compounds were detected and accumulated, naphthalenes were the most persistent.

Depuration of accumulated hydrocarbons began as the hydrocarbon concentration in the water column decreased. When clams were placed in fresh seawater, depuration proceeded rapidly within the 1st wk, after which the rate slowed. Di- and trimethylnaphthalenes were still present in the clams after 2 wk in fresh seawater.

Gilfillan (1973) found that small amounts of oil extract could decrease the amount of energy available for maintenance, growth, and reproduction by decreasing the net carbon balance of the animal. Fong (1976) also found that the respiration rates of small *M. arenaria* were significantly increased after 3-wk exposure to seawater containing 220–370 g oil/L. In contrast, Dunning and Major (1974) reported that exposure to cold seawater extracts of No. 2 fuel oil tended to decrease O_2 consumption by mussels. Avolizi and Nuwayhid (1974) investigated the effects of a crude oil on two bivalve species and they also found a depression of respiratory rates at sublethal concentrations. Higher temperatures and physiological variation may possibly have influenced their results.

The results with *M. arenaria* in this study confirm that low levels of fuel oil cause a marked elevation in the respiratory rates. Higher concentrations cause a depression in the rates. As long as the oil- or water-soluble fraction is present, the rates remain affected. The actual content of hydrocarbons in the water column during the last 3 wk of the test ranged from 0.0 to 1.5 ppm. Even at the highest concentrations tested (100 ppm), as the concentrations of the water-soluble fraction decreased, the respiration rates increased. This apparently reflects a dose–response relationship. Very low sublethal concentrations of oil cause a doubling of the respiratory rates. Further support of a dose–response effect is evidenced in the accumulation of hydrocarbons. The greater uptake of hydrocarbons by clams exposed to 50 ppm may be due to a dose-dependent narcosis. Galtsoff et al. (1935) reported that water-soluble substances of crude oil could produce anesthetic effects on the ciliated epithelium of gills. The 100-ppm oil concentration may have reduced the filtration activity of the clams below that of the clams exposed to 50 ppm. The reduced filtration rate may account for the animals exposed to the higher concentration (100 ppm) accumulating less oil than the animals exposed to the lower concentration (50 ppm).

The mechanism(s) that cause the alterations in respiratory rates is unknown. Concomittant studies on the effects of oil exposure on tissue structure (Stainken 1976a) revealed a depletion of glycogen and generalized leukocytosis particularly evident in the blood sinuses of the pallium and mantle membrane. There was also

an increase in vacuolization of the diverticulum, stomach, and intestine. Effects appeared to be exacerbated at the higher exposure levels. Although the increase in respiratory rates is similar to an uncoupling effect of oxidative phosphorylation, further work on the cellular level is necessary. Altered O_2 consumption may be due to the disruption of normal cell membranal processes (Goldacre 1968) or cellular metabolism.

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ADDITIONS

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METHODS FOR THE SOURCE IDENTIFICATION AND QUANTIFICATION OF OIL POLLUTION

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ABSTRACT: *Petroleum oil analyses frequently are performed by government and industrial organizations and by academic institutions to identify the source of discharge of oil pollutants and to quantify such oils in water, sediments and tissues. This paper presents a concise review of oil analysis methods used by several U.S. agencies, industrial organizations and standard setting societies. Oil analysis methods published by these organizations are evaluated with regard to their convenience and safety for general laboratory use, ability to yield correct quantification data, and correctness in identifying the presence and source of discharge of petroleum oil pollution. Several procedures also are addressed for isolating individual petroleum fractions and hydrocarbons from environmental samples for the purpose of monitoring for the presence of oil pollution in sediments and tissues.*

Although oil spill prevention and control techniques have advanced in the past decade, the discharge of oil to the environment continues to be a serious problem. These discharges may be of acute or chronic duration, and occur from both point and nonpoint sources. Consequently, petroleum oil analyses frequently are performed by government and industrial organizations and by academic institutions to identify the source of discharge of oil pollutants and to quantify such oils in water, sediment and biota. Bentz[10] discussed some of the legal and scientific aspects of analyses for oil in spill identification.

The complex composition and dissemination of petroleum in the environment complicates analyses for oil pollution. Petroleum rapidly undergoes various weathering phenomena, or becomes contaminated with other environmental contaminants. Farrington[33] reviewed many of these analytical problems.

There are a multitude of methods for analyzing oil and its component subfractions. Many are too time consuming, are imprecise, use toxic solvents, and are impractical for routine use. Complicating the analysis of petroleum is the relative lack of standardized methods of analysis. The present standard method is that used for analysis of oil and grease in water and wastewater.[78] This method has been reported in several "official" publications.[3,4,106] Comparison and assessments of the efficacy of oil and grease methods have been reported.[27,94] Although these methods are accurate when analyzing water contaminated by refined nonvolatile oils (such as lubricants, greases, heavy fuel oils), their applicability to water contaminated by crude and fuel oils is questionable. Further problems in oil spill identification and quantification were discussed by Bentz.[11]

The complexity of petroleum composition, its weathering, environmental dissemination and assimilation led to the application of a variety of column, thin layer and high pressure chromatography techniques for sample separation-preparation, and use of ultraviolet and infrared absorption, UV-fluorescence, gas chromatographic and mass spectral techniques for analysis. However, many of the methods are not standardized and interlaboratory data varies. Interlaboratory and intercalibration studies[33,55,73,114] have been reported covering several facets of the problem.

Several frequently used methodologies with various modifications gradually evolved from numerous investigative reports. Multimethod approaches have often been used and Bentz[10] described many of them. Most involve some type of sample solvent extraction, sample cleanup procedure, concentration and analysis by several analytical techniques. Many analysts now use similar techniques with modifications. These approaches are evolving into prestandardized procedures as more interlaboratory and intercalibration exercises are performed.

These methods must ultimately meet several criteria, however, to be useful for spill source identification and quantification of petroleum oil. Dependability of the quantification data and the ability of the method(s) to establish the source of oil discharge are critical for monitoring and damage assessment. Many methods now used employ volumes of toxic solvents (such as benzene, carbon disulfide, and CCl_4) which present difficulties in analyst safety, and problems in proper waste solvent disposal. Some methods are criticized for the time consumed in sample preparation. Those methods ultimately chosen should be practical for routine laboratory use.

This paper presents a preliminary synopsis of oil analysis methods now used by U.S. agencies, industrial organizations and standard setting societies. A more comprehensive review is in preparation. Methods evaluated were examined according to their accuracy in identifying and quantifying waterborne oil, the relative toxicity of solvents used, and the practicality of the method for routine use. Also addressed are several methods for isolating individual petroleum fractions and hydrocarbons from environmental samples to monitor the presence of oil pollution.

Identification of waterborne oils

Establishing the identity and quantity of spilled petroleum oil involves complex methodology. Several reviews have addressed earlier oil identification techniques. Brunnock[6] reviewed mostly European practices for analysis of beach pollutants and Adlard[1] reviewed British and U.S. methods of oil spill analysis. Additional methods for oil identification were discussed by Gruenfeld[50] and Bentz.[9,10]

Many methods now are used by "standard setting" organizations, government, industry and academic institutions to identify and quantify petroleum oil. Oil spill identification methods recommended by the principal standard setting organizations in the U.S. are reviewed in this section. Also discussed are the primary methods used for the quantitative determination of petroleum oil dispersed in water.

Methodology for the identification of waterborne oils covers the broad concepts of sampling and analyzing spilled petroleum for identification and comparison with oils taken from suspect sources of discharge. The general procedure includes sample collection, preservation, preparation, pretreatment, and analysis.

Sample collection. Procedures for sample collection must assure that a representative portion of the oil be taken, from a slick, shore-

line residue or suspect source, for example. Sampling can be complicated by emulsion formation, insufficient oil film thickness, or contamination. The collection procedure must assure that no contamination of the sample occurs. No standard method of sample collection has yet been developed. However, the U.S. Coast Guard (USCG) presented a procedure[105] in which Teflon strips attached to a rake-like device are drawn through an oil slick. The oil adheres to the strips and 10 ml of oil can be collected in one pass through a thin slick.

Some collection procedures that may be implemented routinely in the field are reported by Kawahara[66] and Kreider[70]. More complex procedures are described by the USCG.[7] Several other field techniques for collection of surface oil using aluminum foil[18] and Teflon discs[46] also have been reported.

Sample preservation. Protection of the collected sample against chemical and physical changes during transit or storage is essential to ensure sample integrity. Microbes can alter or deplete the n-alkanes in an oil sample[83] and volatilization and auto-oxidation may affect the sample's composition.[66]

The American Society for Testing and Materials[4] described appropriate sample containers and a sample preservation procedure (ASTM Method D3325) which minimizes contamination and degradation of the collected samples. Narrow mouth borosilicate glass bottles with aluminum or Teflon-lined caps are recommended as sample containers. Metal and plastic containers are unsuitable, because of the introduction of contaminants from the container which may invalidate subsequent trace element analyses. Biodegradation was minimized by storing at 10°C, and displacement of sample bottle air with N_2 or CO_2 prevented auto-oxidation.

Sample preparation and pretreatment. Prior to analysis, it is necessary to separate the sampled oil from extraneous matter (water, sand, debris), and to bring the unweathered suspect oil samples and the weathered waterborne oil samples to physical conditions as comparable as possible. Spilled oils weather as soon as they are exposed to the environment and the changes affect the comparisons of suspect oil to waterborne oil.

ASTM Method D3326[4] incorporates a method combining four procedures for separating oil from water, solids and debris, and for simulating weathering. Procedure A applies to samples of more than 50 ml volume containing significant quantities of hydrocarbons boiling above 280°C. It involves dissolution of the sample in an equal volume of chloroform or dichloromethane and centrifuging to remove water and debris. Simulated weathering involves distilling the sample to 280°C using a nitrogen purge to remove the solvent and volatile hydrocarbons.

Procedure B applies to samples of less than 1 ml of "heavy" oil and uses pentane or hexane as solvents. The extract is dried with anhydrous $MgSO_4$ and volatiles and solvent are stripped in a beaker under a nitrogen stream. Procedure C covers "light" oils containing significant components boiling below 280°C. It only includes separation of oil from the water phase by centrifugation and does not address weathering. Procedure D is for samples containing both petroleum and vegetable-animal derived oils. This procedure includes the separation of petroleum hydrocarbons from the animal-vegetable oils by column chromatography using a silica gel-alumina column.

The USCG oil analysis scheme[105] includes an elaborate oil weathering procedure that simulates natural weathering. Fiberglass troughs with circulating sea water and exposure to the environment are used to weather the suspect oils.

A simulated weathering procedure for minute amounts of oil has been developed[32] by which treatment of 70 mg of an unweathered reference oil yielded a chromatogram virtually the same as that obtained by ASTM Procedure A. The procedure is applicable to a minimum of 0.5 mg of oil and uses a 10 by 30 mm glass vial heated at 40°C for 15 minutes in the presence of an air stream.

Analysis. The analysis of oil samples often involves a multiparameter approach using complementary methods that exploit the complex composition of petroleum. The frequent use of these methods is a reflection of the methods' convenience for general lab use, and correctness in identifying the presence and source of discharge of petroleum oil.

Gas chromatography. The most widely used methods for oil spill identification are based on gas chromatography (GC). Many different columns have been used in an effort to attain resolution of the alkane fingerprint components of oil. Both packed columns using nonpolar liquid phases and various types of capillary columns have been used to effect separations. Flame ionization (FI) and flame photometric (FP) detection are used primarily to measure the eluted components. FP detection using a sulfur specific 394 nm filter produces a profile of the sulfur components present in oil. The combination of a conventional FI detection and the FP detection provides useful fingerprints for identifying oils.

ASTM includes a method (D3328-78) using both packed columns and dual FI/FP detection. Method A (a packed column procedure), uses a 10-foot stainless steel column packed with OV-101 on 60/80 mesh Chromosorb W. Method B (a capillary column procedure) is similar but employs higher resolution support coated open tubular (SCOT) columns. Column effluents from both methods may be detected by FI or split (1:2) between FI and FP detection for sulfur compounds. FP detection can, in certain circumstances, yield chromatograms that distinguish two oils when the FI chromatograms cannot. The matching of samples is based on the premise that identical oils yield identical chromatograms. A detailed discussion of the various features of FI detection oil chromatograms and their use in oil spill identification is provided by Gruenfeld and Frank.[31] FP detection chromatograms have fewer features, but give sulfur profiles which may be used for oil characterization.

The USCG method[105] avoids the use of packed columns and uses both OV-101 and Dexsil-300 SCOT columns. The Dexsil column does not resolve pristane and phytane but does allow operation to high temperatures (350°C) and, therefore, provides additional information on high boiling components from heavy oils. The USCG approach also recommends FI and FP detection for two simultaneous GC profiles.

The use of another high resolution GC column has been recommended[43] for increasing the separations of oil components. This GC fingerprinting technique used a column of lithium chloride supported on diatomaceous silica. The advantages of inorganic packings are high resolution and thermal stability, and no column bleed, thereby permitting the separation of higher boiling oil components.

Fluorescence spectroscopy. Numerous fluorescence spectroscopic techniques using several approaches for obtaining oil fingerprint profiles have been reported. Frank[40] reviewed many of the fluorescence methods employed for oil identification, and a means of comparing fluorescence methods was described earlier.[37,39] Using this mode, the various fluorescence methods are compared and contrasted within the context of a three-dimensional system. This system uses the three interdependent variables inherent to fluorescence spectroscopy: excitation wavelength (x), emission wavelength (y) and fluorescence intensity (z). Within the scope of this system, the fluorescence characteristics of petroleum oils are presented as "total fluorescence spectra," and the spectral information obtained by each technique is described as an appropriate portion of such spectra. Figure 1 shows a hypothetical three-dimensional total oil spectrum (illustrated as mountains by solid lines), and the spectral information obtained by several methods.

The more common approach involves the derivation of emission spectra and use of the spectral profiles as fingerprints. Emission spectra are obtained by fixing a fluorescence spectrometer's excitation monochromator at a selected wavelength and scanning the emission monochromator. In the three-dimensional system in Figure 1, emission spectra (dotted line) represent slices of total fluorescence spectra that are parallel to the y axis. By varying the fixed excitation wavelength, different spectral slices are obtained that vary in their position along the x axis.

Both ASTM Method D3650-78 and the USCG[105] fluorescence methods are based on the emission spectra approach using an excitation wavelength of 254 nm. Thruston and Knight[104] initiated the use of emission spectra for fingerprinting oils, and used an excitation wavelength of 340 nm. Coakley[24] developed a similar method using excitation wavelengths from 280-300 nm. Goldberg and Devonald[47] compared the diagnostic value of various excitation wavelengths (200, 250, 300 and 350 nm), and demonstrated that the 250 nm wavelength used in the ASTM and USCG methods produces the most definitive spectral fingerprints for identifying oils.

The use of synchronously excited spectra for fingerprinting oils has been advocated.[64,75] In this procedure, oil spectra are obtained by scanning both the excitation and emission monochromators in synchronization, and separated by a wavelength interval. In the three-dimensional system, synchronously excited spectra also represent slices of total fluorescence spectra but are oriented at a 45° angle to the x and y axes (dashed line in Figure 1). Synchronously excited

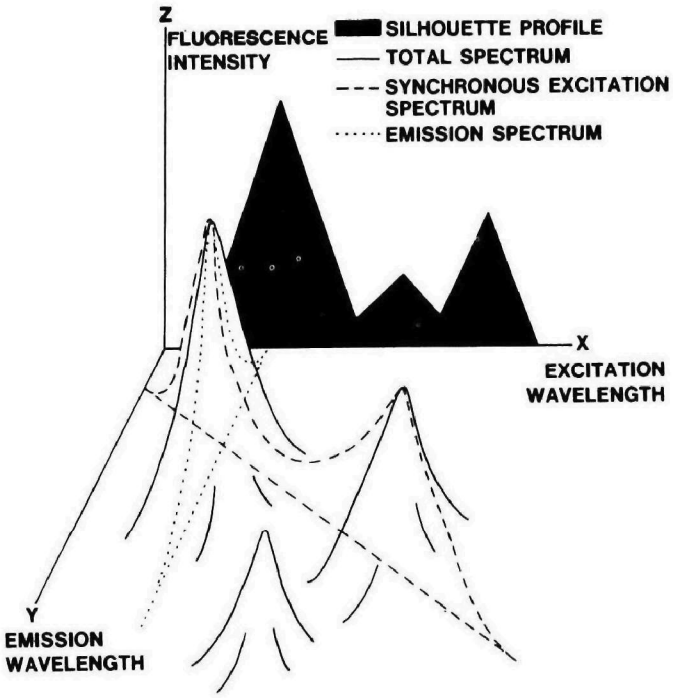

Figure 1. Comparison of emission spectrum, synchronously excited spectrum, and silhouette profile within the context of a total oil spectrum—The emission spectrum includes only one maximum, and the synchronously excited spectrum includes two maxima. The silhouette profile includes all three maxima and represents the "shadow" of the total spectrum.

spectra have been demonstrated[75] to usually contain more spectral information than emission spectra and, in many instances, to contain the same informational content as several emission spectra combined.

The more complex approach of obtaining fluorescence "contour maps" of oils has been described[44,59] as the ultimate in fingerprinting by fluorescence. In this method, a large number of emission spectra derived for each oil undergo computer analysis for conversion into maps. Within the context of the three-dimensional system, contour maps represent total oil spectra reduced to the two-dimensional (x, y) plane (Figure 2). Relative fluorescence intensities within the maps are indicated with concentric lines. Although a maximum amount of spectral information is obtained by this approach, the possible insufficiency of contour maps for fingerprinting weathered oils has been discussed.[48]

A simpler approach for obtaining maximum spectral information from an oil involves the derivation of silhouette profiles of total oil spectra.[37,49,99] This procedure can be performed manually with a routine laboratory spectrometer in a relatively short time. Silhouette profiles of oils are obtained by plotting the maximum fluorescence intensities of oil emission spectra versus excitation wavelengths and connecting the plotted points with straight lines. Figure 1 shows that silhouette profiles represent the shadows of total oil spectra as projected into the x, y plane of the three-dimensional system. The utility of silhouette plotting for discriminating between crude and refined petroleum oils has been shown[37] and weathered oils successfully correlated with unweathered oils. Also demonstrated[99] was the method's utility in identifying weathered lubricating oils in waste treatment effluents, surface waters, and marine organisms.

In addition to these primary methods, several secondary approaches have been employed to increase fingerprinting capabilities. Some analysts[104] have advocated the use of excitation spectra, in addition to emission spectra, as oil fingerprints. Within the three-dimensional system, excitation spectra are the perpendicular counterpart to emission spectra; that is, they are slices of total oil spectra parallel to the x axis (emission spectral slices are parallel to the y axis).

Several analysts advocate a multi-parameter fingerprinting procedure deriving emission and excitation spectra of simple compound groups chromatographically isolated from oils. Drushel and Sommers[28] isolated simple multicomponent fractons by GC and identified fluorescing oil components by comparing emission and excitation spectra of standards and oil fractions. Correlations of oils was performed by comparing the type of compounds identified. McKay and Latham[84] used a similar procedure substituting a complex compound isolation scheme of ion-exchange, gel permeation and thin layer chromatography. Lloyd[76] used gradient elution chromatography and a combination of emission, excitation and synchronously excited spectra to identify individual oil components. High pressure liquid chromatography (HPLC) and emission and excitation spectra have been used to identify polynuclear aromatic oil compounds for use as fingerprinting indices.

Studies on the use of cryogenic temperatures to enhance details in the spectral fingerprints of oils have been conducted by several analysts. Procedures for obtaining "cryogenic temperature spectra" involve the dissolution of oils in selected liquids and cooling the solutions to 77°K with liquid nitrogen. The liquids used are limited to those that form a transparent solid (glass) at that temperature. Drushel and Sommers[28] used mixtures of methylcyclopentane-methylcyclohexane and ether-isopentane-ethyl alcohol (EPA) to prepare glasses at 77°K. Freegarde *et al.*[44] also used EPA for cryogenic fluorescence analysis of oils. Hornig and Eastwood[57] evaluated several solvents for the preparation of solid media and concluded that methylcyclohexane yielded an optimum glass. They also compared fluorescence spectra of crude and refined oils in both solid and liquid methylcyclohexane, demonstrating dramatic improvements in the resolution of spectra derived from the solid medium.

Infrared spectroscopy. Methods measuring the 4000-250 cm^{-1} region frequently have been used for fingerprinting oils. The most useful IR region for fingerprinting oils is the 1500-500 cm^{-1} range, termed the "IR fingerprint region." IR spectra are compared by visual comparison or by comparison of IR absorbance ratios. ASTM Method D3414-75T, for IR analysis of waterborne oils, involves recording spectra between 4000 and 667 cm^{-1}. The spectra of the sample and the reference oils are compared visually or by calculating the ratios of the intensities of a number of bands, such as 810/720 cm^{-1}, and 1600/1375 cm^{-1}.

The USCG uses a similar approach, primarily using an overlay procedure to visually compare spectra. The USCG procedure also includes a very detailed IR procedure for routine use by relatively untrained personnel. Oil weathering effects and possible interferences incurred from sample collection and preparation procedures are thoroughly discussed.

The use of IR spectroscopy for oil spill identification has been developed to a high degree of sophistication using computer and statistical data treatment. Statistical means of evaluating and comparing IR data,[67,81] and several computerized procedures for identifying oils by IR spectra have been reported.[15,23]

Other methods. A number of other methods for fingerprinting oils are used, and may provide useful fingerprints in many instances.

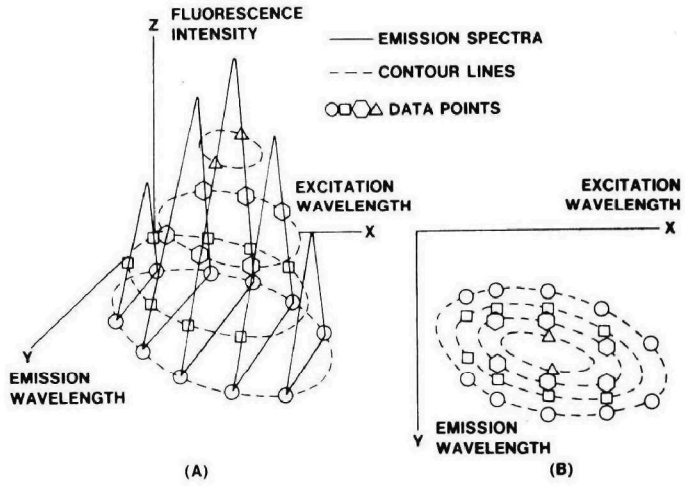

Figure 2. Three dimensional presentation of the derivation of a contour map of an oil having an inverted-cone-shaped total spectrum—The data points used for plotting the map B are obtained from five emission spectra in A.

ASTM Method D3327-74T includes the determination of nitrogen, sulfur and nickel/vanadium contents of oils as fingerprint indices. Nitrogen is determined by the Kjeldahl procedure and sulfur by X-ray spectroscopy or, alternatively, by a high temperature combustion method. The nickel/vanadium content is obtained by either X-ray or atomic absorption spectroscopy.

Other methods[10] include the use of isotope mass spectrometry for determining carbon and sulfur isotope ratios for fingerprinting oils. Weiss[113] and Bentz[9] reviewed many methods, including mass spectrometric, nuclear magnetic resonance (NMR) spectroscopic, and ultraviolet spectroscopic methods, used for oil spill identification.

Quantification of water-dispersed petroleum

The accurate measurement of oil in water, sediment, or tissue necessarily involves a definition of the term "oil," particularly because different quantitative methods measure different sample constituents (that is, alkanes vs aromatics). A broad definition is that oil consists of crude petroleum or its liquid derivatives used as fuels and lubricants. Each analytical technique, however, expands or restricts this definition and establishes an "operational definition" of its own.

The primary methods now available for quantitating oil dispersed in water are classified as gravimetric or spectroscopic techniques. All of these techniques include solvent extraction procedures, followed by the appropriate determinative measurement. In gravimetric techniques, this measurement consists of weighing the extracted residues. In spectroscopic techniques, measurements are performed using either IR, UV or fluorescence spectroscopy. Some methods also incorporate an interference removal procedure for separating petroleum compounds from other non-petroleum organics, thereby increasing the specificity for petroleum hydrocarbons.

The use of appropriate standards is mandatory in all but the gravimetric techniques. Sample measurements usually are related to a standard of a single known concentration or to standards spanning the concentration range of the samples. The choice of standards critically affects the accuracy of results and, in an ideal situation, the standard is identical to the oil being monitored.

Spectroscopic methods. IR spectroscopy is the most commonly used spectroscopic technique for quantitating water-dispersed oils. In an American Petroleum Institute procedure,[2] dispersed oils are extracted from water with carbon tetrachloride (CCl_4). The absorption maxima of oil at 2850 and 2930 cm^{-1} is then measured using 10 mm path length quartz cells. Oil concentrations are determined by comparing the sum of these absorbances to the sum of absorbances derived from a prepared CCl_4 solution containing an accurately known concentration of the oil. In this method, a blend of hydrocarbons (37.5% isooctane, 37.5% cetane, and 25% benzene) is used to approximate the IR absorptivity of an average petroleum oil. This standard solution was used when a portion of the dispersed oil was not available. This hydrocarbon blend also has been used to represent "typical oils."[6]

In an IR method by Beckman Instruments, Inc., 100 ml of dispersed oil in water samples are extracted with two ml of CCl_4. The absorption maxima at 2930 cm^{-1} is then measured using 10 mm path length near IR silica cells. The 2850 cm^{-1} band was not used. Reference solutions are prepared and similarly measured as oil-in-water dispersions of known oil content. A concentration vs absorbance plot is then derived for quantitating dispersed oil in water samples.

The two preceding methods use the same extraction solvent, but differ in most other respects. The American Petroleum Institute method uses a large oil extraction flask and salt and acid are added. Extraction is performed with successive 100 ml portions of CCl_4 and two IR absorption band maxima are then measured. Sample comparison is made to only a single standard solution. The Beckman method uses a small separatory funnel and one two ml portion of CCl_4 to extract a 100 ml sample volume. Acid or salt are not added, and one IR absorption band maximum is measured. A concentration vs absorbance plot derived from several oil in water reference solutions is used for sample determination.

The influence of salt and acid has been discussed elsewhere by one of the authors,[10] who also compared the extraction efficiencies of CCl_4 and Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane). Freon 113 can also be used for IR measurement of oil at 2930 cm^{-1}. One liter of dispersed oil in water samples was extracted with four consecutive 25 ml portions of solvent, using two-liter separatory funnels. Use of Freon 113 is of special interest, because it is less toxic than CCl_4. According to other analysts,[93] CCl_4 is highly toxic (10 ppm TLV) when inhaled or absorbed through the skin, while Freon 113 is much safer (1,000 ppm TLV).

A study of the accuracy and sensitivity of the IR technique when used for the quantitative determination of petroleum oils by single point analysis has been reported.[10] Carbon tetrachloride, Freon 113, and a mixture of these solvents were used. The detection limits of oils by IR, the stability of oil absorptivities during prolonged solution storage, and the utility of these absorptivities for oil identification were also examined.

In an attempt to distinguish petroleum oils from other extractable organics, several analysts have combined chromatographic adsorption techniques with IR measurements. Silica gel adsorption chromatography has been used to separate petroleum hydrocarbons from non-petroleum organics.[18,63,115] Others developed a rapid silica gel adsorption technique which can be easily used for routine analysis.[52,77] In their procedure, three g of activated silica gel are added to the solvent extract and the extracts are stirred for 10 minutes with a magnetic stirrer.

The IR method has now been incorporated into the APHA[3] and EPA[106] procedures. These two methods are essentially the same. Either soxhlet or solvent extractions with Freon are used. ASTM[4] also includes the rapid silica gel adsorption procedure mentioned above.[52,77]

Fluorescence spectroscopy is also frequently used for quantifying oil in water, sediment and tissue. Although more sensitive than IR spectroscopy, fluorescence spectroscopy measures primarily the aromatic constituents of oil. Hornig[58] discussed some advantages of using fluorescence-based methods, noting that these methods are especially useful when the concentration of residual oils in water is less than 10 ppm. Table 1 summarizes the numerous fluorescence approaches that have been used for measuring oil in extracts of water, sediment or tissue.

The oil concentration in the extract is determined by excitation at one of several wavelengths, measurement of the fluorescence intensity at a certain wavelength, and relation of the sample's intensity to the intensity of a reference oil. In a rapid procedure described elsewhere,[16] oil is measured in the water phase after the addition of 2-propanol (which acts as an intermediary solvent between water and oil). By use of the synchronous excitation approach for quantifying oil in water, Rayleigh-Tyndall and Raman radiation interferences may be totally avoided.[38,42] This resulted in increased sensitivity and shorter analysis time.

UV spectroscopy has been sparingly used for quantitating oil in water. Several UV methods have been reported, measuring UV absorption in the 260 or 262 nm regions.[53,88,90] However, some analysts noted that measurements of oil in the 260 nm region can be confused with elemental sulfur.[71]

Gravimetric methods. These methods include solvent extraction, solvent evaporation and gravimetric measurement of the residues. In one technique, 100 ml of benzene is used to extract 2.5 liter portions of water. The benzene then is evaporated and oil residues dried to constant weight. Another also uses benzene as the extraction solvent, but sodium chloride is added to the sample to decrease emulsion problems. That technique was valid for petroleum with boiling points of 140°C or greater. The APHA and EPA standard methods compendia also include gravimetric methods. These are essentially the same, using Freon as the extraction solvent instead of the more toxic benzene.

Other methods. Methods based on chromatography (GLC or HPLC) have infrequently been used for quantifying oil in water. A GLC procedure employing FI detection has been reported in which the results were quantified in terms of individual oil components.[42] GLC has been compared to IR spectroscopy for the quantification of oil in water and proved more suitable because it yielded data about individual oil compounds.[93] Freon has been used as an extraction solvent and samples analyzed by HPLC.[120] Quantitative values however, were given as phenanthrene equivalents. Other analysts recommended the use of total organic carbon (TOC) analysis for quantitating oil in water.[78]

Analysis of petroleum fractions and hydrocarbons

The complex composition of petroleum and its environmental fate often preclude obtaining distinct oil chromatograms, spectra, and so

Table 1. Fluorescence spectroscopic methods for the quantification of oil

Author	Sample medium, extraction solvent	Excitation wavelength (nm)	Emission wavelength at which measurement is taken (nm)
Zitko and Carson[117]	Tissue, hexane	Varies with oil type	Varies with oil type
Zitko[114]	Tissue, hexane	300	360
Levy[74]	Water, CCl_4—evap. residue in hexane	310	360
Zitko and Tibbo[119]	Tissue, hexane	280	310
Cretney and Wong[23]	Water, CH_2Cl_2—evap. residue in cyclohexane	308	383
Keizer and Gordon[48]	Water, CH_2Cl_2—evap. residue in hexane	310	374
Frank[36]	Water, direct in-situ measurements (addition of 2-Propanol)	290	340
Gordon and Keizer[48]	Water, hexane or or CH_2Cl_2—evap. residue in hexane	310	374
Gordon *et al.*[49]	Water, CH_2Cl_2—residue in hexane	310	374
Hargrave and Phillips[54]	Sediment, hexane or CH_2Cl_2—evap. residue in hexane	310, Fluorescence contour maps also used	370
Tanacredi[99]	Water, CCl_4—evap. residue in hexane	290, Fluorescence silhouette profiles also used	Emission maximum between 300-540 nm
Gordon *et al.*[49]	Water, pentane	310, Fluorescence silhouette profiles and synchronous excitation also used	374
Frank[41]	Sediment, 2-propanol/ cyclohexane (60:40 v/v)	Synchronous excitation	Varies with oil type

on. Consequently, fractions of the petroleum are often analyzed as a means of detecting oil pollution. The most common approach has been to isolate, identify and quantitate the aliphatic and aromatic fractions ascribing their presence and composition to oil pollution. These analyses are often complicated to interpret in areas previously or chronically contaminated.

The water soluble polynuclear aromatic hydrocarbons are often analyzed.[13,43] This approach of monitoring the aromatic fraction (PAH) has been used by numerous investigators examining tissues[5,21,30,91,100] and sediments.[80] However, caution has been advised in directly ascribing PAH presence to oil pollution.[34,56] Aliphatic fractions are also analyzed to determine the presence of oil pollution[26,79] and some analysts examine both aromatic and alkane fractions.[20,69,103]

Further complicating the analysis of environmental samples (sediments, tissues, water) for oil pollution, is the problem of background contamination in areas near pollution sources, other contaminants, and the natural occurrence of recent biogenic hydrocarbons. Several criteria are frequently used for identifying the presence of oil pollution in a complex matrix.[35,51,103]

The identification of petroleum and fractions in sediments, tissues and water after an acute or chronic event, often employs similar approaches. Analysis includes sample extraction, treatment of the extract to remove or decrease background interferences, concentration and instrumental analysis. Multimethod approaches are frequently used analyzing both alkane and aromatic fractions. Most methods employ internal standards or recovery data, and results are often reported at the μg/kg level. Aspects of sample extraction and cleanup of extracts are subsequently addressed.

Sediment extraction. A common problem in analyzing sediments for petroleum oil and fractions is the extraction of oil from the complex sediment matrix. Several reports have addressed aspects of these problems, their background and comparative sediment extraction methods.[17,32,35,55,73] A multimethod approach was commonly used. The methods can be categorized as: (1) a caustic chemical digestion process to remove recent biogenic hydrocarbons by saponification, etc., (2) soxhlet extraction, (3) physical agitation-solvent extraction, (4) headspace sampling, or (5) refluxing.

Caustic chemical digestion methods have employed various solvents and either KOH[12,14,29,32,73] or NaOH.[73] Soxhlet extraction procedures predate digestion procedures and have also been used for sediment analysis.[12,17,32,109] Extraction into methanol or benzene-methanol was the most common method.

Various forms of physical extraction-solvent extraction have been reported. Disruption and extraction of wet sediments in solvent has been accomplished using a homogenizer[98] or ultrasonification.[103] Other methods have used variations of a tumbling procedure in which sediments are placed in a container with solvent and tumbled.[17,73,80,110,111,112]

A headspace sampling procedure has been used for analysis of the volatile components in the sediments.[82,114] However, these procedures used additional methods to obtain a total sample.[114] Others concluded that both solvent extraction and headspace methods yielded essentially the same value for low levels of hydrocarbons but analyzed different aspects of the oil (aromatics vs aliphatic).

A simple reflux procedure has been discussed which uses methylene chloride and distilled water while stirring with a magnetic stirrer.[73] Results were considered good for PAH fractions and almost comparable to the digestion procedures.

Tissue extraction. Extraction and isolation of petroleum hydrocarbons from tissues is complicated by the biochemical content of the tissue. The petroleum hydrocarbons may reside within the extra or intracellular matrix or be bound to membranes. Complete analysis must, therefore, ensure complete extraction of the organics from the tissues. Extraction procedures are often coupled with a subsequent procedure to decrease the levels of coextracted biotic hydrocarbons. Aspects of these problems were addressed by Farrington[35] and Pancirov *et al.*[92]

Extraction procedures are based on either caustic digestion, soxhlet extraction or physical disruption-solvent extraction. The most commonly used caustic in digestion procedures has been KOH with various solvents.[14,29,72,87,91,96,102] Other analysts also have used digestion approaches but substituted NaOH instead.[22,80,111,112] The efficiency of these procedures is often enhanced if the tissue is prehomogenized. Soxhlet extraction procedures also have been used, employing various solvents and extraction times.[20,31,112,121]

Other procedures of extracting petroleum hydrocarbons from tissues have used a physical disruption of tissues—solvent extraction approach. A method for naphthalenes and alkylnaphthalenes[89] simply involved homogenizing the tissue in hexane, centrifuging and collecting the hexane supernatant for sample cleanup. A similar procedure was used to analyze clam tissues chronically oiled with Bunker C.[46]

Extract cleanup. Extraction of petroleum hydrocarbons from environmental samples invariably results in coextraction of recent biotic hydrocarbons and contaminating organics. These background hydrocarbons are often removed prior to instrumental analysis. There are several approaches used to cleanup the extract including column chromatography using either alumina, silica gel, florisil or combinations, TLC, solvent partitioning, removal of sulfur, or filtering the sample through specific media.

Column chromatography is the most common procedure. These procedures employ packed columns onto which the concentrated sample is charged, followed by multiple solvent elution to collect various petroleum derived fractions for analysis. Columns have been packed with silica gel,[17,73,80] alumina,[14,90,104] or more commonly with alumina over silica gel.[4,63,87,101,116,121] Sample preparation also has been achieved by merely adding small amounts of silica gel to sample extracts and stirring briefly.[52,97] Other analysts have packed columns with florisil for sample cleanup.[7,29,89]

Other procedures have employed TLC,[14,103,108] solvent partitioning,[7,29] preconcentrating devices,[22,55,88] or filtration media.[69,78]

Extraction of large amounts of sulfur is a common problem in the analysis of sediments. The sulfur compounds will interfere with several types of instrumental analysis and, consequently, are often removed. The most frequent procedure is to percolate the extract through a column of activated copper which removes the sulfur[95,116] or to include the copper as part of a multilayered column.[83]

Ultimately, the sample extract cleanup procedure chosen should enable the petroleum hydrocarbons or fractions to be identified and

quantitated with the least interference from background contaminants.

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A Review of Fluorescence Spectroscopic Methods for Oil Spill Source Identification

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A review and evaluation of analytical techniques and methods for oil spill source identification by fluorescence spectroscopy is presented. The subject is discussed within the context of a three dimensional system which permits the reader to readily visualize and comprehend the many diverse approaches incorporated into the reviewed methods. The three dimensional system utilizes the three interdependent variables inherent to fluorescence spectroscopy, i.e., excitation wavelength, emission wavelength and fluorescence intensity, and portrays the fluorescence characteristics of petroleum oils as "total fluorescence spectra" that are analogous to topographic maps of mountainous regions. Spectral information, derived by each of the methods, is discussed in terms of appropriate portions of such total spectra.

The methods are categorized with respect to the approach used and are evaluated on the basis of five criteria. These criteria address pertinent factors such as diagnostic ability, weathering, quenching, scattering and solvent effects and their influence on a method's utility for oil spill source identification. Conclusions are presented describing optimum techniques.

INTRODUCTION

Increased activity in the prevention and abatement of petroleum pollution has culminated in numerous and diverse analytical methods capable of establishing the identity of the source or origin of an oil spill. The term "Fingerprinting" is used to describe this type of methodology and involves techniques whereby environmentally altered (weathered) petroleum oil, found as waterborne surface slicks and shoreline residues, are matched with virgin oils obtained from suspect sources. The magnitude and variety of publications currently available on this topic have made the selection of appropriate methods difficult and, at best, time consuming. As a consequence, several review papers[1,3,4] have been published on fingerprinting

techniques. These reviews however, do not adequately address fingerprinting techniques based on fluorescence spectroscopy and cite few of the available methods. A state-of-the-art review of methods for fingerprinting oils by fluorescence spectroscopy is therefore presented to compensate for this lack.

Photoluminescence includes both fluorescence and phosphorescence, and is defined as the radiation emitted by a molecule, ion, or atom, that has been excited by the absorption of ultraviolet or visible radiation. The emitted radiation is characteristic of the electronic structure of the emitting substance and serves as the foundation for the analytical utility of luminescence. The two forms of luminescence, are of different analytical importance and are distinguished by observing the lifetimes of their excited states: 10^{-10} to 10^{-7} seconds for fluorescence, and 10^{-3} to 10 seconds for phosphorescence. Fluorescence techniques are currently more useful than phosphorescence techniques for fingerprinting petroleum oils and are therefore exclusively reviewed.

A novel mode of presentation that incorporates a three dimensional system of mutually orthogonal coordinate (cartesian coordinate) axes is used in this paper to illustrate petroleum fluorescence and the appropriate fingerprinting methods. This three dimensional system permits the reader to readily visualize and compare the seemingly wide variety of fluorescence methods and compare basic similarities and subtle differences in the approaches used for performing fluorescence measurements. Petroleum oil fluorescence is characterized within the context of this system in the form of "total fluorescence spectra". Figure 1, demonstrates this concept and illustrates a total spectrum of an oil with three fluorescence maxima. The three interdependent parameters inherent to fluorescence spectroscopy (excitation wavelengths, emission wavelengths, and fluorescence intensity) are simultaneously incorporated in these spectra and are represented by the x, y and z axes, respectively. Total spectra of different oils exhibit different maxima and generally resemble topographical maps of mountainous regions.

The reviewed methods are divided into categories which serve to differentiate the methods on the basis of the approach used for performing fluorescence measurements and to highlight basic method differences and similarities. The categories correspond to approaches which derive the following spectral data: (1) excitation spectra, (2) emission spectra, (3) synchronously excited spectra, (4) contour maps, and (5) silhouette profiles. An additional category is used to describe techniques which combine chromatographic procedures with determinations of the cited spectral data.

The effectiveness of the methods for fingerprinting petroleum oils are

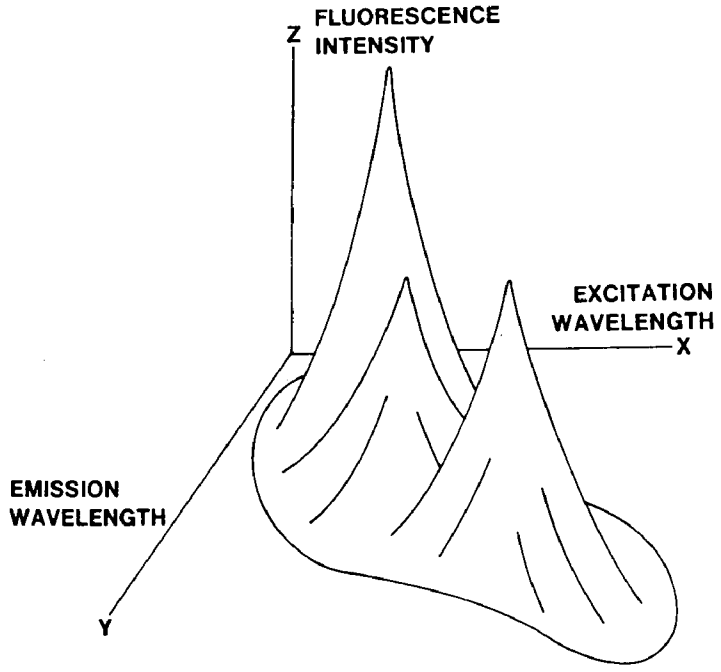

FIGURE 1 Three dimensional presentation of the total fluorescence spectrum of an oil with three maxima represented as mountain peaks.

evaluated on the basis of the five criteria described in Table 1. Compliance with these criteria affirms a method's utility for matching weathered, spill derived, petroleum residues with oils from suspect sources.

Solvent media used for the fluorescence analysis of oils are also examined and, because they perform an important function in all methods, are discussed first.

DISCUSSION

Solvent media

Both liquids and solids have been used as dilution media for reducing the effects of quenching phenomena encountered in the fluorescence analysis of oils. Because quenching impairs spectral resolution and distorts fluorescence spectra, the proper selection of solvent media is an essential function. The criteria used for media selection are primarily based on medium-oil interactions, the media's fluorescence characteristics, solvent properties, and ease of application. Solvents that absorb in the 200–

TABLE I

Criteria used for contrasting the effectiveness of the reviewed techniques for fingerprinting petroleum oils.

Criterion 1—*Ability to discriminate between oils.*

A technique's effectiveness for measuring those parameters that are unique to each oil is thereby evaluated. Aromatic and polynuclear aromatic constituents of oils primarily impart the characteristic fingerprint to oil fluorescence spectra and are therefore the parameters of concern.

Criterion 2—*Ability to correctly match environmentally weathered oils with corresponding unaltered (source) oils.*

Weathering effects—evaporation, dissolution, photodecomposition, oxidation, and biodegradation—incurred by spilled oils and their influence on a technique's fingerprinting ability are thereby evaluated. Evaporation is the main concern in the fluorescence measurement of oils and is responsible for a concentration phenomenon that involves a build-up of principal fluorescing compounds (e.g. polynuclear aromatic compounds) in weathered oil matrices relative to corresponding unweathered source oils.

Criterion 3—*Ability to cope with quenching phenomena.*

This criterion evaluates a technique's ability to circumvent quenching effects. Fluorescence quenching entails any process resulting in a decrease in the true fluorescence efficiency of a compound and is responsible for a shift of emission maxima to longer wavelengths. For oils the most common problem is concentration quenching, characterized by the formation of combinations of excited molecules and molecules in their ground state (excimers).

Criterion 4—*Ability to resolve interferences caused by radiation scattering.*

A technique's ability to avoid the effects of Rayleigh–Tyndall and raman scatter is thereby evaluated. Rayleigh–Tyndall scatter emanates from reflections of excitation radiation and produces intense and readily discernible peaks in emission spectra at wavelengths corresponding to the applied excitation wavelengths. Raman scatter results from an interaction of the excitation radiation with solvent molecules and appears in emission spectra at wavelengths characteristic of the solvent used.

Criterion 5—*Ability to be expediently and routinely applicable.*

This criterion evaluates a technique's utility in oil spill incidents where immediate analytical evidence is mandatory for establishing liability and for enforcement of legislation. Complex and time consuming techniques are of very limited utility.

400 nm region, or deteriorate when subjected to ultraviolet radiation are usually excluded in fluorescence analysis.

Aliphatic hydrocarbon liquids such as cyclohexane, methylcyclohexane, hexane and pentane are the most widely used solvent media for obtaining fluorescence spectra of oils. They are relatively inert, non

fluorescent, solubilize most oils and can be routinely applied. Fluorescent impurities in solvents obtained from commercial sources often present a problem, but extra pure "fluorometric grade" solvents are available or can be readily prepared. Frank and Jeleniewski[10] have demonstrated that cyclohexane of sufficient purity can be prepared by simple distillation.

In addition to the aliphatic hydrocarbons, other liquids have been used and were shown to be superior media for measuring oil fluorescence. Sawicki[27] demonstrated that high viscosity solvents tend to retard the intramolecular twisting of polynuclear-aromatic petroleum components and encourage their fluorescence. Frank[9] demonstrated an increase in the resolution of fluorescence spectra derived from oils in a solvent (dipropylene glycol) 20 times more viscous at room temperature than cyclohexane.

Solid media in the form of frozen liquids have also been used for obtaining fluorescence spectra of oils. Procedures for preparing such media involve the dissolution of oils in selected liquids and cooling the solutions to 77°K (cryogenic temperatures) with liquid nitrogen. The liquids utilized are limited to those that form a transparent solid or "glass" at that temperature. Drushel and Sommers[6] used mixtures of methylcyclopentane-methylcyclohexane, and ether-isopentane-ethyl alcohol (EPA) to prepare clear, transparent glasses at 77°K. Freegarde *et al.*[11] also used EPA for the cryogenic fluorescence analysis of oils. Hornig and Eastwood[17] evaluated several solvents for the preparation of solid media and concluded that methylcyclohexane results in the formation of an "optimum glass". The last authors also compared fluorescence spectra of crude and refined oils in both solid and liquid methylcyclohexane and demonstrated dramatic improvements in the resolution of spectra derived from the solid medium.

Excitation spectra

Excitation spectra of oils are obtained by exciting oil solutions with continuously varying wavelengths of ultraviolet radiation and recording the fluorescence intensity profiles of the emitted radiation. Instrumental procedures for obtaining such spectra require two basic steps: (1) setting a fluorescence spectrometer's emission monochromator at a constant wavelength and (2) scanning the excitation monochromator. The spectra's intensity profiles are often unique and can be used as fingerprints for the comparison of oils.

Within the concept of the three dimensional system, excitation spectra of oils represent slices taken from total fluorescence spectra that are parallel to the x axis. Figure 2 illustrates three such spectra as slices taken through the total fluorescence spectrum of Figure 1. The slices are a

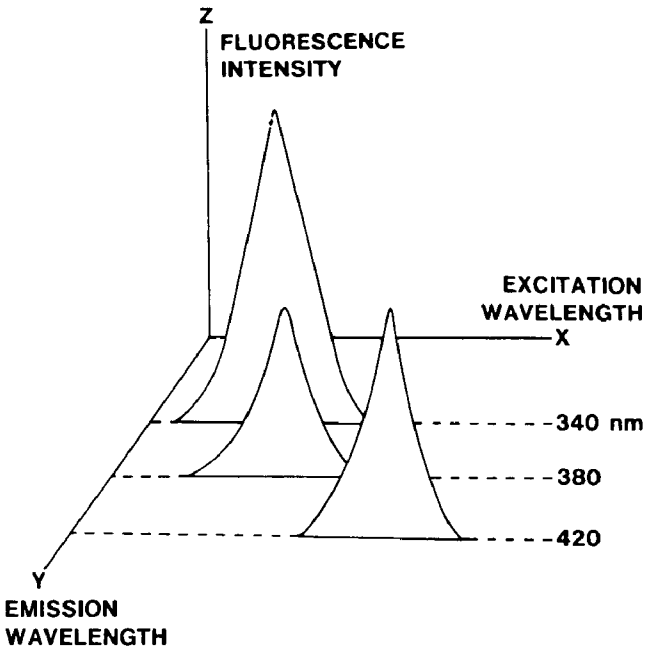

FIGURE 2 Three dimensional presentation of excitation spectra. The spectra represent slices taken at specific emission wavelengths through the total spectrum in Figure 1.

function of x (excitation wavelength) and z (fluorescence intensity) with y (emission wavelength) constant.

The excitation spectra approach has not been used for developing any major methods. However, it has been used in conjunction with other approaches and some authors[19,29] have advocated its potential utility as an ancillary approach for expanding the discriminating ability of other fluorescence methods.

Emission spectra

Emission spectra are derived by exciting oil solutions with a single selected wavelength of ultraviolet radiation and recording the spectral profiles of the emitted fluorescence radiation. This is accomplished by fixing a spectrometer's excitation monochromator at a selected wavelength and scanning the emission monochromator. Spectral envelopes of emission spectra obtained by this procedure also serve as fingerprints for the comparison of oils.

In the three dimensional system, emission spectra of oils represent slices taken from a total fluorescence spectrum and are analogous in this respect to excitation spectra. These slices however, are a function of y (emission

wavelength) and z (fluorescence intensity) with x (excitation wavelength) constant and are parallel to the y axis. Figure 3 illustrates three emission

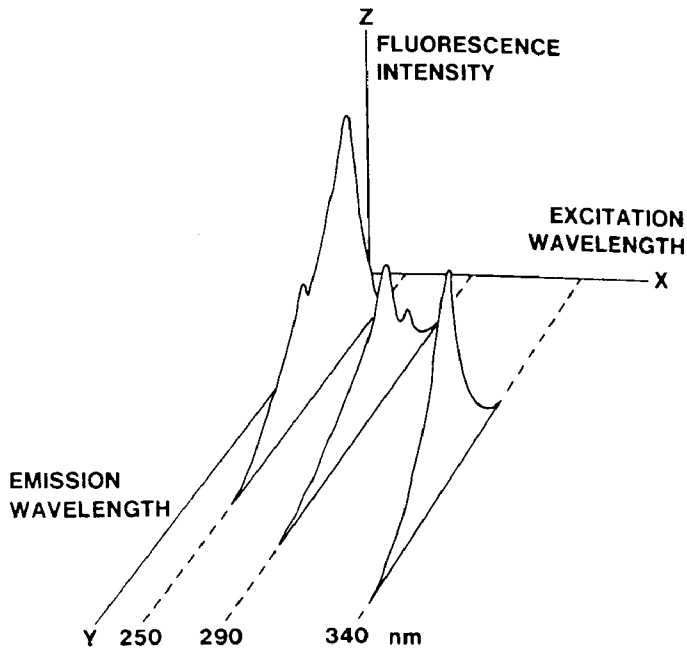

FIGURE 3 Three dimensional presentation of emission spectra. The spectra represent slices taken at specific excitation wavelengths through the total spectrum in Figure 1.

spectra presented as slices of the three dimensional total fluorescence spectrum of Figure 1. Each value of x represents an excitation wavelength and generates a unique slice (emission spectrum).

Thruston and Knight[20] initiated the use of emission spectra for fingerprinting petroleum oils and used 340 nm as the excitation wavelength: this represents the slice where x equals 340 nm in the three dimensional system in Figure 3. The authors developed a method based on the use of emission spectra derived from oils dissolved in cyclohexane at concentration levels of 10, 50 and 100 mg/l. Ratios of the spectra's fluorescence intensities at 386 and 440 nm served as fingerprinting indices for the comparison of pollution and reference oils. The method's differentiation capabilities were demonstrated with indices of several crude and residual fuel oils.

Coakley[5] developed a similar method, also based on the single wavelength excitation approach. Instead of exciting all oils at a constant

wavelength, this author used wavelengths that produce maximum fluorescence intensities in excitation spectra of oils. The excitation wavelength for each oil was determined by manually scanning the excitation monochromator until a maximum emission response was achieved. The wavelength that corresponded to this emission maximum then served as the excitation wavelength for the oil being fingerprinted.

Coakley used only a single concentration of 10 mg/l for oil solutions and correlated emission spectra by visual comparison of their profiles instead of intensity ratios. He demonstrated the correlation of three pollution samples to three source oils, in two separate oil spill incidents: the excitation wavelengths used for these oils were 295 and 314 nm.

Freegarde *et al.*[11] proposed the use of a 250 nm wavelength for exciting oils, which in the three dimensional system in figure 3, yields the slice where x equals 250 nm. These authors, however, presented only emission spectra of four crude oils and did not develop a specific method for fingerprinting oils.

Jadamec and Porro[19] employed a similar wavelength and developed a fingerprinting method based on an excitation wavelength of 254 nm. They used solution concentrations of 10 and 100 mg/l, and also correlated oils by visual comparison of spectral profiles. They obtained emission spectra of a large number of crude and refined petroleum oils and demonstrated their method's fingerprinting ability in eight oil spill incidents.

Of the excitation wavelengths described thus far, it has been demonstrated that the lower wavelengths, such as 250 nm, are superior in discriminating power to wavelengths in the 280–350 nm range. Jadamec and Porro[19] have shown that 254 nm produces a greater variability than 290 nm in oil emission spectra. Similarly, Gordon and Keizer[13] demonstrated that excitation wavelengths in the 300 nm range produce only slight variations in the emission spectra of ten oils examined. Goldberg and Devonald[12] compared excitation wavelengths of 200, 250, 300 and 350 nm and confirmed the diagnostic superiority of the 250 nm wavelength.

Although a solution concentration of 10 mg/l is used in all of the single excitation wavelength methods, several authors suggested the use of additional higher concentrations (e.g., 50 and 100 mg/l) for utilizing the maxima shifts, that accompany concentration quenching of oils, as identification parameters. These higher concentrations however, are not compatible with weathered oils because quenching and weathering effects produce similar spectral distortions.

Interferences caused by Rayleigh-Tyndall and raman scattering, upon excitation at 290–340 nm, may significantly distort emission spectra as is illustrated in figure 4, for the case of a crude oil. The difference between

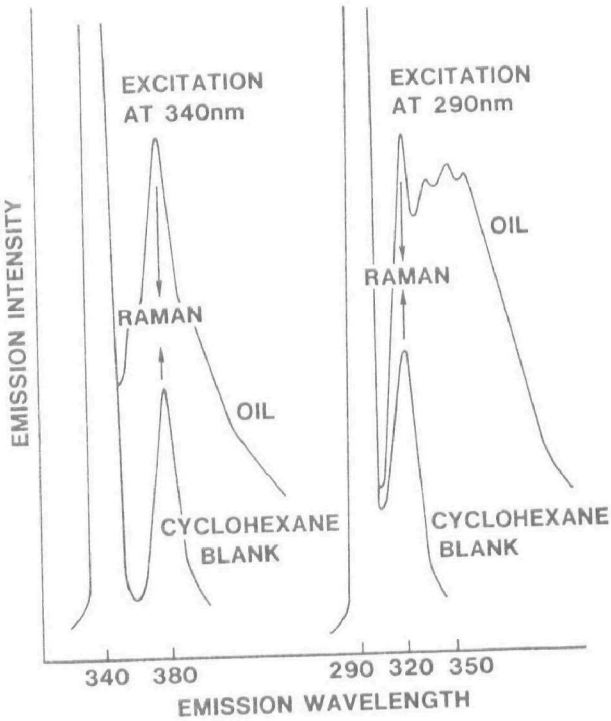

FIGURE 4 Raman scatter interference with the emission spectra of a crude oil. A solution of 5 mg l La Rosa Crude in 99 Mol % Pure cyclohexane was used.

the Rayleigh-Tyndall and raman peaks is constant in terms of frequency but the wavelength difference increases with increasing excitation wavelengths. In the three dimensional system, the combined effect of these scattering phenomena resemble an "interference wedge" that bisects a total oil spectrum (Figure 5). The use of the lower excitation wavelengths, such as 250 and 254 nm, circumvents the distortions caused by the scattering wedge and is therefore preferred. Figure 6 illustrates the extent of coincidence of the wedge with 340 and 250 nm derived spectra.

All of the methods based on the single excitation wavelength approach can be expediently and routinely used for sample analyses. Proportionately little time is expended for the amount of information obtained from oils by these techniques.

Synchronously excited spectra

Synchronously excited spectra are obtained by exciting oils with continuously varying wavelengths of ultraviolet radiation, while simultaneously scanning the emitted radiation. This is performed by scanning

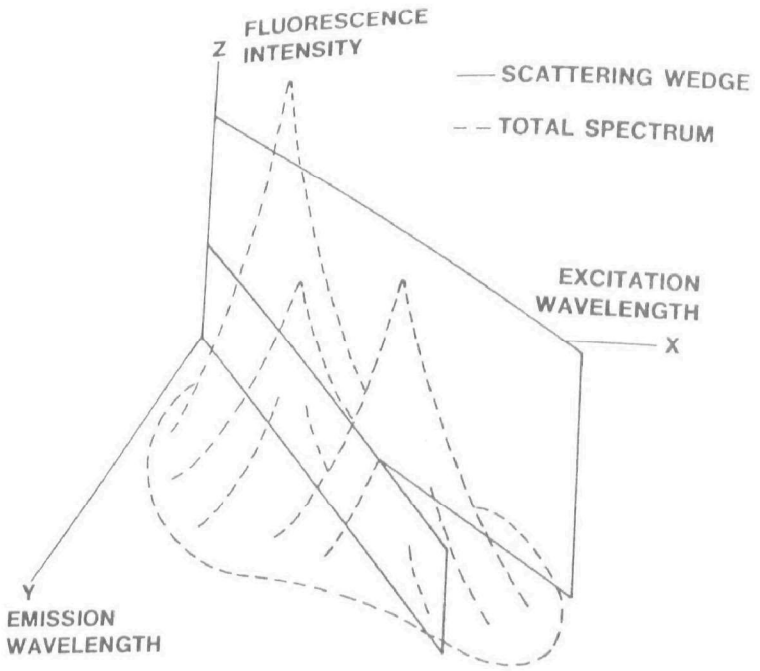

FIGURE 5 Three dimensional presentation of Rayleigh-Tyndall Raman "Scattering wedge" and a total oil spectrum. Rayleigh-Tyndall and Raman scatter predominate in the large and small plane, respectively.

both the excitation and emission monochromators in synchronization and separated by a wavelength interval. Each wavelength interval separating the monochromators determines a unique spectrum, and is in that respect analogous to an excitation wavelength. Like emission spectra, the envelopes of synchronously excited spectra are also used for fingerprinting oils.

Figure 7 illustrates this approach in the three dimensional system. Similar to emission spectra, synchronously excited spectra also represent slices of total fluorescence spectra, but are oriented in planes at an angle (θ) to the x (excitation wavelength) and y (emission wavelength) axes, and are a function of x, y and z. Angle θ is constant and equal to 45°.

Lloyd[22] introduced a method based on this approach for fingerprinting trace quantities of petroleum derivatives encountered in forensic criminology. The author developed analysis procedures for determining spectra of these products and evaluated the use of wavelength intervals ranging from 10 to 50 nm. Solution concentrations were based on the examined products' fluorescence efficiencies but were selected to avoid concentration

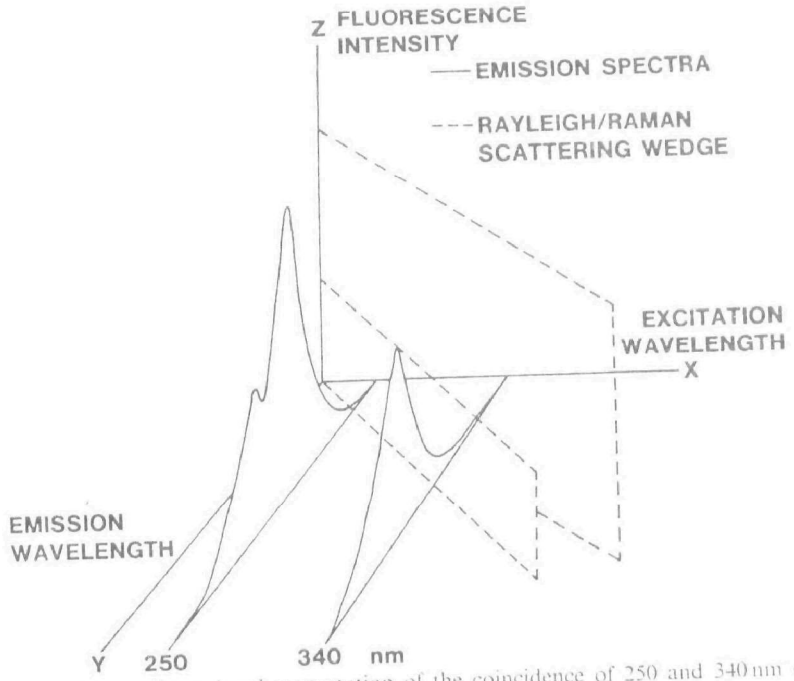

FIGURE 6 Three dimensional presentation of the coincidence of 250 and 340 nm derived emission spectra with the Rayleigh-Tyndall/Raman "scattering wedge".

quenching. Suspect and source materials were correlated by visual comparison of their spectral profiles.

Lloyd[23,24] demonstrated the use of this technique for identifying polynuclear aromatic compounds and for fingerprinting lubricating oils, petroleum fuels, tar derivatives and exhaust soots. He reported that a wavelength interval of 23 nm yielded the most definitive and detailed spectra for a wide variety of examined petroleum products. John and Soutar[21] complemented Lloyd's application of synchronous excitation, demonstrating the use of this technique for the characterization of eight crude oils originating from a number of different geographic locations.

Comparing synchronous excitation to single wavelength excitation within the context of the three dimensional system demonstrates that synchronously excited spectra usually contain more spectral information than emission spectra and in many instances contain the same informational content as several emission spectra combined. Figure 8 compares the two types of spectra and demonstrates a synchronously excited spectrum containing all of the maxima displayed by two emission spectra. The above authors[21,22,23,24] note that this increase in spectral information also increases the diagnostic ability of synchronously excited spectra over the diagnostic ability of emission spectra.

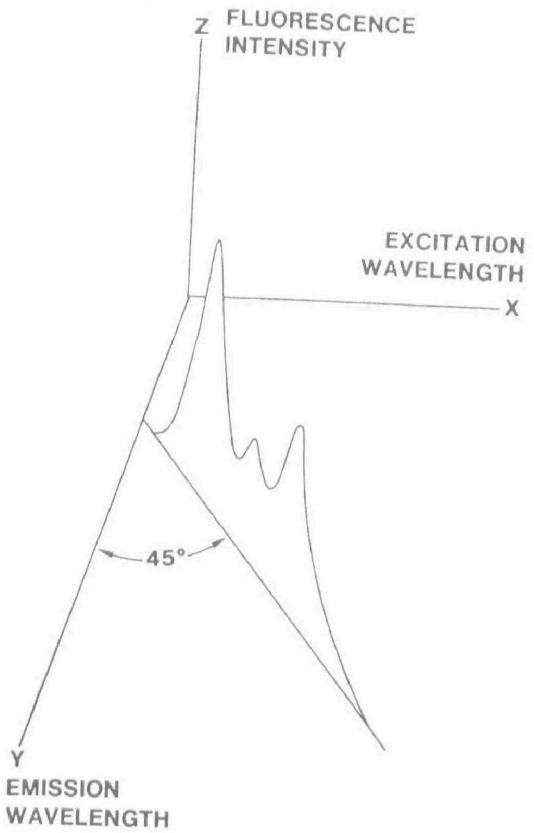

FIGURE 7 Three dimensional presentation of synchronously excited spectrum. The spectrum represents a slice taken at a 45 angle through the total spectrum in Figure 1.

Although it has been demonstrated that synchronous excitation is a viable approach for discriminating between various crude and refined petroleum oils, its general utility for pairing weathered and corresponding unweathered portions of oils has not yet been established. In one oil spill incident, however, Gordon and Keizer[13] obtained conclusive results by the use of this approach.

The effects of quenching and radiation scattering on synchronously excited spectra are similar to those on emission spectra. John and Soutar[21] discussed the concentration dependence of synchronously excited spectra and illustrated spectra derived at concentrations ranging from 0.1–10,000 mg/l. The effects of radiation scattering varies with the selected wavelength interval set between the excitation and emission monochromators. Within the three dimensional system, Rayleigh-Tyndall scatter is analogous to a synchronously excited scan with the wavelength

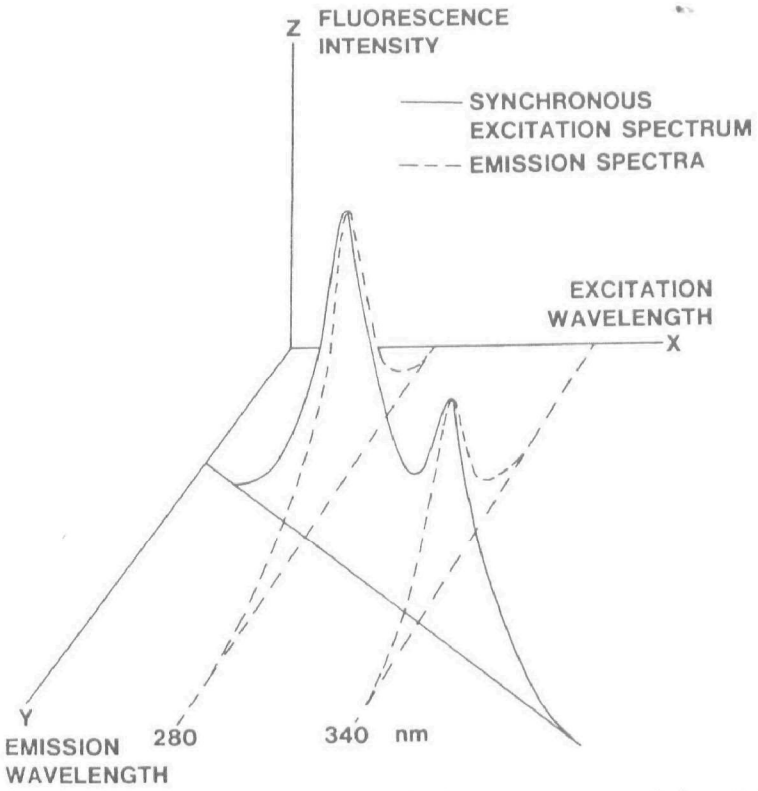

FIGURE 8. Comparison of a synchronously excited spectrum to two emission spectra. The synchronously excited spectrum contains all of the maxima displayed by the emission spectra.

interval set at zero and can therefore be totally avoided by selecting any wavelength interval substantially greater than zero. Raman scatter is similarly avoided by judicious selection of wavelength intervals. Figure 9 demonstrates the feasibility of using a wavelength interval that entirely circumvents interferences caused by the Rayleigh-Tyndall and raman scattering wedge.

Synchronously excited spectra can be readily obtained by routine procedures. An equivalent amount of time is required to derive synchronously excited spectra as emission spectra.

Contour maps

Contour maps of oils are prepared by plotting equal fluorescence intensities of oil emission spectra as a function of excitation and emission wavelengths, and connecting the plotted points. A sufficiently large

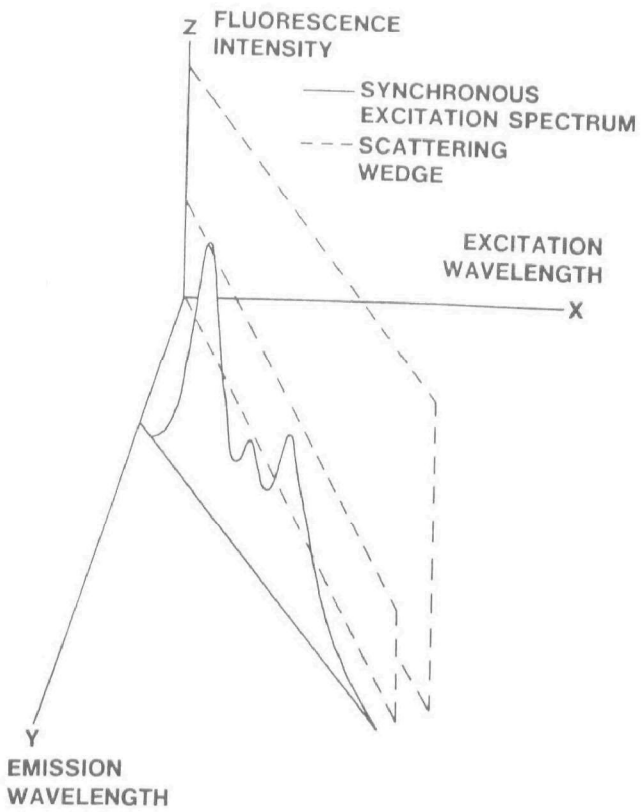

FIGURE 9 Synchronously excited spectrum and Rayleigh-Tyndall-Raman "scattering wedge". This illustrates the feasibility of deriving a spectrum that entirely circumvents interferences caused by the scattering wedge.

number of emission spectra must be used, that encompass the entire fluorescence range of an oil: this requires generally about 30 emission spectra. Figure 10 illustrates this approach in the three dimensional system and demonstrates the derivation of the contour map of an oil having a very simple, inverted cone shaped total spectrum.

Contour maps are a function of x (excitation wavelength) and y (emission wavelength); z (fluorescence intensity) is equal to zero. Comparison of simple maps can be performed visually. The majority of petroleum oil maps, however, are too complex and require computer matching.

Several authors have recommended contour mapping for fingerprinting oils and presented contour maps of various crude and refined oils, but failed to develop specific fingerprinting methods. Freegarde *et al.*[11] initiated the use of this approach for oil analyses and demonstrated the

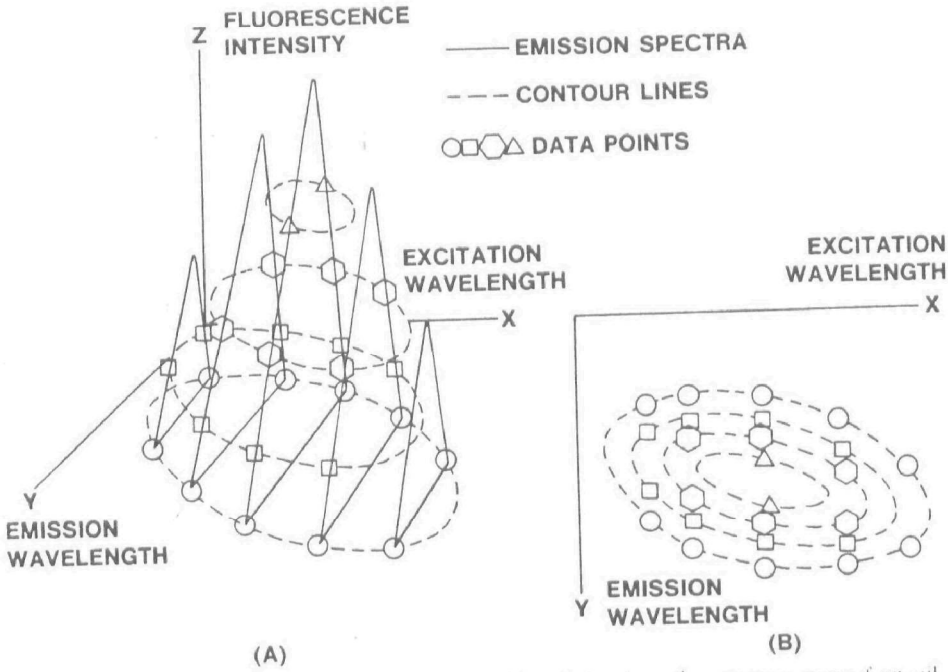

FIGURE 10 Three dimensional presentation of the derivation of a contour map of an oil having an inverted cone shaped total spectrum. The data points used for plotting the map (B) are obtained from five emission spectra (A).

preparation of a contour map from 20 emission spectra of a crude oil. Hornig[15] demonstrated the feasibility of determining contour maps of "neat" oil films and illustrated the variations caused by film thicknesses in the maps of a fuel oil. Hornig and Brownrigg,[16] and Hornig *et al.*[18] discussed the advantages of fingerprinting oils by the contour mapping approach and presented contour maps of various fuel and crude oils dissolved in methylcyclohexane.

The utility of contour maps for fingerprinting weathered oils from spill incidents has not been evaluated. In one experiment. Gordon and Keizer,[13] actually demonstrated that the contour mapping approach is inferior to synchronous excitation in its ability to fingerprint a crude oil exposed to simulated weathering conditions.

The effects of quenching and radiation scattering on contour maps are the combined effects of these phenomena on the emission spectra used as data base. Precautions similar to those used in obtaining emission spectra are therefore necessary.

The preparation of contour maps is very time consuming and tedious when performed manually and requires specialized instrumentation when

computer services are used. Correlation of maps also presents a problem when compared visually and usually necessitates computer assistance. As a consequence, the contour mapping approach is primarily useful as a research tool and is less desirable than the previous approaches for the routine fingerprinting of spill samples.

Silhouette profiles

The use of silhouette profiles for fingerprinting oil represents an approach which combines the three dimensional character of oil fluorescence into a concise two dimensional format. Silhouette profiles of oils are obtained by plotting the maximum fluorescence intensities of oil emission spectra as a function of excitation wavelengths and connecting the plotted points with straight lines. Plotted oil profiles are amenable to direct visual comparison and because of their format are also readily compatible with routine computer software.

Figure 11 illustrates the derivation of a silhouette plot within the three

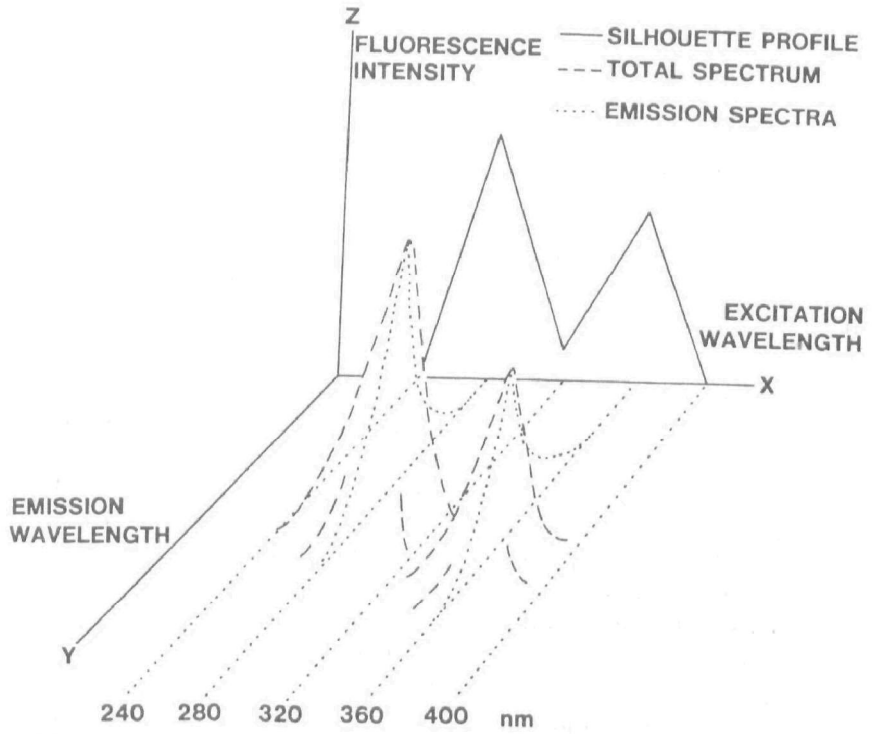

FIGURE 11 Three dimensional presentation of the derivation of a silhouette profile of an oil whose total spectrum contains two maxima. The profile is prepared by plotting the maximum fluorescence intensities of the emission spectra versus the excitation wavelengths and connecting the plotted points with straight lines.

dimensional system. Silhouette profiles are a function of x (excitation wavelength) and z (fluorescence intensity); y (emission wavelength) is equal to zero.

Frank[7] introduced a method based on this approach and demonstrated its utility for fingerprinting oils. In this method, silhouette profiles are obtained by exciting 5 ppm solutions of oil in cyclohexane at 15 wavelengths in 20 nm intervals ranging from 220 to 500 nm. At each of the 15 excitation wavelengths, a rapidly scanned emission spectrum is obtained whose maximum intensity serves as a data point.

Frank demonstrated the utility of silhouette plotting for discriminating between crude and refined petroleum oils and successfully correlated artificially weathered oils with corresponding portions of unweathered oils. Tanacredi,[28] confirmed the fingerprinting capabilities of this approach and demonstrated its utility for identifying weathered lubricating oils in waste treatment effluents, surface waters and marine organisms. Silhouette profiles from these studies were found to retain their identification characteristics despite extensive weathering.

The effects of quenching and scattering on silhouette profiles are similar to those of emission spectra. Frank[7,8] performed several studies to determine the extent of influence on profiles and discussed precautionary procedures.

Silhouette plots of oils are rapidly obtained and can be routinely used for fingerprinting spill samples. In contrast to contour mapping, this approach circumvents the necessity of computer services for data handling and comparisons, and still retains the essence of three dimensional total spectra.

Individual oil component spectra

Fingerprinting oils by use of fluorescence spectra obtained from individual petroleum compounds represents a multi-parameter approach which exploits the complexity of petroleum oil composition. A variety of chromatographic procedures are used to first fractionate oils into simple mixtures comprised of several similar type compounds. Fluorescence spectra of each compound are then obtained and compared to reference spectra in order to establish the compound's identity. This identification procedure is possible because of the spectra's specificity and the ability to selectively obtain spectra of individual mixture components. Correlation of oils is consequently performed by comparison of identified compounds.

Although numerous authors have demonstrated the use of individual oil component spectra for characterizing petroleum oils, this approach has only recently been adopted as a fingerprinting tool. Drushel and Sommers[6]

initiated the use of this approach and demonstrated the characterization of several petroleum distillates. A gas chromatographic (GLC) procedure was used by these authors for isolating simple multicomponent fractions. They then identified many of these components as heterocyclic aromatic compounds from their emission and excitation spectra and in some instances from their phosphorescence spectra.

McKay and Latham[25,26] used this approach for characterizing high-boiling petroleum distillates and adopted a technique utilizing a combination of ion exchange, gel permeation and thin-layer chromatography to isolate fractions of similar compound types. They then identified numerous polynuclear aromatic hydrocarbons and heterocyclic aromatic ring systems from emission and excitation spectra.

Lloyd[24] demonstrated the use of this approach for fingerprinting lubricating oils, gasolines and engine exhaust soots encountered in the forensic investigation of crime. He used gradient elution chromatography for the isolation of fractions and obtained emission, excitation and synchronously excited spectra for the identification of individual compounds. He also determined relative compound concentrations by comparing their fluorescence intensity yields to the intensity yield of an internal standard, added to each isolated fraction. Polynuclear aromatic hydrocarbons and alkylated benzothiophenes were identified and both their identity and relative quantity served as fingerprinting parameters.

Jadamec *et al.*[20] performed a preliminary study on the use of this approach for fingerprinting petroleum oils. The authors used high pressure liquid chromatography to separate major ultraviolet absorbing fractions of four crude and fuel oils, and obtained emission and excitation spectra of compounds in each isolated fraction. They identified a homologous series of polynuclear aromatic hydrocarbons ranging from naphthalene to coronene and suggest their use as fingerprinting indices.

In comparison to the other fluorescence approaches, the individual oil component spectra approach exploits much of the potentially available information that is otherwise obscured in the complexity of "whole" oil spectra, i.e., usual fluorescence spectra are unresolved combinations of individual spectra arising from a large number of fluorescent entities. Several of the above authors,[20,24] have demonstrated that this approach enhances the ability to distinguish between very similar oils. Thus, Lloyd[24] used this approach to identify several oils that are indistinguishable by comparison of their emission spectra.

Jadamec[20] and Lloyd[24] developed the most applicable fingerprinting techniques using this approach. Their method's utility, however, for pairing weathered with corresponding unweathered oils has not yet been demonstrated. It is believed that most of the isolated compounds used as

identification parameters are not significantly degraded by weathering but that their relative concentrations in oil matrices are influenced.

Ready acceptance of this approach for the routine analysis of spill samples is unlikely. The compound isolation and spectra acquisition procedures for compound identification are excessively complex and time consuming and are not amenable for the routine analysis of samples.

SUMMARY AND CONCLUSIONS

The reviewed methods are primarily based on five approaches which derive spectral data that represent specific sections of total fluorescence spectra. Table 2 summarizes the spectral data obtained from these methods and the corresponding approaches within the context of the three dimensional system. Conclusions regarding the methods' relative effectiveness for fingerprinting petroleum oils are based on the methods' compliance with each criterion outlined in Table 1 and are as follows:

Criterion 1 (ability to discriminate between oils) identified several methods as having superior diagnostic abilities over the other reviewed methods. Data presented by authors of the following methods demonstrated their greater resolving power: excitation at 254 nm,[19] synchronous excitation[21,22] contour mapping,[11,18] silhouette plotting,[7] and fluorescence analysis of individual oil component spectra.[20,24]

Criterion 2 (ability to correctly match environmentally weathered oils with corresponding unaltered oils) determined which methods have been successfully applied to "weathered" oils and are suited for use in oil spill incidents. Excitation at 254 nm,[19] and silhouette plotting[7,28] were demoustrated by more than the original authors to correctly match weathered with unweathered oils. Evidence has also been presented[13,22] that synchronous excitation is a potentially viable fingerprinting approach.

Criterion 3 (ability to cope with quenching phenomena) established that some methods[19,29] using oil solutions having concentrations greater than 10 ppm yield distorted spectral features as a consequence of quenching. Spectra of weathered and unweathered portions of the same oils are not equivalent in terms of the spectral distortions caused by quenching and are therefore not useful in fingerprinting spill derived oils.

Criterion 4 (ability to resolve interferences caused by radiation scattering) determined which methods successfully circumvented the spectral distortions caused by Rayleigh-Tyndall and raman scatter peaks. Within the context of the three dimensional system, minimal coincidence of the Rayleigh-Tyndall and raman "scattering wedge" with oil spectra occurred at low excitation wavelengths, such as 250 nm[11,19] and in synchronous excitation.[21,22] The ability to subtract scattering interferences was also demonstrated in the silhouette plotting approach.[7]

TABLE II

Summary of approaches, spectral data and methods

Three dimensional representation of approach		Spectral data	Method (reference)
Description	Coordinate function (F)		
Slices parallel to x (excitation wavelength) axis	$F(x, z); y=$constant	Excitation spectra	19, 29, 30
Slices parallel to y (emission wavelength) axis	$F(y, z); x=$constant	Emission spectra	5, 11, 19, 20, 29
Slices at 45° angles to x (excitation wavelength) and y (emission wavelength) axes	$F(x, y, z)$; angle $\theta=$constant	Synchronous excitation	21, 22, 23
Plotted "peripheries" of total spectra	$F(x, y); z=0$	Contour maps	11, 15, 16, 18
Plotted "shadows" of total spectra	$F(x, z); y=0$	Silhouette profiles	7, 28

Criterion 5 (ability to be expediently and routinely applicable) identified those methods that can be readily implemented in spill incidents where immediate analytical data is mandatory for legislative and damage assessment purposes. Compliance with this criterion, therefore excludes methods based on the contour mapping and individual component spectra approaches.

Excitation at 254 nm,[19] synchronous excitation[21,22] and silhouette plotting[7] most closely comply with the criteria in Table I. These methods have been successfully used by several investigators,[7,19,21,22,28] which in the case of synchronous excitation and silhouette plotting have presented published data. Contrasting these three methods within the context of the three dimensional system demonstrates the variability of spectral information obtained from each technique. Figure 12 illustrates the infor-

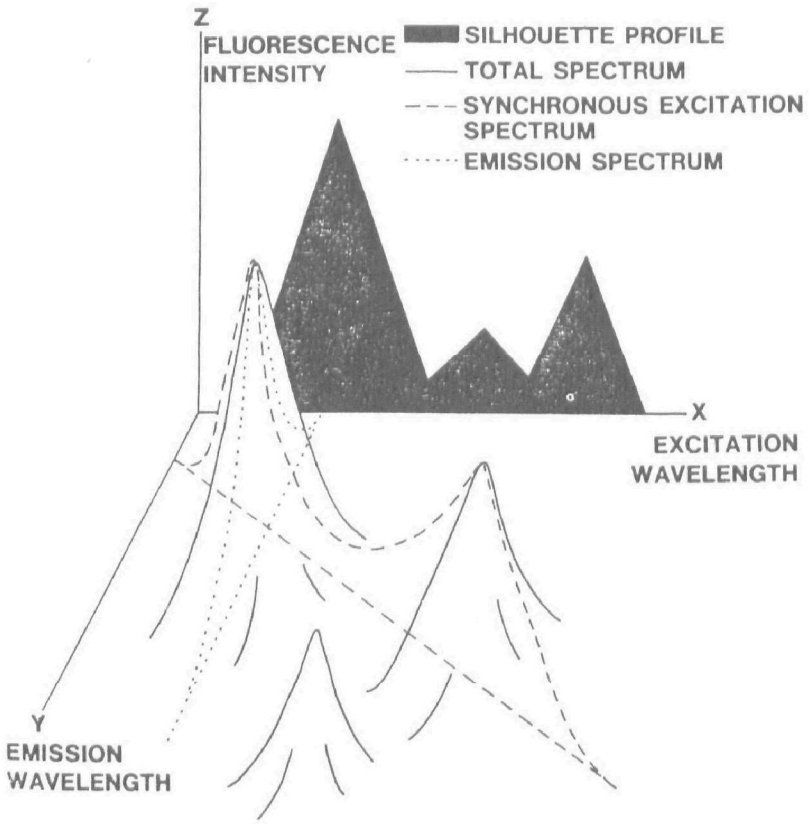

FIGURE 12 Comparison of emission spectrum, synchronously excited spectrum and silhouette profile within the context of a total oil spectrum. The emission spectrum includes only one maximum, and the synchronously excited spectrum includes two maxima. The silhouette profile however includes all three maxima and represents the "shadow" of the total spectrum.

mation obtained by each method from an oil whose total spectrum is shown as three peaks. In this example the single wavelength excitation method derives the least detailed spectrum and includes only one of the available three maxima. Synchronous excitation provides a more detailed spectrum inclusive of two of the maxima. The silhouette profile method, however, provides information that incorporates the essence of the total spectrum, within a two dimensional format and includes all three maxima. Silhouette profiles, therefore yield a fingerprinting parameter broader in scope than either emission or synchronously excited spectra.

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OCCURRENCE OF EXTRACTABLE HYDROCARBONS IN SEDIMENTS FROM RARITAN BAY, NEW JERSEY

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ABSTRACT. *Sediments from 22 sites in the Raritan Bay — Lower New York Bay complex showed that extractable hydrocarbons and percent volatiles increased as the silt-clay content of the sediment increased. The hydrocarbons appeared to be concentrated in the deeper center of Raritan Bay and the Raritan Bay muds, heading down the Bay towards Sandy Hook. Extractable hydrocarbons ranged from 2.2-1098.2 μg/g of dry sediment and the percent volatiles ranged from 0.85-11.39. A peak within the n-C_{11} range and a large "unresolved complex mixture" were characteristic of gas chromatograms. Mixed isomers of benz-pyrene and benz-anthracence and unidentified 1-6 ring polynuclear aromatic hydrocarbons were present.*

INTRODUCTION

Hydrocarbons may enter the estuarine environment by many routes including air pollution fallout, sewage, rivers, dredging and industrial outfalls. The tidal nature of estuaries increases the probability that some hydrocarbons will be retained and deposited. Hydrocarbons present in an ecosystem can frequently be divided into two components: a) those recently derived biogenically from flora, fauna, detritus, and b) those derived from fossil hydrocarbons by anthropomorphic activities or seepage from the earth. Many of the hydrocarbons are potentially dangerous to human health and knowledge of hydrocarbon content and distribution is necessary for management decisions, e.g., dredging impacts, spill treatment effects and alternatives, and use of biotic resources (shellfish).

The Raritan Bay — Lower New York Bay Complex receives hydrocarbons from municipal and industrial wastes, both directly and from tributary waters (Tanacredi, 1977). Unknown amounts of hydrocarbons have entered the bay from oil spills in the Arthur Kill and Raritan River, yacht marinas, ship traffic, and dredging. The Hudson River may also contribute hydrocarbons from the upper New York Bay and New York City environs. DeFalco (1967) reviewed many types and sources of effluents. The circulation patterns (Patten, 1962) and flushing rates in the Raritan Bay — Lower New York Bay Complex tend to retain introduced pollutants.

DeFalco (1967) described general sediment regions of the Bay but particle-size distributions were not reported and extractable hydrocarbons were not quantified with respect to sediment types. Grain-size properties of the sediments may affect the transport and preservation of organic compounds and absolute amounts of sedimentary hydrocarbons considered without other information on the physical properties of the sediments may be of little value (Wehmiller and Lethen, 1975). This study attempted to survey the grain-size distribution, percent volatiles and extractable hydrocarbons of the sediments of the Raritan Bay — Lower New York Bay Complex.

METHODS

Sediments were obtained from the Lower New York Bay — Raritan Bay Complex during June, 1977 (Fig. 1). Samples were not taken near ship channels or dredge spoil areas to avoid overt possibilities of contamination. Benthic surface sediments were sampled with a Petersen Grab (0.1 m^3). Several grabs were taken per station and composited. Sediments for chemical analysis

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ERRATA: The illustrations for Figures 2 and 4 have been switched. The chromatograms depicted in Figure 2 should have the legend of Figure 4, and the chromatograms of Figure 4 should have the legend of Figure 2.

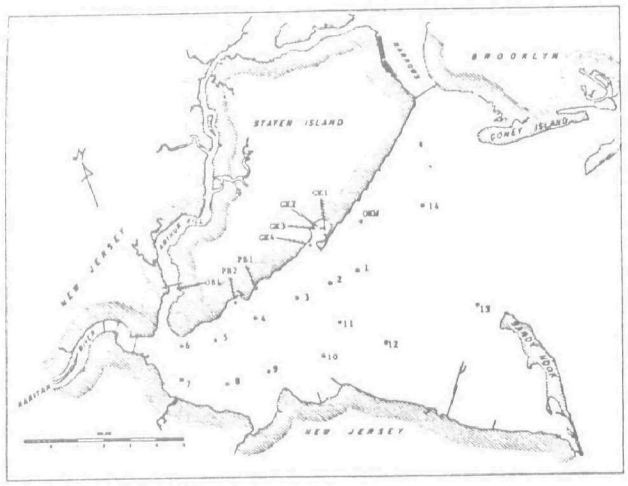

FIGURE 1. Location of sampling sites. Intertidal samples were taken at OB1 (near the Outerbridge Crossing), PB1 (Sequine Point), GK4 (a sand bar at the mouth of Great Kills Harbor) and GK3 (below a storm sewer outfall). Sub-littoral samples were taken at stations 1-14, GK1 and GK2 (in Great Kills Harbor), PB2 (Princes Bay) and OKWD (off the outfall of the Oakwood Sewerage Treatment Plant.

were placed in quart mason jars with tin lined caps, refrigerated at 4°C, and analyzed within two months. All collection and storage materials were washed with "distilled in glass" hexane (Burdick and Jackson, Muskegon, Mich.). Sediments for textural characterization were subsampled from the grab composite. Similar procedures were utilized in the analysis of littoral samples.

Particle-size analyses were performed by sieving 100 g dry weight of sediment through a series of U.S. Standard Wire-cloth sieves (4 mm, 2 mm, 0.5 mm, 0.24 mm, 0.125 mm, and 0.063 mm). Macrodebris (i.e., shells, large rocks) were excluded from samples prior to weighing. Dried congealed sediments (mud) were ground with a mortar and pestle prior to sieving. The sediments were sieved for 30-40 minutes. Sediment fractions were then weighed and relative percentages were calculated.

Percent volatiles of the sediments were measured by placing predried sediment samples in a muffle furnace at 500-800°C for 24 hours. Differences in weight were calculated as percent volatiles. Total extractable hydrocarbons were analyzed using a modification of the method of Giger and others (1974). All material was prewashed with Freon 113 prior to use. Approximately 50 g of sediment from each sample was dried to constant weight at 60°C for two days. Sediments were then transferred to 250 ml screw top Erlenmeyer flasks (sand sediments were separated with a spatula, mud sediments were ground in an agate mortar). Fifty ml of Freon 113 was added to each flask. Flasks were sealed with a tinfoil lined cap and tumbled 10 minutes on a wrist action shaker. The freon extract was then filtered through glass wool into a 100 ml volumetric flask. An additional 50 ml of freon was added to the sediment in the Erlenmeyer flasks and tumbled for another 10 minutes. This supernatant was then filtered through glass wool and added to the first. The combined extracts of each sample were brought to volume and dried with anhydrous sodium sulfate previously baked at 150°C to remove impurities. The extracts were analyzed by Infrared Spectroscopy utilizing 10 and 1 cm cells in a Perkin-Elmer Model 457 Infrared Spectrophotometer. The extracts were scanned from 3200 cm^{-1} to 2700 cm^{-1} and quantitated at 2930 cm^{-1} (Gruenfeld, 1973). Subsamples (25 ml) of selected extracts were analyzed by gas chromatography and fluorescence spectroscopy.

The remaining freon extract in each flask was treated with 2 g of 2% deactivated silica gel to remove non-hydrocarbon interferences (Gruenfeld and Frederick, 1977). The treated extracts were then re-analyzed by IR Spectroscopy similar to the pretreated samples. All extracts were quantitated by comparing the absorbances to a reference mixture of iso-octane and hexadecane in freon. To determine the types of hydrocarbons present and the effects of silica gel treatment, select samples from oil contaminated sites (GK3 and OB1) were analyzed by gas chromatography.

For gas chromatographic analysis, samples were dried under carbon filtered air and brought to 100 μl volume with cyclohexane (Fisher 99 Mol %). A Perkin-Elmer (Model 900) gas chromatograph was used, equipped with a flame ionization detector and a six foot stainless steel column packed with 3% OV-1 on 80/100 mesh of Chromosorb WAW. Operating conditions

TABLE 1. Particle size classification (percent retained on sieves), percent volatiles, and extractable hydrocarbons before (BT) and after (AT) silica gel treatment (μg/g dry sediment).

Sampling site	Particle size (Wentworth) Granules	Very coarse sand	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt-clay	Percent volatiles	Extractable hydrocarbons (BT)	(AT)
1	0.9	3.5	20.8	66.6	6.8	1.1	0.2	1.35	18.4	11.5
2	2.4	2.0	3.1	14.6	39.0	34.2	4.1	3.06	292.3	269.2
3	0.0	0.2	0.4	1.1	4.9	65.3	27.6	5.53	422.5	395.7
4	0.4	0.7	1.4	1.9	4.0	55.9	33.9	8.55	840.0	768.3
5	0.6	2.1	4.4	5.3	12.5	42.7	30.4	11.39	444.9	414.3
6	19.9	14.0	19.7	31.4	13.8	0.6	0.3	0.85	74.8	55.1
7	3.3	4.2	6.0	20.9	24.2	24.7	14.7	3.80	179.3	179.3
8	2.3	3.7	10.4	16.5	13.4	32.7	20.4	5.12	120.0	120.0
9	1.2	3.5	5.5	13.8	24.8	27.7	22.7	5.41	696.8	636.7
10	0.0	0.2	0.7	2.3	26.3	10.0	59.8	7.36	329.5	307.2
11	8.0	7.4	8.8	33.1	17.9	12.8	11.4	3.79	441.0	412.9
12	0.0	1.0	2.7	5.2	10.0	28.7	52.2	8.84	572.0	549.5
13	0.2	1.2	2.7	4.4	52.4	23.2	14.7	5.41	381.4	332.9
14	1.3	1.1	2.4	10.3	36.9	43.4	4.5	1.47	34.8	30.4
GK 1	0.1	0.7	2.5	4.3	10.0	31.9	50.1	7.02	672.6	655.6
GK 2	0.1	0.1	0.4	1.1	21.0	42.5	34.1	7.38	1194.3	1098.2
GK 3	13.7	8.5	16.4	29.7	21.9	6.2	3.5	2.56	715.2	590.2
GK 4	8.0	15.6	36.0	38.4	1.4	0.3	0.2	0.35	7.2	2.2
PB 1	4.5	2.6	5.0	36.6	48.0	2.9	0.4	0.95	14.5	9.6
PB 2	4.8	4.9	9.5	39.6	33.4	5.0	2.8	1.31	109.4	98.3
OB 1	21.7	7.8	16.9	42.8	8.2	1.3	1.0	2.8	737.8	641.3
OKWD	12.8	6.9	16.1	42.3	19.8	1.8	0.3	0.91	19.9	16.6

were: carrier gas; He_2; column temperature; 60°C with a two-minute hold, programmed to increase 8°C/minute to 300°C; injection sample: 1-2 microliters. Sample peak areas were identified by matching to alkane standards.

Sample volumes were then diluted to 5 ml with cyclohexane and analyzed for polynuclear aromatic hydrocarbons (PNA) by synchronous excitation fluorescence spectroscopy (Frank, 1978). Confirmation was obtained by combination HPLC and fluorescence spectroscopy. An ODS reverse phase column (DuPont) and a mobile phase of 50:50 2-propanol/water was used to chromatograph the sample. The predominant constituent was isolated and collected. Both single wavelength (emission) and synchronous excitation spectra were then obtained by fluorescence analysis in a 10 mcl cuvette. Solutions of known PNA standards were similarly analyzed. Retention times on chromatograms and fluorescence spectra were used as identification parameters for the chromatographed sample constituent.

RESULTS

The results of textural analysis, determination of percent volatiles and quantitation of extractable hydrocarbons are presented in Table 1. Values of hydrocarbons are reported before and after silica gel treatment to illustrate the large residual amount of hydrocarbons remaining after treatment. Silica gel treatment removed an average of 14.4% of non-hydrocarbon material from extracts. The extractable hydrocarbons (after silica gel treatment) ranged from 2.2 to 1098.2 mg/g of dry sediment.

Percent volatiles and μg extractable hydrocarbons (after silica gel treatment) were examined to relate their quantitative values to the percent silt-clay fraction of the sediment. Table 2 indicates that the percent volatiles and μg extractable hydrocarbons apparently increase as the silt-clay fraction increases.

Relationships between the silt-clay fraction, percent volatiles and μg of extractable hydrocarbons were analyzed by linear regression. A cor-

TABLE 2. The percent volatiles and μg extractable hydrocarbons (recorded as mean values ± S.E.) determined sediments with different silt-clay fractions.

% Silt-clay fraction	n	% Volatiles	μg extractable hydrocarbons
0.1- 1.0	5	0.88 ± 0.16	19.0 ± 9.31
1.1-10.0	4	2.1 ± 0.42	247.0 ± 124.9
10.1-20.0	3	4.3 ± 0.54	308.4 ± 68.2
20.1-30.0	3	5.3 ± 0.13	384.1 ± 149.3
30.1-40.0	2	10.0 ± 1.42	591.3 ± 177.0
40.1-50.0	0		
50.1-60.0	3	7.7 ± 0.56	504.1 ± 103.1

relation coefficient of 0.64 was found for the regression of the percent silt-clay fraction vs. μg of hydrocarbons. A correlation coefficient of 0.73 was calculated for the regression of the percent volatiles vs. μg of hydrocarbons. However, the calculated standard error of the regression for both of the above indicated considerable scatter around both calculated regression lines. The calculated correlation coefficient (0.85) of the regression between the silt-clay fraction and the percent volatiles indicated a closer relationship. In calculations, data from sites OB1 and GK2 were excluded because of several previous spill events of petroleum oil (OB1) and sewage (GK2).

To determine types of hydrocarbons present and the effects of silica gel treatment, select samples (OB1, GK3, Sites 1, 4, 5, 6, 8, 11 and 12) were analyzed by gas chromatography. Figure 2 illustrates chromatograms of samples from sites OB1 and GK3, before and after silica gel treatment. Although some material was removed by the silica gel, the occurrence of a large "unresolved complex mixture" in the chromatograms indicated the hydrocarbon material was not recently biologically produced (Gruenfeld and Frank, 1977). The chromatogram of site OB1 illustrates residual oils in the sediment after several spill incidents of crude and fuel oils over a period of years. The chromatograms of GK3 indicate the presence of well weathered oil, higher boiling materials and possible sewage derived hydrocarbons from the storm sewer outfall which was located approximately 40 feet from the site. Gas chromatograms of a light Arabian Crude oil and a No. 2 fuel oil are presented in Figure 3 to illustrate non-weathered oil chromatograms.

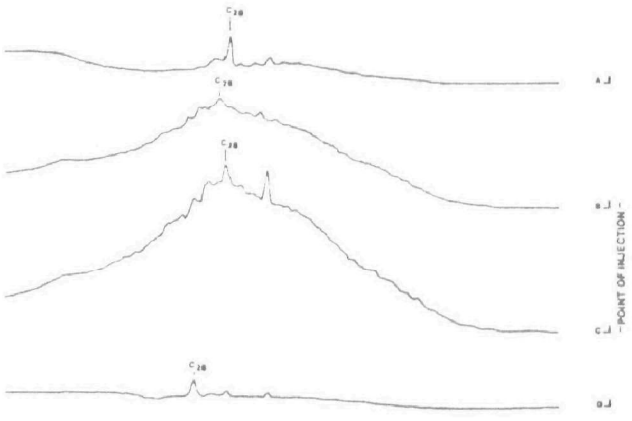

FIGURE 2. Gas chromatograms of Sites OB1 (A) and GK3 (C). Chromatograms B and D are sites OB1 and GK3 after treatment with silica gel. The baseline is drawn below chromatogram D.

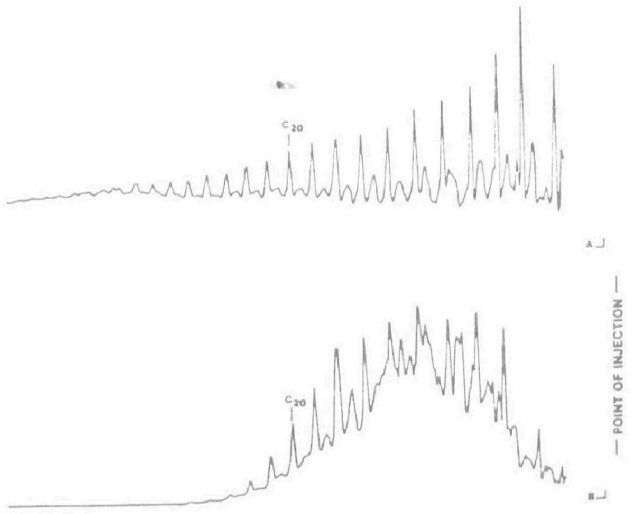

FIGURE 3. Gas chromatograms of Light Arabian Crude oil (A) and No. 2 fuel oil (B).

Figure 4 contains chromatograms from Sites 1, 4, 5 and 6. A large indigenous hydrocarbon content is evident in the chromatograms of Sites 4 and 5. Site 4 was near the "Raritan Bay-West Reach Ship Channel" and the Site 5 sample was from a large mud bank. Sites 1 and 6 were sandy areas and the chromatograms indicate low levels of hydrocarbons present.

Synchronous fluorescence spectra were obtained for samples from Site OB1 and GK3 (before silica gel treatment; (Frank, 1977) and Sites 1, 5, 6 and 8. In analyzing the spectra of OB1 and GK3, benz-pyrene (mixed isomers) were the predominant compounds with possible

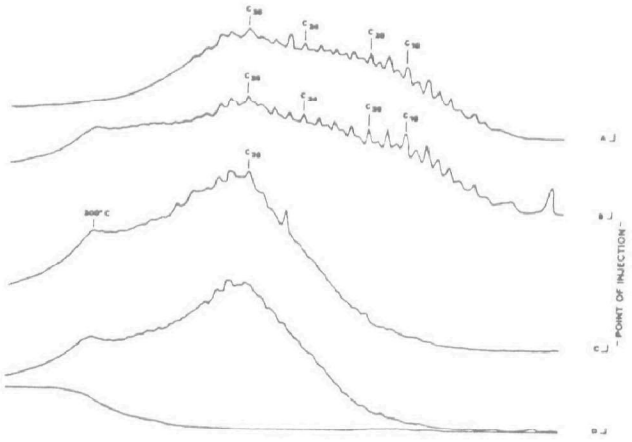

FIGURE 4. Gas chromatograms of Site 1 (A), Site 4 (B), Site 5 (C) and Site 6 (D).

4 and 6 ring compounds present. In the Site 1 spectra, two ring compounds were the most prevalent (with possible three ring compounds present), five ring compounds exhibited a well resolved peak and a six ring compound peak was partially resolved. In the Site 5 spectra, benz-anthracence was predominant with benz-pyrene and other five ring compounds prevalent. The peaks for benz-anthracence and benz-pyrene were well resolved. In the spectra of Site 6, three ring compounds were prevalent, probably anthracence. Five ring compounds were next in prevalence with six ring compounds indicated in the spectra. The spectra of Site 8 indicated a predominance of three ring compounds with 5 and 6 ring compounds present and partially resolved.

DISCUSSION

The particle-size distributions found in this study generally conformed to those found by DeFalco (1967). This indicates that the general bottom sediment distribution is a relatively stable feature of the Bay.

The particle-size distribution and percent volatiles reported here indicates a pattern of movement of sediments in the Bay complex. The sediments entering the Bay from various locations move progressively toward the area bounded by Sequine Point, Great Kills, Keyport and Keansburg. The pattern conforms with earlier descriptions by DeFalco (1967) and Greig and McGrath (1977). The distribution of extractable hydrocarbons reflects this sediment movement with relatively high values at Stations 4, 5, 7, 8, 9, 10 and 11. Generally, sample sites with high percent volatiles were areas with large silt-clay and fine-sand fractions.

Several of the values of μg of extractable hydrocarbons after treatment were higher than others and reflect the area sampled. Site GK2 was sampled from the center of Great Kills Harbor and receives inputs of petroleum hydrocarbons from boat marinas, storm sewers and dredging. Sites GK1 and GK3 also contain hydrocarbons found within semi-enclosed Great Kills Harbor. The hydrocarbon material found at Site OB1 reflects several previous oil spills within the Arthur Kill. This beach was predominantly medium and coarser sands, and oil appeared in bootprints while sampling. When taking sediments, a light crude or fuel type oil appeared at the bottom of the hole. Site 4 was near the "Raritan Bay-West Reach Ship Channel" and the higher hydrocarbon values may reflect settled dredge spoil or ship derived hydrocarbons.

Gas chromatograms frequently exhibited a large 'unresolved complex mixture' indicative of pollution derived hydrocarbons. The Bay receives large amounts of sewage from many sources, and both Hatcher and others (1977), and Goodfellow and others (1977), have reported frequent contamination of sediments by sewage derived coprostanol (an n-C_{28} sterol). Some material eluting in the chromatographs in this study in the n-C_{28} region may also be sewage derived contaminants. However, Wehmiller and Lethen (1975) reported that Delaware estuary sediments showed a significant 'unresolved complex mixture,' and many chromatograms also contained a mixture that peaked in the n-C_{28} to n-C_{30} region. They termed these "high boiling" mixtures which were frequently observed in coarse grained sediments.

The lack of resolution in the chromatograms of this report indicates that a substantial amount of hydrocarbon material is present. The fluorescence spectra indicated that many aromatic compounds were present. Mixed isomers of both benz-pyrene and benz-anthracene were frequently found with other 1-6 ring compounds present.

Hites and others (1977), and Tanacredi (1977), have also reported the occurrence of PNA hydrocarbons in sediments. Although Tanacredi reported that sewers in Jamaica Bay were discharging PNA hydrocarbons, the derivation of aromatic hydrocarbons in this study is not known.

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