AN EVALUATION OF HYDROCARBON EXTRACTION TECHNIQUES FOR TISSUE

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

Colleen Becker

1979

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

Author

Approved, February 1979

Craig L. Smith, Ph.D.

Rudolf H. Bieri, Ph.D.

Howard Kator, Ph.D.

Robert A. Jordan, Ph.D.

Robert E. Black, Ph.D.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS v

LIST OF TABLES. vi

ABSTRACT. vii

INTRODUCTION. 2

EXPERIMENTAL. 10

MATERIALS. 10

STANDARDS. 10

METHODS. 12

Determination of Replicate Numbers. 12

Preparation of Tissue Homogenate. 12

Extraction Techniques 13

Digestion in KOH-methanol. 13

Soxhlet Extraction 14

MacLeod's Procedure. 15

Evaporation of Excess Solvent 15

Column Chromatography 16

Gas Chromatography. 16

Method of Quantification. 17

Preliminary Determination of Efficiencies 18

Statistical Analysis. 19

RESULTS 20

DISCUSSION. 29

TABLE OF CONTENTS (Continued)

Page

APPENDIX. 36

LITERATURE CITED. 71

VITA. 75

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to her major professor, Dr. Craig Smith, for his continued support, guidance and sense of humor throughout the course of this study. Thanks are also extended to Drs. Howard Kator, Rudolf H. Bieri and Robert E. Black for critically reviewing the manuscript.

A special debt of thanks is owed to Dr. Robert A. Jordan for his patience, encouragement, and invaluable critical review of the manuscript in its various stages. Thanks are also extended to Linda Jenkins for typing the manuscript.

Finally, the author wishes to thank her husband, James Schwan, without whose patience, support, and "realistic" sense of humor this project would not have been completed.

LIST OF TABLES

Table		Page
1	Extraction Techniques	9
2	Recovered Weights of Aliphatic Hydrocarbon Fraction Calculated by the External Standard Method.	21
3	Recovered Weights of Aromatic Hydrocarbon Fraction Calculated by the External Standard Method.	22
4	Normalized Recovered Weights of Hydrocarbons.	24
5	Student-Newman-Keul's Tests (SNK)	26
6	Standard Recoveries as Arcsine Transformed Percents from Spiked Blanks for the Complete Techniques.	27

ABSTRACT

The purpose of this research was to evaluate and compare three methods of extraction of hydrocarbons from clam tissue. No significant difference between techniques (KOH alcoholic digestion, Soxhlet extraction, and MacLeod's procedure) was determined for the fractions quantitated by the external standard method and for the arcsine transformed percent recoveries of the internal standards. Significant differences among techniques were determined for fractions quantitated to the internal standards except hexamethylbenzene. Interpretation is difficult because no technique proved to be consistently superior. The three techniques must be considered equivalent since raw recoveries were statistically similar and recoveries normalized to the internal standards did not behave consistently.

This study demonstrates that different interpretations of data can be made based solely upon the quantification method. Nearly all the losses of compounds in the spike blanks were accounted for by three processes: concentration under nitrogen, concentration by rotary evaporation, and column chromatography. A comparison of the standard recoveries for the clam tissue and the spike blanks indicated a 10-20% loss due to the presence of the tissue. Given the observed uncertainties within the techniques studied here, small differences between techniques are not significant. Based upon time and equipment, MacLeod's procedure was the preferable technique.

AN EVALUATION OF HYDROCARBON EXTRACTION TECHNIQUES FOR TISSUE

INTRODUCTION

Potential development of the U. S. Atlantic continental shelf petroleum reserves has increased the need for accurate analytical techniques to determine the concentration and composition of petroleum pollutants in the marine environment. Typical hydrocarbon analytical scheme consists of several distinct operations: collection, storage, extraction, concentration, isolation, identification, and quantification. A key step in most of the analyses of biological samples is the extraction of the hydrocarbons. Many extraction techniques involving the separation of the compounds and isolation from non-hydrocarbons have been developed. These involve extraction with a solvent, steam distillation and gas distillation. Soxhlet extraction involves an extensive extraction by an organic solvent followed by partitioning into a non-polar solvent (Clark and Blumer, 1967; Blumer et al., 1970; Ehrhardt, 1972; Clark and Finley, 1973; Farrington et al., 1973a; Bieri and Stamoudis, 1977). Other procedures include: alcoholic KOH digestion followed by partitioning into a non-polar solvent (Greffard and Meury, 1967; Blaylock et al., 1973; Smith et al., 1977); steam distillation (Ackman and Noble, 1973); headspace analysis of volatilized hydrocarbons (Hertz et al., 1974); grinding, homogenizing, or sonication with an organic solvent (Stegeman and Teal, 1973; Hunter et al., 1974; Mackie et al., 1974); and aqueous alkaline digestion followed by ether extraction (MacLeod et al., 1976; Warner, 1976).

These techniques rely on a disruption of the tissue matrix and cells to gain access to the hydrocarbons, followed by volatilization or organic solvent extraction.

Prior to the analysis for petroleum hydrocarbons, the extracted material must be isolated. A variety of techniques have been used to remove lipids and other non-hydrocarbons from the extract. Thin-layer chromatography is a form of liquid-solid chromatography employing the mechanism of adsorption to retain sample molecules. Thin-layer chromatography is capable of accepting dirty samples and can be used to process a large number of samples simultaneously. One technique based upon the same adsorption mechanism is column chromatography (gravity flow). Thin-layer chromatography and column chromatography, singly and in combination, have been used to isolate hydrocarbon fractions (Koons et al., 1965; Avigan and Blumer, 1968; Sever and Haug, 1971; Barbier et al., 1973; Farrington and Quinn, 1973; Johnson and Calder, 1973; Zsolnay, 1973). High performance liquid chromatography (HPLC) is useful for the separation of aromatic hydrocarbons but its use in conjunction with subsequent gas chromatographic analysis has not been fully developed.

Amounts of total hydrocarbons isolated by chromatography can be quantitated gravimetrically if sufficient material remains after the evaporation of the solvent. One possible disadvantage is the loss of the more volatile hydrocarbons (Farrington et al., 1973a). Another disadvantage, which applies to all bulk measurements, is that the quantitation includes both indigenous and petroleum hydrocarbons in addition to other organic compounds.

As a quantification and identification method, infrared spectrometry shows little promise for detecting small quantities of

petroleum if biogenic hydrocarbons are present. Loss of volatile hydrocarbons is minimal, but the absorption frequencies of indigenous hydrocarbons overlap or coincide with those of petroleum hydrocarbons. A possible exception to this generalization are the aromatic hydrocarbons absorbing IR radiation in the long wavelength region (Brown et al., 1973; Zafiriou, 1973).

UV absorption and UV fluorescence spectrometry detect the presence of aromatic hydrocarbons (Zitko and Carson, 1970; Zitko and Tibbo, 1970; Erhardt and Blumer, 1971; Zafiriou, 1973; Gordon and Keizer, 1974). The former method is based upon absorption of UV light, the latter on emission. Completely saturated compounds show no selective absorption throughout the visible and ultraviolet regions. After molecules have absorbed radiant energy and been excited to a higher electronic state, they must lose their excess energy in order to return to the ground electronic state. Fluorescence is the immediate emission of energy as visible light from a molecule after it has absorbed radiation of a shorter wavelength. Both techniques detect aromatic hydrocarbons but there is little differentiation within a mixture of aromatic hydrocarbons. However, UV and UV-fluorescence scanning of chromatographic separations are useful in determining which fractions contain aromatic hydrocarbons.

The increasing utilization of gas chromatography indicates its wide acceptance as an analytical technique for any sample to which it may be applied. In reality, this means any stable compound which exhibits a measureable vapor pressure at temperatures up to 300°C, as well as any compound of which a stable, volatile derivative may be formed. Conditions of column adsorption and detector sensitivity

also influence the application of this analytical technique. Gas chromatography provides a degree of sensitivity and efficiency which makes it ideal for the analysis of hydrocarbons. It provides an excellent separation and quantification method for petroleum mixtures, along with extensive information on the molecular weight range and the complexity of the mixture. Tentative identifications of resolved and partially resolved compounds are possible if standards are available for superimposition (Koons et al., 1965; Avigan and Blumer, 1968; Sever and Haug, 1971; Youngblood et al., 1971; Iliffe and Calder, 1974; Farrington and Medeiros, 1975; Youngblood and Blumer, 1975; Warner, 1976; Smith et al., 1977).

Mass spectrometry and gas chromatography-mass spectrometry (gc-ms) provide excellent qualitative analyses of petroleum hydrocarbons. The basic function of the mass spectrometer is to produce ions from a sample, separating them according to their mass-to-charge ratios (m/e). Coupling of mass spectrometry with gas chromatography has provided a unique opportunity to begin to identify even complex naturally occurring compounds in petroleums. This technique has been found very effective for aromatic hydrocarbons (Blumer et al., 1970; Sever and Haug, 1971; Youngblood et al., 1971; Hites and Biemann, 1972; Blaylock et al., 1973; Brown et al., 1973; Clark and Finley, 1973; Bieri et al., 1974; Iliffe and Calder, 1974; Youngblood and Blumer, 1975; Warner, 1976; Bieri and Stamoudis, 1977).

Previous evaluations of hydrocarbon extraction techniques have been made on sediments (Rohrback and Reed, 1975) and tissue (Farrington and Medeiros, 1975; MacLeod et al., 1976; Gritz and Shaw, 1977). The evaluation of techniques for sediments considered three aspects of the extraction: 1) sample pretreatment; 2) extraction

techniques (Soxhlet, sonication, homogenization, reflux extraction, and reciprocal shaker table); and 3) choice of solvents. Soxhlet extraction proved slightly more efficient (4%) than the other extraction techniques. Acid-treated, water-washed, freeze-dried sediment extracted with toluene-methanol (3:7) appeared to be the best generally applicable procedure (Rohrback and Reed, 1975).

Farrington and Medeiros (1975) evaluated three methods of extracting hydrocarbons from marine organisms: Soxhlet extraction, homogenization with Na_2SO_4 in a Virtis homogenizer, and KOH-methanol digestion. The amounts of extracted hydrocarbons from spiked subsamples of clam (_Mercenaria mercenaria_) homogenate were determined gravimetrically with Soxhlet extraction statistically slightly more efficient. Gritz and Shaw (1977) using the soft parts of _Saxidomus gigantea_ (butter clam) compared Soxhlet extraction followed by 2 hour saponification to either 2 hour or twenty-four hour alkaline extraction. A further parameter which was investigated was the use of fully activated or partially deactivated alumina-silica gel columns. Similar recoveries of added alkanes were obtained by the three extraction methods. Twenty-four hour saponification and column chromatography on partially deactivated columns was judged the best procedure. Soxhlet extraction followed by two hour saponification did not completely remove methyl esters and was the most laborious technique. Direct two hour saponification was also questionable in the removal of esters and led to severe emulsion problems. Their column chromatography work illustrated the need for caution in arbitrarily assigning compounds to different fractions. Several alkenes which eluted in the first (alkane) fraction when partially deactivated columns were used, appeared partially or totally

in the second (aromatic) fraction when fully active columns were employed. While this situation will cause little problem in heavily polluted samples in which alkenes are only a small percentage of the total hydrocarbons, in unpolluted biological materials where alkenes may be prominent, classification of molecules as alkanes or aromatics based only on column chromatographic behavior must be done with caution. The results from the evaluation study from which MacLeod's procedure was selected are currently being evaluated (D. Brown, personal communication).

An intercalibration of three analytical procedures conducted by Farrington et al. (1973b), using a cod liver lipid standard containing biogenic and petroleum hydrocarbons, indicated similar concentrations of pristane and squalene and levels of contamination of petroleum. The intercalibration concentrated on clean up and gas chromatography procedures, since the lipid had already been extracted from the liver tissue.

The purpose of this research was to evaluate and compare three methods of extraction of hydrocarbons from animal tissue. The organism chosen was the readily available, common guahog clam *Mercenaria mercenaria*. Three procedures tested were Soxhlet extraction (Bieri and Stamoudis, 1977), alcoholic KOH digestion (Smith et al., 1977) and ether extraction (MacLeod et al., 1976); the first two techniques have been used extensively in this laboratory. All the procedures involved a digestion followed by extraction with a non-polar solvent. They differed by the method of extraction and the type of digestion. To eliminate as many outside factors as possible and to concentrate the evaluation solely on the extraction procedures, identical column chromatography and gas chromatography procedures were used to determine

hydrocarbon concentrations. The three analytical procedures are presented in Table 1.

TABLE 1

EXTRACTION TECHNIQUES

Method A	Method B	Method C
(MacLeod et al., 1976)	(Smith et al., 1977)	(Bieri and Stamoudis, 1977)
	Homogenate	
		MeOH-KOH digestion
NaOH digestion (aqueous)	alcoholic KOH digestion (methanol)	Soxhlet extraction (methanol)
ether partitioning	hexane partitioning	pentane partitioning
concentration under N_2	rotovap concentration	rotovap concentration
	column chromatography	
	gas chromatography	

EXPERIMENTAL

Materials

Pentane, hexane, benzene, methanol, and unpreserved diethyl ether, all "distilled in glass", were utilized (Burdick and Jackson, Muskegon, Mich). Diethyl ether with 2% ethanol preservative was not acceptable for MacLeod's procedure. Up to 0.5 ml of ethanol remains in the extract and upon concentration ethanol separates as a second phase. This ethanolic phase may cause bumping and loss of sample. Furthermore, the alcohol may deactivate the silica gel column and nullify the separation of saturated and unsaturated hydrocarbons. Sodium chloride, anhydrous sodium sulfate and sea sand were Soxhlet extracted with hexane and dried at 135°C for 16 hours. The Bio-Sil^{R}A silica gel, 100-200 mesh, was activated by heating for 16 hours at 210°C. All deionized water was extracted with hexane prior to use. All glassware was first washed with soapy water, rinsed with tapwater, dried, placed in dichromate cleaning solution, rinsed with tapwater, deionized water and acetone, and baked at 135°C.

Standards

The standards for the spike blanks and spiked tissues are listed below.

Standard A

compound	g/l
2-methyloctadecane	0.0570
2-methyleicosane	0.0248

compound	g/l
dotriacontane nC_{32}	0.0371
hexamethylbenzene	0.0362
benzo(ghi)perylene	0.0356

The standards for the recoveries of concentration under nitrogen, concentration under rotary evaporator, and column chromatography are listed below.

Standard B

compound	g/l
hexadecane nC_{16}	0.0584
eicosane nC_{20}	0.0242
octacosane nC_{28}	0.0236
dotriacontane nC_{32}	0.0260
1-methylfluorene	0.0236
pyrene	0.0220
benzo(a)pyrene	0.0238

Warner (1976) determined that different spike concentrations varied in their recoveries: a spike concentration of 10 μg/g had recoveries in the range of 65-100%; 2.0 μg/g had a recovery range of 70-103%; 0.4 μg/g had a range of 53-102%; and 0.1 μg/g had a range of 40-90%. With the range of 3-8 μg total extracted hydrocarbons for the tissue samples, another factor besides recoveries should be considered in determining the spike concentration. To maximize the parameters of recoveries versus sample masking, the spike concentrations 0.10-0.28 μg/g were chosen which were therefore close to the lowest concentration (0.10 μg) used by Warner (1976).

Methods

Determination of Replicate Numbers

Prior to the actual tissue analysis, an attempt was made to determine the number of replicates necessary for statistically valid results. The recoveries for the spiked blanks and a 95% confidence level were utilized in the calculations. The number of replicates necessary was dependent upon the power of the test. An error of the first kind (Type I) is made when the experimenter rejects the null hypothesis and it is true. An error of the second kind (Type II) is made when the experimenter accepts the null hypothesis and the alternative is true. When both types of error are considered in the case of a sample of fixed size, it is seen that a reduction in the probability of a Type I error (α) must be accompanied by an increase in the probability of a Type II error (β). In an ideal situation, both α and β are fixed in advance; this determines the required sample size. The power of a test is its ability to detect the alternative hypothesis when it is true ($1-\beta$). It is obviously desirable to have this probability high. In order to optimize the parameters with α at 0.05, nineteen replicates had a power of 70% while nine replicates had 40%. The nine replicates were chosen due to outside limiting factors (limited time and equipment). However, these calculations provide some indication of the limits of any statistical handling of the results. It could not be determined how valid it was to utilize results from spiked blanks for spiked tissue.

Preparation of tissue homogenate

The tissue and fluids of eight medium-sized clams *Mercenaria mercenaria* were homogenized at medium-high speed using a Virtis "45"

homogenizer. To determine the homogeneity of the aliquots, the homogenate was first frozen in hexane-washed metal ice cube trays; three frozen cubes were weighed, then dried overnight in a 125°C oven to a constant weight. The dry weight/wet weight ratio average for the three tissue cubes was 0.14669 ± 0.00364.

Extraction techniques

Digestion in KOH-methanol (Method B)

The technique of Smith et al. (1977) was followed. Ten grams of homogenate (one homogenate cube) were weighed into a tared boiling flask. Fifteen ml of 0.5 N potassium hydroxide in methanol and 50 µl of Standard A were added. The sample was refluxed for four hours, then allowed to cool to room temperature.

The digested mixture was transferred to a separatory funnel; the flask was rinsed three times with methanol into the separatory funnel. Equal volumes of saturated salt solution and hexane were added; the volume was equivalent to the volume of the digested mixture. The mixture was shaken vigorously, and after separation of aqueous and organic layers (usually 0.5-1 hr), the aqueous layer was drained off and the organic layer, including any emulsion, was transferred to centrifuge tubes and centrifuged at 2000 rpm for five minutes. The supernatant was transferred by Pasteur pipet to an Erlenmeyer flask with a ground glass stopper. The emulsion and aqueous layer were returned to the separatory funnel and the extraction repeated three more times. The combined hexane extracts were back-extracted four times with saturated salt solution; the aqueous solutions were centrifuged when necessary. The salt solution was saved and re-extracted

once with hexane. All hexane extracts were combined and dehydrated over sodium sulfate overnight.

Soxhlet extraction (Method C)

The procedure of Bieri and Stamoudis (1977) was followed. Ten grams of homogenate were weighed into a tared 250 ml boiling flask. Fifty µl of Standard A, 5.5 g KOH pellets, 60 g methanol and 20 ml of hexane-extracted deionized water were added. The mixture was refluxed for 2 hours at 83-87°C then allowed to cool to room temperature. It was then transferred into a coarse-fritted glass thimble, which was placed in a Soxhlet extractor containing 200 ml of methanol. Extraction proceeded for 15 hrs at a speed of 1.5-2 cycles per hour. The extract was allowed to cool to room temperature, and the extract removed and saved. Fresh methanol was added and the procedure was repeated; the two methanol extracts were combined. The total extract was transferred to a 2000 ml separatory funnel.

Approximately 300 ml of hexane-extracted deionized water, 16 g NaCl and approximately 80 ml pentane were added. The mixture was shaken thoroughly and the layers allowed to separate, usually 1-2 hrs. The aqueous layer and any emulsion were then drained into a beaker, and the clear pentane layer was transferred to an Erlenmeyer flask equipped with a ground glass stopper. The aqueous portion was returned to the separatory funnel and the procedure was repeated twice. The pentane layer and any emulsion formed in the third extraction were combined with the other two extracts in a separatory funnel and washed three times with 500 ml hexane-extracted deionized water. If any emulsion was present after the third wash, the mixture was shaken with 0.5-1.0 g NaCl and left 15-30 min. The water layer was discarded and

the pentane extract was transferred to an Erlenmeyer flask; the funnel was rinsed well with about 20 ml pentane which was then added to the extract. Ten grams of anhydrous Na_2SO_4 were added, the flask stoppered overnight and the sample allowed to dehydrate.

MacLeod's Procedure (Method A)

Ten grams of homogenate were weighed into a tared 50 ml, teflon-lined, screw-capped centrifuge tube. Six ml of 4N NaOH and 50 µl of Standard A were added and the mixture shaken vigorously for one minute. The tubes were placed in a 30°C water bath for 18 hrs. At the end of this period, the samples were cooled to room temperature (MacLeod et al., 1976).

Fifteen ml of peroxide-free ether were added to the samples, the tubes recapped tightly and shaken vigorously for one minute. The tubes were then centrifuged at 2000 rpm for 10 min. If the ether phase was clear, it was transferred with a Pasteur pipet to a 50 ml glass stoppered centrifuge tube. Approximately 1 g of anhydrous Na_2SO_4 was added, without agitation. The extraction was repeated with 10 ml of ether. The extracts were combined and 3 g of anhydrous Na_2SO_4 were added. The samples were left stoppered overnight (MacLeod et al., 1976).

Evaporation of excess solvent

Sample volumes for KOH alcoholic digestion and Soxhlet extraction were reduced to about 6 ml in a warm water bath using a rotary evaporator connected to a water aspirator for removal of vapors. The concentrated sample was then transferred by Pasteur pipet into 15 ml tubes and further concentrated, in a warm-water bath 30°C for ether

samples and 60°C for all others) under a gentle N_2 stream, to about 1.0 ml.

Column chromatography

A 10 x 300 mm chromatographic column with a sealed-in coarse porosity fritted disc in the bottom was used. The column was packed by pouring a hexane slurry of activated Bio-Sil silica gel into the tube. The silica was settled by vibration to a height of 17.5 cm (about 7 g). About 0.5 cm of Soxhlet-extracted sand was added. The column was washed with about 40 ml hexane. When the solvent level reached the top of the adsorbent, the sample was added using a Pasteur pipet. After the level of the sample had reached the top of the adsorbent, the tube was rinsed with about 1 ml hexane. Collection of the fractions began immediately after the sample was added. First the column was eluted with hexane. The first 5 ml were discarded; the next 15 ml were saved and labeled as the aliphatic fraction. Then the column was eluted with 40/60 (v/v) benzene-hexane mixture; the next 30 ml were collected and labeled as the aromatic fraction. Fractions were concentrated to about 0.2 ml as described above (Smith et al., 1977; Bieri and Stamoudis, 1977).

Gas chromatography (gc)

The gas chromatograph was a Varian 2740 with dual columns, direct data outputs to strip chart recorders in a Hewlett-Packard 3352B laboratory data system. Glass capillary columns, coated by the method of Grob et al. (1977), were 20 m x 0.28 mm with 0.20% SE52, a silicon methyl phenyl liquid phase, and were programmed from 50-240°C at 6°C/minute (then held until nC_{32} detected). The He carrier gas had a

pressure of around 12 psi with a total flow rate of 30 ml/minute. With the splitters open, the He flow into the columns was 6-8 ml/minute. The flame ionization detector had a H_2 flow rate of 30 ml/minute and an air flow of 300 ml/minute at 265°C. Sensitivity was 1×10^{-11} amp/mv.

Response factors of aliphatic and aromatic standards (listed in Tables A24 and A25 Appendix) were used for the quantification of the sample fractions. Two fractions were injected simultaneously as a time saving step. The columns were independently calibrated and the two fractions never compared one to another. With the splitters closed and a 50°C oven, samples were injected; timed three seconds then the syringes were removed. Temperature programming was started one minute after the removal of the syringes. Splitters were opened for each channel when the solvent peak appeared.

Method of quantification

Standards (Tables A24 and A25 Appendix) were first injected into each column; one column was used exclusively for aromatic compounds and the other for aliphatic compounds. Once each peak was identified and assigned a retention index and area, the F factor was calculated for each peak as follows:

$$F = \frac{\text{concentration (mg/}\mu\text{l) x injection volume (}\mu\text{l) x 1000}}{\text{area (microvolt-sec)}}$$

where the concentration was the known standard concentration and the area of the peak under study.

Then for each standard a graph was made for F factor versus retention indices (R.I.).

Peaks in the sample chromatogram were then identified and assigned retention indices and areas. The area for each peak was

multiplied by the F factor (taken from the standard graph for that particular channel and retention index) to give the μgrams injected. This number was then multiplied by the K factor as follows:

$$K = \frac{\text{total volume (ml)}}{1000 \times \text{dry weight (g)} \times \text{injection volume } (\mu l)}$$

where dry weight = dry weight/wet weight ratio x total sample wet weight to give the μg/g dry weight for that peak. The hydrocarbon weight was totaled for all the peaks to get the total extracted hydrocarbons for either the aliphatic or aromatic fraction.

This calculation process was classified as the external standard method which assumes 100% recovery for the extracted hydrocarbons. The internal standard method calculates the theoretical recovery for extracted hydrocarbons based upon the known percent recoveries of added standards.

Preliminary determination of efficiencies

Loss of hydrocarbons is a possible problem at a number of steps critical to the analyses. To estimate the occurrence and the magnitude of loss, extraction efficiencies were determined for each technique using standards in place of samples. Extraction efficiencies were determined for each of the three methods by placing 50 μl of Standard A solution in the appropriate vessel (see extraction procedures above), and treating it as sample. Loss during solvent reduction was estimated by placing a 100 μl aliquot Standard B, diluted to 100 ml, in a boiling flask, and treating it as a sample. Loss during column chromatography was measured by fractionation of a 100 μl aliquot of Standard B that had been diluted to 1.0 ml with hexane. Loss during evaporation under N_2 was estimated by reduction of a 100 μl aliquot

of Standard B (diluted to 1.0 ml in hexane) to about 0.2 ml in a 15 ml glass stoppered centrifuge tube. Standard concentrations were measured by GC and their efficiencies determined.

Statistical Analysis

Parametric techniques were chosen for the statistical analysis of the data, and the data were examined to confirm that they satisfied the underlying assumptions for which the techniques are valid: homogeneity of variance; normal distribution; and random samples. Bartlett's test of homogeneity of variance (Snedecor, 1956) was computed for the extracted recovered weights and for the arcsine transformed percent recoveries for the internal standards. In the handling of the percentages, if the form of the parent population is known to be reasonably close to a distribution for which there is a standard (parametric) theory, or if the data can be transformed so that such is the case, then parametric procedures extract more information from the data than do nonparametric procedures (Steel and Torrie, 1960). Arcsine transformation fulfills this requirement. Another procedure to check the normal distribution of data is a graphic method describing the relationships expressed be the normal distribution available in terms of probability paper. Data which follow the normal probability curve will plot as a straight line on this grid, thus, there is available a quick method of testing the normality of a series of observations. The parametric techniques of ANOVA and Student-Newman-Keul's Test were applied to the evaluation data.

RESULTS

Nine replicates were used for the Soxhlet extraction and KOH alcoholic digestion; thirteen replicates were used for MacLeod's procedure. Bartlett's Tests and the probability graphs indicated that the extracted recovered weights and percent standard recoveries were normally distributed if the #9 KOH alcoholic digestion replicate for extracted recovered weights is not included. The aromatic and aliphatic hydrocarbon fractions of clam tissue extracts as determined by gas chromatography are given in Tables 2 and 3, respectively. These weights were calculated using the external standard method. Mean ($\bar{x}$) recoveries for the aliphatic fraction were 7.04 µg/g dry weight for MacLeod's procedure, 5.24 µg/g dry weight for the Soxhlet extraction and 7.96 µg/g dry weight for KOH alcoholic digestion. For the aromatic fraction, the mean ($\bar{x}$) recoveries were 4.27 µg/g dry weight, 3.73 µg/g dry weight and 3.95 µg/g dry weight respectively. An analysis of variance (ANOVA) was performed on the means for each technique (Table A26 Appendix) and showed no significant difference between techniques ($P<0.05$). An examination of the gas chromatograms indicated that all three techniques were extracting the same or similar compounds. The aliphatic fraction was characterized by the n-alkanes, nC_{22} through nC_{31}. The aromatic fraction had two distinct groups of peaks: one with retention indices between 1700 and 2300; the other with retention indices between 2800 and 3100. The large standard deviation determined for KOH alcoholic digestion

TABLE 2

RECOVERED WEIGHTS OF ALIPHATIC HYDROCARBON FRACTION

CALCULATED BY THE EXTERNAL STANDARD METHOD

(μG/G DRY WEIGHT TISSUE)

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	8.93	2.20	4.81
2	7.45	4.32	9.59
3	8.60	5.03	7.87
4	4.63	7.14	6.12
5	4.92	6.92	4.19
6	3.07	6.58	5.81
7	5.72	3.98	8.73
8	5.87	3.01	4.99
9	7.30	7.98	19.55
10	10.00		
11	9.64		
12	8.33		
13	7.03		
$\bar{x} \pm s$	7.04 ± 2.09	5.24 ± 2.01	7.96 ± 4.72

(spikes not included in recovered weights)

TABLE 3

RECOVERED WEIGHTS OF AROMATIC HYDROCARBON FRACTION

CALCULATED BY THE EXTERNAL STANDARD METHOD

(μG/G DRY WEIGHT TISSUE)

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	4.23	2.56	2.36
2	5.03	3.67	2.47
3	5.19	4.01	5.96
4	4.73	3.92	2.84
5	3.03	4.37	2.95
6	2.88	3.36	2.83
7	4.12	4.11	4.38
8	3.67	3.17	2.78
9	5.87	4.44	8.97
10	2.96		
11	4.93		
12	4.84		
13	4.05		
$\bar{x} \pm s$	4.27 ± 0.94	3.73 ± 0.61	3.95 ± 2.21

(spikes not included in recovered weights)

was due to replicate #9. With this replicate excluded, the standard deviation for the KOH method was similar to those for the other two techniques and still no differences between techniques were detected by ANOVA.

The percent recoveries of the standard compounds after arcsine transformation from the spiked clam tissue were:

Standard	Percent Recovery ($\bar{x} \pm s$)
2-methyloctadecane	54.10 ± 12.53
2-methyleicosane	50.84 ± 12.64
dotriacontane nC_{32}	41.57 ± 9.60
hexamethylbenzene	36.90 ± 7.04
benzo(ghi)perylene	65.54 ± 11.59

(Tables A1-A5 Appendix)

A statistical examination of the standard and indigeneous compounds showed no difference in the recoveries for the three techniques. The standard compounds did, however, show (by ANOVA, Table A27 Appendix) a significant variation in their percent recoveries. The Student-Newman-Keul's Test (SNK Table A6 Appendix) illustrates the differences.

The data listed in Tables 2 and 3 were normalized to 100% efficiency using the internal standard method. In this method, the area of the selected standard was assumed to indicate 100% recovery and the areas for the other peaks were normalized in relation to this standard. The means for the normalized data are listed in Table 4. ANOVA (Tables A28 and A29 Appendix) for the normalized data revealed significant differences between methods for 2-methyloctadecane, 2-methyleicosane, nC_{32}, and benzo(ghi)perylene; there was no significant

TABLE 4

NORMALIZED RECOVERED WEIGHTS OF HYDROCARBONS (μG/G DRY WEIGHT TISSUE)

($\bar{x} \pm s$)

Aliphatic Fraction

Internal Standard	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
2-methyloctadecane*	13.08 ± 3.73	9.08 ± 2.13	15.27 ± 6.05
2-methyleicosane*	13.82 ± 3.91	9.57 ± 2.68	17.28 ± 10.30
nC_{32}*	15.11 ± 3.68	9.46 ± 5.72	10.96 ± 5.88
	Aromatic Fraction		
hexamethylbenzene	12.69 ± 4.26	9.12 ± 1.29	11.84 ± 7.78
benzo(ghi)perylene*	7.21 ± 1.22	5.94 ± 1.08	5.05 ± 2.17

Tables A7-A11 Appendix

*ANOVA indicated significant difference between techniques at the 95% confidence level.

difference in extraction recoveries for hexamethylbenzene. The Student-Newman-Keul's Tests for the four significant fractions illustrate the differences in Table 5.

Spiked blanks were run for each technique to provide an indication of the losses due solely to the presence of tissue. The means ($\bar{x}$) and standard deviations (s) are illustrated in Table 6.

ANOVA (Table 30 Appendix) indicated no significant difference between the extraction recoveries of the three techniques at the 95% confidence level. ANOVA (Table A30 Appendix) indicated no difference between compounds in extraction recoveries for KOH alcoholic digestion and Soxhlet extraction; the SNK Test for MacLeod's procedure is illustrated in Table A19 (Appendix).

Concentration under N_2, concentration under rotary evaporator and column chromatography were evaluated utilizing a spike with standards similar to the ones used for the spiked tissue and blanks. The recoveries for the standards at various steps in the techniques were:

Procedure	Percent Recovery ($\bar{x} \pm$ s
procedure blank	70.4 ± 15.0
column chromatography and concentration under N_2	80.8 ± 6.4
concentration by rotary evaporator and N_2	91.8 ± 4.0
concentration under N_2	94.1 ± 3.0

(Tables A16-A18; A20-A22 Appendix)

ANOVA (Table A31 Appendix) indicated no difference between compounds for the two concentration steps. The SNK for the column chromatography (Table A23 Appendix) showed a significant difference

TABLE 5

STUDENT-NEWMAN-KEUL's TESTS (SNK)

Aliphatic Fraction Normalized to 2-methyloctadecane Internal Standard

Soxhlet Extraction	MacLeod's Procedure	KOH Alcoholic digestion
9.08	13.08	15.27

Aliphatic Fraction Normalized to 2-methyleicosane Internal Standard

Soxhlet Extraction	MacLeod's Procedure	KOH Alcoholic digestion
9.57	13.82	17.28

Aliphatic Fraction Normalized to nC_{32} Internal Standard

Soxhlet Extraction	KOH Alcoholic digestion	MacLeod's Procedure
9.46	10.96	15.11

Aromatic Fraction Normalized to benzo(ghi)perylene Internal Standard

KOH Alcoholic digestion	Soxhlet Extraction	MacLeod's Procedure
5.05	5.94	7.21

Tables A12-A15 Appendix

Those compounds sharing a common underline were not significantly different. Results are in terms of µg/g dry weight tissue.

TABLE 6

STANDARD RECOVERIES AS ARCSINE TRANSFORMED PERCENTS FROM SPIKED BLANKS FOR THE COMPLETE TECHNIQUES ($\bar{x} \pm s$)

Internal Standard	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
2-methyloctadecane	74.0 ± 8.2	77.3 ± 3.3	74.5 ± 5.2
2-methyleicosane	76.0 ± 11.1	79.6 ± 11.3	76.7 ± 10.4
nC_{32}	51.6 ± 11.1	91.1 ± 45.0	55.6 ± 26.2
hexamethylbenzene	54.0 ± 9.2	54.8 ± 6.9	53.3 ± 8.3
benzo(ghi)perylene	78.4 ± 9.9	81.7 ± 5.2	77.4 ± 9.7

(Tables A16-A18 Appendix)

($P<0.05$) between nC_{32} and the two compounds nC_{28} and benzo(a)pyrene.

The reproducibility of gas chromatographic injections for aliphatic and aromatic fractions is illustrated in Tables A24 and A25.

DISCUSSION

This investigation was conducted to evaluate three commonly used procedures for the extraction of hydrocarbons from clam tissue. The total recovered hydrocarbons from MacLeod's procedure (11.31 μg/g dry weight), KOH alcoholic digestion (11.91 μg/g dry weight), and Soxhlet extraction (8.97 μg/g dry weight) indicated that MacLeod's procedure and KOH alcoholic digestion appeared marginally more efficient than Soxhlet extraction but these differences were not statistically significant. Normalization of the recovered hydrocarbon fractions to internal standards produced anomalous results (Tables 4 and 5). Significant differences among techniques were determined for fractions normalized to all standards except hexamethylbenzene. Interpretation is difficult because no technique proved to be consistently superior. The only consistent observation in this evaluation was that Soxhlet extraction was least efficient for extraction of aliphatic hydrocarbons. However, the three techniques must be considered equivalent since raw recoveries were statistically similar and recoveries normalized to the internal standards did not behave consistently.

Commonly used techniques for the quantification of hydrocarbons from environmental samples presently include gravimetric and gas chromatographic determination using either the external standard method or the internal standard method. Gravimetric determination provides a bulk measurement of the extracted hydrocarbons with very

little qualitative data and yields no indication of the efficiency of the extraction of hydrocarbons. Gravimetric methods are most useful where relatively large amounts (10^{-4} g) of each hydrocarbon fraction exist (Clark, 1973). Gas chromatography provides both a separation and a quantitative estimate of specific compounds. Quantification by the external standard method provides no indication of the efficiency of hydrocarbon extraction. The internal standard method provides some indication of extraction efficiencies, however, variations in recoveries of the internal standards themselves induce uncertainties in results. This study demonstrates that *different interpretations of data can be made based solely upon the quantification method.* In previous studies, Gritz and Shaw (1977), Warner (1976), and Blaylock et al. (1973) used the internal standard method; Farrington and Medeiros (1975) used the gravimetric method and MacLeod et al. (1976) used a gravimetric method and a gas chromatographic method utilizing a standard added after extraction and concentration procedures. It is important that several methods of quantification yield the same results before concluding that one extraction technique is more efficient than another.

All of the added hydrocarbon standards were recovered in less than 100% yield. These spike recoveries appear to be similar to recoveries cited in other references (Blaylock et al., 1973; Warner, 1976; Gritz and Shaw, 1977). Direct comparison is not appropriate since different organisms, spike compounds and concentrations were used. The slightly lower levels recovered in this study may be due to the low concentration of spiked standards (0.10-0.28 μg/g). Previous works (Warner, 1976; Gritz and Shaw, 1977) used concentration

levels two to ten times those utilized in this study. Warner (1976) demonstrated that recovery yield decreases with decreasing standard concentration. Preliminary work in this laboratory indicated that the selected standard concentrations gave adequate recovery and showed little interference with the indigenous hydrocarbons. The concentration of the spike was chosen to be similar in magnitude to individual compounds in the sample so that detector nonlinearity would not be troublesome.

Nearly all the losses of compounds in the spike blanks were accounted for by three processes: concentration under nitrogen, concentration by rotary evaporation, and column chromatography. Hexamethylbenzene and nC_{32} had the lowest recovery yields. Hexamethylbenzene is a comparatively low boiling hydrocarbon which might be susceptible to volatilization, however, previous work (Blaylock et al., 1973) has indicated higher recoveries for compounds with lower boiling points than hexamethylbenzene. Since hexamethylbenzene was not used in the evaluation of the steps within the procedures (concentration under nitrogen, rotary evaporation, and column chromatography), there is no way of determining where the losses occurred. *A comparison of the standard recoveries for the clam tissue and the spike blanks indicated a 10-20% loss due to the presence of the tissue with hexamethylbenzene having one of the higher losses.* This indicated a possible interference with extraction by the tissue matrix. Low nC_{32} yields were determined for the spiked blanks and spiked tissue samples which underwent column chromatography, but not for the samples concentrated only under rotary evaporation and nitrogen. This would suggest that silica gel in the column chromatography procedure

retains the higher molecular weight compounds, however, previous work (Gritz and Shaw, 1977) did not report low recoveries of nC_{32}.

A possible factor in the determination of losses may be the low concentrations of standards used in this study. For example, a given procedure may retain a constant amount of compound regardless of the original concentration. This loss would be minimal where the original concentration is large with respect to that loss but highly significant when magnitudes are similar.

The variability between replicates may be partially due to the gas chromatography procedure. For the n-paraffins larger than nC_{25}, the reproducibility of gas chromatographic injections may vary as much as 12-22%. This could be due to a change in sensitivity with increasing molecular weight or a change in the shape and size of peaks at the upper end of the chromatogram (Clark, 1973). However, the aromatic standards (Table A25, Appendix) varied from only 1.5-9%, excluding benzo(ghi)perylene which varied more than 50%. Benzo(ghi) perylene is one of the higher molecular weight compounds examined. The explanation for this variability is probably the same as for the larger n-paraffins. MacLeod et al. (1976) checked the reproducibility of replicate gas chromatographic sample injections and found variations of 10-17% for nC_{24} to nC_{29} with nC_{30} varying 51% and nC_{31}, 38%. Grob and Grob (1978) have reportedly eliminated this problem with on-column injections onto the glass capillary columns. Gas chromatographic injections in this study were highly reproducible for most compounds. The variability within the recovered hydrocarbon weights ranged from 17-60%. Blaylock et al. (1973) found that variation in recovery of the individual n-alkanes was two to eight times lower for the high

concentration levels than for the minimum and intermediate concentration levels. Again, this indicates the importance of the effect of concentration levels on the results. *Given the observed uncertainties within the techniques studied here, small differences between techniques are not significant.* Choice of one technique over another based on these small differences (i.e. Rohrback and Reed's (1975) recommendation of one technique over another based on a 4% difference) is not valid.

This evaluation attempted to concentrate on just a few techniques and factors influencing the efficient extraction of hydrocarbons from organisms. The selection of the three techniques (Soxhlet extraction, MacLeod's procedure, and KOH alcoholic digestion) was based upon several factors: previous utilization in the laboratory (Bieri and Stamoudis, 1977; Smith et al., 1977); utilization in previous evaluation studies (Blaylock et al., 1973; Farrington and Medeiros, 1975; Gritz and Shaw, 1977); and previous determination of high recoveries and simplification of techniques (Warner, 1976; MacLeod et al., 1976). Similar yields were expected due to the basic similarities between techniques (an alkaline digestion followed by an organic solvent extraction) and the results of previous evaluations (Farrington and Medeiros, 1975; Gritz and Shaw, 1977). Alkaline digestion prior to Soxhlet extraction was performed to totally disrupt the cell for easier access to hydrocarbons contained within the cell. It was felt that Soxhlet extraction would not give complete recovery as hydrocarbons associated with the cell lipids may not be adequately separated from the tissues (Blaylock et al., 1973). Selection of clam tissue was indicated by the previous utilization of shellfish

for hydrocarbon extraction studies (Blaylock et al., 1973; Farrington and Medeiros, 1975; Gritz and Shaw, 1977). This study encompassed procedures of past evaluations by including alkaline digestion and Soxhlet extraction (Farrington and Medeiros, 1975; Gritz and Shaw, 1977).

This study has tried to improve on previous evaluations by demonstrating the need to evaluate techniques using several quantitative methods, by the use of a larger number of replicates, and by the application of statistical tests for significance of results.

An infrequently mentioned difficulty is the formation of persistent emulsions during the extraction procedure. In this study, recoveries for Soxhlet extraction and KOH alcoholic digestion were not lowered where an emulsion was present compared to when it was absent. At the present time, the most satisfactory treatment consists of extensive time consuming, solvent-solvent partitioning. Emulsions appear to be due to a number of factors. Blaylock et al. (1973) found that the addition of excessive water tended to reduce the extraction efficiency because of emulsion formation during the solvent extraction procedure. Gritz and Shaw (1977) and previous work in this laboratory indicated that normal alkane solvents such as hexane or pentane caused emulsion problems. The use of diced rather than homogenized tissue reduced the formation of an emulsion without affecting hydrocarbon recovery (Gritz and Shaw, 1977). No emulsion was formed in this study with the use of an aqueous rather than an alcoholic digestion mixture. One problem which may occur with MacLeod's procedure and KOH alcoholic digestion is the formation of a gel upon solvent evaporation. This highly viscous material can

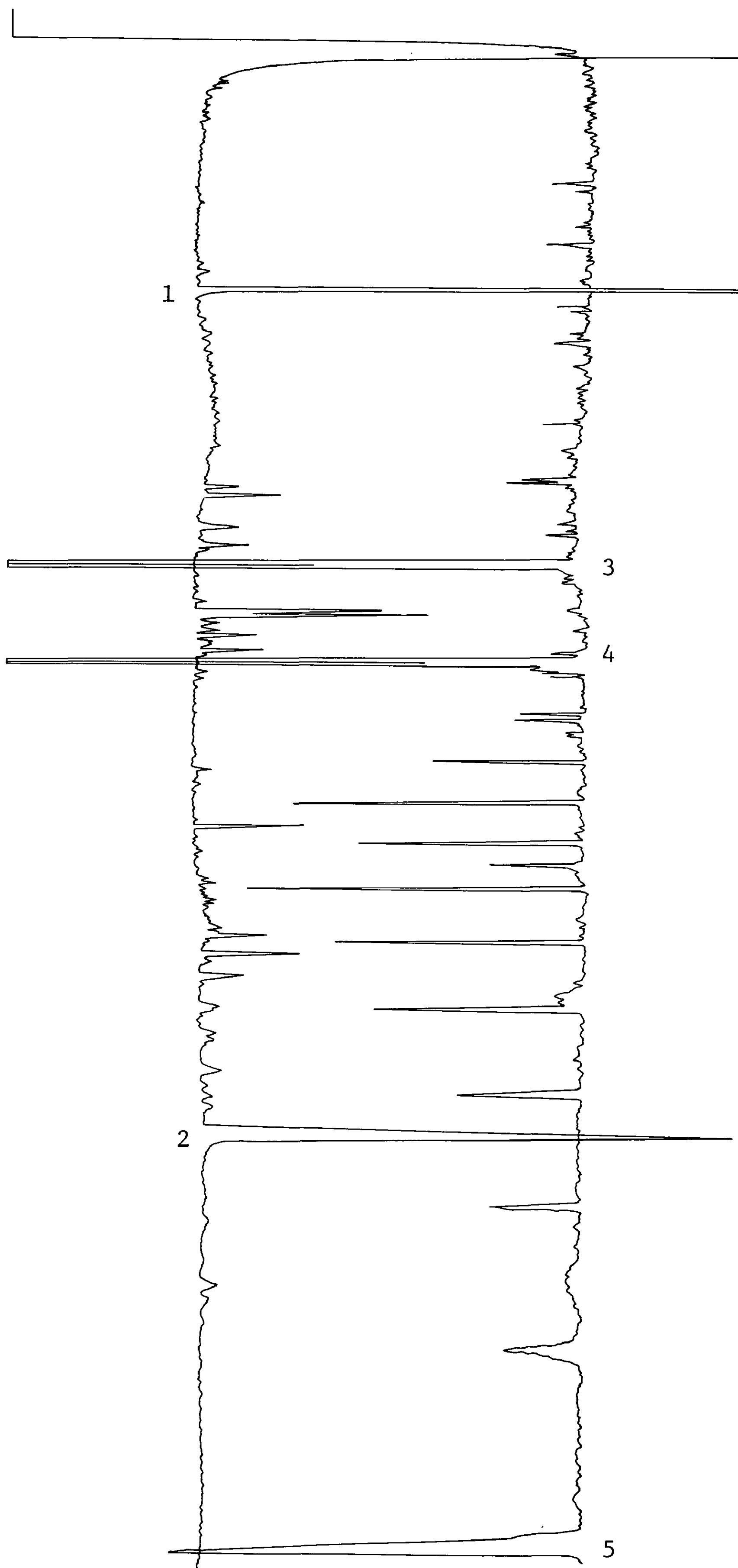
plug an adsorption chromatography column, preventing solvent flow. However, the sample gel can be dissolved in methylene chloride and filtered through a bed of chromatographic silica gel prewashed with methylene chloride. The methylene chloride solvent is then displaced by hexane for adsorption chromatography (MacLeod et al., 1976).

Drawing of conclusions from the results in any evaluation of hydrocarbon extraction techniques should always be done with the correct perspective. The results of this study and previous work illustrate that the determination of the most efficient procedure may be influenced by the selection of a given spike, concentration or quantification method. Any conclusion drawn should be conditional upon the factors that influence the results of the evaluation.

Other factors become pertinent in influencing a decision when all three techniques extract the same relative amounts. When time and equipment are considered, it becomes readily apparent that MacLeod's procedure is the preferred technique. By using aqueous caustic for digestion, there was no need for extraction steps to remove alcohol prior to the silica gel chromatography. The entire digestion and extraction steps were conveniently carried out in a single screw-cap centrifuge tube. Soxhlet extraction and KOH alcoholic digestion required either extensive time or equipment or both.

Additional work is needed to generalize the evaluation of these techniques. The effect of different tissue types and standard concentrations are worthy of study. The formation of emulsions or gels presents a difficulty within these techniques which also deserves further study.

A P P E N D I X



1
3
A
B
4
2
5

Figure 1A. Gas chromatogram of the (A) aromatic fraction and (B) aliphatic fraction of clam tissue extracted by MacLeod's procedure, with the standardppeaks 1) hexamethylbenzene, 2) benzo(qhi)perylene, 3) 2-methyloctadecane, 4) 2-methyleicosane, and 5) nC_{32}.

TABLE A1

ARCSINE TRANSFORMED PERCENT RECOVERY FOR 2-METHYLOCTADECANE

FROM SPIKED CLAM TISSUE

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	61.30	42.70	47.98
2	62.37	41.39	53.23
3	57.57	51.23	49.17
4	40.98	63.02	41.35
5	23.73	71.05	40.61
6	42.98	67.15	45.23
7	51.30	47.25	53.78
8	73.09	49.26	50.30
9	43.20	73.21	66.70
10	66.12		
11	61.60		
12	81.19		
13	57.11		
	$\bar{x} \pm s$	54.10 ± 12.53	

TABLE A2

ARCSINE TRANSFORMED PERCENT RECOVERY FOR 2-METHYLEICOSANE

FROM SPIKED CLAM TISSUE

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	55.38	41.25	48.63
2	67.41	46.78	49.56
3	61.29	48.72	50.23
4	38.79	62.75	38.47
5	20.94	53.37	37.26
6	31.63	67.17	43.17
7	37.63	45.21	52.01
8	69.83	43.31	50.64
8	69.83	43.31	50.64
9	42.98	59.28	45.10
10	71.46		
11	61.20		
12	76.90		
13	57.87		
	$\bar{x} \pm s$	50.84 ± 12.64	

TABLE A3

ARCSINE TRANSFORMED PERCENT RECOVERY FOR nC_{32}

FROM SPIKED CLAM TISSUE

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	46.74	27.38	37.91
2	42.03	38.43	38.92
3	62.63	39.01	41.03
4	39.62	41.25	34.71
5	36.62	45.83	29.18
6	36.78	39.38	32.78
7	28.92	40.17	42.62
8	60.55	36.04	30.48
9	38.22	47.21	35.60
10	64.59		
11	54.95		
12	57.68		
13	41.54		
	$\bar{x} \pm s$	41.57 ± 9.60	

TABLE A4

ARCSINE TRANSFORMED PERCENT RECOVERY FOR HEXAMETHYLBENZENE

FROM SPIKED CLAM TISSUE

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	50.11	36.60	41.43
2	45.73	32.68	30.48
3	34.87	45.63	32.01
4	28.54	45.03	41.94
5	26.20	51.19	34.25
6	34.21	38.52	32.35
7	35.12	48.76	35.68
8	36.81	31.00	36.10
9	36.85	42.38	30.30
10	39.07		
11	36.51		
12	20.80		
13	33.04		

$\bar{x} \pm s$ 36.90 ± 7.01

TABLE A5

ARCSINE TRANSFORMED PERCENT RECOVERY FOR BENZO(GHI)PERYLENE

FROM SPIKED CLAM TISSUE

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	57.48	74.16	90.13
2	57.25	66.45	78.18
3	69.50	57.09	83.30
4	60.39	71.13	70.25
5	42.91	67.29	69.79
6	57.63	48.12	63.29
7	69.30	63.78	71.92
8	61.83	53.34	66.70
9	67.60	72.83	93.94
10	47.90		
11	54.40		
12	69.90		
13	54.06		
	$\bar{x} \pm s$	65.54 ± 11.59	

TABLE A6

STUDENT-NEWMAN-KEULS' TEST FOR THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF SPIKED CLAM TISSUE

	Ms	df
error	118.57936	150

$S_{\bar{x}} = \sqrt{118.57936/31} = 1.9557967$

$Wp = P \times S_{\bar{x}}$

treatment	hexamethylbenzene	nC_{32}	2-methyleicosane	2-methyoctadecane	benzo(ghi)perylene
$\bar{x}$	36.91	41.57	50.85	54.10	65.58

P	2	3	4	5
q(P,150)	2.77	3.31	3.63	3.86
Wp	5.42	6.47	7.10	7.55

65.58-36.91 = 28.61*
65.58-41.57 = 24.01*
65.58-50.85 = 14.71*
65.58-54.10 = 11.48*

54.10-36.91 = 17.19*
54.10-41.57 = 12.53*
54.10-50.85 = 3.25
50.85-36.91 = 14.94*
50.85-41.57 = 9.28*
41.57-56.91 = 4.66

*significant

Those compounds sharing a common underline were not significantly different.

TABLE A7

ALIPHATIC HYDROCARBON FRACTION (μG/G DRY WEIGHT TISSUE)

NORMALIZED TO 2-METHYLOCTADECANE INTERNAL STANDARD

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	14.57	5.15	10.02
2	11.94	10.43	18.02
3	14.94	9.82	16.00
4	11.30	11.33	14.80
5	20.73	9.74	10.32
6	7.13	9.80	12.84
7	11.15	8.42	16.23
8	8.03	6.12	9.92
9	16.90	10.90	29.31
10	15.12		
11	15.64		
12	10.25		
13	12.31		
	$\bar{x}$ 13.08	$\bar{x}$ 9.08	15.27

significant

TABLE A8

ALIPHATIC HYDROCARBON FRACTION (μG/G DRY WEIGHT TISSUE)

NORMALIZED TO 2-METHYLEICOSANE INTERNAL STANDARD

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	16.12	5.33	9.89
2	11.05	9.23	19.35
3	14.03	8.21	15.67
4	11.94	11.38	15.91
5	23.49	12.97	11.25
6	9.70	9.79	13.46
7	15.20	8.80	16.78
8	8.41	6.95	9.85
9	16.98	13.46	43.35
10	13.99		
11	15.75		
12	10.83		
13	12.15		
	$\bar{x}$ 13.82	9.57	17.28

significant

TABLE A9

ALIPHATIC HYDROCARBON FRACTION (µG/G DRY WEIGHT TISSUE)

NORMALIZED TO nC_{32} INTERNAL STANDARD

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	19.10	9.35	6.23
2	17.73	9.57	6.35
3	13.73	10.28	14.52
4	11.69	9.50	8.18
5	13.41	9.53	10.11
6	8.35	8.53	8.63
7	19.81	10.23	10.27
8	9.69	8.79	9.12
9	19.10	9.40	25.20
10	15.48		
11	17.54		
12	14.44		
13	16.92		
	$\bar{x}$ 15.11	9.46	10.96

significant

TABLE A10

AROMATIC HYDROCARBON FRACTION (μG/G DRY WEIGHT TISSUE)

NORMALIZED TO HEXAMETHYLBENZENE INTERNAL STANDARD

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	8.44	6.99	5.10
2	10.99	11.23	8.11
3	14.88	8.79	18.62
4	16.56	8.71	6.77
5	11.56	8.54	8.61
6	8.39	8.72	8.74
7	11.73	8.43	12.75
8	9.97	10.23	7.70
9	15.92	10.48	29.60
10	7.58		
11	13.50		
12	23.26		
13	12.26		
	$\bar{x}$ 12.69	9.12	11.84

not significant

TABLE A11

AROMATIC HYDROCARBON FRACTION (μG/G DRY WEIGHT TISSUE)

NORMALIZED TO BENZO(GHI)PERYLENE INTERNAL STANDARD

Replicates	Techniques		
	MacLeod's Procedure	Soxhlet Extraction	KOH Alcoholic Digestion
#1	7.36	3.45	2.62
2	8.79	5.52	3.16
3	7.47	7.02	7.15
4	7.83	5.51	4.04
5	7.05	6.49	4.23
6	5.00	6.98	4.47
7	5.95	6.44	6.09
8	5.94	5.94	4.17
9	8.68	6.10	9.54
10	6.18		
11	9.06		
12	6.92		
13	7,49		
	$\bar{x}$ 7.21	5.94	5.05

significant

TABLE A12

STUDENT-NEWMAN-KEULS' TEST FOR THE ALIPHATIC HYDROCARBON FRACTION

NORMALIZED TO 2-METHYLOCTADECANE INTERNAL STANDARD

	Ms	df	
error	17.7322257	28	$S_{\bar{x}} = \sqrt{MSerror}\sqrt{\frac{n_1 + n_2}{2n_1,n_2}} = 1.2911$
			$S_{\bar{x}} = \sqrt{17.7322257/9} = 1.40365$
			$Wp = P \times S_{\bar{x}}$

treatment	Soxhlet Extraction	MacLeod's Procedure	KOH Alcoholic Digestion
$\bar{x}$	9.08	13.08	15.27

P	2	3
qα(P,28)	2.90	3.50
Wp	4.07	4.91
Wp	3.74	

15.27 - 9.08 = 6.19*

13.08 - 9.08 = 4.0*

*significant

Those compounds sharing a common underline are not significantly different.

TABLE A13

STUDENT-NEWMAN-KEULS' TEST FOR THE ALIPHATIC HYDROCARBON FRACTION

NORMALIZED TO 2-METHYLEICOSANE INTERNAL STANDARD

	Ms	df
error	38.94647	28

$$S_{\bar{x}} = \sqrt{38.94647 \sqrt{\frac{9+13}{2(9x13)}}} = 1.9135396$$

$$= \sqrt{38.94647/9} = 2.08023$$

$$Wp = P \times S_{\bar{x}}$$

treatment	Soxhlet Extraction	MacLeod's Procedure	KOH Alcoholic Digestion
$\bar{x}$	9.57	13.82	17.28

P	2	3
qα(P,28)	2.90	3.50
Wp	6.03	7.28
Wp	5.54	

17.28 - 9.57 = 7.71*

*significant

Those compounds sharing a common underline are not significantly different.

TABLE A14

STUDENT-NEWMAN-KEULS' TEST FOR THE ALIPHATIC HYDROCARBON FRACTION

NORMALIZED TO nC_{32} INTERNAL STANDARD

	Ms	df	
error	15.686578	28	$S_{\bar{x}} = \sqrt{15.686578/9} = 1.32017$
			$S_{\bar{x}} = \sqrt{15.686578}\sqrt{\frac{9+13}{2(9x13)}} = 1.2144$
			$Wp = P \times S_{\bar{x}}$

treatment	Soxhlet Extraction	KOH Alcoholic Digestion	MacLeod's Procedure
$\bar{x}$	9.46	10.96	15.11

qα(P,28)	2.90	3.50
Wp	3.52	4.25
Wp	3.83	4.62

15.11 - 9.46 = 5.65*

15.11 - 10.96 = 4.15*

10.96 - 9.46 = 1.5

*significant

Those compounds sharing a common underline are not significantly different.

TABLE A15

STUDENT-NEWMAN-KEULS' TEST FOR THE AROMATIC HYDROCARBON FRACTION NORMALIZED TO BENZO(GHI)PERYLENE INTERNAL STANDARD

	Ms	df	
error	2.328833456	28	$S_{\bar{x}} = \sqrt{2.328833456 \sqrt{\frac{13+9}{2(13x9)}}} = .46792109$
			$S_{\bar{x}} = \sqrt{2.328833456/9} = .50868399$
			$Wp = P \times S_{\bar{x}}$

treatment	KOH Alcoholic Digestion	Soxhlet Extraction	MacLeod's Procedure
$\bar{x}$	5.05	5.94	7.21

qα(P,28)	2.90	3.50
Wp	1.47	1.78
Wp	1.35	1.64

7.21 - 5.05 = 2.16*

7.21 - 5.94 = 1.27

5.94 - 5.05

*significant

Those compounds sharing a common underline are not significantly different.

TABLE A16

ARCSINE TRANSFORMED EXTRACTION RECOVERIES FOR THE SPIKED BLANKS

UTILIZING MACLEOD'S PROCEDURE (%)

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
hexamethylbenzene	49.1	51.9	58.7	67.3	43.3	54.0± 9.2
benzo(ghi)perylene	65.8	76.4	91.8	84.2	74.1	78.4± 9.9
2-methyloctadecane	72.3	67.8	67.9	74.5	87.9	74.0± 8.2
2-methyleicosane	79.5	77.6	62.0	70.1	91.9	76.2±11.1
nC_{32}	50.3	34.7	51.3	65.3	56.7	51.6±11.1
$\bar{x} \pm s$	63.1±13.4	61.6±18.2	66.3±15.4	72.2±7.5	70.7±20.6	

(percentages after arcsine transformation)

TABLE A17

ARCSINE TRANSFORMED EXTRACTION RECOVERIES FOR THE SPIKED BLANKS

UTILIZING KOH ALCOHOLIC DIGESTION

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
hexamethylbenzene	50.2	46.8	45.1	63.2	61.2	53.3± 8.3
benzo(ghi)perylene	85.7	64.3	69.9	84.4	83.1	77.4± 9.7
2-methyloctadecane	68.2	70.4	76.9	76.1	81.3	74.5± 5.2
2-methyleicosane	73.7	69.4	73.4	72.1	95.2	76.7±10.4
nC_{32}	45.8	34.8	32.2	93.1	72.3	55.6±26.2
$\bar{x} \pm s$	64.7±16.5	57.1±15.6	59.5±19.7	77.7±11.4	78.6±12.7	

TABLE A18

ARCSINE TRANSFORMED EXTRACTION RECOVERIES FOR THE SPIKED BLANKS

UTILIZING SOXHLET EXTRACTION

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
hexamethylbenzene	59.2	61.2	55.2	55.1	43.4	54.8± 6.9
benzo(ghi)perylene	80.3	84.5	73.1	84.7	85.9	81.7± 5.2
2-methyloctadecane	79.2	79.3	71.6	77.5	79.3	77.3± 3.3
2-methyleicosane	83.6	91.2	60.7	82.3	80.6	79.6±11.3
nC_{32}	71.2	63.7	53.2	103.2	164.5	91.1±45.0
$\bar{x} \pm s$	74.7±9.7	75.9±13.1	62.7±9.1	80.5±17.2	90.7±44.5	

TABLE A19

STUDENT-NEWMAN-KEULS' TEST FOR THE SPIKED BLANKS

UTILIZING MACLEOD'S PROCEDURE

	Ms	df
error	44.812728	20

$S_{\bar{x}} = \sqrt{44.812728/5} = 2.9937511$

$Wp = (S_{\bar{x}} \times q\alpha)$

Compound	nC_{32}	hexamethylbenzene	2-methyloctadecane	2-methyleicosane	benzo(ghi)perylene
$\bar{x}$	45.98	47.37	59.69	61.41	62.90

P	2	3	4	5
$q\alpha(P,20)$	2.95	3.58	3.96	4.23
Wp	8.83	10.72	11.86	12.66

62.90-45.98 = 16.92*
62.90-47.37 = 15.53*
62.90-59.69 = 3.21
61.41-45.98 = 15.43*
61.41-47.37 = 14.04*
61.41-59.69 = 1.72

59.69-45.98 = 13.71*
59.69-47.37 = 12.32*
47.37-45.98 = 1.39

*significant

Those compounds which share a common underline are not significant.

TABLE A20

ARCSINE TRANSFORMED PERCENT RECOVERIES FOR CONCENTRATION UNDER NITROGEN

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
nC_{16}	83.3	87.9	92.1	87.8	91.7	88.5± 3.5
1-methylfluorene	87.0	92.3	91.3	93.5	95.2	91.8± 3.0
nC_{20}	97.7	100.6	92.6	100.1	97.4	97.6± 3.1
pyrene	98.2	98.3	89.4	90.6	93.6	94.0± 4.1
benzo(a)pyrene	95.8	93.2	90.1	98.8	98.2	95.2± 3.6
nC_{28}	98.7	101.0	93.1	92.8	97.3	96.5± 3.5
nC_{32}	92.7	88.5	90.4	86.2	119.5	95.4±13.6
$\bar{x} \pm s$	93.7±6.0	94.5±5.4	91.2±1.3	92.8±5.2	98.9±9.3	

TABLE A21

ARCSINE TRANSFORMED PERCENT RECOVERIES FOR CONCENTRATION UNDER ROTARY EVAPORATION

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
nC_{16}	81.3	79.9	93.7	86.3	89.3	86.1± 5.6
1-methylfluorene	93.2	93.0	96.6	89.3	92.1	92.8± 2.6
nC_{20}	92.4	96.1	92.1	95.0	99.3	94.9± 2.9
pyrene	88.6	88.2	89.5	93.7	98.1	91.6± 4.2
benzo(a)pyrene	86.3	88.5	96.3	86.1	97.6	90.9± 5.5
nC_{28}	93.0	95.4	98.9	89.4	91.4	93.6± 3.6
nC_{32}	92.1	78.6	114.3	83.7	96.3	93.0±13.7
$\bar{x} \pm s$	89.5±4.4	88.5±7.0	97.3±8.1	89.0±4.1	94.8±3.8	

TABLE A22

ARCSINE TRANSFORMED PERCENT RECOVERIES FOR COLUMN CHROMATOGRAPHY

Standards	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
nC_{16}	76.3	77.4	73.3	78.1	74.3	75.8± 2.0
1-methylfluorene	87.5	87.3	81.1	71.2	79.1	81.2± 6.7
nC_{20}	85.1	86.3	82.1	73.2	78.2	80.9± 5.3
pyrene	86.2	79.5	89.0	83.2	82.3	84.0± 3.6
benzo(a)pyrene	92.3	79.9	80.2	81.5	91.2	85.0± 6.1
nC_{28}	88.3	84.2	92.1	75.7	86.3	85.3± 6.1
nC_{32}	61.2	80.1	77.4	76.3	73.2	76.6± 7.3
$\bar{x} \pm s$	82.4±10.5	82.1±3.8	82.1±6.4	77.0±4.2	80.6±6.4	

TABLE A23

STUDENT-NEWMAN-KEULS' TEST FOR ARCSINE TRANSFORMED PERCENT

RECOVERIES FOR COLUMN CHROMATOGRAPHY

	Ms	df
error	17.4465	28

$S_{\bar{x}} = \sqrt{17.4465/5} = 1.86$

$Wp = P \times S_{\bar{x}}$

Compound	nC_{32}	nC_{16}	nC_{20}	1-methylfluorene	pyrene	benzo(a)pyrene	nC_{28}
	59.25	60.60	64.31	64.60	66.56	67.62	67.80

P	2	3	4	5	6	7
q(α,28)	2.90	3.50	3.84	4.10	4.30	4.46
Wp	5.42	6.54	7.17	7.66	8.03	8.33

67.80-59.25 = 8.55*
67.80-60.60 = 7.2
67.62-59.25 = 8.27*
67.62-60.60 = 7.02
66.56-59.25 = 7.31

*significant

Those compounds which share a common underline are not significant.

TABLE A24

RESPONSE FACTORS (F FACTORS) FOR ALIPHATIC HYDROCARBON STANDARDS

(µG/MICROVOLT-SECONDS)

Compounds	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
nC_{13}	1.64	1.41	1.41	1.41	1.42	1.45 ± 0.10
nC_{14}	1.32	1.38	1.40	1.39	1.37	1.37 ± 0.03
nC_{15}	0.99	1.30	1.34	1.40	1.38	1.28 ± 0.16
nC_{16}	1.67	1.55	1.53	1.59	1.55	1.57 ± 0.05
nC_{17}	1.45	1.74	1.65	1.64	1.67	1.63 ± 0.11
nC_{18}	1.59	1.57	1.54	1.54	1.56	1.56 ± 0.02
nC_{19}	1.78	1.66	1.69	1.61	1.67	1.68 ± 0.06
nC_{20}	1.68	1.75	1.68	1.67	1.70	1.69 ± 0.03
nC_{21}	1.71	1.70	1.73	1.70	1.72	1.71 ± 0.01
nC_{22}	1.55	1.59	1.59	1.49	1.57	1.56 ± 0.04
nC_{23}	1.91	1.91	1.88	1.88	1.88	1.89 ± 0.02
nC_{24}	1.38	1.39	1.59	1.39	1.40	1.43 ± 0.09
nC_{25}	2.27	1.91	2.67	2.15	2.07	2.21 ± 0.28
nC_{26}	1.93	2.00	2.50	1.81	2.09	2.06 ± 0.26
nC_{28}	2.54	2.86	3.09	2.05	2.57	2.62 ± 0.39
nC_{29}	1.66	2.63	2.77	1.89	2.00	2.19 ± 0.48
nC_{30}	3.01	3.06	3.02	3.18	3.01	3.03 ± 0.09
nC_{31}	2.84	3.16	4.14	2.82	2.84	3.16 ± 0.56
nC_{32}	2.01	3.75	3.67	2.34	3.47	3.22 ± 0.59

TABLE A25

RESPONSE FACTORS (F FACTORS) FOR AROMATIC HYDROCARBON STANDARDS

(μG/MICROVOLT-SECONDS)

Compounds	Replicates					
	#1	#2	#3	#4	#5	$\bar{x} \pm s$
1-methylnaphalene	0.77	0.99	0.97	0.99	1.01	0.95 ± 0.09
biphenyl	1.25	1.40	1.48	1.44	1.47	1.41 ± 0.09
hexamethylbenzene	1.45	1.47	1.54	1.54	1.52	1.50 ± 0.04
dibenzothiophene	1.73	1.80	1.90	1.83	1.87	1.83 ± 0.07
phenanthrene	1.66	1.73	1.77	1.71	1.77	1.73 ± 0.05
anthracene	1.78	1.74	1.87	1.82	1.82	1.80 ± 0.05
1-methylphenanthrene	1.79	1.72	1.91	1.73	1.72	1.77 ± 0.08
fluoranthene	2.47	2.19	2.18	2.11	2.15	2.22 ± 0.14
pyrene	1.80	1.83	1.88	1.82	1.85	1.84 ± 0.03
chrysene	1.97	1.96	2.03	1.97	1.99	1.98 ± 0.03
benzo(a)pyrene	2.36	2.58	2.44	2.30	2.37	2.41 ± 0.11
benzo(ghi)perylene	3.07	1.27	1.20	1.18	1.21	1.58 ± 0.83

TABLE A26

ANOVA OF RECOVERED WIEGHTS OF ALIPHATIC HYDROCARBON FRACTION EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	34.7859	17.3929	1.847
Within Techniques	28	263.5803	9.4135	
Total	30	298.3663		

ANOVA OF RECOVERED WEIGHTS OF AROMATIC HYDROCARBON FRACTION EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	1.6018	0.8009	0.4265
Within Techniques	28	52.5690	1.8774	
Total	30	54.1708		

TABLE A27

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF THE ADDED STANDARDS EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	76.0657	38.0328	0.6322
Within Techniques	154	35883.3835	233.0089	
Total	156	35959.4492		

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF THE ADDED STANDARDS: 2-METHYLOCTADECANE, 2-METHYLEICOSANE, nC_{32}, HEXAMETHYLBENZENE, AND BENZO(GHI)PERYLENE

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Compounds	4	15532.5147	3883.128	32.70*
Within Compounds	150	17811.4194	118.742	
Total	154	33343.9342		

*significant at the 95% confidence level

TABLE A28

ANOVA OF THE ALIPHATIC FRACTION RECOVERED WEIGHTS NORMALIZED TO 2-METHYLOCTADECANE EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	178.8058	89.4029	5.04*
Within Techniques	28	496.5023	17.7322	
Total	30	675.3081		

ANOVA OF THE ALIPHATIC FRACTION RECOVERED WEIGHTS NORMALIZED TO 2-METHYLEICOSANE EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	268.6736	134.3368	3.44*
Within Techniques	28	1090.5013	38.9464	
Total	30	1359.1749		

TABLE A28 (Continued).

ANOVA OF THE ALIPHATIC FRACTION RECOVERED WEIGHTS NORMALIZED TO nC_{32} EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	181.9136	90.9568	5.87*
Within Techniques	28	417.6588	15.4688	
Total	30	599.5724		

*significant at the 95% confidence level

TABLE A29

ANOVA OF THE AROMATIC FRACTION RECOVERED WEIGHTS NORMALIZED TO HEXAMETHYLBENZENE EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	69.7002	34.8501	1.36
Within Techniques	28	715.6501	25.5589	
Total	30	785.3503		

ANOVA OF THE AROMATIC FRACTION RECOVERED WEIGHTS NORMALIZED TO BENZO(GHI)PERYLENE EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	25.7050	12.8525	5.51*
Within Techniques	28	65.2073	2.3288	
Total	30	90.9123		

*significant at the 95% confidence level

TABLE A30

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF THE SPIKED BLANKS (2-METHYLOCTADECANE, 2-METHYLEICOSANE, nC_{32}, HEXAMETHYLBENZENE AND BENZO(GHI)PERYLENE) EXTRACTED BY MACLEOD'S PROCEDURE, SOXHLET EXTRACTION AND KOH ALCOHOLIC DIGESTION

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among Techniques	2	1581.31546	790.657	2.29
Within Techniques	72	24796.3944	344.394	
Total	74	26377.709		

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF THE SPIKED BLANKS (2-METHYLOCTADECANE, 2-METHYLEICOSANE, nC_{32}, HEXAMETHYLBENZENE AND BENZO(GHI)PERYLENE)

Sources of Variation	df	Sums of Squares	Mean Squares	F
Among compounds	4	6574.8445	1643.7111	5.81*
Within compounds	70	19802.8653	282.8980	
Total	74	26377.709		

*significant at the 95% confidence level

TABLE A31

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF ADDED SPIKES AFTER CONCENTRATION UNDER NITROGEN

Source of Variation	df	Sums of Squares	Mean Squares	F
Among Compounds	6	288.6017	48.1002	1.28
Within Compounds	28	1046.9280	37.3902	
Total	34	1335.5297		

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF ADDED SPIKES AFTER CONCENTRATION BY ROTARY EVAPORATION

Source of Variation	df	Sums of Squares	Mean Squares	F
Among Compounds	6	245.6788	40.9464	0.954
Within Compounds	28	1200.7880	42.8852	
Total	34	1446.4668		

TABLE A31 (Continued).

ANOVA OF THE ARCSINE TRANSFORMED PERCENT RECOVERIES OF ADDED SPIKES AFTER COLUMN CHROMATOGRAPHY

Source of Variation	df	Sums of Squares	Mean Squares	F
Among Compounds	6	621.9788	103.6631	3.273*
Within Compounds	28	886.8080	31.6717	
Total	34	1508.786		

*significant at the 95% confidence level

LITERATURE CITED

Ackman, R. G. and D. Noble. 1973. Steam distillation: a simple technique for recovery of petroleum hydrocarbons from tainted fish. J. Fisheries Research Board of Canada. 30: 711-714.

Avigan, J. and M. Blumer. 1968. On the origin of pristane in marine organisms. J. Lipid Research 9: 350-351.

Barbier, M., D. Joly, A. Saliot and D. Tourres. 1973. Hydrocarbons from seawater. Deep Sea Research 20: 305-314.

Bieri, R. H. and V. C. Stamoudis. 1977. The fate of petroleum hydrocarbons from a No. 2 fuel oil spill in a seminatural estuarine environment. Pages 332-344 in Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Douglas A. Wolfe (ed.). Pergamon Press, Inc. New York.

Bieri, R. H., A. L. Walker, B. W. Lewis, G. Loser and R. J. Huggett. 1974. Identification of hydrocarbons in an extract from estuarine water accomodated No. 2 fuel oil. Pages 149-153. in NBS Spec. Publ. 409. Marine Pollution Monitoring (Petroleum). Proceedings of a Symposium and Workshop at NBS. Gaithersburg, Maryland. May 13-17.

Blaylock, J. W., P. W. O'Keefe, J. N. Roehm and R. E. Wildung. 1973. Determination of n-alkane and methylnaphthalene compounds in shellfish. Pages 173-177 in Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills. API/EPA/USCG. Washington, D. C. March 13-15.

Blumer, M., G. Souza and J. Sass. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Marine Biology 5: 195-202.

Brown, R. A., T. D. Searl and J. J. Elliott. 1973. Distribution of heavy hydrocarbons in some Atlantic Ocean waters. Pages 505-520 in Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills. API/EPA/USCG. Washington, D. C. March 13-15.

Clark, R. C., Jr. 1973. Methods for establishing levels of petroleum contamination in organisms and sediment as related to marine pollution monitoring. Pages 96-101 in Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills. Preprints. API/EPA/USCG. Washington, D. C. March 13-15.

Clark, R. C., Jr. and M. Blumer. 1967. Distribution of n-paraffins in marine organisms and sediments. Limnology and Oceanography 12: 79-87.

Clark, R. C., Jr. and J. S. Finley. 1973. Techniques for analysis of paraffin hydrocarbons and for interpretation of data to assess oil spill effects in aquatic organisms. Pages 161-172 in Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills. API/EPA/USCG. Washington, D. C. March 13-15.

Ehrhardt, M. 1972. Petroleum hydrocarbons in oysters from Galveston Bay. Environmental Pollution 3: 257-271.

Ehrhardt, M. and M. Blumer. 1971. The Identification of oil spills by means of gas chromatographic and spectrometric data. Presented before the Division of Water, Air and Waste Chemistry American Chemical Society. Los Angelos. March 28-April 2.

Farrington, J. W. and G. C. Medeiros. 1975. Evaluation of some methods of analysis for petroleum hydrocarbons in marine organisms. Pages 115-121 in Conference on the Prevention and Control of Oil Pollution. San Francisco. March 25-27.

Farrington, J. W. and J. G. Quinn. 1973. Petroleum hydrocarbons and fatty acids in waste water effluents. J. WPCF 45: 704-712.

Farrington, J. W., J. M. Teal and P. L. Parker. 1973a. Petroleum hydrocarbons. Chapter 1 in Strategies for Marine Pollution Monitoring. E. D. Goldberg (ed.). Contribution #3631 Woods Hole Oceanographic Institution.

Farrington, J. W., J. M. Teal, J. G. Quinn, T. Wade and K. Burns. 1973b. Intercalibration of analyses of recently biosynthesized hydrocarbons and petroleum hydrocarbons in marine lipids. Bulletin of Environmental Contamination and Toxicology. 10: 129-135.

Gordon, D. C., Jr. and P. D. Keizer. 1974. Estimation of petroleum hydrocarbons in sea water by fluorescence spectrometry: improved sampling and analytical method. Fisheries and Marine Science. Environment Canada. Technical Report #481.

Greffard, S. and J. Meury. 1967. Note sur la pollution en rade de Toulon par less hydrocarbures cancérigènes. Cah. Oceanogr. 19: 457-468.

Gritz, R. L. and D. G. Shaw. 1977. A comparison of methods for hydrocarbon analysis of marine biota. Bulletin of Environmental Contamination and Toxicology. 17: 408-415.

Grob, K., G. Grob, and K. Grob, Jr. 1977. The barium carbonate procedure for the preparation of glass capillary columns; further informations and developments. Chromatographia 10: 181-187.

Grob, K. and G. Grob. 1978. On-column injection on to glass capillary columns. Journal of Chromatography. 151: 311-320.

Hertz, H. S., S. N. Chesler, W. E. May, B. H. Gump, D. P. Enagonio and S. P. Cram. 1974. Methods for trace organic analysis in sediments and marine organisms. Pages 197-199 in Marine Pollution Monitoring (Petroleum): Proceedings of a Symposium Sponsored by: The Intergovernmental Oceanographic Commission (IOC-UNESCO), the World Meteorological Organization (WMO), United States Department of Commerce. Publication No. 409 of the U. S. National Bureau of Standards, U. S. Government Printing Office. Washington, D. C. No. 0303-01359.

Hunter, L., H. E. Guard and L. H. DiSalvo. 1974. Determination of hydrocarbons in marine organisms and sediments by thin layer chromatography. Pages 213-216 in Marine Pollution Monitoring (Petroleum): Proceedings of a Symposium Sponsored by: The Intergovernmental Oceanographic Commission (IOC-UNESCO), the World Meteorological Organization (WMO), United States Department of Commerce. Publication No. 409 of the U. S. National Bureau of Standards, U. S. Government Printing Office. Washington, D. C. No. 0303-01359.

Iliffe, T. M. and J. A. Calder. 1974. Dissolved hydrocarbons in the eastern Gulf of Mexico Loop Current and the Caribbean Sea. Deep Sea Research 21: 481-488.

Johnson, R. W. and J. A. Calder. 1973. Early diagenesis of fatty acids and hydrocarbons in a salt marsh environment. Geochimica et Cosmochimica Acta 37: 1943-1955.

Koons, C. B., G. W. Jamieson and L. S. Ciereszko. 1965. Normal alkane distributions in marine organisms; possible significance to petroleum origin. Bulletin of the Am. Assoc. Petrol. Geologists 49: 301-304.

Mackie, P. R., K. J. Whittle and R. Hardy. 1974. Hydrocarbons in the marine environment I. n-alkanes in the Firth of Clyde. Estuarine and Coastal Marine Science 2: 359-374.

MacLeod, W. D., D. W. Brown, R. G. Jenkins, L. S. Ramos and V. D. Henry. 1976. A pilot study of a petroleum hydrocarbon baseline investigation for Northern Puget Sound and Strait of Juan De Fuca. Report submitted to Puget Sound Energy-Related Research Project, Marine Ecosystems Analysis Program. Environmental Research Laboratories. NOAA Technical Memorandum ERL MESA-8.

Rohrback, B. G. and W. E. Reed. 1975. Evaluation of extraction techniques for hydrocarbons in marine sediments. Publication No. 1537. Institute of Geophysics and Planetary Physics. University of California at Los Angeles.

Sever, J. R. and P. Haug. 1971. Fatty acids and hydrocarbons in Surtsey Sediment. Nature 234: 447-450.

Smith, C. L., W. G. MacIntyre and C. W. Su. 1977. Baseline studies of hydrocarbons from the Mid-Atlantic Bight. Chapter 9 in Middle Atlantic Outer Continental Shelf Environmental Studies: Volume II. Chemical and Biological Benchmark Studies. VIMS Special Report in Applied Marine Science and Ocean Engineering No. 178.

Snedecor, G. W. and W. G. Cochran. 1956. Statistical Methods. Iowa. The Iowa State University Press. 534 pp.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. N.Y. McGraw-Hill. 481 pp.

Stegeman, J. J. and J. M. Teal. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster, Crassostrea virginica. Marine Biology 22: 37-44.

Warner, J. S. 1976. Determination of aliphatic and aromatic hydrocarbons in marine organisms. Analytical Chemistry 48(3): 578-583.

Youngblood, W. W. and M. Blumer. 1975. Polycyclic aromatic hydrocarbons in the environment: homologous series in soils and recent marine sediments. Geochimica et Cosmochimica Acta 39: 1303-1314.

Youngblood, W. W., M. Blumer, R. L. Guillard and F. Fiore. 1971. Saturated and unsaturated hydrocarbons in marine algae. Marine Biology 8: 190-201.

Zafiriov, O. C. 1973. Petroleum hydrocarbons in Narragansett Bay II Chemical and Isotopic Analysis. Estuarine and Coastal Marine Science I: 81-87.

Zitko, V. and W. V. Carson. 1970. The characterization of petroleum oils and their determination in the aquatic environment. Fisheries Research Board of Canada. Technical Report #217.

Zitko, V. and S. N. Tibbo. 1970. Fish kill caused by an intermediate oil from coke ovens. Fisheries Research Board of Canada. Biological Station of St. Andrew N.B. Mimeographed Report.

Zsolnay, A. 1973. Hydrocarbons and chlorophyll: a correlation in the upwelling region off West Africa. Deep Sea Research 20: 923-925.

VITA

Colleen Gail Becker

Born in New Haven, Connecticut, February 12, 1952. Graduated from Lyman Hall High School, Wallingford, Connecticut in June 1970. Received a Bachelor of Arts degree from Quinnipiac College, Hamden, Connecticut in May 1974, with a major in biology.

In September 1974, the author entered the School of Marine Science, the College of William and Mary as a graduate student.