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DREDGED MATERIAL RESEARCH PROGRAM

TECHNICAL REPORT D-77-26

ASSESSMENT AND SIGNIFICANCE OF SEDIMENT-ASSOCIATED OIL AND GREASE IN AQUATIC ENVIRONMENTS

by

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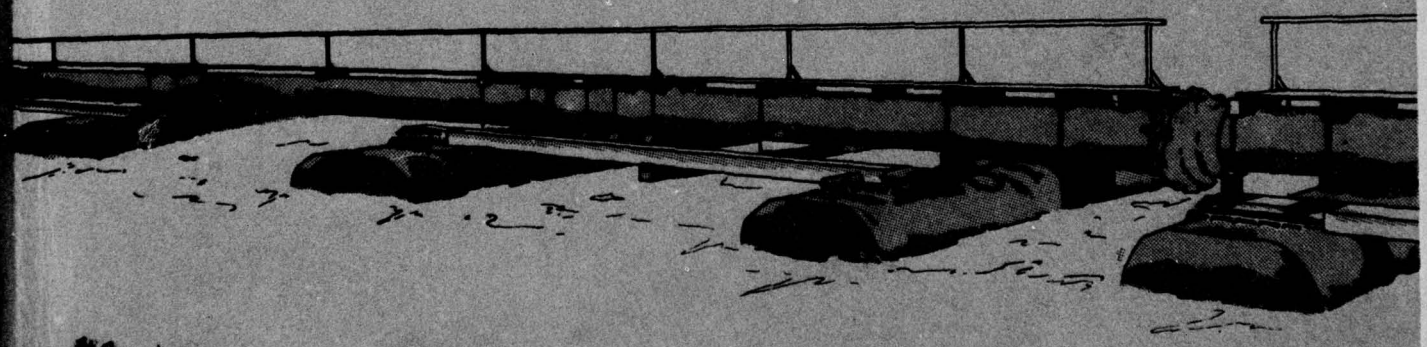

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1. The technical report transmitted herewith was undertaken as Work Unit 1D11 of Task 1D, Effects of Dredging and Disposal on Aquatic Organisms, of the Corps of Engineers' Dredged Material Research Program (DMRP). Task 1D is a part of the Environmental Impacts and Criteria Development Project (EICDP), which has a general objective of determining on a regional basis the direct and indirect effects on aquatic organisms due to dredging and disposal operations. The study reported on herein was part of a series of research contracts developed to achieve the EICDP general objective.

2. The objectives of this research were (a) to determine release to the water column and decompositional loss of petroleum hydrocarbons associated with dredged sediments and (b) to determine the potential for uptake of sediment-associated hydrocarbons into the tissues of organisms. The test species were exposed to oil-contaminated dredged sediments for 5- and 30-day periods under conditions simulating worst-case dredging and disposal effects. Organisms were either resident on the sediments, on a screen positioned above the sediment, or on a screen above the sediment with sediment mechanically stirred into the water column at 4-hour intervals. Animals were observed daily for mortality and gross behavioral changes. Water, sediment, and tissue samples taken prior to testing and at the end of the experiment were analyzed for hydrocarbon content by thin-layer chromatography, gas chromatography, and fluorescence spectrophotometry.

3. Characterization of test sediments showed hundreds or thousands of parts per million hydrocarbons, elemental sulfur, and polar compounds. The hydrocarbons were primarily alkanes and highly alkylated aromatic hydrocarbons similar to those in lube oil. Minor mortality of the benthic organisms occurred in test tanks and was not significantly greater than that of control tanks, and no overt behavioral impairment was observed. Release of water-soluble hydrocarbons was minimal during the experimental periods, in the few parts per billion range. Parts-per-million levels of hydrocarbons in the water column were the result

of their adsorption on suspended fine sediments. Biodegradation of sediment-associated hydrocarbons was remarkable for its absence over 30-day experimental periods. Analytical measurements showed no evidence for uptake of hydrocarbons by organisms that were not in direct contact with sediments. Uptake of hydrocarbons was minor compared to total oil content of the sediments.

4. It was concluded that with the dredged materials tested, hydrocarbons were tightly bound to the sediment matrix, that the hydrocarbons underwent minor (if any) biodegradation, and that the hydrocarbons were not readily transferred from sediments to the tissues of the estuarine and freshwater species tested. Evaluation of the standard elutriate test showed it to be of questionable value in evaluation of oil and grease release, primarily due to adsorption of these residues on the walls of the elutriation apparatus.

5. The information and data published in this report are contributions to the further understanding of the complex nature of sediment, water, and chemical/biological interactions and establish a baseline from which to develop meaningful evaluations for the selection of an environmentally compatible disposal alternative. It is expected that the methodology employed in this study and the resulting interpretation of the chemical/biological interactions will be of significant value to those persons concerned with CE dredged material permit programs.

JOHN L. CANNON
Colonel, Corps of Engineers
Commander and Director

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A review of the literature suggests that the term "oil and grease", as used in descriptive and regulatory context for dredged material disposal, is a broadly based term defined by the methodology used in its determination. Oil and grease contains numerous natural and petroleum derived hydrocarbons, plus fats, oil, and waxes of both natural and anthropogenic origin. Also included in oil and grease are trace quantities of fat-soluble materials such as DDT and PCB. Elemental sulfur is a contaminant often included in oil and grease determinations
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20. ABSTRACT (Continued)

by virtue of its solubility in some organic solvents. Although some oil and grease fractions are readily degraded by microbiological action, many petroleum hydrocarbons are resistant to decomposition. Little is known concerning the effects of sediment-associated oil and grease on organisms and ecosystems, although in the case of certain oil spills it is known that toxic oily residues can be retained over periods of years in sediments with little abatement of their toxic properties.

Experimental studies using dredged material contaminated with oil and grease show low toxicity for mussels, clams, crabs, and snails. Minor uptake of hydrocarbons into organism tissues occurred, compared with levels of contaminants in the dredged material tested. In test periods lasting up to 30 days, very little biodegradation of oily residues occurred, suggesting the oil and grease value to be of little use in the prediction of BOD loading. Evaluation of the standard elutriate test with regard to oil and grease suggested this test to be of little value as the small amounts of oil released from the sediments were rapidly adsorbed to the walls of the test vessels.

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EXECUTIVE SUMMARY

One of the major parameters in determining the environmental quality of dredged sediments is their content of "oil and grease". Since this parameter is used in regulatory criteria for the discharge of dredged material, it was of interest to the U.S. Army Corps of Engineers to determine the meaning of the oil and grease measurements, and obtain preliminary experimental information on possible transfer of oil and grease residues from contaminated dredged material into representative aquatic organisms occurring near dredging activities. An extensive literature search and review was carried out to list and evaluate methods used for the determination of oil and grease, to identify components of sedimentary oil and grease and their levels under natural and contaminated conditions, and to describe the potential effects and environmental fate of sediment associated oil and grease residues. A set of preliminary experiments was conducted to determine if, under "worst case" conditions, oil and grease residues (hydrocarbons) could be taken up in significant quantity by the tissues of aquatic organisms intimately exposed to oil and grease contaminated dredged material.

The literature review suggested the term "oil and grease" to be unspecific, representing an ill-defined measurement that refers to different mixtures of compounds based on the methodology used for their determination. Unless identical methods are used, oil and grease measurements are not intercomparable. Oil and grease constituents include naturally produced fat-soluble substances, as well as an extremely broad array of petroleum derivatives and synthetic organic chemicals, some of which are carried as trace contaminants with the major fractions by virtue of their fat solubility. These include the numerous polynuclear aromatic compounds, some of which may have toxic or carcinogenic properties. Elemental sulfur is often included in the

total oil and grease determination based on its solubility in some organic solvents, and is an extremely variable source of error in the determination. Naturally occurring oil and grease components range from a few parts per billion into a few hundred parts per million in highly enriched natural sedimentary sites of accumulation. Effluents of technological development produce sediments contaminated with hundreds of parts per million oil and grease, sometimes ranging into the tens of thousands of parts per million.

Since the term oil and grease includes all fat-soluble components of the organic matter in aquatic sediments, discussion of the fate and effects of oil and grease in aquatic environments must be defined by the specific components of interest. Most available data have arisen from literature concerning the origin of petroleum and the effects of petroleum spillage in the environment.

Waste and spilled oil and grease residues are readily adsorbed on finely particulate sedimentary materials by mechanisms that are as yet poorly understood. These adsorptive processes aid in the transferral and deposition of lipophilic substances into aquatic sediments. Components of sedimentary oil and grease are decomposed to various degrees by the action of sunlight (photolysis), and by microorganisms. Non-petroleum hydrocarbons are most susceptible to microbiological decomposition, with petroleum derived compounds often very resistant to attack, particularly under anaerobic conditions in buried sediment.

When toxic hydrocarbon residues are associated with sediments, such as in some well-documented oil spill cases, sedimentary association promotes long-term retention of the oil, and retention of toxic properties of the residues over periods of years. No general conclusions can be made from the small amount of data in the literature concerning the uptake and disposition of oily sediment residues in

metazoans, based on the large number of compounds and equally large number of species potentially affected. A small amount of data suggests that certain higher metazoans possess enzymatic systems which help degrade hydrocarbons, although these systems sometimes confer carcinogenicity on molecules such as the polynuclear aromatics. The ultimate fate of sedimentary oil and grease, aside from microbiological decomposition, is probably permanent sedimentary deposition in deep ocean basins.

Experimental studies were carried out to determine in a preliminary manner if hydrocarbons could be taken up from dredged material that were heavily impacted with these major components of "oil and grease". Sediments used for exposure were from the Duwamish Waterway, near Puget Sound, Wash., containing over 400 ppm hydrocarbons, and from New York Harbor, containing over 1500 ppm hydrocarbons.

Estuarine mussels, crabs, snails, and freshwater clams were exposed to the dredged material in three experimental arrangements for periods ranging from 4 to 30 days. Organisms were maintained in direct contact with dredged sediments on a screen above settled dredged sediments or on a screen with sediments stirred into the water column at regular intervals. Mortality of organisms was monitored routinely. Samples of water, sediments, and organisms were taken at the beginning and at the end of established exposure periods, and analyzed by thin-layer chromatography, gas chromatography, and/or fluorescence spectro-photometry.

The standard elutriate test (minus filtration step) was used with one sediment sample to determine its performance in relation to sedimentary oil and grease content.

Experimental results showed little or no release of soluble hydrocarbon residues to the water column, and that hydrocarbons in

the water column were invariably associated with fine sediment fractions. Essentially no degradative loss of hydrocarbons was found for aerobic or anaerobic sediments in experiments lasting up to 30 days.

Mortality of organisms upon exposure to dredged material from all areas was no greater than that of controls containing uncontaminated reference sediments.

Experimental evidence suggested insignificant uptake of hydrocarbons by all test organisms incubated in the presence of Duwamish River sediments which contained almost 500 ppm total hydrocarbons. Mussels exposed for 30 days to heavily contaminated New York Harbor (Bay Ridge) sediments (6000 ppm hydrocarbons) showed an uptake of less than 5%, and shore crabs exposed for two weeks showed no significant uptake of hydrocarbons. Mussels and crabs exposed for 4 days to New York Harbor (Perth Amboy) sediments containing 2000 ppm total hydrocarbons showed average uptakes above background of about 50 and 70 ppm, respectively.

The results show that selected estuarine and freshwater organisms can be exposed to dredged material that is contaminated with thousands of ppm oil and grease, without experiencing mortality for periods up to 30 days. It appears that uptake of hydrocarbons from the heavily contaminated sediments was minor when compared to the hydrocarbon content of the sediments, and to literature results describing exposure of uncontaminated organisms in field conditions where total hydrocarbon uptake ranged to several hundred ppm.

Gas chromatography (GC) of selected samples provided qualitative results unavailable with the thin-layer method; the most demonstrative GC results were obtained when examining aromatic hydrocarbon fractions. Each species (mussel, crab, clam) showed unique chromatographic patterns. Small increases in certain peaks occurred when comparing GC traces of aromatic hydrocarbon content of organisms before and after

30-day incubations in Duwamish River dredged material. The only striking difference, however, was found in comparing chromatographs of aromatic hydrocarbons between initial (background) and 30-day incubated mussels. Thus, although significant quantitative changes could not be demonstrated over the experimental exposure period, it was apparent that subtle qualitative changes might be occurring, at least in the mussels.

A salient result was that the oil and grease content of the sediments was not altered by biodegradation, suggesting it could not cause high BOD, and that the residues were inert. The validity of using the oil and grease parameter as a measure of sediment quality and BOD is open to serious question.

PREFACE

Sediments containing oil and grease from a wide array of sources commonly settle in waterways subject to dredging by the U. S. Army Corps of Engineers. As with other sediment-associated contaminants, such as heavy metals, little is known concerning the release of oil and grease from sediments during dredging, or the possible environmental impact of such residues on organisms occurring in the vicinity of oil-impacted dredged areas. Furthermore, the effect of depositing oil and grease-contaminated sediments into uncontaminated aquatic disposal or land disposal sites is unknown regarding the ability of organisms in such uncontaminated areas to take up potentially toxic or mutagenic oily residues from suspended or deposited sediments released at disposal sites.

This report contains a literature survey made to review knowledge on the content, levels, fate, effects and methods of analysis of oil and grease with particular emphasis on the oil and grease fraction associated with natural or simulated sedimentary materials. The experimental section of this report details preliminary experiments made to begin laboratory evaluation of the potential transfer of oil and grease residues (hydrocarbons) from actual dredged material into tissues of selected test species under simulated environmental conditions.

The research conducted herein was as Work Unit No. 1D11 of the Office, Chief of Engineers Dredged Material Research Program (DMRP), Environmental Effects Laboratory (EEL) of the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

Dr. Neylan A. Vedros, Director of the Naval Biosciences Laboratory, University of California, Berkeley, was Principal Investigator on this project. Research was carried out under the immediate supervision of Dr. Louis H. DiSalvo, Head, Marine Sciences Division. Dr. Harold E. Guard was responsible for all chemical analyses, and was assisted by Research Associates Ms. Nina Hirsch and Mr. James Ng. Various technical details and library searches were carried out by John Wyman, LTJG, MSC, USNR, Ms. Janet Coffey, and Ms. Terry Coughlin. Report prepara-

tion was done by Miss Phyllis Butterworth and Ms. Caro Hopper.

The contract was monitored by Dr. H. E. Tatem and Dr. R. Peddicord under supervision of Dr. Robert M. Engler, Manager of the Environmental Impact and Criteria Development Project, DMRP, and Dr. John Harrison, Chief, EEL.

The Commander and Director of WES during the study was COL J. L. Cannon. Mr. F. R. Brown was Technical Director, WES.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

U. S. customary units of measure used in this report can be converted to metric (SI) units as follows:

Multiply	By	To Obtain
inches	0.0254	meters
feet	0.3048	meters
miles (U. S. statute)	1.609344	kilometers
acres	4046.856	square meters
cubic yards	0.764555	cubic meters
gallons (U. S. liquid)	0.003785412	cubic meters
tons (short)	907.1847	kilograms

ASSESSMENT AND SIGNIFICANCE OF SEDIMENT-ASSOCIATED OIL AND GREASE IN AQUATIC ENVIRONMENTS

PART I: REVIEW OF LITERATURE

Introduction

1. The term oil and grease has been classically used to refer to the organic fraction in natural waters and sediments that is preferentially soluble in organic solvents.[1] In contrast to the more easily definable classes of water contaminants such as the heavy metals, and bacteria, oil and grease may include thousands of organic compounds with various chemical, physical, and toxicological properties. The oil and grease fraction in sediments may include fats, oils and waxes of vegetable or animal origin, hydrocarbons of natural origin, petroleum derivatives, organic chemicals, pesticides, detergents, and soaps, as well as elemental sulfur. Due to the variable nature of fractions appearing in the oil and grease extractions, many investigators prefer the term total extractable matter to allow for the presence of substances that can neither be classified as "oil" nor "grease". Over twenty methods are in existence for the determination of oil and grease, some of which are reviewed in the next section on Methods of Analysis.

2. Among the extractable organics, plant and animal materials derive from natural production, death, and decay, as well as from industrial, agricultural, and municipal waste discharge. Due to their lack of toxicity and ease of biodegradation, naturally produced compounds are expected to have exertion of BOD as their major environmental impact. Petroleum residues, for which there is a rapidly increasing body of research literature, appear to be the most quantitatively significant

contributors to long-lasting oil and grease burdens in dredged material. Millions of tons of petroleum derivatives are discharged into the nation's waterways each year.[2] Different fractions of waste and spilled petroleum have various effects ranging from obvious esthetic effects and direct organism kills, to potential long-term sublethal effects, with damage to wildlife and commercially valuable resource organisms. A number of major reviews have been published in recent years on petroleum in natural environments (references 2-9) and are the source of a number of important research reports listed in the literature review. Non-petroleum organic chemicals occur in municipal, industrial, and agricultural effluents and may include quantitatively small, nevertheless toxic or carcinogenic substances, which are incorporated into aquatic sediments. Biochemically produced elemental sulfur, although fairly inert, is a variable constituent of "oil and grease", which introduces significant and unpredictable errors into gravimetric determinations of organic materials.

3. The function of this review was to document the occurrence of oil and grease residues in aquatic sediments, the composition of the oil and grease residues, and the availability of components of the oil and grease fraction to aquatic ecosystems when benthic sediments are disrupted or deposited in disposal sites during dredging operations. The literature survey was conducted parallel to laboratory experiments designed to give preliminary information on some of these areas of interest.

4. The following literature review includes:

   a. Methods used to assay for oil and grease residues, with primary emphasis on the petroleum-derived hydrocarbons.

   b. Components of the oil and grease fraction and their levels of occurrence in aquatic sediments.

   c. Fate and movement of sediment-sorbed oil and grease residues, and their known environmental effects.

5. Literature for the years 1970 through 1976 was searched, using the following key words: oil and grease, oil pollution, petroleum, hydrocarbons. The search included Biological Abstracts, Chemical Abstracts, Pollution Abstracts, the Environmental Index, Water Resources Abstracts, and Government Reports Index.

6. Computer searches were ordered through February 1977 from the Defense Documentation Center, Naval Oceanographic Data Center (ENDEX), and Automatic Subject Citation Alert (ASCA) from the Institute for Scientific Information, Philadelphia.

Methods of Analysis of Oil and Grease in Sediments

7. The quantities "oil and grease" and "grease" are defined as the solvent extractable material, which is measured by the detection method and the work-up employed. The composition of the oil and grease depends on the method of extraction, solvent used, and type of sample. The significance of oil and grease measurement is related to the methodology. This section discusses: 1) standard methods for samples containing solids (sludge methods), 2) the effects of variation of preservation methods, sampling procedures, and extraction methods on results, and 3) supplementary, but not standard, methods for determining oil and grease components, primarily petroleum constituents.

8. Some components of oil and grease are measured appropriately by other standard methods. For example, oil and grease fractions often contain detergents, nitrilotriacetic acid, organic carbon, phenolics, and vanadium. The appropriate standard methods specified for these substances are not discussed here.

Standard methods for samples containing solids

9. Several standard methods for determination of oil and grease in water and wastewater have been published by the U. S. Environmental Protection Agency (EPA),[10] the American Society for Testing and Mate-

rials (ASTM),[11] and by the American Public Health Association (APHA).[1,12] The API has reviewed 21 different methods as summarized in Table 1.[13] However, only two standard methods for oil and grease applicable to sediments have been published. One method for sludge is APHA method No. 209;[12,13] the other was published by a Conference on Clean Air and Water in Western Europe (CONCAWE).[14] In the APHA method, sediment is dried with magnesium sulfate monohydrate and Soxhlet extracted with trichlorotrifluoroethane, bp 47.5°C (Freon 113 or Freon TF). A precision of 4.6% (relative standard deviation) was obtained on six replicate samples. This high precision is not typical of that to be expected generally (see below).

10. In the CONCAWE[14] method, the oil and grease is extracted from an acidified sample with carbon tetrachloride. The non-hydrocarbons are removed by shaking with Florisil, and the hydrocarbons are determined by infrared (IR) absorbance. This method measures only the hydrocarbon fraction of oil and grease.

11. Extensive examination of oil and grease methods[13,15] for water samples has demonstrated that these methods are neither precise nor very accurate. These conclusions are also pertinent to measurement of oil and grease in sediment. Inaccuracies of the methods, which make intercalibration of methods impossible, result from the operational definition of the term "oil and grease". Solvent extracts of sediments may contain sulfur, volatile hydrocarbons in addition to vegetable oils, animal fats, and non-volatile hydrocarbons but not elemental sulfur.

12. A comparison of infrared and gravimetric measurements has been reported by the American Petroleum Institute (API).[13] On these particular samples the IR method gave uniformly higher results by a factor of approximately 2.4 (Table 2) probably due to the presence of volatile components in these samples. The opposite results would be expected for samples high in elemental sulfur. Precision of these

Table 1

Summary of Analytical Methods for Determination of Oil & Grease*

Type	Method Designation	Reference*	Status	Conditions of Extraction	Extractant	Adsorbent	Costs
Gravimetric Methods	Chloroform Extractable Matter	ASTM D-1178	Discontinued	pH 3-4	Chloroform	None	Moderate
	Solvent Extraction	ASTM-2778	Limited Use	pH Variable	Any Solvent	None	Moderate
	Soxhlet Extraction	EPA Manual 00550	Wide Use	pH - 2 Soxhlet	Hexane or Freon-113	None	Moderate
	APHA Oil & Grease	APHA - 137	Wide Use	Acidic	Freon or Pet. Ether	None	Moderate
	Hydrocarbon and Fatty Matter	Std. Methods P. 413	Limited Use	Follow Method 4	Freon-Hexane	Alumina	Moderate
	Reflux Distillation Method	ASTM D-1340	Discontinued	Acidic	Benzene, Others	None	Moderate
	Petroleum Ether Extraction	a)	Limited Use	No pH Adj.	Petroleum Ether	None	Expensive
	Hexane Extractable Material	ASTM D-1891	Discontinued	pH-4	Hexane	None	Moderate
Volumetric Methods	Flocculation - Extraction Method	API 732-53	Limited Use	pH-4	Ether	$Fe(OH)_3$Floc	Inexpensive
Instrumental Methods	Oil and Grease - Freon Extractables	EPA - 00560	General Use	pH-2	Freon 113-IR	None	Expensive
	Hydrocarbons in Water or Soil	Concawe I/72	Limited Use	Acidic	CCL_4 (IR)	Florisil	Expensive
	Combined Methods	API O & G Comm	Proposed	pH < 7 $\geq$7	CCL_4 (IR)	None	Expensive
	Petroleum Hydrocarbons	1975 EPA Study	Proposed	Acidic	CCL_4 (IR)	Silica Gel	Expensive
	Volatile and Non-Volatile Oily Material	API-733-58 & Lit.	Limited Use	Acidic	CCL_4 (IR)	None	Expensive
	Ultraviolet Fluorescence		Limited Use	None	CCL_4-Hexane (UV)	None	Expensive
	Ultraviolet Absorption		Limited Use	Acidic	Hexane (UV)	None	Expensive
	Gas Chromatography	Concawe III-72	European Use	Acidic	CCL_4 or Pentane (GC)	Florisil	Expensive
	Visible Color Method	Hach Handbook	General Use	None	3-4 Solvents	None	Inexpensive
	Color Comparator	Champion Chem. Co.	Very Limited	None	Special Chemical	None	Inexpensive
Sampling		EPA, ASTM, APHA	Wide Use	pH Control, Refrigeration, Time-Dependent, Glass Bottles			
Continuous Monitors	Ultraviolet		Specific Applications	None	As received	None	Moderate
	Visible						Inexpensive
	Infrared						Expensive
	Fluorescence						Expensive
	Light Scattering						Moderate

*See reference 13 for citations.

Table 2

Comparative Results of "Oil and Grease" Values from Infrared and Gravimetric Finishes*

	"Oil and Grease" mg/ℓ			
Measurement:	Infrared		Gravimetric	
Solvent:	CCl_4	Freon	CCl_4	Freon
Samples from location 1	185 ± 57	160 ± 57	84 ± 27	57 ± 5
rel. std. dev.,%	31	36	32	9
Samples from location 2	128 ± 13	117 ± 20	53 ± 9	55 ± 2
rel. std. dev., %	10	17	17	4

* From reference 13.

measurements ranged from 4 to 36% relative standard deviation.

13. The precision and accuracy of the Freon extraction-gravimetric detection method,[10] and the Freon extraction-infrared detection method[10] were examined in a "round-robin" test conducted by ASTM.[15] A summary of these has been prepared by Engineering-Science, Inc. (Table 3).[16] The analytical variability for the Freon-extraction gravimetric determination and Freon extraction-infrared determination procedures is considerable and increases with a decrease in actual oil and grease content (Tables 4,5). Infrared detection is more precise (lower variability) than the gravimetric method. The precision decreases less with decreasing oil concentration making the IR method considerably more precise at concentrations in the 1-10 mg/l range. This precision represents interlaboratory variation, which is likely greater than that obtained on replicate samples by a single laboratory. The IR method consistently gave values higher by a factor of 1.25 to 1.52 when compared with the gravimetric method. Again, this difference might be reversed in analysis of sulfur-containing sediment samples. The accuracies of the gravimetric and IR methods depend on the composition of the oil and grease. The gravimetric method gives low values if volatile components are present and high if elemental sulfur is present. Conversely, the IR method does not detect sulfur. The IR method requires selection of a standard oil. This selection usually requires some preliminary qualitative evaluation of the samples in order to choose a relevant standard oil or prepared mixture of known hydrocarbons.

14. While standard methods for determination are simple, these methods are neither precise nor accurate. Considerable variability exists between laboratories, methods, and oil samples. Composition of "oil and grease" is a function of extraction, work-up, and determination methods. Nevertheless, "oil and grease" determinations are the basis for decision making by regulatory agencies, and are strongly entrenched in water-quality standards. Scientific studies on fate and effects

Table 3. Oil and Grease Analytical Method Comparison: Relative Analytical Yield*

Group	Average Round-Robin Results**		Yield
	Gravimetric	Infrared	Infrared/ Gravimetric
1	201.5	252.6	1.25
2	206.5	262.6	1.27
3	90.4	130.5	1.44
4	75.1	111.8	1.49
5	31.7	48.2	1.52
6	17.4	23.4	1.34

*From reference 16.
**All data within ±2 standard deviation of "true value".

Table 4. Analytical Variability: Freon Extraction-Gravimetric Detection Procedure*

"True" Oil and Grease Concentration mg/l	95% Confidence Level Analytical Error mg/l	
	Low	High
100	63.00	158
50	27.00	93
25	11.00	59
15	5.10	44
10	2.80	36
5	0.90	29
1	0.03	38

*From reference 10.

Table 5. Analytical Variability: Freon Extraction-Infrared Detection Procedure*

"True" Oil and Grease Concentration mg/l	95% Confidence Level Analytical Error mg/l	
	Low	High
100	78.00	129.0
50	38.00	67.0
25	18.00	35.0
15	11.00	21.0
10	7.00	15.0
5	3.20	7.7
0	0.56	1.8

*From reference 10.

generally monitor or determine some component fraction of the "oil and grease" to circumvent the inaccuracies in these general methods.

Effects of variation of methods on results

15. Preservation methods. The APHA method[1] suggests "every possible precaution must be taken to obtain a representative sample", but no specific precautions are specified for sludge or sediment samples. Samples may be preserved with 1 ml H_2SO_4 for each 80 g of sample; chloroform or sodium benzoate should not be used as preservatives.[1] The EPA specifications[10] for oil and grease in water samples should be followed for sediments, if possible. The use of glass containers, storage at 4°C or with added H_2SO_4, and holding times of up to 24 hr, are recommended. Effects of preservation methods on oil and grease have been examined for oil field waters by API.[13] The limited data obtained indicate that changes in oil and grease levels occur within 5 to 7 days in unrefrigerated samples and that acidification tends to increase oil and grease values. These studies provide reason for concern with preservation methods for sediments.

16. Sampling methods. Hummel and Krizek[17] report experiences in the use of various equipment for sampling bottom sediments, dredged material from hoppers, slurries from discharge pipes, and landfill material. These authors' conclusions are summarized in Table 6. Sampling of suspended sediments is a more complicated problem. Kirchner[18] reports validation of a trap method for collecting sediment in lakes; however, this method cannot be used for collection of sediment for oil and grease determinations since a dye is added as an indicator of turbulence. Schultz[19] comes to the conclusion that "the considerable variation in sediment concentration, which exists in tidal waterways -- especially in those cases where material is predominantly silt and clay particles -- makes it impractical, if not impossible, to measure the quantity of sediment discharge".

Table 6

Sampling Methods for Various Sediments*

1.	Soft fill material	Hand-operated piston sampler
2.	Soft harbor sediment	Ekman dredge and flap-valve sampler
3.	Fine-grained sediment with high water content	Flap-valve sampler
4.	Dredged material from hopper	Additional R & D needed
5.	Dredged material from discharge pipe	Additional R & D needed

* From reference 17.

17. In practice several methods are used for collection of suspended sediment. All must be considered unproved in regard to whether such samples provide representative samples of suspended sediment without affecting oil and grease loads. A unique "biosampler" has been employed by DiSalvo and Guard[20] using the blue mussel, Mytilus edulis, to deposit suspended sediment into collection tubes.

18. While measured oil and grease levels are affected by sampling procedures, only a single study describing this type of effect is available.[21] Farrington and Quinn[21] compared fatty acids in recent sediments sampled either by a Foster Anchor Dredge, which collects the upper 8 cm, or by a Phleger corer, which takes 50-cm cores. Dredge samples contained larger amounts of all fatty acids than core samples as a result of decreasing fatty acid abundance with increasing depth. Dredge samples contained more surface sediment and, hence, more fatty acids than the 50-cm core samples. A similar effect would be expected for the total "oil and grease" measurements that do not include sulfur. The reverse would be expected in situations where the deeper sediments are anoxic and where methods that include sulfur are used.

19. Extraction methods. Determination of oil and grease in sediments is more complicated than in water because sediments may contain considerable amounts of elemental sulfur and asphaltic materials not present generally in water samples. These materials are soluble to various degrees in organic solvents. Extraction methods using equilibration with a limited amount of solvent (e.g., shaking) minimize the quantity of low-solubility material extracted. Repetitive extraction, as with Soxhlet apparatus, improves recovery of sulfur and asphaltenes. The solvent used can affect the composition and amount of total extract. Properties of commonly used solvents are listed in Table 7. Elemental sulfur is readily soluble in carbon disulfide but nearly insoluble in

Table 7

Properties of Common Extraction Solvents

Solvent	Boiling Point °C	General Properties
Chloroform	61	Dissolves asphaltenes. Not flammable, high toxicity. Decomposes to produce phosgene on storage unless stabilized.
Freon 113	48	Not flammable, low toxicity, precipitates asphaltenes. May be used for IR determination.
Hexane	69	Flammable, moderate toxicity, precipitates asphaltenes.
Petroleum ether	30-60	Similar to hexane.
Carbon tetrachloride	77	Not flammable, high toxicity, may be used for IR determination, dissolves asphaltenes.
Benzene	80	Flammable, high toxicity, dissolves asphaltenes.
Toluene	111	Flammable, moderate toxicity, dissolves asphaltenes.
Methanol	65	Flammable, moderate toxicity, poor hydrocarbon solvent, especially if wet.

hexane. Asphaltenes are defined as the pentane insoluble fraction of petroleum. Asphaltenes are soluble in chloroform, benzene, and other aromatic solvents but insoluble in saturated hydrocarbons (pentane, hexane, cyclohexane, etc.) and in Freon 113. An APHA method[12] specifies the use of trichlorotrifluoroethane, bp 47°C (Freon 113). The use of hexane in the earlier version[1] has been dropped, since it creates a fire hazard. Freon appears to extract somewhat less oily material than hexane.[13] This difference is likely less than interlaboratory variability.

20. Ferguson [22] has examined factors affecting extraction of hydrocarbons and total extractable material from sediment samples. In this study wet sediment samples were pulverized and extracted in a specially designed extractor with recycling solvent (benzene) at 80°C for 24 hr. Grinding sediments to smaller particle size range increased yield of extractable material. Extraction of hydrocarbons was complete with median particle sizes of 16 μm or less, while removal of organic matter was enhanced at even lower median particle size. Therefore "total extractable organics" is not a good measure of efficiency of hydrocarbon extraction, nor is the amount of hydrocarbon extracted a good measure of extraction efficiency for oil and grease. When the influence of extraction time was studied, an exponential curve typical of a repetitive extraction process was obtained, with 10 hr being required for 90% extraction of extractable material. Extraction with benzene was as effective for hydrocarbons as 80:20 benzene:methanol. The use of the mixed solvent removes excessive amounts of non-hydrocarbon materials including sodium chloride which interferes with subsequent analyses. Additionally, the composition of non-azeotropic solvent mixtures frequently changes during extraction. For these reasons mixed solvents are not recommended.

21. Ferguson [22] also discusses common problems in extraction methods. Soxhlet extraction was inefficient due to channeling of solvent through the sediment layer. This conclusion, however, does not necessarily

reflect on the standard Soxhlet extraction method where sediment is dried prior to extraction. Channeling of solvent through dried sediment may or may not be significant. Repeated extraction of sediment with refluxing required large amounts of operator time to change solvent as required for efficient extraction. As a result, a special unattended device was developed for repeated extraction of a sediment-solvent slurry at the boiling point using recycled solvent.

22. The precision obtainable in a single laboratory in gravimetric determination of sediment hydrocarbons after separation by column chromatography is a function of sediment hydrocarbon concentration.[22] The relative standard deviation of the mean ranged from 30% at 10 ppm to 10% at 2,000 ppm. The loss of high boiling hydrocarbons, tetralin (bp 207°C) and mixed dimethylnaphthalenes (bp 260°C), during evaporation of benzene at 70°C has been examined.[22] The higher boiling dimethylnaphthalenes were recovered quantitatively, whereas, 20-40% of the tetralin was lost depending on evaporation time. More highly volatile compounds will be lost entirely during solvent evaporation.

23. Size of extract was also found to affect losses during work-up of samples; larger losses occurred with small samples.[22]

24. Several techniques for extraction of organic material and hydrocarbons from sediment have been evaluated by Rohrback and Reed.[23] Seven pre-extraction techniques, five extraction methods, and a series of solvents and solvent mixtures were examined. Although considerable variability was observed in amount and composition of hydrocarbon fractions obtained, the authors suggested that Soxhlet extraction of acidified, water-washed and freeze-dried sediment using toluene: methanol (3:7) for 100 hr be used for extraction of hydrocarbons.

25. Walker et al. [24] have compared four extraction procedures

using benzene: 1) Soxhlet method, 2) mixing solvent and sediment with moderate stirring, 3) mixing solvent and sediment with sonication, and 4) repeated shaking of solvent with sediment on a reciprocal shaker. Method 4 yielded the greatest amount of extractable material. Using this method the yield of total extractable material obtained with benzene was greater than with hexane or chloroform. The hydrocarbons in the benzene and chloroform extracts contained relatively more alkanes than in the hexane extracts, but these differences are not significant.

26. Summary of extraction methods. The standard method for oil and grease using Freon for extracting dried sludge is recognized as empirical. Precisions of only 20-30% are not unusual, and the efficiency has not been studied. The standard method is not used for measurement of total extractables or for determination of petroleum hydrocarbons. Numerous methods have been reported, and supporting data are often inadequate or conflicting.

27. It may be concluded that it is important to have good contact between solvent and sediment. Vigorous stirring, shaking, sonication, and drying and grinding aid in getting good contact. Mixed solvents generally give more total extractables than single solvents. The efficiency of extracting various fractions of the total extractable is not necessarily related. Water-miscible solvents, e.g., acetone-methanol, result in inclusion of water-soluble materials such as sodium chloride in the total extractable fraction.

Methods for components

28. Several oil and grease methods have been developed for monitoring gross efficiency of sewage treatment processes for removal of animal fats and vegetable oils. From this beginning, standards for oil and grease in effluent and receiving waters as well as in dredged

material have evolved. High levels of oil and grease in sediments, while possibly indicative of fats and vegetable oils, often reflect high petroleum inputs since both sewage treatment and natural biodegradation reduce the amount of animal fats and vegetable oils. To measure the petroleum content of sediments, hydrocarbons are assayed separately from the majority of the other components. To this end numerous methods have been developed to measure petroleum hydrocarbons independently of other oil and grease components. Some of these methods have evolved out of basic geochemical studies on petroleum origin, while others were developed to measure petroleum contamination. Concurrently, methods have been developed to determine the fatty acid components of the polar lipids in sediments. Several reviews of methods for determination of petroleum hydrocarbons are available.[25-28] The commonly used methods are summarized in Table 8.

29. Gravimetric methods. These methods have been used for measurement of total hydrocarbons [APHA, standard method 12] and for various chromatographic fractions such as alkanes, aromatics, and polar compounds obtained from soils.[29] Generally milligram amounts are required, but with use of electronic microbalances, microgram quantities can be measured, although considerable error may be encountered.[30] Gravimetric methods do not measure volatile petroleum components. Sulfur, if present, is included both in total hydrocarbon measurements and the saturated or alkane fraction unless samples are desulfurized.[31]

30. Gas chromatography (GC) - FID. Petroleum hydrocarbons have been determined using gas chromatography with flame ionization detectors (FID) by a large number of investigators.[32-39] Packed columns,[32-34] support-coated, open-tubular (SCOT) columns,[35] and high performance capillary columns[36] have been used. Packed columns accommodate the largest sample volume but resolution is sacrificed, and capillary columns offer the best resolution of components but require the injected sample to be split so as not to overload the column. The

Table 8

Techniques for the Determination of Petroleum Components in Sediments

	Technique	Components Determined	Sensitivity	Pretreatment	Accuracy/* Precision	References
1.	Gravimetry	non-volatile hydrocarbons and hydrocarbon fractions	µg	chromatography desulfurization	E/G	10-14,30,31
2.	Gas chromatography-FID	n-alkanes, isoprenoid alkanes total alkanes and aromatics	ng	chromatography desulfurization	F/G	33-40
3.	Gas chromatography-FPD	sulfur-containing compounds	pg	chromatography desulfurization	F/G	41-42
4.	GC/MS	n-alkanes, iso and cyclo-alkanes, mono-, di-, and tri-aromatics, etc. individual compounds	ng	chromatography desulfurization	Qual	43
5.	Fluorescence emission	aromatic hydrocarbons-primarily those containing 3 or more rings	pg	chromatography desulfurization	P/E	47-49
6.	Mass spectrometry	n-alkanes, ring no. of cycloalkanes ring no. of aromatics, sulfur content		chromatography	Qual	44-46
7.	Thin-layer chromatography	saturated hydrocarbons and unsaturated hydrocarbons	mg	none	F/F	31, 51
8.	UV adsorption	aromatic hydrocarbons	mg	chromatography desulfurization	P/E	52
9.	IR adsorption	total hydrocarbons	mg	none unless polysaccharides present	F/G	10,14,53-55
10.	High pressure liquid chromatography	aromatic hydrocarbons	mg	none	P/G	56,57

*E - excellent, G - good, F - fair, P - poor, Qual - primarily a qualitative method.

SCOT columns provide an intermediate alternative.

31. Gas chromatographic methods have been used for both low molecular weight hydrocarbons, C_1-C_{10},[38,39] and higher molecular weight hydrocarbons, C_{12}-C_{36}.[32-37] The high molecular weight components, including asphaltic materials, cannot be determined by this method.

32. Farrington et al.[33] have carried out intercalibration of GC methods after varied pretreatment of samples including saponification, no saponification, column chromatography, and thin-layer chromatography among three laboratories. Measurement of oil-spiked cod liver oil gave values of 9% relative error and 6% relative standard deviation for a distillate cut of Southern Louisiana crude oil, and values of 69% relative error and 34% relative standard deviation for Wilmington crude. Relative standard deviation on hydrocarbons from tuna meal was 12%, pristane in cod liver lipids 10%, and pristane in tuna 80%.

33. Gas Chromatography - FPD. Sulfur containing petroleum constituents such as thiophene, C_5H_5S, have been determined selectively by gas chromatography using a flame photometric detector (FPD).[40,41] Petroleum hydrocarbons are not detected by the FPD.

34. Gas Chromatography - MS. Gas chromatography - mass spectrometry (GC/MS) methods have been reviewed by Eglinton et al.[42] The addition of mass spectrometry detection to gas chromatography enormously expands the amount of obtainable data. Mass spectrometry enables measurement of classes of compounds, such as n-alkanes, cycloalkanes, monoaromatics, and diaromatics, to name a few. Individual compounds may be measured in complex mixtures, and the structure of major components can often be determined. The major disadvantage of GC/MS is the high cost of equipment and operation.

35. Mass spectrometry. Several studies have used mass spectrometry alone to characterize chromatographic fractions of hydrocarbons.

Bean et al.[43] have used it for determination of ring content, sulfur content, and molecular weight. Others[29,44,45] have used mass spectrometry for analysis of n-alkanes and cycloalkanes in soil and sediment extracts.

36. Fluorescence emission. Fluorescence spectrophotometry has been used for estimation of petroleum oils in sediments.[46-48] This technique employs the fluorescence of the aromatic compounds, primarily those polynuclear aromatic hydrocarbons in petroleum. The saturated hydrocarbons and alkylated monoaromatics are not usually determined by this method. Therefore standards must be selected carefully to accurately measure oil. However, Hargrave and Phillips[46] report that fluorescence patterns of sediment extracts do not resemble fresh oil, so that some inaccuracy results from use of fresh oil standards. A new total luminescence method is being developed[49] that will alleviate the problems associated with the fact that different oils have quite different fluorescence spectra.

37. Thin-layer chromatography. A method for estimation of hydrocarbons from environmental samples using thin-layer chromatography has been described by Hunter and co-workers[30,50] and applied to analyses of hydrocarbons from marine tissues and sediments.[50] This method allows determination of both alkane and aromatic hydrocarbons without pre-separation by liquid chromatography. Sulfur is not detected by this method. Polar lipids such as triglycerides are not measured but may be qualitatively observed. The average relative error on determination of Kuwait crude oil in a sediment matrix was 25%.[50]

38. Ultraviolet (UV) absorption. Petroleum oils have been quantified by UV-absorption by the aromatic hydrocarbons.[51] Natural UV absorbing compounds must be separated prior to determination. The problem of selection of a standard is similar to that encountered with fluorescence emission, i.e., petroleum oils exhibit vastly different absorptivities. Mineral oil for example is nearly transparent to UV

while bunker fuels absorb intensely throughout the UV range. Natural forms of elemental sulfur also absorb UV and must be removed.

39. Infrared (IR) absorption. Several groups[52-54] have used an IR method derived from the standard method [1] for oil in water. The IR method is capable of measuring petroleum hydrocarbons (both volatile and non-volatile) as well as proteins, polysaccharides, and other natural material containing $\geq$C-H groups. Mark et al.[54] have developed a method of correcting IR measurements of petroleum oil for the presence of biological material.

40. High pressure liquid chromatography (HPLC). Recently, HPLC methods have been reported for determination of types of aromatic hydrocarbons. Zsolnay [55] detected the aromatic hydrocarbons separated by HPLC using a UV-absorption detector, and Miles et al.[56] have used a more sensitive fluorescence emission detector on a similar HPLC method. The addition of HPLC to UV or fluorescence detection provides additional information regarding the types of aromatic hydrocarbons present. These methods suffer the same problems of quantitation as do the UV absorption and fluorescence emission techniques. Mineral oil cannot be detected and the extent of interference of sulfur has not been investigated.

41. Isolation and analysis of n-alkanes. The n-alkanes may be isolated by urea adduction[44] or by absorption on molecular sieves.[57] The resultant n-alkanes may be analyzed by gas chromatography[49] or by mass spectrometry.[44]

42. Removal of sulfur. Many methods for determination of hydrocarbons also detect elemental sulfur which is often present in extracts of anoxic sediment. Blumer[31] has developed a simple procedure for removal of sulfur from sediment extracts. In this method the extract

is passed through a small column of freshly precipitated copper powder and the elemental sulfur reacts to form copper sulfide, removing it from the extract.

Components of Oil and Grease and Their Levels in Aquatic Sediments

43. As a precautionary note, inferred from the preceding Methods section, "oil and grease" values from different environments may have different meanings, based on the methodology used to determine the value. Thus, literature values expressed as "oil and grease" or "total extractable residue" are not strictly comparable, although of interest in relative terms.

Naturally occurring components

44. Oil and grease residues may be found in sediments wholly uncontaminated by man. The most comprehensive data on these residues are found in the literature of organic geochemistry, and most often in the literature produced through research efforts to determine the natural origin of petroleum.

45. Organic material in anaerobic depositional environments shows the greatest degree of preservation. Shallow Black Sea bottom sediments contained up to 0.4% dry wt of saturated and unsaturated fatty acids, with palmitic acid most abundant.[58] Chlorin and porphyrin pigments were abundant at up to 0.16% dry wt. There were at least 15 sterols with levels reaching to 0.25% dry wt. A variety of carotenoids were present, reaching 40 ppm. The dominant polycyclic aromatic hydrocarbons present reached levels of 1 ppm, with perylene being dominant. Simoneit,[59] working with recent Holocene era cores from the Black Sea, found up to 2.5% solvent-extractable matter, a significant fraction of which was sulfur. The remaining material consisted of a complex suite of carboxylic acids and hydrocarbons. The anaerobic conditions in the Black Sea preserved many individual compounds apparently without their biodegra-

dation, and since deep cores showed similar values to shallow cores, the possibility of contamination by recent civilization was ruled out. Over 50 different components of the alkane fraction were recognized, as well as more than 20 methyl esters, 30 acid esters, and 40 branched cyclic acid esters. This author suggested a terrestrial origin for these organic compounds, arriving in the environment adsorbed to turbidite clays.

46. Surficial sediments not impacted by man in Lake Huron[60,61] (an aerobic depositional environment) were found to contain relatively low levels of fatty acids, fatty alcohols, and hydrocarbons in the solvent extractable fraction. Levels of total hydrocarbons ranged from 6 to 200 ppm with levels of total fatty acids from 5 to 10 times higher than those of either total n-alkanes or total fatty alcohols.

47. Sterols form only 60 to 300 ppm of the total organic carbon of natural marine sediments[62] and thus are a minor component of total extractables in natural sediments, which was reaffirmed by discovery of low levels of cholesterol, campesterol, β-sitosterol, and sigmasterol in marine sediments.[63] Sedimentary sterol content in an uncontaminated region of the Mississippi Gulf Coast showed major variations in levels of the different sterols from one habitat to another.[64]

48. Normal, monomethyl branched chain, isoprenoid long chain alcohols, and dihydrophytol have been found at 6 to 30 ppm dry sediment in recent marine sediments.[65]

49. Petroleum reservoirs in the earth, as well as oil sands and shales containing organic compounds considered to be precursors of petroleum suggest in advance that hydrocarbon residues in benthic sediments would be the longest lived organic compounds of "oil and grease", based on their general resistance to biodegradation. Kvenvolden[66] presents a valuable short resume of the early work in organic geochemistry, which

traces initial discovery of hydrocarbons in modern sediments.

50. There is a wealth of literature supporting the existence, both in marine and fresh water, of significant fractions of hydrocarbons in natural sediments remote from technological development. In core samples analyzed by Smith,[67] 65 samples from 31 locations contained a variety of hydrocarbons, ranging from 9 to 11,700 ppm. Recent origin of some samples was determined by ^{14}C analysis. Recent sediment samples analyzed by Smith contained ranges from 9 to 800 ppm total hydrocarbons with typical values less than 200 ppm. No sharp distinction was found between freshwater and marine sediments although freshwater sediments were characteristically higher in total organic matter content. This author discussed the possibility of bacterial synthesis of hydrocarbons within sediments, as supported by Han and Calvin.[68] Hydrocarbons may be absorbed or adsorbed onto suspended particulate matter in aquatic environments and deposited in benthic sediment (discussed below). Meyers[61] studies of sediments remote from petroleum contamination showed total hydrocarbon levels ranging to a maximum of 200 ppm dry wt. In examining hydrocarbon content in sediment cores, no difference could be noted between surficial and deeply buried sediments many decades in age.

51. Hydrocarbons in uncontaminated estuarine muds from Choctawhatchee Bay, Florida, showed bitumen (benzene soluble components) ranging from 60 to 470 ppm with an average of 170 ppm or about 0.5% of the organic carbon present.[69] Approximately 50% of the surface mud bitumen fraction consisted of hydrocarbons. The distribution of hydrocarbons in these muds ranged from C_{19} to C_{33} in a remarkably uniform manner, showing a marked predominance of odd over even carbon number molecules. This is a commonly noted characteristic difference between modern and fossil hydrocarbons, which characteristically show an even distribution of carbon number molecules.[70] No qualitative differences were found between surface samples and samples from the bottoms of 60-cm cores, although there was a slight decrease in hydrocarbons recorded with depth of burial.

52. Lighter molecular weight hydrocarbons (C_4 - C_8) are probably not retained in recent sediments due to volatilization.[71]

53. A large volume of literature available on $> C_{15}$ hydrocarbons in naturally occurring recent sediments, organisms and fecal material has been summarized by Meinschein[72] as follows:

a. Concentrations of olefinic hydrocarbons, principally terpenes, greatly exceed the concentrations of these compounds in fossil organic matter.

b. Alkane hydrocarbons are principally isoprenoids, steranes, and n-paraffins which structurally resemble biological acids and alcohols; relative abundance of alkanes of any given carbon numbers differ significantly from relative abundances of analogous acids and alcohols in most samples.

c. Concentrations of alkanes in fecal and sediment extracts are similar, and are an order of magnitude greater than the concentrations in biological lipids.

d. Aromatic compounds are found in only trace quantities in biological lipids, and if found in sediments, usually occur in sediments buried in excess of a few feet.

e. Non-alkyl substituted polycyclic aromatic compounds are characteristic of recent sediments, whereas the alkyl substituted analogs of these compounds are common in fossil organic material.

54. Average hydrocarbon compositions summarized by Meinschein[72] for samples of recent clay muds range from 29 to 105 ppm hydrocarbon, with extreme values at 12 and 352 ppm. Reviewing the information on non-isoprenoid aliphatic hydrocarbons in biological systems, Kolattukudy[73] concluded that 15 million tons of these hydrocarbons were synthesized in the sea annually by phytoplankton and that 25 to 100 million tons were produced on land. Presumably a significant fraction of the

estimated 100 million tons of plant hydrocarbons are available for eventual transport to aquatic environments, although little is known concerning their biogenic and biochemical transformations.

55. Koons and Monaghan[74] suggest a production of 6×10^6 metric tons of hydrocarbons in the ocean yearly, which does not differ greatly from the estimate by Kolattukudy.[73] Koons and Monaghan[74] review information on submarine oil seepage, estimating this input to be about 0.6×10^6 metric tons per year.

56. Trace amounts of benzopyrene, found in sediment cores at 2-m depth, exceeded 500 years in age as found by ^{14}C dating. This predates the technological use of fossil fuels.[75] Several reports suggest the probability that polynuclear aromatic hydrocarbons are produced by incomplete combustion in natural fires.[76-78] Biochemical reworking of these hydrocarbons may result in the production of their alkyl homologues in natural substrates[77] where they usually occur at the parts per billion (ppb) level. Aromatic hydrocarbons containing from three to eight rings, and their nitrogen containing analogs, are among the most potent mutagens and carcinogens known, and it has been speculated that these naturally occurring compounds may have had a role in evolutionary processes by causing mutations.[77,78]

57. Comprehensive reviews are available that discuss the occurrence of polynuclear aromatic hydrocarbons (PAH) occurring as extractable residues of water and sediments.[79,80] Sedimentary values for PAH ranged over 5 orders of magnitude. Values for Mediterranean Sea sediments contained 1.4 to 15 ppb benzo(a) pyrene on a dry weight basis.[79]

58. In contrast to the preceding views,[76-78] Hites[81] concluded that there was no biosynthesis of polycyclic aromatic compounds.

He hypothesized that combustion products of fossil fuels (rather than plant fuels) followed by sedimentary deposition, and microbial modification accounted for the presence of alkylated PAH derivatives in aquatic sediments.

Introduced components

59. Components of the oil and grease fraction introduced by man, and thus considered "contaminants", fall into three major categories, based on amount, biodegradability, and potential environmental effects, as listed in Table 9 .

60. The materials of biological origin are expected to form a transitory component of the oil and grease fraction based on their relative ease of biodegradation by bacteria, both in secondary waste treatment facilities[82,83] and in ambient environments.

61. Petroleum (fossil) hydrocarbons, and their sulfur, oxygen, and nitrogen containing analogs are probably the most important general fraction of oil and grease. This category is represented both by the complex constituents in the diverse crude oils,[84] which are spilled, and by altered hydrocarbons and related compounds released to the environment in oil refining activity. Transport and usage of refined products further releases primary as well as waste hydrocarbons, and combustion products into the environment.

62. Oil and grease, if not removed by skimming or sludge removal in waste treatment plants, may constitute an average of 20 mg/l in dissolved, colloidal, or suspended form.[85] Given the more than eight billion gallons of wastewater discharged into coastal waters and existence of physical and biological mechanisms for the transfer of dissolved and suspended residues into bottom sediments (see Fate Section below), municipal wastewater is a significant source of oil and grease

Table 9

Artificially Introduced Components Which May Occur in Oil and Grease Fraction of Contaminated Aquatic Sediments

Origin Component	Molecular Wt Ranges	Relative Biodegradability	Potential Environmental Effects
Biogenic*			
oils, fats waxes, soaps	100 - 700	good	B, S, G
Fossil			
saturated	16 - 500		
alkane		fair	B, ?
naphthene		poor	?
unsaturated	28 - 300		
olefinic		fair	?
aromatic		poor	T, M, C**
N,S,O, analogs of hydrocarbons	48 - 300	poor	T, M, C
Synthetic, non-trace			
detergents	200 - 400	fair to good	B, S
Synthetic, trace			
chlorinated hydrocarbons	300 - 800	poor	T, M, C
plasticizers	200 - 500	poor	T, M, C
Other (pesticides, etc.)		?	?

*Including chemically modified products of biogenic origin.
**When metabolized.

M - Metabolic effects
C - Carcinogenicity, mutagenicity
B - Biochemical oxygen demand
S - Surfactant effects
G - Physical effects
T - Toxicity

contamination in coastal sediments. Marine disposal of sewage sludge could carry large quantities of oil and grease directly into bottom sediments. Digested sewage sludge may contain up to 100 g/kg mineral oil residue.[83] Grossling[86] has suggested that over 1 million tons of spent lubricating oils are discharged into the oceans yearly. Coastal petroleum hydrocarbon inputs from all sources have been estimated at approximately 2.4 million tons per year,[2] and the need for abatement has been recognized. For example, the administrator of EPA Region IX currently specifies that no dredged material for disposal within San Francisco Bay will exceed 1900 mg/kg oil and grease.[87] In its water-quality criteria guidelines, the EPA has suggested that specific bioassay testing be carried out on each effluent in question; virtual freedom from any floating oil and grease residues is desirable.[88]

63. There is apparently no reference data that exhaustively characterize the components of contaminant oil and grease residues in aquatic sediments. Analyses have been made on lipophilic contaminants in river water,[89] with the major components identified, including both naturally occurring and manmade compounds. Presumably many of these compounds can be adsorbed on particulates and deposited in sediments. These data (reference 89) suggest the presence of well over 100 compounds including C_{15}-C_{31} alkanes, alkyl naphthalenes, alkyl anthracenes, or phenanthrenes, pyrene, fluoranthene, and alkyl phthalates in river water.

Comparative levels of natural and contaminant hydrocarbons

64. Voluminous data have been produced through efforts to establish the levels, and identify the origins of the hydrocarbon fractions of oil and grease residues in aquatic sediments.

65. Research on a spill in Buzzards Bay, Mass.[90,91] showed that oil residues persisted in the sediments for several years after the spill. Natural background levels of total hydrocarbons at uncontaminated sites were 50 to 70 ppm, with levels in contaminated sediments up to

1170 ppm. Determinations were carried out by gas chromatography and were aided by prior knowledge of the spill components. Monitoring of the introduced sedimentary oil and grease components was carried out by comparing analyses of the original oil with that recovered from impacted sediments.

66. Sediments from Narragansett Bay, remote from contaminant inflows and urbanization, showed 50 to 60 ppm total hydrocarbon, whereas samplings obtained in upper Narragansett Bay showed up to 3560 ppm total hydrocarbons.[92] These hydrocarbons were determined to be of fossil origin using ^{14}C dating and characteristic of gas chromatograms.[93] Petroleum-derived and indigenous hydrocarbons of Lake Zug, Switzerland, were differentiated by infrared and gas chromatographic analysis,[94] with levels of indigenous hydrocarbons reaching 900 ppm near urbanization and about 50 ppm at uncontaminated stations remote from petroleum usage.

67. Up to 6510 ppm petroleum hydrocarbons were found in manifestly contaminated Baltimore Harbor sediments, decreasing in amount with core sample depth to baseline values of 11 ppm at 120 cm.[95] A complex suite of petroleum-derived hydrocarbons was monitored in this study, leaving no doubt as to the origin of the oil and grease in the samples. A comparative survey of three aquatic sediments from three freshwater lakes differing in exposure to hydrocarbon input in northwestern Washington showed hydrocarbon levels of aliphatic hydrocarbons up to 1500 ppm in the most urbanized lake, 500 ppm in a less urbanized lake, and less than 30 ppm in a remote lake.[96] All lakes showed similar hydrocarbon values at core depths that represented sediment deposition predating the advent of petroleum usage. These results were supported by isotope analysis for age determination.

68. Shelton and Hunter[97] reported total hydrocarbons for sediments

from eight New Jersey Rivers ranging from 0.12 to 38.3% on a dry weight basis. The lower end of the range represented presumably uncontaminated rivers, and the high end, the most seriously contaminated riverine sediments. Tissier and Oudin[98] compared analyses of a number of littoral sediments from the north coast of France and found that hydrocarbons from sediments contained an overwhelming fraction of asphaltene-like compounds with small proportions of alkanes and aromatics. In contrast, oil contaminated sediments contained large proportions of alkanes and aromatics relative to the asphaltene fraction. High molecular weight substituted aromatics in sediments were considered to be the best indicators of oil contaminated conditions.

Fate and Effects of Sediment-Associated Oil and Grease Residues

69. The fate of lipophilic residues in aquatic sediments has been examined in developing the theoretical basis for explanation of petroleum genesis. In general, it is proposed that organic compounds formed in nature are deposited in sedimentary environments, become buried, undergo diagenesis under pressure and increased temperatures over geological time periods, and migrate to petroleum source beds.[72,99] The earliest steps in this process describe the fate of oil and grease residues within the human time frame and are most conveniently treated under four hierarchical categories as follows:

1. physical-chemical interaction
2. microbiological processes
3. metazoan processes
4. ecosystem cycling

70. The following discussion applies to lipophilic materials of both natural and contaminant origin.

Physical and chemical interactions

71. Oil and grease residues are essentially insoluble in water, although there are a number of residues in petroleum that are slightly water soluble.[100] These are known to have serious effects on metazoans,[101] although solubility predisposes to rapid dissolution of these materials, particularly in water bodies that are well mixed due to currents, tidal exchange, and wind-driven turbulence. Contaminant oil and grease may enter aquatic environments in free or associated forms. Massive oil spils are an example of the "free" form, in which wind and wave energy may break up the spill and allow formation of oil particulates, which may sink in the water after losing volatile components. "Bound" oil and grease residues are those that are absorbed or adsorbed onto other organic or inorganic materials, such as the oil and grease residues from municipal waste effluents.[85]

72. The National Academy of Sciences (NAS) survey report[2] on petroleum in the marine environment lists as one of the ultimate fates of petroleum its "incorporation into sediments" without describing the complicated dynamics of physical-chemical interaction of sediment-associated oil and grease. Petroleum oils adhere to diverse inorganic particles based on particle size, irrespective of mineral composition. Smaller particles adsorb larger amounts of the oil based on a simple surface area relationship. As an example, 1 g of silt was shown to be capable of adsorbing as much as 2.5 ml oil.[102] Diatomaceous earth has been shown to take up as much as 0.95 g motor oil at a salinity of 9% with adsorption decreasing with decreasing salinity.[103] Evidence obtained in work with hexadecane, eicosane, anthracene, and motor oil adsorbed onto bentonite clay and natural sediments suggested that the uptake of these hydrocarbons was proportional to their solubility in water (with increase in temperature) as well as particle size, and was retarded by the presence of organic matter on sedimentary particles.[104] Once the hydrocarbons had become associated with the sediments they were not readily eluted with water, although only a weak physical adsorption

was involved. Thus, once oily materials are adsorbed to sediments they are not readily returned to the water column.

73. Similar studies made on fatty acids[105] showed similar results, with adsorption reduced at higher temperature as fatty acids became more soluble. Increasing presence of organic matter also relatively decreased adsorption. Adsorption was attributed to the weak van der Waal forces and to hydrogen bonding. Using natural sediments from Narragansett Bay, increased presence of sedimentary organic matter decreased fatty acid uptake by a factor of 1.6. When the water was not saturated with hydrocarbon (dodecane), it was not adsorbed to montmorillonite clay[106] and it was suggested as a general case that at concentrations exceeding the solubility of the hydrocarbons, clay particles were very likely to show affinity for small oil phase particles as a direct adhesion phenomenon.

74. Although physical-chemical theory has been applied to problems of sediment sorption of oil and grease residues, knowledge of these processes is limited. The physical-chemical basis for sediment sorption and particle adhesion has been reviewed by Engineering-Science.[109] Since adsorption requires the presence of a polar group in the compounds, the non-polar oil and grease residues (e.g., hydrocarbons) are probably held weakly in sediment matrices, probably residing in the sediment by virtue of adhesive properties due to preferential wetting of the sediment by the oil.[16] It is well known that in field sampling of sediments in contaminated waters that oil slicks are often seen when disturbing sediments in situ, probably representing release of oil not directly in contact with sediment particle surfaces. Effective oil sinking agents have a large specific surface area, and are wetted by oil in the presence of water.[107] Absorption of oily residues may occur into pore spaces of certain particulates, as well as absorption into interparticular spaces of tightly aggregated particles.[108] In view of the great diversity of oil and grease residues, and the variety of naturally occurring sediment

types and conditions, a monumental research effort would be required to begin definition of mechanisms for each combination of sediment/oil-grease association.

75. Studies made on trace contaminants of oil and grease help define the fate of the overall oil and grease burden. This has primarily been based on interest in the fate of pesticides in the environment, particularly since it is known that DDT occurs as a contaminant in the more abundant oil contaminants.[109] Inferential evidence has suggested that DDT adsorption to suspended clay and sediment particulates occurred by simple adsorption-desorption equilibrium, whereas adsorption to humic acid occurred by hydrophobic bonding to lipophilic fractions associated with humic acid, and by capillary absorption within the pores of the humic polymer.[108] After reviewing literature on DDT adsorption to particle surfaces, Hargrave and Phillips[110] concluded that particle diameter served as the best operational way to integrate the variety of surface-related parameters describing rates of adsorption of hydrophobic organic compounds. As DDT was biodegraded within Severn Estuary sediments (England), its breakdown products became more and more tightly associated with the sediment particles and cell debris in situ.[111] Studies on toxaphene in lake sediments have suggested that this material is directly sorbed onto benthic sediments from the water and co-deposited with toxaphene-laden algal blooms and other particulate matter. Laboratory studies demonstrated that sorption of the toxaphene was an irreversible process, and that significant leaching of the toxaphene from the sediments by water was improbable.[112] Adsorption of lindane (a neutral molecule) to sediments was attributed to van der Waal's forces and hydrogen bonding. Lindane adsorption was influenced primarily by sediment suspension concentration and organic matter content of the sediment, as was sediment particle size.[113] Consistent with this work, it was later shown[114] that lindane was much more easily leached from aquifer sands than was dieldrin (a polar material) based on the weak attractive

energies between the lindane and the sediment particles.[114] For both lindane and dieldrin, adsorption increased with increasing concentration of pesticide.

76. Sayler and Colwell[115] showed that both mercury and PCB's could be significantly partitioned from the water column by contaminant oils and suspended sediments. This partitioning of toxic contaminants by oils and sediments could affect sedimentary processing of these contaminants by microorganisms.[116]

Microbiological processes

77. Oil and grease/sediment complexes are widely transported throughout water bodies[21] entering into environmental sediment transport pathways from which they are subsequently deposited. Numerous species of bacteria exist in the water column, and particularly within sedimentary systems which are capable of degrading oil and grease residues as determined in laboratory culture trials.[117-123] Although chemical and photochemical oxidation of some oil and grease residues are probable under well-aerated conditions, it is probable that the major net loss of hydrocarbons and other constituents of oil and grease is through microbiological attack aided by sedimentary processing by metazoa.[129]

78. The variety of hydrocarbons and other residues in oil and grease fractions is bewildering, as are the biochemical mechanisms of their degradation;[84] discussion of this topic is beyond the scope of this review. Alkanes are the most readily degraded hydrocarbons, followed by the isoalkanes, cycloalkanes, and aromatic hydrocarbons (summarized by Lee[122]). The simpler compounds are degraded to CO_2 and H_2O in the presence of oxygen. More complex molecules may be degraded to intermediate products (fatty acids, hydroxy derivatives).[124] Bacteria oxidize PAH to cis-diols while at least one species of fungus and mammalian enzymes oxidize PAH to (carcinogenic) trans-diols.[125] Auto-

oxidation and initial bacterial attack increase polarity of some oil and grease residues, rendering them more water soluble and more amenable to adsorption and further biodegradation steps.

79. There have been no studies found to date on the comprehensive evaluation of the biodegradation of contaminant oil and grease fractions in any given sediment. Therefore, the microbiological fate of oil and grease residues must be extrapolated from the numerous studies made on differing aspects of the problem. Early studies concentrated on the role of microbiological involvement in the genesis of petroleum[118,126] while the more recent studies responded to the necessity for determining the fate of spilled petroleum in aquatic environments.[9]

80. It is instructive to note that of 109 abstracts concerning the microbiological fate of petroleum residues in the water environment, only five dealt with petroleum/sediment complexes.[127] Since oily residues of plant and animal origin are readily biodegraded, as evidenced by data from sewage treatment,[83] the following discussion primarily concerns hydrocarbons of petroleum origin.

81. Virtually all kinds of hydrocarbons and their nitrogen and sulfur containing analogs are susceptible to some microbial modification under favorable conditions.[86] Bacteria migrate through sediments, release surfactants that aid in emulsification of oil and grease, dissolve sedimentary particles holding hydrocarbons, and may physically crowd oil residues from particle surfaces.[128] Under pure culture conditions with abundant supplies of hydrocarbons, bacteria have been observed to completely overgrow oil microdroplets, vacuolize hydrocarbons, and produce unusual giant cell variants in response to the hydrocarbon substrate.[129] The fragmentary information about lab and in situ breakdown of hydrocarbons and other substrates by bacteria suggest major

limiting factors on hydrocarbon breakdown to include:[119]

a. oxygen availability: Most hydrocarbon decomposition requires the presence of molecular oxygen.

b. dispersion characteristics of the substrate: Oil and grease residues are more susceptible to attack when adsorbed to finely divided substrate particles.

c. temperature: Although psychrophilic hydrocarbon digesters have been reported, a general rule is that the higher the temperature (within reason), the faster the rate of hydrocarbon decomposition.

d. salinity: Different hydrocarbon digesting microorganisms have been found to show different salinity requirements.

e. turbulence: Turbulence favors hydrocarbon breakdown, based on diffusivity of gases and nutrients into the milieu.

f. organic matter: Oxidizable organic matter may enhance or retard certain hydrocarbon degradations.

g. substrate concentration: In some cases hydrocarbon degradation is retarded by lack of concentration of substrate, and in other cases excess substrate may inhibit it.

h. predation: Bacterial feeders may slow the hydrocarbon degradation by removing produced bacteria from the substrate as they grow.

i. nutrients: Hydrocarbon degrading organisms require nitrogen and phosphorus for growth.

Although a number of workers suggest that sulfur, nitrogen, and iron compounds may function as electron acceptors in oxidation pathways,[130-132] others affirm that molecular oxygen is required for the initiating steps in hydrocarbon oxidation.[133-135] It is almost universally agreed, however, that if there is hydrocarbon biodegradation under anaerobic conditions in the environment, it will occur at extremely slow rates.

82. The most recent and comprehensive studies on breakdown of oil in aerobic and anaerobic environments have been carried out by

Shelton and Hunter.[136,137] These authors recognized that most oil contaminants are sedimented by natural sorptive agents, which result in the contamination of aquatic sediments with oil near urbanized or industrial areas. In aerobic freshwater-sediment microcosms they[136] showed continuous linear oxygen consumption equal to that estimated for naturally metabolizing benthic communities.[138] The hexane extractable oils in the sediments decreased by less than 4% in the 33-week experiment, with gas chromatographic data on the hexane extraction fraction showing no significant qualitative change in the oil. Under anaerobic conditions[137] hexane extractables dropped by 11% and benzene extractables showed no decline. These authors discounted the possibility of more rapid bacteriological activity under anaerobic conditions in favor of a hypothesis that under anaerobic conditions, intermediate biochemical products were formed, which were more soluble, and therefore more readily leached into the water than under aerobic conditions. Functional group analysis showed loss of oxy-compounds (aldehydes, ketones, carboxylic acids) under anaerobic conditions.

83. Ludzack et al.[139] had previously found no evidence of biological degradation of hydrocarbons in samples of oil and grease contaminated river mud, incubated in the laboratory for 125 days under anaerobic conditions, and seeded daily with fresh sewage.

84. In marine systems, extremely low rates of hydrocarbon breakdown have been found for east coast continental shelf sediments[140] containing petroleum wastes and maintained under anaerobic conditions. Under what might be considered optimal conditions for bacteriological hydrocarbon breakdown under environmental conditions, Johnston[141] was unable to demonstrate any more than 10% decomposition of Kuwait crude oil in well-oxygenated sand columns. The meticulous studies of Lee and Ryan[124] demonstrated that bacteria in the water column were capable of degrading a wide range of hydrocarbon types, but that the rates of

degradation were so slow as to be of questionable importance in ocean waters.

85. Review of the literature emphasized that although many bacterial species have shown activity in hydrocarbon degradation under enriched conditions (e.g., references 120,121,123,142), the probability of occurrence of significant environmental degradation of these important oil and grease residues within sediments is very low. The authors of this report have conducted studies that provide further empirical verification of this hypothesis (see Part II).

Metazoan processes

86. The small amount known about the fate of oil and grease residues in macroorganisms has been derived from effect studies of oil contamination, with a minor part of this information on sediment-associated oil and grease. Thus, generalizations in this area are highly tentative.

87. Biomass of macroorganisms in environments is so small compared to the mass of sediments and water, that a priori it may be assumed that the tissue compartment will be a quantitatively insignificant reservoir of environmental oil and grease. In general, macroorganism interaction with the fate of oil and grease residues can be categorized as 1) mechanical activity or 2) metabolic activity.

88. Mechanical activities of macroorganisms include suspension feeding, construction of tubes and burrows, fecal pellet deposition, and other activities in which macroorganisms affect suspended and deposited sediments.[143] Conover[144] speculated that zooplankton "could be the single most important natural agent leading to eventual dispersal and degradation of oil spills" in the open sea. This was based on his demonstration that a large population of zooplankton in Chedabucto Bay, Canada, was able to graze on spilled oil and incorporate it into fecal

pellets that were then sedimented to the Bay bottom. Benthic organisms, primarily small arthropods, polychaete worms, and bivalve molluscs carry out deposit and suspension feeding activities, and in so doing, digest organic materials and mix benthic sediments.[143] Such organisms are of cardinal importance in bringing oxygen to anaerobic sedimentary layers, and indirectly aiding bacterial oxidation processes, in unusual cases to depths of 3 m.[145]

89. With regard to metabolic activities, it is well known that various aquatic organisms accumulate lipophilic compounds in aquatic environments, and a few studies have begun to demonstrate the metabolism (or lack thereof) of some hydrocarbons by certain organisms.[124] Reviews of the fate and effects of hydrocarbons on organisms have been made elsewhere.[2,127,146,147] Only recently has information begun to emerge on the fate of sediment-sorbed oil and grease residues in organisms. Fossato and Canzonier[148] exposed mussels (Mytilus edulis) to suspensions of clay particles to which diesel fuel had been adsorbed. In experiments lasting up to 41 days it was found that fuel residues were taken from the clay particles and accumulated in tissues of the organisms to levels in excess of 10^3 times the concentration of oil in the water; elimination of a significant portion of the accumulated oils occurred although depuration was incomplete.

90. This study[148] is of interest in that their results demonstrated a case of simple charge-discharge (first order) kinetics, characteristic of (for example) an electrical capacitor. The curves depicted in this paper suggested that the uptake into the mussels was a passive process of partitioning of weakly adsorbed hydrocarbons from sediment surfaces into mussel tissues, where they were passively held until the experimental exposure was stopped. Depuration occurred in a logarithmic manner as passively retained hydrocarbons were lost from the compartment. It is further remarkable that the limiting value of hydrocarbon uptake found was between 300 and 400 ppm hydrocarbon, the

approximate value found by DiSalvo et al.[149] in studies of hydrocarbon uptake by mussels in a polluted estuary. Fossato and Canzonier[148] noted physiological stress in the mussels, although recovery was rapid after stress was removed. Physiological stress as well as the uptake and discharge of hydrocarbons is probably all based on the conservative properties of the hydrocarbons absorbed, as it is likely that bivalve molluscs have no enzymes capable of modifying hydrocarbons.[124]

91. Methodology for following the total oil/grease fraction through an organism or ecosystem is unavailable. Anderson et al.[150] considered naphthalenes to be valuable models for the study of petroleum residues in organisms due to their toxicity, known accumulation in organisms, and long retention times in organisms previously tested.[147] Using sipunculid worms, Anderson et al.[151] found minor uptake of sediment sorbed naphthalenes within two days following exposure. Not enough information was provided to evaluate uptake discharge kinetics, however, the results suggest that, compared to Mytilus, the organisms tested had little reservoir capacity for the retention of significant amounts of hydrocarbons.

92. In studies of hydrocarbon dynamics in the polychaete worm Neanthes arenaceodentata, Rossi and Anderson[152] noted the uptake of water soluble hydrocarbon fractions into lipid rich tissues. Passive storage of most of the hydrocarbons occurred in eggs, which upon release underwent normal development, losing their hydrocarbon burden after 40 days, possibly through metabolic activity.

93. Fragmentary knowledge concerning the metabolism of hydrocarbons by metazoans suggests that where hydrocarbon metabolism occurs, it is not a rapid process, and is probably mediated by inducible enzymes.[124] Oil and grease residues, particularly the nonpolar compounds, probably move through metazoan food webs with minor chemical change, and variable impact, depending on specific compounds and organism

species. A monumental research effort would be required to begin to document the spectrum of potential physiological effects of oil and grease residues.

Effects on organisms

94. The effects of a wide variety of oil and grease constituents on aquatic organisms, populations, and ecosystems, have been reviewed in detail by Hyland and Schneider.[146] This review was primarily concerned with the toxic effects of spilled oil, and suggested that the long-term impact of this oil once associated with oceanic bottom sediments would probably be significant. This area of the literature has provided the smallest number of references for this review.

95. Interest in this area of endeavor is increasing, with awareness of the importance of particulate adsorption of lipophilic materials in marine organic matter dynamics.[153] Attachment of oil and grease residues to particulate matter introduces a new variable into the effects of these residues on environmental systems; the sedimentary particles tend to weigh down floating residues and introduce them to water column circulatory pathways, aid their deposition in benthic substrates, present them to filter-feeding organisms that would otherwise not come in contact with spilled oils, and increase the surface area of spilled oils.

96. A most instructive set of studies of impact of a known oil contaminant introduced to an environment where it became associated with sediments was that of the Falmouth oil spill. Wherever the oil was detected in sediments, there was a kill of organisms, with almost total kills in the most heavily contaminated areas.[154] Nearly a year after the spill, sediment-associated oil continued to have effects on the growth of marsh grasses, and it appeared in the tissues of organisms associated with the marsh area.[155] Benthic faunal evaluations made 4 and 5 years after the spill[156] showed lower population densities of benthic organisms in affected marsh areas, with persistent lower animal diversity in inshore and offshore affected areas.

97. In addition to the inherent toxicity of the spilled oil, effects of the oil becoming associated with the sediments were:

a. retention of the oil in the (sensitive) environment over an extended period of time;

b. weak sediment-particle association of sediment with oil, such that oil was available to organisms in the environment over a long period of time;

c. spread of oil-containing benthic sediments over broad areas extending outward from the original impact area, based on normal sedimentary movements;

d. retardation of biodegradation of oil buried in sediments due to anaerobic conditions.

98. Thus, sedimentary association with oil residues served to prolong toxic effects of the spilled oil, rather than allow for its evaporation, dilution, and photochemical oxidation which might have occurred if the spill had remained floating in open water. Efforts by man to sink floating, spilled oils, should be considered environmentally undesirable.

99. An adverse behavioral effect which might have been caused by conditions similar to those in the Falmouth or Chedabucto Bay oil spills was demonstrated in the laboratory by Prouse and Gordon,[157] who exposed the polychaete Arenicola marina to sediment-sorbed No. 2 fuel oil. At the highest concentration (200 µg/g sediment) the worms left their burrows. At lower concentrations, worms failed to carry out normal sediment working processes, showing apparent sublethal effects of the oils. Sediments ingested and passed by the worms showed lower hydrocarbon content than surrounding sediments, suggesting either metabolism or net uptake of sedimentary hydrocarbons by the worms. The oil used was known to have high toxicity for marine life, although weathered oil showed sublethal effects similar to those of the fresh oil.

100. In laboratory studies, Rossi[158] demonstrated that the marine Polychaete worm Neanthes arenaceodentata was capable of taking up naphthalenes dissolved in seawater, but that this organism was unable to take up the same naphthalenes after they had been adsorbed to sediments. Sediments used for exposure contained 10 ppm naphthalenes, and tissue levels of worms living in and feeding upon sediments contaminated with these compounds remained below 0.1 ppm over an exposure period of 28 days. Thus, not only the types of compounds are important in exposure experiments, but also the availability of the compounds after adsorption onto the sediments. DDT residues, which can be considered part of the sedimentary oil-grease burden, were shown to be taken up from natural estuarine detritus by fiddler crabs. The uptake of this residue was the apparent cause of disorientation in the crabs, which would be fatal to them in the natural environment.[159]

101. Certainly, no generalizations can be drawn from the few studies of sediment-associated oil and grease that now exist. In some cases the nature of the oily residues and the type of sediment association allows for uptake and toxic effects on biota. In other cases, the converse is true. New data and discussion on this point are presented in the Experimental Section, Part II, of this report.

Ecosystems level processes

102. The ultimate fate of sediment-sorbed oil and grease residues is speculative. Perhaps this consideration is best envisioned by considering hypotheses concerning petroleum genesis. A fundamental assumption in this field of study is that certain hydrocarbons produced in nature undergo limited chemical or biological degradation and undergo sedimentary deposition. These organic materials are thought to undergo geological processing over long time periods in the formation of oil. A brief, although informative, summary of these poorly understood processes is given by Kallio.[84] Environmental fate of oil and grease residues, whether of contaminant or natural origin, probably follows similar

pathways into environmental sinks, after having cycled through typical sedimentary pathways.

103. On the local level, there is good documentation for transfer of sediment-sorbed oil and grease down rivers and into coastal estuaries[92,160] where the sediments become entrained into coastal sediment transport systems. A study by the Naval Oceanographic Office has begun description of the fate of oil and grease contaminated dredged material when released offshore.[161] About 571,000 cu yd* of dredged material from the U.S. Naval Station at Mayport, Florida, was disposed of approximately 7 miles offshore at a depth of about 45 ft. Oil and grease content of the dredged material was evaluated at the time of the disposal and at eight months post-disposal. Although the dredged material had shifted spatially, it could still be recognized by gross visual appearance when compared with natural sediments at the site. The oil and grease content had not changed markedly. These results suggest that the oil and grease was not chemically or biologically degraded, and that the oil and grease content of the sediment was a fairly conservative property as would be heavy metal or DDT content.

104. Long-term studies of spilled oil which has become associated with sediments in shallow water environments provide some insight into local cycling of sediment-associated hydrocarbon residues. Vandermeulen and co-workers[162,163] investigated the site of a major oil spill in Nova Scotia and found large amounts of the weathered bunker fuel still associated with beach sediments after five and seven years. The main route of reentry of oil to the Chedabucto Bay system appeared to be via contaminated sediments, which were found to be in a dynamic equilibrium

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* A table of factors for converting U.S. customary units of measurement to metric (SI) units is presented on page 15.

with surrounding water. Oil stranded at the high tidemark appeared to be moving into sediment association entrapment, followed by slow release to bay water under tidal and wave action. Loss of alkanes was substantial in the beach sediments although cycloalkanes and aromatics were essentially unaltered.

105. Perhaps the best known and best documented work on local environmental fate of hydrocarbon residues associated with sediments is that of Blumer et al.[154] and other workers [155,164] who have studied the fate of the Falmouth oil spill, which occurred in West Falmouth Harbor, Massachusetts, in September 1969. Initial measurements after the spill showed stranding of oil on surface sediments of the marsh and mixing of oil with sediments down to 10 m of water depth.[154,164] Oil was slowly released from the sediments in the months after the accident. Very slow degradation of the oil was in evidence in the first five months of its residence in the sediments. Alterations that were seen in sedimentary oil samples probably occurred before the oil was intimately adsorbed onto sediment particles.[155] The more heavily oiled sediments showed lower rates of biodegradation than those more lightly contaminated, with the more toxic components most resistant to such degradation. In the year following the accident, oil impacted sediments spread for miles seaward of the accident site down to water depths of 42 ft, and reached about 5000 acres of offshore bottom and 500 acres of marshes and tidal rivers. In inshore areas, oily residue was detected as much as 50 cm beneath sediment surfaces. Biodegradation was slow, with degraded oil offshore apparently being replaced by the more toxic, less degraded oil from inshore areas Oil degradation in marsh sediments was negligible after 8 months.[155] Two years after the spill, the oil residues persisted in marsh and offshore sediments. Although significant degradation of the alkane fraction[90] had occurred, the sediment associated oil was still qualitatively recognizable as the original spilled oil. Five years after the spill, the oil could still be recognized in marsh and

offshore sediments although biodegradation appeared to have progressed further, and quantitative statements about total residual amounts were not offered.[156] Thus, it is seen that the fate of a sediment-associated oil spill included entrainment of oil-laden sediments into offshore sediment transport processes coupled with biodegradation.

106. Kolpack's[165] efforts to construct a simulation model of the fate of environmentally discharged oils included bottom sediments as a major compartment. Although bottom sediment is the potential recipient of the largest majority of oil, there was little firm information to allow an estimate, by his identified mechanisms of dissolution and biodegradation, of the loss of oil from this reservoir.

107. Harvey and Steinhauer,[166] following waterborne PCB through worldwide ecosystems, suggested that these could act as model tracers for materials such as hydrocarbons, fatty esters, halogenated metabolites, and organometallic mercury or arsenic compounds. Unfortunately these authors did not deal with particulate PCB.

108. An example of the ultimate fate of recalcitrant oil and grease residues is given by a study of DDT and PCB residues in ocean sediments as documented for the Santa Barbara Basin.[167] These residues were found accumulated in sediment layers in a deep, low-oxygen basin, relatively free of animal life, which might perturb the sediment structure. A remarkable depositional history of these substances was recorded, correlating depositional concentration with the years of increasing production of these compounds. Woodwell,[168] in his broad survey of the environmental fate of DDT, has speculated that the ultimate fate of DDT would be deposition in deep ocean basins. Thus, using compounds such as DDT as tracers, a fundamental assertion can be made that oil and grease residues that are not biodegraded probably have a similar fate, and thus enter or re-enter the proposed theoretical initial stage of petroleum genesis on a geological time scale.

PART II: EXPERIMENTAL

Introduction

109. Organisms and communities in freshwater and marine environments may be exposed to suspended and deposited dredged material, which is often contaminated with oil and grease. Little is known concerning the impact of oil and grease residues on such organisms, even though oil and grease content is used in regulatory criteria for disposal of dredged material. It was of interest to the Corps of Engineers to undertake preliminary studies with grossly contaminated sediments under laboratory conditions to determine if, under "worst case" conditions, there was measurable transfer of oil and grease residues from the sediments into organism tissues.

110. Although results of laboratory simulations are difficult to extrapolate to actual dredging situations in regard to predicting the real impact of oil and grease on population dynamics, the proposed experiments allowed acquisition of data that could be used to provide information enabling more pertinent questions to be posed in the development of future research.

111. Encompassed within the scope of four different experiments, three different strategies of exposure to dredged material, four species of aquatic organisms (three salt water, one fresh water), and dredged sediments from three different removal sites were investigated. This regime provided a broad range of experimental variables covering some of the variation found at dredging sites and a few types of organisms potentially affected by dredging activity.

112. No studies were carried out on rates of uptake, depuration, or accumulation since it was not known a priori if accumulation of hydrocar-

bons would occur. The experiments completed represent as simply as possible an estimate of before and after conditions. For controls, organisms were exposed to reference sediments that contained little or no hydrocarbon residue. In some cases, tissues of maintenance organisms were analyzed to determine baseline hydrocarbon values. These animals were from the same stocks as the experimental animals and were maintained in the laboratory where the experiments were conducted although they were never exposed to sediment.

113. Standard methods were used to characterize oil and grease content of the sediments tested, and advanced analytical methods were used to further define the general nature of the oil and grease fraction. Three analytical methods were used to evaluate experimental results of animal exposure to the contaminated sediments.

Organisms and Sediments

114. Organisms were chosen for study on the basis of availability and mode of feeding. Mussels, Mytilus edulis, were selected as representative filter-feeding organisms. They are known to remove suspended particulates from water and carry on passive uptake of available hydrocarbons. Mussels have no known metabolic pathways for degradation of hydrocarbons.[169] Crabs, Hemigrapsus oregonensis, were chosen for their known habits of feeding on deposited debris, as well as their close association with sediments through contact with gill surfaces. Crabs in contact with petroleum hydrocarbons probably develop the enzymatic capability for detoxification of at least some of these hydrocarbons.[170] The snail, Acanthina spirata, was chosen to represent a carnivorous species in which any accumulation of hydrocarbons would not be associated with a sedimentary mode of feeding. Freshwater clams, Corbicula sp., were chosen for the same reasons as the mussels, except for the freshwater habitat.

115. Table 10 presents a listing of organisms employed in the experiments and some of their characteristics. Mussels, crabs, and snails were collected at the low tide line, and freshwater clams were purchased from a local bait dealer. Organisms were maintained in the laboratory for periods of one to four days prior to the start of the first three experiments and two months prior to the last experiment to allow flushing of environmental gut content, and acclimation to conditions under which experiments were conducted. Based on prior experience, no adverse effects were expected in transferring these organisms to laboratory maintenance conditions.

116. Oil and grease contaminated sediments used in the experiments were obtained from the Duwamish River, Seattle, Washington, and the New York Harbor. Duwamish sediments were obtained as a subsample from U. S. Army Engineer Waterways Experiment Station (WES) personnel in Seattle, Washington, on 20 February 1976. New York sediments were obtained from personnel of the New York District Office (CE), Jersey City, New Jersey, in October 1976. In all cases, sediments for experiments were placed in solvent-washed metal cans, returned to the laboratory within 12 hr, and maintained at 4°C until used.

117. Living worms were routinely observed in Duwamish sediment up to four months after their return to the laboratory. No living organisms were ever detected in the New York Harbor sediments. A sample of sediment from Oakland Middle Harbor was obtained for comparative purposes and was maintained under seawater at 15°C for one year. Reference sediments were prepared by removing oil and grease residues, including petroleum hydrocarbons, from a San Francisco Bay (clay-silt) sediment by exhaustive Soxhlet extraction with chloroform. The reference sediments for experiments 1 and 2 were vacuum-dried at 60°C, and those for experiment 3 were ashed at 300°C.

118. An experiment was conducted using an internal standard (fluoranthene) as a methods check, and to determine some characteristics of

Table 10

Organisms Used in Studies of the Uptake of Oil and Grease Residues from Contaminated Dredged Material

Species	Common Name	Size Range cm	Mode of Feeding	Origin	Water Type
Mytilus edulis	Blue mussel	6-9*	Suspension	Tomales Bay (inner, middle)	Seawater
Corbicula sp.	Bait clam	4-5	Suspension	Sacramento R.	Fresh Water
Hemigrapsus oregonensis	Green shore crab	1-2**	Selective deposit	Tomales Bay San Francisco Bay	Seawater
Acanthina spirata	Snail	approx 2	Carnivore	Tomales Bay	Seawater

*Maximum shell length.

**Maximum carapace width.

uptake of this hydrocarbon from sediments. Fluoranthene spiked New York Harbor (Perth Amboy) sediment was prepared by adding 36.7 mg fluoranthene in Freon to a slurry of 1600 ml sediment in 4 ℓ seawater. The mixture was stirred vigorously until the Freon had evaporated (approx 4 hr). The final fluoranthene concentration was 69 µg/g dry sediment.

Oil and Grease Determinations

119. Prior to experimentation, sediments were analyzed for oil and grease by standard methods[1] using hexane for extraction, and were analyzed for hydrocarbons by a standard method,[1] followed by desulfurization on a copper column.[31] The sulfur content of the hydrocarbon extract was then determined by difference.

Experimental Setups and Procedures

120. Organisms were exposed to dredged material in three static physical arrangements as diagrammed in Figure 1. Organisms were placed directly in the sediments (SED), 5 cm above the sediments on screens (SCR), or 30 cm above the sediments on a screen through which passed a stirring rod tipped with a paddle used to stir the sediments into the water column for 2 min every 4 hr (STR). In experiments 1 to 3, each of these treatments (SED, SCR, STR) was replicated three times for the contaminated sediment and one time for the reference sediment (Ref). In experiment 4, the STR configuration was established only with contaminated sediments, using two replicates containing either 10 mussels or 30 crabs each. These were stirred once per day for 2 to 3 minutes.

121. Containers were all-glass cylinders 45 by 22 cm, having a total volume of 17 ℓ. Stainless steel screen (0.5-cm mesh) was used to support organisms in SCR and STR configurations and the paddles in the STR tanks were stainless steel. Tanks were filled with water to a volume of 10 ℓ and were maintained by the addition of water throughout the experiments.

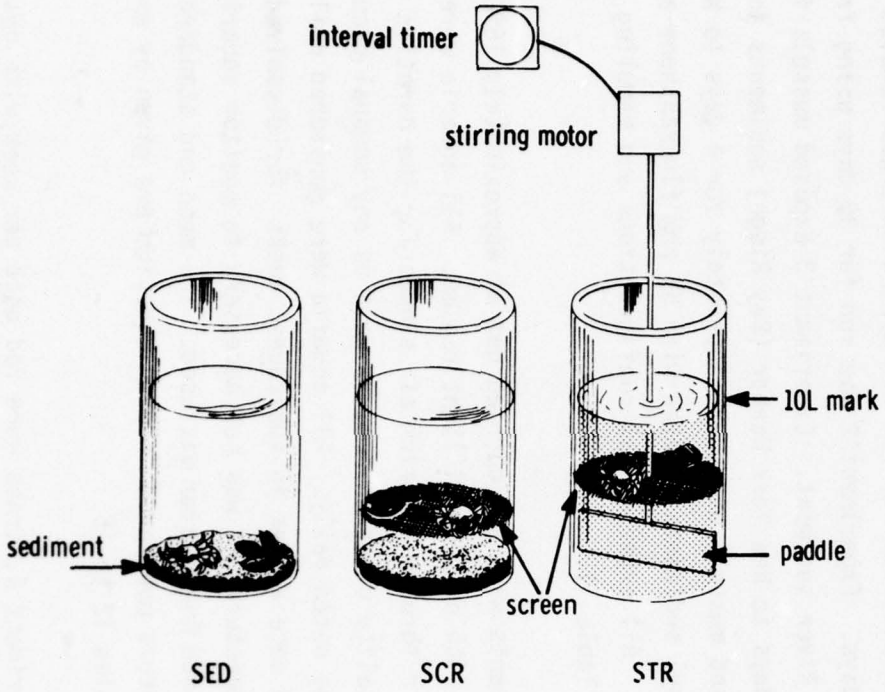

Figure 1. Configuration of aquaria used for the exposure of aquatic organisms to oil and grease containing dredged material.

Water for experiment 1 was obtained from an oceanic water supply at Pigeon Point, California. Seawater in subsequent experiments was 3-month aged San Francisco Bay water passed through a 10-μ mesh fiber filter. Water in experiment 2 (freshwater clams) was tap water, which had been aerated in the laboratory for 48 hr.

122. Experiment 1, using three species of saltwater organisms and Duwamish River sediment, was run for 30 days, with crabs removed from the experiment at 17 days. Experiment 2 was run for 30 days using freshwater clams in Duwamish River sediment. Experiment 3 exposed mussels for 27 days and crabs for 13 days to New York Harbor (Bay Ridge) sediments in seawater. Experiment 4 exposed mussels and crabs separately for 4 days to New York Harbor (Perth Amboy) sediments treated with 69 ppm fluoranthene as an internal standard. All experimental configurations and sampling times are summarized in Table 11.

123. Experiments were all carried out at approximately 16°C in a cooled laboratory with no special light regime. All aquaria were aerated with compressed air through aquarium air stones for the duration of all experiments. Mortality of organisms, as well as any unusual occurrences in the aquaria, was noted daily. All aquaria were monitored daily for the first week and once a week in subsequent weeks for dissolved oxygen, salinity, and temperature; it was not necessary to monitor aquaria salinity in experiment 2 since fresh water was used. The mean and standard deviation of the parameters monitored in each aquarium are given by experiment and aquaria in Tables 12 to 15.

124. In experiment 1, crabs were fed once per week with oyster or mussel tissue at approximately 10 g per aquarium. Snails were fed by placing a barnacle-encrusted rock in each tank for 3 days, once a week, and mussels were fed every 3 days by exchanging 500 ml of the tank water column with 500 ml of a mixed phytoplankton culture. In experiment 2,

Table 11

Experimental Configurations and Sampling in Determination of Oil and Grease Residues Taken Up by Organisms Exposed to Dredged Material

Expt No.	Treatment	Sediment Type Amount/Tank	No. Tank Replications within Treatment	Organism and Number per Tank	Duration of Exposure days	Sampling Scheme days			Method of Analysis
						Water	Sediment	Tissue	
1	SED,SCR STR	D + R 250 g wet wt	3+r	Mussels, 10 Crabs, 10 Snails, 10	30 17 30	0,17,30	0,30	0, 17(crab), 30	GC,TLC,F
2	SED,SCR STR	D + R 450 g wet wt	3+r	Clams, 20	15,30	0,15,30	0,30	0,15,30	GC,TLC,F
3	SED,SCR, STR	N + R 300 ml	3+r	Mussels, 10 Crabs, 10	27 13	0,1,7,33	0,33	0,27	TLC
4	STR	N(+Fl) 126 g dry wt	2/species	Mussels, 10 Crabs, 30	4 4	0,2,4	0,4	0,4	TLC,F

key:

Treatment
- SED - Organisms in sediment
- SCR - Organisms on screen 5 cm above sediment
- STR - Organisms on screen 30 cm above stirred sediment.

Sediment Type
- D - Duwamish River, Washington
- N - New York Harbor, New York
- R - Reference sediment
- Fl - Fluoranthene spiked sediment.

Tank Replications
- r - reference tanks.

Method of Analysis
- GC - Gas chromatography
- TLC - Thin-layer chromatography
- F - Fluorescence spectrophotometry.

Table 12

Experiment 1. Parameters in Aquaria in Which Crabs (Hemigrapsus oregonensis), Mussels (Mytilus edulis), and Snails (Acanthina spirata) were Exposed to Oil and Grease-Containing Dredged Material for 30 Days (17 Days for Crab) in Seawater, with Duwamish River Sediment

		30-Day Mean and Standard Deviation Values			
Treatment*	Tank	Temperature °C	Dissolved Oxygen mg/l	pH	Salinity o/oo
SED	A-1 (Ref)**	15.7 ± 0.4	6.9 ± 0.5	7.8 ± 0.2	29.0 ± 0.6
	A-2	15.9 ± 0.3	6.7 ± 0.6	7.8 ± 0.2	29.0 ± 0.9
	A-3	15.9 ± 0.5	5.9 ± 1.2	7.8 ± 0.3	28.3 ± 0.4
	A-4	16.4 ± 0.4	6.0 ± 1.2	7.7 ± 0.2	28.6 ± 0.5
SCR	B-1 (Ref)	15.8 ± 0.6	6.7 ± 0.5	7.9 ± 0.2	29.0 ± 0.5
	B-2	15.9 ± 0.5	5.5 ± 0.9	7.6 ± 0.2	28.4 ± 0.7
	B-3	15.9 ± 0.5	7.0 ± 0.6	7.8 ± 0.2	28.6 ± 0.5
	B-4	16.4 ± 0.4	5.9 ± 1.3	7.6 ± 0.2	28.5 ± 0.5
STR	C-1 (Ref)	16.2 ± 0.5	5.9 ± 1.0	7.7 ± 0.2	28.5 ± 0.5
	C-2	16.0 ± 0.5	6.3 ± 0.7	7.7 ± 0.1	28.6 ± 0.5
	C-3	16.2 ± 0.6	6.8 ± 0.7	7.7 ± 0.2	28.3 ± 0.6
	C-4	16.3 ± 0.5	6.6 ± 0.6	7.7 ± 0.2	27.8 ± 0.5

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.
**Ref - Reference sediment.

Table 13

Experiment 2. Parameters in Aquaria in Which Clams (Corbicula sp.) were Exposed to Oil and Grease-Containing Dredged Material for 30 Days in Fresh Water, with Duwamish River Sediment

Treatment*	Tank	30-Day Mean and Standard Deviation Values		
		Temperature °C	Dissolved Oxygen mg/l	pH
SED	A-1 (Ref)**	15.0 ± 0.5	9.5 ± 0.4	8.2 ± 0.2
	A-2	15.0 ± 0.5	9.5 ± 0.5	8.0 ± 0.1
	A-3	15.0 ± 0.3	9.3 ± 0.5	7.8 ± 0.1
	A-4	15.0 ± 0.5	9.5 ± 0.5	8.0 ± 0.1
SCR	B-1 (Ref)	15.0 ± 0.4	9.6 ± 0.5	8.3 ± 0.2
	B-2	15.0 ± 0.4	9.4 ± 0.6	8.1 ± 0.2
	B-3	15.5 ± 0.4	9.0 ± 0.8	7.8 ± 0.1
	B-4	15.5 ± 0.5	8.9 ± 0.8	8.0 ± 0.3
STR	C-1 (Ref)	15.0 ± 0.6	9.3 ± 0.5	8.2 ± 0.2
	C-2	15.0 ± 0.3	8.5 ± 0.8	7.6 ± 0.3
	C-3	15.3 ± 0.4	8.9 ± 0.6	7.8 ± 0.2
	C-4	15.3 ± 0.4	9.4 ± 0.5	8.0 ± 0.2

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.
** Ref - Reference sediment.

Table 14

Experiment 3. Parameters in Aquaria in Which Mussels (M. edulis) and Crabs (H. oregonensis) were Exposed to Oil and Grease-Containing Dredged Material for 27 and 13 Days in Seawater, with New York Harbor (Bay Ridge) Dredged Material

		30-Day Mean and Standard Deviation Values			
Treatment*	Tank	Temperature °C	Dissolved Oxygen mg/l	pH	Salinity o/oo
SED	A-1 (Ref)**	14.5 ± 0.8	5.7 ± 1.8	7.9 ± 0.2	34.1 ± 0.8
	A-2	15.1 ± 0.9	6.3 ± 1.5	7.7 ± 0.2	32.8 ± 0.6
	A-3	14.9 ± 0.9	5.4 ± 1.0	7.9 ± 0.2	33.2 ± 0.5
	A-4	14.9 ± 0.9	6.2 ± 1.5	7.9 ± 0.2	33.2 ± 0.6
SCR	B-1 (Ref)	15.2 ± 0.9	5.7 ± 3.1	7.9 ± 0.1	32.8 ± 0.7
	B-2	15.0 ± 0.9	6.7 ± 1.2	7.9 ± 0.1	32.8 ± 0.5
	B-3	15.2 ± 1.0	5.5 ± 0.9	7.9 ± 0.2	32.7 ± 0.6
	B-4	15.0 ± 0.9	5.9 ± 2.7	7.8 ± 0.2	32.8 ± 0.4
STR	C-1 (Ref)	14.9 ± 0.9	6.4 ± 0.4	7.9 ± 0.1	33.8 ± 0.5
	C-2	15.0 ± 0.9	6.1 ± 1.2	7.9 ± 0.3	33.2 ± 0.6
	C-3	15.0 ± 0.9	6.9 ± 0.6	7.8 ± 0.1	33.1 ± 0.6
	C-4	15.1 ± 0.9	6.2 ± 0.7	8.1 ± 0.2	33.0 ± 0.7

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.
**Ref - Reference sediment.

Table 15

Experiment 4. Parameters in Aquaria in Which Mussels (M. edulis) and Crabs (H. oregonensis) were Exposed to Oil and Grease-Containing Dredged Material for 4 Days in Seawater, with New York Harbor (Perth Amboy) Dredged Material

		Four-Day Mean and Standard Deviation Values			
Treatment*	Tank	Temperature °C	Dissolved Oxygen mg/l	pH	Salinity o/oo
STR	Mussel #1	15.8 ± 0.4	6.2 ± 1.0	7.8 ± 0.2	33.0 ± 0.4
	Mussel #2	15.8 ± 0.4	6.9 ± 0.7	7.9 ± 0.2	32.7 ± 0.5
STR	Crab #1	15.9 ± 0.5	7.6 ± 0.4	8.0 ± 0.1	32.8 ± 0.6
	Crab #2	15.8 ± 0.5	7.7 ± 0.2	8.0 ± 0.1	32.9 ± 0.7

Note: Fluoranthene tracer added to sediment prior to experiment at 69 µg/g dry wt sediment.

* STR - Organisms on screen 30 cm above stirred sediment.

clams were fed once a week with yeast. In experiment 3, crabs were fed only at the beginning of the experiment and observed for feeding behavior. No attempts were made to feed mussels in experiments 3 and 4, or crabs in experiment 4.

Sampling Procedures

Equipment

125. Glassware was either kilned at 500°C for 2 hr or detergent-washed and exhaustively rinsed with glass-distilled chloroform (Burdick and Jackson Co., Muskegee, Michigan) to remove trace organic contaminates. Aluminum foil, which was used to line caps of sample jars, cover glassware, and provide clean surfaces, was kilned. Stainless steel utensils were detergent-washed and solvent-rinsed.[171]

Water samples

126. Water samples were collected after suspended sediment had been allowed to settle, by repeatedly lowering a 250-ml beaker into the tank. Approximately 500 ml was removed from each tank. Initial water samples were collected prior to addition of sediments and represent a composite of all tanks. Subsequent water samples from each set of replicated tanks containing contaminated sediment were pooled. Tanks containing reference sediments were sampled individually. In addition, in experiment 3, water samples were collected 24 hr after the addition of sediment and prior to the addition of organisms, and water in the SCR tanks was sampled after the water column had been clarified as a result of mussel-filtering action for 7 days. The final water sample in experiment 3 was taken after the tank had settled 5 days post-experiment. In experiment 4, the water column was sampled 48 hr after the addition of the sediment. A drop of chloroform was added to prevent biodegradation and the samples were frozen when extraction was not undertaken the same day.

Organism samples

127. All live organisms in each tank were removed at the time of sampling, except in experiment 2, where half of the organisms were sampled (two samples of 4 to 5 clams, each) at the midpoint of exposure (15 days). In addition, tissue from freshwater clams maintained in the laboratory (from which test organisms had been selected) without sediment was sampled to compare the Soxhlet and Tissumizer extraction methods. Single samples were collected from each tank in experiments 1 and 3, and duplicate samples were collected from each tank in experiments 2 and 4. Living organisms were removed from aquaria and allowed to purge themselves of sediment over a 24-hr period in clean water. Organisms were externally rinsed with distilled water at the time of sampling the tissues. Entire crabs were taken for analyses. Mussels and clams were externally scrubbed, shucked, and partially drained, and the byssal threads were removed from mussels and discarded, with the remaining body tissue taken for analysis. Snails were removed from the shell after boiling in distilled water for 2 to 5 min. The organism samples were minced, weighed, and stored at -50°C.

Sediment samples

128. Samples of initial sediment were obtained prior to addition to experimental tanks. Final sediment samples were collected from sediment remaining on bottom of aquaria after water had been removed by siphoning. Sample replication and storage was the same as with organism samples.

Analytical Procedures

Extraction

129. Tissue and sediment samples were lyophilized for approximately 24 hr. Dry samples were extracted either in a Soxhlet apparatus for 24 hr with 80- to 150-ml glass-distilled chloroform (B & J Co.) or using a high-speed disintegrator (Tissumizer, Tekmar Co., Cincinnati, Ohio, Model No. SDT 182) for 1 to 2 min with 150 ml chloroform. In some cases,

sediment samples were divided into subsamples for extraction and analysis. Water samples were extracted in 2-ℓ separatory funnels with 50 ml of chloroform three times, either immediately after sampling or after thawing. Chloroform was removed from tissue, sediment, and water extracts using a rotary evaporator and the remaining extract was maintained at 4°C until analysis.

Saponification

130. Prior to instrumental analysis, it was found necessary to remove excess non-hydrocarbon lipids from clam and mussel tissues by saponification and column cleanup. Saponification included heating of each extract with 6 ml of 4 to 6 N NaOH for each (dry) extract for 8 hr at approximately 80°C. Hydrocarbons were then extracted from the residues with 5-ml portions of glass-distilled grade hexane (B & J Co.) three times. When samples showed a thixotropic appearance, indicative of continued presence of biological lipids, the hexane extract of the saponification was passed through a 2- by 2.5-cm syringe containing approximately 10 ml activated Silica Gel G (Baker Chemical Co.) and eluted with three bed volumes of chloroform. Satisfactory extracts were then analyzed for hydrocarbon residues as diagrammed in Figure 2.

Contamination control

131. Extreme caution was imperative throughout the experimental studies to maintain immaculately clean glassware and contaminant-free solvents for all analyses and laboratory procedures in attempts to avoid artifacts in analytical procedures. A large number of initial procedure checks using glass-distilled solvents were carried out in all analytical systems to locate and eliminate sources of contamination where possible. All solvents, Soxhlet extractors, and Tissumizer were routinely checked for contamination before use. Periodic procedural blanks were run to monitor unexpected contamination.

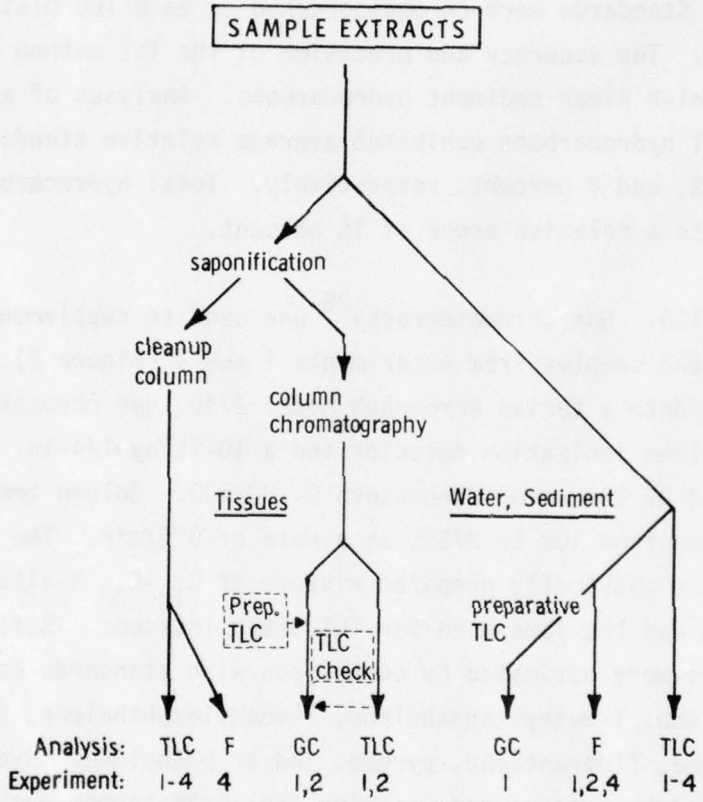

Figure 2. Flow chart of analysis for determination of hydrocarbons in water, sediment, and tissue in Experiments 1 - 4, using gas chromatography (GC), thin-layer chromatography (TLC), and fluorescence spectrophotometry (F).

Hydrocarbon determination

132. Thin-layer chromatography (TLC) was carried out in all experiments using methods modified from Hunter.[30] The "alkane" fraction, containing the n-alkanes, isoalkanes, and cycloalkanes, was quantitated by comparison with a gravimetrically prepared standard alkane mixture containing alkanes separated from three American Petroleum Institute standard oils (South Louisiana Crude, Venezuelan Bunker, and Kuwait Crude). Standards were chromatographed on each TLC plate along with unknowns. The accuracy and precision of the TLC method was established for Duwamish River sediment hydrocarbons. Analyses of alkanes, arenes, and total hydrocarbons exhibited average relative standard deviations of 13, 12, and 9 percent, respectively. Total hydrocarbons were determined with a relative error of 15 percent.

133. Gas chromatography[26] was used to supplement TLC analyses on selected samples from experiments 1 and 2 (Figure 2). Samples were injected onto a Varian Aerograph Model 2740, gas chromatograph, equipped with a flame ionization detector and a 10-ft by 1/4-in. SS column packed with 1.5% OV 101 on HP Chromosorb G-100/120. Column temperature was programmed from 100 to 275°C at a rate of 6°C/min. The alkane standard was a gravimetrically prepared mixture of C_{12}-C_{30} n-alkanes. The arene standard was the same used for TLC determinations. Boiling point ranges of arenes were estimated by comparison with standards containing naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, phenanthrene, anthracene, fluoranthene, pyrene, and triphenylene. Hydrocarbons were quantified by cutting and weighing the chromatogram peaks and comparing them with peaks derived from standards having comparable retention times. Data for the alkane fraction were reported as ppb per gram dry weight sample (sediment or tissue), and plotted on semi-log paper to form a "fingerprint"[172] for intercomparisons of samples. Purity of samples that were analyzed by GC was checked by TLC.

134. The following methods of interpreting the GC data for alkanes were attempted, consistent with recent methodology:[26,172]

a. total n-alkanes
b. unresolved complex mixture (UCM)
c. other resolved peaks (ORP)
d. total alkane (n-alkanes, cycloalkanes, isoalkanes)
e. carbon preference index (CPI) for C_{12-20} and C_{20-30}
f. peak/UCM ratios

135. For the arene fraction, the total concentration per sample dry weight and selected peak/UCM ratios were calculated.

136. Visual inspection of the gas chromatograms was made to determine similarities and differences of hydrocarbon profile with time, within species, between species, and between organisms and sediment. Chromatograms were observed with respect to boiling point range, presence and absence of corresponding peaks, and shape and extent of the unresolved complex mixture (UCM). Fluorescence spectrophotometry was carried out by preparative TLC of samples on Silica Gel G Uniplates (Analtech, Newark, Delaware). The plates were developed with hexane, the arene-containing region (R_f values of 0.05 to 0.78) was scraped off, and the arenes were extracted with 4.0 ml chloroform (B & J Co.). In experiment 4, only the fluoranthene region on the plate was extracted. The fluorescent intensity of the chloroform solution was determined at 460 nm with excitation at 365 nm on a Perkin-Elmer 204 A Fluorimeter. The fluorescent intensity was compared to fluoranthene standards prepared by preparative thin-layer chromatography. The amount of fluorescent arenes were calculated in µg fluoranthene equivalents per g dry weight.

Elutriate Test

137. A modification of the standard elutriate test[173] was carried out to determine at what levels oil and grease residues could be transferred to the water column. One hundred ml of the contaminated sediment (Bay Ridge, Perth Amboy) was shaken vigorously with 400 ml of three-month aged, high-quality oceanic seawater obtained at Pigeon Point, California. The mixture was allowed to settle for one hour and the supernatant was decanted and centrifuged. The filtration step of the standard method was omitted. The remaining sediment, centrifuged elutriate, and in one case, the centrifuge jars, were extracted with chloroform and analyzed for hydrocarbons by thin-layer chromatography using methods as described above.

PART III: RESULTS AND DISCUSSION

Oil and Grease Determinations by Standard Methods

138. The results of sediment analyses were typical of oil-contaminated sedimentary environments where elemental sulfur is produced from H_2S by bacterial and inorganic processes. The sediments contained 0.07 to 0.88 percent oil and grease (Table 16). As shown in Table 17, the New York Harbor sediments from Bay Ridge contained the highest oil and grease load (8755 µg/g), the Duwamish River sediments (1224 to 2301 µg/g) ccntained intermediate levels, and the lowest level was found in the Oakland Middle Harbor sediment (748 µg/g). The oil and grease from each sediment consisted of hydrocarbons, sulfur, and polar materials (presumably natural fats and oils). The sulfur content of fresh samples was similar to the desulfurized hydrocarbon content except in the Bay Ridge sediments, which contained more than twice as much desulfurized hydrocarbon as sulfur. The ratio of polar fats and oils to hydrocarbons was quite variable with a range of 0.7 (Bay Ridge) to 3.6 (Oakland Middle Harbor, aged). Although the composition varied, the levels of hydrocarbons, sulfur, and polar materials were well correlated with oil and grease levels with correlation coefficients calculated to be 0.984, 0.976, and 0.961, respectively.

139. Results of the analysis of Oakland Harbor sediment, after maintenance in an aquarium for one year (Table 17), show an increase in sulfur content, producing a higher total oil and grease value than originally found. The hydrocarbons in the aged sample were lower by 66 percent, and there was a slight increase in polar material content. Oakland Harbor sediments were not used in further experimentation, and thus, no additional data have been generated on this material. The one-year result was interesting, not as a valid simulation of in situ environmental conditions, but as an example of the inability of indigenous microflora to fully degrade the hydrocarbons. This result was expected, based on knowledge of microbial hydrocarbon breakdown under anaerobic conditions as discussed in the

Table 16

Characteristics of Sediments Obtained for Study

Sample	Dry Solids* % sediment	Volatile Residue* % dry solids	Oil and Grease** % dry solids	Hydrocarbons† % oil and grease
Duwamish River (Slip 1)	49.0	7.1	0.12	52
Duwamish River	36.6	6.4	0.23	37
Oakland Middle Harbor	51.6	7.8	0.07	62
New York Harbor (Bay Ridge)	30.1	10.2	0.88	68
New York Harbor (Perth Amboy)	34.4	13.0	0.45	63

*APHA (1971) Method 224G[1]
**APHA (1971) Method 209C[1]
†APHA (1971) Method 209D[1]

Table 17

Determination of Oil and Grease and Derivative Parameters in Sediments Obtained for Study of Oil and Grease Uptake from Dredged Material

µg/g dry weight

Sample	A Oil and Grease*	B Total Hydrocarbons*	C Hydrocarbons Desulfurized**	(B-C) Sulfur	(A-B) Polar Materials
Duwamish River (Slip 1)	1224	637	338	299	587
Duwamish River	2301	854	413	441	1447
Oakland Middle Harbor	748	462	275	187	286
Oakland Middle Harbor (aged)†	1050	710	93	617	340
New York Harbor (Bay Ridge)	8755	5930	4145	1785	2825
New York Harbor (Perth Amboy)	4525	2850	1580	1270	1675

* APHA (1971)[1]

** Blumer (1957)[31]

† One year in the laboratory aquarium at 15°C

literature review. It was instructive to note the rise in sulfur content, which in addition to demonstrating active microbiological activity, showed the typical interference by elemental sulfur in obtaining meaningful estimates of actual lipophilic materials by means of standard oil and grease methodology.

Aquarium Parameters and Organism Mortality

140. Laboratory conditions optimal for survival of the test organisms were maintained throughout experiments 1, 2, and 4. A power outage occurred over one weekend during experiment 3, which affected aquarium parameters and resulted in serious effects on the organisms under test. Summaries of the environmental parameters are given in Tables 12 through 15. Aside from experiment 3, there was little temperature or salinity fluctuation. For all experiments the oxygen concentration in the water was normally above 60 percent saturation (saturation values were 9.6 mg/ℓ for saltwater and 8.0 mg/ℓ for freshwater). The pH always remained slightly above neutrality and generally showed fluctuations of less than 0.2 pH units. In general, sediments in SED and SCR tanks showed formation of a surface oxidized layer (brown) and a subsurface reduced layer (black). Stirred tanks showed variable sediment profiles, and water developed a permanent turbidity after 4 to 5 days into the long-term experiments.

141. Under normal laboratory conditions, all organisms appeared to be in healthy condition, with mussels forming byssal attachment threads and carrying out filtering activity. Crabs and snails were motile, with apparently unimpaired feeding behavior. Mortality of organisms in experiments 1-3 is summarized in Table 18. Mortality data were difficult to record in stirred tanks, due to lack of visibility in the water. In some cases, mussels were so bound by byssal threads that dead organisms were not discovered until the end of the experiment.

Table 18

Organism Mortality in Experiments 1, 2, and 3

Treatment**	Tank	Experiment 1 Crabs (17 days)* Mortality %	Experiment 1 Mussels (30 days)* Mortality %	Experiment 2 Clams (30 days)* Mortality %	Experiment 3 Crabs (13 days)* Mortality %	Experiment 3 Mussels (27 days)* Mortality %
SED	A-1, Ref†	0	60	10	100	100
	A-2	30	10	5	10	0
	A-3	10	40	0	100	100
	A-4	10	0	0	100	100
SCR	B-1, Ref	20	50	5	80	100
	B-2	10	0	10	10	0
	B-3	20	0	0	100	100
	B-4	0	0	10	70	100
STR	C-1, Ref	20	20	5	100	100
	C-2	20	0	10	100	100
	C-3	30	20	25	10	0
	C-4	60	20	15	100	100

Note: Original number of organisms/tank was 10, 20, and 10 for each species in experiments 1, 2, and 3, respectively.

* ( ) - length of exposure.

** SED - Organisms in sediment; SCR - Organisms on screen 5 cm above sediment; STR - Organisms on screen 30 cm above stirred sediment.

† Ref - Reference sediment.

142. There were no snail mortalities in experiment 1. Mortality of crabs and mussels in reference sediment tanks in experiment 1 remains unexplained although it may have been due to some toxic residue left in the sediment after removal of oil and grease. There was no clear trend in mortality, and it was assumed that the few mortalities which occurred over this 30-day experiment were not caused by toxic properties of the Duwamish dredged material. Experiment 2, using the same sediments, produced survival of organisms exposed to contaminated sediments essentially no different from that of organisms in reference sediments.

143. Results of experiment 3 were complicated by elevated temperatures which prompted spawning of the mussels resulting in organic fouling of water and associated low oxygen values. Since one replicate (tank) in each treatment survived with no mussel mortality and low crab mortality, it was suggested that mortality was not associated with toxic properties of the dredged material. This suggestion was further confirmed in experiment 4 which showed 100 percent survival of crabs and mussels in all tanks over the four-day test period. There was some spawning in experiment 4, but the water cleared within 48 hr with no ill effects on water quality.

144. In general, exposure of the organisms to the dredged material constituted a limited benthic bioassay. Mortalities were irregular. None were recorded prior to the fourth day. Most mortality was in evidence after a week or more of exposure to aquarium conditions indicating a time dependent factor. This evidence is most clear in experiment 4 where there was no mortality in 96 hours. The highest mortalities occurred in STR tanks, but not enough data were generated to determine if this were a toxic effect, or a physical effect due to exposure to suspended sediment. Experiment 2 with freshwater clams was closest to "ideal" over the 30-day test period.

Hydrocarbon Determination: Thin-Layer Chromatography

145. Thin-layer chromatography was chosen for its flexibility and ability to process large numbers of samples rapidly on a quantitative basis. Both arene and alkane hydrocarbons are determined in a single analysis; no preseparation of fractions is required. Alkane values include all saturated hydrocarbons above C_{10}; arene values include arenes from C_{10} to about C_{30}. Neither sulfur or halogenated hydrocarbons are measured. Although major amounts of olefins (alkenes) were not anticipated, long-chain olefins would have been included in the alkane value and alkylated olefins would have been included in the arene value. All values reported represent the "best determination", based on operator experience. Average values, in some cases, exclude out-lying numbers obtained as a result of obvious sample contamination or analytical error.

Water

146. Levels of total hydrocarbons in the water column, including dissolved and those associated with suspended sediment, were routinely in the parts per billion range (Tables 19 to 21). In experiments 1 and 2 maximum values of 158 and 91.5 ppb, respectively, were found after 30 days in the STR tanks. Additional sampling in experiments 3 and 4 indicated that levels of hydrocarbons associated with suspended sediment in the water column were higher in all treatments prior to the addition of organisms than in the STR tanks after thirty days. In experiment 3 the striking drop in hydrocarbon levels in the water of the SCR tanks from 363 ppb to only 6 ppb within 7 days, demonstrates the ability of mussels to redeposit suspended sediment (Table 21). No outstanding differences were noted between seawater and fresh water (Tables 19 and 20).

147. Based on data obtained from SCR tanks in experiment 1, the maximum possible hydrocarbon dissolved in the water was 14 ppb or 0.3 percent of the total sedimentary hydrocarbon available. In the SCR tanks of experiment 1, a low alkane/arene ratio of waterborne hydrocarbons.

Table 19

Hydrocarbon Determinations on Water from Experiment 1 by Thin-Layer Chromatography (TLC) and Fluorescence Methods

Tank Series*	Time days	Hydrocarbon Concentration µg/l (ppb) Alkanes	Arenes	Total	Alkanes/ Arenes	PAH** ng/l (ppt)
	0	9.6	nd†	9.6	--	3.4
	0	11.4	nd	11.4	--	--
SED	17	33	7	40	4.7	106
SED, Ref	17	nd	nd	nd	--	15
SED	30	10	3	13	3.3	17
SED, Ref	30	nd	nd	nd	--	9
SCR	17	6	8	14	0.8	14
SCR, Ref	17	nd	nd	nd	--	0.2
SCR	30	2	8	10	0.2	13
SCR, Ref	30	nd	nd	nd	--	4
STR	17	108	50	158	2.2	19
STR, Ref	17	7	nd	7	--	29
STR	30	66	12	78	5.5	140
STR, Ref	30	13	nd	13	--	50

* SED - Organisms in sediment; SCR - Organisms on screen 5 cm above sediment; STR - Organisms on screen 30 cm above stirred sediment; Ref - Reference sediment.

** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.

† nd - Not detectable, < 5 ppb for hydrocarbons.

-- - Not determined.

Table 20

Hydrocarbon Determinations on Water from Experiment 2 by Thin-Layer Chromatography (TLC)

Tank Series*	Time days	Hydrocarbon Concentration µg/l (ppb) Alkanes	Arenes	Total	PAH** ng/l (ppt)
$\bar{X}$ ± SD	0	4.6 ± 0.1	nd†	4.6 ± 0.1	17 ± 9
SED	15	4.3	nd	4.3	16
SED, Ref	15	4.8	nd	4.8	nd
SED	30	7.3	nd	7.3	5
Sed, Ref	30	4.8	nd	4.8	--
SCR	15	nd	nd	nd	14
SCR, Ref	15	4.4	nd	4.4	11
SCR	30	1.3	nd	1.3	4
SCR, Ref	30	2.4	nd	2.4	nd
STR	15	13.5	nd	13.5	11
STR, Ref	15	nd	nd	nd	nd
STR	30	73.4	18.1	91.5	84
STR, Ref	30	8.1	nd	8.1	167††

* SED - Organisms in sediment.
SCR - Organisms on screen 5 cm above sediment.
STR - Organisms on screen 30 cm above stirred sediment.
Ref - Reference sediment.

** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.

† nd - Not detectable, < 3 ppb hydrocarbons; < 4 ppt (parts per trillion) PAH.

†† - Suspected contamination.

-- - Not determined.

Table 21

Hydrocarbon Determinations on Water
from Experiments 3 and 4 by Thin-Layer Chromatography (TLC)

Tank Series*	Time days	Hydrocarbon Concentration µg/ℓ (ppb) Alkanes	Arenes	Total	PAH** µg/ℓ (ppt)
	0	1	nd†	1	--
Experiment 3					
SED, SCR & STR	1, $\bar{X}$ ± SD	274 ± 22	88 ± 11	363± 12	--
SED, SCR & STR, Ref	1	5	nd	5	--
SCR	7	6	nd	6	--
SCR, Ref	7	nd	nd	nd	--
STR	33	94	33	127	--
STR, Ref	--	--	--	--	--
Experiment 4					
	2	48	2	50	3.8
STR - Crabs	4	22	nd	22	1.8
STR - Mussels	4	8	nd	8	2.7

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment
Ref - Reference sediment.

** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.

† nd - Not detectable, < 1 ppb.
-- - Not determined.

(Table 19) supported this assumption (arenes are more soluble in water); in the STR tanks higher alkane/arene ratios of water column hydrocarbons were similar to deposited sediment ratios (Table 22) suggestive of insoluble hydrocarbon fractions.

148. Water column results suggested that during dredging, insignificantly small amounts of hydrocarbons would be leached from these oil-impacted sediments, and that only limited amounts of sediment-sorbed hydrocarbons would potentially be biologically available, depending on the amount and duration of turbidity maintained in the water surrounding and downstream from the dredging activity.

Sediments

149. Analytical results on sediments on a before-and-after basis for experiments 1 through 4 are listed in Tables 22 through 24. There were no major differences in hydrocarbon concentration between initial and final sediments except for experiment 2 (Table 23), which showed an increase in total hydrocarbons after the 30-day incubation period, primarily in the alkane fraction of the STR tanks. This increase is as yet unexplained, but may be due to inhomogeneity of the sediment used, lubricating oil contamination from the laboratory compressed air, or microbial synthesis of alkanes. Lubricating oil contains few arenes, and there was little change in the amount of sedimentary arenes. Slight increases were recorded in reference sediments of experiments 1 and 2. Since absolute hydrocarbon increases were minimal in reference sediments, contamination of experimental sediments from external sources was ruled out.

150. No significant differences were noted in the sediments between initial and final hydrocarbon content in experiments 3 and 4 performed with New York Harbor sediments (Table 24). This observation is of great interest in that the presence of oil and grease residues in sedimentary materials has primarily been considered in the past to cause high biochemi-

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Table 22

Hydrocarbon Determinations on Experiment 1 Sediments by Thin-Layer Chromatography (TLC) and Fluorescence Spectrophotometry

Tank Series*	Time days	Hydrocarbon Content µg/g dry wt (ppm), $\bar{X} \pm SD$ Alkanes	Arenes	Total	Alkanes/ Arenes	PAH** µg/g
Duwamish River Sediment						
	0	364 ± 20	122 ± 24	486 ± 17	3.0	1.75 ± 0.51
SED	30	388 ± 93	132 ± 36	520 ± 84	2.9	1.73 ± 0.30
SCR	30	316 ± 13	92 ± 23	408 ± 10	3.4	1.80 ± 0.04
STR	30	435 ± 88	112 ± 36	547 ±122	3.9	1.66 ± 0.13
Reference Sediment						
	0	10	nd†	10	--	0.20
SED	30	27	nd	27	--	0.22
SCR	30	29	nd	29	--	0.19
STR	30	40	nd	40	--	0.19
Procedural Blank		< 3	< 1.5	< 4.5	--	--

* SED - Organisms in sediment
SCR - Organisms on screen, 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.
† nd - Not detectable, < 8 µg/g hydrocarbons.
-- - Not determined.

Table 23

Hydrocarbon Determinations on Experiment 2 Sediments by Thin-Layer Chromatography (TLC) and Fluorescence Spectrophotometry

Tank Series*	Time days	Hydrocarbon Content µg/g dry wt (ppm), $\bar{X}$ ± SD Alkanes	Arenes	Total	PAH** µg/g, $\bar{X}$ ± SD
Duwamish River Sediment					
	0	367 ± 23	126 ± 27	493 ± 11	1.7 ± 0.6
SED	30	389 ± 38	137 ± 34	526 ± 61	1.9 ± 0.5
SCR	30	405 ± 68	156 ± 13	561 ± 63	1.5 ± 0.6
STR	30	546 ± 35	169 ± 20	715 ± 25	2.2 ± 0.4
Reference Sediment					
	0	3 ± 2	nd†	3 ± 2	0.09± 0.03
SED	30	17	nd	17	0.10± 0.03
SCR	30	12 ± 1	nd	12 ± 1	0.05
STR	30	13	nd	13	0.04

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.
** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.
† nd - Not detectable, < 8 µg/g.

Table 24

Hydrocarbon Determinations on Experiment 3 (Bay Ridge, N.Y.) and Experiment 4 (Perth Amboy, N.J.) Sediments by Thin-Layer Chromatography (TLC) and Fluorescence Spectrophotometry

Tank Series*	Time days	Hydrocarbon content µg/g dry wt (ppm), $\bar{X}$ ± SD			Fluoranthene µg/g
		Alkanes	Arenes	Total	
Experiment 3					
	0	4530 ± 251	1500 ± 289	6030 ± 406	- -
STR	33	4711 ± 397	1270 ± 294	5981 ± 692	- -
Experiment 4					
	0	1591	620	2211	38.7
STR-M #1	4	1615	590	2205	35.7
STR-M #2	4	1240	504	1744	35.1
STR-C #1	4	1397	546	1943	37.7
STR-C #2	4	1468	624	2092	36.4
$\bar{X}$ ± SD	4	1430 ± 174	566 ± 78	1995 ± 235	36.7±1.8

*STR - Organisms on screen 30 cm above stirred sediment
M - Mussels, Mytilus edulis
C - Crabs, Hemigrapsus oregonensis.
-- - Not determined.

cal oxygen demand (BOD). The lack of biodegradation, even under conditions of aeration, in the presence of marine organisms and a rich sedimentary bacterial flora, showed that hydrocarbons found adsorbed to sediments in the environment are highly resistant to degradation (see literature review). The authors therefore suggest that oil and grease content of dredged material cannot be used as an a priori indication of high sediment BOD.

Organisms

151. Hydrocarbon levels in organisms from experiments 1 through 4 are listed in Tables 25 through 28. In experiment 1, mussels showed no striking evidence of uptake of hydrocarbons from dredged material, and in the presence of low oil and grease reference sediments, a slight decrease in hydrocarbon content was recorded (Table 25). Crabs showed an approximate doubling of hydrocarbon content with the major contribution coming from the alkane fraction (Table 25). Snails showed elevated levels of hydrocarbons, especially in the STR configuration, at the end of the experiment (Table 25). Hydrocarbon accumulation in snails was based on single analyses due to the small amount of available tissue. If this snail uptake is real, it may have occurred by direct absorption of hydrocarbons that had formed a film on the tank walls (see Elutriate Test results, page 118). In experiment 2 (Table 26) no change in hydrocarbon levels was observed in the clams in the SCR tanks at 15 days, with a slight increase after 30 days, and no differences between experimental and reference clams. Clams in SED and STR tanks showed elevated levels of hydrocarbons compared with clams in reference sediments (Table 26).

152. Determinations made on a pooled tissue sample from a group of (non-experimental) clams maintained in the laboratory were used for comparing Soxhlet and Tissumizer extraction methods (Table 26). Both methods showed similar results, although the Tissumizer method was more rapid and cost effective.

Table 25

Hydrocarbon Analyses by TLC of Mussels (Mytilus edulis), Crabs (Hemigrapsus oregonensis), and Snails (Acanthina spirata) Exposed to Duwamish River Dredged Material in Experiment 1

Tank Series*	Time days	Organisms per Samples	Hydrocarbon Content µg/g dry wt (ppm), $\bar{X} \pm SD$ Alkanes	Arenes	Total	PAH** µg/g
Mussels						
	0	12	143	35	178	0.23
SED	30	5,8,9	35 ± 12	58 ± 31	93	0.36
SED, Ref	30	3	48	47	95	--
SCR	30	9	84	54	138	0.15
SCR, Ref	30	4	35	< 6.5	< 41.7	--
STR	30	6,6,7	110 ± 20	52 ± 10	162	0.39
STR, Ref	30	7	63	15	78	--
Crabs						
	0	12	14	2	16	0.10
SED	17	7,8,9	22 ± 2	7 ± 3	29 ± 2	0.10
SED, Ref	17	10	8	3	11	--
SCR	17	10, 10	21 ± 4	14 ± 11	35 ± 14	0.05
SCR, Ref	17	9	19	10	29	--
STR	17	5,8,9	25 ± 4	6 ± 2	30 ± 5	0.05
STR, Ref	17	10	10	5	15	--
Snails						
	0	12	< 6	< 4	< 9	1.5
SED	30	23	14	7	21	2
SED, Ref	30	10	4	4	8	1
SCR	30	30	C O N T A M I N A T E D			
SCR, Ref	30	10	6	2	8	3
STR	30	20	28	37	65	0.76
STR, Ref	30	9	C O N T A M I N A T E D			

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment
Ref - Reference sediment.

** PAH - Polynuclear aromatic hydrocarbons as fluoranthene.
-- - Not determined.

Table 26

Hydrocarbon Analyses by TLC of Freshwater Clams (Corbicula sp.) Exposed to Duwamish River Dredged Material in Experiment 2

Tank Series*	Time days	Hydrocarbon Content µg/g dry tissue (ppm), $\bar{X}$ ± SD Alkanes	Arenes	Total
	0	41	39	80
SED	15	76 ± 16	61 ± 22	137 ± 24
SED, Ref	15	42	33	75
SED	30	34 ± 3	42 ± 4	76 ± 6
SED, Ref	30	57	43	100
SCR	15	46 ± 14	39 ± 8	85 ± 7
SCR, Ref	15	81	33	114
SCR	30	86 ± 23	55 ± 17	141 ± 26
SCR, Ref	30	60	52	112
STR	15	66 ± 19	70 ± 15	136 ± 30
STR, Ref	15	51	60	111
STR	30	59 ± 20	64 ± 3	123 ± 22
STR, Ref	30	18	26	44
Maintenance Organisms				
Soxhlet Extraction	15 & 30	56	61	117
Tissumizer Extraction	15 & 30	48	65	113

* SED - Organisms on sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment
Ref - Reference sediment.

Table 27

Hydrocarbon Analyses by TLC of Crabs (Hemigrapsus oregonensis) and Mussels (Mytilus edulis) Exposed to Bay Ridge (N.Y.) Dredged Material in Experiment 3

Tank Series*	Time days	Organisms per Sample	Hydrocarbon Concentration µg/g dry tissue (ppm), $\bar{X} \pm SD$		
			Alkanes	Arenes	Total
Mussels					
	0	5,5,5	25 ± 17	22 ± 4	46 ± 20
SED	27	9	72	121	193
SCR	27	10	23	32	55
STR	27	10	108 ± 56	180 ± 73	289 ± 128
Crabs					
	0	19,18,17	62 ± 5	11 ± 2	73 ± 5
SED	1	10	58	22	80
SCR	1	10	24	5.2	30
STR	1	10	70	22	92

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

Table 28

Hydrocarbon Analyses by TLC of Crabs (*Hemigrapsus oregonensis*) and Mussels (*Mytilus edulis*) exposed to Perth Amboy (N.J.) Dredged Material in Experiment 4

Tank Series*	Time days	Organisms per Sample	Hydrocarbon Content µg/g dry tissue (ppm)			PAH** µg/g
			Alkanes	Arenes	Total	
Mussels						
	0	6	17	46	63	0.21
STR #1	4	5	22	65	87	21.4
	4	5	28	76	104	16.9
STR #2	4	5	18	86	104	21.0
	4	5	20	143	163	18.2
	4	$\bar{X} \pm SD$	22 ± 4	93 ± 35	115 ± 33	19.4 ± 2.2
Crabs						
	0	20	19	6	25	0.10
	0	20	21	7	28	0.03
STR #1	4	15	47	21	68	0.9
	4	15	59	34	93	0.6
STR #2	4	15	66	42	108	0.8
	4	15	85	35	120	0.3
	4	$\bar{X} \pm SD$	64 ± 16	33 ± 9	97 ± 22	0.7 ± 0.2

* STR - Organisms on screen 30 cm above stirred sediment.

** PAH - Polynuclear aromatic hydrocarbons (added fluoranthene internal standard) as fluoranthene.

153. In experiment 3 (Table 27) mussels in SED and STR tanks showed marked increases in hydrocarbon content after exposure to dredged material. In most cases, arenes were enriched in these tissues compared with alkanes. Mussels in SCR tanks showed essentially no differences from mussels prior to exposure. Crabs in this experiment showed no significant uptake of hydrocarbons, with an apparent drop in hydrocarbons in the SCR tank. Since these values are based on one sample from each of the surviving tanks, these results are not as well founded as results of replicated experiments. Two replicated results in experiment 4 suggested minor uptake of hydrocarbons from sediments by both mussels and crabs (Table 28); fluoranthene added to sediments as an internal standard was taken up by both crabs and mussels.

Summary; Hydrocarbon determination by TLC

154. In all cases, changes in hydrocarbon content of organisms were small in comparison to the hydrocarbon contents of the sediments to which they were exposed. Cases of accumulation and depuration of hydrocarbons were observed. In the case of mussels and crabs, contact with the sediment was required for accumulation. The snail results may point to an unexamined area of concern as particulate oil may have been released from the sediment and adsorbed on the walls of the exposure container. Since these snails are strict carnivores, any uptake of hydrocarbons was expected to be by direct adsorption through the foot, rather than by feeding. Only in experiment 3 (mussels exposed to Bay Ridge sediments) is there accumulation of the magnitude (hundreds of ppm) that is found in petroleum-impacted environments. It is hypothesized that uptake of petroleum hydrocarbons as reported elsewhere,[20] occurred by uptake of particulate oils in the water column, and not from sediment-associated oil. Interpretation of the small variations frequently observed is limited since the lack of replications has not allowed determination of the variation to be expected in subpopulations of organisms.

Hydrocarbon Determination: Gas Chromatography

155. Gas chromatographic analysis of selected sediment (experiment 1) and tissue (experiments 1 and 2) samples was undertaken primarily to assess qualitative changes within the alkane and arene fractions of sediment and tissue hydrocarbons during the course of the laboratory experiments. Gas chromatography (GC) allows further resolution of the fractions quantitated by TLC enabling subtle changes in hydrocarbon fractions to be observed. Numerous methods for quantifying compositional changes were employed. The most successful are included. The data from the GC analyses must be considered semi-quantitative since no replication of samples or analyses was attempted. The hydrocarbon levels of selected samples determined by GC are presented in Tables 29 through 31. The qualitative aspects are discussed for alkane fractions and arene fractions below. References to figures and tables are made to elucidate the visual interpretation of chromatograms (not included in this report) that follows.

Alkane fraction

156. In the chromatograms of Duwamish River sediment alkanes, initially and after 30 days, there was a series of medium-sized resolved peaks from C_{13} to C_{30} with three dominant n-alkane peaks at C_{25}, C_{27}, and C_{29} above a broad, unresolved complex mixture (UCM) centered at C_{25}-C_{27}. This typical pattern was reflected in the n-alkane "fingerprints" (Figures 3-6) and in the carbon preference index (CPI_{20-30}) that ranges from 3.4 to 4.9 (Table 32). The relative composition of the alkane fraction was the same in initial sediment and the sediments in the SED and STR tanks after 30 days, but was different in the 30-day SCR sample which had a much smaller UCM (Table 29). The 30-day STR sample had the highest hydrocarbon concentration, which is the result of a larger UCM (Table 29). Except for these changes in the amount of UCM, all Duwamish River sediments appeared the same. The pattern of alkanes was typical of extensively

Table 29

Analyses of Hydrocarbons in Sediments of Experiment 1 by Gas Chromatography (GC)

Tank Series**	Time days	Hydrocarbon* Content µg/g dry wt (ppm) n-ALK	ORP	UCM	ΣALK
Reference Sediment					
	0	0.43	0.14	3.90	4.47
SED	30	0.89	0.10	12.6	13.6
SCR	30	1.14	0.16	19.5	20.9
STR	30	1.31	1.59	28.3	31.2
Duwamish River Sediment					
	0	12.2	11.7	106	130
	0	10.8	4.53	126	141
SED	30	6.71	2.40	128	137
SCR	30	9.52	3.62	14.5	28.2
STR	30	14.4	5.08	324	344

Note: All values are the result of a single GC analysis

* n-ALK- n-Alkanes
ORP- Other Resolved Peaks
UCM- Unresolved Complex Mixture
Σ ALK- Total Alkanes.

**SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

Table 30

Analyses of Alkanes in Organisms of
Experiments 1 and 2 by Gas Chromatography (GC)

Tank Series**	Time days	Hydrocarbon* Content µg/g dry wt (ppm) n-ALK	ORP	UCM	ΣALK
Experiment 1					
Mussels					
	0	2.37	3.62	76.6	82.8
SED	30	1.36	0.96	15.1	17.5
SCR	30	0.86	1.15	10.4	12.4
STR	30	0.89	1.29	26.2	28.5
Crabs					
	0	0.36	0.42	0.46	1.25
SED	17	1.55	0.95	12.5	15.2
SCR	17	1.31	0.75	1.96	4.03
STR	17	0.66	0.07	0.83	1.55
Experiment 2					
Clams					
	0	--†	--	--	--
SED	30	0.42	1.77	41.0	43.3
SCR	30	0.39	2.93	70.6	74.3
STR	30	0.61	2.49	53.2	56.6

Note: All values are the result of a single GC analysis.

*n-ALK - n-Alkanes
ORP - Other Resolved Peaks
UCM - Unresolved Complex Mixture
Σ ALK - Total Alkanes.

** SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

† -- - Not determined.

Table 31

Summary of Hydrocarbon Analyses by Gas Chromatography of Organisms and Sediment from Experiments 1 and 2

Tank Series*	Time days	Organisms/ Sample	Hydrocarbon Content µg/g dry wt (ppm) Alkanes	Arenes	Total
Experiment 1					
Mussels					
	0	6	82.8	34.8	117.6
SED	30	9	17.5	45.9	63.4
SCR	30	9	12.4	25.3	37.7
STR	30	6	28.5	66.4	94.9
Crabs					
	0	12	1.25	6.81	8.06
SED	17	7	15.2	13.6	28.8
SCR	17	11	4.03	3.81	7.84
STR	17	8	1.55	10.6	12.15
Duwamish River Sediment					
	0		130	43.5	173.5
Experiment 2					
Clams					
	0		--**	--	--
SED	30	5	43.3	21.4	64.7
SCR	30	5	74.3	50.4	124.7
STR	30	4	56.6	55.2	111.8

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

** -- - Not determined.

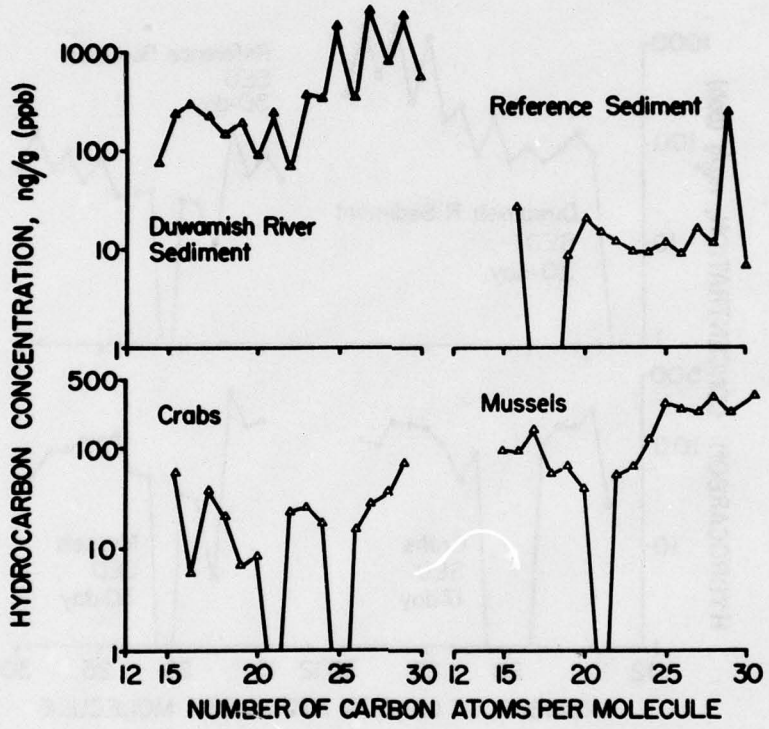

Figure 3. Fingerprints of n-alkane fraction of representative samples from experiment I, initial conditions.

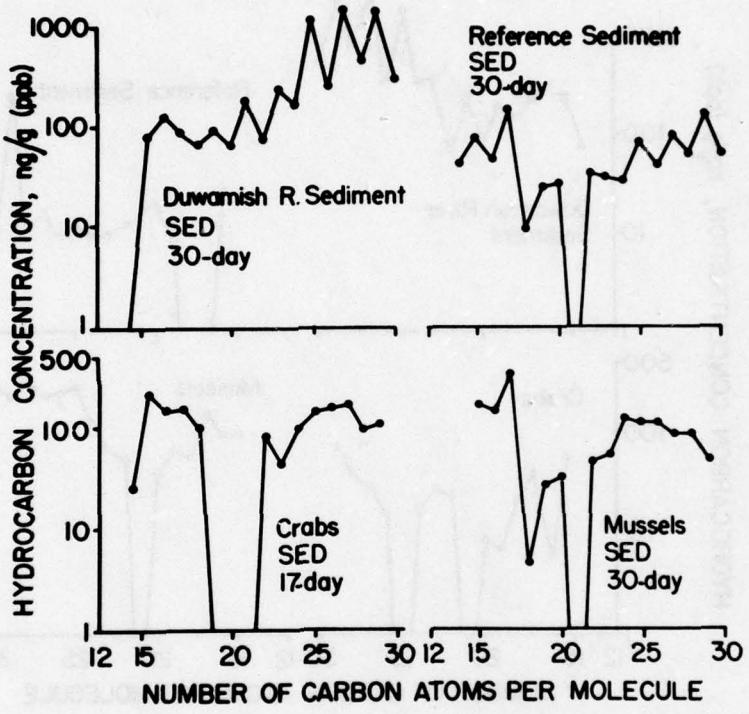

Figure 4. Fingerprints of n-alkane fraction of representative samples from experiment I, 17and30-day results, SED treatment.

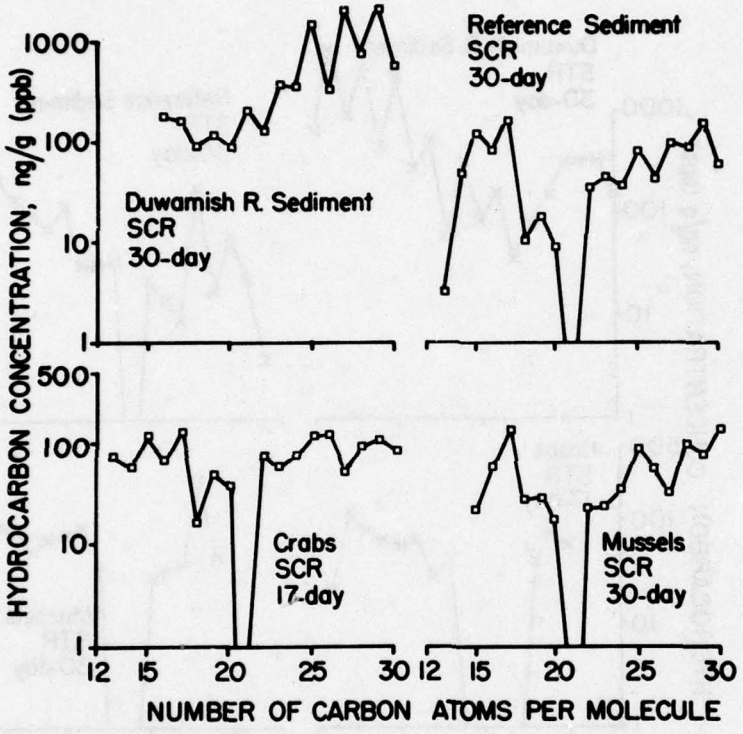

Figure 5. Fingerprints of n-alkane fraction of representative samples from experiment I, 17-and 30-day results, SCR treatment.

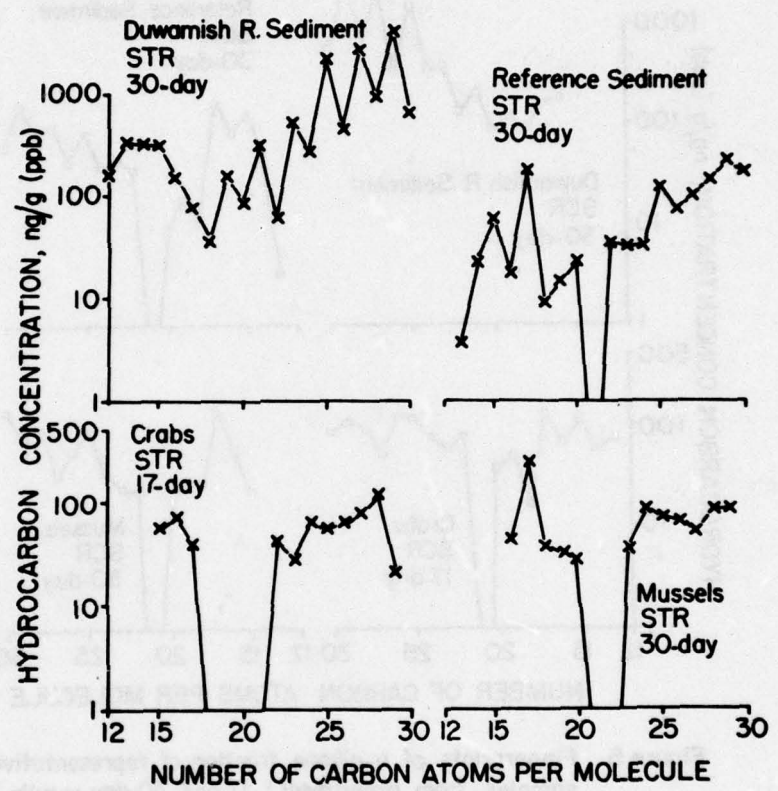

Figure 6. Fingerprints of n-alkane fraction of representative samples from experiment I, 17-and 30-day results, STR treatment.

Table 32

Analytical Data for Gas Chromatographic Analyses of Organism and Sediment Alkanes from Experiment 1

Tank Series*	Time days	CPI** 12-20	CPI 20-30	ORP† $C_{21}/n\text{-}C_{22}$
Reference Sediment				
	0	0.5	5.5	0
SED	30	2.3	1.7	6.4
SCR	30	2.0	1.6	3.2
STR	30	4.8	1.2	25.8
Duwamish River Sediment				
	0	1.2	4.2	0
SED	30	1.1	4.3	0
SCR	30	0.9	3.4	0
STR	30	1.4	4.9	0
Mussels				
	0	2.0	0.9	43.2
SED	30	2.2	0.8	7.0
SCR	30	2.2	0.8	11.2
STR	30	2.6	1.0	16.5
Crabs				
	0	3.5	1.3	13.1
SED	17	1.4	1.1	6.1
SCR	17	2.3	0.8	10.3
STR	17	1.3	0.6	0.7

* SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

** CPI - Carbon Preference Index.

† ORP - Other Resolved Peaks.

weathered petroleum oil with the possible inclusion of some recent biogenic n-alkanes in the C_{25}-C_{29} region.

157. To assess the extent of biodegradation that occurred during the thirty-day exposure period, a more detailed analysis of chromatograms of initial and final Duwamish River sediment alkanes was completed. Based on the assumption that n-alkanes are biodegraded more rapidly than iso- and cycloalkanes, biodegradation would be reflected by a decrease in the ratio of n-alkane peak height/UCM height after 30 days. The results of this type of analysis are summarized below.

Range of n-Alkanes	30-Day Change in Ratio of peak ht/UCM ht	Evidence for Biodegradation
C_{15} -C_{19}	Decrease	Slight (most in STR)
C_{19} -C_{24}	None	None
C_{25} -C_{30}	Decrease	Slight (most in STR)

Biodegradation was minimal, only occurring to a slight extent in certain alkane regions. The lack of any striking changes in the Duwamish River sediment alkanes and the minimal extent of biodegrdation of the most labile components is consistent with an earlier observation that indigenous microflora are incapable of fully degrading sediment-associated hydrocarbons.

158. The initial reference sediments had small resolved peaks ranging from C_{16}-C_{30} above a small UCM. The alkane fractions of final sediments from the SED, SCR, and STR tanks contained a UCM with a maximum at C_{25}-C_{27} and a series of medium peaks that ranged from C_{13}-C_{30}. In the range C_{13} to C_{21} inclusive, there were certain characteristic peak sequences formed by components other than n-alkanes, especially in the vicinity of C_{15}, C_{16}, C_{17}, and C_{18}. This distinguishing pattern was present in all final reference sediment alkanes, but was not seen in the n-alkane fingerprints (Figures 3-6). There was only a slight predominance of n-alkanes at C_{25}, C_{27}, and C_{29}, resulting in a low CPI_{20-30} of

1.2 to 1.7 (Table 32). Although the total alkane concentration changed, the relative amounts of n-alkanes, ORP, and UCM did not (Table 29). During the experiment, all reference sediments regardless of treatment acquired an alkane load that exhibited the same basic composition. Some of these hydrocarbons were probably adsorbed from the crabs (see below), while others may have been the result of uncontrolled laboratory contamination.

159. No clear trends were evident when comparing the mussel alkane fractions. The alkanes of the initial mussel sample contained components that ranged from C_{15}-C_{30} with resolved peaks above a UCM centered at C_{25}-C_{26}. The chromatograms of the 30-day samples from the SED and SCR tanks were not similar to the initial mussels but contained a characteristic sequence of peaks in the C_{15}-C_{20} region that was absent in the initial sample. The chromatogram of the STR tank sample did not contain this characteristic sequence and therefore vaguely resembles the initial sample. The CPI did not show any change in the course of the experiment (Table 32), but a change was evident in the alkane "fingerprints" in the SED and SCR treatments (Figures 3-6). The alkane profiles of the initial and 30-day samples showed little similarity to either the reference or Duwamish River sediment, suggesting that uptake, discharge, or exchange of selected components did not occur. These results are consistent with the TLC results and probably reflect an unusually large natural variability in the mussels used.

160. Initial and final SED and SCR crab samples gave chromatograms that showed similar peak patterns that ranged from C_{13}-C_{17}, with outstanding ORP sequences near C_{15} and C_{17}. The UCM of the initial sample was a single small hump at C_{29}, whereas in the final SED sample the UCM extended from C_{15}-C_{30} with a maximum at C_{24}. In the SCR sample, the UCM was smaller and extended from C_{16}-C_{30}. The final STR chromatogram extends from C_{13}-C_{30} with a very small UCM and small, poorly resolved peaks. Although there were slight differences between chromatograms, there was no

basic change in alkane composition of the crabs after 17 days in the SED and SCR tanks. There were some changes observed in the STR tanks. Initial and final crab alkanes were not similar to reference or experimental sediment alkanes. The hydrocarbon profile of the Duwamish River sediment was not observed in the crab alkanes at the end of the experiment. Whatever alkanes accumulated in the reference sediment during the course of the experiment did not accumulate in the crabs.

161. In both mussels and crabs there was a pronounced similarity between chromatograms of the alkane fraction after 30 days in the SED and SCR treatments and only a slight resemblance to the STR samples. This is of interest since the test organisms were in direct contact with the sediment in the SED tanks and were in the water column suspended above the sediments in the SCR series. The alkane fractions of organisms exposed to stirred dredged material were different, yet cannot be readily related to the sediment alkane profiles.

162. In experiment 1 a large ORP was present in the C_{21} region, both in the 30-day reference sediments and in all crabs and mussels. This component was not observed in either the initial condition reference sediments or the contaminated sediments. Initial condition crabs and mussels contained more of this C_{21} ORP than the 17- and 30-day organisms while the 30-day reference sediments contained more than the initial reference sediments. The C_{21}-ORP/n-C_{22} ratio reflected these differences (Table 32). These changes suggest that this compound was transferred from both organisms to the sediments during the course of the experiment. The appearance of this component was masked by the large amount of other alkanes in the n-C_{21} region in the Duwamish River sediment. The reference sediment in the STR tank contained the largest relative amount of this C_{21}-ORP. This result is consistent with the fact that crabs, which represent the larger portion of the organism dry weight in the STR tank, exhibited the lowest

C_{21}-ORP/n-C_{22} ratio. These results are the first documentation of passive transport of hydrocarbon burden from crab tissue to an external sedimentary environment.

163. All three clam alkane fraction (SED, SCR, STR) chromatograms have a large UCM centered at C_{19} with many medium-sized resolved peaks ranging from C_{13}-C_{30} and an outstanding group of ORP between C_{19} and C_{20}. The alkane composition of the clams is about the same after 30 days regardless of the treatment used (Table 30, Figure 7). The clam chromatograms did not appear similar to the reference or Duwamish River sediment chromatograms (experiment 1); no indication of incorporation of sediment alkanes was found.

Arene fraction

164. The chromatogram of the initial Duwamish River sediment has almost no UCM and just a few poorly resolved, small peaks. This result probably reflects a low arene content in this sediment.

165. All chromatograms of crab arene fractions contained components boiling in the range 275 to 535°C and generally had a small UCM with relatively small, uniform peaks resolved above it. There are some dominant resolved peaks and small changes in the UCM. There were no striking differences in arene composition during the exposure with different treatments; however, the appearances and disappearances of some peaks suggested that slight exchange of material occurred.

166. The mussel arene chromatograms had a boiling point range of 270 to 505°C with several large peaks resolved above the UCM. There were more resolved components after 30 days in all treatments. These components were different than those initially in the mussel arene fraction. Appearance and disappearance of resolved peaks after 30 days was evident. Some changes in shape and extent of UCM were noted, suggesting there was a change in the arene composition during the experiment in all treatments.

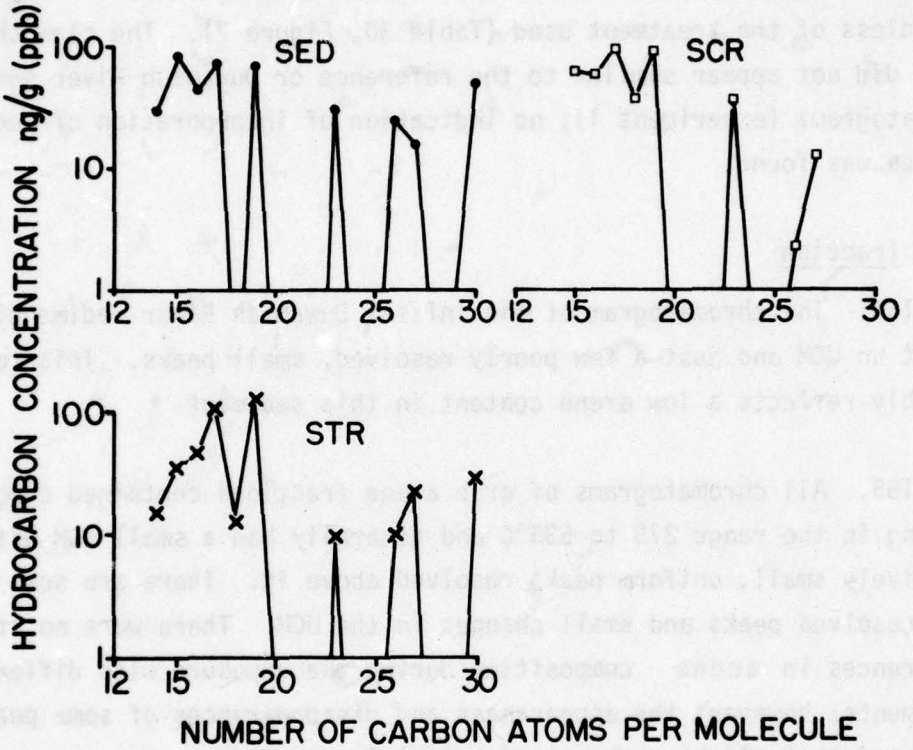

Figure 7. Fingerprints of n-alkane fraction of clam samples from experiment 2, 30-day results, all treatments.

167. All three clam arene chromatograms were similar with many uniform, medium-sized peaks (280 to 490°C bp) resolved above a large UCM that is centered at 370 to 395°C bp. The complexity of these chromatograms contrasts sharply with those of the mussels and crabs above.

168. Although no attempts were made to identify specific aromatic compounds, it was noted that all samples contained resolved components with retention times typical of polynuclear aromatic hydrocarbons. Further research on the aromatics would be desirable, because they include water-soluble components which may be toxic and readily accumulated by some organisms.

Comparison of Thin-Layer and Gas Chromatographic Analyses

169. A comparison of the results of quantitative determination of hydrocarbons in sediments used in experiment 1 by GC and TLC is presented in Table 33. Figure 8 shows the correlation of GC and TLC results for mussels, crabs, and clams from experiments 1 and 2. Results in Table 33 show a good correlation for the reference sediment hydrocarbons only, with poor correlations between these methods when evaluating Duwamish River sediments. This lack of correlation points out that GC data and TLC data are not necessarily intercomparable. Each of these methods is probably internally consistent for similar mixtures of hydrocarbons, but differences will occur as a function of composition of the mixture being determined. For example, the GC method does not detect hydrocarbons above C_{30}, while the TLC method includes most of these. The TLC method does not measure highly chlorinated hydrocarbons, which are included in the GC results. Figure 8 shows the same case occurs with hydrocarbons from tissue extracts. Only the results for the arene fractions showed a significant correlation (t-test of correlation coefficient, $\alpha = 0.10$). Most of the GC quantitation supports the TLC results. GC analysis of

Table 33

Comparison of Total Alkane Analysis of the Sediment Samples from Experiment 1, using Gas Chromatography (GC) and Thin-Layer Chromatography (TLC)

Tank Series†	Time days	Hydrocarbon Content µg/g dry wt (ppm) GC*	TLC**
Reference Sediment			
	0	4.47	12
SED	30	13.6	26
SCR	30	20.9	29
STR	30	31.2	40
Duwamish River Sediment			
	0	130	333
	0	141	359
SED	30	137	538
SCR	30	28.2	538
STR	30	344	467

* Result of a single GC analysis.

** Reference Sediment - single TLC analysis
Duwamish River Sediment - average of multiple TLC analyses.

† SED - Organisms in sediment
SCR - Organisms on screen 5 cm above sediment
STR - Organisms on screen 30 cm above stirred sediment.

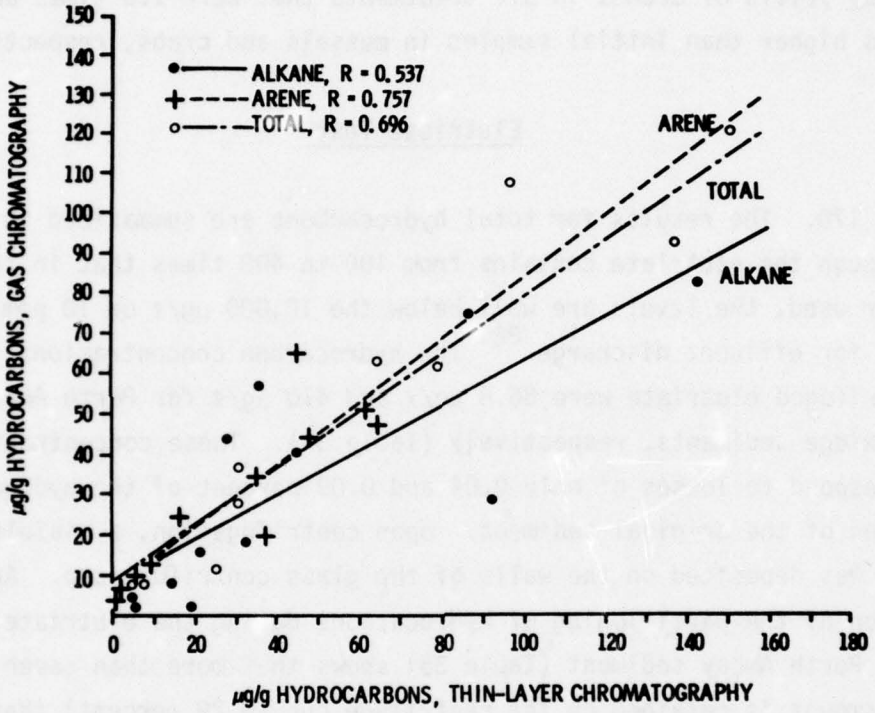

Figure 8. Comparison of GC and TLC results on organism samples from experiments 1 and 2 .

mussels from experiment 1 showed a drop in alkane concentration over 30 days (Table 30); this also appeared in TLC results (Table 25) for SED and SCR, but only slightly in the STR tanks. Crabs showed a small increase in alkanes by the TLC method which was not as clear in the GC results. The increases in hydrocarbon content of the reference sediment by GC show excellent correlation to TLC results that indicate a loss of alkanes from organisms. Mussel and crab arenes analyzed by GC showed increases in the SED and STR tanks and decreases in the SCR tanks after 30 days (mussels) and 17 days (crabs). TLC analysis showed 30- and 17-day levels of arenes in all treatments that were 1.6 times and 3 to 7 times higher than initial samples in mussels and crabs, respectively.

Elutriate Test

170. The results for total hydrocarbons are summarized in Table 34. Although the elutriate contains from 100 to 400 times that in the seawater used, the levels are well below the 10,000 µg/ℓ or 10 ppm acceptable for effluent discharge.[88] The hydrocarbon concentrations in the centrifuged elutriate were 86.8 µg/ℓ and 410 µg/ℓ for Perth Amboy and Bay Ridge sediments, respectively (Table 34). These concentrations correspond to losses of only 0.04 and 0.09 percent of the hydrocarbon burden of the original sediment. Upon centrifugation, a visible oily film was deposited on the walls of the glass centrifuge cup. An examination of the partitioning of hydrocarbons during the elutriate test with Perth Amboy sediment (Table 35) shows that more than seven times the amount is retained on the centrifuge cup (0.29 percent) than remains in the centrifuged elutriate (0.04 percent). The amount of oil released from the sediment, while being much larger than background, is less than 1 percent of the sediment-associated hydrocarbons.

171. These results illustrate a major problem in using the elutriate test to estimate release of oil and grease. An often major part of the oil and grease released is in the form of oil particles. These particles adhere to the glass surfaces of separatory funnels and centrifuge cups.

Table 34

Summary of Elutriate Test Results for Total Hydrocarbons

Sample	Hydrocarbon Concentrations	
	Perth Amboy	Bay Ridge
Original sediment, µg/g dry solids	2204 ± 34	6394 ± 148
Recovered sediment, µg/g dry solids	2385 ± 341	6214 ± 299
Centrifuged elutriate, µg/l	86.8 ± 0.07	410 ± 63
Seawater used, µg/l	0.9	0.9

Table 35

Partition of Total Hydrocarbons in the Elutriate Test with Perth Amboy Sediments

Sample	Percent of Total Hydrocarbons
Original sediment	100
Recovered sediment	100
Centrifuged elutriate	0.04
Centrifuge cup	0.29

They are removed by filtration. Finally, oil particles, which may be released when bottom sediments are disturbed, may be broken up and readsorbed during vigorous shaking with sediment in an elutriate test. Results from any elutriate test must be considered as minimum estimates.

PART IV: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

172. These experiments must be considered preliminary and of an extreme nature because organisms were confined within a static water mass with constant exposure to contaminated dredged material for periods up to 30 days. In spite of burdens of oil and grease, and perhaps contaminant heavy metals, no serious mortalities of organisms occurred which could be attributed to contact with the sediments.

173. It is concluded that the (weathered) hydrocarbon residues associated with the sediments tested were highly resistant to biodegradation, suggesting that they were inert and thus, non-toxic, and that they could not exert biochemical oxygen demand either in situ or when dispersed into the water column. It is not know whether this is a property of the organic material itself, or a property of the organic-sediment complex.

174. Hydrocarbon release to the water column was minor compared to total oily matter in the sediments tested, being in the parts per billion range. The hydrocarbons remaining in the water column were associated with suspended sediment. The standard elutriate test was of no value in predicting availability of oil and grease because most of the small amount of oil released was adsorbed to the walls of the extraction vessels and not retained in the test water. Available information suggests that the elutriate test, as presently established, is inapplicable to oil and grease assays. This test is adapted for assay of water-soluble components potentially released from sediments. Literature review and experimental results have shown that extremely low levels of water-soluble components are released from contaminated sediments when they are dispersed in aquatic environments. Research will be required in the future to estab-

lish new test procedures to evaluate the water-quality impacts of any water solubles released from oil and grease fractions.

175. It is concluded from overall examination of the thin-layer chromatography data for all experiments that uptake of hydrocarbon residues into organism tissues occurred with difficulty, and then only from the most heavily contaminated sediments. In these cases, contact with sediments was required for uptake by crabs and mussels.

176. Exhaustive examination of gas chromatographic data from two 30-day experiments with different species showed no outstanding changes in composition or concentration of the saturated hydrocarbon fraction of Duwamish River sediment or organism tissue, and it is concluded that the transfers observed were minor (experiments 1 and 2). Several subtle changes evidenced in the arene fraction will be of interest in future research.

177. Gas chromatographic and thin-layer chromatographic results were mutually supportive of the general conclusions. Thin-layer chromatography appears to be the method of choice in future survey work where large numbers of samples are analyzed for statistical validity, while gas chromatographic analysis can provide more information on the composition of hydrocarbon fractions, if desired.

178. The literature search demonstrated the need for improvements and standardization in measuring the fat-soluble components of dredged material. Standardization is of cardinal importance in development of regulatory criteria, so that all determinations of the parameter measure the same contaminants and are intercomparable between stations and laboratories. Standard procedures might include more than one measurement; for example, oil and grease assessment may require measurement using two or more different extracting solvents in separate assays since the

different solvents remove different types of oily residues. The standard method when applied to sediments should include a desulfurization step to remove this source of error.

179. Research should be carried out on the variability of the oil and grease levels within stations, and within the same load in a hopper dredge. Variability should also be determined for the oil and grease parameter at different levels down through core samples at dredging sites. It appears unreasonable to evaluate the quality of potential dredged material based on the oil and grease values obtained for the surface layer of sediment alone, which may differ significantly from underlying layers of sediment. Field methods for sediment sampling require standardization.

180. Dredged material from several representative major sites should be exhaustively analyzed to determine specifically which compounds constitute the oil and grease fraction, for comparison with known toxicity or mutagenicity effects. In cases where these are not known, they should be tested, with particular reference to the polynuclear aromatic hydrocarbons.

181. Levels of hydrocarbons below C_{12} should be measured in representative sediments. Uptake and sublethal effects of this fraction should be investigated if this fraction is found to occur in sediments subjected to dredging.

182. Available data suggest the hypothesis that the oily residues taken up by organisms are those not tightly bound to sediment particles. It is of interest to determine the capacities of adsorption/absorption of naturally occurring sediments for pollutant oils, and the degree to which they are bound to the particles. There is apparently a phenomenon of entrapment of oils which do not become bound tightly to particles, and

it appears that this oil is available for uptake by organisms. Methods for measuring sorbed and entrapped oil separately should be developed.

183. Experimental studies should be carried out in which a fully characterized mixture of oily residues adsorbed to sediments (comparable to an oil and grease value determined in polluted areas) is tested against organisms, such that overall mortality, selected sublethal effects, and interference in reproduction can be monitored over extended periods of time. Available toxicity and mutagenicity data argue for prime attention to be made to aromatic fractions of the oil and grease load. Synergistic and antagonistic effects of oil and grease with other pollutants should be examined.

184. Research should also be carried out on the biodegradability of well-defined oil and grease mixtures, with determination of the effect of sediment association on the decomposition process. It is of importance to determine if toxic biodegradation products can be produced in anaerobic sediments which may affect further biodegradation or ultimately be released into the water column by dredging, thus becoming available to the aquatic food web and ultimately to man.

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APPENDIX A: NOTATION

BOD	Biochemical oxygen demand
bp	Boiling point
CPI	Carbon preference index
DDT	1,1,1- Trichloro- 2,2- bis (p-chlorophenyl) ethane
DO	Dissolved oxygen
FID	Flame ionization detector (GC)
FPD	Flame photometric detector (GC)
GC	Gas chromatography
GC/MS	Gas chromatography/Mass spectrometry
HC	Hydrocarbon
HPLC	High pressure liquid chromatography
IR	Infrared
OG	Oil and grease
ORP	Other resolved peaks
PAH	Polynuclear aromatic hydrocarbons
PCB	Polychlorinated biphenyl
ppb	Parts per billion
ppm	Parts per million
R_f	Movement of the zone from origin/Movement of advancing liquid front from origin

NOTATION

SED	Organisms in sediment
SCR	Organisms on screens 5 cm above sediment
STR	Organisms on screens 30 cm above stirred sediment
SCOT	Support-coated open-tubular (GC column)
SD	Standard deviation = $\sqrt{\frac{\Sigma y^2}{n-1}}$
TLC	Thin-layer chromatography
UCM	Unresolved complex mixture
UV	Ultraviolet

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

DiSalvo, Louis H
Assessment and significance of sediment-associated oil and grease in aquatic environments / by Louis H. DiSalvo ... [et al.], Naval Biosciences Laboratory, Naval Supply Center, Oakland, Calif. Vicksburg, Miss. : U. S. Waterways Experiment Station ; Springfield, Va. : available from National Technical Information Service, 1977.
146, 2 p. : ill. ; 27 cm. (Technical report - U. S. Army Engineer Waterways Experiment Station ; D-77-26)
Prepared for Office, Chief of Engineers, U. S. Army, Washington, D. C., under Interagency Agreement WESRF 76-86 (DMRP Work Unit No. 1D11)
References: p. 125-146.

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