Sources, Effects & Sinks of Hydrocarbons in the Aquatic Environment

Proceedings of the Symposium
American University
Washington, DC
9-11 August 1976

AIBS

The American Institute of Biological Sciences

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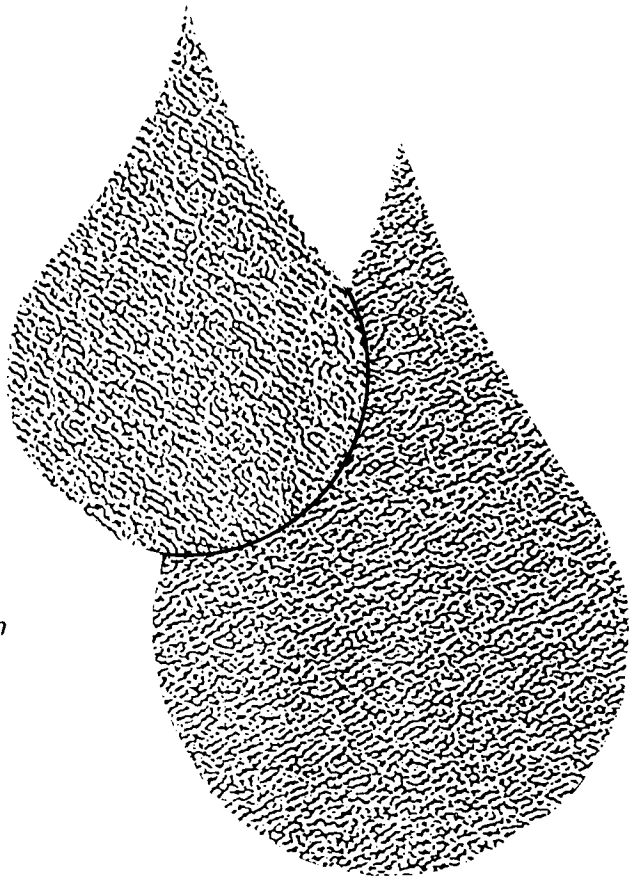

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SYMPOSIUM ON
SOURCES, EFFECTS AND SINKS OF HYDROCARBONS
IN THE AQUATIC ENVIRONMENT

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ACKNOWLEDGMENTS

It is entirely appropriate to acknowledge the substantial efforts and contributions which went into making the symposium so successful. Firstly, to the sponsoring agencies, not only for their financial assistance but also in providing members to the Program Committee and for speakers. To the Program Committee whose members carefully designed the program and selected our excellent speakers. To the invited speakers, all of whom have generously given of their time in the preparation and the presentation of the substance of the program.

The American Institute of Biological Sciences has been most effective in planning, arranging, and coordinating the symposium. Particular thanks are to Donald Beem, Head of Special Science Programs, to Patricia Russell, Staff Biologist, and to Judy Ross, all of whom put in many hours in doing all that was required to see that the meeting ran smoothly and efficiently.

F. T. Weiss

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BEHAVIORAL, PHYSIOLOGICAL AND MORPHOLOGICAL
Session Chairman: Gilles LaRoche

SYMPOSIUM ON
SOURCES, EFFECTS AND SINKS OF HYDROCARBONS
IN THE AQUATIC ENVIRONMENT

WELCOMING STATEMENT

Richard Trumbull
Executive Director
American Institute of Biological Sciences

It is my pleasure to extend a welcome to this Symposium on Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment on behalf of the participating agencies, the American Petroleum Institute and the American Institute of Biological Sciences. It is rather appropriate, I believe, that this first concentrated look at the effects of current practices and procedures upon biological systems involve the industrial, governmental and academic R & D worlds. All of us have much to gain through increasing our understanding of the larger ecological picture. We are considering the long-range as well as the short-range effects. We now recognize the more insidious effects of hydrocarbon penetration of water fowl eggs as well as upon the dramatic oil-coated fowl themselves.

We have a clearer understanding of our own need for vigilance in tapping new resources, in transport and in waste disposal. We have a new awareness of the potential inadvertent ecological costs as we reduce and/or induce pressures in recovery and transport. Over the next few days we hope to establish through the topics and scope of variables being discussed why the planning committee believed this to be the time to get it all together.

We are very happy to have an audience of this size, reflecting interest in and concern about this important topic. We earnestly ask your active participation to make the product more significant and helpful to those who must make decisions and achieve a balance between the advancement of technology and the maintenance of ecology.

INTRODUCTORY REMARKS

Sidney R. Galler
Deputy Assistant Secretary for Environmental Affairs
U.S. Department of Commerce

Ladies and gentlemen, in participating in the opening of what I hope will come to be known in retrospect as a key meeting on the subject of hydrocarbons in the environment, I am reminded of something that Sir Ritchie Calder was supposed to have said: "Science produces knowledge, not wisdom. Wisdom is knowledge tempered with judgment." Calder's comment takes me back to the 17th century when Newton, Hook, Boyle, Wrenn, and the other founding members of the Royal Society chose as the motto for their new group "*nullius inverba*," "put not faith in words." Both Calder and his spiritual ancestors recognized that neither pious polemics or omphaloskepsis on the one hand, nor hard data alone derived from scientific research can impart the wisdom that society requires to maintain and enhance its physical and spiritual environment. That wisdom can only come from conclaves such as this where scientists and other learned persons can come together to share their knowledge gained from creative endeavor and their judgments gained from practice and experience.

This symposium is not intended to be positional in nature. It was not designed to provide soap boxes for pious moralizing or the presentation of theoretical abstractions devoid of any semblance of reality. Rather, this meeting was planned to provide a warm, congenial and secure intellectual environment to facilitate the exchange of knowledge and ideas free from the threats of harangue and purple prose.

A symposium such as this one should provide an important linkage between research and practice, between discovery and application and, most importantly, it should enlarge the boundaries of our collective wisdom so that we, as representatives of our communities and society as a whole, can accelerate the processes needed to create and maintain the conditions under which man and nature can exist in productive harmony and fulfill the social, economic, and other requirements of present and future generations of Americans, to quote from the National Environmental Policy Act and, may I add, the conditions needed for all of mankind to live in productive harmony with nature on a global scale.

This symposium will fall short of its intended goal if the discussions on the Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment lose sight of what should be the ultimate goal of all of our efforts and all of our contributions--namely, to enhance the quality of life for our citizens and for all mankind. It is the maintenance and improvement of the total human environment that should serve as the singular point of reference for our efforts. Those efforts become moot, of no consequence, if we lose our battle to protect and maintain the global ecosystem which yields the life support capacities for human existence and, indeed, all life forms. At the same time, we must not lose sight of the fact that winning the battle to protect the global biosphere alone insures only the survival of the human species. The concomitant objective must be to maintain and enhance the flow of materials, services, and most importantly, intellectual creativity to insure an adequate supply of food, shelter, clothing, and the amenities, the sufficiency of which, in the aggregate, adds the dimension of quality to human existence, driving the human ecosystem, as it were, to provide the productive basis for meeting the social, economic, and other requirements of global society.

I will be an eager listener in this symposium and hope that upon its conclusion I will be more knowledgeable and perhaps even a little wiser than I am at this opening session. I wish you well in your endeavors.

SESSION I

SOURCES AND DISTRIBUTION

Chairman
Fred T. Weiss
Shell Development Company

AN ESTIMATE OF THE AMOUNTS OF OIL ENTERING THE OCEANS

Bernardo F. Grossling
U. S. Geological Survey
Reston, Virginia

AN ESTIMATE OF THE AMOUNTS OF OIL ENTERING THE OCEANS

Bernardo F. Grossling
U. S. Geological Survey
Reston, Va.
August 2, 1976

The amounts of crude oil and some of its liquid products which enter the world oceans are estimated for the 1972 level of economic activity. Spent lubricants contribute about 17.1 million barrels per year, oil industry accidents not more than 6.4 million barrels per year, tanker cleaning operations 2.6 million barrels per year, natural seeps offshore contribute 1.5 to 44 million barrels a year, and natural seeps onshore (about 6 to 176 million barrels per year) a further unknown amount. By comparison, world crude production for 1972 was 20,561 million barrels, of which about 10,000 million barrels was transported by tankers.

Introduction

Certain oil pollution incidents that have occurred in recent years, at a time of enhanced environmental concern, have raised the question of how much crude oil is being released to the world's oceans by man's activities. Crude oil and its liquid products enter the oceans: a) as the result of the normal outcome of industrial and other users practices, b) as the result of accidents, and c) because of release from natural oil seeps. Activities responsible for accidents which have been mentioned are: well drilling, oil field operations, oil land transportation, and oil oceanic transportation.

The oil pollution of the oceans consists of oil spilled into the oceans themselves, or of oil spilled on land and which flows into the oceans from land areas, or oil which enters the oceans from the atmosphere. Crude oil or its traces, when spilled on the ground or in the water, can remain for many days or even a few years. The lighter components evaporate first, other fractions are weathered, and the heavier ones remain

longer. Of the liquid refined products, lubricating oils are of special interest here because of the manner of disposal when they have been used. Another important factor, as we shall see, is the manner of cleaning of oil tankers.

Specifically, I will attempt to estimate the yearly release of crude oil and some of its long-lived oil products into the oceans for the level of economic activity of 1972. The year 1972 was selected as a base year for this estimation because of the availability of certain background studies for the year 1968-1972 and 1969-1973, to be mentioned later. For later years one has to consider that the release from user practices and accidents continued to increase from year to year in parallel to the increase in economic activity. One can then scale up the foreseen amounts of oil released in accordance to suitable economic activity indexes.

The discussion will be restricted to the petroleum industry (exploration, production, transportation, refining), and to some ultimate-user practices. That is, the release of oil products from industrial activities other than petroleum industry will not be included. Also, as the scope of this paper is long-range effects, the estimation of annual overall averages is needed rather than the statistical distribution of releases in individual spills.

What I am aiming at is the assessment of the overall annual impact on a worldwide basis of oil-related activities. Locally, rather quite small amounts of oil spilled may be serious. But this local issue is beyond the scope of this paper.

The subject will be discussed under three categories: release from certain user practices, release from accidents, and natural release. The main problem in this undertaking is how to make reasonable estimates for the various quantities, which in light of the paucity of certain data we will bridge by making inferences from other available data.

Perspective on the Amounts of Crude Oil and Oil Products Which are Involved

To set an initial perspective of the relative importance of the amounts of crude oil and liquid oil products we can consider first the world and U.S. production figures of these substances, as indicated in Table 1 for 1974.

Not all of the substances in Table 1 are significant for our purposes. First, as we are interested in only long-term effects, we can eliminate those substances which evaporate quickly when released into the environment, namely: gasoline, distillate fuel oil, kerosene and jet fuel. However, one should note that some of these leave small residues after evaporation which may be of ecological importance. Thus there still remains to be considered: crude oil, residual fuel oil, and lubricating oils. Moreover, because of their relatively high viscosity and manner of use one can exclude bitumen and road oil, which are not shown in Table 1.

Table 1

CRUDE OIL PRODUCTION AND PETROLEUM PRODUCTS DEMAND, 1974 [1]

($\times 10^6$ bbl)

	U.S.	World
Crude oil production	3,202.6	20,537.7
Demand:		
Residual fuel oil	963.2	4,525.8
Gasoline (motor and aviation)	2,402.4	4,163.4
Distillate fuel oil	1,015.9	3,695.2
Kerosine and jet fuel	427.0	1,126.6
Lubricants (including grease)	56.7	153.7
	4,865.2	13,664.7
International tanker trade		11,200 [2]

[1] U. S. Bureau of Mines, International Petroleum Annual 1974, March 1976.

[2] Estimated from data in B P Statistical Review of the World Oil Industry 1974, British Petroleum Company Ltd.

Crude oil has to be considered because it is the largest of the annual amounts involved (Confer Table I) and because accidents during the exploration, production, and transportation phases may lead to spills. Residual fuel oil is commonly used as ship and industrial fuels, and is the petroleum product used in greater quantity in the world. Residual fuel oil can lead to spill incidents because of ship accidents, and to a minor extent by industrial accidents. Lubricating oils create a problem when they are used because they have to be disposed somehow. Road oil accounts for a small part of the total production of products and by its nature is meant to be released to the environment.

Therefore, we will focus only on: crude oil, residual fuel oil, and lubricating oils. The 1972 production figures for these substances [1] are:

	($\times 10^6$ bbl)		
	US	Non-Communist World	World
Crude oil	3,455.4	15,347.6	18,598.0
Residual fuel oil	292.5	4,426.7	n.a.
Lubricants	65.3	148.7	n.a.

Release of Oil Because of Certain User Practices

Release of spent lubricating oils. Lubricating oils are used in internal combustion engines and various mechanical devices, and also in certain industrial processes. The amount of lubricating oil consumed in relation to the total consumption of petroleum products for the United States has been decreasing slightly from year to year as shown in Table 2. To a first approximation, lubricating oils account for one percent of the total petroleum products consumed. For 1972, the demand of lubricating oil was 52.81 million bbl, equivalent to 0.88 percent of the total product demand.

When examining the U.S. consumption by industrial sector, Table 3, we find that industry accounts for 54 percent and automotives for 44 percent of the lubricating oil sales. On this basis, the lubricating oil demand for 1972 would be distributed as follows:

	($\times 10^6$ bbl)
Industry	28.5
Automotives	23.4
Aviation	0.9
	52.8

Of the 1972 industry lubricating oil demand, about 2/3 (that is 19 $\times 10^6$ bbl) is for lubrication, and about 1/3 (that is 9.5 $\times 10^6$ bbl) is for processing. Some of the processing applications are: metal quenching, heat reduction, and rust prevention.

Table 2

U.S. DOMESTIC DEMAND OF LUBRICATING OIL IN RELATION TO THAT OF PETROLEUM PRODUCTS [1]

	Petroleum products ($\times 10^9$ bbl)	Lubricating oil ($\times 10^6$ bbl)	Lubricating oil, % of total demand
1958	3.315	39.47	1.19
1959	3.450	42.88	1.24
1960	3.536	42.68	1.21
1961	3.579	41.53	1.16
1962	3.736	43.61	1.17
1963	3.851	43.58	1.13
1964	3.959	45.79	1.16
1965	4.126	47.12	1.14
1966	4.325	48.95	1.13
1967	4.585	44.12	0.96
1968	4.902	48.47	0.99
1969	5.160	48.78	0.95
1970	5.364	49.69	0.93
1971	5.553	49.32	0.89
1972	5.990	52.81	0.88
1973 [2]	6.317	59.17	0.94
1974 [3]	6.078	56.67	0.93

[1] De Golyer and MacNaughton, Twentieth Century Petroleum Statistics 1975.

[2] U. S. Bureau of Mines, International Petroleum Annual 1973.

[3] U. S. Bureau of Mines, International Petroleum Annual 1974.

Table 3

DISTRIBUTION OF SALES OF LUBRICATING OIL, 1965 [1/]

Category of user	(× 10^6 bbl)	%
Industrial	33.19	53.9
Automotive	27.29	44.4
Aviation	1.05	1.7
Total	61.53	100

[1/] Am. Petroleum Inst. and Ad. Soc. of Lubrication Engrs, Industrial Oily Waste Control, 1970, p. 6.

To estimate the consumption of lubricating oils for the various types of automotive users we can use the relative consumptions of fuel as shown in Table 4 for the United States. For instance, passenger cars would account for 70 percent of the lubricating oil consumption by these users. On this basis we can estimate the 1972 U. S. lubricating oil consumption for the automotive users as follows:

	$\times 10^6$ bbl
passenger cars	16.4
trucks and combinations	6.8
busses	0.2
	23.4

During engine operation, part of the lubricating oil is burned and part is carried out by the exhaust gasses as semi-oxidized products. The amount depends on the age and condition of the engine, and may amount to about ten percent for new engines and increase to more than 20 percent for old engines. Overall, we surmise that a figure of about 15 percent of the gross lubricant intake is burned during engine operation. Another portion leaks during the engine life, and it may amount to about five percent. Hence only roughly 80 percent of the gross lubricant intake has to be disposed of when the lubricant is changed. On this basis, the amount of crankcase oil to be disposed of for 1972 in the United States can be estimated at 18.7 million barrels for all automotive users, of which 13.1 million barrels is for passenger cars.

Most automobile crankcase oil is received by gasoline service stations when the oil is changed. When the mobile units are parts of a fleet, the point of reception is the corresponding fleet maintenance station. The various ways in which crankcase oil is presently being disposed of are the following:

a) used as fuel,
b) used as road oil,
c) re-refined,
d) impounded in artificial reservoirs, and
e) released to sewage, flood water systems, or simply poured on the ground surface.

The questions of economics obviously must be already being considered in the ways of disposal a), b), and c) above. Of the fraction released as waste, part is retained in the underground and part flows eventually to the oceans.

The quality of used crankcase oil varies greatly. Some may consist of pure mineral oil with only small amounts of free carbon and some dirt, for instance, as generated by crankcases of a fleet of trucks. These crankcase oils seem to be effectively disposed of already. Taxi fleets, bus companies, truck fleets, large general contractors, farmer cooperatives, and railroads usually have their engine oils re-refined. But some re-refined crankcase oil must be disposed of for other uses or wasted. Re-refining of highly aqueous or emulsified crankcase oils is not worthwhile, and the crankcase oil may have be incinerated or

Table 4

U.S. MOTOR FUEL CONSUMPTION BY USE, 1972

	$\times 10^6$ bbl	%
Passenger cars	1749	69.9
Trucks and combinations	731	29.2
Busses	21	0.9
	2501	100

Source: Statistical Abstract of the United States, 1974, p. 561.

disposed of some other way. Crankcase oil when used as fuel in coal-fired boilers is simply misted over the burning coal. But low-flash point oils may cause "flashbacks" or explosions when burned with coal or fuel oil.

Waste of crankcase oil implies some negative environmental effects and also implies a certain economic waste of resources. Oily waste released or entering water bodies may either form slicks or floating scums, sink to the bottom, or dissolve in the water. The floating fraction creates fire hazards and can impair the recreational value of water bodies. The fraction that sinks to the bottom (because some lubricating oils are actually heavier than water) can kill animals and plants. The fraction dissolved or emulsified can be a toxic agent and may deplete the oxygen content. Oily waste can contain alkaline cleaners, metallic and non-metallic solids, soaps, fats, detergents, emulsifiers, oxidation products, or chemical additives. Emulsifiable lubricating oils contain fat, soap, or various other additives to enhance their working properties. Excessive oily waste may interfere with the operation of municipal water disposal plants.

Reclamation of the fraction of crankcase oil now being wasted could be a sequel to the collection of crankcase oil at service stations. Thereby product reclamation is easier, and gives the opportunity of segregating the oily waste as to type.

The questions of how to dispose of such crankcase oils, as well as other oily wastes, have been examined by task forces of the American Petroleum Institute and American Society of Lubrication Engineers [2/] [3/].

On that basis, I estimate that about 30 percent of the automotive crankcase oil is currently being released as waste. So that in the United States roughly 5.6 million barrels of crankcase oil could be recovered or burned per year. This figure can be compared with the 1972 consumption of residual fuel oil which is 925.6 million barrels. So the recovery of the crankcase oil now being wasted is roughly equal to 0.6 percent of the consumption of residual fuel oil.

Therefore to obtain the annual amount of crankcase oil that pollutes the oceans, one needs to reduce the gross amount of lubricating oil consumed per year by: 1) amount burned during engine operation (about 15 percent), 2) amount that leaks during engine life (about 5 percent), 3) amount already effectively disposed (incinerated, impounded, re-refined), (about 56 percent), and 4) fraction of the waste either absorbed and retained by soils and rocks, or otherwise destroyed.

Estimation of world lubricant demand. Now we come to the problem of obtaining the world lubricant demands for industry, automotives and aviation for 1972. No such data seems to be available. To estimate the world industrial lubricant demand from the U.S. industrial lubricant demand I will use for projection the industrial and public electricity consumption data which is available. To estimate the world automotive lubricant demand from the U.S. automotive lubricant demand I will use for projection the total number of vehicles, data on which is given in

Table 5. To estimate the world aviation lubricant demand from the U.S. aviation lubricant demand I will use for projection the jet fuel demand, data on which can be obtained. These activity indexes - namely industrial and public electricity consumption, number of vehicles, and jet fuel demand - for the United States and the world are indicated in Table 6. The resulting amounts of lubricants wasted for the United States, rest of the world except Sino-Soviet, and for the world are given in the third column of Table 7. A resumé of the preceding lubricant estimation procedure is given in Appendix A.

Ocean input of land lubricant waste. Now we come to the difficult question of how much of this lubricant waste released on land reaches the oceans. First, we have to consider the weathering of the oil. The low boiling point compounds are evaporated rather rapidly. In about ten days all the hydrocarbons containing less than about C_{15} would be thus evaporated. For an average crude oil an evaporation loss of roughly fifty percent would occur.

Some of the oil will be dissolved in the surface and underground waters, and in this manner it would eventually reach the oceans. When at the surface, biodegradation would deplete the soluble low boiling aromatics. Finally some of the oil will be retained in the soils and rocks as an absorbed layer,

What is the overall effect of the above factors is hard to estimate. A great deal depends on the time of transit from the point of spill to the ocean and on the manner of transit. For this preliminary report I will assume that overall about 50 percent of the oil waste reaches the oceans. In this manner I have estimated the oil waste inputs to the oceans given in the last column of Table 7.

Release of oil from tanker cleaning practices. Oil is released to the oceans as the result of certain cleaning practices of tankers. These releases are very significant because of the magnitude of the annual volume transported. About 56 percent of the total world crude production enters international trade. For instance, in 1972 of the 18.6 billion barrels of crude oil production, 10.2 billion barrels was hauled by tankers - mainly from the Middle East, Venezuela, Nigeria, Libya, Algeria, Indonesia - to consuming countries in Europe, Asia, and the Americas. The remaining 8.2 billion barrels was consumed in the producing countries themselves, or processed for export as refined products.

In response to the increasing need for overseas imports of crude oil the world tanker fleet has been increasing both in number of vessels and average deadweight, as shown in Table 8 for the 1940-1973 period. During this period the number of vessels 2,000 gross tons and over increased by an average of three percent per year, and their average tonnage by five percent per year.

During 1972, the 10.2 billion barrels (1.39×10^9 metric ton) in international trade were hauled by 4,336 vessels. That is an average of 320,000 metric tons per vessel. If we consider the average vessel deadweight, namely 50,941 ton, we obtain that on the average each vessel would be loaded up 6.28 times per year, and that during the year

Table 5

WORLD VEHICLE POPULATION, 1972

(registrations)

	Millions of vehicles	
	Passenger cars	Trucks and buses
United States	97.0	21.6
Western Europe	76.6	9.5
Asia	16.5	13.0
Other Americas	16.7	6.1
Eastern Europe	5.6	6.3
Oceania	5.6	1.3
Africa	3.2	1.5
World totals	221.2	59.3

1/ Statistical Abstract of the United States 1975, p. 570.

Table 6

ACTIVITY INDEXES FOR ESTIMATES OF SPENT LUBRICANTS

	U.S.	World	Ratio
a) Electricity consumption, industrial and public, $\times 10^9$ KWH [1]	1,721.1	5,219.5	3.03
b) Number of motor vehicles, 1972, $\times 10^6$	118.6 [2]	280.5 [2]	2.37
c) Jet fuel demand, 1971, $\times 10^3$ bbl/d [3]	1,010	2,633	2.61
d) Gasoline demand, 1972, $\times 10^6$ bbl [4]	2,350.7	3,986.7	1.70

[1] United Nations, World Energy Supplies 1968-1971, Table 21, 1973.

[2] From Table 5

[3] Estimated from the 'kerosene + jet fuel' data in reference [1] and data in Office of Oil and Gas, U. S. Department of the Interior, 1971 Petroleum Supply and Demand in the Non Communist World, 1973, p. 25.

[4] U. S. Bureau of Mines, International Petroleum Annual 1972.

Table 7

ESTIMATES OF SPENT LUBRICANTS RELEASED, 1972

Industry	Lubricants Demand ($\times 10^6$ bbl)	Waste ($\times 10^6$ bbl)	Waste into Oceans ($\times 10^6$ bbl)
United States	28.5	est. 6.8	est. 3.4
World	est. 86.4	est. 20.6	est. 10.3
Automotive			
United States	23.4	est. 5.6	est. 2.8
World	est. 55.5	est. 13.3	est. 6.5
Aviation			
United States	0.9	est. 0.22	est. 0.13
World	est. 2.4	est. 0.56	est. 0.28
World totals		est. 34.46	est. 17.1

Table 8

WORLD TANK SHIP FLEET [1]

(Vessels 2,000 gross tons and over)

Year	Number	Deadweight (10^6 ton)	Av. deadweight (ton)
1940	1,637	17.58	10,739
45	1,768	21.67	12,257
1950	2,056	26.96	13,113
55	2,681	41.62	15,524
1960	3,264	65.78	20,153
65	3,436	93.17	27,116
1970	4,002 [2]	167.94 [2]	41,964
71	4,207 [2]	193.89 [2]	46,087
72	4,336 [2]	220.88 [2]	50,941
1973	4,563 [2]	256.72 [2]	56,261

[1] Dept. of Transportation, Energy Statistics, September 1973.

[2] FEA, informal communication.

there would have been 27,230 trips with tanks empty in need of being cleaned before the next cargo.

In order to make an estimate of the amount of oil which would be released to the oceans by tanker practices I will review the procedure now preferred for cleaning the tanks.

First, let us consider a fully loaded tanker which travels on a long route. To minimize oil pollution a load-on top (LOT) procedure is used to clean the tanks. At the start of the trip back, after the oil cargo has been unloaded, about 1/3 of the tanks are to be filled with sea water to serve as a ballast. Tankers usually have from about 20 to about 35 tanks. That is, first about 6 to 12 of the tanks would be filled with water without having previously cleaned them. Then another third of the tanks are cleaned with jets of water and the oily water is put into a special tank called the "slop tank". This cleaned 1/3 of the tanks is then filled with sea water. After two to five days the water in the first third of dirty tanks is discharged overboard from the bottom until what remains is mostly the overlying oil; then the oil with remains of water is added to the slop tank. The first third of the tanks is then washed with water jets, and the dirty water is put into the slop tank. Near the end of the trip, the oil would have had time to separate from the water in the slop tank; so that the underlying water may be discharged overboard until almost only oil remains. Before reaching the loading dock, the second third of the clean tanks which were filled with sea water are emptied. Thus the tanker arrives to the loading dock with 2/3 of the tanks clean and 1/3 of the tanks with oil remains. The tanker is then reloaded. In this manner in each trip 2/3 of the tanks would be cleaned.

With the above procedure, the oil pollution on each return trip would consist of: a) the discharge overboard of the water with some oil remains from the one third of the dirty tanks which were filled with water, and b) the discharge overboard of the water with some oil remains from the slop tank which contained the washings of 2/3 of the tanks.

Now the question is how much oil would remain in a tank after the cargo has been unloaded. A bottom pool of about four inches would account for about five percent of the volume of a typical tank. The oil coating on the tank walls and inside beams, and small pools in structural recesses would at most account for 0.1 percent of the volume of the tank. As to effectiveness of the water and oil separation in the slop tank and in the dirty tank after several days, I will assume that five percent of the oil is dumped overboard with the water.

With the above assumptions, the fraction of the oil cargo which would be disposed overboard with the LOT procedure would be

$$1/3 \times 0.05 \times 0.05 + 2/3 \times 0.05 \times 0.05 = 0.00025$$

that is 0.025 percent.

The new international standards established by the Inter-Governmental Maritime Consultative Organization (IMCO) require all tankers over 150 tons to use load-on-top procedures. That was not required in 1972. However, since what we are interested in is an estimate of the oil pollution of the oceans with current technology, I will estimate the oil pollution of the oceans from tanker cleaning operations for the 1972 level of economic activity, assuming that the new standards had been in effect then.

Therefore, since the total amount of oil hauled by tankers in 1972 was about 10.2 billion barrels, then the resulting oil intake of the oceans from cleaning operations would be about 2.6 million bbl (about 360,000 metric tons per year).

The above estimate may be compared with the Porricelli *et al* estimate (1971) [4] of 1.94 million barrels (265,000 metric ton) from LOT operations plus 5.15 million barrels (702,000 metric tons) from non-LOT operations.

Release from Accidents

Some activities of the petroleum industry lead to accidental releases of crude oil and of its liquid products. As mentined before, the liquid products to be considered are: residual fuel oil and lubricating oils; of which only the first is significant in relation to petroleum industry accidents. The second product was considered in a previous section on certain user practices.

Although gas release is ecologically important, it is beyond the scope of this report. Briefly we note that accidental gas releases can have detrimental environmental effects. In part this is because hydrogen sulphide is often a component of natural gas. The natural gas itself can have detrimental effects. Leaks from buried gas pipelines can soak with gas the adjoining ground and kill trees when their root systmms are within the gas soaked volume. When significant amounts of gas are released, either by accident or design, they are often burned thus contributing to thermal pollution.

To estimate the annual release of crude oil from accidents in the world one would need a survey of what has happened in terms of number of accidents and amounts spilled in various countries. I will use to this effect some results of a report that Diane T. Nielsen [5], of the U. S. Geological Survey, is completing on all the petroleum industry accidents worldwide - comprising exploration, production, pipelines, refineries - which have been reported in the Oil and Gas Journal for the period 1968-1972.

It should be noted that in the Oil and Gas Journal the report of incidents of pollution in foreign countries is more scant than for the United States. Perhaps this is because oil pollution incidents - aside of tanker accidents - may receive less attention abroad and also because of the lower levels of petroleum activity abroad. For this reason I intend to base a world estimate of crude oil releases by accidents based on a projection of the accident data for the United States.

The reported oil spills according to major categories for 1968-1972 for the United States, as taken from the Nielsen report and from reports of the Smithsonian Center for Short-Lived Phenomena, are as shown in Table 9. The largest spill corresponds to an offshore well blowout.

To make a world estimate of the crude oil released from similar sources elsewhere, assuming that the spill statistics are the same as for the United States, a suitable activity index has to be identified.

The oil pollution contribution of well drilling. The drilling of exploratory and development wells can lead to pollution when blowouts occur. A blowout is an uncontrolled flow of reservoir fluids through a well drilled into the reservoir. Oil field operations can lead to oil pollution by accidents such as rupture of pipes, failure of valves, and other control elements, overflow of tanks, and rupture of tanks.

The basic activity index for these operations is the number of wells completed each year, which can be summarized as shown in Table 10 for the period 1968-1972. For this period the number of wells completed in the world, aside of Communist countries, was 182,691, of which 143,103 were in the United States, that is 78.3 percent of the wells. Therefore an estimate of the pollution incidents from petroleum drilling based on the United States data should give a good estimate for the world.

The types of well accidents which can lead to oil pollution are: blowouts, other kinds of uncontrolled oil flow, and subsurface blowouts, For the 1968-1972 period the number of these accidents, which were reported in the Oil and Gas Journal, is given in the tabulation which follows:

	Blowout	Subsurface blowout	Other uncontrolled oil flow	Cumulative reported oil spill (bbl)
United States	39	2	3	650,000-700,000
Other countries	21	1(?)	0	9,770

In addition to the above amounts of oil spilled, the Oil and Gas Journal reports of six other incidents in the United States and one abroad of slicks and spills, but without an indication of the amounts spilled.

The reported amounts of oil spilled, in barrels, because of well accidents in the United States consist of the following list of individual spills:

640,000-690,000
12,000-20,000
5,476
4,000
2,500
2,362
240
4.5
3

Table 9

SOURCES OF REPORTED CRUDE OIL SPILLS FOR THE UNITED STATES 1968-1972

	Onshore		Offshore		
Amount (bbl)	Well	Pipeline	Well	Pipeline	Source Unknown
640,000-690,000 [1]			X		
25,000				X	
12,000-20,000 [2]			X		
7,400				X	
6,785		X			
6,000				X	
5,476 [3]			X		
4,000			X		
3,800				X	
3,700				X	
2,500	X				
2,362			X		
1,000				X	
1,000				X	
240 [4]			X		
200				X	
<100					X
4.5			X		
3			X		

[1] Of this amount, only 32,000-35,000 bbl was spilled without being burned.

[2] Estimated from data reported by the Smithsonian Center for Short-Lived Phenomena, Event 105-70.

[3] Dr. Alan Allen estimated the oil spilled during the first four months of this accident at about 71,000 bbl.

[4] Spill of diesel fuel from sunken rig.

Table 10

NUMBER OF PETROLEUM WELL COMPLETIONS 1/

Region or Country	1968	1969	1970	1971	1972
United States	30,939	30,815	27,408	26,077	27,864
Canada	3,120	3,329	3,103	3,198	3,673
Latin America	2,354	2,123	2,398	2,456	2,276
Oceania	377	502	718	649	763
Africa	661	759	707	619	648
Middle East	481	410	303	332	502
Western Europe	350	407	303	334	448
Far East	248	368	247	216	206
Totals Free World	38,530	38,713	35,187	33,881	36,380
U.S.S.R.	3,000				
Other Eastern Europe	885				
Total E. Europe	3,885				
World Total	42,415				

1/ *World Oil*, August 15, 1970-1974.

This distribution shows that the bulk of the amount spilled is concentrated in very few of the total number of spills. For instance, about 96 percent of the total amount reported spilled is in just one spill [6].

Here we meet the difficulty of how to judge the occurrance of such very large events, either in a different period in the same region, or elsewhere in the world in the same period of time. First, it should be acknowledged that one occurrence in a five-year period provides a very flimsy basis on which to predict its recurrence. Also there is no firm hold on the upper side of the amount that could be spilled, nor on how exceptional the event may be. That being said, we can however proceed to speculate having a specific purpose in mind. Rather than to increase the size of the exceptional event, one could argue that it is more reasonable to somehow diminish the weight or consideration given to the exceptional event. And at this point a purposeful bias may be useful. What I propose to do is to include the exceptional event as a working hypothesis, and make forecasts on that basis of the amounts spilled. If under the hypothesis, which might be called a bias to exaggerate, the amounts to be spilled because of well accidents turn out to be not too significant in comparison of other sources of pollution, then the hypothesis would have been useful. On the other hand, if it were to turn out that the amounts to be spilled, under the exaggerating bias, were relatively too large then one would have to somehow judge the probability of the largest spill being smaller than the one observed.

The total amount reported spilled by the Oil and Gas Journal, as complemented from other sources, in the United States because of 1968-1972 well accidents is about 695,000 barrels. The total number of well completions in the same period is 143,103. This gives an average spill of

4.86 bbl of oil spilled per well completed.

However, a separate analysis should be made of onshore and offshore operations. The previously mentioned oil spill data for the United States, in barrels, are split in onshore and offshore as follows:

onshore	offshore
2,500	640,000-690,000 [7]
	12,000-20,000
	5,476
	4,000
	2,362
	240
	4.5
	3

This indicates that the cumulative oil spills for offshore for the 1968-1972 period, namely 660,000-720,000 bbl, is about two orders of magnitude larger than for onshore. Therefore, in relation to the 2,921 offshore wells which were completed in the United States in the same period, one

obtains:

236 bbl average oil spill per offshore well completed.

In 1972 for the world, with the exclusion of Communist nations which do not publish well statistics, the number of wells completed during the year was about 10,000 offshore, of which 965 were completed in the United States. If we assume an average spill of 240 bbl per offshore well completed, then one would obtain for the world an oil spill of about 2,400,000 bbl from offshore oil field operations for the 1972 level of activity.

An estimate could be made, in similar way, for onshore oil field operations. The number of wells completed onshore in the world in 1972 was about 32,641 of which 26,326 were in the United States. The reported rate of spill per onshore well for the United States in the period 1968-1972 was about 1/10 bb. In this manner one would obtain for the world that the oil spill from onshore oil field operations would be negligible in comparison to the offshore operations, for the 1972 level of activity. However, the lack of adequate reported data is an impairing factor. Perhaps the oil spills in the ocean are more noticeable, and have to be reported.

If one were to take the rate of oil spill per offshore well as indicative of the rate of spill of onshore wells, which may go unreported, one would obtain an oil spill figure of about 7,800,000 bbl from onshore oil field operations in the world.

Release of oil from tanker accidents. As was mentioned before, a large part of the world crude oil production enters international trade and is transported by ocean-going tankers. For instance, in 1972 of the 20.561 billion barrels of crude oil produced in the world, about 11.2 billion barrels were transported overseas. To move this amount of oil the world tanker fleet undertook thousands of trips during the year. A simple calculation, based on the number of vessels over 2,000 gross tons and their deadweight, indicates that the number of trips loaded with cargo was of the order of 27,000 during 1972.

A worldwide survey of tanker accidents for the period 1969-1973 undertaken by J. C. Card, P. V. Ponce, and W. D. Snider of the U. S. Coast Guard 8/ provides valuable statistics on these accidents. The polluting and non polluting accidents, classified as to type of accident, are summarized in Table 11. Of a total of 3,183 tanker accidents there were only 452 which were polluting.

The average of 90.2 polluting accidents per year should be viewed in relation to the average of 4,200 tankers in operation and their 27,000 loaded trips per year.

The oil outflow that resulted from these tanker accidents is summarized in Table 12. The average outflow per year for the 1969-1973 period is 1,395,900 bbl. As the average number of polluting tanker accidents per year was 90.2, then the average outflow per tanker accident

Table 11

NUMBER OF 1969-1973 TANKER ACCIDENTS IN THE WORLD [1/]

(Vessels greater than 2,000 GRT)

Accident	Non-polluting	Polluting	Total
Grounding	667	123	790
Collision	618	126	744
Structural failure	421	94	515
Ramming	427	46	473
Breakdown	344	11	355
Fire	180	17	197
Explosion	73	31	104
Others	1	4	5
	2,731	452	3,183

[1/] J. C. Card, P. V. Ponce, and W. D. Snider, U. S. Coast Guard Report, Tankship Accidents and Resulting Oil Outflows, 1969-1973, 1974.

Table 12

OIL OUTFLOW FROM 1969-1973 TANKER ACCIDENTS IN THE WORLD [1]

Accident	Outflow (bbl) [2]
Structural failure	2,486,000
Grounding	1,692,000
Collision	1,357,000
Explosion	695,000
Breakdown	219,000
Ramming	100,000
Fire	22,000
Others	402,000
	6,973,000

[1] J. C. Card, P. V. Ponce, and W. D. Snider, U. S. Coast Guard Report, Tankship Accidents and Resulting Oil Outflows 1968-1973, 1974.

[2] Metric tons converted to barrels by multiplying by 7.33.

was 15,466 bbl. Moreover, the average number of tankers in operation during 1969-1973 was 4200.2, so that the annual amount of outflow because of accidents was 332 bbl per tanker in operation. In this manner, we can estimate the amount of oil outflow for 1972 because of tanker accidents, for the average conditions in the 1969-1973 period. Considering that the number of tankers in operation during 1972 was 4,336, the expected amount of oil outflow would be 1,340,000 bbl.

Release of oil from pipeline accidents. Pipeline accidents have resulted in significant amounts of oil spilled throughout the world, as summarized in Table 13 from the Nielsen report, previously mentioned. The 1968-1972 totals for the world are:

onshore	62,000-71,000 bbl
offshore	248,000
	310,000-320,000 bbl

Thus the oil spill per year would be:

onshore	12,400-14,200 bbl
offshore	50,000 bbl
	62,000-64,000 bbl

Release of Oil from Natural Seeps

Natural oil seeps are known both onshore and offshore. Geologists look for them because they may give a clue of the presence of hydrocarbons in a basin. However, the search for offshore seeps is not complete, in particular as to the published record.

An ESSO team of investigators [9] estimated the probable range of the amount of oil discharged from submarine seeps in the world to be 1.5 to 44 million barrels (0.2 to 6.0 million metric tons) per year, with a "best" estimate of 4.4 million barrels (0.6 million metric tons) per year. The method of estimation was based on a worldwide classification of the likely geologic environments and a projection from the rate of known seeps.

One should add to this the oil reaching the oceans from seeps on land. As the petroleum prospective area onshore are about four times those offshore, we could take for the onshore oil seeps four times the previously mentioned estimates for offshore.

What fraction of this onshore oil seepage reaches the oceans is difficult to guess. Most onshore oil seeps are rather local events and with a small flowrate. The oil for the most part would seem to be weathered or be retained by local soils. This is probably because the lighter hydrocarbons tend to escape first, and what is left are the boiling, more viscous fractions.

Table 13

REPORTED PIPELINE OIL SPILLS 1968-1972 [1]

(bbl)

Onshore		Offshore	
US	Other world	US	Other world
6,785	50,000	25,000	100,000
	2,600-12,000	7,400	100,000
	2,500	6,000	
	one massive (?)	3,800	
		3,700	
		1,000	
		1,000	
		200	

[1] Nielsen, D. T., report in preparation.

Resumé of Ocean Oil Intake Estimates

The various estimates of the amounts of oil spilled for a 1972 level of activity in the world which I have made or analyzed in this report arranged in decreasing order of the amount of oil spilled, are summarized in Table 14. Also the estimate of the oil intake of the oceans because of subsurface seeps, made by Wilson et al (1973), is included in the Table. The amounts which reach the oceans from land sources are assumed to be 1/2 of the gross amounts on land, and are listed in the last column of Table 13.

These estimates show that natural oil seepages and spent lubricants account for about 60 to 90 percent of the total oil intake of the oceans. Offshore petroleum exploration and production activities contribute less than three percent of the total oil intake of the oceans. Most of the oil spill in offshore wells is burned, so that a small part of the amount spilled is left as ocean intake. However, even if we make the unfavourable assumption that all the oil spilled in offshore wells enters the oceans, it is seen that the contribution of offshore oil well accidents is small.

Regulation may still decrease the oil impact of the disposal of spent lubricants, of tanker operations, and of petroleum industry accidents.

The estimates which I have discussed, based on publicly available information, provide a perspective of the relative importance of various sources of overall ocean pollution.

Table 14

RESUME OF THE OCEAN-OIL-INTAKE ESTIMATES

(For a 1972 level of activity)

Source	Amount spilled ($\times 10^6$ bbl)	Ocean intake ($\times 10^6$ bbl)
Industrial spent lubricants	20.6	10.3
Automotive " "	13.3	6.5
Onshore oil well accidents	$\leq$7.8	<3.9
Tanker cleaning operations	2.6	2.6
Offshore oil well accidents	$\leq$2.4	<2.4
Tanker accidents	1.4	1.4
Aviation spent lubricants	0.6	0.3
Offshore pipeline accidents	0.05	0.05
Onshore " "	0.015	0.007
Natural submarine oil seepage		1.5-44
Natural onshore oil seepage	6 -176	?
	about	29-71
For comparison:		
Crude oil production, 1972	20,561	
Tanker transportation, 1972	10,000	

Appendix A

Basis for Estimation of World Lubricating Oil Waste

U.S.(1972):

lubricating oil demand = 0.88% of total petroleum products demand.

U.S.(1965):

		($\times 10^6$ bbl)
lubricating oil demand	industry	28.5
	automotive	23.4
	aviation	0.9
		52.8

Estimate, U.S.(1972):

		($\times 10^6$ bbl)
industry lubricating oil demand	for lubrication	19
	for processing	9.5
		28.5

Assumption:

Automotive lubricating oil demand ~ automotive fuel consumption

U.S.(1972):

		($\times 10^6$ bbl)
Automotive lubricating oil demand	passenger cars	16.4
	trucks and combinations	6.8
	busses	0.2
		23.4

Estimate, U.S.(1972):

		($\times 10^6$ bbl)
Automotive lubricating oil demand	burned or leaked	4.7
	residual	18.7
		23.4

Assumptions:

Lubricant demand for industry ~ (industrial + public) KWH

Lubricant demand for automotives ~ number of vehicles

Lubricant demand for aviation ~ jet fuel demand

References

1. Bureau of Mines, International Petroleum Annual, 1972, Tables 1 and 3.

2. Am. Petroleum Inst. and Am. Soc. of Lubrication Engrs., Industrial Oily Waste Control, 1970

3. Am. Petroleum Inst., Final Report of the Task Force on Used Oil Disposal, 1970, 144 pp.

4. J. D. Porricelli; V. F. Keith; and R. L. Storch; "Tankers and the Ecology, Trans. of the Society of Naval Architects and Marine Engineers, Vol. 79, 1971.

5. D. T. Nielsen, The 1968-1972 Accident Record of the Petroleum Industry as Interpreted from the Oil and Gas Journal, a report being completed.

8. Tankship Accidents and Resulting Oil Outflows, 1969-1973, 1974 report.

9. R. D. Wilson, P. H. Monaghan, A. Osanik, L. C. Price, and M. A. Rogers, "Estimate of Annual Input of Petroleum to the Marine Environment from Natural Marine Seepage", Trans. Gulf Coast Assoc. of Geol. Soc, 23rd Annual Convention, October 24-26, 1973.

Comments

6. Fire and blowout, Shell Platform B, Bay Marchand field, offshore Louisiana, December 1, 1970.

7. Of this amount, only 32,000-35,000 was spilled without being burned.

DISCUSSION

WEISS: Do you know of any specific locations where there have been large-scale spills many years ago, as indicated in your discussion?

GROSSLING: We have a complete list of locations. In Southern California alone there were probably 10 or 20, which occurred in the Coast Range area. In Mexico, for instance, there was an enormous spill in a coastal area many years ago, 1904.

SAGER: In this particular spill in Mexico or in the ones in California, are those land blowouts you are talking about which occurred 30 or 40 years ago?

GROSSLING: The one in Mexico occurred right on the coast (Dos Bocas, Lake Tamiahua). At one spot in California, for instance, the Lake View gusher, they had to build a dam to contain the oil. The dam broke, and there was a river of oil that reached the ocean. So, the bulk of the oil reached the ocean in both cases.

SAGER: When they reach the oceans, then, are they distributed globally, do you think, or would that be considered a local incident?

GROSSLING: I have not examined that aspect. The local impact is a very important one that requires a special, different type of analysis. When you look at the total picture, you see that the oil was absorbed into the system. It is not presently there in California. As I mentioned, there were many in Southern California in the 1910's through 1920's.

LASDAY: Would you give a further elaboration of the basis for your assumption that 50 percent is the figure that enters the ocean from land spills?

GROSSLING: That is very difficult. Actually, as I explained, I have not done any detailed analysis of that question. But if you looked at a map, for instance, for the locations of industrial centers or centers of population in the world, it is seen that most of them are close to the water. They are very close to the water's edge throughout the world. So, if anything should spill into a sewage system, it is usually drained into the ocean. The waste would move very quickly. So, the 50 percent figure is probably an underestimation. It is important to establish what happens if you dump a barrel of oil somewhere in the foothills of the Rockies, of course much less than 50 percent will reach the ocean.

GUZIAK: I had a similar question about your Table 14 concerning natural onshore oil seepage. To me it seems very high, and you also have no figure on the ocean intake for that.

GROSSLING: I have made estimates of the extent of sedimentary areas of the world. I have measured sedimentary areas worldwide, onshore and offshore, and I have taken simply the proportion of the sedimentary areas onshore to offshore. So, this is the only basis, and I said that in the text of the paper. If I accept that, I come to a very large figure. I have the same query that you have. So, the question, you see, gives two possibilities: one is that the figure for the offshore may be exaggerated; and, secondly, that there is a systematic geological reason for greater oil seepages offshore. Pursuing this question could reveal a systematic effect, and also something about the geology of the continental shelves.

WARD: You have had a difficult task estimating this worldwide release of oil into the oceans. It occurs to me that an equally useful figure might be an estimate of the amount of petroleum hydrocarbons entering the fresh water systems of the United States. I have a feeling that you are almost there and with a little extra work you might be able to estimate that, because the fresh water and the salt water systems are different.

WEISS: We are going to review some of those items. I think General Whipple will do that in his discussion on the inland sources. So, we will have a chance to get to that.

BRUBAKER: It is my understanding from what you said that perhaps the effects that the biologist might be concerned with would be due to something that may have occurred years and years ago in another era. Is this based on your mass balance type of calculations?

GROSSLING: The observation I made was more or less the following: that I found so many instances of massive leaks in the past 100 years, incidents of pollution, so to speak, at times when there had been no environmental concerns. I have been in many of these areas myself afterwards, and I don't recognize any sign of a "Death Valley," so to speak. So, I wonder whether these environmental effects really remain for more than a few years. In 50 years, certainly, they disappear completely. You don't find anything in Southern California or in Mexico. So, I thought that since these spills are very well documented, one could make a good environmental study of them. Really what has happened? You have an experiment already; they are all from the 1880's so to speak to the 30's and 40's. You have a whole sequence in different habitats. I was merely suggesting that it might be a good research topic.

BRUBAKER: Was there any consideration given to a recycling of any spent oils or cleanup of any oil accidents, this sort of thing?

GROSSLING: Right. Recycling was considered. 1972 was chosen as a base level of economic activity. Everything can be projected to later years simply by using indices which I have in here.

The United States, since it accounts for about half of the fleet of the world's automobiles, could do a great deal to improve the situation by recycling. Elsewhere it would be very difficult. People in other places are not as concerned about it.

THE STATISTICAL PICTURE REGARDING DISCHARGES OF PETROLEUM HYDROCARBONS IN AND AROUND UNITED STATES WATERS[1]

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

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Two statistical data bases regarding discharges of Petroleum Hydrocarbons are available as a basis for any study on the subject: The Coast Guard's Pollution Incident Reporting System and the National Academy of Science's statistical compilation.

The Coast Guard Data indicate over 13,000 pollution incidents annually within the United States and suggest two distinct pollution problems: the small chronic discharges and the infrequent major discharges with five sources accounting for 90 percent of the volume.

On a global basis, the 1975 National Academy of Sciences study, "Petroleum in the Marine Environment", found that about 45 million barrels of PHC entered the ocean annually.

The Coast Guard data provides a detailed U. S. budget while the NAS information provides the best global budget.

OVERVIEW OF EXISTING OIL POLLUTION BUDGETS

Any broad study of the input, fates, and effects of petroleum hydrocarbons (PHC) in the aquatic environment, whether it be a symposium or an environmental impact analysis, must have a reliable data base to work from. In the case of navigable United States waters, the Federal Water Pollution Control Act as amended by the Water Quality Improvement Act of 1970 provided the United States Coast Guard with authority to create a "Pollution Incident Reporting System" (PIRS) nationwide.[1] This system came into being in late 1971 and requires the reporting of all pollution incidents involving discharges of oil and other hazardous substances to

the closest appropriate Federal authority for inclusion in the central data base maintained at Coast Guard Headquarters. However, the PIRS system for petroleum hydrocarbons is restricted to U. S. waters and pertains only to non-continuous discharges that create a visible signature on the water's surface. Obviously, there are many other types of input of petroleum hydrocarbons into the aquatic environment, e.g., from atmospheric fallout, industrial and municipal outfalls, tanker operation on the high seas, and natural seeps. Acquisition of this broader type of input data is, of course, much more subjective than in the case of the pollution incident data incorporated into the PIRS system by force of Federal law.

If one omits mention of the numerous and comprehensive studies of oil spill statistics incorporated in the evolving "gray literature" of environmental impact analyses, it can likely be said that there have been four definitive studies published in the past six years regarding the influx of petroleum hydrocarbons into the ocean as a whole. These were generated by the Summer Study of the Massachusetts Institute of Technology entitled, "Man's Impact on the Global Environment" (MIT, 1970),[2] the University of Oklahoma technological assessment of outer continental shelf oil and gas operations (Kash, 1973),[3] the U. S. Coast Guard (1973)[4] environmental impact statement for the upcoming International Convention for Prevention of Pollution from Ships, and the National Academy of Science study (Wilson, 1975)[5] of petroleum in the marine environment. Bates and Pearson (1975)[6] have analyzed these four global budgets and concluded that the study accomplished between 1972 and 1974 and edited by Wilson (1975) for the National Academy of Science (NAS) is probably the most reliable. At first glance, it might be concluded that the Coast Guard's PIRS system and the National Academy of Science's statistical compilation of PHC inputs might be easily correlated one with the other. After all, much of the background data used in the NAS effort was derived from United States data and multiplied by a factor of 3.3 to give the global picture in view of the fact that the United States uses about 30 percent of the world's output of petroleum. Unfortunately, the two statistical data bases do not mesh directly for they have been assembled for separate reasons and differ in scope and type of reporting. Accordingly, for the purposes of this conference, the remaining portion of this paper is restricted to delineating the nature of these data bases in order that workers in the broad field of petroleum pollution may be familiar with just what these statistical assemblages bases do--and do not--provide.

THE NAS BUDGET OF PETROLEUM IN THE MARINE ENVIRONMENT

The NAS PHC budget was derived by a 13-man team consisting of geochemists, engineers (naval, sanitary, chemical and petroleum specialties), and geologists from four different countries (United Kingdom, Canada, Sweden, and the United States). Although most of the panel's work was done in mid-1973, the panel primarily used

world petroleum production and transport statistics for 1971, supplemented by domestic oil spill data for the 1970-1972 period. The panel concluded that approximately 6.1 million metric tons of petroleum hydrocarbons entered the ocean from all sources each year. This budget breaks down as follows:

TABLE I

BUDGET OF PETROLEUM HYDROCARBONS ENTERING THE OCEAN*

SOURCE: INPUT RATE (mta)	Best Estimate	Probable Range
Man-Made:		
Marine Transportation		
LOT Tankers	0.31	0.15 - 0.4
Non-LOT Tankers	0.77	0.65 - 1.0
Dry Docking	0.25	0.2 - 0.3
Terminal Operations	0.003	0.0015 - 0.005
Bilges/Bunkering	0.5	0.4 - 0.7
Tanker Accidents	0.2	0.12 - 0.25
Non-Tanker Accidents	0.1	0.002 - 0.15
TOTAL	2.1	
River Runoff	1.6	----------
Atmospheric Rainout	0.6	0.4 - 0.8
Urban Runoff	0.3	0.1 - 0.5
Coastal Municipal Wastes	0.3	----------
Coastal (Non-Refining)		
Industrial Wastes	0.3	----------
Coastal Refineries	0.2	0.2 - 0.3
Offshore Production	0.008	0.08 - 0.15
Natural:		
Offshore Seeps	0.6	----------
GRAND TOTAL	6.113	

*NOTE: Data Source is Table 1-5 of NAS Study (1975)
mta: millions of metric tons per annum
LOT: Loan-On-Top Tankers Equipped with Slop Tanks

This estimated value of 6.1 million metric tons of PHC entering the ocean annually also approximates the amount of biogenic hydrocarbon now believed to be forming locally in the ocean each year as the

result of planktonic activity. The NAS budget can also be broken down either in gallons or by major contributor percentage-wise as follows:

TABLE II

CONTRIBUTORS TO THE INTRODUCTION OF PETROLEUM HYDROCARBON INTO THE OCEAN

CONTRIBUTOR	PERCENT OF TOTAL CONTRIBUTION (APPROXIMATE)	MILLIONS OF U.S. GALLONS/ANNUM*
Marine Transportation	34	656
River Run-Off	26	493
Atmospheric Rainout	10	185
Natural Seeps	10	185
Municipalities	10	185
Industrial Wastes	8	154
Offshore Production	2	25
TOTAL		1,883

*Assumes 308 gallons (U.S.) to 1 metric ton of crude oil

Just how valid are these PHC pollution statistics today in view of the fact that they are basically derived from data at hand in the 1970-1972 period? Much has happened since then--there has been a drastic lay-up of tankers due to the tripled price of crude oil; several very large crude carriers, such as the METULA, have gone aground; major tanker operators have begun introducing the "crude-washing" technique to replace the "water-washing-down" technique for cleaning tanker walls and bottoms; the Intergovernmental Convention for the Prevention of Pollution from Ships (Gray, 1975)[7] will ultimately drastically limit the amount of oil that can be pumped overboard during an entire cruise, and there has been increased operator, enforcer, and social awareness of the need to curtail both intentional and accidental discharges of PHC into the ocean. As a consequence of these and other related considerations, such as the introduction of "oil fingerprinting" and automobile catalytic converters, it can be postulated that the approximate value of 6.1 million metric tons of PHC entering the ocean during the early 1970's will probably prove to be close to the peak annual load before this load starts tapering off to the 4.57 million metric ton annual load postulated by the NAS study for the early 1980's. But what does the Coast Guard's PIRS system show to be actually happening in the United States within the 1971-1974 time frame?

THE POLLUTION INCIDENT REPORTING SYSTEM (PIRS) OF THE COAST GUARD

This system, which has been undergoing continual upgrading since inception in 1971, is described in full within the Coast Guard Manual, CG-450 (1976). As shown in Table III, the system generates data relating to actual accidental or intentional discharges of oil and hazardous substances, the nature of the response and clean-up activities (including their cost), and the penalty actions taken.

Evidence to date suggests that fully 90 percent of the nation's pollution incidents are now being reported to this system. As a result, the system not only assists in measuring the effectiveness of the Coast Guard's Marine Environmental Protection Program (Leotta and Wallace, 1973;[8] U. S. Coast Guard, 1975)[9] but also allows appropriate response to frequent inquiries from the Congress, industry, academic institutions, and the public.

POLLUTION INCIDENTS IN AND AROUND U. S. WATERS DURING 1974*

During calendar 1974, the PIRS system acquired data on 13,966 pollution incidents involving 16.9 million gallons of materials of which 93 percent was petroleum hydrocarbon as indicated in Table IV. The 1973-1974 data suggest that 90 percent of the volume of oil discharged into U. S. waters via pollution incidents during this time frame has been included in the resulting data base. These data suggest that there are two distinct pollution problems: the small chronic discharge and the infrequent but major discharge. Five sources account for over 90 percent of the volume discharged in major pollution incidents, namely: pipelines, tank ships, tank barges, marine facilities, and onshore production and storage facilities. Tables V, VI and VII present the statistics for drawing these conclusions. Area-wise and as indicated in Table VIII, the Gulf Coast experiences 32 percent of the incidents, followed by the Atlantic Coast experiencing 25 percent of the incidents. Coastal waters experience fully 70 percent of the incidents, as shown in Table IX, while inland waters and the contiguous zone experience 20 percent and 9 percent of the incidents, respectively. High seas incidents were minor during 1974, being only 1 percent of the cases. Volume-wise, however, inland waters experienced more spill in terms of gallonage than did coastal waters, the two values being 8.9 million and 7.9 million gallons, respectively.

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*U. S. waters are defined as being U. S. navigable waters out to three miles from the coastline, into the tributaries of these navigable waters, into the contiguous zone extending from 3 to 12 miles offshore, and into waters or onto shorelines such as to threaten the navigable waters or the contiguous zone of the United States.

TABLE III

CATEGORIES OF THE POLLUTION INCIDENT REPORTING SYSTEM

DISCHARGES	RESPONSES	PENALTY ACTION
Time of Occurrence	Record Identification	Record Identification
Location	Transaction Code	Transaction Code
State	Removal Undertaken By	Card Number
Water Body	Equipment	No Coast Guard Penalty Action - Reason
Source	Personnel	Initiating Agency
Source Identifier	Duration of Response	Authority
Cause	Amount Recovered	Action Taken Against (Party)
Operation	Cost of Cleanup	Action Date
Material		Referral to U. S. Attorney
Quantity		Referral to Commandant/Other Agency
Affected Resources		Action by U. S. Attorney
Weather Notifier		Penalty, Fine, or Settlement Assessed
Anticipated Response		Imprisonment
		Suspension, Revocation, or Probation
		Hearing or Trial
		First Appeal
		Second Appeal
		Civil Action Appealed to U. S. Court
		Penalty, Fine, or Settlement Collected
		Case Closed

TABLE IV

TYPE OF MATERIAL DISCHARGE, 1974

	Number of incidents	% of total	Volume in gallons	% of total
Crude oil	3,639	26.0	9,028,262	53.0
Gasoline	545	4.0	1,045,603	6.0
Other distillate fuel oil	322	2.0	1,824,130	11.0
Solvent	44	0.0	13,114	0.0
Diesel Oil	1,833	13.0	1,120,862	7.0
Asphalt or residual fuel oil	1,127	8.0	1,908,752	11.0
Animal or vegetable oil	57	0.0	27,316	0.0
Waste oil	1,094	8.0	111,900	1.0
Other oil	2,774	21.0	728,497	4.0
Liquid chemical	222	2.0	913,027	5.0
Other pollutant (Sewage, dredge, spoil, chemical wastes, etc.)	162	1.0	31,792	0.0
Natural Substance	105	1.0	1,528	0.0
Other Material	199	1.0	104,709	1.0
Unknown material	1,843	13.0	56,816	0.0
TOTAL	13,966	100.0	16,916,308	100.0

TABLE V

POLLUTION INCIDENTS
DISCHARGE VERSUS SIZE (1974)

Volume	Number of Incidents	% of Total	Volume in Gallons	% of Total
Unknown	3,598	26.0	-	0.0
0-49 gal.	6,984	50.0	66,457	5.0
50-99 gal.	787	6.0	49,710	5.0
100-499 gal.	1,342	10.0	276,589	2.0
500-999 gal.	341	2.0	219,689	1.0
1000-2499 gal.	366	3.0	563,797	3.0
2500-4999 gal.	189	1.0	664,862	4.0
5000-9999 gal.	158	1.0	1,059,494	6.0
10,000-49,999 gal.	142	1.0	2,859,652	17.0
50,000-99,999 gal.	27	0.0	1,831,129	11.0
100,000-999,999 gal.	30	0.0	7,056,929	42.0
Above 1 Million gal.	2	0.0	2,268,000	13.0
TOTAL	13,966	100.0	16,916,308	100.0

TABLE VI

SOURCES OF POLLUTION INCIDENTS (1974)

	Number of Incidents	% of Total	Volume in Gallons	% of Total
VESSELS				
1. Dry cargo ships	346	2.0	89,717	1.0
2. Dry cargo barges	31	0.0	1,270	0.0
3. Tank ships	973	7.0	1,434,168	8.0
4. Tank barges	833	6.0	2,468,724	15.0
5. Combatant vessels	278	2.0	39,552	0.0
6. Other vessels	1,265	9.0	253,007	1.0
TOTAL	3,726	26.0	4,286,438	25.0
LAND VEHICLES				
1. Rail vehicles	51	0.0	453,964	3.0
2. Highway vehicles	294	2.0	313,943	2.0
3. Other/unknown vehicles	28	0.0	17,641	0.0
TOTAL	373	2.0	7,855,480	5.0
NON-TRANSPORTATION-RELATED FACILITIES				
1. Onshore refinery	155	1.0	772,634	5.0
2. Onshore bulk/storage	281	2.1	1,011,543	6.0
3. Onshore production	383	3.0	877,010	5.0
4. Offshore production facilities	2,006	14.0	153,771	1.0
5. Other facilities	819	6.0	653,148	4.0
TOTAL	3,644	26.0	3,468,106	20.0
PIPELINES	557	4.0	6,205,039	36.0
MARINE FACILITIES				
1. Onshore/offshore bulk cargo transfer	367	4.0	1,286,289	8.0
2. Onshore/offshore fueling	93	1.0	35,946	0.0
3. Onshore/offshore nonbulk cargo transfer	41	0.0	6,569	0.0
4. Other transportation-related marine facility	98	1.0	3,538	0.0
TOTAL	599	6.0	1,332,342	8.0
LAND FACILITIES	200	1.0	235,209	1.0
MISC/UNKNOWN	4,867	35.0	603,626	4.0
TOTAL	13,966	100.0	16,916,308	100.0

TABLE VII

CAUSES OF POLLUTION INCIDENTS (1974)

	Number of Incidents	% of Total	Volume in Gallons	% of Total
Hull/tank rupture/leak	952	0.7	4,861,431	29.0
Transportation pipeline rupture/leak	286	0.2	4,519,102	27.0
Other structural failure	269	0.2	196,517	0.1
Pipe rupture/leak	1,499	11.0	2,302,546	14.0
Hose rupture/leak	268	0.2	207,507	0.1
Valve failure	510	0.4	367,109	0.2
Pump failure	184	0.1	127,435	0.1
Other rupture/leak	39	0.0	9,265	0.0
Other equipment failure	1,409	10.0	605,461	0.4
Tank overflow	1,094	0.8	1,590,014	0.9
Improper valve operation	176	0.1	210,199	0.1
Improper hose handling	144	0.1	10,643	0.0
Other improper equipment handling/operation	342	0.3	94,666	0.1
Other personnel error	571	0.4	354,053	0.2
Bilge pumping	290	0.2	49,503	0.0
Ballast pumping	31	0.0	2,364	0.0
Other intentional discharge	316	0.2	292,193	0.2
Natural or chronic phenomenon	380	0.3	241,410	0.1
Unknown	5,206	37.0	858,086	0.5
TOTAL	13,966	100.0	16,916,308	100.0

TABLE VIII

GENERAL AREAS OF POLLUTION INCIDENTS (1974)

	Number of incidents	% of total	Volume in gallons	% of total
Atlantic Coast	3,517	25.2	3,028,599	18.0
Gulf Coast (West of Long. 83° 15')	4,470	32.0	3,864,219	23.0
Pacific Coast	2,715	19.4	493,454	3.0
Great Lakes	449	3.2	599,252	4.0
Inland U. S.	2,815	20.2	8,923,639	52.0
TOTAL	13,966	100.0	16,916,308	100.0

TABLE IX

TYPE OF LOCATION, POLLUTION INCIDENTS (1974)

	Number of Incidents	% of Total	Volume in Gallons	% of Total
Inland Waters				
1. Roadsteads	225	2.0	126,156	1.0
2. Ports	617	4.0	404,499	2.0
3. Beaches	250	2.0	2,510,414	15.0
4. River areas	826	6.0	1,472,486	9.0
5. Non-navigable areas	897	6.0	4,415,172	26.0
TOTAL	2,815	20.0	8,924,727	53.0
Coastal Waters (including Great Lakes)				
1. Bays, estuaries and sounds	2,519	18.0	321,928	2.0
2. Ports	4,783	34.0	5,432,169	32.0
3. Beaches	297	2.0	174,551	1.0
4. River areas	1,681	12.0	1,571,627	9.0
5. Non-navigable areas	223	2.0	302,237	2.0
6. Open Waters (Great Lakes or territorial sea)	251	2.0	108,016	1.0
TOTAL	9,754	70.0	7,907,828	47.0
Contiguous zone	164	9.0	24,706	0.0
High Seas	1,233	1.0	52,347	0.0
TOTAL	13,966	100.0	16,916,308	100.0

TRENDS IN THE OCCURRENCE AND NATURE OF POLLUTION INCIDENTS IN AND AROUND U. S. WATERS BETWEEN 1971 AND 1974

An introductory macro-view of oil pollution trends is given in Table X for the calendar years 1971 through 1974. It is important to reiterate that these are reported discharges only. Reported pollution incidents are a suitable surrogate for actual pollution incidents. For example, the sizeable increase in the number of reported discharges between 1972 and 1973 is largely caused by the more stringent reporting requirements and penalties introduced by the 1972 Amendments to the Federal Water Pollution Control Act. Volume statistics must also, like most statistics, be handled with care. For example, in 1972, almost half of the total volume reported was the result of the flooding of the Schuykill River in Pennsylvania by Hurricane Agnes. Also of interest is the skewness resulting from 76 percent of all reported incidents accounting for only 1 percent of the total spill volume. In fact, the actual number of incidents that should have been reported is probably an order of magnitude greater than shown by the data for who knows how many quarts of waste motor oil go down sewer and storm drains during a given year? Nevertheless, there does appear to be a leveling off in both the number of spills and the volume discharged between 1973 and 1974. The 1975 data will be available shortly to either confirm or deny this conclusion for the 3-year trend.

TABLE X

POLLUTION TRENDS FOR PHC DISCHARGES, 1971 - 1974

CATEGORY	1971	1972	1973	1974
Number of Oil Discharges	7,522	8,380	11,003	11,440
Volume of Oil Discharges (US Gallons)	8,635,000	16,765,000	15,143,000	15,802,000
Average Volume Of Discharge (US Gallons)	1,148	2,000	1,349	1,381

SUMMARY

Needless to say, the data base chosen to work from must be carefully selected with the objective in hand and a full understanding of the character of the data and its collection.

Even though the NAS figures cannot be supported by the attempted correlation of other data bases they provide a useful understanding of the total problem of oil pollution.

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DISCUSSION

WEISS: In one of the charts, you spoke of oil spill fingerprinting as being effective. Would you like to say a few words about how you use oil spill fingerprinting?

BATES: Well, we have found that about 30 percent of the oil spills reported to Washington have been mystery spills, namely, nobody would own up to them, and so we had to go ahead and clean them up. The Key West spill is the famous one, and that was a case in which we were able to get a match which we think is far better than 99 percent. The key on this one is whether the spiller has to clean it up. Only the chemistry could have provided a true match.

SIVA: You mentioned that you were collecting information on response, spill response, and on the effectiveness of such information on refining spill response plans. I am wondering if you have any data on the effects of different cleanup techniques in various marine environments?

BOYD: Yes, we do. We have a section in the Marine Environmental Protection Program that deals specifically with response. By contacting us we can give you reams of information on that.

GRIFFITH: Effective December 1, we will report to PIRS [Polluting Incidents Reporting System] on any spills on navigable waters, and we have been informally advising the EPA on inland spills where they furnish the on-scene coordinator, at the same time that we have been reporting to the Coast Guard. Will the Coast Guard then assume the responsibility, or shall we continue to communicate directly with the EPA?

BOYD: EPA refers their cases to us. The PIRS and the SPCC [Spill Prevention Control and Countermeasures] system involve our working together. We have an EPA liaison person right in our office.

GALLER: Dr. Bates or Ms. Boyd, perhaps even earlier Dr. Grossling, do you know of any work that is under way, as part of your statistical manipulations, to develop biological equivalency values? I have heard some very interesting data so far about gallons, barrels, tons of oils that get into the aquatic environment from various sources, but in one sense they are not really terribly informative except in the grossest possible way.

Obviously, certain kinds of hydrocarbons have a biological importance greater than other kinds of hydrocarbons, depending on chemical constituents, location of the spill, et cetera. Is there enough of a data base now to start working on biological equivalence values along with the pounds and gallons and tons?

BATES: I think I know what you are after, Dr. Galler. It is something you and I talked about a lot, namely: Is there some sort of biological indicator that you can introduce at the site of a spill to develop, as you say, some idea of biologic impact on a spill? Frankly, we are not as far along in that area as I hoped we were. We are still primarily using the eyeball as

the prime sensor for when to quit cleaning up a spill. This is the thing, at least, in the Coast Guard that makes the Commandant and others wonder whether we are using quite the right indicator.

Right now the law says that we are to restore the area to its original condition, and that means we can clean a bird on the National Pollution Cleanup Fund, but we cannot buy a new bird. So, there still needs to be some clarification of responsibilities, but actually EPA has had the biologic part of the spill business.

WEISS: That is a very good question, and perhaps it could be asked again, as we go through the biological aspects.

LASDAY: In your references to the inputs to the oceans you have had two items on which I would appreciate a little further elaboration. The first was that rainfall accounts for about 10 percent of the input of hydrocarbons into the oceans. Are there any experimental data which are the basis of that, or is this just pure hypothesis? Of course there are photochemical mechanisms that are going to be working on those hydrocarbons that do get into the atmosphere. As a parallel to that, biological activity is a very potent degrading mechanism. I wonder if this is factored into the estimates of the amounts of hydrocarbons that actually reach the oceans from the inputs to the rivers.

BATES: In the case of fallout, if you look at the National Academy of Sciences report that was published early last year, you will find the references we used. There is some literature, but considering the importance of the area, it was rather surprising. In some of the earlier estimates, for example, the MIT study in 1970 actually used a figure 10 times higher than we ended up using for the reasons you are pointing out. We believe that there is a lot of degradation of hydrocarbons in the air by solar radiation. As far as river data are concerned, again our data were primarily from rivers like the Mississippi and the Rhine and a few others, near their mouths.

HYDROCARBON IN SEWAGE AND URBAN RUNOFF - DELAWARE ESTUARY

by

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ABSTRACT

Research has been conducted to identify and measure the sources of hydrocarbons entering the Delaware Estuary. The most important sources of petroleum pollution have been industrial wastes; but currently mandated treatment measures will greatly reduce such outputs, especially those of petroleum refineries. Municipal sewage effluent has been sampled and analyzed. Methods have been developed to sample storm runoff from urban areas at frequent intervals and to use the analysis of these samples to estimate total annual petroleum loadings. Major petroleum pollution above water supply intakes is hazardous. Evaluation of sources appears likely to change pollution control strategy.

SCOPE OF RESEARCH

This paper reports on an investigation of sources of petroleum through an NSF-RAN research project "The Petroleum Industry in the Delaware Estuary," now in its third year*. The program of controlling water pollution from petroleum, like other water pollution control, has been directed almost entirely towards point sources, in this case, mainly the petroleum refineries. Through the usual process of NPDES permits, the refineries have been induced to construct treatment plants which will very largely reduce the present output of petroleum pollution to the estuary. The research described has been so as to characterize and estimate all of the principal sources of petroleum including petroleum in urban runoff and various effluents.

PETROLEUM REFINING

The Delaware Estuary is a major petroleum refining area. Its seven refineries process about a million barrels of crude oil daily.

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* Grant Env. 74-14810 A03. The project director is Wm. Whipple, Jr.. The chief scientist is Dr. Ruth Patrick, of the Philadelphia Academy of Natural Sciences. Drs. J. V. Hunter and S. L. Yu are among the principal investigators.

At the present time, the refineries release about 27,000 pounds of oil and grease daily in their process waters, which amount is expected to be reduced to about 2000 pounds when presently planned waste treatment processes are completed.*

A number of samples taken from the effluent of the ARCO refinery in Philadelphia were analyzed by methodology indicated in the next paragraph following, by means of which particulate and soluble fractions were each analyzed for content of aliphatic, aromatic and polar material. The sum of the aliphatic and aromatic material from both fractions, taken as measuring the hydrocarbons, averaged 1.38 mg/l for the seven samples analyzed in 1976, with a range from .33 to 3.23, Eight samples taken during 1974 and 1975 averaged 2.02 mg/l. Since the effluent permit is based on an effluent limitation of 10 mg/l, it may be seen that this treatment plant is performing very well. This analysis indicates the probability that the seven refineries should be able to meet their present effluent limitations readily. It has been proposed by officials of the State of New Jersey that this effluent limitation should be reduced from 10 mg/l to 1 mg/l. This proposal is now in abeyance, and should be considered in perspective with other sources of petroleum pollution.

ANALYTICAL PROCEDURES

The method used for analysis was designed so that it would be equally applicable to stormwaters and to effluents. To accomplish this it was first necessary to separate the hydrocarbons, or oil, associated with the suspended matter in effluents and stormwaters from constituents which might be considered "soluble." This was done by passing the water through a continuous Sharples Super Centrifuge at a flow rate of 100 ml/min and operating at 15,000 rpm. This will remove particles down to 1μ.

The particulates are removed from the centrifuges and dried with anhydrous $MgSO_4$. The "soluble" hydrocarbons are then passed through an activated carbon column (approximately 40 grams of carbon per 5 gallons of sample). The carbon is then dried under slightly reduced pressure. At this point in the analysis it is possible to treat both the "soluble" constituents adsorbed on carbon and the $MgSO_4$ dried particulate oil in an analogous manner. This consisted of successive extractions with hexane, benzene, and a 1/1 methanol/chloroform mixture. This results in three extracts for the soluble portion and three extracts for the particulate portion.

Since these extracts contain material other than hydrocarbons, it is next necessary to separate the hydrocarbons from the other solvent soluble material in the extracts. This was accomplished using the activated silica column chromatographic procedure of Rosen and

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* One of the refineries also releases large but indeterminate quantities of oil and grease in its "cooling water," which is not included in the above estimates, either present or future.

Middleton[2]. This consists of dissolving the extract in hexane, putting it into an activated silica column, and successively eluting it with hexane, benzene, and a 1/1 methanol/chloroform mixture. This procedure is designed so that the aliphatic (saturated) hydrocarbons are eluted with the hexane, the aromatic hydrocarbons with the benzene, and polar materials (relatively high molecular weight esters, alcohols, acids, etc.) with the chloroform methanol mixture. The solvent is then evaporated under reduced pressure, and the weight of the hydrocarbon residue is measured. This weight, plus the sample volume employed, allowed immediate conversion to concentration in units of mg/l. For the stormwater samples involving analysis of each sample obtained, the flow data which were known for each sample were employed to calculate a weighted average concentration. The composite stormwater sample and refinery effluent samples were accepted as composites representative of flow.

This procedure defines petroleum oil as the hydrocarbons isolated by the chromatographic procedures; that is, the sume of the aliphatic and aromatic fractions.

OTHER INDUSTRIAL SOURCES

A check was made of NPDES* permits of 45 other major industries bordering the estuary. In some cases the figures were revised after clarification by the permittees. The present load of oil and grease in effluents is estimated as about 12,000 pounds per day. The total future load is expected to be a little over 8000 pounds, of which 4900 would be from a single industrial plant. A major qualification in use of these figures is that they incorporate revised estimates for one large plant based upon the use of new analytical technology**. The figures cited in the official permit for that plant were many times larger, both for present and future loadings.

For most of the plants the required reductions of oil and grease are not as great as has been required by the permits for the oil refineries. It should be noted that the limitations of "oil and grease" apply mainly to hydrocarbons in some cases, and in other cases may include various other kinds of fats and oils.

TRIBUTARY PETROLEUM LOADINGS

Samples were taken of the Lower Schuylkill River, during a winter period (February 1976), when the discharge at the gage upstream varied between 2740 and 6000 cfs, the annual average being 2789. The average concentration of hydrocarbons was .23 mg/l. If this concentration were representative of the mean flow year around, the annual average loading from the Schuylkill River would be 3400 pounds a day. However, the point of sampling is adjacent to two oil refineries and probably includes petroleum from their effluents. Moreover, it may include

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* "National Pollution Discharge Elimination System," administered by EPA.

** Extraction with "Freon" -113, followed by infrared analysis.

benthal demand resulting from past oil spills.

Another much smaller tributary which drains part of Trenton, Assunpink Creek, was sampled during two storm events. It drains 91 square miles of which 22% is forest, and 54% cropland. The mean concentration at the mouth during the smaller storm was .67 mg/l, and during the larger storm 1.58 mg/l. The loadings of hydrocarbons during these two storm events were 235 pounds and 3690 pounds respectively. Low flows in this creek have a relatively small petroleum content.

SEWAGE PLANT EFFLUENTS

Practically all of Trenton's wastes discharge into a single waste treatment plant which has an average daily flow of just under 20 mgd. The plant provides secondary treatment with fast-rate trickling filters, but only removes 60% of the biological impurities because of heavy fouling from industrial wastes. On three occasions, multiple samples were taken in a bucket from a highly turbulent trough, and immediately transferred into glass or pyrex containers. The total hydrocarbons averaged 1.06 mg/l from the particulates and .43 mg/l from the soluble fraction, a total of 1.49 mg/l. For a plant of 20 mgd, this amounts to 248 lbs/day.

Probably the main significance of this sewage treatment plant hydrocarbon loading is that this plant discharges its treated wastes into waters which are later treated for municipal use, including the process of chlorination. The much larger sewage plants of Camden and Trenton, some of which give advanced primary treatment only, add additional loadings; but the Trenton plant is the only one upstream of a municipal water supply intake.

URBAN RUNOFF

The first investigation of urban runoff in Northern Philadelphia undertaken covered a 13 foot diameter storm sewer which drains 1520 acres of fairly well kept, mainly residential neighborhood. A staff gage was first set and calibrated by measuring flows and corresponding gage heights during several storms, at a point in the channel below the end of the sewer. The average concentration of the hydrocarbons detected during three storms ranged from 2.18 mg/l to 4.04 mg/l, with no obvious relationship between runoff volume and hydrocarbon concentration. The storm producing the smallest runoff volume also produced the highest average hydrocarbon concentration, but the lowest average hydrocarbon concentration was produced by the storm producing the intermeediate runoff volume. About 79 percent of the total hydrocarbons were associated with the particulate fraction. Most of the hydrocarbons were aliphatic (i.e., saturated hydrocarbon) averaging about 66 percent of the total.

Each of the three storms during which stormwater runoff was studied was quite different in duration and in the shape of the

stormwater hydrograph. These differences may be outlined as follows:

Date	Duration, Seconds	Description
9/3/74	4500	One major peak at 1800 seconds.
10/16/74	7500	Two approximately equal peaks, one at 1200 seconds and one at 2600 seconds.
4/7/75	5700	Two peaks, a smaller peak at 900 seconds and a much large peak at 3900 seconds

An example of one of the storm hydrographs is presented in figure 1. Since the hydrocarbon concentration is known at each point on the hydrographs for the first two storms sampled, a plot can be made of hydrocarbon loading (pounds/second) as a function of time. An example of such a curve is presented in figure 2. As might be expected, suspended solids loading follows the flow rather well. Hydrocarbon loading is more variable, but tends to peak at peak flow. If the areas under these curves are integrated, the total number of pounds of hydrocarbons discharged during the storm can be calculated. In the case of the final storm, the sample represented a flow composite, obtained by taking for analysis a portion of each sample proportionate to the discharge at the time the sample was taken from the river. The loading can be calculated from the stormwater runoff volume and concentration. When this is done, the following results are obtained:

Date	Volume Million Gallons	Pounds Discharged	Pounds per Million Gallons
9/3/74	3.53	77.9	22
10/16/74	1.57	28.6	18
4/7/75	0.46	15.4	33

In view of the extreme variability of the quantities measured, it is interesting to note from the last column that the petroleum contents per million gallons of flow for all three storms show so little variation.

The latest urban runoff data to become available covers two storms on the lower portion of the Assunpink Creek, with drainage in Trenton, totalling 2.38 square miles. It is highly urbanized, with 33% single family housing, 3% multiple family, 26% industrial and 22% commercial development. The petroleum pollution data were

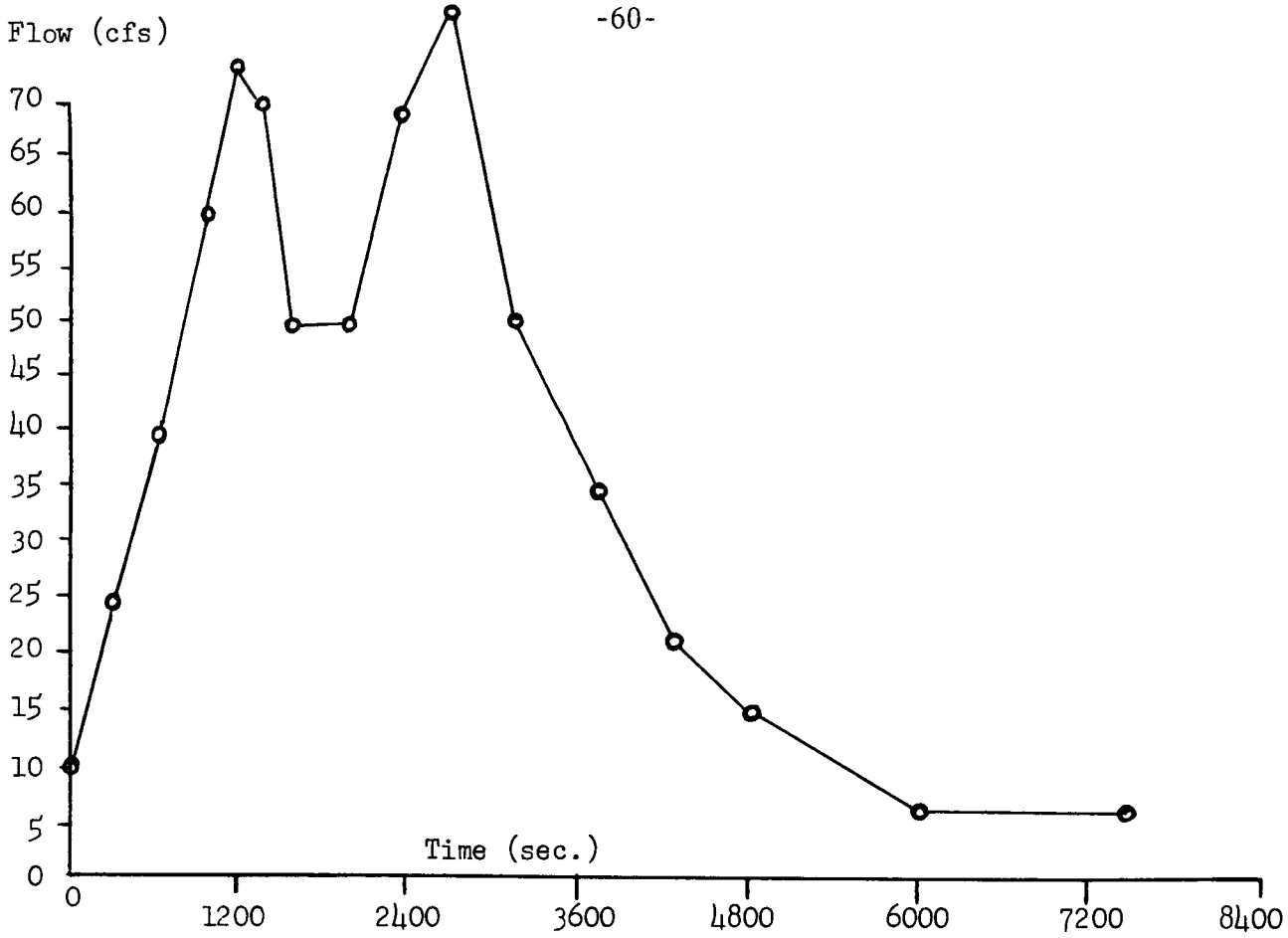

FIGURE 1. Storm 10/16/74

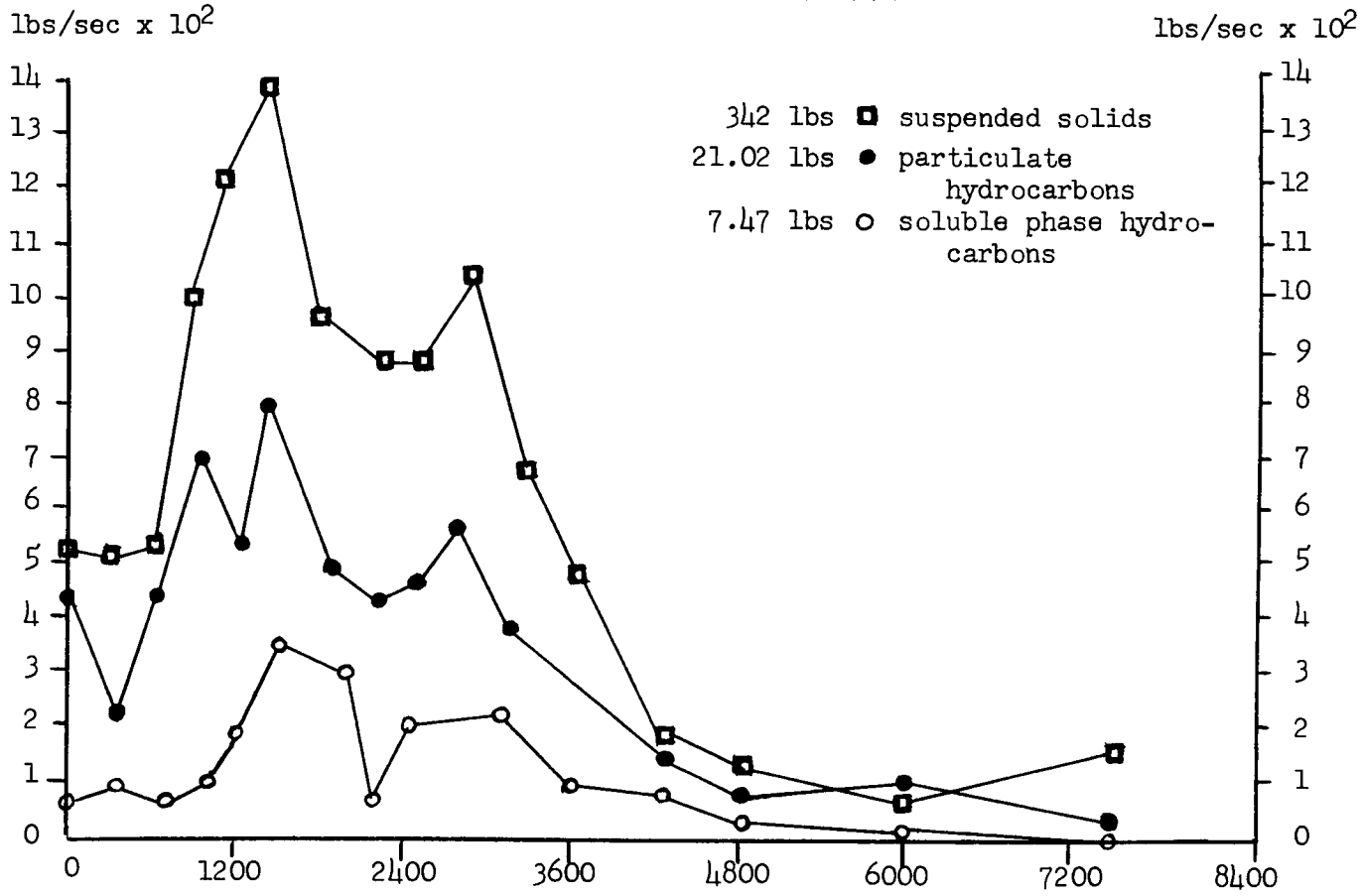

FIGURE 2. Storm 10/16/74

obtained from three sampling points, an upper and a lower point, on the creek, and a third station near the mouth of Pond Run, a tributary which enters between the first two stations.* Numerous observations were taken during each storm; and the flow and petroleum loading at the upper station and at Pond Run were subtracted from the lower station. There is considerably less accuracy to this method of measuring the pollution loading, as compared to sampling at single point, since the difference has to be obtained between two large loadings each with a considerable probable error. Obviously, the inherent probability of error in computing the difference is considerably greater than that of each of the individual estimates. The results for these two storms indicated concentrations and loadings as follows for the urban area between the sampling points

Date	Storm flow Million Gallons	Pounds Loading	Mean Concentration
6/5-6/75	160.7	2932**	2.19 mg/l
10/7/75	20.6	109	0.64

In this case by far the greater concentration of hydrocarbons occurred with the greater storm.**The larger storm was sampled at two points only; therefore the area included the entire Pond Run drainage as well as the wholly urban area covered by the other.

A preliminary estimate of the petroleum loading from stormwater runoff in the Delaware Estuary has been made. This was based on the weighted mean concentration of petroleum of 2.63 mg/l for the large storm sewer and 2.01 mg/l for the Assunpink. The average concentration is 2.32 mg/l. To estimate the runoff volume, the following equation was used:

$$D = C_p + (C_i - C_p) \sum_{i}^{L} X_i F_i$$

in which

C_p = runoff coefficient for previous areas
C_i = runoff coefficient for impervious areas
X_i = area in land use i as a fraction of total drainage area
F_i = fraction of land use i that is impervious
L = total number of urban land uses

* For one storm the Pond Run Station was not sampled. Therefore a larger area was included.

Using values suggested in current references, a C value of 0.65 was obtained for the sewer area. For a mean rainfall of 43 inches expected annually for the Philadelphia area, a runoff value of 27.9 inches was obtained. Applying this value, an average of about 70 lbs of petroleum daily for the 13 ft sewer area was obtained.

If the same estimate loading is applied to the entire Philadelphia metropolitan area (about 287 square miles), a petroleum loading of 7400 lbs/day could be expected to enter the Delaware Estuary from urban runoff.

This estimate is preliminary. Other tributaries and other storm sewers in the Trenton and Philadelphia areas are being tested; and more sophisticated models will be used for analysis. However, there seems to be no doubt that urban runoff, including runoff from industrial and commercial sites is a major source of petroleum hydrocarbons in metropolitan area waterways.

SPILLS

There are many spills in the Delaware Estuary, some of them of large size. However, we have no estimate as yet of the average pollution from this source.

DISCUSSION

The sources of petroleum pollution outlined above are not all independent. The hydrocarbon loading in the tributaries comes largely from urban and industrial runoff, and also contains urban and industrial treatment plant effluents.

From the fragmentary results so far it does not appear that municipal waste treatment plant effluents will constitute a really major source after treatment now planned is completed.

The location of petroleum sources is of great importance. This is obviously true from the viewpoint of ecological damage, since in a slow moving tidal estuary much of the petroleum entering the water seems to be dissipated within a very few days. An even more important aspect is location with respect to muncipal water supply intakes. In the Delaware Estuary about 5000 pounds of petroleum may legally enter the estuary upstream of a major water supply intake. Although only a small portion of the river flow enters the intake, and much of the hydrocarbon content is presumably removed in particulate form by sedimentation, this is still a situation which urgently requires more investigation.

The clarification as to what are the actual major sources of petroleum hydrocarbons should change pollution control priorities. Under present laws, policies, and permit decisions the petroleum refineries are moving to comply with a reduction of effluent loadings to about 8% of its former level, which corresponds to

"best allowable treatment" under PL 92-500. The next step, presumably to be taken by 1983 would impose an even stricter standard, as yet undefined.

Industries other than petroleum appear to have been treated much more leniently, since the percentages of reduction in effluent loadings of petroleum are much less, and the total loadings permitted are much greater.

There are no limitations of petroleum pollution required of municipal treatment plants or for the urban and industrial runoff, other than that of the oil companies. Such action could be required under PL 92-500 to meet water quality standards but such a program has not been spelled out.

Also there is no differentiation in degree of treatment required on account of location of the source. As soon as sources can be more accurately located and estimated it will be possible to develop a program more effective environmentally as well as more cost effective. This will require a much more detailed examination of where the petroleum in urban runoff actually comes from, and also of the relative toxicity of various components.

ACKNOWLEDGMENT

This research was supported by the National Science Foundation, RANN Program. It includes results of research conducted by the three co-authors and Ass't. Professor T. J. Tuffey. The research program includes numerous other important aspects not alluded to in this paper. Some of the data given in this paper have been previously published. 3

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DISCUSSION

GRIFFITH: Mr. Whipple, do you know of anyone who has checked the water that goes into the main at those two treatment plants for the presence of chlorinated hydrocarbons at normal operating levels of chlorination?

WHIPPLE: No, we have not started to do it, and I don't happen to know. Of course, in the famous case of New Orleans, they checked what was going into New Orleans drinking water.

KALLIO: When you speak of hydrocarbons in a liter of water, how are these hydrocarbons determined?

WHIPPLE: The hydrocarbons are measured by the process that I have indicated, and then they are determined, as nearly as we can, by processes of gas chromatography.

KALLIO: If the extraction process extracts all the hydrocarbons present in crude oil, then the gas-liquid chromatograph, at those temperatures, at least will leave a great number of them undetected. They simply become part of the column at temperatures less than, let us say, 400 degrees. These are extremely low figures that our speaker is referring to.

WHIPPLE: Do you mean low figures of content?

KALLIO: Yes, that is correct. I am saying that your figures are essentially minimal because of the temperature of the determination.

WHIPPLE: I had not talked about characterization, and I had not really intended to. We are using a number of techniques for characterization including mass spectrographic analysis, and we do think that we are getting all the hydrocarbons in the process that we use with the three different solvents.

I would like to ask Rick Larson to remark on this. He is working on the same project.

LARSON: I think that Professor Hunter's method at Rutgers uses a combination of liquid and gas chromatographic methods. The liquid chromatographic method is essentially a gravimetric and quantitative method. But, in addition to this, he uses such things as infrared GC to characterize each of these samples qualitatively. Most of the effort has been put into the hexane soluble fraction, but I think the overall combination of methods gives a roughly quantitative number.

GARTMAN: Would you say that the urban runoff loading of petroleum is similar to the nutrient runoff loading that you found in inland waters in that the point source input is not as important as a diffuse source?

WHIPPLE: Well, that is certainly true for nutrients. It is also true, to a very large extent for biochemical oxygen demand, that the non-point sources (in particular, urban runoff in the rivers that we have studied) usually turn out to give more pollution than the point sources once you pass the stage of secondary treatment.

SOURCES AND DISTRIBUTION OF HYDROCARBONS IN THE ENVIRONMENT

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Sources and distribution of hydrocarbons in the aquatic environment include the daily activities of the Nation's population. There are natural occurring hydrocarbons from decaying plants, industrial discharges from oil dependent industries, and there are discharged hydrocarbons from municipally-operated sewage treatment systems. These discharges represent the three basic sources of hydrocarbons; animals, mineral, and vegetable.

In addition to hydrocarbon sources, the distribution of these sources are widespread. Petroleum refinery production, refining, and petrochemicals are predominately located in the Southern United States, while steel production is predominant in the Northern United States. Municipal hydrocarbon discharges are, of course, from every village, town, and city.

Good morning!

It is a pleasure to attend this symposium and present a brief discussion on the sources of hydrocarbons in our environment. The subject area is really easy, and the answers are simple. Sources of hydrocarbons are animal, mineral, and vegetable in origin, and their distribution includes every farm, hamlet, city, and most industries. This presentation will attempt to point out some of the highlights concerning sources and distributions of hydrocarbons.

When asked to present this topic, it appeared simple, because the best known sources are oil wells, service stations, refineries, and sewage treatment plants. The first step, of course, is to define hydrocarbons, and the place to look is in Daniel Webster's Dictionary.

Webster's definition for hydrocarbon is, "An organic compound containing only carbon and hydrogen and often occurring in petroleum, natural gas, coal, and bitumens."[1] That definition, while entirely correct seemed too restrictive because an important test for sewage treatment plant operation is a solvent extraction test for oils and greases. Since Mr. Webster's definition was too brief, the water chemist's Bible, which is appropriately entitled Standard Methods for the Examination of Water and Wastewater[2] was consulted. The thirteenth edition of this compendium of analytical procedures states, with respect to the subject of hydrocarbons, "Groups of substances with similar physical characteristics are determined quantitatively, based on their mutual solubility in the solvent used. Grease may, therefore, be said to include fatty acids, soaps, fats, waxes, oils, and any other material which is extracted by the solvent from an acidified sample and which is not volatilized during evaporation of the solvent." Using this broader definition, it is possible to discuss many hydrocarbon sources because we have extended the specific "hydrocarbons" test definition to include all compounds containing carbon and hydrogen.

While this is the definition selected to use, it has several areas of apparent concern. The first concern is the "mutual solubility," the second is "any other material extracted by the solvent," and the third is "not volatilized during evaporation of the solvent." In 1975, the American Petroleum Institute (API) reviewed the most common analytical procedures to determine oil and grease (hydrocarbons). This survey[3] cited 20 methods available to determine hydrocarbons in water. Furthermore, these 20 procedures used 11 different solvents or solvent combinations to quantitatively determine the oils present in waters. Since each of these solvents exhibit different solubility characteristics, extract different quantities of materials, and volatilize at different temperatures, another problem area was added to this assignment: the problem of data acceptability for hydrocarbon loads being discharged into our environment; because we can vary the reported discharges depending on the analytical procedure we use to determine hydrocarbons in water. This analytical variability can cause uncertainty when discussing how much oil is in our Nation's waters. To compound the problem even further, there is analytical variances between different analysts. Several years ago, a study was conducted at 17 different petroleum refineries. This study consisted of splitting oily water samples between the Robert S. Kerr Environmental Research Laboratory and the refinery laboratory; each laboratory used the soxhlet extraction method (EPA 00550) to determine the quantity of oil in

the samples.[4] A total of 69 samples were analyzed during this study, and the Kerr Laboratory practiced extensive quality control to provide a valid data base for comparison. The results--22% of the refiners reported less oil than the EPA base results, and 78% reported higher results. The percentage differences from stated EPA base value was 45% of the results reported by the industrial participants differed by 0 to 50% of the base concentration values, 36% of the participants results differed by 50 to 100%, and 19% of the cooperating analysts reported results that were 100 to 1000% different from the EPA base values. If the variances are this large for highly trained industrial technicians, one can only surmise the problems associated with less technical industries.

Now that the shortcomings of measuring hydrocarbons in the aquatic environment have been discussed, the next step is to indicate the sources of hydrocarbons being discharged to the environment.

People automatically picture industry as the source of oil pollution to our national waters, so this will be the initial point for discussion. First, there is oil production, then petroleum refineries which produce the marketable products on which other industries and individuals have become so dependent.

Aside from accidental spills from storage tanks, or ruptured pipelines, most production sites are relatively clean. The produced brine and oil are separated by various processes, and the brine is either injected for secondary recovery, disposal, or discharged into lined evaporation pits. In some Colorado/Wyoming production areas, after the oil is separated from the produced water, the water is discharged for consumption by cattle, and this is the main water source for cattle ranchers.

In a study conducted by the Kerr Laboratory with the Offshore Operators Committee[5] to determine the effectiveness of treatment systems used on platforms, it was determined that the oil in treated discharges varied from 1.0 mg/l to 380 mg/l with an average of 44 mg/l for 25 locations represented by 144 samples. The Hexane extraction-gravimetric procedure was used.

The next industrial step is the petroleum refining industry.

In the continental U.S., there are about 250 petroleum refineries located from Buffalo, New York, to Los Angeles, California. The predominant locations are the Gulf Coast and Southwestern states. These refineries range in size from 1,000 barrels/day (b/d) crude oil input to about 200,000 b/d, and all together, they process an estimated 14 million barrels of crude oil each day. During this processing, oil comes into contact with water either directly or indirectly and the water borne oil is separated by detention methods prior to treatment. A study was conducted with the American Petroleum Institute (API) in 1972 to determine the efficiency of the activated sludge treatment process in removing contaminants.[6] It was determined that the median concentration of oil and grease by the hexane extraction-gravimetric method of the treated refinery wastewaters was 11 mg/l. This amounts to an estimated daily discharge of 2.9 lb. of oil for each 1,000 barrels of oil refined at the plant. Nationwide, using 14 million barrels of oil as the daily crude charge, the refiners are discharging 406,000 lbs. of oil or about 1,300 barrels of oil daily into our waters.

The marketable oil products produced by refining crude oil can be separated into four major categories.

Lubricating oil production is estimated to be about 2.2 billion gallons this year. Automobile lube oil represents nearly half of the marketable oil, and therefore, is one of the major potential sources of oil to contaminate the Nation's water resources. In EPA's report on waste oil to Congress in April 1974,[7] it was estimated that 1.1 billion gallons of waste oil would be generated during 1972. Assuming a conservative estimate of 25% of the generated waste oil is discharged to either the sewer or dumped on a vacant lot, the consumers would be the source for 280 million gallons of waste oil annually, or nearly 18,000 barrels of oil per day.

EPA's Industrial Wastes Program[8] identified six industries as major dischargers of oil. Petroleum production and petroleum refining were two of these industries, the other four were chemical and allied products, blast furnaces and steel, food and kindred products, and textile mills.

In the chemical and applied products industry, the Nation's largest industrial water user, the anticipated level of free and floating oil varied with the chemical produced. For example, if a plant produced benzene, toluene, and xylene, the anticipated oily discharge would be 0.1 lb/1,000 pounds of production; butadiene producers would

anticipate a level of 2 lb/1,000 pounds of production; and manufacturers of phenol would contribute 0.5 lb/1,000 pounds of production. Iron and steel industry contributes oil from hot forming and cold rolling operations, and the expected oily discharges from these operations are estimated to be 0.9 and 1.87 lb of oil, respectively, per ton of steel.

Food and kindred products include such industries as slaughter houses, and tuna fish and vegetable canneries. These industries are so diverse in location and operations that is doubtful that reliable information exists to assess their oily discharges.

The textile industry includes fabric mills, dyeing and finishing, knitting, textiles, floor covering, and thread mills. It is estimated that the average oil discharge from the textile industry is 50 mg/l primarily from wool scouring and dyeing operations.

Municipal sources for oily discharges resemble a Rand McNally map because virtually, every village, town, and city has a sewer system. The oily discharges vary dependng on industrial contribution, service station ordinances, pre-treatment requirements for industry, and management of the treatment system.

Oil and grease is a natural constituent of sewage. Fecal material alone contains as much as 25% grease. Add to this the domestic kitchen wastes, restaurant wastes, laundry soaps, automobile washes, garages, etc., and it becomes apparent that the quantities of oil and greases discharged to the sewers are quite large.

Prior to reaching the sewage treatment plant, now more politely referred to as the Publicly Owned Treatment Works (POTW), oils and greases cause clogging and coating of sewer pipes and lift stations. At the POTW, further problems develop by clogging screens and interfering with screening operations, scum draw-off systems, and sludge pumps. The oily scum coats the walls of treatment systems or causes foaming problems, interferes with oxygen transfer, and is responsible for serious problems in a covered digestor.

Slugs of oils, such as a dump from a large service station, will cause a major problem to the POTW by coating the biological mass, thereby, providing a decrease in oxygen transfer which in turn decreases the effectiveness of aerobic systems to remove biologically oxidizable material, interferes with floc settling rate, and thereby increases

the BOD load to the receiving stream and stream beds, and coats the river banks with unsightly contaminants. If these conditions occur frequently, the dissolved oxygen in the receiving water becomes depleted and, fish kills will occur.

Concentrations of oil and greases discharged by POTW's vary widely depending on treatment system design, operation, maintenance, and analytical methods. In a comparative study of three plants, the primary clarifier effluents averaged 35-40 mg/l (using the hexane/soxhlet extraction method), 35-40 mg/l in a trickling filter effluent, and about 50 mg/l in the effluent from activated sludge treatment systems. The results for the separatory funnel/chloroform analytical method are less oil and grease concentrations (20-30), and the freon-infrared analytical procedures reported were near 0 mg/l.[9]

In addition to industrial and municipal sources for hydrocarbons, there is the natural occurring sources. Oil seeps, which will be discussed in a later presentation, metabolic products of fish and wildlife, benthos organisms, and plant life are the most notable for this hydrocarbon contribution source.

During 1972, there were 760 reported fish kills, 597 of which the pollution source was specified and for 163 fish kills, the cause was unknown.[10] It is impossible to determine the role that oil and grease discharges contributed toward these fish kills. These fish kills were attributable to the sources found in Table 6.

In summarizing this presentation, it is important to realize the concentration values reported for oil and grease contaminated water discharges should be scrutinized carefully depending on the analytical procedure used and the quality control observed.

Hydrocarbon contamination does not specifically refer to petroleum oil, but encompasses the organic spectra. Pesticides, ethylene glycol, steroids, and soaps are generally reported as oil and grease, dependent on their solubility with the solvent.

Hydrocarbon contamination exists from agriculture, nature, industry, and municipality sources. Three of these sources are directly related to human activities, therefore, the major hydrocarbon contamination is a result of our own daily activities.

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DISCUSSION

LASDAY: I would like to hear a little elaboration on the extremes of the variability in the oil and grease sample you did, both in offshore productions and production locations and refineries, and what you think the implication of this is for needs in analytical method development. This has a lot to do with the regulatory picture, obviously, and it is a real problem. I would just like a little further elaboration on that.

MYERS: I would be glad to. Not having any definite figures right here and not anticipating that particular question, I will digress a

minute. We further conducted a study with 13 refiners in Oklahoma, and we looked at several different parameters: the BOD, COD, oil and grease, ammonia and cyanide, or sulfide. As chemists we are very aware of the analytical variances that can occur in the laboratory. When you go to the literature to try to pick up data, you have to be very careful because depending on the method that was used you may get a skewed effect.

We have also run, I think, five different analytical methods and tried to compare those, and we are not getting any kind of good comparison at all. It appears to me that the road we are going to travel now is one of specific organic contaminants, related to toxicity, and if this is true then we are going to use more specialized instrumental methods. We will be looking at GC mass spec data, and we will be looking at some bioassay methodology. That is the way I view it going in the future.

WEISS: That really opens up a whole discussion, and I think someday we could have a very good symposium on that subject. I think the difference is between absolute methods and empirical methods.

TABLE 1. API REVIEW OF ANALYTICAL METHODS

GRAVIMETRIC METHODS

	REFERENCE	METHOD
1.	ASTM D-1178	CHLOROFORM EXTRACTABLE MATTER
2.	ASTM-2778	SOLVENT EXTRACTION
3.	EPA MANUAL-00550	SOXHLET EXTRACTION
4.	APHA - 127	APHA OIL & GREASE
5.	STD. METHODS P. 413	HYDROCARBON & FATTY MATTER
6.	ASTM D-1340	REFLUX DISTILLATION METHOD
7.	"CALIFORNIA" METHOD	PETROLEUM ETHER EXTRACTION
8.	ASTM D-2891	HEXANE EXTRACTABLE MATERIAL

VOLUMETRIC METHODS

9.	API 732-53	FLOCCULATION-EXTRACTION METHOD

TABLE 2. API REVIEW OF ANALYTICAL METHODS

INSTRUMENT METHODS

	REFERENCE	METHOD
1.	EPA - 00560	OIL & GREASE-FREON EXTRACTABLES
2.	CONCAWE I/72	HYDROCARBONS IN WATER OR SOIL
3.	API O & G COMM	COMBINED METHODS
4.	1975 EPA STUDY 13	PETROLEUM HYDROCARBONS
5.	API-733-58 & LIT.	VOLATILE & NON-VOLATILE OILY MATERIALS
6.	LITERATURE REF.	ULTRAVIOLET FLUORESCENCE
7.	LITERATURE REF.	ULTRAVIOLET ABSORPTION
8.	CONCAWE III-72	GAS CHROMATOGRAPHY
9.	HACH HANDBOOK-1973	VISIBLE COLOR METHOD
10.	CHAMPION CHEM. CO.	COLOR COMPARATOR

CONTINUOUS MONITORS

11.		ULTRAVIOLET VISIBLE INFRARED FLUORESCENCE LIGHT SCATTERING

TABLE 3. SPLIT SAMPLING STUDY

17 LOCATIONS - 69 SAMPLES

LESS THAN EPA VALUES	MORE THAN EPA VALUES	DIFFERENCE FROM STATED VALUES		
%	%	0-50%	50-100%	100-1000%
22	78	45	36	19

TABLE 4. WATER USING INDUSTRIES

INDUSTRY	NO. PLANTS	WATER USED ANNUALLY GALLONS(10^9)	OILY DISCHARGER
1. CHEMICAL & ALLIED PRODUCTS	1125	9416	YES
2PRIMARY METALS	841	7780	NO
3. PETROLEUM & COAL PROCESSING	-	7290	YES
4. PETROLEUM REFINING	250	7279	YES
5. PAPER & ALLIED PRODUCTS	619	6522	NO
6. BLAST FURNACES & STEEL	-	6504	YES
7. FOOD & KINDRED PRODUCTS	2345	1346	YES
8. TEXTILE MILLS	684	328	YES

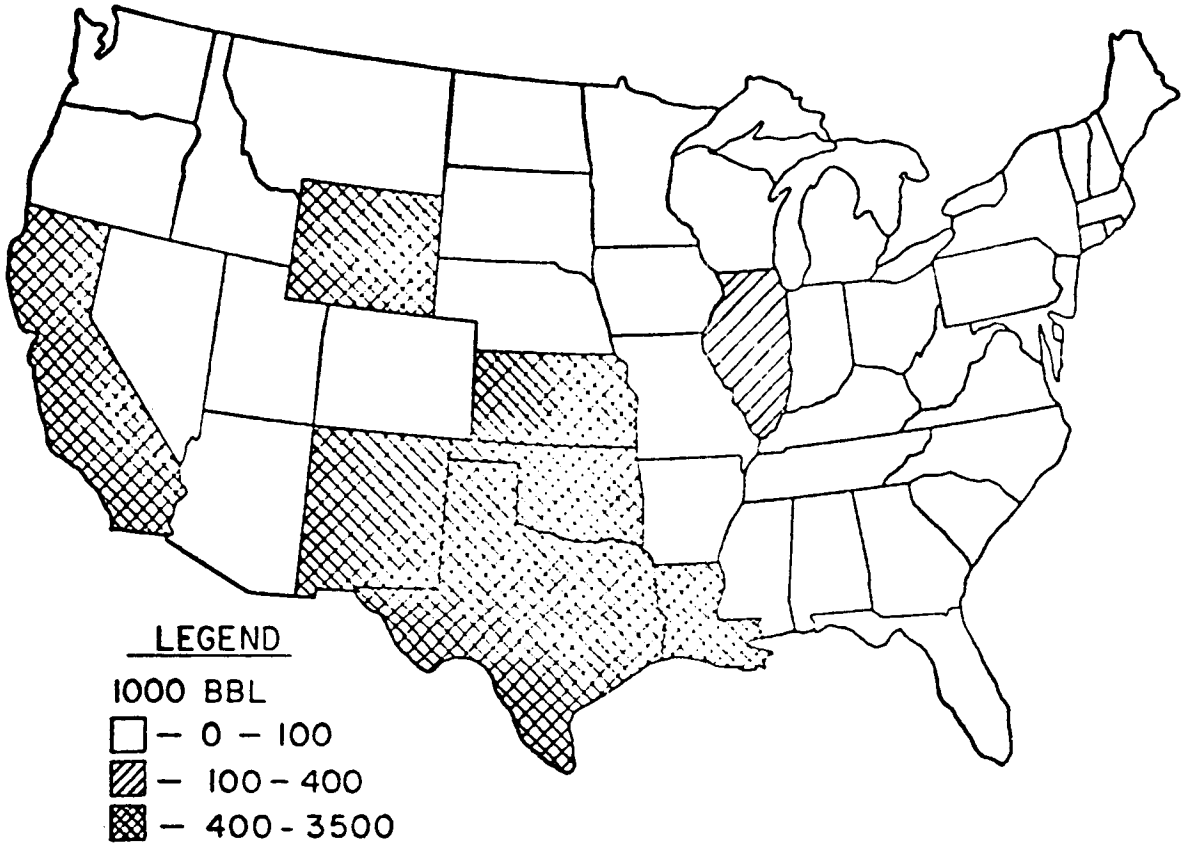
TABLE 5. DOMESTIC LUBE OIL PRODUCTION

	NEW OIL SALES - - - - -GALLONS X 10^6	WASTE OIL GENERATED - - - - -
AUTOMOTIVE LUBE OILS		
SERVICE STATIONS	270	170
AUTO SUPPLY/GARAGES	60	38
NEW CAR DEALERS	102	92
COMMERCIAL ENGINES	90	57
AUTO FLEET	136	68
FLACTORY FILLS	60	54
DISCOUNT STORES	168	37
COMMERCIAL ENGINE FLEET	200	100
	1086	616
INDUSTRIAL		
HYDRAULIC	325	137
METAL WORKING	150	105
RAILROADS	60	32
GAS ENGINES	62	56
AVIATION & OTHER	137	64
	734	394
OTHER INDUSTRIAL		
PROCESS OILS	310	31
ELECTRICAL	57	51
REFRIGERATION	10	5
	377	87
U.S. GOVERNMENT	37	18
	2234	1115

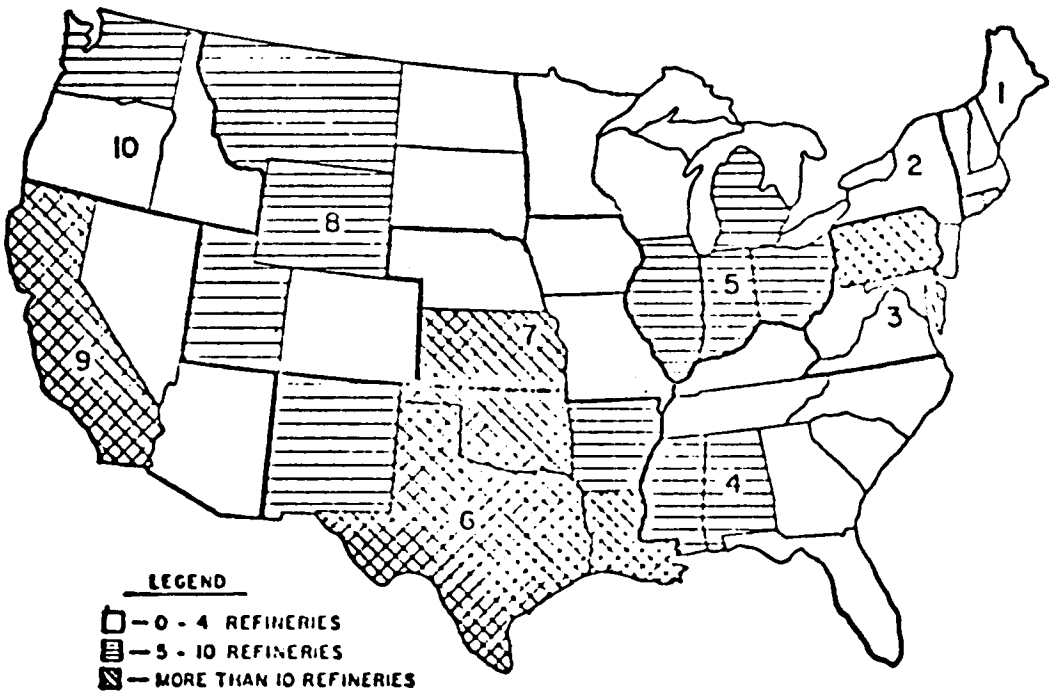
TABLE 6. IDENTIFIABLE SOURCES OF FISH KILLS

	Fish Killed	
Agriculture		
Pesticides	1,500,147	
Fertilizers	30,944	
Manure	276,464	
		1,807,555
Industrial		
Mining	30,328	
Food Products	328,268	
Paper Products	720,667	
Chemicals	825,641	
Petroleum	345,518	
Metals	128,860	
Combinations	1,255,528	
Other	779,580	
		4,414,390
Municipal		
Sewage Systems	3,696,990	
Refuse Disposal	37,799	
Water Systems	3,203	
Swimming Pools	46,075	
Power	4,576,527	
		8,360,594
Transportation		
Rail	14,610	
Truck	300,006	
Barge or Boat	23,750	
Pipeline	118,160	
		456,526
Other		1,028,869
Unknown		1,369,284
1972 Fish Kills Total		17,431,218

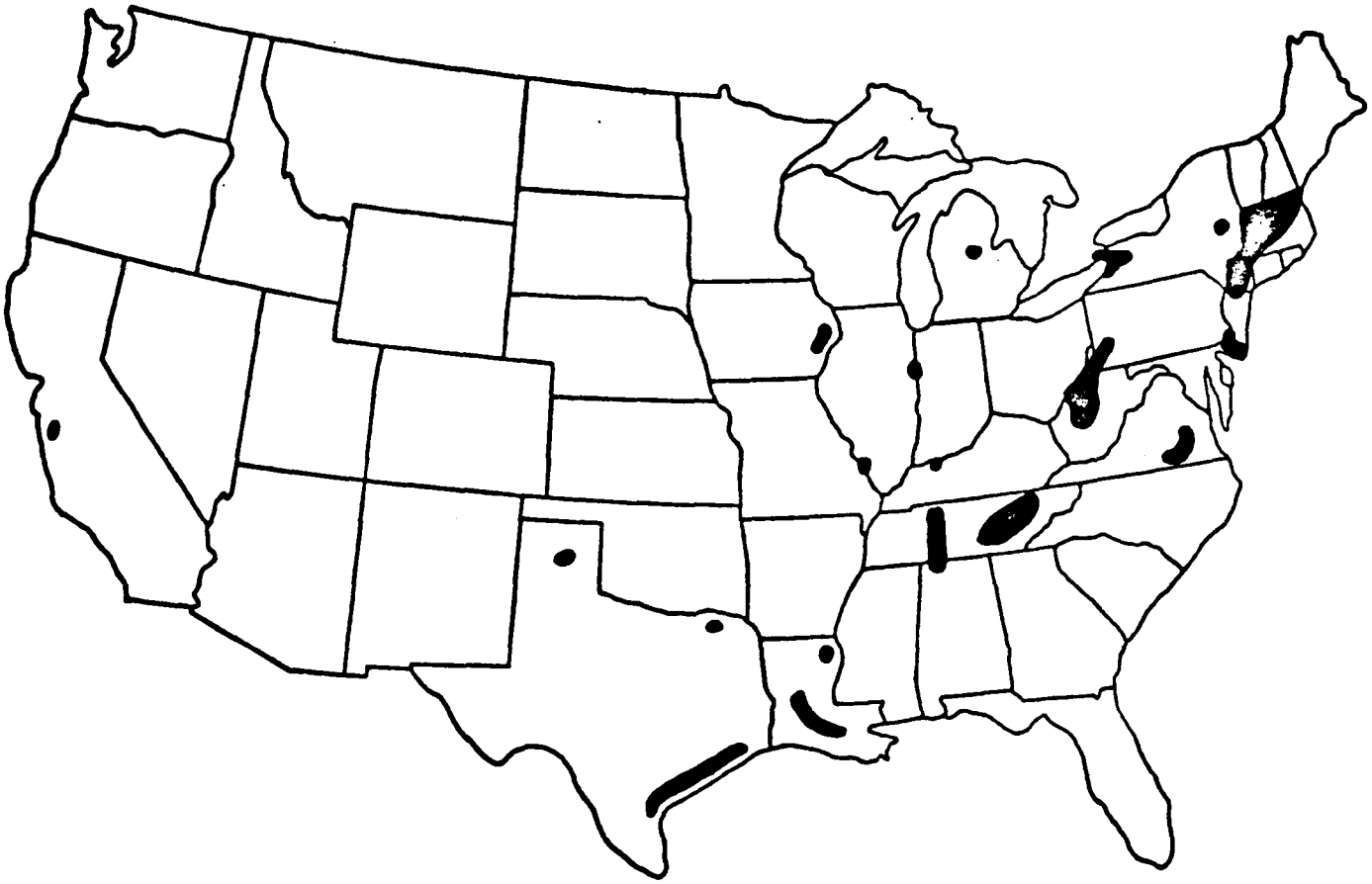
LOCATION OF PETROLEUM PRODUCTION INDUSTRY



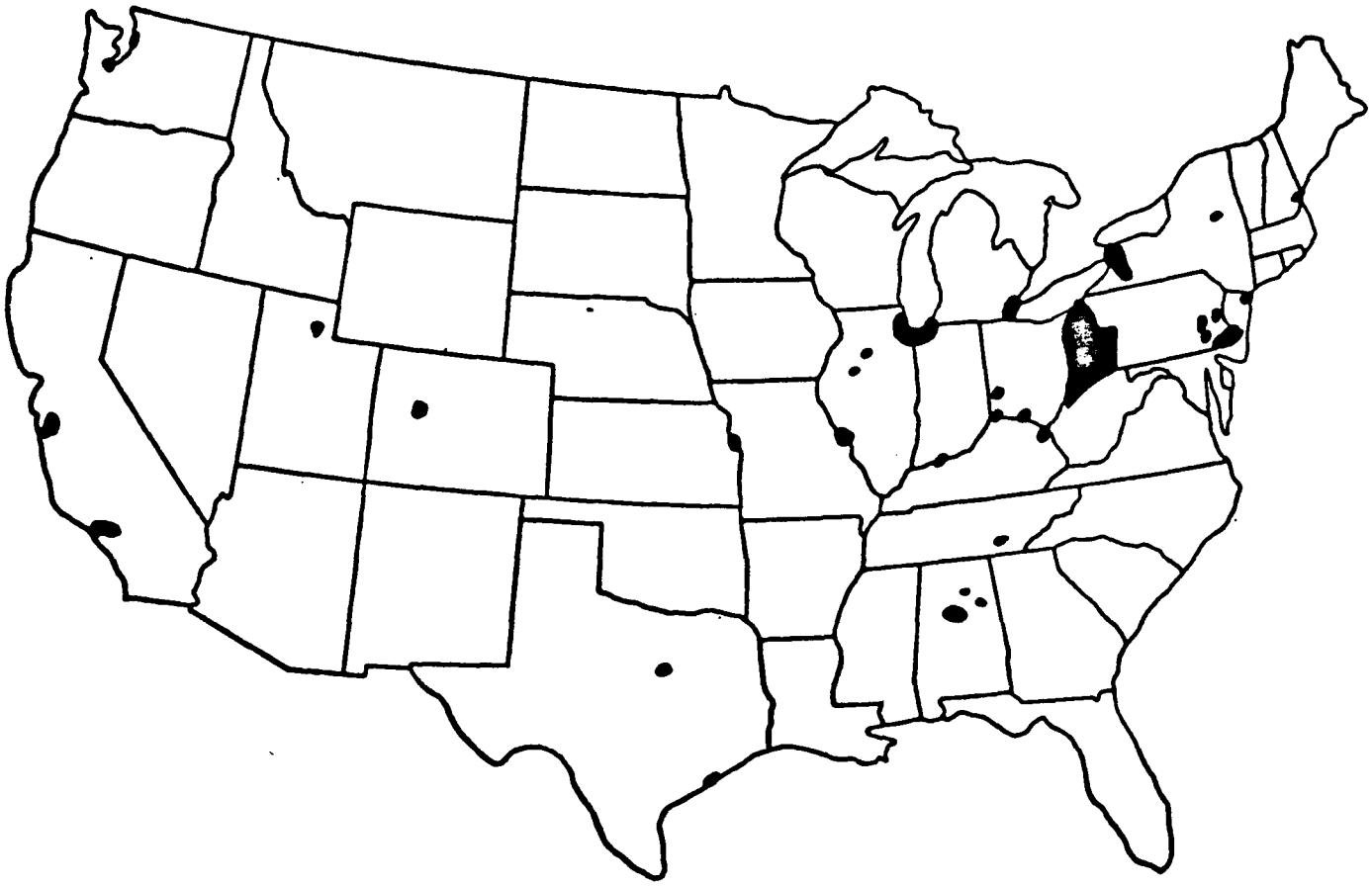
DISTRIBUTION OF PETROLEUM REFINERIES



LOCATION OF MAJOR ORGANIC CHEMICAL INDUSTRIES



LOCATION OF MAJOR BLAST FURNACES & STEEL MILLS



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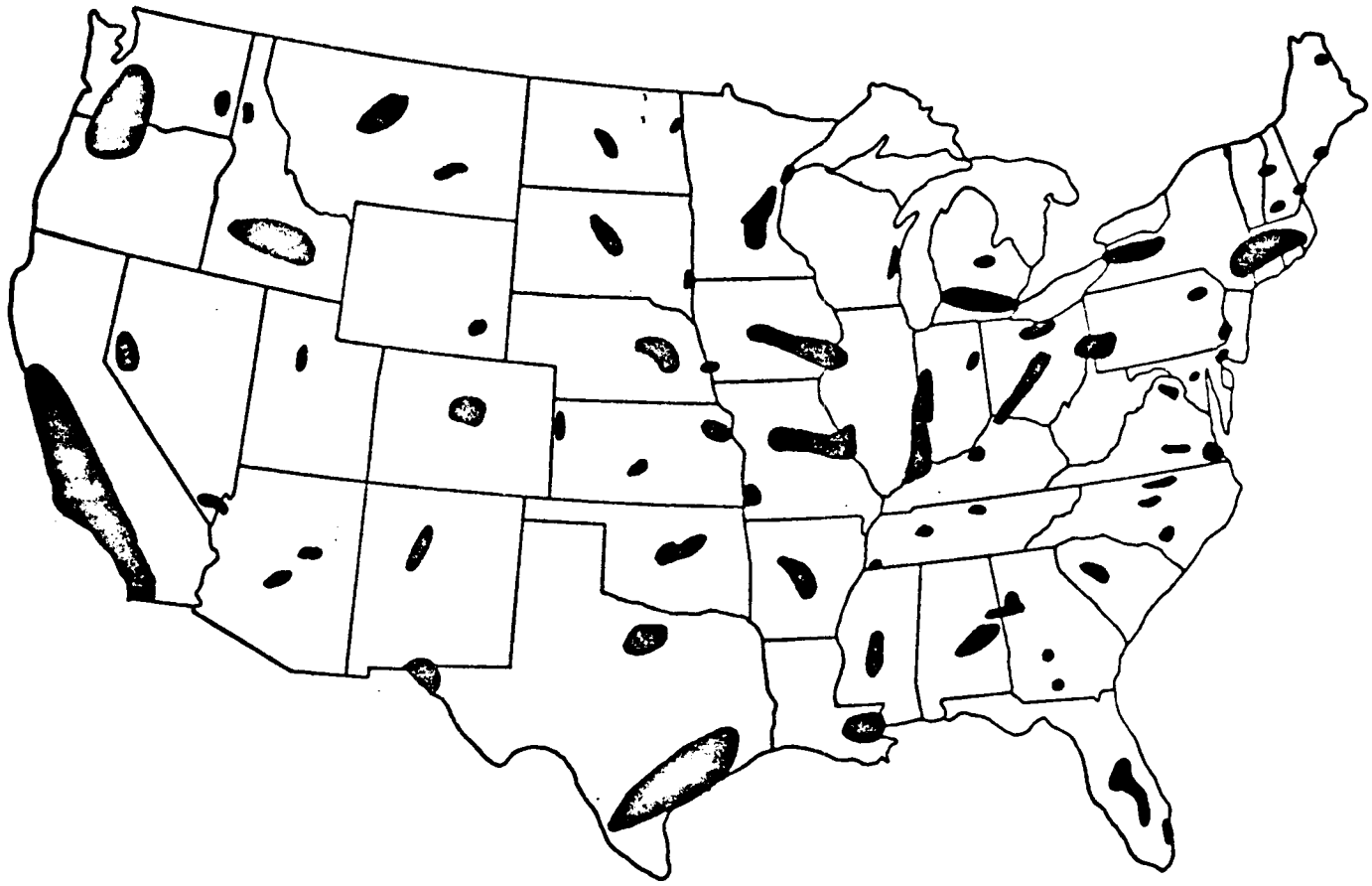

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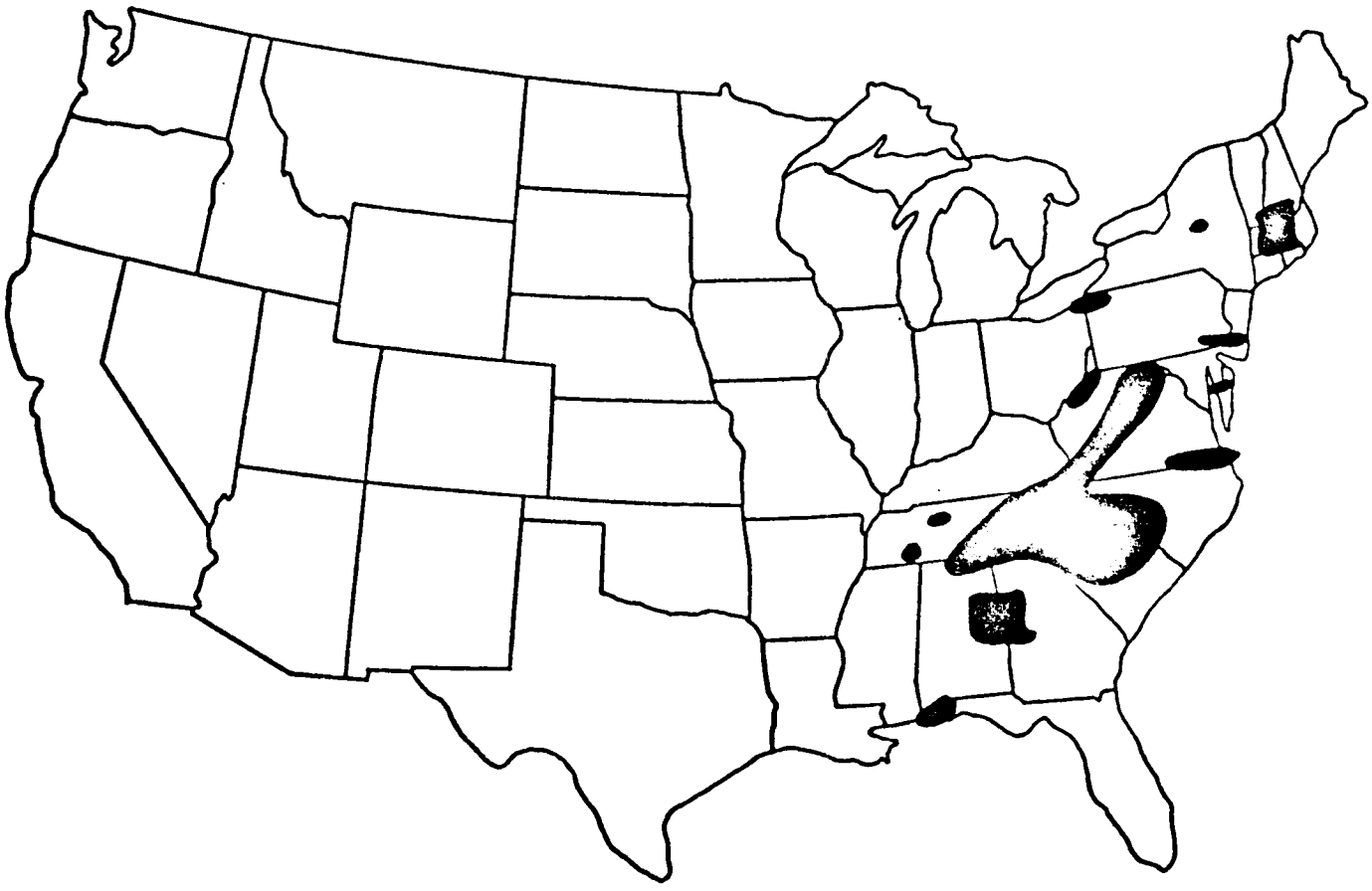

INPUT OF HYDROCARBONS FROM SEEPS AND RECENT BIOGENIC SOURCES

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ABSTRACT

A portion of the hydrocarbons now residing in the world's oceans results from natural sources. Input from submarine seepage is estimated to be 0.6×10^6 metric tons/yr and from biochemical synthesis in the oceans, 6×10^6 metric tons/yr. An unknown amount of hydrocarbons are transported to the oceans from sources on land.

The most abundant hydrocarbon classes in submarine seepage are cycloalkanes, branched alkanes, normal alkanes, and aromatics, in that relative order. In contrast, the most abundant classes of biogenic hydrocarbons are alkenes, normal alkanes, and branched alkanes, in that order. Cycloalkanes and aromatics are quite low.

INTRODUCTION

A portion of the hydrocarbons now residing in the world's oceans results from natural sources. Proper assessment of the current "hydrocarbon load" carried by the oceans must take into account these naturally introduced hydrocarbons in addition to those hydrocarbons contributed by man through exploration, production, refining, manufacturing and transportation of petroleum and/or petroleum products. In this paper we attempt to (1) present our best estimates of the input amounts from the different natural sources and (2) qualitatively describe and contrast the chemical compositions of the hydrocarbons introduced from the different natural sources.

We have grouped the natural sources into three quite broad classifications, based on processes by which hydrocarbons enter the marine environment: submarine seepage, biochemical synthesis in marine organisms, and land sources. Each of these three will be discussed. However, the first two will be treated in much greater detail, primarily because the data, limited as it is, is much better with these.

SUBMARINE SEEPAGE

General

Natural seepages have been described throughout man's written history, as evidenced by biblical references, the works of Herodotus[1], and the writings of Marco Polo[2]. In the early days of petroleum exploration, seepages on land were often used as exploration tools. This led to an active search for them as well as an accurate identification of their location and even limited descriptions of their characteristics. In comparison, accounts of submarine seep occurrences are scarce. A literature survey identified 190 reported submarine seeps worldwide. These are plotted on Figure 1. Most of these appear to be concentrated in the Middle East, around the edge of the Pacific Ocean, and the Caribbean-Gulf of Mexico area.

As noted by Wilson et al.,[3] the limited record of submarine seeps is likely due to (1) difficulty of observation and (2) less extensive exploration of offshore areas compared with onshore areas. The residual evidence of intermittent submarine seepages, so apparent on land, is hidden by water. Scuba divers have observed seeps offshore California that rarely break water[4]. These types of seeps form tar flows or mats on the ocean floor[5,6]. In addition, the dispersing effect of the oceans makes both observation and location of marine seeps problematical.

Even in areas of known submarine seeps, detailed studies will no doubt reveal additional seeps not previously identified. Recent experience off southern California shows that prior to the detailed studies of Wilkinson[7,8], only a few marine seeps were reported, including those at Coal Oil Point and at Santa Monica Bay. When this area was studied in greater detail, the number increased to 50 or 60 confirmed seeps.

Geographic and Geologic Occurrence of Seeps

Study of land seeps is essential to determine marine seepage. Seismic data, drilling, and other geologic data have shown that similar geologic conditions exist on land and in the oceanic areas adjacent to the continents. As a result, natural seepage from both areas can be expected to behave in a similar manner and to be functions of the same geologic and geochemical parameters.

The locations of land seeps in addition to the submarine seeps are shown on Figure 1. Although the coverage is not likely complete, the distribution of seeps covers broad geographical areas. It is clear, however, that there are geographical areas with many seeps as well as areas where few, if any, seeps occur. These differences are dependent on geological and geochemical conditions.

While oil seeps occur almost exclusively in sedimentary rocks or in metamorphic or igneous rocks closely associated with sediments, they are found in a number of different geologic situations. Seeps may originate

from breaching or truncation of an oil reservoir, from leaching along faults, from fractures associated with diapirs or intrusions of shale, salt, or igneous rocks, or as direct emanations from prolific source rocks.

All seepage areas are not necessarily commercial petroleum provinces or indicative of reservoired petroleum. For example, despite extensive seepage in the northern half of Cuba and on the Philippine Islands, little petroleum has been found to date[9]. Link[10] considered one of the main seepage types to be "seeps of oil found associated with beds and formations in which the oil was formed".

The Coal Oil Point seep offshore southern California is generally considered to be an exceptionally large seep with estimated flow rates from about 2500 tons/yr[11] to 47,000 tons/yr[12]. There is evidence of other large seeps. Huge blocks of asphalt, one as large as 20 tons in weight, have been found floating in the Dead Sea[13]. Asphalt Lake on Trinidad Island had a stream of asphalt, sediment and water 5 to 6 meters deep that flowed continually to the sea before mining of the asphalt commenced. The 120-billion-ton Athabasca tar sands are the remains of a giant seep.

Comparison of the onshore and offshore seep frequency data (Fig. 1) with the worldwide seismicity (Fig. 2) indicates a strong correlation between areas of high seepage and areas of current tectonic activity and structuring. This association of seepage and structure is demonstrated by a comparison of adjacent parts of the Persian Gulf basin. The Zagros Mountains in the northeastern portion of the basin are highly structured and are the site of recent earthquake activity. Prolific seepages in this region of Iran and Iraq resulted in numerous oil discoveries[1]. South and southwest into the Mesopotamian Valley, the sediments are characterized by much less deformation with only moderate seepage activity[14]. The Arabian platform in the southwesternmost portion of the basin shows no recent earthquake activity, exhibits little recent structuring, and has little or no seepage[15]. Ghawar, the world's largest oil field, is on the Arabian platform of the Persian Gulf basin. Correlation is not between size of commercial petroleum reserves and seepage activity, but between degree and timing of geologic structuring and seepage activity.

Other examples also confirm the correlation between seepage and structuring. Southern California and southern Alaska have high seepage, are highly structured, and have experienced considerable recent earthquake activity. By contrast, Western Canada and the Sirte Basin in Libya show little recent structuring or earthquake activity and have low present-day oil seepage.

Information on types of structuring and relative deformation of the marine areas is substantially more complete than is information on seep frequencies or flow rates. As a result, the differences in structuring along continental margins in conjunction with the available data on seeps provide a more comprehensive basis for estimating the amount of petroleum seeping into the sea.

Geologic Considerations for Estimating Marine Seepage

Several geologic assumptions are required for estimating marine seepage:

1. Based on onshore occurrences, there are more seeps than have been observed in offshore basins.

2. Factors that determine total seepage in an area (number of seeps per unit area and daily flow rate for each seep) are related to the structural style of the area and to the stage of basin evolution. For example, a high potential for seepage would occur with strike-slip faulting associated with high incidence of earthquakes, tight compressive folding associated with high incidence of earthquakes, igneous activity, and thick geochemically mature Tertiary sediments. On the other hand, a low potential for seepage would occur with pull-apart margins of the Atlantic type, little indication of recent structuring, little or no earthquake activity, and older sediments or geochemically immature young sediments. A moderate or intermediate potential for seepage would occur with strike-slip faulting associated with low incidence of earthquakes, trench associated margins with high incidence of deep earthquakes, early active phase of pull-apart margins, growth faulting associated with giant, river-fed submarine fans, and piercement structures (shale, salt or igneous).

3. Within each structural style, seepage (both numbers of seeps and to a lesser degree average seepage rate) is primarily dependent on area of rock and not volume, provided there is sufficient sediment volume and organic matter for the maturation and generation of petroleum.

4. Most marine seeps are clustered within the continental margins in areas where a certain minimum thickness of sediments is exceeded.

5. As with most geologic and geochemical distributions and processes, seepage rates are log-normally distributed.

Classification of Continental Margin Areas

The geologic criteria listed above were used in conjunction with geologic interpretations to classify the continental margins into areas of high, moderate, or low potential seepage (Fig. 3). Maps of sediment thickness were used to limit areas of potential seepage[16]. The minimum thickness of sediments required for seepage used in this study is 1000 meters (which eliminates most of the world's deep ocean basin area). There is, however, a greater likelihood of generation and migration of oil and, hence, seepage in areas where there are thicker sediments. For young sediments, the required thicknesses may be as great as 10,000 to 20,000'. Thus, all areas of potential seepage are not equivalent geochemically, even if they are of similar structural styles. Because of these variations, maturation-sediment thickness factors, 0.1 to 1.0,

were determined for all the areas. If 60+% of an area has sediment thicknesses of over 20,000', that area is given a factor of 1.0. If an area has no sediment thicknesses greater than 20,000' and only 0-4% of its area with sediments thicker than 10,000', it is rated 0.1. An intermediate area, rated 0.5, might have 10-19% of its area with 20,000'+ sediments and 30-39% with 10,000'+ sediments.

Localities of High, Low and Moderate Seep Potential

Offshore southern California meets the geologic criteria for high seepage potential. It is an area of high structural complexity dominated by strike-slip faulting. It exhibits considerable earthquake activity and recent structural deformation. Estimates by different workers for seepage in the nearly 1000 sq mi area from Point Conception to Point Fermin range from about 5000 tons/yr to more than 45,000 tons/yr with some indication of seasonal variability. In the calculations that follow, the lower rate of 5000 tons/yr is used.

The stable, central portion of the Western Canadian Basin at the present time meets the geologic criteria for low seepage potential despite the presence of the Athabasca tar sands, which attest to the existence of substantially higher seepage rates in the past. The basin lacks significant recent strike-slip faulting or compression, is not highly structured, and has a low incidence of earthquake activity. The high degree of observational and geologic control in this basin suggests that any large seeps will have been found and reported. The basin has an area of 1,000,000 square miles. Fifteen major petroleum seeps have been identified and an accumulated flow rate of 100 barrels per day was estimated by Wilson et al[3]. These observations suggest that seepage here is approximately 5 tons/yr per 1000 square miles.

Classification of moderate potential seep areas both onshore and offshore is based on the relative extent that the various geologic conditions are met compared to high and low potential seep areas. Compared to high seep areas, these areas exhibit lesser degrees of structural complexity and recent earthquake activity. However, moderate seepage areas possess the required geologic criteria for seepage to a greater degree than low potential seep areas. For example, in the Persian Gulf, the Mesopotamian Valley with some reported seeps (and located between the high seep potential Zagros Mountains and the low seep potential Arabian shield) is interpreted as a moderate potential seep area. Other onshore areas of moderate potential and with known seeps are the Alaskan North Slope and Magdalena Valley of Colombia.

Calculations of Seepage

To determine marine seepage, flow rates of 5000 tons/yr/1000 sq mi for areas of high seepage, 150 tons/yr/1000 sq mi for areas of moderate seepage and 5 tons/yr/1000 sq mi for areas of low seepage were used.

Remember that each gross area of potential seepage was multiplied by the appropriate maturation factor (0.1 to 1.0) to arrive at a seepage prone area to be used in the seepage calculations. The high and low rates are taken from the Southern California and Western Canadian examples, respectively, and it is assumed that seepage in areas of moderate potential is the geometric mean of the high and low rates. For log-normal distributions, the geometric mean is the median.

Although the geological conditions for "high," "moderate," and "low" localities match those for the "type of localities," e.g., Southern California and Western Canada, it is unknown whether the flow rates selected are typical of such areas. While these rates are based on observations and will be in the statistical population of rates, they may fall anywhere within the distribution curve.

Total seepage estimates can be made assuming that the above rates fall at different points within the log-normal distribution. Thus, three probability levels were considered - P_{16}, $P_{1.0}$ and $P_{0.1}$ — for each of the three seepage areas. From the resulting curves, a mean seepage rate for high, moderate and low areas was derived. Mean seepage is tabulated by area and for each case in Table I.

For the total seepage calculations, the area in each of the "high," "moderate," and "low" potential localities was summed for the world's oceans, shown in Table II. These world ocean seepage-prone areas were multiplied by the various statistically calculated mean flow rates. The world total and regional breakdown among the Pacific, Atlantic, Indian, Arctic and Southern oceans are summarized in Table III.

Determination of Likely Annual Submarine Seepage

Determination of annual marine seepage really depends upon how well the southern California and western Canada seepages represent other 1000 sq mi areas of similar geology in the world.

Case I considers the three flow rates at the 16 percentile and yields an estimated seepage of 6 million metric tons/yr. This value appears to be an upper limit based on present knowledge. In this case, it is required that there be 175 blocks of 1000 sq mi each where seepage is 5000 tons/yr (100 bbl/day), the rate used for offshore southern California. There is a body of opinion which believes that seepage rates exceeds 20,000 tons/yr (400 bbl/day) and may approach 50,000 tons/yr (1000 bbl/day). If these higher rates for California are correct, then it is possible that either 175 undocumented 1000 sq mi areas with 5000 tons/yr or fewer areas with higher rates may exist.

Case II places the type locality flow rates at the 1.0 percentile and yields an annual worldwide marine seepage of 0.6 million metric tons/yr. The 1.0 percentile means that in 99 out of 100 areas of high, moderate and low potential seepage, the flow rates will be less than 5000, 150 and

5 tons/yr per 1000 sq mi for each respective type seepage area. In this case mean flow rates for high, moderate and low seepage areas are approximately 400, 2.5 and 0.4 tons/yr/1000 sq mi, respectively. This estimate requires that there be eleven 1000 sq mi offshore areas in the world with 5000 tons/yr flow rates. Observations suggest 5000 tons/yr/1000 sq mi may occur in offshore portions of Trinidad, Eastern Venezuela and Ecuador-Peru[17]. Based on the various geologic and geochemical considerations outlined, as well as the limited seep data available, the estimate of 0.6 million metric ton/yr appears highly reasonable in view of current available knowledge. This estimate recognizes that both offshore California and western Canada are geologically representative of high and low potential seep areas, respectively. However, it indicates their seepage rates are not typical but have only a 1 in 100 probability of occurring elsewhere in the world. In this regard, this estimate, although considered most reasonable, may be somewhat conservative.

Case III, with flow rates at the 0.1 percentile, yields a minimum estimate. This case places the rate of 5000 tons/yr/1000 sq mi as the highest seepage rate worldwide. Since actual seepages in the offshore California area may be higher than the assumed value, and at least a few other areas equivalent to offshore California apparently exist, the calculated estimate of 0.2 million metric ton/yr represents a minimum value.

Comparisons with Other Submarine Seepage Estimates

In view of limited observations of submarine seeps, only a few cursory estimates of total worldwide annual marine seepage have been made prior to the one presented here.

A National Academy of Science (1971) panel[18] estimated natural submarine seepage to be less than 100,000 metric tons/yr. Blumer[19] estimated that submarine seepage is "orders of magnitude" less than the oil pollution caused by man, which he places at 5×10^6 tons/yr. Two orders of magnitude less would be 50,000 metric tons/yr.

The best estimate of submarine seepage, 0.6 million metric tons/yr presented in this paper, is appreciably higher than these earlier estimates. However, since it is based on geologic and geochemical considerations, we believe that it is the best estimate possible with the limited amount of observation data available on underwater seeps.

BIOCHEMICAL SYNTHESIS IN MARINE ORGANISMS

General

Many marine organisms synthesize hydrocarbons through certain specific biosynthetic pathways. Usually certain molecular types and sizes are preferred for individual families or genera of organisms. For example,

many marine algae produce hydrocarbons with relatively high concentrations of certain medium-weight normal alkanes or paraffins, such as n-pentadecane (C_{15}) and n-heptadecane (C_{17}) and polyunsaturated C_{17}, C_{19}, and C_{21} olefins[20,21]. The sulfate-reducing bacterium *Desulfovibrio desulfuricans* produces sizable quantities of C_{25} to C_{35} normal alkanes with no preference for an odd or even number of carbon atoms[22].

Among the marine animal kingdom zooplankton extracts have been shown to contain appreciable quantities of the C_{19} isoprenoid branched alkane pristane, C_{19} isomeric mono-olefins, and C_{20} isomeric diolefins[23]. Octocorals (alcyonarians) have been shown by Ciereszko et al.[24] to contain sizable quantities of sesquiterpenes, a series of isomeric bicyclic hydrocarbons, molecular formula $C_{15}H_{24}$, carrying methyl and isopropyl groups. Ackmann[25] has reported that marine fish oils (herring, sand launce, cod liver, and gray cod liver) contain certain normal alkanes (C_{15}, C_{16}, C_{17}, C_{18}, and C_{19}) in addition to pristane. Interestingly enough, three of the four fish oils have n-C_{18} as the most abundant n-paraffin homolog present.

From the limited amount of data present on the natural hydrocarbons of marine organisms, it appears that different organisms may have completely different assemblages of hydrocarbons. Also, the complexity of each assemblage may be quite variable. That is, certain organisms may contain only a few individual hydrocarbon compounds (e.g., marine algae) while others may contain many individual hydrocarbon compounds (e.g., marine bacteria, marine octocorals).

Amounts of Hydrocarbons Found in Marine Organisms

As described above, hydrocarbons of different size and structure are constantly being formed by marine organisms. The total amount of hydrocarbons generated each year in the sea will be a rather large number simply because the organic productivity of the sea is large. Neither the organic productivity in the ocean nor the hydrocarbon contents of many marine organisms are known precisely. These are the two factors required to estimate biogenic hydrocarbon production.

Hydrocarbons have been identified in many marine organisms by a number of workers, but few have made quantitative measurements. Such measurements have been made on some marine algae, and these data are summarized in Table IV. None of these authors mentioned possible petroleum contamination; therefore, these probably represent indigenous biogenic hydrocarbons.

Measurements on planktonic algae are reported by Clark and Blumer[26] and shown as Item 2 on Table IV. Their algae were grown in the laboratory in petroleum-free environments. They report values for individual species ranging from 34 to 120 ppm on a dry weight basis, average of 72 ppm.

In this same paper Clark and Blumer[26] report a similar range of hydrocarbon values for benthic algae collected from natural waters. This suggests that the hydrocarbon content of planktonic and benthic algae may be similar. Later, Youngblood et al.[21] reported hydrocarbon contents for 23 benthic algae with values ranging from 20 to 4810 ppm. Included in the Youngblood study were six of the same benthic species examined by Clark and Blumer. The hydrocarbon contents of two of these species were within a few parts per million of the earlier analyses. However, the values obtained for the four other species varied by factors of 3 to 14 between the studies. This suggests that the hydrocarbon content of a species may vary considerably.

The studies of Gelpi et al.,[27] Han et al.,[28] and Winters et al.[29] show higher hydrocarbon contents in the algae they investigated. The average hydrocarbon content for the 49 marine algae reported on by all these investigators is 400 ppm (dry weight basis).

From the data discussed above, it seems likely that a hydrocarbon content of 50 ppm dry weight for marine algae would be a minimum value. This is below the average for planktonic algae reported by Clark and Blumer[26]. Other studies yield higher values, so it is possible that the proper average hydrocarbon content may be as high as 400 ppm.

Organic Production in the Oceans

Photosynthesis by planktonic algae is the main source of organic matter in marine and oceanic basins[30]. Datsko[31] has shown that phytoplankton account for nearly two-thirds of the annual production (dry weight) of organic matter in the Caspian Sea.

There are several estimates of annual organic production in the world's oceans[31,32,33,34,35]. The average value for these five estimates is 40×10^9 tons of organic carbon/year, with a range from 20×10^9 to 70×10^9 tons org.C/yr. It is probable that these latter estimates set the lower and upper limits for the production of organic carbon in the world's oceans.

Estimate of Annual Biogenic Production of Hydrocarbons

A value for the total organic production in the sea and a value for the average hydrocarbon content of marine life are required to estimate biogenic hydrocarbon production. Table V shows the estimates presented in this paper.

The average organic productivity estimate of 40×10^9 tons organic carbon/yr combined with hydrocarbon content estimates of 400 ppm dry weight (average of 49 analyses) and 50 ppm dry weight (minimum of analyses) gave estimates of annual biogenic production of hydrocarbons of 16×10^6 metric tons/yr and 2×10^6 metric tons/yr, respectively. The true input

of hydrocarbons from this source likely falls within this range. It certainly does not seem unreasonable to set the true biogenic input of hydrocarbons close to the National Academy of Science[36] estimate of petroleum hydrocarbons going into the marine environment, that is, approximately 6 million metric tons/yr.

LAND SOURCES OF HYDROCARBONS

General

There are several possible processes by which naturally produced hydrocarbons derived from land sources might be transported into the marine environment. However, these transport processes are very poorly understood and any sort of quantitative estimate of total hydrocarbons being transferred from land to sea is extremely speculative.

The National Academy of Science[36] estimated that river runoff into the marine environment contributes about 1.6 million metric tons/yr of "petroleum hydrocarbons". This estimate is based on results of carbon-chloroform extracts (CCE) on river waters. This test does not discriminate between petroleum hydrocarbons derived from man's release of petroleum and petroleum products into the environment and those hydrocarbons derived from geochemical processes such as the weathering and erosion of soils and sediments containing naturally produced hydrocarbons. Farrington and Meyers[37] believe that the input amounts of these naturally derived hydrocarbons are low because of slow degradation of these hydrocarbons during the weathering process.

Other natural chemical synthesis processes which are sources of hydrocarbons have been mentioned by Farrington and Meyers[37]. Forest fires inject an estimated 6 million metric tons/yr of hydrocarbons into the atmosphere. An unknown portion is eventually delivered to the marine environment. There are also geochemical reactions occurring during the maturation of organic matter in sediments which yield hydrocarbons. These hydrocarbons could enter the marine environment by submarine exposure of sediments or diffusion out of the sediments. Again, Farrington and Meyers[37] state that the rates of input for these sources are probably small.

Because of the extreme lack of knowledge about the processes involved in the transfer of naturally derived hydrocarbons from land sources to the marine environment, no estimate of this hydrocarbon input is made.

CHEMISTRY OF SEEPAGE AND BIOGENIC HYDROCARBONS

General

There are chemical similarities and differences between hydrocarbons found in seepage oils and hydrocarbons synthesized in marine organisms. This chemistry must be considered in any hydrocarbon analyses of waters,

sediments, and biota tissues exposed to inputs from these two sources as well as anthropogenic sources (discharges of petroleum hydrocarbons in exploration, production, transportation, refining, etc. of petroleum and/or petroleum products).

Seepage Oil Hydrocarbons

The types of hydrocarbons found in seepage oils will be typical of crude oils, in general. There will be three main classes: alkanes, cycloalkanes, and aromatics.

In an average seepage oil, the alkanes (paraffins) would be the second most abundant main class of hydrocarbons present, about 30% by weight of the total oil. However, seepage (and crude) oils vary tremendously in composition and one could have oils in which the alkanes predominate or, at the other extreme, the alkanes could be essentially absent in a particular oil. Within the alkanes there are two subclasses: normal alkanes (n-alkanes), with the carbons arranged in a straight chain, and branched alkanes (isoalkanes), with the carbons arranged in branched chains. Among the isoalkanes a very important group is the isoprenoid alkanes, a homologous series containing repeating saturated isoprene units. Farnesane (C_{15}), pristane (C_{19}), phytane (C_{20}) are important individual hydrocarbons in this group.

The cycloalkanes (naphthenes) would be the most abundant class of hydrocarbons found in an average seepage oil, about 50%. Here again, the variability between different oils is great, so the contents of cycloalkanes among different samples might range from 20 to 80%. The cycloalkanes are a complex mixture of saturated hydrocarbons containing substituted (with alkyl groups) and unsubstituted rings (usually 5 or 6 carbons to each ring). The alkyl-substituted cycloalkanes are more abundant than the parent unsubstituted cycloalkanes. The number of rings per molecule range from one to as high as six or greater. In most samples the one- or two-ring cycloalkanes are the most abundant. Two important classes of multi-ring cycloalkanes found in seepage oils are the steranes, C_{27}-C_{30} four-ring cycloalkanes with carbon skeletons identical to lipid sterols found in biota, and triterpanes, C_{27}-C_{32} five-ring cycloalkanes related in structure to triterpenes, also found in biota.

The third most abundant hydrocarbons in seepage oils are the aromatic hydrocarbons. In an average oil the aromatics might constitute 15-20% by weight of the oil. However, here again the range between different samples is great. Some heavy seepage oils might approach 40% aromatics. The aromatics are a complex mixture of hydrocarbons, each member of which contains at least one six-carbon unsaturated ring structure (described as an aromatic ring). Aromatics include mono-, di-, tri-, and tetraalkyl benzenes, naphthalenes (2-ring), and polynuclear (3+ ring) hydrocarbons with multiple substitutions. Also included in this class are hydrocarbons sometimes designated as naphthenoaromatics, because of the mixed nature of the molecule — i.e., part aromatic, part cycloalkane. These naphtheno-

aromatics are also highly alkyl substituted. The unsubstituted parent compounds are present only in trace quantities.

Seepage oils (and crudes) would not be expected to contain unsaturated alkene and cycloalkene hydrocarbons. These hydrocarbons are found only in refined petroleum products such as gasoline, jet fuel, kerosene, etc.

Biogenic Hydrocarbons

Farrington and Meyers[37] have reviewed the hydrocarbon classes native to organisms: alkenes (olefins), n-alkanes, branched alkanes, cycloalkanes and cycloalkenes, and aromatic hydrocarbons.

Alkenes are often the most abundant class of hydrocarbons found in organisms, particularly marine organisms. Squalene ($C_{30}H_{50}$) is a major constituent of basking shark liver oil and cod liver oil. Isoprenoid C_{19} and C_{20} mono-, di-, and tri-olefins are present in zooplankton and certain fishes. Several straight-chain C_{15}, C_{17}, C_{19}, and C_{21} mono- to hexa-olefins are present in marine algae.

N-alkanes are synthesized by both land and marine organisms. Odd-carbon-number chains (e.g., C_{21}, C_{23}, C_{25}, C_{27}, etc.) predominate, although even-carbon number chains (e.g., C_{22}, C_{24}, C_{26}, C_{28}, etc.) are present. In many instances one or two odd-carbon-chain n-alkanes predominate over all others. In marine phytoplankton, n-C_{15}, n-C_{17}, n-C_{19}, and n-C_{21} are most abundant, while in marsh grasses and *Sargassum*, n-C_{21}, n-C_{23}, n-C_{25}, n-C_{27}, and n-C_{29} predominate. Bacteria apparently produce equal amounts of even-carbon-number and odd-carbon-number n-alkanes between n-C_{25} and n-C_{32}.

Branched alkanes are also found in marine organisms. Pristane, the C_{19} isoprenoid alkane, is the most abundant alkane in some fishes. Phytane, the corresponding C_{20} isoprenoid alkane, is noticeably less abundant than pristane. Phytane has been reported in bacteria. Several monomethyl branched alkanes have been identified in marine organisms.

In comparison with the classes of biogenic hydrocarbons discussed above, the cycloalkanes, cycloalkenes, and aromatics are significantly less abundant in marine organisms, with the exception of the carotenes. The carotenes are $C_{40}H_{56}$ polyolefinic cyclohexenes found in considerable quantities in many organisms. Some polynuclear aromatic hydrocarbons may be synthesized by marine microorganisms. The aromatic hydrocarbons native to organisms contain at most one or two substituents.

Characteristics of Seepage and Biogenic Hydrocarbons Useful for Distinguishing Them

Most organic geochemists believe that crude oils or seepage oils that are being discussed in this paper are derived directly or through

chemical conversion from organic compounds which were once laid down in marine sediments in the geologic past. Many investigators have found similar types of compounds or hydrocarbons (e.g., n-alkanes, pristane) in crudes and present-day unpolluted marine organisms, sediments, and waters. Thus, the problem of distinguishing seepage hydrocarbons from biogenic hydrocarbons evolves to choosing those individual hydrocarbons or class of hydrocarbons which most clearly differentiate petroleum and biosynthetically produced materials.

Farrington and Meyers[37] note that petroleum contains a much more complex mixture of hydrocarbons with a much greater range of molecular structures and molecular weights than has been reported for hydrocarbons native to organisms. Also, petroleum contains many homologous series in contrast with biogenic hydrocarbons which have few. In petroleum adjacent members of a homologous series usually are present in nearly the same concentration. The unity ratio of even- and odd-numbered n-alkanes is an example. This complexity and predominance of homologous series can be used in a somewhat subjective manner to distinguish petroleum hydrocarbons from biogenic hydrocarbons.

Certain alkylated aromatic, naphthenoaromatic, and naphthenic hydrocarbons appear to be good choices for distinguishing petroleum hydrocarbons from biogenic ones. Examples of these are the alkylbenzenes, -indanes, -tetralins, and -naphthalenes in the carbon number range C_{15} to C_{35}. These hydrocarbons are relatively abundant in most crude and seepage oils, but are not known to be synthesized to any great extent by marine organisms. Also, these hydrocarbons are apparently resistant to biodegradation than certain other classes of hydrocarbons particularly the paraffins. Higher ring alkylated naphthenes, such as the four-ring steranes and five-ring triterpanes, are probably good choices, too.

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DISCUSSION

SWEENEY: Why do you have a heading called "lipids" and then speak of straight chain materials?

KOONS: The hydrocarbons are part of the lipids. Lipids, to me at least, are fraction extracted from organisms that include soluble organics, and they would contain materials like hydrocarbons. The major components are triglycerides of free fatty acids, but I lump hydrocarbons in with the total lipid fraction. I should have said "biosynthesized hydrocarbons." That would have cleared it up.

BRUBAKER: You didn't consider the possibility of rainout or washout hydrocarbons in the ocean?

KOONS: No. That was one of the areas that I don't think we have a good handle on as far as calculating. Max Blumer of Woods Hole has done some calculating on the amount of hydrocarbons that are thrown into the atmosphere by burning, and it is a tremendous amount of hydrocarbons that are released by forest fires. Well, what happens to these? We just don't have good numbers on how much of these would wind up in the marine environment, and so we did not try to come up with any estimates of this. But that is lumped under the third category of natural sources.

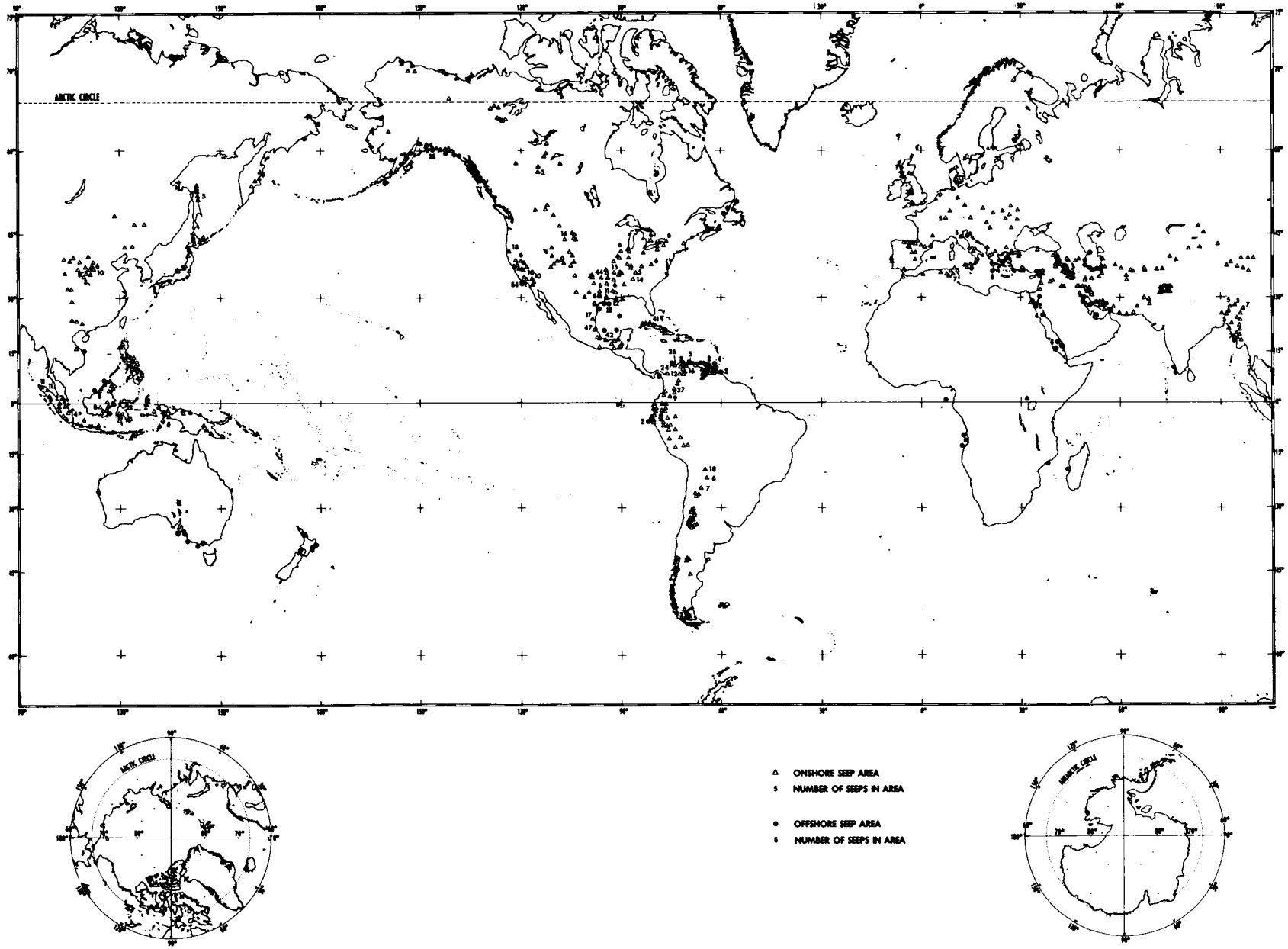

FIG. 1 LOCATION OF OFFSHORE AND ONSHORE SEEPS

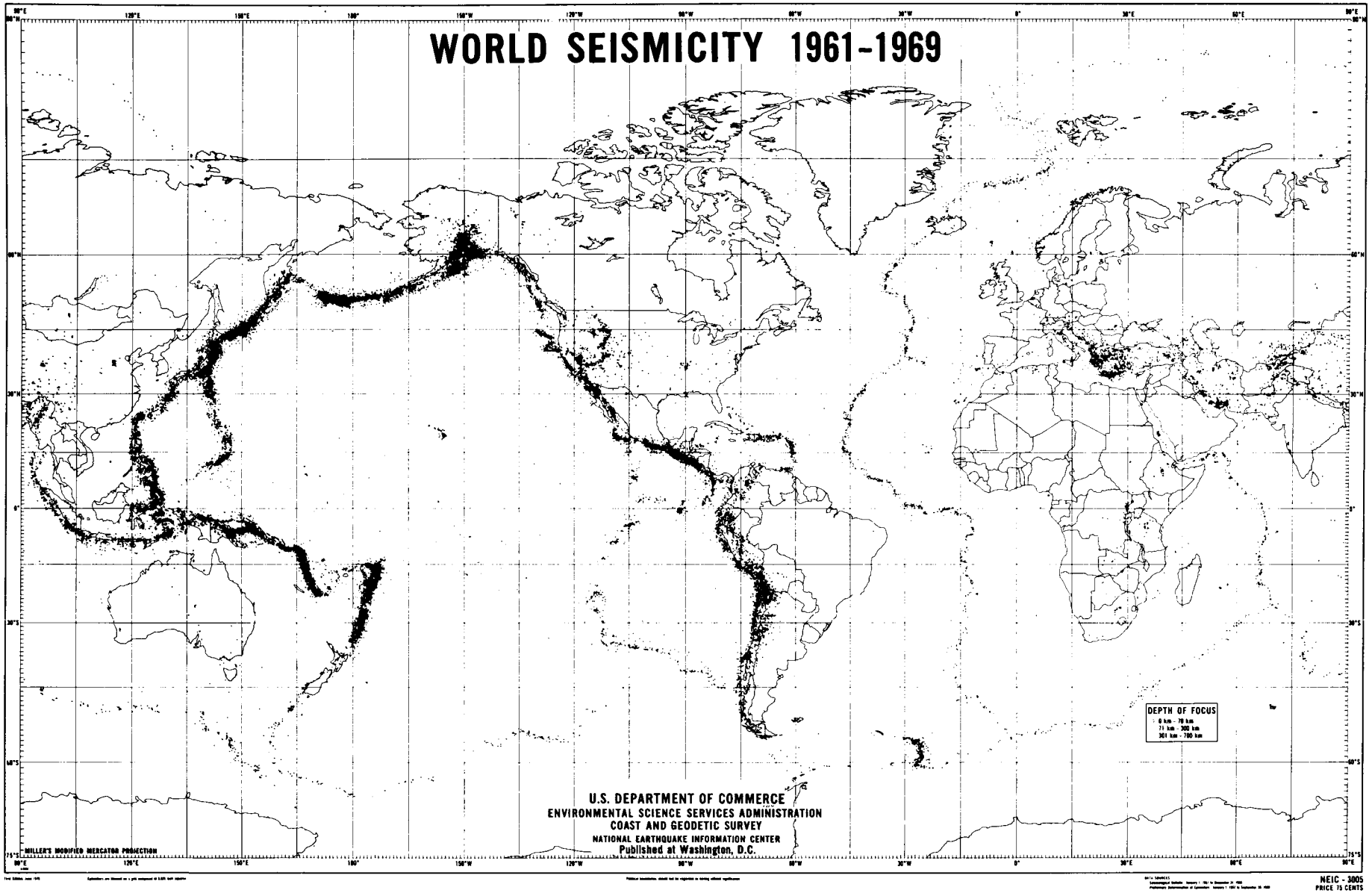
WORLD SEISMICITY 1961-1969
DEPTH OF FOCUS
0 km - 70 km
71 km - 300 km
301 km - 700 km
U.S. DEPARTMENT OF COMMERCE
ENVIRONMENTAL SCIENCE SERVICES ADMINISTRATION
COAST AND GEODETIC SURVEY
NATIONAL EARTHQUAKE INFORMATION CENTER
Published at Washington, D.C.
MILLER'S MODIFIED MERCATOR PROJECTION
NEIC - 3005
PRICE 75 CENTS

FIGURE 2

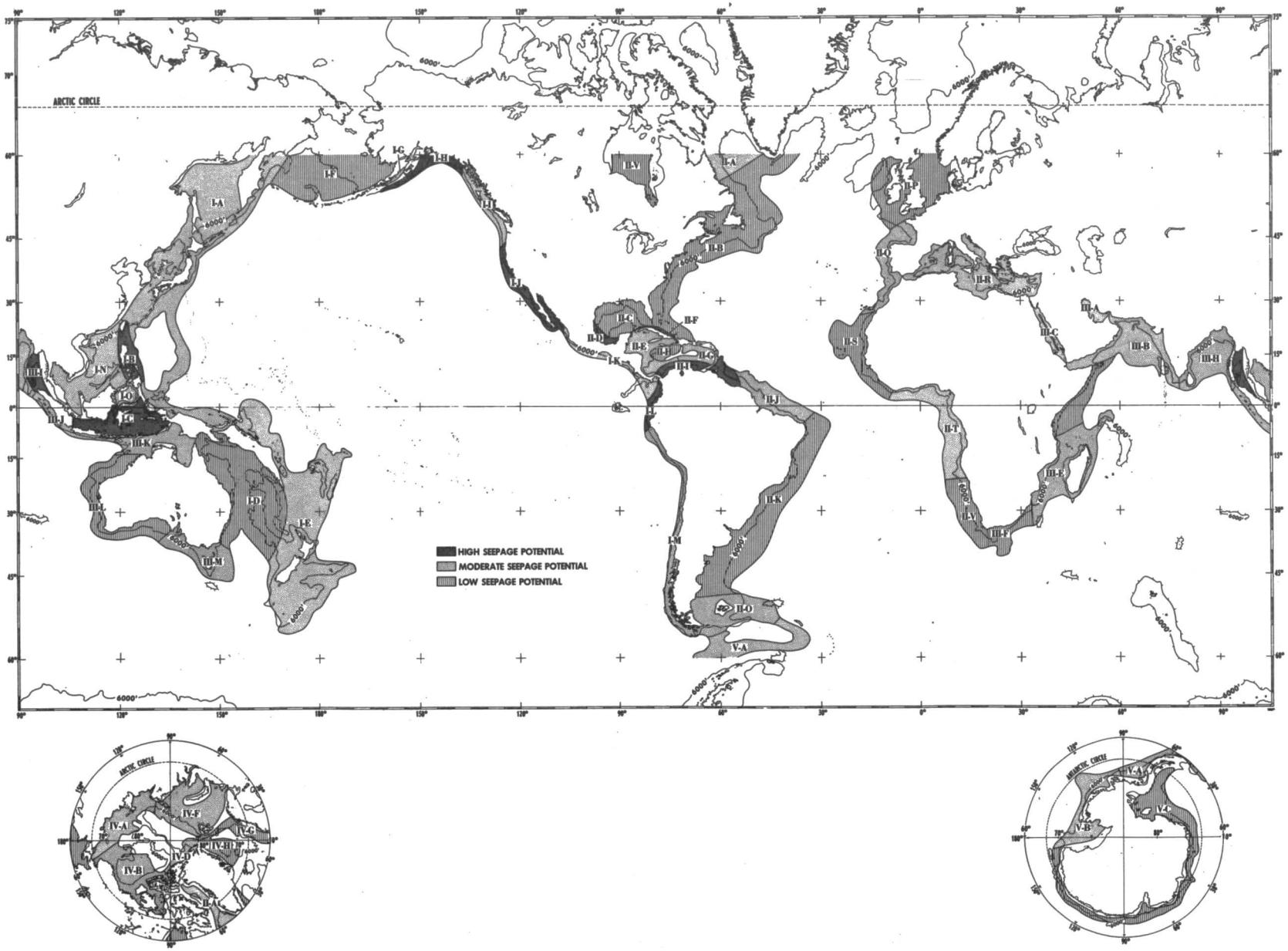

FIG. 3 AREAS OF HIGH, MODERATE AND LOW SEEPAGE POTENTIAL

TABLE 1

MEAN SEEPAGE RATES

Seepage Potential Areas	Type Locality Seepage Rate Tons/Yr/1000 sq mi	Mean Seepage Rate, Tons/Yr/1000 sq mi		
		Case I P_{16}	Case II $P_{1.0}$	Case III $P_{0.1}$
High	5000	4100	400	100
Moderate	150	125	2.5	0.5
Low	5	4	0.4	0.1

TABLE II

SEEPAGE-PRONE AREAS OF THE WORLD'S OCEANS

Ocean	High Potential Seepage Area (1000 sq mi)	Moderate Potential Seepage Area (1000 sq mi)	Low Potential Seepage Area (1000 sq mi)
Pacific	568	2715	1241
Atlantic	381	3030	3289
Indian	145	2318	880
Arctic	---	1648	718
Southern	---	142	134
All Oceans	1094	9853	6262

TABLE III

SUMMARY OF WORLDWIDE SEEPAGE RATES:
REGIONAL BREAKDOWN BY OCEANS
(metric tons/year)

	Case I P_{16}	Case II $P_{1.0}$	Case III $P_{0.1}$
Pacific	2.83×10^6	2.69×10^5	0.689×10^5
Atlantic	2.06×10^6	1.96×10^5	5.04×10^4
Indian	9.30×10^5	8.85×10^4	2.28×10^4
South	1.88×10^4	1.74×10^3	4.51×10^2
Arctic	2.14×10^5	2.30×10^3	5.20×10^3
World	6.05×10^6	0.558×10^6	0.148×10^6

TABLE IV

HYDROCARBON CONTENT OF MARINE ALGAE

	Hydrocarbon Content ppm (Dry Weight)
Twelve algae (Clark and Blumer, 1967)	8-120 (n-alkanes) Avg. 47
Four algae (Clark and Blumer, 1967)	34-120 (n-alkanes) Avg. 72
Two algae (Gelpi et al., 1968)	1000-3000 Avg. 2000
Four algae (Han et al., 1968)	250-380 Avg. 325
Four algae (Winters et al., 1969)	500-1200 Avg. 850
Twenty-three algae (Youngblood et al., 1971)	0.5-4810 Avg. 450

Avg. 49 samples 400 ppm

TABLE V

ESTIMATES OF BIOGENIC HYDROCARBON PRODUCTION

Organic Productivity Tons/Yr, Org. C	HC Content of Marine Organisms, ppm	Biogenic HC Production, Tons/Yr
40×10^9	400	16×10^6
(Average of Estimates by Datsko, 1959; Winberg, 1960; Ryther, 1969; Bolin, 1970; Borgorov, 1971)		
	50	2×10^6

HYDROCARBON POLLUTION MONITORING - A NATIONAL IMPERATIVE

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HYDROCARBON POLLUTION MONITORING - A NATIONAL IMPERATIVE

Over the past few years, mainly since the passage of the National Environmental Policy Act in 1969, the people of this country have become more acutely aware of the quality of their environment and what they contribute to the whole picture through everyday living, industry, and farming activities. As a Nation, we are more concerned with these problems, and potential problems than any other Nation in the world as exemplified by the number of agencies involved in the study, regulation, detection, and prevention of pollution. Every cabinet level agency is engaged in one or more of these activities including the Postmaster General's Office. We are concerned with particulate matter, trace metals, hydrocarbons, nutrients, noxious gases, solid waste, acid waste, cellar dirt, and even noise. In short, we have become a nation with an imperative mission, to maintain our status among the industrial nations of the world and simultaneously to ensure the integrity of our environment so that our scion may also enjoy the qualities of life. Not an easy task in any sense, we have attacked it vigorously; the U.S. Government spends billions of dollars annually in environmental research and protection activities. The Sixth Annual Report of the Council on Environmental Quality[1] (1975) shows that more than $4.2 billion was spent on pollution abatement activity in Fiscal Year 1975 and that figure was to jump to $4.7 billion in Fiscal Year 1976. Untold billions more were spent by state and local governments and industry for the same purpose.

How do we best deal with the problem of environmental protection? There are several avenues of approach that one can take in controlling pollution: i.e. eliminate the use of the pollutants in question; identify the sources and control the introduction of pollutants into the environment; prevent their dissemination once introduced; or understand and negate the effect of the pollutants. In order for any of these approaches to be effective, there must be an equally effective monitoring program. If you ban the use of certain pollutants then you must monitor manufacturer's production or retail outlets; if you want to control discharge, then you must monitor potential sources; if you want to control dispersion once pollutants are introduced, you must monitor their movement once they are in the environment for evidence of their presence. There are presently in operation monitoring systems for each category mentioned.

What will be discussed in this paper are some of the larger hydrocarbon monitoring programs in effect at the present time and what their objectives are. From there to a more philosophical discussion of what environmental monitoring is, or perhaps should be. Finally, to a discussion of some of the problems that we are facing in environmental monitoring of petroleum hydrocarbons, and how we might solve them.

There are basically four programs in effect at the present time which are oriented toward monitoring petroleum hydrocarbons in the environment: one conducted by the U.S. Environmental Protection Agency (EPA); one by the U.S. Coast Guard; that conducted by the U.S. Geological Survey; and that conducted by the United Nations through the Intergovernmental Oceanographic Commission (IOC-UNESCO).

Since EPA is basically a regulatory agency whose primary function is enforcement of environmental air and water quality standards, their petroleum hydrocarbon monitoring is slanted toward identifying the substances of concern and controlling their release into the environment. It includes examination of the atmosphere, soils, water, and tissue as well as critical determination of health effects. The EPA program is strongest in the areas of air and water quality (primarily fresh water). They have only more recently ventured into the offshore for special projects such as ocean dumping and sewage outfalls. Their monitoring objectives are to observe in a systematic and continuous way the environmental quality parameters (including physical-chemical, biological, and microbiological) for the purpose of providing a sound data base for development of criteria for regulatory and operating programs. Their concern is abiding, and in fact they maintain an Office of Monitoring Systems. In October of 1973 the Office sponsored a "Seminar on Methodology for Monitoring the Marine Environment," the proceedings of which is now a publication of EPA[2]. Air quality standards, water quality standards, effluent guidelines, automobile emission standards, are but a few of the fruits of their efforts. Billions of dollars are spent annually by EPA on determining what are, and are not, harmful substances, whether or not they are entering the environment, how to prevent them from getting into the environment (technology assessment, regulations, R&D, and inspection), and monitoring to ensure compliance.

The U.S. Coast Guard (USCG) has the responsibility for detecting hydrocarbon pollution events in the coastal marine environment and cleaning them up as rapidly as possible. They are also charged with attempting to determine who is responsible for a polluting event and assessing damages against the guilty party. Coast Guard has initiated

two programs to achieve this mission: The Pollution Incident Reporting System (PIRS)[3] and the Transportation Induced Pollution Surveillance System (TIPS). PIRS is exactly what its name implies, a system whereby polluting events occurring within the jurisdictional limits of the USCG are reported immediately to the Regional Headquarters and investigated to assess the nature and extent of the damage. The Coast Guard maintains all statistics on polluting events in the PIRS, and publishes yearly summaries. They use the data to make recommendations for up-grading technology or for modifying regulations regarding operations within their purview. The Coast Guard also maintains an airborne surveillance system that is designed to detect petroleum hydrocarbons in coastal waters and to estimate the volume and nature of the spill and the direction in which it is moving. They have, for several years, been using a system called the Airborne Remote Surveillance System (ARSS) which consists of infrared and ultraviolet line scanners that are flown in Albatross (HU-19) Aircraft on routine missions over the coastal areas. At present, an average of two missions per week are flown (more often in heavily traveled shipping areas) concentrating on high density shipping lanes and offshore production areas. Only one of the ARSS systems is still operational; they are soon to be replaced by the Airborne Oil Surveillance System (AOSS) which was described in detail in a paper[4] presented by at the Oil Spill Conference in San Francisco in March, 1975. Briefly, it consists of a passive microwave imager, low light level television system, infrared and untraviolet line scanners, and both side-looking and front-looking radar. The system has recently undergone a comprehensive field testing, the results of which are published in Coast Guard Technical Report[5], CG-D-90-75. The system is being converted to a C-130 which will be operational in the spring of 1977. USCG hopes to have a number of the AOSS systems in the air in small jets by the end of 1977.

Basically the Coast Guard is conducting a combination of the second and third types of monitoring programs that were mentioned at the beginning of this paper; namely, to control dispersion and movement and also to control discharge from the source.

The U.S. Geological Survey (USGS) conducts a petroleum monitoring program in the form of inspections of offshore oil and gas exploration and production facilities to ensure compliance with existing Department of the Interior Operating Regulations and Operating Orders. This is also an example of the second type of monitoring program intended to control discharges by proper use and maintenance of the best available technology. Where the USGS inspectors find the

operators in non-compliance they either issue a warning, which carries a mandatory period in which the operator must come into compliance, or they may shut-in all activity depending on the severity of the violation.

In 1974, the Intergovernmental Oceanographic Commission (IOC) of the United Nations expanded their Integrated Global Ocean Station System (IGOSS)[6] Program to include a Marine Pollution Monitoring Pilot Project[7]. The Pilot Project, actually begun in January of 1975, was aimed toward the development of an organizational framework and toward gaining experience in the development of a larger program of monitoring the discharge and distribution of petroleum hydrocarbons in the marine environment. A number of currently available publications describe the program in detail; however, it would be useful to summarize the status of that project. Results of the first year of the IGOSS Pollution Monitoring Program show that 1475 reports[8] were received by the National Oceanographic Data Center of NOAA including 103 reports observing pollutants, 446 Tar Ball Observations, and 280 Dissolved/Dispersed Hydrocarbon Reports. The most recent meeting of the IGOSS Marine Pollution Monitoring Committee in June of 1976 resulted in the recommendation that the program be continued for two more years because of its success to date[9]. This is a major step forward in our understanding of the worldwide distribution of petroleum hydrocarbons in the marine environment, and will hopefully lead to some type of viable regulatory program to control discharges.

What of the nature of these programs? Are they truly monitoring programs? Are they indicative of the monitoring programs of the future? If not, why not? Upon investigation of this subject, it came to light that the Atomic Energy Commission (AEC, now ERDA), the Defense Department, and the Public Health Service have been at the task of monitoring longer than any other agencies. Although the contaminants are different, the example that follows is valid and quite analogous to the situation involving petroleum hydrocarbons. In the 1940's the Federal Government through the aforementioned agencies assumed the responsibility of monitoring the impact of weapons testing on the environment. What were the contaminants of concern? What were the critical pathways in the environment? What effects do they have? How do we detect changes in the ambient levels of the contaminants with which we are concerned? The first tack that was taken was to conduct an "Inventory Program"

which measured the levels of the pollutants at many different points in the ecosystem. This was an expensive but necessary first step in the evolution of a monitoring program. It was soon realized that certain factors are more critical than others in determining the effects of changes in levels of radionuclides. When the critical pathways were examined it was then determined where the most likely step to monitor was located. In this case it is most instructive to note several results.

It was found, quite by accident, that iodine-131, a product of weapons testing, was a very easy isotope to detect and quantify. It was also found that this isotope of iodine goes from the atmosphere to the pasture grass, is ingested by cows where it is concentrated in the milk and ultimately into the human thyroid. The easy answer then was to monitor the levels of radioactive iodine in cow's milk rather than measuring levels in air samples and pasture grass or human thyroids because cows acted as real-time integrators of the influx of radioactive iodine.

One other example is instructive. It was determined early in the program that strontium was a bone-seeking element and cesium was a tissue-seeking element; they both acted similarly with regard to critical pathways and maintained a more or less constant ratio. Strontium is more expensive and time-consuming to determine than cesium, so it was decided that only cesium would be routinely measured with an occasional check on strontium to reaffirm the premise. So far this approach has been highly successful in monitoring the levels of radionuclides.

What does this all have to do with developing monitoring programs for petroleum hydrocarbons? At present, we are at a stage comparable to the radionuclides program of the late 1940's. We are taking a shotgun approach to the problem in that we are measuring ambient levels of hydrocarbons in the air, water, sediments, organisms, and even human tissue. We are looking at the full range of hydrocarbons. Some people advocate looking at the low molecular weight compounds, others the high molecular weight compounds; some the aromatics, others the aliphatics; some advocate using gas chromatography-mass spectrometry techniques, others insist that fluorescence techniques are cheaper and just as reliable. The answers simply do not exist at the present time although there has been, and is continuing to be, some excellent research to answer these questions.

This analogy is not intended to imply that the problem of monitoring petroleum hydrocarbons will be simple. Far from it!! Not only are hydrocarbons, similar to those found in petroleum, produced in nature, but also they are degraded quite readily and altered into compounds not readily associated with a petroleum origin. There are also natural sources of petroleum hydrocarbons as well as man-related sources.

The lesson to be learned from the radionuclide experience is that any successful monitoring program must have the following characteristics:

I. It must reflect an understanding of the nature of the system. A knowledge of the basic interrelationships among components and the key pathways within the system is essential to the development of an effective monitoring program. We may in fact be attending to details when the problems lie in our understanding of the system. We must understand what the system is like if we are to measure the critical factors. The truth is we don't understand the system well enough, nor do we understand the movement of petroleum hydrocarbons through the system.

II. Whatever observational elements are chosen for a monitoring system, they are only valuable if they are sensitive to significant change that can be related to the activity being monitored, and they give results that will permit response within the lifespan of the polluting event, and they are quick, easy, and reliable measurements to make.

III. The approach must be multi-faceted. It must include collection and analysis of real-time field data, laboratory and controlled field studies on effects, benchmark studies (at least in the early stages), and modelling for predictive purposes.

We are in the late infant stages of petroleum hydrocarbon pollution monitoring. We have the capability of detecting large volumes of such substances, but we don't have a rapid, reliable technique for determining the presence of small amounts of petroleum hydrocarbons. The National Science Foundation, the American Petroleum Institute, the U.S. Environmental Protection Agency, the Bureau of Land Management[10], and many others are working on the critical questions that will permit us to design an approach that meets the criteria of a viable monitoring program.

Too frequently we are moving down parallel pathways at great manpower and monetary expense, resulting in a diluted effort that comes too late to be effective. We must work toward a cooperative program of study that takes into account the requirements of all agencies interested in petroleum hydrocarbons.

A national program of petroleum hydrocarbon monitoring is an imperative. The magnitude of the problem will not diminish, what must change is the approach we are taking.

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DISCUSSION

WHIPPLE: I would like to ask if the monitoring system that is envisaged here would be something that would be taken over by one of the agencies or how it might be patterned between the various agencies who have a certain part of the responsibility now.

MONASTERO: I don't know. I think that the agencies are making giant strides toward the development of a unified program of environmental monitoring. We have had some excellent cooperation.

We are kind of under fire right now because we at BLM are the most visible at the moment. We have organized a committee called the OCS, Outer Continental Shelf Environmental Studies Advisory Committee. It has on it the agencies that I mentioned, as well as the cooperation of the U. S. Geological Survey, the Fish and Wildlife Service, the National Science Foundation, and the governors of the 21 coastal states. They advise us on the kinds of programs that we carry out in studying the environmental impact of offshore exploration and development of hydrocarbons, i.e., studying the petroleum hydrocarbons in the marine environment.

It is the same problem that you are facing all the time with any program. What has to be done, I guess, is either you get the focus by having one agency receive the bulk of the money to do the work or you get some kind of an administrative mandate handed down that says that you will do the work. That is just about the only way you get it done.

If you have any suggestions, I would certainly appreciate them.

SESSION II

CYCLING

Chairman
James N. Butler
Harvard University

(Dr. Bostwick H. Ketchum was originally scheduled as chairman but was unable to attend the Symposium. However, he provided valuable assistance in organizing the Session.)

RECENT BIOGENIC HYDROCARBONS

BIOGENESIS OF NONISOPRENOID ALIPHATIC HYDROCARBONS

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BIOGENESIS OF NONISOPRENOID ALIPHATIC HYDROCARBONS

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ABSTRACT

Nonisoprenoid aliphatic hydrocarbons are widely distributed in both the animal and plant kingdoms. n-Alkanes, methyl branched alkanes, and n-alkenes with odd numbers of carbon atoms constitute the major hydrocarbons. Probable mechanisms for the biogenesis of hydrocarbons in bacteria, algae, higher plants and insects are discussed. *In vivo* and *in vitro* studies strongly suggest that elongation of a fatty acid followed by decarboxylation give rise to the n-alkanes and most of the olefins found in biological systems. Direct evidence for decarboxylation of an exogenous fatty acid to the corresponding alkane is presented. Experimental evidence show that *iso*-alkanes and *anteiso*-alkanes are most probably derived by decarboxylation of the appropriately branched acids which are synthesized from branched starters derived from branched amino acids. Internal methyl branches of the alkanes in algae are derived from S-adenosylmethionine whereas such branches might be derived from methylmalonyl-CoA in insects. Dianteisoolefins in certain bacteria might be derived by a head-to-head condensation between two fatty acid derivatives.

INTRODUCTION

Aliphatic non-isoprenoid hydrocarbons are widely distributed among the organisms which presently live in the biosphere, even though biogenic origin of petroleum has not been established. It is difficult to estimate the amount of such hydrocarbons produced by living organisms. It has been estimated that 2.5 to 10 x 10^{10} tons of dry material is produced per year by land plants alone (1). From the analytical results obtained with a wide variety of plants it appears that hydrocarbons constitute at least 0.1% of the dry matter. With this approximation, it would appear that 25 to 100 million tons of aliphatic non-isoprenoid hydrocarbons are produced per year by land plants alone. Based on the estimate that 3 x 10^{10} tons of phytoplankton are produced per year in the oceans (2), an additional 15 million tons of hydrocarbons would be produced per year by these organisms, assuming that hydrocarbons constitute 0.05% of the dry weight. This estimate, based on analyses of some of the marine algae, is probably conservative. Another major source of biological hydrocarbons is the insect population, but considering the great diversity of insects and the uncertainty concerning the source of the hydrocarbons found in them, it is difficult to estimate the quantity of hydrocarbons produced by insects, although their contribution must be substantial.

Even though it is widely accepted that the predominance of odd-chain hydrocarbons is a hallmark of recent biogenesis, the mechanism of biogenesis of hydrocarbons has been largely ignored. In this paper I shall briefly summarize the current state of our knowledge concerning biogenesis of non-isoprenoid aliphatic hydrocarbons. The distribution of biogenic hydrocarbons is indicated in Table 1 and is discussed in an extremely brief manner only as an introduction to the biogenesis. A fairly comprehensive coverage of this subject can be found in a recent book on the chemistry and biochemistry of natural waxes, which deals with distribution as well as biogenesis of hydrocarbons in marine organisms (2), insects (3), fungi and algae (4), higher plants (5,6), and bacteria (7). Other reviews on the distribution and biogenesis of hydrocarbons are also available (8,9,10,11).

OCCURRENCE AND DISTRIBUTION OF ALIPHATIC HYDROCARBONS

Hydrocarbons occur in essentially every living organism from microbes to man (12). The occurrence of alkanes as a biological component was originally recognized in the plant waxes almost half a century ago; during the past decade alkanes have been also found in a variety of internal tissues of animals (13-20). In humans, alkanes have been found in arterial tissues and plaques, lymph nodes, spleen, liver, meninges, and meningiomas (21-27). Since the 1940's, there has been a conspicuous increase in follicular lipidosis and hydrocarbon accumulation in lymph nodes in humans (25). Certain diseases, including cancer, appear to cause accumulation of hydrocarbons in human tissues (22,23,24, 26,27,28,29). Fungal infection can alter the pattern of hydrocarbon synthesis in some organisms (30). Even biological membranes have been found to contain hydrocarbons (31). Thus, hydrocarbons are indeed bonafide biological components.

Among biological hydrocarbons, n-alkanes appear to be the dominant group. In lower organisms such as algae n-C_{17} or n-C_{15} predominate (4), whereas in higher organisms, such as higher plants and animals, alkanes from C_{23} to C_{33} are dominant (5). In most cases odd chain hydrocarbons are by far the major components, although even-chain alkanes are invariably present in smaller quantities. In many organisms, particularly in plants, one or a few alkanes predominate; in *Brassica*, for example, n-C_{29} constitutes more than 90% of the hydrocarbon fraction, whereas in *Pisum sativum* n-C_{31} is the dominant (> 90%) alkane (5). Most probably C_{29} and C_{31} are the major alkanes of higher plants.

Branched hydrocarbons are also quite widely distributed in biological systems, although in most cases they are quantitatively less significant than the n-alkanes. The most common types of branches are 2-methyl (*iso*) and 3-methyl (*anteiso*). The chain lengths of such branched hydrocarbons are in the same range as that of n-alkanes. Among the *iso*-alkanes, odd carbon numbers usually predominate, whereas even carbon numbers dominate the *anteiso*-alkanes. The biochemical reason for this distribution will be apparent from the ensuing discussion of their biogenesis. Internally branched hydrocarbons are also found in biological systems. For example, in some lower organisms, such as the

blue-green algae, a significant proportion of the hydrocarbons have methyl branches in or near the middle of the carbon chain (32,33). Multiple methyl-branched hydrocarbons are also found in some organisms, although they are more rare and quantitatively less significant than the other types of hydrocarbons. For example, in certain insects a variety of multiple methyl-branched hydrocarbons have been identified (3).

A variety of unsaturated aliphatic non-isoprenoid hydrocarbons have been found in living organisms. They constitute major components of the hydrocarbon fraction only in lower organisms such as bacteria (7) and algae (4), although their distribution is almost as widespread as that of alkanes. The chain length range of the unsaturated hydrocarbons and the occurrence of methyl branches in them are similar to those of alkanes. In the monounsaturated hydrocarbons, the double bond has been found in almost every conceivable position up to Δ^{10}, and *cis* as well as *trans* double bonds have been observed, although the former is more prevalent than the latter (34). In some relatively rare cases, such as *Sarcina lutea*, the double bond is near the middle of the carbon chain and both ends of the olefin have methyl branches (35). The chain length range of the diunsaturated hydrocarbons, which seem to be substantial components of the hydrocarbon fraction only in algae (4) and certain insects (3), is similar to that of the alkane fraction, and the position and the geometry of the double bonds in these olefins can be explained by reasonable biogenetic schemes discussed in a following section. Tri- and tetraunsaturated hydrocarbons are usually only minor components, and their occurrence appears to be limited to algae. Polyunsaturated hydrocarbons are of very limited occurrence, but a striking example of such an olefin is the predominance of all-*cis*-3,6,9,12,15,18-heneicosahexaene in the hydrocarbon fraction of a variety of algae, especially from marine phytoplankton (36,37,38).

BIOSYNTHESIS OF n-ALKANES

In higher plants n-alkanes constitute the most common component of the surface wax, which often occurs in crystalline form on the surface (Fig. 1). These surface waxes can be readily extracted with little contamination from internal lipids by a brief (10-30 sec) dip of the plant tissue in an organic solvent such as chloroform. Subsequent thin-layer chromatographic analysis provides a hydrocarbon fraction which upon gas-chromatography often reveals a simple pattern of chain length distribution (Fig. 2). Furthermore, in rapidly expanding tissues such as leaves, biosynthesis of alkanes occurs during a relatively short period of time so that exogenous precursors can be incorporated quite readily into alkanes. For these reasons, much of our knowledge concerning biogenesis of alkanes is derived from biosynthetic studies on higher plants.

Occurrence of hydrocarbons, approximately twice as long as the usual fatty acids, together with the corresponding ketones and secondary alcohols with their functional group in the middle of the chain, prompted

the early workers to propose a head-to-head condensation pathway for the biosynthesis of hydrocarbons and their oxygenated derivatives (discussed in Ref. 7). According to this pathway (Fig. 3), two molecules of C_{15} acid would condense head-to-head, with decarboxylation of one of them to give n-nonacosan-15-one. This ketone upon reduction would produce n-nonacosan-15-ol, which on dehydration followed by reduction would give rise to n-nonacosane, one of the most prevalent hydrocarbons in the plant kingdom. The presence of the postulated intermediates, namely the ketone and the secondary alcohol in *Brassica* wax supported this hypothesis. However, this hypothesis was soon abandoned because C_{15} acid was not known to occur in plants, and the presumed intermediates, the ketones and the secondary alcohols, could not be found in many plants which contained hydrocarbons. However, the recent discoveries of the widespread occurrence of odd-chain acids in plants, coupled to the demonstration of very active α-oxidation systems in young leaves, revived the classical head-to-head condensation hypothesis. The experimental evidence which supported such a head-to-head condensation in the biosynthesis of corynomycolic acid (39) strengthened a similar argument for alkane biogenesis. However, no direct experimental tests had been performed to test the validity of the head-to-head condensation hypothesis until the mid 1960's.

In vivo Experiments

The finding that exogenous precursors were readily incorporated into n-C_{29} alkane in young broccoli (*B. oleracea*) leaves provided an opportunity to test the head-to-head condensation hypothesis (40). The following experimental results (discussed more fully in Ref. 7) ruled out this hypothesis. If α-oxidation of C_{16} acid is involved, as shown in Figure 3, the carboxyl carbon of this acid would be lost, but the remaining carbons could be incorporated into the C_{29} compounds. However, [1-^{14}C]C_{16} acid labeled the C_{29} compounds just as effectively as did [U-^{14}C]C_{16} acid or [16-^{14}C]C_{16} acid. Double-labeling experiments confirmed that the intact carbon chain of C_{16} acid was incorporated into the C_{29} compounds. Similar experiments with [1-^{14}C-9,10-^{3}H]stearic acid showed that the intact carbon chain of this acid was also incorporated into the C_{29} compounds in *B. oleracea* (Table 2) (41). With the same techniques it was shown that exogenous C_{16} and C_{18} acids were incorporated into C_{31} alkane in pea and spinach leaves without involving any loss of their carboxyl carbon. Furthermore, time-course experiments with *B. oleracea* showed that hydrocarbons had higher specific activities at all times than the ketones or secondary alcohols. This finding is inconsistant with the head-to-head condensation hypothesis according to which of the ketones and secondary alcohols are intermediates in the synthesis of hydrocarbons and therefore should have higher specific activities than the hydrocarbons. Furthermore, exogenous labeled n-nonacosan-15-one failed to be converted into n-nonacosane in *B. oleracea* and roses. This experimental evidence prompted us to abandon the classical head-to-head condesation hypothesis and to look for alternative pathways.

Incorporation of the intact carbon chain of C_{16} acid into hydrocarbons and the observation that C_{18} acid was incorporated nearly three times as fast as C_{16} acid into hydrocarbons in *B. oleracea*, pea and spinach leaves, led to the proposal of an elongation-decarboxylation hypothesis for the biosynthesis of paraffins in plants (92). According to this hypothesis (Fig. 4), palmitic acid, the usual end product of fatty acid synthetase, becomes the substrate for an elongation system which adds C_2 units from malonyl-CoA until the chain length reaches C_{30} or C_{32} and then decarboxylates this acid releasing the hydrocarbon.

The following experimental evidence strongly supports this elongation-decarboxylation pathway:

1. Double-labeling experiments showed that C_{12}, C_{14}, C_{16}, and C_{18} acids were incorporated into alkanes without any degradation of their carbon chains.

2. The labeled hydrocarbon formed in a given tissue is the same irrespective of the chain length of the labeled precursor (C_2-C_{22}) used. The elongation-decarboxylation hypothesis provides the best explanation for this observation.

3. Tissues which synthesized labeled hydrocarbons from radioactive fatty acids also contained labeled very long chain acids, including appropriately branched acids where branched alkanes were formed, and a variety of experimental evidence summarized elsewhere (10) strongly suggested that the elongation process was directly related to biogenesis of alkanes.

4. In *Alium porrum*, distribution of ^{14}C incorporated from [U-^{14}C]acetate into fatty acids and alkanes showed a precursor-product relationship consistent with the elongation-decarboxylation mechanism (43). Specific activities for each acid and the corresponding alkane also support this mechanism (Fig. 5).

5. Blockage at the terminal step of alkane synthesis by chemical inhibitors in *P. sativum* resulted in an accumulation of even chain compounds one carbon longer than the alkane. Inhibition of incorporation of labeled acetate into C_{31} alkane by dithioerythritol in pea leaves was accompanied by an increase of labeled C_{32} aldehyde, which suggested that this aldehyde was derived from a C_{32} precursor of C_{31} alkane (44). In addition, a concentration of trichloroacetic acid that inhibited C_{31} alkane synthesis, specifically inhibited the formation of C_{32} aldehyde but not the synthesis of C_{26} and C_{28} aldehydes and alcohols. The relationship between C_{32} aldehyde and C_{31} alkane was also demonstrated by the presence of large quantities of C_{32} aldehyde (35% of the aldehyde fraction) in young pea leaves which synthesize C_{31} alkane most rapidly. Older leaves, which did not synthesize much alkane, contained mainly C_{26} and C_{28} aldehydes, with very small amounts of C_{32} aldehyde (< 10%).

6. Genetic blocks of alkane synthesis at the decarboxylation step in *B. oleracea* and *P. sativum* also provide strong support for the elongation-decarboxylation mechanism (45,46,47). In the gl_4 mutant of *B. oleracea*, C_{29} alkane synthesis was blocked and C_{30} acid accumulated. Incorporation of labeled acetate into the alkanes in this mutant was only 2% of that observed with normal *B. oleracea*, whereas no difference in the labeling of the very long acids and aldehydes was detected. In the *P. sativum* mutant, wsp, significant decreases in C_{31} alkane and C_{31} secondary alcohol were accompanied by an accumulation of aldehydes, particularly the C_{32} aldehyde.

7. Exogenous [1-^{14}C]C_{24} acid gave rise to C_{25}, C_{27}, C_{29}, and C_{31} alkanes in *A. porrum*, but no n-C_{23} alkane could be detected, whereas 27% of the label contained in the alkane fraction derived from [G-^{3}H]C_{24} acid was in C_{23} Alkane (48).

8. Recently, direct conversion of exogenous labeled long chain fatty acids into alkanes containing one carbon atom less than the precursor acid has been demonstrated in several plant tissues. In *Nostoc muscorum*, which synthesizes n-C_{17} as the major alkane, exogenous labeled n-C_{16} acid gave rise to n-C_{15} alkane and n-C_{17} alkane, the latter most likely by elongation followed by decarboxylation (49). In the same organism exogenous n-C_{18} acid was converted to n-C_{17} alkane. This system can be taken as a primitive model for the more sophisticated elongation-decarboxylation systems that evolved in the higher plants. It was demonstrated that exogenous [G-^{3}H]C_{30} acid was directly converted into C_{29} alkane in *B. oleracea* (50). In *A. porrum*, [G-^{3}H]C_{24} acid was readily converted into C_{23} alkane, whereas [1-^{14}C]C_{24} acid labeled only C_{25} and longer alkanes, but not C_{23} alkane. [G-^{3}H]C_{24} acid was converted into longer acids and the corresponding alkanes at rates slower than that of its conversion into C_{23} alkane (Fig. 6). Thus, it appeared that both direct decarboxylation and elongation followed by decarboxylation occurred in this tissue. The most convincing *in vivo* evidence for the decarboxylation mechanism was obtained with the flower petals of *Vicia faba*, a tissue which contains three major n-alkanes, C_{27}, C_{29}, and C_{31}. In this tissue, exogenous [G-^{3}H]C_{28} acid gave rise to labeled C_{27} alkane, the direct decarboxylation product, as well as labeled C_{29} and C_{31} alkanes, expected from elongation followed by decarboxylation (51). [G-^{3}H]C_{30} acid underwent decarboxylation to C_{29} alkane, and elongation followed by decarboxylation to give C_{31} alkane (Fig. 7). On the other hand, [9,10,11-^{3}H]C_{32} acid produced only labeled C_{31} alkane. Further evidence for elongation-decarboxylation mechanism was provided by the observation that trichloroacetate, an inhibitor of elongation process, inhibited the conversion of [G-^{3}H]C_{28} acid into C_{29} and C_{31} alkanes, but not its direct decarboxylation into C_{27} alkane. In addition, dithioerythritol, an inhibitor of the decarboxylation process, did, in fact, inhibit the conversion of C_{32} acid into C_{31} alkane.

Synthesis of Alkanes by Cell-Free Preparations

Cell-free preparations from excised epidermis of pea leaves and from whole leaf extracts catalyzed the decarboxylation of [9,10,11-^{3}H]C_{32} acid to alkanes in the presence of ascrobic acid and oxygen (Table 3) (52). The products were identified by radio gas-liquid chromatography as n-C_{31} alkane and n-C_{30} alkane, suggesting that both direct decarboxylation and α-oxidation followed by decarboxylation occurred (Fig. 8). Imidazole, an inhibitor of α-oxidation, inhibited the formation of both n-C_{30} alkane and n-C_{31} alkane from n-C_{32} acid, and synthetic α-hydroxy C_{32} acid was converted to n-C_{31} alkane. Therefore, this hydroxy acid was suggested to be an intermediate in the conversion of C_{32} acid to C_{31} alkane. Alkane formation by the cell-free preparation was also inhibited by p-chloromercuribenzoate, dithioerythritol, ethylenediamine tetraacetate, and o-phenanthroline. The decarboxylase activity was located in the 100,000 xg supernatant. All experimental evidence thus far obtained suggests that an intermediate in the α-oxidation process is an intermediate in the conversion of the fatty acid to the corresponding alkane. However, the mechanism of decarboxylation of a fatty acid to an alkane, and the nature of the intermediates involved in this process, remain to be elucidated.

Biosynthetic Relationships Among Alkanes and Their Oxygenated Derivatives

The elongation-decarboxylation mechanism for alkane biosynthesis does not explain the occurrence of the secondary alcohols and ketones of the same chain length as the major alkanes in plant waxes. Experiments with [1-^{14}C,2-^{3}H]palmitic acid strongly suggested that the ketone and the secondary alcohol are probably derived from the corresponding alkane (53). Direct evidence for this conclusion was obtained when it was shown that exogenous labeled nonacosane was directly converted into secondary alcohol and ketone, while exogenous labeled secondary alcohol was converted only into the corresponding ketone (54).

The nature of the reaction that introduces the oxygen into a long-chain alkane is not well understood. One possibility is a dehydrogenation, of the type observed with lower alkane homologs in bacteria, followed by hydration of the olefin to give a secondary alcohol. The recent finding that secondary alcohols of *B. oleracea* comprise both nonacosan-14-ol and the 15-ol would be consistent with this mechanism (55). Alternatively, a mixed function oxidase-type enzyme could hydroxylate a specific carbon at the center of the alkane chain. In accordance with this hypothesis, incorporation of low levels of ^{18}O from molecular oxygen into the ketone was detected in *B. oleracea*. Furthermore, the conversion of hydrocarbon into ketone in *B. oleracea* required molecular oxygen, and this reaction was inhibited by chelating agents such as phenanthroline. As is expected of a mixed function oxidase reaction involving Fe^{+2}, the inhibition by phenanthroline was at least partially reversed by exogenous Fe^{+2}. The experimental evidence thus far obtained indicates that alkanes are hydroxylated to secondary alcohols, which in turn are oxidized to ketones, in plants such as *B. oleracea*. In other plants, such as pea, the latter oxidation step is missing and therefore no ketones are found.

Chemical degradation of the labeled secondary alcohol fraction derived from exogenous n-[R-^{3}H]nonacosane in *B. oleracea* leaves (55) showed that the exogenous alkane was converted into a mixture of nonacosan-14-ol and nonacosan-15-ol in an approximate ratio of 2:3. The naturally occurring secondary alcohol fraction also contained the same two positional isomers, in approximately the same ratio. However, the labeled ketone fraction derived from the alkane was exclusively nonacosan-15-one, which is the only naturally occurring major ketone. It is not clear why nonacosan-14-ol is not converted into the corresponding ketone. If the hydroxylation occurs at the C_{30} acid level, and the reaction is specific for C-16 position of the acid, the major secondary alcohol would be the 15-ol. The lack of absolute specificity could result in hydroxylation at C-15 and C-17 positions of the acid and both of the resulting hydroxy acids would give the 14-ol on decarboxylation. In any case the oxidation of the secondary alcohol function, whether it occurs prior to or after decarboxylation, must be specific for the C-16 position of the acid or the C-15 position of the secondary alcohol.

The above conclusions concerning the biochemical conversion of alkanes to secondary alcohols have been confirmed also in insects. Thus, labeled n-alkanes administered via the diet or to the surface of the grasshopper *Melanopus sanguinipes* were converted to secondary alcohols, which were subsequently esterified (56,57). Shorter chain alkanes C_{21}, C_{23}, and C_{25} were more readily converted to secondary alcohols than the longer alkanes C_{27} and C_{29}. This chain length specificity is reflected in the natural surface lipid composition of this insect, in that the major alkanes are C_{27} and C_{29} whereas the major secondary alcohols are shorter, C_{21}, C_{23}, and C_{25}. A mixed function oxidase was suggested to be involved in the conversion of alkanes into secondary alcohols in this insect, as was previously suggested by the results obtained with higher plants. Thus, incubation of *M. sanguinipes* with $^{18}O_2$ or $H_2{}^{18}O$ followed by mass spectrometry of the isolated secondary alcohols showed that the oxygen of the secondary alcohol originated from O_2 and not from H_2O. The only case, where a different mechanism for secondary alcohol synthesis has been suggested is that of the bacterium *S. lutea*, in which hydration of an olefin was postulated as a mechanism for secondary alcohol biosynthesis (58).

BIOSYNTHESIS OF BRANCHED HYDROCARBONS

Iso- and *Anteiso*-Branches

The most probable mechanism for the biosynthesis of *iso*- and *anteiso*-hydrocarbons would appear to be by elongation and decarboxylation of the appropriately branched fatty acid. Such acids could be derived from the appropriately branched starter acids, generated from the branched amino acids. Thus, valine could give rise to C_4 *iso*-branched starter acid, which would generate *iso*-C_{16} acid. This acid upon elongation should give *iso*-branched very long acids with even number of carbon atoms, and upon decarboxylation these acids should generate *iso*-alkanes with an odd number of carbon atoms (Fig. 9).

Similarly, isoleucine would generate *anteiso*-branched C_5 starter pieces, which should give *anteiso*-branched fatty acids with an odd number of carbon atoms and *anteiso*-alkanes with an even number of carbon atoms (Fig. 9). This hypothesis explains the observed distribution of naturally occurring *iso*- and *anteiso*-alkanes, as in the former group odd chain alkanes, and in the latter group even chain alkanes, predominate as predicted.

In support of this hypothesis, labeled valine and isobutyric acid fed to tobacco plants (59) or their excised leaves (60) and to *A. porrum* (61) were incorporated preferenctially into odd chain *iso*-alkanes. In tobacco leaves, *iso*-branched C_{16} to C_{28} even chain fatty acids were also detected. Similarly, exogenous labeled isoleucine gave rise to labeled even chain *anteiso*-alkanes as well as to labeled odd chain *anteiso*-branched C_{15} to C_{29} acids in tobacco leaves (Fig. 10). These results strongly suggest that branched fatty acids derived from the branched starters undergo elongation and decarboxylation to give the branched alkanes.

Formation of branched hydrocarbons is apparently regulated by the enzyme system that synthesizes hydrocarbons. Tracer studies showed that plants such as *B. oleracea* which do not synthesize branched hydrocarbons can synthesize branched acids shorter than C_{20} from the branched starters (62). However, such acids were not incorporated into hydrocarbons, although they were incorporated into other surface lipid components such as wax esters. These conclusions are also supported by the more recent analyses which showed that the *B. oleracea* wax esters contained branched chains whereas hydrocarbons of the same plant did not (47). An interesting mutant of *B. oleracea* was discovered which contains, in addition to the normal hydrocarbons, *anteiso*-C_{30} hydrocarbon (63). Biochemical studies with such mutants could reveal the nature of the specificities involved in hydrocarbon synthesis, but such studies have not been reported.

Origin of Internal Methyl Branches

As indicated in an earlier section, internally methyl branched hydrocarbons are significant components in certain algae and insects, and they have been reported to be minor components also in other organisms. The internal branch could be introduced either by methylation of an olefinic function after the synthesis of the aliphatic chain (Fig. 11) or by substitution of a molecule of methylmalonyl-CoA for one malonyl-CoA during the synthesis of the aliphatic chain (Fig. 12). Experimental evidence obtained with algae strongly suggests the former mechanism (33,49), whereas the latter was suggested to be responsible for the biogenesis of internally branched alkanes in insects (64).

If the 1:1 mixture of 7- and 8-methyl C_{17} alkane found in the blue-green algae is formed by methylation of the appropriately unsaturated fatty acid precursor, vaccinic acid might be expected to be the precursor of these alkanes. This unsaturated acid was identified as a component of the cellular lipids of this organism, and exogenous tritiated vaccinic

acid was shown to be incorporated preferentially into methyl branched C_{18} alkane (49). Further evidence for the methylation pathway was obtained when it was found that in both *N. muscorum* (49) and *Anabaena variabilis* (33) exogenous [methyl-^{14}C]methionine specifically labeled the methyl branched C_{18} alkanes. The mechanism of methylation was investigated using exogenous [methyl-$^{2}H_3$]methionine. Mass spectrometry of the deuterated methyl branched C_{18} alkane, metabolically derived from the labeled methionine, showed that the intact CD_3 group was contained in the alkane. Thus, the methylation step most probably does not involve a cyclopropyl intermediate. A cell-free preparation from *A. variabilis* incorporated the methyl group from S-[methyl-^{14}C]adenosyl methionine into branched C_{18} alkanes (65). The apparent Km for S-adenosylmethionine was 1.1×10^{-4} M. The pH optimum was 7.0, and a partial dependence on NADPH could be demonstrated. Activity was inhibited by Cu^{2+}, Zn^{2+}, EDTA, dithiothreitol, each at 1 mM, and by 0.1% solutions of the detergents Triton X-100, sodium deoxycholate, sodium dodecyl sulfate, and cetylpyridinium chloride. As the methyl group of S-adenosylmethionine was transferred to an endogenous olefinic compound, and not to any added olefinic methyl acceptors, the nature of the endogenous acceptor remains abscure. Furthermore, the sequence of methylation and decarboxylation has not been elucidated. In any case, the evidence for a methylation pathway for the biogenesis of internally branched C_{18} alkanes in algae appears convincing.

Biosynthetic studies with a cockroach, *Periplaneta fuliginosa*, in which internally branched monomethyl alkanes (mainly 13-methylpentacosane) constitute almost 60% of the hydrocarbons, suggested that the methyl branch might be derived from methylmalonyl-CoA (64). In this organism, exogenous [methyl-^{14}C]methionine was not incorporated into alkanes, whereas labeled propionic acid, the expected precursor of methylmalonyl-CoA, gave rise to methyl branched alkanes. However, no proof that the methyl carbon of propionate was indeed converted into the methyl branch of the alkane was provided. Even though convincing experimental evidence for the involvement of methlmalonyl-CoA in the synthesis of internally branched alkanes is not available, the evidence thus far obtained with cockroaches suggests such a possibility. There is ample precedent to show that methylmalonyl-CoA can indeed replace malonyl-CoA during the synthesis of aliphatic chains in other systems (66,67). Further work is needed to establish that methylmalonyl-CoA is involved in the synthesis of internally branched alkanes in the cockroach.

BIOSYNTHESIS OF UNSATURATED HYDROCARBONS

Elongation of an appropriately unsaturated fatty acid followed by decarboxylation could give rise to most of the olefins thus far identified in biological systems. For example, *cis*-9-nonacosene and *cis*-6,9-nonacosadiene, the two major olefins of the cockroach, *Periplaneta japonica* (3), could be formed by elongation of oleic acid and linoleic acid, respectively, with malonyl-CoA as the C_2 donor, followed by decarboxylation (Fig. 13). The position, geometry, and the number of double

bonds in these two acids, which are the most dominant unsaturated acids in the lipids of most nonphotosynthetic organisms, are appropriate for the precursor role. However, no direct experimental evidence concerning the biosynthesis of these unsaturated hydrocarbons is available.

Another type of diunsaturated hydrocarbon found in certain algae, such as *Botryococcus braunii*, contains a double bond at the terminal carbon (68). Elongation of oleic acid to Δ^3 unsaturated C_{28}, C_{30}, and C_{32} acids followed by decarboxylation could give rise to the major diunsaturated hydrocarbons of this organism (Fig. 14). The position of the nonterminal double bond is in agreement with this suggestion, and mechanistically, this decarboxylation would be more favorable than the decarboxylation of the saturated acid. However, no direct evidence is available concerning the biogenesis of these olefins.

The unique polyunsaturated hydrocarbon, all-*cis*-3,6,9,12,15,18-heneicosahexaene, which appears to be widely distributed in algae has not been subjected to biosynthetic studies. However, analysis of the fatty acids and hydrocarbons in a variety of photosynthetic bacteria (Pseudomonadales and Hyphomicrobiales), blue-green algae (Cyanophyta), red algae (Rhodophyta), yellow-green algae (Xanthophyta), green algae (Chlorophyta), euglenids (Euglenophyta), dinoflagellates (Pyrrhophyta), cryptomonads (Cryptophyta), diatoms (Bacillariophyta), golden algae (Chrysophyta), brown algae (Phaeophyta), and protozoa (Ciliata) showed that, in most every case, the presence of the all-*cis*-3,6,9,12,15,18-heneicosahexaene was accompanied by the presence of docosa-all-*cis*-4,7,10,13,16,19-hexaenoic acid (37). The obvious structural relationship between the acid and the olefin, and the correlation between the presence of these two components, very strongly suggest that the $C_{21:6}$ olefin is derived by decarboxylation of $C_{22:6}$ fatty acid. However, direct experimental evidence for this hypothesis has not been reported.

The only class of olefins which has been subjected to biosynthetic studies is that of *Sarcina lutea*, which synthesizes mainly dianteiso branched 14-nonacosene. With this bacterium it was shown that the branches originated from the starter pieces derived from isoleucine, as already shown in other systems (7). Repetition of the labeling experiments, similar to those performed with higher plants, indicated that under certain conditions exogenous [1-^{14}C]palmitic acid did not label the hydrocarbons nearly as rapidly as did [16-^{14}C]- and [9,10-^{3}H]-palmitic acid. When the incorporation of exogenous palmitic was done in the presence of acetate the carboxyl carbon of the C_{16} acid was incorporated into the hydrocarbon, whereas in the absence of acetate the C_{16} acid was incorporated into the olefin with the loss of the carboxyl carbon. This observation suggested the possibility of a head-to-head condensation type mechanism in the biosynthesis of the olefins in this organism. This concept, originally formulated in attempts to consider alternate pathways for hydrocarbon synthesis in plants (12), involves condensation of two biochemically disimilar fatty acid derivatives with a decarboxylation of specifically one type of derivative. With *S. lutea*, it was found that the presence of acetate in the medium resulted in incorporation of the exogenous acid into olefin preferentially via the nondecarboxylating derivative (7). Chemical degradation of the

labeled olefin derived from specifically labeled palmitic acid showed that the double bond was introduced between C-1 and C-2 of the fatty acid which was incorporated without decarboxylation into the olefin.

Even though a cell-free preparation from *S. lutea* was shown to catalyze biosynthesis of olefins, the nature of the decarboxylating and nondecarboxylating derivatives which participate in the hypothetical condensation process remains obscure. Incorporation of [1-^{14}C]palmitic acid and [16-^{14}C]palmitic acid into hydrocarbons required ATP, Mg^{+2}, CoA, NADPH, and pyridoxal or pyridoxamine phosphate (69). Some experimental results suggested that the nondecarboxylating fatty acid derivative is a neutral plasmalogen and that the vinyl ether somehow condenses with the decarboxylating derivative in such a way that C-2 of the latter becomes attached to C-1 of the former conserving the olefinic functions of the vinyl ether (Fig. 15). Since these tentative conclusions were published little progress appears to have been made in defining the chemical nature of the intermediates involved in olefin synthesis in this organism.

CONCLUSION

In spite of the omnipresence of hydrocarbons in biological systems, the biogenesis of hydrocarbons remains a largely neglected area of biochemistry. Even though hydrocarbons represent some of the simplest organic molecules, their extreme hydrophobicity poses difficult biochemical problems. However, recent years have seen some progress in our understanding of hydrocarbon biogenesis but as discussed in this paper, gaps in our knowledge are so extensive that we are only barely beginning to see how hydrocarbons are generated by living organisms.

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DISCUSSION

UZIAK: How were the different samples prepared? For example, one can grind up a whole leaf, but with the cockroach, did you just take the whole cockroach and homogenize it or what? What solvent was used for extraction?

KOLATTUKUDY: The cockroach came from Blomquist's Laboratory in Mississippi. What they normally do is take the integument out and float them on the precursor fatty acid or inject into the cockroach the radioactive sample. These are the two techniques they have thus far used. They have not gotten a cell-free system up to now which will do the same job. That is, if you grind it up, the activity is lost.

The extraction solvent is a mixture of chloroform and methanol. Identification is made by gas chromatography and mass spectrometry with radio-gas chromatography for identification of the radioactive products.

TABLE 1. ALIPHATIC NONISOPRENOID HYDROCARBONS OF BIOLOGICAL ORIGIN

Hydrocarbon	Range	Major	Source & Comments
Saturated			
n-alkanes	C_{21}-C_{33}	C_{29},C_{31}	Almost every living organism, higher plants & fungi, insects bacteria; also found in animals but probably from the diet in most cases.
	C_{13}-C_{31}	C_{15},C_{17}	Algae; many algae contain hydrocarbons only up to C_{21}.
Iso-alkanes (2-methyl)	C_{21}-C_{33}	C_{27},C_{29},C_{31}	Higher plants and probably almost every living organism. In most cases not major components.
Anteiso-alkanes (3-methyl)	C_{22}-C_{32}	C_{28},C_{32}	Probably most living organisms especially higher plants; in most cases not major components.
Internally methyl branched	C_{21}-C_{33}	---	Minor components in most cases. Insects, higher plants.
	C_{18}		Blue green algae, 7- and 8-methyl alkanes significant components.
Unsaturated			
Alk-1-ene	C_{19}-C_{31}	C_{27},C_{29}	Sugar cane, roses, *Senedesmus* not major components.
Alk-2-ene (*trans*)	C_{20}-C_{33}		Sugar cane.
Alk-3-ene (*cis*)	C_{17}-C_{33}	C_{27},C_{29},C_{31}	Rose petals.
Alk-5-ene Alk-7-ene (*cis*) Alk-9-ene	C_{17}-C_{33}	C_{27},C_{29},C_{31}	Roses, probably as minor components in other plants, algae.
Alk-10-ene	C_{15}-C_{33}	C_{31},C_{33}	Significant component in sugar cane hydrocarbon.

TABLE 1. (cont.)

Hydrocarbon	Range	Major	Source & Comments
Alka-dienes	C_{21}-C_{27}	C_{21},C_{27}	Significant component in certain algae.
		C_{29}	Significant component in certain insects.
Alka-trienes and tetraenes			Algae.
Polyunsaturated	C_{21}	C_{21}	Major hydrocarbons of many algae, most of them, but not limited to marine planktons.

NOTE: The hydrocarbons found internally in animals such as in liver and in milk are most probably derived from the diet. However, in cases such as wool wax, hydrocarbons may not be exclusively from the diet although biosynthetic studies are lacking. In many cases sources shown are only examples.

TABLE 2. EFFECT OF VARIOUS COFACTORS AND INHIBITORS ON THE CONVERSION OF *n*-DOTRIACONTANOIC ACID (C_{32}) AND α-HYDROXY DOTRIACONTANOIC ACID TO ALKANES IN THE EPIDERMAL EXTRACTS OF PEA LEAVES

ASSAY CONDITIONS	RELATIVE RATES OF ALKANE FORMATION FROM	
	n-Dotriacontanoic Acid	α-Hydroxy *n*-Dotriacontanoic Acid
+ Ascorbic Acid	100.0	100.0
- Ascorbic Acid	26.8	13.6
- O_2	15.7	40.6
+ Parachloromercuribenzoate (10^{-3} M)	32.4	62.4
+ Dithioerythritol (5×10^{-3} M)	11.0	----
+ Imidazol (5×10^{-4} M)	11.0	----
+ o-Phenanthroline (2×10^{-4} M)	0.0	----
+ Ethylenediamine tetraacetate (10^{-3} M)	40.0	----

Data taken from ref. 52.

TABLE 3. INCORPORATION OF [1-^{14}C-9,10-^{3}H]STEARIC ACID INTO THE PARAFFINS OF SPINACH, PEA, AND BROCCOLI LEAVES

	Incorporation of Radioactivity into Paraffins		
	^{3}H	^{14}C	^{3}H:^{14}C
		(DPM × 10^{-5})	
[1-^{14}C-9,10-^{3}H]Stearic Acid	100	8	12.5
Tissue:			
Spinach	0.55	0.043	12.8
Pea	5.0	0.40	12.5
Broccoli	12.8	1.02	12.6

Data taken from ref. 41.

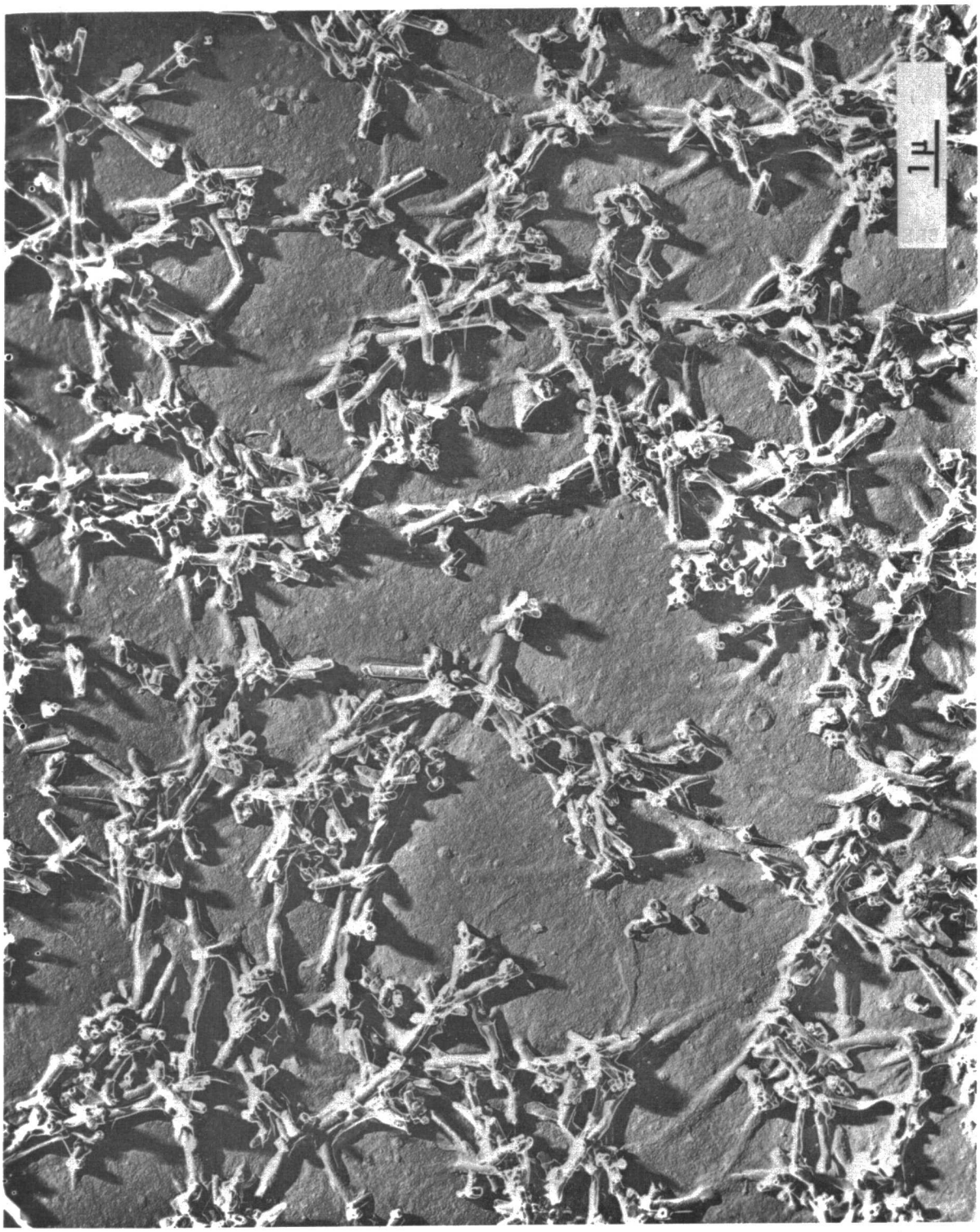

Figure 1 (A). Electron micrographs of carbon replica of *Picea abies* needle surface (X 13,300). (Courtesy of Drs. B. E. Jinuper and N. D. Hallum)

Figure 1 (B). Electron micrographs of carbon replica of *Eucalyptus papuana* leaf surface (X 12,000). (Curtesy of Drs. B. E. Jinuper and N. D. Hallum)

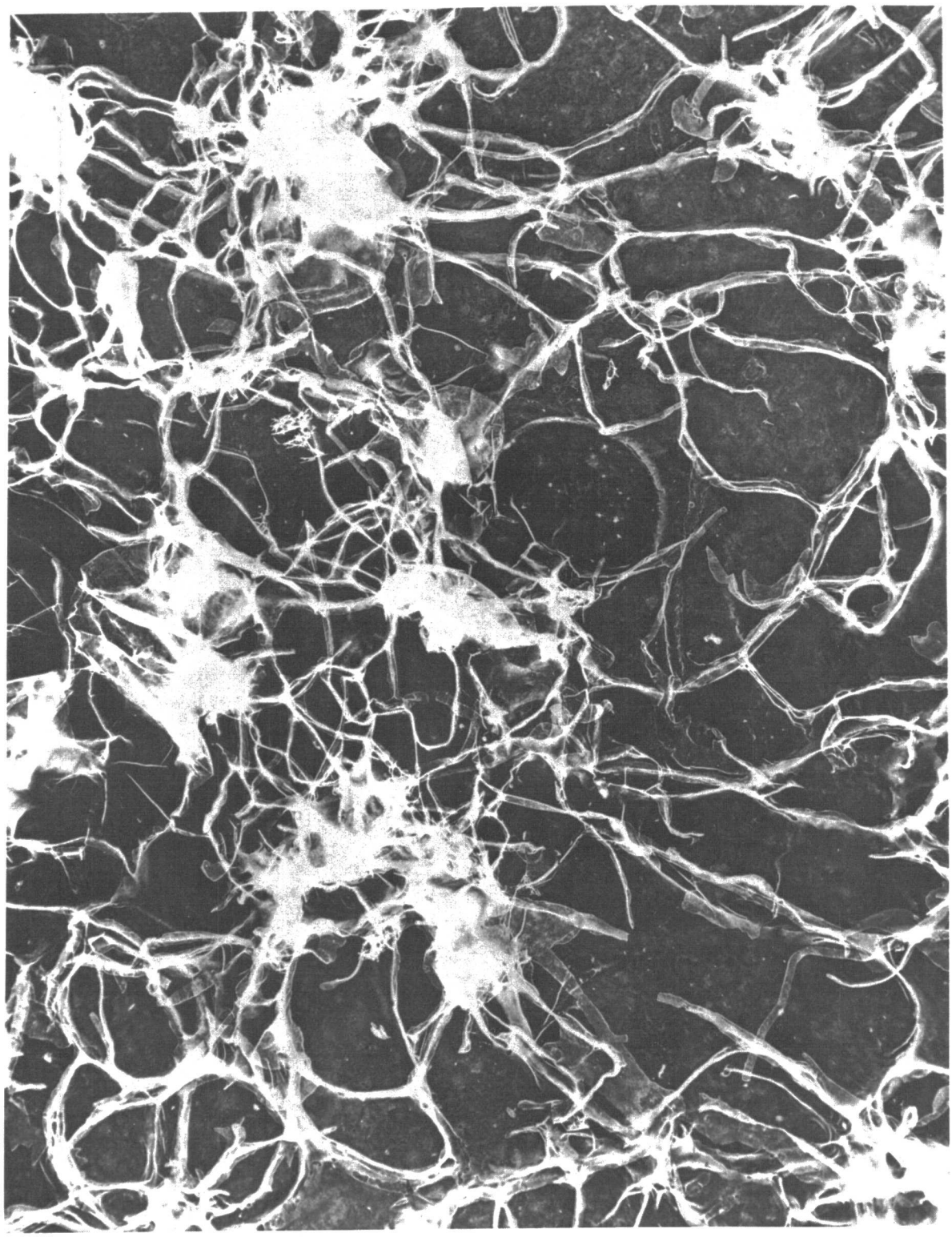

Figure 1 (C). Electron micrograph of carbon replica of *Leptospermum laevegatum* leaf surface (X 15,000). (Courtesy of Drs. B. E. Jinuper and N. D. Hallum)

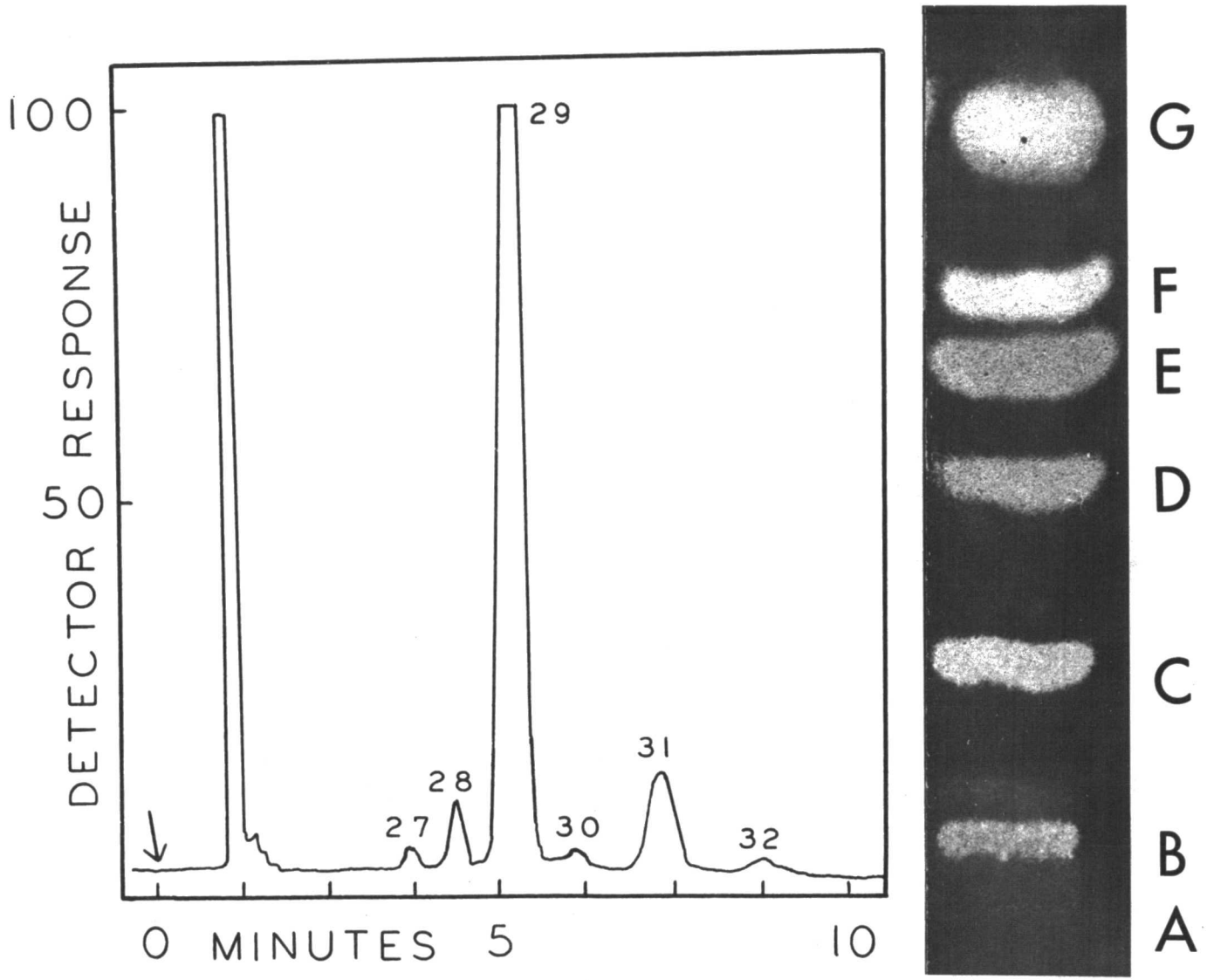

Figure 2. (Right) Thin-layer chromatogram of leaf surface lipid of *Brassica oleracea* on silica gel G, with benzene as the solvent. (A) Fatty acids; (B) primary alcohol; (C) secondary alcohol; (D) aldehydes; (E) ketones; (F) wax esters; (G) hydrocarbons. (Left) Gas-liquid chromatogram of hydrocarbons eluted from the silica gel represented by spot G on the thin-layer chromatogram. The number of carbon atoms in each n-paraffin is shown on each peak (12).

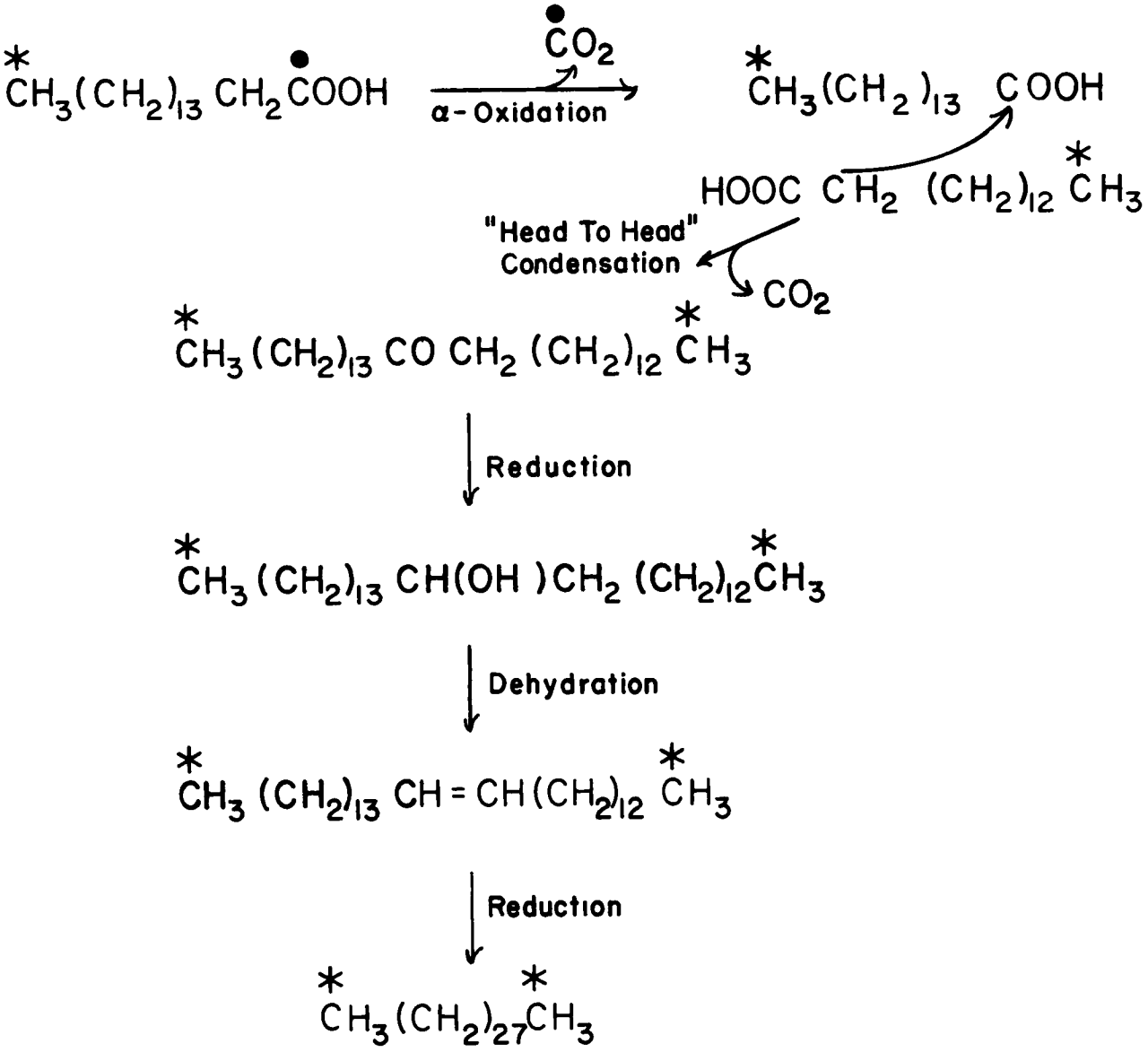

Figure 3. A head-to-head condensation mechanism proposed for the biosynthesis of hydrocarbons in plants. Expected fate of the carboxyl carbon and methyl carbon of the precursor acid is marked.

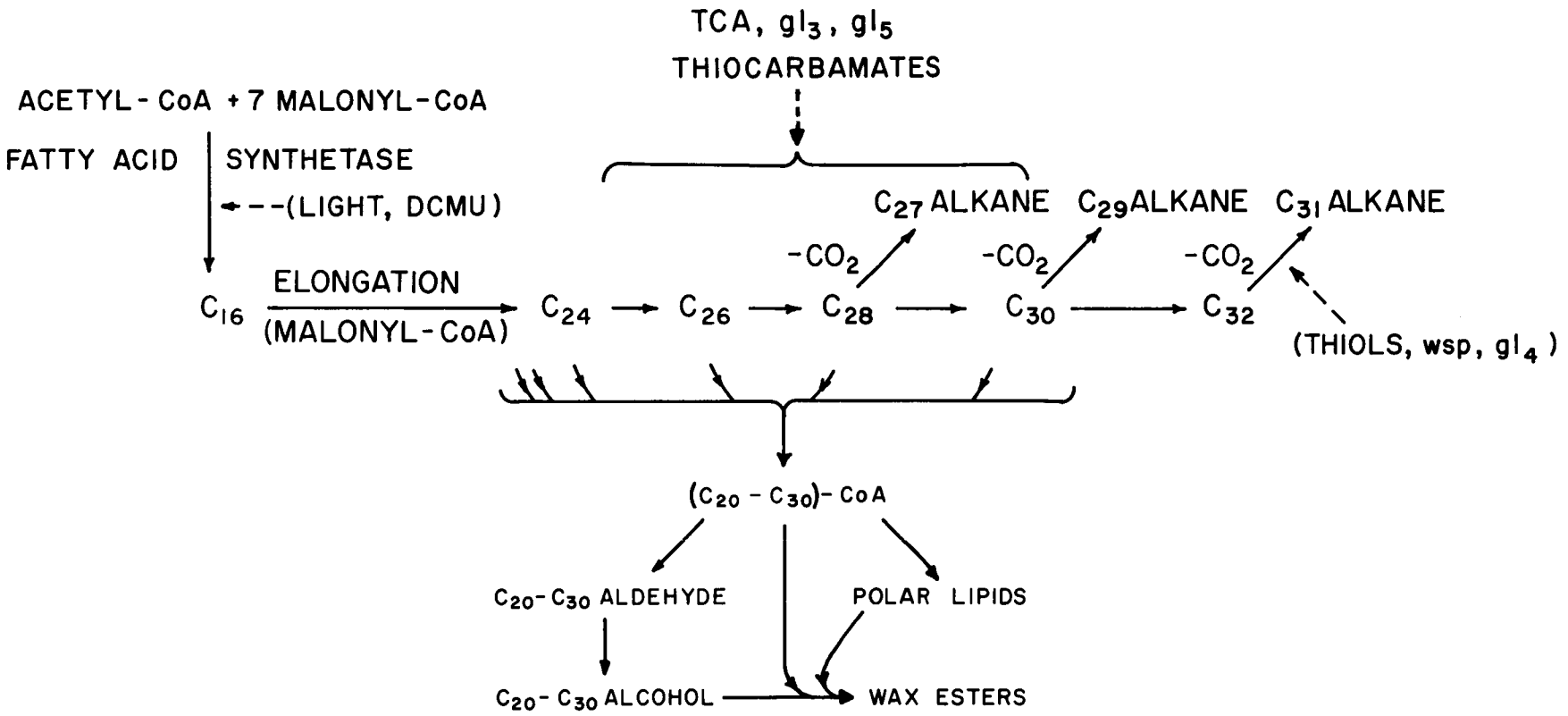

Figure 4. An elongation-decarboxylation pathway for the biosynthesis of alkanes and its probable relationship with the biosynthesis of other wax components. The steps which are most probably influenced by light, chemicals, and certain mutations are indicated; gl_3, gl_4, and gl_5 are mutants of *B. oleracea* and wsp is a mutant of *P. sativum*.

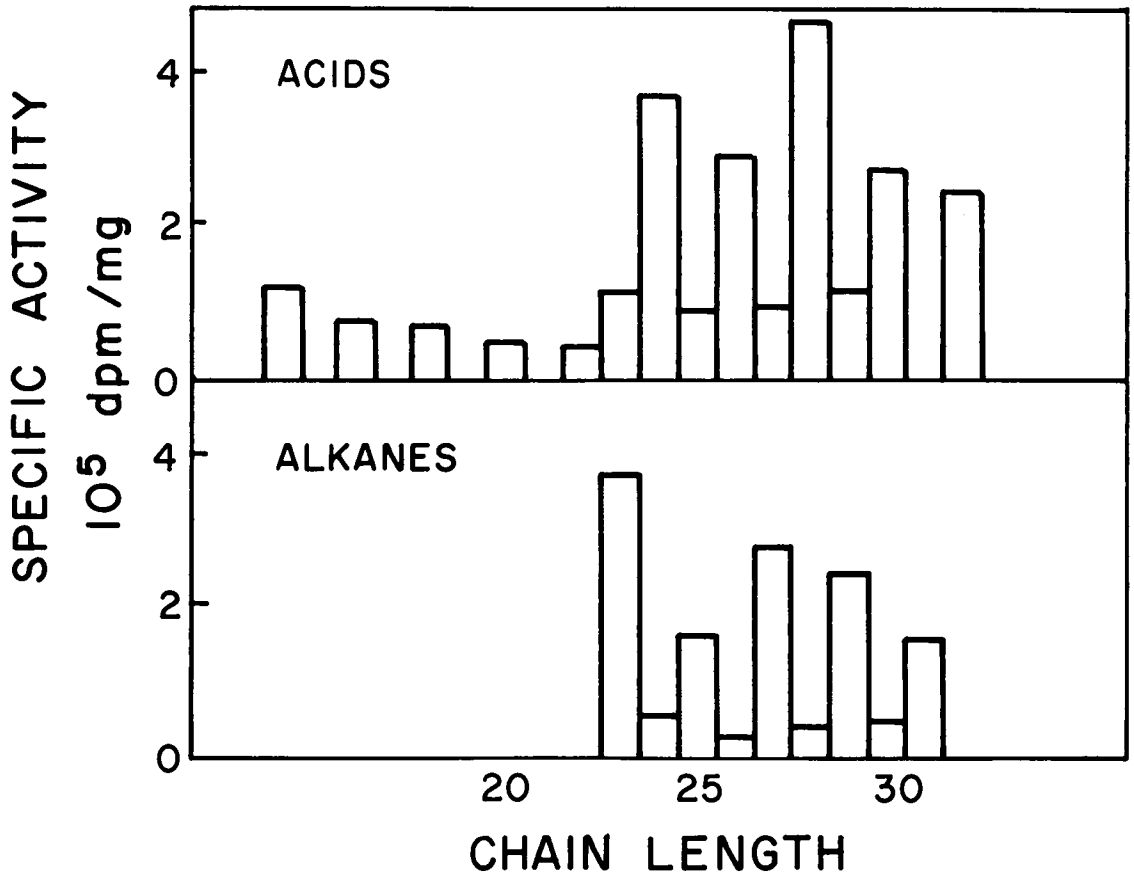

Figure 5. Specific radioactivity of fatty acids and alkanes isolated from *A. porrum* leaf slices which metabolized [U-^{14}C] acetate (61).

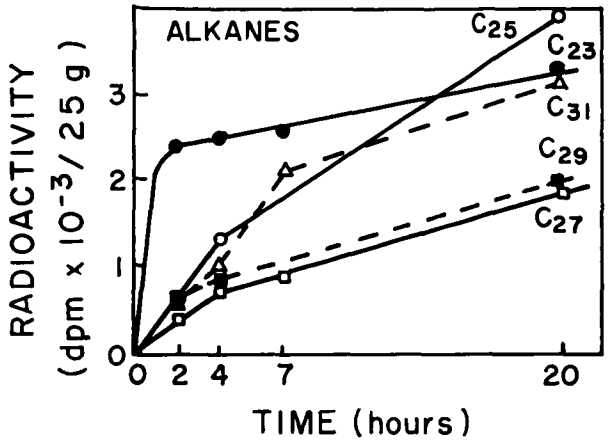

Figure 6. Time-course of incorporation of [G-^{3}H]C_{24} acid into alkanes in leaf slices of *A. porrum* (48).

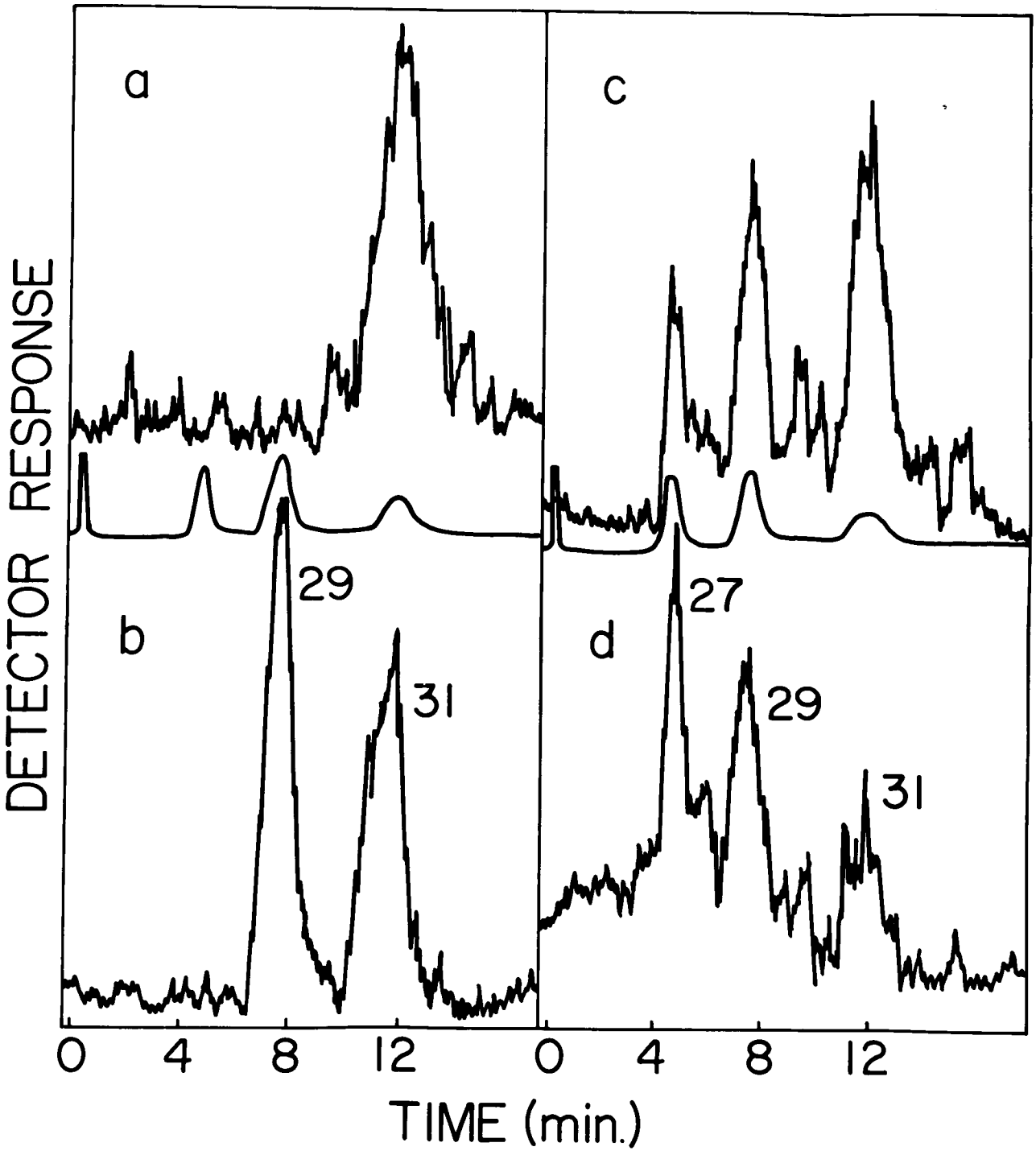

Figure 7. Radio gas-liquid chromatograms of the alkane fractions isolated by TLC from the lipids of *V. faba* flower petals which were incubated with: (a) n-[9,10,11-^{3}H]dotriacontanoic acid (C_{32}); (b) n-[G-^{3}H]triacontanoic acid (C_{30}); (c) n-[G-^{3}H]octacosanoic acid (C_{28}); and (d) n-[G-^{3}H]octacosanoic acid in the presence of trichloroacetate. The middle traces represent the flame ionization detector response. The number on each peak represents the chain length of the n-alkane (51).

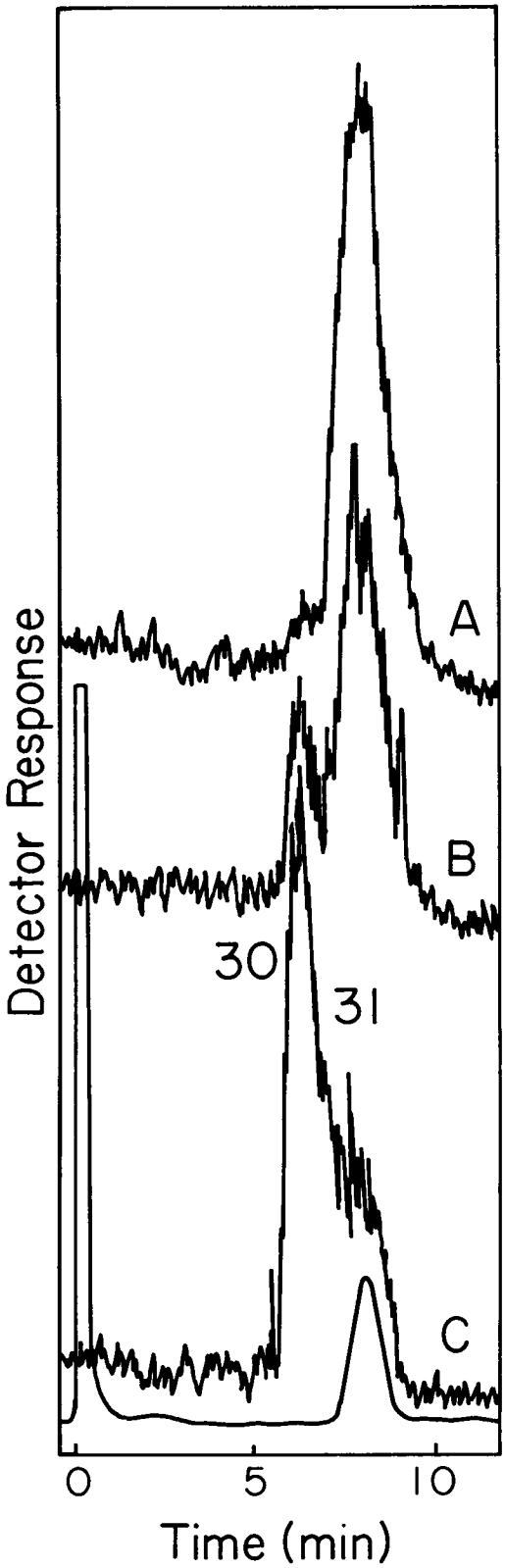

Figure 8. Radio gas-liquid chromatograms of alkanes synthesized from n-[9,10,11-^{3}H]dotriacontanoic acid by cell free preparations of epidermis (A), whole leaves in the presence (B), and absence (C) of p-chloromercuribenzoate. The number of each peak represents chain length. The bottom tracing is flame ionization detector response (52).

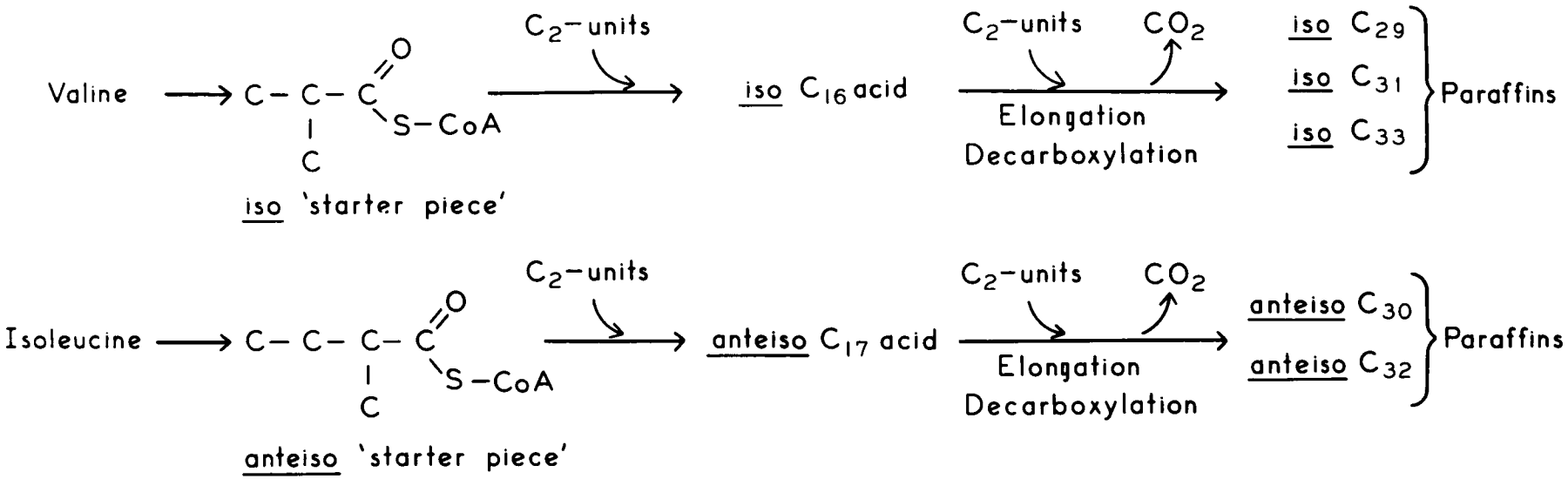

Figure 9. Proposed pathway for the biosynthesis of *Iso* and *Anteiso* fatty acids and alkanes.

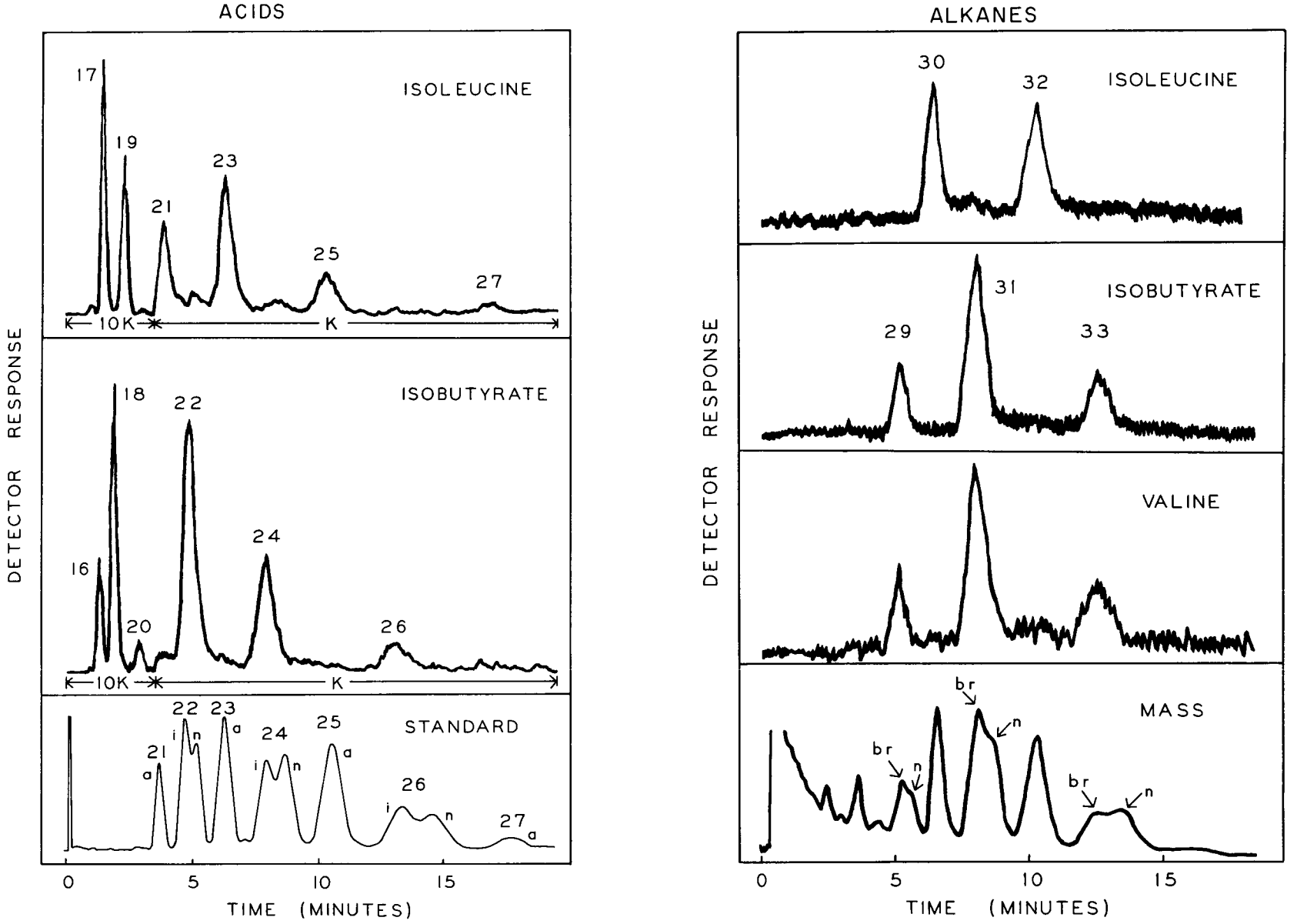

Figure 10. Radio gas-liquid chromatograms of methyl esters of fatty acids (left) and alkanes (right) from the lipids of tobacco leaves which had metabolized the labeled substrates shown on each tracing for 16 to 24 hrs. The flame ionization detector response shown in bottom of each figure was obtained with authentic standards; br, branched; n, normal; i, *Iso*; a, *Anteiso* (60).

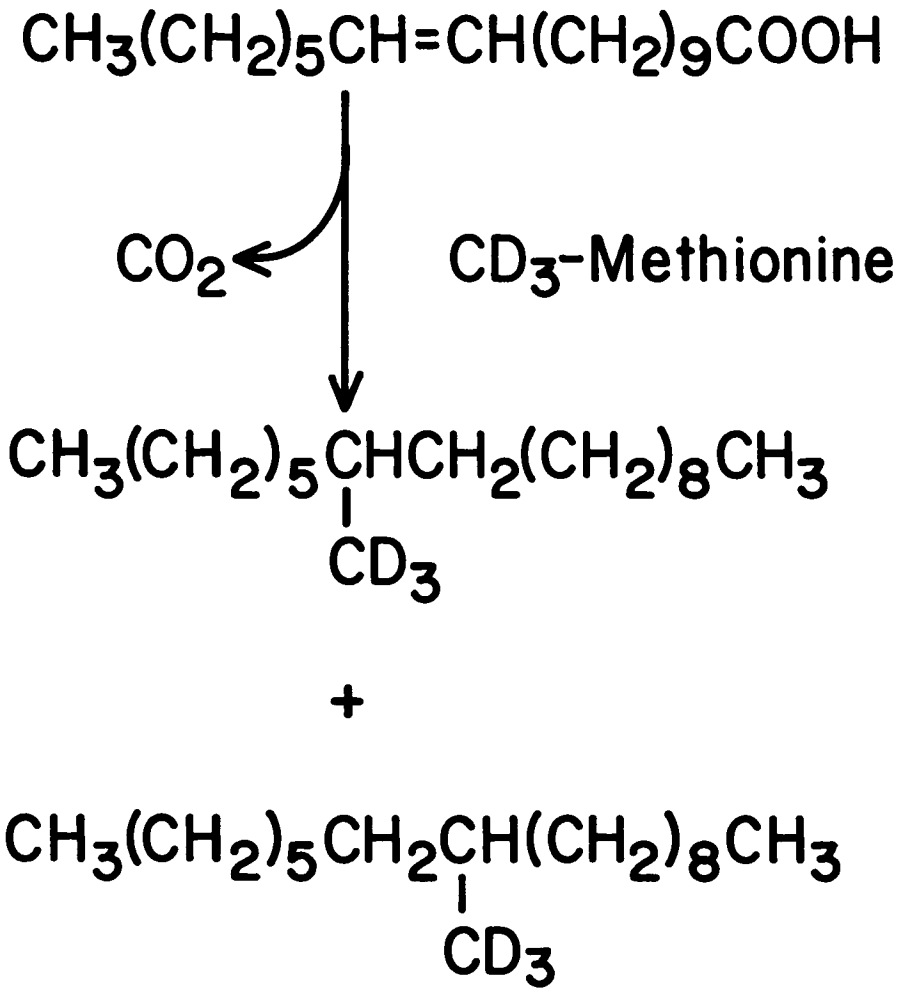

Figure 11. Biosynthesis of internally methyl branched hydrocarbons in algae.

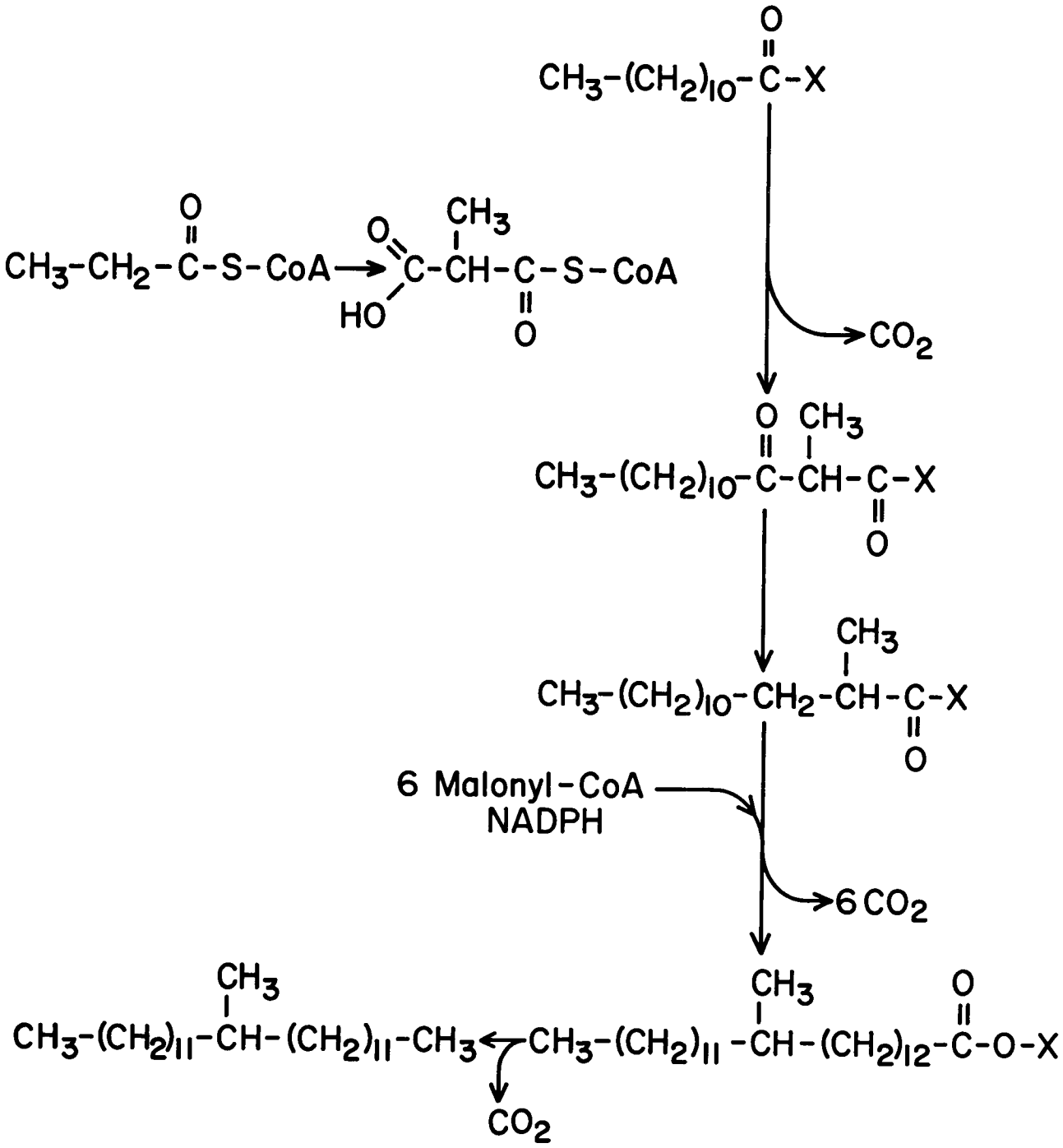

Figure 12. A possible biosynthetic pathway for internally methyl branched hydrocarbons in insects.

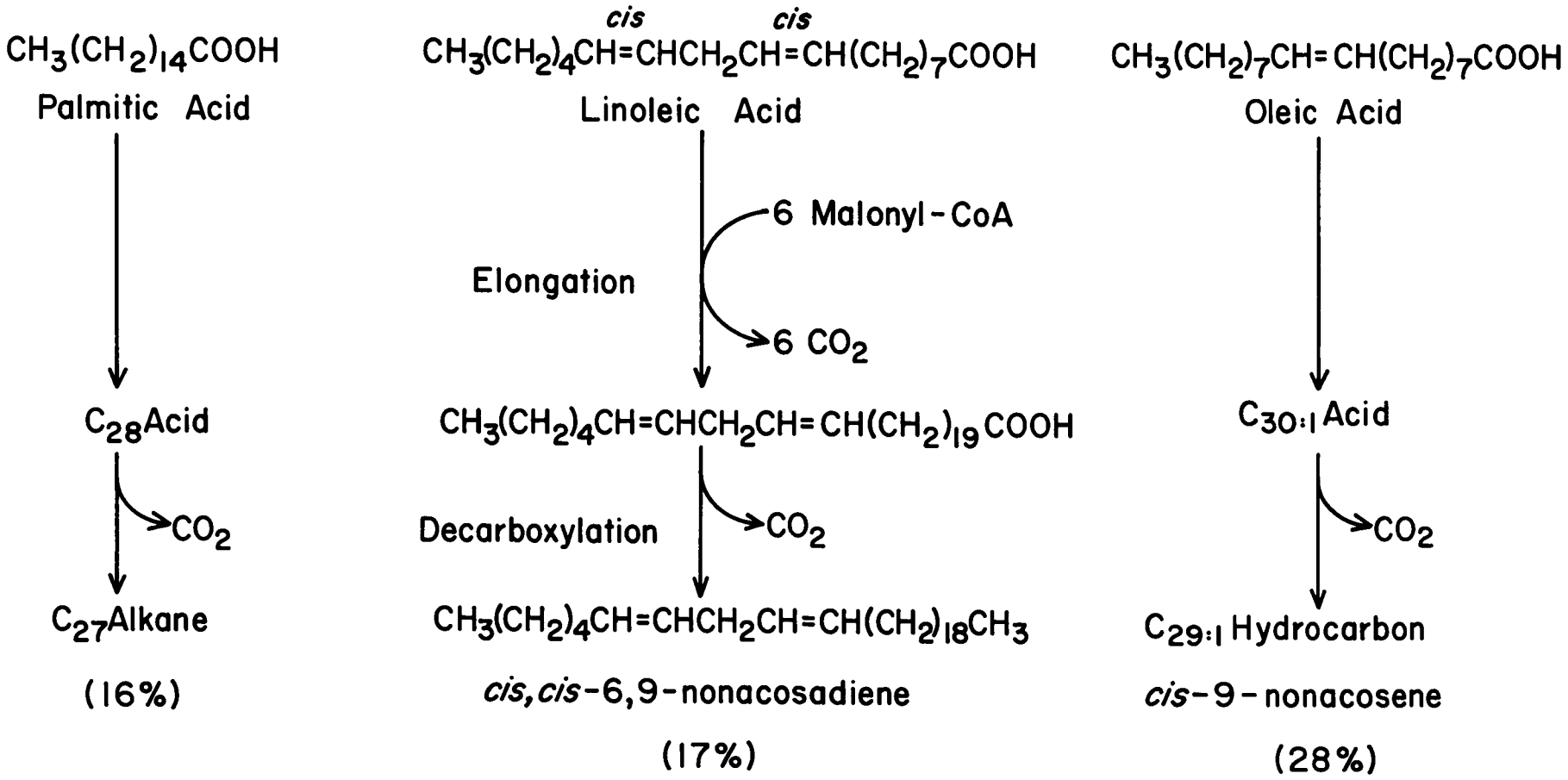

Figure 13. Probable pathways for the biosynthesis of hydrocarbons in *Periplaneta japonica* (cockroach).

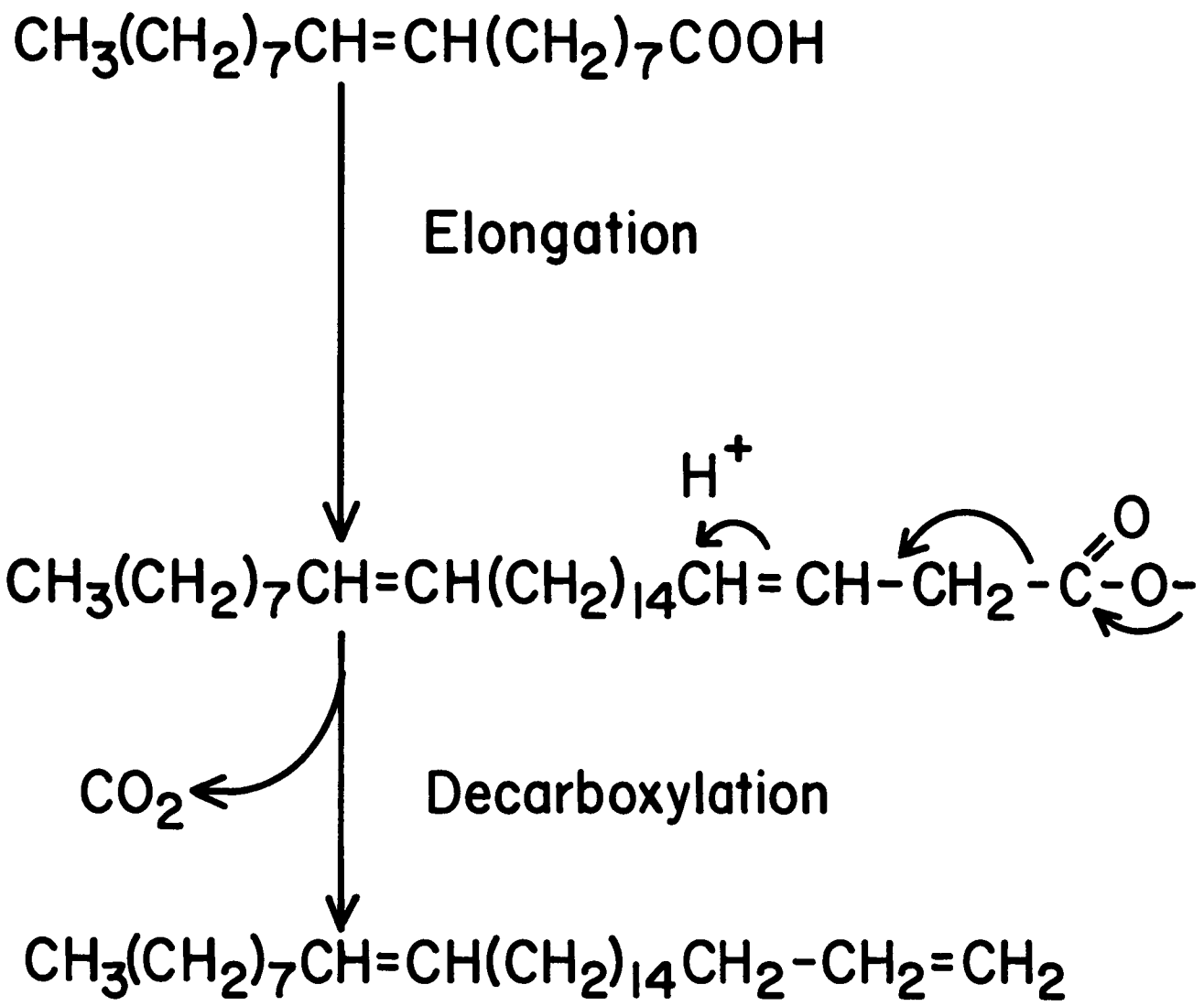

Figure 14. A possible mechanism for biosynthesis of diunsaturated hydrocarbons in *Botryococcus braunii*.

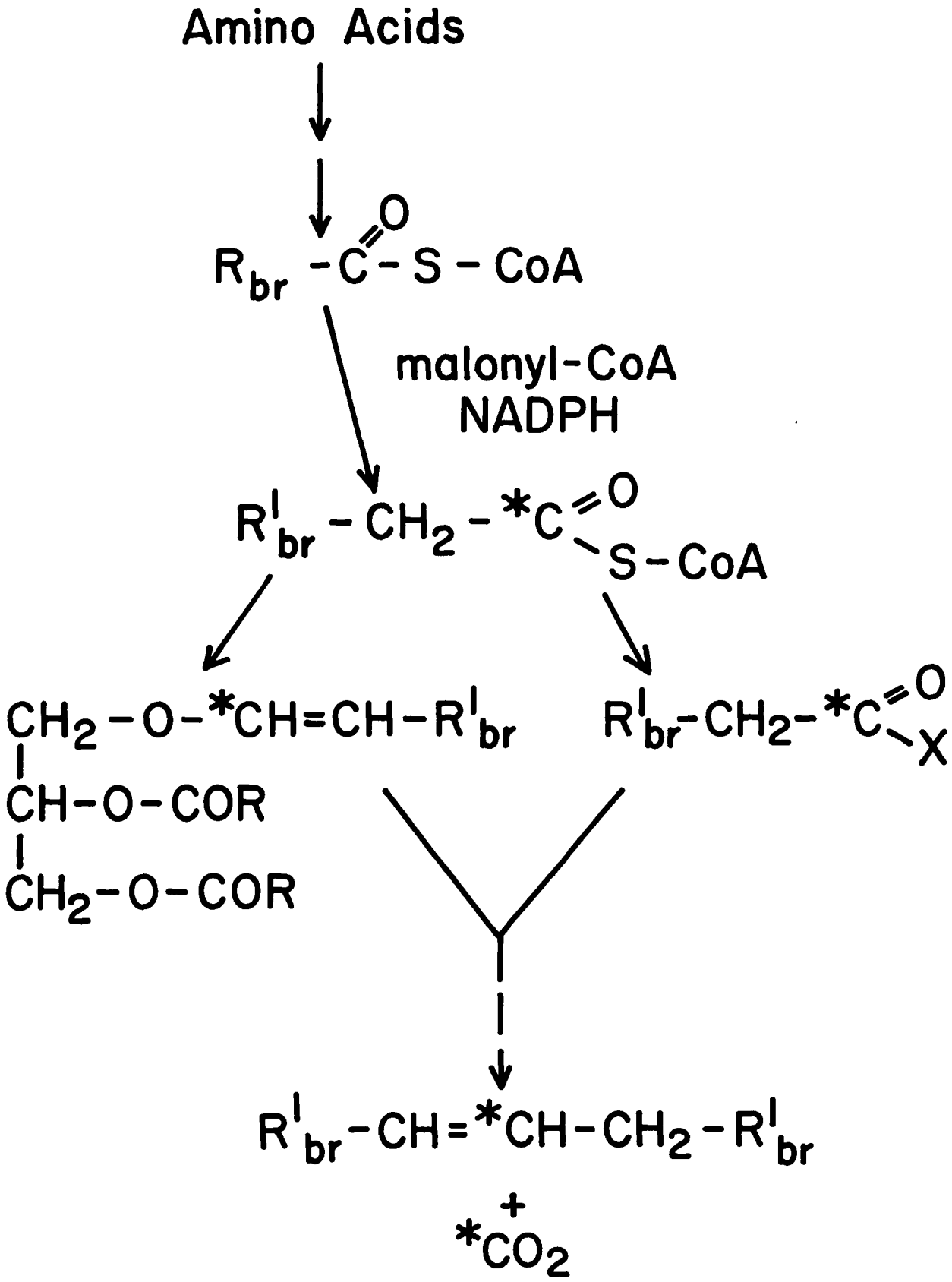

Figure 15. A proposed pathway for the biosynthesis of dianteiso olefins in *S. lutea*.

HYDROCARBONS FROM ZOOPLANKTON OF THE EASTERN GULF OF MEXICO

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Hydrocarbons from Zooplankton of the Eastern Gulf of Mexico

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Introduction

The sale of oil and gas leases along the entire U. S. outer-continental shelf (OCS) and heightened public awareness of the potential harmful impact of petroleum-related activities, resulted in the initiation of environmental baseline and monitoring studies in the lease areas, under the sponsorship of the U.S. Department of Interior, Bureau of Land Management. The first of these studies was the MAFLA (Mississippi-Alabama-Florida) program in the northeast Gulf of Mexico. To date, four sets of seasonal samples have been collected and analyzed, the last three of which were identical with regard to locations, measurements and techniques. My laboratory participated in the program by analyzing for hydrocarbons in water, suspended particulates, neuston and zooplankton. The latter samples are the subject of this report.

Methods

Zooplankton were collected by oblique tows using 0.5 meter, 202 micron nets with a 5:1 length to width ratio. The zooplankton were removed from the cod end (without washing the net), placed in glass vials with Teflon-lined caps and frozen. In the laboratory, samples were thawed and foreign material was removed under a 30 power dissecting microscope. A known weight of oven dried (50^{o}C) samples was refluxed in a 1:1 mixture of benzene and methanolic KOH for four hours.

The mixture was then filtered through pre-combusted Whatman GF/F filters to remove debris and the benzene layer was removed from the filtrate following addition of 1 part of distilled water. After two

additional extractions of the aqueous phase with benzene, the extract was reduced to dryness and weighed. The residue was taken up in hexane and applied to a pre-washed alumina/silica gel column (1:5 V/V ratio, activity one) and eluted with two column volumes of hexane (saturated or non-polar hydrocarbon fraction) and two column volumes of benzene (unsaturated/aromatic or polar hydrocarbon fraction). The hexane fraction was reduced to small volume and the benzene fraction dried and taken up in a small volume of hexane for gas chromatographic (GC) analysis.

Primary GC analysis was done with 2.2 mm ID x 2 m stainless steel columns packed with 4% FFAP on Gas Chrom Z, 80/100 mesh. Retention times were converted to retention indices utilizing known standards of n-alkanes. Peak areas were automatically integrated and converted to weight by applying GC response factors calculated from quantitative normal and isoprenoid alkanes and aromatics. These calculations as well as calculations of peak ratios, odd-even preference, wt. % composition and concentration were done by a computer program which produced both paper and magnetic tape output for submission to a central data bank.

Glassware was washed in detergent, soaked in acid, rinsed with distilled water and oven dried. Solvents were doubly distilled. Periodic blanks were run and rejected if material with retention index greater than 1200 was present.

Results and Discussion

A series of 15 stations along four transects in the MAFLA area (Fig. 1) were sampled in June/July 1975, September 1975 and January/February 1976. The zooplankton biomass collected averaged 91 mg dry weight per m^3 in summer, 18 mg dry weight per m^3 in fall and 13 mg dry weight per m^3 in winter (Table 1). Total lipid content was nearly constant at 38-50 mg/g dry wt. The total hydrocarbon content (sum of all integratable peaks in both hexane and benzene fractions) averaged 212 µg/g dry wt. in summer, 135 µg/g dry wt. in fall and 719 µg/g dry wt. in winter. In laboratory studies, Lee et al. (1971) determined that the total lipid content of a Calanus sp. was related to the concentration of phytoplankton carbon fed to it. At 100 µg of phytoplankton carbon per liter, the copepod contained 120 mg/g of total lipid. The lower total lipid in zooplankton from the MAFLA area may be a reflection of a low standing stock of phytoplankton. The concentration of Chl *a* averaged less than 0.5 µg/l (Iverson, 1976) and concentration of POC averaged less than 200 µg/l (Knauer, 1976) during the 3 sampling periods.

Visual inspection of chromatograms from summer 1975 indicated that the zooplankton hydrocarbons fell into three compositional patterns, which were differentiated primarily by the unsaturated/aromatic fraction. The same groupings recurred in fall and winter. The first group, A (Fig. 2), is characterized by high concentrations of pristane and variable amounts of n-alkanes in the C21-C32 region. (Blumer et al, 1963). The higher n-alkanes are generally not as abundant as in this sample. Two peaks with retention indices of 1950 and 1976 appear frequently. These may be the phytadienes originally reported by Blumer and Thomas (1965). The benzene fraction of group A samples contained a group of peaks with retention indices from 2000 to 3200. There was considerable variation in the composition from station to station and season to season but the retention index range mentioned above was not exceeded. The concentration of total hydrocarbon averaged 250 µg/g dry wt. A peak in the benzene fraction at RI ∿ 3055 corresponds to squalene (Blumer, 1967). This peak has at least one other component which is resolved from squalene on a non-polar column (SP2100).

The second group, B (Fig. 3) contained very low amounts of hydrocarbons, primarily pristane in the hexane fraction and a peak at RI=2350 in the benzene fraction. The total hydrocarbon content averaged 29 µg/g dry wt.

The last group, C (Fig. 4), is most interesting. The hexane fractions were much like those of group B, containing pristane and little else. The benzene fractions contained a group of peaks in the 2000-3200 retention index range although they were generally fewer in number and lower in concentration than those in group A. The interesting feature is the group of peaks with retention index 3400 and greater, to an estimated 4000. The same peaks seem to be recurring in this RI range; a pair at 3415 and 3450, a pair at ∿ 3600 and a very large peak at ∿ 3800. Total hydrocarbon content was 640 µg/g dry wt. The higher retention index peaks in the benzene fraction account for the bulk of the total hydrocarbon weight. The identity of these components is still a subject of investigation.

The three zooplankton hydrocarbon groupings recurred in each of the three sampling periods. In summer (Fig. 5) the C group was most abundant, occupying the offshore stations in transects 2, 3 and 4. The A group occurred in transect 1 and 2 stations of transect 2 while the B grouping was limited to the

inshore stations of transects 2 and 4. In fall (Fig. 6) the B group was dominant and occupied all the inshore stations. The C group appeared offshore in transects 1 and 3, while the A group appeared only at the 2 outermost stations on transect 4. In winter, (Fig. 7) the B group was not present and the A group occupied the nearshore stations of transects 1, 2 and 3 as well as 1 offshore station on each of transects 3 and 4. The C group occupied the nearshore stations of transect 4, but was in its usual offshore spot on the other transects.

The 3 hydrocarbon compositions could be the result of three factors:

a) different biosynthetic hydrocarbons from different zooplankton species
b) different hydrocarbons taken up from different food sources or water masses
c) different biosynthetic hydrocarbons resulting from environmental variation (e.g. Temperature)

The taxonomy of the zooplankton was determined by Maturo and Caldwell (1976). A first level examination showed that the major zooplankton groupings occurred in nearly every sample at all seasons. Thus the hydrocarbons in the A and C group must be due to very lipid rich minor components of the zooplankton if taxonomic variation is responsible for observed hydrocarbon variations. This may be more likely than it first seems because the hydrocarbon extraction was done on a bulk zooplankton sample, while taxonomy was performed on a sample that had been split from 7 to 11 times. The splitting could have diluted a minor yet lipid rich component.

Neither dissolved hydrocarbons nor those on suspended particulates bear any relation to the zooplankton hydrocarbons (Calder, 1976) and thus the zooplankton hydrocarbons do not appear to have been taken up from different external sources.

Because the C group was generally found offshore it came from waters generally deeper, colder and more saline. Yet the inshore stations in winter were just as cold and saline as the offshore stations in summer (Rinkel, 1976) and contained the A, not the C group. Temperature and salinity variations do not seem to cause the zooplankton to alter their biosynthetic hydrocarbon content.

Because the hydrocarbon groups do display spatial patterns, rather than random distribution, they must be the result of general circulation phenomena. Hydro-

carbon analysis of the major zooplankton groups (e.g. copepods, jellies, etc.) might be the best way of clarifying these observations.

Tar balls were ubiquitous in neuston samples and on rare occasion were found in a zooplankton sample. When seen they were removed before analysis. None of the zooplankton analyzed showed any evidence of either fresh or weathered petroleum. For comparison, Fig. 8 shows the chromatogram of a contaminated neuston sample.

Conclusions

1. Zooplankton biomass in the MAFLA area is high in summer, low in fall and winter.

2. Total lipid did not vary with season, but total hydrocarbon was much higher in winter. Because of greater biomass the standing crop of zooplankton total hydrocarbons was greatest in summer.

3. The hydrocarbon composition fell into 3 groups, most definitively characterized by the benzene fraction. The same 3 groups recurred in each sampling season in spatial configurations which appear to be controlled by general circulation phenomena.

4. There was no evidence for fresh or weathered petroleum in zooplankton.

Acknowledgements

This research was supported by the U. S. Dept. of Interior, Bureau of Land Management under contract number 08550-CT5-30. Excellent technical assistance was provided by L. Griffin, W. Teehan, S. Horlick, C. Byrne, D. Wynne, and D. Siebert.

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E. Rinkel, M. Salinity and temperature profiles.

Table 1: Gravimetric Data - Seasonal

	Summer	Fall	Winter
Zooplankton Biomass mg dry wt./m^3	91	18	13
Total lipid extract mg/g dry wt.	49.9	37.7	49.8
Total Hydrocarbon µg/g dry wt.	212	135	719
Total hydrocarbon µg/m^3	19.3	2.4	9.4

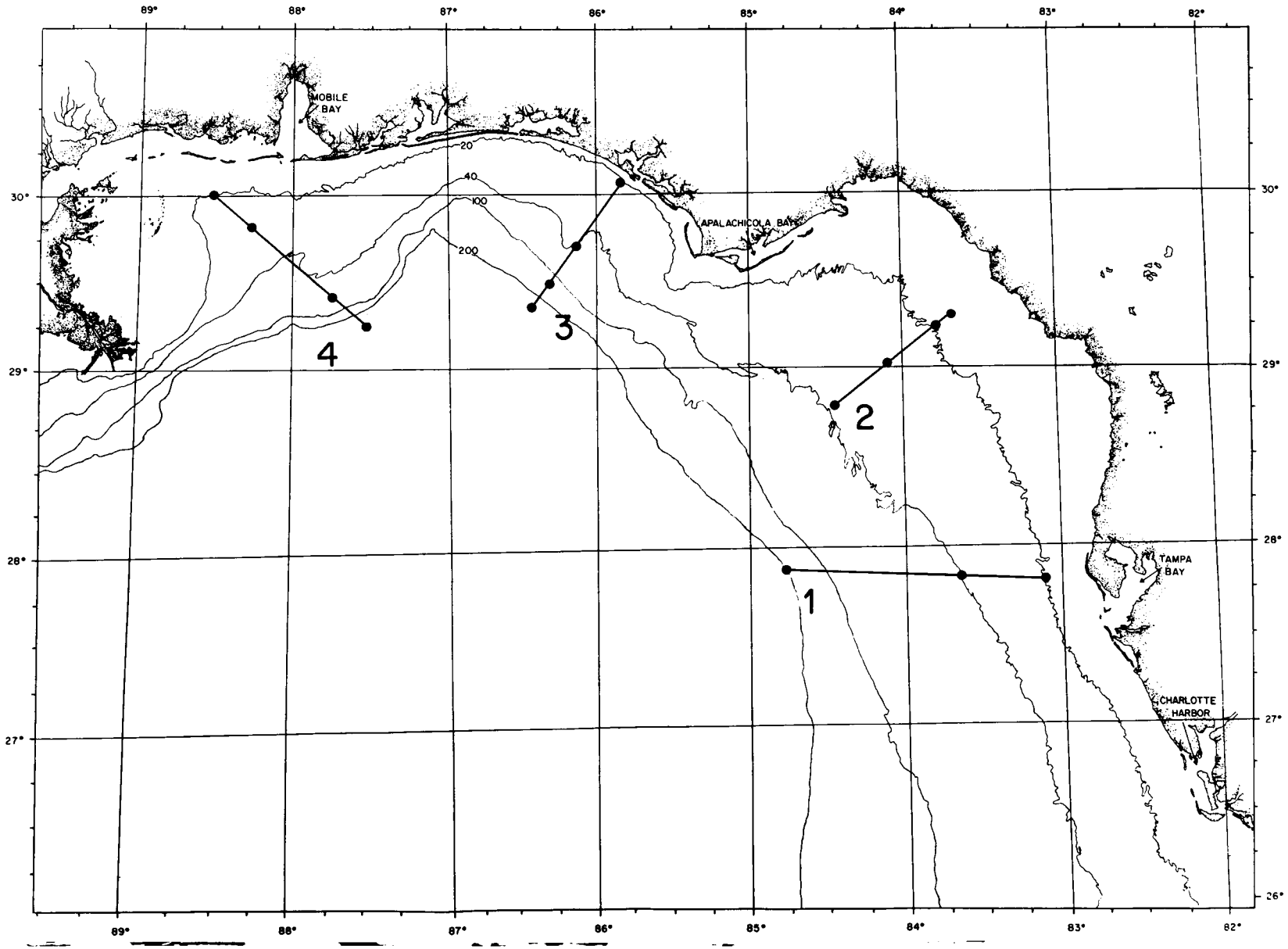

Fig. 1. Transect and station locations in the MAFLA area.

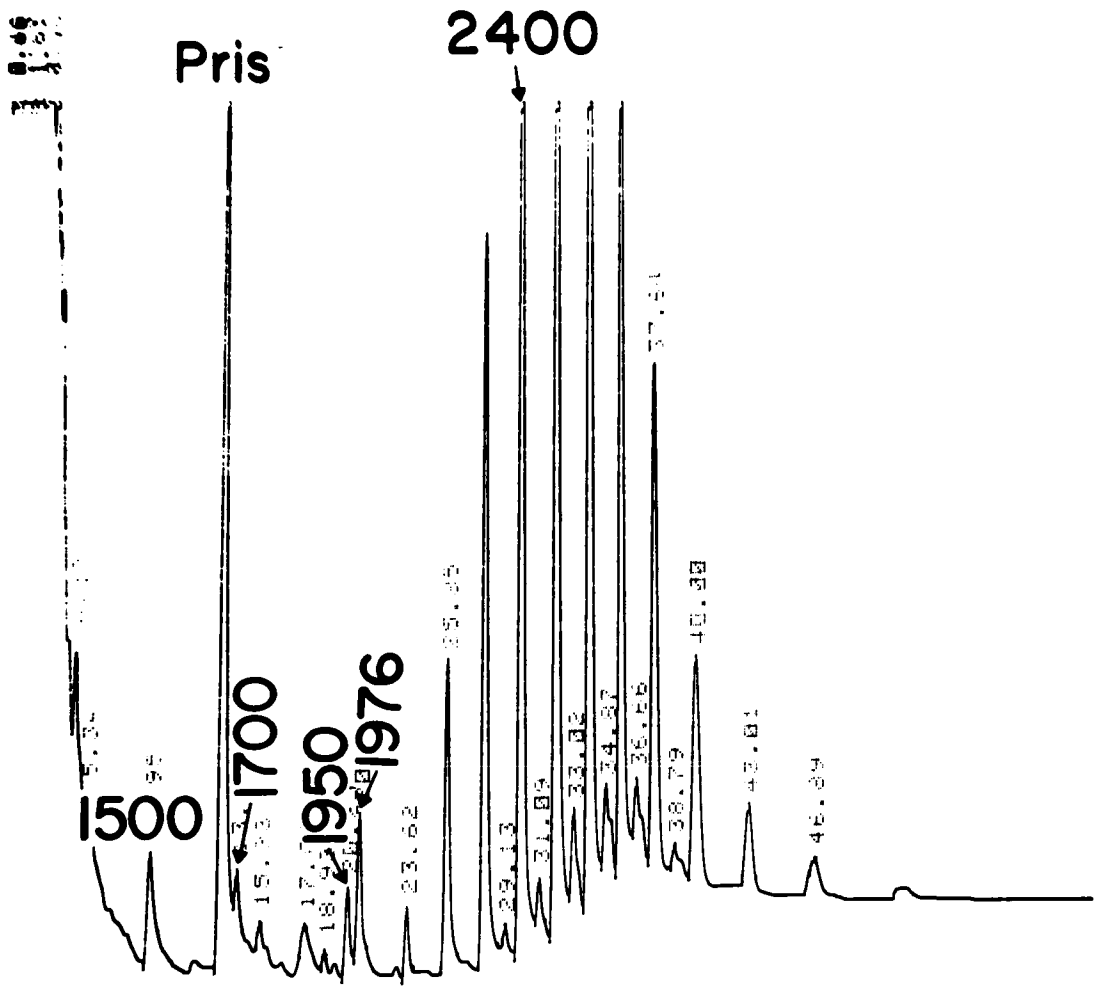

Fig. 2A. Zooplankton hydrocarbons, Group A, Station 1102, hexane fraction, summer 1975.

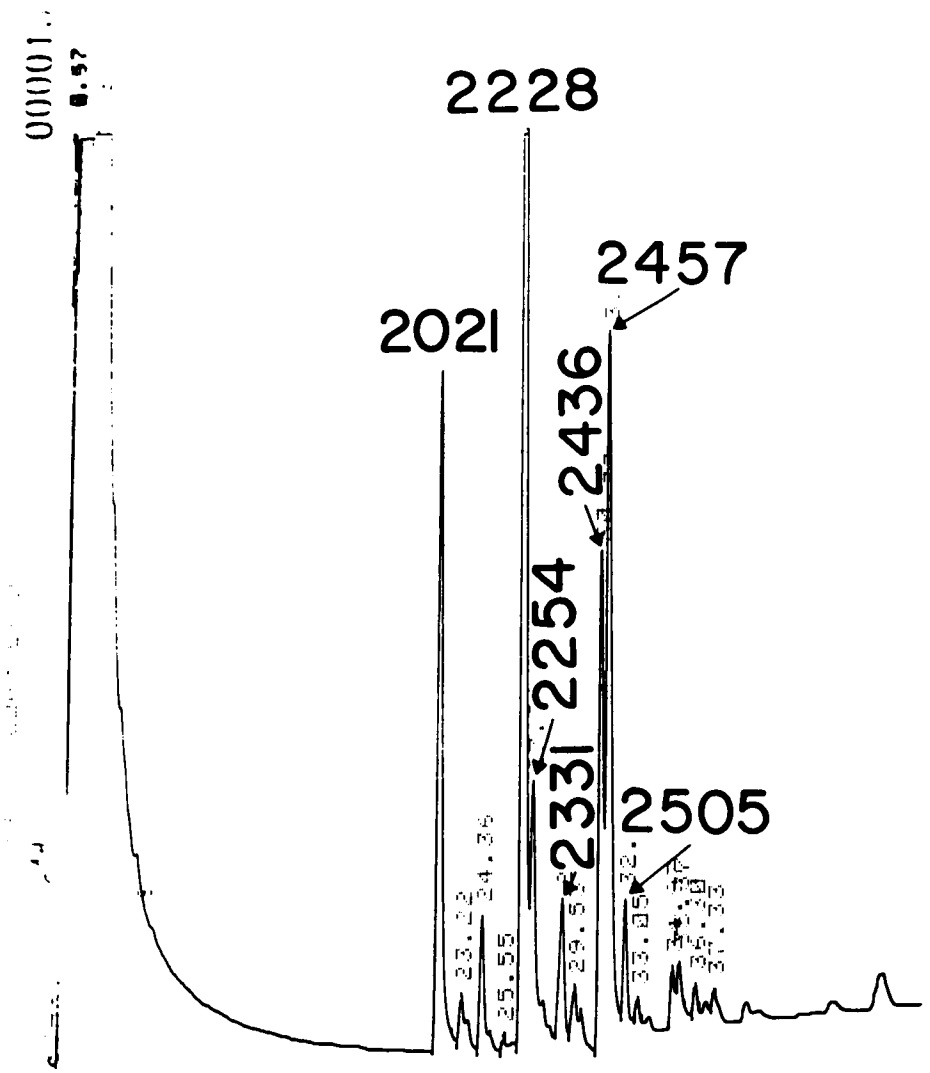

Fig. 2B. Zooplankton hydrocarbons, Group A, Station 1102, benzene fraction, summer 1975.

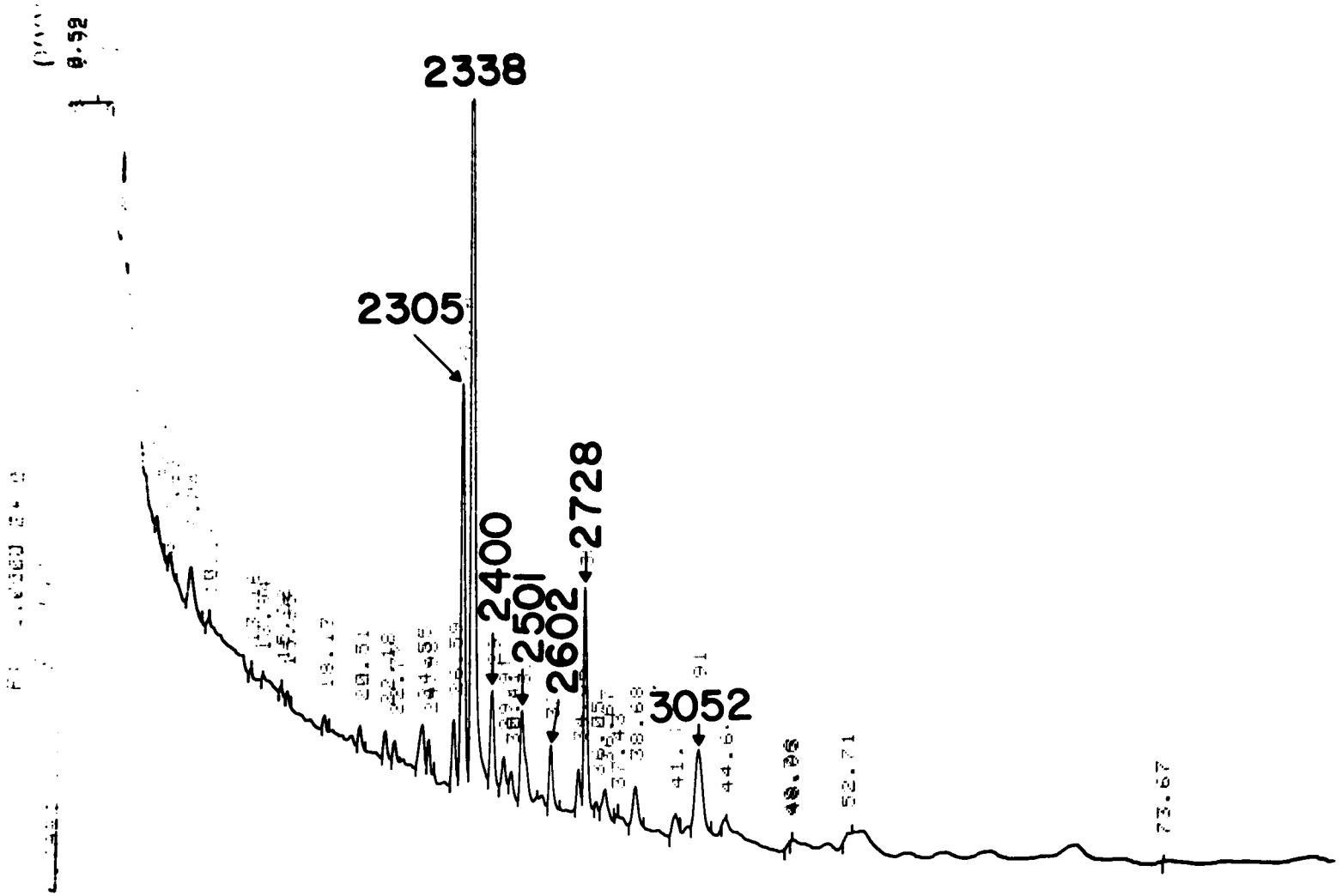

Fig. 2C. Zooplankton hydrocarbons, Group A, Station 1415, benzene fraction, fall 1975.

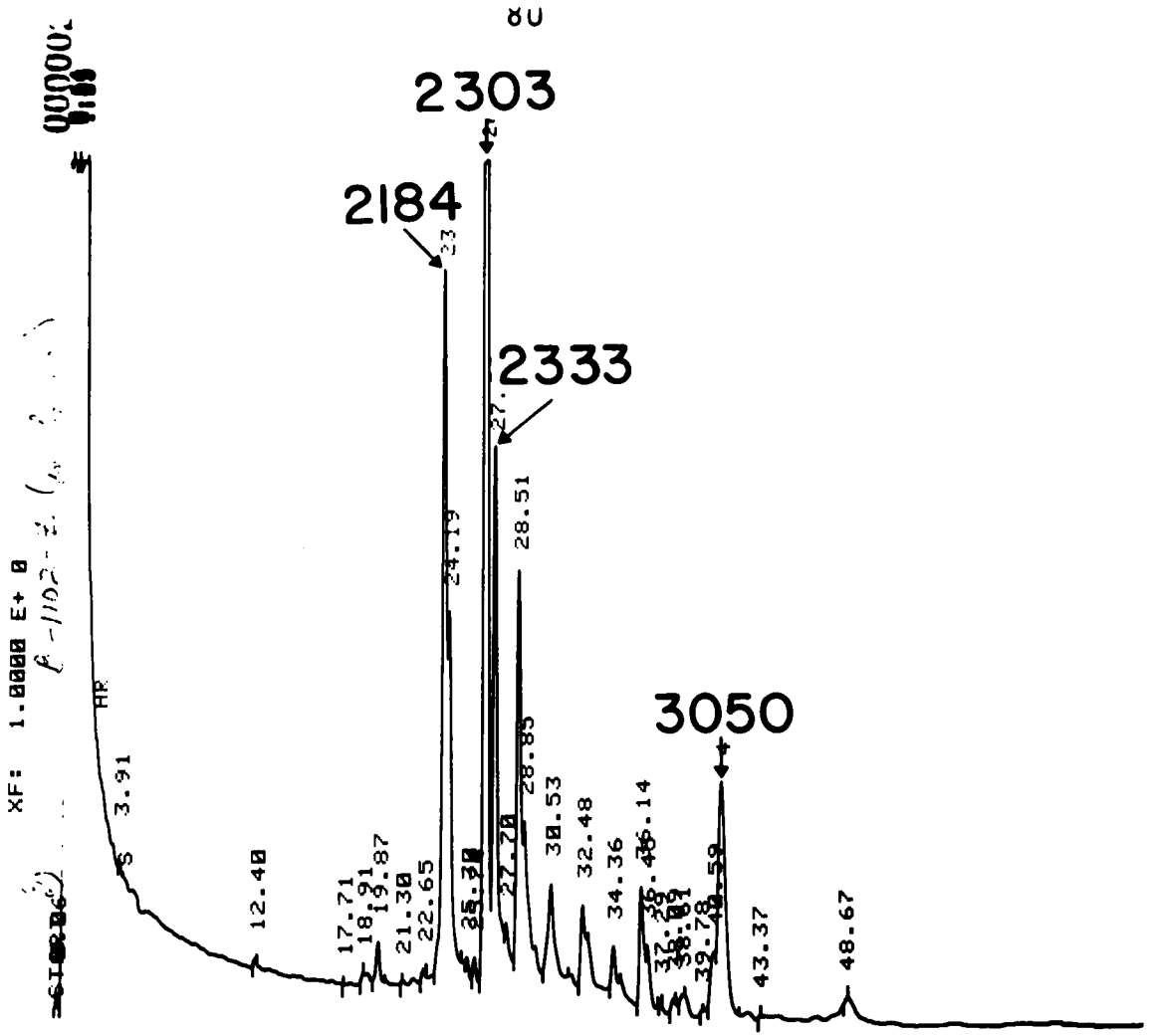

Fig. 2D. Zooplankton hydrocarbons, Group A, Station 1102, benzene fraction, winter 1976.

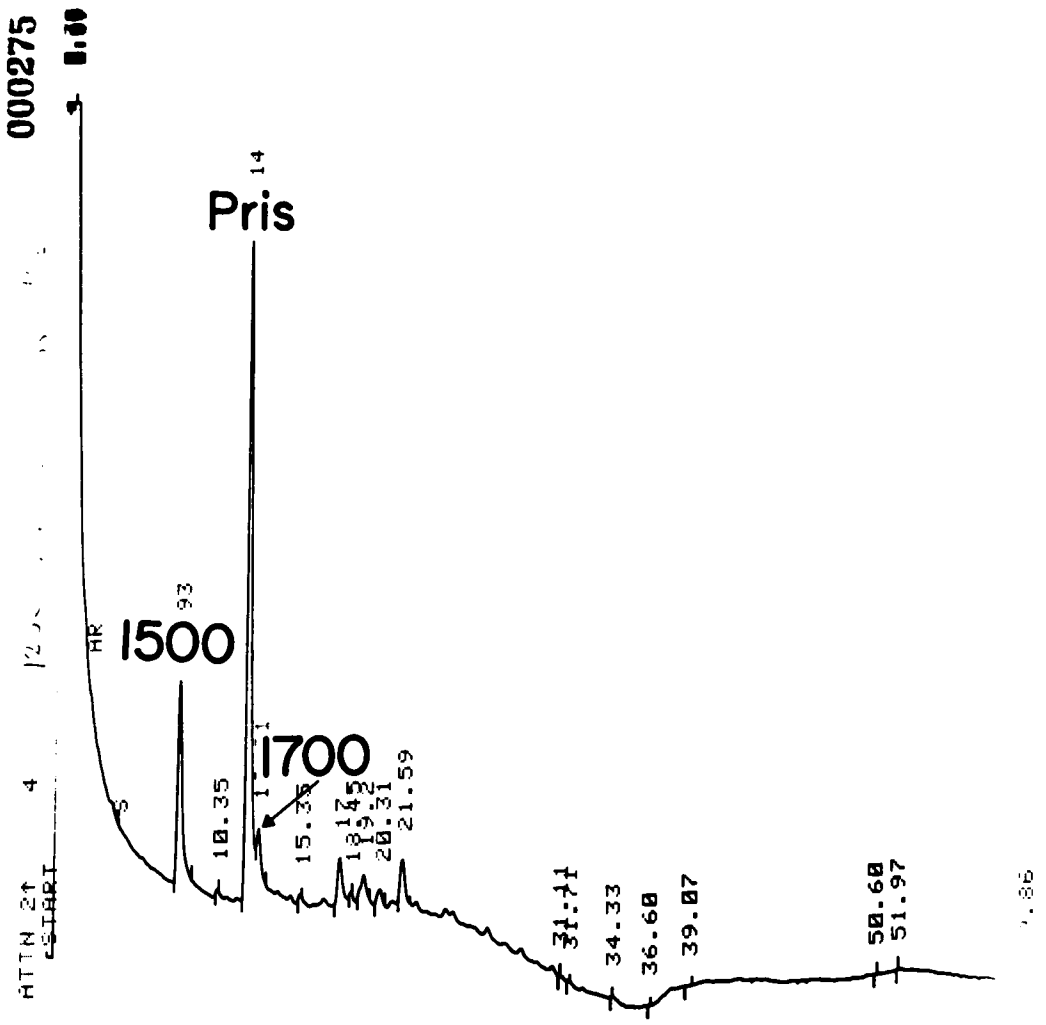

Fig. 3A. Zooplankton hydrocarbons, Group B, Station 1205, hexane fraction, summer 1975.

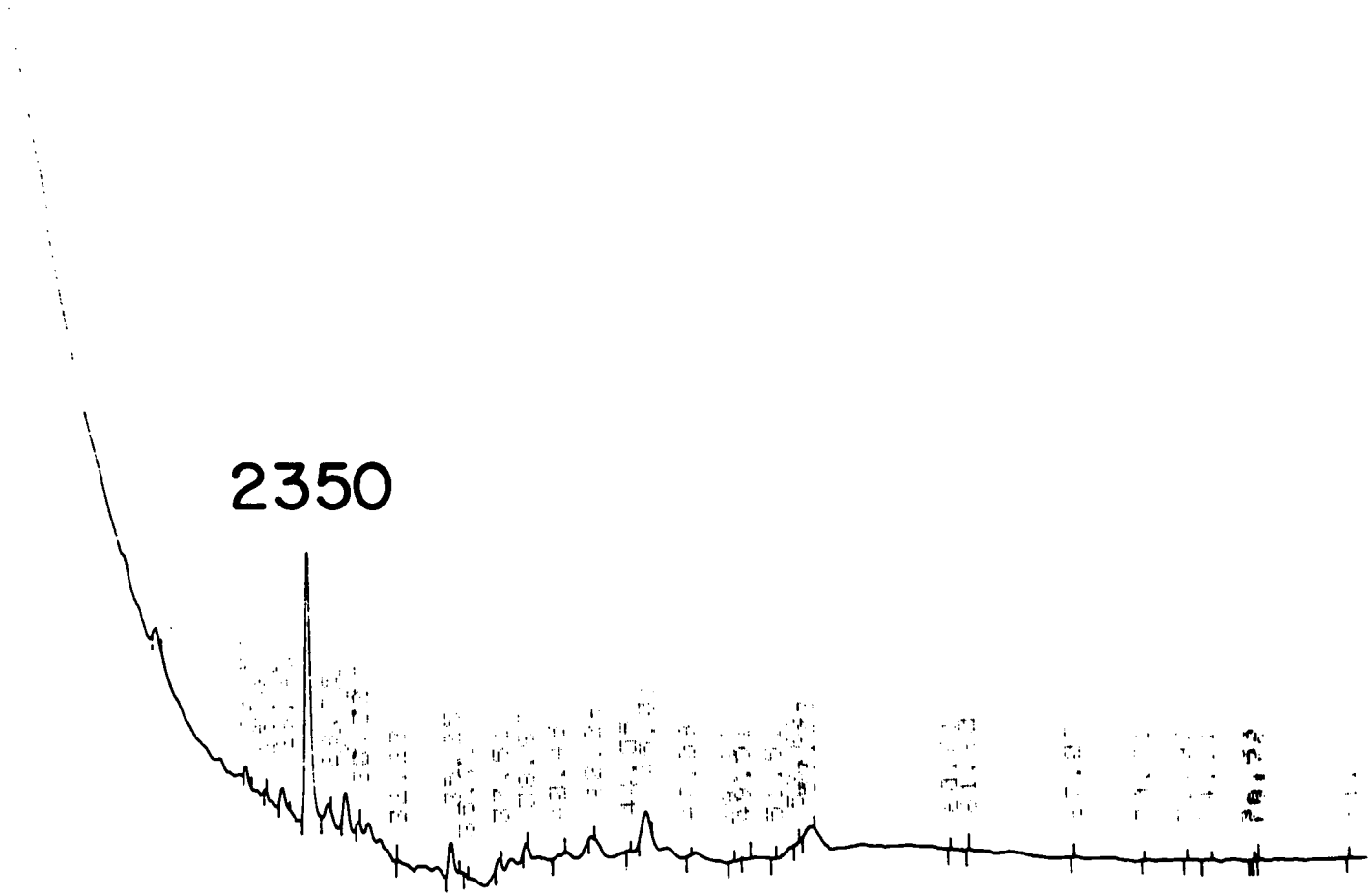

Fig. 3B. Zooplankton hydrocarbons, Group B, Station 1205, benzene fraction, summer 1975.

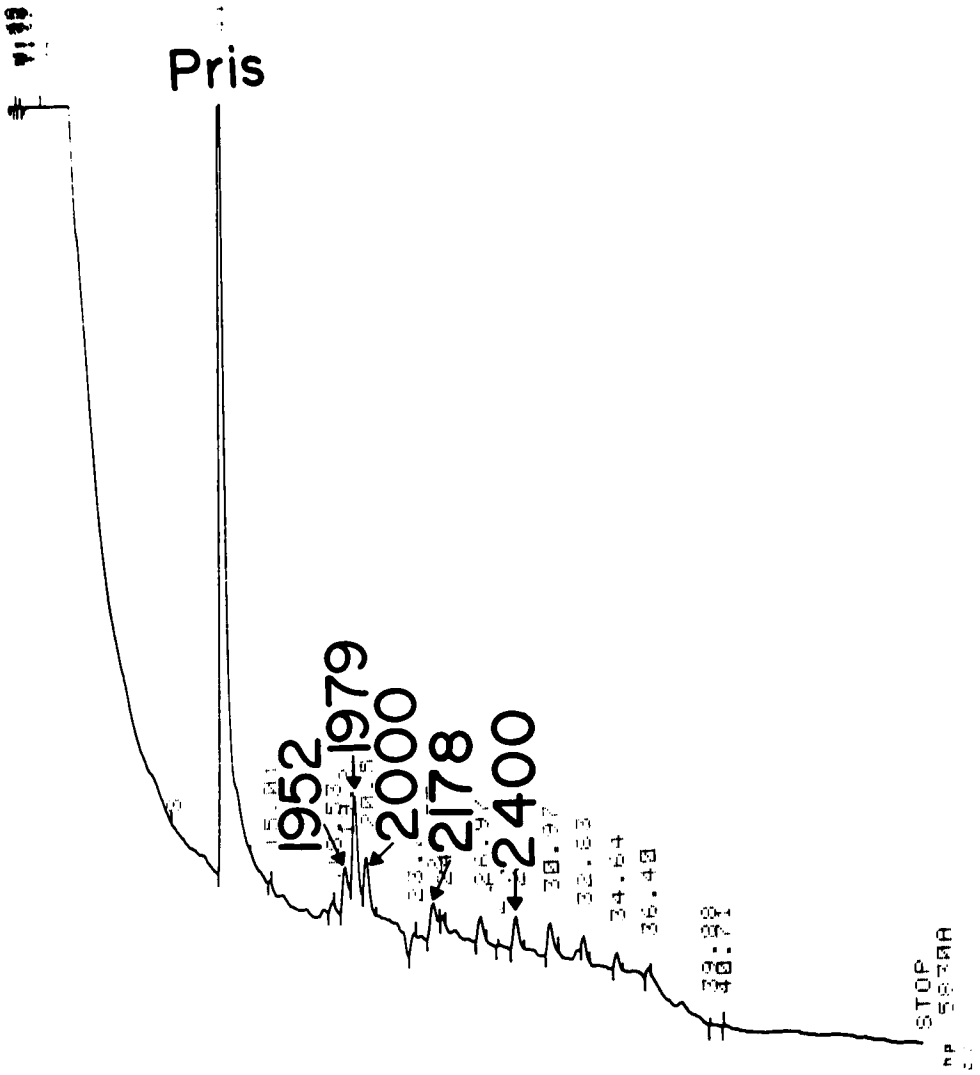

Fig. 4A. Zooplankton hydrocarbons, Group C, Station, 1309, hexane fraction, winter 1976.

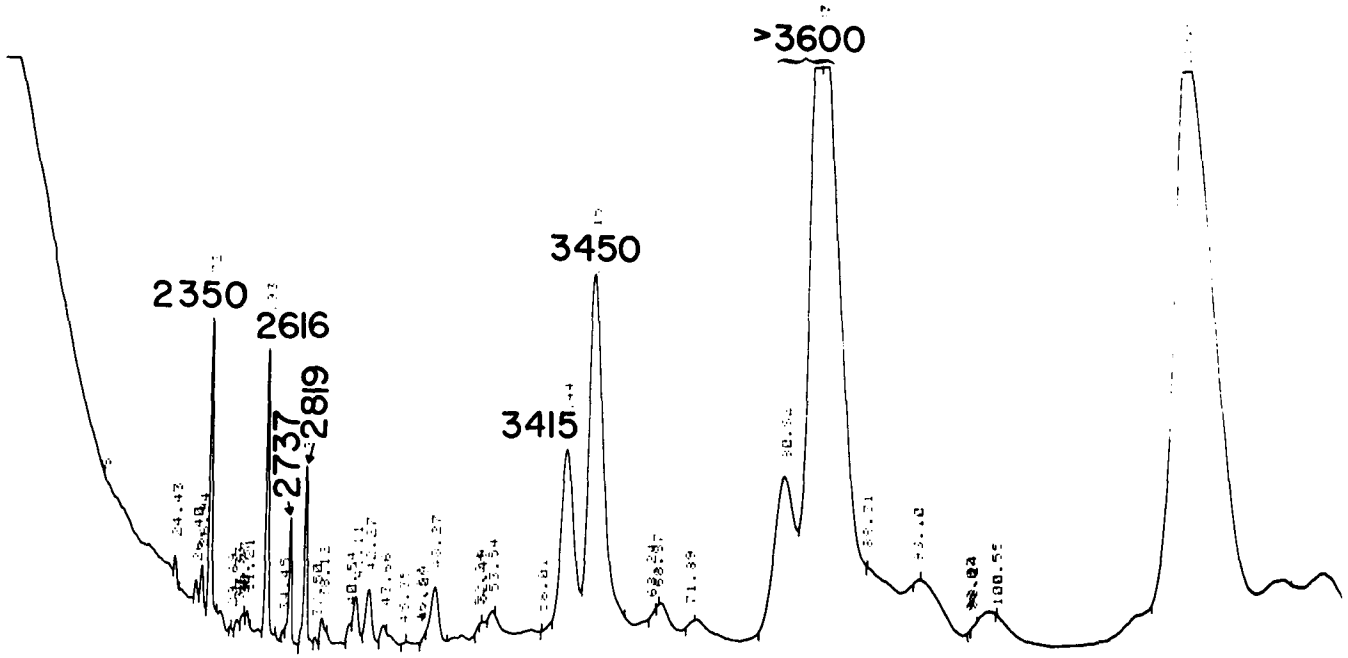

Fig. 4B. Zooplankton hydrocarbons, Group C, Station 1415, benzene fraction, summer 1975.

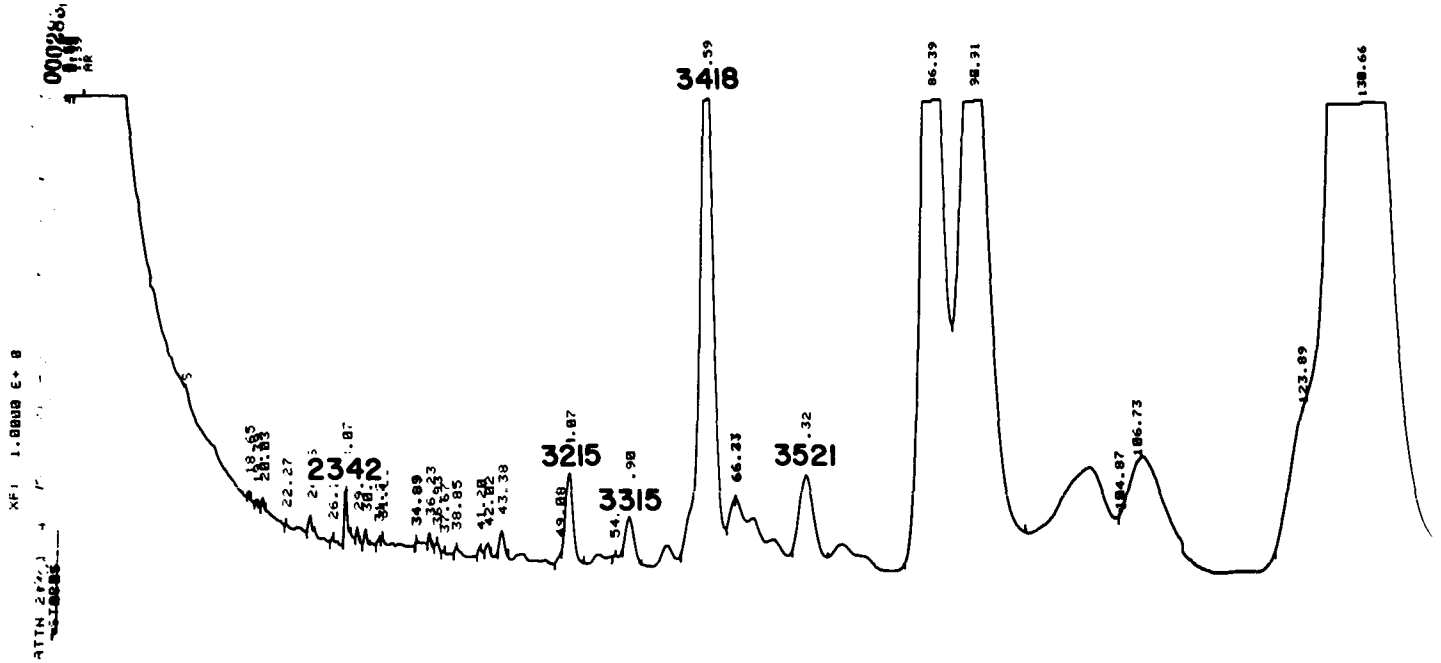

Fig. 4C. Zooplankton hydrocarbons, Group C, Station 1309, benzene fraction, fall 1975.

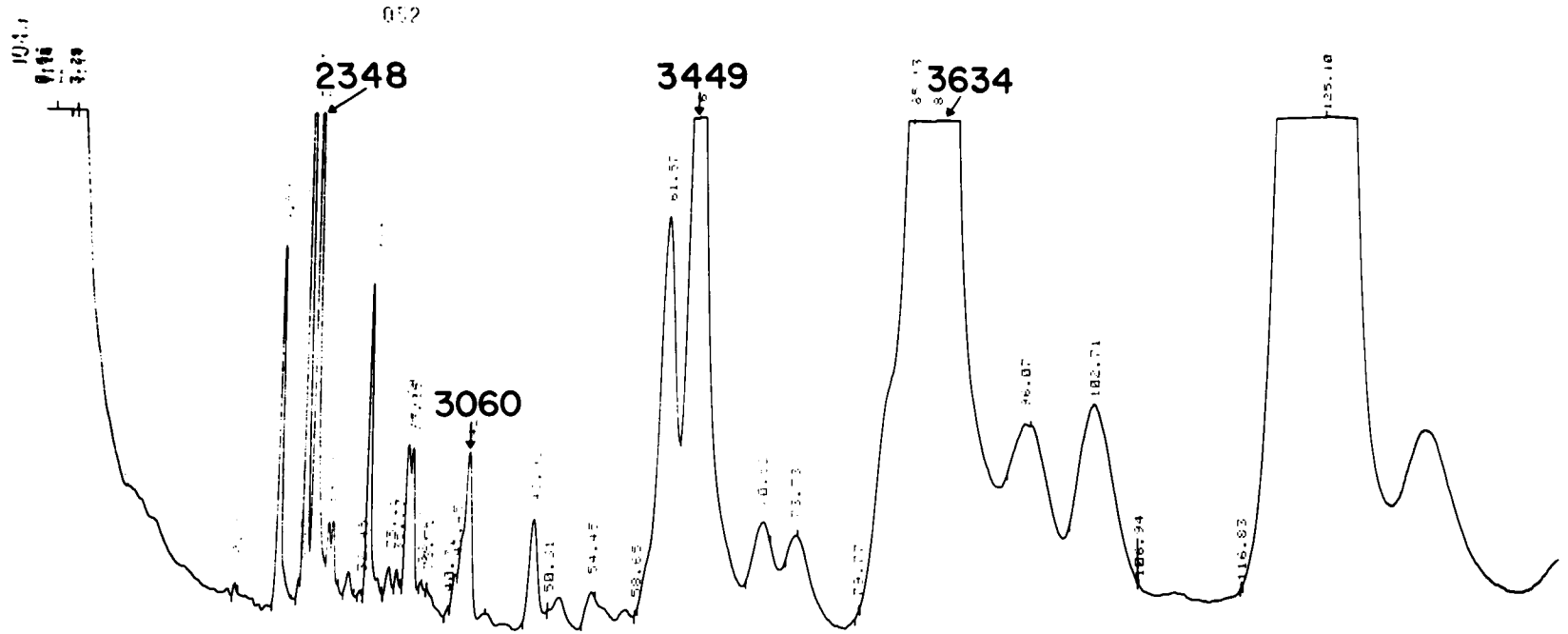

Fig. 4D. Zooplankton hydrocarbons, Group C, Station 1309, benzene fraction, winter 1976.

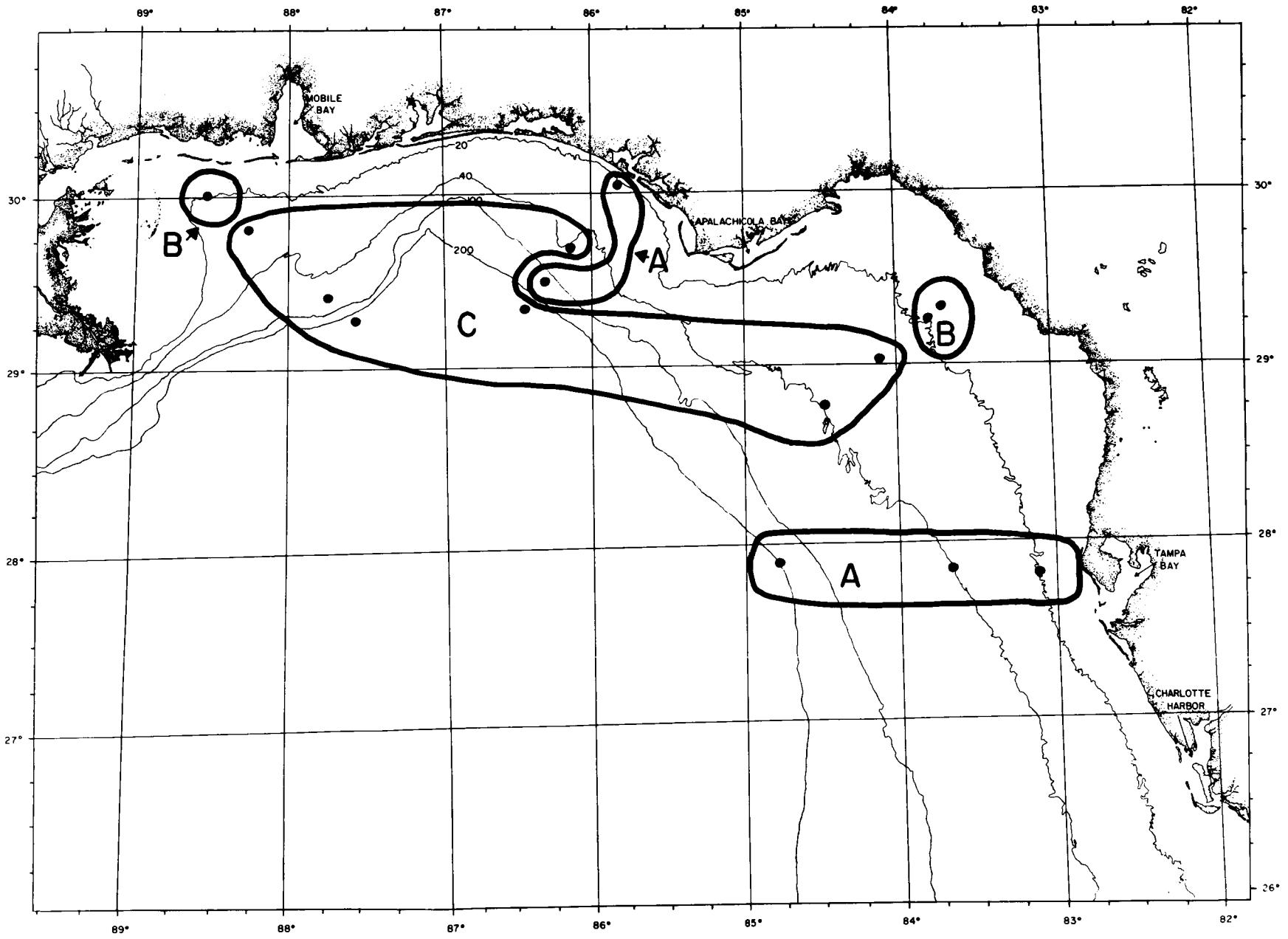

Fig. 5. Zooplankton hydrocarbon group distribution, summer 1975.

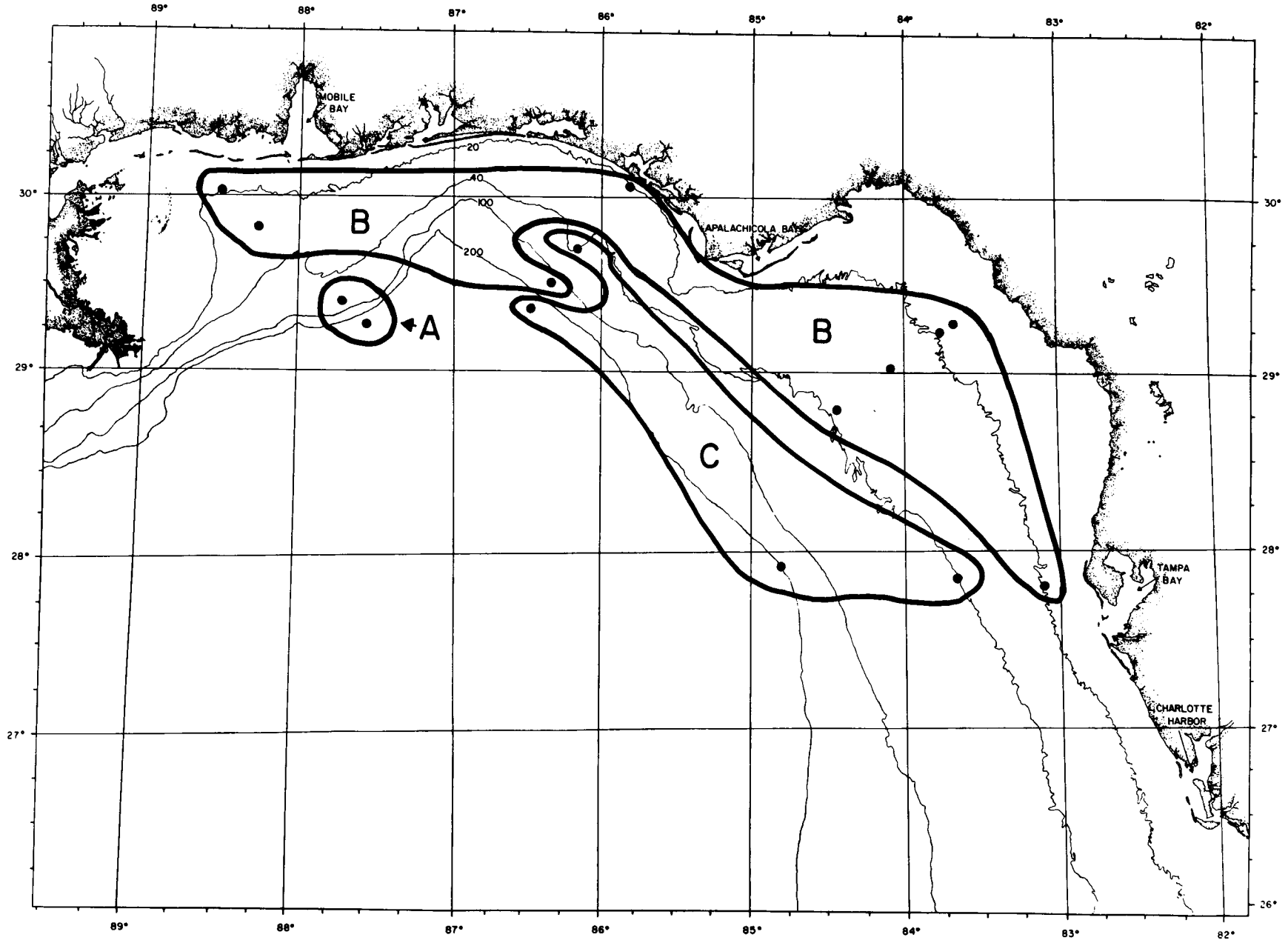

Fig. 6. Zooplankton hydrocarbon group distribution, fall 1975.

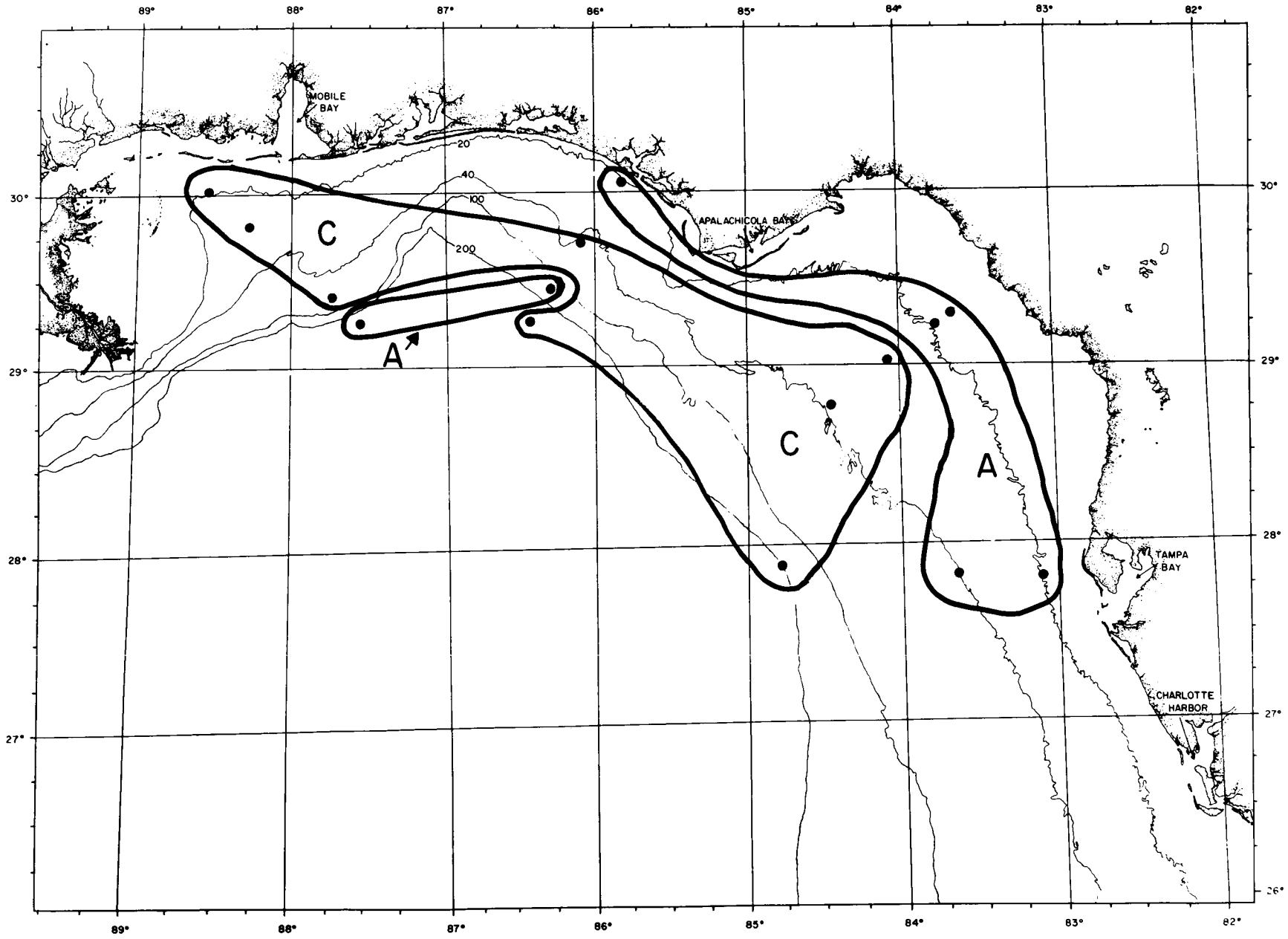

Fig. 7. Zooplankton hydrocarbon group distribution, winter 1976.

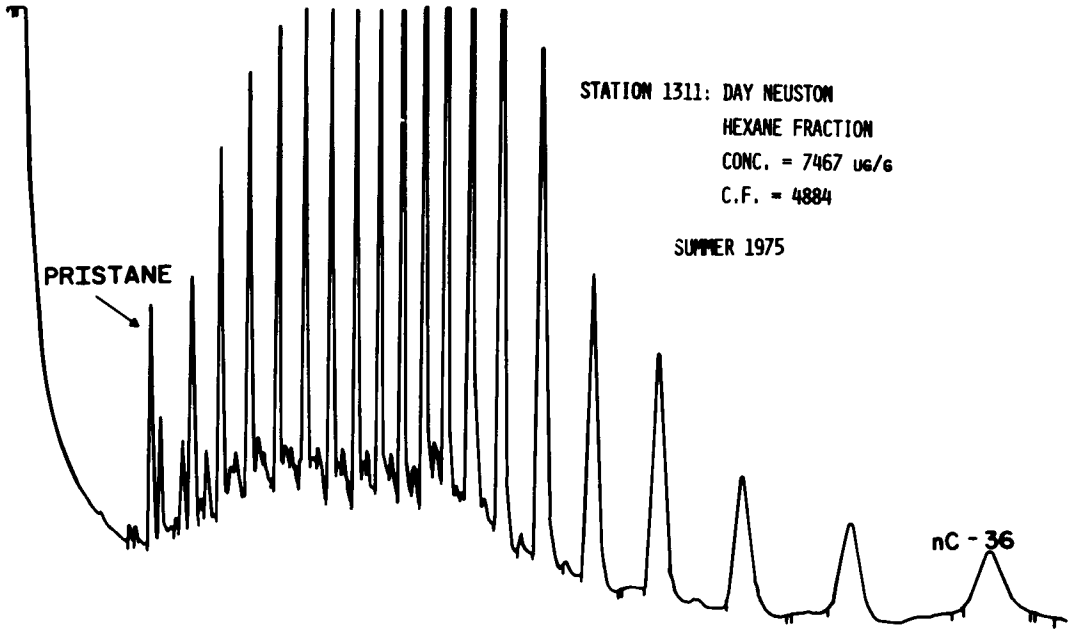

Fig. 8A. Tar ball contaminated neuston sample, hexane fraction.

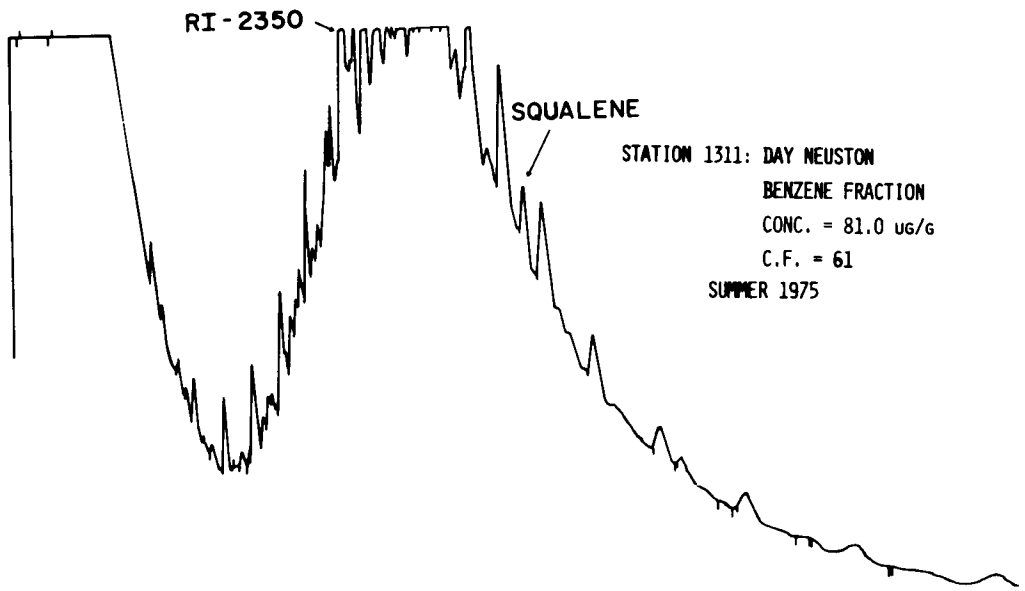

Fig. 8B. Tar ball contaminated neuston sample, benzene fraction.

METABOLISM OF BENZO(a)PYRENE AND OTHER XENOBIOTICS BY MICROSOMAL MIXED-FUNCTION OXIDASES IN MARINE SPECIES

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Benzo(a)pyrene and other compounds were metabolized by hepatic microsomal preparations from several species of teleosts and elasmobranchs. Hepatopancreatic microsomes from several crustaceans were essentially devoid of similar activity. Significant variability in the rates of *in vitro* metabolism of xenobiotics was noted among different species and among individuals of a given species. The oxidative metabolism of xenobiotics by marine species is catalyzed by a microsomal mixed-function oxidase system. The enzymes of this system, cytochrome P-450 and NADPH-cytochrome *c* reductase, were solubilized from hepatic microsomes from the little skate, *Raja erinacea*. These enzymes, along with a lipid-containing fraction, were shown to be required for the reconstitution of skate mixed-function oxidase activity.

INTRODUCTION

Animals in marine environments are exposed to a variety of foreign organic chemicals from a number of sources. Contamination of the oceans results from the discharge of industrial wastes directly into waterways, from runoff water containing pesticides and herbicides used in agriculture, from municipal discharges, and from other sources including accidental and natural releases of crude oil.

The introduction of hydrocarbons into the marine environment via oil tanker accidents and cleaning procedures and off-shore drilling leaks is a major concern of those who are investigating the effects of pollution on marine organisms. The ability of marine species to metabolize hydrocarbons, especially polycyclic aromatic hydrocarbons, into excretable and/or activated products is an important factor in the disposition and possible toxic effects of these compounds.

A number of marine species contain oxidative drug-metabolism systems. We have investigated hepatic and extrahepatic mixed-function oxidase (MFO) activities in a number of teleosts, elasmobranchs, and crustaceans found in the coastal waters of Maine and Florida [1-4]. The MFO system of marine vertebrates is similar to that found in mammals. The activity is located primarily in the 100,000 g subcellular fraction (microsomes) prepared from liver, the enzymes of the system appear to be cytochrome P-450 and NADPH-cytochrome *c* reductase, and NADPH and O_2 are required for activity. Both the mammalian and marine systems are inhibited by cytochrome *c* and carbon monoxide. Generally, MFO activity in hepatic microsomes from marine species is less than 1/10 of the activity present in rat or rabbit hepatic microsomes [4].

The present paper summarizes some of our findings concerning the activity, inducibility, and tissue distribution of MFO activity in several marine species. In addition, evidence is presented to show that the components of the hepatic MFO system from the little skate, *Raja erinacea*, are indeed cytochrome P-450 and NADPH-cytochrome *c* reductase and that they are similar to the components of the mammalian hepatic MFO systems.

METHODS

Standard methods were used for the determinations of the rates of benzo(a)pyrene hydroxylation[5,7], benzphetamine N-demethylation[6], and 7-ethoxycoumarin O-deethylation[7]. The reactions are catalyzed by the microsomal MFO system as follows:

$$AH + 2e^- + 2H^+ + O_2 \quad AOH + H_2O$$

When the substrate (AH) is benzphetamine or 7-ethoxycoumarin, the hydroxylation reaction results in N-demethylation and O-deethylation, respectively. The assay for benzo(a)pyrene hydroxylation primarily measures the amount of 3-hydroxybenzo(a)pyrene produced. 3-Hydroxybenzo(a)pyrene is a major metabolite of the reaction but many additional metabolites (arene oxides, dihydrodiols, and quinones) are also formed. Incubation conditions for these reactions in preparations from marine species have been reported[4].

Cytochrome P-450 concentrations were determined by the method of Omura and Sato[8,9] and NADPH-cytochrome *c* reductase activity by the method of Williams and Kamin[7]. Hepatic cytochrome P-450 from the little skate was solubilized from microsomes using sodium cholate (1 mg per mg protein). The cytochrome was partially purified by chromatography on DEAE-cellulose and hydroxylapatite. NADPH-cytochrome *c* reductase was solubilized using sodium deoxycholate and was partially purified by chromatography on DEAE-cellulose.

RESULTS AND DISCUSSION

Microsomal Mixed-Function Oxidase Activity in Marine Species

Hepatic Cytochrome P-450 Concentrations. Hepatic microsomal cytochrome P-450 concentrations were determined for a number of teleosts and elasmobranchs (table 1). The stingray, *Dasyatis sabina*, had a cytochrome concentration of 0.59 ± 0.23 nmoles per mg protein which compares favorably with the hepatic concentration of cytochrome P-450 in some mammals. The lowest P-450 concentrations observed, 0.17 ± 0.01 nmoles per mg protein in the winter flounder, *Pseudopleuronectes americanus*, were about 1/10 the concentration in rabbit liver and about 1/5 that of rat.

Hepatic Mixed-Function Oxidase Activity in Teleosts. Significant variations were found in the *in vitro* MFO activity of several teleost species (table 2). The rates of hydroxylation of benzo(a)pyrene ranged from 0.004 and 0.02 units per min per mg protein in the king of Norway, *Hemitripterus americanus*, to 5.03 units per min per mg protein in the sheepshead, *Archosargus probatocephalus*. In addition to the wide range of activity among the species, individual variation was also large (e.g., 1.62 ± 1.91 for the rate of benzo(a)pyrene hydroxylation as determined from eleven individual drum, *Pogonius cromis*). The species variation for benzphetamine N-demethylation rates (about 10-fold) was less than that observed for benzo(a)pyrene hydroxylation (table 2). However, individual variation for this reaction was as great as that observed for benzo(a)pyrene hyddroxylation (table 2). In species with rates of benzo(a)pyrene hydroxylation greater than 1.0, the relative ratios of benzo(a)pyrene hydroxylation to benzphetamine N-demethylation were between 3.2 and 4.3. On the other hand, in species with benzopyrene hydroxylation rates of less than 1.0, the benzo(a)-pyrene-benzphetamine ratios were less than 1.0.

Table 1.

CYTOCHROME P-450 CONTENT OF HEPATIC MICROSOMES FROM VARIOUS MARINE VERTEBRATE SPECIES

Species	Locale	Cytochrome P-450 Content (nmoles/mg microsomal protein)
TELEOSTS		
Winter Flounder (*Pseudopleuronectes americanus*)	Maine	0.17 ± 0.01 (3)[1]
Sheepshead (*Archosargus probatocephalus*)	Florida	0.29 ± 0.12 (13)
ELASMOBRANCHS		
Dogfish Shark (*Squalus acanthias*)	Maine	0.23, 0.29 (2)
Large Skate (*Raja ocellata*)	Maine	0.36, 0.41 (2)
Little Skate (*Raja erinacea*)	Maine	0.32 ± 0.08 (4)
Stingray (*Dasyatis sabina*)	Florida	0.59 ± 0.23 (7)

[1]Mean ± S.D. (N).

Table 2.

HEPATIC MICROSOMAL-FUNCTION OXIDASE ACTIVITY IN SEVERAL MARINE ELASMOBRANCHS OF MAINE AND FLORIDA

Species	Locale	Mixed-Function Oxidase Activity	
		Benzo(a)pyrene Hydroxlase[1]	Benzphetamine N-Demethylase[2]
Large Skate *Raja ocellata*	Maine[3]	0.30 ± .09 (3)[4]	1.49 ± .41 (3)
Little Skate *Raja erinacea*	Maine	0.17 ± .10 (10)	1.07 ± .19 (9)
Thorny Skate *Raja radiata*	Maine	0.12 ± .02 (3)	0.45 ± .14 (3)
Dogfish Shark *Squalus acanthias*	Maine	0.07 ± .02 (3)	0.15 ± .05 (3)
Stingray *Dasyatis sabina*	Florida[3]	0.79 ± .46 (24)	1.07 ± .75 (11)

[1]Fluorescence units formed/min/mg protein.

[2]Nmoles HCHO formed/min/mg protein.

[3]Maine species assayed at 30°C, Florida species at 35°C.

[4]Mean ± S.D. (N).

Hepatic Mixed-Function Oxidase Activity in Elasmobranchs. The rates of benzo(a)pyrene hydroxylation in hepatic microsomes from elasmobranchs were generally lower than the rates observed for the teleost species, whereas the rates of benzphetamine N-demethylation were quite similar (table 3). The relative ratios of benzo(a)pyrene hydroxylation to benzphetamine N-demethylation were all less than 0.75 for the elasmobranchs. Less variation in reaction rates was observed with elasmobranchs as compared to the teleosts. However, variation within a given species was pronounced (e.g., 1.07 ± 0.75 nmoles product per min per mg protein for the N-demethylation of benzphetamine in the stingray).

Hepatopancreatic Mixed-Function Oxidase Activity in Crustaceans. Hepatopancreatic microsomes were prepared from four species of crustaceans and examined for MFO activity (table 4). Benzo(a)pyrene hydroxylase activity was detected in several preparations from the spiny lobster, *Panulirus argus*, and the blue crab, *Callinectes sapillus*. This activity was not detected in preparations from the rock crab, *Cancer ittorus*, or the lobster, *Homerus americanus*. Benzphetamine N-demethylation activity was not detected in any of the preparations examined.

The near absence of detectable MFO activity in the crustaceans may be the result of inhibitors released during preparation of the microsomes[4].

Tissue Distribution of Microsomal Mixed-Function Oxidase Activity in the Little Skate, *Raja erinacea*. Eight tissues from the little skate were examined for MFO activity (table 5). Benzo(a)pyrene hydroxylation was detected in all tissues except the spiral valve. However, the activities were low in all tissues when compared to the liver. Benzphetamine N-demethylation activity was detected in microsomes from kidney (30% of hepatic activity) and gill and stomach lining (10% of hepatic activity) but not in microsomes from spleen or spiral valve.

Induction of Hepatic Mixed-Function Oxidase Activity in the Sheepshead, *Archosargus probatocephalus*. Mammalian hepatic MFO activity can be induced by a number of compounds administered *in vivo*. Generally, these compounds belong to one of two classes. One class (type I inducers) increases a great number of MFO activities and induces the formation of increased concentrations of cytochrome P-450. Phenobarbital is an example of a type I inducer. The second class (type II inducers), comprised mainly of polycyclic aromatic hydrocarbons, increases a few MFO activities, including benzo(a)pyrene hydroxylation, and induces the formation of cytochrome P-448[10]. An apparent correlation between the formation of cytochrome P-448 and increases in benzpyrene hydroxylase activity has been observed in a number of studies, e.g., [10-13]. We have been unable to demonstrate that phenobarbital is an inducer of MFO activity in marine species. However, repeated treatment of several species with 3-methylcholanthrene has resulted in significant increases in the hepatic microsomal hydroxylation of benzo(a)pyrene. This effect in the sheepshead is shown in table 6. Significant increases in benzo(a)pyrene metabolism were seen 6, 9, and 13 days following treatment with 3-methylcholanthrene on days 1 and 3. Less pronounced increases were also observed for the O-deethylation of 7-ethoxycoumarin. These increases, along with the lack of effect on benzphetamine N-demethylation, were the expected results. However, the lack of either a qualitative or quantitative effect on hepatic cytochrome P-450 remains to be explained (table 6).

Table 3.

HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE ACTIVITY IN SEVERAL MARINE TELEOSTS OF MAINE AND FLORIDA

Species	Locale	Mixed-Function Oxidase Activity	
		Benzo[a]pyrene Hydroxylase[1]	Benzphetamine N-Demethylase[2]
Mummichog *Fundulus heteroclitus*	Maine[3]	4.10 ± 2.10 (3)[4,5]	1.13 ± 0.76 (3)[5]
Winter Flounder *Pseudopleuronectes americanus*	Maine	2.54 ± 1.67 (7)	0.59 ± 0.13 (3)
Eel *Anguilla rostrata*	Maine	0.21[5]	0.44[5]
King of Norway *Hemitripterus americanus*	Maine	0.004, 0.02 (2)	0.16 ± 0.04 (3)
Sheepshead *Archosargus probatocephalus*	Florida[3]	5.03 ± 1.39 (20)	1.57 ± 1.10 (18)
Drum *Pogonias cromis*	Florida	1.62 ± 1.91 (11)	0.45 ± 0.09 (5)

[1]Fluorescence units formed/min/mg protein.

[2]Nmoles HCHO formed/min/mg protein.

[3]Maine species assayed at 30°C, Florida species at 35°C.

[4]Mean ± S. D. (N).

[5]Liver from three or more individual animals pooled prior to microsome preparation.

Table 4.

MICROSOMAL MIXED-FUNCTION OXIDASE ACTIVITY IN HEPATOPANCREAS OF MAINE AND FLORIDA CRUSTACEANS

Species	Locale	Mixed-Function Oxidase Activity	
		Benzo[a]pyrene Hydroxylase[1]	Benzphetamine N-Demethylase[2]
Rock Crab *Cancer ittorus*[3]	Maine	< 0.01[4] (2)	< 0.06[4] (2)
Lobster *Homerus americanus*	Maine	< 0.01 (3)	< 0.06 (3)
Spiny Lobster *Panulirus argus*	Florida	< 0.01 - 0.04 (15)[5]	< 0.06 (4)
Blue Crab *Callinectes sapillus*	Florida	< 0.01 - 0.01 (3)	< 0.06 (3)

[1] Fluorescence units formed/min/mg protein.

[2] Nmoles HCHO formed/min/mg protein.

[3] Separate pools of hepatopancreas from 3 crabs were pooled.

[4] Lowest activity that could be accurately assayed with the procedures used.

[5] Range (N).

Table 5.

BENZO(a)PYRENE HYDROXYLASE AND BENZPHETAMINE N-DEMETHYLASE ACTIVITIES IN MICROSOMES FROM SEVERAL ORGANS OF THE LITTLE SKATE RAJA ERINACEA

Organ	Benzo(a)pyrene Hydroxylase[1]	Benzphetamine N-Demethylase[2]
Liver	0.17[3]	1.25
Kidney	0.03	0.35
Spleen	0.03	<0.01[4]
Pancreas	0.03	---[5]
Gill	0.02	0.12
Stomach Lining	0.02	0.12
Heart	0.02	---
Spiral Valve	<0.003[4]	<0.01

[1]Fluorescence units formed/min/mg protein.

[2]Nmoles HCHO formed/min/mg protein.

[3]Results are from assays on tissues pooled from three skates (prior to homogenization).

[4]Lowest activity that could be assayed with the procedures used.

[5]Not measured due to insufficient microsomal protein.

Table 6.

EFFECT OF REPEATED 3-METHYLCHOLANTHRENE (3-MC) ADMINISTRATION ON THE HEPATIC MICROSOME MIXED-FUNCTION OXIDASE SYSTEM IN SHEEPSHEAD (*ARCHOSARGUS PROBATOCEPHALUS*)[1]

Parameter	Control	3-MC (Day 6)	3-MC (Day 9)	3-MC (Day 13)
Cytochrome P-450 Content (nmoles/mg protein)	0.28 ± 0.09 (7)[2]	0.44 ± 0.17 (6)	0.54 ± 0.28 (4)	0.37 ± 0.13 (5)
Benzo(a)pyrene Hydroxylase	1.41 ± 0.49 (7)	15.21 ± 8.19 (6)	10.98 ± 3.76 (4)	14.48 ± 5.63 (5)
7-Ethoxycoumarin O-Deethylase	0.05 ± 0.03 (7)	0.23 ± 0.08 (6)	0.58 ± 0.33 (4)	0.24 ± 0.06 (5)
Benzphetamine N-Demethylase	1.10 ± 0.42 (7)	1.03 ± 0.40 (6)	1.08 ± 0.31 (4)	0.79 ± 0.17 (5)
Microsomal Protein Yield (mg/g liver)	9.7 ± 1.3 (7)	13.1 ± 5.3 (6)	11.1 ± 1.5 (4)	10.5 ± 1.2 (5)

[1]Treated by I.P. injection with 20 mg/kg 3-MC in corn oil on days 1 and 3. Animals then sacrificed on day 6, 9 or 13.

[2]Mean ± S.D. (N).

[3]Fluorescence units formed/min/mg protein.

[4]Nmoles product formed/min/mg protein.

Solubilization and Reconstitution of the Hepatic Microsomal Mixed-Function Oxidase System from the Little Skate, *Raja erinacea*

Preparation of Skate Hepatic Cytochrome P-450. Hepatic microsomes from the little skate were solubilized with sodium cholate and applied to a column of DEAE-cellulose. The cytochrome P-450 was eluted from the cellulose with a buffer solution containing the nonionic detergent Emulgen 913. Two fractions (I and II) containing cytochrome P-450 were obtained in this manner. These fractions were then chromatographed on a column of hydroxylapatite. Fraction II was found to contain a pigmented impurity which resembled melanin. This impurity was separated from the cytochrome by the hydroxylapatite procedure. The cytochrome P-450 preparations obtained from fractions I and II were combined and passed through a column of Porapak Q to remove unbound detergent. The final preparation contained 3.0 nmoles cytochrome P-450 per mg protein as compared to a microsomal concentration of 0.26 nmoles per mg protein. The carbon monoxide difference spectrum of the dithionite-reduced cytochrome preparation is shown in figure 1. The spectrum, with a peak absorption at 450 nm, is the same as the spectra obtained with mammalian cytochrome P-450. The absolute spectra of skate cytochrome P-450 (figure 2) are also similar to the spectra of mammalian hepatic cytochrome P-450.

Preparation of Skate Hepatic NADPH-Cytochrome *c* Reductase and Lipid Fractions. Skate hepatic microsomes were solubilized with sodium deoxycholate and applied to a column of DEAE-cellulose. Fractions containing NADPH-cytochrome *c* reductase activity were eluted with a linear gradient of KCl (0 to 0.5 M) in phosphate buffer. The NADPH-cytochrome *c* reductase preparations contained about 500 units of activity per mg protein. (One unit equals one nmole cytochrome *c* reduced per min.) Skate hepatic microsomes contain about 60 units of reductase per mg protein. Fractions containing lipid required for maximum MFO activity in the reconstituted system were obtained by further elution of the DEAE-cellulose with 0.6 M KCl.

Reconstitution of Mixed-Function Oxidase Activity Using Components Solubilized from Hepatic Microsomes of the Little Skate. Figure 3 shows that the maximum activity obtained with the soluble skate MFO system required cytochrome P-450, NADPH-cytochrome *c* reductase, and lipid. Cytochrome, reductase, or lipid alone were inactive. Cytochrome plus reductase without added lipid formed a partially active system (25% of maximum). Saturation of the O-deethylase activity with respect to cytochrome was achieved when constant amounts of reductase (130 units per ml) and lipid (0.08 mg/ml) were used (figure 3). Thus, we were able to demonstrate that the components of the skate hepatic MFO system are the same as those observed in mammalian hepatic MFO systems[14].

CONCLUSION

Examination of a number of marine vertebrates for their ability to metabolize certain xenobiotics *in vitro* disclosed a great deal of both species and individual variation. It is difficult to assess the factors which contribute to species variation but individual variation may result

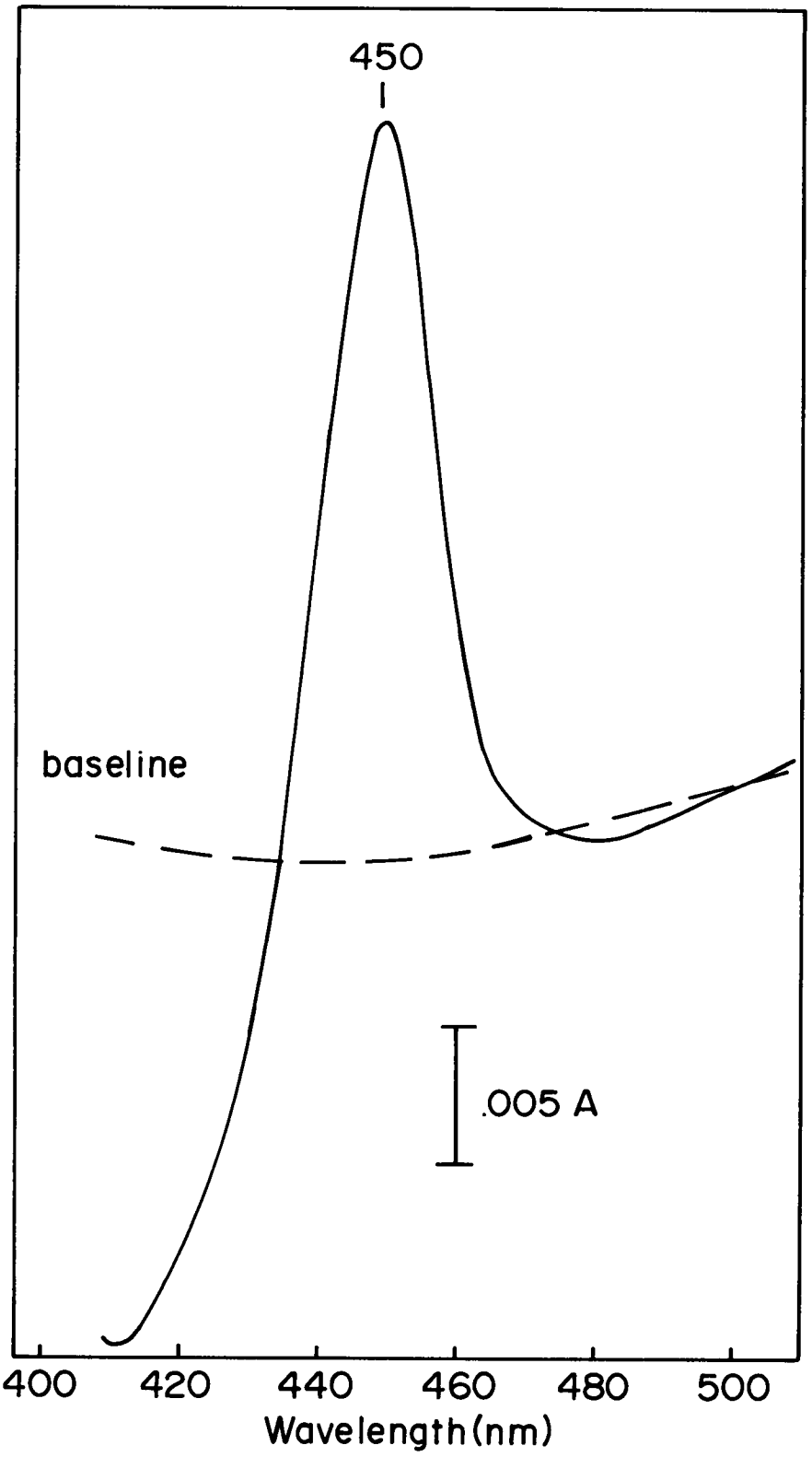


Fig. 1. CO-difference spectrum of dithionite-reduced, partially purified cytochrome P-450 from skate hepatic microsomes. A preparation containing 3.0 nmol of cytochrome P-450 per mg protein was dissolved in 0.1 M phosphate buffer, pH 7.7, containing 30% glycerol and 0.1 mM EDTA. The final concentration of cytochrome P-450 was 0.30 μM and the protein concentration was 0.097 μg per ml.

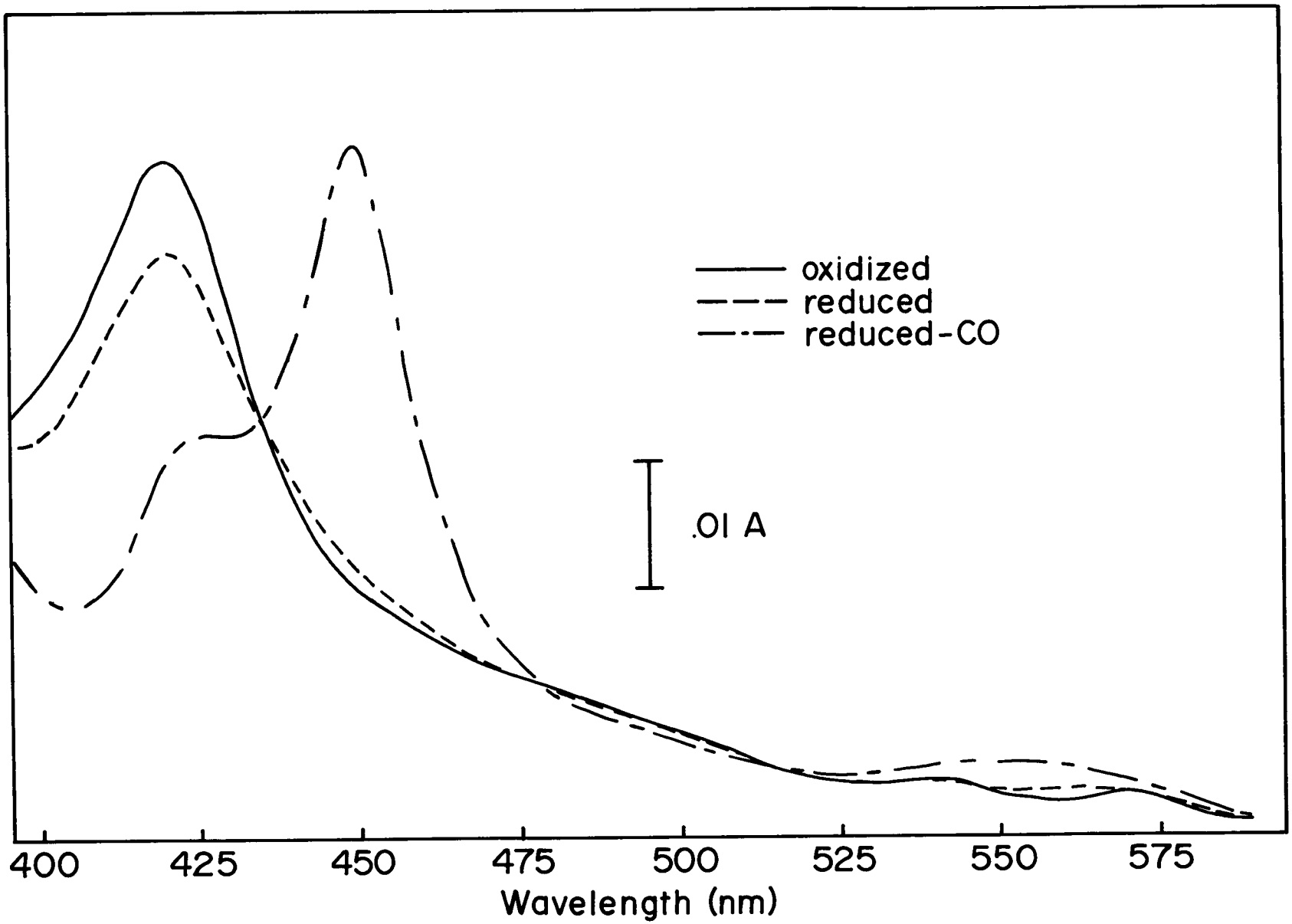
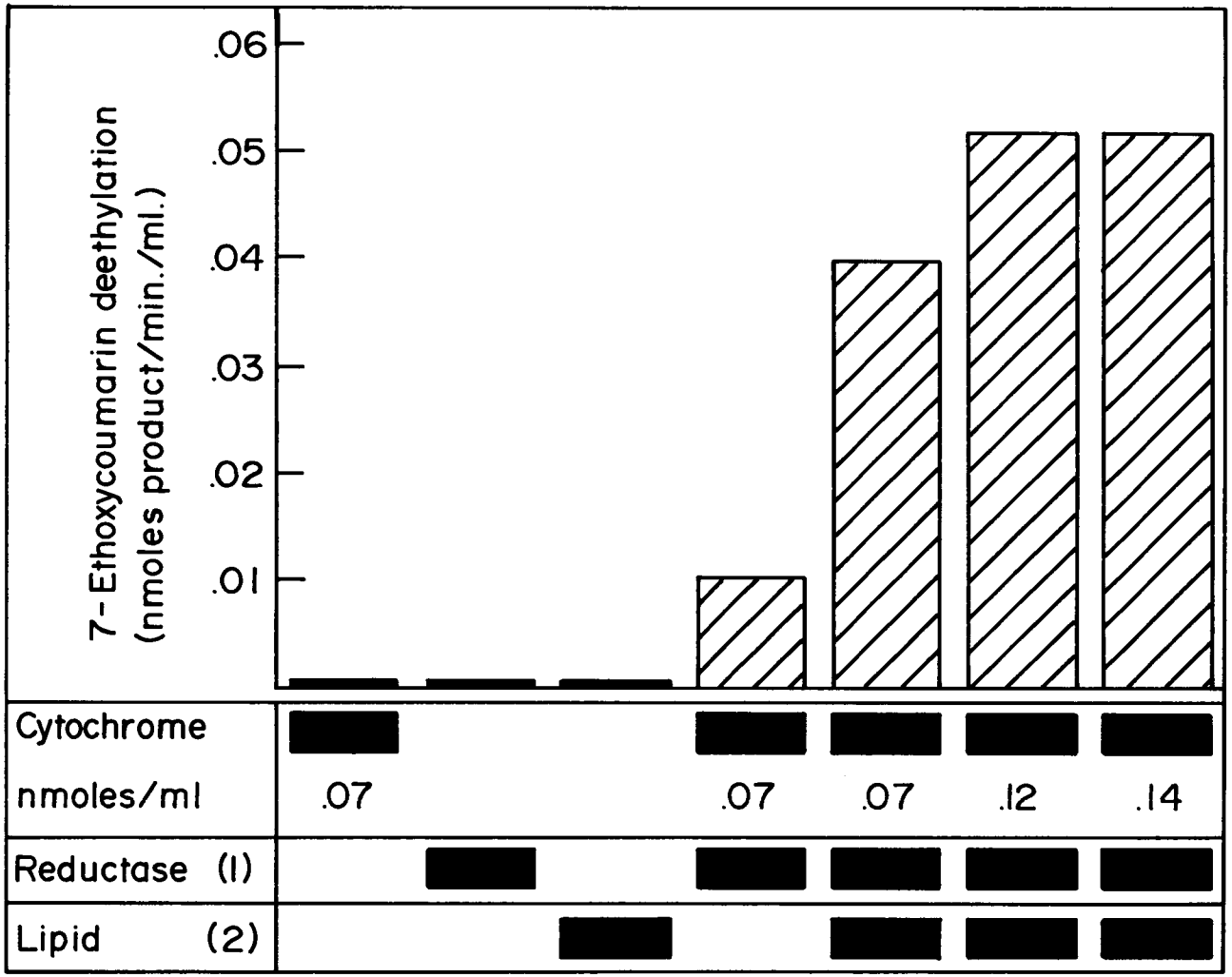


Fig. 2. Absolute spectra of partially purified skate hepatic cytochrome P-450. ————, oxidized; —— —— ——, dithionite-reduced; —— – —— –, dithionite-reduced plus carbon monoxide. The conditions were those described for Figure 1.



1. 130 units/ml
2. .08 mg/ml

Fig. 3. 7-Ethoxycoumarin O-deethylation activity in incubations containing solubilized components of the skate hepatic microsomal mixed-function oxidase system. Reductase, cytochrome, and lipid concentrations were those noted. The incubation volumes were 2.5 ml.

from exposure to different amounts of inducers. Experiments using selective inhibitors of induced and uninduced forms of cytochrome P-450 are now underway to determine if this is the case with the little skate.

The hepatic MFO systems of marine animals appear to be quite similar to those found in mammals. One exception to this generalization is the lack of response of the fish MFO systems to phenobarbital. In any case, it is clear that a number of marine species have the ability to oxidatively metabolize xenobiotics. Other species, like the crustaceans examined, may not have efficient MFO systems or may be lacking in activity altogether. The possible consequences of the absence or presence of MFO activity in different marine species are not clear.

Pollution of the marine environment with crude oils can result in both temporary high concentrations and long-term low concentrations of aromatic hydrocarbons. It is known that many aromatic hydrocarbons require metabolic activation before their toxic effects are manifested. It is also true that such compounds must be biotransformed before they can be effectively excreted. Both activation and excretion pathways may be initiated by the microsomal MFO system. The absence of MFO activity may result in the storage of potentially dangerous substances is species which are used as a food source for man. On the other hand, active MFO systems in some species may generate carcinogenic, mutagenic, or toxic metabolites from environmental contaminants. The result could be depletion of certain economically important species and/or significant changes in some ecosystems.

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DISCUSSION

BRUBAKER: Is it reasonable to conclude from your presentation that the mixed function oxidase system is a species-equivalent reaction found in animals and man? If so, what are the environmental health implications of changes caused by such xenobiotics? If the system can be sufficiently quantified, is it feasible to consider this system as a bioequivalent indicator that was mentioned this morning for assessing pollution?

PHILPOT: First, I think it is important in the description of the system that we no longer refer to the biological oxidation of xenobiotics as degradation per se. I think it is quite clear now that a number of carcinogens, benzopyrene being one of them, and a number of mutagens require the system in order to be activated. Thus, even though the system degrades compounds into excretable products, we now know that it also activates compounds into metabolites, which may be capable of doing biological damage.

There is some question about the possibility of using marine organisms as "an early warning system" for pollution. The problem there stems from the fact that we don't know what the baseline is. In many of the species that are bottom dwellers and inshore dwellers the variability from animal to animal is quite significant. This may be due to the fact that they are exposed to different levels of inducer compounds in their environment, whereas the activities in animals that are in the deeper waters, particularly in Maine, appear to be a lot more standardized. So one of the things that we are in the process of doing is setting up aquaria to culture these particular species or some of these species so that we can try to make a determination of what their activities are in the absence of compromising compounds. I think if we can do that there at least is a possibility that you might be able to collect enough numbers, say, in an area of an oil spill or an area of PCB contamination, so that you could differentiate between, say, the epicenter of the spill and an area which the material had not reached yet.

SOME TERPENOIDS FROM MARINE ORGANISMS

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The terpenoids from marine organisms show sufficient significant variation with source that they can be used as chemotaxonomic markers. Some typical examples of terpenoids from different marine phyla are reviewed, together with some known degradation schemes.

The ambition of marine chemists is to define the molecular species, both inorganic and organic, which exist in the oceans and elucidate the interactions between these species. Unfortunately, this is a very difficult task, expecially in the case of organic chemicals. Seawater contains less than 2.5 mg C/liter of dissolved organic carbon, which probably consists of many thousands of individual component compounds. The separation of individual components, first from the seawater itself and then from other similar compounds, is considered too demanding at present.

We must therefore approach the problem of describing the organic molecules in the oceans in an oblique manner by examining molecules which enter and leave the marine environment. Geochemists are compiling a list of organic constituents in marine sediments[1], while marine natural products chemists are elucidating the structures of compounds which are expected to enter the oceans[2]. I wish to put the case for examining terpenoids in more detail, since they can most easily be traced to specific organisms or groups of organisms.

Naturally-occurring organic molecules are classified according to function as primary or secondary metabolites. Primary metabolites such as fatty acids, sterols, amino acids, sugars, vitamins, etc., are those compounds which are required for life processes. The secondary metabolites are those compounds to which no general function has been assigned. A few secondary metabolites have been ascribed species-specific roles, but the majority of the secondary metabolites have no known function. Since they show great variation from organism to organism, they can be used as chemotaxonomic markers.

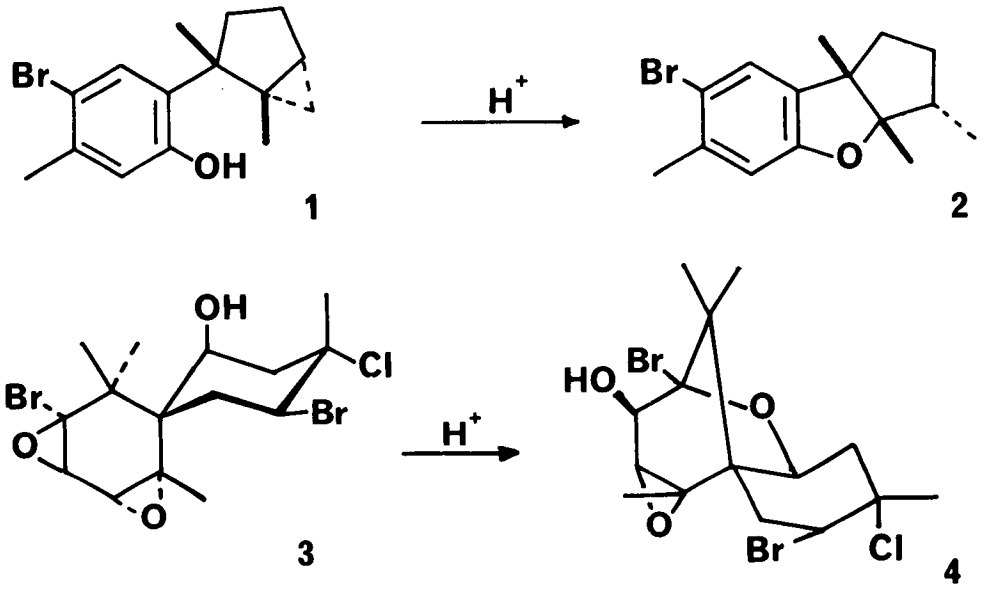
Among the secondary metabolites, terpenoids are one of the most variable classes. Organic chemists first studied terpenoids because they were constituents of the essential oils of perfumery. Early researchers established that terpenoids normally contained multiples of five carbon atoms and classified the terpenoids accordingly as monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}), and carotenoids (C_{40}).

Sterols are terpenoid primary metabolites. Almost all sterols have the same tetracyclic ring system with variations in the carbon skeleton of the side chain and in oxidation patterns. The sterols from marine organisms have been studied in detail with the objective of establishing chemotaxonomic relationships, particularly among sponges[3]. Despite the extensive research which has resulted in the identification of many new sterols, a chemotaxonomic scheme based on sterols has not materialized. Recent studies using radiolabelled precursors suggest that sponges are not capable of de novo biosynthesis of sterols[4] but do modify dietary sterols such as cholesterol. There is a growing suspicion that invertebrates normally obtain sterols from dietary sources ultimately depending on photosynthetic organisms. Given these circumstances, the discovery of sterols or sterol derivatives in a sediment does not clearly define the source of the sterol.

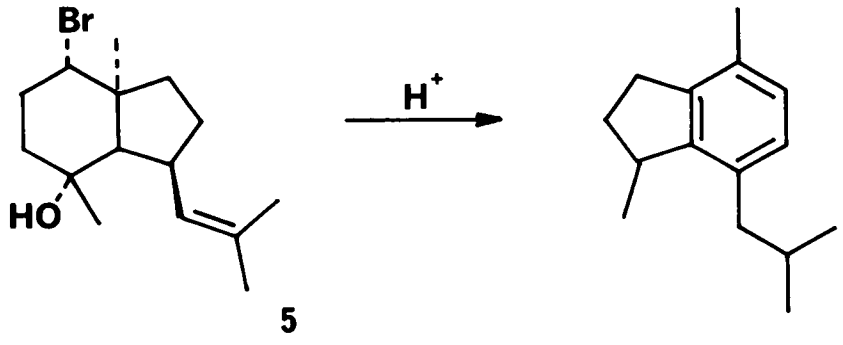
With the exception of sterols, obvious quantities of terpenoids have been found in algae and a few invertebrate phyla. Herbivorous molluscs such as the sea hare Aplysia californica consume large quantities of algae and store terpenes from these sources in a digestive gland[5]. Starfish and sea cucumbers (Echinoderms) both use triterpenoid glycosides as toxins, but research suggests that the triterpene portions are dietary components[6]. Soft corals and gorgonians sometimes contain as much as 3% of dry weight as diterpenes, but the same corals also have symbiotic photosynthetic zooxanthellae which appear to be responsible for terpene biosynthesis[7]. Sponges contain many terpenes[8], but again, de novo biosynthesis of terpenes has not been observed. Just as plants are the most abundant source of terrestrial terpenoids, so many of the marine terpenoids are from algal sources.

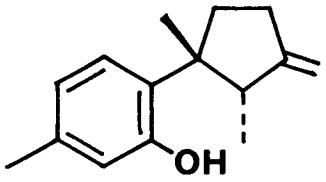
The most unusual feature of marine natural products is the large number of compounds which contain covalently-bound halogen atoms. The first halogen-containing terpenes were found in a sea hare, Aplysia kurodai[9]. However, this was not the true source of the halogenated sesquiterpenes. Several species of red algae of the genus Laurencia contain laurinterol (1), a powerful antimicrobial agent[10], which can undergo a facile acid-catalyzed rearrangement to aplysin (2), a metabolite of A. kurodai. Laurencia species have been investigated by many groups throughout the world and have been shown to contain a wide variety of metabolites, including some

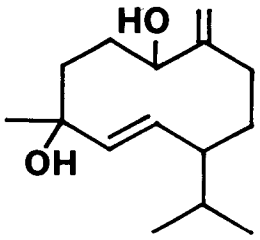
chamigrene derivatives which contain both bromine and chlorine. Acid-catalyzed rearrangements, such as the rearrangement of prepacifenol epoxide (3) to johnstonol (4), are known in the chamigrene series[11]. The same rearrangement can occur in the digestive gland of A. californica. In both these rearrangements, the more stable ethers were formed without skeletal rearrangement.

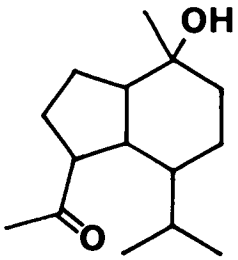


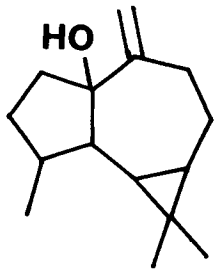
Oppositol (5), isolated from Laurencia subopposita, undergoes acid-catalyzed rearrangement to an aromatic compound[12]. This type of rearrangement, involving loss of halogen and skeletal rearrangement to an aromatic system, requires slightly more severe acid conditions but is autocatalytic. The same alga, Laurencia subopposita, also contains a variety of non-halogenated sesquiterpenes of different skeletal classes but, by current standards, this is unusual[13a].



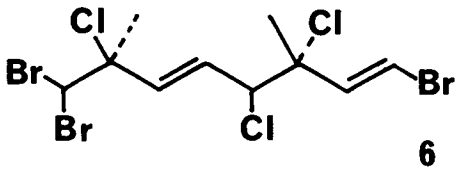








We have carried out two studies of the terpenoid constituents of the digestive glands of sea hares, which we assume to be part of a chemical defense system. Our results indicated that the halogenated sesquiterpenes in Aplysia californica could all be found in local species of Laurencia[5]. We also isolated a series of halogenated monoterpenes which were originally metabolites of Plocamium cartilagineum and Plocamium violaceum. The first halogenated monoterpene to be described contained three chlorine and three bromine atoms (72% halogens by weight)[14]. This highly halogenated monoterpene 6 proved to be the first of a series of halogenated linear monoterpenes from Plocamium cartilagineum[15]. Plocamium violaceum contains a series of monocyclic halogenated monoterpenes of two skeletal types[16]. Violacene-2 (7) undergoes a facile dehydrohalogenation to 2,4-dimethyl-2'-chlorostyrene,which bears little resemblance to a terpene, although the skeletal rearrangements are not unusual.



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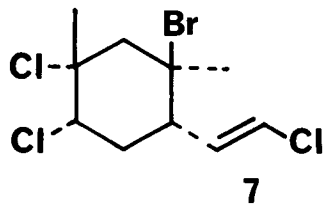
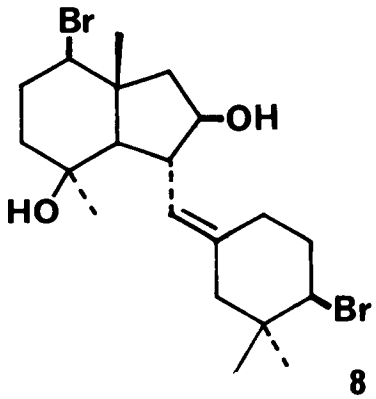


7

Halogenated diterpenes have also been found in Laurencia species. The skeleton of irieol (8) is reminiscent of oppositol (5) but contains an additional bromine atom in the additional ring[17]. A quite unrelated ring system is found in concinndiol (9)[18], which has a sesquiterpenoid counterpart in snyderol (10)[19].



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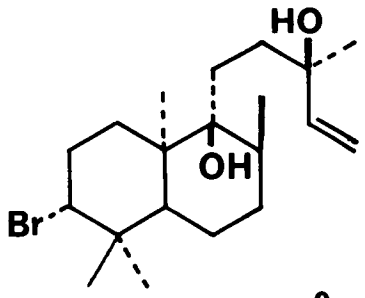

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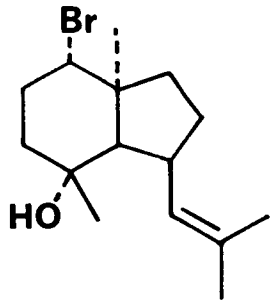

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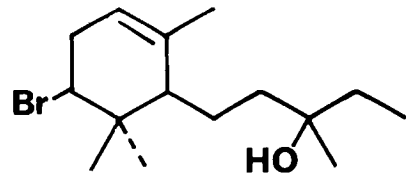


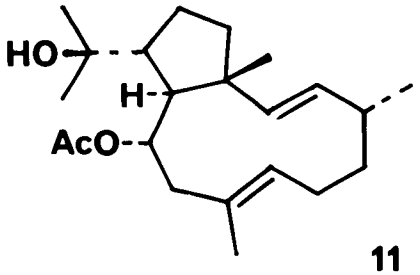
10

Our second sea hare study was on Dolabella californica, which was found to contain a series of 15 diterpenes, all having the same carbon skeleton[13b]. We have been unable to find an alga containing the diterpene 11 or relatives, but we expect that the compounds originate in a brown alga, since the only known sources of non-halogenated diterpenes are the brown algae.

Brown algae of the family Dictyotaceae contain sesquiterpenes, diterpenes and compounds having a mixed biosynthetic origin. It is not uncommon to identify phytol among the metabolites of an alga, but phytol is usually regarded as a primary metabolite, since it is a common degradation product of chlorophyll.

The linear diterpene crinitol (12) has been isolated from Cystoseira crinita[20] and the bicyclic diterpene pachydictyol A (13) from Pachydictyon coriaceum[21]. A bicyclic sesquiterpene, zonarene (14), was found in Dictyopteris zonaroides[22]. The same alga also contains zonarol 15, having a bicyclic sesquiterpene portion joined to a hydroquinone moiety[23]. The brown alga Taonia atomaria contains two

quinonoid diterpenes, taondiol (16) and atomaric acid (17)[24]. These two molecules are related by way of a series of 1,2 migrations along the backbone of the molecule. I cannot claim that terpenoids are the major secondary metabolites of marine algae, but they may be the most easily differentiated chemotaxonomic markers, for few have terrestrial counterparts.



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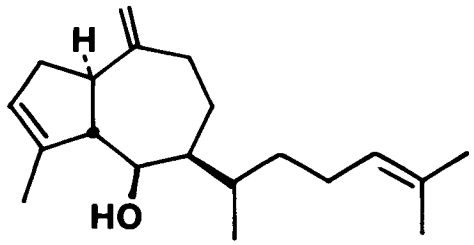

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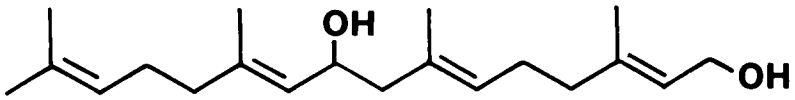

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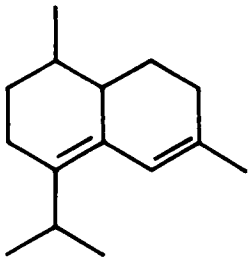

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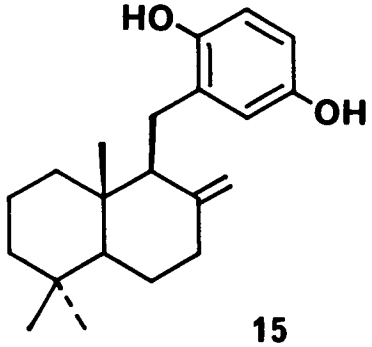

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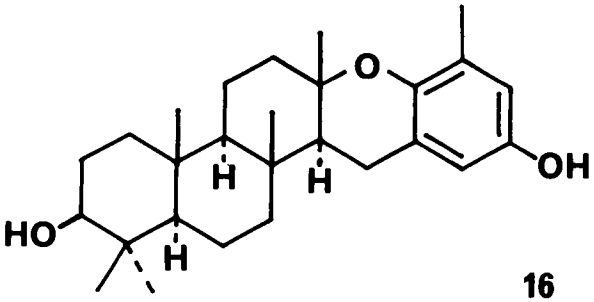

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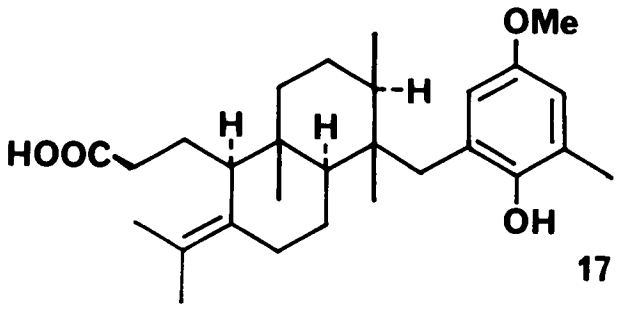
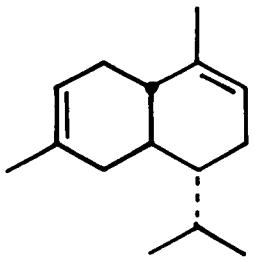


17

The terpenoids of soft corals and gorgonian corals from tropical waters are obvious to the chemist as soon as the organism is removed from the ocean. Gorgonians such as Pseudoplexaura crassa have a terpene-like odor, and when they are squeezed the expressed liquor contains crystals of a diterpene, crassin acetate (18). When Ciereszko found cadinene (19) and crassin acetate (18) in the gorgonian, a carnivorous invertebrate, he correctly surmised that the symbiotic zooxanthellae played a part in their synthesis[25]. He found that isolated zooxanthellae contained >3% cadinene and 8% crassin acetate. Examination of other gorgonians revealed that they contained many other sesquiterpenes which were identical to common terrestrial sesquiterpenes except that they are optical enantiomers. The large ring diterpenes called cembranolides are the most typical metabolites of gorgonians and soft corals, but they are found in large quantities only in tropical corals, presumably because the gorgonians of temperate waters lack zooxanthellae.



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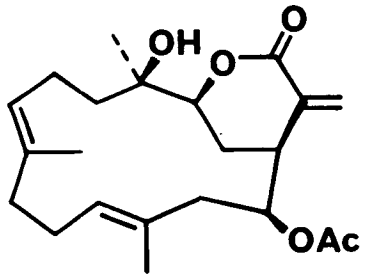
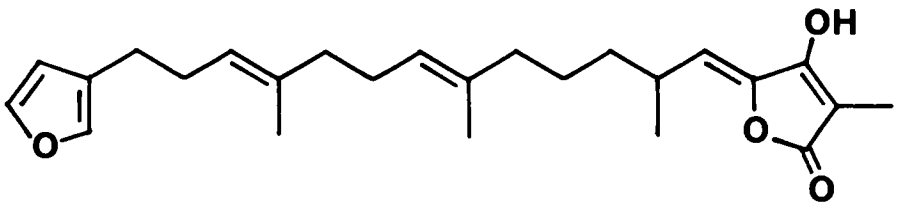


18

Sesterterpenes are the least frequently encountered class of terpenes, yet many sesterterpenes and abbreviated sesterterpenes (C-21) have been found in the family Spongidae (sponge). A recurring feature of sponge terpenes is the presence of one or more furan rings. The sesterterpenes, such as variabilin (20), from Ircinia variabilis, contain a tetronic acid functionality[26]. The C-21 difurans, such as furospongin (21), from Spongia officinalis[27], are believed to be degradation products of sesterterpenes, resulting from oxidative cleavage of a tetronic acid as shown. Several sponges contain furanosesquiterpenes, the simplest example being dendrolasin (22), from Oligoceras hemorrhages[28]. There are also cyclic sesquiterpenes, such as pleraplysillin (23), from Pleraplysilla spinifera[29], and cyclic sesterterpenes, exemplified by scalarin (24), from Cacospongia scalaris[30]. The largest terpenoids found in sponges are the polyprenyl quinones (25), from Ircinia species[31], which are reminiscent of ubiquinones.



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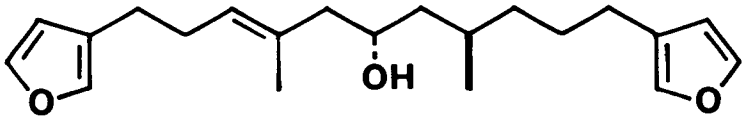

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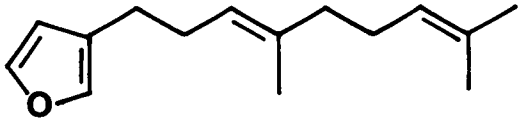

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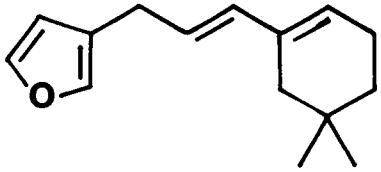

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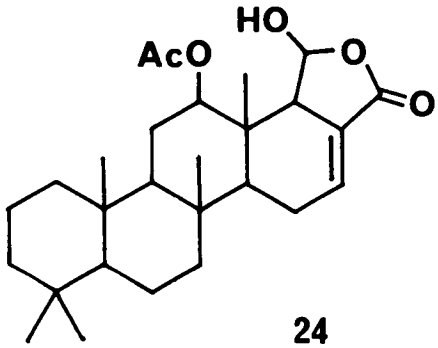

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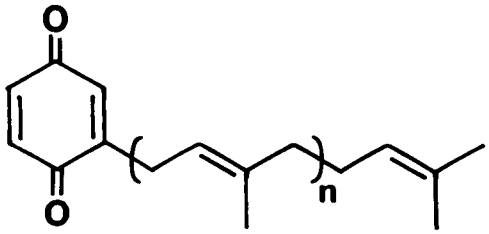

n = 3, 5, 6, 7

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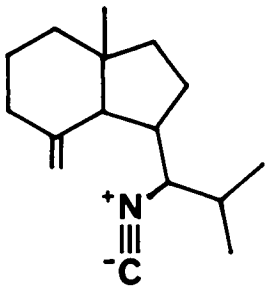

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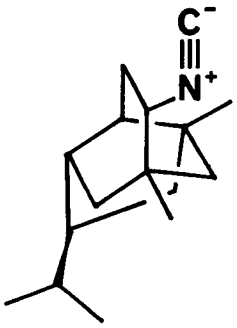

27

The most intriguing class of terpenoids is a group of isonitriles found in sponges. Both sesquiterpene and diterpene isonitriles are known, but there does not seem to be any biosynthetic connection between the various carbon skeletons. It is interesting to note that axisonitrile-1 (26)[32] has the same carbon skeleton as oppositol (5) and that this carbon skeleton has not been encountered in terrestrial species. The nudibranch Phyllidia varicosa secretes a toxic mucus containing an isonitrile (27) which it obtains from the sponge Hymeniacidon sp., on which it feeds[33].

Although less than 1% of marine organisms have been examined, some interesting chemotaxonomic patterns are emerging among the terpenes. It is too early to say whether terpenoids can be followed through the marine food chain. We have shown that we can trace the food source for nudibranchs and sea hares, but no attempt has been made to take this approach further. The structural similarities between halogenated terpenoids and some halogenated industrial materials is striking and may imply that the halogenated materials will be degraded in the oceans. There is certainly evidence that halogenated products from marine organisms can be found in seawater under certain circumstances[34].

The geographical distribution of marine organisms is expected to result in variations in the classes of terpenoids in the seawater and sediment. For example, the tropical reef areas will contain mainly terpenoids from corals and sponges, while terpenoids in temperate coastal regions will be mainly the halogenated terpenes from red algae and non-halogenated terpenes from brown algae. The phytoplankton, which might be expected to produce terpenes in the open ocean, have not been studied. Terpenoids clearly show enough variation to be used as chemotaxonomic markers. Future research will show whether they can be traced through the marine environment into the sediments.

ACKNOWLEDGEMENTS

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DISCUSSION

RUDELL: The structures of some of your terpenoids, just by looking at them, seem to be sort of broad. They could do almost anything. Some of them resemble estrogen, some phenols, and so forth. What do these animals do with these terpenoids?

FAULKNER: That is next year's research. We are trying hard to find out what the functions of molecules are. This is an area of natural products research which was really ignored until the insect people started working on chemical communication, chemoreception. They started finding out the function of molecules which were previously thought of as just being something of mild interest. It is really not possible to give most of these compounds a function in life, except to say that an awful lot of them are toxic. A lot of the phenols, for instance, are pretty unpleasant compounds. Some phenols can kill larval barnacles somewhere in the region of 10 parts

per billion. So that is a very toxic compound probably involved in the chemical defense of the coral.

There are all sorts of functions that we are finding, but whether they are the real intended function I don't know.

BROWN: In one of the samples that we analyzed from someplace in the middle of the Pacific Ocean we found a very strange compound or compounds. We are quite sure that this was material quite high in molecular weight, but it was polychlorinated. We thought that we had found another man-made hazardous chemical, but it did not match up with anything that we know of so far as what is manufactured. This would appear to be a biogenic-type hydrocarbon or organic compound.

COPPAGE: Do any of the peaks mimic toxaphene?

FAULKNER: Not that I know of. The compounds are not identified. They are just halogenated compounds found, and that is really all you can tell from gas chromatographic analysis. So, we really don't know what any of those were.

As for whether any marine natural products mimic any pollutants, I would say that there was a very good chance that they might, but you would have to find the individual mimic. I cannot just say that there is a mimic. We would have to go and look for one.

PETROLEUM HYDROCARBONS:
CRUDE OIL, REFINED PRODUCTS AND WASTE PRODUCTS

THE VARIETY OF PETROLEUMS AND THEIR DEGRADATIONS

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Petroleums are all different from each other. The reasons, the processes, the alterations by which these changes are arrived at are noted. The implications of these are directed toward environmental studies of oil spills and their effects on the biosphere.

The first oil well was drilled in 1859, an event which the American Petroleum Institute has equated with the invention of the wheel and the achievement of the atomic chain reaction. Edwin L. Drake produced oil on that date from a well 69 1/2 feet in depth near Titusville, Pennsylvania and subsequent drillings which followed the original well ushered in the so-called age of kerosene. In the first forty to fifty years of commercial oil production, apart from a number of empirical physical tests which were developed little was learned about the chemical nature of crude oil. Indeed, it was not until 1927 that the American Petroleum Institute commenced its now famous Research Project 6 which was charged with investigating the number and nature of hydrocarbons in petroleum. A Midcontinent crude was considered by the Project's Advisory Committee to be a suitable representative oil and to this end, in 1928, 600 gallons of crude from the Brett No. 6 well in the Ponca City field in Oklahoma were delivered to the project for examination. Ensuing data based on this petroleum have become famous as Ponca City crude. The well was plugged and abandoned in 1936 and so has not been an article of commerce for decades.

In 1952, some 300 man years and 141 publications later API Project 6 summarized its findings.[1] After heroic use as well as refinements of the then available classical methods--distillation, adsorption, extraction, clathrate formation, synthesis of reference compounds, in addition to development of physical measurement

tests--a total of 130 hydrocarbons had been identified, all having boiling points below 300° C. Clearly, only light ends of petroleum had been described at this point; a very considerable amount of crude still remained a chemical mystery. It is significant that a large number of the hydrocarbons identified in API 6 have been shown to be degradable by bacterial action during the intervening years.

For a variety of reasons interest in heavier fractions of petroleum continued to increase and in 1967 API Research Project 60 was started for the purpose of characterizing the heavy ends of crude oils. Whether there was any irony in choosing a project number an order of magnitude greater than 6 is moot, but as will be seen, it would not have been misplaced. Much of the information gathered by API 60, which was terminated in 1972, was of course, obtained with analytical techniques several generations more sophisticated than those available to API 6. The summary of data from API60, published in 1974,[2] clearly demonstrated that the number and types of compounds in the heavier ends are many orders of magnitude more numerous and complex in character than those enumerated by API 6. It is now accepted that the number of individual compounds in petroleum is in the hundreds of thousands, more probably approaching a million discrete compounds; the implications are several.

It may be useful to examine some basic aspects of petroleum before entering into a description of its chemical character. There is no really good, widely accepted definition of petroleum; virtually every definition proposed has so many exceptions or caveats appended as to make it unusable. The writer was once told, during a meeting of petroleum scientists, that when the organic material in source rock becomes liquid enough to migrate it may be considered petroleum. While simplistic this convention will be followed despite the fact that it may be less than a satisfactory definition to the purist.

There is general agreement among geochemists that the carbon and hydrogen of petroleum are derived from biological (primarily microbial) debris most of which grew or accumulated in aquatic environments. Material escaping early aerobic degradation accumulated in an inorganic matrix and, over geological time subsided into the earth's crust where, under the weight of the overburden water was expelled resulting in an organic containing shale-like rock. The organic matter was converted to an insoluble material of largely unknown structure called kerogen. Exposure of the organic material to relatively modest temperatures and pressures over geological time

resulted in the generation of a petroleum-like oil by a process which might be likened to destructive distillation. The "oil" at this stage left the shale and by largely unknown mechanisms migrated and accumulated in a variety of porous sedimentary rock structures. These accumulations which develop under special geological conditions are what we now refer to as oil reservoirs. Both the oil and the reservoir rock were buried ever deeper in the earth over millions of years thus exposing them to higher temperatures over longer periods of time resulting in further changes in the chemical make-up of the crude. Although this account is far from complete and as devoid of landmarks as a Kafka novel it will suffice here.

Given the conditions which obtain, the processes which occur coupled with the vast expanses of time involved in the genesis of crude oil it should not be surprising that the original biological materials have become rearranged into a bewildering array of compounds most of which have no counterpart in modern biological systems. And the situation is even more complex for at any stage from the time the oil leaves the shale source to the time it is produced by man a variety of changes take place in the nature of the oil through the intervention of a number of processes - thermal alteration (or thermal maturation); microbial alteration; water washing (or leaching); inspissation; separation of gas and liquid phases; to name a few. Each process changes the nature and composition of crude oil in a (now) more or less predictable way. Of the processes named thermal and microbial alteration are probably the most important; time strictures dictate that only these two will be considered in what follows.

Crude oils are generally considered to be a complex mixture of hydrocarbons ranging in molecular weight from that of methane to (possibly) 100,000. By purely empirical methods crudes may be separated into three fractions namely oil, resins, and asphaltenes. The so-called oil fraction consists of hydrocarbons with small but varied contents of nitrogen, sulfur and oxygen containing materials which will be referred to later. The hydrocarbon portion which represents from close to 100% to, in extreme cases 85% of the total oil is composed of three classes of hydrocarbons - alkanes, cyclo-alkanes and aromatics. It may seem this simplifies matters, but the complexity is still formidable.

Alkanes or paraffins range in amount from practically zero to close to 100% of the oil fraction in unusual oils. In typical oils, if indeed, there are such entities, the paraffin content is about 20% of which half is normal and half is branched. While it is possible that all

possible isomers of a paraffin may be present most iso-paraffins show rather simple arrangements of methyl substituents. The normal paraffins range from methane to about C_{40} although there are wide variations among crudes. Chemically and microbiologically the paraffins have undergone intensive study over the years and appear to be well understood. Cycloparaffin contents also vary among crudes but generally account for about 50% of the oil fraction, most being cyclopentane or cyclohexane types although compounds having 7 and 8 membered rings occur in small amounts. The rings may be fused with other rings, cycloparaffins may be fused with aromatic rings in addition to which aliphatic substituents may be present. The possible number of isomers, homologs and analogs is thus tremendous. There does not appear to be a predominant isomer of any carbon number in fractions which boil above the gasoline range. Cycloparaffins represent the most difficult analytical problem in unravelling petroleum composition, largely a situation which is apt to continue because these materials yield, on refining, stable, high quality, non-toxic products, or to put it another way, there is no incentive to undertake massive efforts to isolate or analyze components of the group.

Aromatics most generally do not account for more than 20% of the oil fraction but represent a considerable problem because they have poor viscosity-temperature behavior, are toxic to plants and animals in addition to which they oxidize easily and in any process involving heat form carbonaceous deposits. A great deal of effort has gone into the study of these materials and these studies are apt to continue. Polynuclear aromatic compounds with 9 rings have been identified, but undoubtedly this by no means represents a maximum size. Large-sized polynuclear aromatics unquestionably fall into the resin range as well. Again, as in the case of the cycloparaffins the aromatic rings may fuse with not only other aromatic rings but naphthenes as well, all such large molecules having a complex diversity of short aliphatic substituents with no predominant pattern except that the more thermodynamically stable forms will exceed the less stable possibilities.

Asphaltenes are high molecular weight materials held in colloidal suspension in the oil; they make up from zero to twenty or so percent of the total crude. The asphaltenes represent a compound class having a presently unknown structure despite considerable research directed at this feature. There is evidence that asphaltenes from different crudes differ somewhat although they conform to the definition of asphaltenes, i.e., that material which precipitates (out of colloidal suspension) when an excess of a paraffin (methane, propane, pentane, heptane) is added to the crude oil. It should be noted

that the definition, however empirical, works well with crude oil, but applied to other complex mixtures of organic compounds will define merely pentane (say) insoluble material. The two cases are not synonymous. Molecular weights for asphaltenes have never been accurately determined presumably because of the peculiar nature of their solubility characteristics. Molecular weights from 500 to 100,000 have been claimed; the commonly quoted value of 10,000 seems more reasonable. More sulfur, nitrogen, and oxygen occur in the asphaltene fraction than in resins or oils. In addition, organically complexed nickel and vanadium occurs in the asphaltene molecules. It should be noted also that despite the presence of oxygen, nitrogen and sulfur, the asphaltene fraction does not consist of a collection of small, pentane insoluble compounds containing these elements, rather, asphaltenes are thermally induced condensation-polymers of lower molecular weight aromatics.

Resins are not well characterized but are known to be highly aromatic, probably contain materials which might simplistically be called junior asphaltenes--most likely very large polynuclear aromatic hydrocarbons are included. It seems likely that apart from asphaltene, future characterizing of crude oils will be carried out by the more logical technique of defining boiling point ranges.

Nitrogen, sulfur and oxygen-containing compounds also appear to a greater or lesser extent and, again, there is wide variation among crudes in this regard. Only sulfur will be considered here in an attempt to hold the discussion within reasonable grounds. Sulfur reacts with hydrocarbons across the entire boiling point range, both aliphatic and aromatic sulfur-containing analogues are known to occur. The sulfur content of crude oil ranges from near zero to an extreme case in which it represents 12% of the total crude. Since sulfur is found in all boiling point ranges it is clear that a very large number of S-heterocyclics are present. For reasons which are not clear, the majority of S-heterocyclic compounds contain only one sulfur atom although hydrocarbon analogues with two or more sulfur atoms are undoubtedly present. Apart from some very small sulfur heterocyclic compounds (benzthiophene, dibenzthiophene) little is known about microbial degradation of these substances; evidence, indeed, suggests they may be quite refractory to microbial degradation.

Given, then, the complicated process by which petroleum is formed, the several ways it can be altered prior to production, the astronomical number of hydrocarbons and hydrocarbon heteroanalogues it should come as no surprise that crude oils differ from

each other widely depending on source material, age, geological constructs and in situ alterations. Crudes differ from reservoir to reservoir and, in many cases, crudes from different parts of the same reservoir show vast differences. From what appears to be maximal chaos the geochemical community has created some order in the situation and much recent geochemical study is directly applicable to environmental work.

There can be no question, however primitively the nature of crude oil is understood, that no artificial crude for laboratory study can be constructed. Data from such mixtures will provide information about hydrocarbon mixtures but not about crude oils. Studies of microbial degradation of hydrocarbons limited to a one hydrocarbon-one bacterium system are restricted to those hydrocarbons available. The sad fact is that most of the recent analyses of crude oil composition have been carried out by methods which destroy the molecules being analyzed. Thus, while in terms of classes of compounds, we know a great deal about petroleum composition we possess only (compared to the total crude) a tiny number of the individual components. It can be asked how many of the individual components need to be studied? Metabolic and toxicity studies are best carried out with single compounds for unambiguous results. If priorities dictate that we need to have far more of the individual compounds of crude oils it will necessitate heroic separation or synthetic efforts, perhaps new generations of instruments or entirely new approaches. The best mass spectral methods employed today, such as those used in API 60, were developed by the oil industry and are the basis for our present knowledge of crude oil in terms of classes of compounds--each class, at least in the complicated ones, composed of scores perhaps hundreds of isomers and homologues. The cost of the development of these techniques is not known but it clearly must have been considerable. A greater investment will be required if the need for individual compounds is thought to be necessary.

More than two decades of intensive research on microbial metabolism of hydrocarbons has demonstrated that the ability to attack and degrade a wide variety of hydrocarbons is a common and widespread character in the microbial world. Extensions of these studies to crude oils, however incomplete the analytical work involved, have further demonstrated that microbial degradation is the major force in restoring the environment after an oil spill. These two statements have some interesting implications. By actual count in the relevant literature a total of 50 hydrocarbons have been subjected to microbial metabolic studies.[3] Compared with the total number of different

hydrocarbons in a crude oil it seems a miniscule effort. But it is also obvious that the total effort will never be completed given the almost astronomical number of hydrocarbons in petroleum. At what point should these efforts be considered more or less complete? Studies in which microbial degradation of crude oils in laboratory situations have been carried out in efforts to assess the role of bacteria in biodegradation of oil spills suffer from several shortcomings. Many of the studies have used vaguely defined crudes as experimental material. For example, the oil involved in the Torrey Canyon incident was Kuwait oil--a light oil with a high paraffin content and medium sulfur (1.6 - 2.0 percent). Biodegradation of the lighter ends proceeded rather rapidly and certainly the bulk of the crude can be shown to biodegrade in the laboratory. But consider another possibility. Jobo, a Venezuelan oil, an article of active commerce is only 57% volatile in the best of mass spectrometers. There are no normal paraffins in this oil; there is 7.7% asphaltenes and about 2% sulfur. Moreover, the specific gravity of the oil is greater than that of sea water (for the cognoscenti the API density is 8-10 API^{o}). In an oil of this sort there is no place wherein bacteria can grasp a metabolic foothold. Consider, if you will, another example. Recluse, Moorcroft and Altamont are all midcontinent oils. The first two are from the Powder River Basin and Altamont is derived from the Uinta Basin of Utah. Analysis shows that Altamont has almost 100% paraffin content. Both the Powder River Basin crudes, which were probably derived from the Cretaceous shales which surround the basin, differ considerably--for example, Moorcroft has no paraffin content whereas Recluse shows about 20% paraffins. It follows, then, that environmental studies should define very carefully the crudes used in studies. Perhaps, a spectrum of standard crudes, all articles of commerce, all well analyzed (as are those in API 60: Wasson, Texas; Wilmington, California; Boscan, Venezuela; Red Wash, Utah; Recluse, Wyoming; Gato Ridge, California; and Gach Saran, Iran) should be chosen for further study. At the very least this choice would assist in correlating data.

Environmental microbiologists might consider, with some profit, those aspects of organic geochemistry which apply to petroleum modifications in the reservoir. The two generally accepted processes which bring about changes in reservorial oils are thermal alteration and microbial degradation. Thermal alteration causes a redistribution of hydrogen resulting in an increase of the light aromatic and paraffinic sectors of the oil to produce a significantly lighter crude. Sulfur, oxygen and nitrogen containing compounds are much less thermodynamically stable than their hydrocarbon counterparts and decompose under thermal stress resulting in considerable decrease in the NSO

fraction. These, perhaps, oversimplified compositional changes in the oil under thermal action, are highly dependent upon the temperatures and times involved. At higher temperatures all the petroleum liquids are destroyed leaving only dry gas (methane). At intermediate temperatures, as noted the changes result in improved crude. As burial increases so also does temperature and pressure. The increased pressure forces light hydrocarbons and methane into solution in the oil upsetting phase equilibria and resulting in the precipitation of asphaltenes thus providing another increment in crude improvement. The other important process resulting in major changes in crude oil composition, namely microbial alteration, produces an entirely different pattern of modification. Since the primary reaction by which microorganisms breach the pure hydrocarbon structure requires gaseous oxygen microbial alteration in reservoirs can take place only where meteoric waters have brought oxygen (and other nutrients) into contact with the oils. Thus, microbial alteration of crudes in reservoirial situations may be widespread but not the rule, depending on geology and other factors. Alteration of crude oil by microbial action results in rather specific changes quite distinct in its own right. Paraffins from C_1 to about C_{40} disappear and light naphthenes and light aromatics are destroyed. Whether paraffins, light aromatics and light cyclo-paraffins are degraded sequentially or simultaneously is irrelevant to this discussion. The upshot, in any case, is a heavier oil. Nitrogen, sulfur, and oxygen compounds increase. Whether the increase is because of simple concentration (by removal of hydrocarbons) or by contribution of microbial activity has never been documented; the more likely situation is the former. Crudes which have been microbially altered show an increase in asphaltene content, probably by concentration since, apart from suggestion, it has never been shown that microbes produce asphaltenes. The oil density becomes heavier, the viscosity increases and, in general, oils degraded in this fashion are poorer quality crudes. Microbially degraded crudes are widespread but not ubiquitous - Boscan, Jobo and the Athabasca tar sands represent a few well known examples of crudes which have been microbially degraded in situ. Many giant oil fields (producing one million barrels or more per year) are, in fact, already biodegraded yet are active products of present day trade. Thus, environmental studies of microbial action on oil spills, seeps, and the like, demand considerable knowledge of the specific crude involved.

There are other processes, as noted, which bring about compositional changes but thermal and microbial modifications represent the major natural effects on oil. Clearly, the two processes bring about differences in the nature of the crude and are recognizable one from the other. The more interesting notion is that, apart from a few obvious differences which unquestionably relate to more rapid evaporation

and dissolution in water columns on the surface, the changes brought about by biodegradation in the reservoir are identical to those which occur on the surface. Thus, tremendous amounts of potential pollutant exist in the normal channels of commerce, which is already severely biodegraded. Perhaps we should (at long last) accept that microorganisms have little difficulty in degrading completely the light ends of crude oils (in the case of light crudes probably the entire oil) and do some serious work on the fate and effects of the heavy ends. Such studies will require considerably more than routine efforts but are prerequisite to an understanding of the effects of a total crude oil on the biosphere. It may be argued that there is no effect given the amount of natural oil seepage in the world (currently estimated at 0.6 million metric tons per year) but no analytical data exist on the persistence or nonpersistence of the heavier, possibly nonbiodegradable fractions of these seep oils.

Most studies of crude oil degradation by bacteria suffer from a number of shortcomings--inadequate analytical procedures, repetetive experiments, rediscovery of old principles, and the like. There may be cogent reasons for investigating the metabolism of single compounds in crude oils, such as some of the higher molecular weight sulfur heterocyclic materials, variously substituted polynuclear aromatics and very long chain paraffins. If that is the case, such studies should be carried out however heroic the isolations or syntheses of the compounds become. Chronic, as opposed to acute, exposures of the biosphere to crude oils should be extended but with the proviso that the crudes employed be well identified and these should be materials of modern day commerce or seepage. To put it another way, the material ignored (or thrown away) from most experiments involving microbial degradation of crude oils are still items of commerce subject to spills and sources of pollution. Thus, experimental studies on petroleum are fraught with hazard, not the least of which is ignorance of the real nature of the almost infinite variety of crude oil.

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MICROBIAL DEGRADATION OF CARCINOGENIC HYDROCARBONS AND RELATED COMPOUNDS

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ABSTRACT

The carcinogenicity of several polycyclic aromatic hydrocarbons is thought to be due to metabolic activation by the target organism. Thus, mammals incorporate one atom of molecular oxygen into these compounds to form arene oxides. The latter compounds are electrophiles that can react with cellular constituents. In contrast, bacteria incorporate both atoms of molecular oxygen into aromatic hydrocarbons. The first detectable products are *cis*-dihydrodiols which are then rearomatized prior to enzymatic fission of the aromatic nucleus. Recent results confirm that certain species of fungi produce oxygenated metabolites similar to those formed by mammalian microsomes.

INTRODUCTION

Aromatic hydrocarbons are ubiquitously distributed throughout the environment. Although a biosynthetic origin of this class of compounds has been suggested[1] it is generally accepted that these compounds are produced by the pyrolysis of organic material. The advent of the industrial revolution led to increased use of fossil fuels as sources of energy with a concommitant increase in the amounts of hydrocarbons liberated to the environment, a situation that has continued to the present day. However, pyrolytic processes have been occurring throughout geological time. For example, in the generation and maturation of petroleum thousands of different aromatic hydrocarbons are formed by the degradation of organic material that was produced by living organisms[2].

The temperatures associated with the pyrolysis of organic compounds dictates the types of aromatic compounds that are produced. This concept has been elegantly developed by Blumer, Youngblood and their associates at the Woods Hole Oceanographic Institution[3,4]. The high temperatures (~2,000° C) associated with the operation of the internal combustion engine produce polycyclic hydrocarbons that contain very few alkyl substituents. In fact unsubstituted mole-

cules are the predominant forms. At intermediate temperatures (400-800° C) such as those found in wood fires, the abundance of alkyl side chains increases with decreasing temperature. Petroleum is formed at relatively low temperatures (100-150° C) and the most abundant polycyclic aromatic hydrocarbons contain two or three alkyl substituents[5]. From these observations it is clear that there are several sources of aromatic hydrocarbons in the environment. These include incomplete combustion of coal, wood and petroleum and also the deposition, accidental or otherwise, of crude petroleum and its refined products.

Since all living organisms have been in contact with polycyclic aromatic hydrocarbons throughout geological periods of time it is not surprising to find that enzyme systems have evolved that will oxidize this class of compounds. However, we must be aware that of the thousands of aromatic molecules that are found in crude petroleum only a fraction of one percent has been investigated in terms of metabolism by living organisms.

MAMMALIAN OXIDATION OF AROMATIC HYDROCARBONS

The relationship between pyrolysis products and cancer was first reported in 1776[6]. Since that time several different polycyclic molecules have been implicated as chemical carcinogens[7]. The molecular basis of chemical carcinogenesis is an area of intensive investigation and a detailed analysis of current theories is beyond the scope of this presentation. However, some of the results are germane to a discussion of the mechanisms used by microorganisms to degrade aromatic molecules. At this time it is generally accepted that the carcinogenic properties of certain polycyclic aromatic hydrocarbons are only manifested after metabolic activation by microsomal monooxygenases. The initial oxidation products are arene oxides[8] which, due to their electrophilic character, can bind to nucleophilic sites in cells. The metabolism of benzo[a]pyrene by mammals leads to the formation of several oxygenated products. The reaction sequence shown in Figure 1 depicts the formation of 7,8-dihydro-7,8-dihydroxybenzo[a]-pyrene-9,10-oxide[9] which binds to DNA[10] and has been shown to be a powerful mutagen[11].

The incorporation of one atom of molecular oxygen into the parent hydrocarbon to give benzo[a]pyrene-7,8-oxide is catalyzed by a cytochrome P-450 enzyme system[12]. Hydration of the oxide to yield *trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene is a property of the enzyme epoxide hydrase[13]. These two enzymatic reactions, epoxidation followed by hydration, are observed in the mammalian metabolism of many different aromatic hydrocarbons.

BACTERIAL OXIDATION OF AROMATIC HYDROCARBONS

The ability of certain strains of bacteria to degrade simple aromatic hydrocarbons to carbon dioxide and water is a well established phenomenon. In addition, many organisms exist that can partially oxidize aromatic hydrocarbons when an alternative growth

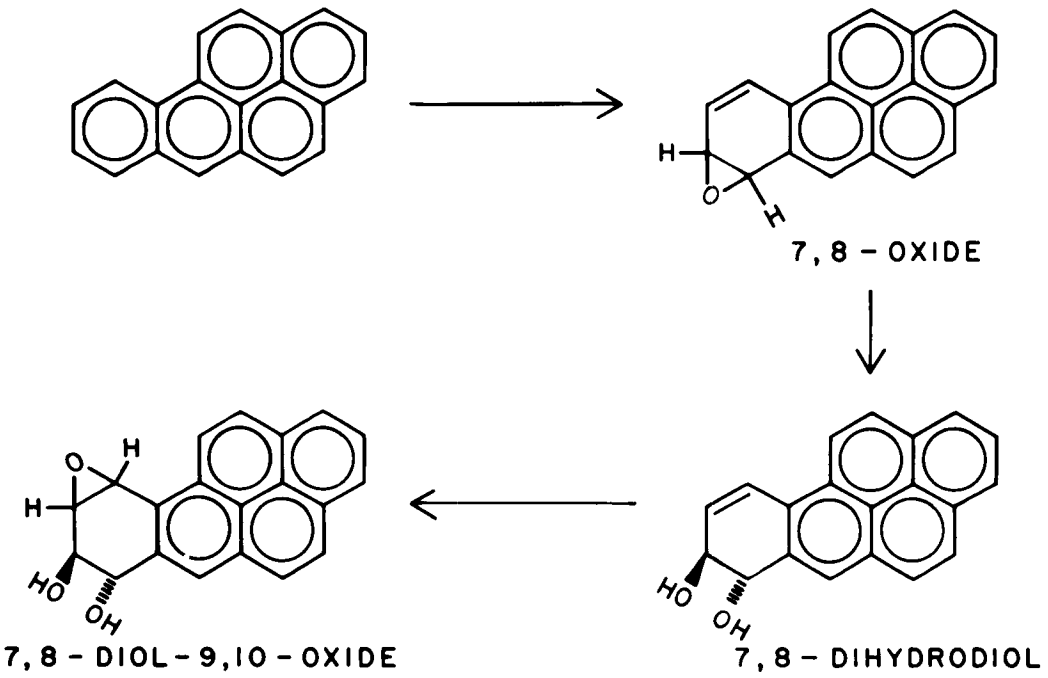


Figure 1. Metabolism of Benzo[a]pyrene by Mammalian Microsomes (only one of several sequences is shown).

substrate is available. This phenomenon is termed co-oxidation and has been elegantly developed by Raymond and his colleagues[14]. Figure 2 gives two examples of cooxidation. Growth of a *Nocardia* species on hexadecane in the presence of ethylbenzene led to the accumulation of phenylacetic acid in the culture medium[15]. A different strain of *Nocardia* oxidized *p*-xylene to α,α-dimethylmuconic acid[16]. These results clearly show that bacteria can initiate oxidation of substituted aromatic hydrocarbons at the alkyl group or by direct oxidation of the aromatic nucleus. Another consequence of these observations is an extension of the field of microbial hydrocarbon metabolism. Once oxygen is incorporated into an aromatic hydrocarbon, phenols and aromatic carboxylic acids are produced. The literature on the microbial degradation of these two groups of compounds is extensive and several excellent reviews are available[17,18,19]. Thus, the only reactions in the degradation of aromatic hydrocarbons that are dictated by chemical structure are those whereby molecular oxygen is incorporated into the hydrocarbon molecule.

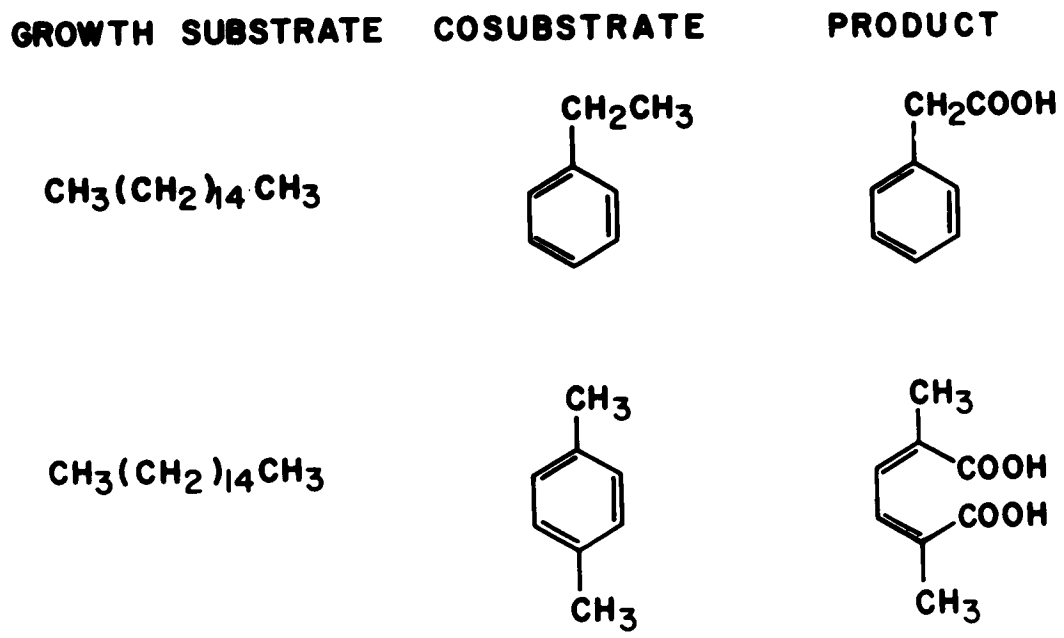


Figure 2. Bacterial Co-oxidation of ethylbenzene and *p*-xylene.

INITIAL REACTIONS IN THE BACTERIAL OXIDATION OF SIMPLE AROMATIC HYDROCARBONS

Table I shows the types of aromatic hydrocarbons that are known to be susceptible to microbial oxidation. Again it must be emphasized that this is an extremely small percentage of the compounds that are present in the environment. One common feature of the mechanisms used by bacteria to degrade these types of compounds is the introduction of two hydroxyl groups into the aromatic nucleus. The hydroxyl groups may be *ortho* to each other as in catechol or *para* to each other as in gentisic acid[20]. Dihydroxylation appears to be an essential requirement for the subsequent enzymatic fission of the benzene ring. Thus, recent studies on the bacterial degradation of aromatic hydrocarbons have been directed towards the elucidation of the initial oxygenation reactions[21]. At one time it was thought that bacteria used the same reactions as those described above for mammalian microsomes. However, results obtained in our laboratories have revealed the existence of a different oxygenation mechanism.

Benzene oxidation by *Pseudomonas putida*. A strain of *P. putida* was isolated from a polluted creek by virtue of its ability to grow with ethylbenzene. This organism also grew with benzene and toluene. Washed cell suspensions rapidly oxidized benzene, catechol and *cis*-benzene dihydrodiol (*cis*-cyclohexa-3,5-diene-1,2-diol). Surprisingly, *trans*-benzene dihydrodiol, which is the compound formed

TABLE I.

Aromatic Hydrocarbons Known to be Oxidized by Microorganisms

MONOCYCLIC	POLYCYCLIC
Benzene	Pyrene
Toluene	Benzo[a]pyrene
Xylenes	Benzo[a]anthracene
Tri and tetramethylbenzenes	Dibenzo[a]anthracene
Alkylbenzenes	Benzperylene
Cycloalkylbenzenes	Perylene
DICYCLIC	**TRICYCLIC**
Naphthalene	Phenanthrene
Methylnaphthalenes (mono and di)	Anthracene
Ethylnaphthalenes	

during the oxidation of benzene by mammalian microsomes, was not attacked[22]. A mutant strain of *P. putida* was obtained by treatment of the parent compound with nitrosoguanidine[23]. This organism, *P. putida* 39/D, when grown on glucose in the presence of benzene accumulated *cis*-benzene dihydrodiol in the culture medium. The hydroxylated metabolite was isolated in crystalline form and shown to be identical in its chemical properties to a synthetic sample of *cis*-benzene dihydrodiol. Subsequent studies with $^{18}O_2$ showed that both oxygen atoms in the enzymatically-formed dihydrodiol were derived from molecular oxygen. Cell extracts prepared from the wild type organism contained a dehydrogenase that oxidized *cis*-benzene dihydrodiol to catechol. These results established the pathway shown below (Figure 3) for the initial reactions used by *P. putida* to degrade benzene.

The mutant organism oxidized a variety of monocyclic aromatic compounds to *cis*-dihydrodiols (Table II).

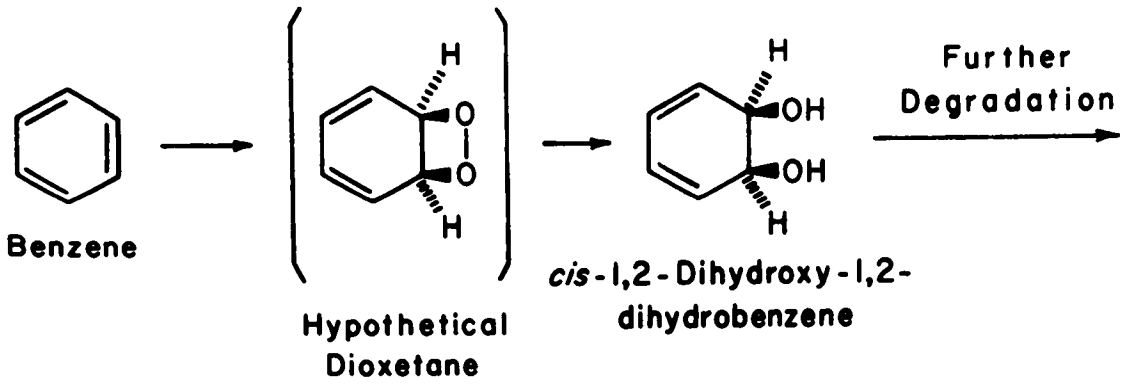


Figure 3. Initial Reactions in the Degradation of Benzene by *P. putida*.

TABLE II.

Aromatic Compounds oxidized to *cis*-Dihydrodiols by *P. putida* 39/D

Benzene	Toluene
Fluorobenzene	*p*-Chlorotoluene
Chlorobenzene	*p*-Fluorotoluene
Bromobenzene	*p*-Bromotoluene
o-Dichlorobenzene	Trifluoromethyltoluene
m-Dichlorobenzene	Ethylbenzene
p-Dichlorobenzene	Phenylethanol
Cyanobenzene	Acetophenone
	p-Xylene

Naphthalene oxidation by bacteria. The unexpected formation of dihydrodiols from monocyclic aromatic hydrocarbons led us to investigate the biodegradation of naphthalene. Previous studies had indicated that this compound is metabolized through *trans*-1,2-dihydroxy-1,2-dihydronaphthalene[24] (*trans*-naphthalene dihydrodiol), which is the same compound produced by mammalian microsomes. A different strain of *P. putida* that can grow with naphthalene was isolated from soil. Mutation of this organism produced a strain, *P. putida* 119 that accumulated a dihydrodiol in the culture medium. When this metabolite was isolated and characterized by conventional chemical techniques it was shown to be the *cis*-isomer[25]. Studies with isotopic oxygen showed that both atoms of oxygen in the dihy-

drodiol were derived from a single molecule of atmospheric oxygen[26]. An enzyme was purified from naphthalene-grown cells of the parent organism that oxidized *cis*-naphthalene dihydrodiol to 1,2-dihydroxy-naphthalene[27]. This enzyme was specific for the optical isomer produced by the mutant organism. The pathway used by microorganisms to degrade naphthalene is shown in Figure 4. The compounds shown in brackets have been firmly identified. It should be pointed out that the sequence from 1,2-dihydroxy-naphthalene was elucidated by Evans and his colleagues at the University of North Wales[28].

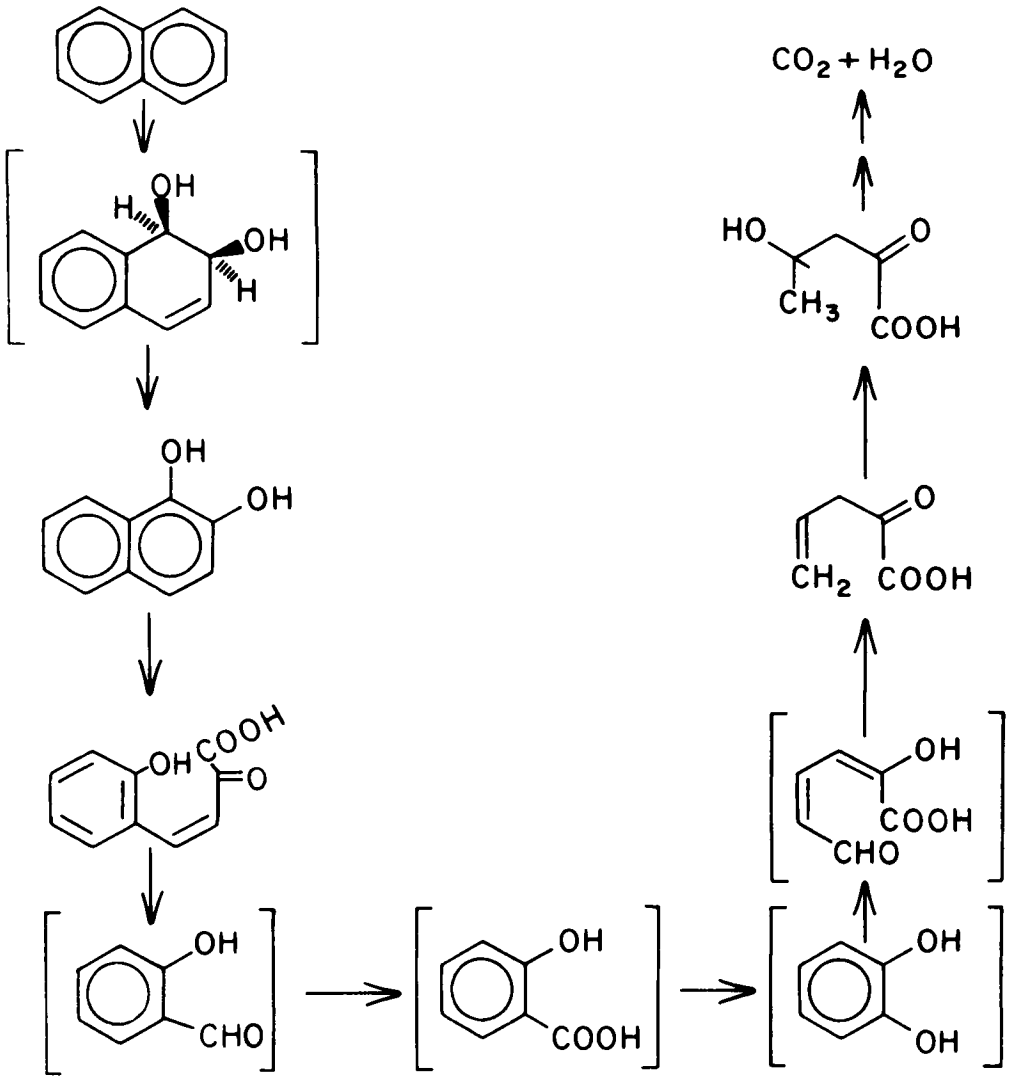


Figure 4. Metabolic Pathway for the Degradation of Naphthalene by Certain *Pseudomonas* species.

Bacterial oxidation of polycyclic hydrocarbons. Little is known about the microbial degradation of aromatic hydrocarbons that contain more than three aromatic rings. We have been unable to isolate organisms that will grow with these compounds. However they are oxidized by bacteria. Cells of a mutant strain (B836) of *Beijerinckia* that oxidizes biphenyl[29], phenanthrene[30] and anthracene[31] to *cis*-dihydrodiols were grown with succinate in the presence of biphenyl. When these cells were incubated with benzo[a]pyrene a neutral product accumulated in the culture medium[32]. High pressure liquid chromatography resolved the product into a major and a minor component. The major product was identical, with the exception of optical activity, to a synthetic sample of *cis*-9,10-dihydroxy-9,10-dihydrobenzo[a]pyrene. The minor product was identified as *cis*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene. The parent strain of *Beijerinckia* oxidized benzo[a]pyrene to acid products that have yet to be identified. The major pathway for benzo[a]pyrene degradation is shown in Figure 5.

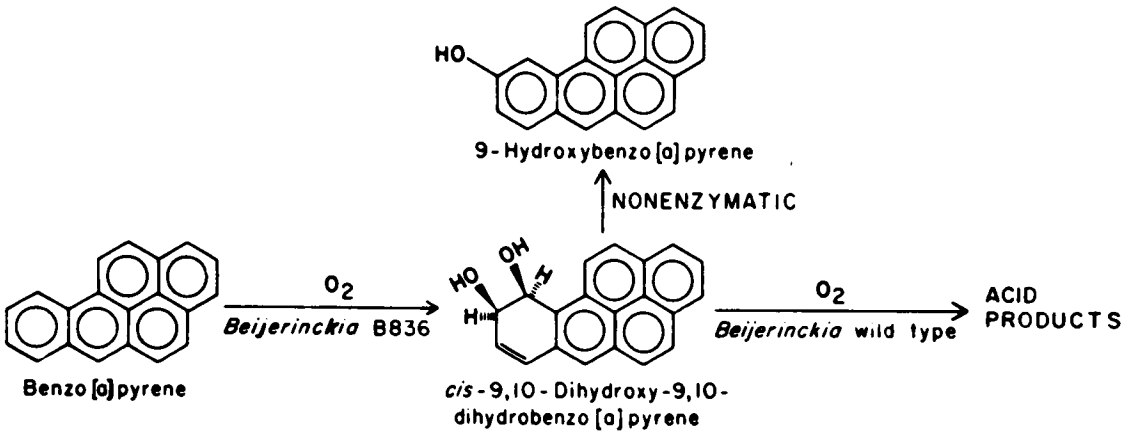
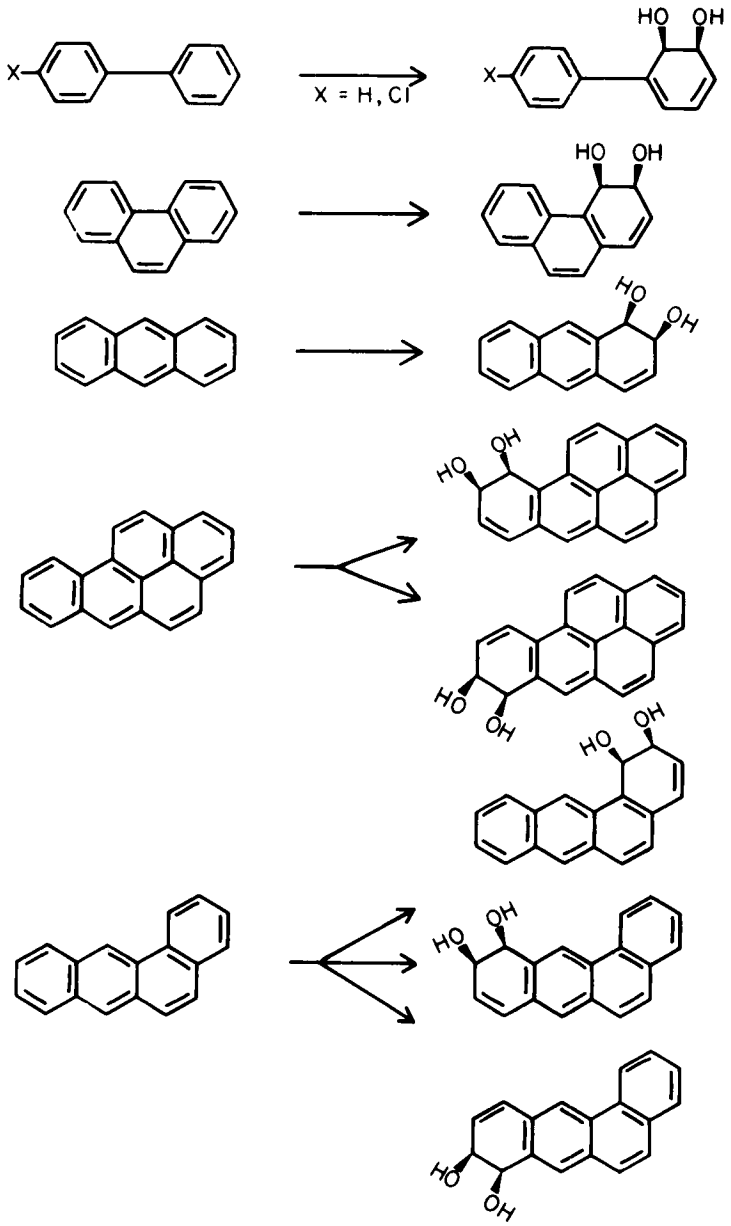


Figure 5. Oxidation of Benzo[a]pyrene by *Beijerinckia* B836.

Metabolism of benzo[a]anthracene by *Beijerinckia* B836 led to the eventual isolation and identification of three *cis*-dihydrodiols. These products together with other dihydrodiols formed by this organism are given in Figure 6.



cis- DIHYDRODIOLS PRODUCED BY Beijerinckia B-836

Figure 6

CONCLUSIONS

Evidence has been obtained that bacteria are capable of oxidizing aromatic hydrocarbons that range in size from benzene to benzo[a]pyrene. Whether or not larger molecules, particularly the highly condensed ring structures that are found in petroleum, are subject to biodegradation remains to be established.

Mammals initiate the oxidation of aromatic hydrocarbons by the enzymatic incorporation of one atom of molecular oxygen to form physiologically-reactive arene oxides. Further metabolism of the oxide intermediates gives *trans*-dihydrodiols as one class of mammalian metabolites. In contrast, bacteria incorporate both atoms of molecular oxygen into aromatic hydrocarbons and *cis*-dihydrodiols are the first detectable products. Further oxidation of *cis*-dihydrodiols leads to the formation of catechols which are the substrates for enzymatic fission of the aromatic nucleus. The differences between the mechanisms used by mammals and bacteria to degrade aromatic hydrocarbons are shown in Figure 7. However, the differences may not reside at this level. Fungi have been reported to degrade naphthalene through *trans*-naphthalene dihydrodiol[33] and we have recently shown that a strain of *Cunninghamella elegans* oxidizes naphthalene to α-naphthol, *trans*-

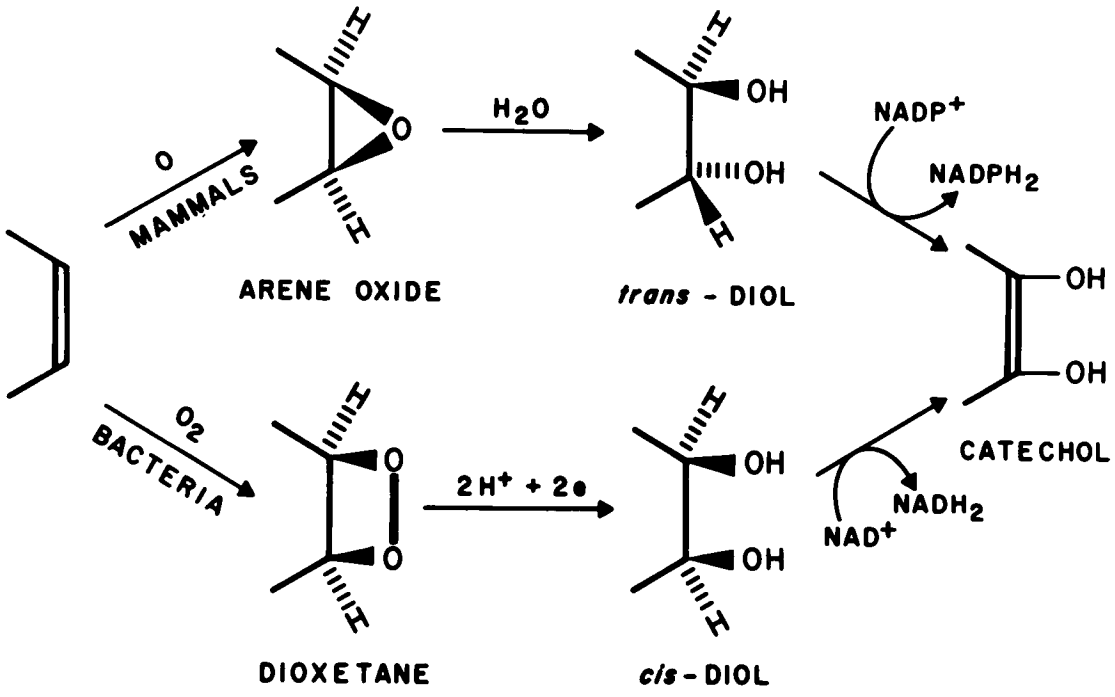


Figure 7. Initial Reactions in the Oxidation of Aromatic Hydrocarbons by Mammals and Bacteria.

naphthalene dihydrodiol and 4-hydroxytetralone (unpublished results). It is attractive to speculate that eucaryotic cells oxidize aromatic hydrocarbons through arene oxides and *trans*-dihydrodiols while procaryotic organisms utilize the *cis*-dihydrodiol pathway. However such a rigid demarcation line is unlikely to occur in nature. It is possible that organisms that grow with aromatic hydrocarbons do so by the *cis*-dihydrodiol mechanism. In this way they would avoid the production of potentially mutagenic intermediates. The arene oxide pathway could have evolved for the detoxification of natural products (non-hydrocarbon) found in the environment and it is an unfortunate evolutionary accident that the enzymes will also oxidize aromatic hydrocarbons. These hypotheses can only be evaluated when a diverse range of living organisms have been examined for their ability to oxidize aromatic hydrocarbons.

ACKNOWLEDGEMENTS

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DISCUSSION

HERBES: Dr. Gibson, do you have any feeling for what the mass balance of benzopyrene or benzanthracene was in your experiments?

GIBSON: The mass balance we recovered was mostly unchanged. There was very little metabolism. In terms of benzanthracene, we got 30 percent conversion; for benzopyrene, I believe it was on the order of 2 percent--very low.

HERBES: Do you believe that the diol and then the phenolic metabolites are released in that form to the environment as opposed to being metabolized more completely to carbon dioxide?

GIBSON: I believe that the diol is metabolized further. The only reason we can isolate the diol is because we have a mutant strain. The wild-type organism produces acid products. With a polycyclic ring, I don't know if the organism would stop. I know it will metabolize one ring, but whether it will then go on to the second ring and go further all the way I don't know at this stage.

NONVOLATILE HYDROCARBONS IN THE PACIFIC OCEAN

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GEOSECS #90

NONVOLATILE HYDROCARBONS IN THE PACIFIC OCEAN

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Abstract

Extractable organics and nonvolatile (C_{14+}) hydrocarbons were measured in Pacific Ocean water along tanker routes and the tracks of the GEOSECS voyage. Hydrocarbon concentrations fit a log-normal distribution having median values of 2 and 0.8 parts per billion (ppb) for surface and subsurface (-3, -10 m) water, respectively. The hydrocarbons were complex mixtures which seemed to be of both petroleum and biogenic origin. Data from this and other studies indicate that a "natural" background of 1.5 ppb occurs in surface water.

INTRODUCTION

The vitality of the world's oceans is of widespread interest and concern. An extensive scientific effort is being carried out to evaluate the level of this vitality. One area of technical interest is the organic constituents and, in particular, the organics which may be introduced via man's activities. Included in these latter organics are petroleum hydrocarbons. In order to evaluate the present and future status of hydrocarbons, it is important to obtain baseline data. In response to this need, our laboratory and others have measured the concentrations of nonvolatile ($\sim C_{14+}$) hydrocarbons in the Atlantic Ocean.[1,2,3,4,5,6,7] Areas of the Baltic Sea[8], Mediterranean[4], and Indian Ocean[4] have also been studied.

Little is known about hydrocarbons in the Pacific Ocean, however, where only limited study of nonvolatile hydrocarbons has been reported.[9,10] Volatile hydrocarbons (C_1-C_4) were measured by Swinnerton and Lamontagne[11] and tar ball measurements have been carried out by Wong, Green and Cretney.[12,13]

In order to obtain baseline data on the Pacific Ocean, an extensive study* was carried out to measure and characterize nonvolatile hydrocarbons.

*This study was jointly funded by the National Oceanic and Atmospheric Administration and the U.S. Maritime Administration.

Additional insight was obtained from an extractable organic measurement which includes the hydrocarbons and other organics, such as esters and acids. Concentrations of volatile hydrocarbons were also observed although these results will be published at a later time.

This study began in mid-1973 when plans were carried out to collect samples during the GEOSECS cruise of 1973-74. Starting in February, 1974, sampling from aboard Exxon Corporation tankers was begun. Some results of sampling along tanker routes have been previously reported.[14]

SAMPLING AND ANALYSIS

Details of the sampling and analysis methods have been previously described.[15] A brief summary follows, however.

Sampling

In sampling from a tanker a bucket swinging from a boom was used to scoop water from the surface as the tanker traveled at its regular speed of approximately 14 knots. At about the same time a deeper sample at approximately 10 m was taken. This sample was obtained by drawing water directly from the sanitary pump line into the extraction bottle. Large volumes of water continuously flow through this line (~150 gal/min) during a voyage, and the thoroughly flushed line (20 volume changes per minute) gives a representative sample.

Bucket samples were collected off the R/V Melville in a manner similar to that followed for tankers. A sample was taken as the vessel was moving into a station. A deeper sample was collected from an uncontaminated sea water line whose intake is at a depth of approximately 3 m. At five stations, profile samples were taken at depths from 10 to 3000 m. Profile sample results will be described at a later date.

Periodic sample blanks were taken during all voyages. Each sample measurement was corrected for the appropriate blank value.

Method of Analysis

Starting point of the method is to extract the water with carbon tetrachloride. For a tanker sample, 8 ℓ of water were extracted immediately after collection aboard the ship. In the case of GEOSECS samples, five gallon glass jugs were filled and then spiked with CCl_4. These jugs were then shipped to our laboratory for the analysis.

The CCl_4 extract is examined by infrared spectrophotometry to measure total extractable organics. Following this, the carbon tetrachloride solution is evaporated to a small volume, placed on a dry silica gel column, and hydrocarbons are eluted as fractions. These fractions are combined and after being placed once again in carbon tetrachloride are again analyzed by infrared to obtain a total hydrocarbon content. Detailed compositional information can be obtained by further analysis based on UV spectrophotometry, gas chromatography, and mass spectrometry.

The method is sensitive to six micrograms. Its precision is 12 relative per cent (1 sigma variation) and its absolute accuracy appears to be 25 to 50 relative per cent at concentrations of 1 to 2 ppb.

Sampling Voyages

More than 300 water samples were taken along 17,000 miles of tanker routes. The actual mileage was considerably more than this because, as shown below, round-trip sampling was twice carried out along the same tanker route as summarized below:

- San Francisco to Cook Inlet, Alaska - sampled round trip in February and September, 1974, from aboard the Exxon Newark.
- San Francisco to Vancouver to the Panama Canal - sampled one way in May-June, 1974, off the Exxon Lexington.
- San Francisco to Singapore, one way on a ballast voyage of the Esso Antwerp, October-November, 1974.

The GEOSECS voyage by the R/V Melville began in August, 1973, from San Diego and was concluded at San Diego in June, 1974. Tracks covered in this voyage were from San Diego to Hawaii, to Adak in the Aleutians, to Tokyo, to Hawaii, to Pago Pago, to Wellington, down to Antarctica, to Tahiti, and finally a return to San Diego.

The total number of samples taken and the various measurements applied to them are summarized in Table 1.

Table 1

Summary of Samples and Their Analyses

Measurement	No. Samples GEOSECS	Tanker
Extractable Organics	242	336
Nonvolatile Hydrocarbons	177	187
Composition of Hydrocarbons	57	50

EXTRACTABLE ORGANICS AND NONVOLATILE HYDROCARBONS IN SURFACE AND SUBSURFACE WATER

It is beyond the scope of this manuscript to present the individual sample data which are included in the Pacific Ocean sampling report. These measurements are broken down by voyage as shown in Figures 1 and 2. Here, median values for surface and subsurface samples are geographically shown on maps.

Referring to Figure 1, it will be noted that, without exception, the surface water is higher in extractable organics compared with subsurface

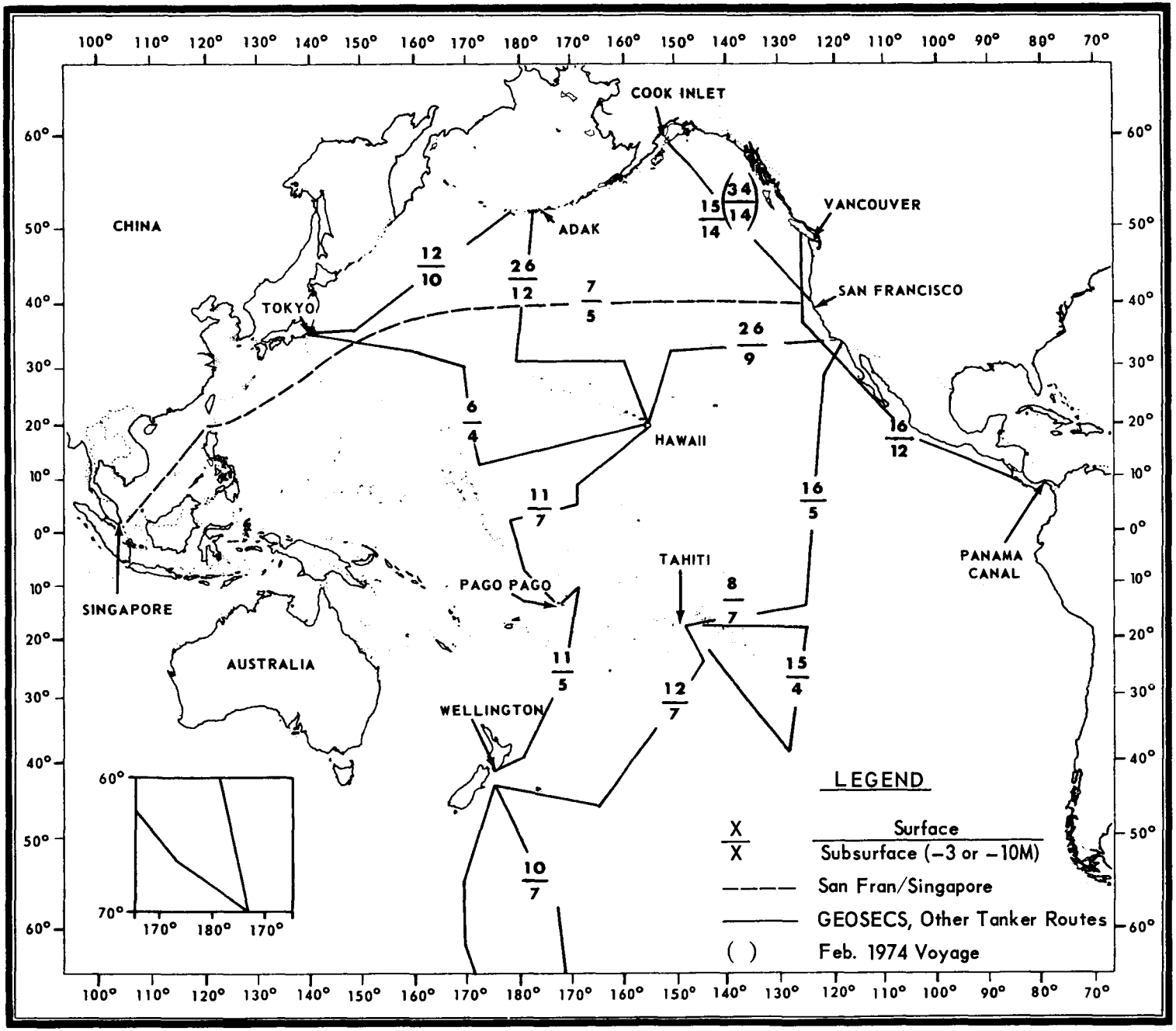

Figure 1. Median Values (ppb) of Extractable Organics in Surface and Subsurface Water of Pacific Ocean

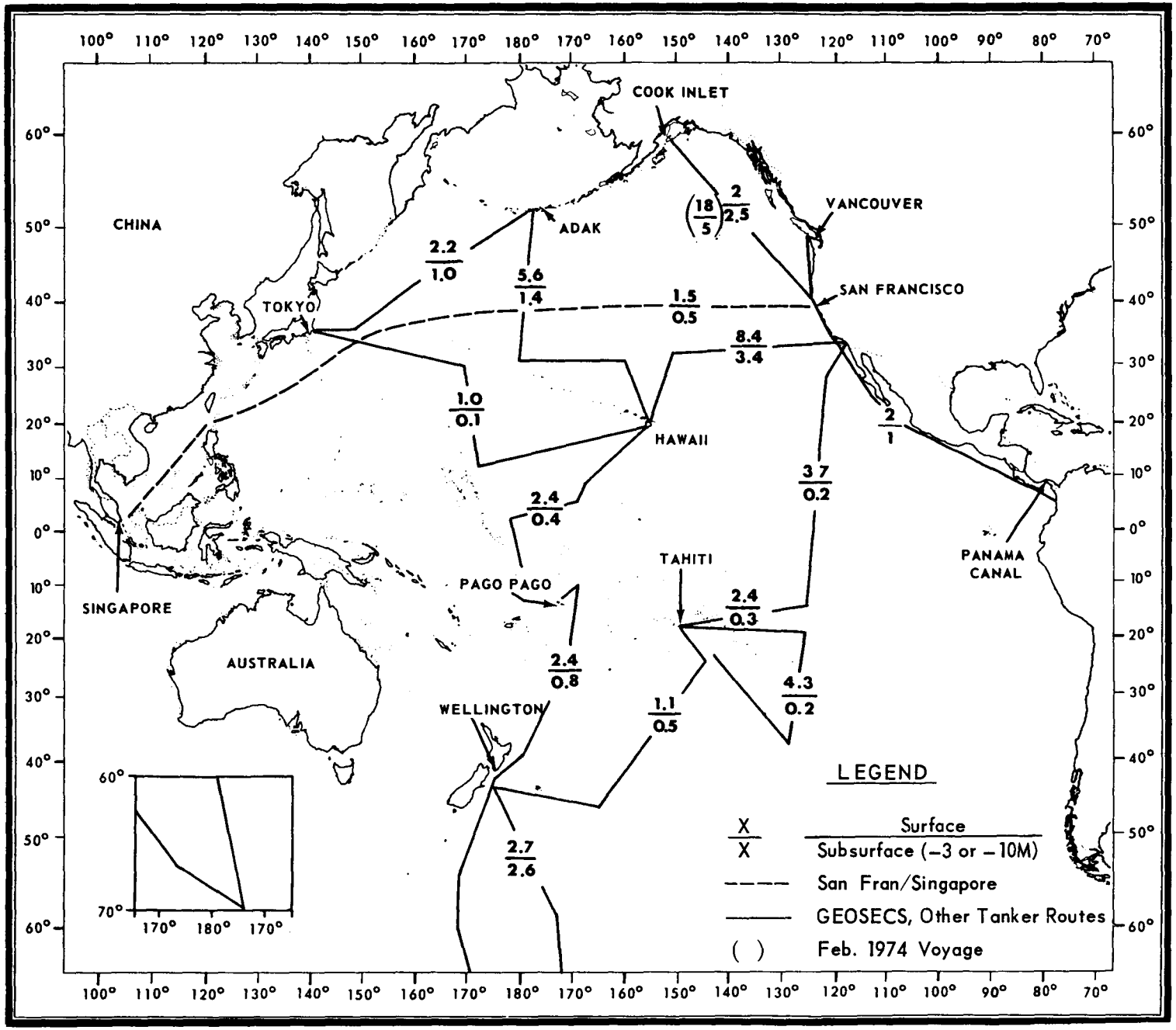

Figure 2. Median Values (ppb) of Nonvolatile Hydrocarbons in Surface and Subsurface Water of Pacific Ocean

water. In two instances, the ratio between surface and subsurface is just slightly >1 as, for example, 15/14 for the September voyage between San Francisco and Cook Inlet and 12/10 for leg III of the GEOSECS voyage. The highest concentrations are in the northeast sector of the Pacific, including GEOSECS legs I and II and the February voyage from San Francisco to Cook Inlet. As will be pointed out later, this latter voyage sampled water which was statistically different from all other samplings in the Pacific.

Figure 2 shows median hydrocarbon values. For surface and subsurface water, leg III had median values of 2.7/2.6 and the September/San Francisco/Cook Inlet values were 2/2.5. These are the only waters in which approximately equal concentrations were observed for surface and subsurface water. In all other cases, the surface water is significantly richer in nonvolatile hydrocarbons as compared with the subsurface. On average, the concentrations in the Southern Pacific are lower than in the northern half.

It should be pointed out also that higher concentrations of extractable organics and hydrocarbons occurred for GEOSECS leg I (San Diego to Hawaii) than was found approximately a year later in nearby water that was sampled in the first part of the San Francisco/Singapore tanker voyage. This is one of a number of observations which suggest that hydrocarbons in ocean water are transitory in nature.

Overall Median Values

In a study of the Atlantic Ocean it was found that total extractable organics and the nonvolatile hydrocarbon data fit a log-normal distribution. A similar observation was made of the Pacific Ocean tanker routes except for the February voyage between San Francisco and Cook Inlet. With this same exception, all of the Pacific Ocean data fit a log-normal distribution. Median concentrations and one-sigma variations are tabulated in Table 2.

Table 2

Median Concentrations in Pacific Ocean
of Extractable Organics (EO) and Nonvolatile Hydrocarbons (HC)

	Median (ppb)	1σ Range
Surface		
EO	13	7 to 49
HC	2	0.8 to 5
Subsurface		
EO	8	4 to 15
HC	0.8	0.3 to 2

COMPOSITION OF HYDROCARBONS

The combined IR, GC, and MS measurements of the analytical method provide a hydrocarbon type analysis, the concentration and carbon number distribution of n-paraffins and the carbon number range. A presentation of part of this information is illustrated in Figure 3 for the hydrocarbons found in surface water at GEOSECS station 201. The gas chromatogram of the sample is the upper trace whereas the lower trace is the combined background effect from the GC column bleed and the analytical silica gel column blank. N-paraffin peaks are identified by carbon number. The lined area between the chromatogram and background traces, on the other hand, identifies the smear of unresolved hydrocarbons which are the bulk of those found in most samples.

Analyses of some 364 Pacific Ocean samples provide some interesting insights as to composition. Inspection of the data showed, for example, that there was no systematic difference between surface and -10 m of water. Of particular interest is the insight provided as to origin of the hydrocarbons.

N-Paraffins

A number of investigators have shown that n-paraffins may biodegrade in one or two days.[16,17] In view of this and our own observation that low n-paraffin concentrations are often encountered even along busy tanker routes, n-paraffin concentrations may be considered as an indication of hydrocarbon age.

A summary of data from the GEOSECS and tanker voyages is shown in Table 3.

Table 3

N-Paraffins in Pacific Ocean Hydrocarbons

GEOSECS Stations	Wt. %	Tanker Route	Wt. %
201-239 - North Pacific	5	San Francisco/Cook Inlet	
336-347 - North Pacific	<1	February	2
		September	<1
246-285 - South Pacific	6		
301-334 - South Pacific	1	San Francisco/ Vancouver/Panama Canal	7
		San Francisco/ Singapore	5

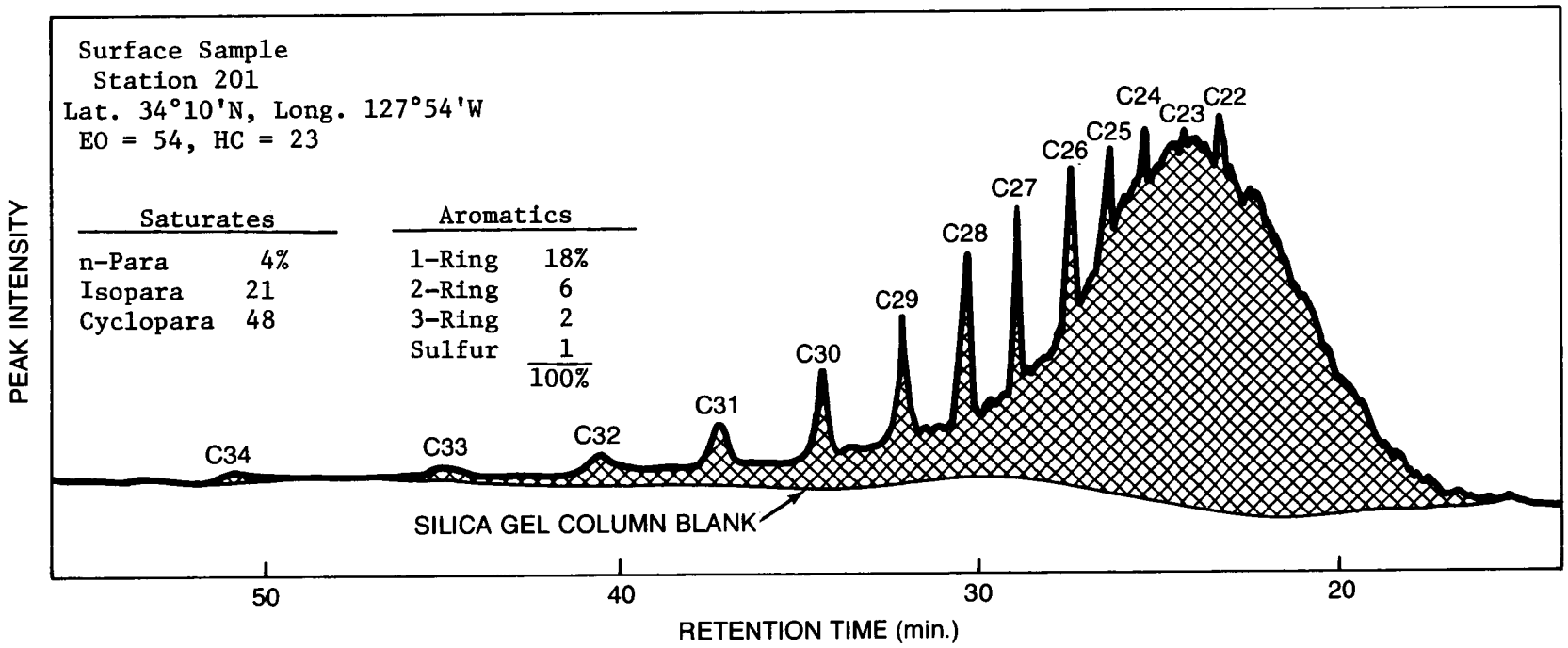

Figure 3. Hydrocarbons at Station 201

On the average, relatively high concentrations (5 to 6%) are observed for stations 201 to 285 with low values for all the later stations, 301 to 347. Both groups of stations cover northern and southern areas of the Pacific and thus the n-paraffin contents do not appear to relate to geographic location. Stations 201-285 were sampled between August, 1973, and February, 1974, and the n-paraffin data suggest that fresh hydrocarbons were found in water during this time.

Relatively high concentrations were observed for the San Francisco/Vancouver/Panama Canal tanker voyage. Much of the water between Vancouver and the Panama Canal is in an upwelling region and since sampling was done during May and June when upwelling would occur, a maximum quantity of biogenic organics might be expected. This could account for the relatively high n-paraffin concentrations of this voyage.

High n-paraffin values were also observed for the San Francisco to Singapore voyage. This occurred in October and November, 1974--a time of year for which comparable values were observed for GEOSECS sampling in 1973.

Overall Composition

In 99% of cases for which hydrocarbon composition was determined, the hydrocarbons were found to be complex mixtures of paraffins, cyclo-paraffins and 1-, 2- and 3-ring aromatics. A summary is shown in Table 4.

Table 4

Composition of Pacific Ocean Hydrocarbons

	San Francisco/ Cook Inlet (Feb.)	All Other
	Wt. %	
Isoparaffins	10	19-27
Cycloparaffins	48	43-60
Aromatics	36	15-22
Aromatic Sulfur	4	2-5

Except for the San Francisco/Cook Inlet voyage of February, 1974, there is a good deal of similarity among the compositions. The hydrocarbons from the San Francisco/Cook Inlet voyage are significantly higher in aromatic content as compared with compositions observed for all other sampling.

Petroleum is a complex mixture of hydrocarbons such as shown in Table 4 and the assumption may be made that the hydrocarbons found in Pacific Ocean water originate from petroleum. This interpretation is not a straightforward one, however. Ship operations might explain some of the

hydrocarbons found along tanker routes but hardly those found in the remote areas sampled during the GEOSECS voyage.

Of pertinence to this discussion are some hydrocarbon measurements made of a wilderness lake in Ontario, Canada.[18] Data for the lake water are shown in Figure 4. This sampling was done in May, 1975, at a time shortly after ice out when water turnover would have recently occurred. Significant quantities of n-paraffins were found but a smear of hydrocarbons is also evident.

Additional evidence that a smear of hydrocarbons may originate from nature was presented at this symposium by Meyers.[19] Sediment from a pristine area in Lake Huron was shown to contain hydrocarbons of complex composition.

Hydrocarbons Associated with High Lipid Content

Pacific Ocean water generally contains CCl_4 extractable organics (lipids-organic acids, esters, hydrocarbons) at concentrations below 30 ppb. Significantly higher quantities were observed for a few samples. By looking at data for these samples it was felt that some insight might be gained as to whether or not hydrocarbon content relates to high lipid content. The results, as summarized in Table 5, appear to indicate two factors at work. In samples for which median EO concentrations of 61 and 69 are observed, it is reasonable to assume that the hydrocarbons of concentrations 1 and 4 ppb, respectively, are associated with the high lipid content and are thus primarily of biogenic origin.

Table 5

Hydrocarbons Vs. Extractable Organics of > 39 ppb

	No. Samples	Median, ppb EO	HC
GEOSECS	6	46	14
San Francisco/Cook Inlet - Feb.	5	49	23
San Francisco/Cook Inlet - Sept.	2	69	4
San Francisco/Vancouver/Panama Canal	4	61	1

The high lipid contents associated with the hydrocarbon concentrations of 14 and 23 ppb may be attributed to a behavior which has generally been noted in ocean sampling studies. Qualitatively, it has been observed that a high hydrocarbon concentration always has a high lipid content. This is attributed to the fact that these hydrocarbons are in particulate form and serve as a sink since they selectively dissolve other lipids from the surrounding water phase.

Obvious Biogenic Hydrocarbons

In addition to compound class information, the analyses identify individual hydrocarbons in terms of well defined peak(s) in a gas chromatogram. This might include one or more n-paraffins, isoprenoids (pristane,

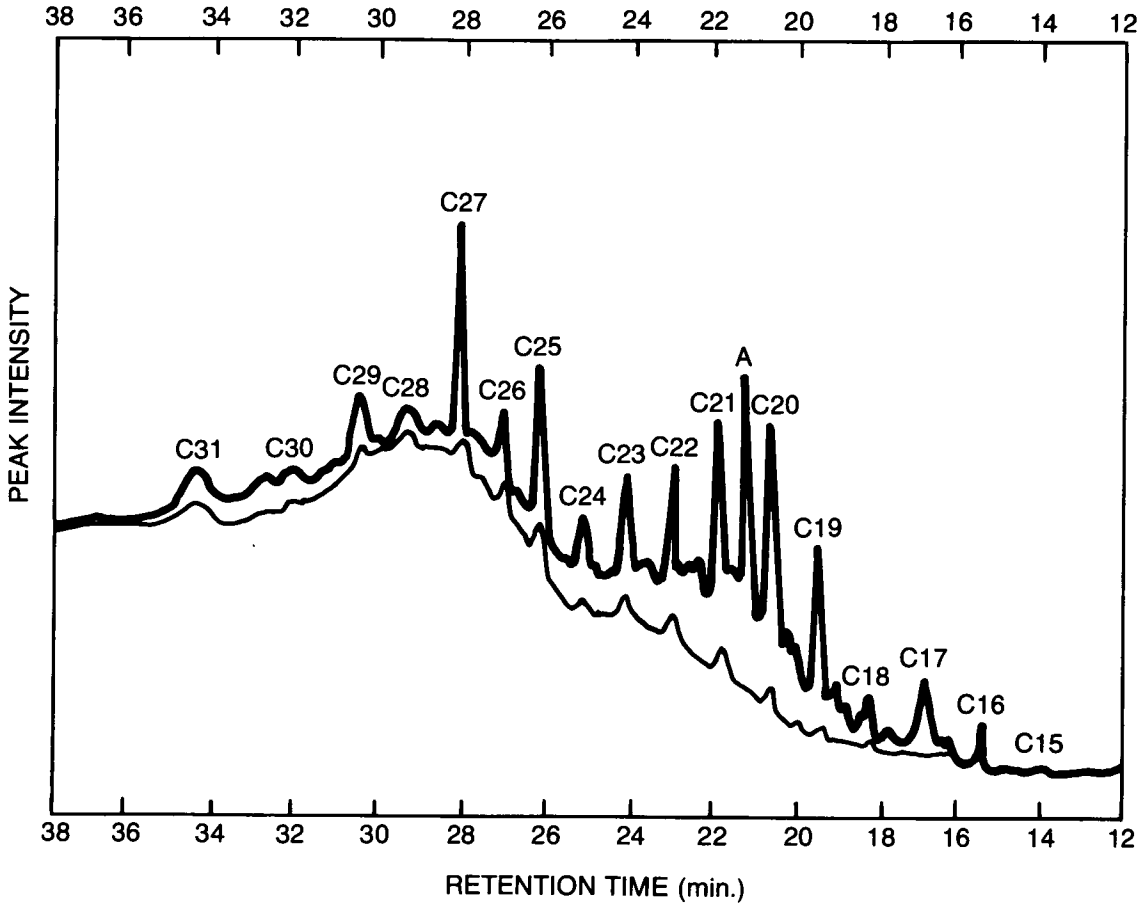

Figure 4. Hydrocarbons from a Wilderness Lake

phytane), polyolefins, triterpanes and steranes. It is apparent that some of these hydrocarbons are of biogenic origin and may serve as indicators that nature has furnished a portion of the hydrocarbons in ocean water. Not enough is known to suggest how these indicators may be used to estimate total hydrocarbons from nature, but it is helpful at this point to observe the frequency and general concentrations of these indicators in ocean water.

To demonstrate the observed frequency of these hydrocarbons, Table 6 presents a summary of their occurrence. Among the n-paraffins, nC_{15} is the most frequently observed biogenic indicator. It appeared as a spike in seventeen of forty-five GEOSECS samples and was relatively intense (0.5-5% of total hydrocarbons) in four of the samples.

Table 6

Occurrence of Individual Hydrocarbons in Ocean Water

Hydrocarbon	No. Samples GEOSECS	Tanker
	45	55
nC_{15}	17	8
nC_{31}	3	16
Other odd carbon normals	9	9
Pristane/Phytane	10/12	34/37
Olefins	18	19

Of particular interest also are hydrocarbons which occur as a fractional carbon number, such as: $C_{19.3}$, $C_{19.7}$, $C_{20.4}$, $C_{22.5}$, $C_{24.7}$, and $C_{25.7}$. It is believed that some or all of these hydrocarbons are olefins.

Pristane and phytane occur much more frequently along tanker routes, lending support to the thought that petroleum derived hydrocarbons are more priminent along such routes as compared with less frequently traveled waters.

ORIGIN OF HYDROCARBONS

At least three different methods have been proposed for differentiating between petroleum and biogenic source hydrocarbons.

In one, it is assumed that biogenic hydrocarbons represent 10% of the total lipids in ocean water.[4] Any hydrocarbons above this relative amount are attributable to petroleum. This method worked reasonably well for Atlantic Ocean water but is not applicable to the Pacific Ocean.

Farrington, et al[20] proposed that in the gas chromatogram of hydrocarbons from water or sediment, the entire envelope represents petroleum type hydrocarbons. Biogenic hydrocarbons, on the other hand, are

the n-paraffins or other single hydrocarbon peaks which usually can be recognized as being non-petroleum in nature. This method would consistently predict that only minor proportions of biogenic hydrocarbons ever occur in ocean water. As noted by the authors, this method ignores published data that some bacteria, mussels, yeasts, and fungi contain complex mixtures of iso and cyclic hydrocarbons.

The basis of a third method is that a low concentration of hydrocarbons persist as a natural background. This appears to be the most promising way to gauge contributions from nature versus those from petroleum. We would propose to define nature in a more literal manner than commonly considered and include not only biogenic activity in local water but ground water runoff and atmospheric fall-out. Ground water and the atmosphere may include hydrocarbons from both petroleum and biological activity so that a mixed source is proposed as the "natural" background. A surprisingly similar background level of approximately 1.5 ppb can be attributed to surface water of the Atlantic[4] and Pacific Oceans, as well as the wilderness lake previously discussed.

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DISCUSSION

BUTLER: On the North Pacific one finds a fair amount of pelagic tar, particularly near Japan. In the South Pacific all the reports I have seen have indicated that there is no pelagic tar at all. You can tow a net for hours and not pick up any tar. Your surface hydrocarbon samples showed just about the same amount of hydrocarbons in the North Pacific as in the South Pacific. I wondered whether there was any sign of particularly high hydrocarbon levels near Japan or whether there is no correlation at all with pelagic tar.

BROWN: There seems to be a slight correlation. Let me put it differently. Our data seem to fit in well with parts of the pelagic tar data. In other cases it does not seem to correlate.

BUTLER: You did find higher hydrocarbons near Japan?

BROWN: No, I did not say that. In sampling from San Francisco to Singapore we sampled in an area which would cover part of the tanker routes from the Middle East to Japan. There was no correlation, really, in our findings and the pelagic tar content along that particular part.

FRIIS: Have you analyzed any samples from the deep ocean, say, 1000 meters or something like that, and compared those to the composition of the surface samples?

BROWN: Yes. We have sampled and measured concentrations and analyzed quite a number of samples in profile tests down to 4000 meters in both the Atlantic and the Pacific, and we still find a smear of hydrocarbons regardless of where the sample is taken. There is an indication that the aromatic concentration might be a little bit lower for the deeper water.

FRIIS: How much lower? What is the percentage of the surface water as opposed to deep water with the aromatics?

BROWN: In the Pacific Ocean the aromatic concentration averaged out at around 15 percent. In the profile samples we sometimes find zero percent, sometimes 4 percent, and sometimes something like 15 percent. It is very unusual to find a sample with no aromatics.

GOULD: Can you speculate on the physical nature of the hydrocarbons that you have analyzed? Are they dissolved hydrocarbons? Are they absorbed

on particulate matter? Are they contained in plankton that happened to be floating near the surface? Do you have any data of that type?

BROWN: We don't have any data of that type. These hydrocarbons are identified as total dispersed simply because we don't know where they come from, whether they are from solution or particulate matter or what.

PETROLEUM POLLUTION: CHEMICAL CHARACTERISTICS AND BIOLOGICAL EFFECTS

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Two approaches, biological effects of specific petroleum molecules on marine organisms and a baseline survey of the level of petroleum hydrocarbons in biota, water and sediments, have been used to study the problem of petroleum pollution.

The subject matter of this symposium, petroleum and the environment, has only recently become the focus of public and scientific interest. This interest is the result of two national concerns: more petroleum and a healthy environment. The search for more petroleum is well underway and in most respects is based on extensions of proven technology. The overriding environmental question in these activities is to what extent is petroleum recovery and use having a deleterious effect on plants and animals.

The scientific community has been called upon to answer this important question. Various national programs in which many of us participate have been launched to deal with this question at specific levels. The marine biological scientific community is being asked to explain how a complex ecosystem is being impacted by stresses when in fact the mode of operation of a natural marine ecosystem is not well understood. Similar statements are true for other fields, for example, transport of petroleum would be easier to evaluate if the transport of natural organics was understood. My reason for stating these generalizations is to urge that petroleum pollution studies be done not as isolated, special studies, but as extensions of marine biology, organic geochemistry, or other appropriate fields.

The theme of my talk is transport in the water column. In order to show our particular approach to this problem I will mention some work on the chemical composition of the water-soluble fraction of oils and the biological effects of the water-soluble fractions on organisms.

The water-soluble fraction (WSF) of oil is prepared by layering an oil over seawater and gently mixing. This yields a solution of oil in water at a concentration of from 1-25 mg/lt (ppm), depending on the type of oil used. When oil is spilled on the ocean a similar liquid-liquid extractor is set up. This process of solution and mixing is an important transport mechanism. Since the components of oils vary greatly in solubility, one would expect the water-soluble fraction to have a com-

position unlike that of the parent oil. We have investigated the chemical composition of the WSF of several petroleums and fuel oils. Fuel oils have been given special attention because of their toxicity[1,2,3].

Four fuel oils kindly supplied by the Exxon Corporation and the API No. 2 fuel oil have been characterized with respect to WSF. The water solubles (WSF) were prepared by addition of 1 part of oil to 8 parts of water (usually algal culture medium) in a bottle with a bottom drain and containing a magnetic stirring bar. The bottle was gently stirred for 24 hours at room temperature. The water phase, sampled from the bottom, was placed in a liquid-liquid extractor and the organic compounds extracted into benzene. The benzene extract was used for gas chromatographic and gas chromatography-mass spectrometric analysis[4]. The striking effect that water as the transport medium has on the chemical composition of oil is reflected in Table 1; the asphaltics, oxygen and nitrogen containing compounds, which make up around 0.5 percent of the whole oils, account for at least 20 percent of the WSF. Aromatics, as represented by the naphthalenes, are only slightly depleted in the WSF. Paraffins are almost excluded from the WSF due to their very low solubility[5]. This low level of dissolved paraffin is consistent with values we have measured for the Texas shelf as part of a large survey of hydrocarbon levels sponsored by the Bureau of Land Management.

Table 1. Identification and concentration of major components of four fuel oils: whole oil vs. WSF

Oil	Montana	Baytown	New Jersey	Baton Rouge
Whole Fuel Oil				
% paraffins	57	53	51	57
% aromatics	33	38	38	38
% asphaltics	0.3	0.3	0.6	0.3
% recovery	90	91	90	95
Water Soluble Fraction				
Total organics, mg/lt	16	19	14	9
naphthalenes				
mg/lt	1.5	1.8	2.5	1.6
%WSF	9.3	9.5	17.9	17.8
phenols				
mg/lt	2.3	4.1	2.0	1.1
%WSF	14.4	21.6	14.3	12.2
anilines				
mg/lt	2.6	.72	.27	.02
%WSF	16.3	3.8	1.9	.2
indoles				
mg/lt	.79	.09	.32	.05
%WSF	4.9	.5	2.3	.6

The detailed chemical analyses of these four WSFs of the American Petroleum Institute #2 fuel oil are given in Table 2.

Table 2. Identification and concentration of major components in the water soluble fractions of four fuel oils, mg/l.

Major Components	Montana	Baytown	New Jersey	Baton Rouge	API
1,2,4 Trimethyl Benzene	.37	.56	.42	.50	.22
C_3-Benzene[a]	.23	.23	.21	.29	.16
Indan + C_4-Benzene	.22	.26	.13	.11	.18
Methylindan	.25	.15	.13	.07	.25
Naphthalene	.64	.75	.66	.39	.87
o-Toluidine	.37	.34	.12	.04	.14[b]
p-Toluidine	.14	--	.02	--	--
m-Touidine + 2,6 Dimethylaniline	.53	.24	--	--	--
1-Methylnaphthalene	--	--	--	--	.36
2-Methylnaphthalene	.33	.51	.84	.48	.51
2,4 Dimethylaniline	.24	--	--	--	--
1-Methylnaphthalene	.20	.30	.46	.28	--
2,5 Dimethylaniline	.30	.04	.03	--	--
2,6 Dimethylphenol + C_2-Aniline	.19	.13	--	.04	.10[b]
3,5 Dimethylaniline + dimethylnaphthalenes	.33	--	--	--	.35
2,3 Dimethylaniline + Dimethylnaphthalene + C_3-Aniline	.26	.07	.08	.04	--
Dimethylnaphthalene + 3,4 Dimethylaniline + C_3-Aniline	.32	.08	.16	.08	.07
o-Cresol + 2,4,6 Trimethylphenol + dimethylnaphthalene	.42	1.16	.46	.25	.54
2,6 Dimethylnaphthalene	.14	.11	.12	.05	--
m + p Cresol + 2,4 + 2,5 Dimethylphenol	.96	1.95	.60	.32	1.33
2,3 Dimethylphenol + C_3-Phenol	.18	.12	.14	.08	.46
3,5 Dimethylphenol + C_3-Phenol	.51	.60	.45	.24	.63
3,4 Dimethylphenol + 2,3,5 Trimethylphenol	.06	.15	.21	.13	.39
C_3-Phenol	.09	.06	.10	.02	.05
Indole + Methylindole	.24	.03	.07	--	.12
Methylindole + Dimethylindole	.35	.02	.11	.06	.07
Methylindole + Dimethylindole	.15	.02	.08	--	--
Dimethylindole + C_3-Indole	.05	.02	.06	.05	--
Perinaphthenone	--	--	--	--	.20
Total Organics by G.C.	12.8	12.9	10.5	7.0	--
Total Identified Organics	8.07	7.90	5.66	3.52	7.63
Methylnaphthalenes	.53	.81	1.30	.76	.87
Dimethylnaphthalenes	.31	.24	.55	.41	.33
Phenols	2.33	4.12	1.96	1.08	3.54
Anilines	2.57	.72	.27	<.02	.19
Total Organics by weight	16	19	14	9	15

[a]Notation C_2, C_3 or C_4 indicates parent compound plus 2, 3 or 4 additional saturated carbon atoms in side chains of unspecified chain length.

[b]The API oil contains benzothiophene in the o-Toluidine peak; 2,6 dimethylphenol peak contains some methylbenzothiophene.

If solution and mixing is a major transport mechanism for oil, then the rates of these processes will be important. Our preliminary studies show that different types of compounds move into the water phase at different rates. The rate is related to the number and type of polar groups on molecules. The data in Figure 1 was obtained by varying the equilibration time in the preparation of a series of WSFs. While all compounds reach equilibrium level in 20 hours, the fact that some are transported in 3 to 6 hours may be significant in the early hours of an oil spill. While highly volatile compounds will be quickly lost to the atmosphere, it is not likely that these polar substances, once in water, will be quickly lost by that pathway. Polar compounds are likely to be subject to microbial decomposition as well as association with particulate matter. Whether these are competing or mutually reinforcing processes is not clear. There are a good deal of recent research results dealing with microbial metabolism of hydrocarbons. The interaction with and transport by particles of these polar molecules is a pathway that needs further study.

Supporting evidence for differential rates of transport and of the actual abundance of polar compounds in oil is given in Table 3. In this experiment the same oil was equilibrated with several water layers for 24 hours. The rapid and preferential transfer of polar compounds such as phenols and toluidines to the water phase is consistent with chemical considerations. This phenomenon means that events of oil on water represent a changing process with short-term and long-term transfers. One should remember that the chemical identity of only about two-thirds of the WSFs is known. Rapid transfer of highly toxic compounds which may be present in an oil in low concentration may cause biological effects that would be missed in long-term studies.

Table 3. Concentration of selected compounds in water soluble fractions prepared by successive equilibration[4].

Compound	1st Equilibration 0 - 24 h	2nd Equilibration 24 - 48 h	4th Equilibration 72 - 96 h
1,2,4 Trimethylbenzene	100%[a]	98%	99%
Naphthalene	100%	94%	92%
2 Methylnaphthalene	100%	98%	102%
1 Methylnaphthalene	100%	99%	98%
2,6 Dimethylnaphthalene	100%	104%	104%
Indole + Methylindole	100%	106%	67%
o-Toluidine	100%	50%	11%
m-Toluidine	100%	52%	10%
2,4 + 2,5 Dimethyl-phenols, m + p cresol	100%	57%	14%
3,5 Dimethylphenol + C-3 phenol	100%	49%	13%

[a]Concentration expressed as percentage of the concentration present in the 1st equilibration (0 - 24 h).

These and other chemical characterization studies have been used to support the development of a biological-effects program. Our specific approach to this complex problem is to isolate chemical fractions from

petroleum, especially from the WSF, and to test the fractions for biological effects. In cases where plants or animals react to a fraction further work is done to isolate and prove structure on the toxic agent. Petroleum components that are toxic to one organism may show little activity toward another. Nevertheless, the concentration ranges for biological effects of specific substances on various organisms have been established in closely controlled experiments. The microalgae have been used to measure differential growth responses when exposed to pure compounds or WSF. Table 4 shows that the WSF has a clear effect on the rate of growth and survival of algae at the ppm level. Following this lead, Winters, Batterton, and Van Baalen have found a case where a single compound phenolen-1-one (perinaphthenone), isolated and characterized from the API No. 2 fuel oil, was toxic to microalgae. The possibility that a few specific molecules may be the biologically active components of oil means that it is necessary to identify such substances, describe their transport mechanisms and learn their fate if the biological effect of the flow of petroleum molecules through ecosystems is to be understood. If, indeed, it can be shown that only a few molecule types are active then it will be reasonable to investigate the mode of action of these on organisms at the physiological and biochemical level.

A second and perhaps equally useful approach to the study of the import of petroleum on the environment is the measurement of the levels of heavy hydrocarbons in the sea. Our laboratory has been making such measurements for several years, beginning with the International Decade of Ocean Exploration Baseline Program and presently as part of the Bureau of Land Management Outer Continental Shelf Program[6]. The goal of these programs is to establish the present-day level of heavy hydrocarbons in biota, water and sediment so that future trends may be evaluated. Aside from the complications due to the complexity of the required chemical analysis, there is a real problem in data interpretation. That well-known problem is to distinguish natural-product hydrocarbons from petroleum-derived materials. This problem has not been fully resolved but the even-odd ratios of normal paraffins, the presence of aromatics, the preserve of unresolved humps, pristane-phytane ratios and similar parameters do promise a solution.

The samples we have studied, all from the Gulf of Mexico, are too many to be appropriate for this symposium. Nevertheless, some real numbers will serve to bring the program into focus. Twelve stations off the South Texas coast have been sampled three times during the year (Figure 2). Chemical analyses of these materials were done according to the usual techniques of extraction, saponification, purification by liquid-solid chromatography, gas-liquid chromatography (GLC) and GLC-mass spectrometry[7].

The identification and quantification of traces of heavy hydrocarbons dissolved in seawater is very difficult, not unlike the situation for trace metals in seawater. For this reason the average of a number of values is best used to provide the levels. The values given in Table 5 indicate three general trends, a decrease in concentration with distance from shore, a slight increase in concentration during the spring and fairly constant levels for the four transects. The non-paraffin fraction, benzene eluate, of most of these samples does not contain significant levels of aromatic hydrocarbons, suggesting that the paraffins are natural products rather than oil-derived.

Table 4.

GENERATIONS PER DAY OF MICROALGAE GROWN AT 30°C IN PRESENCE OF 50% WATER SOLUBLES FROM NO. 2 FUEL OILS.

STRAIN DESIGNATION	CONTROLS	FUEL OILS			
		BAYTOWN	BATON ROUGE	MONTANA	NEW JERSEY
PR-6 (BLUE-GREEN)	4.6 ± 0.2	No Growth (7 days)	3.9 (31 hr. lag)	No Growth (7 days)	1.9 (150 hr. lag)
580 (GREEN)	2.7 ± 0.2	2.9 (55 hr. lag)	2.7	2.7	No Growth (9 days)
N-1 (DIATOM)	4.9 ± 0.2	4.3 (16 hr. lag)	4.9	1.4 (81 hr. lag)	4.6 (4 hr. lag)

Table 5. Average total n-paraffin concentration in seawater, microgram/liter.

Station	One	Two	Three
I.	.31*	.25	.21
II.	.85	.51	.24
III.	.27	.25	.21
IV.	.27	.23	.13

*These values are the average for three seasons, winter, spring, and summer; the seasonal average for all stations are, winter - .13, spring - .64, summer - .23.

A summary of the hydrocarbon levels in zooplankton collected at the stations shown in Figure 2 are given in Table 6. The large nC_{17}

Table 6. Average of hydrocarbon levels in zooplankton, South Texas Outer Continental Shelf, 1974-1975.

	Winter		Spring		Fall	
	µg/g	Rel.%	µg/g	Rel.%	µg/g	Rel.%
nC_{15}	3.2	2.7	6.0	5.7	1.8	4.7
nC_{16}	1.0	0.8	0.8	0.8	0.2	0.5
nC_{17}	18.5	15.5	39.6	37.6	8.8	23.2
nC_{18}	3.6	3.0	2.0	1.9	1.1	2.9
nC_{19}	3.0	2.5	2.0	1.9	1.6	4.2
nC_{20}	2.0	1.7	1.2	1.1	0.6	1.6
nC_{21}	0.7	0.6	0.6	0.6	1.3	3.4
nC_{22}	8.1	6.8	3.0	2.8	3.3	8.7
Pristane	73.9	61.9	49.1	46.6	17.8	47.9
Phytane	0.7	0.6	0.1	0.1	0.05	0.1
Phytadine	4.7	3.9	1.0	0.9	1.4	3.7

and pristane values indicate that the patterns are biogenic. Fewer than five percent of the zooplankton have a pattern of paraffins that is characteristic of petroleum contamination. This is in sharp contrast to hydrocarbon data for 36 samples of neuston collected at the same time. Twelve of these had patterns of paraffin hydrocarbons that were clearly petroleum-derived, which is good evidence that micro-tarballs are present. The non-paraffin fraction of the zooplankton contains a variety of interesting lipids including squalene, diolefins and, isoprenoids.

For several years our laboratory has been concerned with the chemistry of organic matter in Recent sediment, especially for the Gulf of Mexico. Thus we have a good deal of information on the occurrence and significance of fatty acids, fatty alcohols, stable carbon isotopes, total carbon and kerogen in Gulf sediments. The Bureau of Land Management hydrocarbon study has been an excellent way to add to this understanding. We would submit that useful environmental insights are most

likely to result when the overall organic geochemistry of sediment is considered. The calculations in Table 7 illustrate where the big gaps are for an "idealized" Gulf sediment.

Table 7. Material balance in an idealized Gulf of Mexico sediment[8].

Dry weight	16 g
Total organic carbon	100.mg
Non-lipid carbon	95.mg
Total lipid carbon	5.mg
Total non-saponifiables	3.mg
Total fatty acids	.4mg
Total sterols	.1mg
Total fatty alcohols	.1mg
Total saturated hydrocarbon	.03mg

Analysis of n-alkanes for 34 samples taken from the BLM stations shown in Figure 2 established that there are no seasonal trends in abundance or patterns, and no significant overall trends. The odd-even ratio of n-alkanes has been a useful parameter in petroleum geochemistry and promises to be useful in the pollution geochemistry of hydrocarbons.

The use of odd-even ratios to distinguish petroleum from natural product hydrocarbons is based on the fact that many natural product patterns show strong odd carbon predominance, while petroleums often have odd-even ratios of near one[9]. The odd-even parameter we have used, the OEP, was developed by Scalan[10]. The summary of OEP values given in Figure 3 indicates that the sedimentary hydrocarbons of the area are "natural" except for stations 1 and 2 on line IV. There, the near unity OEP values may be due to oil seeps or ocean dumping.

In general, based on hydrocarbon levels and patterns in water, zooplankton, and sediment, the South Texas Outer Continental Shelf shows little petroleum pollution. The neuston does show frequent petroleum contamination, perhaps by micro-tarballs. This favorable baseline suggests that the area could be usefully monitored.

In this presentation we have tried to demonstrate that while oil pollution is very complex, real advances can be made through several approaches.

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DISCUSSION

BUTLER: When you did these studies on the water soluble components of fuel oil, did you try to distinguish between truly dissolved material and emulsified material?

PARKER: We feel that we are dealing entirely with material that is in solution because of the way that we prepare the sample. The stirring is very gentle and never makes a mixing vortex. Also, the fact that we don't find any appreciable concentration of normal paraffins in our samples evidently confirms this.

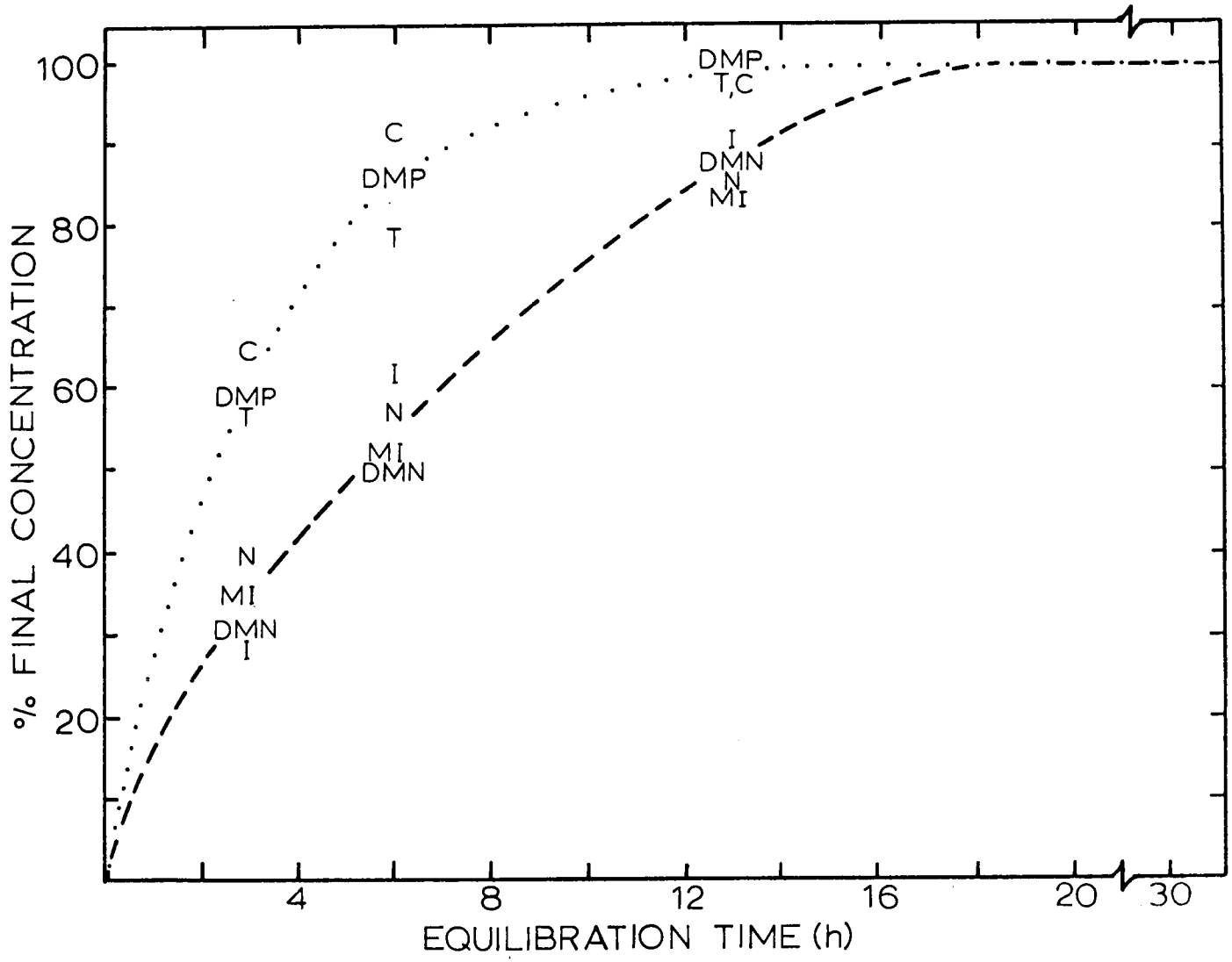

Figure 1. Rate of solution of water-soluble components. Concentrations expressed as percentage of equilibrium concentration. C: o-cresol + 2,4,6 trimethylphenol; DMP: 2,4 + 2,5 dimethylphenol, m + p cresol; T: o-toluidine; N: napthalene; MI: methyl indan; DMN: dimethylnapthalene; I: indole + methyl indole.

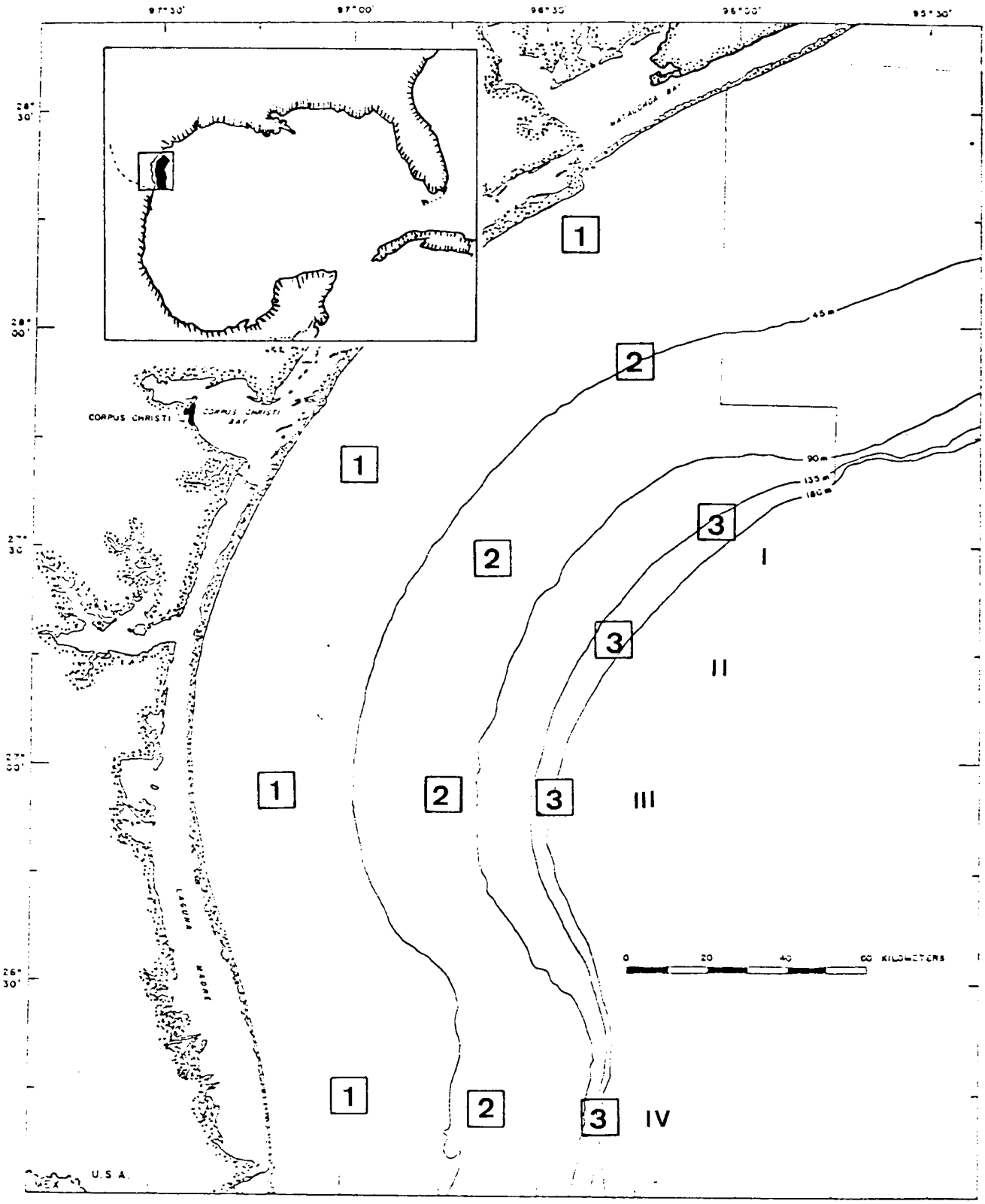

Figure 2. Station locations for Bureau of Land Management South Texas Outer Continental Study, 1974-75.

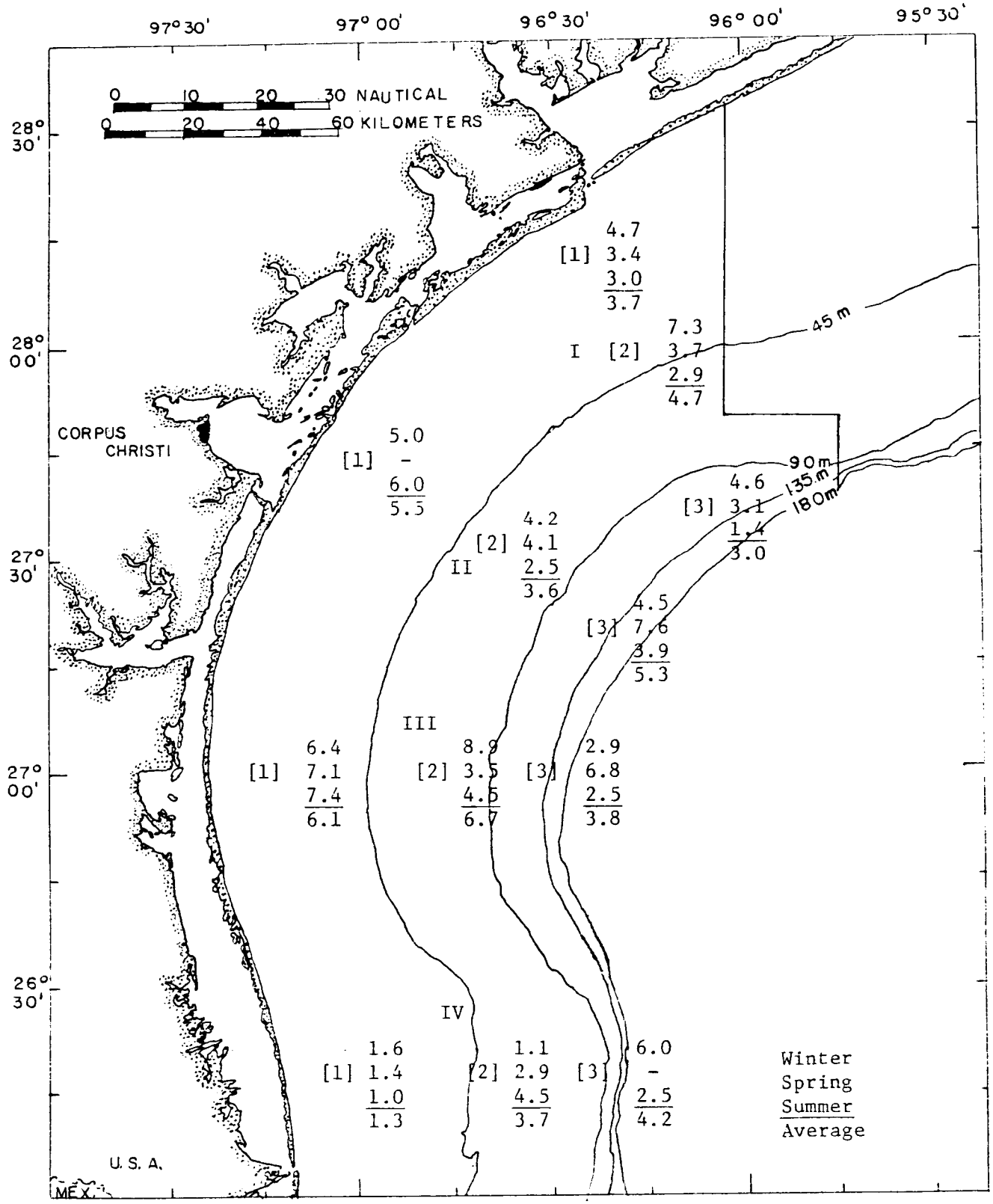

Figure 3. The odd-even preference (OEP) index for sediment n-paraffins, taken during 1974-75.

SOURCE AND DISTRIBUTION OF HYDROCARBONS IN SURFACE WATERS OF THE SARGASSO SEA

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ABSTRACT

Pelagic tar and surface water samples from the Sargasso Sea were analyzed for hydrocarbons using gas liquid chromatography and infrared spectrometry. Based on these analyses, small particles of weathered tar (0.3 μm to 1.0 mm diameter) are suggested as a major source of hydrocarbons in the unfiltered water samples. These particles are probably formed by weathering of large tar lump surfaces and contain relatively large amounts of cycloparaffins. Previous estimates of pelagic tar may be very low because they have not included the smaller size range of these particles.

INTRODUCTION

Pelagic tar has been observed in the North Atlantic,[1,2] Mediterranean Sea,[1] North Pacific,[3] Carribean Sea[4] and Gulf of Mexico,[4] with the Sargasso and Mediterranean Seas usually having the highest concentrations. These tar lumps are sometimes so abundant that they foul Neuston nets.[1] In general, the chemical properties of pelagic tar are somewhat similar to those of crude oil, although various weathering processes can cause some alterations.[2-5] The presence of paraffinic wax in tar lumps indicates their origin is probably from tanker operations.[2-6]

Butler et al.[2] reviewed all the available information as of the end of 1972, regarding the occurrence, probable sources, and potential fate of pelagic tar found in the Sargasso Sea. They reported the concentration of tar ranged from 2 to 40 mg/m^2 on a wet weight

basis, and estimated the apparent lifetime of this material on the surface of the ocean to be approximately a year or more. (The estimated residence time of pelagic tar in the North Pacific is also about one year.)[3] In order to provide additional information on the source, distribution, and chemical nature of hydrocarbons in Sargasso Sea surface waters, we analyzed eight samples of pelagic tar and seventeen pairs of water samples using gas liquid chromatography and infrared spectrometry.

EXPERIMENTAL

Pelagic tar samples were collected on R/V TRIDENT cruise 123 from the Azores to Bermuda in September 1972 (Table 1). All samples were collected from a rubber raft which was rowed at least 100 m upwind of the R/V TRIDENT to avoid sample contamination from the ship. The tar lumps (∿1 to 5 cm dia.) were found floating freely or attached to Sargassum and collected from the top 30 cm of the ocean surface with a stainless steel screen (1.0 mm mesh) used in the collection of surface microlayer samples,[7] or a rope net. The tar samples were then wrapped in aluminum foil and stored at -20° to 0°C until analyzed, within 3 months of collection.

(Table 1)

Samples of the frozen tar (∿200 mg, wet weight) were placed in pre-weighed 25 ml centrifuge tubes. The samples were either whole tar particles if sufficiently small (∿1 cm dia.), or a representative sub-sample of larger tar particles. The tar samples, in the centrifuge tubes, were brought to room temperature (20-25°C) and weighed to give the wet weight, then dried to constant weight in a vacuum oven (60 mm Hg total pressure) at 40°C to give the dry weight. The dried tar samples were extracted by adding 5 ml of methanol and 10 ml of benzene to each centrifuge tube, flushing with nitrogen, sealing with a Teflon-lined screw cap, and heating in a boiling water bath for 30 minutes, shaking every 10 minutes. The centrifuge tube was cooled, 8 ml of distilled water was added and the tube shaken. The aqueous-methanolic phase and benzene phase were separated by centrifugation and the latter removed and saved. The aqueous-methanolic phase was extracted twice more with 10 ml portions of benzene. The combined benzene fractions were placed in a 50 ml volumetric flask and brought to volume with benzene. These tar extracts were then stored at room temperature in amber glass bottles with Teflon-lined screw caps.

Aliquots (1 ml) of each pelagic tar extract, after addition of 15 to 30 μg n-docosane ($n\text{-}C_{22}$) internal standard, were analyzed for total hydrocarbons using thin layer chromatography (TLC) and gas liquid chromatography (GLC) procedures as described elsewhere.[8,9] Procedural hydrocarbon blanks were less than 1% of the sample values,

thus eliminating the need for correction. Aliquots (30 ml) of the extracts were also dried to constant weight to obtain the benzene extract weight.

The infrared (IR) spectrum of each extract was measured after removing the benzene. One drop of solution was placed on a miniature KBr window and, after the benzene evaporated, a second drop was added. The process was continued until a film (∿1 mg of residue) covered the window. Spectra were measured on a Perkin-Elmer Model 521 spectrometer using a 6X beam condenser.

Water samples were also collected on R/V TRIDENT cruise 123 as well as two other cruises to the Sargasso Sea in 1973. As in the case of the pelagic tar, the water samples were collected from a rubber raft and consisted of surface microlayer samples (top 100-300 μm of the ocean surface) and subsurface samples (20-30 cm below the surface). After addition of internal standard ($n\text{-}C_{22}$), the unfiltered samples were extracted with chloroform or methylene chloride. The total hydrocarbons in the extract were isolated by TLC and analyzed by GLC and IR spectrometry. Complete details of the collection, storage and analytical procedures have been reported by Wade and Quinn.[7]

RESULTS AND DISCUSSION

Pelagic Tar

The individual weight percentages based on the wet weight of tar as well as the average values, which will be used to compare the different samples, are given in Table 2. The pelagic tar samples averaged 32% water (range of 11 to 44%) and 68% dry weight material. Butler et al.[2] found that tar lumps from the Atlantic Ocean typically contain about 25% water by weight (range of trace to 36%). (Table 2)

An average of 53% of the wet tar was soluble in the benzene extract (range of 31 to 89%) and this material accounted for 78% of the average dry weight of the samples. Jeffrey et al.[4] reported an average of 63% of the dry weight of tar was soluble in benzene. The benzene insoluble fraction of tar may include inorganic salts, non-organic debris and high molecular weight organic material.

The wet tar samples averaged about 16% total hydrocarbon material as determined by our chromatographic procedures (TLC and GLC) and this fraction was 24% of the dry weight and 31% of the benzene extract weight. The remaining fraction of the extract may include non-hydrocarbon organic material or hydrocarbons not measured by our procedures (e.g. $<n\text{-}C_{14}$ and $>n\text{-}C_{38}$). For example, Jeffrey et al.[4] found their pelagic tar samples contained an average of 26% asphaltenes based on the dry weight of tar.

The hydrocarbon weight % determined by GLC is divided into resolved and unresolved components. The latter components (measured as a broad envelope) average 79% (range of 67 to 97%) of the total hydrocarbons and include a complex mixture of aromatic and cyclo-paraffinic components. The resolved components (peaks) account for an average of 21% (range of 3 to 33%) of the total hydrocarbon weight and are predominantly n-paraffins with small amounts of branched paraffins and isoprenoid hydrocarbons. The gas chromatograms of three of the tar samples (Nos. 1, 4, and 7) are shown in Figure 1 (A, B, and C respectively). Chromatogram A shows a small percentage of peaks (∿3%) and is representative of samples 1, 2, 6 and 8 with a range of 3 to 8% resolved components. Chromatogram B (sample 4) indicates a large percentage of peaks (∿33%) and is unique in its hydrocarbon distribution. Chromatogram C shows an intermediate percentage of resolved components (∿24%) and is representative of samples 3, 5 and 7 with a range of 24 to 33% resolved components. These variations in the amount of resolved and unresolved components are probably due to different source materials, ages, and weathering histories of the tar samples as will be discussed later. (Figure 1)

The sample hydrocarbons had a boiling range (Apiezon L column) from n-C_{14} to n-C_{38} with most samples having their major fraction between n-C_{14} and n-C_{35}. The presence of pristane and phytane in several samples (e.g. chromatogram C) was indicated by coinjection with authentic compounds on both polar (FFAP) and non-polar (Apiezon L) columns. These isoprenoid hydrocarbons are more resistant to biological weathering than the n-paraffins.[2] In one sample, the presence of small amounts of lower molecular weight n-paraffins (e.g. n-C_{14} to n-C_{20}) was also detected (e.g. chromatogram B). The ratio of odd to even chain n-paraffins for several samples (e.g. chromatograms B and C) was approximately 1.0. A reliable correction for the natural level of n-C_{22} present in the samples was made by subtracting one-half of the combined peak areas of n-C_{21} and n-C_{23} from the peak area of n-C_{22}.[10] This technique gave results in good agreement with the measurement of natural n-C_{22} in samples by analysis with n-C_{20} internal standard.

GLC analyses of the benzene extracts of tar before and after TLC, showed that the latter procedure does not significantly affect the qualitative or quantitative distribution of hydrocarbons in the extracts. In addition, experiments in our laboratory[10] showed that of the measured weight, approximately 40% of a #6 fuel oil and 30% of API bunker "C" fuel oil[11] were analyzed by the GLC methods used in this study. (TLC isolation procedures did not affect the analyses of these fuel oils.) Recovery of API #2 fuel oil[11] ranged from 70-87% (using TLC) and approximately 95% by direct GLC analysis without TLC. These findings indicate that #6 and bunker "C" fuel oils contain polar or high molecular weight compounds (>n-C_{38}) not analyzed by our GLC methods, while #2 fuel oil contains low boiling compounds (<n-C_{14}) that are lost during the analytical procedures (e.g. TLC and evaporation of solvents). Thus, it is important to note that GLC analyses do not measure all of the hydrocarbons present in some oil products.

IR spectra of petroleum can be used to provide information on the n-paraffin and aromatic content of oils.[12,13] A band at ∿720 cm^{-1} is due to n-paraffins, whereas a band at ∿1600 cm^{-1} is due to aromatic hydrocarbons. In addition, bands at 740, 810, and 870 cm^{-1} are also indicative of aromatics; the first is due to 4 adjacent hydrogens on a ring, the second to 2 adjacent hydrogens, and the third to isolated hydrogens. These latter three bands are characteristic of petroleum, and their relative ratios have been used to identify this material.[14,15]

The IR spectra of the benzene extracts showed that all, except samples 3 and 5, contained aromatic hydrocarbons. Spectra of representative samples (1, 4, and 7) are shown in Figure 2 (A, B and C respectively). Except for the carbonyl band at ∿1700 cm^{-1}, spectra A and B are similar to crude oils found throughout the world. The low aromatic and high paraffin content of sample 7 is found only in a few crude oils. (From infrared spectra of 120 samples of crude oil, we have found only one similar to this sample.)[16] (Figure 2)

Comparison of the chromatograms in Figure 1 with the spectra in Figure 2 indicates that GLC and IR analyses give similar trends in the amount of n-paraffins present in the three samples. In sample 1 (A) both methods indicate a very small amount of n-paraffins, i.e., the resolved peaks in the chromatogram are very small and the 720 cm^{-1} band in the spectrum is very weak. On the other hand, both the chromatograms and the spectra of the other two samples indicate much larger amounts of n-paraffins.

As previously indicated, the major source of pelagic tar in the Sargasso Sea is probably the operational discharge of crude oil sludge by tankers.[2-6] Our GLC (Figure 1) and IR (Figure 2) analyses of this tar indicate three distinct hydrocarbon distribution patterns. Different source materials, ages, and weathering histories of tar probably account for these variations. For example, the GLC distribution of hydrocarbons shown in Figure 1B may be that of a relatively new sample of tar which is rich in paraffinic wax. Under certain conditions, this material may be weathered to produce the distribution seen in Figure 1A which results from a loss of n-paraffins and aromatic hydrocarbons as well as an increase in carbonyl compounds (Figures 2B and 2A). The hydrocarbon distribution shown in Figure 1C may result from preferential microbial degradation of lower molecular weight n-paraffins in a paraffin rich tar containing small amounts of aromatics.

Surface Waters

The results of our analyses of unfiltered water samples have been reported in a previous publication.[7] We found the total hydrocarbon concentration of the surface microlayer samples ranged from 14 to 559 µg/l with an average value of 155 µg/l. The corresponding values

for the subsurface samples were 13 to 239 µg/l with an average of 73 µg/l. In twelve out of seventeen sample pairs, the surface microlayer had a higher concentration than the subsurface. Large tar lumps were observed at many of our stations but only the eight previously described samples were collected for analysis. Therefore, we have no information on the relationship of these lumps to the hydrocarbon concentration in the water samples. In general, the higher hydrocarbon values were found in water samples taken close to Bermuda.

The combined water samples contained an average of 11% resolved hydrocarbon components with a range of 3 to 21%. A representative chromatogram of these samples is shown in Figure 3. A comparison of this chromatogram and those in Figure 1 indicates that the distribution of hydrocarbons in the water samples is somewhat similar to that in tar samples 1, 2, 6 and 8 (Figure 1A) in terms of the boiling range, ($n\text{-}C_{14}$ to $n\text{-}C_{32}$), and the small amount of resolved components (e.g. n-paraffins). Analyses of these hydrocarbons by IR also showed relatively small quantities of n-paraffins and aromatics. Thus, our measurements indicate that a substantial amount of these hydrocarbons are cycloparaffins and are in agreement with the findings of Brown and Huffman.[17] Based on the available data and present criteria,[6] we suggest that most of the hydrocarbons in our water samples have a petroleum origin.

(Figure 3)

We also filtered a few water samples through a Gelman A glass fiber filter (0.3 µm particle size retention) and observed small black particles on the filters.[7] Analyses of the filters and filtrates showed that almost all of the hydrocarbons were retained on the filters. In order to provide additional information on the source of these particles, we added large (>1 cm) pelagic tar lumps to filtered Narragansett Bay water and shook this mixture at room temperature over a period of 4 weeks. We then filtered the mixture through a 1.0 mm mesh screen and found that the larger size particles (>1.0 mm) still retained the basic GLC features of the original tar (Figure 4A). The smaller particles (<1.0 mm) contained mostly unresolved hydrocarbon components (Figure 4B) and in this regard were similar to the water samples.

(Figure 4)

CONCLUSIONS

Small particles of tar were produced by the artificial weathering of large pieces of tar in our laboratory. We have also seen similar black "tar-like" particles on the filters (0.3 µm) used to filter some of our water samples collected with a 1.0 mm mesh screen. The hydrocarbon distribution in the artificially produced particles was somewhat similar to that of the unfiltered water samples as well as the "tar-like" material on the above filters. Based on this information, we suggest that a major source of hydrocarbons (mostly

cycloparaffins) in our unfiltered Sargasso Sea water samples are particles of weathered pelagic tar in the size range of <1.0 mm down to 0.3 μm in diameter.[7] These particles are probably formed by biological, chemical and physical weathering of large tar lump surfaces. We also suggest that previous estimates[2] of pelagic tar may be very low because particles less than 300 μm diameter were not included. The recent work of Morris et al.[18] supports this hypothesis since they found "tar-like" particles (10 to 500 μm dia.) in the Sargasso Sea and estimated their total mass in the water column to 100 m is about four times the standing crop of larger pelagic tar lumps at the ocean surface. It is clear from both of these studies that additional information on the distribution, fate and effects of small pelagic tar particles is needed to properly assess their impact on the marine environment.

ACKNOWLEDGEMENTS

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DISCUSSION

GORDON: I certainly agree with the concern expressed over the sampling procedures. In regard to sampling, though, I was wondering whether there might be any chances of picking up hydrocarbons from the atmosphere during sampling if it has taken several hours to obtain 16 liters of water. During that time I imagine quite a few hundred liters of air are passing through your sampling screen.

QUINN: We had a chance to look at atmospheric hydrocarbons on some of the cruises, and the boiling point distribution of the hydrocarbons from the atmospheric samples is quite a bit different from that of the water samples. Secondly, based upon other people's work, in two hours we would not be collecting very many hydrocarbons on our screen. The diameter of the screen is 1 millimeter. Most of the hydrocarbons are in the gaseous phase, so they would pass through the screen, not be trapped on it.

LARSON: I noticed in the infrared spectra of some of your tar samples a fairly strong carbonyl band. Did you attempt to characterize these carbonyl compounds at all?

QUINN: The tar extracts that we used for the infrared were not separated on thin layer chromatography to isolate the compounds. This band was in infrared spectra of the entire tar extract. It is quite common to see carbonyl-type compounds in these extracts because of various weathering processes.

TABLE 1

Sampling Locations for Collection of Pelagic Tar on R/V Trident Cruise 123.

Sample No.	Date Collected	Position (N-W)
1	9/13/72	$34^{o}29'-35^{o}39'$
2	9/26/72	$31^{o}46'-64^{o}50'$
3	9/23/72	$31^{o}48'-62^{o}44'$
4	9/25/72	$31^{o}46'-64^{o}50'$
5	9/21/72	$32^{o}06'-55^{o}02'$
6	9/19/72	$32^{o}24'-46^{o}53'$
7	9/19/72	$32^{o}24'-46^{o}53'$
8	9/22/72	$31^{o}55'-59^{o}18'$

TABLE 2

Weight Percentages Based on Wet Weight on Pelagic Tar.

Sample No.	Dry Weight (%)	Benzene Extract Weight (%)	Hydrocarbon Weight (%) Resolved Components	Unresolved Components	Total
1	69	56	0.4	12.1	12.5
2	58	51	0.6	9.5	10.1
3	56	56	5.0	15.9	20.9
4	89	89	8.9	17.9	26.8
5	78	45	8.8	17.3	26.1
6	62	33	0.9	10.1	11.0
7	59	31	3.0	9.7	12.7
8	76	59	0.6	11.4	12.0
Average	68	53	3.5	13.0	16.5

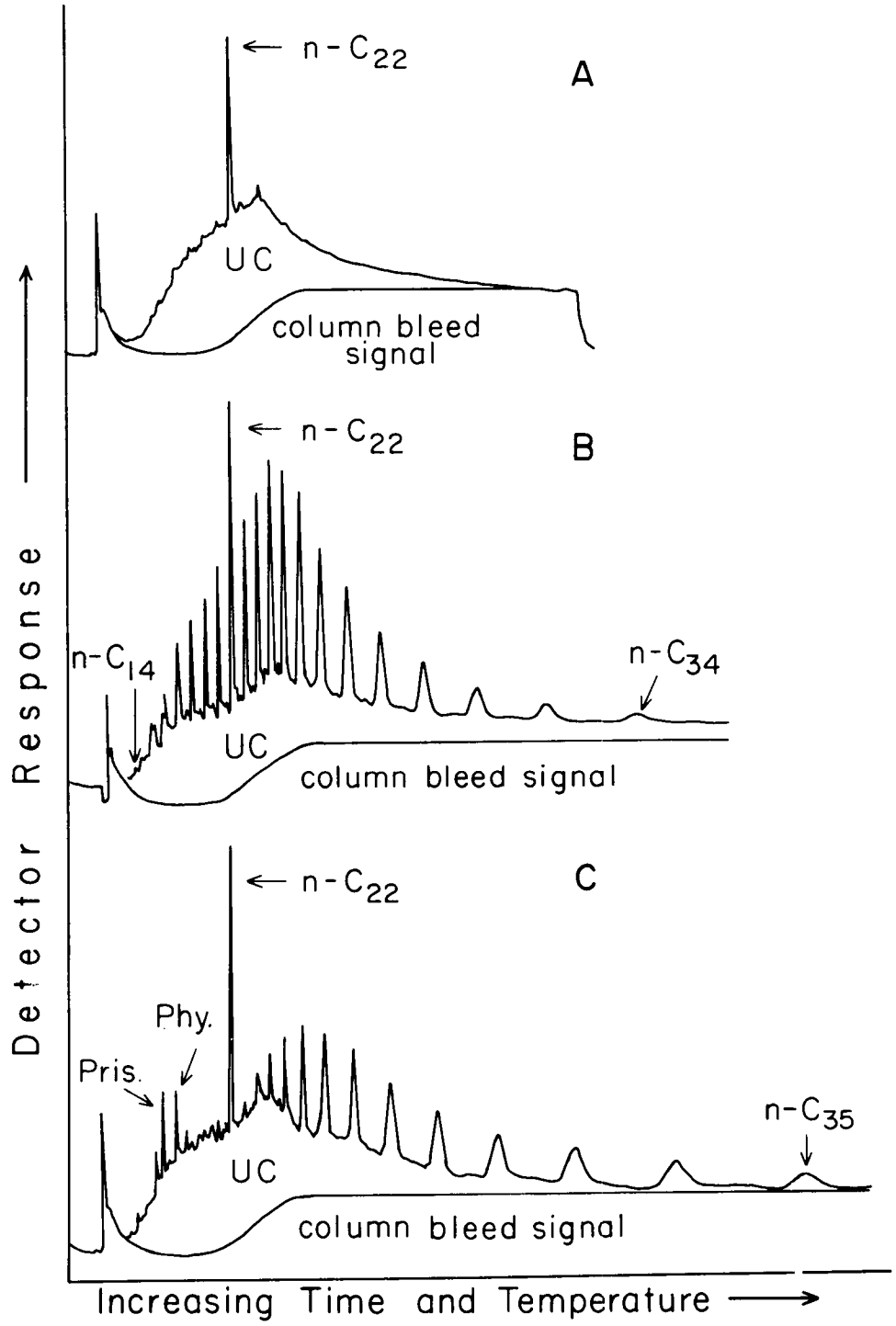

Figure 1. Gas chromatograms of hydrocarbons in three pelagic tar samples.
A: sample 1, B: sample 4, C: sample 7.
$n\text{-}C_{22}$ - n-docosane internal standard; UC- unresolved components, $n\text{-}C_{14}$, $n\text{-}C_{34}$, $n\text{-}C_{35}$ - resolved n-paraffins noting carbon number; Pris - pristane; Phy - phytane. The GLC column was 1.8 m x 2.2 mm i.d. stainless steel containing 3% Apiezon L on Chromosorb W (HP), 80/100 mesh. It was programmed from 150°C to 280°C at 6°C/min with a N_2 carrier gas flow of 10 ml/min and held at 280°C until $n\text{-}C_{38}$ eluted.

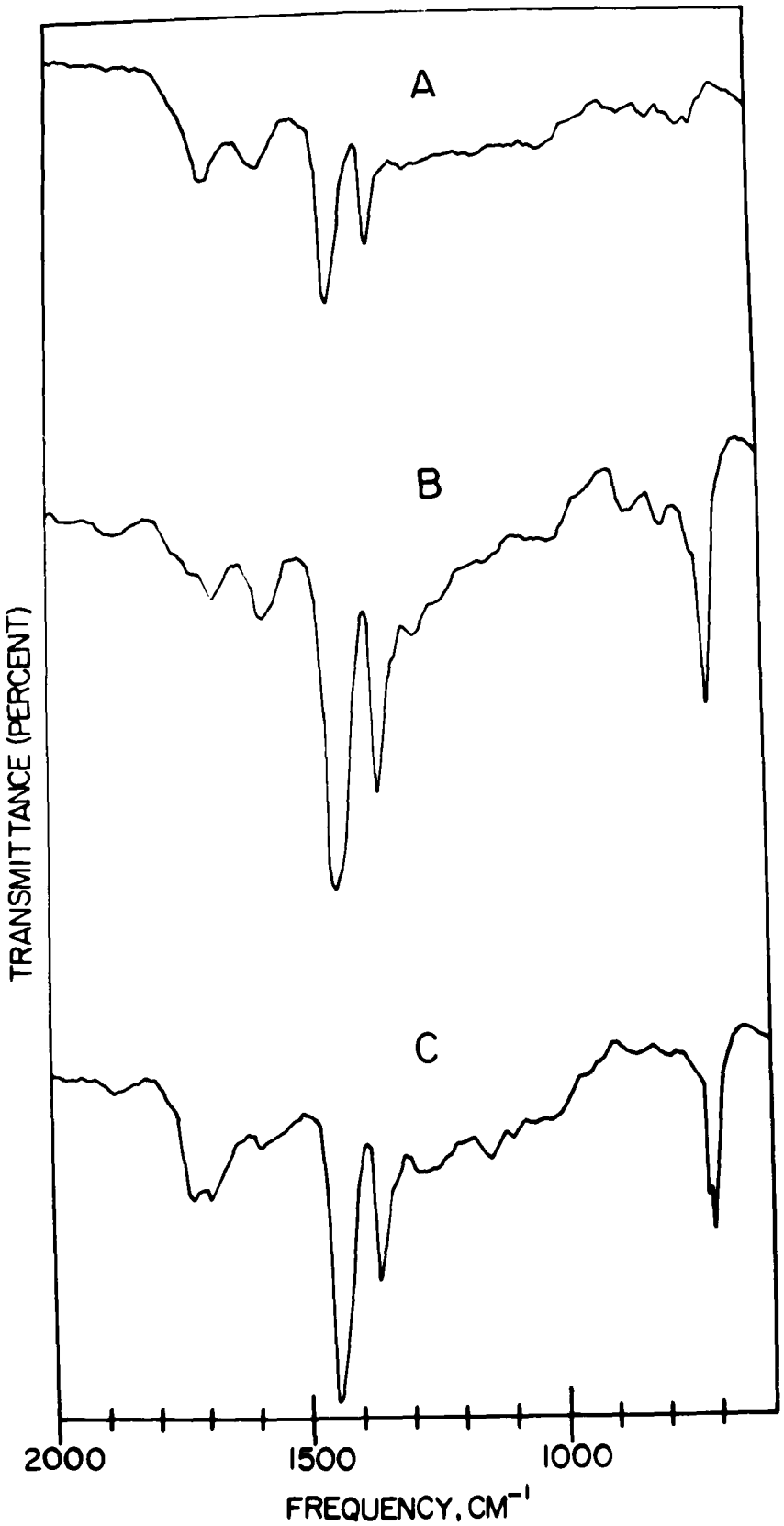

Figure 2. Infrared spectra of benzene extracts from three pelagic tar samples.
A: sample 1, B: sample 4, C: sample 7.

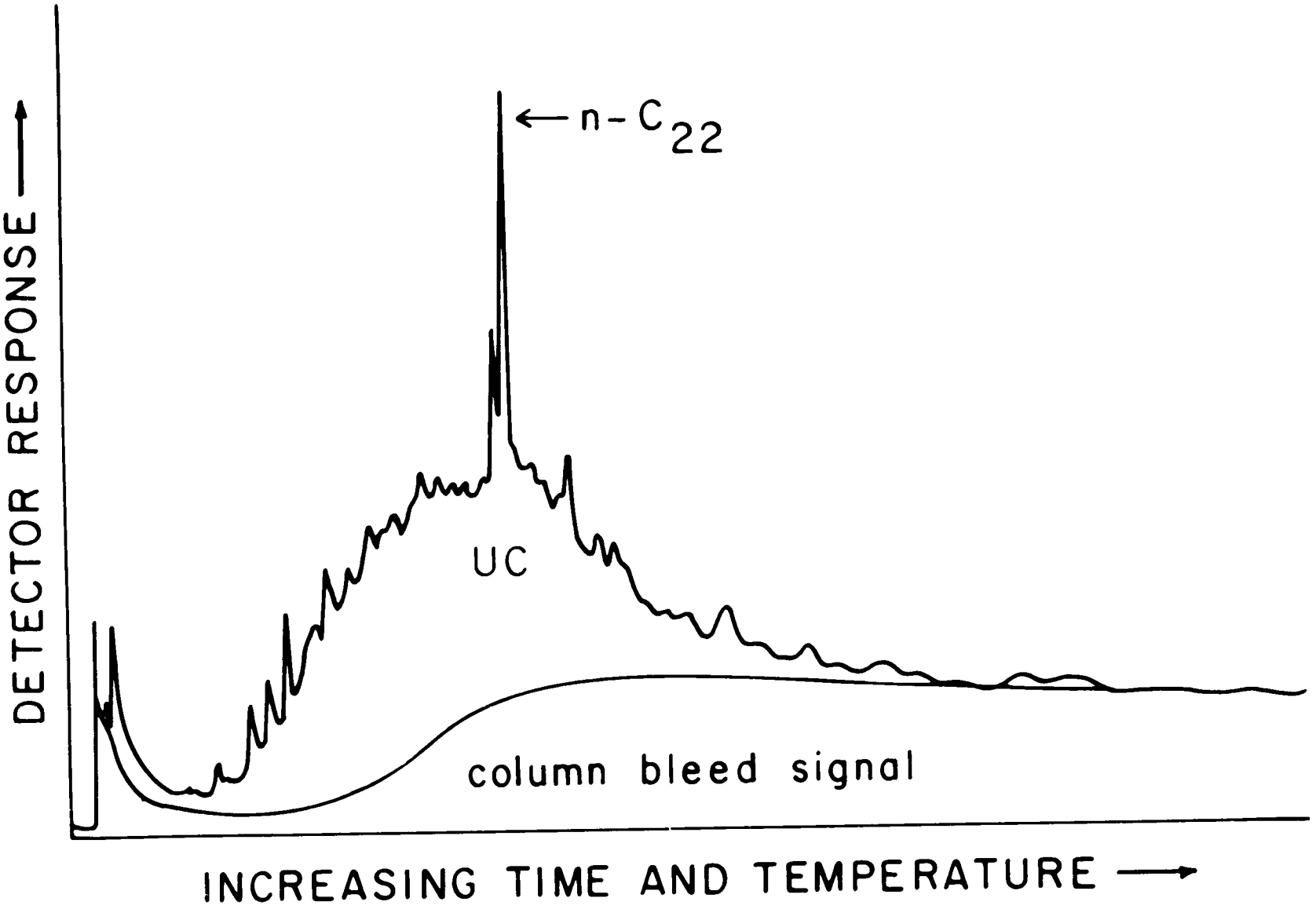

Figure 3. Gas chromatogram of hydrocarbons in a subsurface water sample. The GLC column was 1.8 m x 2.2 mm i.d. stainless steel containing 12% FFAP on Chromosorb W (HP), 80/100 mesh. It was programmed from 150°C to 250°C at 8°C/min with a N_2 carrier gas flow of 15 ml/min and held at 250°C until n-C_{38} eluted.

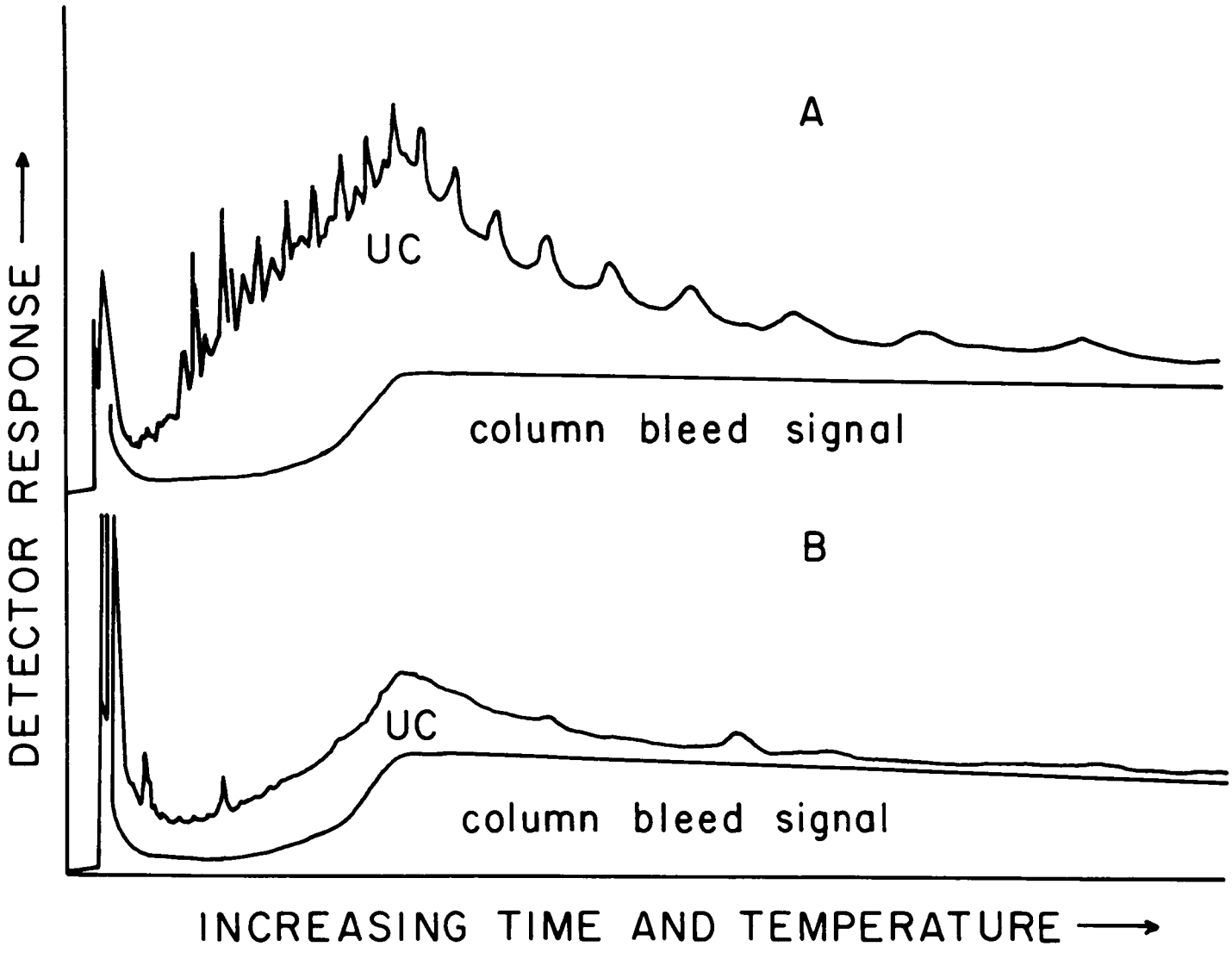

Figure 4. Gas chromatograms of hydrocarbons from pelagic tar samples used in a laboratory experiment.
A: >1.0 mm dia. particles;
B: <1.0 mm dia. particles;
GLC conditions were the same as described in Fig. 3.

THE FATE OF PETROLEUM IN THE OPEN OCEAN

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THE FATE OF PETROLEUM IN THE OPEN OCEAN

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Petroleum spilled at sea, by operational discharges or by accident, is dispersed and degraded. Evaporation and dispersion in the water are most rapid, but do not remove petroleum compounds from the environment. Degradation occurs by photochemical oxidation and biological processes. Physicochemical dispersion processes can be represented by quantitative models, but degradative processes are poorly understood under field conditions. The ultimate fate of petroleum at sea is not known with any certainty at present. Amounts comparable to the residue from many years' spillage are found in pelagic tar, fine particles dispersed in the upper water column, and in abyssal surface sediments.

INTRODUCTION

In view of the relatively large amount of oil spilled in the world oceans (several million tons per year[1]) and its potential effects on living organisms and aesthetic values, it is important to assess its fate; to determine how long it can be expected to last, and what forms it may eventually take. We have studied some aspects of this problem on the open ocean, where the processes might be expected to be simpler than on shorelines, estuaries, embayments, or inland waters.

Petroleum enters the open oceans primarily as operational discharge of ballast by tankers which do not use the load-on-top system.[1] A smaller amount is the result of bilges from other vessels, or accidents, although the latter may receive much wider notice because of their

spectacular character. Thus we are concerned primarily with spills of crude oil and crude oil sludge, and much less with refined products such as diesel fuels or gasoline.

DISPERSIVE PROCESSES

Evaporation and dispersion in solution are the most rapid processes, decreasing in rate as time proceeds and the most easily volatile or soluble materials are removed from the spilled oil. Agitation by surface waves increases the rates. Dispersion (distinct from true solution) is greatly increased in the presence of surface-active agents, whether these are deliberately added detergents or natural compounds found in the water.

Evaporation removes the most volatile (lowest molecular weight) compounds: pentadecane ($C_{15}H_{32}$) is the lowest normal alkane commonly found in weathered oils[2,3,4] and it is rare to find hydrocarbons lower than C_{12} in seawater extracts. These more volatile fractions compose 20% to 50% of most crude oils, 75% or more of refined petroleum fuel, and 10% or less of residual oils such as Bunker C. A simple mathematical model of evaporation based on vapor pressure data and one adjustable parameter[5] gives a quantitative description of the evaporation of crude oils.

Non-polar hydrocarbons (such as alkanes) are essentially insoluble except for the lightest members of the series; the compounds which preferentially dissolve are the more polar, usually aromatic or heterocyclic, ones. Even with dispersion in the water column included, this process removes less than 15% of a crude.[6,7,8]

Other dispersive processes include sorption of hydrocarbons on surfaces, such as sediment or phytoplankton[9,10] and subsequent sedimentation. Slicks can be transferred into the atmosphere as aerosols as well as by evaporation, leading to a preferential loss of surface active compounds. However, the residence time in the atmosphere is probably short[11], and aerosol formation ultimately leads to redeposition on the ocean surface in another place.

DEGRADATIVE PROCESSES

None of the above processes actually <u>removes</u> petroleum compounds from the environment, but only redistributes them. The ultimate fate of environmentally dispersed

petroleum is poorly understood. In shallow coastal waters, burial in sediments and metabolism by organisms plays a major role, but in the open ocean there is no opportunity for a slick or emulsion or tar lump to come in contact with sediments except by sinking to abyssal depths; and contact with organisms, even microbes, is relatively rare. The processes of biodegradation are poorly understood compared to the physicochemical processes of dispersion, and even qualitative predictions are difficult without good field data.

Photochemical oxidation requires ultraviolet light and hence is limited to the ocean surface or a few centimeters beneath; for a compound to be photolyzed, it must absorb ultraviolet light, and thus those compounds with a high extinction coefficient (aromatic, heterocyclic) tend to be more easily oxidized than alkanes.[12] The initial products (acids, alcohols, ketones, aldehydes) are more easily metabolized by microorganisms or macroscopic plankton than are the hydrocarbons themselves, and thus degradation by biological processes may be aided by photolysis. In some cases, however, photolysis can produce polymers which are more resistant to degradation than the original material.[13,14]

Microbial oxidation has been so often demonstrated in the laboratory that it is sometimes assumed to be well understood in the field; but there are very few studies under open ocean conditions.

Some laboratory studies[15,16] show that microbial degradation starts preferentially on lower molecular weight compounds and proceeds to higher molecular weights. With a mixed culture, low molecular weight alkanes and aromatics degrade first. Higher molecular weight alkanes then become degraded. Some microbial species specifically attack certain aromatic and naphthenic compounds[18], particularly those with side chains. Asphaltenes are resistant and may persist for years.[19,20,21] Estimates of degradation rates under natural conditions are 1 to 10 $mg/m^3 day$, a factor of 100 lower than obtainable in the laboratory.[21,22] Indeed, there is some question whether hydrocarbon-utilizing bacteria are concentrated enough even to maintain these rates. Open ocean water off Hawaii failed to yield cultures in 75% of the water samples.[23]

FATE OF RESIDUES

The oceans, particularly the Mediterranean and North Atlantic, have been well documented with respect to the presence of pelagic tar[4,9,24] which is deduced (from its high iron content and highly paraffinic gas chromatogram) to be the residue of crude oil and crude oil sludge. Estimates of the lifetime of this tar have been made[25] on the basis of a mass balance to be of the order of several months to a year. However, this is consistent with the evaporative weathering model (ratios of paraffins in the range C_{15}-C_{20}) only if the diffusion rates are smaller than expected[26] or if the lumps collected are but fragments of the original lumps from which weathering began.[5] Both of these hypotheses are probably applicable, but complicate the quantitative dating of tar lumps.

A further complication is the disintegration of tar lumps to give tiny (1 μm to 1 mm) fragments with chromatograms similar to those of tar lumps, but which are dispersed to more than 100 m depth in the water column.[25] The amount found in the Sargasso Sea near Bermuda is about four times the pelagic tar on the surface above the same water column. Distinction of these fine particles from dissolved or emulsified hydrocarbons has not always been made because of the large water samples used for extraction and the possibility of contamination during filtration.

Whether the ultimate fate of petroleum residues in the open ocean is biodegradation or sedimentation remains to be determined. Sedimentation can be biologically aided: if copepods ingest tar particles and these particles are incorporated in fecal pellets, their sedimentation rate can be greatly increased. Certainly tar lumps which acquire an excessive load of barnacles could become heavy enough to sink under their own weight. On the other hand, the few sediment samples obtained from the top 5 cm of the deep ocean floor[25,27] show hydrocarbon distributions which appear to be a mixture of biogenic and petroleum hydrocarbons[25]. Their concentration (of the order of 1 μg/g or 50 mg/m^2) is comparable to that found near the surface of the ocean (10 mg/m^2 pelagic tar, 40 mg/m^2 particles in the water column). However, because of the vagaries of this preliminary sampling, it is not clear how deeply buried the material analyzed actually was, or what the ratio of petroleum to biogenic hydrocarbons might be.

Pelagic tar thus appears to be about one year's worth of residues, the upper 100 m of water contains several

years' worth of residues, as do the surface sediments. Since major inputs to the Sargasso Sea date from the change in tanker routes in 1967, this standing stock of petroleum residues is comparable to what might be expected if little further degradation took place.

CONCLUSION

It is clear that the dispersal of spilled petroleum is relatively rapid at sea, and except for floating residues (tar lumps) the resulting material is not easily detected. Table 1 summarizes the pathways for the environmental fate of a typical crude oil at sea.[28] The long-term degradation processes may be biological; this is most likely if the petroleum hydrocarbons are highly dispersed. However, the involvement of living organisms in such a large scale chemical process (5×10^{10} g/year in the Sargasso Sea) will almost certainly modify the ecosystem in a direction which favors hydrocarbon-tolerant and hydrocarbon-utilizing species. Unfortunately the requisite ecological studies are so difficult and time-consuming that it will be a long time before we know precisely what this impact is. Ironically, by that time petroleum will probably have become so expensive that its deliberate discharge into the sea will have become a thing of the past.

ACKNOWLEDGEMENTS

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Table 1

PATHWAYS FOR THE ENVIRONMENTAL FATE OF CRUDE OIL[28]

Pathway	time scale (days)	%
Evaporation	1 - 10	25
Solution	1 - 10	5
Photochemical	10 - 100	5
Microbial	50 - 500	30
Disintegration & sinking	100 - 1000	15
Residue	> 100	20
		100

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Note to References 9, 10, 12, 21, 22, 25, 26: These were presented at a Workshop on Petroleum Hydrocarbons in the Marine Environment, held Sept. 1975 at the Marine Laboratory, Aberdeen Scotland. The proceedings will be published in 1976 as a volume of Journal du Conseil by the International Council for the Exploration of the Sea.

DISCUSSION

PUGH: Jim, in your studies you talked about finding tar in the gut of some of the Sargassum community fishes. In your studies, did you look to see whether any of this tar had been assimilated into the tissues?

BUTLER: We tried to look at some of the animals just from the tissue point of view but I don't think we made a clear distinction. I wasn't actually doing any of the dissecting, so I am not sure exactly how clear a distinction was made between the gut contents and the tissue contents of these little animals.

To do the work precisely, one would have to dissect every animal. And I think the labor involved in that is so great that nobody really wanted to sit down and do it. So we have not made a clear distinction there. We can infer it, but I don't think that even the animals which were clean are necessarily only tissues. You have to work with much larger animals to do that effectively.

PARKER: Those zones of inhibition that you showed with the tar lumps on the blue-green mats, are they very abundant?

BUTLER: Those rings around the tar patches you find on the higher dry zones of the beach are wet only during storms. As soon as you move down toward the water you come through a zone where the algae and the tar seem to be in balance. Then you get to a lower zone where that yellow-green algae grows over the tar lumps and actually obscures them.

SPEAKER: That slide you showed looked like it had a ring around it.

BUTLER: The first one?

SPEAKER: The yellow-green one.

BUTLER: The yellow-green looked like it had a ring around it? Actually, maybe it wasn't such a good picture. As I remember it, it was actually sort of like the mat was growing over it and obscuring it. We had a little, small patch of tar which was visible. If you peeled the mat back, you would see tar under the mat for a way, and then it would be rock.

I am sure it has some effect on the algae. There certainly are places where the rock is just completely covered with tar and nothing can grow.

LEINONEN: I would just like to know if you can remember an item from the differential equation from your model. Can you remember a number for k?

BUTLER: It was 10 in whatever units it was.

LEINONEN: And was X, I assume, a small fraction?

BUTLER: X over X-naught would be a fraction.

LEINONEN: What are the units of X-naught?

BUTLER: It doesn't make any difference because you are measuring a ratio of the weather component to the amount which was originally present. It is the model that assumes that all components weather independently of whatever else there is, which is surely a very bad approximation.

LEINONEN: Is that reasonable?

BUTLER: It seems to work.

GORDON: I am intrigued with your observation on the tar particles in sea water and wondered if you could give a little bit more information on the concentrations in the deeper water. As you know, the work I have done in deeper water, where we are using fluorescence as an indicator, indicates, that the concentrations in the deeper water are certainly less than a microgram per liter.

It also would be interesting to compare these concentrations to the total particulate organocarbon, which out in that area is in the upper 100 meters, as on the order of, maybe, 15 to 25 picograms of carbon per liter.

BUTLER: The greatest number of particles, averaging 6.4 per liter, and the greatest weight, 1.0 micrograms per liter, occurred at the 50 meter depth. This is near the bottom of the seasonal thermocline.

Just below the surface, where one might expect a large number of particles from disintegration of surface tar lumps, only small particles (less than 140 microns) were found, and the lowest concentration by weight, 0.03 micrograms per liter, also occurred. So they are small compared to the fully extracted water samples.

TOXIC HYDROPEROXIDES: PHOTOCHEMICAL FORMATION FROM PETROLEUM CONSTITUENTS

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ABSTRACT

Hydroperoxides were the principal oxygenated species formed upon short-term long wavelength ultraviolet irradiation of a #2 fuel oil. These substances were produced from benzylic hydrocarbons, were relatively water-soluble, and suppressed the growth of yeast cultures at low (ca. 10^{-4} M) concentrations. Some of their breakdown products (carbonyl compounds, acids, and phenols) were also growth inhibitors.

INTRODUCTION

Although spills of crude oils and other petroleum products have been matters of serious public concern at least since the Torrey Canyon spill of 1967, the behavior of petroleum films under environmental conditions is still not thoroughly understood. Because oil products vary widely in their chemical composition and physical properties, they respond differently to biological processes and the action of wind, waves, and sunlight. Some petroleum constituents, especially n-alkanes, are readily degraded by microbial species, but little is known of the environmental fates of aromatic, alicyclic, and heteroatom-containing compounds.

A few studies of photochemical transformations of some oils have been reported. Among the products are organic acids[1,4], esters[1], carbon dioxide[1], oxygenated aromatics[2], sulfoxides[3], and carbonyl compounds.[5]

Very recently it has been shown that some oil products, after irradiation, become increasingly toxic to algae[6], marine

invertebrates, and fish.[7] We report here studies of a #2 fuel oil and some of its hydrocarbon constituents which help to elucidate the chemical basis of this toxicity.

EXPERIMENTAL

Irradiation conditions: An Ace-Hanovia 200-W UV lamp equipped with a tubular Pyrex filter was used. Intensity was adjusted to simulate natural summer sunlight at 40° latitude by using a Blak-Ray long-wave UV meter calibrated to an Eppley pyrheliometer.

Hydroperoxide percursors: A #2 fuel oil was purchased from a local distributor. Individual compounds were obtained from Aldrich and recrystallized.

Hydroperoxide analysis: Cumene hydroperoxide was pruchased from Mc/B, t-butyl hydroperoxide from Aldrich. Hydrogen peroxide was J. T. Baker "30%" (actual concentration, established iodometrically, 26.3%). Tetralin hydroperoxide was synthesized by air oxidation of tetralin.[8] Quantitative hydroperoxide analysis was by an iodometric method.[9] Thin-layer chromatographic (TLC) behavior was monitored on silica gel, using 1% KI or N,N-dimethyl-p-phenylenediamine as detection reagents.

Partition experiments: Organic hydroperoxides (approx. 0.4 mmole) were dissolved in 50 ml 2:1 (v:v) hexane:benzene in a separatory funnel and shaken thoroughly with an equal volume of glass-distilled water. After allowing the funnel to stand for 90 min., portions of the water and organic layers were removed for quantitative hydroperoxide analysis.

Growth inhibition studies: Samples (0.25 ml of oil or 2.5 micromoles of individual compounds) were added to 25-ml cultures of baker's yeast (Saccharomyces cerevisiae) immediately after inoculation of the medium with ca. 1×10^6 log phase cells. Growth was measured turbidimetrically at 600 nm, and expressed as a percentage relative to untreated control cultures.

The medium consisted of 10 g glucose, 3 g $(NH_4)_2 SO_4$, 3 g $KH_2 PO_4$, 250 mg $MgSO_4$, 250 mg $CaCl_2$, 10 mg inositol, 2.2 ug biotin, 1.0 mg calcium pantothenate, 1.0 mg pyridoxine HCl and 1.0 mg thiamine HCl in 1:1 distilled water.

RESULTS AND DISCUSSION

Fuel oils are distillates which contain little or no low-boiling material. They are usually reduced in sulfur (0.1-0.7%) relative to crude oils (up to 5%, or rarely higher), but are enriched in aromatics (including two-, three-, and four-ring compounds) and phenols.

Irradiation of a #2 fuel oil containing 23% aromatics and 600 ppm phenols led to a virtually linear (r=0.980) increase in peroxide content. There was no sign of an initial induction period. Regression analysis indicated an apparent rate constant of 2.09×10^{-7} l/mole sec. Growth-inhibitory activity also increased, with an especially dramatic rise between 15 and 24 hr. of irradiation (Fig. 1). Other classes of oxygenated products (phenols, carbonyl compounds, acids) also increased, but after 96 hr. irradiation, their molar concentrations were much lower than those of peroxides. No significant changes were noted in control #2 fuel oil samples not exposed to light.

Thin-layer chromatography (Fig. 2) showed that the peroxides formed were relatively nonpolar. Hydrogen peroxide was not detectable. Their high reactivity toward aqueous KI indicated that they were probably hydroperoxides.[11] Such compounds are known to be important in thermally-induced autoxidations. To assess whether they could be produced photochemically,eight

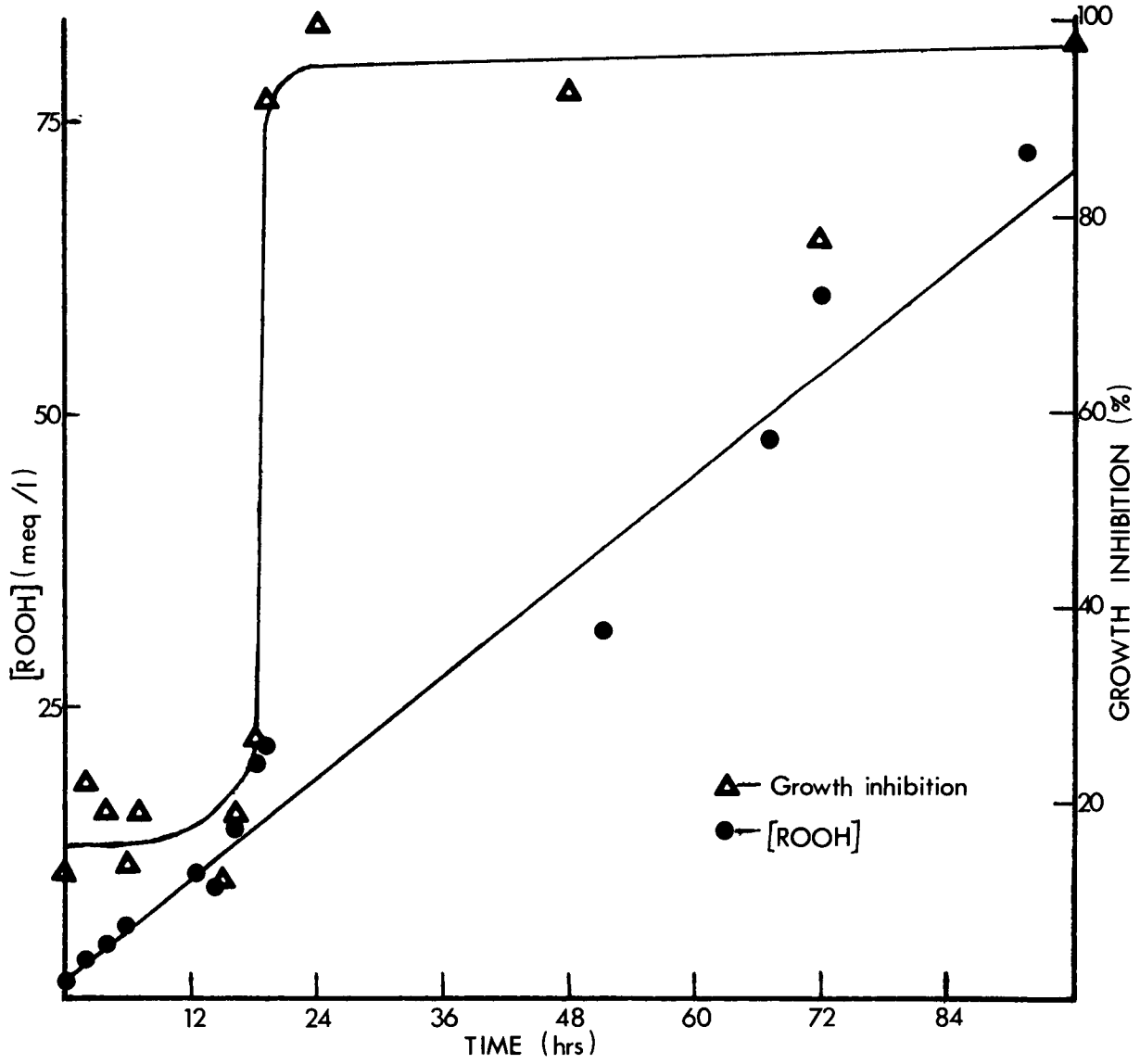

Fig. 1 Hydroperoxide formation and production of substances inhibitory to yeast growth by #2 fuel oil irradiation

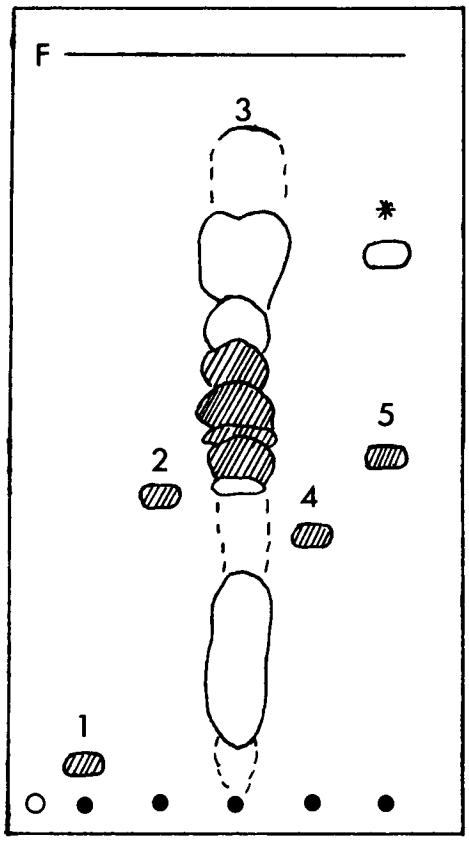

Fig. 2. Peroxides in fuel oil and standard hydroperoxides. Silica TLC, developed successively with hexane and 5:1 benzene:ether. O, origin: F, solvent front.

1 Hydrogen peroxide
2 Cumene hydroperoxide
3 Fuel oil (118 hr. irradiation)
4 t-Butyl hydroperoxide
5 Tetralin hydroperoxide
(*, tetralin impurity)

Hatched areas: compounds giving positive N,N-dimethyl-p-phenylenediamine test.

hydrocarbons known to occur in distillate fuel oils were individually irradiated in heptane solution with long wavelength UV. Hydroperoxide formation was monitored by TLC. The results, summarized in Fig. 3, indicate that only hydrocarbons having benzylic methylene groups constrained in a ring system afforded hydroperoxides on irradiation. Tetralin was previously shown to be the most reactive aralkyl hydrocarbon (of nineteen tested) toward thermally generated peroxy radicals; it was about twice as reactive as fluorene or indan, five times as reactive as cumene, and 67 times as reactive as toluene.[12]

Hydroperoxides, as reactive species, can be destroyed by numerous pathways, some of which are indicated in Fig. 4. Of particular importance is homolytic fission of the O-O bond. This reaction is favored by the low bond energy (ca.35 kcal/mol) of this linkage; it is also known to occur upon long-wavelength UV irradiation.[13] The reaction gives two equivalents of free radicals, including the extremely reactive and cytotoxic hydroxyl radical. The process has a high likelihood of initiating free-radical chain reactions. The behavior of irradiated #2 fuel oil gives evidence that such a mechanism is indeed involved. In addition to the linear increase

in hydroperoxide concentration, prolonged (90-120 hr.) irradiation afforded increasing concentrations of phenols and carbonyl compounds, likely decomposition products of hydroperoxides. During this period, hydroperoxide concentration declined somewhat, but growth inhibition by the irradiated oil remained high (even after thiacyclohexane treatment) indicating that secondary irradiation products were also contributing to toxicity.[10]

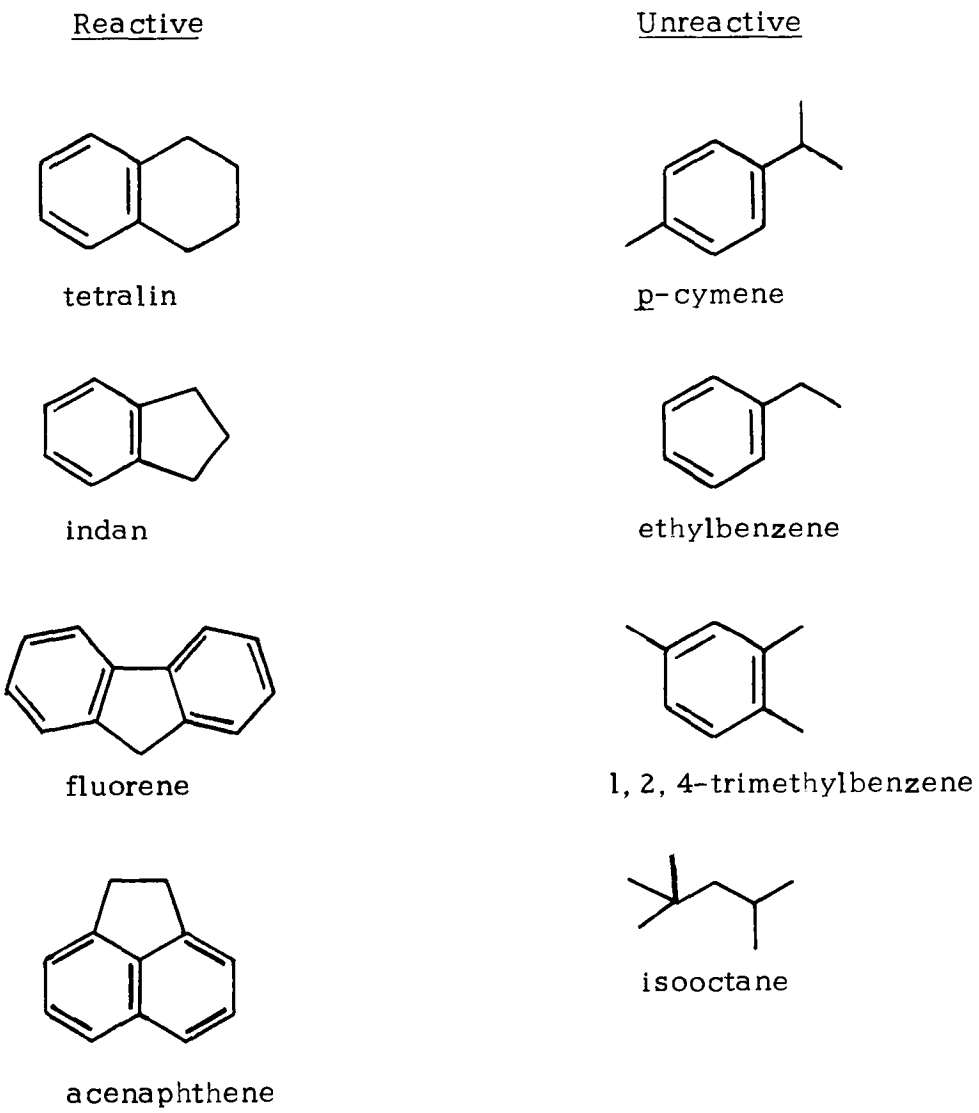

Fig. 3. Fuel oil hydrocarbons and their reactivity toward photochemical oxidation, as measured by hydroperoxide formation

We also showed by TLC and gas chromatography that irradiation of tetralin afforded a complex mixture of products, including 1-tetralol, 1-tetralone, and other more polar and higher-boiling substances.

Hydroperoxides are far more soluble in water than their parent hydrocarbons; for example, the solubility of cumene hydroperoxide in water is 13.9 g/l.[14] In order to assess the possible degree of water extractability of the peroxides from a spill, we carried out experiments on their partition between a "model fuel oil" (2:1 hexane:benzene) and water.

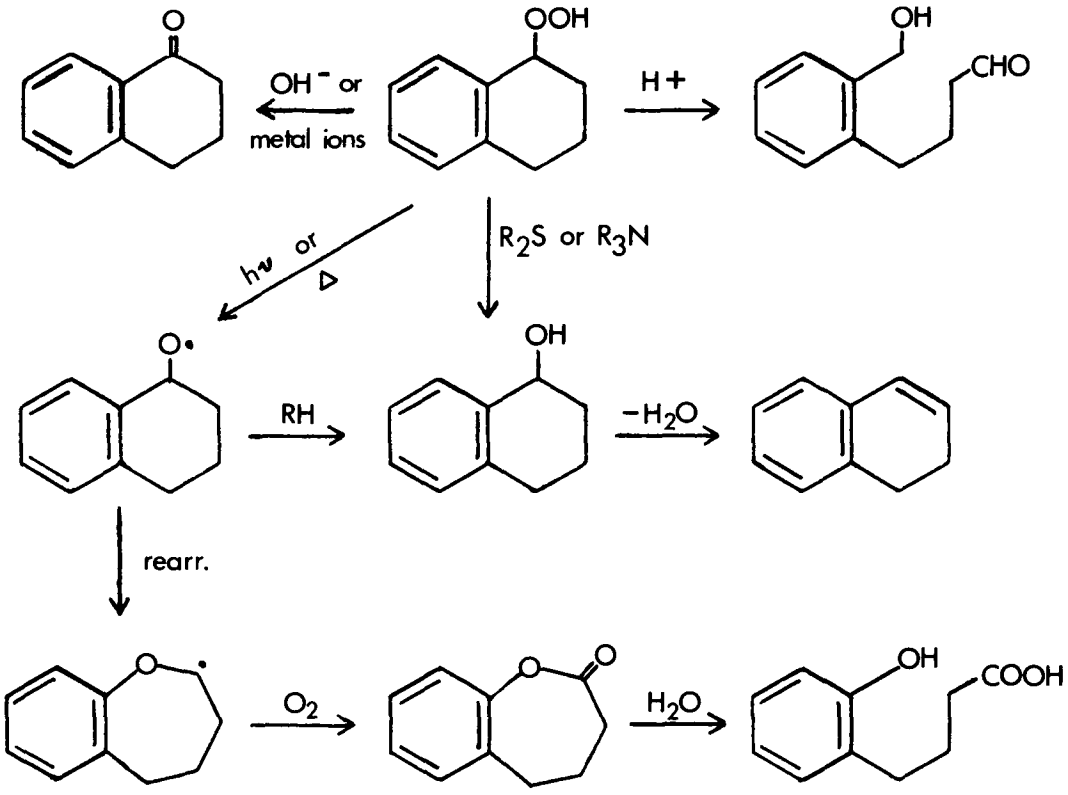

Fig. 4. Degradation pathways for organic hydroperoxides, exemplified by tetralin hydroperoxide

The extractability of the three individual organic hydroperoxides was 77% for t-butyl hydroperoxide and 8% and 11% for tetralin and cumene hydroperoxides, respectively. The irradiated fuel oil was also shaken with water in a similar experiment; 6% of the peroxide was extractable.

Little is known about the potential toxicity of hydroperoxides to aquatic organisms. Cumene hydroperoxide has been shown to be toxic to some algae at 2 ppm (1.3×10^{-5} M).[15] In our tests of the yeast inhibitory activity of the individual hydroperoxides, we found that tetralin hydroperoxide was highly active at less than 10^{-4} M (Fig. 5). Cumene, t-butyl, and hydrogen peroxide all were less active.

There are a number of possible explanations for the observed high toxicity of some hydroperoxides. The compounds may react oxidatively with thiol or amino groups of enzymes; may decompose to radicals which attack membrane lipids; or may destroy important metabolic substrates.

CONCLUSION

There has been a tacit belief that water pollution by oil can be measured by determining the concentration of hydrocarbons in a body of water. It is increasingly apparent that hydrocarbons are not environmentally dormant. The energy of sunlight in the presence of oxygen is sufficient to transform numerous hydrocarbons into derivatives having remarkable chemical and biological activity. It should also be recognized that nonpolar gases (such as oxygen) are concentrated by surface films of nonpolar liquids, making reactions involving them more probable. When methods for control and cleanup of oil spills are considered, photochemical effects should be taken into account.

ACKNOWLEDGEMENT

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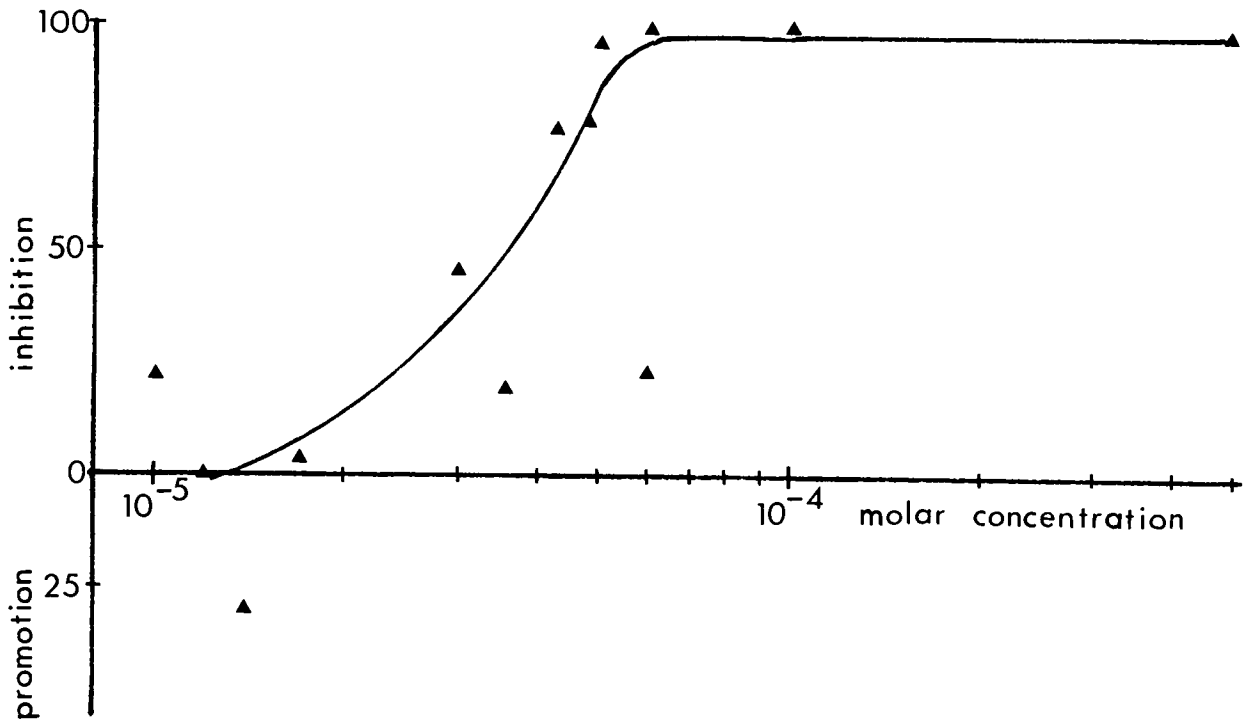

Fig. 5. Tetralin hydroperoxide effect on yeast growth.

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DISCUSSION

LASDAY: Have you done any comparisons of your UV irradiation with sunlight irradiation? How have you made certain that you weren't also getting bacterial-induced changes?

LARSON: As far as the sunlight irradiation is concerned, we are just starting these experiments now. We started the laboratory irradiations during the winter in Pennsylvania, which is a bad time to put things outside, but we are now looking at these.

They seem to be at least qualitatively similar. Dr. Scheier's group at the Academy of Natural Sciences, Philadelphia, has also done some fish toxicity experiments on a solar-irradiated system. He does find similar things. The question of bacterial contamination or of bacterial oxidation was ruled out by including a dark control in all of our experiments and finding no significant increase in hydroperoxides or, indeed, in other oxygenated products.

BRUBAKER: I was curious about the observations of the survivors of these toxicity experiments, whether there were any mutations, etc., since you based your hypothesis on free radical radiation-induced damage.

LARSON: No, we didn't look at them microscopically. What we did observe was that there was no sign of any growth at any time after we had applied the higher concentrations of irradiated oil. So they definitely were killed.

GIBSON: I understand that para-naphthaquinone was isolated from a No. 2 fuel oil and tested for toxicity against a blue-green algae, a diatom, and a green algae. It was very toxic, as I remember, for the blue-green but not for

the green and not for the diatom. So here you have a ketone that is possibly toxic, too, in addition to hydroperoxides.

LARSON: Yes, we observed this, too. For example, fluorenone, which is the product that we do observe, has a relatively high toxicity toward yeast, but not as high as some of the hydroperoxides. Some of the phenolic compounds and the acids are toxic as well.

SPEAKER: When we isolated para-naphthaquinone we thought it was fluorenone for a long time since they have similar properties. I was wondering, are you sure that you don't have para-naphthaquinone?

LARSON: No, we are not at all sure we don't have para-naphthaquinone. We observed a very wide range of carbonyl compounds from the irradiation. We tried to separate these as well as we could by column chromatography, and we got a fraction that had a UV spectrum that looked very much like fluorenone. We injected this sample into the GC, and we got a peak which appeared at about the same time as fluorenone. So we think we have fluorenone as well as some other materials, but there may be many, many other kinds of carbonyl compounds as well.

RUDDELL: I have a number of related questions. First of all, in your partition experiments, where you took oil and then layered your water on top, were you able to detect small molecular weight carbonyls, sulfoxides, quinones, and related compounds of that nature that you might expect during this photoreaction?

LARSON: Yes. We looked very hard for compounds of this sort, especially quinones, since we thought that these might well represent some of the toxic compounds. We have a spray reagent for quinones. We also looked at these things by infrared, and we don't believe that quinones are formed, at least in this reaction.

Sulfoxides are probably not present in high concentrations. I believe the fuel oil has very little sulfur in it. Right away we saw formation of hydroperoxides. We attribute this to the absence of much sulfur in the oil.

RUDDELL: The second part of my question is, do I understand you to mean that if you layer water on top and then irradiate, you don't get formation of quite as many peroxides?

LARSON: No, you get about the same amount of peroxides, but they disperse. Quite a large percentage of them disperse into the water layer and react there. We get peroxides in both types of experiments.

Just for experimental simplicity, most of the experiments I described were done without water present.

SEDIMENTS-SOURCES OR SINKS FOR PETROLEUM HYDROCARBONS?

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SEDIMENTS - SOURCES OR SINKS FOR PETROLEUM HYDROCARBONS?

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ABSTRACT

Reports of distributions of petroleum and indigenous hydrocarbons in Recent sediments and of processes participating in the incorporation of hydrocarbons into sediments are reviewed. Horizontal and vertical distributions of hydrocarbons in Lake Huron sediments are presented as examples of natural, non-petroleum distributions. In these sediments, virtually no change over a period of several centuries occurs in hydrocarbons buried below a depth of 10 cm. It is concluded that burial in sediments can effectively remove petroleum hydrocarbons from further interaction with the environment, although a certain amount of these materials appears to be released from surficial sediments.

A topic of considerable importance in oil pollution studies is the role of sediments. Do sediments help to clean up petroleum spills by adsorbing hydrocarbons and thereby remove them forever from the water column, or do sediments prolong the impact of spills by slowly releasing adsorbed hydrocarbons back to the overlying water and thus act as a source of chronic oil pollution? While a single definitive conclusion is not presently possible, several conditional statements can be derived from a review of studies of hydrocarbons in sediments.

Numerous investigators have reported the presence of petroleum hydrocarbons in Recent sediments. As part of a continuing study, Blumer and Sass show that number 2 fuel oil incorporated into coastal marine sediments by an oil spill off West Falmouth in Buzzards Bay, Massachusetts, was still present two years later.[1] Although total concentrations of hydrocarbons had been reduced over this period, the overall composition of the fuel oil had been well preserved. In a similar study, Bunker C fuel oil was found in the sediments of Chedabucto Bay, Nova Scotia, 26 months after a tanker grounding.[2] Oil levels in fine-sized sediments showed little decrease over this period, but some reduction was observed in coarse sediments. These studies show that even in surficial sediments oil degradation is slow.

In contrast to situations like the West Falmouth and Chedabucto Bay incidents in which single, major spills were the sources of petroleum pollution, coastal waters in many areas are subject to constant, low-level inputs of petroleum hydrocarbons which can become incorporated into underlying sediments. For example, the

sediments of Narragansett Bay, Rhode Island, contain a very complex mixture of hydrocarbons unlike those present in marine organisms.[3] Gas chromatographic patterns of these sediment hydrocarbons suggest a petroleum origin and such an origin is supported by ^{14}C dating of the hydrocarbons, which shows them to be much older than their host sediments.[4] The probable source of these petroleum hydrocarbons is from discharge of domestic sewage and industrial effluents into this bay, with eventual transfer from the water column to the sediments. In a similar study, petroleum-like hydrocarbons were found associated with sewage sludge in sediments from Boston Harbor, Massachusetts.[5]

Sediments from near the densely populated northern shores of Lake Zug, Switzerland, have concentrations of hydrocarbons four to six times higher than those in sediments from near less populated areas.[6] Chromatographic patterns of the enriched hydrocarbons are similar to those of petroleum products. Chronic, low-level inputs of petroleum-derived hydrocarbons from land-based sources are suspected in this case because no large-scale petroleum transport occurs on the lake and there are no recorded spills.

A petroleum origin of sediment hydrocarbons is usually inferred from gas chromatographic analysis. Resolved hydrocarbons are quantified and identified, and relationships between recent biogenic, altered biogenic, and nonbiogenic compounds are determined. The amount of nonresolved hydrocarbons, indicated by a baseline hump in chromatograms, is also used to determine the presence of petroleum. Included in this complex, unresolved mixture are aromatic hydrocarbons. Because aromatic as well as aliphatic hydrocarbon components of petroleum can become incorporated into sediments, direct measurement of florescence patterns of hydrocarbon extracts of sediments has been used to detect petroleum residues in beach sands and marine sediments.[7]

The incorporation of petroleum hydrocarbons into sediments is primarily the result of absorption or adsorption onto sinking particulate materials, although the existence of small tar particles more dense than sea water has been reported.[8] Meyers found that the amount of n-alkanes sorbed by marine sediments depended upon grain size.[9] Equal weights of smaller-sized particles sorbed more hydrocarbons than did those of larger-sized particles. This probably reflects differences in surface areas for a given weight of sediment, although mineralogical factors could also be important. In sediments from Bedford Basin, Nova Scotia, the highest concentrations of petroleum hydrocarbons were found associated with silt-sized sediments,[7] indicating the role of particle size in accumulating hydrocarbons. All marine sediments are porous and therefore have effective surface areas greater than those indicated by their mean diameters.[10] Therefore, absorption, as well as adsorption, of hydrocarbons is likely to be important. Destruction of sediment organic matter by hydrogen peroxide treatment can increase porosity by as much as 70%[10] and can also double the uptake of hydrocarbons by marine sediments.[9] Evidently absorption may be responsible for as much as half of hydrocarbon sorption by sediments.

The heats of adsorption of eicosane and anthracene onto montmorillonite clay have been determined to be 3 kcal/mole and 12 kcal/mole, respectively.[11] These values indicate weak, nonchemical

attractions between petroleum-type hydrocarbons and minerals. However, three resuspensions of a marine sediment sample in salt water removed only 15% of the amount of number 2 fuel oil which initially was sorbed onto the sample.[9] It appears that partitioning of petroleum between sea water and sediment particles strongly favors hydrocarbon-sediment associations.

In addition to petroleum hydrocarbons, hydrocarbons derived from recent biological or other natural sources are present in sediments. While it is not always possible to distinguish between natural and pollutant hydrocarbons, studies of hydrocarbon distributions in areas believed to be free of petroleum contamination are helpful in learning the probable fate of hydrocarbons in sediments. In this approach, the behavior of hydrocarbons buried for hundreds and sometimes thousands of years can be used to project the possible long-term behavior of petroleum hydrocarbons under similar sedimentary conditons.

As part of a broader investigation of the diagenesis of organic compounds in Recent sedimentary environments, the distribution of hydrocarbons was determined in sediments from Lake Huron, Michigan. Sedimentation rates in this lake are on the order of 1-2 meters per thousand years. Thus large amounts of sediments representing relatively short periods of geological time were collected and studied. Samples were obtained by Peterson grab in transects across sedimentary basins and by coring in the deeper parts of the basins. Thus, both horizontal and vertical distributions of hydrocarbons in Lake Huron were obtained.

The distributions of normal alkanes in surface sediments from the basin transects shown in Table 1 appear to be influenced by both the sediment texture and the character and amount of organic matter incorporated into sediments. In two transects across the Goderich Basin in southern Lake Huron, the highest concentrations of n-alkanes were found in sediments from the basin centers. These areas had both the finest sediment particles and the highest levels (3-4%) of total organic carbon. The nearshore slope sediment sample from the central basin transect showed nearly the same concentration of n-alkanes as the basin center samples but less total unsaturated hydrocarbons and had an organic carbon level of 2%. Other sediment samples from this basin were composed of sands and were low in both n-alkanes and unsaturates and in total organic carbon. These data support the statement that a total organic content and hydrocarbon uptake in sediments are related.[12] Such a relation exists primarily because the level of total organic carbon can be an indication of the effective sorptive surface area of the sediment particles.

However, the actual concentration of hydrocarbons present in sediments does not necessarily relate directly to the effective sorptive surface area. No agreement between petroleum hydrocarbons and either sediment texture or the amount of sediment organic carbon could be found in a study of coastal marine sediments.[7] In the Lake Huron study, the levels of n-alkanes given in Table 1 from the transect of Saginaw Bay show an agreement with particle size but not total organic carbon. The surface sediments in this transect are finer than those from the Goderich Basin and have organic carbon levels of 2-3%, yet their hydrocarbon concentrations are higher than those found in the Goderich Basin transects. Apparently, both grain size and the nature of the available organic matter are important in

influencing the hydrocarbon content of sediments.

The carbon preference index (CPI), or ratio of amounts of odd- to even n-alkanes, of the ten Lake Huron samples presented in Table 1 ranged from 2.5 to 4.3 and averages 3.3. Other Recent samples that have been analyzed have yielded CPI values of 4.0 from the Mississippi delta and 2.6 from Tanner Basin, California,[13] a range of 1.6 to 6.0 and an average of 2.9 for samples from the Beaufort Sea,[14] and of 1.5 for a continental slope sediment off Africa.[15] Analysis of American Petroleum Institute reference petroleum samples by this laboratory yielded CPI values of 1.0 for southern Louisiana crude oil and 0.9 for number 2 fuel oil. Therefore, CPI data indicate the n-alkane compositions from Lake Huron surface sediments are similar to those from other natural sediments and dissimilar to those in petroleum.

Figure 1 shows chromatograms of the aliphatic hydrocarbon and unsaturated hydrocarbon fractions obtained from the surface sediment sample from the center station of the central Goderich Basin transect. Figure 2 gives similar chromatograms from Station 3 in the Saginaw Bay transect. The aliphatic hydrocarbons from both locations display a homologous series of n-alkanes superimposed on an unresolved complex mixture of hydrocarbons. The most abundant n-alkane in both distributions is C_{27}, followed by C_{29}. Such dominance of long-chain hydrocarbons is common in Recent sediments,[13, 15] but rare in older sediments.[13] The only major medium-chain n-alkane present in these samples is C_{17}, which is the major alkane constituent of many phytoplankton.[16] Based upon the absence of the homologous series of non-biogenic n-alkanes usually found in contaminated sediments,[5, 6] Lake Huron surface sediments appear to contain predominantly natural hydrocarbons from a non-petroleum source. The unresolved complex mixtures of hydrocarbons present in both examples and indeed in all ten samples are most likely due to microbial activity *in situ*. These mixtures were larger in Saginaw Bay samples than in Goderich Basin samples and may reflect more land runoff into the Bay and higher biological productivity in the water column.

The distributions of unsaturated hydrocarbons from the two locations are qualitatively similar as shown in Figures 1 and 2. While none of these hydrocarbons have been identified yet, it is probable polyolefinic hydrocarbons typical of aquatic algae[17, 18] are present. In addition, polycyclic aromatic hydrocarbons whose presence has been reported in a variety of Recent sedimentary and soil environments[19] are likely to be in these distributions.

The sediments of Lake Huron accumulate rapidly and contain hydrocarbon compositions which appear to be uncontaminated by petroleum. Thus, they offer an opportunity to study changes in sediment hydrocarbons over historically long but geologically short periods of time.

Examination of hydrocarbon distributions obtained from a core taken in Saginaw Bay showed only small changes in total hydrocarbons over a 4 cm vertical distance representing about a decade of sediment accumulation. The chromatograms from the 0-1 cm section are given in Figure 2 and from the 3-4 cm section in Figure 3. Total n-alkane concentrations do not change, nor are there any large-scale qualitative changes in the aliphatic fraction of hydrocarbons. In the aromatic fraction, however, there is an almost complete loss of short-retention time hydrocarbons. Separation of hydrocarbons on the non-polar OV-101 columns used in this study is based largely on hydrocarbon

vapor pressure and compounds having short retention times are likely to have relatively low molecular weights and therefore be somewhat more soluble in water. Thus, the changes in the two aromatic fraction compositions may reflect preferential losses of water-soluble hydrocarbons over time.

The hydrocarbon content of a 50 cm vertical section of sediment from Goderich Basin is presented in Figure 4. This length of core represents about 300 years of sediment accumulation. While an overall slightly decreasing trend in concentration exists, no truly significant change with depth is found. The contribution of n-alkanes to total resolvable aliphatic hydrocarbons shown in Figure 5 ranges between 60 and 80% of the total and averages 70%. No consistent depth trend is evident. These data suggest that this sedimentary environment has received a fairly steady input of hydrocarbons from a non-changing source for the past 300 years. This is consistent with the contention that man's activities have not had a significant impact on the hydrocarbons in Lake Huron sediments. These compounds are from natural, biological sources and any changes in hydrocarbon distributions are due to natural processes. Moreover, these distributions suggest the expected fate of petroleum hydrocarbons in similar environments.

Only small vertical changes are observed in Figure 4 below a depth of 10 cm. CPI values of n-alkanes decrease from 3.2 at the sediment surface to 2.3 at 48 cm depth. Nearly all this decrease occurs in the first 8 cm of sediment, as shown in Figure 6. Also, the ratio of n-heptadecane to pristane decreases from 2.7 to 1.3 in the top 8 cm but varies little below this point. Bacteria attack straight-chain hydrocarbons in preference to branched hydrocarbons,[1] so this ratio is a sensitive indicator of bacterial degradation of hydrocarbons. Thus, it appears that a certain amount of alteration and degradation of hydrocarbons occurs, but only in surface sediments. Furthermore, that portion of total sediment hydrocarbons which undergoes degradation over geologically short periods is very small. Assuming the chemical processes occurring in Lake Huron are representative of similar sedimentary regimes, burial of hydrocarbons evidently can lead to their preservation and isolation from biological processes.

It must be stressed, however, that evidence from the Lake Huron and other investigations indicates that hydrocarbons in surface sediments can interact and exchange with surrounding environments via several processes. For example, in the months following the West Falmouth spill, the area of sediment contamination steadily expanded until it was many times that originally affected by the spill.[20] Evidently the scouring action of tides, currents, and waves redistributed contaminated sediments. Thus, sediment particles can become transport agents for petroleum hydrocarbons in the benthic environment even though they may effectively remove these pollutants from the pelagic environment by adsorption or absorption.

In addition to reworking of sediments by physical forces, there can also be reworking by burrowing benthic organisms. Bioturbation is generally assumed to be important only to a sediment depth of 5-10 cm, but an extreme case to depths greater than 3 m has been

reported.[21] Any sort of sediment reworking counteracts the effects of burial and re-exposes hydrocarbons to continued biological and chemical interactions with the sediment-water interface.

As indicated by the n-heptadecane-to-pristane ratio change with depth in Lake Huron samples, bacterial attack can degrade biogenic hydrocarbons in surface sediments. This can also happen to petroleum hydrocarbons. At one location contaminated by the West Falmouth spill, the n-heptadecane-to-phytane ratio of sediment hydrocarbons decreased from 2.0 to 0.2 over a two-year period.[1] Even so, the rate of such degradation is usually slower under natural conditions than under optimized laboratory conditions, and the character and fate of degradation products is not well-understood.

The degradation of petroleum also involves the partial dissolution of some constituents in water.[1, 9, 11] Dissolution is enhanced by the presence of dissolved organic matter[22] which can act to solubilize normally insoluble hydrocarbons. Prediction of the solubility behavior of petroleum hydrocarbons is complicated, however, by solute-solute interactions which cause deviations in actual water solubilities and which are influenced by the total petroleum composition.[23] Therefore, solubilities of hydrocarbons depend upon the type of oil, the amount of dissolved organic matter, and such environmental factors as salinity and temperature. Nonetheless, it can be safely assumed that some portion of the total hydrocarbons is lost from sediments by resuspension and reworking.

The goal of this paper is to decide whether sediments act to moderate or to prolong the impact of an oil spill. In approaching a conclusion, observations made over a year after the Chedabucto Bay spill should be mentioned. While Bunker C oil was still present in the sediments, no evidence of oil could be found in the waters of the Bay.[24] Although Bunker C oil is more viscous than most petroleum products, other oils probably behave in a similar way. Therefore, the evidence presented here strongly suggest that sediments act as a sink for petroleum hydrocarbons. Although a portion of the hydrocarbons can be released from surface sediments by biological, chemical, and physical processes, deeper burial in sediments appears to remove petroleum-type hydrocarbons from interaction with the environment.

This review would not be complete without identifying areas in which research is critically needed. Only a limited number of studies have been done on the hydrocarbon geochemistry of Recent sediments. Little is known about the transport physics of sediment particles having high organic contents. The interaction of organic matter and trace elements is poorly understood and may be important in mobilizing toxic materials, both organic and inorganic, in sediments. Further research in areas such as these will greatly enhance our ability to predict the impact of future oil spills on aquatic systems.

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DISCUSSION

BUTLER: When you took these cores, did you notice any living organisms, worms or things like that, in the top 4 or 5 centimeters? One would expect that any kind of interstitial fauna would really stir up the top few centimeters of the sediment.

MEYERS: Yes, that is an important point. Bioturbation is an important process of reworking the sediments. In the Great Lakes, again, one is fortunate because lake sediments just don't have the same amount of bioturbation as marine sediments.

The fauna are much smaller. It occurs, there is no question about it. Some of the trace metal work that one of the other people involved with this reseach has done indicates that, probably, reworking of the sediments amounts to about 5 centimeters in depth.

SPIES: Is there any chance that the high levels of hydrocarbons in the surface sediments resulted from inclusion of organisms in your samples? Did you take any pains to separate organisms from your samples?

MEYERS: We sift all our sediments prior to analysis. Probably, some organisms are definitely included but it is material that would pass a 1 millimeter sieve.

SPIES: Could your samples have included some interstitial things, then?

MEYERS: Yes.

Table 1

Hydrocarbons in Surficial Sediments from Lake Huron Basins

Basin	Sediment Description	TOC %	Normal Alkanes µgm/gm	CPI	Range	Total Unsaturates µgm/gm
Goderich, south						
nearshore slope	fine sand	0.34	2.1	2.5	C_{14}-C_{35}	3.7
basin center	silty clay	3.15	11.9	4.3	C_{14}-C_{37}	46.5
mid-lake ridge slope	fine sand	0.09	1.6	3.2	C_{14}-C_{35}	2.2
Goderich, central						
nearshore slope	fine sand	1.95	10.4	3.1	C_{14}-C_{35}	3.7
basin center	silty clay	4.26	12.8	4.1	C_{14}-C_{35}	35.2
mid-lake ridge slope	fine sand	0.02	1.7	2.8	C_{14}-C_{38}	3.5
Saginaw Bay						
station 1, head of bay	clay	n.d.*	25.7	2.6	C_{15}-C_{35}	73.0
station 2	clay	2.30	22.6	3.0	C_{15}-C_{35}	89.4
station 3	clay	2.78	19.9	3.6	C_{15}-C_{35}	64.0
station 4, mouth of bay	clay	2.54	27.5	3.6	C_{15}-C_{35}	176.0

* not determined

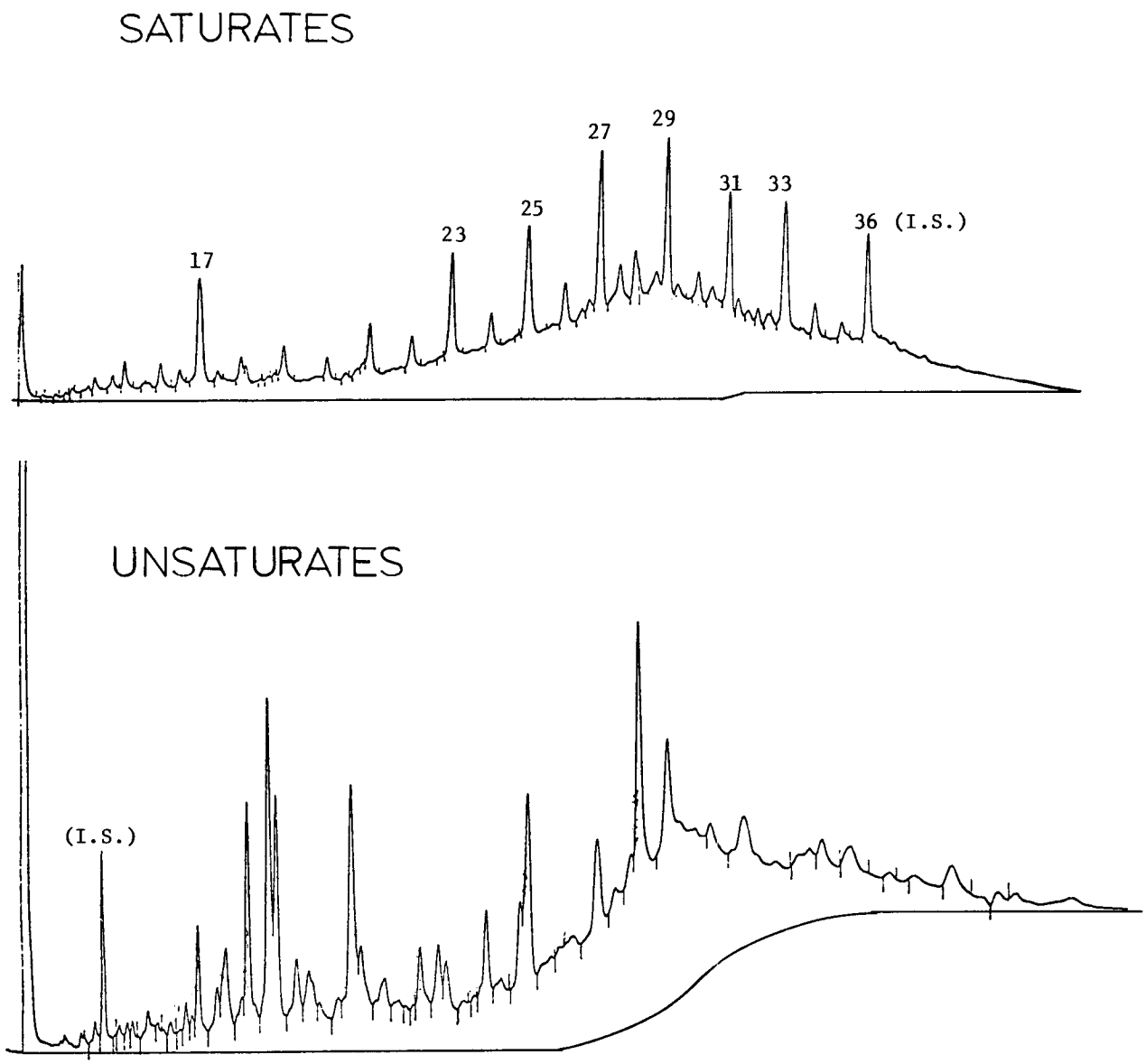

FIGURE 1. CHROMATOGRAMS OF HYDROCARBONS FROM SURFICIAL SEDIMENTS, GODERICH BASIN, LAKE HURON. NORMAL ALKANES ARE LABELED BY CARBON NUMBERS. RETENTION TIMES OF BOTH FRACTIONS ARE SIMILAR. GC CONDITIONS: 4 m x 2.1 mm ID 3% OV-101 ON SUPELCOPORT 80-100 COLUMN, 150-325°C AT 4°C/MIN. INTERNAL STANDARD INDICATED BY I.S.

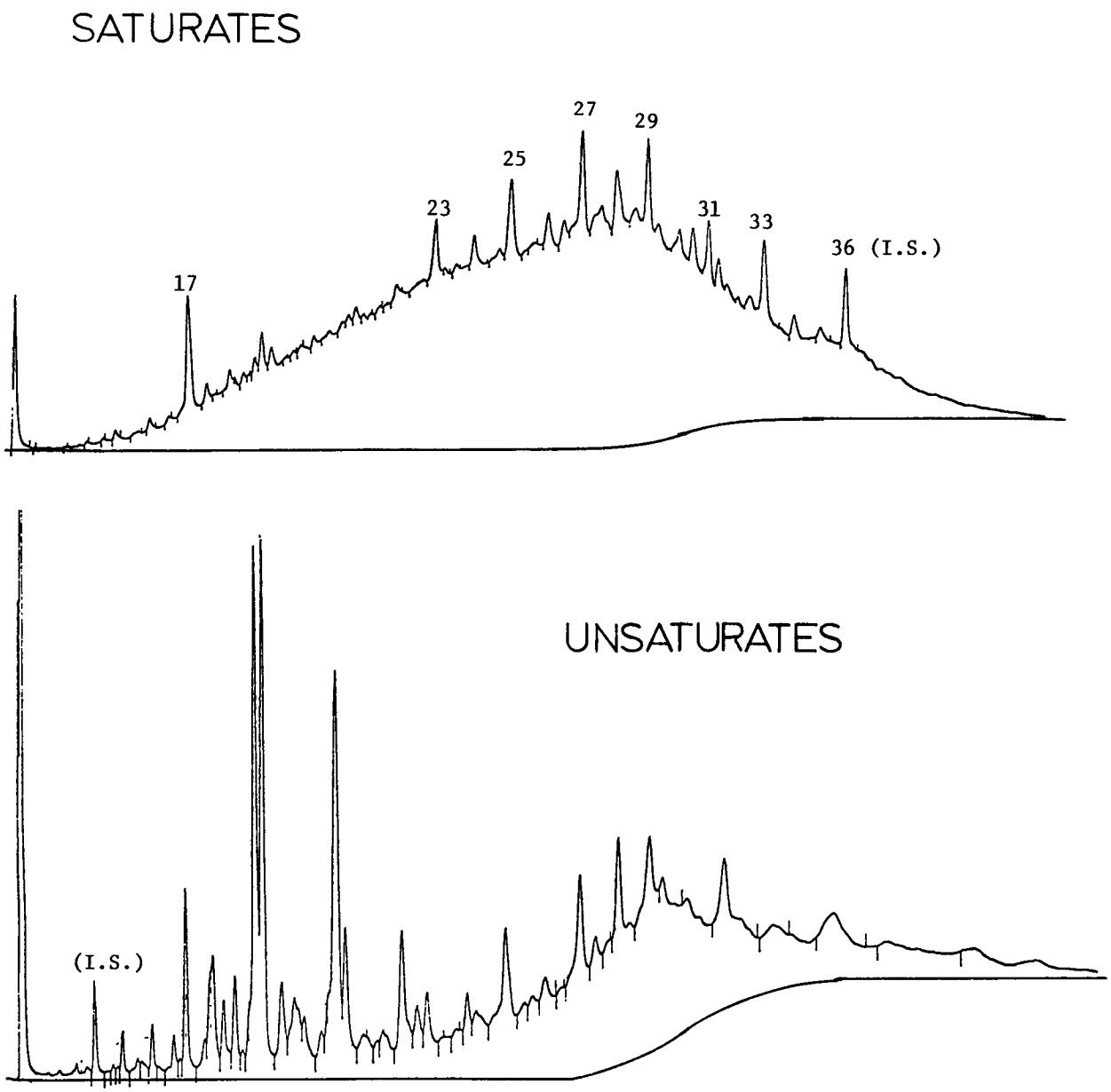

FIGURE 2. CHROMATOGRAMS OF HYDROCARBONS FROM SURFICIAL SEDIMENTS, SAGINAW BAY, LAKE HURON. NORMAL ALKANES ARE LABELED BY CARBON NUMBERS. RETENTION TIMES OF BOTH FRACTIONS ARE SIMILAR. G.C. CONDITIONS: 4 m x 2.1 m ID 3% OV-101 ON SUPELCOPORT 80-100 COLUMN, 150-325°C AT 4°C/MIN. INTERNAL STANDARD INDICATED BY I.S.

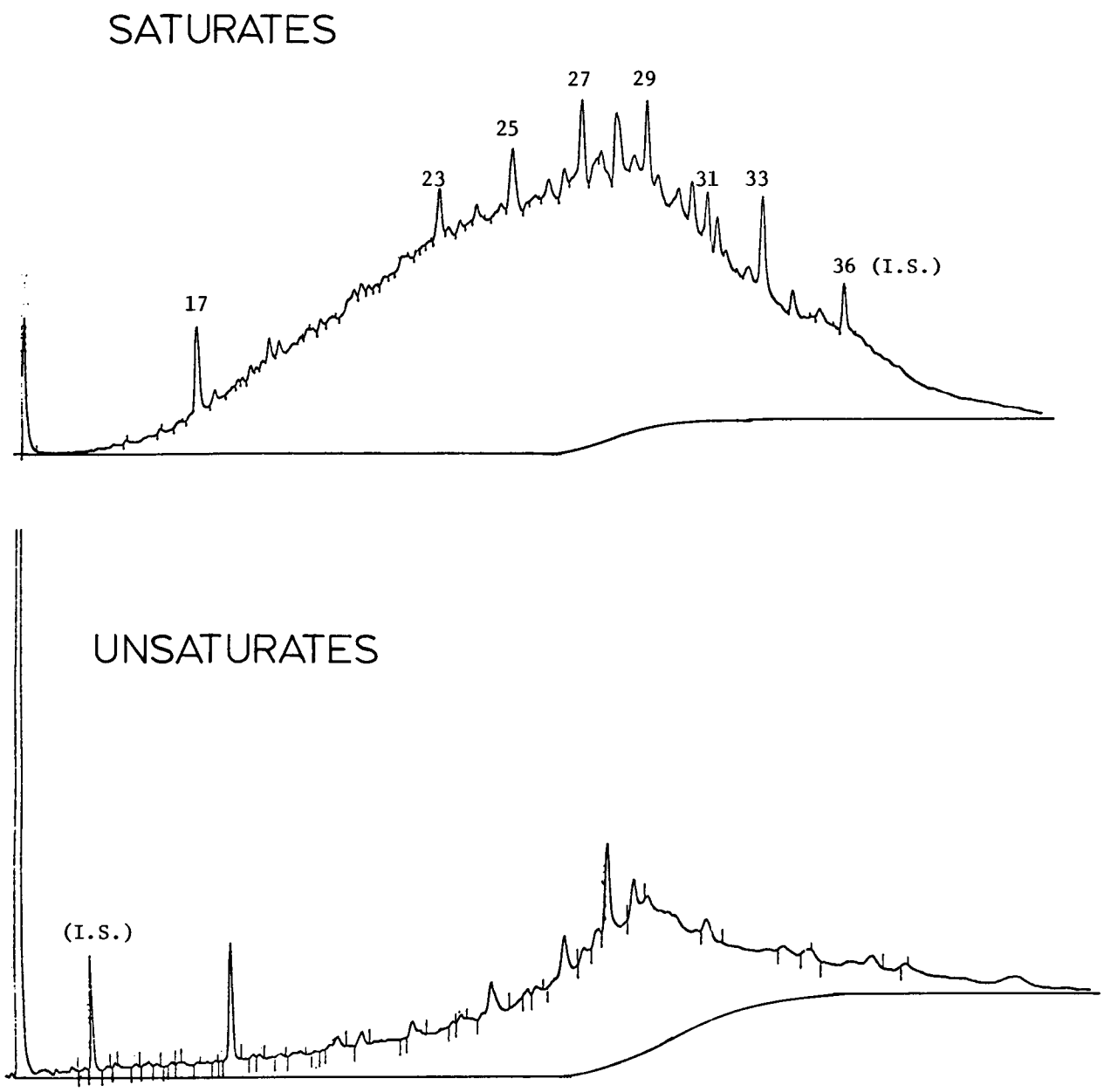

FIGURE 3. CHROMATOGRAMS OF HYDROCARBONS FROM SUBSURFACE SEDIMENTS (3-4 cm), SAGINAW BAY, LAKE HURON. NORMAL ALKANES ARE LABELED BY CARBON NUMBERS. RETENTION TIMES OF BOTH FRACTIONS ARE SIMILAR. GC CONDITIONS: 4 m x 2.1 mm ID 3% OV-101 ON SUPELCOPORT 80-100 COLUMNS, 150-325°C at 4°C/MIN.

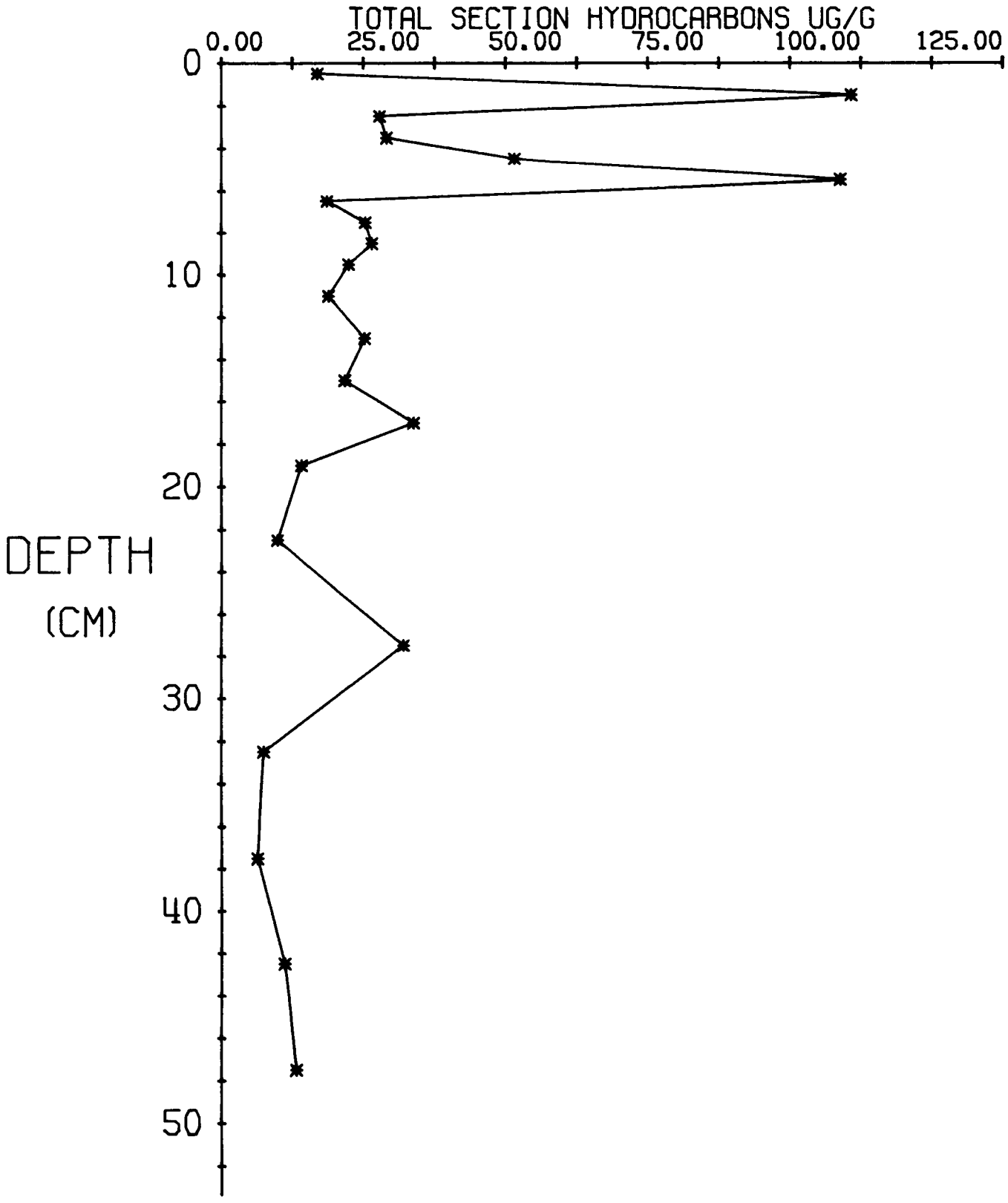

FIGURE 4. DISTRIBUTION OF TOTAL HYDROCARBONS WITH DEPTH IN GODERICH BASIN SEDIMENTS, LAKE HURON.

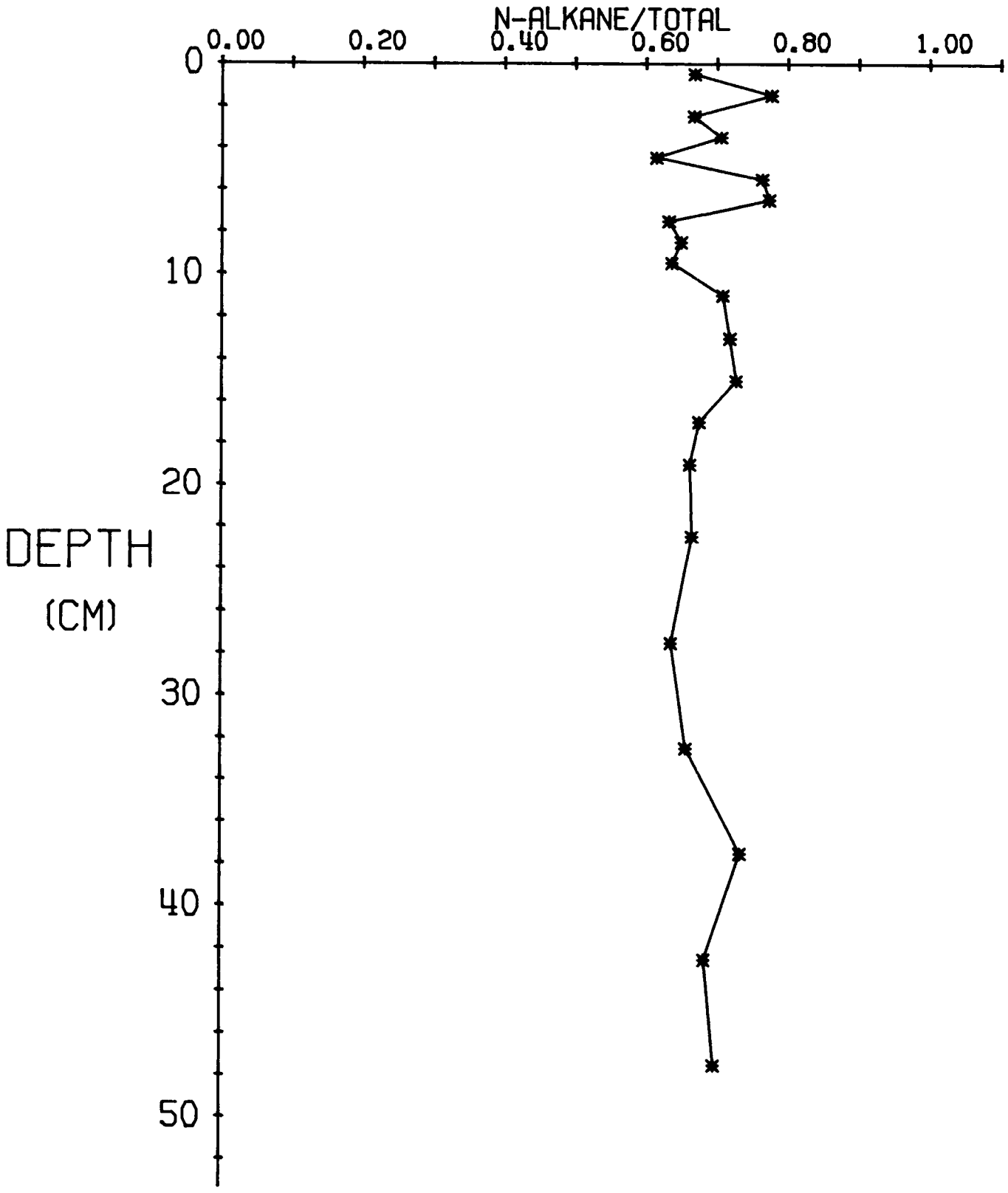

FIGURE 5. CONTRIBUTION OF N-ALKANES TO TOTAL SATURATED HYDROCARBONS IN GODERICH BASIN SEDIMENTS, LAKE HURON.

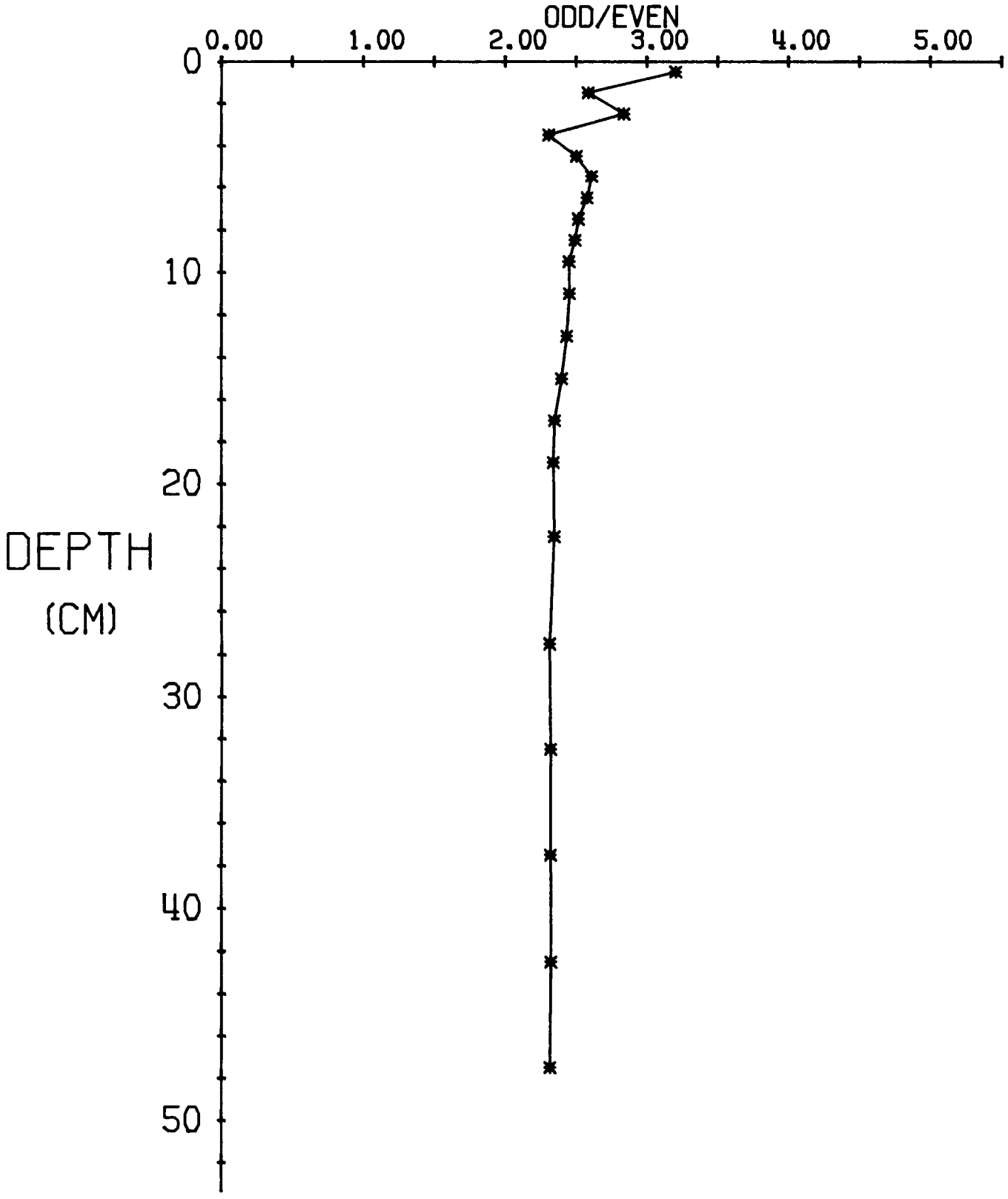

FIGURE 6. CARBON PREFERENCE INDEX IN GODERICH BASIN SEDIMENTS, LAKE HURON.

SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE AQUATIC ENVIRONMENT

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Abstract

Recent studies in our laboratory and elsewhere have shown that several soils and marine sediments from the New England region contain very complex mixtures of polycyclic aromatic hydrocarbons (PCAH) and their alkyl homologs. The geochemical significance of this fact is not yet clear. Important questions such as identification of sources, modes of transport, and mechanisms of diagenesis are just beginning to be addressed. Our studies have ruled out biosynthesis as a major source and have indicated that anthropogenic sources followed by in situ modificaton of the PCAH distribution is a valid hypothesis.

A. POLYCYCLIC AROMATIC HYDROCARBONS IN RECENT SEDIMENTS

Recent studies have shown that several soils and marine sediments from the New England region contain a very complex mixture of polycyclic aromatic hydrocarbons (PCAH) and their alkyl homologs.[1,2,3] For example, sediment from the Charles River Basin contains PCAH with at least eleven different aromatic ring structures and abundant alkyl substituted derivatives thereof, in some cases containing up to 15 carbon atoms in the alkyl groups.[2]

In addition to the Charles River sediment, we have analyzed several other soils and sediments, and the remarkable feature of these results is that the same qualitative PCAH distribution is found in each case. (There is one exception which we will discuss later.) The complexity and wide-spread distribution of these PCAH mixtures has been a surprise, revealed only through the application of very sophisticated analytical techniques centered around mass spectrometry. This finding is even more interesting when one remembers that many PCAH are known chemical carcinogens.

B. SOURCES OF PCAH IN NATURE

Different sources of PCAH in these sediments have been suggested. These include (a) biosynthesis; (b) petroleum spillage; and (c) combustion from mobile and stationary sources including refuse burning, forest fires, and industrial activity (all of which lead to air particulate formation). One of our goals has been to evaluate these possibilities.

1. Biosynthesis

There have been suggestions that at least some PCAH can originate from biosynthesis by algae,[4] by plants,[5] or by various bacteria.[6] Thus, it was suggested[2] that the complex mixtures of PCAH found in the Charles River Basin could originate from the biosynthetic activity of anaerobic bacteria. Careful experiments in our laboratory, however, have indicated that this is an unlikely possibility and that bacteria accumulate PCAH but do not biosynthesize them.[7]

2. Petroleum Spillage

Polycyclic aromatic hydrocarbons may also originate from petroleum. PCAH mixtures from petroleum are quite deficient in the unsubstituted species; the most abundant alkyl homolog usually contains three or four carbon atoms.[3] Since the PCAH homolog distribution in sediments is monotonically decreasing, the PCAH in sediments could not originate directly from petroleum.

3. Combustion: Air Particulates

Because combustion-produced PCAH transported by air are one of the most likely sources of polycyclic aromatic hydrocarbons in nature, we investigated the distribution of PCAH alkyl homologs in samples of urban air particulates. In general, there is a considerable difference in relative homolog abundance between the air particulate samples and the sediment samples.[8,9] Because of this difference, Blumer and Youngblood[3] have concluded that the polycyclic aromatic hydrocarbons in these recent sediments could not have come from the simple deposition of such anthropogenic airborne particulates but instead originate from forest fire particulates. We feel this conclusion is not correct for three reasons:

a) Effect of Combustion Conditions. The general formation mechanism of PCAH in combustion systems has been thoroughly investigated and there is now agreement that qualitatively similar PCAH mixtures are produced almost regardless of the fuel type and the combustion conditions.[9,10]

Quantitatively, however, the distribution of alkyl homologs can be quite different depending on flame zone temperature. Low temperatures (such as 1100° K, as in a cigarette) will yield a soot with quite abundant alkyl substituted PCAH. Very high temperatures (such as 2200° K, as in a carbon black furnace) will yield soot completely devoid of alkyl PCAH.[11] Extended periods of time ($\sim 10^6$ years) at very low temperature (450° K) will yield PCAH mixtures in which the unsubstituted species is not at all abundant; for example, in petroleum.

It is, thus, clear that the slope of the PCAH alkyl homolog distribution curve can give information on the formation temperature of these compounds (given that there has been no subsequent modification). Based on these considerations, Blumer and Youngblood[3] concluded that the PCAH in recent sediments were produced at modest combustion temperatures and that the fuel giving rise to such temperatures was wood (as in a forest fire); thus the sedimentary PCAH were of natural origin. There is a fallacy in this line of thought; namely, wood is not unique in its combustion temperature. Other, exclusively anthropogenic fuels, also burn at these moderate temperatures, coal being a noteworthy example. In fact, experiments in our laboratory show that the burning of wood gives a distribution of PCAH alkyl homologs more similar to that of Boston's air particulates than to that of recent sediments; whereas the burning of coal gives a distribution of PCAH alkyl homologs which is quite similar to that of recent sediments.[9]

Therefore, it seems that the PCAH alkyl homolog distribution can give some information on formation temperature but this fact is not sufficient to uniquely define the fuel or the exact source.

b) Modification of PCAH After Deposition. It seems reasonable that there are natural mechanisms which can modify a PCAH homolog distribution after the mixture has been deposited in the soil or sediment. One such mechanism could be differential water solubility of the higher alkyl homologs vs. the unsubstituted species. Several workers have shown that the logarithm of water solubility is a linear function of the number of carbon atoms for several alkyl benzenes and other hydrocarbons.[12,13] Extrapolating somewhat, we would expect that the various alkyl homologs of PCAH systems would have water solubilities distributed in a similar fashion. We, therefore, suggest the following mechanism for transition of a typical air particulate homolog distribution into that observed in sediments: After airborne particle deposition on soil or in water, the lowest homologs (including the unsubstituted species) continuously fractionate into the water phase to an extent proportional to their carbon number as described above. The remaining species, which accumulate on the particulate matter and in the sediment, are therefore devoid of the lowest homologs, thus increasing the relative abundance of the higher homologs.

As a preliminary test of this hypothesis, we examined PCAH in water and sediment samples from the Charles River Basin system and compared them to Boston's urban airborne particulate PCAH. The slope of the air particulate homolog plot was intermediate between that found in the water and in the sediment.[8] This indicates that the water is enriched in the lowest homologs, while the sediment is enriched in the higher homologs relative to the air particulates. These preliminary data support our hypothesis that PCAH homolog distributions can be extensively modified, in a regular fashion, by simple natural processes.

c) Geographical Distribution of PCAH in Soils and Sediments. The astute reader will note that the soil and sediment samples on which the above discussion is based are all taken from the New England region of the United States. It is obviously risky to base any firm conclusions about sources upon such a small portion of the globe. Therefore, in a preliminary effort to expand the geographical distribution of this data base, we have examined the PCAH content of a soil from a site located in rural South Carolina. This sample is dominated by C_2 and C_4 alkyl substituted phenanthrenes or anthracenes. The other PCAH ring systems (such as pyrene or fluoranthrene) are not abundant in this sample, and the normal, rather uniform, distribution of PCAH alkyl homologs has completely vanished leaving only the even carbon number alkyl substituted species.

Although the source of these PCAH in this South Carolina soil is not yet clear, one thing is perfectly obvious: The source of PCAH in the New England region is different from that in South Carolina. If this source were forest fires one would expect to see a similar PCAH distribution in all wooded areas, this includes New England and South Carolina. Since this is not the case, anthropogenic sources seem to be indicated. Such sources would be expected to be quite variable depending on the human activity in the region and it, therefore, seems likely that a rural area such as the Southeastern United States would have a considerably different emission pattern of PCAH than the highly industrialized and urbanized Northeast. While we realize that one sample is not a sufficient study of geographical distribution, these data do indicate that such studies are of considerable importance if one is to distinguish among sources of PCAH.

C. CONCLUSIONS

It is clear that PCAH and abundant PCAH alkyl homologs are widely distributed in nature, but the geochemical significance of this fact is not yet clear. Important questions such as identification of sources, modes of transport, and mechanisms of diagenesis are just beginning to be addressed. Our work to date was among the first to completely identify the complex PCAH mixtures present in a marine sediment. Our

subsequent studies have ruled out biosynthesis as a major source and have indicated that anthropogenic sources followed by in situ modification of the PCAH distribution is a valid hypothesis. It is, however, only a hypothesis. Much work remains to be done on this interesting class of compounds.

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DISCUSSION

HERBES: You mentioned at the end the possibility of in situ modification as sort of an open area for speculation. Have you considered the possibility of bacterial degradation at differential rates preferentially attacking the less alkylated compounds?

HITES: We have considered that as a possibility plus an additional possibility of differential bioaccumulation. It would seem that that may be equally likely. There is a whole list of these which can be investigated. We are looking thoroughly at the differential water solubility question. I think that is a good thing to look at first.

HERBES: That would be coupled, too, with the increased absorptive potential, I think, of the more highly alkylated compounds which would accumulate.

LASDAY: How do you explain the preferential deposition of the airborne substituted PNA's from the midwestern air when those that you have shown present in the Boston region are not deposited into the sediments?

HITES: Well, I think it is well known that there is a certain amount of airborne transport of particulate and it could well be that the particulate generated in the Boston area are the ones that we measured. They never have a chance to land on Boston, but are transported by air up to Nova Scotia.

But, instead, the particulate from e.g. Indianapolis could well be transported into the Boston area. I am assuming that the ones we measured in the air never get a chance to land back on the surface.

CALDER: I wonder if you had prepared any subsurface sediments by the same technique to look at distributions which would predate any sort of coal-burning or man-induced activities.

HITES: We have. We are just starting to do some preliminary experiments, and we have looked at one sediment which is about 300 years old from Buzzards Bay. We could not find any polycyclics. But we are doing more research in that area.

METABOLISM OF PETROLEUM HYDROCARBONS IN MARINE SEDIMENTS

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ABSTRACT

The degradation of petroleum hydrocarbons in aquatic sediments result from the interaction of microfauna, meiofauna and macrofauna. Populations of hydrocarbon degrading microbes are high in areas of petroleum input, resulting in a rapid degradation of the alkanes with slower attack on isoalkanes, cycloalkanes and aromatic hydrocarbons. In the process of feeding, the interstitial community (meiofauna), and the benthic macrofauna expose deeper sediments to the water-sediment interface where there is more microbial activity. The polychaete worms take up hydrocarbons from the sediment and have an active enzyme system in the lower portion of their intestine which metabolizes these compounds. Hydrocarbons on resuspended sediments are recycled by passage through filter feeding bivalves. Bacteria and animals metabolize aromatic hydrocarbons by different mechanisms with bacteria producing cis-diols while animals degrade them to trans-diols. Since bacteria use hydrocarbons as a carbon source there is ring cleavage of the diols with eventual degradation to carbon dioxide. Animals excrete the diols or their conjugated products.

INTRODUCTION

Oil spills and the wastes of various industrial processes are some of the contributors of hydrocarbons to aquatic sediments. Sediments from a Norwegian fjord had high concentrations (up to 1000 mg/kg) of polycyclic aromatic hydrocarbons due to wastes from an aluminum production plant[1]. Blumer and Sass[2] have continued to find petroleum-derived hydrocarbons in sediments from Buzzards Bay, Massachusetts, for many years after a spill of fuel oil #2. Sewage effluents and small oil spills were the sources of the high hydrocarbon concentrations in the sediments of Narragansett Bay, Rhode Island[3,4]. The observed biological degradation of hydrocarbons in the sediments of these areas is due to microfauna, meiofauna and macrofauna. This paper will discuss the importance of each of these groups of organisms in the metabolism of sediment hydrocarbons.

MICROBES

Hydrocarbon degrading microbes have been isolated from various types of marine sediments[5,6,7]. After introduction of oil into sediments there is a large increase in the hydrocarbon degrading microbial population[8,9,10]. Hughes and McKenzie[11] added oil to sediment and followed oil degradation by taking sediment cores for two years. The surface layer of oil was degraded but oil below the surface remained unchanged, indicating that microbial degradation takes place at the water-sediment interface. Only slow hydrocarbon reduction occurred in anaerobic muds[12] and it was speculated that the sulfate-reducing bacteria, Desulfovibrio, was responsible for the degradation which was observed[10].

The alkanes are rapidly degraded by sediment microbes, followed by slower attack on isoalkanes, cycloalkanes and aromatic hydrocarbons[13,14]. In oil spill areas the hydrocarbon degrading microbes were able to metabolize both aliphatic and aromatic hydrocarbons[8,9]. Different crude and refined oils would be expected to show different rates of degradation because of variations in the relative amounts of different petroleum components. In one experiment, hydrocarbon microbes were allowed to act on two crude oils (South Louisiana and Kuwait) and two refined oils (bunker C and fuel oil #2). The South Louisiana was the most susceptible to microbial degradation and bunker C was the least degraded in the 28 day study[15]. The high concentration of high molecular weight aromatic hydrocarbons in bunker C may explain its resistance to degradation.

Hydrocarbon microbes generally use hydrocarbons as a carbon source so that carbon dioxide is the final product. The intermediate products are fatty acids for alkanes and hydroxy derivatives for the aromatics. Cytoplasmic sequestering of hydrocarbons has been demonstrated in bacteria associated with tar balls and droplets of oil, suggesting active transport of hydrocarbons into the cells[5]. Bacteria metabolize polycyclic aromatic hydrocarbons to cis-diols while animals degrade them to trans-diols. For example, an aquatic Beijerinckia species degraded benzo(a)pyrene to cis-9,10,dihydroxy-9,10-dihydrobenzo(a)pyrene[16]. Vertebrate animals oxidized benzo(a)pyrene and other polycyclic aromatic hydrocarbons to trans-dihydrodiols[17]. A bacterial metabolite from naphthalene was cis-1,2-dihydroxy-1,2-dihydronaphthalene[18], while in mammals trans-1,2-dihydroxy-1,2-dihydronaphthalene was produced[19]. Thus bacteria and animals use a very different enzyme system for degrading aromatic hydrocarbons.

In addition to bacteria, hydrocarbon degrading yeast and filamentous fungi have been isolated from marsh sediment along the coasts of Louisiana[20] and North Carolina[21]. A well characterized species is the filamentous fungi, Cladosporium resinae, which occurs in estuarine sediments, and can use alkanes as the sole carbon source[22]. Alkanes were degraded to carbon dioxide with alcohols and acids as the intermediate products in this oxidation.

MEIOFAUNA

In addition to microbes, aquatic sediments also contain a large interstial community called the meiofauna and composed of harpacticoid copepods, nematodes, turbellarians and small polychaetes[23]. Many species from these groups are deposit feeders which are thus directly exposed to hydrocarbons in the sediments. Calanoid copepods can metabolize hydrocarbons[24,25] and presumably harpaticoid copepods also possess a hydrocarbon-degrading system. Polychaete worms, particularly, Capitella capitata, are associated with areas of high oil input[26,27]. Except for the polychaetes, Streblospio benedicti and Polydora ciliata, most benthic organisms were absent from sediments near oil field brine effluent in Trinity Bay, Texas[28]. Detritus associated with sediment is used for nourishment by many benthic polychaetes. Much of this detritus is formed in the water where hydrocarbons can adsorb to it[29]. Rossi and Anderson[30] has shown uptake by detritus-bound methylnaphthalene by the polychaete, Neanthes arenaceodentata, followed by rapid discharge of methylnaphthalene and its metabolites. Cell-free extracts of Capitella capitata have arylhydrocarbon hydroxylase activity and living animals will take up benz(a)anthracene from the sediment with subsequent metabolism to 5,6-dihydro-5,6-dihydroxbenz(a)anthracene[31].

The possible role of nematodes and turbellarians in degradation of sediment hydrocarbons has not been explored. McIntyre et al.[23] have proposed that in the sandy beach area which they studied, all the organic matter passed through bacteria before being utilized by the meiofauna. In this case cytoplasmic sequestering of hydrocarbon by bacteria[5] would be an important process for the entrance of hydrocarbon to the meiofauna.

MACROFAUNA

Certain benthic species of molluscs, crustaceans, large polychaete worms and spinculid worms, referred to as the macrofauna, may play a role in hydrocarbon degradation in the sediments. As noted earlier, microbes are most effective in hydrocarbon degradation when working at the water-sediment interface. Many of the benthic animals rework the sediment so that hydrocarbon adsorbed to this sediment would be more exposed to microbial action. Tidal flow causes resuspension of fine sediments with their associated hydrocarbons. These resuspended sediments can be taken in by benthic filter feeders, such as clams, mussels and oysters. The clam, Macoma, feeds directly on organic matter in the sediment[32]. The work of Farrington and Quinn has shown the importance of bivalves in the uptake of hydrocarbons from sediments[3]. There is no evidence of hydrocarbon metabolism by these bivalve molluscs[33,34], but the discharge of hydrocarbon in the feces and pseudofeces would allow attack by microfauna and meiofauna.

Oil uptake from the sediment has been shown for the brown shrimp, Crangon crangon[35], and for the sipunculid worm, Phascolosoma agassizii[36]. Benthic decapods are able to rapidly metabolize petroleum hydrocarbons with subsequent excretion of hydrocarbon metabolites[37,38].

Many of the large polychaetes injest sediment during their feeding. Recent work has shown that polychaetes are able to metabolize petroleum hydrocarbons[30,31]. When benz(a)anthracene was incorporated into sediment containing Nereis succinea and Nereis virens, the major metabolite produced by the worms was 5,6-dihydro-5,6-dihydroxybenz(a)-anthracene (Figure 1). Presumably an epoxide intermediate is produced during this reaction as occurs in mammals. The hydroxylated and conjugated products of these oxidation reactions are subsequently excreted by the worms. Aryl hydrocarbon hydroxylase activity was associated with the lower portion of the intestine with little or no enzyme activity in the pharynx, esophagus or upper portion of the intestine (Table 1)[31]. Subcellular fractionation of the intestine indicated that the enzyme was localized in the microsomal fraction and enzyme activity required oxygen, reduced pyridine nucleotide and magnesium. The association of the enzyme activity with the microsomes indicates that bacteria in the polychaete were not responsible for the observed hydrocarbon degradation since bacteria would be removed in the low speed centrifugation (8000 x g) carried out before preparation of the microsomal fraction (100,000 x g). Worms exposed to oil-contaminated sediment for two days showed induction of aryl hydrocarbon hydroxylase activity.

DISCUSSION

The various filter feeders, grazers and deposit feeders of the meiofauna and macrofauna utilize the organic matter of the sediment. In this process they may expose deeper sediments to water-sediment interface where there is more microbial activity. The pathway of polycyclic aromatic hydrocarbon metabolism in animals involves hydroxylation and subsequent conjugation reactions, but no ring cleavage, so that the excreted products retain the aromatic ring. Bacteria, using aromatic hydrocarbons as a carbon source, carry out ring cleavage after hydroxylation with eventual degradation to carbon dioxide. Bacteria would also completely degrade the hydrocarbon metabolites excreted by benthic animals. The involvement of both microbes and animals in hydrocarbon degradation in marine sediments may be similar to their symbiotic association in recycling organic material in terrestrial sediments.

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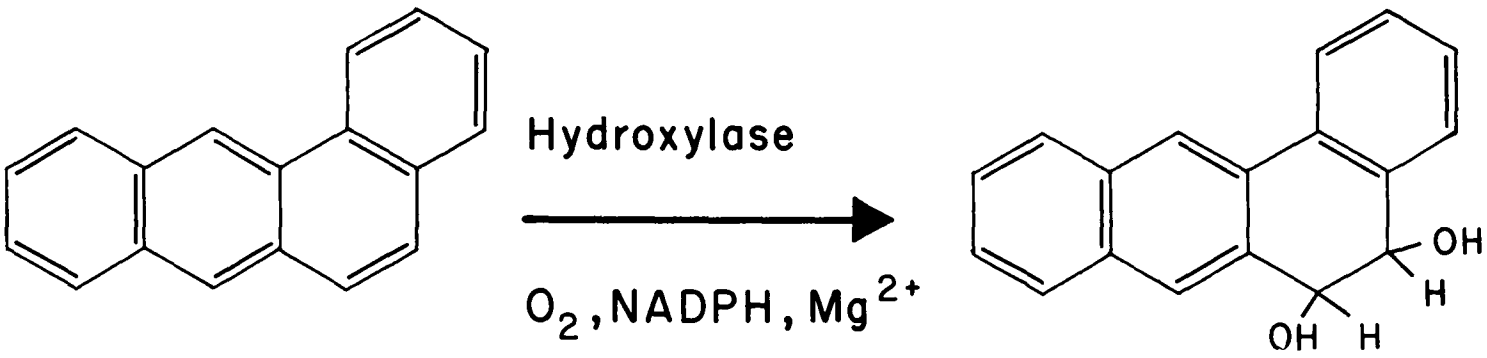
Table 1

Tissue Distribution of Aryl Hydrocarbon Hydroxylase in the Polychaete Worm, Nereis virens

The methods used for the extraction and assay of the polychaete tissues were similar to those described by Nebert and Gelboin[39]. One unit of hydroxylase activity is defined as that amount catalyzing the formation in a 60 minute incubation period at 28°C of hydroxylated product causing a fluorescence equivalent to that of 1×10^{-12} moles of 3-hydroxybenz(a)pyrene.

Tissue	Specific Activity (enzyme units/mg protein)	Total Activity (enzyme units)
Pharynx	0	0
Esophagus	0	0
Upper portion of intestine	35	150
Lower portion of intestine	230	13,200



Benzanthracene

trans-5,6-Dihydro-5,6-dihydroxybenzanthracene

Figure 1 Metabolism of benz(a)anthracene by tissue extracts from the intestine of the polychaete worm, Nereis virens

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DISCUSSION

LIGHT: Have you observed the role of the protozoans relative to degradation of oils? There has been some work done in Wales. Andrews and Floodgate observed ciliates ingesting oil, but they also found that bacteria were in the oil globules. I was wondering whether you had done any work with protozoa at all?

LEE: No, we have not done any work with protozoans. We have done some work with tintinnids up in Canada, pelagic tintinnids, but no work with sediment protozoans at all. I believe George Floodgate didn't see anything either in terms of metabolism by protozoa. They did sequester hydrocarbons but there was no evidence that they actually metabolized the hydrocarbons.

NEFF: Do you have any real evidence of accumulation of hydrocarbons from sediments, other than the fact that they induce the enzymes? Have you seen uptake from sediments directly into the animals?

LEE: We have seen uptake into the gut, and I don't believe we have seen any of what you would call accumulation in tissues. It seems to be more of a passage through the gut. I don't think we have done enough experiments to say whether there actually is accumulation of hydrocarbons within the tissues of the polychaete.

NEFF: Well, this is what we see. We can account for all the accumulated hydrocarbons in terms of what is actually in the gut contents. So you cannot say that the animals are absorbing anything.

LEE: Right. I wasn't implying that they were accumulating it.

GORDON: I really have more of a comment than a question. Dr. Vandermeulen of our Laboratory has also been working with the aryl hydrocarbon-hydroxylase system and, as you said, has not been able to find it in mollusks. He was looking in particular, at Mya arenaria exposed to the oil spill in Nova Scotia for about five years and couldn't find any activity there as well.

So it doesn't seem to be inducible either in that regard.

LEE: I think that the bivalves possibly have such an efficient filtering system that they just are pumping out hydrocarbon as a part of, you might say, detoxification system. They remove a great deal of it just by filtering such huge volumes of water that, rather than using a metabolic system, they seem to pump out most of the toxicants.

I think there is really quite a bit of evidence now, as you mention. Several laboratories have looked unsuccessfully for hydrocarbons metabolized by bivalves. In our laboratory, we allowed bivalves to take up radioactive napthalene, and then they depurated most of it. But a small percentage was retained, and over a three-month period, there was no evidence that metabolites of napthalene were produced.

ELEMENTS OF MASS BALANCE RELATIONSHIPS FOR OIL RELEASED IN THE MARINE ENVIRONMENT

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ABSTRACT

The composition of oil released in the marine environment and the processes and parameters which influence the composition, quantity and spatial distribution of hydrocarbons in the environment are the controlling factors in determining the fate of an oil spill. Rate data derived from the literature were used to construct a three dimensional computer simulation model in order to obtain mass balance calculations for oil released under a variety of conditions. The importance of more than 15 different processes and 40 environmental parameters in influencing the fate of an oil release depends on the interactions which take place under the specific conditions that describe a particular situation.

INTRODUCTION

Petroleum hydrocarbons are injected into the marine environment by a variety of pathways including natural seepage, pipeline leaks, well blowouts, tanker operations, runoff, discharge with sewage effluent and motor boat activity. The input from natural seepage in some areas has probably taken place during a considerable length of geological time although good evidence to document this is not presently available. Emery[1], for example, detected detrital tar fragments in cores obtained from the uppermost layers of sediment in the Santa Barbara Basin, California. The time of deposition of this material was at least several thousand years before the present time and is indicative of natural seep activity in that area long before man commenced utilizing petroleum as a major source of energy. The input of petroleum hydrocarbons to the marine system from the other sources increased as this form of energy became a more significant factor in the industrialized world. Within the last decade there has been an increasing concern about potential problems associated with the introduction of petroleum hydrocarbons to the ocean. This concern is directed toward the influence on the biota and the somewhat equivocal area that includes recreational

considerations, asthetics and quality of life. Studies involving various aspects of the problem have also increased commensurately.

Previous work can be classified in the general categories of basic research, studies of oil spill incidents and studies of specific aspects of petroleum in the environment. Some attempts to synthesize the data and form conclusions from the latter two types of studies were not particularly successful because the results were not consistent. Part of the problem could be attributed to poor analytical work or inadequate supporting information and the ensuing confusion and criticism led to additional studies. However, additional work quite emphatically pointed out that the problem was exceedingly complex.

The fate of a particular oil spilled depends on a combination of factors such as type of oil involved, volume released, residence time in a given area of the environment and on the environmental conditions at the site of the spill. The large number of variable factors involved and the significant influence of environmental conditions created a situation wherein comprehensive general principles could not be readily defined from the results of a few major studies. Within the general classification of previous work that was mentioned previously, one can further subdivide the emphasis into studies on fate and on effects of petroleum hydrocarbons in the environment. Although the interest in studies concentrating on the effects of petroleum hydrocarbons on the biota may be of prime importance from an environmental viewpoint, it is necessary to acquire an understanding of the fate of oil after it is released in order to formulate effective experimental plans to study the affects of various petroleum components on the biota.

Our review of the available literature[2,3] indicated that the development of a general three dimensional computer simulation model based on mass balance concepts would provide a means of organizing the data from a large variety of studies to gain a better understanding of the problem. This organizational framework would also provide a method for outlining the gaps in the present knowledge and could assist in predicting the fate of an oil spill under given conditions at a particular location. The following description of the approach and concepts used to develop a mass balance simulation model for the fate of released oil in the marine environment outlines the basic framework of this work.

APPROACH

The basis for the design of the model was to emphasize mass balance relationships by accounting for the type and amount of hydrocarbon components released in the environment for as long as possible after an oil spill. To do this the oil was characterized by carbon number and chemical class. In addition, the environment was subdivided into five major divisions which represent well-defined natural units. The site of injection for the majority of oil into the environment is at the water

surface although other avenues of injection are known to occur. For example, oil from an offshore well blowout or pipeline leak usually enters the water column before it reaches the water surface. In order to maintain limits on the number of computations and to simplify the situation somewhat, the initial design of the model was based on a release at the water surface.

One area of particular interest is the movement or trajectory of an oil slick on the water surface. This factor was also a major consideration in the model design because it involved a decision on the method for denoting spatial variations within the reservoirs. A Lagrangian approach, utilizing trajectories within reservoirs, was selected as the means of establishing three dimensional variations. This approach was favored over the more common Eulerian approach, which involves specification of variables at the intersections of a spatial grid, because it is orders of magnitude cheaper to run on a computer and because the field measurements available do not warrant utilizing the grid system. Inasmuch as the process calculations affecting the fate of oil are dependent on the ambient conditions at the geographic location of the oil mass, the model has a routine for calculating the horizontal movement of oil in the water surface and water column reservoirs. This routine also serves as a means of transferring oil to the nearshore reservoir and determines contact with the shoreline.

In order to attempt mass balance calculations one must establish the factors which modify oil and the rate at which these modifications take place. It is evident that a large number of variable factors affect the fate of oil with respect to time after release.[2,4] The most rapid changes in the character of an oil spill occur during the initial stage of a spill and the rate of change generally decreases with time. This means that attempts to simulate oil spills must utilize short calculation intervals, on the order of a second, during the early history of a spill. However it is also desirable, from the standpoint of computer costs, to increase the interval between calculations if the variations take place over periods of hours. These considerations imply that the changes which take place can be represented by calculations for the computational time unit selected. An advantage to a simulation model based on mass balance relationships is that one can strive to account for the amount and type of oil in the different reservoirs of the environment. This approach also allows one to check the validity of the computations by comparing the values with available measurements from actual spills.

OIL COMPOSITION

Crude oil is a complex mixture of several tens of thousands of compounds. Even refined products, which are processed to obtain selected portions of crude oil, are complicated mixtures that have not been completely defined.[5] This situation poses a difficult problem in terms of establishing a classification scheme that is amenable to mass balance calculations. The scheme that was selected reflects the type of information

that is usually available from chemical analyses and represents the processes involved in determining the fate of oil. It is a two part classification based on chemical classes and on carbon number. Previous reviews[2,4,6] show that environmental factors are extremely important in determining the fate of oil released at sea. Furthermore, many of the natural processes acting on the oil do not act uniformly on all fractions. This preference for certain fractions also occurs at widely varying times in the life history of an oil mass. The four chemical classes that were selected as being representative of the major processes involved are: paraffins, naphthenes, aromatics, and asphaltics.

The second classification was based on carbon numbers because some of the processes affect a particular range of molecular weights to a greater degree than they affect the entire spectrum of petroleum hydrocarbons. In addition, carbon number assignments usually can be made from distillation boiling point or gas chromatographic information. Most of the work done on elucidating the chemical composition of petroleum has been directed toward the light or volatile fraction because the analytical techniques available are more applicable to this type of material than to the heavy or more residual components.[5] The reaction rates for modification of petroleum by environmental processes is also faster for the light end of the spectrum than for the higher molecular weight components. Consequently, the carbon number classification scheme usually used in the model is for groups of five carbon numbers up to a maximum of carbon number fifty. All material with a carbon number larger than fifty is presently lumped into one group. This two part classification scheme yields a 4 x 11 or 44 unit matrix which is accounted for during each computational step used in a model run.

RESERVOIRS

The first step in organizing the rate information obtained from the literature was to segregate the available knowledge into simplified units. This was done by subdividing the environment into the following distinct units: water surface, water column, bottom sediments, atmosphere and the nearshore zone. The boundary of the water surface reservoir extends downward from the air/water interface to a maximum depth of ten meters. Below this, the water column reservoir extends downward to the sediment/water interface and the bottom sediment reservoir represents the area from the water/sediment interface downward. In the latter instance one is usually interested in the upper few centimeters of the sediment layer.

The fifth, or nearshore, reservoir is the area between the surf zone and the winter berm. Petroleum hydrocarbons which are introduced to the environment in the water surface reservoir may be transferred to any one of the other reservoirs by means of the processes which act on the petroleum. One example involves material, primarily the light hydrocarbons, which is evaporated and transferred to the atmospheric reservoir.

This is principally a fractionation process wherein the light hydrocarbons go into the atmospheric reservoir and the residual or heavier components temporarily remain in the water surface reservoir.

The water surface reservoir is the most complex of the various compartments or reservoirs considered in the model and the complexity is primarily a reflection of the large number of processes and parameters that are operative in that area. A portion of the hydrocarbons, mainly the light hydrocarbons, can also enter the water by the process of dissolution. The initial transfer from the water surface to the upper layer of the water surface reservoir usually takes place during a short period of time following a release of oil. If high energy conditions exist, such as at the time of high winds and large waves, a portion of the total fraction of the hydrocarbons can be driven down into the water below the surface by mixing. Some of this material can then be transferred into the water column below by the process of settling. If the downward trajectory through the water column is maintained, oil particles can then be transferred to the bottom sediments. This process of transferring from the water surface to the water column and eventually to the bottom sediment reservoir can also be accomplished by a variety of other factors or processes which are described in greater detail in later sections.

The computational scheme of the model follows a similar organization to account for the material that is transferred from one reservoir to another reservoir or is lost from the system. In some instances the processes which can potentially act on the hydrocarbons are different in some of the reservoirs but in other cases the processes are similar. For example, the process of dissolution can take place in the water surface, water column, bottom sediment and the nearshore reservoirs. Although a process may act on the oil in several reservoirs, such as the last example for dissolution, the rate at which this reaction takes place may be different in the various reservoirs. The intent of this design is to characterize the history of the various fractions of petroleum after they are released at the water surface up to the time that they are removed from the environment. It is also a bookkeeping method to describe the amount of the various fractions in each of the subdivisions of the environment at a given instant in time after oil has been released at the water surface.

PROCESSES AND PARAMETERS

Mechanisms that cause modification of oil composition and properties of oil or result in a transfer of oil between reservoirs are defined as processes. These mechanisms include evaporation, dissolution, mixing, advection, diffusion, adherence and more than a dozen others. The rate or effect of these processes are influenced by environmental agents called parameters. Some examples of parameters are: air temperature, water temperature, wind speed, wave height, sediment grain size, and

composition of the oil. Computer simulations of an oil spill involving various processes requires information about the initial oil release such as geographic location, oil type, and amount of oil released. In addition, the values and time variations of certain environmental variables are necessary.

A comprehensive simulation of an oil spill involves specifying the initial environmental conditions and all subsequent environmental changes which occur during the course of a particular incident. After this information is supplied as input to the model, the changes which occur with respect to time are accounted for during the course of the computations. The magnitude of change brought about by processes acting on the oil in the environment are represented by algorithms. The changes which the process algorithms produce reflect a response to the pertinent environmental parameters. Accordingly, these algorithms produce changes in the model which represent the quantity, character, and physical location of an oil mass with respect to time. A complete outline of the operative processes and parameters is beyond the scope of this paper. Therefore, only a representative portion will be mentioned as a means of indicating the major interactions that determine the fate of oil released in the marine environment.

The greatest number of interactions occur in the water surface reservoir and several processes commence to act on the oil immediately after a release at the water surface. Spreading is one of the initial processes and it's rate is influenced by parameters such as composition of the oil, specific gravity, temperature, viscosity, and surface tension. Spreading then influences the surface area of the oil, which in turn affects the thickness of oil, evaporation, dissolution, and microbial degradation. The mixing of oil into the water, emulsification and photochemical degradation are controlled somewhat by the thickness of the oil. Likewise the process of diffusion is influenced by the amount of material made available through the process of dissolution. As mentioned previously, some parameters are integral components of more than one process. For instance, oil composition influences the processes of dissolution, spreading, evaporation, and in some cases microbial degradation. The physical energy supplied to a system by winds and waves plays an important role in the processes of evaporation, mixing, and emulsification. Changes brought about by the process of microbial degradation can first of all be categorized by the density and characteristics of the microbial population. The dynamics of this situation are influenced by such parameters as temperature, nutrients, dissolved oxygen and composition of the oil. Superimposed on this general outline of processes affecting oil on the water surface is the process of advection. Three parameters which affect the movement of oil on the water surface are: wind speed and direction, water current speed and direction, and tidal currents.

Fewer processes are active in the water column reservoir as compared to the water surface reservoir. Those processes in the water column which tend to produce the greatest changes include: diffusion and transport, sedimentation, adherence, and

microbial degradation. The magnitude of change brought about by these processes is commonly a function of the water depth because the residence time of a particle of oil is related to the transit time through the water column. Important parameters in this reservoir are: water current speed, density of the oil particles, size and settling rate of oil particles, water density (which includes temperature and salinity factors) and water depth. Parameters influencing microbial degradation in the water column are essentially the same as those influencing microbial degradation in the water surface reservoir. These include: surface area of the oil, water depth, dissolved oxygen and nutrient concentrations, and population density.

The rate of reactions which take place in the bottom reservoir is generally slower than in any of the other reservoirs. However, the range of activity is extremely large because highly dynamic and well oxygenated environments may produce significant changes in time periods which are on the order of days or weeks. On the other hand, zero energy conditions in an anoxic environment, such as a restricted deep water basin, tends to enhance the possibility of preserving petroleum hydrocarbons incorporated in the bottom sediments. Important processes in this reservoir are: transport, dissolution, diffusion, bioturbation or mixing of sediments, burial and microbial degradation. The first three processes are influenced by the activity of the bottom water. Mixing of hydrocarbons with bottom sediments is essentially a function of the type and density of the benthic biota although the water current dynamics at the sediment/water interface can be important. Burial of petroleum results when there is a high rate of detrital sediment accumulation or when burrowing benthic organisms transport hydrocarbons into the underlying sediment. The most important parameter controlling the rate of microbial degradation of petroleum in the bottom sediments, assuming that organisms capable of degrading petroleum are present, is the availability of oxygen. In a normal marine situation the concentration of dissolved oxygen is higher at the sediment/water interface than it is in the interstitial water. Therefore, the highest rate of microbial degradation in the bottom reservoir is in the uppermost layer of bottom sediments.

The nearshore environment can be an extremely dynamic area if the physical energy supplied by wind, wave and tidal activity is high. This area did not receive all of the attention it deserves in this study because time constraints and other factors dictated that most of the initial effort be expended on the water surface and water column reservoirs. The processes which were considered are: oil deposition, burial, evaporation, photochemical degradation, microbial degradation, entrapment and mixing. Some of the important parameters affecting these processes are: tidal range, beach slope, surface area of oil, wave height, water currents, temperature and microbial population density.

Adequate measurements for developing some of the process

algorithms for the atmospheric reservoir were not available when this study commenced. At the present time this reservoir is treated essentially as a sink in that hydrocarbons transferred to the atmosphere from the water surface or nearshore environments are not returned. Examples of a possible mode of return include precipitation or settling of particles injected by spray and bubble bursting. The present emphasis involves parameters associated with the process of diffusion such as air temperature, wind speed and eddy diffusivity.

ENVIRONMENTAL CONDITIONS

Two factors exert the greatest influence on the reaction rates and magnitude of hydrocarbon transfer between reservoirs following a release of oil at the water surface. Oil composition, the first factor, is important because it defines the type of material available for modification and thereby is an important consideration in determining the influence of each process of degradation during the history of an oil spill. For example, a refined number two fuel oil has a higher proportion of light components than a crude oil or a refined number six oil. If all three types of oil were released under identical conditions in a temperate climate, the percentage of each oil that would generally be available for the process of evaporation would follow the sequence: number two oil > crude oil > number six oil. Patterns for other processes such as dissolution, photochemical degradation or microbial degradation are also influenced by the composition of the oil.

Although the range of variation in the composition of natural and refined oils is very large, the potential effects of variations in environmental conditions are even greater. However, the effect of some variations such as the concentration of suspended sediments and nutrients are not clearly understood. On the other hand, the effect of temperature, wind speed and dissolved oxygen are more evident. Even a cursory knowledge of oceanography is sufficient for one to determine that many environmental parameters are independently variable. An appreciation of the significant influence that some environmental parameters exert on the rate at which processes can modify oil and thereby determine the fate of the various fractions of petroleum allows one to recognize the complexity of the problem.

It is also somewhat dangerous to assume that an understanding of the fate of oil released at sea can be gained by considering "average" conditions. The more realistic approach to this problem is to assign importance to the various processes and parameters after considering the effects of interaction which can take place in a dynamic environment. Those process reactions which involve the light or more volatile fraction of a petroleum will tend to occur earlier in the life history of a spill than the process reactions involving the heavier or residual segments of petroleum. However, any assignment of the order of reactions and more specifically the volume of petroleum that is degraded or transferred to the various environmental

reservoirs following a release in the open ocean is dubious if the environmental conditions at the site of release are not specified. Several general but simple examples of this type of situation are outlined for illustrative purposes.

If a crude oil containing a significant portion of material that is less than carbon number 15 is released at a deep water site under a clear sky with a water temperature of 20°C, air temperature of 30°C, wind speed of 2.5 m/sec and a wave height of 1 meter, then the processes of evaporation and dissolution will remove a large portion of the volatile fraction from the petroleum on the water surface during the first 24-72 hours following the time of release. Subsequently, the processes of photochemical and microbial degradation will exert a greater degree of relative importance in modifying the petroleum remaining at the water surface. Other processes that may be involved in the fate of this particular release are mixing, emulsification and settling.

To further illustrate the importance of changing several environmental variables, consider the following situation. A crude oil identical to the one mentioned in the previous example is released over the continental shelf at a water depth of less than 50 meters under an overcast sky with a wind speed of 10 m/sec, wave height of 3 meters, water temperature of 20°C and air temperature of 20°C. The rate of evaporation in this example will also be high. However, the total amount of hydrocarbons removed at the water surface will probably be less than in the previous example because the greater amount of physical energy imparted to the system by the higher winds and waves will promote the process of mixing. The ensuing transfer of particles of crude oil containing the entire spectrum of available hydrocarbons downward into the water column will increase the significance of dissolution and microbial degradation because of the increased surface area of oil exposed to the water. A high rate of mixing may also transfer relatively unaltered oil to the bottom sediments in shallow water.

The importance of mixing, as just one of the significant processes affecting the fate of oil released at sea, also can be outlined in the last example of this type. In this case the crude oil released is also the same as in the first two examples. The other conditions are: water depth of 200 meters, overcast sky, wind speed of 20 m/sec, wave height of 7 meters, water temperature of 3°C and air temperature of -5°C. In comparison to the other examples, these conditions will reduce the rates at which processes such as spreading, evaporation, and microbial degradation influence the oil at the water surface. In contrast, the effectiveness of the process of mixing could be increased to the point that a major portion of the oil released at the water surface is transferred to the water column. The subsequent distribution and fate of this oil would then depend on factors such as subsurface currents and settling rate of the oil particles.[7]

A host of more subtle variations could exist throughout many parts of the world ocean and the ensuing influence on the fate of an oil release would be more difficult to illustrate.

A considerable amount of work remains to be done on this subject but hopefully the approach used in this study will provide a framework for achieving a better understanding of the fate of petroleum hydrocarbons released in the marine environment.

SUMMARY

The fate of oil released in the marine environment depends on a large number of variable factors that influence the composition, quantity and spatial distribution of hydrocarbons in the environment. This problem was studied by designing a three dimensional computer simulation model that was based on mass balance relationships derived from a review of the available literature. A classification system utilizing the carbon numbers and chemical classes of petroleum as well as five major subdivisions or reservoirs of the environment constituted the basic framework for evaluating a variety of situations. The quantity, character and physical location of an oil mass with respect to time (Figure 1) are highly dependent on the operative processes and parameters in a given situation. Consequently, the fate of an oil release involves a consideration of the interactions which reflect the specific conditions that characterize a particular dynamic environment. The rate at which processes such as spreading, evaporation, mixing, dissolution and microbial degradation determine the history of an oil release depends on the influence of more than 40 different parameters such as air temperature, water temperature, wind velocity, water currents, solar radiation, microbial populations and dissolved oxygen.

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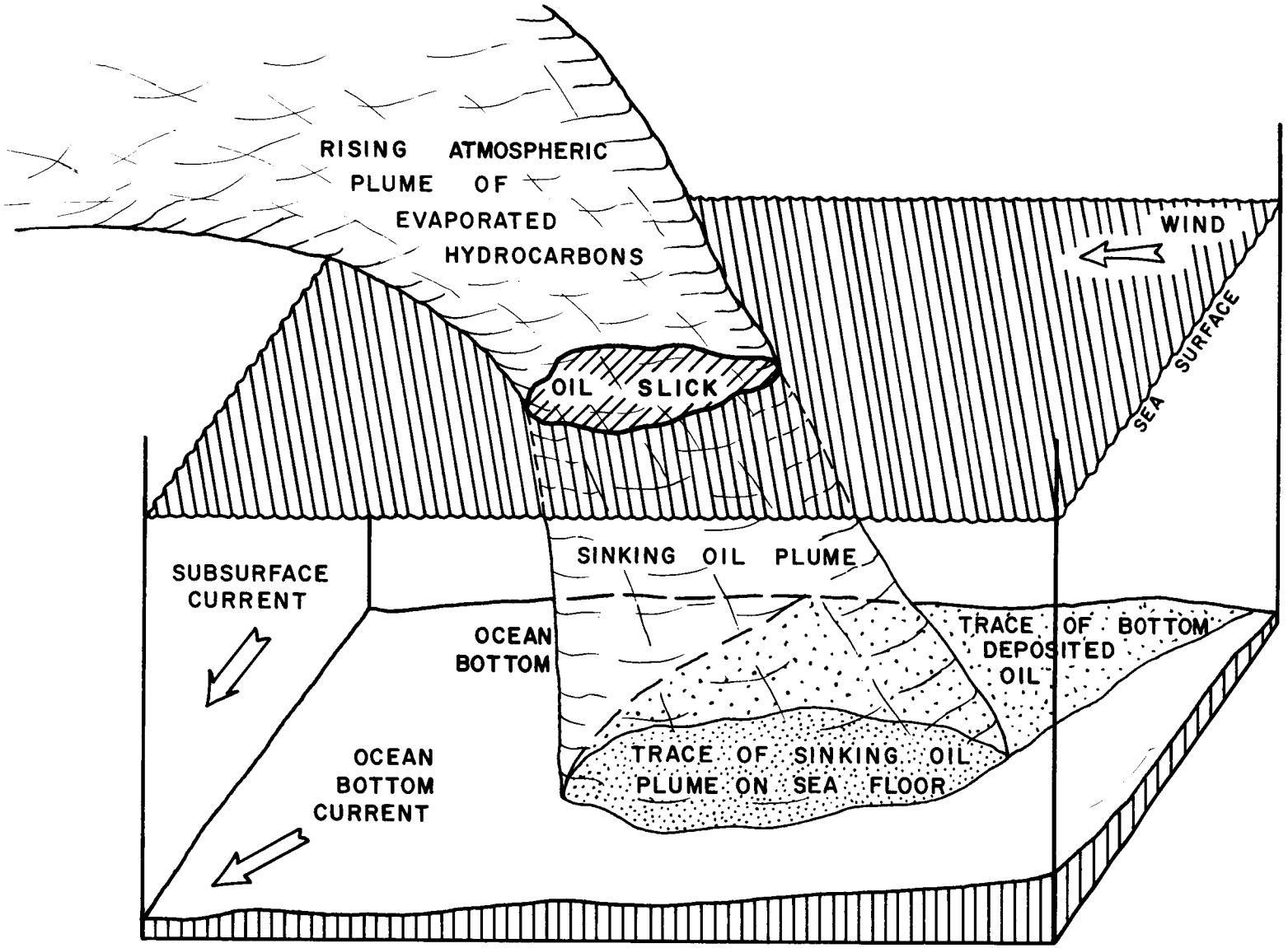

FIGURE 1

BLOCK DIAGRAM SHOWING THE FATE OF AN OIL SLICK

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HYDROCARBON UPTAKE BY DEEP SEA BENTHOS

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INTRODUCTION

The uptake of hydrocarbons by aquatic animals can be examined by comparing the distribution of these compounds in the animals themselves and their environment. An animal's environment is usually taken as the source of its body burden of hydrocarbons since, as far as we know, animals synthesize relatively few hydrocarbons. Since hydrocarbons are only slightly soluble in water, their concentrations in that part of the environment are difficult to measure. Because of this environment data is usually restricted to sediments and neighboring organisms.

This approach contrasts with experimental uptake studies which can provide more precise information on the exact routes and rates of uptake. However, survey data can give a much broader picture. It can include environments from which it is currently very difficult to maintain experimental animals and can measure what actually happens in nature where complications not considered in experiments may be the determining factors in uptake.

We have already published survey data, mainly on non-aromatic hydrocarbons in marshes[1] and shallow coastal waters[2]. In general, although the body burden in benthic animals does reflect the hydrocarbons found in the sediments they are living on and in, some significant differences do exist. Animals often exhibit a discrimination against the heavier end of the range of naphthenes found in chronically polluted sediments. Whether this is due to the routes and mechanisms of uptake or to subsequent metabolism by the animals we do not know, but suspect the former. Immediately after the West Falmouth marsh system was polluted by a massive spill incident, a general lack of discrimination against the pollutant hydrocarbons occurred. This was followed by the development of a very high degree of discrimination in some species, while others exhibited no change over the four years study period.

Analyses of the Sargassum community seem to indicate that all members of this surface living, pelagic community are contaminated with petroleum products in quantities sufficient to mask the occurrence of

natural hydrocarbons, except in the algae themselves[3]. We did not analyze tar balls for this study but from the data of Butler et al.[4], we can conclude that, in these animals we again see a preferential inclusion of the lighter fraction of the contaminant naphthenic hydrocarbons.

In this paper I present the preliminary results[5] of analyses of some sediment and benthic animals collected from 5500 m to 5800 m depth at about 25°N, 62°W at the northern part of the Nares Abyssal Plain on cruise AII-85 from Woods Hole Oceanographic Institution in September 1974.

METHODS

Sediment was collected with 8 inch diameter sphincter cores. Animals were collected with beam trawls in tows which lasted several hours. The trawl mesh was not coated with preservatives and great care was used to keep it clean. The animals were removed from the trawl upon recovery, washed with ethanol and frozen in solvent rinsed glass containers capped with solvent rainsed aluminum foil. In the shore laboratory, tissues were excised from inside the animal using care to prevent contamination of one tissue by another. Gut analyses included both gut and contents in the case of the fish. In the holothurians we tried to collect only the gut contents without any tissue.

Analytical methods are those detailed in Farrington et al.[6,7,8]. They consist of chemical extraction, column chromatographic separations, gas-liquid chromatography before andafter hydrogenation on columns of varying polarity, and GC-mass spectrograph-computer analysis. Retention indices in this paper are all measured on Apiezon L. In this discussion I will restrict myself to results obtained with that portion of the extract eluted from the chromatographic column with one column volume of pentane - the non-aromatic fraction for unsubstituted hydrocarbons - and to results of Harvey and Steinhauer[9] for DDT's and PCB's.

RESULTS

The saturated hydrocarbons in the surface sediments (Fig. 1) are almost entirely normal alkanes from about 18 to 33 carbon atoms in length. There is a preponderance of odd-numbered chain lengths that closely resembles those found in higher plants, such as those that grow on land or in salt marshes. The C-15 and C-17 compounds characteristic of algae are lacking. The amount present, 0.36 ppm dry weight (Table 1) is one or two orders of magnitude below that found in sediments on the shelf.

The analysis of sediment from the holothurian gut was really an attempt to sample the surfacemost sediment, assuming that is what these animals ingest. Unfortunately, the amount of material was very small and is difficult to distinguish from the blank (Fig. 1), except that the peaks with retention indices of 2211 and 2625 are present in the gut contents and not the blank. As these reflect the predominant peaks in the holothurians themselves, we cannot be certain whether we are seeing

their source in the topmost sediment or contamination of our sample with gut tissue from the cucumbers.

The holothurians resemble each other rather closely in their hydrocarbon content and do not reflect the sediment very closely. The principal hydrocarbons present are a n-C-22 alkene and a branched alkane (Table 2). The second species displays the definite presence of an unresolved complex mixture (UCM) extending from retention indices of about 1700 to 2400 (Fig. 1) which accounts for a large fraction of the difference in total hydrocarbon found in the two animals. This latter cucumber also had slightly more DDT and five times as much PCB as the other. This was one of the few specimens which we analyzed for DDT's before saponification and in which we could distinguish DDT from DDE. We found almost three times as much of the parent compound p,p'-DDT as the daughter p,p'-DDE.

The galatheid showed a very large UCM hump in the chromatogram resulting in a picture (Fig. 2) very similar to that for a degraded tar lump of relatively low retention indices[4], with perhaps a small admixture of sediment hydrocarbons.

The rattail (Nematonurus armatus, the only species for which we have a positive identification as yet) has been the subject of our most detailed analysis. It contained a mean of about 50 µg/g set weight of hydrocarbons mostly in the liver (Table 1).

The gut contents included a mass of small white, egg-shaped objects about 1 cm long and several squid beaks. All were extracted together. The most conspicuous hydrocarbon was pristane (Fig. 2) which was twenty times as abundant as the next most abundant compounds (R.I. 2330 and 1466) which have not yet been identified (Table 2). There were also a number of olefins, n-alkanes from C-24 to 33 in about equal abundance and enough DDE to just show up in the flame detector.

The muscle in contrast contained only a small amount of pristane; the most abundant hydrocarbons were the normal alkanes from C-30 to 26 followed by p, p'-DDE (Table 2). Total hydrocarbon concentration in the muscle was twice as much on a wet weight basis or fourteen times as much on a lipid basis as that in the gut and its contents.

The rattail liver chromatogram showed one major peak, that of p,p'-DDE (Fig. 3), the mass spectrum ion plot which I show in Fig. 4, along with the authentic standard. The next most abundant hydrocarbon in the liver is an as yet unidentified compound containing chlorine, which may be a metabolic product of DDE. The 1 ppm of DDE present in the liver made it the most abundant hydrocarbon in the fish. Unfortunately, we do not know how much was present as the parent compound.

DISCUSSION

There is relatively little one can say with certainty on the basis of the analyses of so few animals but a number of points do emerge. The sediment hydrocarbons appear to have come from plants growing either on or near land. If this is true they have come a considerable distance and have been exposed to chance of degradation for an appreciable period without showing much change in their relative abundance. The fact of their lower concentration in comparison with shallow water sediments could be either to a uniform degradation of all members of the series or to dilution by other types of sediment without hydrocarbons. The lack of the typical algae compounds must mean that they are more readily used by benthic organisms.

The lack of algal hydrocarbons is emphasized by the evidence of rapid transport of organic materials from the surface to the bottom, a distance of 5000 m. Such transport is necessary to explain the large amount of total DDT's found, especially the relatively large fraction still present as the parent compound, which is readily degraded to DDE by organisms and has only a short lifetime in surface waters of the ocean[6]. The petroleum evidence found in one holothurian and the galatheid probably arrived on the bottom as a tar ball either sinking by itself or after having been eaten and carried to the bottom when the animal died and sank.

We see no reason to change our earlier conclusions concerning haphthenic uptake. Although we cannot be sure what sort of hydrocarbon distribution the animals encountered in the tar particles on the bottom, most tar balls on the surface have a distribution which extends farther toward the heavy end[4] than the distribution we found in the benthic animals.

Animals selectively absorb hydrocarbons from their environment. The selection may occur partly by selection of food. If food is a principal source, then our data, especially that for the rattail, indicate the composition is further modified by selective absorption and/or subsequent metabolism. For example, it seems likely that the petroleum compounds we found must have been absorbed through the gut, i.e., eaten as or along with food. If there was some other mode of uptake I would expect to find the oil in all of the animals on the assumption that the contamination is not great enough for some to have induced metabolic pathways for ridding themselves of it. On the other hand, the pristane abundant in the rattail gut is present in the body in relatively small amounts. Either the gut contents we found represent an unusual meal or the fish metabolizes the pristane.

There is a strong predominance in the sediment of odd carbon chain lengths in the alkanes. This is lacking in the fish gut or muscle. I find it difficult to believe that either the rattail or its microorganisms are selectively metabolizing the odd carbon chain lengths. The distribution could indicate petroleum contamination although no strong case can be made for this, especially since in the other cases of apparent petroleum contamination, the alkanes are mostly gone. Perhaps the fish of

their gut flora produce these alkanes in the distribution found. They might also be absorbed through the gills. The n-alkanes found in open ocean waters have a uniform distribution in surface waters and though no data are presented there is no indication that the distribution changes upon approach to the bottom though the total hydrocarbon content of the water does seem to rise in the only such sample analysed[11]. Even if there is an increase in near bottom water as a result of equilibration with the sediment, perhaps the solubilities of the alkanes in water phase is so low that the differences in concentration in the sediments are not reflected in the dissolved fraction.

As far as I am aware these are the first analyses of hydrocarbons in the bodies of animals from the deep waters of any mid-ocean region. They are interesting in themselves and show that even these remotest of animals are not safe from contact with pollutants. They even more clearly illustrate the rudimentary state of our knowledge about the details of hydrocarbon uptake in most animals.

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DISCUSSION

BUTLER: Do you think it is always a good idea to take the gut contents out of animals and analyze them separately?

TEAL: If you are trying to find out what the animal was absorbing from its food, obviously, it is a good idea to take the gut contents out and analyze them separately.

As I pointed out, we didn't really quite do that. We tried, in the case of the holothurians. I think, probably, the best evidence is that we didn't succeed there, either.

Sample	µg/g wet	µg/g lipid	µg UCM / g wet	ng/g wet DDTk	ng/g wet DDE	ng/g wet PCB
sediment 0-2 cm	0.36*			0.09	0.03	2.5
holothurian #1	7.7	2900		na	0.36	1.2
holothurian #2	22.2	11500	7.9	0.37	0.13	6.6
rattail gut	.43	12		na	5.7	3.2
muscle	.89	172		na	31.3	6.0
liver	470.0	1000		na	2610.0	1230.0
galatheid	7.7	470	15.4	na	440.0	86.0

Table 1. Hydrocarbons found in material collected from 5500 to 5800 near 25°N, 62°W at the northern part of the Nares Abyssal Plain.

* - dry weight basis.
na - saponified before analysis so data for DDT not available.
UCM - unresolved complex mixture.

Specimen	Ap.L. R. I.	µg/g wet	Identification
holothurian #1	2211	0.16	n-C22 alkene
	2625	0.33	branched alkane
	2695	0.20	n-C27
	2600	0.07	n-C26
rattail gut	1690	0.44	pristane
	2330	0.02	?
	1466	0.017	?
	2309	0.018	olefin
rattail muscle	3000	0.031	n-C30
	2891	0.031	n-C29
	2800	0.027	n-C28
	2692	0.024	n-C27
	2600	0.020	n-C26
	2174	0.017	DDE
rattail liver	2180	1.13	DDE
	2118	0.34	chlorinated

Table 2. Concentrations and identifications of the most abundant hydrocarbons in some abyssal plain animals.

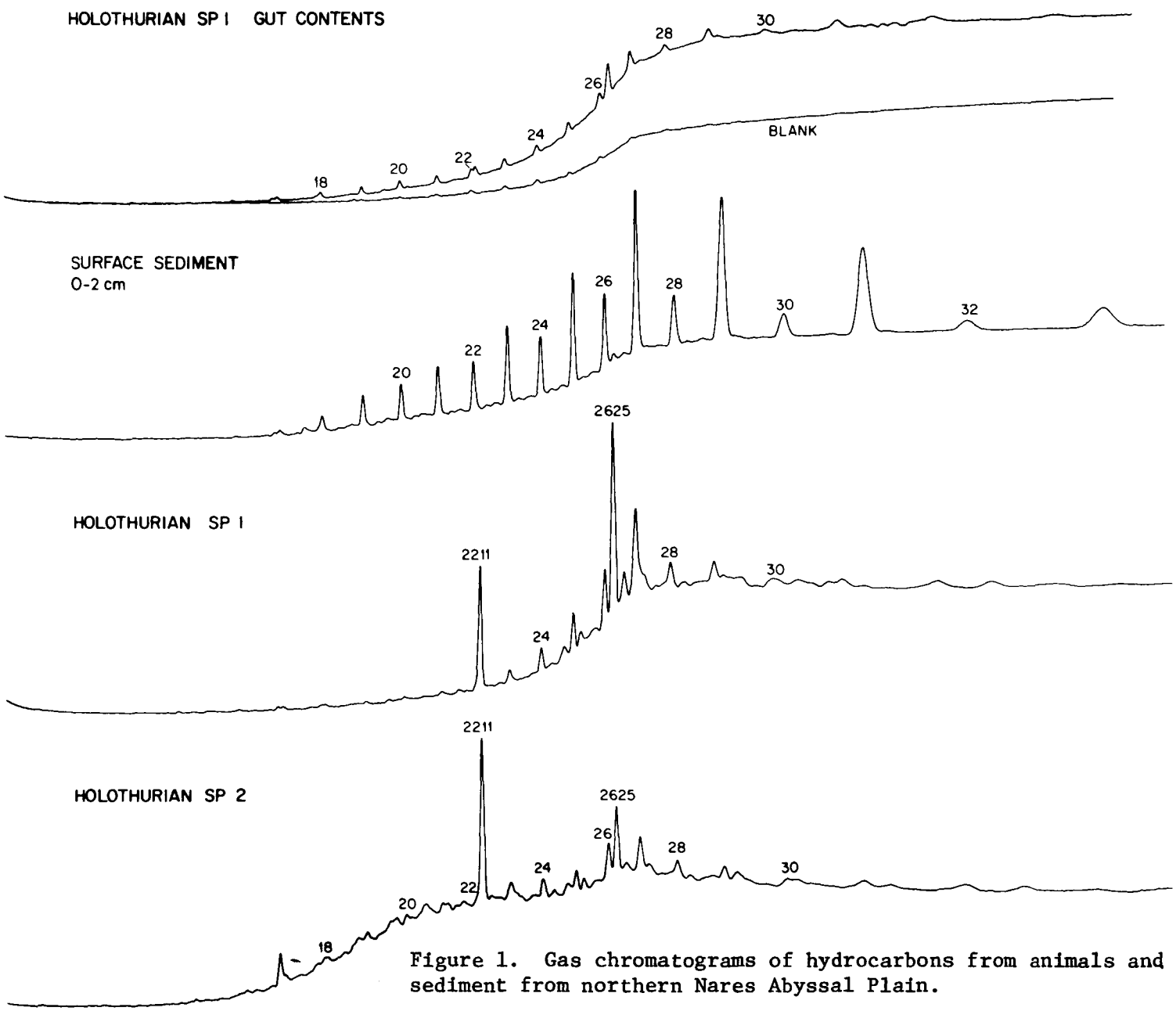

Figure 1. Gas chromatograms of hydrocarbons from animals and surface sediment from northern Nares Abyssal Plain.

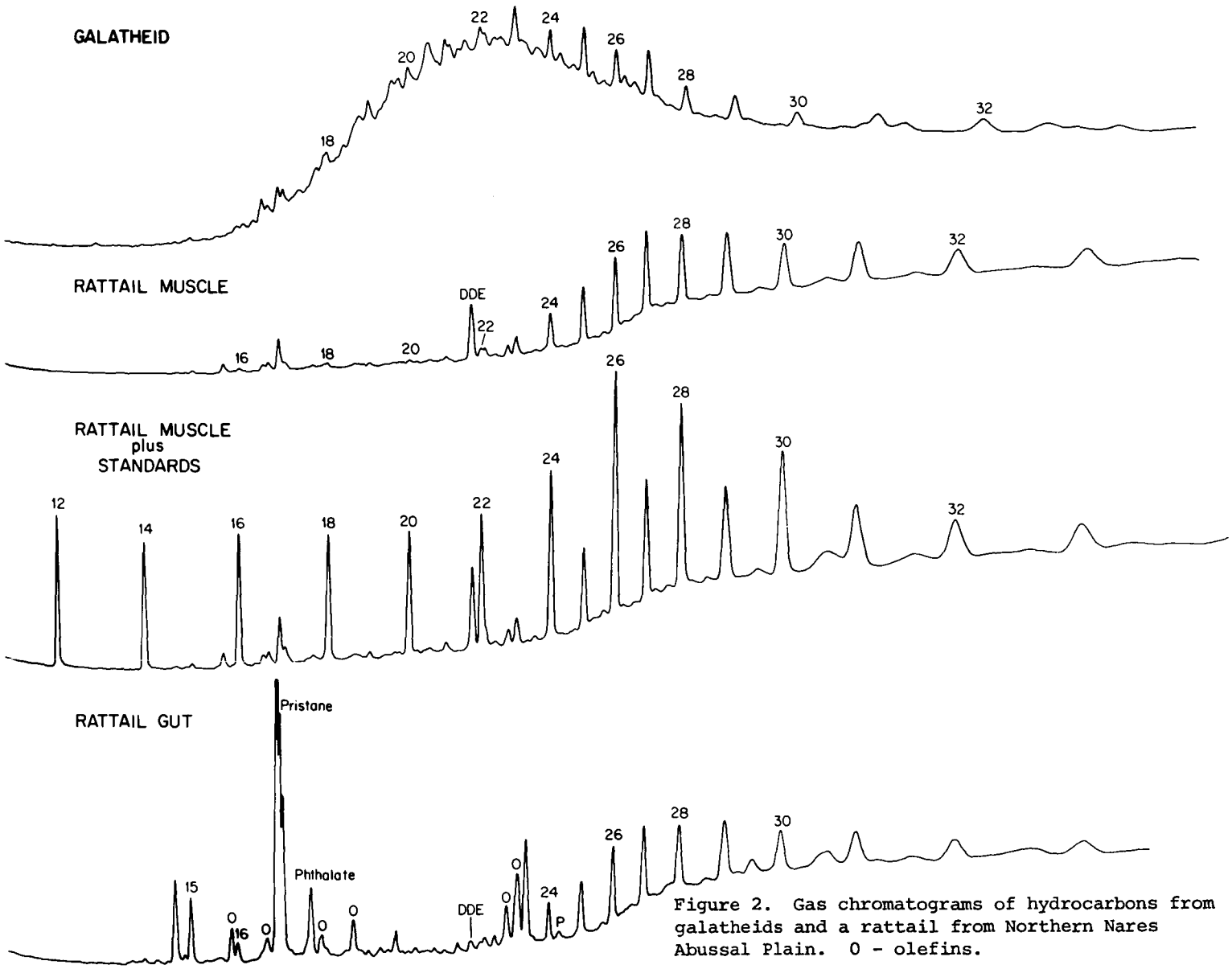

Figure 2. Gas chromatograms of hydrocarbons from galatheids and a rattail from Northern Nares Abussal Plain. O - olefins.

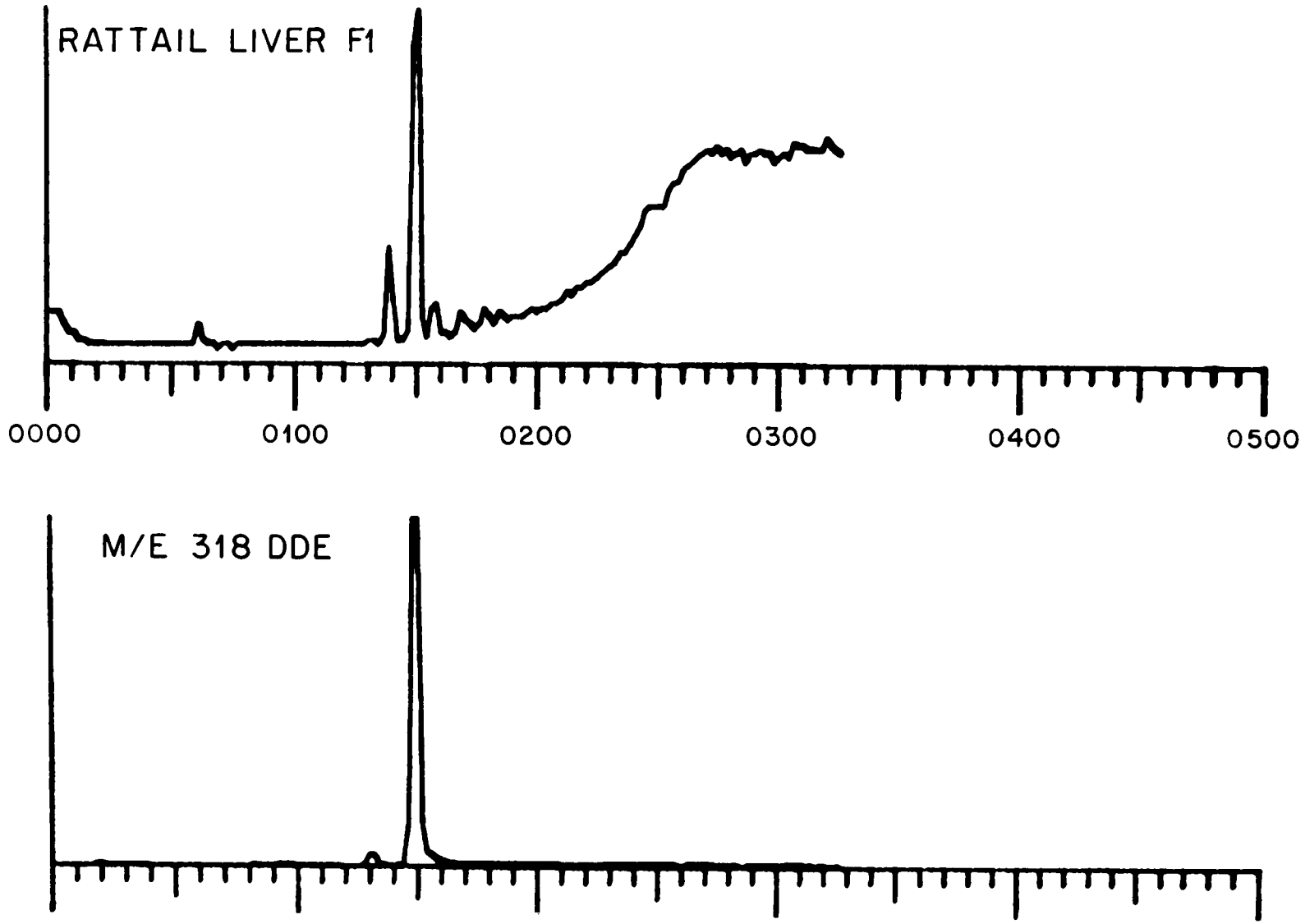

Figure 3. Reconstructed gas chromatograms of hydrocarbons from rattail liver from Nares Abyssal Plain. Top is complete reconstructed GC made with GC-MS-computer system. Bottom constructed with mass range limited to M/E = 318.

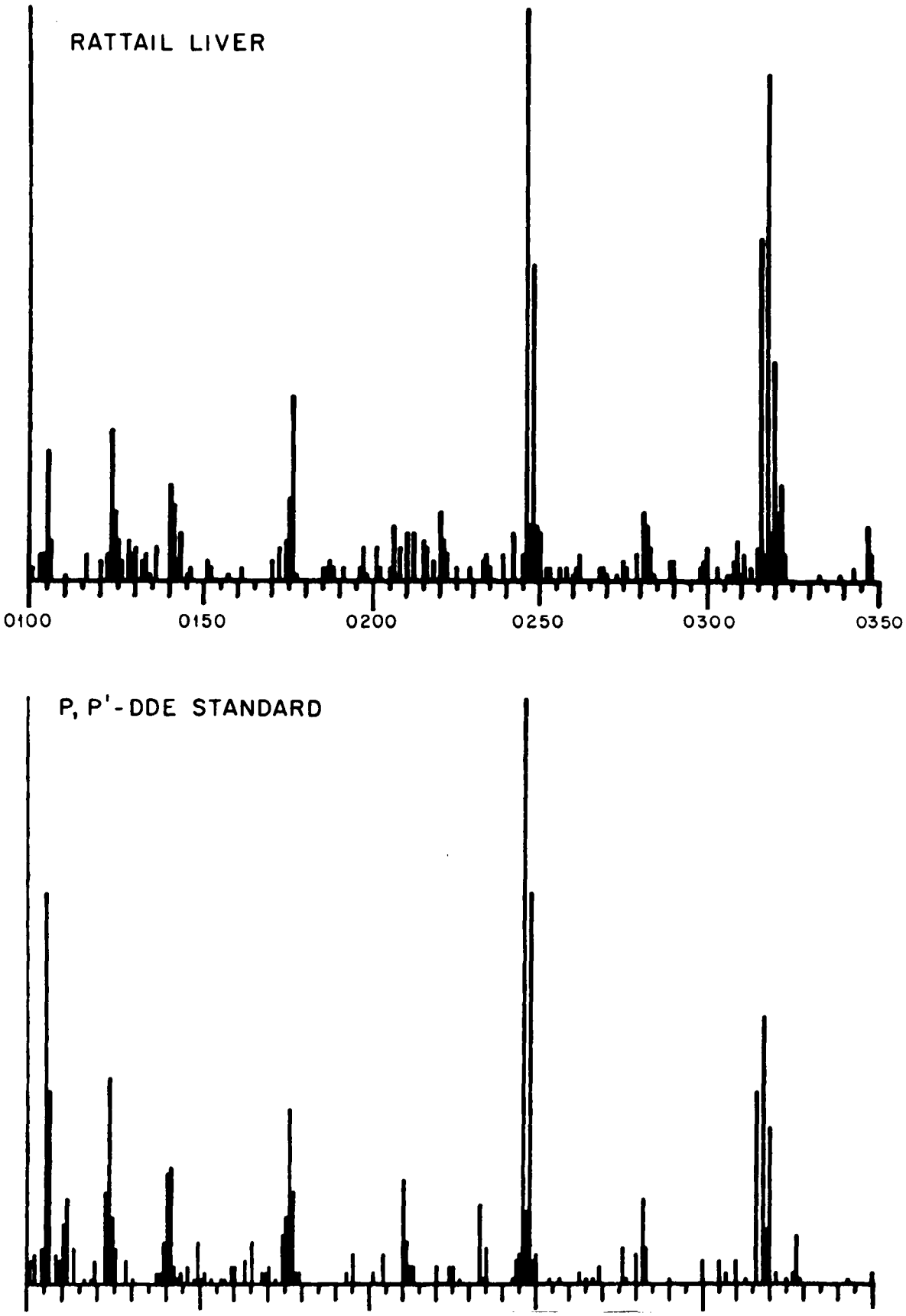

Figure 4. Top-ion plot of principal hydrocarbon from rattail liver collected from Nares Abyssal Plain. Bottom - ion plot from DDE standard.

SESSION III

EFFECTS OF HYDROCARBONS ON BIOLOGICAL SYSTEMS: BEHAVIORAL, PHYSIOLOGICAL AND MORPHOLOGICAL

Chairman
Gilles LaRoche
McGill University

EFFECT OF NO. 2 FUEL OIL, NIGERIAN CRUDE OIL AND USED CRANKCASE OIL ON THE METABOLISM OF BENTHIC ALGAL COMMUNITIES

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ABSTRACT

No. 2 Fuel Oil, Nigerian Crude Oil, and used crankcase oil were studied for effects on the metabolism of benthic algal communities. Exposure depressed net community primary productivity but degree of effect was dependent on the kind of oil and concentration of exposure. Used crankcase oil became associated with the algae in greatest amounts but No. 2 Fuel Oil exerted greatest toxicity. Recovery of community function took place in all instances. Blue green algal development was fostered to varying degrees.

INTRODUCTION

There is an expanding literature concerning the effects of hydrocarbons on algae. Many recent reports describe laboratory experiments in which individual or complex mixtures of water soluble hydrocarbons were studied for effects on the photosynthesis [1,2] and growth [3,4,5,6] of single species. Some [7] have also studied entire oils. In a few instances effects on mixed populations were studied either in the laboratory [8] or in the field [9]. These physiological studies indicated that hydrocarbons present in the water column may have a wide range of effects (both stimulatory and inhibitory) on phytoplankton. However, many workers have noted the difficulty of extrapolating findings from the laboratory to natural environments.

Most post-oil spill investigations have consisted of observations of effects on animals and macrophytes. For marsh areas, interest has usually centered on the effects of petroleum on higher plants [10] with little attention to other primary producer organisms. The contribution of algal primary productivity to the energetics of marsh areas can be significant. Gallagher and Daiber [11] estimated that as much as one third of the primary productivity of a Delaware salt marsh was contributed by benthic algae. It has also been noted that studies of oil spills emphasized the rocky intertidal zone and that sheltered areas where oil may persist have received little study [12]. Although not exclusively, studies have tended to focus on marine forms because of the suspicion that major oil contamination will occur in the marine environment.

In the experiments described here the effects of oil on benthic algal communities were studied. The communities were obtained from a marsh bordering Oldmans Creek approximately 5 km from the Delaware River. The area is flooded with water of extremely low salinity (< 0.5 ppt) and is in relatively good condition as judged by species diversity. Experiments were conducted under conditions as natural as possible and yet permitting control of selected variables.

MATERIALS AND METHODS

General procedure. Communities of benthic algae and underlying sediment were dug from the marsh and transferred to plastic trays (surface area 337 cm^2, 5 cm deep) with small holes (1 mm diameter) drilled in the bottom. The communities were placed in microcosms (two trays per microcosm) in a greenhouse laboratory located approximately 45 min. from the collection site. Microcosms were made from animal trays (45 cm long x 23 cm wide x 13 cm deep) by constructing a drain in the bottom. Four microcosms were held in a water bath (105 cm long x 57 cm wide x 18 cm high). Water from a nearby stream was used to keep the microcosms and reservoirs of Oldmans Creek water at temperatures typical for the season of year.

The microcosms were flooded with approximately six liters of water from Oldmans Creek and drained twice daily to simulate tidal cycles using a system of pumps and solenoid valves activated by timer clocks. Water and air temperatures were monitored using maximum-minimum thermometers (Brooklyn Thermometer Co., Farmingdale, N.Y.). Communities received solar radiation but the photoperiod in the late fall experiment was lengthened by 2 hr. using floodlights and in the summer experiment intense solar radiation was attenuated by the use of gray nylon window screening to simulate light levels reaching the benthic algae under marsh vegetation.

Oil was added to the microcosms to simulate spills by layering the oil on the water while the microcosm was flooded. The algae were exposed directly to the oil on the next drain cycle. The drain was shielded so that the floating slick did not leave the

system on the first emptying and the algae were repeatedly exposed. Nigerian Crude Oil (obtained courtesy of Sun Oil Co., Marcus Hook, PA.), No. 2 Fuel Oil (obtained from a local distributor), and used crankcase oil (obtained from a local service station) were used.

Chemical analyses. The water in the microcosms was monitored according to Standard Methods [13] for total alkalinity, PO_4-P, NO_3-N, NH_3-N, and SiO_2 concentrations. Hydrocarbons dissolved in the water and associated with the benthos were monitored daily or several times weekly by infra-red spectrophotometry (Acculab 2, Beckman Instruments, Fullerton, CA). Water samples (500 ml) were collected as the microcosms drained on one tidal cycle each day and were extracted with 50 ml CCl_4. Concentrations of aliphatic and aromatic hydrocarbons were determined on 32 ml aliquots from absorbance measurements at 2930 cm^{-1} and 3040 cm^{-1}, respectively using standard curves of the test oil in CCl_4. Samples of the benthos (five replicates, 15-200 mg dry weight) were collected at each sampling time, dried at room temperature, weighed and extracted in 5 ml CCl_4. Aromatic concentrations were generally low, and the total values reported are principally aliphatic hydrocarbons.

Biological analyses. At intervals, trays of benthos were placed in Plexiglas respirometer chambers with 21.5 liters Oldmans Creek water. The chambers were closed and the water in them recycle using submersible pumps. Community metabolism was measured by monitoring dissolved oxygen changes electrometrically (Model 3000-S6 D.O. meter with flow-through chamber, Rexnord Instruments, Malvern, PA) with continuous recording of the output (Model M recorder, Leeds and Northrup, Fort Washington, PA). The chambers were similar in design to those of McIntire, et al., [14] and were housed in a plywood water jacket through which water from a stream near the laboratory was passed continuously to maintain temperatures appropriate for the time of year. Measurements of net community primary productivity were made by illuminating the community for 3 hr. with fluorescent lights with a spectrum approximating solar radiation (Duro-Test Corp., North Bergen, NJ) and having an output of 165 uEinsteins/m^2/sec. (Li-COR Quantum Sensor, Model LI 185, Lambda Instruments, Lincoln, NB). The lights were turned off and respiration was measured for 3 hr. (R_3). Gross productivity (P_g) was estimated by summing the net productivity and respiration values and a Photosynthesis/Respiration ratio (P_g/R_3) value was computed. Chlorophyll a concentrations were determined on five 1 cm^2 subsamples from each tray according to Lorenzen [15].

Statistical analysis. The Scheffe Multiple range test was used to test for statistically significant differences in nutrient concentrations ($p=.01$), hydrocarbon concentrations associated with benthos, chlorophyll a concentration, net community primary productivity estimates, respiration estimates, and P/R ratios (all at $p=.05$) between treatments.

RESULTS

No. 2 Fuel Oil. Autumn experiment. No. 2 Fuel Oil was applied at 1:100 and 1:1000 (v:v) oil:water ratios to study effects at different concentration ranges. Both unextracted oil and oil previously extracted with water for 48 hr. to lower the concentration

of water soluble hydrocarbons were used at the 1:1000 ratio. The sediments were initially covered with an algal community dominated by the filamentous xanthophyte Vaucheria with filamentous blue green algae and diatoms (Coscinodiscus, pennate genera) interspersed to a much lesser extent. Water temperature in the microcosms ranged from 6-18 C. Nutrient concentrations in the microcosms are presented in Table 1. Statistical analysis (p=.01) indicated no significant difference in concentrations (averaged over the course of the experiment) between microcosms for any nutrient but PO_4-P. Phosphate in the 1:100 microcosm was significantly different from the control but not from the other microcosms receiving oil.

Hydrocarbons leached rapidly from the applied oil slicks and generated maximum concentrations of approximately 6 mg/l in the water in all microcosms one day after application (Fig. 1). Concentrations of water soluble hydrocarbons declined rapidly and reached those in the control microcosm in three weeks.

Hydrocarbon concentrations associated with the algal communities exposed to the 1:1000 applications were little different from those of the control mat (usually 5-10 mg/g dry weight) despite a visible sheen of oil associated with the mats in the experimental microcosms. Mean daily concentrations as high as 50 mg/g were associated with the community following the first 1:100 application. The mean concentration for the 1:100 microcosm for the period October 19-November 7 was 27.2 mg/g which was significantly different from the mean concentrations in the other microcosms (5.8-8.6 mg/g) over the same time period but no significant difference existed between the other three. (Scheffe test, p=0.05).

Hydrocarbon concentrations were only slightly elevated following the second application of oil on November 9 and subsequently no significant differences existed between microcosms. Rapid oxidation and volatilization of hydrocarbons may have occurred because floodlights were used at this time or because hydrocarbon degrading bacterial populations had developed.

Chlorophyll a concentrations of the communities are shown in Fig. 2. The estimate of total chlorophyll a present on the tray was obtained by multiplying values/cm^2 by the area of algal colonization visible to the naked eye. Mean concentrations for the period following the first 1:000 application were slightly lower than the control (38.8 ug/cm^2 for the extracted oil and 33.7 ug/cm^2 for the unextracted vs. 48.8 ug/cm^2 for the control) and the growth in these microcosms appeared less luxuriant than in the control. The concentration on the trays receiving the 1:100 application for the period October 19-November 7, averaged 29.3 ug/cm^2. These mats became flacid and sank into the sediment so that by the third day of exposure the sediment appeared bare although the texture of the mat could be felt on sampling. Microscopically the Vaucheria on the 1:100 trays appeared both plasmolyzed and ruptured. Statistical analysis (p=0.05) indicated the data could be grouped into two subsets within which there was no significant difference: (1) the control and the two 1:1000 applications, and (2) the microcosms treated with oil. Only the control and 1:100 applications were significantly different. Following the second application of oils data were in same range and the same conclusions were drawn from statistical analysis.

Seventeen days after the experiment started small patches of algae were visible on the 1:100 tray but the dominant recolonizing organisms were blue green algae of the genera Oscillatoria, Schizothrix, and Microcoleus and several diatom genera. The communities in the other microcosms were still dominated by Vaucheria although it was in poorer condition in the 1:1000 U microcosm and Schizothrix and pennate diatoms were more important in the 1:000 E community than they were initially.

Metabolism estimates were made at 13-17 C but for the sixth and ninth measurements which were made at 8-10 C. Net community primary productivity was similar for the control and 1:1000 applications (Fig. 3) and ratios of photosynthesis to respiration (P_g/R_3) were invariably >1.0 indicating that autotrophic metabolism overrode the respiratory demand of the community and there was a net addition of organic matter to these communities through photosynthesis. Three days after the 1:100 application there was no net community primary productivity and the P/R ratio was < 1.0; these differences were statistically significant from the other communities ($p=.05$). Following the second application patches of blue green algae were on the 1:100 tray and P/R values ≥ 1.0 were obtained. There were no statistically significant differences between treatments, however, the absolute value for net community production on the 1:100 treatment was significantly lower than for other communities. Mean respiration values for the different treatments in periods following each oil application were not different statistically.

No. 2 Fuel Oil. Winter experiment. A similar experiment was conducted during the winter months by applying No. 2 Fuel Oil at 1:1000, 1:500, and 1:100 oil:water ratios. Water temperature was 0-12 C following the first application of oil in January and 2-15 C following a second application on March 9. Air temperature was 8-45 C in the greenhouse following March 9. The sediments were bare when collected although diatoms were present in them. Nutrient concentrations were measured during the period March 13-March 26 when visible algal development had occurred. Total alkalinity was the only chemical species for which a significant difference in concentration occurred between microcosms ($p=0.01$). The control was significantly different from the 1:1000 and 1:500 application but not the 1:100 microcosm.

Concentrations of water soluble hydrocarbons were maximal 3-4 days after the first application (Fig. 4); the value for the 1:1000 application was less than before but that for the 1:100 application was much greater. Concentrations declined to the level found in the control microcosm in about five weeks. Concentrations obtained after the second application on March 9 were not as high which may be the result of warmer water and air temperatures and acclimated bacterial populations.

Hydrocarbon concentrations associated with the benthos were only half those in the previous experiment at equivalent applications. Less oil was associated with the benthos when algal mats were absent or of small proportion. Following the first application statistical significance could be attached to the difference in concentration between the control and 1:100 treatment.

Mean hydrocarbon concentrations could be grouped into two subsets: (1) control, 1:1000, and 1:500 treatment, and (2) 1:500 and 1:100 treatment. Following the second application the 1:100 treatment was statistically different from all others ($p=0.05$).

Approximately half way through the experiment patches of algae grew on the sediments which were visible to the naked eye as a thin film but aerial estimates could not be made. The development was first noted on the control trays and then on the oil exposed trays in the order 1:1000, 1:500, and 1:100. Vaucheria and Oscillatoria were dominant in the communities. Chlorophyll a concentrations (Fig. 5) reflected the sparser colonization compared with the fall experiment (11.5 ug/cm^2 vs. 50 ug/cm^2 for the control microcosm). After the second oil application a decrease in chlorophyll a concentration occurred. The means of all points for the periods following each application showed no statistically significant differences between microcosms ($p=.05$).

The metabolism of these communities is compared in Fig. 6. Measurements were made initially at 3-8 C. Because water temperatures were 10-13 C during the first measurement when algae were visible on the trays, all subsequent metabolism measurements were made at temperatures in this range. Differences between the mean net community productivity and P/R values for each treatment for the periods following oil applications were not statistically significant ($p=.05$). However, the influence of oil exposure on the development of algal populations was reflected in metabolism measurements. There was no net oxygen evolution at the start of the experiment in any microcosm. Net oxygen production and P/R ratios > 1.0 were obtained at the same time in the control and 1:1000 oil treatment where hydrocarbons were generally 0-5 ug/g dry weight. The 1:500 treatment (generally 3-7 mg/hydrocarbons/g sample, maximum 10 mg/g) and 1:100 treatment (generally 3-15 mg/hydrocarbons/g sample, maximum 20 mg/g) introduced longer lag periods before autotrophic metabolism dominated. Respiration rates between microcosms were not different statistically; thus, the results represent primarily effects on the algal photosynthesis.

Comparison of No. 2 Fuel Oil, Nigerian Crude Oil, and used crankcase oil. Summer experiment. The effects of oils on a Vaucheria dominated community under the same environmental conditions were compared. The oils were applied at a 1:1000 oil:water ratio because the thickness of the slick in the microcosms and the concentrations generated were considered more environmentally realistic. Water temperature ranged from 14-31 C and air temperature was 33-52 C. In this experiment, duplicate microcosms (series I and II) were used with each oil. The Scheffe test ($p=0.01$) indicated a significant difference in concentration for only one nutrient; total alkalinity concentrations in microcosm series I (Table 1). This data could be grouped into two subsets within which no significant differences existed (Control, Nigerian, crankcase) or (Nigerian, crankcase, No. 2); the concentration in the No. 2 Fuel Oil microcosm was significantly greater than in the control.

Maximum concentrations of water soluble hydrocarbons in experimental microcosms were considerably lower than in previous experiments (Fig. 7) which presumably resulted from higher water and air

temperatures. Concentrations declined to levels in the control microcosm after 2-3 weeks. Hydrocarbons associated with the mats averaged 3.8 mg/g dry weight in the control microcosm (Series I and II combined), 15.1 mg/g in the No. 2 Fuel Oil microcosms, and 16.5 mg/g in the Nigerian Crude microcosms. These elevated concentrations were not, however, statistically different ($p=0.05$) from the control. In contrast, the average hydrocarbon concentration of the algae exposed to used crankcase oil was 54.4 mg/g which was different statistically ($p=.05$) from the other microcosms. Samples were taken from a 1 cm^2 area in this experiment so that data could be expressed on an aerial basis.

Control mats had luxuriant growths of Vaucheria and the chlorophyll a concentrations were higher in this experiment than in the previous ones. Although statistically significant differences in the overall chlorophyll a concentrations could not be demonstrated between treatments the concentration on the mats exposed to Nigerian Crude Oil declined to approximately 50 percent of the initial levels although Vaucheria dominance persisted through the experiment. The concentration in the used crankcase oil microcosm changed little or increased and Vaucheria dominance was unchanged. The concentration on the No. 2 Fuel Oil trays decreased more in microcosm II which was exposed to greater hydrocarbon concentrations than in microcosm I but the Vaucheria mat on both trays was noticeably thinner. By the tenth day of the experiment patches of blue green algae (Oscillatoria, Schizothrix, and Microcoleus) developed to macroscopically visible proportions on the oiled trays (most extensively with No. 2 Fuel Oil) but by the end of the experiment the Vaucheria mat was luxuriant again in all microcosms and blue green algae were not visible to the naked eye.

Net community primary productivity increased through the experiment under all treatments but the levels achieved where oils were applied were lower on a given day compared to the control (Fig. 9). This effect was least pronounced with used crankcase oil even though this application resulted in greatest hydrocarbon association with the algae. No. 2 Fuel Oil retarded productivity the most.

DISCUSSION

The experiments reported here along with those of Copeland and Dorris [16] and Ganning and Billing [17] are among the first to examine experimentally the effects of oil on the metabolism of entire communities. It is of interest that Ganning and Billing reported increased community respiration with increased concentration of oil exposure but in our studies this was not observed.

The simulated spills with No. 2 Fuel Oil at 1:100 oil:water ratios generated concentrations of 20-50 mg hydrocarbons/g dry weight. Prolonged exposure to these concentrations led to complete destruction of mats of Vaucheria. Samples taken from the 1:1000 treatments shortly after application were occasionally in this range and a thinning of the mat occurred in the fall experiment and patchy destruction in the summer experiment but Vaucheria dominance was retained. Recovery (or community

development in the winter run) occurred in all experiments although the communities recolonizing the trays were dominated by different genera (notably filamentous blue green algae). In the summer experiment Vaucheria eventually outcompeted these algae and completely dominated the community as it did originally. However, the initial Vaucheria biomass was greater than in other experiments.

From the experiment with different oils it is clear that concentration is only one factor involved in toxicity. Hydrocarbon concentrations associated with algae exposed to used crankcase oil were higher than the concentration found with any other oil and yet toxic effects were least pronounced of any oil used in the experiment. Qualitative differences in hydrocarbon composition are important. If the toxic compounds were water soluble they must be effective in short time periods or extracted continuously from the slick. Because direct contact with the algae was permitted, lipid soluble toxic substances may have been more readily absorbed. Photochemical and bacterial modifications of hydrocarbons certainly occurred during the experiments and no attempt was made to control volatilization. In some preliminary experiments we have observed that water soluble hydrocarbons at concentrations of 1.0-3.0 mg/l depressed net oxygen production but the effects were transitory. Other experiments are in progress to investigate effective constituents.

The concentration to which the communities were exposed in the 1:100 experiments were greater than those found associated with algae and sediment along the banks of the Delaware River. In one collection concentrations ranged from 0.5-14.1 mg/g dry weight (n=24) and in another 0.8-29.7 mg/g (n=25). Thus, concentrations in the 1:1000 applications were typical of areas in a polluted environment but hydrocarbon composition probably differed.

Algae form an important base of the food web and the benthic algae in shallow water areas may be particularly important because of the role these areas play as breeding and nursery grounds for fish and shellfish. One effect of oil exposure was to alter species composition to varying degrees. Because organisms are related in an intricate food web in which feeding preferences may be important, alteration of species composition at the primary producer level may have an eventual impact on the fisheries of an area [18]. Algae are also important agents in the reoxygenation of water. Although we have used oxygen production as a tool for estimating relative amounts of primary productivity our data show that the reoxygenation of water is impaired on oil exposure.

ACKNOWLEDGEMENTS

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Table 1. Concentrations of selected nutrients in microcosm water.

Experiment	No. Estimates	Microcosm	Concentration (mg/l) (mean ± standard deviation) Total Alkalinity	NO_3-N	NH_3-N	PO_4-P	SiO_2
No. 2 Fuel Oil Autumn (10-11/75)	16	Control	45.3 ± 6.7	1.15 ± 0.18	0.025 ± 0.014	0.017 ± 0.007	
		1:1000 U	48.9 ± 4.6	1.07 ± 0.36	0.037 ± 0.051	0.024 ± 0.010	
		1:1000 E	45.1 ± 4.8	1.08 ± 0.21	0.023 ± 0.017	0.020 ± 0.009	
		1:100 U	44.1 ± 4.7	1.13 ± 0.52	0.077 ± 0.100	0.032 ± 0.017	
No. 2 Fuel Oil Winter (1-3/76)	7	Control	40.9 ± 3.8	1.41 ± 0.22	0.097 ± 0.047	0.026 ± 0	4.2 ± 1.0
		1:1000	30.6 ± 4.6	1.57 ± 0.47	0.059 ± 0.045	0.023 ± 0.004	3.4 ± 1.4
		1:500	30.6 ± 4.9	1.32 ± 0.29	0.081 ± 0.067	0.029 ± 0.006	3.4 ± 1.1
		1:100	31.9 ± 5.6	1.58 ± 0.44	0.082 ± 0.059	0.027 ± 0.007	4.3 ± 1.6
Comparison Summer (6-7/76)	7	Control I	49.1 ± 4.1	0.64 ± 0.24	0.045 ± 0.015	0.046 ± 0.018	3.0 ± 0.6
		Nigerian I	53.6 ± 8.2	0.70 ± 0.16	0.071 ± 0.077	0.045 ± 0.018	2.2 ± 1.0
		Crankcase I	53.7 ± 5.1	0.66 ± 0.15	0.094 ± 0.158	0.046 ± 0.018	2.8 ± 0.6
		No. 2 I	62.7 ± 8.5	0.74 ± 0.26	0.135 ± 0.117	0.053 ± 0.017	2.4 ± 0.9
		Control II	47.7 ± 6.1	0.71 ± 0.16	0.039 ± 0.014	0.054 ± 0.032	2.8 ± 0.4
		Nigerian II	58.7 ± 8.8	0.71 ± 0.16	0.068 ± 0.049	0.051 ± 0.026	3.2 ± 0.7
		Crankcase II	53.7 ± 5.1	0.69 ± 0.15	0.185 ± 0.149	0.049 ± 0.020	2.8 ± 0.9
		No. 2 II	52.9 ± 11.2	0.71 ± 0.21	0.110 ± 0.161	0.054 ± 0.029	2.9 ± 10.0

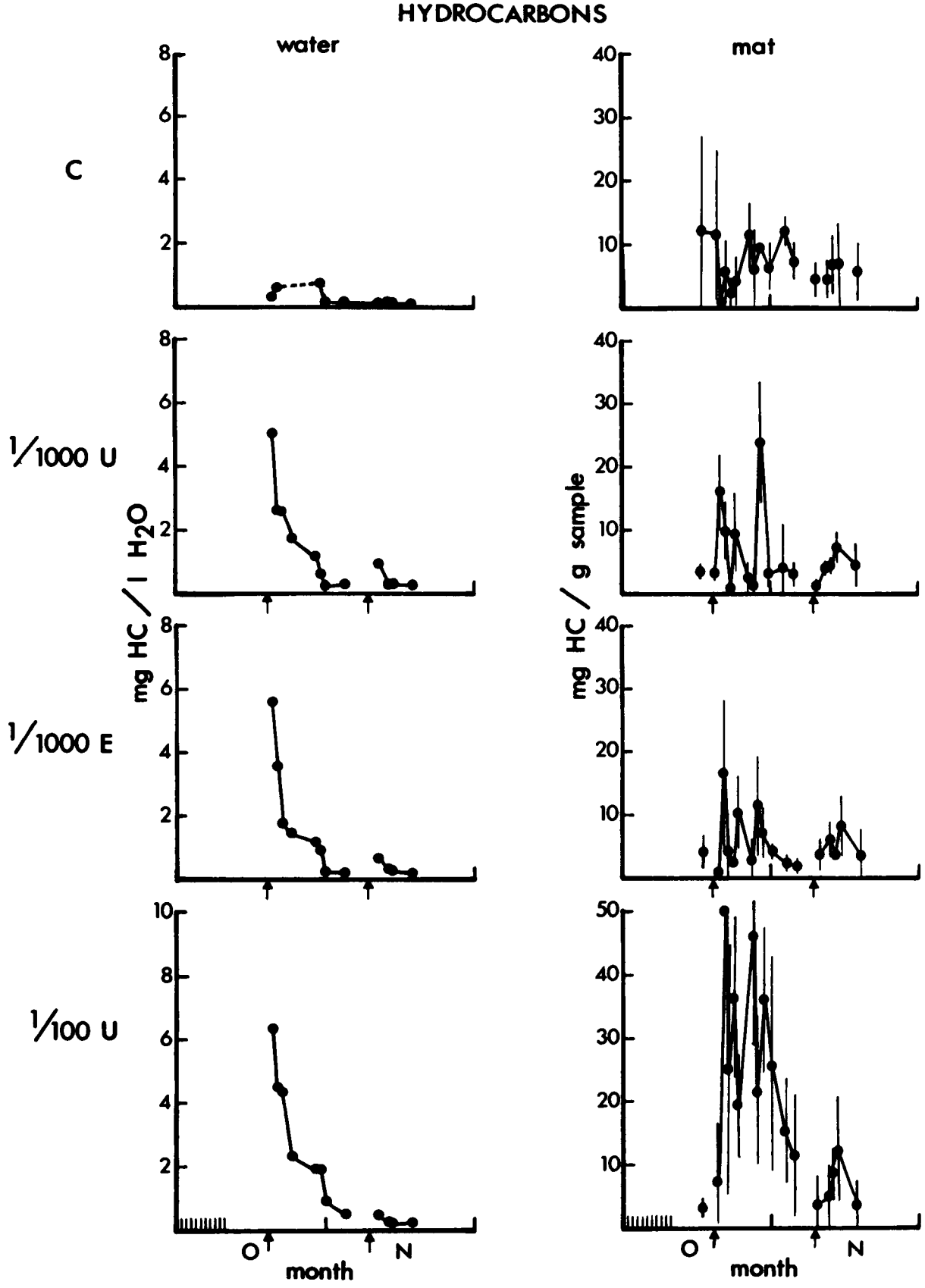

Fig. 1. Water soluble hydrocarbons and hydrocarbons associated with benthic communities (x + s.d., n = 5). No. 2 Fuel Oil, Autumn experiment, C = control; 1/1000 U=1:1000 oil:water ratio, unextracted oil; 1/1000 E=1:1000 oil:water ratio, extracted oil; 1/100 U=1:100 oil:water ratio, unextracted oil. Arrows indicate oil application.

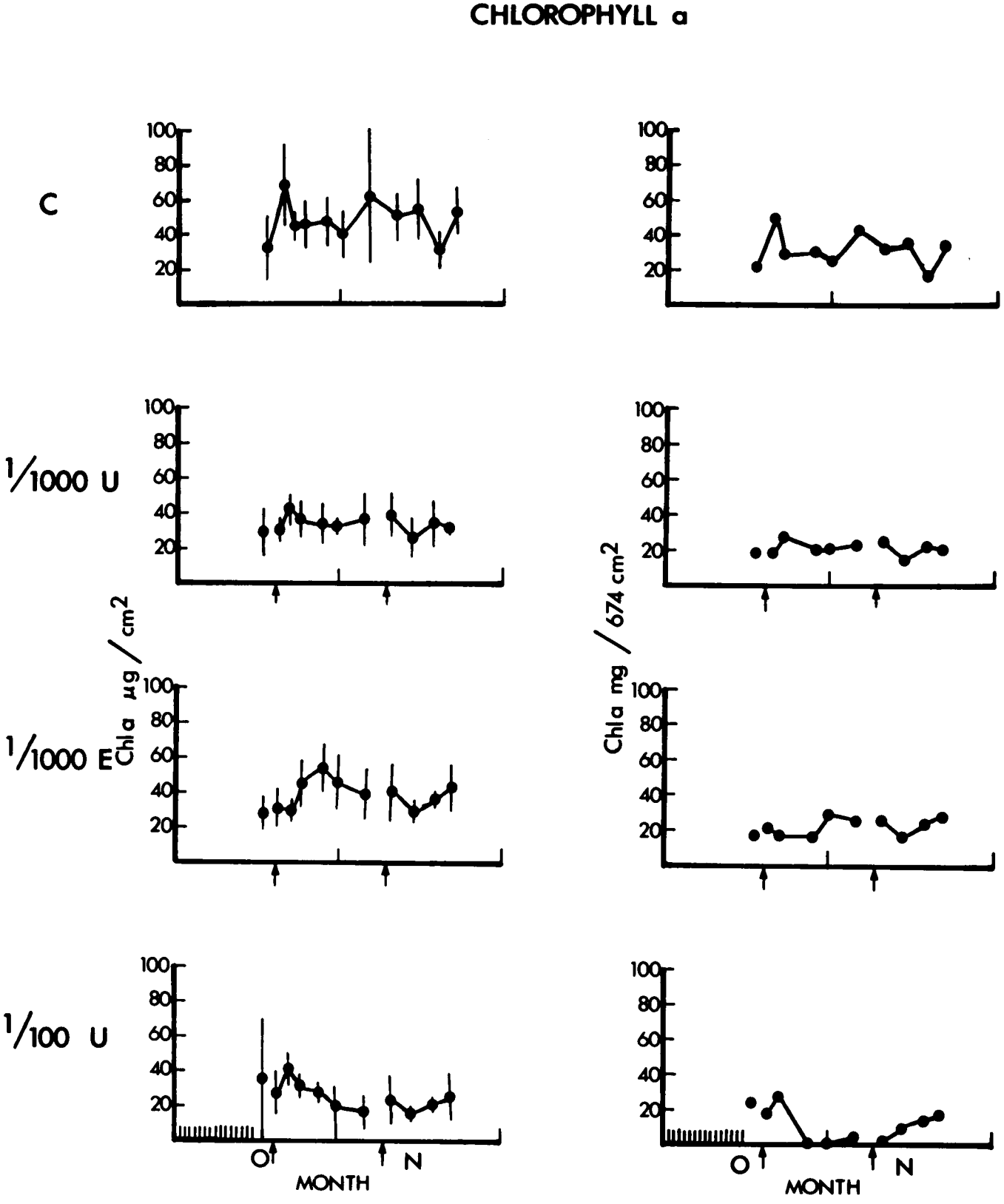

Fig. 2. Chlorophyll a concentration of benthic communities; ug/cm^2 ($x \pm$ s.d., n = 5) and mg/total tray areas in microcosm. No. 2 Fuel Oil, Autumn experiment. Designations as in Fig. 1.

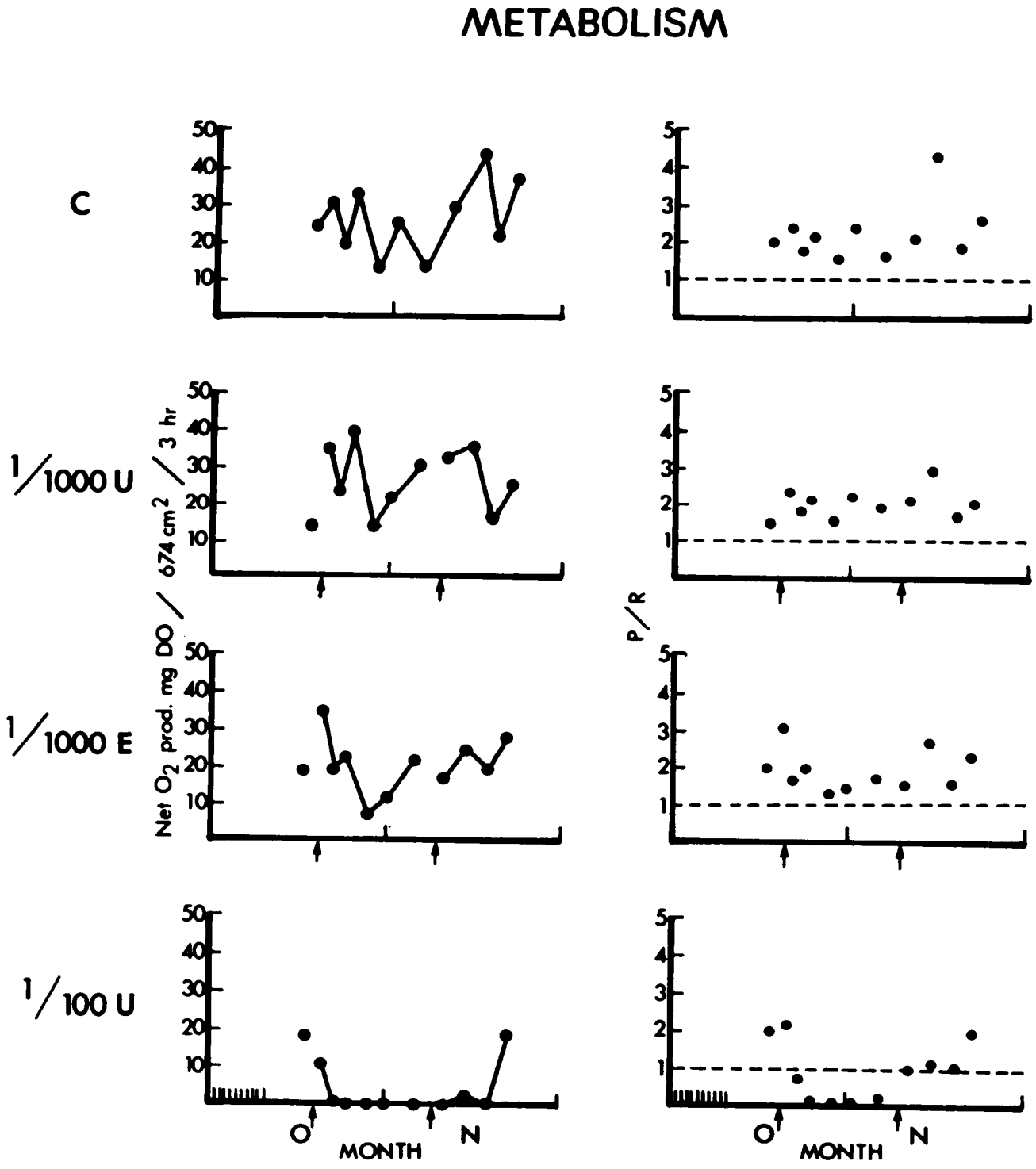

Fig. 3. Metabolism estimates for communities exposed to No. 2 Fuel Oil, Autumn experiment. Designations as in Fig. 1.

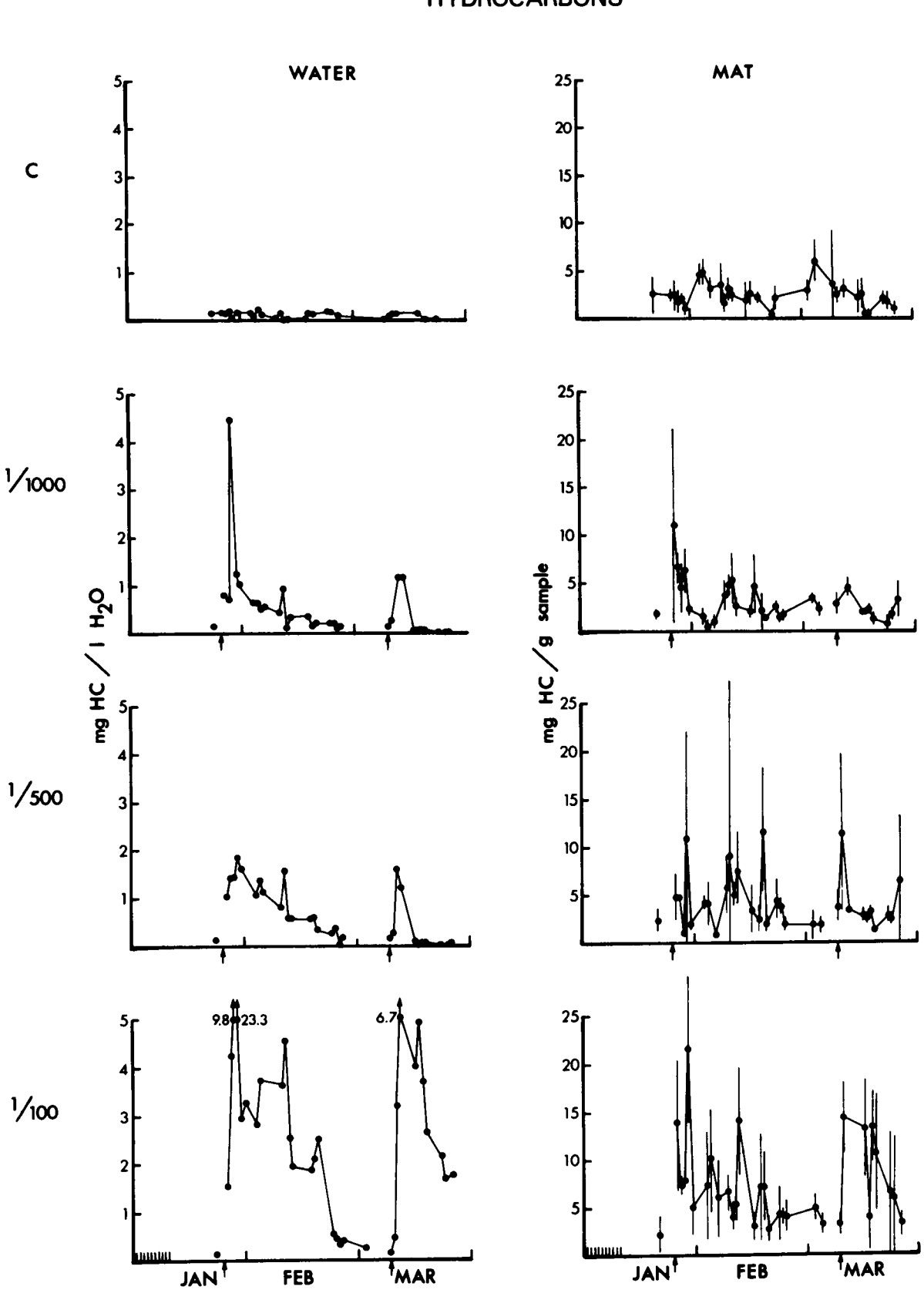

Fig. 4. Water soluble hydrocarbons and hydrocarbons associated with benthic communities (x + s.d., n = 5). No. 2 Fuel Oil, winter experiment. C = control; 1/1000, 1/500, 1/100 indicate oil:water ratio used. Arrows indicate oil application.

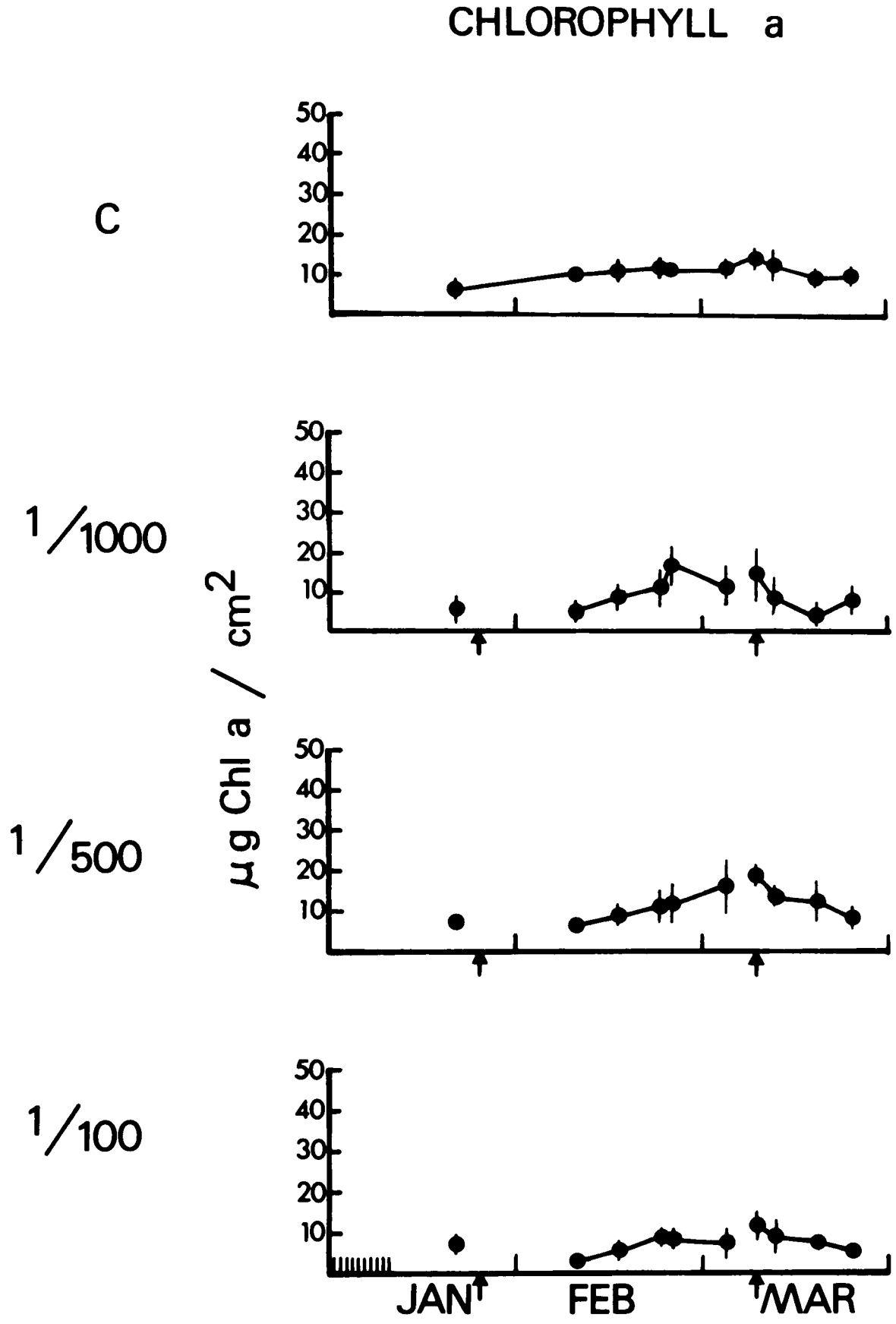

Fig. 5. Chlorophyll a concentration of benthic communities; ug/cm^2 (x + s.d., n = 5) and mg/total tray area in microcosm. No. 2 Fuel Oil, winter experiment. Designations as in Fig. 4.

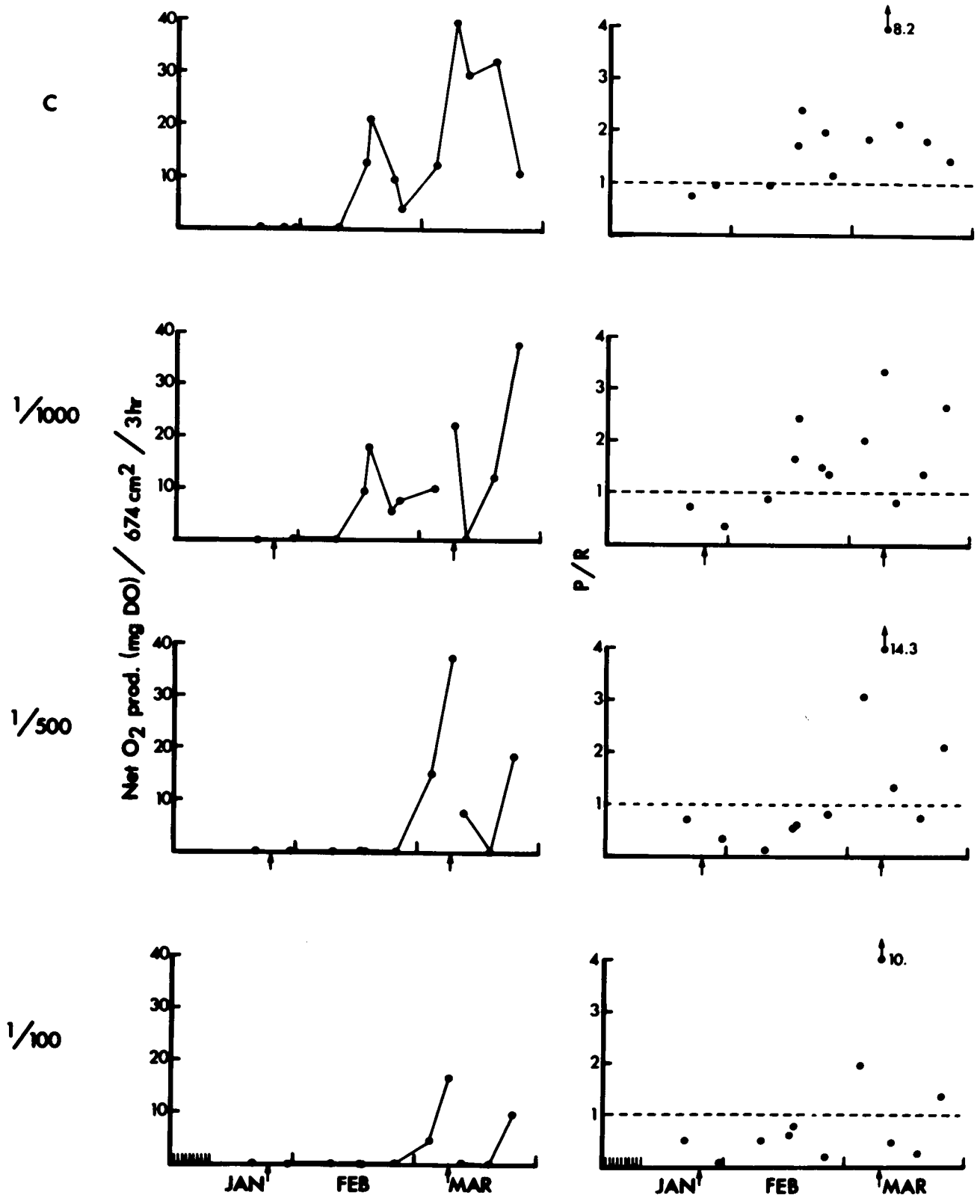

Fig. 6. Metabolism estimates for communities exposed to No. 2 Fuel Oil, winter experiment. Designations as in Fig. 4.

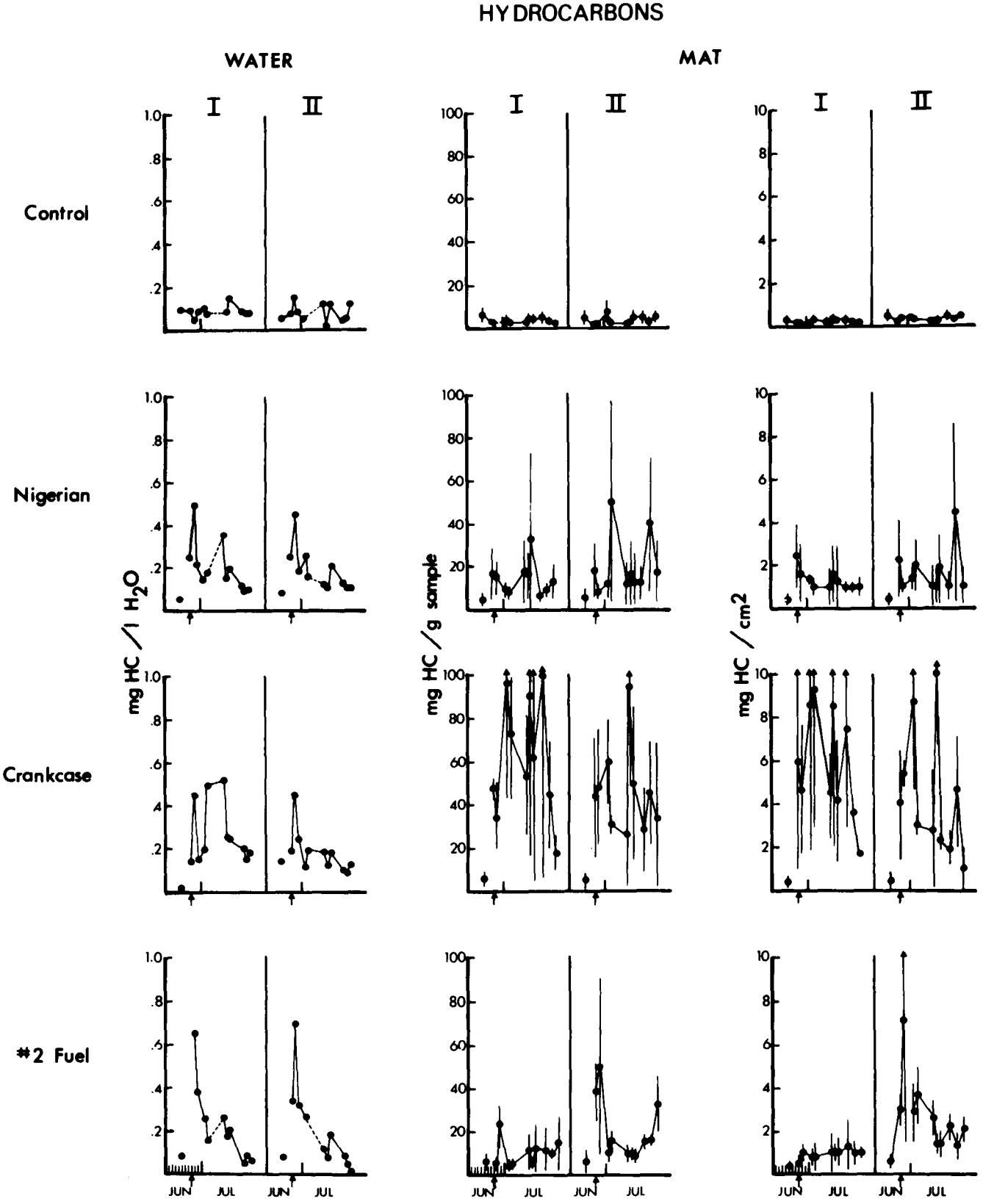

Fig. 7. Water soluble hydrocarbons and hydrocarbons associated with algae. Comparison experiment, summer. Oil applied at 1:1000 oil:water ratio. Arrows indicate oil application.

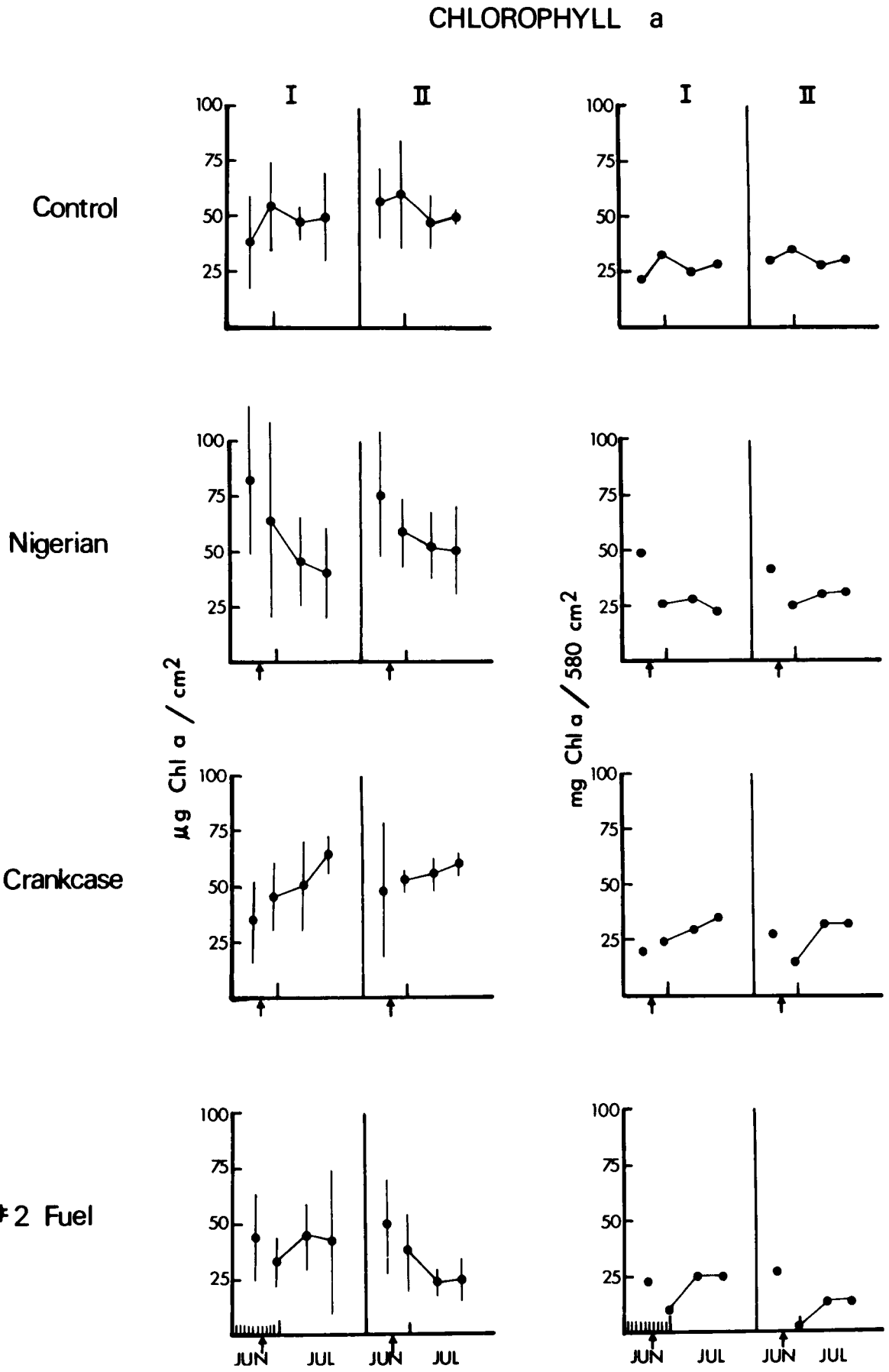

Fig. 8. Chlorophyll a concentrations of benthic communities; ug/cm^2 ($x \pm$ s.d., n = 5) and mg/total tray area in microcosm. Comparison experiment, summer. Designations as in Fig. 7.

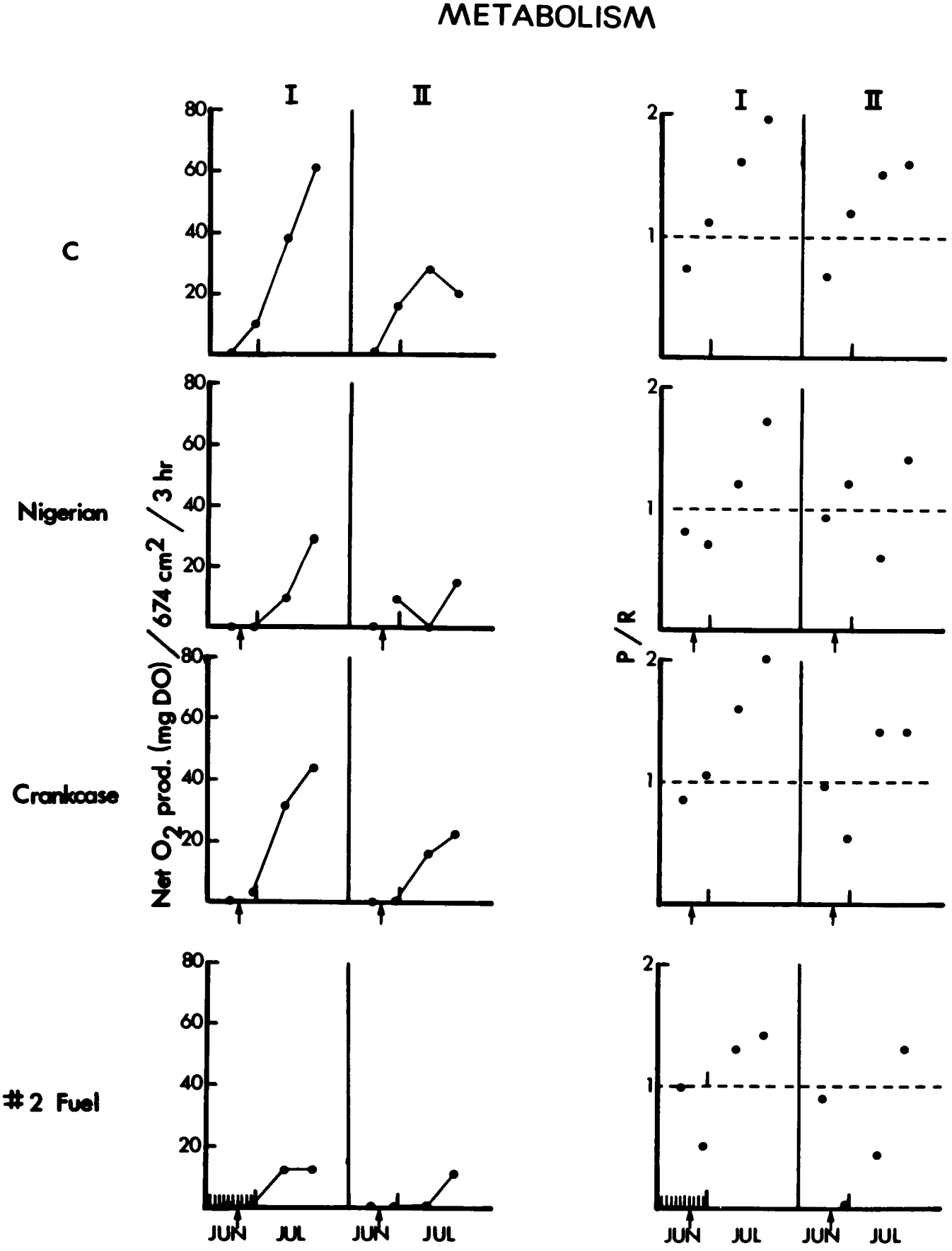

Fig. 9. Metabolism estimates for communities exposed to Nigerian Crude oil, used crankcase oil, and No. 2 Fuel Oil. Summer experiment. Designations as in Fig. 7.

TOXICITY OF COOK INLET CRUDE OIL AND NO. 2 FUEL OIL TO SEVERAL ALASKAN MARINE FISHES AND INVERTEBRATES

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ABSTRACT

We used a 96-hour static bioassay method to determine the TLm's (median tolerance levels) of 27 different invertebrate and vertebrate Alaskan marine species exposed to WSF's (water-soluble fractions) of Cook Inlet crude oil and No. 2 fuel oil. Concentrations of oil in the exposure doses of the WSF's were determined by infrared spectrophotometry.

The two different oils were about equally toxic--No. 2 fuel oil being somewhat more toxic than the Cook Inlet crude oil to some of the species. Fish were consistently among the more sensitive species with 96-hour TLm's from 0.81 to 2.94 ppm. Some invertebrates were as sensitive as fish, while others were quite resistant. Intertidal invertebrates were consistently among the most resistant species.

It appears that Alaskan marine species may be slightly more sensitive than similar species residing in more temperate regions. However, the differences in observed sensitivity may be due to the greater toxicity of oil at lower temperatures (because of greater persistence of hydrocarbons) rather than to actual increases in the sensitivity of the animals.

INTRODUCTION

Although there have been many studies of the toxicity of oil to various marine species using the 96-hour static bioassay method, very few of these studies have accurately determined the concentration of oil present in the test water. The usual method of determining the concentration of oil in test water has been to simply note the volume of oil added. Recent evidence[1,2,3] indicates that mixing energy, mixing duration, salinity, and possibly other factors play a critical role in determining how much oil is actually transferred into the water column. Because of the importance of these factors, most of the literature dealing with the acute

toxicity of oil is characterized by a very wide latitude in the reported toxic concentrations of oil, even when the same test animal has been used.

Recent studies[2,4,5,6,7] have used some method of chemical analysis, either IR (infrared) or UV (ultraviolet) spectrophotometry, or gas-liquid chromatography to determine the oil concentration actually in the test water. However, all of these studies have examined the response of animals in either subtropical or warmer temperate regions.

The development of petroleum resources in subarctic areas has stimulated our interest in the relative sensitivity of marine species to oil pollution in these areas. Fisheries in these areas are typically very productive, so that any substantial alteration of the ecosystem due to oil pollution could lead to considerable economic loss. Thus, our present study has two main objectives: (1) to identify especially sensitive species in subarctic areas, and (2) to compare, in a rough and general way (limitations of the 96-hour static bioassay method will not allow a more exact comparison), the sensitivity of cold-water inhabitants with the sensitivity of animals from warmer environments.

METHODS

Collection and Holding of Animals

Most animals were collected in or near Auke Bay in southeastern Alaska. Saffron cod, collected near Nome, and two species of limpets, collected at Cape Yakataga, were shipped to Auke Bay. Subtidal animals were collected by divers and in shrimp or crab pots. Animals were held in running seawater at 3.7°-11.0°C and at salinities of 26-30°/$_{oo}$ (depending on season). Animals were fed and appeared healthy.

Source of Toxicants

Cook Inlet crude oil was supplied by Shell Oil Company*; No. 2 fuel oil was purchased from the local Standard Oil dealer.

Preparation of Water-Soluble Fractions

The WSF's (water-soluble fractions) were prepared by slowly mixing 1% oil in seawater (1 liter oil/100 liters seawater) for 20 hours at ambient seawater temperatures (4°-12°C depending on season). Rheostats controlled the stirring speed of motor-driven propellers so that oil particles stayed in the upper third of the container. After 20 hours of mixing, the mixture stood for three hours to allow the oil to separate from the WSF. The WSF was then siphoned from under the slick. Based on analysis of this stock solution by UV spectrophotometry, the WSF was diluted to the concentrations desired for the experiments. Since there was no slick, analysis of individual exposures and the addition of animals could be done directly.

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*Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Exposure of Animals

Adult animals were separated according to species and held for at least one week in the laboratory for acclimation. Representative individuals were weighed for estimates of wet weight. Individual test containers (18-liter glass bottles) received no more than one gram of tissue/liter of oil-water mix. Five to six concentrations of oil were used for each assay with 8-12 animals per dose. The test containers were continuously aerated. Test temperatures ranged from 4°-12°C depending on season. Mortality was recorded daily for 96 hours. Death was defined as "no visible motion, even when prodded."

Determination of Oil Concentration of WSF's

The concentrations of the WSF's were determined by IR spectrophotometry[8]. Water samples of WSF's were extracted with 1,1,2-trichloro-1,2,2-triflouroethane, and the absorbance of the extract at 2930 cm^{-1} was measured on a Beckman Acculab 1 IR spectrophotometer. This method is particularly sensitive to paraffins, since absorbance at 2930 cm^{-1} is due mainly to methyl and methylene CH stretch[9]. The method was calibrated by measuring absorbances of known dilutions of the crude oil. Results are expressed as ppm of oil (by weight).

Statistical Analysis of Data

The TLm's and associated 95% fiducial limits were calculated by probit analysis[10] where possible. In situations where there was not enough data for probit analysis, TLm's were calculated by the method of Doudoroff et al.[11]

RESULTS

Table 1 summarized the 24- and 96-hour TLm's for each of the 27 species tested with WSF's of both Cook Inlet crude oil and No. 2 fuel oil. The species tested fell conveniently into three groups: sensitive vertebrates, sensitive invertebrates, and resistant invertebrates. Among the more sensitive species, the TLm's due to exposure to WSF's of Cook Inlet crude oil are all nearly identical (Table 1). This is also true of the TLm's resulting from exposure to No. 2 fuel oil, except that king crabs seem to be unusually resistant to the fuel oil.

For most of the species tested that were sensitive enough for us to calculate a TLm, there were no significant differences between the two oils. Exceptions were scallops, scooter shrimp, and pink salmon fry, which were all more sensitive to No. 2 fuel oil than to the Cook Inlet crude oil. Species that were highly resistant to Cook Inlet crude oil were also highly resistant to No. 2 fuel oil.

In nearly all of the 96-hour bioassays, regardless of the oil used or species tested, most of the deaths occurred within the first 24 hours. This is reflected by the fact that the difference between 24-hour and 96-hour TLm's for a given oil and species is usually very small (Table 1). The slope functions were similar for most of the species tested, indicating that among

the sensitive species the tolerance distribution of the animals is similar regardless of species and oil used in the test.

DISCUSSION

We have measured oil concentrations in our static bioassays by IR spectrophotometry, a method which is sensitive to paraffin hydrocarbons. Although recent evidence indicates that aromatic hydrocarbons are most responsible for acute toxicity, the bulk of the quantitative oil toxicity literature that is available reports oil concentrations measured by IR. There is a general correlation between the concentration of oil as measured by IR and the concentration of aromatics in a given WSF, but relative concentrations will be influenced by the oil used, temperature, salinity, mixing energy, etc; making cross comparisons of data derived from the two analytical methods quite difficult.

Interpretation of the results of our 96-hour static acute bioassays is complicated by the fact that the concentration of oil in exposure water declines with time. In a study of the stability of oil in WSF's of Cook Inlet crude oil[12], we learned that in 96 hours evaporation and bacterial oxidation reduced the concentration of paraffins and aromatics in the WSF to about 15% of the initial concentration. This reduction is reflected in our acute bioassays by the slight differences between 24-hour and 96-hour TLm's for most species.

Another important observation we made is that delayed mortality may be significant for some invertebrates that survived the 96-hour static bioassays. We measured the delayed mortality of scallops and hermit crabs. Scallops continued to die up to four weeks after 96-hour exposures to WSF's of crude oil, while delayed mortality in hermit crabs was not significant[13]. Delayed mortality has also been observed[14] in other bivalves.

These two complications combined, suggest that the TLm concentrations we found are the maximums to produce 50% mortality within 96 hours of exposure. If the dose level could be kept constant for the 96-hour exposure, and if delayed mortality were taken into account, the TLm for most of the species we tested would undoubtedly be lower. Despite these complications there are two generalities to be made concerning the relative sensitivities of the animals tested: (1) Fish are consistently among the more sensitive species tested, and (2) Invertebrates tested appear to fall into one of two groups--sensitive or resistant.

Although fish have the ability to induce detoxifying enzymes[15,16], these enzymes apparently do not confer resistance to acute exposures. In fact, the catabolic intermediates of these enzymatic reactions could be more toxic than the unmetabolized aromatics, thus accounting for the increased sensitivity of fish. Alternatively, these enzymes may be effective at detoxifying aromatic compounds, but do not have enough time to be induced before mortal damage is done on exposure to high doses of the WSF. Our 96-hour static bioassays may be conveniently considered as being effectively 24-hour (or even 12-hour) bioassays because of the declining oil concentrations. Thus, nearly all of the damage to the animals would occur during the first few hours of exposure, and there would not be

enough time for fish to induce detoxifying enzymes.

The sensitive group of invertebrates was comprised of species from the subtidal environment, while the resistant group of invertebrates tested in our study was dominated by intertidal species. The sensitive group of invertebrates was about as sensitive as the fish to acute exposures of WSF's.

In their natural habitat, resistant intertidal species are periodically subjected to natural stresses such as low salinity, high and low temperatures, and dehydration. The severity of these stresses depends on height in the intertidal zone and other factors such as tides, seasons, and weather. Although each species has some degree of regulation and tolerance to natural stresses, these animals have adapted to the intertidal environment principally by insulating themselves temporarily from the environmental stress. Effective insulation is possible because metabolic demands of food and oxygen can be temporarily decreased. Thus blood flow through the gills is diminished, etc. Many of these organisms have exoskeletons that may aid in physical isolation from the environment. Both of these factors would help the animals survive exposure to the 96-hour bioassays, because high concentrations of oil in the WSF persist for only a few hours. If this is in fact the case, then ecological niche would be an important criterion in predicting resistant species.

We have summarized quantitative acute toxicity data in Table 2 for Alaskan species from this study and for several other species from warmer climates from studies by Anderson et al.[2], Vaughan[4], Bean[5], Rossi et al.[6], and Vanderhorst et al.[7] All of these studies have used comparable analytical techniques and, with the exception of the studies by Vaughan, Bean, and Vanderhorst et al., similar mixing and exposure procedures. Although the Alaskan species (other than intertidal invertebrates) appear more sensitive, these studies are not directly comparable since the other studies used different oils and were tested at different temperatures. Chemical studies at our laboratory indicate that oil hydrocarbons persist somewhat longer in solutions at lower temperatures[12] and this persistence may account for toxicity differences. In other studies at our laboratory, bioassays on pink salmon fry and shrimp with toluene, naphthalene, and WSF's at different temperatures[17] indicate that some differences in toxicity at different temperatures do exist, but are not sufficient to cause an order of magnitude increase at low temperatures from those of high temperatures.

It seems likely that animals adapted to colder temperatures will have sensitivities approximately equal to their counterparts in warmer climates. Increases in toxicity in colder waters are probably related to the increased persistence of hydrocarbons resulting from decreased volatility and rates of biodegradation. Therefore, differences in TLm's between cold and warm water forms are probably due to differences in toxicity resulting from greater hydrocarbon persistence rather than to differences in sensitivity of the species.

Caution should be used in extrapolating these data to a spill situation. Laboratory experiments are simple single-variable systems rather than the multivariable system found in the natural environment. Most important, however, toxic effects from spills have not been correlated with quantitative measurements of oil concentrations existing in the water. Therefore, volumes of oil spilled in the environment cannot be related to toxic concentrations measured analytically in the laboratory.

There are certain deficiencies in the 96-hour bioassay method as a tool for measuring differences in sensitivity between different species, or even between members of the same species tested under slightly different conditions. The utility of the method rests upon two primary assumptions. First, the test species must achieve equilibrium with the toxicant before 96 hours of exposure. By equilibrium with the toxicant, we mean that the probability of additional mortality occurring with further exposure to the toxicant approaches zero. Different animals will require different lengths of exposure before an equilibrium is achieved. If this condition is not met, then the relative sensitivities of the species tested could vary with the choice of exposure duration. Secondly, to estimate the concentration of the toxicant that the animals have equilibrated with, one must assume that the concentrations have been constant. If the concentration declines with time, then one would no longer know with what concentration of toxicant a particular species actually acheives equilibrium, and the relative sensitivities of the species tested could again vary with the choice of exposure duration.

In static exposures to oil, the oil concentration declines with time, making it impossible to determine the concentration of toxicant with which the animals achieve equilibrium. It seems likely that most animals that cannot insulate themselves from the environment achieve equilibrium rapidly and reflect this in the similarities of their sensitivities. But even if the oil concentration was constant and all the species tested achieved equilibrium with the toxicant well before 96 hours, one would still want to know how the TLm changes with duration of exposure to the WSF for each species being tested: only then could one make unequivocal comparisons of species sensitivity.

Nevertheless, quantitative 96-hour static bioassays are useful for comparing toxicity of various oils and as first determinations of relative sensitivity of species. The next generation of studies should observe animals for longer periods of time to take into account the problem of delayed mortality and should have constant dose exposures so that the animals can achieve an equilibrium with a known concentration of oil. Achieving constant dose exposures with complex mixtures of hydrocarbons will be a challenge.

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Table 1. Median tolerance limits (TLm--ppm of oil as measured by IR[2930^{cm-1}]) of 27 Alaskan species to water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil. For each oil, the 24- and 96-hour TLm's with associated 95% fiducial limits are given, along with a slope function (SF) and their associated 95% fiducial limits. Intertidal species are indicated by (IT).

Species	Cook Inlet crude oil				No. 2 fuel oil			
	24-hour		96-hour		24-hour		96-hour	
	TLm	SF	TLm	SF	TLm	SF	TLm	SF
FISH								
Pink salmon, *Oncorhynchus gorbuscha*	4.13 3.51-4.84	3.35 2.36-4.33	2.92 2.65-3.22	8.35 5.65-11.03	0.89 0.82-0.97	11.82 5.15-18.50	0.81 0.72-0.92	9.81 3.35-16.27
Dolly Varden smolts, *Salvelinus malma*	3.25 1.27-8.27	3.14 0.04-6.32	2.94 1.25-6.90	2.50 0.20-4.8	-- --	-- --	2.29 2.10-2.61	11.47 10.89-12.94
Saffron cod, *Eleginus gracilis*	2.48 2.34-2.64	18.42 3.88-32.96	2.28 2.06-2.53	17.38 0.36-34.40	>4.56 --	-- --	2.93 2.38-3.61	6.01 2.15-9.87
Tube-snouts, *Aulorhynchus flavidus*	-- --	-- --	1.34 1.14-1.58	9.64 2.92-16.3	-- --	-- --	-- --	-- --
SENSITIVE INVERTEBRATES								
Dock shrimp, *Pandalus danae*	0.95 0.60-1.50	2.96 1.48-4.45	0.81 0.50-1.33	3.02 1.42-4.62	1.68 1.08-2.61	2.34 0.18-4.51	1.11 0.67-1.86	2.16 0.24-4.09
Humpback shrimp, *Pandalus goniurus*	2.31 1.99-2.69	8.15 4.72-11.58	1.98 1.69-2.32	7.40 4.02-10.78	-- --	-- --	1.69 1.54-1.85	10.41 5.50-15.31
Scooter shrimp, *Eualus fabricii*	2.52 1.27-5.02	5.32 1.27-5.02	1.46 1.07-2.00	1.46 1.05-5.11	0.91 0.82-1.22	9.57 5.04-14.11	0.53 0.48-0.59	12.41 6.09-18.73
Coonstripe shrimp, *Pandalus hypsinotus*	2.87 2.62-3.14	12.03 6.16-17.90	2.72 2.49-2.98	12.93 6.71-19.16	-- --	-- --	-- --	-- --
Pink shrimp, *Pandalus borealis*	2.89 2.52-3.31	9.90 4.70-15.09	2.43 2.13-2.77	12.45 4.93-19.97	0.38 0.28-0.52	-- --	0.21 0.15-0.30	-- --

Table 1. Continued.

Species	Cook Inlet crude oil				No. 2 fuel oil			
	24-hour		96-hour		24-hour		96-hour	
	TLm	SF	TLm	SF	TLm	SF	TLm	SF
SENSITIVE INVERTEBRATES--continued								
Scallops, *Chlamys* sp.	3.83 3.51-4.17	10.49 6.72-14.25	3.15 2.95-3.36	16.16 9.45-22.87	1.01 0.91-1.11	11.11 5.92-16.30	0.80 0.72-0.90	9.03 5.10-12.95
King crabs, *Paralithodes camtschatica*	5.16 4.05-6.59	4.79 2.15-7.42	4.21 3.58-4.97	6.87 3.37-10.37	-- --	-- --	5.10 4.51-5.68	16.09 --
RESISTANT INVERTEBRATES								
Hermit crabs (IT) *Pagurus hirsutiusculus*	--	--	3.1	--	--	--	>5.59	--
Amphipod, *Orchomene pinguis*	>7.40	--	>7.40	--	--	--	>1.34	--
Isopod (IT), *Idothea-wosnesenskii*	--	--	>8.99	--	--	--	>5.59	--
Mysid, *Acanthomysis pseudomacropsis*	--	--	>8.99	--	--	--	>0.95	--
Barnacle (IT), *Balanus glandula*	>8.51	--	>8.51	-	--	--	--	--
Sea cucumber (IT), *Eupentacta quinquesemita*	--	--	>6.9	--	--	--	>2.28	--
Sea cucumber (IT) *Cucumaria* cf. *vega*	--	--	>14.7	--	--	--	>2.11	--
Littleneck clam (IT) *Protothaca staminea*	--	--	>14.7	--	--	--	>2.11	--

Table 1. Continued.

Species	Cook Inlet crude oil				No. 2 fuel oil			
	24-hour		96-hour		24-hour		96-hour	
	TLm	SF	TLm	SF	TLm	SF	TLm	SF
RESISTANT INVERTEBRATES--continued								
Mussel (IT), *Mytilus edulis*	>5.15	--	>5.15	--	>3.11	--	>3.11	--
Limpet (IT), *Notoacmaea testudinalis scutum*	--	--	3.65	3.23	>4.19	--	5.04	4.73
	--	--	2.62-5.08	--	--	--	3.49-7.27	--
Limpet (IT), *Notoacmaea* sp.	>5.15	--	9.59	0.68	>1.77	--	4.27	1.08
	--	--	1.24-74.42	--	--	--	2.56-7.15	--
Chiton (IT), *Ischnochiton stelleri*	--	--	--	--	>1.13	--	1.24	1.84
	--	--	--	--	--	--	0.65-2.35	--
Chiton (IT), *Katharina tunicata*	--	--	--	--	1.03	--	0.44	--
	--	--	--	--	--	--	0.25-0.77	--
Snail (IT), *Littorina sitkana*	>20.97	--	>20.97	--	--	--	--	--
Snail (IT) *Margarites pupillus*	--	--	--	--	>1.13	--	>1.13	--
Whelk (IT), *Nucella lima*	>20.97	--	>20.97	--	--	--	--	--

Table 2. Summary of acute toxicity of several crude oils and No. 2 fuel oil to several marine species. Data are from four studies, each using at least two different crude oils. Ranges of TLm's are reported in ppm of oil as measured by IR (2930 cm^{-1}).

Study and species tested	Temperature range (°C)	96-hour TLm's	
		Crude oils	No. 2 fuel oil
Vaughan[4]* Table 2			
Two fish species	8	15-65	--
Bean et al.[5]*			
Coonstripe shrimp, juvenile salmon (flow-through)	10-11	6.6-24.9	--
Coonstripe shrimp (static)	8	1.3-4.9	--
Vanderhorst et al.[7]			
Coonstripe shrimp (flow-through)	10.5	--	0.8
Anderson et al.[2]			
Three crustacean species	18-22	6.6->19.8	1.3-4.9
Three fish species	18-22	5.5- 19.8	3.9-6.3
Rossi et al.[6]			
Two polychaete species	20	9.8-12.5	2.3-2.7
This study			
Four fish species	3.6-10.2	1.16-2.94	0.81-2.15
Five shrimp species	3.5-5.4	0.65-2.72	0.53-1.69
One crab species	3.8-7.8	4.21	5.10
One scallop species	3.9-7.4	3.15	0.8
Four limpet and chiton sp.	--	3.65-9.59	0.44-5.04
Twelve invertebrate species	3.6-10.2	>3.1-14.7	>0.95-5.59

*These studies do not report TLm's directly in the form of IR ppm, so the values presented are measured from raw data. Some of the tests are flow-through and others are static. Different mixing procedures were used and the data may not be directly comparable with ours.

INTERACTIONS BETWEEN THE DEPOSIT FEEDING POLYCHAETE *ARENICOLA MARINA* AND OILED SEDIMENT

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ABSTRACT

The deposit feeding polychaete *Arenicola marina* can be a sensitive indicator of the effects of oil pollution on marine ecosystems. Concentrations of oil in both water and sediment commonly occurring in spill situations can force worms to surface or stop feeding activity. Lower concentrations can reduce the rate of cast production and presumably feeding. Oil concentrations in casts are substantially lower than in unworked sediment indicating that the working activity of *Arenicola* can be an important factor in the weathering of oil in sediment. These results are preliminary and are being expanded with further experiments.

INTRODUCTION

It is well documented that a major portion of oil entering the marine environment, especially in coastal areas, becomes incorporated into sediments where decomposition is very slow. In some environments, spilled oil is still present after five years (1,2) and shows signs of persisting considerably longer. In contrast to pelagic organisms, benthic animals spend most of their lives attached to or living in the bottom so their mobility is severely restricted. For these reasons, it is generally agreed that the real impact of an oil spill is best assessed by studying benthic organisms.

Polychaete worms are a dominate group in most soft bottom benthic communities and their reaction to oil pollution has been studied by several investigators. Their response to the West Falmouth oil spill has been studied in considerable detail (1, 3 and 4). The behavior of two cirratulid polychaetes in an intertidal mudflat before and after a fuel oil spill has been investigated (5). Several investigators have studied the response of polychaetes to oil accomodated in seawater (6 - 8). Recently, the response of *Arenicola marina* to oil sprayed on sandflats has been studied (9). These studies have produced conflicting results, some indicating high tolerance to oil and others suggesting much sensitivity.

Deposit feeding polychaetes are also important in the concern of oil pollution because of the considerable amounts of sediment that they rework (10,11). This activity could have a pronounced effect on the weathering rate of oil in sediment since it increases exposure of oil to oxygen, nutrients and micro-organisms.

In the spring of 1975, we started a study of the interactions between oil in sediment and the deposit-feeding polychaete, *Arenicola marina*. *Arenicola* was selected because of its local abundance, its adaptability to experimental manipulation, and its high rate of sediment working. This paper represents a progress report of the results obtained to date and indicates the expected direction of further experiments.

NOTES ON *ARENICOLA*

Arenicola is a large, stout worm (up to 10 cm long and 4 g wet weight) that occurs in the vicinity of the low tide mark. It is very abundant on the North American Atlantic coast north of Long Island and in northern Europe. Closely related species occur in the North Pacific. The burrowing and feeding habits of *Arenicola* and related species have been studied by numerous investigators (12 - 15). In brief, the worm occupies an L-shaped burrow. Sediment is ingested at the deep end (about 10 cm) and deposited as casts at the surface. Daily cast production can reach 15 g wet sediment. Excavated sediment is

replaced by sediment collapsing from above which produces a funnel-like depression at the surface. Water, mainly for ventilation, is pulled down into the burrow through the tail-shaft. The abundance and distribution of the worm is affected by both organic content and grain size of the sediment. Nutrition is derived from ciliates, flagellates, nematodes and bacteria. Population density can reach $100/m^2$.

METHODS

Arenicola and sediment were collected at low tide from an intertidal sand flat at Petpeswick Inlet, about 45 km east of Halifax, N.S. The site is relatively remote and free of oil pollution. All experiments were run in the laboratory using No. 2 fuel oil from the API reference collection (this oil was selected because of its high toxicity to marine organisms, 8).

Sediment (control and oiled) was mixed well and placed in 2 l glass beakers (filled to capacity). Each experiment had four replicates of control and oiled sediment. The beakers were placed in a tank of running seawater approximately 25 cm deep (6 - 8 cm of seawater above the sediment surface). Worms were washed with seawater to remove sediment adhering to their outer surface, weighed (wet), and one placed on the sediment of each beaker. Within minutes, the worms burrowed into the sediment. At daily intervals, casts and unworked surface sediment were collected with a spatula after removing beakers from the tank and decanting off seawater. After weighing the casts wet, casts and unworked sediment were analyzed (wet) for oil content by fluorescence spectroscopy (16). The estimates of oil concentration obtained using this method should be quite accurate since a known oil is being measured and can be used for calibrations. Background fluorescence measured in control sediment was subtracted, so calculations of oil concentrations are based on fluorescence response attributed to oil only.

In Experiments 1 - 7, different volumes of fresh oil were mixed with 8 l of sediment to produce concentrations averaging less than 250 mg oil/g sediment. In Experiment 8, an attempt was made to study the response of *Arenicola* to weathered oil. Oil was weathered by pouring 150 ml directly on 10 cm of sediment placed in a 1 x 1 m tank. The tank was filled with clean seawater and drained daily to simulate tides while an overhead bank of fluorescent bulbs supplied light and a fan provided a constant breeze over the tank. A control tank containing sediment without oil was exposed to the same conditions. Sediment was collected at regular intervals for experiments using the same methods detailed above.

In Experiment 9, the response of *Arenicola* to oil accomodated in seawater was observed. Fresh sediment, 10 cm deep, was placed in two 1 x 0.5 m tanks which were subsequently filled with running seawater 4 cm above the sediment. Six worms were

added to each tank. After 5 days, the seawater was shut off and 10 ml of oil was added to one tank. Both tanks were aerated with an air hose. Seawater samples were collected through a syringe needle floated by corks and analyzed for oil using fluorescence spectroscopy (17).

Selected unworked sediment and cast samples from Experiment 8 were dried and analyzed for carbon and nitrogen using a Perkin-Elmer Model 240 elemental analyzer.

RESULTS

Effects of Oil on *Arenicola*

Fresh Oil in Sediment. High concentrations of oil in sediment can drive worms to the surface or prevent them working sediment and producing casts (Table 1). This behavior was not observed in experiments having oil concentrations of about 25 µg/g sediment or less. Worms never emerged from control beakers and never more than one control worm in any given experiment failed to produce casts. This behavior in the natural environment probably leads to death. Worms that surface are easy prey to predators (fish, birds, etc.) while those that stay in their burrows without working sediment will starve.

The size of the cast produced is affected by all the sediment oil concentrations investigated (Table 2) (since the size of the cast is directly related to worm size, which varied in these experiments by a factor of about three, daily cast weight is divided by worm wet weight). At the highest oil concentrations (about 200 µg/g sediment and higher), the size of cast produced dropped about 90% compared to control casts. This difference decreased with decreasing oil concentrations and was only 5% at about 6 µg/g sediment (which is probably not significantly different). From these observations, it can be concluded that the feeding rate of worms was reduced in a similar manner. The form as well as the size of the cast was affected by the presence of oil in the sediment. Casts produced from oiled sediment were much thinner and tended to fall apart much more easily. This difference became less noticeable at the lower oil concentrations.

Weathered Oil in Sediment. High mortality and reduced working rates, in control as well as oiled sediment, spoiled the results of this experiment. The sediments were obviously undergoing changes while contained in tanks during the weathering process which were unfavorable to *Arenicola*. Despite these problems, it is clear that oil weathered on and in sediment for up to two months still affects the behavior of *Arenicola* (Table 3). Both cast production and cast size were reduced compared to controls.

Oil Accomodated in Seawater. *Arenicola* can also be affected by oil accomodated in seawater (Table 4). In the experiment conducted, cast production was affected at a concentration of 0.7 mg oil/l seawater after just 5 hr. Worms began emerging from the sediment by 22 hr when concentrations reached about 5 mg/l.

At the end of the experiment (3 days), all six worms in the oiled tank had quit the sediment and were dead on the surface, while control worms behaved normally.

Effects of *Arenicola* on Worked Sediment

In all experiments conducted, the estimated oil concentrations were substantially lower in casts than unworked sediment at the time of sampling (Table 5). The absolute difference decreased with decreasing oil concentration in the sediment. The percent removed ranged between 20 and 100%, with the lowest percentages associated with the lowest oil concentrations. These results indicate that *Arenicola* exerts a profound influence on oil concentrations in sediment.

There were no significant differences in either carbon or nitrogen concentrations as a result of working in both control and oiled sediment. Oil concentrations were too low to be seen against background carbon concentrations.

DISCUSSION

Our results suggest that *Arenicola* can be affected by both oil accomodated in the overlying water and incorporated into sediment. It is probable that in the case of a spill on water, worms would first be potentially affected by oil accomodated in seawater, which is pumped into their burrows during ventilation periods. Our preliminary data suggest that No. 2 fuel oil concentrations in excess of about 1 mg/l will drive worms from their burrows and cause death (Table 4). Concentrations in this range do occur in surface water near freshly spilled oil (for example, 18). The effects of sub-lethal concentrations (less than 1 mg/l) are unknown. The TLm values of the water soluble fraction of the same No. 2 fuel oil at 48 and 96 hr are reported to be on the order of 2 - 3 mg/l for the polychaetes *Neanthes arenaceodentata* and *Capitella capitata* (8), suggesting that the sensitivities of these polychaetes to water accomodated oil may not be very different from *Arenicola*.

If not immediately affected by high oil concentrations in seawater (which are generally of short duration, 18), *Arenicola* can be affected by oil as it becomes incorporated into sediments by various potential pathways. Concentrations in excess of about 100 µg oil/g sediment of fresh oil can force worms to leave their burrows or halt their feeding activity (Table 1). Oil concentrations tested, including those as low as about 10 µg oil/g sediment, reduced the rate of cast production (Table 2) and presumably feeding activity. Similar but less detailed results were obtained with oiled sediment subjected to weathering (Table 3). The oil concentrations producing these effects are typical of sediments polluted with oil (16).

Field studies (9) have also demonstrated that *Arenicola* can be adversely affected by oil (Kuwait crude). Spraying oil on sediment (0.2 l/m^2) over *Arenicola* beds caused a 25 - 50% reduction in population density. Successive spillages could wipe out entire populations (20 - 25 worms/ 0.25 m^2). On the day following the spill, feeding activity in up to 75% of the worms was depressed. A majority of the worms gradually recovered normal feeding activity over a period of about a month. Recolonization of polluted sediment was also affected.

The available data indicate that *Arenicola* is more sensitive to oil pollution than the cirratulid polychaetes *Cirriformia tentaculata* and *Cirratulus cirratus*. The mortality, growth and spawning of the latter worms were not visibly affected by a spill of fuel oil that blanketed at low tide the flat they inhabited (5). This variance is difficult to explain, but could be due in part to anatomical and behavioral differences in the worms. Cirratulid polychaetes are surface deposit feeders and respire through branchiae which are spread in the surface layer of mud. However, it is clear that both cirratulids (5) and *Arenicola* (9) are affected by dispersants.

Observations made during July 1976 (19) in Chedabucto Bay, N.S. indicate that *Arenicola* do occur in sediment that is visibly polluted with oil remaining from the *Arrow* spill of 1970 (Bunker C). The behavior of these worms and the chemistry of their environment will be examined this fall.

The apparent decrease in sediment oil concentrations as a result of working (Table 5) could result from several processes working singly or perhaps in combination. Selection of sediment particles does occur during feeding (15) and worms might avoid patches of sediment with the highest concentrations of oil (despite thorough mixing, the distribution of oil in sediment is quite heterogeneous). It is possible that oil is being assimilated through the gut (worm tissue has not yet been analyzed). The casts and presumably the guts of worms are rich in micro-organisms (15) so it is conceivable that part of the drop in concentration can be caused by increased re-mineralization of oil. And finally, since feeding, digestion and defecation all take place in an aqueous medium, loss by solution is also quite probable. The relative importance of these processed will be examined in further experiments.

Assuming that the removal of oil from sediment observed is real and that similar rates would occur in the natural environment, a population of 10 - 100 worms/m^2 could completely remove 100 µg oil/g sediment in about a year's time.

All deposit feeding organisms probably affect the weathering of oil in sediment. Through their activities, sediment is being continually oxidized and the growth of micro-organisms is stimulated. A concept of gardening has in fact been proposed (15). A supression of working activity by organisms through oil pollution could reduce the level of benthic production.

SUMMARY

1. Deposit-feeding invertebrates, such as the polychaete *Arenicola marina*, can be sensitive indicators of the long term effects of oil pollution on marine ecosystems.

2. High concentrations of oil in seawater and in sediment can force *Arenicola* to surface (where death by predation is probably imminent) or to stop ingesting sediment and producing casts.

3. At lower concentrations of oil in sediment where working occurs, cast size (and therefore feeding rate) is reduced. The reduction appears to be related to sediment oil concentration.

4. Oil concentrations in casts were substantially lower than in unworked sediment indicating that the working activity of *Arenicola* (and presumably other deposit feeders) can be an important factor accelerating the weathering of oil in sediment.

5. The results presented here are only preliminary. Additional studies for the next six months will concentrate on: a) obtaining more detail on the effects of weathered oil on the behavior of *Arenicola*; b) changes in hydrocarbon composition and concentrations due to working activity; and c) field studies of *Arenicola* populations in Chedabucto Bay, N.S.

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DISCUSSION

GRIFFITH: Since oil is a nutrient, could it be that the reduced eating observed was due to the fact that they were already getting a sufficient amount of nutrients?

GORDON: It is a possibility, but I doubt it. We were looking at the total organocarbon in the sediment as well and, really, the oil in terms of carbon is only a percent or so of the total carbon that is present. In fact, you really couldn't see the oil in the carbon data, and you actually cannot see any difference in the organocarbon concentration before and after ingestion.

Also, the worms feeding habits have been worked out fairly well, and it is feeding mostly on small bacteria and nematodes. So I don't think it is because it is eating the oil.

HERBES: Do you have any feeling for what concentration of oil in the sediments would be toxic or how far below the acute toxicity you are seeing these changes in behavior?

GORDON: Well, I guess what we would call an acute toxicity might be at those concentrations where the organisms are forced to leave the sediment. In the higher concentrations we were looking at, which were averaging on the order of, say, 200-300 milligrams of oil per gram of sediment, some worms were dying. Those concentrations were also driving some worms to the surface where, if they were still alive, they wouldn't last very long due to predation by fish or sea birds.

So, really, in terms of the work where we were looking at oil accommodated in sea water, it appeared that concentrations on the order of a milligram or so of oil in a liter of sea water were sufficient to drive the worm up to the surface as well.

HERBES: Do you know if there have been any similar studies on the effects of oil on fresh water benthic burrowing organisms?

GORDON: I don't know of any. I was interested in the work of Phil Meyers this morning. He indicated that the rate of bioturbation, at least in Lake Huron, was much, much lower than we find in the marine environment.

Table 1. Emergence of worms from sediment and the number of worms producing castings at termination of each experiment (4 to 7 days). Four worms in each set. The ratio of castings produced to worms is 1:1.

Experiment	Mean Oil Conc. (µg/g sediment)	Number of Emerging Worms		Number of Casts Produced	
		Control	Oiled Sediment	Control	Oiled Sediment
1	249.2	0	1	4	1
2	184.1	0	2	3	2
3	111.6	0	0	4	2
4	87.1	0	1	3	2
5	25.9	0	0	3	3
6	16.6	0	0	4	3
7	6.4	0	0	3	4

Table 2. Size of casts produced by *Arenicola* in control and oiled sediment. Average of all replicates in each experiment. Weight of cast divided by initial wet weight of worm to correct for worm size.

Experiment	Mean Oil Conc. (µg/g sediment)	g cast/g worm/day		% Difference
		Control	Oiled	
1	249.2	1.97	0.25	87
2	184.1	4.24	0.29	93
3	111.6	1.26	0.27	79
4	87.1	2.84	0.63	78
5	25.9	6.39	1.10	83
6	16.6	5.08	1.92	38
7	6.4	6.80	6.45	5

Table 3. Results of experiment using weathering oil in sediment. Four worms in each set. Experiments lasted 3 - 6 days. The ratio of castings produced to worms is 1:1. Sediment oil concentration ranged between 200 - 500 µg/g sediment.

Days Weathering	Number of Casts Produced		Cast Weight(g/g worm/day)	
	Control	Oiled	Control	Oiled
1	3	1	3.4	0.9
25	3	1	0.6	0.6
63	2	0	2.2	0.0

Table 4. The effects of oiled seawater on the behavior of *Arenicola*. Time indicates hours elapsed after oil added to seawater overlying sediment containing worms. Six worms in each set. Casts removed at each observation time.

Time (hr)	Worms	Mean Aqueous Oil Conc. (mg/l)	Number of Emerging Worms	Number of Casts Produced
Initial	Control		0	6
	Oiled	0.0	0	6
2	Control		0	0*
	Oiled	0.8	0	0*
5	Control		0	3
	Oiled	0.7	0	1
22	Control		0	6
	Oiled	4.8	2	1
29	Control		0	6
	Oiled	40.1	2	1
46	Control		0	6
	Oiled	7.6	3	0
75	Control		0	6
	Oiled	7.3	6 (all dead)	0

* Casts removed at start of experiment and no further production during first two hours.

Table 5. Apparent loss of oil while sediment is worked by *Arenicola*. Numbers in parentheses represent range of replicates. All replicates are averaged and control concentrations subtracted.

Experiment	µg oil/g sediment					
	Sediment		Cast		Difference	%
1	249.2	(111.6 - 386.9)	105.5	(64.6 - 159.5)	143.7	58
2	184.1	(109.3 - 354.1)	76.6	(0.0 - 164.3)	107.5	58
3	111.6	(58.1 - 150.4)	44.2	(14.4 - 68.0)	67.4	60
4	87.1	(50.2 - 142.7)	12.9	(2.8 - 23.6)	74.2	80
5	25.9	(0.0 - 54.5)	0.0	(0.0 - 28.8)	25.9	100
6	16.6	(3.2 - 34.3)	11.3	(7.9 - 23.9)	5.3	32
7	6.4	(0.0 - 17.0)	5.1	(2.4 - 7.7)	1.3	20

ASPECTS OF THE EFFECTS OF PETROLEUM HYDROCARBONS ON INTERMEDIARY METABOLISM AND XENOBIOTIC METABOLISM IN MARINE FISH

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ABSTRACT

Aspects of normal intermediary metabolism and xenobiotic metabolism are considered in relation to low levels of petroleum contamination. Petroleum hydrocarbons appear to be associated with altered patterns of lipid metabolism, characterized by a net decline in lipogenesis, in hepatic tissue of *Fundulus heteroclitus* or *Stenotomus versicolor* contaminated either in the environment or experimentally at less than 200 ppb. There also is an association between environmental contamination and low lipogenesis rates in gill, muscle and brain in *F. heteroclitus*.

Various properties of cytochrome P-450 mixed-function oxidases in fish, including EPR characteristics, indicate a basic similarity with the system in mammals. Treatment of teleost fish with 3-methylcholanthrene or 5,6-benzoflavone results in induction of mixed-function oxidase activity. Evidence exists suggesting induction of mixed-function oxidases occurs in fish environmentally contaminated by petroleum, although in at least some cases the appearance of increased activity can be ascribed to factors other than induction.

INTRODUCTION

Studies of the biological effects of petroleum in fresh or marine waters have, until recently, been directed principally at identifying lethal levels and those effects resulting from exposure of organisms to barely sublethal concentrations of hydrocarbons. Although the appearance of altered behavioral or physiological parameters in such highly stressed individuals is of interest[1] it is not possible to extrapolate from findings obtained under such circumstances to the effects produced by less stressful conditions, and in general the effects at lower concentrations of a toxicant are less overt.

The toxic potential of low concentrations of a given hydrocarbon or mixture of hydrocarbons may be discerned by examining effects on biological function

of the organism at cellular and subcellular levels. Analysis of metabolic processes in various tissues, and the nature of any changes resulting from exposure to petroleum, is a first step in determining whether tissue function may be impaired by petroleum as well as the significance of such impairment. In this contribution we describe and discuss some aspects of metabolic function in tissues of fish exposed to low levels of petroleum, dealing first with aspects of normal intermediary metabolism, and secondly with xenobiotic metabolism.

METHODS

Fish used in these studies, *Fundulus heteroclitus* (killifish) and *Stenotomus versicolor* (scup), were collected from local Cape Cod waters as described in the text. Experimental exposure of some groups to petroleum hydrocarbons was performed in a flow through system described by Stegeman and Teal[2], using a concentration of between 180 and 200 µg of total hydrocarbon (API #2 fuel oil) per liter. Treatment of animals with 5,6-benzoflavone or 3-methylcholanthrene was accomplished by intraperitonial injection of the drug in a corn oil suspension.

Analysis of metabolic function was carried out using tissue slices incubated with ^{14}C labelled metabolic intermediates according to previous procedures and monitoring appearance of ^{14}C in lipid and respired to CO_2[3]. Benzo[a]pyrene hydroxylase was assayed in microsomal preparations or in postmitochondrial supernatant preparations using a procedure similar to that of Nebert and Gelboin[4] and cytochrome P-450 was estimated according to the method of Omura and Sato[5]. Electron paramagnetic responance spectra of cytochrome P-450 from *Stenotomus versicolor* were determined at 1.6°K according to Chevion *et al.*[6]. (A complete account of the EPR studies is in preparation[6].)

INTERMEDIARY METABOLISM

Normal hepatic metabolism in mammals is known to be affected by a variety of lipophilic drugs and xenobiotics and it is not unreasonable to expect the same to be true of fish. Thus, in a study of changes in metabolic processes resulting from low level contamination by hydrocarbons we looked first for changes in liver function. The parameters examined included the rates at which glucose-1-^{14}C, glucose-6-^{14}C or acetate-1-^{14}C were incorporated into ^{14}C-lipid or $^{14}CO_2$ by liver slices in a two hour *in vitro* incubation[3].

Initial studies on *Fundulus* collected from two separate marshes, one being the site of the 1969 West Falmouth oil spill (Wild Harbor Marsh) and the other being uncontaminated (Great Sippewissett Marsh), indicated a distinct difference in the hepatic function between fish from these two environments[3]. This difference was characterized by a lower net rate of hepatic lipogenesis in fish collected from the Wild Harbor Marsh, but this distinction was evident, however, only in the incorporation of ^{14}C-acetate into lipid. Respiration of labelled CO_2 from acetate, principally via the tricarboxylic acid cycle, was not significantly different in the Wild Harbor fish, nor were glucose-1-^{14}C or glucose-6-^{14}C preferentially metabolized to CO_2 or incorporated into lipid by hepatic tissue from either group of fish.

These results prompted an exposure of *Fundulus* from both Wild Harbor and Sippewissett marshes and an additional species of fish, *Stenotomus versicolor* from

Hadley Harbor, Massachusetts, to petroleum hydrocarbons in a flow through experimental system. Analysis of hepatic metabolic function revealed that the rate of acetate incorporation into lipid was markedly reduced in the *Stenotomus* and the *Fundulus* from Sippewissett which were exposed to oil, but not in the *Fundulus* from Wild Harbor (Table 1). The distinction between the populations of *Fundulus* in response to experimental exposure was evidently a feature of the substantially lower rate of lipogenesis in the Wild Harbor fish, as indicated by the control values for *Fundulus*. This difference between the control values for Wild Harbor and Sippewissett fish is qualitatively similar to that reported previously[3]. There was little or no consistent difference observed between the experimental and control groups of *Stenotomus* or *Fundulus*, from either location, in the rate of incorporation of glucose-1-^{14}C or glucose-6-^{14}C into CO_2 or lipid. Similarly there was no consistent difference between experimental and control animals in the metabolism of acetate-1-^{14}C to CO_2.

In spite of the fact that only two of the experimental groups exhibited a net decrease in the rate of lipogenesis, all three groups exposed to oil displayed a somewhat lower % hepatic lipid. In addition, histological examination of liver from the two experimental and two control groups of *Fundulus*, at the light microscopic level, demonstrated an apparent disappearance of hepatic lipid vacuoles in the oil-treated animals in both groups (Table 1). The lower liver lipid content and the loss of lipid vacuoles suggests that the decreased net lipogenesis may be due in part to increased mobilization and perhaps utilization of some portion of the lipid pool, possibly triglycerides, in these oil-exposed fish. This suggestion is supported by an observed decrease in net synthesis of triglycerides in some contaminated fish[3]. The same study[3] indicated altered patterns of hepatic phospholipid and cholesterol metabolism in fish exposed to petroleum.

Examination of metabolism of acetate-1-^{14}C in extrahepatic tissues of *Fundulus* from Wild Harbor and Sippewissett provided additional information concerning the differences between fish from these areas. In gill, muscle and brain tissues the incorporation of acetate into tissue lipid was lower by 40-50% in the Wild Harbor fish, a difference in the same direction as that observed in liver. There was no difference in metabolism of acetate to CO_2 in either gill or brain while in muscle there appeared a two-fold increase in $^{14}CO_2$ respired in *Fundulus* from the contaminated marsh, suggesting an increased TCA cycle activity. As with liver, it is tempting to attribute these differences to the petroleum hydrocarbons present in Wild Harbor.

Although the above results indicate there are apparent metabolic differences between fish living in uncontaminated and petroleum contaminated marshes, and that certain similar metabolic alterations can be seen in fish experimentally exposed to low levels of petroleum, it is difficult to define the true nature or significance of these observed effects. While environmental and experimental results are thus far consistent, suggesting a decrease in net lipogenesis and alterations in patterns of lipid synthesis, it is possible that such consistency is fortuitous and that additional factors may be involved. As an example, the apparent affects of petroleum on hepatic lipid synthesis are similar to those observed in fish starved for a week or more[7], suggesting a route whereby the observed effect of petroleum may be secondary in nature, with a primary effect being on food availability, nutrient absorption, etc. At present it is clear only that petroleum is one among those factors which may affect normal intermediary metabolism. It is not yet clear whether the physiological changes observed in fish in conjunction with low levels of

petroleum contamination are merely compensatory or whether they have some pathological significance representing certain dysfunction given continued exposure.

XENOBIOTIC METABOLISM

The duration and intensity of action of biologically active foreign compounds are to a large extent determined by their biological half-life. Accordingly, a principal aspect of hepatic function which is important in a consideration of fates and effects of petroleum hydrocarbons in fish is the biotransformation or metabolism of hydrocarbons. This process, in concert with patterns of uptake and of disposal of hydrocarbons by other routes, must play a significant role in determining the half-life of petroleum compounds in fish.

Cytochrome P-450 is a generic name applied to a class of hemoproteins which serves as the terminal oxidase in the mixed-function oxidase system involved in the biotransformation of many drugs, steroids, and compounds such as petroleum hydrocarbons. In addition to the cytochrome this microsomal electron transport system is comprised of a flavoprotein, NADPH cytochrome c reductase, and a phospholipid component[8]. In mammals the system is localized primarily in the microsomal fraction of the cell, principally in the liver.

The occurrence in fish of a microsomal hemoprotein analogous to mammalian cytochrome P-450 is by now a well recognized phenomenon. As with the mammalian protein, cytochrome P-450 from fish exhibits a characteristic absorption maximum at 450 nm when reduced and ligated to CO (Figure 1). Analysis of the heme iron of cytochrome P-450 from the teleost fish *Stenotomus versicolor* by EPR spectroscopy yields g-values at around 2.4, 2.24, and 1.9 (Table 2), values which are also characteristic of cytochrome P-450 from other sources[9]. In addition to these properties of the hemoprotein component, fish mixed-function oxidases are known to carry out a wide range of catalytic functions similar in scope to the mammalian system[10,11,12]. These features, in conjunction with the resolution of the cytochrome, reductase and phospholipid components of the mixed-function oxidase system from the elasmobranch *Raja erinacea*, and reconstitution of the active system[13], clearly establish the similarity between mammalian and fish mixed-function oxidases.

A great number of compounds are known to stimulate the activity of mixed-function oxidases, as described in a landmark review almost 10 years ago by Conney[14]. Included among inducing compounds are polycyclic aromatic hydrocarbons, typified by 3-methylcholanthrene which induces high levels of benzo[a]pyrene hydroxylase activity and a form of cytochrome P-450 which in the reduced, CO-ligated state is characterized by an optical absorption maximum at about 448 nm rather than 450 nm[15].

Treatment of the teleost fish *Stenotomus versicolor* with either 3-methylcholanthrene or an inducer of a similar type, 5,6-benzoflavone, resulted in a moderate induction of benzo[a]pyrene hydroxylase activity (Table 3). However, neither inducer caused increases in the amount of cytochrome P-450 present, nor was there a shift in the absorbance maximum of the reduced, CO-ligated species away from 450 nm. Similar results have been obtained by other investigators for different fish species[16]. This situation is in sharp contrast to the response of mammals to polycyclic aromatic hydrocarbon inducers, and presents a most intriguing topic for further investigation.

Induction of mixed-function oxidases in fish chronically exposed to environmental contamination by petroleum is a matter of some importance in a consideration of the disposition and effects of petroleum hydrocarbons in these animals. Recent reports have described population differences in levels of mixed-function oxidase activity in several species of fish[17,18,19]. In a similar investigation we have observed population differences in benzo[a]pyrene hydroxylase activity in *Fundulus heteroclitus* sampled both from contaminated and uncontaminated marshes (Table 4). Although there appears to be a relationship between the type of environment in which the fish were living and the capacity to metabolize xenobiotics, and also an effect of sex on this activity, most but not all of the differences expressed in Table 4 were found to be a feature of a strong correlation between size and mixed-function oxidase activity, normalized to body weight, in these samples[20]. Thus, it appears that chronic environmental contamination by petroleum may result in "induced" levels of mixed-function oxidase activity in certain populations[17,18], yet the above findings, and the data of Pederson *et al.*[19], strongly suggest that factors other than induction may explain some apparent population differences.

In addition to the interesting questions concerning such population variation there are numerous other questions regarding cytochrome P-450 mixed-function oxidases in fish. Such questions concern the multiplicity of cytochromes P-450 in lower vertebrates, time-dose relationships and the fraction(s) of petroleum which may be involved in putative induction, synergistic effects of various pollutants, and how induction may affect both the metabolism of endogenous substrates, such as steroid hormones, and also the ability to activate chemical mutagens and carcinogens.

This latter topic is of particular interest as it involves the binding of metabolites to macromolecular constituents of the cell and the carcinogenic action of some polynuclear aromatic hydrocarbons contained in petroleum. There is now substantial evidence in mammals linking the carcinogenic action of these hydrocarbons to their metabolites rather than the parent compound, and in particular to diolepoxides such as 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene 9,10-oxide[21]. These products of mixed-function oxidase activity may therefore be more dangerous than the unmetabolized hydrocarbon, and an enhanced rate of metabolism following induction will not necessarily result in a reduction of toxic potential. There is strong evidence indicating that fish are quite capable of metabolically activating potential carcinogens[22,23], and such activation of some environmental contaminants may be linked to a greater incidence of neoplasia in fish from contaminated regions[24].

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DISCUSSION

LAROCHE: On the last slide you showed no difference between the males and females of Wild Harbor. Did you notice if those females were ripe at all on autopsy?

STEGEMAN: They were.

BRUBAKER: Did you say that your level of oil was 200 micrograms per liter?

STEGEMAN: That is right. That works out to about 2/10 parts per million; that is right.

BRUBAKER: How did you arrive at that?

STEGEMAN: This is a system that we have used for some time, and it is added at that level. We use a shallow tank in a flow-through system and at the end of the system, right by the drain, water is drawn from beneath the surface and away from the sides and extracted to measure the amounts of hydrocarbon.

BRUBAKER: My question was: How did you arrive at that number?

STEGEMAN: Oh, why did we use that? It seemed a little bit higher than what might occur in waters, for instance, near a seep. I don't know what the levels are at the Coal Oil point, but I am sure they are in the neighborhood of a tenth part per million. Yet it was not so low that we wouldn't see anything at all.

BRUBAKER: The next question I have concerns your comments about energy metabolism and so forth. Do you observe any changes in the mitochondrial structure?

STEGEMAN: Yes, there were some changes we saw in the mitochondrial structure; there were enlarged mitochondria in the oil-exposed fish.

BRUBAKER: Finally, was there evidence of hyperplasia and neoplasia or increased cell division?

STEGEMAN: No.

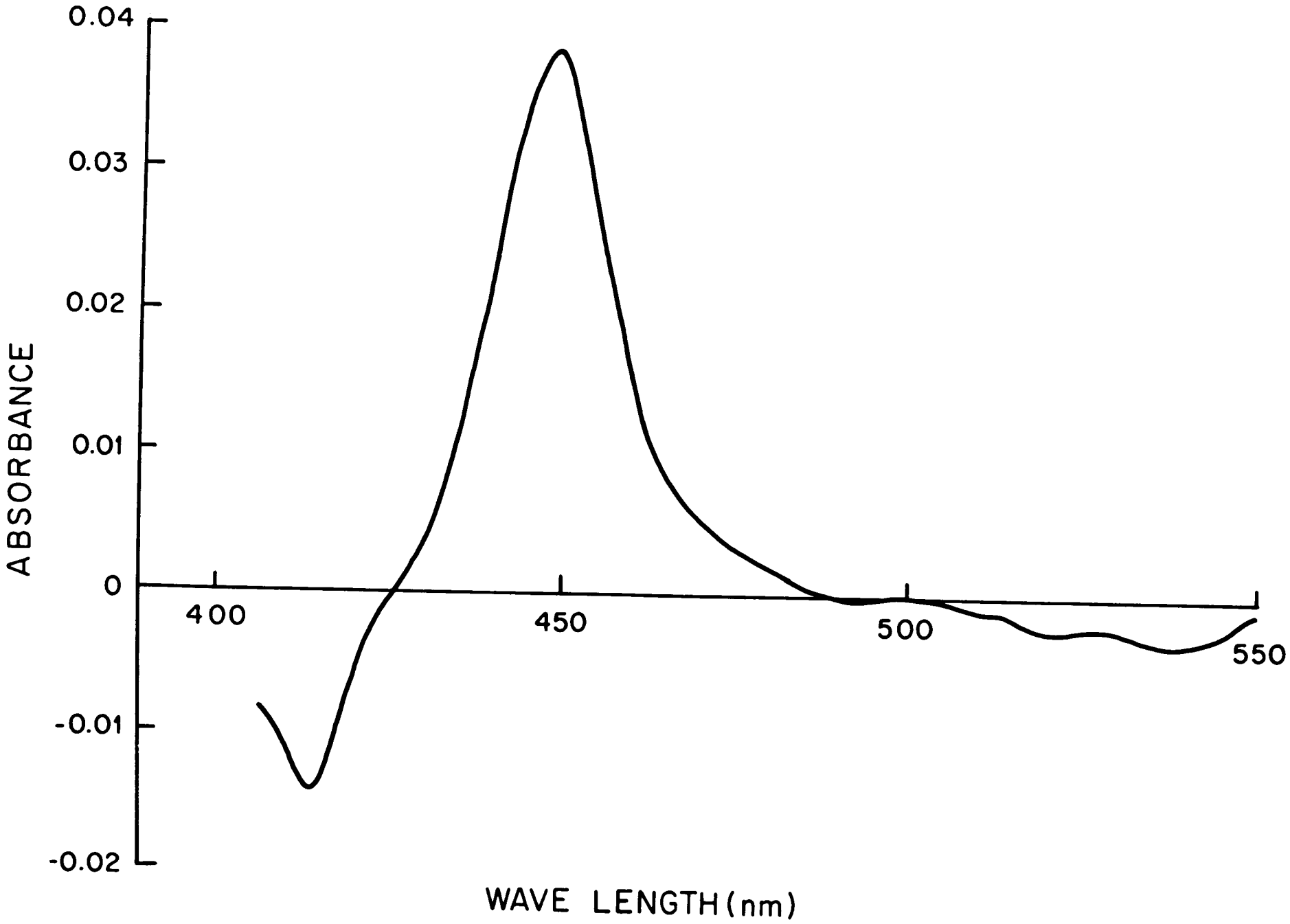

Fig. 1. Carbon monoxide difference spectrum of hepatic microsomes prepared from *Stenotomus versicolor*. Protein concentration was 1-2 mg/ml. Spectra determined in Tris-Cl buffer, pH 7.4, using a Cary 118-C

Table 1. Incorporation of acetate-1-^{14}C into hepatic lipid and hepatic lipid content in *Stenotomus versicolor* and *Fundulus heteroclitus*.

Sample	Days treatment[a]	Control				Experimental			
		N[b]	cpm/100 mg tissue[c]	% liver lipid	Lipid vacuoles per cell[d]	N[b]	cpm/100 mg tissue[c]	% liver lipid	Lipid vacuoles per cell[d]
S. versicolor	30	3	2895(±434)	1.8(±1.1)	-	3	179(± 23)[f]	1.1(±0.12)[g]	-
F. heteroclitus (Sippewissett)	5	5	1885(±305)	3.1(±0.3)	13.2	6	250(±150)[f]	2.4(±0.2)[e]	1.5[g]
F. heteroclitus (Wild Harbor)	5	11	440(±311)	2.4(±0.4)	12.8	11	300(±145)	1.8(±0.3)	0.6[g]

[a]Continuous exposure in a flow-through system with 180-200 µg #2 fuel oil (API)/liter.

[b]Samples were of mixed sexes, pooled, and the numbers in parentheses indicate S.E.M. of 2 to 9 replicates.

[c]cpm of lipid extracted following 2 hr incubation with acetate -1-^{14}C at 21(±1)°C.

[d]Values given are averages of visible lipid vacuoles counted in 25 randomly selected hepatocytes in histological preparations from each group.

[e-g]Significantly different from control at $P \leq 0.10$ (e); $P \leq 0.01$ (f); $P \leq 0.001$ (g). Values determined using 2 x 2 contingency tables and Fisher's exact test, Student's t-test or a one-way anova and F test.

Table 2. EPR[a] parameters of cytochrome P-450 from *Stenotomus versicolor*.

Sample	Low spin g values
Hepatic microsomes[b]	2.411, 2.249, 1.920

[a]EPR spectroscopy performed at 1.6°K.

[b]Microsomes prepared in Tris-HCl, pH 7.4, from untreated animals.

Table 3. Hepatic microsomal cytochrome P-450 and benzo[a]pyrene hydroxylase in treated *Stenotomus versicolor*[a].

Sample	N (pooled)	Reduced, CO-ligated absorption maximum (nm)	Cytochrome P-450 specific content (nmol/mg microsomal protein)	Benzo[a]pyrene hydroxylase (units/mg microsomal protein)[c]
Control	6	450	0.352	126
5,6-benzoflavone[b]	5	450	0.387	217
3-methylcholanthrene[b]	3	450	0.393	219

[a]Data derived from Chevion *et al.*[6]

[b]Animals received a total of 150 mg/kg over 8 days, injected I.P.

[c]Activity was determined in isolated microsomes and units are expressed as 3-OH-benzo[a]pyrene equivalents produced per minute.

Table 4. Hepatic benzo[a]pyrene hydroxylase activity in populations of *Fundulus heteroclitus*.

Population[a]	N (pooled)	Activity[b] per mg PMS protein (pmol/min)	Activity[b] per g body weight (pmol/min)
1. Monomoscoy	♂ (15)	56.9	62
	♀ (7)	34.1	96
2. Little Sippewissett	♂ (5)	67.0	105
	♀ (5)	65.0	142
3. Great Sippewissett	♂ (11)	75.9	139
	♀ (5)	49.3	189
4. Wild Harbor	♂ (7)	67.9	128
	♀ (4)	46.8	151

[a]Populations 1 and 2 were from uncontaminated marshes; populations 3 and 4 from marsh areas with known sources of petroleum and/or chlorinated hydrocarbon contamination.

[b]Activity was detetermined in post-mitochondrial supernatants and is expressed as 3-OH-benzo[a]-pyrene equivalents, (p moles) produced per minute per mg PMS protein, or normalized to body weight.

THE EFFECTS OF PETROLEUM HYDROCARBONS ON AQUATIC BIRDS

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Office of Biological Services

and

Environmental Protection Agency

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Recently petroleum hydrocarbon compounds have been positively identified in avian tissue and their structures confirmed utilizing gas chromatography/mass spectrometry techniques. Our analytical studies are utilizing these instruments to establish extraction procedures, check recoveries, and quantitate petroleum hydrocarbons in different avian tissues. Eggs and birds from coastal sites are being collected for residue analysis in the future.

The possible effects of petroleum on avian reproduction are receiving special attention. Earlier work suggested incubating eggs were sensitive to oil, and we have found dose-related mortality after application of only microliter amounts of crude oil in two separate hatching studies. Apparently some of the components of petroleum are highly embryotoxic at very low concentrations.

Our studies with adult mallards will compare physiological responses in ducks fed sublethal concentrations of crude oil with those fed a reconstituted aromatic mixture containing representative components in crude oil. Food chain studies with invertebrates and mallards are also being conducted to examine the possible effects of indirect petroleum hydrocarbon pollution in aquatic birds.

Disastrous spills, like the one last spring on Chesapeake Bay, receive media attention and kill thousands of waterfowl, but account for less than 10 percent of the petroleum spilled into the aquatic environment. The largest source of oil pollution results from the discharge of petroleum effluents during normal usage.[1] Increased exploration, processing, transportation, and consumption of petroleum will lead to unavoidable increases in low level oil pollution. However, the physiological effects of sublethal concentrations of petroleum hydrocarbons in aquatic birds are largely unknown.

The analytical methodology for detecting and quantitating petroleum hydrocarbons in avian tissues is in the early developmental stages and standardization of techniques has not been accomplished. However, there have been some reports of residues of petroleum hydrocarbons in tissues of birds from oil spill areas. For example, brain and muscle of an immature herring gull from the West Falmouth oil spill site contained total hydrocarbon contents of around 500 ppm as compared to 10 ppm in these tissues from another immature gull collected 15 km away in a clean area.[2] Planimetry of gas chromatograms from pentane eluates of the tissues were compared with those of Number 2 fuel oil. Snyder, et al.[3] collected tissues from three different aquatic birds at the San Francisco Bay oil spill. Two separate laboratories in Texas and Massachusetts analyzed the samples by gas chromatography and identified petroleum hydrocarbons in a composite sample of liver, kidney, fat, heart and brain of an oil-exposed common murre, in the liver and kidney of an oil-exposed surf scoter, and the liver of an oil-exposed western grebe. Both laboratories failed to detect petroleum hydrocarbons in an unexposed common murre. Comparison with gas chromatograms of Bunker C fuel oil indicated the murre tissues contained 8820 ppm of the $C_{15}+$ saturated hydrocarbon fraction, the surf scoter tissues 1200 ppm, and the grebe tissue 9100 ppm.

We will be using South Louisiana crude oil in our studies at Patuxent Wildlife Research Center. It represents a possible pollutant in the Mississippi Flyway, and is the product most likely to be encountered by mallard or black ducks -- two species readily available to us. Patuxent chemists, Dr. Martha Gay and Mr. Andrew Belisle, supported by a contractor, Dr. John Laseter, University of New Orleans, are establishing the most efficient extraction procedures, checking recoveries, and quantitating petroleum hydrocarbon fractions in avian tissues. Combined gas chromatography and mass spectrometry analysis of crude oil, clean duck feed, and duck feed containing 100 ppm crude oil were carried out and the mass spectra analyzed. The presence of oil in the treated feed was indicated by i) the almost gaussian distribution of the low molecular weight n-alkane hydrocarbons (paraffins), ii) which were superimposed over a biological pattern of odd carbon dominance, and iii) peaks with the same retention times as those in the crude oil gas chromatograms. The gas chromatograms of the clean duck feed were free of the n-alkane pattern indicative of the presence of oil. Sensitivity was checked by spiking liver tissue with 2,000, 200, or 20 ppm of crude oil, extracting with pentane and pentane/benzene mixtures, and analysis by gas chromatography/mass spectrometry. Three fractions were collected from a fluorisil column containing aliphatic hydrocarbons in fraction 1, and aromatic hydrocarbons in fractions 2 and 3. Saturated aliphatic hydrocarbons in oil were clearly discernible even at 20 ppm. Aromatic compounds identified included alkyl benzenes, benzocyclo-alkanes, and alkyl napthalenes. Both the aliphatic and aromatic fingerprints were obtained in 15g of liver spiked with 20 ppm crude oil.

The biological research underway at Patuxent Wildlife Research Center is directed at monitoring the environment by analysis of avian samples collected at coastal sites of proposed or ongoing petroleum development, and experimental studies on waterfowl.

Representative species of waterfowl killed in the 1976 Chesapeake Bay oil spill were collected and frozen. Egg collections have been made from 15 sites along the southern coast of Alaska extending to the outermost islands of the Aleutian chain. Other egg collections are planned along the northern and western coast of Alaska from Prudhoe Bay to Nome.

One of the matters we are most concerned with is the possible effects of petroleum contamination on avian reproduction. Seabirds nesting in the outer continental shelf area would be particularly vulnerable to petroleum pollution since many of the species have a low reproductive potential and are heavily concentrated on the nesting grounds. In prototype experiments by Drs. Peter Albers and Robert Szaro dose-related mortality of mallard eggs has been observed after application of only microliter amounts of crude oil. These studies were extended and application of microliter amounts of crude oil to eider eggs collected from Maine again resulted in high mortality. Reports in the literature that preceded these studies all indicated that incubating eggs were sensitive to the effects of petroleum products. Herring gull colonies have been controlled by spraying eggs with an oil emulsion.[4] In this case saturation of the eggs with oil probably cut off the oxygen supply needed for embryogenesis. Rittinghaus[5] spoke of an oil spill that resulted in feather contamination in brooding hens, and clutches of eggs that failed to hatch because of too much oil contamination. Mineral oil was applied to the overall surface of artificially incubated mallard eggs by Hartung[6] and again mortality that occurred was somewhat related to dosage. Mortality was almost certainly caused by interruption of gaseous exchange between egg and environment. In a study on herbicide toxicity one group of pheasant eggs was sprayed heavily with No. 1 diesel fuel and none of the 57 fertile eggs hatched.[7]

Mortality in the egg-oiling experiments at Patuxent was believed to be caused by toxic components in the crude oil and not interruption of gaseous exchange, since eggs treated with propylene glycol exhibited normal hatching success. Albers and Szaro also found that eggs treated with a mixture of paraffins found in crude oils showed only small decreases in hatching success. These results suggested the toxic components, which were highly embryotoxic at very low concentrations, might be aromatic compounds.

Gulls from Lake Ontario were examined by Fox, et al.[8] because they displayed almost totally depressed reproduction and represent a top level consumer in the food chain. One of the factors suspected to be involved was poor embryonic survival that may be attributable to the presence of embryotoxins. In an analysis for unknown pollutants 1 kg of whole body lipid was extracted and 14 polynuclear aromatic hydrocarbons separated on gas chromatography. Compounds identified from lipid extracts whose structures were confirmed by gas chromatography/mass spectrometry included napthalene, 2-methyl-napthalene, acetonapthalene, and biphenyl. Other compounds identified by gas chromatography included anthracene, 2-methyl-phenanthrene, pyrene, benz(a)fluorene, methyl-pyrene, benz(a)-pyrene, benz(e)pyrene, perylene, 9,10-diphenylanthracene, and carbazole. These are components of petroleum hydrocarbons and are definitely not of biogenic origin. The high molecular weight polynuclear aromatic hydrocarbons were present at least at 100 micrograms/gm lipid.

Crude oils also contain high concentrations of metals. The Committee on Biologic Effects of Atmospheric Pollutants have reported that different types of crude oils contain as high as 1400 ppm vanadium.[9] We have completed the initial phases of a kinetic study of vanadium and found this crude oil component significantly altered lipid metabolism in mallard hens (White and Dieter, unpublished results). Normal cholesterol concentrations in blood of non-laying hens averaged 119 mg/dl compared to 38 mg/dl in laying hens. However, in laying hens fed 100 ppm vanadyl sulfate the average cholesterol concentration at 3, 6, 9, and 12 weeks was no different than that in non-laying hens, averaging 91 mg/dl. Similar alterations in lipid metabolism occurred in a pilot study of mallard hens fed 10,000 ppm crude oil. They also laid fewer eggs than normal. Preliminary information of this sort is being gathered for incorporation into a large scale, chronic reproductive study.

Reproductive responses in waterfowl are not the only demonstrable effects of sublethal concentrations of petroleum hydrocarbons. One of the adaptive responses that occurs when waterfowl are exposed to salt water is an increase in water uptake by the small intestine to compensate for osmotic water loss from tissues.[10] Crocker, et al.[11] used an *in vitro* system of sacs of small intestine and found that ducklings dosed with 0.2 ml of Santa Barbara crude oil failed to absorb water at a rate commensurate with undosed ones adapted to salt water. Using similar *in vitro* techniques the same authors were able to show that the increase in intestinal water uptake developed over prolonged exposure to salt water was abolished after a single dose of crude oil. In a followup study[12] this *in vitro* bioassay system was used to compare effects of crude oils from eight different locations. It was found that each oil inhibited development of the adaptive response (increased water transfer after 4 days salt water exposure) and that maximal inhibition was due to Kuwait crude and South Louisiana crude oils. Dr. Neil Holmes, University of California, Santa Barbara, has contracted with us to extend these studies with pen-reared mallard ducks. Initially his group will study electrolyte balance and adrenal hormone responses in sea water adapted mallards fed South Louisiana crude oil in their food. Their previous work with adult Pekin ducks suggested that adaptation to sea water was impaired by crude oils and mortality occurred when a cold stress was imposed.[13]

Physiological studies at Patuxent Wildlife Research Center will evaluate the effects of petroleum hydrocarbons on hepatic, cardiac, and renal function. Mrs. Nancy Coon and Dr. John Patton will be comparing responses in mallard ducks fed sublethal concentrations of South Louisiana crude oil with those fed a reconstituted aromatic mixture (RAM) containing representative components in the crude oil. The crude oil will be fed at 2500 and 25,000 ppm, and because South Louisiana crude oil contains 16% aromatics, the RAM will be fed at 400 and 4000 ppm aromatics in a paraffin mixture. The aromatics employed are ethyl benzene, 1,2,3,4-tetrahydronaphthalene, dimethylnaphthalene, 2,3,3-trimethylindolenine, acenaphthylene, acenaphthene, phenanthrene, 2-methylbenzothiazole, dibenzothiophene, and 2,6-dimethylquinoline. The paraffins consist of tridecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4,6,6-pentamethyl heptane, 2,2,4,4,6,8,8-heptamethylnonane, 2,6,10,14-tetramethylpentadecane, and decahydronaphthalene. The effects of petroleum hydrocarbons on hepatic function are under close scrutiny because the liver represents

the primary site of detoxification and excretion of the toxic compounds present in crude oils. Hartung and Hunt[14] measured hepatic function of Pekin ducks 24 hours after dosage with 3 to 24 ml/kg of diesel oil. They obtained a dose-related increase in plasma aspartate aminotransferase activity at all treatment levels, and increased bromsulphalein dye retention at doses of 12 and 24 ml/kg.

Dr. Patton has adapted the mammalian indocyanine green clearance test for use in birds. It is a sensitive measure of hepatic function in which removal of the dye from blood is carried out entirely by the liver. Injection of known amounts of dye into the jugular vein and withdrawal of serial, timed blood samples from the alar vein has proven to be an accurate, reproducible method to measure half-life of dye, disappearance rate of dye, plasma volume, blood volume, plasma clearance, and hepatic blood flow. In addition tissue-specific enzymes that appear in the circulation because of discrete organ damage will be measured. These include plasma aspartate aminotransferase originating from the liver, ornithine carbamyl transferase originating from the kidney, and hydroxybutyric acid dehydrogenase originating from the heart. Because high concentrations of vanadium are present in crude oils and this metal alters lipid metabolism in ducks, triglyceride and cholesterol concentrations will also be measured in the crude oil feeding study.

Food chain studies are also being conducted at the Center to study the possible effects of indirect petroleum hydrocarbon pollution in aquatic birds. The evidence for uptake and depuration of petroleum hydrocarbons by invertebrates are conflicting,[15] and the evidence for bioaccumulation is incomplete. Dr. I. Barry Tarshis is doing preliminary feeding studies with several species of clams, snails, and crayfish to select a suitable food item for waterfowl that can be readily obtained, but ultimately can be reared in a clean, laboratory environment. Initially we hope to establish the kinetics of South Louisiana crude oil transfer from water to invertebrate to duck. Radioactive tracer methodology will help to identify the uptake, accumulation, and loss rates in the system. Once the transfer rate of petroleum hydrocarbons through the system is established biological responses to expected environmental concentrations can be evaluated.

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DISCUSSION

LAROCHE: Thank you. Did I understand you correctly that you intend to reconstitute some oils from paraffins and aromatics?

DIETER: I may have phrased that a little badly. What I meant to say was that we hope to use representative compounds that are present in crude oil, and we have selected 10 paraffins and 10 aromatics for our reconstituted mixture. We know that these compounds are present in the crude oil we are trying to look at, and the results we get will merely be those of these 20 compounds.

LAROCHE: If you are to reconstitute these things, you realize that even if they are, supposedly, chemically pure, you may have impurities. The responses may not at all compare to any realistic exposures to, let us say, a commonly occurring crude oil.

DIETER: All right. We realize that the individual compounds may not be 100 percent pure, and these are being analyzed to see what degree of purity we have. We know, for instance, that 1, 2, 3, 4-tetrahydronaphthalene supplied by one of the reputable chemical companies is only 99 percent pure. So we are subjecting this chemical or this mixture to GC mass spec analysis to try to find out what we have.

In relation to the second point in your statement, we realize that the responses we get may not be realistic in terms of what you might find in the environment. That is why we are comparing the responses to a similar group of animals fed the crude oil. What this gives us, in addition to some effect of the reconstituted mixture on biological response, is that it provides our analysts with tissues from animals that have been fed this mixture. That is a little analytically simpler, and we may be able to advance our analytical techniques and our understanding in this area faster than we would working with the whole crude oil.

BRUBAKER: I have a question on the effect on hatching. Is that just an effect on hatching itself or was there also an effect observed in the chicks following hatching?

DIETER: The surviving chicks that we did obtain did not appear abnormal in any way at all. So the effect was apparently very swift and occurred very early, within a very short time after application. The majority of the mortality occurred within about four days after the application. Thereafter, the mortality trickled along. The chicks that hatched appeared totally normal.

BRUBAKER: Five microliters, for example, were the microliter samples straight oil? I mean, it wasn't diluted with anything?

DIETER: That was straight oil.

BRUBAKER: The second question I have concerns your outline of your pathology protocols. Is that going to be structured in accord with any observed effects, or do you have some particular observed effect already in mind which you are trying to document? Why are you measuring hepatic, renal, and cardiac functions?

DIETER: The reason we are using this approach is because the liver is the primary site of detoxification of these products. Therefore, we are concentrating on the liver. By using the plasma enzyme assay tests and looking at specific enzymes that originate from either the heart or the kidney, we can very easily at the same time take a look at whether or not any pathology is occurring in these particular organ systems from petroleum hydrocarbons at the concentration that we are employing.

Another very good reason for using this protocol is that all of these systems, the methodology and everything, has been worked out. It has been completed in other studies on other types of environmental contaminants and we are well familiar with the techniques employed.

We have been conducting prototype experiments with very small numbers of birds, feeding them different types of crude oils and, indeed, we have found indications that some of these variables will respond. Therefore, we are homing in on these particular ones.

HAY: I would like to ask you two questions. One, are you going to use salt water adaptive birds like Dr. Holmes did at the University of California, Santa Barbara?

DIETER: No. Our birds will not be salt water adapted at all.

HAY: You won't be able to make any comparisons then?

DIETER: No. Dr. Holmes has been concerned with the in vitro systems on water uptake and electrolyte uptake by intestinal preparations. He has received a contract from the Fish and Wildlife Service and will be conducting studies with salt water adapted mallard ducks, measuring adrenal response and electrolyte changes, and the effect of oil on this system.

HAY: One of the big impacts on migratory birds, of course, in the past has been oil spills on sea birds, not on mallards. Are you going to do work of this nature on sea birds?

DIETER: The only work we are going to be doing immediately on sea birds will be some analytical work on collections of birds or eggs from proposed sites of petroleum development or oil spill sites.

HAY: This is for baseline data?

DIETER: This is baseline data. We hope to complete work on the prototype species, the mallard or the redhead or the black duck first, before we try to get into further work with a pelagic bird and a salt water bird. We do have the possibility of using eider ducks, which are sea ducks, for some of our early physiological work.

I would say that the ducks we are using initially, though, are exposed to estuarine environments as they travel down the Mississippi flyway and winter in the Gulf Coast area.

HAY: The possibility of birds getting fresh oil with a lot of aromatics in them, of course, would occur primarily in the spill situations. Are you going to consider the use of any weathered oil in any of your experiments?

DIETER: Not initially. We are talking about the possibility of using weathered oil for some of the nonphysiological experiments, but initially what I have outlined will carry us through, at least for the first year.

BRUBAKER: Just from that discussion, is there a significant probability that mallard nests would receive 5 microliters or more of crude oil or the oil that you are concerned about?

DIETER: The reason that we are looking at the effects of crude oil on hatchability is the possibility that ducks might be exposed to oil either around the fringes or directly from an oil spill, get the oil on their breast feathers, and come back and expose the eggs. If you look at the field situation from that standpoint, an egg might very well have more than 5 microliters or more than 50 microliters of oil applied to it.

GOULD: Could you explain the rationale for your protocol of the detailed experiments you are planning? For example, why do you choose 25,000 parts per million of oil in the feed? Is there any justification based upon any real life situations that you anticipate in which 25,000 parts per million would be in the feed? Or are your experiments just exploratory?

DIETER: The reason we chose these particular levels of crude oil, 25,000 and 2500 parts per million, is that these are the levels that Dr. Holmes was using when he found a response in white Peking ducks that had been adapted to salt water and exposed to oil and then stressed by a decrease in temperature.

We thought that we would pick levels that would mimic those for which he had already found responses. The reason why we are using, then, 4000 and 400 parts per million of our reconstituted mixture is because one of the oils that we are concentrating on, South Louisiana crude, has 16 percent aromatics in it.

BEHAVIOR AND PATHOPHYSIOLOGY OF SEALS EXPOSED TO CRUDE OIL

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ABSTRACT

Ringed seals, Phoca hispida and harp seals, Phoca groenlandica were exposed to oil both in the field and in the laboratory. They were either placed into crude-oil-covered water, brush-coated with oil, or given oil by mouth. Twenty-four hour surface exposure to light crude oil was damaging only to the eyes of healthy seals, whereas stressed seals died within 71 minutes of exposure. Oil in quantities reasonably expected to be ingested during an oil spill was not irreversibly harmful. Evidence is presented to show that the consequences of an oil spill ultimately depend on the season of spill, productivity of the area, and the variable health status of a seal population.

This report is modified from the original publication; Geraci & Smith; Direct and Indirect Effects of Oil on Ringed Seals (Phoca hispida) of the Beaufort Sea. J. Fish Res. Board Can. (In press Sept. 1976).

INTRODUCTION

Recently, the search for petroleum in the Canadian arctic has increased. Oil reserves in the nearshore areas of the southeastern Beaufort Sea have already been proven. Tentative approval to start drilling in the further offshore areas has been given for the summer of 1976. As the intensity of exploration increases, so does the prospect of major blowouts and oil spills. Our study was designed to evaluate the behavioral, physiological and pathological consequences of crude oil contact and ingestion on wild ringed seals under field and laboratory conditions. Ten years of data on ringed seal population structure (Smith 1973; Smith et al. 1973) plus stress indicators, (Geraci and Smith 1975; Smith and Geraci 1975) are used to extend the findings to include the probable consequences of large-scale offshore oil exploitation on seal populations.

MATERIALS AND METHODS

Brown's Harbour, on the east side of Cape Parry, ($70^{o}05'30"N$, $124^{o}22'30"W$) was the site chosen for capturing ringed seals, Phoca hispida in August and September 1974. During this period 96 seals, 32 of which were live, were caught by the method described by Smith et al. (1973). All live seals were placed in holding pens measuring 3.6 m^2, constructed of pipe and chain-link fencing. Pens were located in a small saltwater pond near the netting site. Norman Wells (N.W.T.) crude oil was used in the ringed seal oiling experiments.

Immersion Studies

Prior to immersion in oil, each of six seals was immobilized with ketamine (Geraci 1973), blood samples were taken, and a sonic temperature telemetry pill was administered. Seals were then placed in a pen 2.4 X 2.4 X 1.2 m high that had a plywood floor and sides which allowed water to circulate through a 3-cm opening 35 cm below the oil-water interface. Seals were in the pen for 12 h before oil was introduced. Body temperatures were monitored every 3 h and blood samples were drawn to establish control values. Crude oil, sufficient to create a 1-cm thick surface layer (60 liters), was poured into the pen. Sea ice was then added to cool the water to about 8 C. Seals were left for 24 h, then removed, sampled, examined, photographed, and placed in a clean-water pen. They were monitored continually, and a subsample was killed by gunshot at 2-day intervals and necropsied.

Next, a small group of ringed seals was taken to holding facilities at the University of Guelph, Ontario, where a second immersion study was conducted. There, the effects of low level ingestion of crude oil were also assessed. The oil immersion study at Guelph was carried out in pools 3 X 3.6 X 1.2 m deep, containing water of 24‰ salinity, at about 13 C. The three seals used were apparently in good health and eating Atlantic herring, Clupea harengus harengus, and rainbow smelts, Osmerus mordax. After 2 mo. of acclimation in captivity, they were exposed to oil in the same manner as in the field experiment.

Nine, 3-4 wk-old whitecoat harp seals, P. groenlandica, were used to assess the effects of oil coating on temperature regulation in pups. The harp seal study was carried out in March 1975 on the Magdalen Islands. Core body temperatures were monitored with the aid of a YSI telethermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) fitted with an internal rectal probe. Temperatures were recorded every 4 h for 48 h prior to oiling and continued for up to 4 days after . Six seals were designated as experimentals and three as controls. The experimental seals were well coated by brushing Norman Wells crude oil onto the hair over the entire body. The next day, as a measure of assurance they were recoated in the same manner using crude oil from Mildale, Saskatchewan. Four experimental seals and two controls were killed 3 days after oiling and the remaining ones a day later. All the animals were weighed before and at the end of the experiment. Postmortem examinations were carried out on the dead seals.

Ingestion Studies

Ingestion experiments were carried out to evaluate the effects of seals swallowing a substantial amount of oil during a spill. Seals are not carrion feeders, and any oil which they might consume from live contaminated prey would probably not exceed 5 ml/day. Using this rationale, a low level, cumulative oil-ingestion study was carried out on five ringed seals at Guelph. Blood samples were drawn 6 times during 20 days prior to the experiment. Each seal was then given 5 ml/day of Norman Wells crude oil for 5 consecutive days. Oil was placed in 1-ml capsules and inserted into the food fish. Blood samples were taken at frequent intervals for 4 wk after ingestion of oil. Blood was processed for hematology and blood chemistry.

A high level, single-dose ingestion study was carried out on 2-3 wk old fasting harp pups. Seals were divided into two groups, each containing six experimental and one control animal. One group was fed 75 ml of Norman Wells crude oil, the other 25 ml, as a single dose. One seal from each group was killed 1, 2, 4, 6, 8, and 10 days after ingestion of oil. Controls were also killed on the 10th day. Blood samples were drawn from all seals 6-8 h before ingestion of the oil, and again just prior to death.

Analytical Techniques

Blood samples throughout this study were drawn, preserved, and analyzed by the methods described by Geraci (1971), Geraci and Engelhardt (1974), and Geraci and Smith (1975).

Blood was analyzed for plasma chemical constituents by multichannel autoanalyzers. (Technicon Instruments Corp., Ardsley, New York), according to the methodology outlined in Technicon bulletins.

Enzyme activities were determined kinetically and colorimetrically using commercially available kits. Details of the analyses are given in Smith and Geraci (1975).

RESULTS

Immersion Studies

In the field experiments the oil formed a uniform film on the surface of the water as soon as it was introduced into the holding pens. For the first 2-3 min, the six animals continued to move about the surface with no apparent recognition of the oil. Because of the swimming movements, the oil quickly became churned into the whole water column, though most of it still remained on the surface. Within 3 min, the heads of the seals were darkened with oil. As they continued to swim about, the hair of the back became oiled, and within 20 min. the abdominal hairs became stained. (Fig. 1)

Seven or eight minutes after the oiling, one of the seals began to lacrimate excessively, and would open and close its eyes. It jumped from the surface and shook its head vigorously. Soon, eye irritation became apparent in the other seals. (Fig. 2) They lacrimated profusely, yet at first there was no attempt to close their eyes and avoid the oil. Twenty minutes into the study, however, some of the seals seemed to have difficulty keeping their eyes open; the conjuctiva of the eyes were obviously reddened and inflamed. Breathing rate of these same seals appeared to increase, and two of them stretched their necks out of the water and shook their heads. The animals were also observed to force air through their nostrils making an audible sound when at the surface. This general behavior seemed to persist throughout the first 4 h by which time all of the seals were lacrimating and squinting.

Throughout the remaining exposure period, five of the seals remained submerged most of the time, the sixth and most aggressive of the group would remain on the surface and continue its agonistic behavior towards approaching persons or other seals. When on the surface all of the seals showed varying degrees of arching of the back, a behavior that was not observed in the control group nor in the experimental group prior to oiling.

Twenty-four hours after the introduction of the oil, the seals were removed from the pen and examined. All showed obvious signs of eye disturbances, characterized by blinking, squinting, lacrimation, and severe conjunctivitis with swollen nictitating membranes; some evidence of corneal erosions and ulcers were also noted. (Fig. 2, Table 1)

Within 3 h of being placed into a clean-water holding pen most of the eye squinting subsided, there was less lacrimation, the seals remained quiet and calm, and body quivering and arching of the back was no longer detectable. After 20 h all appeared to be in good health. Their hair coats were clean and the eyes showed no signs of irritation. By the 3rd and 4th days, there was scarcely visible evidence that the seals had been contaminated by oil.

Temperatures, which were monitored throughout the oil-exposure study, remained stable with a mean core temperature of 37.7 C (SD = 0.66).

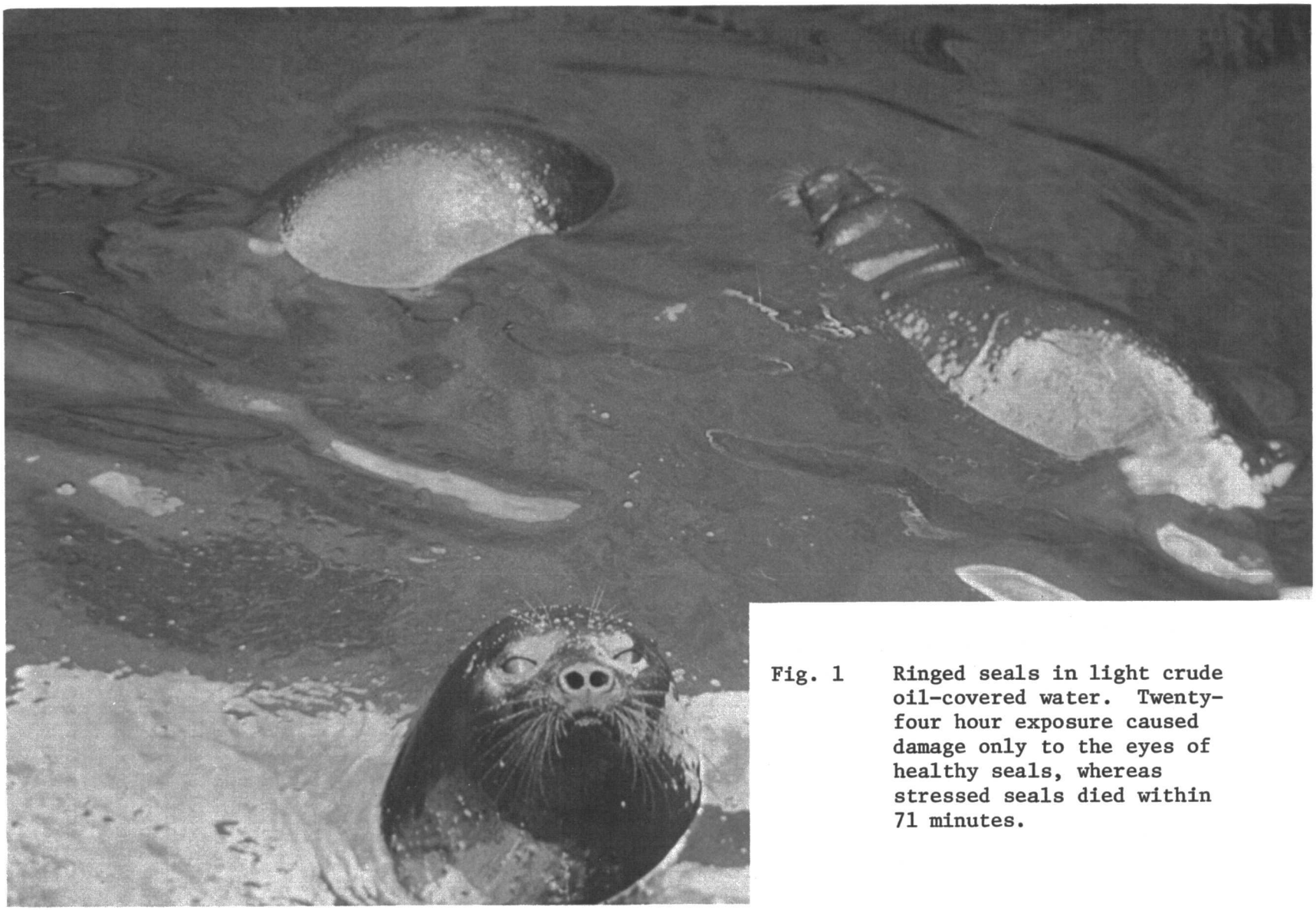

Fig. 1 Ringed seals in light crude oil-covered water. Twenty-four hour exposure caused damage only to the eyes of healthy seals, whereas stressed seals died within 71 minutes.

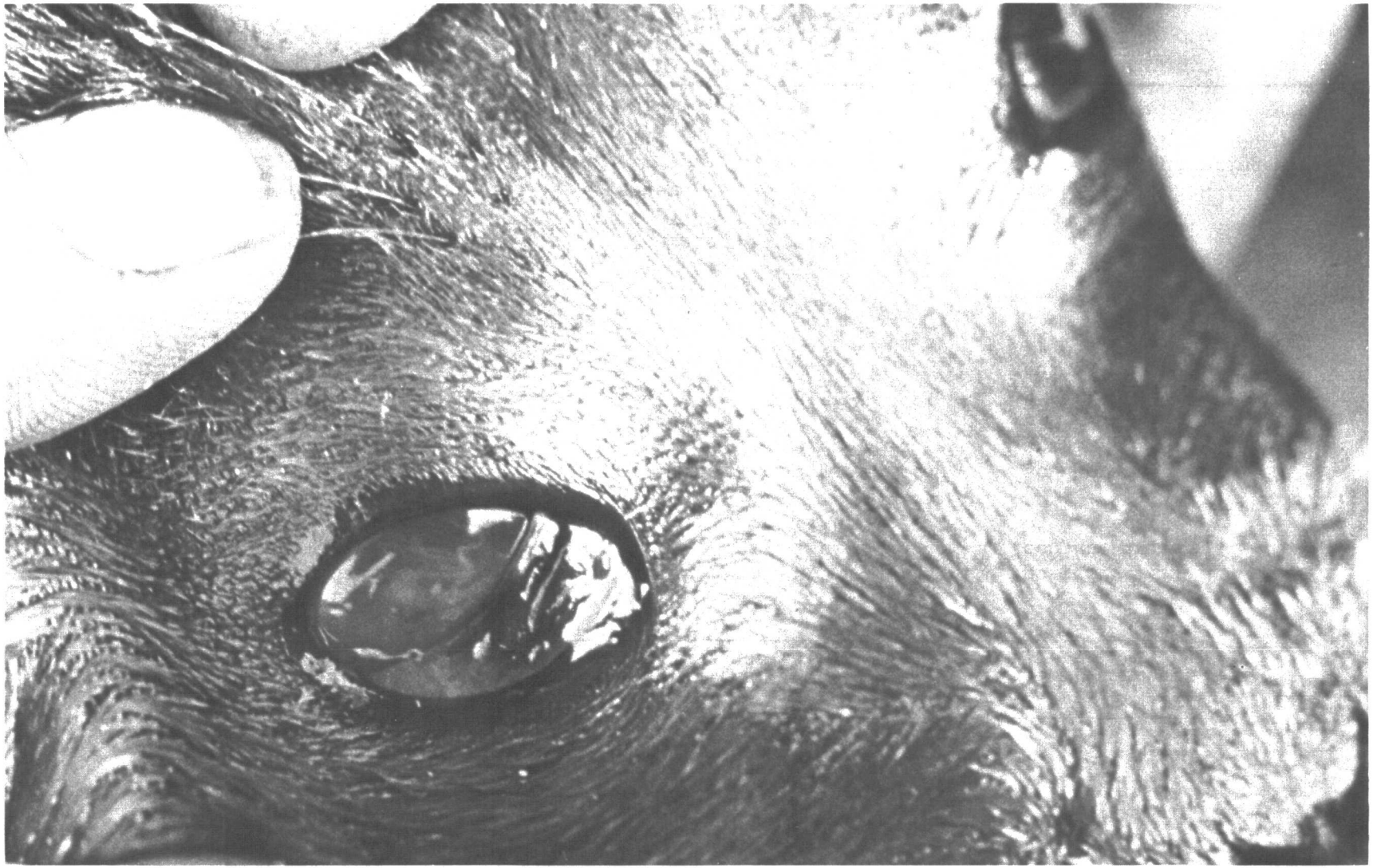

Fig. 2 Conjunctivitis in the eye of a ringed seal after 24 hr exposure in light crude-oil-covered water. Nearly all of the seals had evidence of eye inflammation, which regressed soon after they were placed in clean water.

Table 1. Condition of ringed seals at the time of removal from 24 hours exposure in crude oil-covered water

Seal #	Lids	Lacrimation	Conjunctivitis	Cornea	General comments
1	blinking	moderate	severe	shallow ulcer 1 cm x 1.5 cm right eye	most severe conjunctivitis of all seals
2	closed	severe	moderate	2 mm shallow ulcer left eye	head drawn back; arching of back
3	open	moderate	severe	diffuse ulcer 5 x 5 mm right eye	slight body quivering
4	blinking	moderate	moderate		
5	squinting	slight	moderate		
6	firmly closed	moderate	severe	pinpoint, erosion, left eye	arching of back some body quivering

Hematologic and plasma chemical analyses taken throughout the oil immersion study revealed few consistent patterns of change. Plasma levels of calcium, blood urea nitrogen, glucose, cholesterol, bilirubin, potassium, chloride, protein, albumin, and inorganic phosphorus were unaffected by exposure. There were significant but inconsistent changes in uric acid levels in three of the seals. Sodium levels dropped below 147 mEq/liter in 4 out of 20 samplings in six seals, twice before oiling, and twice 24 h after oiling.

Eight plasma enzymes were analyzed. In four seals, glutamic oxalacetic transaminase, an enzyme of muscle and liver origin, decreased from abnormally high levels at the beginning of the experiment, to normal values at the end. In the two remaining seals, the pattern was reversed. Creatine phosphokinase, also a muscle enzyme, decreased in five out of the six seals. The remaining enzymes alkaline phosphatase, lactic dehydrogenase, glutamic pyruvic transaminase, sorbitol dehydrogenase, gamma-glutamyl transferase, and leucine amino peptidase remained within normal limits throughout the experiment, as did all of the blood cellular elements. Individual values for all the parameters considered are detailed in Smith and Geraci (1975, tables 5-10).

Three ringed seals were used in a laboratory immersion study. Norman Wells crude oil was poured into the holding tank and came into contact with the seals within 2 min. Almost immediately, all three animals began to shake vigorously. Eye blinking and frequent audible exhalations were observed. This behavior continued for 15 min. during which time the seals remained underwater for unusually long periods. Twenty minutes after the oiling, a haul-out platform was provided. The seals made no attempt to climb onto it. Swimming movements were uncoordinated. One animal made determined attempts to leave the pool, thrashed on the surface along the edge, dived, and died 21 min. after oiling.

Twenty minutes after the oil was introduced, the two remaining seals stopped thrashing and became quiet on the surface of the pool, but milder trembling and forced exhalation continued. The second seal died 60 min. after the oiling. The behavior of the last seal remained essentially unchanged until it thrashed about briefly and died 71 min. after contact with the oil.

Hematologic and plasma chemical studies performed before oiling and immediately after death revealed significant differences in six parameters. Uric acid increased from a range of 2.8-4.2 to 5.4-5.7 mg/100 ml. Hydrocortisone decreased from 80-150 to 55-70 µg/100 ml. Potassium, increased from 3.6-4.1 to 6.6-7.8 mEq/liter. The white blood cell count and the total eosinophil count remained unchanged in the seal that died after only 21 min. of exposure, whereas the two remaining seals showed marked total white cell reductions of from 50 to 75%, and eosinophil counts decreased to 15-33% of the pre-oiling values

In the field oil-coating study conducted on harp seal pups there were no significant differences in core temperatures between pre- and post-oiled seals or between the experimental and control groups. (Fig. 3) No

Fig. 3 3-week old harp seal "whitecoat" pups before and after brush-coating with light crude oil. There were no thermoregulatory problems observed in these post-weaned seals which depend on blubber and not hair for insulation.

behavioral changes were observed during the experiment. All seals showed a weight loss through the study period during which time they were not fed. There were no pathological changes which could be attributed to oil coating.

Ingestion Studies

There were no obvious deletrious effects or behavioral alterations in any of the experimental animals throughout the study. Of all the blood parameters analyzed, only creatine phosphokinase showed changes. Three of the four animals had appreciably higher levels after ingestion.

When oil was fed in a single high dose to harp seal pups in the Magdalen Islands, its presence was noted on the anus and on the hind flippers 1½ h later. This occurred in the low dose (25 ml) and high dose (75 ml) groups. Most of the oiled seals fell asleep within 6-8 h after ingestion, at which time the controls had already been asleep for 4 h. Ten hours after oiling, one highly dosed animal appeared to be unusually unresponsive to manipulation. This behavior was not apparent on subsequent observations. For 12 h from the time oil excretion was first noted, the pelts were stained yellow from rolling in oil-covered snow. When they were moved to clean snow, the pelts became almost white again within 24 h. Aside from these findings, all observations made hourly up to 24 h after ingestion of oil, and at 3 h intervals thereafter, revealed no significant differences in behavior or health between the control and experimental seals.

During the high dose study, plasma levels of muscle-based enzymes, CPK, GPT, and aldolase showed a definite and significant pattern of decline from the beginning to the end of the experiment. This pattern occurred in the high and low dose groups as well as the controls. Individual values are shown in Smith and Geraci (1975, tables 18 through 25). Sorbital dehydrogenase showed a single peak of high activity 48 h after high-dose administration, reflecting transient liver damage.

Significant hematologic findings were confined to packed cell volume which increased with time in control as well as experimental animals. The increases, which reflect hemoconcentration or dehydration, were in the order of 0.5-50.6% within the 10-day experimental period. This finding was consistent with the 5.7-24.5% weight loss in all the animals during the experimental period and reflects the fact that they were not fed during the study. Necropsy on the seals did not reveal significant lesions.

DISCUSSION

The present study shows that up to 75 ml of ingested crude oil is not irreversibly harmful to seals. The liver is generally regarded as a prime target organ for hydrocarbon damage in mammals. Effects of such damage have been well documented (Cornelius and Kaneko 1963). If sufficient quantities of these hepatotoxic substances are administered, liver

enzymes are released into plasma and are detectable. Degree and duration of enzyme release is generally a function of the quantity and toxicity of the substance(s). Geraci (1972a) induced measurable liver damage in gray seals, Halichoerus grypus, using 5 and 10 ml of carbon tetrachloride, a rather potent fraction. In the present studies, there was only transient liver enzyme release. If there was liver damage, it was negligible. The release and subsequent decline of muscle-based enzymes during the study reflect the vigorous capture handling activity which was imposed on the seals: it was transient and not related to oil. Similarly unrelated was the observed weight loss and hemoconcentration which are physiologically natural events associated with postweened fasting whitecoat pups.

Surface contact with oil had a far greater impact on the seals than did absorption. Such direct effects of oil can be categorized as physical, physiological, and behavioral. The simple physical fouling of birds and fur bearing mammals has been well documented (Hartung 1967). The picture in marine mammals is not clear. Literature does exist indicating that seal deaths have been associated with Bunker C fuel oil (Warner 1969; Anon 1970). However, the reports do not convincingly indicate that the observed mortality was directly linked to contact with oil. Postmortems were not performed and data were not provided for comparison of the natural mortalities in years when no oil spills occurred. Other reports indicate that although large numbers of seals had come into contact with oil, no mortalities were observed (Hess and Trobaugh 1970; Morris 1970; Muller-Willie 1974). Some evidence also exists that gray seals actively avoid oil slicks (Anon 1970). LeBoeuf (1971) and Brownell and LeBoeuf (1971) in reliable investigations of the crude oil spillage in the Santa Barbara Channel observed no deaths of seals that could be related to oil. In spite of this, popular press and scientific review papers (Nelson-Smith 1970) referring to the incident imply that seal deaths were actually caused by the oil.

In the present study, oil fouling with Norman Wells crude oil did not cause any mechanical damage such as sticking of the flippers to the body or the plugging of body openings. It should be pointed out that Norman Wells crude oil, which is similar to Beaufort Sea oil, is relatively light, highly volatile, and of low viscosity. It is unlikely that such an oil can be compared to the more viscous crudes and to fractional products such as Bunker C, in its physical fouling characteristics.

Hair in postweaned phocid seals contributes little to their overall insulation (Irving and Hart 1957); no thermoregulatory problems were expected and none were observed. Core body temperatures of oiled seals showed no trends indicating increased thermal conductivity, such as occurs in other fur-bearing mammals (McEwan et al. 1974). This was also true in the oil-coating studies on whitecoat harp seals which were between 2 and 4 wk old, even though Øritsland and Ronald (1973) showed that the white lanugo does provide protection against skin cooling by the wind. It may be significant that the pups in the present study had already developed blubber layers of between 2.5 and 5.0 cm, which apparently was enough to prevent surface heat loss. Seals between the time of birth and the laying down of the blubber are more dependent on hair for insulation and, presumably, would be vulnerable to the thermal effects of oil coating. In

the event of an under-ice oil spill the ringed seal pups, which are born in early April and occupy subnivean birth lairs (Smith and Stirling 1975), could be exposed to an oil-fouled ice surface and a layer of oil at the water-ice interface for the whole of the suckling period. It is unlikely that the adult female seal would be able to move the pup away from the contaminated area until it had been weaned.

Eye damage was a significant finding in the field oil study. At least some of the damage appears to have been done by volatile components of the oil. Nearly all of the investigators experienced eye irritation when exposed to the pungent fumes in the seal pen. The eye inflammations in the seals subsided soon after they were placed in clean water. Continued exposure to oil may have resulted in more severe and possibly permanent eye disorders. Nelson-Smith (1970), quoting an unidentified source, states that oil damage in seals frequently includes severe eye irritation, and makes reference to a female seal, now blind, which was rescued during an oil spill. Eye damage and blindness are observed in wild and captive seals (King 1964; Ridgway 1972), and the occurrence in nature need not be linked with oil or other noxious substances. Nevertheless, oil is irritating and damaging to eyes and the severity of damage is likely to be related to exposure time and to the concentration of volatile components.

Divergent results were obtained from the ringed seal field and laboratory immersion studies. Apart from eye damage and behavioral changes, at least one seal in the field study showed some histologic evidence of kidney damage. The lesions may be related to an attempt to concentrate and/or excrete oil or its metabolites via the urinary system. This route has been confirmed by Engelhardt, Geraci,and Smith (unpublished data), who observed the presence of high kidney and urine oil concentrations, which persisted at decreasingly lower levels for 6 days after immersion. The ultimate consequences of the kidney lesions can be assessed only with a long-term study.

Seals in the Guelph immersion study provide a clue to an important factor which could complicate the effects of an oil spill. They responded to oil with nearly convulsive behavior and died in just over an hour. These findings point to stress, with death triggered only by the presence of oil. Stress is a rather common condition of phocid seals. Generally, it has been regarded as a disease of captivity. The exact mechanism is unknown, but it is probably related to adrenal insufficiency (Geraci 1972b). Affected animals become uncoordinated, display muscle quivering, and have electrolyte disturbances. When further stressed in any way, they often die with little or no warning (Geraci 1972a, b). The Guelph study adds oil to the growing list of factors that can trigger the death of stressed seals.

Recently, we have shown that stress also occurs and can be assessed hematologically in wild ringed seals (Geraci and Smith 1975). The electrolyte disturbances, which characterize the condition, have been found in emaciated and late molting animals (J. R. Geraci and T. G. Smith unpublished data). Assuming that animals so stressed respond as do captive stressed seals, then any severe disturbances, including contact with oil, would conceivably have a selective effect on the populations, eliminating

those animals in poor condition. Typically, this would include isolated seals that are diseased and heavily parasitized.

All animals in the 1974 and 1975 catches were in poor physical condition as reflected by their lower average weight and extended molting period. There was also a significantly lower number of pups, possibly indicating that the generally poor feeding conditions also affected the annual production of young. (Smith and Geraci, 1975) During years such as these, the effects of an environmental disturbance would presumably be more widespread, affecting entire age-classes and weakened segments within the population.

Other factors related to the life history of seals bear on the ultimate consequences of a spill. Ringed seals in the Beaufort Sea and Amundsen Gulf appear to depend on the vast offshore and inshore areas. The largest harvest is taken at Holman in the Amundsen Gulf. Preliminary calculations based on population estimates and reproductive rates,indicate that the Holman harvest depends on a larger area than the Amundsen Gulf for its sustenance. Direct tagging evidence shows movement of seals between the Beaufort Sea and Amundsen Gulf, to a point as far west as Point Barrow and the eastern Siberian coast. Thus, a spill in a given area such as from the proposed Canmar drill sites, could affect the harvest in areas as far east as Holman or as far west as the hunting villages along the Alaskan north slope.

In conclusion, it may be said that the direct effects of an oil blowout or spill, to which healthy seals are briefly exposed may cause transient though severe eye damage. Such brief exposure can likely occur only in open water, where seals are free to move in and out of a contaminated area. Such conditions exist in tropical and some temperature regions. However, seals inhabiting the polar regions are at most, only briefly exposed to open water. Most of their life is spent in and around fast ice. Should their restricted breathing space become fouled, permanent eye disorders will likely result. Irrespective of habitat, preweaned seals will be more susceptible to the thermal effects of oil than postweaned seals, which rely on blubber rather than hair for insulation. Consequently, oil fouling during the birthing and nursing months may have a more serious effect on that age class, especially if the spill occurs in a region of high seal production. Adverse environmental conditions which result in low food production may further complicate the effects of oil by selectively affecting the stressed seals in poor nutritional conditions. Certainly, any model attempting to predict the consequences of oil fouling must take into account the season of spill, the productivity of the area, and the variable health status of the population. In fragile environments like the Beaufort Sea, the mere presence of a large offshore oil field of the type already in existence in the North Sea, may well affect the seasonal pattern of movement of seals, and in turn reduce the ability of these mobile populations to survive adverse natural conditions.

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PETROLEUM HYDROCARBONS AND THEIR EFFECTS ON MARINE ORGANISMS, POPULATIONS, COMMUNITIES, AND ECOSYSTEMS

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ABSTRACT

Analysis of available data from bioassays conducted on adult stages of a wide variety of marine organisms reveals lethal effects from soluble fractions of petroleum and petroleum products in the 1 to 100 part per million range. However, for the more sensitive larval and juvenile life stages lethal effects from oil may occur at lower levels, 0.1 to 1 ppm. Sublethal responses, regardless of life stage, indicate that oil will adversely impact certain ecologically and commercially important species in the low part per billion range, i.e., 1 to 10 ppb.

Strict control is suggested for oil development and related activities in certain shallow, highly productive, continental shelf regions, and in relatively confined, ecologically important wetlands and estuaries. Soft-bottom, coastal benthic communities are vulnerable to impact, and recovery from spilled oil could be relatively slow where oil tends to persist or where community stability is extremely low. Bird populations are highly susceptible to lethal and adverse effects, if contact with oil occurs. Local breeding populations of many organisms (including ichthyoplankton and meroplankton) may become heavily impacted, especially in certain confined coastal areas. In general, populations and/or communities of pelagic fish, mammals, phytoplankton, and zooplankton are considered low risk in terms of susceptibility

to effects of petroleum. Little is known of the ecological effects of petroleum on neuston or in coral and polar ecosystems.

INTRODUCTION

In recent years marine scientists have shown a greater concern for the impact of petroleum and petroleum products on the health of the world's oceans and coastal areas. The words "Torrey Canyon" and "Santa Barbara," together with Thor Heyerdahl's observations of tar balls and slicks in the open Atlantic have heightened the awareness of the American public as well as its scientific community on the possible impact of petroleum products on marine life. This concern seems to be somewhat warranted in view of America's growing dependence on petroleum products--the average U. S. citizen utilizes some 7,800 gallons of petroleum per year--and the consequent demand for offshore oil development and related nearshore activities centered around transportation and processing.

Arriving from a host of sources, much of this petroleum ultimately finds its way to the marine environment (Table 1). Approximately 16% of the total amount spilled is introduced naturally or accidentally. However, by far, the largest percentage of petroleum is introduced as a result of intentional discharges which are capable of being regulated through government policy. With increasing supply and demand, an increase in the rate of petroleum pollution is inevitable, unless such regulations exist.

The determination of a permissible level of petroleum contaminants in the marine environment should be based foremost on careful consideration of their potential impact to vulnerable and sensitive marine life forms. It is the purpose of this paper to review and synthesize available data, and provide an up-to-date summary of the ecological effects of petroleum and petroleum products on marine life, from single organisms to intact ecosystems.

EFFECT OF PETROLEUM ON MARINE ORGANISMS

Marine toxicology is a science in its infancy. As a result, many of the earlier attempts to determine the toxic potential of petroleum and petroleum products to marine life have produced confusing and often erroneous conclusions. For example, lack of standardized techniques with respect to type of oil, test organism, or duration of exposure has introduced much confusion in the interpretation of reported effects. In addition, bioassays have been performed in the laboratory under static conditions, with little attempt made to simulate natural conditions which influence the behavior of the test organisms. Rarely were analytical techniques employed to measure the

exact amount of oil in solution. Instead, investigators reported the amount of oil initially added to aquaria on a volume to volume basis. Hence, concentrations causing biological effects were often over-estimated.

Death has often been used as a measure of biological response and has been reported in a form such as the LD-50, i.e., the concentration of oil responsible for the death of 50 percent of the test population over a given time period. The literature is replete with references to such values (see review in Moore et al.[1]). Too often these LD-50 values were calculated for resistant species that were easy to manage in the laboratory, while fragile species were often excluded from studies. Obviously, "safe" levels of oil contamination for all marine organisms lie well below the LD-50 values calculated for tolerant species.

The authors of recent reviews[1,2,3,4,5] on the subject of oil pollution have synthesized much of this fragmentary information, and as a result a great deal more has been learned concerning types of biological responses at the organism level and the fractions of oil responsible for such effects. Accordingly, Moore[2] has identified five major categories of effects from oil on individual organisms: 1) direct lethal toxicity; 2) sublethal disruption of physiological or behavioral activities; 3) the effects of direct coating by oil; 4) incorporation of hydrocarbons in organisms which may cause tainting of edible species and/or accumulation of potentially carcinogenic polycyclic aromatic hydrocarbons in food chains; and 5) changes in biological habitats.

Lethal toxicity refers to interference with subcellular, cellular, and physiological processes (e.g., disruption of membrane activities) leading directly to organism death.[2] As a result of the literature evaluation by Moore et al.[1] and their analysis of the type of compounds causing toxicity in variously reported bioassays, it was determined that soluble aromatic hydrocarbon derivatives (mono- and dicyclic aromatics, and naptheno-aromatics) are the primary cause of organism mortality. Low molecular weight paraffins can cause narcosis, while certain heterocyclic compounds are lethally toxic; but concentrations capable of causing such responses are extremely high and are unlikely to result from oil spills.[2,6]

Table 2, which was adopted with modifications from Moore et al.[1] and Moore,[2] summarizes the minimum concentrations of soluble aromatic hydrocarbon derivatives (S.A.D.) necessary to cause lethal toxicity for a wide variety of marine organisms. Minimum lethal concentrations for particular categories of organisms are based on Moore et al.[1] estimates of S.A.D. in solution from relevant bioassays that were reported in the literature. The factor of ten uncertainty observed for each category of organisms in Table 2 is a result of the confusion created when interpreting results from such a large number of bioassays performed prior to the development of standardized techniques. Table 2 also reveals estimated maximum percent S.A.D. for several types of petroleum products. Thus, refined products such as No. 2 Fuel oil,

which contain a high content of S.A.D., are considerably more toxic than equal amounts of crude oil or residual products.

Apparently, lethal effects of S.A.D. appear at the 1 to 100 part per million range for the adult forms of most marine organisms. Crustaceans and certain benthic organisms, especially burrowers, are the most sensitive (1 to 10 ppm), while fish and bivalves are moderately sensitive (5 to 50 ppm), and gastropods and marine flora the least sensitive (10 to 100 ppm). Lethal toxicity from S.A.D. may occur at lower concentrations, 0.1 to 1 ppm, for the more sensitive larval and possibly juvenile life stages.

Sublethal effects result from cellular and physiological interferences, usually leading to some form of abnormal behavior, particularly disruption of normal feeding and reproductive patterns.[2] Such behavior is often dependent upon sensitive levels of communication via chemical cues, which may be disrupted by very low levels of petroleum hydrocarbons in solution. Sublethal responses may indirectly result in lethal effects. Table 3, which was adopted with updated modifications from the National Academy of Sciences,[5] summarizes sublethal effects of petroleum on marine organisms. From this synthesis, it is apparent that many organisms respond to sublethal effects of S.A.D. in the part per billion range, particularly between 10 and 100 ppb. However, several citations from Table 3 reveal sublethal responses in the low part per billion range, i.e., 1-10 ppb. Included are a wide variety of effects across animal and plant kingdoms and components thereof. Such effects include: delaying cellular division in phytoplankton;[7] producing abnormal fish spawn;[8] reducing chemotactic feeding responses in snails[9] and in crabs;[10] inhibiting mating responses of male crabs to sex pheromones;[10] decreasing filter feeding activity of mussels;[11] and decreasing survival and fecundity in worms.[12] The ecological implications, as well as the exact mechanisms causing sublethal responses are still poorly understood.

The effects of direct coating of oil include disruption of physiological or behavioral processes, resulting from smothering, entanglement of appendages or filtering devices, and dislodgement of sessile organisms from their substrates.[1,2,3,4] Residual oil fractions are implicated as the primary cause of such effects. The effects of direct coating are most significant along exposed shorelines populated by attached or relatively immobile species (barnacles, mussels, limpets, snails, algae, etc.).

Incorporation of petroleum hydrocarbons in marine organisms may result in tainting of edible species and/or accumulation of potentially carcinogenic polycyclic aromatic fractions into marine food chains.[2] Exposure of some animals, particularly filter feeding bivalves and fish, to as little as 1 ppb dissolved petroleum hydrocarbons can result in tainting.[2] Humans can taste petroleum hydrocarbons in animal tissue at concentrations between 5 and 50 ppm.[2,13] The belief that oil can induce cancer in marine organisms is based on the following reasoning: 1) polycyclic aromatic hydrocarbons have been identified as

carcinogenic agents; 2) they are widely distributed over the ocean; 3) they are found in the residual fractions of crude oil; and 4) they have been found to concentrate in animal tissues.[2] Incidence of cancer has been evidenced among clams sampled from oil spill sites.[14,15] However, there has been no conclusive evidence to date positively implicating oil as the direct cause of the observed neoplasms.

Much of the oil spilled in the marine environment ultimately finds its way to bottom sediments. As a result, bottom habitats are potentially subject to alterations in their chemical and/or physical integrity. Benthic organisms are highly dependent on such integrity. To fully understand the effects of these alterations, one must search beyond the organism level of response for shifts in species composition and distribution, functional community changes, etc.

EFFECT OF PETROLEUM ON MARINE POPULATIONS, COMMUNITIES, AND ECOSYSTEMS

As demonstrated in Tables 2 and 3, individual organisms are killed by soluble oil fractions in the 0.1 to 100 part per million range, and may exhibit sublethal responses at very low concentrations (1 to 10 ppb). However, the ecological significance of these responses are manifested only by consideration at or above the population level of biological organization. As expressed by the U. S. Council on Environmental Quality,[16] an individual organism may be proven extremely sensitive to oil in the laboratory; but under natural conditions, due to effective reproductive and dispersal strategies, or high rates of birth, maturation and immigration, an entire population may recuperate rapidly from a catastrophe. Similarly, the expected rapid recovery of a community of organisms proven resistant in the laboratory may be seriously delayed under natural conditions as a result of restrainable interactions, i.e., competition for food and space, dependence on a specific food source, or the ability to compete with other recovering species.

To better visualize the impact of a stress on a system as a complex as a total aquatic ecosystem, one might imagine the latter as a giant, delicately balanced artist's mobile. The mobile, similar to an ecosystem, has both hidden, functional balance and visible structure (the weighted components). Small perturbations may momentarily set the weighted components into motion. However, if the system is well balanced, i.e., stable, fluctuations gradually lessen until the original state of equilibrium is reached. It is possible for several components, each serving as another's counterbalance, to be removed simultaneously with little or no change in the system's stable configuration. Yet, if just one strategically positioned component is removed, stability may be lost, and the original state of equilibrium may never be regained.

"Stability" seems to be the key word. It is the ability of a system to maintain or return to its initial state after an external

perturbation.[17,18] Determining the stability of a system then helps one to predict its susceptibility and long-term behavior in response to perturbations. Of course, the behavior of a natural system to a perturbation (such as oil) is dependent on factors other than the system's stability, for example the persistence of the perturbation, etc. Boesch[18] describes stability as being dependent upon "resistance," the ability to withstand stress without change, and "resiliency," the speed at which the system returns to a state of equilibrium following the perturbation.

Many aquatic ecologists believe that communities subjected historically to a constancy of abiotic factors (temperature, salinity, oxygen, etc.) have developed through time the most extreme, complex biological interactions.[19,20] Member species have become highly specialized, contributing to speciation. The resulting communities are "biologically accommodated" and are characterized by a high diversity of species. In contrast, communities subjected to rigorous and variable abiotic factors (fluctuating temperatures, salinities, or periods of light and darkness; atmospheric exposure; breaking surf; etc.) may be more controlled by their physical environment. They are composed of a relatively few eurytolerant species and are characterized by low diversity.

According to Copeland,[21] and supported by Boesch,[18] those systems already accustomed to "energy requiring stresses" (low diversity systems) are better able to resist additional human disturbances, e.g., petroleum, than those adapted to relatively constant environments (high diversity systems). This hypothesis contradicts the earlier belief[22,23] that the more complex a community (i.e., the more numerous its species and the more intricate their interrelationships), the more stable it is. The earlier hypothesis was based on the assumption that effects of stress in complex communities would be buffered, since many alternate sources of food and/or community strategies would be available. Which hypothesis is correct remains to be proven.

Other than hypotheses, we now have very little first-hand information of how various populations, communities, or ecosystems respond to oil contamination. What little is known has been learned from post-spill studies, surveys of chronically polluted areas, or from the few experimental field studies involving artificial oiling of natural or simulated "ecosystems." Interpretation of results from the former two methods has been gravely limited due to the lack of precise pre-spill control information to use for comparison. The experimental field approach is recommended since it corrects for the limitation memtioned above and still emphasizes the importance of natural environmental and biological variables in influencing the behavior of both the oil and the subjected communities. For a review of studies following major spills, see National Academy of Sciences.[5]

Damages from oil contamination result from either single spills or from low-level continuous discharges. In the spill situation, the exposed community will often experience an initial impact, the extent

of which depends largely on conditions of the exposure--amount of oil, type, degree of weathering prior to exposure, degree of spreading, cleanup techniques, and the resistance of the organisms. Less resistant individuals may die, usually as a result of chemical poisoning or complications from direct, physical coating--suffocation, entanglement or dislodgement.[4] Changes in population densities, age-distribution and species abundance and distribution may result from initial mortalities. Shifts in the relative sizes of interacting populations may also occur, favoring an increase in some as a result of relaxed pressures in the absence of others. The latter is exemplified by the increases in algal populations in some contaminated areas, resulting from the disappearance of grazers[24,25,26] and the invasion of oil-devastated areas by opportunistic polychaetes.[27,28] Oil may also alter the physical or chemical integrity of the habitat,[16] making it undesirable for recolonization and thus delaying recovery.

Results gathered thus far have revealed that marine communities subjected to oil spills are able to recover, although the time required has ranged from weeks for rocky shores at Santa Barbara[29] to several years for soft-bottom, offshore and marsh communities at West Falmouth.[30]

The rate of recovery depends on the extent of the initial impact; the persistence of the oil; and inherent factors of the community governing its resiliency (effectiveness of reproductive and dispersal strategies in replenishing numbers), and the extent of biological interactions such as predation, parasitism, competition and commensalism.[4,16] Recovery is usually thought to be complete if the internal dynamics of each population--density and stable age distribution (structure)--and the species interactions (function) have been restored to pre-spill levels.[16]

Continuous, low-level contamination results from frequent exposure to oily waste discharges from refineries, sewage treatment facilities, oil ports, petrochemical plants, etc.; or from exposure to persistent oil fractions left over from a spill. In such cases, initial impacts are not as significant as the gradual structural or functional changes which develop through time. This has been exemplified by the effects of persistent oil following the West Falmouth spill,[27,28,30,31] pollution from (oily) wastes in Los Angeles Harbor,[32,33] and pollution from refinery effluents in Milford Haven, England.[34]

For management purposes, it is extremely important for decision makers to understand how stable various types of marine systems are in response to oil. Such information can be used to help predict the ecological impact of an oil-related operation while it is still in the planning stages. Unfortunately, there is too little known presently to make definitive conclusions for every system type. Nonetheless, an attempt is made herein to review on a broad scale what little is known, or what can be theoretically expected in terms of responses to initial impact and rates of recovery. Estimates for recovery are often conjectural. Accordingly, Table 4 summarizes the responses of marine populations and communities to oil, while Table 5

summarizes the responses of some major ecosystems to the effects of oil.

Effect of Petroleum on Marine Populations and Communities

Plankton.--The "passively"drifting plankton appear to be threatened primarily by the chance event of contacting a floating slick. Individuals may die from chemical poisoning within the toxic plume, or as a result of complications from physical coating by the slick itself. The consequent loss of large populations could have a local effect on productivity.

Generally, however, dramatic changes in plankton as a result of oil contamination have not been observed. Smith[35] observed minor population changes in the ichthyoplankton and phytoplankton following the Torrey Canyon spill, but in general the plankton were apparently unaffected. Likewise, no apparent changes in phytoplankton or ichthyoplankton were reported following the Santa Barbara blowout.[29,36] Furthermore, zooplankton were found to be unaffected by brine effluents from oil fields in Texas estuaries.[37]

It must be pointed out that plankton populations are usually characterized by wide spatial and temporal variations. This has made it extremely difficult to discern effects from spills, where precise control or prespill information has not been available. However, using the experimental, field approach, an interesting effect was observed in an artificially oiled, natural marsh community.[38] Here, the application of crude oil caused an initial decrease in zooplankton, accompanied by massive phytoplankton blooms. As the oil began to disappear, zooplankton rapidly increased in response to the availability of food.

With exceptions, the recovery of phytoplankton and zooplankton communities, particularly in large bodies of water, is apparently rapid. These species are widely dispersed, reproduce rapidly and grow quickly to maturity, such that pre-spill population densities and/or stable age-distributions are soon restored.[16] However, in estuarine nursery grounds or other confined areas small localized breeding populations--particularly the larval forms of some fish (ichthyoplankton), or crustaceans and molluscs (meroplankton)--may become severely impacted, and complete recovery may take several years.[16]

Neuston.--Little is known of the ecological effects of oil on neuston, assemblages of organisms living at or near the surface of the sea. Yet, much concern has been voiced for these unique communities since they appear as being quite vulnerable to contact with either a floating slick or ubiquitous tar lumps. Burns and Teal[39] have reported the contamination of pelagic Sargassum communities with petroleum hydrocarbons in the central North Atlantic. However, study of the possible ecological effects of oil was not considered.

Since the ecology of neustonic communities is poorly understood and

case studies of oil impact are rare, it is difficult to predict their recovery rates.

Benthos.--In comparison to pelagic communities (plankton and most fish), benthic communities are particularly vulnerable to impact of spilled oil, since bottom substrates accumulate oil and since many of the species (represented largely by attached plants and small invertebrates living in or on the substrate) are sessile or relatively immobile and hence cannot escape the pollution. Initial damages from oil have been reported on rocky shores,[26,29,35,40,41] along soft-bottom intertidal shores,[42,43,44,45] and in subtidal substrates.[26,27,35,44]

As Boesch et al.[4] have pointed out, mortalities have resulted from chemical poisoning (e.g., Tampico Maru and West Falmouth spills) or from the effects of physical coating, such as loss of purchase on substrates or suffocation (e.g., Santa Barbara and San Francisco Bay spills). The loss of individuals may result in decreases in densities or changes in age distributions within populations, or changes in the abundance and distribution of species.

On several occasions oil was reported to have affected the functional relationships between different, interacting species. For example, after the Tampico Maru spill, extensive kelp beds developed in the absence of grazing abalones and sea urchins, which normally kept the sporelings in check.[26] Following the Torrey Canyon spill, once grazing limpets and periwinkles were killed by a combination of the oil and toxic emulsifiers, dense growths of green algae developed, followed by brown rockweeds.[24,25] Similarly, under temporary noncompetitive conditions, the opportunistic polychaete, Capitella capitata, invaded oil-devastated areas following the West Falmouth spill.[27,28] Additionally, Clark et al.[46] reasoned that normally occurring anemones were few in number along a rocky shore exposed to an oil spill in Wreck Cove, Washington, since algae, as evidenced by their abundance, had outcompeted the former for space.

Rates of recovery for oil-impacted benthic communities have ranged from weeks on rocky shores at Santa Barbara[29] to half a decade in soft-bottom, offshore and marsh stations at West Falmouth.[30] With exception (Tampico Maru spill), intertidal communities appear to have been affected the least and to have recovered the fastest--usually within two years.[4] As Boesch et al. point out, rapid recovery occurs since: 1) all but the most resistant fractions of oil are quickly removed by waves, reducing chronic stress, 2) the species reproduce rapidly to replenish what is lost, and 3) the species generally have acquired mechanisms for protection which increases resistance and minimizes the initial impact.[4]

The recovery rates for soft-bottom, intertidal communities may be slower than for their rocky counterparts, since in the case of the former, the oil may persist for longer periods of time in the unconsolidated substrate. Energy from waves breaking along a beach will

aid in removing oil-laden sediments from a sandy or muddy beach; however, in such an event the pollution is only relocated to an offshore area.

In comparison to intertidal communities, full recovery has been extremely slow for some subtidal communities (e.g., West Falmouth). This is largely due to the oil's persistence once incorporated into the subtidal sediments. Here, the oil may remain for long periods of time relatively free from bacterial attack or mechanical dispersion. In addition, it is possible that many subtidal communities are not as stable--resistant or resilient--as communities which have adapted to the extremely variable and rigorous conditions found in intertidal areas. Of course, it is recognized that one may find stable as well as unstable subtidal communities, and rates of recovery will be faster or slower depending on their degree of stability. Yet, for now, it is convenient to conjecture that in comparison to some other broad categories reviewed herein, subtidal communities require relatively longer recovery periods.

Benthic communities have also exhibited changes in response to chronic low-level inputs of oil. For example, Crapp[34] noted that in areas around refinery outfalls in Milford Haven, fucoid algae were more abundant than molluscs, which normally graze upon the algae, while in unpolluted areas the molluscs were more abundant. Similarly, Reish[32,33] surveyed sections of Los Angeles Harbor which were apparently receiving refinery wastes and found an absence of species, except for the very resistant polychaete, Capitella capitata. When Los Angeles refineries ceased discharging wastes, the diversity of species increased in previously uninhabited areas.

Fish.--In many cases, fish have the ability to avoid an oil-impacted area,[4,26] particularly if the area is not restricted in size (e.g., in offshore waters). Some fish kills have been reported following major spills--Argea Prima[47] spill and the West Falmouth spill[44]--or in artificially oiled ponds.[38,48] However, generally the oil has been confined to relatively small and shallow bodies of water, so that exposures were assured. Boesch et al.[4] pointed out that fishes may be more resistant than other organisms to the toxic effects of oil, since their surfaces are coated with oil repellent mucous.

In general, populations of pelagic fishes, that are by chance affected by a spill, will probably recover quickly since larval and adult immigrants will rapidly replace individuals that were eliminated. Benthic fishes may be threatened if oil should accumulate in bottom sediments at levels capable of inducing sublethal or lethal responses. Apparently the greatest threat is to local breeding populations which congregate in small spawning and nursery grounds. In such a case, there is a greater chance for exposure since the grounds are often represented by relatively narrow waterways. Furthermore, the larval stages are particularly sensitive to chemical poisoning. If a local population is reduced it may take years to recover.

Birds.--Some populations of sea birds, including the auks, penguins and diving ducks, are particularly threatened by oil spills.[5] For example, since many are flightless (or weak fliers) and dive to collect their food or to avoid a disturbance, their chances of contacting a slick are increased. They are also highly gregarious--which means that there is potential for a small local population to be lost all at once. Some species are extremely rare or near extinction and local losses may have an impact on the entire species.

Bird kills were reported following the Torrey Canyon spill, Southwest England;[35] the Esso Essen spill, South Africa;[49] the Santa Barbara spill, California;[29] the San Francisco Bay spill, California;[41] and on several other occasions.[5,50] Causes of death include: 1) disruption of feather surfaces leading to either drowning as a result of the loss of buoyancy, or pneumonia as a result of the loss of thermal insulation; 2) ingestion of toxic oil droplets from excessive preening; and 3) "accelerated starvation" as a result of increased metabolic activity (to compensate for loss of body heat) coupled with a decrease in feeding.[4]

Since birds are relatively long-lived and are not very prolific, replenishment of losses is often extremely difficult.[16] Recovery could be a very slow process.

Marine Mammals.--Despite much publicity and over-reactions from humans, it appears that oil has not caused serious effects on entire populations of marine mammals. In comparison to other animals, marine mammals are not particularly abundant. Hence, contact with oil occurs rather infrequently. Also, throughout most of the year many mammals are quite mobile and thus are apparently capable of avoiding heavily polluted areas. A breeding population of seals on an oiled beach represents an obvious exception, as well as small fur bearers living in coastal wetlands chronically polluted with oil.

Although incidents of oiling have been reported,[3] conclusive evidence of individual mortalities due to oil pollution is rare, if not lacking entirely. Possible harmful effects, however, include ingestion of toxic oil droplets during grooming; loss of thermal insulation and/or waterproofing as a result of coating; and irritation of eyes and exposed mucous membranes.[3,4] Eye irritations were reported after the Arrow spill[51] and after a spill of diesel oil off Alaska.[52]

If an entire population (conceivably a small breeding population on or near an oiled beach) were lost, then recovery could be a very slow process. Individuals are long-lived and are not extremely prolific. Also, the number of reproductive events per given time period can be small since breeding occurs rather infrequently and is often dependent upon complicated behavior. However, there is no supportive evidence for population losses as a result of oil pollution. On the contrary, oiled individuals among a population of elephant-seal pups on San Miguel Island survived and later dispersed in a normal manner.[53]

Effect of Petroleum on Marine Ecosystems

Open Oceans.--Researchers have in the past concentrated on studying effects of oil in coastal or inland regions, mainly as a result of their vulnerability to spilled oil and their biological or economic significances. Consequently, the ecological effects of oil in the open ocean is poorly understood. Yet, based on what little is known, it is felt that some general conclusions can be drawn.

For the most part, catastrophic effects are not expected in the open ocean environment, primarily as a result of the rapid dispersion and degradation of the oil, and the general low vulnerability of open ocean organisms to contact with oil. For example, damages to small populations of phyto- and zooplankton depend mostly on the chance event of encountering a floating slick. Individuals that do encounter the slick may be eliminated; however, once contact occurs and organisms are killed, numbers are generally quickly restored as a result of fast rates of reproduction and immigration. Adult pelagic fish are generally capable of avoiding a spill although they may ingest toxic oil droplets. It is not likely that dispersed oil would accumulate in open ocean sediments at levels toxic to benthic organisms, since oil would lose much of its toxicity before reaching the bottom.[16]

On the other hand, some communities, particularly the surface-dwelling neuston, are obviously threatened by contamination from floating oil. It is significant that floating tar lumps have been found along vast stretches of the ocean's surface.[54]

Outer Continental Shelf (OCS).--Concern for potential environmental impact has been expressed for certain OCS areas in the Atlantic and Gulf of Alaska, which are now being considered for the development of oil and gas resources. For example, the U. S. Council on Environmental Quality (CEQ)[16] has reported that high environmental risk is involved in the development of Northern Baltimore Canyon, Southwest Georgia Embayment, and the Gulf of Alaska; although less risk is involved in the development of Central and Southern Baltimore Canyon and George's Bank. It must be noted that CEQ's ranking of OCS environmental risks is based primarily on the probability of oil spills reaching the shore and impacting biologically productive coastal wetlands and estuaries or intensively used recreational beaches, rather than on potentially damaging effects that the oil may have on offshore communities. This illustrates the fact that very little is known of the ecological effects of disturbances in offshore, OCS areas.

Based on what little is known, some generalities have been drawn.[16] First, the impact of oil on entire planktonic populations should be slight, although fractions thereof might be eliminated as a result of coating or exposure to the toxic wake of a slick. Rapid recovery--within the life spans of the individual organisms--occurs since once the oil has dispersed, reinvading individuals from unaffected

neighboring areas are generally sufficiently numerous to restore population densities to prespill levels, and since individuals reproduce and grow to maturity rather rapidly. However, extreme caution must be exercised in developing certain OCS areas (e.g., Georges Bank) that are frequented by breeding and migratory populations of commercially or ecologically important species. Whole year classes of organisms may be affected by oil spills or by chronic lethal exposures, which may jeopardize the strength of the overall stocks.

Benthic communities in OCS areas may be moderately affected if oil reaches the bottom. It is conceivable that in most offshore areas oil would not accumulate in bottom sediments at levels high enough to cause dramatic alterations in the structure or functioning of the communities existing there. Much of the oil from an offshore spill is widely dispersed and somewhat detoxicated before reaching the bottom, such that it is not likely that any given area would become heavily coated with extremely toxic oil fractions. However, again, in shallow OCS areas (such as Georges Bank) low levels of soluble oil fractions could cause tainting of commercial shellfish or affect the behavior of certain commercially or ecologically important species.[11,56,57]

Moderate rates of recovery are expected for benthic communities in the OCS areas, and is dependent largely on the persistence of the oil once incorporated in the soft substrates. CEQ predicts a two to three year recovery period for sandy offshore substrates, and longer periods for substrates containing larger proportions of silt or mud.[16]

The CEQ report concludes that many populations of fish living in OCS areas are generally not threatened from spills, since they are free to move about and thus can usually avoid the oil. However, certain OCS fishes that migrate inland to breed and spawn--alewife, striped bass, salmon--may be threatened by nearshore spills, which could interrupt spawning migrations, disrupt complex breeding behavior, or affect sensitive larval forms aggregated in their nursery grounds. According to CEQ, some short-lived species such as pink salmon, which have a two-year life span, if subjected to severe losses in a given larval class, may in turn suffer significant losses in terms of population densities for the overall stock, and require a few years to recover. Longer-lived species can apparently sustain the loss of an entire year class without serious stock reduction. Studies to delineate temporal and spatial patterns for breeding and larval populations of OCS fishes are urgently needed to assess the potential impact of petroleum development in these areas.

According to CEQ, those species of fish considered potentially most vulnerable to OCS-related oil spills include: 1) winter flounder, sand lance, mummichog, alewife, and salmon for the Northern New England area (Maine to Cape Cod); 2) summer and winter flounder, tautog, sand lance, anadromous smelt, alewife, and striped bass for the Southern New England area (Cape Cod to Sandy Hook); 3) hogchoker and summer flounder for the Middle and South Atlantic area (Sandy Hook to Cape Canaveral); and 4) breeding populations of several salmon and

groundfish species in the Gulf of Alaska.

Open Estuarine Areas, Bays, Channels, Harbors.--The effect of petroleum and petroleum byproducts on open estuarine areas, bays, channels, and harbors deserve considerable attention, since these areas continue to serve as depositories for oily substances from a host of sources--boat traffic, tanker on- and off-loading practices, refineries, sewage treatment outfalls, accidental spillage due to collision or grounding, etc. Farrington and Quinn[58] reported that bottom sediments in the upper part of Narragansett Bay, Rhode Island, which receive large inputs from sewage treatment plants, tanker off-loading practices near oil storage points, and general boat traffic, are polluted with oil at concentrations ranging from 800 to 3,560 ppm (dry weight).

In such chronically polluted areas oil may gradually reduce the size of some populations of fish and benthic species, and induce changes in species abundance and distribution. This is exemplified by the observations of Reish[32,33] where he noted an absence of normally occurring benthic species near refinery outfalls and an overabundance of the very resistant, opportunistic polychaete, Capitella capitata. Single spills may cause similar effects, especially where the oil has a tendency to persist in subtidal sediments, prolonging the toxic effects (e.g., West Falmouth spill). The oil spilled at West Falmouth, as well as in Chedabucto Bay, Canada, persisted for several years. Oil spilled in Casco Bay and Muscongus Bay, Maine, has persisted for 11 years.[59]

Spilled oil may have a particularly significant impact on small, local breeding populations, especially fish and shellfish, which characteristically migrate in and out of these rather confined coastal areas. As noted earlier, larval and possibly juvenile forms are quite vulnerable to the toxic effects of oil. In this respect, the immediate impact of a major oil spill will depend largely on the time of year, i.e., whether animals have migrated to these areas and are breeding or spawning.

Recovery from spills in these areas is dependent on flushing characteristics of the body of water; shoreline characteristics, i.e., how consolidated the sediments are, wave exposure, or how confined the areas are as a result of the shoreline contour; routes of oil to benthos; and how stable the communities are against disturbances. In regard to the latter, Boesch[18] has found that, depending on the constancy and level of salinity, bottom communities from some portions of estuaries are more stable in response to disturbances than from other areas. For example, he characterized subtidal benthic communities appearing in polyhaline portions of a temperate estuary as being more diverse and less stable in response to disturbances than those communities found in mesohaline and oligohaline portions of the estuary. The latter are usually composed of relatively few species with wide physiological tolerances. Similarly, with regard to constancy in temperature, some tropical estuarine communities (which experience

little variation in other physical parameters as well) may be less stable in response to disturbances than temperate estuarine communities composed of species which have developed defensive mechanisms to cope with natural seasonal variations.[21]

Recovery may be a slow process for a local breeding population of fish or shellfish impacted by oil. Where individual year classes are heavily impacted, several years may be required to numerically restore the overall stock to prespill levels.

Wetlands.--An oil spill may have a significant impact on a wetland, e.g., salt marshes in temperate regions or mangrove swamps in the tropics. In the first place, these areas are extremely important biological resources. They are highly productive; form the basis of detrital food chains; provide habitat, breeding, and nursery grounds for fish and wildlife; act as sediment and nutrient traps; and serve as the important transition between terrestrial and marine systems.

Secondly, these shallow estuarine environments are particularly vulnerable to spills, as they are unquestionably subjected to a large amount of oil-related activities. Also, once spilled the oil has a good chance of reaching either the shore or bottom, since wetlands are typically shallow and confined by their shape and size. Oil which has not washed ashore, may be deposited in subtidal sediments via particulate matter, and may remain there, under typical anaerobic conditions, for long periods of time. Also, oil has a tendency to coat oleophilic detritus which may then be ingested by detritivores.[4]

Several post-spill reports illustrate detrimental effects of oil spilled in wetland environments. For example, damages to mangrove communities were reported following the Argea Prima spill in Puerto Rico[47] and the Witwater spill in the Canal Zone.[60] Damages to salt marsh communities have occurred as a result of the Chryssi P. Goulandris spill in Milford Haven,[24] the Arrow spill in Chedabucto Bay,[43] and the West Falmouth spill in Massachusetts.[30]

Experimental attempts are now being made in the field to evaluate the effects of oil, mostly on salt marsh communities. After artificially oiling a natural estuarine tidal pond, Lytle[38] reported reduction in productivity of marsh plants, drastic changes in diversity and density of fish populations, and imbalances between numbers of zooplankton and phytoplankton as a result of the initial impact of the oil. After surveying marsh grass communities around refineries, and areas that were artificially oiled, Baker[61] noted that marsh grasses recovered well from a single spill, but that continuous or repeated exposure could be harmful. Particularly sensitive were shallow-rooted plants with little or no food reserves, in comparison to perennials with large food reserves and resistant characteristics at the cellular level.

Oil has a tendency to persist in shallow estuarine sediments,

e.g., after the West Falmouth spill, which may prolong toxic effects and delay recovery. However, in many cases once the oil has been removed recovery may occur at a moderate rate--few to several years. This is because estuarine organisms typically are "r-strategists," i.e., they reproduce and grow to maturity rapidly;[18] and secondly, similar to many intertidal species, these shallow water inhabitants have to some extent developed resistant adaptations to cope with inconsistencies in the physical environment. On the other hand, if Copeland's[21] "response to disturbance" model is true then some of the more diverse and complex communities, particularly those in tropical mangrove swamps, may reveal slower rates of recovery. Mangroves are particularly vulnerable to clogging or interference with the holes in their aerial root system.[62] Odum and Johannes[62] point out that mangrove trees generally require a minimum of twenty years to recover.

Coral Reefs.--The threat of contaminating coral reefs with oil is increasing. While a complex of refineries continues to discharge effluents over reefs along the South coast of Puerto Rico,[63] offshore drilling has begun in the vicinity of Indonesia, and exploration for oil has commenced on the Great Barrier Reef of Australia.[4] Yet, little is known of the ecological effects of oil on coral reef communities.[4,64]

Several examples summarize much of what is known. Following the Witwater spill in the Canal Zone in 1968, a qualitative survey was conducted to assess the impact on the coral community.[60] However, no evidence was found suggesting that severe damages had occurred. On the other hand, Cerame-Vivas et al.[65] noted that the discharge of refinery wastes along the South coast of Puerto Rico caused structural changes in a nearby coral community. Likewise, during field experiments, Birkeland et al.[66] found that hermatypic corals exposed to Bunker C showed reduced and varied growth between heads. The latter finding was significant in the sense that a coral's success in the community--ability to tolerate grazing and predation, and to compete for space--is dependent largely upon its growth.[67] Johannes et al.[68] have found that oil damages corals while they are exposed to air.

Coral reef communities are extremely complex and are characterized by high diversity.[19,20,21] If Copeland's[21] "response to disturbance" model is true, then coral reef communities would be relatively unstable in response to oil. Initial impacts from oil may be great and recovery may be extremely slow. Without conclusive data, such a statement is strictly conjectural at this time. However, Copeland[21] reviewed several incidents, revealing that coral communities respond drastically to either dredge spoils, turbidity, or organic loading.

Polar Ecosystems.--There is a paucity of information concerning effects of oil in polar regions.[69,70] This is a serious inadequacy since it is generally conceded that oil spilled in polar waters will have severe and long-lasting ecological effects.[69,71] Reasons include: 1) polar organisms have slow growth rates, extended life cycles,

longer reproductive periodicity, and narrow ranging dispersal stages;[72,73] and 2) at low temperatures, one might expect a slow rate of dispersion and biological degradation of the oil.[74]

Such a conjecture may apply well to the more environmentally constant polar areas--self contained arctic lakes[75] or the bottoms of deep bodies of water. Yet, application to some low-diversity, coastal polar communities, existing under extreme conditions (variable temperatures, exposure to ice, shifts in periods of light and darkness), is questionable since theoretically[21] these communities may already have adapted to harsh, natural conditions in such a way that the effects from additional disturbances may be minimized.

CONCLUSIONS

Analysis of available data from bioassays conducted on the adult stages of a wide variety of marine organisms reveals lethal effects of soluble aromatic hydrocarbon fractions in the 1 to 100 part per million range. For the more sensitive larval and juvenile forms, lethal effects may occur at lower levels, typically between 0.1 and 1 ppm. Major sublethal responses of commercially or ecologically important species (e.g., certain shellfish) are noted in the low part per billion range, i.e., between 1 and 10 ppb.

We have learned that basic biological differences exist between various populations and communities of organisms or between major ecosystem types. We also suspect that due to such differences, various populations, communities or ecosystems are not the same in their susceptibility to or ability to recover from effects of oil. However, it must be stressed that data are not available at this time to provide a sufficient basis for accurately distinguishing varying degrees of response to oil. One must recognize that predicted responses according to population, community or ecosystem type, that are summarized herein, are subject to uncertainty.

However, based on data collected thus far, strict control is suggested for oil development and related activities in certain highly productive, continental shelf regions, and in wetlands and estuaries, where ecologically and commercially important species seek shelter, food and breeding grounds. Soft-bottom, coastal benthic communities are vulnerable to impact, and recovery from spilled oil could be relatively slow where oil tends to persist or where community stability is extremely low. Bird populations are highly susceptible to lethal and adverse effects, if contact with oil occurs. Local breeding populations of many organisms (including ichthyoplankton and meroplankton) may become heavily impacted, especially in certain confined coastal areas. In many cases, populations and/or communities of pelagic fish, mammals, phytoplankton, and zooplankton are considered low risk in terms of susceptibility to effects of petroleum. Fish and mammals are highly mobile and can thus usually avoid spills. If populations of plankton

happen to encounter a floating slick in open waters, and individuals are eliminated, densities and age-distributions are generally quickly restored through effective reproductive and dispersal mechanisms. Little is known of the ecological effects of petroleum on neuston or in coral and polar ecosystems.

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DISCUSSION

SIVA: I think we all agree that we have come a long way from the LD_{50} static bioassay but I think, also, that you must agree that that was definitely the place we had to start. We all definitely need information on effects of oil on total ecosystems. To me, the only way to get this information, since we have our spills in areas where we haven't had very good baseline data previously, is to study a system and then experimentally spill oil in it.

I would like your opinion of that approach. What would be your approach to the problem and what is EPA doing along this line to get a handle on total ecosystem effects?

SCHNEIDER: As far as the types of studies to be done presently, as I mentioned, the large marine ecosystem work that we are doing is now aimed at chronic effects instead of individual spills.

That is the work being done in our large tanks at the University of Rhode Island, and it really isn't URI that is doing it. A large number of workers everyplace from Woods Holes to Skidaway Institute is included. We are pushing microcosm experiments done on a fairly large and grand scale. The tanks are 25 or 30 feet high, 8 feet in diameter, complete with a benthic community and running a semi-flow-through arrangement.

We are using as our baseline, in this case, Narragansett Bay as a whole. The first thing we are doing is tuning our microcosms, as we have done on the smaller ones already, to existent systems in the Bay; then when we understand and recognize that our microcosms are mimicking natural systems, so we feel free to dose them.

Initially, we are going to be dosing at the 100-part-per-million range. As far as spilled oils are concerned, we have done, and we have funded, and we are still carrying out spilled oil experiments in controlled systems, and I think that they are very important. The main things to look for here are the recovery of those systems. How rapidly do they get back to their individual structural and functional relationships that have existed?

I think terrestrial ecologists and aquatic ecologists have expended an immense amount of money in individual experiments trying to look at complex interactions. Ecosystems study should be done in a holistic manner -- looking at total systems parameters, rather than individual species parameters within that.

TABLE 1

SOURCES OF PETROLEUM IN THE MARINE ENVIRONMENT[a]

	Natural	Intentional	Accidental
Natural Seeps	10%		
Offshore Production			1%
Transportation			
LOT tankers		5%	
Non-LOT tankers		13%	
Dry docking		4%	
Terminal operations			
Bilges bunkering		8%	
Tanker accidents			3%
Non-tanker accidents			2%
Coastal Refineries		3%	
Atmosphere		10%	
Coastal Municipal Wastes		5%	
Coastal (nonrefining industrial wastes)		5%	
Urban Runoff		5%	
River Runoff		26%	
Total	10%	84%	6%

[a]Recalculated from National Academy of Sciences.[5]

TABLE 2

SUMMARY OF LETHAL TOXICITY[a]

Class of Organisms	Estimated Conc. (ppm)[b] of S.A.D.[c] Causing Toxicity	Estimated Amount (ppm) of Various Petroleum Substances Containing Equivalent Amounts of S.A.D.				
		#2 Fuel Oil (Est. Max. % S.A.D. = 1-30)	Crude Oil (Est. Max. % S.A.D. = 0.1-10)	Kerosene (Est. Max. % S.A.D. = 1-20)	Dispersant (BP 1002) (Est. Max. % S.A.D. = 1-20)	Residual (Est. Max. % S.A.D. = 0-1)
Flora	10-100	50-500	10^4-10^5	10^2-10^3	10^2-10^3	10^3-∞ (no effect)
Finfish	5- 50	25-250	10^4-10^5	50-500	50-500	500-∞
Larvae (all species)	0.1-1.0	0.5- 5	10^2-10^3	1- 10	1- 10	10-∞
Pelagic Crustaceans	1- 10	5- 50	10^3-10^4	10-100	10-100	10^2-∞
Gastropods	10-100	50-500	10^4-10^5	10^2-10^3	10^2-10^3	10^3-∞
Bivalves	5- 50	25-250	10^4-10^5	50-500	50-500	500-∞
Benthic Crustaceans	1- 10	5- 50	10^3-10^4	10-100	10-100	10^2-∞
Other Benthic Organisms (Polychaetes, etc.)	1- 10	5- 50	10^3-10^4	10-100	10-100	10^2-∞
Birds			← Coating →			

[a] Adopted with modifications from Moore.[2]

[b] Based on a review of the literature by Moore *et al.*[1] and their estimates of S.A.D. in the bioassay solutions.

[c] Soluble aromatic hydrocarbon derivatives (mono- and dicyclic aromatics, naptheno-aromatics).

TABLE 3

SUMMARY OF SOME SUBLETHAL EFFECTS OF PETROLEUM PRODUCTS ON MARINE ORGANISMS[a]

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Marine Flora	Marsh Plants (Festuca rubra, Distichlis maritima)	Baker, in Cowell, 1971[76]	Crudes and refinery effluents.	Single or successive coatings with crude.	Inhibition of germination and growth. Repeated coatings cause disappearance of some plants (increasing order of tolerance: shallow rooted plants, shrubby perennials, filamentous green algae, perennials, perennials with large food reserves).
	phytoplankton (Chlorella vulgaris; Chlamydomonas angulosa)	Kauss et al., 1973[77]	Crude Naphthalene	1 ppm 3 ppm	Suppress growth. Reduction of bicarbonate uptake (i.e., photosynthesis).
	phytoplankton (diatoms and dinoflagellates)	Mironov, 1970[7]	"Oil"	10^{-1} 10^{-4} ppm	Inhibition or delay in cellular division.
	phytoplankton (Asterionella japonica)	Aubert et al., 1969[78]	Kerosene	3 ppm; 38 ppm	Depression of growth rate.
	phytoplankton (Phaeodactylum tricornutum)	Lacaze, 1967[79]	Kuwait crude	"1 ppm"	Depression of growth rate

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Marine Flora Continued	phytoplankton (Monochrysis lutheri)	Strand et al., 1971[80]	Kuwait crude; dispersant emulsions	20-100 ppm	Inhibition of growth; reduction of bicarbonate uptake at 50 ppm.
	phytoplankton (Phaeodactylum tricornutum, Skeletonema costatum, Chlorella sp., Chlamydomonas sp.	Nuzzi, 1973[81]	Extracts of outboard motor oils, No. 6 Fuel Oil, No. 2 Fuel Oil.	1 ppm	Inhibition of growth with No. 2-stimulation with No. 6 and outboard motor oil.
	phytoplankton (mixed natural samples)	Gordon and Prouse, 1973[82]	Venezuelan crude, No. 2 and 6 Fuel oils.	10-200 mg/ℓ (ppm)	Stimulation of photosynthesis at 10-30 µg/ℓ, decrease in photosynthesis at 100-200 µg/ℓ No. 2 fuel oil.
	kelp (Macrocystis angustifolia)	Wilber, 1969[83]	Toluene	10 ppm	75% reduction in photosynthesis within 96 hr.
	lichen (Lichen pygmaea)	Brown, 1972[84]	Kuwait crude, BP 1002	0.1-100 ppm	1 ppm emulsifier decrease total C^{14} fixation.
	Spartina marsh grass	Lytle, 1975[38]	Crude	Poured into pond.	Decrease in productivity.
	Algae (Ulva lactuca, Grateloupia dichotoma, Polysiphonia opaca)	Davavin et al., 1975[85]	Crude	.1-10 mℓ/ℓ (100-10,000 ppm)	Complete inhibition of biosynthesis of DNA and RNA at higher conc. for Ulva.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Larvae and eggs	Pink Salmon fry (Onchorhynchus gorbuscha)	Rice, 1973[86]	Prudhoe Bay crude	1.6 ppm	Avoidance effects; could have effect on migration and behavior.
		1975[87]	Prudhoe Bay crude	0.73 ppm	Decrease in growth
	Black sea turbot (Rhombus maeoticus)	Mironov, 1967[8]	"Oil"	0.01 ppm	Irregularity and delay in hatching--resulting larvae deformed and inactive.
	Plaice larvae (Pleuronectes platessa)	Wilson, 1970[88]	BP 1002	0-10 ppm	Disruption of phototactic and feeding behavior.
	Cod fish larvae (Gadus morhua)	Kühnhold, 1970[89]	Iranian crude	Aqueous extracts from 10^3 ppm, 10^4 ppm	Adverse effect on behavior leading to death.
	Lobster larvae (Homarus americanus)	Wells, 1972[90]	Venezuelan crude	6 ppm	Delay molt to 4th stage.
	Sea Urchin larvae (Strongylocentrotus purpuratus)	Allen, 1971[91]	Extracts of Bunker C	0.1-1 ppm	Interference with fertilized egg development.
	Barnacle larvae (Balanus)	Mironov, 1970[7]	"Oil"	10-100 µℓ/ℓ (ppm)	Abnormal development.
	Crab larvae (Pachygrapsus marmoratus)	Mironov, 1970[7]	"Oil"	10-100 µℓ/ℓ (ppm)	Initial increase in respiration.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Larvae and eggs (Continued)	Polychaete larvae (Sabellaria spinulosa)	Smith, 1968[35]	BP 1002	0.5-1 ppm	Abnormal irritability in larval revealed by stiffening out of median setae.
	Oyster larvae (Ostrea edulis)	Simpson, 1968[92]	BP 1002	1 ppm	Inhibition of growth
	Herring larvae (Clupea harengus)	Kühnhold, 1969;[93] 1971[94]	Crude	5 ppm	High % deformed larvae (stunted tail and constricted yolk mass) among hatching eggs; normal hatched larvae became narcotized within 2 days after hatching and died by 3rd.
	Herring and anchovy larvae (Clupea pallasi; Engraulis mordax)	Struhsaker, et al., 1974[95]	Benzene	15-45 ppm	35-45 ppm causes delay in development of eggs and produces abnormal larvae; 10-35 ppm causes delay in development of larvae; decrease in feeding and growth, and increase in respiration.
Fish	Chinook salmon (Onchorhynchus tshawytscha, striped bass (Morone saxatilis)	Bracksen and Bailey, 1973[96]	Benzene	5, 10 ppm	Initial increase in respiration.
	Menidia menidia	Gardner, 1975[97]	Crude (whole fractions) (water-soluble) (water-insoluble)	140 ppm (v/v) 12 ppm estimated 588 ppm (v/v)	Histological damage to chemoreceptors.
	Cyprinodon variegatus, Lagodon themboides, Micropogon undulatus	Steel and Copeland, 1967[98]	Petrochemical wastes	0.2-2.0 ppm in addition to 0.4-4.0 phenol	Respiratory inhibition.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Fish (Continued)	Lagodon rhomboides	Wohlschlag and Cameron, 1967[99]	Petrochemical wastes	50% of wastewater	Respiratory inhibition.
	Ictalurus natalis	Todd, 1972[100]			Alteration of social behavior.
	Menidia menidia	Gardner et al., 1975[101]	Waste motor oil	>20 ppm	Incidence of lesions in vascular systems (pseudobranch, heart, arterial system).
	Siganus rivulatus	Eisler, 1975[102]	Dispersant Crude	.010 mℓ/ℓ (10 ppm) .3-10 mℓ/ℓ (300-10,000 ppm)	Reduction in blood hematocrit. Increase in somatoliver index.
Crustaceans	Lobster, (Homarus americanus)	Blumer et al., 1973[103]	Crude, Kerosene	10 ppm	Effects on chemoreception, feeding times, stress behavior, aggression, grooming.
	Barnacle, (Pollicipes polymerus)	Straughan, 1971[29]	Crude--Santa Barbara	Field study after blowout	Apparent decrease in adult brooding; no recruitment in oiled areas.
	Lobster (H. americanus)	Atema and Stein, 1972,[56] 1974[57]	LaRosa Crude	Extracts; whole oil at 1:100,000 (10 ppm)	Delay in feeding with whole crude fractions.
	Crab, (Pachygrapsus crassipes)	Kittredge, 1971[104]	Crude	Dilutions of diethyl ether extracts (1:100)	Inhibition of feeding.
	Crab, (Uca pugnax)	Krebs, 1973[105]	No. 2 Fuel oil	Field observations after W. Falmouth spill	Adverse effects on sexual behavior. Mortalities in heavily-oiled areas.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Crustaceans (Continued)	Copepod (Calanus helgolandicus)	Spooner and Corkett, 1974[106]	Suspended oil droplets in lab vessels	10 ppm	Decrease in feeding and metabolic activity among survivors, based on amount fecal pellets deposited by controls vs. experimentals.
	Benthic amphipods (Gammarus oceanicus, Onisimus affinis) isopod (Mesidotea entomon)	Percy, 1976[107]	3 Crudes	Oil-soaked object; oil tainted food	Avoidance of oil masses and oil-tainted food for amphipods; neutral response for isopod.
	Crab, (Pachygrapsus crassipes)	Kittredge et al., 1975[10]	Naphthalene	1 ppb	Inhibition of feeding (reduction in intensity of response)
			Crude	(extracts)	Inhibition of feeding and response to sex pheromone (male mating stance).
Molluscs	Mussel (Mytilus edulis) Modiolus demissus	Gilfillan, 1973,[108] 1975[109]	Crude	1 ppm	Reduction in carbon budget (increase in respiration; decrease in feeding and assimilation).
	Snail (Nassarius obsoletus)	Blumer et al., 1973[103]	Kerosene	Saturated extract diluted 10^{10}	40% reduction in chemotactic perception of food.
	Snail (Nassarius obsoletus)	Jacobson and Boylan, 1973[9]	Kerosene	0.001-0.004 ppm	Reduction in chemotactic perception of food.
	Clam (Mya arenaria)	Barry and Yevich, 1975[14]	No. 2 Fuel oil	Collected from field	Gonadal tumors.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Molluscs (Continued)	Oyster (Crassostrea virginica)	Mackin and Hopkins, 1961[110]	Bleedwater	Spray	Reduced growth and glycogen content.
	Snail (Littorina littorea)	Perkins, 1970[111]	BP 1002	30 ppm	Significant inhibition to growth.
	Oyster (Crassostrea virginica)	Menzel, 1948;[55] in Nelson-Smith, 1973[3]	"Oil"	0.01 ppm	Marked tainting.
	Mussel (Mytilus edulis)	Blumer et al., 1971	No. 2 Fuel oil	Collected from field after spill.	Inhibition in development of gonads.
	Gastropod drill (Drupa granulata), Mussel (Mytilus variabilis)	Eisler, 1973[112]	Iranian crude, dispersant	10 mℓ/ℓ (10,000 ppm)	Decrease in predation rate of drill on mussel when exposed to crude; decrease in fecundity of drills when exposed to dispersant.
	Oyster (Crassostrea virginica), Scallop (Aquipectins irradians)	Gardner et al., 1975[101]	Waste motor oil	> 20 ppm	Incidence of lesions in branchial efferent vein, mantle, and gastro-intestinal tract of oyster; and in mantle, gill, and kidney of scallop.
	Mussel (Mytilus variabilis)	Eisler, 1975[102]	Dispersant Iranian crude	.020 mℓ/ℓ (20 ppm) 3 mℓ/ℓ (3000 ppm)	Decrease in ability to attach to surfaces
	Mussel (Mytilus edulis)	Gonzalez et al., 1976[11]	No. 2 Fuel oil, water-accommodated-fractions	10 ppb- 1 ppm	Decrease in filter feeding activity; and byssal thread attachment at the higher concentrations.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 3 Continued

Type of Organism	Species	Reference	Type Petroleum Product	Concentration	Sublethal Response
Molluscs (Continued)	Oyster (Crassostrea virginica)	Kittredge et al., 1975[10]	Naphthalene	1 ppm	Irritation of gill cilia.
	Snail (Littorina littorea)	Hargrave and Newcombe, 1973[113]	Bunker C, dispersant	.750-80 ppm	Increase in crawling and respiration rates (decreased in response to undefined conc. of dispersant or dispersant plus oil).
Other Benthic Invertebrates	Polychaeta (Capitella capitata)	Bellan et al., 1972[12]	Detergent	0.01-10 ppm	Decrease in survival, fecundity.
	Octocoral (Heteroxenia fuscescens)	Eisler, 1975[102]	Crude	10 mℓ/ℓ (10,000 ppm)	Reduction in tentacular pulsation.
	Hermatypic corals (Porites furcata)	Birkeland and Reimer, 1976[66]	Bunker C, diesel	exposed to oil layer	Exposure caused decrease in growth and variation of heads.
	Corals (Pavonia, Psammocora, Porites)	Reimer, 1975[114]	Bunker C, diesel	exposed to oil layer	Caused prolonged "mouth opening" responses, followed by reduction in feeding.

[a]Adopted with updated modifications from National Academy of Sciences.[5]

TABLE 4

SUMMARY OF EFFECTS OF OIL ON POPULATIONS AND COMMUNITIES

Community or Population Type	Expected Degree of Initial Impact		Expected Recovery	
Plankton	Light to Moderate:	Impact dependent on chance event of contacting floating slick. Decrease in population densities may have effect on local productivity. Greatest danger to small local breeding populations composed of larval fish.	Fast to Moderate:	Effective reproductive and dispersal mechanisms for most phyto- and zooplankton in open waters (populations dense, widely dispersed; individuals ubiquitous, prolific, grow quickly to maturity). Local breeding populations of larval fish and shellfish may take much longer to recover.
Neuston	Unknown:	Chance of contact high since communities exist on or near surface. Contamination reported, but effects unknown.	Unknown:	Ecology poorly understood.
Benthic Communities		Mortalities lead to decrease in population densities and age distributions; changes in species abundance and distribution; imbalances between interacting populations.		
Rocky Intertidal	Light: (with exceptions, e.g., Tampico Maru spill)	Hardiness of organisms. Most damage from coating leading to suffocation or loss of purchase on substrates.	Fast:	Oil rapidly removed by waves. Populations rapidly restored since individuals grow and reproduce rapidly.
Sandy or Muddy Intertidal	Moderate:	Impact increased by persistence of oil in unconsolidated substrates. Chance for greater mortalities since infaunal organisms may be more sensitive than rocky intertidal organisms that have developed defense mechanisms for living in rigorous and variable environments.	Moderate:	Persistence of oil in sediments prolongs toxic effects.

TABLE 4 Continued

Community or Population Type		Expected Degree of Initial Impact		Expected Recovery
Subtidal, Offshore	Heavy:	Impact increased by persistence of oil in unconsolidated substrates. Chance for greater mortalities since many subtidal organisms may be more sensitive than rocky intertidal organisms that have developed defense mechanisms for living in rigorous and variable environments.	Slow:	Persistence of oil. Possibly, slow rate of biological succession for complex, highly structured communities found in some subtidal areas where abiotic factors have been historically constant.
Fish	Light to Moderate:	Possibility of avoiding spills; some resistance offered by mucous coating. Greatest danger to local breeding populations in confined waterways (increased chance of contact; sensitive larval forms present; adults display complex breeding behavior) or benthic fish in heavily polluted substrates.	Fast to Moderate:	Effective reproductive and dispersal mechanisms for most pelagic populations (fast immigration of larvae and adults). Local breeding populations may take much longer to recover.
Birds	Heavy:	Mortality from ingestion of oil droplets and coating (loss of body heat and buoyancy). Mortalities lead to decrease in population densities.	Slow:	Individuals long-lived; low fecundity; gregarious behavior increases chances of losing entire population.
Mammals	Light:	In comparison to other groups, marine mammals not extremely abundant along most coasts. Impact dependent on chance event of small population contacting floating slick. Due to mobility, most mammals can probably avoid heavily-polluted areas. Conclusive evidence of mortalities, due to oil pollution, is rare. Possible effects include ingestion of toxic oil droplets during grooming; loss of thermal insulation and/or waterproofing, due to coating; and irritation of eyes and exposed mucous membranes. Eye irritation reported after Arrow spill[51] and spill in Alaska.[52]	Slow, if Population Seriously Affected:	Individuals long-lived; low fecundity--hence, time for recovery increased. Also, some mammals near extinction. However, no supportive evidence for loss of entire populations as result of oil pollution.

TABLE 5

SUMMARY OF EFFECTS OF OIL ON SOME MAJOR ECOSYSTEMS

Type Environment		Expected Initial Impact		Expected Recovery
Open Ocean	Light:	Impact on pelagic phyto- and zooplanktonic organisms dependent on chance event of contacting floating slick. Many organisms (particularly fish) may avoid spill. Neuston communities (surface dwellers) may be affected. Not likely that oil would accumulate in open ocean sediments to lethal or sublethal levels.	Fast:	Rapid dispersion and degradation of oil. Effective reproductive and dispersal mechanisms for most pelagic organisms (fast immigration of larvae and adults).
Outer Continental Shelf	Light to Moderate, e.g., George's Bank and Gulf of Alaska	Impact on phytoplankton and zooplankton populations light. Spawning population of fish larvae severe. Moderate impact on benthic systems if oil reaches the bottom.	Fast to Moderate:	Fast recovery for phytoplankton and zooplankton because of rapid regeneration times. Moderate recovery to benthic systems if oil reaches bottom.
Open Estuarine Areas, Bays, Channels, Harbors	Moderate to Heavy:	Chronic oil may depress populations of fish and some benthos; or induce changes in species abundance and distribution. Spilled oil effects dependent on time of year (spawning, migration, etc.) and oil's persistence.	Fast to Slow:	Dependent on flushing characteristics, route to benthos, shoreline characteristics, and community stability. Individual year classes of larval fauna may be severely impacted.
Wetlands: Marshes and Mangroves	Heavy:	Potential serious threat as result of vulnerability to spills and significance of estuarine functions (nursery and breeding grounds; high productivity; basis of detritus food chain). Several effects noted: faunal mortalities leading to decreases in population density, changes in species abundance and distribution; damage to marsh grasses after repeated exposure, and decrease in productivity; damage to mangroves and neighboring grasses.	Moderate to Slow:	Persistence of oil in sediments prolongs toxicity. Yet, once oil removed, biological succession may be moderate in some areas, since generally organisms reproduce and disperse fairly rapidly. Mangroves particularly complex and may take long to recover. Marsh area at West Falmouth still slightly affected 5 years after spill.
Special Ecosystems: Coral Reefs	Unknown:	Some reports of lethal damage to corals exposed to air and sublethal effects on growth and behavior of individuals. One report of altered community structure from refinery wastes in Puerto Rico.[65]	Unknown:	Recovery could be slow due to structural complexity of coral communities.

TABLE 5 Continued

Type Environment	Expected Initial Impact		Expected Recovery	
Polar Ecosystems	Unknown:	Some reports of effects in arctic lakes and on individual organisms.	Unknown:	In general, recovery could be slow since (1) spilled oil may persist longer at colder temperatures, and since (2) polar organisms have slower growth rates, extended life cycles, longer reproductive periodicity and narrow ranging dispersal stages. Yet some low-diversity coastal communities existing under rigorous and variable conditions may recover rapidly since organisms have already adapted to harsh conditions.

THE IMPACT OF OIL ON MARINE LIFE: A SUMMARY OF FIELD STUDIES

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ABSTRACT

Extensive field studies on the effects of chronic low level exposure of oil to marine life have been conducted at Santa Barbara; Lake Maracaibo, Venezuela; Bermuda; and Timbalier Bay, Louisiana, in the Gulf of Mexico. No measurable effects have been observed on such indicators of the health of the local marine communities as population levels of various organisms; species diversity; and size, growth rate, or reproducibility of various organisms. Moreover, there is no evidence of adverse effects such as abnormal growths and biomagnification of petroleum fractions in the food chain.

INTRODUCTION

Surprisingly, the volume of literature concerning the effects of oil on marine life is extensive. Several comprehensive summaries of the literature have been published.[1,2,3,4] Whether one reviews the literature directly or the summaries that are available, it is apparent that most of the published work concerns laboratory work. Only a few field studies have been conducted.

Laboratory work has proven generally unsatisfactory because its results correlate poorly with field observations. Fortunately, in recent years, some work has been directed toward investigating the effects of chronic exposure of oil on marine life under field conditions. The results of five such field studies are summarized below.

NATURAL OIL SEEPS AT COAL OIL POINT, SANTA BARBARA

Straughan and her associates at the University of Southern California recently concluded a three-year study concerning the sublethal effects of natural chronic exposure of oil to marine life. Her laboratory was the marine waters at Coal Oil Point near Santa Barbara, California, where natural oil seeps have been known to exist for centuries. The natural seepage there is 50-100 barrels per day.[5] Extensive control data were obtained from studies conducted at Pismo Beach (north of Santa Barbara), Gull Island (near Santa Barbara), and Santa Catalina Island (near Los Angeles).

The major conclusions of this study are:

1. All organisms are present that would be expected to be in that environment if oil seepage was not there.
2. Exposure to the natural oil seepage has no effect on either the growth rate or reproductivity of the resident organisms.
3. No abnormal growths in organisms were observable either by external examination or by dissection.
4. There is no evidence of bioaccumulation (increase in concentration) of hydrocarbons by transfer up the food chain.

CHRONIC EXPOSURE OF MARINE ORGANISMS TO TAR BALLS, BERMUDA

A relatively recent phenomenon is the influx of tar balls washing ashore along the Bermuda coastline.[7,8] The Bermuda Biological Station for Research has just concluded a two-year study to determine whether the influx of tar balls was having any effect upon the local intertidal life.

Control data were obtained by studying beaches that were slightly impacted. Also used were the extensive baseline data developed by annual studies extending back to the 1890's.

The major conclusions concerning marine life inhabiting the intertidal zone are:[9]

1. There is no measurable effect of tar influx on the number of organisms of any species at any locality.
2. All species identified as native to Bermuda shores prior to the tar ball influx still remain.
3. Exposure to the tar influx has no effect upon the reproductivity of the organisms.
4. Size of organisms is not affected by the exposure to tar influx.

These results provide an insight into the potential threat of offshore spills washing ashore to intertidal life. This is especially valid for spills, if they should occur, from platforms in areas 50 or more miles offshore in outercontinental shelf waters. Such potential production areas exist along the Atlantic Coast. The weathered oil that would wash ashore would be similar in composition and properties to the tar balls appearing on the Bermuda beaches.

LAKE MARACAIBO,
VENEZUELA

A three-year study was completed in 1974 by Battelle-Northwest Laboratories (Richland, Washington) on the impact of offshore production in Lake Maracaibo, Venezuela. Approximately 6500 wells have been drilled in this lake over a period spanning four decades. Sites in those sections of the lake where oil production does not occur were also studied to obtain control data.

The major conclusions from this study are:

1. Despite significant discharges of oil into Lake Maracaibo from production of oil and from natural seeps, both laboratory and field data show that the presence of oil has caused no discernible damage to the local ecosystem.

2. Although low concentrations of oil exist in the lake water, there is no evidence of a buildup of hydrocarbons in selected commercial species of fish or shrimp.

3. Although fisheries data are limited, no evidence exists that suggests this important renewable resource has yet been diminished.

4. However, discharge of nonpetroleum wastes, both domestic and industrial, are approaching such levels as to impair water quality. The biological resources of the lake may decline in future years.

TIMBALIER BAY,
GULF OF MEXICO

The Gulf Universities Research Consortium (GURC) studied the impact of offshore drilling and production of oil on the estuarine and marine environment in the coastal waters of Louisiana, specifically, in Timbalier Bay. The study involved 23 principal scientists from the 20 Gulf Coast universities that comprise GURC. Conducted over a period of two years, 1972-1974, and at a cost of 1.5 million dollars, this study is undoubtedly the most comprehensive study concerning the effects of chronic exposure of oil to marine life ever attempted.

Timbalier Bay contains about 400 oil and gas wells. The first well was drilled in 1937. Several stations

within this area were studied. Stations were also studied in adjoining areas where there never had been oil drilling or production in order to obtain control data.

Many observations and conclusions may be derived from this work, namely:[12,13,14]

1. Seasonal changes, especially in temperature and salinity, have a far more significant effect on species diversity of marine life and on the population of a given species than does the presence of low-level concentrations of oil.

2. Even the effects of other less important natural phenomena, such as floods, upwellings, and turbidity, affect the ecosystem markedly. Their influences completely obliterate whatever effect, if any, results from exposure to oil.

3. No known biological hazard could be related to any compound or material used in drilling and production.

4. Timbalier Bay has not undergone significant ecological change. Every indication of good ecological health is present.

5. Evidently, the platforms have increased the total quantity of marine life. These structures provide surfaces where planktonic larvae of organisms such as barnacles, mussels, sea anemones, and other forms of sessile marine life may settle and flourish to become high productive, complex communities.

SURVEY OF MARINE LIFE UNDER OFFSHORE PLATFORMS IN SANTA BARBARA CHANNEL

Platforms Hilda and Hazel were constructed in the Santa Barbara Channel in 1959 and 1960. During their construction, a survey revealed that the surfaces of these structures quickly became encrusted and a complex marine community including sessile, benthic and pelagic forms developed.[15]

A year's survey was initiated early in 1975 to assess the extent and complexity of the marine community under the two platforms constructed in the Santa Barbara Channel in 1959 and 1960. This study was conducted by the Southern California Coastal Water Research Project under the direction of the Scripps Institution of Oceanography near San Diego.

The major observations resulting from this survey are:[16]

1. A highly complex community has developed under each platform. Communities on either the soft or hard bottom control areas are far less complex and far less abundant.

2. The pelagic fish population inhabiting the area under the platforms is estimated to be 20,000-30,000 per platform.

3. Positively identified are at least 50 species of fish, 110 species of intertebrates living on or near the structures, and 77 species of worms inhabiting the nearby sediments.

4. All sea life appears to be extremely healthy. Mussels 8-10 inches in length are numerous; larger ones have been observed.

5. Every available underwater surface of the platforms is heavily encrusted with mussels, barnacles, aggregate anemones, or other types of sessile sea life.

6. Drill cuttings were deposited at the base of the platform. Being sterile, they did not support marine life for two to three years after the platforms were constructed. Today this pile is covered by a depth of 37 inches of shells and now supports a teeming community of seastars, anemones, nudibranches, and other benthic organisms.

CONCLUSIONS

The major conclusions that may be derived from the field studies conducted to date are:

1. Low-level chronic exposure to crude oil has, at most, negligible effect on marine life.

2. Platforms provide a structure whereby a thriving, highly complex community of marine life can develop.

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DISCUSSION

SAGER: It is interesting that marine biologists don't start right off and talk about succession like terrestrial ecologists.

LEMON: The important ecological process of succession has occurred in all times, including the present, and we must look toward the same thing in the future. Succession takes place about as often as evolution in a very general sense. So individual problems that we might be anxious to understand or solve must relate to a time schedule. I have lectured my classes ad infinitum on terrestrial succession and then wanted to talk about succession in fresh or salt water habitats.

As much as I have been able to learn, the same processes occur in the ocean, but they are markedly compressed. In fact, an area suddenly laid bare by filling or blasting or anything quite drastic will be suddenly reoccupied by what one would refer to, in terms of land succession, as six or eight stages of succession. But they will come so fast, maybe in one season, that we have pioneers and climax species crowding each other.

I am sure that healing or curative processes may occur in response to any ocean disturbance. Whether that is worth any worry or not depends on how much time we want to think about; whether it be a generation, a millenium, or a few centuries.

SAGER: I agree with you, but I think that one of the reasons for the rapidity of the time schedule may involve the aquatic ecosystem's fluidity, mobility, and motility, which are not demonstrated in a land system. Therefore, it is easier and quicker for the bodies of water to be filled in.

EFFECTS OF PETROLEUM ON SURVIVAL, RESPIRATION AND GROWTH OF MARINE ANIMALS

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ABSTRACT

Petroleum products vary tremendously in their toxicities to marine animals. The relative toxicity of an oil is, in most cases, directly correlated to its content of aromatic hydrocarbons. The most toxic aromatics are the phenanthrenes. There is a wide interspecies variation in the sensitivity of marine animals to petroleum. Estuarine and benthic species are often, but not always, more tolerant than oceanic species. Larvae and juveniles are often, but not always, more sensitive than the adults. Both respiration and early growth and development of marine animals are affected by exposure to sublethal concentrations of oil. These sublethal responses are variable and in most cases are rapidly reversible when the animals are returned to oil-free sea water.

INTRODUCTION

As a result of the rapid increase in recent years in the volume of petroleum shipped by sea and in the exploitation of offshore oil and gas deposits, there is a growing concern about the impacts of spilled oil on marine organisms and on the marine environment as a whole. Several attempts have been made to estimate the annual influx of petroleum to the marine environment from natural and anthropogenic sources. The best estimate for natural marine oil seepage is 0.6 million metric tons per year (MTA)[1]. The most reliable estimate of the annual influx of petroleum to the marine environment from all sources is 6.2 MTA[2]. The major anthropogenic sources of marine petroleum are those associated with marine transportation (2.2 MTA) and surface runoff from land (1.6 MTA). Much of this influx occurs in the biologically productive estuaries and near-shore marine waters.

A major research effort has been initiated in this country and abroad to assess the impact of these petroleum discharges on marine

organisms and ecosystems. Much of the laboratory research to date has been concentrated on acute toxicity bioassays with one or a few oils or petrochemicals and selected species of marine organisms. Such bioassays, while important, are of limited value in assessing the potential impact of long term low level petroleum contamination on marine ecosystems. Studies of sublethal biological effects of petroleum are potentially more useful in this regard[3]. The purpose of this paper is to review some of the results of studies of the lethal and sublethal effects of oil on marine organisms conducted in our laboratory during the past several years.

Sensitivity of Marine Animals to Oil: Influence of Oil Composition

A considerable volume of published information is available concerning the acute toxicity of different crude and refined petroleum products to marine organisms. However, comparisons of the relative toxicities of different petroleum products and the sensitivity to oil of different marine species are difficult because a variety of methods were used to introduce petroleum into the water and in many cases the actual concentration of hydrocarbons in the aqueous phase of the exposure medium was not measured[4]. Petroleum is an extremely complex mixture of literally thousands of different hydrocarbons and related hetero-compounds. Different crude and refined oils vary tremendously in the relative concentrations of different components and as a result show substantial variability in solubility, dispersibility and persistance in sea water[5,6]. As a result, bioassays in which results are reported in terms of oil added are of little comparative value. For laboratory bioassay and biological effects studies, the simplest reliable techniques for the analysis of petroleum hydrocarbons in sea water are infrared analysis for total hydrocarbons[7] or a method for measuring soluble aromatics, such as ultraviolet spectrophotometry[8] or fluorescence spectroscopy[9].

When these parameters are taken into consideration, several trends emerge. Crude petroleums are generally less toxic to marine organisms than refined products. However, crude oils from different sources vary tremendously in their toxicity and some may be more toxic than certain highly refined light distillate products such as JP-8 jet fuel[10]. Recent evidence strongly indicates that in most cases the acute toxicity of a petroleum is directly correlated to its content of soluble aromatic derivatives[4]. The different aromatic hydrocarbons commonly encountered in oil vary substantially in their toxicity to marine animals (Table 1). The monoaromatics are the least toxic. Acute toxicity increases with increasing molecular size until the 4 and 5 ring compounds are reached. These polycyclic aromatic hydrocarbons, presumably because of their large molecular size and low aqueous solubility, are not acutely toxic. However, they may show substantial chronic toxicity, since some are known to be potent carcinogens. Alkylation of the aromatic nucleus seems to increase the toxicity of the parent compound. Thus, in both the benzene and naphthalene series, toxicity increases with increasing degrees of alkylation. The most toxic hydrocarbon evaluated is 1-methylphenanthrene.

To test the hypothesis that the relative toxicity of an aromatic hydrocarbon is related to the rate at which it is accumulated from sea

Table 1. Comparative acute toxicity of 15 aromatic hydrocarbons present in petroleum to 2 species of marine invertebrates. 96h LC50= concentration in ppm lethal to 50% of test animals in 96 hours.

Aromatic Hydrocarbon	Palaemonetes pugio (grass shrimp)	Neanthes arenaceodentata (polychaete worm)
	96 h LC50	
Benzene	27.0	-
Toluene	9.5	-
Xylenes	7.4	-
1,2,4 Trimethylbenzene	5.4	-
Naphthalene	2.4	3.8
2-Methylnaphthalene	1.1	-
2,6-Dimethylnaphthalene	0.7	2.6
2,3,6-Trimethylnaphthalene	-	2.0
Phenanthrene	-	0.6
1-Methylphenanthrene	-	0.3
Fluorene	-	1.0
Fluoranthrene	-	0.5
Chrysene	-	*
Benzo(a)pyrene	-	*
1,2,5,6-Dibenzanthracene	-	*

*Not lethal at its maximum solubility in sea water.

water and the degree to which it is retained in the tissues following exposure, uptake and release of 4 aromatic hydrocarbons by the clam Rangia cuneata was investigated. Groups of 10 clams were exposed to each hydrocarbon for 24 hours. Five clams were then sacrificed for analysis and the remaining 5 clams were returned to hydrocarbon free sea water for 24 hours before being sacrificed. Aqueous and tissue aromatic hydrocarbons were measured by ultraviolet spectrophotometry[8]. Phenanthrene was the aromatic hydrocarbon accumulated most rapidly and released most slowly (Table 2). Chrysene and benzo(a)pyrene were accumulated more slowly but were also released relatively slowly. The rapid release of naphthalene from the clam tissues probably masked a similarly rapid uptake during exposure, since both influx and efflux of these compounds undoudtedly occur simultaneously. These observations may partially explain the relatively high acute toxicity of phenanthrene. Its uptake/release kinetics favor its rapid accumulation in the tissues of marine animals. Chrysene and benzo(a)pyrene, because they are accumulated much more slowly, have a relatively low acute toxicity. However, because of their slow release from the tissues of exposed animals, they have a potential for high chronic toxicity. The lower toxicity of naphthalene may be related to the rapidity with which it is released from the tissues of exposed animals.

The acute chemical toxicity of petroleum to marine animals is probably due primarily to those components of the oil which go into solution or are accomodated in the water column. The solubility of aromatic hydrocarbons in water decreases substantially with increasing molecular weight[11]. Thus, the water-soluble fractions (WSF) of oil usually contain relatively high concentrations of mono- and diaromatic hydrocarbons and very low concentrations of higher molecular weight polycyclic aromatic hydrocarbons[6]. The actual composition of the WSF will depend on the composition of the parent oil. Therefore much of the acute toxicity of most crude and refined oils can be attributed to the naphthalenes and, to a lesser extent, to the benzenes present.

Sensitivity of Marine Animals to Oil: Species Differences

Marine animals vary tremendously in their sensitivity to oil. Table 3 summarizes data on the toxicity of the WSF of Southern Louisiana crude oil to 10 species of marine animals representing 3 phyla. Water soluble fractions were prepared by gently mixing one part oil with 9 parts artificial sea water for 20 hours[6]. The concentrations of total hydrocarbons in the exposure solutions were measured by infrared analysis[7]. The polychaete worms tested are relatively tolerant. The pelagic species Platynereis dumerilii is somewhat more sensitive than the 2 benthic species, Neanthes arenaceodentata and Capitella capitata. The crustaceans studied show substantial differences in their sensitivity to the WSF of Southern Louisiana crude oil. The opossum shrimp Mysidopsis almyra, which is an estuarine species, is more sensitive than the oceanic prawn Leander terruicornis. However, 2 other estuarine shrimp, Palaemonetes pugio and Penaeus aztecus are extremely tolerant. The three fish species tested are common sympatric estuarine species from the Texas coast, yet the silverside minnow Menidia beryllina is substantially more sensitive than either the gulf killifish Fundulus similus or the sheepshead minnow Cyprinodon variegatus.

The early life stages of marine animals are usually thought to be

Table 2. Accumulation and release of different aromatic hydrocarbons by the clam Rangia cuneata.

Aromatic Hydrocarbon	Naphthalene	Phenanthrene	Chrysene	Benzo(a) pyrene
Exposure Concentration(ppm)	0.071	0.089	0.066	0.052
Tissue Concentration after 24 hr Exposure (ppm)	0.43±0.1	2.85±1.1	0.54±0.3	0.45±0.1
Bioaccumulation Factor [Tissue]/[Water]	6.1	32.0	8.2	8.7
Tissue Concentration after 24 hr Depuration (ppm)	0.15±0.02	2.47±1.2	0.40±.15	0.38*
% Released in 24 hours	66	13	26	16

*Only one sample analyzed

Table 3. Concentration of the water-soluble fraction of Southern Louisiana crude oil (ppm total hydrocarbons) lethal to 50% of the test animals in 48 (48 h LC50) or 96 (96 h LC50) hours.

Species	48 h LC50	96 h LC50
Polychaeta		
Sargassum worm, Platynereis dumerilii	12.3	9.5
Errant benthic worm, Neanthes arenaceodentata	13.9	12.5
Sedentary benthic worm, Capitella capitata	16.2	12.0
Crustacea		
Opossum shrimp, Mysidopsis almyra	8.7	--
Prawn, Leander terruicornis	10.2	6.0
Grass shrimp, Palaemonetes pugio	>16.8	>16.8
Brown shrimp postlarvae, Penaeus aztecus	>19.8	>19.8
Teleosti		
Silverside minnow, Menidia beryllina	8.7	5.5
Gulf killifish, Fundulus similus	16.8	16.8
Sheepshead minnow, Cyprinodon variegatus	>19.8	>19.8

more sensitive to pollutant stress than are the juveniles or adults. However, this is not always the case. Postlarvae of the brown shrimp Penaeus aztecus are significantly more tolerant to the WSF of No. 2 Fuel Oil than are either the early or late juvenile stages (Table 4). However, the postlarvae and juveniles of the closely related white shrimp Penaeus setiferus don't show this differential sensitivity and both stages are more sensitive than P. aztecus. The grass shrimp Palaemonetes pugio shows the opposite trend. The larvae are significantly more sensitive to the WSF of No. 2 Fuel Oil than are either the postlarvae or adults.

These data indicate that we cannot predict a priori the relative sensitivity of an animal to oil based on its habitat or life stage. Each species responds differently to pollutant stress.

Sublethal Effects of Oil: Respiration

Acute toxicity bioassays in which the end point is death are useful primarily as a first step in the evaluation of the environmental impact of a pollutant. Such bioassays are not sufficient for assessing the potential long term environmental hazards of a pollutant. The elucidation of the sublethal responses of marine animals to pollutant stress has the potential of providing information that is more ecologically meaningful[3]. There are two general types of sublethal effects studies and each provides a different type of information. In the first, the effects on some biological function of brief exposure to relatively high concentrations of the pollutant are investigated. This approach may provide valuable information about the mode of toxic action of the pollutant. In the second type of sublethal effects study, the test animals are exposed continuously for all or a critical portion of their life cycle to low concentrations of the pollutant and the effects of this exposure on such processes as growth, behavior or intermediary metabolism are measured. This approach is the only one by which delayed or long term effects of a pollutant can be assessed. Therefore, it is the most valuable in predicting the environmental impact of a pollutant. We have conducted both types of sublethal studies and examples of each will be described here.

Groups of opossum shrimp, Mysidopsis almyra, were exposed to several concentrations of the water-soluble fraction (WSF) or oil-in-water dispersion (OWD) of No. 2 Fuel Oil. The WSF and OWD were prepared as described previously[6]. Oxygen consumption by the shrimp was measured in closed all glass respirometer chambers by the micro-Winkler technique[12] during or immediately after exposure to the oil-water mixtures. The shrimp respond to brief exposure to the WSF of No. 2 Fuel Oil by an elevation of respiratory rates above control values (Figure 1A). There is an approximately 2-fold increase in respiratory rate with rising exposure concentrations between 0 and 20% WSF. Those animals maintained in 30% WSF while respiration was measured show a lower rate than the 20% WSF exposure group.

A similar pattern of increasing respiratory rates with increasing exposure concentration is seen in shrimp following a 2 hour exposure to OWDs of No. 2 Fuel Oil (Figure 1B). Again, respiratory rates of shrimp exposed to the highest OWD concentration (10 ppm total hydrocarbons) are below those of shrimp exposed to 5 ppm OWD.

It is interesting to note the close correlation between the concentrations of total naphthalenes in the WSF and OWD which result in

Table 4. Comparative toxicity of the water soluble fraction of No. 2 Fuel Oil to different life stages of three marine crustaceans. (Concentrations in ppm of total hydrocarbons).

Species	Description	96 h LC50 (ppm) and 95% C.I.
Penaeus aztecus (Brown shrimp)	Postlarvae (2.23 mg dry wt.)	6.6 (6.1 - 6.9)
	Early juveniles (29.2 mg dry wt.)	3.7 (3.0 - 5.1)
	Late juveniles (225.8 mg dry wt.)	2.9 (2.2 - 3.8)
Penaeus setiferus (White shrimp)	Postlarvae (0.33 mg dry wt.)	1.4 (0.9 - 21.)
	Juveniles (77.8 mg dry wt.)	1.0 (0.8 - 12.)
Palaemonetes pugio (Grass shrimp)	Larvae (<3 weeks old)	1.2 (1.0 - 1.5)
	Postlarvae (4-5 weeks old)	2.4 (2.1 - 2.8)
	Adults (>5 weeks old)	3.5 (2.4 - 4.9)

the highest respiratory rates. In both cases respiratory rates reach a maximum at approximately 0.4 ppm total naphthalenes in the exposure medium.

It should be noted that 24h LC50 values for this species and this oil are approximately 40% WSF and 2 ppm OWD[6]. Thus the respiratory rate changes observed represent immediate responses to near lethal pollutant stress.

An experiment was conducted with the grass shrimp Palaemonetes pugio to test the hypothesis that the respiratory response of crustaceans to oil exposure is related to the accumulation of petroleum hydrocarbons in the tissues of the affected animals. If this is true, then respiratory rates would be expected to return to normal if the animals are returned to oil-free sea water and allowed to depurate the accumulated hydrocarbons. Since earlier measurements had indicated that the level of nutrition has an important effect on the respiratory rate of this species, care was taken to control the feeding regime of all test animals. As part of the experiment, the effect of 48 hours starvation on respiratory rate was examined before the animals were exposed to an OWD of No. 2 Fuel Oil (5 ppm total hydrocarbons and 0.1 ppm total naphthalenes, approximating the 24h LC50 for this species). Respiratory rates of 8 groups of 12-14 shrimp were measured immediately before and after 5 hours exposure to the OWD. In addition, a large group of animals was exposed to the same OWD, and 2 groups of 10 animals were sampled at different times during exposure and 72 hours after return to oil-free sea water for tissue naphthalenes analysis[8].

The results of this experiment are summarized in Figure 2. While 48 hours starvation cause a 20% reduction in respiratory rate, 18 hours with food available is sufficient to allow the respiratory rate to return to the control value. Exposure to the OWD of No. 2 Fuel Oil for 5 hours causes a respiratory depression similar in magnitude to that produced by starvation. This decrease in respiratory rate is not the result of nutritional stress since the animals were fed immediately before exposure and no significant decrease in respiration is seen following only 5 hours starvation. The shrimp were then returned to oil-free sea water with adequate food for 7 days, after which their respiratory rates were measured again. At this time respiratory rates had returned to the control values. Naphthalenes uptake and release data, also summarized in Figure 2, show that tissue naphthalenes concentrations, which reached a peak of 2.0 ppm after 5 hours exposure to the OWD, had decreased to background levels in 72 hours.

In the two experiments reported here the respiratory response of 2 species of crustaceans to oil exposure is dramatically different. The response of the opossum shrimp to oil is an elevation of oxygen consumption while that of the grass shrimp is a decrease in respiratory rate. Other investigators have observed similar interspecies variations in the response of marine animals to oil or petrochemicals. Steed and Copeland[13] reported that the shrimp Penaeus aztecus and P. duorarum exhibited opposite respiratory responses when exposed to petrochemical effluents. P. aztecus decreased oxygen consumption while P. duorarum increased oxygen consumption during exposure to petrochemical waste concentrations approximately one-third the 48h LC50 value. In the same study, sheepshead minnows Cyprinodon variegatus showed depressed respiratory rates at low effluent concentrations and elevated rates at high sublethal concentrations. Respiratory rates of snails, Littorina littorea were increased in the presence of dispersed bunker C oil[14]. The respiratory rates of two species of mussels, Mytilus and Modiolus were also increased during exposure to the WSF of crude oil[15]. However,

respiratory rates of two other bivalves Brochiodontes and Donax were depressed when these mollusks were exposed to a light Arabian crude oil[16].

We have shown in the experiments described earlier that the respiratory response of grass shrimp to oil is transitory and respiratory rates return to normal if the animals are returned to oil-free seawater and allowed to release the accumulated hydrocarbons. Brockson and Bailey[17] observed a similar transitory respiratory response in juvenile salmon and striped bass exposed to 10 ppm benzene. Respiratory rates were elevated during exposure and returned to normal after 10 days in clean sea water.

Thus marine animals do exhibit a respiratory response to exposure to sublethal concentrations petroleum and petrochemicals. However, this response is variable and often reversible after the termination of exposure. Petroleum hydrocarbons may influence respiration directly at the cellular level by their interactions with cellular membranes[18, 19], or indirectly by modifying behavior and activity. Thus, the respiratory responses observed are usually the sum of several superimposed responses of the animals. Despite these difficulties, respiration can be a simple yet sensitive indicator of sublethal pollutant stress if adequate controls and sufficient replicates are run to establish some measure of variance.

Sublethal Effects of Oil: Growth

Marine animals that are exposed acutely or chronically to low concentrations of petroleum or other pollutants may exhibit subtle sublethal responses which are difficult to quantitate and interpret in short-term studies. In chronic low level exposure situations, sublethal responses may not occur until the pollutant has accumulated in the tissues to toxic levels. Because the toxic effects of a pollutant may be delayed or may develop slowly, long-term studies are required to adequately define its ecological impact. Development and growth are biological processes which are readily quantitated. Their rates represent the product of many interacting physiological and biochemical processes and therefore should be sensitive indices of pollutant induced perturbations[20]. We have therefore studied the effects of petroleum on the development and growth of several species of marine animals.

Juvenile polychaete worms, Neanthes arenaceodentata (mean initial wet weight, 0.02 g/worm) were exposed continuously for 28 days in a flow-through system to 3 concentrations of the WSF of No. 2 Fuel Oil. Mean exposure concentrations were 60, 95 and 180 ppb (µg/l) total naphthalenes measured, corresponding to 180, 310 and 600 ppb total aqueous hydrocarbons, respectively. These concentrations are all well below the 96 h LC50 value of 2.7 ppm total hydrocarbons for this species[21].

Growth rates were suppressed by all three WSF concentrations. This effect was most noticeable after 28 days exposure (Figure 3). Analysis of variance verified that just prior to exposure, the worms were equal in weight ($p=0.05$). After 14 days of exposure, only those juveniles exposed to the two highest WSF concentrations were growing at rates significantly slower than that of control animals ($p=0.05$). After 28 days exposure, the final weights of worms grown in all three WSF concentrations were significantly lower than that of controls worms ($p=0.05$).

The ecological significance of these results is difficult to assess, especially in light of the observation that equal numbers of females just

becoming gravid were present in the control and WSF exposure aquaria on the 28th day of exposure. Thus, although the presence of the WSF apparently retarded growth, it did not seem to retard the development of sexual maturity or egg maturation.

In another experiment, the effects of a WSF of No. 2 Fuel Oil (270-340 ppb total naphthalenes, 520-850 ppb total hydrocarbons) on growth of grass shrimp Palaemonetes pugio larvae was investigated. The exposure and control groups of larvae were maintained in petri dishes containing the appropriate exposure medium. Exposure media were changed and larvae were fed newly hatched brine shrimp Artemia salina daily. After 12 days the WSF-exposed larvae were significantly smaller than the controls ($p<0.001$) (Figure 4). On the 12th day of the experiment, the larvae exposed to the WSF were transferred to petri dishes containing oil-free sea water for five days. During this time the growth rates of the experimentals was similar to that of the controls. The experimental shrimp were then exposed for two days to the WSF and then returned to oil-free sea water for 10 days before the final weight determinations. The final weight determinations revealed that the exposed larvae recovered from the effects of exposure and grew at an accelerated rate so that final weights of the control and experimental animals were not significantly different ($p<0.05$). These results can be explained in part by the observation made earlier in this paper, that young larvae of this species are very sensitive to oil and sensitivity decreases as the larvae grow to juvenile and adult life stages (Table 3).

To better define the effects of oil on crustacean growth, mud crabs Rhithropanopeus harrisii were exposed continuously from hatching for 6 months to WSFs of No. 2 Fuel Oil. Observations were made of mortality, molting rate and final weight. This growth study was conducted at a temperature of 25°C and salinity of 15 o/oo. There were one-hundred larvae in each exposure group (0, 0.16, 0.31, 0.63, 0.94 and 1.26 ppm total aqueous hydrocarbons). Animals were censused and fed Artemia nauplii and the exposure mixtures were changed daily.

Larval development of R. harrisii is characterized by four zoeal stages, followed by a megalops stage. The megalops then molts to the first crab stage. The larvae in the control, 0.16 and 0.31 ppm exposure groups all had similar survival to the megalops stage ($\sim$90%). In the 0.63, 0.94 and 1.26 ppm exposure groups survival to the megalops stage was 76, 30 and 6% respectively. Most mortalities occurred between days 4 and 6 of exposure, the approximate time of the first zoeal molt.

The time required to reach the megalops stage was different for the different exposure groups (Figure 5). At low WSF concentrations, the mean time of molting to the megalops was decreased relative to that of controls. At high concentrations, mean time to the megalopal molt was greater than that of the controls. Zoeal molting was highly synchronized in the control and low concentration exposure groups. At the higher exposure concentrations, this synchrony was partially abolished, resulting in wide variations in intermolt periods among individuals in these groups.

Relatively few mortalities occurred among the megalops and crab stages at any exposure concentration. In addition, the mean duration of the megalops and first crab stages was not greatly affected by exposure to No. 2 Fuel Oil WSFs (Figure 6). There was a tendency for the mean duration of the megalops to increase with increasing WSF concentration between 0 and 0.63 ppm WSF, but the differences were not significant. At 0.94 and 1.26 ppm WSF, the mean duration of the megalops was only slightly longer than that of the controls. The mean duration of the

first crab stage was longer than that of the controls at all exposure concentrations. However, there was no correlation between exposure concentration and duration of the first crab stage and only in the 0.16 and 0.63 ppm exposure groups were the differences from controls significant.

The mean size of the crabs in different instars after six months continuous exposure to the WSFs were determined (Table 5). The mean size of the controls at any stage was consistently greater than that of animals exposed to 0.16, 0.31 or 0.63 ppm WSF. All the surviving crabs, with the exception of the ninth stage individuals, exposed to 0.94 and 1.26 ppm WSF were larger than the corresponding controls. This may be due in part to the small sample size in these two exposure groups. However, the largest individuals in these groups were larger than any in other exposure groups or the control.

In this study, the first zoeal stage or R. harrisii was the stage most sensitive to oil. Later larval stages, the megalops and the crab stages were much less sensitive to the No. 2 Fuel Oil WSFs. This may in part be an artifact of the experimental design, since the most sensitive individuals would be eliminated early in the exposure period. Thus, only the heartiest, most resistant individuals survived long enough to be exposed at the megalops and later molt stages. However, Katz[22] exposed each zoeal stage of the crab Neopanope texana separately to a WSF of a light Venezuelan crude oil and showed that larvae first exposed at the first zoeal stage had a much higher mortality than groups first exposed at later zoeal stages. Katz also reported delayed molting among those crabs that survived oil exposure. In the experiments reported here, zoeal molting rate and thus time to the megalops stage was decreased by low and increased by high concentrations of the WSF. It would appear, as Epifanio has suggested[23], that changes in larval molting rate may be a sensitive index of sublethal pollutant stress in decapod crustaceans. However, later molt stages and intermolt periods were less sensitive to pollutant-induced perturbation. After six months exposure, the mean weights of crabs exposed to low concentrations of WSF were less than that of controls and those of crabs exposed to the 2 highest concentrations were greater than the mean weight of the controls. However, the small differences observed are of uncertain ecological significance.

CONCLUSIONS

Petroleum products vary tremendously in their toxicities to marine organisms. However, several generalizations can be made. Refined products are usually more toxic than crude oils. The relative toxicity of an oil is, in most cases, directly correlated to its content of aromatic hydrocarbons. The acute toxicity of aromatic hydrocarbons increases with increasing molecular size from benzene to phenanthrene. However, the four and five ring aromatics are not acutely toxic. The alkyl analogues are more toxic than the parent compounds. These chemical structure/biological activity relationships appear to be related to differences in the aqueous solubility and bioaccumulation potential of these hydrocarbons.

There is a wide interspecies variation in the sensitivity of marine animals to petroleum. Estuarine and benthic species are often, but not always, more tolerant than oceanic species. Larvae and

Table 5. The mean size (mm) of each instar of Rhithropanopeus harrisii after six months of continuous exposure to various concentrations of WSF of No. 2 Fuel Oil.

Stage	Concentrations of WSF #2 Fuel Oil % WSF and ppm total aqueous hydrocarbons					
	0	2.5% (0.16)	5% (0.31)	10% (0.63)	15% (0.94)	20% (1.26)
4	-	3.07±0.47	4.15±0.35	2.50±0.14	-	-
5	5.63±1.23 mm	3.69±0.61	4.54±1.01	3.91±0.68	5.90±0.85	-
6	5.23±0.57	4.12±0.51	4.83±0.54	4.42±0.79	6.63±0.75	-
7	5.53±0.90	4.97±0.81	5.12±0.50	4.59±0.84	7.41±0.96	6.97±1.61
8	6.04±0.77	5.09±0.69	5.91±0.69	5.10±0.54	6.62±0.88	-
9	6.89±0.84	6.0*	6.13±0.29	5.8*	6.57±0.25	6.8*
10	-	4.8*	-	7.0*	-	-

*Absence of a standard deviation indicates only one animal was present at this stage.

juveniles of many species are more sensitive than adults. However in some species such as the brown shrimp Peaneus aztecus the young juveniles are much more tolerant to oil than are the late juveniles or adults. Species and age differences in sensitivity to oil may be related to qualitative or quantitative differences in their capabilities for metabolizing and excreting petroleum hydrocarbons. Several species of marine fish[24,25] and crustaceans[26,27] are known to be able to metabolize aromatic hydrocarbons. However little quantitative information is available concerning the rates at which these detoxification processes go on in different species and different life stages of marine animals.

Some important conclusions can be drawn from the sublethal studies reported here. The concentrations of aqueous petroleum hydrocarbons required to elicit significant sublethal respiratory and growth responses are close to the acutely toxic concentrations for the species studied. Growth perturbations appear, in some cases, to decrease in magnitude during the course of long term exposure to WSFs of oil. In the growth and respiration experiments with Palaemonetes reported here, sublethal responses rapidly returned to normal when the shrimp were returned to oil-free sea water. Thomas and Rice[28] and Rice et al.,[29] observed transitory changes in the breathing and coughing rate of pink salmon Onchorhynchus gorbuscha fry during exposure to concentrations of the WSF of Prudhoe Bay crude oil equivalent to 20% of the 96 h LC50 concentration. Breathing and coughing rates rose to a maximum in 3 to 6 hours after the beginning of exposure and then began to drop back toward the control rate. When the fish were returned to oil-free sea water breathing and coughing rates rapidly returned to normal.

These findings all strongly suggest that marine animals have some ability to acclimate to and recover from sublethal exposure to oil. Acclimation may involve the induction or increase in activity of hydrocarbon-metabolizing enzymes in response to exposure to hydrocarbons. Support for this hypothesis is provided by the recent studies of Pedersen et al.,[30] and Lee et al.[31]. Pedersen et al., showed that hepatic benzo(a)pyrene monooxygenase could be induced in several strains of rainbow trout by intraperitoneal injection of 3-methylcholanthrene. Lee et al., showed that this enzyme could also be induced by benzanthracene in several tissues of the blue crab Callinectes sapidus and the polychaete worm Nereis sp.

It must be stressed that, in most cases, the concentrations of hydrocarbons required to elicit measurable acute or sublethal responses in the marine animals investigated are well above those ordinarily encountered in solution in sea water even after a major oil spill. McAuliffe et al.,[32] reported that the highest aqueous hydrocarbon concentration under an oil spill from an offshore production platform was 200 ppb. Dissolved non-polar hydrocarbon concentrations (mostly alkanes) ranging from traces to 75 ppb were reported in the offshore waters of the Gulf of Mexico, Caribbean Sea and Florida Strait by Iliffe and Calder[33]. Thus, pelagic species are unlikely to be seriously affected by spilled oil. However, local physical and weather conditions at an oil spill or discharge site may favor incorporation of large concentrations of petroleum hydrocarbons into bottom sediments[34]. Little is known about the bioavailability and toxicity to benthic animals of sediment adsorbed petroleum hydrocarbons. Because of the great ecological and economic importance of near shore marine benthic communities, these problems deserve special attention.

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DISCUSSION

GOULD: Could you please comment on whether or not your data on uptake and depuration of pure hydrocarbons in clams are applicable to complex mixtures such as crude oils and refined petroleum products? Could synergistic or physical factors, due to the presence of so many other compounds in such oils, alter your mechanisms and, perhaps, invalidate them?

NEFF: We have some data on accumulation of total hydrocarbons from crude and refined oils. As an example, the biomagnification factor for 24 hours for naphthalene was 6.2. From fuel oil the biomagnification factor for the same species for naphthalene was, I think, 5.8 to 6. So the results are essentially identical.

I was amazed at how close the two values were. It appears that, concerning the influx or uptake, these hydrocarbons are independent of one another. I would have expected some interaction between different hydrocarbons, but all the data I have seen so far seem to indicate that they are partitioning into the phases or into the organisms independently of one another.

The rates of uptake are dependent on various physical factors of the individual hydrocarbons, and there doesn't seem to be any competition; at least I haven't seen any evidence of it. Maybe, under certain circumstances, we will.

RICE: We have done quite a bit of testing with shrimp and crab larvae and get quite similar results. Among about eight different species of crab larvae that we have tested, the range is approximately an order of magnitude of sensitivity; some are quite difficult to kill.

We only have done one species where we did different instar stages, and the sensitivity increased from stage 1 through about stage 6.

LAROCHE: I am glad to see that you are working on growth effects and this kind of thing because, as far as I am concerned, the 96-hour TL_{50} can be a bit of a hoax on some occasions. It may not really show the things that we are meant to see.

For instance, I remember when I was with EPA at West Kingston, we showed a similar TL_{50} to No. 2 fuel oil for silver side, and the fundulus, and there was quite a discrepancy. Fundulus was a very resistant species.

But we soon realized that at about 1 part per million of that particular No. 2 fuel oil, we had mechano-and chemoreceptor damage. So it is all fine and good to say the species would survive. If it cannot survive in the environment, it is not worth very much.

NEFF: Yes, I agree. You have got to do the long-term studies.

LAROCHE: Not necessarily long term as much as trying to identify sensitive means of detecting physiological damage.

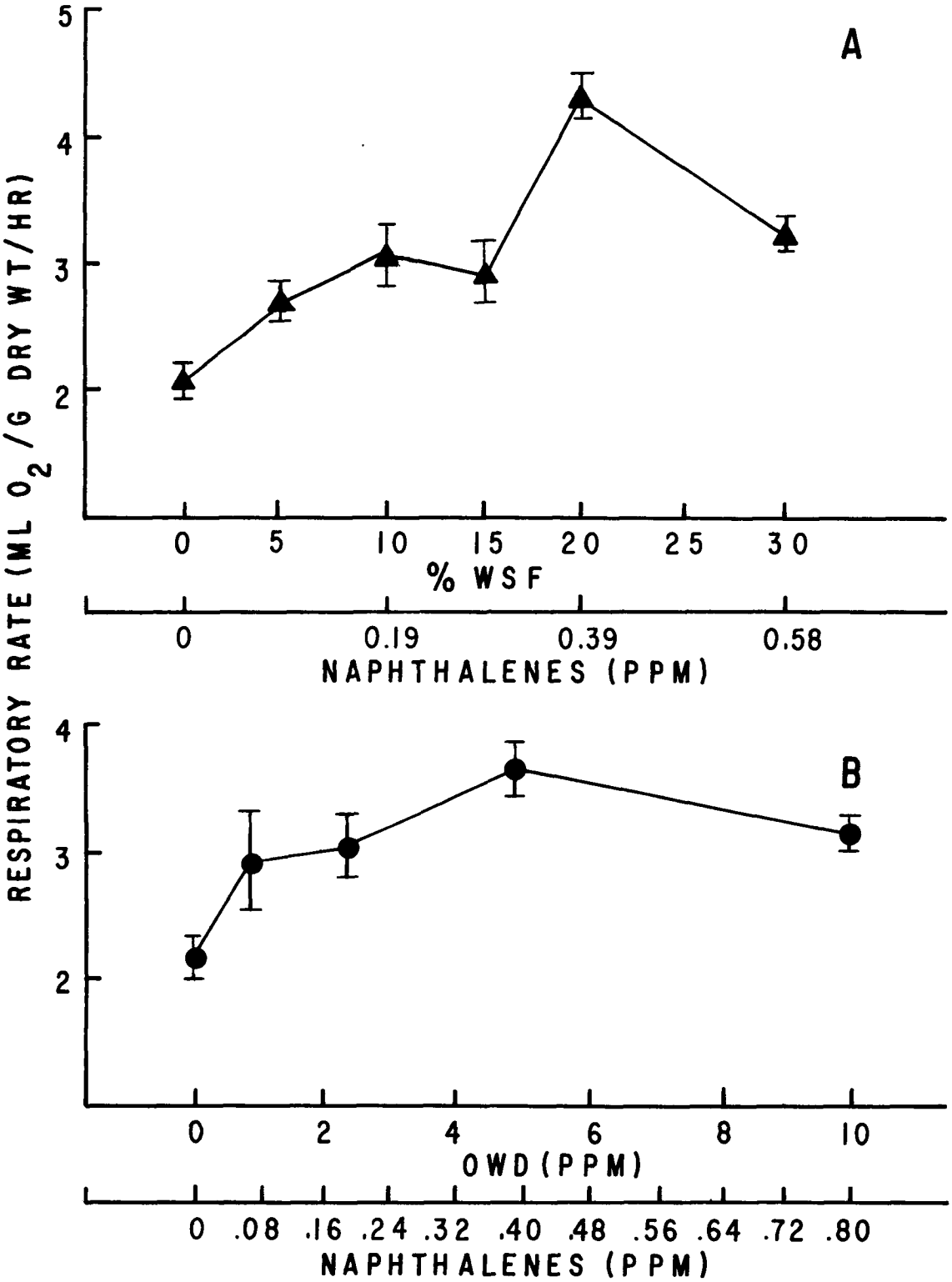

Figure 1. Mysidopsis almyra. The effect of exposure to No. 2 Fuel Oil on the respiratory rate of opossum shrimp Mysidopsis almyra. Total naphthalenes concentrations in the exposure mixtures are given in the abcissa. Vertical bars represent standard errors of the mean.

A. Measured during exposure to the WSFs of No. 2 Fuel Oil (5 animals/container and 5 containers/concentration).

B. Measured in oil-free sea water after a 2 hour exposure to OWDs of No. 2 Fuel Oil (10 animals/container and 3 containers/concentration).

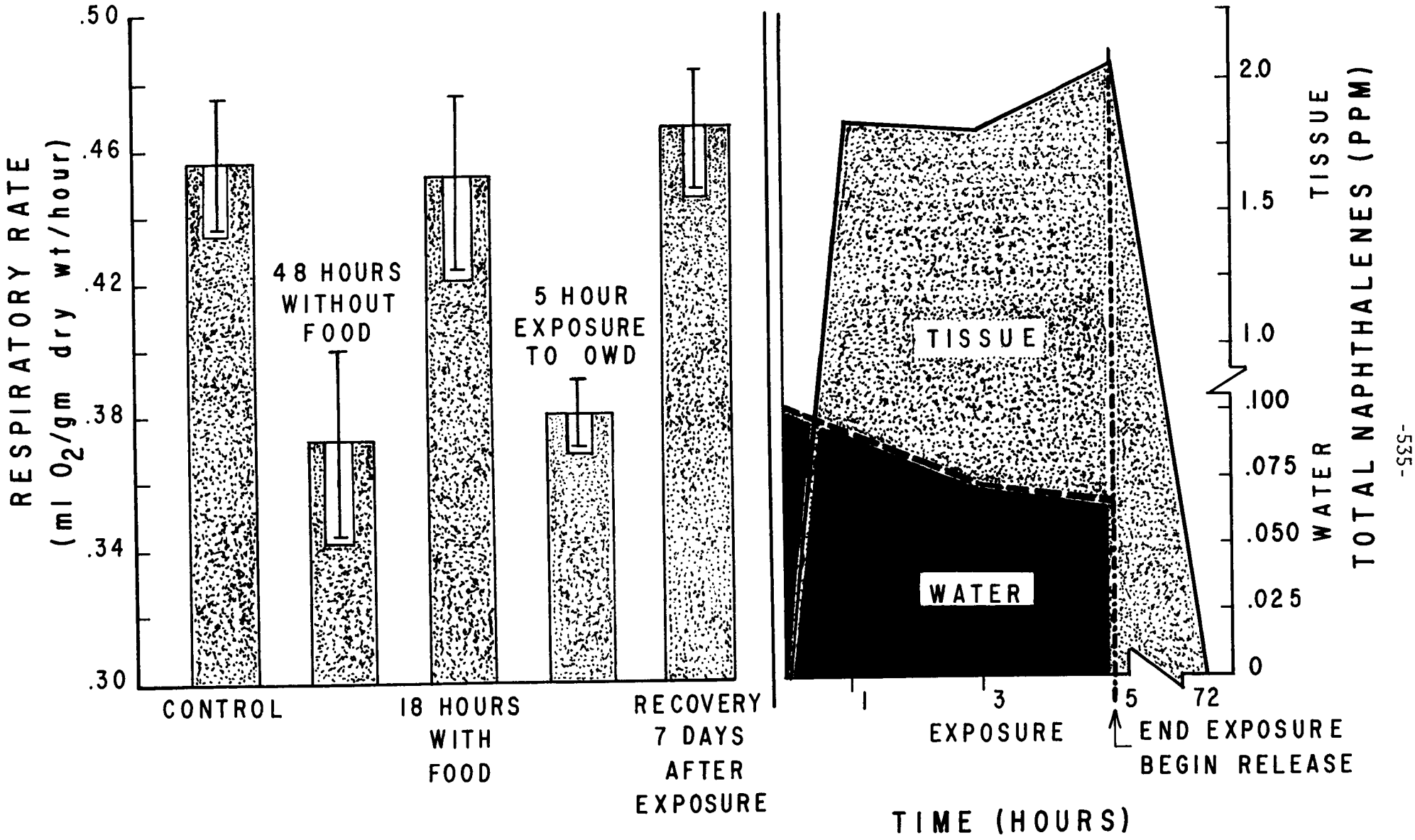

Figure 2. Palaemonetes pugio. Respiratory rates of grass shrimp P. pugio following 48 hours starvation, following 5 hours exposure to an OWD of No. 2 Fuel Oil (3.3 ppm total aqueous hydrocarbons) and after 7 days recovery in oil-free sea water. Concentrations of total naphthalenes in the exposure water and tissues of exposed shrimp are also given. Respiratory rates are mean values plus standard deviations obtained from 8 groups of 12 to 14 individuals each. Two groups of 10 animals each were analyzed for total naphthalenes at each sampling time.

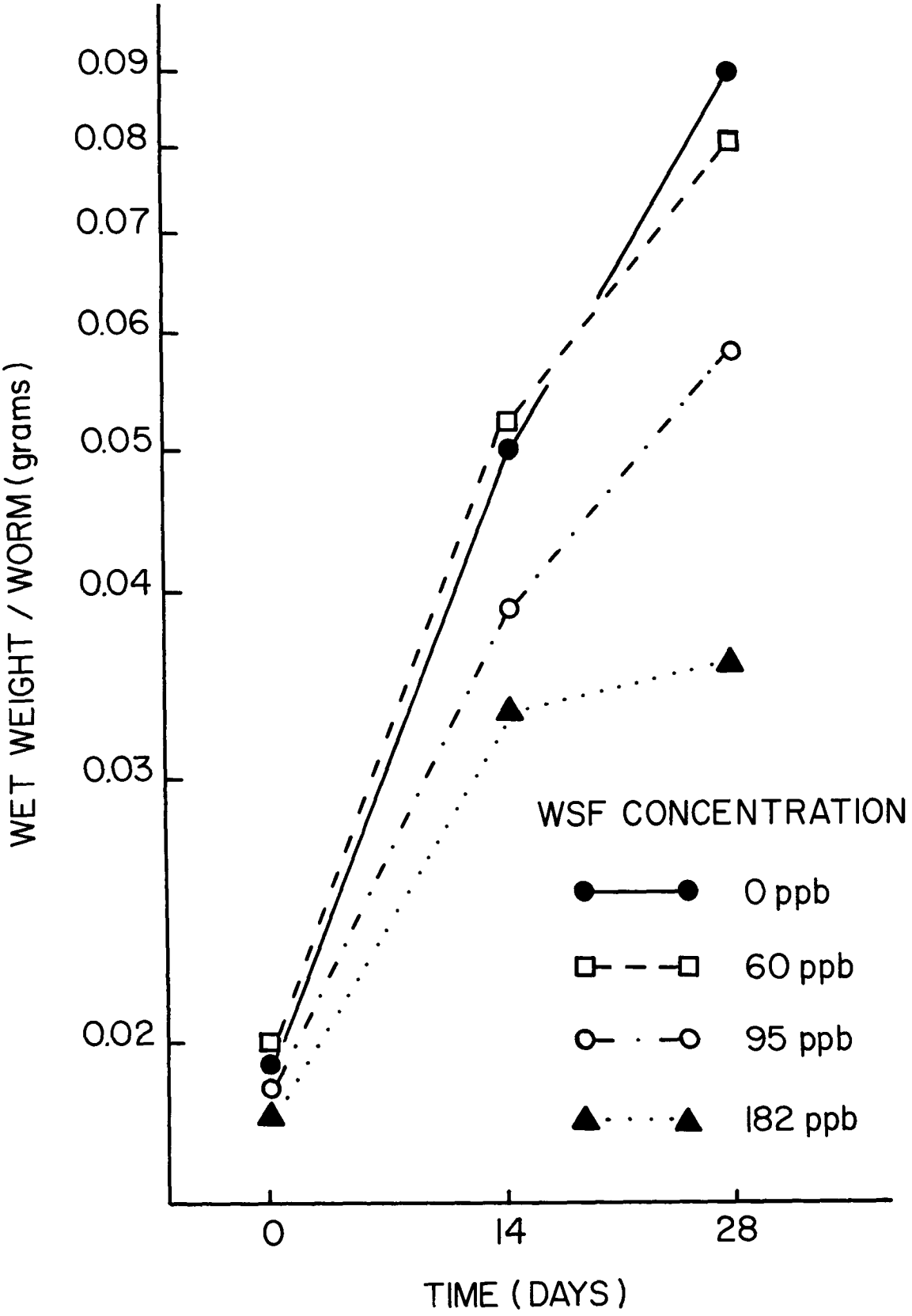

Figure 3. Neanthes arenaceodentata. Growth of juvenile N. arenaceodentata during continuous exposure to WSFs of No. 2 Fuel Oil. Symbols represent mean values for n number of samples ± S.D. (vertical bars). Each sample was composed of 20 animals. Exposure concentrations of 60, 95 and 182 ppb total naphthalenes are equivalent to 3, 5 and 10% WSF respectively.

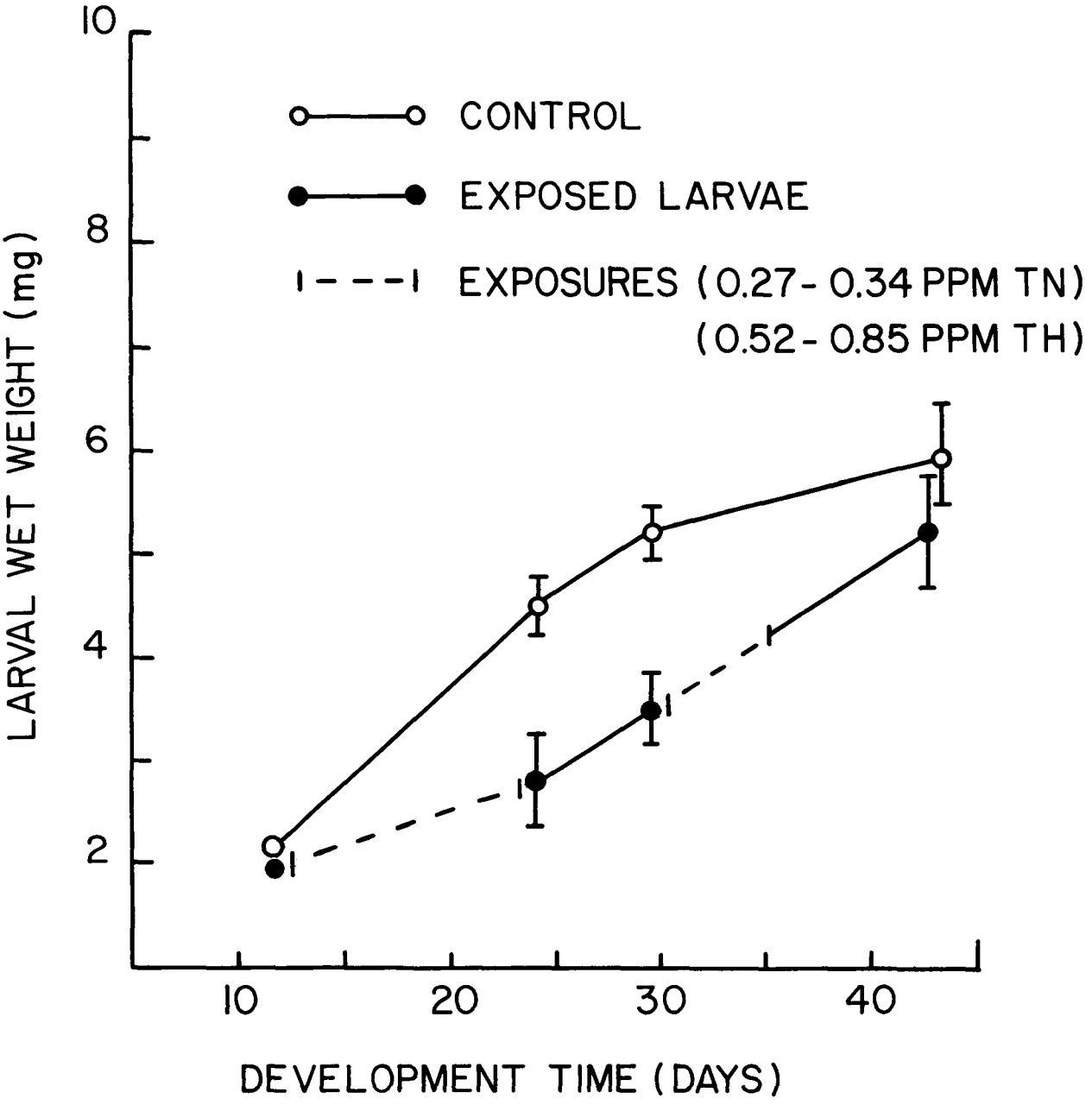

Figure 4. Palaemonetes pugio. Growth of larval grass shrimp P. pugio exposed initially for 12 days to a dilute WSFof No. 2 Fuel Oil, returned to oil-free sea water for 5 days, exposed again for 2 days to the WSF, and then returned for 10 days to oil-free sea water. Exposure concentrations are given in ppm total naphthalenes (TN) and total hydrocarbons (TH). Each data point represents the mean individual weight of 10-12 shrimp. Vertical bars are standard deviations.

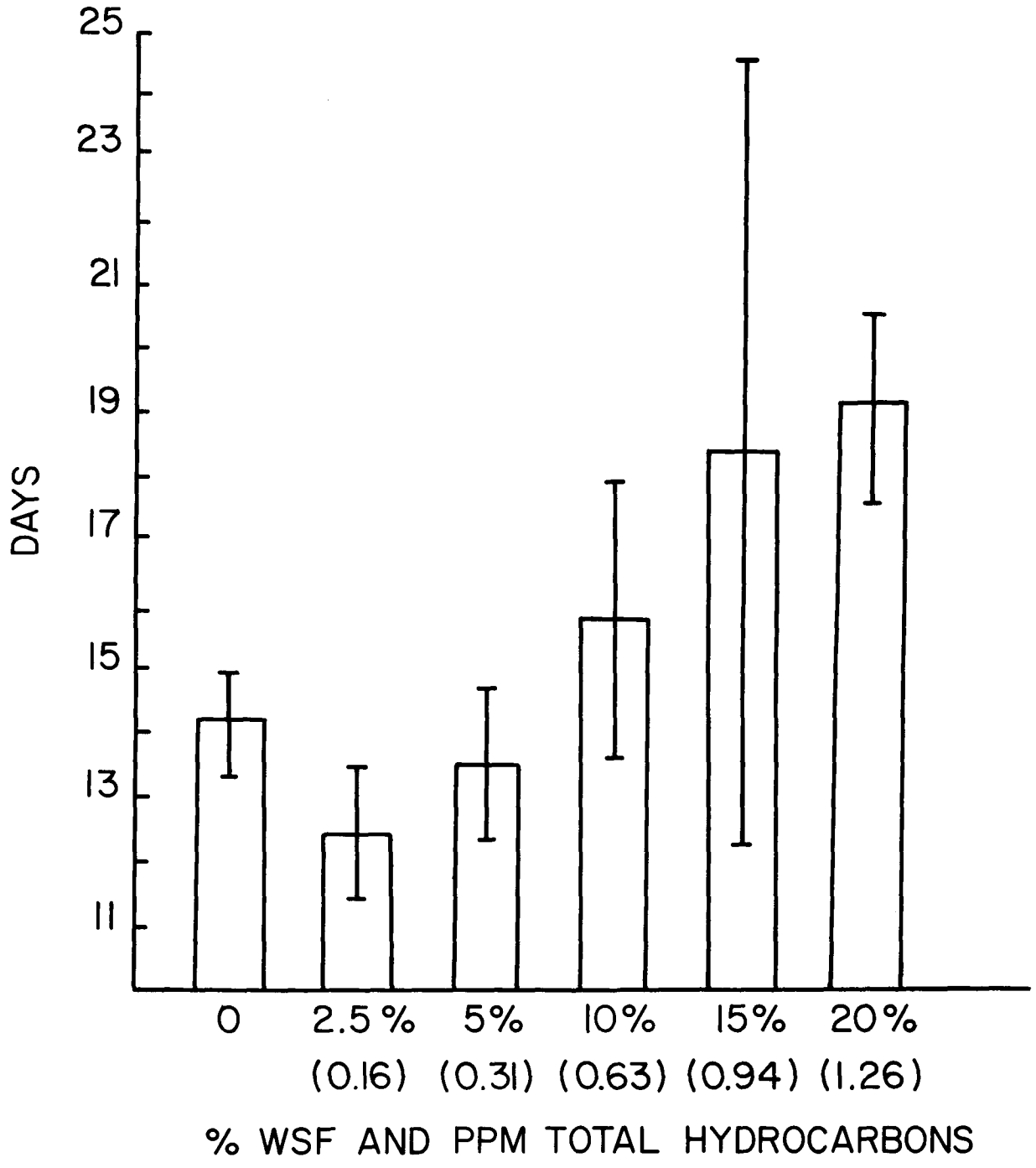

Figure 5. Rhithropanopeus harrisii. The mean (plus standard deviation) duration of larval development of R. harrisii from hatching to the molt to the megalops stage during continuous exposure to WSFs of No. 2 Fuel Oil.

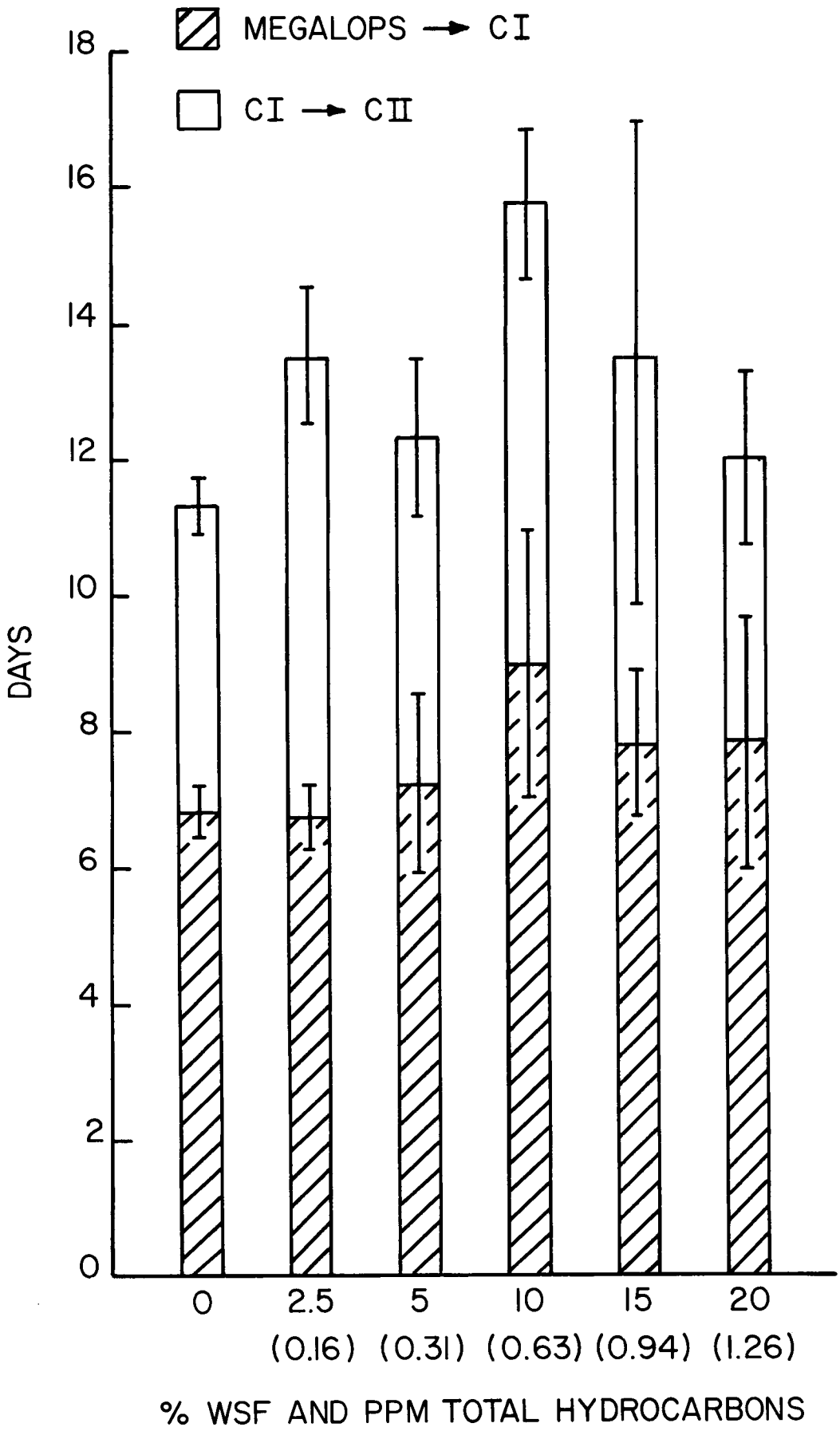

Figure 6. Rhithropanopeus harrisii. The effect of No. 2 Fuel Oil WSFs on the mean duration of the megalops and first crab stage of R. harrisii. Vertical bars represent standard deviations.

SESSION IV

SOCIOLOGICAL IMPLICATIONS OF HYDROCARBON USE

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LONG-TERM SOCIETAL IMPLICATIONS OF HYDROCARBON USE PATTERNS

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To address such a complicated subject briefly we must be modest in our aims. I propose to set forth, first, three facts about which there will be little cause for dissent--then to examine some major uncertainties regarding how those facts should be interpreted. In the process of following through this argument together I hope we might come to a clear understanding of the choices before the nation.

FACT NUMBER ONE: THE ECONOMY RUNS ON LOW ENTROPY

As Georgescu-Roegen[1] has pointed out, the conventional portrayal of the economic process as a self-sustaining, circular flow between "production" and "consumption" (or in more elaborated form in input-output tables) is seriously misleading in one respect. It encourages neglect of the fact that the economy requires inputs from the environment--energy and resources--and spews back into the environment waste heat and waste materials. This important general property of the economy is related to the Second Law of Thermodynamics.

This law, the Entropy Law, states essentially that (a) all kinds of energy are gradually transformed into heat, and heat flows by itself only from a hotter to a cooler region (never the reverse), so that it tends to get more and more dissipated and unavailable to do mechanical work; and (b) matter, too, is subject to an irrevocable dissipation (with one exception, to be mentioned just below). Entropy is a measure that can be thought of as an index of unavailable energy (energetic entropy) or of material dissipation (material entropy). The tendency of order (e.g., the arrangement of materials in a house) to become disorder (e.g., scattered by a tornado) can be counteracted through the directed use of available energy (e.g., by picking up the fragments, transporting them to the original spot, and reassembling them). This is the exception noted above--energetic low entropy can be exchanged for material entropy, thus reversing the natural tendency toward dissipation.

The low entropy required as inputs to the economy is in the forms of high quality energy (mainly from naturally concentrated hydrocarbons and the sun) and concentrated material resources (e.g., ores, hydrocarbon feedstocks). The economy exudes high entropy in the form of waste heat and rubbish. Life processes in the environment restore some of this waste to a low-entropy form (e.g., plants converting carbon dioxide to plant structure); the remainder accumulates. The central point is that the greater the economic activity the greater the amount of low entropy required and the greater the amount of high entropy cast forth into the environment. This is true despite recycling (which uses up low entropy) and "pollution control" which may convert noxious wastes into less undesirable ones, but does not eliminate the waste. As Georgescu-Roegen describes the inexorability of the situation, "'Bigger and better' motorcycles, automobiles, jet planes, refrigerators, etc., necessarily cause not only 'bigger and better' depletion of natural resources but also 'bigger and better' pollution."

Thus it is no accident, but rather a fundamental characteristic of the economy, that material resource use and energy use have risen essentially proportionally with measures of economic activity such as the GNP. All but a few percent of the energy use has been from hydrocarbons, and will continue to be so well toward the end of the century, despite the longer-term promise of nuclear and solar energy. (Since less than ten percent of hydrocarbons are used for chemical feedstocks and other non-energy uses, it will not distort unduly if we focus on energy use from here on.)

It is also fundamental, that whatever is accomplished in the way of making hydrocarbon waste products less noxious, the total amount of waste of one form or another will tend to go up with total economic activity (including the activity of pollution control).

FACT NUMBER TWO: INDUSTRIAL-ERA TRENDS DERIVE FROM A PARTICULAR WORLDVIEW

Among the trends characteristic of industrial society, which have accounted for its benefits and achievements but also lead toward the most basic problems, are the following:

- o Industrialization of production, i.e., subdividing work needed to produce goods and services into elemental increments, and organizing and managing these increments toward the goals of productivity and efficiency;

- o Automation, the further organizing of work so that it can be performed by energy-driven, self-operating machines;

- o Rising influence of science, i.e., the search for materialistic knowledge guided by the principles of objectivity and causality and embodying the prediction and control values of technological exploitation;

- o New concentrations of power, especially economic power in the expanding industrial corporations and associated financial institutions, and intellectual power in the scientific and technological elite;

- o Rising levels of education with strong emphasis on preparation for entering the industrialized economy;

- o Pragmatic values predominating, with the individual free to seek his own self-interest, as he defines it, in the marketplace;

- o Material progress, both as an observable trend and as a declared goal, implying man's expanding control over nature and his unlimited ability to understand the universe from the data provided by his physical senses.

As Mumford[2], White[3], and others[4] have argued, these trends are intimately related to an underlying image of man-in-the-universe, involving materialistic values, scientific principles of objectivity and causality, focus on the outer world (in contrast to the medieval inner-world focus), and an ethic of man dominating the rest of nature. Numerous signs of challenge to this paradigm have been evident in the last decade.

FACT NUMBER THREE: THESE TRENDS HAVE LED TO A "NEW SCARCITY"

The industrial-era trends have brought us to a "new scarcity" of:

- o Fossil fuels and other sources of energy,

- o Mineral and nonmineral resources,

- o Natural fresh water,

- o Arable land and habitable space,

- o Waste-absorbing capacity of the natural environment,

- o Resilience of the planet's life-supporting ecosystems.

Although they are somewhat interdependent and exchangeable, we are simultaneously approaching the planetary limits for all these resources. This is not necessarily to say that shortages in all of them are imminent, but neither are the limits infinitely far away.

The new scarcity differs fundamentally from age-old scarcities of food and shelter. The latter were "solved" in the past through geographical expansion and technological advancement. The new scarcity is more of a consequence of technological and industrial advances.

QUESTIONS AND UNCERTAINTIES

As a consequence of the above developments, concern has been growing over various questions. What are ultimate resolutions to the problems represented in the "new scarcity"? What are wise hydrocarbon use patterns for the future? What are our options in the long-term?

Manifestly there are major uncertainties involved in attempting to arrive at answers to these questions--uncertainties of two types. One type is the technical uncertainties, the kind we are used to resolving through research. The other type is uncertainties about future public attitudes, value commitments, preferred life ways, and interest-group political actions. This second type of uncertainty we typically leave out of our calculations. As a result, time and again in recent years careful forecasts made with the best quantitative data have been confounded by unexpected changes in these "soft" variables. Examples include changes in attitude with respect to:

- o Family size, unexpectedly bringing U.S. population growth below replacement fertility rates in the mid-1970s;
- o Environmental quality, which, reflected in legislation and public actions, delayed large construction projects and hence affected both energy supply anticipations and economic forecasts;
- o Desirability of urban/suburban life, resulting in a net out-migration from urban areas for the first time this century, contrary to demographic forecasts;
- o Science and technology, resulting in major departures from past trends in federal funding of basic research, affecting all post-SST technology forecasts, and bringing an unprecedented insistence by the public to be involved in major scientific decisions;
- o U.S. world actions and responsibilities, resulting in the halting of the Vietnam War and a drastic change in the conditions under which the U.S. might mount any future overseas military action.

Part of the reason that attitudinal uncertainties are so difficult to anticipate in our planning is that the evidences of future attitude shifts are so sensitive to the observer's interpretation.

THREE VIEWS OF OUR THREE FACTS

Thus consider the three "facts" outlined above, each of which represent propositions that all informed persons could agree upon--more or less, and up to a point. When the three are considered together and implications drawn, there are violent disagreements.

The first perception is that which might be inferred from federal energy policy thus far. The entropy argument simply doesn't seem relevant. The situation it describes, where the energy used in the process of getting out resources mounts higher and higher--as does the pile of refuse from the

workings of the economy--is a picture of some far distant time. Meanwhile, we may develop fusion power, or something else, which will push it still further off. For the time being there is lots of coal, and when people realize what the issues really are, they will put up with a little environmental degradation to keep the economy rolling and unemployment down.

The benefits of a high-consumption, high-technology society as compared with any feasible alternative are obvious and generally agreed to. Furthermore, continued high growth (in economic output and energy use) provides the best hope for raising the nation's poor, and the poor of the world, to a higher state of material and social well-being. Thus, without neglecting efforts at energy conservation and environmental protection, we must keep energy supply capabilities up to meet projected energy demands.

A second perception finds the entropy argument an interesting one, but only one of many (and not even the most compelling) pointing to the need for drastic cutbacks on energy demand. Social, environmental, and ecological costs of continued energy use expansion are becoming increasingly intolerable. Expanding use of hydrocarbon feedstocks to provide nonbiodegradable fibers, plastic gimmicks, and detergents have exacerbated the environmental problem. Energy demands--and that means demands on economic output generally--need to be reduced greatly. This can be accomplished through voluntary choice and cultural change, made more equitable by supporting legislation.

Thus we need more understanding of and acting on the virtues of voluntary frugality, "doing more with less"--of the simple life, in community, engaging together in a search for meaning and commitment; pursuing handcrafts and gardening with "appropriate technology"; identifying with nature, fellow wo/man, and future generations.[5] There must be a "fairness revolution" in the world, with the rich nations learning to consume less and the poor nations achieving a more equitable redistribution of the earth's resources.[6] The planet cannot stand the resource binge of the industrialized nations, and it is not clear that humanity can stand it either. This is not turning our backs on the achievements of modern technology--it is redirecting technology to different ends. Ends which are less guided by such economic criteria as profits and return on investment, and more guided by humane values and goals for the individual and society.

A third perception is in contrast to both the first two. It perceives the entropy argument as fundamental and the "new scarcity" as a sign the industrial era is approaching its end. The industrial period, with trends as indicated earlier, is most properly in a historical sense considered a brief (two centuries or so) transition period following the long pre-industrial period during which man's control over his external environment was very limited, and preceding a period in which that environment is very much more a matter of social choice (implemented through technology). The industrial period is not the beginning of endless (cancerous) growth; rather it is a step in the development of man. It could be, in John Platt's happy phrase, "the step to man"[7].

The environmental and resource crisis; the growing sense of careening ahead faster and faster with less and less consensus on where it is worth getting to; the widespread alienation and anomie; the growing challenges to the legitimacy of corporate economic power concentrations and scientific-technological-manipulative intellectual power concentrations--all these are signs of a forthcoming wrenching around of society, a reorganization around a new trans-industrial paradigm. The characteristics of the reorganization are far from clear, but they will emerge out of the nature of the challenging forces.

Perhaps the salient characteristics of this new pattern are represented by a shift in emphasis

FROM	TOWARD
Economic individualism	Reassertion of the brotherhood of man
Isolated, exploitative attitude toward nature	Unitive, stewardship attitude toward nature; ecological ethic taken for granted
Subservience of other values to economic values	Reassertion of transcendent social values and relegation of economic values to a subservient, instrumental role
Discounting the future by economic logic	Direct involvement with the welfare of future generations
The predominant quest for knowledge (science) biased in favor of knowledge leading to technology	A more balanced search for understanding both of the physical universe and of man's spiritual being and his relation to the whole

LEGITIMATION OF ALTERNATIVE REALITIES

It is important to stress that these three perceptions are literally that--three ways of seeing based essentially on the same data. Anthropologist Ruth Benedict[8] introduces the concept of patterns of perception in her Patterns of Culture as her basic analysis tool for understanding and comparing societies. She emphasized the potential utility of this concept in illuminating goals, political conflict, and decision making in modern society.

We introduce it here as an aid to understanding some of the conflict surrounding energy-related issues, and also hopefully as a tool to help improve communication and reduce conflict--to lift the issues to a more fruitful plane of discourse than adversary confrontation. If the various perceptions of the issues surrounding crucial energy decisions can be (a) legitimated, and then (b) explored together in the public dialogue, it may be possible to move toward (c) establishing consensus on which perception, with

the actions that follow from it, is most accordant with a long-term desirable future.

PREFERABILITY OF PERCEPTIONS

At the same time it is necessary to honor different perceptions of our energy situation (since each "fits" the observations of his environment as made by the person holding that view), it is also important to note that these patterns of perceptions are not equal in their consequences. They lead the society to a different future. Thus the choice among them is not arbitrary; in the long run they are not equally serviceable. So it is ultimately extremely important which one society chooses. Yet one of them cannot be proven, in the ordinary sense of the term, to be "right" and another "wrong".

There appear to be at least three tests that can be applied--not to whether a picture of reality is "correct", but to whether it seems to be a wholesome one for a society to hold. These are:

1. Does the view in the long term lead toward societal or system adaptability, and hence toward survivability? There are certain laws of nature and universal properties of systems that a society ignores at its peril. After all, the laws of thermodynamics, the fundamental principles of ecosystem behavior and adaptability, do obtain--regardless of the opinions of men. Some conditions for adaptation, for preservation of options, are ineluctable--they operate regardless of whether or not they are included in a society's picture of reality.

2. Does the view lead toward fruition of the long-term trend of human civilization? Does it tend to move us in the direction of such traditional values and goals as democratic liberation from oppression by institutions, reverence for Nature, the brotherhood of man, and man's further spiritual development?

3. Is the view compatible with whatever can be discovered to be man's most fundamental nature? Among the powerful criticisms of the day is the protest that "Homo economicus" is not man's most fundamental nature.

SOCIAL CHOICES PREVAIL OVER ECONOMIC RATIONALITY

From what we have seen, the nation's choice of future hydrocarbon use patterns involves far more than technical or even economic criteria. The most basic goals of society are at the heart of the issues. And yet it is precisely here that our present society is most confused. Subtly but steadily, economic goals have gradually substituted for social goals and economic rationality has come to prevail over social rationality. We have gotten it backwards. What are properly a means--technology and the economy--have been elevated to the rank of ends. The plurality of values and norms that characterize political rationality have been over-shadowed by the single-valuedness of economic logic.

And so the examination of what may have appeared to be a technical issue--future hydrocarbon use patterns--has led us to several related but more fundamental issues: the need to legitimate and deal with alternative perceptions of reality, the critical nature of our present energy decisions, and the need to reestablish the precedence of social choices over economic ones.

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SESSION V

PANEL DISCUSSION

Chairman
Martha Sager
American University

SESSION V: PANEL DISCUSSION

Martha Sager, Bernardo F. Grossling,
Willis W. Harman, Gilles LaRoche,
Edward W. Mertens, William Sullivan

SAGER: In planning this symposium, the Steering Committee was most interested in devoting the last period of time to two ideas: to crystallize the disparate perspectives, positions, and items, which have been presented overall, and to elicit this cohesion not only from members of the panel, but also from members of the audience.

We are most interested in your participation with us in this discussion. Now let me introduce the panel members, some of whom you have heard from and some who are new to you. On my far right is Dr. Bernardo Grossling, who was our opening speaker. Dr. Grossling is a research geophysicist in the Office of Director, U.S. Geological Survey Research Division. He is a native of Chile, but he has been in the United States as a citizen for 20 years. He is a graduate of Cal Tech and London University. On Monday morning he introduced and presented for us the idea of quantity. That is, how much are we talking about? What quantities are of concern when we speak of spills, sinks, seeps, and naturally occurring hydrocarbons in the aquatic ecosystem of the ocean?

Next, let me introduce William Sullivan from the Department of Commerce, from whom you have not yet heard. As an economist, he will be presenting the economic perspective of cleanups in relation to cost-benefit, cost-effective ratios as these discussions arise within the panel. He is seated beside Willis Harman.

Dr. Harman is at Stanford University in a fascinating department called Engineering Economic Systems. He is also a member of Stanford Research Institute, and for ERDA he has just finished a study called "Solar Energy Assessment for the Next Half-Century."

I have known Willis on the Commerce Technical Advisory Board which we are on together, and I have tremendous respect for his ability to review overall situations and give total discussions of philosophies concerning the effects of all kinds of activities on human beings.

Next is Ed Mertens. Mr. Mertens is a chemist and Senior Research Associate at Chevron Research in Richmond, California. He has been very active in environmental research and is Chairman of the API Committee on Fate and Effects of Oil in the Environment.

Directly beside me is Gilles LaRoche from McGill University--a comparative physiologist and biochemist. Dr. LaRoche spent many days organizing portions of the symposium program and contacting speakers. I would like to thank him for his special efforts which have benefitted all of us.

I am Martha Sager, an ecologist and limnologist by profession and a professor here at The American University. I am also with the Effluent Standards and Water Quality Information Advisory Committee to the Environmental Protection Agency, which is a statutory committee under the Water Act.

I would like to ask you, Mr. Sullivan, if you would like to open this section with some remarks about the economics of oil and aquatic ecosystems.

SULLIVAN: I would like to start by referring to some of the comments Dr. Harman made earlier, as a preface to what I have to say. Discussing the world view underlying society's approach to solutions of environmental problems can be applied to many other types, such as safety and health problems. But economics is very relevant, no matter which world view you are discussing.

You cannot escape costs. Costs must be dealt with, perhaps even more so if you want a frugal lifestyle or, in some sense, want to have an ecologically sound lifestyle.

What I am going to address today is the process we are using to make economic decisions about environmental matters, in particular, shortcomings that exist in the identification of problems and in the development of solutions to these problems. To make a fairly hard statement, all too frequently it seems the policies are based on emotional reactions to unproven hypotheses, which are implemented in panic and enforced in blind ignorance.

The issues that I think can cover many of the shortcomings are how information is presented publicly, how we react to it, and the use or lack of use of benefit-cost analysis. We use public reaction as a motivator, which is proper. The problem arises when we seem to use it as the sole criterion for motivation to take certain actions.

Referring again to Dr. Harman, the underlying world view is important, as demonstrated by public reaction. But just because people prefer something does not necessarily mean that it should be done. Other rationales, I think, must be considered. There are other competing objectives, which must be balanced.

We produce tremendous confusion in our public reaction. Today we say we should do this. Tomorrow we say we should do that. We don't know where we are going. The extent to which the public approaches biased estimates of risk blows things out of proportion. We don't really know; we are not really evaluating what a particular threat means to society in terms of risk: What population is exposed to this risk? How real is it? How large is it relative to other competing problems in the same area?

This approach has many, many costs associated with it, both psychic and real. I won't really discuss psychic costs extensively. By prejudicing our approach to problems, we are incurring costs. The government frequently takes the position that, "Well, we will do this now. If it is wrong, we can modify it later, and possibly the review process will catch the problems. Then, of course, we have the last resort of the courts." We have no idea of what we are expending in terms of dollars, resources, people, and others when we take the viewpoint, "Oh, we can modify it later. We can use the courts."

SAGER: A couple of thoughts: First of all, the identification of the problems. We can go back again to the three gestalt theories that were presented, i.e., that problems are seen differently by different people. So the identification of a problem is a difficult thing to assess.

But when we see an oil spill, everybody says to himself, "Well, that is a problem." No matter whether it is the A, the B, or the C, the problem can be identified as that oil spill, which is, perhaps, one of the reasons that these things do get such great publicity. All three groups can see a problem there.

The extent of the problem is a little different. Yesterday, in the Wall Street Journal, there was an editorial called, "Ecology's Missing Price Tag." It is based on an economic assessment of what the overall costs will be to industry to meet the Clean Air Act Amendments; it has not yet gotten to the Water Amendments.

Some of the statements in that article fit directly here. One stated that there is a wide range of environmental objectives society must choose to pursue, but each one is associated with some real resource costs. Society must decide which environmental controls are worth the expenditure they require and which are not.

All during this seminar, for the last two days, we have been hearing about disparate, fractionated, fascinating, extensive analyses into fragments of the total issue of what is happening with oil spills. Perhaps, what we thought was happening that was so tremendous, horrendous, and holocaustal in terms of destroying the aquatic ecosystems is actually not true. If we could decide that which would be shown by eventual research under the cleanups and so on, then, indeed, we wouldn't have to spend tremendous sums of money on monitoring systems to find the source! Remember we had a presentation on monitoring systems which described four new ones to be put out, satellite surveillance, and so on.

If the ecosystem is going to recover in a given period of time and become productive again, once the accident occurs, maybe we wouldn't have to worry so much about spotting each accident. On the other hand, maybe we would find ways to clean it up more rapidly and have less impact occur at that particular time. I think that is an interesting speculation.

A particular attitude on which I wanted to comment is controversial. I always hate the idea of public this and public that because I don't think there is any difference in the public, the consumer, the government, the legislature, and so on. We are all citizens, and I dislike intensely using "public reaction." It is people reaction because I don't see how we can separate one group from another.

SULLIVAN: The issue is, in fact: To what extent should we allow decisions of science, decisions based on advanced hypotheses, to be based on motivation?

Let me make a few brief comments about the use of cost-benefit analysis. Did you have a reaction?

GROSSLING: I would like to make a comment since we are a panel here. First, to make the record clear on the question of spills, in my first talk I dealt with the broad problem; I think I can show that the overall effect is not important.

The local effect is very important. Our position in the Geological Survey, as one of the agencies that controls or supervises this, is that none should occur. That is really the position, and we are trying to tighten up everything that way.

But when we reflect on the broad problem, we are leaving the question of spill itself and addressing more what Dr. Harman said this morning. He presented a picture of choices of views of the world - A, B, or C - and the nature of the problem was one of the citizens having an attitude towards one or the other or the other (A, B, or C).

I have looked a great deal at the question of energy and economic development on a comparative basis. My feeling is that over the next 50 years, for the Untied States and the developed nations, it isn't a question of choice. There is no choice anymore. I think they are so heavily dependent on petroleum that the question isn't what people would like it to be; it is what is viable.

There has to be a massive transition from one form to another. I don't think we can dictate what we would like the choice itself to be in that short time. In the long run, yes. We have to look at the hard realities of economics. It isn't a question of choice, what we would like it to be, what would be really nice.

Someone will have to decide whether a standard of living from $5,000 per capita will fall to $300 per capita or not, if we suddenly remove the oil input. So the problem of viability really bothers me very much.

MERTENS: I would like to make a few comments about the problem of defining a problem. Earlier in the presentations of the day before yesterday, there was quite a discussion on the inputs and the different sources of oil in the sea. One of the inputs is the release of reclaimed lubricating oil or used lubricating oil that is normally flushed down the drain in many localities and municipalities. Eventually it finds its way into the waterways and gets out into the ocean.

If we remove all the lubricating oil that is discharged in this manner, we will reduce the amount of oil entering the sea by a third. Well, what have we done? Have we really solved the problem? I wonder whether that lubricating oil is actually harming the marine life in any way whatsoever. By the time it gets to the sea it is, first of all, in a very dilute form. Secondly, by its very nature, lubricating oil is a quite innocuous substance. It does not have a very high toxicity. It is rather readily biodegraded since it is mainly paraffinic.

So what are we doing when we take out all the lubricating oil? Are we solving a nonproblem? We could use the amount of money that we are using to take the oil out to solve some problem that is actually real. I am not saying that we don't have a problem, but I am not satisfied that we do yet. This is the sort of thing I think we have to take a look at. What are our problems, really?

SULLIVAN: I have a couple of comments I would like to make about cost-benefit analysis, the use or lack of use of it. Too many policies are being determined without an assessment of benefits and costs. We are just not doing it. The typical objection is that benefits are not very quantifiable and cost estimates vary a lot. But, all things considered, I think that we must perform cost-benefit analysis, not necessarily to find out a cost-benefit ratio, but at least to apply the discipline and look at a problem in terms of what the benefits and the costs are.

SAGER: Let us pick up on the lubricating oil problem. Could you do a cost-beneft?

SULLIVAN: Well, you can do a cost-benefit analysis of any problem that you wish to do. You have to start with a defined problem, of course, and a policy option or control option that you wish to bring forth.

But let me get to the second point, Martha. The second problem, if cost-benefit analysis is used, is that we treat costs and benefits very differently. We don't treat them symmetrically. We say there are many nonquantifiable benefits, which is true. Therefore, we say that the benefits are greater than we can estimate.

Then we turn around with cost and say the only costs that exist are those that can be estimated, that we can measure. We have not been willing to concede that there are also unmeasurable or nonquantifiable costs. These are the costs I was referring to. For example, what are the costs of using public reaction as a policy mechanism?

What cost do we impose upon society when making a wrong decision? There are costs involved in an error of risk estimation. There are many, many items that are not being considered, and they really must be considered. Otherwise we will not get what we could get for the dollars we spend.

SAGER: Well, the EPA presentation by Dr. Schneider here this morning said that ecologists need mathematical data and further information which they don't have. I told him privately I disagree with this. If ecologists followed his logic and they do not have proper data, then how is the economist going to figure out the benefits to the ecosystem in relation to the cost for cleanup if there are no standards on which to base the rationale?

When we are talking about cost-benefit, we are talking about quantification. I happen to be a person who doesn't believe that if I say something is good--take the words "good, better, best"--if I say something is good, let us say we attach the number 85 to it. If I say it is better, it is 95, and if I say it is best, it is 99. It doesn't make it any different whether I say 85, 95, or 99 or if I use the descriptive words "good, better, best."

I am not always sure that I think quantification is the true answer to anything. It is just a different way of describing things. But in cost-benefit analysis we are looking for quantification, aren't we?

SULLIVAN: We are attempting to compare benefits and costs.

SAGER: So how can cost-benefit in an ecological sense be established? I don't want you to tell me how it could be done right now because that would take too long. But you say that it can be done, and obviously it has been done.

SULLIVAN: We can do much more than we are doing now; that is what I am saying. We can go through the discipline, we can look at these, get some feeling for them, so that we can at least make better judgments.

SAGER: Let me refer to a news article which states that the cost of meeting the Clean Air Act standards would be approximately 57 cents per pound of emissions reductions. This means the cost-benefits ratio for the Clean Air Act Amendments for industry would be 33.5:1.

Then, when we get 33.5:1, we have to go back to the National Environmental Policy Act, to which Dr. Galler referred yesterday. We have to say to ourselves: Is a benefit of 1 to the aquatic ecosystem on a scale -- if it is a scale of 100 -- is the benefit of 1 worth the expenditure of time, technological resource, human resource, and everything in terms of protecting the aquatic ecosystem or the natural environment for future generations?

SCHNEIDER: In Schumacher's book, Small is Beautiful, (one of our speakers, in a way, alluded to many of his principles today), he has about a three-page description saying the cost-benefits in natural systems are a ridiculous, inane process to go through. We are trying to quantify nonquantifiable terms. I think that in many ways you cannot go through cost-benefit analysis to do this.

If we went back 50 or 100 years ago and did a cost-benefit on Central Park, you would never have Central Park there. It would be filled with oil refineries or apartments buildings and things of that sort. So the difficulty in attempting to do cost-benefit analysis in natural systems (if you insist on going through that process, which I would rather not see done) would be to go through and try to quantify nonquantifiable aspects.

The Louisiana State Fisheries Board recently came out with a journal, which came across my desk, in which they tried to give the price per pound for various sport fisheries. My deputy director explained to someone recently that he caught two bluefish last year that cost him $4,000 apiece. That came out to be equivalent to so many hundreds of dollars per pound.

So these are things that are very difficult to quantify, and I think that you are playing a very difficult mental game in going through that process. Maybe we should go with our previous speaker (I know this sounds somewhat esoteric) and get some type of transcendental thinking to determine what the values are to society, rather than go through standard economic exercises.

SULLIVAN: Do you feel that we should assess the benefits but also that we should go ahead and take actions that have considerable economic costs?

SCHNEIDER: No. I think that you should look at the benefits as well as the risks in going through this. My friends from BLM know that I am pushing very strongly for good risk analysis.

SULLIVAN: Yes, EPA has been moving very favorably in that direction recently.

SCHNEIDER: We are pushing for that very hard. However, the problem you end up with is a problem that Odom has whenever he tries to look at a nuclear generating station versus the energetics of a marsh system. When you look at the total number of jobs, dollars produced, et cetera, of a large nuclear power plant on a 1,600 acre marsh, the economics are always in favor of the large nuclear generating facility. You will never win.

SULLIVAN: That's if you do not try to assess benefits but you assert that they are always present. The action is always adverse from an economic viewpoint.

SCHNEIDER: The total economics generally end up being that you have so many jobs and so many billion dollars worth of electricity being produced. At the same time you are dropping off a so-called insignificant fraction of salt marshes.

The problem is, as we play one salt marsh against a large nuclear power plant, the salt marsh always loses. But if you were to sum up the totality of loss of salt marshes and try to equate that into the total ecosystem process, you might be able to look at the total impact.

SULLIVAN: I grant that. In fact, it is really what I am saying, that you must assess what the benefits and the costs are. I am not saying that you can necessarily put a dollar value on this or that you necessarily come out with a benefit-cost ratio. I would not advocate that because it is a very uncertain process.

But we are talking about using economic resources for preservation and protection of other types of resources; presumably there is a tradeoff of some type involved. You would want to assess in some way, to the extent that you possibly could.

LANGLOIS: In response to that observation, I would like to suggest that an important question should be asked: Who is to determine costs and/or benefits? If one uses purely economic values (i.e., industrial values) for the calculation of costs associated with environmental cleanup, while relying on a different set of values for estimating the benefits (i.e., the esoteric value of a stable ecosystem), the cost factor may be artificially inflated compared to the benefits. For example, the indirect costs of allowing deterioration to continue are often not even considered. If policy decisions are going to be made on the basis of dollar costs of cleanup versus deterioration, we must somehow confront this problem of cost assessment based on strikingly different sets of values. It seems to me that we are not yet at a place, sociologically or technologically, where we can quantify economic benefits of healthy ecosystems, much less measure cost-effectiveness of pollution abatement.

BATES: I think you are really getting in a bunch of quicksand when you start playing cost-effectiveness, in government operations at least; I don't know about industrial.

Our main mission, supposedly, is saving human life and we have some 40,000 rescues on mag tape. If we know where you are and you are in trouble or something, we pick you up in 28 minutes. The problem we have is that the Office of Management and Budget cannot tell us what a human life is worth.

In fact, we have just come out with a rather good report trying to study what a human life is worth; if anybody is interested in this game of saving humans, I think it is about the best there is. It is a lot different from what FAA and your airline ticket has been saying in the past, which is about $7,500 (and then it got escalated to $80,000).

But, in the cleanup costs, as I said in my first paper, the Coast Guard is pretty pragmatic. We have the national pollution cleanup fund. Congress set up the revolving fund at a $20 million level. We have gone through that. But the whole basis is strictly to use the eyeball to try to get it back the way it was.

Another assumption made is that the spiller cleans it up, and we have certainly found time and time again this is much too slow. All the stuff does get into the marine ecosystem. I think that if there is demonstrated biologic impact, the dialogue needs to start with Congress again because the spiller really cannot move fast enough to contain the spill. In that case, it really gets back to being an almost air delivery operation using the Coast Guard. As I said, if we know you are in trouble, our response time is about 27 minutes.

The original R&D concept concerning petroleum spills was that containment would be accomplished within four hours anywhere in the country with the appropriate equipment. This meant a lot of air delivery--actually by parachute. The spillers are getting smart from the point of view that Congress only holds them for $100 a ton liability. That is about 30 cents a gallon. After, that, they throw it back on the taxpayer.

We have had two cases of independent companies now. One had the largest bird kill yet by a local petroleum company, which occurred down here on the Chesapeake Bay on February 2. But in both cases, as soon as they exceeded their $100 a ton, they just said, "Federal government, it's your baby." The same thing happened in St. Lawrence, which we are still cleaning up at the cost of something like $15 a gallon.

Those are pretty hard numbers. I think this panel should get at the whole problem of speed of response. Which is more important, the concept that the biggest penalty in spilling something should be cleaning it up or essentially not let the spill get as big as it can?

BRUBAKER: We have a split in two government representatives here. Some of the comments I have with respect to the cost-benefit analysis, having some 10 years experience in the government, involve several factors. First, Mr. Merten's concern over problem definition is, I think, basic. Secondly, I think the reality of the circumstances is that some of the legislation that has passed calls for inflationary impact statements. That gets close to cost-benefits. And third, I am particularly intrigued by the representative from the Department of Commerce who wants to gauge the public response in defining his cost terms and place that in perspective.

Only recently funds were ordered to the victims of the flood in Idaho. A dam had broken, and the survivors experienced some of the stress, which amounted to behavioral changes that involved sleeplessness and so forth. The courts ordered damage funds for such damage.

We are all in a gray area, once we get beyond the data base, when we are asked to make judgments here. It would be interesting to have a legal representative to see how, in fact, they are going to construct the limits and definition of ecological damage functions as well as health damage functions. The Congress so far has, in my opinion, been trying to lay the groundwork for exposure assessment, ecosystem vis-a-vis man.

My impression so far is that other difficulties with hydrocarbons in the environment constitute a bigger problem than oil spills. These amount to immediate, acute reactions, some of which are quantifiable in terms of biological effects. We are rightly concerned about residual long-term effects, not as readily quantifiable, and we may be going too hard, too fast in the cost-benefit approach.

LAROCHE: Dr. Sager, I would like to remind us that the keynote speaker actually made it fairly clear that it is not total volume of oil spill that really matters but rather the biological costs of the spills. We know fairly well what an oil spill does to real estate and to the outside of birds and boats or ships. But we really have very little knowledge as to what it costs in terms of biological systems, in terms of an adequate crop for survival.

This is an aspect that should be considered. I really don't think that there can be any kind of sensible cost analysis data base on something where the standards are still eluding us. I think that it would be rather vital that we look at the discussion along this angle, for a moment anyway.

GROSSLING: There is a question of semantics here. The objection to cost-benefit analysis depends on who you are. If a person is an economist, that is very proper and the right way to do it. If a person is an eologist, he will think that you cannot put a value on a marsh, on a dead fish, and so forth. So he would not like to have a cost-benefit analysis.

There is no escape from having a rational decision. You have to reduce everything to some scale. Otherwise, you see, you will be irrational. You will be doing things unilaterally. That is economics in a broad, classical sense, which says that economics is a science of choices, interactions, and decisions.

Whether to be more or less conventional in the use of dollars is immaterial. You have to develop a framework of decision in which you can really put a marsh against economic benefit. I still hold that economics, in the broad, classical sense, is not the limited view of an industrialist, that he doesn't see spilloff of side effects. So I think it is necessary that we evolve a rationale of decision.

In the United States I don't think there is any choice from the statutory point of view because the National Environmental Policy Act (NEPA) requires the environmental impact statements to provide that sort of balance. The challenge is really to academicians and/or theoreticians to develop the explicit rationale of decision in which the marsh has its proper value.

I call that economics, but in a broad, classical sense. Unfortunately, both limited views deserve criticism: the ecologist who thinks that the marsh is beyond measurable value and the industrialist who thinks only in terms of his profit - and - loss statement. There is a broader economics that encompasses the whole thing.

I don't think we have a choice, that what we have to do -- the proper word, of course -- is cost-benefit analysis. It is done in social development projects, for instance. You balance, in this case, the environment against economics. In social development projects, the world bank has to balance economic development against political and social benefits.

So you really do have to balance things that are very opposite; it does require a common framework of reference, and unfortunately the word "dollar" has sort of an unethical value. I don't think it has. You could use anything you wanted to, but one common framework is necessary.

LAROCHE: I really don't think that you can use cost-benefit analysis unless you have all the facts in front of you. Right now we are dwelling on a series of hypotheses, a series of anomalous figures based almost exclusively on something that is referred to in the biological business as 96-hour TL50. Now, if you were to do economics and not be allowed to use the figures 8, 9, and 3, for instance, what kind of economic factor or numbers would you come up with?

GROSSLING: Well, you see, this is again a question of semantics. You have to go, for instance, with a type of rationale of decision, the utility function, in which you really have decision makers and people who make choices to weigh the thing.

But you really require a quantitative factor of decision. Otherwise, you will say my values are immeasurable and you have to act on that. Then if every person decides, if every group takes that position, they will split apart as a society, as a work community. We have to make decisions jointly. On that, we more or less vote, we do something to some scale.

I don't think ecologists and environmentalists can take the position that their values are immeasurable. It is up to them to develop a persuasive measure that is really a proper counterpart to the economic benefit, which is easy to measure. You cannot escape from that; you cannot say I am beyond measure.

LAROCHE: I think we are back to your comment that this is semantic. I didn't say that we can put all the money in the world on something like this. What I am saying is that we do not have the value or the baseline or the standards to apply. Therefore, how can you make any kind of system without knowing this?

SULLIVAN: I think I can speak to that. One, we would like to try. The first step we need to take is to determine what the benefits are. What are these benefits that are continually asserted to exist?

SAGER: I think we are making those decisions as a nation; in 1970 we passed the National Environmental Policy Act. For the first time, then, as a nation we are beginning to put a value on the natural environment.

We really had not put a value on the natural environment prior to that time. We had technological gross national product values. I am talking about us as a whole, not disparate groups of small bird lovers and things. But as a nation now we do have an environmental policy. This means we are making the first step toward evaluating, if you like to use the word "cost-benefit," we are beginning to evaluate not the benefits but the values of our natural resources, all of them.

We are beginning to do that because we now have a national law. That national law made our Federal Government responsible for the protection of the natural environment, not only for now but for future generations.

What we are doing and what we did at this whole conference was to take one section of the natural environment--the aquatic ecosystems, mainly the marine aquatic ecosystems--and we said, "What happens to oil, or hydrocarbons, I should say, in the marine environment after a spill? What happens to it?"

We now have bits and pieces of biochemistry and intermediary metabolic activities and all different kinds of patches here, intense, descriptive, really beautiful academic evaluations of what happens to these little bits and pieces. This is a very big step forward in identifying what is the value of the marine ecosystem in relation to the rest of the natural environment, which includes ourselves.

So I think that, nationally, we are moving forward towards an evaluation, an economic cost-benefit analysis, if you like, of what is the value of the marine ecosystem in relation to the total natural environment and supportive systems, which then support man and his endeavors. We are on the track, and I am agreeing with Bernardo here on the idea that you must have something when you are talking about costs, but it doesn't have to be dollars cost.

When we first started with effluent discharges under Public Law 92-500, I was trying to say cost-effective reductions, rather than cost-benefit reductions. I thought if we could tell if we spend $5.00 we could judge how effective it was in helping the ecosystem at the end of that point source. I thought effective might be a better word to use than benefit, but that is solely semantic, also.

I don't think that we are in a negative phase of our evaluations. This conference has shown a very positive way in which the academic and professional scientific community is at least beginning to point out, quantify, and identify some of the ways in which we can measure the value of an aquatic ecosystem.

LAROCHE: We are, in other words, in the process of identifying the problem. At this point I think it would be rather premature to have an economic tag on this and not have a reliable or definitive economic policy before the problem has been identified.

BRUBAKER: I would like to go back to Mr. Merten's concern about problem definition here in terms of the last three days. What is the panel's judgment of hydrocarbons in the environment? With particular reference to petroleum spills, how much do we know? What have we learned? There should be some kind of summary statement; maybe we should have done this before we got into cost-benefit.

LAROCHE: The only thing I can speak for, naturally, is the biological aspects. I cannot give a value for the real estate problem or the private property. Concerning the ecological damage, we are in the process of defining some realistic standards. This is the positive aspect. We are in the process of identifying the problem.

The main problem at this point is to identify the toxic products of these oils from an ecological point of view. I am not talking about anything else. Some of these products are beginning to be understood; their interaction with each other or with various conditions are beginning to be understood on a toxicological basis.

But this again remains to be done and completed. We have witnessed through the speakers who have been on the podium here that there are hundreds of thousands of products within the crude oils. Certainly not all of them are as toxic to various species. A few of them most likely are.

But this remains to be established.

SAGER: For my own education at this conference, I was pleased to have the presentations about the secondary metabolic intermediary products and the utilization of those by the various organ systems that were presented. I was impressed by the fact that so many of the components of hydrocarbons were taken into various organ systems, broken down, and utilized either as energy sources or for the production of other intermediate metabolic products. That use in the organ system has yet to be identified, but its toxicity to the organ system was clearly presented as not posing a problem.

KREBS: I would like to comment on Dr. Sager's remarks that organisms have been shown to metabolize oil hydrocarbons and even use them as a source of nutrition, I have the following comments:

Dr. Sager's suggestion that organisms have been shown to metabolize oil hydrocarbons and even use them as a source of nutrition is very misleading; it even suggests a beneficial aspect of oil inputs. In perspective, only bacteria and some fungi have been shown to utilize oil as a source of nutrition. All other organisms in which intermediary metabolism of oil has been documented do so in an attempt to detoxify these hydrocarbons, generally by changing the compounds into excretable form (see Lee's paper). Often these intermediate products of oil hydrocarbon metabolism are in themselves still toxic to a great number of other organisms, though perhaps more accessible to microbial attack and degradation. Only a few groups of organisms possess the physiological ability to metabolize oil hydrocarbons to even a limited extent, and many other important groups, for instance decapod crustaceans, have been shown to possess such limited powers of oil metabolism as to be of little value in their survival.

HAY: Dr. LaRoche mentioned that our keynote speaker said the total cost we have to pay for these oil spills was the real thing we have to evaluate.

LAROCHE: Pardon me, the biological costs.

HAY: The biological costs, that is right. Then our next speaker, Dr. Grossling, came along and said (and I would like him to amplify a little bit on this) that we have already had some mammoth oil perturbations to the environment historically, natural as well as man made.

Some of these major castastrophes, historically, ought to be studied now that they are over with to determine the bottom line, the net cost we had to pay from a biological standpoint. I would like him to elaborate. Is the Geological Survey going to do this, or who should be doing it?

LAROCHE: Actually, I am speaking strictly for the biological systems in this case, you are studying something after it has happened. For instance, you have a spill at a place that hasn't been studied before. I suppose you could gather some information as to how the thing is deteriorating after the spill, but you really don't know what it was before. I am not suggesting that it should be brought back to what it was before, but you have no idea what really is going on in there. A fair amount of the studies that will have to be done will be to understand what happens in smaller systems, in medium-size systems, and eventually in the environment.

In many instances, we are trying to jump too fast. Tell me what we should do. You can pick up the tar, for instance, but you may not be able to pick up other material. I am putting this with a big question mark. I don't know that there will be any substance remaining that is toxic and will destroy the environment beyond the mere smearing of heavy oil, for instance. We don't know this element at the moment. Does that answer your question?

HAY: But you always have controlled areas in marine spills. You always have an area that is very much one that has just had an impact, so you do have a natural control area.

LAROCHE: I don't know if you are a biologist or anything like that, but there is, to begin with, no such thing as standard sea water. I can ask anyone here who is an oceanographer, chemical or otherwise, there is no such thing as standard sea water.

When you speak of control, you are speaking about one area that is intact, let us say. You are speaking of an identical area, experimentally now, an identical area where there has been a damage, an introduction of one element, one variable not 20. That is the only way that we can deal with the problem.

Eventually, as we get to understand the variables, we can study them in twos, threes, and more, but you have to proceed systematically in order to be able to understand. Otherwise, it may take you an infinitely long time to resolve the problems at hand.

BARBER: I would like to respond to Keith's point here about always having control areas in the marine environment. If we turn to the estuaries, I don't know where we would find controls. We have virtually no estuary, unless you can find a few virgin ones in Alaska, that is still relatively a natural unstressed system. Almost everything has been pushed somewhere, either by timber cutting, change in flow, contamination, pollution, dredging, change in salinity. It is pretty hard to find a system that you can be sure is really a valid system without those unbalancing factors.

One thing that has struck me about all these discussions about hydrocarbons in the marine environment is that we are, I think, overlooking the fact that our use of petroleum has doubled in something like the last 15 years. Almost all of our use of manufactured petroleum products and their derivatives has come along in the last 100 years.

So we are looking at these spills and trying to assess what their impacts are, but almost invariably, particularly in the estuaries, it is a spill on top of some sort of hydrocarbon exposure which has been going on and growing. Therefore, finding this baseline to measure against is very difficult. In looking at these total systems, how they hold together, and how we are going to make them work, we must ascertain what is going to be the effect of the chronic exposure to hydrocarbons at the rate that they are now exposed.

We have only reached these higher levels, in most cases, in recent times, certainly in the last 50 years. What is going to be the result of 50 years' exposure at today's level?

GROSSLING: I would like to answer that question. Something was referred to me and I would also like to make a comment on that. First, are we going to make available the information of what has happened in the past? As I mentioned before, the '68-'72 accidents that have occurred worldwide in the petroleum industry will be published by Diane Nielsen of the Geological Survey. Then you will know exactly when it happened and how it happened, as documented in the public record. You will have a whole account of accidents which have occurred. Beyond that period and in retrospect, there have been many, many incidents of blowouts leading to oil pollution in the United States alone, mostly in California. Judging by the sample that we have, my feeling is that there have been about 3,000 since the 1900's.

Throughout the world, I would say there have been 5,000 to 10,000. There are massive ones, million barrels and so forth. As of now, the volume of petroleum is much greater than before, it is true. But the incidence of blowout is very, very small in comparison to what it used to be. Through the forties, as I mentioned, 1 percent of the wells had a blowout.

Now it is a fraction per thousands. It is very, very small. It has come down by about a factor of 100. So despite the fact that we produce so much more oil, the total impact per year is less. Beyond that, if we look at the geological record (and it is very easy to make a projection from that), we have 30,000 oil fields discovered in the United States. There are probably 60,000 total discovered and undiscovered. In the world itself there are probably half-a-million oil fields that exist in the earth from the beginning, except that some were destroyed oilgenic cycles. If you looked at the average rate, an oil field is exposed on the average of 100 to 1,000 years; it is broken up to the surface and exposed, and the oil is destroyed.

How many hundred million to five-hundred million barrels? If it happens to be a billion barrels, then you have a rate of input into aquatic environment which is 10 or 100 times what it is now. This has been going on for 300 million years. That record has to be looked at, and it isn't an experiment which we started in 1875, '57 whatever it was, the Drake well. This was started long, long before, and it has to be looked at. It can be documented.

It is a foolproof argument because of your oilgenic cycle--sediments are piled, organic matter is formed, and they come up and are destroyed. It is inescapable, they come up and are destroyed. The seeps that we hear are part of that game. For the broad picture, you can make a balance sheet, and the balance sheet is that the average input of oil on the broad marine environment must have been at least on the order of now or more.

We are not really increasing the level which can be demonstrated always by the geologic record. The local impact is different.

BRUBAKER: In that context, you say that there are a million years worth of oil--for lack of a better word, "contamination"--of the marine environment. Did you say that?

GROSSLING: But of course. You see, the oilgenic cycle, the people who are not geologists know what it is, involves the pressure of the earth's crust, 10,000 5,000 feet of sediments are deposited and are pressed, oil fields are produced, and then this area is destroyed. They come up and the ocean destroys them. This has been going on for 300 or 100 million years. There are oilgenic cycles which are 300, 400 million years old, and the oil field would destroy it.

BRUBAKER: I asked that because it seems to me, over the time frame and from a biological point of view, there has certainly been (1) survival in many of the species and (2) adaptation, which relates to some of the concerns that Dr. Schneider mentioned in Darwinism and functional evolution.

LANGLOIS: I am a bit concerned about the disparity between the research reports presented earlier at the conference and the panel comments. It seems very misleading to suggest that research findings have shown that marine ecosystems can easily and quickly undergo ecological succession in response to major perturbations, or that they have repeatedly done so. To suggest this negates the ecosystem complexity implicit in concepts of succession; ignores the wide variability shown by different marine ecosystems; and represents, in my opinion, a misinterpretation of the experimental evidence.

Specifically, the impact of petroleum hydrocarbons today is markedly greater than in past geological times, especially in given segments of the environment, e.g., estuaries. Furthermore, the type of hydrocarbons released into marine environments may be quite different from those in natural seeps. Secondly, the impact of hydrocarbon loading must take into account the totality of hydrocarbons impinging on a given marine ecosystem, not just those associated with an isolated instance or a single effluent. Lastly, decisions about the impact of petroleum on marine ecosystems must consider the fact that as petroleum use rises, and as transport of oil from one location to another becomes more extensive, the resultant, and collective, impact of these contacts will probably enhance the levels of deterioration and will certainly reduce the time frame allowed for ecosystem recovery. In those areas where chronic, long-term, exposure exists, there may be simply not enough time for any recovery mechanisms to operate.

BRUBAKER. I am a little surprised that we don't have a better handle on conductance of the biology here. If it is true that we cannot find standard sea water, I think many of the professional biologists here are perfectly capable of conducting good quality research. The biology here is one in which, if we are examining survival and health of species of concern, we can do that in their indigenous environment in the sample to be taken from that.

The point I wanted to make was that, if we cannot define a standard sea water, what other parameters need we define for standardization and integration of this emerging data base, which we are generating here for the cost-benefit analysis?

LAROCHE: I said that we cannot find a standard sea water, but I didn't say that we couldn't carry on an experimental setup. You can use the same water and assume the background, oil or whatever you have there, as background. Then add one component or change one variable at a time.

I think we have to live in the world today. I am firmly convinced of this. We cannot go back 300,000 years and pretend that we are living there. We are not.

KREBS: I would like to speak to Dr. Grossling's remarks suggesting that 300 million years of natural seeps have provided us with a useful model upon which to predict the impact of oil hydrocarbons on the marine environment today and in the future.

First of all, there is reason to believe this phenomenon of natural coastal seepage has not been a continuous release at relatively constant rates through geologic time. Rather, from Dr. Koons talk, it is obvious that natural marine seeps are largely associated with seismic activity in coastal areas. The amount of coastline in the world has undergone rapid expansion over only the last 100 million years due to Continental Drift, with a concomitant increase in seismic activity. Erosion as a mechanism of release of oil from geologic formations is primarily a terrestrial source. The fate and impact of oil in terrestrial environments probably is drastically different from that occurring in the marine environment due to their extreme differences.

Lastly, the nonseep marine inputs today are not qualitatively similar to past inputs of wholly crude oils. Much of today's inputs are refined oils with generally greater toxicities than past or present crudes from natural seeps (see Neff's paper). The scale of inputs is increasing and is projected to increase at logarithmic rates through the end of this century. Thus, these inputs will be neither qualitatively nor quantitatively similar to historic inputs. Quantitatively, the nonseep inputs will be far more ubiquitous in their distribution and orders of magnitude greater than any the marine environment has experienced historically. These will also be nonrandom, often occurring in naturally highly productive, but generally already chronically pollution-stressed, environments, primarily estuaries and wetlands. It is these chronic additive effects that have yet to be assessed and for which we need a predictive model.

SAGER: One of the interesting things, to close the discussion, is that the sciences have only recently, in the last 50 years, been able to utilize microdissection techniques, physical, chemical, and biological. That is why we are at this threshold of being able to identify particular intermediary metabolic byproducts and products within the organ system because we have to keep pace with electronics and so on.

I know a poem by Archibald MacLeish which I think is very fitting with which to close this session. I will not read the whole poem, but as our discussions have progressed here today, I was really struck by the relationship. This is taken from the "Epistle to be Left in the Earth" by Archibald MacLeish*, which he wrote when the first world's fair was held in Chicago. I am going to quote some lines from it:

Each man believes in his heart he will die
Many have written last thoughts and last letters
None know if our deaths are now or forever
None know if this wandering earth will be found

We lie down and the snow covers our garments
I pray you
you (if any open this writing)
Make in your mouths the words that were our names
I will tell you all we have learned
I will tell you everything
The earth is round
there are springs under the orchards
The loam cuts with a blunt knife
beware of
Elms in thunder
the lights in the sky are stars
We think they do not see
we think also
The trees do not know nor the leaves of the grasses
hear us
The birds too are ignorant
Do not listen
Do not stand at dark in the open windows
We before you have heard this
they are voices
They are not words at all but the wind rising
Also none among us has seen God
(...We have thought often
The flaws of sun in the late and driving weather
Pointed to one tree but it was not so)
As for the nights I warn you the nights are dangerous
The wind changes at night and the dreams come

* Modern American Poetry 1942. Harcourt Brace and Co., Inc., Chicago, Ill.

SAGER: I will turn the meeting to Fred Weiss.

WEISS: I cannot do any better than that. I did write out some closing remarks, but I am not going to use them. All I want to do is thank the speakers, the audience, our panel, Martha for doing such a beautiful job, and Gilles for his work. Don Beem and Pat Russell deserve special thanks for all the hard work they have done in getting us organized. And if Sid Galler were here, I would thank him for turning the hurricane away from us because I am sure the National Oceanographic and Atmospheric Administration must be doing something like that.

Now the meeting is formally adjourned and thanks to all for coming.

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