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# Hydrocarbon pollution of edible shellfish by an oil spill*

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## Abstract

A spill of 650,000 to 700,000 l of No. 2 fuel oil has contaminated the coastal areas of Buzzards Bay, Massachusetts (USA). Gas chromatography demonstrates the presence of this oil in the sediments of the affected area. Two months after the accident, essentially unchanged oil is still being released from the sediments. The presence of the same pollutant is demonstrated in whole oysters *Crassostrea virginica* and in the adductor muscle of the scallop *Aequipecten irradians*. A presumably biochemical modification leads to a gradual depletion of the straight chain and, to a lesser extent, of branched chain hydrocarbons. This does not result in detoxification, as the more toxic aromatic hydrocarbons are retained in the organisms several months after the accident. Scallops from an uncontaminated area contain hydrocarbons in lesser amounts and of very different molecular weight and type distribution; they are accountable entirely from biological sources.

## Introduction

Hydrocarbons are universal components of the marine environment. Synthesis by marine organisms results in a surprising variety of hydrocarbons (Blumer et al., 1963, 1964; Blumer and Thomas, 1965a, b; Blumer, 1967; Blumer et al., 1969) that spread through the environment because of their relatively great stability in the food chain and their resistance to degradation in seawater and in marine sediments (Blumer and Snyder, 1965; Blumer, 1969a). Hydrocarbons from pollution by fossil fuels and oil products are being found in increasing amounts; it has been estimated that at least 1 million metric tons of oil and oil products are released annually into the ocean, mostly in shipping lanes and in biologically productive coastal regions (Blumer, 1969a, b, c). Hydrocarbons in fossil fuels and pollution differ from the natural, biogenic hydrocarbons in organisms. They are more toxic, mostly because of the higher content in aromatic hydrocarbons, and are less readily degraded by bacteria.

The persistence of oil slicks on the high seas and the stability of biogenic hydrocarbons in the food chain have suggested that pollutants might remain in the ocean for long time periods. A possible incorporation of dispersed fossil fuels into the food chain might eventually contaminate food products derived from the sea.

An opportunity to test these assumptions came with a recent oil spill near Woods Hole, Massachusetts. On September 16, 1969, the barge "Florida", transporting 14,000 barrels (2,220,000 l) of No. 2 diesel fuel oil[1] (aromatic hydrocarbon content 41%) came ashore off Fasset's Point, West Falmouth, in Buzzards Bay, and released an estimated 650,000 to 700,000 l fuel oil along the shores of West and North Falmouth, Massachusetts (Hampson and Sanders, 1969). A strong SW gale carried much of the oil towards West Falmouth Harbor and Wild Harbor. Both Harbors were closed off by floating booms. After 2 to 3 days, the wind shifted to NE and the remaining oil slick moved out of the Buzzards Bay Area. Detergents were applied in limited areas for a short time. After the spill, a drastic kill of fish, worms, crustaceans and molluscs was noticed almost immediately, before detergents had been applied. It extended into the inshore areas upstream from the floating booms. Oil was incorporated into the sediments to at least 10 m of water depth, probably because of the intense mixing of oil and water by the gale force winds. Studies were initiated by biologists at this Institution and at the Marine Biological Laboratory, Woods Hole, to assess the immediate damage. Investigations will be extended over at least 1 year in order to study the effect of the spill on the faunas and to determine the fate of the oil in the organisms and sediments.

Some of the productive shellfish beds upstream from the affected areas had remained viable; however, they were closed to the taking of oysters and scallops because of possible contamination. On September 25, 1969, taste tests suggested that shellfish could be taken safely and the areas were reopened. However, commercial exploitation yielded scallops with objectionable "oily" taste; this led again to a closing of the shellfish areas on September 27, 1969. At that time a joint effort

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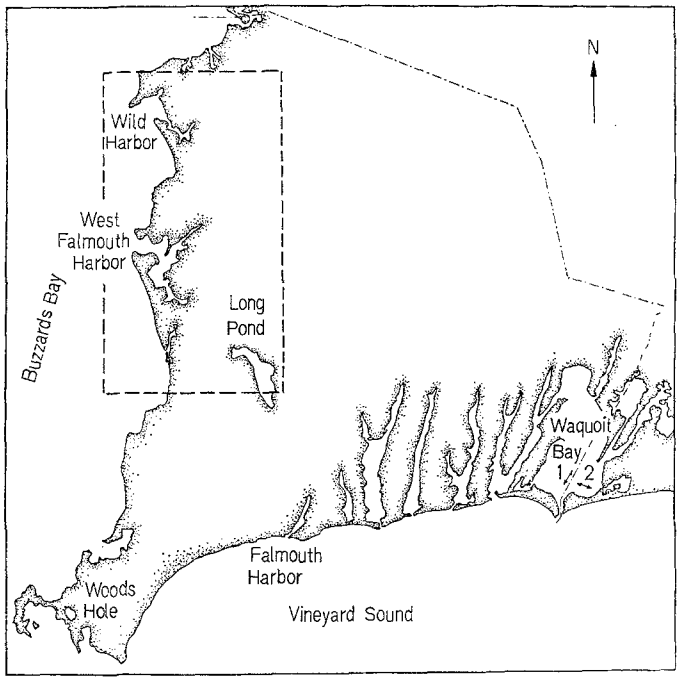


Fig. 1

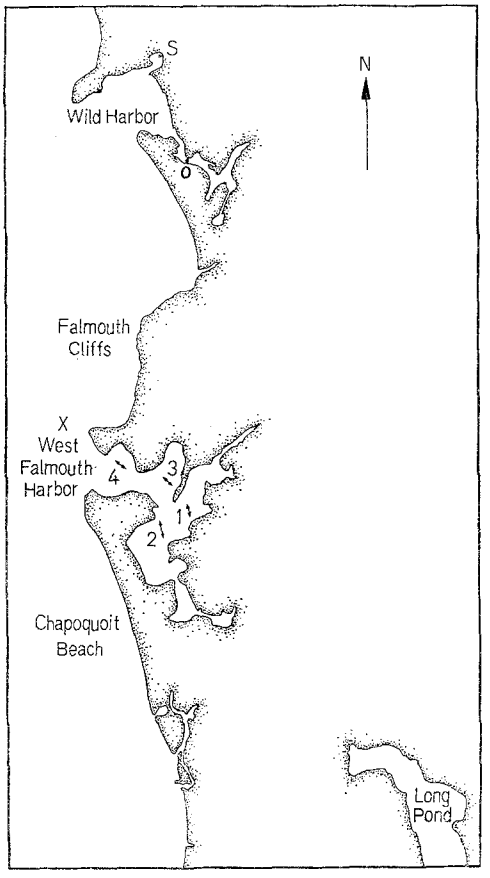


Fig. 2

Fig. 1. Southwestern Cape Cod, Massachusetts (USA). Uncontaminated oysters *Crassostrea virginica* were taken at sites 1 and 2 in Waquoit Bay. For site of accident and contaminated samples see detail, Fig. 2

Fig. 2. Site of accident (X). Samples taken at Wild Harbor. S sediment; O oysters *Crassostrea virginica*. Scallop (*Aequipecten irradians*) samples from West Falmouth Harbor (1—4)

between the Town of Falmouth and this laboratory was initiated to determine the possible pollution of oysters and scallops by this accident and to advise on further closing or reopening of the shellfish areas.

Scallops *Aequipecten irradians* were collected in West Falmouth Harbor on November 4 and oysters *Crassostrea virginica* in Wild Harbor River on November 12 (Figs. 1 and 2). For comparison, uncontaminated scallops were collected on the same date from Waquoit Bay on the South Shore of Cape Cod, Massachusetts.

## Experimental methods

### *Extraction*

Immediately after collection, the animals were taken to the laboratory. They were shucked as for commercial use, but care was taken to avoid contamination. The whole oysters, excluding the shell but including any liquid, and the scallops (the adductor muscles of *Aequipecten*) were weighed and transferred to paper thimbles that had been Soxhlet-extracted for 24 h with redistilled methanol.

The samples were extracted with refluxing methanol for a total of 20 h. This was interrupted by 3 soaking periods, 35 h in total, during which the specimens remained in the methanol-filled thimbles at room temperature. Methanol in the receiving flasks was changed once to minimize foaming and bumping.

The extracts were combined and solids were removed by centrifugation; these solids were then extracted by vigorous stirring with pentane, followed by centrifugation. The extraction was repeated 3 times. The original extracts, freed from solids, were extracted in separatory funnels with 4 batches of pentane. These pentane extracts and those from the solids were combined, washed with water to remove the remaining methanol, dried with extracted sodium sulfate, and concentrated on a rotary evaporator below room temperature. The vacuum was removed immediately when

a drop in pressure indicated that the pentane had been removed.

All solvents used were analytical grade and were redistilled in all glass stills through packed columns.

### *Chromatography*

Samples were chromatographed from a minimum volume of pentane on a column, packed in pentane, consisting of 3 parts of silica gel (Davison, grade 922, through 200 mesh, activated at 120 °C and then deactivated with 5% water, by weight) and 2 parts of alumina (Harshaw, 0102-P, activated at 250 °C and then deactivated with 5% water). The silica gel was packed first as an even layer and the alumina was packed over it as a separate top layer. The dual column gives better retention of higher molecular weight polar materials than silica gel, and better resolution of the hydrocarbons from nonhydrocarbons than alumina alone. Deactivation of the column with water prevents the formation of hydrocarbon artefacts from biogenic nonhydrocarbons, e.g. the formation of phytadienes from phytol (JOHNSTONE and QUAN, 1963; BLUMER and THOMAS, 1965b). The column dimensions were chosen for a minimum adsorbent to sample ratio of 50. The columns were eluted with 4 column volumes of n-pentane.

The suitability of the chromatographic technique for the analysis of No. 2 fuel oil was checked; a sample of the oil involved in the accident at West Falmouth was chromatographed and gave a recovery of 98.7 ± 1%. Analysis for other, heavier, oils might require a technique modified for the elution of the higher molecular weight and more polar components by mixtures of pentane with benzene.

The sediment extracts contained elemental sulfur in addition to the hydrocarbons, this was removed on a column of precipitated copper (BLUMER, 1957). The chromatographic eluates were dried and taken up in a known quantity of carbon disulfide. A small aliquot was evaporated on an aluminum pan and weighed on a Cahn Gram Electrobalance. The remainder of the $CS_2$ solution was used for gas chromatography. The hydrocarbon composition of the No. 2 fuel oil involved in the accident was analyzed by column chromatography. The oil (80.0 mg) was charged to a column of activated silica gel (15 ml) in pentane and eluted with pentane (15 ml, 15 ml, 30 ml), and with pentane containing 10, 30 and 50% benzene (30 ml, 30 ml, 50 ml). The 6 fractions were freed from solvent, weighed and analyzed by UV spectrophotometry in pentane. Fraction 1 was saturated and accounted for 58% of the total oil. Fraction 2 (0.8%) was mixed saturated and aromatic, while the remaining fractions (41%) were fully aromatic and increased in aromatic ring numbers from benzenes in fraction 3 to naphthalenes in 4, and phenanthrenes in 5. The weight of fraction 6 was negligible.

### *Gas chromatography*

The equipment consisted of a Varian Aerograph 600 D gas chromatograph, with linear temperature programmer, 1 mv recorder and automatic attenuator. Columns were 10 ft., 1/8″ o.d. packed with 2.2% Apiezon L on Chromosorb W, 70 to 80 mesh, acid washed, siliconized. For injection, the column was cooled below 100 °C; after elution of the $CS_2$ the oven temperature was programmed from 100° to 300 °C at 6 C°/min. Duplicate analyses were run, alone and after the addition of an internal standard containing the n-paraffins from decane to eicosane.

### Discussion of chromatograms

The gas chromatogram (Fig. 3 A) of the No. 2 fuel oil involved in the accident shows the presence of normal paraffins approximately from decane ($C_{10}H_{22}$) to docosane ($C_{22}H_{46}$), with a maximum in the $C_{12}$ to $C_{15}$ range. The estimated boiling range of the oil is 170° to 370 °C, with the largest fraction distilling between 200° and 300 °C. In addition to the straight

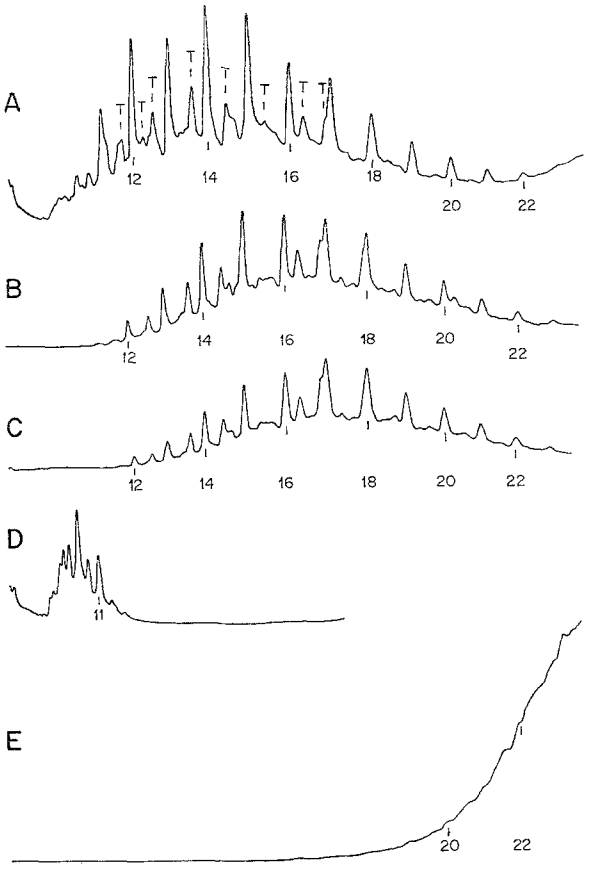


Fig. 3. Gas chromatograms. (A) No. 2 fuel oil; (B) sediments in Wild Harbor Basin, 12 days after accident; (C) oil recovered from water in Wild Harbor Basin, 2 months after accident; (D) 2-cycle outboard motor oil; (E) SAE No. 10 Lubricating oil. T marks position of isoprenoid alkanes

chain alkanes, several other homologous series are evident. Next in intensity are peaks which we assign to the isoprenoid alkanes from $C_{13}H_{28}$ to $C_{19}H_{40}$ (pristane). Higher homologues, especially phytane and the $C_{21}$ equivalent, may be present but are not resolved by this column from the $C_{18}$ and $C_{19}$ straight chain alkanes. Minor and only partly resolved peaks represent other branched or cyclic alkanes. In addition, a broad unresolved background from cycloparaffins and aromatics is present: this is typical for chromatograms of crude oil or crude oil distillates that are analyzed on a column of relatively low efficiency.

Sediments in Wild Harbor Basin (Fig. 2) (water depth 10 ft. 3.3 m) were sampled 12 days after the accident. The chromatogram of the total hydrocarbon fraction is reproduced in Fig. 3 B. The similarity with the chromatogram of No. 2 fuel oil is striking. The carbon number range, boiling point distribution, and relative contribution of different isomers is nearly identical. The major difference is a general decrease of the lower molecular weight hydrocarbons in the oil recovered from the sediment; these hydrocarbons are more readily soluble and should be depleted in an oil that has been in contact with seawater for an appreciable length of time. Conversely, these hydrocarbons are the most immediately toxic fraction of the oil, and their dissolution may be responsible, in part, for the lethal effect of the oil on the faunas. We also notice a rather striking reduction in the ratios of the lower boiling normal alkanes to the branched alkanes. For instance, the ratio of n-tetradecane to 2,6,10-trimethyldodecane (retention index 1358) has decreased from 2.7 to 2.0, and similar changes are evident throughout the $C_{12}$ to $C_{17}$ range. This may reflect preferred bacterial attack upon the straight chain paraffins (McKENNA and KALLIO, 1964) rather than solubilization. The normal alkanes are good growth substrates for specialized bacteria, but they are less water-soluble than the branched isomers.

Oil continued to be released from the sediments for a long time after the accident. Water with a patchy oil film was sampled in Wild Harbor Basin on November 21, 1969, more than 2 months after the spill. The chromatogram of the extracted oil (Fig. 3 C) shows a pattern very similar to Fig. 3 A and B. The carbon number range extends from $C_{12}$ to $C_{22}$, and the peaks of normal and branched alkanes are superimposed over a broad background of unresolved hydrocarbons. The lower boiling hydrocarbons are further, but not drastically, depleted; little additional change in the ratio of normal to isoprenoid alkanes is noticed. This suggests that the principal alteration may well have taken place immediately after the accident and before the oil became incorporated into the sediment.

Other possible hydrocarbon pollutants of this area were analyzed. A two-cycle outboard motor oil (Fig. 3 D) has a much lower boiling range, while automotive (SAE No. 10) lubricating oil (Fig. 3 E) contains only traces of hydrocarbons boiling below n-eicosane. Lubricating oils are dewaxed in processing; consequently they do not show the predominance of the straight chain hydrocarbons that we find in some, but not all, crude oils and in their straight run distillates. Because of the complexity of branched, cyclic and aromatic isomers and homologues present, the low resolution gas chromatogram of the lubricating oil presents a featureless broad envelope.

Floating oil particles and tar balls are now encountered on all oceans (HORN et al., 1969; NOSHKIN and CRADDOCK, 1968) and may be another source of inshore pollution. In most cases they result from crude oil spills rather than from losses of refinery products, and have a much wider molecular weight distribution. For comparison, a typical tar ball (from the Mediterranean) was subjected to our analytical procedure; the chromatogram (Fig. 4 A) shows a wide carbon number distribution from $C_{12}$ to beyond $C_{31}$, typical for a full range crude oil.

The hydrocarbons from oysters taken in Wild Harbor River on November 12, 1969, (Fig. 4 B) 2 months after the accident, extend from $C_{12}$ to about $C_{23}$ and show the same general features as the No. 2 fuel oil and the oil recovered from the sediments. Normal and branched paraffin peaks are superimposed over a broad and unresolved background. Especially impressive is the close agreement in the region of the chromatogram above n-heptadecane. Below that we note a further decrease in relative peak heights and an additional shift in the ratios of normal to isoprenoid alkanes. The ratio of n-tetradecane to 2,6,10-trimethyldodecane is now close to unity. The progressive alteration, in the same direction as that already observed in the oil from the sediments, is not unexpected though the extent of change is remarkable. Compared to the oil within the sediments, which is protected from further solubilization, the oil now in the shellfish may have been exposed to the seawater for a much longer time, for instance on top of the sediments, in the water column, or in the shellfish itself.

The greater relative height of the unresolved background in Fig. 4 B suggests a greater resistance of the more toxic cyclic-aromatic fraction.

The gas chromatograms of all scallops taken in West Falmouth Harbor on November 13, 1969, are nearly identical; typical is the chromatogram in Fig. 4 C. The carbon number range again extends from just below $C_{13}$ to somewhat above $C_{20}$. The progressive alteration already evident in the oil incorporated into the sediments and into the oysters has gone much farther here; the steep slope of the chromatogram below pentadecane suggests increased dissolution of the lower molecular weight hydrocarbons. Straight chain hydrocarbons have disappeared almost completely, and are now minor components relative to the isoprenoid alkanes. Even these have decreased in concentration relative to the unresolved background of the

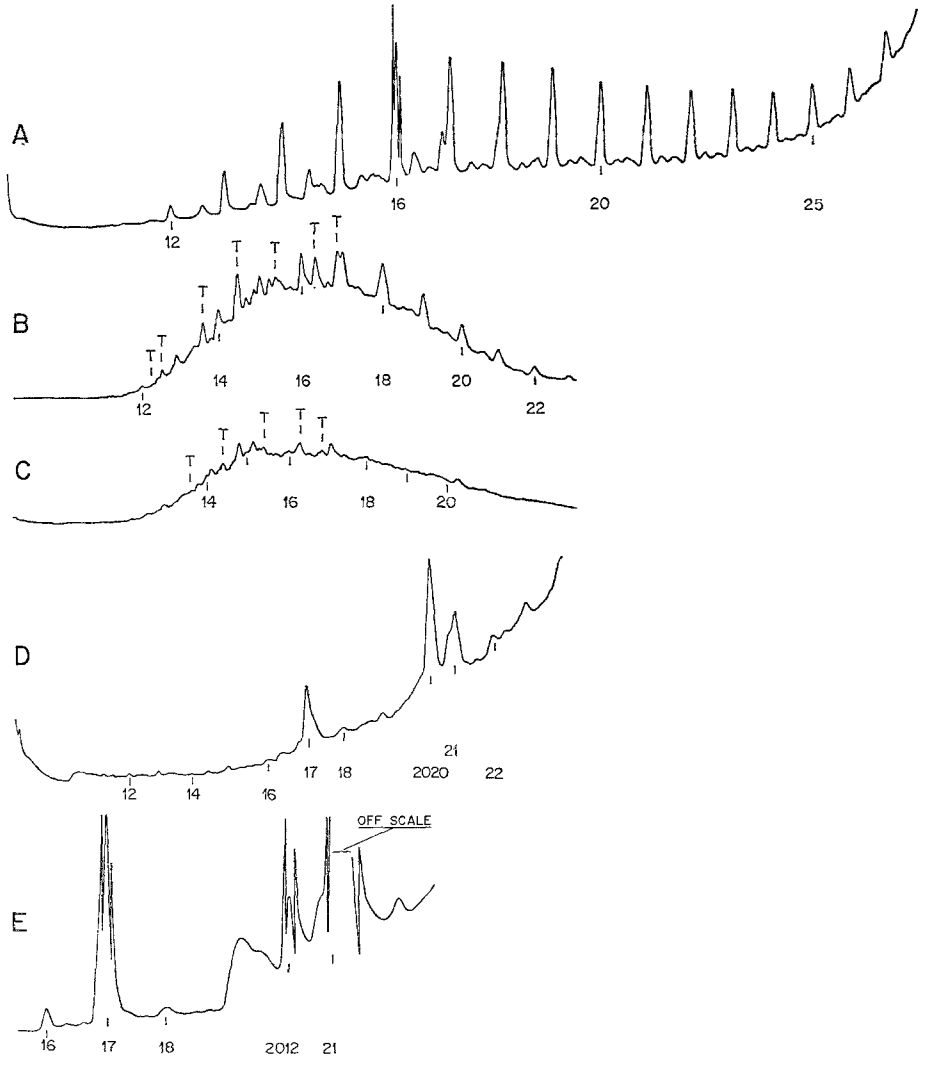


Fig. 4. Gas chromatograms. (A) tar balls, n-hexadecane added as standard; (B) oysters *Crassostrea virginica*, Wild Harbor River; (C) scallops *Aequipecten irradians*, West Falmouth Harbor; (D) oysters, Waquoit Bay; (E) *Rhizoselenia setigera*. Chromatograms 4A, 4D—E at different column lengths or program rates. T marks position of isoprenoid alkanes. *Note:* To demonstrate the presence of natural hydrocarbons in uncontaminated scallops (D) a much larger sample had to be chromatographed (see Table 1)

cyclic hydrocarbons. In spite of the alteration there is no doubt that the scallops are contaminated by fuel oil; this is evident from the boiling point distribution, from the correlation with the branched alkanes in the fuel oil, and from a comparison with hydrocarbon concentrations and type distributions in scallops from another, unpolluted area.

Scallops from Waquoit Bay (Fig. 4 D) show a very different hydrocarbon distribution. Normal alkanes are present only in small amounts, except for normal heptadecane and normal heneicosane. The high peak at retention index 2020 is an n-heneicosahexaene. This olefin and the $C_{17}$ and $C_{21}$ n-alkanes are abundant in many species of marine algae (CLARK and BLUMER, 1967); in fact *Rhizoselenia setigera* (Fig. 4 E), a winter diatom in this area, has a hydrocarbon distribution which strongly resembles that of the scallops from Waquoit Bay. The presence of olefinic hydrocarbons, of strongly predominant $C_{17}$ and $C_{21}$ n-alkanes, the absence of isoprenoids outside the $C_{19} - C_{20}$ range, and the lack of an unresolved envelope from cyclic saturates and aromatics is a clear indicator of biological origin and of the absence of pollution in the scallops from Waquoit. The contamination of the scallops from West Falmouth, on the other hand, is evident and has completely obscured the natural hydrocarbon pattern except for a possible slight evidence for n-heptadecane and the $C_{21}$ hexaolefin.

Table 1. *Hydrocarbon content of scallops Aequipecten irradians and oysters Crassostrea virginica*

| Number of sample and species | Location | Number of individuals | Wet weight (g) | Hydrocarbon content (mg/100 g wet weight) | Aliquot used for chromatograms 4B—4D (g) |
|---|---|---|---|---|---|
| 1 scallop | West Falmouth Harbor | 10 | 37 | not determined | |
| 2 scallop | West Falmouth Harbor | 11 | 39 | 1.4 | 0.31 |
| 3 scallop | West Falmouth Harbor | 10 | 60 | 1.1 | |
| 4 scallop | West Falmouth Harbor | 10 | 60 | 0.74 | |
| 5 scallop | Waquoit Bay | 11 | 91 | 0.55 | 5.2 |
| 6 scallop | Waquoit Bay | 12 | 36 | 0.23 | |
| 7 oyster | Wild Harbor River | 7 | 110 | 6.9 | 0.07 |

The hydrocarbon content of the animal specimens was determined gravimetrically (Table 1); the same table also lists the corresponding wet weight aliquot needed to give the chromatograms in Fig. 4 B, C, D; these were run at the same instrument sensitivity and are therefore directly comparable. It would be difficult to establish the presence of hydrocarbon pollution from the weight data alone, especially in the case of the scallops; the gas chromatograms, on the other hand, tell a conclusive story. The apparent discrepancy may come from the presence in the weighed fraction of higher boiling hydrocarbons, or of nonhydrocarbons which are not chromatographically evident. In either case, gravimetric determination of pollutants in contaminated animal species is unselective and looses its power as the contamination level approaches the natural background level. A selective analytical technique, like gas chromatography, can still detect the presence of contaminants at or below the background level and should therefore be much preferred.

## Conclusions

Hydrocarbons are much more universally distributed throughout nature than was realized a few years ago. They are synthesized by most, if not all, living organisms and, because of their relatively great stability, they may spread from their sources through the environment, in the food web, in the water masses or on the surface of particulate matter. This poses a serious problem in tracing hydrocarbon pollution through the environment since pollutants are always accompanied by natural hydrocarbons. However, a distinction is possible through careful analysis of the characteristic differences between hydrocarbons from organisms and from oil pollution, both in molecular size and in type distribution. Crude oil and oil products are wide range mixtures, that contain molecules of different size in fairly even distribution; organisms on the other hand possess specific biosynthetic pathways which favor the production of hydrocarbons in preferred size ranges. Thus, the *Calanid* copepods contain almost exclusively hydrocarbons with 19 carbon atoms arranged in a branched chain; many algae contain very high concentrations of n-pentadecane, n-heptadecane and other normal paraffins in the virtual absence of the other straight chain hydrocarbons which are abundant in fossil fuels.

Other differences exist in the molecular type distribution: oil and oil products are rich in the toxic aromatic hydrocarbons and in cycloparaffins; they also contain isoprenoid hydrocarbons ranging from about $C_{11}$ to $C_{22}$ and beyond. Oil pollution — except from certain cracking products — is devoid of the olefins which are so abundant in most organisms. In contrast to oil products, organisms are limited in their isoprenoid hydrocarbon content to compounds containing 19 and 20 C atoms.

It is evident that a distinction between pollutants and natural hydrocarbons at low concentration levels is not made easily by analytical techniques that rely only on the properties of hydrocarbon mixtures, such as the weight of a fraction, its fluorescence or absorption in the ultraviolet, visible, or infrared region of the spectrum. Gas chromatography separates a hydrocarbon mixture into individual components according to their boiling point and their structural type; therefore, it can provide a much more meaningful distinction between fossil fuels and recent biogenic hydrocarbons.

The gas chromatographic techniques used in this study establish the identity of the hydrocarbons in the shellfish with No. 2 fuel oil with a relatively low effort. Considerable sophistication of the techniques is possible in more demanding cases, at greater cost in time and equipment, through the use of more efficient columns and of multi-column techniques, possibly followed by mass spectral identification of critical components.

Our previous work had established the refractory nature of biogenic hydrocarbons in the marine food web. It had then been suggested that hydrocarbons from oil pollution might be incorporated into marine

organisms where they would persist in a similar manner. This work now demonstrates that oil pollutants can be incorporated into marine organisms which are harvested for human consumption. It is especially interesting to find the oil derived from this spill not only in the whole organism of the oysters, but also incorporated into the adductor muscle of the scallops. Thus, what we found earlier for the biogenic hydrocarbons is realized also for hydrocarbons from pollution: hydrocarbons ingested by marine organisms become part of their lipid pool and are not necessarily concentrated in a specific organ. Together with the high resistance of hydrocarbons towards metabolism, this may have the serious consequence that oil pollution is not readily lost from a contaminated organism after removal of the source of pollution. It has been an accepted and effective practice to transfer shellfish contaminated by certain toxins and pathogens to clean water for detoxification. There is a high probability that this will not be effective for the elimination of hydrocarbon pollutants.

Another critical finding is the persistence of essentially unaltered fuel oil in the sediments 2 months after the spill. We expect that the continued presence of this oil will substantially delay the repopulation of the affected area; in addition, the gradual release of the oil with its high content of strongly toxic aromatic hydrocarbons from the sediments into the water column may pollute the water and the shellfish long after the actual accident.

The extent of modification of the oil in the sediments and in the organisms poses interesting questions for further research. Aside from modification caused by solubility effects, the change in oil composition in the sediments after 2 months is small[2]. We would have expected to see a larger extent of biological, e.g. bacterial, modification, resulting in the disappearance of the straight chain hydrocarbons. Did the modification proceed at the normal rate for this environment and this time of the year, or was it delayed because the bacterial flora has been adversely affected by the spill? On the other hand, alteration of the hydrocarbon composition in the oysters and especially in the scallops has been considerable. The mechanisms that bring about these changes are not understood; they might operate in the water column, in the planktonic organisms upon which the shellfish feed, or in the oysters and scallops themselves. However, the alteration of the hydrocarbons observed in the shellfish is not favorable to an eventual detoxification; the least toxic hydrocarbons disappear while the more toxic cyclic hydrocarbons are retained.

The continuing study of the long term effect of this oil spill on the ecology of the coastal regions of West Falmouth will attempt to answer many of these remaining questions.

[2] Four months after the accident the hydrocarbon concentration in the sediments remains unchanged.




## Summary

1. An oil spill (650,000 to 700,000 l of No. 2 fuel oil) has contaminated the coastal waters of Buzzards Bay, Massachusetts, USA. The presence of the oil in the sediments is demonstrated by gas chromatography.

2. Essentially unchanged oil is still being released from the sediments 2 months after the accident.

3. The same oil pollutant is present in whole individuals of *Crassostrea virginica* and adductor muscles of *Aequipecten irradians*.

4. A presumably biochemical modification leads to a gradual depletion of the straight chain and, to a lesser extent, of branched chain hydrocarbons. This does not, however, lead to detoxification, since the more toxic aromatic hydrocarbons are retained in the scallop several months after the accident.

5. Uncontaminated *Aequipecten irradians* contain hydrocarbons in lesser amounts, and of very different molecular weight and type distribution than contaminated individuals.


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