

FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Work-

shop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

A. D. McINTYRE
K. J. WHITTLE

BIOGENIC HYDROCARBONS AND PETROLEUM FRACTIONS

C. BOCARD, C. GATELLIER, N. PETROFF, PH. RENAULT
and J. C. ROUSSEL

Institut Français du Pétrole, 1 et 4 avenue Bois-Préau, 92506 - Rueil-Malmaison, France

Knowledge of the differences between biogenic hydrocarbons and those from petroleum is reviewed, and the problems of making this differentiation are discussed. The value of ^{14}C techniques in some cases is considered.

Whitmore (1944) calculated that from the estimated world-wide annual production of kelp alone, the hydrocarbon content (0.03 % dry weight) would amount to 6×10^7 barrels of oil, or 9×10^6 tons, if not consumed in the carbon cycle. Oakwood (1946) reported that hydrocarbons could be found in the unsaponifiable fraction of lipids from a variety of algae and bacteria and that the hydrocarbon fraction exhibited optical rotations as crude oils did. Using techniques of elution chromatography, Smith (1952) succeeded in finding liquid and low melting hydrocarbons in recent sediments (about 31 mg of petroleum-like material/100 mg of sediments, dry weight basis) at various depths in Gulf of Mexico. It was calculated that a cubic mile of sediments would contain 2×10^6 tons of "oil". Stephens (1956) found in sediments naphthenic, paraffinic and aromatic hydrocarbons, the average age of which was about 4000 years based on radioactive ^{14}C . In sediments off the coast of California, chlorophyll derivatives, particularly phaeophytin and spectrally similar pigments were compared with the hydrocarbon content (Orr et al, 1958). The weight ratios of hydrocarbons to pigments in phytoplankton ranged between 0.02 and 0.08; in basin sediments the ratio ranged between 2 and 8. That change in ratio of two orders of magnitude between plankton and sediment could be attributed to a preferential destruction of chlorophyll relative to hydrocarbons.

More recently Zsolnay (1973) attempted to correlate the concentrations of hydrocarbons in sea water and the abundance of phytoplankton present. An upwelling region is ideal for such a study, since a great variety of pigment concentrations can be found within a small geographical area; there is a lower probability that any correlation is due to other factors such as might occur by samples being taken over a greater time span or over a larger area. One can conclude that the hydrocarbons in the water of the euphotic zone in the region studied are largely produced *in situ* by the

phytoplankton. However, it seems that the hydrocarbons of planktonic algae are few and of simple structure compared to those of ancient sediments and fossil fuels (Blumer et al, 1971). Crude oils contain a wide range of isomeric structures covering many homologous series and they are rich in isoparaffins, cycloalkanes and aromatics but they contain no olefins; from algae on the other hand, the hydrocarbon fraction contains a large proportion of normal paraffins, often with one or two compounds exceeding all others by orders of magnitude; it contains olefins and only few, if any, isoparaffins, cycloparaffins or aromatics.

There has been some increase during the last few years in the number of laboratories analyzing marine samples for hydrocarbons in the presence of recently biosynthesized hydrocarbons (Farrington et al, 1974). Oil consists of a mixture of hundreds of hydrocarbons, the proportions of which vary strongly with the type of oil. In order to obtain results quickly and in a relatively simple way, infra-red spectrometry (I.R.) is one of the techniques employed most often for the determination of oil in water (Carlberg and Skarstedt, 1972). In the extraction of the sample not only petroleum hydrocarbons are isolated but also organic substances of other origins such as humic compounds, lignin, animal and vegetable fats and surface active agents. By chromatography through a polar column (i.e. florasil) the interfering organic substances can be eliminated. However, the I.R. method is not capable of distinguishing between non-polar compounds of different origins. The most commonly used methods for the identification of the source of oil pollution are gas liquid chromatography (G.L.C.) and trace metal analysis. G.L.C. is particularly suited to the identification of the source of crude oils, distillate, fuel oils and other petroleum fractions which contain significant concentrations of low boiling constituents; it is subject to some ambiguities which may arise when the volatile compounds have evaporated; then an identification of crude oil pol-

lutants by means of trace metal contents (nickel and vanadium) can be suitable.

Such analyses are much more difficult than those made for other polluting chemical products such as pesticides, detergents or phenolic compounds because we are faced with a highly complex mixture of products (Tissier and Oudin, 1973). The sediment is dried, crushed and Soxhlet extracted with chloroform. After separation of the heaviest products (asphaltenes) by precipitation in hexane, a liquid chromatography gives three fractions, i.e. non aromatic hydrocarbons, aromatic hydrocarbons and resins. The samples which have been analysed by that method were collected on the French coast of the English Channel in Normandy and in the Seine Bay and the presence of polluting hydrocarbons was revealed on chromatograms of total saturated hydrocarbons by a reduction of the odd/even predominance. In fact, it has been pointed out previously that the hydrocarbons in sediments show a strongly odd carbon number preference while the paraffins in petroleum do not (Blumer and Sass, 1972) and that the aromatic fraction from recent sediments is much simpler than the corresponding fraction from petroleum.

Have we to concur with these authors in the view that hydrocarbons are not universal, if minor, components of all marine plants and animals of sea water and marine sediments?

A more complete knowledge of the natural hydrocarbon background would be valuable for application of hydrocarbon analysis to the study of marine processes and for tracing hydrocarbon pollution at concentration levels approaching those of the natural background. For instance, the saturated and unsaturated hydrocarbon content of a benthic marine algae like *Ulva lactuca* can be as high as 0.5 % (dry weight basis) (Youngblood et al., 1971). 3–4 Benzopyrene and other carcinogenic hydrocarbons have been detected in numerous plant species. This poses the pertinent question whether such polynuclear aromatic hydrocarbons (PAH) content represents extraneous contamination or does it represent synthesis of PAH by the plants and bacteria. Both processes are probably widely operative (Mallet, 1972). Pristane and other hydrocarbons such as normal alkanes (C_{15} , C_{16} , C_{17} , C_{18} , C_{19}) and homologues of unknown structures have been found in some freshwater and marine fish oils (Ackman, 1971). With regard to aromatic hydrocarbons, isopropyltoluene is one of the substances derived from a natural source other than coal or petroleum. It is a constituent of many essential oils (Gerarde, 1960) and was obtained commercially from old stumps of the longleaf and slash pines (*Pinus palustris* and *Pinus caribaea*). Essential oils distilled from the flowering plants thyme and origanum also contain pseudo cymene. In the produc-

tion of pulp from pine wood by the sulphite process of making paper, sulphite wood turpentine and p. cymene have been important by-products. Sea animals and fish were examined for hydrocarbons: unsaponifiable fractions containing different alkanes and isoprenoids were found in sharks, whales and herring. In general, marine organisms do not have the odd number carbon preference which is common in higher organisms. It seems plausible that certain hydrocarbons like pristane found in sharks and whales come from their diet of plankton and nekton.

In contrast to the knowledge of the lipid extractable material of higher plants and green algae (Gerarde and Gerarde, 1961), little or no information is available on most micro-organisms. The only bacteria reported to have measurable amounts of hydrocarbons are *Serratia marinarum*, *Vibrio ponticus* and *Sarcina lutea* (Tornabene, 1967). The *Sarcina* species contain hydrocarbons from C_{10} to C_{36} with no predominance of hydrocarbon chains. Aliphatic hydrocarbons are 0.25 % of the cell mass. As in the case of the fatty acids, the aliphatic hydrocarbons in *S. lutea* show a distribution having a predominance of neither even nor odd carbon numbered chains. Some components are identified as branched hydrocarbons with substituents at either iso or ante-iso positions. Moreover, it has been well reported that culturing conditions play an important role in the nature and distribution of the components of the lipid extractable material.

The bulk of the organic matter in the ocean is autochthonous, produced by the vital activity and death of the animal, plants and bacterial population of the water column and mud. It may be noted that the greater part of the organic matter in ocean waters is in dissolved form (DOC), its quantity being up to 4000 times the organic matter present in the living organisms in the water (Kriss et al., 1967). The problem of the molecular nature of the dissolved organic matter in the ocean, and especially in the deep sea, is similar to, but more difficult than the identification of humus in soils. Trace amounts of C_1 – C_4 hydrocarbons, C_{20} – C_{33} n-alkanes and pristane have been identified in sea water. Unhappily, in many cases, there were no total DOC determinations made on the same water samples from which the organic compounds were isolated. It is of interest to consider the relative importance of the total hydrocarbons in the chloroform extracts of different samples collected from coastal and open sea waters by Barbier (1973). They represent between 10 and 20 % for tropical waters, and from 40 to 55 % for the Antarctic.

The ^{14}C activity of DOC can be used to distinguish between two classes of organic carbon: petrochemical industrial wastes from fossil petroleum which contains no ^{14}C , and agricultural and municipal wastes and bio-

production within the aquatic system, which supply modern biological material in the form of decaying animal and vegetable matter.

This organic material has recently been in equilibrium with the atmosphere and thus contains the modern atmospheric ^{14}C content. As an example, the apparent "age" of the dissolved organic matter at 2000 m in the north east Pacific Ocean is approximately 3400 years. This time is of the same order of magnitude for a calculated residence time of DOC from input and output fluxes assuming a steady-state situation (Williams, 1971).

A basic assumption in such a study is that sources of organic carbon with an intermediate ^{14}C content are negligible. This is certainly erroneous, not only in sea water, as pointed out above, but also in rivers, regarding the age of naturally derived humic material. Kolle (1974) used the simplified method to evaluate the pollution of sediments in Bodensee. The ratio of recent hydrocarbons to polluting oil, calculated in carbon, varies from 14 to 36 %. In fact all the difficulties we have to get over, lie in the extractive phase, to ensure that the sample has not been polluted by some petrochemical carbon. This was a time consuming problem. More experimental details will be soon published.

REFERENCES

- Ackman, R. G. 1971. Pristane and other hydrocarbons in some freshwater and marine fish oils. *Lipids* 6: 520-22.
- Barbier, M. 1973. Hydrocarbons from sea water. *Deep Sea Res.* 20: 305-14.
- Blumer, M. et al., 1971. Hydrocarbons of marine phytoplankton. *Mar. Biol.* 8: 183-89.
- Blumer, M. & Sass, J. 1972. Indigenous and petroleum derived hydrocarbons in a polluted sediment. *Mar. Pollut. Bull.* 3: 92-94.
- Carlberg, S. R. & Skarstedt, C. B. 1972. Determination of small amounts of non-polar hydrocarbons in seawater. *J. Cons. int. Explor. Mer.*, 34(3): 506-15.
- Farrington, J. W. et al., 1974. Analyses of hydrocarbons in marine organisms. In *Marine Pollution Monitoring*. NBS special publication 409 pp.
- Gerarde, H. W. 1960. Toxicology and biochemistry of aromatic hydrocarbons. Edited by Elsevier. London 329 pp.
- Gerarde, H. W. & Gerarde, D. F. 1961. The ubiquitous hydrocarbons (presented at the 65th Annual Conference of the Association of Food and Drug Officials of the U. S. Washington D. C. June 18-23, 1961).
- Kolle, W. 1974. Untersuchungen der Mineralöl-Belastung der Bodensee-Sedimente. *Kernforschungszentrum Karlsruhe* - June 1974 p. 8-16.
- Kriss, A. E. et al. 1967. Microbial population of oceans and seas. Translated from Russian by Syers K. Edited by Arnold. London 350 pp.
- Mallet, L. 1972. Pollution des milieux vitaux par les hydrocarbures cancérigènes. Edited by Maloiné. Paris 195 pp.
- Oakwood, T. S. 1946. Annual report of progress. Fundamental research on the occurrence and recovery of petroleum. *Am. Petrol. Inst. N. Y.* 92-102.
- Orr, W. L. et al. 1958. Preservation of chlorophyll derivatives in sediments off southern California. *Bull. Am. Ass. Petrol. Geol.* 42: 925-62.
- Smith, P. V. 1952. Preliminary note on origin of petroleum. *Bull. Am. Ass. Petrol. Geol.* 36: 411-13.
- Stevens, N. P. 1956. Origin of petroleum. A review. *Bull. Am. Ass. Petrol. Geol.* 40: 51-61.
- Tissier, M. & Oudin, J. L. 1973. Characteristics of naturally occurring and pollutant hydrocarbons in marine sediments. *Proceed. Joint. Conf. Prevention a. Control oil Spills. API* 205-14.
- Tornabene, T. 1967. Distribution and synthesis of hydrocarbons and closely related compounds in microorganisms. PhD thesis/ University of Houston.
- Whitmore, F. C. 1944. Annual report of progress. Fundamental research on the occurrence and recovery of petroleum. *Am. Petrol. Inst. N. Y.*, 124.
- Williams, P. M. 1971. The distribution and cycling of organic matter in the ocean. In *Organic compounds in aquatic environments*. Ed. by Marcel Dekker. New York 638 pp.
- Youngblood, W. W. et al. 1971. Saturated and unsaturated hydrocarbons in marine benthic algae. *Mar. Biol.* 8: 190-201.
- Zsolnay, A. 1973. Hydrocarbon and chlorophyll: a correlation in the upwelling region of West Africa. *Deep Sea Res.* 20: 923-25.