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 Workshop 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

Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171: 27–32. 1977.

SAMPLING AND EXTRACTION METHODS AND THEIR ASSOCIATED PROBLEMS

P. R. Mackie, R. Hardy and K. J. Whittle
Torry Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen AB9 8DG, U.K.

Because of the heterogeneity of the marine ecosystem a variety of sampling and extraction techniques have to be used to obtain relevant fractions for hydrocarbon or petroleum oil component analyses. Considerable care has to be taken to obtain representative samples and also to minimise sample contamination.

The application of *n*-alkane analyses to these problems is described and the problems in interpretation are highlighted.

INTRODUCTION

Petroleum crude oils and petroleum products are complex mixtures whose analysis in the marine environment poses considerable difficulties. For large quantities such as in an oil spill, a number of adequate methods can be used to confirm their identity. At lower concentrations, however, one is usually restricted to the measurement of a single component or group of components. From such measurements extrapolations are necessary in order to estimate the amount of petroleum in the sample.

It is not the purpose of our paper to discuss methods for and interpretation of extrapolations of this kind but rather to deal with the question of sampling and, in particular, the means of obtaining a representative sample. To do this, a standard method of analysis has to be used and we have chosen to measure the amount of *n*-alkanes present.

EXPERIMENTAL

A sampling site at Loch Ewe on the north-west coast of Scotland was chosen because it is remote from industrial centres, is not close to any major shipping lanes and was thus felt to represent a comparatively unpolluted area. Conversely, Port Seton is situated on the Firth of Forth which carries a large volume of shipping and on whose banks are major urban and industrial areas including a large refinery; it is typical of an area where petroleum input is likely to be high.

SAMPLING AND EXTRACTION

SURFACE FILM

The surface film was sampled by laying a stainless steel screen (67×100 cm, 200 mesh) on the sea surface, rolling the screen up and placing it on top of an upright glass beaker inside a glass extraction tube to hold it clear of the bottom of the tube which was then stoppered. Care was taken to minimise handling of the screens.

An aliquot of cyclohexane (5 µl) containing squalane (5 µg) was then placed on the screen by means of a syringe. Pentane (150 ml) was poured over the screen and a condenser connected to the head of the tube. The bottom of the tube was heated in a water bath, causing the pentane to reflux (3 hours) thus bathing the screen in vapours and vapour condensate but keeping it isolated from the boiling solvent. After cooling, the pentane was separated from the water layer, which usually amounted to 30 ml, and then concentrated to about 1 ml on a rotary film evaporator. The subsequent work-up procedure was essentially that described by Mackie et al, 1974.

The series of screens from Port Seton were given an additional wash with chloroform/methanol (1:1) (100 ml) after the pentane extraction. Distilled water (50 ml) was shaken with the chloroform/methanol in order to separate the chloroform. The latter was then washed with a further 50 ml distilled water and the organic phase removed on a rotary evaporator. The residue was dissolved in an aliquot of pentane (1 ml) which was worked-up as previously described (Mackie et al, 1974).

SUB-SURFACE WATER

The sample of sub-surface water (5 litre) was obtained by opening and closing glass Winchester bottles under the surface, allowing the bottles to fill at a depth of 1 m. The sample was then filtered through a nominal 20 µm stainless steel screen prior to the addition of the internal standard, [squalane (1 µg) in cyclo-

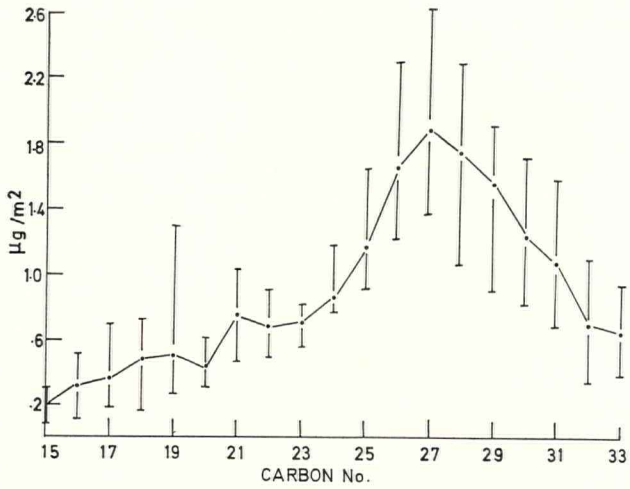

Figure 15.[1] Surface film from Loch Ewe.

[1] The figures 15–20 are a plot of the arithmetic mean of the values of the individual *n*-alkanes. The vertical lines show the range of values. The lines joining each mean value have been inserted to make the alkane distribution easier to follow visually. They have no real meaning.

hexane (10 µl)], and subsequent extraction with 3×50 ml aliquots of pentane (Mackie et al, 1974). Manipulations were reduced to the absolute minimum since vessel-to-vessel transfers can result in considerable losses.

SEDIMENT

Intertidal cores were taken at low tide since no divers were available. At Port Seton, cores 40 mm deep were obtained with both a 100 mm and a 45 mm diameter perspex corer. At Loch Ewe the wide cores only were taken to a depth of 70 mm. All cores at both sites were sampled within an area of 2000 cm². It would have been desirable to have collected cores of uniform length but this was impractical due to the varied nature of the beaches.

The cores were extracted by the method of Mackie et al (1974).

FISH

A batch of twenty codling caught by hand line in Loch Fyne were placed in a large tank and fed a squid diet over a period of 10 months. Samples of three, and in one instance four, fish were withdrawn at approximately 2 monthly intervals, sacrificed and the muscle and liver tissues excised. After mincing, the internal standard [squalane (1 µg in the case of the muscle, 5 µg in the case of the liver) in cyclohexane (10 µl)] was added and the sample treated according to Mackie et al (1974). All samples were analysed at the same time.

Table 2. *n*-Alkane content of Port Seton surface films (in µg/m²)

Carbon number	1	2	3	4
15	0·42	0·03	0·03	0·02
16	0·20	0·11	0·15	0·18
17	0·23	0·29	0·29	0·40
18	0·28	0·25	0·21	0·20
19	0·48	0·26	0·19	0·34
20	0·65	0·41	0·38	0·61
21	1·31	1·02	1·02	1·18
22	2·12	2·10	2·46	2·35
23	2·02	2·83	3·87	4·35
24	2·35	3·68	5·47	5·87
25	2·64	4·03	6·16	6·20
26	3·35	4·44	6·75	6·29
27	3·20	3·76	5·80	5·60
28	3·10	3·67	4·51	4·28
29	2·80	2·76	3·34	3·49
30	2·74	1·70	2·87	2·63
31	2·59	1·83	2·22	2·09
32	1·79	0·74	1·35	1·36
33	1·13	0·53	1·08	0·85
Σ_{33}^{15}	33·39	34·44	48·16	48·29
Pristane (PR)	1·77	0·88	0·66	1·17
Phytane (PH)	0·04	0·12	0·09	0·09
PR:PH	43:1	7:1	7:1	13:1
% Standard recovered after extraction with pentane	54	53	55	61
% Standard recovered after re-extraction with chloroform:methanol	95	93	93	96

EFFICIENCY OF EXTRACTION

The following experiments were carried out to determine the efficiency of the various extraction procedures.

SURFACE FILM

Pentane (10 µl) containing a North Sea crude oil (10 µg) was added with a syringe to the surface of water contained within a large shallow pyrex dish. After allowing the pentane to evaporate the film was sampled with a 67×100 mm piece of mesh previously described. After correction for the area of the mesh in relation to the area of the vessel a routine recovery of 75 ± 2 % was obtained. No difference was observed using tap, distilled or sea water. The oil recovered had an identical GLC profile in the region of interest (C_{15}–C_{33}) to that of the starting material.

SUB-SURFACE WATER

Because of the difficulties of preparing a homogeneous solution of alkanes in water, recovery experiments were carried out using solutions of benzene in sea water. These solutions were prepared in a ther-

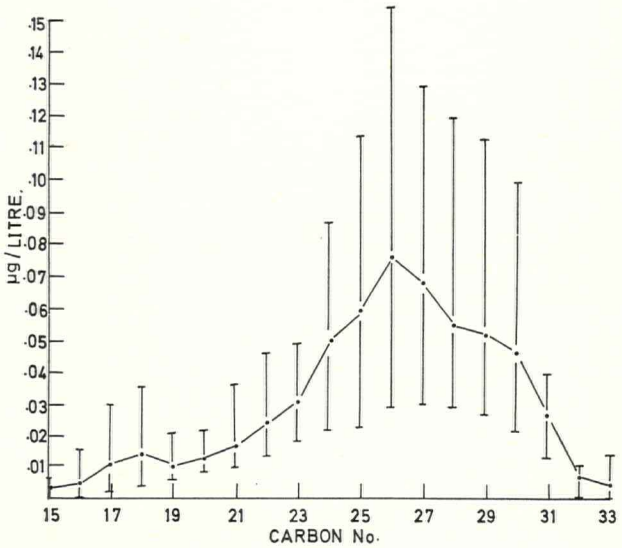

Figure 16. Sub-surface water from Loch Ewe.

mostated bath maintained at 10° by slowly stirring filtered sea water under benzene ensuring that a vortex did not form during the stirring. The dissolution of the benzene was monitored by pumping the chilled sea water through a flow cell in a spectrophotometer set at a wavelength of 254 nm and the concentration of benzene in the underlying water was measured from the steady absorption value. The water, free of overlying benzene, was then extracted with three, 50 ml, portions of pentane as described above. Recoveries of benzene in the first, second and third extractions were 90, 7 and 2 % respectively. Since the alkanes of interest are less soluble than benzene it is assumed that their recovery would be at least as good as that of benzene.

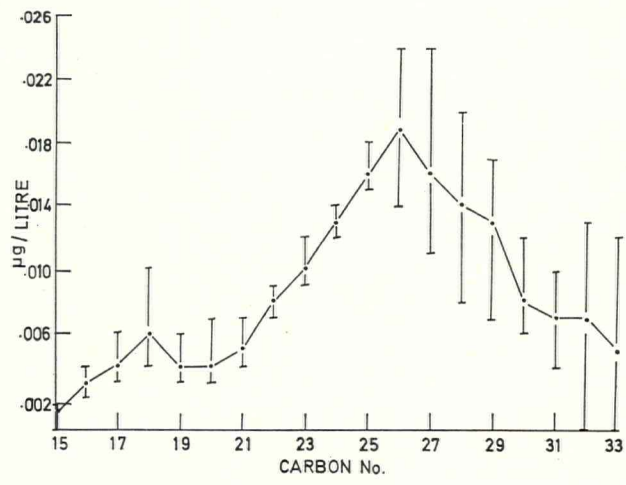

Figure 17. Sub-surface water from Port Seton.

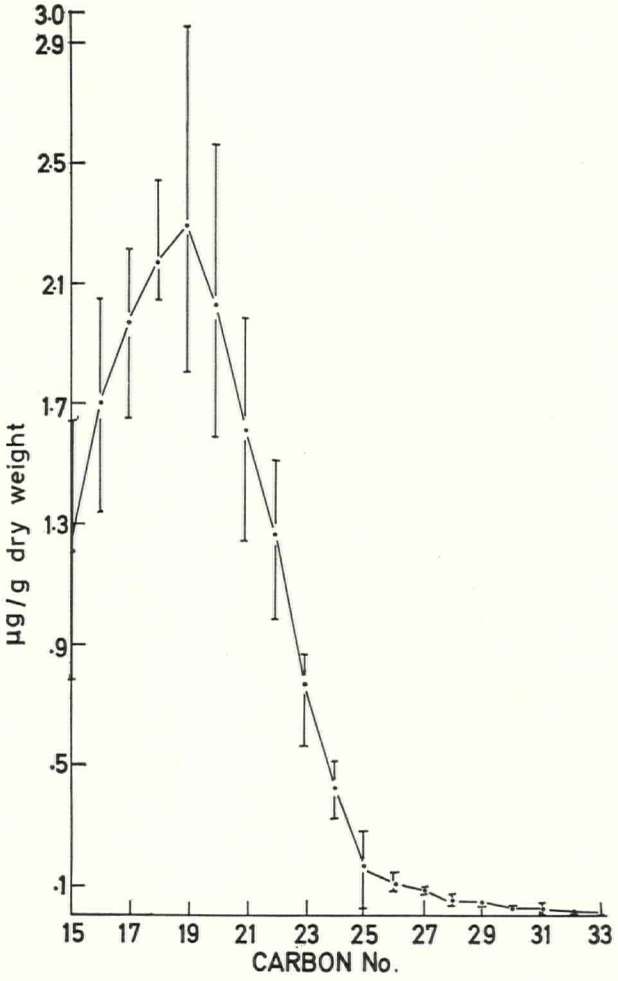

Figure 18. Sediment from Port Seton (wide core).

SEDIMENT AND ANIMAL TISSUES

Known amounts of oil added to sediments and tissues and extracted as described earlier gave recoveries of 95 %. This method of testing is not wholly satisfactory, however, since added oil will simply give a surface coating on the material, whereas hydrocarbons already present in the tissue are more likely to be in a dispersed state and thus more difficult to extract.

RESULTS AND DISCUSSION

SURFACE FILM

The Loch Ewe results obtained by extracting the screens with pentane only are shown in Figure 15. The range of values for each individual alkane are shown (results of 7 determinations) and the graph is plotted through the arithmetic mean. The variation in quantity of individual *n*-alkanes (average coefficient of variation 37 %) is quite marked and greater than that for the

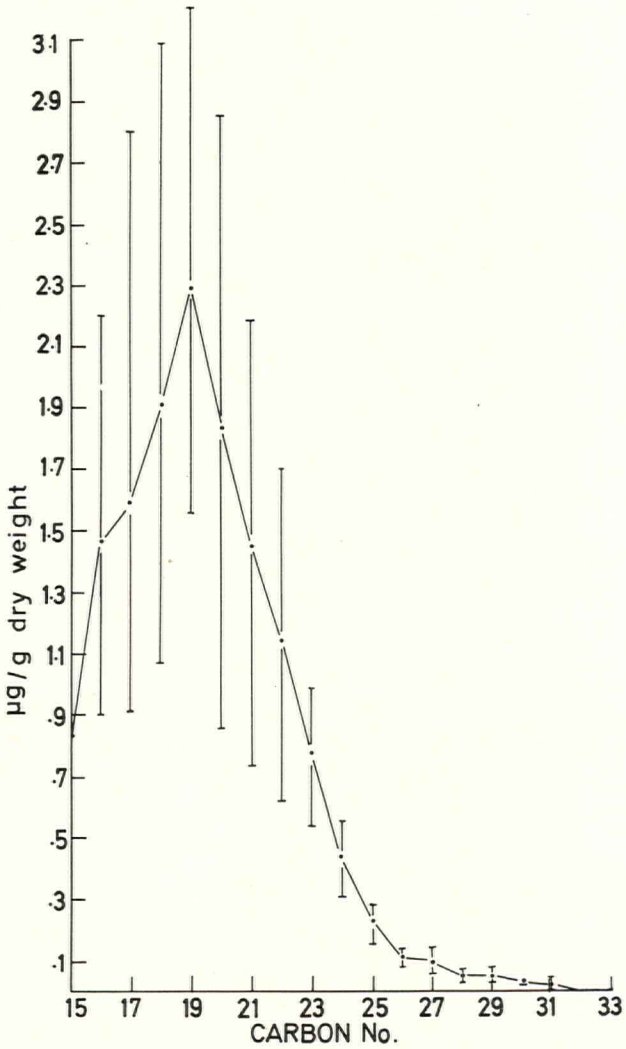

Figure 19. Sediment from Port Seton (narrow core).

total quantity of *n*-alkanes (coefficient of variation 27 %) and, as expected from such observations, the envelope patterns were not identical. This would seem to indicate that the surface film, even within a small area, was not homogeneous with respect to single *n*-alkanes but was more so for the total. In this set of experiments recovery of the internal standard, squalane, was low (60 %).

The results obtained from the analysis of the Port Seton screens which were reextracted with chloroform/methanol in the manner described earlier are shown in Table 2. It is clear that total recovery of squalane was better than 90 %. The samples from the first and second extractions were analysed separately but the total quantities are recorded in the Table. It was disturbing to note that samples taken close to one another did

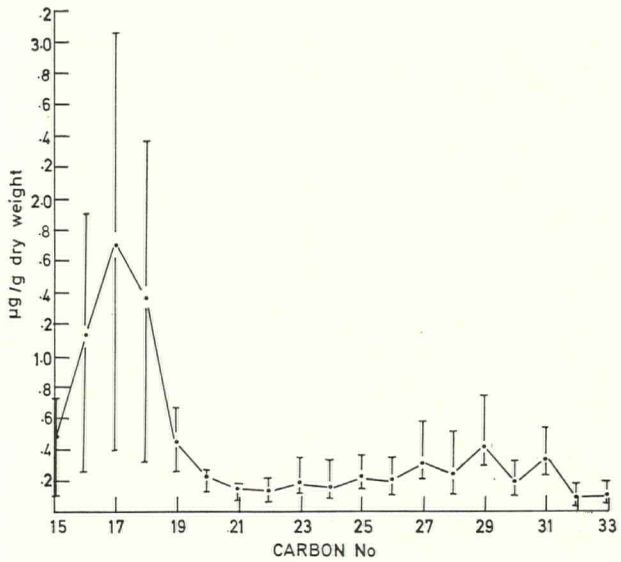

Figure 20. Sediment from Loch Ewe (wide core).

not give identical gas chromatographic patterns and, at least in this work, it appeared that extraction with pentane alone did not extract the alkanes and squalane to the same extent. The results on the four samples taken show a peculiar division almost as though two separate films were present. We conclude that erroneous values can be obtained if care is not taken to

Table 3. *n*-Alkane content and composition of cod flesh and liver (µg/g wet wt)

Carbon number	62 days		42 weeks	
	Flesh	Liver	Flesh	Liver
15	0·002	0·10	0·001	0·06
16	0·002	0·07	0·002	0·03
17	0·002	0·07	0·004	0·11
18	0·007	0·04	0·006	0·01
19	0·003	0·04	0·007	0·04
20	0·004	0·05	0·004	0·06
21	0·006	0·05	0·004	0·04
22	0·007	0·07	0·006	0·04
23	0·011	0·12	0·009	0·13
24	0·015	0·12	0·014	0·08
25	0·019	0·17	0·020	0·20
26	0·020	0·27	0·023	0·21
27	0·019	0·54	0·023	0·32
28	0·015	0·47	0·018	M*
29	0·013	0·40	0·014	0·24
30	0·008	0·12	0·010	0·08
31	0·008	1·38	0·009	2·36
32	0·006	0·03	0·006	0·02
33	0·006	0·19	0·004	0·45
Σ	0·173	4·30	0·184	4·48
Pristane	0·008	0·22	0·012	0·24

*M – masked by squalane

Table 4. Σ_{33}^{15} and Pristane in codling kept under identical conditions (μg/g wet wt)

		Flesh				Liver			
62 Days	Σ	0·375	0·173	0·151		4·16	1·64	4·30	
	Pristane	0·009	0·008	0·008		0·17	0·19	0·22	
126 Days	Σ	0·179	0·166	0·182		2·81	3·51	1·35	
	Pristane	0·084	0·031	0·031		0·27	0·45	0·30	
175 Days	Σ	0·140	0·155	0·167		5·58	5·63	3·52	
	Pristane	0·005	0·008	0·005		0·15	0·17	0·12	
245 Days	Σ	0·186	0·229	0·198		8·72	7·42	7·33	
	Pristane	0·008	0·017	0·012		0·28	0·30	0·29	
294 Days	Σ	0·097	0·096	0·120	0·184	2·20	4·48	3·84	3·11
	Pristane	0·012	0·010	0·011	0·012	0·06	0·24	0·11	0·10

	Σ	PR	Σ	PR
Minimum value	0·096	0·005	1·35	0·06
Maximum value	0·375	0·084	8·72	0·45
Arithmetic mean	0·175	0·017	4·35	0·21
Coefficient of variation	34%	118%	49%	48%

extract all the hydrocarbon material and that furthermore the composition and content of the surface film can be heterogeneous even within such small sampling areas. If the amounts of alkanes in the surface film are to be determined in future by sampling with this screen procedure then extraction methods should be examined further. Laboratory produced oil films are not wholly satisfactory as a test system since the hydrocarbons in natural conditions are closely associated with more polar lipid material which is not readily soluble in pentane. Experiments designed to find more suitable extraction methods are under way.

SUB-SURFACE WATER

The results (Figs. 16 and 17) are presented in the same form as for the Loch Ewe surface film. Again there are considerable variations in the concentrations of individual and total *n*-alkanes although the variations in the former are again more pronounced. We did, however, obtain an efficient extraction of both the alkanes and the squalane. This was shown by re-extracting the samples with chloroform; although a large quantity of more polar lipid material was obtained no hydrocarbons were present. Both the Loch Ewe and Port Seton extracts have essentially the same envelope with the main peak at C_{26}. On the basis of these analyses it would seem that alkanes are not distributed homogeneously in the water. Perhaps this is to be expected if they are present, absorbed or adsorbed on particulate material.

SEDIMENT

It must be stressed that the cores analysed in this experiment were taken from the intertidal zone at low tide. Thus, the variations discussed here are probably not the same as those encountered in open sea sediments, since it is likely that the intertidal zone is subject to more disturbance. It is immediately obvious from the results presented in Figures 18–20 that the sediments from the two areas are quite different. The Port Seton samples do not possess the odd carbon atom predominance normally associated with recent sediments. This is not due to any masking effect since all alkanes having high carbon numbers are present at very low concentrations.

The carbon preference indices at Loch Ewe vary from 1·3 to 2·1 with a mean value of 1·6 (coefficient of variation 24 %) while those at Port Seton are lower and much more constant (1·0 to 1·3 with a mean of 1·1 and a coefficient of variation 8 %).

In the limited repetitive sampling carried out in this study considerable variation between samples was observed. It is concluded therefore that values from single samples represent only that particular sampling site and

probably provide only an approximate indication of the levels in the general sampling area.

FISH

The results of two single fish samples, including both liver and muscle tissue analyses near the beginning and at the end of the period of holding are given in Table 3. The total *n*-alkane and pristane levels for all the fish are presented in Table 4. There is still considerable variation although less than with the water and sediment samples. No analyses have been done on several individual wild fish caught at the same time where the scatter might be expected to be greater because of the variable environmental conditions.

The difference in *n*-alkane content and profile between the flesh and liver is immediately obvious with the liver containing an order of magnitude more *n*-alkanes than the flesh.

One can conclude from this work that even when samples are taken from small well defined sites or from fish that have been kept under identical conditions a considerable scatter in the *n*-alkane levels is obtained. For greater precision it would be essential to carry out considerably more analyses. Although this is of interest from the point of view of determining the likely scatter in the results replicate sampling on such a scale would be impractical in pollution monitoring. It can be questioned whether for many purposes greater precision is required since the values observed in this work and elsewhere are low. Thus, it would be relatively easy to detect the presence of large inputs of oil, as for example from tanker washings which at present probably enter the sea at a mean oil concentration of around 100 µg/ml (IMCO, 1973).

REFERENCES

IMCO 1973. "The environmental and financial consequences of oil pollution from ships". Preparation for International Marine Pollution Conference 1973. Report of Study No. VI submitted by the UK, Appendices 1, 224, p. 1.18, Programmes Analysis Unit, Didcot, UK.

Mackie, P. R., Whittle, K. J. & Hardy, R. 1974. Hydrocarbons in the marine environment I. *n*-Alkanes in the Firth of Clyde. Estuar. & Coast. Mar. Sci., 2, 359–74.