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: 143–146. 1977.

PETROLEUM TAINTING IN FISH

P. Howgate[1], P. R. Mackie[1], K. J. Whittle[1], J. Farmer[1],
A. D. McIntyre[2] and A. Eleftheriou[2]

[1] Torry Research Station, P.O.Box 31, 135 Abbey Road, Aberdeen AB9 8DG, U.K.
[2] Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen, AB9 8DG, U.K.

"Petroleum" taints in fish flesh do not necessarily arise by petroleum contamination. Such taints are well known in certain fisheries and have been traced to natural dietary components. Tainting of the flesh which is due to petroleum contamination, however, is usually accompanied by the presence in the flesh of hydrocarbons derived from the contaminating source, but these hydrocarbons are not necessarily responsible for the taint. Experimental tainting studies on benthic organisms by using sediment contaminated with a North Sea crude oil are described.

INTRODUCTION

Hydrocarbons can be assimilated into the tissues of fish either from the environment or diet. Thus, from the work of Ogata and Miyake (1970), Shipton et al. (1970), Connell (1971, 1974), Deshimaru (1971), Mackie et al (1972) and Motohiro and Inoue (1973) it is clear that, following contamination of the environment with crude oil, petroleum products or refinery effluent, both aliphatic and aromatic hydrocarbons can be assimilated into the muscle tissues of fish such as mullet (*Mugil japonicus* and *M. cephalus*), yellow tail (*Seriola quinqueradiata*), trout (*Salmo trutta*), Black Sea bream (*Mylio macrocephalus*) and chum salmon (*Onchorhyncus keta*). At the same time, the muscle tissues of these fish were found to be tainted. However, only in the case of grey mullet, caught in the vicinity of a petroleum refinery complex, were Ogata and Miyake (1970) able to show clearly that a hydrocarbon (toluene) deposited in the muscle was largely responsible for the offensive odour. They were able to reproduce the effect in eels kept in similarly contaminated water.

Deshimaru (1971) fed yellow tail for 13 days on a diet containing 1 % crude oil. After 8 days a slight, oily taste was perceptible in the muscle and oil-derived hydrocarbons were detected in analyses of the head space vapours of the tissue. Sidhu et al. (1972) reported that mullet became tainted in sea water containing 5 µg/ml kerosene whilst Deshimaru (1971) in his series of experiments found that yellow tail were tainted after 5 days in sea water containing 50 µg/ml crude oil.

Thus, although some species of fish become tainted in the presence of crude oil or petroleum products and it can be shown that petroleum derived hydrocarbons are associated with the muscle tissue, only in the one case so far, Ogata and Miyake (1970), has it been established clearly that a hydrocarbon (toluene) was also responsible for the taint. We cannot and should not conclude therefore that hydrocarbons as a class are responsible for "oily taints". Indeed, similar taints have in the past been wrongly attributed to petroleum, being confused with those arising from dimethyl sulphide (DMS), the source of the well known "petroleum odour" of canned salmon (Motohiro, 1962). He showed that the DMS was formed by the thermal decomposition of dimethyl-β-propiothetin (DMP). DMP occurs naturally in phytoplankton and is passed on to fish in the food chain via particular zooplankton and, by a similar mechanism, accounts for the "blackberry" condition of Labrador coastal cod and the "gunpowder" condition of fish in certain Norwegian and Greenland waters (Ackman et al, 1967).

The work reported subsequently in this paper describes the assessment for tainting of plaice (*Pleuronectes platessa*), Norway lobster (*Nephrops norvegicus*) and brown shrimp (*Crangon crangon*), kept in large tanks for up to 10 days on a sandy bottom, the surface layer of which was coated with a North Sea crude oil. These are the preliminary results of a programme to examine substances present in the oil which are likely to cause tainting.

MATERIALS AND METHODS

Test groups of plaice (57), Norway lobster (126) and brown shrimp (623) were each kept in sea water tanks (4×2×1·5 m) containing a layer (7—8 cm) of sea water-washed beach sand (water depth, 0·3 m),

as were control animals. Three further tanks were prepared in the same way for subsequent treatment with oily sand.

OILY SAND PREPARATION AND EXPOSURE OF ANIMALS

A homogeneous 10 % (v/v) oil-in-sand mixture was prepared for each tank by adding a solution of either a North Sea or a topped Kuwait crude oil in pentane (1:1, v/v) to dry beach sand in a cement mixer. The mix was turned out and spread thinly in the open air to allow the solvent to evaporate (1–2 h). It was then scattered through the water on to the bottom of a tank to provide as even a layer as possible about 0·5 cm thick. Some oil separated from the sand at this stage. Sea water was constantly trickled into the tanks so as not to disturb the oily sand layer. The system was allowed to leach to waste for 24 hours, any floating oily scum being removed via an overflow.

The test animals were transferred to their respective tanks after setting aside sufficient numbers for a zero time test. The effects of the Kuwait oil were tested only on shrimp. The Norway lobsters were caged individually to prevent cannibalism. Animals were withdrawn daily up to 10 days as mortality and numbers allowed, washed in running fresh water and subsequently handled only with forceps or tongs. The plaice were gutted and the crustaceans headed. After 10 days, the plaice gills (but not skin) and the crustaceans' tails had a slight, oily smell. The samples were wrapped carefully in clean, aluminium foil, frozen and stored at –30°C until required for the tasting sessions.

TRAINING OF THE PANEL OF ASSESSORS

Prior to assessing the samples, a panel of assessors was selected and trained to recognise samples artificially contaminated with the same North Sea crude oil. It consisted of 4 staff directly associated with the project and 4 who were experienced in tasting fish and were known to be discriminating assessors. Initially, the panel was familiarised with the flavour and odour of the oil either dissolved in deodorised squalene at concentrations between 50 µl and 25 ml/litre or as aqueous extracts. The aqueous extracts, which it was thought might be analogous to the material leached from the oily sand, were prepared by slowly stirring for 24 hours a layer of oil above water (1:5, v/v) without forming a vortex. 25 % of the aqueous layer was removed and diluted in binary fashion to 1/128th of the original concentration. The panel's performance in ranking the samples was monitored by the rank correlation test of Snedecor and Cochran (1967). Subsequently, the panel was asked to rank cooked fish samples contaminated with the oil. These were prepared by adding 2 ml aliquots of varying concentrations of oil in pentane to 100 g portions of skinless plaice fillets. The solvent was allowed to evaporate and the fillets were then cooked for 30 min in closed glass casseroles on a steam bath. The cooked portions were mixed throughly and sub-divided for presentation to the assessors.

It was assumed that the response of an assessor to the stimulus presented obeyed Weber's law (Amerine et al, 1965), i.e. equal ratios of concentration produce equal differences in response. Increments in concentration (×2 or ×3) were selected in the range 5 to 270 µl/kg of oil in fillet. Poor discrimination between samples occurred below 10 µl/kg and this was accepted as the threshold level of detection. As expected the best correlations between the assigned and known rankings were obtained for three-fold increments. Based on these findings an intensity scale was drawn up in unit steps from 0 to 3 corresponding to concentrations of 10, 30, 90 and 270 µl/kg according to the relationship:

$$S = 2{\cdot}096\ (\log_{10} C - 1{\cdot}0)$$

where S is the score and C is the concentration in µl/kg. The equation is that expression of Weber's law proposed by Fechner (Amerine et al, 1965) to relate physical stimulus to subjective response.

The panel was next trained to use this scale to quantify prepared oil-tainted plaice fillet samples by reference to standards at the same concentrations as above. They were screened for discrimination by comparing their scores with the expected scores. The pooled mean standard deviation for the selected best assessors was 0·78.

ASSESSMENT OF TEST SAMPLES

The test samples were thawed. No oily odours were associated with the plaice but they were associated with the shellfish. Nevertheless, the fish were carefully filleted to prevent cross contamination in handling. Similar precautions were taken in peeling the shellfish. In some cases it was necessary to combine samples from consecutive exposure periods on the oily bottom to provide sufficient material for tasting. Each species was assessed at a separate taste panel session. For this purpose the sample (skinless, filleted plaice or shelled crustacean meat) was cooked in the manner described earlier, thoroughly mixed and presented in coded, random order to a panel of 5. Reference standards prepared and treated as above from a further batch of the appropriate species were presented at the same time.

RESULTS AND DISCUSSION

The mean panel scores are given in Table 46 and it should be noted that all panel members recorded the

Table 46. Mean panel scores for oily taint assessment of animals kept on a sandy bottom contaminated with a North Sea crude oil

Sample	Treatment (days on oily bottom)	Treatment mean score	Standard error of a mean score	Equivalent oil concentration µl/kg
Plaice.....	0	0·10		< 11
	1/2	2·40		
	3/4	2·40	0·35	
	5/6	2·70		160[1]
	7/8	2·60		
Norway lobster...	0	0·50		17
	1/2	2·25		118
	3/4	2·92	0·32	247
	5/6/7	>3·00		>270
	9/10	2·17		109
Brown shrimp....	0[2]	–		–
	2	2·25		118
	3	2·38	0·39	137
	4	1·88		79
	1[3]	0·38		15

[1] Only the mean value for 1–8 days is given since the individual mean scores are not significantly different.

[2] The commercial reference sample was slightly tainted so the panel was told for reference that the 0 day sample was such.

[3] For comparison these shrimps had been kept on a bottom treated with topped Kuwait crude oil.

intensity of the 5/6/7 day Norway lobster sample as greater than that of the most concentrated standard. It may be generally concluded from the results that, after exposure of 1—2 days on the contaminated bottom, the muscle tissues of the organisms were tainted at a level equivalent to 100—150 µl/kg whole North Sea crude oil in the muscle. The comparison between the two crude oils used in the shrimp experiments is interesting. After one day exposure to the topped Kuwait crude oil, the shrimps were tainted only to a level just above the apparent threshold value. This is to be expected if the volatiles which were removed in topping the oil are components of the taint. Incidentally, this crude was found to be much less toxic to *Crangon* than the North Sea crude. The latter was found to be most toxic to *Crangon*, less so to *Nephrops* and not at all to *Pleuronectes* under the experimental conditions described. The tendency for the apparent taint concentration to decrease with time during two of the trials may be due to continued leaching from the oily bottom to waste, a reduction in concentration of taint substances in the water and partial depuration from the tissue.

As a general note of caution in examining the nature of the taint it is worth pointing out that the assessors recognised subtle differences in the characteristics of the odour and flavour between the standards and test samples and between the aqueous extracts and solutions

Table 47. Adsorption chromatography and odour assessment of an aqueous extract of North Sea crude oil

Elution pattern from activated silicic acid	Odour assessment	
% ether in pentane mixtures	Pentane extract of aqueous oil extract	Pentane extract of water (blank)
0	nd	nd
2	nd	nd
10	nd	nd
25	tar (asphalt)	v. sl. leather
50	tar, creosote (sharp)	*v. sl. bakelite
100	sl. sweet, sl. tar	*sl. bakelite

nd = not detected
v = very
sl = slight
* typical faint odour of the solvent residue.

in squalene. Thus the "equivalent oil concentrations" given in Table 46 must be regarded as nominal concentrations.

Chemical analysis of a sub-sample of the animals used for tasting in order to determine the class of compounds responsible for the taint is not yet complete. However, we have been able to make a cursory examination of the nature of the components responsible for odour of aqueous extracts of whole North Sea crude oil prepared as described earlier. Most of the discernible odour of the aqueous extract was removed by solvent extraction with pentane. After concentration the pentane soluble material was subjected to adsorption chromatography on activated silicic acid and eluted with pentane followed by successive, increasingly polar mixtures of di-ethyl either in pentane (Table 47). The eluates were concentrated to a residue free from the characteristic odour of the solvents and their odours were compared with blanks obtained in the same way from a pentane extract of water uncontaminated with oil (Table 47). Capillary gas-liquid chromatography of the pentane extract of oil-containing water demonstrated the presence of aliphatic hydrocarbons. However, the compounds responsible for the "tarry" odours were eluted from the column with eluent mixtures too polar to have been expected to elute aliphatic, mono-, di- and tricyclic aromatic hydrocarbons.

If present, the aromatics which do have characteristic odours were at concentrations below the odour threshold. The results suggest that the "tarry" element of the odour of aqueous extracts of North Sea oil is too polar to be a hydrocarbon. Because of the differences noted by the taste panel between the odours of the various samples, it is too early to state whether this "tarry" note is a feature of the odour of the tainted

plaice or crustacean flesh. Clearly, comparative chemical analysis of the tainted fish and shellfish, both with the crude oil and aqueous extracts of it, must be coupled with taint assessment and evaluation.

REFERENCES

Ackman, R. G., Hingley, J. & May, A. W., 1967. Dimethyl–β-propiothetin and dimethyl sulphide in Labrador cod. J. Fish. Res. Bd Can., 24(2): 457–61.

Amerine, M. A., Pangborn, R. M. & Roessler, E. B., 1965. Principles of Sensory Evaluation of Food, 256–66, Academic Press, New York.

Connell, D. W., 1971. Kerosene-like tainting in Australian mullet. Mar. Pollut. Bull., 2(12): 188–89.

Connell, D. W., 1974. A kerosene-like taint in the sea mullet (*Mugil cephalus*) I. Composition and environmental occurrence of the tainting substance. Aust. J. Mar. Freshwater Res., 25: 7–24.

Deshimaru, O., 1971. Studies on the pollution of fish meat by mineral oils I. Deposition of crude oils in fish meat and its detection. Bull. Jap. Soc. Sci. Fish., 37(4): 271–301.

Mackie, P. R., McGill, A. S. & Hardy, R., 1972. Diesel oil contamination of brown trout (*Salmo trutta*). Environ. Pollut., 3: 9–16.

Motohiro, T., 1962. Studies on the petroleum odour in canned chum salmon. Bull. Fac. Fish. Hokkaido Univ., 10(1): 2–65.

Motohiro, T. & Inoue, N., 1973. *n*-Paraffins in polluted fish by crude oil from "Juliana" wreck. Bull. Fac. Fish. Hokkaido Univ., 23(4): 204–08.

Ogata, M. & Miyake, Y., 1970. Offensive odour substance in the evil-smelling fish from the sea polluted by petroleum and petrochemical industrial waste. Report 1. Identification of offensive odour substance. Acta. Med. Okayama, 21: 471–81.

Shipton, J., Last, J. H., Murray, K. E. & Vale, G. L., 1970. Studies on a kerosene-like taint in mullet (*Mugil cephalus*) II. Chemical nature of the volatile constituents. J. Sci. Fd Agric., 21(8): 433–6.

Sidhu, G. S., Vale, G. L., Shipton, J. & Murray, K. E., 1972. Nature and effects of kerosene-like taint in mullet (*Mugil cephalus*). *In* Marine pollution and sea life, pp. 546–50. Ed. by M. Ruivo, Fishing News (Books) Ltd., London.

Snedecor, G. W. & Cochran, W. G., 1967. Statistical Methods, 6th edition, pp. 194–95. Iowa State University Press, Iowa, USA.