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.

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FATE OF HYDROCARBONS IN FISH

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Juvenile herring were fed an experimental diet of squid containing [^{14}C]—labelled hexadecane and benzo[*a*]pyrene and the distribution of activity in various tissues and organs was assayed 43–45 h later. 24–25% of the activity fed was recovered in the tissues and organs examined but the distribution of activity in these was quite different for each hydrocarbon. Most of the hexadecane recovered was found in the muscle but most of the benzo[*a*]pyrene remained in the stomach.

INTRODUCTION

A review of the information available on the biological fates of petroleum hydrocarbons in the marine environment may be found in the report of the Workshop sponsored by the Ocean Affairs Board of the National Research Council-National Academy of Sciences (NAS, 1975). The literature specifically concerned with fish was reviewed briefly by Corner et al (1976) and included a summary of their current experiments on the fate of hydrocarbons in fish. Related information, but especially relevant to the problem of tainting in fish, is given in this volume by Howgate et al (1977).

Whittle et al (1977), in their report of the results for the UK survey of hydrocarbons in marine organisms, pointed out the difficulties in establishing the origin of the *n*-alkanes found in the organisms, including fish, which were collected from a variety of areas some of which were thought to suffer chronic input of petroleum hydrocarbons. In order to assist the interpretation of such data, we initiated an examination of the production, assimilation, transfer and metabolism of hydrocarbons at various trophic levels. Some aspects of this work, which is not yet complete, have been described elsewhere in this volume (Murray et al, 1977). This paper briefly reports some preliminary results of feeding [^{14}C]-labelled hexadecane and benzo[*a*]-pyrene to juvenile herring.

EXPERIMENTAL

Young herring were caught in Loch Etive and reared in the laboratory by Dr John Blaxter, SMBA, Oban, Scotland. When used in our experiments they were 107 –152 mm long with a body weight of about 10 g and a muscle lipid content of 3·5 %. A group of 15 were trained to accept cubes of squid (3 mm, coated with gelatin) dropped into the aquarium individually.

Aliquots (25 µl) of benzene containing approximately 5 µCi of either *n*-[1-^{14}C]-hexadecane or [7, 10-^{14}C]-benzo[*a*]pyrene were evaporated at room temperature onto the cubes of squid which were then coated with gelatin. Some of each type of cube were set aside as reference standards.

With 3 observers present it was possible to recognise and track within the group, 3 individual fish which accepted the squid. (The success of this observation was monitored later by identifying the labelled component in the stomach of a fish). Then, 30 minutes after 3 fish had each eaten a piece of squid containing the appropriate hydrocarbon they were transferred to one of 2 tanks set aside for the trials. The next three were then fed and the sequence repeated. Each group was killed 43–45 h after feeding. The fish were carefully dissected and, after storage at –30°C, the organs and tissues of each group (Table 44) and the reference squid pieces were extracted with solvent as described by Mackie et al (1974). However, to improve the efficiency of the extraction procedure, each liquid phase was washed with fresh opposite phase, the residual tissue was re-extracted and the phases washed in the same way. Like phases were bulked. The [^{14}C]-activity of the various extracts was assayed and the lipid extracts fractionated by adsorption chromatography on deactivated silicic acid, TLC or gas-liquid chromatography. These methods and the techniques of radioassay are described by Murray et al (1977).

RESULTS AND DISCUSSION

The results of the assay of the fractions from extraction of the tissues and organs from each experiment are shown in Table 44. The activity of each of the component fractions obtained from the various tissue samples is given in terms of percentage of total activity

Table 44. The activity recovered in the fractions of the solvent extraction of the tissues and organs of herring fed with ^{14}C-labelled hydrocarbons showing percentage distribution

*Tissue or organ	45 h after feeding Hexadecane						43 h after feeding Benzo[*a*]pyrene					
	lipid		aqueous		residual		lipid		aqueous		residual	
	A	B	A	B	A	B	A	B	A	B	A	B
Stomach	95·70	2·81	0·55	3·04	3·75	1·49	97·56	87·10	0·27	7·59	2·17	31·26
Pyloric caecae	97·52	8·98	0·59	10·22	1·89	2·36	41·99	1·29	16·74	16·22	41·27	20·48
Liver	95·61	7·41	0·59	8·60	3·80	3·99	14·29	0·06	22·21	2·96	63·51	4·34
Bile	61·39	0·08	37·28	8·67	1·33	0·02	11·56	0·05	61·65	8·32	26·79	1·85
Intestine	96·36	7·09	1·67	22·96	1·98	1·97	73·75	11·25	12·26	58·90	13·99	34·41
Mesenteric fat	99·07	7·75	0·08	1·11	0·86	0·91	36·46	0·02	16·25	0·24	47·29	0·36
Muscle	90·15	58·61	0·31	37·30	9·54	83·99	16·11	0·08	26·58	4·19	57·31	4·62
Brain	91·70	0·33	4·26	2·86	4·03	0·20	49·38	0·01	16·05	0·07	34·57	0·08
Gills	94·51	6·95	0·38	5·25	5·11	5·09	40·42	0·14	13·60	1·50	45·98	2·60
The extract fractions summed as % of total activity recovered in fish	92·66		0·50		6·84		91·42		2·91		5·67	
% of total activity recovered from squid food	93·53		3·44		3·03		99·45		0·30		0·25	
% recovery of the activity fed			25						24			

*includes contents where appropriate

A % distribution between the fractions of the extract for each tissue

B % distribution between the tissues for a particular fraction of the extract

(i) recovered from the fish and (ii) present in the tissue. After filtration of each aquarium tank, 0·2 % and 5·4 % of the activity fed was recovered as "tank solids" (perhaps faeces) for the hexadecane and benzo-[*a*]pyrene experiments respectively. Also given in Table 44 is the distribution of activity in the fractions from extraction of the labelled squid cubes. The percentages for the lipid fraction are typical of the values obtained from recovery experiments when tissue lipids are extracted (Mackie et al, 1974). Only 24—25 % of the activity fed may be accounted for, most of which was recovered in the tissues and organs.

The broad conclusion from Table 44 is that most of the activity in the tissues examined is present in the lipid fraction. In the hexadecane trial the activity in the bile lipid (61·4 %) is considerably less than that in the other tissues (90·1—99·1 %); the remainder of the activity in the bile is largely in the aqueous fraction (37·3 %) but it represents only 0·04 % of the total activity recovered in the fish. The greater proportion of

Table 45. Distribution of activity in the lipid extracts by area measurement of active peaks identified by radio-TLC scanning of the tissue lipid extracts

Solvent system	Hexane		1% ether in hexane	
Hydrocarbon used	Hexadecane		Benzo[*a*]pyrene	
Radio/TLC peak, Rf	0·62	0·0	0·16	0·0
Sample				
Hydrocarbon std. . . .	100	–	84	16
Squid pellet	>99·5	<0·5	>99·5	<0·5
Stomach	100	–	57	43
Pyloric caecae	77	23	35·5	64·5
Liver	65	35	–	100
Bile	100	–	–	100
Intestine	73·5	26·5	38	62
Mesenteric fat	100	–	–	–
Muscle	100	–	–	–
Brain	100	–	–	*
Gills	<0·5	>99·5	–	*

std = standard
– = no activity detected
> = greater than
< = less than
* = some activity detected, not quantified

the lipid activity is found in the muscle (54·3 % of the activity in the fish) whilst mesenteric fat, liver and brain tissues are 7·2 %, 6·9 % and 0·3 % respectively and stomach, pyloric caecae and intestine together accounted for 17·5 %. The major non-lipid activity is found in the muscle residue representing 5·7 % of the activity recovered in the fish.

The major feature of the benzo[*a*]pyrene results is the recovery in the stomach lipid of 79·6 % of the total activity present in the fish. The largest proportion of the remainder of the activity is found in the lipids of the intestine (10·3 %) and the residues of the stomach, pyloric caecae and intestine (4·9 %). In contrast to the hexadecane result, most of the activity recovered in the bile is in the aqueous fraction but it represents only 0·2 % of the total activity recovered in the fish. Again, in contrast to the hexadecane result, only 0·07 % of the lipid activity in the fish is recovered in the muscle and 0·05 % in liver. Despite the careful extraction procedure, a relatively large proportion of activity remains with the residual fraction in many of the tissue extracts.

Using the radio-TLC technique the lipid fractions from the experiments were separated with hexane or 1 % diethyl ether in hexane as solvent and compared with authentic [^{14}C]-labelled hydrocarbon reference standards. Activity in the fractions from the hexadecane experiment coincided with authentic *n*-hexadecane but, in some cases, (pyloric caecae, intestine, liver and gills) a second active peak (Rf 0·0 in hexane), present in varying proportions (23 %, 26·5 %, 35 % and 99 % respectively by area measurement), was noted (Table 45). This component, which may well be a metabolite, was not present in significant quantities in the [1-^{14}C]-hexadecane standard or in the lipid extract of the reference squid/hexadecane samples made up as the original food. The liver lipid extract was chromatographed on a small column of deactivated silicic acid and, after elution of the [^{14}C]-hexadecane with hexane, the secondary activity was eluted with diethyl ether. Radio-TLC of the ether concentrate after double development in chloroform suggested that the activity coincided with authentic long chain fatty acid. Although this system resolves fatty acids from fatty alcohols, neither individual acid nor alcohols are separated within their respective groups. The presence of a substance of this nature was confirmed by radio-autography of a 2-dimensional TLC of the ether concentrate after double development with chloroform in the first dimension and single development with 20 % diethyl ether in hexane in the second dimension. Preliminary examination of the methyl ester derivatives of this fraction suggested the presence of small amounts of C_{14}, C_{16} and C_{18} normal fatty acids and the corresponding monoenoic acids.

Radio-TLC of the benzo[*a*]pyrene lipid extracts in 1 % ether in hexane showed that two active components could be present in varying proportions (Table 45), one of which coincided with authentic benzo[*a*]-pyrene whilst the other (Rf 0·0) was usually the major component in tissues other than the stomach. This component, which may be a metabolite, seems to have similar properties to an impurity present in the original benzo[*a*]pyrene and also detected in the treated squid cubes but its presence in the tissues cannot be explained simply on the basis of accumulation of the impurity present initially.

It is clear that in the hexadecane trial significant proportions of the fed hydrocarbon were deposited in the muscle but less so in the mesenteric fat. It would be valuable to compare rates of accumulation and rates of clearance during depuration of the hydrocarbons in these two tissues since the survey results of Whittle et al (1977) show that the alkane distribution in these tissues is likely to be quite different. Benzo[*a*]pyrene was also detectable in both tissues although at a very low level but its distribution, compared with hexadecane, is complicated by its slow release from the stomach. In the case of the hexadecane trial, 18·1 % of the total activity recovered in the fish was found in the stomach, pyloric caecae and intestine, the percentage for stomach alone being 2·7 %, whereas by comparison in the benzo-[*a*]pyrene trial, 98·4 % of the total was found in the same organs, the percentage for stomach alone being 81·6. Since there is no evidence to the contrary, it

seems reasonable to assume that the two groups of fish may be compared legitimately, indeed, each fish in each group was fed approximately the same quantity of squid and hydrocarbon, the subsequent period of digestion was similar, inspection of stomach content, gut and gall bladder showed no obvious differences between the two groups in the passage of food through the alimentary system and, the total recovery of activity in each trial was very similar. Thus the differences found in the manner and rate of absorption and assimilation between hexadecane and benzo[*a*]pyrene from the stomach appears not to be attributable simply to a slower rate of digestion. Retention, then, seems to imply strong adsorption or binding to the stomach wall, but not absorption across it even although about 40 % of the activity recovered in the stomach had been converted to a more polar form than the original benzo[*a*]pyrene. The effect of this retention is to reduce the absorption and consequently the assimilation of benzo[*a*]pyrene compared with hexadecane when both are ingested with food. This contrasts sharply with the rapid absorption of benzo[*a*]pyrene across the surface of the gills of marine fish noted by Lee et al (1972) in animals exposed to benzo[*a*]pyrene in the aquarium water.

Distributions in the tissues contrast with those briefly described for codling (*Gadus morhua*) by Corner et al (1976) in which the extragastric activity found 96 hours after feeding showed that hexadecane and benzo[*a*]pyrene either had been excreted or, were *en route* to excretion with little or no tendency for deposition in muscle tissue. In the codling experiments the benzo[*a*]-pyrene-fed fish accumulated activity in the bile of the gall bladder. Between 85 % and 40 % of the activity was still present in the stomach and, in direct contrast with the herring, none was recovered from the muscle tissues. The results of the experiments on both species only provide information on the changes which had occurred after ingestion of the hydrocarbons by the time the fish were removed for analysis and provide little information on the sequence of these changes. Thus analyses at intervals after ingestion would be more useful both for comparative purposes and to provide some further information to determine clearly whether the differences between the two species are due largely to their different lipid metabolism.

Whittle et al (1974), on the basis of the results of feeding oil to codling as well as comparative analyses of planktonivorous fish and their diet, proposed that hydrocarbon deposition in fish tissues depended on the location of the neutral lipid stores in species according to the arrangement of their lipid metabolism. This provides a rationale for some of the features of tainting in fish (Corner et al, 1976; Howgate et al, 1977). Additional support for this proposal may be adduced from the survey data on marine organisms presented earlier in this volume (Whittle et al, 1977) in which the tendency was noted for high *n*-alkane values to be associated with high lipid contents in the tissues and, in general, for liver concentrations to be higher than those in muscle. It is important to note, that the hydrocarbons were deposited in the herring muscle even though the lipid content of the tissue in the fish used was at the low end of the range for this species. This seems to emphasise the view (Whittle et al., 1974) that in fish with herring-type lipid metabolism some dietary hydrocarbons are quickly assimilated in the muscle, whilst fish with a cod-type lipid metabolism do not assimilate dietary hydrocarbons in the muscle but, instead, in the liver, and with some discrimination. We suspect too that the turnover rate of hydrocarbons in the herring muscle is more rapid than hitherto expected but that the rate of depuration in cod liver is less so.

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