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THE FATE OF n-ALKANES IN MARINE ORGANISMS

by

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Summary

The significance of the n-alkane distribution in the tissues of fish in relation to the content of these compounds in their diet is discussed.

Introduction

There can be no doubt that all marine organisms, pelagic, demersal or benthic, are exposed to hydrocarbons of petroleum origin whether the source be accidental spill, eolar input or persistent loss and wastage from industrial practice. In assessing the effects of hydrocarbon pollution of the marine environment on these organisms several questions arise.

What is the nature and extent of the hydrocarbon input?

Are these compounds assimilated and metabolised by the organisms?

Do hydrocarbons or metabolites accumulate in the food chain?

Is there a toxic effect?

Unlike many other organic pollutants (Widmark et al. 1972), components of some classes of petroleum derived hydrocarbon pollutants are identical to those produced naturally by marine organisms (Blumer et al. 1971; Youngblood et al. 1971; Avigan and Blumer 1968) although this cannot be said for the complex array of aromatic compounds present in certain crudes, fuel oils and other products. As might be expected, estimates of the input vary (Blumer et al. 1972; Jeffery 1971; SCEP 1970), since at best, such estimates by their very nature must be imprecise (Whittle et al., in press; Duce et al. 1974). Nevertheless, evidence of the input remains in reports of tar-balls, tainting of fish and shellfish and fouling of fishing gear and amenities. Considerable expenditure is required to report and make good the damage. Concern has led amongst other things to the establishment of load-on-top techniques for bulk transport and to careful control at major handling centres such as Milford Haven.

Studies on marine species of the toxic effects of crude oils and petroleum products, of dispersing agents and the like used to counter spillages and of the combined effects of both have resulted in the development of rather less toxic dispersants as well as concern for the effects on larval stages of marine life.

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We deal in this paper with the 2nd and 3rd questions posed initially; that is, with the assimilation, metabolism and accumulation of hydrocarbons in marine organisms and, in particular in this study, of n-alkanes in fish.

Results and Discussion

Our data have originated basically from two sources: firstly, from detailed survey work in UK coastal waters and the North Sea, some of which was reported to ICES last year (Whittle et al. 1973), and from instances of opportunity; secondly, from controlled feeding experiments. Details of the extraction methods and chromatographic techniques are given elsewhere (Mackie et al., in press).

So far our analyses of n-alkanes in organisms from the survey samples (Mackie et al., in press) have shown no evidence of accumulation at higher levels of the food chain and the levels of individual compounds are so low in fish flesh for instance (1-100 ppb) that it is very difficult to determine to what extent they are derived biologically or from petroleum. However, it is clear from analyses of planktonivorous species such as mackerel (Scomber scombrus) and sprat (Sprattus sprattus) that the array of dietary hydrocarbons can be assimilated unchanged into the flesh lipids although the relative proportions might be altered, as can be shown by following the alkane fraction right through the simple food chain – plankton, sprat and mackerel (Fig. 1). Unlike the n-alkanes, the branched alkane, pristane, shows some tendency to accumulate in these fish (Table 1) and lends some support to the idea that it is fairly stable in the food chain and so not subject to rapid turnover.

From area to area the composition of the plankton will vary, as it will at a particular site with season and this is reflected in the differing n-alkane distribution of mixed plankton samples from different areas at the same time of year and from the same area at different times of year (Table 2). The n-alkane patterns of the individual zooplankton species from a particular location are also quite specific (Table 2). Thus the n-alkane pattern in the diet of planktonivorous species is likely to vary considerably. Since we have noted above the good correlation between dietary and flesh alkanes of herring and mackerel from different areas and at different times of the year, even with an intermediate member of the food chain present in the latter case, it is likely that the flesh patterns will always closely reflect the dietary pattern. In turn, it follows that the turnover rate of the n-alkanes in the flesh keeps pace with the changing feed pattern, representing a dynamic system rather different from the case of pristane.

Rapid acquisition of an alkane pattern virtually identical to that of a diesel oil spill was noted in a sample of tainted trout (Salmo trutta) flesh (Mackie et al. 1972). Tainted mullet (Mugil cephalus) flesh was shown to have volatile components similar to those in kerosene (Shipton et al. 1970). It was reported to ICES last year (Blackman and Mackie 1973) that the n-alkanes of a topped crude oil in the diet of plaice (Pleuronectes platessa) were deposited in the flesh but were eliminated within 20-30 days of withdrawal of the oil.

Our controlled feeding experiments with cod (Gadus morhua) suggest that a rather different mechanism operates with this species. For six months a topped crude oil at a level of 1 mg/day was included in the diet of codling. The oil was withdrawn and recovery followed for a further six months. Analyses showed that the oil derived n-alkanes were not assimilated in the flesh to any discernible extent even after six months feeding, in contrast to the examples described above. Instead, rather

2

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dramatic changes were seen in the livers of these fish. Analysis of numerous field samples has shown that fish livers, compared to the flesh, generally show a characteristically high predominance of odd carbon number n-alkanes over even numbered members as measured by the Carbon Preference Index (Table 3). The n-alkane content of the livers of the oil-fed fish increased greatly (Table 4) and the odd carbon predominance present initially and maintained by the control fish was supplanted by a more even distribution akin to that of normal flesh (Fig. 2). Although the n-alkane levels diminished when oil was withdrawn a persistent fraction remained even after six months (Table 4) which maintained the abnormal liver n-alkane profile. By comparison with the n-alkane pattern of the fed oil it was possible to calculate the percentage retention in the liver of individual n-alkanes during oil feeding and find that the abnormal profile resulted from a 100-fold greater retention of the higher homologues (C25-C26) over the lower homologues (C16-C17) as shown in Figure 2. A similar low retention of the shorter chain compounds was noted when the diet of codling was spiked with 1^{14}-C-hexadecane. Furthermore, no evidence was found of deposition in the flesh or of metabolism of the hexadecane in the liver or gut.

Conclusion

With the information to hand, two clear patterns emerge of the way in which fish handle dietary or environmental n-alkanes. One group which is mostly comprised of species tending to store fat in the flesh assimilates n-alkanes into the muscle tissue. From a practical point of view we can suggest that the n-alkanes acquired in the diet and probably those acquired either directly or indirectly from the environment although deposited rapidly in the flesh lipids will just as rapidly be replaced by the changing dietary and/or environmental pattern. The cod is an example of the second group in which fat is deposited primarily in the liver and the n-alkanes where assimilated from the diet are found in the liver and not in the muscle. If in both groups the compounds in petroleum responsible for tainting behave in the same way as the n-alkanes the flesh of group one-type fish is much more likely to become tainted than that of group two.

It appears to us that n-alkane deposition in fish occurs at the sites of lipid deposition. The data available do not enable us to determine clearly whether the mechanism of deposition is an active or a passive one but the low retention of the labelled hexadecane and the apparent discrimination in retention amongst the homologues suggest that the latter mechanism is less likely.

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Table 1

Pristane and n-alkane content of plankton and planktonivorous fish

Date	Sample	Hydrocarbon, μg/100g wet wt	
		$\sum_{33}^{18}$ n-alkanes	pristane
April '71	Clyde, mixed plankton	3 246	13 708
April '71	Herring flesh	1 178	36 750
Aug. '73	L. Ewe, mixed plankton	701	256 080
Aug. '73	Whole sprat	463	965
Aug. '73	Mackerel flesh	188	2 831
Aug. '73	Mackerel liver	315	1 435

Table 2

The n-alkanes of plankton samples

Sample	Location	Date	Carbon No. 15	16	17	18	19	20	21	22	23	24	25	26	27	28	n-alkanes 29	30	31	32	ug/g dry wt 33
Mixed	Cruden Bay	7/73	ND	TR	.75	.33	.58	.75	1.33	2.62	5.16	7.34	9.07	9.59	9.32	7.41	6.32	4.01	5.62	3.08	2.97
Mixed	Burghead	7/73	TR	TR	.95	1.14	2.28	2.66	4.75	5.32	8.74	7.03	11.78	9.5	20.14	7.98	15.77	4.94	16.91	4.18	9.12
Mixed	Dunbar	6/72	ND	TR	4.50	3.22	6.43	7.07	7.72	8.68	10.61	12.86	16.07	18.0	17.36	15.11	12.54	9.32	10.61	6.43	7.07
Mixed	Port Seaton	6/72	65.9	62.0	54.3	65.9	64.0	60.1	52.3	44.6	52.3	38.8	36.8	34.9	34.9	23.3	27.1	17.4	17.4	9.7	5.8
Mixed	Loch Ewe	5/72	ND	ND	.14	.17	.14	ND	.11	.17	.42	.45	.42	.42	.67	.56	.34	ND	ND	ND	ND
Mixed	Loch Ewe	7/73	.30	.04	2	.06	.37	.09	.10	.06	.06	.06	.05	.05	.04	.03	.03	.02	.03	.01	.01
Pseudo-* Calanus	Loch Ewe	6/74	.23	.29	.21	.14	.17	.28	.45	.47	.68	.47	.68	1.05	1.37	1.34	1.23	1.17	1.14	.56	.22
Cladocera*	Loch Ewe	6/74	.05	.03	.01	.05	.01	.02	.02	.04	.04	.11	.10	.11	.15	.61	.71	.58	.49	.35	
			.05	.03	.01	.05	.01	.02	.04	.04	.11	.10	.11	.15	.61	.71	.58	.49	.35	.06	.04

ND - not determined

TR - less than .001

* in ug/100 animals

Table 3

A comparison of the Carbon Preference Index* (CPI) for fish livers and flesh

	Gadus morhua	Pleuronectes platessa	Squalus acanthias	Scomber scombrus	Notothenia rossii
Liver	2.44	1.86	2.15	2.40	2.61
Flesh	1.08	1.02	0.97	1.16	1.03

$$* \quad CPI = \frac{\left(\frac{\sum_{20}^{21}}{\sum_{30}^{31}} + \frac{\sum_{22}^{21}}{\sum_{32}^{31}} \right)}{2}$$

Table 4

Change in n-alkanes in the livers of codling fed with crude oil at 1mg/day

Days	Liver $\sum_{C33}^{C15}$ n-alkanes (μg/g tissue), mean values			
Oil Feeding	Control Fish	N	Oil-fed Fish	N
62	3.37	3	21.37	3
126	2.56	3	13.70	3
175	4.91	3	29.91	3
Recovery-oil withdrawn	Control Fish	N	Oil-fed Fish	N
70	7.82	3	16.12	3
119	3.41	4	13.30	5

FIGURE 1

ALKANES FROM PLANKTON SPRAT AND MACKEREL

Conditions: 50m, 0.25mm i.d. stainless steel column coated with Dexsil.
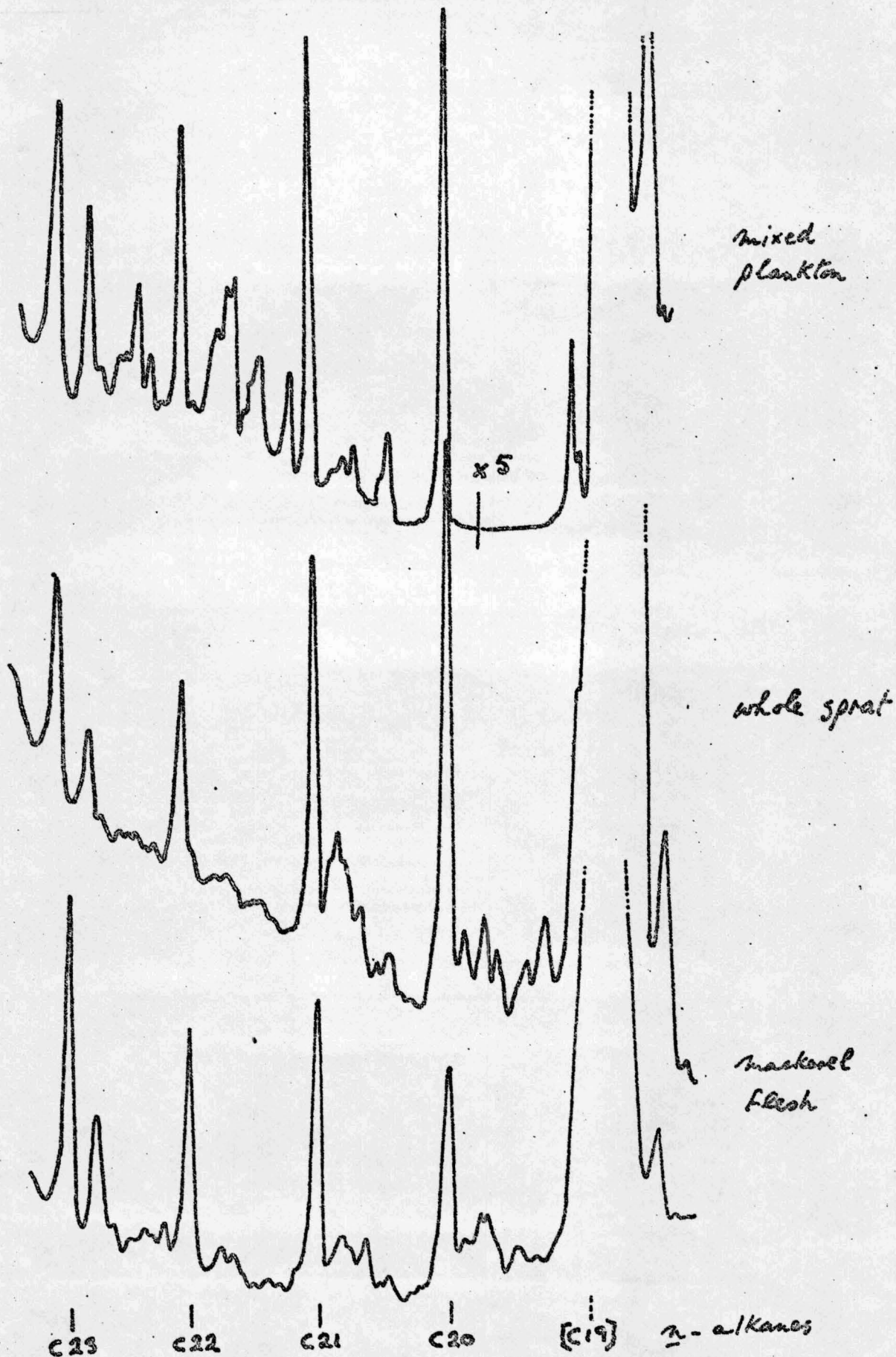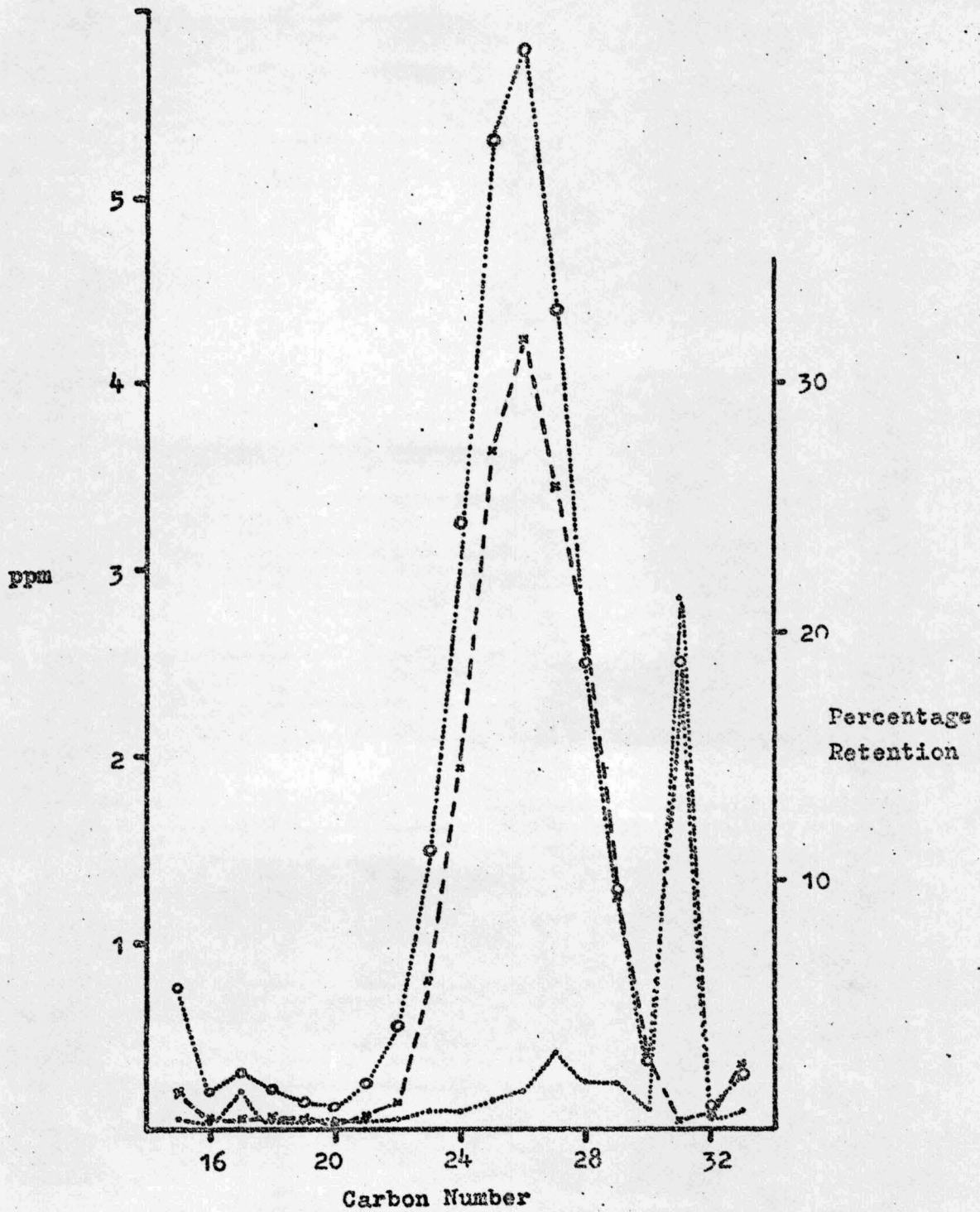Carrier gas nitrogen, 2.5 ml/min.

mixed plankton

x 5

whole sprat

mackerel flesh

C23   C22   C21   C20   [C19]   n-alkanes

FIGURE 2

DISTRIBUTION OF n-ALKANES IN OIL-FED AND CONTROL LIVERS OF CODLING
AND
PERCENTAGE RETENTION OF EACH ALKANE IN THE LIVER

x – – x   Percentage Retention ⎫
•——•   Control Liver   ⎬ after 175 days feeding
o——o   Oil-fed Liver   ⎭ values means of three fish