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
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Rapp. P.-v. Réun. Cons. int. Explor. Mer, 171: 202–211. 1977.

AN ASSESSMENT OF THE POTENTIAL CARCINOGENIC HAZARD OF PETROLEUM HYDROCARBONS IN THE MARINE ENVIRONMENT

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Crude petroleums are known to contain low concentrations of a number of substances mainly of the polycyclic aromatic hydrocarbon type, which can, on prolonged and intimate contact, cause skin cancer in man. Similar substances are present in the environment as byproducts of pyrolysis etc. and as natural constituents of certain vegetable foodstuffs.

These substances have only a very limited solubility in water and at such low concentrations should cause little hazard, but there is a possibility of their concentration in the food chain.

This paper attempts to examine all aspects of this problem in the light of published research in a variety of disciplines.

INTRODUCTION

The fact that certain substances in the environment can cause cancer in animals and in man has been recognised for at least 200 years since Pott's (1775) well-known description of Scrotal Cancer in chimney sweeps, and his attribution of this to the soot with which they came into contact. Over the intervening two centuries many chemical carcinogens have been identified (e.g. Bergel, 1974, Searle, 1970), some of these, particularly heterocyclic nitrogen compounds, have been shown to be very potent, whilst others only induce cancer after long and intimate contact. Amongst the latter class of substances are certain fractions derived from crude petroleums.

MINERAL OIL CANCER

In order to get the matter into perspective, a few points follow about oil-induced cancer. The incidence in the U.K. is very small compared with cancer attributed to other environmental carcinogens. For example, during the ten years 1950—59, Fife (1962) reported (for industries other than cotton) 79 notified cases of skin cancer due to mineral oil; of these 23 proved fatal, i.e. less than 2·5 per annum; however, in the one year 1967 deaths from lung cancer were reported to be in excess of 23 500 for men alone. It should also be noted that unfractionated crude oil is not known to have caused skin cancer. The cancers, which have been reported to have been induced by oil, have been caused by contact with certain fairly high boiling fractions into which the potential carcinogens have been concentrated. However, there is little doubt that a definite, if small, hazard does exist and this paper attempts to assess the significance of this hazard in respect of the marine environment.

Mineral oil cancer appears to be caused by direct contact and is normally a skin cancer usually associated with growths on the scrotum and less often of the hands and forearms. The use of mineral oils in food is illegal in many countries, but exemptions occur, as in the U.K., where a mineral oil content of up to 0·2 wt per cent is allowed (Statutory Instruments, No. 1073, 1966) to cover absorption from surfaces with which the food has necessarily been in contact e.g. from food processing machinery. Such oil content must consist only of highly refined white oils as defined in the regulation. Medicinal grade white oil is used internally as a mild aperient and in some pharmaceutical preparations. Occasional objections have been raised against its use internally on the grounds of carcinogenicity. But there is no reliable evidence that cancer can be caused by medicinal white oil (Biske et al, 1973).

The induction period between first contact with a carcinogen and the first signs of malignant growth is often lengthy, and in the case of mineral oils is normally found to be 20—50 years. Henry (1946) has reported cases where contact was as short as 6—7 years and as long as 70 years. Fuller details of the medical background of this problem have been published by several authors (e.g. Kipling, 1969).

NATURE OF CARCINOGENS IN MINERAL OIL

Work to identify the chemical nature of the carcinogens in mineral oil was initiated by the Medical Re-

search Council in 1948 under the auspices of the Carcinogenic Action of Mineral Oils (CAMO) Committee. A detailed report of the work of this committee was published in 1968 (SRS 306, Medical Research Council 1968). The chemical implications of this work were summarized by King (1969).

"The active material appears to be in materials boiling above 350°C (although its presence in lower boiling fractions is possible due to azeotropism), it is still present in fractions boiling at 420°C. It may be extracted with solvents such as acetone-water (which shows its general aromatic character) and it appears with the polycyclic aromatic fraction during adsorption chromatography."

"By a process of repeated chromatography, complexing with picric acid and trinitrobenzene and by fractional crystallization, over 40 chemical compounds were isolated from mineral oil fractions, many for the first time. As a result of these chemical studies it is possible to make further observations as to the nature of the active compounds, although no single, highly potent, carcinogen was identified. Several of the compounds separated were members of the same families as very potent carcinogens and it would appear that the total activity of the oil may be due to the combined effect of several individually rather weak carcinogens."

"Some of the compounds which were isolated and identified are shown in the table (see Table 69). Three ring compounds are generally not active carcinogens and the dimethyl anthracenes isolated were all non-active; however, it should be remembered that 9,10-dimethyl anthracene is active. 1,2-Benzanthracene whilst reported to be itself only weakly active is the parent of several known carcinogens. As an example of this, the 7-methyl-1,2-benzanthracene also isolated and positively identified, is weakly active to rabbit skin, but apparently not to mouse skin. Another member of this family is 6,7-dimethyl-1,2-benzanthracene, which was isolated but not, unfortunately, positively identified; this material is also a known, if somewhat weak carcinogen. (It is perhaps worth noting that although it has never been identified in the oil fractions, the standard carcinogen which was used in this work is also a member of this family, 9,10-dimethyl-1,2-benzanthracene). The activity of chrysene, which has been identified, is variously reported in the literature and it may be slightly active. Again several of the dimethyl derivatives of chrysene, but not any of the ones found and identified in the oil, are active. Of the heterogeneous compounds separated from the oil only, 1,3,6,7-tetramethyl dibenzthiophene is known to be weakly active."

Since the publication of the MRC work a number of other workers have succeeded in isolating benzo[*a*]pyrene [3,4-benzpyrene; henceforward referred to as B[*a*]P[1])] (e.g. Catchpole et al, 1971). This is a well-known potent carcinogenic hydrocarbon which is widespread in the environment (as will be shown later) and has been identified in coal tar distillates, cracked petroleum fractions and the combustion products of coal and oil.

Thus it seems highly probable that carcinogen(s) present in mineral oil are mainly polycyclic aromatic hydrocarbons (PCAH), although the presence of active hetrocyclic compounds (sulphur or nitrogen), compounds cannot be ruled out completely. In the remainder of this assessment it will be assumed that the hazard is due entirely to PCAH and, in particular, to B[*a*]P. It should be remembered, however, that there are apparently several carcinogens of this type present and there may also be promotors of co-carcinogens (SRS 306 supplement, to be published) present, all of which may serve to enhance the effect of the individual carcinogen(s).

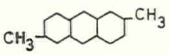
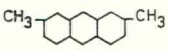
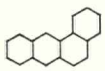
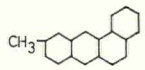
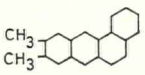
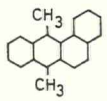
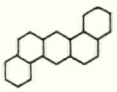
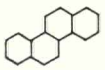
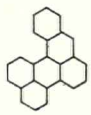
AMOUNT OF CARCINOGEN PRESENT IN CRUDE OIL

Not all the PCAH present in petroleum are carcinogens and although it is possible, by more or less complicated chemical and/or physical analysis, to obtain an estimate of the concentration of either all the PCAH or of certain specific individual components, it is virtually impossible at present to determine the total concentration of all the carcinogen(s) present, except by rather imprecise biological testing. By a careful consideration of the biological tests, the CAMO committee (SRS 306, Medical Research Council 1968) made an estimate for Kuwait oil, expressing the total carcinogenicity of the oil in terms of the standard carcinogen used in their work, 7,12-dimethyl benz[*a*]anthracene (DMBA). This amounted to approximately 100 parts per million (ppm) on a weight basis (or about 95 µg/ml). Note, however, that this estimate was based on the summation of the activity of various fractions of the crude, since no activity was found by skin painting test animals with the crude itself. This may have been because a concentration of about 100 µg/ml is just about the lower limit of sensitivity for the biological tests. Note also, that the activity is expressed in terms of DMBA but, if more potent materials or promotors or co-carcinogens are present, then the concentration would be lower.

Gräf and Winter (1968) reported concentrations of B[*a*]P equal to 1·66 µg/ml in Venezuelan crude, but it is not known if the method of analysis used did, in fact, indicate all the B[*a*]P present, since Catchpole et al

[1]) As an aid to the non-chemist, and particularly since several different naming conventions are used in the literature, the structural formulae of the polycyclic aromatic hydrocarbons etc. mentioned in the text, are given in Table 69.

Table 69. Structural formulae of

	(?)	Benzene
	(–)	Toluene
	(i)	Anthracene
	(i)	2,6-Dimethylanthracene
	(i)	2,7-Dimethylanthracene
	(i)	9,10-Dimethylanthracene
	(i, ?)	Benz[*a*]anthracene (1,2 Benzanthracene)
	(?)	10-Methylbenz[*a*]-anthracene (7 Methyl-1,2-benz-anthracene)
	(w)	9,10-Dimethyl benz[*a*]-anthracene (6,7-Dimethyl-1,2-benz-anthracene)
	(m)	7,12-Dimethylbenz[*a*]-anthracene (9,10-Dimethyl-1,2-benz-anthracene)
	(s)	Dibenz[*a*, *h*]anthracene (1,2,5,6,-Dibenzanthracene)
	(i)	Phenanthrene
	(?)	Chrysene
	(i)	Pyrene
	(s)	Benzo[*a*]pyrene (3,4-Benzpyrene)
	(w, ?)	Benzo[*e*]pyrene (1,2-Benzpyrene) (4,5-Benzpyrene)
	(i)	Perylene
	(–)	1,2-Benzperylene
	(–)	Benzo[*ghi*]perylene (1,12-Benzperylene)

Note:

The nomenclature used is in accordance with IUPAC standards. The names given in brackets are in accordance with other standards which have been used by authors quoted and are sometimes used in the main text (e. g. where the author is quoted directly).

(1971) using a similar technique only "removed" about one third or less, of B[*a*]P when this material was added, in known concentrations, to samples prior to analysis.

OIL DERIVED CARCINOGENS IN THE MARINE ENVIRONMENT

The amount of carcinogen directly released to the environment from oil sources must, of course, depend on the amount of oil discharged. Chandler (1974) estimated conservatively that (assuming good practice by all countries etc.) some 75 000 tonnes of crude per annum should be discharged at sea[2]); at 100 µg/ml carcinogen concentration, this would imply about 7500 kg/annum of carcinogen discharged — worldwide — to the marine environment. (Total area of the world's oceans is approximately $1{\cdot}5 \times 10^8$ sq miles).

[2]) Other estimates of discharge are much higher; Wilson (1974) estimating about 6×10^6 tonnes/yr i. e. about 100 times more than Chandler, but even this figure still gives average carcinogen concentrations in terms of µg m^2 $year^{-1}$.

OTHER ENVIRONMENTAL SOURCES OF PCAH

Hydrocarbons of all types occur widely in nature (e.g. Badger et al, 1960) for example in deposits of petroleum (those which are exploited by man being our main concern), as seepages of such deposits particularly submarine seepages through the sea bed, in exhaust gases of petrol and diesel engines, in coal gas, in snuff, in tobacco smoke, in atmospheric dust, and also as a significant proportion of the composition of marine and land plants and animals. Their presence in marine plants and animals is, perhaps, not surprising since most

compounds referred to in the text

(i) Anthanthene

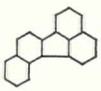
(–) Coronene

(i) Acenaphthylene

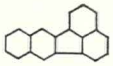
(i) Fluorene

(i, ?) Fluoranthene

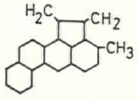
(m) Benzo[*b*]fluoranthene
(3,4-Benzfluoranthene)



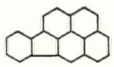
(m) Benzo[*j*]fluoranthene
(10,11-Benzfluoranthene)



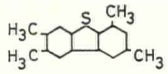
(?) Benzo[*k*]fluoranthene
(11,12-Benzfluoranthene)



(s) 3-Methylcholanthrene



(w) Indeno[*1,2,3-cd*]pyrene
(indeno pyrene)



(w) 1,3,6,7-Tetramethyldibenz-
thiophene

The symbol, in parenthesis, beside the structural formulae, indicates the generally accepted carcinogenic status of the compound. A double symbol indicates that different authorities disagree on classification. This indication is given merely as a guide to help the understanding of the text and is not meant to be used for any other purpose.

(–) – no information
(i) – inactive
(?) – activity doubtful
(w) – weakly active
(m) – moderately active
(s) – strongly active

crude oil probably originated from the organic remains of plants and animals in shallow, organic rich, marine sediments (e.g. Knorr, 1957). What is surprising is the amount of hydrocarbon found in comparatively recent sediments, and the PCAH content of such hydrocarbons. Smith (1954) estimated that dried marine sediment (from the Gulf of Mexico and from off California) might contain as much as 11 700 µg/ml of extractable hydrocarbons. The aromatic fraction might amount to between 8 and 14 % of this, with one sample of the aromatics showing 15 % with 3 or 4 rings (i.e. potential carcinogens). Similarly, Orr and Emery (1956) found a hydrocarbon content of between 2·3 % and 18·6 % of the extractable organic matter in marine sediment from areas off the southern California coast.

Smith (1954) reports work in the Esso laboratories which showed phytoplankton with a concentration of 2000 ppm (2 g/kg) of dry weight of hydrocarbons (paraffin, naphthene and aromatic). Taking the 1929 value of the Encyclopaedia Britannica of 6400 tonnes/sq. mile of ocean surface as the annual production of phytoplankton in the sea, Smith estimated an annual hydrocarbon production by this means of 13 tonnes per sq. mile of ocean surface per year. This may be compared with Wilson's estimate which amounts to 0·4 tonnes/sq. mile on average of oil discharged at sea each year. Numerous hydrocarbons (including aromatics) have been detected in a great variety of plants (leaves, flowers and fruit) at concentrations of from 100 µg/kg (0·1 ppm) to more than 10 g/kg (1 %) (Gerarde and Gerarde, 1961/2). Thus, as estimated by Zobell (1963), if the average concentration of hydrocarbons in marine plants is only 10 mg/kg (10 ppm), with a world production of such plants of 3×10^{11} tonnes per annum,

there is an annual production of 3×10^6 tonnes of hydrocarbons. This estimate is probably conservative comparing it with the value of 2×10^9 tonnes/year of phytoplankton hydrocarbon production based on Smith's (1954) figures. The natural production of such a large amount of hydrocarbon material cannot be ignored in any discussion of the impact of hydrocarbons on the environment. The extent to which these materials contain polycyclic aromatics and consequently constitute a health risk is important.

Mallet et al (1960) detected B[*a*]P in marine flora and fauna but considered that this was due to contamination by the air aerosol, itself contaminated by PCAH formed in combustion processes. However, B[*a*]P has been found in a variety of foodstuffs, mainly of vegetable origin and Table 70 contains a list taken from Grimmer (1966), who identified many other polycyclic hydrocarbons, e.g. anthracene, phenanthrene, pyrene, fluoranthene, 1,2-benzanthracene, chrysene, 3,4-benzpyrene, 1,2-benzpyrene, perylene, anthanthene, 1,2-benzperylene, 1,2,5,6-dibenzanthracene, coronene (see Table 69 for structural formulae). These materials occurred in varying proportions with, for example 266 µg/kg of fluoranthene in one sample of cabbage, up to a total of about 800 µg/kg for the sum of these PCAH (again in cabbage). Controlled experiments by Borneff et al (1968) showed PCAH to be present in small amounts in all samples of asparagus, cauliflower and filamentous algae which had been grown carefully in PCAH free nutrient media. In similar experiments growing *Chlorella* in the laboratory on a nutrient media, free of demonstrable quantities of such hydrocarbons, they detected the presence of benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, indenopyrene, benzo[*j*]fluoranthene, bezno[*k*]fluoranthene, benzo[*ghi*]perylene, fluoranthene and pyrene. Gräf and Diehl (1966) also reported the presence of eight PCAH, of which five are recognised to be carcinogens, in vegetable matter. They stated that the "normal" content of 10 to 20 µg/kg of dry substance could increase 3 to 5 fold during the drying process, i.e. when the plants become yellow.

Table 70. (Grimmer, 1966)

	B[*a*]P µg/kg
1) Cabbage	12·8–24·5
2) Lettuce	2·8–12·8
3) Spinach	7·4
4) Leeks	6·6
5) Barley, wheat and rye	0·2– 4·1
6) Tea	3·7– 3·9
7) Roasted and grilled meat	0·2– 0·6
8) Roasted coffee	0·3– 0·5
9) Tomatoes	0·2
10) Milk (in bottles)	0
11) Milk (in cartons)	0
12) Butter	0

These findings would appear to indicate that PCAH are synthesised by plants but conclusive evidence is required from radio-tracer techniques. It is worth noting too that hydrocarbons with high carcinogenic potency in animal tests are also particularly effective plant growth factors (Gräf and Nowak, 1966) and it may be assumed that PCAH are either plant growth hormones or their precursors, the growth promoting efficiency corresponding essentially to the carcinogenicity. Thus, PCAH should be included with the group of "natural" materials with definable biological functions in the vegetable kingdom. These compounds are either eaten by animals or man (see later) or are transferred to the soil by dead plants.

Several workers have found B[*a*]P in the soil. Shabad (1968) found 191 mg/kg in soil polluted by car exhausts, but much less in country areas. Blumer (1961) detected B[*a*]P in soil samples from non-industrial areas and concluded that pyrolysis as in forest fires could not be the sole source. The PCAH are found consistently in water which has seeped through the soil (Borneff et al, 1968), and hence may reach the marine environment via land drainage.

PCAH IN THE MARINE ENVIRONMENT

The situation to be considered then, is one where mineral oil may add PCAH to a marine environment already "contaminated", albeit by natural causes. The problem is to assess whether or not this extra burden is significant. The direct effects of skin contact with contaminating oil will not be considered since such contact, though unpleasant in the short term, is unlikely to be long enough or intimate enough to produce cytotoxic effects. We should look rather at the possible contamination of the marine food chain with a relatively insoluble material, the carcinogenic content of which is far above the normal level.

SOLUBILITY OF PCAH IN WATER

Since direct contact of oil spills etc. with higher animal or plant life is likely, in the short term, either to be lethal to the organism, or to render it unpalatable as food (or both) to men or other organisms, there is little likelihood of dangerous substances entering the food chain by this means. The more insidious sub-lethal contamination due to solution in sea water is perhaps of greater importance, although it should be noted that Mertens and Haxby (1975) found no evidence that abnormal growths in marine organisms, or in man, result from oil spills.

Low molecular weight hydrocarbons, particularly

aromatics such as benzene and toluene have appreciable solubilities in water at normal temperatures, of the order of 0·01 Mol % (approximately 500 mg/litre). PCAH are usually described as insoluble in water but would in fact appear to have individual solubilities perhaps of the order of 15 μg/litre (a figure extrapolated approximately from data given by Griswold and Kasch (1942)). Boylan and Tripp (1971) reported a total hydrocarbon concentration in a water extract of Kuwait oil of 1·453 mg/litre, but much of this was low molecular weight material (and there is at least a possibility that some of this material was present as an emulsion and not as a true solution). The individual compounds described in the sea water extracts of Kuwait oil were present in concentrations of 15—30 μg/litre and these were methyl naphthalenes with much lower molecular weights than the PCAH which might be carcinogens. Boylan and Tripp reported larger concentrations with sea water extracts of Kerosene but again the molecules involved were not large enough to be carcinogens.

These values may be taken to be saturation values if the substances are present in true solution and thus are considered to be maximum concentrations; they are very small but they are significant due to the very large volumes of water involved, for example Dean (1968) estimated that at a solubility of 1 mg/litre (1 ppm) the *whole* of the Torrey Canyon oil could have dissolved in a patch of sea 20 miles square by 500 ft deep. The concentrations are in fact of the same order as the concentrations to be expected from natural hazards and it is difficult to believe that carcinogens from spills (or seepages) or crude petroleum can add significantly to the natural hazard. It must be remembered, however, that the safe concentration of any carcinogen is zero.

The next problem is to trace the fate of PCAH in contact with water and to assess the chances of significant build up in the food chain and the hazard to health so induced.

FATE OF PCAH IN THE MARINE ENVIRONMENT

Hydrocarbons are usually considered to be fairly stable materials. Crude petroleum was formed in geological time and appears to be but little altered during the aeons it has remained undisturbed.

However given the right conditions, which really means an aqueous environment (solution or adequate interface) and sufficient nutrient (phosphorus and nitrogen), many hydrocarbons are degraded by many species of microorganisms. Under marine conditions however, Berridge et al (1968) considered such attack would be very slow and that normally virtually no aerobic degradation of oil occurs. Davies and Hughes (1968) discussed two possible pathways for such degradation in the case of substituted benzenes and stated that similar routes were applicable to PCAH. Zobell (1963) reported that there was no direct evidence of any marine bacterial species being directly injured by oil pollution and, in fact, the bacterial population in polluted water was often several millions per ml. Since bacteria are eaten by numerous animal species such a situation could be beneficial to the food chain, as the polluted water becomes progressively enriched by bacteria, protozoa and higher animals. Laboratory experiments have shown that oil oxidizing bacteria convert 30—40 % of the carbon in hydrocarbon to cell substance or protoplasm, i.e. for each gram of oil oxidized some 300—400 mg of animal food might be manufactured. However, the oxidation of an oily substance requires a mixed population of bacteria since they tend to be type specific. The rates of oxidation depend on temperature, degree of agitation etc., but Zobell estimates that rates of between 100—960 m^3 day^{-1} (100—960 μg $litre^{-1}$ day^{-1} may be obtained. Such rates would require about 300 μg—4·0 mg O_2 $litre^{-1}$ day^{-1} and since sea water rarely contains more than 10 mg/litre O_2 these higher degradation rates could be limited by the amount of oxygen available. Bridie and Bos (1971) concluded that a more likely limitation to growth was the lack of nutrients, particularly phosphorus and nitrogen. They obtained rates, for a topped Kuwait crude, in general agreement with Zobell's (1963) figures when no extra nutrients were added, but many times greater on the addition of small amounts of ammonia and phosphate. Oil under adverse conditions, e.g. on the sea bed where oxygen availability might be a limiting factor, might persist for a long time but small quantities of oil on the surface or in solution should degrade fairly quickly under the right conditions i.e. it might be assumed that a sample of oil with a total solubility of 1·453 mg/litre, and degrading at between 100 μg and 1 mg $litre^{-1}$ day^{-1}, would completely disappear in 2 to 14 days under reasonable conditions.

However, in the absence of oxygen and/or suitable nutrients the oil may persist for months, and in this case if shellfish come into contact with the oil before it is degraded what is the prognosis?

Recent work reported by Mertens and Haxby (1975) shows that, while marine organisms subjected to sublethal concentrations of oil do accumulate hydrocarbons, they rapidly depurate these materials once the contamination is removed.

To quote almost directly from Zobell:

"Oysters and other shellfish"

"Only where oil pollution is severe and prolonged are oysters, mussels, clams, cockles, and other shellfish

of economic importance injured, and then primarily in beds which occur between high and low tide levels. In some cases the marketability of shellfish is adversely affected by an oily taste or odour. In their field and laboratory investigations, Galstoff et al (1936) found no direct correlation between mortality of oysters and the presence of crude oil, but a water-soluble fraction in crude oil narcotized the ciliated epithelium of the gills, thereby inhibiting the feeding mechanism of the oysters. The extensive studies of Mackin (1950) demonstrated that oysters are extremely tolerant of crude oil overlaying the water, emulsified in the water, or periodically sprayed on the oysters."

"Nevertheless, trade journals, court reports, and private communications document numerous localized instances of oyster, cockle and other shellfish beds being seriously affected by oil pollution. At times large numbers of oysters and cockles in certain beds along the French and English coasts have been killed by crude or fuel oil, and many more have been rendered unpalatable due to tainting of the flesh."

Cahnmann and Kuratsune (1957) analysed oysters collected from water moderately polluted with oil, but with no evidence of tar pollution. The PCAH values obtained which are given in Table 71 amount to about 1 mg/kg in all. They assumed that the quantities of PCAH found would vary with habitat, but that oysters, along with other shellfish, were able to take up PCAH from polluted surroundings. Results published by Mallet et al (1960) showed concentrations of B[*a*]P of about 1·2 µg/kg in the flesh of oysters selected at random from the Paris market. This figure is lower than that reported by Cahnmann and Kuratsune (1957), and appears at about the same level as for grain in the data of Grimmer (1966) in Table 70. Kraybill (1974) has recently quoted values for the B[*a*]P content of oysters of from 0·1 to 8 µg/kg and Zobell (1971) gives values up to 2·2 mg/kg for invertebrates from the Adriatic coast of Italy.

Simpson (1968) has stated that oil alone will not harm crustaceans or sub-littoral molluscs and is most unlikely to harm intertidal molluscs. It can, however, cause serious tainting which is noticed if it comes into contact with the shells of commercially valuable intertidal molluscs. Smith (1954) reports work done in the Esso laboratories where oysters showed a hydrocarbon content based on the dry weight of the oyster of 45 ppm (45 mg/kg) paraffin-naphthene analysing as $C_{72 \cdot 5}H_{125}O$ and thus consisting predominately of polycyclic-naphthenic type molecules. The small aromatic fraction possessed a bright blue fluorescence.

Thus it appears that shellfish normally contain PCAH in small quantities, with reported values as high as 1 mg/kg for oysters harvested in moderately polluted water, but it is not known if such fish were tainted or not (i.e. whether they were likely to be eaten). Cole (1971), suggested that such shellfish were likely to be so tainted that they were inedible. It is difficult to tell from the literature at what level food becomes inedible. Halstrick (1942) reports a threshold limit for taste at 4 mg/litre and for smell at 2·5 mg/litre, whilst Zimmerman (1955) found a taste of kerosene at 200 µg/litre. Knorr (1957) found that such materials could be detected in distilled water at concentrations of 10 µg/litre by both taste and smell and placed the threshold at perhaps 1 µg/litre. However the temperature of the water has a distinct influence on detection levels.

Table 71. Approximate amount of PCAH in extract of 1 kg of shucked oysters from moderately polluted waters (Cahnmann and Kuratsune, 1957)

Compound	Approx. amount µg
Benzo[*ghi*]perylene	1– 5
Benzo[*a*]pyrene	2– 6
Benz[*a*]anthracene	< 10
Benz[*k*]fluoranthene	8– 12
Benzo[*e*]pyrene	< 20
Chrysene	20– 40
Pyrene	100– 140
Fluoranthene	600–1000

PCAH IN HUMAN FOOD

Earlier it was shown that many foods contain PCAH and in particular B[*a*]P. Borneff and Fabian (1966) estimated an average human intake from such sources as 10 mg/year but, of course this will vary widely with diet and the level of "industrial" pollution. For example, it is found that samples of vegetables from industrial districts contain perhaps ten times more carcinogens than those from country areas. However, many normal foods contain a definite level of PCAH independent of locale. The average range of B[*a*]P is 10—20 µg/kg dry substance, but seeds, fruits and tubers (with a high starch content) contain only 1—10 % of the PCAH of green plants (Gräf and Diehl, 1966).

Table 72 taken from Dungal (1961) lists the concentrations of various individual PCAH in smoked food. (Note: These figures are on a "wet" basis). Of these PCAH, fluorene and anthracene are not considered carcinogenic but the total value of PCAH as given for smoked mutton is nearly 300 µg/kg. It should be noted that the figures quoted are for food smoked in Iceland. The cod and redfish were commercially smoked for only a few hours, whereas the mutton and trout were smoked by farmers, the mutton possibly for months and trout for weeks. Grimmer (1966) also gave some information on smoked food. He quoted B[*a*]P

Table 72. PCAH found in smoked food—Dungal 1961 (concentration in μg/kg wet material)

	Mutton	Trout	Cod	Redfish
Acenaphthylene	137·7	83·0	0	4·5
Fluorene	20·6	31·1	0	0
Phenanthrene	86·5	41·3	0	5·0
Anthracene	19·8	13·1	1·3	1·5
Pyrene	5·9	4·9	0·7	3·0
Fluoranthene	4·6	0	0·5	4·0
1,2-Benzpyrene	0	0	1·9	0·3
3,4-Benzpyrene	1·3	2·1	0·5	0·3
Total	276·4	175·5	9·4	18·6

contents in μg/kg for smoked fish (0·1—0·8), "cottage" ham (0·1), and sausage (0·2), (the skin of smoked sausage gave a value of 0·4, with very low levels on the inside of the sausage.)

Thus, is the intake of PCAH from shellfish for instance, especially if this is increased by "oil contamination", likely to add significantly to the diet of humans? *If* the figure of 1 mg/kg were taken as the norm for shellfish, and assuming the normal oyster weighs about 30 g (1 oz) then Borneff and Fabian's (1966) "normal intake" of 10 mg/year would amount to about 30 dozen oysters. But to sound a note of caution it is believed that the figure of 1 mg/kg is rather high and that the figure for unpolluted waters would be lower. It is not known if such shellfish are likely to be eaten, nor is it known if there is any health hazard at the "normal intake" level of 10 mg/year.

HEALTH HAZARD CONNECTED WITH INGESTED PCAH

It has already been stated that generally the use of mineral oils in food in the U.K. and in many other countries is illegal, due largely to the fear that such materials may cause cancer. How real is this fear in relation to PCAH ingested from sources other than oil? It is not possible to answer this question explicitly but a few pointers may be gleaned from the literature. Boyland (1964) stated that PCAH are generally, but not always, local in their action; when applied to the skin they give rise to epithelomata, on implantation in the bladder they result in bladder cancer and, on injection, induce sarcomata. Other carcinogens often produce tumours in tissues remote from the site of application. On this basis, it would be expected that PCAH would, on ingestion, give rise to gastric cancer, and a number of workers have investigated such a correlation. Dungal (1961) induced gastric cancers in rats by feeding them on smoked mutton and trout and he correlated the high incidence of gastric cancer in parts of Iceland with the consumption of large amounts of smoked foods which contain significant percentages of PCAH. In a comprehensive epidemiological investigation of gastric cancer, Wynder et al (1963) concluded that the factor of smoked food consumption was compatible with the high incidence of gastric cancer in Iceland and Slovenia and with the low incidence in the USA but could not account for the prevalence of this disease in Japan. They also concluded that the diet of those populations with a high incidence of stomach cancer is relatively high in the consumption of potatoes, rice and bread, from which those groups derive most of their calories. The high level of such starchy food components in the diet of populations with a high rate of gastric cancer seems to be of importance, although not necessarily by itself. Hakama and Saxen (1967) also pointed to the high incidence of gastric cancer being connected with a low intake of fresh vegetables and fruits and a high consumption of starchy foods. It should be remembered that Borneff and Fabian (1966) showed that starchy foods contain rather less PCAH than green plants.

Really this appears to leave the case against PCAH on ingestion as nonproven. Certainly PCAH are metabolised by animals (including man). Many of these metabolic processes (Boyland, 1964) are probably concerned with the defence of the body against carcinogens rather than with carcinogenicity itself, they are inactivating processes which permit substances to be quickly and safely excreted. These processes are carried out by the microsomes, one of the functions of which is the conversion of lipid soluble toxic materials into water ionized products that can be excreted by the kidneys. Boyland (1964) stated that none of the many known metabolites of PCAH were carcinogenic. However, recent results have indicated that the proximate metabolites of B[*a*]P and Benz[*a*]anthracene contain the moiety 1,2-epozy-3,4-dihydroxy-tetrahydronaphthalene, and this group may react as a carbonium ion with DNA and perhaps be the ultimate carcinogenic agent (Hulbert, 1975). There have been many reports that hydrocarbons pass through the body unabsorbed and unchanged (e.g. Gerarde, 1960) — but it is known, (Zobell, 1950) that hydrocarbons can be assimilated under certain conditions. There has also been some evidence in recent years of B[*a*]P linkage to DNA (Umans et al, 1969) together with some indication that such linkage might be correlated with carcinogenic activity. Also the binding of PCAH, in particular 7,12-dimethyl benz[*a*]anthracene, 3-methylchloranthene, B[*a*]P and dibenz[*a*,*h*]anthracene, to macromolecules in cultured human bronchi cells under certain circumstances has been reported (Harris et al, 1974). The recent paper by Hulbert (1975) linking the metabolites of B[*a*]P and benz[*a*]anthracene with DNA has already been mentioned.

CONCLUSIONS

To update the conclusion of Dean (1968), it seems that the dangerous portion of petroleum consists of the higher boiling aromatic fractions. A number of polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene, which is of course, generally used as a "marker" for carcinogenicity, have been identified in small concentrations in crude oils. These compounds have a very low solubility in sea water but have been identified in certain shellfish. However benzo[*a*]pyrene and other PCAH are relatively abundant in many foods including fresh vegetables and smoked foods and it would appear that the risk to the health of a member of the public by spillage of oil at sea is probably far less than that which he usually encounters by eating a range of popular foods.

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