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Tainting of Atlantic Salmon (*Salmo salar*) by Petroleum Hydrocarbons During a Short-term Exposure

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A set of three short-term exposures was conducted to explore concentrations of Flotta North Sea light crude oil dissolved in seawater which could lead to tainting in Atlantic salmon (*Salmo salar*). The water-soluble fraction (WSF) of Flotta light crude was prepared by stirring the oil with cold seawater. The main components of the WSF were low-boiling aromatics, although these were only a small proportion of the starting oil. The fish (average size 367 g; lipid content of the muscle 3.6% wet wt) were exposed to three levels of the WSF for 6 h. A sensory panel evaluation of taint was conducted, and the results were compared with hydrocarbon analyses performed by capillary gas liquid chromatography. Although the time of exposure was short, flavours of experimental fish samples were significantly ($p < 0.01$) different from those of control samples, even at the lowest exposure concentration of 0.45 ppm WSF. The muscle uptake is discussed as a reflection of fat content which was much less than in market sized salmon. Muscle and liver bioaccumulate mainly toluene and naphthalene.

Salmon farming operations are a valuable fishery resource, but before salmon reach the market, they are held in seawater cages for nearly 2 years. An important environmental threat to marketing this live fish is hydrocarbon pollution, particularly because many salmon farms are near the routes of oil tankers.

Each year, an estimated 4 million t of petroleum enter the marine environment through sea and land based discharges, atmospheric fall-out and other events (Clark & McLeod, 1977). Petroleum aromatic hydrocarbons, particularly because they are relatively soluble in water (Siron *et al.*, 1987) are potentially toxic or tainting components of crude oil, and depending on concentration, they are also carcinogenic (Andelman & Snodgrass, 1974; Rice, *et al.*, 1977). Several studies have been conducted with salmonids to assess the effect of hydrocarbons in respect to their behaviour, physiology, pathology and toxicology (Purdy, 1989; Malins, 1982; Morrow *et al.*, 1975; Hawkes *et al.*, 1980), but there is no information available in the literature about the tainting of this fat-rich fish by a petroleum water-soluble fraction (WSF). Our primary objective was to perform a series of three short-term exposures to different concentrations of petroleum dissolved in seawater that could lead to tainting in Atlantic salmon (*Salmo salar*), and study the relationship between the lipid content and hydrocarbon uptake by fish tissues. Depuration was not attempted since the fish were smaller than market size and had only a modest (~4% wet wt) muscle lipid content.

Materials and Methods

Test media

Flotta North Sea crude oil, obtained from the Dartmouth N.S. refinery of Esso Petroleum Canada, was used to prepare the water-soluble fraction (WSF) for the tainting exposures. It was stirred in a 1000 l stainless steel mixing vessel equipped with a powerful mechanical stirrer, a bottom drain, and a jacket cooling-water system. Crude oil and seawater in a ratio of 1:299 (v/v) were stirred for 24 h and allowed to settle for an additional 48 h. The WSF was kept cool by circulating cold water through the outer jacket of the mixing vessel. The WSF was prepared immediately prior to its use in exposure experiments.

Exposure system

The exposure experiments were conducted at the Aquatron laboratory, Dalhousie University, in 355 l fibreglass tanks with clear plastic covers. The drainage system was designed so that the overflow water came from the bottom of the tank to ensure good mixing of the exposure water. Exposures were run using a flow-through system with well-oxygenated seawater at a flow rate of 250 ml min^{-1}. Diluent seawater was bubbled with pure oxygen to a supersaturated condition in a large constant-volume head tank. The oxygen level in the exposure tanks was monitored hourly, and the flow of oxygen to the head tank adjusted to maintain an oxygen level of 70–100% saturation in the exposure

tanks. The flow of WSF and diluent seawater was monitored and adjusted manually so as to keep conditions uniform for 6 h.

Fish handling and experimental design

Groups of three salmon (average wt 367 g) from the reserve fish of ongoing diet studies (Li *et al.*, 1990) were placed in three tanks, and exposed to different levels (0.45, 0.87, and 1.54 ppm) of the WSF for 6 h. A control group was kept in clean seawater.

When the trial was completed, the salmon were killed by a blow on the head and then washed and placed in plastic bags on ice for transport to the Department of Food Science at the Technical University. The fish were immediately gutted, skinned, filleted and frozen in polyethylene bags at −40°C until required for sensory panel assessment and other analyses.

Isolation and recovery of hydrocarbon

The hydrocarbons of the flesh were extracted by the steam distillation procedure of Ackman & Noble, 1972. The efficiency of the steam distillation technique for the recovery of hydrocarbons was studied using a mixture of eight hydrocarbon standards. The hydrocarbons were dissolved in ethanol and 1 ml of this solution was used to spike 50 g of salmon fillets by injecting several aliquots with a microsyringe and allowing these to diffuse into the muscle for 1 h. The flesh was then minced in a food processor and immediately steam distilled. The percentage recoveries showed the highest recovery for the higher molecular weight hydrocarbons. The final correction factors, which also incorporate the GLC system response factor, are given in Table 1.

TABLE 1

Recovery of hydrocarbons from injected salmon muscle. Final correction factors.

Hydrocarbon	Correction factors	
	Recovery*	Final†
Toluene	7.42	7.12
p-Xylene	6.74	6.13
0-Xylene	4.87	4.43
Isopropylbenzene	7.58	7.13
1,3,5-Trimethylbenzene	4.53	4.22
1,2,4-Trimethylbenzene	3.60	3.28
1,2,3-Trimethylbenzene	2.74	2.66
1-Methylnaphthalene	1.58	1.44

*Recovery correction factor = 100/% recovery of hydrocarbon from steam distillation procedure (see also text).

†Final correction factor = recovery corr. factor/GLC response corr. factor.

Analyses of the WSF were performed on seawater samples from the test tanks. The hydrocarbons in the WSF were extracted according to the Murray extraction procedure (Murray, 1979; Murray *et al.*, 1984), employing the correction factors from a published report (Ernst *et al.*, 1989).

Gas Chromatography and Gas Chromatography/Mass Spectrometry

The hydrocarbon analyses were conducted with a Perkin Elmer 8420 capillary gas chromatograph equipped with a flame ionization detector (FID) and a split injection system. The chromatography was conducted on a DB-1 methyl silicone fused silica capillary column (60 m, 0.25 mm ID, 0.25 μm film thickness).

Conditions and temperatures were: FID 280°C; injector 280°C. The column temperature was programmed as follows: initial temperature 45°C held for 15 min; increased at a rate of 13°C min^{-1}; final temperature 280°C, held for 30 min. The carrier gas was helium at a pressure of 20 psig and hydrogen and air pressures were 13 and 23 psig, respectively.

The concentration of hydrocarbons in the samples was calculated with respect to the internal standard n-heneicosane (C_{21}), and corrected for GLC response, combined with the recovery efficiency of the steam distillation technique for muscle or with that for the Murray extraction method of WSF analyses. Blank readings were made daily and all solvents were glass distilled. GC/MS analyses were performed using a Finnigan MAT 700 Ion Trap Detector interfaced with a Perkin Elmer 990 GC. The chromatography was executed on a SPB-5 column (30 m × 0.25 mm) fused silica capillary column (Supelco). The initial column temperature was 45°C held for 16 min, then programmed at a rate of 12°C up to 280°C and held for 24 min. The helium carrier gas pressure was 10 psig. The system was controlled by a PC AT computer which handled all data storage and processing. The specimen components were identified by comparison of retention times with that of external standards and with the mass spectra library.

Sensory evaluation of taint

The sensory panellists were staff and students of the Canadian Institute of Fisheries Technology (CIFT) and had experience in sample tasting. The fillets stored at −40°C were thawed, and then minced in a Cuisinart food processor. Approximately 15–20 g of flesh were formed into a patty, placed in a covered glass petri dish, and cooked in a microwave oven before presentation to the taste panellists (Ernst *et al.*, 1989). Each panellist was presented with four samples: one control and each of the three exposed, and was asked to examine the odour and taste of samples and to state the amount of difference found compared to the reference sample. The sensory panel results were evaluated statistically by analysis of variance (Larmond, 1977).

Lipid analysis

The lipids of the fillets and livers were extracted according to the procedure of Bligh & Dyer (1959). The quantitation of the natural lipid classes was performed by TLC/FID. The procedure employed was basically that described by Parrish & Ackman (1985), using an Iatroscan TH-10 Mark III analyser equipped with a flame ionization detector (FID) and a SP4200 integrator. The air flow was 2 l min^{-1} and the detector hydrogen flow rate 160 ml min^{-1}. The scanning speed was 0.42 cm s^{-1}. The silica gel Chromarods employed for the TLC separations were type S-III.

The lipid sample was dissolved in chloroform, and spotted on the rods using 1 μl microcap pipettes

(Drummond Scientific Co., Broomall, PA, USA). Phosphatidylcholine, tristearin, 1,2-dipalmitin, palmitic acid and free cholesterol were used for the calibration of the Chromarods. The solvent mixture employed was hexane:diethylether:formic acid (97:3:1 v/v/v) for 55 min. Identifications were made by comparison with authentic standards.

Results

Water-soluble fraction

Figure 1 is a typical GLC chromatogram of the hydrocarbons recovered from the WSF used for the experiment. The main components of this fraction were low-boiling aromatics (~60%), though these were minor components in the starting oil. Aliphatics were present in low concentrations. The GC profile showed the presence of toluene, methyl and ethylbenzenes and the xylenes as the major components. Among other aromatics of interest were complex patterns of substituted naphthalenes. The main polymethylbenzenes appeared to be 1,2,4- and 1,2,3-trimethylbenzene. The WSF did not show higher polynuclear aromatics (PNAH) of mass greater than methyl and dimethyl naphthalenes. Apart from aromatics and alkanes, the WSF showed a high level of methylhexane, almost equal to that of toluene.

Tainting test

There were no mortalities during the 6 h exposure. Fish behaved normally, though they were disturbed by people closely observing the tanks. A low stress response of Atlantic salmon to crude oil exposure and handling had been previously reported (Aabel & Järvi, 1990). The hydrocarbon levels in the exposure and head tanks dropped markedly during the experiment. The average decrease was 56%, of which some seemed to adhere to the fibreglass surfaces. A portion may have evaporated.

Sensory evaluation

The results of the sensory panel evaluation are shown in Table 2. Salmon in all of the trials were found to be tainted to a level of significance of 1% for all samples. The level of significance between the salmon exposed to 0.45 and the other two concentrations was also 1%. The level of significance between the 0.87 and 1.54 ppm samples was 5%. These results correlate well with the hydrocarbon analysis performed by GLC (Table 3).

Hydrocarbon levels in muscle, seawater and liver

Figure 2 compares the levels of the principal polluting hydrocarbons found in the muscle at different

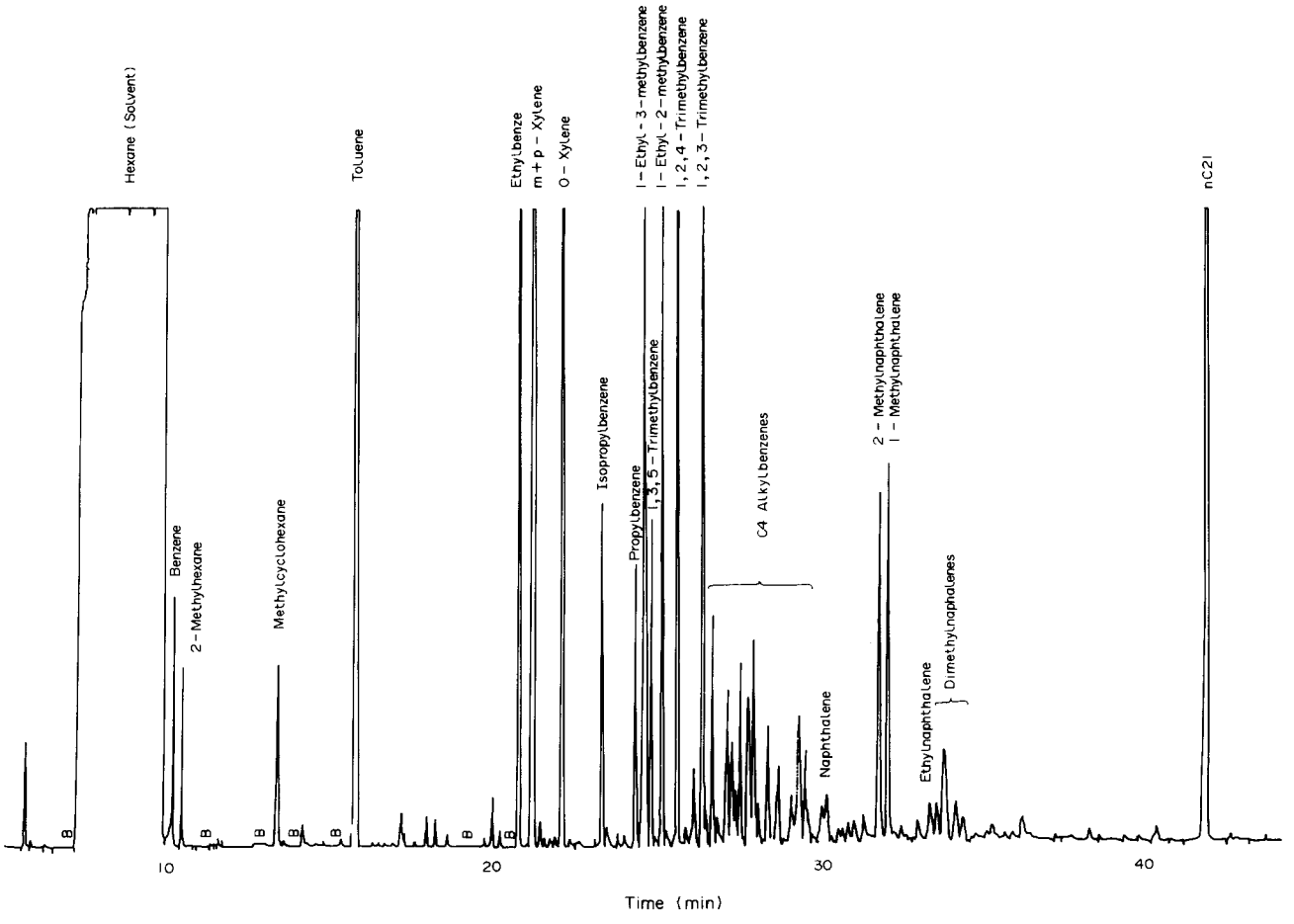

Fig. 1 Typical gas chromatogram of the WSF of Flotta crude oil used in exposure studies with salmon. Heneicosane (C21) was used as an internal standard. Note that benzene and 2-methylhexane are recorded with an attenuation 2-fold less than the rest of the chromatogram. C4 Alkylbenzenes = several tetramethyl- and propyl-methyl-benzenes.

TABLE 2

Sensory panel evaluation of fillets.

Hydrocarbon exposure level (actual ppm)	No. of panelists	% Identifying the odd sample	Level of significance (%)
0.45	10	100	1
0.87	10	100	1
1.54	10	100	1

TABLE 3

Hydrocarbons in seawater and in tissues of salmon exposed for 6 h to WSF.

Tank	Seawater ppm	Muscle ppm	Liver ppm	Total accumulation* Muscle	Total accumulation* Liver
Tank no. 1	0.45	13.52	14.56	30.0	32.4
Tank no. 2	0.87	25.63	33.03	29.5	28.0
Tank no. 3	1.54	31.29	ND	20.3	ND
Control	–	0.9	1.9	–	–

*Ratio between the concentration of hydrocarbon in seawater and concentration in the tissue.
ND: Not determined.

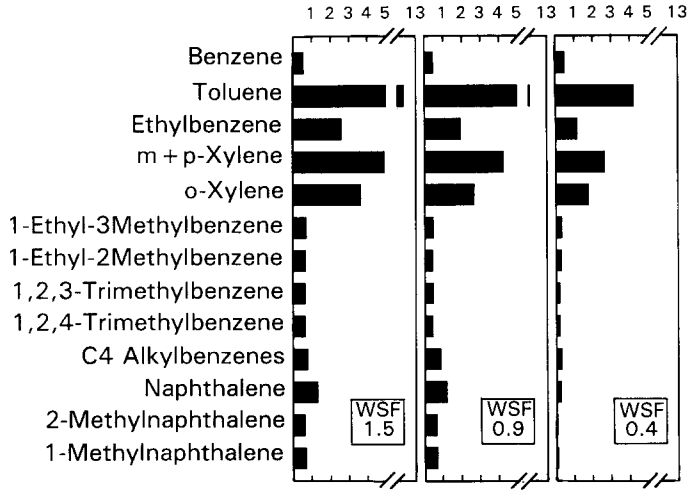

Fig. 2 Major aromatics in salmon muscle (ppm). C4 Alkylbenzenes = several tetramethyl- and propyl-methyl-benzenes.

exposure levels. The hydrocarbon composition of the tissues analysed was qualitatively the same as that of the WSF, although the components were in different concentrations (Table 4).

The uncontaminated seawater used in this study was examined for background levels of hydrocarbons by Murray extraction and gas chromatography. The seawater samples had no detectable levels of petrogenic hydrocarbons. The control salmon showed 1 ppm and 2 ppm of total hydrocarbon in muscle and liver, respectively. These background levels were, therefore, subtracted when calculating the total hydrocarbon levels in the tainted group tissues.

Lipid analysis

The fillets and livers were analysed by the Bligh and Dyer method for lipid content. The results, summarized in Table 5, showed a lipid content in flesh of about 4% (wet wt) which is typical for smallish salmon such as those used in these experiments. The average total wt of the salmon was 367 g. The lipid analysis of liver showed

TABLE 4

Bioaccumulation* of hydrocarbons in liver and muscle of salmon exposed for 6 h to WSF.

Hydrocarbon	WSF 0.45 ppm Liver	WSF 0.45 ppm Muscle	WSF 0.87 ppm Liver	WSF 0.87 ppm Muscle	WSF 1.54 ppm Muscle
Toluene	1.8	6.9	2.6	5.7	6.2
Xylenes	0.2	0.6	1.8	2.1	2.1
Alkylbenzenes	4.2	2.7	1.9	1.3	1.4
Naphthalene	8.1	5.1	7.2	9.3	8.0
Methylnaphthalenes	1.4	0.9	2.1	2.6	1.6
Dimethylnaphthalenes	3.3	0.1	1.5	1.0	0.1

*Calculated as the ratio of individual hydrocarbon concentration to the total hydrocarbon concentration in the tissue divided by the equivalent ratio in the exposure water.

TABLE 5

Lipids in muscle and liver of experimental salmon.

Tissue	Total lipid (g% wet wt)	% Triacylglycerol	% Polar lipids
Muscle	3.98 ± 0.35	77.73 ± 6.58	21.31 ± 6.65
Liver	5.36 ± 0.64	91.06 ± 1.01	5.45 ± 1.13

Values are the average of four samples ± standard deviation of the mean.

a higher concentration of lipids and of triacylglycerol than flesh.

Discussion

The hydrocarbons of the WSF exhibited a pattern similar to those previously reported for other crude oil WSFs (Ernst *et al.*, 1987; Goksøyr *et al.*, 1991), being dominated by monoaromatic compounds, primarily benzene, toluene and the xylenes. These low-boiling aromatics are the most soluble constituents of petroleum, and, besides its tainting effect, are the primarily toxic agents of WSF towards fish (Moore & Dwyer, 1974). The alkanes present in the parent oil were almost absent in the WSF, due to their low solubility in water. They are virtually odourless and tasteless and do not contribute to the tainting.

In general, the sensory panel results indicate that, although the time of exposure was short, odour and tainting were significantly different from that of control samples, at all three concentration levels. The fish from the lowest concentration group were tainted to a lesser extent than the other two groups. At this concentration level, the sensory panellists were able to find the tainting significantly different ($p < 0.01$) from the higher concentration levels. From the sensory panel results, it is also inferred that the threshold concentration of Flotta crude WSF which will taint salmon flesh is lower than 0.45 ppm.

The confirmation of tainting in flesh was performed by GC analysis which showed that the predominant hydrocarbons were the same as those that dominate the WSF chromatograms, although the different hydrocarbons are accumulated to different levels, such that the concentration in the fish does not necessarily represent the relative concentrations of the components in the environment (Hardy *et al.*, 1974). The GC

analysis is a good complement to the sensory panels, because the latter is a subjective sensory perception. Tainting can only be evaluated using sensory tools such as odour and taste, while the GC analysis, though it can give a detailed composition of the tainting hydrocarbons, can only assist in determining whether the flesh is tainted or not. GC is, however, not subject to fatigue, is available 24 h day^{-1}, and promotes inter-laboratory comparisons.

The bioaccumulation of hydrocarbons in our experimental condition was mainly reflected in the levels of toluene, alkylbenzenes and naphthalene found in muscle and liver. These aromatics have been reported to impart offensive odour to other fish (Ogata & Miyake, 1973). The hydrocarbons with the highest bioaccumulation were toluene and naphthalene in both tissues, and naphthalene was in general more readily accumulated than toluene. This was also reported by other authors (Heath, 1987). Ogata & Miyake (1973), studying tainting in the grey mullet (*Mugil japonicus*), concluded that toluene was largely responsible for taint and suggested that it was deposited in the flesh via blood from the gill, a mechanism which could also be suggested in this case.

The fact that the bioaccumulation of hydrocarbons is greater than that in other fish species, such as cod (Ernst *et al.*, 1987), is presumably related to the higher concentration of lipids in salmon muscle. If we compare this with a lean-muscle species like cod (*Gadus morhua*) for which a detailed analysis of tainting by WSF of petroleum has been recorded in our laboratory (Ernst *et al.*, 1987), we observe that cod has the major site of lipid storage located in the liver (60% fat) while muscle has only 0.75% lipid (w/w) (Dambergs, 1964; Morris & Culkin, 1989). The hydrocarbon uptake from the WSF in cod exposed for 8 h to 3 ppm WSF, was 0.7 ppm (Ernst *et al.*, 1987) which is 20–45 times less than in the present experiment. The muscle of the small salmon examined in the present experiment had 4.0% lipid (w/w), and the liver 5.4% (Table 5). This marked difference in muscle lipid may very well account for the low level of hydrocarbon uptake shown by cod muscle. Furthermore, the triacylglycerols, that may be responsible for the accumulation of hydrocarbons, are more than 100 times greater in the salmon used in these experiments than in cod, though these small fish are still leaner than market-sized farmed salmon which typically have 10–14% muscle fat (Polvi, 1989; Roch *et al.*, 1988). It is generally accepted that hydrocarbons are very soluble in adipocytes, so that the more fat the animal contains, the more it is susceptible to hydrocarbon pollution (Whittle *et al.*, 1977; Whittle & Mackie, 1976; Hardy *et al.*, 1974; NRC, 1983). The uptake of hydrocarbon is also related to the behaviour of the fish. The major routes by which hydrocarbons present in the WSF are available to fish are either via gills or through drinking water (Corner *et al.*, 1976). The drinking rates of fish depend upon the need to maintain a more or less constant osmotic balance; thus the marine teleosts such as salmon drink heavily to compensate for the water loss across the gills. In this respect salmon are again more likely to absorb hydrocarbons than cod, because they are very active swimmers.

Another factor that should be taken into account is the blood circulation rate of the fish. The pollutants increase the ventilation rates in salmonids (movement of water over the gills) (Heath, 1987; Nakano & Tomlinson, 1967) which in turn alters the pattern of blood flow through the gills, increasing the blood levels of catecholamine (Randall, 1970). One could pose the question of whether the blood flow would be enough to account for the hydrocarbons accumulated in the tissues. If we suppose a flow of 15–30 ml min^{-1} kg^{-1} (Randall, 1970), we could estimate a total flow volume of 1.98–3.96 l in 6 h. The hydrocarbons in the blood are most likely to be transported by the triglyceride-rich very low density lipoproteins (VLDL), which may have an approximate concentration of 1.7 g l^{-1} plasma (Babin & Vernier, 1989). This means that a total of 3.4–6.7 g of VLDL would have been circulated through the body during the experiment. Thus, the amount of VLDL present is enough to carry the polluting hydrocarbon to the different tissues, even if only 1×10^{-3}% of the weight of the particle is made of hydrocarbon.

Conclusions

It has been shown that salmon are capable of taking up important quantities of hydrocarbons from the environment in a short period of time without lethal effects. These results, though preliminary in the sense that the thresholds of toxicity and of tainting were not examined, indicate that salmon exposed to dilute crude petroleum WSF may gain taint without any lethal effect, which is a very important issue in the aquaculture of salmon. Salmon may look healthy, and still be badly tainted. Work is continuing in this laboratory in order to determine the tainting threshold, toxicity, depuration times, uptake, transport and fate of hydrocarbons in this valuable high fat fish species. This knowledge could also be used to assess other biological parameters. In view of the present results with a moderate fat level, it is felt that market-size salmon would retain petroleum tainting for extended periods compared to the lean-muscle species of fish.

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