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INVESTIGATION OF TASTE TAINTING IN SALMON FLESH IN THE RIBBLE CATCHMENT

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INVESTIGATION OF TASTE TAINTING IN SALMON FLESH IN THE RIBBLE CATCHMENT

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SUMMARY

This report presents the findings of the first phase of an investigation into the cause(s) of taints in salmonid fish in the River Ribble, commissioned by the North West Region of the Environment Agency. There have been reports of tainting in fish taken from both the estuary and the freshwater river for many years, but the contaminants involved and their source and transport pathway are unknown. Tainting by phenols has been of specific concern in the past.

The work programme comprised: examination of tainting reports; collection of salmonids; their submission for taste testing; literature review; analysis of fish flesh using gas chromatography-mass spectrometry (GCMS) and analysis of river bed sediments.

From enquiries, three common descriptors of the 'taint' were identified: disinfectanty; diesely; and muddy. The incidence of taints appears transient/irregular and may therefore relate to the incidence of discharges and specific threshold concentrations of pollutants. The literature review showed that a wide range of organic compounds including many industrial chemicals, and others which are naturally occurring, can taint fish flesh.

Taste testing confirmed the presence of tainted salmon and trout in the Ribble Catchment. It identified a low incidence of 'untainted' fish but demonstrated the 'taint' to be not specific to one tainting substance. Differences were found both between the species and fish from different parts of the catchment. Overall, most fish exhibited an unpleasant flavour, though this may have been influenced to some extent by the fact that most were sexually mature. The worst tainting was found in trout from the river Calder: a soapy/chemical aftertaste. An unpleasant earthy/musty flavour distinguished the salmon from the trout. Phenol was shown to have been a minor issue during the present study, whilst no hydrocarbon taints were identified.

Examination of tissue from the eight salmon exhibiting the worst taints revealed the presence of aromatic hydrocarbons, but no phenolic compounds. Other notable substances present in the fish were siloxanes and benzophenone. Data from sediment analysis is presented which shows the main compounds present to be aromatic and polyaromatic hydrocarbons, that concentrations at two locations R. Darwen and R. Calder were significantly higher than at other sites, and that some phenolic compounds were detected at low levels.

A paucity of fish flesh taste descriptors linked to specific compounds prevented an obvious correlation to be made between the tastes observed and the organic compounds detected. Descriptors frequently used by the taste testing panel (e.g. earthy, musty, astringency, chemical) cannot be linked to any of the compounds identified in the tissue analyses. No taste information was available from the literature on siloxanes. Aromatic hydrocarbons though present in tissue and sediments were not identified as tainting.

Recommendations are made for further research to confirm the nature of the taste taints (particularly the earthy tainting in the salmon and soapy/chemical flavours River Calder trout) and examine their origin. Surfactants/anti-foaming agents and siloxanes are specifically identified for further research.

KEYWORDS

Salmon, Trout, Ribble, Tainting, Taste Testing, Sediment, Analysis.

1. INTRODUCTION

1.1 Background

Taste tainting in Salmonids (salmon/sea trout/brown trout) has been reported by anglers on the River Ribble since at least 1980, but the frequency and magnitude of complaints has increased in recent years and the issue was highlighted for action in 1990 in The Ribble Fisheries Management Plan (NRA 1990).

The contaminants involved and their source and transport pathway is unknown. There are, however, several industrial sources which discharge to tributaries (directly or via sewage treatment works) and contaminated land sites within the catchment. Appropriate research was therefore required to establish the cause of the taste taint and undertake the necessary steps to identify ways in which it could be eliminated.

The description of tastes and flavours can be very individual specific and the use of trained taste panels is necessary to reliably categorise them. Tissue analysis may then be applied to attempt to identify the contaminant(s).

In 1996 the Agency requested tenders for a one year investigation into the occurrence of Salmonid flesh tainting in the River Ribble catchment. The following questions were identified as being of relevance:

- Is there a continuing taste taint problem in the Ribble catchment and what is its nature?
- Who has made complaints?
- Which species of fish are affected (salmon, brown trout, sea trout, eels)?
- What is the contaminant(s) that is causing the problem and to what extent does it exist throughout the Ribble catchment and fishery?
- Where is the release site(s) of the contaminant?
- What is the upper tissue concentration level of the contaminant(s) in Salmonids above which the taste taint manifests itself?

Assuming that the contaminant(s) had been identified, along with its source, it was considered appropriate to provide a guideline discharge consent value for the contaminant(s), which if implemented, would prevent the manifestation of the taste taint.

1.2 Work programme

WRc identified a large number of variables which might affect the outcome of this investigation, including the availability of fish samples, the likelihood of obtaining tainted fish (especially if the problem was intermittent) and the prospects of then being able to identify the contaminant(s). A two phase approach was proposed, as follows:

Phase I

The proposed programme comprised the following components:

- sampling of migratory fish (salmon);
- submission of fish for taste testing;
- literature review on causes of taste tainting,
- analysis of fish flesh using gas chromatography-mass spectrometry (GCMS),
- review of potential sources of pollution.

Phase II

Two outcomes of Phase I were considered, and the following actions recommended.

- Insufficient tainted migratory fish caught to enable identification of contaminant(s)

Non-migratory fish population (brown trout) or salmon smolts in spring 97 should be sampled for taste testing, or the study should be extended to encompass the migratory run in summer 97.

- Tainting contaminants successfully identified

A sampling and analysis programme should be conducted to identify the source of the contaminant(s). The extent of this programme would be dependent on the degree to which this had been established in Phase I. The type of sampling (e.g. water quality, caged fish) would depend on the contaminant and the pattern of contamination.

Phase II would also include establishing taste thresholds for the contaminant(s), and propose consent conditions to alleviate the problem.

1.3 Modifications to programme

The start date for the project had been planned by the Agency for early 1996/97 but delays in letting the contract resulted in Phase I beginning in September 1996. This meant that the fishing season was almost over, and problems were envisaged in the collection of fish samples. To counter this, sampling of non-migratory salmonids (brown trout) was included in Phase I.

The collection of sediment samples (the surface layer laid down over summer 1996) for chemical analysis was originally agreed by the Agency for inclusion in Phase II, but with the high flows of autumn/winter imminent it was agreed that these should be collected at the start of Phase I in September 1996 and stored until required for analysis.

Preliminary Phase I findings on taste testing and tissue analysis, and developments concerning discharges to the catchment caused The Agency to review its approach to the tainting problem in March 1997, and it was decided to include the sediment analysis in Phase I.

The extended Phase I is reported here and a decision is awaited on future work.

1.4 The Ribble Catchment

1.4.1 General

The Ribble catchment is one of the largest in the North West, draining a catchment area of 2128 km^2 and covering a distance of 110 km from source to mouth (Figure 1.1).

The Ribble originates in the Pennines at an altitude of 422 m and is joined in its middle reaches by two major tributaries which differ distinctly in character. The River Hodder rising in the moorland of the Forest of Bowland drains a predominantly agricultural catchment, as does the Upper Ribble. The River Calder, in contrast, has historically suffered severely from industrial pollution and although in recovery, continues to be affected by minewater, contaminated land runoff, and industrial and sewage discharges.

In its lower reaches below these confluences, Hodder Foot and Calder Foot, the Ribble meanders through natural flood plains with mature grazing before reaching Preston and its tidal limit on the western edge of the city.

Two major tributaries, the Darwen and the Douglas, join the Ribble in its estuarine reaches and both have historical and current problems relating to industrial pollution.

1.4.2 Effluent disposal

The principal consented discharger to the Ribble Catchment is North West Water Ltd (NWW) with 42 wastewater treatment works (WwTW) throughout the catchment (NRA 1995). These vary in size from 20 000 to >300 000 population equivalent. Past policies encouraged the discharge of trade effluent to sewer and direct consented discharges of industrial effluent to the Ribble catchment are now rare. For certain of its customers NWW sets a discharge to sewer consent to protect its own STW discharges. Some 330 combined sewer overflows operate during periods of intense rainfall when there is a high dilution capacity in receiving waters.

There are several trade effluent discharges consented from quarries (limestone), and discharges from abandoned coal mineworkings affect water quality in parts of the catchment.

Agriculture predominates over much of the catchment and spillages of silage, slurry, sheep dip etc. cause serious pollution incidents, whilst diffuse pollution from fertiliser, pesticides and animal waste is an additional issue. Consented discharges are permitted from fish farms.

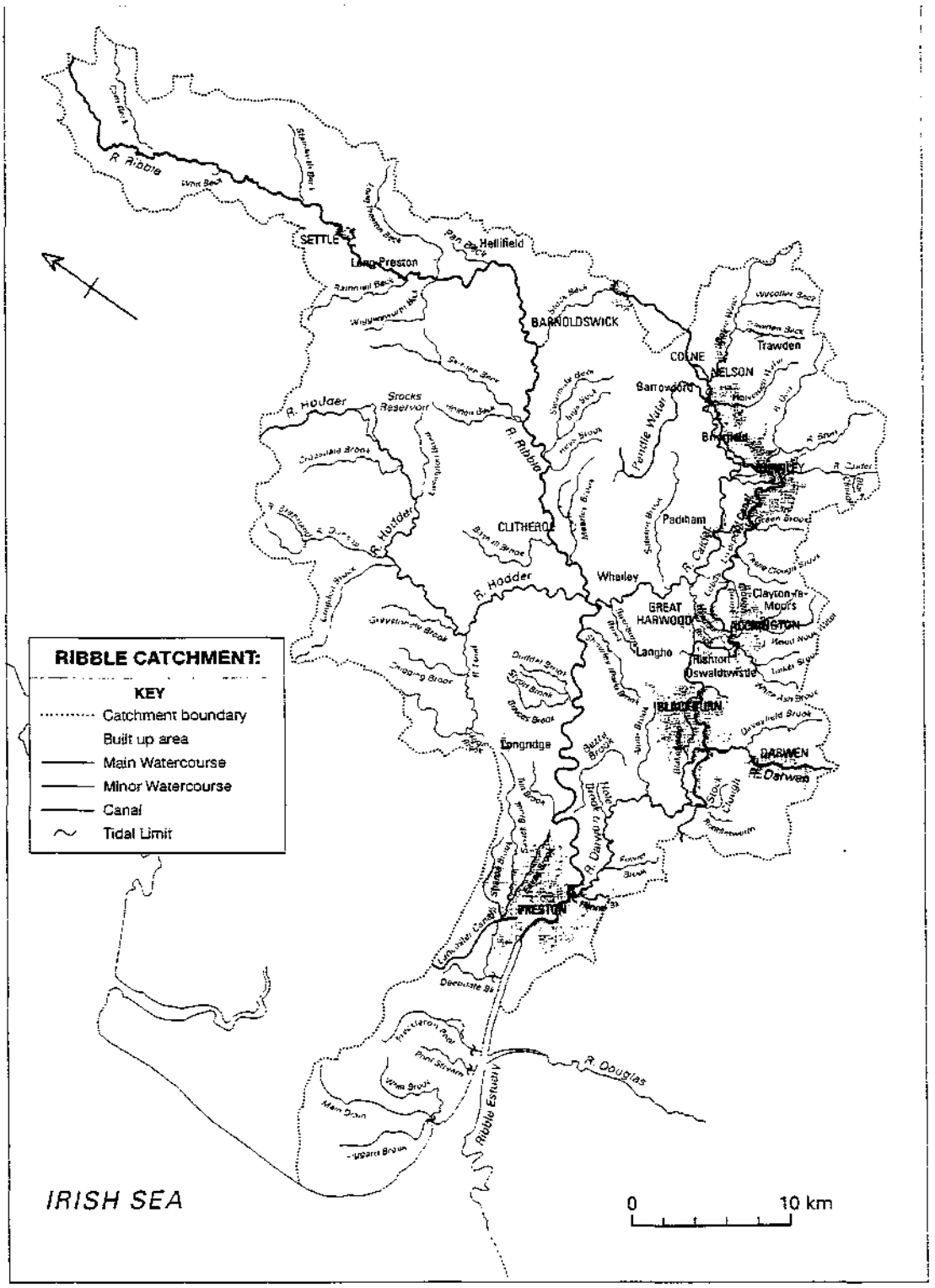

Figure 1.1 The Ribble Catchment (from NRA 1995)

1.4.3 Fisheries

The River Ribble and its tributaries support major recreational fisheries and the Ribble Estuary supports commercial drift net fisheries for salmon and sea trout, as well as bass and mullet.

The Ribble migratory salmonid fishery is in the order of 500 - 700 salmon and 500 migratory trout a year. The drift net catch in the Ribble Estuary peaks and ends in August for salmon (c. 250) and is minimal for sea trout (mainly June/July). Rod catches of salmon increase throughout the fishing season with appreciable numbers being caught in August and September, and a peak of about 100-150 in October. The rod catch of migratory trout, however, tends to peak in August (200-300), rapidly dropping off in September.

Rod fishing is undertaken throughout the Ribble and Hodder, with occasional salmon taken on the Calder.

Historically, poor water quality from the River Calder and other industrialised areas affected the fisheries, but since its recovery began greater use has been made by salmon of the river below Calder Foot (the Big Ribble) as a lying up area (NRA 1995). The Big Ribble now contributes a major portion of the total rod catch but the River Hodder is regarded as having better sea-trout and multi-sea winter salmon fisheries than the River Ribble (NRA 1995).

The fisheries of the River Ribble can be divided into three distinct sections: the Estuary (commercial); the Big Ribble and the Upper Ribble (above Calder Foot/Hodder Foot).

Brown trout are found throughout the river system, though the major rod fisheries are located in the Hodder and the Middle/Upper Ribble.

2. INITIAL ASSESSMENT

2.1 The tainting problem

Consultations with Agency Fisheries/Pollution Staff and the examination of available records revealed that the majority of reports of tainting related to the Estuary and all parts of the River Ribble, particularly the Big Ribble, with fewer reports implicating the River Hodder.

Officially the current controversy started in early 1991, when a Mr Hargreaves submitted a sample of 'tainted' salmon flesh to the former National Rivers Authority (NRA). Analysis of the tissue revealed the presence of 1.92 mg kg^{-1} (ppm) of phenol. At the time there was no record of a phenol spill, although one company in the catchment produced effluent containing p-chlorophenol. This company discharged a treated waste under a consent from NWW via the foul sewer to Hyndburn STW on the River Calder. WRc toxicologists were consulted at the time and reported that concentrations of 15-25 mg l^{-1} of phenol in water could cause tainting of fish flesh. NRA Pollution Control staff arranged for water samples to be tested and a maximum concentration of 0.06 µg l^{-1} phenol was found, which was considerably below the levels required to cause tainting.

There are claims of tainting from anglers prior to the date of this incident, but official records of these were unavailable. A member of the Agency Fisheries Staff, with over 20 years of salmonid angling experience in the Ribble catchment, reported that he had been forced to curtail his angling effort in the catchment because of tainting. He described the tainting as commonly being a 'diesel or disinfectant' smell that could be smelled on the hands after handling freshly caught fish or following washing/gutting. He commonly fishes other rivers and has never experienced the same 'taint'. A specific case he cited was having to refund a restaurant for a 25 lb salmon sold about 1980 because it tasted of diesel. In the mid 1990s he was given two fish from estuary which had an 'oily' flavour rather than the usual 'diesel-disinfectant' taint.

Another member of staff has questioned whether additives put into STW effluent to improve the odour could have a tainting effect. He described a 'muddy' taint, as if the fish had picked up the smell/flavour of the river when there was die back of vegetation occurring. It was also suggested that this taint might be more prevalent in fish that had been holding in the Big Ribble, waiting for a spate to take them upstream. However, tainted 'silver' fish with sea lice (i.e. freshly arrived from the sea) have also been reported.

There have been few 'official' taint complains about fish in the last year but, a Pollution Inspector reported hearing of 2-3 tainted fish taken from Big Ribble.

To summarise, three types of taint have been commonly reported, referred to as:

a) "disinfectanty"

b) "diesely"

c) "muddy".

A request for information on past tainting experiences was forwarded by letter to major angling clubs in the Ribble catchment but no returns were forthcoming.

2.2 Discharges/Problem areas identified by Agency Pollution Control Staff

Agency Pollution Control Staff were consulted about the known spill and discharge sources in the Ribble catchment, and their observations are summarised below.

R. Calder Catchment

Phenols	The only known potential source of phenols/p-chlorophenol in the Ribble Catchment is located at Oswaldtwistle. This chemical works discharges via the Hyndburn STW to the River Calder. In 1995 a serious p-chlorophenol pollution and fish kill occurred in the Calder. A legal case is pending, alleging that the licensed discharger discharged above consented limits to Hyndburn STW.
Mineworkings	Historically, old mineworkings in the catchment have been used as a dump for chemical waste, so the groundwater may be polluted and could be a potential source of surface water pollution.
Oil/diesel	Many oil pollution (diesel or light oil) incidents - as frequent as one a week in the catchment.
STWs	Hyndburn STW (the origin of the p-chlorophenol incident); Burnley STW (cocktail of trade effluents including chemicals); Colne WWTW (cocktail).
degreaser/citrus oils/detergents/dyes	Responsible for various different pollution incidents.
Plating waste	Toxic metals - discharged through STWs.
Storm overflows	Possibly contain trade effluent.

R. Darwen

Paint	A paint spill was recorded in 1996 and a salmon was handed in smelling of paint. One paint manufacturer has a consented storm overflow (COD sampled when it discharges).
White spirit	A wallpaper manufacturer has had losses of white spirit.
Blackburn WwTW	Solvents and aromatic compounds.

R. Douglas

R. Tawd	Solvent spills reported in the past.
Wiggin WwTW	Trade effluents, PCP.
Starvale WwTW	PCP

Hodder and Upper Ribble Catchments

In the Upper Ribble two tributaries were specifically identified as possible sources of tainting substances viz. Mearley Brook and Stock Beck. These, and additional potential sources of pollution are listed below.

Farm pollution	Can occur in Tun Brook, Duddel Brook, Stydd Brook, Boyces Brook, Showley Brook, Wigglesworth Beck.
Bezza Brook	Airfield activities (de-icing chemicals and road drainage) and brewery failing on BOD (occasionally discharge goes white).
STW	Wigglesworth Beck.
Sheep dip	Langden Brook.
Trade effluent	Mearley Brook flows through Clitheroe. Clitheroe STW receives trade effluent. There have been complaints downstream (yellow foam - fish gasping). Intermittent toxic problems from surface drains. Prone to oil pollution - three incidents per year on average. Cement works discharges to Brook via a side stream (Pimlico Brook) which also carries industrial waste (consented discharge).
Abattoir	Swainside Brook
Effluent	Stock Brook is Class 3 due to poor dilution or sewage effluent from Barnoldswick which has a range of factories, some using dyestuffs and PCP. Some unexplained fish kills downstream. R. Loud - Higgin Brook receives some toxic effluent. Suffered a major fish kill in the past. Rafts of fungus and some low DO incidents.

2.2.1 Literature search

A literature search was undertaken to establish which types of organic chemicals are known to cause tainting problems in fish. Particular attention was paid to the types of compounds known or suspected to be present in the Ribble catchment, and to compounds causing taints similar to those described in fish from the catchment.

A variety of organic compounds, both naturally-occurring and synthetic, can cause taints or off-flavours in fish and much of the literature relating to uptake and release of environmentally occurring odorous compounds by fish published prior to 1982 is summarised in a review which appeared in 1984 (Persson 1984). This suggests that, of the three potential routes for the uptake of odorous substances by fish, for freshwater fish uptake via the gills is the most important route; for saltwater fish the main uptake route is via the alimentary canal. Skin absorption is not considered to be important for freshwater fish.

Rates of uptake of odorous compounds by fish may be very rapid and, depending upon various factors such as water concentration, may occur within minutes; however, rates of release are very much slower (days). For example, a 15 minute exposure was sufficient to produce a detectable taste in rainbow trout exposed to water containing 100 $\mu g\ l^{-1}$ 2,4-dichlorophenol; at a concentration of 10 $\mu g\ l^{-1}$ 2,4-dichlorophenol, exposure for a period of 1.27 hours produced a taint (Shumway and Palensky 1973). Tabulated information on sixty six compounds, fifty seven of which have been found to produce taints in fish, is included in Persson's review.

More recent information (together with some earlier data, where relevant), grouped according to chemical type, is presented below.

Petroleum-derived products

The relationship between odorous compounds in a petroleum refinery wastewater and an oily taste in rainbow trout (*Salmo gairdneri*) was investigated some time ago (Krishnaswami and Kupchanko 1969). The wastewater was diluted with clean water to give water samples of varying threshold odour numbers, and fish maintained in these samples for varying lengths of time. Unfortunately, no chemical analysis was undertaken on the wastewater so it is not possible to relate the tainting to specific organic chemicals or to their concentrations in the diluted water samples. However, it was concluded that there was a relationship between the threshold odour number (TON) of the samples and the occurrence of an oily taste in the fish when the TON was above 0.25 and that the fish developed an oily taste/flavour within 24 hours in samples where the TON was >0.25. It has been suggested that the intensity of the oily flavour in fish differs with the types of hydrocarbons - saturated hydrocarbons (alkanes) do not produce a taint, while unsaturated hydrocarbons (alkenes) and aromatic hydrocarbons produce a strong flavour (Motohiro 1983).

A more recent study on the tainting of Atlantic salmon (*Salmo salar*) (Heras *et al.* 1992) found that salmon can take up significant quantities of hydrocarbons from the environment in a short period of time, and that following exposure to the water soluble fraction of dilute crude petroleum, salmon may look healthy but still be badly tainted. The water soluble fraction of the crude oil used in this study contained mainly monoaromatic compounds (primarily benzene, toluene and xylenes), and this group of compounds was found at concentrations of up to 5 ppm in salmon muscle following exposure of the fish for six hours to different levels (0.4 - 1.5 ppm) of this oil fraction in water. Other hydrocarbons detected at lower levels in the fish included C_3- and C_4-alkylbenzenes, naphthalene and methyl naphthalenes. It was concluded that tainting occurs at lower hydrocarbon concentrations than those used in this study, and that larger salmon ("market-sized") with a moderate fat level would retain petroleum tainting for longer time-periods.

Results from a study of tainting of rainbow trout by diesel fuel in water, based only on taste testing (Davis *et al.* 1992), showed that fish held in tanks containing concentrations of diesel fuel in water as low as 0.06 mg l^{-1} (triangular taste testing procedure) to 0.08 mg l^{-1} (three-alternative forced choice (3-AFC) procedure) became tainted. That such low levels of diesel cause tainting is surprising, although bioaccumulation over the period of exposure presumably leads to far higher concentrations in the fish. In terms of aromatic hydrocarbon content, diesel fuel contains % levels of various volatile compounds such as benzene, toluene, xylenes and other alkyl benzenes, most of which are appreciably water soluble.

A study into depuration of oil taints from rainbow trout (Davis 1995) noted that increasing exposure time to diesel oil (from 1 day to 4 days) had a greater influence on the intensity of tainting than increasing the diesel concentration by a factor of ten (without increasing exposure time). The depuration times found in this study (up to 10 weeks), are very much longer than those noted by some workers (e.g. 11 days for oil taints in salmon (Heras *et al.* 1993)), but similar to those found by other workers (25-35 days for trout (Lockhart and Danell 1992)). This suggests that low continual background levels of hydrocarbons in a river or other water body are more important than occasional short-term high levels (such as might be expected from intermittent spills) with respect to tainting of fish, and that exposure times of a few days lead to prolonged tainting even if the fish move to cleaner sites.

Phenolic compounds

Phenolic compounds, particularly halogenated phenols and phenol methyl ethers (anisoles), often produce characteristic taints in beverages (including water) and foods. For some phenolics (e.g. chlorophenols and particularly bromophenols and halogenated anisoles) the taste thresholds are extremely low.

As noted by Persson (1984), there is contradictory information relating to phenol itself in the literature. Several authors found no tainting in fish exposed to 25 mg l^{-1} phenol, while others report that provided sufficient exposure time is allowed phenol causes taints at 1 mg l^{-1}. It appears that phenolic tastes/odours are only evident in cooked fish as many phenols are present in fish as the sulphate esters, which have no odour. However, other authors (Koning and Hrudey 1992) detected phenol, cresols and dimethylphenols in fish fillets extracted using Soxhlet extraction with dichloromethane and analysis by GCMS. The concentrations of these compounds found in tainted fish were in the range 14 - 165 µg kg^{-1}.

Alkylphenols (various isopropyl-phenol, diisopropyl-phenol and isopropylmethyl-phenol isomers) and thiophenol have been shown to be the principal compounds contributing to tainting of walleye pike (*Stizostedion vitreum*) in a Canadian river (Lindsay and Heil 1993). Taste descriptors used included chemical/petrol/phenolic and burnt rubber/sulphurous. 2,4-diisopropyl phenol present in fish at 3 µg kg^{-1} produced a phenolic/smokey taste, but at 30 µg kg^{-1} the taste was described as petroleum-like. Paper-like and cardboard-like descriptors were associate with thiophenol (20 µg kg^{-1}), 2-isopropyl phenol (100 µg kg^{-1}) and 3-isopropyl phenol (10 µg kg^{-1}). The alkylphenols varied in their flavour potency and qualities, they seemed to show synergistic interactions, e.g. alkylphenols alone were modestly unpleasant, whereas when thiophenol was also present the flavour was extremely unpleasant. It was suggested that the origin of the alkylphenols in these fish was their diet of insects which had acquired the tainting compounds from river sediments.

Tabulated data from the literature in Persson's review provide information on estimated threshold concentrations (ETC) of various other phenolic compounds as follows:

- chlorophenols (o-, m- and p-) - ETC range 0.015 - 0.06 mg l^{-1};
- cresol (methyl phenols; o-, m- and p-) - ETC range 0.12 - 2 mg l^{-1};
- dichlorophenols (various isomers) - ETC range 0.0004 - 0.023 mg l^{-1};
- trichlorophenols (2,4,6-) - ETC range 0.001 - 0.052 mg l^{-1};
- xylenols (dimethyl phenols; various isomers) - ETC range 1 - 5 mg l^{-1}.

The values for the ETC ranges given above refer to freshwater fish.

A report on the tainting of fish (including trout and salmon) by chlorinated anisoles and veratroles showed that tainting correlated significantly with the levels of these compounds (Paasivirta *et al.* 1987). Taste descriptors used for these compounds include muddy, sweet, bitter sweet and musty, but no data are presented on the taste thresholds of these compounds in fish.

Unfortunately there is little information relating to brominated phenols or anisoles - these compounds have much lower taste/odour thresholds in water than do chlorophenols. Published data relating to tainting of fish by bromophenols generally relate to marine fish and saltwater shrimps and prawns. For example, it has been reported (Whitfield *et al.* 1995) that 2-bromophenol and 2,6-dibromophenol produced off-flavours in prawns at concentrations of 2 ng g^{-1} and 0.06 ng g^{-1} respectively, and that the flavour threshold for 2,6-dibromophenol in several marine fish species is 0.06 ng g^{-1}.

Another report dealing with concentrations of polybrominated anisoles in marine fish, shellfish and sediments in Japan (Watanabe *et al.* 1983) gives data on the levels detected, but does not provide information on whether these levels led to tainting.

It has been noted (Whitfield 1988) that phenolic taints may be enhanced by the presence of detergents or kerosene.

Geosmin and 2-methyl isoborneol

These two compounds (which have extremely low taste/odour thresholds) are produced by various species of algae and actinomycetes and have been responsible for many incidents of tainting described as muddy, earthy or musty, although most incidents (e.g. Lovell 1983, van der Ploeg *et al.* 1992) relate to pond culture of fish rather than fish in rivers. Fish appear to be very sensitive to 2-methyl isoborneol (e.g. rainbow trout responded to a concentration of about 2×10^{-5} ng l^{-1}) but behavioural experiments showed that freshwater fish do not avoid waters contaminated with this compound and therefore are easily tainted by it.

Miscellaneous organic compounds

As noted earlier a variety of organic compounds, including the following, can cause taints in fish:

- mono- and sesqui-terpenes (Juttner 1992);
- diphenyl oxide (Branson *et al.* 1979);
- acrolein and 2,4-D (DMA salt) (Folmar 1980);
- butyl acetate and other esters (Juttner 1992; Persson 1984);
- o-dichlorobenzene (Persson 1984);
- thiols (Persson 1984).

Mono- and sesquiterpenes are compounds which may result from wood processing, or may be leached from trees by rain. Others can be produced by microbial action, and some are used as odorants in detergents. The commonest are limonene, bornyl and isobornyl acetate, menthol, α-terpineol and 4-terpineol. Geosmin, mentioned earlier is also a terpene.

Butyl acetate is a common solvent often found in the rivers, and other esters (acetates and propionates) were found in a river following a pollution incident traced to a paint factory.

Although there are obviously considerable data relating to uptake of organic compounds by fish directly from water, another route for tainting is the intake of food which may contain compounds which cause taints. It could be argued that this route is not significant for migratory salmon unless contaminated food is consumed prior to migration, and even if this was the case, depuration times of several weeks would presumably mean that salmon taken early in the season would be the most tainted.

Summary

Several factors which are of relevance have emerged from this literature review. These are listed below.

- Most fish tainting tests are done on cooked fish; therefore the information may not be relevant to fish thought to be tainted when they are caught.
- The medicinal/TCP tainting caused by chlorophenols and bromophenols is only detectable after processing or cooking because the chlorophenols and bromophenols are present in raw fish in the form of the sulphate esters which have no smell. Sources of halogenated phenols could include:
  - silage run-off waters treated with chlorine
  - cooling waters
  - upland raw waters treated with chlorine

- Chloro- and bromo-phenols in rivers may be biomethylated to the corresponding chloro- and bromo-anisoles which are likely to have a musty/earthy/mouldy taint. These compounds might be detectable in the water because they are odorous at concentrations of a few ng l^{-1}.

- The most likely substances to induce petrol or "diesely" flavours are alkylaromatic hydrocarbons such as toluene, xylenes, ethylbenzene, C_3-alkylbenzenes, C_4-alkylbenzenes, naphthalene, indene, indane, methyl- and dimethylnaphthalenes and also alkylcyclohexanes. For alkylbenzenes odour threshold concentrations in water range from just below 1 µg l^{-1} to about 1 mg l^{-1}. Potential sources of petrol/fuels in water include the following:

  - petrol station leakage
  - illegal dumping of waste oil
  - refinery effluents
  - diesel/petrol spillages

- There is relatively little information relating to chemicals dissolved in water tainting fish. The more usual cause of fish tainting appears to be their food, and in particular the eating of insects which are contaminated by polluted sediments, rather than the fish absorbing chemicals from the water.

3. FISH AND SEDIMENT SAMPLING

3.1 Fish

3.1.1 Factors affecting the collection of fish

Introduction

A strategy for the collection of fish flesh samples was derived after consideration of the following main factors:

- the technical requirements of taste testing and tissue analysis;
- the known occurrence of 'tainted' fish;
- means of obtaining fish;
- the financial resources available for fish purchase, taste testing and tissue analysis;
- the remaining duration of the rod season;
- the cessation of the commercial fishing season.

Technical requirements for taste testing and tissue analysis

The SOAEFD Marine Laboratory at Aberdeen, which was sub-contracted to undertake the taste testing, proposed that the ideal number of fish portions per taste panel sitting was ten. These portions should be derived from individual fish, but replicates from the same fish could be used if necessary. For reference purposes a small number of untainted control fish was desirable. For each tasting, the Marine Laboratory required one 200 g steak per fish, taken from behind the dorsal fin. WRc Analysis also required one additional steak 200 g per fish for tissue analysis. For the smaller non-migratory trout, obtaining two 200 g samples was not expected to be feasible; for these, fish flesh from behind the dorsal fin to the tail to be was divided into two portions. For chemical tissue analysis either individual fish, or if insufficient flesh were available, bulked flesh from several fish could be used.

It was considered inadvisable to mix fish of different species at a taste panel sitting, or in bulked samples for tissue analysis.

Occurrence of tainted fish

Since most reports of tainting concerned salmon, it was concluded that of the two migratory species available, flesh from salmon rather than sea trout would be examined in Phase 1, whenever possible.

The tainting problem had been mainly reported from the estuary and the Big Ribble, and to a lesser extent from the Upper Ribble. It was thought highly unlikely that rod caught salmon could be obtained from the Douglas, Darwen or Calder. It was therefore concluded that salmon should be obtained from the Estuary, Big Ribble and Upper Ribble and, if available, from the Hodder.

There was also a need to sample salmonids from the Calder, since it was considered to be the most likely source of tainting. In the absence of a migratory salmonid fishery it was decided that brown trout should be collected from this river, and compared with brown trout from the Upper Ribble.

Means of obtaining fish

Consultation with Agency staff confirmed that the principle means of obtaining fish would have to be the purchase of rod and net caught fish offered voluntarily to the Project. The capture and killing of adult salmon by the Agency using other means was not considered to be acceptable to local fishing interests. In particular, a fish trap owned by the Agency in the Upper Ribble at Waddow Hall could not be used for this purpose as agreements only existed for the removal of salmon for egg stripping purposes. However, trapping of brown trout was considered feasible and acceptable.

For salmon, it was not considered feasible to prescribe whether the sampled fish should be silver (freshly arrived from sea, and referred to locally as 'bars of silver') or coloured fish that had been in the river for some time (locally termed 'kippers), due to the imminent closure of the angling season.

Financial resources available for fish purchase, taste testing and tissue analysis

The costs of fish purchase, taste testing and tissue analysis were used to define the maximum number of fish that could be sampled within the available budget.

Duration of remaining rod season

By the time the project was let there was only about one month left of the 1996 rod fishing season. Following consultations with the regional Fisheries Staff and the Agency Contract Manager it was concluded that it should be feasible to obtain the required rod-caught fish.

Cessation of the commercial fishery

The cessation of the commercial fishery, which occurred as the contract started, precluded the collection of fresh estuarine fish for the Project. However, enquiries revealed that frozen fish were still available from stock held by fishermen.

3.2 Fish sample requirements

3.2.1 Numbers and location

Having considered the factors described in Section 3.1, the organisation of the fishery (Section 1.3) and the known potential sources of tainting substances in the Catchment (Section 2.2) a plan for the sampling of catches was derived. This comprised the collection of Salmon in batches of 10 from the Estuary, Big Ribble and Upper Ribble (Table 3.1). If possible three silver salmon from the Hodder would be supplied to MLA as taste controls and should any Calder caught salmon be made available up to 5 would be taken.

Since it was thought unlikely that Calder Salmon would be available a requirement was identified for 10 brown trout (non-migratory) from the Calder and 10 from Big Ribble. Three trout from the Hodder, if available, would again be used as taste controls.

Table 3.1 Summary of fish requirements

Species	Fishery	Number
Salmon	Estuary	10
	Big Ribble	10
	Upper Ribble	10
	Calder	up to 5
	Hodder	3
Trout	Calder	10
	Upper Ribble	10
	Hodder	3

Any non-migratory brown trout obtained were to be retained pending a decision on whether to use them, once the salmon had been taste tested.

3.2.2 Collection of fish

It was appreciated from the outset of the work that the collection of fish samples should be undertaken within the Ribble Catchment by either a local commercial outlet or Agency staff, and the co-operation of the regional Fisheries Staff based at Bamber Bridge, Preston was offered.

The collection was jointly organised by WRc and the Area Fisheries Staff. WRc provided the necessary paperwork, letters to angling clubs, protocols, payments to anglers/netsmen and organised refrigerated transport to Aberdeen. Area Fisheries Staff provided local contact with anglers/netsmen, the collection of fish and their storage prior to despatch to Aberdeen.

To facilitate this arrangement WRc provided:

- a sampling protocol;
- receipt form;
- letter to angling clubs/anglers.

WRc wrote to each of the major salmon angling clubs on the Ribble advising them of the project and requesting their assistance in informing their members of the offer to purchase fish and the means of handing them over. Further personal contacts were made by Area Fisheries Staff. Despite the ending of the commercial netting season Area Fisheries Staff were able to obtain whole-frozen Ribble Estuary salmon from stock still held by commercial netsmen.

Provenance

Particular attention was paid to the provenance of fish which was facilitated by the experienced fisheries staff. For each fish the following information was obtained:

a) Name, address and telephone number of angler/netsmen (and of supplier if different from above).

b) Date and time caught.

c) Time elapsed from capture to freezing.

d) Location caught (including grid reference).

e) Condition ('kipper' or 'silver bar', presence of lice etc).

on a signed proforma.

Payment of anglers/suppliers

An essential part of the trust that was needed with fish suppliers was prompt payment. Copies of the receipts issued to suppliers/anglers by Area Fisheries Staff were forwarded to WRc by fax for payment within 1-2 days.

3.2.3 Sample preparation

A sampling protocol was designed following consultation with the Marine Laboratory in Aberdeen to ensure (a) that the fish samples arrived at the lab in the best possible condition for the taste panel, (b) that a sample was also preserved for chemical analysis at WRc, and (c) that the required information was recorded.

Fish supplied frozen by angler/netsmen

The fish supplied whole were left in whatever wrapping (if any) they had been frozen in. The package, or unwrapped fish, was wrapped in aluminium foil upon receipt by The Agency and frozen in a freezer at a temperature of about -18 °C. Once wrapped in aluminium foil, the fish could be placed in a plastic bag or other suitable container and labelled.

It was impracticable to remove fish steaks prior to transport and therefore whole fish were supplied to the Marine Lab. Their staff removed sufficient flesh for their requirements, re-froze the fish, and supplied WRc with a steak.

Fish supplied unfrozen

Where practicable fish were prepared as follows before freezing in aluminium foil. Two steaks were cut from behind the dorsal fin, separately wrapped in aluminium foil and placed in a labelled bag or plastic sample pot.

Labelling of samples

Each fish and the samples removed from it were labelled SALMON or TROUT and given a separate identifying code:

Estuary caught:	E1 - E10
Big Ribble caught	BR1 - BR10
Upper Ribble caught	UR1- UR10
Calder	C1 -
Hodder	H1 -

Where steaks were removed they were labelled with the fish identifier and A or B, so that sample A was used for taste testing and sample B was used for analysis.

3.2.4 Fish samples obtained

Following a considerable effort by the Agency's Fisheries Staff the sample requirements were partially met (Table 3.2). However, the numbers obtained were considered adequate for the taste testing and analysis to be performed.

Table 3.2 Summary of fish obtained

Species	Fishery	Number planned	Number obtained
Salmon	Estuary	10	10
	Big Ribble	10	9
	Upper Ribble	10	2
	Calder	up to 5	-
	Hodder	3	1
Trout	Calder	10	5
	Upper Ribble	10	3
	Hodder	3	-

The ten Estuary fish were obtained from commercial netsmen's stock in trade supplied by two netsmen (5 each). The remaining salmon were purchased from anglers.

The five Calder trout were netted by Agency Staff and the three Upper Ribble trout were taken by Agency staff from the Waddow Trap.

The location where each fish was taken is shown in Figure 3.1 and details of fish are given in Appendix B.

3.3 Sediment sampling

Sediments were collected from fifteen sites in the catchment (Figure 3.2), selected to represent the full length of the River Ribble, its principal tributaries and those minor tributaries identified in Section 2.2 as being of concern, and from the estuary.

Sampling was undertaken over a two day period (17.9.96-18.9.96) before the first major spate flows of Autumn.

At each site replicate samples of 100 cm^3 of surface sediment (from the top 2 cm) were obtained from the river bed. Samples were collected in glass screwtop jars. The jars were capped with aluminium foil before the lids were screwed down. Each set of replicate samples was stored in a separate freezer to minimise the risk of accidental loss.

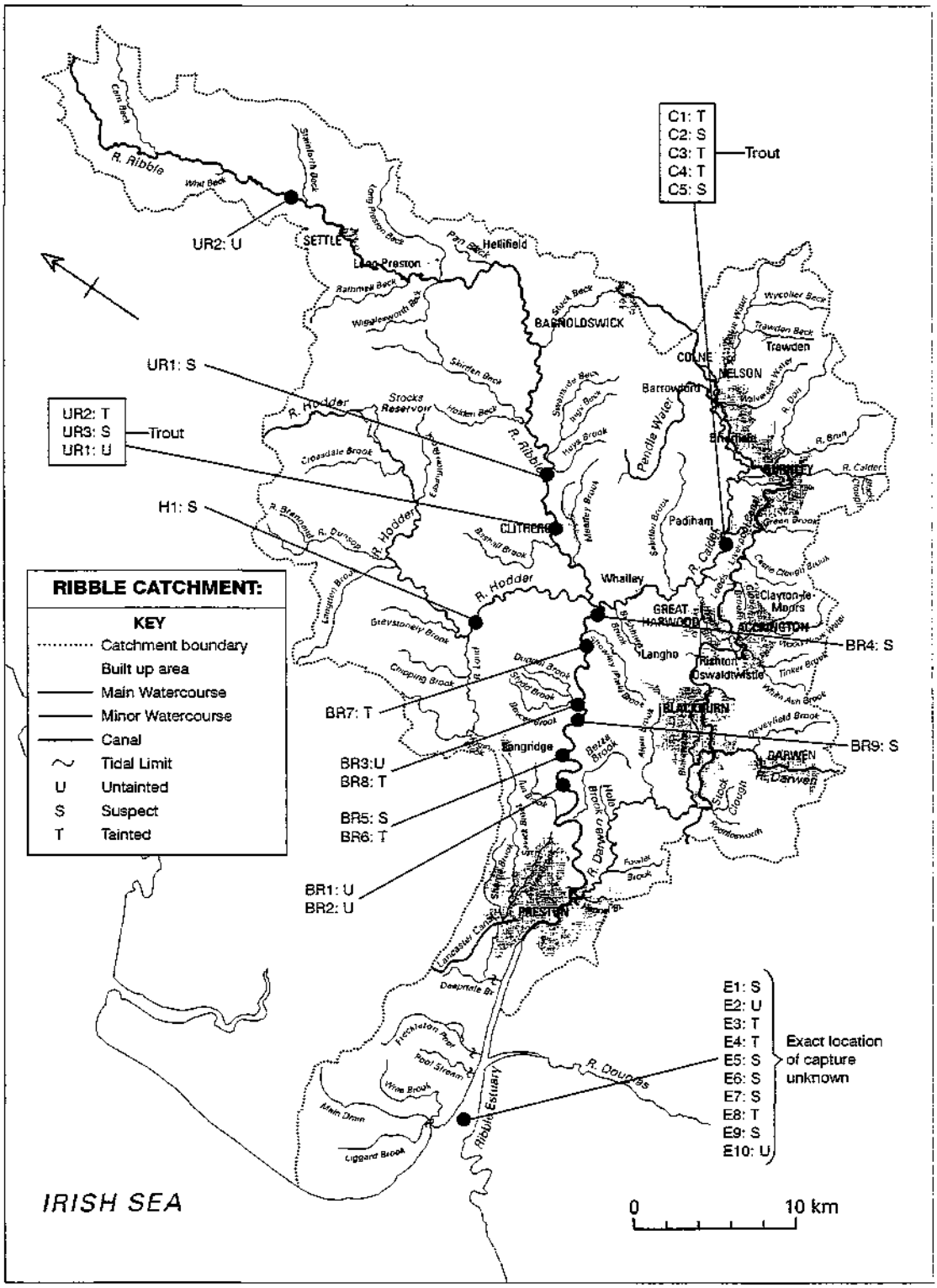

Figure 3.1 Location of capture of each fish

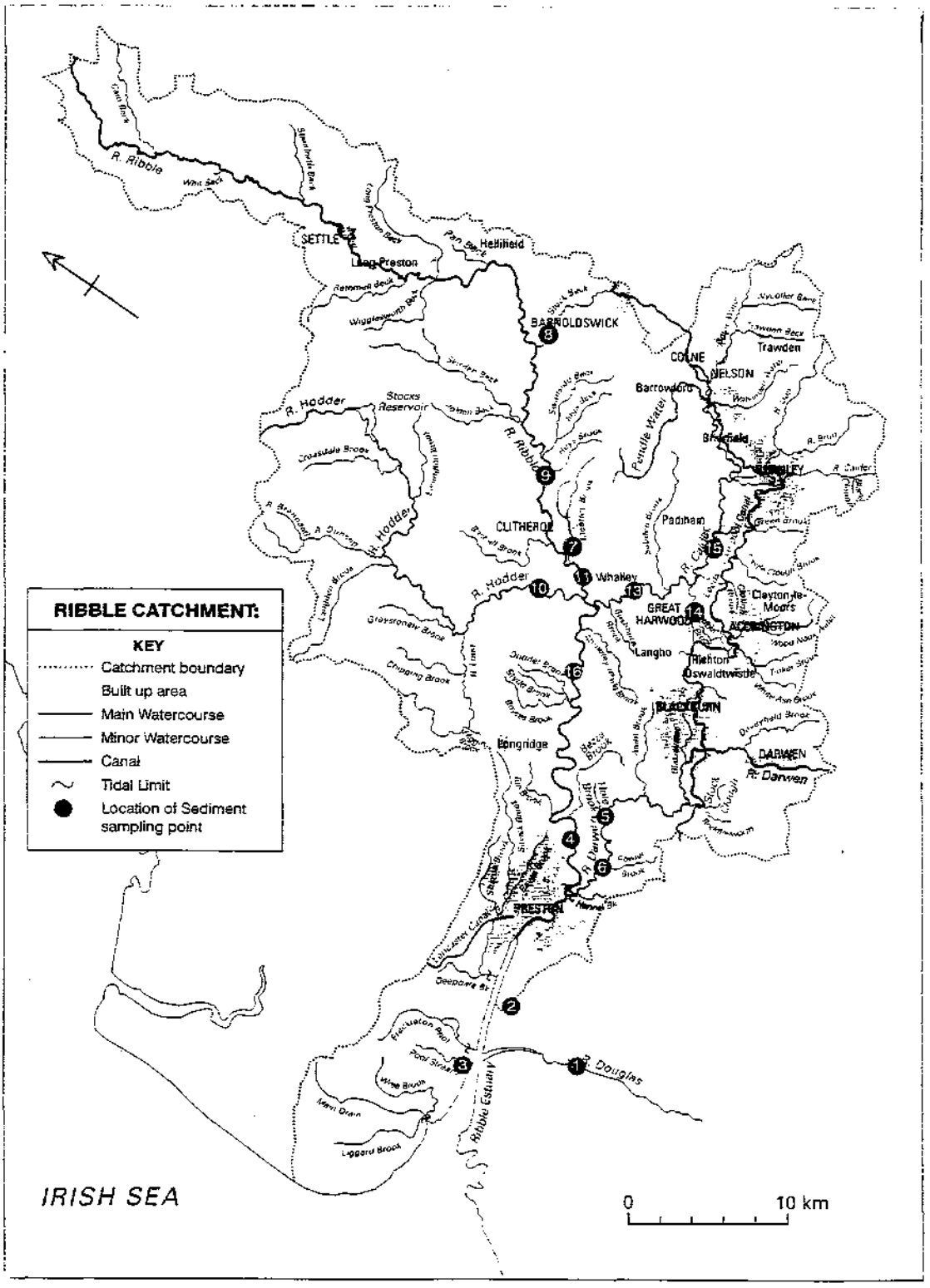

Figure 3.2 Location of sediment sampling points

4. TASTE TESTING

4.1 Introduction

Taste testing of all samples was undertaken by the SOAEFD Marine Laboratory Aberdeen (MLA), which has taken over this function from the former Torry Food Science Laboratory.

MLA was supplied with two batches of fish: firstly the 22 salmon and then, following testing and reporting on these, the 8 trout.

Transportation of the fish from The Agency in Preston to MLA was undertaken by a commercial contractor in refrigerated road transport.

4.2 Tasting methodology

4.2.1 Preparation of fish samples

Salmon

The twenty two salmon samples comprised 12 whole fish containing guts and 10 steaks (one containing guts). Each sample was prepared and cooked prior to tasting using the following protocol:

- A steak, approximately 2 cm thick, was taken from behind the dorsal fin of each whole fish whilst frozen.
- The frozen guts and kidney tissue were removed from all the steaks. The steaks were then rinsed in clean potable water.
- The steaks were allowed to thaw at ambient temperature and once thawed, the skin was removed.
- The steaks were placed in coded glass casseroles and cooked by microwave heating until a temperature of 65 °C was reached.
- The samples were kept warm on electric hot plates during each tasting session.

Trout

The procedure preparation procedure as described for salmon was followed with the exception that the steaks were cut slightly thicker due to the smaller size of the fish.

4.2.2 Taste panels

The samples of salmon were tasted by a panel of 8 assessors experienced in sensory assessment of fish and taint assessment. The samples were tasted by a reduced panel of 4 trained assessors and one trainee, due to staff sickness.

4.2.3 Scoring of samples

A 'taint' can be described as an odour or flavour that is foreign to the product being sampled and this can be either pleasant or unpleasant. Tainting is recorded using standard descriptors to define the flavours/textures a panellist experiences and a numerical score to quantify the severity of the taint.

MLA uses a numerical score on a scale from 0 to 5 where:

0 = no taint;

1 = a slight taste;

5 = extremely strong flavour.

Any score >0 is considered to be a 'positive' result. A fish is deemed to be 'tainted' if 50% or more of the taste panel's scores are positive. In cases where the percentage is less than 50 but equal to or greater than 20, the sample is regarded as 'suspect'. Scores <20 are deemed 'untainted'. This system can also be applied to batches of fish using the cumulative positive and negative results from each panellist for each fish.

For each fish the individual scores from each panellist were also combined to give a 'mean panel score' which is used as a measure of the 'degree of tainting' of that fish, on the same 0 - 5 scale.

4.3 Results

4.3.1 Tasting results for each Salmon

Estuary Salmon

The 10 estuary salmon were tasted at a single sitting by the 8 panellists and the results for each fish are shown in Table 4.1. Only 2 fish were considered untainted (positive scores <20%), five were suspect (+ve scores ≥ 20 <50) and three (E3, E4 and E8) were tainted (+ve scores ≥ 50%). Mean panel scores ranged from 0.2 - 0.4 for the suspect fish and from 0.6 - 1.6 in the tainted fish.

All 10 fish were described as having an unpleasant 'earthy' flavour, 7 left an 'aftertaste' in the mouth and 5 gave a sensation of 'astringency'. The aftertaste was described as 'chemical' in 3 cases. A plastic/PVC flavour was recorded in 2 fish and in the worst fish (E8) a Dettol-like flavour was recorded.

Nine fish were described as having a 'dry texture' (described also as 'tough' in 5 cases) and the other (E7) as having a 'stale odour'.

There were no reports of either a 'phenolic' or an 'oily/dieselly' flavour in the 10 estuary fish.

Table 4.1 Taste testing results for individual salmon from the Estuary

Fish	Tastings	No. positive	% Positive	Mean panel score	Description
E1	8	2	25 Suspect	0.2	Very strong earthy, musty flavour. Slight astringency and after-taste. Tough dry texture.
E2	8	1	12.5 Untainted	0	Slightly earthy, cardboard flavour. Tough dry texture.
E3	8	4	50 Tainted	0.8	Very strong earthy, cardboard, old leather, PVC, plastic flavour. Slightly tough, dry texture
E4	8	4	50 Tainted	0.6	Very strong earthy, unpleasant flavour. Slight astringency and after-taste. Dry texture.
E5	8	2	25 Suspect	0.2	Slightly earthy, some astringency and chemical aftertaste. Dry texture.
E6	8	3	37.5 Suspect	0.4	Strong earthy, musty flavour. moderate astringency and lingering after-taste. Dry texture
E7	8	2	25 Suspect	0.4	Earthy, leathery, slight chemical after-taste, stale odour.
E8	8	7	87.5 Tainted	1.6	Very strong earthy unpleasant flavour. Strong astringency and chemical aftertaste. Dettol like flavour. Tough, dry texture
E9	8	3	37.5 Suspect	0.3	Slightly earthy, painty, some after-taste. Dry texture
E10	8	1	12.5 Untainted	0	Earthy, unpleasant flavour. Tough dry texture.

Big Ribble Salmon

The 9 Big Ribble salmon were tasted at a single sitting by the 8 panellists and the results for each fish are shown in Table 4.2. Three fish were considered untainted (positive scores <20%), 3 were suspect (+ve scores ≥ 20 <50) and 3 (BR6, BR7 and BR8) were tainted (+ve scores ≥ 50%). Mean panel scores ranged from 0.3 - 0.8 for the suspect fish and from 0.7 - 1.6 in the tainted fish.

Table 4.2 Taste testing results for individual salmon from the Big Ribble

Fish	Tastings	No. positive	% Positive	Mean panel score	Description
BR1	8	1	12.5 Untainted	0	Earthy, some sweetness, slightly tough dry texture.
BR2	8	1	12.5 Untainted	0	Earthy, musty, mossy flavour with some sweetness. Slightly tough dry texture
BR3	8	1	12.5 Untainted	0	Earthy, moderate sweetness, slightly tough dry texture.
BR4	8	2	25 Suspect	0.3	Earthy, musty, slight astringency, slightly tough dry texture.
BR5**	8	2	25 Suspect	0.3	Slightly earthy, musty, sour, bitter fish oil.
BR6	8	6	75 Tainted	1.0	Musty, leathery, slight chemical taste, sour, bitter, unpleasant.
BR7	8	6	75 Tainted	1.6	Old leather, PVC, plastic flavour, garden chemicals, earthy.
BR8	8	4	50 Tainted	0.7	Earthy, leather, PVC, plastic flavour, garden chemicals, strong initial taste
BR9	8	3	37.5 Suspect	0.8	Phenols, slightly perfumed, bitter,, unpleasant aftertaste, soft, slimy texture

** Steak contained guts and some of the unpleasant odours may have been spoilage odours related to the gut contents.

Seven of the 9 fish were described as having an 'earthy' flavour and one more as having a musty flavour 7, whilst the remaining fish (BR9) had a 'phenol/perfumed/bitter' taste. BR9 was also the only Big Ribble salmon that left an 'aftertaste' in the mouth.

The three untainted fish were described as having a 'sweet' flavour; this was not reported in the others. A slight 'astringency' was reported in only one fish. Six of the 9 fish, including the three untainted, had a tough or leathery texture.

All three tainted fish gave a 'chemical' flavour, described as 'garden chemicals' in two of them. These two (BR7 and BR8) also gave a PVC/plastic taste.

There were no reports of an 'oily/dieselly' flavour in the Big Ribble salmon.

Other Salmon

The two Upper Ribble salmon and single fish from the Hodder were tasted by the panel of 8 assessors (Table 4.3). One Upper Ribble and the Hodder fish were 'suspect'. Both Upper Ribble fish exhibited the earthy/musty flavour, whilst the Hodder fish was described as 'bitter with an eggy odour and soft, slimy texture.

None had 'chemical, phenol, plastic or oily/diesel' flavours.

Table 4.3 Taste testing results for individual salmon from the Upper Ribble and Hodder

Fish	Tastings	No. positive	% Positive	Mean panel score	Description
Hodder 1	8	2	25*	0.5	Bitter, eggy odour, soft, slimy texture
Upper Ribble 1	8	2	25*	0.6	Earthy, musty slightly sour off-flavours.
Upper Ribble 2	8	1	12.5	0	Earthy, musty slightly off-flavours.

General observations on the salmon by MLA

The report provided by MLA (Appendix B) provides some additional general observations, within its discussion section, on the taste of the salmon:

"the nature of the taint described by the panel is not specific to one chemical or tainting substance but that the main conclusion is that the fish tasted were particularly unpleasant. Although earthy, musty flavours are characteristic of salmon, the intensity at which these flavours occurred was objectionable and would render the fish unfit for human consumption."

and that:

"The majority of the whole fish supplied (mainly Estuary) were female in an advanced state of sexual maturity. Fish of this condition are not normally consumed as the flesh is of poor eating quality and this will have added to the unpleasantness of the fish as they were very dry and tough in texture."

4.3.2 Taste results for each trout

Calder Trout

The five estuary salmon were tasted at a single sitting with the three Upper Ribble trout by five panellists and the results for each fish are shown in Table 4.4.

Table 4.4 Taste testing results for individual trout from the R. Calder

Fish	Tastings	No. positive	% Positive	Mean panel score	Description
C1	5	3	60	1.2	Strong earthy, musty flavour with a strong chemical, soapy taste. Very unpleasant
C2	5	2	40*	0.6	Sour, dirty dishwater odour, musty, soapy flavour. Tough texture.
C3	5	5	100	2.6	Strong earthy and chemical phenol type flavour
C4	5	4	80	0.4	Sour, musty, earthy off-flavours, soapy and phenolic.
C5	5	2	40*	0.6	Earthy, musty slightly unpleasant

None were untainted; two were suspect and three were tainted. Trout C3 stands out as being the only fish in the study to be scored 100% tainted and had the highest MPS of 2.6. The flavour of this fish, together with Trout C4 were given a 'phenolic' descriptor whilst the tainted C1 had a very unpleasant strong chemical, soapy taste. In all 3 of the 5 fish were described as 'soapy'.

None were described as having an 'aftertaste' at the time of sampling but after the panel sitting to taste the trout, all the assessors reported an unpleasant and lingering soapy, chemical like after-taste which persisted for several hours (even after drinking coffee).

None reported a sensation of 'astringency' or the flavours of 'PVC/plastic' or 'oily/dieselly'.

Upper Ribble Trout

Of the three Upper Ribble trout taken in the Waddow Trap one was untainted one was suspect and one was tainted (Table 4.5). The latter fish (Trout UR2) was described as very earthy with a slight chemical taste.

Two fish were described as slimy or soft textured.

None of the assessors reported a sensation of 'astringency' or the flavours: 'PVC/plastic, phenols or oily/dieselly'.

Table 4.5 Taste testing results for individual trout from the Upper Ribble

Fish	Tastings	No. positive	% Positive	Mean panel score	Description
UR1	5	0	0 Untainted	0	Bland, slightly soapy flavour
UR2	5	3	60 Tainted	0.8	Very earthy, slight chemical taste, slimy texture
UR3	5	1	20 Suspect	0.6	Unpleasant, slight off-odour, soft texture

General observations on the trout by MLA

The report provided by MLA (Appendix B) provides some additional general observations, within its discussion section, on the taste of the trout:

The nature of the taint as described by the panel was similar to that of the salmon but with an additional soapy, chemical like flavour which resulted in a very persistent after-taste. Again the majority of the fish were female in an advanced state of sexual maturity adding to the impression that these were unpleasant and of very poor eating quality.

4.4 Tasting results for batches of fish

The data from individual tastings (one sample by one assessor) combined to provide summary scores for the different batches of fish (Tables 4.6). For the 80 samplings of the Estuary salmon (10 fish x 8 assessors) 29 were positive giving a batch score of 36% (suspect). An identical score was derived for the Big Ribble salmon.

Calder trout, by contrast, gave 64% positive results and can be deemed 'tainted'. The Upper Ribble batch of trout was 'suspect' like the two batches of salmon with a 27% score. These results, albeit from a limited number of fish suggest that fish from the Calder are more tainted than those from the Upper Ribble.

Table 4.6 Summary data for batches of fish

Batch	No. Fish	Tastings	No positive	% Positive
Estuary salmon	10 Salmon	80	29	36% (Suspect)
Big Ribble salmon	9 Salmon	72	26	36% (Suspect)
All freshwater caught salmon*	12 Salmon	96	31	32% (Suspect)
Calder trout	5 Trout	25	16	64% (Tainted)
Upper Ribble trout	3 Trout	15	4	27% (Suspect)

* Big Ribble (9) + Hodder (1)+ Upper Ribble (2)

4.5 Summary

The nature of the taint described by the panel is not specific to one chemical or tainting substance, and differences are apparent between the two species and the batches of fish from different parts of the Catchment (Table 4.7).

Table 4.7 Summary of taints reported for each batch of fish

Batch of Fish	Earthy or musty	Soapy	Phenol	PVC/ Plastic	After taste	Oily or Diesely	Astringency	Chemical
Estuary salmon	✓ 10/10	✗	✗	✓ 2/10	✓ 7/10	✗	✓ 5/10	✓ 3/10
Big Ribble salmon	✓ 8/9	✗	✓ 1/9	✓ 2/9	✓ 1/9	✗	✓ 1/9	✓ 3/9
Upper R. Hodder salmon	✓ 2/3	✗	✗	✗	✗	✗	✗	✗
Calder trout	✗	✓ 3/5	✓ 2/5	✗	✓*	✗	✗	✓ 2/5
Big Ribble trout	✓1/3	✗	✗	✗	✗	✗	✗	✓ 1/3

* None were described as having an 'aftertaste' at the time of sampling but afterwards all the assessors reported an unpleasant and lingering soapy, chemical like after-taste which persisted for several hours.

The almost universal presence of and earthy/musty flavour distinguishes the salmon from the trout. MLA concluded that these fish tasted particularly unpleasant.

A soapy flavour distinguishes the Calder trout and this occurred in association with a chemical taste. All the samples were checked for 'TCP' (chemical) smell before cooking and none were positive. After preparation, chemical tastes were observed in some individuals from most batches of fish but the descriptors differ: two Big Ribble salmon having a 'garden' chemical taste; whilst a 'Dettol' flavour was described for the worst Estuary salmon (E8).

Two Calder trout exhibited a 'phenolic' taste but elsewhere the incidence of this taste was low (one Big Ribble salmon only). Plastic/PVC was reported in some salmon from both the Estuary and the Big Ribble.

Aftertastes were particularly bad in the Calder trout (chemical/soapy) and the Estuary salmon (chemical or undefined). The Estuary salmon also frequently exhibited astringency.

The taste panels were experienced in assessing hydrocarbon tainting but did not encounter any characteristic flavours in any sample. MLA also noted that none of the raw samples of fish (before preparation) smelt of diesel.

MLA considers that, since most of the sample fish were female in an advanced state of sexual maturity this contributed to their general unpleasantness and made them of poor eating quality. However, all fish were taken during the open fishing season and preserved frozen (in the case of the estuary fish these were in stock ready for sale) and the taste/texture can be assumed to be typical of that experienced by consumers of Ribble fish.

5. TISSUE ANALYSIS

5.1 Introduction

The scope of the fish tissue analysis was limited by the available resources and concentrated on salmon as this species was of greatest concern. Tissue samples from the eight fish that were considered by the tasting panel to exhibit the highest Mean Panel Scores (worst taints) were examined by Gas Chromatography-Mass Spectrometry (GCMS). The salmon selected were: BR6, BR7, BR8, BR9, UR1, E3, E4 and E8.

5.2 Methodology

Portions of the salmon flesh which had been taste tested at the Marine Laboratory in Aberdeen were delivered to WRc using refrigerated transport, and were immediately placed in freezer at -18 °C upon receipt. Samples (20 g) were then taken for analysis.

The samples were ground with anhydrous sodium sulphate and loaded into Sohxlet extraction thimbles. A mixture of deuterium labelled internal standards was added (so that the concentration of each internal standard was 50 $\mu g\ kg^{-1}$) and the samples extracted with dichloromethane for about 16 hours. Each extract was then reduced in volume to 1 ml and analysed using GCMS.

The GCMS system was used in general survey mode, i.e. the aim of the analysis was to identify as many as possible of the detected compounds. A 60 m fused silica GC column was used, and the initial temperature of 30 °C was maintained for 4 minutes following injection of the extract (1 µl), then linearly programmed at 8 °C min^{-1} to 300 °C. This final temperature was maintained for 20 min. The mass spectrometer was operated in electron impact mode, with a scan speed of 0.5 sec $decade^{-1}$ over the mass range 20-700 amu, so that a mass spectrum was obtained approximately each second.

The mass spectra obtained from compounds detected as discernible peaks on the total ion current chromatogram were examined and where possible the compounds were identified. In cases where mass spectra were not recognised by the operator, they were searched (using the mass spectrometry data system) against a library of over 300 000 spectra held on disc.

Following this initial GCMS analysis, four of the extracts were re-analysed following removal of acidic compounds. Many such compounds occur naturally in salmon flesh at relatively high levels, and it was considered that these could potentially mask the presence of the compounds which gave rise to the tainting. The acidic compounds were removed by re-diluting the extracts with dichloromethane to about 50 ml and shaking with 50 ml sodium bicarbonate (1 M) solution. The dichloromethane extracts were then concentrated to 1 ml for re-analysis by GCMS.

5.3 Results

The compounds identified in the initial GCMS analyses of eight samples of salmon flesh considered to be those exhibiting the worst taints by the tasting panel are given in Table 5.1.

Table 5.1 Compounds identified from GCMS analysis of extracts from R. Ribble salmon

Compound / Sample	UR1	BR9	BR8	E3	E4	BR6	BR7	E8
%Taint	25	37.5	50	50	50	75	75	87.5
Toluene	-	-	✓	✓	-	✓	✓	✓
Tetrachloroethylene	-	-	✓	-	-	✓	✓	-
d_5-Chlorobenzene	✓	✓	✓	✓	✓	✓	✓	✓
d_{10}-Xylene	✓	✓	✓	✓	✓	✓	✓	✓
C_2-Alkylbenzenes (Xylenes)	-	-	-	-	-	✓	✓	✓
C_3-Alkylbenzenes	-	-	-	-	-	✓	-	✓
2,4-Heptadienal	✓	✓	✓	✓	✓	✓	✓	✓
Octamethylcyclotetrasiloxane	✓	✓	✓	✓	✓	✓	✓	✓
Benzylalcohol	✓	✓	✓	✓	✓	✓	✓	✓
d_8-Naphthalene	✓	✓	✓	✓	✓	✓	✓	✓
Decamethylcyclopentasiloxane	✓	-	✓	-	✓	✓	✓	✓
Dodecamethylcyclohexasiloxane	-	-	✓	-	-	✓	✓	-
Tetradecamethylcycloheptasiloxane	-	-	-	-	-	-	✓	✓
Nicotinamide	✓	✓	✓	✓	✓	✓	✓	✓
Pristane	✓	✓	✓	✓	✓	✓	✓	✓
Benzophenone	✓	-	✓	✓	-	✓	✓	✓
Myristic acid	✓	✓	✓	✓	✓	✓	✓	✓
Palmitic acid	✓	✓	✓	✓	✓	✓	✓	✓
Methyl eicosapentenoate	✓	✓	✓	✓	✓	✓	✓	✓
Stearic acid	✓	✓	✓	✓	✓	✓	✓	✓
Methyl octadecenoate	✓	✓	✓	✓	✓	✓	✓	✓
Cholesteryl acetate	✓	✓	✓	✓	✓	✓	✓	✓
Cholesterol	-	-	-	-	-	✓	✓	✓
Vitamin E	✓	✓	✓	✓	✓	✓	✓	✓

✓ Present

\- Absent (i.e. not detected above c. 25 ppb)

As can be seen from this table, various compound classes and types were identified. Some, such as the fatty acids (myristic, palmitic and stearic acid), the vitamins (nocotinamide (one of the B group vitamins) and vitamin E) and cholesterol and cholesteryl acetate occur naturally in salmon flesh. Others, such as toluene and other aromatic hydrocarbons (C_2- and C_3-alkylbenzenes), do not and are presumably present due to contamination. Several siloxanes were also detected, and these are also considered to be contaminants.

The following general conclusions can be drawn:

- aromatic hydrocarbons (toluene, xylenes and C_3-alkylbenzenes (i.e. trimethylbenzene isomers, ethylmethylbenzene isomers or propylbenzenes)) were detected in the majority of the fish considered to be most tainted (BR6, BR7, E8 - taint scores 75%, 75% and 87.5% *respectively*);
- although tetrachloroethylene (a chlorinated solvent used for cleaning and de-greasing purposes) was detected in three out of four of the Big Ribble fish (BR6, BR7 and BR8) it was not detected in fish caught in other areas of the catchment;
- phenolic compounds (apart from d_5-phenol which was used as an internal standard and added to the fish sample prior to extraction) were not detected in any of the fish analysed; this was not unexpected given that "phenolic" was not a commonly used descriptor by the taste panel, and the contradictory evidence in the literature relating to the detection of phenols in uncooked fish (due to their presence as sulphates which would not be detected by GCMS);
- various siloxanes were detected in several of the fish; these compounds have a wide variety of uses, and are included in some anti-foaming formulations;
- benzophenone (an aromatic ketone) is used as a fixative for heavy perfumes so it may be relevant to the tainting problem; this compound was detected in seven of the fish analysed, but it is not known whether it occurs naturally in salmon flesh or whether its presence is due to industrial usage.

Although these analyses produced some evidence that aromatic hydrocarbons could be involved in the tainting of fish flesh a decision was taken to re-analyse four of the extracts following removal of the acids. As noted earlier (5.2) these could hinder the detection of other compounds, and their presence in the extracts at relatively high levels also adversely affected the chromatographic performance of the GCMS system. The extracts re-analysed were from the three most tainted fish (BR6, BR7 and E8) and the least tainted of the eight fish (UR1), and the results are tabulated in Table 5.2.

As can be seen from this table, many more compounds were detected following removal of the acids from the extract. Again many of these (e.g. the methyl esters of unsaturated fatty acids) occur naturally in salmon flesh. However, many others do not, and in terms of numbers of compounds detected there does appear to be some correlation with tainting, i.e. fewer compounds (12) which are considered to be contaminants were detected in the least tainted fish. The highest numbers and concentrations of aromatic hydrocarbons (toluene, xylenes, C_3- and C_4-alkylbenzenes, methyl naphthalenes and C_2- and C_3-alkylnaphthalenes) were detected in the most tainted fish (E8). Intermediate numbers/concentrations of hydrocarbons were detected in fish BR6 and BR7, while in the least tainted fish no aromatic hydrocarbons were detected. This does not of course rule out the possibility that some were present at concentrations lower than the limits of detection (about 10 $\mu g\ kg^{-1}$) for these analyses.

Table 5.2 Compounds identified in Ribble catchment salmon extracts following removal of acids

Compound identity	Sample = %Taint	UR1 25	BR6 75	BR7 75	E8 87.5
Toluene		-	✓	✓	✓
Tetrachloroethylene		-	✓	✓	-
d_5-Chlorobenzene (I.S.)		✓	✓	✓	✓
Hexamethylcyclotrisiloxane		✓	✓	-	✓
d_{10}-p-Xylene (I.S.)		✓	✓	✓	✓
C_2-Alkylbenzenes (Xylenes)		-	✓	-	✓
Acetophenone		-	-	-	✓
Benzaldehyde		✓	✓	✓	✓
2,4-Heptadienal		✓	✓	✓	✓
p-Dichlorobenzene		-	-	-	✓
Octamethylcyclotetrasiloxane		✓	✓	✓	✓
C_3-Alkylbenzene isomers		-	✓	-	✓
C_4-Alkylbenzene isomers		-	-	-	✓
Decamethylcyclopentasiloxane		✓	✓	✓	✓
Benzyl alcohol		✓	✓	✓	✓
Unknown 54,96,81,67		-	✓	-	-
Unknown 54,96,81,68		-	✓	-	-
d_8-Naphthalene (I.S.)		✓	✓	✓	✓
Naphthalene		-	-	-	✓
Benzothiazole		-	✓	-	✓
2-Phenoxyethanol		-	✓	-	✓
Methylnaphthalene isomers		-	✓	-	✓
Unknown 43,42,71,57		-	✓	-	-
Unknown 145,160,131,118		-	-	-	✓
Dodecamethylcyclohexasiloxane		✓	✓	✓	✓
C_2-Alkylnaphthalenes		-	✓	-	✓
C_3-Alkylnaphthalenes		-	-	-	✓
Tetradecamethylcycloheptasiloxane		✓	✓	-	✓
Diethyl phthalate		-	✓	-	-
Benzophenone		✓	✓	✓	✓
Nicotinamide		✓	✓	✓	✓
Unknown 81,137,197,212		-	✓	-	-
Unknown 145,187,230,107		-	✓	-	-
Pristane		✓	✓	✓	✓
Methyl myristate		✓	✓	✓	✓
Dibutyl phthalate		-	✓	-	-
Methyl palimtoleate		✓	✓	✓	✓
Methyl palmitate		✓	✓	✓	✓
Mehtyl linolenate		✓	✓	✓	✓
Methyl oleate		✓	✓	✓	✓
Methyl stearate		✓	✓	✓	✓
Methyl eicosadienoate		✓	✓	✓	✓
Methyl eicosenoate		✓	✓	✓	✓
Methyl eicosanoate		✓	✓	✓	✓
Di-(2-ethylhexyl)adipate		✓	✓	-	-
Methyl docasadienaote		✓	✓	✓	✓
Methyl docosenoate		✓	✓	✓	✓
Methyl docosanoate		✓	✓	✓	✓
Dinonyl phthalate		✓	✓	-	-
Squalene		✓	✓	-	-
Cholesterol		✓	✓	✓	✓
Vitamin E		✓	✓	✓	✓

✓	Present
-	Absent (i.e. below limit of detection of about 10 ppb)
I.S.	Internal standard

As noted earlier (Tables 4.3 and 4.4) the taste descriptors used for the three most tainted fish were as follows:

- E8 - very strong earthy unpleasant flavour; strong astringency and chemical aftertaste; "Dettol-like" flavour;
- BR6 - musty; slight chemical taste; sour; bitter; unpleasant;
- BR7 - old leather; PVC; plastic flavour; garden chemicals; earthy.

Unfortunately, it is not known whether any of these descriptors could apply to cooked fish contaminated by aromatic hydrocarbons as most of the data in the literature merely refer to fish as being tainted or untainted. "Dettol-like" flavour could be taken to indicate the presence of chlorinated phenols or xylenols (dimethylphenols), but none of these compounds were detected in the uncooked fish. "Earthy" and/or "musty" are descriptors often used to describe the tastes produced in water by algal-related compounds such as geosmin and 2-methylisoborneol, but again these types of compounds were not detected.

Although there is no obvious correlation between the taste descriptors used for the cooked fish and the organic compounds detected in the raw fish, the descriptors "oily" or "diesely" have been used to describe fish considered tainted when caught, and the hydrocarbons detected in the most tainted fish may well give rise to this type of taint. The other compound class which may be of some relevance is the siloxanes, but there does not appear to be any information in the literature on their tainting potential.

6. SEDIMENT ANALYSIS

6.1 Methodology

Sediments were collected in September 1996. These were stored in freezers at -18 °C until required for analysis (March 1997). Seven sediments were analysed. These were from the following sites:

Site 1 R. Douglas

Site 3 Ribble estuary from opposite bank (west) of confluence with R. Douglas

Site 4 R Ribble upstream of confluence with R. Darwen ("Big Ribble")

Site 6 R. Darwen

Site 9 R. Ribble upstream of confluence with R. Hodder and R. Calder ("Upper Ribble")

Site 13 R. Calder, downstream of Hyndburn STW

Site 15 R. Calder, upstream of Hyndburn STW

The sediments were allowed to thaw overnight before sub-samples of approximately 10 g wet weight (dry weight in the range 4.42 - 8.66 g) were removed. These were spiked with a mixture of deuterium-labelled internal standards (added at 50 $\mu g\ kg^{-1}$ wet weight) and Soxlet extracted for about 16 hours (overnight) with dichloromethane (about 250 ml). The dichloromethane extracts were reduced to 1 ml for analysis by GCMS. The GCMS conditions used were identical to those used for the analysis of the salmon extracts.

The dry weights of sediments extracted were as follows:

Site 1	7.12 g;	Site 3	4.42 g;	Site 4	8.66 g;	Site 6	6.07 g;
Site 9	5.07 g;	Site 13	7.22 g;	Site 15	5.67 g		

6.2 Results and discussion

The compounds detected in the sediment extracts are listed in Table 6.1. Over 250 compounds were detected, although not all have been fully identified. For example, twelve C_4-alkylbenzene isomers were detected, but there are over 20 possible isomers if tetramethylbenzenes, ethyldimethylbenzenes, diethylbenzenes, methylpropylbenzenes, methyliso-propylbenzenes and butylbenzenes are considered. As there is no information in the literature relating to the fish-tainting potential of any of these compounds it did not appear worthwhile establishing the exact identities of those isomers detected.

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment

Compound identity	Scan number(s)	Site 1	Site 3	Site 4	Site 6	Site 9	Site 13	Site 15
Toluene	250	✓	✓	✓	✓	-	✓	✓
Butyric acid	293	-	-	-	✓	-	-	-
Unknown 41,69,39,43	306	-	✓	✓	-	-	-	-
Unknown 41,69,39,43	359	-	-	✓	-	-	-	-
d_5-Chlorobenzene	374	✓	✓	✓	✓	✓	✓	✓
Chlorobenzene	376	-	✓	-	-	-	-	-
Isovaleric acid	391	✓	-	-	✓	-	-	✓
2-Methylbutyric acid	413	✓	-	-	✓	-	-	-
Xylene isomers	411;426;463	-	✓ 3	✓ 3	✓ 3	✓ 3	✓ 3	✓ 3
d_{10}-p-xylene	418	✓	✓	✓	✓	✓	✓	✓
C_3-Alkylbenzene isomers	563;576;579;588;602;625;665	-	✓ 1	✓ 8	✓ 7	-	✓ 6	✓ 6
Dimethyl trisulphide	568	✓	-	-	-	-	-	✓
d_5-Phenol	593	✓	✓	✓	✓	✓	✓	✓
Phenol	596	✓	✓	✓	✓	✓	✓	✓
p-Dichlorobenzene	640	-	✓	-	✓	-	✓	✓
n-Decane	653	✓	-	-	✓	-	✓	✓
Benzyl alcohol	662	✓	✓	✓	✓	-	✓	
2-Ethylhexanol	673	-	-	-	-	-	-	✓
Undecane isomer	688	-	-	-	✓	-	✓	✓
C_4-Alkylbenzene isomers	666;673;705;719;718;731;747; 757;783;799;805;847	-	-	✓ 16	✓ 12	-	-	✓ 8
Butylcyclohexane	696	-	-	-	-	-	-	✓
p-Cresol	728	✓	✓	✓	✓	✓	✓	✓
Phenylethyl alcohol	772	✓	-	-	-	-	-	-
Undecene isomers	772,777,838	-	-	-	✓	-	-	-
Dimethylphenol isomers	773,828,855	✓ 4	-	-	✓ 3	-	-	-
n-Undecane	792	-	-	✓	✓	✓	✓	✓

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

2-Methyldecalin	807	-	-	-	✓	-	-	-
C_5-Alkylbenzene isomers	826;835;846;853;857;865;876; 891;898;906;917	-	-	-	✓ 11	-	-	-
1-Methyldecalin + Dodecane isomer	830	-	-	-	✓	-	-	-
2-Piperidinone	838	-	✓	-	-	-	-	-
Pentylcyclohexane	838	-	-	-	✓	-	-	-
d_8-Naphthalene	875	✓	✓	✓	✓	✓	✓	✓
Naphthalene	878	✓	✓	✓	✓	✓	✓	✓
Tridecane isomer	907	-	-	-	✓	-	-	-
3-Ethyl-4-methyl-1H-2,5-dione	913	-	✓	-	-	-	-	-
n-Dodecane	920	-	-	✓	✓	✓	✓	✓
Tridecane isomer	940	-	-	✓	✓	-	-	✓
Tridecene isomer	956	-	-	-	✓	-	-	-
C_3-Alkylphenol isomer	963	✓	-	-	-	-	-	-
Hexylcyclohexane	969	-	-	-	✓		-	✓
Indole	982	✓	-	-	✓	✓	-	✓
p-Bromophenol	991	-	✓	-	-	-	-	-
t-Butylphenol	1005	✓	✓	-	-	-	-	-
Tridecane isomer	1011	-	-	✓	✓	-	✓	✓
2-Methylnaphthalene	1017	✓	✓	✓	✓	✓	✓	✓
Tetradecane isomer	1024	-	-	-	✓	-	-	-
1-Methylnaphthalene	1035	✓	✓	✓	✓	✓	✓	✓
n-Tridecane	1039	-	-	✓	✓	✓	✓	✓
Tetradecane isomer	1062	-	-	-	✓	-	-	-
$C_{15}H_{24}$ isomers	1091;1128;1134;1153;1166;1171	-	-	-	✓ 6	-	-	-
Biphenyl	1108	-	-	-	✓	-	-	-
Pentadecene isomer	1128	-	-	✓	✓	-	✓	✓
C_2-Alkylnaphthalenes	1136;1144;1159;1163;1180;1196	✓ 5	-	✓ 9	✓ 6	✓ 5	✓ 5	✓ 6

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment

Compound identity	Scan number(s)	Site 1	Site 3	Site 4	Site 6	Site 9	Site 13	Site 15
Toluene	250	✓	✓	✓	✓	-	✓	✓
Butyric acid	293	-	-	-	✓	-	-	-
Unknown 41,69,39,43	306	-	✓	✓	-	-	-	-
Unknown 41,69,39,43	359	-	-	✓	-	-	-	-
d_5-Chlorobenzene	374	✓	✓	✓	✓	✓	✓	✓
Chlorobenzene	376	-	✓	-	-	-	-	-
Isovaleric acid	391	✓	-	-	✓	-	-	✓
2-Methylbutyric acid	413	✓	-	-	✓	-	-	-
Xylene isomers	411;426;463	-	✓ 3	✓ 3	✓ 3	✓ 3	✓ 3	✓ 3
d_{10}-p-xylene	418	✓	✓	✓	✓	✓	✓	✓
C_3-Alkylbenzene isomers	563;576;579;588;602;625;665	-	✓ 1	✓ 8	✓ 7	-	✓ 6	✓ 6
Dimethyl trisulphide	568	✓	-	-	-	-	-	✓
d_5-Phenol	593	✓	✓	✓	✓	✓	✓	✓
Phenol	596	✓	✓	✓	✓	✓	✓	✓
p-Dichlorobenzene	640	-	✓	-	✓	-	✓	✓
n-Decane	653	✓	-	-	✓	-	✓	✓
Benzyl alcohol	662	✓	✓	✓	✓	-	✓	
2-Ethylhexanol	673	-	-	-	-	-	-	✓
Undecane isomer	688	-	-	-	✓	-	✓	✓
C_4-Alkylbenzene isomers	666;673;705;719;718;731;747; 757;783;799;805;847	-	-	✓ 16	✓ 12	-	-	✓ 8
Butylcyclohexane	696	-	-	-	-	-	-	✓
p-Cresol	728	✓	✓	✓	✓	✓	✓	✓
Phenylethyl alcohol	772	✓	-	-	-	-	-	-
Undecene isomers	772,777,838	-	-	-	✓	-	-	-
Dimethylphenol isomers	773,828,855	✓ 4	-	-	✓ 3	-	-	-
n-Undecane	792	-	-	✓	✓	✓	✓	✓

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

2-Methyldecalin	807	-	-	-	✓	-	-	-
C_5-Alkylbenzene isomers	826;835;846;853;857;865;876; 891;898;906;917	-	-	-	✓ 11	-	-	-
1-Methyldecalin + Dodecane isomer	830	-	-	-	✓	-	-	-
2-Piperidinone	838	-	✓	-	-	-	-	-
Pentylcyclohexane	838	-	-	-	✓	-	-	-
d_8-Naphthalene	875	✓	✓	✓	✓	✓	✓	✓
Naphthalene	878	✓	✓	✓	✓	✓	✓	✓
Tridecane isomer	907	-	-	-	✓	-	-	-
3-Ethyl-4-methyl-1H-2,5-dione	913	-	✓	-	-	-	-	-
n-Dodecane	920	-	-	✓	✓	✓	✓	✓
Tridecane isomer	940	-	-	✓	✓	-	-	✓
Tridecene isomer	956	-	-	-	✓	-	-	-
C_3-Alkylphenol isomer	963	✓	-	-	-	-	-	-
Hexylcyclohexane	969	-	-	-	✓		-	✓
Indole	982	✓	-	-	✓	✓	-	✓
p-Bromophenol	991	-	✓	-	-	-	-	-
t-Butylphenol	1005	✓	✓	-	-	-	-	-
Tridecane isomer	1011	-	-	✓	✓	-	✓	✓
2-Methylnaphthalene	1017	✓	✓	✓	✓	✓	✓	✓
Tetradecane isomer	1024	-	-	-	✓	-	-	-
1-Methylnaphthalene	1035	✓	✓	✓	✓	✓	✓	✓
n-Tridecane	1039	-	-	✓	✓	✓	✓	✓
Tetradecane isomer	1062	-	-	-	✓	-	-	-
$C_{15}H_{24}$ isomers	1091;1128;1134;1153;1166;1171	-	-	-	✓ 6	-	-	-
Biphenyl	1108	-	-	-	✓	-	-	-
Pentadecene isomer	1128	-	-	✓	✓	-	✓	✓
C_2-Alkylnaphthalenes	1136;1144;1159;1163;1180;1196	✓ 5	-	✓ 9	✓ 6	✓ 5	✓ 5	✓ 6

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

n-Tetradecane	1150	-		-		✓		✓		-		✓	
2-Phenylethyl formate	1185	-		✓		-		-		-		-	
Pentadecadiene isomer	1190	-		-		-		✓		-		-	
Tetradecene isomer	1202	-		-		-		✓		-		-	
Pentadecane isomer	1219	-		-		✓		✓		-		✓	
Acenaphthene	1224	-		-		-		✓		-		✓	
C_3-Alkylnaphthalene isomers	1250;1266;1271;1277;1292; 1297;1310;1332	-		-		✓	8	-		✓	6	✓	7	✓	7
n-Pentadecane	1254	-		-		✓		✓		-		✓	
Dibenzofuran	1258	✓		-		-		✓		-		✓	
Thymine	1279	✓		-		-		-		-		-	
(1-Butylhexyl)benzene	1285	✓		-		-		✓		-		✓	
(1-propylheptyl)benzene	1293	✓		-		-		✓		-		✓	
Diethylphthalate	1311	✓		-		-		-		-		-	
(1-ethyloctyl)benzene	1313	✓		-		-		✓		-		-	
Fluorene	1325	✓		-		-		✓		✓		✓	
n-Hexadecane	1353	-		-		✓		✓		-		✓	
Benzophenone	1354	✓		✓		-		-		✓		✓	
Methyldibenzofuran isomers	1363;1377	-		-		-		✓	2	✓	2	✓	3	✓	3
(1-Pentylhexyl)benzene	1375	✓		-		-		✓		-		✓	
(1-Butylheptyl)benzene	1380	✓		-		-		✓		-		✓	
(1-Propyloctyl)benzene	1390	✓		-		-		✓		-		✓	
Heptadecane isomer	1402	-		-		✓		-		-		✓	
(1-Ethylnonyl)benzene	1410	✓		-		-		✓		-		-	
C_4-Alkylnaphthalene isomers	1414;1429;1433;1457;1471; 1482;1503	-		-		✓	7	-		-		-	
$C_{15}H_{16}O$ isomers	1416;1421;1451;1458;1467	-		-		-		✓	5	-		-		✓	5
Nonylphenol isomers	1430-1482	✓		-		-		✓		-		-	

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

Hexathiepane	1434	✓	✓	-	✓	✓	-	-
n-Heptadecane	1447	-	-	✓	-	✓	✓	✓
Pristane	1455	-	-	✓	-	-	✓	✓
$C_{15}H_{16}$ isomers	1448;1470;1474;1484;1488; 1492;1499	-	-	✓ 7	-	-	✓ 4	-
(1-Pentylheptyl)benzene	1466	✓	-	-	✓	-	✓	✓
(1-Butyloctyl)benzene	1470	✓	-	-	✓	-	✓	✓
(1-Propylnonyl)benzene	1482	✓	-	-	✓	-	✓	✓
(1-Ethyldecyl)benzene	1502	✓	-	-	✓	-	-	✓
d_{10}-Phenanthrene	1503	✓	✓	✓	✓	✓	✓	✓
Phenanthrene	1508	✓	✓	✓	✓	✓	✓	✓
Anthracene	1516	✓	✓	✓	✓	✓	✓	✓
n-Octadecane	1534	-	-	✓	✓	✓	✓	✓
Carbazole	1541	-	-	-	✓	-	-	✓
Phytane	1542	-	-	✓	-	-	-	-
Nonadecane isomer	1546	✓	-	-	✓	-	✓	✓
$C_{16}H_{18}$ isomers	1550;1562;1575;1581	-	-	-	-	-	✓ 4	-
(1-Pentyloctyl)benzene	1552	✓	-	-	✓	-	✓	✓
(1-Butylnonyl)benzene	1558	✓	-	-	✓	-	✓	✓
Dibutyl phthalate isomer	1562	-	✓	-	-	✓	-	-
(1-Propyldecyl)benzene	1570	✓	-	-	✓	-	✓	✓
Galaxolide isomer	1572	✓	-	✓	✓	-	✓	✓
Galaxolide isomer	1582	✓	-	✓	✓	-	✓	✓
(1-Ethylundecyl)benzene	1590	-	-	-	✓	-	-	✓
Phytol	1601	-	-	-	-	-	-	✓
Methylphenanthrene/anthracene isomers	1608;1613;1621;1628;1632	✓ 5	-	✓ 5	✓ 5	✓ 5	✓ 5	✓ 5
Nonedecane isomer	1618	-	-	✓	✓	-	✓	✓
4H-cyclopenta-[def]-phenanthrene	1628	-	-	✓	✓	-	✓	✓
Dibutylphthalate isomer	1638	-	✓	-	✓	✓	-	-

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

9,10-Anthraquinone	1656	-		-		-		✓		-		-		-	
2-Phenylnaphthalene	1665	✓		-		-		✓		-		✓		✓	
C_2-Alkylphenanthrenes/anthracenes (7)	1686;1700;1708;1722;1726;1730;1738	✓	6	-		✓	9	✓	7	✓	7	✓	6	✓	7
n-Eicosane	1698	-		-		✓		✓		-		✓		✓	
Fluoranthene	1742	✓		✓		✓		✓		✓		✓		✓	
Dimethoxyanthracene isomer	1772	-		-		-		✓		-		-		-	
$C_{20}H_{40}O$ isomer	1775	-		-		-		-		✓		-		✓	
Pyrene	1782	✓		✓		✓		✓		✓		✓		✓	
Dimethyldibenzodithiophene isomer	1788	✓		-		-		-		-		-		-	
$C_{16}H_{10}O$ isomers	1789;1800;1812;1829	-		-		-		✓	4	-		✓	4	-	
Methylpyrene/fluoranthene isomers	1835;1853;1866;1874;1891;1896	✓	6	✓	6			✓	6	✓	6	✓	6	✓	6
n-Docosane	1846	-		-		✓		✓		-		✓		✓	
Unknown 43,57,55,83	1897	-		-		-		-		✓		-		-	
n-Tricosane	1915	-		-		✓		✓		✓		✓		✓	
$C_{18}H_{22}O_2$ isomer	1918	-		-		-		✓		-		-		-	
$C_{18}H_{14}$ isomers	1938;1949;1958;1972;1986	✓	5	-		-		✓	5	-		-		-	
Triphenylene	1977	✓		-		-		-		-		-		✓	
n-Tetracosane	1982	-		-		✓		✓		-		✓		✓	
Chrysene/Benzanthracene isomers	2018;2023;2041	✓	3	✓	2	✓	4	✓	3	✓	3	✓	4	✓	3
Unknown 43,57,55,83	2031	-		-		-		-		✓		-		✓	
n-Pentacontane	2046	-		-		✓		✓		✓		✓		✓	
Dioctylphthalate isomer	2050	-		-		-		✓		-		-		-	
Di-(2-ethylhexyl)phthalate	2057	✓		✓		✓		✓		✓		✓		✓	
Methylchrysene isomers	2064;2095;2102;2109;2119;2127;2139	✓	5	-		✓	4	✓	7	-		-		✓	7
Unknown 300,31,5,302,272	2074	-		-		-		-		-		✓		-	
Hexacosane isomer	2086	-		-		-		✓		-		-		-	
n-Hexacosane	2110	-		-		✓		✓		-		✓		✓	

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

Unknown 57,43,82,55	2121	-		-		-		-		✓		-		-
Unknown 57,43,71,55	2163	-		-		-		-		✓		-		-
n-Heptacosane	2176	-		-		-		-		✓		✓		✓
Benzpyrene/Benzfluoranthene isomers	2236;2258;2292;2303;2321	✓	4	✓	4	✓	5	✓	5	✓	5	✓	5	✓ 5
Squalene	2264	✓		-		✓		✓		-		-		✓
Unknown 43,57,82,69	2265	-		-		-		-		✓		-		-
Unknown 43,57,55,83	2314	-		-		-		-		✓		-		-
n-Nonancosane	2326	-		-		-		-		✓		✓		✓
Decyloctylphthalate	2327	-		-		-		✓		-		-		-
Unknown 43,57,82,55	2441	-		-		-		-		✓		-		-
Didecylphthalate	2513	-		-		-		✓		-		-		-
n-Untriacontane	2517	-		-		-		-		✓		✓		-
Coprostanol	2519	✓		-		-		✓		-		-		✓
Vitamin E	2544	-		-		-		-		-		-		✓
Cholesterol	2560	✓		✓		✓		✓		✓		✓		✓
Hexadecyl tetradecanoate	2581	-		✓		-		-		-		-		-
Benzo[ghi]perylene	2606	✓		-		✓		✓		✓		✓		✓
Unknown 43,57,82,69	2677	-		-		-		-		✓		-		-
Indeno[1,2,3-cd]pyrene	2684	✓		-		✓		✓		✓		✓		✓
Hexadecyl pentadecanoate	2709	-		✓		-		-		-		-		-
Steroid (M.Wt. 416)	2769	-		-		-		✓		-		-		✓
Steroid (M.Wt. 414)	2825	-		-		✓		✓		✓		✓		✓
Hexadecyl hexadecanoate	2863	-		✓		-		-		-		-		✓
Unknown 81,95,205,109	2908	-		-		-		-		✓		-		-
Octadecyloctadecanoate	3094	-		-		-		✓		-		-		-
Dibenzpyrene isomers (2)	3127;3166	-		-		-		✓		-		-		-
Steroid ($C_{30}H_{50}O$)	3206	-		-		-		✓		-		-		-

Table 6.1 Organic compounds identified in sediment samples from Ribble catchment (cont.)

Sediment samples taken from following sites:

Site number	Location
1	R. Douglas
3	Ribble estuary
4	R. Ribble upstream of R. Darwen confluence
6	R. Darwen
9	R. Ribble upstream of R. Hodder confluence
13	R. Calder downstream of Hyndburn STW
15	R. Calder upstream of Hyndburn STW

✓	Present at a concentration > 20 micrograms per kg (20 ppb)
-	Not detected

Where numbers are shown in the detected column, these indicate the number of isomers detected

C_3-Alkyl benzene isomers include trimethylbenzenes, ethylmethylbenzenes and propylbenzenes
Similarily, C_2-Alkylnaphthalenes include dimethylnaphthalene isomers and ethylnaphthalenes

In comparative terms, the sediments containing the highest concentrations of organic compounds amenable to GCMS analysis were from sites 6 and 15 (R. Darwen and R. Calder upstream of Hyndburn WwTW respectively), and contamination by compounds present in treated sewage effluent (as gauged by the concentrations of linear alkyl benzenes (LABs)) was also highest at sites 6 and 15. Surprisingly, the concentrations of LABs were almost an order of magnitude higher at site 15 (R. Calder upstream of Hyndburn WwTW) compared to site 13 (R. Calder downstream of Hyndburn WwTW). The same conclusion can be drawn if the concentrations of two of isomers of galaxolide (two "musk" compounds used as fragrances in various consumer products such as cosmetics, soaps and domestic detergents) are considered - the highest levels were found at sites 6 and 15.

Not surprisingly, complex mixtures of aromatic and polyaromatic hydrocarbons (PAH) were found at all sites. These ranged from naphthalene and alkyl-substituted naphthalenes to benzpyrenes and benzfluroanthenes. Taking phenanthrene as an example, in terms of concentrations found at the various sites, the highest level was found at site 6 (28.3 mg kg^{-1} dry weight; R. Darwen) and the lowest level at site 9 (0.075 mg kg^{-1} dry weight). In decreasing concentrations of phenanthrene, the site order was 6(28.3) >15(19.4) >13(0.55) >4(0.22) >1(0.093) >3(0.088) >9(0.075) mg kg^{-1} dry weight, i.e. the least polluted sediments with respect to PAH were from the upper Ribble and the Ribble Estuary. As can be seen the variation in concentrations is quite high, with the highest concentration being almost 400 x greater than the lowest concentration found. A similar picture emerges if fluoranthene or pyrene are taken as being representative of PAH levels, with sites 6 and 15 being the most contaminated and sites 3 and 9 being the least contaminated.

Some phenolic compounds were found in all the sediments, but generally concentrations were relatively low. For phenol itself, concentrations were in the range 0.11 - 0.42 mg kg^{-1} dry weight. The highest concentration was at site 4 (Big Ribble) and the lowest at site 15 (R. Calder upstream of Hyndburn WwTW). The site order was 4(0.42) >3(0.26) >1(0.24) >9(0.18) >6(0.17) = 13(0.17) >15(0.11) mg kg^{-1} dry weight. In contrast to the PAH there was little variation in these concentrations, with only a factor of 4 x between the highest and lowest levels. p-Cresol (4-methylphenol) was detected at all sites, with the highest levels at sites 15 and 6. The lowest levels (about 10 x lower) were at sites 3 and 4. This compound occurs naturally and is found in silage liquor. Assuming that the behaviour of p-cresol (in terms of extraction, analysis etc.) is the same as that of phenol, the highest concentration detected was 3.5 mg kg^{-1} dry weight and the lowest was 0.3 mg kg^{-1}. p-Bromophenol was detected only at site 3 (Ribble estuary) and t-butylphenol only at sites 3 and 4 (the estuary and the "big" Ribble). Low levels of dimethylphenol (xylenol) isomers were detected at sites 1 and 6 (R. Douglas and R. Darwen), and a C_3-alkylphenol detected at site 1. For all of these latter phenolic compounds, the concentrations detected were low (<1 mg kg^{-1}).

Other compounds which may be of relevance to tainting problems were as follows:

- p-dichlorobenzene was detected at sites 3, 6, 13 and 15; this is an odorous compound which has domestic uses and can be found in treated sewage effluent. However, the levels detected were relatively low.

- benzophenone, which is used as a fixative for heavy perfumes, was detected at sites 1, 3, 9 and 13 at low concentrations.

- two galaxolide isomers were detected at sites 1, 4, 6, 13 and 15. As noted earlier, this is a "musk" fragrance which is widely used. Unfortunately there does not appear to any information on its tainting potential, although some time ago WRc investigated a fish tainting problem in a river to which effluent from a manufacture of these compounds was discharged. This work was spread over several years, and at the time it was not possible to prove conclusively that galaxolide was involved.

To summarise the data obtained from the analyses of sediments, in terms of both numbers detected and concentrations found, hydrocarbons predominated. Several phenolic compounds were detected, but their concentrations were relatively low. No p-chlorophenol was detected.

7. DISCUSSION

Taste tainting in Salmonids caught within the Ribble catchment has been a problem for almost twenty years, and possible longer. Parts of the catchment have suffered severely from industrial pollution in the past and there are numerous discharge sources and potential for accidental spills. In the recent past spillages have involved p-chlorophenol, paint, white spirit, solvents and petroleum products such as diesel and oils.

The literature search has shown that a wide range of organic compounds can taint fish flesh. Two major classes of compounds that are relevant to the Ribble Catchment are hydrocarbons and phenols, but there are numerous other substances including many industrial chemicals and others, which are naturally occurring (e.g. geosmin and 2-methyl isoborneol), that can give rise to taints in fish.

The degree of tainting does depend on the concentrations of the various compounds involved, but it is obvious that for some substances the taste thresholds are extremely low. It is worth bearing in mind that most fish tainting is detected in cooked fish, although there have been reports of freshly caught fish from the Ribble catchment smelling oily.

The three main descriptors used in complaints by the public in relation to cooked fish appear to have been as follows: "disinfectanty", "diesely" and "muddy". It appears from the eyewitness accounts and reports of tainting that the incidence of these is transient/irregular and they may therefore relate to the incidence of discharges and specific threshold concentrations of pollutants. This may explain the absence of 'diesel' taints and very low incidence of 'phenolic' and chemical taints in the samples tasted.

The taste testing of fish has demonstrated that many salmon caught in the Ribble catchment are tainted. An important finding is that the incidence of untainted salmon was low and that tainted or suspect salmon were caught throughout the catchment. Of the ten salmon caught in the estuary, only two were considered to be untainted, and all were described as having an unpleasant earthy flavour. In the Big Ribble, three salmon were considered untainted, three were thought to be suspect and the remaining three were tainted. Of the two salmon sampled from the Upper Ribble one was suspect, as was the single fish sampled from the River Hodder.

From these results it is unclear whether the cause of the salmon taint is due to Ribble specific factors or factors related to the marine phase of the salmon's lifecycle. If the cause is marine related then it would follow that salmon from other rivers would be similarly tainted. Reports of tainting from other rivers were not sought in this study, but such an investigation, including taste testing of salmon from other nearby rivers, would help establish the facts more clearly.

Taste testing of trout from the Upper Ribble (taken from the Waddow Trap) and Calder gave similar results to the salmon, with the majority being suspect or tainted. The earthy/musty taint reported in the salmon distinguish them from the trout samples that were tasted, in particular the soapy/chemical taint of those from the River Calder. It is, of course, possible that the unpleasant soapy/chemical flavour of the trout masks other taints and that the trout were exposed to the same chemicals as the salmon.

The report of the taste testing panel that a contributing factor in the general unpleasantness of the fish (salmon and trout) was their advanced state of sexual maturity, must be considered. On the other hand, all of these fish were taken during the open fishing season and must therefore be considered representative of the general quality of fish from the catchment.

Analysis of fish tissue using GCMS did not allow an obvious correlation to be made between the taste descriptors used for the cooked fish and the organic compounds detected. The main compounds detected (apart from those considered to be normally found in salmon flesh) were aromatic hydrocarbons, yet 'hydrocarbon' taints (oily/diesely?) were not reported in the taste tests. However, no taste descriptors for these types of compounds were found in the literature survey and therefore one cannot be prescriptive about how their 'taste' might be manifest in the Ribble samples. Descriptors frequently used by the taste testing panel (e.g. earthy, musty, astringency, chemical) could not be linked to any of the compounds identified in the tissue analyses. No phenols were detected in any of the samples of fish tissue analyses. No taste information was available from the literature on siloxanes, several of which were detected in the fish analysed.

Analysis of sediments taken from various sites in the catchment showed that the main compounds detected by GCMS were aromatic and polyaromatic hydrocarbons. Concentrations at two locations (R. Darwen and R. Calder upstream of Hyndburn WwTW) were significantly higher than at other sites. Some phenolic compounds were detected, including phenol itself and p-cresol. Concentrations of the former were in the range 0.11 - 0.42 mg kg^{-1} dry weight of sediment, while for the latter (which may be naturally occurring) they were in the range 0.3 - 3.5 mg kg^{-1}. Dimethylphenol isomers were detected at low levels at two sites (R. Darwen and R. Douglas), p-bromophenol at one site (the estuary) and t-butylphenol at two sites (the estuary and the Big Ribble). No p-chlorophenol was detected at any of the sites.

It should be pointed out that GCMS analysis does have limitations in terms of the types of compounds that can be successfully analysed. Provided their boiling points are within the range 50-500 °C and they are effectively extracted from water or sediment samples using relatively non-polar organic solvents, many organic compounds will be detected using GCMS. However, some compound classes such as surfactants cannot be analysed using GCMS; for these, other more specialised MS techniques are necessary. The other relevant point with respect to the chemical analysis is that no water samples have been analysed, and although the sediment analyses do provide some information on the compounds present in the water, this is selective in nature as some organic compounds are strongly adsorbed onto sediments while others are not and would therefore not be detected by sediment analysis.

The main problem in linking analytical results with taste descriptors observed in the sample fish is the subjective nature of tasting and the general lack of information in the literature on the tastes of organic chemicals in cooked fish. There is considerably more information on taste descriptors and taste thresholds of organics in water, but it is not clear how this can be applied to fish (either raw or cooked). For example, in water samples earthy musty odours are caused by algal products such as geosmin, 2-methyl isoborneol or methoxypyrazines, which have very low taste thresholds (less than 50 ng l^{-1}). Although these compounds also taint fish, the concentrations at which they do so is unclear and they were not detected in either the fish or sediment samples analysed. It is therefore not known whether the sensitivity

of the analyses undertaken for this work (c. 25 µg kg^{-1} in the case of fish) was sufficiently high. If not, specific analyses for these compounds would be necessary, if they are considered to be of interest.

In summary, the taste testing has confirmed the presence of tainted salmon and trout in the Ribble Catchment and it can be concluded that there is no one specific cause or 'taint'. Phenol, which triggered interest in Ribble tainting in the early 1990's, appears to have been a minor issue during the present study, whilst hydrocarbons, despite being identified in fish tissue and sediment, could not be positively attributed a taint. The cause(s) of the high incidence of earthy tainting of the salmon and soapy/chemical taint in trout from the River Calder warrants further investigation, in particular, the incidence (concentration) and nature of tainting caused by surfactants/anti-foaming agents, and specifically siloxanes.

8. CONCLUSIONS AND RECOMMENDATIONS

1. There is no doubt that the Salmonids taken from the Ribble catchment during the course of this project were generally tainted and that a range of 'taints' were found. The incidence of tainted/suspect/untainted salmon was similar in both estuarine and freshwater-caught specimens. Overall, the most 'tainted' batch of fish examined by taste panellists was trout from the River Calder.

2. Phenol, which triggered interest in Ribble tainting in the early 1990's, was used as a taste descriptor by a small number of panellists for a few fish. However, phenols were not detected in any of the fish tissue samples analysed although low levels of phenolic compounds were found in sediment samples. The levels were not considered sufficiently high to cause taints.

3. Hydrocarbons were the most commonly observed organic compounds in both fish and sediment samples, but could not be related to any 'taste' descriptor reported by the tasting panels.

4. The strong chemical/soapy aftertastes reported following taste testing of trout may relate to the presence of surfactants in some effluents and discharges. It is worth noting that it has been reported that the presence of surfactants may enhance tainting. Additional data are required relating to the use of surfactants, particularly in the river Calder. It is recommended that the relationship between surfactants and soapy/chemical taint is further examined.

5. It is recommended that it should be established whether the tainting is related to the use of anti-foaming formulations at WwTWs. It appears (EA pers. comm.) that on average these formulations may be present at about 0.5 mg l^{-1} in rivers downstream of sites where they are used, and it is understood that these formulations may contain siloxanes. Several siloxanes were identified in tainted fish flesh, but there is no information on the tainting potential of siloxanes. It is recommended that any further investigation of tainting in the Ribble examines siloxanes and that the manufacturers of these products be approached to obtain relevant taste threshold information.

6. The taste panel commented that as most of the sample fish were females in an advanced state of sexual maturity, this may have contributed to their general unpleasantness, and so have biased the tainting data obtained. However, all the fish were obtained during the fishing season and the 'taint' can consequently be considered typical of that experienced by consumers of late season caught Ribble fish. To confirm these observations and to address the question of whether the salmon became tainted during their marine phase it is recommended that:

   a) further taste testing should be undertaken to ascertain whether salmon caught at an earlier state of maturity in the Ribble catchment are similarly tainted.

   b) a comparative taste testing should be carried out with salmon from a neighbouring estuary (e.g. the Lune).

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APPENDIX A FISH RECEIPT AND RECORD SHEET

A1 FORM OF RECEIPT FOR FISH SAMPLES

This receipt is issued for the purchase of adult Ribble salmon for the Environment Agency's Taste Tainting Project.

Name of angler/supplier _____

Licence Number _____

Address _____

_____

Telephone Number _____

Weight of (each) fish _____

Total Price £_____

Signed _____ Date _____

Signed for EA _____ Date _____

Name of issuing officer _____

_____ cut _____

This receipt is issued for the purchase of adult Ribble salmon for the Environment Agency's Taste Tainting Project.

Date _____

Price £_____ for _____ fish (number)

Issued to _____

Issued for EA by _____(signed)

_____(name)

A2 FISH RECORD SHEET

SALMON or TROUT (DELETE) Ref Number _____

Name of angler/supplier _____

Received for EA by _____ Date _____

Licence Number _____

Address _____

_____

Telephone Number _____

Weight of fish _____

Date of capture _____

Time of capture _____

Time from capture to freezing _____

Location (including grid reference if available) _____

Condition _____

A3 LETTER TO ANGLING CLUBS/ANGLERS

Dear Sir,

Request for assistance with the Environment Agency's Project: Taste Tainting of Salmon Flesh in the River Ribble Catchment.

The Environment Agency has contracted WRc (formerly the Water Research Centre) to undertake a study of the issue of salmon flesh tainting in the River Ribble Catchment, beginning in September 1996. This investigation will initially comprise taste and chemical analyses of salmon flesh, and a review of past complaints and scientific literature. The objectives are to identify both the causal agent(s) and source(s).

To undertake this exercise we require to obtain between 20 and 30 rod caught salmon before the close of the 1996 season. These will be purchased from anglers and we would therefore be grateful for your assistance in advertising this programme to your members.

1. Supply of fish

The fish will be required whole (gutted or ungutted) and can be supplied either frozen or unfrozen. Fish should be wrapped in aluminium foil, not plastic.

2. Collection

Anglers with fish to supply are to contact the EA Fisheries Section at Preston via the Agency's 24 hour Communication Centre The fish will be collected by mutual arrangement by the EA's Fisheries Section at Preston.

3. Payment

The weight and price of the fish will be agreed and a receipt issued. A price of £2.50 per lb. will be paid. Payment will be provided by cheque within seven days.

4. Conditions

Fish will only be accepted from anglers with a valid licence and who are prepared to sign a statement that the fish was taken on the Ribble and give the exact location, date etc. (see below).

5. Numbers of Fish

The total requirement for fish is:

Big Ribble (Hodder Foot to tidal limit)	10 salmon
Upper/Middle Ribble (Above Hodder Foot)	10 salmon
Hodder	3 salmon
Calder (if available)	*up to 5 fish*

6. Information required

Name/address/telephone/licence number of angler

Date and time of capture

Time elapsed to freezing

Location (including grid reference if available)

Tainting reports

We are also seeking to collate information on past tainting experiences from individual anglers. Anyone willing to participate should contact me at the address given on this letter.

The Environment Agency Project Manager for this Project is Philip Williams of the Pollution Control Department and support from the Fisheries Section is organised by Jon Shatwell, Area Fisheries, Recreation and Ecology Manager. Both are located at the EA Central Area office in Preston (01772 339882).

APPENDIX B SUMMARY DATA FOR FISH COLLECTED

Salmon

Identity	Date caught	Time to freezing (hr)	Weight (lb)	Sex (M/F)	Whole/ steak	Comments
BR1	1.10.96	5.25	4.5	F	S	Between coloured/silver
BR2	1.10.96	5.25	15	F	S	Between coloured/silver
BR3	5.10.96	2	15.5	F	S	Ripening
BR4	7.10.96	6	13	F	S	Coloured
BR5	7.10.96	11	18	F	S	-
BR6	8.10.96	11	7	F	S	-
BR7	9.10.96	8	4.5	-	W	-
BR8	16.10.96	6	7.5	-	W	-
BR9	30.10.96	10	18	F	S	Silver
H1	24.10.96	26	8.5	F	S	-
UR1	7.10.96	3	10	F	S	Coloured
UR2	19.10.96	30	6	M	S	Coloured
E1	96 season	)	19	)	W	-
E2	96 season	)	15	)	W	-
E3	96 season	) <12*	9	)mainly F	W	-
E4	96 season	)	13	)	W	-
E5	96 season	)	13	)	W	-
E6 - E10	96 season	)	10-15	)	W	-

* E xact details unknown

Trout

Identity	Date caught	Time to freezing (hr)	Weight (lb)	Sex (M/F)	Whole/ steak	Comments
C1	14.10.96	4	)-	M	W	Good condition
C2-5	14.10.96	4	)1.5-2.5	F		Good condition, ripe
UR1-3	7/8.11.96	2	)	F	W	Good condition, ripe

APPENDIX C REPORT ON TASTE TESTING BY SOAEFD MARINE LABORATORY, ABERDEEN

SOAEFD MARINE LABORATORY

RESTRICTED - COMMERCIAL

SOAEFD MARINE LABORATORY, ABERDEEN

File F65/C167/14
C167.16

Taint Assessment of River Ribble Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*)

Water Research Centre, Henley Road
Medmenham, Marlow, Bucks, SL7 2HD

6 February 1997

The results are based on analyses of samples supplied by the client and as received by the Laboratory. The validity of any implication of the characteristics of a batch from the characteristics of a sample will depend on the sampling procedure used.

Signed: N J Shepherd

Nicky J Shepherd

Signed: Colin Moffat

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BACKGROUND

The River Ribble runs a course through an area of England which has a lot of industrial activity resulting in a high degree of effluent discharge. There have been several reports or complaints of fish from these waters having an unpleasant taste or taint. The Water Research Centre (WRC) were asked to investigate the problem and requested the Marine Laboratory Aberdeen (MLA) to assess samples of fish from the Ribble for the presence of a taint.

The exact nature of the tainting substance(s), if indeed there was a taint, was not known due to the extensive range of compounds being released into the river. However, a *p*-chlorophenol spill was reported two years prior to this investigation and was a possible source of taint.

For the first phase of the study, WRC arranged for samples of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) to be taken from different reaches of the river, the exact location and quantity being dependent on the fishing conditions and the availability of material. Once caught, the fish were frozen until enough material was gathered for the MLA to assess.

The MLA requested that all fish were frozen as soon as possible after capture and that they should be gutted and wrapped in aluminium foil and placed in labelled polythene bags.

PROCEDURE

The first consignment, 22 salmon samples, was delivered to MLA on 19 November 1996. The samples were frozen and comprised of 12 whole fish, all containing guts, and 10 steaks (one containing guts). The samples were identified by labels which contained varying degrees of information relating to the size of the fish, date of capture, delay before freezing and the name of the angler/netsman. This information was available on separate data sheets for 12 of the samples.

Preparation of Salmon Samples for Cooked Assessment

- A steak, approximately 2 cm thick, was taken from behind the dorsal fin of each whole fish whilst still frozen.
- The frozen guts and kidney tissue were removed from all the steaks which were rinsed clean in potable water.
- The steaks were allowed to thaw at ambient temperature and once thawed, the skin was removed.
- The steaks were placed in coded glass casseroles and cooked by microwave heating until a centre temperature of 65 °C was reached.
- The samples were kept warm on electric hot plates during each of the two tasting sessions.

The samples were tasted by a panel of eight assessors experienced in the sensory assessment of fish and taint assessment.

A second consignment of eight whole trout was delivered to MLA on 17 December 1996. The labelled samples were frozen and all contained guts.

Preparation of Trout Samples for Cooked Assessment

The procedure followed for the preparation of samples for assessment was the same as that for the salmon with the exception that the steaks were slightly thicker due to the smaller size of the fish.

Due to ill health, the samples were tasted by a panel reduced in numbers to four experienced assessors and one trainee.

A sample is deemed to be tainted if 50% or over of the panel scores for the sample are positive. These scores range from 1 ("slight") to 5 ("extremely strong") see Appendix 1. In cases where the percentage is less than 50 but equal to or greater than 20, the sample is regarded as "suspect". A single asterisk * is shown when the sample is deemed to be "not tainted".

RESULTS

Table 1. Summary of salmon tasting data

Sample code	Sample type	Date caught	No of assessors	No Tastings	No of positive taints	% positive taints	Mean panel score
E1	Whole fish	Not known	8	8	2	25.0	0.2
E2	Whole fish	Not known	8	8	1	12.5	*
E3	Whole fish	Not known	8	8	4	50.0	0.8
E4	Whole fish	Not known	8	8	4	50.0	0.6
E5	Whole fish	Not known	8	8	2	25.0	0.2
E6	Whole fish	Not known	8	8	3	37.5	0.4
E7	Whole fish	Not known	8	8	2	25.0	0.4
E8	Whole fish	Not known	8	8	7	87.5	1.6
E9	Whole fish	Not known	8	8	3	37.5	0.3
E10	Whole fish	Not known	8	8	1	12.5	*
BR1	Steak	01 10 96	8	8	1	12.5	*
BR2	Steak	01 10 96	8	8	1	12.5	*
BR3	Steak	05 10 96	8	8	1	12.5	*
BR4	Steak	07 10 96	8	8	2	25.0	0.3
BR5	Steak	07 10 96	8	8	2	25.0	0.3
BR6	Steak	08 10 96	8	8	6	75.0	1.0
BR7	Whole fish	09 10 96	8	8	6	75.0	1.6
BR8	Whole fish	16 10 96	8	8	4	50.0	0.7
BR9	Steak	30 10 96	8	8	3	37.5	0.8
H1	Steak	24 10 96	8	8	2	25.0	0.5
UR1	Steak	07 10 96	8	8	2	25.0	0.6
UR2	Steak	19 10 96	8	8	1	12.5	*

Table 2. Summary of trout tasting data

Sample code	Sample type	Date caught	No of assessors	No of tastings	No of positive taints	% positive taints	Mean panel score
C1	Whole fish	14 10 96	5	5	3	60.0	1.2
C2	Whole fish	Not known	5	5	2	40.0	0.6
C3	Whole fish	14 10 96	5	5	5	100.0	2.6
C4	Whole fish	14 10 96	5	5	4	80.0	1.9
C5	Whole fish	14 10 96	5	5	2	40.0	0.4
UR1	Whole fish	07 11 96	5	5	0	0.0	*
UR2	Whole fish	07 11 96	5	5	3	60.0	0.8
UR3	Whole fish	08 11 96	5	5	1	20.0	0.6

Table 3. Salmon taste panels - a summary of the descriptors used by assessors

Sample code	% Positive taints	Description of sample
E1	25.0	Very strong earthy, musty flavour. Slight astringency and after-taste. Tough, dry texture.
E2	12.5	Slight earthy, cardboard flavour. Tough dry texture.
E3	50.0	Very strong earthy, cardboard, old leather, PVC, plastic flavour. Slightly tough, dry texture.
E4	50.0	Very strong earthy, unpleasant flavour. Slight astringency and after-taste. Dry texture.
E5	25.0	Slightly earthy, some astringency and chemical aftertaste. Dry texture.
E6	37.5	Strong earthy, musty flavour. Moderate astringency and lingering after-taste. Dry texture.
E7	25.0	Earthy, leathery, slight chemical aftertaste, stale odour.
E8	87.5	Very strong earthy unpleasant flavour. Strong astringency and chemical after-taste. Dettol like flavour. Tough, dry texture
E9	37.5	Slightly earthy, painty, some after-taste. Dry texture.
E10	12.5	Earthy, unpleasant flavour. Tough dry texture.
BR1	12.5	Earthy, some sweetness, slightly tough dry texture.
BR2	12.5	Earthy, musty, mossy flavour with some sweetness. Slightly tough dry texture.
BR3	12.5	Earthy, moderate sweetness, slightly tough dry texture.
BR4	25.0	Earthy, musty, slight astringency, slightly tough dry texture

Sample code	% Positive taints	Description of sample
BR5	25.0	Slightly earthy, musty, sour, bitter fish oil flavour.[+]
BR6	75.0	Musty, leathery, slight chemical taste, sour, bitter, unpleasant.
BR7	75.0	Old leather, PVC, plastic flavour, garden chemicals, earthy.
BR8	50.0	Earthy, leather, PVC, plastic flavour, garden chemicals, strong initial taint.
BR9	37.5	Phenols, slightly perfumed, bitter, unpleasant aftertaste, soft, slimy texture.
UR1	25.0	Earthy, musty, slightly sour off- flavours.
UR2	12.5	Earthy, musty, slightly off- flavours.
H1	25.0	Bitter, eggy odour, soft, slimy texture.

[+]The steak contained guts and some of the unpleasant odours may be spoilage odours related to the gut contents.

Table 4. Trout samples - summary of the descriptors used by assessors

Sample code	% Positive taints	Description of sample
C1	60.0	Strong earthy, musty flavour with a strong chemical, soapy taste. Very unpleasant.
C2	40.0	Sour, dirty dishwater odour, musty, soapy flavour. Tough texture.
C3	100.0	Strong earthy and chemical phenol type flavour.
C4	80.0	Sour, musty, earthy off-flavours, soapy and phenolic.
C5	40.0	Earthy, musty slightly unpleasant.
UR1	0.0	Bland, slightly soapy flavour.
UR2	60.0	Very earthy, slight chemical taste, slimy texture.
UR3	20.0	Unpleasant, slight off-odour, soft texture.

NB After tasting the trout samples all assessors reported an unpleasant and lingering soapy, chemical like after-taste which persisted for several hours.

DISCUSSION

A "taint" is described as an "odour or flavour foreign to the product" and makes no reference to whether the taint is pleasant or unpleasant. The taints observed by the panel for the samples supplied are discussed below. The salmon and trout are discussed separately.

Salmon

The samples which were labelled E1-E10 were estuarine fish, BR1-BR9 and H1 were from an area of the river known as the "Big Ribble" which is fresh water and the two samples UR1 and UR2 are from the upper reaches of the Ribble.

If the results from these three areas are looked at in more detail it can be seen that of the estuarine fish, three are deemed to be "tainted" and 5 "suspect", the same number are deemed "tainted" from the "Big Ribble" whilst only three are "suspect". Of the two "upper reach" fish 1 is regarded as being "suspect" whilst the other is deemed "not tainted". From these results it may be concluded that the fish from the estuarine region are the worst affected. The nature of the taint described by the panel is not specific to one chemical or tainting substance but the main conclusion is that the fish tasted were particularly unpleasant. Although earthy, musty flavours are characteristic of salmon, the intensity at which these flavours occurred was objectionable and would render the fish unfit for consumption.

The majority of the whole fish were female in an advanced state of sexual maturity. Fish of this condition are not normally consumed as the flesh is of poor eating quality and this will have added to the unpleasantness of the fish as they were very dry and tough in texture.

Trout

The samples which were labelled C1-C5 were from an area known as the Calder whilst samples UR1-UR3 were from an area known as Waddow Hall.

Of the five Calder fish tasted, three are deemed to be "tainted" and two are regarded as "suspect". Only three fish from Waddow Hall were assessed and the results show that one sample is deemed to be "tainted" one sample is regarded as "suspect" and third is deemed "not tainted".

The results of this limited survey of trout from the two areas, suggests that fish from the Calder are more tainted than those from Waddow Hall. The nature of the taint as described by the panel was similar to that of the salmon but with an additional soapy, chemical like flavour which resulted in a very persistent after-taste. Again the majority of the fish were female in an advanced state of sexual maturity adding to the overall impression that these were unpleasant and of very poor eating quality.

The general statement which can be made from the assessment of the samples of salmon and trout supplied is that they all had an unpleasant flavour and were of poor eating quality which would lead one to conclude that the waters from which these fish were taken is not clean enough to support fish of good eating quality with a flavour profile characteristic of the species.

APPENDIX 1

Marine Laboratory, Aberdeen - Sensory Unit - Taint Assessment Scoring Sheet

Name: .....

Ref. No: .....

Date: .....

Species: .....

Taste the samples in comparison with your experience of the typical flavour of the species under test and rate the intensity of any taints on the scale below. Please add any comments about the nature of the taint.

0 = absence of taint
1 = slight
2 = moderate
3 = strong
4 = very strong
5 = extremely strong

Sample No	Intensity	Comments
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		