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Tainting of Fishery Resources

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TAINTING OF FISHERY RESOURCES

W. Gordon Tidmarsh[1]
R. Ernst[1]
R. Ackman[2]
T. Farquharson[2]

[1]Martec Limited
Halifax, Nova Scotia

[2]Canadian Institute of Fisheries Technology
Halifax, Nova Scotia

Scientific Advisor: Dr. J. Payne

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The authors take full responsibility for any errors or omissions.

PREFACE

Martec Limited, in association with the Canadian Institute of Fisheries Technology, was contracted under the Environmental Studies Revolving Funds Effects Monitoring Priority Subject Area to undertake a review of the real or potential concerns for tainting of fishery resources by hydrocarbons as a result of offshore oil and gas exploration and development activity. The request for proposals for the project appeared in the ESRF Update II (3) dated July 3, 1984. The terms of reference for the study are presented below:

"Objective

The objectives of the study are to evaluate real or potential concerns for the tainting of fisheries resources (including shellfish) resulting from oil spills or hydrocarbon development activities, to assess the effectiveness of the various methods and procedures used to date, and to identify possible research/monitoring options for dealing with tainting.

Statement of Work

Case histories of fisheries tainting by petroleum hydrocarbons will be documented through literature searches and communication with various national and international resource agencies, oil developers, and individuals. An important component of the study will be the identification of fishery closures that have resulted from either tainting or fear of tainting. The second part of the

review will include an evaluation of the adequacy of scientific knowledge for dealing with concerns related to tainting. Included will be the assessment of methods (e.g. taste panels, analytical techniques) used to resolve the problems of human perception of tainting. The value of carrying out selected laboratory studies with representative commercial species in order to obtain an appreciation of dose-response relationships for fish tainting will be assessed using case studies to show the effectiveness related to specific hydrocarbon impacts (e.g. onshore, offshore). Also to be assessed will be the value of including fish tainting as part of strategies for effects monitoring at offshore oil and gas exploration and development sites.

Much of the required information will not be available through regular literature searches and the successful applicant will be expected to carry out a comprehensive survey of the appropriate agencies, developers, individuals, etc., in countries that have experienced major oil spills or have fishery/oil pollution concerns."

The project team and their responsibilities were as follows:

Mr. W.G. Tidmarsh - Project Manager;
Dr. R.G. Ackman - Scientific Coordinator;
Ms. R. Ernst - Research Biologist; and
Ms. T. Farquharson - Food Science Technologist.

The report is presented in five parts plus seven appendices. The introduction (Part 1) describes the objectives

and scope of the review, and the methods used to acquire the information presented in this document. Part 2 contains a definition of tainting, and identifies the hydrocarbon compounds responsible for tainting. Part 3 identifies known case histories of tainting and procedures currently in place in Canada and other jurisdictions to identify and monitor instances of tainting. The major sensory and chemical analytical methods used to detect the presence of off-flavours and levels of hydrocarbons in tissues, together with a review of research in the field, are presented in Part 4. This part includes a discussion of proposed methods and procedures which could be adopted for dealing with a potential tainting situation associated with offshore oil and gas activities. In Part 5 a series of recommendations for future studies related to tainting are presented.

The literature used in the review is presented in two sections. References Cited contains literature cited in the report. Literature reviewed but not specifically referred to in the report is presented in Appendix A.

SUMMARY

Tainting is the development of an atypical flavour in fish caused by natural spoilage or by the assimilation of contaminants into fish tissue. Exposure of fish to sublethal levels of lipid-soluble anthropogenic hydrocarbons in the C_6-C_{26} range either in the environment or present in the diet can cause a taint, but the severity depends on the types, concentrations, and behaviour of the hydrocarbons present, and the species affected. Tainting is a complex problem because a taint is a subjective assessment which cannot be properly verified using chemical analytical techniques, and it is often impossible to establish a direct relationship between the presence of hydrocarbon contaminants and the presence of a taint. Yet the potential economic costs associated with possible tainting can be significant.

Tainting is a potentially important issue in Canada where offshore oil and gas activity coexists with rich offshore and coastal fisheries on both the east and west coasts. Because tainting or fear of tainting is a concern to both the oil and fishing industries, and the economic risks are potentially high, the present review was initiated to place the concerns associated with tainting in perspective, and benefit from the experiences in other countries.

Although tainting, or fear of tainting, arising from oil pollution has caused severe economic losses to fishermen in various parts of the world in the past, it has not received coordinated scientific and regulatory attention until recently. Tainting incidents have for the most part been poorly documented. Generally, it has been found that tainting has been more prevalent in confined, shallow coastal embayments where sessile organisms or lipid-rich

mobile species have been exposed to anthropogenic hydrocarbons from either chronic discharges or high concentrations from an acute spill persisting in the water column or sediments. Experience in laboratory tests has yielded conflicting evidence on the risk and degree of possible tainting of fish, and the actual taint-producing hydrocarbons present.

Available case histories of major oil spills or well blowouts reveal few instances where fisheries have been officially closed due to tainting or fear of tainting. Fishermen avoid areas if tainting, or threat of tainting, is present because their catch may not be saleable or may be condemned by inspectors. Fear of tainting can have as serious an economic effect on a fishery as a confirmed incident.

Regulatory procedures for managing potential tainting of fish resources are not well established in most countries. With the exception of the United Kingdom, legislation for closing fisheries because of confirmed contamination or tainting is lacking. In general, control is exercised by health authorities who act only after contamination is reported and who can condemn tainted fish as inedible and therefore unmarketable.

Because a taint is a subjective sensory perception, it can only be evaluated by using sensory tools such as odour and taste. Standard methods for determining taint have not been adopted internationally. Chemical analysis can only assist in determining the possible types of hydrocarbons responsible for a taint but cannot be used to determine whether a product is tainted or not.

Based on experience in the food science industry, four types of sensory methods were assessed as tools for evaluating hydrocarbon taint.

The triangle test has been determined to be the best method. In this test, the panelist is presented with three samples, two of which are the same, and is asked to identify the odd sample. The triangle test has also been recommended for taste testing by the Working Group on the Evaluation of Hazardous Substances Carried by Ships (GESAMP 1983).

Tissue analysis of a tainted product is recommended to support the findings of sensory evaluations by "finger-printing" and quantifying the hydrocarbon present compared to control samples. Many methods to determine the hydrocarbon content in fish tissues are available. The three basic steps common to all methods are: extraction of hydrocarbons and lipids, isolation of the hydrocarbon fraction and analysis of appropriate fractions. The analytical method that has been recommended for use is based on steam distillation which rapidly recovers the most likely taint-causing hydrocarbons from the tissue matrix. These are then concentrated for analysis by gas-liquid chromatography, with or without additional analytical technology such as mass spectrometry, and UV and IR absorption.

The study revealed the lack of standard internationally accepted sensory evaluation and analytical tools for assessing possible fish tainting. Recommendations were made on the development of a series of standard methods that could be applied in the event of an oil spill or well blowout where fish tainting may be a concern. In addition, scientific understanding of several specific questions related to tainting is lacking. These include:

- Threshold levels of hydrocarbons causing tainting;

- Depuration rates in organisms after exposure;

- Potential tainting associated with the discharge of oil-based drilling muds and production water, the application of dispersants, and the presence of sunken oil; and

- Baseline levels of taint-producing anthropogenic hydrocarbons in selected species of commercial importance.

Recommendations for studies to fill these deficiencies have been made.

RESUME

Le poisson est dit avarié ou détérioré lorsque sa saveur devient anormale par suite de détérioration naturelle ou parce que ses tissus ont assimilé des substances contaminantes. La détérioration peut résulter de l'exposition du poisson à des niveaux sublétaux d'hydrocarbures anthropogéniques liposolubles de la gamme C_6-C_{26}, présents soit dans l'environnement, soit dans l'alimentation du poisson, mais la sévérité d'une telle dégradation dépend des types, des concentrations et du comportment des hydrocarbures présents, et de l'espèce affectée. La détérioration du poisson par les contaminants est un problème complexe, car elle ne se reconnaît que sur la base d'évaluations subjectives qui ne peuvent être vérifiées avec précision par les techniques de la chimie analytique; il est souvent impossible d'établir un rapport direct entre la présence d'hydrocarbures contaminants et les cas de détérioration. Et cependant, les détriments économiques potentiels associés aux possibilités de détérioration du poisson, peuvent être significatifs.

La détérioration du poisson par les contaminants est un problème potentiellement sérieux au Canada, car des activités d'extraction de pétrole et gaz sous-marins coexistent avec de riches pêcheries côtières et hauturières le long des côtes ouest et est du pays. La contamination du poisson, ou la crainte de cette contamination, inquiète aussi bien l'industrie pétrolière que l'industrie de la pêche, et les risques économiques sont potentiellement élevés; c'est pour ces raisons que la présente étude a été entreprise: son but est d'examiner les problèmes de détérioration de divers points de vue, et de profiter de l'expérience accumulée dans d'autres pays.

La détérioration du poisson par des contaminants, ou la crainte de détérioration, provoquées par la pollution pétrolière, a causé dans le passé de graves pertes économiques aux pêcheurs dans diverses parties du monde. Cependant, ce n'est que récemment que cette détérioration a été l'objet d'efforts coordonnés en matière d'études scientifiques et de règlements. La plupart des cas de détérioration par contaminants ont été mal documentés. En général, on a trouvé que cette détérioration a été plus fréquente dans des échancrures côtières resserrées et peu profondes, où des organismes sessiles, ou des espèces mobiles riches en lipides, ont été exposés à des hydrocarbures anthropogéniques provenant soit de décharges chroniques, soit, en fortes concentrations, de déversements intenses et persistants dans la colonne d'eau ou dans les sédiments. L'expérience accumulée grâce aux tests en laboratoire a fourni des données contradictoires sur le risque et l'intensité des détériorations possibles, et sur la nature exacte des hydrocarbures présents dans le milieu et capables de provoquer ces détériorations.

Les exemples connus de grands déversements de pétrole ou d'éruptions de puits de forage, révèlent peu de cas de fermeture officielle des pêcheries pour cause de détérioration, constatée ou redoutée, par les contaminants. Les pêcheurs évitent les régions affectées par ces détériorations ou menaces de détériorations, car leurs prises pourraient être invendables ou condamnées par les inspecteurs. La crainte des détériorations possibles peut exercer sur une pêcherie un effet économique aussi grave qu'une détérioration confirmée.

La plupart des pays n'ont pas encore établi de dispositions réglementaires fermes pour contrôler la détérioration

potentielle des ressources halieutiques par les contaminants. Sauf au Royaume-Uni, il n'y a pas de législation pemettant la fermeture des pêcheries lorsque la contamination, ou la détérioration par les contaminants, sont confirmées. En général, le contrôle est exercé par les autorités sanitaires, qui n'agissent qu'après que la contamination ait été signalée, et qui peuvent condamner le poisson avarié comme immangeable et par conséquent invendable.

La détérioration par les contaminants est une perception sensorielle subjective et ne peut donc être évaluée que par des essais sensoriels, tels que ceux basés sur l'odeur ou la saveur. A l'échelle internationale, on n'a pas encore adopté de méthodes standardisées pour déterminer cette détérioration. L'analyse chimique ne peut qu'aider à identifier les types possibles d'hydrocarbures responsables des détériorations, mais ne peut être employée pour determiner si un produit est avarié ou non.

Sur la base de l'expérience accumlée dans les sciences de l'alimentation, on a évalué quatre types de méthodes sensorielles permettant d'estimer le degré de détérioration par les hydrocarbures.

Le test triangulaire a été reconnu comme étant la meilleure méthode. Dans ce test, on présente à chacun des essayeurs trois échantillons, dont deux proviennent du même matériel, et on lui demande d'identifier celui des échantillons qui diffère des deux autres. Le test triangulaire a également été recommandé pour les essais gustatifs par le Groupe de Travail sur l'Evaluation des Substances Hasardeuses Transportées par Navires (GESAMP 1983).

L'analyse des tissus d'un produit avarié est recommandée pour renforcer les résultats des évaluations sensorielles: cette analyse identifie avec précision les hydrocarbures présents, et en mesure les quantités, par comparaison avec des échantillons témoins. Il existe de nombreuses méthodes pour déterminer le contenu en hydrocarbures des tissus de poisson. Les trois étapes fondamentales communes à toutes ces méthodes sont: l'extraction des hydrocarbures et lipides, l'isolement de la fraction des hydrocarbures, et l'analyse des sous-fractions appropriées. La méthode analytique recommandée est basée sur la distillation par entraînement à la vapeur, laquelle récupère rapidement, à partir de la matière tissulaire, les hydrocarbures les plus capables d'avoir causé la détérioration. Ces hydrocarbures sont ensuite concentrés pour être analysés par chromatographie de partage gaz-liquide, avec, éventuellement, analyses supplémentaires par des techniques telles que la spectrométrie de masse et l'absorption ultra-violette et infra-rouge.

La présente étude a révélé qu'aucune méthode standardisée sensorielle ou analtyique d'évaluation de la détérioration du poisson n'est acceptée internationalement. Des recommandations sont présentées en vue de développer une série de méthodes standardisées qui pourraient être utilisées lorsqu'il y a danger de détérioration due poisson par suite de déversement de pétrole ou d'éruption de puits de forage. En outre, plusieurs problèmes spécifiques, liés à la détérioration par les contaminants, n'ont pas encore été résolus sur le plan scientifique. Ces problèmes comprennent:

- En ce qui concerne les hydrocarbures: les niveaux minimums capables de causer des détériorations;

- Les vitesses de dépuration des organismes après que ceux-ci aient été exposés aux contaminants;

- Les possibilités de détérioration associées aux décharges de boues de forage et d'eaux de production des forages pétroliers, à l'application de dispersants, et à la présence de pétrole enfoui;

- Les niveaux normaux de base, chez des espèces sélectionnées, des hydrocarbures anthropogéniques qui causent les détériorations.

L'on a présenté des recommandations sur les recherches à effectuer en vue d'éliminer ces déficiences.

1.0 INTRODUCTION

The effect of elevated levels of anthropogenic hydrocarbons, commonly termed oil pollution, on marine and freshwater systems has attracted widespread attention from the general public, and the scientific and regulatory communities, for almost two decades. This interest has stimulated a considerable amount of pure and applied research and resulted in the production of a voluminous literature on the subject. Originally, almost all of the work was directed toward assessing the behaviour of hydrocarbons, and their direct effects on organisms and ecosystems. More recently, increased attention has been given to more subtle, second order effects such as the influence dissolved and particulate hydrocarbons taken up by commercially exploited fish species potentially could have on human health and resource exploitation.

GESAMP (1977) identified two potential effects of oil pollution on humans. The first is the possible accumulation of carcinogens such as polycyclic and heterocyclic compounds and polynuclear aromatic hydrocarbons in exposed organisms, and their transmittal up the food web. The second is the loss of marine foods as a result of reduced production in exploited populations or uptake of hydrocarbons by exposed organisms rendering their tissue unpalatable, or impairing flavours normally associated with finfish or shellfish species. This effect is commonly termed "tainting".

It is a very complex problem which has not received a great deal of attention from the scientific community until recently (GESAMP 1983) although officials in Canada are becoming more sensitive to the potential issue, e.g. Sable

Island Environmental Assessment Panel (1983) report.

Tainting of seafoods can arise from either the spoilage of improperly handled produce or the exposure of living organisms to specific contaminants present in the aquatic environment. The latter is of greatest concern because it can result in substantial economic losses to fishermen (GESAMP 1977), yet currently there are no standard methods for assessing or verifying a tainting situation (Howgate pers. comm.). Economic losses as a result of perceived or actual tainting are impossible to evaluate because of inadequate documentation, and technical data on the subject are sparse (GESAMP 1982).

Fear of tainting arising from an oil pollution incident can be as serious a problem as an actual tainting event. Consumer resistance to suspect produce, closures imposed by regulatory authorities, and embargoes on harvesting activities by producers for fear of gear damage or lack of markets can all cause severe economic dislocations. Unlike exposure to carcinogens, the presence of tainted produce is readily detectable by human sensory mechanisms so the risk to public health is reduced. Therefore, tainting per se is more an economic problem than either an environmental or public health concern and is as much a perceptual problem as a real concern.

Although it is only one element of the hydrocarbon contamination problem, real or potential tainting associated with petrogenic hydrocarbons, i.e. condensate and crude oil, has been of increasing concern to fishermen because of the increased exploration, production, and transportation of oil at sea in recent years (GESAMP 1977). Until recently, this concern has not been matched by concerted scientific or

regulatory effort at national levels or within the international community to develop mechanisms for dealing with the problem.

Potential tainting of fish resources arising from potential oil spills or well blowouts associated with offshore oil and gas activity has been recognized as a priority subject area by the Effects Monitoring Committee of the Environmental Studies Revolving Funds. Given the fragmented sources of information on the subject, the committee identified a requirement for a scoping study to identify experience with the problem of tainting in other jurisdictions, evaluate case histories, and identify sensory and analytical methods used to identify and evaluate potential tainting situations. This report contains the results of this review.

1.1 OBJECTIVES AND SCOPE

At the outset, the study had three general objectives. These were:

1. To identify case histories of situations where tainting or fear of tainting as a result of hydrocarbon contamination has occurred;

2. To determine how questions related to tainting are handled in other jurisdictions; and

3. To assess the sensory (organoleptic) and chemical analytical methods used, at present, to confirm or deny the presence of a taint.

Because of the complexity of the subject, the avenues of inquiry were fairly carefully defined to insure that the

general objectives were not obscured by the myriad of issues associated with the presence of hydrocarbons in the environment and their effects on fish. Therefore, subjects such as hydrocarbon toxicities, general bioaccumulation, and public health issues were not addressed, although they do have some relationships with tainting. The investigation was confined to hydrocarbons and did not address other materials used in offshore oil and gas exploration and production activities such as well work-over fluids, which, if lost or discharged into the environment in significant quantities, conceivably could result in very localized tainting situations. Finally, the investigations generally concentrated on tainting as it relates to offshore oil and gas exploration and development, and did not delve deeply into tainting associated with refinery effluents or bulk chemical transportation.

These constraints were established to insure that the results of the study were clearly focussed on the subject of real or potential tainting of fishery resources related to offshore oil and gas development. The study was designed to show what is known about the subject and where the major deficiencies in present knowledge lie.

1.2 METHODOLOGY

To achieve these objectives, two avenues of inquiry were followed. The first involved a detailed review of relevant literature on the subject of tainting and potential methods of evaluation. The literature on sensory and analytical methods is substantial but information on case histories, jurisdictional questions, and incipient tainting levels of hydrocarbons is hard to locate. Some is located in the grey literature but, as indicated earlier, there is inadequate documentation of incidents of tainting, fishery closures,

compensation claims, or condemnation of tainted fish by regulatory agencies.

In an attempt to augment published sources of information, contacts were made with over 60 people or organizations world-wide in order to obtain general information about tainting incidents associated with oil spills or oil and gas activities, and the regulatory process established in various countries for monitoring and evaluating a potential tainting situation. Much of the information received through this process was anecdotal although in some cases copies of legislation, papers, and procedures were forwarded in replies. From these sources, it has been possible to piece together a general indication of tainting incidents and how they have been managed in different jurisdictions.

The report, therefore, contains a mixture of literature reviews, and general information about tainting incidents, some of which cannot be readily confirmed. This confirms GESAMP's (1982) finding that the general subject of fish tainting is inadequately documented.

2.0 PERSPECTIVE ON TAINTING

2.1 INTRODUCTION

This section of the document presents a perspective on potential fish tainting caused by exposure to anthropogenic hydrocarbons associated with offshore oil and gas activity. It contains a definition of the term tainting, and identifies how a taint can be acquired by finfish and shellfish. It provides the background for the documentation of case histories of hydrocarbon-associated tainting incidents and methods used for identifying tainted material, which are presented in later sections of the report.

2.2 DEFINITION

The flavour of a food is determined by a blend of taste and odour evoked by a substance in or near the mouth. As a sensory perception, experience as well as preferences can greatly influence a determination as to whether a food is "off". In fish, the flavour can be strongly influenced by the diet of the living organism prior to harvesting, as well as by natural degradation or spoilage after death. Therefore, separating flavours associated with natural processes, compared with those resulting from exposure to contaminants such as hydrocarbons released into the environment, can be difficult.

From the point of view of marketability of fish and shellfish and acceptance or rejection of a product by inspectors and the public, factors such as raw odour, tissue or shell colour, tissue texture, and general aesthetic quality of the product are the primary factors to consider. In rare cases, the potential presence of an "off-flavour" or taint may not

be detectable until the product is prepared for eating or is actually consumed. With respect to contamination of finfish or shellfish by hydrocarbons, external contamination or fouling, as well as a taint, may be cause for product rejection. External contamination of an organism does not necessarily mean that tainting of the underlying tissue has occurred (GESAMP 1977).

These factors make it difficult to develop an entirely satisfactory definition of the term "tainting". For the purposes of this study, a basic definition developed by the GESAMP Working Group on the Evaluation of the Hazards of Harmful Substances Carried by Ships (GESAMP 1985) is considered as generally applicable for finfish or shellfish prior to processing.

> "(Tainting is) the development of a flavour or odour in the organism when caught or harvested which is not typical of the flavour or odour of the organisms themselves."

It should be noted that this definition does not make a statement about the nature of the atypical flavour or odour or attempt to quantify the degree of the taint. The importance lies in whether the flavour or odour of the product is altered, not whether a contaminant impairs or improves the flavour. It also does not attempt to cover the related public health questions associated with the exposure of fish and shellfish to chronic or acute sources of contaminants where bioaccumulation of hazardous substances may be significant but the flavour or odour associated with the organism is not affected.

2.3 TAINTING MECHANISMS

A taint or acquisition of an unnatural odour or taste in organisms exposed to dissolved or particulate hydrocarbons in the water column can arise from four mechanisms (Connell and Miller 1981). These are:

- absorption of hydrocarbons on the skin;
- absorption of dissolved hydrocarbons from the water through the skin;
- absorption of dissolved hydrocarbons through the gills, i.e. respiration; and
- ingestion of food contaminated with petroleum.

The mechanism varies from situation to situation depending upon the species exposed, the type and concentration of hydrocarbons present, and exposure time.

While tainting may occur as a result of absorption through the skin, the more usual modes of uptake are through respiration and/or ingestion (Connell 1974). Days or weeks of exposure are usually required to acquire a taint, if ambient concentrations of hydrocarbons are low, but if concentrations are high, tainting may require only a day or so. The taint can be eliminated by placing the organism in an uncontaminated environment. This depuration process can take weeks or even months to complete.

Tainting of fish as a result of absorption to the surface of fish has been observed when a catch has accidentally come in contact with oily water from storage tanks (Stansby 1978) or from fouled nets (Ackman pers. comm.). The contaminant and to some extent the acquired odour can be removed by washing in running water and detergents although a residual odour

may remain after cleaning. As indicated above, external contamination, however, does not necessarily mean that tainting has occurred (GESAMP 1977).

2.4 FACTORS AFFECTING THE ACQUISITION OF A TAINT

The threshold level of exposure necessary to cause a taint in finfish and shellfish depends on the chemical composition of the hydrocarbon contaminant and on the species affected.

2.4.1 Hydrocarbon Type

Tainting of fish resources has been associated with losses of diesel fuel (Mackie et al. 1978), crude oil (Motohiro and Inoue 1973), Bunker C (Shenton 1973; Scarratt 1980a,b), and gasoline (Kerkhoff 1974) into the environment, as well as with refinery effluents (Nitta 1972; Connell 1974).

It is difficult to unequivocally identify, by chemical analytical techniques, compounds in crude oil or distillates that exactly match hydrocarbons isolated from tainted fish. This is due to metabolic processes of organisms that alter the chemical characteristics of the contaminant. However, it is known that the principal components of crude and refined oil causing tainting include the phenols, dibenzo-thiophenes, naphthenic acids, mercaptans, tetradecanes and methylated naphthalenes (GESAMP 1977). These compounds are volatile and soluble in water. Their solubility makes them more available to aquatic organisms. Due to their lipid solubility they are readily transferred by partitioning into the blood and tissues of organisms. There is some evidence to indicate that fish preferentially absorb hydrocarbons with chain lengths in the range of C_{22} to C_{30} with a maximum at C_{26} (Hardy et al. 1974). Although these may be minor

constituents of the hydrocarbon contaminants present, they may, in fact, be the principal cause of tainting.

The chemical verification of tainting is also difficult due to hydrocarbons produced by the living organisms themselves, termed biogenic hydrocarbons. Pristane, some n-alkanes and isoprenoid hydrocarbons are widespread in the food chain (GESAMP 1977) and overlap to a large extent with petrogenic hydrocarbons.

2.4.2 Fish Species and Lipid Content

Each species of finfish and shellfish has its own characteristic flavour (McGill et al. 1984). The flavouring components occur in both the lipid and the protein of the fish. Ordinarily, however, in the absence of putrification the most volatile and flavourful components are the chemicals dissolved in the lipid (Stansby 1978). It is generally accepted that there is a greater storage and persistence of aromatic and polynuclear hydrocarbons in lipid-rich than in lipid-poor tissues of most marine species (GESAMP 1977). Whittle and Mackie (1975) have suggested that the amount of "free" lipids present in a fish indicates the susceptibility of that animal to tainting.

Although organochlorine pesticides and PCB residues are not usually classed with compounds considered to be tainting as defined earlier, they share the same affinity for lipid, especially depot fat, as do hydrocarbons such as napthalene. The organochlorines can be unequivocally measured and distribution data are normally reliable. There is an extensive literature on the distribution of organochlorine pesticide and PCB residues in fish and other organisms (e.g. Addison 1976).

Addison, Fletcher et al. (1972), in an experiment with a short-term feeding of Aroclor 5460, found distributions to be as shown in Table 2-1.

Since the liver of the cod is usually 30-50% fat, the muscle <1% lipid, the affinity of this mixture for lipid is well illustrated. In order to decide on species and parts of fish to be sampled for suspected tainting by hydrocarbons, the lipid content and distribution must be taken into account.

2.4.2.1 Finfish

In this review three basic types of finfish will be briefly discussed: cod, haddock, etc. with edible part lipids in the order of 1.5% by weight; sole, halibut, etc. with edible part lipids in the 1.5-6.5% by weight range; and capelin, herring, mackerel, etc. with edible part lipids ranging up to 20% of total wet weight. Table 2-2 lists the majority of commercially valuable fish species from eastern Canadian waters and the lipid (or fat) contents.

The term "lipid" includes all types of material recoverable by extraction of tissue with an organic solvent such as chloroform, benzene, diethyl ether, acetone, etc. Figure 2-1 shows the structures of major classes of lipids found in all edible fish species. In practice the ideal solvent for biochemical studies is a 2:1 mixture of chloroform and methanol variously described as a (modified) Folch et al. (1957) procedure or a Bligh and Dyer (1959) system. The merits of these systems have been reviewed by Hopkins et al. (1984).

These lipids, and several others of lesser importance, are found in the membranes that enclose every fish body cell.

TABLE 2-1

Mean Aroclor 5460 Concentrations in Four Fish

Sample	In tissue (µg/g wet weight)	In lipid (µg/g)
liver	16.7	135
muscle	0.11	25.7
blood	5.52	704
gill	2.9	218

TABLE 2-2

Species of fish of commercial interest or likely to be caught in emergency fishing operations conducted after petroleum-related incidents, in the North-west Atlantic. The fat or lipid content is given as averages and/or ranges of percentages of wet weight, usually of edible muscle, but in some cases of whole fish bodies.

Species	Edible Part	Lipid Content
Alewife (see gaspereau)		
Bass, striped (Roccus(Morone) saxatilis)	muscle, raw	3.1 2.3-4.5
Butterfish (Poronotus triacanthus)	muscle, raw	11.2 5.1-17.3
Capelin (Mallotus villosus)	spawning, body gutted	1.9-3.0
	spawning(?)	4.1
	whole, raw	2.1-10.3
	fall, whole, raw	2.7-18.4
	spawning(?), muscle, raw	3.1 2.6-3.4
Catfish (see wolffish)		
Cod, atlantic (Gadus morhua)	muscle, raw	0.6 0.1-1.2
Cusk (Brosme brosme)	muscle, raw	1.2 0.8-1.5
Dogfish, spiny (Squalus acanthias)	muscle, raw	5.2 0.2-10.0
Dolphin (Coryphaena hippurus)	muscle, raw	1.4 1.3-1.5
Eel, conger (Conger oceanicus)	muscle, raw	11.6
Eel, freshwater (Anguilla rostrata)	muscle, raw	13.3 11.6-14.9

TABLE 2-2 (Continued)

Species	Edible Part	Lipid Content
Flounder, summer (*Paralichthys dentatus*)	muscle, raw	0.4 0.1-1.0
Flounder, winter (*Pseudopleuronectes americanus*)	muscle, raw	0.6 0.2-1.4
Flounder, witch (*Glyptocephalus cynoglossus*)	muscle, raw	0.6 0.1-1.0
Flounder, yellowtail (*Limanda ferruginea*)	muscle, raw	0.3 0.1-0.4
Gaspereau (alewife, blueback herring) (*Alosa pseudoharengus*)	muscle, raw	6.8 2.9-15.2
Goosefish (Monkfish) (*Lophius* sp.)	muscle, raw	1.4 1.0-2.0
Haddock (*Melanogrammus aeglefinus*)	muscle, raw	1.3 1.1-2.7
Hake, red (white, squirrel, ling) (*Urophycis chuss*)	muscle, raw	0.5 0.4-0.6
Hake, silver (Whiting) (*Merluccius bilinearis*)	muscle, raw	1.3 0.2-2.4
Hake, spotted (*Urophycis regius*)	muscle, raw	0.8
Halibut, Atlantic (*Hippoglossus hippoglossus*)	muscle, raw	6.2 3.9-8.5
Herring, Atlantic (*Clupea harengus*)	muscle, raw	9.8 2.4-20.2
	sardine muscle, raw	2.0 1.2-2.5

TABLE 2-2 (Continued)

Species	Edible Part	Lipid Content
Lamprey, sea (*Petromyzon marinus*)	muscle, raw	15.5 13.0-18.8
Ling (see hake, red)		
Lumpfish (*Cyclopterus lumpus*)	muscle, raw	5.7 3.7-7.6
Mackerel, Atlantic (*Scomber scombrus*)	muscle, raw	9.6 0.7-24.0
	dark meat	11.8 8.7-18.3
Newfoundland turbot (Greenland halibut) (*Reinhardtius hippoglossoides*)	muscle, raw	11.6 10.8-12.4
Plaice, American (*Pleuronectes platessa*)	muscle, raw	1.5 0.3-2.3
Pollock (Boston bluefish) (*Pollachius virens*)	muscle, raw	0.8 0.2-2.0
Redfish (*Sebastes marinus*)	muscle, raw	3.1 0.6-8.4
Salmon, Atlantic (*Salmo salar*)	muscle, raw	5.5 0.2-14.5
Sand lance (*Ammodytes americanus*)	whole body	7.2 6.3-8.1
Saury, Atlantic (billfish) (*Scomberesox saurus*)	muscle, raw	6.1 1.4-12.7
Sculpin (sea raven) (*Hemitripterus americanus*)	muscle, raw	5.0
Shad (*Alosa sapidissima*)	muscle, raw	11.2 3.0-17.2
Skate (*Raja* sp.)	muscle, raw	1.3 0.5-6.1

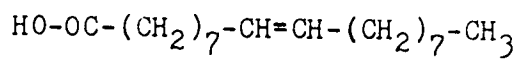
TABLE 2-2 (Continued)

Species	Edible Part	Lipid Content
Smelt (*Osmerus mordax*)	muscle, raw	2.0 0.9-2.4
Sole (see flounder, plaice)		
Sturgeon (Acipenseridae)	muscle, raw	1.9
(*Acipenser oxyrhynchus*)	steak section	7.2
Turbot (see Greenland turbot)		
Weakfish (*Cynoscion regalis*)	muscle, raw	1.5
Wolffish, Atlantic (catfish) (*Anarhichas lupus*)	muscle, raw	2.8 2.1-3.0

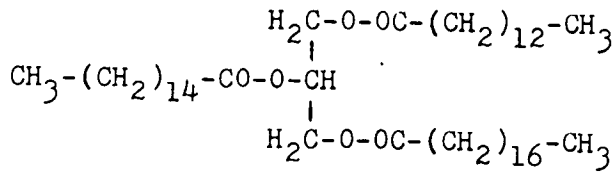
Source: Taken primarily from NOAA Technical Memorandum NMFS F/SEC-11 (V.D. Sidwell, author), Washington, D.C., Jan. 1981, 432 pages. Additional data from publications of R.G. Ackman.

FIGURE 2-1

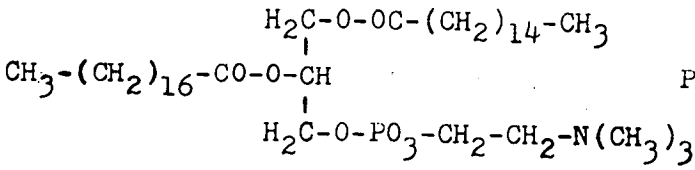
Structure of Major Classes of Lipids Found in all Edible Fish Species



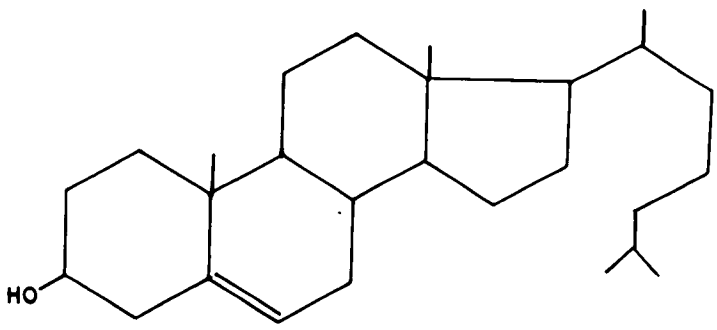
FATTY ACID



TRIGLYCERIDE



PHOSPHOLIPID



STEROL (CHOLESTEROL)

For practical purposes the edible muscle of the cod Gadus morhua may be taken as having the basic minimum lipid composition (see Table 2-3). In practice this lipid is largely insoluble in non-polar solvents such as hexane because of the polarity of the phosphoric acid moiety, which may be regarded as "hydrated" with dozens of water molecules and hence not truly soluble in hexane or petroleum ether and similar non-polar solvents, although some is transferred in micelle form. Thus menhaden Brevoortia tyrannus mince yielded 8.2% total lipid by chloroform-methanol extraction (15% polar, 85% triglyceride), but only 3.2% (98% triglyceride) with petroleum ether extraction (Ackman et al. 1976). Clearly the triglyceride represents lipids into which tainting hydrocarbons circulating in the bloodstream can partition and be stored within the fish.

Table 2-4 shows an example from another and more extreme case, that of mackerel S. scombrus with over 20% lipid in flesh. Because 89% of the lipid is liquid triglyceride the chloroform-methanol extract can also be called a fat or oil, whereas the chloroform-methanol extract from cod is a soft whitish solid.

As far as acquisition of a taint by fish is concerned, the distribution of fats in and under the skin is of paramount importance. In some species up to 50% of the fat can be in the subdermal layer (Figure 2-2). If hydrocarbons are deposited in this fat under the skin, rough handling could reveal the presence of a taint on the outside of the fish, whereas, in fact, the hydrocarbons are deposited in a part often included in the "edible part" of the organism.

Figure 2-2 also shows the seasonal variation in lipid content in capelin and mackerel. This variation may in turn

TABLE 2-3

Lipid Class Distribution in Flesh Lipids of Atlantic Cod (w/w%)

Compound	Male	Female
Sterol esters	2.2	2.8
Triglycerides	4.1	3.6
Cholesterol	6.3	6.5
Phosphatidyl ethanolamine (PE)	1.64	17.5
Phosphatidyl choline (PC)	60.0	61.2
Other	11.0	8.4
Total lipid in flesh (w/w%)	0.59	0.62
Total lipid in liver	53.0	30.6

Source: Addison et al. 1968.

TABLE 2-4

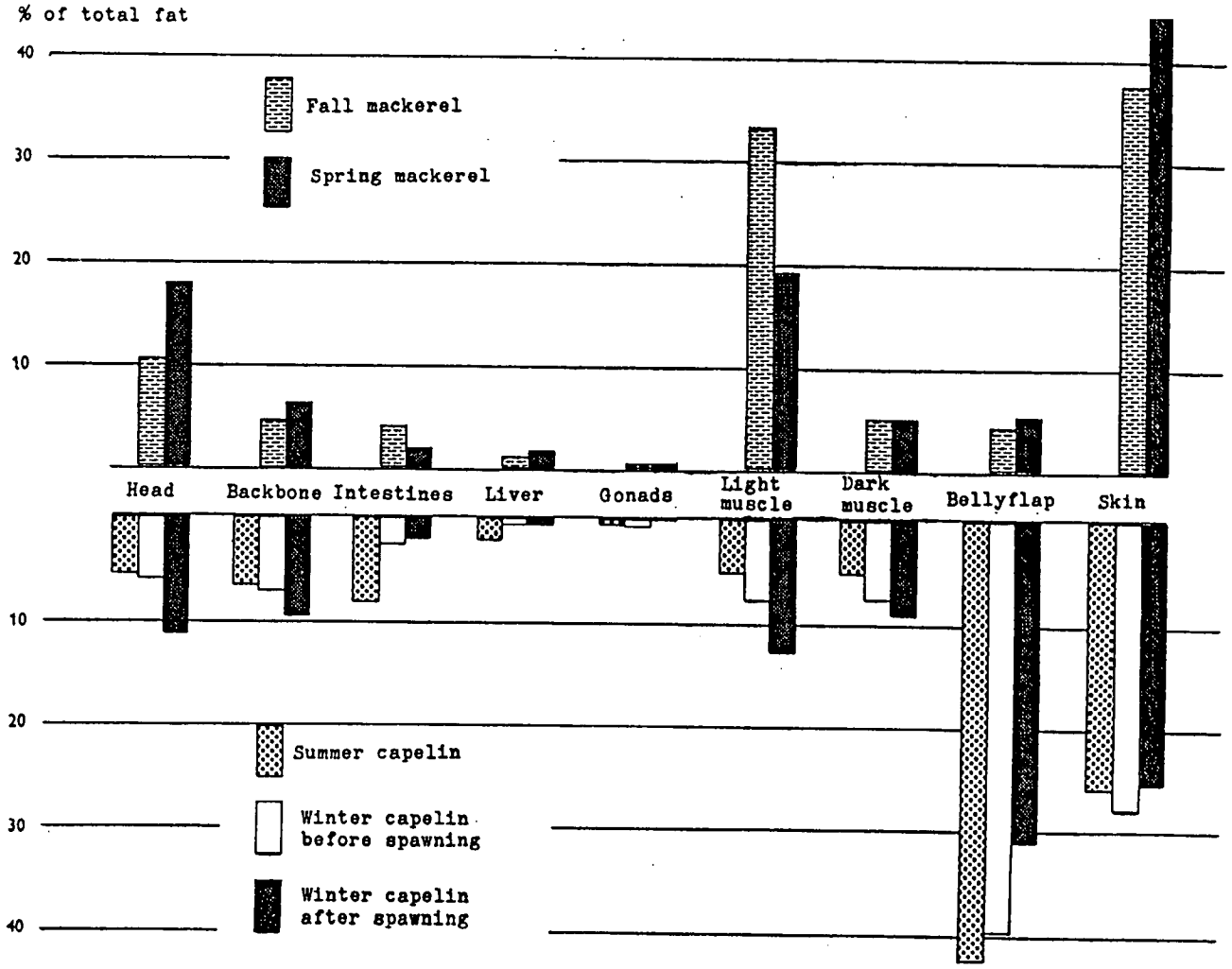
Lipid Class Distribution in Flesh Lipids of Atlantic Mackerel (w/w%)

Compound	December Male Flesh	Male Liver	December Female Flesh	Female Liver
Sterol ester	-	4.4	-	8.8
Triglyceride	89.4	61.2	89.7	43.8
Cholesterol	2.4	1.1	0.9	4.0
Phospholipids (PE+PC)	3.5	8.7	4.6	11.0
Other	4.7	24.6	5.4	32.4
Total lipid in sample	24.1	24.9	21.6	23.8

Source: Hardy and Keay 1972.

FIGURE 2-2

Distribution of Fat in Mackerel and Capelin



Source: Ackman 1980.

cause a seasonal variation in the potential for tainting to occur, or in the threshold concentration at which a taint would become evident.

2.4.2.2 Shellfish

Lipid contents of edible shellfish (bivalves and crustacea) are presented in Table 2-5. It is important to recognize that in bivalves the main energy reserve is glycogen. Triglycerides are at most one half of the total lipid present. Invariably, the lipid content is in the range of 0.5-3.0% by weight, which is relatively low compared to many finfish (see Table 2-2).

In most animals, deposited fats (usually triglycerides) are morphologically recognizable entities, although small amounts of triglycerides are also found in cell membranes. In bivalves obvious and discrete depot fat deposits do not occur, although fat globules have been reported in mantle vesicular cells of Crassostrea virginica (Galtsoff 1964). In Crassostrea gigas nearly 40% of the lipid of the mantle margin and gonad was present as triglyceride (Allen and Conley 1982). Otherwise, it is usually reported (Barber and Blake 1981) that lipid is stored, at least temporarily, in the digestive diverticula of molluscs, for example in the sub-Antarctic limpet Nacella (Patinigera macquariensis) (Simpson 1982). It is believed that in bivalves such as the Iceland scallop Chlamys islandica (Sundet and Vahl 1981), the Atlantic deep-sea scallop Placopecten magellanicus (Robinson et al. 1981), or the scallop Chlamys hericia (Vassallo 1973), as well as in C. gigas (Allen and Conley 1982), modest reserves of lipid are transferred to the gonad at maturation (c.f. Barber and Blake 1981). In the Japanese prickly scallop Chlamys nipponensis the lipid content (wet

TABLE 2-5

Lipid Content (w/w%) of Edible Parts of Shellfish
(Bivalves and Crustacea)

	Percent Lipid
Clam meat	1.6
Oyster meat	2.0
Scallop meat	0.2
Crab meat	1.9
Lobster meat	1.5
Shrimp, canned, drained	1.1

Source: Ackman 1976.

weight) of visceral organs was 4.6%, while that of the remaining soft parts was only 1.5% (Hayashi and Yamada 1973). It has been reported for a variety of other types of marine shellfish that visceral lipids are invariably at least twice the percentage found in the remaining parts of the animal body (Kochi 1975).

Most questions on the proportions of different lipid classes in the various organs of bivalves remain unanswered, but Allen and Conley (1982) showed that in contrast to the approximately 50:10:40 proportions of phospholipid: sterol: triglyceride in C. gigas mantle, the gill and adductor muscle both had proportions of approximately 80:12:8. The digestive diverticula differed from both with proportions of 78:7:15. Langdon and Waldock (1981) found that starved, hatchery-reared and dextrin-fed C. gigas spat had high proportions of phospholipid relative to triglyceride. Ratios of up to 8:1 were observed, and it is possible that the low levels of triglyceride indicated a poor nutritional state (Swift et al. 1980). With most of the other diets triglyceride and phospholipid approached the proportions of 1:1 which was also reported in wild but well-fed O. edulis and C. virginica adults (Watanabe and Ackman 1974), in wild Arctica islandica (Ackman et al. 1974) and Mesodesma mactroides (De Moreno et al. 1976), and also in several of the species studied in Gardner and Riley (1972).

In the edible parts of crab, shrimp and lobster the lipid is also of the same order of magnitude. For example in the meat of the Queen (snow) crab Chionoecetes opilio the recoverable lipid is 0.75%, but of this neutral lipid was only 0.19%, and only 2/3 of that was triglyceride (Addison, Ackman et al. 1972). Other figures for lipids are 1.9% for crab meat, 1.5% for lobster meat, and 1.1% for shrimp meat

(Table 2-5). However the tips of lobster claws may be a little higher in lipid, perhaps 2%. The organization of tissue in these shellfish is such that lipid is concentrated in the hepatopancreas, which is usually about 1/2 fat. However, this organ is not now recommended as edible because of the heavy metal content.

The distribution of assimilated hydrocarbons will thus be quite different in the two types of shellfish. In the molluscs a substance such as naphthalene in a short "pulse" can be assimilated but is widely distributed throughout the animals. Possibly phagocytosis is involved. Both gills and digestive gland (hepatopancreas) may have initial high levels and are included in the edible part (Widdows et al. 1983). In the lobster, crab, etc., either longer-term exposure or a pulse exposure can lead to accumulation in the hepatopancreas. This interferes with biochemical functions such as cholesterol conversions (O'Hara et al. 1985). From the potential tainting standpoint, the storage of hydrocarbons in the lipid of the inedible hepatopancreas is of importance as it may maintain long-term low level contamination in the edible muscle. In this respect, the crustacea resemble fish, for example cod, where the main fat storage organs are not commonly eaten.

3.0 FISHERY CLOSURE CASE HISTORIES AND REGULATORY PROCEDURES

3.1 INTRODUCTION

Episodes of tainting of fishery resources as a result of exposure to petroleum hydrocarbons have been reported in the literature as early as the 1940's (Menzel 1947, 1948 from Stansby 1978); however, details on the extent and verification of an incident and fishing restrictions imposed due to tainting are scant (GESAMP 1982). Reports of incidents of tainting are often based on opportunistic investigations and consequently are very general in nature.

There are few data presented in the literature about the hydrocarbon contaminant levels responsible for reported cases of tainting. Tainting has often only become evident some time after the fact. Similarly, assessment methods used to determine a taint are rarely presented. It is, therefore, difficult to establish, on the basis of international experience, rules or practices that may be appropriate for the Canadian situation.

The presence of a chronic discharge or acute spillage of hydrocarbons can interfere with a fishery in two ways. The resource itself may become tainted as a result of direct exposure, or there may be a high risk of fixed or mobile fishing gear becoming fouled which could result in uncontaminated fish acquiring a taint. Both situations can lead to serious losses of income to fishermen.

When examining reported cases of tainting associated with hydrocarbons, it is important to distinguish between tainting occurring as a result of hydrocarbon uptake from

contaminated water and sediments and organisms fouled by contact with fishing gear oiled as a result of interaction with a surface slick or subsurface globules of oil. On occasion, fish caught in the vicinity of a spill have been rejected by buyers or inspecting officers on the grounds of suspected tainting, although the organism may only be fouled (Anon. 1967; Grainger et al. 1980). For example, inspecting officers examining lobster for Kurdistan oil rejected and confiscated lobsters that had any visible traces of oil on the carapace or among the walking legs whether or not there were measureable traces of oil within the lobster tissue (Scarratt 1980a).

There have been instances when an area has been closed to fishing for extended periods of time, particularly where sediments have become contaminated and shellfish are continually exposed to elevated hydrocarbon levels through filter feeding (Mayo et al. 1974). In most oil spill situations, however, a form of self-imposed closure takes place as fishermen generally tend to avoid areas where their gear and catch would become fouled.

The area around the site of an oil spill may also be closed to fishing or marine traffic for safety reasons or to facilitate countermeasures. Such a zone was imposed around the Uniacke wellsite on the Scotian Shelf near Sable Island during the blowout in 1984. Thus, a possible tainting situation would go unnoticed through an indirect closure.

More recently, warnings of a potential tainting situation have been issued to fishermen when the situation warrants. In January 1985 a fuel hose ruptured spilling 65 metric tons of diesel while refueling a jackup rig on the Scotian Shelf. A notice was issued to fishermen advising them about

the spill and warning about the possibility of fish becoming tainted, but no reports of contamination were received (OSIR 1985).

For various reasons, it appears that it has rarely been necessary to close a fishery because a stock has knowingly become tainted.

In the following subsections, reports of tainting, suspected tainting and fishing disruptions due to oil pollution incidents have been tabulated and discussed in relation to the methods employed to assess the situation and measures taken to protect the affected fishery. This information was obtained through a literature search and through extensive correspondence with research scientists and government agencies in Canada and overseas. These same sources provided general information on the regulatory procedures used in various jurisdictions for dealing with a potential tainting situation.

3.2 DOCUMENTATION OF TAINTING

Publications and communications reporting examples of tainting, or potential tainting situations, have been divided into three categories depending on the source of the hydrocarbon contaminant:

- industrial discharges;
- marine shipping incidents; and
- offshore oil and gas development activities.

These reports were then screened for taste observations, assessment methods, chemical verifications of observations, and indication of any fishing disruptions or restrictions

imposed as a result of tainting identified and associated with an incident.

From the point of view of this study, tainting associated with marine transportation, and offshore oil and gas activities, was of primary interest.

3.2.1 Industrial

Table 3-1 lists publications reporting tainting of fish or shellfish resulting from hydrocarbons contained in effluent discharges from refineries and industrial operations.

A kerosene-like taint in sea mullet (Mugil cephalus) and, to a lesser extent, in sea bream (Mylio australis Gunther), has been reported on numerous occasions in Australia (Sidhu et al. 1970; Vale et al. 1970; Connell 1974; Connell et al. 1975). This has resulted in a restriction of fishing effort and condemnation of quantities of mullet (Connell 1974). Sidhu et al. (1970), determined through taste panel observations that cooking the fish made the taint more obvious and that dark meat and fatty layers had a stronger taint than white meat. They reported that raw, whole fillets from contaminated and uncontaminated fish were indistinguishable by appearance or smell, but the taint became apparent when the fillets were minced. Hydrocarbons from tainted mullet oil, hydrocarbons from muds from an estuary below a refinery, and commercial kerosene were examined by gas chromatography-mass spectrometry and infra-red spectrometry and proton magnetic resonance spectrometry and a close similarity was observed. From this evidence, it was assumed that refinery effluents were the cause of the observed tainting.

TABLE 3-1

Publications Reporting Examples of Tainting and Potential Tainting Situations in Fish and Shellfish from Industrial Sources of Hydrocarbons

Hydrocarbon	Tainting	Taste Panel	Chemical Verification	Species	Fishing Disruptions	Reference
Hydrocarbon-bearing effluent	Kerosene-like			sea mullet	Restriction of fishing effort, condemnation of catches	Connell 1974
Kerosene-like mixture (n-alkanes)	Kerosene-like	"Subject-ively tested"	GC; GC/MS	bream	Not an economic problem	Connell et al. 1975
Kerosene	Kerosene-like	Yes	GLC; GC/MS; infra-red, proton magnetic resonance spectrometry	mullet		Sidhu et al. 1970
Industrial waste	Yes	Yes	GC	mullet	Withdrawn from sale	Vale et al. 1970
Toluene; aromatic and aliphatic hydrocarbons	Offensive odour	No	GC; MS; thin layer chroma-tography; i.r., u.v. absorption	eels, grey mullet		Ogata and Miyake 1973
Petroleum refinery and industrial waste	Offensive odour Bitter taste		No	grey mullet, eel, flatfish, squilla clams	Unmarketable	Nitta 1972
Oil refinery wastewater	Oily taste	Yes	No	rainbow trout		Krishnaswami and Kupchanko 1969
Wastewater discharge	Yes	Yes	No	fish	Fishing (sport) curtailed because of poor eating quality	Thomas 1973
Oil, hydrocarbides	Taste of oil	No	No	mussels, oysters, fish	Controls imposed on fishing	Panella 1968
Industrial wastewater	Yes; in scallop held <500 m from discharge	Yes	No	scallops		Dr. J. Davis (pers. comm.)

Similar incidents of tainting of mullet and other fish have been reported in Japan (Nitta 1972; Ogata and Miyake 1973; Stansby 1978). Fish caught in the vicinity of industrial complexes had offensive odours and had become unmarketable. Ogata and Miyake (1973) confirmed that toluene imparts an offensive odour to fish and that other aromatic hydrocarbons (benzene and o-, m- and p-xylene) and some aliphatic hydrocarbons were also substances responsible for the tainting.

Krishnaswami and Kupchanko (1969) reported numerous taste tests which confirmed that the tainting of rainbow trout (Salmo gairdneri) in the Bow River, Alberta, was a result of petroleum refinery wastewater discharged into the river. Similarly, Thomas (1973) reported that a taste panel could differentiate between fish held upstream or downstream from a source discharging wastewater into the Ohio River.

The Department of Agriculture and Fisheries in Scotland continually monitors shellfish contamination around a refinery wastewater outfall through taste panel observations. Scallops held in cages within 500 m of the outfall have become tainted, but those caught outside this zone remain unaffected (Dr. J. Davis pers. comm.). A 500 m fishing exclusion zone around the outfall is enforced to avoid damage to fishing gear and the discharge pipe. Secondarily, this zone prevents the harvest of contaminated shellfish.

3.2.2 Shipping Accidents

Reported incidents of tainting as a result of tanker spills are more numerous but frequently further testing and analysis have not been conducted and the case remains an isolated report by a fisherman or buyer. For the reasons cited in

the introduction, tainting of exposed finfish and shellfish may have gone unnoticed in other situations.

3.2.2.1 Finfish

There have been numerous reports of finfish tainting, or potential tainting, as a result of shipping accidents (Table 3-2). Accidents involving the Torrey Canyon off the Scilly Islands (Simpson 1968 from Clarke 1973); the Dona Marika in Milford Haven (Blackman et al. 1973; Golob 1975); the Amoco Cadiz on the coast of Brittany (Chassé 1978); the Metula in the Strait of Magellan (Golob 1975); the Juliana outside Niigata Harbour (Motohiro and Inoue 1973); the British Mallard (Palmork 1974 from Connell and Miller 1981); and the Eleni V off the Norfolk coast (Blackman and Law 1980) have all been reported to have resulted in finfish tainting. Some of these incidents have been more carefully reviewed than others.

Following the Torrey Canyon accident, Simpson (1968) confirmed tainting of nearshore sea-trout and mackerel populations on the basis of taste tests. In this case, GESAMP (1977) indicates that the reported tainting resulted from dispersant usage rather than the weathered crude oil itself.

Although fishermen reported tainting of mullet, chum salmon (Oncorhynchus keta), and black sea bream (Mylio macrocephalus) as a result of the Juliana wreck, this was not confirmed by comparative taste tests conducted by Motohiro and Inoue (1973). Hydrocarbon fractions isolated from fish caught in the area did, however, have a strong crude oil odour and gas chromatographic analysis of tissue samples revealed peaks similar to n-paraffins.

TABLE 3-2

Publications Reporting Examples of Tainting and Potential Tainting Situations in Fish and Shellfish from Shipping Accidents

Hydrocarbon	Incidence	Tainting	Taste Panel	Chemical Verification	Species	Fishing Disruptions	Reference
Kuwaiti crude (122,000 t) and dispersants	Torrey Canyon	Yes; for about 3 weeks			lobster	Sales resistance; fishing resumed after confirmation of no contamination	Anon. 1967 Portman 1970
	Torrey Canyon	Yes			mackerel, sea trout, lobster, crab, plaice		Simpson 1968 from Clarke 1973
Petrol (3,000 t)	Dona Marika	Public report of tasting of petrol			mackerel		Blackman et al. 1973
Petrol	Dona Marika	Yes			finfish		Golob 1975
#2 diesel fuel	Florida	Oily taste	Yes, "taste tests"	GLC - established identity of hydrocarbon in shellfish with #2 fuel oil	oysters, scallops	Closed initially due to contamination and later reclosed due to oily taste	Blumer et al. 1970
#2 fuel oil	Florida	Yes	Yes; for depuration studies	GC	oysters, clams quahogs, mussels, scallops	Closure due to contamination for 2 years	Blumer et al. 1971
Crude (223,000 t)	Amoco Cadiz	Yes; taste and smell of oil			crabs, mullet, mackerel, lobster, oyster	Oyster industry shut down	Chassé 1978 Cross et al. 1978 Laubier 1978
	Amoco Cadiz	Taste of oil			lobster, crabs	Inferred; fishing resumed May, 1978	Lezlise and Raguenes 1981
Arabian light crude	Metula	Reported by fishermen			mussel, finfish	Self imposed due to taste problem. Nat. Health Service determined fish not fit for human consumption (for 5 months)	Golob 1975
Gasoline	Wemeldinge	Inedible because of oily taste	Yes (detection limit 5 ppm)	column chromatography	mussels	Normal harvesting curtailed 6 weeks	Kerkhoff 1974

TABLE 3-2 (Continued)

Hydrocarbon	Incidence	Tainting	Taste Panel	Chemical Verification	Species	Fishing Disruptions	Reference
Arabian light crude	Betelgeuse	Yes	Yes	Yes	scallop	Catches rejected by buyers, fishing disrupted by pollution and clean-up process	Grainger et al. 1980
Crude oil (6,000 t)	Juliana	Fishermen report that fish were inedible due to crude oil taint	Yes, and found no abnormal appearance, flavour or smell	GC - little hydrocarbon found in salmon, C13-C20 found in mullet and bream	salmon, mullet, Black Sea bream		Motohiro and Inoue 1973
Iranian crude	Northern Gulf	Unpalatable oily taste		GC	clams	Clam-growing area could not be harvested for 2 years	Mayo et al. 1974
Iranian crude oil	Northern Gulf	Oily taste		GC - 200 ppm in clams 10 years later	clams, lobsters	Could not be harvested for 2 years due to an oily taste	Shenton 1973
#6 oil	Barge (STC-101)	As reported by oyster ground leaseholders			oysters	Little commercial crabbing in 1976	Roland et al. 1977
Bunker C	Maine Maritime Academy Ship	Oily taste			clams	Clamming prohibited for 6 weeks due to an oily taste, preventing marketing	Shenton 1973
Bunker C (1,500,000 gallons)	Arrow	No	Yes; to determine if the meat may become tainted		lobster		Wilder 1970
Diesel oil (2,200 t)	British Mallard	Oily and kerosene flavour reported by fishermen over 2 month period	Yes; confirmed taint	CG/MS confirmed taint	herring, flounder, sea trout, salmon, haddock, saithe		Palmork 1974 from Connell and Miller 1981
Bunker C	Kurdistan	Some oily off-flavour	yes	Spectro-fluorescence	lobster	Confiscation of lobsters visibly contaminated with oil	Scarratt 1980b; Vandermeulen and Scarratt 1979

TABLE 3-2 (Continued)

Hydrocarbon	Incidence	Tainting	Taste Panel	Chemical Verification	Species	Fishing Disruptions	Reference
Heavy fuel (5,000 t)	Eleni V	No	Yes			fouling of fishing gear	Blackman and Law 1980
Kuwaiti crude	Universal Leader				periwinkles, scallops, mussels, sea urchins	Moratorium placed on fishing in <20 ft. water (2 months)	Golob 1975
Bunker C	Afran Zodiac				scallops, clams, sea urchins	Marketing and fishing in <20 ft. water prohibited (10 months)	Golob 1975
Diesel oil (2,000 l)	Spill into a river (N. Ireland)	Strong diesel fuel taint	Yes	GC; UV and fluorescence spectroscopy	trout		Mackie et al. 1972
#6 fuel oil (7.7 million gallons)	Argo Merchant	900 interviews with fishermen resulted in no reference to tainting	No	Yes	fish, shellfish	One scalloper reported one fouled tow was discarded as unmarketable	Sherman and Busch 1978
JP-5 jet fuel and #2 fuel (10,000 gallons)	Long Cove			GC	clams	Unmarketable due to prolonged oil contamination. Loss of $4 million a year (1973 values)	Shenton 1973
Iranian crude (2,000 t)	Klakken	Fish not tainted as determined by organoleptic tests	Yes	Yes	saithe	Contaminated 2 seine nets	Grahl-Nielson et al. 1976
5,000-6,000 t	Antonio Gramse	No oil taste or smell observed	Yes		fish		Pfister 1980
Oil	Transhuron					Temporary stoppage of fishing by fishermen on seeking the oil slick	Gopakumar 1985 (pers. comm.)
Oil (3,000 t)	Bahrain coast	Isolated cases of tainting were reported Some resistance among public to the consumption of fish				Oil prevented fishing efforts in the area	Linden 1984
Diesel 2,200 t and dispersant	Tanker ground 1(Norway)	Yes	Yes	GC/MS	cod, saithe, haddock, herring, flounder, sea trout, salmon		Palmork and Wilhelmsen 1974 from GESAMP 1977

Flavour degradation in finfish was also reported after the Metula grounding in the Strait of Magellan. Light Arabian crude oil was spilled and the fish caught in the vicinity were subsequently determined to be unfit for human consumption (Golob 1975). It was not reported how such a decision was reached. The same author also reports that there were public complaints of finfish tasting of petrol after the Dona Marika spill, but these were not confirmed by authorities. In this incident, tainting from refinery effluents, as opposed to the tanker spill, could possibly have caused confusion.

After the Eleni V accident, fishing gear was fouled, and as a result, tests were conducted to determine if demersal fish had been tainted. The taste tests conducted at the Torrey Research Station did not find any tainting by oil in samples of cod, sole, plaice, and black dab (Blackman and Law 1980).

3.2.2.2 Shellfish

Reported tainting of shellfish as a result of hydrocarbon spills from shipping incidents is more frequent, especially among filter-feeding sessile species. The situations tend to be more thoroughly assessed in the literature than finfish tainting incidents. This is due to the fact that shellfish tend to be concentrated in coastal areas where hydrocarbons do not disperse as readily as in open water or become more readily trapped in sediments. Also the fisheries for these organisms are often intensive and of relatively higher value.

The Torrey Canyon and the Amoco Cadiz spills remain the most spectacular in terms of size and public awareness. Increased public attention to large marine oil spills may

cause shellfish marketing problems even when there is not a demonstrated tainting. For example, it has been reported that after the Torrey Canyon spill sales and prices fell dramatically in Paris, even though much of the shellfish was from other waters (Korringa 1968 from Nelson-Smith 1973). The only actual reports of shellfish tainting were from lobsters held in pots near beaches which were treated with detergents and from a few caught within about a mile of this area (Anon. 1967).

Crustaceans (lobsters (Homarus sp.), spiny lobsters, edible crabs (Cancer pagurus), and spider crabs (Maia squinado)) were mainly free of taint in offshore waters after the Amoco Cadiz incident. However, the inshore oyster culture industry (Crassostrea gigas) was badly damaged and shut down due to contamination (Chassé 1978; Cross et al. 1978). More than 9,000 tonnes of oysters were contaminated at a level of 150-400 ppm total hydrocarbon (wet weight) (Laubier 1978).

Contamination and taint in shellfish (oysters, soft-shell clams, quahogs, and scallops) after the grounding of the barge Florida in Buzzards Bay and the tanker Northern Gulf in Maine led to lengthy closures of those industries (Blumer et al. 1970, 1971; Shenton 1973; Mayo et al. 1974). A probable loss of 209 metric tons of edible clam meats, representing a two year market, can be attributed to the wreck of the Northern Gulf (Mayo et al. 1974).

In Canada, fear of tainting of lobsters after the Arrow spill in Chedabucto Bay and the Kurdistan spill in the Cabot Strait prompted experiments simulating these incidents. In the case of the Arrow, Wilder (1970) concluded that the meat and tomalley of lobsters in Chedabucto Bay would not become tainted by oil. Some of the lobsters taken in areas con-

taminated by Kurdistan oil and subjected to taste evaluations by trained taste panelists were found to have slight oily off-flavours, but they were not considered serious enough to intervene in the fisheries (Scarratt 1980a). After the incident, spectrofluorometric analysis demonstrated that high concentrations of hydrocarbons may occur in lobsters captured in areas known to have been contaminated by oil (Scarratt 1980b). It was fortuitous that both accidents occurred outside the normal lobster fishing season in the areas most affected.

In the Netherlands, mussels became inedible because of their oily taste after the Wemeldinge spilled 80 tons of gasoline and harvesting was curtailed for 6 weeks (Kerkhoff 1974). Following the Betelgeuse disaster in which oil leaked into Bantry Bay, Ireland, for over a year, shellfish were taste-tested to determine whether a taint was present. Some panelists found slight tainting while others found the scallops to be of poor eating quality (Grainger et al. 1980). As a result of the incident, the shellfish industry (periwinkles, scallops and clams) was seriously affected and some catches were rejected by buyers.

3.2.3 Offshore Oil and Gas Development

Incidents involving tainting or potential tainting of a fishery as a result of offshore oil and gas development activities are presented in Table 3-3. The following subsections discuss this evidence in terms of chronic discharges, pipeline failure and well blowouts.

TABLE 3-3

Publications Reporting Examples of Tainting and Potential Tainting Situations in Fish and Shellfish from Offshore Oil and Gas Development Sources of Hydrocarbons

Type of Incident	Hydrocarbon	Tainting	Taste Panel	Chemical Verification	Species	Fishing Disruptions	Reference
Louisiana offshore operations	Drilling mud containing diesel	Yes			oysters		St. Amant 1973
North Sea operations	Production water drilling muds	No	Yes	No	finfish		Dr. J. Davis (pers. comm.)
Onshore pipeline fracture; Turut Bay	Arabian light crude	Tainting within fouled traps in the bay. No tainting of fish in the open bay.			fish, shellfish	Traps unusable for 6 weeks	Golob 1975 Spooner 1970
Louisiana - storage tank pipeline rupture	Crude (4,000 - 5,000 gallons)				oysters	Long-term damage to oyster beds	Anon. 1982
Funiwa 5 blowout		Yes			crabs, periwinkles		OSIR 1980a&b
Ekofisk Bravo blowout	Crude (14,000 t)	Taint close to the threshold level of a trained taste panel	Yes	Yes	haddock, plaice, lemon sole, gurnard		Whittle et al. 1978
Ekofisk Bravo blowout	Crude (14,000 t)	No	Yes	Yes	cod, haddock, plaice	Yes	Mackie et al. 1978
Ekofisk Bravo blowout						No closures	O. Ostvedt (pers. comm.)
Ixtoc I blowout	Oil and gas 140 million gallons oil	No	No	No	shrimp	Fishing banned	Golob and McShea 1980
Uniacke blowout	Gas/Condensate (480 m^3)	No	Yes	No	cod, haddock, halibut		Martec Limited 1984
Nowruz blowout	Crude (4,000 - 7,000 barrels/ day)					Interference with important fisheries	Anon. 1983
Hasbah 6 blowout						Fouled fishing gear	OSIR 1980d&e
Santa Barbara		No tainting reported				Two harbours were temporarily closed and fishermen reluctant to foul gear consequently fishing effort was reduced	Nelson-Smith 1973

3.2.3.1 Chronic Discharges

Diesel oil used in oil-based drilling muds and oily water discharges constitute a significant input of oil into waters around offshore rigs operating in the North Sea (Read and Blackman 1980; Davis et al. 1981; Blackman and Whitehead 1982). Dr. J. Davis (pers. comm.), however, has indicated that taste tests conducted on fish caught in the vicinity of North Sea production platforms have revealed no evidence of tainting. In the Buccaneer oilfield in the Gulf of Mexico, approximately 200 g/day of alkanes are discharged into the sea from each of the two production platforms (Middleditch et al. 1979). In Louisiana coastal waters the addition of oil through brine discharge is thought to be about twice that caused by accidental spillage (Environmental Protection Service 1978). Complaints of an oily taint in oysters obtained from areas near drilling rigs in the State of Louisiana were reported as early as 1947 (Menzel 1947 from Stansby 1978).

Oil-based drilling muds containing light and middle distillates can cause a taint in shellfish located near a platform. Oily taste generally occurs in oysters when the substrate exceeds 500 ppm of hydrocarbon (St. Amant 1973). In the North Sea, significant offshore contamination is found only in the sediments close to platforms where oil-based drilling muds have been used (Davis et al. 1981), but enhanced concentrations can occur up to several miles around some platforms (McIntosh et al. 1983). Preliminary results from experiments conducted by Hardy et al. (unpublished data from Whittle and Mackie 1976) show that within 1 or 2 days of exposure of plaice to bottom sediments contaminated with slightly weathered North Sea crude oil, the fish were tainted and taste differences could be recognized by a

trained taste panel. The tainted fish contained hydrocarbon levels corresponding to 200-250 ppm crude oil in the flesh.

3.2.3.2 Pipeline Failures

There are few incidents of pipeline failures where tainting has occurred and been addressed. In India, an onshore pipeline fracture in Tarut Bay was blamed for tainting shrimp within fouled traps (Golob 1975) although there was no tainting of fish caught in the open bay (Spooner 1970).

A pipeline rupture in a tank farm resulted in 4,000 to 5,000 gallons (15-19 m^3) of crude oil being spilled into water off Pelican Island, Louisiana. The Louisiana Wildlife and Fisheries Commission found contamination in oysters and, based on these findings, a $1 million lawsuit was filed against Exxon, U.S.A. for long-term damage to beds in an 800 acre area around the site (Anon. 1982).

3.2.3.3 Blowout

Tainting of fishery resources has occurred as a result of well blowouts, but only in shallow, confined waters. The Funiwa 5 blowout in the Niger River Delta in 1979 resulted in crabs and periwinkles becoming tainted and inedible (OSIR 1980c).

Taste tests were conducted on plaice, haddock, lemon sole and gunard from the Ekofisk Bravo wellsite area in the North Sea after that blowout. The tests demonstrated that the level of any taint present was close to the threshold of a trained panel and it was concluded that it would be of little concern to the average consumer and would probably

have gone unnoticed (Whittle et al. 1978). Dr. J. Opstvedt (pers. comm.) of the Institute of Marine Research, Norway, confirmed that there were no closures of commercial fishing activities as a result of the Ekofisk Bravo blowout.

Similarly, taste tests were conducted on cod, halibut and haddock caught in the immediate vicinity of the Uniacke wellsite on the Scotian Shelf after that blowout. Tainting was not reported in samples tested for both Shell Canada Resources (Martec Limited 1984) or the Department of Fisheries and Oceans (Zitko et al. 1984).

There were no reports of tainting as a result of the Ixtoc I blowout in the Gulf of Mexico; however, fishing was banned in oiled areas (Golob and McShea 1980). Up to 3,000 U.S. commercial fishermen, primarily shrimpers and crabbers, have claimed at least $155 million for future decreases in catch size as a result of the blowout.

3.2.4 Summary

As indicated above, specific information about tainting incidents and the requirement for fishing closures is sparse. Tainting has been reported in a number of situations but it is difficult or impossible to establish what actions were taken, and by whom, when an apparent tainting incident has arisen. The sensory and analytical methods used are rarely reported.

It is evident from the foregoing discussion that the risk of tainting is higher in coastal waters than in offshore areas. Where blowouts at offshore wellsites have occurred and oil or condensate released during the accident has not reached the coast, fish tainting as a result of hydrocarbon uptake

from the dissolved or particulate material in the water column has not been apparent. The hydrocarbons are rapidly dispersed in the environment and concentrations rapidly decrease to levels below which tainting will occur. In coastal areas, the hydrocarbons do not disperse as quickly and can become trapped in sediments. These factors substantially increase the risk of finfish and shellfish resources acquiring a taint as a result of a spill incident.

Of particular interest from the oil spill countermeasures standpoint is the finding that dispersant usage after a spill has been responsible for fish resources in coastal areas becoming tainted. This finding warrants consideration when a potential application for dispersant usage is being considered during a spill event.

3.3 REVIEW OF STANDING PROCEDURES TO MONITOR INSTANCES OF TAINTING

3.3.1 Introduction

To determine how instances of real or potential tainting are handled in other jurisdictions, letters were sent to established contacts in 19 countries requesting information on fishery closures due to tainting from petroleum-associated activities and requesting the names and addresses of official bodies responsible for managing a tainting situation. A copy of this letter is included as Appendix B. Responses to these requests generated additional contacts. In all over 80 individual contacts were made. A complete list of names and addresses of those contacted is included as Appendix C.

In general, it appears that most countries contacted do not have firmly established protocols for dealing with a potential tainting incident by imposing closures on affected fisheries. On the other hand, health authorities usually ensure that contaminated fish is not used as human food which, secondarily, limits fishing in polluted areas. This leaves the actual decision of when and where to fish largely to the individual fishermen.

The United Kingdom stands out as the only country in the survey which is addressing the issue of tainting through use of imposed fishery closures. At present, there is a bill before the House of Lords on this matter.

The current procedures to monitor and regulate instances of tainting in effect in the countries surveyed are detailed in the following subsections.

3.3.2 Canada

In Canada, the Fish Inspection Division of the Department of Fisheries and Oceans (DFO) is responsible for inspecting fish catches on board fishing vessels, at the dock-side and potentially in the factory or market-place. Fish are only inspected for taint per se when an actual complaint from health authorities is received. A catch may, however, be condemned at dock-side on the basis of odour or fouling by petroleum products (F. Allen, Fisheries Inspection, DFO pers. comm.). In the event that fish from a particular area are suspected of being contaminated or tainted, fishermen are notified through the Regional Director General's Office of DFO. They are advised of the potential problem of tainted fish from a specific area and warned that their catches may not pass fisheries inspection.

The Fisheries Management Division of DFO has sole responsibility for fisheries closures. They may, however, only close or regulate the fishery in a given area on the basis of conservation of the stocks. Unilaterally, DFO is not able to close a fishery on the basis of a real or potential contamination problem (G. Stevens, pers. comm.), but in consultation with Public Health Officials, closures are imposed where incidents such as paralytic shellfish poisoning occur.

3.3.3 United Kingdom

In the United Kingdom evaluation of pollution of commercial fishing areas is the responsibility of the Ministry of Agriculture, Fisheries and Food (MAFF). At present, however, there is no legislation actually in place for closing fisheries or fishing areas because of pollution by petroleum or other products. As is the case in Canada, various MAFF laboratories can advise fishermen to avoid certain areas or certain species. The fishermen usually heed these warnings as they will otherwise waste their efforts and land an unsaleable product. Unwholesome fish is the concern of local health authorities and fishermen may be restricted from selling fish under the Food and Drug Act (Dr. R. Hardy, and Mr. P.C. Wood, pers. comm.).

As mentioned above, new legislation is currently before the House of Lords in the form of the Food and Environment Protection Bill which will grant powers to Ministers to stop the taking of foodstuffs, including fish and shellfish, contaminated as a result of spillage of toxic material. This could mean a ban on fishing or the taking of tainted fish or shellfish (P.C. Wood, pers. comm.). Pertinent portions of this Bill are presented as Appendix D.

3.3.4 Scandinavia and Finland

The situation in Finland is similar to that in Canada. According to the law on foodstuffs the health authorities ensure that contaminated fish is not used for human or animal consumption. There is no statute, however, that would directly prohibit fishing in the case of an oil pollution incident (A. Voipio, pers. comm.). The superior authority in the case of an oil pollution incident is the Ministry of the Environment which delegates tasks to the appropriate authorities.

The Directorate of Fisheries in Norway forwarded copies of legislation and regulations for continental shelf activities. Issues pertaining to fishery-related conflicts or interactions are not addressed in these regulations.

Similarly, Sweden has no specific legislation concerning fisheries and fishery closure associated with oil pollution (T. Gustavsson, pers. comm.).

In Denmark the agency responsible for taking action in a tainting incident is the National Agency of Environmental Protection. W. Schmidstdorff of the Ministry of Fisheries forwarded copies of regulations concerning dumping of materials from offshore installations; however, the issue of a potential tainting problem and policing this problem are not specifically addressed in the regulations (see Appendix E).

3.3.5 Germany

In the Federal Republic of Germany, legal matters related to coastal fisheries are handled by the individual states (K.

Tiews pers. comm.). For example, in the state of Schleswig-Holstein legal closures would be ordered and enforced by government health and/or water authorities.

3.3.6 Other European Countries

Some responses were received from letters sent to Belgium and France; however, no defined mechanisms for dealing with tainting problems could be determined. In the Amoco Cadiz incident, local public health officials were responsible for monitoring the quality of shellfish harvested (IFREMER 1984). No responses have been received on this subject from the Netherlands, Poland or Italy.

3.3.7 South Africa

The Department of Marine Development is responsible for the closure of fisheries in the event of contamination in South African waters. This responsibility is laid out in rather loose terms in Section 10 of the Sea Fisheries Act No. 58 of 1973 (see Appendix F).

Provisions are made in local oil spill contingency plans for the closure of areas in the event of an oil spill (Molden pers. comm.). This is based on an analyses of types and quantities of the hydrocarbons released in an incident.

3.3.8 India

In India there is no legislation in place for temporary stoppage of fishing by any agency due to contamination by hydrocarbons (Gopakumar pers. comm.).

3.3.9 Argentina

In Argentina the Maritime Resources Secretariat is the official body responsible for the closure of a fishery (Dr. Boschi pers. comm.). It could not be determined, however, whether this body has the authority to effect closures due to potential or real contamination that may be responsible for tainting. The National Health Service becomes involved where the suitability of product for human consumption comes into question.

3.3.10 New Zealand

Various jurisdictions and bodies from the Ministry of Agriculture and Fisheries (MAF) to the Health Department and the local Water Boards (Branson pers. comm.) are responsible for overseeing pollution matters. The Fishery Management Division of MAF, however, is the responsible authority for actually closing a fishery in the event a potential spill poses a risk of tainting.

3.3.11 Australia

In Australia, there does not appear to be any government legislation covering tainting of fisheries products and responsibilities for detecting such contamination are spread over many federal and state agencies. At the federal level, the responsible body is the Division of Fisheries of the Department of Primary Industry (J. Volkman pers. comm.). State health commissions also become involved if tainted fish are bought by consumers.

3.3.12 United States

Inquiries made to various organizations in the United States regarding regulatory mechanisms for dealing with potential tainting situations have proven unfruitful. It is generally understood that responsibility would be assumed by state agencies and local health authorities. Standardized procedures have not been adopted.

3.3.13 Other

Some of the correspondents in Japan and the U.S.S.R. have answered the letters of inquiry; however, no insight has been gained into how their countries would handle a tainting incident. The answers usually made reference to other organizations or offices who might provide the information.

3.3.14 Summary

From the foregoing review, it is evident that regulatory responsibility is often fragmented. Public health authorities generally are responsible for insuring that contaminated products are kept off the market. It appears that there are few mechanisms available to responsible agencies for actually closing an area to fishing either permanently or until contaminant levels are reduced to the point where tainting is no longer a concern. The exception is the United Kingdom.

Where major spills of hydrocarbons from tanker incidents or offshore well blowouts have occurred, large exclusion zones established to ensure public safety and to facilitate clean-up or well-capping activities have, as a side-effect, led to a temporary fishing closure. These closures are not

enforced after the emergency has passed and there are no established procedures for determining the quality of finfish or shellfish resources exposed to petrogenic hydrocarbon contaminants before the area is reopened.

4.0 ASSESSMENT METHODS

4.1 INTRODUCTION

Tainting is the development of a flavour or odour atypical of that normally associated with the product. A taint can only be evaluated by sensory methods. Sensory testing, however, is subjective and is difficult to quantify. Chemical analysis of the product will yield quantitative results, but will only identify the types of hydrocarbons present and will not always identify the taint-producing agent. Chemical analysis will not, by itself, determine whether a product is tainted or not.

To date there has only been limited research to establish a relationship between sensory testing and chemical analysis. In the case of hydrocarbon contamination, case histories provide little guidance in this area because the methods used are often only very generally described in available reports. However, methods to assess product quality have been developed in the food sciences and these are suitable for the evaluation of tainted finfish or shellfish.

In the following subsections general sampling procedures, and major sensory and analytical methods that could be employed to evaluate a tainting situation are reviewed.

4.2 SAMPLING

Field sampling and tissue preservation techniques are of prime importance in conducting either sensory evaluations or chemical analysis to identify a tainting incident. The sampling procedure adopted in a field situation will depend

on the individual situation, i.e. equipment available, type of spill or chronic exposure, season and species present.

At least ten fish of each commercially important species present should be obtained near the source of the hydrocarbons during and, if possible, following the event. Control samples should also be collected from an area determined to be free of the hydrocarbon contaminant. Sampling should be reasonably close in time and space to avoid potential seasonal and geographic flavour differences within the same species. Biological information such as age, sex, size, physiological status, parent stock, locality and season should be documented for each specimen sampled (Zitko 1984). Additionally, a visual inspection and raw odour evaluation for surface contamination of the fish are desirable as soon as the catch is brought on board. In the case of hydrocarbon contamination, raw odour is an important first step in the evaluation of taint given the acuteness of the olfactory sense to the presence of atypical hydrocarbons.

In handling the catch, consideration must be given to possible cross-contamination. Individual specimens should be kept separate and remote from potential hydrocarbon contaminants on the vessel. The samples should be placed in sterile and chemically inert bags and placed on ice or, if possible, glazed with water and frozen at -35°C. The draft guidelines for evaluating threshold values for fish tainting prepared by the Working Group on the Evaluation of Hazardous Substances Carried by Ships (GESAMP 1983) recommend that the fish be gutted immediately, be allowed to bleed, and be stowed in ice prepared from potable water at a ratio of at least one part ice to three parts fish by weight. Care must

be taken to ensure that knives used for preparing the catch are properly cleaned (Zitko 1984).

If this fish is being held on ice sensory evaluation should be carried out within 48 hours of harvesting to avoid any possibility of tainting from natural degradation of the specimens to be evaluated.

4.3 SENSORY EVALUATION METHODS

Sensory evaluation methods involve using the senses of taste and odour to evaluate the quality of a product. The basic methods include tests for differences or preference using trained or untrained people assembled in a taste panel. Specific methods include the triangle test, multiple comparison, hedonic and category scaling. The method chosen depends on the number of samples, quantity of product available, the information desired, and the qualifications of the panel members.

Before describing the methods used, a brief review of threshold levels of compounds causing taint and sensory detection limits is presented.

4.3.1 Threshold Levels of Tainting

As indicated earlier, the threshold levels of some contaminants responsible for causing tainting are highly variable. Connell and Miller (1981) list the concentrations of various chemical compounds causing tainting (see Table 4-1). The data indicate that levels as low as 0.1 ppb can cause a taint but the range for most compounds is above 100 ppb. Phenolic compounds as a group are the most potent taint producers evident in Table 4-1.

TABLE 4-1

Threshold Concentrations of Chemical Compounds Causing Tainting

Chemical	Estimated Threshold Level in Water (µg/l)
Acetophenone	500
Acrylonitrile	18,000
N-Butylmercaptan	60
o-Chlorophenol	0.1-15
p-Chlorophenol	10-50
Cresol	70
m-Cresol	200
o-Cresol	400
p-Cresol	120
o-Dichlorobenzene	250
2,3-Dichlorophenol	84
2,4-Dichlorophenol	1-14
2,5-Dichlorophenol	23
2,6-Dichlorophenol	35
Dimethylamine	7,000
Diphenyl oxide	50
Ethylbenzene	250
Ethanethiol	240
Ethylacrylate	600
Formaldehyde	95,000
Guaiacol	82
Kerosene	100
Kerosene plus kaolin	1,000
2-Methyl-4-chlorophenol	75
2-Methyl-6-chlorophenol	3
Naphtha	100
Naphthalene	1,000
Naphthol	500
2-Naphthol	300
Oil, emulsifiable	15,000
Phenol	1,000-10,000
Phenols in polluted rivers	20-150
o-Phenylphenol	1,000
Pyridine	5,000-28,000
Pyrocatechol	800-5,000
Pyrogallol	20,000-30,000
Quinoline	500-1,000
p-Quinone	500
Styrene	250
Toluene	250
2,4,6-Trichlorophenol	3-50

Source: Connell and Miller 1981.

GESAMP (1977) and Stansby (1978) indicate that few studies to establish threshold levels of hydrocarbon components have been undertaken. The level of contaminants at which a taint becomes apparent varies with the species affected and the contaminant present, but it is generally between about 5 and 200 ppm in tissues (GESAMP 1977). Howgate (pers. comm.) has indicated that little work has been done on threshold levels of tainting in recent years, but that it may be the subject of a forthcoming project carried out by ECETOC.

Some of the more relevant literature on tainting as well as the assessment methods used to determine the taint are briefly described below.

A kerosene-like taint has been studied in the grey mullet (Mugil cephalus) (Vale et al. 1970; Connell 1974, 1978) and in the Australian bream (Mylio australis gunther) (Connell et al. 1975). The fish were indistinguishable by odour but the taint became evident when minced. This effecthas also been reported by Deshimaru (1972) in his study on the rearing of yellowtail (Seriola dorsalis) in water containing 10 and 50 ppm crude petroleum oil in the water and 1% in the diet. Fish exposed to 50 ppm acquired an oily flavour on the fifth day whereas those exposed to 10 ppm did not develop an oily flavour until the thirteenth day and then only slightly. Yellowtail reared on a diet of 1% crude oil obtained the objectionable flavour on the eighth day.

Ogata and Miyake (1975) tested eels exposed to petroleum for 1, 3 and 7 days. In all cases, taste panelists detected both an obnoxious odour and taste. Diesel oil contamination in brown trout (Salmo trutta L.) was studied by Mackie et al. (1972). Diesel odour and taste were generally noticeable in the contaminated trout as well as the hydrocarbon

fraction isolated from the fish.

Kuusi and Suihko (1983) obtained sensory data over a period of 12 years on fish in Finland. Panelists were usually able to distinguish the taste of oil even when present with another off-flavour. Kuusi and Suihko recommend the use of a trained panel for the evaluation of fish taint from the stand-point both of economics and of reliability.

Taste and odour associated with hydrocarbon contamination in shellfish have been studied by Motohiro and Iseya (1976a), Howgate et al. (1977) and Wilder (1970). Motohiro and Iseya added various hydrocarbons from 0-300 ppm to scallop muscle. A taint from a mixture of hydrocarbons or toluene was evident at 200 ppm, from xylene or crude oil at 100 ppm but from the bland n-tetradecane or n-hexadecane taint was difficult to discern at 300 ppm. Howgate et al. treated plaice, Norway lobster and brown shrimp and found objectionable flavours after only short periods of exposure, while Wilder tested Atlantic lobster meat and hepatopancreas and found that lobsters immersed in seawater containing 1000 ppm bunker C, or 1000 ppm bunker and 1000 ppm dispersants acquired a very objectionable oily flavour. Sensory studies by Murray (pers. comm.) indicate that fish with over 200-300 ppm hydrocarbons are generally rejected during sensory evaluation.

Stansby (1978) and Motohiro (1983) have written reviews of petroleum flavour pickup in fish and Johnston (1976) has studied the problem of marine pollution in relation to commercial fisheries. Johnston reports that seafood requires 10-50 ppm contaminant to produce an objectionable taste of oil. Much of the literature reports sensory evaluations of fish by simply stating that the fish did or did

not taste of oil and comments concerning methods or data are often not presented.

Tables 4-2 and 4-3 (Motohiro 1983) present taste threshold concentrations of various crude oils and aromatic hydrocarbons in scallop adductor muscle. These results confirm that aromatic hydrocarbons and unsaturated alkanes cause strong flavours in the flesh of fish whereas saturated hydrocarbons do not tend to produce a taint.

4.3.2 Organoleptic Evaluation Methods

Substantial research has been conducted on organoleptic (taste and odour) evaluation methods (Larmond 1970; Stone et al. 1974; Shaw et al. 1983). The methods used to determine the flavour quality of a fish product are described and evaluated below.

4.3.2.1 Triangle Test

The triangle test has been recommended by GESAMP (1983) for evaluating threshold values for fish tainting. This method can also be used for field samples to evaluate the presence or absence of a taint as defined in Section 2.2.

Each sample of test material is presented to the members of a taste panel as a triangle test where the material in question is compared with a reference (zero concentration). If the test material is referred to as A and the reference as B, then there are two combinations of presentation - AAB and BBA. The series of samples to be tested should be presented to each panelist in the two combinations in random order.

TABLE 4-2

Taste Threshold Concentrations of Crude Petroleum Oil and its Products in Scallop Adductor Muscle

Samples	Concentrations (mg/kg)							
	0	10	30	50	100	200	300	400
Crude oil	-	-	-	-	+	+	+	+
n-Tetradecane	-	-	-	-	-	-	-	+
n-Hexadecane	-	-	-	-	-	-	-	+
Toluene	-	-	-	-	-	+	+	+
Xylene	-	-	-	-	+	+	+	+

+: detectable
-: undetectable

Source: Motohiro 1983.

TABLE 4-3

Taste Threshold Concentrations of Some Petroleum and Aromatic Hydrocarbons

Samples	Concentrations (mg/l)				
	500	400	300	200	100
Triethylbenzene	+	+	+	x	
Ethylbenzene	+++	+	+	+	-
n-Propylbenzene	++	++	+	+	
1-Decene	++	+	+	-	
1-Octene	+	-			
n-Hexane	-				
n-Pentane	-				

-: Negative
x: Threshold
+: Slight
++: Moderate
+++: Strong

Source: Motohiro 1983.

The panelist is presented with a set of three identical receptacles containing at least 15 g of sample. The receptacles should be coded with a 3-digit random number. The panelist is told that two samples are identical and is asked to indicate the odd one. A choice should be made even if the selection is by guessing.

An example of a simple score sheet that would be used in evaluating a difference only is shown in Figure 4-1. Figure 4-2 shows an expanded triangle test score sheet in which the panelist is also asked to grade the degree of difference and acceptability of the sample.

4.3.2.2 Hedonic Scale

The second evaluation method, the hedonic scale, is useful in flavour analysis because it provides both information on acceptability levels and an indirect ranking of the samples. The samples, however, are rated on a basic personal preference for the taste whether that be a typical or natural flavour for the product, or an unnatural or atypical flavour. Although five or nine point versions of this scale exist, Shaw et al. (1983) found the five point version more acceptable for use with fish products. An example of a five point score sheet is shown in Figure 4-3.

4.3.2.3 Multiple Comparison Test

The third method involves multiple comparison tests. In these tests, a known reference or standard is labelled R and is presented to the panelist with several coded samples. The panelist is asked to score the coded samples in comparison with the reference sample and asked to compare and grade the difference (Larmond 1970).

FIGURE 4-1

Triangle Test -
Difference Analysis Questionnaire

Here are three samples for evaluation. Two of these samples are duplicates. Separate the odd sample for difference only.

Samples	Check Odd Sample
____	____
____	____
____	____

Source: Shaw et al. 1983

FIGURE 4-2

Triangle Test -
Analysis and Degree of Difference Questionnaire

Here are three samples for evaluation. Two of these samples are duplicates. Separate the odd sample for difference only.

Samples	Check Odd Sample
____	____
____	____
____	____

Indicate the degree of difference between the duplicate samples and the odd sample.

Slight ____ Much ____

Moderate ____ Extreme ____

Acceptability:

Odd sample more acceptable ____

Duplicate samples more acceptable ____

Comments:

Source: Shaw et al. 1983.

FIGURE 4-3

Hedonic Scale Questionnaire

Taste samples and place a check at the point best describing your feelings about the sample.

Sample #	Sample #
__ Like extremely	__ Like extremely
__ Like moderately	__ Like moderately
__ Neither like/dislike	__ Neither like/dislike
__ Dislike moderately	__ Dislike moderately
__ Dislike extremely	__ Dislike extremely

Please share your comments concerning your choice.

Source: Shaw et al. 1983.

An example of the score sheet for a multiple comparison test is shown in Figure 4-4.

4.3.2.4 Category Scaling

For category scaling methods of sensory evaluation, the panelists should be trained in the perception of the odour/ flavour in question. In this test the panelist is asked to score or rank the samples using descriptive terminology and a reference sample as a guide or representation of the most acceptable quality. The samples are evaluated for flavour, odour and general acceptability. An example of the form is shown in Figure 4-5.

4.3.2.5 Data Analysis

The generated data can be analyzed in several ways. Results from sensory evaluations of fish are frequently analyzed non-parametrically (Shaw et al. 1983). O'Mahony (1981) discusses the advantages and disadvantages of non-parametric analysis and Basker (1981) shows that detectable differences can be shown by non-parametric techniques.

The GESAMP EHS Working Group is preparing draft guidelines describing a statistical method for recording and evaluating concentration-response data from tainting (GESAMP 1985). This method, however, would be designed for analyzing data from taste tests involving fish exposed to a known concentration of a contaminant.

4.3.3 Basic Test Panel Requirements and Conditions

Several basic elements have been identified which can influence the quality of the results obtained from panels assem-

FIGURE 4-4

Multiple Comparison Questionnaire

You have samples of (type of fish). One is marked "R" for reference. Please indicate whether each sample has more of a tainted flavour or odour, or less than the reference. Then please indicate the degree to which the sample is different.

Please complete the form - even if you are not quite sure.

Odour	#	#	#
More chemical odour than R	___	___	___
Equal to R	___	___	___
Less chemical odour than R	___	___	___
Amount of difference			
None	___	___	___
Slight	___	___	___
Moderate	___	___	___
Much	___	___	___
Extreme	___	___	___

Comments:

Source: Larmond 1970.

FIGURE 4-5

Category Scaling Questionnaire

Sample "R" is of high quality. Become familiar with its characteristics and note its assigned scores.

Evaluate each coded sample for flavour, odour and acceptability. Retaste "R" whenever necessary.

Flavour	R
1) very fresh flavour	x
2) tasteless	
3) slightly chemical off-flavour	
4) moderately chemical off-flavour	
5) extremely chemical off-flavour	

Odour	R
1) fresh sea tang	x
2) odourless	
3) slightly off-odour	
4) moderately off-odour	
5) extremely off-odour	

Acceptance	R
1) perfect	x
2) good	
3) fair	
4) borderline	
5) unacceptable	

Comments:

Source: Burns and Ke 1984.

bled to evaluate whether fish or fish products are, in fact, tainted.

Selection of panelists should be done on the basis of their capability to detect differences. Panel members should have experience in evaluating the quality of fish and be familiar with the normal flavour of the species of fish used in the test. Age and smoking are not considered to be deterrent to membership but panelists should be advised not to smoke in the two hours before a panel and not to ingest highly flavourful food in the same period. People preparing the samples or setting up the experiment should not be on the panel.

GESAMP (1983) recommends that panel members should not be selected for acuity to the chemical (hydrocarbon) under test. They recommend using the triangle test with 8 panelists. Similarly, the hedonic test does not require that panel members be selected for their sensitivity to the odour or flavour of the hydrocarbons. Training of panelists for these tests involves a familiarization with the method and score sheet that will be used.

Other methods (multiple comparison and category scaling), however, require that the taste panel be trained to evaluate the flavour to be assessed. Training methods would vary depending on the type of test chosen and the type and concentration of hydrocarbon expected. A refresher training session is advised if the panel has been previously trained for this determination but there has been some modification in the procedure adopted, or a great deal of time has elapsed from the last testing.

The testing area should be such that the panelists have some

privacy and are free from distractions (noise, etc.). Natural white fluorescent lighting is recommended. Kniper (1973) suggests that judges be subjected to only two taste panels per day and spaced at least six hours apart to avoid organoleptic fatigue. The sample (if possible) should be presented in a glass container to avoid the faint perfuming often found in plastic and paper products (Woyewoda pers. comm.). The panelists should be provided with water or some other rinse for use between testings and instructed to use the same procedure with each sample.

GESAMP 1983 (see Appendix F) recommends that the flesh from all the fish should be washed, passed through a coarse mincer, thoroughly mixed and cooked in one of the following ways:

a. Casserole method. 150 g of material is placed in a casserole with a loose fitting lid. The casserole is suspended over or in boiling water or steam for a period just sufficient to cook the fish. This period should be determined from prior experimentation, and

b. Boil-in-the-bag method. 150 g of material is placed in a plastic bag intended for cooking foods in boiling water. The bag should be weighted and suspended in boiling water or steam for a period just sufficient to cook the fish. This period should be determined from prior experimentation.

Samples should be presented to the panelist warm as detectable odour and flavour differences tend to diminish with cooling.

4.3.4 Summary

The sensory evaluation methods available to identify fish tainting involve comparing the taste and odour of a product with a known standard or a control. While the methods of scoring are subjective, non-parametric statistical analysis can be used to identify differences.

Based on the definition of tainting presented in Section 2.2, the triangle test is thought to be the most suitable for determining whether fish are tainted as a result of exposure to hydrocarbons. This choice has been made because it attempts only to answer the question "Is there a difference?". This conforms with GESAMP's (1983) recommendation.

As far as sample preparation is concerned, it would appear that the "boil-in-a-bag" method is preferable because it is easier to standardize and results should be more readily reproducible. Variations in oven temperature and sample handling associated with the casserole method could inadvertently bias the results.

4.4 TISSUE ANALYSIS

4.4.1 Introduction

Muscle, skin, gills, or internal organs such as the liver can be analyzed chemically to determine hydrocarbon concentrations. As a procedure to support sensory evaluations, the chemical analysis should concentrate on the same tissues (e.g. fish fillet, whole oyster, scallop muscle, etc.) that are consumed.

In the following subsections the basic methods currently employed are discussed followed by a review of the literature on tissue analysis as it supports taste panels.

4.4.2 Methods

Many methods have been developed to analyze for hydrocarbon content in tissues (Zitko and Carson 1970; Yamamoto 1973). In simplified form, most analytical methods involve recovery of hydrocarbons and lipids from the protein-water matrix, isolation of the hydrocarbon fractions, and analysis of these fractions.

Three basic recovery methods can be considered: steam distillation, solvent extraction such as the chloroform-methanol system of Bligh and Dyer (1959), and tissue digestion with alkali (sometimes referred to as saponification).

The steam distillation method involves the direct distillation of the ground tissue sample with steam and collection of the distillate, which is then solvent extracted. The solvent volume is then reduced to concentrate the hydrocarbon for gas chromatography (GC) or liquid chromatography (LC) analysis.

The Bligh and Dyer technique uses chloroform-methanol to extract the lipids (which include the hydrocarbons) from the samples via a homophasic solution step. The water in the tissue is an integral part of the system and not a problem as would be the case with water immiscible solvents such as hexane or dichloromethane. The separated chloroform layer can be concentrated for analysis.

In the saponification method the sample is digested with

alkali and then the non-saponifiables (which contain the hydrocarbons) are recovered by solvent extraction into hexane or dichloromethane for analysis by GC or LC. The low boiling-point solvents are preferred to facilitate concentration steps.

Even with careful handling the quality of fish will deteriorate. Tokunaga et al. (1981) noted that the dark muscle of fatty fish was especially prone to produce aldehyde acids and amine, and similar volatiles. In principle these could interfere with any isolation procedure but alkali saponification has the advantage of retaining volatile acids if steam distillation is used. Alkali does cause the polymerization of aldehydes. They may be partially recovered from the system before steam distillation but not extraction. Most systems inevitably include the amines but these are likely to be mostly the very volatile dimethyl and trimethylamines and can be recognized. Alkali digestion combined with steam distillation warrants further investigation for recovery of volatile hydrocarbons of interest in detecting fish and shellfish tainting.

Chromatography can be used to isolate the hydrocarbons present in the extract from the first and third methods. The first or steam distillation method yields a relatively clean sample. The hydrocarbons are minor in the natural non-saponifiable compounds which can include sterols, fatty alcohols, and biogenic hydrocarbons such as squalene. The chloroform extract contains total lipids in which hydrocarbons are a very minor proportion. Thin layer chromatography (TLC) is a clean-up procedure which can be used to separate the less volatile hydrocarbons from other lipid components.

In considering these techniques it is imperative to consider the volatility of the hydrocarbons which are associated with tainting. Table 4-4 shows that the most likely flavour components of the aromatic series have boiling points between 80 and 242°C. In concentrating solutions of hydrocarbons or liquids in solvents such as methylene chloride (BP 40°C), chloroform (BP 62°C), pentane (BP 36°C) or hexane (BP 69°C) it is imperative that great care be taken to avoid losses of components such as xylene. Moreover, for the cyclic hydrocarbons, a high boiling point gives a false impression of security. The familiar smell of naphthalene (BP 218°C) is an indication of the high vapour pressure. For the same reason these components are particularly suited to recovery by steam distillation. The vapour pressure of the aliphatic hydrocarbons such as C_{12} and C_{14} is quite adequate to also concentrate these hydrocarbons (see GLC charts for diesel oil and diesel oil steam distillate, Ackman and Noble 1973). Nash (1984) has recently recommended steam distillation for the isolation of a wide variety of stable pesticides, most with molecular weights approximately the same as the aromatic hydrocarbons discussed above.

The separated hydrocarbons can be isolated and analyzed by a convenient technique such as gas chromatography. Column chromatography can be used to separate hydrocarbons from the lipid components. The total sample is applied to a column of silicic acid and washed with aliquots of solvents. Hydrocarbons are feebly retained by silica gel and collection of the early-eluting fractions for further analysis may be all that is required. Recently Ligocki and Pankow (1984) reported oxidative losses on silicic acid of anthracene and related hydrocarbons due to peroxides in the solvents. High performance liquid chromatography (HPLC) can be used to either clean up an extracted sample or to actually separate

TABLE 4-4

Boiling Points (°C)
of Selected Aliphatic and Aromatic Hydrocarbons

Carbons	Aliphatic		Aromatic	
6			Benzene	80
7			Toluene	111
8	Octane	125		
8			Xylene	140
10	Decane	174		
12	Dodecane	216		
10			Naphthalene	218
11			1-Methylnaphthene	245
14	Tetradecane	254		
16	Hexadecane	287		
18	Octadecane	316		
20	Eicosane	343		

various hydrocarbon fractions for identification.

The above techniques usually separate the hydrocarbons as a mixture. To study the individual components, gas chromatography (GC) is used. These hydrocarbons can then be confirmed by looking at their mass spectra by gas chromatography/mass spectrometry (GC/MS) (Lee 1976; Hardy et al. 1977; Ramos and Prohaska 1981; Zitko 1984).

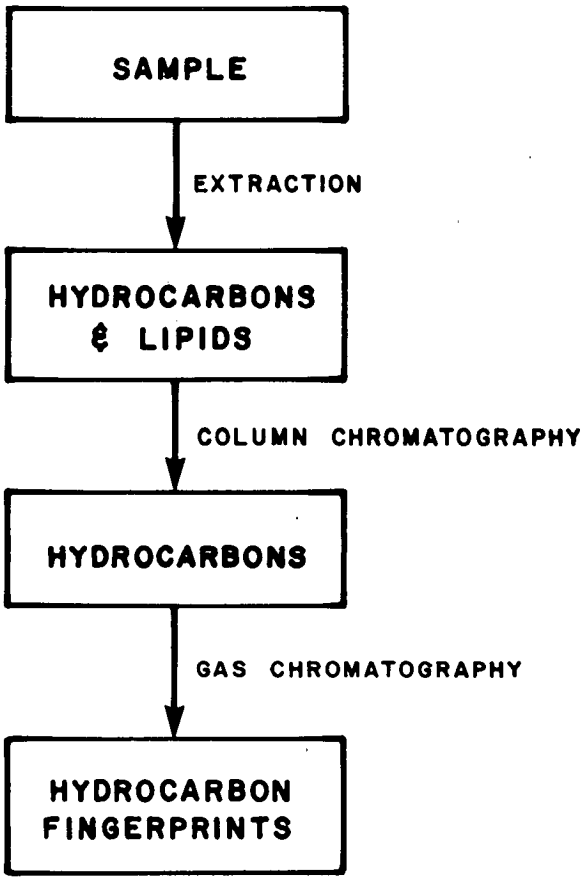
Flow charts in Figure 4-6 (Dewitt et al. 1982), Figure 4-7 (Vassilaros et al. 1982) and Figure 4-8 (Desideri et al. 1984) identify methods for hydrocarbon analysis ranging from simple (Figure 4-6) to more complex (Figure 4-8). The extraction procedure of Desideri et al. (1984) separates the hydrocarbons into 6 distinct fractions, Vassilaros et al. (1982) into 3 fractions and Dewitt et al. (1982) into 1 fraction.

Ramos and Prohaska (1981) suggest NaOH digestion followed by solvent extraction of tissue samples. These extracts are filtered through a silica gel column to remove interfering compounds followed by a Sephadex LH-20 gel column and elution of the polyaromatic hydrocarbon (PAH) fraction, which is then subjected to GC and GC/MS. The method gives a relatively pure PAH fraction and good recoveries.

Donkin and Evans (1984) use a method of steam distillation to recover hydrocarbons from water and steam distillation incorporating NaOH for tissues. The distillates can be either directly analyzed by GC or further cleaned up by HPLC (both normal-phase and reverse-phase using a UV detector). The steam distillation method also gives good recoveries of hydrocarbons from tissue.

FIGURE 4-6

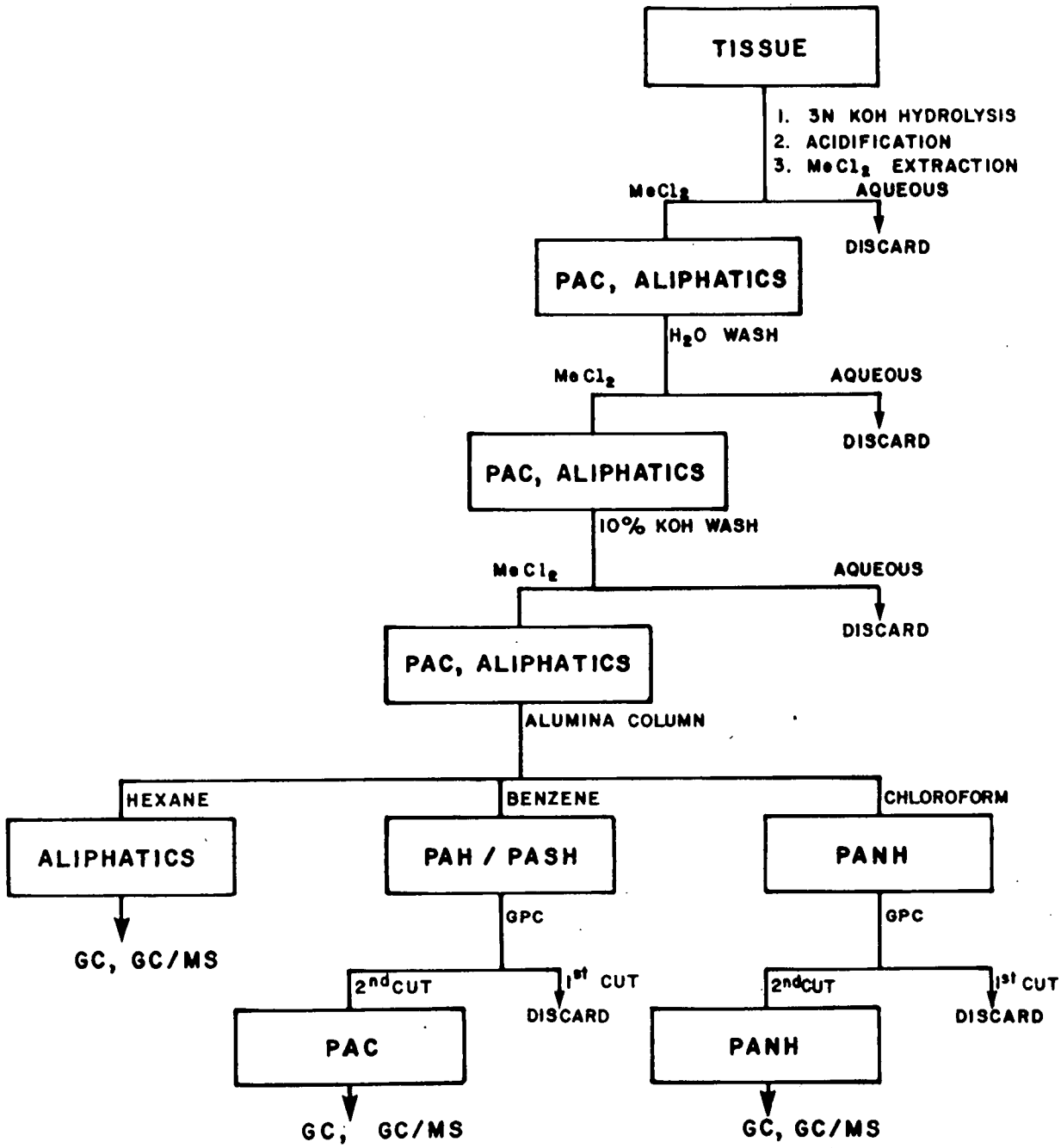
Extraction and Analysis of Hydrocarbons I



SOURCE: DEWITT et al. 1982

FIGURE 4-7

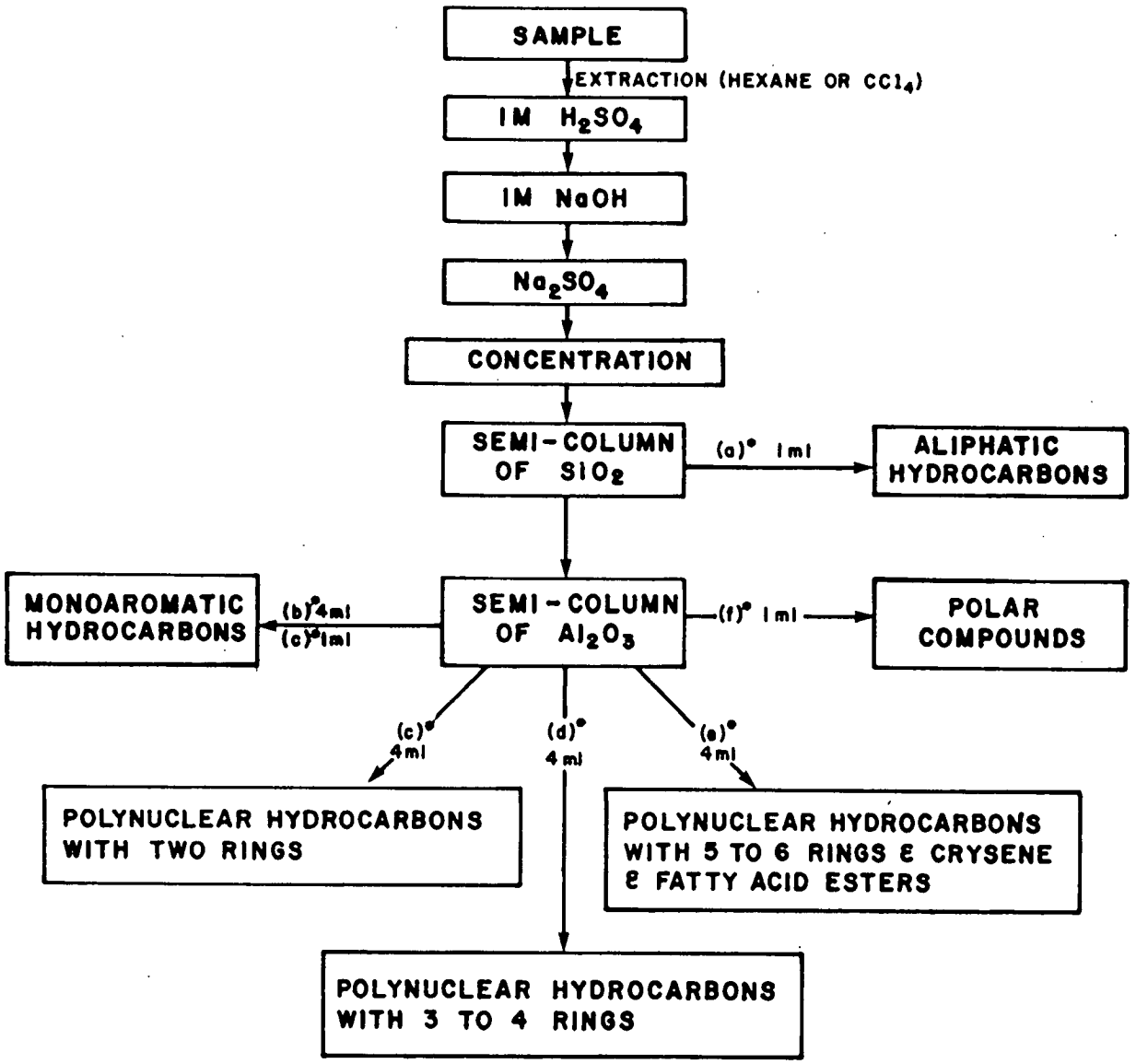
Extraction and Analysis of Hydrocarbons II



SOURCE: VASSILAROS et al. 1982

FIGURE 4-8

Extraction and Analysis of Hydrocarbons III



*Eluents:

(a) n-pentane

(b) n-pentane - carbon tetrachloride 8:2

(c) n-pentane - dichloromethane 9:1

(d) n-pentane - dichloromethane 7:3

(e) n-pentane - dichloromethane 2:8

(f) methanol

Source: Desideri et al. 1984.

Steam distillation has an advantage in that it does not introduce additional hydrocarbon contaminants to the analysis. In column chromatography the surfaces of silica gel, Florisil, etc. are very absorptive and can acquire hydrocarbons from the atmosphere, for example from automobile exhaust, or even from cigarette smoke. The use of large volumes of solvents, followed by concentrations from several ml to a few l, can create "background" problems. Procedural problems are encountered in all methods. Blanks can correct for this to a certain extent, but they double the workload.

Aromatic hydrocarbons were also studied by Sinkkonen (1982) using alkaline digestion, solvent extraction, column chromatography, HPLC (reversed phase-UV) and GC/MS analysis. Papers by Ackman and Noble (1973), Saito et al. (1979), Yasuda and Fukamiya (1978) and Teal et al. (1978) give similar or slightly different procedures for identifying hydrocarbons in whitefish, sardines, oysters, clams and sediment samples.

A new method for hydrocarbon fractionation utilizes the Iatroscan TH-10 Mark III (TLC/FID). Heavy crude oils, synthetic fuels and diesel exhaust particulates extracted from tainted samples can be analyzed in this manner if their boiling points are above 200°C (Poirier and George 1983) or 300°C (Obuchi et al. 1984).

High performance liquid chromatography is a relatively new method which is gaining in popularity for its reproducibility and speed. HPLC would seem to be a good method to separate hydrocarbon types for further analysis by either GC, GC/MS or additional HPLC. Tables 4-5 and 4-6 summarize some of the work done using HPLC for hydrocarbon analysis.

TABLE 4-5

Summary of Hydrocarbon Analysis by HPLC
Showing Column Type and Detector Used

Author(s)	Column	Mobile phase	Detector
Tate (1982)	Partisil 5 or 10 µm	Dry hexane	RI
Tong & Karasek (1984)	Spherisorb 10 µm	Dichloro-methane Acetonitrile Hexane	UV 254 nm
Alexander et al. (1982)	Radial Pak C18 cartridge	Acetonitrile water gradient	UV
Black et al. (1981)	PE PAH-10 reversed phase	Acetonitrile water gradient	Fluorescence 300 EX, 420 EM UV 254 nm
Colin & Vion (1983)	Radial Pak silica cartridges	Fluorocarbon FC-72	RI
Fodor & Newman (1975a&b)	Porasil Microporasil	Hexane, heptane 2,2,4-trimethyl pentane	RI UV 254 nm
Lichten-thaler & Oreld (1983)	Partisil 5 µm Rad Pak 10 µm	n-pentane cyclohexane	RI
O'Donnell (1982)	LiChrosorb	Methanol-water	Fluorescence
Sirota & Uthe (1981)	Vydac201TP reverse phase	Acetonitrile water	Fluorescence 280 EX, 389 EM UV 254 & 265 nm
Symons & Crick (1983)	Rad-Pak C18	Acetonitrile water	Fluorescence UV

TABLE 4-6

Summary of Hydrocarbon Analysis by HPLC
on Different Materials

Author(s)	Hydrocarbon type	Samples analyzed
Tate (1982)	Olefins, saturates, total aromatics, naphthalenes	Kerosene, aviation fuel, diesel, light mineral oils
Tong & Karasek (1984)	PAH	Diesel exhaust
Alexander et al. (1982)	Aromatics	Refinery effluent, soot, marine biota
Black et al. (1981)	PAH	Fish and sediments
Colin & Vion (1983)	Saturates, olefins, aromatics	Gasolines and kerosene
Fodor & Newman (1975a&b)	Saturates, olefins, aromatics	Crude oil
Lichtenthaler & Oreld (1983)	Aliphatics, aromatics	Marine biota, sediment and water
O'Donnell (1982)	PAH	Water extracts
Sirota & Uthe (1981)	PAH	Lobster, clams and mussels
Symons & Crick (1983)	PAH	Refinery effluent

Other papers concerning hydrocarbon analysis using HPLC include Zsolnay (1974), Albaiges and Grimalt (1982), Choudhury (1981), Gibson et al. (1981), Ho et al. (1982), Katz and Ogan (1981), Marsh and McNair (1981) and May et al. (1981).

HPLC has some limitations due to the detector. The popular UV detector is not especially sensitive for aliphatic hydrocarbons, although satisfactory for aromatic hydrocarbons. The alternative refractive index (RI) detector limits changing solvent as a participating factor in the analysis. Fluorescence detection is more expensive but improves the sensitivity.

There is no doubt that HPLC has a definite role to play in examining the less volatile hydrocarbons especially the polycyclic aromatics of health concern, when found in fish (Lawrence and Weber 1984). These authors used time saponification, extraction, column chromatography, liquid-liquid partitioning and then HPLC. Calibration indicated better than 70% recovery. "Fresh" haddock gave an indication of several lower aromatic hydrocarbons but, quote, "untreated (not smoked) fish extracts exhibited a large number of interfering peaks". Lobster meat showed more of these but several polycyclic aromatics were found to be present in the 0.2 - 2.3 ppm range. A lobster "spread", partly made with tomalley, had much more, as previously reported. This paper is an interesting one as it is based on Canadian fish samples, and is the "state of the art" for HPLC at the time of writing.

After HPLC the collected fractions would usually be subjected to GC or GC/MS for identification. Table 4-7 identifies a variety of GC and GC/MS methods that have been used for

TABLE 4-7

Summary of Hydrocarbon Analysis by GC and GC/MS

Author	Type of GC or GC/MS	Column & Temp.	Samples analyzed
Berthou & Friocourt (1981) Berthou et al. (1981)	Carlo Erba Nermag R-10-10B	OV-1,73, OV-225 SE-52 40-280C	Amoco Cadiz oil, oyster extracts, aromatics and aliphatics
Ho et al. (1982)	Girdel 3000	SE-52 120-279C	Saturated and olefinic water extracts
Tan & Heit (1981)	HP 5985 GC/MS	SE-30	PAH and sediment cores
Laub et al. (1981)	Carlo Erba 4160	SE-52,BBBT 220C	PAH
Blaylock et al. (1973)	Hewlett-Packard 1704	SE-30 50-259C	Methyl naphthalenes and clam tissue
Farrington et al. (1976)	HP 700	APL 80-280C	Tuna meal and cod liver oil
	Varian 1200 & 1700	APL 80-290C	
Krahn & Malins (1982)	HP 5840A Finnigan 3200	DB-5 90-300C	Fish livers and fuel
Warner (1976)	Varian 1840 Finnigan 1015	OV-1&17, SE-30 60-300C	Clam tissue - aliphatic and aromatic hydro-carbons
Murray & Lockhart (1981)	PE900	Dexsil 400 100-250C	Water and fish tissue - crude oil and diesel

TABLE 4-7 (Continued)

Author	Type of GC or GC/MS	Column & Temp.	Samples analyzed
Mackie et al. (1978)	PE F11	Dexsil 300 150-310C	Alkanes in fish and sediment
	Varian 1400	OV-101 195C	
Motohiro & Inoue (1973)	Shimadzu GC-4APF	SE-30 70-210C	n-Paraffins in fish
Motohiro & Iseya (1976a&b)	Hitachi 063	SE-30 80-240C	n-Paraffins in scallops and sediments

hydrocarbon analysis.

There is no doubt that capillary (wall-coated open-tubular) GLC is the preferred state of the art for hydrocarbon analysis, with or without mass spectroscopy backup. A significant improvement in "availability" of capillary GLC followed the introduction in 1979 of flexible fused silica as a cheap, convenient, inert and widely available column material. Later the introduction of "bonded" (actually cross-linked is a more correct term) liquid phases opened new possibilities for higher temperature operations, extended column life, and less bleed as an interference problem in mass spectroscopy. Examples of techniques and lists of retention times can be found in Hiatt (1983), Ogata and Fujisawa (1983) and Vassilaros et al. (1982), to mention only a few of various recent papers.

4.4.3 Tissue Analysis in Support of Taste Panels

There are very few papers which deal with both tissue analysis and taste panels concurrently. Most papers deal with some limited aspect of the subject or are of a very general nature. The following papers that have approached this subject are reviewed briefly.

Berg (1983) studied salmon and compared results from sensory assessments to those obtained by headspace gas chromatography (Hewlett-Packard Model 5840-FID) and GC/MS (Finnigan 4021). Sensory analysis data were supported by the GC, GC/MS data. Grey mullet with a kerosene-like taint were subjected to chemical and sensory analysis (Shipton et al. 1970; Connell 1974). The volatile constituents were isolated from the tissue samples by steam distillation and further subjected to hydrogenation, urea adduction and

treatment with chlorosulphonic acid followed by GC and GC/MS analysis. The chemical analysis results were similar to those produced by commercial kerosene. They supported the sensory analysis that showed a kerosene-like odour or taste in contaminated fish. Connell et al. (1975) also studied the kerosene-like taint of Australian bream using similar methods.

Sensory analysis of yellowtail was tested by Deshimaru (1972) in conjunction with head space and GC analysis. Brown trout (Mackie et al. 1972) contaminated by diesel oil was tested organoleptically and chemically. Flesh samples were extracted using the Bligh and Dyer (1959) method followed by silicic acid column chromatography for hydrocarbon separation. The hydrocarbon fraction was further analyzed by UV and fluorescence spectroscopy, GC and GC/MS.

Oysters and scallops contaminated by the Florida spill were studied by Blumer et al. (1970). The samples were reported to have an oily taste which was substantiated by chemical analysis (Soxhlet-extraction, column chromatography and GC). Ogata and Miyake (1973), Ogata and Ogura (1976), Ogata et al. (1977), Ogata et al. (1979) and Ogata (pers. comm.) have conducted numerous experiments using eels, green fish and short-necked clams. The objectionable odour and/or taste present in these organisms were corroborated by GC analysis confirming the presence of aromatic hydrocarbons (benzene, toluene), unsaturated aliphatic hydrocarbons, paraffins and organic sulphur compounds.

4.4.4 Summary

The review presented above indicates the wide range of sample preparation and chemical analytical techniques that are available for determining hydrocarbon concentrations in tissues of finfish and shellfish. Results of a limited number of investigations have shown that chemical analysis can be used to support sensory evaluations.

At present, the recommended method for chemical analysis of tissue involves mincing the sample and using steam distillation, followed by hexane extraction of the distillate, drying and concentration. Hydrocarbon determinations should be made using capillary GC-MS. This method has the advantage of speed and simplicity. These are prime considerations where an oil spill could be having an adverse effect on local fisheries because of a perception that the exploited stocks may be tainted. However, further work to refine the techniques for tissue analysis would be beneficial.

5.0 RECOMMENDATIONS

As indicated throughout this report tainting of finfish and shellfish by petrogenic hydrocarbons has not been extensively documented or completely researched by the scientific community. Much of the information is anecdotal. In the following subsections further studies are recommended to provide a strong scientific basis for conducting evaluations of possible tainting in the event of a spill or blowout associated with offshore oil and gas activities.

5.1 METHODOLOGY

In Subsections 4.3.4 and 4.4.4 of this report, specific methods are proposed for conducting taste panels and tissue analysis on fish products suspected of being tainted by hydrocarbons. These are based on general experience gained in food science technology. These methods should be specifically tested and refined for their application to a potential tainting situation of locally exploited species. The methods should be refined to satisfy the following objectives:

- Reproducibility of the results from event to event; and

- To meet any legal requirements oil and gas industry compensation programs may have for attributable and non-attributable damage.

Sampling and sample preparation methods should be evaluated to insure that the samples will be kept as fresh as possible and uncontaminated from outside sources. The finfish and shellfish used for taint testing would be exposed to hydrocarbon contaminants at concentrations sufficient to produce

a taint. Test conditions and exposure levels to artificially tainted live specimens have been proposed by GESAMP (1983) and the European Chemical Industry Ecology and Toxicology Centre (ECETOC 1984) (see Appendix G1 and G2). These should be used as the basis for program development, but the species chosen should be representative of those that will potentially be at risk as a result of an accident. Only crude oil or condensate need be used as the test media.

Holding conditions should be suitable to insure the health of fish used in the study program. Some considerations in this regard are volume of water, temperature, salinity and oxygen concentration. GESAMP (1983) recommends an exposure period of at least 24 hours under static conditions providing the concentration of the test substance does not drop below 50% of the initial levels. Semi-static holding conditions or continuous flow systems are alternatives which may offer suitable alternatives to maintain the concentration of the test substance, where low oxygen concentrations are expected or for tests of longer duration.

The first level of assessment of a potential tainting incident is a total sensory evaluation which involves visual impressions, raw odour grading and taste testing. The samples would be examined for surface fouling and graded for raw odour. A standardized score sheet should be developed to record the impressions at this level of the evaluation.

Methods for conducting taste tests are well documented and score sheets have been developed to record the impressions of panelists. Experimental taste tests should be conducted, however, with particular attention being paid to sample preparation and cooking methods suitable for local species. Specimens used for these tests could either be contaminated

through exposure which allows discrimination by chemical class as discussed above, or by mixing the contaminant directly with the flesh which does not allow for modification by biological processes. The assumption is made that the body burdens to elicit a response from a taste panel will be the same whether the natural assimilation process is used, or the contamination is mixed in post mortem. In experiments conducted by Howgate et al. (1977) the contaminant was added to skinless plaice fillets and the solvent allowed to evaporate prior to cooking. However, in experiments with lobster taint, the contaminant was added to the meat after cooking and blending (Scarratt 1980b).

GESAMP (1983) recommends coarse grinding of the flesh and then cooking by either a casserole or "boil-in-a-bag" method (see Appendix G1). Scarratt (1980b) prepared lobster for taste tests by boiling whole live lobsters in a 3% brine for 8 minutes. The tail meat was coarsely blended prior to tasting and the hepatopancreas was removed and tested separately. The question of whether it is better to present a taste panel with a minced or natural product should be determined because of the potential for a perceived bias on the part of panelists.

The procedures used to choose and train a taste panel should also be reviewed to insure that a panel can be assembled and prepared in minimal time in the event of an incident requiring a tainting evaluation. This would best be done by maintaining a permanent program of taste panels at a selected facility in Canada. The program would involve periodic training or refresher sessions for panelists supported by concurrent laboratory assays.

The second level of assessment is the analysis of the quan-

tity and type of hydrocarbon present in the flesh. Chemical analysis should generally be employed in the event a positive taint is identified during the sensory evaluation. These analyses should be used in an attempt to identify contaminating substances. The proposed sample preparation and analytical procedures identified in Subsection 4.4.4 should be tested and any procedural problems resolved. The procedures developed would be fully documented.

The study would determine the fastest and simplest technology for sample extraction and recovery. As an example a comparison of steam distillation with and without prior alkali digestion of tissue is needed. Vassilaros et al. (1982) have listed work done up to that time on digestion but did not explore steam distillation. It is possible that different routes would be taken with obviously fatty fish on the one hand, and lean white fish on the other. Shellfish would also have to be considered.

The result of this study should be the production of a manual to ensure that standardized methods are used in evaluating potential tainting situations associated with offshore oil and gas activities. This manual should be reviewed periodically to ensure that the proposed chemical analytical techniques remain the most appropriate.

5.2 CORRELATION OF TASTE PANEL RESULTS WITH TISSUE ANALYSIS

In Section 5.1, it has been recommended that tissue analysis be used to confirm evidence of tainting determined by the first level sensory evaluation. A review of the literature has revealed very few papers which deal with both tissue

analysis and sensory evaluation concurrently. Further research should be done to correlate the results of the two procedures and to determine the threshold level of hydrocarbons in the tissue which can be perceived by a trained taste panel. These tests should be done following the program recommended in Section 5.1 using the probable types of crude oil and condensate that may be exploited in Canadian offshore areas.

5.3 EXPOSURE LEVELS AND DEPURATION

Threshold concentrations causing tainting should be determined from preliminary range-finding tests for several representative species of finfish and shellfish. This could include but not necessarily be limited to cod or haddock, plaice, herring, capelin, as well as scallops and mussels. GESAMP (1983) recommends that this value should be lower than the 24 h LC_{50} by a factor of 10.

Once the threshold exposure and tissue hydrocarbon levels necessary to cause a taint have been determined, test organisms should be transferred to uncontaminated aquaria and sampling continued to determine depuration rates in each species, and the concentration at which a taint is no longer detectable.

5.4 BASELINE SURVEY

Petrogenic hydrocarbons are introduced into the marine system from many sources including natural seeps, atmospheric fallout, surface runoff, industrial waste discharges, marine transportation and accidents, and offshore oil and gas production and well blowouts. The annual global input has been estimated in the range of 6 million metric tons

(GESAMP 1977). It follows, therefore, that the baseline level of hydrocarbons in fish populations arising from petrogenic sources will vary in space and time.

The majority of the analytical papers cited refer to samples taken in nearshore areas. Little is known about the offshore fishing areas. The offshore fish are accepted by consumers as "normal" in flavour. There is a need for an assessment of the baseline occurrence of hydrocarbons of the type of interest to this paper, particularly the aromatics.

It is suggested that the representative fish populations in offshore Canadian waters potentially at risk as a result of offshore oil and gas activity be sampled and chemically analyzed for hydrocarbon levels in the tissues. Sampling would be designed to mimic natural situations and should concentrate on sedentary species as they may be more vulnerable to hydrocarbon contamination as a result of routine discharges from exploration and production facilities, or an accident. This information would provide an indication of background levels to be expected in these populations in the event of a spill. It would also serve to indicate how much contamination populations at risk can tolerate before a taint becomes evident.

Before this survey is launched it would be necessary to have the sensory evaluation and analytical methodology as described in Section 5.1 in place.

5.5 MONITORING UPTAKE FROM PRODUCTION WATER

There is evidence to indicate that petrogenic hydrocarbons are accumulating in the waters and sediments around some North Sea oil and gas platforms (Oppenheimer et al. 1977).

A number of potential sources may be responsible but one to consider is the release of production water containing dissolved hydrocarbons.

The volume of production water discharged is usually small in the initial life of a field, but levels increase as the field is depleted. Regular monitoring of the levels of hydrocarbons in finfish in the vicinity of producing platforms would provide a warning of a possible chronic tainting situation of local fishery resources. Again, sampling would be designed to account for the natural movement of fish populations present in the area. This could be incorporated into a comprehensive monitoring effort.

5.6 DISPERSANT USAGE AND TAINTING

Dispersants used to assist in the clean-up after the Torrey Canyon incident were blamed for the tainting that emerged in inshore finfish and shellfish populations. Shipton et al. (1970) pointed out that the solvent fraction of first generation dispersants contained compounds known to cause tainting. Although these solvents are not used in the second generation dispersants, their usage increases the risk of tainting simply by moving the oil from the surface into the water column and sediments and making the hydrocarbons more readily available to finfish and shellfish populations in the vicinity (GESAMP 1977).

The question of how the use of second generation dispersants may affect the acquisition of a taint has not been addressed. Although the second generation dispersants use surfactants that should not cause a taint in exposed fish, this should be confirmed experimentally. In addition, threshold levels of tainting from dispersed oil as compared

to oil mechanically mixed into the water column should be determined.

The results of this evaluation will be useful to decision-makers should the question of dispersant usage arise during a major oil spill or well blowout incident.

5.7 OIL IN SEDIMENTS AND TAINTING

Oil can be introduced into sediments as a result of weathering and sinking after a spill, or as a result of the discharge of cuttings from oil-based drilling muds. The oil from these two sources will be in a different physical form. Sunken oil will exist as globules or mats on the seabed, while oil on cuttings will be adsorbed to particle surfaces. In either case, the oil present could result in a long-term potential tainting situation, if concentrations are high enough, particularly in a low energy environment where the oil is likely to remain for an extended period.

The literature does not indicate clear cause-effect relationships between oil in sediments and taint in exposed organisms. Howgate et al. (1977) found off-flavours in plaice after 1 or 2 days of exposure to fresh crude mixed into bottom sediments, while this same effect has not always been apparent in other investigations (e.g. Wilder (1970) working with lobster). In the case of oil-based drilling muds, Davis (pers. comm.) indicates that tainting has not been a concern around drilling platforms in the North Sea despite the fact that diesel oil has been used extensively in oil-based drilling muds in the past. In Canada, drilling muds used offshore contain variable amounts of aromatics (Addison pers. comm.).

While the probability of sunken oil existing in offshore areas in sufficient concentrations to cause tainting is low, and the aromatics responsible for tainting are largely absent in drilling muds used in the Canadian offshore, there are a number of uncertainties that should be investigated to better define the risk of tainting associated with oil on the seabed. A series of laboratory experiments using cod, a species of flatfish, scallops and mussels should be conducted in which organisms would be exposed to representative concentrations of sunken oil (i.e. Hibernia crude) and oiled drill cuttings for varying periods of time. The organisms would be sacrificed and subject to taste panel evaluations and chemical analysis to demonstrate what, if any, effect these types of oil are having on taste.

The oil-based mud component of the recommended program could be undertaken in conjunction with a study on the sublethal effects of oil-based drilling muds sponsored by the Interdepartmental Panel on Energy Research and Development (Addison pers. comm.) which is currently under way.

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APPENDIX A

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APPENDIX B

EXAMPLE OF LETTER SENT

Dear Sir:

Among my responsibilities is the preparation of a report on the closure of fisheries for food or food products, either fish or shellfish, for reasons of either real, suspected, or feared "tainting" from petroleum-associated activities.

There are well-documented cases such as the Falmouth spill in the U.S.A., and the Amoco Cadiz tanker wreck off Brittany, but even these tend to gloss over or ignore the economic realities of effects on the local fishing industry. Sometimes there are reports of fish condemned as having "petroleum flavors" which are never linked to identifiable events. The problem is that there are often different jurisdictions and bodies responsible for officially closing fisheries in different countries, so I must ask if, in addition to giving me the benefit of your personal knowledge of any of these events, you could list for me the names and addresses of the senior management of official bodies with this responsibility. Please note that "petroleum activities" includes all oil and gas drilling activities. People tend to forget that these can lead to low-level, long-term local effects, due to the diesel oil consumed during rig operations, as well as to spectacular accidents.

Your cooperation as soon as possible would be greatly appreciated, as I know that the festive season will soon be upon us and there are many items put off at that time.

Yours very truly,

R.G. Ackman
Professor

APPENDIX C

NAMES AND ADDRESS OF PEOPLE CONTACTED

CANADA

Dr. R.F. Addison
Marine Ecology Laboratory
Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, N.S.
B2Y 4A2

Mr. F. Allen
Fisheries Inspection Division
Dept. Fisheries and Oceans
Halifax, N.S.

Dr. R. Danell
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba
R3T 2N6

Dr. P. Eaton
Environmental Protection Service
Queen's Square
Dartmouth, N.S.

Dr. D.A.J. Murray
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba
R3T 2N6

Mr. R. Simmons
Environmental Protection Service
Queen's Square
Dartmouth, N.S.

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

CANADA (Continued)

Mr. G. Sirota
OceanChem Ltd.
1000 Windmill Road
Dartmouth, N.S.

Dr. J. Sprague
University of Guelph
Guelph, Ontario

Mr. G. Stevens
Regulation Unit
Dept. Fisheries and Oceans
Halifax, N.S.

Mr. A. Woyewoda
CIFT
Technical University of Nova Scotia
P.O. Box 1000
Halifax, N.S.

UNITED KINGDOM

Dr. S.M. Barlow
International Association of Fish Meal Manufacturers
Hoval House, Orchard Parade
Mutton Lane
Potters Bar, Herts
England
EN6 3AR

Dr. Q. Bone
Marine Biology Association of the U.K.
Plymouth Laboratory
Plymouth, Great Britain

NAMES AND ADDRESS OF PEOPLE CONTACTED

UNITED KINGDOM (Continued)

Mr. P.D. Chaplin
Sea Fish Industry Authority
Sea Fisheries House
10 Young St
Edinburgh EH2 4JQ
UK

Commodity News Service (International) Inc.
72 Fleet Street
London EC 4
England

Dr. John Davis
Department of Agriculture and Fisheries
Aberdeen
Scotland

Dr. E. Edwards
The Shellfish Association of Great Britain
Fishmongers' Hall
London Bridge
London EC4R 9EL
UK

Dr. R. Hardy
MAFF
Torrey Research Station
P.O. Box 31
135 Abbey Road
Aberdeen AB9 8DG
Scotland

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

UNITED KINGDOM (Continued)

Dr. D.L. Holland
NERC Unit of Marine Invertebrate Biology
Marine Science Laboratories
Menai Bridge, Gwynedd
Great Britain

Dr. P. Howgate
MAFF
Torrey Research Station
P.O. Box 31
135 Abbey Road
Aberdeen AB9 8DG
Scotland

The Institution of Environmental Health Officers
Chadwick House,
Rushworth Street
London SE1 0QT
UK

Dr. J.R. Maxwell
University of Bristol
School of Chemistry
Bristol, England
BS8 1TS

Dr. R.J. Morris
National Institute of Oceanography
Brook Road, Wormley
Gadalming, Surrey
England
GV8 5VB

Dr. M. Nauke
International Maritime Association
4 Albert Embankment
London SE1 7SR
UK

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

UNITED KINGDOM (Continued)

Dr. W.D. Pocklington
Food Commodities Division
Laboratory of the Government Chemist
Cornwall House, Stamford Street
London SE1 9NQ
UK

Dr. S.A. Reed
Seven Seas Health Care
Marfleet, Kingston upon Hull
UK
HU9 5NJ

Mr. P.C. Wood
Ministry of Agriculture, Fisheries and Food
Directorate of Fisheries Research
Fisheries Laboratory Remembrance Avenue
Burnham-on-Crouch, Essex
UK

SCANDINAVIA AND FINLAND

Mr. Klaus Bender
Miljostyrelsen
Strandgade 29
DK-1401 Kobenhavn K
Denmark

Dr. O. R. Braekkan
Norwegian Fish Research Institute
Vitamin Laboratory
Bergen, Norway

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

SCANDINAVIA AND FINLAND (Continued)

Dr. A. Bruce
National Food Administration Nutrition Laboratory
Statens Livsmedelsverk
P.O. Box 622
S-751 26 Uppsala, Sweden

Commissioner on Fisheries
Commission of the European Communities
Rue de la Loi 200
B-1049 Brussels
Belgium

Director
Ministry of Environment
Department for Protection of Environment and Nature
Kaikukatu 3, 00530
Helsinki 53, Finland

Director
National Food Administration
P.O. Box 622
S-751 26 Uppsala
Sweden

The National Swedish Environmental Protection Bd.
Box 1302
S-171 25 Solna
Sweden

Director
Netherlands Institute for Sea Research
P.O. Box 59
1797 SH Texel
The Netherlands

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

SCANDINAVIA AND FINLAND (Continued)

Director
Netherlands Institute for Sea Research
P.O. Box 59
1797 SH Texel
The Netherlands

Mr. T. Gustavsson
National Board of Fisheries
Box 2565
S-403 17 Goteborg
Sweden

Dr. T. Hattula
Food Research Laboratory
Technical Research Center of Finland
SF-02150
Espoo 15, Finland

Dr. L. Hiisivirta
Siltasaarenkatu 18 A, PB223
SF-00531 Helsinki 53
Finland

Secretary-General
International Council for the Exploration of the Sea
Paloegarde, 2-4
1261 Copenhagen
Denmark

Dr. P. Johansen
Greenland Fisheries and Environment Research Institute
Tagenscej 135
DK-2200 Copenhagen N
Denmark

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

SCANDINAVIA AND FINLAND (Continued)

Dr. T. Kuusi
Food Research Laboratory
Technical Research Center of Finland
SF-02150
Espoo 15, Finland

Dr. O. Linden
Swedish Environmental Research Group
S-371 03 Karlskrona
Sweden

Dr. O.J. Ostvedt
Institute of Marine Research
Directorate of Fisheries
P.O. Box 1870
N 5011 Nordnes
Bergen, Norway

Mr. H. Pitkanen
Ministry of Agriculture and Forestry
Fisheries Department
Vironkatu 7, 00170
Helsinki 17, Finland

Dr. Walther Schmidtsdorff
Ministry of Fisheries
Technology Lab
Technical University, Building 221
2800 Lyngley, Denmark

Dr. P.L. Schuller
Director, Food Inspection Service
for the Area of Goes
Evertsenstraat 17
4461 XN Goes
The Netherlands

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

SCANDINAVIA AND FINLAND (Continued)

Dr. A. Voipio
Institute of Marine Research
P.O. Box 33
SF-00931 Helsinki 93
Finland

GERMANY

Dr. H. Hoffmeister
Fischereiamt
Wischhoffstr 1-3
D-2300 Kiel 14
Federal Republic of Germany

Dr. C. Osterroht
Institut fur Meereskunde an der Universitat Kiel
Dusternbrooker Weg 20
D-2300 Kiel 1
Federal Republic of Germany

Dr. P. Pohl
Institut fur Pharmazeutische Biologie der Universitat
2300 Kiel, Grasweg 9
West Germany

Dr. K. Tiews
Bundesforschungsanstalt fur Fischerei
Palmaille 9
D-2000 Hamburg 50
Federal Republic of Germany

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

OTHER EUROPEAN COUNTRIES

Dr. G. Forneris
Instituto Zooteenica Generala e Speciale della
Facolta di Medicina Vetarinaria
Universita di Torino
Torina, Italy

M. LeDirector
IFREMER
Centre de Nantes
Rue de l'Ile d'Yeu
44000 Nantes
France

The Director
IFREMER
66 Avenue Jena
75016 Paris
France

Mr. P. Mayzaud
Universite P. et M. Curie
C.E.R.O.V.
Station Zoologique
Boite Postale 28
06230 Villefranche-sue-Mer
France

Dr. L. Stodolnik
Akademia Rolincza
Instyt Technologii Zywosci
Pochodezenia Morskiego
Kazimierza 3
71-550 Szczecin
Poland

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

SOUTH AFRICA

Mr. A. Moldan
Sea Fisheries Research Institute
Marine Development
Private Bag X2, 8012 Rogge Bay
Cape Town, South Africa

Dr. A.A. Spark
Laboratory Analytical Services
99 Hope Street, 1st Floor
Cape Town 8000
Republic of South Africa

Dr. J.P.H. Wessels
Fishing Industry Research Institute
University of Cape Town
Private Bag
Rondebosch 7700, C.P.
Republic of South Africa

INDIA

Dr. C.V.G. Reddy
National Institute of Oceanography
Dona Paula
Goa 403004
India

Dr. K. Gopakumar
Central Institute of Fisheries Technology
Indian Council of Agricultural Research
Willingdon Island
Cochlin 682 029
India

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

INDIA (Continued)

Dr. A. Ghosh
Department of Chemistry
Bose Institute
93/1 A.P.C. Road
Calcutta 700 009
India

Dr. R. Sen Gupta
Assistant Director
National Institute of Oceanography
Dona Paula
Goa-403 004, India

ARGENTINA

Dr. R.J. Pollero
Instituto de Fisiologia
Facultad de Ciencias Medicas
Calles 60 y 120
1900 La Plata
Argentina

NEW ZEALAND

Director
Fisheries Management Division
Ministry of Agriculture and Fisheries
Private Bag
Wellington
New Zealand

Dr. D.R. Body
Dept. of Scientific and Industrial Research
Applied Biochemistry Division
Private Bag
Palmerston North
New Zealand

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

NEW ZEALAND (Continued)

Mr. N.E. Jarman
General Manager
NZ Fishing Industry Board
Private Bag
Manners Street Post Office
Wellington
New Zealand

AUSTRALIA

Director
Fisheries Division
Commonwealth Dept. of Primary Industry
Edmund Barton Building
Broughton Road
Canberra Act 2600
Australia

Dr. A.A. Johnson
CSIRO
Division of Food Preservation
P.O. Box 43
Ryde, NSW
Australia 2112

Mr. W. Murray
Queensland Fish Board
Colmslie Road
Colmslie, Queensland 4171
Australia

Dr. G.S. Sidhu
CSIRO
Division of Food Preservation
P.O. Box 43
Ryde, NSW
Australia 2112

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

AUSTRALIA (Continued)

Dr. J.K. Volkman
CSIRO Division of Oceanography
G.P.O. Box 1538
Hobart, Tasmania 7001
Australia

UNITED STATES

Director
University of Texas
Marine Sciences Research Institute
Port Aransis, Texas 78373
USA

Dr. A.T. Bimbo
Director of Research
Zapata Haynie Corporation
P.O. Box 175
Reedville, VA 22539
USA

Dr. D. Chamberlain
Atlantic Richfield
Los Angeles, CA

Dr. Y. H. Edmondson
Limnology and Oceanography
Department of Zoology NJ-15
University of Washington
Seattle, Washington 98195
USA

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

UNITED STATES (Continued)

Dr. J.W. Farrington
Woods Hole Oceanographic Institute
Department of Chemistry
Woods Hole, Mass. 02543
USA

Dr. Andrea Hall
Biologist
American Petroleum Institute, Washington, D.C.

Dr. D.H. Hwang
Associate Professor
Louisiana State University
Baton Rouge, Louisiana 70803
USA

Dr. Richard R. Lee
Skidaway Institute of Oceanography
P.O. Box 13687
Savannah, GA 31406
USA

Dr. N. Maciolek-Blake
Assistant Program Manager
Georges Bank Benthic Infauna Monitoring Program
Battelle
New England Marine Research Laboratory
397 Washington St
Duxbury, Mass. 02332
USA

Dr. J. Pearce
National Marine Fisheries Service
Sandy Hook Laboratory
Highlands, NJ
USA

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

OTHER

Dr. M. Kayama
Laboratory of Marine Biochemistry
Department of Applied Biological Sciences
Hiroshima University
2-17 Midori-cho
Japan

Dr. M. Maeda
Ocean Research Institute
University of Tokyo
Nakano, Tokyo 164
Japan

Dr. Y. Miyake
Okayama Prefectural Research Institute
of Fisheries
35 Kashino, Ushimado-cho
Oku-gun, Okayama-ken, 701-43
Japan

Dr. M. Ogata
Dept. Public Health
Okayama University Medical School
2-5-1 Shikata-Cho
Okayama City
700 Japan

Dr. Masaaki Takeuchi
Research Division, Fisheries Agency
Ministry of Agriculture, Forestry and Fisheries
1-2-1 Kasumigaseki
Chiyoda-ku, Tokyo
100 Japan

APPENDIX C (Continued)

NAMES AND ADDRESS OF PEOPLE CONTACTED

OTHER (Continued)

Dr. G.E. Shulman
South Sea Research Institute
Maximov Street
Sevastopol
USSR

Dr. V.E. Vaskovsky
Institute of Marine Biology
Far East Research Centre
Academy of Sciences of the USSR
Vladivostok 690022
USSR

APPENDIX D

FOOD AND ENVIRONMENT PROTECTION BILL [H.L.]
[As Amended in Committee]

An Act to authorize the making in an emergency of orders specifying activities which are to be prohibited as a precaution against the consumption of food rendered unsuitable for human consumption in consequence of a release of substances; to replace the Dumping at Sea Act 1974 with fresh provision for controlling the deposit of substances and articles in the sea....

Part I

Contamination of Food

Emergency order etc.

1.(1) If in the opinion of a designating authority-

(a) There has been or many have been a release (whether or not accidental) of substances of such descriptions and in such quantities and such circumstances as are likely to create a hazard to human health through human consumption of food; and

(b) In consequence food which is in an area-
(ii) Of sea within British fishery limits;
or which is derived from anything in such an area, is, or may be, or may become, unsuitable for human consumption,

the designating authority to whom it so appears may by statutory instrument make an order designating that area and containing emergency prohibitions.

(2) In this Act-

"designating authority" means the Ministers or either of them;

"emergency order" means an order under this section-

(a) which designates an area; or

(b) which amends or re-enacts an order which designated an area;

"emergency prohibitions" means the prohibitions specified in Schedule 1 to this Act; and

"designated area" means an area designated by an emergency order.

SCHEDULE I

EMERGENCY PROHIBITIONS

PART I

ACTIVITIES THAT MAY BE PROHIBITED IN A DESIGNATED AREA

1. An emergency order may prohibit any of the following in the designated area-

(d) fishing for and taking fish;

PART III

ACTIVITIES THAT MAY BE PROHIBITED THROUGHOUT THE UNITED KINGDOM

3. An emergency order may prohibit any of the following anywhere in the United Kingdom or in United Kingdom waters-

(b) the landing of fish or other forms of aquatic produce which were taken from waters in the designated area after a time so specified;

(d) the supply, or the possessing for supply, to purchasers or others of any food or feeding stuff, or anything from which food or feeding stuffs could be derived, which was in the designated area after a time so specified;

APPENDIX E

(unofficial translation)
REGULATIONS CONCERNING DUMPING OF MATERIALS
FROM OFFSHORE INSTALLATIONS - DENMARK

Proclamation no. 394 of July 17, 1984 from the ministry of environment.

Regulation concerning dumping of matter and materials into the ocean from certain offshore installations.

According to section 32, section 42 article 2 and section 61 in law no. 130 of April 9, 1980 concerning protection of the marine environment the following laid down:

Chapter 1.
Definitions and area of use.

Section 1. An offshore installation is in this regulation platforms and other installations used in connection with the search for, production of, drilling for, and recovery of, submarine minerals, and also pipelines and other installations for the transport of these minerals.
Article 2. Drillship will be considered as an offshore installation, when these are carrying out searches for, production of, drilling for, or recovery of submarine minerals.

Section 2. Submarine minerals are in this regulation hydrocarbons and other raw materials in the subsoil under the ocean floor.

Section 3. Dumping in this regulation includes all transfer of matter or materials to the sea, when as a direct result

of the search for, including well testing and recovery of, including production drilling, raw material in the subsoil under the ocean floor.

Section 4. The regulation is in effect for offshore installations which operate in Danish territorial water or on the Danish continental shelf.

Chapter 2.
Application.

Section 5. Dumping of matter or materials from an offshore installation may take place only after approval from miljostyrelsen (a part of the ministry of environment).
Article 2. The owner or the user of the offshore installation must make an application, which is the basis for the approval from miljostyrelsen.

Chapter 3
Search for hydrocarbons.

Section 6. Application for a dumping license in connection with the search for hydrocarbons shall include information about the following items:

1) Type of offshore installation, geographical position, and the purpose of the search.

2) Ecology of the area, including physical and chemical conditions, and biological resources. The information can be used on already existing research results.

3) The amount, the composition, the biodegradability, toxicity and removal methods of the drilling mud.

4) Use of chemicals: Amount, composition, analysis, detoxification, and disposal methods.

5) Cuttings: Amount, the most likely composition, analysis, detoxification, and disposal methods.

6) Environment protection arrangements in connection with well testing.

Section 7. Miljostyrelsen can put down conditions in the license mentioned in section 5.
Article 2. If nothing is stipulated in the license, this is in effect as long as the search on this fixed position is continued, see however section 10.

Chapter 4.
Production drilling and recovery of hydrocarbons.

Section 8. An application for a dumping license in connection with production-drilling and recovery of hydrocarbons shall include information about the following areas:

1) Type of offshore installation, geographical position, and the purpose of recovery.

2) Ecology of the area, including physical and chemical conditions, and biological resources. The information can be based on already existing research results.

3) The amount, the composition, the biodegradability, toxicity, and disposal methods of the drilling mud.

4) Cuttings: Amount, the most likely composition, analysis, detoxification, and disposal methods.

5) Production water and displacement water: Amount, composition, analysis, detoxification, and dumping methods.

6) Use of chemicals: Amount, composition, analysis, detoxification, and dumping methods.

Section 9. Miljostyrelsen can put down conditions in the license mentioned in section 5.

Chapter 5.
Control and the like.

Section 10. Miljostyrelsen can, if it finds it is necessary in connection with research, production drilling, or recovery, require that the owner or the user of an offshore installation carry out investigations and arrangements to control and preserve the environment of the surrounding sea.
Article 2. Miljostyrelsen can put down directions for this work, among these if necessary allowing the work to be done at the owner's or the user's expense.

Section 11. It is forbidden to throw tools, materials, and waste from the research, production drilling, or recovery operations into the sea.
Article 2. All larger equipment, which is used on the offshore installation or is transported to or from this installation, shall be marked according to the regulations from miljostyrelsen.

Section 12. It is the duty of the owner or the user of an offshore installation to inform miljostyrelsen about the end of the search, production drilling, or recovery.

Chapter 6.
Control and withdrawal of the license.

Section 13. Miljostyrelsen ensures that the rules in this regulation are kept.

Section 14. The owner or the use of an offshore installation shall give all the information which is necessary for miljostyrelsen to control offshore installation activity affected by this regulation. Miljostyrelsen can as far as necessary for this control require from the owner or the user of the offshore installation the sending in of samples, raw data, work results, interpretations, evaluation and technical and economic information.

Section 15. If there is any change in the conditions on which the license is based, the owner or the user of the offshore installation shall immediately inform miljostyrelsen.
Article 2. Miljostyrelsen can at any time change or withdraw a license given by this regulation, if the conditions in this regulation or in pursuance of this regulation are not kept, or if anything in the application given is untrue or misleading information, or major changes in the circumstances.

Chapter 7.
Penalty and start date.

Section 16. If not guilty of a higher penalty under another law, the penalty for the person, who

1) Dumps matter or materials from an offshore installation, without license from miljostyrelsen, see section 5.

2) Ignores the conditions put down in license according to section 7 and section 9.

3) Fails to hand in information or samples according to section 14 and section 15.

4) Violates the rules about control and detoxification after sections 10-12.

is fined or sent to ordinary imprisonment for up to one year.

Section 17. For violations of the rules mentioned in section 16 a penalty can be placed on the owner or the user of the offshore installation, even if the violation was not intentional or negligent.

Section 18. For violation done by corporations, co-operative societies and the like, the penalty can be placed on the company.

Section 19. The regulation is in effect from October 1, 1984, and is used in the search for, production, drilling, and recovery, of all which is given by license after this date. For existing installations the regulation is effective after renewal of a production license, however for not less than 1 year after this regulation is effective.

The ministry of environment, July 17, 1984.

Christian Christensen/Peter Skak-Iversen.

APPENDIX F

STATUTES OF THE REPUBLIC OF SOUTH AFRICA - FISHERIES

SEA FISHERIES ACT
NO. 58 OF 1973

10. Protection of fish and restrictions on landing of fish and supplying of ships' stores. - (1) The Minister may by notice in the Gazette make regulations -

(a) Prohibiting for an indefinite period or for a specified period, and either generally or in a specified area, the catching or disturbing of fish or fish belonging to a specified species, or the catching or disturbing of any such fish by a specified person or persons belonging to a specified category or any person other than a specified person or persons belonging to a specified category;
[Para. (a) substituted by s. 6(a) of Act No. 61 of 1979.]

(b) Prohibiting the landing of fish or fish belonging to a specified species in any specified area at any place other than a specified place;

(c) Prohibiting the conveyance or the removal from one place to another of any fish or fish products without the written authority of the director and otherwise than subject to such conditions as he may determine;

(d) Regulating or prohibiting the supply of ships' stores, excluding medical supplies, to any fishing boat or any vessel registered in any foreign State and used as a fishing boat or factory, and imposing a levy for the benefit of the State Revenue Fund on stores supplied to such fishing boats and vessels, and prescribing how and by whom any such levy shall be collected.

[Para. (d) substituted by s. 4 of Act No. 99 of 1977.]

(2) Regulations under subsection (1)(a), (b) and (c) may grant exemption from the provisions thereof, whether or not subject to any conditions, in respect of specified quantities of fish caught by any person for his own use or for any other specified purpose.

[Sub-s. (2) substituted by s. 6(b) of Act No. 61 of 1979.]

APPENDIX G

RECOMMENDED GENERAL GUIDELINES FOR EVALUATING TAINT

G1 - DRAFT GUIDELINES FOR EVALUATING THRESHOLD VALUES FOR FISH-TAINTING - GESAMP 1983

INTRODUCTION

These Guidelines are only intended for the purpose of evaluating the hazards of harmful substances carried by ships and relate specifically to the column A "T"-rating in the hazard profiles as developed by the Working Group and approved by GESAMP (e.g. Reports and Studies No. 17). The following factors are important and should be taken into account in considering the design, conduct and interpretation of tainting tests and the test results.

The Guidelines consist of two parts which do not necessarily need to be undertaken within a single laboratory or institution:

Part 1: Exposure of Fish
Part 2: Assessment of Threshold Concentrations for Fish Tainting

1. EXPOSURE OF FISH

1.1 General Considerations

- Water solubility
- Vapour pressure
- Purity of the substance
- Octanol/water partition coefficient

- Chemical stability of substance in water and light
- Molecular weight of substance to be tested
- Method used for chemical analysis of concentrations in water

1.2 Test Organisms

The Working Group suggests that commercial fin fish with a moderate fat content (5-10%) and which can be maintained in aquaria should be used as test animals. Acceptable species are, e.g. Salmo gairdneri (rainbow trout), Mugil cephalus (mullet). Sufficient fish should be exposed to give 20 grams of wet flesh, preferably obtained from several individual specimens for each test concentration. More fish flesh is required for the reference.

1.3 Exposure Concentrations

There should be at least three exposure concentrations and a "blank" (zero concentration) for the final test, spaced by a factor of preferably $\sqrt{10}$ (0.32) made up without using any adjuvants (such as solvents, emulsifiers or dispersants). Exposure maximum concentrations are to be found from a preliminary range finding test. They should be lower than the 24 h LC_{50} by a factor of 10.

The concentration of the test substance in the water should be determined by chemical analysis as necessary to allow estimation (calculation?) of the actual concentrations to which the fish were exposed during the whole of the exposure period.

1.4 Test Conditions

The test should be carried out preferably with seawater. The exposure period should be at least 24 hours.

Static conditions are acceptable provided that during the exposure period the test substance concentration will not drop below 50% of the initial concentration.

Semi-static (discontinuous renewal) conditions or continuous flow systems could be an alternative, e.g. in case of high losses of test substance from the water phase or when low oxygen concentrations are expected.

The oxygen concentration should be high enough to keep the fish healthy. Aeration is allowed unless by this the concentration of the test substance drops below 50% of the initial value.

The temperature should be suitable for the species chosen.

The volume of water used should be at least 1 litre of water per gram of fish in a static system (or the equivalent in a semi-static or continuous system) per 24 hours.

Only fish behaving normally at the end of the exposure period should be used by the taste panel.

2. EVALUATION OF TAINT

2.1 Harvesting of the Fish

At the end of the exposure period the fish should be harvested from the tank and allowed to die by suffocation or killed by a blow to the head. The fish shall be gutted immediately and allowed to bleed. If the fish are not to be tasted within two hours they should be stowed in crushed ice prepared from potable water at a ratio of at least one part of ice to three parts of fish by weight. The fish shall be tested within 48 hours of harvesting.

2.2 Preparation of Samples

All utensils and equipment used for preparing and holding samples should be free of taints. Where the same equipment is used successively to prepare samples the lowest concentrations should be processed first then successively to the highest concentration, cleaning the equipment between each sample.

The fish may be washed briefly in potable water to remove blood, slime or ice. The flesh, including the belly flaps, should be removed and freed from skin and membranes. The flesh from all the fish should be passed through a coarse mincer and thoroughly mixed.

2.3 Cooking

The fish should be cooked in one of the following ways:

.1 Casserole method. 150 g of material is placed in a casserole with a loose fitting lid. The

casserole is suspended over or in boiling water or steam for a period just sufficient to cook the fish. This period should be determined from prior experimentation; or

.2 Boil-in-the-bag method. 150 g of material should be put into a plastic bag intended for cooking foods in boiling water. The bag should be weighted by putting into it glass rods or weights and the bag should be closed loosely and clipped. The bag should be suspended in boiling water or steam for a period just sufficient to cook the fish. This period should be determined by prior experimentation.

2.4 Selection of Assessors

At least eight assessors should be used. They should have experience in evaluating the quality of fish and be familiar with the normal flavour of the species of fish used in the test. They should not be specially selected for acuity to the chemical under test.

2.5 Conduct of Test

Considering the samples as containing increasing concentrations of the test chemical the concentration at which tainting occurs is determined by taste threshold test essentially the same as that described in ASTM 679-79*.

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* Standard practices for determination of odour and taste thresholds by a forced-choice ascending concentration series method of limits. American Society for Testing and Material, Philadelphia, U.S.A.

Each sample of test material is presented to the assessors as a triangle test in comparison with the reference (zero concentration). If the test is referred to as A and the reference as B then there are two combinations of presentation - AAB and BBA. The test should be conducted so that at each test concentration each combination should be presented as far as possible an equal number of times. In addition, for each assessor, the series of samples should be presented in the two combinations in random order.

The assessor is presented with a set of three identical receptacles containing at least 15 g of sample in each in one of the two combinations. The receptacles should be coded with a 3-digit random number. The assessor is required to indicate the odd sample. The assessor should make a choice even if the selection is by guessing. The test samples are presented in the sets in increasing concentration in the test environment.

2.6 Recording and Calculating the Result

The outcome of each test is recorded as an incorrect, 0, or a correct, +, selection. For each assessor the outcomes are expressed as a series of correct or incorrect judgements. Reading down from the highest concentration to the lowest the threshold for that assessor is the geometric mean of the lowest concentration with a correct assessment and the next lowest concentration with an incorrect assessment ignoring any lower concentrations with a correct assessment.

[Example here]

3. DATA AND REPORTING

A full description of the test method used should be given for the exposure part and for the tainting evaluation. Concerning the exposure period, at least the results of all chemical analysis and the test temperature should be given.

The panel threshold is the geometric mean of the assessors' thresholds.

The size of the panel and the individual threshold values should be reported together with the panel threshold values.

APPENDIX G

RECOMMENDED GENERAL GUIDELINES FOR EVALUATING TAINT

G2 - DRAFT GUIDELINES FOR EVALUATING THRESHOLD VALUES FOR FISH-TAINTING - ECETOC TASK FORCE 1984

1. Introduction

These Guidelines are only intended for the purpose of the Working Group on the Evaluation of the Hazards of Harmful Substances Carried by Ships and relate specifically to the column A "T"-rating in the hazard profiles as developed by the Working Group and approved by GESAMP (e.g. Reports and Studies No. 17). The following factors are important and should be taken into account in considering the design, conduct and interpretation of the list and the test results.

It is recommended that the evaluation be carried out in three stages:

1. Preliminary assessment of taint.

2. Definitive test to determine the threshold concentration for fish tainting.

3. Assessment of taint retention.

No taint detected at Stage 1 will require no further testing. A taint present at the first stage will require Stages 2 and 3 to be carried out in sequence.

The Guidelines consist of two parts which do not

necessarily need to be undertaken within a single organization:

Part 1: Exposure of Fish
Part 2: Assessment of Threshold Concentrations for Fish Tainting

Part 1: EXPOSURE OF FISH

2. General Considerations

- Water solubility
- Vapour pressure
- Purity of the substance
- Octanol/water partition coefficient
- Chemical stability of substance in water and light
- Method for chemical analysis in water
- Molecular weight of substance to be tested

3. Test Organisms

The Working Group suggests that commercial fin fish with a moderate fat content (5-10%) and which can be maintained in aquaria should be used as test animals. Acceptable species are, e.g. Salmo gairdneri (rainbow trout), Mugil cephalus (mullet). At each exposure concentration sufficient fish should be exposed to give a minimum of 200 grams of wet flesh (approximately 500 grams of fish), obtained from a minimum of five individual specimens. More fish flesh is required for the reference.

The test population should come from the same source and have been fed a bland diet. They should be sampled prior to testing for any strong or unusual flavour.

4. Exposure Concentrations

The preliminary assessment requires only one exposure concentration at one tenth of the 24 hour LC50. The definitive test should have a minimum of five exposure concentrations spaced by a factor of $\sqrt{10}$ (0.32). A blank (zero concentration) must be run with each test. No adjuvants (e.g. solvents, emulsifiers or dispersants) should be used in the preparation of the test solutions as these may add strong or unusual flavour to the flesh.

The concentration of the test substance in the water should be determined by chemical analysis as necessary to allow estimation (calculation?) of the actual concentrations during the whole of the exposure period.

5. Test Conditions

The test should be carried out in seawater. The exposure period should be at least 24 hours.

Continuous flow test systems are recommended but static or semi-static (discontinuous renewal) are acceptable provided that during the exposure period the concentration of test material does not drop below 50% of the initial value.

The oxygen concentration should be high enough to keep the fish healthy. Aeration is allowed unless by this the concentration of the test substance drops below 50% of the initial value.

The test should be carried out at 15 ± 1°C.

The water provided should be at least 1 litre of water per gram of fish in a static system (or the equivalent in a semi-static or continuous system) per 24 hours.

Only fish behaving normally at the end of the exposure period should be used by the taste panel.

Part 2: EVALUATION OF TAINT

1. Harvesting of the Fish

At the end of the exposure period the fish should be harvested from the tank and allowed to die by suffocation or killed by a blow to the head. The fish shall be gutted immediately and allowed to bleed. If the fish are not to be tasted within two hours they should be stowed in crushed ice prepared from potable water at a ratio of at least one part of ice to three parts of fish by weight. The fish shall be tested within 48 hours of harvesting.

2. Preparation of Samples

All utensils and equipment used for preparing and holding samples should be free of taints. Where the same equipment is used successively to prepare samples the lowest concentrations should be processed first then successively to the highest concentration, cleaning the equipment between each sample.

The fish may be washed briefly in potable water to remove blood, slime or ice. The flesh, including the belly flaps, should be removed and freed from skin and membranes. The flesh from all the fish should be passed through a coarse mincer and thoroughly mixed.

3. Cooking

The fish should be cooked in one of the following ways:

.1 Casserole method. 150 g of material is placed in a casserole with a loose fitting lid. The casserole is suspended over or in boiling water or steam for a period just sufficient to cook the fish. This period should be determined from prior experimentation; or

.2 Boil-in-the-bag method. 150 g of material should be put into a plastic bag intended for cooking foods in boiling water. The bag should be weighted by putting into it glass rods or weights and the bag should be closed loosely and clipped. The bag should be suspended in boiling water or steam for a period just sufficient to cook the fish. This period should be determined by prior experimentation.

.3 Microwave cooking. (It might be possible to include microwave cooking among the cooking procedures but some investigation is required to determine the optimum conditions.)

After cooking, the test material should be dispensed into the containers in which it will be presented to the assessors (Section 5), kept warm and assessed as soon as possible.

4. Selection of Assessors

At least fifteen assessors should be used. They should have experience in evaluating the quality of fish and be familiar with the normal flavour of the species of fish used in the test. They should not be specially selected for acuity to the chemical under test.

5. Conduct of Test

Considering the samples as containing increasing concentrations of the test chemical the concentration at which tainting occurs is determined by taste threshold test essentially the same as that described in ASTM 679-79*.

The material at each test concentration is presented to the panel of assessors as a triangle test in comparison with the reference (zero concentration). Detailed instructions for carrying out the triangle test are given in ISO 4120-1983, 'Sensory analyses - Methodology - Triangular test' or the equivalent national standard. The following is a summary of the procedure.

An assessor is presented with a set of three identical, odour-free receptacles containing at least 15 g of sample. The receptacles are coded with 3 or 4-digit random number. Two samples are identical and the third is different. The assessor is required to select the single sample.

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* Standard practices for determination of odour and taste thresholds by a forced-choice ascending concentration series method of limits. American Society for Testing and Material, Philadelphia, U.S.A.

The pair of identical samples can be from the test material or from the reference but across all the assessors there should be an equal (or near equal in the case of an odd number of assessors) presentation of the 2 possibilities. Further, within each of these 2 possibilities there are 3 ways the receptacles can be ordered when presented to the assessors giving 6 combinations in all. If the test and reference materials are denoted as A and B the 6 combinations are AAB, ABA, BAA, BAB and ABB. As far as possible considering the number of assessors the combinations should be presented an equal number of times and distributed randomly among the assessors.

The assessment proceeds by presenting material from the test concentrations successively to assessors as triangle tests starting with the lowest concentration. At each concentration the proportion of assessors who correctly identify the odd sample is recorded. The assessors should be encouraged to make a decision but in the case that reference and test are so similar that an assessor is unwilling to make a choice this should be recorded as a 'no difference' response. One third of the number of no difference responses (if any) should be allocated to the number of correct responses when calculating the proportion of correct responses.

6. Recording and Calculating the Result

7. Data and Reporting

A full description of the test method used should be given for the exposure part and for the tainting evaluation. Concerning the exposure period, at least the results of all chemical analysis and the test temperature should be given.

The size of the panel and the individual threshold values should be reported together with the panel threshold values.