# METHOD DEVELOPMENT FOR THE ANALYSIS OF TAINTING COMPOUNDS

by

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## 1. RÉSUMÉ À L'INTENTION DE LA DIRECTION

On a effectué une étude de la littérature en vue d'établir une liste des composés qui altèrent les propriétés organoleptiques du poisson, en se basant sur la fréquence à laquelle ces composés sont cités et sur le seuil d'altération perçue. On a choisi, dans la cadre de cette étude, les composés suivants qui serviront de composés modèles lors de la mis au point de méthodes: benzène, toluène, o-xylène, éthylbenzène, tétraméthylbenzène, naphtalène, 2-méthylnaphthalène, di- et triméthylnaphtalènes, thianaphtène, dibenzothiophène, quinoléine, phénol et m-crésol. L'extraction était réalisée par digestion en présence d'une base puis par distillation à la vapeur, et l'extrait séché était analysé directement par CG capillaire/SM. On a élaboré un protocole complet pour le dosage de ces composés d'altération et pour l'analyse quantitative de substituts marqués avec des isotopes.

Des études de récupération realisées avec des tissus de poisson et de mollusque auxquels on avait ajouté les composés d'altération visés ont révélé que la méthode mise au point permet de doser avec précision les composés basiques et non polaires à faible point d'ébullition, mais que le taux de récupération des composés acides (comme le phénol et le m-crésol ) et moins volatils (comme le dibenzothiophène et les composés à point d'ébullition plus élevé) est faible on non satisfaisant. Le taux de récupération des composés basiques est faible, mais on peut les doser de façon sûre en utilisant des analogues perdeutérés.

Les limites de détection pour un échantillon de tissu de 20 g se situent, selon le composé, entre 4 et 80 ng/g peuvent être plus élevées en présence de concentrations de fond plus grandes dans les blancs d'analyse.

## 1. EXECUTIVE SUMMARY

A literature survey and review was carried out to select a list of compounds identified as fish tainting agents based on their frequency of citation and perceived tainting threshold. Based on this survey, the following compounds were selected as model compounds for method development: benzene, toluene, o-xylene, ethylbenzene, tetramethylbenzene, naphthalene, 2-methyl naphthalene, di- and trimethylnaphthalenes, thianaphthene, dibenzothiophene, quinoline, phenol and m-cresol. Base digestion followed by steam distillation was selected as the extraction method with direct analysis of the dried extract by capillary GC/MS. A complete protocol for the determination of these tainting compounds and quantitation by isotopically labelled surrogates was developed.

Recovery studies of fish and mollusc tissues fortified with target tainting compounds indicated that basic and non-polar, low boiling compounds can be determined with good precision by the developed method while acidic (e.g., phenol and m-cresol) and less volatile compounds (e.g., dibenzothiophene and higher boiling) show low or unsatisfactory recoveries. Basic compounds show low recoveries but may be reliably quantitated by the use of perdeuterated analogues.

Detection limits for a 20 g tissue sample are in the 4 to 80 ng/g range, depending on compound, and these limits may be raised by elevated background procedural blank levels.

## 2. OBJECTIVES

The objectives of this project were:

- to select, based on a literature survey, a list of petroleum related compounds identified as fish tainting agents;
- to review existing analytical methods for the determination of tainting compounds; and
- to develop a method for the determination of the selected compounds at the part per billion (ppb) level in fortified fish tissues.

# 3. LITERATURE REVIEW AND METHOD SELECTION

## 3.1 Introduction

Tainting has been defined as the development in an organism of a flavour or odour which is not typical of the flavour or odour of the organism itself (Gesamp, 1977). Potential sources of tainting include hydrocarbons from sites of development of oil and gas resources.

Since the approval of oil and gas development off Canada's East Coast, the public and the fishing industry have expressed concerns about the possible effects of oil spills and chronic releases from the development sites. The literature contains several reports of tainting as a result of exposure of fish to petroleum hydrocarbons (e.g., GESAMP, 1977; Scarratt, 1980; Motohiro, 1983; and Tidmarsh *et al.*, 1986).

However, taint, by definition, is a matter of sensory perception by one or more individuals and it is therefore difficult to monitor and quantify. Chemical analysis of the tainted tissue identifies the types and quantities of various hydrocarbons present but does not necessarily identify the taint producing agent. Also, the level of contaminants at which a taint can be detected varies with the fish species affected and the specific contaminant present.

Other factors complicating the study of taint include the lack of consensus on which hydrocarbon compounds are responsible and the difficulty in monitoring the potential range of compounds in fish tissue. Howgate *et al.* (1977) point out that taints in flesh do not necessarily arise by petroleum contamination. In cases where tainting of the flesh is attributed to petroleum contamination, hydrocarbons derived from the contaminating source may be found in the flesh but these hydrocarbons are not necessarily responsible for the taint.

A literature review has been conducted to determine the current state of knowledge of fish tainting by petroleum hydrocarbons. The objectives of this review were (i) to derive a list of candidate compounds which have been shown to cause taint or to be closely associated with it; and (ii) to select appropriate analytical methods for analyzing fish tissues for the target compounds.

## 3.2 Literature Surveyed

A literature search was conducted using a combination of manual and computer searching techniques. The journals at the Fisheries and Oceans Canada library at the Institute of Ocean Sciences in Sidney, B.C. were surveyed and the following publications were determined to be of possible relevance.

Marine Pollution Bulletin
Analytical Chemistry
Marine Environmental Research
Water Pollution Research
Sea Grant Abstracts
Marine Biology
Marine Chemistry
Journal of Environmental Toxicology and Chemistry.

The 1990 and current 1991 volumes of these publications were searched manually. This revealed some papers of interest but was also useful in developing a strategy for the subsequent computer searches.

Computer searches were conducted of several data bases. A search on the CD ROM version of ASFA (Aquatic Sciences and Fisheries Abstracts - last update December 1990) was conducted. An on-line computer search was conducted of the following data bases: Food Science and Technology Abstracts (last update February 1991), ELIAS (Environment Canada Library Network - last update March 1990), Chemical Abstracts (1982-1986) and Chemical Abstracts 1987 (last update mid-February 1991).

These searches turned up many publications on the topic of fish tainting (see References, Section 19 of this report for a complete listing). Those references providing information relating to the two objectives of the current study are summarized in the following sections, (section 3.3 and 3.4).

## 3.3 Compounds Associated with Petroleum Tainting of Marine Organisms

The review of the literature conducted to determine which compounds have been shown to cause taint or be associated with it identified many specific compounds present in

tainted fish, but few papers have described threshold concentrations of these compounds.

Motohiro and Iseya (1976) conducted laboratory studies to detect organoleptically the taint in scallop caused by various hydrocarbons. They found that n-tetradecane and/or n-hexadecane did not cause a taint at the 0.3 mg/g (300 ppm) level while crude oil, xylene and toluene caused taint when present at the 0.1 to 0.2 mg/g level.

$C_{11}$ - $C_{22}$ n-paraffins, 5-ethyltridecane and 8-propylpenta- decane were detected in brown trout (Salmo trutta L.) caught eleven days after a diesel spill in the area (Mackie *et al.*, 1972).

Shipton *et al.* (1970) determined the composition of a volatile extract from mullet (*Mugil cephalus*) possessing a kerosene taint to be n-tetradecane, naphthalene, 2-methyl-naphthalene, 1-methylnaphthalene and possibly methyl-isopropyl-benzene, 3-(2-methylphenyl)pentane, 2,6-dimethyl-1,2,3,4-tetra-hydronaphthalene and 2,3-dimethyl-1,2,3,4-tetrahydronaphthalene.

Kameda and Yasumoto (1974) found that oily taint fish contained $C_9$-$C_{11}$ hydrocarbons, alkylbenzene, olefins and a trace of compounds containing sulphur.

Connell *et al.* (1975) found kerosene-like hydrocarbons ($C_9$ -$C_{15}$ n-alkanes) in bream fish (Mylio australis) exposed to kerosene and judged to have a kerosene-like taste. Connell (1974) also found a similar mixture of hydrocarbons in tainted sea mullet (Mugil cephalus). These compounds were found at concentration levels ranging from 25 to 500 mg/kg.

Laboratory studies conducted by Ogata and Miyake (1973) identified monoaromatic compounds such as toluene, benzene and o-, m- and p-xylene in tainted fish and eels. Later Ogata *et al.* (1979) conducted laboratory experiments to identify substances transferred to fish and shellfish from petroleum suspension. Eels and short-necked clams were found to take-up alkylated naphthalenes and dibenzothiophene. The clams also contained alkylated dibenzothiophenes.

Paradis and Ackman (1975) analyzed lobster tissue which had been exposed to diesel fuel and were judged to be tainted. They found low levels (2 to 4 ppm) of hydrocarbons in the tissue. Its composition was very similar to that of marine diesel characterized as

primarily straight chain hydrocarbons from $C_9$ to $C_{24}$ superimposed on a broad envelope region of unresolved minor peaks and high levels of pristane and phytane.

GESAMP (1977) reviewed available data for tainting thresholds of various hydrocarbons. They reported a threshold of 5 ppm for kerosene spiked into muscle tissue (Kerkhoff, 1974), 4 to 12 ppm of diesel oil components in lobster (Paradis and Ackman, 1975) to 10 to 30 ppm of crude oil in spiked tissue (Whittle and Mackie, 1976). Kerkhoff (1974; cited by GESAMP, 1977) reported that the middle distillate fraction of crude oil, e.g., diesel fuel contains many of the odorous compounds present in the crude. Diesel in water can be detected nasally at 0.0005 ppm while fuel and crude oils can only be detected at 0.1 to 0.5 ppm.

Brandl *et al.* (1976) simulated the effects of an oil spill on salmon kept in the laboratory. Tainting was observed and the components of significance in the tissue were found to be alkyl-naphthalene and benzene compounds. The taint threshold level of 0.3 ppm naphthalene was reported in this study.

McGill *et al.* (1987) studied dabs (*Limanda limanda*) caught near the Beatrice oil platform. Although contamination from petrogenic hydrocarbons (n-alkanes and polynuclear aromatic hydrocarbons) was apparent, the fish were not found to have a taint.

Connell and Miller (1981) published a review article on the topic of the effects of sublethal concentrations of hydrocarbons in the marine ecosystem and include a list of compounds which can cause tainting of fish flesh and other aquatic organisms. The estimated threshold level of each compound in water concentration that can cause tainting is also given. These data indicate that the chlorophenols, cresols, kerosene and butylmercaptan have the lowest thresholds in water.

From these various publications a list of "hits" has been determined. These are compounds which have been implicated in past studies on tainting. This list of hits or candidate compounds is given in Table 3.2 along with the number of references to that compound and an estimate of the threshold value.

**TABLE 3.2 Compounds Identified as Taints Ranked By Frequency of Citation**

| Compound Name | Compound Type | Citations in Literature |
|---|---|---|
| Naphthalene | PAH | 12 |
| C9-C13 n-Alkanes | Alkane | 8 |
| C1-Naphthalene | Alkylated PAH | 8 |
| Xylenes | Volatile-Aromatic | 7 |
| Toluene | Volatile-Aromatic | 6 |
| C2-Naphthalene | Alkylated-PAH | 6 |
| Propylbenzenes | Alkylated-Benzene | 4 |
| C14-C20 n-Alkanes | Alkanes | 4 |
| Trimethylbenzenes | Alkylated-Benzene | 3 |
| Thiophene | S-Hetero Aromatic | 3 |
| Ethylbenzene | Volatile-Aromatic | 3 |
| 3(2-Methylphenyl)Pentane | Volatile-Aromatic | 3 |
| 2,6-Dimethyl-Tetrahydronaph. | Alkylated-PAH | 3 |
| Dichlorophenols | Chlorophenol | 3 |
| Methyl Isopropylbenezne | Volatile-Aromatic | 3 |
| Dibenzothiophene | S-Hetero PAH | 3 |
| Pyridine | N-Hetero aromatic | 3 |
| n-Butylmercaptan | Mercaptan | 2 |
| 1-Decene | Olefin | 2 |
| Ethanethiol | Mercaptane | 2 |
| Chlorophenols | Chlorophenol | 2 |
| Alkyl Dibenzothiophenes | S-Hetero | 2 |
| C3-Naphthalene | Alkylated-PAH | 2 |
| Dichlorobnezenes | Chlorinated Volatile-Aromatic | 2 |
| Phenol | Phenol | 2 |
| Butylbenzenes | Volatile-Aromatic | 2 |
| Styrene | Volatile | 2 |
| Naphthols | Phenol | 2 |
| Quinoline | N-Hetero Aromatic | 2 |
| Alkyl-Chlorophenols | Chlorophenol | 2 |
| Cresols | Phenol | 2 |
| Acetophenone | OHC | 2 |
| Benzene | Volatile-Aromatic | 2 |
| Fluorene | PAH | 1 |
| Thiophenol | Phenol | 1 |
| Dibenzofuran | PAH | 1 |
| Tetramethylbenzene | Volatile-Aromatic | 1 |
| Butanol | OHC | 1 |
| Diphenyl Ether | Aromatic-Ether | 1 |
| Alkyl Styrene | Volatile-Aromatic | 1 |
| Dimethylamine | Amine | 1 |
| Benzothiophene | S-Hetero | 1 |
| Cyclohexene | Olefin | 1 |
| Pristane | Alkane | 1 |
| Isopropylphenol | Phenol | 1 |

## 3.4 Survey of Analytical Methods for Tainting Compounds

Many of the tainting papers reviewed do not discuss analytical methods and those which do usually only refer to a particular subset of the candidate compounds. Based on the list given in Table 3.2, it is evident that a goal in selecting an analytical method should be that of finding a method suitable for the range of compounds of interest; i.e, the neutral volatile compounds such as the alkanes and aromatics as well as the more polar nitrogen and sulphur containing compounds. A second goal is the selection of a method suitable for the very volatile compounds of interest. This suggests that an ideal method would be one which the distillate or extract should be analyzed directly with the need for solvent removal or a concentration step.

Connell (1974) used an extraction-steam distillation method to recover volatile hydrocarbons (alkanes) from tainted tissue. The material was first serially extracted with ether. The extract was concentrated to a small volume at 100°C, steam distilled and the distillate concentrated again. Alkanes as low in molecular weight as nonane were detected with this procedure.

Ogata *et al.* (1979) used a base digestion extraction procedure for analyzing tainted eel tissue for organic sulphur compounds. The tissue was saponified in alkaline KOH and chromatographed on silica gel to remove interferences. Rotary evaporation was used to concentrate the extracts. Hydrocarbons as low in molecular weight as methylnaphthalene were detected in tissue analyzed by this procedure. More volatile compounds known to be present in the crude oil to which the biota had been exposed (alkylbenzenes) were not detected in the tainted tissue and it was concluded that they had likely been lost by volatilization during the rotary evaporation step of the analysis procedure.

Ogata and Ogura (1976) describe a steam distillation-head space analysis procedure for analyzing fish tissues for a variety of compounds including $C_6$ and higher molecular weight aliphatics, and monoaromatics.The fish flesh was mixed with distilled water and the mixture was steam distilled. The vapour was collected from the distillate by the head space gas method and analyzed directly by GC. The authors point out that this method may have some disadvantages because it assumes that all compounds will partition between the water and vapour phases in the same ratio. In fact more water soluble compounds are more retained in the aqueous phase and a bias in analysis results may occur in which the water soluble compounds such as aromatics are under reported.

McGill *et al.* (1987) described a procedure for monitoring alkanes and aromatics ranging from naphthalene to benzo(ghi)perylene in tainted dabs. Their procedure was a standard solvent extraction, silica column cleanup and gel permeation column fractionation into alkanes and aromatics. Solvent removal was by rotary evaporation. Data collected by this analysis procedure was not able to show a difference between control fish tissue and fish caught in the vicinity of an oil platform. This suggests that an inappropriate suite of compounds may have been monitored and that less volatile compounds should have been considered.

Shipton *et al.* (1970) extracted volatile hydrocarbons from frozen fish tissue by vacuum sublimation. The aqueous sublimate was concentrated and extracted into isopentane which was then concentrated by fractional distillation.

Ackman and Noble (1973) described a direct steam distillation method for isolation of hydrocarbons in the diesel boiling range from fish tissue. The distillate was concentrated to a small volume under a stream of nitrogen. Recovery of compounds as low in molecular weight as $C_{10}$ was reported but the authors also noted a lower than expected proportion of compounds in the $C_{10}$ to $C_{18}$ boiling range, suggesting that some volatility loss may have occurred.

Donkin and Evans (1984) described a steam distillation method for petroleum hydrocarbons in the volatility range encompassed by toluene and pyrene. Recoveries in excess of 80% were reported for hydrocarbons extracted from mussel tissues. The authors showed that saponification improved hydrocarbon recovery from mussel tissue. The steam distillation was carried out in the presence of sodium hydroxide and a two step procedure was used. The first step was a slow distillation designed to recover the volatile hydrocarbons. The distillate was removed after two hours and the procedure continued for fourteen hours at a higher distillation rate to recover "involatile alkanes". The distillates did not require cleanup prior to GC analysis.

### 3.5 Selection of Tainting Compounds

There is no consensus yet in the literature on what compounds cause taint in fish and other marine organisms.

In this study, our approach in selecting a list of candidate compounds for developing an analytical method is therefore dependent on a set of five criteria. These are: (i) the

number of times a compound was found cited for being taint-causing during the literature review; (ii) the estimated or reported activity of each compound as a tainting agent; (iii) the ability to analyze for the compound using readily available lab equipment, (iv) the representation of a particular compound for extrapolation to the rest of the compounds on the list; and (v) the presence of the compound in petroleum or petroleum products.

A list of candidate compounds was developed (see Table 3.3). This list includes volatile aromatic hydrocarbons, phenols, a nitrogen containing molecule (quinoline) and sulphur compounds (thianaphthene and dibenzothiophene). It is felt that these compounds represent the classes of compounds expected to be involved in fish tainting by petroleum sources. Saturated and branched alkanes are also abundant in petroleum products and are readily detected as conspicuous homologues series of peaks in a chromatogram. They are widely distributed in the environment and may be detected in fish from pristine areas. However, this group of compounds have generally high tainting thresholds (i.e., low potential for tainting), and although frequently implicated in tainting, they are unlikely to be the primary taints. Consequently saturated alkanes were not included in the list of candidate compounds.

Compounds with low tainting thresholds generally have a significant vapour pressure at 30°C and include volatile and semi-volatile compounds in the boiling point range of 70°-300°C. After an initial set of preliminary runs, this list was expanded to include additional alkyl naphthalenes and alkylbenzenes. The expanded list is given in Table 3.4.

### 3.6 Selection of an Analytical Method

Using the selected compound list (Table 3.2), an appropriate analytical method could be selected. Due to the volatile nature of most of the candidate compounds a requirement is that the procedure can contain no solvent removal step (such as rotary evaporation). The literature refers to a steam distillation method in several papers. The most promising results were obtained when the tissue was saponified during the distillation. That method (Donkin and Evans, 1984) was therefore selected as the starting point for evaluation in this study. It was realized, however, that the published procedure has only been validated for the non-polar compounds and that it might not perform properly for the more polar nitrogen and sulphur compounds. However, the method might be adapted to accommodate these compounds and work will proceed along

**TABLE 3.3 Initial Lists of Selected Target Compounds for Fish Tainting Study**

| Compound | Tainting Threshold In Water (a) (ppb) | Boiling Point °C |
|---|---|---|
| benzene | 250 - 1000 | 80 |
| toluene | 250 | 110 |
| ethylbenzene | 250 | 136 |
| O-xylene | 250 - 1000 | 144 |
| phenol | 1000 - 10,000 | 182 |
| m-cresol | 200 | 202 |
| naphthalene | 100 | 218 |
| thianaphthalene | | 221 |
| quinoline | 500 - 1000 | 238 |
| 2-methylnaphthalene | | 241 |
| 1,2-dimethylnaphthalene | | 266 |
| dibenzothiophene | | 322 |

(a) Source: Connell & Miller (1981)

## Table 3.4 Extended List of Selected Compounds for Fish Tainting Study

| Compound | Tainting Threshold in Water [(a)] (ppb) | Boiling Point (°C) |
|---|---|---|
| benzene | 250-1000 | 80 |
| toluene | 250-1000 | 110 |
| ethybenzene | 250 | 136 |
| o-xylene | 250-1000 | 144 |
| 1,2,3,4-tetramethylbenzene | - | 204 |
| napththalene | 1000 | 218 |
| 2-methylnaphthalene | - | 241 |
| 1,2-dimethylnaphthalene | - | 266 |
| 2,3-dimethylnaphthalene | - | 268 |
| 1,3-dimethylnaphthalene | - | 263 |
| 2-ethylnaphthalene | - | 251 |
| 2,3,5-trimethylnaphthalene | - | 285 |
| 2,3,6-trimethylnaphthalene | - | 263 |
| phenol | 1000-10000 | 182 |
| m-cresol | 70-400 | 202 |
| quinoline | 500-1000 | 238 |
| thianaphthene | - | 221 |
| dibenzothiophene | - | 332 |

(a) Source: Connell and Miller (1981)

these lines. The target compound list includes the relatively volatile compounds benzene and toluene which normally are determined under sub-ambient capillary gas chromatography (GC) conditions. To simplify the instrumented analysis, a solvent system was chosen that would allow the samples to be run without cryogenic cooling. The proposed GC method is readily implemented on most commercial gas chromatography-mass spectrometry (GC/MS) systems and lends itself to automation.

## 4. METHOD SCOPE AND APPLICATION

A fast and efficient analytical method is required which will allow the detection and identification of tainting compounds at ppb levels in fish tissues. This method should form the basis of an analytical protocol designed to monitor fish tainting in species of commercial interest.

The proposed method is designed to:

1) liberate the wide range of classes of tainting compounds while retaining the low boiling compounds extracted from tissue samples;

2) present neutral, acidic and basic compounds in one fraction for GC/MS analysis;

3) allow extracts to be analyzed with high sensitivity and precision using conventional GC/MS in a configuration found in many laboratories; and

4) provide ongoing sample performance data to qualify each analysis.

The inclusion of acidic compounds required pH adjustment steps and pH monitoring, adding complexity to the method. Results from three initial sets of analyses indicated erratic recoveries for this class of compounds. Subsequently, acidic compounds were dropped from the list of target compounds used to evaluate the method.

## 4.1 Summary of Methods

The determination of the selected fish tainting compounds was carried out by micro steam distillation followed by gas chromatographic-mass spectrometric determination. The steam distillation process is combined with a microextraction using the apparatus described by Kuehl and Dougherty (1980). This concentrates the analytes in a small volume of a volatile solvent (pentane) and the distillate contains negligible involatile material, that may be analyzed directly without the need of a clean-up step or solvent removal. Direct analysis retains the more volatile components in the extract. Analysis by capillary GC/MS in the Multiple Ion Detection (MID) mode provides the highest confidence of correct assignment and, with the use of perdeuterated analogues of the target compounds, allows the method to internally correct for losses and extraction deficiencies of the target compounds.

An initial series of characterization runs with spiked tissue were made using the preliminary method. A number of problems were evident which required some minor revisions to be made to the method during the method development phase of this work. Based on data from those runs, further revisions were made to attempt to improve method performance. Data from all runs were used for method evaluation.

### 4.1.1 Preliminary Method

Homogenized tissues spiked with selected tainting compounds and internal surrogate standards were extracted by steam distillation with pentane as the retaining solvent. The steam distillation process consisted of two steps; a first distillation was carried out under basic conditions (pH >12) for 30-40 minutes. This was followed by adjustment of the sample pH to 2 and a second distillation step for 30 minutes. The two extracts were combined, dried with previously baked sodium sulphate, spiked with recovery standard solution and analyzed by GC/MS.

### 4.1.2 Final Developed Method

A homogenized tissue sample was stirred with 1 M NaOH and allowed to stand at room temperature for two hours, followed by addition of surrogate standard and calcium chloride solution. Steam distillation was then carried out for 1.5 to 2 hours by using 30% dichloromethane in pentane. The combined pentane extracts were dried with

previously baked sodium sulphate, spiked with recovery standard solution and analyzed by GC/MS.

## 5. INTERFERENCES

Interference affecting the peak shape of d7-quinoline was noted during the course of this study also. Some of the target compounds are commonly present in lab and urban atmosphere and can be expected in the procedural blanks. In the course of this method development work, persistently high blanks were found for toluene, which is a common lab solvent. Isotope exchange between deuterium and hydrogen in toluene-d8 and to a lesser extent benzene-d6 was found to affect the quantification of low boiling compounds.

## 6. REAGENTS, MATERIALS AND APPARATUS

All solvents were pesticide grade (BDH, Omnisolv), these included pentane, methanol and dichloromethane.

Distilled water and 6 M HCl solution used for pH adjustment were extracted twice with dichloromethane and twice with pentane before use.

A 6 M sodium hydroxide solution was prepared by dissolving analytical reagent grade pellets in distilled water. The resulting solution was serially extracted twice with dichloromethane and twice with pentane before use.

Boiling chips were previously cleaned by soxhlet extraction.

All glassware used including 500 and 1000 mL round bottom flasks, separatory funnels, steam distillation condensers for low density solvent-water systems, reflux condensers, centrifuge and test tubes were cleaned with detergent and baked in a forced air oven at 350°C for eight hours before use.

The steam distillation apparatus used for this method is based on an exhaustive steam distillation and solvent extraction apparatus (Kuehl and Dougherty, 1980) and is shown in Figure 6.1. The apparatus is mounted on a 1 L round bottom flask and backed up

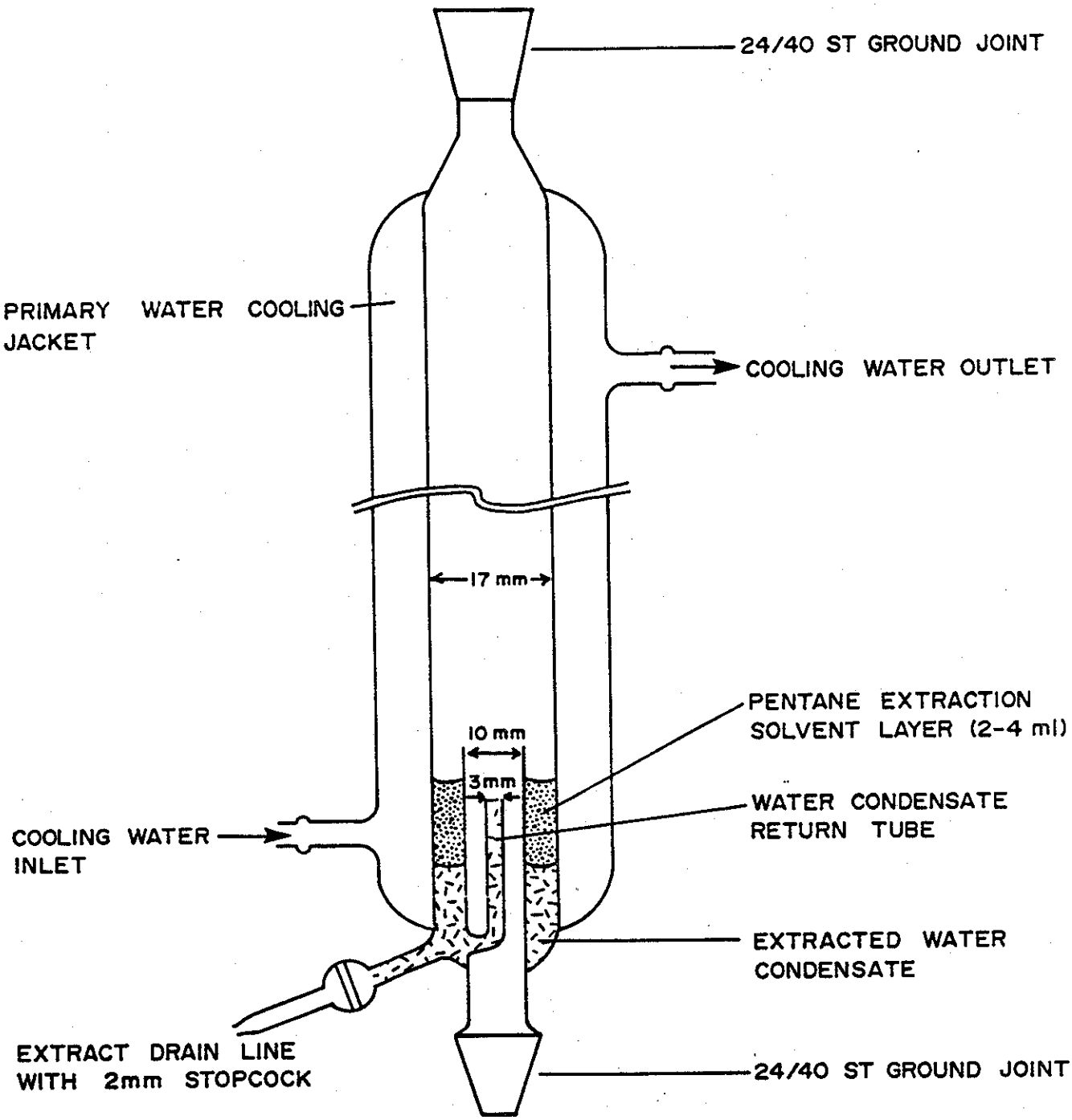



Fig 6.1
Microsteam distillation and
solvent extraction apparatus

with a 20 cm liebig condenser cooled with tap water (10°C). This design of apparatus allows steam distillable compounds to be concentrated in a small volume (2 to 4 mL) of a solvent less dense than water. The extract is withdrawn through the sidearm stopcock at the completion of the extraction.

## 7. SAMPLE COLLECTION, PRESERVATION AND STORAGE.

A selection of locally available fish and mollusc were used as matrices for this study. Two whole mackerel, cod fillets (500 g) and shelled scallops (500 g) were purchased.

Mackerels were gutted, filleted and homogenized in a stainless steel meat grinder. Cod fillets and scallops were also homogenized. Each set of tissue was wrapped in aluminium foil and stored at -10°C. A subsample of mackerel was allowed to spoil by storage at room temperature for approximately 15 days.

## 8. STANDARDS AND SURROGATES

Compounds selected as tainting compounds are referred to as target compounds, chemically labelled compounds used as internal standard surrogates are referred to as surrogates and standards added to extracts after extraction and immediately prior to GC/MS analysis are recovery standards.

Primary standards of each target, surrogate and recovery compound were prepared by weight in methanol and kept under cold storage. Working solutions for target compounds (FT-S) and internal surrogate standards (FT-I1) were prepared by dilution of appropriate primary standards in methanol. The working recovery standard solution FT-R was made by dilution of appropriate primary standards in pentane. The working calibration solution FT-C1 was prepared by mixing appropriate amounts of FT-I1, FT-R and FT-S and dilution with pentane. This solution was used for daily instrument calibration and to obtain relative response factors for each target and surrogate compound. A list of each solution components and their respective concentrations is given in Table 8.1.

## TABLE 8.1 CONCENTRATION OF WORKING STANDARDS - INITIAL SET

| Compound Name | FT-I Surrogate (µg/mL) | FT-C1 Calibration (µg/mL) | FT-R Recovery (µg/mL) | FT-S Spiking (µg/mL) |
|---|---|---|---|---|
| benzene | | 8.22 | | 205.4 |
| toluene | | 26.90 | | 672.7 |
| ethylbenzene | | 8.07 | | 201.6 |
| o-xylene | | 5.09 | | 127.4 |
| phenol | | 11.98 | | 299.4 |
| m-cresol | | 6.38 | | 159.6 |
| naphthalene | | 7.16 | | 179.0 |
| thianaphthene | | 5.87 | | 146.7 |
| quinoline | | 6.12 | | 153.1 |
| 2-methylnaphthalene | | 4.44 | | 111.0 |
| 1,2-dimethylnaphthalene | | 7.71 | | 190.1 |
| 2-ethylnaphthalene | | - | | - |
| 2,3-dimethylnaphthalene | | - | | - |
| 1,3-dimethylnaphthalene | | - | | - |
| 2,3,5-trimethylnaphthalene | | - | | - |
| 2,3,6-trimethylnaphthalene | | - | | - |
| 1,2,3,4-tetramethylbenzene | | - | | - |
| dibenzothiophene | | 2.93 | | 73.2 |
| d6-benzene | 99.7 | 3.99 | | - |
| d8-toluene | 103.1 | 4.12 | | - |
| d8-naphthalene | 100.6 | 4.03 | | - |
| d7-quinoline | 159.6 | 6.39 | | - |
| dibromophenol | 61.0 | 2.44 | | - |
| d5-phenol | | | | |
| d10-pyrene | 103.8 | | | |
| d22-n-decane | | 3.72 | 93.05 | |
| d8-dibenzofuran | | 2.21 | 55.22 | |
| pentane (%) | 0 | 92 | 100 | 0 |
| methanol (%) | 100 | 8 | 0 | 100 |

Additional compounds were added to the target and surrogate list during the course of this work. Subsequently, an expanded set of spiking, calibration and surrogate standards was prepared. These are listed in Table 8.2.

The final calibration solution contained less than 10% methanol in pentane. Preliminary tests demonstrated that up to 10% methanol in pentane was a satisfactory solvent system for GC/MS analysis. Methanol levels higher than 10% resulted is poor chromatography. Attempts to remove methanol by water extraction resulted in stripping of quinoline, phenol and cresol from the calibration solution, therefore this procedure was abandoned, and the calibration standard used as prepared containing 8% methanol.

## 9. DETAILED METHOD DESCRIPTION

### 9.1 Sample Extraction

A steam distillation apparatus consisting of a round bottom flask (1 L or 500 mL) equipped with two condensers was used throughout (Figure 6.1). Heating of the round bottom flask was accomplished by a rheostat-controlled heating mantle. Tap water was run in parallel through both lower and upper condensers.

Twenty grams of homogenized tissue were placed in a 500 mL or 1000 mL round bottom flask, 100 mL of pre-extracted 1 M NaOH were added, the flask stoppered, mixed by swirling and allowed to stand at room temperature for two hours. After digestion, 100 mL of 200 g/L solution of $CaCl_2$ , 200 µL of surrogate standard (FT-I1), boiling chips and 5 mL 10% dichloromethane in pentane were added and steam distilled for two hours. The pentane extract was withdrawn from the collection trap into a 15 mL centrifuge tube and dried over anhydrous sodium sulphate for 5 minutes. Then 100 µL of recovery standard (FT-R) were added, the extract mixed and transferred to an autosampler vial for GC/MS analysis. A flow chart describing this procedure is given in Figure 9.1.

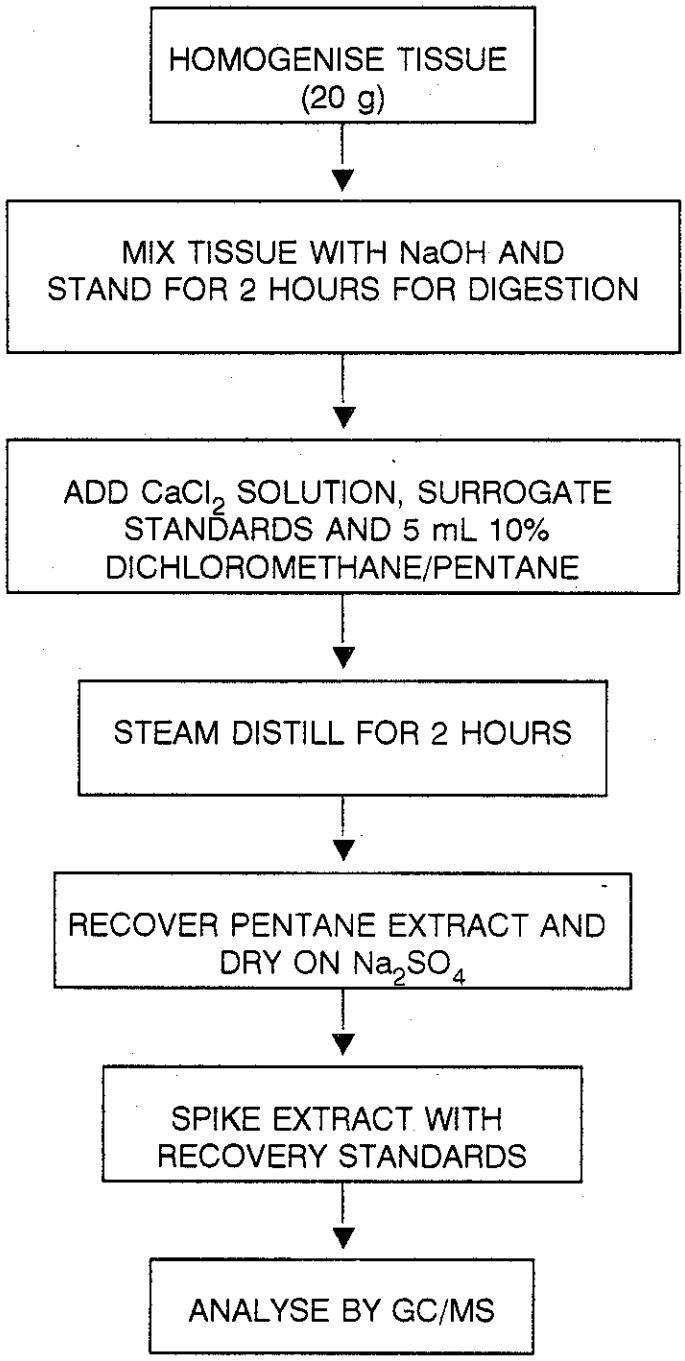
### 9.2 GC/MS Analysis

Extracts were analyzed on a Finnigan Incos 50B GC/MS system consisting of a Varian 3400 gas chromatograph with a 30 m x 0.25 mm, 0.25 µm film fused silica capillary

## TABLE 8.2 CONCENTRATION OF WORKING STANDARDS - FINAL SET

| Compound Name | FT-I2 Surrogate (µg/mL) | FT-C2 Calibration (µg/mL) | FT-R2 Recovery (µg/mL) | FT-S2 Spiking (µg/mL) |
|---|---|---|---|---|
| benzene | - | 6.32 | - | 157.9 |
| toluene | - | 10.65 | - | 266.3 |
| ethylbenzene | - | 6.20 | - | 155.0 |
| o-xylene | - | 3.92 | - | 97.9 |
| phenol | - | 11.70 | - | 292.4 |
| m-cresol | - | 9.81 | - | 245.3 |
| naphthalene | - | 5.51 | - | 137.6 |
| thianaphthene | - | 4.51 | - | 112.8 |
| quinoline | - | 4.71 | - | 117.7 |
| 2-methylnaphthalene | - | 3.41 | - | 85.3 |
| 1,2-dimethylnaphthalene | - | 5.85 | - | 146.2 |
| 2-ethyl naphthalene | - | 5.99 | - | 149.5 |
| 2,3-dimethylnaphthalene | - | 6.02 | - | 150.4 |
| 1,3-dimethylnaphthalene | - | 6.61 | - | 165.2 |
| 2,3,5-trimethylnaphthalene | - | 5.18 | - | 129.4 |
| 2,3,6-trimethylnaphthalene | - | 3.00 | - | 74.0 |
| 1,2,3,4-tetramethyl benzene | - | 3.90 | - | 97.1 |
| dibenzothiophene | - | 2.30 | - | 56.2 |
| d6-benzene | 98.7 | 3.95 | - | - |
| d8-toluene | 102.1 | 4.08 | - | - |
| d8-naphthalene | 99.7 | 3.99 | - | - |
| d7-quinoline | 158.1 | 6.32 | - | - |
| 2,6-dibromophenol | 60.4 | 2.42 | - | - |
| d5 phenol | 178.9 | 7.16 | - | - |
| d10 pyrene | 79.8 | 3.19 | - | - |
| d22-n-decane | - | 2.59 | 64.63 | - |
| d8-dibenzofuran | - | 1.53 | 38.35 | - |
| pentane (%) | 0 | 92 | 100 | 0 |
| methanol (%) | 100 | 8 | 0 | 100 |

**FIGURE 9.1 FINAL METHOD SCHEME**



column (DB-5, J&W Scientific) coupled directly into the source of a Finnigan Incos 50B quadrupole mass spectrometer. Four microlitres (μL) of extract were injected using a CTC A200S autosampler with a 10 μL Hamilton 701 syringe, with the injector operating in split injection mode set at a 5:1 split ratio. The GC was operated using helium carrier gas, (ultra high purity grade) at an injector pressure of 20 psig flushing the injector at 60 mL/min, giving carrier flow rate of 65 cm/sec at 30°C. The injector temperature was held at 250°C and MS transfer line at 300°C. After sample injection the GC oven was held at 30°C for 2 minutes, ramped at 10°C/min to 250°C and held for 5 minutes.

MS data were acquired using the multiple ion detection (MID) mode monitoring 24 ions, with a 0.7 sec scan time. Table 9.1 lists the selected ions for the target compounds, surrogate and recovery standards.

## 10. GC/MS CALIBRATION

Instrument calibration was carried out daily with a calibration standard run before and after each suite of samples or every eight hours which ever comes first. A series of five solutions containing target compounds at increasing concentrations and internal and recovery standards at fixed concentrations was analyzed by GC/MS in order to define the linear working range of the instrument. A relative response factor (RRF) was calculated for each target compound with respect to its selected internal standard in each solution making up the linearity series. As described above, deuterated surrogates were used as internal standards for target compounds when available, while d8-dibenzofuran and d22-n-decane were used as recovery standards to determine surrogate recovery efficiency. RRFs for each target with respect to its surrogate compound in each calibration solution were approximately constant over this range of concentrations, indicating that the response was linear under these conditions. These results are presented in Table 10.1.

## 11. CALCULATION METHODS

### 11.1 Sample Calculations

Relative response factors RRFs were calculated for all target compounds with respect to the surrogates and all surrogates with respect to the recovery standard, and were

**Table 9.1 Characteristic ions for target, surrogate and recovery compounds**

| No. | Compound Name | Type (a) | Quantitation ion | Confirmation ion | Quantitation compound |
|---|---|---|---|---|---|
| 1 | benzene | T | 78 | 77, 82 | 19 |
| 2 | toluene | T | 91 | 92 | 20 |
| 3 | ethylbenzene | T | 91 | 65, 106 | 20 |
| 4 | o-xylene | T | 91 | 65, 106 | 20 |
| 5 | 1,2,3,4-tetramethylbenzene | T | 134 | 120 | 21 |
| 6 | naphthalene | T | 128 | 129 | 21 |
| 7 | 2-methylnaphthalene | T | 142 | | 21 |
| 8 | 1,2 dimethyl naphthalene | T | 156 | 142 | 21 |
| 9 | 2,3 dimethyl naphthalene | T | 156 | 142 | 21 |
| 10 | 1,3-dimethyl naphthalene | T | 156 | 142 | 21 |
| 11 | 2-ethylnaphthalene | T | 156 | 142 | 21 |
| 12 | 2,3,5-trimethylnaphthalene | T | 170 | 156 | 21 |
| 13 | 2,3,6-trimethylnaphthalene | T | 170 | | 21 |
| 14 | phenol | T | 94 | | 26 |
| 15 | m-cresol | T | 107 | 77, 108 | 26 |
| 16 | quinoline | T | 129 | | 23 |
| 17 | thianaphthene | T | 134 | 108 | 21 |
| 18 | dibenzothiophene | T | 184 | | 21 |
| 19 | d6-benzene | S | 84 | 77, 78 | 22 |
| 20 | d8-toluene | S | 100 | | 22 |
| 21 | d8-naphthalene | S | 128 | 129 | 22 |
| 22 | d22-n-decane | R | 66 | 82 | 24 |
| 23 | d7-quinoline | S | 136 | | 24 |
| 24 | d8-dibenzofuran | R | 176 | 142 | 24 |
| 25 | d10-pyrene | S | 202 | | 24 |
| 26 | d5-phenol | S | 99 | | 24 |
| 27 | 2,6-dibromophenol | S | 250 | | 24 |

(a) T = target tainting compound
S = internal surrogate standard
R = Recovery standard

**Table 10.1 Mean Relative Response Factors from Linearity Standard Data**

| Compound | Mean RRF | Std. Dev | RSD % | Concentration Range µg/mL |
|---|---|---|---|---|
| benzene | 0.755 | 0.119 | 15.7 | 4-102 |
| toluene | 0.907 | 0.149 | 16.4 | 13.5-336 |
| ethylbenzene | 0.880 | 0.074 | 8.4 | 4-101 |
| o-xylene | 0.699 | 0.053 | 7.6 | 3.6-64 |
| phenol | 4.034 | 0.567 | 14.1 | 6-150 |
| m-cresol | 2.470 | 0.331 | 13.4 | 3-80 |
| naphthalene | 1.107 | 0.021 | 1.9 | 3.6-89.6 |
| thianaphtalene | 0.707 | 0.035 | 4.9 | 2.9-73.4 |
| quinoline | 1.033 | 0.043 | 4.1 | 3-76.6 |
| 2-methylnaphthalene | 0.907 | 0.056 | 6.2 | 2.2-55.5 |
| 1,2-dimethylnaphthalene | 0.389 | 0.025 | 6.54 | 3.8-95 |
| dibenzothiophene | 0.806 | 0.102 | 12.7 | 1.5-36.6 |
| benzene-d6 | 4.020 | 0.589 | 14.7 | 9.9 |
| toluene-d8 | 2.271 | 0.307 | 13.5 | 10.3 |
| naphthalene-d8 | 1.464 | 0.095 | 6.5 | 10.1 |
| quinoline-d7 | 0.586 | 0.078 | 13.3 | 16.0 |
| 2,6-dibromophenol | 0.149 | 0.007 | 4.6 | 6.1 |
| n-decane-d22 | 0.863 | 0.042 | 4.9 | 9.3 |

determined from the mean of RRFs from calibration standards (FT-C1 and FT-C2) run before and after sample suites. Relative response factors were determined using the following formula:

$$RRF_i = \frac{A_i \times C_s}{A_s \times C_i}$$

where $RRF_i$ is the relative response factor of compound i with respect to surrogate s

$A_i$ is the peak area of the quantitation ion for compound i

$A_s$ is the peak area of the quantitation ion for the surrogate s

$C_i$ is the concentration of compound i in the calibration standard

$C_s$ is the concentration of the surrogate in the calibration standard.

Concentrations of target compounds in samples were calculated using the following formula:

$$Concentration\ of\ i\ (\mu g/g) = \frac{A_i}{A_s} \times \frac{W_s}{RRF_i} \times \frac{1}{Wt}$$

where $A_i$ is the quantitation peak area for compound i

$A_s$ is the quantitation peak area for the surrogate

$W_s$ is the weight of surrogate spiked into the sample in micrograms

$RRF_i$ is the relative response factor of i with respect to s

and $Wt$ is the sample weight in grams

Surrogate recoveries were calculated using the following formula:

$$R_s = \frac{A_S}{A_R} \times \frac{W_R \times 100}{W_S \times RRF_S}$$

where $R_s$ is the percent recovery of surrogate S

$A_S$ is the quantitation ion peak area of surrogate S

$A_R$ is the quantitation ion peak area of the recovery standard R

$W_R$ is the weight of recovery standard added (μg)

$W_S$ is the weight of surrogate added (μg)

$RRF_S$ is the relative response factor of the surrogate S with respect to the recovery standard

$RRF_S$ is calculated from the calibration standard a modified form of the formula given above for $RRF_i$.

### 11.2 Detection Limits

Detection limits were calculated for each compound from procedural blank data using the noise level in the quantitation channel for that compound. This detection limit is the calculated smallest quantity that can be positively detected at three times the height of the average maximum noise. The height is converted to an area by multiplying by the area/height ratio of the nearest surrogate peak and the resulting area is converted to a detection limit using the formula above for the concentration of i. Typical detection limits as seen in the preliminary blank runs were calculated by this method and are presented in Table 11.1.

## 12. QUALITY CONTROL AND ASSURANCE

Reagent blanks were carried through the steam distillation procedure initially in duplicate to determine residual levels of interferences, then subsequently a procedural blank was carried through for each suite of up to five tissue samples analyzed. Persistent and variable levels of toluene (typically 0.2 to 0.5 μg), and to a lesser extent benzene (typically 0.06 to 0.1 μg) were detected in procedural blanks throughout this study. Procedural blanks were generally free of other inferences. All data is blank corrected to correct for background levels of compounds found in blanks.

A mean relative response factor was used for all data to compensate for MS drift and column activity. The instrument was response calibrated by running the calibration standard (i.e., initially FT-C1, FT-C2) immediately before and following each batch of samples or every eight hours, whichever occurs first. The instrument linearity range was determined by running a series of linearity standards with the surrogates at a constant concentration and the target at four levels as described on Table 10.1 in Section 10.

## TABLE 11.1 DETECTION LIMITS

| COMPOUND | DETECTION LIMIT[a] (ng/g) | BLANK LEVEL[b] (ng/g) |
|---|---|---|
| benzene | 6.5 | 70 |
| toluene | 15 | - [c] |
| ethylbenzene | 6.5 | 20 |
| xylene | 6.5 | 20 |
| naphthalene | 4 | 10 |
| 2-methylnaphthalene | 7.5 | 10 |
| 1,2-dimethylnaphthalene | 11 | 11 |
| thianaphthene | 2.5 | 10 |
| dibenzothiophene | 7.5 | 10 |

(a) based on a noise calculation for a 20 g sample
(b) blank level in mackerel tissue, preliminary runs, Set 2.
(c) highly variable and elevated toluene levels due to contamination from lab

## 13. TESTING AND EVALUATION OF THE PRELIMINARY METHOD

### 13.1 Spiked Blanks

A number of spiked blanks were carried through the proposed method to evaluate performance in the absence of a sample matrix. A reagent and procedural blank was carried through with each set.

A 20 mL aliquot of pre-extracted water (2 x dichloromethane, 2 x pentane) in a 500 mL round-bottom flask was spiked with the target compound spike standard FT-S, 200 µL), an aliquot of the surrogate standard (FT-I, 200 µL), 2 mL of pentane and 250 mL sodium hydroxide solution (0.1 M, 4 g/L). Boiling chips were added, the steam distillation started and allowed to distil for 30 to 40 minutes.

Set 1. Steam distillation was carried out as described above. At the end of the 40 minute period the pH of the sample was adjusted to 2 with 6 M hydrochloric acid and the distillation was resumed for an additional 40 minutes. After this period the pentane extract was collected in a centrifuge tube and dried on sodium sulphate.

Set 2. Pentane extracts were collected after basic extraction, this was followed by adjustment of the sample pH to 2 by addition of sulphuric acid, further distillation for 30-40 minutes and pentane collection at the end of this period. The pentane extracts were dried on sodium sulphate.

Dried pentane extracts were spiked with recovery standards and concentrated for GC/MS analysis.

### 13.2 Spiked Tissue

Twenty grams of fish tissue (cod, mackerel, or scallops) were carried through the same procedure as described above for Set 2. The fish samples were spiked with target compounds at concentrations approximately ten times the detection level. A procedural blank and an unspiked tissue sample of each type were also carried through the method.

### 13.3 Spiked and Unspiked Fish Tissue with Extract pH adjustment

Spiked tissue and a spiked blank were carried through as for Set 2 but with a number of adjustments to ensure the extract pH followed that of the distillation blank.

## 14. PRELIMINARY RESULTS

Results from the first series (Set 1, Table 14.1) of steam distillations indicated problems with the recovery of both labelled (d-7) and unlabelled quinoline, and although the phenol surrogate 2,6-dibromophenol was reasonably recovered (mean 76%), cresol and phenol targets were not detectable.

Modifications were made to the procedure so that the distillate from the first base distillation was drawn off and replaced with fresh pentane prior to the acidified distillation step (Set 2, Table 14.2). Similar results to Set 1 were obtained.

Further modifications were also made to the procedure to monitor and adjust the distillate pH to ensure the condensate pH followed that within the distillation flask. Results of Set 3 (Table 14.3) show low and variable 2,6-dibromophenol recoveries (0% for four out of six determinations) and 0% recoveries for cresol and phenol.

## 15. DISCUSSION OF PRELIMINARY RESULTS

The preliminary method gave acceptable recoveries (40% to 120%) and precision (< 20% RSD) for a range of neutral compounds for spiked blanks and fresh fish samples spiked for: benzene, toluene, ethylbenzene, o-xylene, naphthalene, thionaphthene, 2-methylnaphthalene and 1,2-dimethylnaphthalene. Recoveries of dibenzothiophene were more variable (13% to 123% and percent RSD of 40% to 50%) than any other neutral target. Spike recoveries of basic compounds were low and variable.

Adjustment of the pH to recover acidic, neutral and basic compounds gave poor and variable recoveries for phenolic compounds. Investigation of the partition of phenols between water and pentane at pH >1 indicated pentane to be a poorer solvent, with phenol recoveries of less than 15%. The addition of dichloromethane gave high recoveries on a partition test but low phenol recoveries on a steam distillation run.

## TABLE 14.1 RECOVERIES OF TARGET COMPOUNDS AND STANDARDS IN SET 1

| | % Recoveries | | |
|---|---|---|---|
| **Target** | **Blank 1** | **Spiked Blank 1** | **Spiked Blank 2** |
| benzene | NA | 139 | 102 |
| toluene | NA | 104 | 82 |
| ethylbenzene | NA | 104 | 89 |
| o-xylene | NA | 110 | 102 |
| phenol | NA | 0 | 0 |
| m-cresol | NA | 0 | 0 |
| naphthalene | NA | 97 | 83 |
| thianaphthalene | NA | 99 | 82 |
| quinoline | NA | 0 | 0 |
| 2-methylnaphthalene | NA | 95 | 90 |
| 1,2-dimethylnaphthalene | NA | 97 | 84 |
| dibenzothiophene | NA | 123 | 108 |
| **Surrogate Standards** | | | |
| benzene-D6 | 52 | 46 | 45 |
| toluene-D8 | 77 | 67 | 67 |
| naphthalene-D8 | 93 | 92 | 96 |
| quinoline-D7 | 3 | 3 | 5 |
| 2,6-dibromophenol | 68 | 74 | 87 |

NA = not applicable; blank corrected data

## Table 14.2 RECOVERIES OF TARGET COMPOUNDS AND STANDARDS IN SET

| Target | % Recoveries | | | | | |
|---|---|---|---|---|---|---|
| | Reagent Blank 2 | Spiked Blank 3 | Spiked Blank 4 | Mackerel Blank | Spiked Mackerel | Spiked Mackerel |
| benzene | NA | 68 | 68 | NA | 98 | 68 |
| toluene | * | * | * | * | * | * |
| ethylbenzene | NA | 79 | 89 | NA | 99 | ** |
| o-xylene | NA | 79 | 95 | NA | ** | ** |
| phenol | NA | 0 | 0 | NA | 0 | 0 |
| m-cresol | NA | 0 | 0 | NA | 0 | 0 |
| naphthalene | NA | 89 | 100 | NA | 89 | 89 |
| thianaphthalene | NA | 96 | 96 | NA | 96 | 96 |
| quinoline | NA | 0 | 0 | NA | 131 | 0 |
| 2-methylnaphthalene | NA | 90 | 90 | NA | 72 | 72 |
| 1,2-dimethylnaphthalene | NA | 84 | 95 | NA | 53 | 42 |
| dibenzothiophene | NA | 82 | 110 | NA | 14 | 41 |
| **Surrogate Standards** | | | | | | |
| benzene-D6 | 94 | 84 | 83 | 56 | 59 | 63 |
| toluene-D8 | 94 | 79 | 77 | 74 | 79 | 73 |
| naphthalene-D8 | 82 | 78 | 74 | 77 | 83 | 60 |
| quinoline-D7 | 4 | 3 | 3 | 127 | 0 | 0 |
| 2,6-dibromophenol | 58 | 85 | 77 | 52 | 0 | 0 |

NA = not applicable; blank corrected data
* = highly elevated level in procedural blank; not quantitated
** = interference; not quantified

## TABLE 14.3 RECOVERIES OF TARGET COMPOUNDS AND STANDARDS IN SET

| Target | % Recoveries | | | | | |
|---|---|---|---|---|---|---|
| | Cod Blank | Scallop Blank | Spiked 1 Cod | Spiked 2 Cod | Spiked 1 Scallop | Spiked Scallop |
| benzene | NA | NA | 78 | 88 | 98 | 117 |
| toluene | * | * | * | * | * | * |
| ethylbenzene | NA | NA | 79 | 89 | 89 | 89 |
| o-xylene | NA | NA | 79 | 95 | 95 | 95 |
| phenol | NA | NA | 0 | 0 | 0 | 0 |
| m-cresol | NA | NA | 0 | 0 | 0 | 0 |
| naphthalene | NA | NA | 89 | 100 | 89 | 89 |
| thianaphthalene | NA | NA | 96 | 96 | 110 | 96 |
| quinoline | NA | NA | 0 | 0 | 52 | 118 |
| 2-methylnaphthalene | NA | NA | 90 | 90 | 90 | 90 |
| 1,2-dimethylnaphthalene | NA | NA | 95 | 84 | 84 | 84 |
| dibenzothiophene | NA | NA | 27 | 27 | 55 | 82 |
| **Surrogate Standards** | | | | | | |
| benzene-D6 | 71 | 44 | 72 | 62 | 54 | 76 |
| toluene-D8 | 76 | 34 | 68 | 66 | 60 | 87 |
| naphthalene-D8 | 65 | 16 | 70 | 67 | 60 | 78 |
| quinoline-D7 | 0 | 0 | 0 | 0 | 5 | 12 |
| 2,6-dibromophenol | 0 | 0 | 0 | 0 | 19 | 61 |

NA = not available; blank corrected data
* = highly elevated blank; not quantified

The preliminary method was clearly not suitable for phenolic compounds due to unfavourable partitioning of these compounds between pentane and the aqueous phase even at low pH.

## 16. METHOD DEVELOPMENT

### 16.1 Method Revisions

The initial steam distillation runs demonstrated the preliminary method gave acceptable performance for non-polar compounds in the 70°C to 250°C boiling point range. Recoveries of the highest boiling compound, dibenzothiophene, were acceptable in spiked blanks (82%, 110%, 123%, 108%) but poor in spiked tissue (mean of 27% in cod, 55% in mackerel). This indicated some modifications were necessary to improve the recovery of higher boiling compounds. The list of target compounds was expanded to include 2-ethylnaphthalene, 2,3-dimethylnaphthalene, 1,3-dimethylnaphthalene, 2,3,5- and 2,3,6-trimethylnaphthalene, and tetramethylbenzene (Table 8.2). Pyrene-d10 was added to the surrogate standard. (Table 8.2)

The persistent recovery problems were evident for the recovery of phenols and the resulting complexity of the procedure required to retain them impeded method development. Although phenols are acknowledged taints (Table 3.1), they are a minor compound class in petroleum products and in consultation with the scientific authority for this project we agreed to drop phenols from the list of target compounds at this time.

Using the expanded list of target compounds the method was tested using mackerel, cod, scallop and spoiled mackerel, each run in quintuplicate, and sets spiked with target compounds at low (100 to 200 ppb) and high (1 to 4 ppm) levels. In the course of these runs, a number of method revisions were made to correct problems that became evident. These revisions are summarized in Table 16.1.

The rationale for these revisions is described in the following section.

#### 16.1.1 Revision 1. Addition of Dichloromethane to Extraction Solvent

As noted above, poor recoveries of dibenzothiophene were observed in the analysis of spiked tissue although good recoveries were evident in spiked blanks. This matrix effect

## TABLE 16.1 FISH TAINTING METHOD REVISION

| Revision No. | Observed Problem | Possible Cause | Modification | Results Observed |
|---|---|---|---|---|
| 1 | Poor recovery for high boiling point compounds such as dibenzothiophene and pyrene-d10. | Poor solubility in pentane and/or low vapour pressure at 100°C. | Use of better solvent. 30% dichloromethane in pentane. | Improved recovery for dibenzothiophene. |
| 2 | Only one alkylated PAH in selected tainting compounds in spikes. | | Expansion of list byaddition of di- and tri-methylnaphthalene, ethyl naphthalene and tetra-methylbenzene. | |
| 3 | Occasional lower overall recoveries for spoiled high lipid samples. | Presence of dichloromethane increased tendancy for emulsification. | Reduction of dichloromethane to 10% in pentane. | Stable and improved recoveries except pyrene-d10 and dibenzothiophene. |
| 4 | Severe foaming of high lipid samples after digestion. | The presence of free fatty acids resulting in the stabilization of foams. | a. Addition of MeOH to reduce foaming. | a. Reduction of foaming but low recoveries. |
| | | | b. Addition of $CaCl_2$ to precipitate fatty acids. | b. No foaming with satisfactory recoveries. |
| 5 | High recoveries for ethyl benzene, toluene and xylenes. | Base catalyzed exchange of acid deuterium on toluene $d_8$, lowering the concentration of $D_7$ ion used in quantification. | Use of alternate $d_8$ (m/z 100) to quantify toluene. | Spikes recoveries close to 100%. Requires more rugged surrogates, such as $^{13}C$-labelled compounds. |

is attributed to the higher boiling PAH being retained in the tissue and exhibiting a lower vapour pressure, in effect a colligative property, and the compounds are consequently driven off at a slower rate. A longer steam distillation time should improve this and the reflux time was subsequently increased to 1.5 to 2 hours. The extraction efficiency is expected to be improved if the high boilers can be released from tissue by an extraction solvent. Pentane has a very low water solubility and the concentration within the distillation pot at 100°C will be very low.

Dichloromethane, which has a similar boiling point (41°C compared to 36°C for pentane) is more soluble and is expected to improve the extraction. However, being more dense than water, the maximum amount of dichloromethane that can be added to the pentane and still have the distillate separate rapidly as the upper layer in the steam distillation trap is estimated at 30% dichloromethane in pentane. Initial results at 30% dichloromethane showed excellent recoveries of dibenzothiophene for scallop and cod.

### 16.1.2 Revision 2. Reduction of Concentration of Dichloromethane in Solvents to 10%

Recoveries in spoiled mackerel spiked at low levels were erratic for all compounds and generally poor for dibenzothiophene. Large losses of extraction solvent (in excess of 50% loss) were observed and it was noted that a significant emulsified layer was present in the trap. We attribute these poor recoveries to the poor separation occurring in the trap. In order to decrease the density of the trapped solvent and improve separation in the trap, the composition of the extraction solvent was reduced to 10% dichloromethane in pentane. At this level good recoveries were consistently observed for compounds with this modification except for dibenzothiophene (Table 16.13) and indicates the dichloromethane level is too low to assist the extraction of the higher boiling dibenzothiophene.

### 16.1.3 Revision 3. Suppression of Foaming by the addition of Calcium Chloride to the Digest

After digestion, fish samples tended to foam during steam distillation. This was a severe problem for the mackerel (high lipid fish). Foaming can contaminate the collected distillate and cause a significant interference problem for the analysis. Free fatty acids, generated by lipid hydrolysis are likely present and these stabilise foam in the steam

distillation flask. A large 1 L distillation flask gives some protection against foam problems but they still must be continually monitored and heating reduced when necessary. The addition of a small quantity of methanol was found to reduce foaming but resulted in generally low recoveries and is not recommended. The addition of excess calcium chloride (20 g $CaCl_2$ in 100 mL water) to the digest precipitates the fatty acids as sparingly soluble calcium salts and was found to eliminate the formation of stable foams.

16.1.4 Revision 4. Deuterium Exchange of d6-benzene and d8-toluene

Recoveries of benzene, toluene, ethylbenzene and o-xylene were consistently high, 150-300%, although the surrogate for these compounds (d6-benzene and d8-toluene) gave lower recoveries (e.g., Table 14.1). Inspection of the ion ratios of these surrogates indicated partial exchange of the deuterium label had taken place. An alternate ion was used for the toluene-d8 quantitation which reduced the apparent recoveries of the affected target compounds but many recoveries exceeded 100%. The deuterium exchange is believed to be base catalysed and will be most rapid for the surrogate compounds, benzene and toluene. Rugged quantitation of these compounds calls for more stable surrogates ideally the $^{13}C$ labelled analogues or alternately fluorinated analogues such as fluorobenzene.

## 16.2 Results and Discussion of Method Development Work

Following the preliminary work on the original method (Section 15), the method was evaluated by spiking the three types of fish tissue with the expanded list of tainting compounds. Also a batch of mackerel, allowed to spoil by storage at room temperature for 15 days, was spiked and analyzed. Additionally, mackerel was spiked with crude oil (Lago Medio crude) at one level and analyzed in duplicate. For each tissue type, duplicate unspiked tissue samples were also analyzed except for scallops for which only one unspiked sample was analyzed. Spiked tissues were analyzed in quintuplicate at each target compound spiking level, and analyzed using six sets of steam distillation systems.

A summary of method development work is contained in Table 16.2.

## TABLE 16.2 Summary of Method Development - Experimental Work

| Set | Tissue Type | Spike (1) Level | Spiked Conc.(2) Range µg/g | Spike (3) Type | % DCM (4) | Method (5) Revisions |
|---|---|---|---|---|---|---|
| 1. | Scallops | 5 | 0.1-0.2 | T2 | | 1, 4 |
| 2. | Scallops | 100 | 1-4 | T2 | 30 | 1, 4 |
| 3. | Cod | 5 | 0.1-0.2 | T2 | 30 | 1, 4 |
| 4. | Cod | 100 | 1-4 | T2 | 30 | 1, 4 |
| 5. | Spoiled Mackerel | 5 | 0.1-0.2 | T2 | 30 | 1, 3, 4 |
| 6. | Spoiled Mackerel | 100 | 1-4 | T2 | 30 | 1, 3, 4 |
| 7. | Fresh Mackerel | 5 | 0.1-0.2 | T2 | 10 | 2, 3, 4 |
| 8. | Fresh Mackerel | 100 | 1-4 | T2 | 10 | 2, 3, 4 |
| 9. | Scallops | 20 | 0.1 | LMC | 10 | 2, 3, 4 |

(1) Spike level as a multiple of the detection limit
(2) Range of concentration of target compounds in tissue sample
(3) Spike Type T2 = extended list of tainting compound (Table 3.3)
LMC = Lagomedio crude oil
(4) Percentage dichloromethane in solvent charged into steam still
(5) Method Revisions adopted - see Table 16.1

### 16.2.1 Scallop

Five replicate extractions at each spike level were carried out. Tables 16.3 and 16.4 show the recoveries of each of the target compounds.

The spiked scallop data indicated good recoveries of all target compounds although surrogate recoveries were highly variable and occasionally low. The value of internal standards is evident as the target compound recoveries are largely corrected for losses by similar losses of the surrogate against which it is quantitated. Quinoline is a good example of this (Table 16.4), the surrogate (quinoline - $d_7$) recovery is very low and variable (0.2% to 2.9%) yet the target compound, quinoline, is quantitatively determined (87% to 114%). Elevated levels of benzene and toluene were evident in the procedural blank and when small with respect to the amount found, data is corrected for this. For the low level spikes, toluene was at a comparable level to the blank and is not reportable. High recoveries for benzene are attributed primarily to contamination from the laboratory atmosphere above the level found in the blanks, and possibly also to isotopic exchange of the deuterated benzene. The mean recoveries for n-decane-d22, the lower boiling 'recovery' standard was high (86%) and consistent (7% rsd, n = 10) which indicates only minor losses occurred after drying the extract, and presumably the steam distillation step is the principal step where losses occur.

The statistics for target recoveries from high and low level spikes in scallops are presented in Table 16.5.

### 16.2.2 Cod

Five replicate extractions at each spike level were carried out. Tables 16.6 and 16.7 show the recoveries of each of the target compound.

The general trends noted for the scallop are evident in the cod tissue results, although in general individual recoveries are more consistent for cod tissue than scallop. Highly variable background levels of benzene and toluene seriously interfere with spike determination, particularly at the low spike level. One notable difference with the data presented for scallop is that recovery values for the high boiling compounds are higher. Table 16.8 provides average recovery values and precision for each compound in both sets of spiking experiments.

## Table 16.3

### Recovery of target compound from spiked scallop tissue
### Low spike level (1)
### (%)

| Target Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 145 | 123 | 321 | 122 | 140 |
| toluene (2) | | | | | |
| ethylbenzene | 114 | 97 | 61 | 114 | 123 |
| o-xylene | 121 | 100 | 57 | 123 | 109 |
| naphthalene | 112 | 109 | 86 | 110 | 94 |
| thianaphthalene | 111 | 106 | 112 | 106 | 108 |
| quinoline | 138 | 121 | * | 116 | 91 |
| 2-methylnaphthalene | 121 | 105 | 176 | 103 | 116 |
| 1,2-methylnaphthalene | 121 | 108 | 108 | 104 | 120 |
| dibenzothiophene | 118 | 80 | 84 | 85 | 82 |
| 1,2,3,4-methylbenzene | 114 | 108 | 204 | 107 | 110 |
| 2-ethylnaphthalene | 118 | 105 | 132 | 102 | 118 |
| 2,3-methylnaphthalene | 122 | 105 | 110 | 101 | 117 |
| 1,3-methylnaphthalene | 125 | 104 | 110 | 102 | 116 |
| 2,3,5-methylnaphthalene | 125 | 104 | 86 | 99 | 112 |
| 2,3,6-methylnaphthalene | 129 | 101 | 150 | 97 | 114 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 10 | 37 | 19 | 14 | 30 |
| toluene-d8 | 22 | 56 | 6.6 | 45 | 54 |
| naphthalene-d8 | 23 | 61 | 1.8 | 54 | 67 |
| quinoline-d7 | 1.1 | 0.3 | 0.06 | 3.2 | 2.9 |
| pyrene-d10 | 2.4 | 0.3 | 0.04 | 3.2 | 3.7 |
| n-decane d22 | 94 | 79 | 86 | 80 | 82 |

1) 0.1-0.2 µg/g for each compound
2) Not quantified due to high blank values
3) 30% dichloromethane in pentane used for this set
4) * = interference

## Table 16.4

### Recovery of target compound from spiked scallop tissue
### High spike level (1)
### (%)

| Target Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 98 | 127 | 114 | 88 | 84 |
| toluene | 120 | 88 | 69 | 129 | 103 |
| ethylbenzene | 79 | 74 | 87 | 84 | 78 |
| o-xylene | 84 | 77 | 90 | 90 | 83 |
| naphthalene | 101 | 98 | 102 | 107 | 100 |
| thianaphthalene | 100 | 81 | 97 | 106 | 101 |
| quinoline | 102 | 87 | 93 | 105 | 114 |
| 2-methylnaphthalene | 86 | 69 | 89 | 103 | 93 |
| 1,2-methylnaphthalene | 58 | 35 | 78 | 101 | 75 |
| dibenzothiophene | 30 | 10 | 59 | 69 | 36 |
| 1,2,3,4-methylbenzene | 92 | 122 | 86 | 91 | 92 |
| 2-ethylnaphthalene | 70 | 48 | 79 | 103 | 85 |
| 2,3-methylnaphthalene | 62 | 37 | 81 | 105 | 78 |
| 1,3-methylnaphthalene | 66 | 41 | 80 | 104 | 81 |
| 2,3,5-methylnaphthalene | 48 | 24 | 79 | 103 | 64 |
| 2,3,6-methylnaphthalene | 50 | 26 | 82 | 105 | 65 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 32 | 8 | 23 | 20 | 40 |
| toluene-d8 | 58 | 15 | 38 | 44 | 54 |
| naphthalene-d8 | 64 | 8.2 | 39 | 47 | 61 |
| quinoline-d7 | 2 | 0.2 | 2.2 | 2.9 | 1.3 |
| pyrene-d10 | 3 | 0.25 | 3.4 | 2.7 | 1.2 |
| n-decane d22 | 87 | 85 | 94 | 96 | 80 |

(1) 0.1-0.2 µg/g of each compound

(2) 30% dichloromethane used for this set

## Table 16.5

### Average recoveries of target compound from spiked scallop tissue High and low spike levels. (1) (2)

| Compound | Low-level Average % recovery | %RSD | High-level Average % recovery | %RSD |
|---|---|---|---|---|
| benzene | 133 | 9 | 102 | 18 |
| toluene | nq | | 102 | 24 |
| ethylbenzene | 112 | 10 | 81 | 7 |
| o-xylene | 113 | 10 | 84 | 6 |
| naphthalene | 107 | 8 | 102 | 4 |
| thianaphthalene | 108 | 2 | 97 | 2 |
| quinoline | 116 | 16 | 100 | 10 |
| 2-methylnaphthalene | 112 | 8 | 88 | 14 |
| 1,2-methylnaphthalene | 113 | 8 | 70 | 35 |
| dibenzothiophene | 91 | 19 | 41 | 57 |
| 1,2,3,4-methylbenzene | 110 | 2 | 97 | 15 |
| 2-ethylnaphthalene | 111 | 7 | 77 | 27 |
| 2,3-methylnaphthalene | 111 | 9 | 73 | 35 |
| 1,3-methylnaphthalene | 112 | 9 | 74 | 9 |
| 2,3,5-methylnaphthalene | 110 | 10 | 64 | 47 |
| 2,3,6-methylnaphthalene | 111 | 13 | 66 | 13 |

(1) 0.1-0.2 µg/g of each compound.
(2) 30% dichloromethane used for this set
** 1-4 µg/g for each compound.
nq not quantified

## Table 16.6

## Recovery of target compound from spiked cod tissue
## Low spike level (1)
## (%)

| Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene (2) | ** | ** | ** | ** | ** |
| toluene (2) | ** | ** | ** | ** | ** |
| ethylbenzene | 238 | 207 | 207 | 163 | 123 |
| o-xylene | 279 | * | 193 | 198 | 175 |
| naphthalene | 203 | 181 | 130 | 175 | 149 |
| thianaphthalene | 105 | 152 | 117 | 130 | 115 |
| quinoline | * | * | * | * | * |
| 2-methylnaphthalene | 146 | 153 | 117 | 125 | 112 |
| 1,2-methylnaphthalene | 89 | 174 | 126 | 137 | 126 |
| dibenzothiophene | 21 | 169 | 140 | 154 | 146 |
| 1,2,3,4-methylbenzene | 220 | 153 | 114 | 124 | 113 |
| 2-ethylnaphthalene | 119 | 163 | 123 | 134 | 119 |
| 2,3-methylnaphthalene | 107 | 173 | 129 | 138 | 123 |
| 1,3-methylnaphthalene | 117 | 169 | 127 | 133 | 120 |
| 2,3,5-methylnaphthalene | 74 | 183 | 138 | 155 | 132 |
| 2,3,6-methylnaphthalene | 64 | 176 | 135 | 153 | 130 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 11 | 14 | 23 | 17 | 21 |
| toluene-d8 | 7.1 | 13 | 27.6 | 26 | 30 |
| naphthalene-d8 | 4.7 | 33 | 54.8 | 36 | 37 |
| quinoline-d7 | 0 | 2.5 | 3.5 | 1.4 | 1 |
| pyrene-d10 | 0 | 15 | 15 | 4.2 | 5 |
| n-decane-d22 | 102 | 123 | 93 | 93 | 94 |

(1) 0.1-0.2 µg/g for each compound.

(2) ** highly elevated level in procedural blank. Compound not quantified.

(3) 30% dichloromethane in pentane used in this set

* interference; not quantified

## Table 16.7

## Recovery of target compound from spiked cod tissue
## High spike level (1)
## (%)

| Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 139 | 119 | 129 | 292 | 237 |
| toluene | 60 | 68 | 81 | 96 | 78 |
| ethylbenzene | 88 | 104 | 109 | 138 | 111 |
| o-xylene | 93 | 105 | 97 | 130 | 110 |
| naphthalene | 111 | 110 | 98 | 110 | 116 |
| thianaphthalene | 101 | 109 | 108 | 110 | 108 |
| quinoline | 110 | 125 | 118 | 131 | 102 |
| 2-methylnaphthalene | 99 | 108 | 105 | 98 | 103 |
| 1,2-methylnaphthalene | 111 | 120 | 116 | 93 | 107 |
| dibenzothiophene | 75 | 146 | 94 | 51 | 93 |
| 1,2,3,4-methylbenzene | 87 | 95 | 93 | 102 | 97 |
| 2-ethylnaphthalene | 104 | 112 | 110 | 93 | 103 |
| 2,3-methylnaphthalene | 110 | 118 | 112 | 93 | 104 |
| 1,3-methylnaphthalene | 106 | 114 | 112 | 92 | 103 |
| 2,3,5-methylnaphthalene | 111 | 124 | 116 | 81 | 107 |
| 2,3,6-methylnaphthalene | 109 | 125 | 114 | 80 | 105 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 22 | 48 | 26 | 42 | 36 |
| toluene-d8 | 38 | 52 | 29 | 33 | 38 |
| naphthalene-d8 | 48 | 66 | 37 | 45 | 47 |
| quinoline-d7 | 2.3 | 7.6 | 3.1 | 1.4 | 2.6 |
| pyrene-d10 | 3.1 | 32 | 4.7 | 2.1 | 8.3 |
| n-decane d22 | 85 | 102 | 93 | 96 | 112 |

1) 1-4 µg/g for each compound
2) 30% dichloromethane used in this set

## Table 16.8

### Average recoveries of target compound from spiked cod tissue High and low spike levels.

| Compound | Low Level (1) Average % Recovery | %RSD | High Level (2) Average % Recovery | %RSD |
|---|---|---|---|---|
| benzene | 412 | 58 | 183 | 42 |
| toluene | nq | nq | 77 | 18 |
| ethylbenzene | 255 | 47 | 110 | 16 |
| o-xylene | 270 | 51 | 107 | 14 |
| naphthalene | 168 | 17 | 109 | 6 |
| thianaphthalene | 124 | 15 | 107 | 4 |
| quinoline | nq | nq | 117 | 10 |
| 2-methylnaphthalene | 131 | 14 | 103 | 4 |
| 1,2-methylnaphthalene | 131 | 23 | 110 | 9 |
| dibenzothiophene | 126 | 47 | 92 | 38 |
| 1,2,3,4-methylbenzene | 145 | 31 | 95 | 6 |
| 2-ethylnaphthalene | 132 | 14 | 104 | 7 |
| 2,3-methylnaphthalene | 134 | 19 | 107 | 9 |
| 1,3-methylnaphthalene | 134 | 15 | 105 | 8 |
| 2,3,5-methylnaphthalene | 136 | 30 | 108 | 15 |
| 2,3,6-methylnaphthalene | 132 | 32 | 107 | 16 |

(1) 0.1-0.2 µg/g of each compound. 1-4 µg/g for each compound.

(2) nq = not quantified due to interference

### 16.2.3 Spoiled mackerel

The method was then tested using subsamples of mackerel that had been allowed to spoil by storage at room temperature for approximately fifteen days. Twenty g subsamples were spiked in quintuplicate at the low tainting compound spike level (5 x estimated detection limit, 25 µL FT-S2 standard, Table 8.2) and at the high level spike (100 x estimated detection limit, 500 µL FT-S2). Recoveries of tainting compound and surrogates are given in Tables 16.9 and 16.10 for low and high level spikes.

For this matrix, recoveries were erratic even for naphthalene-d8, normally found to give high and consistent recoveries, which fell to 0.7% and 0.1% in two instances, and for quinoline-d7, giving only 0.3% and 0.2% recovery. During steam distillation some emulsification was observed within the solvent trap which occasionally caused extensive loss of the upper pentane layer back to the distillation flask. When this occurs, it is evident that the solvent and low boiling components are rapidly redistilled back to the trap, whereas the higher boiling components are slowly reconcentrated. Poor recoveries of quinoline-d7 are attributed to partition into aqueous emulsified droplets, and being more water soluble, it will be extensively washed out of the trap back to the distillation flask.

Average recoveries for the two spike levels are given in Table 16.11. The tendency for emulsification with spoiled tissue may be due to the release of low boiling polar or surfactant compounds. Consequently conditions were modified (method revision 2) to avoid this problem by the reduction of the level of dichloromethane in pentane in the reduction from 30% by volume to 10%. This solvent composition was used for subsequent steam distillations.

### 16.2.4 Fresh Mackerel (High Lipid Tissue)

Five replicate extractions at each spike level were carried out using 10% dichloromethane. Tables 16.12 and 16.13 show the recoveries of the target compound at low and high spike levels.

As with the scallop and cod data, recoveries were generally satisfactory (Table 16.14), with contamination from background levels evident for benzene and toluene. The generally high (>100%) recoveries for all the low boiling compounds are believed to be

## Table 16.9

### Recovery of target compounds from spiked spoiled mackerel tissue
### Low spike level (1)
### (%)

| Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 116 | 119 | 214 | 390 | 87 |
| toluene | ** | ** | ** | ** | ** |
| ethylbenzene | 128 | 137 | 65 | 87 | 74 |
| o-xylene | 117 | 144 | 37 | 56 | 70 |
| naphthalene | 94 | 114 | * | * | 100 |
| thianaphthalene | 108 | 111 | * | * | 102 |
| quinoline | * | 67 | * | * | 78 |
| 2-methylnaphthalene | 98 | 110 | * | * | 63 |
| 1,2-methylnaphthalene | 89 | 75 | * | * | 26 |
| dibenzothiophene | 14 | 13 | * | * | 12 |
| 1,2,3,4-methylbenzene | 94 | 105 | * | * | 95 |
| 2-ethylnaphthalene | 87 | 84 | * | * | 36 |
| 2,3-methylnaphthalene | 91 | 78 | * | * | 31 |
| 1,3-methylnaphthalene | 88 | 81 | * | * | 32 |
| 2,3,5-methylnaphthalene | 71 | 49 | * | * | 12 |
| 2,3,6-methylnaphthalene | 72 | 53 | * | * | 10 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 22 | 28 | 37 | 7 | 89 |
| toluene-d8 | 35 | 42 | 20 | 3.3 | 81 |
| naphthalene-d8 | 55 | 69 | 0.73 | 0.1 | 40 |
| quinoline-d7 | 4.2 | 5.0 | 0.3 | 0.2 | 3.7 |
| pyrene-d10 | 0.3 | 0.4 | 0.1 | 0.05 | 0.3 |
| n-decane d22 | 84 | 72 | 77 | 80 | 88 |

(1) 0.1-0.2 µg/g for each compound
(2) ** = elevated level in procedural blank; not quantifiable
(3) * = interference or not detected

## Table 16.10

## Recovery of target compound from spiked spoiled mackerel tissue
## High spike level
## (%)

| Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 67 | 69 | 75 | 122 | 106 |
| toluene | 52 | 52 | 61 | 94 | 70 |
| ethylbenzene | 58 | 62 | 62 | 78 | 86 |
| o-xylene | 61 | 70 | 71 | 72 | 85 |
| naphthalene | 96 | 94 | 98 | 107 | 110 |
| thianaphthalene | 99 | 98 | 96 | 92 | 91 |
| quinoline | 51 | 42 | 59 | * | 78 |
| 2-methylnaphthalene | 80 | 89 | 77 | 65 | 76 |
| 1,2-methylnaphthalene | 63 | 71 | 48 | 28 | 47 |
| dibenzothiophene | 6 | 16 | 7 | 3 | 10 |
| 1,2,3,4-methylbenzene | 83 | 87 | 95 | 129 | 102 |
| 2-ethylnaphthalene | 67 | 71 | 57 | 42 | 59 |
| 2,3-methylnaphthalene | 64 | 69 | 49 | 32 | 50 |
| 1,3-methylnaphthalene | 66 | 69 | 51 | 36 | 53 |
| 2,3,5-methylnaphthalene | 43 | 53 | 28 | 14 | 32 |
| 2,3,6-methylnaphthalene | 46 | 52 | 30 | 15 | 34 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 108 | 82 | 69 | 34 | 75 |
| toluene-d8 | 62 | 72 | 62 | 39 | 53 |
| naphthalene-d8 | 51 | 66 | 39 | 12 | 50 |
| quinoline-d7 | 2.6 | 3.5 | 2.2 | 0.5 | 4.2 |
| pyrene-d10 | 0.3 | 0.5 | 0.2 | 0.1 | 0.5 |
| n-decane d22 | 95 | 87 | 83 | 97 | 103 |

(1) 1-4 μg/g for each compound

(2) * interference; not quantitated

(2) solvent system in steam distillation step was 30% dichloromethane in pentane

## Table 16.11

### Average recoveries of target compound from spoiled mackeral tissue High and low spike levels

| Compound | Low-level (*) Average % recovery | %RSD | High-level (*) Average % recovery | %RSD |
|---|---|---|---|---|
| benzene | 107 | 16 | 88 | 28 |
| toluene | nq | nq | 66 | 27 |
| ethylbenzene | 113 | 31 | 69 | 17 |
| o-xylene | 111 | 34 | 72 | 12 |
| naphthalene | 103 | 10 | 101 | 7 |
| thianaphthalene | 107 | 5 | 95 | 4 |
| quinoline | 48 | 87 | 46 | 63 |
| 2-methylnaphthalene | 90 | 27 | 77 | 11 |
| 1,2-methylnaphthalene | 63 | 52 | 51 | 32 |
| dibenzothiophene | 13 | 5 | 8 | 61 |
| 1,2,3,4-methylbenzene | 100 | 6 | 99 | 19 |
| 2-ethylnaphthalene | 69 | 42 | 59 | 20 |
| 2,3-methylnaphthalene | 67 | 48 | 53 | 26 |
| 1,3-methylnaphthalene | 67 | 45 | 55 | 24 |
| 2,3,5-methylnaphthalene | 44 | 68 | 34 | 44 |
| 2,3,6-methylnaphthalene | 45 | 70 | 35 | 41 |

* 0.1-0.2 µg/g of each compound.

** 1-4 µg/g for each compound.

nq not quantified

## TABLE 16.12

### Recovery of target compounds from spiked mackerel tissue
### Low spike level (1)
### (%)

| Target Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 179 | 133 | 146 | 179 | 181 |
| toluene | ** | ** | ** | ** | ** |
| ethylbenzene | 171 | 151 | 122 | 139 | 179 |
| o-xylene | 169 | 148 | 126 | 144 | 184 |
| naphthalene | 126 | 123 | 107 | 107 | 119 |
| thianaphthalene | 117 | 114 | 109 | 96 | 116 |
| quinoline | 70 | 41 | 105 | 78 | 42 |
| 2-methylnaphthalene | 107 | 104 | 96 | 78 | 96 |
| 1,2-methylnaphthalene | 79 | 83 | 73 | 40 | 67 |
| dibenzothiophene | 15 | 29 | 12 | 4 | 15 |
| 1,2,3,4-methylbenzene | 102 | 100 | 101 | 94 | 101 |
| 2-ethylnaphthalene | 86 | 86 | 77 | 56 | 71 |
| 2,3-methylnaphthalene | 94 | 97 | 93 | 55 | 76 |
| 1,3-methylnaphthalene | 135 | 127 | 169 | 101 | 94 |
| 2,3,5-methylnaphthalene | 64 | 69 | 63 | 39 | 51 |
| 2,3,6-methylnaphthalene | 78 | 76 | 67 | 34 | 55 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 36 | 30 | 13 | 17 | 26 |
| toluene-d8 | 53 | 49 | 18 | 25 | 43 |
| naphthalene-d8 | 77 | 74 | 19 | 26 | 69 |
| quinoline-d7 | 22 | 8.2 | 1.7 | 1.8 | 10 |
| pyrene-d10 | 2.0 | 2.0 | 1.1 | 0.9 | 1.2 |
| n-decane d22 | 93 | 93 | 100 | 100 | 98 |

(1) * 0.1 to 0.2 µg/g for each compound

(2) ** elevated toluene levels in procedural blank, ∴ toluene not quantitated

(3) 10% dichloromentane in pentane used

## TABLE 16.13

### Recovery of target compound from spiked fresh mackerel tissue
### High spike level
### (%)

| Compound | Rep #1 | Rep #2 | Rep #3 | Rep #4 | Rep #5 |
|---|---|---|---|---|---|
| benzene | 81 | 71 | 68 | 80 | 88 |
| toluene | 138 | 361 | 248 | 302 | 209 |
| ethylbenzene | 90 | 79 | 69 | 84 | 97 |
| o-xylene | 130 | 104 | 97 | 87 | 95 |
| naphthalene | 105 | 86 | 87 | 97 | 100 |
| thianaphthalene | 102 | 101 | 95 | 94 | 97 |
| quinoline | 35 | 56 | 60 | 66 | 69 |
| 2-methylnaphtalene | 101 | 91 | 77 | 76 | 67 |
| 1,2-methylnaphthalene | 76 | 62 | 48 | 44 | 36 |
| dibenzothiophene | 7 | 7 | 4 | 6 | 5 |
| 1,2,3,4-methylbenzene | 106 | 101 | 98 | 100 | 113 |
| 2-ethylnaphthalene | 85 | 74 | 61 | 60 | 49 |
| 2,3-methylnaphthalene | 82 | 65 | 53 | 49 | 38 |
| 1,3-methylnaphthalene | 135 | 127 | 169 | 101 | 94 |
| 2,3,5-methylnaphthalene | 54 | 38 | 28 | 52 | 42 |
| 2,3,6-methylnaphthalene | 56 | 39 | 29 | 29 | 22 |
| **Surrogate Recoveries** | | | | | |
| benzene-d6 | 36 | 42 | 42 | 32 | 28 |
| toluene-d8 | 50 | 57 | 34 | 31 | |
| naphthalene-d8 | 75 | 67 | 45 | 28 | 19 |
| quinoline-d7 | 5.6 | 5.1 | 3.1 | 1.8 | 1.0 |
| pyrene-d10 | 0.8 | 0.6 | 0.5 | 0.5 | 0.6 |
| n-decane d22 | 100 | 91 | 94 | 91 | 94 |

(1) * 1-4 µg/g for each compound
(2) 10% dichloromethane
(3) * elevated blank and sample values, not quantifiable

## TABLE 16.14

### Average recoveries of target compound from spiked mackerel tissue High and low spike levels. Solvent 10% dichloromethane/pentane

| Compound | Low-level (1) Average | %RSD | High-level (2) Average% | %RSD |
|---|---|---|---|---|
| benzene | 162 | 13 | 77 | 10 |
| toluene | -(3) | | -(3) | |
| ethylbenzene | 152 | 15 | 84 | 13 |
| o-xylene | 154 | 15 | 103 | 16 |
| naphthalene | 117 | 7 | 95 | 9 |
| thianaphthalene | 109 | 9 | 98 | 4 |
| quinoline | 67 | 40 | 57 | 23 |
| 2-methylnaphthalene | 96 | 12 | 83 | 17 |
| 1,2-methylnaphthalene | 68 | 25 | 53 | 30 |
| dibenzothiophene | 15 | 60 | 6 | 20 |
| 1,2,3,4-methylbenzene | 100 | 4 | 104 | 6 |
| 2-ethylnaphthalene | 76 | 18 | 66 | 22 |
| 2,3-methylnaphthalene | 83 | 21 | 57 | 30 |
| 1,3-methylnaphthalene | 125 | 24 | 60 | 26 |
| 2,3,5-methylnaphthalene | 57 | 22 | 34 | 38 |
| 2,3,6-methylnaphthalene | 62 | 30 | 35 | 38 |

(1) 0.1-0.2 µg/g of each compound
(2) 1-4 µg/g for each compound
(3) elevated blank and sample levels for this compound, not reported

an artifact due to deuterium exchange on the surrogate standard. The parent ion ($M^+$,84) of deuterated benzene was initially used to quantify native benzene while the $(M-1)^+$,98 ion of deuterated toluene was used to quantify for toluene, ethylbenzene and o-xylene. The deuterated surrogates partially exchange deuterium for hydrogen during the base digestion procedure, reducing the apparent concentration of deuterated internal surrogate standard, and creating an over estimation in the calculated concentration of native compounds. Subsequently, for the lower boiling compounds, data were calculated against the 100 m/z, the parent ion of toluene-d8, which reduced but did not eliminate this anomaly.

### 16.2.5 Fresh Mackerel and Lago Medio Crude Oil

A final experiment was carried out to check method performance against a source of tainting compounds: a crude oil. Fresh mackerel tissue was spiked with an aliquot of Lago Medio Crude Oil at the level of 12.3 mg/g and distillated/extracted using 10% dichloromethane in pentane. Although this level does not represent a realistic fish exposure situation, it serves as a means of testing for possible interferences and recovery of tainting compounds in native oil.

Table 16.15 shows the concentration of the selected tainting compounds in a sample of Lago Medio crude oil used in these experiments. Table 16.16 shows the recoveries (average of duplicate set) of each of the selected tainting compounds from fresh mackerel spiked with Lago Medio crude oil. The distilled extracts from these oil-spiked samples smelled strongly of crude and the process evidently extracted many of the strongly odiferous compounds. The results on Table 16.16 indicate unsatisfactory method performance particularly for the high boiling compounds. The use of the lower concentration of dichloromethane may partially explain this result, but more work is required using the crude oil.

## TABLE 16.15

## Concentrations of Selected Tainting Compounds in Lago Medio Crude Oil

| Compound | µg/g |
|---|---|
| benzene | 65 |
| toluene | 838 |
| ethylbenzene | 450 |
| o-xylene | 700 |
| naphthalene | 210 |
| thianaphthalene | 49 |
| quinoline | 0 |
| 2-methylnaphthalene | 560 |
| 1,2-methylnaphthalene | 170 |
| dibenzothiophene | 180 |
| 1,2,3,4-methylbenzene | 580 |
| 2-ethylnaphthalene | 180 |
| 2,3-methylnaphthalene | 270 |
| 1,3-methylnaphthalene | 570 |
| 2,3,5-methylnaphthalene | 350 |
| 2,3,6-methylnaphthalene | 540 |

## TABLE 16.16

**Average Recoveries of Target Compound from Mackerel Tissue**
Spiked with Lago Medio Crude Oil at 12.3 mg/g

| Compound | Average % Recovery |
|---|---|
| benzene | 89 |
| toluene | 133 |
| ethylbenzene | 86 |
| o-xylene | 69 |
| naphthalene | 59 |
| thianaphthalene | 35 |
| quinoline | 0 |
| 2-methylnaphthalene | 30 |
| 1,2-methylnaphthalene | 7 |
| dibenzothiophene | 0 |
| 1,2,3,4-methylbenzene | 25 |
| 2-ethylnaphthalene | 14 |
| 2,3-methylnaphthalene | 32 |
| 1,3-methylnaphthalene | 15 |
| 2,3,5-methylnaphthalene | 3 |
| 2,3,6-methylnaphthalene | 3 |

## 17. CONCLUSIONS

A method has been developed which allows a range of base neutral compounds, including many known tainting petroleum hydrocarbons, to be determined in fish tissue samples. The method is applicable to a wide range of tissues for the determination of basic and neutral volatile and semi-volatile compounds in the boiling point range of 80°C to 300°C. Although surrogate recoveries were variable in the 0.1% to 50% range, the method was found to give reliable quantification of selected tainting compounds in the 70 to 100% recovery range. This was accomplished by the use of the internal standard quantification method in which target compound concentrations are calculated against the perdeuterated surrogate internal standards. Detection limits in the range of 25 to 15 ng/g were determined for a 20 g sample size, although the detection limits are initially dependant on background levels of the selected tainting compounds. In this study toluene could not be reliably reported due to elevated and variable levels in blanks and unspiked tissue samples. Similar contamination problems were also observed for benzene although these were not as severe as for toluene and recovery performance data for benzene spikes were satisfactory (80-120%) only at the higher spike levels. For reliable analysis at low detection limits, this method should be carried out in a clean area with minimal working atmospheric levels of the selected tainting compounds.

The base digestion of fish tissue, fresh or spoiled, generates strong and persistent odours and this extraction procedure should be carried out in an efficient fume hood. The recovery of the higher boiling compounds such as dibenzothiophene was poor when samples were extracted with 100% pentane and recoveries were found to be improved by the use of 30% dichloromethane in pentane, although recovery problems were observed with spoiled high lipid samples. A 10% dichloromethane, low dibenzothiophene recoveries were again observed and we concluded the higher level of dichloromethane is desirable.

The method was found to work satisfactorily only for a small number of compounds when spiked with crude oil into mackerel and although the compositional features and odours of the crude oil were evident in the steam distilled extract. More work is needed to clarify this result.

## 18. RECOMMENDATIONS

- The effect of intermediate levels of dichloromethane in the 10-30% range should be investigated to provide the best compromise between high recoveries of the high boilers and consistent surrogate recoveries.

- The use of $^{13}C$ labelled surrogates, which should not be affected by exchange should be investigated.

- The standard method should be characterized further with spiked and unspiked tissue to extend the method performance data. More data on crude oil spike recoveries are needed.

- Real samples reported to be or suspected tainted with petroleum should be analyzed by this method and results compared to taste panel data.

- Work to extend the list of target compounds to include, for example, more alkylated benzenes, naphthalenes and dibenzothiophenes with more isotopically labelled surrogates.

- Investigate alternative sources of solvents free of benzene and toluene.

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