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TAINTING OF FINFISH BY PETROLEUM HYDROCARBONS

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PREFACE

Martec Limited, in association with the Canadian Institute of Fisheries Technology (CIFT), was contracted under the Environmental Studies Revolving [now Research] Funds Effects Monitoring Priority Area to undertake a laboratory study of possible tainting of finfish (Atlantic cod) as a result of exposure to different concentrations of hydrocarbon in the water-soluble fractions of Canadian petroleums. The request for proposals for the project appeared in ESRF IV(1) dated 13 June 1986. The terms of reference for the study are presented below:

"Objective

The objective of the study is to determine the concentrations of petroleum hydrocarbons in water which can lead to tainting problems in marine finfish and to obtain an appreciation of the time to recovery once tainting has occurred. The results of the study will contribute to the promulgation of advice/guidelines for evaluating real or potential concerns for the tainting of fishery resources resulting from either oil spills or hydrocarbon development activities.

"Background

Examples of tainting of fishery resources as a result of exposure to petroleum hydrocarbons have been reported since the early 1940's. Although reports are often based on opportunistic investigation, there have been several accounts of finfish tainting, or potential tainting, as a result of marine transportation accidents within the past few years. Increased public attention to large marine oil spills may cause fish marketing problems even in the absence of demonstrable tainting. Thus government agencies and industry need information for distinguishing concerns related to demonstrable taint on the one hand and to the perception of taint on the other. The main goal of this study is to establish a scientific basis for assessing taint in a representative commercial species, in relation to the occurrence and persistence of soluble petroleum.

"Statement of Work

Exposures: Threshold concentrations of soluble petroleum hydrocarbons in water which will cause taint in codfish will be determined with four different treatments involving two East Coast crudes (one conventional and one waxy-viscous), one Beaufort Sea crude and a drilling mud base oil. The study should determine thresholds for taint as well as recovery from taint (i.e. depuration rates) using standard test protocol. The recovery tests should include fish exposed to 5-10 times the threshold required for taint.

It is recommended that Atlantic cod of comparable size (i.e. in the 700-1,000 g range) and physiological status be used in the treatments.

Chemistry: Some calibration of the system may be required before commencing treatments. The concentration of "total" petroleum hydrocarbons required for taint will be measured using an appropriate analytical technique.

A second goal of the study is to identify the particular compounds in fish flesh which may be responsible for taint. Hydrocarbons will be extracted from tissues of tainted fish and analyzed for fractional hydrocarbon composition by an appropriate method.

Sensory Analysis: After treatment, fish should be prepared for taste panel analysis of fresh and frozen samples, using standard methods. The ESRF study on "Tainting of Fishery Resources" (Report No. 21) is a source document for taste panel methods.

Output: An interim report is due after threshold levels and depuration times have been assessed for two of the oils. At the end of the study, a final report suitable for publication by ESRF will be prepared."

The project team and their responsibilities were as follows:

Mr. W.G. Tidmarsh - Project Manager;
Dr. R.G. Ackman - Scientific Coordinator;
Ms. R.J. Ernst - Exposure System Design and Monitoring;
Ms. T.E. Farquharson - Taste Panel Evaluations; and
Dr. W.M.N. Ratnayake - Tissue and Water Sample Analysis.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (Continued)

LIST OF ILLUSTRATIONS (Continued)

LIST OF PHOTOGRAPHS

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The authors take full responsibility for any errors or omissions.

SUMMARY

Tainting in fish is the development of an atypical flavour caused by natural spoilage or by the assimilation of contaminants into the edible fish tissue. The main goal of this study was to establish a scientific basis for assessing taint in a representative commercial finfish, in relation to the occurrence and persistence of soluble petroleum hydrocarbons.

A series of short- (24 h) and long-term (3 and 7 d) exposure studies was conducted on a laboratory scale (a) to evaluate the threshold concentrations of petroleum hydrocarbons in sea-water which can lead to tainting problems in Atlantic cod (Gadus morhua) and (b) to obtain an appreciation of the time to recover (depuration period) once the tainting has occurred.

Four different oils were used in this study, namely Amauligak, Brent, Hibernia, and Conoco. The first three were crude oils and Conoco was a base oil, a middle distillate. The hydrocarbon profiles of the three crude oils were more or less similar to each other. The main components were normal-, cyclic-, and branched-alkanes with minor levels of low-boiling aromatic hydrocarbons. The n-alkanes generally ranged from C_{11} to C_{31}. The Conoco base oil was completely different from the three crude oils; the main components were hydrocarbons of moderate boiling point, probably cyclic and branched alkanes. The content of n-alkanes was very low and the low-boiling aromatic hydrocarbons were completely absent.

Initially a stock of water saturated with hydrocarbons (generally referred to as the water-soluble fraction (WSF)) was prepared with each of the four oils, as follows. The sea-water and oil were mixed, in a ratio of 99:1 (v/v), for 24 h with mechanical stirring in a 1,100-L stainless-steel vessel, and were allowed to settle for 48 h for separation of the aqueous and oil phases. The hydrocarbon concentration of the aqueous phase, i.e., the WSF, of the three crude oils ranged from 15-21 ppm, whereas the WSF from the Conoco base oil was relatively less concentrated (~4 ppm). The main components of the WSF from the three crude oils were low-boiling aromatics, although these were minor components in the starting oils. Alkanes were almost absent. These low-boiling aromatics were completely absent in Conoco WSF; its main components were hydrocarbons of moderate boiling point, probably cyclic and branched alkanes.

Sea-water was spiked with various volumes of the WSF concentrate to obtain a series of contaminated sea-waters. The hydrocarbon concentrations of the contaminated sea-waters ranged from 0.03 to 3.00 ppm. The exposure studies were conducted by exposing the fish to contaminated waters for a specified period and then subjecting the fish to sensory evaluation in a triangle test in which the flavour and taste of the fillets were compared to fillets from a control group of fish held in uncontaminated water. In the depuration trials, the fish were placed in fresh sea-water immediately after the exposure period and were tested by taste panels for tainting after 1, 4, 7, and 14 d.

From the sensory evaluation it was concluded that the threshold hydrocarbon levels in sea-water which will impart a taint in cod fillets within a 24-h exposure period are in the range of 0.4 to 3.0 ppm for Hibernia, 0.3 to 0.8 ppm for Amauligak, below 0.5 ppm for Brent, and 0.7 to 1.2 ppm for Conoco. For the crude oils, a hydrocarbon concentration above 3 ppm adversely stressed the cod and levels near 8 ppm had an immediate lethal effect.

Depuration trials were carried out with WSF from only two oils; Amauligak and Hibernia. The fish were exposed for 8 and 24 h to concentrations of 3.0 and 2.6 ppm hydrocarbon, respectively, for Amauligak WSF and to corresponding values of 1.8 and 2.4 ppm for Hibernia. After the 8-h and 24-h exposure periods the fish were allowed to depurate in fresh, uncontaminated sea-water. In all four exposures, the fish were tainted within the 8-h or 24-h exposure period and the taint depurated within 24 h, except in the fish exposed to Amauligak WSF for 24 h. The taint of these fish, exposed at a concentration of 2.6 ppm hydrocarbon, depurated within 1 to 4 d.

Bioaccumulation of the hydrocarbons in the fillets was studied only in fish exposed to Hibernia WSF in the depuration trials. The fish exposed for 8 h and 24 h had 0.70 and 0.50 ppm hydrocarbon and these levels dropped to 0 and 0.08 ppm, respectively, after 24 h. These values correlated extremely well with the corresponding sensory evaluations. The taste panelists were able to detect the contaminated fillets (after 8-h or 24-h exposure with no depuration) easily whereas the depurated fish could not be distinguished from the control.

Long-term exposure studies were carried out with WSF of the same two oils used for depuration, namely Amauligak and Hibernia. The fish were exposed for 3 or 7 d at much

lower hydrocarbon levels than the threshold values already discussed with the short-term (24-h) exposure period. The sensory evaluations indicated that some of the fish tainted mildly and it was concluded that a concentration in the range of 0.1 to 0.2 ppm hydrocarbon will impart a taint to cod when exposed for 3 or more days.

The lipid content of the fish exposed to Amauligak WSF during the long-term trial was found to be around 0.6 to 0.8% (wet weight) which was not different from those of the control fish held in uncontaminated sea-water. This result indicates that the hydrocarbons in the water had no influence on the lipid metabolism of cod. Fish with lean muscle, such as cod, have limited lipid reserves in the muscle to absorb or accumulate extraneous organic matter.

Generally, it was determined that a 24-h exposure to a crude oil WSF of approximately 0.5 ppm hydrocarbon will impart a taint to cod and that exposures to lower levels (<0.2 ppm) will taint cod after 3 d of exposure. To place this study in perspective it is recommended that the probability of these levels of contamination existing under environmental conditions should be assessed.

RÉSUMÉ

Le poisson est dit avarié lorsqu'il s'y developpe une saveur atypique causée par la détérioration naturelle ou par l'assimilation de contaminants dans les tissus comestibles. Le but principal de cette étude a été d'établir une base scientifique d'évaluation des niveaux d'avarie chez un poisson commercial représentatif, en rapport avec la présence et la persistance d'hydrocarbures solubles du pétrole.

Nous avons mené, a l'échelle du laboratoire, une série d'expériences d'exposition à court terme (24 h) et à long terme (3 et 7 jours), afin (a) d'évaluer les seuils de concentration d'hydrocarbures de pétrole dans l'eau de mer, qui puissent produire des problèmes d'avarie chez la morue franche (Gadus morhua) et (b) d'obtenir une appréciation du délai de rétablissement de la saveur normale (période de dépuration) après que l'avarie se soit produite.

Quatre pétroles différents ont été utilisés dans cette étude: d'Amauligak, de Brent, d'Hibernia, et Conoco. Les trois premiers étaient des pétroles bruts, alors que le pétrole Conoco était un distallat moyen. Les profils d'hydrocarbures des trois pétroles bruts étaient plus ou moins semblables entre eux; les composants principaux étaient des alcanes normaux, cycliques et ramifiés, avec de faibles niveaux d'hydrocarbures aromatiques à bas point d'ébullition; les n-alcanes s'échelonnaient généralement de C_{11} à C_{31}. Le distillat Conoco était tout à fait différent des trois pétroles bruts; les composants principaux étaient des hydrocarbures à point d'ébullition modéré, probablement des alcanes cycliques et ramifiés; le contenu en n-alcanes était très faible, et les hydrocarbures aromatiques à bas point d'ébullition étaient complètement absents.

Initialement, l'on a préparé, à partir de chacun des quatre pétroles, un stock d'eau saturée en hydrocarbures (généralement désigné comme "fraction hydrosoluble" (FHS)). Les FHS ont été préparées comme suit. L'eau de mer et le pétrole ont été melangés (en proportion volumétrique de 99:1) pendant 24 h, par agitation mécanique, dans un reservoir d'acier inoxydable de 1,100 litres. Ensuite, ce melange a été laissé en repos pendant 48 h pour obtenir la séparation de la phase aqueuse et de la phase pétrole. La concentration en hydrocarbures de la phase aqueuse (c.à.d. de la FHS) des trois pétroles bruts, allait de 15 à 21 ppm, alors que la FHS du distillat Conoco

était relativement moins concentrée (~4 ppm). Les composants principaux des FHS des trois pétroles bruts étaient des aromatiques à bas point d'ébullition, qui n'étaient que des composants mineurs dans les pétroles initiaux respectifs. Les alcanes étaient presque absents. Les aromatiques à bas point d'ébullition étaient complètement absents dans la FHS du pétrole Conoco, dont les composants principaux étaient des hydrocarbures à point d'ébullition modéré, probablement des alcanes cycliques et ramifiés.

L'eau de mer a été additionnée de divers volumes de ce concentré FHS afin d'obtenir une série d'eaux de mer contaminées. Les concentrations en hydrocarbures des eaux de mer contaminées allaient de 0.03 à 3.00 ppm. Les expériences d'exposition ont été menées en exposant les poissons à des eaux contaminées pour une période spécifiée, et en soumettant ensuite le poisson à des évaluations organoleptiques consistant en un test triangulaire au cours duquel la saveur et le goût des filets ont été comparés à ceux de filets d'un groupe contrôle de poissons maintenus dans de l'eau non contaminée. Dans les essais de dépuration, les poissons ont été placés dans de l'eau de mer fraîche immédiatement après la période d'exposition, et leur état d'avarie a été testé par des jurys de dégustateurs après 1, 4, 7 et 14 jours.

Les évaluations organoleptiques ont permis de conclure que les hydrocarbures présents dans l'eau de mer provoqueront l'avarie de filets de morue, après exposition de 24 h, au-dessus de seuils allant de 0.4 à 3.0 ppm pour Hibernia, 0.3 à 0.8 ppm pour Amauligak, inférieurs à 0.5 pour Brent, et allant de 0.7 à 1.2 ppm pour le pétrole Conoco. Pour les pétroles bruts, une concentration d'hydrocarbures supérieure à 3 ppm provoquait des troubles physiologiques visibles chez les morues, et les niveaux proches de 8 ppm avaient un effet léthal immédiat.

Les essais de dépuration ont été menés avec des FHS de deux pétroles seulement: d'Amauligak et d'Hibernia. Les poissons ont été exposés pendant 8 ou 24 h à des concentrations d'hydrocarbures de 3.0 et 2.6 ppm respectivement pour la FHS d'Amauligak, et de 1.8 et 2.4 ppm respectivement pour la FHS d'Hibernia. Après exposition de 8 ou 24 h, on a laissé les poissons se dépurer dans de l'eau de mer fraîche, non contaminée. Dans toutes les quatre expériences d'exposition, les poissons sont devenus avariés au cours des périodes d'exposition de 8 ou 24 h, et l'avarie a été éliminée déjà après 24 h de dépuration, sauf

chez les poissons exposés à la FHS d'Amauligak pendant 24 h. L'avarie de ces derniers poissons (exposés à une concentration d'hydrocarbures de 2.6 ppm) a été dépurée après 1 à 4 jours.

La bioaccumulation des hydrocarbures dans les filets a été étudiée seulement chez des poissons exposés à la FHS d'Hibernia dans les essais de dépuration. Les poissons exposés pendant 8 et 24 h avaient 0.70 et 0.50 ppm d'hydrocarbures, respectivement, et ces niveaux sont tombés à 0 et 0.08 ppm respectivement après 24 h. Ces valeurs présentent une excellente corrélation avec les évaluations organoleptiques correspondantes. Les dégustateurs ont su failement détecter les filets contaminés (après exposition de 8 ou 24 h sans dépuration), alors que le poisson dépuré n'a pu être distingué du poisson de contrôle.

Des expériences d'exposition prolongée ont été menées avec la FHS des deux pétroles utilisés dans les essais de dépuration: les pétroles d'Amauligak et d'Hibernia. Les poissons ont été exposés pendant 3 ou 7 jours à des niveaux d'hydrocarbures beaucoup plus bas que les valeurs seuil déjà discutées pour la période d'exposition à court terme (24 h). Les évaluations organoleptiques ont indiqué qu'une partie des poissons devenaient légèrement avariés, et l'on a pu conclure qu'une concentration d'hydrocarbues de 0.1 à 0.2 ppm avariera les morues exposées pendant 3 jours ou plus.

Le contenu en lipides des poissons exposés à la FHS d'Amauligak dans le test à long terme, s'est trouvé être d'environ 0.6 à 0.8% (poids humide), ce qui ne diffère pas des valeurs chez les poissons de contrôle maintenus dans l'eau de mer non contaminée. Ce résultat indique que les hydrocarbures de l'eau de mer n'ont exercé aucune influence sur le métabolisme lipidique des morues. Les poissons à muscles maigres, tels la morue, n'ont dans leurs muscles que des réserves lipidiques limitées qui puissent absorber ou accumuler les matières organiques étrangères.

L'on a déterminé qu'en général une exposition de 24 h à une FHS de pétrole brut d'environ 0.5 ppm d'hydrocarbures, avariera la morue, et que des expositions à des niveaux plus bas (<0.2 ppm) avarieront la morue après 3 jours d'exposition. Pour mettre cette étude en perspective, nous recommandons d'évaluer la probabilité que ces niveaux de contamination existent dans les conditions de l'environnement.

1.0 INTRODUCTION

1.1 GENERAL

Tainting is the development of an atypical flavour in fish caused by natural spoilage or by the assimilation of contaminants into fish tissue. Tainting as a result of exposure to petroleum hydrocarbons has been reported since the early 1940s. Off flavours have been associated with diesel fuel (Mackie et al., 1972), crude oil (Motohiro and Inoue, 1973), Bunker C (Shenton, 1973; Scarratt, 1980), and gasoline (Kerkhoff, 1974) present in the environment, as well as with refinery effluents (Nitta, 1972; Connell, 1974). Connell and Miller (1981) and, more recently, Tidmarsh et al. (1985) reviewed the reported incidences of tainted finfish and shellfish and discussed factors affecting the occurrence of tainting. Tidmarsh et al. (1985) also assessed the effectiveness of various analytical methods which could be used in identifying a possible tainting situation.

Tainting is a perceptual problem and, as such, experience as well as preference can greatly influence a determination as to whether or not a food is "off". The importance lies in whether the flavour or odour of the product is altered, not whether a contaminant impairs or improves the flavour.

For the purpose of this study, the basic definition of the term "taint" developed by the Joint Group of Experts on Scientific Aspects of Marine Pollution (GESAMP) Working Group on the Evaluation of the Hazards of Harmful Substances Carried by Ships (EHS) (GESAMP, 1985) is endorsed.

> "(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 taint. Screening products, with this definition in mind, would lead to a statement on the threshold concentration of detection and not in recognition of the contaminant. It is important to note that it is the off taste, not the determination of what is causing the off flavour, which would cause the product to be labelled as tainted.

The threshold level of exposure necessary to cause a petroleum taint depends on the chemical composition of the hydrocarbon contaminant and on the species exposed. The principal components of crude and refined oil which cause tainting include the phenols, dibenzothiophenes, naphthenic acids, mercaptans, and methylated naphthalenes (GESAMP, 1977). Some of these compounds are polar, some volatile, and most are somewhat soluble in water and lipids. The fraction that is soluble in water is generally referred to as the water-soluble fraction (WSF). The components expected in the WSF should include cycloalkanes, aromatics, and alkylated aromatics from benzene to methyl napthalenes (Murray et al., 1984). The n-alkanes from C_{11} upwards are almost insoluble in water and should be present in the WSF only in trace levels. Components other than hydrocarbons, such as phenols, anilines, nitrogen, and sulphur compounds, are invariably present in WSF, but were not measured in the present study. The lipid solubility of the above hydrocarbons makes them readily transferred by partitioning into the blood and tissues of organisms. Consequently, it is generally accepted that the amount of "free" lipids present in a fish is an indication of the susceptibility of that organism to acquire a taint (Whittle and Mackie, 1976). Hence, for most groundfish where the lipid content of the edible tissue is low ($\leq$3% wet weight), the susceptibility to acquiring a taint is far less than for pelagic species, such as herring (Clupea harengus) and mackerel (Scomber scombrus), in which the lipid content of the muscle may be as high as 16-20% of the wet weight of edible tissue. The lipid content of the muscle in some species can show marked seasonal variation which is reflected in the tainting potential, or the threshold concentrations at which an off-flavour is noticeable.

The commercial species selected was Atlantic cod (Gadus morhua). Cod is a major fishery in Atlantic Canada. According to the latest available statistics (Anon., 1987), the volume of cod landed along the Atlantic coast (N.S., N.B., P.E.I., Que., Nfld.) in 1986 was 457,270 metric tons compared to 572,394 metric tons of all other groundfish and pelagic fish species landed.

GESAMP recently developed "Draft guidelines for evaluating threshold values for fish tainting" (GESAMP, 1983) which formed the basis for recommendations by Tidmarsh et al. (1985) and stimulated the evaluation of these guidelines by European Chemical Industry Ecology and Toxicology Centre (ECETOC) (1987).

Tidmarsh et al. (1985) concluded that the triangle test was the most suitable method of identifying a tainting situation and that chemical analysis can be used to support the sensory evaluation. They recommended that these methods should be tested and refined for their application to a potential tainting situation of a locally exploited species. They also recommended that threshold concentrations causing tainting and depuration times should be determined for several representative species of finfish and shellfish. These recommendations formed the basis for this study.

1.2 OBJECTIVES AND SCOPE

The main goal of the study was to establish a scientific basis for assessing taint in a representative commercial species, in relation to the occurrence and persistence of soluble petroleum hydrocarbons. The study has four main objectives:

- to identify the threshold concentrations of a water-soluble fraction (WSF) of four different oils that will cause a taint in Atlantic cod (Gadus morhua): the four oils to be used are Brent crude, Amauligak crude, Hibernia crude, and Conoco base oil;

- to determine the length of time for a taint to disappear (depurate) after exposure;

- to assess the effects of long-term exposure (bioaccumulation) at less than threshold concentrations; and

- to determine the hydrocarbon levels in the fish flesh of tainted cod.

Some calibration of the system was required before actual trials were begun. These included:

- the resolution of problems for an appropriate exposure set up;

- determining mixing and setting times and the most efficient mixing ratios of oil to water for the preparation of WSFs;

- the extraction and recovery methodology for gas chromatographic (GC) analysis of water and tissue samples; and

- screening and training taste panelists.

2.0 MATERIALS AND METHODS

2.1 FISH AND FISH HOLDING

2.1.1 Fish Acquisition

The fish were collected by long-lining or hand-lining in 12 to 15 fathoms of water during day trips from Eastern Passage, Halifax, Nova Scotia and held on ship in a flowing sea-water tank. After landing they were immediately transferred, initially to the Lower Water Street Laboratory of Fisheries and Oceans Canada, Halifax and, later, to the Aquatron facility of Dalhousie University, Halifax. The fish were caught from mid-October to mid-November, 1986.

2.1.2 Holding Facilities

The fish were initially kept at the Water Street Laboratory of Fisheries and Oceans in three circular fibreglass holding tanks, 2 m in diameter by 2 m deep in aerated running sea-water drawn from the bottom of Halifax Harbour. It was felt that the maximum capacity of each tank was about 40 fish (a total capacity of 120 fish).

Arrangements were made to hold the additional fish required for the trials at Dalhousie University. Two, large, circular tanks (1.65 m x 1.35 m and 1.80 m x 1.36 m), plus the satellite tank of the Aquatron pool tank, were used to hold an additional 142 fish.

By early November mortalities were experienced, brought on, in part, by crowding (see Section 2.1.3). Also, it was felt that all the fish should be kept at one location with facilities for conducting exposure trials adjacent to the holding tank(s). On 12 November 1986, all the fish were transferred to the pool tank at Dalhousie (Plate 1).

The pool tank is a 685 m^3 circular tank (15.2 m diameter by 3.9 m deep). It is constructed of reinforced concrete with a PVC lining. Sea-water for the pool tank is drawn from the Northwest Arm of Halifax Harbour and passes through four, sand, pressure filters. The flow rate into the pool tank varies between 200 and 300 L/minute depending on the time of day.

The water temperature in the pool tank dropped from 10°C in mid-November to a low of 2°C by mid-February. At this time the temperature was brought back up to 5°C for

PLATE 1 POOL TANK

PLATE 2 WSF MIXING VESSEL

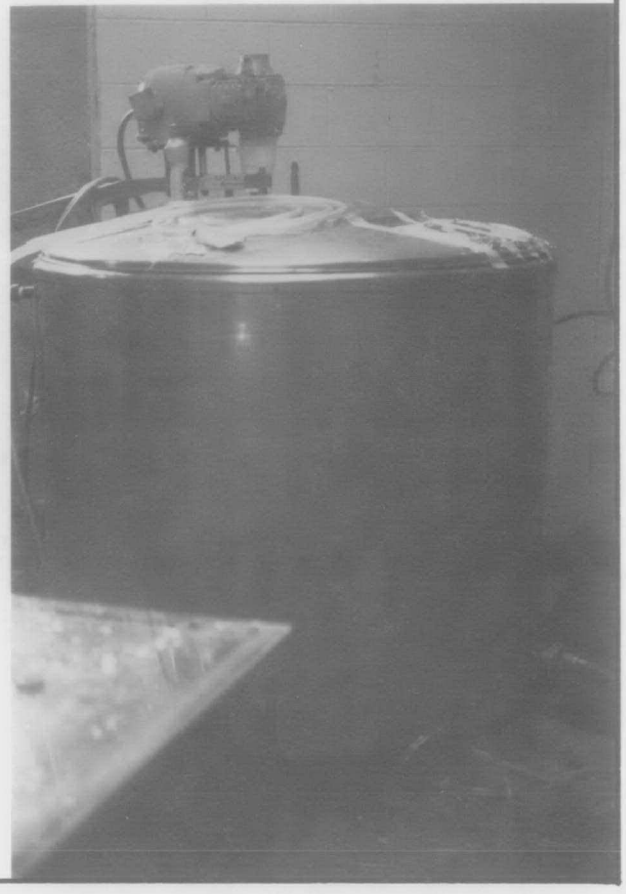


PLATE 3 EXPOSURE TANK

health reasons (see Section 2.1.3). The ammonia-nitrogen (NH_3-N) level was 0.1 ppm. The light cycle was set for 12 h of light and 12 h of darkness. This cycle was upset, however, when trials were being run in the adjoining work area.

The pool tank was vacuumed daily. An air bar was installed which assisted in keeping the water column well mixed and resulted in small particles being drawn down the surface drain.

2.1.3 Fish Health and Nutrition

It was proposed that the fish would be fed a specially prepared pellet with a 3% fat content. This feed was purchased but we were unable to persuade the fish to accept this form of food.

At this time high mortalities were being experienced (an average of 2-4 fish/d). Several of the dead fish were examined by the Fish Health Unit of the Department of Fisheries and Oceans, and were found to have vibriosis, a bacterial infection caused in part by stress, overcrowding, and not feeding. It was recommended that all the fish be innoculated with 0.5 cc of oxytetracycline.

On 12 November, the remaining 118 fish from Lower Water Street were transferred to Dalhousie, were innoculated, and were put in the pool tank. The 142 fish held in the various tanks at Dalhousie were similarly innoculated and were transferred to the pool tank. An additional 74 fish were caught on 12 and 13 November and these fish were also treated before being placed in the pool tank. Five fish died during the next two days, leaving a total of 330 fish.

Once the fish were all settled in the pool tank, the diet was changed from the prepared pellets to chopped herring or mackerel, and beef liver. The fish fed well and a feeding schedule of 20-25 pounds of mackerel every other day and 6 pounds of beef liver once a week was established.

Another five fish died 10 days to 2 weeks after the innoculation program. Examination of these fish showed that vibriosis was still present. It was felt, however, that a second innoculation would be too stressful and, considering the fact that the fish were eating well, no further further treatment was considered.

The fish continued to do well and no further mortalities occurred from late November until mid-February. At that time several fish died and were examined by the Fish Health Unit of the Department of Fisheries and Oceans.

External sea lice (Lepeophtherius sp.) on the skin of the fish were thought to have caused lesions on the tail. Internally, the hind gut was infested with Hexamita sp. which would debilitate and stress the fish; however, there was no systemic infection in the cod. Cultures were negative for any bacterial or fungal pathogens.

It was recommended that the heavily parasitized fish be removed from the pool tank as a precaution against further debilitation of the population as a whole. On 2 March, 99 fish were removed from the tank. Fifty-three of the more "rough" looking fish were sacrificed and filleted for taste panel controls, and the remaining 43 were held for future exposure trials.

On 16 April, 27 fish were transferred to the satellite tank and were held for future trials. In a 24-h period from 19 to 20 April all the fish died. Three of the fish were examined by the Fish Health Unit. There were no external or internal parasites on the fish and bacterial cultures were negative, so disease did not appear to be the cause of death. There were, however, problems with the Aquatron intake over that weekend and the water flow was off or low for an unknown period. The remaining 20 fish in the pool tank were not affected because they were in a much larger volume of water.

2.2 TEST MEDIA

2.2.1 Test Oils

The request for proposals recommended treatments involving two east coast crudes (one conventional and one waxy-viscous), one Beaufort Sea crude, and a drilling mud base oil. Hibernia crude oil was used as a representative waxy-viscous east coast oil. Brent crude was a substitute for a Scotian Shelf light crude oil which was unavailable at the time of the study. Amauligak was used as a representative Beaufort Sea oil and Conoco as the base oil.

Two barrels of Hibernia were obtained from Mobil Oil Canada Ltd., one barrel of Brent from Esso Petroleum, one barrel of Amauligak from Gulf Oil, and two barrels of base oil from Conoco.

Care was taken that the oils did not become weathered prior to use. The barrels were opened a minimal number of times and the barrels were left not less than half full. The oil was removed with a hand pump and was stored in 4-L amber glass bottles until required for preparing WSFs.

Handling procedures for the Hibernia crude followed instructions provided by Mobil Oil Canada Ltd. to ensure sample homogeneity. The barrel was heated to 45°C over several days using heat lamps in a small, insulated box built around the barrel. The oil was then mixed to remove any wax deposited on the wall prior to transferring the oil to the 4-L storage bottles. The 4-L bottles were reheated in a water bath and were mixed in preparation for mixing the WSFs.

2.2.2 Water-soluble Fraction

All water-soluble fractions which were to be used for the exposure trials were prepared in a large 1,100-L stainless-steel mixing vessel equipped with a powerful mechanical stirrer, a bottom drain, and a cooling-water system (Plate 2). The stirrer consisted of a three-blade propeller just above on the bottom of the vessel attached to a vertical shaft and driven by a 2.2 horsepower electric motor mounted on the top of the vessel. The action of the mixing was such that a vortex was produced.

Sea-water and the test oil, in a ratio of 99:1 (V/V), were stirred for 24 h and were allowed to settle for an additional 48 h (see Appendix A for determination of mixing ratios). The WSF was kept cool by circulating cold water through the outer jacket of the mixing vessel.

The WSF was stored in the mixing vessel and was pumped directly out of the vessel from the bottom drain and into the exposure tanks (see Section 2.3.2) as required.

2.3 EXPOSURE TRIALS

2.3.1 General

Short-term exposure trials were run on all four test oils. During these trials fish were exposed to three different concentrations of WSF (approximately 0.25, 0.50 and 2.50 parts per million (ppm) for 24 h. At the end of the exposure period the fish were removed from the exposure tanks and were prepared for taste panel assessment.

Depuration trials were conducted on two of the test oils; Hibernia and Amauligak. These trials consisted of exposing fish to approximately 2.5 ppm WSF for periods of 8 and 24 h. After exposure the fish were transferred to larger tanks containing clear sea-water and were allowed to depurate for 0,1,4,7, and 14 d before being prepared for taste panel assessment.

Long-term exposure trials were also conducted with WSFs of Hibernia and Amauligak crude oils. During these trials fish were exposed to a proposed 0.10 and 0.25 ppm WSF for periods of 3 and 7 d.

Figure 2.1 is a schematic representation of the test system configuration. Exposure tanks and control tanks were plumbed separately to ensure that there was no contamination from the drain system. There was oxygen flow to all tanks and oxygen levels were monitored regularly (see Section 2.3.3). WSF was pumped directly from a bottom drain in the mixing vessel to the exposure tanks. Hydrocarbon concentrations were monitored hourly by measuring the fluorescence of the exposure water as well as by GC analysis of water samples taken at the beginning and end of each trial (see Section 2.3.4).

2.3.2 Exposure Tanks

The short-term and depuration trials in which fish were exposed to a WSF of Amauligak crude oil were conducted using 350-L circular fibreglass tanks fitted with central drains. The exposure water was kept cool by circulating cold water through a hose wrapped around the outside of each tank. The tank was then wrapped with fiberglass insulation and plastic.

The short-term trials using Brent, Hibernia, and Conoco test oils, the Hibernia depuration trials, and all long-term trials, were conducted using 355-L square tanks with corner drains. Plate 3 shows one of the exposure tanks with fish in it. Figure 2.2 is a schematic representation of a tank fitted for an exposure trial. The tanks had 2-cm thick insulated walls and no further means of maintaining the exposure temperatures were employed.

All tanks were covered with 6-mm clear plexiglass tops with a 2-cm strip of foam tape where the plexiglass contacted the tank. A sampling port (4-cm in diameter) was cut in each top and was fitted with a 14-cm tube which opened below the water line, thus exposing only 12.5 cm^2 of

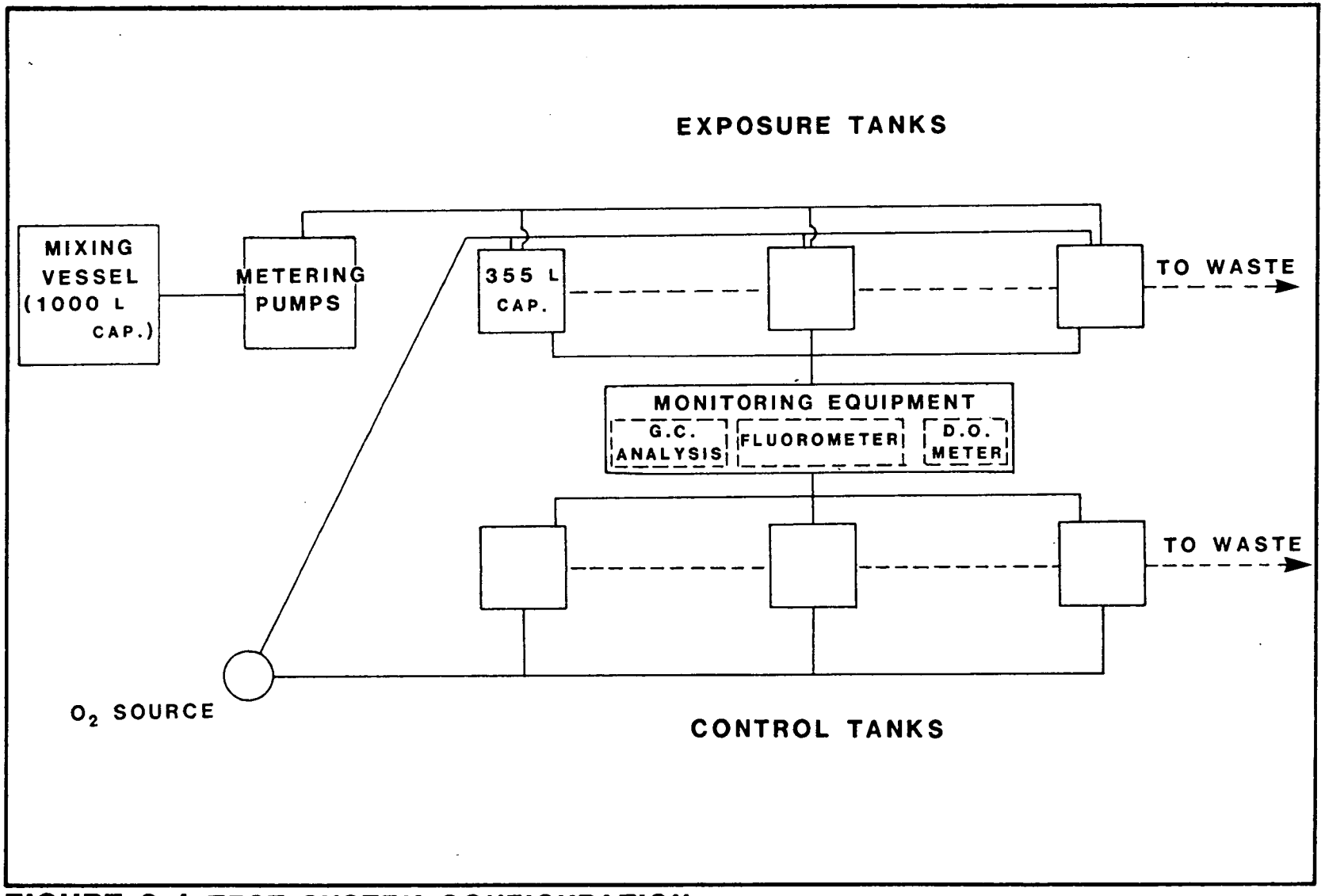


FIGURE 2.1 TEST SYSTEM CONFIGURATION.

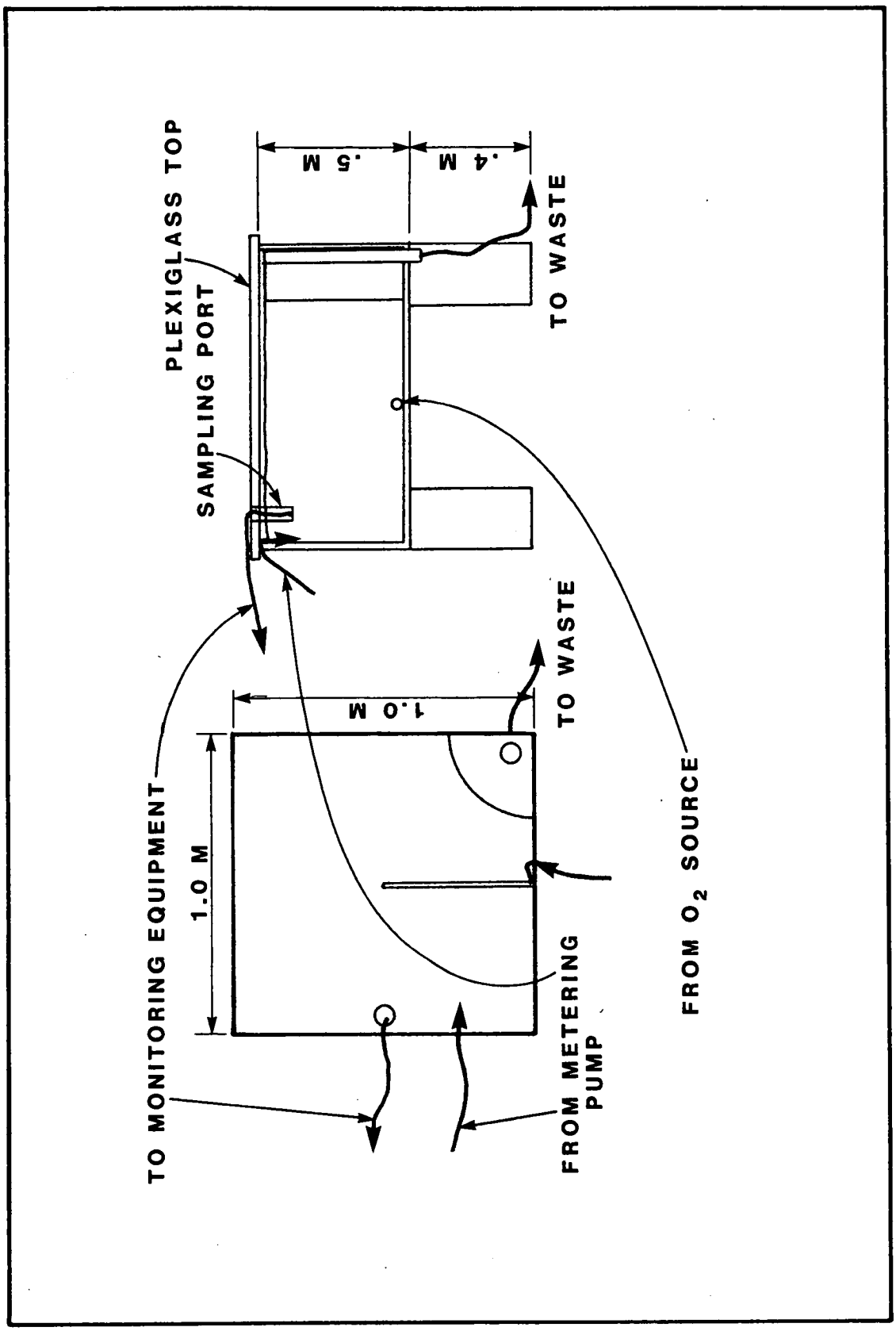


FIGURE 2.2 PLAN AND PROFILE OF EXPOSURE TANK.

water to the air each time the port was opened. When not in use the port was plugged with a rubber stopper. Fish were introduced to the exposure water by lifting up one corner of the plexiglass top and quickly sliding the fish in the tank.

The depuration tanks used were square tanks (1.5 m x 1.5 m and 0.8 m deep) fitted with bottom drains. Water was pumped out of the pool tank and into the depuration tanks to ensure that the fish were exposed to conditions similar to the control population.

2.3.3 Aeration of Exposure Water

Pure oxygen was used to aerate the exposure water rather than compressed air (see Appendix B). The flow of oxygen was controlled by a regulator installed on the oxygen cylinder and a six-way valve which split the flow to the various exposure tanks. A 46-cm bubble wand was fixed to the bottom of each tank with silicon. This wand delivered a fine mist of oxygen bubbles with very little turbulence.

The dissolved oxygen (DO) content of the exposure water during the short-term and depuration trial was measured hourly using a YSI Dissolved Oxygen Meter (Plate 4) and the flow of oxygen to each tank was adjusted to maintain a concentration of between 70 and 90% saturation.

The oxygen level of the exposure water was not monitored as rigorously during the long-term trials and, consequently, the exposure water would frequently become supersaturated with oxygen.

2.3.4 Maintaining the WSF Concentration

A sample of the WSF concentrate was taken directly from the mixing vessel after 48 h settling and was analysed by GC to determine the volatile hydrocarbon concentration. The exposure tanks were then filled using proportional amounts of sea-water and WSF to obtain the desired exposure concentrations. A water sample was taken for GC analysis to check this concentration. The fluorescence of the exposure water was also recorded and WSF was added, as required, to maintain this initial fluorescence. Fluorescence of the exposure water was monitored using a Turner Designs Model 10 Fluorometer fitted with a short-wavelength oil kit and a flow through cell (Plate 5). The calibration of this instrument is detailed in Appendix C.

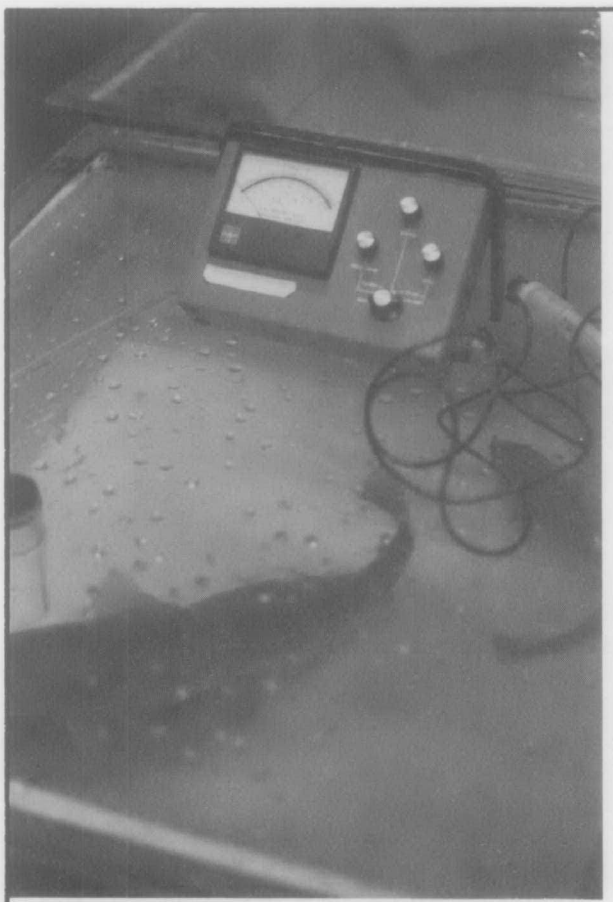
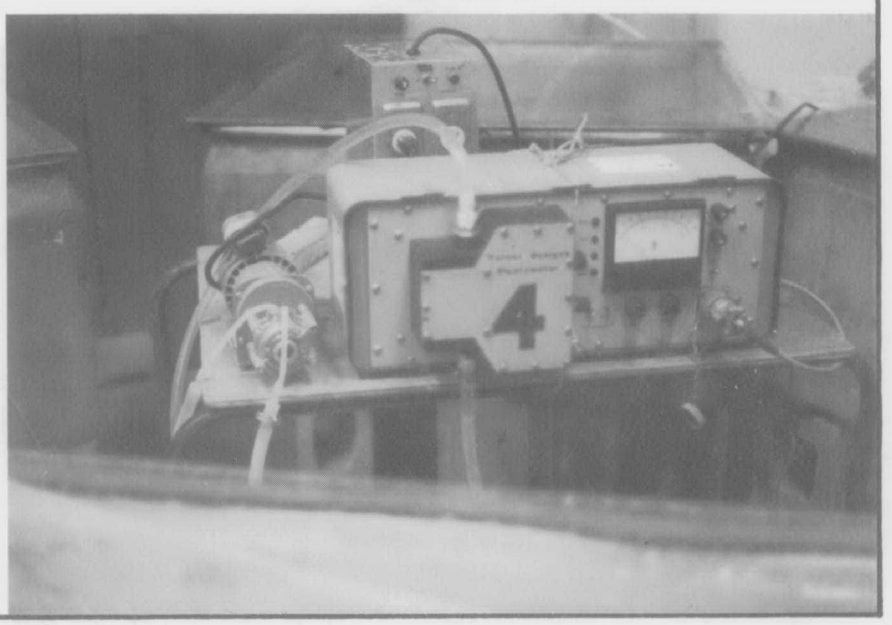


PLATE 4 EXPOSURE TANK WITH AN OXYGEN METER

PLATE 5 FLUOROMETER



A water sample was taken for GC analysis at the end of each trial to check the final hydrocarbon concentration.

The WSF was added by pumping directly out of the mixing vessel into the exposure tanks. Two variable speed peristaltic pumps (Cole-Parmer Masterflex) and one veristaltic pump (Monostat) were used to deliver the WSF. A range of flow rates from 4 mL/min to 2 L/min was possible with this system.

Viton tubing was used with the Masterflex pumps and Tygon tubing with the Monostat pump. All other tubing, before and after the pumps, was FDA, grade A, PVC tubing approved for use in food production. Preliminary tests showed that there was no contamination of sea-water by dissolved matter from the tubing.

The exposure tanks were flushed once and, if necessary, twice daily during the long-term exposures to prevent a build-up of excretory products in the water. After flushing, the hydrocarbon content was assumed to be 0. WSF was added in batch amounts proportional to the initial concentration of the WSF and the losses as observed by the drop in fluorescence of the concentrate (i.e., if there was a 20% loss in fluorescence in the concentrated WSF since it was last analysed by GC, 20% more WSF was added to the exposure tanks than would have been added based on the earlier GC analysis).

2.3.5 Fish Handling

The fish to be used for an exposure trial were removed from the pool tank at least 2 days prior to the proposed trial and were placed in the satellite tank. They were not fed for the 2 days prior to an exposure. When required, the fish were caught with a dip net and then, using cotton gloves, placed directly into the exposure tanks containing the desired concentration of WSF.

When a trial was completed the fish were dipped out of the exposure tank, were washed in clear sea-water, and were placed in plastic bags on ice. Death was by suffocation. The fish were immediately transferred to Canadian Institute of Fisheries Technology (CIFT) where they were gutted, filleted, and frozen at -35°C until required for taste panel assessment.

Fish that were held after an exposure for depuration were tagged just prior to being placed in the depuration

tanks. Spaghetti tags were "shot" into the flesh of the fish directly below the dorsal fin.

2.4 HYDROCARBON ANALYSIS

2.4.1 Gas-Liquid Chromatography

The hydrocarbon analyses were executed on a Perkin-Elmer Sigma-3B gas chromatograph equipped with a flame ionization detector (FID) and a split injection system. The chromatography was on a DB-1 (60 m x 0.25 mm I.D., 0.25 micron) flexible, fused-silica capillary column (polymethylsiloxane phase, chemically cross-linked, and surface bonded; J and W Scientific, Inc., Folson, California).

Conditions and temperatures were FID, 280°C; injector, 280°C. Column temperature was programmed as follows: initial temperature 45°C, held for 15 min then programmed at 13°C per min up to 280°C and held for 30 min. The carrier gas (H_2) pressure was 16 pounds per square inch gauge (psig) and the hydrogen and air pressures of the FID were 22 and 30 psig, respectively.

The concentration of hydrocarbons in the samples was calculated with respect to the internal standard, n-heneicosane (C_{21}). This standard was an ideal internal standard as it is not readily volatile and, therefore, the evaporation losses are minimal. It eluded on the column without interfering with any of the petrosenic hydrocarbons.

The peak areas were recorded and integrated on an LCI-100 computing integrator (Perkin-Elmer, Norwalk, Connecticut). The areas of all the peaks, excluding those contributed by hexane and the blank run, were summed and the concentrations were calculated from the ratios to the internal standard.

2.4.2 Crude Oils

Crude oil (20 mg) was dissolved in distilled hexane (1 mL) and 1 µL of this solution was analysed by capillary gas-liquid chromatography (GC) as described.

2.4.3 Water-soluble Fraction

Water samples for hydrocarbon analysis were collected by siphoning exposure water from the tanks into either amber- or foil-wrapped glass bottles. The bottle was

rinsed with the sample water, then was filled to overflowing, and was covered with tin foil before the top was put on. When possible the sample was analysed immediately, otherwise it was held at 5°C until analysed.

Analysis of trace amounts of organic compounds in water usually requires recovery into a small volume of solvent before determination by GC. In most methods the solvent phase requires further concentration which usually causes serious losses of more volatile compounds. Thus, methods of analysis which avoid the solvent concentration step are preferable for the analysis of trace levels of organic compounds in water.

In the present study, the microextraction technique, described by Murray (1979) (see also Murray and Lockhart, 1981 and Murray et al., 1984), which avoids the concentration step, was used to extract the hydrocarbons from the WSF. The microextraction procedure is efficient and simple and the results are reproducible. The method uses only 1 mL of hexane for extraction of dissolved organic matter in water. The extraction procedure is described below.

An extraction flask of capacity slightly over 1 L was constructed with a side arm and a capillary tube at the top-centre of the flask. This extraction flask was the same as that described by Murray (1979). The WSF (980 mL) was placed in the flask and the mixture was shaken with hexane (1 mL) for 2 min. The flask was stoppered and the layers were allowed to settle for a minimum of 15 min in a domestic refrigerator (~5°C) to permit the hexane droplets dispersed throughout the aqueous phase to collect on the surface. The hexane layer was directed to the centre capillary tube by adding distilled water (70-80 mL) through the side arm, while tilting the flask at an angle of about 45°. One µL of the hexane layer from the capillary tube was taken into a 10 µL Hamilton GC syringe, as well as 1 µL of a hexane solution of n-heneicosane ($n\text{-}C_{21}$; 0.508 µg/µL) and both were injected into the GC. Quantitative analyses in all cases were based on the use of $n\text{-}C_{21}$ as the internal standard.

The areas of the peaks, excluding those contributed by hexane, were integrated and concentrations were calculated from the ratio to the internal standard. These experimentally determined concentrations were corrected for the recovery efficiency of the microextraction technique.

Though the microextraction technique is efficient and reproducible, as with any other extraction the percent recovery of hydrocarbons is not 100% (Murray and Lockhart, 1981). Therefore, the experimentally determined concentrations have to be corrected for 100% recovery. Because the extractibility of hydrocarbons from the WSF depends on the chemistry of the hydrocarbon, therefore, recoveries vary with the type of hydrocarbon. Hence, a thorough study should investigate the recovery of each of the components present in WSF. This study requires complete identification of all of the components present in the hydrocarbon profile of WSF and ready availability of authentic standards.

Murray and Lockhart (1981) have investigated the percent recovery of a number of hydrocarbons, using the same type of microextraction flask and techniques as those used in the present investigation. These hydrocarbons included ethyl benzene, trimethyl benzene, isopropyl methyl benzene, naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, and 2,3-dimethyl naphthalene which are generally representative of those found in the water-soluble fractions of crude oils and petroleum products. The recoveries were around 40% for these hydrocarbons (Murray and Lockhart, 1981). Therefore, this 40% recovery factor was used in the present investigation for correcting the experimentally determined concentrations of WSF.

2.4.4 Fish Fillet

The hydrocarbons in the fillets of cod were initially examined by the microextraction procedure of Murray and Lockhart (1981). This procedure was found to be unsuitable for examining low levels of hydrocarbon in cod tissue. None of the fish tissues analysed, even those from fish found to be tainted by sensory analysis, showed any peaks of hydrocarbon origin when analysed by GC. The Murray and Lockhart (1981) procedure probably works well with high levels of hydrocarbons (2-5 ppm), but apparently not with the very low levels encountered in the present study. Having failed with the microextraction procedure we then tried the steam distillation procedure of Ackman and Noble (1972), which was successful in the detection of hydrocarbons in the cod fillets. An advantage of the steam distillation procedure over the microextraction procedure, is that it allows the use of 60 g of sample as opposed to 5 g.

The two procedures used to analyse the fish fillets for hydrocarbon content are outlined below.

Murray and Lockhart method. About 5.0 g of mascerated fish muscle was accurately weighed in a 100-mL beaker. The fish muscle was stirred with 10 mL of CH_2Cl_2 using a glass rod and was then covered with aluminum foil and was left to stand for 15 min. The slurry was passed through a coarse, stainless-steel sieve and 7.8 mL of CH_2Cl_2 extract was collected. Five mL of the CH_2Cl_2 was cleaned by passing it through a column (30 cm x 1 cm) of anhydrous sodium sulphate (3 g, prewashed with CH_2Cl_2) and Florisil (10 g, prewashed with CH_2Cl_2 and activated at 100°C overnight). The compounds were eluted with CH_2Cl_2 to give a 5-mL eluate. This eluate was transferred to the microextraction flask (see Section 2.2.3.1) and 100 mL of acetone and 850 mL of water were added to produce a homogeneous phase. This solution was then extracted with 1 mL of hexane and was analysed by GC along with the n-C_{21} internal standard as described previously (see Section 2.4.1).

Ackman and Noble method. The distillation apparatus consisted of a 500-mL round-bottomed flask, a Barrett-type distilling receiver, and a gooseneck tube attached to a graduated collection flask, with the addition of an overflow to return excess solvent to the flask, and a water condenser. The water condenser was connected to the graduated collection flask of the Barrett-type distilling receiver. A fresh, pre-rinsed (with CH_2Cl_2), coarse, glass wool plug was placed at the inside joint of the gooseneck tube and the 500-mL round-bottomed flask prior to each distillation of hydrocarbons from the fish muscle. This technique prevented any foam from being transferred directly into the graduated collection flask. Bumping of water was minimized with the help of a few glass beads. The whole apparatus was rinsed with distilled water and acetone and dried shortly before use.

Distilled water (270 mL) was placed in the 500-mL flask and was heated to boiling using an electric heating mantle. The condensate was collected into the graduated collection flask. The distillation was terminated after 20 mL of condensate was collected. The flask of the distillation apparatus was cooled in ice and the inside of the condenser was rinsed with CH_2Cl_2. The water condensate and CH_2Cl_2 were discarded. Minced meat (30-60 g) from cod fillet was added to the remaining water (250 mL) in the distillation flask. The distillation was again commenced and was continued until 20 mL of condensate was collected. The flask of the distillation apparatus was cooled in ice, the condenser was rinsed with CH_2Cl_2 (2 mL) into the collected condensate, and the whole was drained into a

separatory funnel. The CH_2Cl_2 and the aqueous layers were allowed to separate. The CH_2Cl_2 extract was collected, was dried over Na_2SO_4, and an aliquot (1 µL) was analysed by GC.

2.5 TASTE PANELS - SENSORY EVALUATION OF TAINT

2.5.1 Preparation of Fish Fillets

The fish from the experimental tanks were caught and immediately transferred to the pilot plant of CIFT. Soon after their arrival the fish were filleted, and any cod worms present in the tissues were removed by hand. The fillets were individually wrapped in polyethylene bags and were stored at -35°C. Taste panels were conducted within 48 h of exposure to the WSF as recommended by GESAMP (1983). However, when this was not possible the fillets were stored for a longer period, to a maximum of 5 d.

The taste of the fish exposed to WSF was evaluated with respect to a control group of fish (see Section 2.5.2). The fish from the main pool tank at Dalhousie (see Section 2.1.2) served as the control fish for most of the exposure studies. About 60 fish were removed from the main pool and filleted. The fillets stored for a maximum of five weeks at -35°C and were used as required.

The fish to be used for controls for the range-finding trial and the Amauligak short-term exposure were taken from the main pool and kept for 24 h in exposure tanks under exactly the same conditions as the exposed fish, except that the sea-water was not spiked with WSF. The fish from this control group were filleted and the fillets were stored exactly as those of the corresponding exposed fish.

From each experimental or control group three or four fish were used for sensory evaluation. The thawed fillets were cut into strips and were minced in a Cuisinart food processor for 10 to 15 s which ensured a homogeneous sample. A sample of about 25 to 30 g was formed into a patty, placed in a covered glass petri dish, and cooked in a microwave oven for 45 s.

2.5.2 Sensory Evaluation of Samples by Triangle Test

The triangle test was used to detect tainting and to establish the threshold levels in the exposed fish. This method was recommended by Tidmarsh et al. (1985) and GESAMP (1983). The triangle test presents the panelist with three

samples; two are identical and one is different (two controls + one exposed or two exposed + one control) and asks the panelist to select which is the odd sample. If there are 16 panelists, eight are presented with two controls and one exposed sample and eight receive two exposed samples and one control. The petri dishes are placed on a tray in different triangular formats. The score sheet used is shown in Figure 2.3. In addition to determining the odd sample, the panelists were asked to indicate the degree of difference and the acceptability of odd or duplicate samples. Further, they were asked to comment on what they considered to be the difference in the sample (extended triangle test - Jellinek (1985)).

The sensory evaluations were conducted at the taste-testing facilities of CIFT. The testing area had good ventilation for removal of cooking odours to prevent bias in the results. The panelists were in individual booths and, therefore, they were not influenced or biased by the other panelists. The testing area was illuminated with fluorescent light. Each panelist was given a glass of water and a glass of a mixture of equal parts of water, Diet 7-Up, and Schweppes Club Soda. The panelists were asked to rinse the mouth by taking a sip of water or the mixture of liquids between the samples tested.

2.5.3 Taste Panelists

Each panel had 14 to 18 panelists. All the panelists were members (staff and students) of the CIFT and had experience in sample tasting as they had participated in taste panels conducted previously by CIFT. Most of the members had participated in at least five previous taste panel studies.

Nevertheless, before the present taste panels the panelists were familiarized with the triangle test and with the taste and odour of the petroleum hydrocarbons to be tested. Fish exposed to a WSF prepared from Brent crude oil were used for this familiarization trial.

2.5.4 Evaluation of Results

The taste panel results were evaluated statistically as described by Larmond (1977).

The panel results presented in Section 3 of this report were patterned according to that of Jellinek (1985). In the triangle test there is a 1/3 chance of

TRIANGLE TEST - COD

NAME _____

DATE _____

One of these three samples is different from the other two.

1. Test the samples in order indicated and identify the odd sample.

Code	Check Odd Sample
____	_____
____	_____
____	_____

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

Slight _____

Moderate _____

Much _____

Extreme _____

3. Acceptability:

Odd sample more acceptable _____

Duplicates more acceptable _____

4. Comments:

FIGURE 2.3 TASTE PANEL QUESTIONNAIRE.

obtaining correct results. Table 2.1 shows a typical example of the minimum number of correct responses for various levels of significance for a wide range of panelists. As can be seen from the table, as the number of panelists increases the number of correct responses to obtain a significant difference decreases.

2.6 DETERMINATION OF LIPID CONTENT

The lipid content of the cod fillets was determined according to the procedure of Bligh and Dyer (1959).

About 100 g of cod fillet was weighed and was transferred to a covered glass Waring Blendor jar. Chloroform (100 mL) and methanol (200 mL) were added and the mixture was blended for 2 min. A further 100 mL chloroform was added and the mixture was again blended for 30 s. Then 100 mL of water was added to the blend and was mixed briefly. The blend was then filtered through a Buchner funnel under water suction. The Blendor jar was rinsed with chloroform (2 x 15 mL) and the rinsings were poured into the Buchner funnel. When filtering was nearly complete, the cake was squeezed dry by pressing with the bottom of a small beaker to obtain a complete recovery of the lipid. The cake was rinsed with chloroform (2 x 15 mL) and was pressed dry again. The filtrate was transferred to a 1-L separatory funnel and was allowed to stand to separate the organic and aqueous layers completely.

After the layers had separated, the chloroform layer containing the lipids was collected in an Erlenmeyer flask and was dried with anhydrous sodium sulphate. The chloroform layer was then evaporated in a preweighed, round-bottomed flask, first on a rotary-evaporator under low pressure in a 60°C water bath and finally on a mechanical vacuum-pump. The recovered lipid was weighed and the percent lipid was calculated.

TABLE 2.1

Statistical Chart
Triangle test, difference analysis

Number of tasters	Number of correct answers necessary to establish level of significance			Number of tasters	Number of correct answers necessary to establish level of significance		
	• 5%	•• 1%	••• 0.1%		• 5%	•• 1%	••• 0.1%
7	5	6	7	57	27	29	31
8	6	7	8	58	27	29	32
9	6	7	8	59	27	30	32
10	7	8	9	60	28	30	33
11	7	8	9	61	28	30	33
12	8	9	10	62	28	31	33
13	8	9	10	63	29	31	34
14	9	10	11	64	29	32	34
15	9	10	12	65	30	32	35
16	10	11	12	66	30	32	35
17	10	11	13	67	30	33	36
18	10	12	13	68	31	33	36
19	11	12	14	69	31	34	36
20	11	13	14	70	32	34	37
21	12	13	15	71	32	34	37
22	12	14	15	72	32	35	38
23	13	14	16	73	33	35	38
24	13	14	16	74	33	36	39
25	13	15	17	75	34	36	39
26	14	15	17	76	34	36	39
27	14	16	18	77	34	37	40
28	15	16	18	78	35	37	40
29	15	17	19	79	35	38	41
30	16	17	19	80	35	38	41
31	16	18	19	81	36	38	41
32	16	18	20	82	36	39	42
33	17	19	20	83	37	39	42
34	17	19	21	84	37	40	43
35	18	19	21	85	37	40	43
36	18	20	22	86	38	40	44
37	18	20	22	87	38	41	44
38	19	21	23	88	39	41	44
39	19	21	23	89	39	42	45
40	20	22	24	90	39	42	45
41	20	22	24	91	40	42	46
42	21	22	25	92	40	43	46
43	21	23	25	93	40	43	46
44	21	23	25	94	41	44	47
45	22	24	26	95	41	44	47
46	22	24	26	96	42	44	48
47	23	25	27	97	42	45	48
48	23	25	27	98	42	45	49
49	23	25	28	99	43	46	49
50	24	26	28	100	43	46	49
51	24	26	29	200	80	84	89
52	25	27	29	300	117	122	127
53	25	27	29	400	152	158	165
54	25	27	30	500	188	194	202
55	26	28	30	1,000	363	372	383
56	26	28	31	2,000	709	722	737

Source: Larmond (1977).

3.0 RESULTS

3.1 TEST MEDIA

3.1.1 Hydrocarbon Profiles of the Test Oils

The chromatograms in Figures 3.1 to 3.4 illustrate the complex nature of the hydrocarbons in the four oils. Tentative identifications have been made of some of the components from retention time data of known hydrocarbons. Pristane and phytane were easily identified from authentic standards. These two multi-branched hydrocarbons eluted soon after n-heptadecane (n-17) and n-octadecane (n-18) respectively and served as helpful points of identification. All the four crude oils showed n-alkanes from C_{10} to C_{24}. The major hydrocarbons centred around the n-C_{12} to n-C_{17} alkanes. The other components in this region included the branched alkanes and cyclic alkanes. The components that eluted between the hexane solvent peak and n-C_{10} were mostly aromatic hydrocarbons. This area usually includes the simple or volatile aromatics, such as benzene, toluene, ethyl benzene, m-, p-, and o-xylenes, isopropyl benzene, n-propyl benzene , naphthalene, and the various methyl naphthalenes (Murray et al., 1984). Identification of the component hydrocarbons was beyond the scope of this project, nevertheless, the more common aromatic hydrocarbons, namely benzene, toluene, and xylenes, were tentatively identified by comparing the GC retention times with authentic standards. The positions of three xylene isomers are marked on chromatograms for Amauligak and Hibernia (see Figures 3.2 and 3.3).

The hydrocarbon profiles of the four oils were different from each other. Brent crude oil (see Figure 3.1) showed higher levels of n-alkanes of chain lengths ranging from n-C_{10} to n-C_{21}. The content of volatile aromatics was low. Amauligak (see Figure 3.2) showed the presence of higher levels of low-boiling hydrocarbons including the volatile aromatic hydrocarbons. Among the alkanes, the tentatively identified pristane was a major component. Hibernia crude oil was quite viscous compared to the other three oils and it exhibited a wider range of hydrocarbons, ranging from the low-boiling aromatics to long-chain alkanes at least up to C_{34} chain length (see Figure 3.3).

Conoco base oil was completely different from the three crude oils. It was a colourless liquid, in contrast to the dark, more viscous crude oils. Conoco oil had only trace levels of the simple, low-boiling aromatics and early

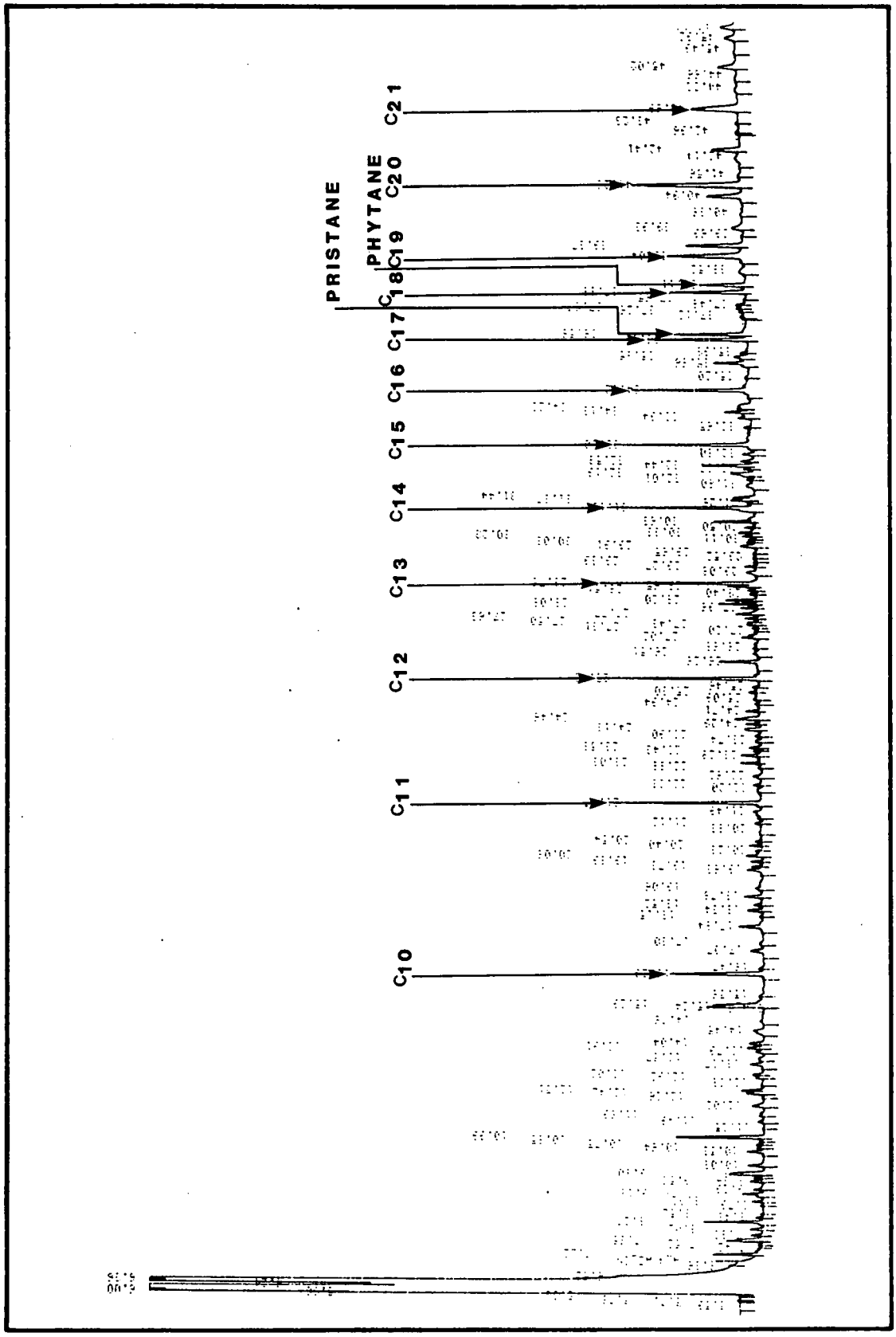


FIGURE 3.1 GC PROFILE OF BRENT CRUDE OIL.

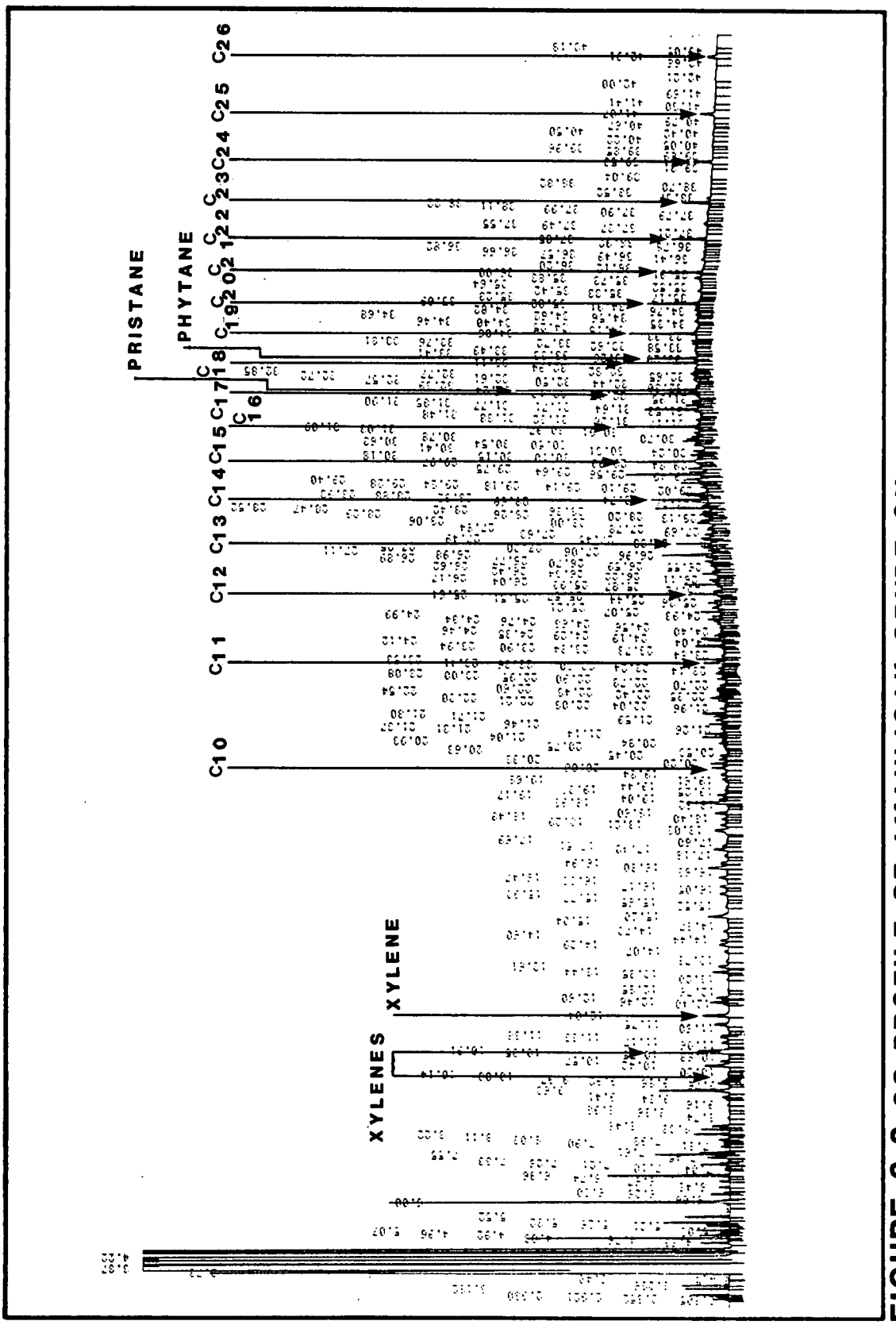


FIGURE 3.2 GC PROFILE OF AMAULIGAK CRUDE OIL.

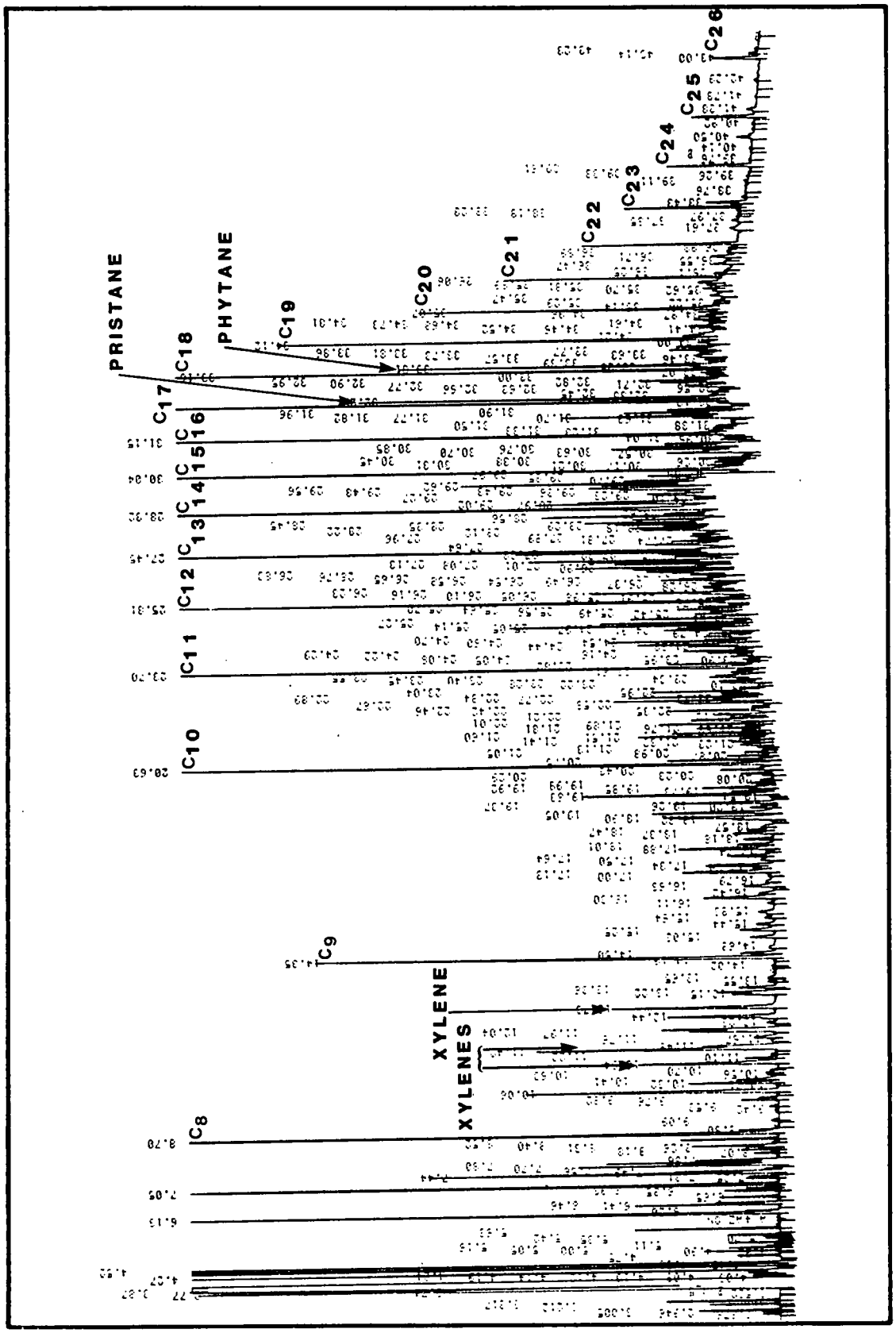


FIGURE 3.3 GC PROFILE OF HIBERNIA CRUDE OIL.

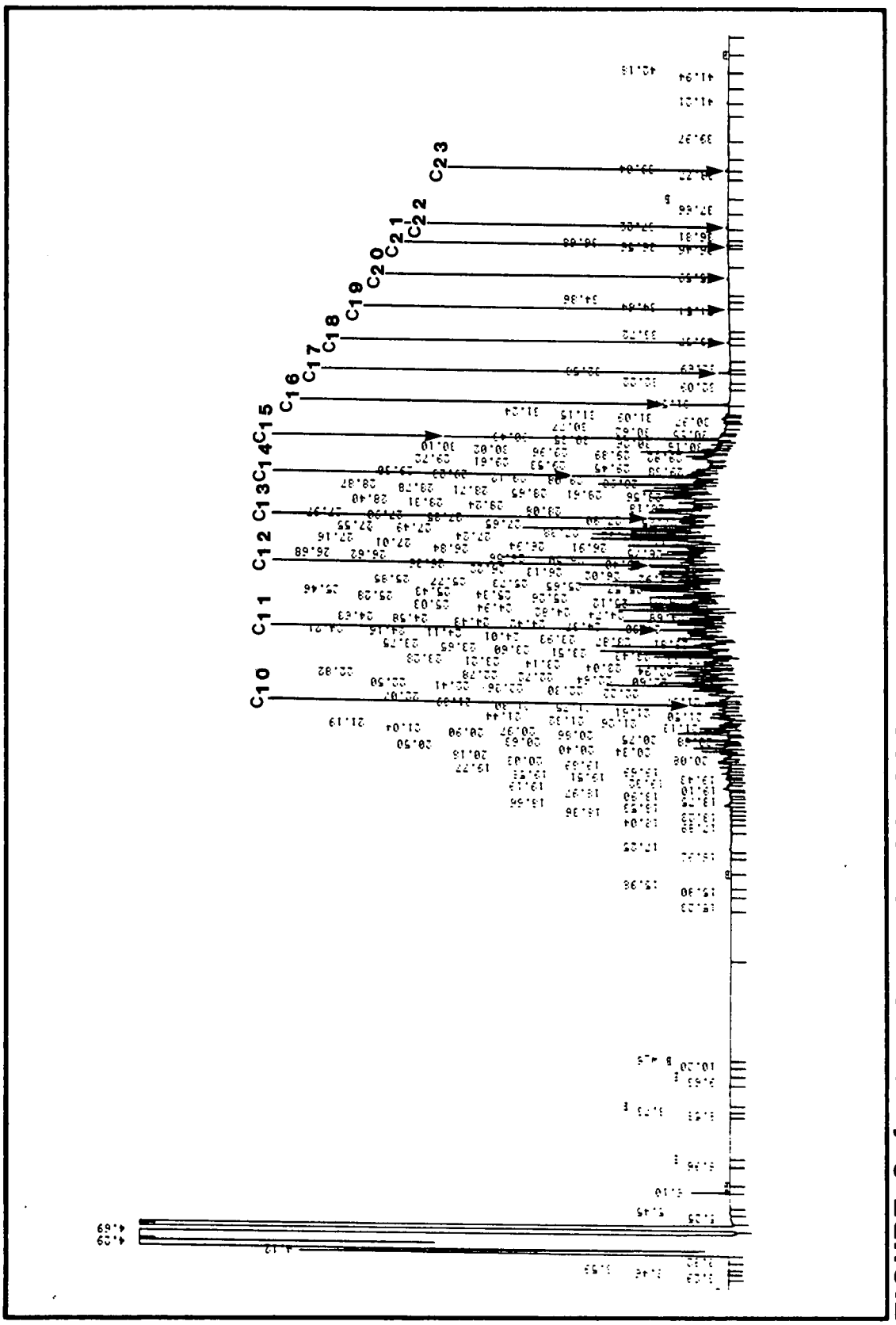


FIGURE 3.4 GC PROFILE OF CONOCO BASE OIL.

eluting alkanes, as could be seen from the virtual absence of peaks in between the solvent peak and the n-C_{10} peak (see Figure 3.4). The major components of Conoco oil eluted between n-C_{10} and n-C_{17}, and after n-C_{17}, the peaks fall away. The major component had a retention time on GC equivalent to that of n-C_{18}.

3.1.2 Hydrocarbon Profiles of the WSFs

The hydrocarbon profiles of the starting stock WSF concentrate of the four oils prepared in the large-scale vessel (see Section 2.2) are shown in Figures 3.5 to 3.8. In most respects the hydrocarbon profiles of the WSF of Amauligak (see Figure 3.6) and Hibernia (see Figure 3.7) were similar to each other. However, the hydrocarbon profiles of these two WSFs are completely different from the starting oils (compare with Figures 3.2 and 3.3). Most of the major components found in the parent, starting oil were absent or found in very low levels in the WSF. This result is quite clear for the higher alkanes, beyond C_9, because these alkanes have very low solubility in water. The components that are enriched in the WSF were the minor components in the parent oil. Identification of the hydrocarbon components of the WSF was not attempted in this project, however, the presence of benzene and toluene in the WSF fractions of Amauligak and Hibernia was confirmed by comparing the GC retention times of authentic standards. The hydrocarbon profile of the WSF prepared from Brent crude oil (see Figure 3.5) differed slightly from the profiles of WSFs of Hibernia and Amauligak. The WSF of Brent showed some new components which were not detected in the WSF of Hibernia and Amauligak, especially in the first half of the chromatogram. From the retention time, toluene was tentatively identified as the major component in WSF of Brent crude oil. Here again there was no correlation of WSF and the starting oil. The water-soluble hydrocarbons were minor components in the starting oil.

The hydrocarbon profile of the WSF from Conoco base oil (see Figure 3.8) was completely different from the other three oils. Like the starting oil, the WSF showed the complete absence of low-boiling alkanes and aromatics. The WSF hydrocarbons eluted between n-C_9 and n-C_{13} and may be branched alkanes and/or cyclic alkanes as these types of alkanes have slightly higher solubility in water than n-alkanes (Connell and Miller, 1981).

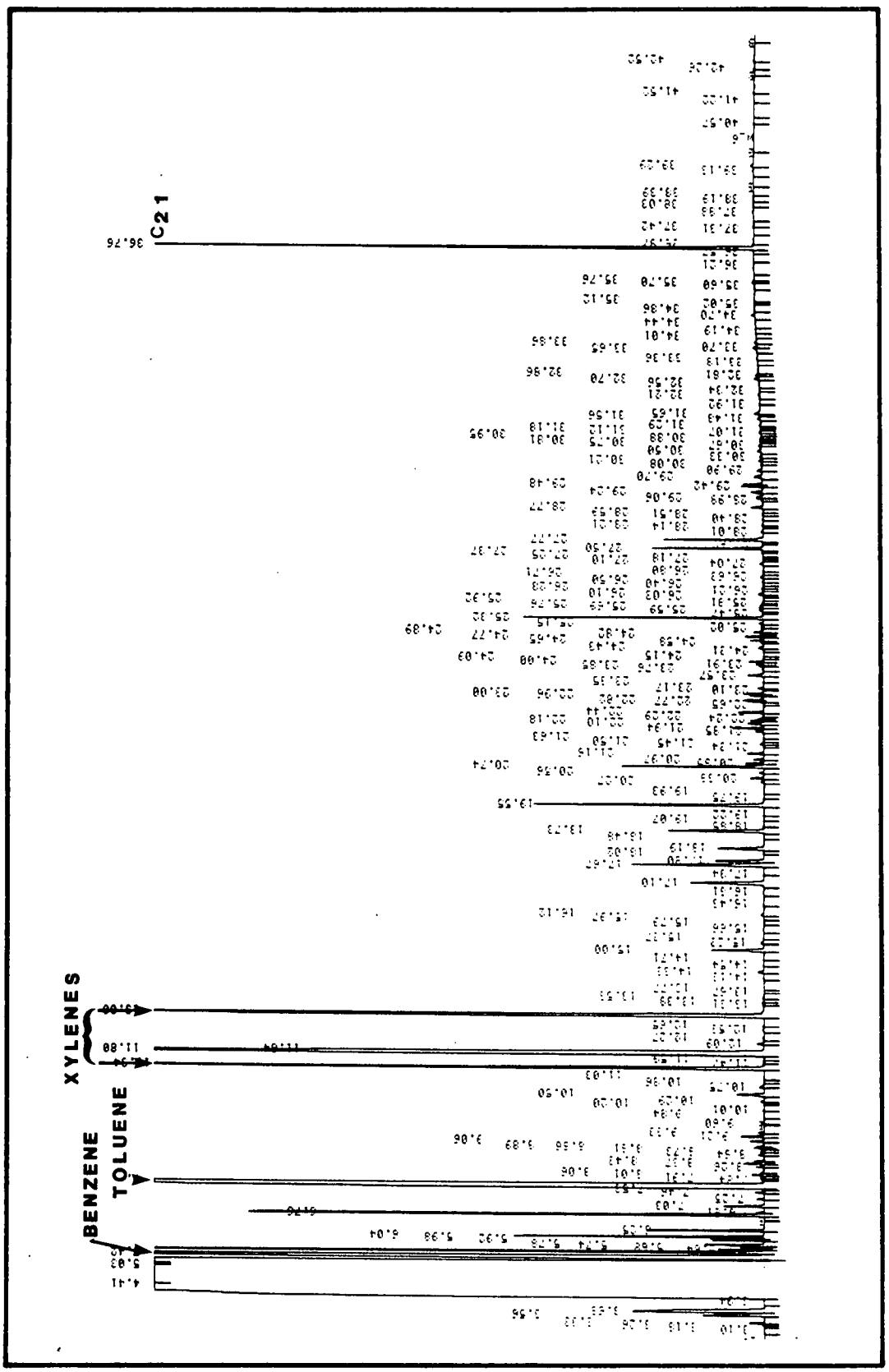


FIGURE 3.5 GC PROFILE OF STOCK WSF CONCENTRATE PREPARED FROM BRENT CRUDE OIL IN LARGE SCALE. C_{21} IS ADDED INTERNAL STANDARD.

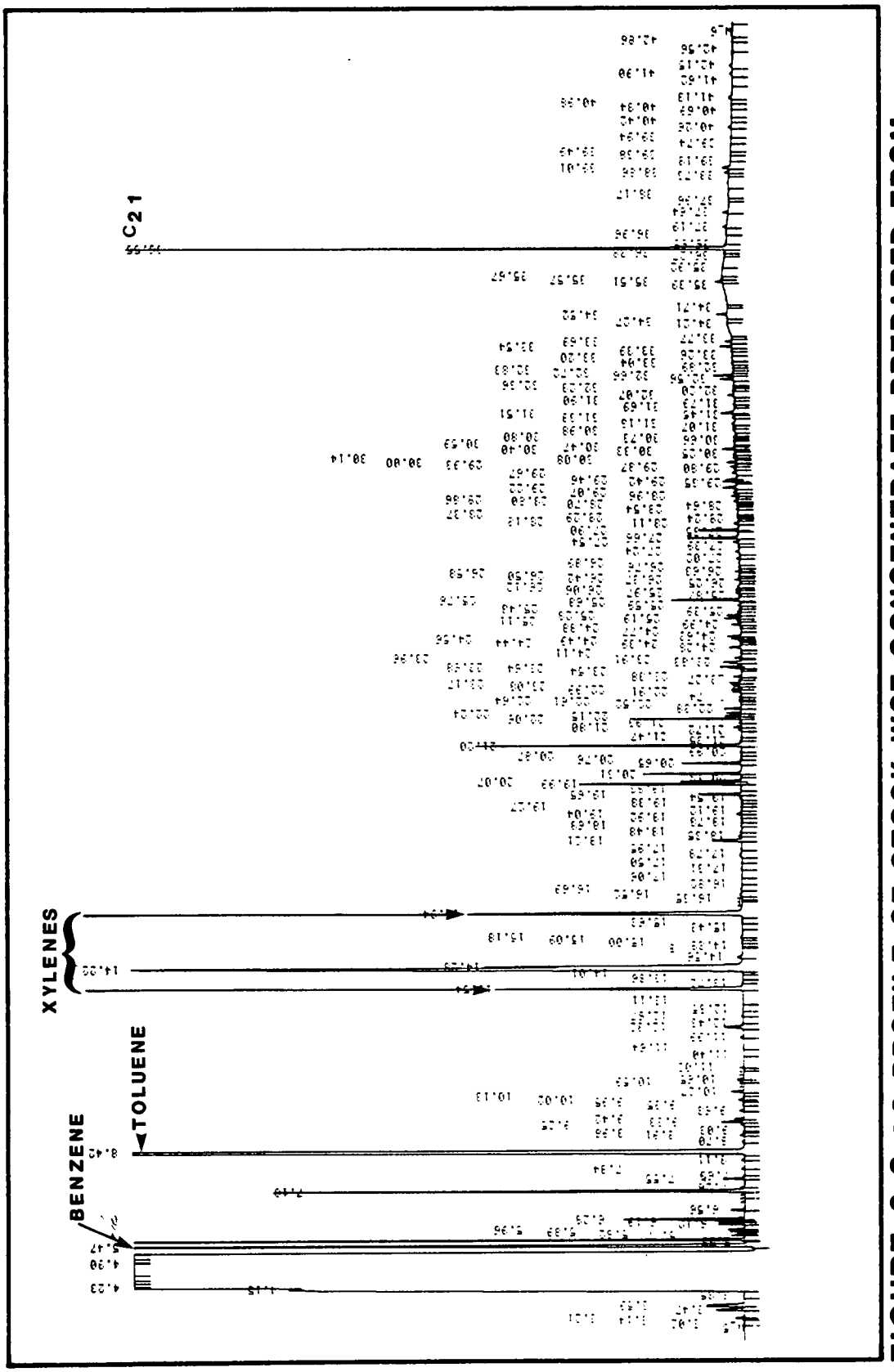


FIGURE 3.6 GC PROFILE OF STOCK WSF CONCENTRATE PREPARED FROM AMAULIGAK CRUDE OIL IN LARGE SCALE. C_{21} IS ADDED INTERNAL STANDARD.

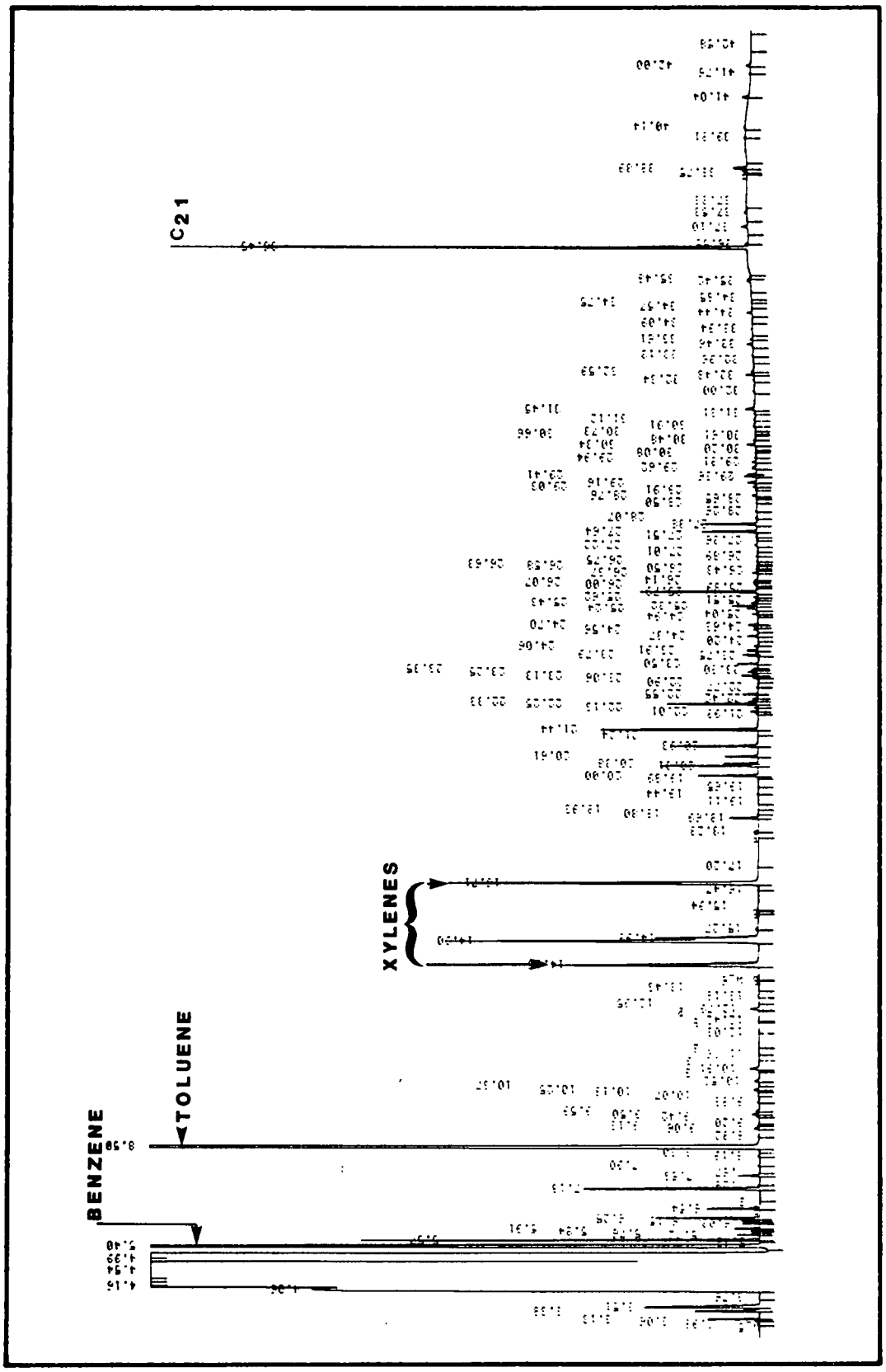


FIGURE 3.7 GC PROFILE OF STOCK WSF CONCENTRATE PREPARED FROM HIBERNIA CRUDE OIL IN LARGE SCALE. C_{21} IS ADDED INTERNAL STANDARD.

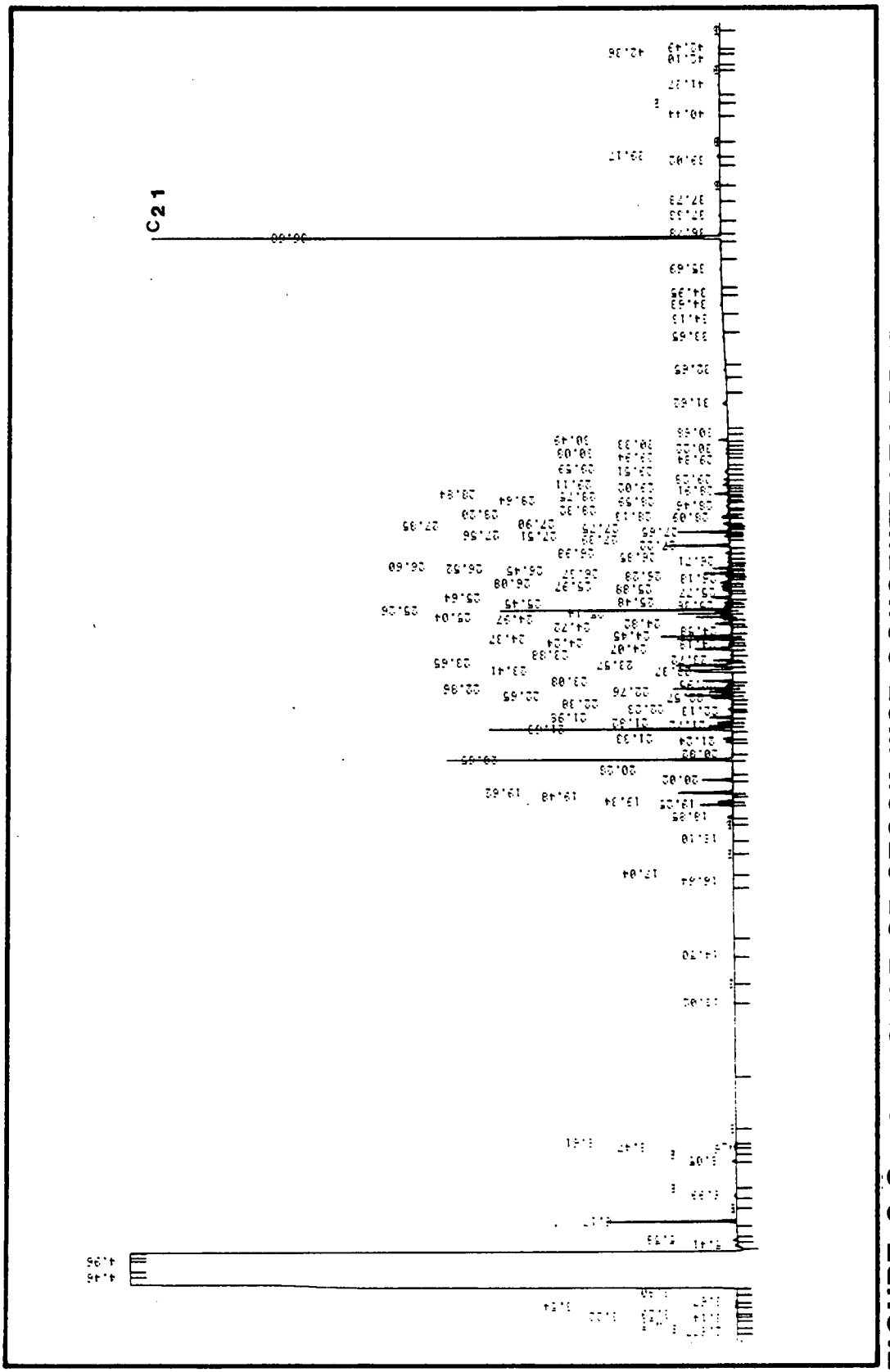


FIGURE 3.8 GC PROFILE OF STOCK WSF CONCENTRATE PREPARED FROM CONOCO BASE OIL IN LARGE SCALE. C_{21} IS ADDED INTERNAL STANDARD.

3.1.3 Hydrocarbon Concentrations of the Stock WSF

The hydrocarbon concentrations of the stock WSF concentrates prepared from the four oils are given in Table 3.1. The concentrations of the WSF from the three crude oils, Brent, Amauligak, and Hibernia were more or less the same, ranging from 15 to 21 ppm. The high value of 33.4 ppm obtained for one of the preparations of Brent was unusual and could have resulted from some dispersed oil in the WSF rather than dissolved oil. Because of this uncertainty in the actual concentration, this batch of WSF was used only for the range-finding trial. A subsequent preparation and analysis resulted in a stock concentration of 17 ppm.

The WSF prepared from Conoco base oil was completely different from the three crude oils. The WSF concentration was relatively low (see Table 3.1).

3.1.4 Hydrocarbon Profiles of the Exposure Water

The hydrocarbon profiles of some of the spiked (with stock WSF) sea-water used for the exposure studies are illustrated in Figures 3.9 to 3.12. The hydrocarbon profiles of spiked sea-water were similar to that of the corresponding stock WSF concentrates (see Figures 3.5 to 3.8). This result indicates that there is no preferential loss of any of the component hydrocarbons after dilution.

3.1.5 Background Hydrocarbon Levels

The sea-water used for making the WSF, the fish food (mackerel), and the cod fillets from the control experimental tanks were examined frequently during the exposure studies for background levels of hydrocarbons; by the microextraction technique (sea-water) or the steam distillation method (fish tissues). Representative chromatograms from these three sources are shown in Figures 3.13 to 3.15.

The blank sea-water sample (see Figure 3.13) had no detectable levels (<0.01 ppm) of petrogenic hydrocarbons. Peaks observed in the chromatogram were those contributed by the extracting solvent; hexane.

The fish food (mackerel) showed, other than the solvent peaks (CH_2Cl_2), a major peak of longer retention time but eluting before the internal standard n-C_{21} (see Figure 3.14). This peak was tentatively identified as pristane by comparing the GC retention time with those of

TABLE 3.1

Hydrocarbon content of stock WSF concentrates prepared on the large scale

Oil	Hydrocarbon content (ppm)		
Brent	33.43[d]	17.00[a]	-
Amauligak	19.05[a] (13.35[e])	17.20[d]	21.0[c]
Hibernia	15.48[ab]	20.08[c]	-
Conoco	11.75[a]	-	-

These WSF were used for the following exposure studies:

[a] Short-term exposure
[b] Depuration
[c] Long-term exposure
[d] Range-finding
[e] Amauligak depuration trials. The WSF of 13.35 was obtained by allowing the unused portion of the 19.05-ppm WSF to settle for 5 d.

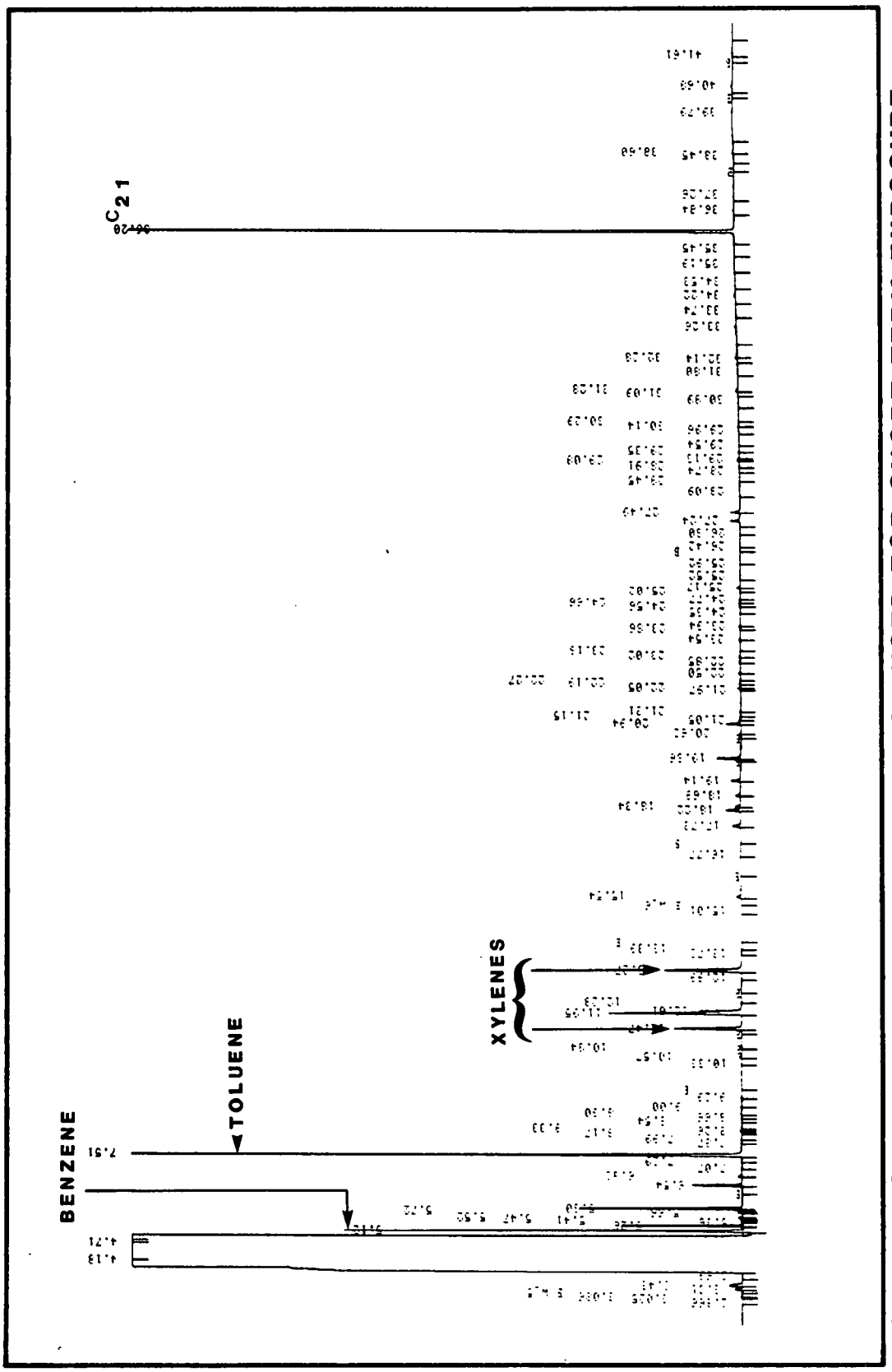


FIGURE 3.9 GC PROFILE OF BRENT WSF USED FOR SHORT TERM EXPOSURE STUDIES. CONCENTRATION IS 2.98 PPM. C_{21} IS ADDED INTERNAL STANDARD.

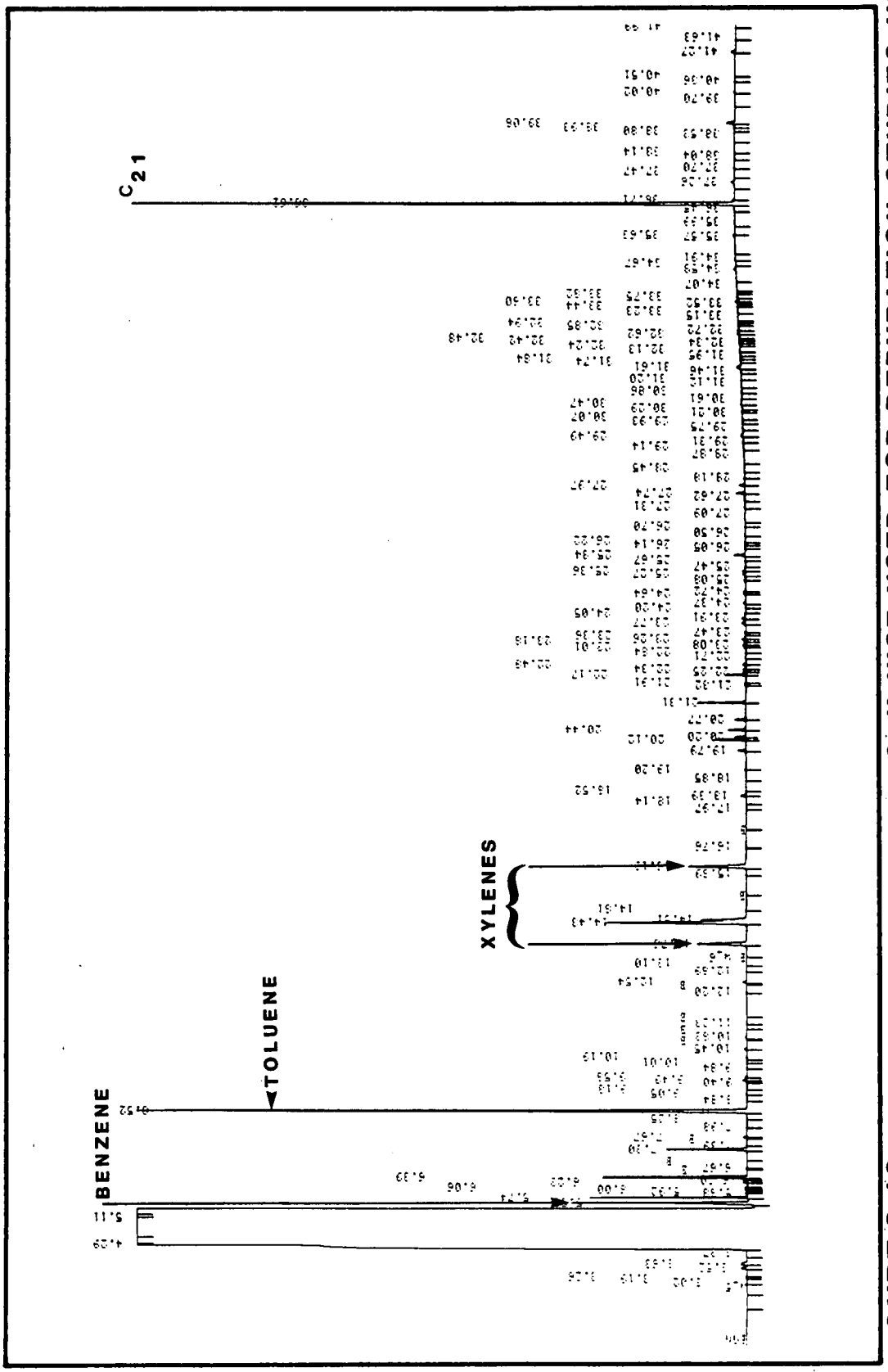


FIGURE 3.10 GC PROFILE OF AMAULIGAK WSF USED FOR DEPURATION STUDIES IN WHICH FISH WERE EXPOSED FOR 8 H. CONCENTRATION IS 3.05 PPM. C_{21} IS ADDED INTERNAL STANDARD.

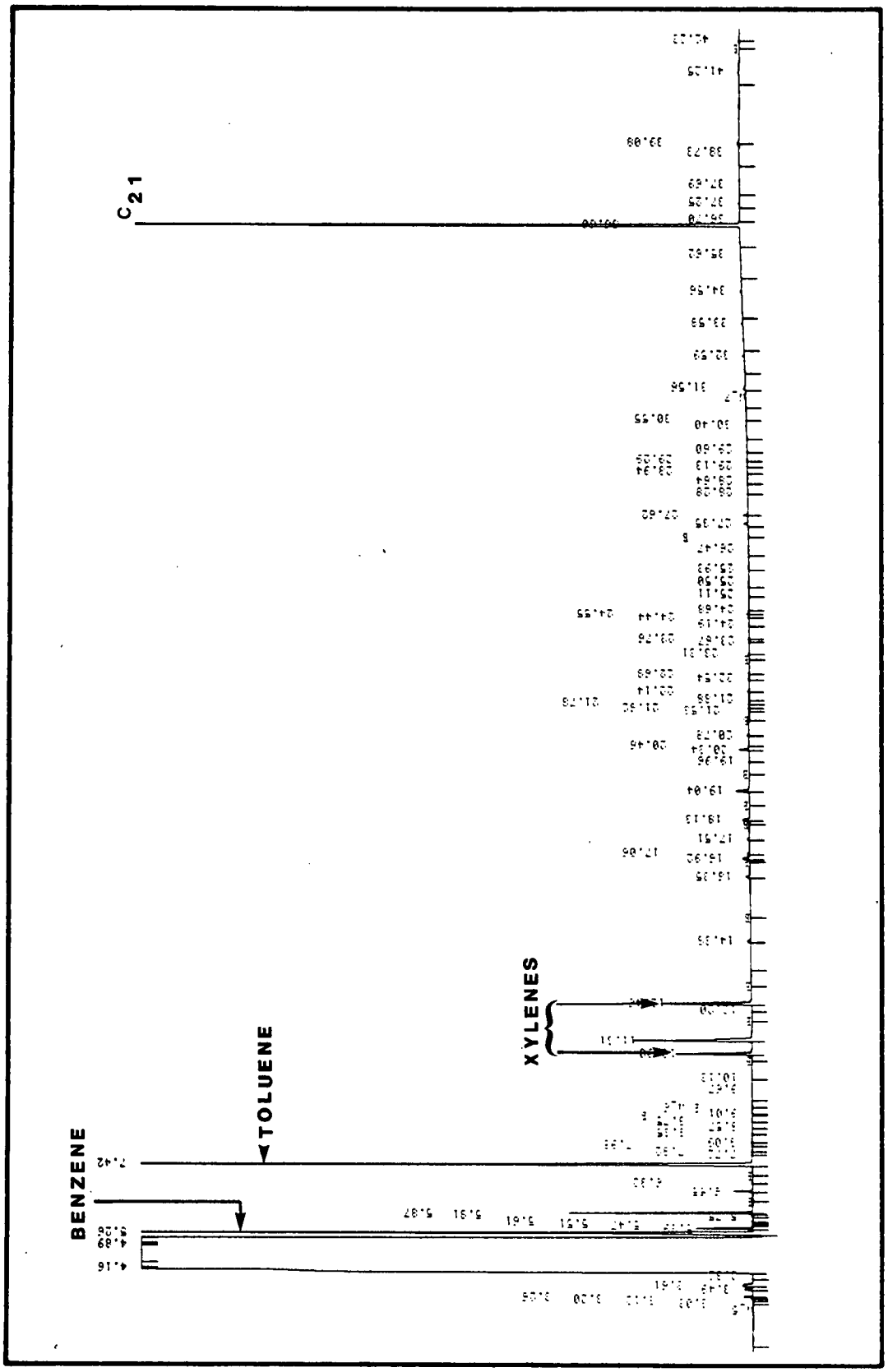


FIGURE 3.11 GC PROFILE OF HIBERNIA WSF USED FOR DEPURATION STUDIES IN WHICH FISH WERE EXPOSED FOR 24 H. CONCENTRATION IS 3.23 PPM. C_{21} IS ADDED INTERNAL STANDARD.

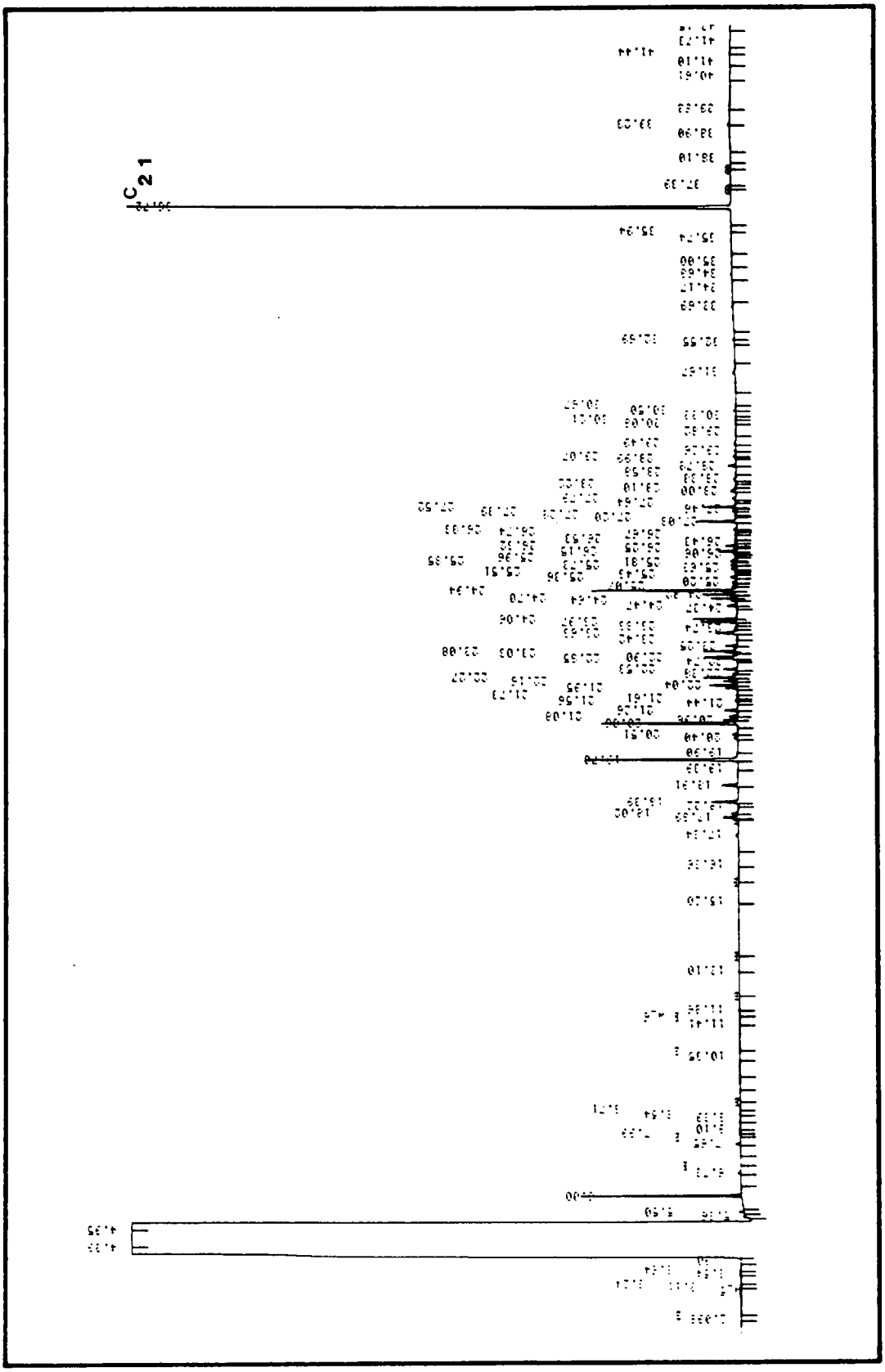


FIGURE 3.12 GC PROFILE OF CONOCO WSF USED FOR SHORT-TERM EXPOSURE STUDIES. CONCENTRATION IS 1.93 PPM. C_{21} IS ADDED INTERNAL STANDARD.

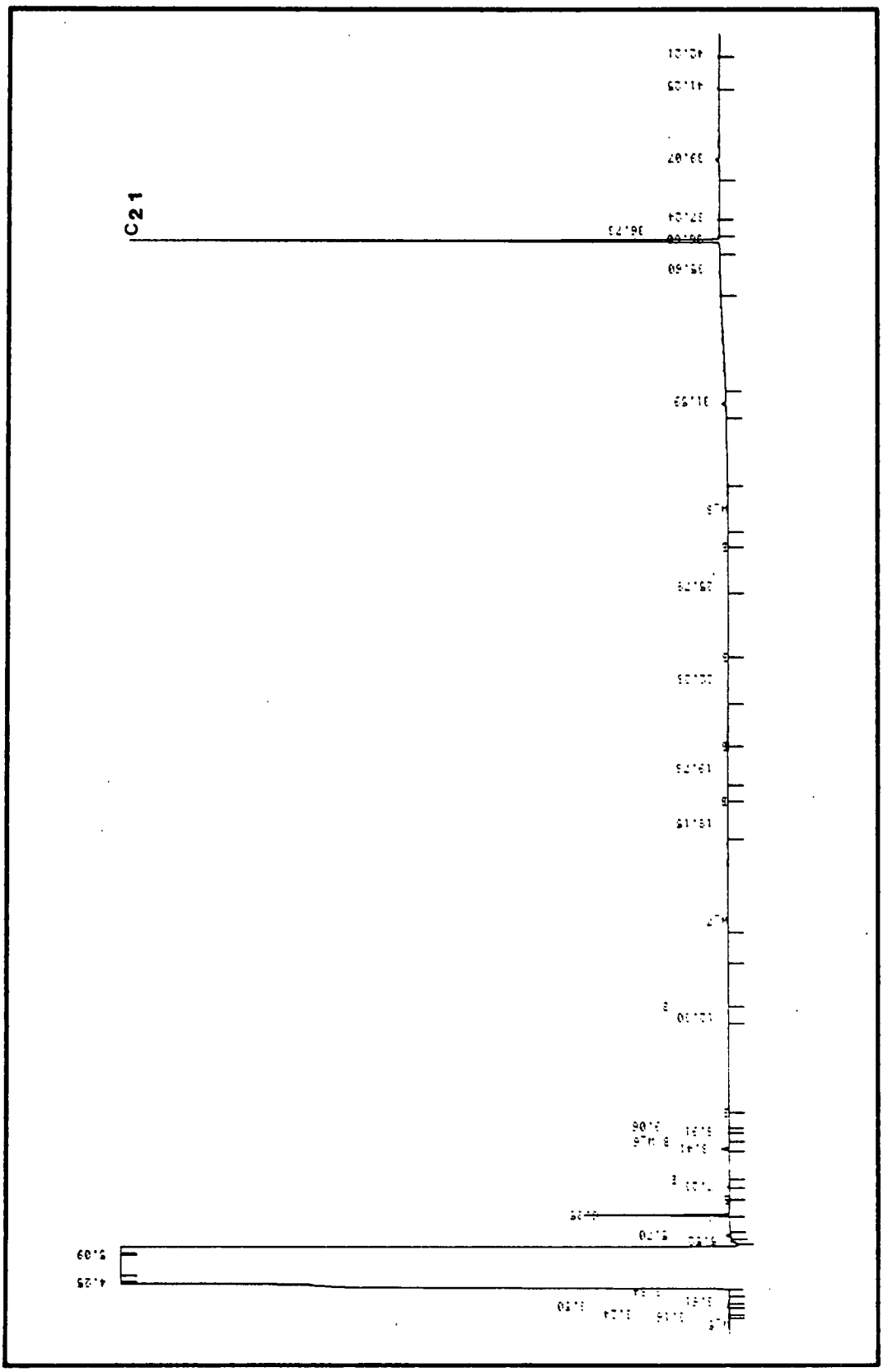


FIGURE 3.13 HYDROCARBON ANALYSIS OF SEA-WATER. C_{21} IS ADDED INTERNAL STANDARD.

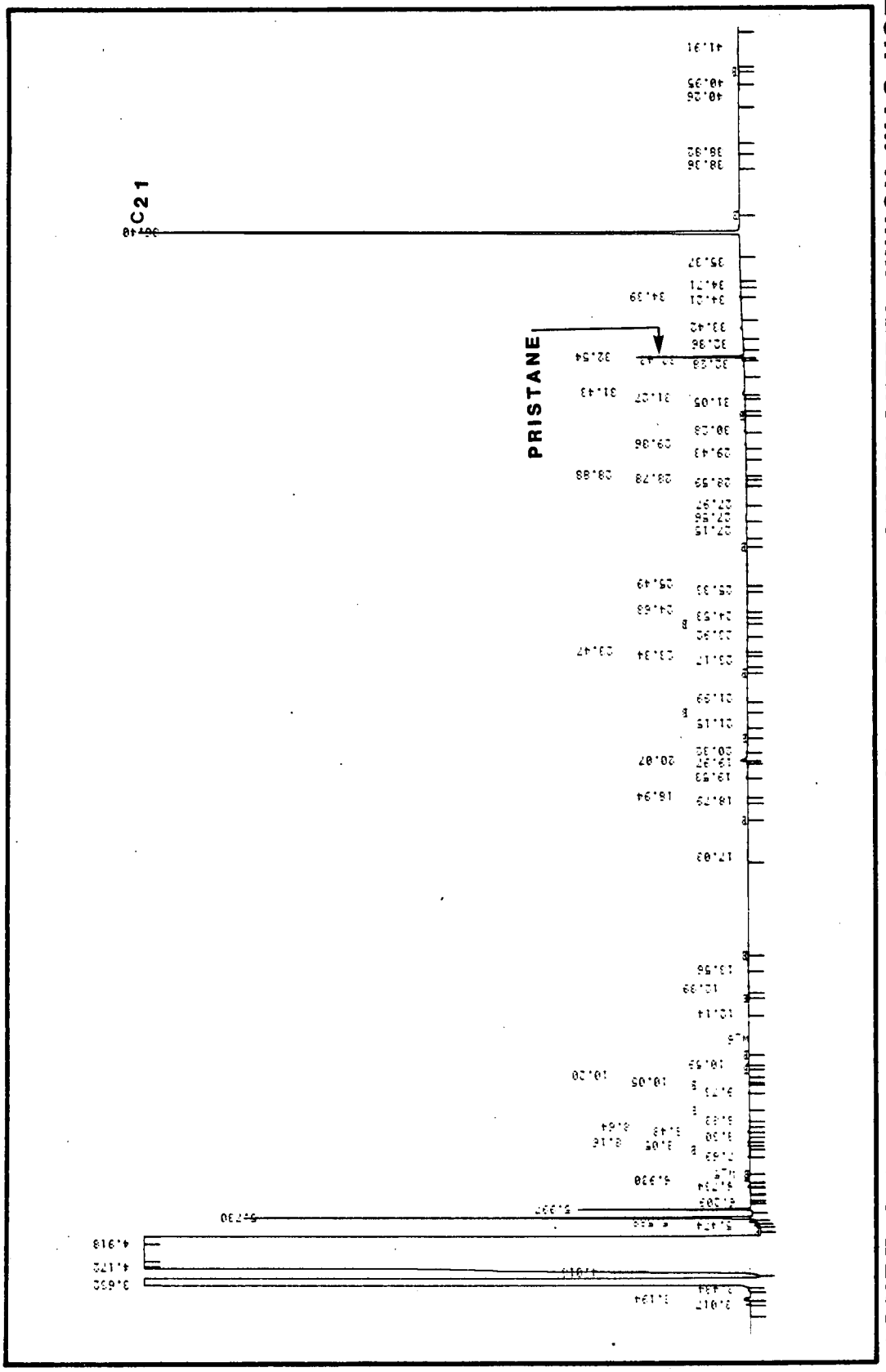


FIGURE 3.14 GC TRACE OF HYDROCARBONS FROM MACKEREL WHICH WAS USED AS THE COD DIET. C_{21} IS ADDED INTERNAL STANDARD.

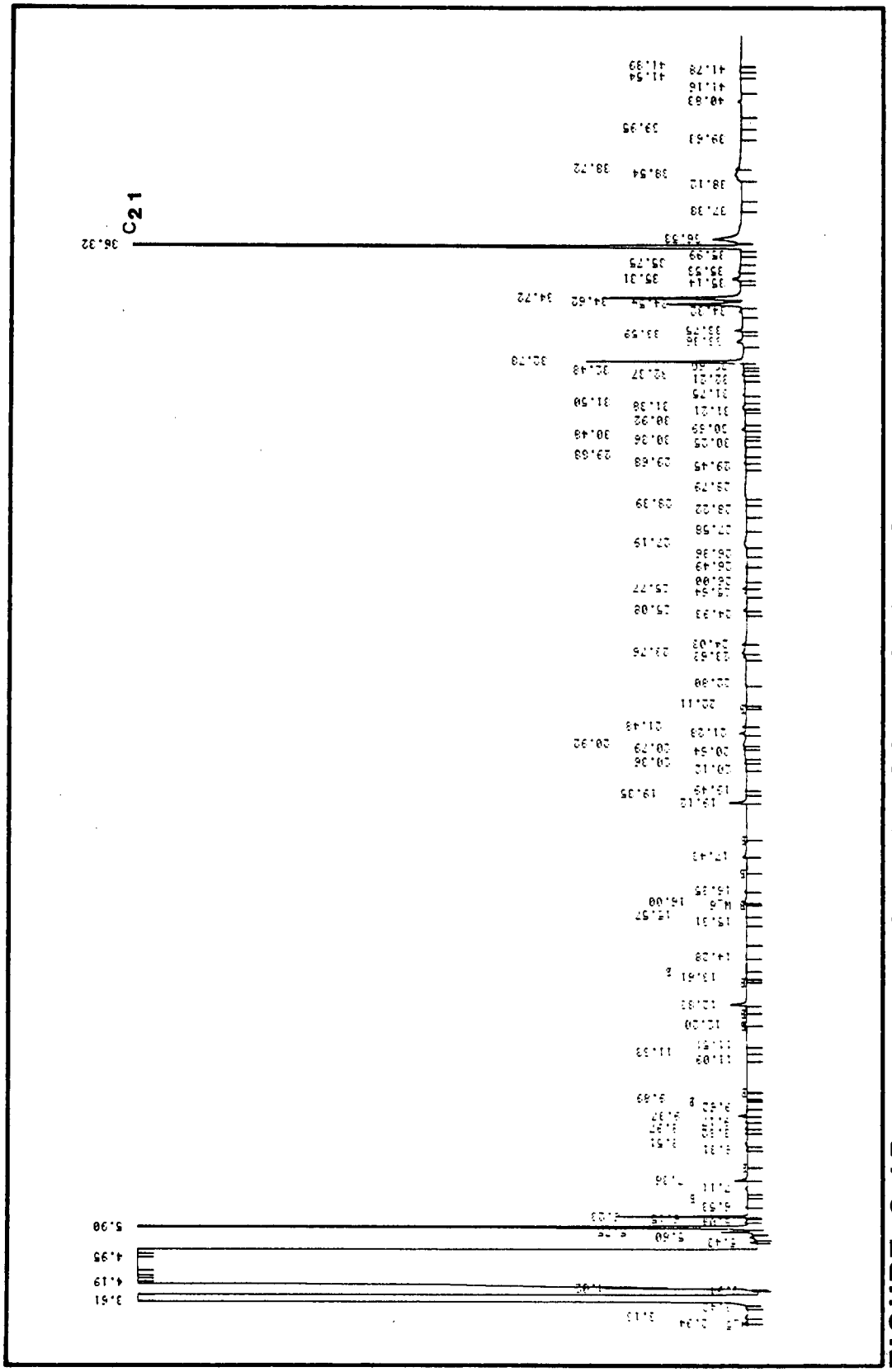


FIGURE 3.15 GC TRACE OF HYDROCARBONS FROM COD FILLETS WHICH WERE USED AS CONTROLS. C_{21} IS ADDED INTERNAL STANDARD.

an authentic standard. A number of minor peaks were also observed, but their concentration was too low to affect the hydrocarbon profiles of sea-water and cod tissues significantly.

The fish fillets from the control group showed three major peaks and a number of minor peaks (see Figure 3.15). The three major peaks eluted close to the n-C_{21} internal standard, but did not correspond with any of the petroleum-type hydrocarbons (comparing the retention times with the hydrocarbon profile of the four oils). Two of the peaks had retention times close to pristane and phytane. From the retention time data it was concluded that these components were not petrogenic hydrocarbons, but perhaps some other organic matter, such as volatile bases characteristic of cod fillets. Fortunately, these three peaks had slightly longer retention times than the hydrocarbon peaks in the WSF (see Figures 3.5 to 3.8) and, therefore, did not interfere at all with quantitative analysis of hydrocarbons in fish tissues from the tainted groups.

Apart from the three major peaks, there were a number of minor and trace-level peaks in the fish fillets. Some overlapped with the hydrocarbons in fish tissues from the tainted groups. These background levels were, therefore, subtracted when calculating the hydrocarbon levels in the tainted group tissues.

Only trace levels of pristane were detected in fish fillets from the control group. Pristane is the major hydrocarbon in copepods and other zooplankton, and occurs in trace amounts in the lipids of most marine fish (Ackman, 1971). The almost complete absence of pristane in the fish fillets showed that the exogeneous pristane (from the mackerel diet) was not directly deposited on the cod muscle tissues but presumably is taken up by the fatty liver.

3.2 SHORT-TERM EXPOSURES

3.2.1 Brent Crude Oil

WSFs of Brent crude oil were used during the initial trials to determine the exposure system conditions (see Appendix B). Brent crude was also used for a range-finding trial to determine exposure concentrations for subsequent trials of all oils. The following subsections describe the results of both this range-finding trial and the short-term exposure to a WSF of Brent crude oil.

3.2.1.1 Exposure conditions.

a) Range-finding trial: The temperature (Temp.), dissolved oxygen in percent saturation (DO), and hydrocarbon concentration in the exposure tanks during the range finding trial are presented in Figure 3.16. The hydrocarbon concentration is based on a GC calibration of the fluorometer (see Appendix C). After 11 h of exposure there was a malfunction with the fluorometer power box. The hydrocarbon levels could not be monitored so all WSF flows into the exposure tanks were turned off and the trial became static.

The WSF stock solution was assumed to have about 17 ppm of hydrocarbon based on earlier small-scale mixing trials (see Appendix A). The exposure water was spiked accordingly for a proposed 0.10-ppm exposure in Tank 1 and 1.00 and 10.00 ppm of hydrocarbon in Tanks 2 and 3, respectively.

Subsequent GC analysis of the WSF indicated that the stock solution was actually 33.21 ppm of hydrocarbon, or almost double that which was anticipated. Water samples taken from each tank at time 0 were analysed and the initial exposure concentrations were, in fact, 0.20, 2.05, and 8.78 ppm of hydrocarbon, respectively (Table 3.2).

The fish in Tank 3 appeared considerably more disturbed than in the other tanks. They swam frantically around the tank and, after half an hour, began turning on their sides. It appeared that the hydrocarbon level was toxic. The tank was flushed with clean sea-water until the fluorescence was reduced either to half or to an assumed 5.00 ppm. A water sample was taken and subsequent GC analysis showed an actual exposure concentration of 6.71 ppm of hydrocarbon.

The fish showed signs of recovery for the next 4 h, after which they began swimming on their sides again. The tank was flushed and then brought back up to the "5-ppm" fluorescence level. One fish died after 11 h exposure and the three remaining fish died after 17 to 19 h exposure. The fish were removed from the tank, gutted, and held for taste panel analysis. The final hydrocarbon concentration in Tank 3 by GC analysis was 2.30 ppm.

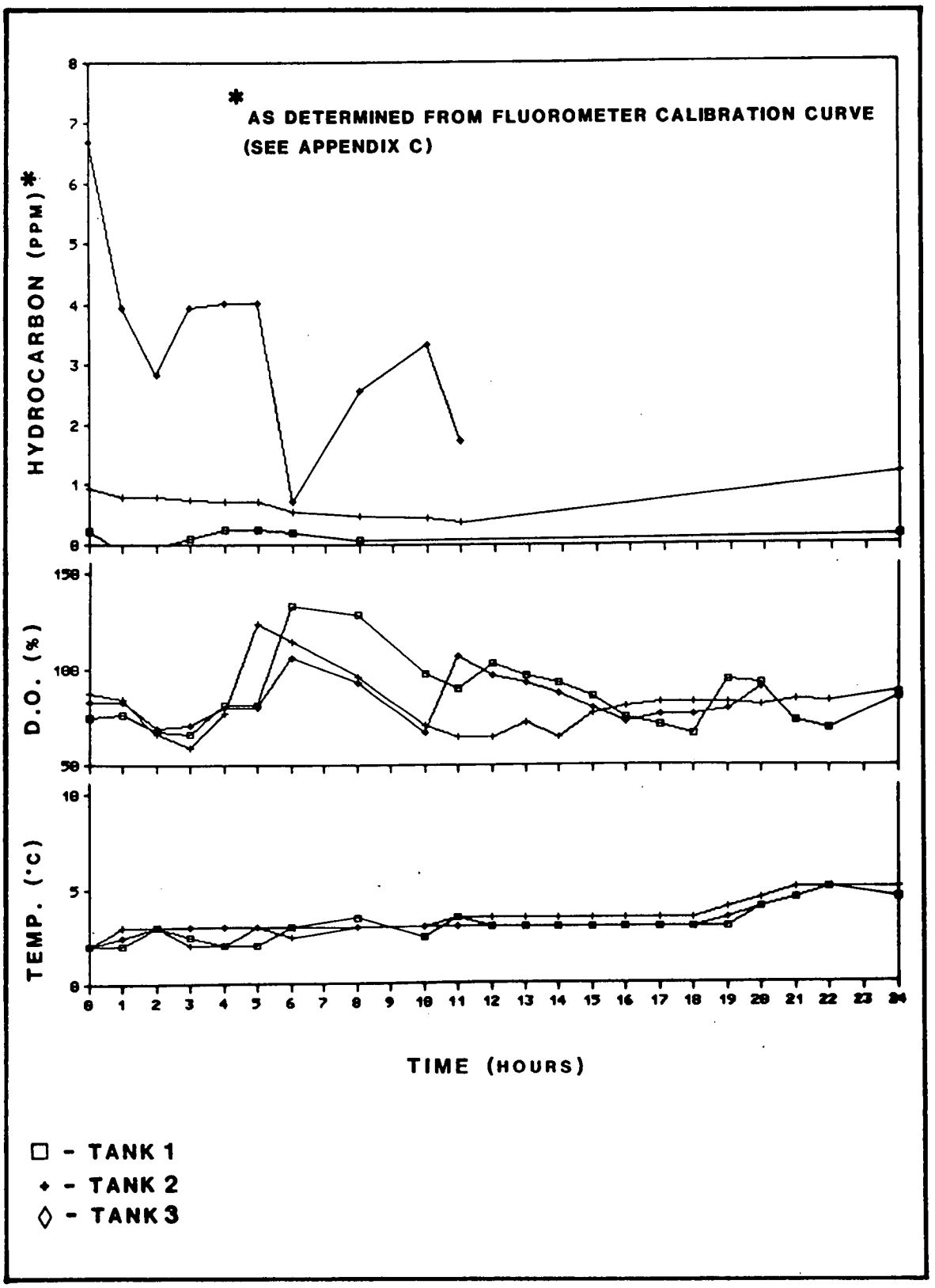


FIGURE 3.16 SHORT-TERM RANGE-FINDING TRIAL EXPOSURE CONDITIONS.

TABLE 3.2

Hydrocarbon concentrations (GC analysis) - short-term exposure trials

Trial	Time	Hydrocarbon (ppm)		
	(h)	Tank 1	Tank 2	Tank 3
a) Range-Finding				
	0	0.20	2.05	8.78
	1	-	-	6.70
	24	0.04	0.30	2.30
b) Brent				
	0	0.53	0.50	2.98
	16	-	-	2.48
	24	0.13	1.05	-
c) Amauligak				
	0	0.28	0.75	1.78
	24	0.03	0.29	2.70
d) Hibernia				
	0	0.21	0.42	1.41
	24	0.12	0.39	3.40
e) Conoco				
	0	.69	.74	1.93
	24	.47	.14	1.21

The temperature in the tank rose 2°C over the 19 h of exposure and the dissolved oxygen level was maintained between 66 and 96% saturation.

Tank 2 was the mid-range exposure level. The hydrocarbon concentration based on the fluorometer calibration was just under 1.00 ppm. GC analysis of water samples taken at the beginning and the end showed a loss in hydrocarbons from 2.05 to 0.30 ppm.

The water temperature rose 3°C over the 24 h and the DO was maintained above 60% saturation, however, it rose above 100% for 2 h.

Tank 1 was the lowest exposure level. Fluorometric monitoring indicated an exposure concentration of less than 0.5 ppm. GC analysis of water samples confirmed this with an initial concentration of 0.20 ppm and an end concentration of 0.04 ppm of hydrocarbon.

The temperature conditions were similar to the other two tanks with an increase from 2 to 5°C over 24 h. The oxygen level was maintained above the proposed lower limit of 60% saturation; however, it rose to supersaturated levels for several hours during the first half of the exposure period.

b) Short-term exposure trials: Based on the range-finding trial the exposure levels proposed for subsequent short-term trials were 2.50, 0.50, and 0.25 ppm of hydrocarbon.

A fresh batch of Brent WSF was prepared and analysed before the trial began. The hydrocarbon content was 17.00 ppm. The exposure tanks were spiked with the appropriate volumes of stock WSF. GC analysis of the resulting exposure waters showed 0.53, 0.50, and 2.98 ppm of hydrocarbon in Tanks 1, 2, and 3, respectively (see Table 3.2).

Exposure conditions during this trial are presented in Figure 3.17. The hydrocarbon concentrations, based on the fluorometer calibration, were relatively constant in Tanks 1 and 2 at approximately 0.50 and 0.90 ppm, respectively. There was more variation in Tank 3: during the first several hours there was a gradual decrease from approximately 4.00 to 3.50 ppm. This level was supported by the Time 0 GC analysis of 2.98 ppm. The flow of WSF was increased to compensate

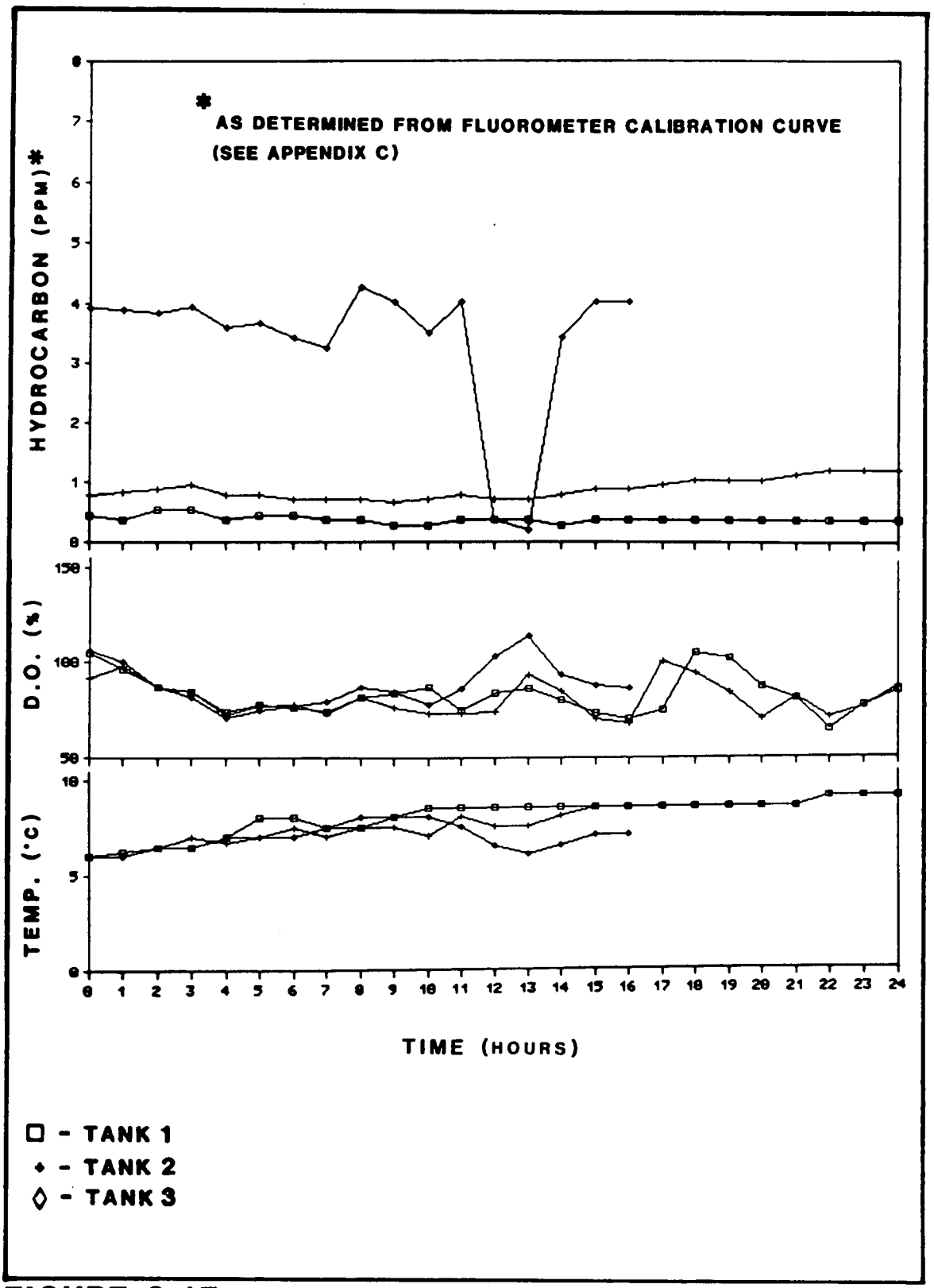


FIGURE 3.17 BRENT SHORT-TERM TRIAL EXPOSURE CONDITIONS.

for this gradual loss in fluorescence; however, after 11 h the fish began showing signs of stress. The tank was flushed with clean sea-water and two of the three fish appeared to recover. The WSF flow was reconnected to resume the initial exposure levels. All three fish died over the next 3 h. A water sample taken at the end of this exposure was analysed at 2.48 ppm of hydrocarbon.

The water temperature increased 3°C over the 24 h of exposure in Tanks 1 and 2 and 2°C after 11 h in Tank 3. The decrease in Tank 3 was caused by the flushing when the fish were stressed. The higher exposure temperatures for this trial over the previous range-finding trial were because the temperature of the water in the holding pool tank had been increased for health reasons (see Section 2.1.2).

The dissolved oxygen levels in the exposure tanks were monitored hourly (see Figure 3.17). The level was maintained between 70 and 80% saturation except for a couple of hours when the water became supersaturated.

3.2.1.2 Taste panel results.

a) Range-finding trial: The taste panel results are presented in Table 3.3. The fish exposed to the highest concentration of hydrocarbons (Tank 3) tasted significantly different from control fish (at a confidence level of 0.1%) as 13 of the 16 panelists were able to detect the odd sample. The fish from Tank 1 and 2 were not significantly different from the control fish. Even though Tank 2 fish had higher levels of hydrocarbons than Tank 1, only 2 of the 14 judges were able to pick out the odd sample.

b) Short-term exposure trials: The fish in the three tanks exposed to Brent crude oil WSF were judged to be significantly different from the control fish (Table 3.3); the fish in Tanks 2 and 3 gave a confidence level of 0.1% whereas those in Tank 1 gave a 5.0% confidence level. In Tanks 2 and 3, 12 out of 16 and 12 out of 14 panelists, respectively, were able to determine the odd sample while in Tank 1 only 9 out of 15 detected the odd sample.

TABLE 3.3

Taste panel results of short-term exposure trials

Trial	No. of panelists	No. of correct results	No. of incorrect results	Level of confidence (%)
Range-finding				
- Tank 1	15	5	10	NS *
- Tank 2	14	2	12	NS
- Tank 3	16	13	3	0.1
Brent				
- Tank 1	15	9	6	5.0
- Tank 2	16	12	4	0.1
- Tank 3	14	12	2	0.1
Amauligak				
- Tank 1	17	7	10	NS
- Tank 2	16	10	6	5.0
- Tank 3	18	16	2	0.1
Hibernia				
- Tank 1	16	6	10	NS
- Tank 2	16	5	11	NS
- Tank 3	16	14	2	0.1
Conoco				
- Tank 1	18	9	9	NS
- Tank 2	16	5	11	NS
- Tank 3	18	14	4	0.1

* Not significant.

3.2.2 Amauligak Crude Oil

3.2.2.1 Exposure conditions. The stock solution of Amauligak WSF had 19.00 ppm hydrocarbon; the exposure water was spiked accordingly. This trial was run before the holding water was heated. Consequently, initial water temperatures were between 1°C and 2°C with an increase of 3°C over the 24 h of the trial. The dissolved oxygen levels were maintained in the 60 to 100% saturation range except for a low of 55% in Tank 1 at 3 h, high readings in Tank 2 at 12 and 13 h, and high readings in Tank 3 at 20 h.

Figure 3.14 presents the conditions of the exposure water during the Amauligak short-term trial. Tank 1 was the lowest concentration at approximately 0.40 ppm hydrocarbon; however, levels dropped considerably towards the end. GC results for water samples confirmed this trend with a drop from 0.28 to 0.03 ppm over the 24 h of exposure. The fluorescence after 10 h was, in fact, very high and erratic because of high turbidity caused by faeces in the tank. The tank was flushed and WSF added to resume a fluorescence level comparable to that observed at hours 8 and 9.

GC analysis of water samples taken at the beginning and end of the trial from Tank 2 were 0.75 and 0.29 ppm, respectively. This trend in hydrocarbon levels was observed in the fluorescence of the exposure water (see Figure 3.18).

Tank 3 was the highest exposure level. There was marked fluctuation in the fluorescence in this tank; however, all readings were well above the two lower concentrations. GC analysis of the exposure water showed an increase in hydrocarbons over the 24 h from 1.78 to 2.70 ppm.

The fish in Tank 3, the highest concentration, were observed to be more active than in the other tanks. They tended to swim near the surface, whereas in Tanks 1 and 2 the fish remained on the bottom. Some parasites had fallen off the fish in Tank 3 and were observed on the bottom as early as 5 hours into the trial.

3.2.2.2 Taste panel results. The results from the taste panel analysis of fish from the Amauligak short-term trial are presented in Table 3.3. The fish from Tanks 2 and 3 produced a significant difference compared to the control fish, with Tank 3 giving a 0.1% level of confidence (16 of

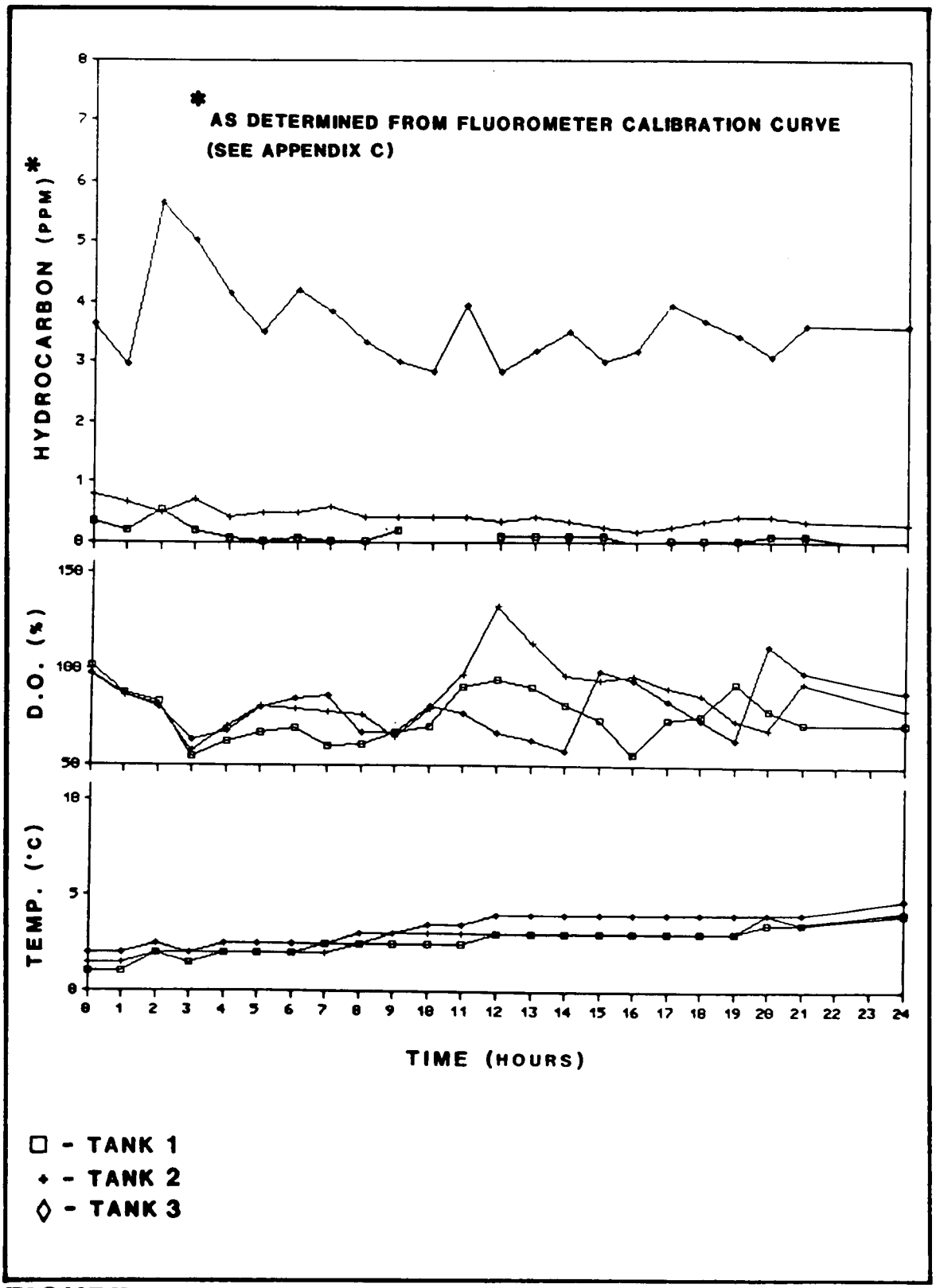


FIGURE 3.18 AMAULIGAK SHORT-TERM TRIAL EXPOSURE CONDITIONS.

18 panelists detecting the odd sample) and Tank 2 giving a 5% level of confidence (10 of 16 panelists detecting the odd sample). The taste of the fish from Tank 1, which had a much lower level of hydrocarbons than the other two tanks (see Table 3.2), was not significantly different from the control group.

3.2.3 Hibernia Crude Oil

3.2.3.1 Exposure conditions. The hydrocarbon content of the stock WSF of Hibernia crude oil was 15.50 ppm. Exposure tanks were prepared and GC analysis of the water showed that initial hydrocarbon levels were 0.21 ppm in Tank 1, 0.42 ppm in Tank 2, and 1.41 ppm in Tank 3 (see Table 3.2). Figure 3.19 presents the results of the hourly monitoring of conditions.

The hydrocarbon levels, based on the fluorometer calibration, were relatively constant over the 24 h of exposure: at about 0.20 ppm in Tank 1, 0.40 ppm in Tank 2, and about 2.00 ppm in Tank 3. GC analysis of the 24-h water sample from Tank 3 showed a marked increase in hydrocarbon levels. This increase may have been caused by a dirty sample bottle as there was no marked increase in the fluorescence in Tank 3 toward the end of the trial, which would have been anticipated if there were an increase in hydrocarbon content of that magnitude.

The water temperature increased 3°C over the exposure period. The oxygen levels were kept above 65% saturation, however, supersaturated conditions existed in Tanks 1 and 3 for 5 h during the first half of the exposure.

3.2.3.2 Taste panel results. The panelists testing the fish exposed to a Hibernia crude oil WSF in Tank 3 detected a signficant difference compared to the control fish, with a 0.1% confidence level when 14 of 16 panelists chose the odd sample (see Table 3.3). The fish exposed in Tanks 1 and 2 produced no significant difference in taint compared to the control fish.

3.2.3.3 Lipid content of tissues. The fillets from the fish exposed to Hibernia WSF from Tanks 2 and 3 (see Table 3.2) were examined for their lipid content and were compared with the control fish. Analyses of the control group of fish were duplicated. The lipids from the fillets were extracted with $CHCl_3$/MeOH according to the Bligh and Dyer procedure (see Section 2.6). The results are given in Table 3.4. The lipid contents of the control group samples

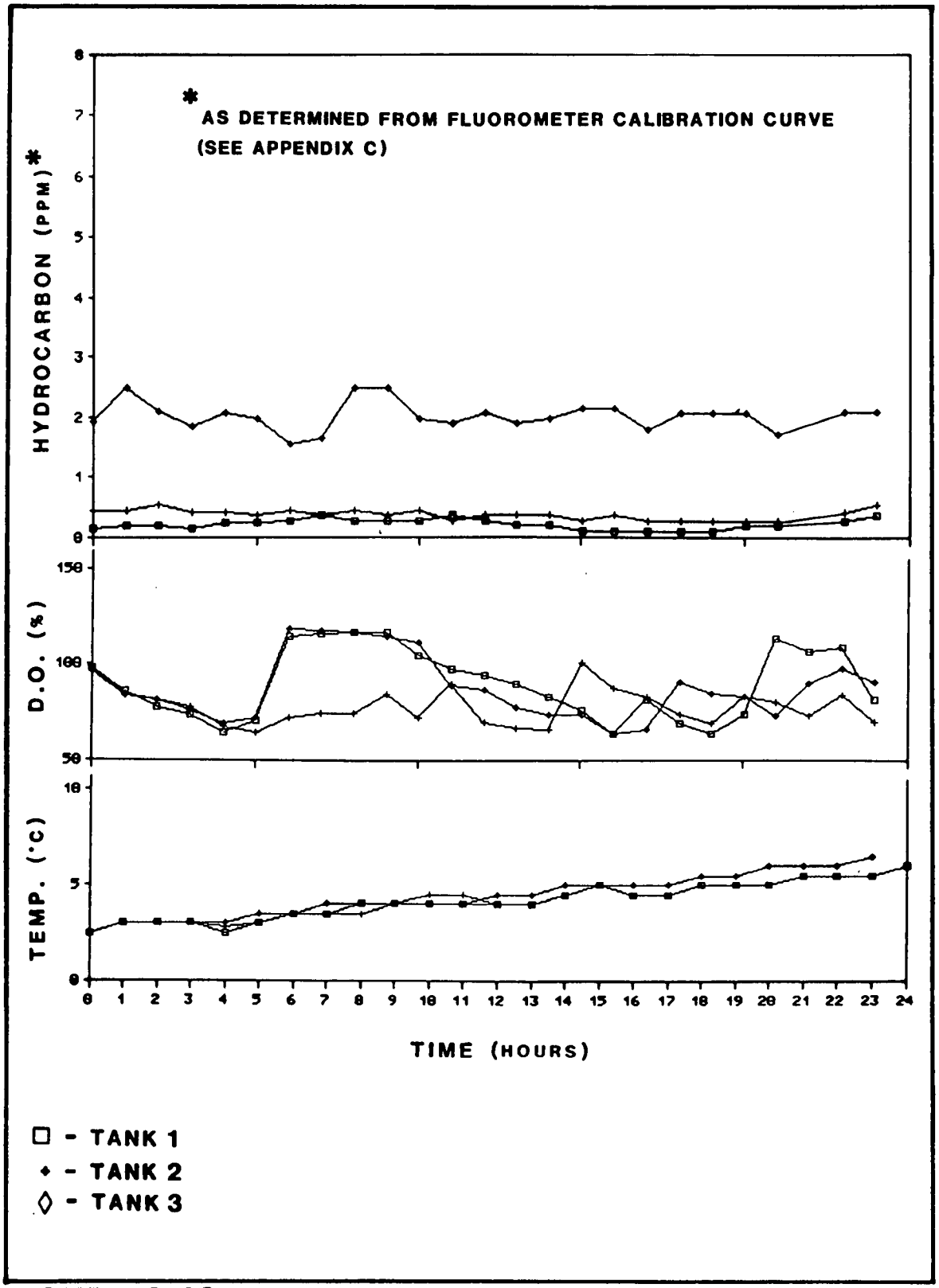


FIGURE 3.19 HIBERNIA SHORT-TERM TRIAL EXPOSURE CONDITIONS.

TABLE 3.4

Lipid content - Hibernia short-term exposure

Percent lipid (w/w)			
Exposed fish		Control fish	
Tank 2[a]	Tank 3[a]	Sample 1	Sample 2
0.82	0.69	0.78	0.72

[a] Refer to Table 3.1 for further experimental conditions.

were 0.78% and 0.72%, with an average of 0.75%. The fish exposed to hydrocarbons had about the same levels of lipid. These lipid levels are quite normal for healthy mature Atlantic cod (Jangaard et al., 1967). These results indicate that the lipid content of fish fillet is not affected when the fish are exposed to hydrocarbons in the range of 2 to 3 ppm for 24 h.

3.2.4 Conoco Base Oil

3.2.4.1 Exposure conditions. The response of the fluorometer to change in the experimental conditions during this trial did not follow the pattern established in earlier trials with the crude oils. The flow rate of WSF to the highest exposure level (Tank 3) was increased continually throughout the exposure trial, however, there was little change in the fluorescence (Figure 3.20). After 10 h the fish were showing signs of stress despite a seemingly low hydrocarbon level based on the fluorescence. Conversely, the fluorescence in Tanks 1 and 2 rose and remained constant despite no additional WSF being added. These tanks (1 and 2) were diluted after 5 h in an attempt to re-establish the initial fluorescence. Tank 2 was spiked with 4 L of WSF at 10 h into the trial to check the response of the fluorometer. The fluorescence of the exposure water increased for a few hours then dropped to, and remained at, the level prior to the spiking.

Table 3.2 presents the results of the GC analysis of water samples taken from each tank at the beginning and end of the trial. The conditions in Tank 1, although high, appear to have remained fairly constant at 0.69 and 0.47 ppm. The hydrocarbon levels in Tank 2 showed more variation with a drop from 0.74 to 0.14 ppm after 24 h.

The hydrocarbon level in Tank 3 was considerably lower (1.93 ppm) than anticipated based on the dilution ratios. The WSF concentrate was 11 ppm of hydrocarbon. Tank 3 was filled half with sea-water and half with WSF. WSF was added at what was considered a high rate (compared to previous trials) and there was still a 37% loss in hydrocarbon over the 24 h of exposure (see Table 3.2).

The GC analysis of the stock WSF concentrate showed that the hydrocarbon profile was similar to the crude-oil hydrocarbon profiles. Further, it was different from those of the subsequent water samples from the three exposure tanks (see Table 3.2) and from the WSF prepared in a laboratory scale (see Appendix A and Figure 3.8). These results

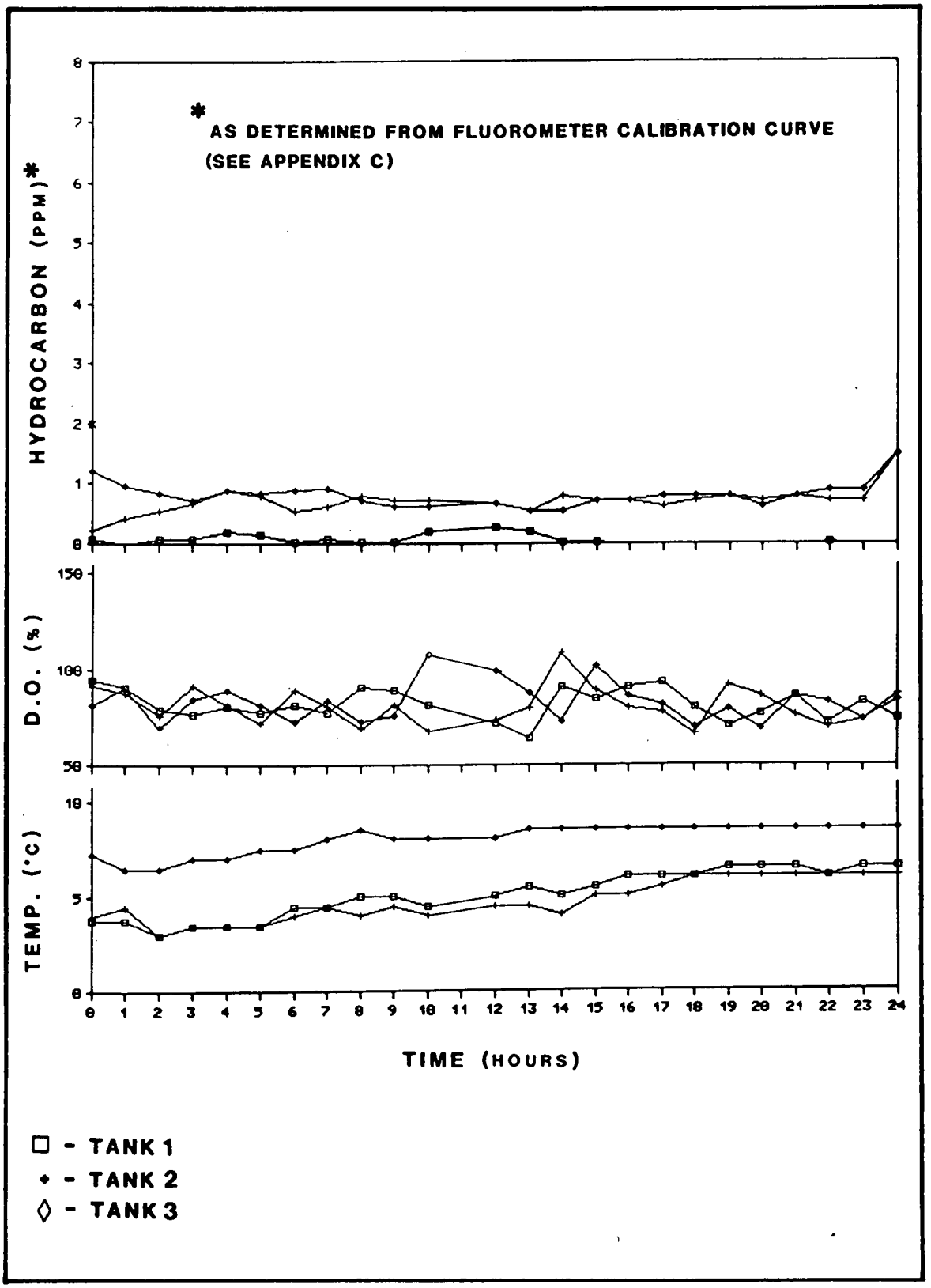


FIGURE 3.20 CONOCO SHORT-TERM TRIAL EXPOSURE CONDITIONS.

indicate that the WSF sample used for GC analysis is not a true water-soluble fraction and was not representative of the stock WSF. It was rationalized that the flask to which a small portion of WSF was taken for analysis may have been contaminated with the starting oil or oil droplets were present in the sample.

The temperature in Tank 1 was higher than in Tanks 2 and 3, however, it was not higher than that observed during subsequent trials. An increase of 2.0°C and 2.5°C over 24 h was recorded for each exposure tank. The oxygen levels were maintained between 65% and 100% saturation in all three tanks.

3.2.4.2 Taste panel results. A significant difference was found in the fish exposed to Conoco oil at the highest exposure concentration (Tank 3), compared to the control fish, giving a 0.1% confidence level. At this exposure 14 of the 18 panelists were able to detect the odd sample (see Table 3.3). The fish exposed in Tank 1 and Tank 2 were not significantly different from the control fish.

3.3 DEPURATION TRIALS

Depuration trials were conducted with two of the oils; Amauligak and Hibernia. Three tanks were prepared for each trial at a proposed hydrocarbon concentration of 2.50 ppm. Water samples were taken for hydrocarbon analysis by GC at the beginning and end of each trial. Water samples were a pooled sample of about 1/3 L from each of the exposure tanks. Duplicate samples were taken. The results are presented in Table 3.3. Figures 3.21, 3.22, 3.23, and 3.24 present the conditions in each exposure tank.

3.3.1 Amauligak Crude Oil

3.3.1.1 Exposure conditions.

a) Eight hour exposure: The average hydrocarbon concentration, as determined by the fluorometer in the three exposure tanks after 1 h was 3.00 ppm except for a spike at 5 and 6 h in two of the tanks (see Figure 3.21). GC analysis of pooled water samples showed an average hydrocarbon level of 3.06 ppm with little loss (2.1%) over the 8-h exposure period (Table 3.5).

   The temperature in the exposure tanks varied betweeen 4.5°C and 6.0°C and the oxygen levels were similar between tanks except for an increase in Tank 3 for the last 3 h.

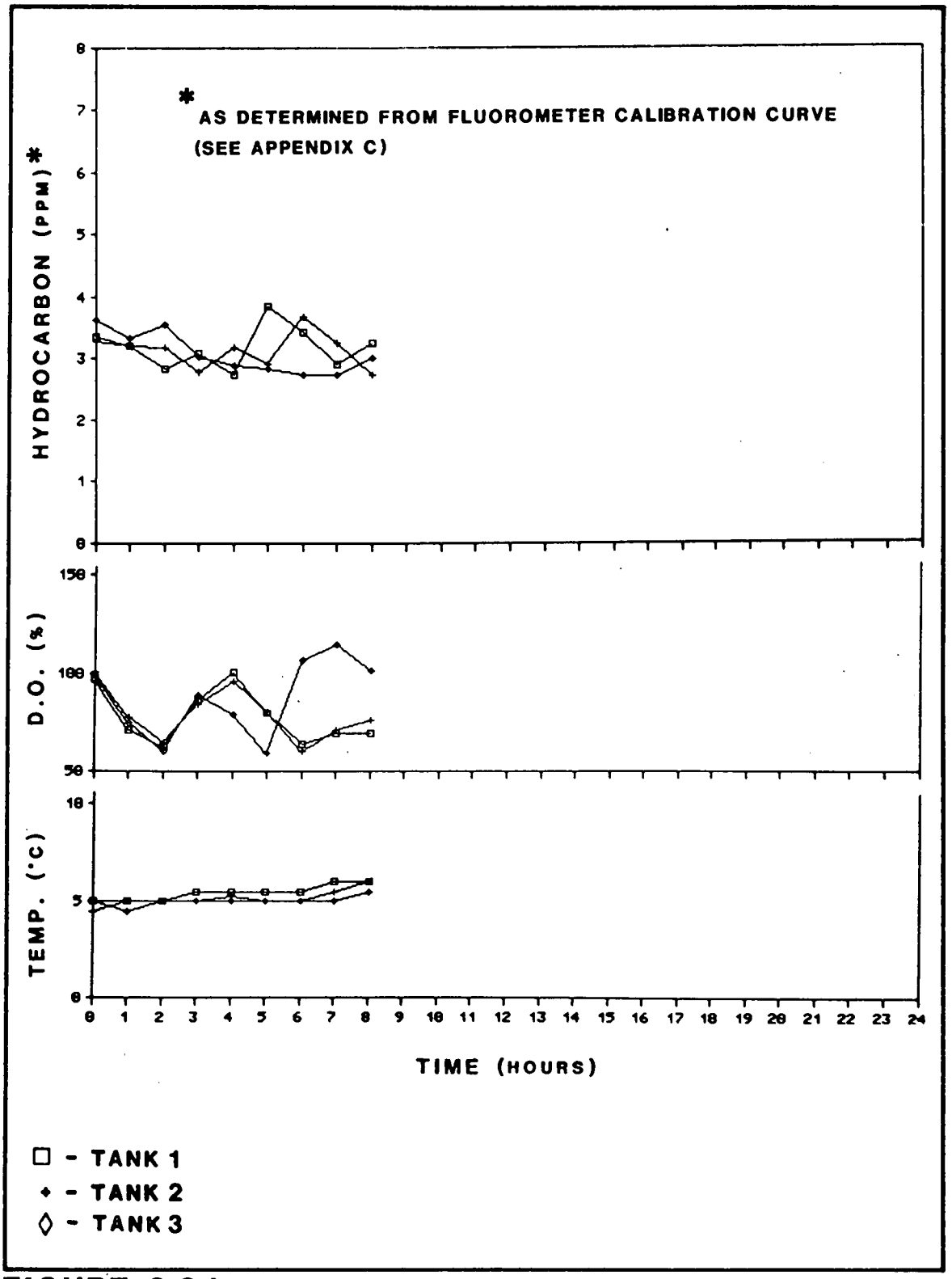


FIGURE 3.21 AMAULIGAK DEPURATION TRIAL - CONDITIONS DURING 8-H FISH EXPOSURE.

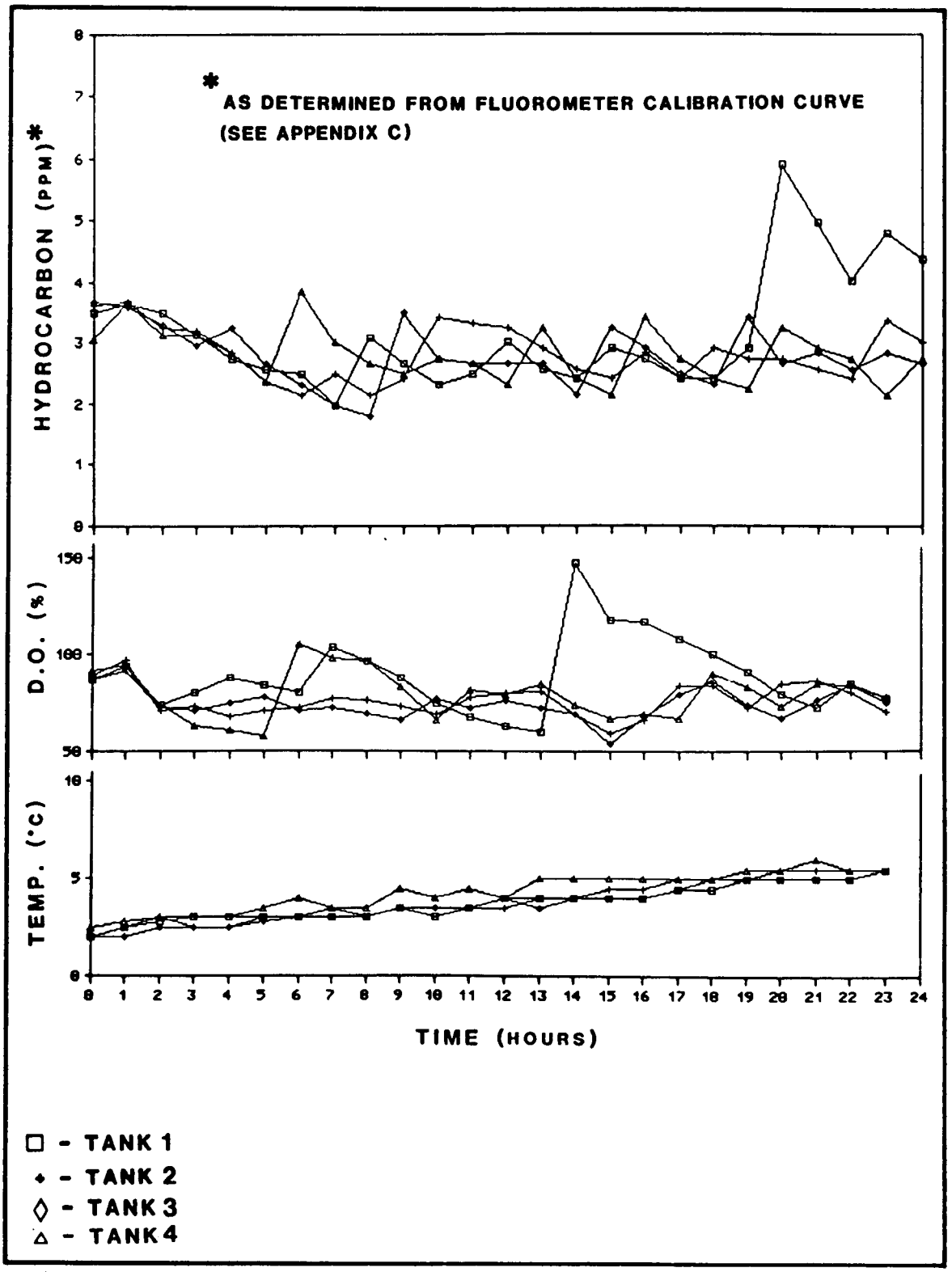


FIGURE 3.22 AMAULIGAK DEPURATION TRIAL - CONDITIONS DURING 24-H FISH EXPOSURE.

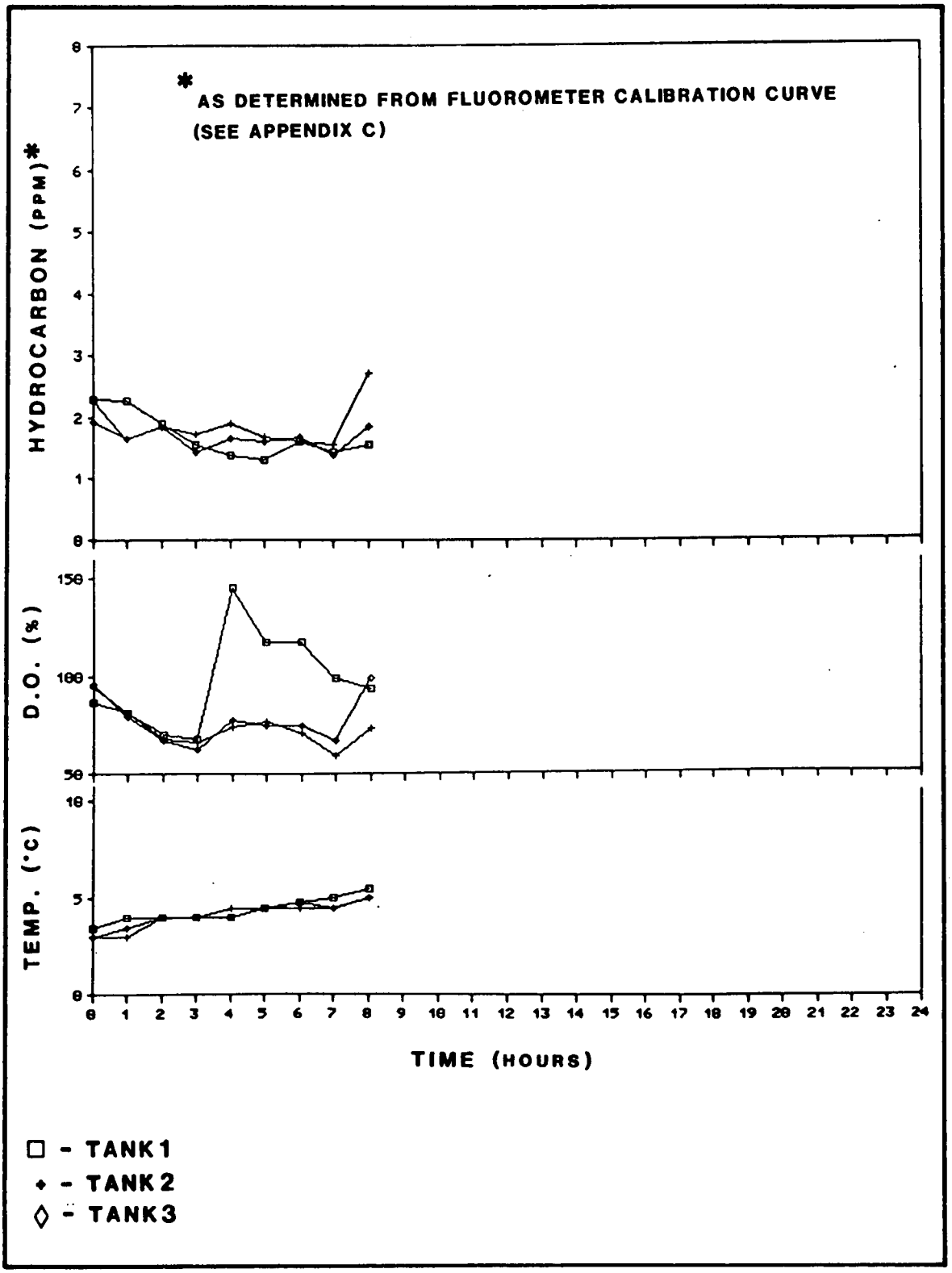


FIGURE 3.23 HIBERNIA DEPURATION TRIAL - CONDITIONS DURING 8-H FISH EXPOSURE.

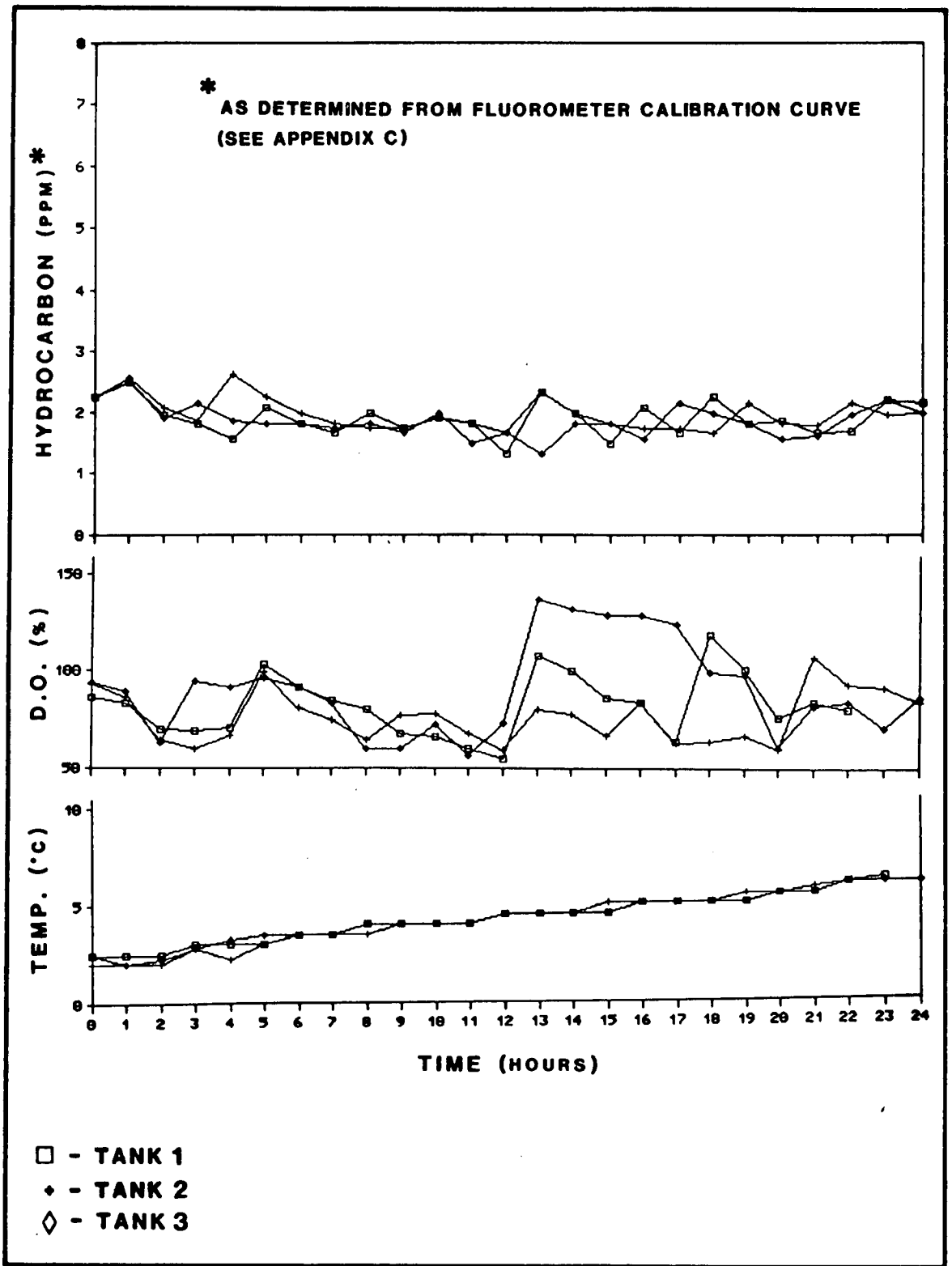


FIGURE 3.24 HIBERNIA DEPURATION TRIAL - CONDITIONS DURING 24-H FISH EXPOSURE.

TABLE 3.5

Hydrocarbon concentrations (GC analysis) - exposures for depuration trials

	Hydrocarbon (ppm)					
	8-h exposure			24-h exposure		
Time (h)	Sample 1	Sample 2	Average	Sample 1	Sample 2	Average
a) Amauligak						
0	2.68	3.43	3.06	3.05	2.98	3.02
8	3.50	2.45	2.98	-	-	-
24	-	-	-	2.35	1.93	2.14
b) Hibernia						
0	1.50	1.67	1.59	1.86	1.93	1.90
8	1.85	2.16	2.01	-	-	-
24	-	-	-	3.23	2.55	2.89

b) Twenty-four-hour exposure: Figure 3.22 presents the conditions in the exposure tanks during this trial. The average hydrocarbon concentration, based on the fluorescence of the exposure water, was 3.00 ppm except for a rapid increase in Tank 1 after 20 h. There were faeces and pieces of semi-digested food in the tanks. The water was turbid causing interference in the fluorometer and, therefore, fluorescence is probably not a reflection of the hydrocarbon concentration in the tank. The fish were showing signs of stress, swimming near the surface with their mouths out of the water. The tank was flushed, but the water was still turbid and the fluorescence remained high and unsteady. The other three exposure tanks were fairly turbid by the end of the trial. Parasites were observed on the bottom of Tanks 1 and 3.

GC analysis of pooled water samples showed average hydrocarbon concentrations of 3.02 ppm at Time 0 and 2.14 ppm after 24 h.

The temperature increased 3°C over 24 h and the oxygen levels were within 60 to 100% saturation except for Tank 1. This tank became supersaturated (148%) at 14 h and took several hours to return to a level similar to the other tanks.

3.3.1.2 Taste panel results. In the depuration trials the fish were exposed to a WSF of Amauligak crude oil for 8 h and 24 h and then depurated up to 14 d with taste panel analyses conducted at 0, 1, 4, and 7 d. The 14-d samples were not subjected to sensory evaluation as the taste panel results of the 4- and 7-d samples indicated a complete depuration of hydrocarbons.

a) Eight-hour exposure: The fish that were tested after 8 h of exposure without depuration (Day 0) gave a 0.1% level of confidence (14 of 16 panelists detecting the odd sample; Table 3.6) with respect to the control fish, whereas those tested after 1, 4, and 7 d of depuration showed no significant difference.

b) Twenty-four-hour exposure: A significant difference was found in those fish exposed for 24 h and tested without depuration (Day 0) and those depurated for 24 h (Day 1). The fish depurated for 24 h gave a confidence level of 5% (9 of 14 panelists detecting the odd samples) and those not depurated a confidence level of

TABLE 3.6

Taste panel results of depuration trials

Depuration time	No. of panelists	No. of correct results	No. of incorrect results	Level of confidence (%)
AMAULIGAK				
8-h Exposure				
- Day 0	16	14	2	0.1
- Day 1	16	6	10	NS *
- Day 4	15	8	7	NS
- Day 7	16	4	12	NS
24-h Exposure				
- Day 0	17	12	5	1.0
- Day 1	14	9	5	5.0
- Day 4	14	6	8	NS
- Day 7	16	5	11	NS
HIBERNIA				
8-h Exposure				
- Day 0	16	14	2	0.1
- Day 1	17	8	9	NS
- Day 4	16	6	10	NS
- Day 7	16	4	12	NS
24-h Exposure				
- Day 0	17	13	4	0.1
- Day 1	15	7	8	NS
- Day 4	17	8	9	NS
- Day 7	15	4	11	NS

* Not Significant.

0.1% (12 of 17 panelists detecting the odd sample) (see Table 3.6). After 4 and 7 d of depuration the taste was not significantly different from the control fish.

3.3.2 Hibernia Crude Oil

3.3.2.1 Exposure conditions.

a) Eight-hour exposure: The fluorescence of the exposure water over the 8-h exposure reflected an average exposure level of 3.00 ppm hydrocarbon (see Figure 3.23). GC analyses of water samples taken at time 0 and 8 h, however, showed an initial exposure concentration of 1.59 ppm of hydrocarbon, increasing to 2.01 ppm after 8 h (see Table 3.5). A marked increase in fluorescence observed in Tank 2 during the last hour. The increase was rapid and was only observed in one tank which suggests that it was due to increased turbidity and not an increase in hydrocarbon concentration (see also Appendix C).

   Temperature and oxygen levels were similar between tanks except for one tank (Tank 1) in which supersaturation occurred after 4 h.

b) Twenty-four-hour exposure: The fluorescence of the exposure water over the 24 h reflected an average exposure level of 2.00 ppm of hydrocarbon (see Figure 3.24). GC analysis of water samples taken, however, revealed a starting concentration of 1.90 ppm increasing to 2.89 ppm by the end of the trial.

3.3.2.2 Taste panel results. Hibernia crude oil depuration trials were conducted for 8- and 24-h exposures with depuration up to 14 d. Panels were conducted at 0, 1, 4, and 7 d.

a) Eight-hour exposure: The fish tasted without depuration (Day 0 sample) gave a confidence level of 0.1% with 14 of the 16 panelists able to determine the odd sample (see Table 3.6). Those fish depurated for 1, 4, and 7 d produced no level of significance compared to the control group of fish.

b) Twenty-four-hour exposure: Thirteen of 17 panelists detected the odd sample (see Table 3.6) in those fish exposed for the 24 h and not depurated (Day 0 sample) giving a confidence level of 0.1%. Fish depurated for 1, 4, and 7 d gave no significant difference when compared to the control fish.

3.3.2.3 Lipid content of tissues. The fillets of the fish exposed to a WSF of Hibernia crude oil for 8 h and depurated for 0 h, and also fillets of the fish exposed for 24 h and depurated for 0 and 24 h were analysed by the Bligh and Dyer method (see Section 2.6) for lipid content and were compared with control fish. The results are given in Table 3.7. Here again, as previously observed for the short-term exposure (see Section 3.2.3.3), the lipid levels were typical of normal Atlantic cod indicating that the hydrocarbon in the water had no observable influence on the lipid contents of edible tissues.

3.3.2.4 Hydrocarbon levels in fish fillets. The hydrocarbon levels in the fish fillets from the Hibernia depuration study, as determined by the steam distillation procedure (see Section 2.4.4) are given in Table 3.8. As mentioned previously (see Section 3.1.3) the control fish showed three major peaks of non-petrogenic origin and a number of minor or trace level peaks, but of petroleum origin (see Figure 3.15). The concentration of these peaks was found to be 0.19 ppm of hydrocarbon. These background peaks were substracted in calculating the hydrocarbon levels in the exposed fish.

The fillets from fish exposed to a WSF of Hibernia for 8 h were found to contain a hydrocarbon level of 0.67 ppm (see Table 3.7). The hydrocarbon profile (Figure 3.25) of these fish fillets was quite close to that of the stock WSF concentrate of Hibernia (see Figure 3.7). After depuration for 24 h, the fish which had been exposed for 8 h showed a similar hydrocarbon concentration to that found in the controls. The GC hydrocarbon profile is given in Figure 3.26.

The fillets from fish exposed to a WSF of Hibernia for 24 h were found to contain a hydrocarbon level of 0.48 ppm and after 24 h depuration the level dropped to 0.08 ppm. The corresponding GC charts are given in Figures 3.27 and 3.28, respectively. Here again the hydrocarbon profiles are similar to the stock WSF of Hibernia.

3.4 LONG-TERM EXPOSURES

3.4.1 General

Long-term exposure trials were conducted with two of the crude oils, Amauligak and Hibernia, to assess potential bioaccumulation of petroleum hydrocarbons leading to development of a taint in the test fish.

TABLE 3.7

Lipid content - Hibernia depuration trials

Percent lipid (w/w)		
Exposure time (h)/Depuration time (h)		
8/0	24/0	24/24
Exposed Fish [a]		
0.74	0.65	0.84
Control Fish		
0.77	0.82	0.70

[a] - Refer to Table 3.3 for further experimental conditions.

TABLE 3.8

Hydrocaron levels in fish fillets -
Hibernia depuration trial

ppm Hydrocarbons				
Exposure time (h)/Depuration time (h)				Control Fish
8/0	8/24	24/0	24/24	
0.67	0.0	0.48	0.08	0.19

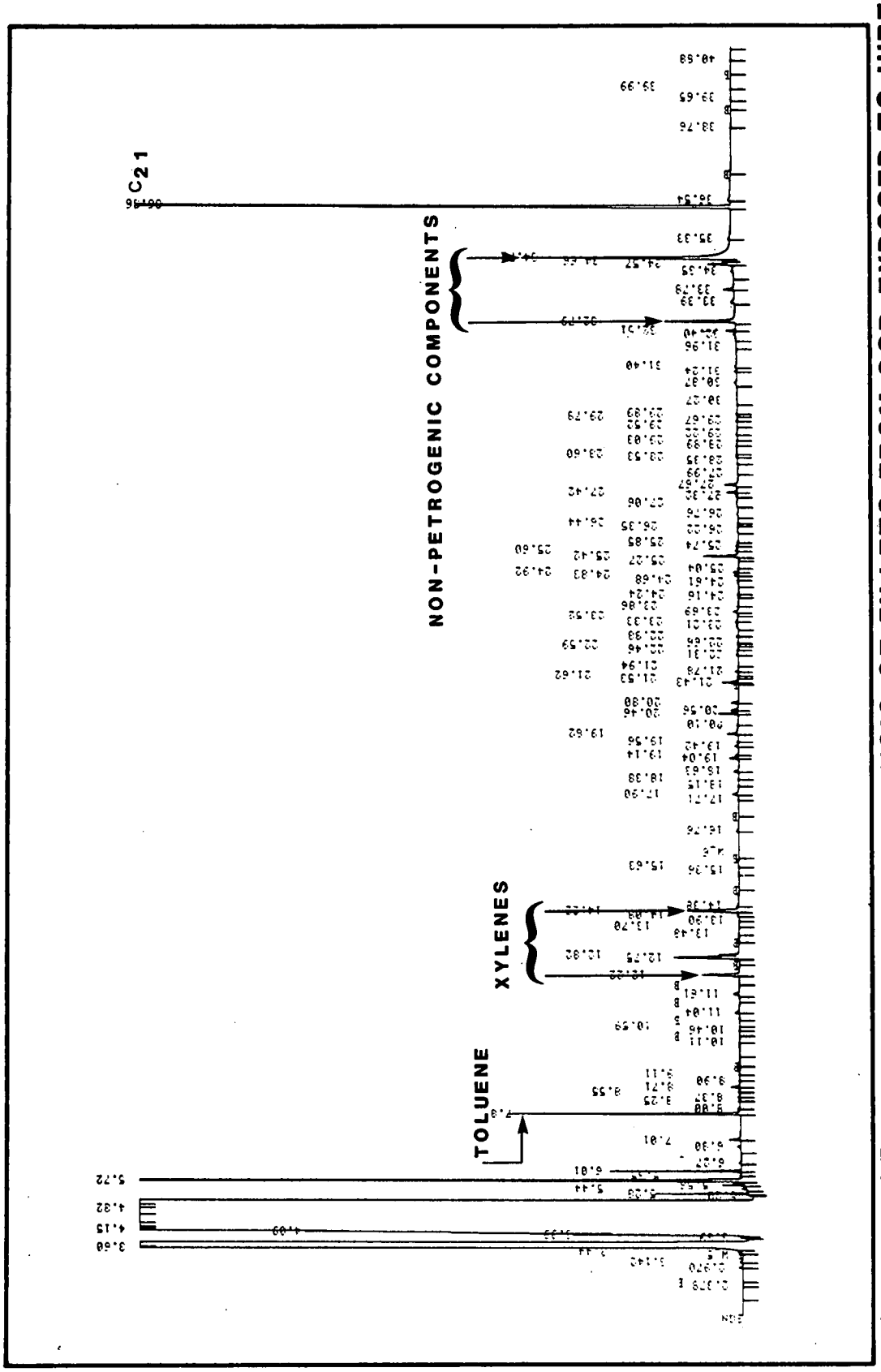


FIGURE 3.25 HYDROCARBON ANALYSIS OF FILLETS FROM COD EXPOSED TO HIBERNIA WSF FOR 8 H AND DEPURATED FOR 0 H. C_{21} IS ADDED INTERNAL STANDARD.

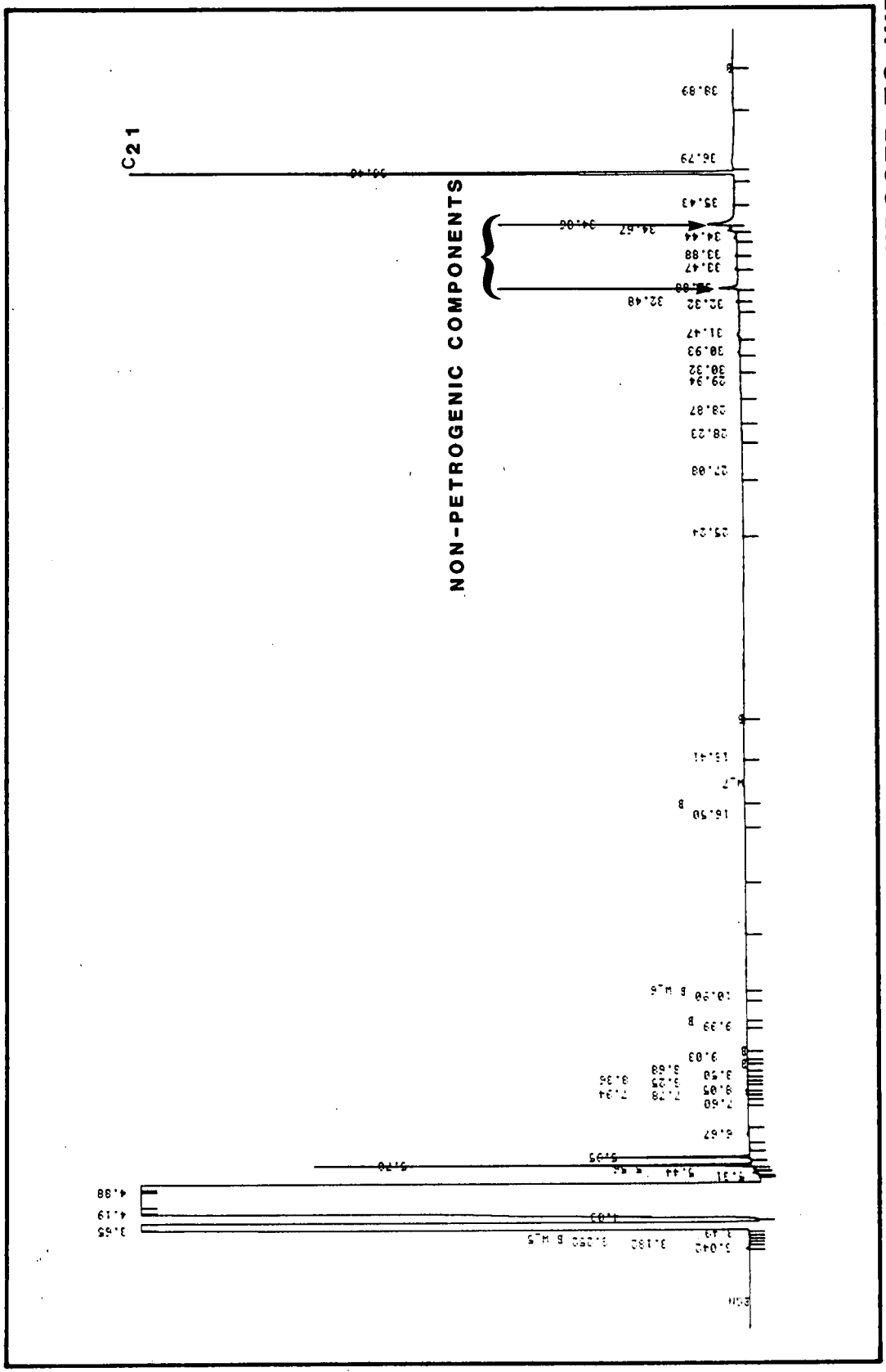


FIGURE 3.26 HYDROCARBON ANALYSIS OF FILLETS FROM COD EXPOSED TO HIBERNIA WSF FOR 8 H AND DEPURATED FOR 24 H. C_{21} IS ADDED INTERNAL STANDARD.

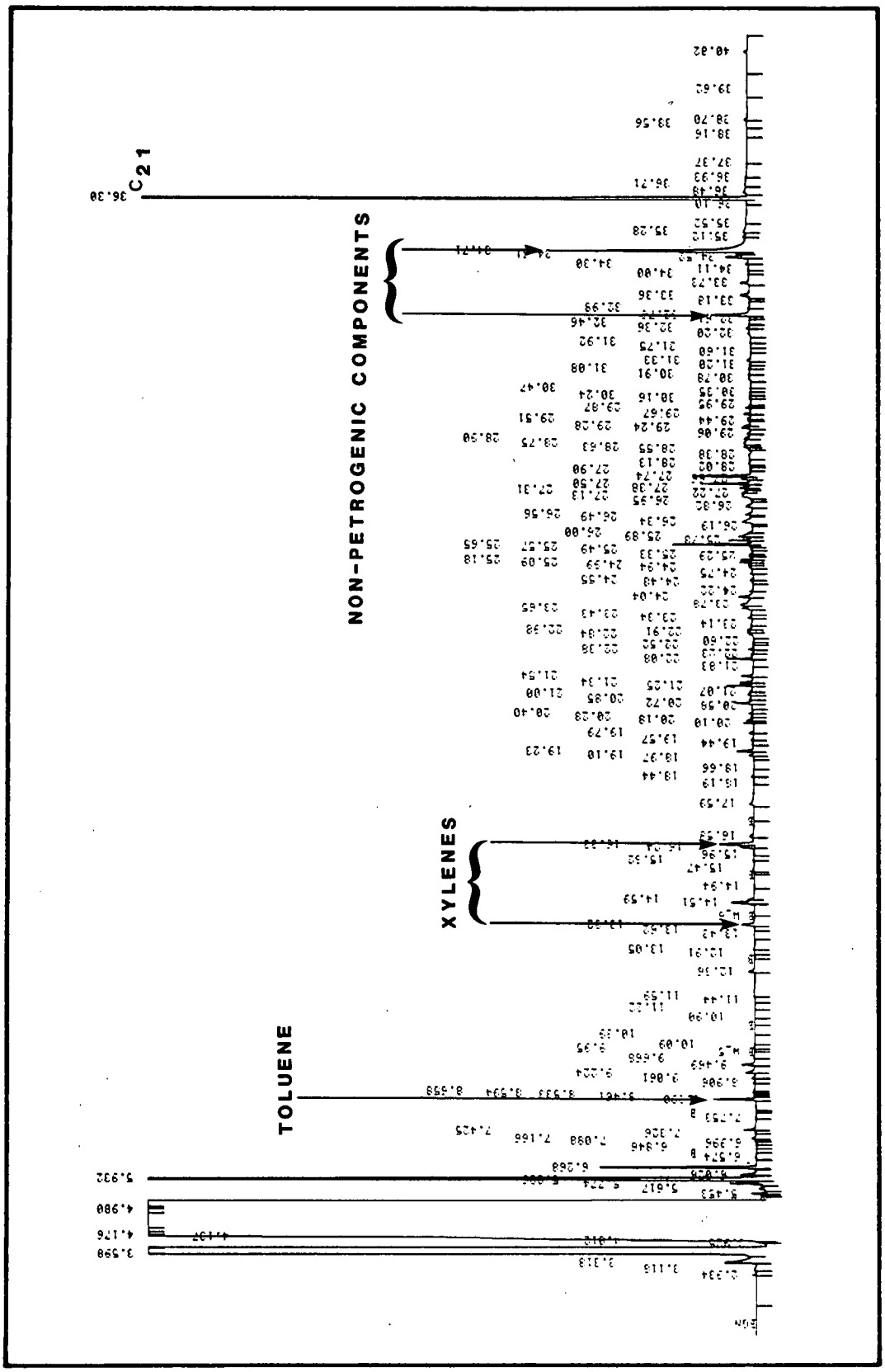


FIGURE 3.27 HYDROCARBON ANALYSIS OF FILLETS FROM COD EXPOSED TO HIBERNIA WSF FOR 24 H AND DEPURATED FOR 0 H. C_{21} IS ADDED INTERNAL STANDARD.

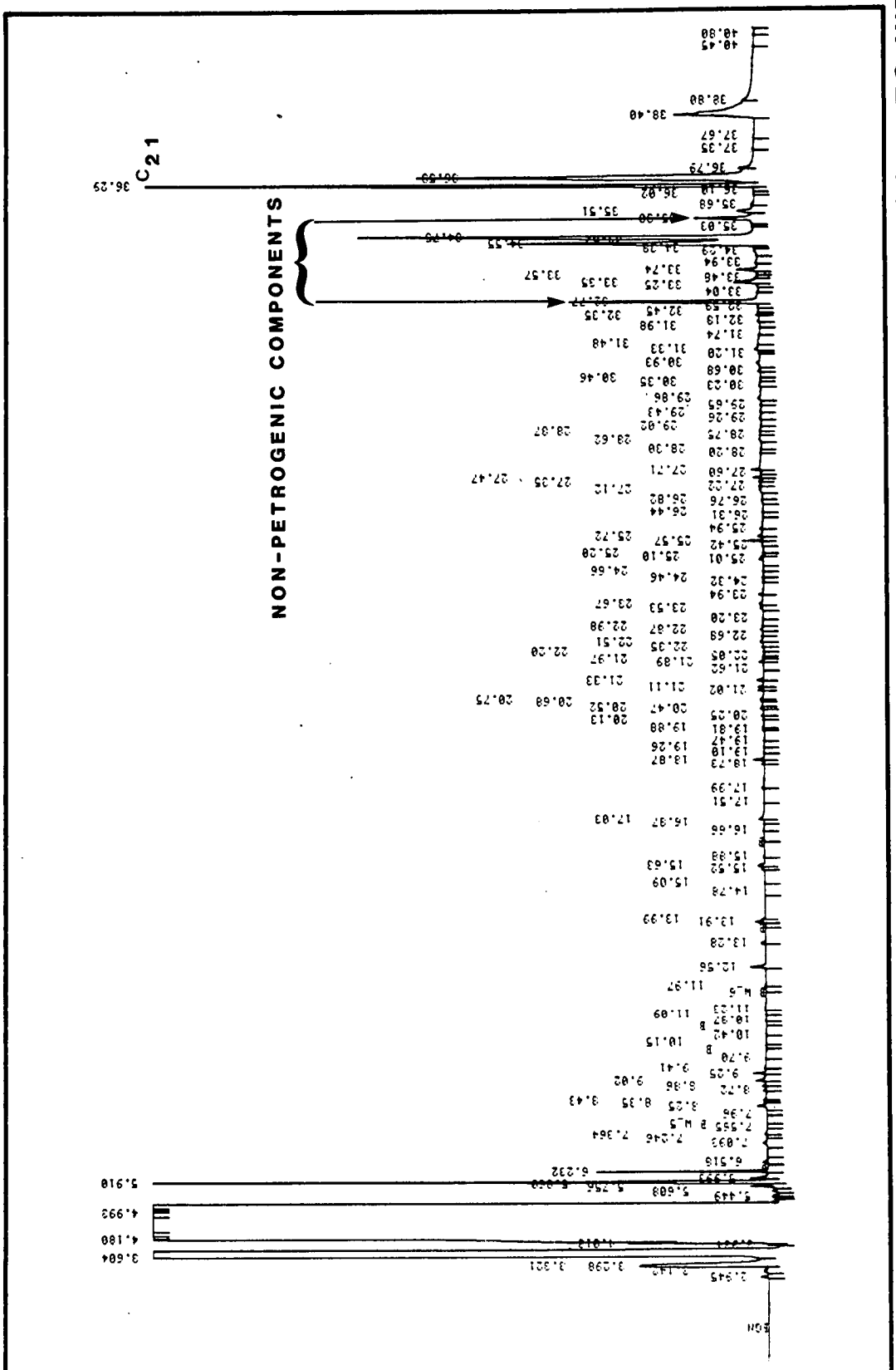


FIGURE 3.28 HYDROCARBON ANALYSIS OF FILLETS FROM COD EXPOSED TO HIBERNIA WSF FOR 24 H AND DEPURATED FOR 24 H. C_{21} IS ADDED INTERNAL STANDARD.

It was recommended in the proposal (Martec, 1986) that the oils be tested at concentrations of 0.5 and 0.1 times the threshold levels determined in the short-term trials, for periods of one and two weeks. Experience from the short-term trials indicated that the lowest exposure would be very difficult, if not impossible, to monitor and that the fish would be overly stressed if kept in the small exposure tanks for two weeks. Therefore, a range-finding trial was conducted with WSF prepared from Amauligak crude oil to test the suitability of various exposure levels and the periods for the actual long-term exposures (see Section 3.4.3).

It was decided, based on this range finding trial, to expose the fish to 0.10 and 0.25 ppm of hydrocarbon for periods of 3 and 7 d. GC analysis of water samples taken throughout the long-term trials, however, indicate that the two exposure levels were not significantly different (Table 3.9). The results of the long-term exposures are, therefore, interpreted as replicates.

3.4.2 Control Group

3.4.2.1 Exposure conditions. Control tanks were run concurrently with the range-finding trial (see Section 3.4.3) and the Hibernia long-term trial (see Section 3.4.4) to monitor the background fluorescence caused by the fish and/or their excretory products in the water and to assess the validity of using the fluorometer to describe the exposure conditions. The fish from these tanks were used in taste panels with control fish from the pool tank to ensure that there was no tank effect, i.e., that the test fish did not develop an atypical flavour from being held in a small tank for up to 7 d.

The control tank during the range-finding trial was monitored more closely than that of the Hibernia trial (Figure 3.29). There was a gradual increase in the fluorescence of the exposure water over the first 24 h. The fluorescence decreased after flushing the tank each day, however, it did not drop to the same level each day. Consequently, a stepwise increase in the background fluorescence was observed for the first 5 d.

There was a marked decrease in the fluorescence during the last 2 d. This decrease may have been caused by a general decrease in excretory products in the water. The fish would not accept food while in the exposure tanks and the amount of faeces in the tanks decreased over the period of the trial.

TABLE 3.9

Hydrocarbon concentrations (GC analysis) - long-term exposure trials

Trial	Time (h)	Hydrocarbon (ppm) Tank 1	Tank 2	Tank 3	Tank 4
a) Range-finding trial					
	0	0.25	0.35	0.75	
	23	0.23	-	-	
	46.5	0.28	0.15	-	
	66.5	-	-	0.13	
	71.5	0.38	-	-	
	166	-	0.05	-	
Average exposure level		0.29	0.18	0.44	
b) Amauligak					
	0	0.09	0.25	0.30	0.22
	66	0.15	0.05	-	-
	70.5	-	-	0.13	0.15
	163	0.13	0.05	-	-
Average exposure level		0.12	0.12	0.22	0.19
c) Hibernia					
	0	0.10	0.10	0.10	0.07
	67	-	0.10	-	-
	68	-	-	0.15	0.25
	71	0.07	-	-	-
	163	-	-	0.23	0.09
Average exposure level		0.09	0.10	0.16	0.14

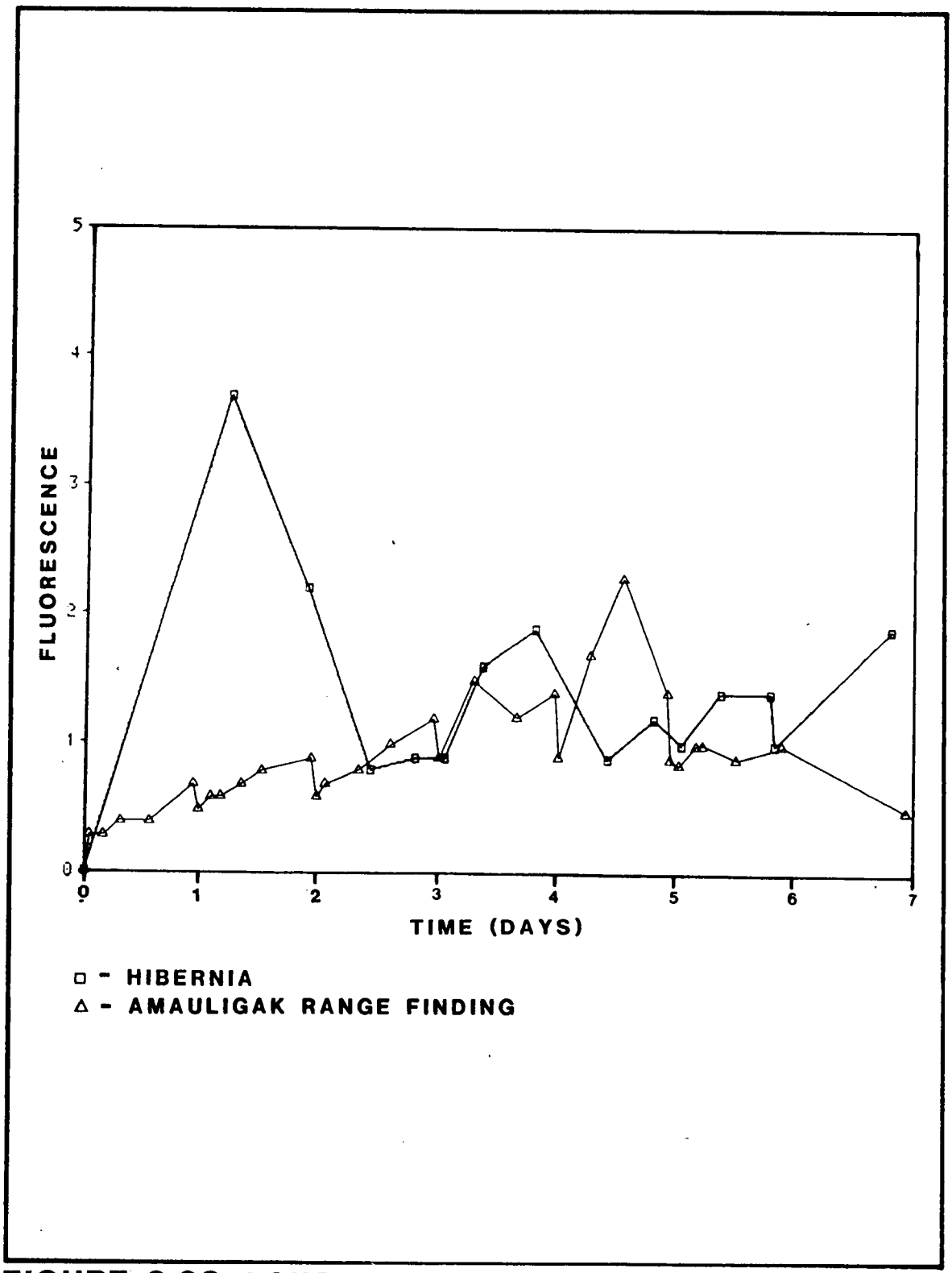


FIGURE 3.29 LONG-TERM TRIALS - BACKGROUND FLUORESCENCE IN CONTROL TANKS.

The background fluorescence in the control tanks exhibited more variation between tanks and over time than the variation in fluorescence that would be expected between the proposed exposure concentrations. This fluctuation in the background fluorescence made using the fluorometer to monitor the hydrocarbon concentrations in the exposure tanks impractical at the proposed levels. Consequently, the conditions in the exposure tanks during the long-term trials are described from the results of the GC analysis alone.

3.4.2.2 Taste panel results. A taste panel was conducted to evaluate if there was any difference between the taste of the fish from the main pool tank which had been stored at -35°C for about 21 d and a control group of fish which had been held in small tanks, exactly the same as those used for exposure studies, for 7 d.

The taste panel results are given in Table 3.10. Of the 16 panelists participating only eight were able to determine the odd sample. Therefore, these results indicated that there was no significant difference between the taste of the fish from the main pool tank and the control fish from the small tanks. Further, these results indicated that there is no detrimental effect from using fish from the main pool as controls as opposed to fish held under conditions similar to the exposed fish, but without addition of WSF.

3.4.3 Amauligak Crude Oil

3.4.3.1 Exposure conditions. Two long-term trials were run using a WSF of Amauligak crude oil. The first trial was a range-finding trial in which fish were exposed to about 0.23 ppm of hydrocarbons for 3 d and 7 d as well as a level of about 0.40 ppm of hydrocarbon for 3 d. The second was a long-term exposure to about 0.10 ppm of hydrocarbon for 7 d and about 0.20 ppm of hydrocarbon for 3 d.

a) Range-finding trial: The concentration of hydrocarbons in the stock solution of Amauligak WSF used for this trial was 17.2 ppm. Table 3.9a presents the results of GC analysis of water samples taken throughout this trial. Conditions in Tank 1 appear to have been relatively constant with an average exposure level of 0.29 ppm of hydrocarbon. An overall loss of hydrocarbon in Tank 2 resulted in an average exposure level of 0.18 ppm of hydrocarbon. Similarly, hydrocarbon levels dropped over the 3-d of exposure in Tank 3, however,

TABLE 3.10

Taste panel results of long-term trials

Trial	No. of panelists	No. of correct results	No. of incorrect results	Level of confidence (%)
Range-finding trial				
- Tank 1	15	9	6	5.0
- Tank 2	15	12	3	0.1
- Tank 3	15	8	7	NS *
Amauliqak				
- Tank 1	15	9	6	5.0
- Tank 2	16	9	7	NS
- Tank 3	14	7	7	NS
- Tank 4	14	9	5	5.0
Hibernia				
- Tank 1	16	7	9	NS
- Tank 2	15	7	8	NS
- Tank 3	17	2	15	NS
- Tank 4	17	10	7	5.0
Control	16	8	8	NS

* Not significant.

the average exposure level (0.44 ppm of hydrocarbon) was well above the two lower exposure levels.

Figure 3.30 presents the temperature and dissolved oxygen conditions in the exposure tanks during the range-finding trial. The temperature fluctuations follow the initial pattern observed in the background fluorescence of the control tanks. A gradual increase in the temperature each day is followed by a drop when the tanks were flushed. The DO conditions are more random. In most instances the exposure water became supersaturated overnight. The tanks were flushed in the mornings, however, the DO levels remained high and the oxygen flow would be shut off. It often took several hours for the DO to drop to levels that necessitated turning the oxygen on and/or the oxygen was always turned on overnight when the tanks were not monitored as closely.

b) Long-term exposure: The concentration of hydrocarbons in the stock solution of WSF used in this trial was 21.0 ppm. Table 3.9b presents the results of GC analyses of water samples taken throughout this trial. The fish in Tanks 1 and 2 were held for 7 d at an average exposure level of 0.12 ppm of hydrocarbon. The conditions in Tank 1 were the most consistent over the 7 d whereas hydrocarbon levels in Tank 2 began relatively high, dropped 80% by the third day, and remained low on the seventh day.

The fish in Tanks 3 and 4 were held for 3 d at slightly higher hydrocarbon levels. The exposure conditions were similar at the end of the trial, however, levels in Tank 3 were higher than in Tank 4 at the beginning of the trial. The average exposure level was 0.22 ppm of hydrocarbon in Tank 3 and 0.19 ppm in Tank 4.

The temperature and DO conditions in these tanks over the 3 and 7 d are presented in Figure 3.31. They follow the same general patterns seen in the range-finding trial described. The overall temperature increase was 4 to 5°C, however, it was no greater than 3°C for any 24-h period. DO levels were greater than 100% saturated for most of the trial and only dropped to the high 60s for brief periods in Tanks 2 and 6.

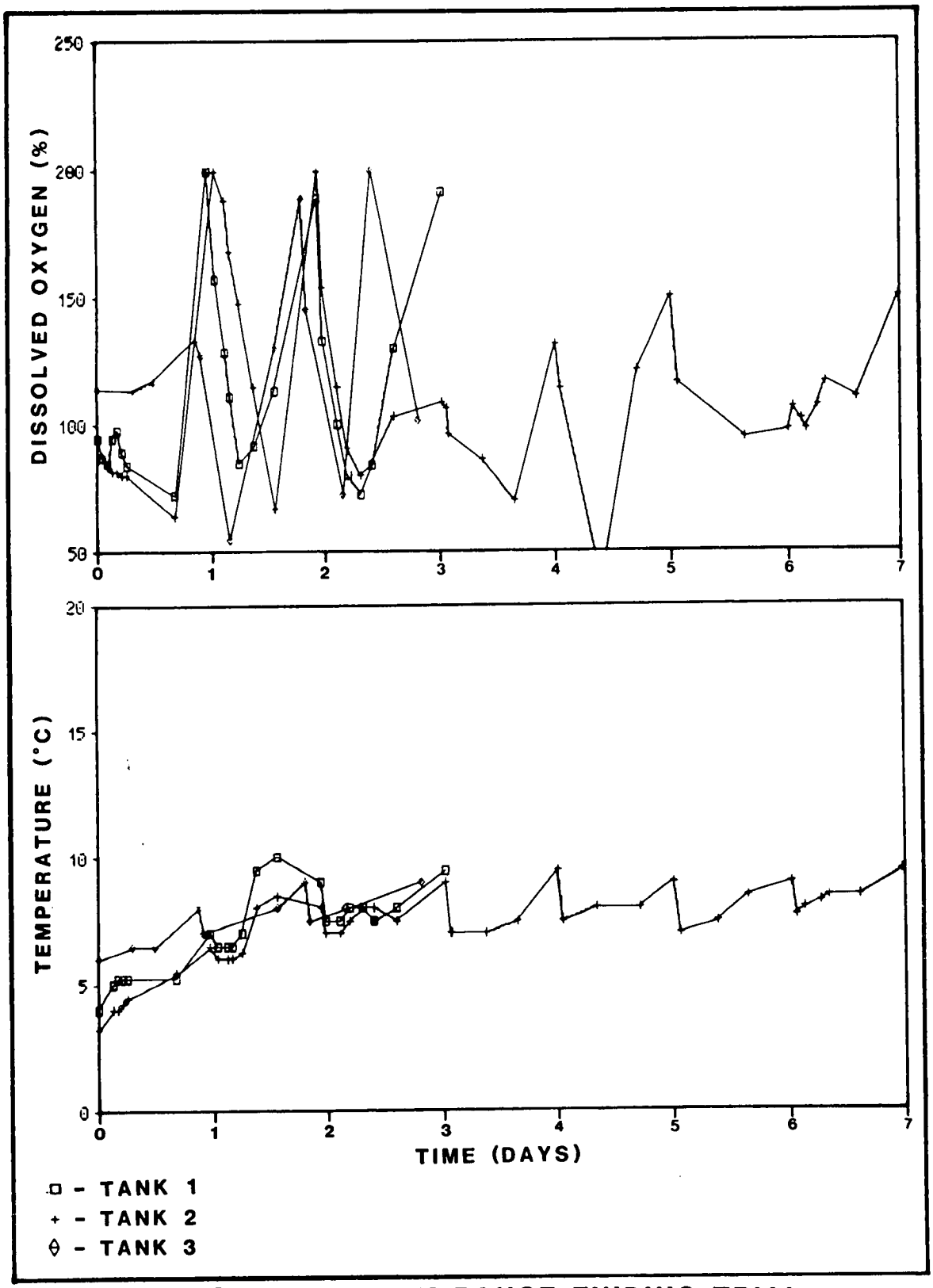


FIGURE 3.30 LONG-TERM RANGE-FINDING TRIAL EXPOSURE CONDITIONS.

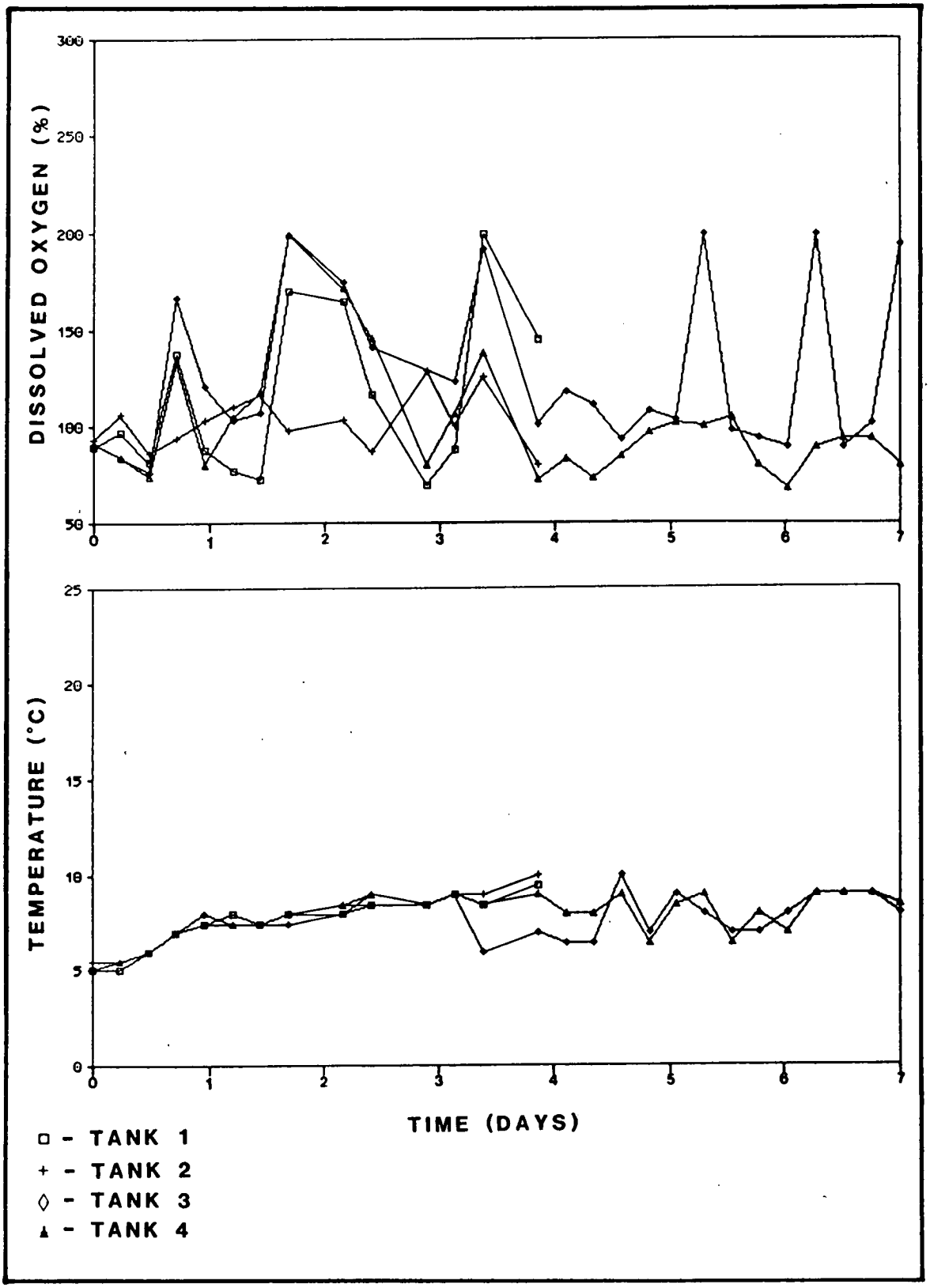


FIGURE 3.31 AMAULIGAK LONG-TERM TRIAL EXPOSURE CONDITIONS.

3.4.3.2 Taste panel results.

a) Range-finding trial: In the range-finding trial of the long-term exposure to Amauligak crude oil WSF, the fish exposed for 71.5 h and 166 h gave a significant difference in taste when compared to the controls (see Table 3.10). Tank 1 gave a confidence level of 5.0% with 9 of 15 panelists detecting the odd sample and Tank 2 gave a confidence level of 0.1% with 12 of 15 panelists detecting the odd sample. The fish exposed in Tank 3 for 66.5 h showed no significant difference in taste when compared to the control fish.

b) Long-term exposure: The fish exposed to WSF in Tanks 1 and 4 were significantly different in taste at a 5% confidence level with respect to the control fish (see Table 3.10). In Tank 1 the cod were exposed to WSF for 7 d with 9 of 15 panelists determining the odd sample and in Tank 4 the cod were exposed to WSF for 3 d with 9 of 14 panelists determining the odd sample. The taste panelists did not detect a significant difference in the taste of the fish exposed to WSF in Tanks 2 and 3 compared to control fish.

3.4.4 Hibernia Crude Oil

3.4.4.1 Exposure conditions. The hydrocarbon content of the stock solution of Hibernia WSF used for this trial was higher than that observed for the short-term exposure (20-25 ppm compared to 15 ppm). The hydrocarbon concentrations in the exposure tanks are presented in Table 3.9c. The fish in Tanks 1 and 2 were exposed for 3 d at an average exposure level of 0.09 and 0.10 ppm of hydrocarbon, respectively. In both tanks the hydrocarbon levels were fairly constant.

The fish in Tanks 3 and 4 were exposed for 7 d at an average exposure level of 0.16 and 0.14 ppm of hydrocarbon, respectively. There was an overall increase in the hydrocarbon levels in Tank 3 over the 7 d, whereas in Tank 4 the trial began and ended at approximately 0.08 ppm, however, there was a three-fold increase (0.25 ppm) at the mid-point of the trial.

Figure 3.32 presents the temperature and DO levels in the exposure tanks throughout the trial. The initial temperature in the exposure tanks is higher than in previous trials because the exposure water sat for several hours prior to the trial actually commencing. The maximum temperature range was 2.5°C over any 24-h period.

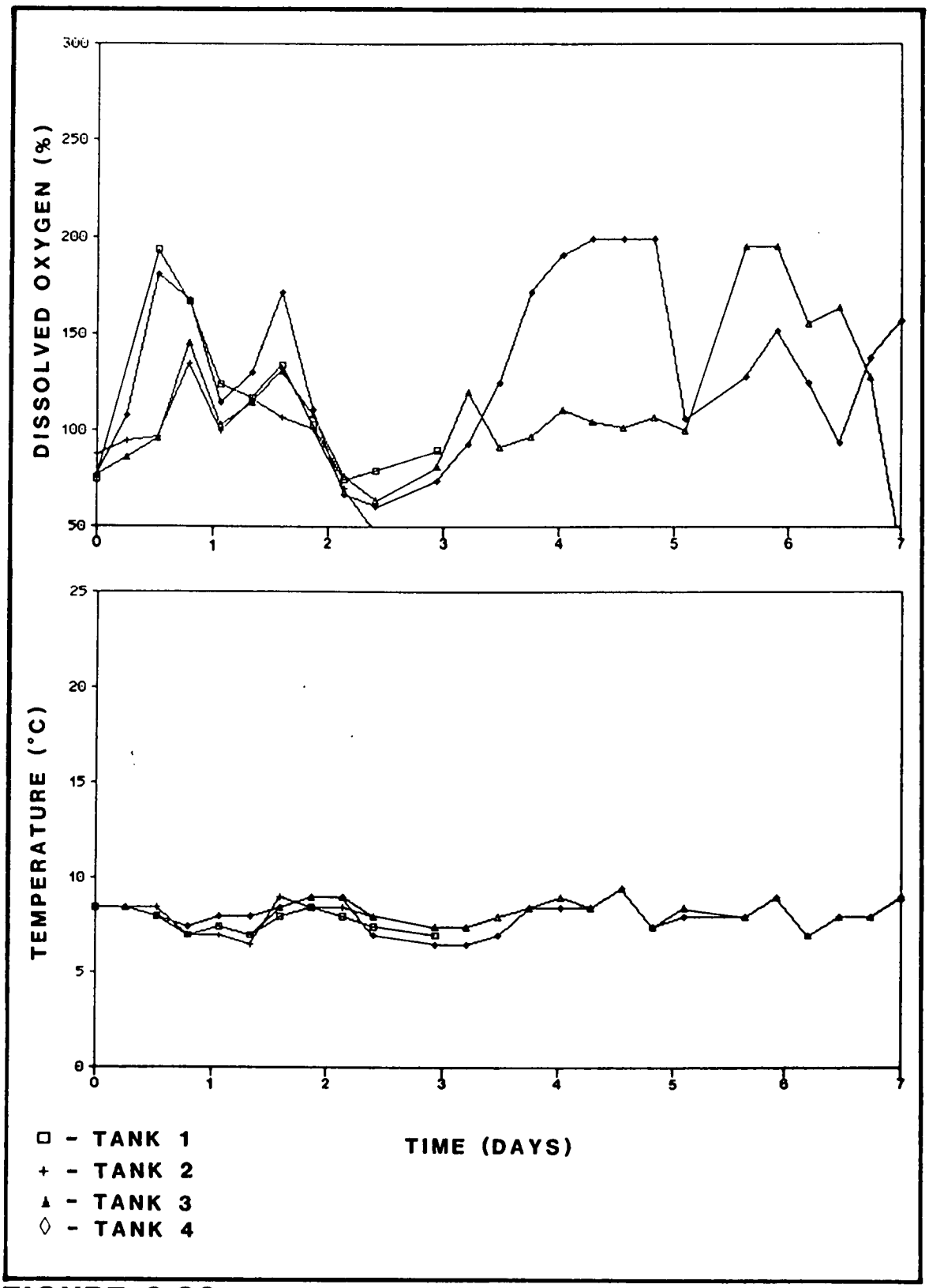


FIGURE 3.32 HIBERNIA LONG-TERM TRIAL EXPOSURE CONDITIONS.

The oxygen flow to all four tanks was terminated after 50 h of exposure and compressed air was used to aerate the tanks. Insufficient air was bubbled into Tank 2 and the DO level dropped to less than 50% saturation over the next 17 h. Two of the three fish in that tank were found dead. The flesh was in good condition and the fish were used in the taste test.

A new cylinder of oxygen was connected after 20 h of using compressed air and DO levels were brought back up to saturated levels. The flow of oxygen to Tank 4, however, was too low during the last 11 h of exposure and the DO level dropped from 128 to 37% saturation. Again, two of the three fish were found dead, however, the fish were used for the taste tests.

3.4.4.2 Taste panel results. Long-term exposure of fish to a WSF of Hibernia crude oil produced taint at a confidence level of 5% (10 of 17 panelists detecting the odd sample) for those fish exposed in Tank 4 for 7 d. The fish exposed in the other three tanks, two for 3 d and one for 7 d, did not develop a taint detectable by taste panel analysis.

4.0 DISCUSSION

4.1 UPTAKE AND PERSISTENCE OF TAINT

4.1.1 Threshold Values and Toxic Levels

4.1.1.1 Brent crude oil. The taste panels results (see Table 3.3) indicated that the cod exposed to Brent WSF were tainted in all the three different concentration levels. The fish from the lowest concentration group were tainted to a lesser extent than the other two, higher concentration groups. During all the exposure studies, the concentrations of the WSF fluctuated during the entire exposure period. Nevertheless, by looking at the GC and the fluorometer results it could be safely assumed that the average hydrocarbon concentration of the lowest exposure group was 0.5 ppm. At this level, the taste panelists were able to detect a taint and found fish of this group significantly different from the unexposed fish at a 5% confidence level. Above 0.5 ppm, the taint is much more severe as the confidence level moved up to 0.1%. From the taste panel results it appears that the threshold concentration of Brent crude oil in sea-water which will taint cod in 24 h is about 0.5 ppm or perhaps slightly lower.

The fish exposed to the most concentrated WSF (~8 ppm) of the Brent showed immediate signs of stress. Fish exposed to between 2.5 and 3.0 ppm of hydrocarbon showed signs of stress after 11 h of exposure and died within the next 3 h. Thus exposure to hydrocarbon levels in excess of 2.5 ppm of Brent crude oil is toxic to cod.

4.1.1.2 Amauligak crude oil. The sensory evaluation results of fish exposed to Amauligak WSF showed that they were tainted at the two higher concentration levels, which ranged from 0.75 to 0.29 ppm and from 1.78 to 2.70 ppm (see Table 3.2). However, the fish exposed to the lowest level, the concentration of which ranged from 0.28 to 0.03 ppm, were not tainted and their taste could not be distinguished from the control fish. Therefore the threshold concentration of Amauligak hydrocarbons which will impart a taint to cod within 24 h is in the range of 0.29 to 0.75 ppm. Levels of Amauligak hydrocarbons above this range will definitely taint the fish.

The fish exposed to the highest concentration of Amauligak WSF showed signs of stress and behaved differently from the fish in tanks with lower concentrations. It would appear that sea-water contaminated with Amauligak

crude oil above 2 ppm of hydrocarbon may have a toxic effect on cod exposed for longer than 24 h.

4.1.1.3 Hibernia crude oil. In contrast to the other experiments, the concentrations of the Hibernia WSF in the exposure tanks were maintained at a reasonably constant range throughout the 24-h exposure period. Based on the GC and fluorometer results the average concentrations of the three exposure tanks were 0.2, 0.4, and 2 ppm.

Out of these three concentrations, the fish exposed to the 2 ppm of hydrocarbon level were tainted and tasted significantly different, at a 0.1% confidence level, from the control fish. The taste of the fish from the other two groups was not different from the controls. Based on these results, it is concluded that the threshold levels of Hibernia crude oil which will taint cod in 24 h is between 0.5 and 2 ppm. The fish in all 3 exposure tanks did not appear stressed. Hydrocarbon levels of Hibernia WSF as high as 2 ppm do not appear to be toxic to cod over a 24-h exposure period.

4.1.1.4 Conoco base oil. With Conoco base oil, the fluorometer was not helpful in monitoring the hydrocarbon concentrations of WSF during the exposure period. The response of the fluorometer did not change even when the concentration of hydrocarbons in the water was increased by spiking with stock WSF of Conoco. This response could be rationalized as resulting from the presence of very low levels of aromatic hydrocarbons in Conoco. GC analysis of the Conoco oil, as well as the stock WSF concentrate proved that Conoco has relatively low levels of aromatic hydrocarbons and is composed of branched and cyclic alkanes. The complete analysis of Conoco hydrocarbons was not attempted by us, but analytical data supplied by Conoco oil company (see Appendix E) supports these statements.

With Conoco oil, the fish exposed to a concentration level of 1.93 to 1.21 ppm of hydrocarbons were found to be tainted by sensory evaluation (see Table 3.3). The fish exposed to concentration ranges of 0.74 to 0.14 ppm and 0.69 to 0.47 ppm were not significantly different from the control fish. Thus the threshold level of Conoco which will taint cod in 24 h is between 0.74 and 1.21 ppm.

The fish exposed to the 1.91 and 1.21 ppm level of hydrocarbons showed signs of stress after 10 h of exposure. Thus the toxicity level of Conoco may be in this range.

4.1.2 Depuration

Depuration trials were carried out with two oils; Amauligak and Hibernia. The fish were exposed for 8 or 24 h to concentration levels much higher than the threshold values already discussed and were allowed to depurate in fresh, uncontaminated sea-water for 14 d. For Amauligak, the average concentration for the 8-h exposure tank was 3.03 ppm and that of the 24-h exposure tank was 2.63 ppm. The corresponding values for the Hibernia exposures were 1.80 and 2.38 ppm.

For both oils, the fish were tainted in the 8- and 24-h exposure period. The fish depurated within 24 h except for those fish exposed to a WSF of Amauligak crude oil for 24 h. In this case the confidence level of the taste panel results dropped from 1 to 5% within 1 d and then after 4 d sensory evaluation showed no significant difference from the control fish. Thus, fish exposed to Amauligak at a concentration of 2.63 ppm would have depurated within 1 to 4 d.

4.1.3 Long-term Exposure Studies

The long-term exposure studies were carried out with WSFs of Hibernia and Amauligak crude oil. The fish were exposed for 3 or 7 d at a much lower hydrocarbon level than the threshold levels observed in the earlier short-term exposures. The sensory evaluation results indicated some of the fish were mildly tainted (the confidence level was only 5%). This set of experiments was an excellent example of threshold values for long-term exposures. For both oils, the threshold hydrocarbon concentrations were in the range of 0.1 to 0.2 ppm, and were found to impart a taint when fish were exposed for three or more days.

4.1.4 Bioaccumulation

Because the major aim of the project was to define the threshold levels of hydrocarbons through sensory evaluation, only a few fish were subjected to chemical analysis. Bioaccumulation of hydrocarbons was studied only in those fish subjected to depuration trials with Hibernia.

The fish exposed for 8 and 24 h had hydrocarbon levels of 0.67 and 0.48 ppm in the fillets and these levels dropped to 0 and 0.08 ppm, respectively (see Table 3.8) after a 24-h depuration period. These results correlated extremely well with the corresponding sensory evalu-

ation results. The panelists were able to detect the contaminated fillets (0 day sample) very easily while the fillets from depurated fish could not be distinguished from the control.

GC analysis showed that the hydrocarbon profiles of the tainted fish (see Figures 3.15 and 3.27) were exactly the same as the hydrocarbon profiles of the Hibernia WSF (see Figure 3.7). Further, with the 8-h exposure fish (see Figure 3.15) the relative proportions of the component hydrocarbons were more or less the same as that of the WSF (see Figure 3.8), but this result is not exactly true for the 24-h sample (see Figure 3.27). In the 24-h sample, the concentration of the early eluting components had decreased. This observation probably indicates that, in the early stages of exposure, the hydrocarbons accumulate directly in the fillet, but after a further period these early eluting hydrocarbons were metabolized selectively by enzyme induction. As excretion into the water is unlikely, transfer to the fatty (liver) organ tissue is possible. The latter theory could have been examined with analysis of organ tissues.

4.1.4.1 Lipid content of fillets. Examination of the lipid content of the fillets of exposed fish and control fish (see Table 3.7) indicates that hydrocarbons in the water had no measurable influence on the lipid metabolism. Previous workers (Vale et al., 1970) have observed that tainted fish had slightly higher levels of lipids in the various tissues and generally concluded that hydrocarbons in the water do interfere with lipid metabolism. This finding is true for fatty fish but not necessarily for lean fish. The lean fish have limited lipid reserves in the muscle to absorb or accumulate extraneous organic matter.

4.2 ASSESSMENT OF TEST GUIDELINES

4.2.1 General

The GESAMP guideline for the assessment of tainting (GESAMP, 1983) and the ECETOC test guidelines (ECETOC, 1987) formed the basis for establishing the exposure system used in this study. Deviations from these guidelines were necessary, however, to accommodate the size of fish being exposed and the volumes of WSF that could feasibly be prepared for any one trial (see Appendix B).

4.2.2 Test Organisms

GESAMP (1983) and ECETOC (1987) recommend using a test organism which fulfils the following criteria:

- are a major source of food;
- are readily available, i.e., laboratory-reared or commercially farmed;
- are maintained relatively easily under laboratory conditions; and,
- are used extensively in aquatic toxicology.

Atlantic cod were selected as the test fish for this study because they represent a significant percentage of the east coast fishery. Using cod, however, necessitated obtaining wild fish and acclimatizing them to laboratory conditions. The stress of being caught (most of the fish had hook wounds) and transported, of crowded conditions, and of diet changes led to considerable mortalities during the early part of the study.

ECETOC (1987) recommend that fish to be used in the test should be acclimatized to water of the quality to be used in the test for at least 7 d before they are used. This recommendation, however, refers to rainbow trout (Salmo gairdneri Rich.) that have been laboratory reared. Experience from this study suggests that, when using wild fish for a study, up to several months should be scheduled for an acclimatization period to ensure that exposure does not occur while the fish are in a stressed condition.

Whittle and Mackie (1976) have suggested that the amount of "free" lipids present in a fish indicates the susceptibility of that animal to tainting. To ensure the comparability of data the fat content of the edible portion of the fish to be used for testing should be determined as part of a tainting study. Ideally fish with moderate fat content should be used as an indicator species in tainting studies.

The edible muscle of cod may be taken as having the basic minimum lipid composition (<1% by weight) compared to other commercial species, such as halibut and sole (which are in the range of 1.5 to 6.5% by weight) and capelin, herring, and mackerel, with edible-part lipids ranging up to 20% of total wet weight (Ackman, 1980; Sidwell, 1981).

Again, cod was the recommended species for this study because it represents a significant part of the east coast fishery and not because of its fat content. This choice should be taken into consideration when applying the results of this study to the susceptibility of other, more fatty fish to taint.

Other factors that affect the lipid content of finfish and, therefore, may affect the potential for tainting or the threshold concentration at which a taint would become evident are the season and sexual maturity of the test population.

Knowledge of the previous diet of the fish is important in ensuring that no strong or atypical flavour results from the diet to mask the taint from the hydrocarbon exposure. The test fish were not fed during the exposure to eliminate the possibility of uptake of taint from the hydrocarbon absorbed onto the food, and they were not fed during the 48 h prior to the study to reduce faecal contamination of the water. There was, however, faecal contamination in the exposure tanks and in some instances semi-digested food was found in the tank. The food (mackerel) was analysed for hydrocarbon content to ensure that there was no contamination of the exposure water from this source.

4.2.3 Test Conditions

4.2.3.1 Temperature. ECETOC (1987) and GESAMP (1983) recommend that the water temperature should be controlled at 15° ± 2°C for rainbow trout (S. gairdneri) or an appropriate temperature for other species. It was found that by using insulated tanks, and by keeping the laboratory facilities relatively cool, that the water temperature rose about 3°C but not greater than 5°C during the 24-h exposure period. If possible thermostatically controlled exposure tanks and/or a flow-through test system should be used to maintain optimal water temperatures.

4.2.3.2 Dissolved oxygen. ECETOC (1987) recommend that the dissolved oxygen level should be maintained above 60% saturation. Oxygen levels can be maintained by adopting a flow-through system using well-aerated dilution water or, by aerating the actual exposure water if this does not cause unacceptable losses (<50%) in the test substance. This problem is addressed at length in Appendix B. It was determined that, when using large fish, a flow-through system would require a volume of test solution in excess of

what could be practically prepared and, that aerating the tanks with compressed air would result in unacceptable losses of the test solution. Aerating the exposure tanks with pure oxygen was used throughout this study and is recommended for future studies. If monitored rigorously it is possible to maintain suitable oxygen levels with limited losses of the test solution.

Although hourly monitoring during the short-term exposures was possible, it became impractical during long-term exposures. Consequently, the exposure water frequently became supersaturated with oxygen. According to G. Iwama[1] exposing fish to super-saturated water for periods up to a week would not stress the fish. At worst, the fish would ventilate at a lower rate resulting in less test solution passing over the gills.

4.2.3.3 Exposure concentrations.

a) Monitoring: GESAMP (1983) and ECETOC (1987) recommend analysis of the test substance in the exposure medium at the beginning and at the end of the exposure period to check the stability and that exposure concentrations should be within ±50% of its initial concentration. Ideally, more GC analyses would describe the exposure conditions more accurately. Because of the turn-around time with this technique, however, GC analysis is not a useful tool for monitoring the exposure water in terms of making adjustments in maintaining the desired concentrations. A "headpsace" technique probably could be adapted to permit frequent on-site GC analyses, but the data reduction would have to be automated as well. Conversely, fluorometry of the crude oil WSF yields an immediate response, however, confidence is limited particularly at low concentrations. The relative changes in the hydrocarbon levels as observed by fluorometry are only accurate if there is little or no contamination of the water by the fish themselves (see Appendix C).

b) Threshold level trials: Short-term trials were designed to determine the threshold concentrations of hydrocarbon required to elicite a taint. ECETOC (1987) recommend that the initial test, or limit test, should be carried out at a concentration of 10 ppm, or one-

[1] G. Iwama, Biology Dep., Dalhousie University, Halifax, Nova Scotia, personal communication, 1987.

tenth of the 24-h LC_{50}, or the limit of water solubility level, whichever is the lowest. During the short-term range-finding trial using a WSF of Brent crude oil it became evident that concentrations in the order of 10 ppm hydrocarbon were toxic and would kill the fish within the first hour of exposure. An upper limit of 2.5 ppm of hydrocarbons was chosen and, in subsequent trials with Brent and other oils, was found to taint the fish in the 24 h of exposure.

In the initial range-finding trial, a lower limit of 0.05 ppm of hydrocarbons was attempted. Because of the background fluorescence in the exposure water it was impossible to monitor and maintain exposure levels at that low level. A lower-level exposure of 0.25 ppm of hydrocarbons was chosen for subsequent short-term trials. It appears that the detection limit of the monitoring instrument may be the crucial factor for exposures to low levels of hydrocarbon. For the oils used in this study, the exposure levels of approximately 0.25 ppm, 0.5 ppm, and 2.5 ppm resulted in an upper- and lower-limit test (i.e., tainted and not tainted) and would be the recommended exposure concentrations for any additional exposures of fish to WSFs of crude oil.

c) Depuration trials: Depuration trials were designed to show the length of time exposed fish retain a taint after being removed to clear water. The length of time to depurate depends on the degree to which the fish were tainted, which, in turn, depends on the exposure concentration and the exposure time. For this study fish were exposed to five times the threshold level (2.5 ppm of hydrocarbons) for 8 or 24 h. All experimental groups lost their taint within 24 h except the Amauligak 24-h exposure.

Short-term exposures (i.e., $\leq$24 h) at lower hydrocarbon levels would presumably depurate in less than 24 h. Fish exposed to levels higher than 2.5 ppm begin to show signs of stress. Therefore, it is not recommended that exposure concentrations be changed for future short-term depuration trials. Depuration trials in which fish are exposed to similar and/or lower hydrocarbon levels but for longer periods may show different depuration times and would be an interesting addition to this study (see Section 5).

d) Long-term exposure trials: Long-term exposures were included in this project to assess potential bioaccumulation of petroleum hydrocarbons leading to development of a taint in test fish. Two oils were tested at half the threshold level (0.25 ppm of hydrocarbons) for two periods (3 and 7 d). Three of the eight groups of exposed fish tested as tainted. It appears that this exposure level is just border-line for imparting a taint after prolonged exposure, and lower exposure levels would not taint the test fish over this period. Exposures at lower levels for longer periods may be a valid exercise. However, an accurate means of monitoring the exposure water would be required as well as a flow-through test system to improve water quality for the extended test periods.

4.2.3.4 Duration of exposure.

a) Threshold level trials: ECETOC (1987) endorse an exposure period of 24 h as recommended by GESAMP (1983). In a real-spill situation some organizms might be exposed to high concentrations for short periods, others to low concentrations for long periods. Lockhart[1] found Arctic char tainted after 3 h exposure to 3 ppm whole oil. In this study cod were tainted after 8 h exposure to about 2.5 ppm of hydrocarbons, after 24 h exposure to about 0.5 ppm, and after 7 d exposure to 0.25 ppm.

In the interest of reporting a threshold tainting concentration comparable to other studies, however, a test period of 24 h should be established as a standard exposure period.

b) Depuration trials: There was little difference in the results of taste panels conducted on fish exposed to 8 h versus 24 h. For high-level exposure an 8-h exposure period appears to be adequate. Trials conducted with lower exposure levels, however, would necessitate a longer test period to ensure that test fish were tainted before depuration.

The sampling periods for depuration were 0, 1, 4, 7, and 14 d. For short-term exposures of cod at relatively high exposure concentrations as seen in this study, the sampling period for depuration should be adjusted to 0, 8, 16, 24, and 48 h. Lower-level expo-

[1] D.W. Lockhart, Fisheries and Oceans, Winnipeg, Manitoba, personal communication, 1986.

sures for longer periods, however, may take longer to depurate. The sampling periods for depuration in this instance may have to extend into the 1- and 2-week period as originally intended for this study.

c) Long-term trials: Fish exposed to hydrocarbon levels of half the threshold concentration were found, in some instances, to be tainted after 3 d. If, however, exposure concentrations are even lower, a longer exposure period should be employed to investigate the possible bioaccumulation of petroleum hydrocarbons in the fish flesh.

4.2.4 Sensory Evaluations

The taste of fillets from exposed fish was evaluated in comparison with those from control fish. The triangle test was selected to determine the threshold level of taint as recommended by GESAMP (1983). The triangle test is an ideal sensory evaluation test for taint because it directly indicates the acceptance or non-acceptance of a tissue by the panelists, and, therefore can be easily used to determine the threshold level of taint.

The fish was cooked in a microwave oven. This method was chosen over the two other methods (boil-in-the-bag and casserole) proposed by GESAMP (1983) and we recommend it for any future studies. The microwave cooking procedure minimizes the loss of hydrocarbons from the fish as the cooking is conducted in a closed, glass, petrie dish. After cooking the samples were presented to the panelists without further handling. This method also minimized the loss of hydrocarbons to the atmosphere. The other two methods involve more handling which could potentially introduce more error in the results.

4.2.5 Hydrocarbon Analysis

4.2.5.1 Gas-liquid chromatography. The hydrocarbon analyses were executed on a DB-1 fused-silica capillary column, which has a chemically cross-linked and surface-bonded polymethyl siloxane liquid phase. Under the temperature program used the DB-1 column gave excellent separation of the petroleum hydrocarbons. We therefore recommend the use of a DB-1 column or a column of similar polarity for separation of hydrocarbons. The internal standard used, n-C_{21}, was found to be ideal for quantitation as it did not seriously interfere with the hydrocarbons in the WSF or in the tainted fillet samples.

4.2.5.2 Water-soluble fraction. The water-soluble fractions were extracted by the microextraction procedure of Murray and Lockhart (1981). We found this procedure a simple and reproducible technique for the extraction of hydrocarbons.

The recovery efficiency of this technique is comparatively better than other extraction techniques discussed in the literature (Tidmarsh et al., 1985). Murray and Lockhart (1981) have studied the recovery of a number of hydrocarbons and found them to be about 40%. The experimentally determined hydrocarbon ppm levels were corrected to 100% recovery, using this 40% recovery factor.

4.2.5.3 Fish fillets. Hydrocarbon analyses of the fish fillets was attempted by two techniques as outlined in Section 2.6. It was found that the technique of Murray and Lockhart (1981) was unsuitable for isolation of low levels of hydrocarbon from cod tissues. We therefore recommend the steam distillation method of Ackman and Noble (1972).

5.0 RECOMMENDATIONS

Further studies are recommended that would expand upon this study to provide a strong scientific basis for evaluating the possibility of a tainted seafood in the event of a spill or blowout associated with offshore oil and gas activities.

5.1 METHODOLOGY

The limitations of the methods used became evident during this study (as discussed in Section 4.4). Some adjustments were made to the methods during the study, however, further changes are recommended.

5.1.1 Identification and Recovery of Component Hydrocarbons in a Test Oil

The recovery efficiency of hydrocarbons from a WSF depends on the physio-chemical properties of the hydrocarbon and, therefore, recovery varies with the type of hydrocarbon. It is recommended that all the major and medium-sized components in the test oil be identified and that the percent recovery of each of these components be determined for the extraction procedure used. The identification could be carried out by comparing the retention behaviour on GC with authentic standards and by GC/MS. Sea-water would then be spiked with various levels of these standards and the percent recovery determined. These individual values could then be used to correct to 100% recovery for each of component hydrocarbons present in the experimental water and/or tissue sample. Identification of the component hydrocarbons in the water and tissue would give a better understanding of the component(s) responsible for taint.

5.1.2 Long-Term Exposure Test System

Ultimately, the type of test system chosen for an exposure will depend on the mixing capacity for WSF. In the short-term studies, in which a relatively high exposure level was employed, a static system was necessary to conserve the amount of WSF required. In the long-term studies, however, this type of system resulted in poor water quality. The water became very turbid which interfered with monitoring and, therefore, with maintaining the hydrocarbon levels particularly at the relatively low exposure levels of the long-term studies.

For any future long-term studies in which finfish are exposed to a hydrocarbon WSF it is recommended that the feasibility of employing a flow-through test system should be readdressed. A flow-through system would improve the water quality which would, in turn, render fluorometric monitoring more effective and would also be less stressful to the fish. Because the concentration of the stock WSF decreases over time, increasingly higher proportions of WSF to diluent water would be required each day. To provide enough WSF it may be necessary to have facilities for preparing several batches of WSF in series. Another possibility which could be examined is some form of continuous-flow mixing apparatus.

5.2 OILED SEDIMENTS AND TAINTING

As recommended by Tidmarsh et al. (1985) the possibility of a commercial species becoming tainted from contaminated sediment should be assessed.

The test oil would be mixed directly into the sediment and allowed to weather a pre-determined length of time before the test organism is introduced. Water samples should be analysed for the type and quantity of hydrocarbons present throughout the exposure period to describe accurately the exposure conditions. The test organism would be sacrificed, prepared for taste panel analysis, and a subsample of tissue held for hydrocarbon analysis if the product proved to be tainted.

This type of study could be easily run parallel to any other exposure studies using a WSF of the oil in question because the problems associated with preparing sufficient volumes of WSF would not be compounded. At the same time, the extensive background analyses required to identify the component hydrocarbons and respective percent recoveries of the test oil would be applicable to both types of exposure trials.

5.3 TEST ORGANISMS AND TYPE OF EXPOSURE

The threshold level of exposure necessary to cause a taint depends on the type of hydrocarbon contaminant and on the species exposed. In this study the threshold level (in a 24-h exposure), the depuration time (after 8 h and 24 h of exposure) and the long-term effects of a low-level exposure were examined for four different test oils but only for one test species.

It is recommended that several other commercial species be examined in this manner to assess more accurately the implications a major oil spill or blowout may have on the local fisheries. Cod is a relatively lean fish; we recommend that similar studies be done using another finfish representative of the more "fatty" species. A flatfish would seem to be a practical compromise for availability, lipid content, and size. Studies should also be done on either clams, mussels, or oysters in which the gut as well as the muscle tissue is consumed, on scallops in which only the adductor muscle is eaten and, on a representative, commercially fished crustacean, such as lobster or crab.

In this study the tainted cod were found to be depurated in less than 24 h for three of the four exposures. In the fourth exposure the tainted fish depurated between 1 and 4 d. ECETOC (1987) cite several authors who report rapid depuration times (from 12 to 48 h) from finfish tissue after exposure to various chemicals. Conversely, there have been several reports of tainted shellfish as a result of hydrocarbon spills from shipping incidence in which the shellfish were considered unfit for eating for several weeks to months after the incident (Blumer et al., 1970, 1971; Shenton, 1973; Mayo et al., 1974; Kerkoff, 1974; Grainger et al., 1980). Tainting studies involving shellfish should therefore include an additional type of trial in which the long-term low-level exposure is followed by a depuration phase.

5.4 AVOIDANCE REACTIONS OF FISH TO HYDROCARBON CONTAMINATION

In this study the fish exposed to the higher levels of hydrocarbons exhibited distinctly different behaviour patterns compared to the fish in the lower exposure levels and in the control tanks. In some instances it appeared that the fish would have come completely out of the tank, if possible, to avoid the exposure water. Thus, given the opportunity, the fish would probably not remain in waters with high levels of hydrocarbons long enough to become tainted. We recommend, in the light of these observations, that a behaviour study be implemented which would indicate at what level of exposure the fish detect the contaminant and move away from its source.

If the fish were found to exhibit an avoidance reaction to hydrocarbon contamination at levels that are known to produce a taint, the probability of the fish becoming

tainted under environmental conditions would be reduced and, as such, the information should be considered when assessing the risks of a tainted fishery (see Section 5.5).

5.5 HAZARD ASSESSMENT

ECETOC (1987) points out that to assess the likelihood that an oil that causes tainting under experimental conditions will cause tainting under real environmental conditions, the experimental and environmental conditions must be compared. A major factor in this type of assessment is predicting the nature and extent of hydrocarbons that would be in the sea after an accidental spill or blowout. We recommend that a literature search and/or a model be developed to address this issue and thus determine the probability of fish being exposed to hydrocarbon levels over the specific periods known to cause a taint in cod. This type of information would put the results of this study in perspective with regard to the concerns of a tainted fishery resource as a result of oil exploration and development in Canadian waters.

APPENDICES

APPENDIX A

RESOLUTION OF WSF MIXING RATIOS

The water-soluble fraction (WSF) refers to that fraction of oil which dissolves in water. Different laboratories have used a variety of methods to prepare WSFs, with ratios of oil to water ranging from 1:6 to 1:1000 (Murray et al. 1984). The wide range of oil to water ratios reported by others has produced a range of concentrations of hydrocarbons in the WSF. Murray et al. (1984) have reported a concentration range of 1.7 to 72.1 ppm hydrocarbon for many crude oils and petroleum products.

The concentration of hydrocarbons in the WSF depends on the:

- type of crude oil;
- ratio of oil and water;
- rate at which the oil and water are agitated or mixed;
- length of mixing; and,
- settling time required to achieve a stable distribution of hydrocarbons between the aqueous and oil phases.

Lockhart et al. (1984) examined in detail the above parameters and concluded that an oil to water ratio of about 1:100 produced a WSF saturated with hydrocarbons for many types of oils. Using high quantities of oil yields a WSF with a higher concentration of hydrocarbons, but the yields reported are low in comparison with the increased amount of oil used. Lockhart et al. (1984) have generally recommended a mixing time of 24 h with a setting time of 48 h to attain equilibrium or a stable distribution of hydrocarbons. The method of mixing seems to matter relatively little with any type of mechanical stirring or shaking producing a suitable WSF.

Based on Lockhart's results it was decided to use an oil to water ratio of 1:99 for preparing WSF from the four test oils. Before doing so, however, we investigated, on a laboratory scale, the suitability of adopting a 1:99 ratio by comparing it with a WSF prepared using a higher ratio of oil to water, namely 1:49.

The WSF was prepared in the laboratory scale by adding known amounts of oil and sea-water to a 6 L Erlermeyer flask and mixing with a magnetic stirring bar for 24 h in a 5°C cold room. The top of the flask was covered with aluminum foil and then sealed with parafilm. The whole mixture was transferred to a 6 L separatory funnel and allowed to settle for 48 h at 5°C. At the end of the settling period a 980 mL sample of the WSF (bottom layer) was withdrawn into a micro extraction flask and the hydrocarbons in the WSF were extracted and analysed by GC as described in Section 2.4.

The concentrations of hydrocarbons in the WSF prepared from the four test oils are given in Table A.1. These results confirmed those of Lockhart et al. (1984). Both the oil to water ratios of 1:49 and 1:99 essentially saturate the water although 1:49 gives a slightly higher level (except for Brent crude oil). Based on these results we decided to use an oil to water ratio of 1:99 for preparing all WSFs used in the tainting trials.

TABLE A.1

Hydrocarbon content of WSF
prepared on laboratory scale

Oil/water Crude oil	Hydrocarbon content (ppm) 1:99[a]	1:49[a]	1:99[b]	1:49[b]
Brent	5.8	11.9	14.6	12.3
Amalaugak	9.2	10.1	23.0	25.3
Hibernia	9.6	10.4	23.9	25.8
Conoco	1.6	2.0	4.0	4.9

[a] Uncorrected values.
[b] Corrected for the recovery efficiency of the microextraction technique.

APPENDIX B

RESOLUTION OF PROBLEMS IN AN EXPOSURE SYSTEM

B.1 GENERAL

A series of tests and trials were designed to determine the most suitable exposure system (see Section 1.2) which would meet the following criteria for:

a) maintaining suitable water quality, specifically oxygen and ammonia levels; and,

b) minimizing the hydrocarbon losses.

GESAMP 1985 recommends that dissolved oxygen levels in the exposure water remains above 60% saturation and that the concentration of the test material does not drop below 50% of the initial value. Castell[1] recommends that ammonia levels should ideally be less than 1 ppm but should not exceed 5 ppm.

Trials in which the water and air flow rates required to meet the first criteria were conducted at the Fisheries and Oceans Lower Water Street Laboratory in Halifax. Subsequent trials in which WSF losses were monitored were conducted at the Aquatron facilities of Dalhousie University.

B.2 WATER QUALITY IN EXPOSURE TANKS

B.2.1 General

A series of three trials were undertaken to determine the water and/or air flow that would be necessary to maintain the desired oxygen and ammonia levels in an exposure tank containing 4-5 kg of fish. Trial #1 was a static test, Trial #2 employed a controlled air flow and Trial #3 a controlled water flow. The dissolved oxygen and ammonia levels were monitored throughout these trials. The air or water flow rates were determined by the dissolved oxygen level in the water.

The oxygen levels were measured using a Fisher Digital Oxygen Meter. Water samples were analysed for ammonia nitrogen (NH_3-N) after "The Direct Nesslerization Method" (Standard Methods for the Examination of Water and Wastewater, 15 Edition, 1980, APHA).

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[1] R. Castell, Fisheries and Oceans, Halifax, Nova Scotia.

B.2.2 Static Tank Test

This short initial trial was run to determine the rate at which the fish used up the available oxygen in the water. Five fish were placed in an exposure tank with no additional air or water flow. The dissolved oxygen (DO) level was monitored every 15 minutes and ammonia samples taken hourly (see Table B.1).

TABLE B.1

Static tank test

Time elapsed (h)	Temperature (°C)	DO (ppm)	DO (% saturation)	NH_3-N (ppm)
0	5.5	10.2	101.0	0.1
.5	5.4	8.0	79.2	
.75	5.4	7.9	78.2	
1.0	5.4	7.3	72.3	0.15
1.25	5.3	6.7	66.3	
1.5	5.2	6.2	61.4	
1.75	5.2	5.8	57.4	
2.0	5.3	5.4	53.5	0.30
2.25	5.3	5.1	50.5	
2.5	5.5	4.2	41.6	
2.75	5.4	3.8	37.6	0.3
3.0	5.2	3.3	32.7	

The oxygen level of the water dropped to 60% saturation within the first hour and a half and the ammonia level rose to, and remained constant at, 0.3 ppm after 2 h. The trial was terminated at 3 h when the DO level dropped to just above 30% saturation.

B.2.3 Controlled Air Flow

The second trial was designed to determine the flow of air that would be required to maintain the test conditions at or above a DO level of 60% saturation. Air was bubbled into the tank and measured as mL of water displaced per minute. Table B.2 presents these results. A flow rate greater than 1,800 mL/min and less than 2,500 mL/min was necessary to maintain the oxygen level in the exposure tank at or above 60% saturation. Ammonia levels rose to a high of 1.15 ppm NH_3-N after 20 h.

TABLE B.2

Controlled air flow

Time elapsed (h)	Temperature (°C)	DO (ppm)	DO (% saturation)	NH_3-N (ppm)	Air flow (mL/min)
0	6.4	9.8	100	0.45	100
.5	6.7	7.5	76	---	100
1.0	6.6	6.1	62.9	0.35	50
1.0	6.6	6.1	62.9	---	750
1.5	6.5	5.5	56.1	0.5	700
2.0	6.4	5.0	51.0	---	700
2.0	6.4	5.0	51.0	0.5	2500
2.5	6.4	6.3	65.3	0.5	2400
3.0	6.6	6.7	68.4	---	2500
3.5	6.5	6.9	70.4	0.5	2500
21.0	6.6	5.8	59.2	1.15	1800
22.0	6.6	5.6	57.1	1.05	2500

B.2.4 Controlled Water Flow

Trial #3 was designed to determine the flow rate of aerated water required to maintain an oxygen level of 60% saturation for 4.5 kg of fish in the exposure tanks. A constant head tank was bubbled vigorously with compressed air and water flow from this tank was controlled by clamping the outflow tube. The dissolved oxygen level was monitored and the flow rate adjusted accordingly.

Ammonia samples were not taken during this trial. Levels were not expected to exceed those observed in the previous trial (Trial #2) when there was no water exchange.

Table B.3 presents the results of this trial. A flow of aerated water (to 100% saturation) of between 1.8 and 2.0 L/min was required to maintain the dissolved oxygen level in the exposure tank.

B.3 HYDROCARBON LOSSES IN EXPOSURE TANKS

B.3.1 General

In the range-finding trials, it was proposed that fish would be exposed to hydrocarbon concentrations of 10, 1.0 and 0.1 ppm for 24 h. A flow rate of 2 L/min would require

TABLE B.3

Controlled water flow

Time elapsed (h)	Temperature (°C)	DO (ppm)	DO (% saturation)	Flow rate (L/min)
0	5.9	7.07	71.2	1
.5	6.8	5.5	56.7	1
1.0	6.7	5.0	51.5	1
1.5	6.8	4.1	42.3	1
2.0	6.8	3.8	39.2	1
8.5	6.1	4.24	43.3	1.05
9.0	6.7	5.2	51.1	1.05
22	6.6	3.6	37.1	.925
46	6.5	2.8	28.6	.8
47	6.8	3.45	35.6	2.0
53.5	6.5	4.8	49	1.8
54.25	7.0	4.5	46.4	1.8
68	7.1	5.7	58.8	1.6
68.5	7.1	5.35	55.2	1.6
80	7.6	6.2	66.7	2.1
80.5	7.7	5.8	60.4	2.1

2,880 L of water per tank plus a proportional amount of WSF. Assuming an average WSF concentration of 35 ppm over 1,000 L of WSF would be required per trial assuming no losses. At this time the proposed mixing vessel for preparing the WSF was 380 L. Three batches of WSF would have had to be mixed, settled and stored - a total of 9 d preparation - for one trial.

It was, therefore, concluded that a flow-through system was impractical for the trials. A static system in which the oxygen level was maintained through bubbling and WSF was added, as required, to maintain the hydrocarbon levels was proposed. A series of trials were run to monitor the hydrocarbon losses due to aeration and uptake by the fish.

WSFs of Brent crude oil were prepared at CIFT, stored in 45 gallon plastic barrels and transported to the Dalhousie University Aquatron where the trials were conducted. Hydrocarbon losses were monitored by changes in the fluorescence using a Turner 10 Fluorometer fitted with a short wavelength oil kit and by GC analysis of water samples. Oxygen levels were monitored using a YSI Model 54 dissolved oxygen meter.

B.3.2 Static Test Trial

The first of these trials observed the hydrocarbon losses from a covered exposure tank over 24 h. One hundred percent (100%) WSF was put into an exposure tank, the plexiglass top fitted in place and changes in hydrocarbon content monitored with no air or water flow. Table B.4 presents these results. A 30% loss in fluorescence was observed, however, no losses were observed by GC analysis.

TABLE B.4

Hydrocarbon losses - static trial

Time (h)	Fluorescence	Temperature (°C)	GC (ppm)
0	26.1	11	20.05
1	25.9	11	
3	25.0	11	
8	20.9	11	20.95
19.5	19.6	10.5	
24	18.3	10.5	20.70

B.3.3 Hydrocarbon Losses - Controlled Air Flow

During the second trial hydrocarbon losses due to bubbling air through the WSF was monitored. An air flow of just above 2 L/min was adopted based on the results of previous trials (see B.2.2). A 43% loss in total hydrocarbon was observed by GC analysis over the 24 h (see Table B.5). A comparable loss in fluoroescence, however, is unavailable due to a failure in the fluorometer.

TABLE B.5

Hydrocarbon losses - controlled air flow

Time (h)	Fluorescence	Temperature (°C)	GC (ppm)	Air Flow (mL/min)
0	25.9	5	10.90	2,250
1	23.4	5		
8		6	10.30	
24		7	6.15	2,300

B.3.4 Hydrocarbon Losses - Fish Uptake

The next trial was designed to observe any additional losses that may be incurred by fish uptake. The test system was prepared as in the previous trial with an air flow of about 2 L/min and five fish (about 4.5 kg).

The fish showed immediate signs of stress upon being placed in the tank. They swam frantically for the first minute then began turning on their sides. An oxygen measurement was taken and was found to be only 4.0 ppm or 31% saturation (see Table B.6). This was due to the fact the exposure tank had been filled with 100% WSF which had been mixing and settling in closed containers for 3 d. (At this time the correction factor for the recovery for the GC analysis was not finalized and we were under the assumption that the hydrocarbon content of the WSF was considerably less.)

TABLE B.6

Hydrocarbon losses - fish uptake
(Air flow 2 L/min)

Time (h)	Fluorescence	Temperature (°C)	GC (ppm)	NH_3-N (ppm)	DO (ppm)	DO (% saturation)
0	10.7	5.0	14.17	0.08	4.0	31 *
1	8.5	5.0			9.2	72
2.5		5.0			9.3	73 **
3.5	5.1	5.0				
4	4.4	5.5			9.0	71
5		5.5			9.2	73
8	4.4	6.0	0.07		9.0	72
23.5	4.7				5.0	40 ***
24	4.6	7.0	0.05	2.15	8.6	70

* Fish were dying - air flow increased.
** One almost dead fish removed.
*** Air bar found disconnected; was reconnected.

During earlier trials, however, the oxygen level had, on occasion, dropped into the 30% saturation range and the fish did not show outward signs of stress. The air flow was immediately increased (>2 L/min) and the fish showed signs of recovery. One fish, however, died after 2.5 h of

exposure despite an increase in the oxygen level to 73% saturation.

The hydrocarbon losses were 99% as measured by GC analysis and 57% by fluorometry. These increased losses were thought to be caused by the substantial increase in air flow (the water was being vigorously bubbled) that appeared necessary to revive the fish.

B.3.5 WSF Flow Rate

The next trial was designed to determine the flow rate of WSF that would be necessary to maintain the hydrocarbon levels with this increased air flow.

An exposure tank was prepared, filled with WSF and the oxygen level brought up to 68% saturation before the fish were introduced. WSF was decanted into collapsible 20 L bags and then pumped out of the bag through a cooling coil and into the exposure tank. The flow rate was 0.5 L/min for the first 1.5 h and 0.8 L/min for the remaining 2 h (see Table B.7). A 48% loss in fluorescence was observed over this time period and an 81% loss in aromatic hydrocarbon. Aromatic hydrocarbon in this trial was measured by UV spectrophotometry at 254 nm and compared against a standard (Irving diesel) curve.

As in the previous trial, the fish showed signs of stress within the first 40 min and the air flow was increased. The air tubing became disconnected from the air bar on several occasions due to the increased air pressure and the activity of the fish. In order to reconnect the air bar the top had to be lifted. This could have contributed to the observed hydrocarbon losses.

B.3.6 Air Versus Oxygen

It became apparent that an air flow of 2-3 L/min was not adequate and that the increase in air flows contributed to an unacceptable loss of hydrocarbons. Table B.7 shows a continual loss in fluorescence of the exposure water despite a WSF flow rate of 0.8 L/min. WSF flow rates at or greater than 0.8 L/min would require in excess of 1,152 L/d for one exposure trial. The next trial, therefore, was designed to determine if hydrocarbon losses could be minimized by bubbling pure oxygen into the tank as compared to compressed air.

TABLE B.7

WSF flow rate

Time (h)	UV (ppm)	Fluoro-meter	Temp. (°C)	DO (ppm)	DO (% satu-ration)	WSF flow (L/ min)	Comments
0	3.3	17.4	5.0	8.7	68	0.5	Air bar off Air stone installed
.3		15.2				0.5	
.6		15.2	5.0	8.3	65	0.5	Fish stressed Increase air flow
1.0		14.2		7.6	59	0.5	Increase air flow
1.25		13.6	5.0	7.5	58	0.5	Fish still stressed Re-install air bar
1.4		13.3		8.5		0.5	
1.6			5.5	8.5	67		
1.8		11.5		8.5	64	0.8	
2.0							
2.2		10.4		8.6	68	0.8	WSF pump leak - flow off for 5 min
2.5		9.6		8.6	68	0.8	
2.9		9.2	6.0	8.7	69	0.8	
3.1							Air bar off
3.3							Air bar off
3.4		8.5	6.0	8.6	69	0.8	
3.6	0.6	9.05	6.0	8.2	65	0.8	Air bar off

Two exposure tanks were prepared and aerated to 90% saturation with air in one tank and oxygen in the other before the fish were introduced. In both tanks, however, the fish showed immediate signs of stress and were removed after 1.5 hours. The fish were placed in the satellite tank where they recovered.

It was now becoming clear that it was the WSF concentration levels, and not the oxygen levels that were stressing the fish and that by increasing the air flow as in earlier trials, the hydrocarbons were being driven off, lowering the concentrations to nontoxic levels, and the fish were then showing signs of recovery.

The trial was continued, however, without fish in the tank for 21.5 h. The air and oxygen flow rates were left at their original settings and changes in fluorescence and hydrocarbon monitored. Table B.8 presents the results of this trial. A 39% loss of fluorescence and a 98% loss of total hydrocarbons was observed in the tank bubbled with compressed air compared to a 17% loss in fluorescence and a 7% loss of hydrocarbons (GC analysis) in the tank bubbled with oxygen.

At this point the correction factor for the recovery of hydrocarbon in the GC analysis was finalized. Exposure concentrations were double what was initially expected which confirmed the conclusions that fish were being exposed to toxic concentrations of WSF.

B.4 SUMMARY

This series of test trials were designed to determine the final configuration of the exposure system. A flow-through/dilution system was eliminated because the volume of WSF that would be required exceeded our mixing capacity (see B.3.1). Similarly, a semi-static system using compressed air to maintain adequate oxygen levels would exceed the WSF mixing capacity (see B.3.6). The test system configuration which minimized the WSF losses while maintaining adequate oxygen levels in the exposure water was that which used pure oxygen.

An 1,100 L mixing tank was obtained and installed at the Dalhousie facility adjacent to the exposure tanks. For future trials WSF was prepared at Dalhousie, stored in the mixing tank and pumped directly out of the mixing tank and into the exposure tanks thus eliminating losses due to handling and transportation.

TABLE B.8

Air versus oxygen

	Air					O_2				
Time (h)	Temp. (°C)	DO (ppm)	DO (% saturation)	Fluorescence	GC (ppm)	Temp. (°C)	DO (ppm	DO (% saturation)	Fluorescence	GC (ppm)
0	6	10	102	15.3	22.18	6	12	122	16.8	28.37
0.25	6	10.5	107			6	13.4	137		
.50		8.75	89	13.8			13	133	16.3	
.75							12.4	126		
1.0		8.2	84	13.6			13.5	138	16.6	
1.25 *							13.4	137		
1.5							12.5	128	16.1	
2.0		9.4	96	12			11.2	114	16.1	
3.0		10	102	11.9			12.4	126	15.8	
4.0							12.4	126	15.6	
9.5	6.5	12	124	13.0		6.5	18.2	187	11.4	
21.5	7	12	125	9.3	0.45	7.0	17.5	182	13.9	25.74

* All fish removed.

The final test system configuration was a semi-static system in which the oxygen level was maintained by bubbling pure oxygen into the system and the hydrocarbon levels were maintained by pumping "pure" WSF into the system. Both the oxygen and the WSF flow rates would be controlled by the conditions in the individual exposure tank. The DO levels would be monitored hourly and the oxygen flow rate adjusted to maintain a level of between 60 and 90% saturation. The exposure water would be spiked with proportional amounts of WSF to obtain the desired exposure levels just prior to introducing the fish. The fluorescence of the exposure water would be recorded and then monitored hourly. Additional WSF would be pumped into the system, as required, to maintain this initial fluorescence.

APPENDIX C

FLUOROMETER CALIBRATION

C.1 GENERAL

A Turner Designs Model 10 flow-through fluorometer was used to monitor the hydrocarbon levels in the exposure water. The fluorometer was fitted with a short wavelength oil accessory kit.

Fluorometric analysis is based on the quantitation of the ability of fluorescent materials to absorb light at one wavelength and convert it into light at a longer wavelength. Fluorometric analysis of hydrocarbons accentuate the polynuclear portion of the oil in question. A correlation between fluorescence and GC analysis is expected to be rough especially between different oils and between weathered versus unweathered oil (B. Phillips[1]).

The sensitivity of the fluorometer is affected by the light which reaches the light detector that is not related to the material being analysed for, and which may vary from sample to sample (Turner Designs Ltd., 1976). The primary sources of such unwanted light are:

- interference from fluorescent materials other than the one being analysed for, and
- interference from light scattered by particulate material in the sample (turbidity).

Both these sources of interference became apparent during this project. The fluorescence of exposure water in control tanks increased as soon as fish were introduced to the tank. When there were faeces and/or semi-digested food present in the tanks there would be a marked increase in the fluorescence of the water. If the water was visibly very turbid the fluorescence would fluctuate randomly.

The response of the fluorometer to varying concentrations of oil was examined under five conditions. The first three responses were to one type of oil with no fish present. The last two calibrations examine the fluorometric response to varying concentrations of different oils, over time and with fish present in the water.

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[1] B. Phillips, Turner Designs Ltd., La Jolla, California.

C.2 LINEAR RESPONSE OF THE FLUOROMETER

The response of the fluorometer was checked for linearity by measuring the fluorescence of a serial dilution of a water-soluble fraction (WSF) of Brent crude oil. Figure C.1 presents the results of this calibration. The top graph is a plot of all samples read on the X10 scale of the fluorometer while the lower one is of samples read on the more sensitive (X31.6) scale. The X31.6 scale was used to measure the fluorescence of the exposure water throughout the trials. Figure C.1 shows a linear relationship between the fluorescence and the percent WSF of the sample ($r = 0.99$ and 0.98, respectively).

C.3 FLUOROMETER CALIBRATION WITH GC ANALYSIS

The fluorometer was then calibrated against gas chromotography (GC) analysis of samples of a WSF of Brent crude oil. Figure C.2 shows the results of this calibration. The fluorescence was read on the X3.16 scale and each point represents an average of four fluorescence readings of the sample which was analysed by GC. Only three points are available. These points, however, confirm the linear response of the fluorometer particularly to WSFs as high as 20 ppm.

This calibration, however, is only representative of one type of WSF with no weathering or other tank effects. A calibration curve derived from actual exposure water samples was felt to be more indicative of the types and concentrations of hydrocarbon that would be encountered throughout an exposure trial.

Water samples were analysed for hydrocarbon content by GC at the beginning and end of each trial (see Appendix D). A comparable fluorescence was also recorded. Linear regressions were run on these pairs and the resulting equations used to transform all fluorescent readings to ppm hydrocarbon.

The fluorescence of water held in an exposure tank with no fish present remained constant at 0 over a 24 h period. The fluorescence of water in which fish are present, however, rose within the first hour by approximately 0.2 and continued to rise to between 0.75 and 1.25 after 24 h. This background increase in fluorescence appeared to be caused by excretory products of the fish. Faeces and sometimes semi-digested food were observed in the tanks.

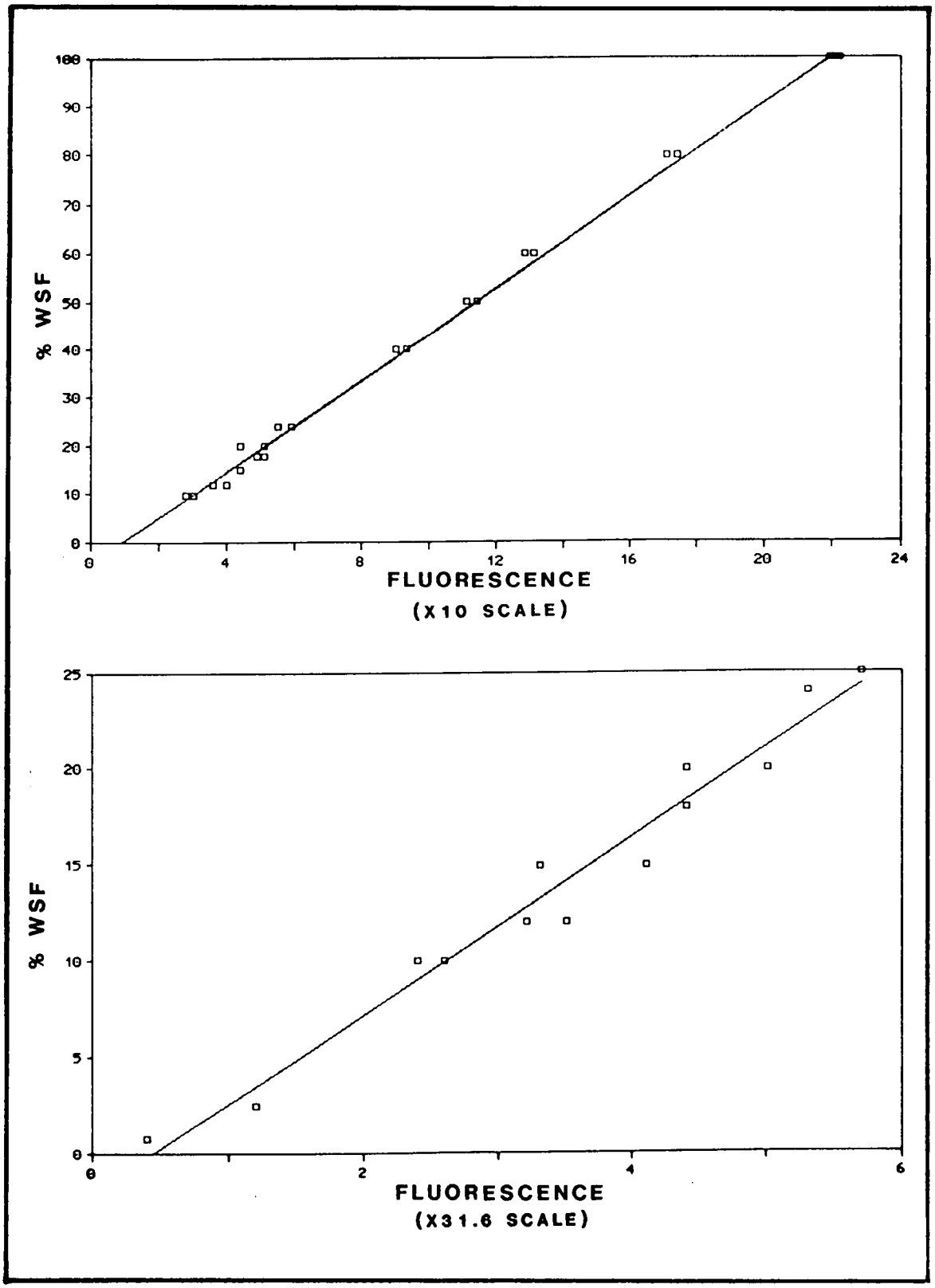

FIGURE C.1 LINEAR RESPONSE OF THE FLUOROMETER.

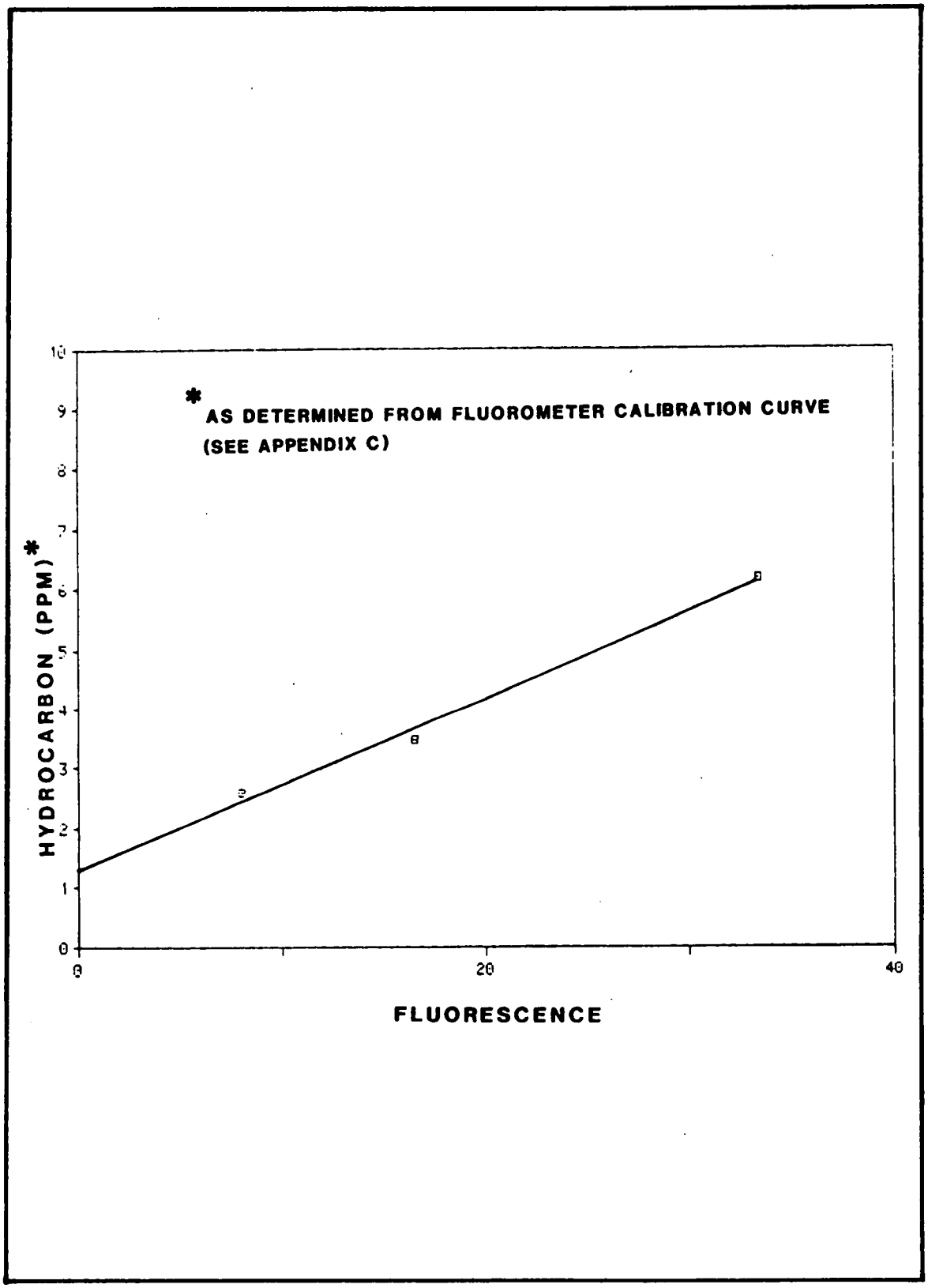

FIGURE C.2 FLUOROMETER CALIBRATION - WSF OF BRENT CRUDE OIL.

This variation in background fluorescence was often greater than the differences expected in the fluorescence between exposure concentrations. Two calibration curves were therefore prepared: one prepared from, and to be used for, time 0 fluorescences; and, one for all times greater than 0 and up to 24 h.

Figure C.3 shows the linear regression of these points. The top graph is a plot of all readings taken at Time = 0 and is described by the equation Y = .7105 X -.0572 (r = 0.93 and n = 26). This equation has been used to transform all Time = 0 fluorescent readings taken during the trials to ppm hydrocarbon.

The lower graph is a plot of all readings taken between 1 and 24 h of exposure and is described by the equation Y = 0.8512 X -.400 (r = .84 and n = 17). The regression shows more variation than that for time 0 due to the range of background fluorescence observed over time and between tanks. This equation has been used to transform all fluorescent readings taken between 1 and 24 h to ppm hydrocarbon.

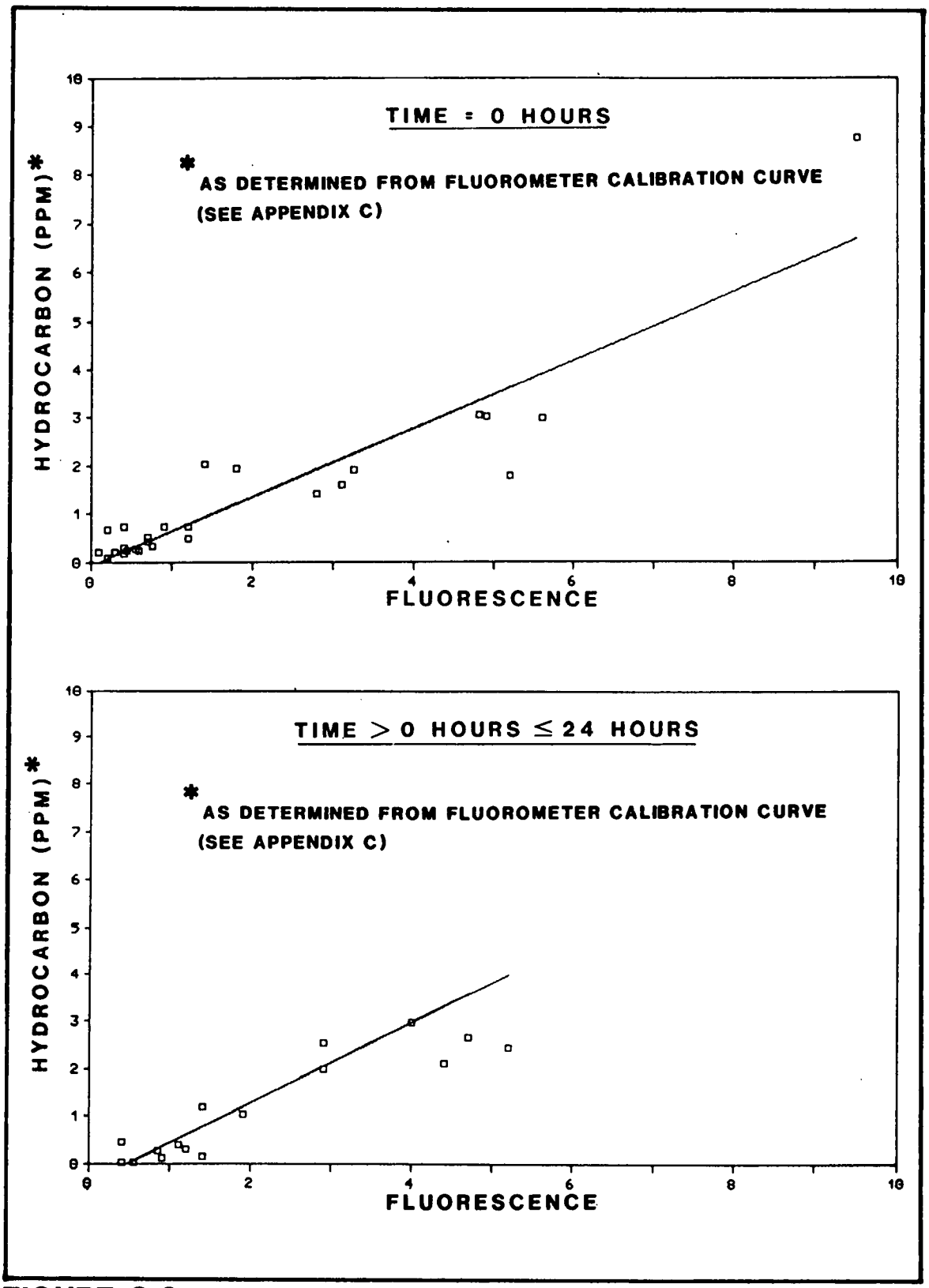

FIGURE C.3 FLUOROMETER CALIBRATION - WSF OF ALL OILS.

APPENDIX D

TABLES OF EXPOSURE CONDITIONS

TABLE D.1
Brent short-term range-finding trial

Time (h)	TANK 1				TANK 2				TANK 3				TANK 4			TANK 5			TANK 6		
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence
0	2.0	75	0.40	0.23	2.0	88	1.40	0.94	2.0	83	9.5	6.69	1.5	96		1.5	93		3.5	96	
1	2.0	76	0.30	-0.14	3.0	85	1.40	0.79	2.5	83	5.1	3.94	1.5	77	0.2	1.5	79	0.2	3.5	84	0.10
2	3.0	68	0.40	-0.06	3.0	66	1.40	0.79	3.0	69	3.8	2.83									
3	2.5	66	0.60	0.11	2.0	59	1.35	0.75	3.0	71	5.1	3.94	2.0	109	0.6	2.0	88	0.6	2.5	94	
4	2.0	82	0.75	0.24	2.0	77	1.30	0.71	3.0	80	5.2	4.03	2.0	93	0.8	2.0	76	0.7	3.0	84	0.70
5	2.0	82	0.75	0.24	3.0	124	1.30	0.71	3.0	80	5.2	4.03	2.0	87		2.0	76	0.7	3.0	84	0.70
6	3.0	133	0.70	0.20	2.5	115	1.10	0.54	3.0	106	1.3	0.71	2.5	77		2.5	64		3.0	66	
7													2.5	52		2.0	91		2.5	67	
8	3.5	129	0.55	0.07	3.0	96	1.01	0.46	3.0	93	3.5	2.58	3.0	78		3.0	118		3.0	93	
9																					
10	2.5	98	0.40	-0.06	3.0	71	1.00	0.45	3.0	67	4.4	3.35	3.0	66		3.0	119		3.5	85	
11	3.5	90	0.20	-0.23	3.5	65	0.90	0.37	3.0	107	2.5	1.73									
12	3.0	103			3.5	65			3.0	97			3.5	49		3.0	93		3.5	68	
13	3.0	97			3.5	72			3.0	93			3.5	137		3.5	94		4.0	70	
14	3.0	93			3.5	65			3.0	88			4.0	117		3.5	77		5.0	69	
15	3.0	86			3.5	77			3.0	80			4.0	109		4.0	72		5.0	81	
16	3.0	75			3.5	81			3.0	72			4.0	101		4.0	66		5.5	84	
17	3.0	71			3.5	83			3.0	76			4.0	80		4.0	73		6.0	90	
18	3.0	66			3.5	83			3.0	76			4.0	78		4.0	83		6.0	87	
19	3.0	95			4.0	83			3.5	79			4.0	70		4.0	83		6.0	78	
20	4.0	93			4.5	82			4.0	91			4.0	82		4.0	89		6.0	67	
21	4.5	73			5.0	85							4.0	89		4.0	84		6.0	74	
22	5.0	69			5.0	84							5.0	79		5.0	81		6.0	85	
23													5.0	59		5.0	59		6.0	86	
24	4.5	86	0.55	-0.16	5.0	89	1.20	1.17													1.25
									At 0.5 h fish were dying; sea-water added to reduce fluorescence to half. At 4.0 h fish again stressed and more sea-water added. Pieces of "food" found in the tank.				Tank has small leak; sea-water was added at 14 h. Pieces of "food" were found in the tank.			Pieces of "food" were found in the tank.			Pieces of "food" were found in the tank.		

TABLE D.2

Brent short-term trial

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	6.00	105	0.7	0.44	6.00	92	1.20	0.80	6.0	106	5.60	3.92
1	6.25	96	0.9	0.37	6.00	98	1.45	0.83	6.0	100	5.05	3.90
2	6.50	87	1.1	0.54	6.50	87	1.50	0.88	6.5	87	5.00	3.86
3	6.50	85	1.1	0.54	7.00	85	1.60	0.96	6.5	82	5.10	3.94
4	7.00	74	0.9	0.37	6.75	72	1.40	0.79	7.0	71	4.70	3.60
5	7.50	78	1.0	0.45	7.00	77	1.40	0.79	7.0	75	4.80	3.69
6	7.50	76	1.0	0.45	7.50	78	1.30	0.71	7.0	77	4.50	3.43
7	7.50	74	0.9	0.37	7.00	73	1.30	0.71	7.5	79	4.30	3.26
8	7.50	82	0.9	0.37	7.50	82	1.30	0.71	8.0	87	5.50	4.28
9	7.50	84	0.8	0.28	7.50	76	1.25	0.66	8.0	85	5.20	4.03
10	8.00	87	0.8	0.28	7.00	73	1.30	0.71	8.0	78	4.60	3.52
11	8.00	75	0.9	0.37	8.00	73	1.40	0.79	7.5	86	5.20	4.03
12	7.50	84	0.9	0.37	7.50	74	1.30	0.71	6.5	103	0.90	0.37
13	7.50	86	0.9	0.37	7.50	93	1.30	0.71	6.0	114	0.70	0.20
14	8.00	80	0.8	0.28	8.00	85	1.40	0.79	6.5	93	4.50	3.43
15	8.50	73	0.9	0.37	8.50	70	1.50	0.88	7.0	88	5.20	4.03
16	8.50	70	0.9	0.37	8.50	68	1.50	0.88	7.0	86	5.20	4.03
17	8.50	75	0.9	0.37	8.50	100	1.60	0.96				
18	8.50	105	0.9	0.37	8.50	94	1.70	1.05				
19	8.50	102	0.9	0.37	8.50	84	1.70	1.05				
20	8.50	87	0.9	0.37	8.50	70	1.70	1.05				
21	8.50	81	0.9	0.37	8.50	82	1.80	1.13				
22	9.00	65	0.9	0.37	9.00	71	1.90	1.22				
23	9.00	77	0.9	0.37	9.00	76	1.90	1.22				
24	9.00	85	0.9	0.37	9.00	87	1.90	1.22				
									Fish stressed at 11 h. Tank was flushed with clear sea-water. WSF added at 14 h and one fish died. The two other fish died after 16 h.			

TABLE D.3
Amauligak short-term trial

Time (h)	TANK 1				TANK 2				TANK 3				TANK 4			TANK 5			TANK 6		
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence
0	1.0	102	0.55	0.33	1.50	97	1.20	0.80	2.00	98	5.20	3.64	1.0	109	0.40	2.25	112	0.00	2.25	112	0.00
1	1.0	88	0.70	0.20	1.50	86	1.30	0.67	2.00	87	3.95	2.96	2.0	82		3.00	77		2.00	82	
2	2.0	83	1.10	0.54	2.00	82	1.10	0.50	2.50	80	7.10	5.64	2.0	79	0.40	3.00	67	0.50	3.00	75	0.35
3	1.5	55	0.70	0.20	2.00	58	1.35	0.71	2.00	63	6.40	5.05	2.0	65		3.00	71		2.00	69	
4	2.0	62	0.55	0.07	2.00	70	1.00	0.42	2.50	68	5.35	4.15	2.0	72	0.40	3.00	71	0.75	2.50	70	0.85
5	2.0	67	0.50	0.03	2.00	81	1.10	0.50	2.50	80	4.60	3.52	2.0	76		3.00	93		2.50	87	
6	2.0	69	0.55	0.07	2.00	79	1.10	0.50	2.50	85	5.40	4.20	2.5	81		2.50	73		2.50	85	
7	2.5	60	0.50	0.03	2.00	78	1.20	0.59	2.50	86	5.00	3.86	2.5	86		2.50	64		2.50	83	
8	2.5	61	0.50	0.03	2.50	76	1.00	0.42	3.00	67	4.40	3.35	3.0	78		3.00	65		2.50	70	
9	2.5	67	0.70	0.20	3.00	65	1.00	0.42	3.00	67	4.00	3.00	3.0	71		3.50	68		3.00	71	
10	2.5	70	3.30	2.41	3.00	79	1.00	0.42	3.50	81	3.80	2.83	3.0	74		4.00	78		3.00	86	
11	2.5	91			3.00	97	1.00	0.42	3.50	77	5.10	3.94	3.0	88		4.00	87		3.00	90	
12	3.0	95	0.60	0.11	3.00	133	0.90	0.34	4.00	67	3.80	2.83	3.5	93		4.50	83		4.00	85	
13	3.0	91	0.60	0.11	3.00	114	1.00	0.42	4.00	63	4.20	3.18	3.5	86		4.50	79		4.00	81	
14	3.0	82	0.60	0.11	3.00	97	0.90	0.34	4.00	58	4.60	3.52	3.5	78		5.00	73		4.00	72	
15	3.0	74	0.60	0.11	3.00	95	0.80	0.26	4.00	99	4.00	3.00	3.5	72		5.00	67	0.60	4.00	63	
16	3.0	56	0.40	-0.06	3.00	97	0.70	0.18	4.00	95	4.20	3.18	3.5	68	0.10	5.00	71		4.50	88	0.1
17	3.0	74	0.50	0.03	3.00	91	0.80	0.26	4.00	84	5.10	3.94	3.5	76		5.00	82		4.50	85	
18	3.0	76	0.50	0.03	3.00	87	0.90	0.34	4.00	74	4.80	3.69	3.5	83		5.00	84		4.50	78	
19	3.0	93	0.50	0.03	3.00	74	1.00	0.42	4.00	64	4.50	3.43	3.5	90		5.00	100		4.00	73	
20	3.5	79	0.60	0.11	4.00	69	1.00	0.42	4.00	113	4.10	3.09	3.5	87		5.00	89		4.00	68	
21	3.5	72	0.60	0.11	3.50	94	0.90	0.34	4.00	99	4.70	3.60	4.0	82		5.00	84		4.50	70	
22																					
23																					
24	4.0	72	0.40	-0.06	4.25	80	0.85	0.30	4.75	89	4.70	3.60	4.5	88	0.25	6.00	71	1.30	5.00	83	0.25
	High turbidity in the tank at 10 h caused high reading. Tank was flushed and WSF added to bring the fluorescence up.								Fish generally more active and stay near the surface compared to the other tanks. Some parasites have fallen off. Faeces in the tank.												

TABLE D.4

Hibernia short-term trial

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	2.5	98	0.30	0.16	2.50	98	0.70	0.44	2.5	96	2.80	1.93
1	3.0	86	0.70	0.20	3.00	85	1.00	0.45	3.0	84	3.40	2.49
2	3.0	78	0.70	0.20	3.00	82	1.10	0.54	3.0	82	2.95	2.11
3	3.0	74	0.65	0.15	3.00	78	0.95	0.41	3.0	76	2.65	1.86
4	2.5	65	0.75	0.24	2.75	68	0.95	0.41	3.0	69	2.90	2.07
5	3.0	71	0.75	0.24	3.00	65	0.90	0.37	3.0	72	2.80	1.98
6	3.5	115	0.80	0.28	3.50	72	1.00	0.45	3.5	119	2.30	1.56
7	3.5	116	0.90	0.37	3.50	75	0.90	0.37	3.5	118	2.40	1.64
8	4.0	117	0.80	0.28	3.50	75	1.00	0.45	4.0	117	3.40	2.49
9	4.0	117	0.80	0.28	4.00	85	0.90	0.37	4.0	115	3.40	2.49
10	4.0	105	0.80	0.28	4.50	72	1.00	0.45	4.0	112	2.80	1.98
11	4.0	98	0.90	0.37	4.50	90	0.80	0.28	4.0	89	2.70	1.90
12	4.0	95	0.80	0.28	4.00	70	0.90	0.37	4.0	87	2.90	2.07
13	4.0	90	0.70	0.20	4.00	67	0.90	0.37	4.5	78	2.70	1.90
14	4.5	83	0.70	0.20	4.50	66	0.90	0.37	4.5	74	2.80	1.98
15	5.0	76	0.60	0.11	5.00	101	0.80	0.28	5.0	74	3.00	2.15
16	4.5	64	0.60	0.11	4.50	88	0.90	0.37	5.0	64	3.00	2.15
17	4.5	82	0.60	0.11	4.50	83	0.80	0.28	5.0	66	2.60	1.81
18	5.0	69	0.60	0.11	5.00	74	0.80	0.28	5.0	91	2.90	2.07
19	5.0	64	0.60	0.11	5.00	69	0.80	0.28	5.5	85	2.90	2.07
20	5.0	74	0.70	0.20	5.00	83	0.80	0.28	5.5	83	2.90	2.07
21	5.5	113	0.70	0.20	5.50	80	0.80	0.28	6.0	73	2.50	1.73
22	5.5	106			5.50	73			6.0	90		
23	5.5	109	0.80	0.28	5.50	84	0.95	0.41	6.0	98	2.95	2.11
24	6.0	82	0.90	0.37	6.00	70	1.10	0.54	6.5	91	2.95	2.11

TABLE D.5

Conoco short-term trial

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	3.75	95	0.20	0.08	4.00	92	0.40	0.23	7.25	82	1.80	1.22
0.5	4.50	102	0.40	-0.06	4.25	101	0.70	0.20	7.00	108	1.75	1.09
1	3.75	91	0.45	-0.02	4.50	88	0.95	0.41	6.50	91	1.60	0.96
2	3.00	79	0.55	0.07	3.00	76	1.10	0.54	6.50	70	1.45	0.83
3	3.50	77	0.55	0.07	3.50	92	1.25	0.66	7.00	85	1.30	0.71
4	3.50	81	0.70	0.20	3.50	82	1.50	0.88	7.00	89	1.50	0.88
5	3.50	78	0.65	0.15	3.50	72	1.40	0.79	7.50	82	1.45	0.83
6	4.50	82	0.50	0.03	4.00	89	1.10	0.54	7.50	73	1.50	0.88
7	4.50	78	0.55	0.07	4.50	81	1.20	0.62	8.00	84	1.55	0.92
8	5.00	91	0.50	0.03	4.00	69	1.40	0.79	8.50	73	1.30	0.71
9	5.00	89	0.50	0.03	4.50	82	1.30	0.71	8.00	76	1.20	0.62
10	4.50	82	0.70	0.20	4.00	68	1.30	0.71	8.00	108	1.20	0.62
11												
12	5.00	72	0.80	0.28	4.50	74	1.25	0.66	8.00	99	1.25	0.66
13	5.50	64	0.70	0.20	4.50	80	1.10	0.54	8.50	88	1.10	0.54
14	5.00	91	0.50	0.03	4.00	109	1.40	0.79	8.50	73	1.10	0.54
15	5.50	85	0.50	0.03	5.00	89	1.30	0.71	8.50	102	1.30	0.71
16	6.00	91	0.40	-0.06	5.00	80	1.30	0.71	8.50	86	1.30	0.71
17	6.00	93	0.40	-0.06	5.50	78	1.20	0.62	8.50	82	1.40	0.79
18	6.00	80	0.40	-0.06	6.00	66	1.30	0.71	8.50	70	1.40	0.79
19	6.50	71	0.40	-0.06	6.00	92	1.40	0.79	8.50	79	1.40	0.79
20	6.50	77	0.40	-0.06	6.00	86	1.30	0.71	8.50	69	1.20	0.62
21	6.50	86	0.40	-0.06	6.00	76	1.40	0.79	8.50	86	1.40	0.79
22	6.00	72	0.50	0.03	6.00	70	1.30	0.71	8.50	83	1.50	0.88
23	6.50	83	0.40	-0.06	6.00	74	1.30	0.71	8.50	74	1.50	0.88
24	6.50	75	0.40	-0.07	6.00	87	1.40	0.79	8.50	84	1.40	0.79
	Exposure water was diluted with sea-water at 5 h. At 10 h WSF was added to check response of fluorometer.				Exposure water was diluted with sea-water at 5 h.				Fish look uncomfortable at 10 h.			

TABLE D.6

Amauligak depuration (8-h exposure)

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	5.0	97	4.80	3.35	4.50	101	4.70	3.28	5.0	99	5.20	3.64
1	5.0	71	4.25	3.22	5.00	78	4.25	3.22	4.5	75	4.40	3.35
2	5.0	62	3.80	2.83	5.00	65	4.20	3.18	5.0	60	4.65	3.56
3	5.5	87	4.10	3.09	5.00	85	3.75	2.79	5.0	89	4.05	3.05
4	5.5	101	3.70	2.75	5.25	96	4.20	3.18	5.0	79	3.85	2.88
5	5.5	80	5.00	3.86	5.00	81	3.90	2.92	5.0	59	3.80	2.83
6	5.5	64	4.50	3.43	5.00	60	4.80	3.69	5.0	107	3.70	2.75
7	6.0	69	3.90	2.92	5.50	71	4.30	3.26	5.0	115	3.70	2.75
8	6.0	69	4.30	3.26	6.00	76	3.70	2.75	5.5	102	4.00	3.00

TABLE D.7

Amauligak depuration (24-h exposure)

Time (h)	TANK 1				TANK 2				TANK 3				TANK 4			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	2.00	87	5.00	3.50	2.00	89	5.20	3.64	2.00	87	5.25	3.67	2.50	92	4.40	3.07
1	2.50	94	4.75	3.64	2.50	97	4.80	3.69	2.00	92	4.70	3.60	2.75	95	4.75	3.64
2	2.75	74	4.60	3.52	3.00	71	4.30	3.26	2.50	73	4.35	3.30	3.00	74	4.15	3.13
3	3.00	80	4.15	3.13	2.50	73	4.25	3.22	2.50	71	3.95	2.96	3.00	63	4.15	3.13
4	3.00	88	3.70	2.75	2.50	68	3.80	2.83	2.50	75	4.30	3.26	3.00	61	3.80	2.83
5	3.00	84	3.50	2.58	3.00	71	3.25	2.37	2.75	78	3.60	2.66	3.50	58	3.25	2.37
6	3.00	80	3.40	2.49	3.00	72	3.00	2.15	3.00	71	3.20	2.32	4.00	105	5.00	3.86
7	3.00	103	2.80	1.98	3.50	77	3.40	2.49	3.00	72	2.80	1.98	3.50	98	4.00	3.00
8	3.00	96	4.10	3.09	3.00	76	3.00	2.15	3.00	69	2.60	1.81	3.50	96	3.60	2.66
9	3.50	88	3.60	2.66	3.50	73	3.30	2.41	3.50	66	4.60	3.52	4.50	83	3.40	2.49
10	3.00	75	3.20	2.32	3.50	69	4.50	3.43	3.50	77	3.70	2.75	4.00	66	3.70	2.75
11	3.50	68	3.40	2.49	3.50	78	4.40	3.35	3.50	72	3.60	2.66	4.50	82	3.60	2.66
12	4.00	63	4.00	3.00	3.50	80	4.30	3.26	4.00	76	3.60	2.66	4.00	79	3.20	2.32
13	4.00	60	3.50	2.58	4.00	81	3.90	2.92	3.50	72	3.60	2.66	5.00	85	4.30	3.26
14	4.00	148	3.30	2.41	4.00	69	3.50	2.58	4.00	69	3.00	2.15	5.00	74	3.30	2.41
15	4.00	118	3.90	2.92	4.50	59	3.30	2.41	4.00	54	4.30	3.26	5.00	67	3.00	2.15
16	4.00	117	3.70	2.75	4.50	66	3.80	2.83	4.00	68	3.90	2.92	5.00	69	4.50	3.43
17	4.50	108	3.30	2.41	5.00	84	3.30	2.41	4.50	79	3.40	2.49	5.00	67	3.70	2.75
18	4.50	100	3.30	2.41	5.00	84	3.90	2.92	5.00	86	3.20	2.32	5.00	90	3.30	2.41
19	5.00	91	3.90	2.92	5.00	72	3.70	2.75	5.00	74	4.50	3.43	5.50	83	3.10	2.24
20	5.00	79	7.40	5.90	5.50	85	3.70	2.75	5.00	67	3.60	2.66	5.50	73	4.30	3.26
21	5.00	72	6.30	4.96	5.50	86	3.50	2.58	5.00	76	3.80	2.83	6.00	85	3.90	2.92
22	5.00	85	5.20	4.03	5.50	80	3.30	2.41	5.00	84	3.50	2.58	5.50	84	3.70	2.75
23	5.50	77	6.10	4.79	5.50	70	4.45	3.39	5.50	75	3.80	2.83	5.50	78	3.00	2.15
24			5.60	4.37			4.00	3.00			3.60	2.66			3.70	2.75
	Water turbid causing high reading at 20 h. Tank flushed. Fish remain near the surface. Food in the tanks as well as parasites.				Water turbid at 23 h.				Parasites in the tank.				One of the fish appeared stressed right from the beginning.			

TABLE D.8

Hibernia depuration (8-h exposure)

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	3.50	87	3.30	2.29	3.0	95	3.30	2.29	3.00	96	2.80	1.93
1	4.00	82	3.15	2.28	3.0	82	2.40	1.64	3.50	79	2.40	1.64
2	4.00	70	2.70	1.90	4.0	68	2.65	1.86	4.00	67	2.65	1.86
3	4.00	68	2.30	1.56	4.0	66	2.50	1.73	4.00	62	2.15	1.43
4	4.00	146	2.10	1.39	4.5	74	2.70	1.90	4.00	78	2.40	1.64
5	4.50	118	2.00	1.30	4.5	77	2.45	1.69	4.50	75	2.35	1.60
6	4.75	118	2.35	1.60	4.5	71	2.35	1.60	4.75	75	2.45	1.69
7	5.00	99	2.15	1.43	4.5	59	2.30	1.56	4.50	67	2.10	1.39
8	5.50	94	2.30	1.56	5.0	73	3.65	2.71	5.00	99	2.65	1.86

TABLE D.9

Hibernia depuration (24-h exposure)

Time (h)	TANK 1				TANK 2				TANK 3			
	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)	Temp. (°C)	DO (%)	Fluor-escence	Hydro-carbon (ppm)
0	2.50	86	3.25	2.25	2.00	93	3.25	2.25	2.50	94	3.25	2.25
1	2.50	83	3.40	2.49	2.00	86	3.50	2.58	2.00	89	3.50	2.58
2	2.50	70	2.75	1.94	2.00	65	2.90	2.07	2.25	63	2.70	1.90
3	3.00	69	2.60	1.81	2.75	60	2.65	1.86	2.75	95	3.00	2.15
4	3.00	71	2.30	1.56	2.25	67	3.55	2.62	3.25	92	2.65	1.86
5	3.00	103	2.90	2.07	3.00	99	3.10	2.24	3.50	96	2.60	1.81
6	3.50	92	2.60	1.81	3.50	81	2.80	1.98	3.50	92	2.60	1.81
7	3.50	85	2.40	1.64	3.50	75	2.60	1.81	3.50	83	2.50	1.73
8	4.00	80	2.80	1.98	3.50	65	2.50	1.73	4.00	60	2.60	1.81
9	4.00	68	2.50	1.73	4.00	77	2.50	1.73	4.00	60	2.40	1.64
10	4.00	66	2.70	1.90	4.00	78	2.70	1.90	4.00	72	2.80	1.98
11	4.00	60	2.60	1.81	4.00	68	2.60	1.81	4.00	56	2.20	1.47
12	4.50	55	2.00	1.30	4.50	59	2.40	1.64	4.50	73	2.40	1.64
13	4.50	108	3.20	2.32	4.50	80	3.20	2.32	4.50	137	2.00	1.30
14	4.50	100	2.80	1.98	4.50	78	2.80	1.98	4.50	132	2.60	1.81
15	4.50	86	2.20	1.47	4.50	67	2.60	1.81	4.50	129	2.60	1.81
16	5.00	84	2.90	2.07	5.00	84	2.50	1.73	5.00	129	2.30	1.56
17	5.00	64	2.40	1.64	5.00	63	2.50	1.73	5.00	124	3.00	2.15
18	5.00	119	3.10	2.24	5.00	64	2.40	1.64	5.00	99	2.80	1.98
19	5.00	101	2.60	1.81	5.00	67	3.00	2.15	5.00	98	2.60	1.81
20	5.50	76	2.65	1.86	5.50	60	2.60	1.81	5.50	61	2.30	1.56
21	5.50	64	2.40	1.64	5.50	107	2.55	1.77	5.50	82	2.35	1.60
22	6.00	84	2.45	1.69	5.75	93	3.00	2.15	6.00	84	2.75	1.94
23	6.25	80	3.05	2.20	6.00	92	2.75	1.94	6.00	71	3.05	2.20
24			3.00	2.15	6.00	84	2.80	1.98	6.00	86	2.80	1.98
	At 23.5 h 2 fish were stressed. One was removed and sacrificed as a "Day 0" sample. The other was transferred to the depuration tank and quickly recovered.				Air bar had a break in it resulting in fewer but larger bubbles.							

TABLE D.10

Amauligak long-term range-finding trial

	TANK 1			TANK 2			TANK 3		
Time (h)	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence
0.0	4.00	95	0.60	3.25	95	0.75	6.00	114	0.90
1.0		87	1.05		88	0.95			
2.0		85	1.05		84	0.95			
3.0	5.00	95	1.05	4.00	82	0.95			
4.0	5.25	98	0.80	4.00	82	0.80			
5.0	5.25	89	0.90	4.25	80	0.85			
6.0	5.25	84	0.90	4.50	80	0.70			
7.0							6.50	113	0.90
11.5							6.50	117	1.10
16.0	5.25	72	0.80	5.50	64	0.65			
20.5							8.00	134	1.70
21.5							7.00	127	1.70
23.0	7.00	200	1.05	6.50	200	0.80			
24.5	6.50	157	1.35	6.00	200	1.25			
26.5	6.50	129	1.40	6.00	188	1.25			
27.5	6.50	111	1.40	6.00	168	1.20	7.50	55	1.80
29.5	7.00	85	1.35	6.25	148	1.20			
32.5	9.50	92	1.35	8.00	115	1.30			
37.0	10.00	113	1.40	8.50	67	1.20	8.00	130	1.70
42.5							9.00	189	2.20
43.5							7.50	146	1.90
46.0	9.00	189	1.80	8.00	200	2.30			
47.0	7.50	133	1.70	7.00	154	2.00			
50.0	7.50	100	1.70	7.00	115	2.10			
51.0							8.00	72	2.60
52.0	8.00	80	1.70	7.50	91	2.05			
54.5	8.00	72	1.60	8.00	80	1.80			
57.0	7.50	84	1.70	8.00	85	1.50	7.50	200	2.30
61.5	8.00	130	1.70	7.50	103	2.00			
66.5							9.00	102	2.35
71.5	9.50	191	2.60	9.00	109	2.00			
72.5				7.00	106	2.05			
73.0				7.00	96	2.00			
80.0				7.00	86	2.00			
86.5				7.50	70	2.10			
95.0				9.50	132	2.30			
96.0				7.50	114	1.90			
103.0				8.00	47	2.30			
112.0				8.00	121	1.80			
119.0				9.00	150	2.00			
120.5				7.00	116	1.70			
128.0				7.50	42	2.40			
134.5				8.50	95	1.20			
143.5				9.00	98	1.80			
144.5				7.75	106	1.40			
146.0				8.00	102	1.50			
147.0					98				
149.5				8.25	107	1.60			
151.0				8.50	116	1.50			
157.5				8.50	110	1.70			
166.0				9.50	150	1.65			
				At 46 h water was very turbid and pieces of semi-digested food were found on the bottom.			One fish died at 51 h.		

TABLE D.11

Amauligak long-term

Time (h)	TANK 1			TANK 2			TANK 3			TANK 4		
	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence
0.0	5.0	89	0.10	5.5	93	0.40	5.0	91	0.20	5.0	91	0.45
4.5	5.0	97	1.20	5.5	106	1.50	5.5	84	0.70	5.5	84	1.20
9.5	6.0	82	1.20	6.0	86	1.30	6.0	76	0.90	6.0	74	1.20
19.0	7.0	138	1.25	7.0	94	1.30	7.0	167	1.50	7.0	133	1.35
24.5	7.5	88	2.45	7.5	103	1.30	8.0	121	1.90	7.5	80	1.70
27.5	8.0	77	1.50	7.5	110	1.20	7.5	103	1.05	7.5	105	1.10
34.0	7.5	72	1.45	7.5	116	1.40	7.5	107	1.00	7.5	118	1.30
43.0	8.0	170	1.65	8.0	98	1.45	7.5	200	1.75	8.0	200	1.80
44.0			1.90			1.90			2.00			2.30
46.0	8.0	165	1.90	8.5	103	1.75	8.0	175	1.95	8.5	172	2.25
48.0	8.5	116	1.95	8.5	87	1.70	9.0	141	2.20	9.0	146	2.35
49.0			1.55			1.55			1.75			2.05
52.5	8.5	69	1.90	8.5	129	1.90				8.5	80	1.90
57.0	9.0	88	1.90	9.0	100	1.80	9.0	123	1.50	9.0	106	1.70
66.0	8.5	200	2.35	9.0	126	2.30	6.0	192	2.00	8.5	138	2.60
68.0			2.25			2.20			2.25			2.60
70.5	9.5	145	2.20	10.0	80	2.90	7.0	101	2.30	9.0	72	2.55
76.0							6.5	118	1.50	8.0	83	2.65
80.5							6.5	111	1.60	8.0	73	2.60
91.5							10.0	93	1.70	9.0	85	1.80
92.0							7.0	108	1.65	6.5	97	1.70
							9.0	103	1.70	8.5	102	1.80
105.0							8.0	200	1.80	9.0	100	2.50
115.0							7.0	98	1.70	6.5	105	2.60
124.0							7.0	94	1.60	8.0	80	2.50
128.5							8.0	89	2.50	7.0	68	2.70
140.0							9.0	200	2.40	9.0	89	1.70
148.0							9.0	89	2.10	9.0	94	2.00
153.5							9.0	102	1.80	9.0	94	1.60
163.0							8.0	194	2.10	8.5	80	1.45

TABLE D.12

Hibernia long-term exposure

Time (h)	TANK 1			TANK 2			TANK 3			TANK 4			TANK 5		
	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence	Temp. (°C)	DO (%)	Fluor-escence
0.0	8.5	75	1.15	8.5	88	0.80	8.5	77	1.70	8.5	77	1.00			
5.0			1.30	8.5	95	0.85	8.5	108	1.80	8.5	86	1.20			
10.0	8.0	194	1.20	8.5	97	0.85	8.0	181	1.70	8.0	96	1.20			
19.0	7.0	167	1.00	7.0	135	1.05	7.5	168	1.00	7.0	146	1.00	7.5	147	
29.0	7.5	124	1.50	7.0	100	0.80	8.0	115	2.10	7.5	103	1.10	6.5	93	3.70
33.5	7.0	117	1.70	6.5	116	1.00	8.0	130	1.70	7.0	115	1.10	6.0	112	
45.0	8.0	134	1.30	9.0	107	1.10	8.5	172	1.90	8.5	131	1.40	6.5	129	2.20
50.5	8.5	103		8.5	101		9.0	111		9.0	109		7.5	91	
58.0	8.0	75	1.40	8.5	70	2.10	9.0	67	1.70	9.0	76	1.50	7.5	72	0.80
67.0	7.5	79	1.10	8.0	47	1.90	7.0	61	1.15	8.0	64	1.25	6.0	76	0.90
68.0									1.10			1.00			
71.0	7.0	90	0.70				6.5	74	0.70	7.5	82	0.85			
73.0							6.5	93	0.80	7.5	120	1.00	5.5	76	0.90
80.5							7.0	125	1.00	8.0	92	1.30	6.5	62	1.60
91.0							8.5	172	1.10	8.5	97	1.50	7.5	107	1.90
97.5							8.5	191		9.0	111		7.5	131	
106.0							8.5	200	1.20	8.5	105	1.40	7.5	173	0.90
115.5							9.5	200	1.30	9.5	102	1.40	8.0	181	1.20
117.0							7.5	200	1.15	7.5	107	1.30	7.0	129	
121.0							8.0	106	1.15	8.5	100	1.55	7.0	81	1.00
121.5									1.00			1.05			
129.0							8.0	128	1.20	8.0	196	1.30	7.5	107	1.40
139.0							9.0	152	1.00	9.0	196	1.25	8.5	127	1.40
140.0							7.0	125	0.85	7.0	156	1.10	7.0	102	1.00
145.0							8.0	94	0.90	8.0	164	1.00	7.5	93	
152.0							8.0	138	0.80	8.0	128	1.00	7.5	142	
163.0							9.0	157	1.00	9.0	37	1.60	9.0	178	1.90

APPENDIX E

GCMS DATA, LVT (TYPICAL) CONOCO OIL

Molecular Group	Area %[1]
C_9-C_{13} Normal Paraffins	2.04
C_{14}-C_{17} Normal Paraffins	0.74
C_9-C_{12} Branched Paraffins	15.92
C_{13}-C_{15} Branched Paraffins	24.76
C_{16}-C_{18} Branched Paraffins	2.68
C_9-C_{12} Monocyclic Paraffins	24.75
C_{13}-C_{16} Monocyclic Paraffins	11.71
C_9-C_{15} Bicyclic Paraffins	2.40
C_9-C_{11} Alkyl Benzenes	6.80
C_{12}-C_{15} Alkyl Benzenes	2.65
C_{10}-C_{12} Tetralins	4.06
C_{13}-C_{15} Tetralins	1.02
C_{10}-C_{12} Naphthalenes	0.13
C_{13}-C_{14} Naphthalenes	0.01
Biphenyls	0.01
Acenaphthalenes, Fluorenes, Phenalenes	0.00

[1] For this type of sample, "area %" is a good approximation of weight %.

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