THE UNIVERSITY OF MANITOBA

WATER SOLUBLE FRACTIONS OF CRUDE OILS AND PETROLEUM PRODUCTS: ANALYSIS AND INVESTIGATION OF FISH TAINTING BY GAS CHROMATOGRAPHY.

by

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ABSTRACT

Water soluble fractions of crude oils and petroleum products were prepared and analysed by two gas chromatographic methods. A headspace technique was used to measure the more volatile hydrocarbons and a microextraction technique was used for the less volatile fraction. The results showed that the crude oils and the petroleum products all produced a qualitatively similar water soluble fraction. The chromatograms gave the same fingerprint of aromatic hydrocarbons with some quantitative differences. Diesel and fuel oils showed extra peaks in the higher boiling range while gasoline gave a water soluble fraction which was richest in both the very volatile and less volatile fractions.

A modified purge and trap method was developed to measure volatile hydrocarbons in fish tissues using a headspace concentrator apparatus and a small charcoal trapping column. Laboratory reared rainbow trout were treated in water containing crude oil, diesel oil and various volatile organic compounds known to be in a water soluble fraction. Muscle samples from these fish and from "naturally" contaminated whitefish and burbot were analysed using this method and the results compared with sensory evaluations from a taste panel.

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I. Introduction

The large scale use of crude oils and petroleum products and the serious environmental effects of known oil spills such as the Argo Merchant, December 1976, the Amoco Cadiz, March 1978 and the Ixtoc 1 blowout June 1979, have served to make governments, industry and the public at large aware of the risks in the transportation and processing of such materials. An accident involving a crude oil, or any liquid petroleum product such as diesel and fuel oils or gasoline presents two problems which must be fully appreciated to realize the implications of an oil spill into an aqueous environment.

The first problem resulting from a crude oil spill is the sticky slick which floats on the surface and which develops into a "chocolate mousse" under the action of wind and waves. This is the formation of a frothy emulsion of oil and water caused by the constant wave motion which envelops and coats everything with which it comes in contact. Bellier and Massart (1979) noted that a "chocolate mousse" was formed after the break-up of the Amoco Cadiz and could be removed from the water surface best with vacuum tank trailers. During the first few days the slick "weathers" and loses much of the extremely volatile material to the atmosphere. Sivadier and Mikolaj (1973) demonstrated that a natural seep oil lost the majority of its volatile fraction in one to two hours under moderate wind and sea conditions.

If an oil spill occurred under Arctic conditions, "weathering" would be inhibited since a layer of ice would prevent the free passage of volatile material to the atmosphere. It was reported by Dickens et al (1981) that oil and gas collected on the underside of sea ice where they remained unless vented. The oil would surface at the spring break-up when the normal weathering process would continue. Low temperatures would inhibit the

solution of the soluble organics but they would nevertheless form a water soluble fraction (WSF) capable of causing trouble to the fishing industry. This has already happened where a spill of a refinery effluent from the Suncor oil extraction plant in 1982 under the ice on the Athabasca River appears to have been the cause of the closure of the commercial fishery the following summer. (Province of Alberta vs Suncor, 1983).

Eventually a crude oil slick will become dense enough to form tarry balls which will either sink and become part of the sediment or will retain a neutral buoyancy and remain in the water column. Morris and Butler (1973) noted that the degradation of such tar lumps would take times of the order of years because of the substantial content of high melting point waxes and asphaltenes.

The second problem caused by a crude oil spill is much less obvious, but perhaps just as serious. This occurs when the soluble organic compounds from the oil dissolve in the water and are distributed throughout the water column to form a WSF. Although this fraction is present only in relatively small concentrations, it is the components in this fraction which are in most intimate contact with fish and other pelagic organisms. The effects of these dissolved compounds on fish can be observed in two ways; the WSF from a crude oil is toxic to fish and it is responsible for unpleasant taste and odors in edible fish muscle.

The toxicity of a substance to fish or aquatic organisms can be defined as that concentration which will kill 50 % of a given species in a stated time, usually 96 h. To determine this LC 50, a range of concentrations of the toxic material must be prepared and the test species placed in these solutions. Observations are made on a regular basis and mortality times

noted. The LC 50 can be calculated from a computer program using a linear regression analysis or from mean survival times at the various concentrations and extrapolated to 96 h. With a complex mixture of organic compounds found in crude oils and their WSFs, the final result must be interpreted with caution as it represents the cumulative effect of many chemicals. Any reduction in the complexity of the mixture would help to simplify this problem and the use of a WSF instead of a crude oil is a step in this direction. A further simplification would be to measure the LC 50 of individual compounds in the WSF.

The concept of using a WSF in toxicity studies has been developed by many authors and the various methods used to prepare WSFs are summarised in Table 1. The oil/water ratios vary from 1:6 to 1:1000, and both stirring and shaking have been used to mix the two phases for up to 72 hours. Separating times also vary from 10 minutes to 24 hours with little or no explanation or reasoned argument regarding the techniques used.

The wide range of oil/water ratios used by the many authors to prepare WSF's has produced a range of concentrations of volatile organics found in solution. Stirring and shaking are approximately equivalent in preparing a WSF but it is important that enough time be allowed for insoluble particles or droplets to coalesce and separate.

The methods of analysis used by the authors in Table 1 include GC, GS/MS, Spectroscopy (UV,IR and Fluorescence), Scintillation Counting, High Pressure Liquid Chromatography (HPLC) and Total Organic Carbon (TOC) analysis. In many cases a particular method can measure only one group of compounds and discrepancies must necessarily result when data from different methods are compared. These authors have reported the toxicities of their

Table 1. Summary of Methods used to Prepare Aqueous WSF's from Various Oils showing Ratios and Mixing Parameters.

Senior Author	Year	Oil/Water Ratio	Mixing Method	Mixing Time	Separating Time
Boylan	1971	1:6	Stir	12 h	2 h
Lee	1978	1:8	Stir	24 h	10 min
Byrne	1977	1:8	Shake	12 h	24 h
Giddings	1981	1:8	Stir	16 h	Filter
Keck	1978	1:9	Stir	48 h	-
Anderson	1974	1:9	Stir	20 h	1-6 h
Pearson	1981	1:9	Stir	20 h	4 h
Scheier	1976	1:20	Stir	72 h	-
Ostgaard	1983	1:20	Stir	18 h	-
Tarshis	1981	1:20	Stir	20 h	1-6 h
Widdows	1982	1:80	Stir	18 h	6 h
Smith	1979	1:84	Stir	24 h	12 h
Moles	1979	1:100	Stir	24 h	3 h
Katz	1973	1:100	Stir	24 h	1 h
Maher	1982	1:100 1:1000	Gentle Oscillation	-	-
Christiansen	1978	1:200	Stir	24 h	Centrifuge
Kappeler	1978	1:1000	Shake	10 min	Centrifuge
Renzoni	1975	1:1000	Shake	30 min	3 h

WSFs to the various species tested but no precautions were taken to preserve the volatiles in solution or to maintain the concentration of the toxic material during the whole exposure time.

A continuous flow apparatus was designed by Nunes and Beneville (1979) to deliver a uniform solution of the water soluble components of a crude oil without losses of volatile compounds. Water was percolated slowly and continuously through a layer of oil to form the WSF without any emulsion problems. The WSF was withdrawn from the bottom of the apparatus while the oil layer was maintained at a constant level with an overflow and replenished with a metering pump. The WSF was analysed by gas chromatography (GC) and the concentrations of the low molecular weight aromatics in the WSF and in clam tissue were determined.

There have been few systematic comparisons of the WSF's from crude oils and petroleum products. Boylan and Tripp (1971) compared Kuwait and Louisiana crude oils and showed the similarity of the seawater extracts. They also identified many substituted benzenes and aromatics in an extract of kerosene. Winters et al (1976) compared the water solubles from four test oils but did not demonstrate any similarities. They did identify by gas chromatography/mass spectrometry (GC/MS) similar compounds to those described by Boylan and Tripp (1971) in their water soluble fractions as well as many nitrogenous compounds and phenols. Kappeler and Wuhrmann (1978) investigated the microbial degradation of the water soluble fraction of a gas oil and demonstrated the presence of a similar range of substituted benzenes and naphthalenes using capillary GC/MS. Table 2 lists the compounds identified in these three papers. Both Boylan and Tripp (1971), and Winters et al (1976) used a concentration step in the preparation of their sample for GC analysis

Table 2. Comparison of Compounds Identified in WSFs.

Boylan & Tripp 1971	Winters et al 1976	Kappeller & Wuhrman 1978
-	-	toluene
-	-	ethyl benzene
-	-	3 dimethyl benzenes
6 C_3 benzenes	2 C_3 benzenes	8 C_3 benzenes
11 C_4 benzenes	1 C_4 benzene	14 C_4 benzenes
1 C_5 benzene	-	-
tetrahydronaphthalene	-	tetrahydronaphthalene
naphthalene	naphthalene	naphthalene
2-methylnaphthalene	2-methylnaphthalene	2-methylnaphthalene
1-methylnaphthalene	1-methylnaphthalene	1-methylnaphthalene
biphenyl	-	-
3 C_2 naphthalenes	5 C_2 naphthalenes	8 C_2 naphthalenes
-	1 C_3 naphthalene	1 C_3 naphthalene
3 methyl indanes	-	-
-	6 dimethyl anilines	-
-	o-, m-, p-toluidine	-
-	o-, m-, p-cresol	-
-	5 dimethyl phenols	-
-	5 C_3 phenols	-
-	3 methyl indoles	-
-	2 dimethyl indoles	-

and they were unable to detect toluene, ethyl benzene or the xylenes. Although Kappeller and Wuhrmann (1978) concentrated their methylene chloride extract in a stream of dry nitrogen, they did detect these low boiling compounds in their analyses.

The second effect of the dissolved organic material in a WSF has been to cause unpleasant taste and odor in fish. As clean fish have a bland flavor, any traces of odorous chemicals in the fish muscle have a great effect on how the prepared fish sample tastes. Large spills of crude oil or petroleum products can have a disasterous effect on commercial fishing as well as the lobster, crayfish and crab industries. The loss of commercial sales in these areas can have a far ranging effect on all the people who handle the product from the initial catch to the final sale to the consumer.

The usual technique of assessing the acceptability of a suspect batch of fish has been the taste panel which either rejects or passes the sample. This sensory evaluation is based on the abilities of a trained panel of experts who have sensitive palates and who can detect off-flavors and odors consistently. Samples of control and suspect fish are prepared in a standard manner and are presented to the panel for evaluation. The panel has to determine the acceptability of the samples and to rate them according to taste, odor, texture and aftertaste. These observations are useful to determine how badly a sample is contaminated and whether it can be marketed. Another advantage is that further fishing in that area can be stopped until the fish are acceptable to the consumer. However taste panel evaluations usually require a relatively high degree of expertise and are not always at hand to give immediate results. A chemical analysis would provide a numerical back-up and would perhaps be more readily available to analyse samples.

Ogata et al (1979) analysed eels and clams to identify organics of petroleum origin but their method of preparation included a concentration step using a rotary evaporator. They were able to identify by GC/MS substituted naphthalenes and various dibenzothiophenes in their samples but did not find any of the lower molecular weight volatiles which were present in the original crude oil. Howgate et al (1977) used a panel of trained assessors to taste plaice and shellfish but did not report any chemical analyses of fish samples because they had not been completed. Connell (1978) determined lipids and hydrocarbons in sea mullet and developed a linear relationship between hydrocarbon and lipid content. He extracted the lipids with ether, and then concentrated and weighed the extract. The lipid extract was steam distilled and the distillate extracted with ether. The ether extract was concentrated and weighed to give a kerosene hydrocarbon content. This technique would not be successful in retaining the volatile material and the author reports a 20% accuracy for the hydrocarbon analysis.

Tainting and off-flavours in fish have been reviewed by Reineccius (1979) in which he discussed the chemical and bacteriological causes. A kerosene taint was noted when fish were caught near docks, sewage outlets or heavy industry. It was noted that muddy taints could be purged from fish by keeping them in clean aerated water under fasting for 18 days and that the very rapid absorption of odorants into catfish indicated that transmission was most likely directly through the gills of freshwater fish.

Hiatt (1981) used a vacuum extractor and a cold trap followed by a purge and trap procedure to remove volatile compounds from fish and sediments. He investigated the priority pollutants and reported a wide range of recoveries in a comparison of vacuum extraction, direct purge and trap and a modified

purge and trap.

The purge and trap method of Bellar and Lichtenberg (1974) was used by Berg (1983) to detect the volatile compounds causing off-flavours in Norwegian fish. The volatiles were collected on an activated carbon trap and desorbed directly in the injection port of a GC. A sensory analysis was used to evaluate fresh and smoked salmon samples with a very strong and a faint off-flavor. GC/MS analysis showed that the main components were sulphite compounds from paper mill efffluent, terpenes and terpene derivatives, alkyl- and alkenyl-benzenes and chlorinated compounds.

Steinke (1979) extracted steam distillates of Lake Michigan salmon with ether and analysed with a large bore capillary GC column. Major flavor compounds were collected on Tenax GC and reanalysed on a SE 30 column. Coho salmon were analysed by GC/MS and 89 compounds were positively identified as mainly hydrocarbons, alcohols, and aldehydes as well as PCB's, phthalate esters and numerous trace contaminants. The author noted that holding off-flavored salmon in fresh water for 7 days reduced the intensity of some chemical flavors but earthy-muddy flavors were not removed.

Any correlations which might exist between off-flavors and a chemical analysis have not been well developed and few authors have been able to positively associate an off-flavor with a particular chemical. Ogata and Miyake (1973) determined that fish and eels kept in contaminated sea water acquired a bad smell which they demonstrated was caused, at least in part, by toluene. They also suggested that since benzene and the xylenes all infiltrated rapidly into fish muscle, they might also be responsible for offensive odors.

The work of Veijanen et al (1983) using a two capillary column system, gave a verbal description to peaks in gas chromatograms of contaminants in water and fish samples. The columns were joined in parallel at the inlet and the other ends were connected to a sniffing detector and to the more conventional GC detectors (FID, FPD, ECD, and MS). They used such words as ethereal, irritating, fresh, fishy, sharp, nauseating, musty, unpleasant, bitter, etc. to describe the odors of peaks in the gas chromatogram. Although experiments were conducted with a sniffer/MS in parallel system, they did not offer any identifications of specific odors. They suggested that the use of MS data only, should be considered to give a tentative identification of off-flavors and that GC retention data as well as IR and NMR spectra were required to be positive.

Geosmin (trans-1,10-dimethyl-trans-9-decalol) was isolated and identified by Yurkowski and Tabachek (1974) as the principal compound responsible for the muddy-flavor in fish from saline lakes in Western Canada. These authors noted that placing the muddy-flavored rainbow trout in clean water for 3 - 5 days resulted in a reduction of the flavor to below the taste threshold which they estimated to be 6 ng/g (ppb).

The two effects of a WSF namely toxicity and tainting, would appear to be caused by the dissolved organic material. Existing methods of analysis do not seem to be particularly suitable for the analysis of these volatile compounds so attention was focussed on analytical methods which could measure volatile organic material in both the WSF and in fish tissue.

The purpose of this work was to compare the WSF's prepared from crude oils, fuel oils and gasoline using two analytical GC techniques to measure the dissolved hydrocarbons. A second purpose was to investigate the volatile

compounds in fish tissue which contribute to the tainting and odor problems found after an oil spill in an aquatic system.

In view of the large differences in volatility of the organics present in a WSF, no one method would be capable of measuring everything, and it was decided to divide the analysis of the WSF's into two groups as suggested by Parker et al (1976). The first group was composed of those extremely volatile compounds up to the boiling point of approximately 115 C (toluene 110 C) and these were analysed by a headspace technique. The second group included those compounds with boiling points from 115 - 270 C and these were analysed by a solvent extraction technique. Because the components of this group were still sufficiently volatile to be lost during any conventional concentration step, a microextraction technique was selected (Murray 1979). In this technique a small volume of solvent was used to extract a constant proportion of the dissolved material in the aqueous solution and the solvent layer analysed directly with no concentration step.

A purge and trap technique was developed to measure the volatile organic compounds in fish tissue using a small charcoal trap. The volatiles were purged out of the heated fish tissue and trapped on a small charcoal column. The adsorbed organics were eluted with the minimum volume of solvent and analysed by GC.

II. EXPERIMENTAL

Preparation of Water Soluble Fraction

Preliminary data (Fig.1) indicated that 10 mL of oil per litre of water (1:100) was sufficient to produce a water soluble fraction saturated with most of the compounds identified in the headspace other than benzene and toluene. Figure 1 was derived from headspace analysis of WSF's prepared from various oil/water ratios and the peaks were identified by comparison of retention data of known compounds. For the remainder of the thesis it was decided to use a ratio of 50 mL oil / litre of water (1:20). A 1L separatory funnel was used to mix 1000 mL of distilled water with 50 mL of each oil with vigorous shaking by hand for 5 minutes. The mixture was allowed to separate into the two main fractions for at least 48 hours before analysis so that a complete separation of the two phases occurred. The WSF could be conveniently stored in the separatory funnel under the oil layer with a minimum of headspace until analysed.

Analysis of Water Soluble Fraction

1. Headspace Analysis

A 50-mL glass gas-tight syringe was used to shake 25 mL of the WSF and 25 mL of nitrogen vigorously by hand for 2 min at room temperature. This equilibration was followed by a 5 min settling period with the syringe in a vertical position and the tip uppermost. A portion of the headspace was pushed through the sample loop of a gas sampling valve and injected into the column of a GC at 40 C followed by temperature programming to 220 C at 30 C/min. The areas of the first 12 peaks eluted in 5 min up to toluene were

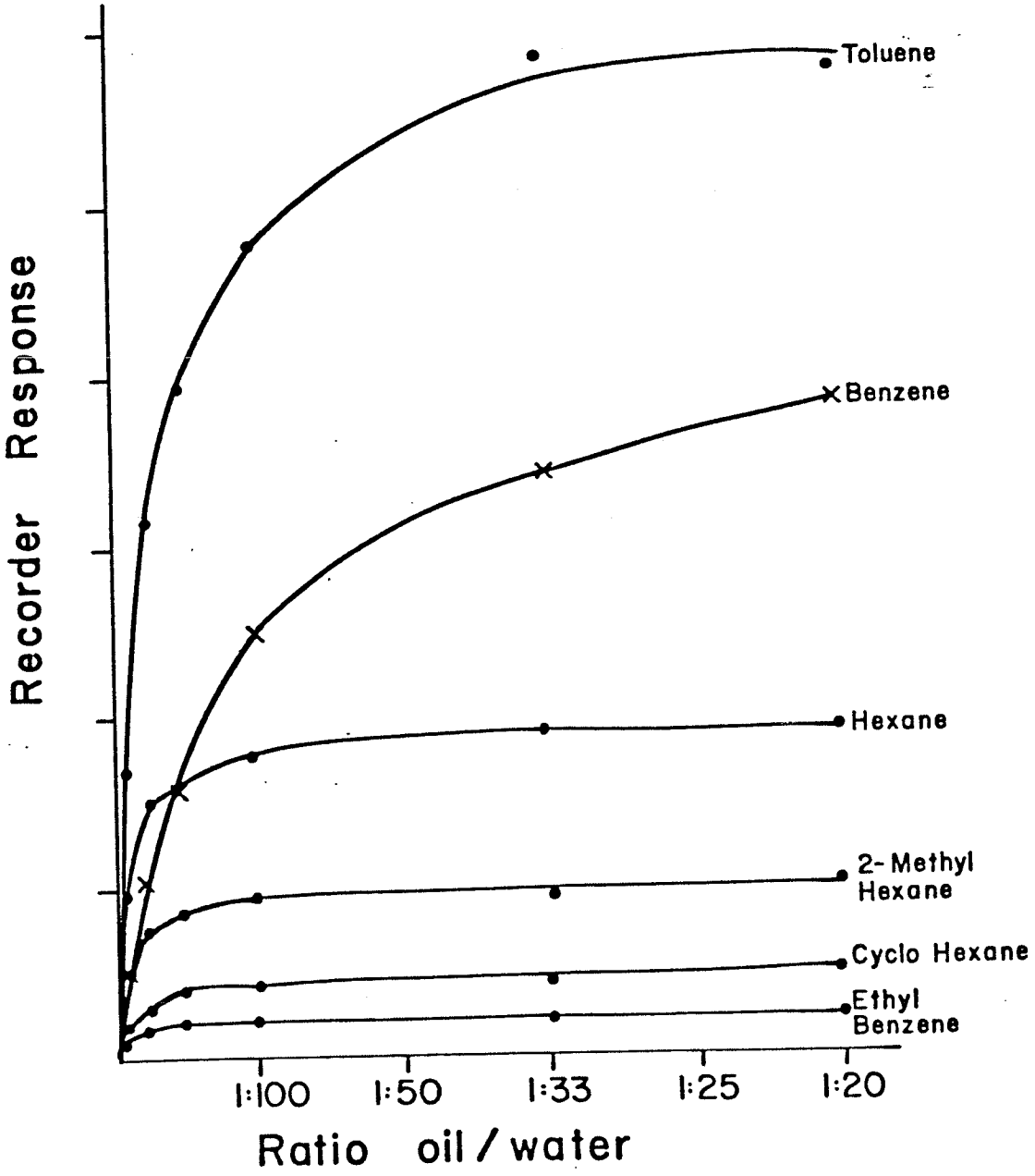

Figure 1. Headspace Analysis of selected Hydrocarbons in WSF's Prepared using Different Oil/water Ratios.

measured with an electronic integrator. The remaining peaks in the chromatogram were eluted with the temperature program but were not measured since the size of the peaks decreased from this point.

Individual gas standards of methane, ethane, propane, butane and iso-butane at a concentration of 100 ppm by volume in helium were purchased from Scientific Gas Products Inc. Analysis of the gas standards using the same sample loop gave an average weight response factor for these compounds. Standards of pentane, hexane, benzene and toluene were prepared by adding individually the appropriate volume equivalent to 10 mg of each to a closed 1 litre flask. The volumes were calculated from the density of the solvents ie. for pentane, 16.0 uL = 10.0 mg. Samples of the gas phase were removed with a gas tight syringe and analysed with the same sample loop to give a weight response factor for these compounds. The alkanes will equilibrate readily out of the aqueous phase so that with one equilibration of equal volumes of water and gas, over 96% was transferred into the gas phase (McAuliffe 1971). With benzene and toluene however, their solubility in water prevent their total transfer to the gas phase. Distribution coefficients for benzene and toluene were calculated from headspace analysis of successive equilibrations (McAuliffe 1971). This distribution coefficient was used to calculate how the benzene and toluene were distributed between the two phases and this ratio applied as a correction factor to the weight response factors calculated from the analysis of gas the standards.

2. Microextraction Analysis

A 1000-mL microextraction flask (Fig 2, Murray 1979) was used to extract 950 mL of the WSF with 1000 uL of hexane by shaking vigorously for 1.00 min and allowing to stand for 10 min to permit the hexane droplets dispersed throughout the aqueous phase to collect on the surface. The flask was tilted at about a 45 degree angle to bring the solvent layer under the capillary tube and distilled water added to bring the solvent up into the centre tube. Following the addition of n-decyl benzene as an internal standard for quantitation, 1.0 uL of the extract was analysed by GC using a non-polar high resolution Dexsil 300 or a DB5 bonded capillary column. Either of these columns gave essentially the same chromatogram of the complex mixture of components in the WSF. The areas of those peaks eluting after toluene were integrated and the concentrations calculated from the ratios to the internal standard using a previously determined 40% recovery factor (Murray 1981). This recovery factor is dependent on the ratio of solvent to water and with a 100 mL flask and 400 uL of solvent the recovery improves to a mean of about 73 % for all compounds.

The sixteen major peaks in the microextract chromatograms of the WSF were identified initially by comparison with standards of known compounds and the identities confirmed by GC/MS. A sample of the microextract of Norman Wells Crude oil was sent to Enviro-Test Laboratories, Edmonton where the tentative identifications were confirmed using a HP 5993 C GC/MS with a DB 5 capillary column. The ion source was at 200 C, the injector at 250 C and the ionization voltage at 70 EV.

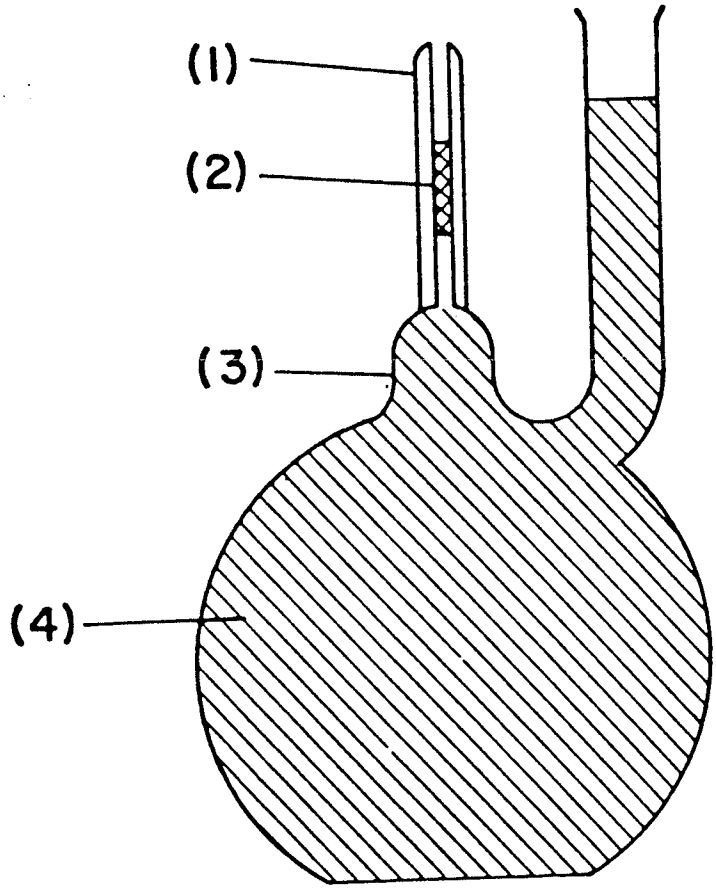

Figure 2. Microextraction Flask. (Murray 1979)

1 - Capillary Neck 2 - Solvent

3 - Modified Volumetric Flask

4 - Water Sample

The twelve major peaks in the headspace chromatogram and the sixteen major peaks in the microextract chromatogram were chosen to compare the WSF's prepared from the crude oils and petroleum products tested.

3. Oil and Grease Analysis (ASTM D3921-80)

A 750 mL aliquot of the WSF was extracted three times with 30 mL of trichlorotrifluoroethane and the combined extracts dried with sodium sulphate and made up to 100 mL with the same solvent. The infrared absorbance was measured with a 1 cm quartz cell using a scan from 3100 to 2700 nm and measuring the transmission minimum at 2920 nm. A mixed standard of iso-octane, hexadecane and benzene was prepared and 1 gm weighed accurately into a 100 ml flask and dissolved in the solvent. Dilutions of this primary standard were analysed in the same way as the sample and an absorbance calibration factor calculated. This factor was used to calculate the concentration of hydrocarbons in the WSF.

Analysis of Fish Tissue

A headspace concentrator apparatus was designed (Fig 3) in which the volatile contaminants were flushed out of a sample and trapped on a small column containing 30 mg of powdered charcoal as in the procedure developed by Grob (1975). The charcoal was held between two small plugs of glass wool in a 3 cm length of 5 mm O.D. glass tubing and occupied a length of 7 mm. The sample of frozen tissue was weighed, chopped into small pieces and placed in the tube. The tube was immersed in a water bath at 70 C, and connected to the charcoal trap, and air was sucked through the system at 60 mL/min. The incoming air was pre-cleaned by passing it through a 5g charcoal trap. After 30 min, the charcoal trap containing the fish volatiles was removed and extracted with 50 uL of carbon disulphide, n-decyl benzene added as an internal standard for quantitation and the sample analysed by capillary GC with a flame ionization detector. This technique allowed the volatiles to be extracted and concentrated onto a small charcoal trap in one step, the trap to be extracted with a very small amount of solvent, and the extract to be injected directly into the GC with no clean-up.

Initial experiments using two charcoal traps in series showed no breakthrough of volatile material when a spiked fish sample was purged at 60 mL/min for 30 min and subsequently only one trap was used. Recoveries were measured by spiking a clean sample of fish muscle with known amounts of a standard mixture of hydocarbons and measuring the amount recovered after 30 min of purge and trap. These recoveries are discussed later.

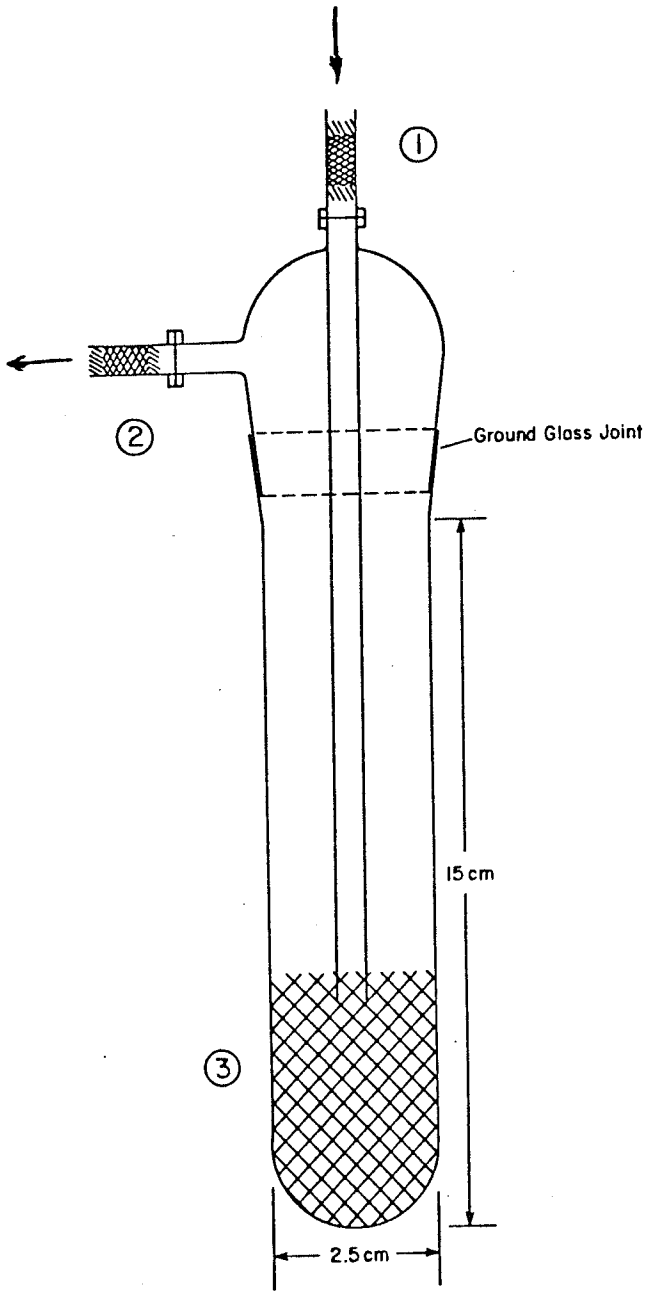

Figure 3 Headspace Concentrator Apparatus.

1 - Charcoal trap to clean incoming air.

2 - Charcoal trap to Collect Volatiles.

(3mm X 7mm charcoal)

3 - Chopped Fish Tissue

Sensory Evaluation Technique

Frozen fish were partially thawed, the intestines and head removed, vacuum sealed in plastic bags and refrozen. Just prior to evaluation the fish were thawed, filleted and the muscle homogenised in a food blender. Small portions (20 g) were wrapped in aluminum foil, coded and steam cooked for 15 min and presented to the panel of experienced judges for rating. Control fish were processed with the test fish and judged at the same time.

Weight Loss Determination

Half petri dishes (40-50 g) were weighed and approximately 10 g of crude oil, diesel oil, fuel oil and gasoline placed in each dish. The dishes were stored in a fumehood at room temperature with the extraction fan at a slow speed and reweighed regularly over a 30 day period. The percentage losses in weight were calculated. The dishes were not covered and any errors caused by the accumulation of dust would be negligible.

Fish Spiking Experiments

Laboratory-reared rainbow trout of about 1 kg weight were acclimated in 150 L tanks at 10 C without feeding for 5 d with fresh running water. The tanks were individually dosed with 100 ppm of Norman Wells crude oil, 50 ppm of P40 Diesel oil and two mixtures of five and six pure volatile hydrocarbons, (analytical standards from Polyscience Corp.) each at a 1 ppm concentration. A single fish was exposed for 4 h in each of the tanks and killed, and fillets of the dorsal muscle prepared for chemical analysis and taste panel evaluation. A control fish was sacrificed at the same time and the samples kept frozen until analysis.

Analysis of WSF for Phenols

The method of Coutts et al (1979) for trace phenols was followed after an initial hexane extraction to remove the regular WSF hydrocarbon peaks. A 250 mL portion of Norman Wells WSF was placed in a 500 mL separatory funnel and 10 g sodium bicarbonate added to convert the phenols to the more hydrophilic phenates and keep them in solution. This was extracted twice with 1.0 mL of hexane and the solvent layer discarded. The acetate esters were formed by adding 0.5 mL of acetic anhydride and they were extracted with two 5 mL portions of methylene chloride. The combined extracts were concentrated by evaporation using a gentle stream of nitrogen to 20 uL and analysed by GC.

Source of Clean Water

Distilled water as received in the laboratory was contaminated and analysis using the microextraction technique showed the presence of phthalate esters. To avoid this contamination, water was taken in clean solvent bottles directly from a tap at the still reservoir.

Gas Chromatographic Conditions

	Headspace Analysis	Microextraction Analysis
G.C.	Hewlett Packard 5750	Varian Vista 6000
Integrator	Infotronics CRS 208	Hewlett Packard 3373 B
Recorder	HP 7127 A 1 mV	HP 7127 A 1 mV
Chart	1.2 cm/min	0.5 cm/min
Injection	Carle Sample Loop	Splitless
Column	3 m 6 mm O.D.	25 m Capillary
Packing	5% Dexsil 300	Dexsil 300 or DB 5
Temp.Prog.	1 min @ 40 C	5 min @ 50 C
	30 C/min-220 C	4 C/min.-250 C
Flow rate	25 mL/min	20.5 cm/sec
Hydrogen	25 mL/min	20 mL/min
Air	200 mL/min	200 mL/min

Venezuelan Bunker C, South Louisiana Crude, Kuwait Crude and #2 Fuel Oil were American Petroleum Institute Reference Standards and the Norman Wells Crude was obtained from Esso Resources Calgary. The remainder of the crude oil samples were obtained from a local refinery. Esso regular gasoline was purchased at a local gas station and the diesel oil samples were originally received from Hay River (N.W.T. Canada) as part of a survey for hydrocarbon contamination.

III. RESULTS and DISCUSSION

The actual ratio of oil to water used to prepare a WSF does not appear to be very significant in that a ratio of 1:100 will produce a WSF which is saturated in most compounds (Fig 1). The important part in the preparation procedure of a WSF is to allow at least 48 h separation time for the suspended particles of oil to coalesce and rise to the surface. After the initial vigorous shaking, the crude oil has been broken up into many tiny droplets and a minimum of 48 h is required for the two phases to separate. Microextraction analysis of Norman Wells WSF after separation times of 1, 6, 24 and 48 h showed a steady decrease of the n-alkanes C12 - C16 in the WSF to levels below the limit of detection (0.5 ppb). The chromatograms in Figure 4 show the presence of an homologous series of n-alkanes present after 1 h separation time which decreases with time so that after 48 h they were no longer detectable. This decrease appears to be due to the slow separation of the two phases. The large peak at 16.2 min was n-decyl benzene which was used as an internal standard for all quantitation. An additional plug of glass wool placed just below the stopcock was found to be helpful in preventing globules of oil sticking to the inside glass surfaces being swept out of the separatory funnel through the stopcock as a sample was withdrawn.

The volatile compounds expected in a WSF would be those with some significant solubility and should include the n-alkanes from methane to heptane, cycloalkanes and the aromatics and alkylated aromatics from benzene to the dimethylnaphthalenes. The n-alkanes from C11 upwards should be present in low concentrations because of their low solubility. Non-hydrocarbons such as phenols, anilines, indoles and sulphur-containing compounds might be expected in a WSF at low concentrations but were not measured by the methods

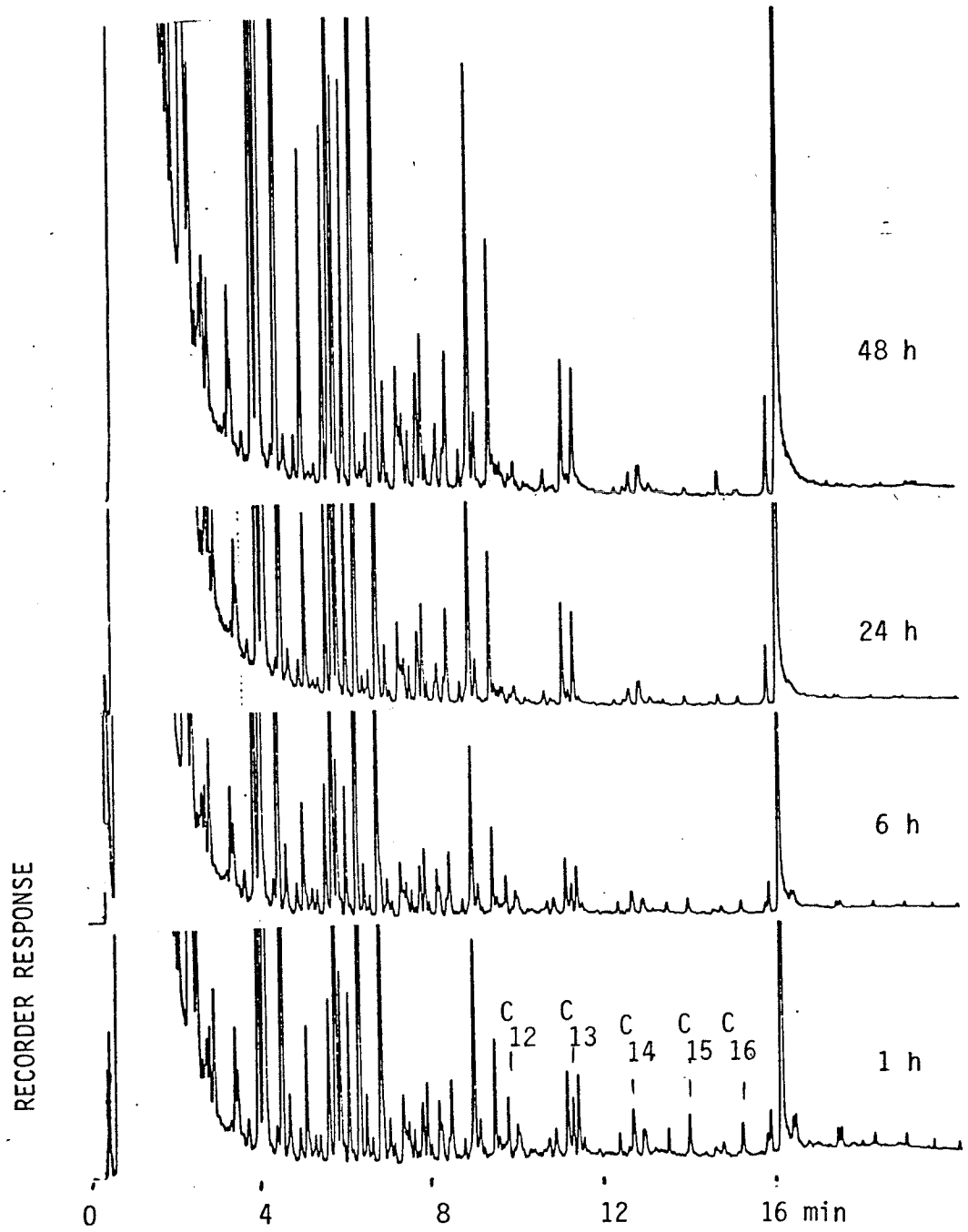

Figure 4. Microextract Analysis.

Gas Chromatograms of Norman Wells WSF after

1, 6, 24, and 48 h separation.

used in this investigation. Nitrogen and sulphur specific detectors could be used to detect such material.

Figure 5 has been included to demonstrate the typical pattern of peaks found in crude oils at a 1:200 dilution in hexane. The regular pattern of n-alkane peaks from C8 to C24 can be seen as well as the typical isoprenoids, pristane and phytane which serve to mark peaks C17 and C18 respectively. The many smaller peaks appearing between the n-alkane series indicate the complexity of the mixture of organic compounds in a crude oil. This chromatogram demonstrates the typical pattern of peaks from a crude oil while chromatograms of WSFs show quite different patterns of peaks for the headspace and microextraction analyses.

Headspace Analysis

Headspace analysis was applied to those volatile compounds in a WSF which equilibrate readily from the aqueous solution into the enclosed headspace above the water sample. There was some overlap between compounds measured by headspace analysis and those measured by microextraction, so a cut-off point was arbitrarily chosen at 5 min after injection of the gas phase in the headspace analysis. This occurred at the valley between the peaks for toluene and ethyl benzene, so that toluene was measured by headspace and ethyl benzene and compounds eluting after this point were analysed by the microextraction technique.

Three headspace analyses were performed on a Norman Wells WSF and duplicate measurements taken from each equilibration to determine the precision of the method. The relative standard deviation (RSD) was calculated for each peak from the six sets of figures and the results ranged from 1.67 -

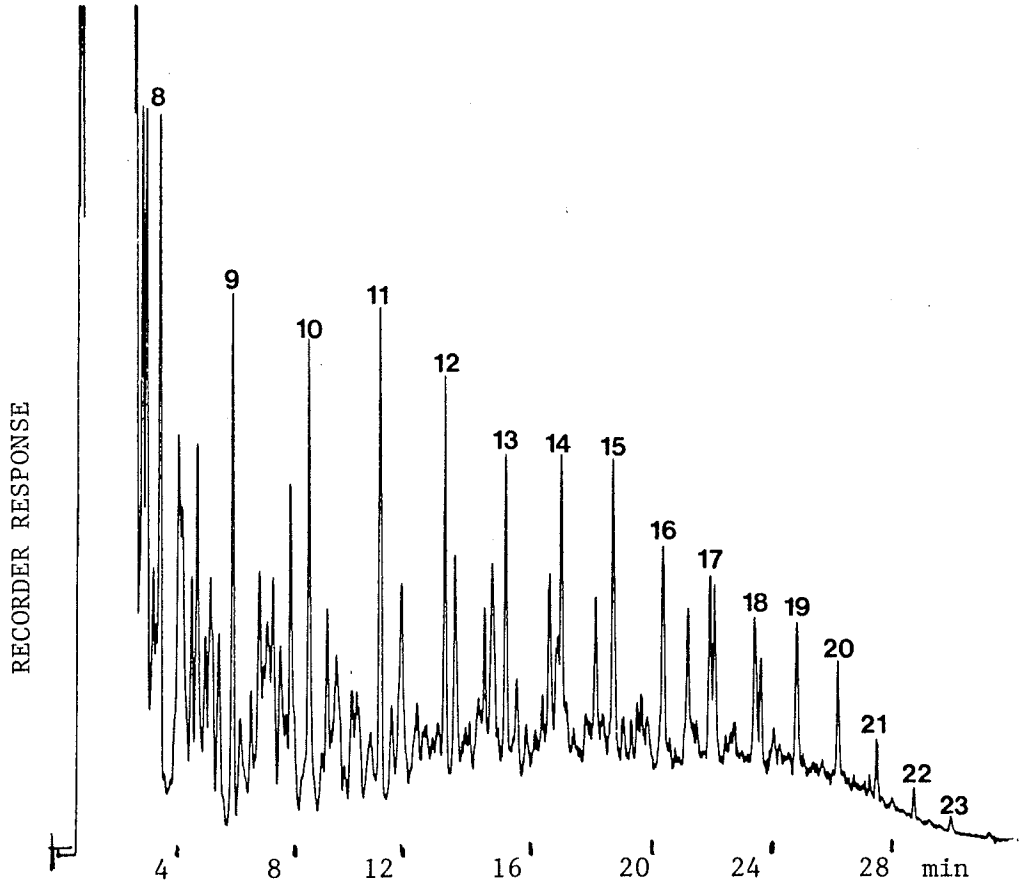

Figure 5. Gas Chromatogram of Whole Norman Wells Crude Oil. (1:200 dilution in hexane)

Numbers refer to Carbon number in n-alkane series.

4.08 % with a mean of 3.42 %. The actual analyses of the WSFs prepared from crude oils and petroleum products used duplicate measurements from a single equilibration.

Figure 6 is a representative chromatogram of the headspace analysis of a WSF of a typical crude oil (Norman Wells). The numbered peaks were identified by comparison of retention data of known compounds but this does not rule out the presence of other unidentified compounds. All the WSF's prepared from crude oils gave very similar chromatograms when analysed by the headspace technique. The WSF prepared from gasoline (Fig. 7) showed some differences in that the peaks for benzene and toluene were much larger than those from crude oils. The other headspace peaks were still present as in the headspace chromatogram of the crude oil WSF. This is also shown in Table 3 where the benzene and toluene concentrations were highest for gasoline

The headspace chromatogram of the diesel oil WSF (Fig. 8) shows some remarkable differences in that there were almost no low molecular weight compounds present until peak number 12 (toluene) which was then followed by a complex series of peaks which could possibly be C2 and C3 benzenes. Headspace analysis was not continued after toluene because of poor separation of GC peaks and a rising baseline which made peak integration inaccurate. Figures 7 and 8 illustrate the particular uses of the distillate cuts in that a gasoline motor requires a volatile fuel while a diesel engine can run on a less volatile fuel.

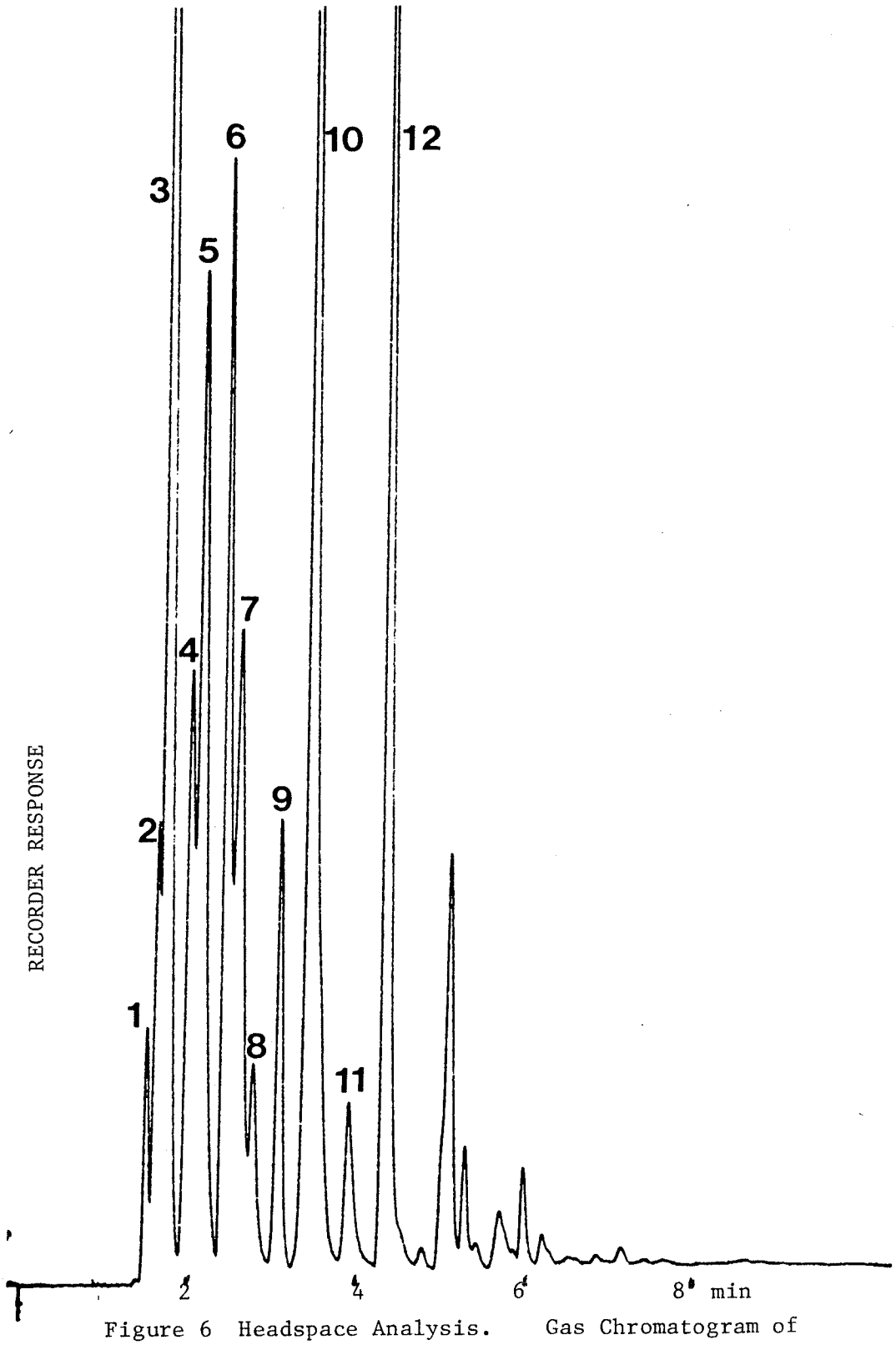

Figure 6 Headspace Analysis. Gas Chromatogram of Norman Wells WSF. 1, methane; 2, ethane; 3, propane; 4, iso-butane; 5, butane; 6,pentane; 7, unknown; 8, 2-methyl pentane; 9, hexane; 10, benzene; 11, unknown; 12, toluene

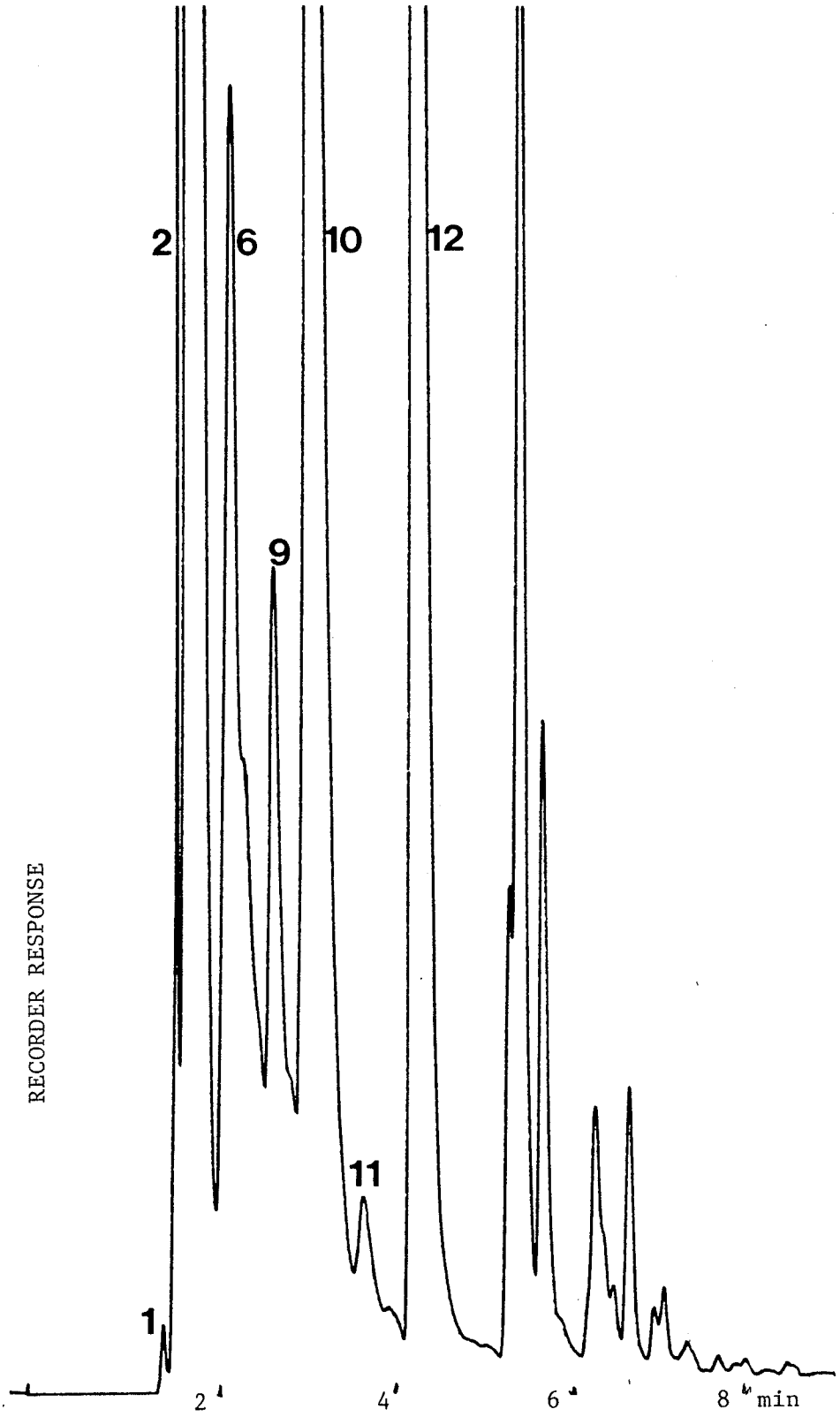

Figure 7. Headspace Analysis. Gas Chromatogram of Esso Regular (Summer) Gasoline WSF.

(See Figure 6 for identification of peaks)

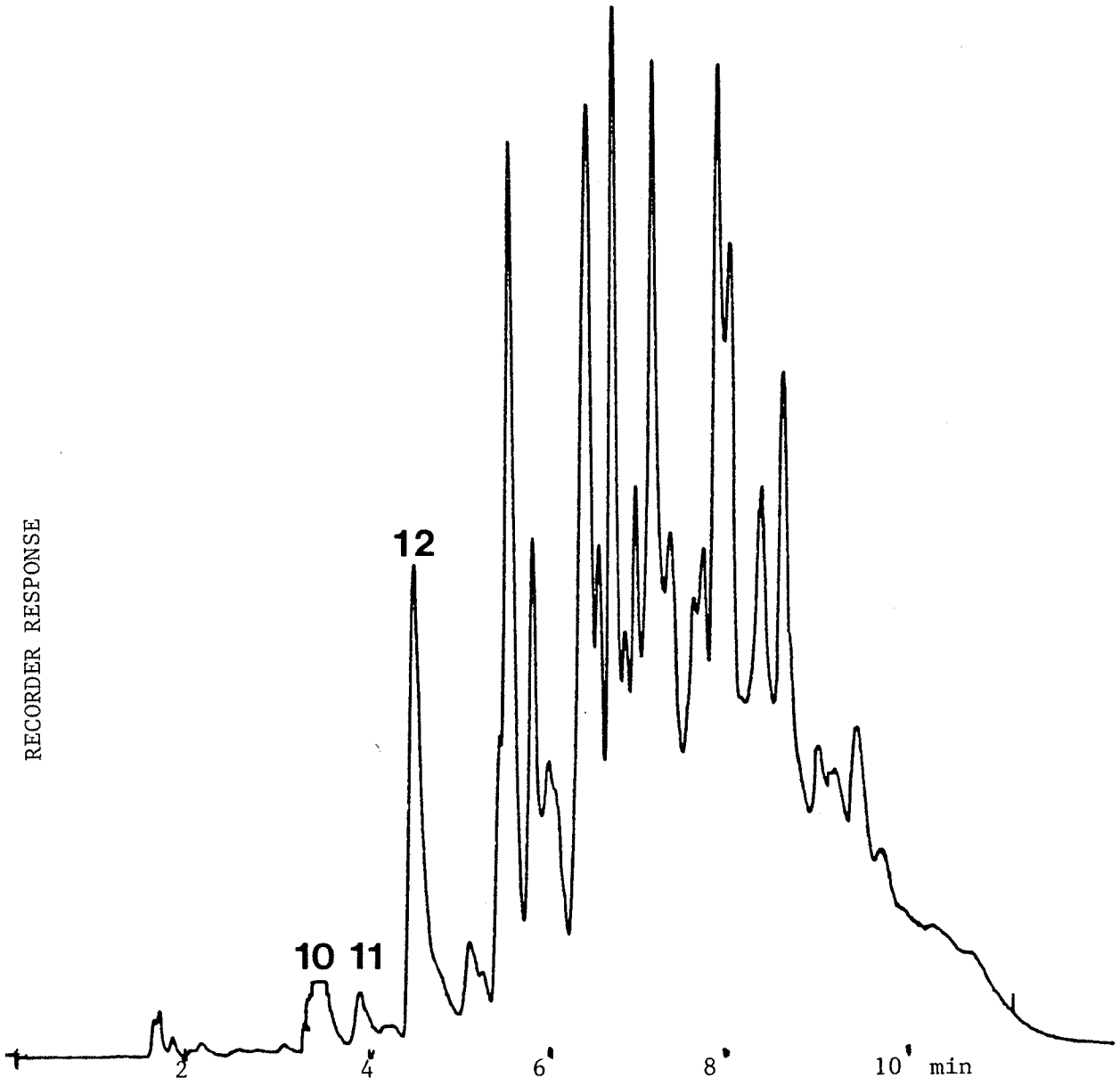

Figure 8. Headspace Analysis. Gas Chromatogram of Gulf P 40 Diesel Oil WSF.

(See Figure 6 for identification of peaks)

Microextraction Analysis

When a solvent has been used to extract dissolved organic material from an aqueous solution, the solvent phase usually requires concentration to a small volume for GC analysis. When the extracted material is volatile with boiling points up to 250 C, significant losses occur with conventional concentration techniques (rotary evaporator, blow down with nitrogen) (Murray 1984). The microextraction flask as shown in Figure 2 was conceived to solve this problem by extracting a large volume of water with a small volume of solvent which could be used directly for GC analysis with no concentration step. Using a 1 L extraction flask it has been shown (Murray and Lockhart 1984) that 30 - 40 % of selected petroleum hydrocarbons were extracted into 1 mL of hexane. With a 100 mL extraction flask the recoveries improve to a mean value of 73.5 % when extracted with 400 uL of hexane. This improvement is the result of a four-fold increase in the solvent to water ratio using the smaller flask. Distribution coefficients have been shown to be independent of concentration of the solute but dependent on the solvent system (Beroza and Bowman 1966). In a solvent/water system the distribution coefficient increases with increasing volumes of solvent (Thrun et al 1980) and the theory of extraction has been developed by Rhoades and Nulton (1980) from the basic Nernst equation to show the amount extracted to be dependent on the solvent/water ratio.

Two microextracts of a Norman Wells WSF were prepared and analysed by injecting 1 uL of each extract three times. Statistical analysis of the six sets of figures produced RSDs for each peak ranging from 2.62 - 8.98 % with a mean RSD of 5.31 % showing the precision of the method. Actual analyses of WSFs by the microextraction method were made by preparing one extract and averaging the measurements from two chromatograms.

The chromatogram shown in Figure 9 is a microextract of Norman Wells Crude WSF and is typical of the pattern of aromatics in a WSF prepared from a crude oil. The numbered peaks have been identified by comparison with retention data of known standards of C2, C3, C4 benzenes and naphthalenes and later confirmed by GC/MS. This pattern of peaks where C2 benzenes were followed by C3 benzenes, C4 benzenes, naphthalene and the 2- and 1-methylnaphthalenes is repeated in extracts of all the crude oils, diesel oils, fuel oils and gasoline, although with some quite significant quantitative differences. The microextract gas chromatogram of gasoline WSF (Fig.10) shows the same pattern but with a large naphthalene peak (No.26). Figure 11 is a chromatogram of a P40 diesel oil WSF and demonstrates the more complex pattern of peaks in the later part of the chromatogram. Peaks in this area have been tentatively identified in this study as dimethyl-naphthalenes by comparison of retention data of known compounds; this interpretation is consistent with that of Kappeler and Wuhrmann (1978). This is similar to the fuel oil WSF chromatogram (Fig.12) where a number of peaks appear in the dimethyl-naphthalene area. Diesel and fuel oils appear to contain relatively high concentrations of naphthalene and substituted naphthalenes.

All the crude oils and the gasoline produced essentially the same pattern of major peaks in both the headspace and microextract chromatograms. The pattern of peaks before and after the cut-off point can be recognised on both chromatograms and a complete analysis obtained by adding the results of the two analyses.

The results given in Table 3 are a summary of Tables 4 and 5 and the numbers in the first two columns represent a total of the hydrocarbons as measured by headspace and microextraction techniques respectively. The third

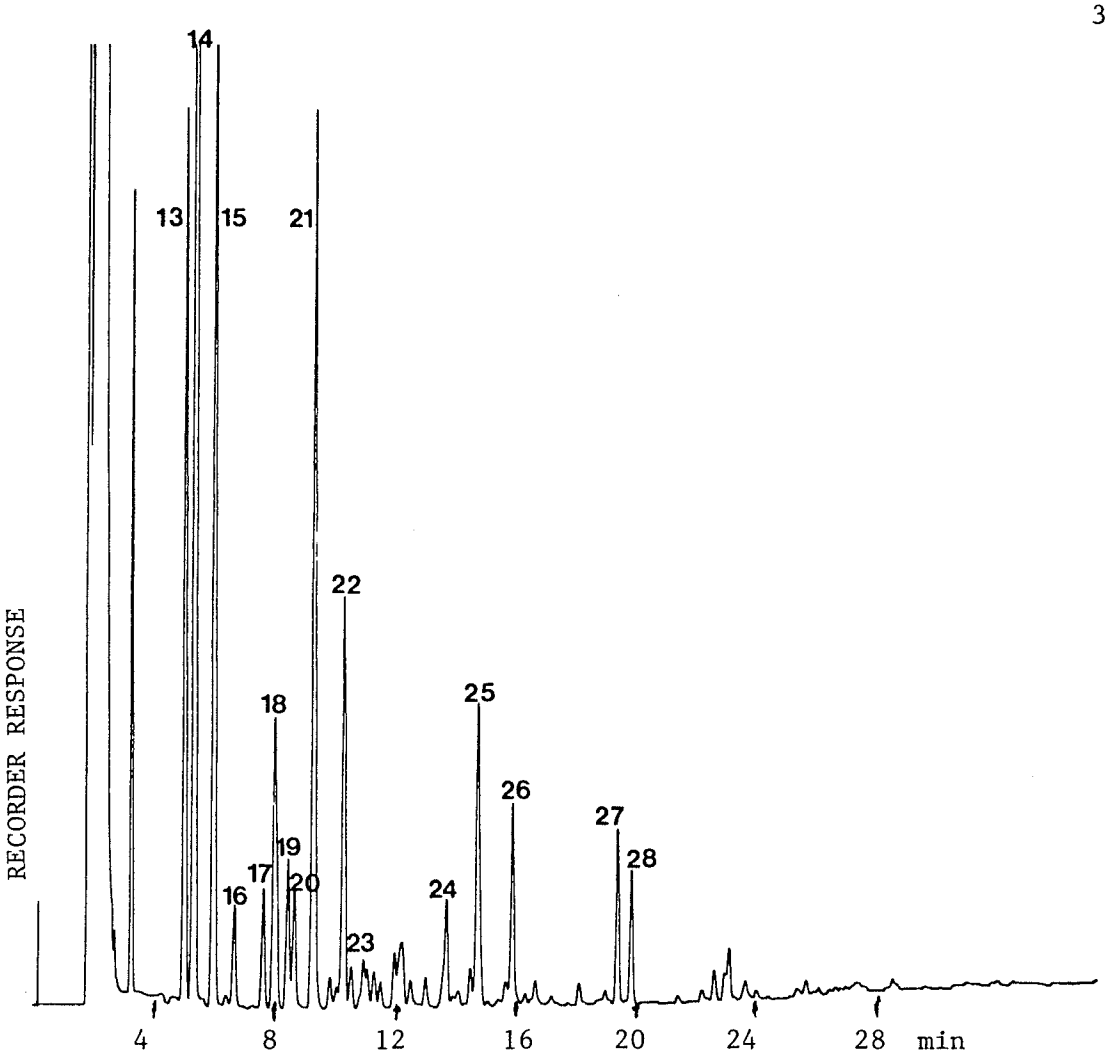

Figure 9. Gas Chromatogram of Norman Wells WSF.

13, ethyl benzene; 14, m- and p-xylene; 15 o-xylene;

16, iso-propyl benzene; 17, n-propyl benzene;

18,19,20,21,22,23, C_3 benzenes; 24,25, C_4 benzenes;

26, naphthalene; 27, 2-methyl naphthalene;

28, 1-methyl naphthalene.

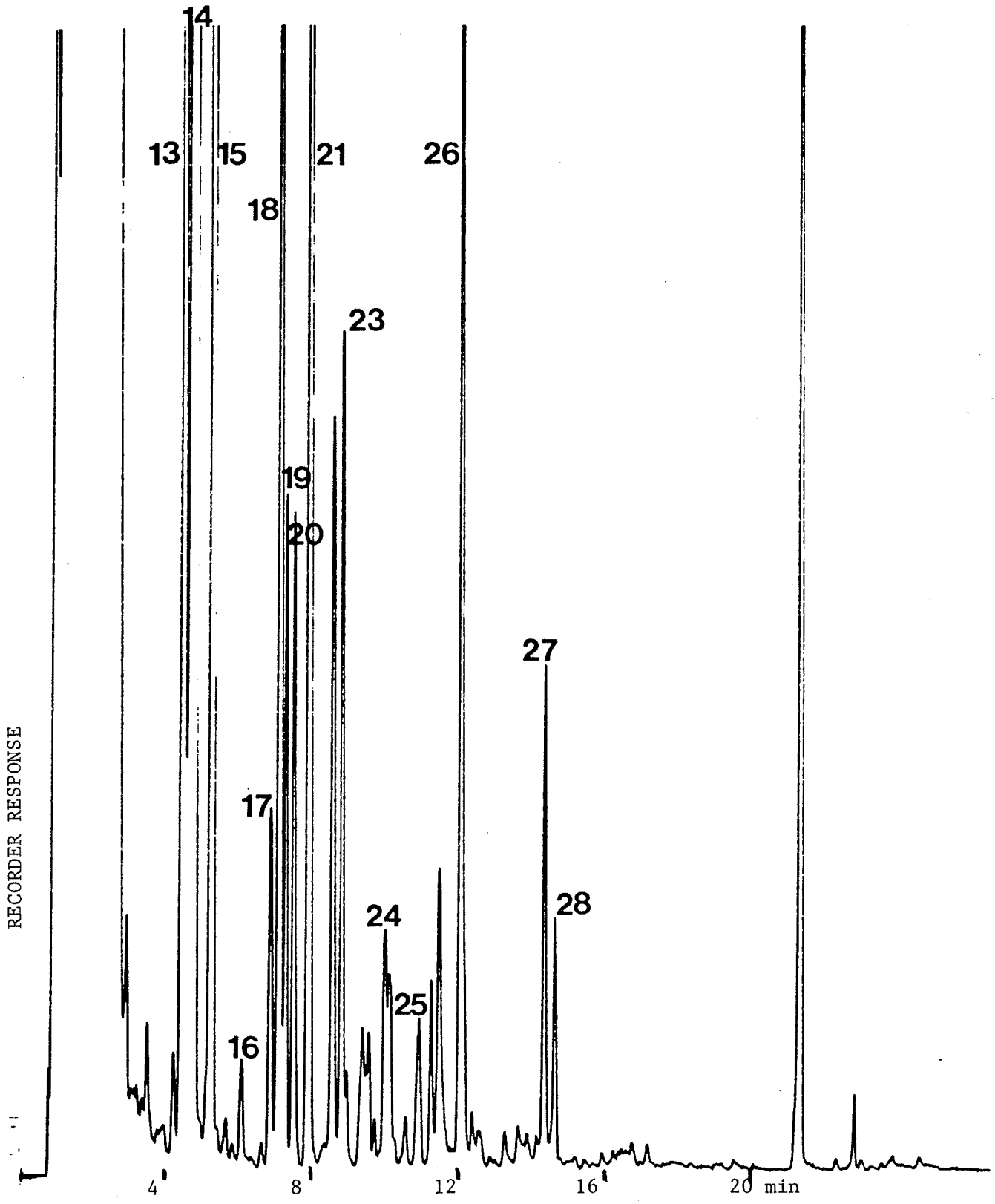

Figure 10. Microextraction Analysis. Gas Chromatogram of Esso Regular Gasoline WSF.

(See Figure 9 for identification of peaks)

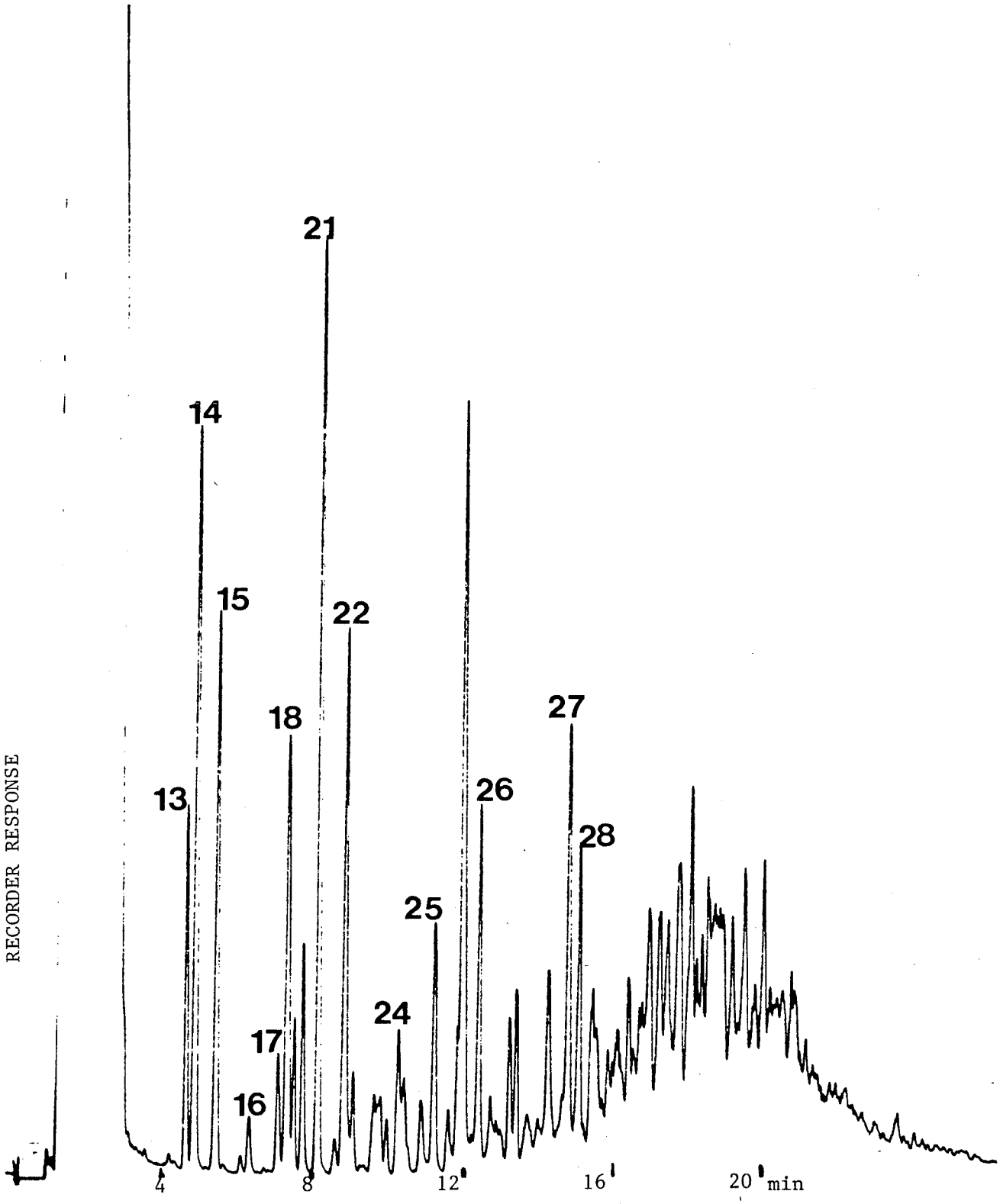

Figure 11. Microextraction Analysis. Gas Chromatogram of P 40 Diesel Oil WSF.

(See Figure 9 for identification of peaks)

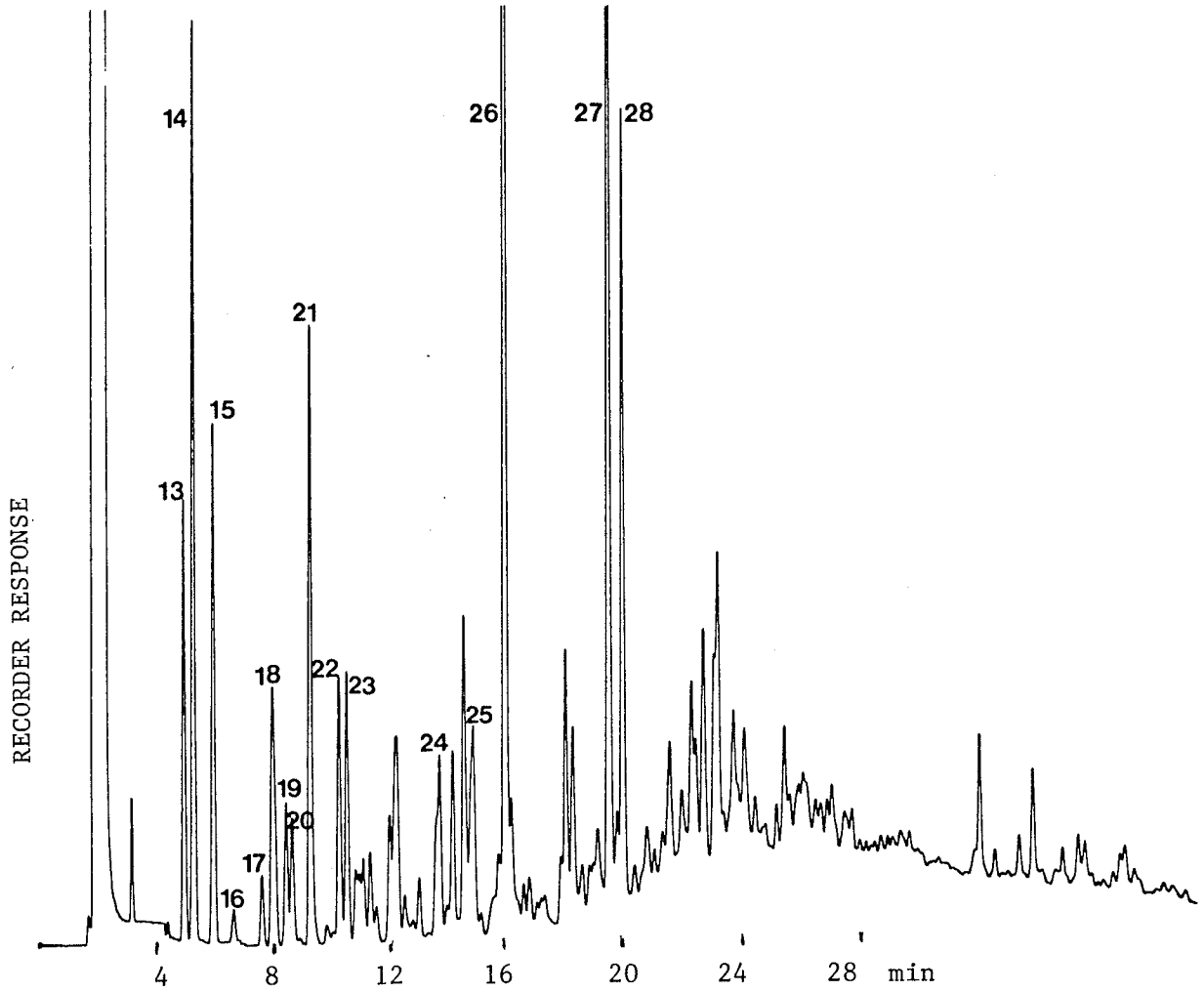

Figure 12. Microextraction Analysis. Gas Chromatogram of #2 Fuel Oil WSF.

(See Figure 9 for identification of peaks)

Table 3. Summary of Analyses for Hydrocarbons in WSFs by Headspace and Microextraction Techniques. (mg/L aqueous solution)

Oil	Headspace	Microextraction	Total
Kuwait Crude	31.5	3.8	35.3
S. Louisiana Crude	33.6	4.3	37.9
G.C.O.S.[a] Synthetic	26.3	6.0	32.3
Saskatchewan L.S.B.[b]	65.6	6.5	72.1
Norman Wells Crude	53.4	6.7	60.1
Wainwright Crude	40.9	5.1	46.0
Alberta Sweet Blend	56.0	7.5	63.5
Syncrude Synthetic	38.2	5.5	43.7
Esso Regular Gasoline	155.6	31.1	186.7
Venezuelan Bunker C	0.0	1.7	1.7
Gulf P20 Diesel	0.0	2.3	2.3
Gulf P40 Diesel	0.6	7.7	8.3
#2 Fuel Oil	3.2	6.1	9.3

a. Great Canadian Oil Sands

b. Light Sour Blend

column is the sum of the headspace and microextraction analyses and presents an overall total for the measured hydrocarbons in a WSF. The numbers in the first column represent concentrations of the extremely volatile compounds which would normally be the first lost to the atmosphere during the "weathering" process. They would not remain in the WSF sample unless precautions were taken to preserve them in solution. Only the benzene and toluene results were corrected using a 20 % recovery factor. The diesel and fuel oils showed low concentrations of volatiles while Bunker C appeared to contribute no volatile material to the WSF as shown by headspace analysis. Headspace analysis of gasoline gave the highest concentration of volatiles which reflects both the properties and use of the material.

Table 4 gives a more detailed picture of the volatiles in the WSFs as measured by headspace analysis. In several cases among the early peaks, overlapping occurred; they were not integrated separately and a total for the two peaks was obtained. Each result was the mean of two determinations and identification of the peaks in the headspace chromatogram was carried out by comparison of retention times of known standards.

The results from the microextraction analyses in Table 3 column 2, range from 1.7-7.7 mg/L with the exception of gasoline which was 31.1 mg/L. Gasoline has the potential to contribute a greater amount of material to a WSF. Table 5 gives a detailed analysis of the compounds in the microextraction chromatogram identified from retention data of known compounds and by a separate GC/MS identification using a WSF prepared from Norman Wells Crude oil. In Figure 9, peak 13 was identified clearly as ethyl benzene and peaks 14 and 15 as dimethyl benzenes. Iso-propyl and n-propyl benzenes were peaks 16 and 17 followed by a number of tri-methyl benzenes

Table 4. Headspace Analyses for Hydrocarbons in WSFs

(mg/L aqueous solution) [a]

Oil	Peak Numbers											
	1	2	3	4	5	6	7	8	9	10	11	12
Kuwait	9.6 [b]		7.5 [c]		1.1	2.4	0.5	0.4	0.1	5.2	0.1	4.6
S.Louis.	1.3	5.4	2.7	3.9	0.9	1.0	0.5	0.0	0.2	11.3	0.2	6.3
G.C.O.S	1.0		7.3		2.0	4.0	1.1	0.5	0.4	5.7	0.1	4.4
Sask.LSB.	12.0		9.2		1.8	3.3	1.1	0.3	0.5	24.1	0.2	13.0
Norman W.	9.1		2.7	11.5	2.1	3.4	1.4	0.5	0.8	14.1	0.3	7.5
Wainwright	0.4	2.0	0.7	3.6	3.5	4.8	1.1	0.4	0.4	15.0	0.2	8.6
Alberta SB.	7.5		1.5	7.7	2.0	3.4	1.1	0.5	0.6	21.1	0.2	10.5
Syncrude	1.3		2.1		10.1	1.7	3.4	1.9	0.5	10.4	0.1	6.5
Reg. Gas	0.0	0.0	0.0	0.8	3.1	2.5		1.8	2.8	70.5	0.2	73.6
Venez.B.C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	5.4
P20 Diesel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P40 Diesel	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
#2 Fuel Oil	0.0	0.1	0.2	0.2		0.0	0.0	0.0	0.0	1.0	0.0	1.6

a. Mean of two determinations.

b. Value for total of peaks 1 & 2 (Overlapping peaks).

c. Highest result for a given peak underlined.

(peaks 18-23). Because of the difficulty of identifying individual isomers by MS, positive identification in this region was not possible. Peaks 24 and 25 were identified as tetramethyl benzenes with no means of naming the particular isomer. Peak 26, however, was identified as naphthalene, and peaks 27 and 28 as 2- and 1- methyl naphthalenes. These GC/MS results were similar to the results of Boylan and Tripp (1971), Winters et al (1976) and Kappeler and Wuhrmann (1978). In the results from the microextraction analysis, gasoline again stands out as being the highest and the most likely to contribute material to a WSF.

The figures given for the microextraction analyses of the diesel and fuel oils and the Bunker C in Table 5 do not represent the total amount present since many peaks were not included. These additional peaks were not present in the crude oil chromatograms. The Venezuelan Bunker C also appeared to be rich in naphthalene and alkylated naphthalenes.

These results suggest that the effects of a crude oil spill in terms of acute toxicity of the WSF may not be as damaging to aquatic life as a spill of gasoline or diesel oil. Gasoline is rich in both the extremely volatile compounds and the less volatile fraction. Diesel oil, #2 Fuel oil and Bunker C contain very little of the extremely volatile fraction but do contain a significant amount of the less volatile fraction. It is not clear which compounds in the WSF are the most toxic but a gasoline WSF could present a greater danger to aquatic life than the WSF from a crude oil. This is because when prepared at the same oil/water ratio, a gasoline WSF contains a two to three fold greater concentration of soluble compounds than a crude oil WSF (Table 3).

Table 5. Microextraction Analyses for Hydrocarbons in WSFs (mg/L aqueous solution) [a]

Oil	Peak Numbers															
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Kuwait	0.5	1.3	0.7	0.0	0.1	0.3	0.1	0.2	0.3	0.1	0.0	0.0	0.1	0.1	0.0	0.0
S.Louis.	0.4	1.7	0.7	0.1	0.1	0.2	0.1	0.1	0.3	0.2	0.0	0.0	0.0	0.2	0.1	0.1
C.C.O.S.	0.6	2.2	1.1	0.1	0.1	0.4	0.1	0.2	0.5	0.3	0.2	0.1	0.1	0.1	0.1	0.0
Sask.LSB.	1.4	2.1	1.0	0.1	0.1	0.4	0.1	0.2	0.4	0.2	0.1	0.1	0.1	0.2	0.1	0.1
Norman W.	0.7	2.4	1.0	0.1	0.1	0.4	0.1	0.1	0.8	0.3	0.0	0.1	0.2	0.1	0.1	0.1
Wainwright	0.4	2.6	0.9	0.0	0.0	0.2	0.1	0.1	0.3	0.1	0.0	0.0	0.0	0.1	0.1	0.0
Alberta SB.	0.9	2.7	1.3	0.1	0.1	0.4	0.2	0.2	0.7	0.3	0.1	0.1	0.2	0.2	0.1	0.1
Syncrude	0.7	1.9	1.0	0.0	0.1	0.4	0.1	0.2	0.4	0.2	0.3	0.1	0.1	0.1	0.1	0.1
Reg. Gas	3.5	7.2 [b]	5.0	0.3	0.7	3.0	1.0	0.9	3.9	1.0	0.9	0.3	1.1	1.5	0.7	0.4
Venez.B.C	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.4	0.5	0.4
P20 Diesel	0.1	0.3	0.2	0.0	0.0	0.2	0.1	0.1	0.3	0.1	0.1	0.1	0.3	0.2	0.3	0.2
P40 Diesel	0.4	0.9	0.7	0.1	0.1	0.6	0.2	0.3	1.2	0.7	0.1	0.3	1.1	0.5	0.4	0.3
#2 Fuel Oil	0.4	0.7	0.4	0.0	0.1	0.3	0.1	0.1	0.5	0.2	0.2	0.2	0.2	1.2	0.9	0.6

a. Mean of two determinations.

b. Highest result for a given peak underlined.

The two GC procedures and the standard IR method for oil and grease (ASTM D3921-80) were compared by analysing the same Norman Wells WSF before and after a 10 min period of aeration at 2 L/min (Table 6). The object of this experiment was to compare the methods and to show how the different methods explained the effects of aerating a WSF. The IR method before aeration yielded a much lower result than the GC total figure. The extraction procedure used for the IR analysis did not appear to recover the extremely volatile fraction as measured by the GC headspace technique. The IR analysis of the aerated Norman Wells WSF gave a higher result than the microextraction procedure, but this difference could be due to non-volatile material in the WSF. The most revealing observation to be drawn from this data was that after only 10 min sparging, 96 % of the dissolved material had been lost to the atmosphere. This would suggest that aeration might be a convenient way of alleviating the effects of a WSF when carried out on a large scale.

The simple weight determination of volatiles demonstrates the wide range of volatiles in the various crude oils and petroleum products tested. The results in Figure 13 show the high percentage of volatiles in a gasoline as well as how quickly they are lost. The crude oils contain the lowest concentration of volatiles leveling off at about 40% or less. Fuel oil appears to contain about 50% volatile material and diesel oil about 80%.

The investigators mentioned in Table 1 in the introduction prepared their WSFs in different ways and reported the toxicity of their WSFs to the various species tested. They analysed their WSFs by UV, IR or Sluorescence spectroscopy. These observed toxicities do not appear to be associated with

Table 6 Comparison of Gas Chromatographic and IR Spectrometric Analyses of Norman Wells WSF before and after Aeration. (mg/L aqueous solution) [a]

	GC Analyses			IR Spectrometry
	Headspace	Microextraction	Total	
Norman Wells WSF	33.0	4.4	37.4	16.5
Norman Wells WSF Aerated 10 min	0.8	0.6	1.4	3.8

a. Mean of two determinations

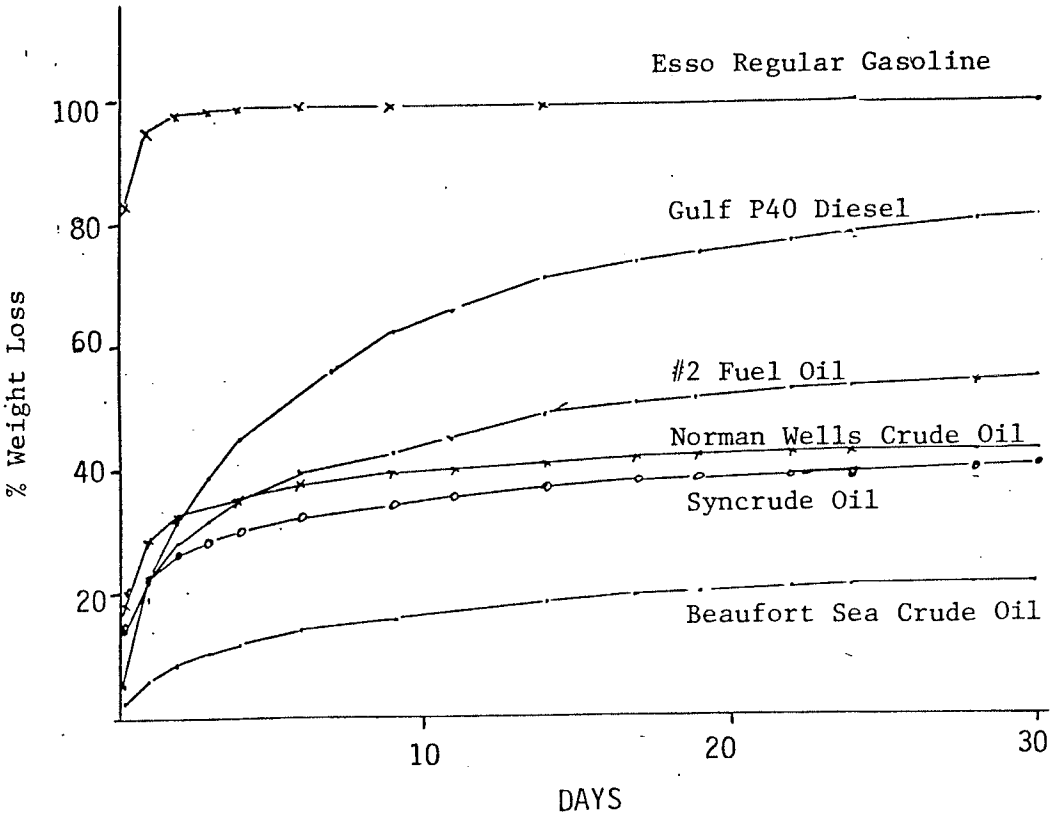

Figure 13. Percent Loss of Weight from Crude Oils, Fuel Oil, Diesel Oil and Gasoline on Standing.

the volatile fraction since no precautions were taken to preserve this material in solution and their analytical techniques were not suited to measure volatile compounds. The toxicity would therefore appear to be associated with the higher boiling components as measured by the microextraction method. This does not rule out however, the possible toxic effects of the volatile components as measured by headspace analysis particularly benzene and toluene. Alternatively the observed toxicities may be due to other compounds better measured by more specific detectors than the FID such as the AFID for nitrogen containing compounds. However the toxicities of some single compounds found in a WSF have been measured by Bobra et al (1983) using a closed system designed to preserve the original concentrations of these volatile toxic compounds.

Examination of the data from the Tables 4 and 5 and comparison of the chromatograms of the various crude oils gave the clear impression that the WSF's were qualitatively very similar, with differences in the relative concentrations of some compounds. Diesel and fuel oils had the same basic pattern of peaks as the crude oils but also had many extra peaks (Fig.12). Other than a comparison of four fuel oils by Winters et al (1976), no systematic comparison of WSF's from a range of crude oils and refined petroleum products had been prepared prior to this present study.

Fish Tainting

The problem of fish tainting was investigated from two points of view. The first was to subject laboratory reared rainbow trout to different chemicals as well as crude and diesel oils at various concentrations. The second was to analyse fish muscle samples which had been exposed to "natural" oil-related exposures. The chromatograms shown in Figures 14 - 19 have been derived by analysis of fish muscle tissue samples using the purge and trap technique. The peaks represent the volatile contaminants from the fish muscle sample.

Recoveries were measured by spiking a 15 g fish muscle with 0.5, 1.0 and 2.5 ug each of selected volatile organic compounds (equivalent to 33, 66 and 167 ppb) and measuring the amount recovered in a 30 min purge and trap. Recoveries were 80.7 % for iso-propyl benzene, 65.2 % for n-propyl benzene, 67.4 % for 1,3,5-trimethyl benzene, 69.8 % for iso-propyl methyl benzene, 63.0 % for n-butyl benzene and 51.2 % for n-hexyl benzene. Statistical analysis of the ratios of these selected organic compounds to the internal standard used to determine the recoveries for the purge and trap method gave a range of 0.81 - 4.47 for the % RSD for each peak. Given a signal to noise ratio of 5, the minimum detectable concentration for single compounds was about 5 ppb (ng/g).

The results shown in Table 7 give the sensory evaluation and the total hydrocarbon analysis of fish samples which had been exposed to hydrocarbons in various ways in the laboratory and these results have not been corrected for recoveries. Figure 14 is a composite of three chromatograms. The bottom chromatogram is of Norman Wells WSF and the top one is from a control rainbow trout muscle. The centre chromatogram is derived from the treated rainbow

Table 7.[a] Taste Panel Results and Chemical Analyses of Control and Treated Rainbow Trout Muscle Samples.

Dosing Conditions	Sensory Evaluation	Total hydrocarbon Analysis (ug/g fish)[b]
Control	Acceptable flavor	0.139
4h in 100 ppm Norman Wells Crude Oil	Petroleum Oil flavor Weak - moderate	0.481
Control	Acceptable flavor	0.021
4h in 1 ppm each 6 volatile hydrocarbons	Diesel-like flavor Moderately intense	2.289
4h in 1 ppm each 5 volatile hydrocarbons	Chemical flavor Moderately intense	1.898
4h in 50 ppm Diesel Oil	Chemical-Oil flavor	0.181

a. See Appendix. P 62 & 64

b. Mean of two determinations.

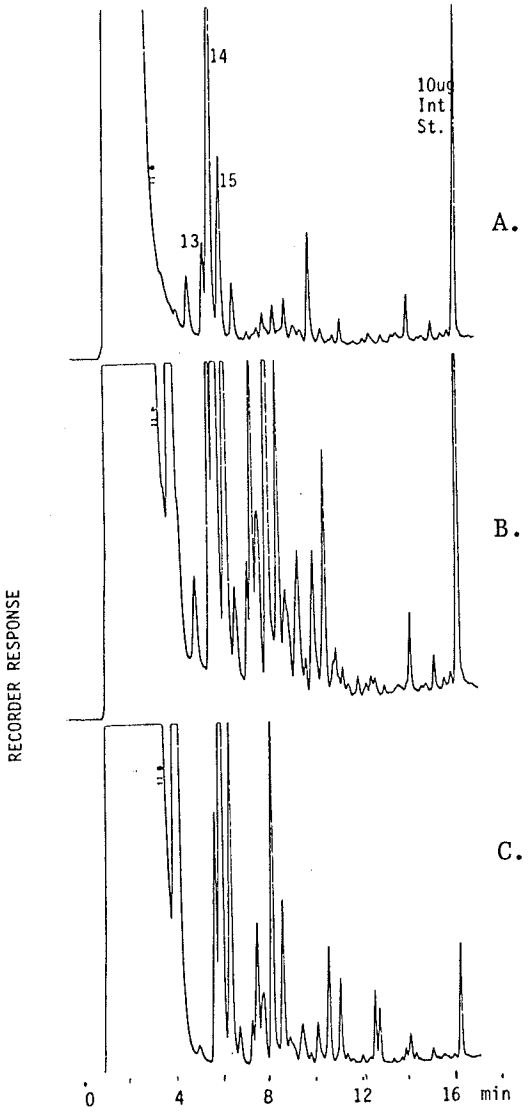

Figure 14. Tissue Analysis Gas Chromatograms.

A. Rainbow Trout Muscle Control

B. Rainbow Trout Muscle treated with 100 ppm Norman Wells Crude Oil

C. Norman Wells Crude WSF.

trout muscle and shows the enhanced pattern of peaks from the Norman Wells WSF. The control fish muscle also shows the presence of ethyl benzene and the xylenes which were contaminants in the original bottle of carbon disulphide solvent. A fresh supply of solvent was much cleaner and showed almost no contamination. However these peaks are much enhanced in the treated sample and occur along with the other hydrocarbon peaks from a typical WSF. This 4 h exposure of fish to a Norman Wells WSF demonstrates how rapidly the muscle could be contaminated by soluble compounds from a crude oil.

Figure 15 shows a chromatogram of a rainbow trout which had been treated for 4 h in water with benzene, toluene, ethyl benzene, o-, m-, and p-xylene at concentrations of 1 ppm each. The bottom chromatogram is a control rainbow trout muscle. The enhanced early peaks can be seen, again demonstrating how quickly these contaminants can be transferred from the water to the fish muscle. This rapid uptake indicates that the mode of transfer was through the gills and that we might expect some tainting to be caused by water soluble material. Benzene is obscured by the solvent peak but the remainder of the compounds appear in the gas chromatogram.

The chromatograms shown in Figure 16 are from rainbow trout which had been exposed for 4 h in water containing 50 ppm of diesel oil and 1 ppm each of iso-propyl benzene, n-propyl benzene, 1,3,5-trimethyl benzene, iso-propylmethyl benzene and n-butyl benzene. The top chromatogram clearly shows the enhanced five peaks while the bottom one shows the pattern of aromatic compounds from a WSF. The low molecular weight volatile compounds appear to invade the rainbow trout muscle rapidly but few peaks appear in the later part of the chromatogram.

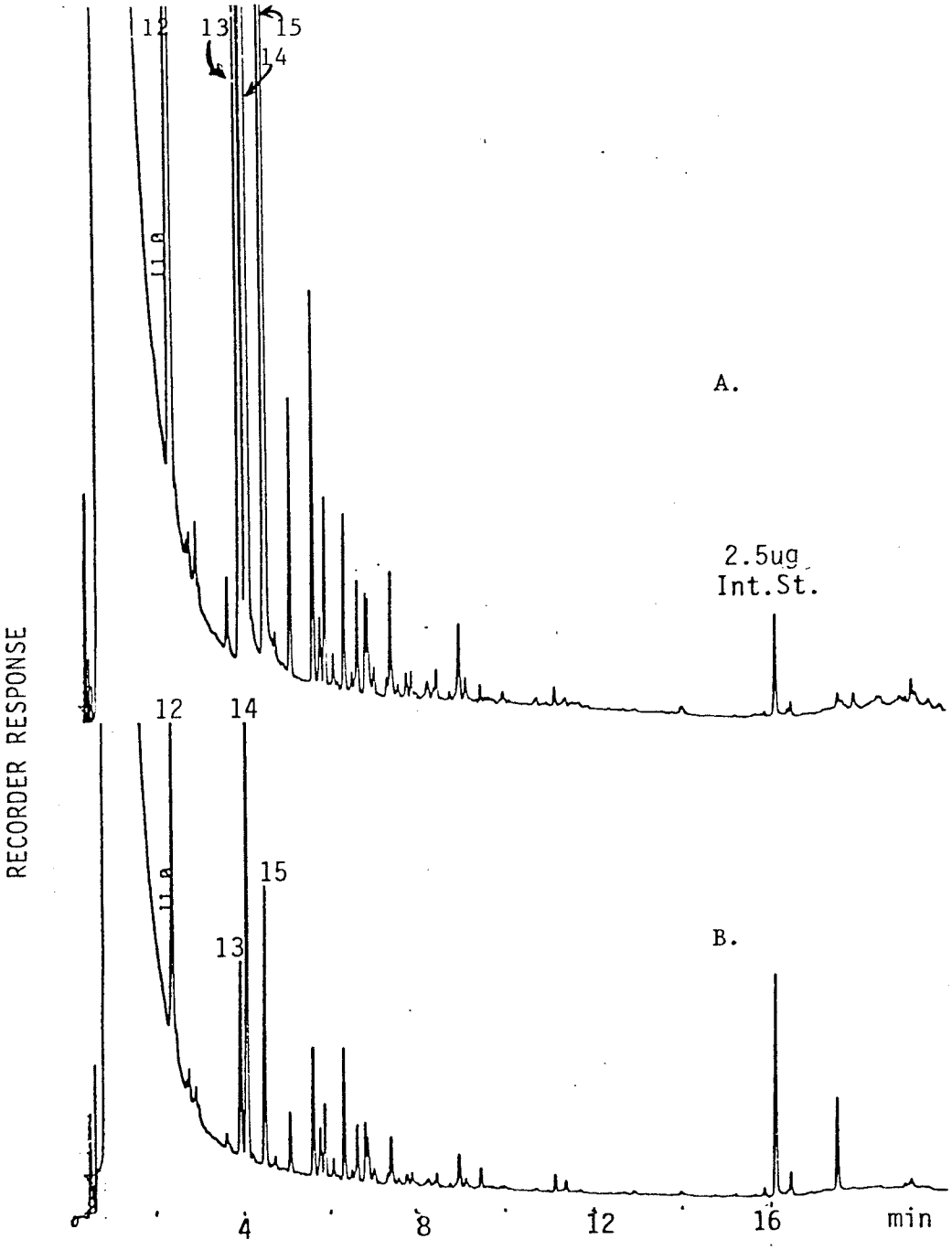

Figure 15. Tissue Analysis Gas Chromatograms.

A. Rainbow Trout Muscle treated with 1 ppm of 10, benzene; 12, toluene; 13, ethyl benzene 14, m-xylene; and 15, o-xylene.

B. Rainbow Trout Muscle Control.

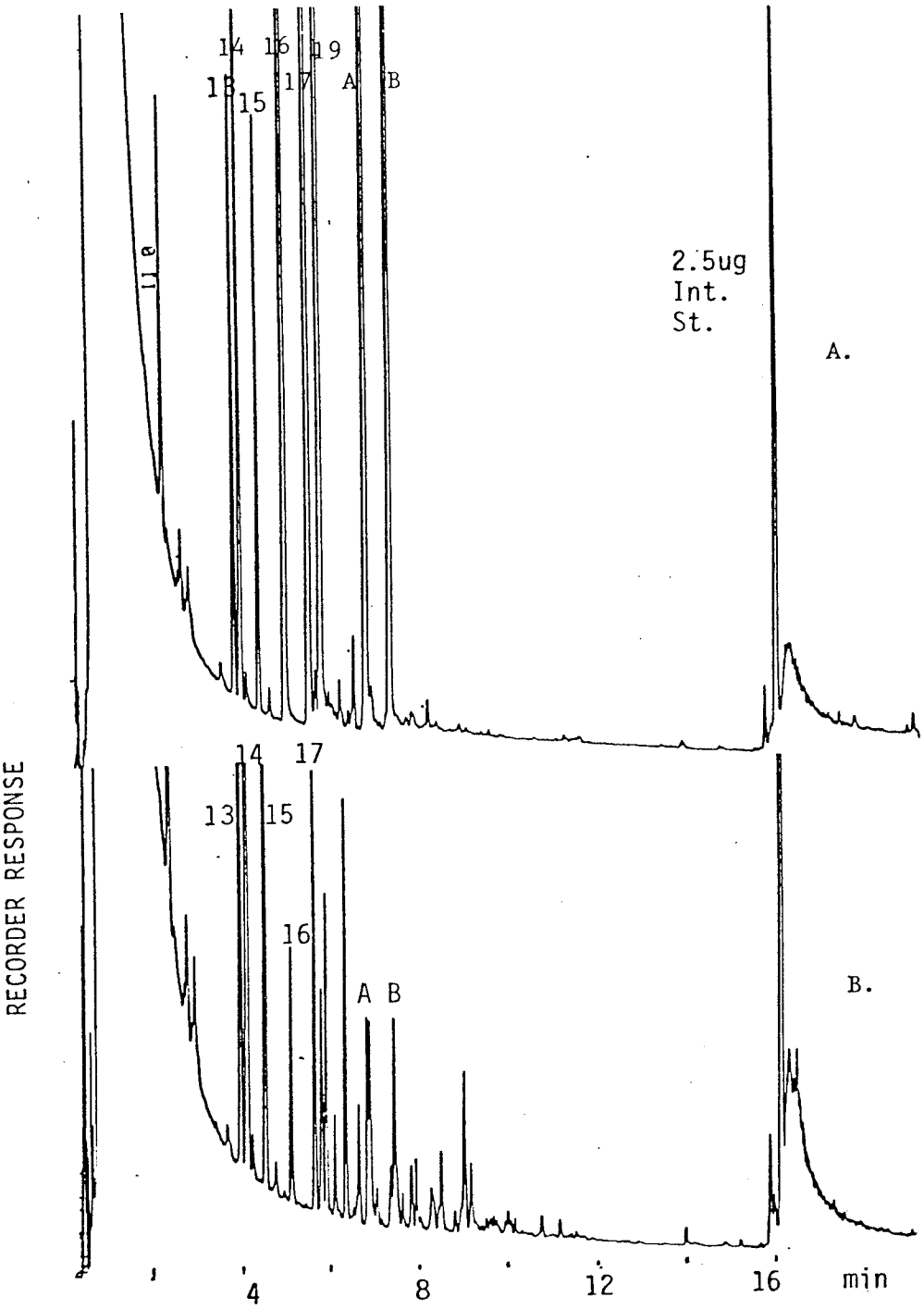

Figure 16. Tissue Analysis Gas Chromatograms.

A. Rainbow Trout Muscle treated with 1 ppm of 16, iso-propyl benzene; 17, n-propyl benzene; 19, 1,3,5,trimethyl benzene; A, iso-propyl methyl benzene and B, n-butyl benzene.

B. Rainbow Trout Muscle treated with 50 ppm of P40 Diesel Oil.

The data presented in Table 8 come from the fish samples connected with "natural" oil spill events. The Fort Good Hope whitefish and burbot were passed as "fit for human consumption" by a trained taste panel with some low level off-flavors. The hydrocarbon analysis detected some contamination, ranging from 6 - 234 ppb total hydrocarbons for the whitefish and 9 - 30 ppb for the burbot. Control whitefish and burbot muscle samples gave 9 and 6 ppb total hydrocarbons respectively. The chromatograms shown in Figures 17 and 18 are of muscle samples from Fort Good Hope and illustrate the type of contamination seen in these whitefish and burbot. The large peak at the end of the chromatograms represents 5 ug of the internal standard, n-decyl benzene. It would appear that the GC analysis was now at least as sensitive as the sensory evaluation technique since low concentrations of organic chemicals can be measured in fish passed as fit for human consumption.

The Cameron River whitefish were condemned as "unfit" with a lingering kerosene aftertaste. Purge and trap analysis of these fish tissues gave a range of 145 - 889 ppb of total hydrocarbons. Figure 19 is a composite of chromatograms of muscle samples of six Cameron River whitefish and shows a different pattern of contamination. Almost none of the early volatile WSF peaks are present and the later peaks visible in the chromatogram correspond to higher molecular weight compounds. This material would probably be less soluble in water than the early volatiles from a WSF and these peaks present an interesting problem as to their mode of entry into the fish. One possibility might be through the digestive system where the fish consumed contaminated food rather than through the gill system. This interpretation might be linked with the taste panel's observation of a lingering after-taste of kerosene.

Table 8. Taste panel Results[a] and Chemical Anlaysis of Contaminated Fish Muscle Samples.

Fish Source	Total Hydrocarbon Analysis (ug/g fish)[b]	Sensory Evaluation
Control whitefish	0.009	-
Control burbot	0.006	-
Fort Good Hope		
Whitefish	0.006 - 0.231	Low levels of off-flavors but fish were fit for human consumption.
Burbot	0.009 - 0.030	
Cameron River		
Whitefish	0.145 - 0.889	Kerosene flavor with lingering after-taste. Not fit for human consumption.

a. See Appendix P 62

b. Mean of two determinations

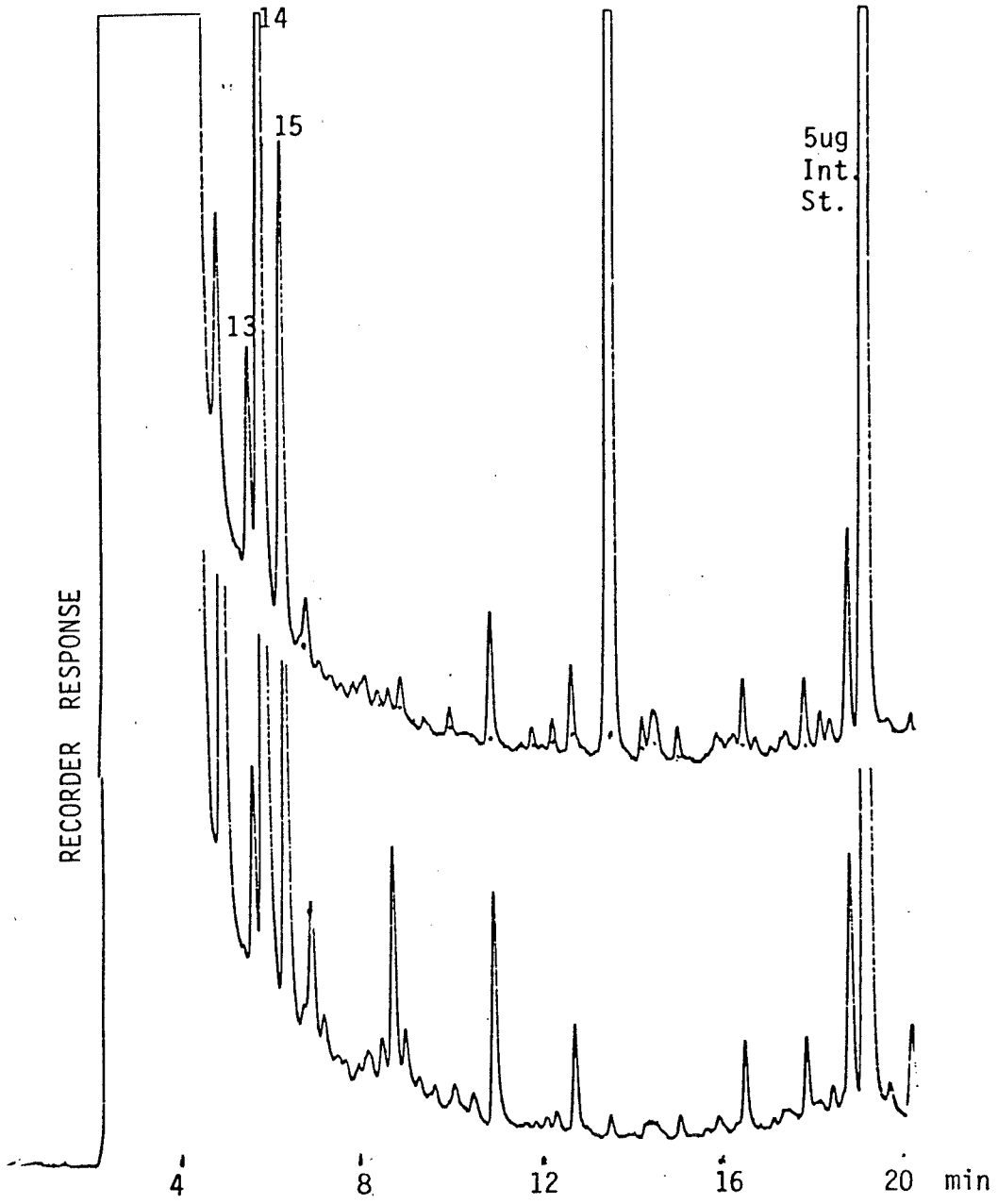

Figure 17. Tissue Analysis Gas Chromatograms
Fort Good Hope Whitefish Muscle Samples, showing range and type of contaminating peaks. These samples were judged fit for human consumption.

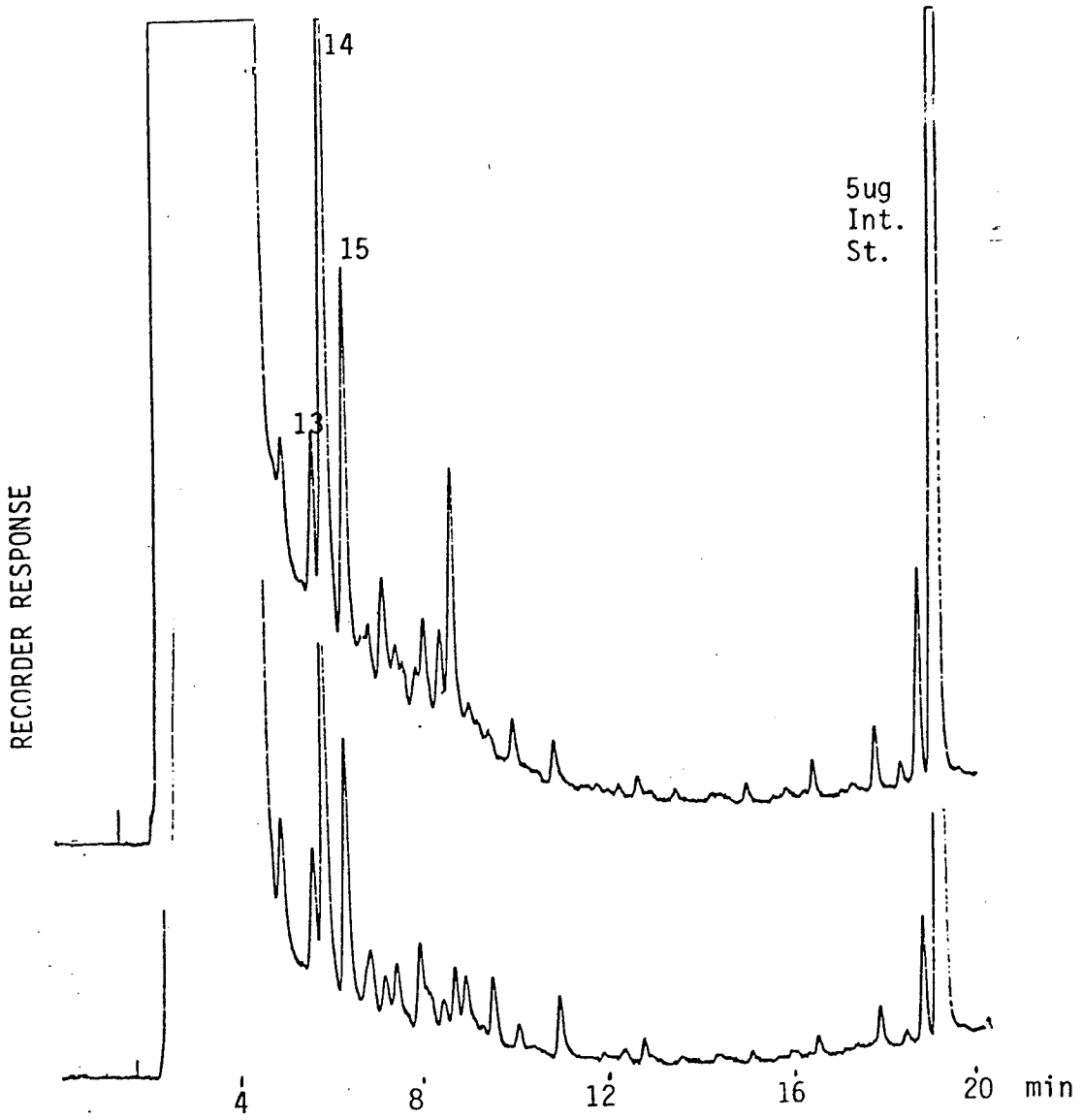

Figure 18. Tissue Analysis Gas Chromatograms.
Fort Good Hope Burbot Muscle Samples, showing range and type of contaminating peaks. These samples were judged fit for human consumption.

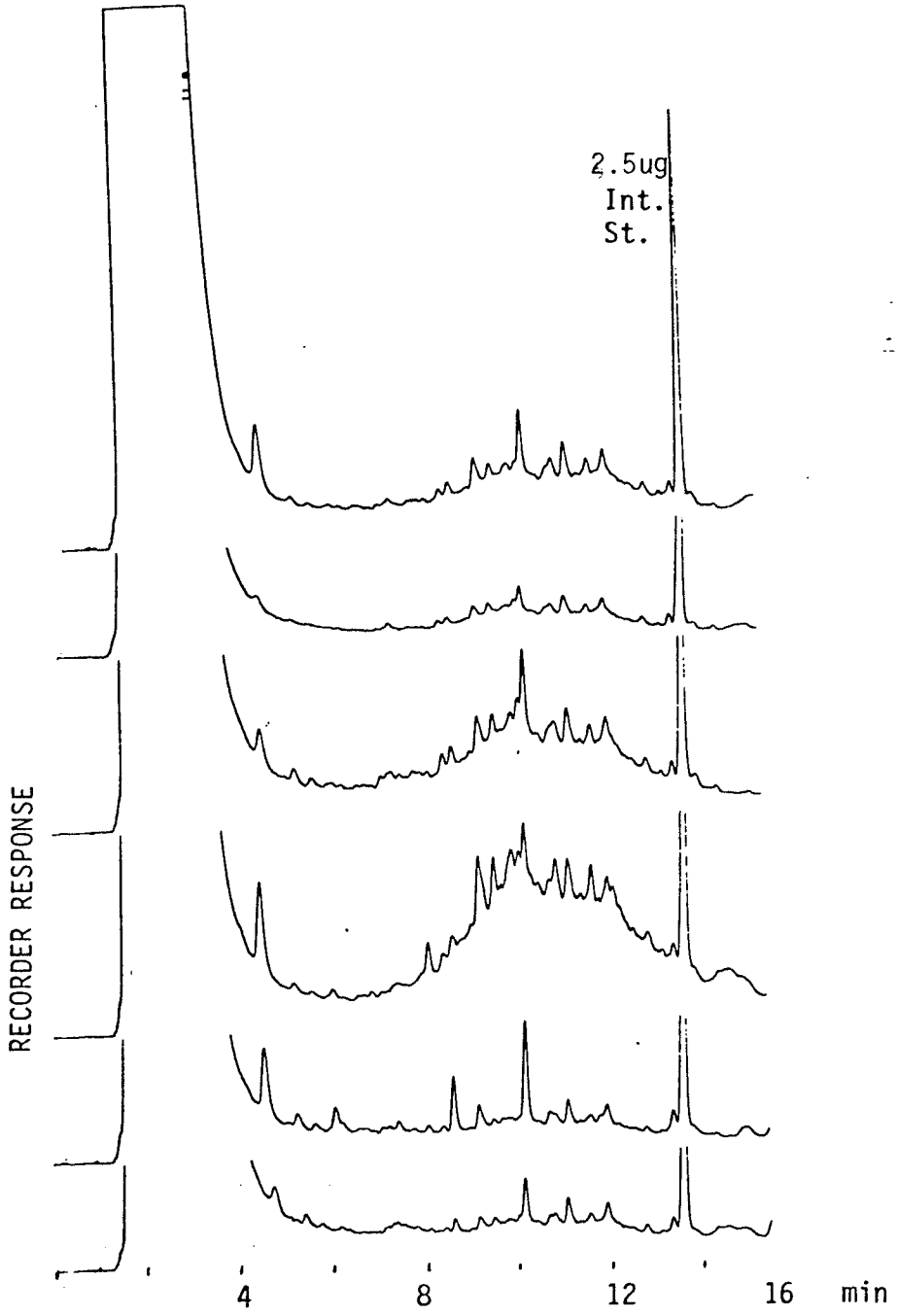

Figure 19. Tissue Analysis Gas Chromatograms. Cameron River Whitefish Muscle Samples, showing range and type of contaminating peaks. These samples were judged unfit for human consumption.

It has been suggested that both losses and chemical changes might occur during the cooking process for taste panel evaluations, so that the taste panel would not detect the same material as the GC analysis. However, the hydrocarbon analysis, although starting with a raw frozen sample, involves heating to 70 C for 30 min which also has the effect of cooking the fish muscle.

Phenol Analysis

An attempt was made to determine phenols using the method of Coutts et al (1979). The chromatograms in Figure 20 show the analysis of Norman Wells WSF and some standard phenols and serve to demonstrate the complex nature of the derivatised extract. Only phenol appears to be present in a significant amount and without the aid of GC/MS available at hand it would be difficult to identify the other peaks present.

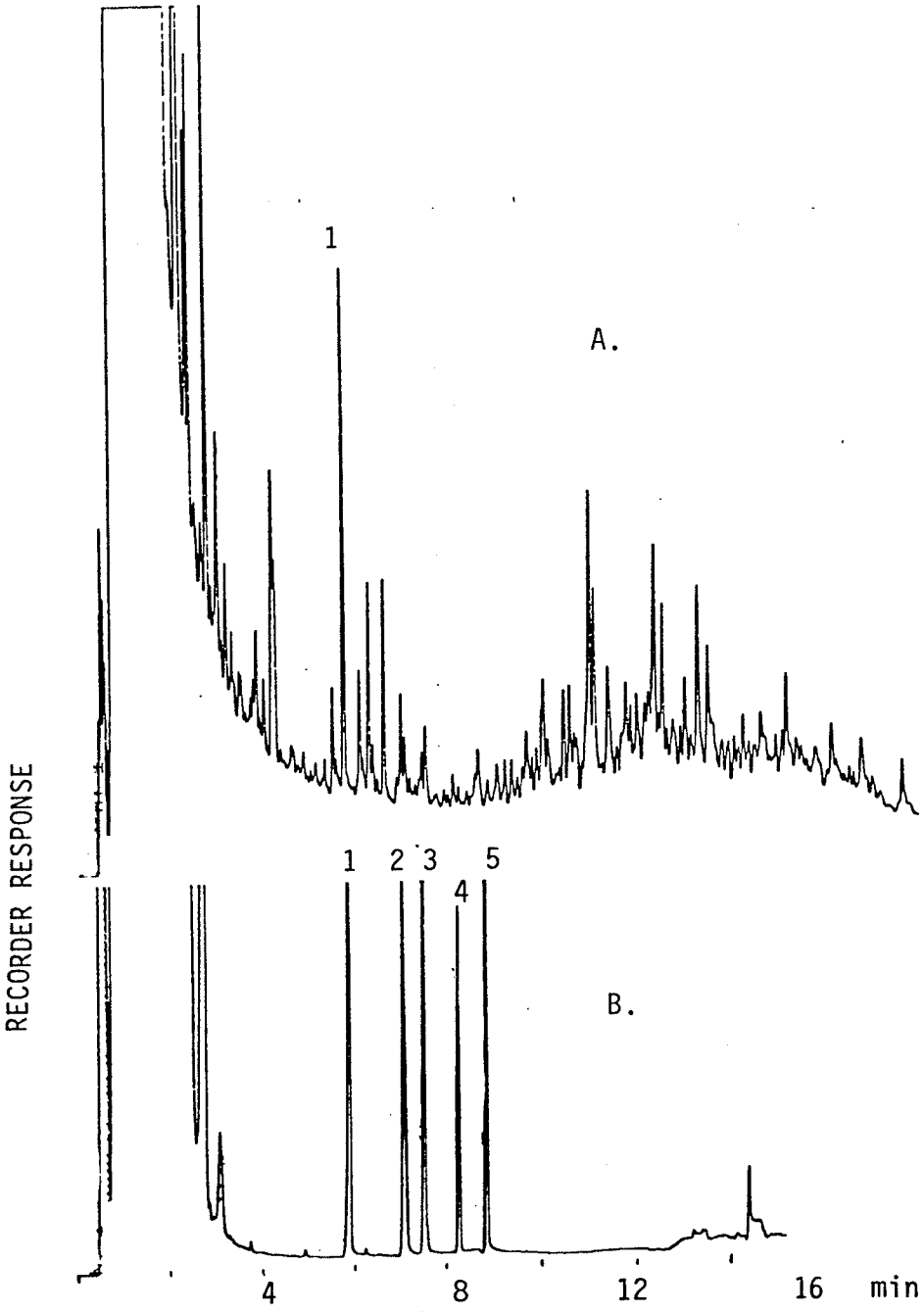

Figure 20. Gas Chromatograms of Phenol Analysis.

A. Norman Wells WSF.

B. Phenol Standards.
1, phenol; 2, o-cresol; 3, m-cresol;
4, 2-ethyl phenol; 5, 3-ethyl phenol.

IV. CONCLUSIONS

No one analytical technique could measure all the components in a WSF prepared from a crude oil or petroleum product. Headspace analysis was particularly suitable to measure the extremely volatile fraction while a solvent extraction was preferable for the higher boiling components.

Crude oils from a wide variety of sources yielded qualitatively similar water soluble fractions as shown by the analytical results from both headspace and microextraction techniques.

Gasoline gave a WSF which contained higher concentrations than the crude oils of both the volatile and the less volatile fractions, while the WSF's from diesel and fuel oils had a more complex pattern of organics in the higher boiling fraction. A reasonable interpretation of this data would be that on purely quantitative grounds, a gasoline spill has the potential to be more acutely toxic than a diesel spill and that both gasoline and diesel could be more toxic than a spill of a similar quantity of crude oil. This would only apply on a short term basis as the weathering process would render a gasoline spill relatively harmless within a few hours.

Fish muscle and liver samples can be analysed using the modified purge and trap procedure to detect less than 5 ppb of total hydrocarbons on a 15 g sample. The results from fish muscle analyses when correlated with taste panel results could lead to a greater understanding of off-flavors in fish and provide a chemical analysis to confirm sensory evaluations. While taste panels are useful in giving an educated opinion regarding off-flavors and odors in fish and other products, a definitive chemical analysis would provide a useful back-up in the event of legal procedings. A further

advantage would be that when a large number of samples required screening for contamination, a chemical analysis could be used to provide rapid results for the hydrocarbons in fish tissues.

There is a need for further study of the lethal and sub-lethal effects of individual compounds known to be in a WSF. Only the recent work of Bobra et al (1983) deals with the problem of volatility while measuring the toxicity of single organic compounds.

A second avenue for investigation would be the taste and odor produced in fish muscle tissue by single organic compounds and the analysis of tainted fish samples to determine which compounds were responsible for off-flavors. Further work is needed to compare recoveries from spiked tissue samples with naturally contaminated fish using labelled compounds and to investigate the possibility of metabolism of organics in the fish tissue and oxidative reactions during the purge and trap step of the analysis.

V. APPENDIXES

SENSORY EVALUATION REPORTS

Government of Canada / Gouvernement du Canada

MEMORANDUM NOTE DE SERVICE

F.J.O. Josephson
Director, Artic Operations

Roberta York
Fishery Products Specialist
Fisheries Development Branch

SECURITY - CLASSIFICATION - DE SÉCURITÉ
OUR FILE/NOTRE RÉFÉRENCE
YOUR FILE/VOTRE RÉFÉRENCE
DATE: January 27, 1984

SUBJECT / OBJET: Sensory evaluation of fish samples from Fort Good Hope area

Samples received: Ten whitefish and nine burbot. The fish were thawed to allow samples to be taken for use in sensory evaluation on December 13th, 1983. At this time the partially thawed samples were wrapped and replaced in frozen storage until used.

Information requested:
Sensory evaluation of fish samples for presence and characterization of any off-flavours which may be present.

Sample treatment: Samples were prepared as individual fillets; homogenized, wrapped and coded using standard procedures. Samples were prepared for sensory panel session by steaming, also according to our standard method.

Sensory panel procedure used:
A laboratory panel of three experienced judges evaluated the samples to obtain a concensus opinion as to the quality of the samples. Extra samples in the form of rainbow trout which had been exposed to an extract of crude oil and a matching untreated control were included in the test situation. A copy of the questionnaire used is attached.

Results:

a) Whitefish - the samples were all found to be fit for consumption. There were comments as to the presence of low levels of off-flavours appearing in several samples, but these were too low to be definetly identified.

b) Burbot - all samples were judged to be fit for consumption. Comments regarding off-flavours present were from the sour flavour associated with a quality deterioration noted by one judge.

c) Rainbow Trout - the samples of untreated control and of treated fish were all correctly identified and the off-flavour in the treated sample characterized as "petroleum oil". The intensity was "weak to moderate".

In summary, the sensory panel was able to perceive off-flabours of petroleum origin when present in the sample of Rainbow Trout. Neither the burbot nor the whitefish contained any of this flavour note. There were comments regarding some low levels of off-flavour which may make it appropriate to evaluate this situation further.

Government of Canada / Gouvernement du Canada

MEMORANDUM **NOTE DE SERVICE**

Derek Murray,
Toxicology.

Roberta York,
Fishery Products Specialist.

SECURITY - CLASSIFICATION - DE SÉCURITÉ	
OUR FILE/NOTRE RÉFÉRENCE	
YOUR FILE/VOTRE RÉFÉRENCE	
DATE	May 3, 1984

SUBJECT / OBJET SENSORY EVALUATION OF EXPERIMENTALLY TAINTED FISH SAMPLES RECEIVED 23/2/84.

SAMPLES RECEIVED: Four fillets of rainbow trout labelled "Control, 1, 2 & 3". Samples wrapped and held in frozen storage until tested.

INFORMATION REQUESTED: General description of flavor in samples.

SAMPLE TREATMENT: Frozen fillets foil-wrapped, coded and steamed to cool for evaluation.

SENSORY EVALUATION PROCEDURES: Informal laboratory panel of two experienced judges evaluated each sample and arrived at an agreed description of each.

RESULTS:

SAMPLE	CONTROL	1	2	3
Appearance	Acceptable	Acceptable	Acceptable	Acceptable
Odor	no off-odor	more diesel than chemical	chemical sl. oil	chemical-oil
Flavor	no off-flavor	diesel-like, moderately intense	chemical, moderately intense	chemical-oil flavor
Texture	acceptable	acceptable	acceptable	acceptable
Aftertaste	none	present as in "Flavor"	as in "Flavor"	as in "Flavor"
Overall Impression	clear	tainted	tainted	tainted
Subsequent Identification of Samples	control	mixture of simple aromatics 1 ppm each	mixture of higher mol. wt. aromatics 1 ppm each	diesel oil at 50 ppb.

The descriptions of flavor given by the informal panel show that different flavor notes were apparent in each of the treated samples. The higher molecular weight aromatics appear to give a more "chemical" flavor, whereas the simple aromatics gave a "diesel-oil like" flavor. The sample in diesel was described as a cross between a chemical and an oil flavor.

MASS SPECTRA

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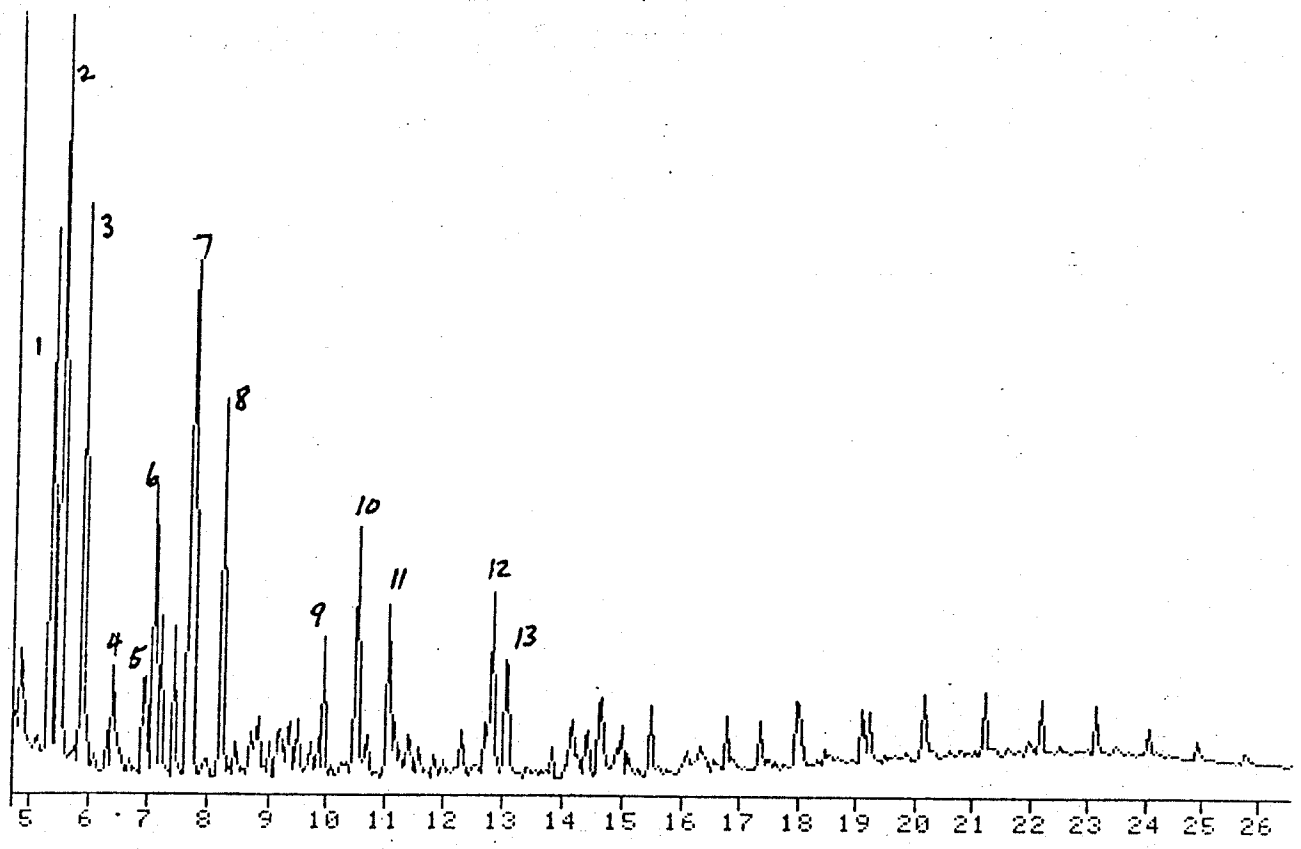
INTSD:2UL,4003-325@10/MIN,DET-1800,

92181



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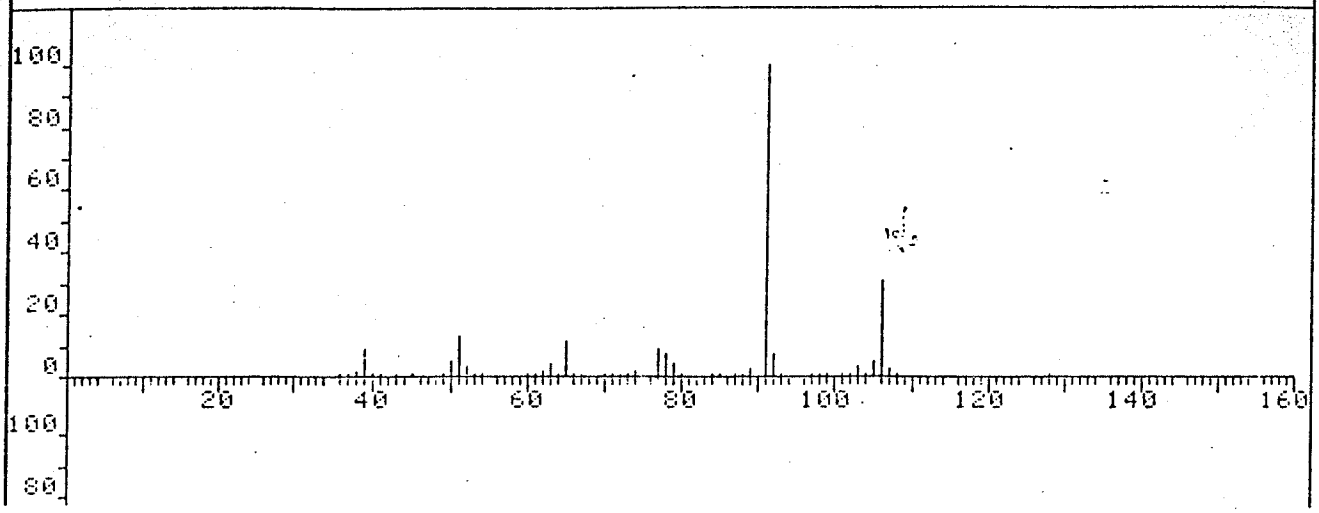
2	38	5.50	262699.	25.29
3	55	5.90	121592.	11.71
4	108	7.12	70742.	6.81
5	113	7.23	31712.	3.05
6	122	7.43	29219.	2.81
7	135	7.73	155769.	15.00
8	157	8.25	88627.	8.53
9	230	9.93	20458.	1.97
10	254	10.48	60780.	5.85
11	278	11.05	34895.	3.36
12	354	12.80	33999.	3.27
13	365	13.07	26523.	2.55

FRN 5032 SPECTRUM 1 RETENTION TIME 5.3
LARGST 4: 91.1,100.0 106.1, 31.0 51.1, 13.1 65.1, 11.2
LAST 4: 106.1, 31.0 107.1, 2.7 108.2, .1 128.2, .1
NORMAN WELLS,WATER SOL. FR. DRN# 5011 PAGE 1



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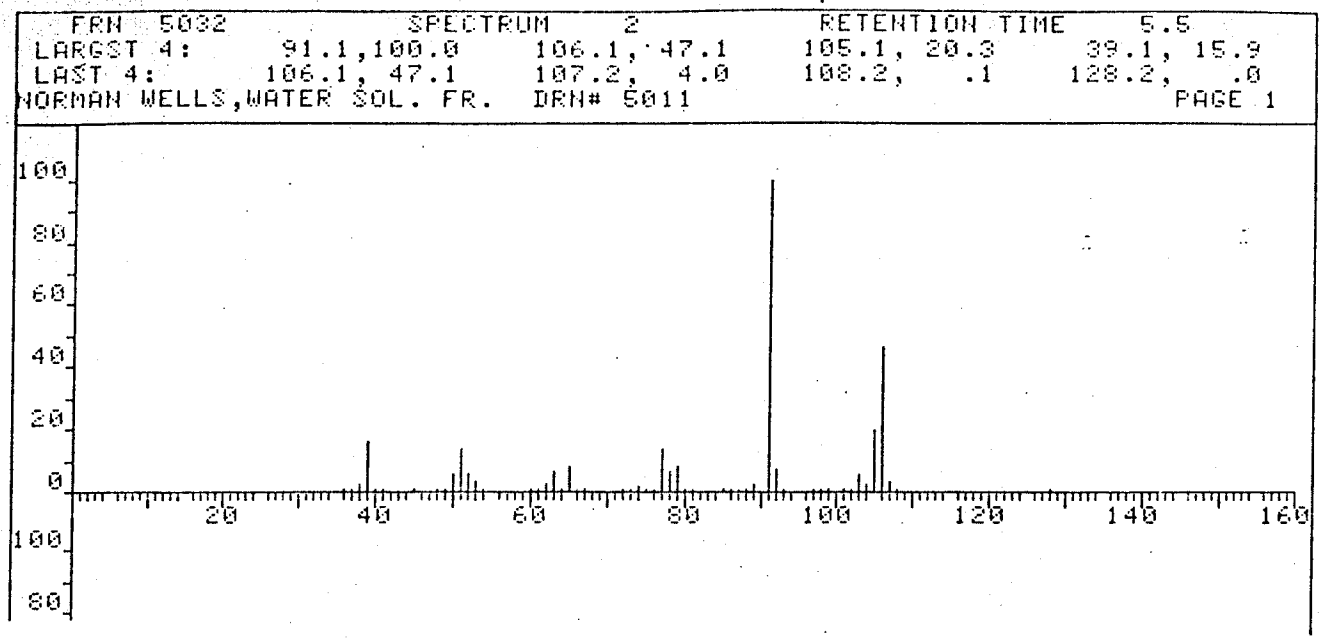
53 PEAKS, 23 SIGNIFICANT MAX K 23.6

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9809 + Benzene, ethyl- (8CI,9CI)
SPEC= 1500 LSN= 1500. MW= 106 C8H10
FRN = 3002 [NBS 1500.] CAS # 0000100414 EPA # 0000001352
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
23.6 10 81% .0 0 0% .0 0 0% MULTIPLIER= 1.00

.9752 Iron, tricarbonyl[(2,3,4,5-.eta.)-2,4-cycloheptadien-1-ol]- (9CI)
SPEC= 2281 LSN= 18118. MW= 250 C10H10FeO4
FRN = 3011 [NBS 18120.] CAS # 0032716704 EPA # 0000012181
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 679
16.3 7 60% .0 0 0% .0 0 0% MULTIPLIER= .75

.9739 Benzene, 3-pentenyl-, (Z)- (9CI)
SPEC= 5516 LSN= 5516. MW= 146 C11H14
FRN = 3002 [NBS 5516.] CAS # 0016487653 EPA # 0000039297
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 666
16.3 7 61% .0 0 0% .0 0 0% MULTIPLIER= .61



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52 PEAKS, 27 SIGNIFICANT MAX K 20.6

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9809 + Benzene, 1,2-dimethyl- (9CI)
SPEC= 1499 LSN= 1499. MW= 106 C8H10
FRN = 3002 [NBS 1499.] CAS # 0000095476 EPA # 0000049297
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
20.6 10 77% .0 0 0% .0 0 0% MULTIPLIER= 1.09

.9809 + Benzene, 1,4-dimethyl- (9CI)
SPEC= 1501 LSN= 1501. MW= 106 C8H10
FRN = 3002 [NBS 1501.] CAS # 0000106423 EPA # 0000020047
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
20.6 10 77% .0 0 0% .0 0 0% MULTIPLIER= .96

.9809 + Benzene, 1,3-dimethyl- (9CI)
SPEC= 1502 LSN= 1502. MW= 106 C8H10
FRN = 3002 [NBS 1502.] CAS # 0000108383 EPA # 0000020044
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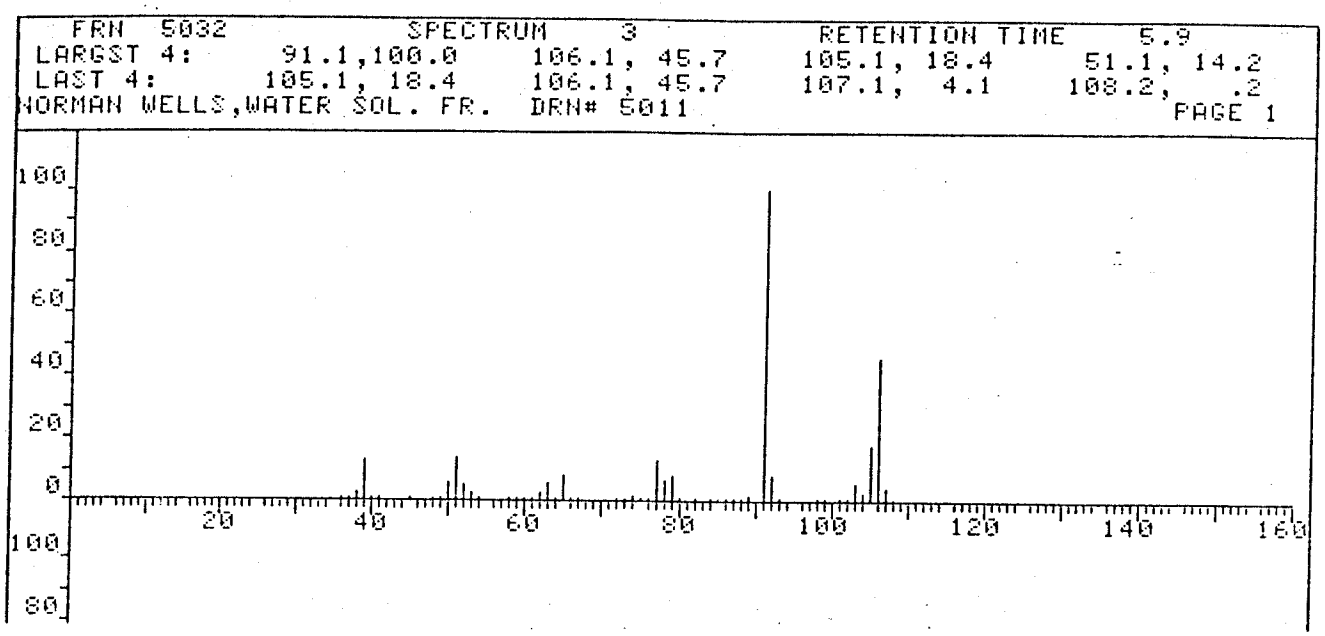

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LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9809 + Benzene, 1,2-dimethyl- (9CI)
SPEC= 1499 LSN= 1499. MW= 106 C8H10
FRN = 3002 [NBS 1499.] CAS # 0000095476 EPA # 0000049297
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
23.5 10 78% .0 0 0% .0 0 0% MULTIPLIER= 1.06

.9809 + Benzene, 1,4-dimethyl- (9CI)
SPEC= 1501 LSN= 1501. MW= 106 C8H10
FRN = 3002 [NBS 1501.] CAS # 0000106423 EPA # 0000020047
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
23.5 10 78% .0 0 0% .0 0 0% MULTIPLIER= .96

.9809 + Benzene, 1,3-dimethyl- (9CI)
SPEC= 1502 LSN= 1502. MW= 106 C8H10
FRN = 3002 [NBS 1502.] CAS # 0000108383 EPA # 0000020044
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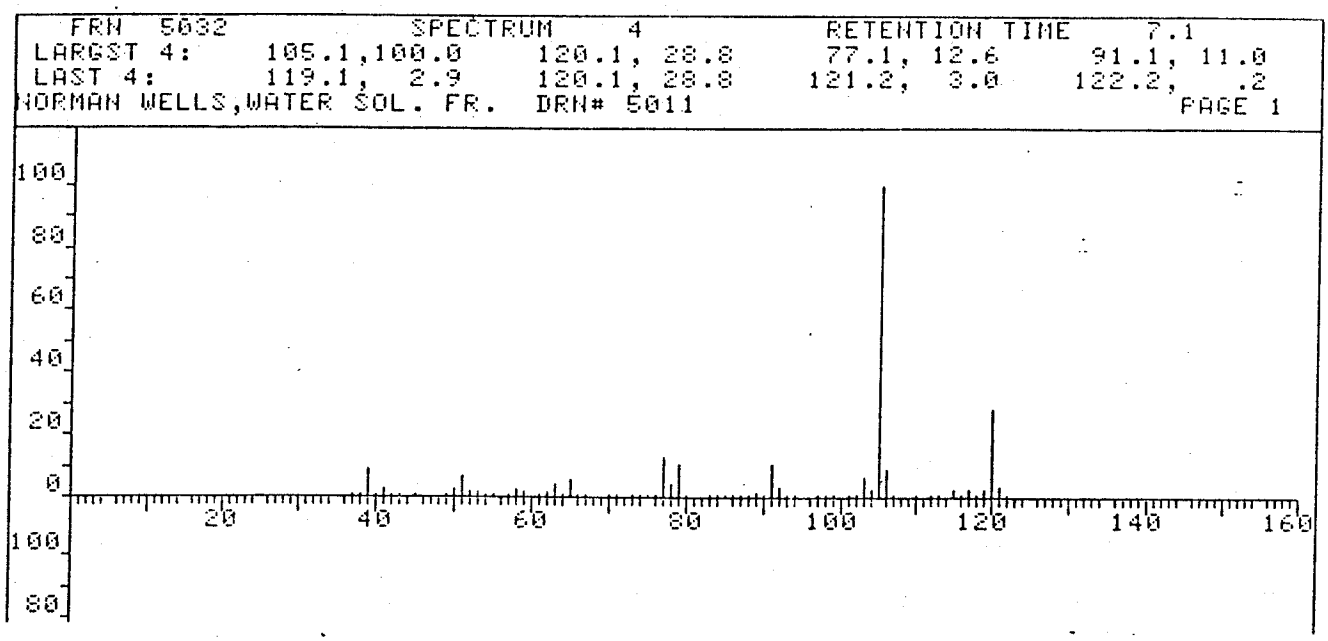

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68 PEAKS, 28 SIGNIFICANT .MAX K 22.6

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9811 + Benzene, 1-ethyl-3-methyl- (9CI)
SPEC= 2596 LSN= 2596. MW= 120 C9H12
FRN = 3002 [NBS 2596.] CAS # 0000620144 EPA # 0000020477
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.6 10 73% .0 0 0% .0 0 0% MULTIPLIER= .96

.9810 + Benzene, (1-methylethyl)- (9CI)
SPEC= 2591 LSN= 2591. MW= 120 C9H12
FRN = 3002 [NBS 2591.] CAS # 0000098828 EPA # 0000020472
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.6 10 74% .0 0 0% .0 0 0% MULTIPLIER= 1.00

.9810 + Benzene, 1-ethyl-4-methyl- (9CI)
SPEC= 2597 LSN= 2597. MW= 120 C9H12
FRN = 3002 [NBS 2597.] CAS # 0000622968 EPA # 0000020479
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.6 10 75% .0 0 0% .0 0 0% MULTIPLIER= 1.00

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LAST 4:      119.1, 11.5    120.2, 46.7   121.2,  4.9   122.2,   .3
NORMAN WELLS,WATER SOL. FR.  DRN# 5011                        PAGE 1
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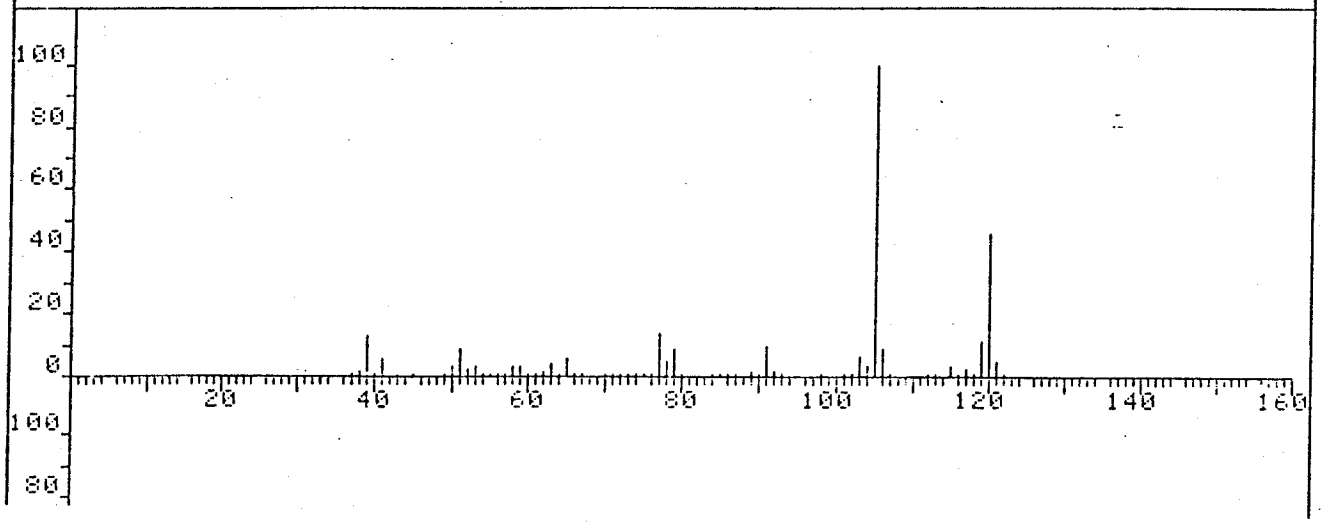


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LIBRARY  3000  27504 SPECTRA SEARCHED,     3 HIT(S)


.9810 + Benzene, 1,2,4-trimethyl- (8CI9CI)
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.9810 + Benzene, 1,3,5-trimethyl- (9CI)
        SPEC= 2593  LSN=  2593. MW= 120  C9H12
        FRN = 3002 [NBS   2593.]  CAS #  0000108678   EPA #  0000002138
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  724
         21.8  10  70%    .0   0   0%     .0   0   0%  MULTIPLIER=  .96

.9809 + Benzene, 1,2,3-trimethyl- (8CI9CI)
        SPEC= 2594  LSN=  2594. MW= 120  C9H12
        FRN = 3002 [NBS   2594.]  CAS #  0000526738   EPA #  0000020465
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  728
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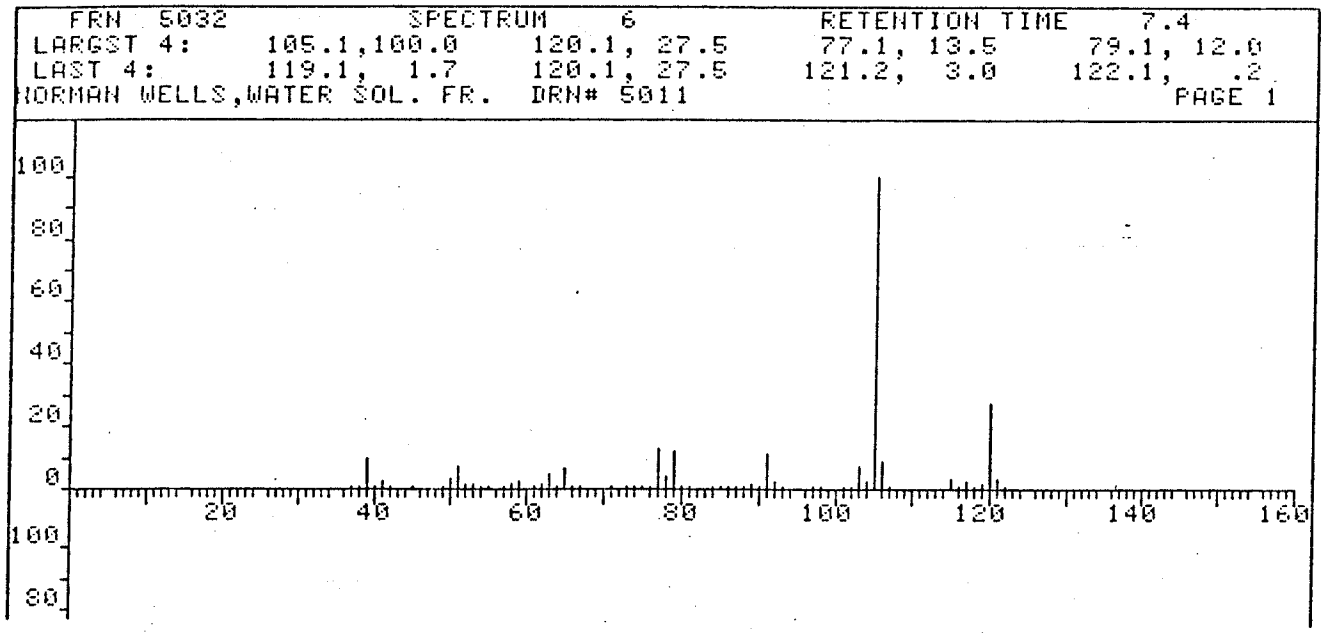

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LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9810 + Benzene, (1-methylethyl)- (9CI)
SPEC= 2591 LSN= 2591. MW= 120 C9H12
FRN = 3002 [NBS 2591.] CAS # 0000098828 EPA # 0000020472
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.7 10 74% .0 0 0% .0 0 0% MULTIPLIER= 1.00

.9810 + Benzene, 1-ethyl-2-methyl- (9CI)
SPEC= 2595 LSN= 2595. MW= 120 C9H12
FRN = 3002 [NBS 2595.] CAS # 0000611143 EPA # 0000002139
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 707
21.7 10 75% .0 0 0% .0 0 0% MULTIPLIER= 1.00

.9809 + Benzene, 1-ethyl-4-methyl- (9CI)
SPEC= 2597 LSN= 2597. MW= 120 C9H12
FRN = 3002 [NBS 2597.] CAS # 0000622968 EPA # 0000020479
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.5 10 74% .0 0 0% .0 0 0% MULTIPLIER= 1.00

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 LAST 4:       121.2,  4.6     122.2,   .2     140.2,   .1     281.1,   .1
NORMAN WELLS,WATER SOL. FR.  DRN# 5011                                PAGE 1
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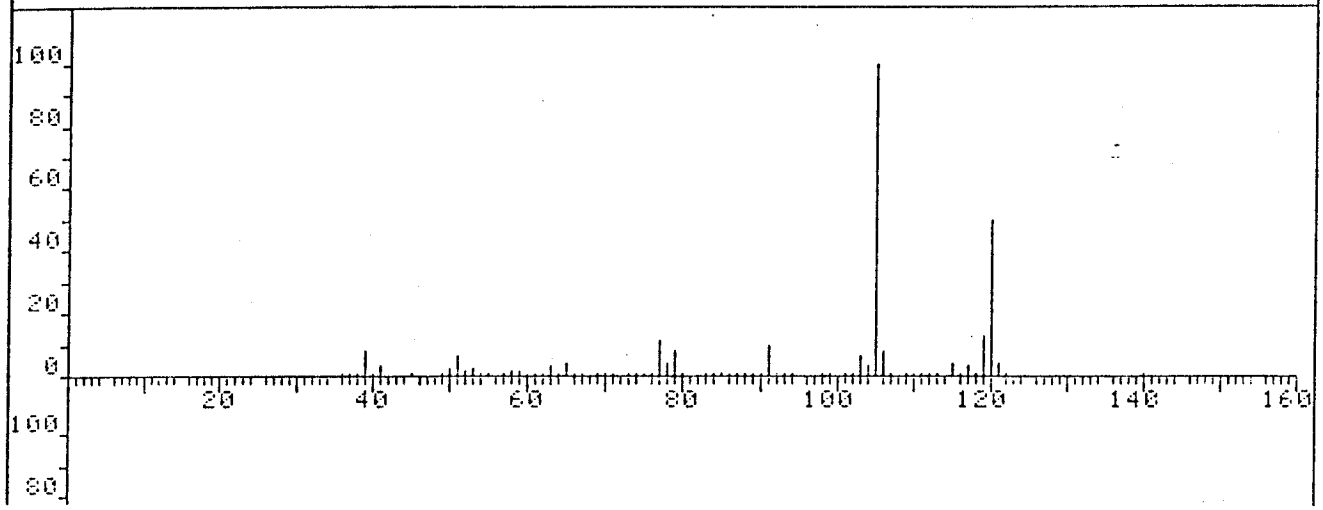


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REF. SPECT #=     7  LSN=      7. MW=    0  FRN= 5032  RET. TIME=    7.7
          72 PEAKS,     29 SIGNIFICANT       MAX K    23.8

LIBRARY  3000  27504 SPECTRA SEARCHED,     3 HIT(S)


.9809 + Benzene, 1,2,4-trimethyl- (8CI9CI)
        SPEC= 2590  LSN=  2590. MW= 120  C9H12
        FRN = 3002 [NBS   2590.]  CAS #  0000095636   EPA #  0000034893
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  719
         23.6  10  76%    .0   0   0%     .0   0   0%  MULTIPLIER=  .99

.9809 + Benzene, 1,3,5-trimethyl- (9CI)
        SPEC= 2593  LSN=  2593. MW= 120  C9H12
        FRN = 3002 [NBS   2593.]  CAS #  0000108678   EPA #  0000002138
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  724
         23.6  10  77%    .0   0   0%     .0   0   0%  MULTIPLIER=  .96

.9808 + Benzene, 1,2,3-trimethyl- (8CI9CI)
        SPEC= 2594  LSN=  2594. MW= 120  C9H12
        FRN = 3002 [NBS   2594.]  CAS #  0000526738   EPA #  0000020465
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  728
         23.6  10  77%    .0   0   0%     .0   0   0%  MULTIPLIER= 1.00
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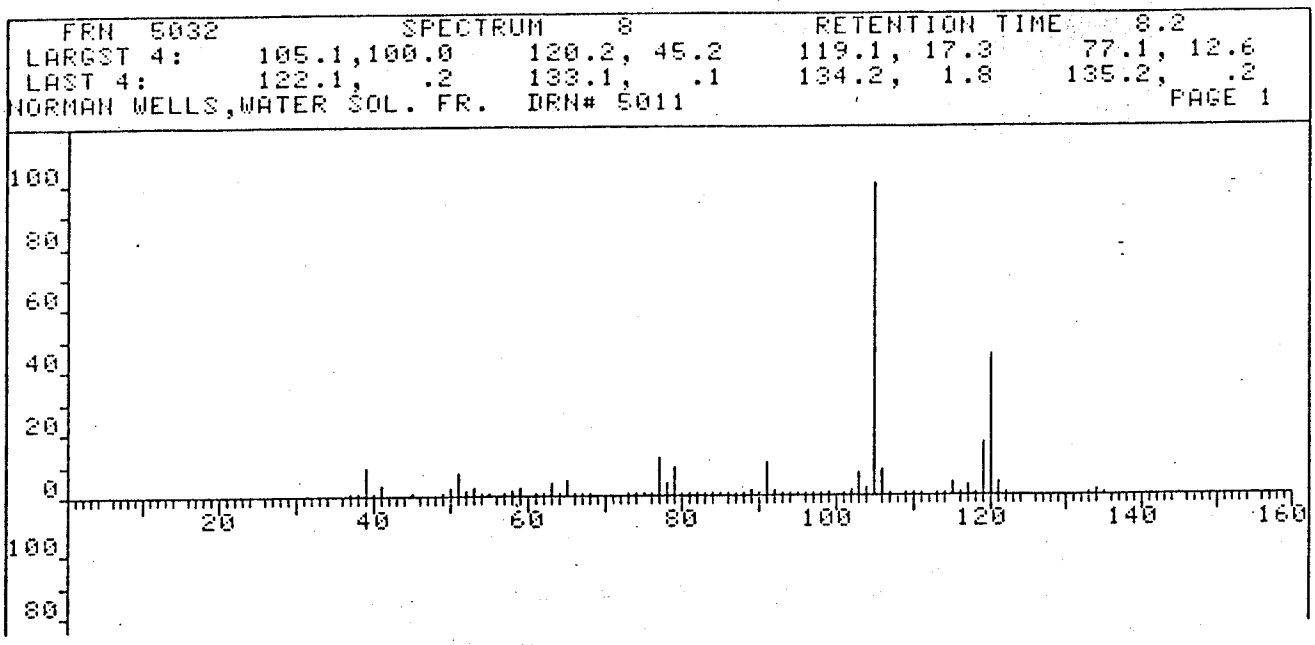


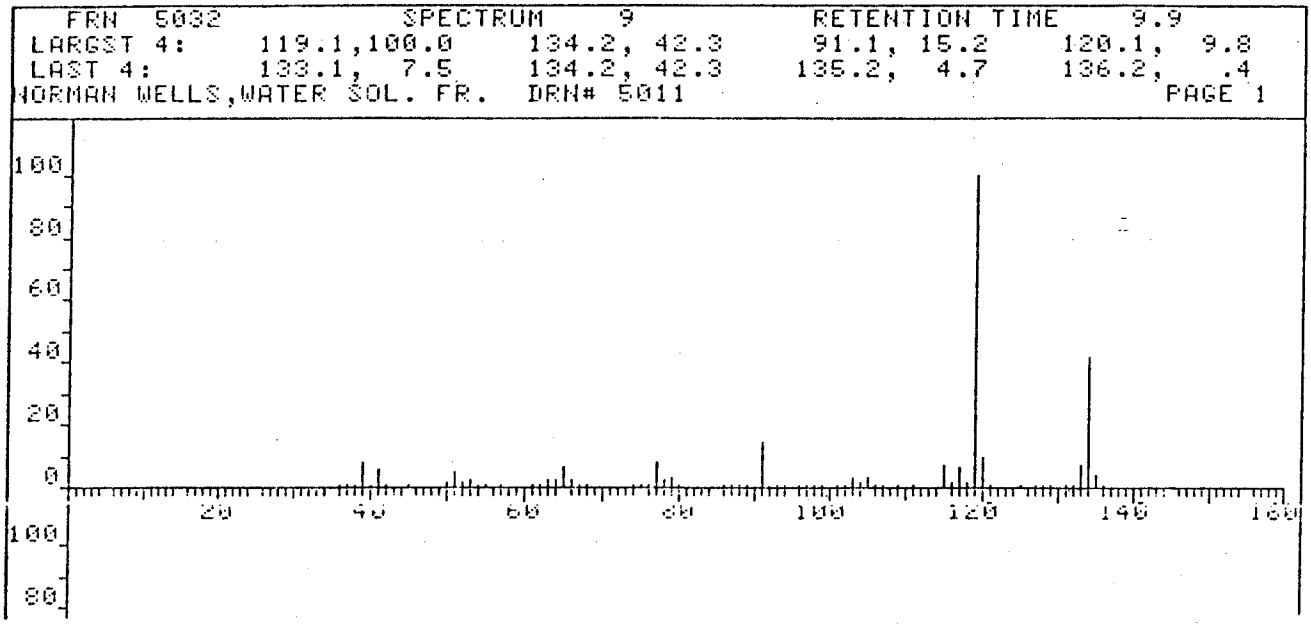
REF. SPECT #= 8 LSN= 8. MW= 0 FRN= 5032 RET. TIME= 8.2
74 PEAKS, 31 SIGNIFICANT MAX K 23.1

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9810 + Benzene, 1,3,5-trimethyl- (9CI)
SPEC= 2593 LSN= 2593. MW= 120 C9H12
FRN = 3002 [NBS 2593.] CAS # 0000108678 EPA # 0000002138
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 724
23.1 10 75% .0 0 0% .0 0 0% MULTIPLIER= .92

.9809 + Benzene, 1,2,4-trimethyl- (8CI9CI)
SPEC= 2590 LSN= 2590. MW= 120 C9H12
FRN = 3002 [NBS 2590.] CAS # 0000095636 EPA # 0000034893
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 719
22.9 10 74% .0 0 0% .0 0 0% MULTIPLIER= .98

.9809 + Benzene, 1,2,3-trimethyl- (8CI9CI)
SPEC= 2594 LSN= 2594. MW= 120 C9H12
FRN = 3002 [NBS 2594.] CAS # 0000526738 EPA # 0000020465
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 720
22.9 10 75% .0 0 0% .0 0 0% MULTIPLIER= 1.00



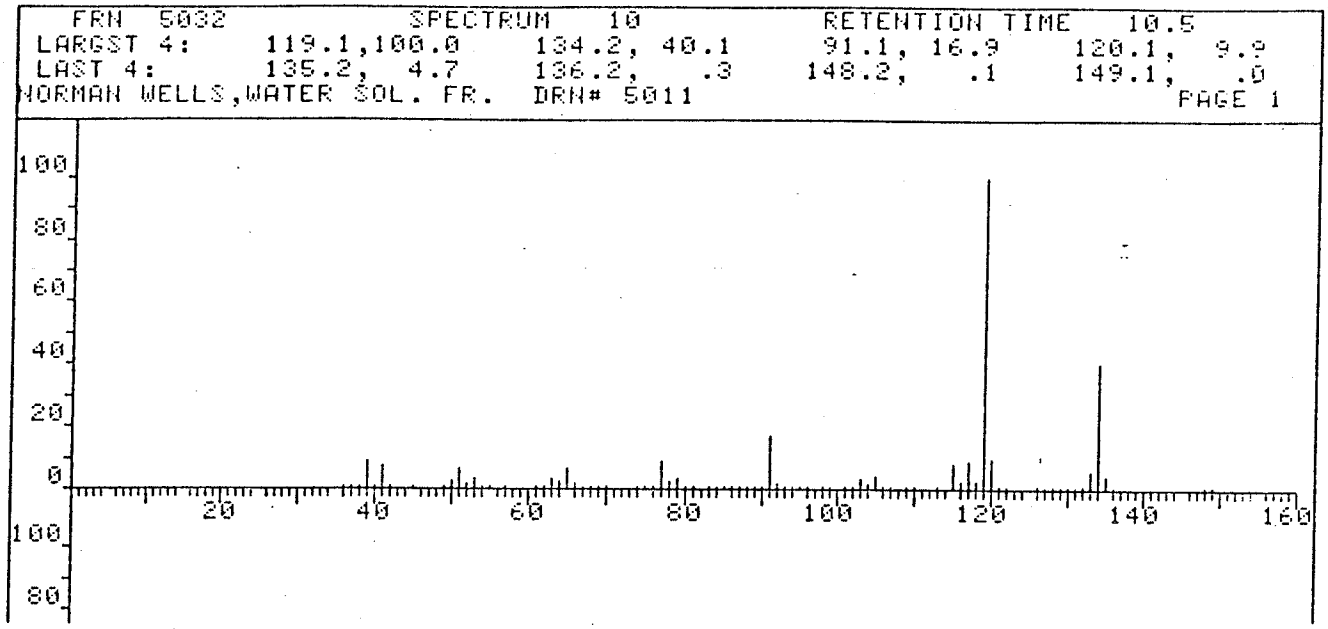
REF. SPECT #= 9 LSN= 9. MW= 0 FRN= 5032 RET. TIME= 9.9
69 PEAKS, 30 SIGNIFICANT MAX K 21.9

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9810 + Benzene, 1,2,3,4-tetramethyl- (8CI9CI)
SPEC= 3920 LSN= 3920. MW= 134 C10H14
FRN = 3002 [NBS 3920.] CAS # 0000488233 EPA # 0000034793
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.7 10 73% .0 0 0% .0 0 0% MULTIPLIER= 1.00

.9810 + Benzene, 1,2,3,5-tetramethyl- (8CI9CI)
SPEC= 3921 LSN= 3921. MW= 134 C10H14
FRN = 3002 [NBS 3921.] CAS # 0000527537 EPA # 0000003139
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.7 10 74% .0 0 0% .0 0 0% MULTIPLIER= .98

.9807 + Benzene, 2-ethyl-1,3-dimethyl- (9CI)
SPEC= 3933 LSN= 3933. MW= 134 C10H14
FRN = 3002 [NBS 3933.] CAS # 0002870044 EPA # 0000020912
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.5 10 72% .0 0 0% .0 0 0% MULTIPLIER= .96



REF. SPECT #= 10 LSN= 10. MW= 0 FRN= 5032 RET. TIME= 10.5
81 PEAKS, 32 SIGNIFICANT MAX K 22.7

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9810 + Benzene, 1,2,3,4-tetramethyl- (8CI9CI)
SPEC= 3920 LSN= 3920. MW= 134 C10H14
FRN = 3002 [NBS 3920.] CAS # 0000488233 EPA # 0000034793
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.5 10 70% .0 0 0% .0 0 0% MULTIPLIER= .99

.9810 + Benzene, 1,2,3,5-tetramethyl- (8CI9CI)
SPEC= 3921 LSN= 3921. MW= 134 C10H14
FRN = 3002 [NBS 3921.] CAS # 0000527537 EPA # 0000003139
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.5 10 71% .0 0 0% .0 0 0% MULTIPLIER= .96

.9808 + Benzene, 2-ethyl-1,3-dimethyl- (9CI)
SPEC= 3933 LSN= 3933. MW= 134 C10H14
FRN = 3002 [NBS 3933.] CAS # 0002870044 EPA # 0000020912
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
22.2 10 70% .0 0 0% .0 0 0% MULTIPLIER= 1.00

```
   FRN  5032              SPECTRUM    11              RETENTION TIME    11.0
LARGST 4:     128.1,100.0      51.1, 11.9     127.1, 11.6     129.1, 10.6
LAST 4:       147.3,   .0     148.2,   .4     149.1,   .0     151.1,   .1
NORMAN WELLS,WATER SOL. FR.  DRN# 5011                              PAGE 1
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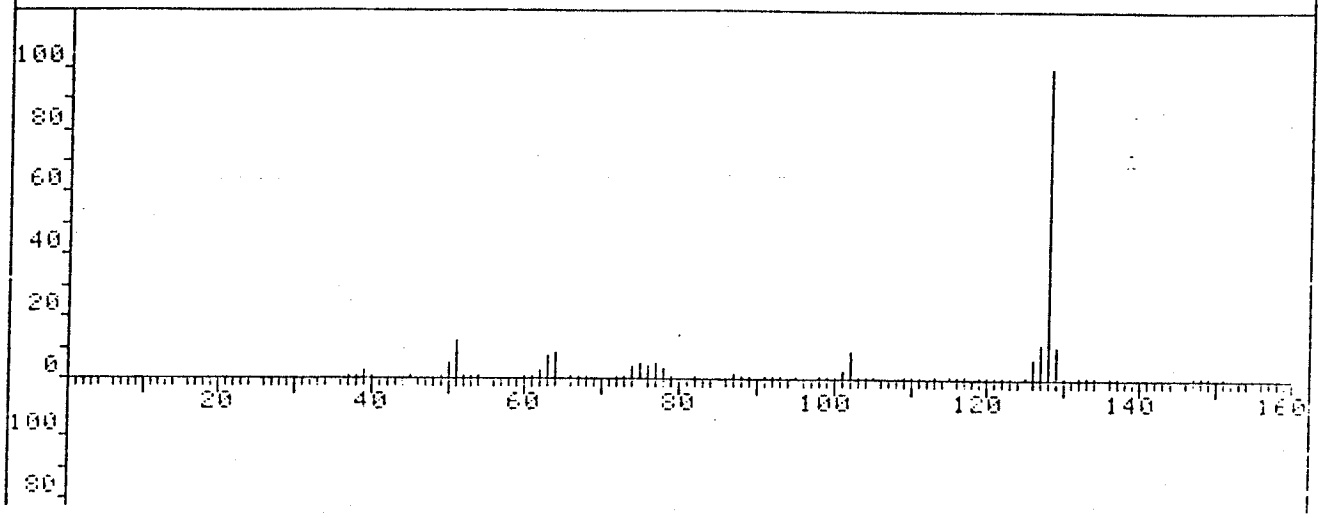


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REF. SPECT #=    11  LSN=     11. MW=    0  FRN= 5032  RET. TIME=  11.0
          72 PEAKS,     22 SIGNIFICANT       MAX K   22.0

LIBRARY  3000  27504 SPECTRA SEARCHED,     3 HIT(S)


.9808 + Naphthalene (8CI9CI)
        SPEC= 3200  LSN=  3200. MW= 128  C10H8
        FRN = 3002 [NBS   3200.]  CAS #  0000091203   EPA #  0000020661
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  728
         21.8  10  77%    .0   0   0%     .0   0   0%  MULTIPLIER= 1.00

.9808 + Azulene (8CI9CI)
        SPEC= 3201  LSN=  3201. MW= 128  C10H8
        FRN = 3002 [NBS   3201.]  CAS #  0000275514   EPA #  0000002628
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=  727
         21.8  10  76%    .0   0   0%     .0   0   0%  MULTIPLIER=  .85

.9791   Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaen-11-one (9CI)
        SPEC= 1260  LSN=  7019. MW= 156  C11H8O
        FRN = 3005 [NBS   7019.]  CAS #  0036628805   EPA #  0000038730
        MATCHING PEAKS   CONTAMINATED   MISSING PEAKS  QUAL INDEX=    0
         19.8   9  70%    .0   0   0%     .0   0   0%  MULTIPLIER=  .85
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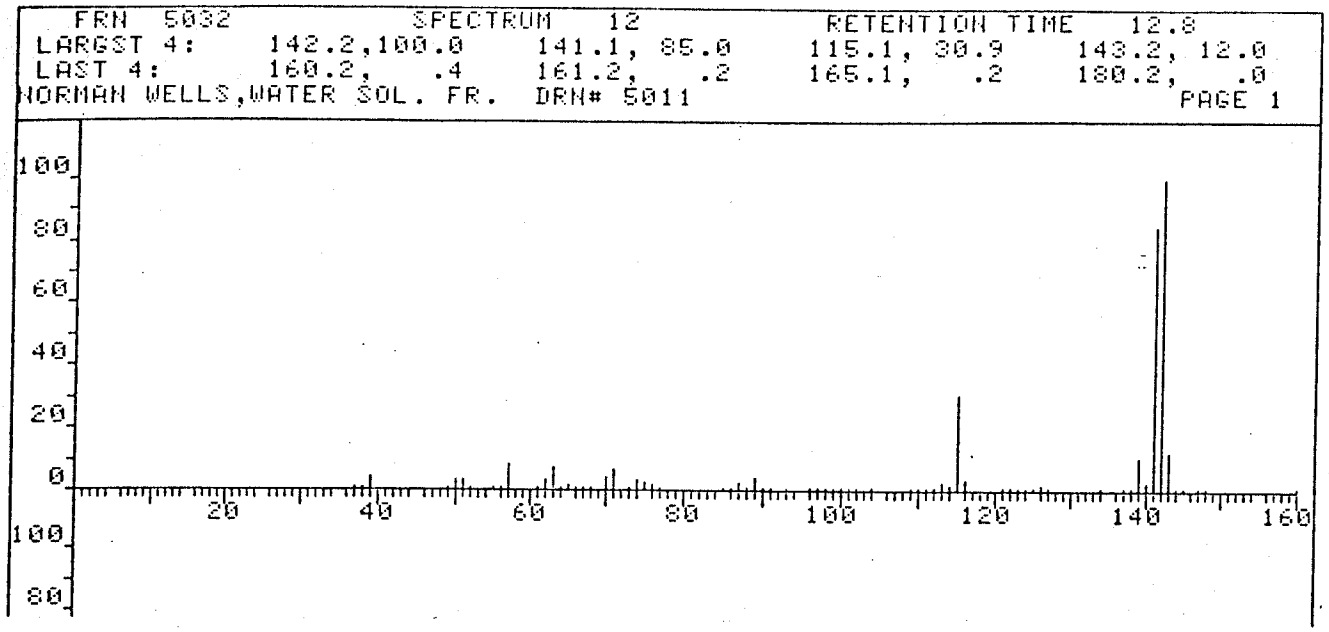


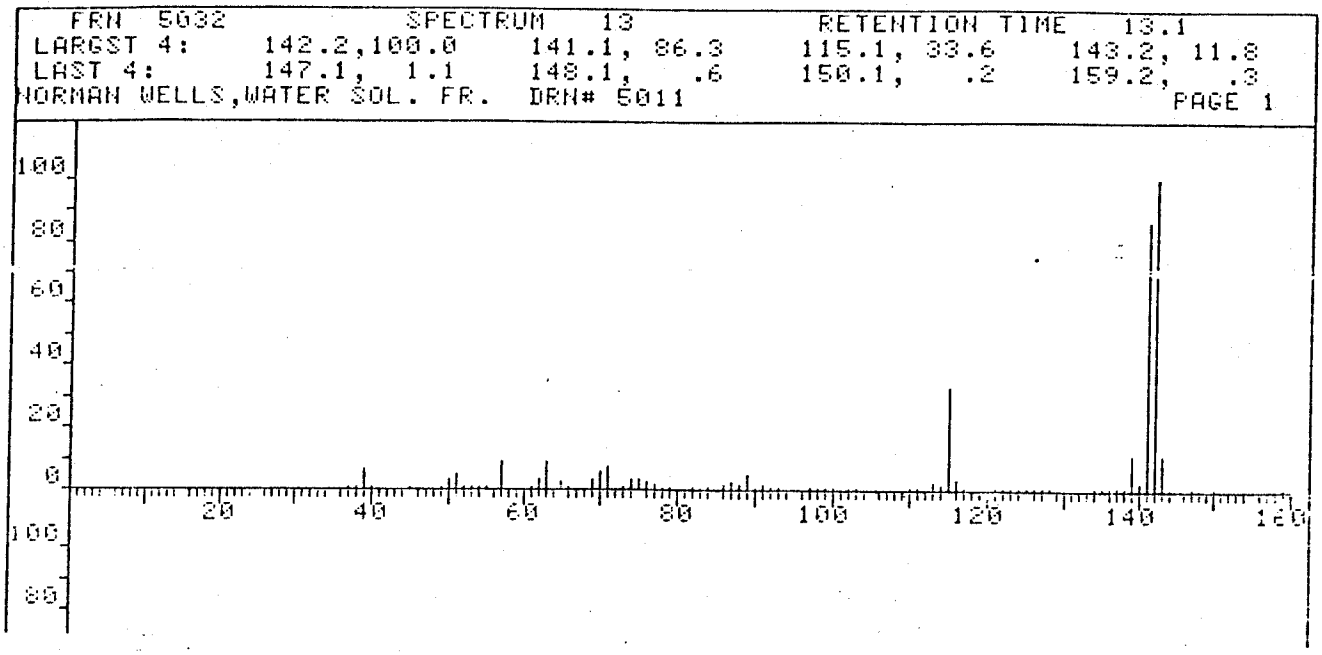
REF. SPECT #= 12 LSN= 12. MW= 0 FRN= 5032 RET. TIME= 12.8
72 PEAKS, 27 SIGNIFICANT MAX K 21.6

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9810 + Naphthalene, 2-methyl- (8CI9CI)
SPEC= 4947 LSN= 4947. MW= 142 C11H10
FRN = 3002 [NBS 4947.] CAS # 0000091576 EPA # 0000021193
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.4 10 78% .0 0 0% .0 0 0% MULTIPLIER= .98

.9808 + Naphthalene, 1-methyl- (8CI9CI)
SPEC= 4946 LSN= 4946. MW= 142 C11H10
FRN = 3002 [NBS 4946.] CAS # 0000090120 EPA # 0000021183
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
21.2 10 79% .0 0 0% .0 0 0% MULTIPLIER= .76

.8684 + 1,4-Methanonaphthalene, 1,4-dihydro- (8CI9CI)
SPEC= 4948 LSN= 4948. MW= 142 C11H10
FRN = 3002 [NBS 4948.] CAS # 0004453901 EPA # 0000003819
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 701
19.2 9 78% .0 0 0% 2.4 1 2% MULTIPLIER= .78



REF. SPECT #= 13 LSN= 13. MW= 0 FRN= 5032 RET. TIME= 13.1
77 PEAKS, 35 SIGNIFICANT MAX K 21.1

LIBRARY 3000 27504 SPECTRA SEARCHED, 3 HIT(S)

.9809 + Naphthalene, 2-methyl- (8CI9CI)
SPEC= 4947 LSN= 4947. MW= 142 C11H10
FRN = 3002 [NBS 4947.] CAS # 0000091576 EPA # 0000021193
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
20.7 10 74% .0 0 0% .0 0 0% MULTIPLIER= .99

.9809 + 1,4-Methanonaphthalene, 1,4-dihydro- (8CI9CI)
SPEC= 4948 LSN= 4948. MW= 142 C11H10
FRN = 3002 [NBS 4948.] CAS # 0004453901 EPA # 0000003819
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 701
20.7 10 74% .0 0 0% .0 0 0% MULTIPLIER= .73

.9807 + Naphthalene, 1-methyl- (8CI9CI)
SPEC= 4946 LSN= 4946. MW= 142 C11H10
FRN = 3002 [NBS 4946.] CAS # 0000090120 EPA # 0000021183
MATCHING PEAKS CONTAMINATED MISSING PEAKS QUAL INDEX= 728
20.5 10 74% .0 0 0% .0 0 0% MULTIPLIER= .77

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