American Petroleum Institute

STEP Strategies for Today's Environmental Partnership

IDENTIFICATION OF ORGANIC TOXICANTS IN TREATED REFINERY EFFLUENTS

Health and Environmental Sciences Department
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Identification of Organic Toxicants in Treated Refinery Effluents

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ASCI CORPORATION/ASCI-DULUTH
ENVIRONMENTAL TESTING DIVISION
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API STAFF CONTACT

Alexis Steen, Health and Environmental Sciences Department

MEMBERS OF THE BIOMONITORING TASK FORCE

Philip B. Dorn, Ph.D., Shell Development Company (Chairman)

W. Raymond Arnold, Ph.D., Exxon Biomedical Sciences, Inc.

Marie T. Benkinney, Mobil Oil Corporation

Janis M. Farmer, BP America R&D

William R. Gala, Ph.D., Chevron Research and Technology Company

Jerry F. Hall, Ph.D., Texaco Research

Michael D. Harrass, Ph.D., Amoco Corporation

Denise J. Jett, Phillips Petroleum Company

Eugene R. Mancini, Ph.D., ARCO

James E. O'Reilly, Exxon Production Research Company

Lawrence A. Reitsema, Ph.D., Marathon Oil Company

C. Michael Swindoll, Dupont Environmental Remediation Svc.

Michael E. Tucker, Occidental Chemical Company

Carl Venzke, Citgo Petroleum Corporation

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ABSTRACT

In this study, effluents from five oil refineries were examined for the presence of nonpolar, organic chronic toxicity following suggested U.S. EPA guidelines for Phase I Toxicity Characterization procedures. The refinery effluent containing the most toxicity from nonpolar organic toxicants was selected for more detailed analyses and identification of these toxicants using Phase II procedures. Extraction and elution conditions were modified to increase chronic toxicity recovery and also reduce the complexity of the nonpolar organic effluent fraction containing toxicity.

Results showed that simple modifications of U.S. EPA guidance for C_{18} solid phase extraction (SPE) procedures combined with proper toxicity testing conditions successfully tracked and, to an acceptable degree, isolated toxicity in an effluent fraction amenable for identification of suspected nonpolar organic toxicants. Toxicity was observed only in 100% effluent concentrations, not in dilutions of the effluents. Further chronic toxicity was not consistently observed in the effluent fractions.

Findings from this study indicated that sources of refinery effluent toxicants were a phenol associated with a jet fuel additive and two brominated organics believed to be reaction products of cooling tower water treatment chemicals, rather than from crude oil constituents.

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EXECUTIVE SUMMARY

Prior to the passage of the Federal Water Pollution Control Act (PL 92-500; *Clean Water Act*) in 1972, refinery wastewater treatment systems were diverse in design and treatment effectiveness. Engineering- and technology-based treatment standards, initially developed under the Act to achieve prescribed effluent concentrations resulted in treatment system upgrades and improved wastewater quality. A subsequent EPA initiative to implement water-quality-based effluent limitations (49 Federal Register 9016), as measured by effluent and receiving water aquatic toxicity tests, substantially expanded and enhanced aquatic toxicity testing capabilities. During this same period, advancements in analytical chemistry and toxicity identification procedures helped identify refinery wastewater constituents and treatment processes which were responsible for observed toxicity. Treatment system upgrades designed to achieve these water-quality-based objectives further improved effluent quality.

This investigation represents the next level of sophistication in effluent quality assessments and similarly reflects a substantial change in the nature and magnitude of refinery effluent toxicity. The focus of this study was the isolation and identification of nonpolar, organic wastewater constituents causing measurable, chronic toxicity in treated refinery effluent. Nonpolar organic toxicants were operationally defined as those adsorbed by C_{18} solid phase extraction (SPE) columns. Effluents from five refineries were selected for screening-level toxicity assessments.

Isolation and identification of the organic compounds responsible for the observed toxicity were accomplished after modifications were made to existing toxicity characterization and identification guidance. Specifically, effluent extraction and elution conditions were modified to reduce the complexity of the organic fraction and to increase recovery efficiency of the chronically toxic fraction. One avenue examined was adjustment of effluent pH before extraction using C_{18} columns. Another avenue was modification of the standard Phase II C_{18} column elution scheme suggested by the U.S. EPA guidance for performing Toxicity Identification Evaluations (TIEs).

The toxicants were neither derived from crude oil or refined product nor were they conventional pollutants associated with refinery wastewater treatment systems. The identified toxicants were a phenol associated with a jet fuel additive and two aromatic brominated organics, believed to be reaction products of cooling tower water treatment chemicals. These compounds exhibited variable, intermittent, and low concentration toxicity and their identification required enhanced fractionation procedures.

None of the effluents tested had sufficient concentrations of total dissolved solids, ammonia, or hydrogen sulfide to be of concern for causing chronic toxicity or interfering with examination of the contribution by nonpolar compounds. Only one of five refinery effluents exhibited organic toxicity of sufficient magnitude to allow subsequent attempts at toxicant isolation and identification. Additionally, levels of chronic toxicity were generally found to be low. These results constitute a broader demonstration of the significant progress during the last 20+ years in refinery wastewater treatment as well as effluent toxicity characterization and identification.

Improvements in refinery effluent quality have been accomplished through treatment enhancements and through better housekeeping practices. Substances such as total dissolved solids, ammonia and hydrogen sulfide, formerly recognized as causing toxicity in refinery effluents, have been largely brought under control. Thus, acute toxicity in refinery effluents is often absent. Chronic toxicity often occurs only at higher effluent concentrations, as demonstrated in this study. Which levels of toxicity are considered of importance in the receiving water depends on the amount and rate of dilution that occurs in the receiving stream. Dilution allowance in the receiving water is usually recognized by regulatory authorities. The type and amount of toxicity identified in this study would be of concern only where available dilution was very low.

Section 1
INTRODUCTION

BACKGROUND

The convergent evolution of aquatic toxicity testing, analytical chemistry, and refinery wastewater engineering has progressed through several levels over the years. Whole effluent aquatic toxicity tests conducted early in this evolutionary process found acute toxicity at relatively low effluent concentrations. These discharges were, and continue to be, complex in chemical composition, and the nature and extent of their toxicity are variable. Prominent inorganic and organic constituents previously identified as responsible for acute and/or chronic toxicity were ammonia, total dissolved solids (TDS), and napthenic acids. Combinations of test species selection and test conditions, treatment system operations and refinery wastewater stream characteristics all played roles in affecting effluent quality. As influences of these conditions were more clearly understood and appropriate enhancements made, the incidence and extent of acute effluent toxicity have generally declined.

More sensitive subacute tests were developed to identify effluent toxicity, which was usually observed at higher effluent concentrations. Treatment system design and operation were also improved to reduce or eliminate sporadic toxicity (e.g., ammonia excursions). Experience illustrated that attention to treatment system operational details and wastewater stream quality (i.e., refinery unit operation) could reduce whole effluent toxicity.

Even after the many improvements that have been made, the more sensitive toxicity tests (largely chronic tests) sometimes reveal measurable chronic toxicity at higher effluent concentrations. The importance of this toxicity to natural receiving systems depends on the degree of dilution occurring. In most situations, sufficient dilution is available in the receiving water. When dilution in the receiving water is very low, regulatory authorities may insist on further toxicity reduction. It was anticipated that nonpolar organic compounds from refinery processes were in the final effluent and would be frequently contributing to observations of chronic toxicity. Information about such toxicants was desired to provide a

better understanding of their contribution to refinery effluent toxicity and to direct efforts towards control and/or reduction. The EPA TIE procedures were used and modified to identify the small amounts of toxicity caused by nonpolar organic compounds in refinery effluents.

OBJECTIVES AND SCOPE

Toxicity characterization procedures with either larval fathead minnow (*Pimephales promelas*) or *(Mysidopsis bahia)* were performed with effluents from five refineries to identify nonpolar organics responsible for chronic toxicity. Test species selected for this study are also commonly used for determining compliance with effluent discharge toxicity limits. Any toxicity caused by more easily recognized substances, such as ammonia, was not of concern.

Characteristics of selected refinery effluents were initially examined to determine suitable effluents for identification of nonpolar organic toxicants. Nonpolar organic toxicants were operationally defined as those adsorbed by C_{18} SPE columns. Desirable effluent characteristics were: 1) consistent presence of measurable chronic toxicity due to nonpolar organic compounds; and 2) a lack of toxicity from compounds other than nonpolar organics. Samples with these characteristics were preferred to minimize difficulties in tracking effluent toxicity through sample manipulations and to reduce the possibility of artifacts from the multiple treatments required to address toxicants belonging to more than one class of compounds.

Historically, several common difficulties have been encountered during identification of nonpolar organic toxicants in refinery effluents. Past problems included: 1) poor recovery of toxicity from C_{18} solid phase extraction (SPE) columns, 2) poor resolution of toxicity during separative steps, 3) failure to recover toxicity following high performance liquid chromatography (HPLC) separation, and 4) inability to adequately simplify effluent fractions containing the nonpolar organic toxicants. Procedures were employed to: 1) simplify the toxic nonpolar organic effluent fraction, 2) achieve sufficient toxicant concentration to allow analytical measurement, and 3) remove water from the fraction to allow analysis by gas

chromatography/mass spectroscopy (GC/MS). GC analyses are often not definitive because of the hydrocarbon content of refinery effluents. To overcome these difficulties, modifications of the U.S. EPA's suggested guidance for Phase II Toxicity Identification Evaluation (TIE) procedures (U.S. EPA 1993) for nonpolar organic compounds were developed and tried.

Section 2
METHODS

GENERAL APPROACH

The initial approach used to screen five refinery effluents for nonpolar chronic toxicity was the U.S. EPA Phase I procedures (U.S. EPA 1991a). The effluent with the most nonpolar chronic toxicity (and the least toxicity from toxicants other than nonpolar toxicants) was selected for detailed toxicant identification using Phase II U.S. EPA procedures (U.S. EPA 1993). Modifications to resolve past TIE performance problems with refinery effluents were made. The modifications are described here and in the Results Section. If an organic compound seemed likely to be a contributor to observed toxicity, additional information was gathered by literature searches, single chemical toxicity exposures, and location of possible sources of the suspect toxicant within the refinery.

INITIAL TOXICITY SCREENS

Initial toxicity screens were performed immediately following sample receipt with each effluent sample using only one of the selected TIE species - either mysids, *Mysidopsis bahia,* or fathead minnows, *Pimphales promelas.* The screening methods for both species are described below. The presence of acute toxicity indicated that the sample was suitable for continued Phase I TIE testing. Generally, test concentrations were 25%, 50%, and 100% effluent, and a control. If the toxicity was sufficient, Phase I TIE procedures were completed for one species.

Fathead Minnow Tests

Dilution water for larval fathead minnow tests was moderately hard reconstituted water (MHRW) prepared following the standard U.S. EPA formula (U.S. EPA 1989). Dilutions were made with the smallest appropriate 14 sized graduated cylinders. Test chambers were 120-ml plastic cups (Plastics Inc., St. Paul, MN). Organisms were obtained from the AScI Corporation/AScI-Duluth Environmental Testing Division's (AScI-DETD) fathead minnow culture or from Environmental Consulting & Testing Inc. (Superior, WI). Organism age at test initiation was either < 24 hours or 24- to 48-hours old. Only organisms from one age bracket were used within any one test. Two concentration replicates each containing ten

fathead minnows were tested. Organisms were fed newly hatched artemia (Biomarine Inc., Hawthorne, CA) two or three times daily. Test solutions were renewed daily for seven days. After seven days, the fathead minnows were euthanized, dried 20-24 hours at 100°C, and weighed.

Mysid Tests

The dilution water for mysids was prepared by dissolving Instant Ocean® or hW-Marinemix® sea salt mixture in Millipore® water to obtain a test salinity of 25 ppt. The dilution water was aerated for at least 24 hours before use. Effluent salinity was also adjusted before testing to 25 ppt with Instant Ocean® or hW-Marinemix® sea salt addition. A minimum of three effluent concentrations (25%, 50%, and 100%) and a control were tested during each screening. Fresh test solutions were prepared each day with the appropriate size graduated cylinders. New test chambers were used each day. Test chambers were 30-ml or 120-ml plastic cups. When the 30-ml cups were used, 20 replicates with one organism in each were tested. When the 120-ml cups were used, two replicates each containing five organisms were tested. Organisms were supplied by Aquatic Research Organisms Inc. (Hampton, NH). The mysids were 2 to 6 days old at test initiation. Organisms were liberally fed newly hatched artemia two or three times daily. At the end of seven days, the mysids were euthanized, dried at 100°C for 20-24 hours, and weighed.

PHASE I METHODS

The methods used for characterization of chronic toxicity are described in U.S. EPA (1991b). One effluent sample each from four of five selected refineries was subjected to a Phase I test battery. The results of the toxicity characterization procedures were used to select the refinery effluent most appropriate for Phase II Toxicity Identification procedures.

PHASE II C_{18} SPE METHODS

Nonpolar organic toxicity was tracked through various separation and concentration steps to ensure the cause of effluent toxicity was present in the fraction subjected to GC/MS analysis. In addition to following EPA Phase II procedures, some modifications were made to the column elution sequence to improve toxicity resolution and tracking. The basic approaches are described below and the rationale for selected changes are presented in the Results Section.

C_{18} SPE Sorption and Elution

Filtered effluent or back-diluted, toxic Phase I methanol eluate was pumped at a rate of 5 ml per minute over a C_{18} SPE column having a capacity sufficient to accommodate the volume of sample treated. Column volume capacity followed manufacturer's suggested guidelines. The standard Phase II elution series (25, 50, 75, 80, 85, 90, 95, and 100% methanol/water) was used to sequentially elute the loaded C_{18} column. Subsamples of the fractions were then diluted and tested for toxicity. The test solutions were prepared to limit concentrations of methanol to less than 1.5% (v/v).

To transfer the effluent toxicity into the methanol phase, whole effluent samples containing measurable chronic toxicity were filtered through a standard glass-fiber filter (Gelman Sciences, Inc., Ann Arbor, MI) and then pumped through a high capacity C_{18} SPE column (Analytichem International, Harbor City, CA) containing 10 g of sorbent. The loaded column was eluted with a large volume of methanol (20-80 ml). The eluate was then concentrated under a nitrogen stream to attain an appropriate concentration factor for use in testing. With the nonpolar toxicants concentrated in methanol, additional manipulations were done to further isolate the toxicant(s) from nontoxic effluent components. To ensure the toxicity in the methanol phase was the same as the toxicity in the whole effluent, the methanol phase was subjected to Phase I TIE procedures. Those findings were compared to whole effluent toxicity characteristics. If the toxicity in both the methanol and the effluent gave similar results, assurance was gained that the toxicant was the same.

Several modifications of the standard elution series were implemented to improve recovery of toxicity, or to increase separation of effluent components eluted in near proximity to the toxicant(s). In several cases, all of the eight methanol/water solutions were not used for column elution to avoid gradual bleeding of toxicity into multiple eluates. Additionally, multiple 100% methanol eluates were collected at the end of the series to increase recovery of highly nonpolar compounds from the column.

Concentration of Toxic Phase II Fractions

The concentration step is necessary to increase the concentration of analytes to detectable levels and to remove water from the fraction. Depending on eluate complexity, concentrated

toxic Phase II fractions with few components were directly analyzed by GC/MS. Fractions with many components were further separated by HPLC before analysis.

Toxic Phase II fractions were diluted 1:10 with Millipore® water and pulled by vacuum through a 1-ml C_{18} SPE column. The column was then purged with nitrogen to remove any residual water. The dried column was eluted with multiple 100 µl aliquots of methanol. The elution volume was measured with a Hamilton® microsyringe. Generally, the column was eluted with 300 µl of methanol. However, when color was still present in the column additional methanol was pulled through until the column was clear. This eluate was then tested at the highest nominal effluent concentration possible while limiting the concentration of methanol in the solution to 1.5% (v/v).

HPLC Separation Techniques

Toxic, concentrated eluates were further separated by HPLC to decrease the number of compounds in each fraction associated with any observed toxicity. A Hewlett-Packard 1050 HPLC including quaternary solvent delivery pump, variable wavelength detector, automatic liquid sampler and HPLC(2D) Chemstation with a Spherisorb® 4.6 mm X 250 mm C_{18} column (5 µm particle size) was used.

Chromatographic conditions for HPLC fractionation were as follows:

Injection volume:	50-450 µl.
Wavelength:	230 nm.
Flow rate:	1.0 ml/minute.
Mobile phase:	Methanol/water.
Solvent gradient:	30% methanol composition at injection linearly increased to 100% at the end of 20 minutes and isocratic for 5 minutes at 100% methanol.

Typically, 25 discreet fractions were collected at 1-minute intervals. The fractions were then tested at the highest nominal effluent concentrations possible while limiting the test solution methanol concentrations to 1.5%. Any of the HPLC fractions discovered to be toxic were concentrated as described above (procedure for concentrating Phase II fractions).

A second method for obtaining HPLC fractions was to inject 5 μl of C_{18} eluate concentrate on the HPLC and record the initial retention times of each major peak using UV detection. Depending on sample availability, 100-450 μl of concentrated Phase II fraction was injected on the column. Each fraction was collected beginning at the initial peak retention time until the initial retention time of the next peak was reached. With each of the collected fractions having a different volume, all fractions were tested at 1.5% methanol. The toxic fractions' volumes were measured with a microsyringe and the toxicity further quantified.

GC/MS Methods

Concentrated toxic eluates from either Phase II or HPLC fractionations were analyzed using GC/MS. An HP system including 5890 gas chromatograph with a RT_x-5 30 M x 0.25 mm capillary column (J & W), 5970 mass spectrometer, 59940 chemstation, and 7673 autosampler was used. GC/MS conditions for the analyses were as follows:

Injector temperature:	250°C.
Transfer line temperature:	280°C.
Temperature program:	Injection at 50°C, isothermal at 50°C for 4 minutes, 10°C/minute to 175°C, 5°C/minute to 275°C, then isothermal at 275°C for 20 minutes.
Carrier gas:	Helium with a column head pressure of 5 psi.
Mass detection range:	50-550 amu.
Scan rate:	1 scan/sec.
Injection volume:	1 µl injected by an autosampler.
MSD calibration:	Autotune using perfluorotributylamine (PFTBA).

Before sample analysis, the GC column and mass spectral detector were checked to make sure they met routine quality control criteria for instrument sensitivity and resolution. An internal standard was added to an aliquot of both the concentrated sample and blank before analysis. The internal standard was 1,4-diiodobenzene in methanol. The blank and concentrate were then injected into the GC/MS for tentative identification and quantitation of sample constituents. The reported concentration for all peaks was determined by comparison to internal standard instrument response. The response factor was assumed to be the same for the internal standard and all the peaks to be quantified. The estimated concentration in the extract was calculated using the following equation:

Concentration in extract (μg/ml or mg/L) = C_i x A_i /A_{int} (Equation 3-1)

Where A_i = Chromatographic Peak Area.
A_{int} = Diiodobenzene Peak Area.
C_i = The concentration of an internal standard in extracts (10 mg/L for concentrate and blank).

Library searches were performed using a Wiley mass spectral library in the HP-UX data base. All chromatographic peaks were corrected for background before performing reverse-searching algorithms. Identifications with quality of fit ≥ 70 were considered reliable.

Section 3
RESULTS

PHASE I RESULTS FOR SELECTED REFINERY EFFLUENTS

Effluents from five selected refineries were fractionated following Phase I and II TIE procedures to identify nonpolar, organic toxicants. The information from the toxicity tests conducted on individual fractions was used to refine the search for nonpolar organic toxicants. Results from modifications to the general TIE procedures are presented to illustrate how to develop and interpret data from effluent-specific approaches.

Samples from Refineries #1 and #2 were evaluated for chronic toxicity to larval fathead minnows. Samples from Refineries #3, #4, and #5 were evaluated for chronic toxicity to mysids. The choice of test species was based on the refinery's NPDES permit requirement. Samples from Refineries #1, #2, #3, and #4 were sufficiently toxic to proceed with Phase I TIE characterization procedures (Tables 3-1 through 3-4). The single effluent sample tested from Refinery #5 did not contain sufficient toxicity to warrant Phase I toxicity characterization procedures.

Phase I results indicated that various amounts of C_{18} extractable toxicity were present in effluent samples from each of the four refineries. The results from each of the Phase I C_{18} SPE methanol eluate tests showed nonpolar organic toxicity was recovered from the columns. Other common toxicity characteristics among the refinery effluents included: (1) substantially increased effluent toxicity at test pH of 6.0, and (2) a slight toxicity reduction following either aeration or filtration.

Refinery #1 effluent was selected for identification procedures because it exhibited the most C_{18}-extractable toxicity. Furthermore, the extractable toxicity was readily recoverable in methanol eluates from the column which provided additional evidence that the toxicity was due to nonpolar organic compounds. Refinery #1 effluent was also free of other classes of toxicants. One principle of tracking TIE toxicity is to distinguish toxicity sources from among multiple potential toxicants. This effluent appeared to have only one type of toxicant.

TABLE 3-1. Chronic Phase I Toxicity Characterization Results for Fathead Minnows Exposed to Refinery #1 Final Effluent

Treatment	Effluent Concentration (%)	7-Day % Survival	Growth as Biomass Wt. (mg)
Whole Effluent	100	0	-
	75	20	0.072
	50	75	0.234
	Control	100	0.365
Filtration	100	40	0.049
	75	75	0.204
	50	85	0.258
	Blank	100	0.384
Aeration	100	5	0.007
	75	35	0.108
	50	74	0.299
	Blank	100	0.338
Post C_{18}	100	90	0.337
	75	100	0.360
	50	100	0.354
	Blank	100	0.364
EDTA	100	0	-
	75	20	0.042
	50	60	0.096
	Blank	100	0.347
$Na_2S_2O_3$	100	5	0.019
	75	10	0.021
	50	53	0.062
	Blank	100	0.370
pH 6.0	100	0	-
	75	0	-
	50	30	0.069
	Blank	100	0.408
pH 8.5	100	0	0
	75	10	0.013
	50	60	0.160
	Blank	100	0.420
Methanol Eluate	200	0	-
	Blank	100	0.316

TABLE 3-2. Chronic Phase I Toxicity Characterization Results for Fathead Minnows Exposed to Refinery #2 Final Effluent

Treatment	Effluent Concentration (%)	7-Day % Survival	Growth as Biomass Wt. (mg)
Whole Effluent	100	33	0.110
	50	94	0.360
	Control	100	0.465
Filtration	100	17	0.057
	50	94	0.394
	Blank	94	0.502
Aeration	100	67	0.270
	50	100	0.464
	Blank	89	0.428
Post C_{18}	100	100	0.511
	50	100	0.471
	Blank	94	0.481
EDTA	100	22	0.101
	50	100	0.389
	Blank	100	0.385
$Na_2S_2O_3$	100	17	0.056
	50	83	0.421
	Blank	89	0.360
pH 6.0	100	0	-
	50	0	-
	Blank	94	0.424
pH 8.5	100	50	0.223
	50	94	0.437
	Blank	100	0.380
Methanol Eluate	200	0	-
	Blank	100	0.438

TABLE 3-3. Chronic Phase I Toxicity Characterization Results for Mysids Exposed to Refinery #3 Final Effluent

Treatment	Effluent Concentration (%)	7-Day % Survival
Whole Effluent	100 Control	14 86
Filtration	100 Blank	57 71
Aeration	100 Blank	43 71
Post C_{18}	100 Blank	71 86
EDTA	100 Blank	43 71
$Na_2S_2O_3$	100 Blank	57 71
pH 6.0	100 Blank	14 100
pH 8.5	100 Blank	43 71
Methanol Eluate	200 Blank	0 86

TABLE 3-4. Chronic Phase I Toxicity Characterization Results for Mysids Exposed to Refinery #4 Final Effluent

Treatment	Effluent Concentration %	96-Hour % Survival	7-Day % Survival	Growth as Biomass Wt. (mg)
Whole Effluent	100	30	30	0.022
	75	60	50	0.059
	50	70	50	0.096
	Control	78	78	0.195
Filtration	100	30	10	0.034
	75	90	70	0.097
	50	70	70	0.072
	Blank	100	88	0.179
Aeration	100	50	40	0.091
	75	90	90	0.117
	50	90	90	0.161
	Blank	100	90	0.179
Post C_{18}	100	70	20	0.008
	75	90	80	0.115
	50	90	60	0.119
	Blank	100	90	0.157
EDTA	100	30	30	0.031
	75	90	80	0.130
	50	70	60	0.104
	Blank	90	80	0.212
$Na_2S_2O_3$	100	30	30	0.006
	75	50	30	0.057
	50	80	80	0.116
	Blank	100	89	0.122
pH 6.0	100	0	0	-
	75	10	10	0.009
	50	60	50	0.058
	Blank	90	90	0.219
pH 8.5	100	20	0	-
	75	60	40	0.044
	50	80	80	0.064
	Blank	90	90	0.151
Methanol Eluate	200	20	10	0.003
	Blank	90	50	0.082

PHASE II NONPOLAR ORGANIC TOXICITY IDENTIFICATION

Early in the study, rapid toxicity degradation was observed in Refinery #1 effluent even under refrigerated storage. The initial screen for toxicity takes four to seven days to complete, and TIE work takes an additional week or more. This toxicity degradation limited the amount of follow-up testing possible with any particular sample. Table 3-5 illustrates degradation of nonpolar organic toxicity. After degradation was confirmed, subsequent effluent samples were extracted with C_{18} and eluated with methanol immediately upon arrival before the initial toxicity screen. Since the toxicity did not readily degrade in methanol, the time within which any particular sample could be tested was extended.

TABLE 3-5. Percentage Survival of Fathead Minnows in C_{18} Concentrates Made from an Effluent Sample That Had Aged for 12 and 19 Days

Test Date	12 Days Post Receipt			19 Days Post Receipt	
Eluates (Percent Methanol)	Blank 4X[a]	2X	4X	Blank 4X	4X
25	90	100	75	100	90
50	90	50	60	100	95
100	80	70	0	100	90

[a] X is the number of times the effluent and/or reagents were concentrated.

To ensure that the toxicity in the methanol phase was the same as the toxicity in the whole effluent, the methanol phase was subjected to Phase I TIE procedures. Those findings were compared to whole effluent toxicity characteristics. If the toxicity in both the methanol and the effluent gave the same results, assurance was gained that the toxicant was the same. The whole effluent from Refinery #1 was always more toxic when tested at pH 6.0 than when tested without pH adjustment. At test pH 6.0, acute as well as chronic toxicity was present in the effluent and the eluate. In contrast, the C_{18}-extracted effluent was not chronically toxic at either natural pH or pH 6.0, indicating removal of all measurable toxicity. This distinction was assurance that the whole effluent toxicity was the same as that observed in the methanol eluate.

pH Modifications of the Effluent Prior to C_{18} Sorption

Two avenues examined to provide further chemical separation and increase toxicity recovery were pH adjustment and alternative methanol/water (Phase II) elution sequences of the C_{18} SPE column to obtain sharper elution of the toxicity. Aliquots of toxic effluent were adjusted to pH 3.0 and pH 9.0, filtered, then pumped through a C_{18} SPE column. Toxicity elution was similar at both pH extremes (Table 3-6). GC/MS analyses of the toxic fraction showed too many constituents to distinguish differences between pH 3.0 and pH 9.0 aliquots (Figure 3-1). The pH modification procedure was not successful in simplifying the toxic eluates, nor was a difference in toxicity recovery observed. This finding was surprising because recovery by C_{18} of pH-sensitive toxicants is usually altered if the effluent pH is changed. Another refinery effluent was examined for pH sensitivity. The Refinery #3 NPDES permit required mysids as test species, and the same pH modification was attempted to see if the mysid toxicity behaved similarly. The data show a similar pattern (Table 3-7). While data from both show some change in toxicity, the change is small relative to the effect of pH change on whole effluent toxicity.

TABLE 3-6. Refinery #1, Fathead Minnow Percent Survival in C_{18} Eluate Employing pH Adjustment Before Extraction

Extraction pH	Blank 15X	3.75X	7.5X	15X
pH*i*	100	100	0	0
pH 3.0	100	100	20	0
pH 9.0	100	100	40	0

TABLE 3-7. Refinery #3, Mysid Percent Survival in C_{18} Eluate Employing pH Adjustment Before Extraction

Extraction pH	Blank 4X	1X	2X	4X
pH*i*	40	0	0	0
pH 5.0	60	40	0	0
pH 9.0	60	80	0	0

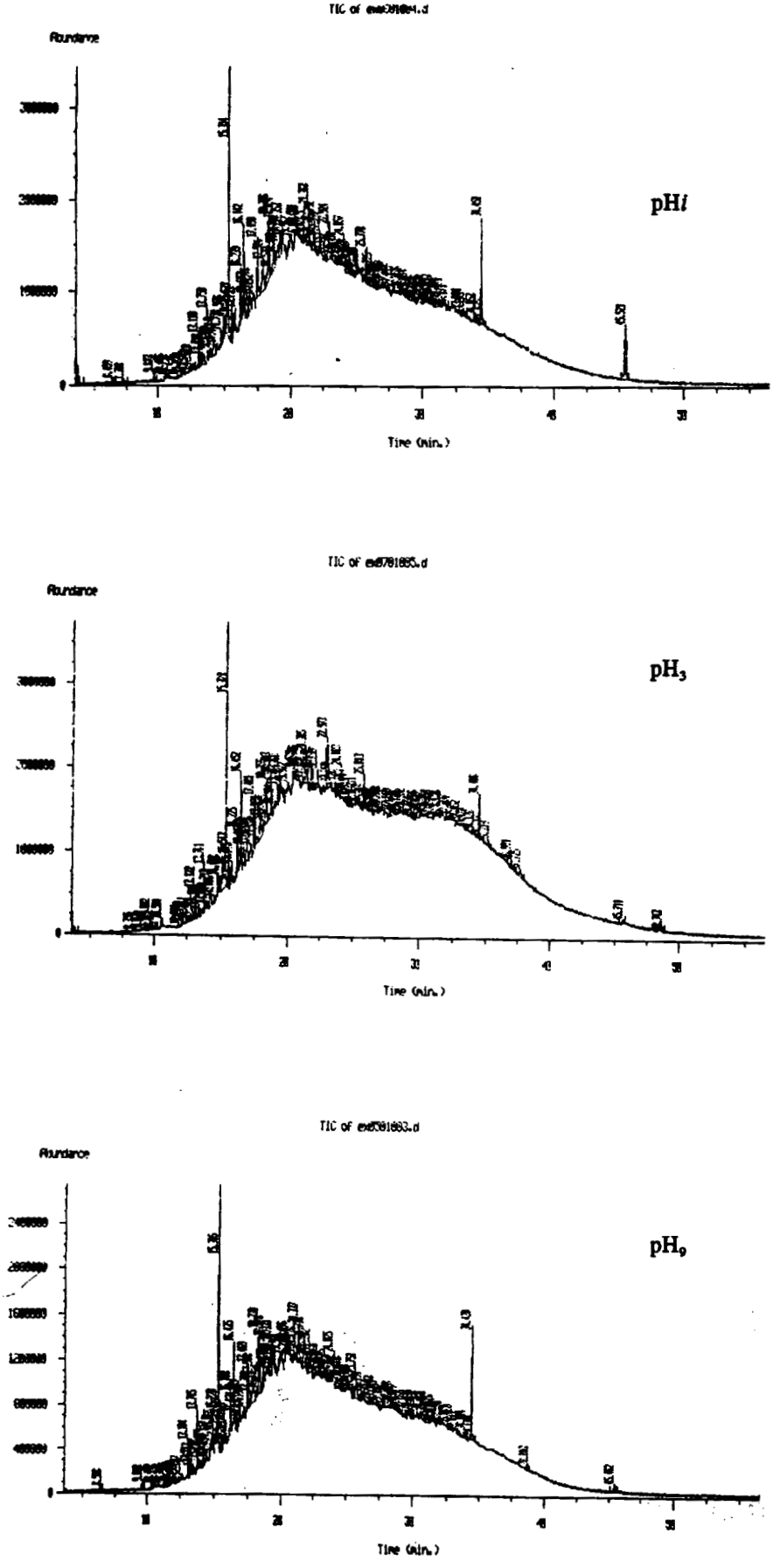


FIGURE 3-1. GC/MS Scans of pH*i*, pH 3.0, and pH 9.0 100% Methanol Toxic Phase II Fraction Concentrates, Refinery #1 - Sample I

Modification of the Standard Phase II Elution Sequence

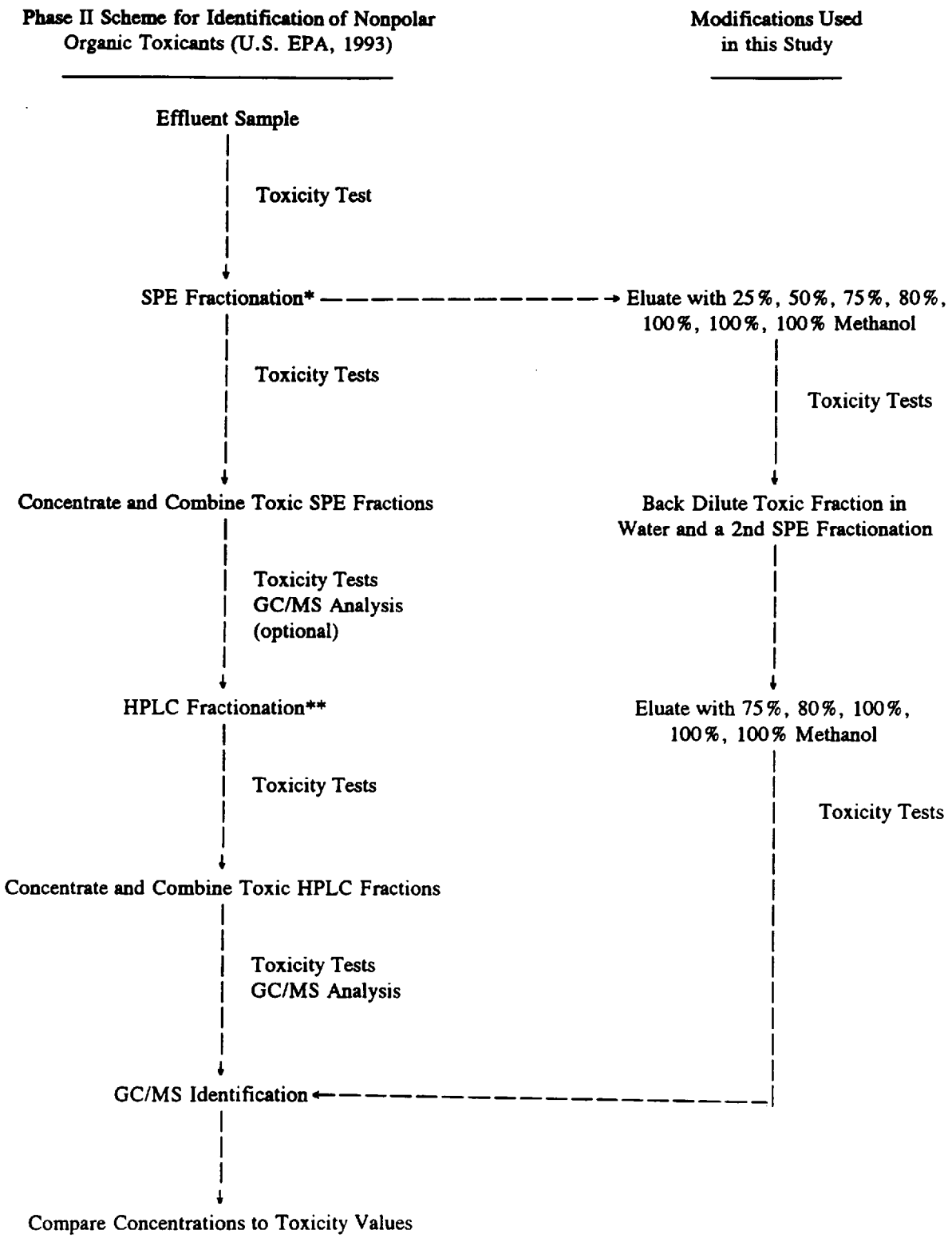
The second avenue to simplify the toxic effluent fraction was modification of the standard Phase II elution sequence. Past experience with Phase II fractionation procedures on refinery effluents indicated that C_{18} toxicity was dispersed through multiple fractions during the eight fraction, methanol/water elution sequence. Table 3-8 shows an example of the poor resolution of toxicity to mysids during a standard Phase II elution sequence for Refinery #3. A different pattern was obtained with Refinery #1 and fathead minnows (Table 3-9). One interpretation of the results from the elution of Refinery #1 effluent was that the toxicant was smeared in many fractions and none were toxic. An obvious approach would then be to reduce the number of fractions.

Figure 3-2 compares the U.S. EPA recommended scheme of fractionation and the one used in this study. The C_{18} columns were eluted with a sequence of 25%, 50%, 75%, 80%, and 100% methanol which was expected to remove some non-toxic constituents from the fractions containing the toxicity as well as to obtain more toxicity in one fraction. Multiple 100% methanol eluates were collected because visible color remained on the column after the first 100% fraction indicating effluent components remained on the column. Testing showed toxicity occurred in the first and second 100% fraction (Table 3-10), and toxicity recovery was nearly complete.

TABLE 3-8. Mysid Percent Survival in Eight Fractions Eluted from C_{18} SPE Columns Using Effluent from Refinery #3

Eluate (% Methanol)	Blank 4X	2X	4X
25	80	Not Tested	60
50	100	100	60
75	80	80	60
80	60	80	0
85	100	100	20
90	100	100	60
95	100	80	80
100	100	60	Not Tested

FIGURE 3-2. Comparison of U.S. EPA and Modified Scheme Used in This Study for Identification of Nonpolar Toxicants



* 25%, 50%, 75%, 80%, 85%, 90%, 95%, 100% Methanol Fractions are Suggested
** 25 Fractions are Suggested

TABLE 3-9. Fathead Minnow Percent Survival in Eight Elutes from C_{18} SPE Columns Using Effluent from Refinery #1

Eluates (% Methanol)	Blank 4X	2X	4X
25	100	100	100
50	100	100	100
75	100	100	100
80	100	40	100
85	100	100	100
90	100	100	100
95	100	100	100
100	100	100	100

TABLE 3-10. Fathead Minnow Percent Survival in Five Eluates from a C_{18} SPE Column that had Previously Been Eluated with 25 and 50% Methanol Using Effluent from Refinery #1

Eluates (% Methanol)	Blank 20X	20X
75	80	100
80	80	100
100 1st	100	0
100 2nd	-	0
100 3rd	-	100

This modified Phase II elution pattern isolated the toxicant(s) into two methanol eluates. The toxic eluate was concentrated, and the concentrate was tested to confirm toxicity. The concentrate was then processed through further separation using the standard HPLC parameters suggested in U.S. EPA Phase II Toxicity Identification guidance. Toxicity tests with the 25 HPLC fractions showed none of the fractions were toxic; therefore, the standard HPLC fractionation procedure did not offer a method to reduce complexity of toxic samples.

Since the Phase II modified elution scheme should have resulted in reduction of sample complexity, the toxic C_{18} concentrates were analyzed via GC/MS without HPLC separation to determine complexity and to search for possible suspect toxicants. Figure 3-3 is the total ion chromatogram obtained and shows dramatic reduction in complexity compared to the ion chromatogram for pH*i* in Figure 3-1. The chromatogram had 23 discernible peaks, 4 of which had a fit >70. One of the four was the internal standard, diiodobenzene. A second peak, a benzenedicarboxylic acid, was common to both the C_{18} concentrate and the non-toxic procedural blank and could be disregarded. Third, an ethanol acetate compound was identified, but it was also detected at essentially the same estimated concentration in a non-toxic eluate obtained from the effluent sample; therefore, it could be eliminated as a probable toxicant. The fourth compound was bis(1,1 dimethylethyl)phenol and could not be discounted as a suspect toxicant.

Because there were still many unidentified peaks, further concentrate separations were performed to reduce the number of peaks associated with toxicity. Since HPLC was not an option (based on above described trials), additional separation using C_{18} SPE was employed to reduce concentrate complexity while retaining effluent toxicity. The concentrate (which did not contain the compounds elutable by 25% and 50% methanol) was back-diluted in water and extracted with another C_{18} SPE column. The column was then sequentially eluted with 75%, 80%, and three 100% methanol/water solutions. Toxicity was again recovered in the first two 100% methanol eluates as was the case during the first elution (Table 3-9). The two toxic 100% eluates were separately concentrated, then tested for toxicity. The eluates were analyzed by GC/MS (Figures 3-4 and 3-5). This second C_{18} extraction and elution sequence greatly reduced the compounds associated with effluent toxicity. [Note the y axis scale is greater in Figure 3-3 (the first extraction) than in Figures 3-4 and 3-5.] No toxicants were identified in the first 100% eluate, but the second 100% eluate contained four identifiable peaks. One peak was the internal standard and two other peaks were propanoic acid and 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl)ester at concentrations which appeared to be insufficient to cause toxicity. The fourth peak was again bis(1,1 dimethylethyl)phenol. Further testing indicated the phenolic compound remained associated with a portion of

effluent toxicity through multiple sample separations and concentrations. The phenolic compound appeared to be a likely toxicant.

FIGURE 3-3. GC/MS Total Ion Chromatogram of Toxic 100% Methanol Fraction Concentrate, Refinery #1 - Sample I

Retention Time (minutes)	Tentative Identification
15.271	Ethanol, 2-(2-butoxyethoxy)-,acetate
16.394	1,4-diiodo-benzene
17.355	bis(1,1 dimethylethyl)phenol
34.340	1,2 Benzenedicarboxylic acid derivative

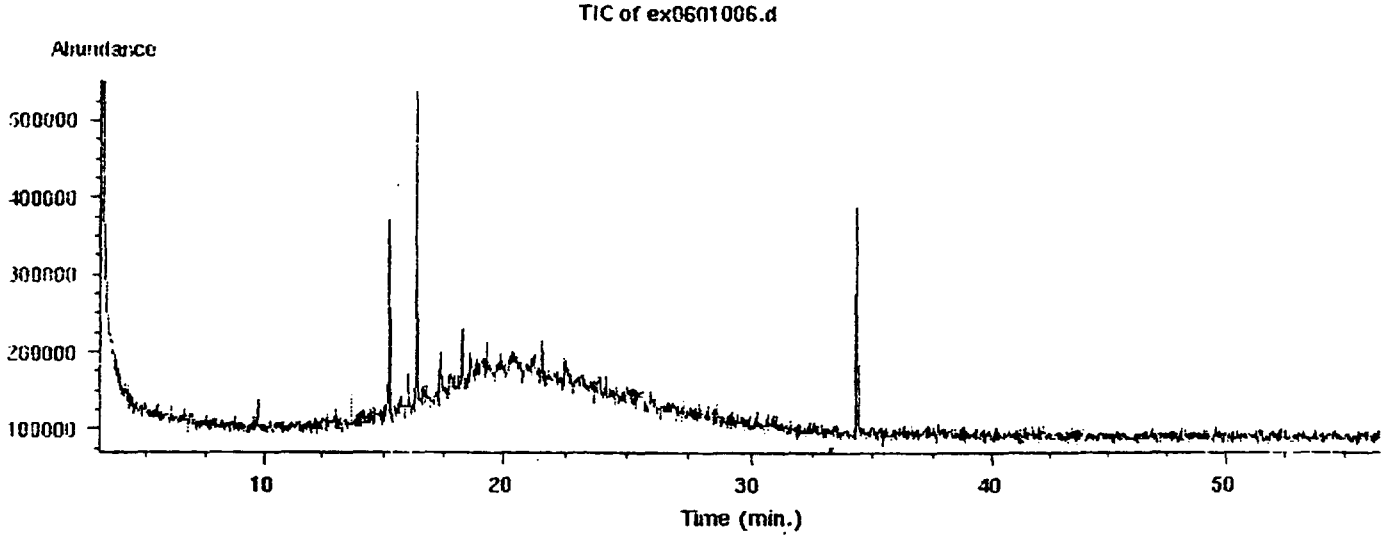


FIGURE 3-4. GC/MS Total Ion Chromatogram for the First Toxic 100% Methanol Fraction Concentrate, Refinery #1 - Sample I

Retention Time (minutes)	Tentative Identification	Amount in Fraction (mg/L)
13.282	unknown	3.11
15.256	unknown	5.87
16.384	1,3 diiodobenzene	10.0
18.243	unknown	11.52

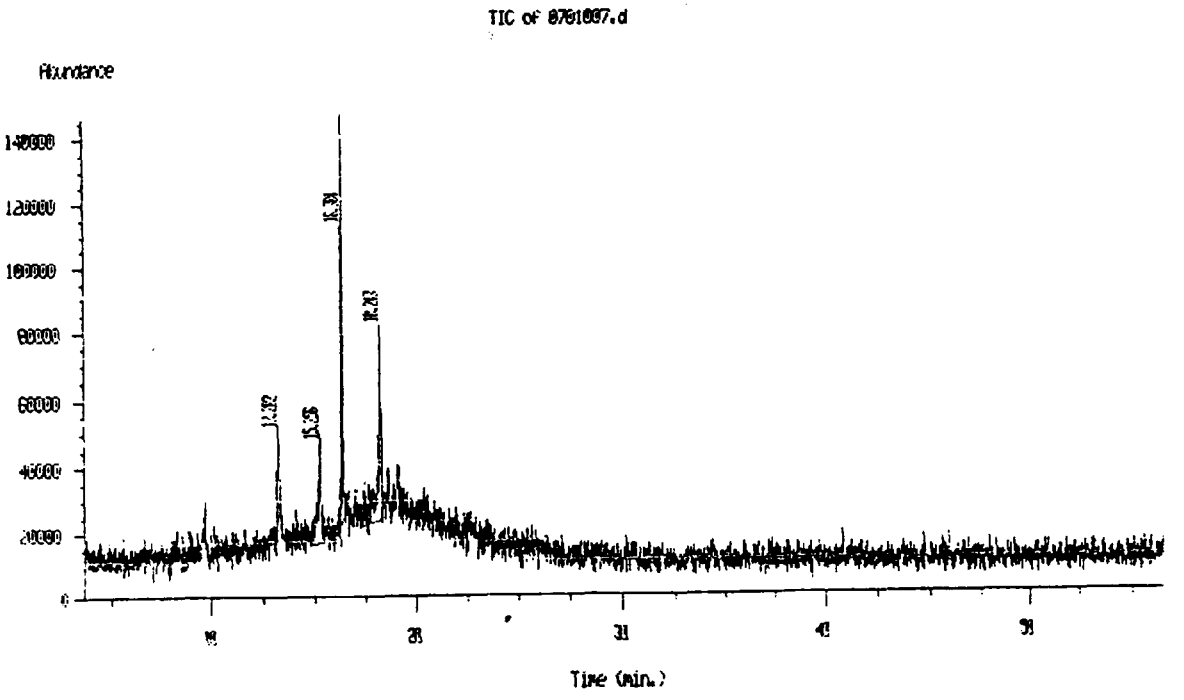
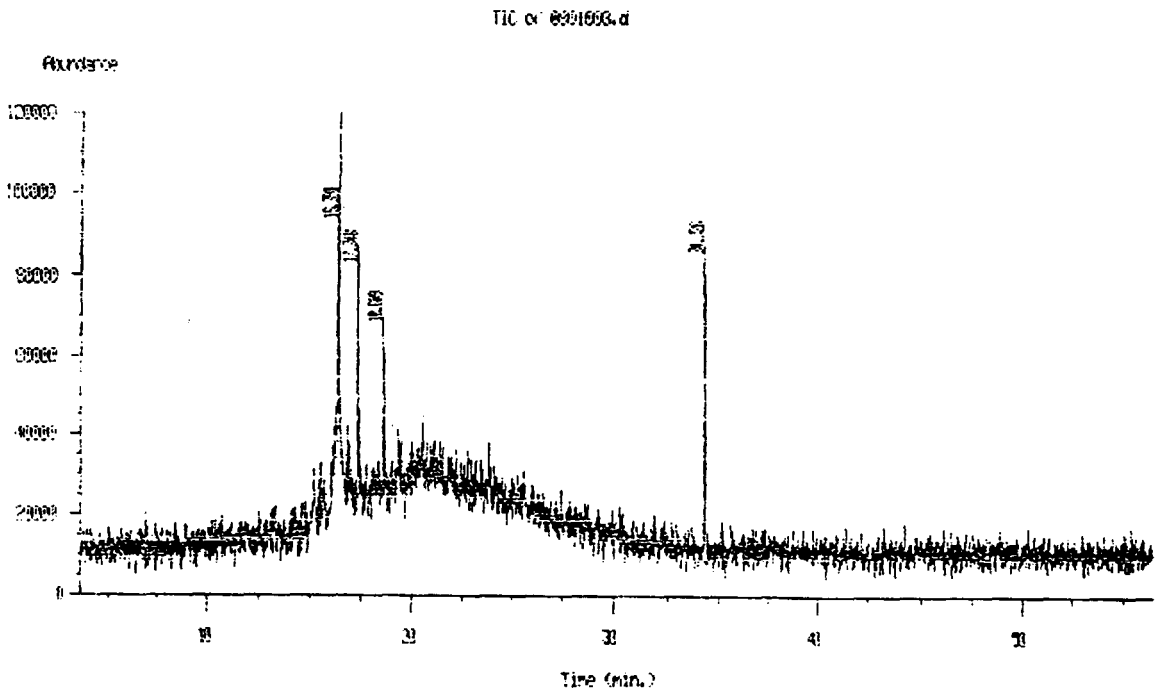


FIGURE 3-5. GC/MS Total Ion Chromatogram for the Second Toxic 100% Methanol Fraction Concentrate, Refinery #1 - Sample I

Retention Time (minutes)	Tentative Identification	Amount in Fraction (mg/L)
16.391	1,4 diiodobenzene	10.00
17.348	2,4-bis(1,1 dimethylethyl)phenol	4.28
18.609	Propanoic acid	2.29
34.326	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	8.29



Phase III Toxicity Confirmation

Effort was then directed towards gathering evidence as to whether the phenol compound was the toxicant. Literature searches did not reveal any information pertaining to the toxicity of that phenolic compound. A sample of the ditert-butyl phenol [bis(1,1 dimethylethyl)phenol] was purchased and the toxicity, GC/MS, and HPLC retention times were all determined.

The 96-hour LC_{50} for larval fathead minnows was 1.77 mg/L. At this stage of evaluation, acute toxicity information was sufficient to confirm tentative identification since acute toxicity was also measured in the eluates. However, the phenolic in the second 100% methanol fraction was only present at 0.07 mg/L in the dilution causing acute toxicity. This discrepancy is large, and since the quantitation in the effluent was not well established, efforts were directed towards better effluent quantitation of the phenolic.

The neat compound possessed essentially the same instrument retention times as the identified suspect in the effluent (Figures 3-6 and 3-7). However, a discrepancy in effluent fraction phenol concentration was discovered when comparing GC/MS quantitation to HPLC quantitation: the phenol concentration determined by HPLC was 100 times greater than the concentration determined by GC/MS. The HPLC value from the fractionated effluent likely represents the summed concentration of a group of compounds very similar in structure to the bis(1,1 dimethylethyl)phenol, each of which could be additive in toxicity and yield higher concentrations than with the phenolic determined by GC/MS. Using HPLC peak retention time separation, the "phenol" peak was separated from other concentrate constituents. The two HPLC fractions were tested for toxicity, and only the "phenol containing fraction" proved to be toxic. While this evidence did not solve the concentration discrepancy, it did continue implicating the phenolic.

A decision was made to obtain a sample of the phenolic from the source contributing to the refinery effluent rather than a reference sample. Refinery personnel traced the source of effluent phenol to two jet fuel additives. Samples of these additives were examined for potency and toxicity characteristics. The 96-hour LC_{50}s for fathead minnow larvae of the bulk additives were between 5 mg/L and 10 mg/L. Both additives were substantially more toxic when tested at pH 6.0, which is consistent with observed whole effluent toxicity characteristics. The toxicities of the additives were

FIGURE 3-6. GC/MS Chromatogram for 58 mg/L 2,4 ditert-butyl phenol and Toxic Fraction Concentrate, Refinery #1 - Sample I

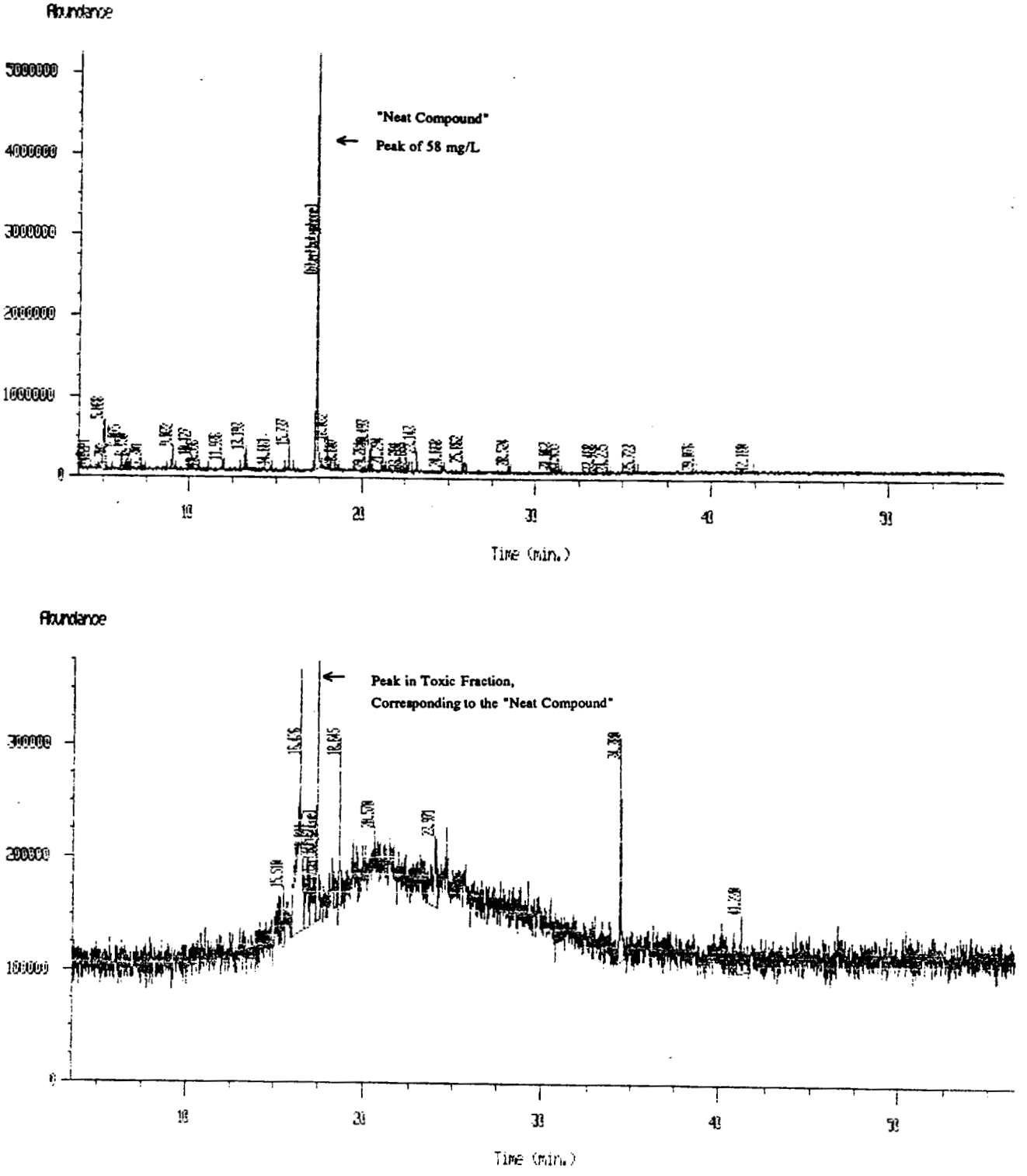
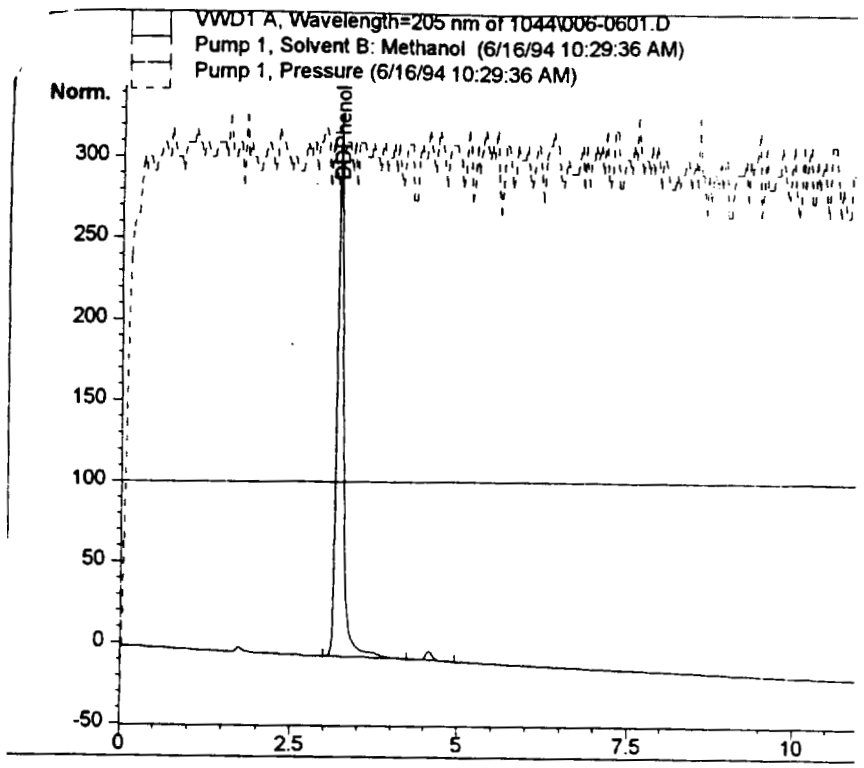
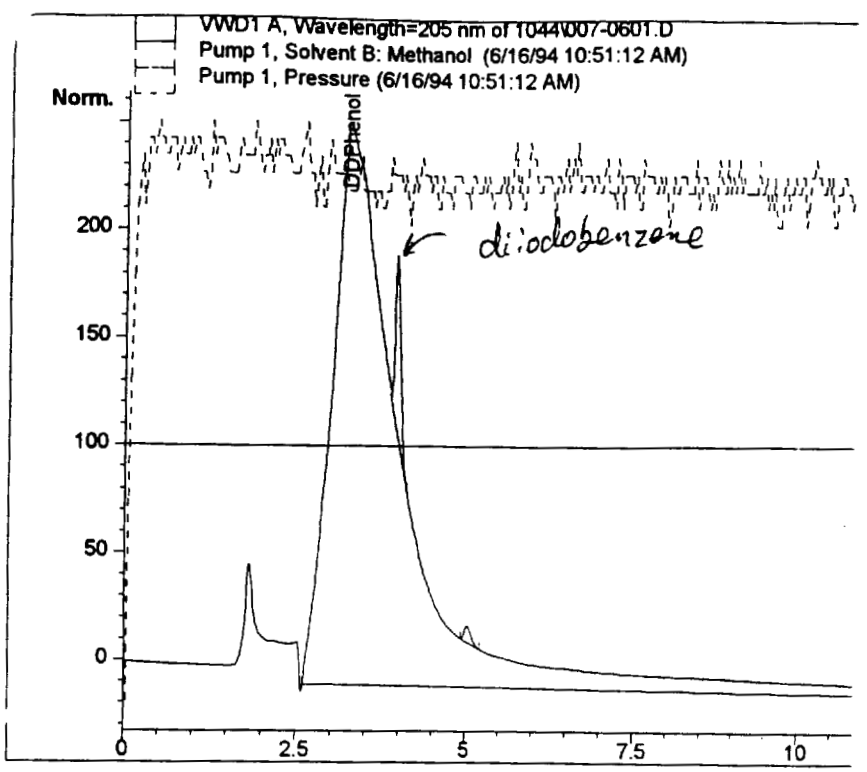


FIGURE 3-7. HPLC Chromatogram for 58 mg/L 2,4 ditert-butyl phenol and Toxic Fraction Concentrate, Refinery #1 - Sample I



"Neat Compound"

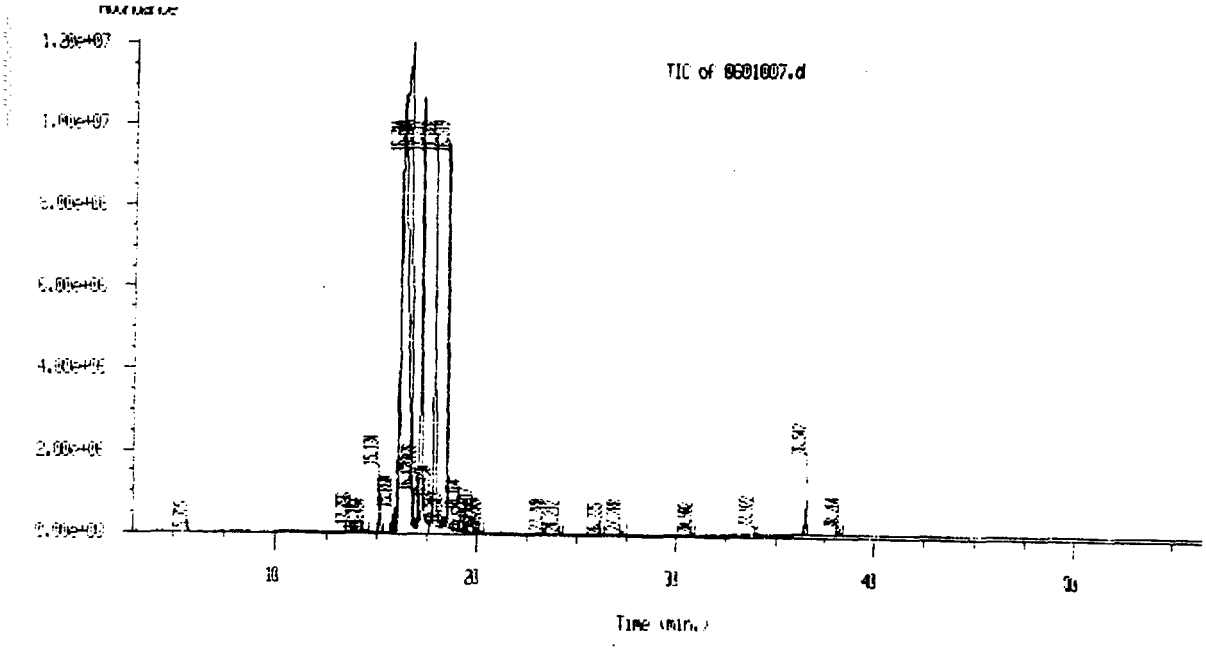
← Peak of 58 mg/L



← Peak in Toxic Fraction, Corresponding to the "Neat Compound"

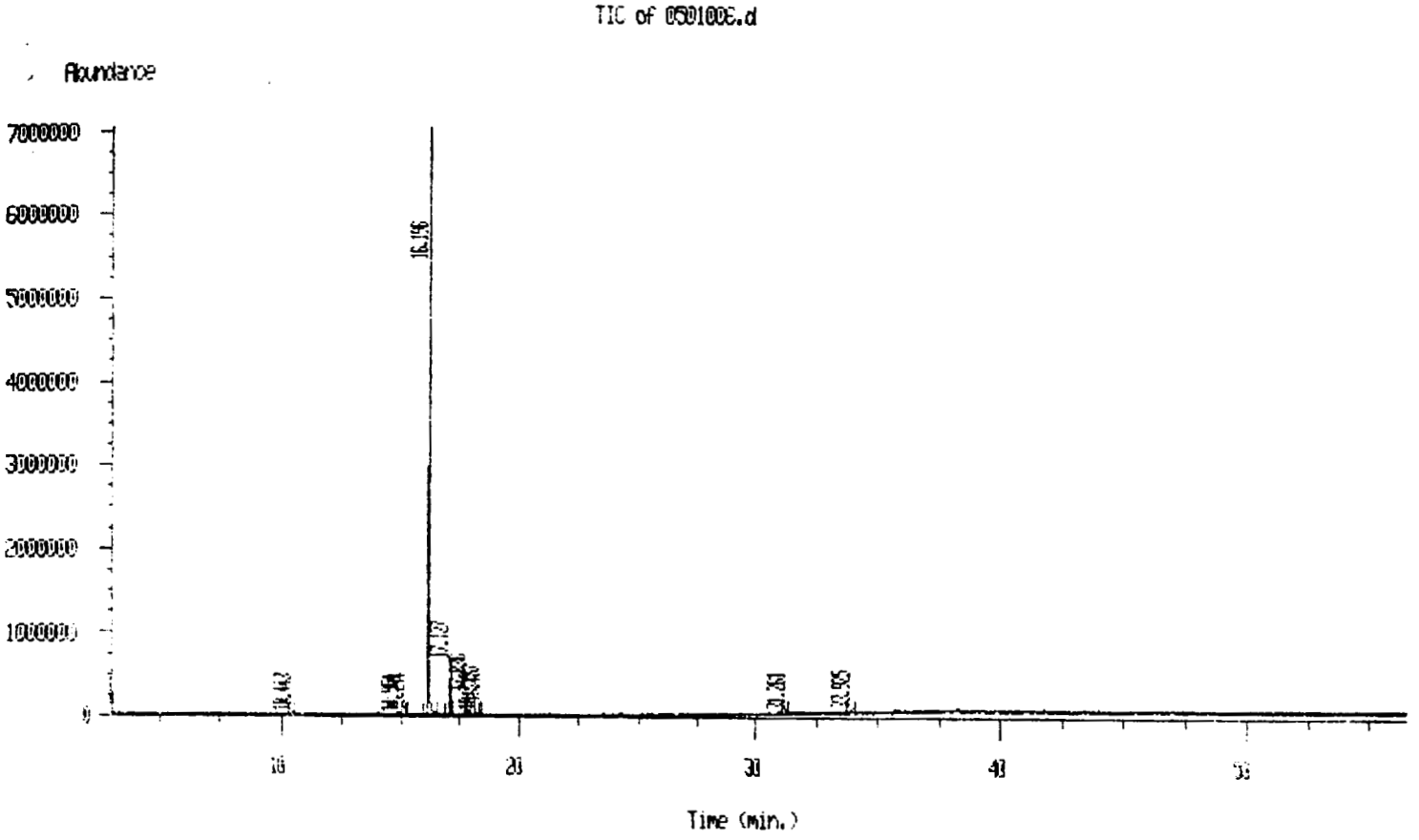
C_{18}-extractable, and the toxicity retained by the column was recovered in the higher Phase II methanol eluates (90%, 95%, and 100% methanol/water) just as was true for the effluent toxicants. GC/MS analysis of the toxic eluates prepared from the additives revealed the major identifiable components were mono-, bis-, and tris(1,1 dimethylethyl)phenol compounds (Figures 3-8, 3-9, and 3-10).

FIGURE 3-8. GC/MS Total Ion Chromatogram of the 90% Methanol Fraction Concentrate of Jet Fuel Additive A and Identified Peaks



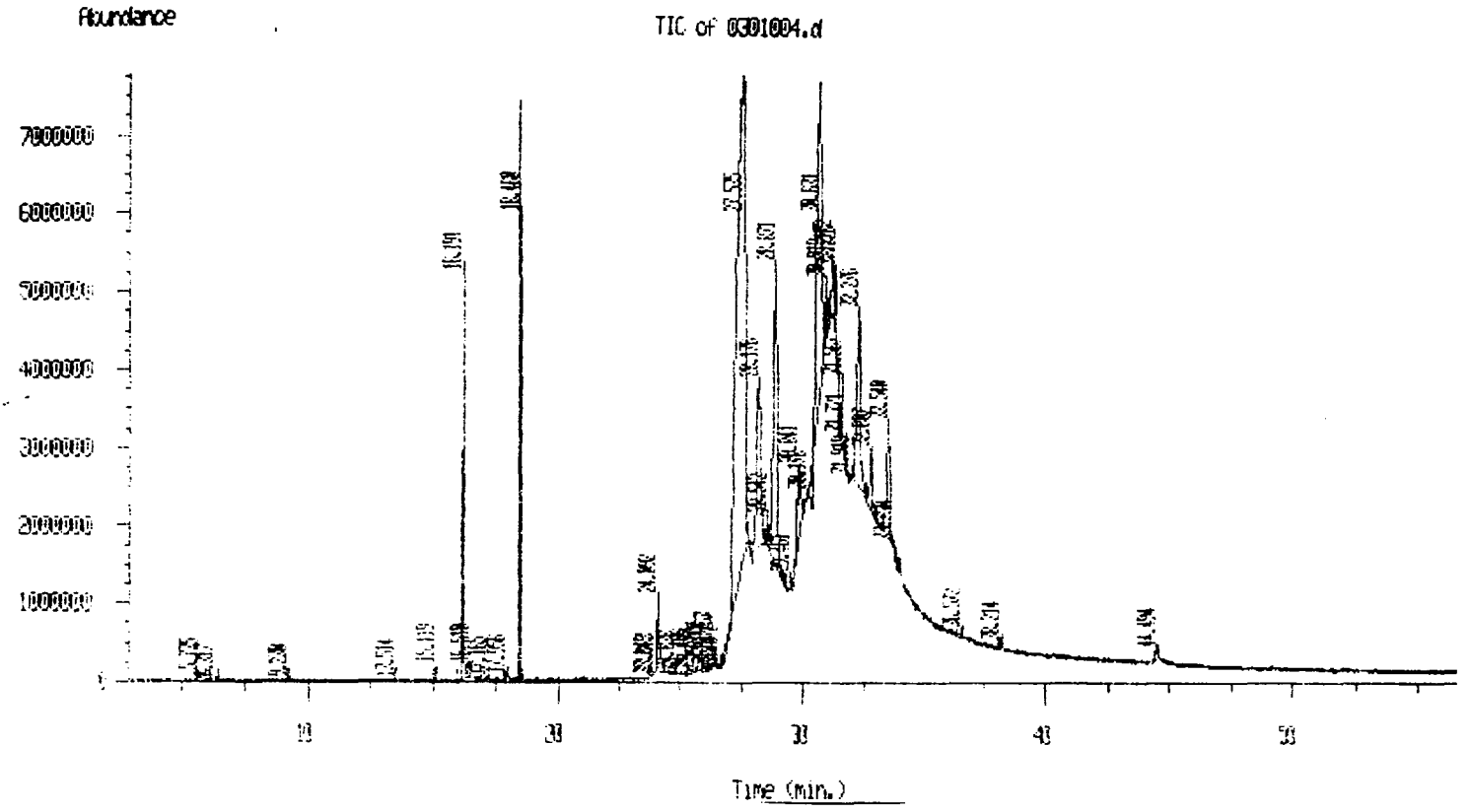
Retention Time (minutes)	Tentative Identification	Amount in Fraction (mg/L)	Amount at LC_{50} (mg/L)
15.136	3-(1,1 dimethylethyl)phenol	42.9	8.0
15.864	2,6-bis(1,1 dimethylethyl)phenol	7.5	1.4
16.686	2,6-bis(1,1 dimethylethyl)phenol	651.0	121.2
16.802	Methoxybis(1-methylpropyl)benzene	7.4	1.4
17.033	2,6-bis(1-methylpropyl)phenol	16.4	3.1
17.216	2,4-bis(1,1 dimethylethyl)phenol	335.5	62.5
17.751	2-(1,1 dimethylethyl)phenol	9.0	1.7
17.886	2,5-bis(1,1 dimethylethyl)phenol	265.9	49.5
18.523	2,4,6-tris(1,1-dimethylethyl)phenol	351.1	65.4
36.541	Rotenalone	40.3	7.5

FIGURE 3-9. GC/MS Total Ion Chromatogram of the 95% Methanol Fraction Concentrate of Jet Fuel Additive A and Identified Peaks



Retention Time (minutes)	Tentative Identification	Amount in Fraction (mg/L)	Amount at LC_{50} (mg/L)
16.199	2,6-bis(1,1 dimethylethyl)phenol	101.64	86.7
17.127	2,4-bis(1,1 dimethylethyl)phenol	10.05	8.6
17.834	2,4-bis(1,1 dimethylethyl)phenol	4.78	4.0

FIGURE 3-10. GC/MS Total Ion Chromatogram of the Combined 95% and 100% Methanol Fraction Concentrate of Jet Fuel Additive B and Identified Peaks



Retention Time (minutes)	Tentative Identification	Amount in Fraction (mg/L)	Amount at LC_{50} (mg/L)
5.739	1,3 dimethyl-benzene	7.24	2.0
15.119	3-(1,1 dimethylethyl)phenol	5.56	1.5
16.189	2,6-bis(1,1 dimethylethyl)phenol	86.96	23.8
18.457	2,4,6-tris(1,1 dimethylethyl)phenol	134.07	36.6
24.095	tridecanoic acid	43.64	11.9
27.539	Heptadecene-(8)-carbonic acid-(1)	1008.43	275.6
28.176	9,12 Octadecadienoic acid, methyl ester, (E,E)-	167.13	45.7
28.538	9,12 Octadecadienoic acid (Z,Z)-	10.91	3.0
28.872	9,12 Octadecadienoic acid, methyl ester (E,E)-	276.95	91.4
32.277	(+,-)-cis-7,9-dimethoxy-1,3-dimethyl-3,4,5,10-tetrahydronaptho	158.47	43.3
32.810	1-Phenanthrenecarboxylic acid derivative	38.87	10.6

These data show several very similar compounds, varying only by the placement of the methylethyl groups. This observation supported the earlier results by HPLC quantitation which indicated a much higher concentration of the phenolics than those obtained by GC/MS. Using the HPLC quantitation, the estimated concentration would be above 1 mg/L, which is in the range of the toxic concentration determined for the ditert-butyl phenol [bis(1,1 dimethylethyl)phenol] that had been purchased.

Other evidence that the toxicant was identified included:

1) Toxicity was always present when the phenol was present and always absent when the phenol was absent.

2) The toxicity of both the whole effluent and jet fuel additive was greater at pH 6.0 than pH*i*.

3) The whole effluent and fraction toxicity was greater at pH 6.0 than pH*i*.

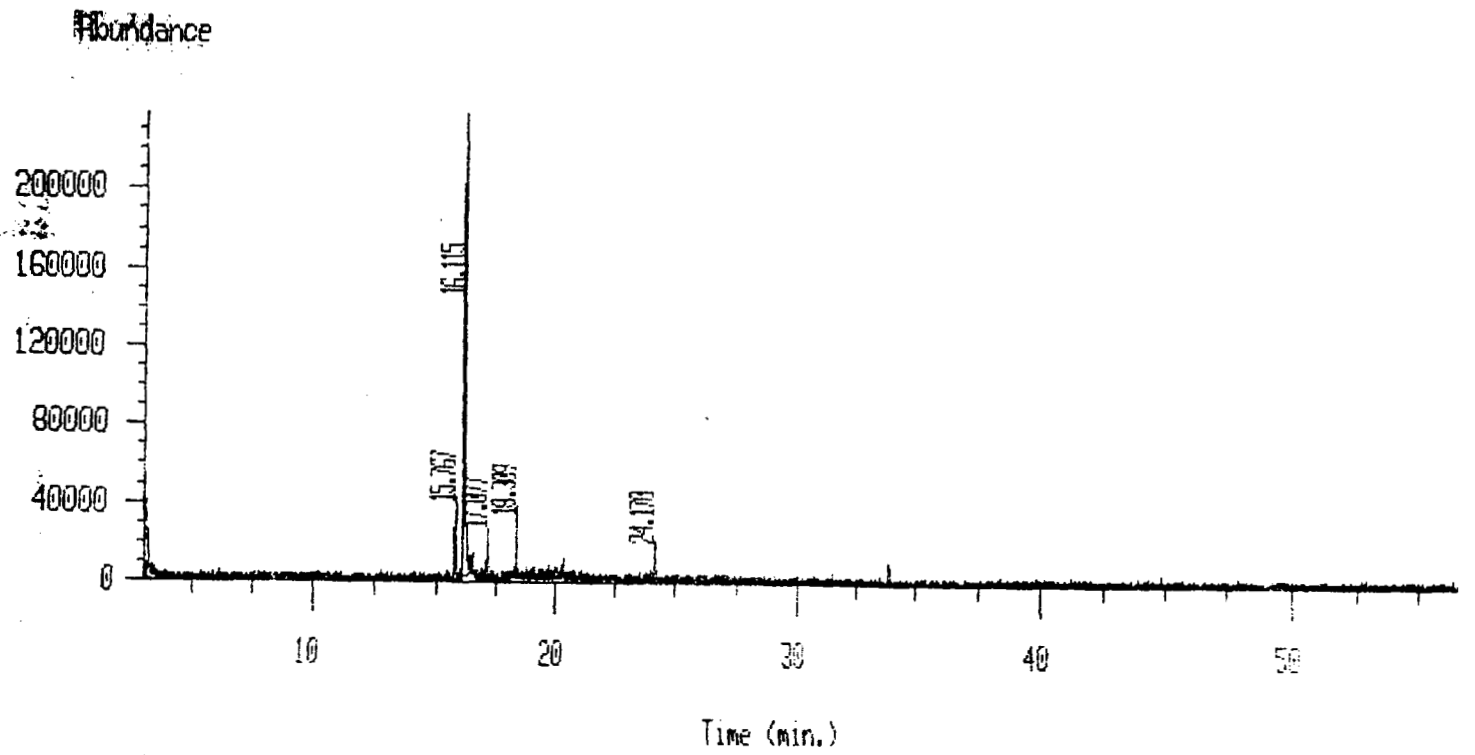
4) The toxicity of the whole effluent and the jet fuel additive was removed by SPE and eluated at the high methanol concentrations.

5) A plausible source of the suspect toxicant was identified in the refinery.

Toxicant Variability

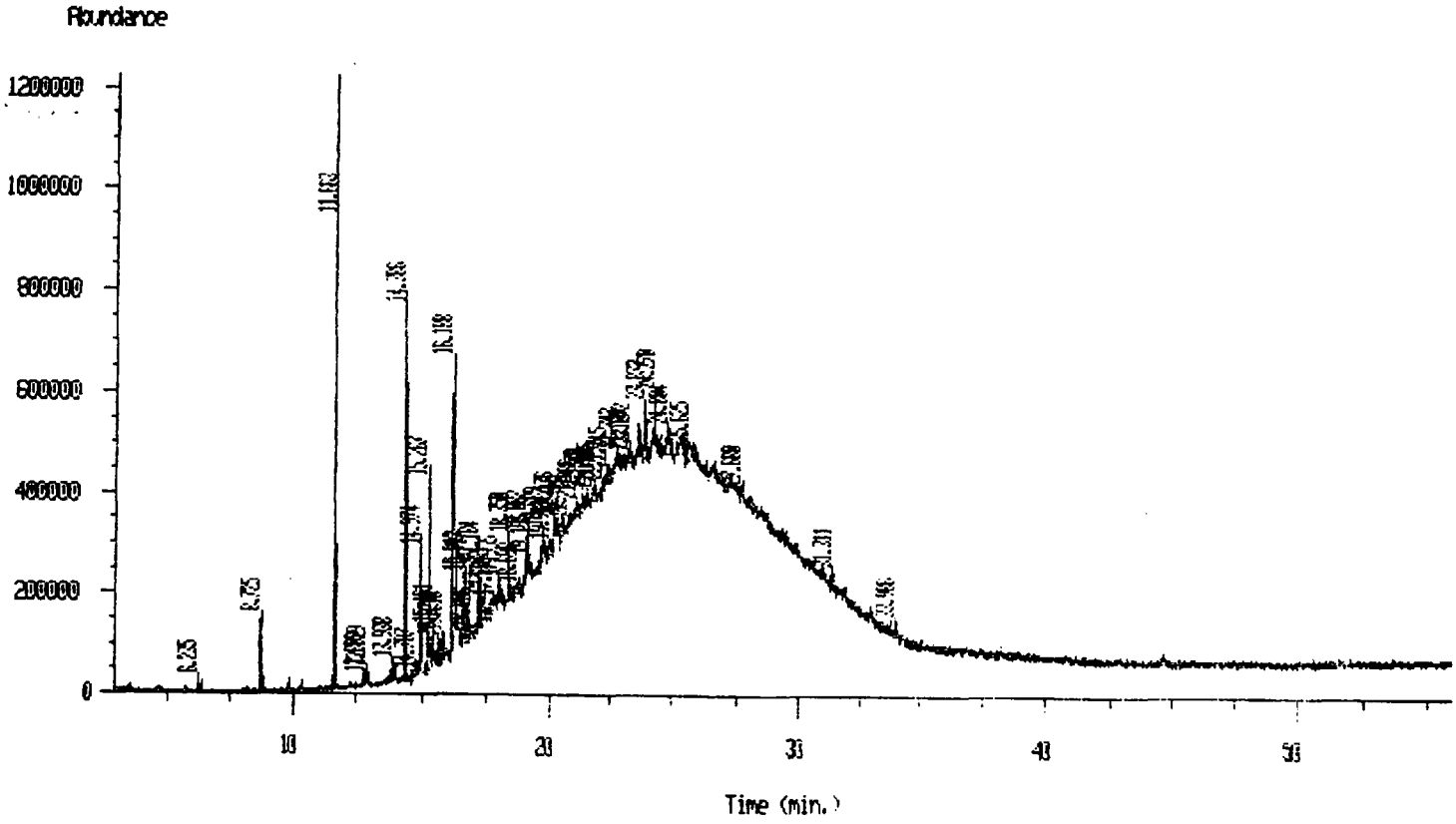
Due to anticipated difficulties in obtaining precise analytical measurements, additional samples were analyzed to obtain further confirming data of the presence of chronic toxicity and the phenolic compound(s). Four additional samples of effluent were screened for toxicity, and then C_{18} extracted using the modified Phase II elution scheme. For the first three samples in this group, toxicity was tracked through the procedure plus the GC/MS results indicating the bis(1,1 dimethylethyl)phenol was present in the toxic fractions. Figures 3-11 and 3-12 are the simplified toxic methanol fractions for the first two samples. Figures 3-13 and 3-14 are the original toxic fractions and the simplified chromatogram after Phase II separation for Samples I and II from Refinery #1. Results of testing with the fourth effluent sample in this sequence indicated a change in toxicity characteristics. The toxicity was still removed from the sample using C_{18} SPE and a significantly greater increase in toxicity occurred at acidic test pH. The difference was that the nonpolar toxicity was recovered in more polar C_{18} SPE methanol eluates (75% and 80% methanol/water), whereas before the toxicity was in 100% methanol eluate. The 75% and 80% eluates were combined and concentrated for GC/MS analysis. The toxic eluate concentrate showed a high degree of complexity and an absence of phenolic compounds.

FIGURE 3-11. GC/MS Total Ion Chromatogram of Simplified Toxic C_{18} SPE Fraction Concentrate, Refinery #1 - Sample II



Retention Time (minutes)	Tentative Identification
15.767	unknown
16.115	diiodo-benzene (internal standard)
17.077	2,6-bis(1,1 dimethylethyl)phenol
18.339	Propanic acid derivative (common C_{18} SPE column contaminant)
24.170	unknown

FIGURE 3-12. GC/MS Total Ion Chromatogram of Simplified Toxic C_{18} SPE Fraction Concentrate, Refinery #1 - Sample III



Retention Time (minutes)	Tentative Identification
8.229	Cyclo tetrasiloxane, octamethyl
11.660	Cyclo pentasiloxane, decamethyl
14.389	Cyclo hexasiloxane, dodecamethyl
15.269	Propanic acid
16.191	1,4 diiodobenzene
17.184	2,4-bis(1,1 dimethylethyl)phenol
19.977	2-(2'-aminophenyl amino) benzyl alcohol
24.271	1,2 benzenedicarboxylic acid, butyldecyl ester

FIGURE 3-13. GC/MS Total Ion Chromatogram of Toxic Eluates Before and After Phase II Separation, Refinery #1 - Sample I

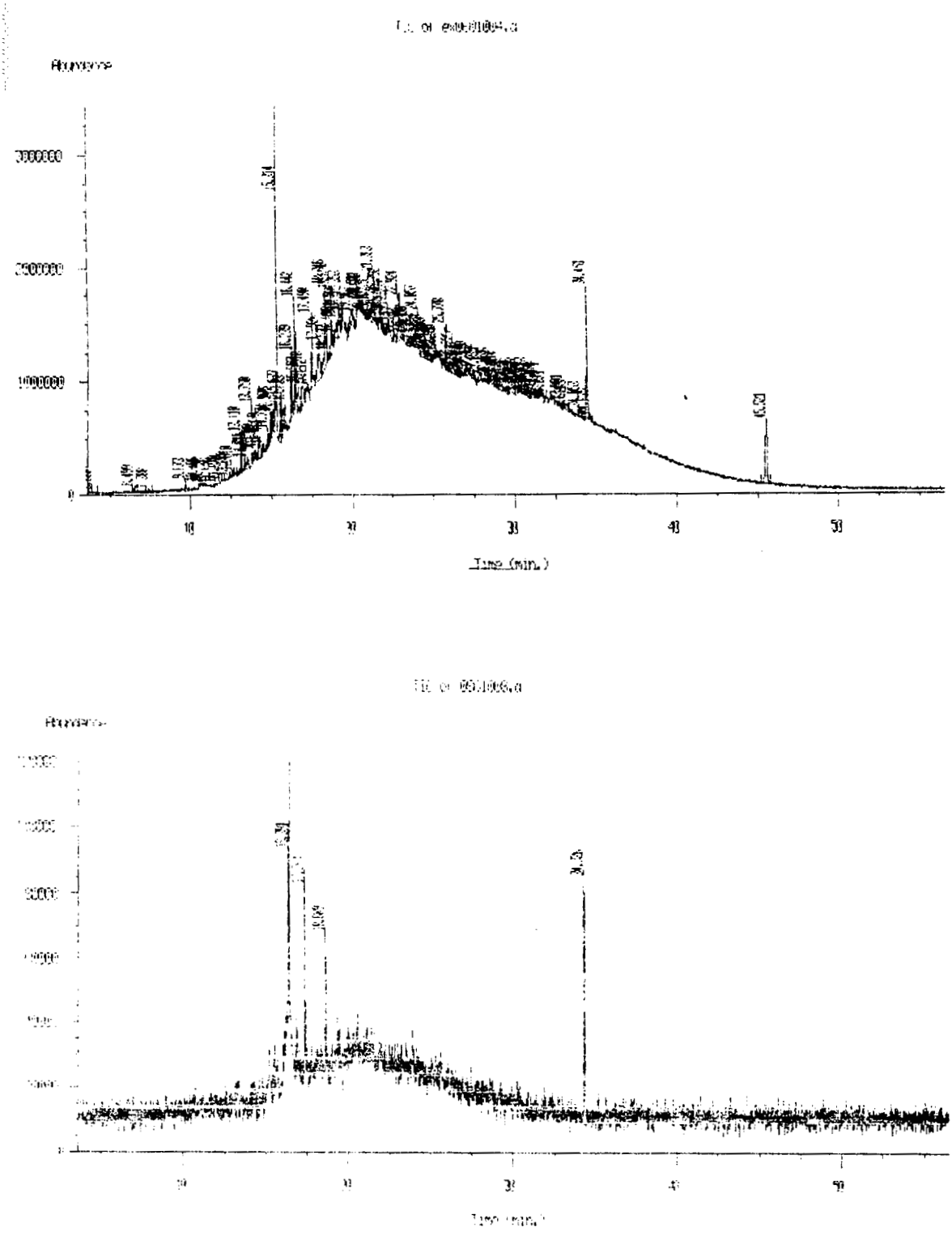
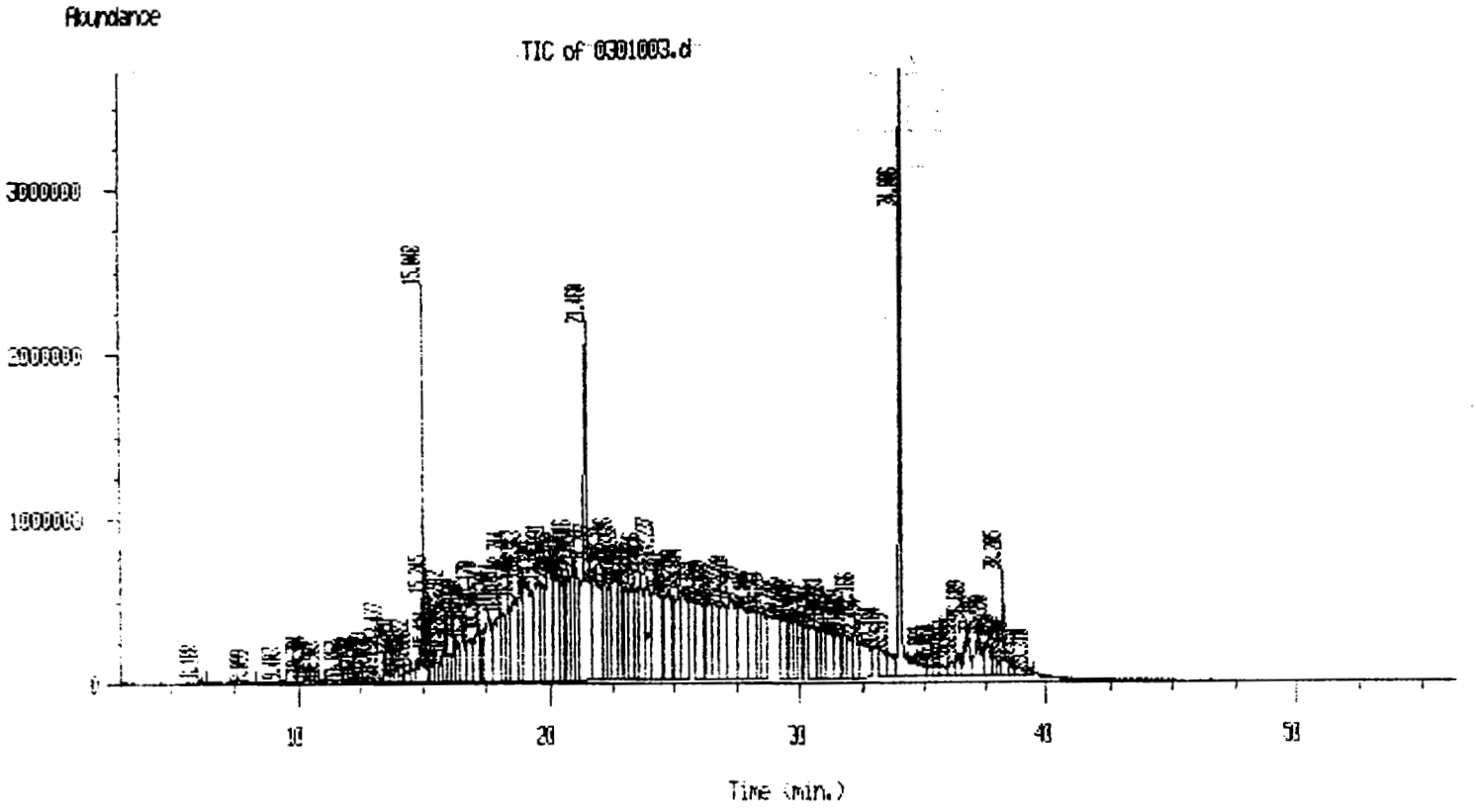
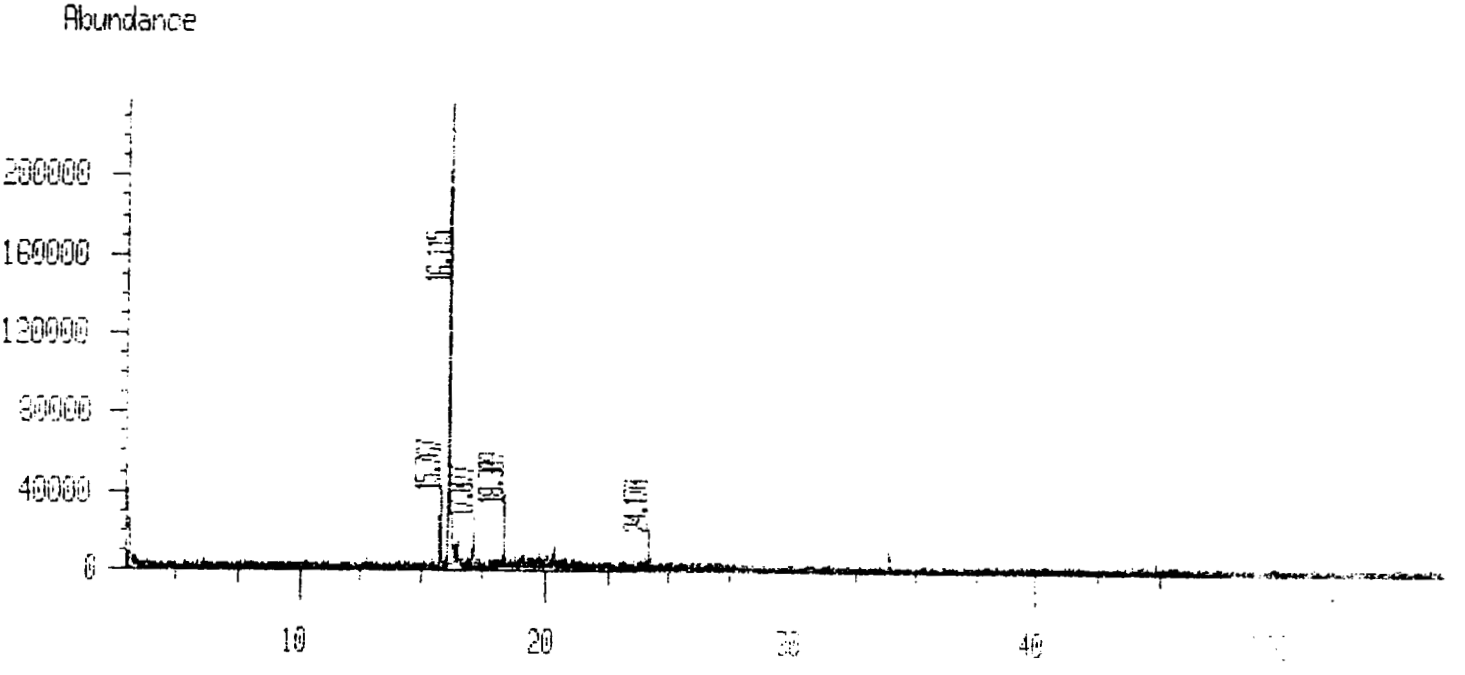


FIGURE 3-14. GC/MS Total Ion Chromatogram of Toxic Eluates Before and After Phase II Separation, Refinery #1 - Sample II



TIC of 0901003.d



Additional chemical separation and attempts to identify the apparent new cause of nonpolar organic toxicity were performed using the standard Toxicity Identification Evaluation HPLC program to reduce the number of compounds associated with toxicity. A test pH of 6.0 was employed to increase the likelihood of detecting toxicity after HPLC separation. Toxicity was recovered in HPLC fractions collected at minutes 15, 16, 17, and 18. Those HPLC fractions were combined and concentrated for GC/MS analysis (Figure 3-15). The sample was complex; however, five dominant peaks were detected, but none of the peaks was identifiable using the Wiley Library. The mass spectra for the four peaks at 25, 26, and 31 minutes are indicative of brominated aromatic compounds likely having similar components (Figures 3-16, 3-17, 3-18, 3-19, and 3-20). The peak with a 44 minute retention time was not identified; however, this peak was detected in a non-toxic procedural blank and can be dismissed as a possible cause of toxicity.

FIGURE 3-15. GC/MS Total Ion Chromatogram of the Concentrated Toxic HPLC Fraction Concentrate, Refinery #1 - Sample IV

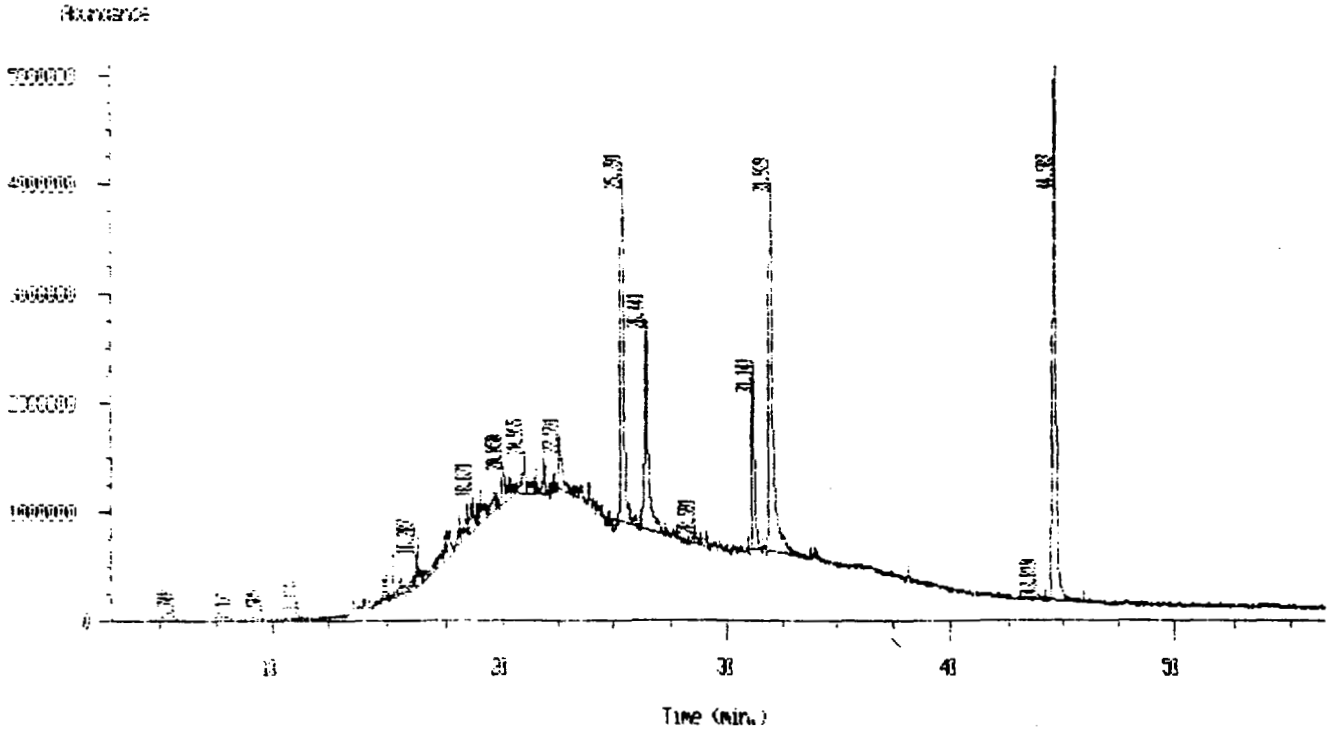


FIGURE 3-16. Mass Spectra of Peak at 25.301 Minutes, Refinery #1 - Sample IV

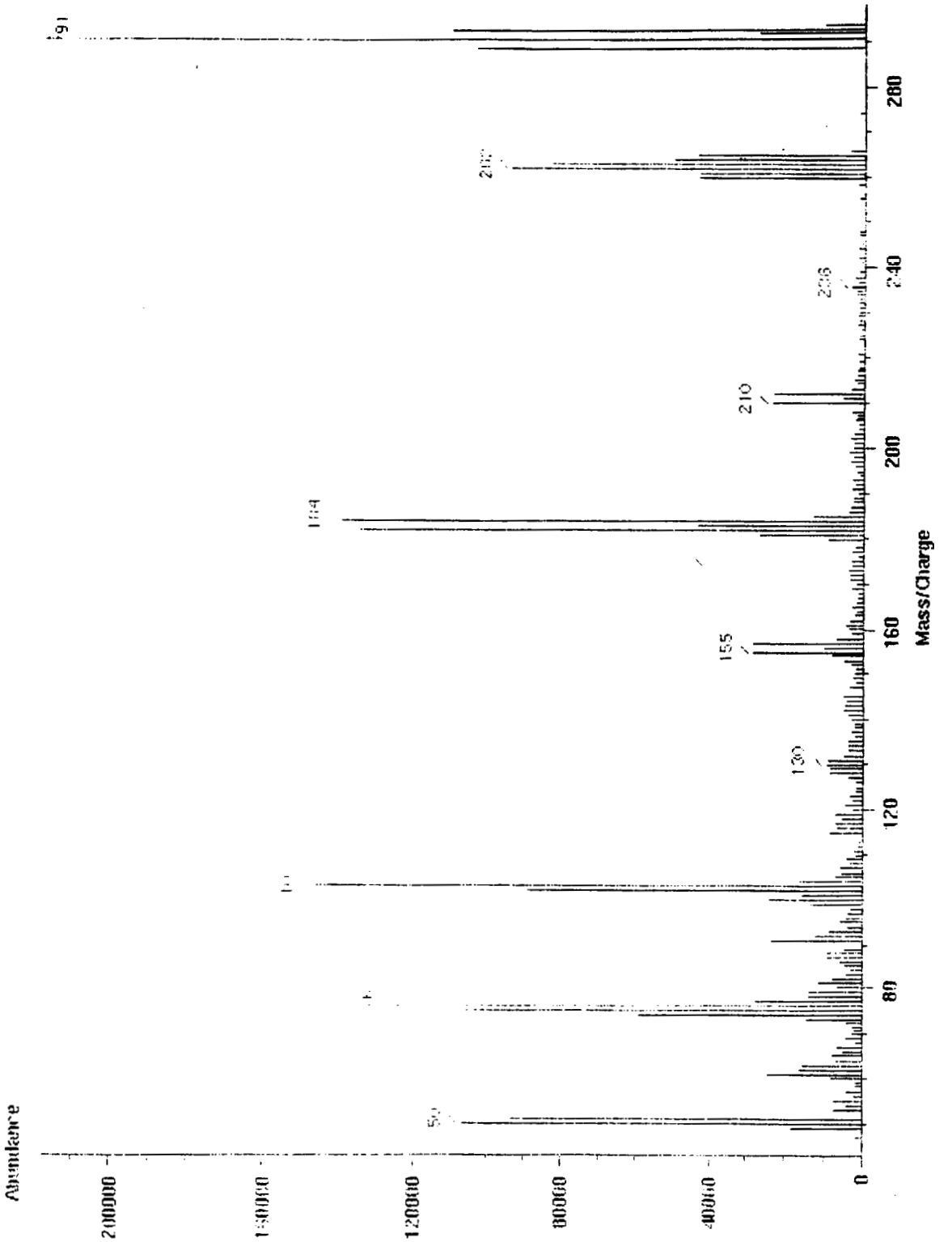


FIGURE 3-17. Mass Spectrum of Peak at 26.350 Minutes, Refinery #1 - Sample IV

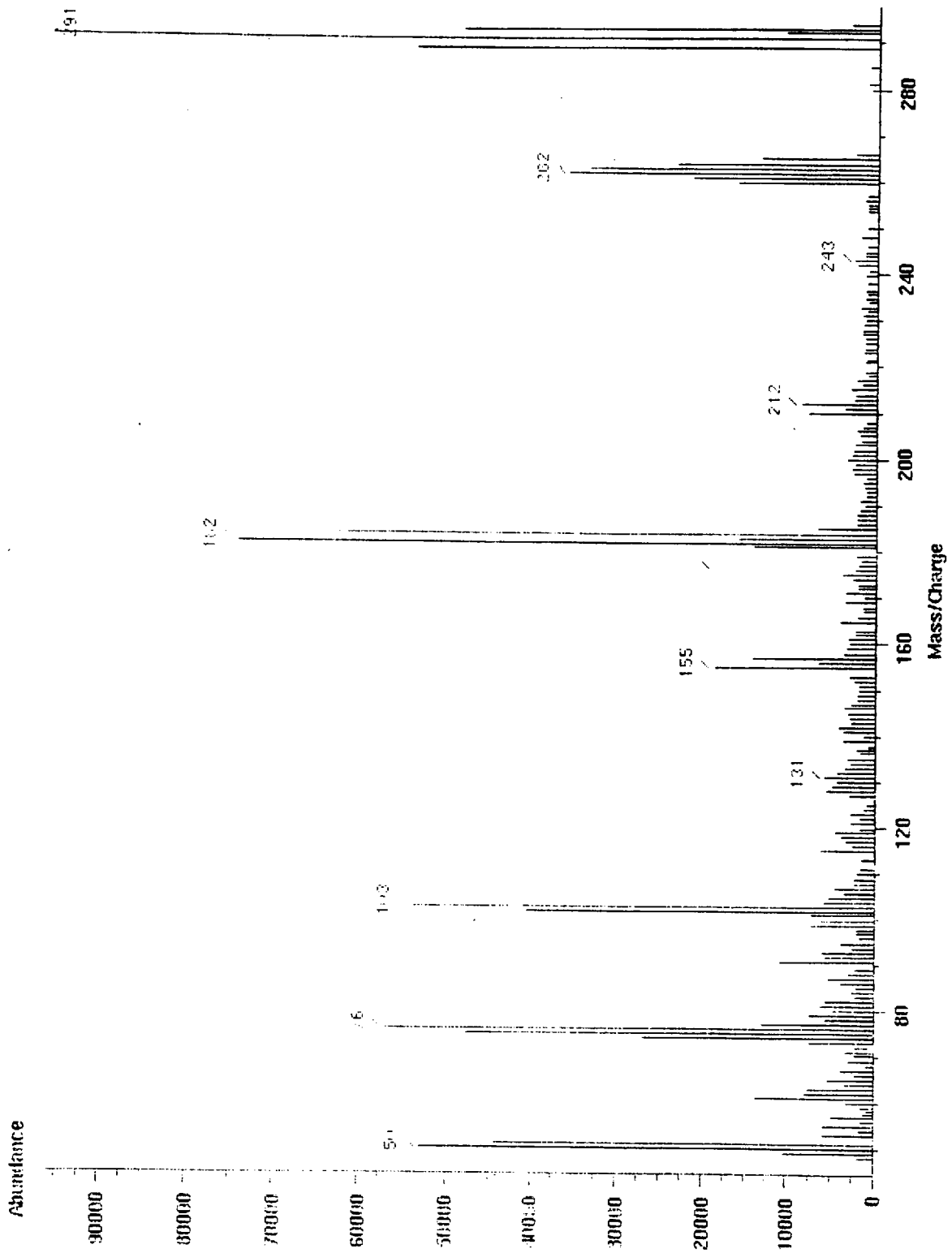


FIGURE 3-18. Mass Spectrum of Peak at 31.066 Minutes, Refinery #1 - Sample IV

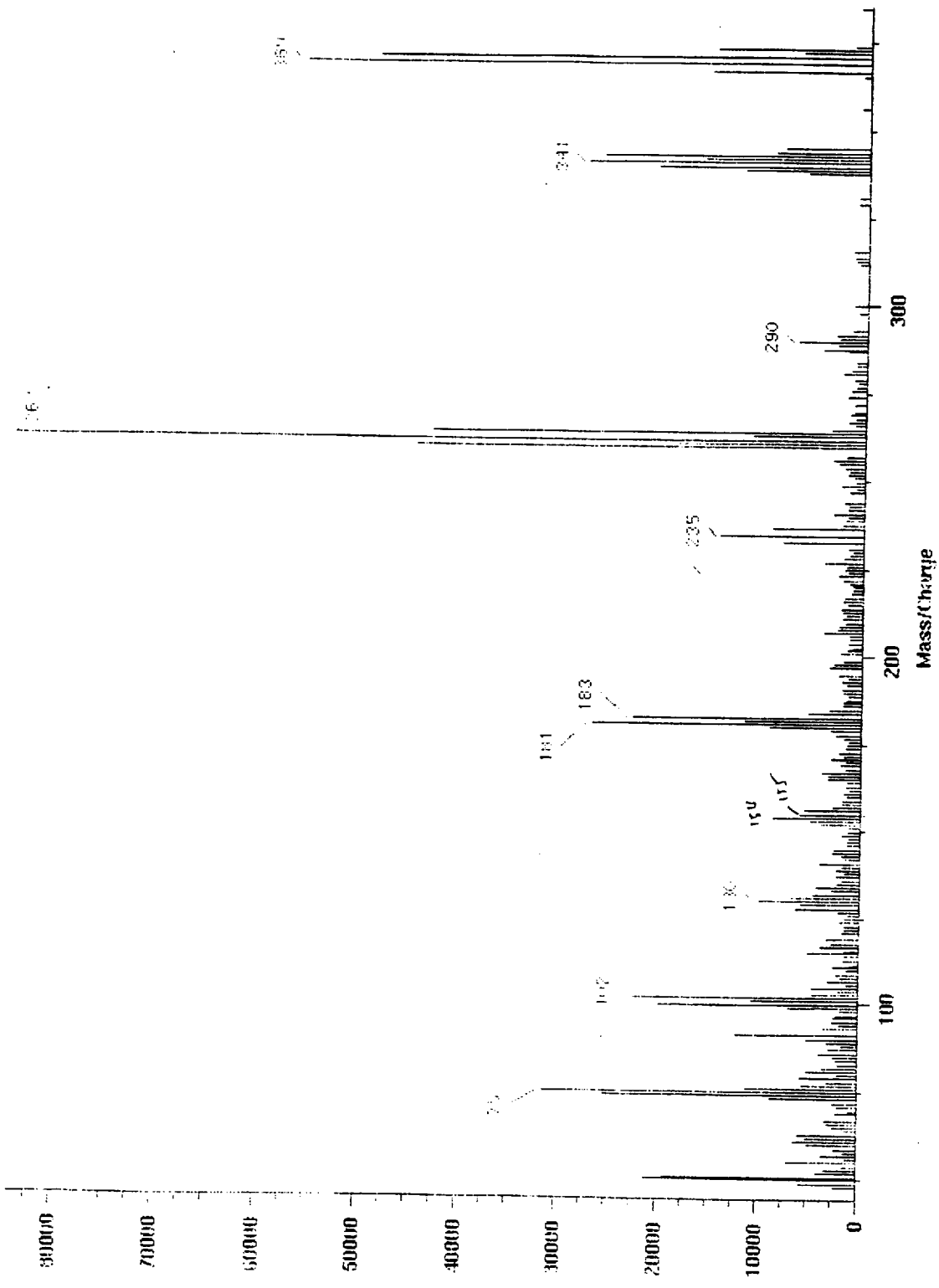


FIGURE 3-19. Mass Spectrum of Peak at 31.835 Minutes, Refinery #1 - Sample IV

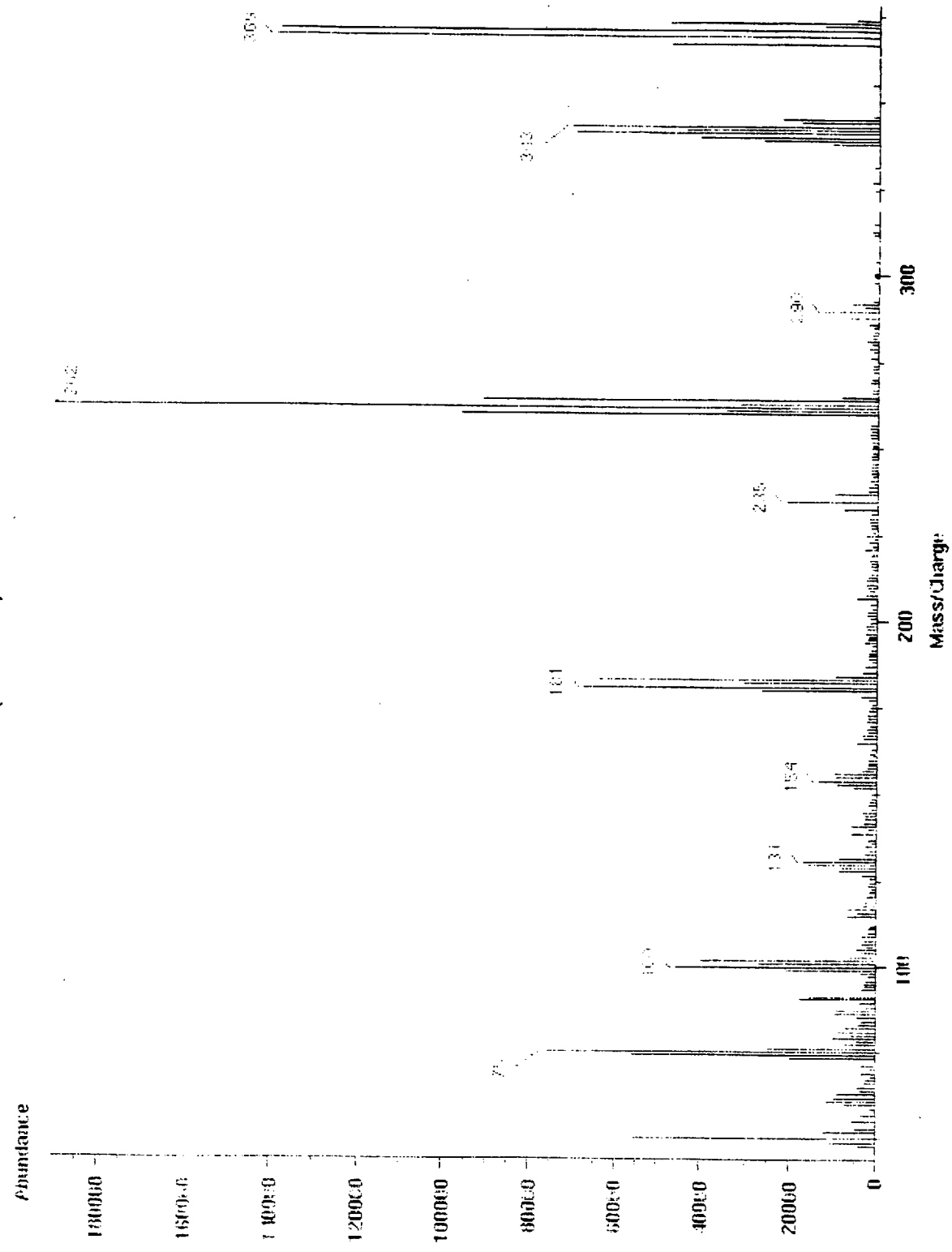
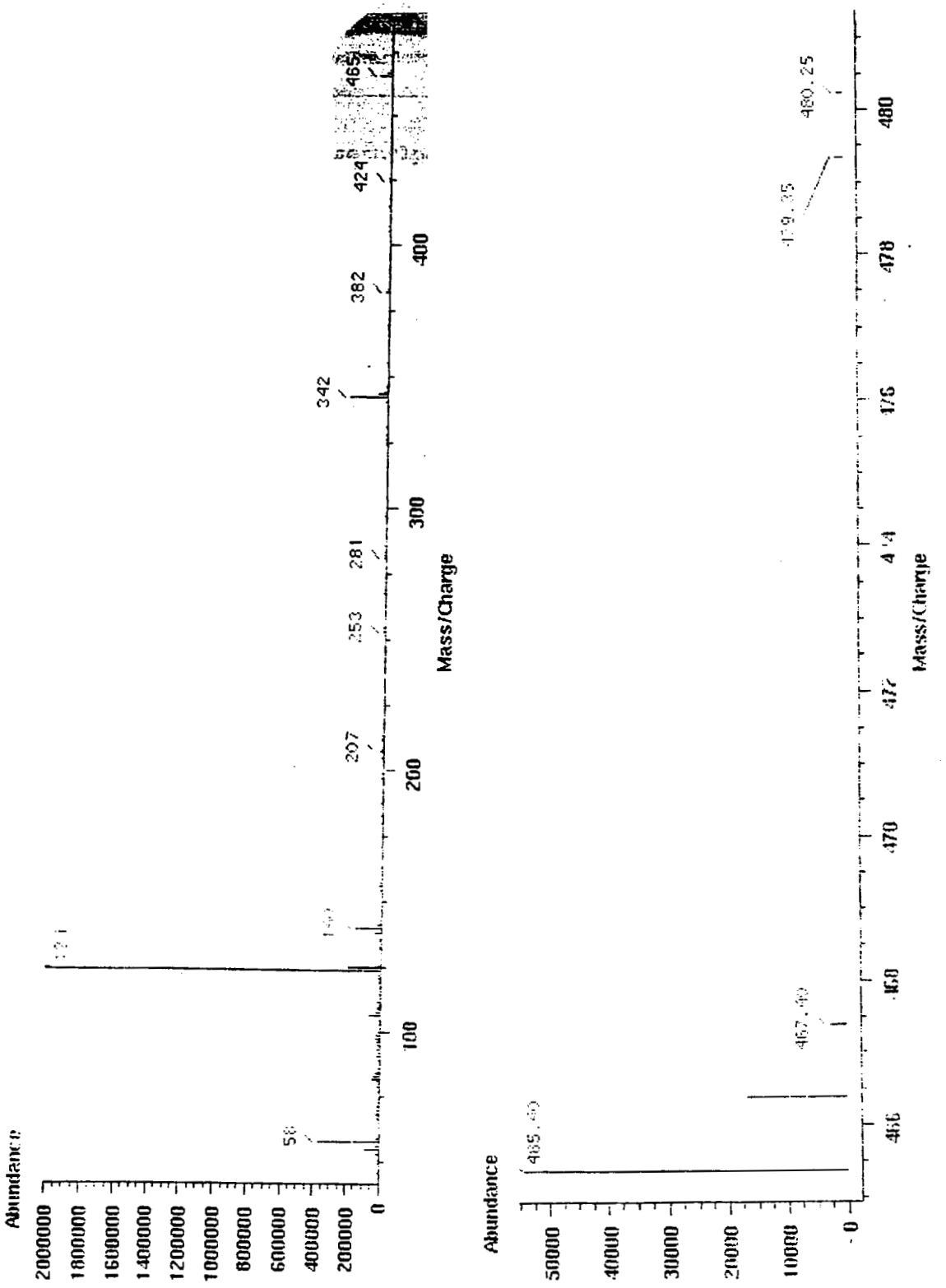


FIGURE 3-20. Mass Spectrum of Peak at 44.587 Minutes, Refinery #1 - Sample IV



The apparent change in toxicants warranted further work to determine variability, and also to reduce the number of compounds associated with toxicity. Effluent toxicity characteristics in a fifth sample were the same as for the preceding sample and nonpolar toxicity was retained through the C_{18} SPE concentration step. A small portion of the toxic concentrate was injected on HPLC and a preliminary UV scan was performed to determine peak retention times. The remaining concentrate was then separated by peak retention times into 15 fractions. Fractions #11 and #12 in the sequence contained a majority of the toxicity recovered after HPLC separation. These fractions were concentrated separately and analyzed by GC/MS following confirmation of toxicity (Figures 3-21 and 3-22). Both chromatograms show the presence of brominated aromatic compounds at retention times of 25.2 and 26.2 minutes. These compounds appeared similar to those present in the previous sample.

The phenolic compounds initially identified in Refinery #1 samples were not detected in the toxic HPLC concentrates. The polarity of the C_{18} extractable toxicants was increased, and the appearance of suspect brominated aromatic toxicants was noted. A possible structure of this compound was constructed from the mass spectra for the four unidentified peaks (Figures 3-23 and 3-24). The molecular formula is $C_9H_9NBr_2$ for the peaks at 25 and 26 minutes. The peaks at 31 minutes have one additional Br, the molecular formula is $C_9H_8NBr_3$. The two peaks for each compound are likely isomers, but the bromides and nitrogen-containing component locations on the benzene ring are not clear. In IUPAC nomenclature the compounds are (x,y dibromo-1-ethylene-z-methylamine) benzene and (w,x,y, tribromo-1-ethylene-z-methylamine) benzene (Figure 3-25). Additional testing would be required to positively identify the brominated aromatic compounds, and to gather evidence to further link them to effluent toxicity. Toxicity to fish and the Phase I characteristics of the pure compounds would need to be determined but were not completed in this study.

The objective to develop an approach for separating toxicants from non-toxicants in the nonpolar fraction was achieved in spite of the unanswered questions regarding the toxic concentrations. Since the phenolic disappeared from the refinery effluent over the course of the study, a complete resolution of the toxic concentration issue could not be completed.

FIGURE 3-21. GC/MS Total Ion Chromatogram of HPLC Fraction #11, Refinery #1 - Sample V

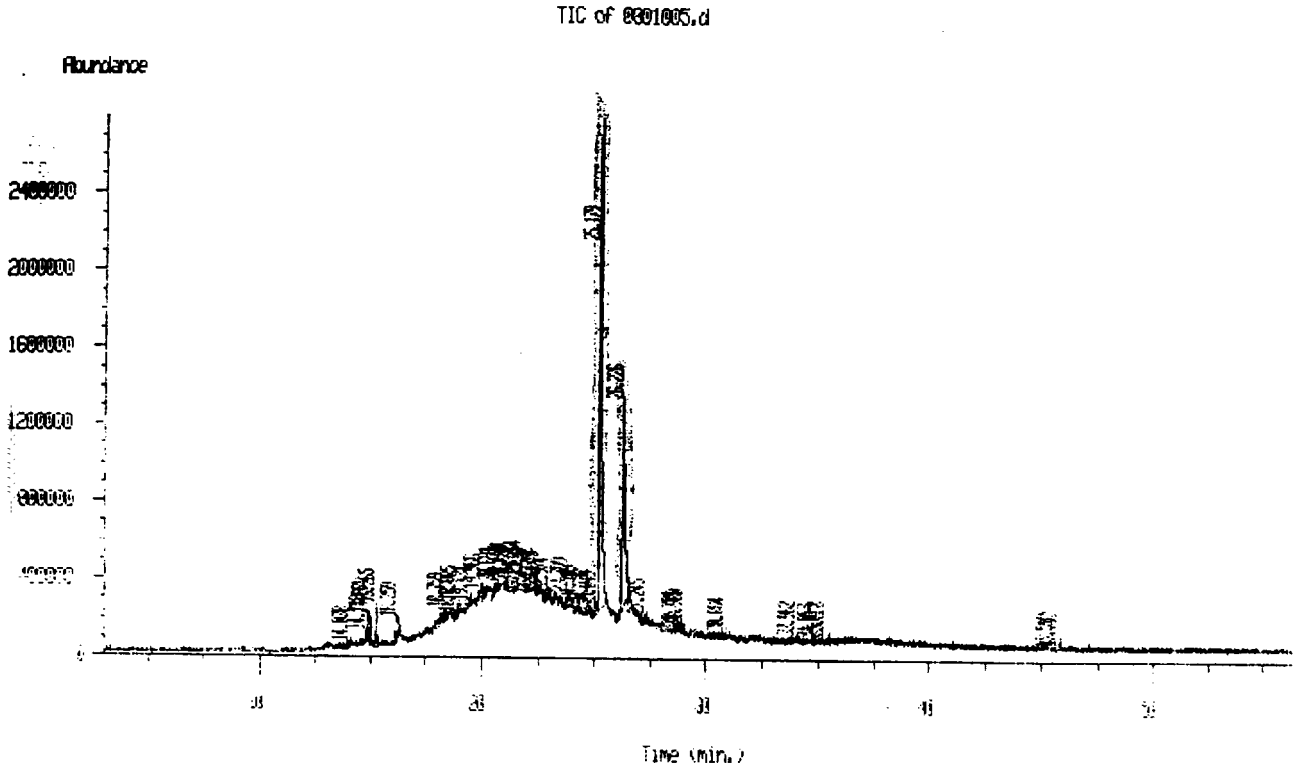


FIGURE 3-22. GC/MS Total Ion Chromatogram of HPLC Fraction #12, Refinery #1 - Sample V

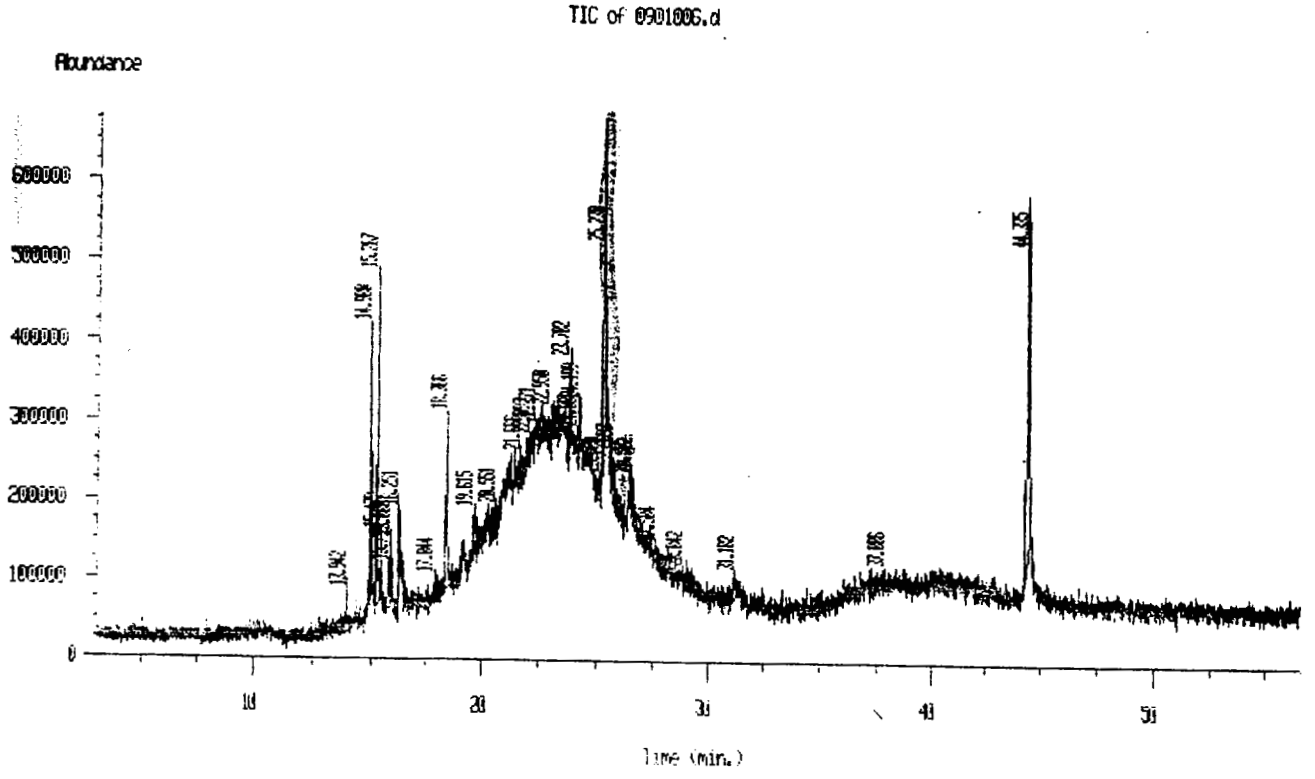


FIGURE 3-23. Mass Spectrum of Peak at 25.384 Minutes with Breakdown of Chemical Components

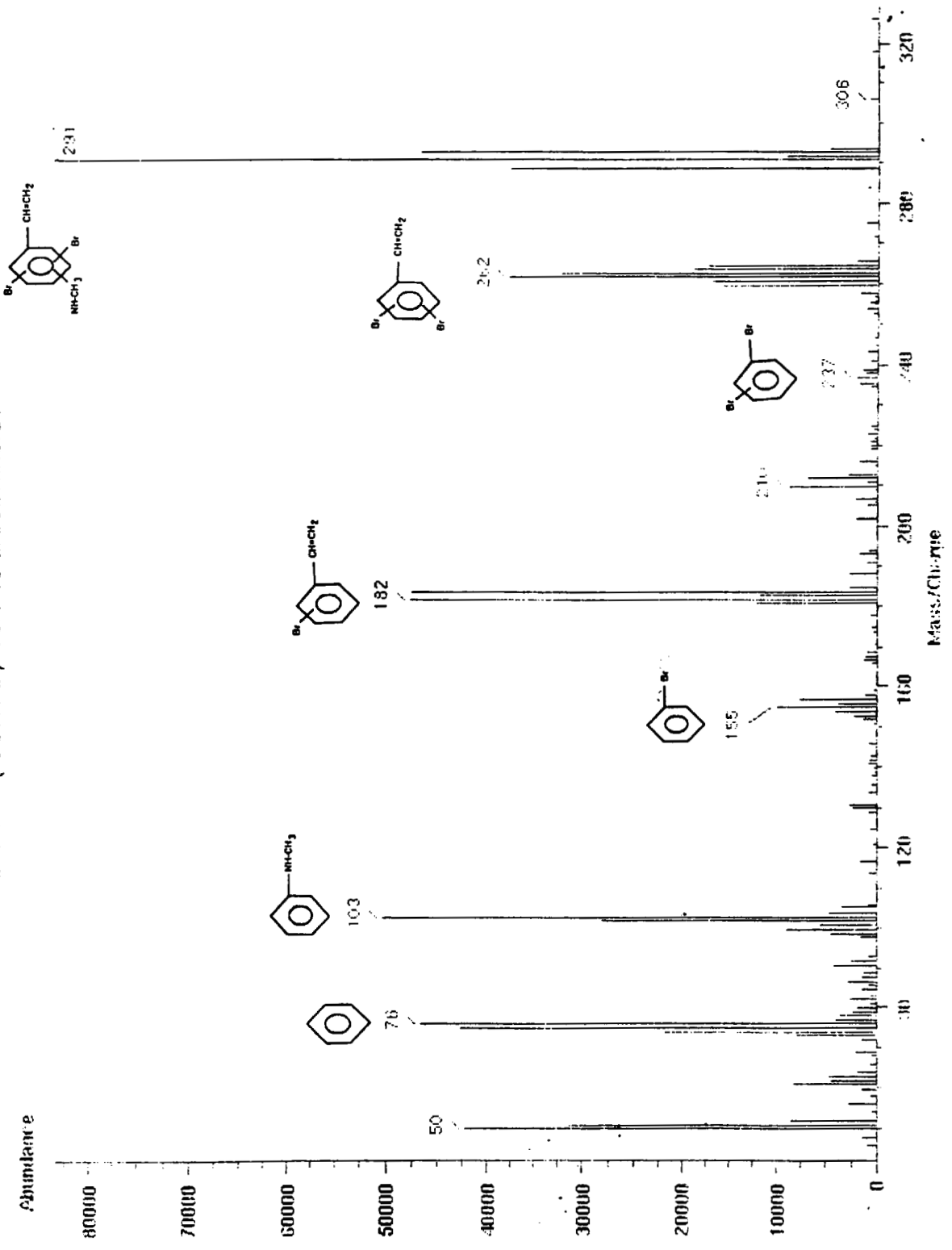


FIGURE 3-24. Mass Spectrum of Peak at 31.042 Minutes with Breakdown of Chemical Components

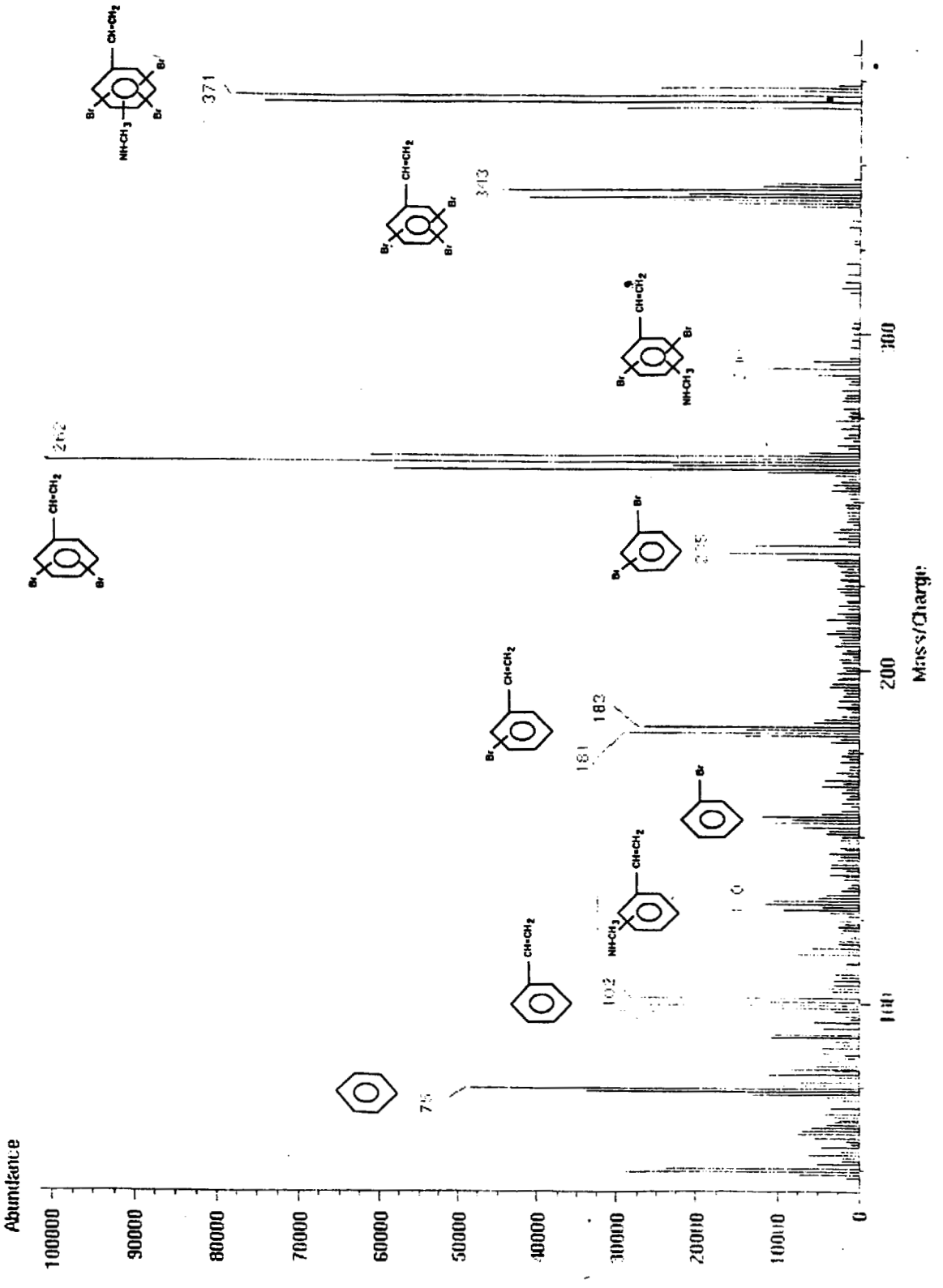
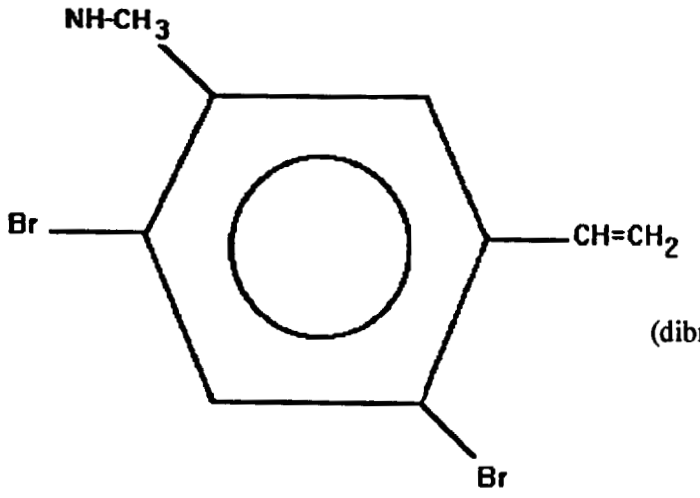
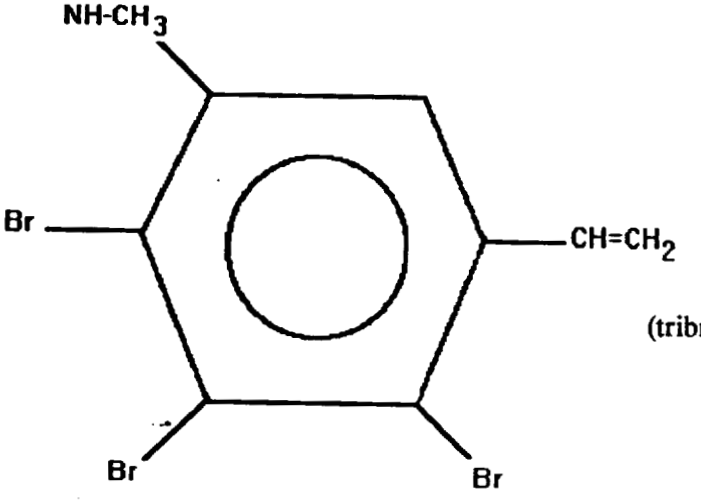


FIGURE 3-25. Structure of Brominated Compounds in Refinery #1 - Samples IV and V



(dibromo-ethylene-methylamine)benzene



(tribromo-ethylene-methylamine)benzene

Section 4
SUMMARY

Effluent samples from five refineries were screened for chronic toxicity caused by nonpolar organics and minimal toxicity from common constituents such as ammonia and total dissolved solids. Of five different refinery effluents tested, four had nonpolar toxicity as measured from Phase I TIE procedures. The one effluent with the most nonpolar toxicity was relatively free of other types of toxicity and was selected for further Phase II characterization. On numerous occasions the selected effluent was not chronically toxic and additional samples had to be collected for evaluation.

The presence of nonpolar toxicity was operationally defined as toxicity removed by C_{18} columns and subsequently recoverable from the column by methanol elution. Data from the Phase I TIEs on the four toxic effluents gave similar results: 1) reduced or no toxicity after C_{18} SPE, and 2) more toxic at pH 6.0 than pH*i* or pH 9.0. The TIE results demonstrated that for Refinery #1 effluent (chosen for further work), C_{18} SPE was the only type of toxicity and, therefore, it could be tested without the complication of any other toxicity sources.

Since even well-treated refinery effluents are still complex mixtures, the first task was to remove as many non-toxic constituents as possible. GC/MS analyses have been notoriously ineffective because of the hydrocarbons present that interfere with other components. Experience has also shown that the toxic components do not separate well into discrete fractions which makes both recovery and clean-up difficult. The problem was resolved by use of C_{18} SPE columns with several modifications tested for performance with refinery effluents. These were: 1) elution with methanol concentrations which usually do not recover toxicants; 2) elution with more than one fraction of 100% methanol; and particularly; 3) elution followed by back-dilution with water and re-chromatographing a second time with a similar elution scheme. The specific elution pattern used in this study is not a broadly applicable one because the toxicants in this effluent were not originating from operations that would occur in all refineries. More broadly applicable is the technique of simple modification of the elution scheme to achieve reduction in sample complexity and allow analytical identification to be achieved.

The bis(1,1 dimethylethyl)phenol, associated with and probably causing the chronic toxicity, could not have been identified in a complex sample. The evidence for this phenolic being the toxicant is: 1) always toxic when present, regardless of sample matrix (whole effluent, C_{18} fractions, and fraction concentrates); 2) more toxic at lower pH values; 3) elution at high methanol concentrations (its log P is relatively high); and 4) the source product from the refinery gave TIE characterization results very similar to those from effluent characterization.

The relationship of the concentration of the phenolic compound to the effluent toxicity was not adequately resolved. The order of magnitude was similar, but comparisons were confounded by the presence of a group of related compounds in the effluent. There are no toxicity data for the related compounds, but the presence or absence of the phenolic compounds was precisely related to the presence or absence of toxicity. Unfortunately, they disappeared from the refinery's effluent before accurate analytical measurements were completed. While subsequent samples were still chronically toxic, this toxicity had different characteristics. Toxicity was recovered at lower methanol concentrations, it could be separated rather well into a few fractions using HPLC, and the effect of lower pH conditions increased toxicity more than for the phenol. The second set of toxicants appeared to be brominated compounds that originated in cooling systems.

One very practical lesson learned is that toxicity may be caused by activities that are not directly related to refining and it may not originate from the crude oil itself. With more sensitive toxicity tests using higher concentrations of effluent, smaller loadings of substances to the effluent stream can cause toxicity. Whether such measured toxicity is environmentally important depends on the dilution factor in the receiving water.

REFERENCES

ASci Corporation/ASci-Duluth Environmental Testing Division Proposal Submitted to American Petroleum Institute, January 1993.

U.S. EPA. 1989. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-89/001. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

U.S. EPA. 1991a. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures. EPA/600/6-91/003. Environmental Research Laboratory, Duluth, MN.

U.S. EPA. 199b. Toxicity Identification Evaluation: Characteristics of Chronically Toxic Effluents, Phase I. EPA-600/6-91/005. National Toxicity Assessment Center. Duluth, MN.

U.S. EPA. 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA-600/R-92/080. Environmental Research Laboratory, Duluth, Minnesota.

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