IDENTIFICATION OF ALACHLOR AND PROPACHLOR DEGRADATION PRODUCTS BY GC/MS ANALYSIS

By

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1982

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1998

IDENTIFICATION OF ALACHLOR AND PROPACHLOR

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ACKNOWLEDGMENTS

I would like to take this opportunity to tell my wife Kathy and my children, Kristina and Kurtis thank you for not giving up on me and always giving me the constant prayers and constructive support that I needed through out the years. Thank you is also in order to my parents Harold and Pat whose inspiration, prayers and constant encouragement gave me the strength to persevere the journey of completing my masters degree. It would be an oversight if I failed to thank my parent in laws Don and Sharon who always checked to see if the coals were still hot or needed a little stoking.

To my principal advisor Dr. Gregory G. Wilber, I thank you for your guidance in the creation of this body of work and of the constant supportive encouragement that you gave. Even though our time of contacts was not the most structured and you not having dealt with a working part-time graduate student until myself, we succeeded and I feel that we both came away with something fulfilling and special.

To Dr. William McTernan and Dr. John Veenstra, thank you for making the time in your schedule to set on my graduate committee. I would also like to thank you for teaching because you both have excellent people and teaching skills and the fire of excitement still burns in you attitudes towards the students.

Special thanks must go out to my long time friends Jim Engman, Terry Smith and Russell Moody for the access of laboratory equipment and technical information; Ray

Cotton and Jerome Bradford for assistance in the preparation of my sample extracts and the use of the extraction laboratory. Without your support and understanding this thesis would not have happened.

I would like to extend to the many others who aided me through out my course of study a truly heart felt thank you.

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CHAPTER I

INTRODUCTION

Herbicides such as alachlor [2-chloro-(2`,6`-diethyl-N-methoxymethyl) acetanilide] and propachlor [2-chloro-N-isopropyl acetanilide] have been favorite pre- and post-emergence herbicides of the agricultural community for more than twenty years. In the midwestern "corn belt" alone, approximately 13 million kilograms (kg) of alachlor was applied in 1994, which reflected a 6 million kg reduction from 1993-1994 (Koplin et al., 1996). Due to the heavy usage of these herbicides, there is an increasing number of water wells that contain various types and amounts of parent herbicides and metabolic by-products (Koplin et al., 1996). A large study of 837 drinking water wells throughout several midwestern states showed that 303 wells contained herbicides or pesticides. Furthermore, 46% of the wells were positive for the alachlor metabolite alachlor-ESA (Koplin et al., 1996). This is significant because 90% of rural households and two-thirds of cities in the United States use groundwater as a drinking water source (McAllister and Chiang, 1994). This drives home the fact that we must understand groundwater contaminant transformations and

regulate the use of the herbicides and pesticides in such a manner that our groundwater resources are not put at risk.

One important component in the process of understanding and predicting the behavior of herbicides once they enter the environment is the many possible transformation products they yield. The Gas Chromatography/Mass Spectrometer (GC/MS) has been given the assignment of analyzing and possibly identifying the various transformation compounds which, to date, have received relatively little attention. The GC/MS creates, by molecular fragmentation, a mass spectral "fingerprint" unique to each compound. The ability of generating and referencing these "fingerprints" makes the GC/MS an excellent tool for this type of research (Thurman et al., 1992). Though the GC/MS is an invaluable tool, it has limitations. These may include background noise in the samples, poor chromatographic separation of compounds, or sample extraction problems.

It is the objective of this thesis to use GC/MS analysis to detect and identify degradation compounds from two different types of experimental reactors spiked with alachlor and propachlor solutions. Specifically, these two reactors were used to investigate various aspects of the environmental fate of alachlor and propachlor. Yanyan Qin (1995), investigating the reactions between acetanilide herbicides and bisulfide, created the bisulfide reactor samples used in this study. Walker (1997), investigating the biotransformation of these herbicides in a variety of biological reactors, generated the nitrate reactor samples. A second element of this study is a comparison of Solid Phase Extraction (SPE) and Liquid-Liquid Extraction (LLE) methods for their usefulness in GC/MS analysis.

CHAPTER II

LITERATURE REVIEW

The herbicides alachlor [2-chloro-(2',6'-diethyl-N-methoxymethyl) acetanilide], also known as Lasso®, and propachlor [2-chloro-N-isopropyl acetanilide], known as Ramrod®, have long been favorite pre-and post-emergent herbicides for the corn and soybean industry. These herbicides have been in use for over twenty years and they have been increasingly noted in the ground water supply of cities as well as private home owners (Koplin et al., 1996). Public health agencies have become increasingly aware of potential health risks that these herbicides pose (Bouwer, 1989). The increasing sophistication of computer modeling, analytical instruments, long term health studies and awareness of the public sector has generated tighter exposure limits and aggressive enforcement of the regulations. With alachlor and propachlor having a toxicological risk, the EPA has set maximum contaminant levels (MCL) in the drinking water supply at 2 ug/L for both pesticides. There is a distinct trend that MCL levels may be decreased to lower levels as

more data are collected and analyzed, and as public pressure is applied to regulatory agencies.

Research is now being directed toward the assessment of degradation compounds. The knowledge base for these degradation compounds is growing rapidly due to the advancements in analytical instrumentation. The past several years have seen the detection limits of many instruments drop ten to one hundred fold (Doherty et al., 1996). The GC/MS is one such instrument, which can now see pesticides down to 10 picograms (Doherty et al., 1996). With these advancements in instrumental analysis, the latest generation of studies can proceed toward identifying degradation products, generating better information for understanding long term health risks of pesticide use, and perhaps, the ultimate establishment of MCLs for these compounds.

Theory of GC/MS

The quadrapole GC/MS has four basic functions to its operation (Barney,1992):

(1) Vaporize and separate the compounds in the sample. This is done in a phase-lined column which is subjected to a temperature program by the GC.

(2) Produce ions from neutral molecules which have been separated in the vapor phase. These ions are

formed by collisions with electrons which are generated by the ion source.

(3) Separation of these ions according to their mass-to-charge ratio. These ions are actually atomic fragments of the molecule and are filtered out by use of oscillating quadrapole magnets known as the mass analyzer.

(4) Detection of the abundance of these separated ions, which is done at the electron multiplier.

The data generated are of two types:

(1) A peak chromatogram, which is abundance of a compound (x-axis) versus retention time (y-axis) in the GC column (Fig. 1).

(2) A distinct time-slice of that peak (a scan) which contains the mass-to-charge ratios of that compound, which acts as a source "fingerprint" of that compound and is known as a mass spectrum (Fig. 2). This mass spectrum in Figure 2 gives several key pieces of information including, the following:

A) The x-axis is molecular mass and the y-axis is abundance

B). The molecular weight of alachlor is 269 AMU.

C) The alachlor molecule fragments into three major masses of 188, 160 and 45 AMU.

D) This mass spectrum or "fingerprint" is unique to alachlor.

The mass spectrum or "fingerprint" of a molecule fragmentation is recorded. The functional groups are then "reassembled" by computer software, following strict rules, to obtain the configuration of the molecule. With the aid of the National Bureau of Standards (NBS, 1987) Revision E database, developed by the EPA, the National Institutes of Health (NIH, 1987) and the Mass Spectrometry Data Center (MSDC, 1987), a compound match is generated with a percent probability. For example, the spectrum in Fig. 3 was identified by the database as alachlor, with a 99% probability based match. To minimize retrieval time from the spectral library, a ten peak criterion algorithm is used. The program selects 10 mass peaks of "significance" from the full mass spectrum; this "significance" is based on mass and abundance. These peaks of "significance" are compared to the condensed 10 peak spectral database, which includes spectra for 38,000 compounds. A listing of the most probable spectrum matches is created with the complete spectral pattern so that a side by side comparison can be made. A mass spectrum can be reproduced very reliably from GC/MS to GC/MS by using standard instrument tuning conditions. The tuning standard set forth by the EPA, which drives the environmental analytical industry, is decafluorotriphenylphosphine (DFTPP) (EPA 1995). The instrument used in this study was operated under the instrument tune parameters set forth by the EPA.

Figure 1. Chromatograph showing abundance versus time for an alachlor sample.

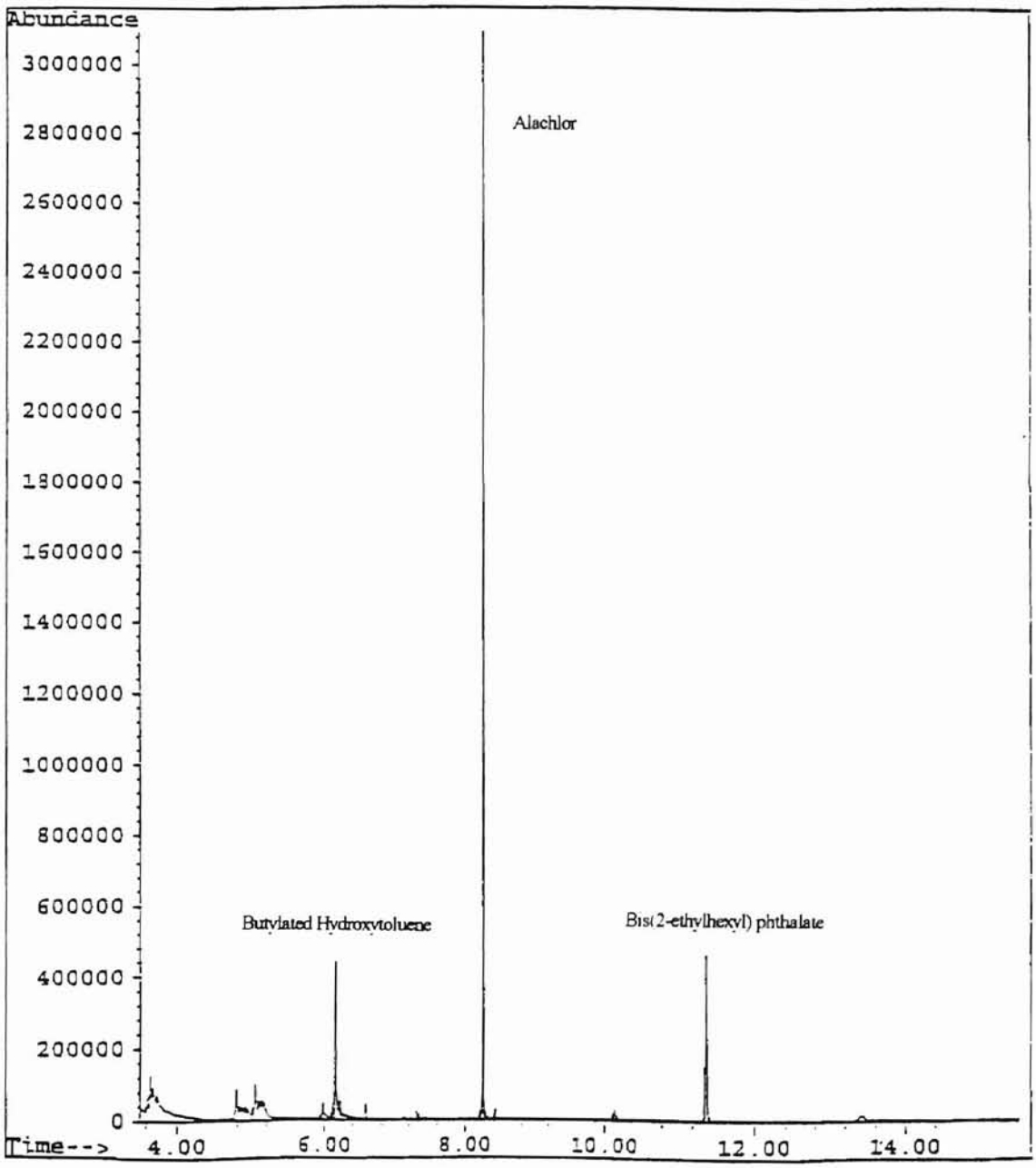


Figure 2. Mass spectral "fingerprint" of alachlor.

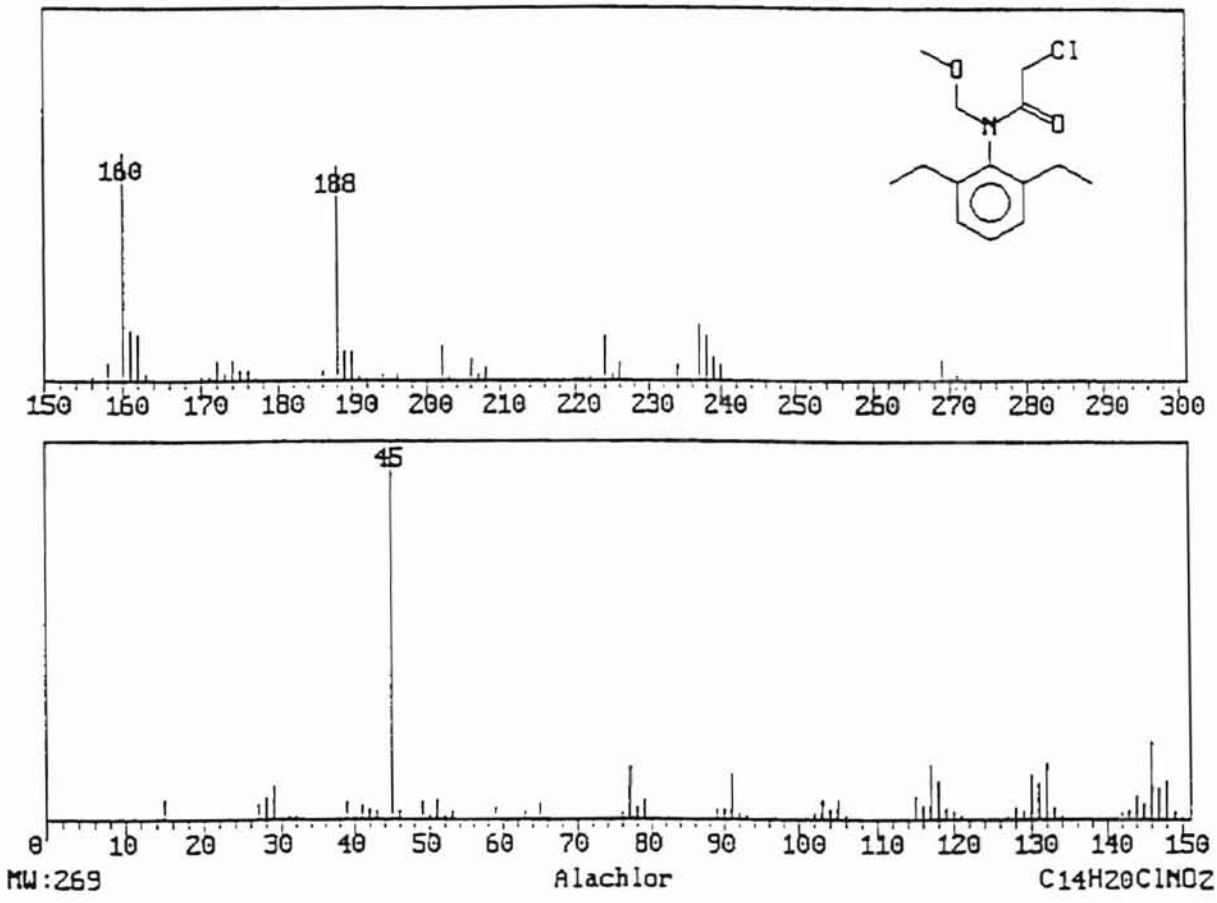
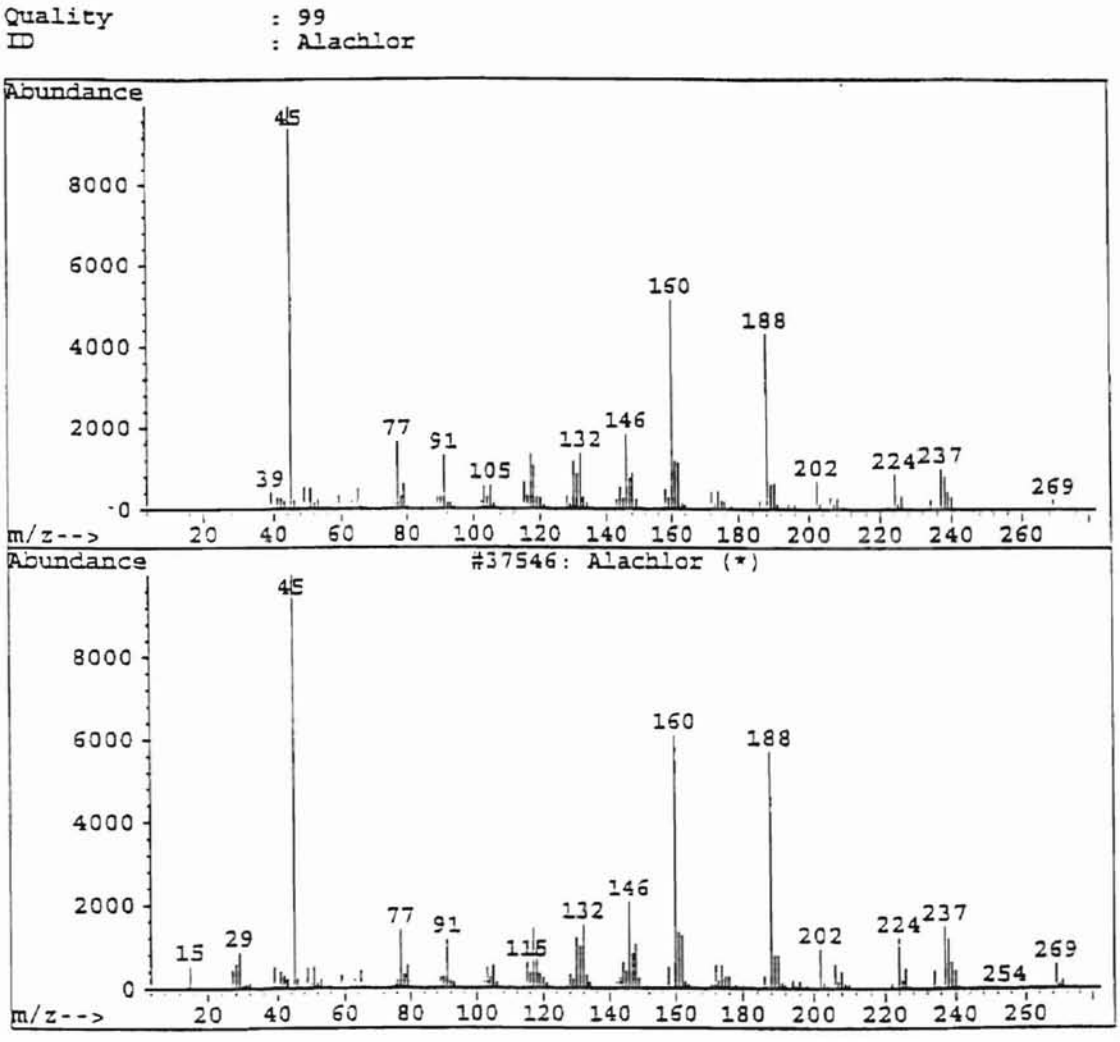


Figure 3. Library match of sample spectra versus alachlor reference spectra.



Applications of GC/MS to Pesticide Fate Studies

The GC/MS has been a useful tool in the identification of degradation compound structures even through qualitative observations of the spectral information. Information such as identification of consistently occurring major herbicide metabolites was being noted in studies as far back as 1992 (Thurman et al., 1992), dealing with surface water run off in corn belt states such as Iowa and Nebraska. Thurman and coworkers (1992) investigated the persistence of herbicides in surface waters and listed two dominant metabolites of atrazine, another common herbicide used predominantly on corn, deethylatrazine and deisopropylatrazine. It was noted in the conclusions of the study that further study should be directed toward the persistent degradation products of herbicides.

A recent study which dealt with tracking a recently introduced herbicide, acetochlor, in surface and rain water from the Blue Earth River basin of Minnesota also centered around GC/MS analysis (Capel et al., 1995). However, this study was primarily an effort to describe the behavior and fate of the parent herbicide, and as such did not confirm any degradation products but did note the existence of degradation products in the samples.

A study of metolachlor, undertaken by Aga et al., (1996), confirmed a "new" sulfonic acid metabolite, metolachlor-ethanesulfonic acid (ESA) by GC/MS. This metabolite, metolachlor- ESA, was analytically confirmed by tandem Mass Spectrometry instrumentation and now is accepted as an indicator of the degradation of metolachlor (Aga et al., 1996). References of the analogous metabolite, alachlor- ESA, had been made

before 1994 (Koplin et al., 1996). A study by Aga et al., (1996) focused on synthesis and identification of the metabolites of alachlor and metolachlor, validating the GC/MS spectral pattern of the compound. Alachlor-ESA is an accepted indicator of alachlor degradation (Aga et al., 1996).

A study done by Potter and Carpenter (1995) have incorporated the unique identification capabilities of the most modern GC/MSs. In their recent study, the synthesis of eleven degradation compounds was performed to achieve reference materials to create a mass spectral database. Of the newly synthesized compounds N - (2, 6 - diethyl phenyl) - N - (methoxymethyl) acetamide, 2 - hydroxy - 2',6' - diethyl - N - (methoxymethyl) acetanilide, Bis (N-methoxymethyl)-2,6-diethylaniline and N-(methoxymethyl)-2,6-diethylaniline were found to exist in this study. Until this time, these spectra had not been associated with an IUPAC name and thus were not present in any commercial GC/MS database. This demonstrates how little is known about this specialized segment of analytical investigation.

Analysis by GC/MS has also been done to samples which had been generated by microbial reactors degrading a similar acetanilide herbicide, metolachlor (Liu et al., 1989). Four metabolites were documented by spectral data and then the structures were theorized. Liu et al. (1991) did further work on metolachlor degradation by a bacterial community, reinforcing their earlier study findings of dechlorination and degradation of the parent compound by microbial metabolism. The authors also listed the Chemical Abstract System (CAS) numbers of nine metabolites which they had hypothesized in their earlier work.

Progress clearly has been made in using GC/MS analysis to identify herbicide degradation products. However, the scarcity of the compounds in existing databases, and the large number of herbicides and their wide variety of products slows the rate of progress. For example, no references of GC/MS analysis of propachlor metabolites after being exposed to a natural system or engineered biological reactor have been found at this time. However, armed with knowledge of the newest identified degradation compounds, another round of studies may begin. How many different products these complex compounds have, only time will tell. The scientific and public health communities are on a steep learning curve when the subject is the "cradle to grave" fate of herbicide compounds and their degradation products.

Extractions of Liquid Environmental Samples

A critical component of any GC analysis is the extraction method used to prepare the sample. EPA protocol has established Method SW 3015 as the Liquid-Liquid Extraction (LLE) technique for liquid samples destined for organic analysis by GC and GC/MS (EPA, 1995). The method is mandated in the preparation of liquid samples if the analytical data is associated with an EPA regulated treatment, storage or disposal of materials. The work of Potter and Carpenter (1995) deals with the synthesis of compounds, which correlate to the spectra of degradation products of alachlor, which were extracted by the standard LLE method. The confidence that these researchers have for this method is

noteworthy, due to the fact that the most popular standard extraction technique used in most herbicide and pesticide studies is the Solid Phase Extraction method (SPE). An advantage of the LLE technique is its ability to extract hydrophilic compounds due to manipulation of the sample's pH. If a compound is polar, water soluble and ionic (the least extractable for SPE), the act of lowering the sample's pH can create a favorable partition coefficient toward the extraction solvent (Markell and Hagen, 1991). The disadvantages of the LLE method is the large sample size required, the large amount of methylene chloride waste or emissions, as well as its being labor intensive and slow.

The vast majority of the samples used for the current study used SPE. This method is quick and can be accomplished in under half an hour while using only a few milliliters of extraction solvents. However, the materials used in the construction of the extraction columns can contribute interferences, such as plasticizers, including phthalates and oligomers (Hagen et al., 1990). With the sophistication of the extraction media, such as resins, CH bonded silica, as well a C18 bonded silica (Thurman et al., 1992), it is inevitable that the EPA will phase out the LLE in favor of SPE technology. The current study includes a brief comparison of these methods.

CHAPTER III

METHODS AND MATERIALS

This chapter focuses on the history of the bisulfide reactor and nitrate reducing reactor samples, the physical attributes of the GC/MS instrument used and a description of two pesticide extraction methods .

Batch Reactors and Samples

As mentioned, most of the samples analyzed in this study were generated in earlier research projects focused on herbicide fate in a variety of reactors. One such study investigated the reaction of acetanilide herbicides with bisulfide ion (Qin, 1995), while another sought to determine the rate of biotransformation of these herbicides by nitrate-reducing cultures (Walker, 1997).

Bisulfide Reactor

A bisulfide reactor, under abiotic conditions, generated the "bisulfide samples" (Qin, 1995). Each reactor contained phosphate buffer (pH 7.2) which had been deoxygenated and batch fed an aqueous solution of alachlor or propachlor at a concentration of approximately 200 ug/ml and a known concentration of bisulfide (0.09-0.9mm). A 1cm thick PTFE-facial silicone septa (Supelco) was crimped onto the reactor with zero headspace. Samples were collected after various durations of reaction time and stored in the dark following extraction.

Nitrate Reactor

A nitrate-reducing biological reactor was operated by Walker (1997). Reactors were seeded with organisms from the Stillwater wastewater treatment plant biotower reactor. Cultures were maintained in an anaerobic medium containing nitrate, acetate, and standard mineral salts for a nitrate-reducing culture. Individual batch reactors, started from the parent culture, were spiked with 100 μg/L of herbicide (alachlor or propachlor). Disappearance of the parent herbicide (alachlor or propachlor) was monitored over time. Samples selected for GC/MS analysis included both those exhibiting relatively minor amounts of herbicide transformation and those whose parent herbicide was no longer

detectable by ECD (electron capture detector) GC analysis. Samples were collected after specific reaction times and stored in the dark following extraction.

Sample Biography

The samples selected for this study were based on the degree to which the parent herbicide had been significantly degraded, creating a probability of detectable concentrations of daughter compounds. The specific samples analyzed in this study, their origin and method of extraction used during sample preparation are listed below.

Table 1. Sample Biography

Name	Sample Description	Sample Source	Reactor Type	Extraction
B1.0	Buffer & Alachlor (8/5)	Qin (1995) MS	Bisulfide	SPE
B1.1	Buffer & Alachlor (8/7)	Qin (1995) MS	Bisulfide	SPE
B2.0	Buffer & Propachlor (8/5)	Qin (1995) MS	Bisulfide	SPE
B2.1	Buffer & Propachlor (8/7)	Qin (1995) MS	Bisulfide	SPE
B3.0	Buffer, Alachlor & Bisulfide (8/5)	Qin (1995) MS	Bisulfide	SPE
B3.1	Buffer, Alachlor & Bisulfide (8/7)	Qin (1995) MS	Bisulfide	SPE

B Table 1, continued

Name	Sample Description	Sample Source	Reactor Type	Extraction
B4.0	Buffer, Propachlor & Bisulfide	Qin (1995) MS	Bisulfide	SPE
4.1	Buffer, Propachlor & Bisulfide	Qin (1995) MS	Bisulfide	SPE
B5.0	Buffer & Bisulfide	Qin (1995) MS	Bisulfide	SPE
B6.0	Alachlor	Crawford (1998)	Bisulfide	LLE
B7.0	Propachlor	Crawford (1998)	Bisulfide	LLE
B8.0	Phosphate buffer	Qin (1995) M.S.	Bisulfide	SPE
N1.0	Ethyl Acetate Blank	Crawford (1998)	Nitrate	SPE
N2.0	Alachlor (0 hours)	Walker (1997)	Nitrate	SPE
N3.0	Alachlor (370 hrs)	Walker (1997)	Nitrate	SPE
N4.0	Propachlor (0 hours)	Walker (1997)	Nitrate	SPE
N5.0	Propachlor (217 hrs)	Walker (1997)	Nitrate	SPE

Extraction Methods

The extraction techniques utilized either Solid Phase Extraction (SPE) with cartridges (C-18), following the method described by Thurman et al. (1992), or a Liquid/Liquid Extraction (LLE) procedure as described in EPA Method 3510C (extraction of semi-volatile compounds) as used by Potter and Carpenter (1995). The LLE extraction was done to selected samples from the bisulfide reactors for the purpose of comparing extraction efficiencies.

Solid Phase Extraction

The SPE method employed was that used by Thurman et al. (1992). PrepSep (C-18) cartridges (Fisher Scientific, Inc.), which contained 360 mg of 40 um bonded silica, were used for the extraction. The cartridge preparation procedures is as follows:

(1) Wash the cartridge with 3ml of methanol.

(2) Wash the cartridge with 3ml of ethyl acetate.

(3) Wash the cartridge with 3ml of methanol.

(4) Wash the cartridge with 3ml of D.I. water.

50 ml samples from the reactors were drawn through the SPE cartridge by a vacuum, air dried, then eluted with 2ml of ethyl acetate. The sample extracts were stored in the dark at 4°C.

Liquid Liquid Extraction

The LLE procedure is outlined in EPA Method 3510C (EPA, 1995) for extraction of semi-volatile compounds in water. This extraction method was used on two bisulfide/alachlor and bisulfide/propachlor samples as an extraction comparison study.

The extraction was performed by pouring 1L of sample into a 2L separatory funnel. No surrogates or internal standards were added to the sample. Using hydrochloric acid the sample pH was lowered to ≤ 2, then 60mls of methylene chloride was added to the funnel and shaken for two minutes. The funnel was placed into a ring stand and the phases are allowed to separate. The methylene chloride layer was drained off the bottom, through Na_2SO_4 into an erlenmeyer flask. This was repeated two more times. Next, the pH was raised to ≥ 12 with sodium hydroxide. Once again, 60mls of methylene chloride was added, shaken and drained. This was done a total of three times. The collected extract was concentrated to a 100ul volume by a nitrogen evaporator and placed into an auto sampler vial with a crimp-top PTFE faced silicone septa, then analyzed.

GC/MS Description

The gas chromatograph (GC) used is a Hewlett Packard (HP) Model 5890 with Electronic Pressure Control, which enables the instrument to run in a split/splitless mode with a constant helium carrier gas flow. Listed below is the GC oven temperature program used in the study. The column is a 25 meter HP Ultra 2 capillary column with an internal diameter of 0.2mm, film thickness of 0.33um, and phase ratio of 150. The mass spectrometer is a HP Model 5970 "MSD" (mass selective detector) of Electron Impact configuration, with the ability of scanning from 10-800 AMU every second using 70ev electron energy. Specific instrument conditions are listed below.

GC/MS Conditions

Mass Range	-	30-450 AMU
Scan Time	-	1 scan per 1.5 second
Initial Temp	-	100°C for 2 minutes
Temp Program 1	-	35°C/min. to 170°C
Temp Program 2	-	20°C/min. to 280°C
Final Temp	-	Hold for 6 minutes at 280°C
Sample Size	-	2ul injection
Injector Temp	-	250°C

Source Temp	-	Factory set at 300°C
Injector Type	-	Split/splitless (Splitless for 2.5 min.)
Pneumatics	-	Electronic pressure control (EPC)
Carrier Gas	-	Helium UHP/Zero Grade w/EPC set for flow of 0.6ml/min.
Run Time	-	15.5 minutes
Source Voltage	-	2200 EMV

GC/MS Software

The system software used was all from Hewlett Packard (Palo Alto, Ca.). It included HP MS Chemstation (DOS, HP G1034C, Rev. C.02.00), EnviroQuant Target Compound software (HP G1032C, Rev. C.00.02) and NIST/EPA/NIH Mass Spectral Library Database (HP G1033A, Rev. C.00.00), Mass Spectral Library of Pesticides (HP G1038A. Rev. A.00.00). The software packages are integrated to function with Windows "95".

CHAPTER IV

RESULTS AND DISCUSSION

The analytical data generated using the methods described in Chapter III are presented and discussed here. The analytical data include sample chromatograms, mass spectra and library matches. The major components of this chapter are general observations, analytical data from the bisulfide (abiotic) reactors and the nitrate reducing reactors, and finally, a side-by-side comparison of Solid Phase Extraction (SPE) vs. EPA Method 3510C Liquid/Liquid Extraction (LLE).

General Observations

It should be noted that the samples were analyzed on the GC/MS system on a "when-available" basis. The system was specifically configured for commercial production

at Laidlaw/USPCI Labs, Tulsa, OK. The necessity of commercial laboratory production hindered the degree to which the instrument parameters could be optimized for the analysis and detection of herbicide metabolites. Parameters of the instrument for these specific environmental samples were set at common and prudent instrumentation settings demanded by production analytical needs.

The time from the extraction of Qin (1995) samples to the time of GC/MS analysis exceeded standard EPA protocol of seventy days. The compounds in the sample could have proceeded to degrade and the end result could be that very few "expected" compound families could be found. This fact could explain the lack of daughter compounds found in the SPE bisulfide samples of Qin (1995). Given that the parent compound was stable, it seems plausible that some reaction products could also be that stable.

A major concern of the study was the amount of background noise that the samples exhibited. This background noise was traced back to the phosphate buffer and bisulfide blank, as revealed by that sample's chromatograph (Fig 4). The baseline is very "active" or "noisy" up to ten minutes into the analysis. The implication of the chromatograph of the phosphate blank is that the constituents of the buffer, solvents or apparatus used are the source of the "noise" present in all of the SPE-extracted samples. A background like this can have several adverse effects on the analytical data, including the following :

(1) It may completely mask either a degradation compound peak or a degradation compound mass spectra.

(2) It may partially mask the degradation compound peak or corrupt the mass spectra; thus the major and minor mass ions will not represent the true compound pattern close enough to give a correct library ID. Although background subtraction can be used, it may leave behind such a weak pattern that the spectra can often be useless.

The background noise is predominantly due to straight chained hydrocarbon molecules, as verified by the indicator mass ions scans (molecular fragments) of masses 43, 57 and 41, 55; these are good indicators of straight chained hydrocarbons, such as heptadecane (Fig. 5). Chromatographs of hydrocarbon-indicating mass ions reveal the abundance of straight chained hydrocarbons in the SPE sample (Fig. 6). Carbon chain lengths of approximately 18 to 22 carbons can be found, as the library searches bear out (Figs. 7 & 8). The SPE cartridge, whose extraction media is a carbon chain length of 18, could be a possible source of the contamination. To determine where the hydrocarbons originate, a comparison with the LLE samples (which did not make contact with the plastic SPE cartridge) was made. Background "noise" exhibited in the LLE propachlor sample chromatograph is similar but not as intense (Fig. 9), again using hydrocarbon indicating mass ions of 43, 57 and 41, 55. In effect, this isolates the buffer and bisulfide solutions as the source of the background noise. Consistent in all samples were the artifacts Bis (2-ethylhexyl) phthalate and butylated hydroxytoluene. The most probable source of phthalates are plastics used in the extraction apparatus or impurities in the compounds of the buffer solution (Hagen et al., 1990). Solvents or solutes would be the most probable

source of the butylated hydroxytoluene artifact. These compounds are noted in the SPE phosphate buffer and bisulfide blank (Fig. 4).

In contrast, the nitrate reactor blank showed a very quiet baseline (Fig. 10) as compared to the phosphate buffer and bisulfide blank (Fig. 4). No major artifacts were present and the few minor peaks that were present were of branched alcohols. These data eliminate the SPE cartridges as source of contaminants, since the same extraction method was used for each. This finding implicates the solvents used in the bisulfide reactor extractions.

Table 2 displays a summary of the major compounds found in each of the samples analyzed. An important point that should be noted is the samples in this study, as well as those in Potter and Carpenter (1995), showed no products which appeared to be chlorinated (Table 2). The only organic chlorine compounds that could be found were those of the parent herbicides. Under certain conditions, dechlorination has been shown to be a primary first step in the reaction pathway of alachlor (Wilber and Parkin 1992). A more detailed discussion of the major products found will be discussed below.

Figure 4. Phosphate buffer and bisulfide blank chromatograph.

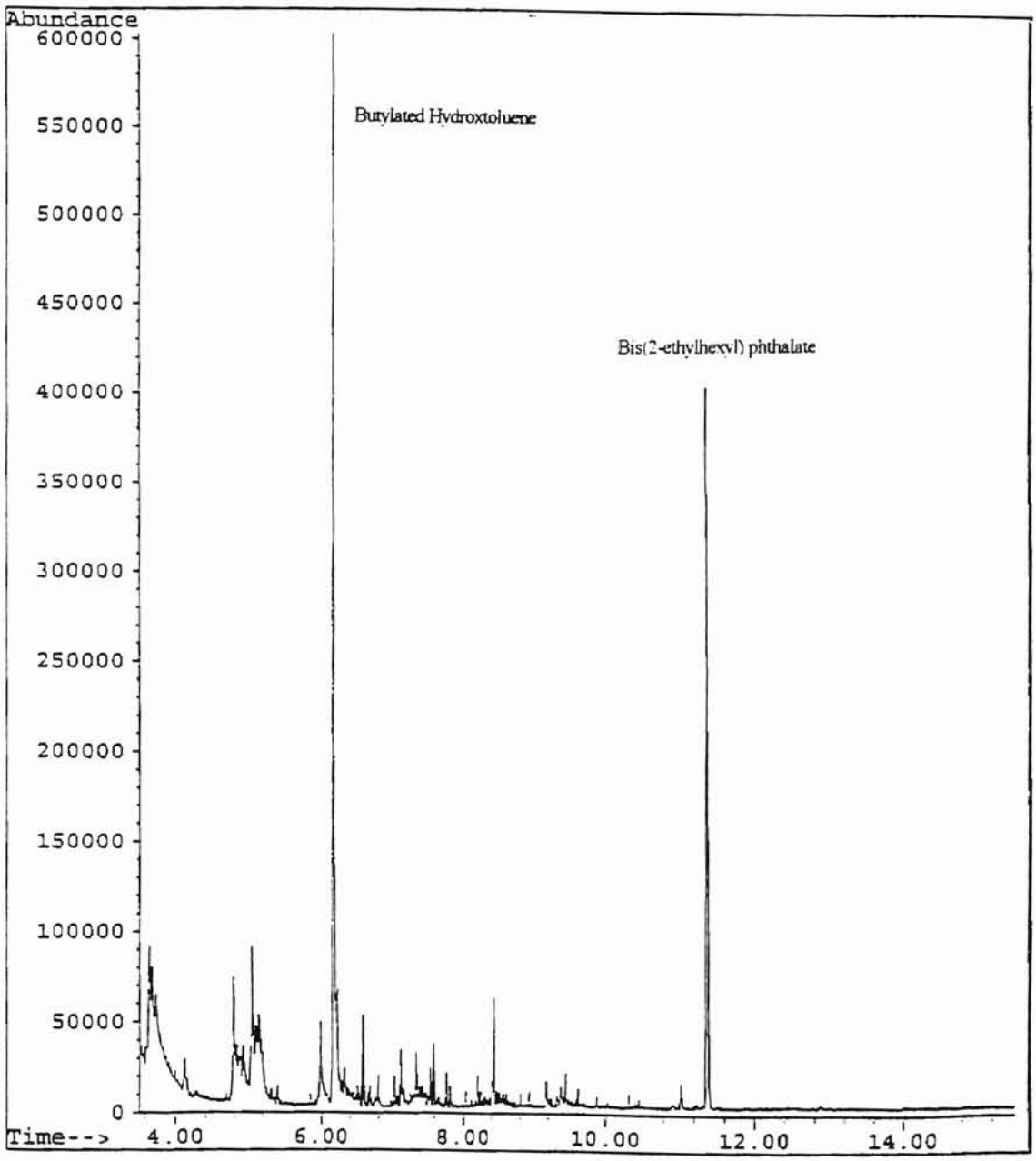


Figure 5. Mass spectral scan of heptadecane.

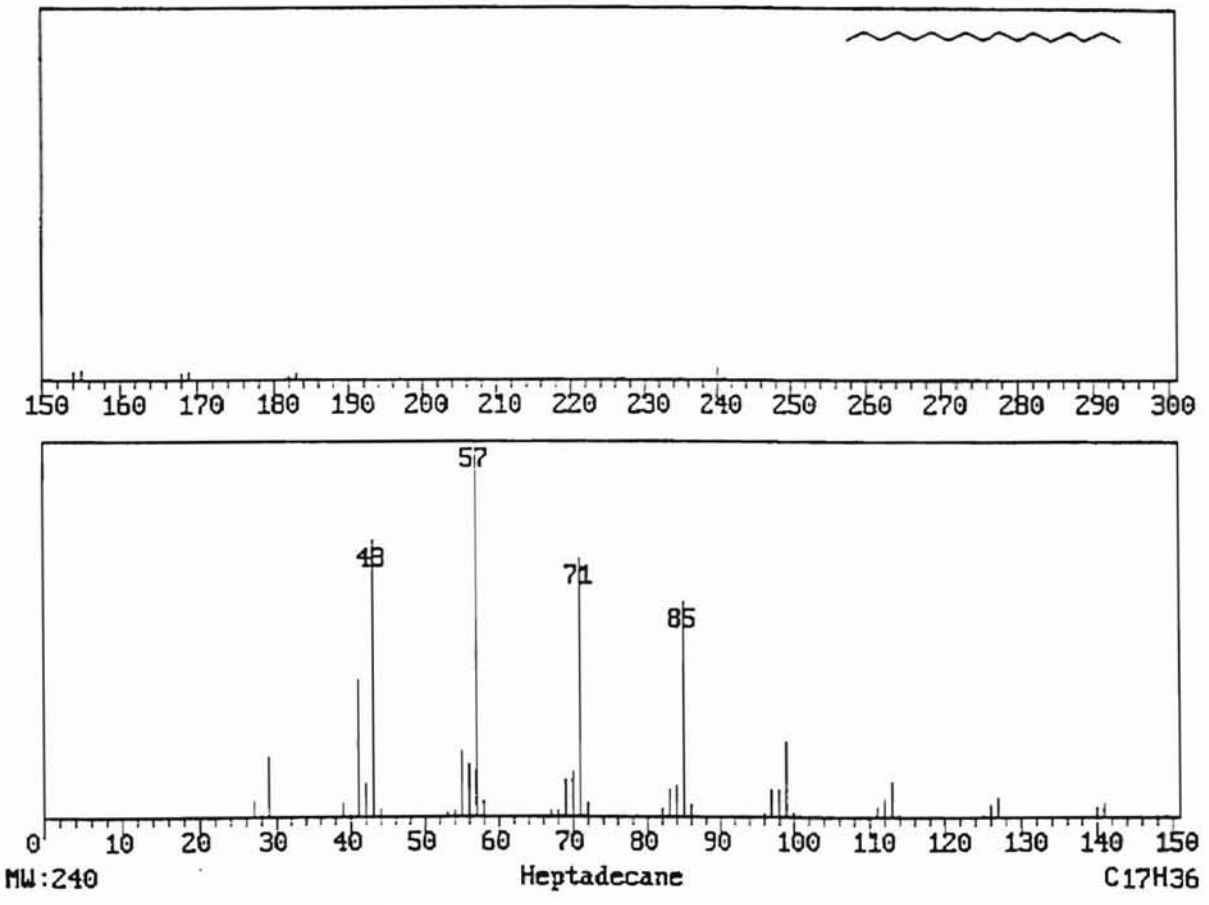


Figure 6. Chromatograph of hydrocarbon mass ions in SPE sample.

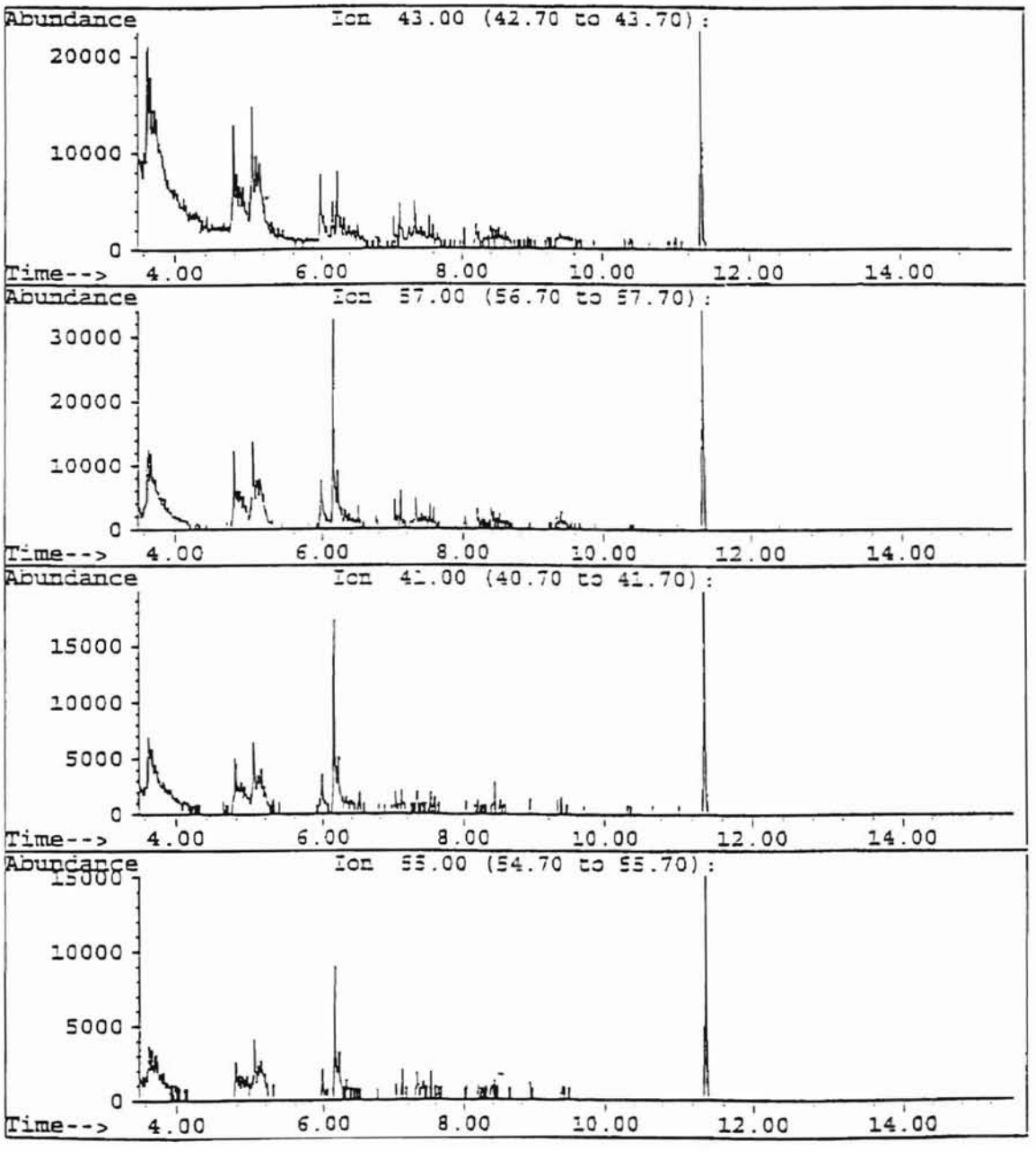


Figure 7. Library spectra of docosane w/spectra scan of phosphate buffer blank

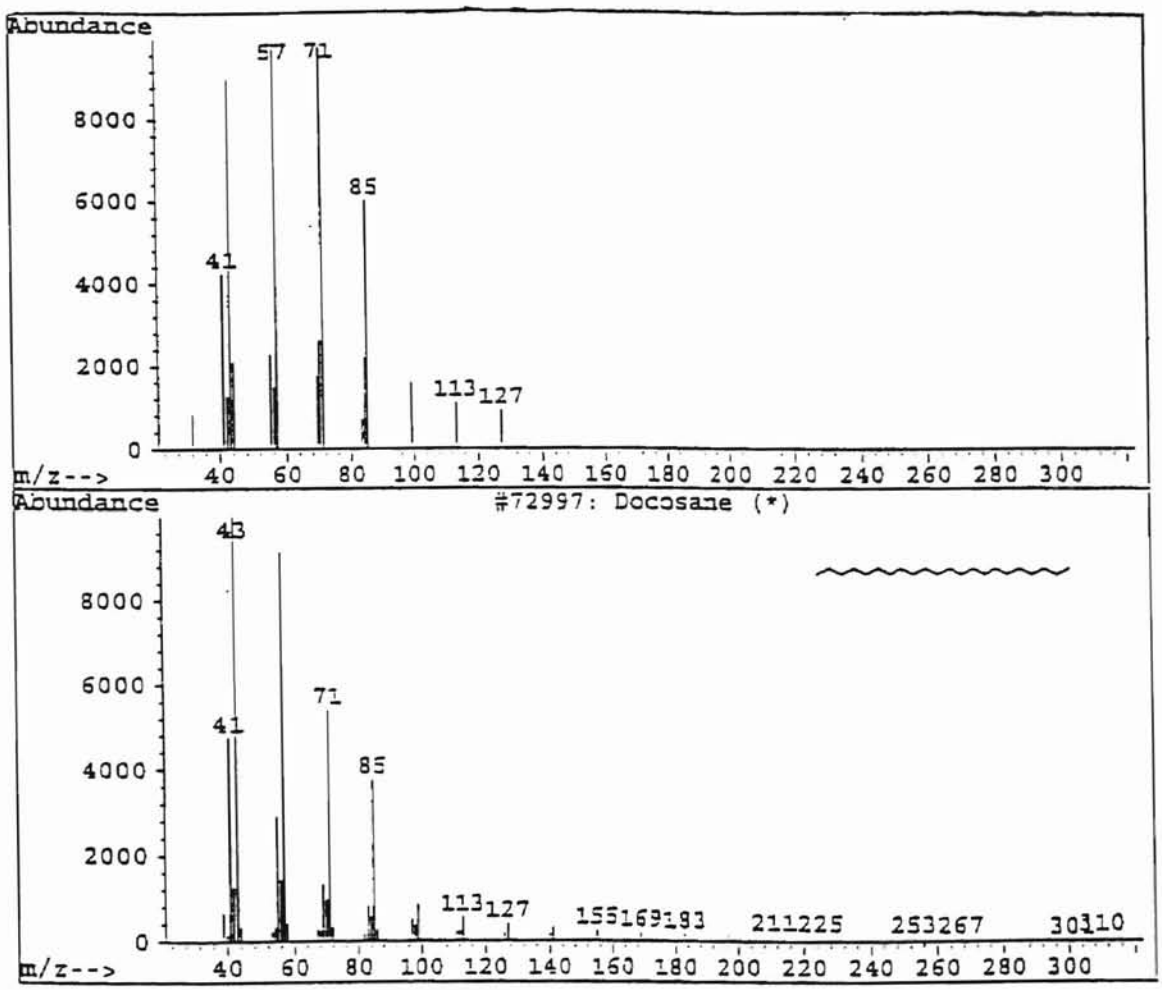


Figure 8. Library spectra of nonadecane with spectra scan of phosphate buffer.

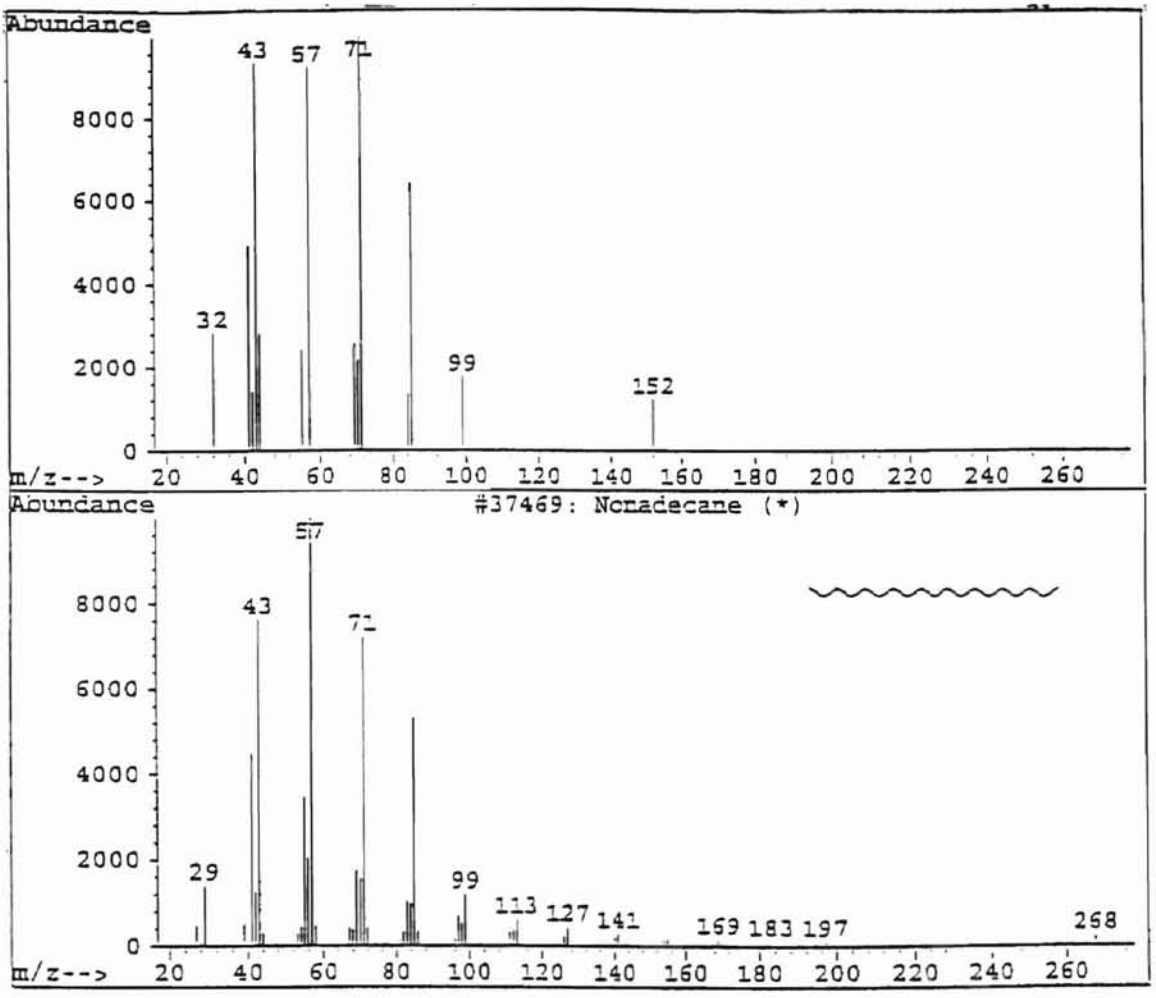


Figure 9. Chromatograph of indicator mass ions of hydrocarbons in LLE sample.

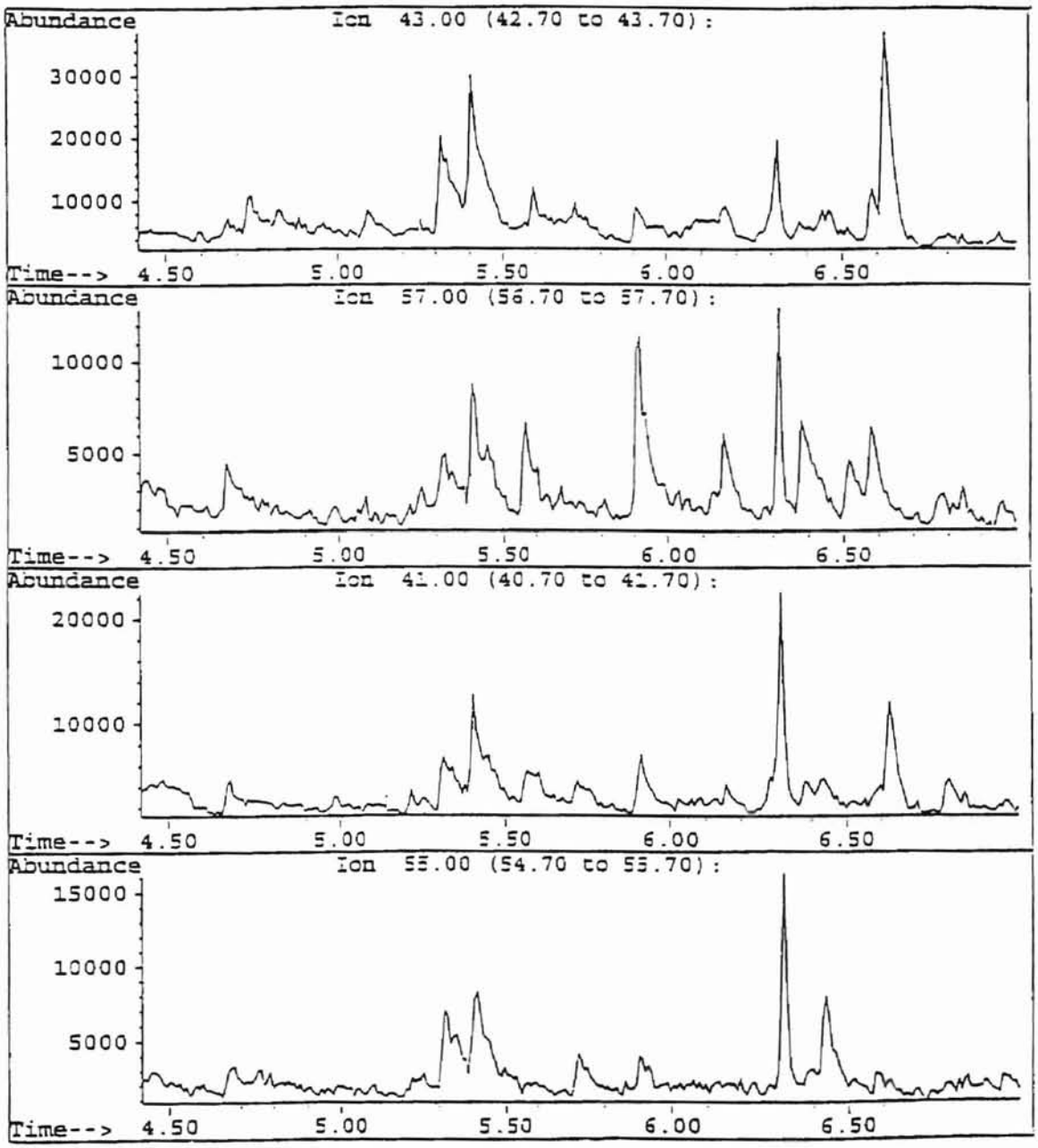


Figure 10. Ethyl acetate blank from nitrate reactor.

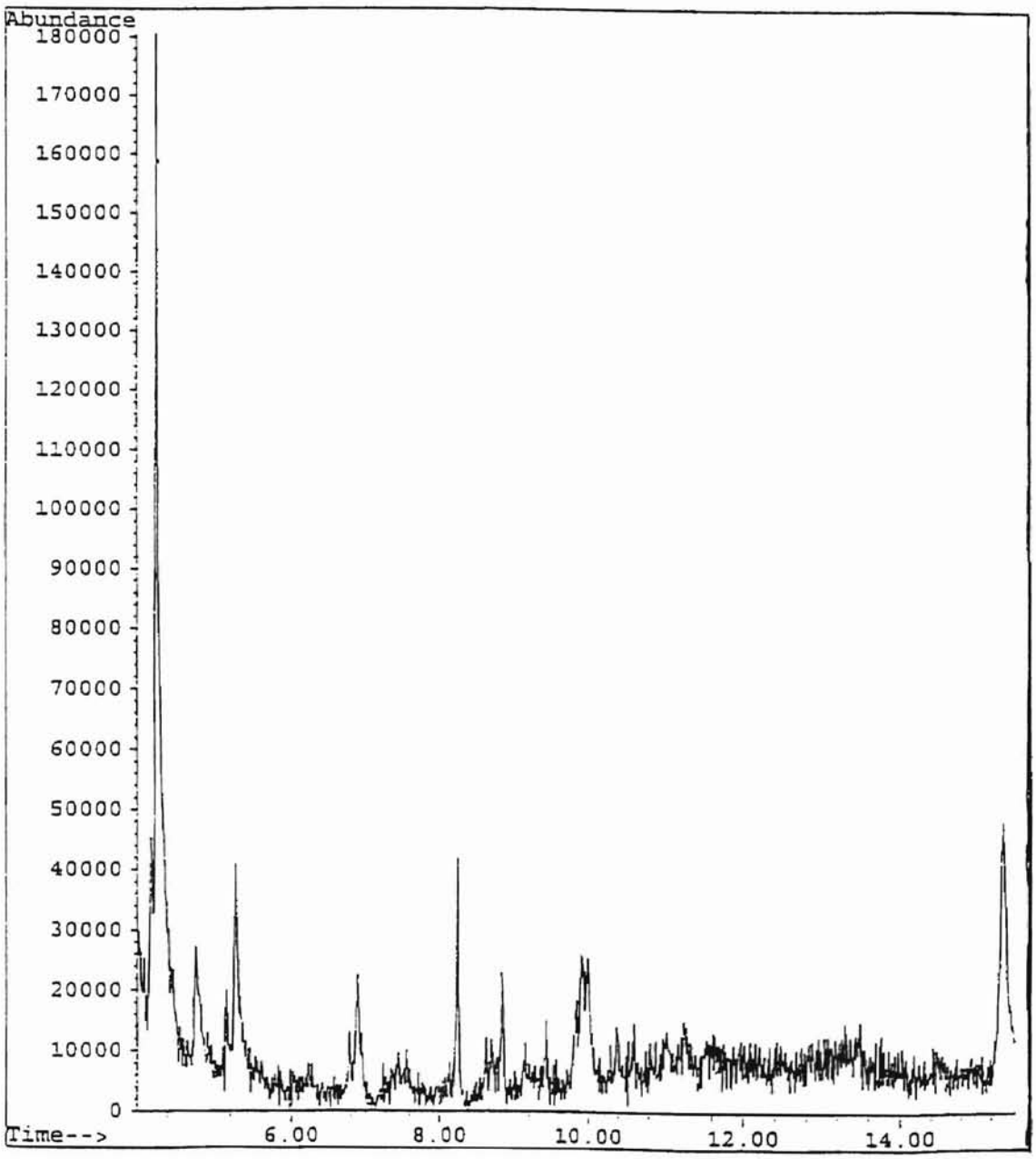


Table 2

TABLE OF ANALYTICAL SAMPLE FINDINGS

Sample Name	Analytical Findings
B 1.0	##
B 1.1	##
B 2.0	##
B 2.1	##
B 3.0	##
B 4.0	##
B 4.1	##
B 5.0	##
B 5.1	##
B 6.0	##
B 7.0	N - (2, 6 - Diethyl Phenyl) - N - (Methoxymethyl) Acetamide
	2 - Hydroxy - 2',6' - Diethyl - N - (Methoxymethyl) acetanilide
	Lenthionine
	Hexathiepane
B 8.0	Lenthionine
	Hexathiepane
B 9.0	##
N 1.0	**
N 2.0	**
N 3.0	2-Ethyl-1-hexanol
N 4.0	**
N 5.0	Propanoic and Benzoic acid, cyclododecane

Common bisulfide background and interferences as noted in bisulfide reactors results section

** Common nitrate background and interferences as noted in bisulfide reactors results section

Bisulfide Reactor Results

A very clear finding of the study by Qin (1995) was the ability of the bisulfide reactor to react with the alachlor and propachlor, resulting in reduced quantities of both parent compounds. The analytical data (measured by SPE and GC/ECD analysis) revealed significant degradation of the parent compounds also. This abiotic degradation of acetanilide herbicides is well documented in the studies of Wilber and Parkin (1992) and Aga et al., (1996). The exposure to the bisulfide reactor yields a predictable set of results. The alachlor peak at 8.26 min. in the "Alachlor, Buffer & Bisulfide" chromatographs (Figs. 11 & 12) and the propachlor peak at 6.75 min. in the "Propachlor, Buffer & Bisulfide" chromatographs (Figs. 13 & 14) show substantial reductions in parent compound over a two day time span. The propachlor peak abundance declined approximately 95% and the alachlor peak abundance decreased approximately 70%. However, there was an absence of degradation products, which was not expected.

Looking at these chromatographs, there is no significant change in other major or minor peak abundance's. The original herbicide mass has not been accounted for. Therefore, it appears that the products are not in a form to be detected. A plausible scenario may include the following:

(1) the metabolites were never extracted because the partition coefficient towards the solvent was poor (Markell and Hagen, 1991).

Figure 11. Chromatograph of alachlor and buffer 8/5/95 (Day 0).

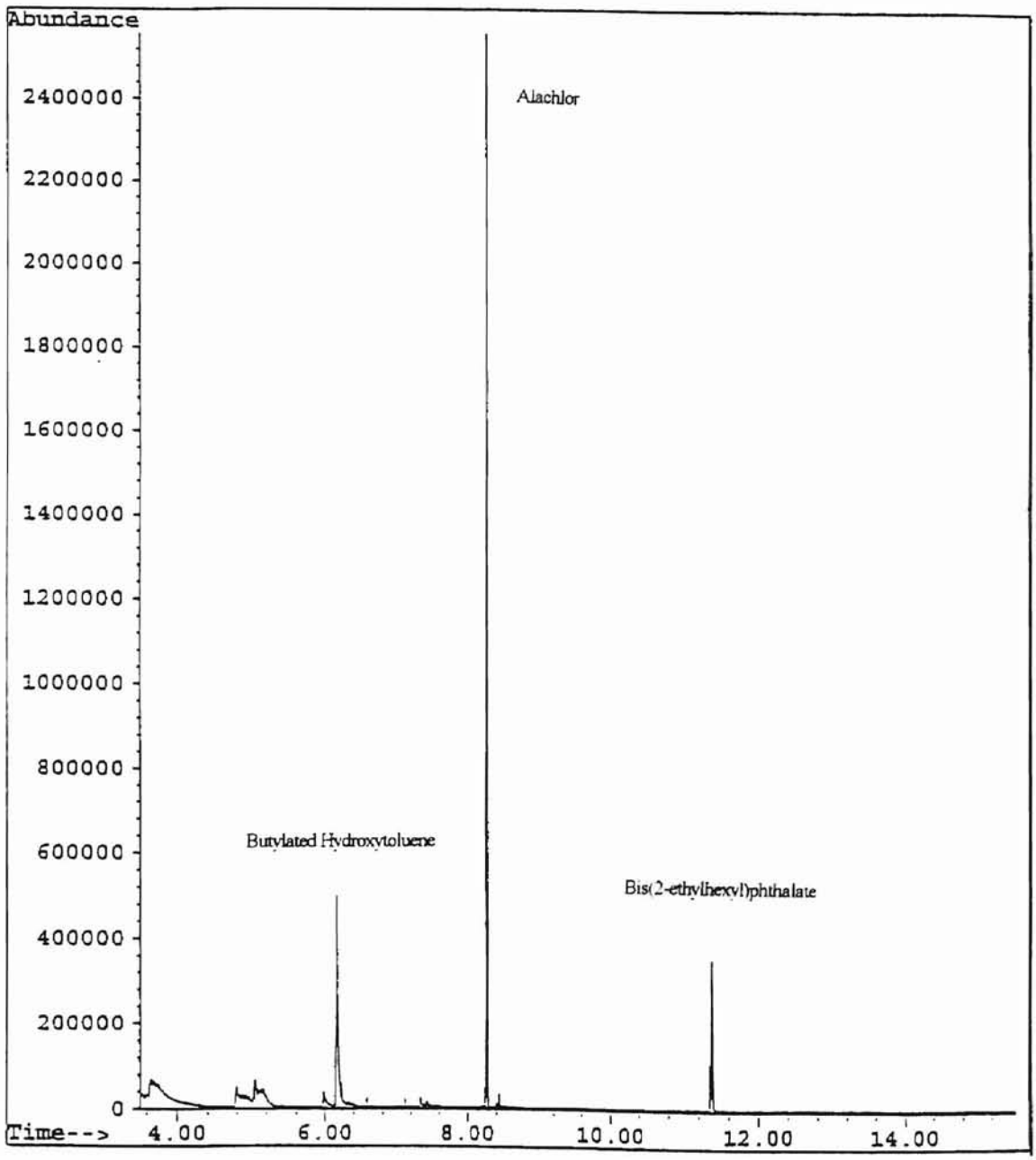


Figure 12. Chromatograph of alachlor and buffer 8/7/95 (Day 2).

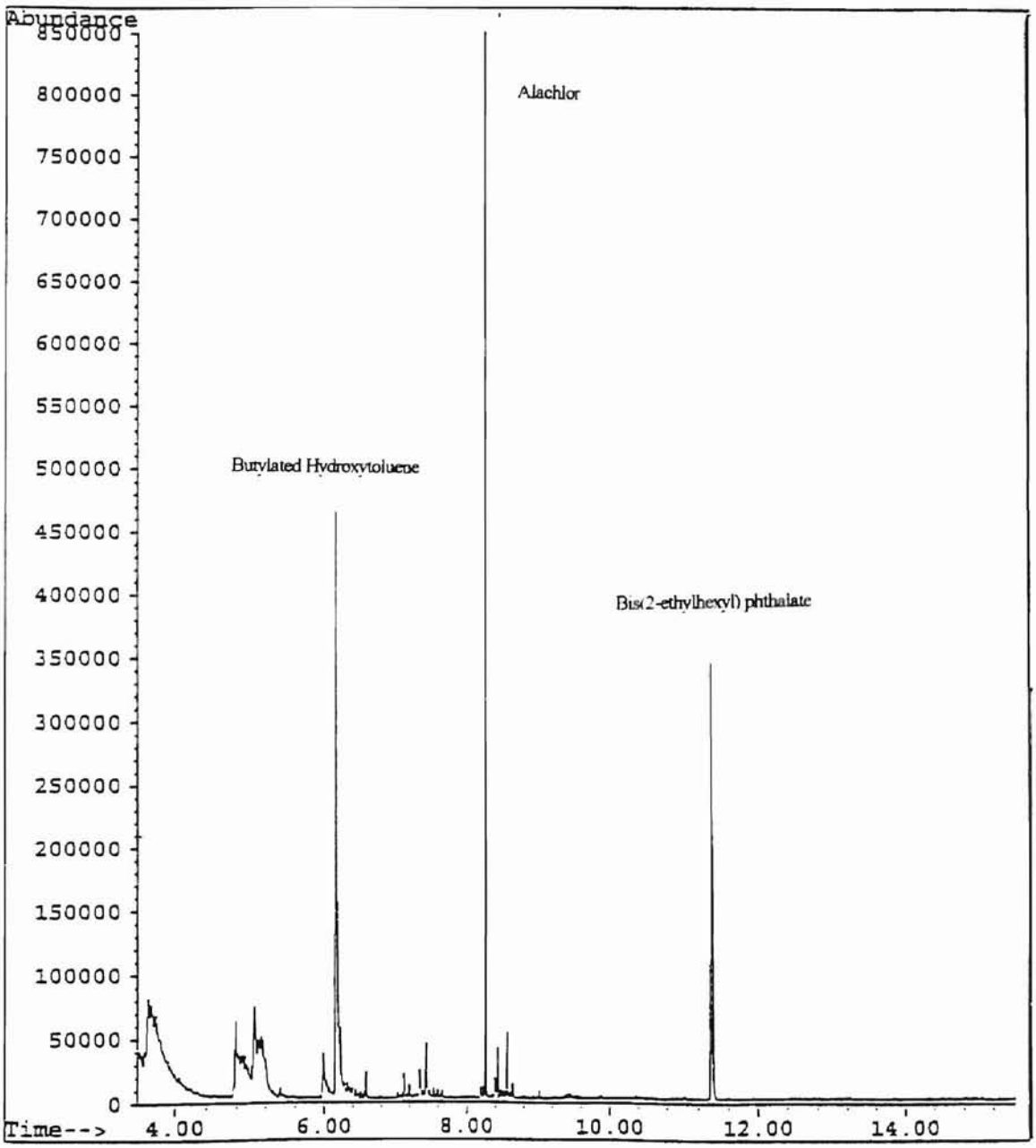


Figure 13. Chromatograph of propachlor and buffer 8/5/95 (Day 0).

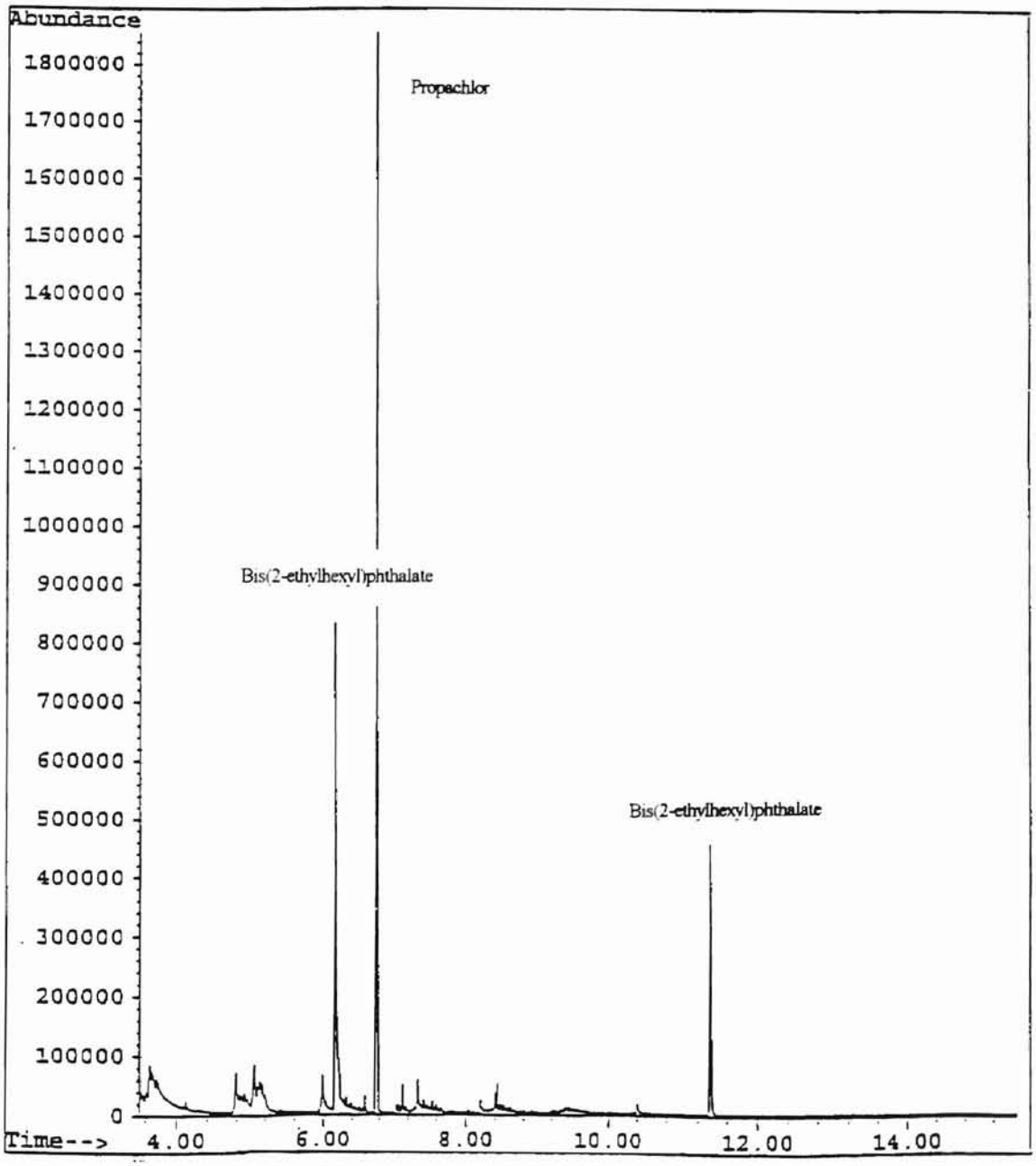
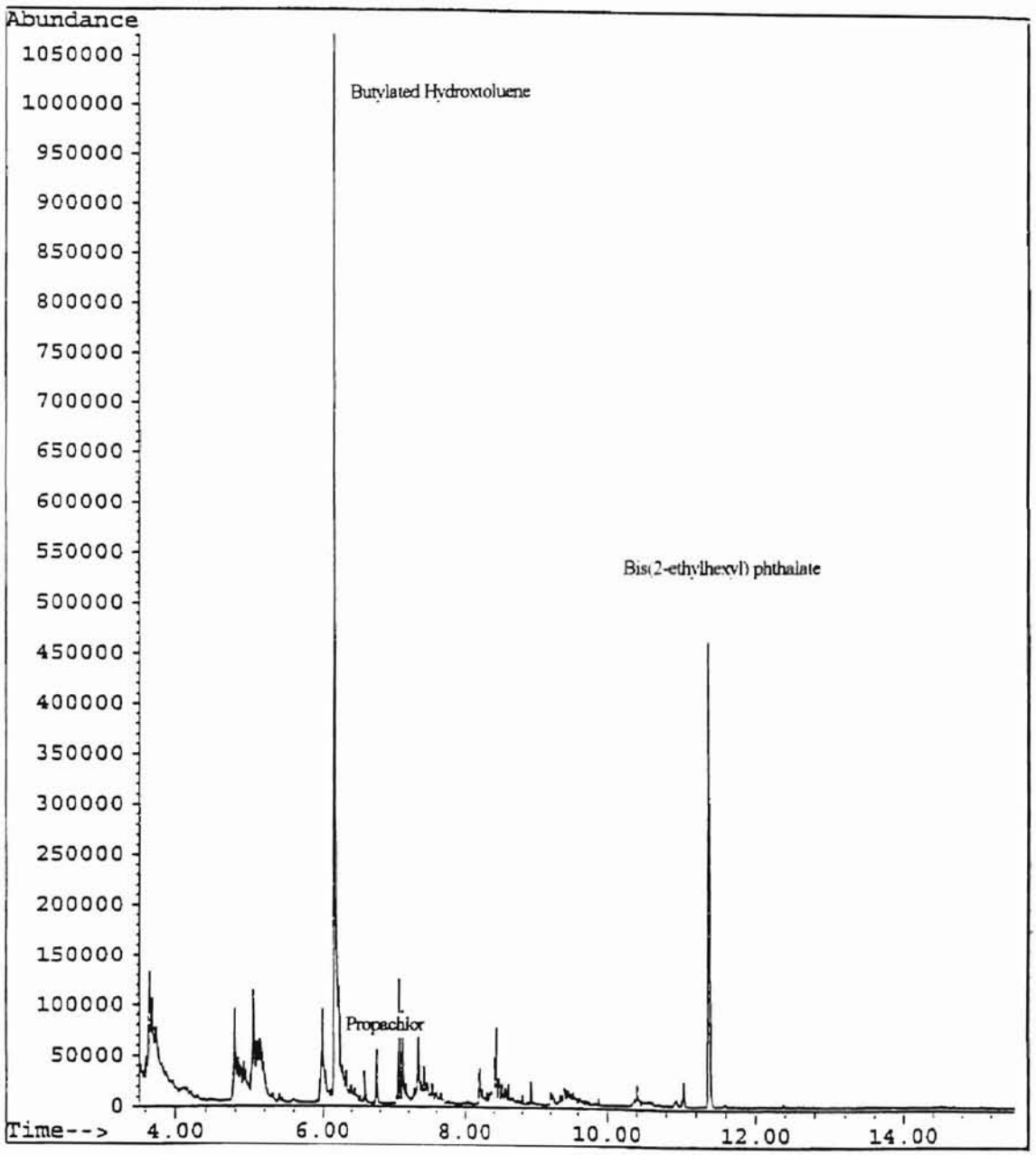


Figure 14. Chromatograph of propachlor and buffer 8/7/95 (Day 2).



(2) the metabolites once extracted onto the C18 media were never re-extracted off the media.

The largest technical barrier preventing the full utilization of the GC/MS analytical data is the absence of reference mass spectra data of newly discovered compounds. Some reference spectral data were generated in the study by Potter and Carpenter (1995), in which the synthesis of twelve degradation products of alachlor was accomplished. In the present study, two degradation compounds from the bisulfide reactor matched the synthesized reference mass spectra as stated in Table 2. These include: N - (2, 6 - diethyl phenyl) - N - (methoxymethyl) acetamide, found in the bisulfide LLE sample and is shown in (Fig. 15) and 2 - hydroxy - 2',6' - diethyl - N - (methoxymethyl) acetanilide, also found in the LLE bisulfide sample (Fig. 16). A comparison of the reference spectra and the sample spectra are shown to have the same major peaks and pattern of minor peaks. Some of the ratios are not in perfect agreement, but this can be directly attributed to impurities in the sample matrix.

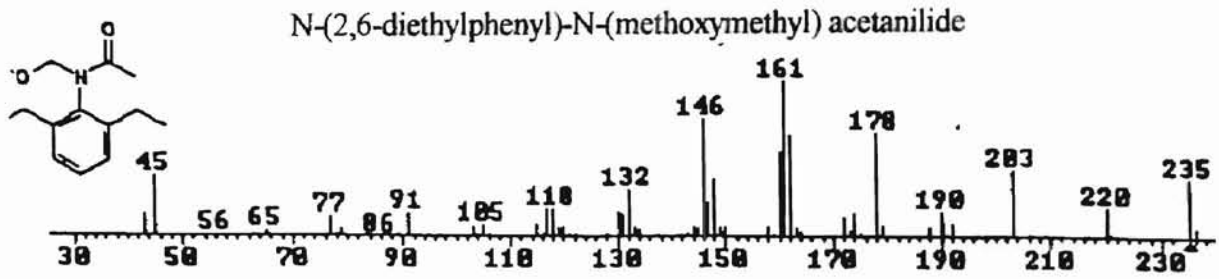
Several near matches to the synthesized reference spectra also were found. For example, Bis (N-methoxymethyl)-2,6-diethylaniline compares closely to another peak from the LLE bisulfide sample (Fig. 17), and N-(methoxymethyl)-2,6-diethylaniline matches reasonably well with yet another (Fig. 18). The comparisons of reference spectra to the sample spectra show very strong similarities which have one or two major deviations of the spectral pattern. These "near matches" imply a degradation pathway that is unique to the

anaerobic bisulfide reactor unlike the natural environment from which Potter and Carpenter (1995) samples were exposed and sampled .

Potter and Carpenters' (1995) reference spectra were created in controlled laboratory conditions geared for the production of pure material. Thus the presence of impurities was minimized. This "pristine" environment was not a paramount concern for the bisulfide reactor samples. The samples generated by Qin (1995) were intended to generate data for degradation kinetics studies of alachlor. Without advanced planning, these samples became the focal point of the present study, which is interested in identifying possible metabolites that could have been created in the bisulfide reactors. This introduces the concern of extract holding times and the type of reactions that may have occurred between components in the extract.

In analyzing the spectra of the above figures, a modest quantifiable reference should be noted. The abundance of the mass spectra of these "matches" and "near matches" range from 17000 to 28000 abundance units, whereas an abundance of 600000 abundance units is noted for the LLE alachlor mass spectra (Fig. 19). This demonstrates the small quantity of metabolic products that was found in the LLE samples, approximately 2% to 4 % of the parent herbicide mass. At this level of abundances, background noise can easily mask or distort the true spectra of a compound.

Figure 15.



Referenced from Potter & Carpenter (1996)

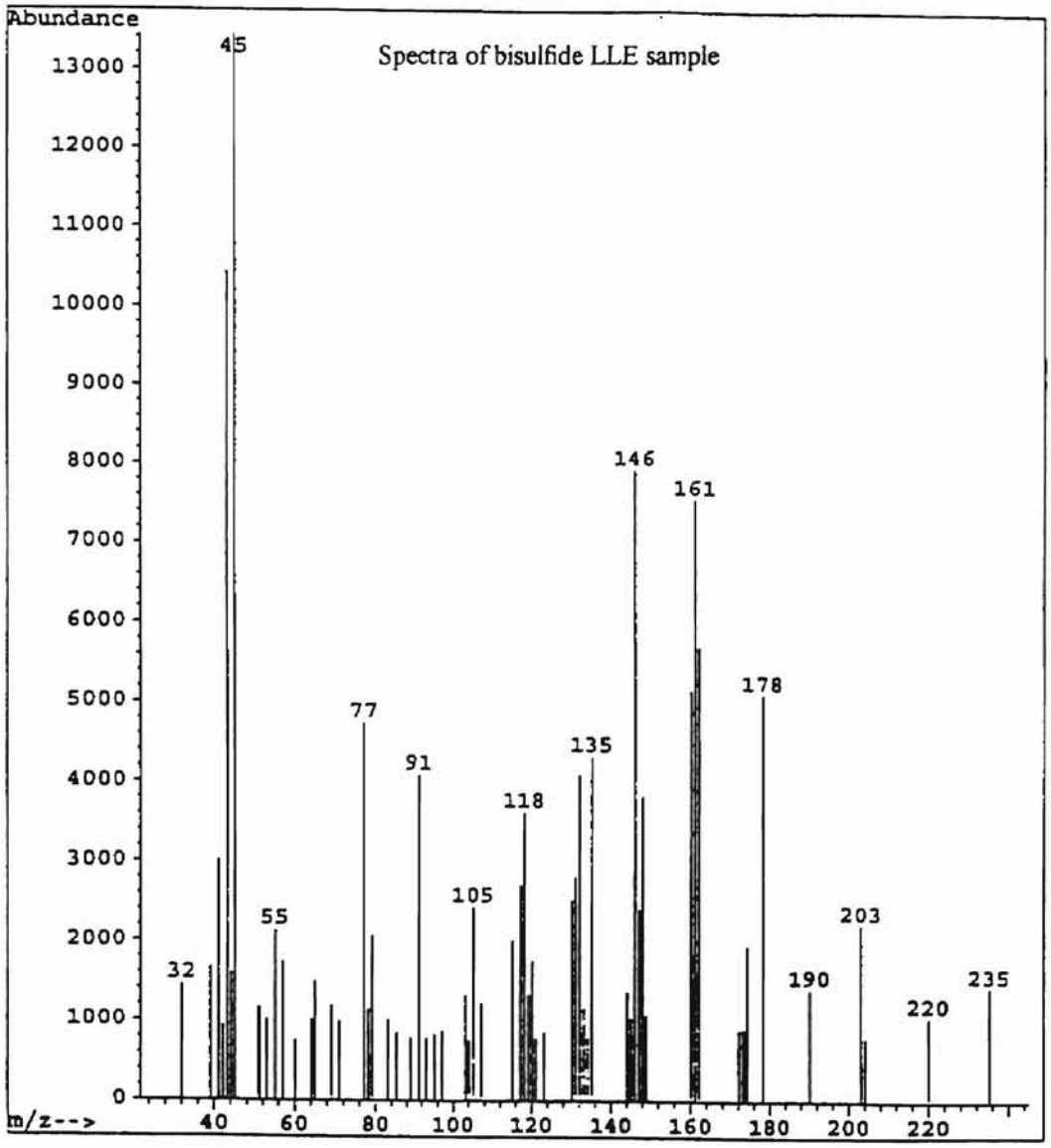
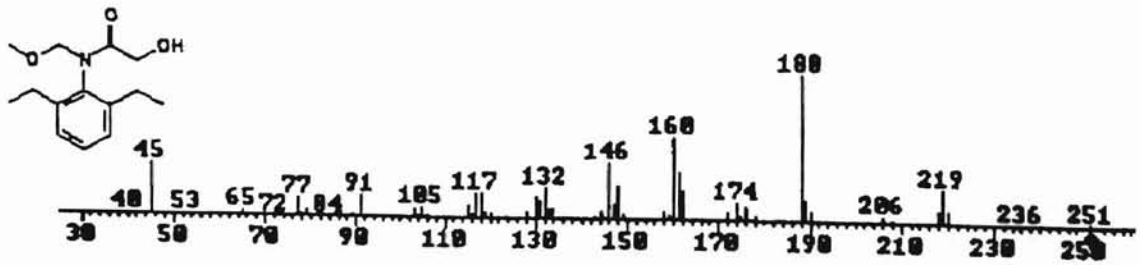


Figure 16.

2-Hydroxy-2',6'-diethyl-N-(methoxymethyl) acetanilide



Referenced from Potter & Carpenter (1996)

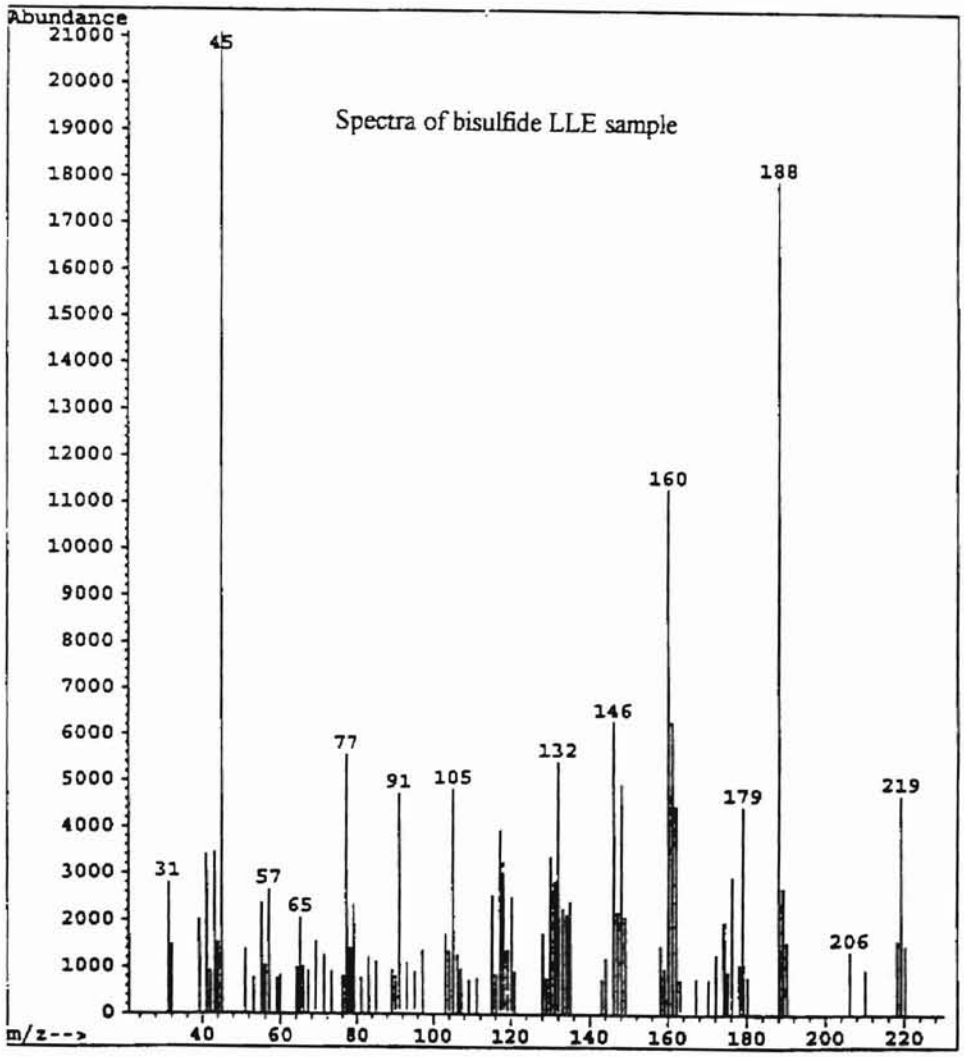
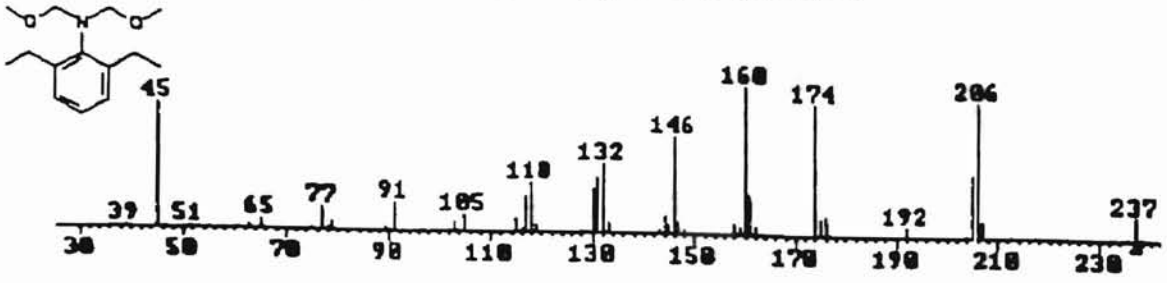


Figure 17.

Bis (N-methoxymethyl)-2,6-diethyl aniline



Referenced from Potter & Carpenter (1996)

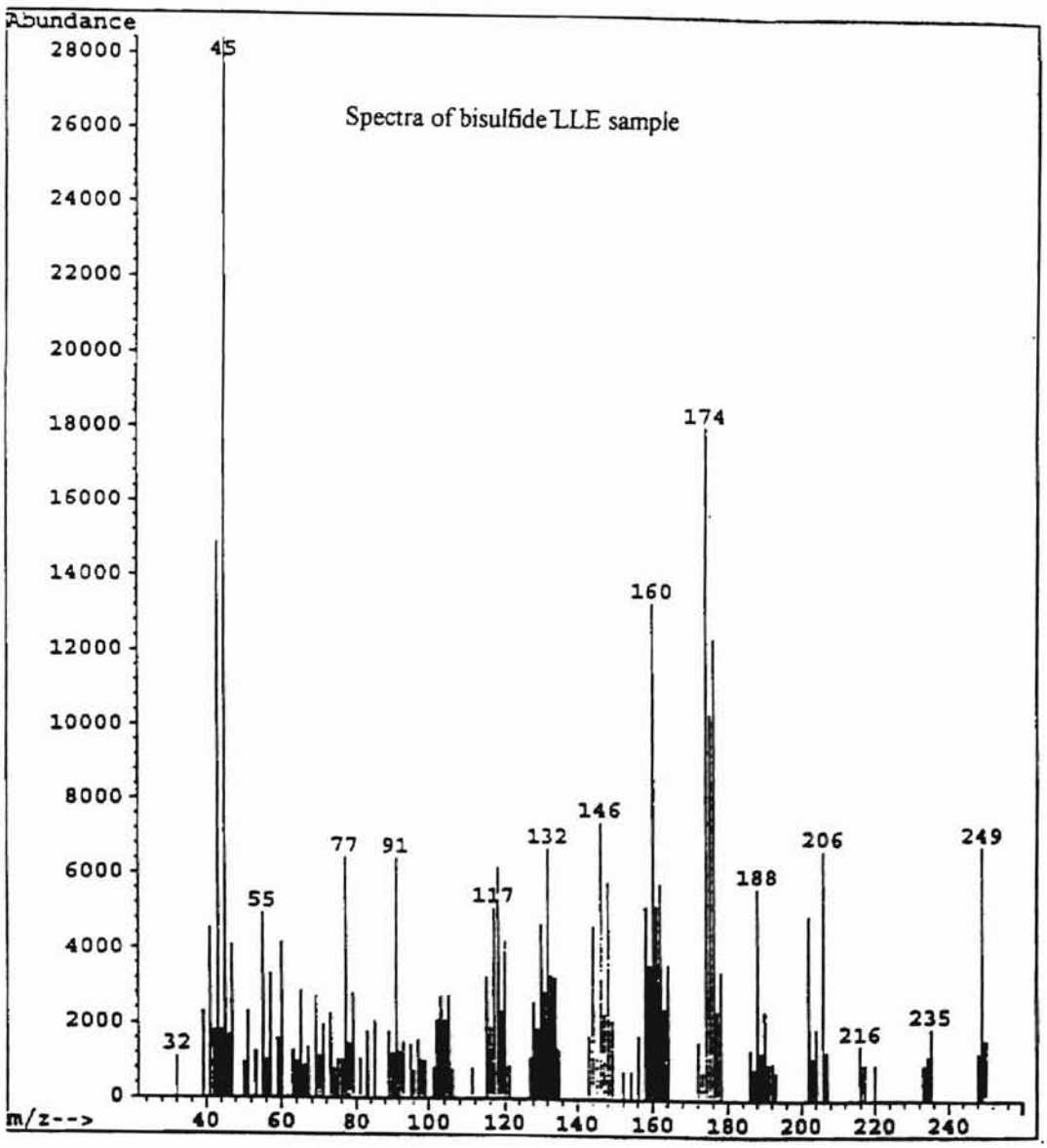
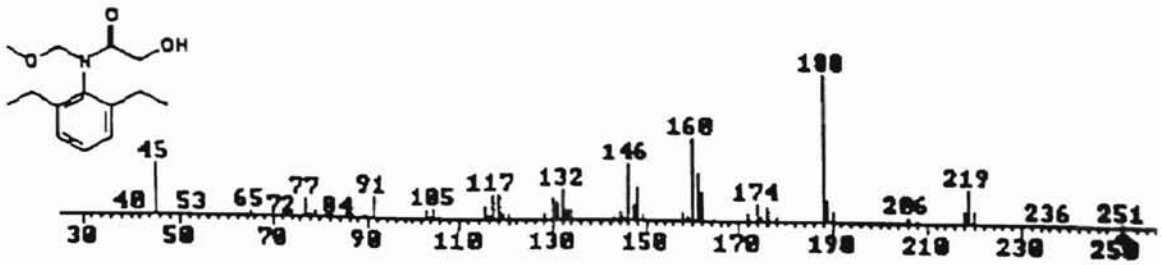


Figure 18.

2-Hydroxy-2',6'-diethyl-N-(methoxymethyl) acetanilide



Referenced from Potter & Carpenter (1996)

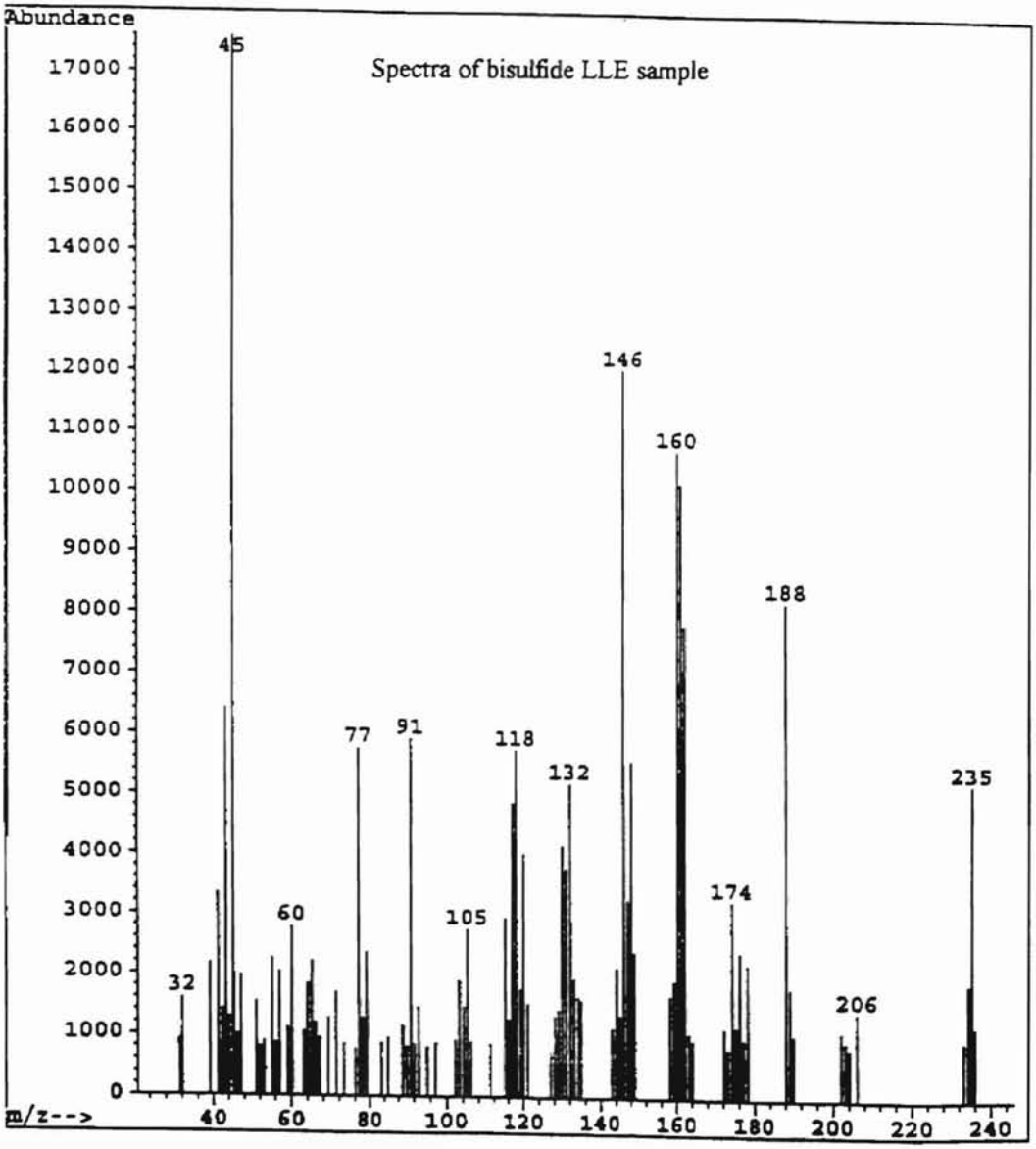
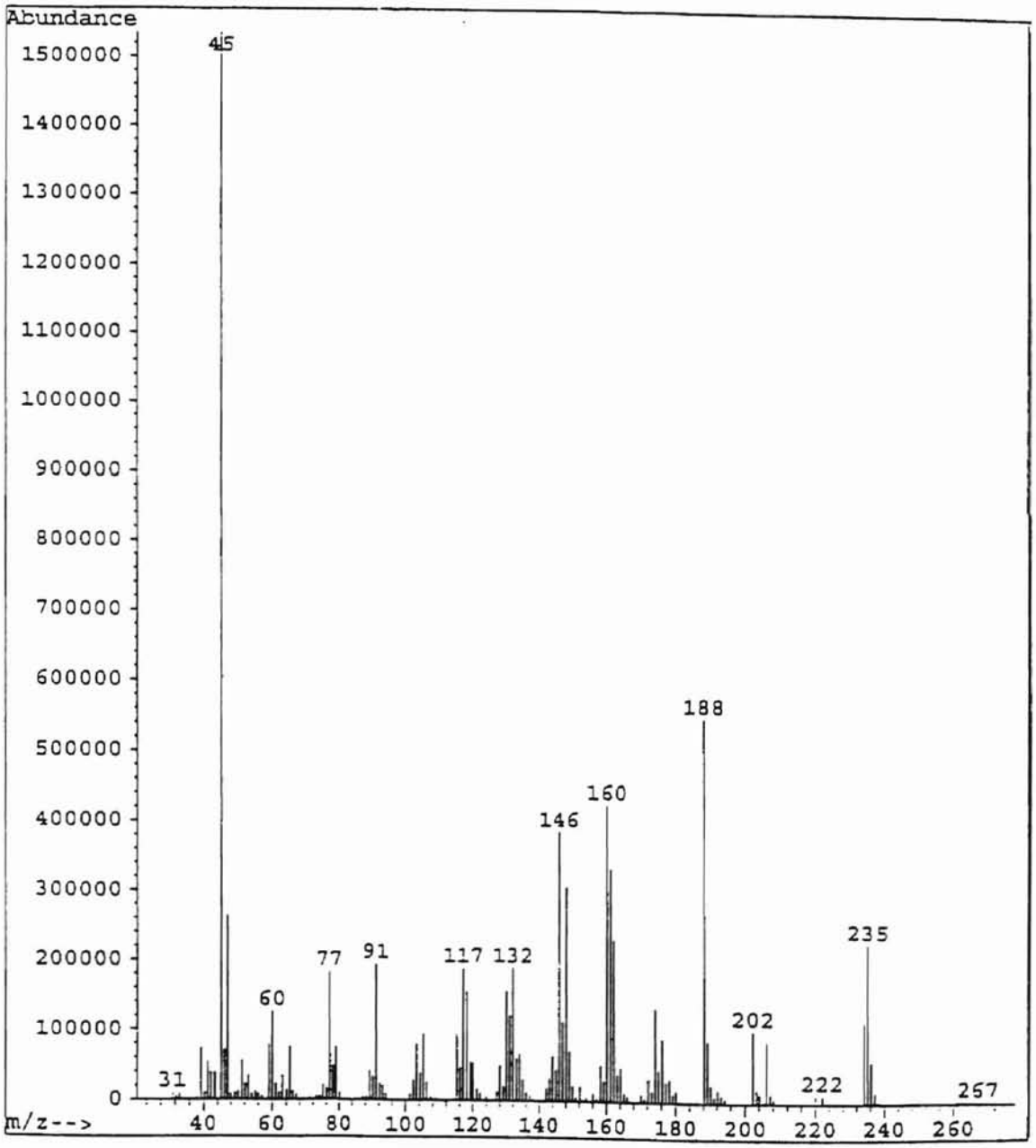


Figure 19. Spectra of LLE alachlor sample.



The reaction pathway to N - (2, 6 - diethyl phenyl) - N - (methoxymethyl) acetamide and 2 - hydroxy - 2',6' - diethyl - N - (methoxymethyl) acetanilide consists of a dehalogenation step. The substitution of the Cl^- with the H^+ or ^-OH ions appears to be a simple Brønstead-Lowry acid/base reaction. This subistution reaction is very probable due to the buffered pH of the solution, giving ample quantities of OH^- and H^+ species to react with and replace the Cl^-.

The existence of similar metabolites in this study and that of Potter and Carpenter (1995) was not necessarily unexpected, despite the fact that Potter and Carpenter's (1995) samples were from dominantly aerobic environments, while the bisulfide reactor was strictly anaerobic, containing significant amounts of sulfide. The same sample extraction process, LLE, was used in both studies, increasing the likelihood of extracting similar compounds, if present. Several factors could have contributed to the fact that these systems had at least two metabolites in common, including the following:

1) The groundwater samples of Potter and Carpenter had a reasonable chance of being exposed to a sulfide (or at least a strictly anaerobic) environment to some degree, thus giving the potential of similar reaction pathways.
2) The groundwater samples and bisulfide samples likely had comparable pHs, which would drive the molecular bond breaking at

Nitrate Reactor Results

The analytical data yielded by the nitrate reducing reactors were not as fruitful as the bisulfide reactors' data. The four reactor samples, two originally fed alachlor and two propachlor, showed marked decrease over time in the herbicide with which they had been spiked, as noted in the earlier study (Walker, 1997). There were several new compounds found, most of which could not be identified. The four samples from the nitrate reactors were extracted using the same SPE method used for the bisulfide reactor samples (Qin, 1995).

The alachlor spiked sample, with 370 hrs exposure time in the reactor (Fig. 20), showed several interesting results. The 370 hr sample showed a new alcohol peak, 2-ethyl-1-hexanol, at 4.4 min., a new peak with a mass of 253 AMU at 9.9 min. that could not be identified (possibly alachlor minus a methyl group) and an alachlor peak at 10.2 min., which had decreased by 80 % from the "time 0" sample. No compounds that could be confirmed as a primary alachlor metabolite such as alachlor ESA nor benzene with nitrogen groups were found.

The propachlor sample with 217 hrs (Fig. 21) of exposure in the reactor revealed several facts. The 217 hr sample showed possible propanoic acid at 8.22 min., possible benzoic acid at 8.3 min., a propachlor peak at 8.43 min. (decreased by 85% from the Day 0 sample), a possible cyclododecane peak at 8.7 min, which had increased 80% (from the day

0 sample), a possible 2,2-dimethoxy-1,2-diphenylethanone at 10.1 min. and a possible match of hexacosane at 11.7 min., which doubled in size from the "time 0" reactor.

Figure 20. Chromatograph of alachlor in nitrate reactor after 370 hrs exposure.

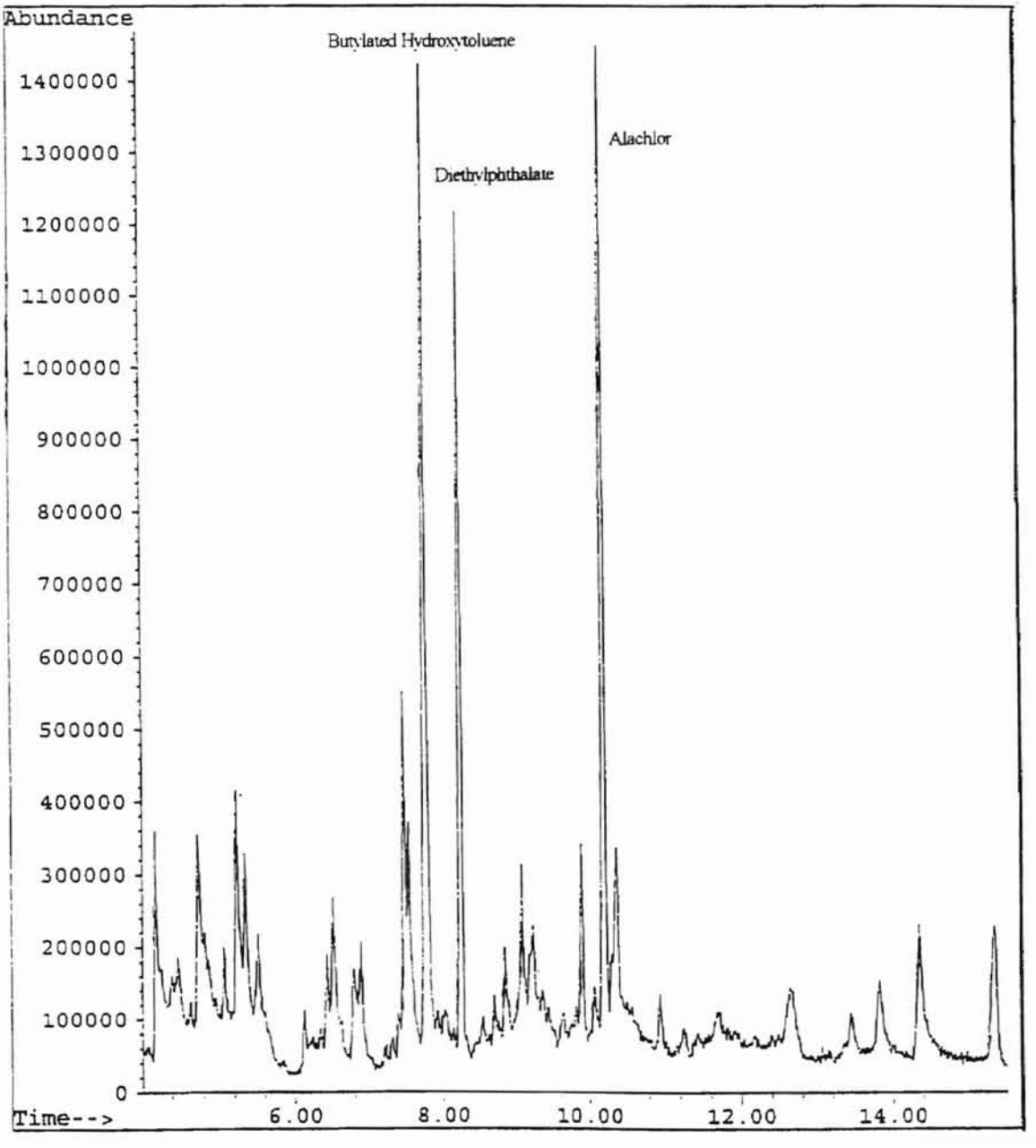
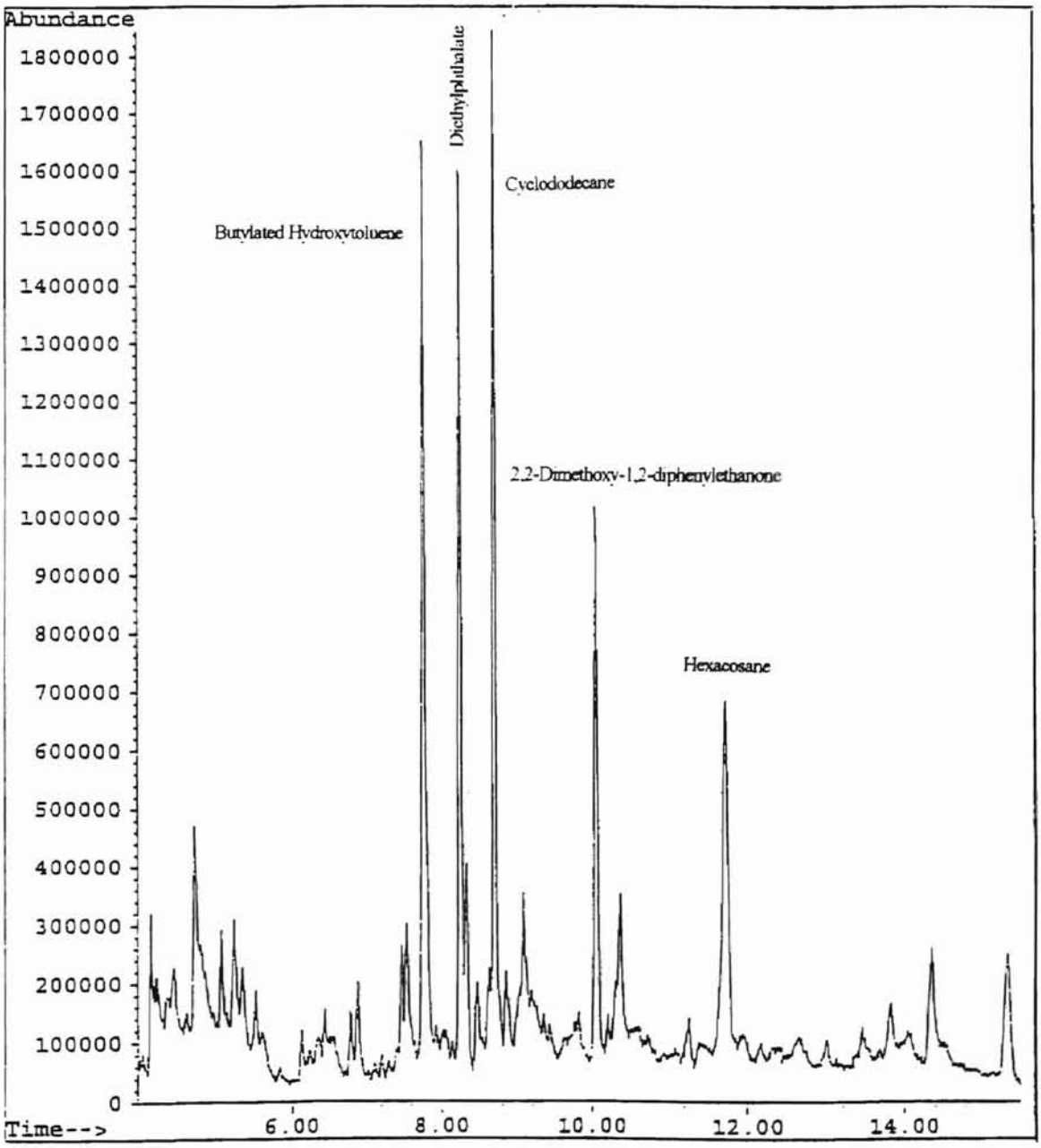


Figure 21. Chromatograph of propachlor in nitrate reactor after 217 hrs exposure.



The data from these samples show two general products, alcohols (nonvolatile neutral compounds) and acids (nonvolatile). Either group of compounds suggests that the system, which was anaerobic, may have supported fermentation reactions, which easily yield these types of products (Grady and Lim, 1980). This system demonstrates total consumption or metabolism occurring to the herbicide substrates, alachlor and propachlor, with such efficiency that very few metabolites could be associated with a parent herbicide. If any aromatic or chlorinated compounds were formed, they apparently were not extracted or recovered by the SPE method used.

Comparison of Extraction Methods

In addition to the alachlor metabolites discussed above, the LLE-extracted alachlor and propachlor samples from the bisulfide reactor contained unique compounds not found in similar samples analyzed using SPE. Two such compounds were cyclic sulfur compounds that were present in both acetanilide herbicide samples. These compounds were lenthionine at (7.03 min.) and hexathiepane at (7.54 min.), as shown in Figs. 22 and 23. Both of these are sulfur and carbon rings. Matches made with the database had the percent probability matches of 91% and 70% (due to minor deviations in the ratios between masses), respectfully. The mass spectral reference pattern is shown with the spectra match in Figs. 24 and 25. It can be stated that after extensive searches these compound were not found in the SPE samples nor in any blanks. It should be noted sulfur compounds only

appeared in samples that initially contained a herbicide and bisulfide; blank samples containing no herbicide, but the typical bisulfide dose, did not form the sulfur-ring compounds.

Figure 22. LLE bisulfide alachlor chromatograph of lenthionine and hexathiepane.

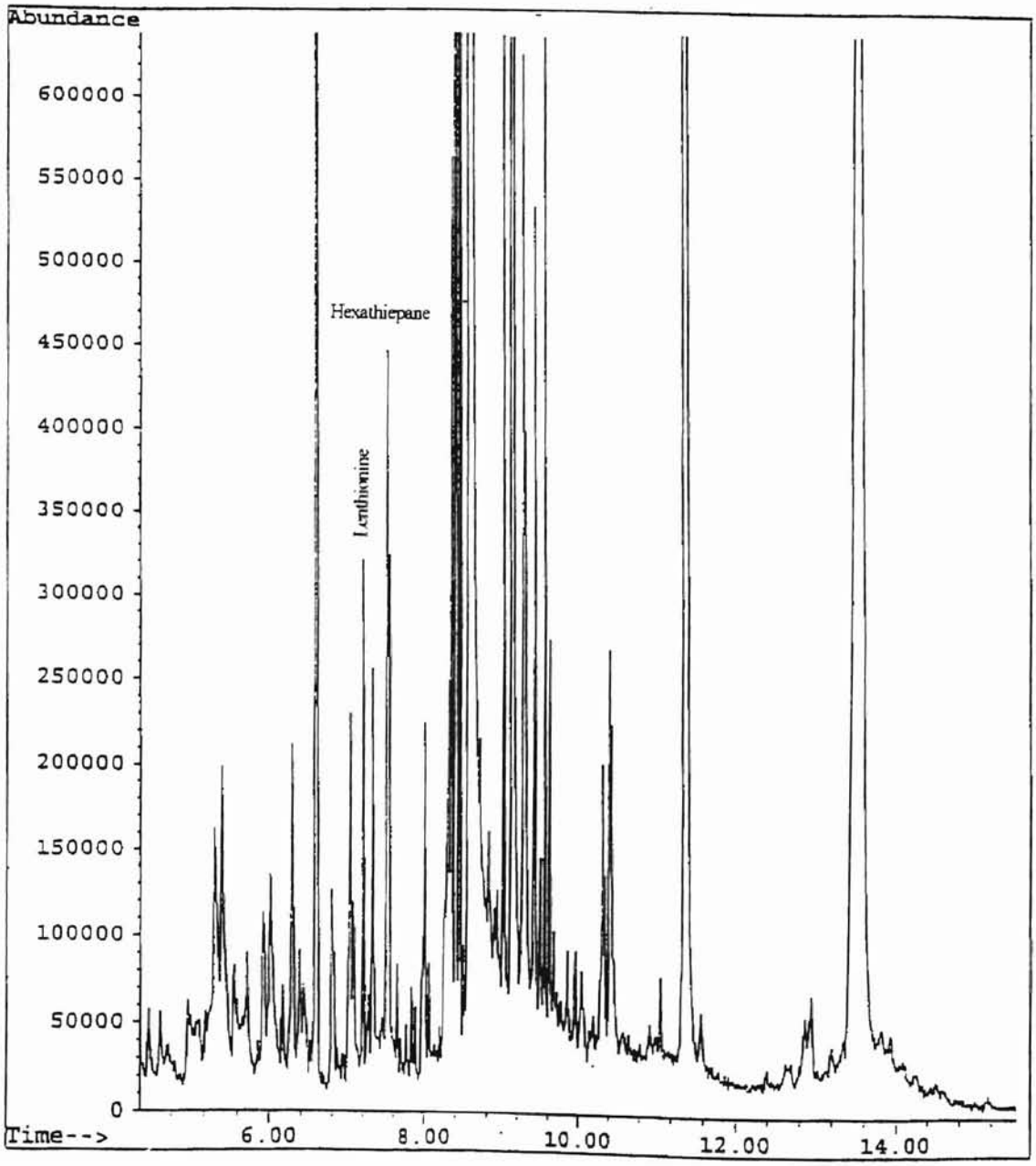


Figure 23. LLE bisulfide propachlor chromatograph of lenthionine and hexathiepane.

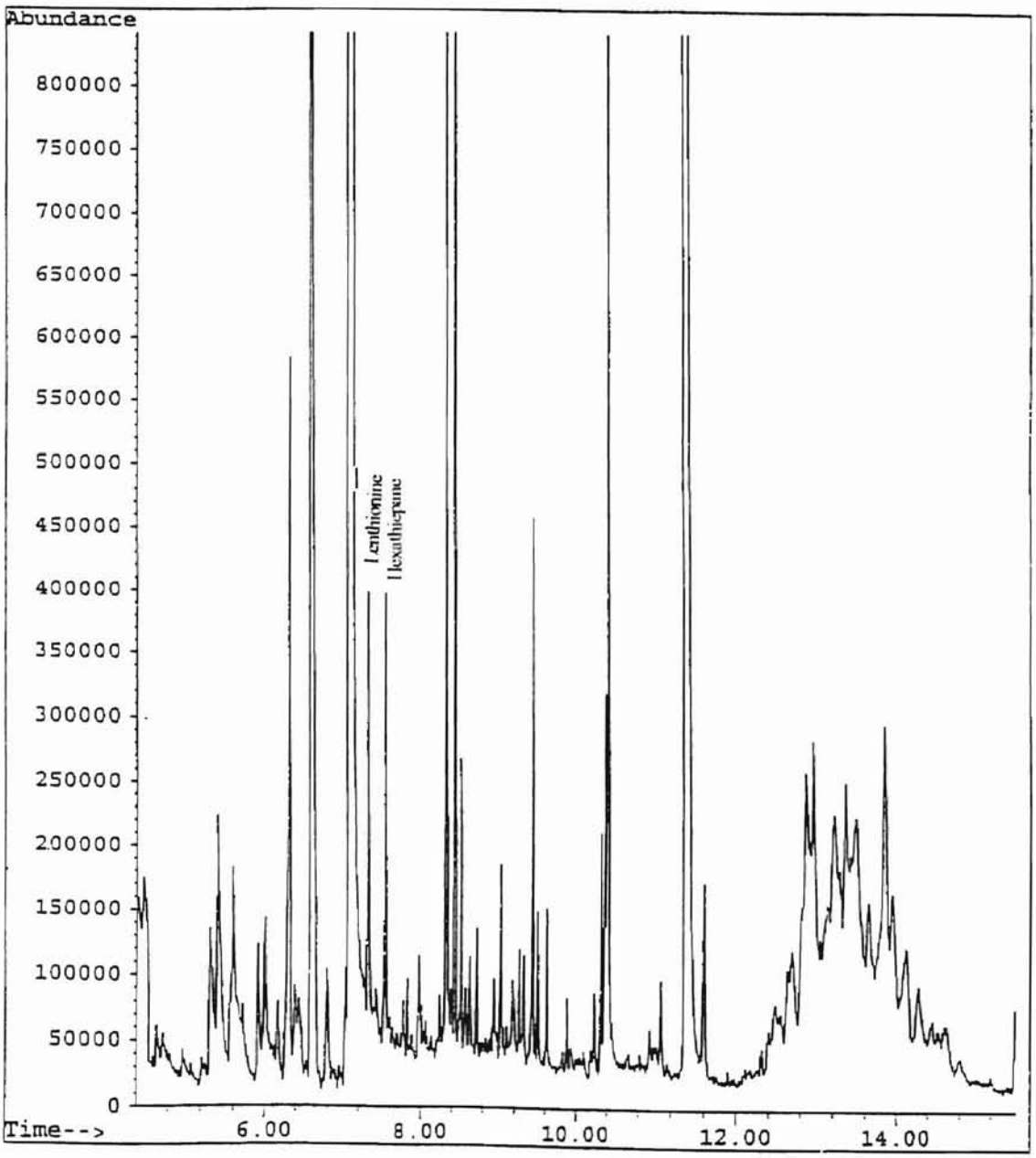


Figure 24. Mass spectra of lenthionine from LLE sample with reference spectra.

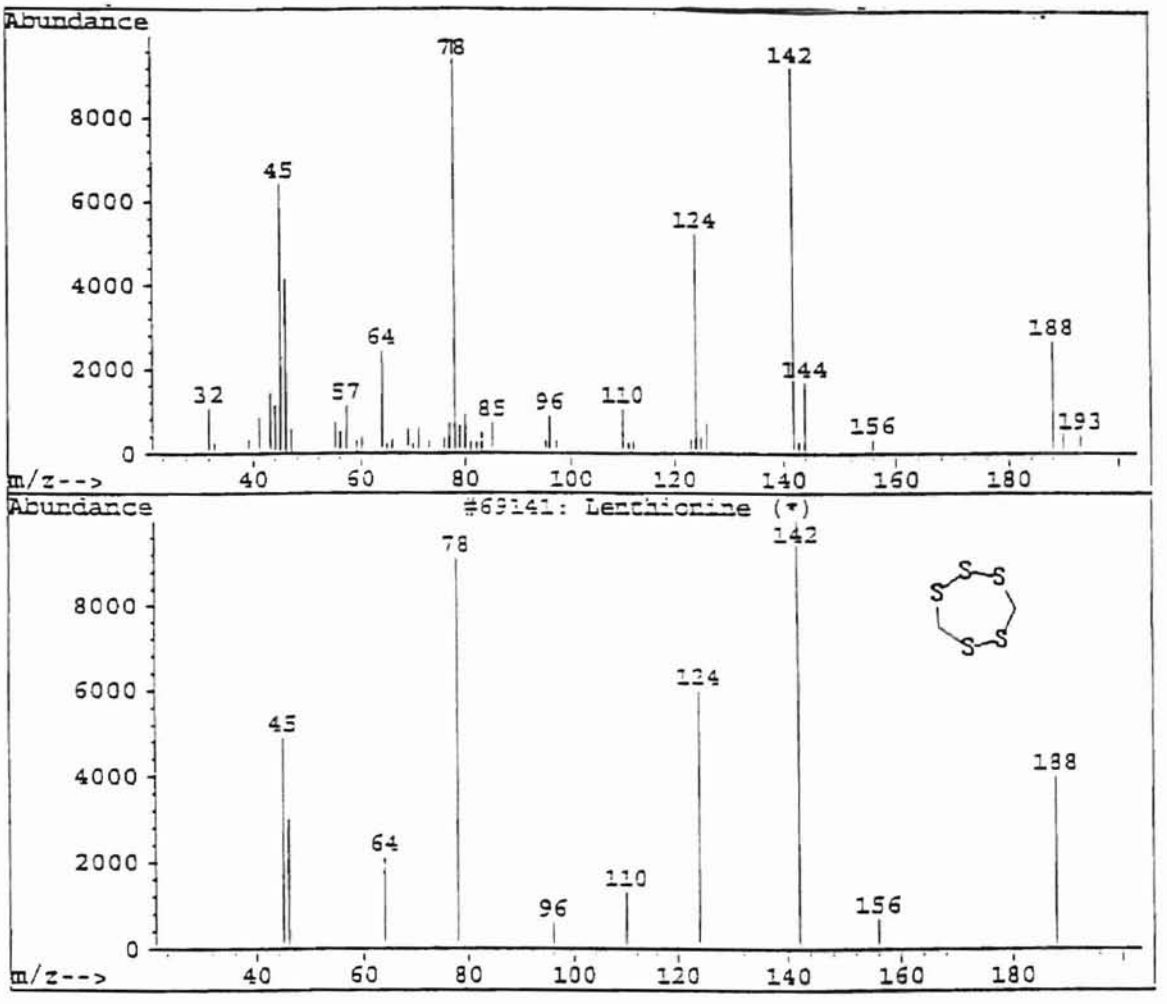
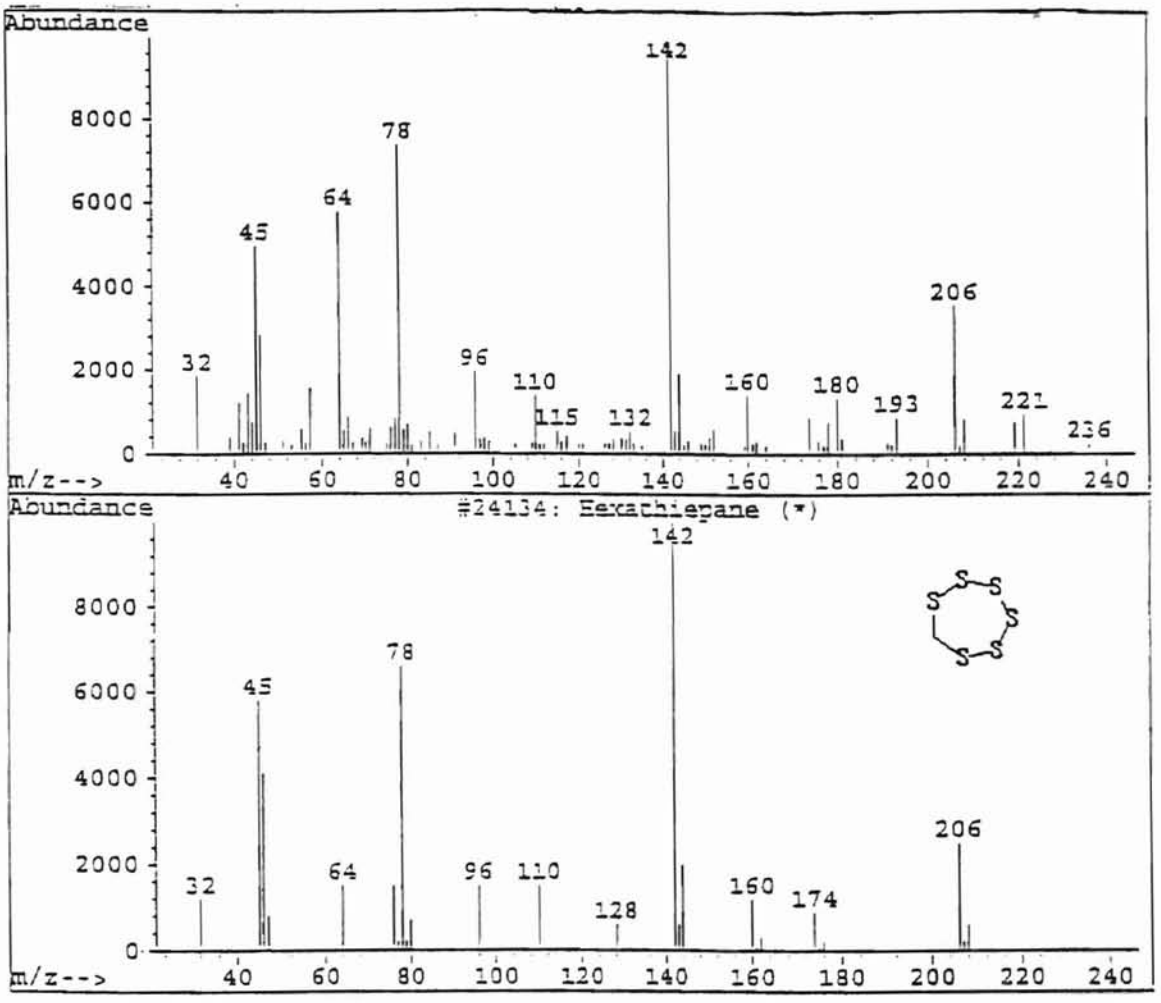


Figure 25. Mass spectra of hexathiepane from LLE sample with reference spectra.



This appears to be a strong indicator that the sulfide is used by the herbicide as a proton donor, but then it is substituted by an intermediate compound leaving the sulfur molecules to combine with other free sulfurs. These sulfur molecules then can bond to form the ring structure. This is supported by the knowledge that sulfur easily bonds to itself.

Why did the LLE extraction method extract two cyclic sulfur compounds while the SPE did not? One possible reason for the appearance of the sulfur compounds could lie in the SPE media, which favors the extraction of compounds having a favorable partition coefficient towards the organic extraction matrix, the C18 material (Markell and Hagen, 1991). It is possible that these sulfur compounds did not efficiently partition out of the water onto the C18 material. Given their cyclic, relatively non-polar structure, this seems unlikely. Thus, it is also a possibility the sulfur compounds have a favorable partition coefficient, which allowed them to be extracted on to the C18 media, but then the compounds were not extracted off the C18 media due to the choice of solvent, ethyl acetate. Reduced sulfur compounds can also be extremely reactive, so it also seems possible the sulfur may have reacted with the solid C18 matrix, rendering it "unelutable".

The number of minor peaks resulting from the LLE extraction were greater than the number resulting from the SPE method, 24 to 10, respectively. This is important since the more compounds that are extracted the better the odds that a compound of interest might be found.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

A qualitative look at degradation products of alachlor and propachlor following exposure to two different reaction media, one abiotic containing bisulfide and the other a biological nitrate-reducing medium, was the major objective of this study. A minor objective of this study was the side-by-side comparison of the Liquid/Liquid Extraction and the Solid Phase Extraction techniques.

The predominant findings of this study are as follows:

* Mass spectral findings from the bisulfide reactors matched newly synthesized reference spectral data (Potter and Carpenter, 1995). Those compounds found include the following: N - (2, 6 - diethyl phenyl) - N - (methoxymethyl)

phenyl) - N - (methoxymethyl) acetamide and 2 - hydroxy - 2',6' - diethyl - N - (methoxymethyl) acetanilide.

* Mass spectral findings of this study also had several "near matches" when compared to the synthesized reference spectral data of Potter and Carpenter (1995). The "near miss" compounds include the following: Bis (N-methoxymethyl)-2,6-diethylaniline and N-(methoxymethyl)-2,6-diethylaniline.

* Liquid/Liquid Extraction is a more conservative method of extraction if there is an uncertainty in the solubilities of the compounds in the matrix. Extracting in both acidic and basic conditions reduces the chances of compounds eluding extraction due to extreme solubility. The Solid Phase Extraction technique may allow compounds of interest to go unextracted due to low partition coefficients.

* The near misses of the spectra produced in the bisulfide samples, when compared to the synthesized reference spectral standards of Potter and Carpenter (1995) indicates that the reactor matrix may have created dynamics which could have produced the different degradation compounds.

* Unknown reactions could have taken place in the Qin (1995) samples from the time of extraction to GC/MS analysis.

* Two cyclic sulfur compounds, lenthionine and hexathiepane, were found and identified only in the LLE samples which had been spiked with a herbicide and exposed to the bisulfide reactor.

* The nitrate reactor produced far fewer compounds that could be considered intermediate degradation compounds.

RECOMMENDATIONS

New concerns and questions have been bought up by this study and further studies are recommended. They include the following:

* A study of the SPE (C-18) media extraction efficiencies of samples that are neutral, acidic and of basic pH, using compounds found in this study as the target compounds. It will be necessary to use quantifiable analytical data to calculate extraction correlation between the pHs.
* Synthesize the "near match" degradation compounds that were found in the study. These could then be used as reference spectra for further study of alachlor degradation in bisulfide and other reactors.
* Run the GC/MS in a Single Ion Mode (SIM) once the major ions of each degradation compound are known or suspected. The act of reducing the number of mass ions that are scanned will increase the detection limits 10 to possibly 100 fold.
* Emulate aquifers which flow through various geological matrix such as bicarbonate, iron or clay soils and document the degradation compounds found after the exposure to the various

geological matrices. These could be tailored to investigate possible herbicide metabolites. Knowledge gained by linking together such limited studies could give new insight to reaction pathways and reaction kinetics.

* Investigate new techniques of increasing the sensitivity of the GC/MS. These could include the Gerstel injection system, which allows up to 1 ml sample injections (Doherty et al., 1996).

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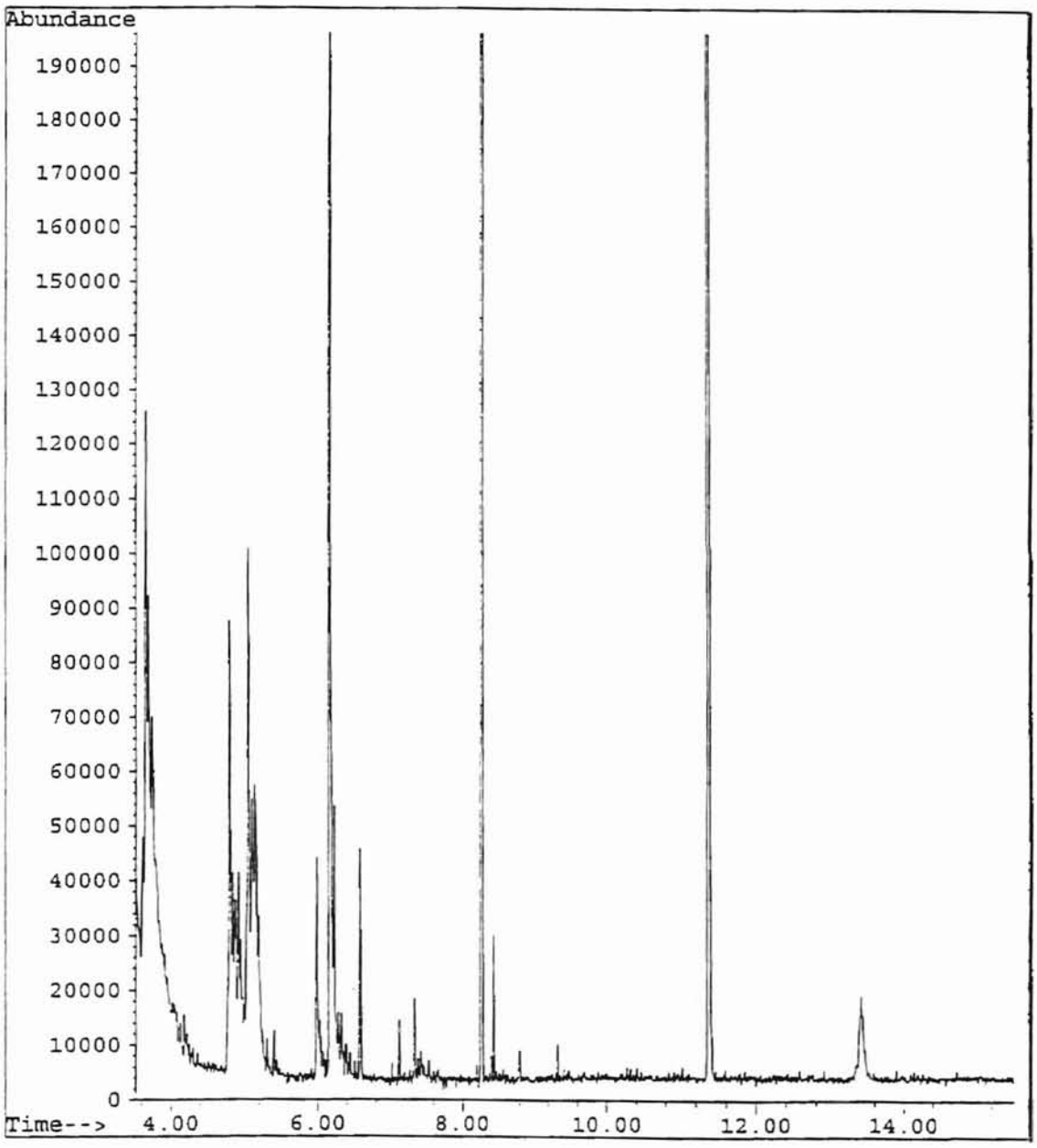
APPENDIX

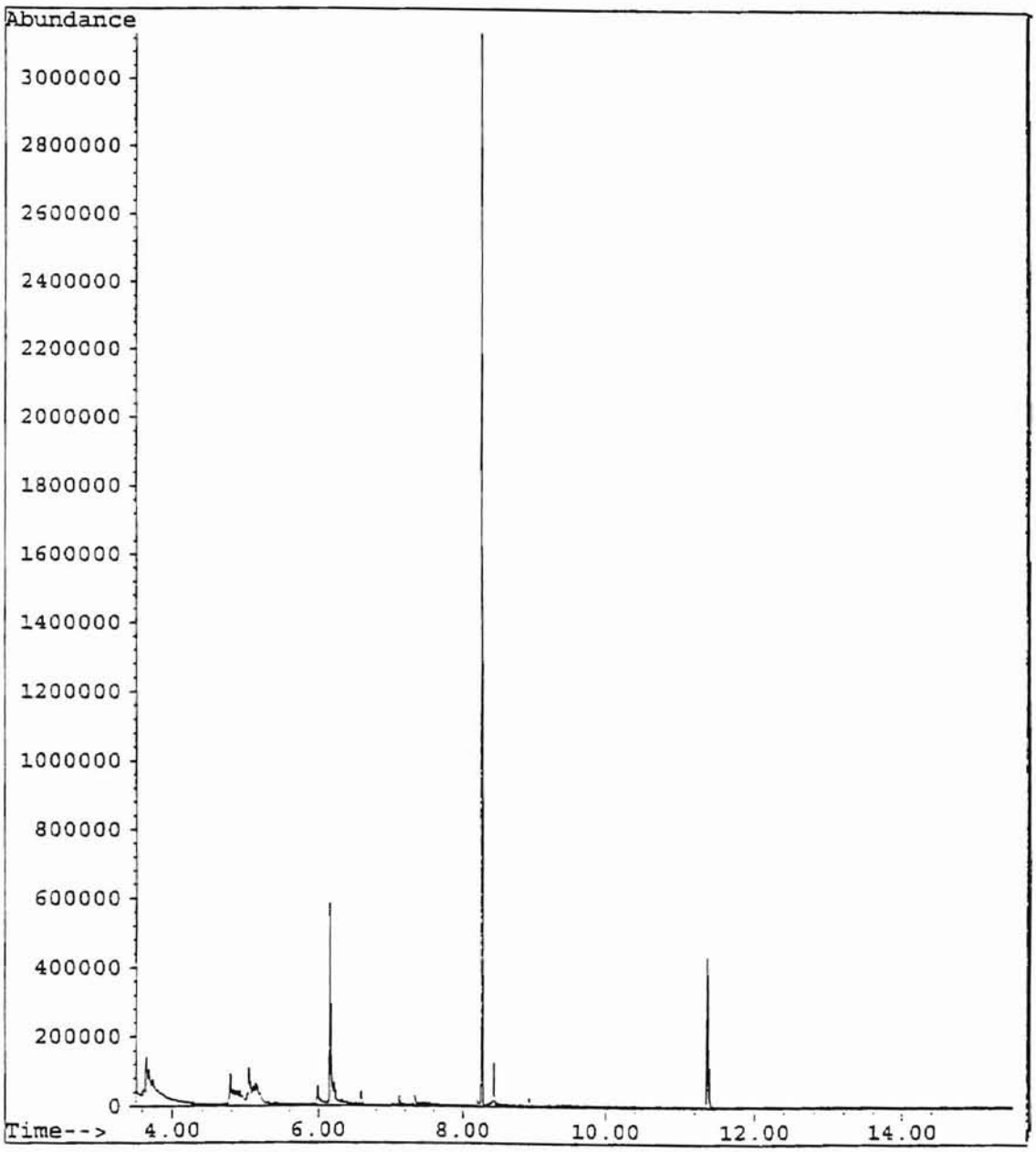
APPENDIX A - Sample B 1.0 Chromatograph

Buffer & Alachlor (8/5/95)



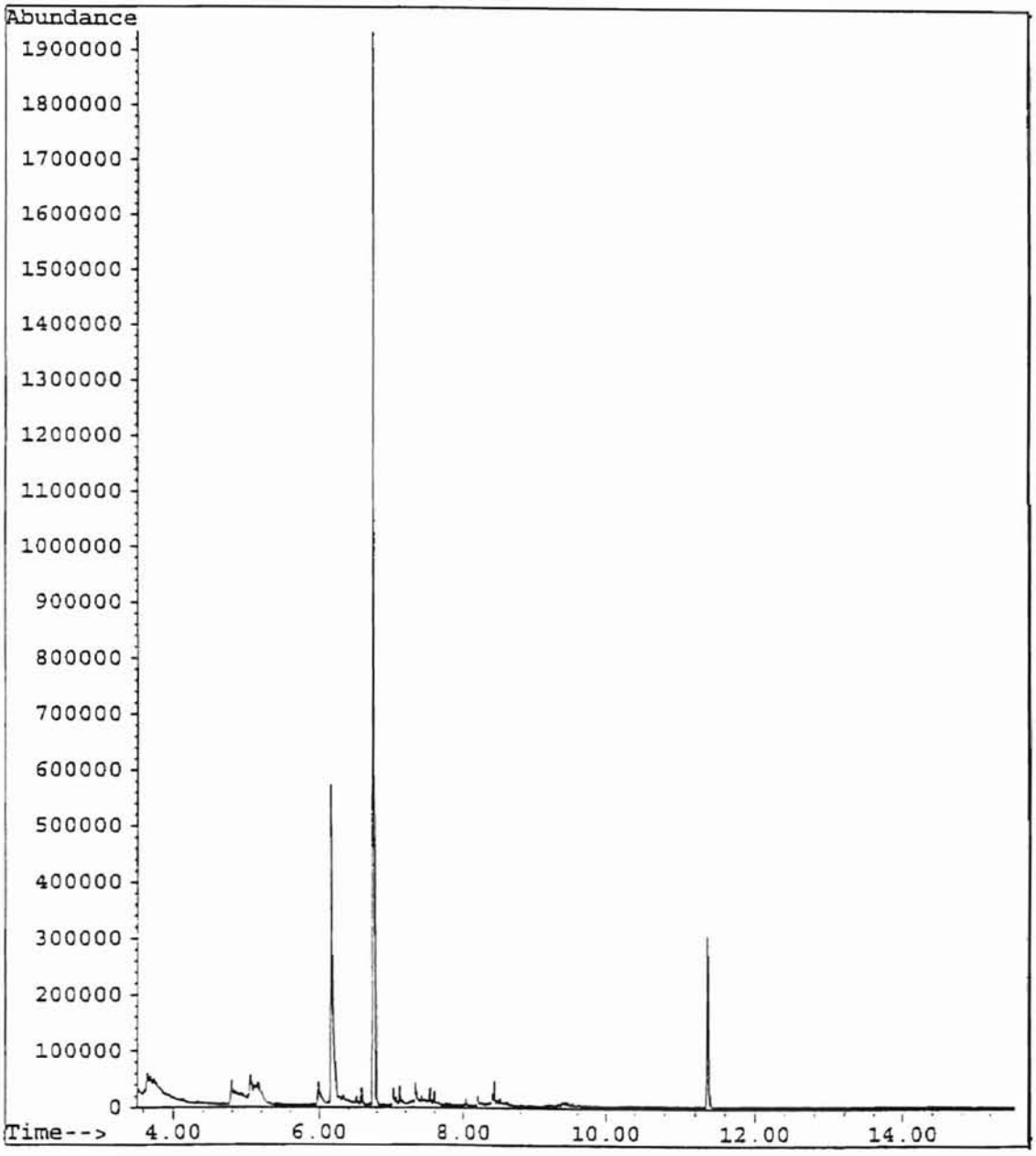
APPENDIX B - Sample B 1.1 Chromatograph

Buffer & Alachlor (8/7/95)



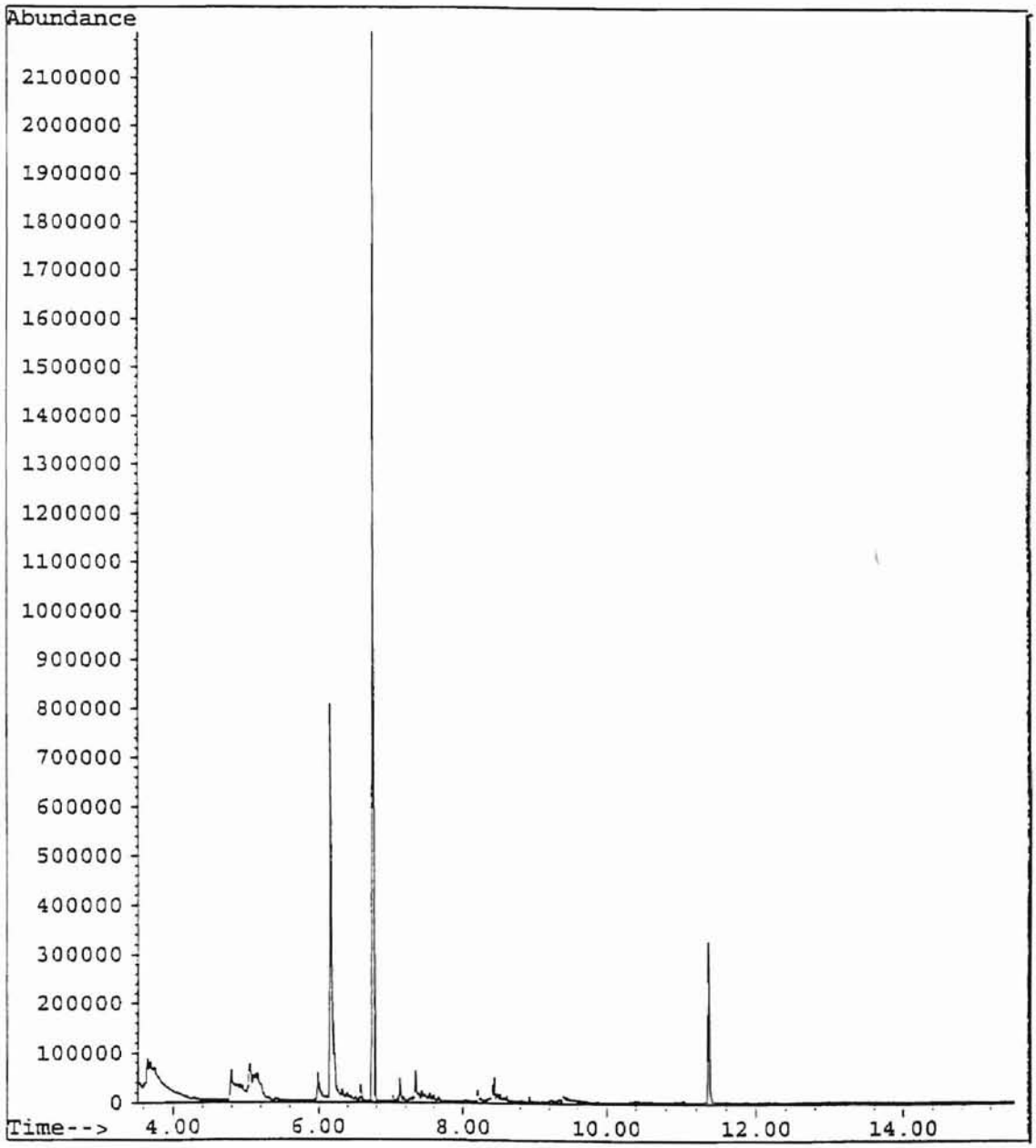
APPENDIX C - Sample B 2.0 Chromatograph

Buffer & Propachlor (8/5/95)



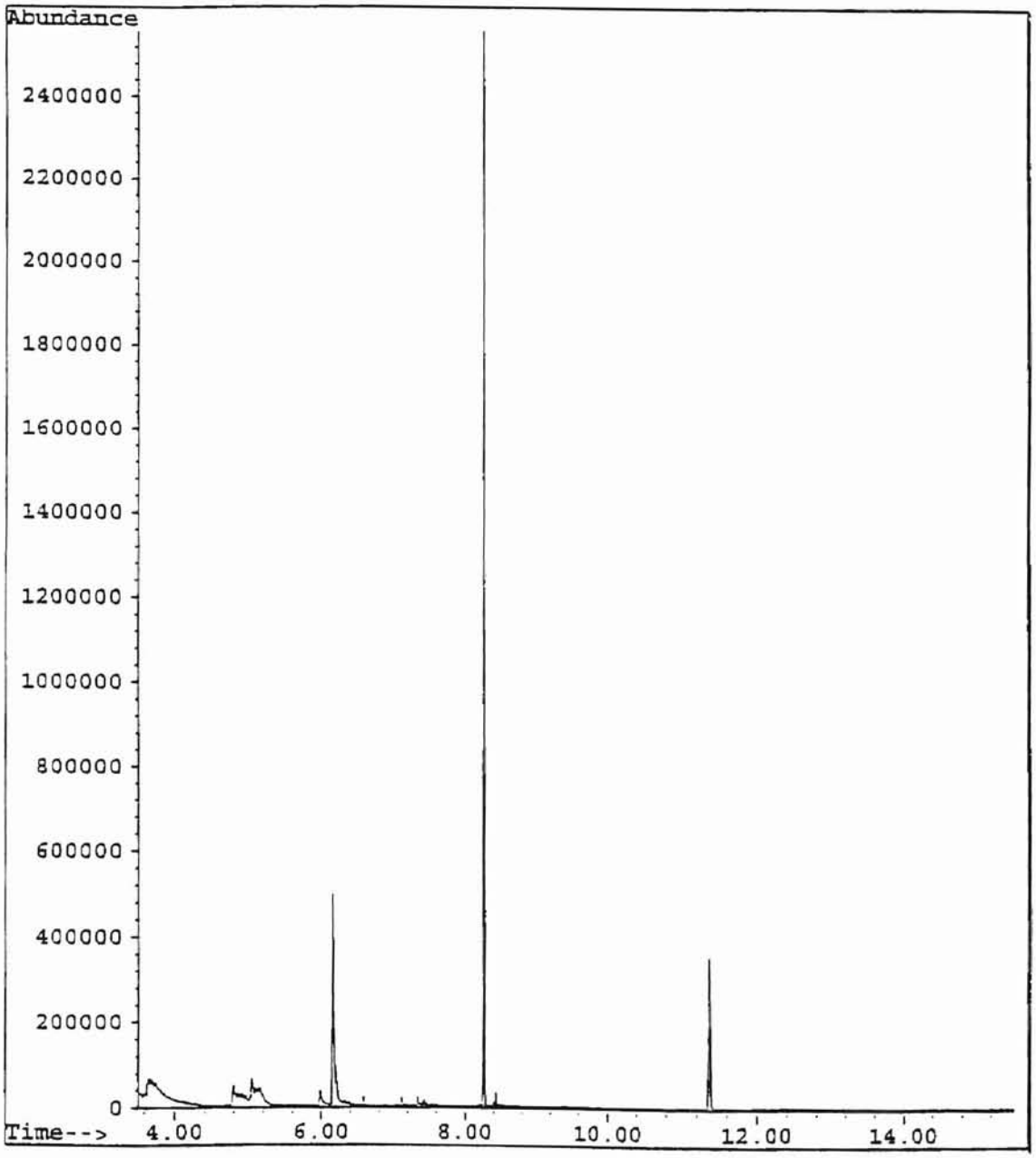
APPENDIX D - Sample B 2.1 Chromatograph

Buffer & Propachlor (8/7/95)



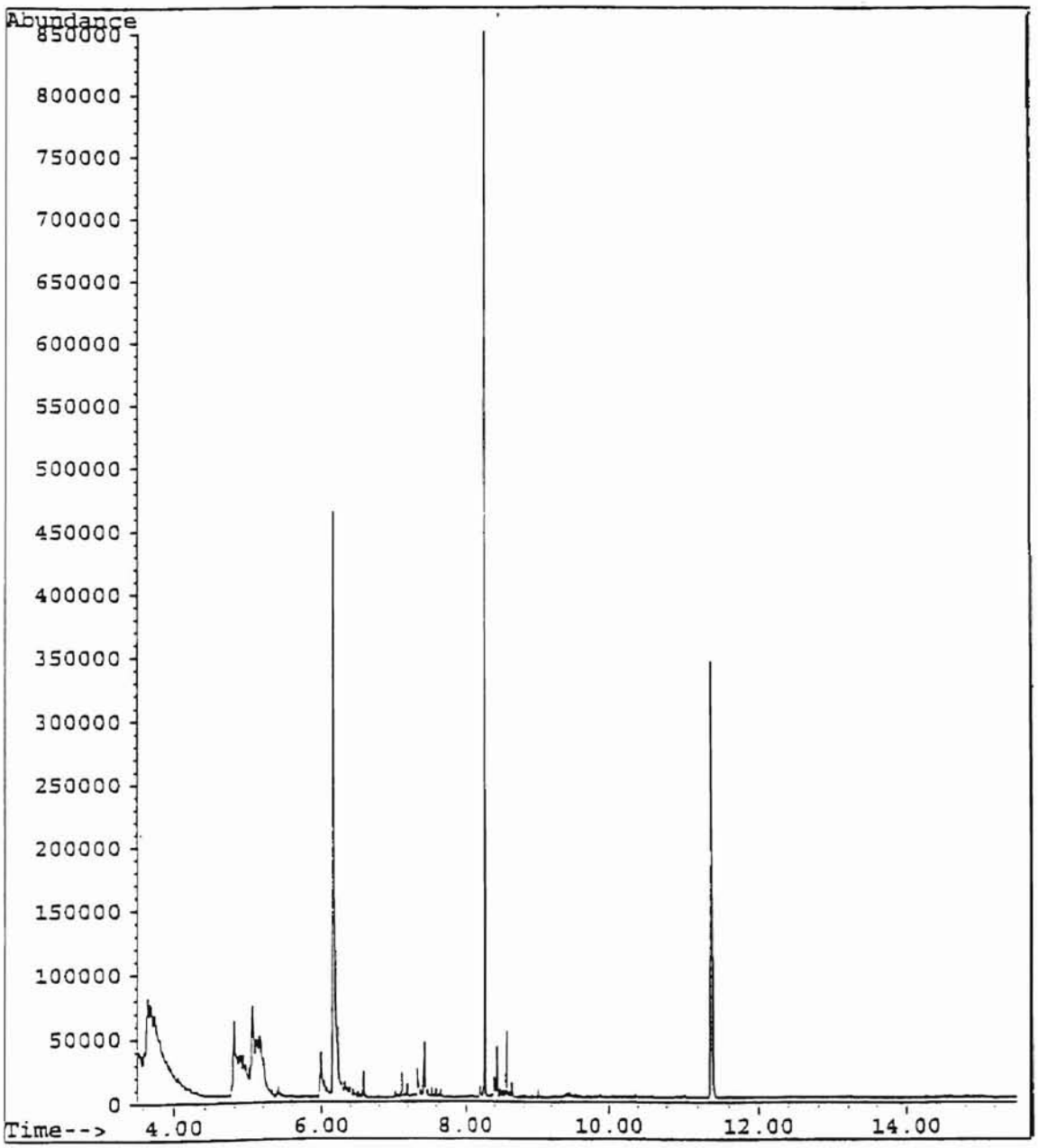
APPENDIX E - Sample B 3.0 Chromatograph

Buffer, Alachlor & Bisulfide (8/5/95)



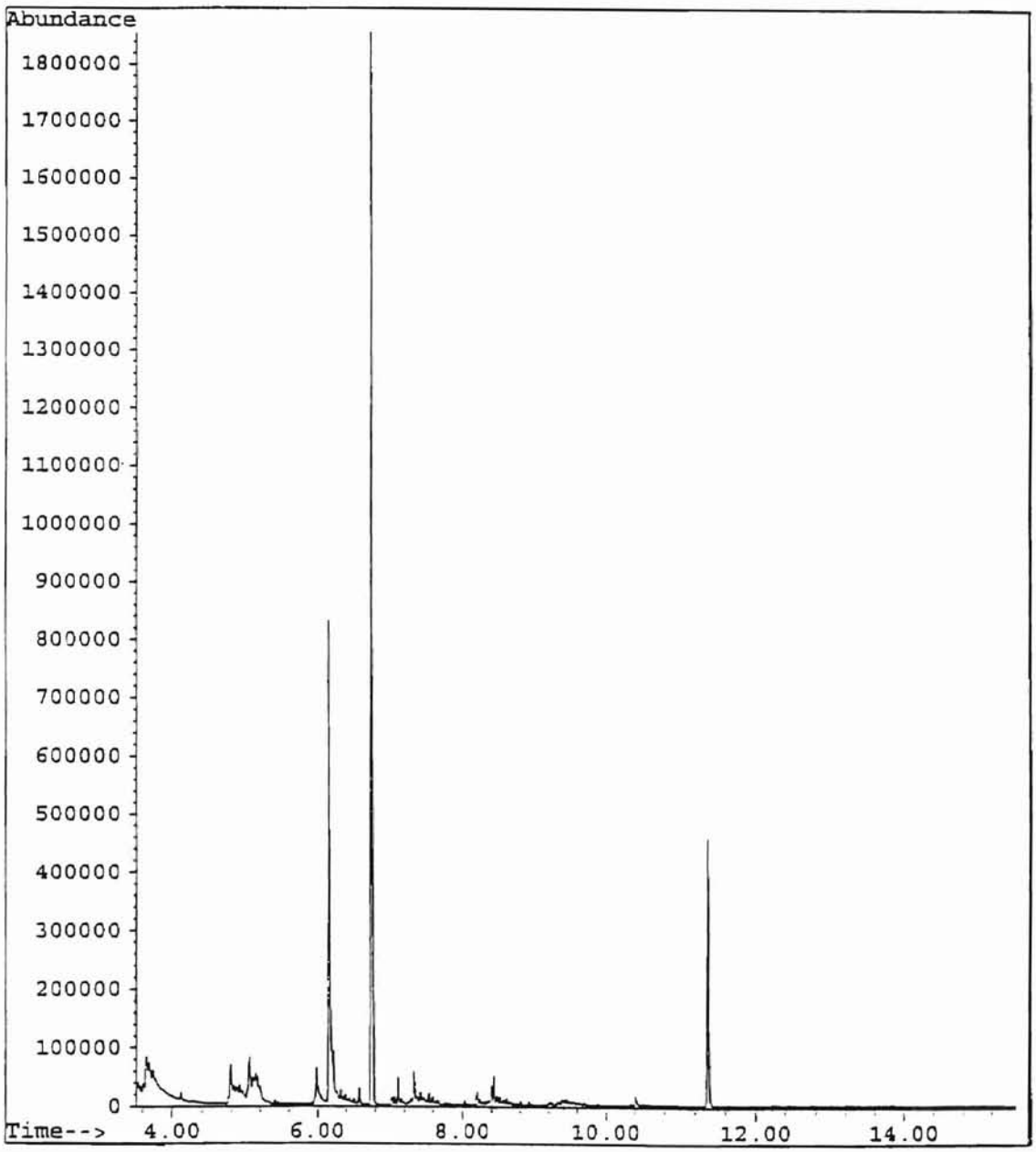
APPENDIX F - Sample B 3.1 Chromatograph

Buffer, Alachlor & Bisulfide (8/7/95)



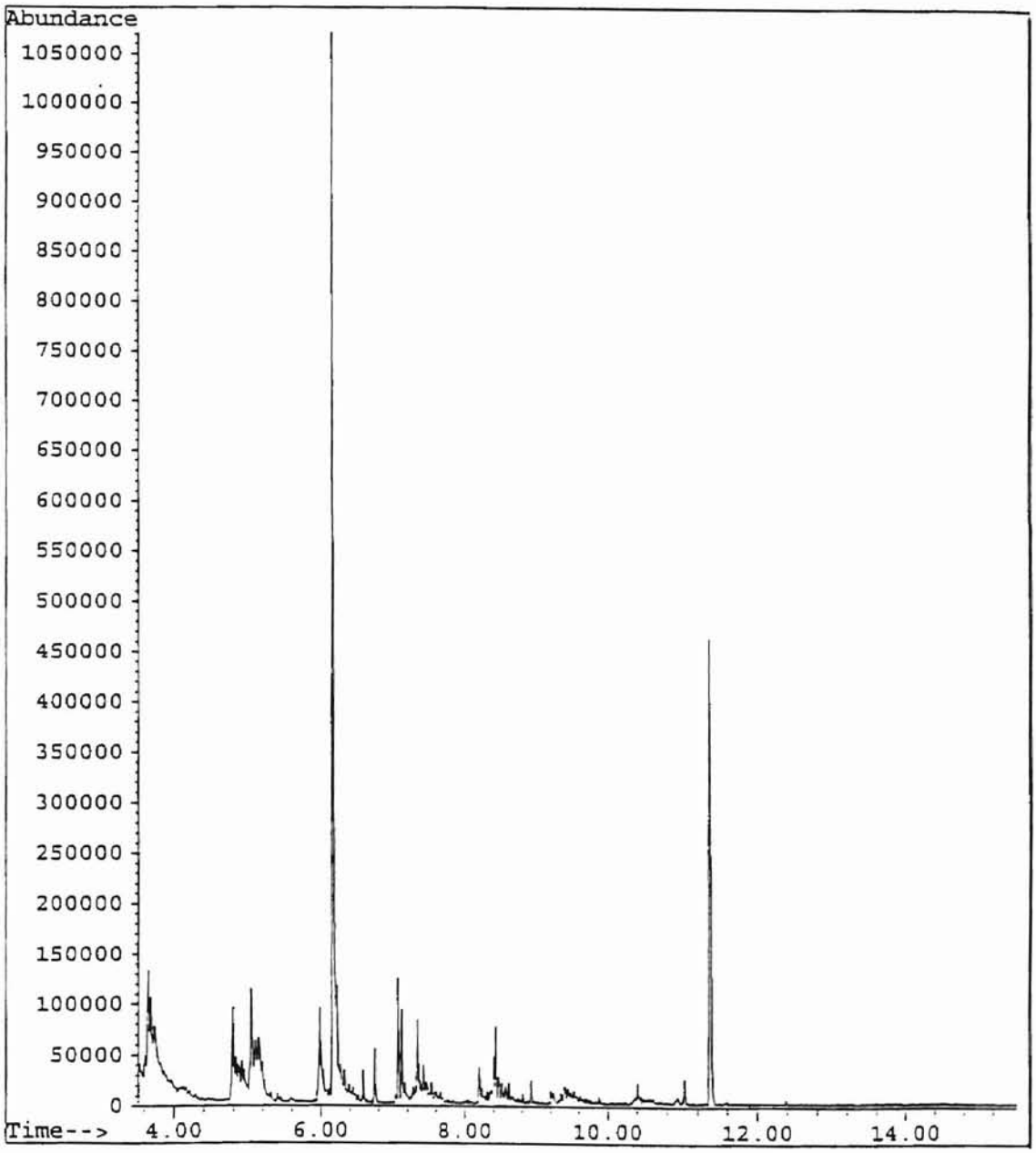
APPENDIX G - Sample B 4.0 Chromatograph

Buffer, Propachlor & Bisulfide (8/5/95)



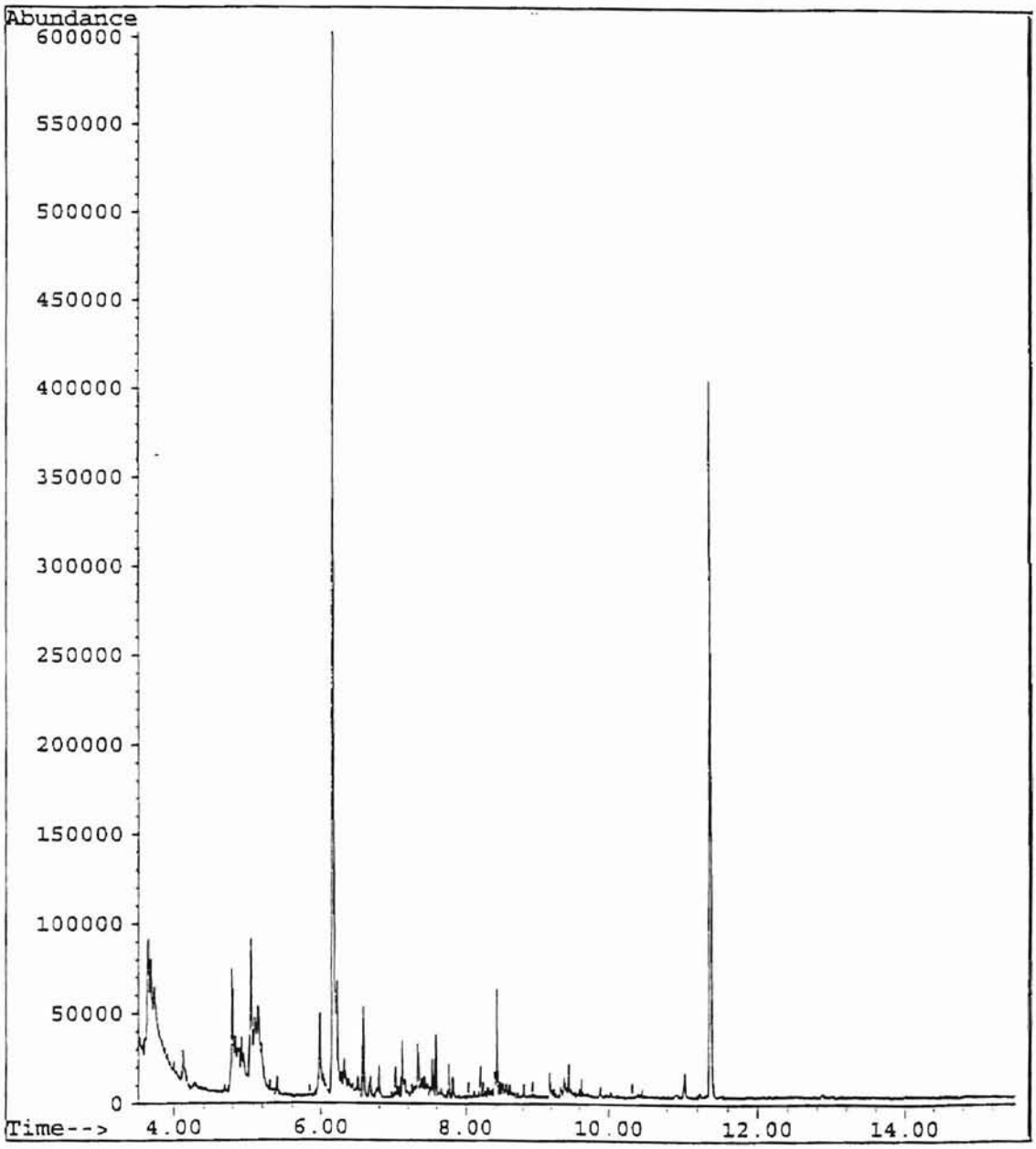
APPENDIX H - Sample B 4.1 Chromatograph

Buffer, Propachlor & Bisulfide (8/7/95)



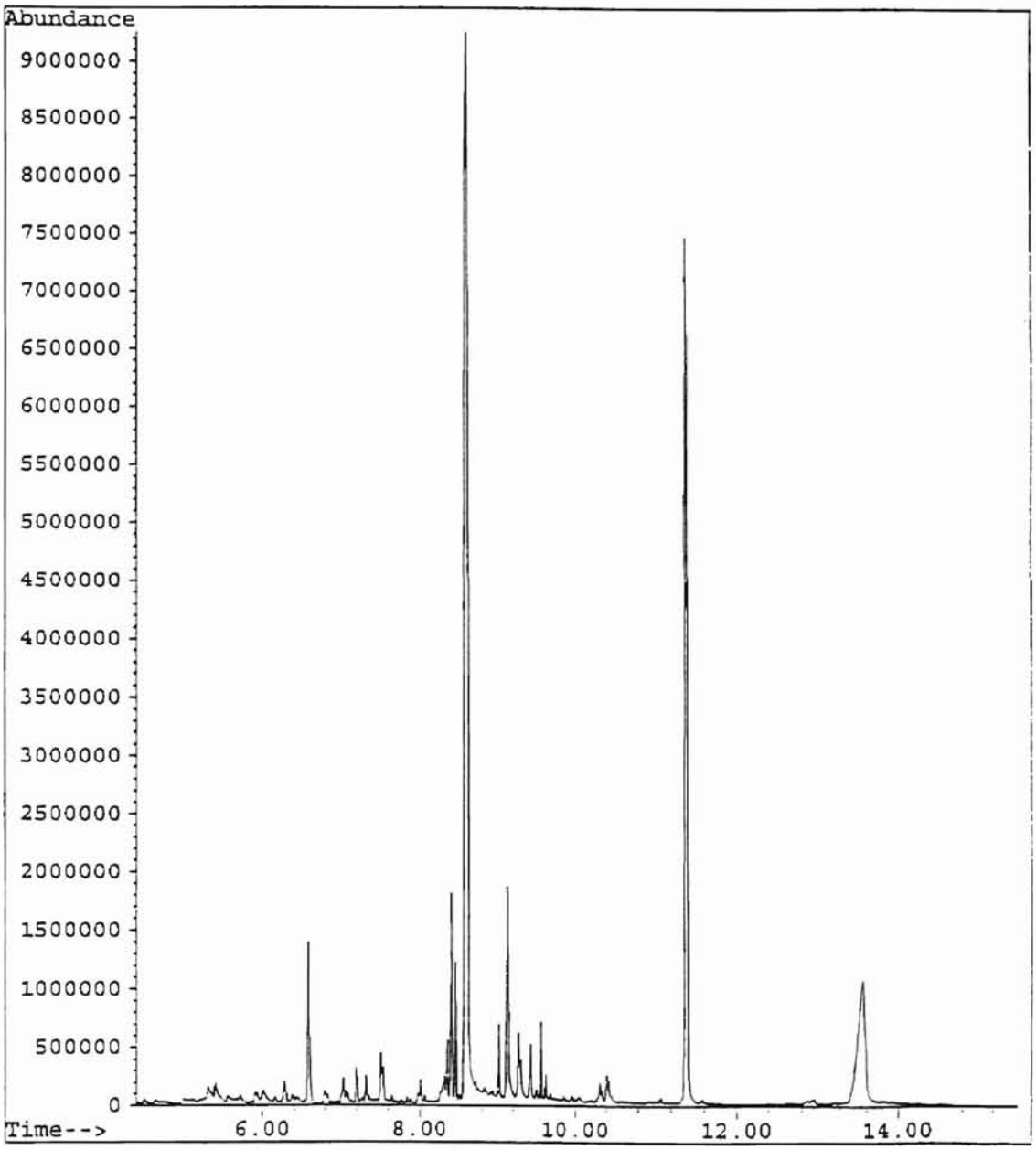
APPENDIX I - Sample B 5.0 Chromatograph

Buffer & Bisulfide



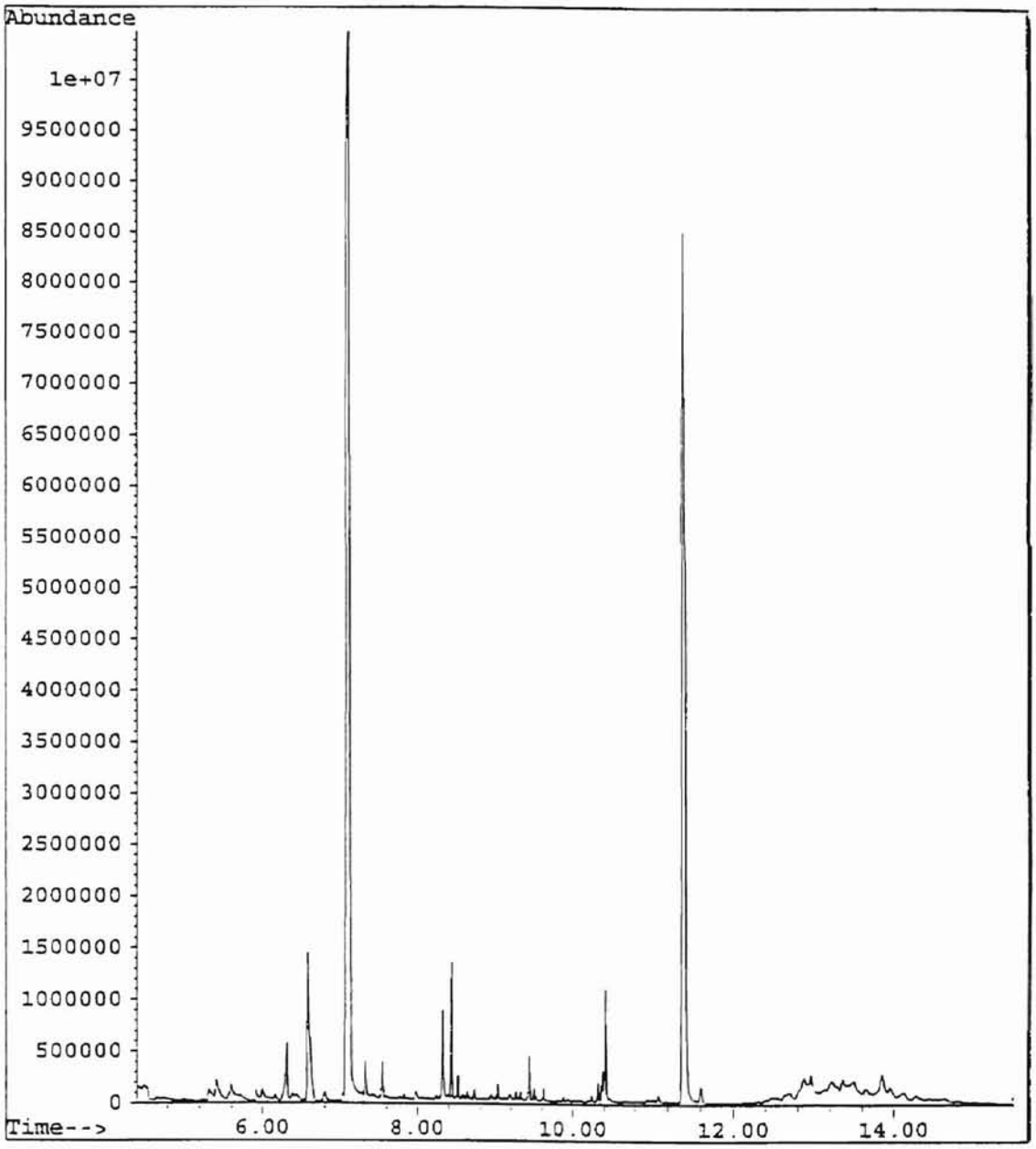
APPENDIX J - Sample B 6.0 Chromatograph

Alachlor LLE



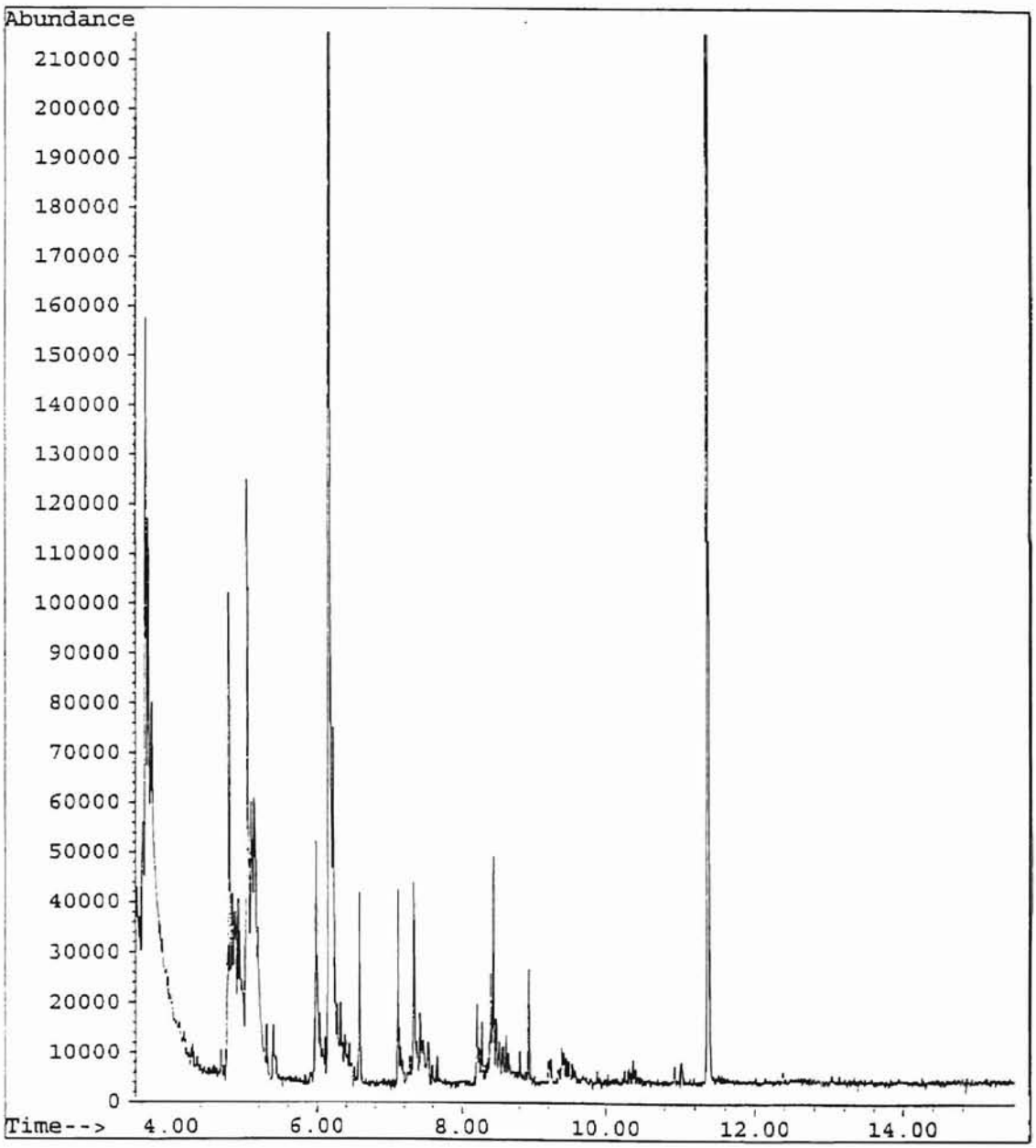
APPENDIX K - Sample B 7.0 Chromatograph

Propachlor LLE



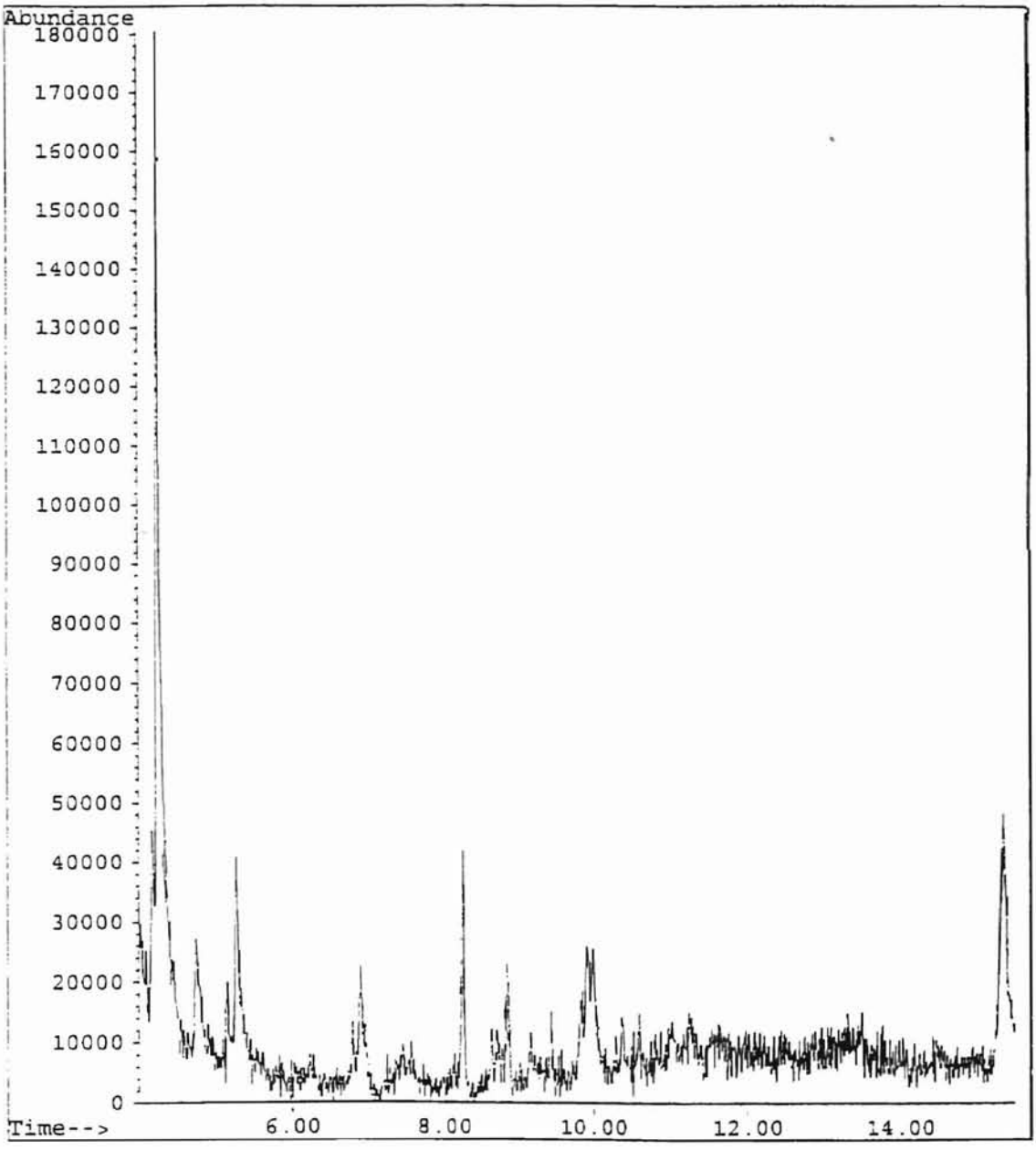
APPENDIX L - Sample B 8.0 Chromatograph

Phosphate Buffer



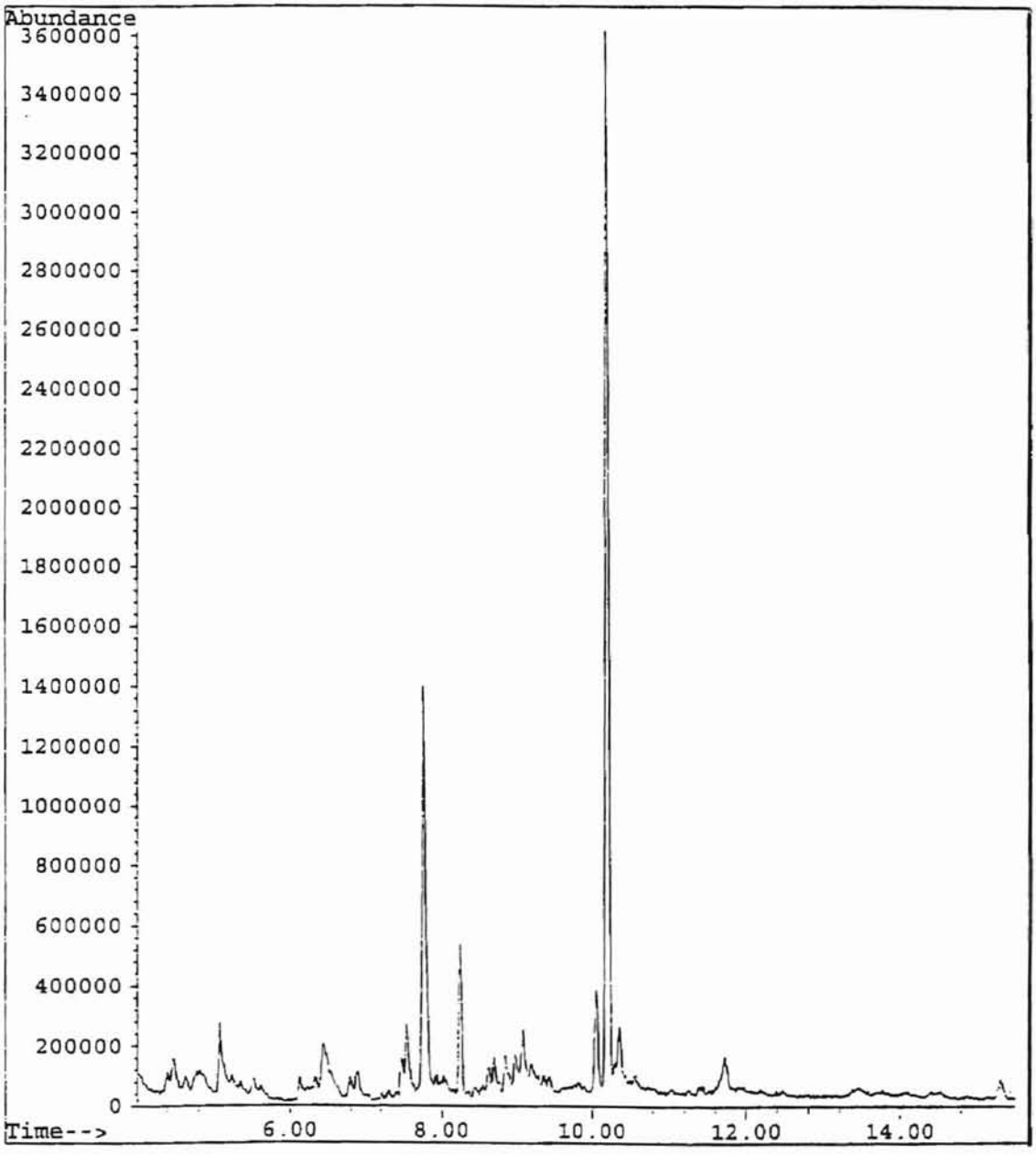
APPENDIX M - Sample N 1.0 Chromatograph

Ehtyl Acetate Blank



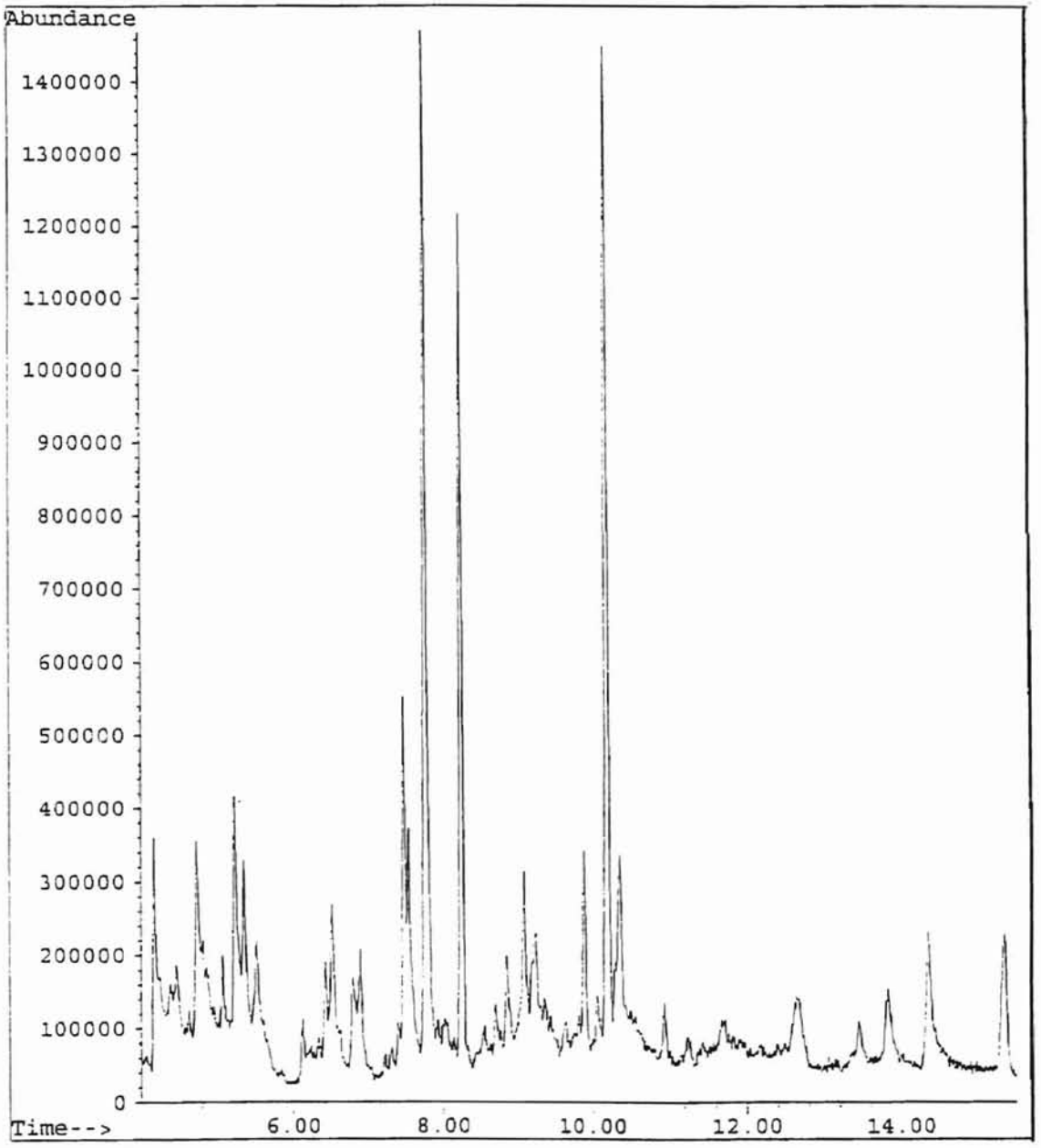
APPENDIX N - Sample N 2.0 Chromatograph

Alachlor (0 hrs)



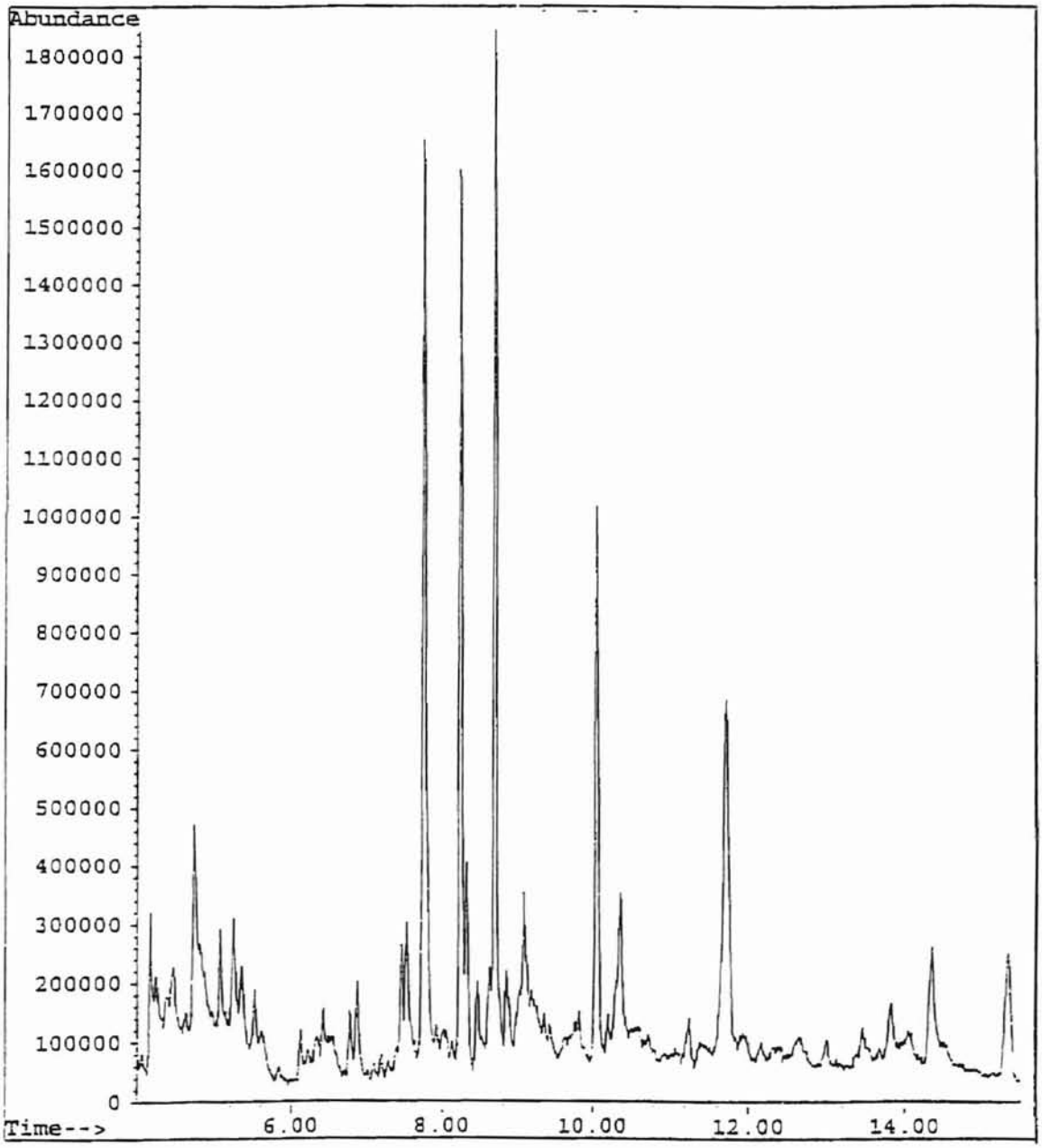
APPENDIX O - Sample N 3.0 Chromatograph

Alachlor (370hrs)



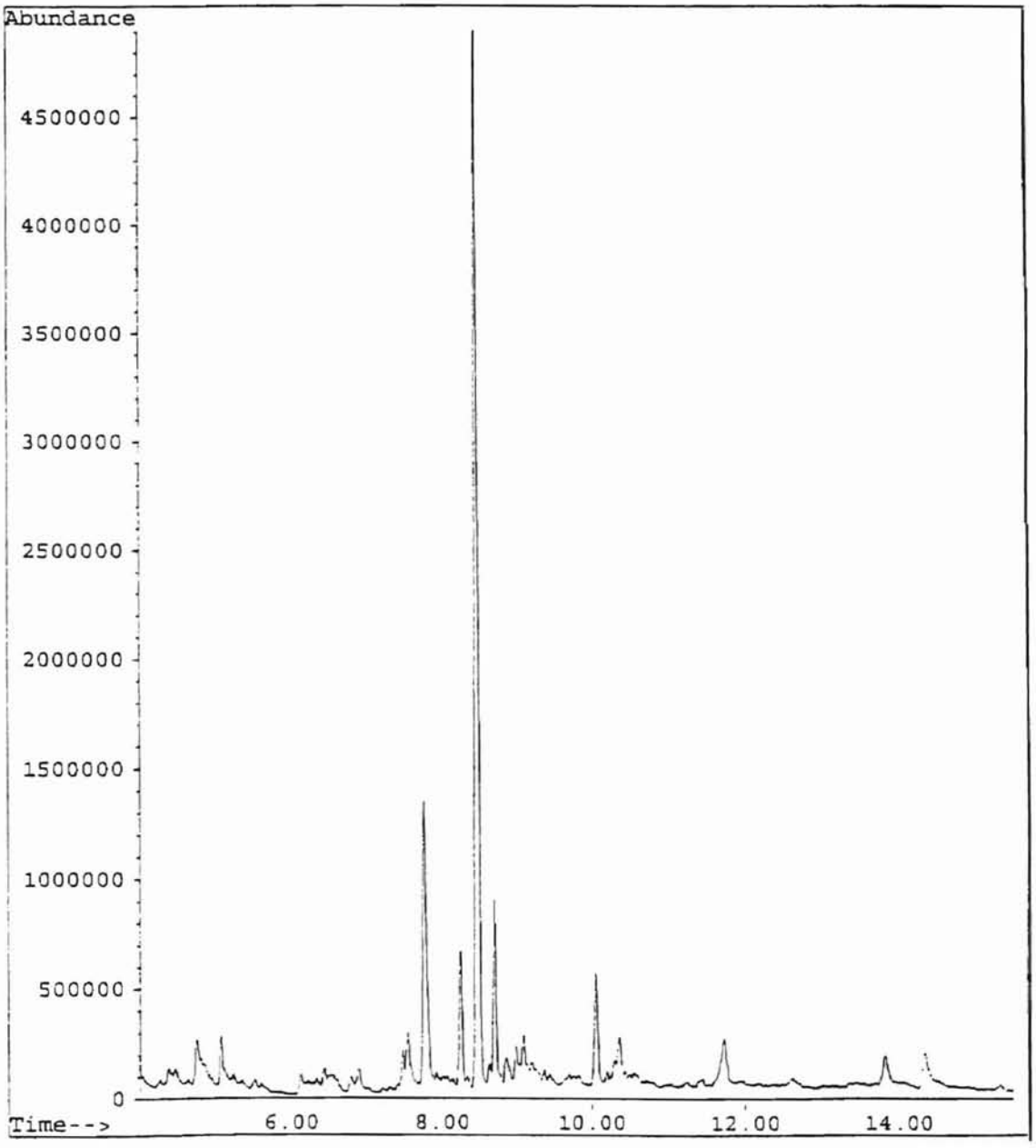
APPENDIX P - Sample N 4.0 Chromatograph

Propachlor (0 hrs)



APPENDIX Q - Sample N 5.0 Chromatograph

Propachlor (217hrs)



VITA

Harold Kenneth Crawford

Candidate for the Degree of

Master of Science

Thesis: **IDENTIFICATION OF ALACHLOR AND PROPACHLOR DEGRADATION PRODUCTS BY GC/MS ANALYSIS**

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Professional Memberships: American Chemical Society, Oklahoma Society of Environmental Professionals.