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Measurement and Chemical Characterization of Vapor-Phase Mutagens in Diesel Exhaust

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Dennis P.H. Hsieh, Norman Y. Kado, Robert Okamoto, Paul A. Kuzmicky, Christine Rathbun and Jerry Ito

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Department of Environmental Toxicology
University of California
Davis, CA 95616

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13. ABSTRACT *(Maximum 200 Words)*

Vapor-phase mutagens are volatile and semi-volatile mutagenic compounds present in numerous emission sources and in ambient air. In this research effort, the undiluted exhaust of a medium heavy-duty diesel truck served as a model comples emission source to test the integration of methods for the collection, extraction, fractionation, bioassay, and chemical analysis of vapor-phase compounds. Samples were collected using adsorbents and were extracted using supercritical carbon dioxide (SFE-CO_2). SFE-CO_2 fractions were directly tested using a microsuspension modification of the Ames ***Salmonella*** mutagenicity assay and chemically analyzed using gas chromatograph/mass spectrometer (GC-MS). The total yield of vapor-phase mutagenic activity collected from the undiluted diesel exhaust was comparable in magnitude to that of the particle-associated mutagenic activity. some of the compound classes found through GC-MS analysis of extracts included: long chain alkanes, alkyl benzenes, naphthalenes, alkyl naphthalenes, phenanthrenes, alkyl phenanthrenes, and other aromatics.

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MEASUREMENT AND CHEMICAL CHARACTERIZATION OF VAPOR-PHASE MUTAGENS IN DIESEL EXHAUST

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Prepared for:

California Air Resources Board
Research Division
2020 L Street
Sacramento, California 95814

Submitted by:

Department of Environmental Toxicology
University of California
Davis, California 95616

Prepared by:

Principal Investigator
Dennis P.H. Hsieh

Co-Investigators
Norman Y. Kado
Robert Okamoto

Research Staff
Paul A. Kuzmicky
Christine Rathbun
and
Jerry Ito

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ABSTRACT

Vapor-phase mutagens (VM) are volatile and semi-volatile mutagenic compounds present in numerous emission sources and in ambient air. The VM are potentially a major class of toxic air contaminants that require special experimental approaches for sampling, recovering and mutagenicity testing due to their volatility. In this research effort, methods for the collection, extraction, fractionation, and concentration of vapor-phase compounds from the undiluted exhaust of a medium heavy-duty diesel truck were developed and integrated with chemical analysis and a sensitive mutagenicity bioassay. A sampling system composed of a Teflon filter, polyurethane foam (PUF) and XAD-4 (XAD) adsorbents, in series, was used to directly trap PM and VM in the diesel truck exhaust. The VM on the PUF and XAD adsorbents were extracted with supercritical carbon dioxide (s-CO_2) into concentrated methanol fractions which were directly analyzed by a specially modified Ames *Salmonella* mutagenicity assay and a gas chromatograph interfaced to a quadrupole mass spectrometer (GC-MS). The PM-associated mutagens were extracted with organic solvents and assayed for mutagenicity. A significant finding was that the mutagenic activity of the VM collected from the undiluted diesel exhaust was comparable in magnitude to that of the PM-associated mutagens and therefore constitutes a substantial portion of the total mutagenicity present in the sampled diesel exhaust. GC-MS analysis of the s-CO_2 fractions from XAD indicated the presence of long chain alkanes, alkyl benzenes, alkyl naphthalenes, unsaturated naphthalenes, and other aromatics. The s-CO_2 fractions from the PUF samples with high mutagenic activity were sub-fractionated using high performance liquid chromatography (HPLC) to facilitate identification of chemical components. The sub-fractions were concentrated by gentle nitrogen blowdown and were analyzed by GC-MS. Compounds found were naphthalene, alkyl naphthalenes, phenanthrene, alkyl phenanthrenes, alkyl substituted aromatics, and long chain alkanes. The analytical procedures developed in this investigation could be optimized to achieve mutagenicity-directed chemical analysis for VM in air.

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ABBREVIATIONS, ACRONYMS and GLOSSARY

BaP	Benzo(a)pyrene
CARB	California Air Resources Board
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
DS	Diesel Study
FID	Flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
HR	Hour
IARC	International Agency for Research on Cancer
LPM	Liters per minute
MSD	Mass selective detection
ML	Milliliter
MIN	Minute
PAH	Polycyclic aromatic hydrocarbons
PBS	Phosphate buffered saline
PCB	Polychlorinated biphenyl
PM	Particulate matter
PSI	Pounds per square inch
PUF	Polyurethane foam
Revertants	The number of mutant colonies detected. Technically, the number of histidine-independent bacterial colonies present that have mutated from histidine dependent growth.
s-CO_2	Supercritical carbon dioxide
SFE	Supercritical fluid extraction
S9	9.000 x g rat liver supernatant
SOF	Soluble organic fraction

TA98	Bacterial tester strain
TA100	Bacterial tester strain
TIC	Total ion chromatogram
Vapor	The gas-phase of substances that are normally in the liquid or solid phase.
VM	Vapor-phase mutagen
VOC	Volatile organic compound
XAD	Macroreticular polystyrene. divinylbenzene copolymer adsorbent resin #4 (XAD-4)

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I. PROJECT SUMMARY

A. Introduction

Vapor-phase mutagens (VM) are those volatile and semi-volatile mutagenic compounds present in numerous emission sources and in ambient air. The VM are potentially a significant class of toxic air contaminants. Volatile compounds exert a relatively high vapor pressure at room temperature (for example, dichloromethane = 440 torr at 25°C) and non-volatile compounds exert low vapor pressures (for example, pyrene = 6.85×10^{-7} torr at 20°C). As the boiling point of a compound decreases, its vapor pressure increases. As the vapor pressure increases, there is a greater tendency for a compound to evaporate, or volatilize. The semi-volatile compounds have vapor pressures and boiling points between those of volatile and non-volatile compounds. Due to their volatility, VM require special experimental approaches for sampling, extracting and mutagenicity testing in order to minimize sample loss. In previous work, we reported on an approach to integrate four procedures for VM: 1) collection on adsorbents, 2) supercritical fluid extraction (SFE), 3) *Salmonella* microsuspension mutagenicity assay, and 4) chemical analysis to measure different classes of model VM (Hsieh *et al.*, 1990; Wong *et al.*, 1991; Kado *et al.*, 1992). Preliminary experiments were also carried out to investigate the presence of VM in diesel engine emissions. The integrated analytical procedure proved to be feasible in the quantitative handling of model VM and there appeared to be significant mutagenic activity in the vapor-phase of diesel exhaust.

Chemical characterization of the mutagenic compounds present in complex environmental mixtures is possible following a mutagenicity-directed chemical analysis procedure (Lewtas, 1988). Briefly, the procedure involves fractionating a complex mixture and determining the mutagenic activity of the various fractions. The mutagenic fractions can be further fractionated and the bioassay used again to monitor the mutagenic activity. As a result, mutagenic compounds can be isolated and identified.

The objective of the current project was to further develop the collection, extraction, bioassay and chemical analysis procedures for the identification and quantitation of VM occurring in the complex mixture of diesel engine emissions.

B. Overview of Methods

1. Sampling and Extraction

The first part of this project required the assembly of a sampling system using commercially available adsorbents. A primary goal was to make the sample collection under "real-world" conditions by trapping VM from diesel truck exhaust, a direct emission source. Our collection train consisted of a Teflon filter to trap PM, followed by pre-cleaned polyurethane foam (PUF) and macroreticular cross-linked polystyrene divinylbenzene coploymer adsorbent resin #4 (XAD), in series, to trap semi-volatile and volatile compounds, respectively. For our studies, we sampled the undiluted exhaust from a medium heavy-duty diesel truck stationed at the University of California at Davis. The truck had a gross vehicular weight of 18,000 lbs equipped with a 7.6 L 6-cylinder diesel engine and was fueled with number 2 diesel fuel.

The VM that were collected on the PUF and XAD adsorbents at a nominal flow rate of 2 liter per minute (LPM) from a pre-warmed engine running at slow idle were extracted using a supercritical fluid extraction (SFE) technique. The PUF and XAD samples were further fractionated by increasing the pressure of the supercritical carbon dioxide (s-CO_2) and each fraction was analyzed directly using a *Salmonella*/microsuspension mutagenicity bioassay and a gas chromatograph interfaced to a quadrupole mass spectrometer (GC-MS). Organics on the filters were extracted with organic solvents and tested in the bioassay.

2. Mutagenicity Bioassay

The most widely used and validated test for airborne mutagenic compounds is the *Salmonella*/microsome test, or the Ames test (Ames *et al.*, 1975). A simple modification of this test known as the microsuspension assay was previously established by Kado *et al.*, (1983; 1986) and is at least 10 times more sensitive than the standard Ames test, based on absolute amounts of material required per sample. The microsuspension assay was optimized for the present studies (Hsieh *et al.*, 1990; Kado *et al.* 1992).

3. Chemical Analysis

Two separate GC-MS temperature programs for the PUF or XAD samples were developed to analyze each SFE fraction. The SFE fractions were extremely complex as seen in the GC-MS total ion chromatograms. To resolve the complex mixture further, PUF samples were subjected to further fractionation. The two most mutagenic PUF SFE fractions (fractions 2 and 3) were pooled and sub-fractionated by high performance liquid chromatography (HPLC). The PUF samples were chosen over the XAD samples for further fractionation because higher boiling point compounds are known to be trapped in the PUF. Each of the HPLC sub-fractions was analyzed by GC-MS to tentatively identify the semi-volatile compounds trapped by PUF. The most mutagenic XAD SFE fractions were analyzed directly by GC-MS.

C. Results and Conclusions

The major findings of this investigation are summarized as follows:

1. Sufficient quantities of PM and vapor-phase compounds were trapped for laboratory analyses by a sampling train consisting of a Teflon filter, PUF, and XAD adsorbents, placed in series. The results indicate the feasibility and usefulness of this sampling device for this research.

2. The trapped vapor-phase compounds were extracted, concentrated, and fractionated (with increasing pressure) into six small volumes of methanol using s-CO_2 under conditions optimized in this study. For the PUF SFE fractions, most of the compounds as determined by GC-MS were detected in fractions 2 and 3. For the XAD samples, the majority of chemicals were detected in fractions 3 and 4.

3. Significant mutagenicity was detected in most of the s-CO_2 extracts of the PUF and XAD samples. High mutagenicity was detected in fractions where the concentration of compounds was highest as shown by GC-MS analysis. There was good agreement between mutagenic activity and compound abundance in s-CO_2 extracts of both the PUF and XAD samples.

4. GC-MS analysis indicated that the SFE fractions from the XAD samples contained long chain alkanes, alkyl benzenes, alkyl naphthalenes unsaturated naphthalenes, and other aromatics. The SFE fractions from the PUF samples were too chemically complex for direct identification of major components. The two most mutagenic PUF SFE fractions were further fractionated by HPLC using a water/acetonitrile gradient program. GC-MS analyses of the PUF HPLC fractions resulted in the tentative identification of a number of organic compounds including naphthalene, alkyl naphthalenes, phenanthrene, alkyl phenanthrenes, alkyl substituted aromatics, and alkanes.

5. The s-CO_2 extracts of PUF and XAD were mutagenic using bacterial tester strains TA98 and TA100 (with and without metabolic activation). Bacterial tester strain TA100 was more sensitive to the PUF and XAD extracts than strain TA98. This difference in tester strain sensitivity was not observed with the filter extracts. The results suggest that the chemical composition of VM is different from that of PM-associated mutagens in the diesel exhaust.

6. The vapor-phase associated mutagenic activity in strain TA100 was approximately equal to the activity associated with the PM. This result indicates that the mutagenicity of VM represents a substantial portion (up to 50%) of the total mutagenicity sampled from the diesel exhaust.

VM in complex environmental mixtures have been previously difficult to analyze due to losses during sample handling and testing. Our work demonstrates the feasibility of an integrated mutagenicity-directed chemical analysis method for routine identification and measurement of this class of airborne mutagens. Using this analytical method, we have shown that VM are likely an important component of vehicular diesel exhaust and as such warrant further investigation.

II. INTRODUCTION

A. Statement of Problem

Vapor-phase mutagens (VM) consist of both volatile and semi-volatile compounds and are potentially a major class of toxic substances present in the ambient air and in vehicular emissions. These compounds can be gases or associated with particulate matter (PM) but are volatilized. This class of compounds are diverse and include halogenated hydrocarbons, aldehydes, and polycyclic aromatic hydrocarbons (PAH), many of which are carcinogenic to animals. It is conceivable that other as yet unidentified VM are present in ambient air, as well as in stationary and mobile source emissions.

Unlike mutagens associated with PM, VM are not routinely monitored due to a lack of methodology that integrates vapor trapping and extraction with bioassay and chemical analysis. Working with VM requires a different approach to trapping, extracting, and analysis compared to methods used for PM. In a previous study of VM by Hsieh *et al.*, (1990), and supported by the California Air Resources Board (CARB), methods were developed that integrated the trapping and extraction of vapor-phase compounds with a sensitive mutagenicity assay and chemical analysis.. In the present study, these methods were applied to the detection and characterization of VM in the exhaust of a diesel truck serving as a model mobile source emission. Dorie *et al.* (1987) reported on the collection of particulate and gaseous samples in a stainless steel dilution tunnel (15.2 m^3/min with a dilution ratio of 15:1). Mutagenic activity of the exhaust was examined with and without the use of ceramic particulate traps. The organic solvent extracts from particles and from volatile organic compounds (VOC) were tested for mutagenic activity using tester strain TA98 (with and without metabolic activation). The solvent extracts with and without metabolic activation appeared to have similar activity based on revertants/m^3. The mutagenic activity of the VOC extract was considerably less than the solvent extracts.

B. Goals and Objectives

The objective of this project was to further validate integrated methods for the collection, quantitative transfer, assay for mutagenicity, and chemical identification of vapor-phase compounds present in a model complex mixture, diesel truck exhaust. This mutagenicity-directed chemical

analysis procedure was also evaluated for assessing the significance of VM as a class of airborne toxic substances.

Our research was focused on the following specific aims:

1. Validation of previously developed methods for the collection, extraction, and concentration of vapor-phase chemicals in a model complex mixture, diesel exhaust.

2. Fractionation of extracted compounds present in diesel vapor-phase using differential supercritical CO_2 extraction and HPLC to facilitate mutagenicity assay and chemical analysis for the compounds in the fractions.

3. Chemical characterization of compounds in the highly mutagenic fractions.

C. Background

Short-term tests for mutagenicity can be used to screen for the mutagenic components of complex environmental mixtures such as diesel exhaust. The *Salmonella*/microsome mutagenicity assay, or the Ames test (Ames *et al.*, 1975), is the most widely used and validated of all assays for mutagenicity. The test has been very useful in directing chemical identification of mutagenic compounds present in environmental mixtures (Lewtas, 1988).

Previously, Kado *et al.* (1983, 1986) developed a simple modification of the Ames test, known as the microsuspension assay, for the detection of mutagenic activity of urine from cigarette smokers and non-smokers and from extracts of airborne PM (Kado *et al.*, 1983, 1986). This assay is 10 to 20 times more sensitive than the standard Ames test for a given amount of mutagen tested. This assay was adjusted for use with vapor-phase compounds in our previous work supported by CARB (Hsieh *et al.*, 1990; Kado *et al.*, 1992).

Much of the information concerning diesel exhaust mutagenicity has been associated with extracts of PM (Slaga *et al.*, 1980). A number of potent mutagens and carcinogens have been isolated and identified using the microsuspension assay in conjunction with chemical analysis (Scheutzle *et al.*, 1982; Lewtas, 1988). Diesel PM was collected on various filter media, and the mutagenic compounds were extracted from the PM with organic solvents (Montreuil *et al.*, 1992). The extracted mutagens were concentrated and exchanged into a solvent that was compatible with the

mutagenicity assay. Ambient airborne PM and its associated mutagenic compounds have been examined by a number of investigators (Talcott and Wei, 1977; Pitts *et al.*, 1977; Moller and Alfheim, 1980; Chrisp and Fisher, 1980; Alfheim *et al.*, 1983; Chrisp *et al.*, 1978). Recently, Arey *et al.*,(1992) used the microsuspension method for determining mutagenic activity in model chamber studies.

Mutagenicity-directed chemical analysis has been instrumental in identifying many important mutagenic compounds present in complex environmental mixtures (Lewtas, 1988). The analysis involves fractionating a complex mixture and determining the mutagenic activity of the various fractions. The mutagenic fractions are further fractionated and the bioassay used further to monitor the mutagenicity in the mutagenic subfractions. In this manner, pure mutagenic compounds can be chemically characterized and identified. Using this technique, 1-nitropyrene and other nitro-PAH have been determined to be potent mutagens and carcinogens (Schuetzle, 1982; IARC, 1984). The mutagenic properties of extracts of PM from diesel engine emissions have been extensively studied and much of the mutagenic activity has been attributed to nitro-PAH (NRC, 1981; Schuetzle and Lewtas, 1986). In addition to PM, unfiltered diesel exhaust also contains many toxic gases or vapors that can cause acute respiratory problems (Slaga *et al.*, 1980). The vapor-phase of diesel exhaust has been associated with lymphoma (cancer of the white blood cells) in animals (Iwai *et al.*, 1986).

The mutagenic activity of volatile compounds emitted from a diesel engine has been studied in a limited scope with conflicting results. Scheutzle and Lewtas (1986) reported that compared to extracts from the PM, 3% of the total direct-acting and about 5% of the total indirect-acting mutagenicity was due to vapor-phase compounds. Egeback (1982) however found that gaseous emissions had greater direct-acting mutagenic activity than that of PM. The discrepancy between the results of these two studies may be attributed to differences in the efficiency of sample collection, preparation, and testing.

Bagley *et al.* (1987) investigated the effect of ceramic particle traps on the chemical mutagens present in diesel exhaust. The authors measured mutagenic activity in both the soluble organic fraction (SOF) and in the volatile organic compounds (VOC) of diesel exhaust. The diesel engine was a medium-duty V-8 and sampling took place in a dilution tunnel. The PM were trapped on a filter, while the volatiles were collected on XAD-2 resin using high volume sampling. A number of

driving modes were evaluated and the bioassay used was the *Salmonella*/microsome test of Ames *et al.* (1975). Based on the number of revertants normalized to kilowatt-hours, the VOC fraction was about one-third the mutagenic activity of the PM extract. A number of compounds were tentatively identified in the VOC fraction including fluorenone, anthracene/phenanthrene ketones, and methyl anthracene/phenanthrene.

Matsushita *et al.* (1986) studied the mutagenicity of PM and PM-free phases of diesel exhaust using a flow-through exposure chamber system to treat *Salmonella* bacteria to the gaseous and vapor compounds of the exhaust. The exhaust from a 2369 cc diesel engine was diluted 10 times in a dilution tunnel. Mutagenic activity was detected under these test conditions without the addition of S9 microsomal enzymes. Bacterial tester strain responses in order of highest response were: TA100, TA104. The authors reported little or no activity when S9 microsomal enzymes were incorporated into their test.

Westerholm *et al.* (1991) studied the chemical composition and mutagenicity of exhaust from a heavy-duty diesel vehicle (14.2 L engine) tested during transient driving conditions. The authors used cryotrapping and XAD-2, and PUF to collect the diluted VOC from the exhaust. Mutagenic activity was detected on the XAD-2 and PUF samples. A number of 3-ringed PAH (substituted and unsubstituted) were tentatively identified in these samples.

The common procedure for trapping trace organics in the air involves passing an air sample through some trapping medium such as an adsorbent or filter. Methods which have been used for trapping VOC from air include condensation into cryogenic traps, absorption into solvent impingers, and adsorption onto solid matrices. A solid matrix was chosen for the present study due to its ease of use and the availability of a number of commercial adsorbents. Examples of solid matrices include XAD-2 and -4 adsorbents. These adsorbents are macroreticular cross-linked polystyrene, divinylbenzene copolymers with high specific surface areas (290-750 m^2/g), small mean pore diameters (50-90 Å) (Nunez and Gonzales, 1984), and large mesh sizes (20-50). VOC that have been collected on the XAD adsorbents include PAH, mutagens from combustion sources, volatiles from diesel exhaust, chlorinated benzenes, PCB, alcohols, alkanes, carbonyls, carboxylic acids and esters, nitrogen and sulfur compounds, phenols, phthalates, and pesticides (Chuang *et al.*, 1987; Bennett *et al.*, 1979; Junk and Richard, 1984; Woodrow and Seiber, 1978; Wehner et al, 1984;

Wong et al. 1988). Moller and Altheim (1983) used XAD-2 resin and found that of the total mutagenic activity present in both the PM and vapor phase, 40-90% of the activity was found in the vapor phase of combusted wood chips, oil, and coal.

Polyurethane foam (white polyether and charcoal gray polyester forms), or PUF, is another adsorbent that has been extensively used in air sampling. Because of its low resistance to air flow, PUF is useful for sampling at high flow rates. PUF has a large sorption surface, is easy to handle and store, and is relatively inexpensive. Westerholm *et al.*(1991) used PUF and XAD-2 to collect diesel exhaust emissions and found that the amounts of PAH emitted in the semi-volatile phase was approximately three times higher than that emitted in the particulate phase. In another study, De Raat *et al.* (1987) used PUF to trap VOC from air, and found mutagenicity in the water and various alcohol extracts of the PUF. Techniques requiring solvent extraction are not suitable for highly volatile mutagens because of considerable losses during sample extraction and concentration. An alternative approach for the extraction and concentration of volatile mutagens is the use of supercritical fluid extraction (SFE).

When a fluid is above its critical pressure and temperature, it possesses gas-like mass transfer properties but behaves like a solvent. Carbon dioxide is commonly used as a supercritical extraction fluid above its critical conditions of 72 atm and 31.1°C. In our previous work, we successfully integrated SFE with a *Salmonella*/microsuspension mutagenicity assay and chemical analysis for model semi-volatile organics (Hsieh *et al.*, 1990; Wong *et al.*, 1991; Kado *et al.*, 1992). SFE has great potential for extracting organics from complex matrices (Hawthorne, 1990) and has several advantages over liquid or thermal extraction. First, the solvating properties can be controlled by controlling temperature and pressure. Second, supercritical fluids are gases at ambient temperatures. Therefore, a concentration step (as required for liquid extraction) is bypassed because the fluid is vented. Third, many supercritical fluids have relatively low critical temperatures (for example, 31°C for CO_2) and are considered inert or have low chemical reactivity.

SFE is a reliable and efficient technique that can expedite the separation and analysis of complex chemical mixtures (Taylor, 1992). This technique has been used for the extraction of organic pollutants from a variety of adsorbents, including Tenax-GC, XAD-2, PUF, and Spherocarb (Hawthorne and Miller, 1986; Hawthorne and Miller, 1987; Schantz and Chester, 1986; Wright *et*

al., 1987). Hawthorne and Miller (1986) reported the SFE of SRM 1650 (National Institute of Standards and Technology reference sample for diesel exhaust PM) and the quantitative recovery of PAH with extraction times as short as 30 min. The same investigators later found that 30 to 60 min of SFE resulted in better recoveries of PAH compared to either a 4 hr sonication of the sample matrix with solvent or an 8 hr Soxhlet extraction (Hawthorne and Miller, 1987). This was due to the fact that supercritical fluids have the solvating power of a normal liquid, but with better mass transfer properties. By varying the extraction pressure, class-specific extractions of alkanes and PAH from diesel exhaust PM were accomplished. Since changes in pressure can influence the solvating power of a supercritical fluid, pressure gradients are commonly used to extract compounds of increasing molecular weight. The SFE-extracted sample can then be easily dissolved in a solvent suitable for bioassay or chemical analysis. SFE can also be directly interfaced with GC, HPLC, or SFC to provide powerful on-line analytical capabilities (Hawthorne and Miller, 1987; Hawthorne *et al.*, 1988; Engelhardt and Gross, 1988; McNally and Wheeler, 1988; Raynor *et al.*, 1988; Vannoort *et al.*, 1990).

While gas chromatography (GC) is the primary analytical method for volatile compounds, mass spectrometry (MS) is one of the most powerful methods for chemical identification. Mass spectral data libraries are readily available to aid in compound identification. A number of investigators have identified previously unknown compounds present in atmospheric samples (Tong and Darasek, 1984; Yasuhara *et al.*, 1984; Arey *et al.*, 1992) and quantitation by GC-MS is possible with the use of 1) isotope dilution, 2) internal standards, and 3) external standards (Tong and Karasek, 1984; Coleman *et al.*, 1983). Isotope dilution and the use of internal standards are the preferred methods of quantitation.

When a component in a mixture is present in low concentrations or there is some chemical interference, selected ion monitoring (SIM) can be used for analysis. SIM is highly selective and sensitive and several studies have reported success using this technique on vapor-phase pollutants (Yasuhara and Fuwa, 1978; Marano *et al.*, 1982; Jonsson and Berg, 1980). If a compound cannot be easily identified using GC-MS, further fractionation might be necessary to facilitate isolation of the compound for mutagenicity-directed chemical analysis.

III. MATERIALS AND METHODS

A. Chemicals

HPLC-grade methanol, ethyl acetate, and acetone were obtained from Fisher Scientific. For mutagenicity experiments, dichloromethane (DCM), methanol (HPLC grade), benzo(a) pyrene (BaP) and dimethylsulfoxide (DMSO, spectrophotometric grade) were from Aldrich Chemical Co. and were used without further purification. For HPLC fractionation, acetonitrile was from Burdick and Jackson and water (HPLC resi-analyzed) was from J.T. Baker Chemical Co.

B. Adsorbents

A sampling train was designed to quantitatively collect different classes of vapor-phase compounds representing a wide range of volatilities. Based on their physical and chemical characteristics, desorption techniques, and feasibility to integrate with the *Salmonella!* mutagenicity assay, polyurethane foam (PUF) and XAD-4 (XAD) adsorbents were selected for trapping diesel exhaust emissions.

PUF was obtained from Hickory Springs Foam Co., North Carolina. The PUF was cut into plugs that were 50 mm in length and 21 mm in diameter. All PUF plugs were pre-cleaned by sequential sonication in methanol (3x) and DCM (3x) and dried under a stream of nitrogen.

Bulk XAD resin was from Rohm and Haas (Philadelphia, PA). The XAD was cleaned extensively to remove potential background interferences by successive 30 min washings with 0.5N HCl, 0.5N NaOH, and distilled deionized water. The XAD was then Soxhlet-extracted with methanol, acetone, ethyl acetate, DCM, and methanol, each for a period of 24 hrs. The resin was dried for 48 hrs under vacuum at 50°C. The cleaned and dried XAD was stored in amber glass jars.

PM was collected on 37 mm diameter Teflon filters with a pore size of 2 μm (Gelman Sciences, Inc., Ann Arbor, MI). The filters were pre-cleaned by sonication in methanol (3x) and DCM (3x) and were dried under a stream of nitrogen. Filters were weighed before and after diesel exhaust sample collection using a Cahn microbalance (Cahn Scientific Instruments, Cerritos, CA).

C. Sampling Apparatus

Each diesel exhaust sampling train consisted of a Teflon filter, two PUF plugs, and one 5 mL bed of XAD, connected in series as shown in Figure 1. The PUF and XAD were packed into 16 x 135 mm open-ended glass culture tubes and were held in place by glass wool plugs. The glass wool plugs were precleaned by solvent extraction with methanol and DCM. Teflon tubing was used to connect both the filter holder assembly to the glass tube and the complete sampling train to a vacuum pump. The vacuum pump used for all sampling experiments was Model 607CA22 (115V, 60Hz, 3.5A) from Thomas Industries Inc. (Sheboygan, WI).

D. Diesel Exhaust Sampling

Undiluted diesel exhaust was sampled from a CARB Mobile Laboratory truck stationed at the University of California at Davis. The Mobile Laboratory truck was a 1988 International Harvester Model DT-466C. The truck had a 6-cylinder diesel engine with a displacement of 7.61 L (466 in^3) and 443.7 horsepower. The truck also had the following specifications: compression: 16.3:1, bore: 109.2 mm, stroke: 135.9 mm, engine weight(without accessories): 1441 lb, low idle: 650 ± 25 rpm, and high idle: 2730 ± 25 rpm. The truck was operated using diesel fuel number 2.

For each sampling experiment, sampling train inlets were positioned approximately 1 ft. from the exhaust pipe in the middle of the exhaust plume. The exhaust was sampled for 15 min to 3 hrs at flowrates ranging from 2 to 10 liters per minute (LPM), depending on the experiment. For each sampling run, both the flowrate and temperature were checked and recorded at 15 min intervals. Sampling trains were placed in duplicate or triplicate, depending on the the sampling run. For each sample collection, at least one complete train served as a blank.

Samples and blanks were wrapped in aluminum foil throughout the sampling period to minimize potential photooxidation. Immediately after each sampling period, the samples and blanks were stored on dry ice and transported back to the laboratory where they were placed in a freezer at -10°C until further processing.

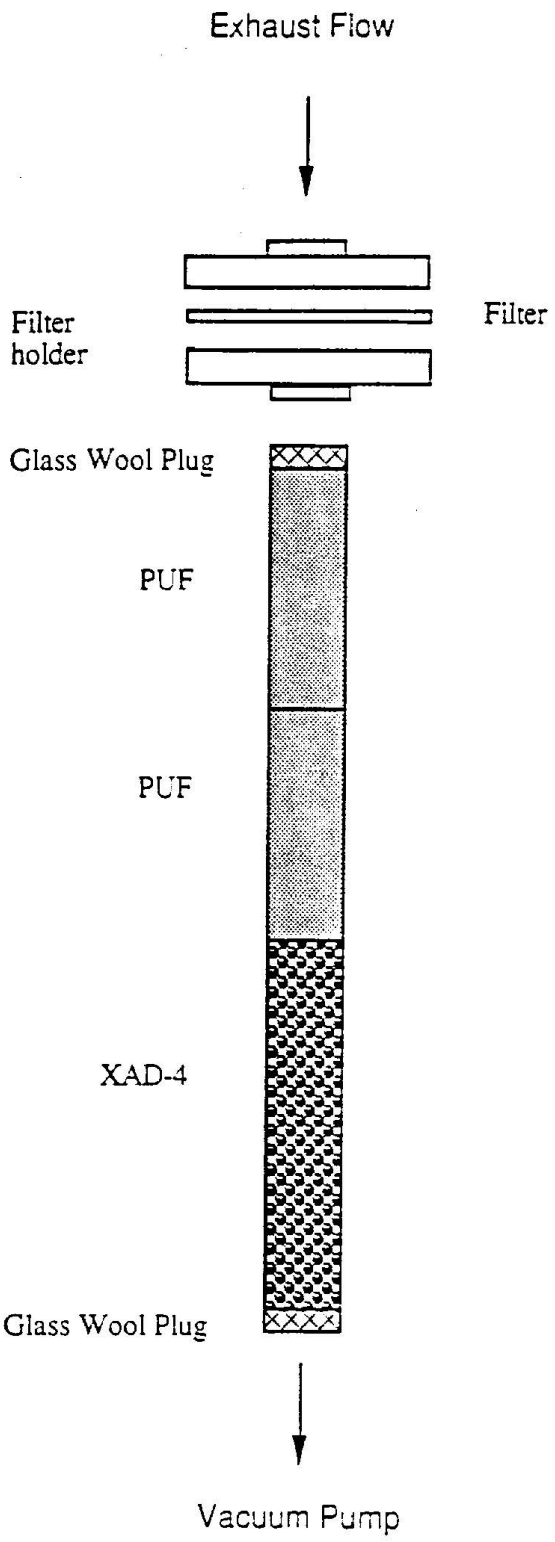


Figure 1. Diesel Exhaust Sampling Train

E. Supercritical Fluid and Solvent Extraction

Supercritical fluid extraction (SFE) of the organic compounds from the PUF and XAD samples was accomplished using liquid carbon dioxide (CO_2) as the extraction solvent at pressures and temperatures above its critical point of 72.9 atm and 31°C. Since CO_2 is a gas at atmospheric pressure, this technique produced an essentially solvent-free concentrated extract which was directly analyzed by both GC-MS and the mutagenicity assay. The SFE apparatus used for all experiments is illustrated in Figure 2.

An Isco Model 260D syringe pump (Lincoln, NE) and SFC grade CO_2 (AGA Specialty Gas, Maumee, OH) under a helium headspace of 1500 psi were used for all supercritical fluid extractions. The PUF and XAD adsorbents were placed in a 3 mL and 5 mL extraction cell (Suprex Corp., Pittsburgh, PA), respectively. The extraction cell was then placed into a GC oven (Varian Aerograph) maintained at 50°C. The syringe pump was operated at pressures ranging from 1200 to 6000 psi and the samples were extracted for 30 or 60 min. A 45 cm length of 25 µm i.d. (375 µm o.d.) deactivated fused silica capillary (Supelco, Inc., Bellefonte, PA) was used as a depressurizing flow restrictor to maintain supercritical conditions within the extraction cell. As the supercritical CO_2 (s-CO_2) exits from the restrictor, it expands to a gas upon reaching ambient pressure. The effluent from the outlet of the capillary was directed into a graduated collection vial with a screw top fitted with a Teflon septum. The collection vial contained approximately 200 to 300 µL of methanol to trap and concentrate volatile chemicals for direct mutagenicity and chemical analyses. Volatile compounds were conserved by placing the collection vial in a dry ice-acetone bath maintained at -20 to -30°C.

Chemicals in filters were extracted with organic solvents for mutagenicity testing. Each filter was placed in a pre-cleaned glass vial or flask and was sequentially mixed with 5 mL of DCM (2x) and methanol (1x) with sonication and shaking. The DCM and methanol extracts were pooled, blown to dryness with nitrogen, and stored in sealed vials at -20°C until tested for mutagenicity, at which time the extracts were resuspended in DMSO.

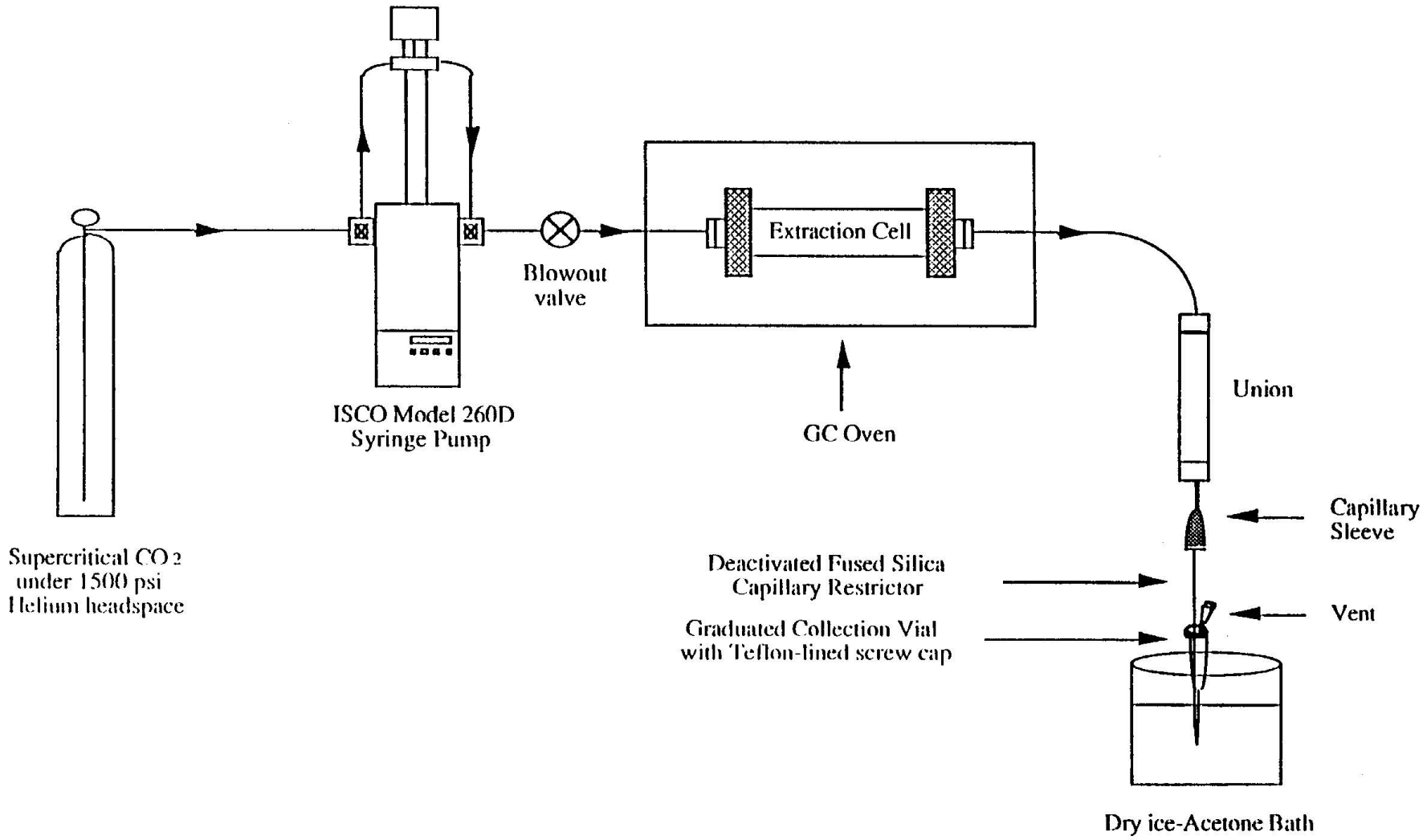


Figure 2. Supercritical Fluid Extraction Apparatus

F. Chemical Analysis

1. GC-MS Analysis

The chemicals in the SFE fractions were analyzed using a Hewlett-Packard Model 5890 Gas Chromatograph (GC) interfaced to a Hewlett-Packard Model 5970 quadrupole mass selective detector (MSD) equipped with an electron-impact positive 70 eV ion source. A ChemStation was used for data processing. The MSD was auto-tuned daily using perfluorotributylamine standard. Samples were introduced onto a 60 m x 0.25 mm i.d. J & W DB-1 capillary column (1.0 µm film thickness; J & W Scientific, Rancho Cordova, CA) by splitless injection.

Two different temperature programs were developed to analyze the PUF and XAD SFE fractions as shown in Table 1.

Table 1. GC-MS Temperature Programs for Analysis of PUF and XAD-4 SFE Fractions

Condition	PUF	XAD-4
column flowrate	1 mL/min	1 mL/min
column temperature	55°C initial 5°C/min to 150°C 5 min hold @ 150°C 3°C/min to 300°C 5 min hold @ 300°C	55°C initial 7°C/min to 100°C 10 min hold @ 100°C 3°C/min to 300°C 5 min hold @ 300°C
injector temperature	250°C	250°C
detector temperature	250°C	250°C
solvent delay	6 min	6 min
run time	80 min	90 min
scan mode	35-300 mass units	35-300 mass units

2. Post-SFE HPLC Fractionation

To reduce the number of peaks in the total ion chromatograms and to facilitate chemical indentification, HPLC of the SFE fractions was performed. The PUF and XAD SFE fractions exhibiting the highest mutagenic activity were pooled and fractionated by reverse-phase HPLC. The HPLC column was a Waters μBondapak C18 column, 3.9 x 300 mm (with guard column). An integrated Waters HPLC system was used for fractionation that included a Model 680 automated gradient controller, dual Model 510 piston pumps, a Model U6K injector, a Model 481 LC spectrophotometer variable wavelength detector (254 nm), and a Hewlett Packard Model 3390A integrator. Fractions were manually collected.

The solvent program (at a flow rate of 1.5 mL/min) was initially 80% water and 20% acetonitrile, followed by a 20 min linear gradient to 100% acetonitrile and held for 20 min. The solvent was then programmed back to the initial conditions. Nine 5 min fractions of decreasing polarity were collected for GC-MS analyses. The HPLC fractions were concentrated for mutagenicity testing by gently blowing down each fraction with nitrogen and mild heating at 30°C.

G. Mutagenicity Testing

A microsuspension procedure previously reported by Kado *et al.* (1983; 1986), which is a simple modification of the Ames test, was used throughout. The assay is approximately 10 times more sensitive than the original Ames test, based on absolute amounts of material added per tube. The procedure was adapted for volatile compounds by sealing the incubation mixture in a closable tube.

Tester strains TA98 and TA100 were kindly provided by Dr. B.N. Ames, Berkeley, CA. For the microsuspension procedure, bacteria were grown overnight in Oxoid Nutrient Broth No. 2 (Oxoid Ltd., Hants, England) to approximately 1 - 2 x 10^9 cells/mL and harvested by centrifugation (5,000 x g, 4°C, 10 min). Cells were resuspended in ice-cold phosphate-buffered saline (0.15M PBS, pH 7.4) to a concentration of approximately 1 x 10^{10} cells/mL.

The S9 (metabolic enzymes) and S9 mix (enzyme co-factors) were prepared according to the procedure of Ames *et al.* (1975). The S9 from Aroclor 1254 pretreated male Sprague-Dawley rats was used throughout and contained 40.3 mg protein/mL, as determined using the modified Biuret method of Ohnishi and Barr (1978).

For the microsuspension assay, the following ingredients were added, in order, to a 12 x 75 mm sterile glass culture tubes kept on ice: 0.1 mL S9 mix, 0.005 mL sample in DMSO, and 0.1 mL concentrated bacteria in PBS (1 x 10^{10} / mL PBS). The mixture was incubated in the dark at 37°C with rapid shaking. After 90 min, the tubes were placed in an ice bath and taken out one at a time immediately before adding 2 mL molten top agar containing 90 nmoles of histidine and biotin (Ames *et al.*, 1975). The combined solutions were vortex-mixed and poured onto minimal glucose plates. Plates were incubated at 37°C in the dark for 48 hrs and counted using an automatic plate counter (Biotran counter, New Brunswick Scientific, Princeton, NJ) Strain markers were routinely determined for each experiment.

H. Quality Assurance and Control

For the sampling and chemical analysis, adsorbent and solvent blanks were analyzed along with the actual samples to determine possible background interferences. Field samples were stored in dry ice during transport to the laboratory where they were stored at -10°C before sample workup and extraction. Blank PUF and XAD samples were routinely extracted and tested to check for any background interferences. All SFE fractions were chemically analyzed immediately following extraction or stored at -80°C until sample analysis and mutagenicity testing were possible.

All mutagenicity assays incorporated both positive and negative controls as well as adsorbent and filter blanks. All chemical and bioassay procedures were carried out in a room fitted with yellow fluorescent lights (G.E. F40Go) to minimize potential photo-oxidation of chemicals.

IV. RESULTS

A. Diesel Study I - Pilot Run

1. Collection of vapor-phase organics and particulate matter

Sampling of undiluted diesel exhaust for the pilot study (DS I) was carried out at the University of California, Davis, using the California Air Resources Board (CARB) Mobile Laboratory truck equipped with a International Harvester 6-cylinder diesel engine. Sampling trains for VM consisted of PUF filters and XAD adsorbent resin placed in series. Each sampling train was preceded by a Teflon filter to trap PM. Exhaust samples were collected from a cold-start engine at an initial flowrate of 8-10 liters/min (LPM). The total sampling time was approximately 15 min. Due to high particle loading of the filters, the flowrates decreased rapidly to approximately 4 LPM after only 5 min of sampling. The average temperature of collection at the filter was 36°C. Three replicate samples were obtained, as well as two adsorbent blanks.

2. Extraction of organics

The organics in each PUF and XAD sample were extracted with s-CO_2. The extraction scheme, as illustrated in Figure 3, was chosen based upon our experience gained from a previous study supported by CARB (Hsieh *et al.*, 1990). Two SFE fractions were sequentially obtained from each sample at 3000 psi for 60 min and at 6000 psi for 60 min. The filters were extracted with DCM and methanol.

3. Mutagenicity measurements

Each SFE fraction was tested for mutagenicity in the *Salmonella* microsuspension assay, using bacterial tester strains TA98 and TA100, both with and without S9 microsomal activating enzymes. Although the sampling period was very short and the flowrate was low, weak mutagenic activity was detected in the first SFE fraction from the first PUF and XAD samples for both TA98 and TA100 (data not shown). The solvent extracts of filters were mutagenic in both TA98 and TA100, with and without S9.

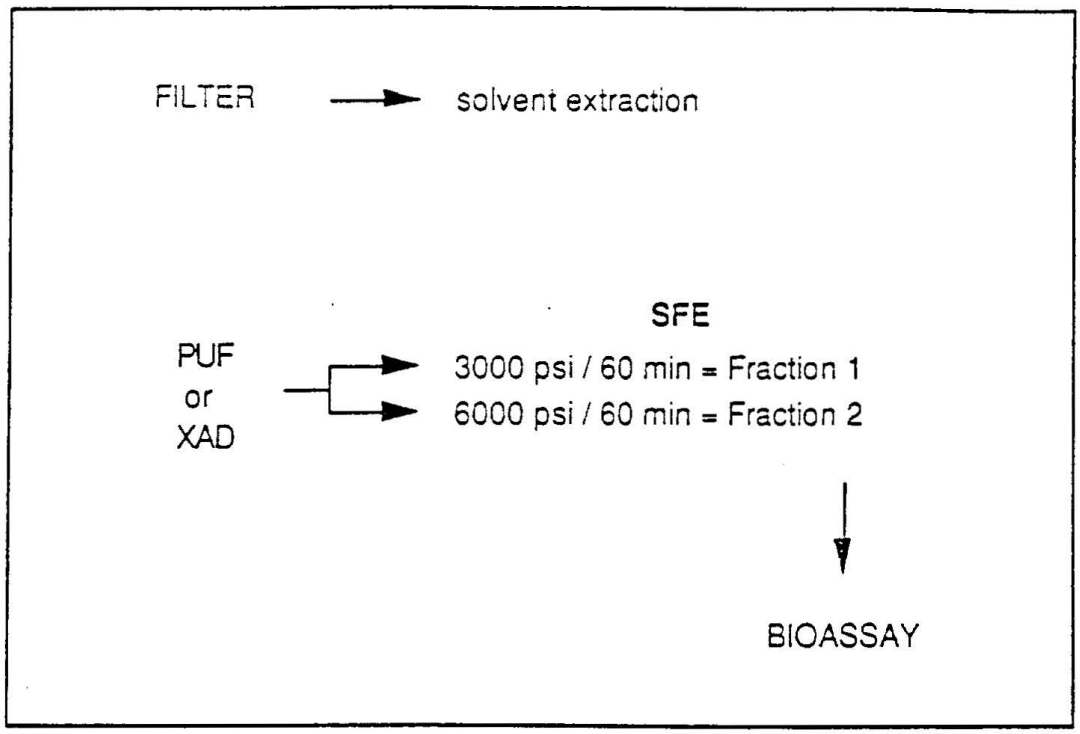


Figure 3. Outline for Diesel Study I - Pilot Analysis

4. Summary

A sampling train that consisted of a Teflon filter and two sections each of PUF and XAD assembled in series was used throughout to trap vapor-phase compounds from diesel exhaust. The compounds adsorbed on PUF and XAD were extracted with s-CO_2 at 3000 and 6000 psi into small volumes of methanol. Weak mutagenic activity was detected in the first SFE fraction from the first PUF sample.

Based on the bioassay data for DS I, it was concluded that a larger quantity of VM would be needed for analyses and hence longer sampling times would be required. To avoid heavy PM loading on the Teflon filters due to sampling exhaust from a cold-start engine, future exhaust samples were collected from a pre-warmed engine.

B. Diesel Study II - Semi-Preparative Run

In order to trap a larger quantity of VM for analyses, longer sampling times were used in Diesel Study II (DS II), and the diesel exhaust samples were collected from a pre-warmed engine to avoid heavy particle loading on the Teflon filters as encountered in DS I.

1. Collection of vapor-phase organics and particulate matter

Sampling of undiluted diesel exhaust was carried out at the University of California, Davis, using the same CARB Mobile Laboratory truck and sampling trains as described in DS I. In this experiment, two separate sampling trains, each maintained at a flowrate of 2 LPM, were used to collect exhaust from an engine that was pre-warmed for 30 min. The average temperature of collection at the filter in the exhaust plume was 40°C. The exhaust was continuously sampled for 60 min before PM loading onto the filter decreased the flowrate and increased the back pressure.

2. Extraction of organics

The filter from Train 1 was extracted with DCM and methanol. S-CO_2 was used to extract each PUF and XAD sample as shown in Figure 4. To extract as many compounds as possible and to provide fractions with higher concentrations of mutagens than obtained in DS I, each sample was extracted at 6000 psi for 60 min followed by another 60 min to provide a second fraction.

Based on the GC-MS analysis of the SFE fractions from Train 1, essentially all compounds were extracted in the first fraction. The compounds present in the first SFE fraction were complex and difficult to analyze. To reduce the complexity of the SFE extracts and to facilitate chemical analysis of individual VM, SFE was used to extract each PUF and XAD sample from Train 2 into six (6) fractions as shown in Figure 4. This extraction scheme also allowed us to determine the utility of SFE as a method of fractionation.

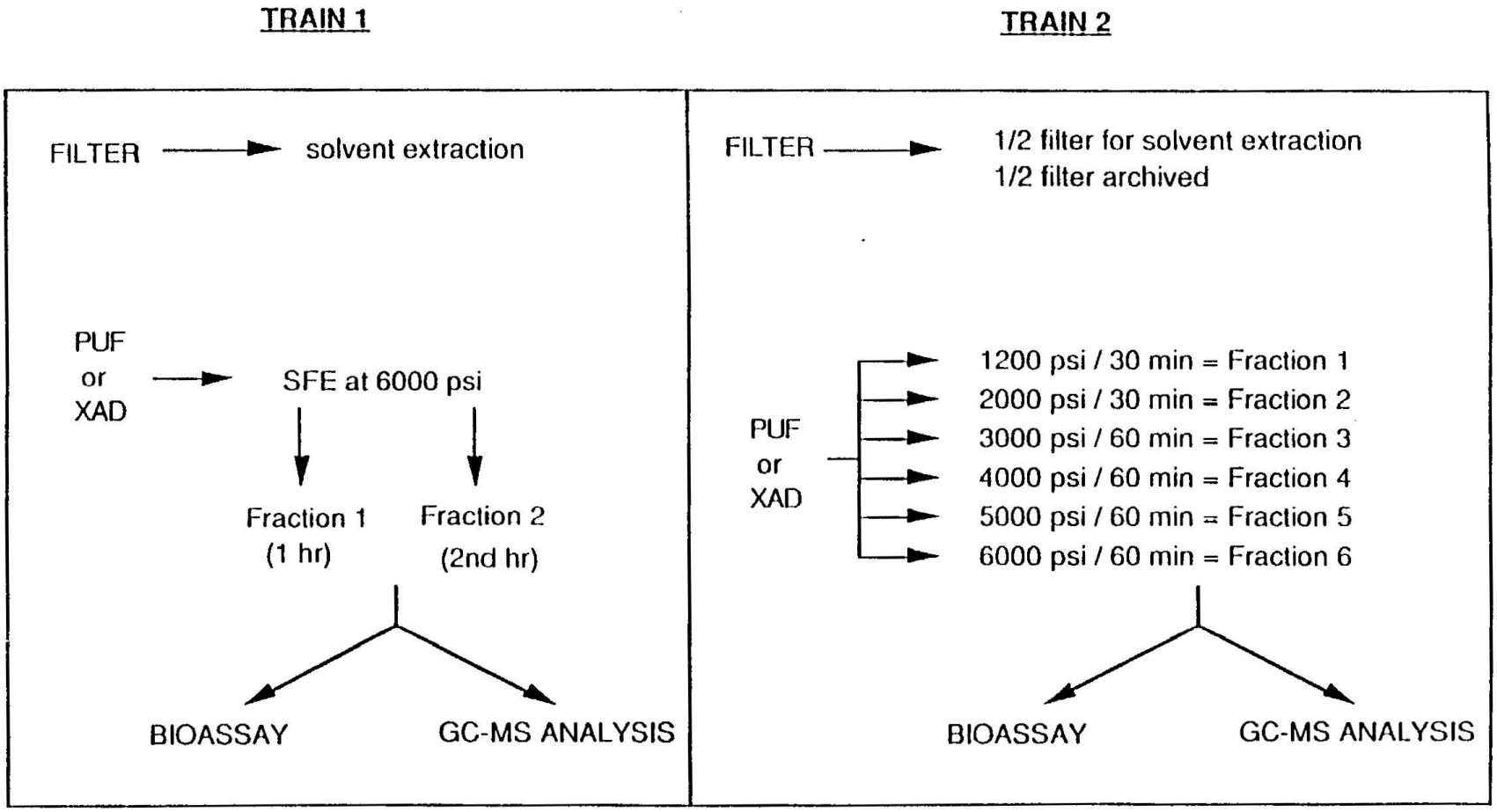


Figure 4. Outline for Diesel Study II - Semi-Preparative Analyses

3. Mutagenicity measurements

a. Train 1

The samples from Train 1 were tested for mutagenic activity with the *Salmonella* microsuspension assay, using tester strains TA98 and TA100, both with and without S9. Greater mutagenicity was observed with TA100. The fractions from PUF appeared to be more mutagenic than the fractions from XAD, and S9 increased the mutagenicity to both tester strains, particularly to TA98 (data not shown).

b. Train 2

Based on the results for Train 1, the SFE fractions obtained from Train 2 were tested using TA100 with S9. The SFE fractions were tested as such and after concentration involving blow-down with nitrogen gas and solvent exchanged into DMSO. Mutagenic activity was detected in both PUF and XAD fractions as shown in Figures 5 and 6. The PUF fractions appeared more mutagenic than the XAD fractions, with the highest mutagenicity found in SFE fractions collected at 2000 and 3000 psi.

Toxicity was observed in the concentrated PUF fractions collected at 2000 and 3000 psi. Increased mutagenic activity was detected in the concentrated XAD fractions, with the highest mutagenicity found in fractions collected at 4000 and 5000 psi. No toxicity was observed in any XAD fraction.

4. Chemical analysis

a. Train 1

Tentative GC-MS analysis indicated that low molecular weight PAH, such as pyrene, fluoranthene and phenanthrene, were present in both PUF SFE fractions from Train 1. The chromatograms for these fractions are shown in Figure 7.

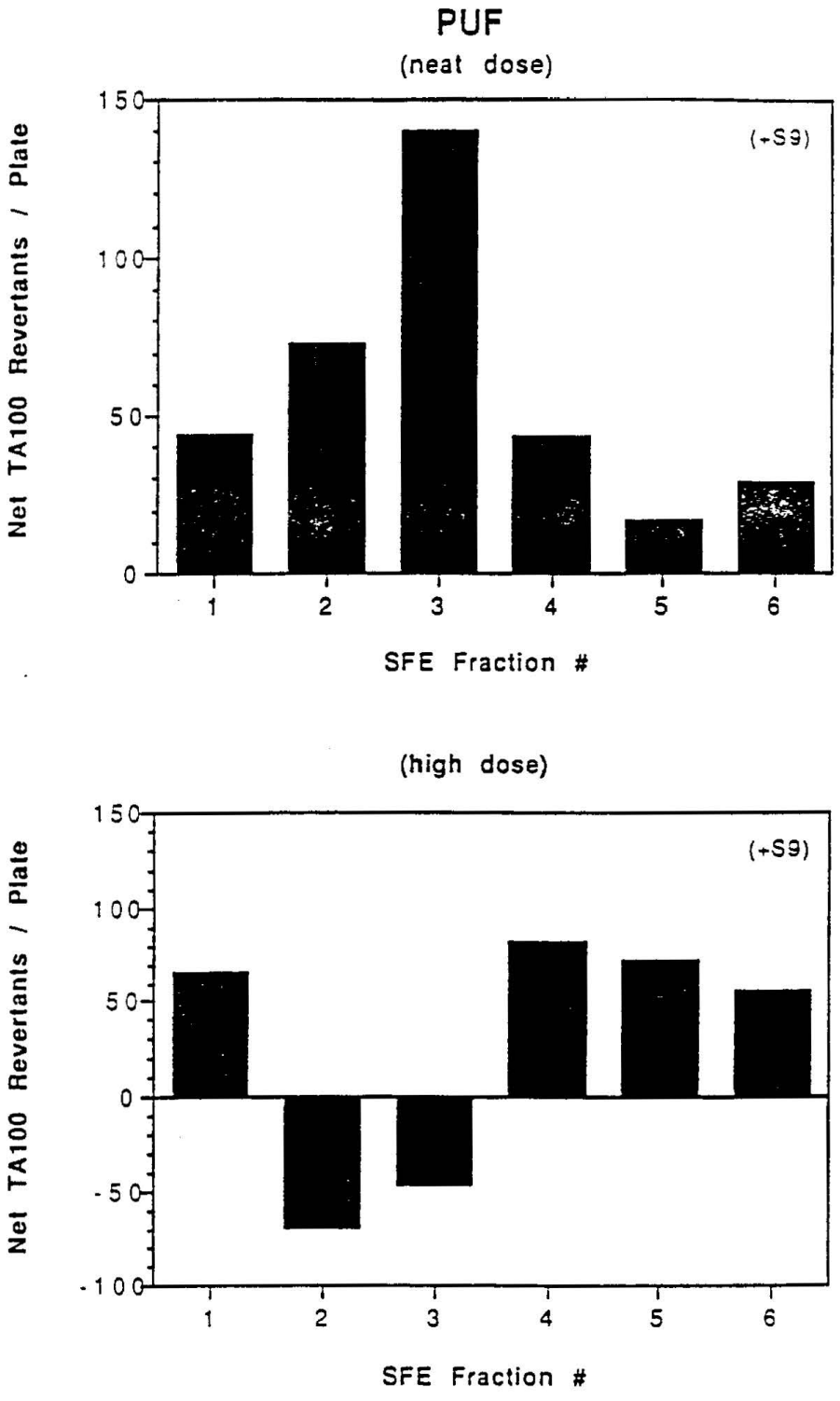


Figure 5. Mutagenicity Profile of PUF SFE Fractions from Diesel Study II Train 2 using TA100 with S9

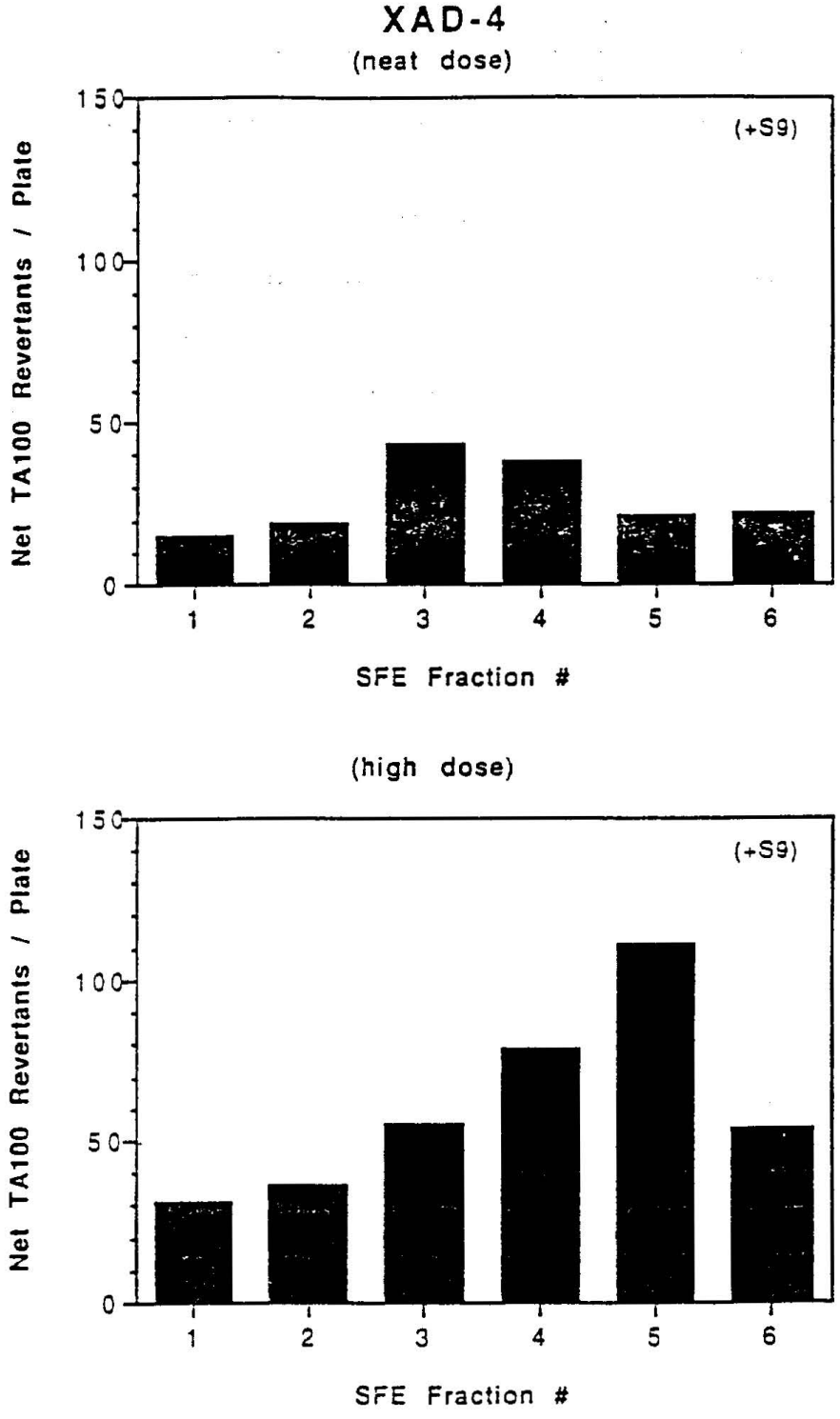


Figure 6. Mutagenicity Profile of XAD-4 SFE Fractions from Diesel II Train 2 using TA100 with S9

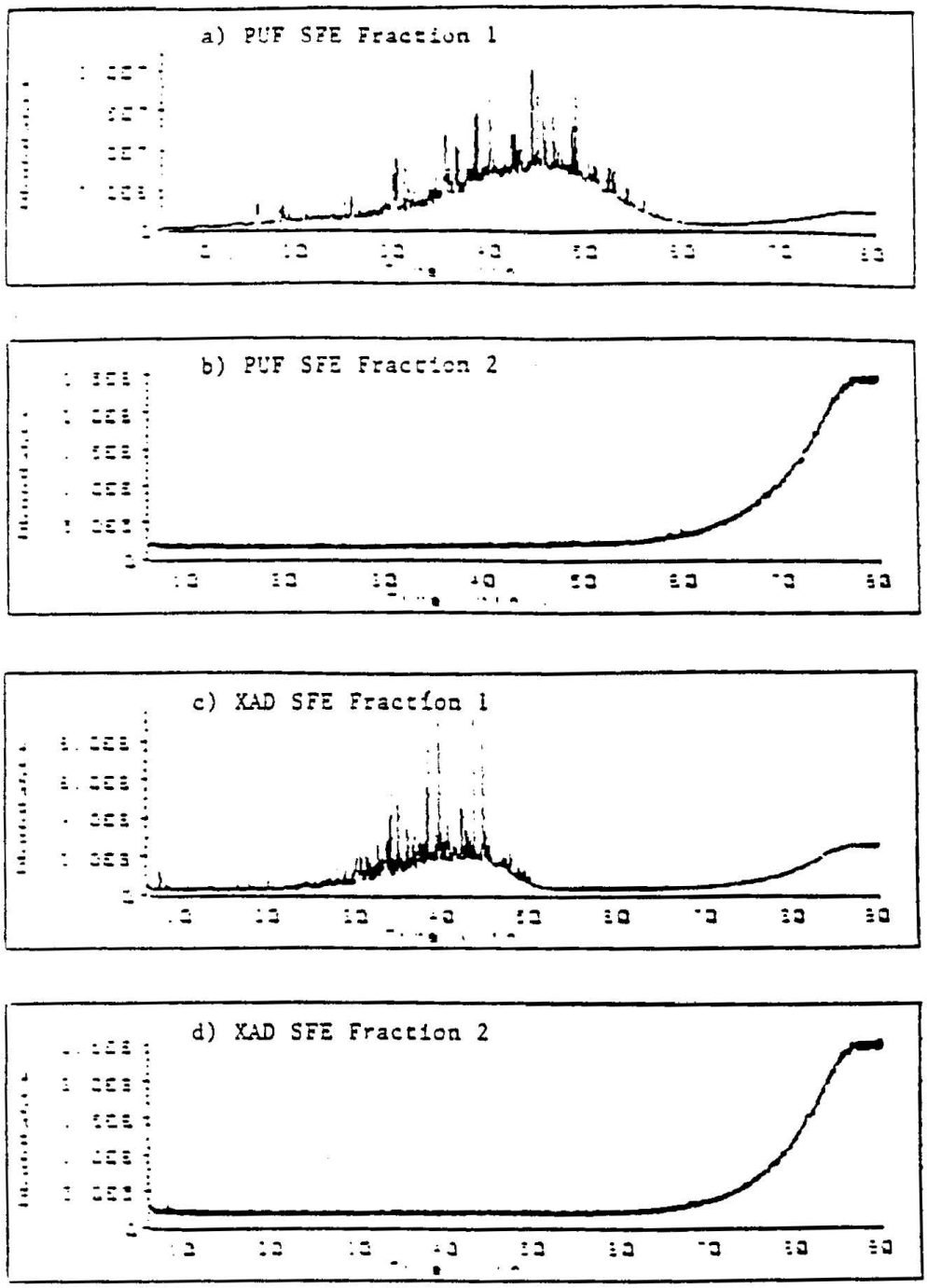


Figure 7. Total ion chromatograms from Diesel Study II Train 1: a) PUF SFE fraction 1, b) PUF SFE fraction 2, c) XAD SFE fraction 1, and d) XAD SFE fraction 2.

b. Train #2

Prior to sample extractions, selected PUF and XAD blanks were subject to the same SFE fractionation procedure to check for possible background chemical interferences. As shown in Figures 8 and 9, the blank SFE fractions did not appear to contain any peaks that would interfere with sample analysis. The results of GC-MS analysis of the PUF and XAD SFE fractions from Train 2 are shown in Figures 10 and 11. These data indicated that almost all compounds were extracted into the first three SFE fractions. A change in chemical composition was observed around 4000 psi. The SFE fractions collected at or below 4000 psi appeared to contain non-polar compounds. The chromatograms for these fractions were complex and contained hundreds of peaks. Fractions collected at pressures higher than 4000 psi appeared to contain more polar compounds. The chromatograms for these latter fractions contained only a few major peaks.

5. Summary

In DS II, larger samples of diesel exhaust were collected to facilitate both mutagenicity assays and chemical analyses. The mutagenicity data for Train 1 indicated that tester strain TA100 was more sensitive than TA98 in the detection of VM in the diesel exhaust samples. There was a difference in mutagenic activity between the compounds extracted from PUF and those from XAD. The GC-MS data indicated that low molecular weight PAH such as naphthalene were present in SFE extracts of both PUF and XAD.

For Train 2, greater mutagenic activity was observed in the PUF fractions than in the XAD fractions. Most compounds were extracted in the first three SFE fractions as detected by GC-MS. The highest mutagenicity was observed in PUF fractions collected at 2000 and 3000 psi. Toxicity was observed in the concentrated PUF fractions collected at these two pressures.

Significant mutagenic activity was also found in concentrated XAD SFE fractions, with the highest mutagenicity found in fractions collected at 4000 and 5000 psi. The highest mutagenicity in the unconcentrated XAD fractions was found in the fractions collected at 3000 and 4000 psi. No toxicity was observed in any SFE fraction of XAD.

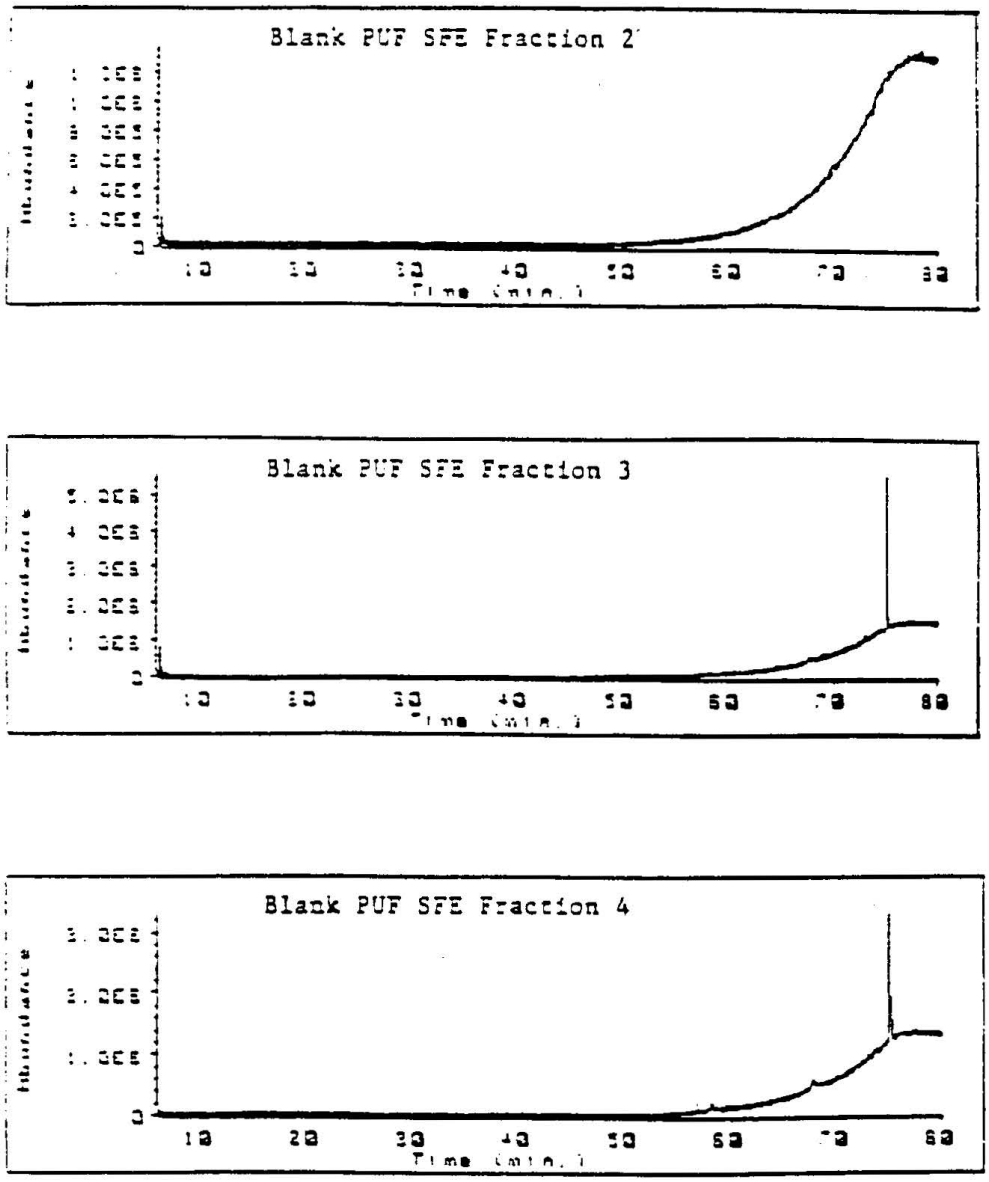


Figure 8. Total ion chromatograms of selected blank PUF SFE fractions from Diesel Study II Train 2.

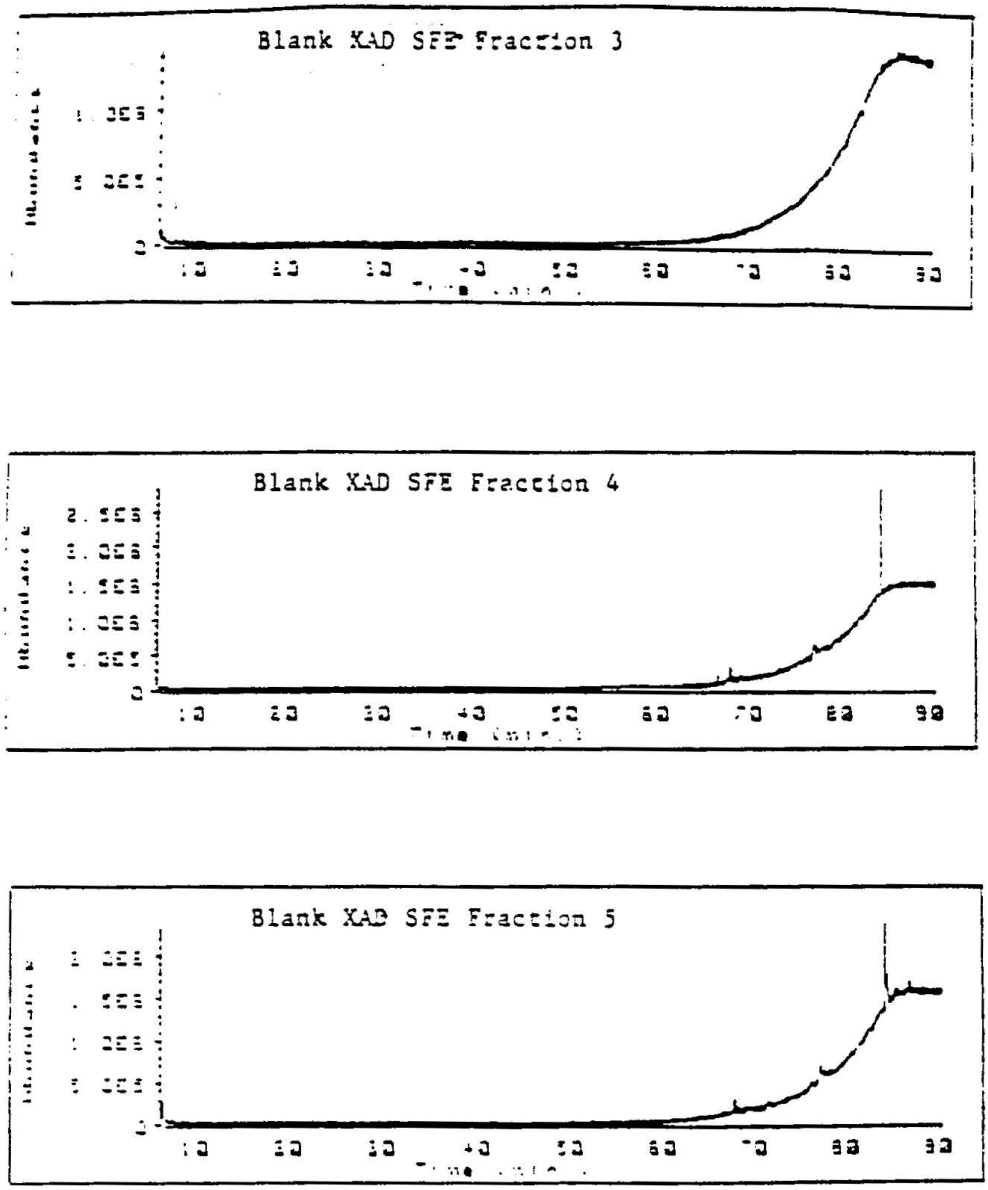


Figure 9. Total ion chromatograms of selected blank XAD SFE fractions from Diesel Study II Train 2.

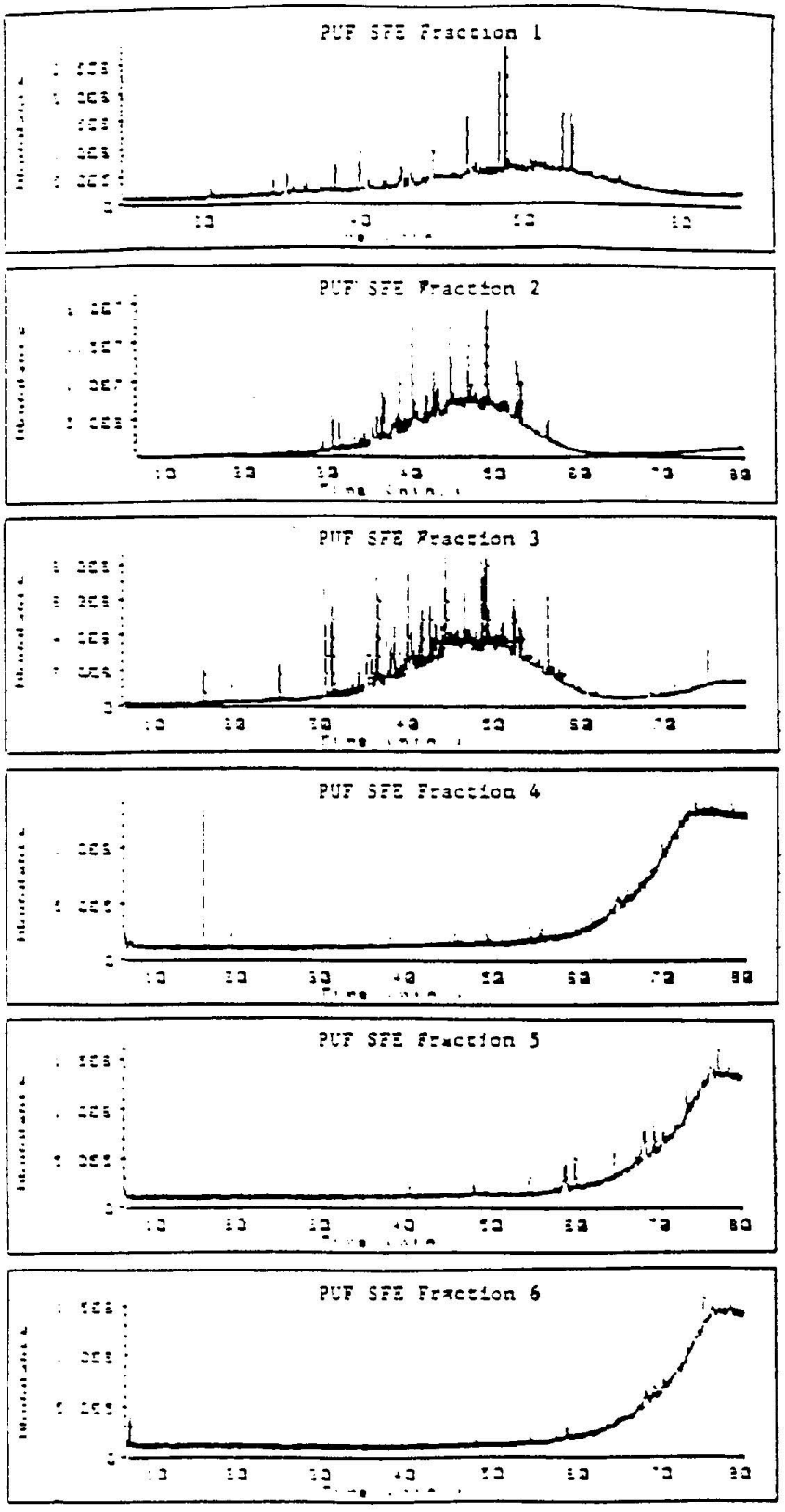


Figure 10. Total ion chromatograms of PUF SFE fractions 1-6 from Diesel Study II Train 2.

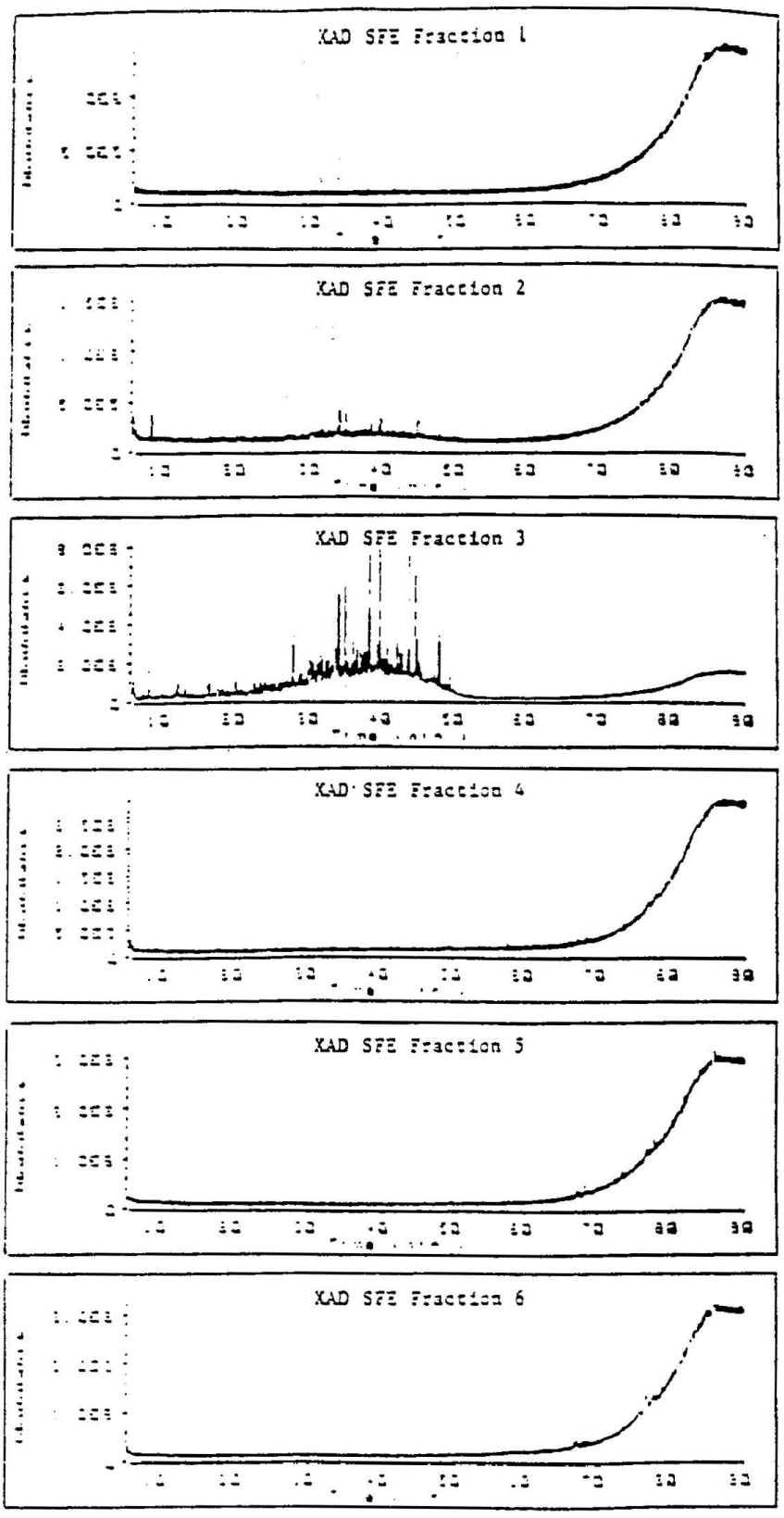


Figure 11. Total ion chromatograms of XAD SFE fractions 1-6 from Diesel Study II Train 2.

C. Diesel Study III - Preparative Run

In order to collect a sufficiently large quantity of VM for mutagenicity-directed chemical analysis, undiluted diesel exhaust was sampled for 3 hr in Diesel Study III (DS III).

1. Collection of vapor-phase organics and particulate matter

Undiluted diesel exhaust samples were collected for 3 hr at a flowrate of 2 LPM. To prevent the accumulation of PM on the filter, an undesirable reduction in airflow, and an increase in back pressure, the sampling train filter was replaced every 60 min. Total PM was that collected by all the filters used for each train. The average temperature of collection at the filter in the exhaust plume was 42°C. Three replicate samples collected with three sampling trains as well as one field blank were obtained.

2. Extraction of organics

Based on the bioassay and GC-MS results obtained for DS II, a similar method was employed to fractionate the DS III samples. SFE was used to extract each PUF and XAD sample from three replicate sampling trains and the field blank into six (6) fractions as outlined in Figure 12.

Organics on the filters of each sampling train were extracted with DCM and methanol. Since each sampling train in DS III employed three whole filters, three filter halves from each train were extracted, pooled, and tested for mutagenicity at three different doses based on the PM mass of each filter half. The remaining filter halves were archived for future analyses.

3. Mutagenicity measurements

Proportionate volumes of each "neat" SFE fraction from two replicate sampling trains were pooled and tested for mutagenicity using tester strains TA98 and TA100, with and without S9. The third sampling train was archived for future analyses. The mutagenicity data for the pooled PUF and XAD SFE fractions are shown in Figures 13 and 14, respectively.

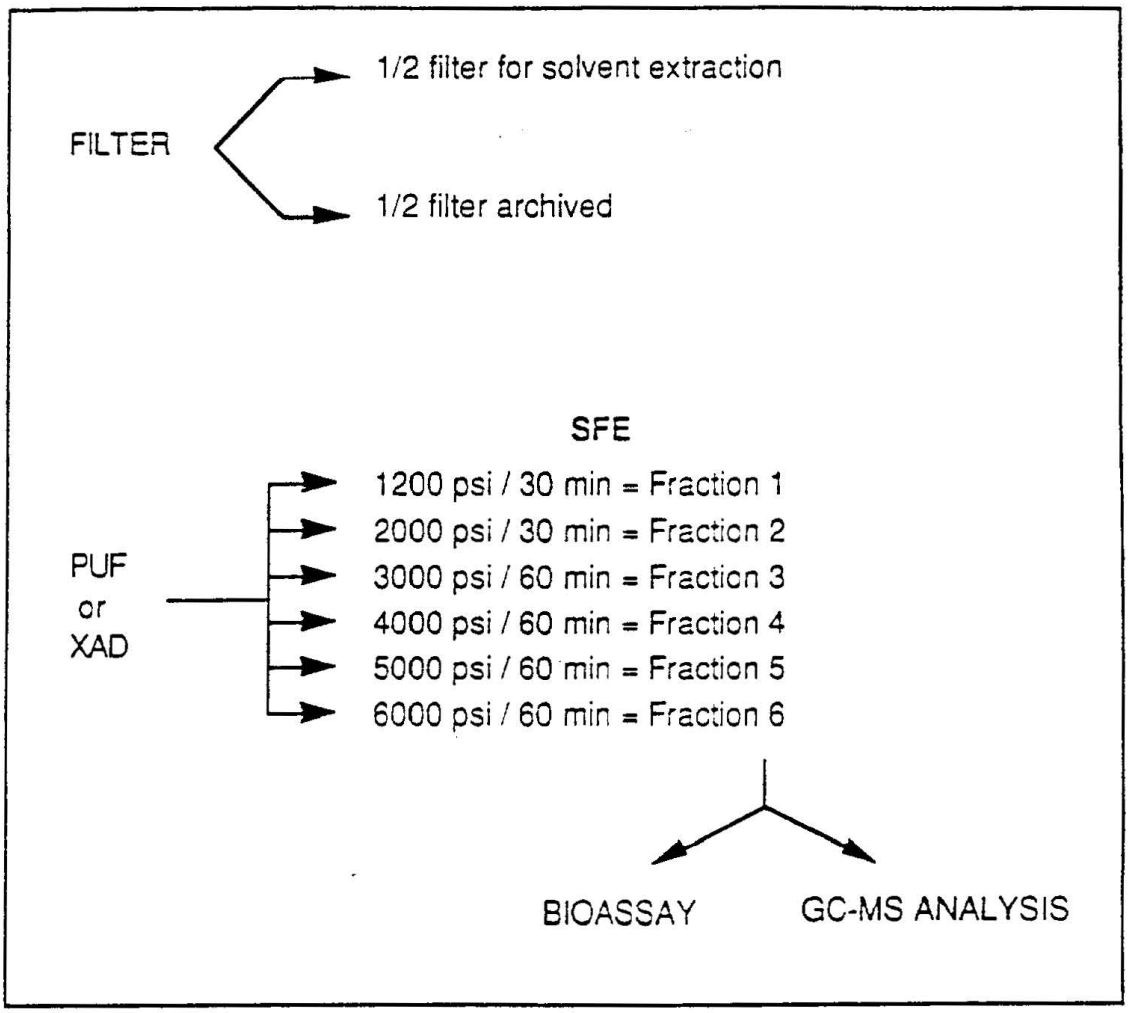


Figure 12. Outline of extractions and analyses for Diesel Study III

PUF

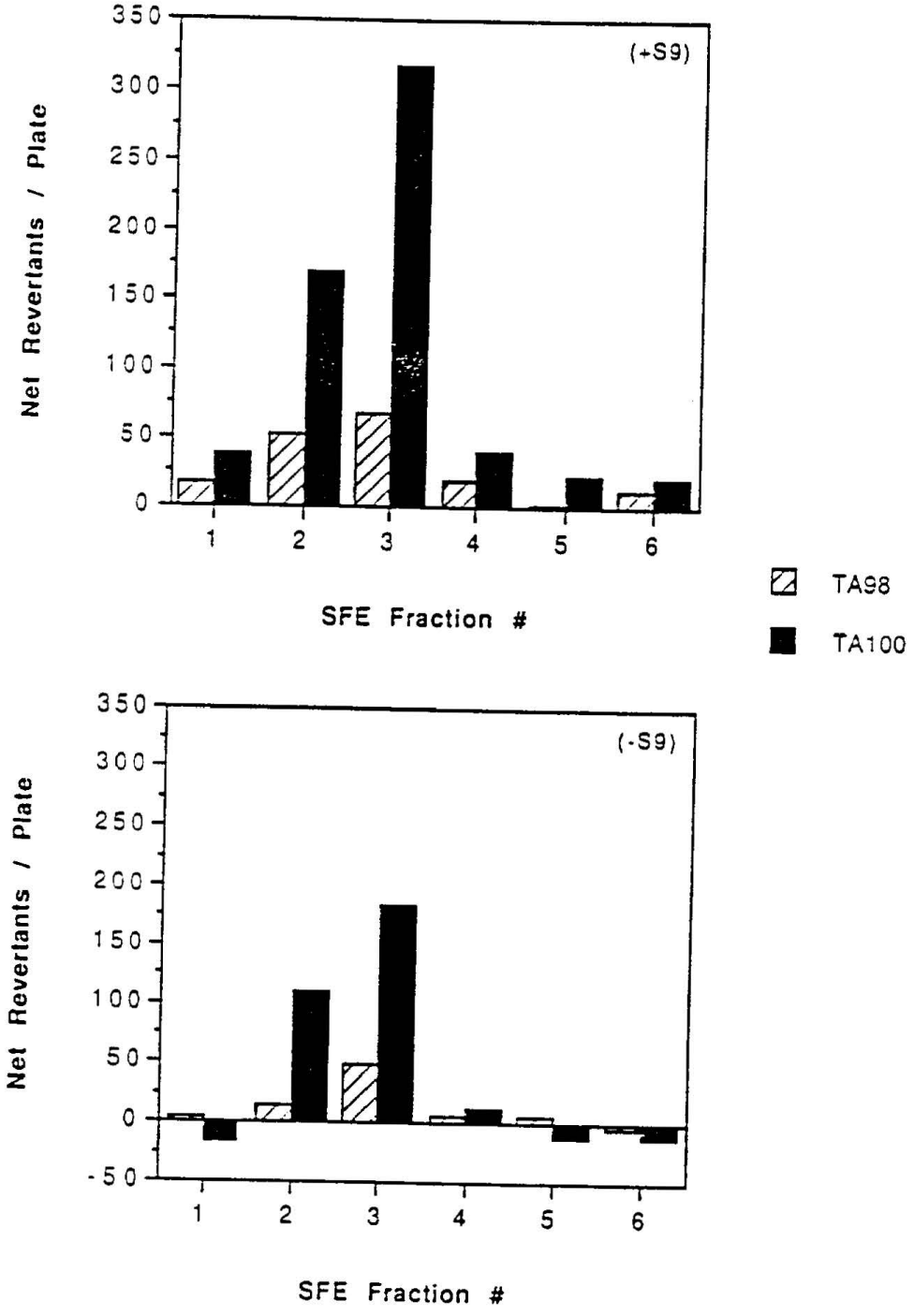


Figure 13. Mutagenicity profile of pooled, neat PUF SFE fractions from two Diesel Study III sampling trains using TA98 and TA100 with and without S9. Data represents the mean of two replicate plates.

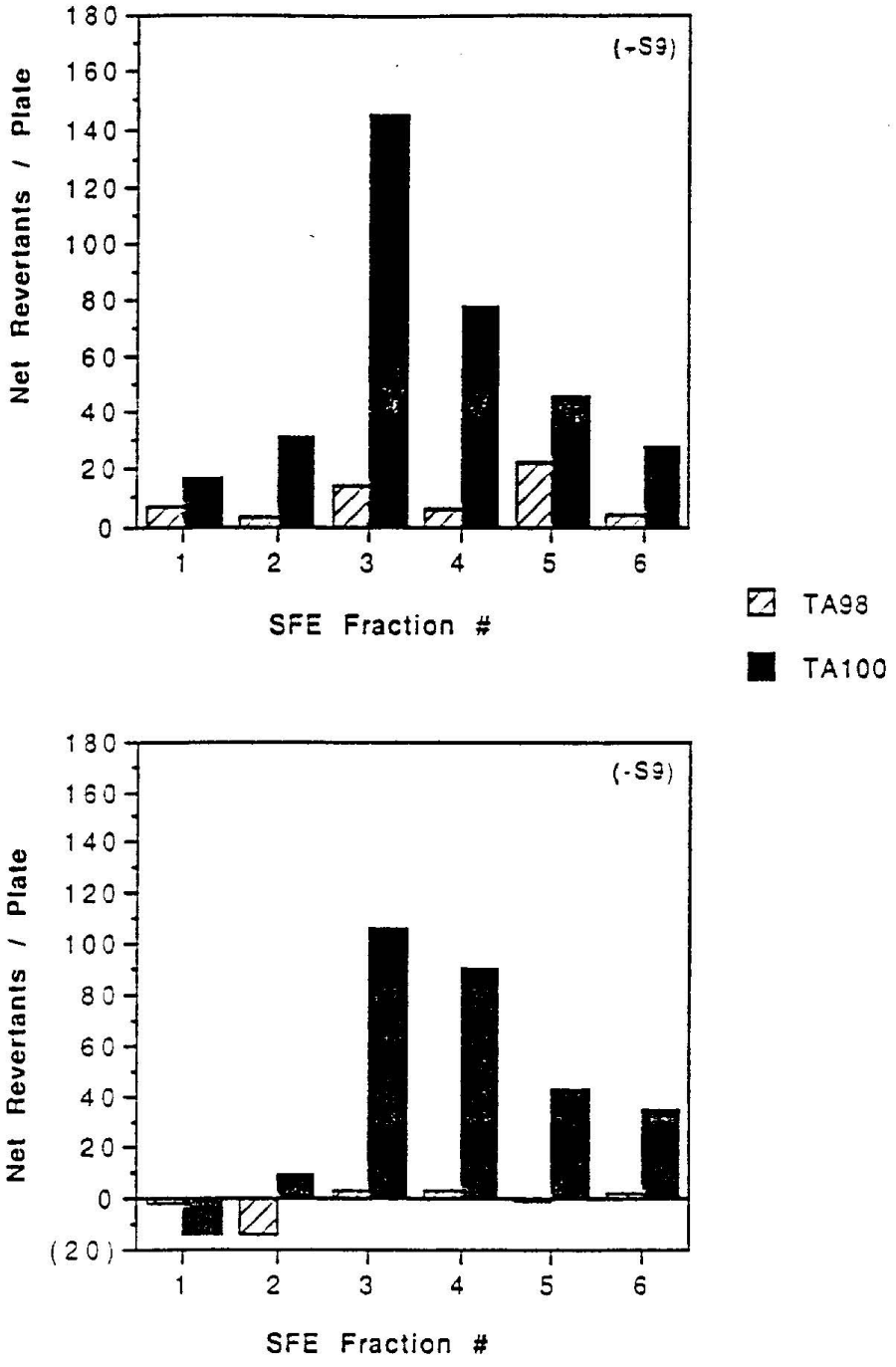


Figure 14. Mutagenicity profile of pooled, neat XAD SFE fractions from two Diesel Study III sampling trains using TA98 and TA100 with and without S9. Data represents the mean of two replicate plates.

The data indicated that the PUF SFE fractions were more mutagenic than the XAD fractions in both tester strains, with the highest levels of mutagenicity found in the PUF fractions collected at 2000 and 3000 psi. Some fractions were slightly toxic to the tester strains without S9. Significant mutagenic activity was detected in the unconcentrated XAD fractions, with the highest mutagenicity observed in the fractions collected at 3000 and 4000 psi. No toxicity was observed in any XAD fraction. As shown in Figure 15, the solvent extracts of the pooled filter halves from each sampling train were mutagenic to both TA98 and TA100, with and without S9.

Using the mutagenic activity data obtained for the pooled filters, PUF, and XAD, the total number of revertants for each sampling train was calculated as shown in Table 2. The data presented in Table 2 was used to determine the percent (%) distribution of mutagenicity among the filter, PUF, and XAD samples collected by each DS III sampling train as illustrated in Figures 16 and 17.

Table 2. Total Revertants for Diesel Study III Sampling Trains 1 and 2

	Total Revertants*			
Train 1	TA98 (+S9)	TA98 (-S9)	TA100 (+S9)	TA100 (-S9)
Filter	122981	80205	90899	59886
PUF	17058	13098	43448	32056
XAD-4	12402	9434	32532	32966
TOTAL	152441	102737	166879	124908
Train 2				
Filter	131330	85650	97070	63952
PUF	19222	14794	49266	36228
XAD-4	13496	10244	35382	35908
TOTAL	164048	110688	181718	136088

* represents the sum of revertants from three filters, six SFE fractions for PUF, or six SFE fractions for XAD-4

Filter Particulate Matter

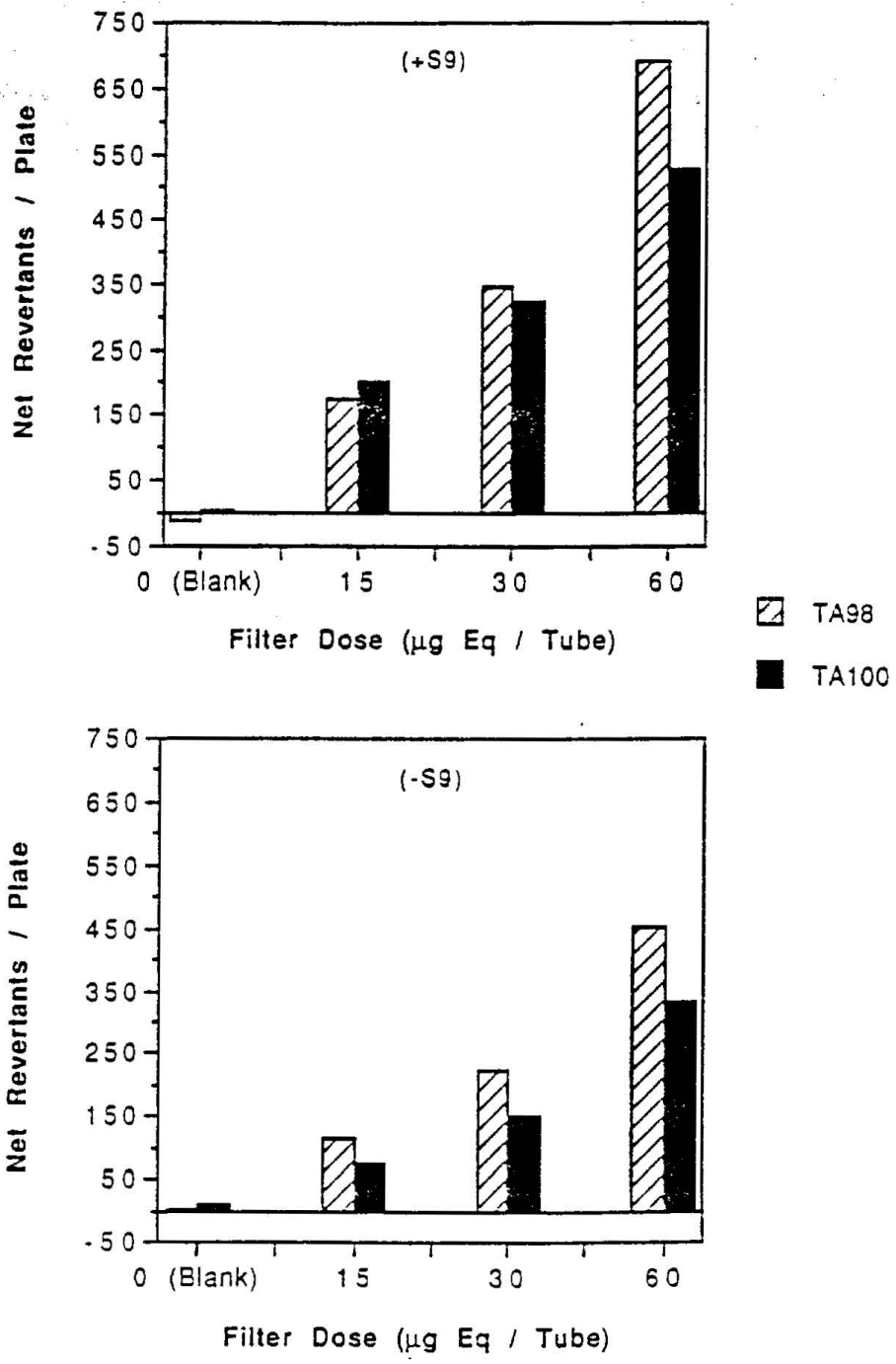


Figure 15. Mutagenicity profile of filter particulate matter extracts from Diesel Study III using TA98 and TA100 with and without S9. Data represents the mean of two replicate plates.

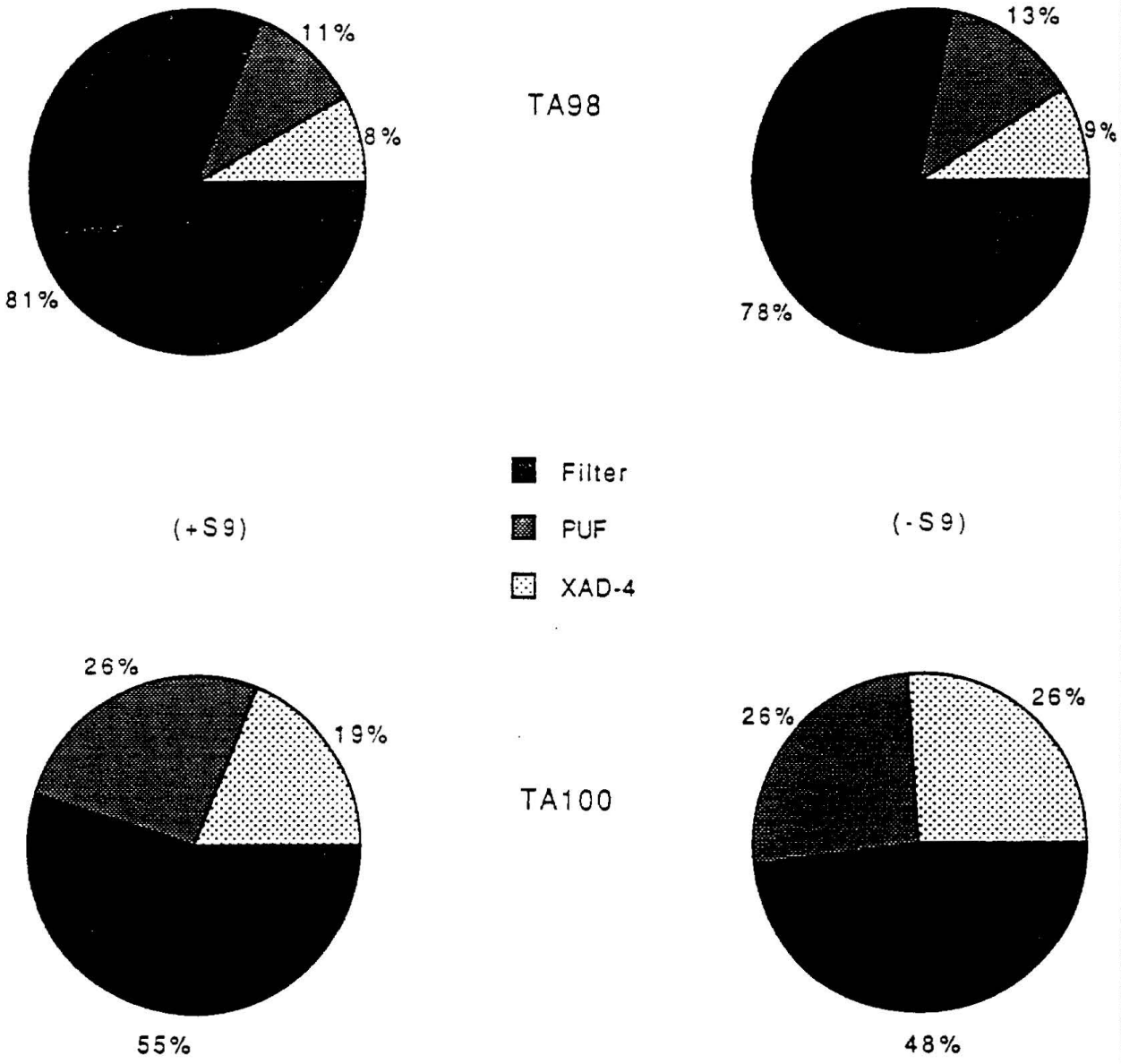


Figure 16. Distribution of percent (%) mutagenicity for Train 1 of Diesel Study III using TA98 and TA100 with and without S9.

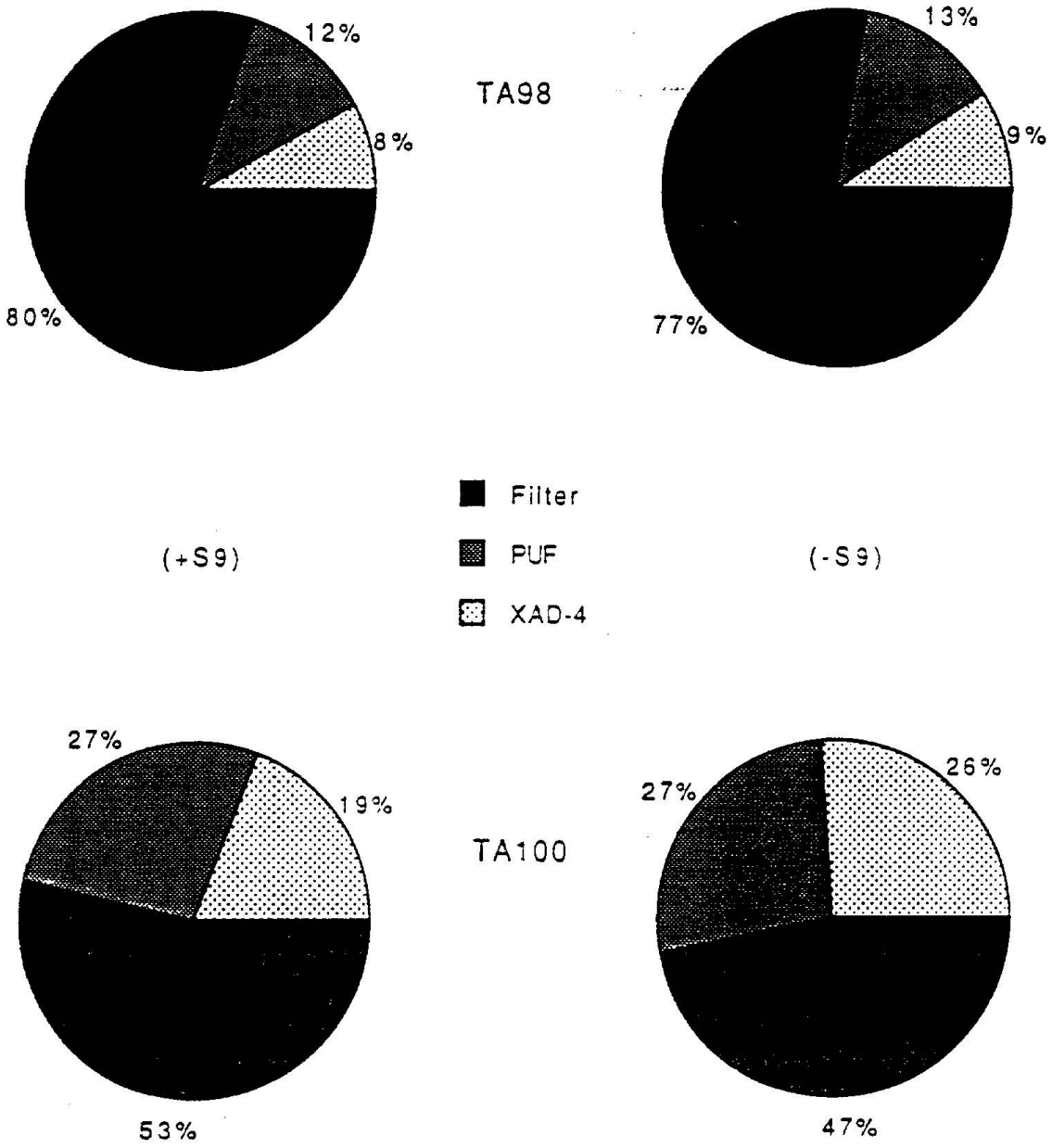


Figure 17. Distribution of percent (%) mutagenicity for Train 2 of Diesel Study III using TA98 and TA100 with and without S9.

4. Chemical analysis

a. SFE Fractions

Total ion chromatograms (TIC) for PUF SFE fractions 1 through 6 are shown in Figures 18 and 19. These chromatograms indicate that a majority of the components were extracted in SFE fractions 2 and 3. The TIC for a pooled PUF blank is shown in Figure 20.

The PUF SFE fractions, as a whole, contain many alkanes and PAHs. A majority of the PAHs found in the PUF fractions were alkylated naphthalenes. SFE fractions 4-6 contain few peaks. Methyl phenol is one of the major peaks found in these fractions. Some major peaks, such as diethylhexyl phthalate, as well as other phthalates may be artifacts since they are also found in the corresponding blank fractions.

TICs for XAD fractions 1 through 6 are shown in Figures 21 and 22. The TICs indicate that a majority of the components were extracted in SFE fractions 3 and 4. Library searches of the major chemical components in each fraction indicate the major components in the XAD SFE fractions to be long chain alkanes and aromatic compounds. The aromatics present in these fractions appear to be alkyl benzenes, alkyl naphthalenes, and unsaturated naphthalenes. Other aromatics such as toluene, ethyl benzene, and *o*-, *m*-, *p*-xylene have been identified by both mass spectra and retention time. Other alkyl benzenes include tri- and tetra-methyl benzenes, diethyl benzenes, and propyl benzenes may also be present in the XAD SFE fractions. In addition, styrene (ethenyl benzene) and other alkenyl benzenes may be present.

Based on the mass spectra, the XAD SFE fractions may contain other classes of aromatics such as indene derivatives, partially saturated naphthalenes, and alkyl naphthalenes. Naphthalene and 1- and 2-methyl naphthalene have been positively identified by both mass spectra and retention time. The presence of alkyl naphthalenes are most likely due to breakthrough from the PUF during sampling.

Many alkanes were found in XAD fractions 3 and 4. Identification of alkanes is difficult using mass spectra alone, since the hydrocarbon spectra are very similar and authentic standards were not available to compare retention times. Most of the alkanes eluted after

naphthalene (boiling point = 218 °C). Since compounds elute in order of their boiling points on a DB-1 column, and based on the boiling range of straight chain (normal) alkanes, the alkanes present in the XAD fractions contain thirteen (13) or more carbon atoms. The more branched the hydrocarbon, the lower its boiling point.

The major components found in the XAD fractions are listed in Table 3. Where positive identification was not possible, chemical components were tentatively identified. The TIC for a pooled XAD blank is shown in Figure 20.

b. HPLC Fractionation

Our analytical results indicate that PUF SFE fractions 2 and 3 had the highest mutagenic activity as well as the highest mass as determined by GC-MS. XAD SFE fractions 3 and 4 also had the highest mutagenic activity as well as the highest mass. Since most of the mass was present in only two of the SFE fractions for each of the adsorbents, SFE did not completely fractionate the chemically complex diesel exhaust samples, indicating the need for an additional method to aid in the chemical characterization of VM present in these SFE fractions. In the present study, HPLC was used to further fractionate these fractions.

Aliquots from PUF SFE fractions 2 and 3 were pooled and a 100 µL aliquot was injected into the HPLC using an acetonitrile/water gradient on a C18 bondpak column and fractions were collected as described in the Methods section. The relatively polar (low molecular weight) compounds eluted in the early fractions and the non polar (high molecular weight) compounds eluted in the later fractions. The HPLC chromatogram of the pooled PUF SFE fractions is shown in Figure 23.

i. Concentration of PUF HPLC Subfractions

Since a 100 µL injection produces nine (9) 7.5 mL HPLC subfractions, it was necessary to concentrate these fractions for mutagenicity testing and GC-MS analysis. Each HPLC subfraction was placed into a water bath at 30°C and concentrated to between 1 mL and 300 µL

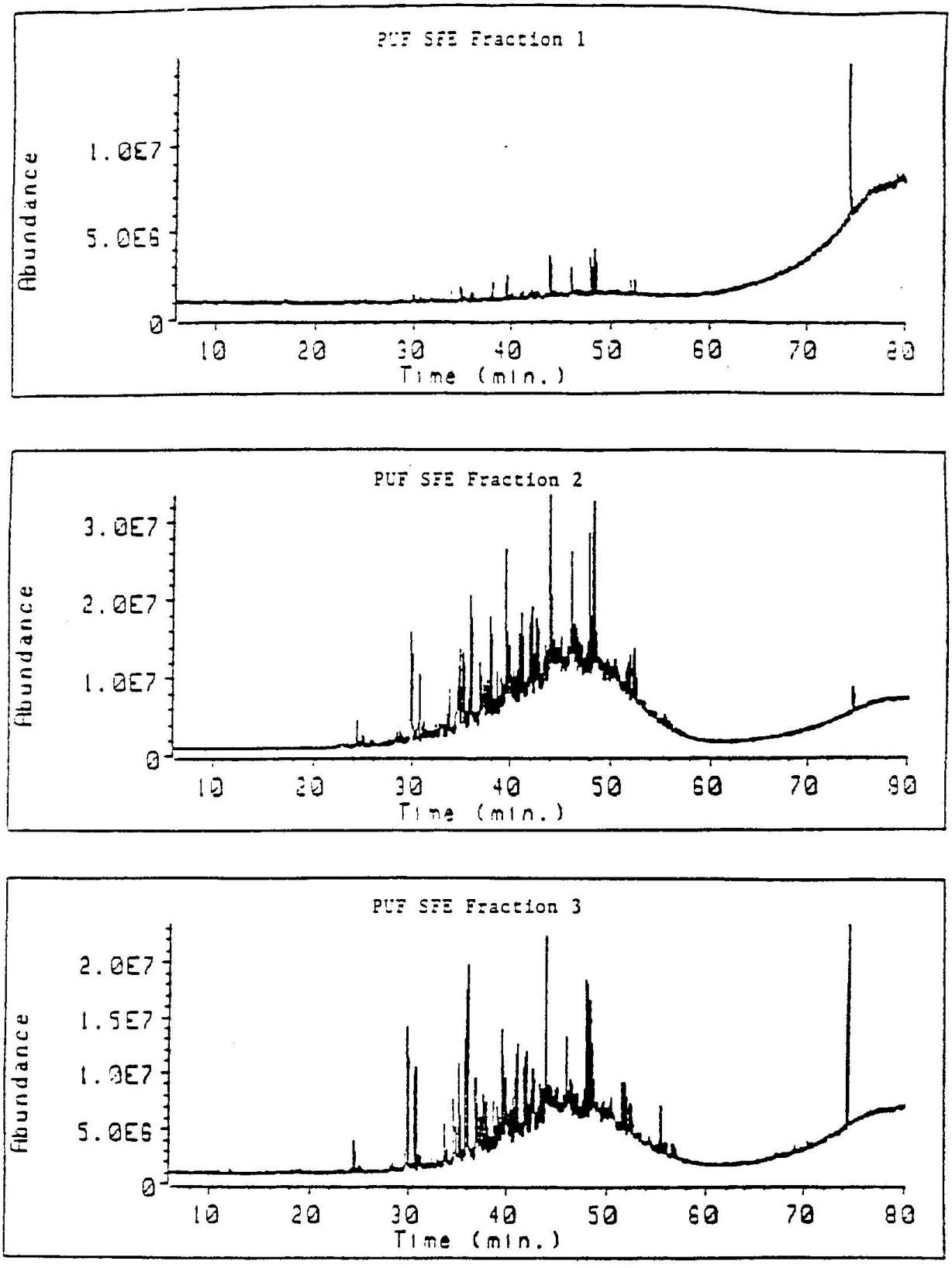
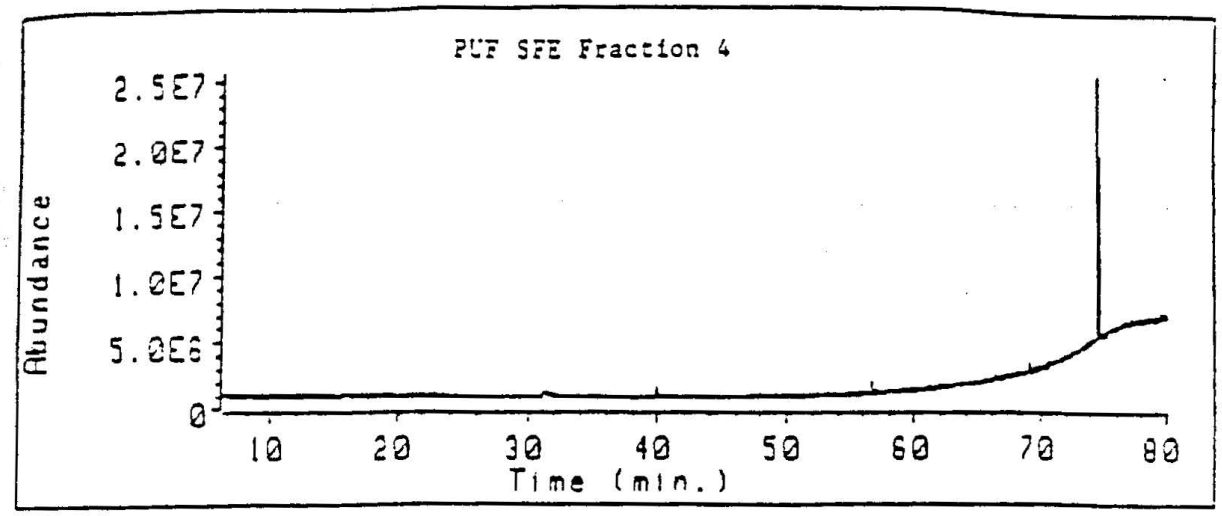
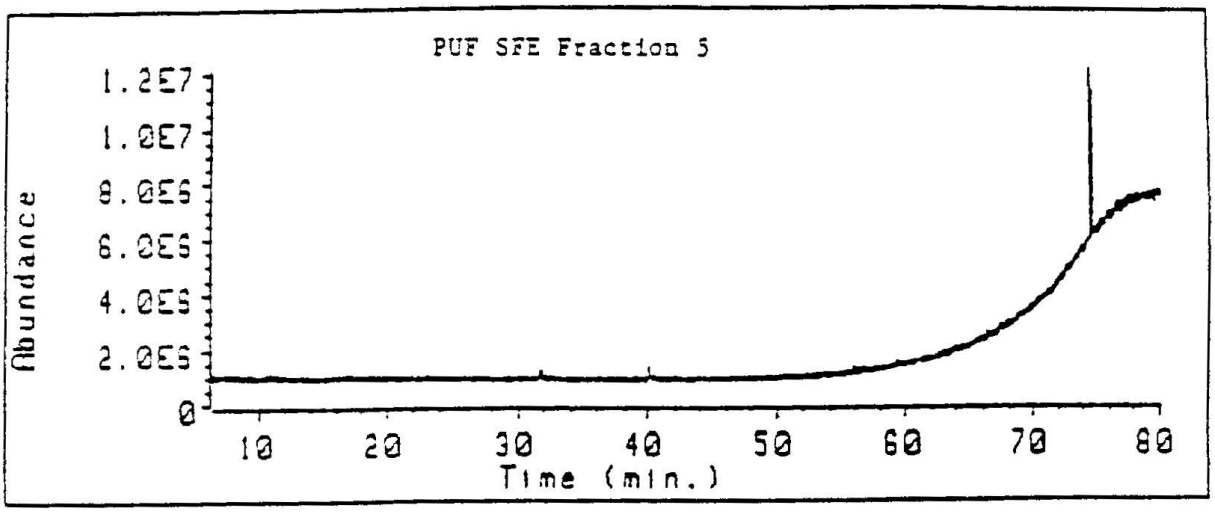


Figure 18. Total ion chromatogram of PUF SFE fractions 1-3.





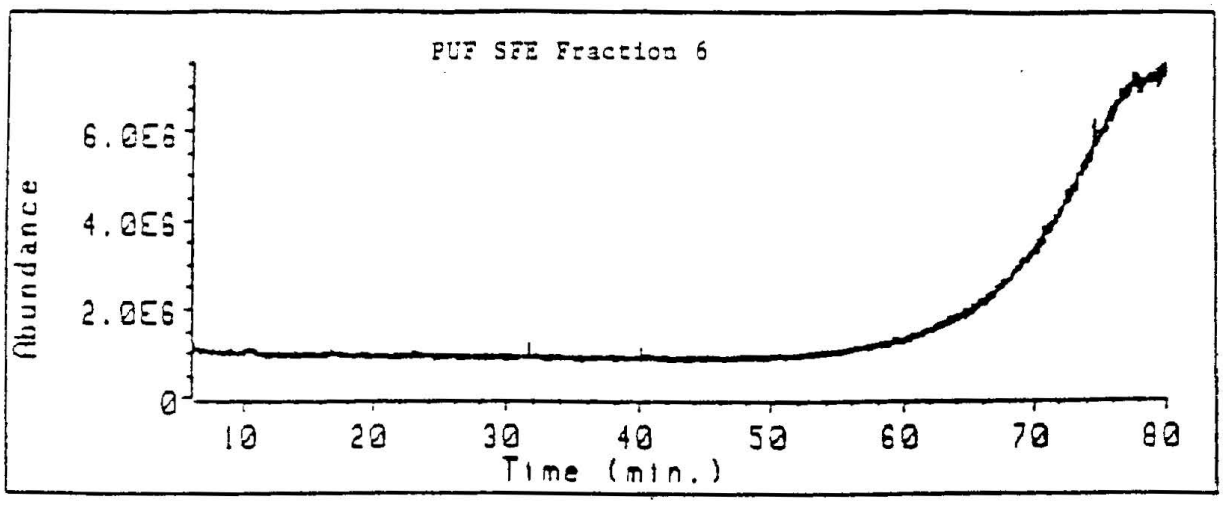
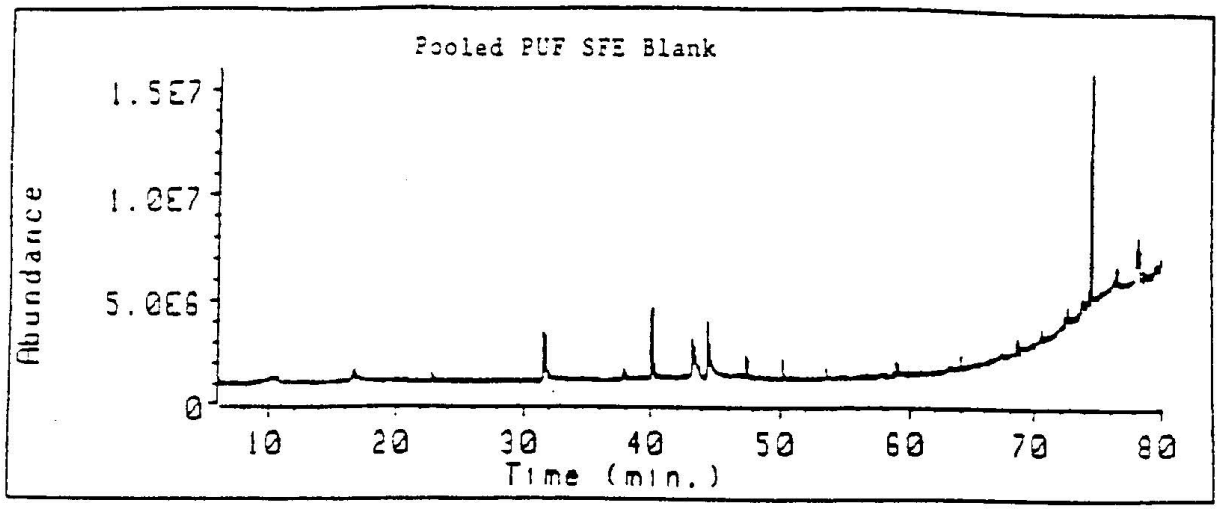


Figure 19. Total ion chromatogram of PUF SFE fractions 4-6



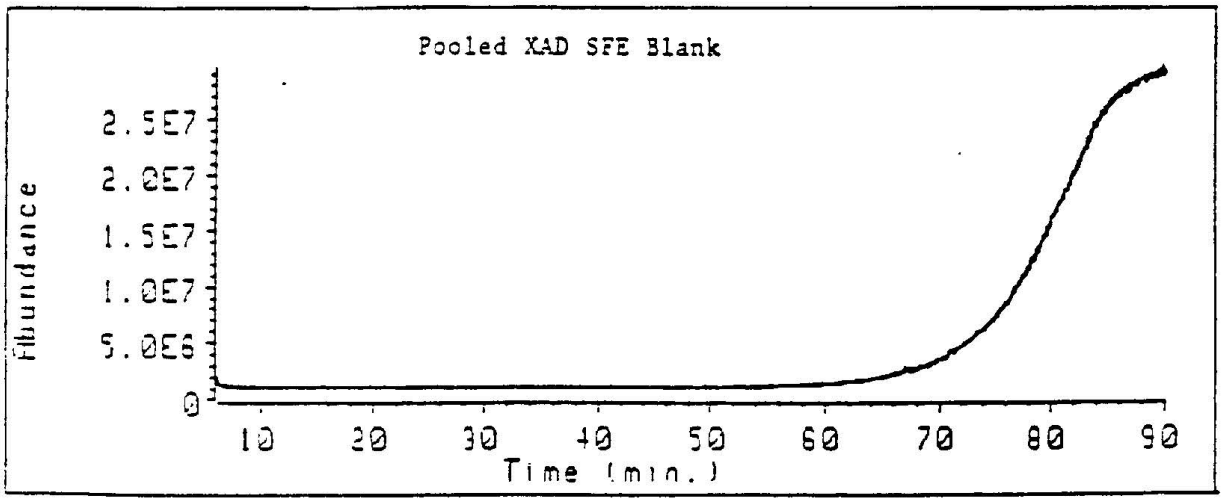


Figure 20. Total ion chromatogram of pooled SFE fractions of PUF and XAD blanks.

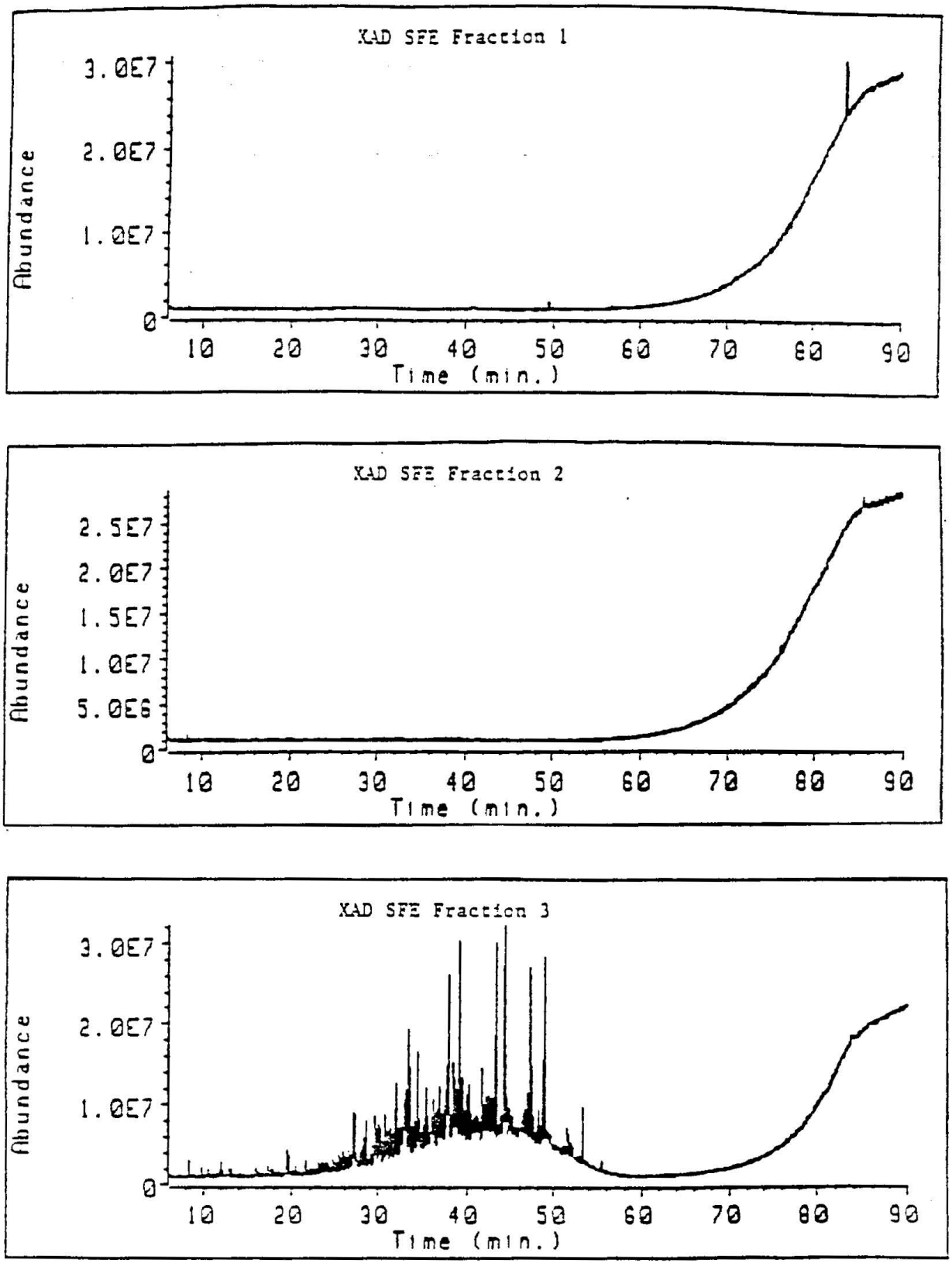
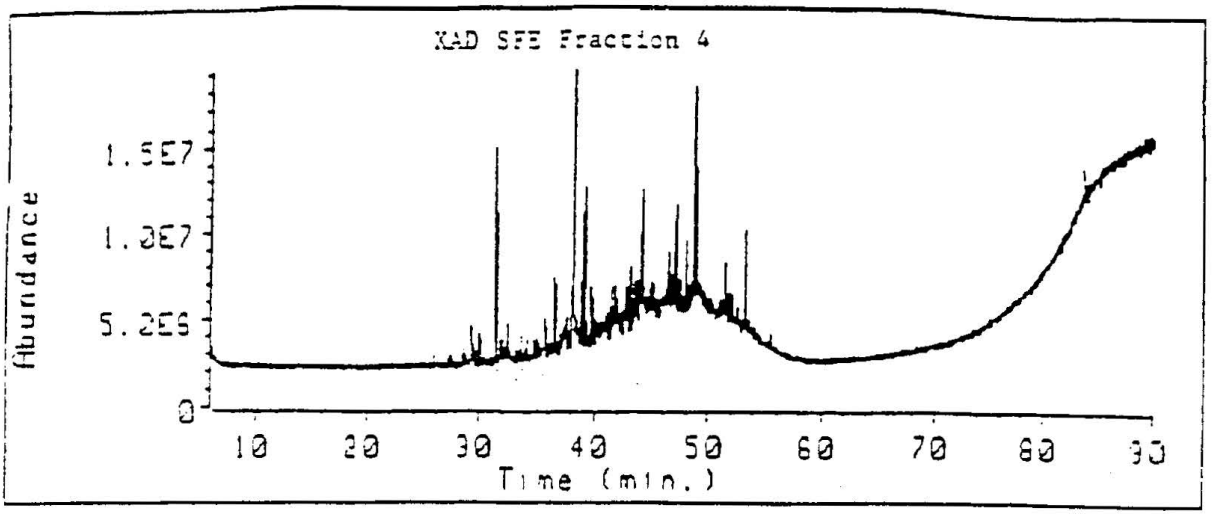
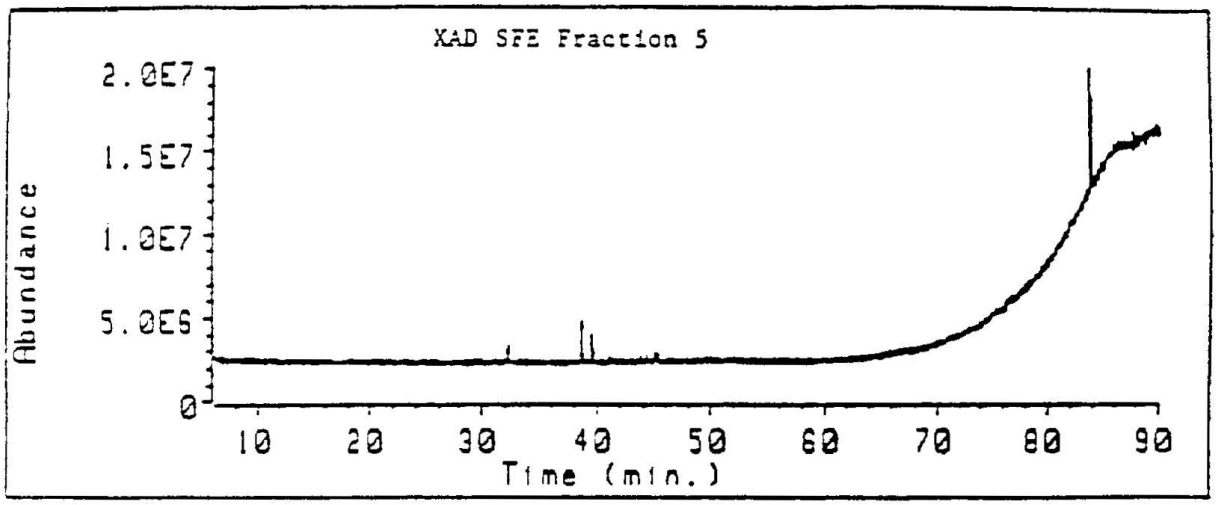


Figure 21. Total ion chromatogram of XAD SFE fractions 1-3.





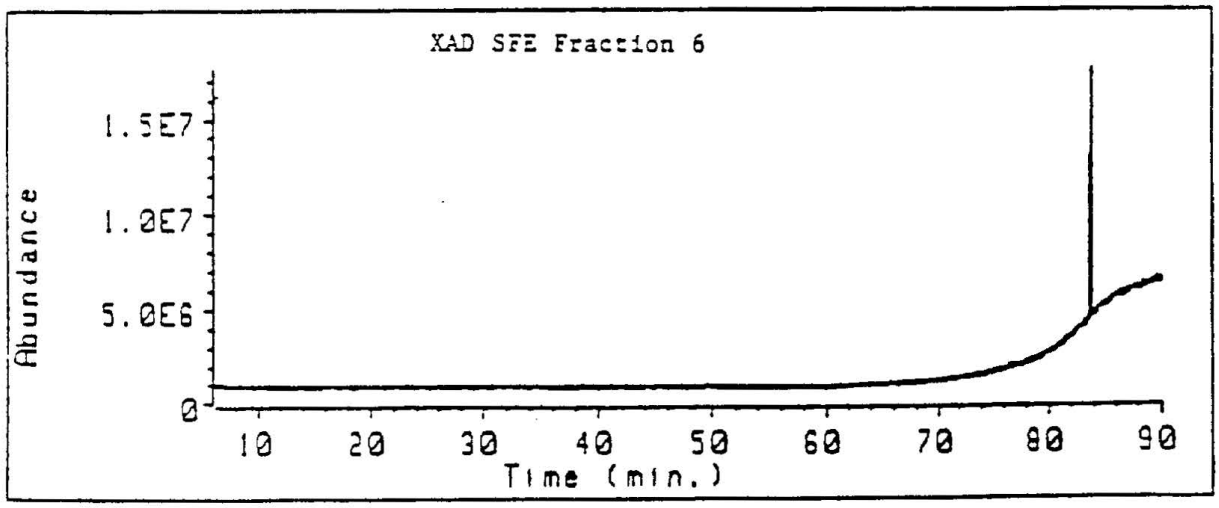


Figure 22. Total ion chromatogram of XAD SFE fractions 4-6.

Table 3. Compounds Detected in the XAD-4 SFE Fractions

Retention Time (min.)	Name or Class of Compound (*positive identification)	Structure # Appendix A	Characteristic Ions
8.339	toluene*	2	77, 91, 121
11.621	ethyl benzene*	2	91, 106
11.999	m-/p-xylene*	2	91, 106
12.843	styrene*	2	104
13.148	o-xylene*	2	91, 106
17.347	alkyl benzene	2	105, 120
17.857	alkyl benzene	2	105, 120
18.636	alkyl benzene	2	105, 120
19.682	alkyl benzene	2	105, 120
21.68	alkyl benzene	2	105, 120
23.912	alkyl benzene	2	91, 105, 134
24.046	alkyl benzene	2	119, 134
25.517	alkyl benzene	2	119, 134
25.947	alkyl benzene	2	119, 134
27.17 27.36	a-methyoxy, methyl ester of benzene acetic acid alkane		77, 91, 121 57, 71, 85
28.099	alkyl benzene	2	119, 134
28.331	alkyl benzene	2	91, 119, 134
28.62	decahydromethyl naphthalene		81, 137, 152
29.52	methyl dihydro indene		117, 132
29.7	decahydro methyl naphthalene		137, 152
30.2	methyl dihydro indene		117, 131
31.47	alkane		57, 71, 85, 126
32.16	naphthalene*		128
33.15	alkyl dihydro indene	12	131, 146
33.61	alkane	6	57, 71, 85
34.55	alkane		57, 71, 85
36.26	alkyl dihydro indene		131
37.06	alkyl tetrahydro naphthalene		131, 146
37.9	alkane		57, 71
38.63	methyl naphthalene*	4	115, 142
38.8	alkene		55, 69, 83, 125, 126
39.22	alkane		57, 71, 85
39.54	methyl naphthalene*	4	115, 142
39.74	dimethyl tetrahydro naphthalene	6	118, 145, 160
39.88	cyclo hexylbenzene	5	104, 117, 160
40.26	alkane		57, 71, 85
41.73	alkane		55, 76, 83
42.53	alkane		57, 71, 95
42.89	alkane		57, 71, 95
43.31	alkane		57, 71, 95
44.1	alkane		57, 71, 95
44.29	alkane		57, 71, 95
44.56	alkene		57, 71, 95
45.12	ethyl / dimethyl naphthalene	4	141, 156
46.37	alkyl octahydro indene	12	
47.35	alkane		57, 71, 95
47.67	alkane		57, 71, 95
48.93	alkane		57, 71, 95
51.44	alkane		57, 71, 95
53.23	alkane		57, 71, 95
83.27	diethylhexyl phthalate*		149

using a gentle stream of nitrogen. Since water and acetonitrile form an azeotrope, acetonitrile was continually added in order to completely remove the water.

Analysis of these concentrated HPLC subfractions revealed the loss of many volatile compounds. Spiking experiments were conducted to determine the cause of compound loss and to optimize the blowdown procedure. Naphthalene, 1- and 2-nitronaphthalene, phenanthrene, and pyrene were spiked into various solutions of acetonitrile and water. All experiments showed a greater loss of the lower boiling compounds such as naphthalene and 1- and 2-nitronaphthalene, compared to phenanthrene and pyrene. The greatest loss measured was that of naphthalene. The loss of naphthalene occurred even under the most gentle nitrogen blowdown conditions. The loss of lower boiling compounds in the PUF fractions was considered acceptable, since naphthalene was one of the most volatile components in the PUF fraction.

Once the nitrogen blowdown procedure was optimized, the PUF HPLC fractions were blown down for further analysis. HPLC fractions 1 through 4 were concentrated to a volume of 1 mL to prevent excess loss of the lower boilers, while fractions 5 through 9 were concentrated to a volume of 300 µL. The more polar HPLC subfractions (1 through 4) required the addition of more acetonitrile than the nonpolar fractions (5 through 9) to remove excess water.

The loss of volatiles was of great concern for the XAD HPLC fractions, since naphthalene is one of the higher boiling components present in these fractions. Before blowing down the XAD HPLC fractions, the blowdown of a model test mixture must be conducted to determine if blowdown is a feasible method for concentrating these fractions. Due to time constraints, the blowdown of a model test mixture and the XAD HPLC fractionation procedure was not completed.

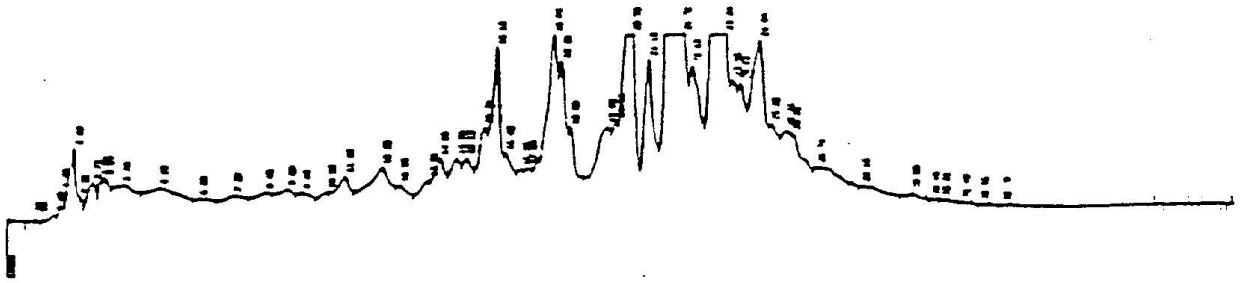


Figure 23. HPLC chromatogram of the pooled PUF SFE fractions.
(UV detector; λ = 254 nm)

ii. GC-MS analysis of the PUF HPLC subfractions

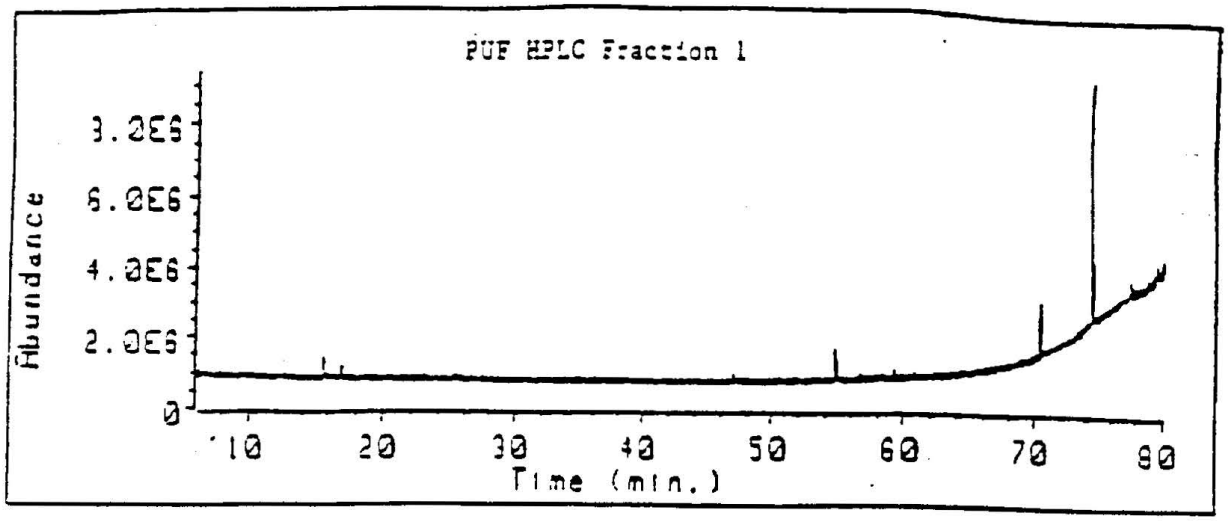
From the combined PUF SFE fractions 2 and 3, a total of nine HPLC subfractions were collected, concentrated, and then analyzed by GC-MS. A 1 μL aliquot was used for GC-MS analysis. Library searches were conducted on each fraction to identify the compounds in each fraction. Whenever possible, mass spectra and retention times of unknown compounds were compared with those obtained with authentic standards. In addition, the characteristic ions of mass spectra were used to determine the chemical structures of compounds.

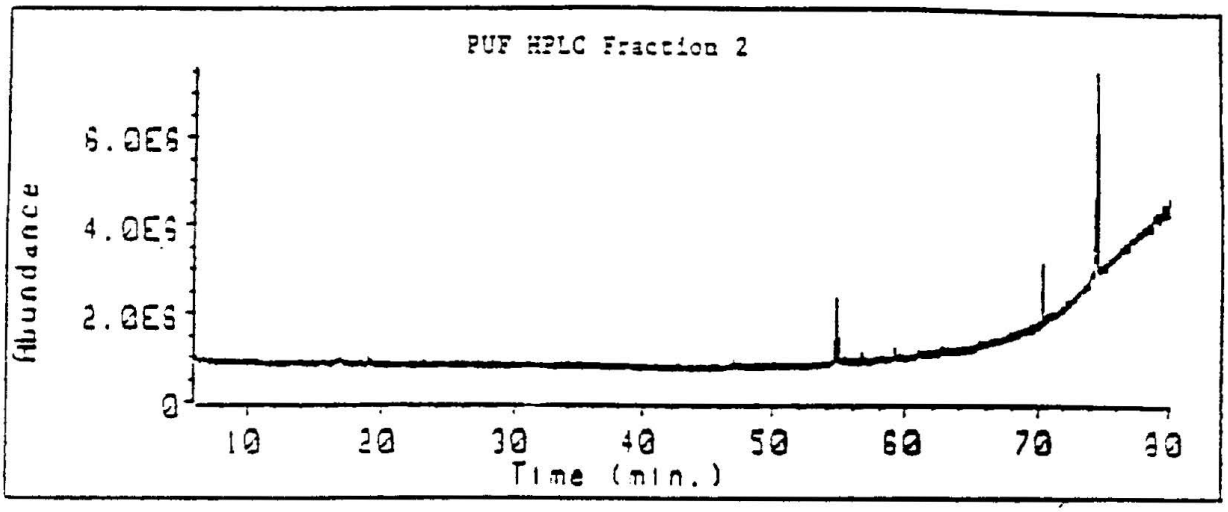
Fraction 1: Fraction 1 is the most polar PUF HPLC subfraction and contains approximately 80% water and 20% acetonitrile. Figure 24 shows the TIC for Fraction 1 after concentration by nitrogen blowdown. The fraction did not contain any major peaks and a library search using the National Bureau of Standards (NBS) library did not result in the identification of unknowns.

Fraction 2: Fraction 2 is the next most polar HPLC subfraction. The TIC for this fraction after concentration did not contain any major compounds. A number of trace components might be present in this fraction, but their detection requires further concentration of the fraction. The library search did not result in any good matches. The TIC for this fraction is shown in Figure 24.

Fraction 3: Fraction 3 contained some measurable peaks, although no good matches were obtained from the library search. Figure 24 shows the TIC for this fraction.

Fraction 4: Fraction 4 contained some peaks. Further concentration of this fraction could reveal a number of trace components. The largest peak in this fraction is naphthalene, as confirmed by mass spectra and retention time. Figure 25 shows the TIC for this fraction.





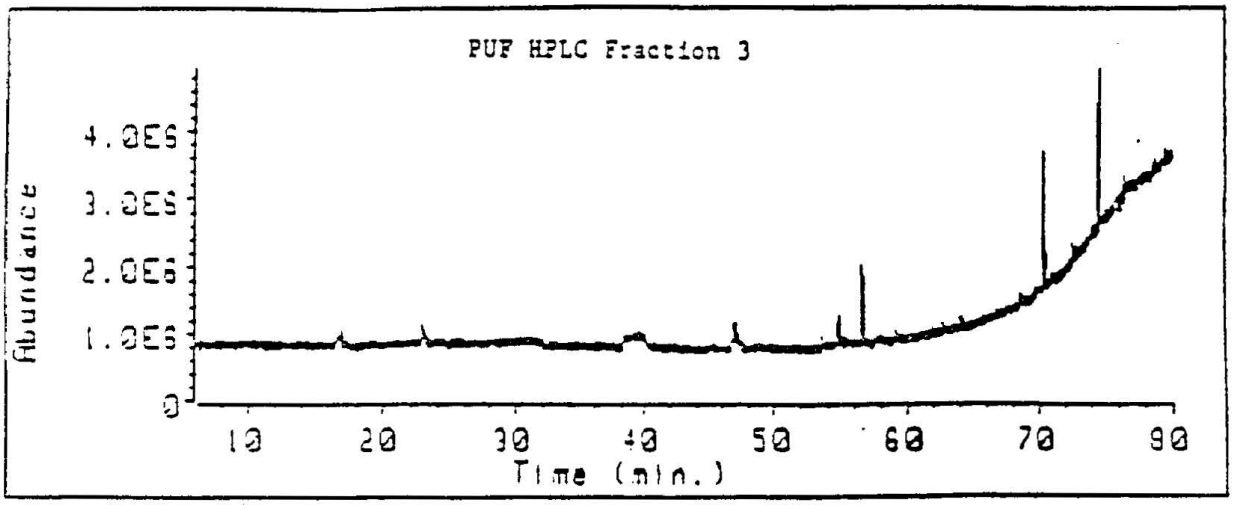


Figure 24. Total ion chromatogram of concentrated PUF HPLC fractions 1-3.

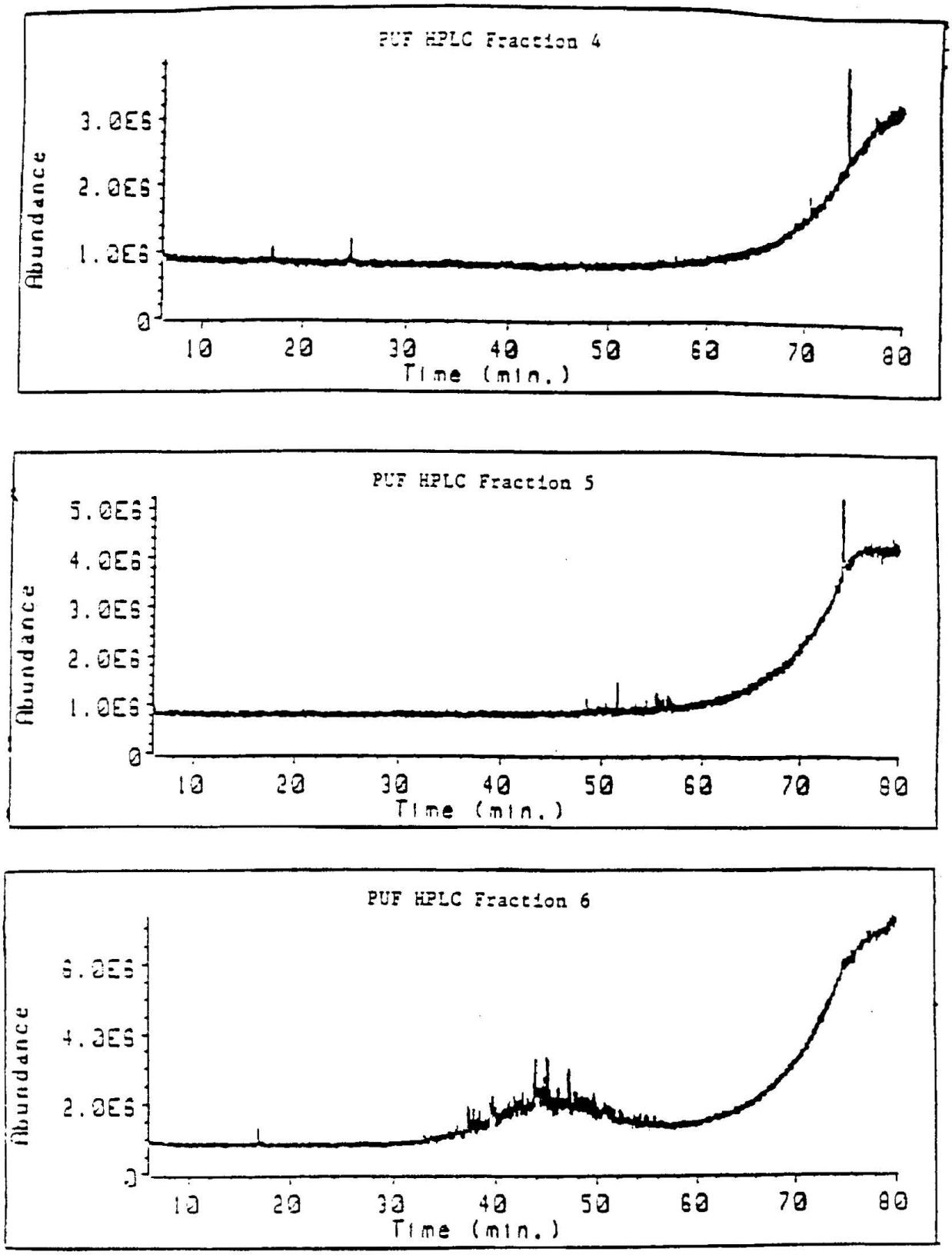


Figure 25. Total ion chromatogram of concentrated PUF HPLC fractions 4-6.

Fraction 5: Most of the compounds in PUF HPLC Fraction 5 were lost during the blowdown procedure due to the incomplete removal of water. During the blowdown, a phase separation occurred between the water and acetonitrile and only the acetonitrile phase was reduced in volume. As a result, many of the compounds soluble in the acetonitrile were lost. The remaining water fraction was yellow in color. The TIC for this fraction is shown in Figure 25.

PUF HPLC Fraction 5 contained a large percentage of the total mass of the PUF fraction. The GC-MS TIC of the unconcentrated Fraction 5 exhibited detectable peaks.

The major compounds in Fraction 5 appeared to be alkyl naphthalenes. The mass spectra were consistent with mono-, di-, and tri-alkyl naphthalenes, although isomers can have almost identical mass spectra unless resolved using authentic standards. The mass spectra of the compounds in Fraction 5 were consistent with methyl and ethyl substituted naphthalenes. Phenanthrene was also found in this fraction, along with alkylated phenanthrenes.

The compounds identified in this fraction are shown in Table 4. Where positive identification was not possible, a tentative identification of chemical class is given. Retention times and key ions used to determine the structure of these compounds are also listed.

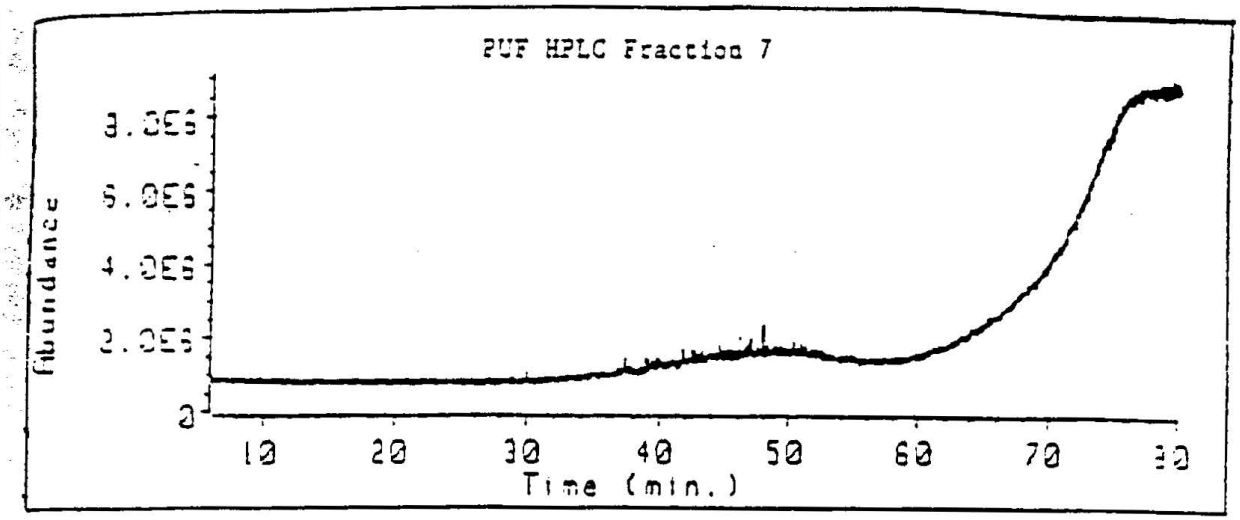
Fraction 6: The TIC for Fraction 6 is shown in Figure 25. The chromatogram shows numerous trace peaks. A library search on this fraction resulted in no good matches. Many of the spectra are of poor quality due to interferences from overlapping peaks. Some of the peaks found in this fraction may be long chain alkyl substituted aromatics.

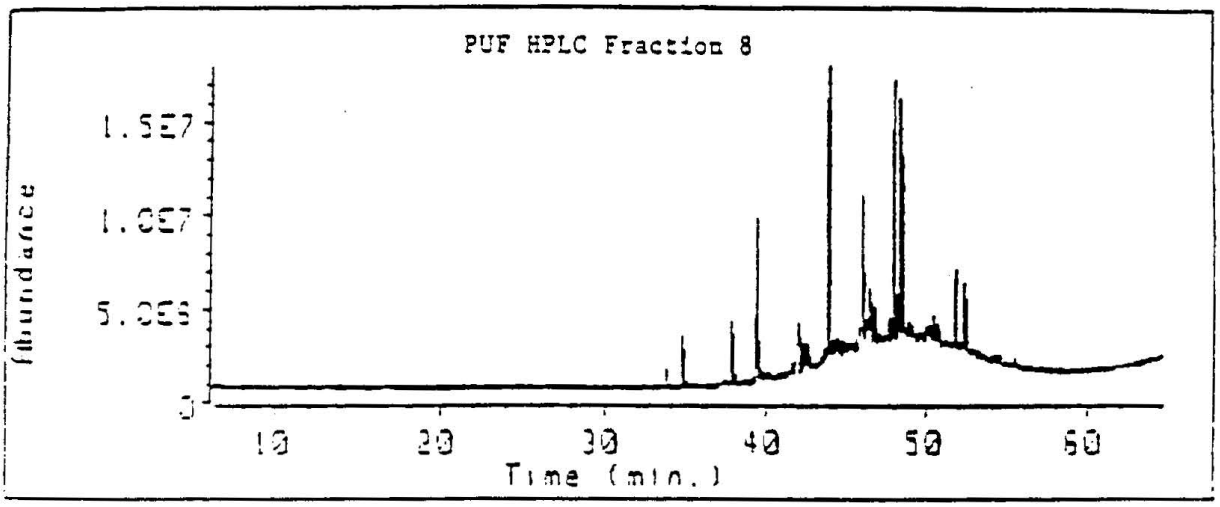
Fraction 7: The TIC for this fraction is shown in Figure 26. Although no major peaks were found in this fraction, there were many trace components present. The analysis of this fraction was difficult since most of the peaks were smaller than those in Fraction 6.

Table 4. GC-MS Analysis of PUF HPLC Fraction 5.

Retention Time (min.)	Name or Class of Compound	Structure # Appendix A	Characteristic Ions
30.03	2-methyl naphthalene	4	115, 142
30.86	1-methyl naphthalene	4	115, 142
33.90	biphenyl	15	154
34.90	ethyl naphthalene	4	144, 156
35.45	dimethyl naphthalene	4	144, 156
36.18	dimethyl naphthalene	4	144, 156
36.36	dimethyl naphthalene	4	144, 156
37.16	dimethyl naphthalene	4	144, 156
37.30	dimethyl naphthalene	4	144, 156
37.89	dimethyl naphthalene	4	144, 156
38.92	methyl biphenyl	16	152, 168
40.16	methylethyl naphthalene	4	155, 170
40.95	trimethyl naphthalene	4	155, 170
41.16	trimethyl naphthalene	4	155, 170
41.42	trimethyl naphthalene	4	155, 170
42.13	trimethyl naphthalene	4	155, 170
42.33	trimethyl naphthalene	4	155, 170
42.83	trimethyl naphthalene	4	155, 170
42.94	trimethyl naphthalene	4	155, 170
43.67	trimethyl naphthalene	4	178
51.95	phenanthrene	4	89
53.00	alkyl PAH		179
53.29	alkyl PAH		179
54.33	methyl phenanthrene/anthracene	14/17	165, 192
55.81	methyl phenanthrene/anthracene	14/17	165, 192
56.93	methyl phenanthrene	14	192

Fraction 8: The TIC for Fraction 8 is shown in Figure 26. Identification of the alkanes in this fraction was difficult without data from authenic standards. Most alkanes have similar mass spectra and alkane isomers are almost impossible to distinguish from each other. The information on the alkanes in Fraction 8 is presented in Table 5.





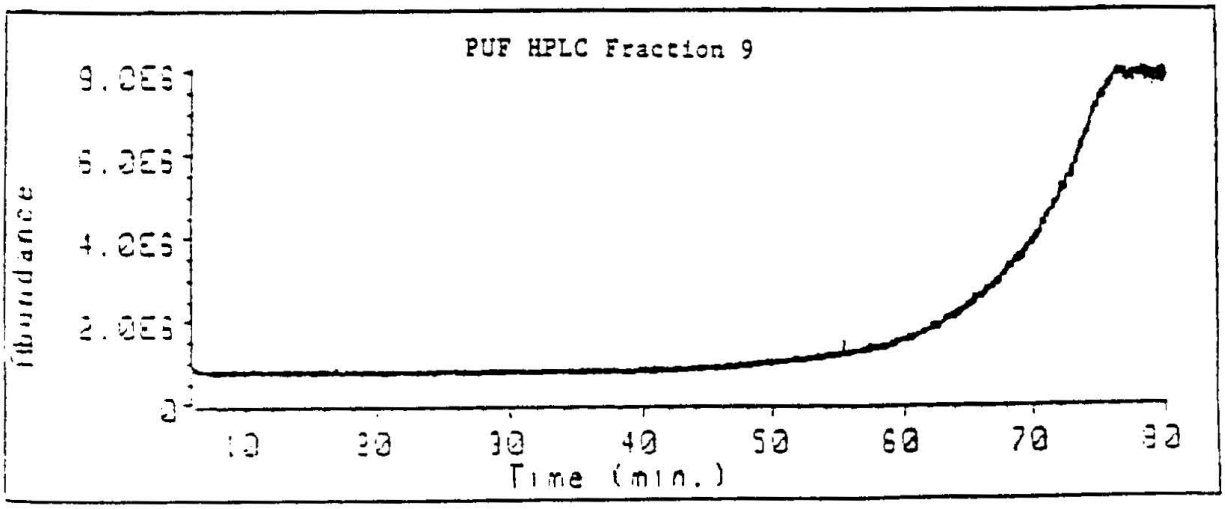


Figure 26. Total ion chromatogram of concentrated PUF HPLC fractions 7-9.

Table 5. GC-MS Analysis of PUF HPLC Fraction 8

Retention Time (min.)	Name or Class of Compound	Characteristic Ions
34.79	alkane	57, 71, 85, 127
37.75	alkane	57, 71, 85, 141
39.44	alkane	57, 71, 85
42.28	alkane	57, 71, 85
42.51	alkane	57, 71, 85, 127, 183
43.83	alkane	57, 71, 85, 127, 183
45.74	alkane	57, 71, 85, 127
45.99	alkane	57, 71, 85, 127, 169, 183
46.81	alkane	57, 71, 85
47.93	alkane	57, 71, 85, 127
48.34	alkane	57, 71, 85, 127, 183
51.78	alkane	57, 71, 85, 127, 183
52.33	alkane	57, 71, 85, 127, 183

Fraction 9: Fraction 9 was the last PUF HPLC fraction collected. The TIC for this fraction is shown in Figure 26 and indicates that most of the components had eluted in HPLC Fractions 1 though 8.

5. Summary

In DS III, sufficiently large samples of undiluted diesel exhaust were collected for 3 hr at a flowrate of 2 LPM by replacing the sampling train filter every 60 min to prevent high PM loading of the filters. Six SFE fractions were obtained from each PUF, XAD, and field blank sample as in DS II.

The highest mutagenicity was found in PUF SFE fractions collected at 2000 and 3000 psi. Significant mutagenic activity was also detected in the XAD SFE fractions, with the highest activity found in fractions collected at 3000 and 4000 psi.

Reverse-phase HPLC was used to sub-fractionate the two most mutagenic PUF SFE fractions to facilitate identification of their chemical components. The PUF HPLC fractions were concentrated for GC-MS analysis. Loss of compounds occurred during the concentration procedure for fraction 5, the PAH fraction. The compounds in the concentrated HPLC fractions were tentatively identified and included naphthalene, alkyl naphthalenes, phenanthrene, alkyl phenanthrenes, alkyl substituted aromatics, and alkanes.

V. DISCUSSION

In this investigation, an interdisciplinary approach was used to develop a mutagenicity-directed chemical analysis procedure for VM collected from diesel truck exhaust. Our goal was to demonstrate the feasibility of specific methods for trapping, extraction and concentration of VM from diesel exhaust and to integrate these methods with an adapted mutagenicity assay and chemical analysis.

The first part of this project required the assembly of a sampling train using commercially available adsorbents. The use of adsorbents was favored over *in situ* detection so that field samples could be conveniently acquired, transported to the laboratory, and analyzed by both the mutagenicity bioassay and chemical analysis. The sampling system consisted of a Teflon filter to trap PM, followed by PUF and XAD adsorbents, in series, to collect VM. Based on previous experience and other reports, at least two different adsorbents are necessary for trapping the wide range of volatile compounds which may be present in the exhaust. PUF was used to trap the moderate and less volatile compounds, and XAD was used to trap the more volatile chemicals.

Volatiles adsorbed on PUF and XAD were extracted with s-CO_2 into a very small volume of methanol as the collection solvent. Using SFE, it was possible to achieve some degree of fractionation for each adsorbed sample by changing the extraction pressure. The multiple fractions so obtained were amenable to analyses by both the mutagenicity assay and GC-MS initially without further concentration. The traditional method of Soxhlet extraction for 6 to 24 hrs has limited utility because it requires large volumes of solvent and a concentration step with an unavoidable loss of volatile compounds (Alfheim *et al.*, 1984, Dorie *et al.*, 1987).

The *Salmonella*/microsuspension mutagenicity assay for VM was based on our previous work, using a culture tube fitted with screw-top cap (Hsieh *et al.*, 1990; Kado *et al.*, 1992). This is consistent with the results of Hughes *et al.* (1984; 1987) who reported that a 1-dram vial was useful in a *Salmonella* pre-incubation procedure for detecting semi-volatile mutagens. Recently, Arey *et al.* (1992) successfully used the same assay to investigate ambient vapor-phase mutagenicity.

In this project, a pilot field study (DS I) was designed and conducted to collect samples of emissions that were expected to contain VM. In DS I, undiluted emission samples were collected from cold-start diesel truck exhaust. The cold-start exhaust contained relatively large quantities of

PM that quickly accumulated on the Teflon filters and severely limited the sampling time and sample size of vapor-phase compounds. No significant mutagenic activity was found in the SFE fractions of PUF or XAD samples due to insufficient amounts of sample. To reduce particle loading on the filters and to increase sample size on PUF and XAD, the exhaust from a pre-warmed diesel engine was sampled in a semi-preparative study (DS II).

Based on the results for one of two sampling trains (train 1) in DS II, almost all SFE-extractable compounds from PUF or XAD were found in the first SFE fraction obtained by continuous extraction at 3000 psi for 1 hr. This fraction contained too many compounds which prevented the identification of the mutagenic components, suggesting a need for further fractionation. The bioassay results indicate that the PUF SFE extract was more mutagenic than the XAD SFE extract.

The second sampling train (train 2) run in parallel with train 1 in DS II, was used to investigate SFE fractionation using six different pressures. The PUF SFE fractions were again found to be more mutagenic than the XAD SFE fractions. The highest mutagenic activity was found in the 2000 psi and 3000 psi SFE fractions of PUF and in the 3000 psi and 4000 psi SFE fractions of XAD, all extracted for one hr. When the two PUF fractions were individually concentrated by gentle blow-down with nitrogen followed by solvent exchange and tested for mutagenicity, toxicity to the bacterial tester strains was observed. This toxicity suggested that there was a net increase in the concentration of certain organics during the blow-down process, despite the loss of some volatiles. Toxicity was not observed in the samples obtained from a preparative study (DS III) with the same treatment. The only difference between the two studies was that the DS II samples were obtained under conditions of much higher humidity and shorter sampling times than the DS III samples. The cause of the toxicity was not investigated. GC-MS chromatograms for both PUF and XAD SFE fractions indicate that, under the experimental conditions, a majority of the trapped compounds were extracted in fractions 2 through 4, with fractions 2 and 3 being the most chemically complex.

Based on DS II results, chemical identification of VM present in the diesel engine exhaust, would require further fractionation of the most mutagenic SFE fractions by HPLC. To obtain a large enough sample for HPLC sub-fractionation, DS III was conducted to sample diesel exhaust for

3 hrs. The DS III samples were extracted and fractionated by SFE following the same scheme as for DS II Train 2. The DS III mutagenicity results were consistent with those for DS II. The PUF neat fractions were more mutagenic than the XAD neat fractions, with the highest mutagenic activity appearing in the 2000 psi and 3000 psi SFE fractions of PUF and in the 3000 psi and 4000 psi SFE fractions of XAD. Overall mutagenic activity of each of these fractions was much greater than that of the comparable fractions from DS II Train 2, most likely due to increased amounts of sample from longer sampling times.

The results for DS III sampling trains 1 and 2, as summarized in Figures 27 and 28, give a comparison of the PM-associated mutagenicity with that of VM (PUF + XAD) in the diesel exhaust. For both TA98 and TA100, the addition of S9 did not significantly change the mutagenicity profile. The mutagenicity observed for TA100 was significantly greater than that for TA98. This result is in agreement with that of Matsushita *et al.*, (1986) where *Salmonella* bacteria were directly exposed to pre-filtered diesel exhaust in a chamber.

Westerholm *et al.* (1991) reported that for solvent extracted PUF and XAD samples, the activities for TA98 were higher with the addition of S9, while the activities for TA100 were higher without the addition of S9. These authors also reported that the contribution of the semi-volatile phase to the total mutagenic activity (particle and semi-volatile associated activities) was approximately 20% in strain TA100 (with and without the addition of S9), approximately 10% in TA98 (-S9), and 37% in TA98 (+S9). The mutagenic activities in the extracts of PM reported were greater without the addition of S9 microsomal enzymes compared to when S9 was added.

In the current as well as in other studies of diesel PM using these tester strains, mutagenic activity is generally greater when S9 is added. We also found approximately 50% of the total mutagenic activity (TA100 either with or without S9) in the vapor-phase. This is higher than reported values and could be due to a number of factors, including: 1) differences in sampling and extraction, 2) differences in the diesel exhaust, and 3) differences in the bioassay procedures used. Another interesting finding was the higher yield of mutagenicity in the PUF compared to the XAD samples. This could be due to a number of factors including: 1) the semi-volatile compounds trapped on PUF are more potent mutagens or are present in relatively higher concentrations,

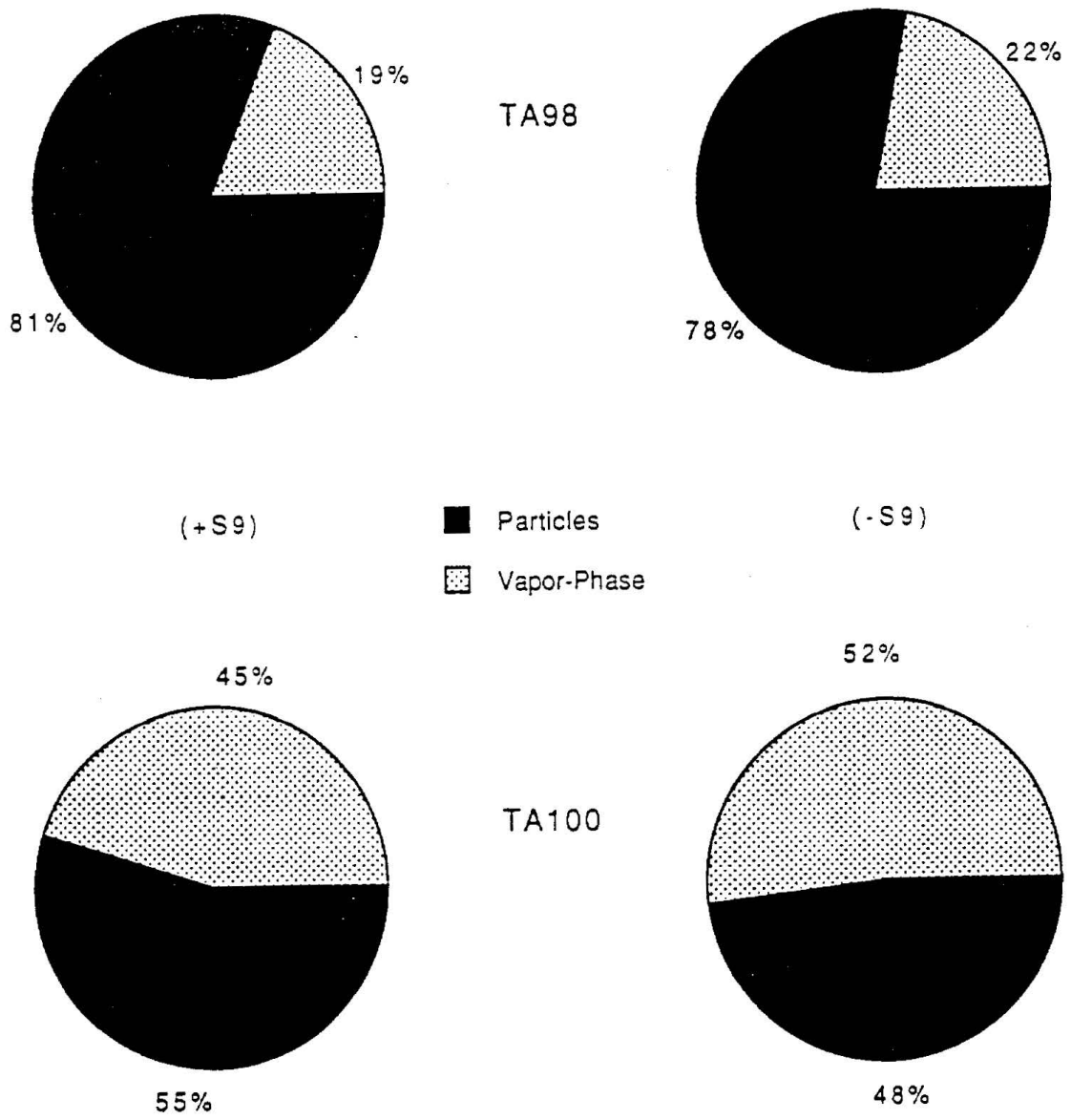


Figure 27. Mutagenicity of Particles vs. Vapor-Phase for Diesel III Train 1 using TA98 and TA100 with and without S9

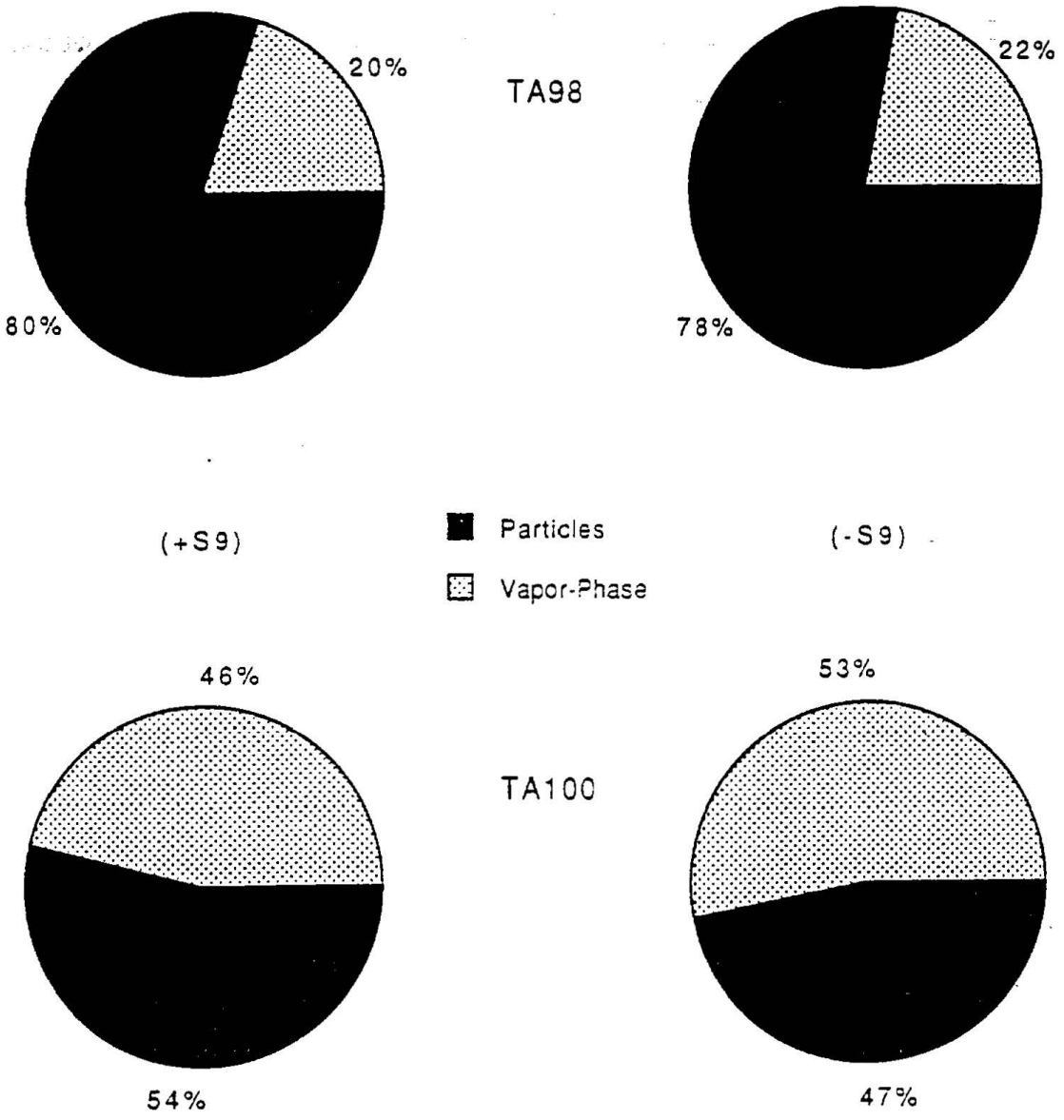


Figure 28. Mutagenicity of Particles vs. Vapor-Phase for Diesel III Train 2 using TA98 and TA100 with and without S9

2) SFE is inefficient for extracting compounds trapped on XAD, and 3) nitrogen oxides present in diesel exhaust are chemically reacting with the adsorbents. The reaction of nitrogen oxides with XAD resin has been reported by Hanson *et al.* (1981). Schuetzle (1983) reported that exhaust gases may react with XAD-2 to form toxic compounds such as quinones. The formation of mutagen artifacts directly resulting from the reaction of nitrogen oxides with XAD cannot be ruled out in our study, although the total contribution of mutagenic activity from the XAD samples is substantially less compared to the PUF samples.

The HPLC sub-fractionation was performed on a pooled sample of the most mutagenic PUF SFE fractions. The HPLC sub-fractions were subsequently concentrated for GC-MS analysis with procedures developed to minimize potential loss of volatile compounds. The loss of compounds has been reported in studies where vapor-phase diesel compounds were extracted using Soxhlet extraction followed by solvent evaporation for sample concentration (Alfheim *et al.*, 1984; Dorie *et al.*, 1987).

With respect to the chemical characterization of diesel exhaust, Bagley *et al.* (1987) chemically characterized compounds in diesel PM and in the vapor-phase as trapped on XAD-2 resin using an ultra-high volume sampler. The lowest boiling point compounds reported included parent and substituted napthalenes, fluorenones, anthracenes, phenanthrenes, and fluorenes. Westerholm *et al.* (1991) reported a number of semi-volatile PAHs collected using PUF and XAD-2 including phenanthrene or substituted phenanthrenes, fluoranthene, pyrene, and anthracene. The authors used volumetric sampling flow rates of 240 and 340 LPM for XAD-2 and PUF sorbents, respectively.

In the current study, substituted naphthalenes, phenanthrenes, and/or anthracenes were tentatively identified. In the PUF SFE extracts, the predominant PAHs were substituted naphthalenes. GC/MS analyses of the PUF extracts indicated that only the alkanes were detected in higher abundance than the substituted naphthalenes. The XAD SFE extracts generally contained lower boiling point compounds. Among the most volatile components identified were toluene, ethyl benzene, m-,p-,o-xylene, and styrene. Other alkylated benzenes as well as substituted naphthalenes were also tentatively identified in the XAD SFE extracts. The substituted benzenes along with alkanes were the major components of the XAD extract.

The chemical classes of the major components of both the XAD and PUF extracts have been tentatively identified. Positive identification of specific compounds must be done by matching the retention time and mass spectra of the unknown component with authentic standards. Rather than identifying all the components in the extracts, the extracts were subfractionated by HPLC and subjected to further chemical analysis. Procedures were developed to minimize potential loss of volatile compounds when concentrating the HPLC subfractions. The loss of compounds has been reported in studies where vapor-phase diesel compounds were Soxhlet extracted followed by solvent evaporation for sample concentration (Alfhein *et al.*, 1984; Dorie *et al.*, 1987).

Pooled PUF SFE extracts were subfractionated by HPLC and concentrated by solvent evaporation. GC/MS analysis of the concentrated HPLC subfractions again confirmed the major components to be hydrocarbons and alkylated PAHs, namely alkyl naphthalenes. These fractions await further bioassay testing and chemical analysis to further reduce the number of candidate mutagens.

The results of the present study indicate that the mutagenicity associated with VM in diesel exhaust is sufficiently significant to warrant further investigation and assessment. The mutagenicity-directed chemical analysis techniques developed in this study will help to further chemically characterize the most potent vapor-phase mutagens present in diesel exhaust and other combustion sources.

VI. RECOMMENDATIONS FOR FUTURE RESEARCH

In the present investigation, a mutagenicity-directed chemical analysis procedure was developed for the identification of major VM in diesel exhaust. The procedure involves collecting vapor-phase compounds on PUF and XAD adsorbents, desorbing and concentrating by SFE, testing for mutagenicity using a microsuspension assay, and qualitatively identifying the major chemical components in the mutagenic SFE fractions by GC-MS analysis. To facilitate GC-MS identification, the two most mutagenic SFE fractions from the PUF sample were pooled and subfractionated by reverse-phase HPLC. When the PUF HPLC subfractions were concentrated, some volatile compounds were lost. In order to further enhance and optimize our analytical capabilities for VM, we offer the following suggestions:

1. Diesel Study III should be repeated to collect a larger sample for the quantitative identification of VM in the diesel exhaust. Further comparisons of PM and vapor-phase compounds should also be investigated using the microsuspension mutagenicity assay and GC-MS analysis.

2. The SFE fractionation methods can be refined by optimizing the extraction pressure in order to facilitate the chemical separation and identification of mutagens in complex diesel exhaust mixtures. The use of SFE solvent modifiers should be evaluated and compared with s-CO_2 for the efficient extraction and fractionation of diesel exhaust VM adsorbed on PUF and XAD. These modifications could result in the elimination of the laborious HPLC subfractionation procedure.

3. To increase the recovery of volatile mutagenic compounds from the HPLC subfractions, the subfraction could be passed over a column containing XAD resin or another suitable adsorbent, extracted by SFE, and transported into a small volume of

solvent for mutagenicity testing and GC-MS analysis. Once the VM have been identified, they could be used as model compounds to further optimize their recovery from diesel exhaust.

4. Tester strains that are insensitive to nitro-substituted PAH should be incorporated into the study of VM. These tester strains indicate the presence of these potent mutagens.

5. To identify more vapor-phase compounds in diesel exhaust, authentic chemical standards can be obtained for the creation of a GC-MS database of authentic standards.

6. Investigations of artifact formation or loss of VM should be incorporated into future studies. One method to investigate this process is to use deuterated chemical standards of similar chemical characteristics of compounds tentatively identified.

7. The analytical procedures that we have developed herein for VM should be applied to and optimized for other emission sources viewed as important by the Air Resources Board such as other mobile sources, or stationary sources such as toxic waste sites and industrial emissions.

Based on results of the present study, VM are an important component of diesel engine emissions and are worthy of continued study to evaluate and better define potential human exposure. Future extensions of our work could include the characterization of VM in other emission sources and in the ambient environment. The analytical methods that we have developed for vapor-phase compounds would help the Air Resources Board to better evaluate health risk to compounds present in complex emissions and in ambient air.

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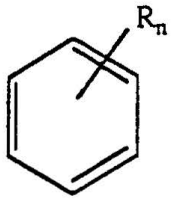
APPENDIX. CHEMICAL STRUCTURES

1. Benzene

2. Alkylbenzene
   R = Me, Et



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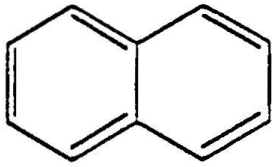

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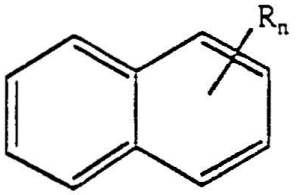

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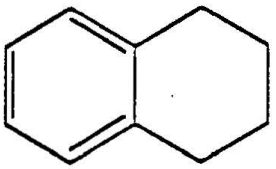

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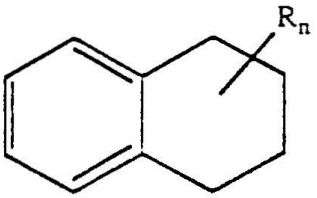
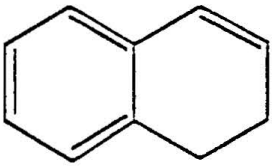


7. Dihydronaphthalene



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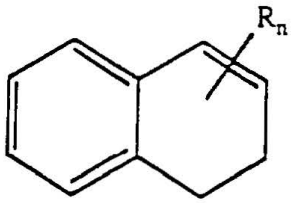
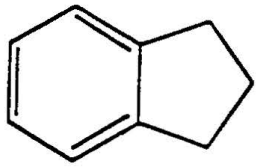
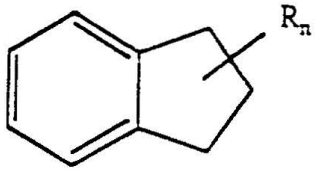


9. Indan



10. Alkyl Indan



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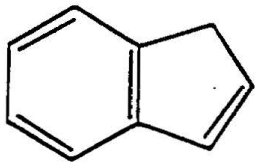

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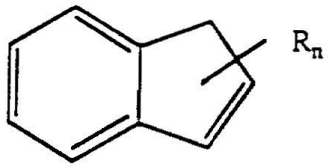

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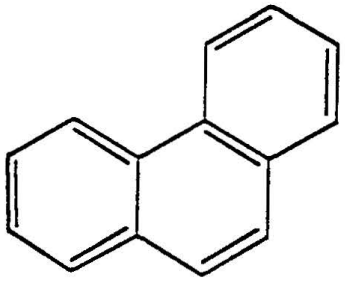

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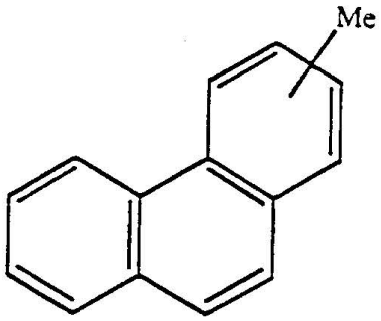
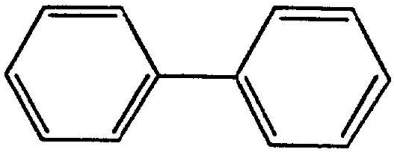


15. Biphenyl



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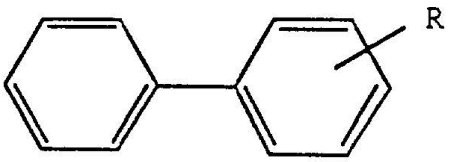

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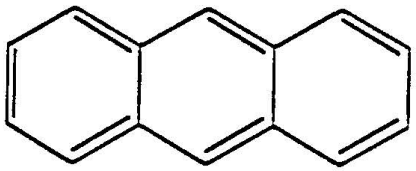
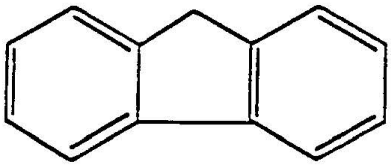


18. Fluorene



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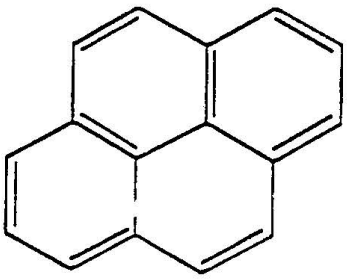

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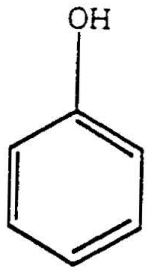

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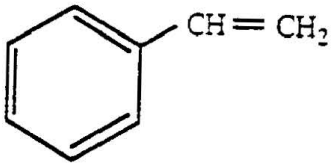

22. Formaldehyde