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Characterization of laboratory simulated road paving-like asphalt by high performance liquid chromatography and gas chromatography-mass spectrometry

Brandon F. Law
West Virginia University

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Characterization of Laboratory Simulated Road Paving-Like Asphalt by High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry

Brandon F. Law

Thesis submitted to the
College of Engineering and Mineral Resources
at West Virginia University
in partial fulfillment of the requirements
for the degree of

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in
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Warren Myers, Ph.D., Chair
Steven E. Guffey, Ph.D.
Paul D. Siegel, Ph.D.

Department of Industrial and Management Systems Engineering
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Abstract

"Characterization of Laboratory Simulated Road Paving-Like Asphalt by High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry"

Prolonged, extensive exposure to asphalt fume has been associated with several adverse health effects. Inhaled polycyclic aromatic hydrocarbons (PAHs) from asphalt fume exposure are of concern. The objective of this study was to characterize both qualitative and quantitative differences between fumes generated at 150 ^{o}C and 180 ^{o}C using a well controlled laboratory road paving fume generation system. Fumes were characterized by (1) total vapor and particulate concentration, (2) simulated boiling point profile and (3) specific PAH content. The mean concentrations of the vapor fractions generated at 180 ^{o}C and 150 ^{o}C were 23.3 mg/m^3 and 11.2 mg/m^3, respectively, demonstrating a statistically significant increase in concentration with increasing asphalt temperature. The mean concentrations of the particulate fractions generated at 180 ^{o}C and 150 ^{o}C were 42.4 mg/m^3 and 28.0 mg/m^3, respectively. The simulated boiling point profile did not show a significant qualitative difference between the fumes generated at the two temperatures. Naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, and chrysene were identified and quantified from the fumes.

Acknowledgments

Of course, I owe a thank you to my friends and family. I owe a thanks to my parents George and Carol for always encouraging me. I owe a big thank you to my wife Diedre and our forthcoming child (this will be your first bedtime story). If it were not for the two of you I might have given up on improving myself and taken the easier road.

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1. Introduction

It is estimated that in the United States 300,000 workers are employed in the asphalt paving industry[1]. Several million tons of asphalt are used each year by the paving and roofing industries[2]. The potential for occupational exposure to road paving asphalt fumes is quite high. However, the health risk to workers is not known. The major route of exposure to asphalt fumes is by inhalation. Low levels of total polycyclic aromatic hydrocarbons (PAHs) present in asphalt fume have been detected through environmental and personal sampling during road paving operations. Enhanced levels of urinary 1-hydroxypyrene, a metabolite of PAHs, have been measured in road pavers[3] and roofers[4].

Asphalt fumes are complex and variable mixtures of inorganic particulate (derived from mineral aggregates) and organic compounds that include aliphatics, PAHs, and heteroatomic compounds containing sulfur, nitrogen, and oxygen[5]. The objective of this study is to characterize both qualitative and quantitative differences between fumes generated at $150\,^{\circ}\text{C}$ and $180\,^{\circ}\text{C}$ using a well-controlled laboratory road-paving fume-generation system. Fumes were characterized by (1) total vapor and particulate concentration, (2) simulated boiling point profile, and (3) specific PAH content.

2. Literature Review

Asphalt is a complex mixture of aliphatic and aromatic hydrocarbons obtained during the crude oil refinement process[5]. Asphalt is defined by the American Society for Testing and Materials (ASTM)[6] as a dark brown to black cementitious material in which the predominating constituents are bitumens that occur in nature or are obtained in petroleum processing. There are some naturally occurring asphalts including native asphalt used in ancient construction[7]. Lake asphalt is another naturally occurring product , first used in the United States for paving in 1874[8]. Rock asphalt deposits contain from 5-25% asphalt and exist as "soft flux" to "hard penetration"[9]. Rock asphalt is ground, sized, placed, and rolled to form pavement surfaces. Rock asphalts are found in Texas, Alabama, Oklahoma, Colorado, California, and Kentucky. Asphalt is also produced by processing petroleum crude oils and its composition can vary depending on the source of the crude oil, refinery processes, additives, and the application practices[10]. It is principally used for road paving (~87%) and roofing (~11%) in the United States[1] . It is known that asphalt contains polycyclic aromatic hydrocarbons (PAHs). However, the fumes that are a by product of road paving applications have not been well characterized and continue to pose analytical problems[11]. These problems are due to the complexity of the mixture, the lack of separation, and the lack of specificity in the assays used.

Problems with asphalt characterization are three-fold: (1) the variations in asphalt manufacturing, (2) the variations in feed stock crude oil, and (3) the variations within a single lot of asphalt. Manufactured asphalts derive their characteristics from the nature of the crude origins and by manufacturing process. There are refineries whose prime function is to produce asphalt, but asphalt is principally a product of integrated refineries. These refineries may select crude for a variety of other product requirements and the residuum produced will vary[12,13]. Feedstock crude may vary significantly from different places in the world, or different places in the same oil field. Another problem with asphalt fume sampling is the variation within the same lot of asphalt (i.e. the mixture is

not homogeneous)[14]. The heterogeneous nature may help explain some difference in analytical results reported between laboratory and field samples. Generally, the laboratory generated fume samples come from static, heated asphalt sources that were stirred. In the field no such stirring takes place and fumes are generated from a non-static source. The fume is generated at the surface of the asphalt and stirring may alter the fume by bring pockets of trap volatilized compounds to the surface.

A variety of extraction and analysis methods are available for PAHs. For example, the NIOSH standard method for total PAHs (PACs) uses a cyano (CN) solid phase extraction (SPE) column followed by liquid:liquid extraction into dimethylsulphoxide (DMSO)[15]. The liquid:liquid extraction transfers the PAHs in a non-volatile solvent appropriate for simple spectrofluormetric analyses but difficult to concentrate for trace analyses.   A complex mixture, such as asphalt fume extract contains compounds causing interference with analyses by having similar physical/chemical properties to the compounds of interest. Several studies have used fluorescence techniques to identify PAH compounds[16,17]. This again poses a problem when applied to a complex mixture. Many compounds fluoresce at wavelengths similar to the compounds of interest. Substituted PAHs may fluoresce with slightly different properties than those of the standard PAHs and thus provide poor qualitative and quantitative estimates of the content of classes of compounds.

The compounds of concern in the present study are PAHs. A PAH is a compound with two or more six- or five-membered rings with annellation either being linear or angled (angles always add to 120°). Just considering the two to eight, six-member ring PAHs there are 1896 possible combinations. EPA Method 610 defines sixteen of these compounds as priority pollutants. The properties of a PAH depend on its size (number of rings) and the topology of the system (type of ring linkage)[18]. Another chemical property of a PAH is the reactivity of its highest occupied molecular orbital. This property makes it a Lewis base and allows it to stabilize according to the Lewis Octet Theory. More specifically a PAH behaves as an electron rich pi-system[17]. PAH compounds are also known to have acutely toxic effects and/or have mutagenic,

teratogenic, or carcinogenic properties[19,20]. Some PAHs are metabolized to carcinogenic species. The classic example is benzo[a] pyrene- 7,8-diol-9,10-epoxide, (see Figure 1). So several of the PAH are procarcinogens. In the substitution reactions typical for PAH compounds, the hydrocarbon acts as a nucleophile; the bases of DNA are also nucleophiles so binding does not take place. The opening of the epoxide ring causes an electrophilic system, which can then react with DNA, causing DNA adducts and transcriptual errors[18,21]. A simplified metabolic pathway can be seen in Figure 2.

Benzo(a)pyrene   benzo(a)pyrene 7,8 diol 9,10 epoxide
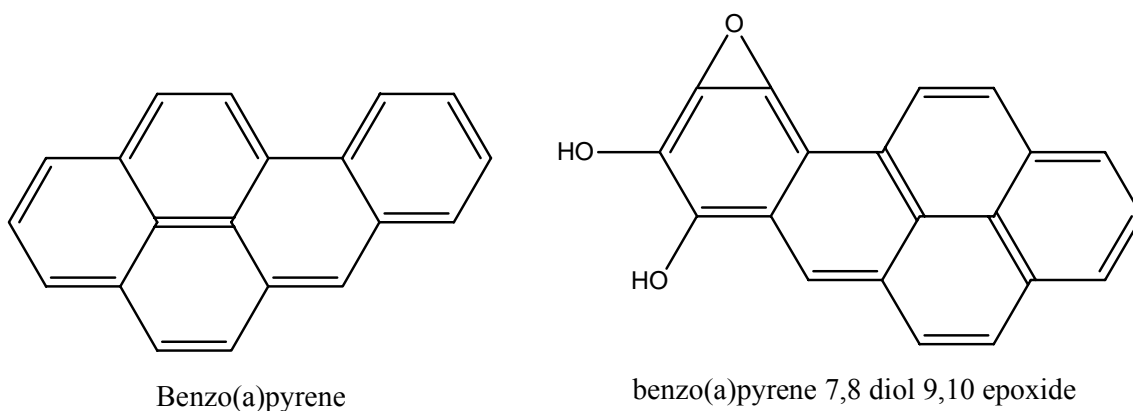
Figure 1- benzo(a)pyrene gets metabolically converted to mutagenic BPDE through several metabolic steps.
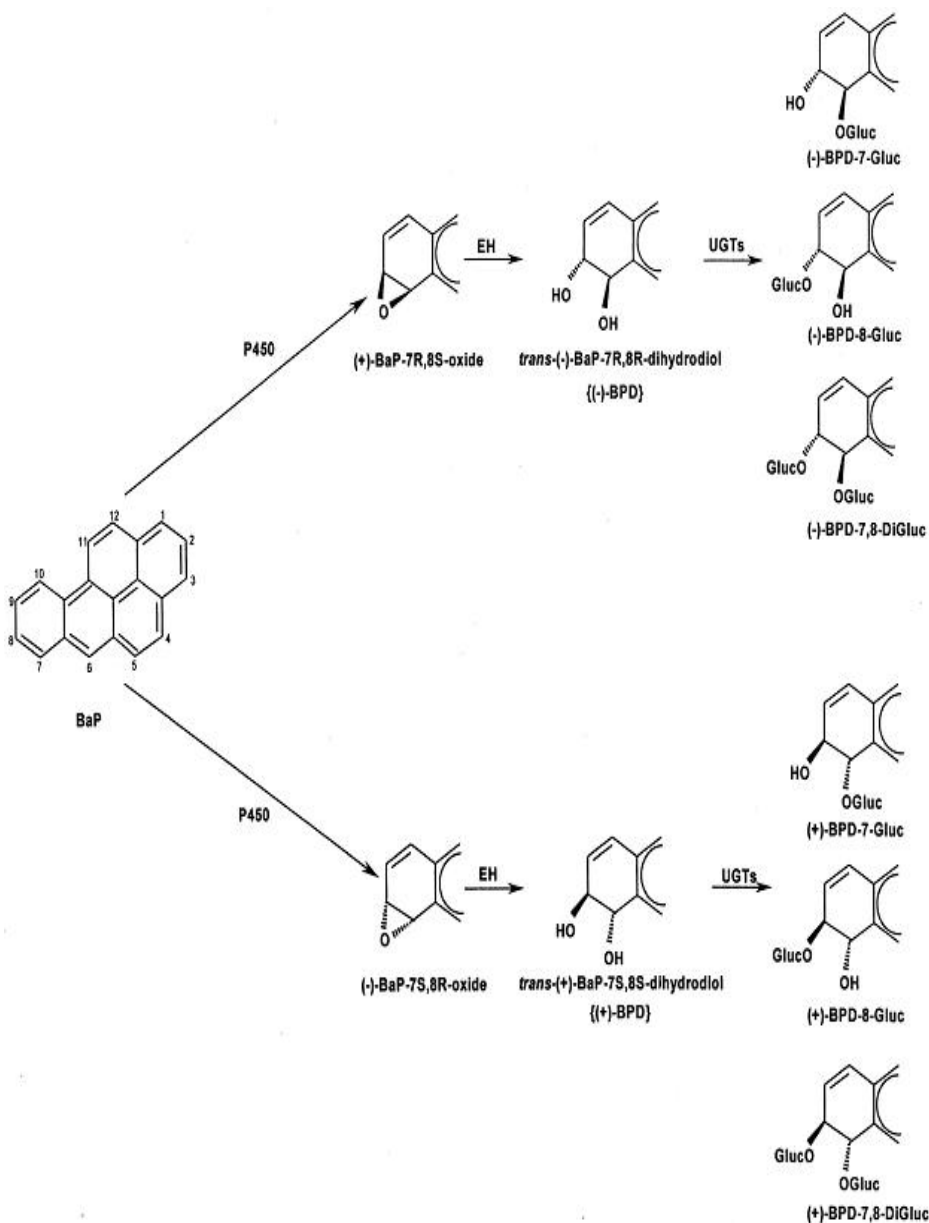
Figure 2- 1A simplified illustration of the metabolism of B[a]P. Copyright ©2002 American Association for Cancer Research[(22)].

PAH compounds are products of incomplete combustion of fossil fuels and other organic material. They are natural constituents in fossil fuels and are therefore present in relatively high concentrations in products of fossil fuel refining[(23,24,25)]. Asphalt is the residue left after all other refined products are processed from crude oil.

The potential health risk due to exposure to asphalt fume from paving operations is not known. A study done by the Asphalt Association in 1975 found no significant air pollution problems were associated with emissions from hot paving operations[26]. Reinke et al.[27] reported that asphalt fume condensates collected from a commercial operation were not mutagenic.

Reinke and Swanson collected paving asphalt fumes from a straight-reduced, vacuum-processed 85-100 grade asphalt cement. They also collected fumes from a storage tank at a hotmix plant maintained at a temperature of 149 °C (300 °F) and from laboratory generation at temperatures of 149 °C and 316 °C (300 and 601 °F). The fume samples were then analyzed for selected PAHs. The results indicate that only two- and three-ring PAHs were present in the fumes from the storage tank and laboratory-generated fumes were predominately two- and three-ring PAHs, and also contained a few three-ring sulfur-PAHs (S-PAHs) and four-ring PAHs. Several of the four-ring PAHs found are carcinogenic, i.e., benz[a]anthracene and chrysene. Methylated chrysenes, pyrenes, and fluoranthenes were also detected and may be of concern because of their structural relationship to known carcinogens. The concentration of four-ring PAHs was highest in fumes generated in the laboratory at the highest temperature. Paradoxically, the concentration of two ring PAHs was lowest in laboratory fumes generated at the highest temperature, most abundant in fumes from the storage tank, and lower in the fumes generated in the laboratory at the ambient temperature of the storage tank[27]. It must also be noted that the analytical methodology consisted only of basic sample extraction into dichloromethane and direct injection onto a tabletop GC-MS. The absence of clean-up and concentration of such a complex mixture raises questions concerning the validity of the results.

Watts and colleagues[28] measured the mean concentrations of airborne particles (2.5 μm) at three different paving sites in the United States and observed an exposure range of 111–389 μg/m^3. Burstyn and coworkers[29] compiled data from several countries and studies and reported that European road paving workers can be exposed to 0.1–2 mg/m^3 of bitumen fume, which may contain 10–200 ng/m^3 benzo[a]pyrene. The study

involved data from many different samples mostly from industrial hygiene surveys for compliance. Asphalt temperatures probably varied greatly between these studies. Measurements of B(a)P and bitumen fume were based on a variety of methods. Again raising questions on the validity of the results. Although normal exposure levels to road paving asphalt fumes are relatively low, long-term health effects associated with chronic exposure may be a concern[30].

The asphalt fume from paving operations has been described as non-mutagenic[31]. Probably the greatest contributing factor to this is the temperature at which road paving asphalt is applied. The heavy PAHs have higher boiling points and thus do not readily volatilize at the lower road paving temperatures temperatures. Several heavy PAHs are known to be mutagenic and carcinogenic. Evidence suggests in some cases PAH genotoxicity increases with size[32]. Smaller, lighter PAHs are generally considered irritants, with the exception of naphthalene. In a two-year naphthalene inhalation study by the National Toxicology Program (NTP), it was concluded there was clear evidence of an increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose in F344/N rats, both male and female[33]. A previous study study showed an increase number of lung cancers in female B6C3F mice[34]. Paving workers are generally not exposed to just asphalt fume. It must be considered that these workers are exposed to diesel exhaust, gasoline engine exhaust, and ultra violet radiation concomitantly with the asphalt fume.

Due to the presence of PAHs in asphalt fume, the potential mutagenic/carcinogenic effects have been the central focus of *in vitro*[35,36], *in vivo* animal[37,38,39], and worker studies[40]. Other health effects may be associated with acute asphalt fume exposure. Norseth et al.[41] reported that, during road repair and construction, asphalt workers experienced abnormal fatigue, reduced appetite, eye irritation, and laryngeal/pharyngeal irritation. The frequency of symptoms reported increased with increasing asphalt fume generation temperature above 150 °C. An epidemiologic study has reported an increase of nonmalignant lung diseases, such as bronchitis and emphysema, among California highway workers[42]. The association

between road paving temperature and potential health effects has not been clearly established. A major road blocks to the study of health risk to asphalt fumes has been the variability of components of asphalt fume and the lack of sensitive, specific analytical methods to properly characterize the exposures.

3. Materials and Methods

3.1 Asphalt Fume Generation, Sample Collection, and Extraction

The asphalt fume generation system was designed to simulate the road paving process. The oven (Grieve, Round Lake, IL) heated three five-gallon buckets of asphalt to a temperature of 190 °C. Liquid asphalt flowed into a custom fabricated storage pan in the bottom of the oven as it was heated. Asphalt temperature was maintained with a single on/off temperature controller and two 500 watt heaters attached to the pan. An asphalt pump (Roper, Commerce, GA), coupled to a 1 HP motor (General Electric 5KCR48UN0102X, @1725 RPM), transferred the liquid asphalt from the pan to a specially designed bitumen kettle. The plumbing between the pump and the kettle was wrapped in 500-watt heat tape to prevent the subsequent cooling of the liquid asphalt. A 750-watt heater was used to maintain the temperature of the asphalt in the kettle. An ultrasonic sensor provided feedback of the asphalt level in the kettle to prevent over-filling. The purpose of the kettle was to maintain a smooth flow of asphalt across the fume generator. A more consistent asphalt flow was obtained by pumping into the bitumen kettle, rather then directly controlling the asphalt pump. Asphalt from the bitumen kettle flowed through a needle valve which was part of a computer feedback system used to regulate asphalt flow. The plumbing between the kettle, needle valve, and generator was wrapped with 500-watt heat tape. Next, the asphalt entered the stainless steel fume generator chamber where it spread over a rectangular plate (6" x 24") that simulated application of asphalt to a road surface. It then passed through a trap and emptied into a container resting on a scale. Incremental scale readings of the container weight were used in a computer controlled feedback loop to achieve a constant mass flow of asphalt (150 mg/min) through the needle valve. A 750-watt heater and an on/off controller were used to maintain an asphalt temperature gradient of 150 °C to 125 °C (180 °C to 150 °C for 180 °C asphalt fume generation temperature) across the generator

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bed. The asphalt was cooled as HEPA filtered air (25 °C, 50 % RH, 20 lpm passed over the bed and entrained the asphalt fume. The inlet and outlet temperatures of the asphalt, as it passed over the plate, were measured with J-type thermocouples positioned 1" from the generator inlet and outlet, respectively. Temperatures through the system were adjusted to obtain the desired asphalt temperature (150 °C or 180 °C) for a given experiment. Fume produced in the generator was transported to the exposure chamber and through the use of a series of heat tapes, gradually cooled to a temperature of around 25 °C. A diagram of the generation system is illustrated in Figure 3.
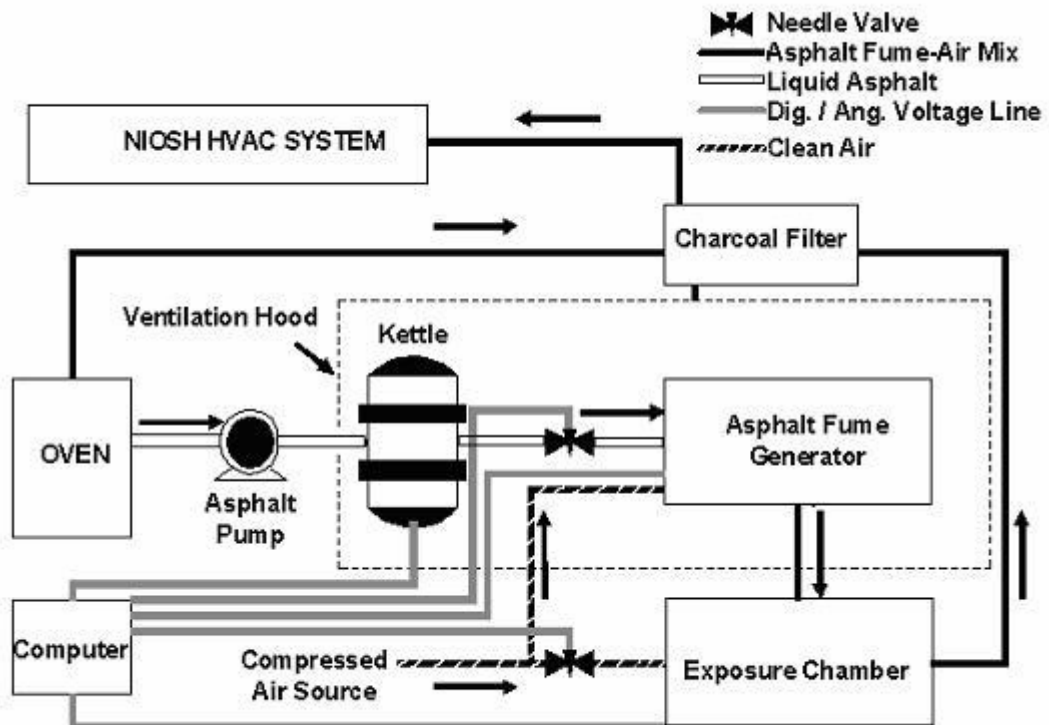
Figure 3- A diagram of the simulated paving asphalt fume generator system.

The sampling train consisted of a 63 mm glass fiber HEPA filter followed by 10g of XAD (Supelco, Bellefonte, PA) resin. Samples were collected at 10 lpm for 240 min. XAD samples were stored at -20 °C in amber glass bottles with polytetrafluoroethylene (PTFE) lined lids until extraction. The mass of XAD recovered from each sampler was determined gravimetrically and recorded. Filters were labeled and stored at -20 °C in the

dark in zipper closed plastic bags. Extraction of XAD and filters consisted of a one-hour gentle vortexing at approximately 100 RPM using dichloromethane (DCM) as the extraction solvent. Filters were extracted in 50 ml of DCM each. XAD samples were extracted in 250 ml of DCM per each XAD sample. Extracts were filtered through 0.2 μm HF membranes (Millipore, Billerica, Ma) using a vacuum flask apparatus. Solvent recovery was measured using a graduated cylinder and recorded. The extracts were stored in amber serum bottles with crimped PTFE lined lids. All crude sample extracts were stored at -20 ^{o}C to prevent evaporation and UV degradation. Breakthrough was not accessed in this set of samples; however, in preliminary runs a small XAD tube was used to sample the effluent air at 1 lpm after the high volume sampler, and breakthrough was not detected. Total fume concentration, simulated boiling point profiles, and naphthalene concentrations were determined using these crude extracts, without additional sample manipulation.

3.2 Purification Methods Development

The preparatory high performance liquid chromatographic (HPLC) method used to separate PAHs from aliphatic interferences is a modification of that used to clean-up diesel fume extracts for PAH analyses reported by Jiao, *et.al*.[43]. This HPLC method employed a poly- divinylbenzene (PDVB) gel solid phase column. To develop the preparatory HPLC method several standards were employed. To simulate asphalt fume extracts a kerosene reference standard and a PAH cocktail were mixed. Kerosene is a good substitute for the aliphatic compounds that interfere with asphalt analysis, but like asphalt, kerosene also contains other compounds. The QTM mixture (Supelco #47930-U) contains 16 PAH compounds which the EPA consider significant. The first attempts at developing this method used an isocratic (constant concentration) flow of different hexane and dichloromethane concentrations. Fluorescent and photo diode array detectors were employed to establish the solvent gradient profile that provided the optimal conditions for fraction collection.

3.3 High Performance Liquid Chromatographic Fractionation for PAHs.

Internal standards, 20 µg, each, of naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{12}, benzo [a] pyrene-d_{12} (Cambridge Isotope Laboratories, Andover MA) were added to 25 ml of each filter sample DCM extract. Twenty micrograms of naphthalene-d_8 and acenaphthene-d_{10} were added to 25 ml of each XAD sample extract. XAD and filter samples were concentrated 125 fold by evaporation under N_2 at room temperature and reconstituted in 200 ul DCM. Aliphatic compounds are retained by PDVB gel using steric features such as size and shape of the molecule, PAH compounds are retained, preferentially, via Lewis acid-base interactions. The method utilizes a 500mm x 10mm, 500 Å poly- divinylbenzene (PDVB) gel HPLC column (Alltech, Deerfield, IL). Concentrated DCM extracts (200 µl), were injected onto the PDVB column in 100 µl aliquots (2 separate HPLC runs each). PAH standards (Supelco, Bellefonte, PA) were prepared and processed in parallel to the asphalt fume samples. Initially, the column was washed with 100% hexane for 25 minutes at 1 ml/min to remove aliphatics, followed by a rapid linear gradient over the next minute to 100% DCM to elute the PAHs. A fluorescent detector set to an excitation wavelength of 254nm and an emission wavelength of 400nm was used to monitor elution of PAHs. Aliphatic compounds were monitored using a GBC LC5000 photodiode array (PDA) scanning the UV range. Fraction collection was done using a manual six-port valve. The valve was opened at 50 minutes post-injection (approximately 1 minute before elution of PAH compounds as determined by preliminary studies using standard QTM PAH standard mixture (Supelco, Bellefonte, PA). The fraction was collected until the fluorescent detector had returned to baseline (approximately 25 ml). This fraction was evaporated to dryness under nitrogen and reconstituted to 100 µl in DCM for gas chromatograpic-mass spectrometry (GCMS) analyses. Table I summarizes the solvent gradient used.

Table I. Summary of HPLC Conditions

0-30 min – 100% Hexane
30-31 min- 100% Hexane to 100% Methylene Chloride
31-75 min- 100% Methylene Chloride
75-76 min- 100% Methylene Chloride to 100% Hexane

XAD and filter PAH fractions were kept in sealed, amber glass vials with PTFE septa at -20 ^{o}C. XAD samples were used to analyze the vapor portion of the fume, especially naphthalene. Naphthalene was identified and quantified from crude DCM extracts because (1) it was lost during solvent evaporation under nitrogen and (2) it was present as sufficient levels in the XAD fraction to analyze directly by GC/MS without further sample clean-up.

3.4 Gas Chromatography and Mass Spectrometry Procedures

The specific compounds were accessed by GC/MS-selected ion-monitoring mode (SIM), using a 5972 series GC/MS, (Agilent Technologies, Palo Alto, CA). The compounds monitored were naphthalene, acenaphthylene, 2-bromonaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo-(b)fluoranthene, benzo(A)pyrene, benzo-(ghi)perylene, indeno(1,2,3-cd)pyrene, and dibenzo(a,h)anthracene. The monitored mass-to-charge ratios (m/z) are listed in Table II. Electron impact ionization (EI, 70eV) MS was used to analyze samples, standards, and blanks. Data acquisition and processing were performed within the ChemStation software suite (Agilent Technologies, Palo Alto, CA). The GC column was an HP-5MS (30m x 0.25mm, 0.25μm film thickness) fused silica capillary (J&W Scientific, Folsom, CA). Seperation by this column is based on the boiling point of the analyte (higher boiling point compounds have greater retention). Analytes were eluted using ultra high purity (UHP) helium as the carrier gas at 1ml/min. Following the 1μl, splitless injection, the GC oven was held at 50 ^{o}C for two minutes; the temperature was then increased by 5 ^{o}C/min to a final temperature of 310 ^{o}C and held for

1 minute (for a total run time of 55 min).   A summary of GC/MS conditions can be
found in Table III.

Table II. Ions Monitored by GC/MS Analysis, SIM Mode.

PAHs	Ions (m/z)	PAHs	Ions (m/z)
Naphthalene	127 127, 102	Benzo[a]anthracene	228, 226, 229
Acenaphthylene	152, 151, 160	Chrysene	228, 229, 226
2-bromonaphthylene	127, 206, 208	Benzo[b]fluoranthene	252, 253, 126
Acenaphthene	153, 154, 152	Benzo[a]pyrene	252, 250, 264
Fluorene	166, 165, 167	Dibenzo [a,h]anthracene	278, 279, 276
Phenanthrene	178, 176, 179	Indeno[1,2,3-cd]pyrene	276, 137, 227
Anthracene	178, 176, 179	Benzo[ghi]perylene	274, 138, 288
Fluoranthene	202, 200, 201		
Pyrene	202, 203, 200		

Table III. Summary of Gas Chromatogrpahy and Mass Spectrometry Conditions

Inlet B Temperature- 250°C

Detector B Temp- 280°C

Initial Oven Temp-50°C

Initial Time- 2.00 min.

Oven Rate- 5°C/min

Final Temp 310°C, 55min

Constant Flow- 1ml/min

Injection Volume- 1ul

Identification of specific PAH compounds present in the sample extracts was
based on the relative abundance ratios of selected ions, as well as, relative retention times
(RRT's) of the target analytes relative to the deuterated internal standards. The ion ratios
of a specific PAH within 25% of its respective standard were required for positive
identification.   Filter, XAD, and solvent blanks were run for all analyses.

Total vapor and particulate fume concentrations and boiling point profiles were
determined using the GC/MS in the total ion monitoring mode, and the above GC
conditions. The total ion chromatogram (TIC) generated by this technique does not
resolve individual compounds from this complex mixture. The sum of the total ions from
the TIC of the unresolved fume eluted from the GC column were integrated to quantify

the fume concentration. The vapor fraction concentration was determined relative to a kerosene standard (47517-u, Supelco, Bellefonte, PA). The particulate fraction was quantified against neat asphalt fume filter DCM extract for which the density was measured to be 1.84 g/ml by gravimetric analyses. The boiling points of naphthalene, acenaphthene, phenanthrene, benz(a)anthracene, and benzo(a)pyrene were plotted against retention time of the respective PAH compounds. The equation of the line ($y = 8.8934x + 86.923$) was used to extrapolate retention time to boiling point to determine the boiling point profiles of the fume fractions. Boiling point profiles and fume concentration were both determined from sample media DCM extracts (without any concentration or cleanup steps prior to GC/MS analysis). A summary of the methods are shown in Figure 4.

3.5 Evaluation of Standards

Three standards were evaluated using response factors compared to asphalt fume filter DCM extract. The analytical standards were chosen by their chemical composition. These standards were a reference kerosene standard (Supelco Inc), aliphatic standard mixture (C_8-C_{36}) (Supelco Inc), and a PAH cocktail (Supelco Inc). Standard curves for each material were developed and an equation for each curve was derived. The concentration of the bulk asphalt fume was determined. After this determination, a response factor was derived by dividing the area under the curve (AUC) for each standard material by each concentration to yield an average AUC/mass of material. The results were averaged and then divided by AUC/mass of bulk asphalt fume.
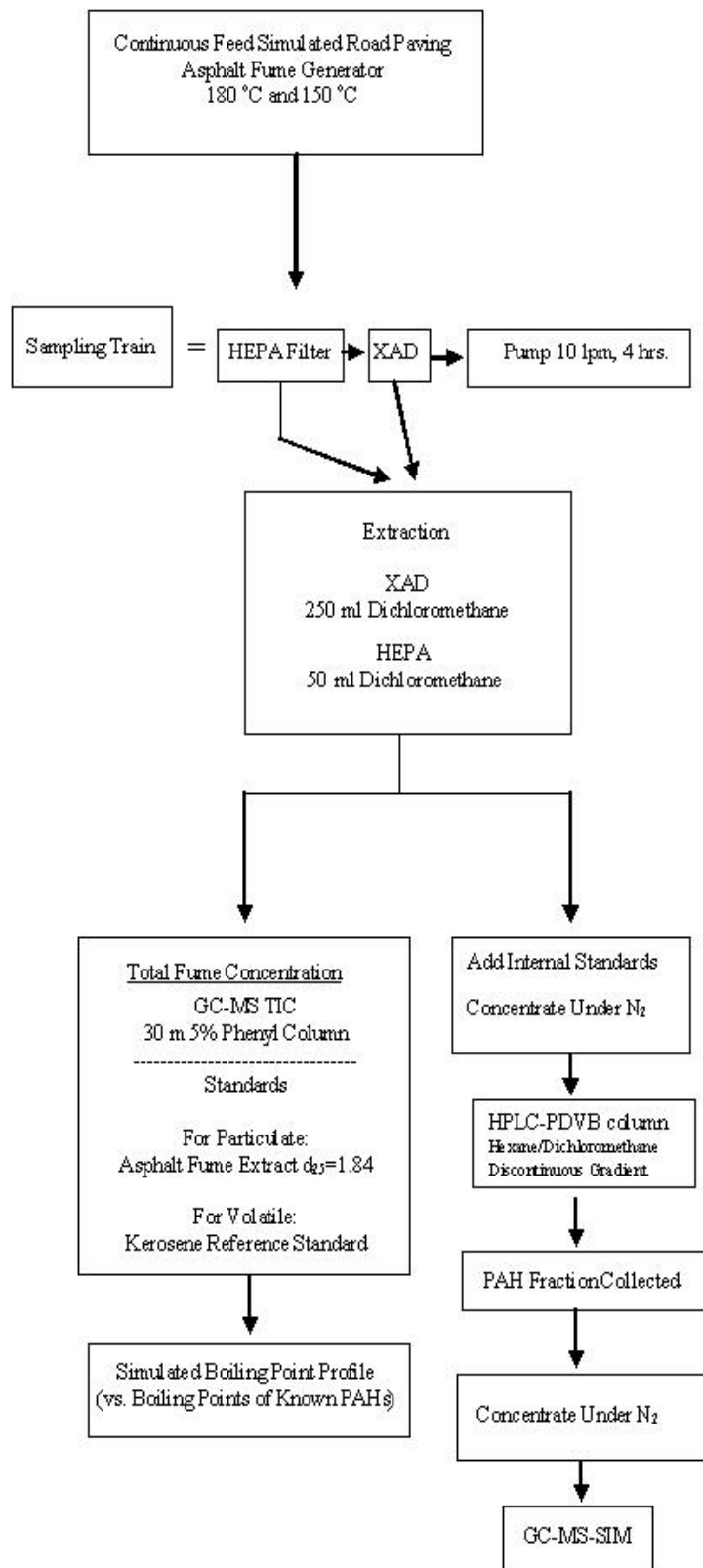
Figure 4- Diagram of the generation, collection, and analysis of the asphalt fume.

3.6 Fluorescence Techniques

Pure mixtures of PAH compounds, including naphthalene, phenanthrene, pyrene, benzo[b]fluoranthene, and benzo[ghi]perylene were made in DCM. Each compound was diluted using DCM to 45ng/ml. Compounds were analyzed using a stand-alone spectrofluorometer (Perkin-Elmer, Wellesley, MA), in both a three-dimensional synchronous scanning mode and a set emission and excitation wavelength mode according to the NIOSH method 5800[15]. Asphalt fume condensate, particulate phase, extracts were diluted 1000 fold and analyzed in the same way. For the set wavelengths method the spectrofluorometer was set to an excitation wavelength of 254 nm and an emission wavelength of 370 nm for 2-4 ring PAHs, and 254 nm excitation 400 nm emission for 4-6 ring PAHs. Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromators in the 250–600 nm range with constant wavelength differences Δλ according to the method of Apicella[44]. Fluorescence intensities were plotted as a function of the excitation wavelength. Four spectra were recorded for each sample with Δλ of 10,

3.7 Statistical Analysis

Most of the statistics were performed using Microsoft© Excel 2002 SP3. Statistical analyses consisted of the student unpaired t-Test with α equal to 0.05 in all instances to show statistical significance. This test was performed to analyze the difference between the groups of data being collected. A regression analysis was performed using SigmaStat v. 3.1 (Sysstat, Point Richmond, CA) on the curves produced for the boiling point profile. This test was done to provide a 95% confidence interval and compare boiling point distribution at the two generation temperatures. The geometric standard deviation was obtained from the boiling point profile curves by dividing the 84.13% of the cumulative ion amount by the mean mass boiling point. One standard deviation was used for error bars.

4. Results

The effect of two different generation temperatures, 150 °C and 180 °C, on the resultant fume was evaluated in the present study. Fume samples were collected from an environmental chamber onto a HEPA filter, followed by XAD resin material. The fumes were comprehensively characterized to allow for evaluation of fumes generated over the road paving temperature range. The particulate (HEPA filter) and vapor (XAD resin) fractions were analyzed from each sample to obtain (1) particulate, vapor and total asphalt fume chamber concentrations, (2) particulate and vapor components simulated boiling point profile, and (3) specific PAH content.

4.1 Standards Comparison and Response Factors

Response factors were developed after evaluating three standards used for quantification of total asphalt fume. Examples of the TIC can be seen in Figures 5-8. For the reference kerosene standard the response overestimated the particulate phase of the asphalt fume concentration by 1.8 times. For the PAH cocktail the curve overestimated the true concentration of the asphalt by 16.7 times. For the aliphatic mixture the overestimation was 1.7 times. The standard curves developed from the bulk asphalt fume, the kerosene reference standard, the PAH cocktail, and the aliphatic standard can be seen in Figures 9-12. Similar comparisons can not be made for the vapor fraction of the asphalt fume, because it is not possible to remove the extraction solvent without losing a substantial portion of the fume.
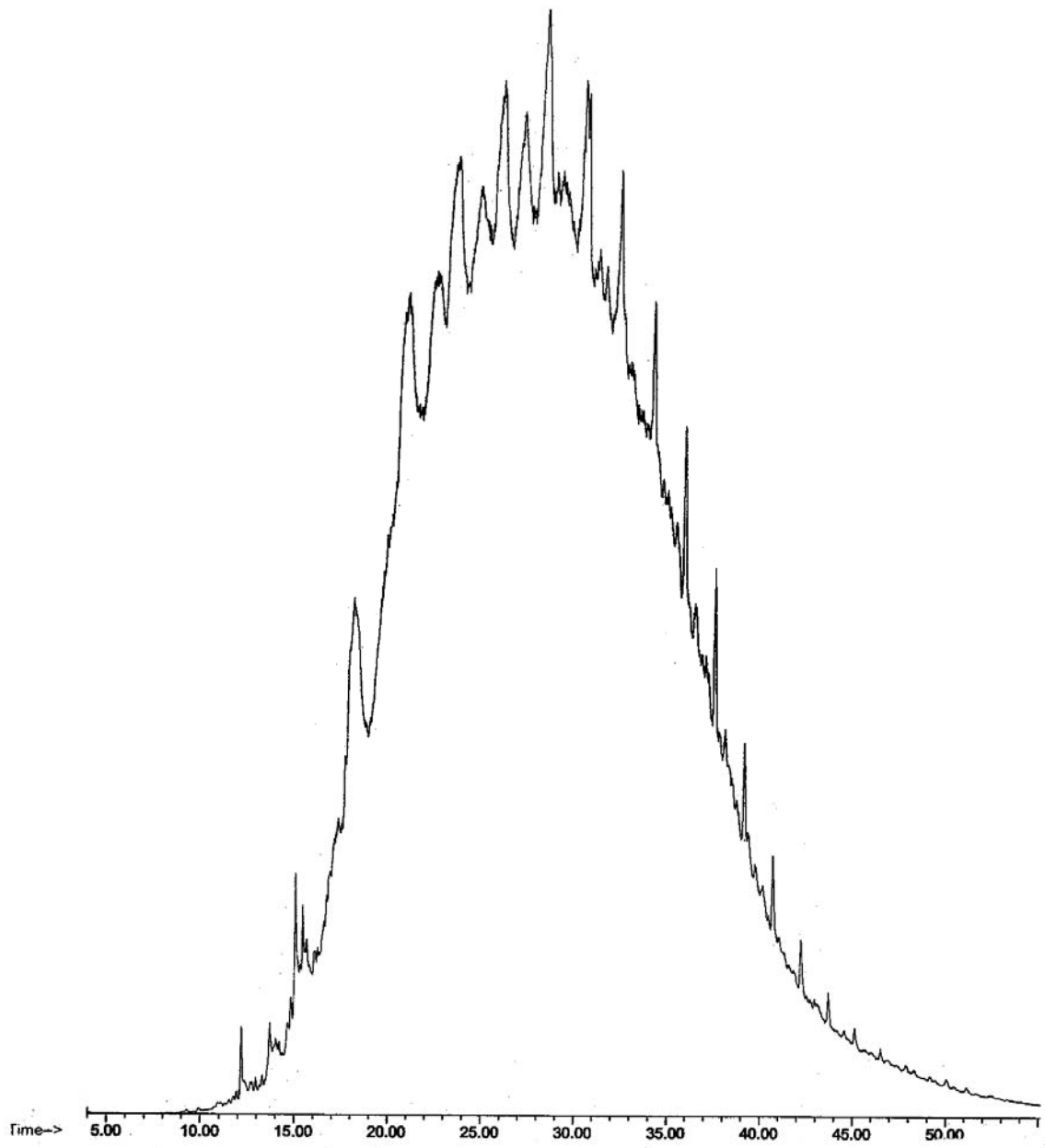
Figure 5- Bulk asphalt fume TIC.
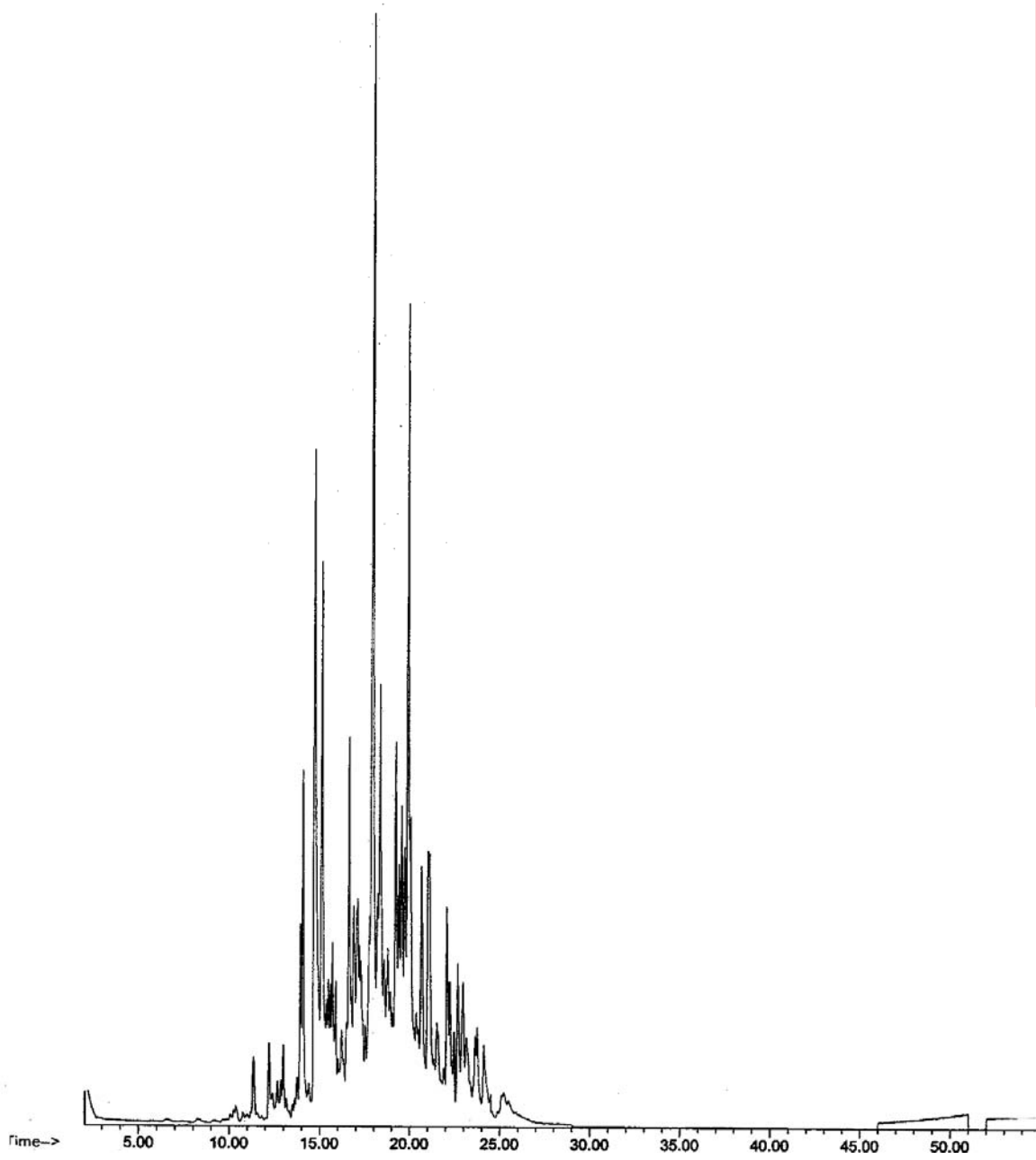
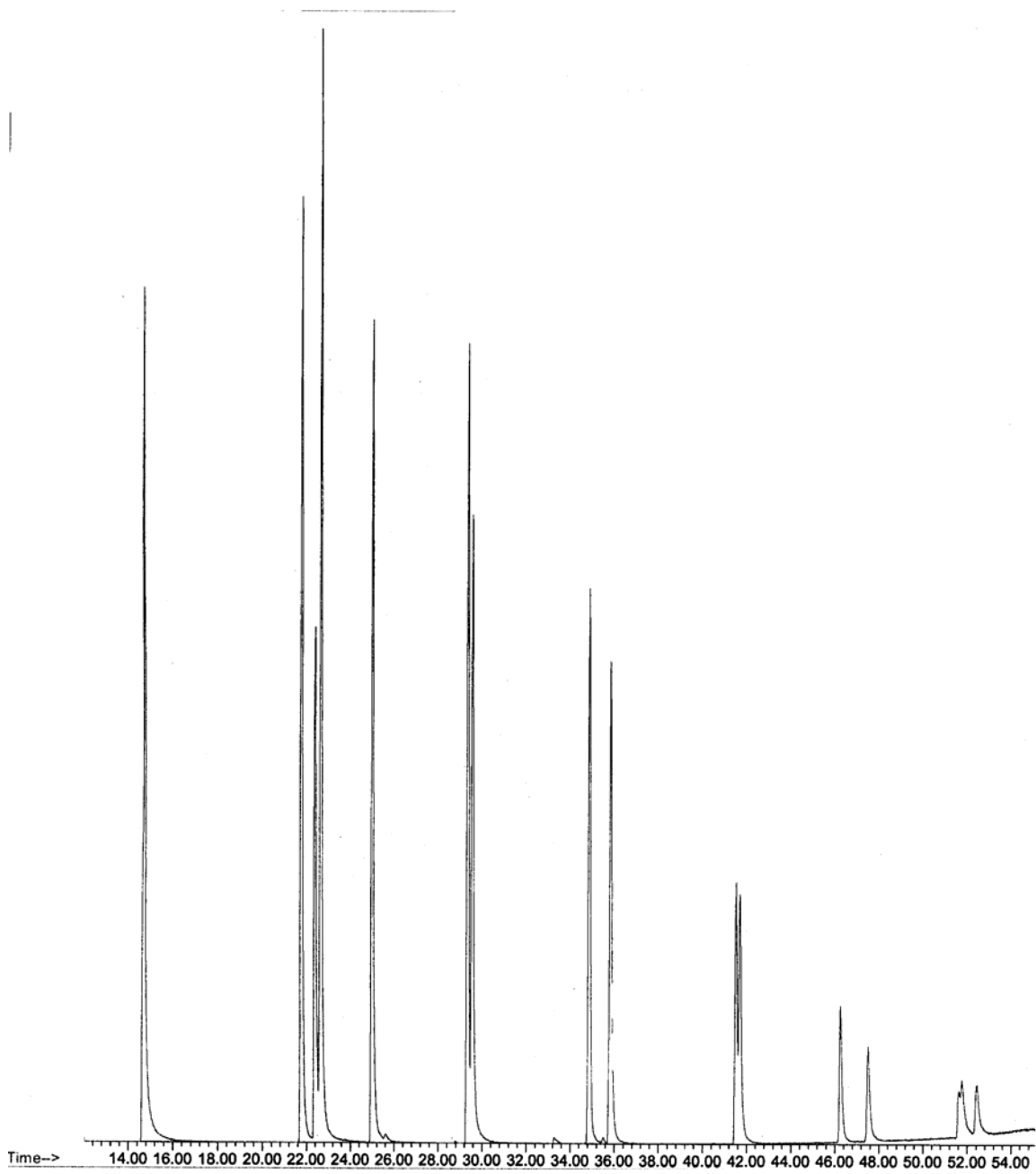**Figure 6- Reference standard kerosene TIC.**
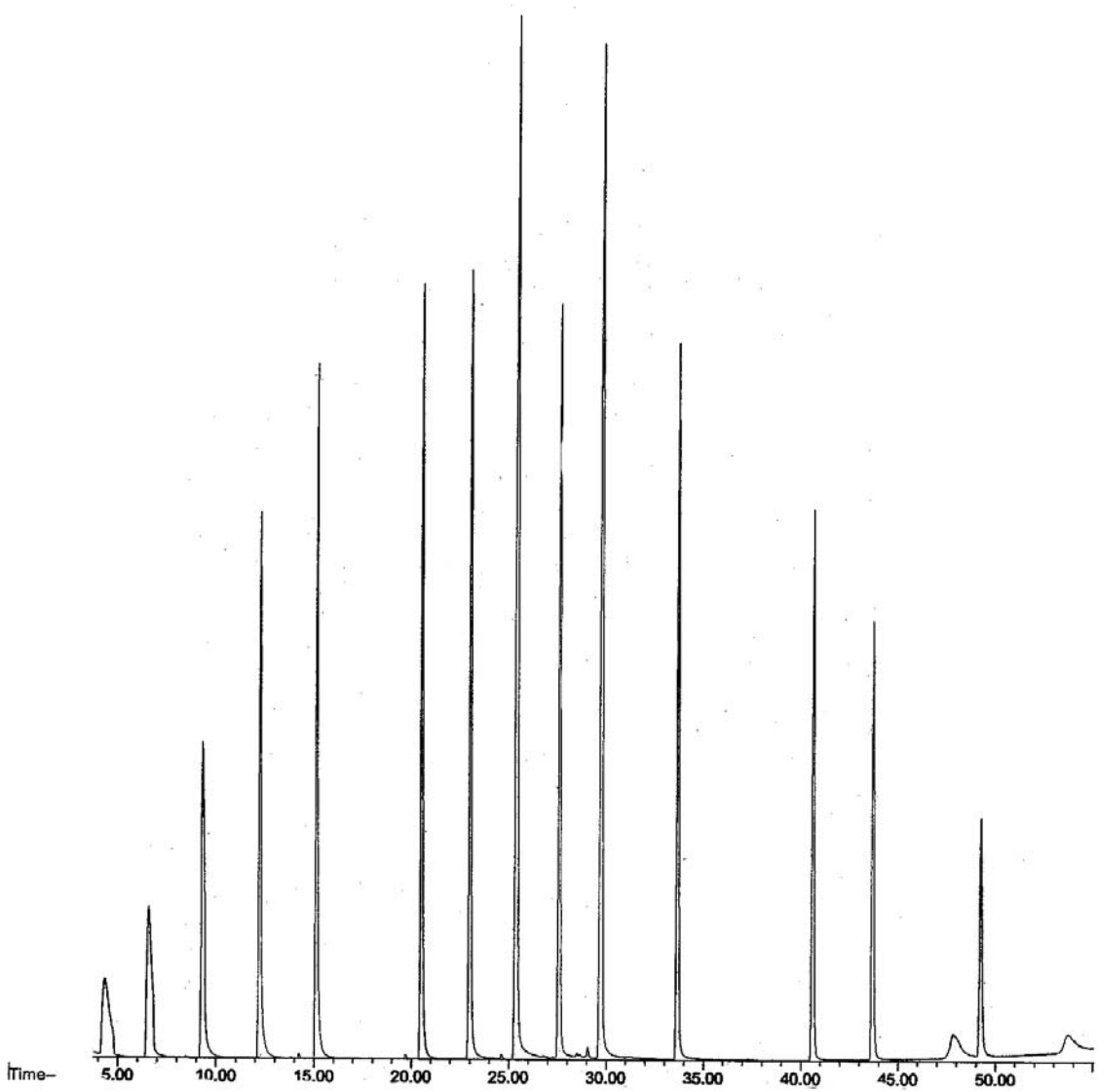
Figure 7- QTM PAH mixture TIC.

Figure 8- Aliphatic standard mixture TIC.
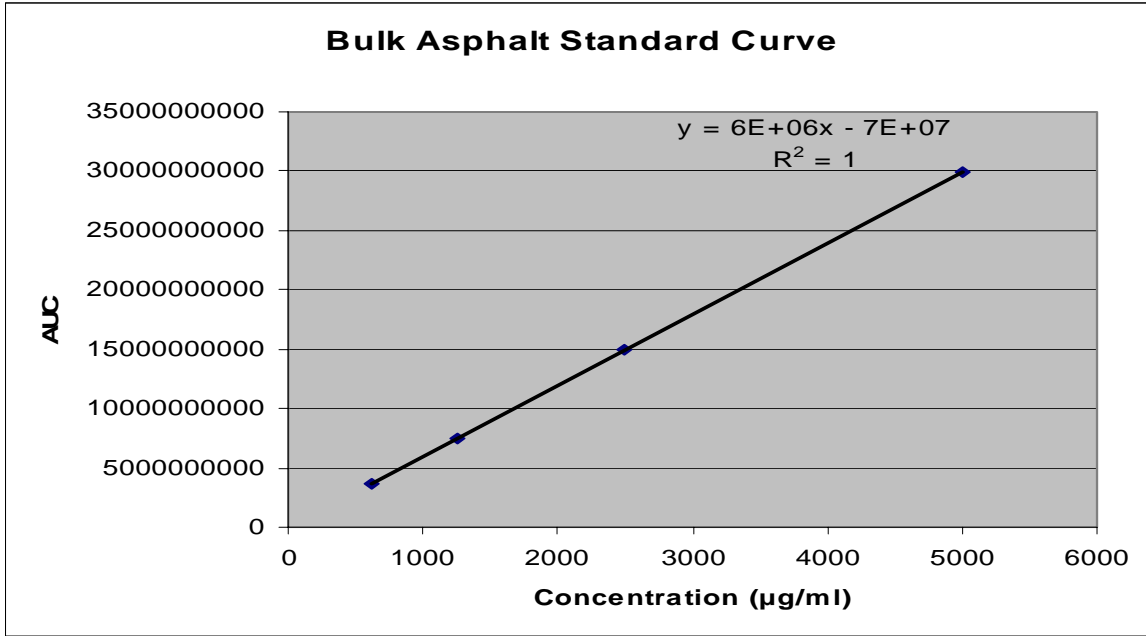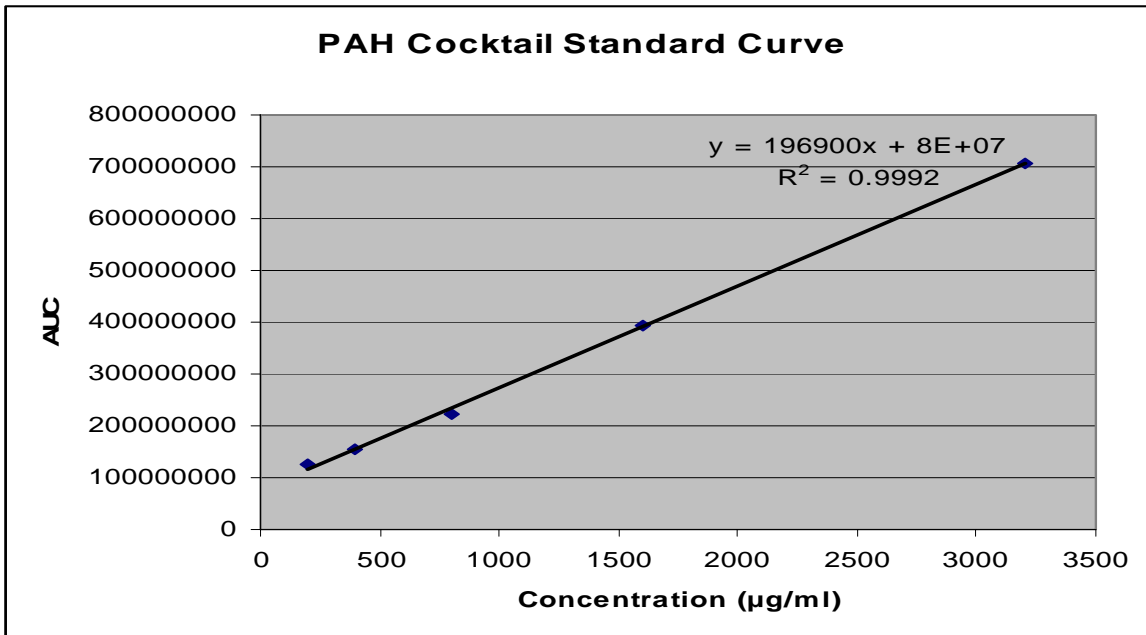
Figure 9- Bulk Asphalt GC/MS Standard Curve

Figure 10- PAH Cocktail GC/MS Standard Curve

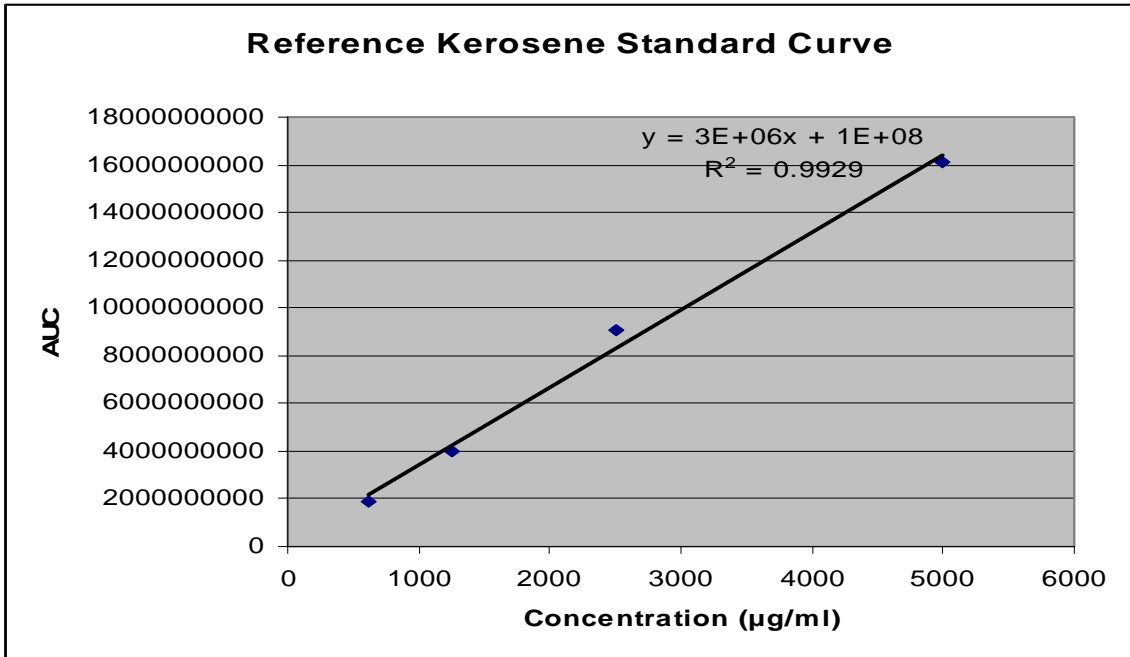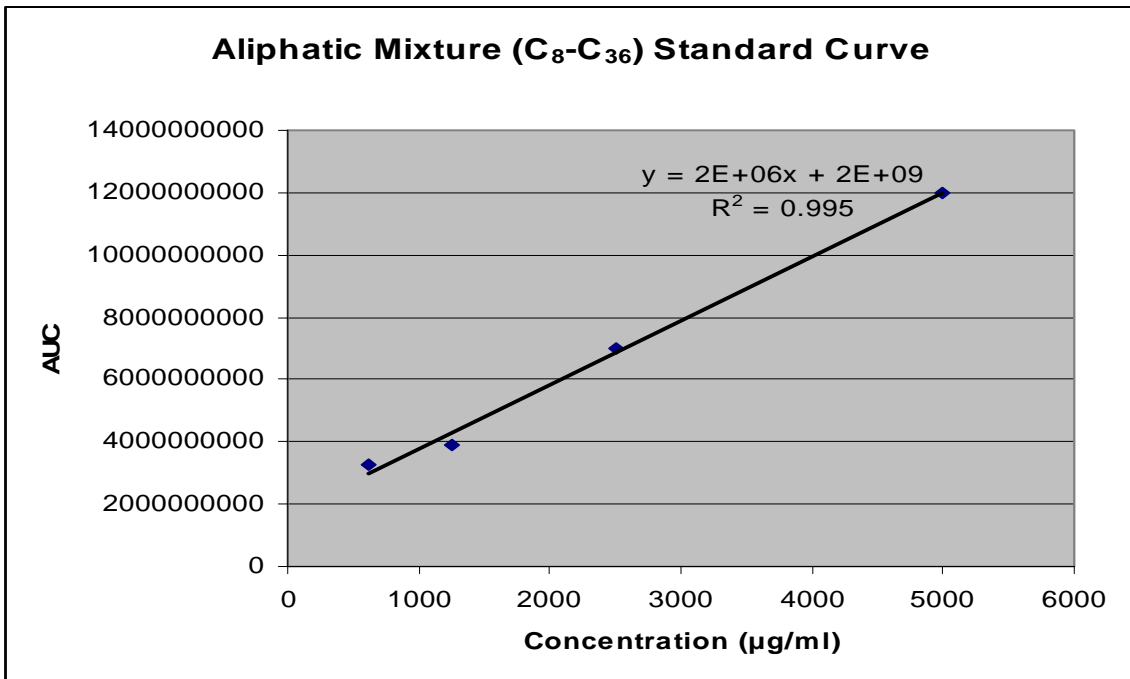**Figure 11- Reference Kerosene GC/MS Standard Curve**

Figure 12- Aliphatic Mixture (C$_8$-C$_{36}$) Standard Curve

4.2 Total, Vapor, and Particulate Asphalt Fume Chamber Concentrations

Increasing the asphalt fume generation temperature from 150 °C to 180 °C caused a significant increase in the total fume air concentration (p=0.02, critical T= 2.4, calculate t= 3.1), Figure 13. The mean total fume concentrations were 65.8 ± 16.9 mg/m^3 for the 180 ^{o}C fume and 39.3 ± 2.6 mg/m^3 for the 150 ^{o}C fume. Both the particulate and vapor fractions were elevated at 180 ^{o}C vs. 150 ^{o}C generation temperatures; however only the difference in the vapor fraction was statistically significant (p= 0.02, critical T value= 2.4, calculated t value= 3.1). The mean concentration of the vapor fractions generated at 180 ^{o}C and 150 ^{o}C were 23.3 ± 6.6 mg/m^3 and 11.2 ± 4.1 mg/m^3 respectively. The mean concentration of the particulate fractions generated at 180 ^{o}C and 150 ^{o}C were 42.4 ± 13.0 mg/m^3 and 28.0 ± 3.5 mg/m^3, respectively (Figure 13).
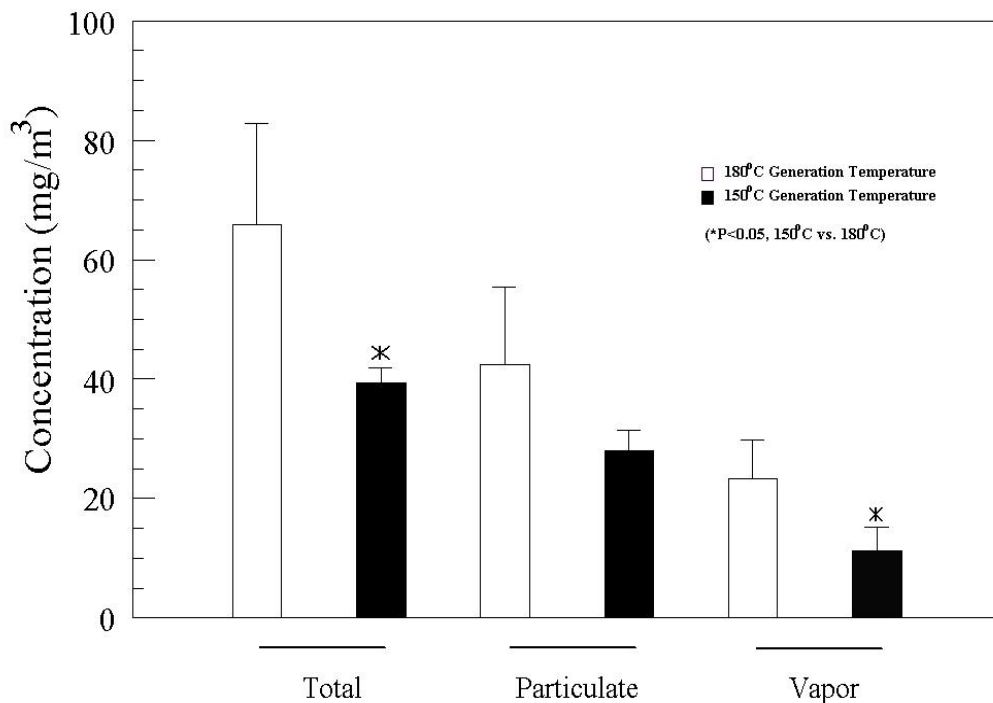

Figure 13- Exposure chamber air concentration of total asphalt fume and the particulate and vapor phases. Samples were collected on HEPA filters and XAD samplers, extracted into DCM and assayed by GC/MS-TIC mode, n=4, each.

4.3 Simulated Boiling Point Comparison

A simulated boiling point profile was developed at both generation temperatures for the particulate and vapor fume fractions. The 5% phenyl GC column separates compounds by boiling point allowing determination of boiling point by elution time relative to known standards.   No significant qualitative shifts in the boiling point profiles were observed between 150 $^{\text{o}}$C and 180 $^{\text{o}}$C for particulate or vapor fractions. Figure 14 contains representative chromatograms of the asphalt fume vapor and particulate fume. Figure 15 is an overlay of the 180 $^{\text{o}}$C and 150 $^{\text{o}}$C filter extract chromatograms. This figure shows how small the difference is between the two fumes. Figure 16 illustrates the same point using the XAD vapor extract. Figure 17 is a plot of the cumulative boiling point curves for both the particulate and vapor fractions of the fume generated at 150 $^{\text{o}}$C and180 $^{\text{o}}$C. The mean mass boiling points (MMBPs) for the vapor component were 229 $^{\text{o}}$C, 1.3 σ_g and 235 $^{\text{o}}$C, 1.25 σ_g for 180 $^{\text{o}}$C and 150 $^{\text{o}}$C generation temperatures, respectively. For the particulate fume fraction, the MMBPs for the 150 $^{\text{o}}$C fume and 180 $^{\text{o}}$C fume were 365 $^{\text{o}}$C, 1.17 σ_g and 372 $^{\text{o}}$C, 1.35 σ_g respectively. A regression analysis of the boiling point profile data was performed for both 150 $^{\text{o}}$C and 180 $^{\text{o}}$C particulate and vapor fume components. The results indicated significant overlap within the 95% confidence intervals for both 150 $^{\text{o}}$C and 180 $^{\text{o}}$C vapor and particulate lines. The slope 95% confidence interval (95%CI) for 150 $^{\text{o}}$C volatile compounds was 0.30 to 0.56, the 95%CI for 180 $^{\text{o}}$C volatile compounds was 0.31 to 0.52. The slope 95%CI for 150 $^{\text{o}}$C particulate compounds was 0.17 to 0.44, the 95%CI for 180 $^{\text{o}}$C particulate compounds was also 0.17 to 0.44.
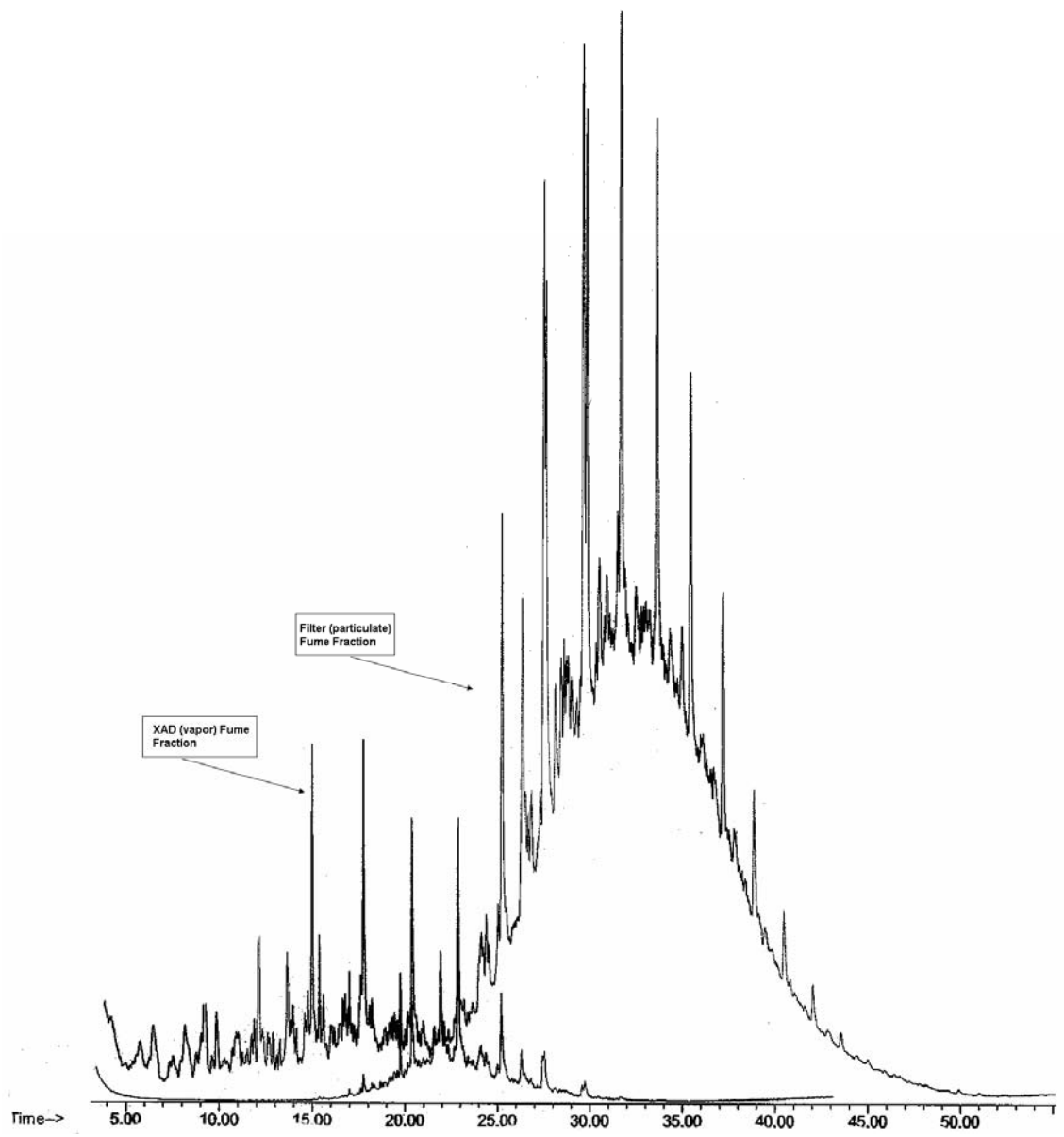
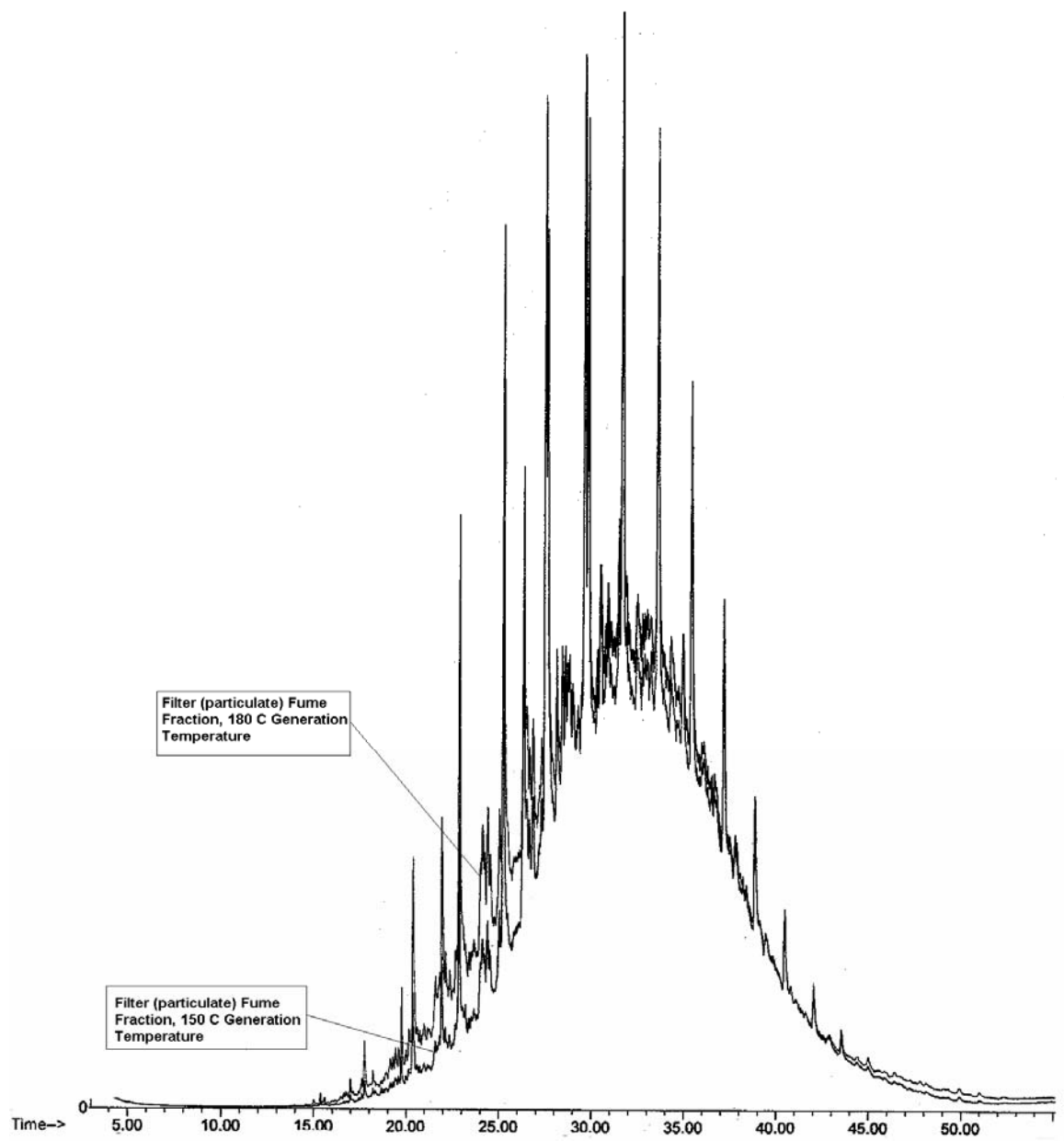**Figure 14- TIC overlay of XAD and filter extracts.**

Figure 15- 180 ^{o}C and 150 ^{o}C filter (particulates) extracts overlaid. This shows a lack of qualititative change.
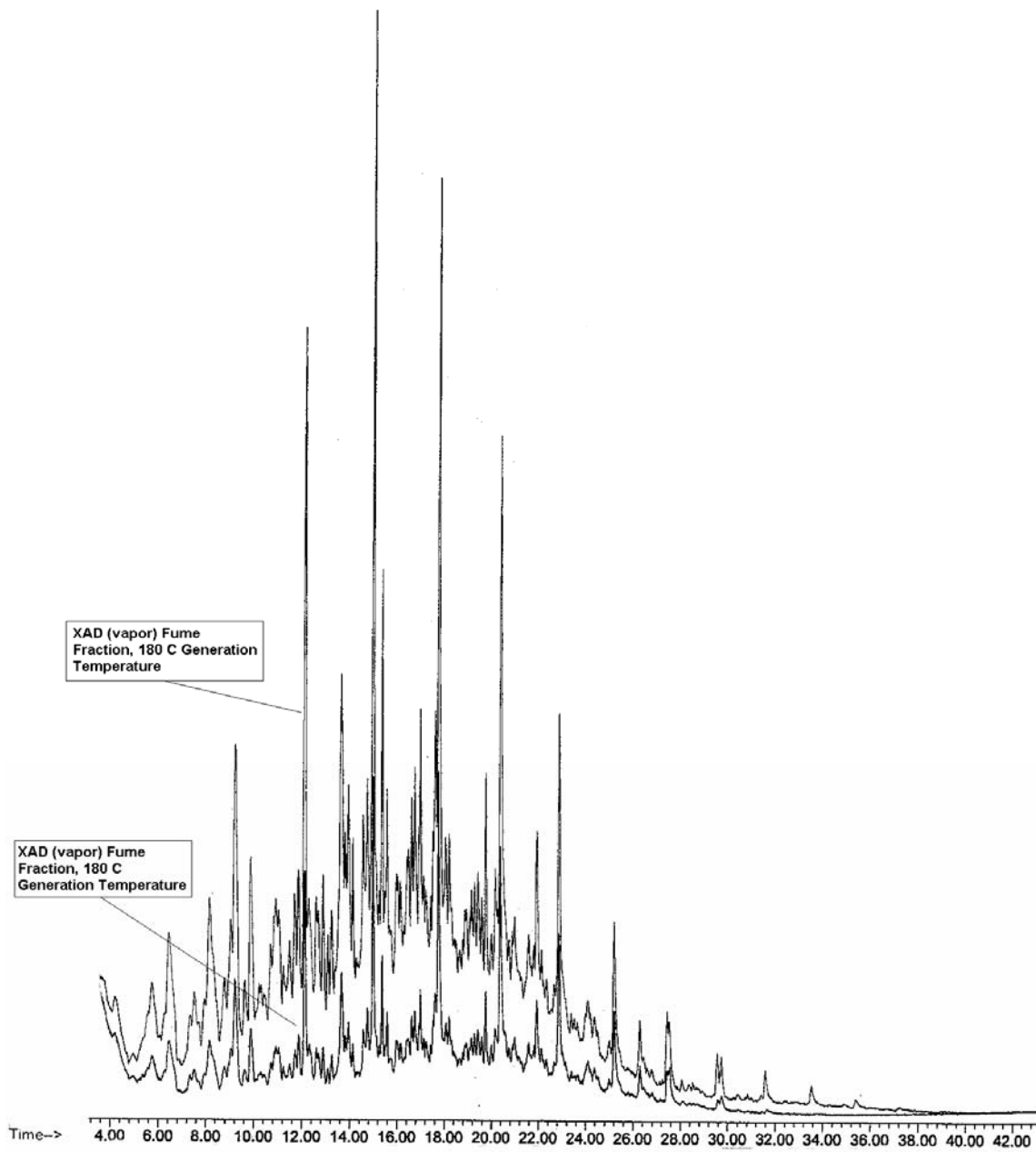
Figure 16- 180 °C and 150 °C XAD (vapor) extracts overlaid. This shows a lack of qualititative change.
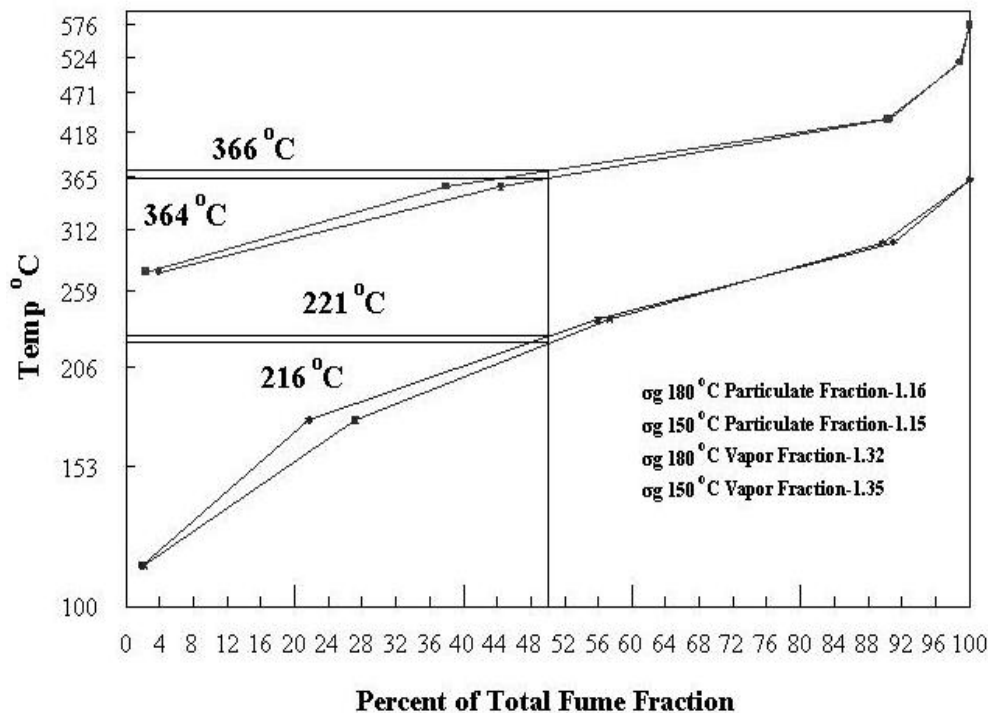
Figure 17- Simulated boiling point profile of 180 °C and 150 °C vapor and particulate fractions. Listed on the figure are the median cumulative boiling points, 366 °C and 364 °C for the particulate fume fractions generated at 180 °C and 150 °C respectively. 221 °C and 216 °C for the vapor fume fraction generated at 180 °C and 150 °C respectively.

4.4 Identification of PAH Compounds in Asphalt Fume Condensate

HPLC fume fractions were collected by utilizing a 100% hexane hold to 100% DCM rapid gradient at a constant 1.5ml/min flow rate. To simulate asphalt, kerosene and a PAH cocktail were mixed. Kerosene is a good surrogate for the aliphatic compounds that interfere with asphalt analysis, but like asphalt, kerosene also contains other compounds. The QTM mixture (Supelco #47930-U) contains 16 PAH compounds which the EPA considers significant. After using various times, gradients, and flow rates the conditions for HPLC were chosen. The surrogate mixtures were also used to establish when the aliphatic compounds and the PAH compounds would elute from the column. This method established that the aliphatic compounds would elute between 25 and 40 minutes and the PAH compounds would start at 50 minutes. These times were

established using a fluorescent detector set to an excitation wavelength of 254 nm and an emission wavelength of 400nm. Aliphatic compounds were detected using a photo diode array (PDA) scanning the UV range. The HPLC runs were started when the autosampler started its pre-injection method of washes. The surrogate mixture was also used to assess what losses occurred during processing and cleanup of the samples. The concern was that semi-volatile compounds such as naphthalene would be lost during the concentration step. Preliminary experiments established that there would be evaporative loss of naphthalene and that concentration and clean-up were not required for its quantification by GC-MS.

The recovery of PAH compounds from the HPLC cleanup method ranged from a low 8% for naphthalene (due to evaporative losses) to a high of 98% for indeno(1,2,3-cd)pyrene, with the larger compounds (4, 5, and 6 ring) having better recoveries than the smaller compounds (2 and 3 ring). The relative recoveries were accounted for by the use of appropriate internal standards. Naphthalene was quantified directly from the sample media extracts to prevent evaporative losses.

Figure 18 is a comparison of a representative fume sample total ion chromatogram (TIC) of the asphalt fume particulate crude DCM extract before and after the cleanup procedure to remove aliphatic compounds. The vast majority of the chemical composition of asphalt fume is aliphatic in nature. Electron impact ionization tends to fragment these compounds into many daughter ions that interfere with identification of the PAH compounds of interest.   Phenanthrene is presented to demonstrate the need and utility of the cleanup procedure. It was observed, as demonstrated in Figure 19, that the 178 m/z of interest was miniscule compared to the interfering ions. After the cleanup, not only was 178 m/z clearly observable, the isotopic distribution for phenanthrene was discernable. It must be noted from the TIC that chromatographic resolution of the PAH compounds of interest was not sufficient to separate individual compounds, even after the extensive clean up. This precluded analyses by non-selective detectors such as the flame ionization detector. Using GC/MS in SIM mode resulted in the identification and quantification of seven PAH compounds of interest. The compounds identified were

naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, and chrysene. Table IV summarizes the asphalt fume concentrations measured for these compounds. Naphthalene was the most abundant PAH identified. It must be noted that a small fraction of the total naphthalene may have also been present at trace levels in the particulate phase; however, interferences in crude extracts and evaporative losses during the cleanup procedure prevent its detection in the particulate phase.

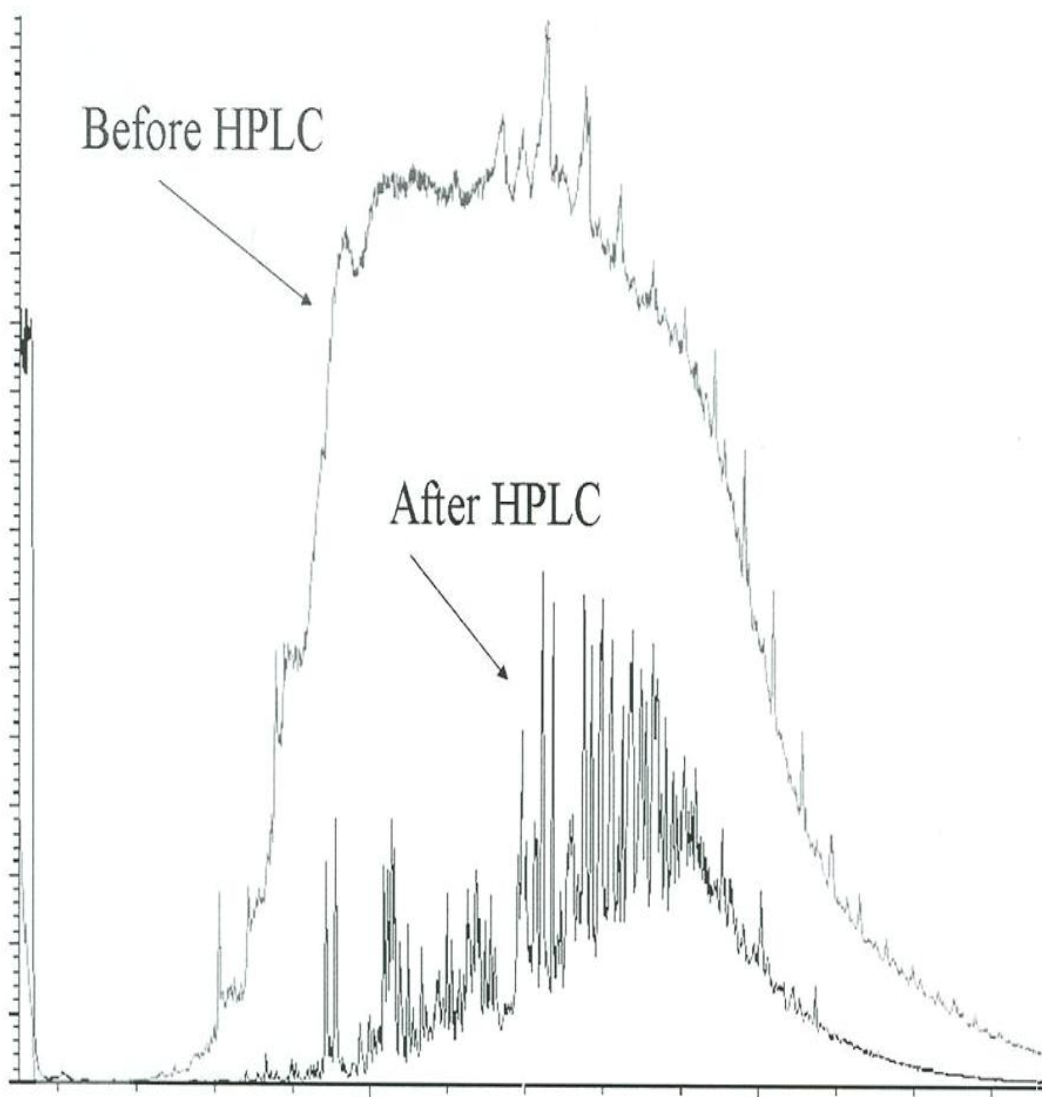**Figure 18- Representative total ion chromatogram of the same fume sample before and after the preparatory HPLC cleanup procedure to remove aliphatics.**
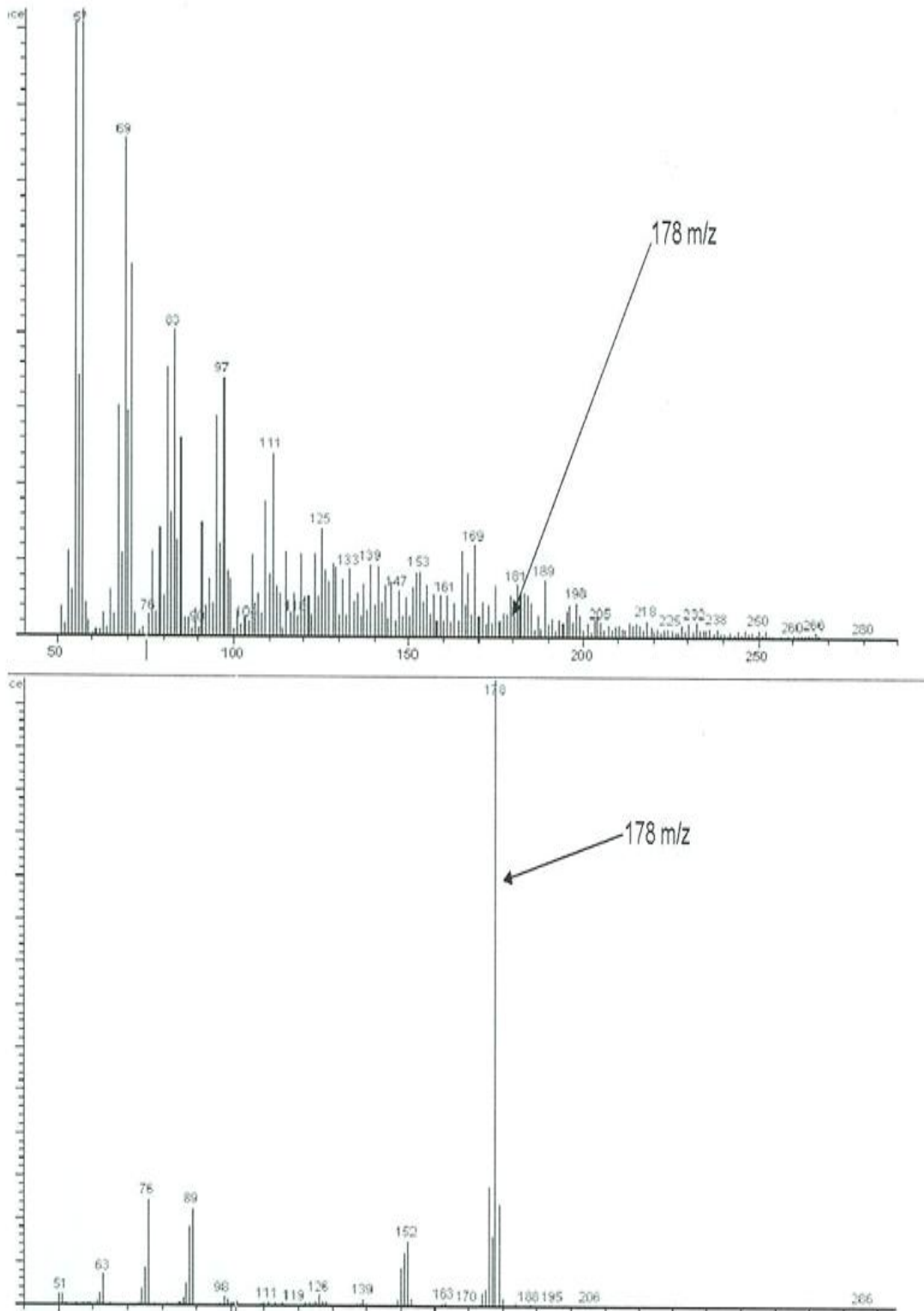
Figure 19- Representative EI-MS spectra are at 29.4 minutes. The top spectrum is the crude extract prior to HPLC cleanup, the bottom spectra is the same fume extract after the cleanup procedure. 178 m/z is the molecular ion for phenanthrene

Table IV. Summary of identified compounds and their concentrations at the150 $^{\circ}$C and 180 $^{\circ}$C.

	Generation Temperature = 180°C				Generation Temperature = 150°C			
PAH	Vapor μg/m^3	Part. μg/m^3	Total μg/m^3	PPM (by weight of total fume)	Vapor μg/m^3	Part. μg/m^3	Total μg/m^3	PPM (by weight of total fume)
Nap.	185.8±53.5	ND	185.8±53.5	2826±813.9	146.3±12.8	ND	146.3±12.8	3726±326.3
Acenap.	1.89±2.7	0.63±0.75	2.52±2.7	34.4±38.5	2.4±1.2	ND	2.4±1.2	60.6±30.9
Fluore.	8.21±9.7	2.63±0.33	10.84±9.7	165.0±151.3	8.7±4.4	1.67± 0.29	10.37±4.4	264.5±182.8
Phenan.	13.4±13.7	10.08±1.13	23.48±13.7	357.2±217.8	20.0±28.5	7.98± 1.10	27.98±28.5	712.6±716.7
Fluora.	ND	0.29±0.39	0.29±0.39	7.8±8.4	ND	1.02± 0.31	1.02±0.31	6.2±9.5
Pyrene.	1.3± 5.9	ND	1.3±5.9	84.9±86.6	3.3±5.9	ND	3.3±5.9	212.3±302.2
Chrys.	ND	1.19±0.78	1.19±0.78	18.1±11.9	ND	1.60± 0.40	1.60±0.40	40.9±10.3

[a]n=4

4.5 Use of Fluorescent Techniques for the Identification and Quantification of PAH Compounds

It was not possible using the described techniques to identify or quantify the class (the number of rings present) of PAH compounds in the asphalt fume samples. Further scrutiny using three-dimensional synchronous scanning fluorescence techniques failed to yield adequate separation to identify compounds by specific ring numbers. Figures 20-25 show some examples of the three-dimensional synchronous scanning fluorescence results. Scans were all run using the same instrument settings, however the software auto-scaled the scans. The scans have areas of specificity; naphthalene has specific peaks at four coordinate sets; 280nm, 50nm; 300nm, 30nm; 350nm, 40nm; and 400nm, 50nm. Specific areas can be seen in all the scans of pure compounds or simple mixtures. Areas of specificity were not discernable when scans were performed on the particulate, vapor, and PAH mixture.

5. Discussion

Quantitative differences were observed in vapor fume fraction concentrations between the 180 $^{\circ}$C and 150 $^{\circ}$C generation temperatures. Crude asphalt, even from the same lot, is a non-homogenous mixture. This adds to the variability when examining both qualitative and quantitative differences. All specific PAH compounds identified in the 180 $^{\circ}$C fume were also present in the 150 $^{\circ}$C fume.

A simulated boiling point profile was developed for the particulate and vapor fume fractions. Again, there was no observable shift in boiling point curves in either fraction. Minor changes could be observed in areas of the curve; however, mean boiling points remained unchanged between generation temperatures. This suggests that fume composition differences noted in published reports generated during road paving operations adhering to industry standards (150 $^{\circ}$C to 180 $^{\circ}$C) are probably not attributable to differences in application temperature.

Prior to running the extracts through the clean-up procedure, it was not possible to identify specific PAH compounds from the filter extracts. The low abundance PAH ions were overwhelmed by the more abundant aliphatic compound ions in the mass spectrum. Using selected ion monitoring resulted in awry specific ion ratios compared to the standards, preventing unequivocal identification and quantification of individual PAHs. When using the present HPLC preparatory (cleanup) method prior to GC/MS-SIM analysis, the m/z and isotopic distribution were prominent, allowing for identification and quantification of the PAH compounds. Seven specific PAH compounds were identified with high confidence in the fume. The compounds identified were two, three, and four ring species. This identification was based on relative retention times (RRTs) and isotopic distribution around the molecular ion peak. RRTs were acceptable if they were within one standard deviation of the mean RRTs from the standards. Two additional compounds, benzo(a)anthracene and benzo(b)fluoranthene, may have been present, however, they were at too low a concentration in the fume to confirm their identity.

Using the National Institute of Standards and Technology library, several substituted compounds were also identified including methyl, dimethyl, and trimethyl naphthalenes; ethyl naphthalenes; methyl and dimethyl fluorenes; methyl anthracenes; methyl phenanthrene; and methyl anthracene. These matches had greater than 90% quality; however, the specific compounds (i.e. position of the methyl groups) were not delineated against standard reference materials. The fume concentrations generated for these experiments were actually much higher than that reported from road paving operations[44]. This allowed for highly concentrated samples for analyses.

Many reports have used less selective techniques such as HPLC fluorescence, GC/MS-SIM based on one ion and GC-flame ionization detectors to identify and measure specific compounds. In this study, monitoring just a single ion and retention time from crude extracts would have indicated the presence 15 of the 16 PAH compounds investigated with the only exception being 2-bromonaphthylene.

In a highly complex mixture with hundreds to thousands of interfering compounds, care should be taken to ensure positive identification. Even when monitoring three m/z at given retention times, these m/z were present in some cases, however upon further scrutiny by using isotopic distributions it became clear that these ions could not be fully attributed to the compounds of interest. Benzo(a)pyrene has been identified in the past using single-ion-monitoring and retention times from asphalt fume extracts. Because of the great variability in asphalt, it was possible that the benzo(a)pyrene was present, but critical assessment of appropriate methodology is often difficult from the published literature. In our studies, after the preparatory HPLC method, the B(a)P m/z were not present above background noise. These results underscore the difficulty associated with assessment of complex chemical exposures, and the requirement of multiple, selective analytical procedures to properly characterize general and specific chemical characteristics of such environments.

The material used in the HPLC column was available in SPE cartridges. The solvent gradient used would easily lend itself to an SPE method and would have the

advantage of multiple samples at a given time. After evaluating many PDVB SPE methods however, the analyte recoveries were not adequate for this purpose. The reason(s) for the drastic difference in analyte retention between the low pressure SPE method and the high pressure HPLC purification were not determined in the present study.

There are numerous sampling, extraction, cleanup, and analysis methods available for identification and quantification of trace PAH compounds. Such methods vary with respect to complexity, utility, and validity for extremely complex mixtures. GC-MS in the absence of prior extensive sample cleanup is plagued with interferences. Reverse or normal phase HPLC-fluorescence detection lacks both chromatographic resolution and detection specificity for this application. Solid-phase extraction techniques employed for cleanup in the procedure for total PAH analyses was examined[15], but were not adequate for specific chemical analyses by GC/MS. Addition of a liquid/liquid partitioning step between hexane and DMSO provided adequate clean-up, but poor and variable recoveries of individual PAH compounds (data not shown). The DMSO solvent prevented further sample concentration in addition to being a poor GC/MS solvent.

The methods developed for this report worked well and allowed for confident identification and quantification of specific PAH compounds, characterization of the boiling point profile of asphalt fume vapor and particulate fractions, and establishing correction factors for standards previously used for quantification of PAH from complex mixtures. The cleanup method allowed for collection of a single fraction, which represents a major improvement of collecting numerous fractions required by normal phase preparatory HPLC sample cleanup procedures. A limiting factor to the analyses provided was the sensitivity and specificity of the MS detector. The GC/MS system is one that is widely used and available in most environmental/industrial hygiene chemistry laboratories. The GC/MS in SIM mode worked well using strict criteria to identify PAH compounds with high confidence, following the preparatory HPLC cleanup to remove the abundant interferences.

In conclusion, it can be said that previous studies, based on the data presented in this study, may be overestimating the concentrations of PAH compounds present in

asphalt fume from paving operations. The boiling point profile of fumes generated show no qualitative difference across industry accepted paving temperature ranges. This data also indicated that fluorescence techniques may have little value in accurately identifying and quantifying compounds based on ring numbers or specific compounds based on standards in complex mixtures.
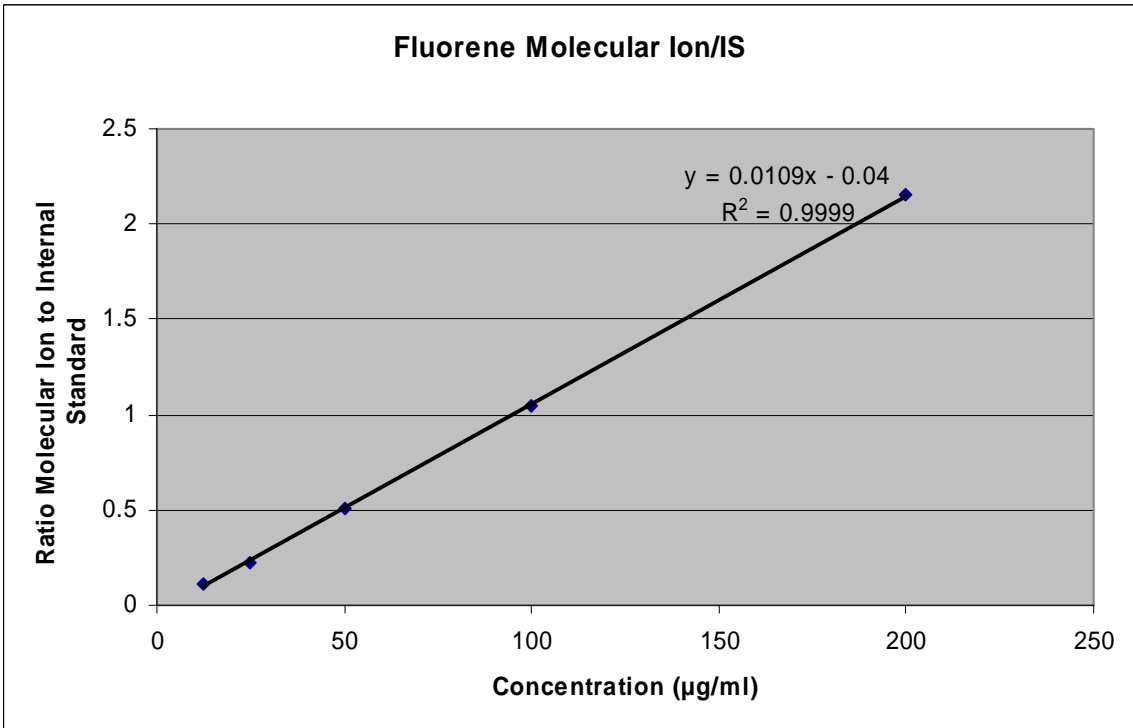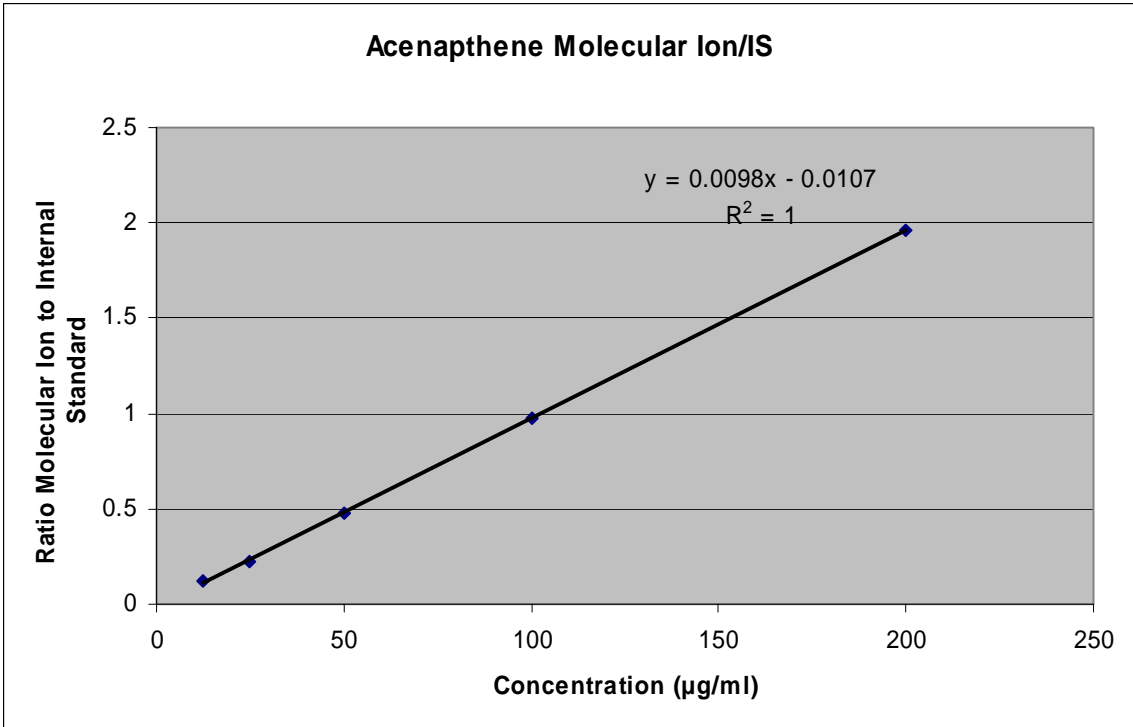
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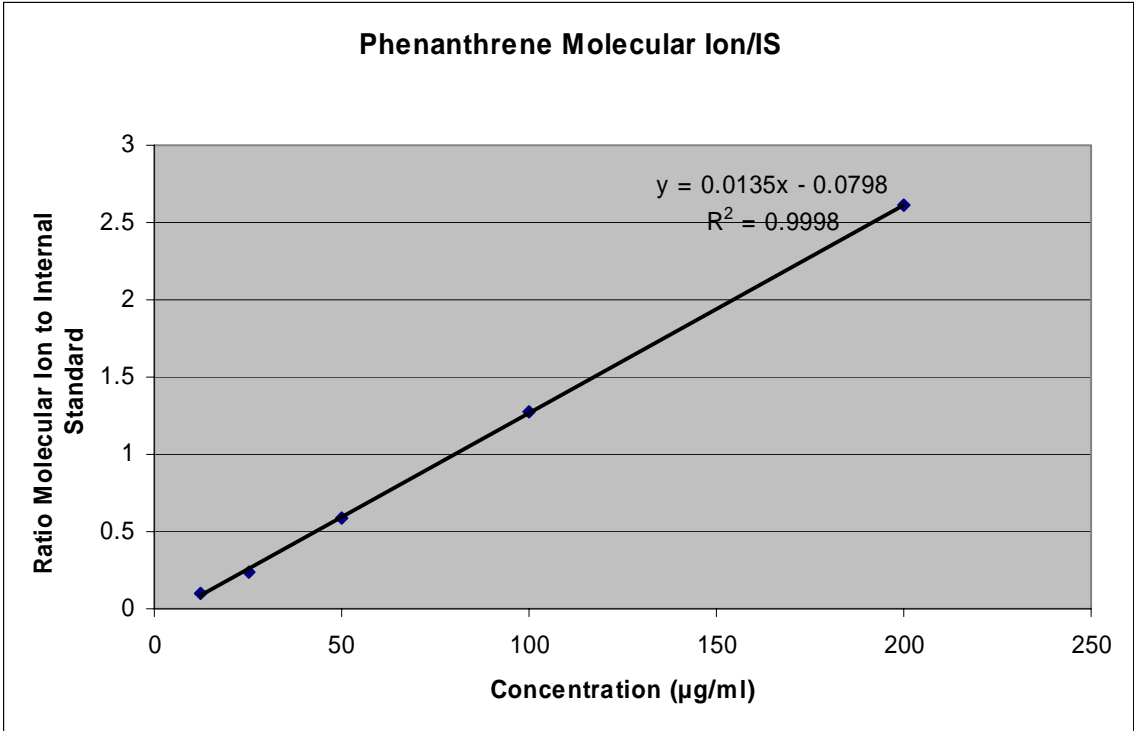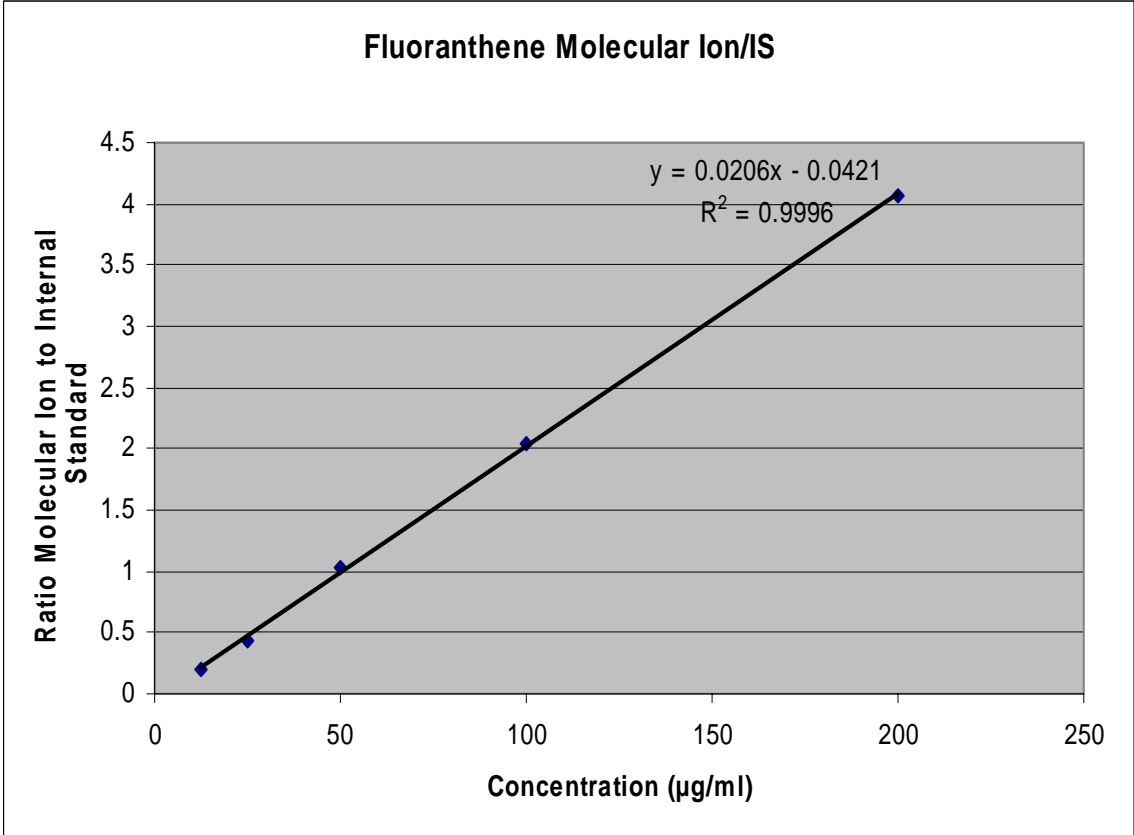
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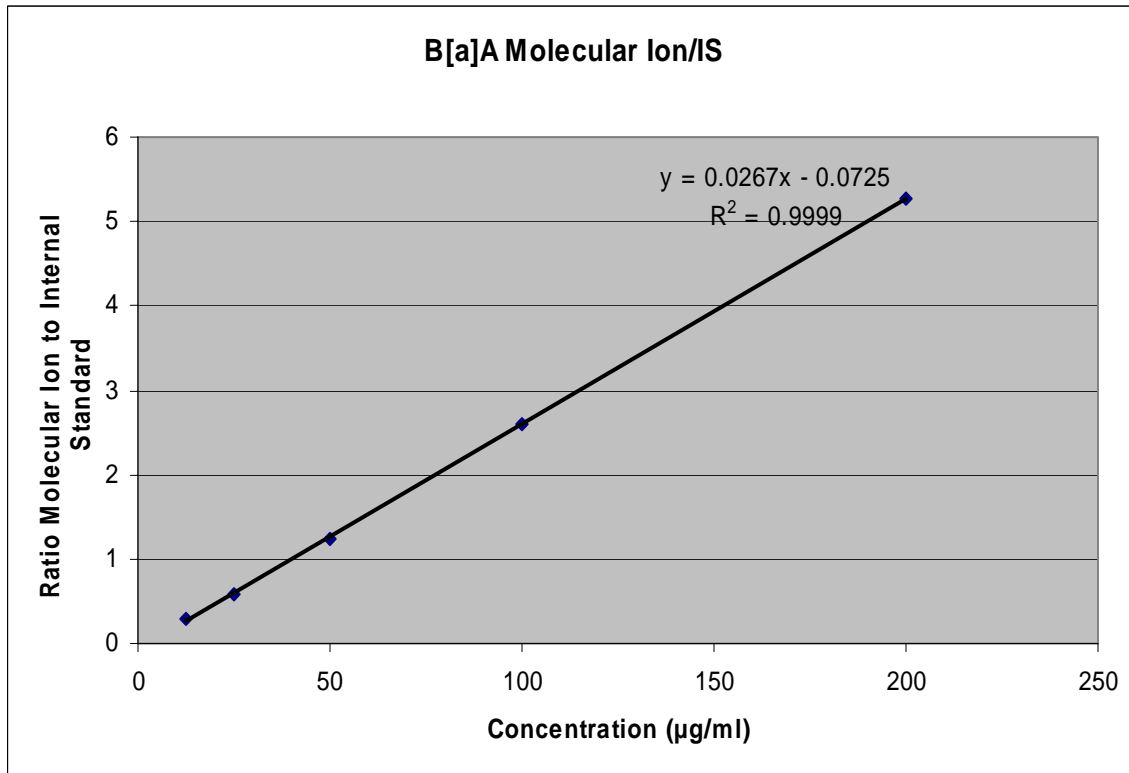
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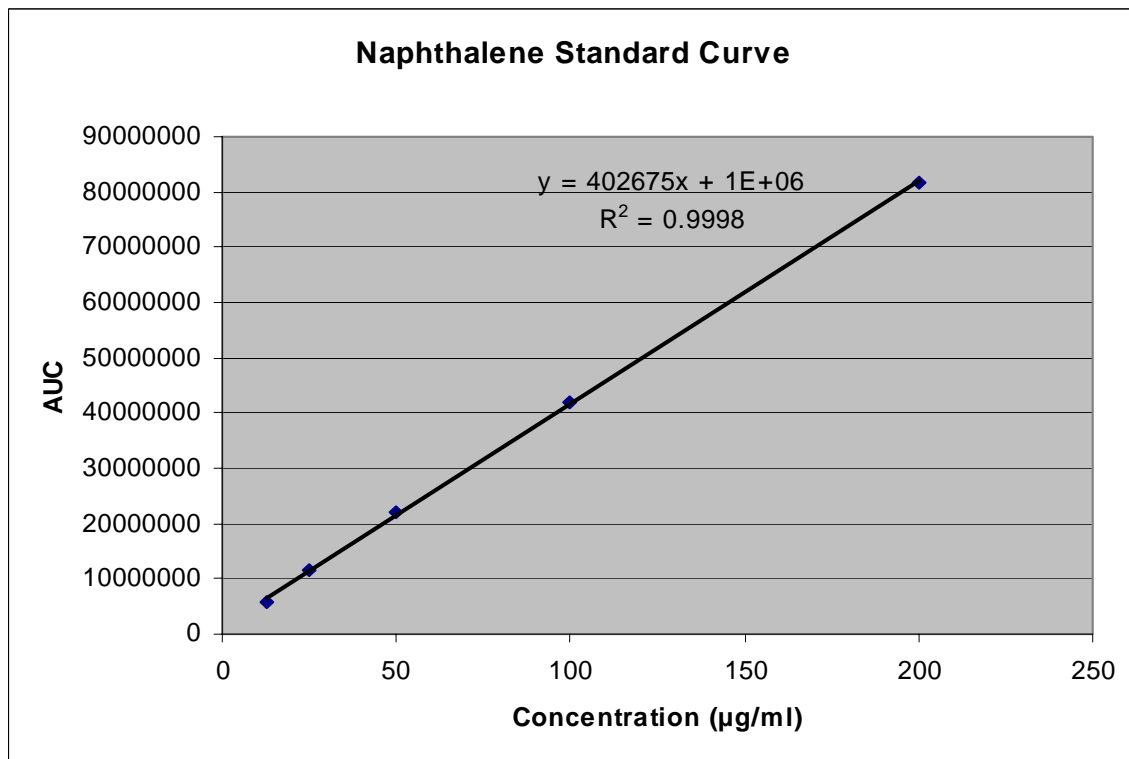
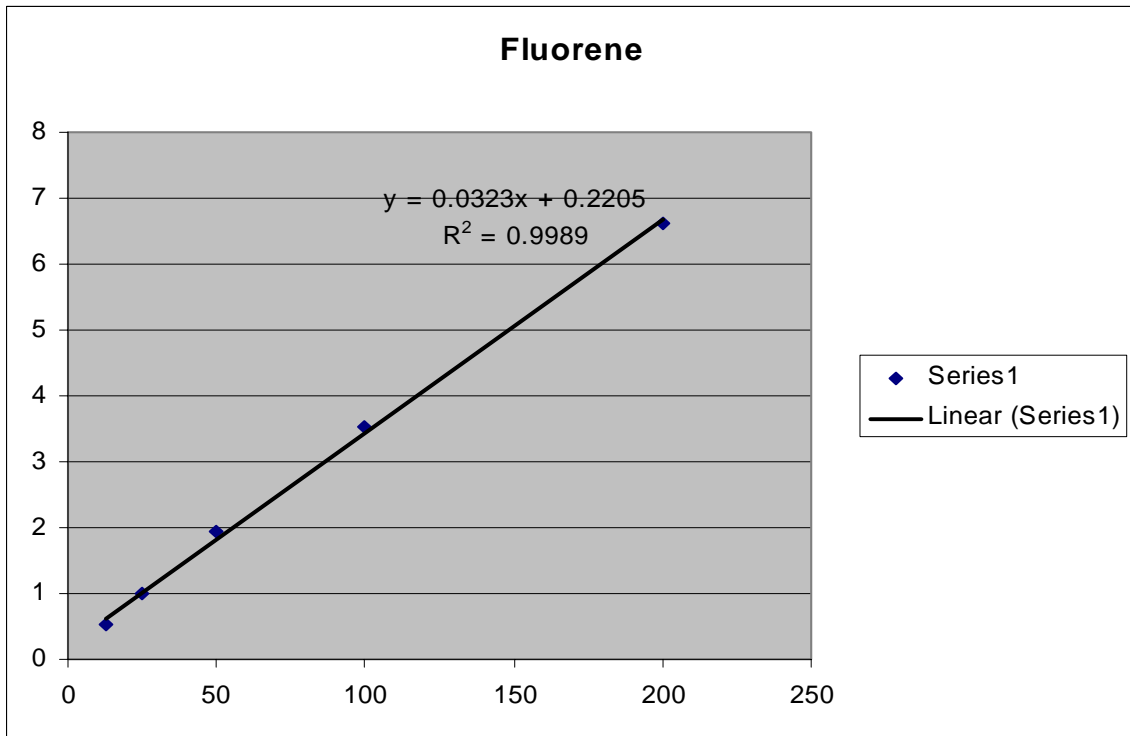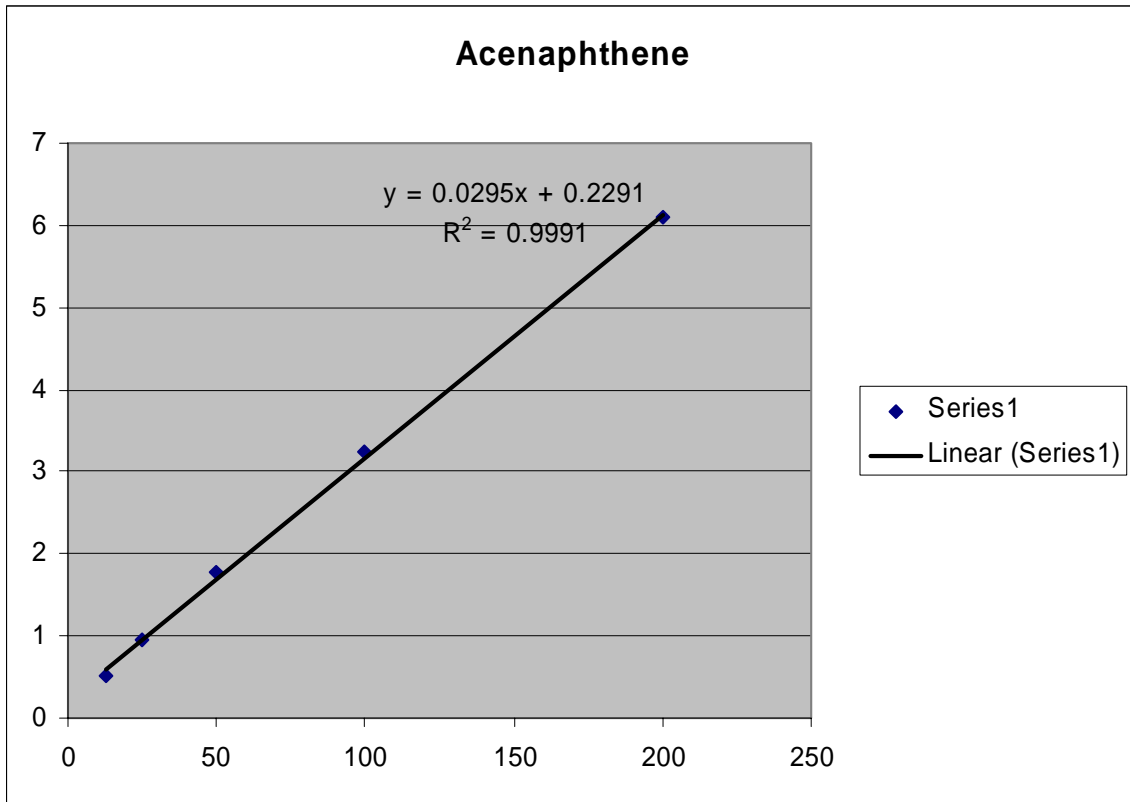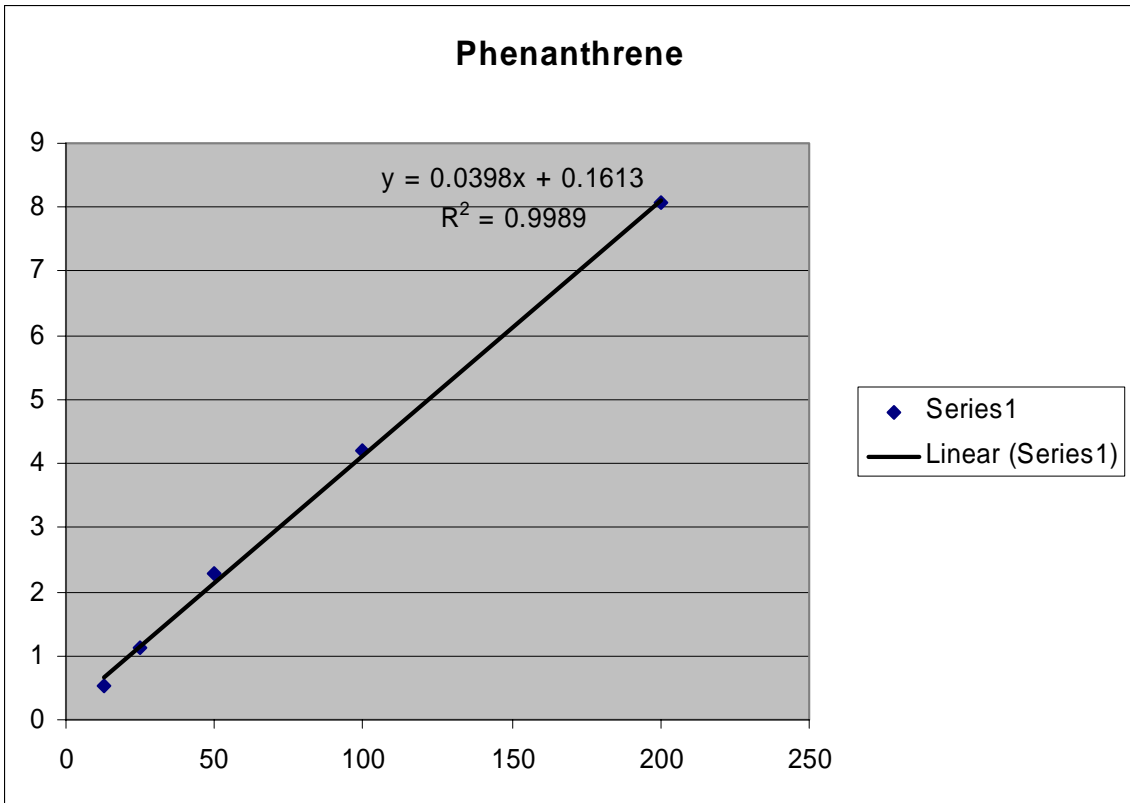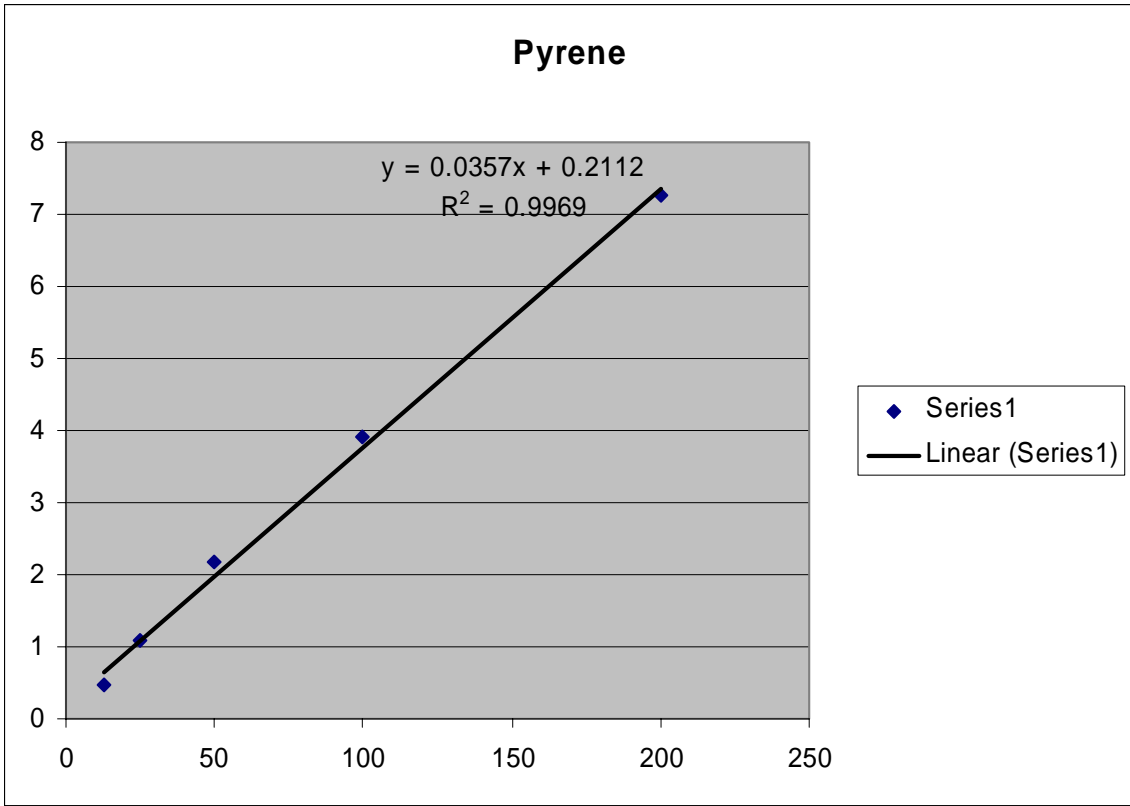**Appendix I. Standard Curves Used for Quantification of Filter Extracts**

Acenapthene Molecular Ion/IS

y = 0.0098x - 0.0107
$R^2 = 1$

Ratio Molecular Ion to Internal Standard

Concentration (µg/ml)

Fluorene Molecular Ion/IS

y = 0.0109x - 0.04
$R^2 = 0.9999$

Ratio Molecular Ion to Internal Standard

Concentration (µg/ml)

Fluoranthene Molecular Ion/IS

y = 0.0206x - 0.0421
$R^2 = 0.9996$

Ratio Molecular Ion to Internal Standard

Concentration (µg/ml)

Phenanthrene Molecular Ion/IS

y = 0.0135x - 0.0798
$R^2 = 0.9998$

Ratio Molecular Ion to Internal Standard

Concentration (µg/ml)

46

B[a]A Molecular Ion/IS

$y = 0.0267x - 0.0725$

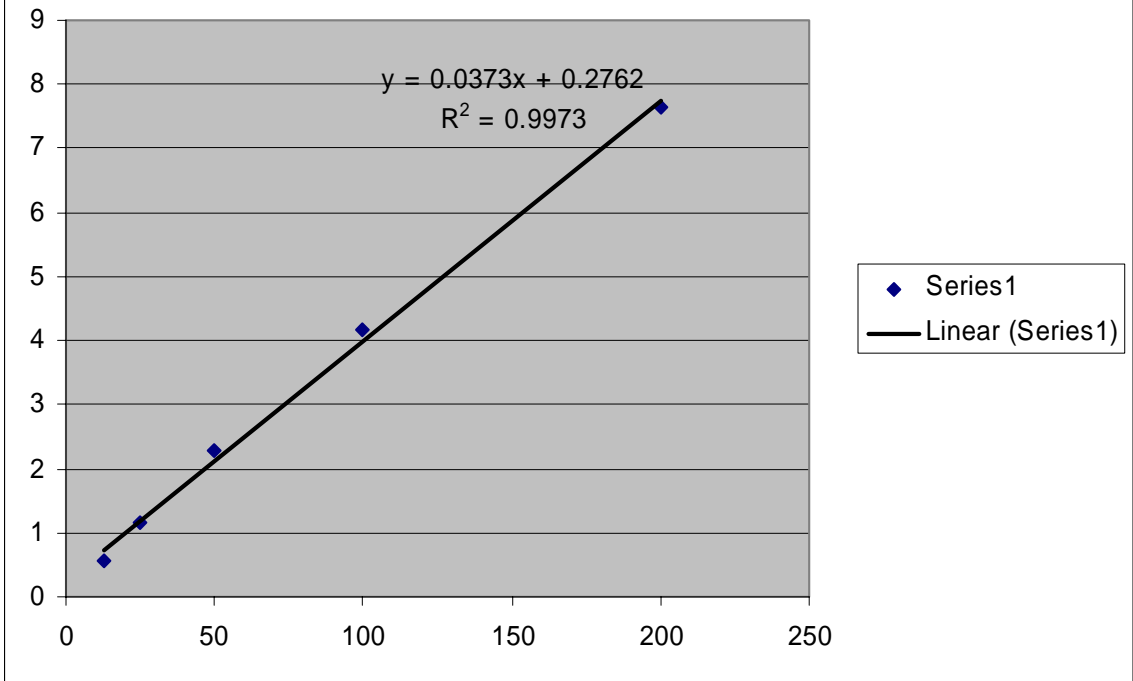$R^2 = 0.9999$

Standard Curve Used for the Quantification of XAD Naphthalene

Naphthalene Standard Curve

$y = 402675x + 1E+06$

$R^2 = 0.9998$

Standard Curves Used for XAD Extract Quantification other than naphthalene

Acenaphthene

$y = 0.0295x + 0.2291$
$R^2 = 0.9991$

Fluorene

$y = 0.0323x + 0.2205$
$R^2 = 0.9989$

Pyrene

$y = 0.0357x + 0.2112$
$R^2 = 0.9969$

Phenanthrene

$y = 0.0398x + 0.1613$
$R^2 = 0.9989$

Fluoranthene

$y = 0.0373x + 0.2762$

$R^2 = 0.9973$

- Series1
- Linear (Series1)

Appendix II. Quality Control

All instrumentation was calibrated with appropriate individual standards and mixes, these results were documented in a lab record. The gas chromatogram with mass spectrometer(GC/MS) was tuned using an autotune sequence built into the software. The autotune was performed for maximum sensitivity once a month and results were printed and logged in the instrument logbook. The instrument was calibrated using a PAH cocktail containing sixteen components, as previously described. Media blanks were prepared and analyzed to ensure there would be little or no contribution from the filter material and adsorbent material used. Blank solvent was also run to ensure no cross-over contamination between samples was present that would interfere with analyses. Three sets of standards were prepared with concentrations in the range of 200 ug/ml to 12.5 ug/ml. The instrument is equipped with an autosampler for consistent sample introduction onto the analytical column. A complete set of standards were placed at the start of the run, a second set was randomly dispersed through the run, and a third complete set was placed after the run. The mean of these three sets was used in the establishment of a quantitative standard curve and each was evaluated to ensure uniform analyese throughout each set of samples. There were four internal standards added to each standard and sample to account for constant losses due to the preparation and analysis procedures. An internal standard is a compound not found in the sample of interest, but has physical chemical similarities to the compounds to be accessed. Specific isotopes of several PAHs were used for this study as described previously. The final step in establishing standard curves for quantification was to calculate a ratio between the molecular ion of an individual compound in the PAH cocktail and the molecular ion of the appropriate internal standard. These ratios were then plotted against concentration. The ratios negate variability due to sample preparation and analyses. An example of the construction of the standard curve can be seen below in Table AII1.

Table AII1

Fluoranthene Concentration ug/ml	Molecular Ion AUC 202	Molecular Ion S. Mean/Molecular Ion IS Mean
200	38453079	4.064738327
100	19341989	2.04457292
50	9729128	1.028431546
25	4120558	0.435569543
12.5	1948672	0.205987192
Internal Standard	**Molecular Ion Mean**	
Chrysene d10	9460161	

Standards were used to establish an average elution time in the preparatory HPLC clean-up method. Since the concern was the class of chemical eluting and not the individual compound the mixture was run several times to find the mean elution time based on a fluorescent peak. Prior to clean-up runs the mixture was injected to establish that the fluorescent detector was working and no major shifts in elution time had occurred. The PAH cocktail was also run after the procedure. Internal standards were introduced prior to this step to negate any recovery varability.

Appendix III. HPLC Information

The system was a GBC LC1150 pump, a Schimadzu RF-551 fluorescence detector, GBC LC5000 photo diode array, GBC LC1650 autosampler, and a GBC LC1460 degasser. The system was controlled using WinChrome V1.32 chromatographic software.
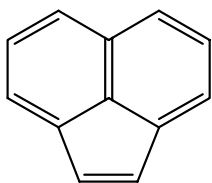
Appendix IV. Gas Chromatograph and Mass Spectrometer (GC/MS) Information

The GC/MS system used was an Agilent 5890 series II plus GC coupled to an

Agilent 5972 MS detector. The system was controlled by Enhanced Chemstation,

V.B.01.00.

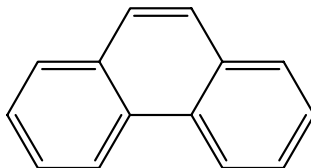**Appendix V. Sixteen PAH Compounds Included in the QTM Cocktail**
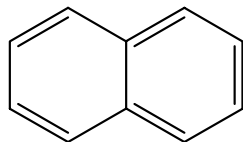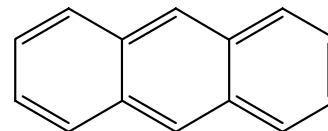

Naphthalene
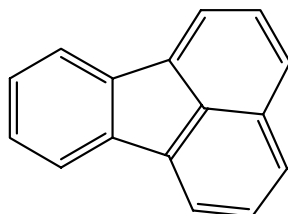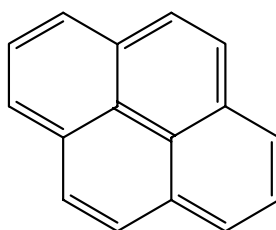

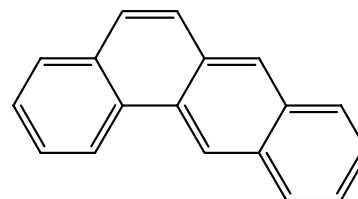
Acenaphthylene
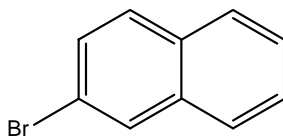
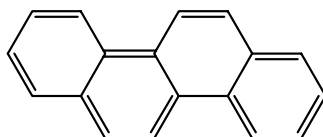2-Bromonaphthalene
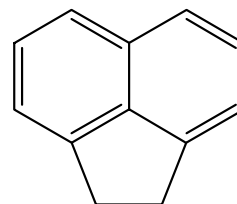
Acenapthene
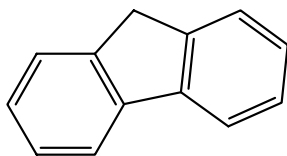
Fluorene

Phenanthrene

Anthracene

Fluoranthene

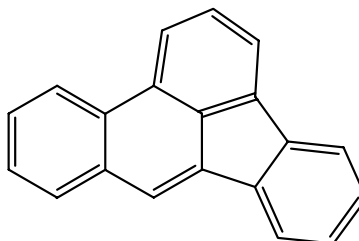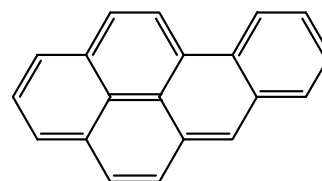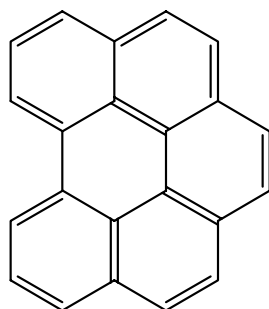
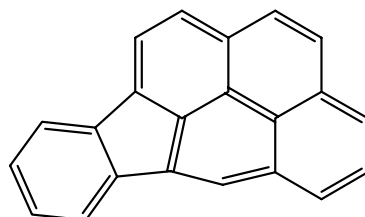
Pyrene

Benzo[a]anthracene
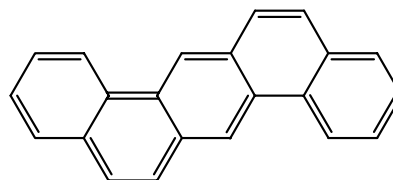

Chrysene

Benzo[b]fluoranthene

Benzo[a]pyrene

Benzo[ghi]perylene

Indeno[1,2,3-cd]pyrene

Dibenz[a,h]anthracene

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