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"Polycyclic Aromatic Hydrocarbons in Particulate Air Pollutants"*

by

Jan E. Harris**
Lycoming College
Williamsport, Pennsylvania

under the supervision of
Dr. Albert E. Gudat

Center for Educational Affairs
Argonne National Laboratory

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous in the environment; some of the PAH are carcinogenic. A method was developed to identify local emissions of atmospheric PAH. Air samples were routinely collected for four-hour periods and extracted with hexane. The concentration of PAH was determined by high performance liquid chromatography with fluorescence detector. Two samples contained 42 ng/M³ of fluoranthene and 8 ng/M³ benzo(a)pyrene, respectively. A study was also performed to determine the degree of degradation of PAH in solution.

Approved for Departmental Honors

Martin A. Fineman

David A. Faug

Dan King

James R. Summers

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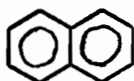
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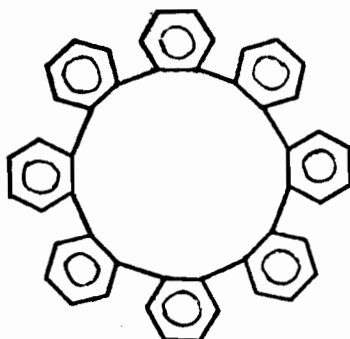
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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are organic compounds containing two or more aromatic rings. They may vary in shape and size from the simple structure of naphthalene



to complex structures such as octophenylene (1).



PAH are ubiquitous in our environment and are present from natural and anthropogenic sources (2). A number of these PAH are carcinogenic (3), and these facts have generated a great deal of interest in discovering the amounts of PAH in the environment, particularly the atmosphere.

PAH can be formed in the combustion of any compound containing carbon and hydrogen (4). Major anthropogenic sources are heat and power generation, refuse incineration, coke production, and motor vehicles. A significant indoor source is tobacco smoking, where PAH are formed in both the sidestream and mainstream smoke. Important natural contributors to background levels of PAH are forest fires, and to a lesser extent plants and certain microorganisms (5).

The first link between air pollution and cancer was discovered by Sir Percival Pott (6) in 1775 who found that chimney sweeps had an unusually high incidence of scrotal cancer, indicating that carcinogenic factors were present in soot. Two of the first pollution related PAH to be identified as carcinogens were dibenzo(a,h)anthracene and benzo(a)pyrene. Since then the list of carcinogenic PAH found in urban air has grown and includes benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, dibenzo(a,i)pyrene, dibenzo(e,i)pyrene, benzo(e)pyrene, dibenzo(h,rst)pentaphene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, and indeno(1,2,3,-cd)pyrene (7). Structures for these compounds can be found in appendix A.

A large amount of work has been done to measure the amounts of these carcinogens in the atmosphere. Earlier methods of analysis relied on thin layer, conventional liquid-liquid, gel, and paper chromatography for separation and on luminescence or absorbance properties for detection (8). More recently these methods have been replaced by gas chromatography-mass spectroscopy (GC-MS) and high pressure liquid chromatography (HPLC) - spectrophotometric detection.

High pressure liquid chromatography using fluorescence for detection is a very promising technique and was the method chosen for this project. A number of recent papers (9-12) indicate good separation of PAH using HPLC and either UV absorbance or fluorescence detection. However, the detection limit appears to be much lower with fluorescence. Williams and Slovin (9) report a detection limit of 40 pg of benzo(a)pyrene using a filter fluorometer, whereas Dong, Locke, and Ferrand (10) report 10 ng of benzo(a)pyrene as their detection limit using a fixed wavelength UV absorbance detector.

Fluorescence also has better selectivity for PAH, since some interfering compounds will absorb light but will not fluoresce. Also, in fluorescence both the excitation wavelength and the emission wavelength can be adjusted to maximize the signal for a certain compound. In absorbance detection only the absorption wavelength can be adjusted.

Many sources of PAH have been identified and these compounds are found in both rural and urban areas. However, often it has not been possible to determine which of the many sources is the main contributor for a given area. The eventual goal of this project was to develop a method to identify local sources with heavy PAH emissions.

EXPERIMENTAL DETAILS

Air samples are collected on glass fiber filters. A short sampling period of four hours is used, ensuring that the wind direction during each sampling period is relatively constant. This also allows six samples to be collected daily from a single sampler, giving a large number of samples in a short period of time. Individual filters are routinely extracted and the amount of certain PAH contained in each discrete sample is quantified by HPLC using a fluorescence detector. The average wind direction for each sample is plotted vs. the concentration of a certain PAH in that sample. This plot should show the direction(s) of the source(s) from the sampler. By using three samplers at different locations, the source(s) can be located using a triangulation approach.

HPLC Conditions

All organic solvents were purchased from Burdick and Jackson Labs, Inc. and were prepared by distillation in glass. All organic solvents were high purity grade. Hexane was of non-spectro grade quality; however, a sample injected into the chromatograph produced only two small and insignificant fluorescence peaks.

Water used in the solvent gradient system was distilled and deionized. The water was filtered through 0.45 μ Millipore filters to remove small particulate matter which could deposit on the analytical column and change its performance characteristics. The water was checked for hydrocarbon content and was judged satisfactory.

Perylene, fluoranthene, and phenanthrene were purchased from Chem Service (practical grade), benzo(a)pyrene was obtained from Eastern Chemicals, naphthalene from Fisher Scientific (Baker reagent grade), and anthracene from an unknown source (unknown purity).

A Varian 8500 high pressure liquid chromatograph was used throughout the experiments. This gradient elution system contains two high pressure syringe pumps and a solvent programmer. The solvent mixture is pumped to the column at a constant flow, and the pressure is adjusted accordingly.

Gradient elution is the process of changing the composition of the mobile phase with time to produce the best separation of the compounds of interest. The initial mixture is composed of enough of the "weak solvent" so that only compounds weakly retained by the column will be eluted. The percent composition of the "stronger solvent" can then be increased to elute more strongly retained compounds. The words "weak solvent" and "stronger solvent" refer to the position of the solvent in the eluotropic series.

The gradient programmer of the Varian 8500 HPLC provides for an almost unlimited variety of gradients. Besides increasing the amount of the strong solvent with time, it can be held constant for certain intervals, or even decreased before increasing again. The rate of change can also be varied greatly. An outline of a possible gradient follows:

Begin at 60% CH₃CN, 40% H₂O
Increase at 2% CH₃CN min⁻¹ for 10 min.
Steady at 80% CH₃CN, 20% H₂O for 5 min.
Decrease at 1% CH₃CN min⁻¹ for 5 min.
Steady at 75% CH₃CN, 25% H₂O for 5 min.
Increase at 5% CH₃CN min⁻¹ for 5 min.
Steady at 100% CH₃CN for 15 min.
Decrease at 10% CH₃CN min⁻¹ for 4 min.

Two sets of solvents were used with the HPLC, acetonitrile/water and methanol/water. Water is the weaker solvent for PAH in both cases. The acetonitrile and water combination worked best in separating PAH

mixtures. The solvents were degassed daily by placing them under vacuum in an ultra-sonic generator for ten to fifteen minutes.

Two types of columns were used, an Aerograph Micropak CH-10 column and an Aerograph Micropak MCH-10 column. Both are stainless steel, packed with 10 micron silica particles to which is bonded a layer of octadecylsilyl groups. In the MCH-10 column, the bonded phase is monomeric and in the CH-10 column the bonded phase is polymeric. The CH-10 column proved much better in separating PAH mixtures and was used for all results shown in this paper. Column temperature was ambient except for one experiment.

An accurate and reproducible injection technique is necessary for good results. A 10 μ l Hamilton syringe was used throughout the experiments. Before filling the syringe with the sample, it was rinsed several times with solvent. This procedure left a "plug" of solvent in the back of the syringe that prevented part of the sample being retained in the syringe after injection. A small air gap was maintained between the solvent plug and the sample. The sample was drawn into the syringe and the volume was measured using the calibration marks on the syringe.

The position at which the sample was injected in the system was found to be important. Pieces of tape were placed over the injection port to leave a small hole for the syringe needle. The glass cylinder of the syringe was rested on this tape and the sample was injected, ensuring that the injection position was reproducible.

Two detectors were used, a Varian Vari-chrom UV-visible spectrophotometer and a Schoeffel F.S. 970 L.C. fluorometer. The Vari-chrom is a double-beam, variable wavelength instrument. Its detection limit for PAH

is about a nanogram, and was not very useful in analyzing the four-hour air filter extracts.

The F.S. 970 fluorometer is a single beam instrument with a monochromator to select the excitation wavelength (190-400 nm) and cut-off filters to select the emission wavelengths. These filters transmit light above their stated wavelengths. The F.S. 970 is equipped with a 2π steradian interception curvette which makes this unit the most sensitive spectrofluorometric detector available.

Corrected emission and excitation spectra of PAH have been reported by Porro, Anacreon, Flandreau, and Fagerson (13). These spectra were used to maximize the response for certain PAH. Examples of these spectra can be found in Appendix B.

It was found that turning on the fluorometer, not including the lamp, for two hours prior to operation partially corrected the drifting of the fluorescence signal.

Peaks were identified by comparing retention times with standards and by co-injection with standards. A calibration curve (Figure 13) shows that the fluorescence has linear response from ten ng (using standard solution #2) to 100 pg (using standard solution #3). The fluorescence response will vary from day to day; therefore, peaks were quantified by direct comparison to a standard run before or after the sample.

Sampling and Extracting

The air sampler was an A.I.S.I. Tape Sampler, made by Research Appliance Company. A diagram is shown in Figure 1. After every four hour sampling period the take-up spool pulls the filter paper through and sampling is then continued on a clean spot.

The standard tape sampler above had to be modified to sample a larger air volume. The pump contained in the sampler was disconnected and a high-volume air pump was attached. A Schmidt Model 2-AP constant flow air pump was connected to the sampler. This pump is designed to keep the air flow constant as the filter becomes loaded with particulates. The pump was adjusted to pull one cubic foot of air per minute.

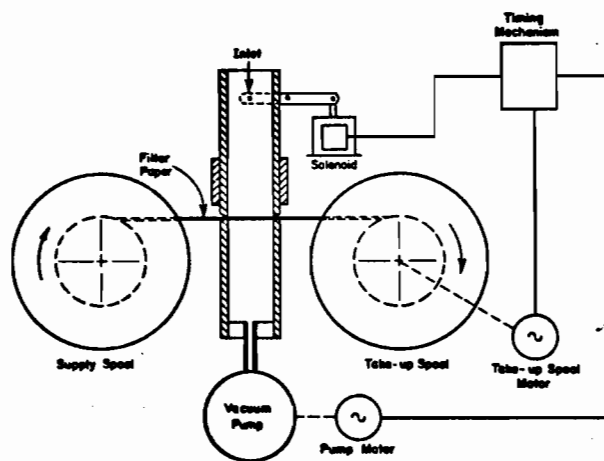


Figure 1. Diagram of A.I.S.I. Sampler

However, because of the large volume now sampled, the fiber glass filter paper had a tendency to bow under the applied vacuum and the paper could not be advanced automatically at the end of the sampling period. A frit, placed beneath the filter paper, prevented this distortion of the paper. Furthermore, a relay was installed that would turn off the pump while the filter paper was being advanced and start the pump afterward for sampling.

The four-hour sampler requires fiber glass filter paper in long-strip format with a width of 38 mm. Unfortunately, this is not a commercial product. For this experiment, 205 x 255 mm sheets of filter

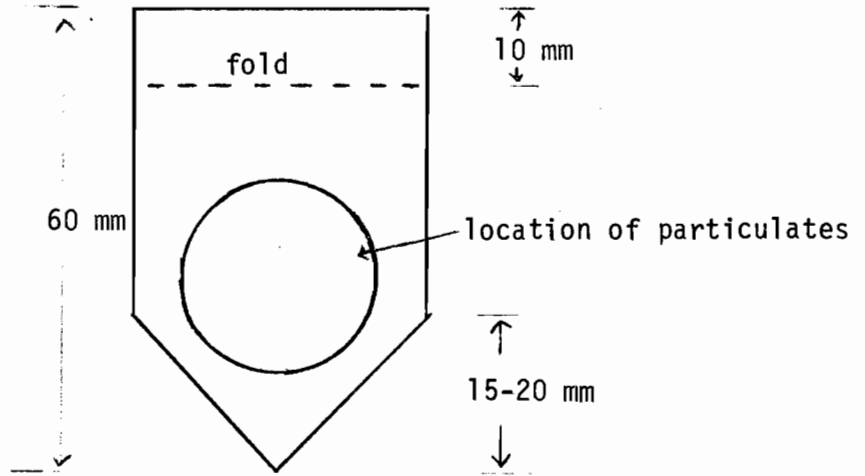
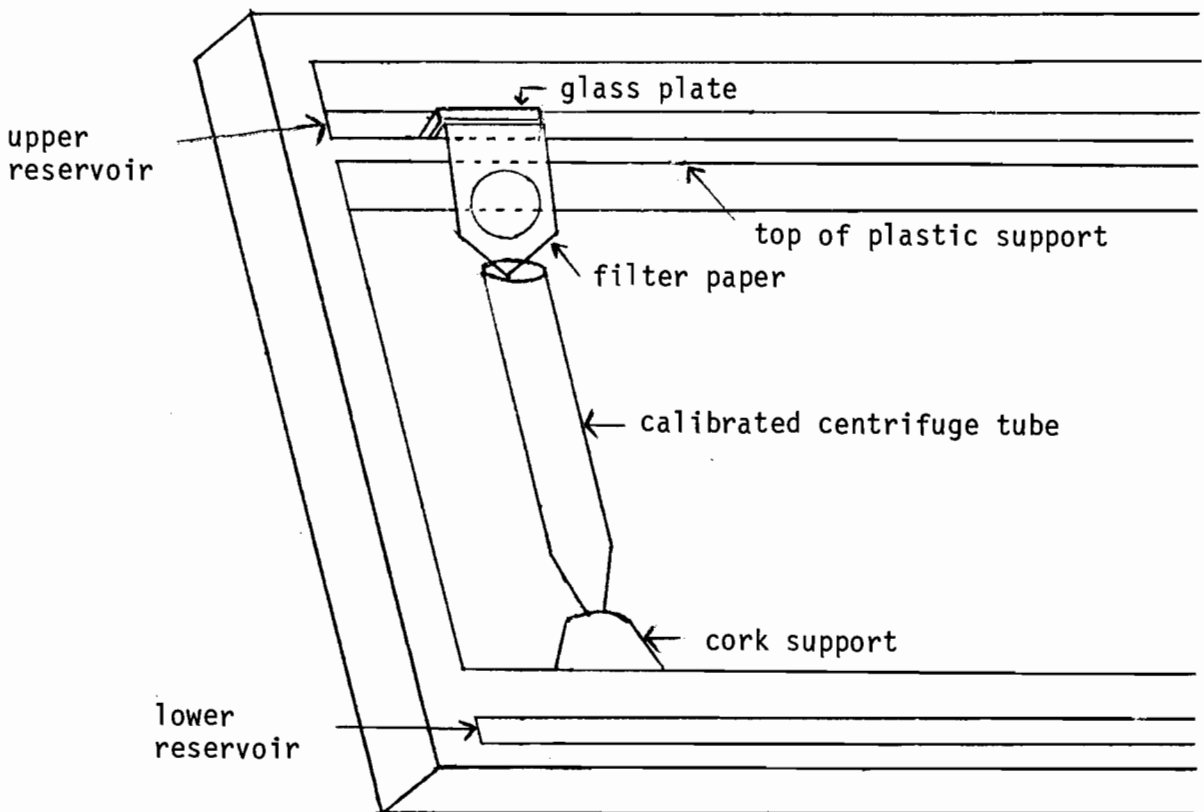


Figure 3 Set-up for Four-Hour Filter Extraction



paper were cut into strips 38 mm x 255 mm; these strips were subsequently taped together end to end with scotch tape. At each splice, one piece of tape was placed on the bottom (smooth) side of the filter paper. Holes were punched along the side of the tape at intervals of 65 mm. These holes activate a microswitch that starts the sampler after every four hour period.

Each four-hour sample was cut and folded as shown in Figure 2. Figure 3 illustrates the position of the filter paper in the extraction box. The glass plate was placed so that the filter paper touched the back side but not the top of the plastic support. Usually four filters were positioned in the box at one time and were extracted simultaneously. Seven to ten ml of solvent was poured into the upper reservoir formed by the back of the box and the plastic support. A small amount of solvent was poured into the lower reservoir and the glass cover was placed over the assembly to prevent excessive evaporation of solvent. Solvent flows through the filter paper, extracting the PAH, and drips into a calibrated centrifuge tube. Solvent must be added to the top reservoir several times during the extraction to compensate for evaporation losses and to facilitate continuous extraction. When the desired volume was collected, the contents of the tube was transferred to a labeled, 1.0 dram screw-cap vial. These samples were then stored in the dark at -6°C.

In some cases, a larger amount of solvent was used than was needed. Beginning November 13th, if a volume of more than 1.5 ml was used, the extract was concentrated under a stream of nitrogen to a smaller volume, usually 1.3 ml.

Preparation of Standards

At the beginning of the project, when benzene was chosen as the solvent for extraction, a set of standards was prepared using benzene

as a solvent. Equal weights of naphthalene, anthracene, fluoranthene, phenanthrene, pyrene and benzo(a)pyrene were used to make solutions with the following concentrations:

- Solution No. 1 - $0.5 \mu\text{g } \mu\text{l}^{-1}$
- Solution No. 2 - $5.0 \text{ ng } \mu\text{l}^{-1}$
- Solution No. 3 - $25 \text{ pg } \mu\text{l}^{-1}$

The solutions were stored in the dark at -1°C .

When hexane was chosen to be the extracting solvent, a set of standards was prepared in hexane. Equal amounts of phenanthrene, fluoranthene, pyrene, and benzo(a)pyrene were used to make solutions with the following concentrations:

- Solution No. 1 - $0.5 \mu\text{g } \mu\text{l}^{-1}$
- Solution No. 2 - $5.0 \text{ ng } \mu\text{l}^{-1}$
- Solution No. 3 - $50 \text{ pg } \mu\text{l}^{-1}$

These solutions were also stored in the dark at -1°C .

RESULTS AND DISCUSSION

Selection of Extraction Solvent

Initially, four solvents were under consideration: benzene, hexane, tetrahydrofuran, and isooctane. All would serve as good extraction solvents; however, in order to select the solvent best suited for our application the following preliminary extraction experiment was performed.

Two filter papers were cut and folded as shown in Figure 2. One was spiked with 100 μl of standard solution No. 2 ($5 \text{ ng } \mu\text{l}^{-1}$), the other was used as a blank. The receiver for the spiked filter paper was changed at regular intervals. The experimental set-up is shown in Figure 3. The amount of benzo(a)pyrene in each receiver was quantified using HPLC and fluorescence detection. One determination was made for benzene and one for hexane; the results are shown in Figure 4.

The results of this experiment indicate that both benzene and hexane extract benzo(a)pyrene efficiently and quickly, almost completely in the first $\text{m}\mu\text{l}$. However, benzene caused a large negative deviation of the fluorescence signal at the beginning of the chromatogram and was rejected for this reason. Tetrahydrofuran (THF) cannot be used because it is a stronger solvent for PAH than the solvents being used in the HPLC analysis; use of THF would lead to varying retention times. Isooctane was rejected because of its high cost.

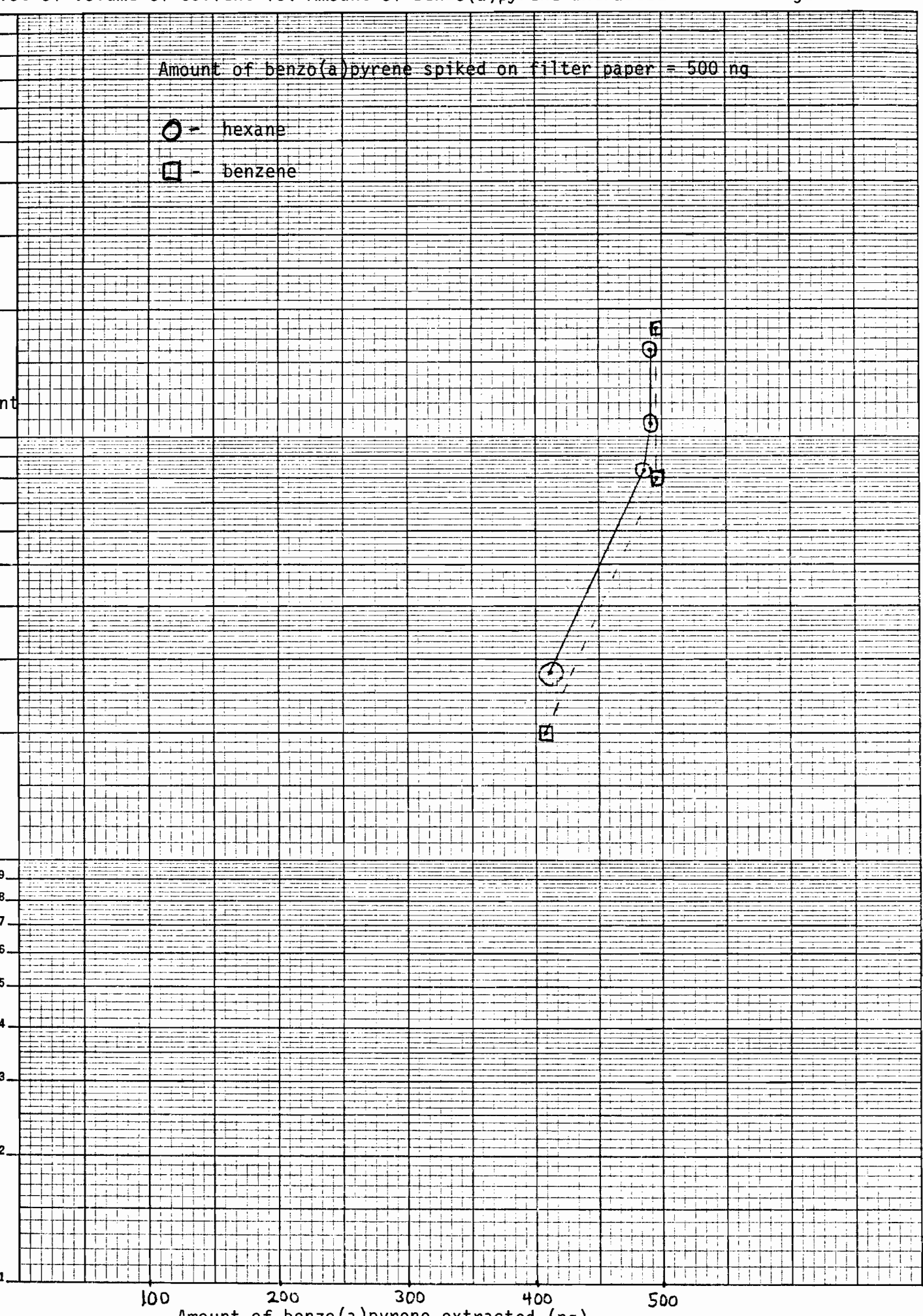
Hexane proved a good solvent in the previous test and was chosen as the extraction solvent for the remaining extractions. After examining Figure 4, it was decided that 1.3 $\text{m}\mu\text{l}$ of hexane should be used in each extraction to ensure complete extraction of PAH.

Degradation of PAH Standards in Hexane

A set of PAH standards in hexane was prepared on November 7th. A

Amount of benzo(a)pyrene spiked on filter paper = 500 ng

- - hexane
- - benzene



chromatograph (Figure 5) of standard solution No. 2 at that time showed the four expected peaks. A small portion of that standard was stored in the dark at room temperature; the rest was stored in a refrigerator at -1°C . On November 16th, chromatograms of these two solutions showed an identical shoulder on the benzo(a)pyrene peak, indicating that degradation of one of the PAH had occurred. One of the chromatograms is shown in Figure 6.

Small portions of standard No. 2 were transferred to small vials and stored in the following locations: the freezer (dark) at -6°C , the refrigerator (dark) at -1°C , a fire closet (dark) at room temperature, and on a shelf in the laboratory (exposed to fluorescent light and indirect sun light). On December 8th, chromatograms were run of these solutions; the results are shown in Figures 7-10. Further degradation has occurred in each vial. Furthermore, the samples corresponding to Figures 5, 6, and 7, even though stored in darkness at -1°C , showed a continued degradation with time.

It seems likely that a factor other than temperature or light is having an effect on the standards. Since the volumetric flasks used in preparing the hexane standards had been cleaned with dichromate cleaning solution and were rinsed several times with distilled-deionized water and several times with hexane, the dichromate adhering to the glass wall may not have been completely removed during the rinses. The residual dichromate probably oxidized the PAH and caused additional peaks to appear in the chromatogram.

PAH are easily photooxidized (14) and Figures 8, 9, and 10 are in agreement with the findings. A large difference in degradation can be seen between Figure 9 and Figure 10. The solution corresponding to Figure 9 was occasionally exposed to room light. Although it shows more degradation

Chromatograms of Standard
Solution No. 2 in Hexane

Figure 5 - Nov. 7th

Figure 6 - Nov. 16th

Figure 7 - Dec. 8th

HPLC Conditions

flow - 1 ml/min.

gradient: plus 2% CH₃CN/min.

from 60% CH₃CN to 100% CH₃CN

Fluorescence Conditions:

excitation wavelength - 289 nm
emission filter:

389 nm for figures 1 and 3
370 nm for figure 2

Compounds in Order of Elution:

1. phenanthrene
2. fluoranthene
3. pyrene
4. benzo(a)pyrene
5. degradation product

Figure 5

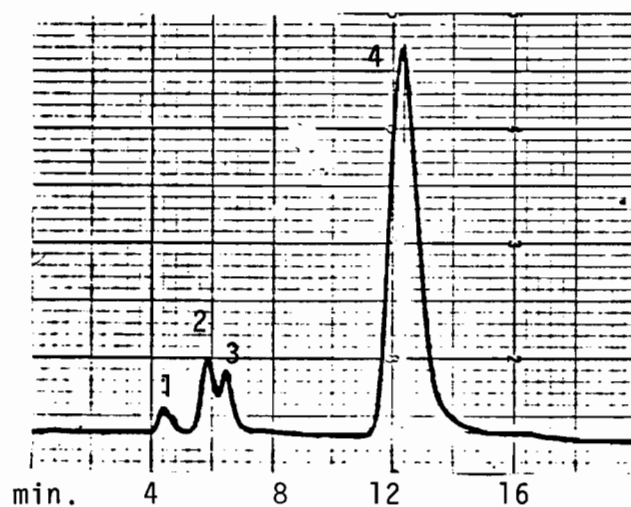


Figure 6

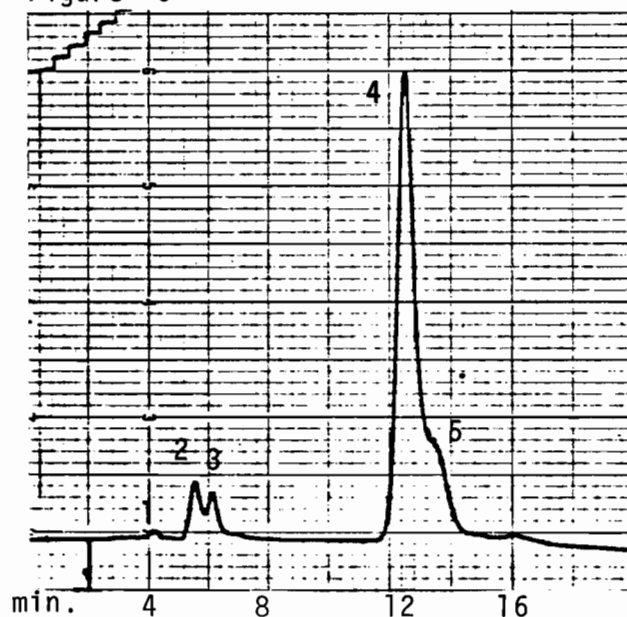
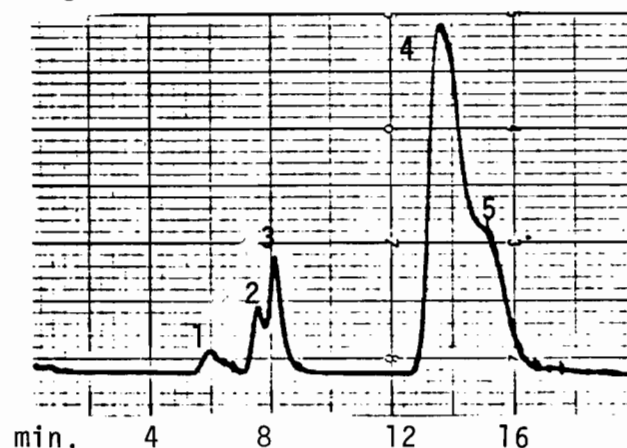


Figure 7



Chromatograms of Standard Solution No. 2 in Hexane. All Run on Dec. 8th.

Figure 8 - standard stored in dark at -6°C

Figure 9 - standard stored in dark at room temperature

Figure 10 - standard stored on shelf

HPLC and fluorescence conditions are the same as for Figure 4

Compounds in order of elution:

1. phenanthrene
2. fluoranthene
3. pyrene
4. degradation product
5. benzo(a)pyrene
6. degradation product

Figure 8

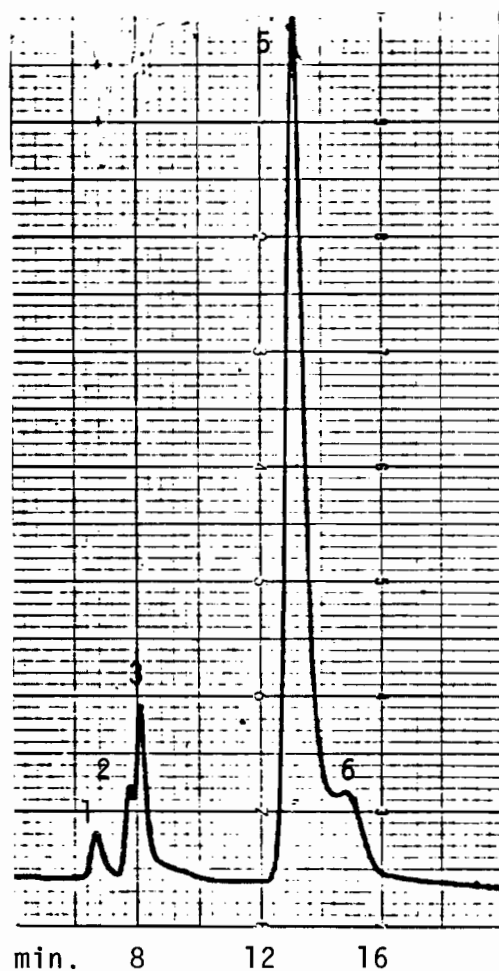


Figure 10

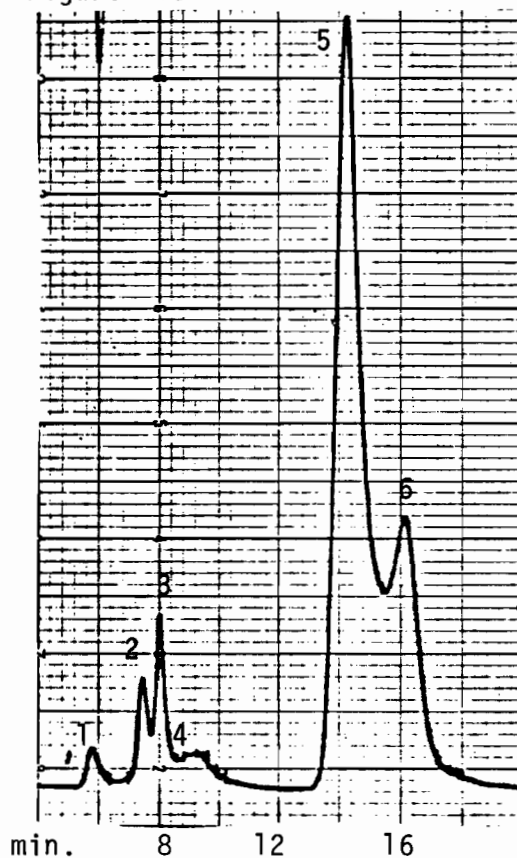
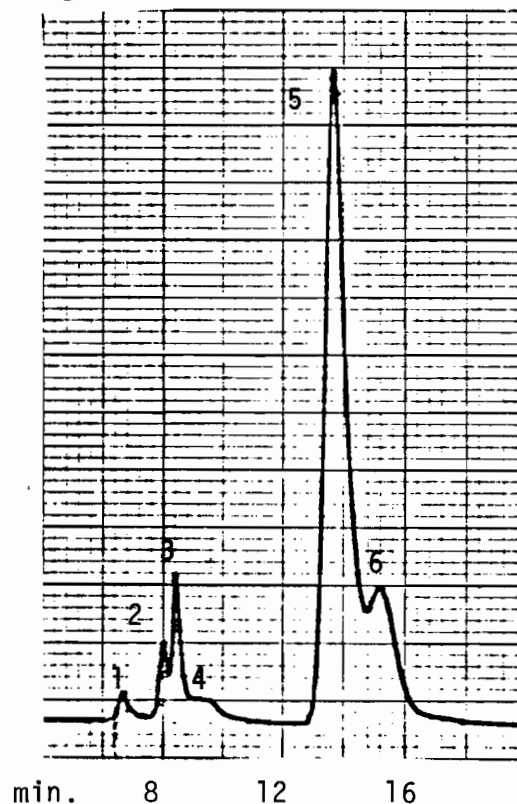


Figure 9



than the solution in Figure 8, it cannot be said whether this is due to the temperature difference or the brief exposure to light.

Quantification of PAH in Four-Hour Air Extracts

Fluoranthene was first chosen as the PAH to be quantified in the air-filter extracts. Fluoranthene elutes relatively early (it was felt this would result in short analysis times) and has its fluorescence maxima at a longer wavelength than pyrene (which elutes close to fluoranthene). By using a filter that transmitted the maximum excitation wavelengths of fluoranthene, but absorbed wavelengths below, the interference by pyrene could be minimized.

A value of 42 ng of fluoranthene per cubic meter of air was calculated for a four-hour air sample collected on November 1st. This was accomplished by comparing peak heights of the fluoranthene peak of the sample and a standard. This value is higher than the average U.S. urban concentration which is reported as four ng per cubic meter of air (10). Unfortunately, several other air samples contained no measurable fluoranthene concentration, and it was decided that a different PAH should be studied. Benzo(a)-pyrene is a more desirable PAH for quantification since it is a proven carcinogen whereas fluoranthene is not.

The benzo(a)pyrene peak (Figure 11) is partially resolved at the chosen experimental conditions and has a retention time of 14 minutes. This peak was identified by two methods: comparison of retention times with a standard (bottom chromatogram) and co-injection of four-hour extract and standard, (middle chromatogram), which gives a quantitative increase in the benzo(a)pyrene peak area. Extract #11.5.9 was run using the same conditions listed in Figure 11. Using a peak--approximation method, a value of 8 ng benzo(a)pyrene per cubic meter of air was calculated.

Figure 11

Top chromatogram - $8\mu\text{l}$ of four-hour extract.

Middle chromatogram - co-injection, $8\mu\text{l}$ of four-hour extract and $4\mu\text{l}$ of standard solution #3.

Bottom chromatogram - $2\mu\text{l}$ of standard solution #2.

HPLC conditions:

flow - 1 ml min.^{-1}
 gradient -
 start at 80% CH_3OH
 $+4\% \text{CH}_3\text{OH min.}^{-1}$ for 2 min.
 steady at 88% CH_3OH for 2 min.
 $-1\% \text{CH}_3\text{OH min.}^{-1}$ for 2 min.
 steady at 86% CH_3OH for 6 min.
 $+1\% \text{CH}_3\text{OH min.}^{-1}$ for 2 min.
 $+8\% \text{CH}_3\text{OH min.}^{-1}$ for 2 min.
 fluorescence -
 excitation wavelength - 289 nm
 emission filter - 389 nm

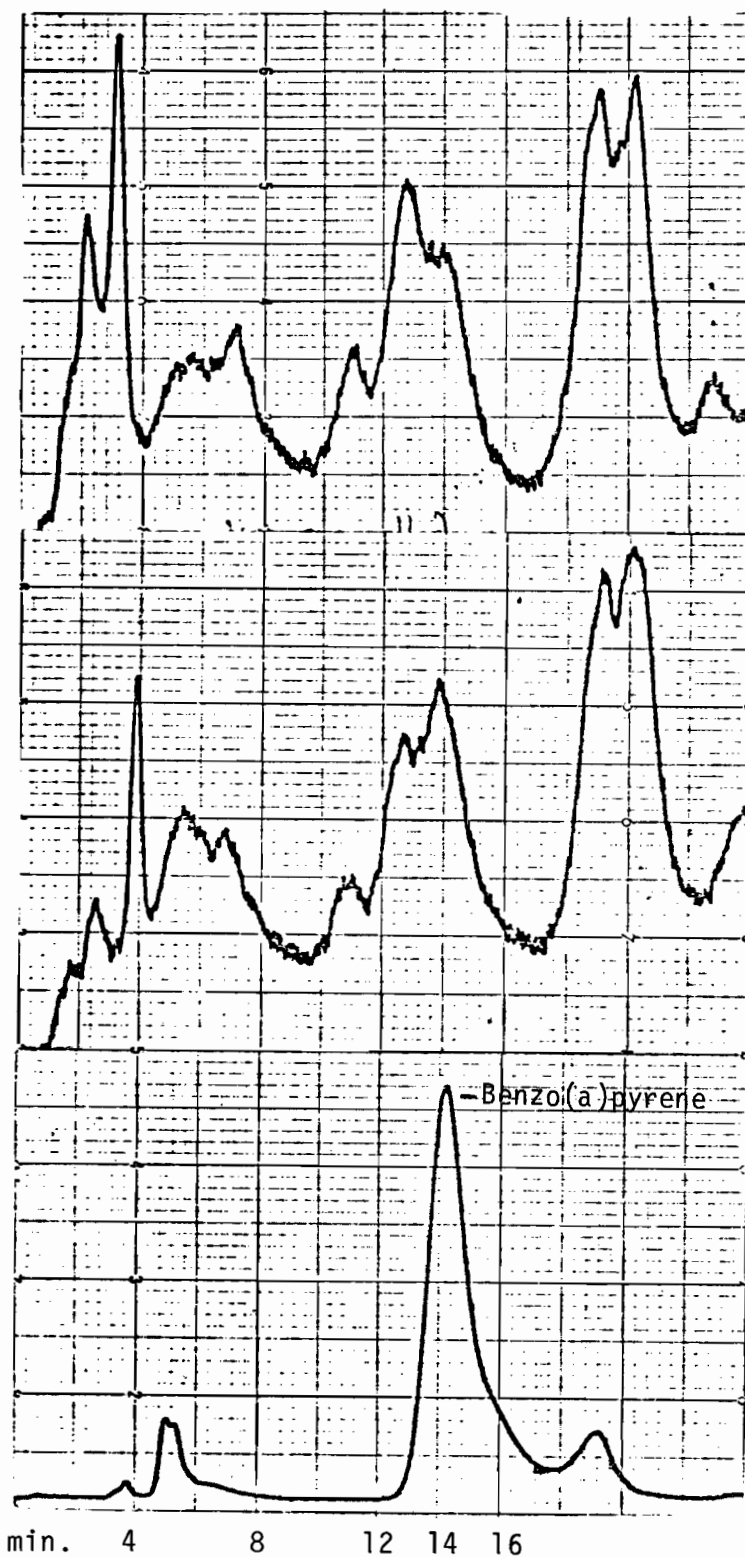


Table 1

Data for Extracted Four Hour Air Filters

The number of the filter relates to the sampling period as follows.

The first number is the month.

The second number is the day.

The third number is the period of the day. 1 is from 12 midnight to 4 A.M., 2 is from 4 A.M. to 8 A.M., 3 is from 8 A.M. to 12 noon, and so on. The letter B stands for blank.

Example: 11.13.5 - November 13th, from 4 P.M. to 8 P.M.

Filters that are grouped together were extracted together.

Filter No.	Volume of hexane used in extraction (mℓ)	Volume of hexane after concentration (mℓ)	Average wind direction (deg)
11.9.5	1.35		250
11.9.6	1.10		242
11.10.1	1.35		230
11.10.4	1.10		247
11.10.5	1.48		275
11.10.6	1.40		285
11.10.B	1.11		
11.11.1	1.26		280
11.11.2	1.60		287
11.11.3	1.50		300
11.11.4	1.75		300
11.13.4	1.80	1.30	172
11.13.5	1.48		167
11.13.6	1.80	1.30	167
11.13.B	1.55		
11.14.1	1.30		157
11.14.2	1.46		155
11.14.3	1.30		185
11.14.B	1.29		
11.14.4	1.35		175
11.14.5	1.31		160
11.14.6	1.30		192
11.15.B	1.40		
11.15.5	1.40		210
11.15.6	1.25		217
11.16.1	1.70	1.30	280
11.16.B	1.40		
11.16.2	1.30		277
11.16.3	1.29		260
11.16.4	1.35		262
11.16.B2	1.10		

Filter No.	Volume of hexane used in extraction (ml)	Volume of hexane after concentration (ml)	Average wind direction (deg)
11.16.5	1.10		245
11.16.6	1.22		252
11.17.1	1.30		245
11.17.B	1.48		
11.17.2	1.40		247
11.17.3	1.70	1.18	247
11.17.4	1.45		267
11.17.B2	1.60		
11.17.5	1.20		270
11.17.6	1.29		277
11.18.B	1.80	1.30	
11.18.1	1.30		277
11.18.3	1.50	1.30	290
11.18.5	1.30		270
11.18.6	1.80	1.31	275
11.19.4	1.26		110
11.19.5	1.32		107
11.19.6	1.29		125
11.20.1	1.80	1.30	157
11.20.2	1.27		175
11.20.3	1.45		187
11.20.B	1.60	1.35	-
11.20.4	1.28		190
11.20.5	1.60	1.30	247
11.21.4	1.30		280
11.21.5	1.60	1.30	285
11.21.6	1.40		320
11.22.1	1.30		125
11.22.2	1.45		32
11.22.B	1.35		-
11.22.4	1.50		75
11.22.5			102
11.22.6	1.50		117
11.23.1	1.30		130
11.23.5	1.30		277
11.23.6	1.50		295
11.24.B	1.55	1.35	-
11.24.1	1.40		305

This is a slightly more than the average urban concentration which is 5.7 ng per cubic meter (10).

It was felt that a better separation was needed for routine analysis. However, this separation was not achieved in the remaining time, partially due to problems to be discussed in the following section.

Many air samples were collected and extracted during the month of November; the data for these extractions is listed in Table 1. Average wind directions were calculated from the weather data provided by the Meteorology Department of Argonne National Laboratory. An example of this listing is found in Appendix C.

Variation of Retention Time

Variations in retention times proved to be a difficult problem with the HPLC. A difference of 0.5 min. was found between the shortest and the longest retention time for benzo(a)pyrene when injecting four identical samples in sequence. Use of the injection technique described earlier did not remedy this situation.

At one point large variations in retention times of several minutes occurred from one chromatogram to the next. Initially, it was thought that the electronics of the programmer were defective, and that a different gradient than the one selected was being produced. A test of the system, however, showed that the programmer was operating properly.

It was proposed that acetonitrile was entering the water pump at certain times. If this happened, the next analysis of the mixture would be run at a higher percentage of acetonitrile than indicated by the programmer and elute PAH more rapidly. This was indeed observed.

It seemed most likely that this mixing of solvents would occur when the pumps were being filled. Thereafter, care was taken to purge the water

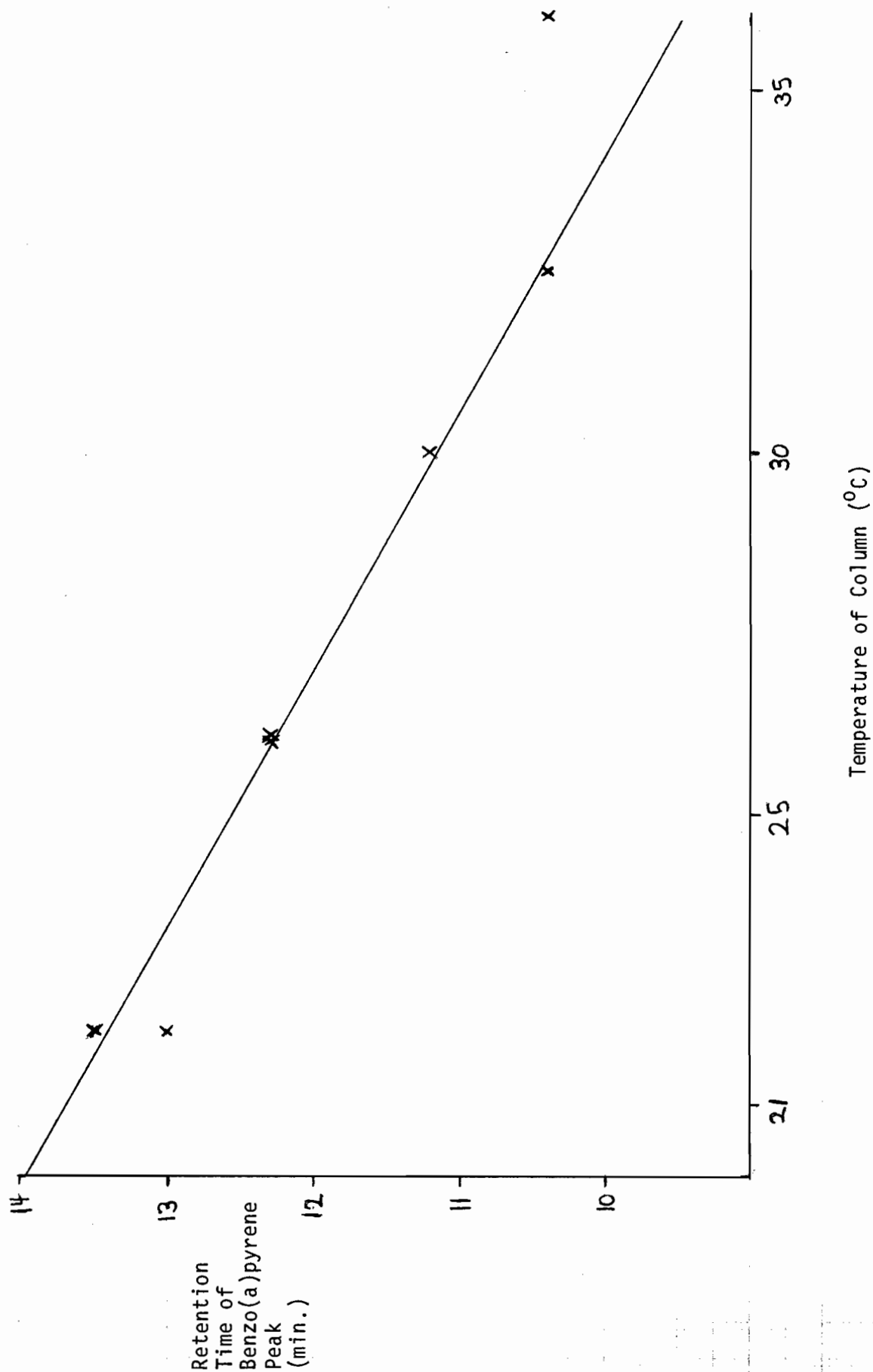
pump completely after refilling. While this may have partially alleviated the problem, it did not eliminate it.

At this point it was decided that changes in column temperature might be the cause of the problem. The following experiment was used to determine the effect of column temperature on retention time.

The column of the HPLC was thermostated by use of a water jacket and a constant-temperature bath. The column was stabilized at the following temperatures: 22°C, 26°C, 30°C, 32.5°C, and 36°C. At each temperature, one or two injections of two μl of standard solution No. 2 were made, and the retention time for benzo(a)pyrene was determined for each injection. A solvent program starting from 60% acetonitrile/40% water and going to 100% acetonitrile at 2% per minute was used. Fluorescence was used for detection; excitation wavelength was 289 nm, emission filter was 389 nm. Results are plotted in Figure 12.

The results of this experiment show a definite decrease in retention time as the column temperature increases, 0.25 minutes/°C. Even though the column was thermostated within a few tenths of a degree, a variation of 0.5 min. occurred for two consecutive injections at 22°C. However, retention times for two injections at 26°C were almost identical. The variation in the retention time for the run at 22°C could be attributed to the fact that the column had not reached its equilibrium temperature. The high value for the 36°C point is probably due to a lower column temperature. Even though the thermometer of the water bath reads 36°C, heat is lost in the transfer tubes and the column is at a lower temperature.

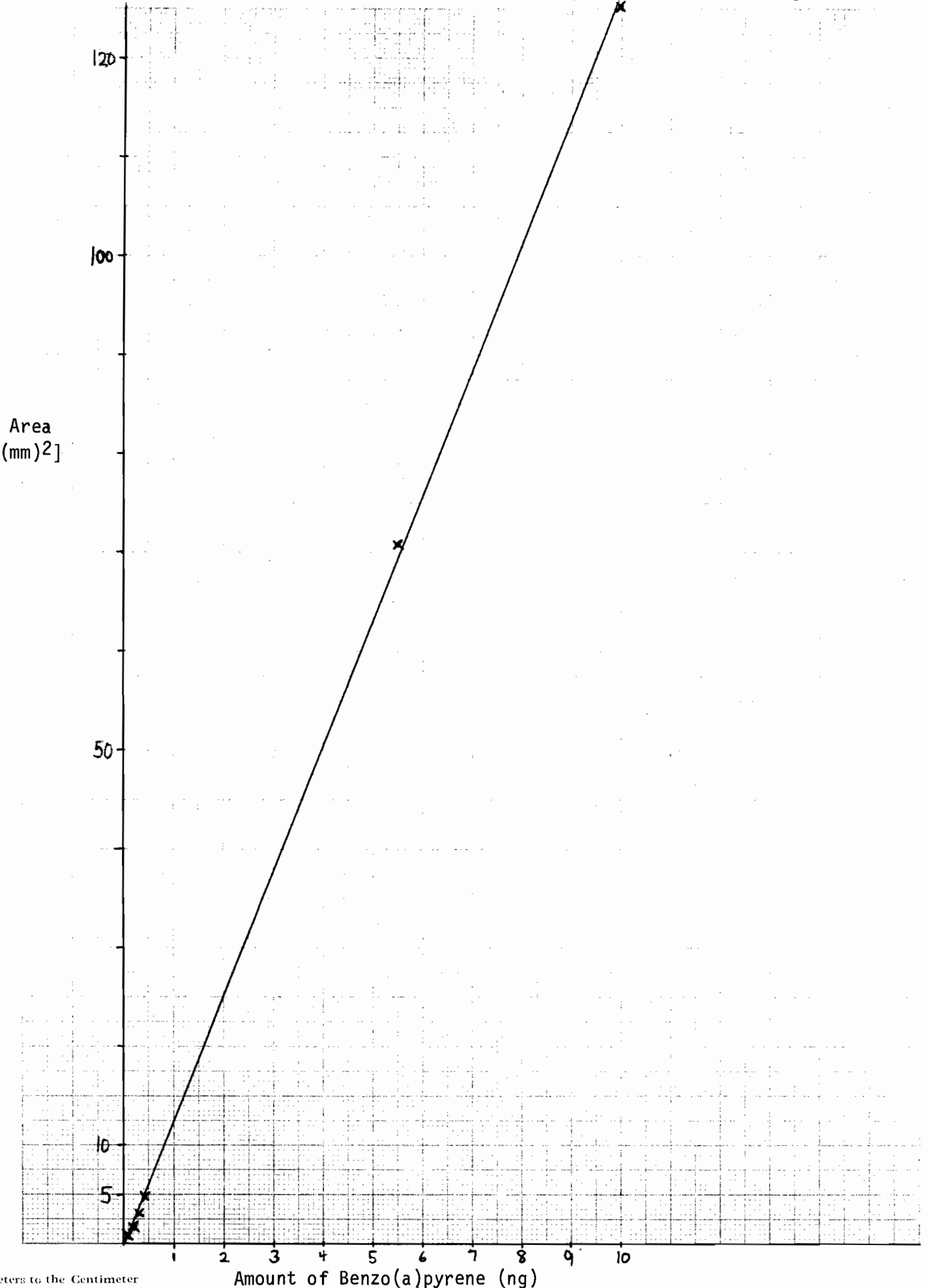
The temperature of the solvents in the morning is generally between 18°C and 20°C. By afternoon, the temperature of the solvents in the reservoirs reaches at least 31°C and perhaps higher. Figure 12 indicates that



refilling the pumps at this time could decrease the retention time by several minutes due to heating of the solvents and, indirectly, the column.

Calibration Plot: Peak Area vs. Amount of Benzo(a)pyrene

Figure 13



RECOMMENDATIONS FOR FUTURE STUDIES

To complete this study, a better separation of the PAH must be accomplished with the HPLC. More air filters must be collected and the benzo(a)pyrene in these and in filters already extracted must be quantified. To aid future workers, the following suggestions are given.

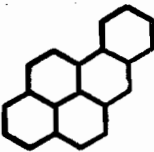
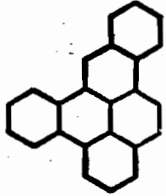
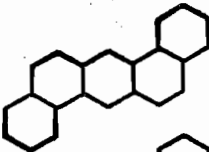
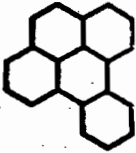
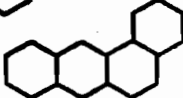
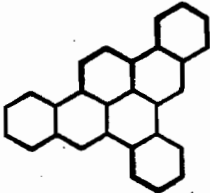
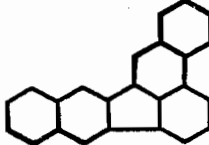
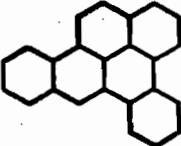
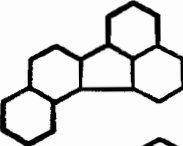

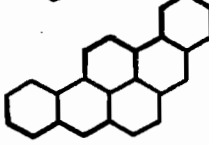
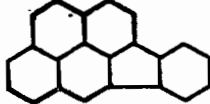
All glassware that comes in contact with PAH extracts or standards should be previously cleaned in boiling, concentrated nitric acid. Nitric acid is removed from glass much more easily than dichromate and numerous washings with deionized-distilled water should accomplish this.

The column should be thermostated during all HPLC runs. A temperature should be selected that is slightly above room temperature at its warmest fluctuation. It is felt that a temperature in the range of 26°C to 28°C would be fine.

An internal standard should be selected which is similar in structure to benzo(a)pyrene, elutes near the benzo(a)pyrene peak, and is not found in air samples. A known amount of this compound should be spiked onto each four-hour filter paper before extraction. Use of an internal standard would correct for two random errors; incomplete extraction of the PAH and variations in the fluorescent response factors of PAH.

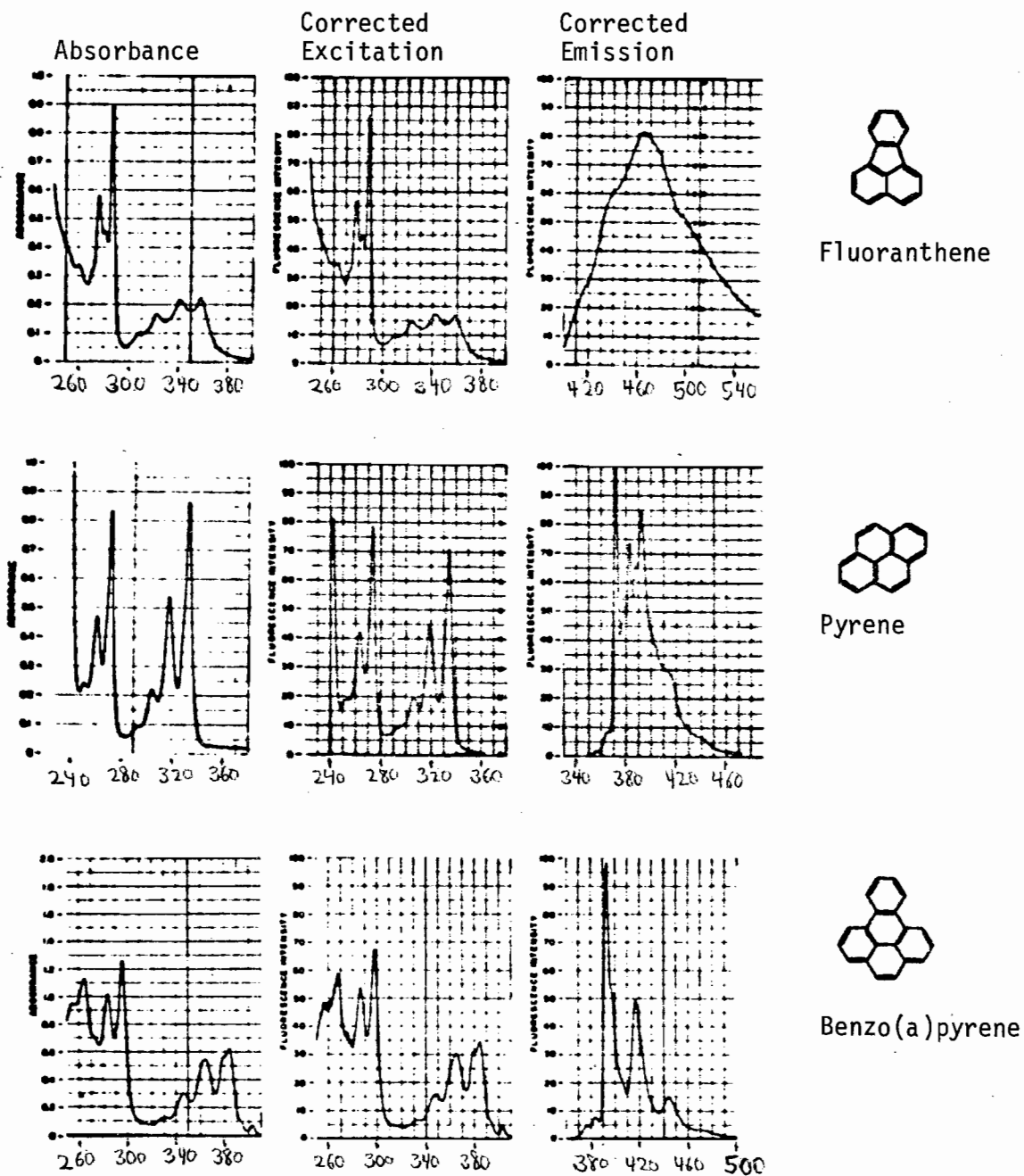
Appendix A

Carcinogenic PAH identified in urban air

Compound	Structure	Compound	Structure
Benzo[<i>a</i>]pyrene		Dibenzo[<i>e,i</i>]pyrene	
Dibenzo[<i>a,h</i>]anthracene		Benzo[<i>e</i>]pyrene	
Benzo[<i>a</i>]anthracene		Dibenzo[<i>h,ret</i>]pentaphene	
Benzo[<i>b</i>]fluoranthene		Dibenzo[<i>a,e</i>]pyrene	
Benzo[<i>j</i>]fluoranthene		Dibenzo[<i>a,h</i>]pyrene	
Dibenzo[<i>a,i</i>]pyrene		Indeno[1,2,3- <i>cd</i>]pyrene	

Source: Compiled from Kornreich 1975; Olsen and Haynes 1969; Fishbein 1973. Taken from Braunstein, Copenhagen; and Pfuderer, "Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview", Oak Ridge National Laboratory, 1977, pg. C-39.

Appendix B Spectra of Several PAH



Taken from Porro, Anacreon, Flandreau, and Fagerson,
 Journal of the AOAC, 56, pg. 614, 618, 619, (1973).

ARGONNE NATIONAL LABORATORY

WEATHER DATA LISTING - 22 NOVEMBER 1977

HR	AIR TEMP.		DEW POINT		R.H. %	WIND		SPEEDS		DRCN.		NET DIFFUSE		SOLAR RADIATION		PRECIP.		SOIL TEMP.		
	--DEGREES	CELSIUS--	DIFF.	POINT		1.5M	6.0M	44.5M	44.5M	METERS/SECOND	DEG.	44.5M	44.5M	WATTS/SQ. METER	LGLY/HR	MM	10CM	100CM	DEGREES	CELSIUS
1	-3.8	0.02	-12.9		45	2.7	3.0	3.6	340	0	0	0.0	0.0	0.00	4.3	13.0	16.3			
2	-3.7	0.01	-12.8		44	2.5	3.1	3.4	30	0	0	0.0	0.0	0.00	4.3	13.0	16.3			
3	-3.6	0.01	-12.6		45	1.5	1.6	2.0	90	0	0	0.0	0.0	0.00	4.2	13.0	16.3			
4	-3.3	0.00	-12.7		44	2.5	2.9	3.7	40	0	0	0.0	0.0	0.00	4.1	13.0	16.3			
5	-3.3	0.13	-12.7		44	1.4	1.6	1.8	40	0	0	0.0	0.0	0.00	4.1	13.0	16.3			
6	-3.1	-0.02	-11.7		47	2.4	2.7	3.3	30	0	0	0.0	0.0	0.00	4.0	13.0	16.3			
7	-2.8	-0.03	-10.9		49	0.0	1.0	1.4	30	0	0	0.0	0.0	0.00	4.0	13.0	16.3			
8	-2.2	-0.10	-9.8		51	1.6	1.6	1.7	30	18	19	1.6	1.6	0.00	3.9	13.0	16.3			
9	-1.3	-0.17	-9.2		51	2.0	2.0	2.0	120	60	62	5.3	5.3	0.00	3.9	12.9	16.3			
10	0.0	-0.18	-7.7		55	2.9	3.2	3.5	160	107	110	9.4	9.4	0.00	3.8	12.9	16.2			
11	1.2	-0.20	-6.5		54	3.2	3.7	4.3	130	128	132	11.3	11.3	0.00	3.8	12.8	16.2			
12	2.3	-0.28	-5.9		54	3.7	4.0	5.6	130	194	203	17.4	17.4	0.00	3.8	12.9	16.2			
13	3.1	-0.30	-7.4		45	3.0	3.5	4.2	90	211	224	19.2	19.2	0.00	4.4	13.2	16.5			
14	3.7	-0.24	-7.8		42	2.7	3.1	3.6	70	106	142	12.4	12.4	0.00	4.4	12.8	16.2			
15	3.8	-0.09	-9.3		37	3.0	3.5	4.6	70	113	116	9.9	9.9	0.00	4.8	12.9	16.2			
16	3.1	-0.01	-6.6		48	3.9	4.8	6.2	70	38	40	3.4	3.4	0.00	4.9	12.8	16.2			
17	2.5	0.23	-6.5		51	3.0	3.7	5.7	90	2	2	0.1	0.1	0.00	5.1	12.9	16.3			
18	1.5	0.33	-6.4		55	2.5	2.9	5.6	100	0	0	0.0	0.0	0.00	5.0	12.9	16.3			
19	1.0	0.27	-8.3		49	3.0	3.4	5.9	110	0	0	0.0	0.0	0.00	4.8	12.9	16.3			
20	0.9	0.09	-7.0		55	3.0	3.5	5.4	110	0	0	0.0	0.0	0.00	4.6	13.0	16.3			
21	1.4	-0.01	-6.2		56	2.9	3.3	4.7	110	0	0	0.0	0.0	0.00	4.5	12.9	16.3			
22	1.4	-0.01	-5.8		58	3.5	4.1	5.9	110	0	0	0.0	0.0	0.00	4.5	13.0	16.3			
23	1.3	-0.02	-5.0		62	3.5	4.1	5.9	120	0	0	0.0	0.0	0.00	4.5	13.0	16.3			
24	1.4	-0.03	-4.4		65	2.4	2.6	4.0	130	0	0	0.0	0.0	0.00	4.5	13.0	16.3			
MEAN	0.1		-8.6			2.6	3.0	4.1		42	44	14	14		4.3	12.9	16.3			
N						24	24	24	24	24	24	24	24		24	24	24			
TOTAL																				
VECTOR WIND														90.0	0.00					

REFERENCES

1. H. M. Braunstein, E. D. Copenhaver, and H. A. Pfuderer, Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview, Oak Ridge National Laboratory, 1977, pg. C-39.
2. Ibid, pg. 6-6.
3. E. Clar, Polycyclic Hydrocarbons, Academic Press, 1964, pg. 133.
4. Committee on Biological Effects of Atmospheric Pollutants, Particulate Polycyclic Organic Matter, National Academy of Sciences, Washington, D.C. 1972, pg. 13.
5. H. M. Braunstein, E. D. Copenhaver, and H. A. Pfuderer, op. cit. pg. 6-4, 6-6.
6. E. Clar, op. cit., pg. 133.
7. H. M. Braunstein, E. D. Copenhaver, and H. A. Pfuderer, op. cit., pg. 9-4.
8. M. A. Fox and S. W. Staley, Analytical Chemistry, 48, pg. 992, (1976).
9. A. T. Rhys. Williams and W. Stavin, Chromatography Newsletter, Vol. 4, No. 2, pg. 28-31, (1976).
10. M. Dong, D. C. Locke, and E. Ferrand, Analytical Chemistry, 48, pg. 368-72, (1976).
11. M. A. Fox and S. W. Staley, op. cit., pg. 992-8.
12. A. M. Krstulovic, D. M. Rosie, and P. R. Brown, Analytical Chemistry, 48, pg. 1383-6, (1976).
13. T. J. Porro, R. E. Anacreon, P. S. Flandreau, and I. S. Fagerson, Journal of the AOAC, 56, pg. 607-20, (1973).
14. Committee on Biological Effects of Atmospheric Pollutants, op. cit., pg. 66.