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ABSTRACT

Title of Thesis: Three Studies on Airborne Polycyclic Aromatic Hydrocarbons

Carol Sophia Eveleens, Master of Science, 1984

Thesis directed by: Dr. Arthur Greenberg, Associate Professor

Three studies have been conducted on polycyclic aromatic hydrocarbons found on airborne particulate matter. The first investigation involves the determination of fly ash "activity" in the reaction of two PAHs adsorbed on its surface. Mass spectrometry is used to monitor the exchange of hydrogen and deuterium atoms between anthracene and anthracene-$d_{10}$. The results indicate that some fly ashes exhibit "activity" in the reactions of PAHs adsorbed on its surface. The second investigation involves the determination of cyclopenta[cd]pyrene (CcdP) and benzo[ghi]flouranthene (BghiF) on samples of airborne particulate material collected from two automobile traffic tunnels. Glass capillary gas chromatography with a precolumn injector system is used to quantitate these two compounds. The results indicate that CcdP is a significant constituent of automobile emissions and that BghiF is present in concentrations much lower than

CcdP. The last investigation involves the attempted identification of benzyl sulfate, the sulfate ester of benzyl alcohol, on ambient air particulate samples. Reverse-phase high pressure liquid chromatography is used to determine the presence or absence of benzyl sulfate on particulate matter. The results are inconclusive at present.

# THREE STUDIES ON AIRBORNE POLYCYCLIC AROMATIC HYDROCARBONS

by
Carol Sophia Eveleens

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science
1984

APPROVAL SHEET

Title of Thesis: Three Studies on Airborne Polycyclic Aromatic Hydrocarbons

Name of Candidate: Carol Sophia Eveleens
Masters of Science, 1984

Thesis and Abstract Approved:

_____ _____
Dr. Arthur Greenberg Date
Associate Professor
Chemical Engineering
and Chemistry

_____ _____
Date

_____ _____
Date

VITA

Name: Carol Sophia Eveleens.

Permanent address:

Degree and date to be conferred: M.S., 1984

Date of birth:

Place of birth:

Secondary education: Rutherford High School 1977.

| Colligiate institutions attended | Dates | Degree | Date of Degree |
|---|---|---|---|
| Rutgers University, Newark | 1977-81 | BA | May 1981 |
| New Jersey Institute of Technology | 1981-84 | MS | May 1984 |

Major: Engineering Science (Chemistry)

Publications: Kebbekus, B., Greenberg, A., Horgan, L., Bozzelli, J., Darack, F., Eveleens, C., and Strangeland, L., "Concentrations of Selected Vapor and Particulate-Phase Substances in the Lincoln and Holland Tunnels," JAPCA, vol. 33, April, 1983, pp.328-330.

Positions held: Research Assistent; NJIT, 323 High Street, Newark, New Jersey, 07102.

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# INTRODUCTION

During the past three decades, many studies have been conducted on the various characteristics of airborne particulate matter. These studies include the extraction and identification of polycyclic aromatic hydrocarbons (PAHs) found on particulates from coal-fired power plants, automobile exhaust condensates, and organic combustion products as well as airborne particulates derived from all of these sources. The present project encompasses three separate studies related to the analysis of polycyclic aromatic hydrocarbons.

The first study involves the determination of induced reactivity between PAH compounds adsorbed on the surface of fly ash. Only a few studies have been conducted on the adsorption and desorption, termed "sorptivity," of PAHs on fly ash. Two of these studies investigated the sorptivity of PAHs on fly ash and the physiological effects of these particulates on body tissue once they are ingested.[1,2] Other studies concentrate on the extraction and recovery procedures of PAHs from fly ash to determine the most accurate quantitative methodologies for particular compounds.[2-5] In the present investigation, hydrogen/deuterium exchange of anthracene-$d_{10}$ is used as a probe in determining the interactions of PAHs on the surface of fly ash. By monitoring the exchange of the hydrogen and deuterium atoms of anthracenes adsorbed on the fly ash surface, the ability to induce reactions between PAH compounds, termed "activity", by a

particular fly ash may be measured.

In the next area of study, the analysis of PAHs found in automobile exhaust is undertaken. This investigation involves the quantitation of two specific PAHs, cyclopenta[cd]pyrene and benzo[ghi]fluoranthene. The samples used are taken from two major automobile routes located between the states of New Jersey and New York, the Holland Tunnel and the Lincoln Tunnel. Detection of cyclopenta[cd]pyrene (CcdP) is normally difficult in ambient air samples because it is readily photodegraded. In the tunnel samples, however, the concentration of CcdP is higher because of the lack of sunlight. The results are thus more indicative of CcdP's actual concentration in automobile exhaust. Benzo[ghi]fluoranthene (BghiF) is determined concurrently with CcdP because it is a co-elutor under the high performance liquid chromatography (HPLC) procedure performed in our laboratory and thus interferes with the quantitation of CcdP in the tunnel samples. The problem of separating these two isomeric compounds and quantitating their concentrations is the motivation for this study.

The third area of study involves the synthesis of a hydroxymethyl sulfate derivative of a PAH and its detection in ambient air samples taken from several urban sites in New Jersey. The idea of investigating the presence or absence of benzyl sulfate on air particulate samples developed from a previous study involving 7,12-dimethylbenz[a]anthracene (DMBA).[6] In that study, the highly carcinogenic derivative

7-hydroxymethyl-12-methylbenz[a]anthracene (7-HMBA) of DMBA was determined to be an active metabolite in rat liver. Although they were only mentioned in the investigation of 7-HMBA, other sulfate esters of arylmethanols have been investigated in the present study. Of these sulfate esters, the least mutagenic towards Samonella typhimurium TA 98 using the Ames Test is benzyl sulfate.[6] Since the sulfate ester of 7-HMBA is more mutagenic than the parent compound, it is worthwhile investigating whether or not such derivatives exist in ambient air. To determine an answer to the question, "Do sulfate esters exist on air particulates?" several samples are collected and prepared for PAH analysis.

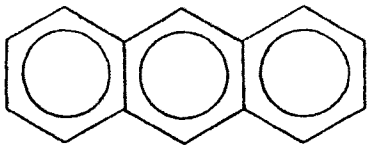
Each of these investigations involves the analysis of PAHs in their parent form or as a derivative. All suggest possible environmental health problems that may be controlled or possibly prevented once the reactions are known and isolated. The following is a report on the findings for each of these studies.

## I. HYDROGEN/DEUTERIUM EXCHANGE OF ANTHRACENES ON FLY ASH

Coal fly ash is a highly adsorptive matrix. It appears to influence the PAH compounds adsorbed on its surface in several interesting ways. First, of the studied PAHs adsorbed on the fly ash samples, all tended to resist photochemical decomposition (photooxidation) when exposed to light.[7] This is in contrast with dissolved PAHs. In solution, photooxidation of PAHs occurs readily when irradiated with a light source.[7] Second, some of the PAH compounds adsorbed on the fly ash surface undergo spontaneous oxidation in the absence of light.[7] These compounds contain a saturated carbon atom attached to an aromatic ring. This benzylic carbon linkage is highly susceptible to oxidative attack.[7] Third, extraction recoveries of PAHs adsorbed on fly ash seem to be dependent on the size of the molecule. More difficulty is encountered in extracting four or more ring membered compounds than three or less.[2,3] The recovery of Carbon-14-labelled benzo[a]pyrene, using ultrasonic extractions with benzene, for example, is quite low (30%).[4] Therefore, in the quantitative analysis of PAHs on fly ash, extraction recovery corrections must be included to give more accurate results.[3] Fourth, in experiments performed by Griest et al[4] using Carbon-14-labelled benzo[a]pyrene ($^{14}$C-BaP) and tritium labelled benzo[a]pyrene ($^{3}$H-BaP) as tracer compounds, there appears to be a $^{3}H$-$^{1}H$ exchange between the $^{3}$H-BaP tracer and the fly ash surface. This exchange is probably caused by the tight binding of the BaP

tracers to the fly ash surface and accounts for the lower extraction recovery of $^{3}$H-BaP tracer to $^{14}$C-BaP tracer. As Griest points out, more than likely there will be an exchange observed for the $^{2}$H-labelled tracers and the fly ash sample as well.[4] Speculation on other fly ash characteristics influencing PAH sorptivity have also been made. These include: the carbon content of the fly ash possibly forming pi-bonding complexes with the adsorbed PAH compounds, or more specifically, dipole-induced dipole interactions between the PAH and the carbon of the fly ash[2] and chemical interaction between "unextractable" PAHs adsorbed on the fly ash with polar surface groups capable of complexing with the adsorbed PAHs.[3,4]

In the present investigation, the idea of hydrogen exchange between anthracene and perdeuterated anthracene adsorbed on fly ash is studied. The object of this investigation is to take several types of fly ash from different coal sites and determine the "activity" (the ability to induce hydrogen/deuterium exchange between adsorbed PAHs), if any, of these fly ashes. To determine the "activity" of the fly ash samples a solution of anthracene (1) and perdeuterated anthracene (2) is prepared and a measured amount mixed with a sample of fly ash. Using mass spectrometry (MS) the induced reactivity of these PAH compounds is measured by comparing the percentages of the m/e values of the fly ash samples to the corresponding m/e percentage values obtained for the control sample.



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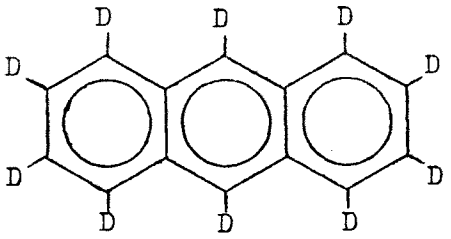


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A. Experimental Procedures for H/D Exchange

1. Samples and Solutions

Fly ash samples from three coal-fired power plants were furnished by Dr. James Grow of the New Jersey Institute of Technology. The coal used in the operation of these power plants came from three coal mines, Nora Coal, Badger Coal, and Upshur Coal, located in New Jersey. One gram samples were analyzed for fly ash "activity". A solution of anthracene plus anthracene-$d_{10}$ was made by dissolving approximately one-tenth of a gram of each compound in 0.500 liter of cyclohexane (HPLC grade). Anthracene and anthracene-$d_{10}$ were purchased from the Aldrich Chemical Company.

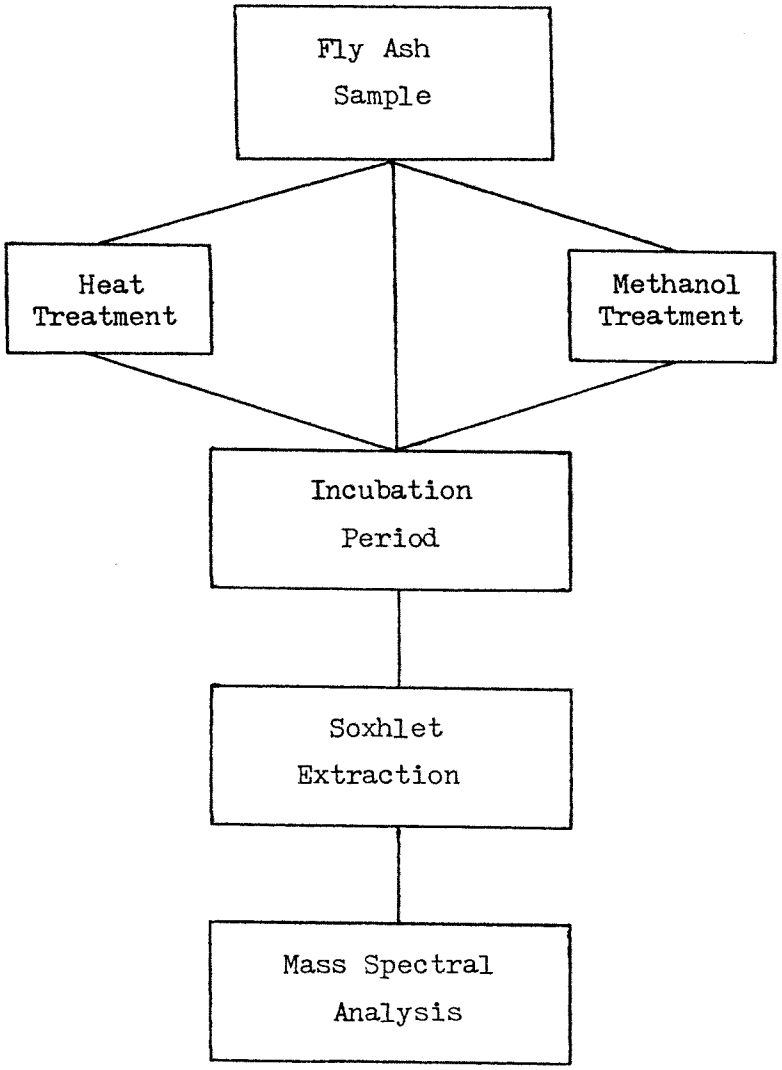
2. Methods of Adsorption

Each trial run on the different fly ash samples is subjected to the same method of adsorption. However, in some cases the fly ash is pretreated with either heat (600$^{o}$C for 6 hrs.) or exhaustive extraction (150 ml of methanol for 24hrs.). The fly ash samples are then incubated for 1 day, 5 days, or 7 days and then extracted with benzene for 6

hours. Figure I is a schematic diagram of the different procedures of adsorption.

Figure I

Schematic Diagram of Fly Ash Analysis




One gram of fly ash is weighed out and placed in a flask. Five milliliters of the cyclohexane solution described above is added to the flask and the slurry stirred magnetically for thirty minutes. (The 5 ml of solution contains approximately 1 mg of each compound.) After 30

minutes, the slurry is rotoevaporated almost to dryness and incubated for a designated time period in a vacuum dessicator at room temperature. The flask is wrapped in aluminum foil to protect it from light. When the incubation period is over, the sample is transferred to a Soxhlet extraction apparatus and extracted with 150 milliliters of benzene for 6 hours. After the extract is rotoevaporated to dryness, the residue is ready for analysis. Control samples of the anthracene solution are 5 ml quantities that have been rotoevaporated almost to dryness, placed in a vacuum dessicator for 24 hours, and then stored in an amber colored bottle until time of analysis by MS.

3. Analysis via Mass Spectrometry (MS)

The "activity" of each fly ash is determined by MS. By monitoring the amount of anthracene, anthracene-$d_{10}$, and their derivatives present in the fly ash sample and comparing these values to control sample values, the "activity" of a particular fly ash measured in terms of H/D exchange can be measured. The mass spectrometry runs were performed by Mr. Michael Lang at Schering Corporation.

B. Results and Discussion of Fly Ash Samples

Griest et al, in their study on extraction and recovery of labelled-BaP tracer compounds, attribute the lower percentages of $^{3}H$-BaP as compared to $^{14}C$-BaP to $^{3}H$-$^{1}H$ exchange between the labelled PAH and the surface of the fly ash.[4] They also presume that a $^{2}H$-$^{1}H$ exchange would occur between a $^{2}H$-labelled PAH and the fly ash. The difficulty of disrup-

tion of the PAH ring system of the tracer compound and the availabilty of free carbon on the fly ash, Griest et al explain, inhibits the exchange of $^{14}C$-$^{12}C$ between the tracer and the fly ash. Therefore, the recovery percentage of $^{14}C$-BaP is greater than the recovery percentage of $^{3}H$-BaP because $^{14}C$-$^{12}C$ exchange between the PAH and fly ash is dependent on the unfavorable disruption of the PAH ring system.[4]

According to the assumption of Griest et al, $^{2}H$-$^{1}H$ (H/D) exchange between tracer PAH compound and fly ash is very possible. If this exchange between fly ash and tracer compound is possible, so is the H/D exchange between two PAHs adsorbed on the fly ash surface. By inducing reactivity between adsorbed PAHs, the fly ash can alter the initial chemical composition of molecules adsorbed on its surface. This can either increase the potential carcinogenic properties or decrease the potential carcinogenic properties of the particulates, both of significant interest in determining the environmental risks fly ash and other particulate matter may impose.

Our present study uses the perdeuterated form of anthracene and anthracene itself in determining the "activity" of fly ash to induce H/D exchange between adsorbed PAH compounds. The "activity" is determined by comparing the percentages of the m/e values obtained for the fly ash samples to the corresponding values obtained for the control group. The results suggest "activity" in certain fly

ash samples and virtually no "activity" in other fly ash samples in the induction of reactivity between adsorbed PAHs. Appendix A displays the actual mass spectrograms obtained during this study. The mass spectra are typical of PAHs in that the base peak is the parent ion. Table I lists each of the sample trials on the three fly ashes, their incubation periods, pretreatment, if any, and possible "activity" in the exchange of H/D atoms of the two

TABLE I

Experimental Results for Fly Ash Samples

| Flyash | Incubation | Pretreatment | Activity | Ion Ratio m/e 178/ m/e 188 |
|---|---|---|---|---|
| Nora 1 | 1 day | none | notable | 1.17 |
| Nora 2 | 1 day | none | notable | 1.00 |
| Nora 3 | 1 day | none | notable | 1.90 |
| Nora 4 | 5 days | none | notable | 1.18 |
| Badger | 1 day | none | slight | 1.00 |
| Badger | 1 day | none | slight | 1.09 |
| Badger | 1 day | none | slight | 1.39 |
| Badger | 5 days | none | slight | 1.00 |
| Badger | 7 days | none | slight | 1.02 |
| Upshur | 5 days | none | slight | 1.00 |
| Upshur | 1 day | 600$^{o}$C, 6hrs. | none | 1.00 |
| Upshur | 1 day | MeOH, 24hrs. | none | 0.99 |

anthracenes. It also lists the ion ratio of the two parent compounds, anthracene (m/e 178) and perdeuterated anthracene (m/e 188).

In the comparison of each of the fly ash sample results, it appears that NORA fly ash exhibits the most notable "activity" in the H/D exchange of anthracenes. BADGER fly ash shows a slight to insignificant "activity" and UPSHUR fly ash exhibits virtually no "activity" at all in H/D exchange.

Looking at the individual percentages of anthracene species present in the mass spectra (Appendix A), several trends are noticed. In each of the samples and the control sample, there is a significant amount of compound present at the mass number of 184.1 in comparison with the other mass values. In comparing all the sample fly ashes and the control sample, the amount of anthracene and anthracene-$d_{10}$ is approximately equal with, anthracene being used as the basis for % computation. The exceptions lie in the second trial of NORA and BADGER fly ashes, here anthracene-$d_{10}$ is used as the basis of % computation. There is a significant percentage of compound present at the mass number of 179.1 and 187.1; these mass numbers probably represent anthracene-$d_1$ and anthracene-$d_9$, respectively. In these two cases, a single H/D exchange probably takes place between the adsorbed compounds. Shifting attention to the corresponding integration values for each of the mass numbers between 178.1 and 188.1, it can be seen that NORA fly ash

exhibits the most "activity" while BADGER and UPSHUR do not exhibit any notable "activity". All conclusions in this respect are based on comparison with the mass number integration values for the control sample.

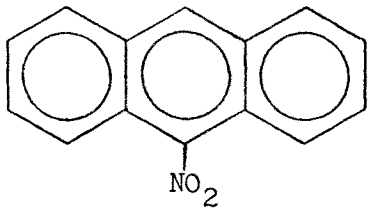
The main problem that arises at this point in the determination of the fly ash "activity" toward inducing reactivity between adsorbed PAHs, is the probable inhomogeneity of the actual size of the fly ash particles in a 1 gram sample. By size-fractioning the fly ash samples into more homogeneous particulate ranges, the inconsistency in experimental results for repetitive runs on the same fly ash sample may disappear and the adsorption of the PAHs onto the fly ash surface may be made more even in distribution. This would lead to a more reproducible answer in determining fly ash "activity". Examples of inconsistent results are shown by comparing the three similar trials of NORA fly ash to each other and the three similar trials of BADGER fly ash to each other. The percentages for several m/e numbers are as follows:

| | NORA | | | BADGER | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| m/e 178= | 100%, | 99%, | 100% | 99%, | 100%, | 100% |
| m/e 179= | 16%, | 30%, | 16% | 9%, | 17%, | 15% |
| m/e 187= | 13%, | 50%, | 39% | 24%, | 14%, | 16% |
| m/e 188= | 86%, | 100%, | 52% | 100%, | 90%, | 71% |

where m/e 178 is the parent ion anthracene; m/e 179 is the specie anthracene-$d_1$; m/e 187 is the specie anthracene-$d_9$

and m/e 188 is the parent ion anthracene-$d_{10}$. At this point, the ability of fly ash to induce H/D exchange is established, but precise quantitation needs further study. The mechanism of "activity" inducing H/D exchange, as yet, is unknown and depends on a number of factors. Examples of these factors include: carbon content of the fly ash, elements on the fly ash capable of complexing with the adsorbed PAH causing retention of the PAHs on the fly ash, extraction and adsorption methods of the PAHs that are capable of giving accurate results, and whether or not the proton transfer step occurs under acidic or basic conditions.

The immediate step to be taken in the analysis of H/D exchange of PAHs adsorbed on fly ash is separation of particle size into more homogeneous groupings. Once this is done, the actual mixing time of the PAH solution and the fly ash should be optimized. The incubation period between adsorption and extraction is monitored very closely, and the solvent used in the extraction of the samples carefully considered. To further assist in the determination of the mechanisms of fly ash "activity", characterization of the fly ash surface by X-ray fluorescence, ESCA, and or SIMS should be performed. In this way, more can be learned as to what and how influencing factors contribute to the interaction of PAHs adsorbed on the fly ash surface. Additionally, it would be worthwhile to see whether 9-nitroanthracene (3) exhibits accelerated or decelerated exchange relative to anthracene. If the exchange is

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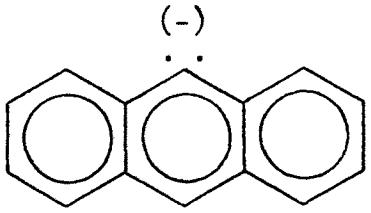
accelerated, presumably a negatively-charged intermediate (4?) is involved. If the exchange is decelerated, presumably a positively-charged intermediate (5?) is involved.

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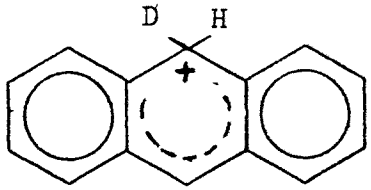


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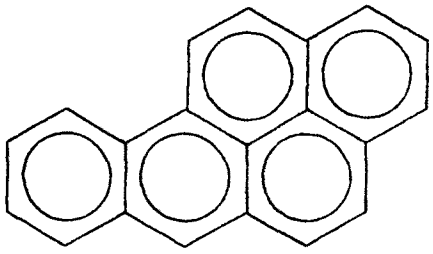
## II. CYCLOPENTA[CD]PYRENE AND BENZO[GHI]FLOURANTHENE IN AUTOMOBILE EXHAUST CONDENSATES

The analysis of PAHs on airborne particulates has been the subject of many investigations in the past three decades.[8-27] One area of study involves the analysis of automobile exhaust condensates for PAHs. Within this group of compounds exists several carcinogenic and procarcinogenic compounds. The PAH most often the subject of analysis is benzo[a]pyrene (BaP, 6). It has been found in a wide variety of environmental samples and it has been proven to be a potent carcinogen. Another PAH recently identified in various particulates and synthesized in the laboratory is cyclopenta[cd]pyrene (CcdP, 7). Its carcinogenic activity is approximately one-fifth that of BaP but it has exhibited highly mutagenic properties.[8] It has been found in higher amounts than BaP, in tunnels and automobile condensates.[9,10]



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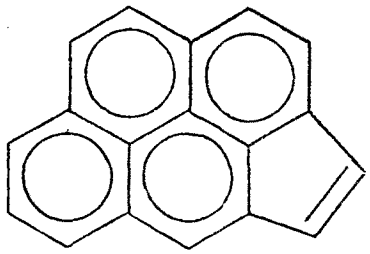

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Cavalieri et al[8,11] have recently performed several experiments involving CcdP. In their first experimental investigation[8], Cavalieri et al administer different dose levels of CcdP by injection into laboratory test mice. Their

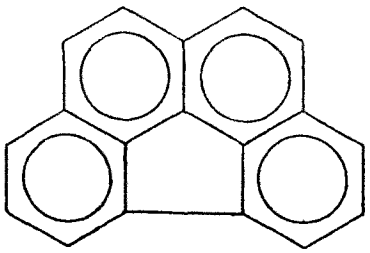
findings show that repeated applications of low dose levels of CcdP cause more incidences of tumors than the same number of applications at higher dose levels. Also, at the low dose level, CcdP exhibits 57% tumorigenicity while BaP exhibits 100% tumorigenicity in laboratory test mice.[8]

The results of the first study of Cavalieri et al led them to investigate the syncarcinogenic effect of CcdP and BaP in test mice.[11] What they found is quite interesting and disturbing considering the abundance of these two PAHs in automobile emissions. When administered simultaneously, BaP and CcdP exhibit a syncarcinogenic effect. Three dose levels of low (2.2 nmoles BaP or 22.2 nmoles CcdP), medium (6.6 nmoles BaP or 66.6 nmoles CcdP), and high (20 nmoles BaP or 200 nmoles CcdP) are determined for each PAH prior to analysis. In the combination of the medium dose of CcdP and the medium dose of BaP, the syncarcinogenic effect is 3 to 7 times the total carcinogenic effect of the separate compounds. When the dosage consists of the low level CcdP and the medium level of BaP or vice versa the effect is 1.2 to 3.8 times the sum effect of the individual PAHs. A dose that combines a high level of either PAH results in a masking of the syncarcinogenic effect of these two PAHs.[11]

The results of the two studies of Cavalieri et al[8,11] coupled with the results of other research[9,10,12] on the relative abundance of CcdP and BaP in automobile exhaust gives rise to concern over the health problems these PAHs impose. CcdP is an elusive compound in ambient air samples

because it is photodegraded. It is studied here because the samples being analyzed are taken from two traffic tunnels. Since the air particulate samples taken from these tunnels are shielded from the sun, photodecomposition of CcdP should be kept to a minimum and determinations of concentrations actually reflecting emission levels of CcdP should be possible.

In the initial quantitation of CcdP via reverse-phase high pressure liquid chromatography (HPLC), a problem arises in the quantitation of CcdP. Large peak areas on the chromatogram indicate concentrations for CcdP much greater than expected. It appears that CcdP is co-eluting with another unknown compound.[13] The HPLC results limit accurate quantitation of CcdP. Quanititation can yield simultaneous determinations of co-eluting PAHs using different detection wavelengths if one knows the identity of the co-elutors. However, further research indicates that glass capillary gas chromatography (GC) is effective in quantitating CcdP since it will separate it from at least one of the HPLC co-elutors (see below), making quantitation of the tunnel samples possible. Although, the capillary GC column conditions employed here failed to completely resolve CcdP from benz[a]anthracene, the latter compound is easily separable using our HPLC conditions. During the time GC was being considered as the new method for analysis, benzo[ghi]fluoranthene (BghiF, 8) was proposed as being the co-elutor of CcdP. T. Alsberg et al[14] note that BghiF elutes almost



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simultaneously with CcdP under certain HPLC conditions. Furthermore, this co-elution was found experimentally under the conditions employed by our laboratory. It is interesting to note that in Alsberg's study the individual fractions of CcdP and BghiF are found to exhibit low mutagenic effects, while the combination of these two fractions with the rest of the sample exhibit mutagenic effects much higher than expected.[14] In other words, the mutagenic effects of CcdP and BghiF are enhanced exhibiting a syncarcinogenic effect.

TABLE II[14]*

Mutagenic Effects of HPLC-Fractioned DCM Extracts

| | DCM ext[a] | A | B[b] | C[b] | A+B | A+C | A+B+C |
|---|---|---|---|---|---|---|---|
| -S9 | 0.56 | - | - | - | - | - | - |
| +S9 | 2.2 | 0.92 | 0.24 | 0.13 | 1.5 | 1.2 | 1.7 |

a. 3 g carbon black extracted 64 h...

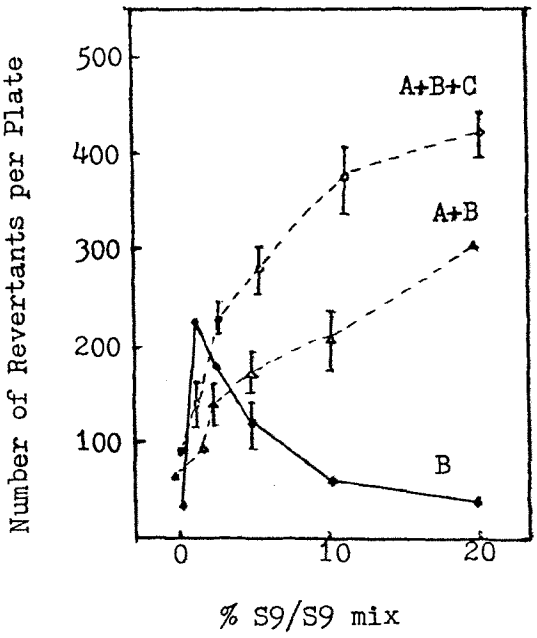
b. B= CcdP fraction, C=BghiF fraction...

*Alsberg, T., et al, "Evaluation of Extraction Methods For Carbon Black: POM Analysis and Mutagenicity Assay," Polynuclear Aromatic Hydrocarbons Physical and Biological Chemistry, Batelle Press, 1982, p. 79.

The values of Table II and the chart of Figure II are taken from Alsberg et al[14] and show the individual and combined effects toward the S9 metabolizing system.

Figure II[14]*

Number of Revertants/Plate vs %S9/S9mix




The mutagenicity of the CcdP fraction, B; the Sample plus CcdP fraction, A+B; and the recombined fractions of CcdP, BghiF, and the Sample, A+B+C in the presence of different amounts of S9 fraction.

*Alsberg, T., p. 81.

A.Experimental Procedure on CcdP and BghiF

1.Collection and Preparation of Samples

The samples used in this analysis are collected by equipping a Dodge Tradesman 200 van with a hooded high volume sampler powered by 110 V generator mounted on the drivers side wheel well. The sampler is mounted on the roof of the van. In this position the generator exhaust vents with the engine exhaust. Gelman Type A glass fiber filters, 100 mm diameter, are used to collect the air particulate samples. (Volumes and sampling data are found in Table III.)

Cyclopenta[cd]pyrene was obtained from the National Cancer Institute Chemical Repository. Benzo[ghi]fluoranthene was obtained from the Commission of European Communities Community Bureau of Reference (BCR, Brussels). Both compounds were used without further purification.

Half of each filter is Soxhlet extracted with 150 ml of cyclohexane for 6 hours. [During the extraction period as well as during the entire procedure, care is taken to protect the samples from all forms of light.] One 1 ml of 1-methyltrypticene, the internal standard, is added to the extract and it is rotoevaporated to approximately 5 ml aliquots. The aliquots are then concentrated to 1 ml under a constant flow of nitrogen gas (prepurified) and dotted on thin layer chromatographic (TLC) glass plates obtained from Analabs. The TLC plates are precoated with Anasil GF a silica gel with 13% $CaSO_4$ binder plus UV flourescent indicator, 254 nm. The layer of silica gel on the TLC plate has a thickness of 250 microns and the size of the glass plate is 20 cm x 20 cm. The TLC plates are developed in a 1:1 solution of toluene and hexane, allowed to dry, and the PAH fraction is located by UV light. The PAH fraction is scraped off and washed with approximately 8 ml of tetrahydrofuran (THF), freshly distilled from lithium aluminum hydride. Samples are concentrated to 1 ml volume and stored at $-15^{\circ}C$ in amber colored bottles until time of analysis.

2. HPLC Preparatory Steps for Analysis

Each of the total sample extracts are subjected to two

reverse-phase HPLC partitioning steps. An initial fraction of 3.5 milliliters is collected from the sample beginning about 2 minutes before the retention time of the CcdP/BghiF peak and ending 1.5 minutes after their peak retention time. This fraction is rechromatographed using an isocratic solvent program that separates the CcdP/BghiF section from the other compounds in the fraction. This second partitioning step is needed to insure that no benzo[a]anthracene (BaA) is present in the fraction to be analyzed by the GC. During standard runs on PAH compounds, it is found that BaA is not completely resolved from CcdP thus causing potential discrepencies in quantitation of CcdP. To avoid this problem the second partitioning step is employed. The two HPLC partitioning programs are:

a. 40% aqueous acetonitrile to 100% acetonitrile in 30 minutes at a flow rate of 1 ml/min, a linear gradient, UV detection at 280 nm and 365 nm, fluorescence detection with an excitation of 360 nm and an emission of >440 nm.

b. 70% aqueous acetonitrile isocratic at a flow of 1.5 ml/min., UV and fluorescent detection is the same as program a.

Note: Because of the insolubilty of PAHs in water, a solvent exchange of acetonitrile is performed on the collected fractions after each HPLC partitioning step.

3. Sample Injection System

Each of the samples are injected onto a precolumn[15]

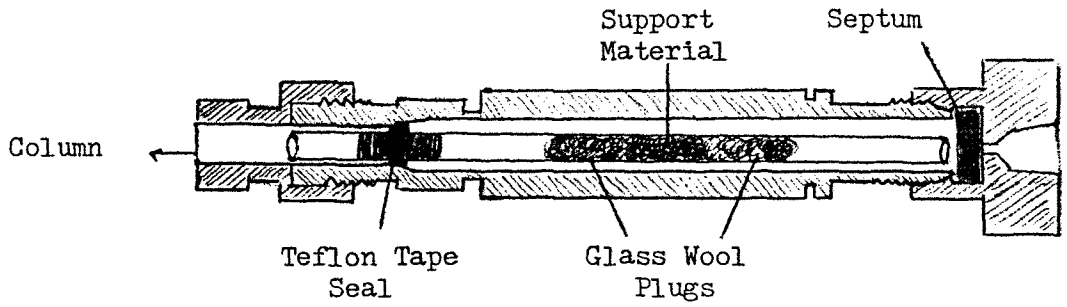
packed with Ultra Bond 20M, a specially treated support material obtained from Alltech Associates, plus two plugs of dimethyldichlorosilane (DMCS)-treated glass wool also obtained from Alltech Associates. The construction of these precolumns is performed in the laboratory as follows: Glass capillary tubes (Kimax size 1.5-2.0 mm diameter by 100 mm length) are silanized with a 50% solution of hexamethyldisilane (HMDS) in toluene and heat treated with a constant flow of zero grade nitrogen gas followed by several hours baking in an oven at 160°C. The silanizing treatment is repeated followed by a 24 hour period of oven treatment. The tubes are rinsed thoroughly with HPLC grade methanol (MeOH) followed by another heat treatment. The capillary tubes are packed with 3 mm of Ultra Bond 20M and 2 plugs of treated glass wool. The packing is placed high enough in the tube so that the lower glass wool plug does not reach the bottom metal fitting of the injector port. Teflon tape capable of withstanding 600°F is wrapped around the lower portion of the precolumn forming a gas tight seal with the metal fitting forcing the carrier gas to flow up and then down through the precolumn. Before being used for analysis, the precolumn is conditioned at 257°C (the temperature of the injector port) for 6-8 hours. Figure III is a schematic diagram of the precolumn.

After the sample has been injected onto the precolumn, the solvent is flushed off by a steady flow of nitrogen gas (zero grade) for fifteen minutes. [A filter trap is

installed in the solvent flushing system as an added precaution against impurities in the tubing.] After flushing off the solvent, the precolumn is ready to be placed in the injector port.

Figure III

Schematic Diagram of the Constructed Precolumn




4. Analysis via GC

A Varian model 3700 Gas Chromatagraph is employed in the analysis of CcdP and BghiF. A Durabond fused silica glass capillary column coated with phase DB-5 by J&W Scientific is installed for analysis. The characteristics of the DB-5 stationary phase are similar to the SE-52 and SE-54 stationary phases. The column is 30 meters long, has an inner diameter of 0.25 mm and a phase thickness of 0.25 um on its inner walls.

The precolumn is placed in the injector port which is being maintained at approximately 257$^{o}$C. With the injector at 257$^{o}$C and the column at 40$^{o}$C, a flow of nitrogen gas is passed through the system thermally releasing and then trapping the sample at the beginning of the column. Once the temperature program begins, each constituent is released and passed through the column to be detected by a flame

ionization detector (FID). The data is integrated and recorded by a Spectraphysics Integrator/Recorder. The program of GC analysis is the following:

Injector=257$^{o}$C, Detector=321$^{o}$C

Column pressure head= 20 PSI

Flow rate @ 280$^{o}$C= 1.0 ml/min

Temperature Program= 40$^{o}$C for 30 minutes, then

4$^{o}$C per minute to 300$^{o}$C

Solvent Flushing Time= 15 minutes

B. Results and Discussion of Tunnel Samples

Table III lists sampling information and experimental

Table III

Sampling Data and Concentrations (ng/m$^{3}$)

For CcdP and BghiF

| Tunnel | Sampling Date | Start Time | End Time | Volume ($m^3$) | Concentration CcdP | BghiF | Ratio CcdP/ BghiF |
|---|---|---|---|---|---|---|---|
| Lincoln | 11/24/81 | 11:53am | 12:38pm | 17.9 | 3.5 | 0.5 | 7/1 |
| Lincoln | 11/24/81 | 4:08pm | 4:40pm | 13.9 | 44.3 | 7.5 | 6/1 |
| Lincoln | 11/25/81 | 10:30am | 11:03am | 12.6 | 6.0 | 1.0 | 6/1 |
| Lincoln | 11/25/81 | 1:52pm | 2:24pm | 12.7 | 18.0 | 1.3 | 14/1 |
| Holland | 11/24/81 | 3:10pm | 3:45pm | 16.6 | 5.0 | 1.0 | 5/1 |
| Holland | 11/25/81 | 12:05pm | 12:45pm | 13.2 | 2.8 | - | - |
| Holland | 11/25/81 | 12:50pm | 1:35pm | 15.1 | 30.0 | 1.7 | 18/1 |

data for each of the tunnel samples. The reported concentrations of CcdP and BghiF range from 2.8 ng/$m^3$ to 44.3 ng/$m^3$ and the concentrations of BghiF range from undetectable to 7.5 ng/$m^3$ for the cubic meters of air sampled as listed in Table III. The fractions of actual sample injected range from (0.09)(total volume sampled) to (0.37)(total volume sampled). In the specific case of the Holland Tunnel sample where no BghiF was detected, the fraction of sample injected is (0.16)(13.2 $m^3$) or 2.1 $m^3$. The results indicate that detection to a fraction of a nanogram per cubic meter can be quantitated using the precolumn technique of injection with glass capillary GC. This method of PAH analysis is highly sensitive and capable of separating and quantitating PAHs that reverse-phase HPLC has difficulty in doing. However, HPLC is needed in separating CcdP from BaA which capillary GC has difficulty in doing. The high concentrations of CcdP, listed in Table III, reinforce the previous conclusions that CcdP is a major constituent of automobile exhaust and that, together with BaP is responsible for a significant percentage of the carcinogenic effect of the PAH fraction. The lower concentrations of BghiF in comparison with CcdP is in keeping with previous reported results.[10,12,16] However, the values reported here for BghiF are somewhat lower than the values reported in earlier studies. Several factors involving the nature of these PAHs and the method of analysis explain why some of the reported concentrations for CcdP and BghiF are so low.

To begin with, CcdP is a member of the highly photoreactive pyrene group of PAHs.[17] In the presence of sunlight or ultraviolet light, CcdP will undergo photooxidation and decomposition to a significant extent. This explains why the detection of CcdP is difficult in ambient air samples. It also explains why larger quantities of CcdP are detected in automobile exhaust condensates taken from traffic tunnels and soot particulates taken from the inside of stacks.[9,10,12,18] During the collection and extraction of the tunnel samples, care is taken in protecting the samples from light, but even with careful handling, it is likely that some of CcdP photoreacts. In addition, CcdP is not a very stable compound in solution and can slowly decompose over a long period of time.[17] Since the samples used in this study are those collected and analyzed in our earlier investigation[13], the samples are approximately a year and a half old. The age of the samples could account for further loss of CcdP. [A note should be made at this time on the use of the old tunnel samples. In our earlier study[13] no results are reported on the concentrations of CcdP because it was co-eluting with one or more other PAHs. Since quantitation of CcdP in these tunnel samples was desirable because few studies have been performed on the concentration of CcdP in automobile exhaust condensates taken from tunnels, they were used in this analysis.] The above factors explain systematic loss of CcdP for each of the samples. To explain the large discrepancies in concentrations of CcdP between samples, a

comparison is made with the reported results of BaP in our earlier study.[13] (BaP is also a member of the photoreactive pyrene group.) The results for BaP range from 11 ng/$m^3$ to 51 ng/$m^3$, a high/low ratio of 4.6/1. CcdP concentrations range from 2.8 ng/$m^3$ to 44.3 ng/$m^3$, a high/low ratio of 16/1. The greater variation for CcdP may be a measure of its greater reactivity.

What accounts for the low concentrations of BghiF? It is not a member of the photoreactive pyrene group and is a relatively stable compound. The explanation lies in the actual method of analysis. With each successive step of fraction partitioning and solvent exchange, it is assumed that all the sample is totally recovered. The possibility does exist that not all the sample is recovered after each injection in the HPLC followed by a solvent exchange. Having to concentrate the fractions to dryness between injections could lead to sample loss. (Particulates adhering to the side of the bottle and not being dissolved in the solvent could account for lower concentrations.) Total loss of sample explains the lower concentrations of both BghiF and CcdP only if the other PAHs in the sample are also lower in concentration. In addition, both compounds are relatively volatile compared to BaP.

Taking into consideration the possible factors influencing CcdP's and BghiF's concentrations, the results are acceptable in a semiquantitative sense. The ratio of CcdP/BghiF in this study is in keeping with the profile

studies of Grimmer et al. In our study, the ratios of CcdP/BghiF fall between 5 and 18. In one report by Grimmer et al[12], the results indicate ratios between 1 and 6 for CcdP/BghiF, while in another report[10], the results indicate ratios between 1 and 13 for CcdP/BghiF. Our study incorporates the range of both ratios reported by Grimmer et al showing that the precolumn injection is a reliable method of analysis for quantities in the nanogram range.

The results are similar to that of other profile studies[10,12], even though some of the concentrations are lower than anticipated. With the addition of the precolumn injection, minute quantities of PAHs found in large amounts of solvent are detected. Broad solvent peak tailing is avoided and sharp peaks chromatographed. Use of this method is not necessary for PAHs easily quantitated by reverse-phase HPLC. It does involve several partitioning steps, but it does quantitate PAHs that are otherwise hard to separate. Alsberg et al[14] also use several HPLC partitioning steps in their determination of CcdP and BghiF.

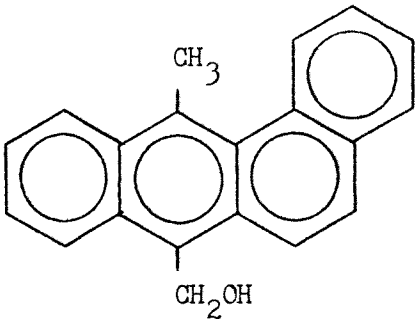
The concentrations, 18 $ng/m^3$, 30 $ng/m^3$, and 44 $ng/m^3$ for CcdP indicate that even with decomposition, CcdP is a major constituent of the PAHs in automobile exhaust. Between the relatively high concentrations of CcdP reported here and the high concentrations of BaP reported in our earlier study[13], one may speculate that these two compounds exert significant influence on the carcinogenic effect of automobile exhaust on the environment. As stated earlier in this

report, these two compounds have been proven to be animal carcinogens[8,11], as well as being mutagenic in several test studies.[8,11] In the absence of CcdP, BaP is a potent carcinogen, but in the presence of CcdP, both PAHs exhibit a syncarcinogenic effect. The accurate quantitation of CcdP is necessary in determining the health problems automobile exhaust may pose to society.

Although the concentrations of BghiF reported here are somewhat low and it has not been shown to be a carcinogen, Alsberg's article[14] gives rise to some speculation as to the role BghiF plays in the carcinogenic effect of automobile exhaust. BghiF does exhibit some mutagenic properties toward the metabolizing system S9 (Table II), about half of that reported for CcdP. When BghiF is combined with CcdP and or the rest of the sample, the mutagenic effects toward S9 is greater than expected. It does not follow an additive rule. This promotes speculation on how the presence of both CcdP and BghiF influence the mutagenic/carcinogenic properties of automobile emission and how the presence of BghiF, CcdP, and BaP together influence the effects of automobile emission on the environment. Much further research is needed in determining the environmental risks of automobile exhaust condensates on human health. Appendix B contains pertinent sections of the chromatograms on each of the tunnel samples.

## III. HYDROXYMETHYL SULFATES

In 1965, E. Boyland and P. Sims reported that 7,12-dimethylbenz[a]anthracene (DMBA) is metabolized in vivo to two monohydroxymethyl derivatives.[28] These two derivatives, 7-hydroxymethyl-12-methylbenz[a]anthracene (9) and 7-methyl-12-hydroxymethylbenz[a]anthracene (10) are reported to induce cancer in laboratory test rats and mice. In a later

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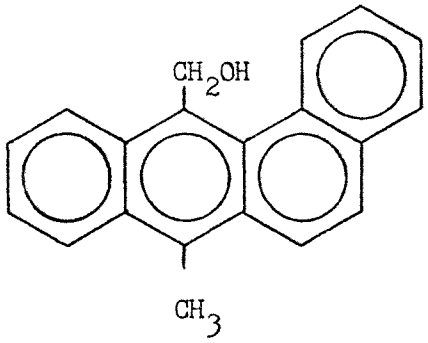


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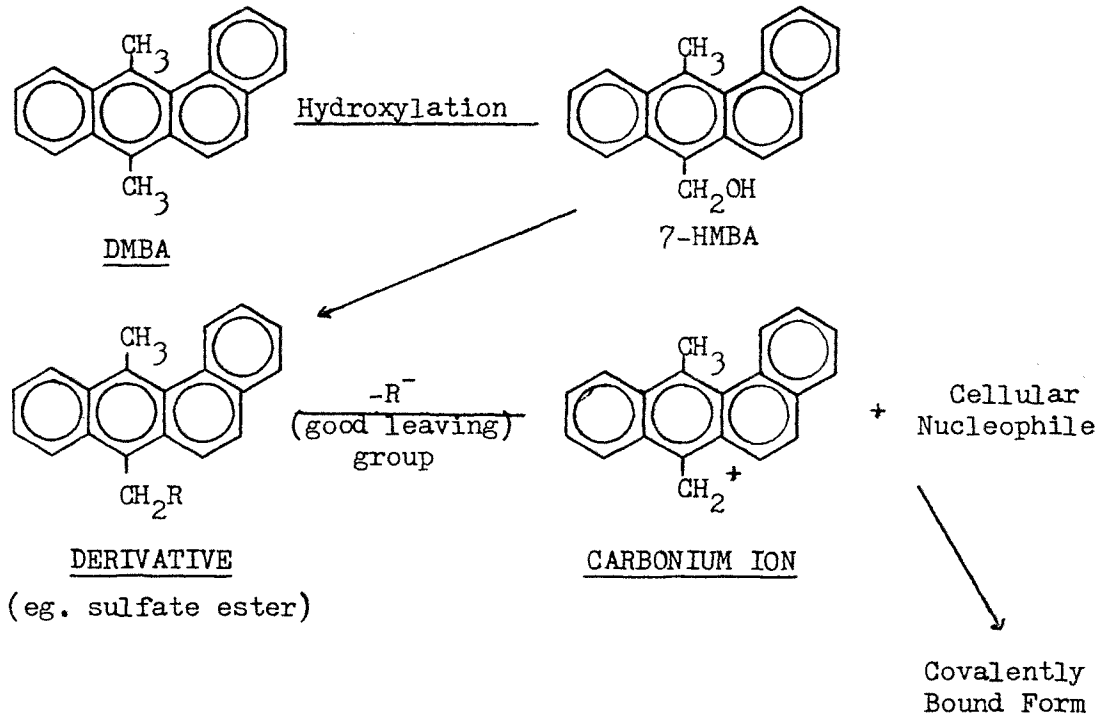
study, Flesher and Sydnor[29] propose a mechanism for the metabolism of certain compounds to the carcinogenic derivative of DMBA, 7-hydroxymethyl-12-methylbenz[a]anthracene (7-HMBA). Figure IV gives the proposed mechanism beginning with DMBA. DMBA is representative of those compounds that, upon hydroxylation, form 7-HMBA, a more potent carcinogen than its parent compound. Once in the form of 7-HMBA, formation of a derivative such as a sulfate ester takes place. These derivatives are expected to have a good leaving group in order to generate a reactive carbonium ion. The carbonium ion would then be able to react with the cellular nucleophile (e.g. DNA) initiating the step necessary in the development of cancer.

Figure IV[29]

Proposed Mechanism for Metabolic Activation of DMBA

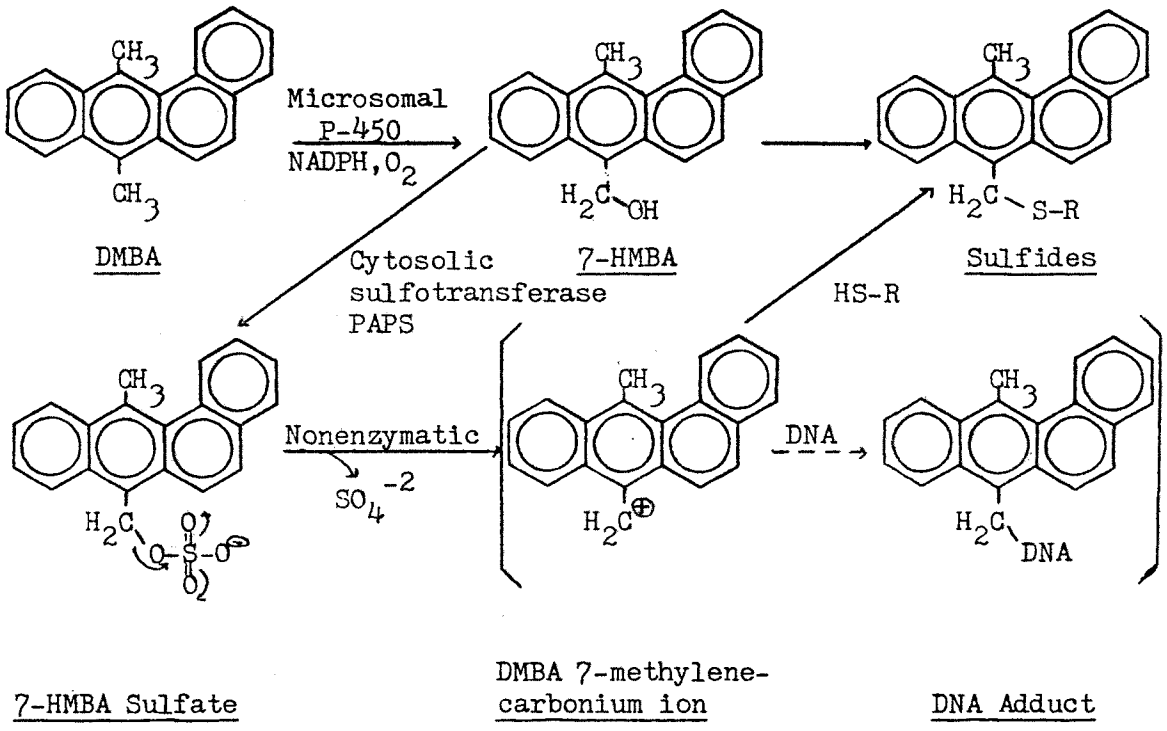



Why the 7-HMBA derivative is more carcinogenic than its parent compound, DMBA, remains unexplained. However, the mechanism for metabolic activation of 7-HMBA has been explained by Watabe et al.[6] Figure V represents the results of their study on DMBA in the presence of rat liver microsomes and a NADPH-generating system.

Watabe et al have synthesized 7-HMBA in the laboratory from DMBA and converted it to a sulfate ester derivative, isolating it as a sodium salt. Several other sulfate esters of arylmethanols have also been synthesized by Watabe et al. These arylmethanols are benzyl alcohol, 1- and 2-hydroxymethylnapthalene and 1-hydroxymethylpyrene. All tested are mutagenic toward S. typhimurium TA 98.[6] The sulfate ester of

Figure V[6]

Metabolic Pathway for the Activation of DMBA in Rat Liver




benzyl alcohol (i.e. benzyl sulfate) is the least mutagenic[6] of these compounds and 1-hydroxypyrene is the most mutagenic.

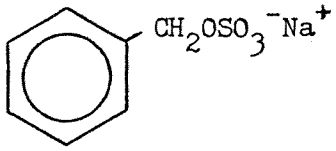
The research of Watabe et al[6] on the synthesis and isolation of several mutagenic compounds led to our question, "Do the sulfate esters of arylmethanols exist in the atmosphere on air particlate matter?". Benzyl alcohol is chosen to be the basis of this investigation because it is nonmutagenic and its parent compound, toluene, is present in the atmosphere in significant concentrations. Using the synthesis outlined by Watabe et al[6], the sulfate ester of benzyl alcohol is synthesized and isolated as its sodium salt.

The samples used in the attempted qualitative analysis of benzyl sulfate were air particulate samples obtained from a random selection of several urban sites in New Jersey. The date of sampling, the collection and the sample preparation is the same for each site.

A. Experimental Procedures for Benzyl Sulfate

1.Synthesis of Benzyl Sulfate(Na salt)

The synthesis of benzyl sulfate(Na salt) (11) in the laboratory follows the procedure outlined by Watabe et al.[6]

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The quantities used in the reaction are double the amounts listed by Watabe et al. Ten millimoles of benzyl alcohol are placed in a reaction flask containing 40 ml of anhydrous pyridine. (The pyridine is dried by refluxing over potassium hydroxide followed by fractional distillation.) Freshly distilled chlorosulfonic acid, 11.0 millimoles, is added to the reaction flask dropwise. The mixture is allowed to react for three hours at room temperature and then neutralized with an aqueous solution of sodium hydroxide, 11.5 Molar. The neutralized mixture is diluted with 10 volumes of ether in three equal portions. The precipitate is collected by filtration and washed thoroughly with ether. The white precipitate is suspended in ethanol, 20 ml, to separate the

ethanol insoluble inorganic salts from the benzyl sulfate. The salts are removed by filtration and the filtrate roto-evaporated to dryness in vacuo at room temperature. The residue is redissolved in 10 ml of ethanol and the solution diluted with 10 volumes of ether. The salt of benzyl sulfate is collected by filtration and the precipitate washed thoroughly with ether and dried in vacuo. The spectroscopic results for benzyl sulfate taken from Watabe et al[6] are: NMR $\delta_{ppm}$ in $CD_3OD$ = 5.06 (methylene H, singlet) and 7.43 (aromatic H, singlet); UV $\lambda_{max}$ in EtOH ($\epsilon$) = 212 (2686), 257 (124); IR $\nu_{max}$ in KBr pellet $cm^{-1}$ = 3050, 3012, 2875, 1497, 1465, 1250, 1201, and 1080. In this study, the spectroscopic results for the NMR and UV tests run on the synthesized benzyl sulfate(Na salt) are comparable to the values determined by Watabe et al.

2. Collection and Preparation of Samples

The samples used in the detection of benzyl sulfate are obtained from high volume samplers placed in several urban sites in New Jersey. The air particulate samples are collected on Gelman Type AE glass fiber filters. The filters are weighed and measured prior to collection. The sampling date of all the filters is January 6, 1983. Each filter is cut into sections and a section is used for analysis. The filter is Soxhlet extracted for 6 hours, first with 150 ml of benzene and second, with 150 ml of acetone. One 1 ml of the internal standard, 1-methyltrypticene, is added and the samples are rotoevaporated to approximately 5 ml aliquots.

The 5 ml aliquots are concentrated under a flow of nitrogen gas to 1 ml. The 1 ml solutions are dotted onto fluorescent silica gel TLC plates and developed in a 1:1 solution of toluene and hexane. A standard of benzyl sulfate is prepared and dotted next to the sample fractions in order to determine the correct area to scrape off and wash with ethanol in two 3 ml increments. Prior to analysis, the solutions are concentrated to a tenth of a milliliter.

3. Analysis by Reverse-Phase HPLC

The determination of the presence of benzyl sulfate(Na salt) is carried out on a Waters gradient HPLC system operated in the reverse-phase mode (Vydac ODS column, separations group) connected to a Spectraphysics Integrator/Recorder. UV detection is at 280 nm and 365 nm with fluorescence at an excitation wavelength of 360 nm and an emission wavelength of >440 nm. The solvent program starts with 80% aqueous acetonitrile reaching 100% acetonitrile in 15 minutes at a linear gradient.

B. Results and Discussion on Benzyl Sulfate(Na Salt)

The general mechanism for the metabolic activation of DMBA, a methyl substituted aromatic hydrocarbon, follows the pathway:

DMBA $\xrightarrow{\text{hydroxilation}}$ 7-HMBA $\xrightarrow{\text{esterfication}}$

Sulfate Ester Derivative $\xrightarrow{\text{Loss of Leaving Group}}$

Reactive Carbonium Ion $\xrightarrow{\text{Binding}}$ DNA-adduct

This pathway is known to be occur in laboratory test mice.[6]

Perhaps, the same pathway for metabolic activation will take place for toluene once it has been ingested by the body. If, however, benzyl sulfate exists on air particulates, then the need for the hydroxlation and esterfication steps of the metabolic activation of toluene is eliminated. Now, if the sulfate ester is ingested into the body, the only step needed in the activation of the metabolite is the generation of the carbonium ion. At this point, the metabolic activation of toluene and the existence of benzyl sulfate on air particulates is only speculation. It is possible that benzyl sulfate may not have sufficient fat solubility to enter cells. The object of this investigation, is to determine the presence and or absence of benzyl sulfate on air particulates.

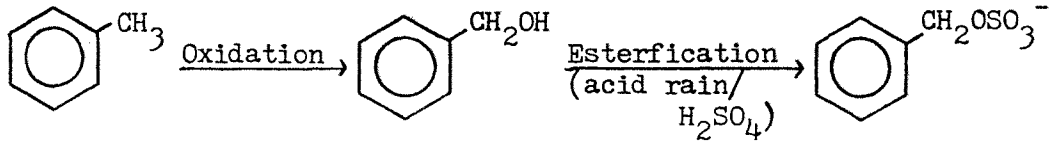
Considering the presence of toluene and what is termed "acid rain" in the atmosphere, it is conceivable that the formation of benzyl sulfate from oxidation and reaction with sulfuric acid can take place (Figure VI). More than likely, it would exist as the sodium salt on the particulates and, when extracted, exist in solution as the sulfate ester $(C_6H_5)CH_2OSO_3^-$. The spectroscopic results on each of the sample extracts indicate that benzyl sulfate may be present on air particulates. The results, however, are not conclusive. They are consistent with but do not prove that sulfate esters are present in the atmosphere.

Figures VII-XVII are portions of the chromatograms indicating the possibilty of benzyl sulfate's presence.

Figure VI

Hypothetical Mechanism for the Formation of

Benzyl Sulfate in the Atmosphere




Toluene — Benzyl Alcohol — Sulfate Ester

However, because of the lack of sufficient retention by the HPLC column and the limited migration (approximately one-third) up the TLC plate, the large peak areas at the beginning of the chromatograms indicate the presence of any polar material. If there were no peaks in this area one could conclude that no benzyl sulfate is present.

Much more work is needed in determining the optimum conditions for the preparation, separation, and identification of benzyl sulfate on air particulate matter. Subsequent work should include the use of a more polar solvent (e.g. methanol) in the extraction of the sample filters and a more polar solvent system in the development of the TLC plates. The change to a more polar solvent system would increase the migration and separation of the benzyl sulfate from the rest of the sample. Further study should also include the use of an HPLC column packed with a more polar material, thus increasing the retention of benzyl sulfate relative to the solvent peak. Comparison of the two

extractions for each sample can not be made until better operating conditions are determined. The results, therefore, are inconclusive and need much further research.

By postulating that benzyl sulfate is present on air particulate matter, an entirely new area of study on PAHs is opened. Assuming that the presence of benzyl sulfate, eventually is established, other sulfate esters of methyl-substituted PAHs can be undertaken. Following the identification of these compounds on air samples, the determination of the metabolic activation of these compounds can be investigated. In so doing, the environmental health problems related to benzyl sulfate, as well as, the other sulfate esters can be analyzed.

Figure VII

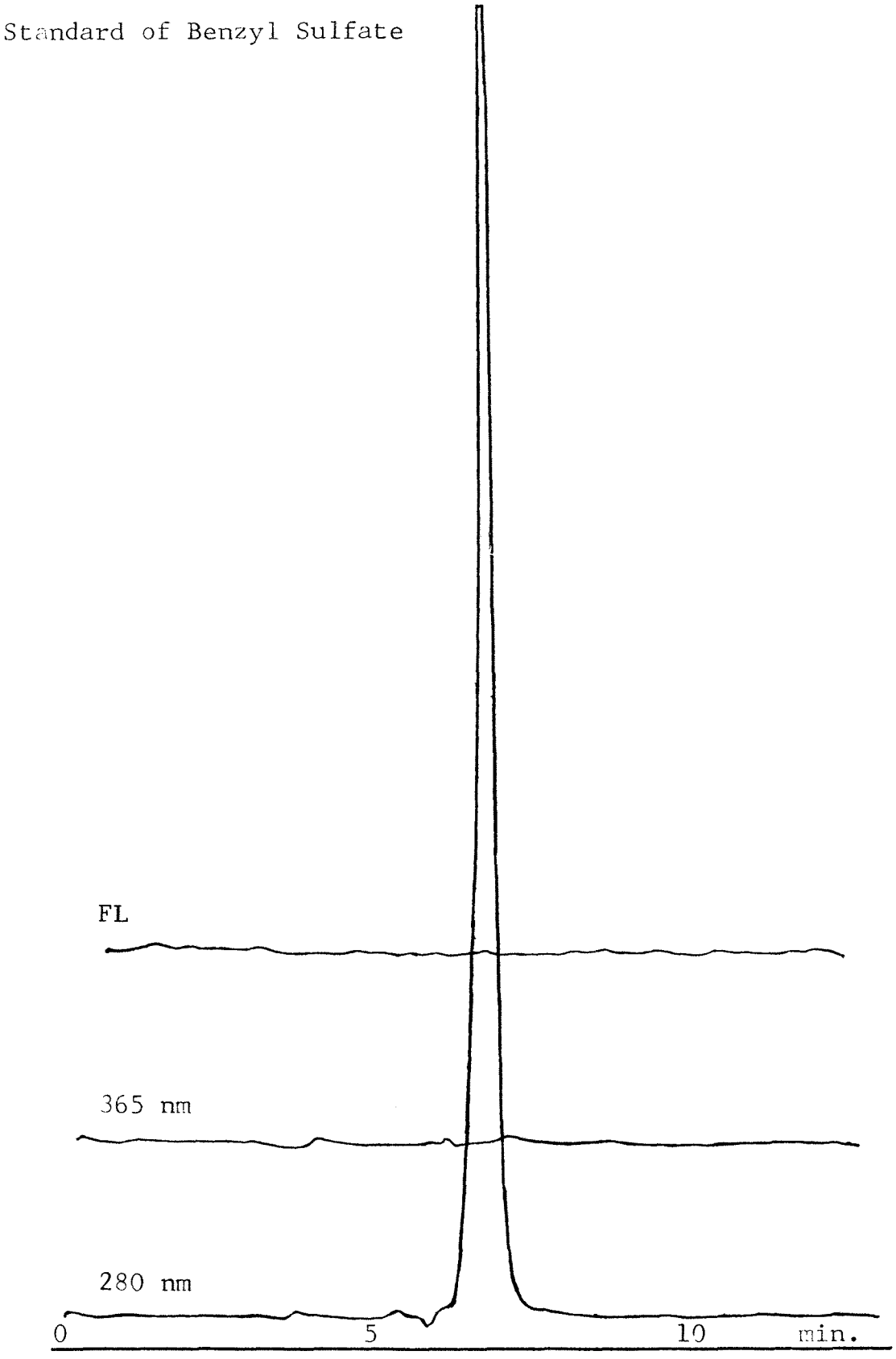


Figure VIII

Ringwood: Benzene Fraction for Benzyl Sulfate

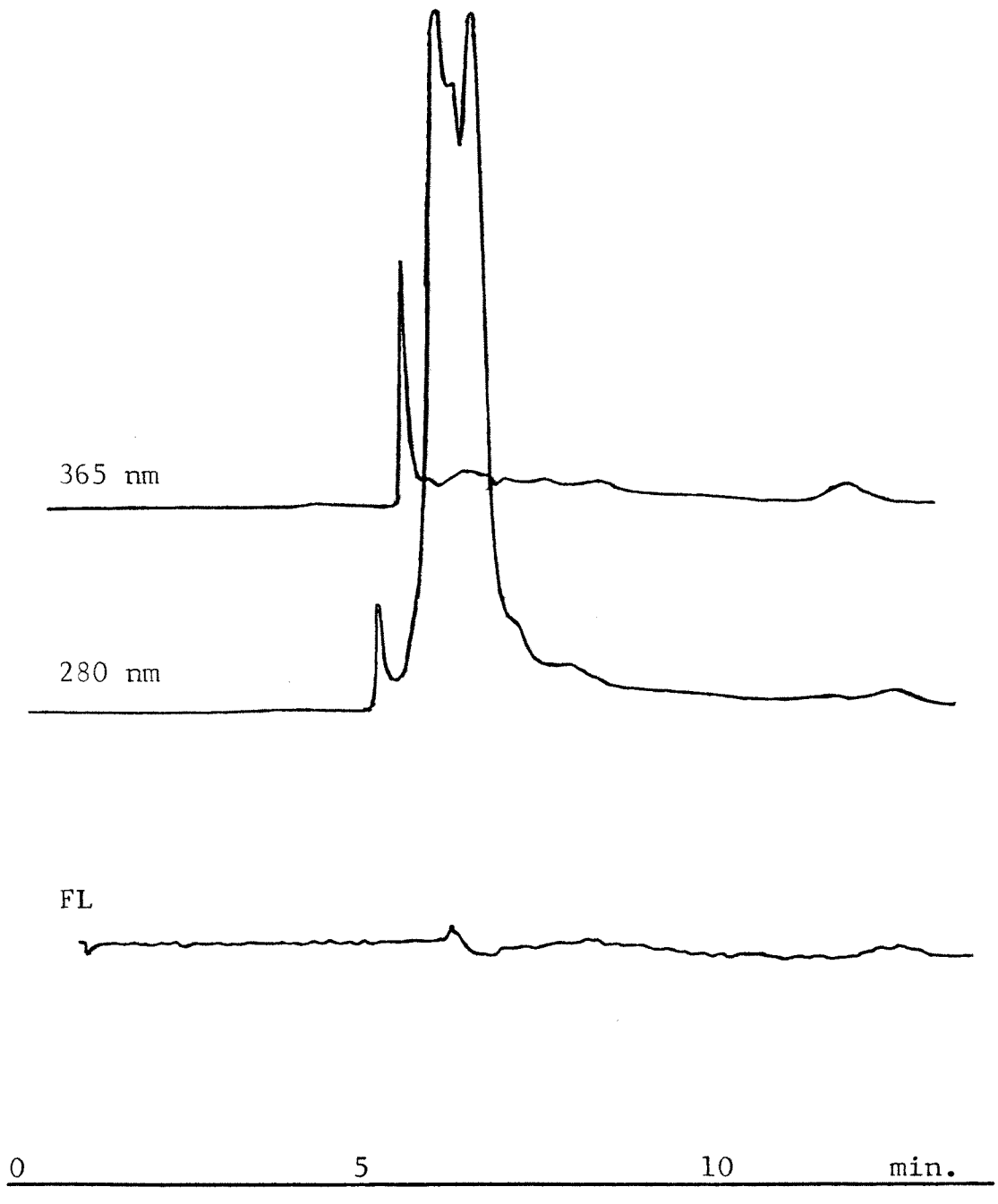


Figure IX

Ringwood: Acetone Fraction for Benzyl Sulfate

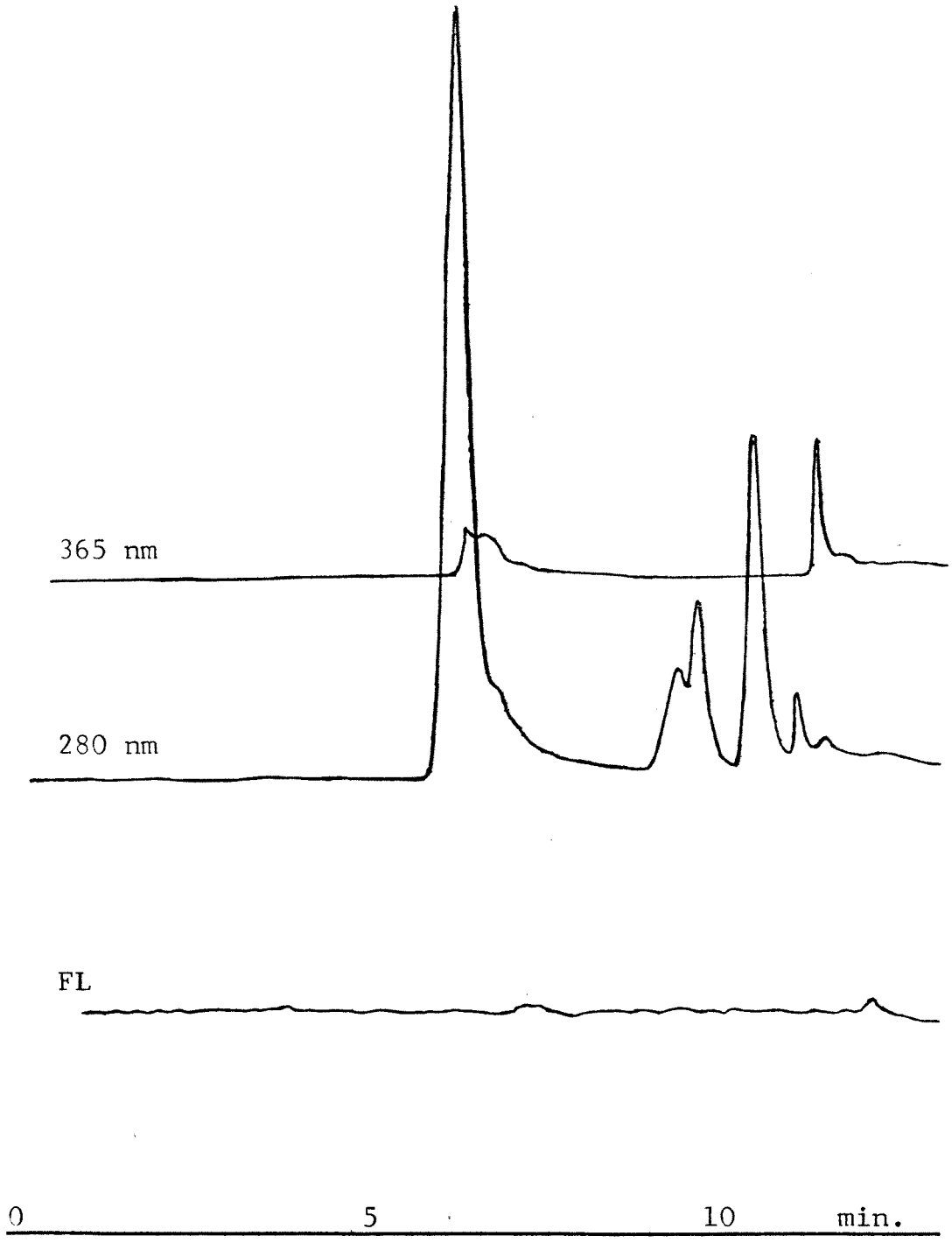


Figure X

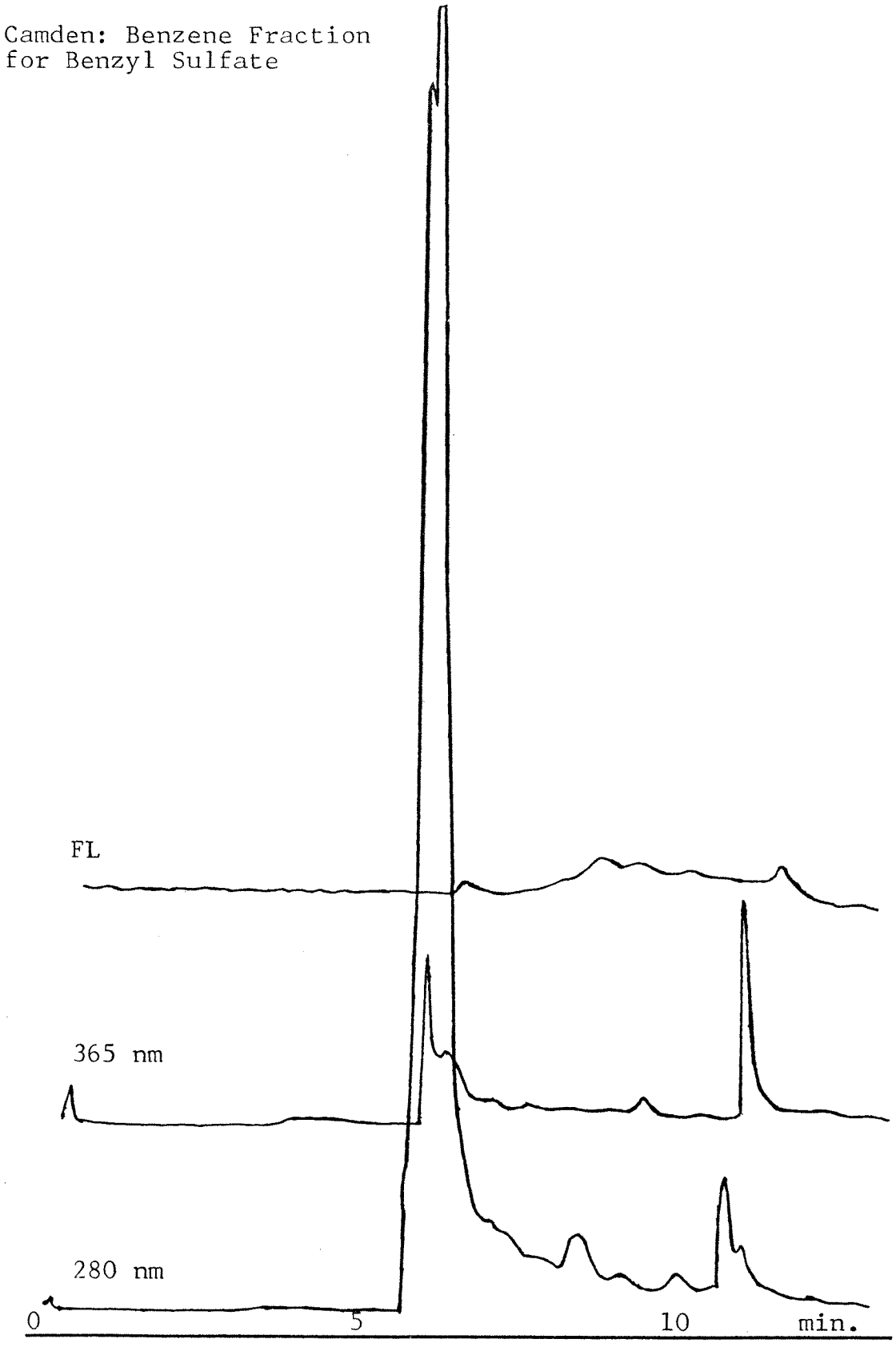

Camden: Benzene Fraction
for Benzyl Sulfate
FL
365 nm
280 nm
0
5
10
min.

Figure XI

Camden: Acetone Fraction
for Benzyl Sulfate

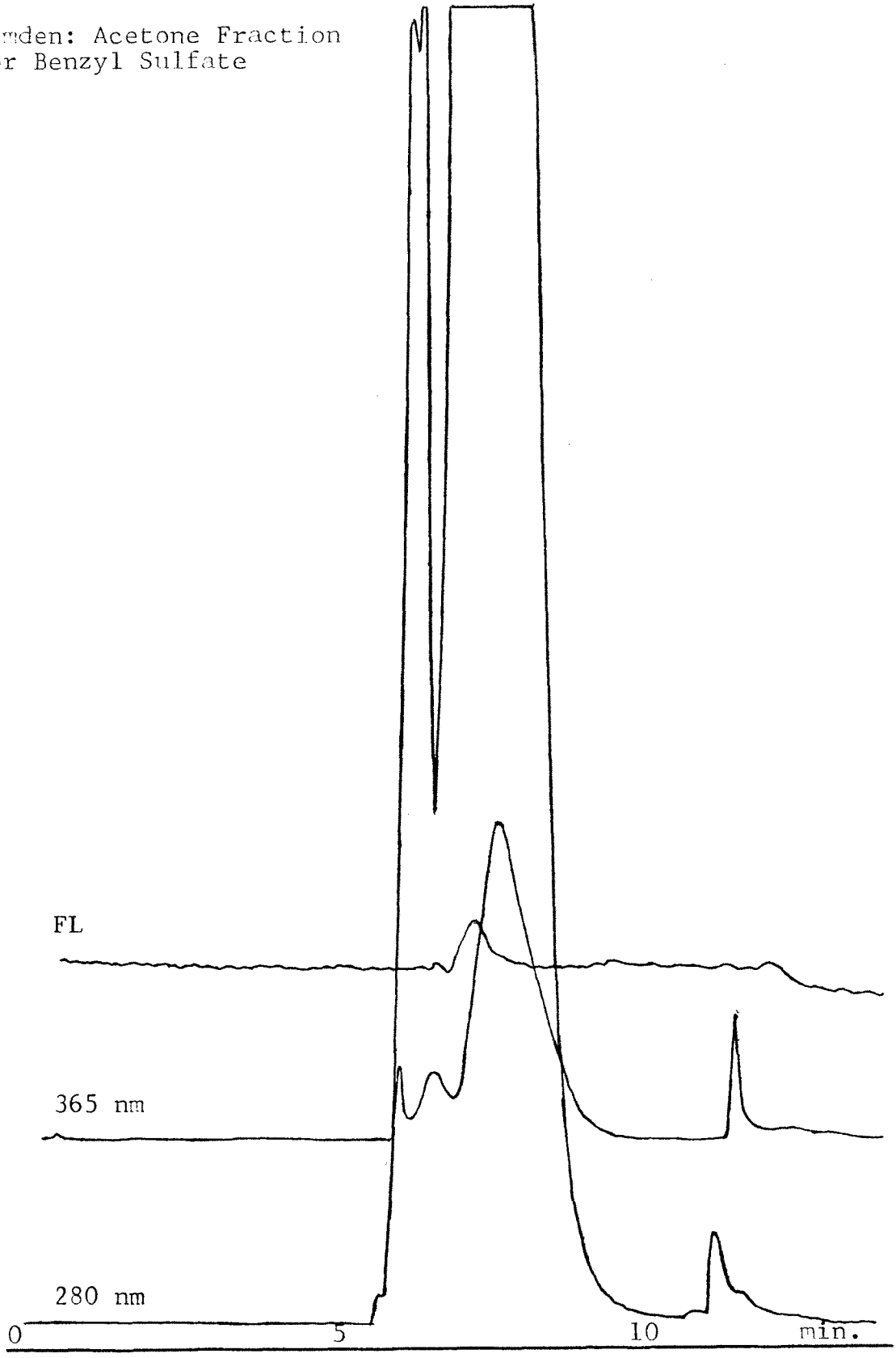


Figure XII

Toms River: Benzene Fraction for Benzyl Sulfate

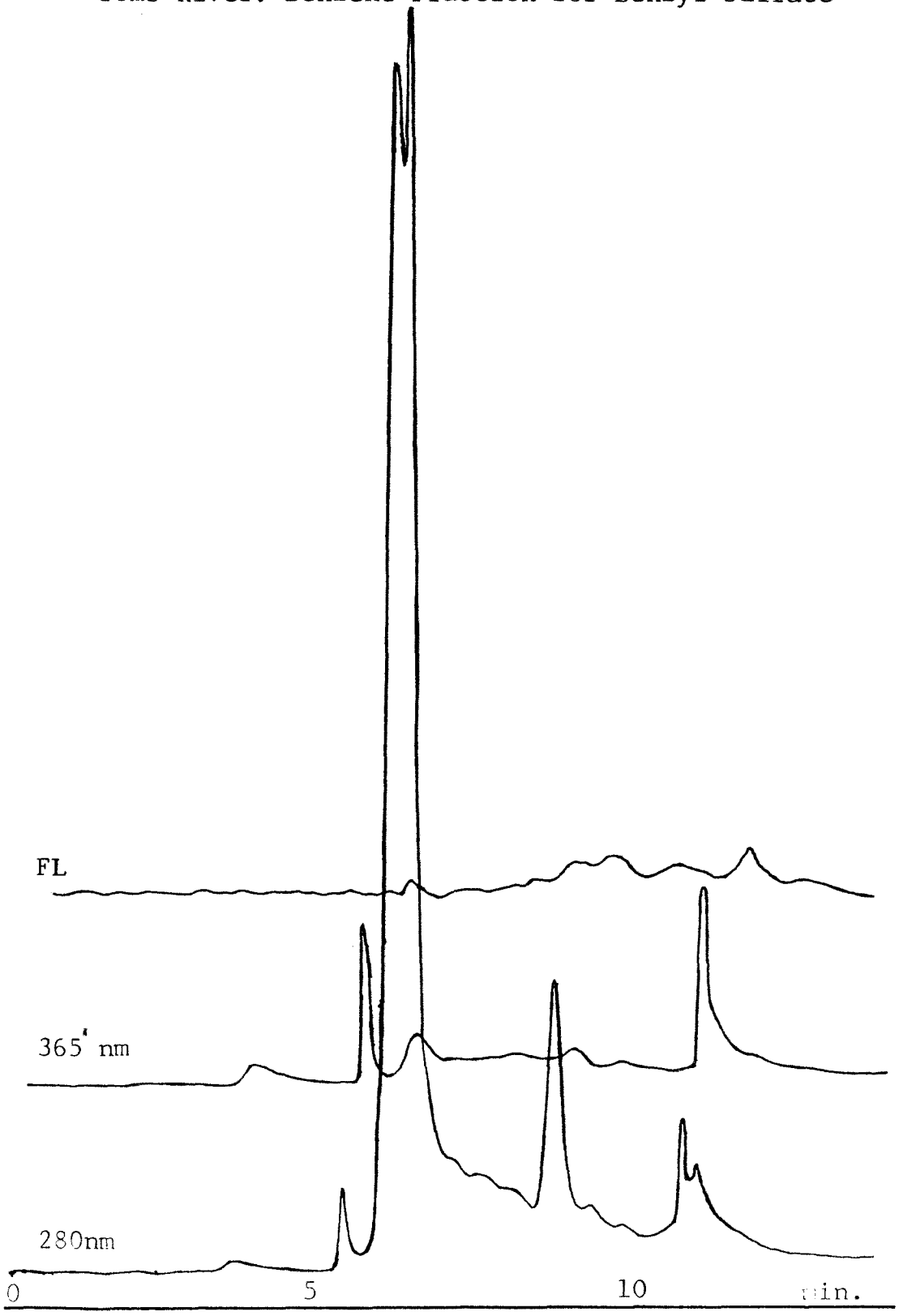


Figure XIII

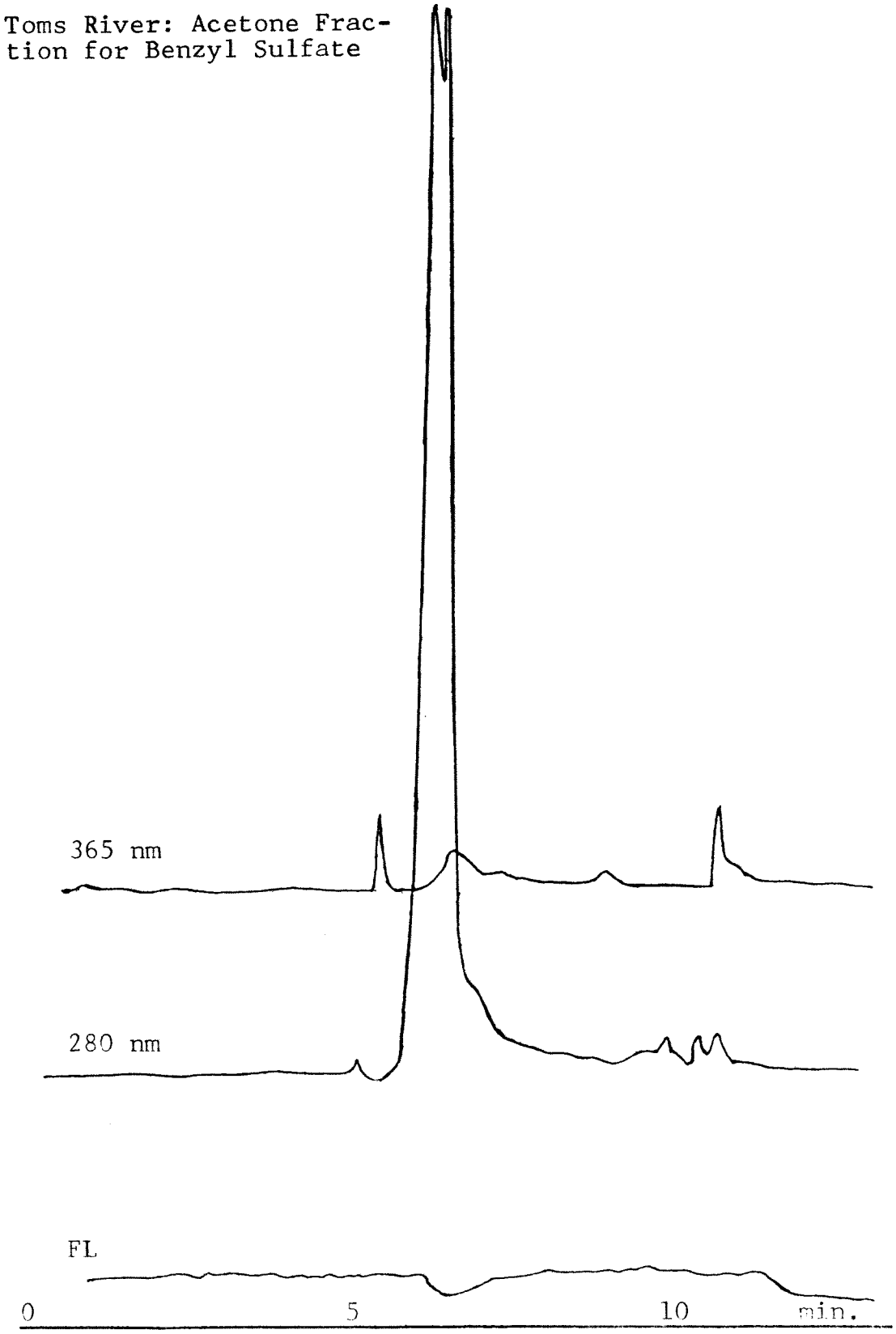

Toms River: Acetone Fraction for Benzyl Sulfate
365 nm
280 nm
FL
0
5
10
min.

Figure XIV

Fairlawn: Benzene Fraction
for Benzyl Sulfate

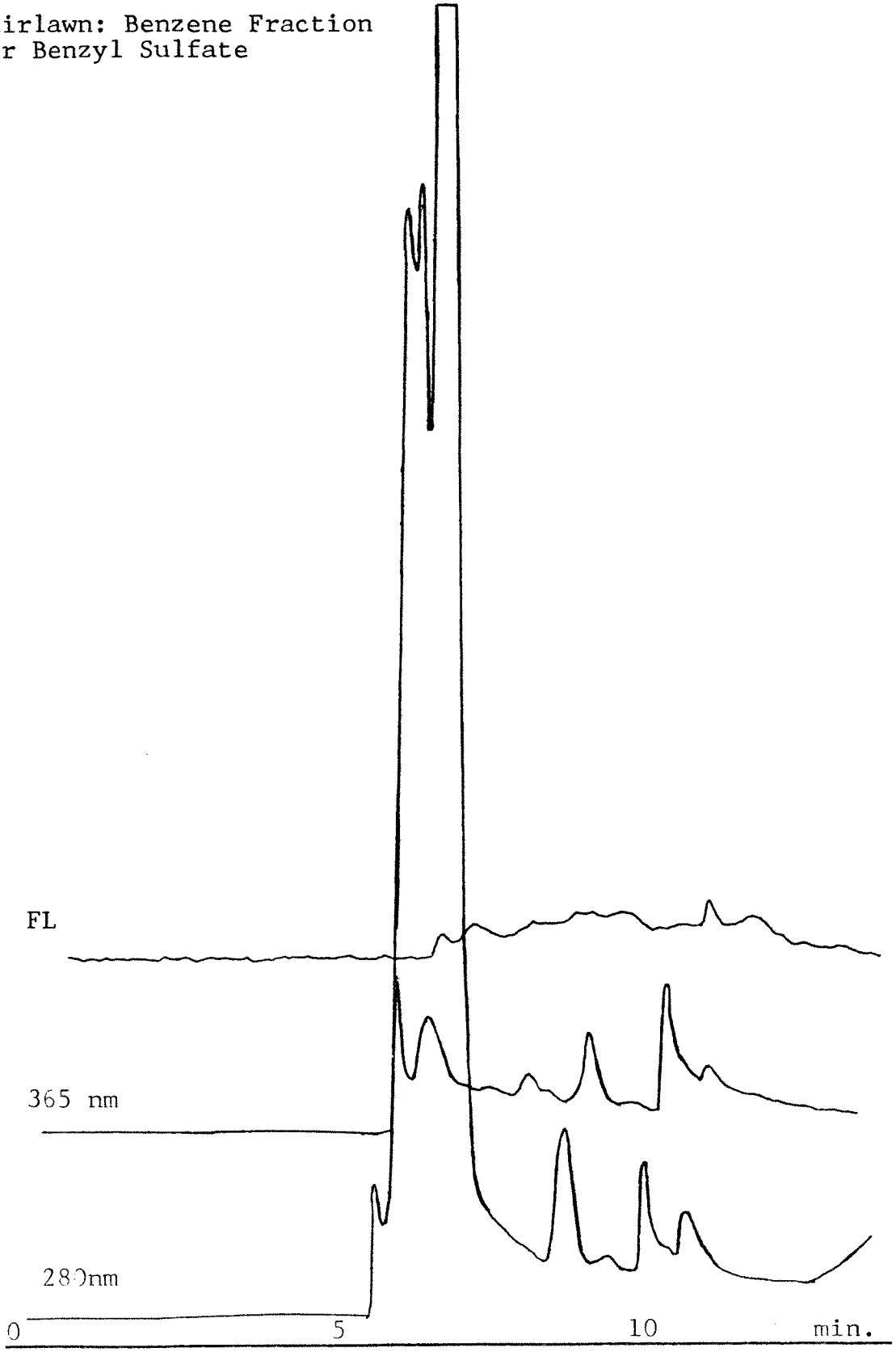


Figure XV

Fairlawn: Acetone Fraction for Benzyl Sulfate

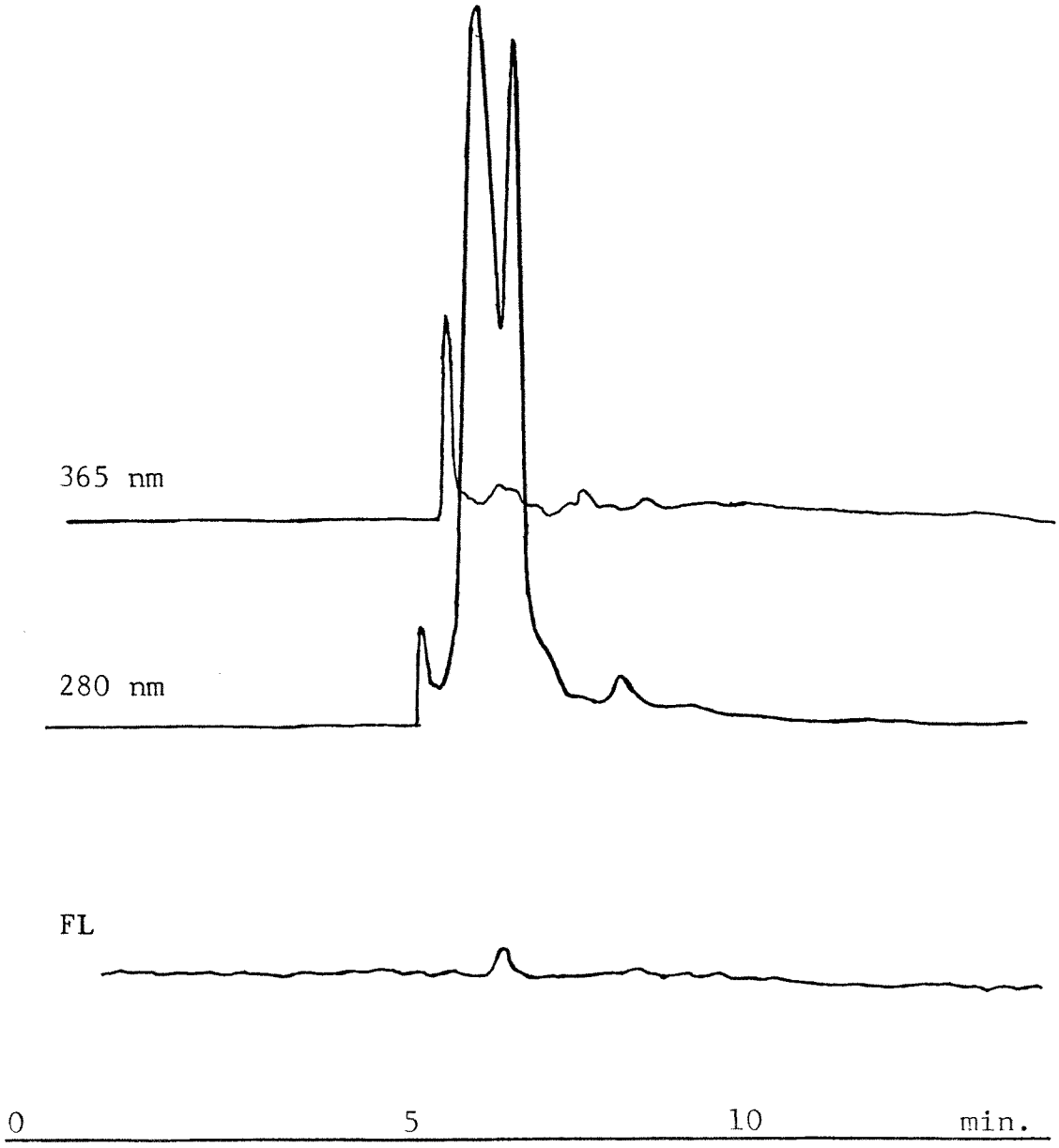


Figure XVI

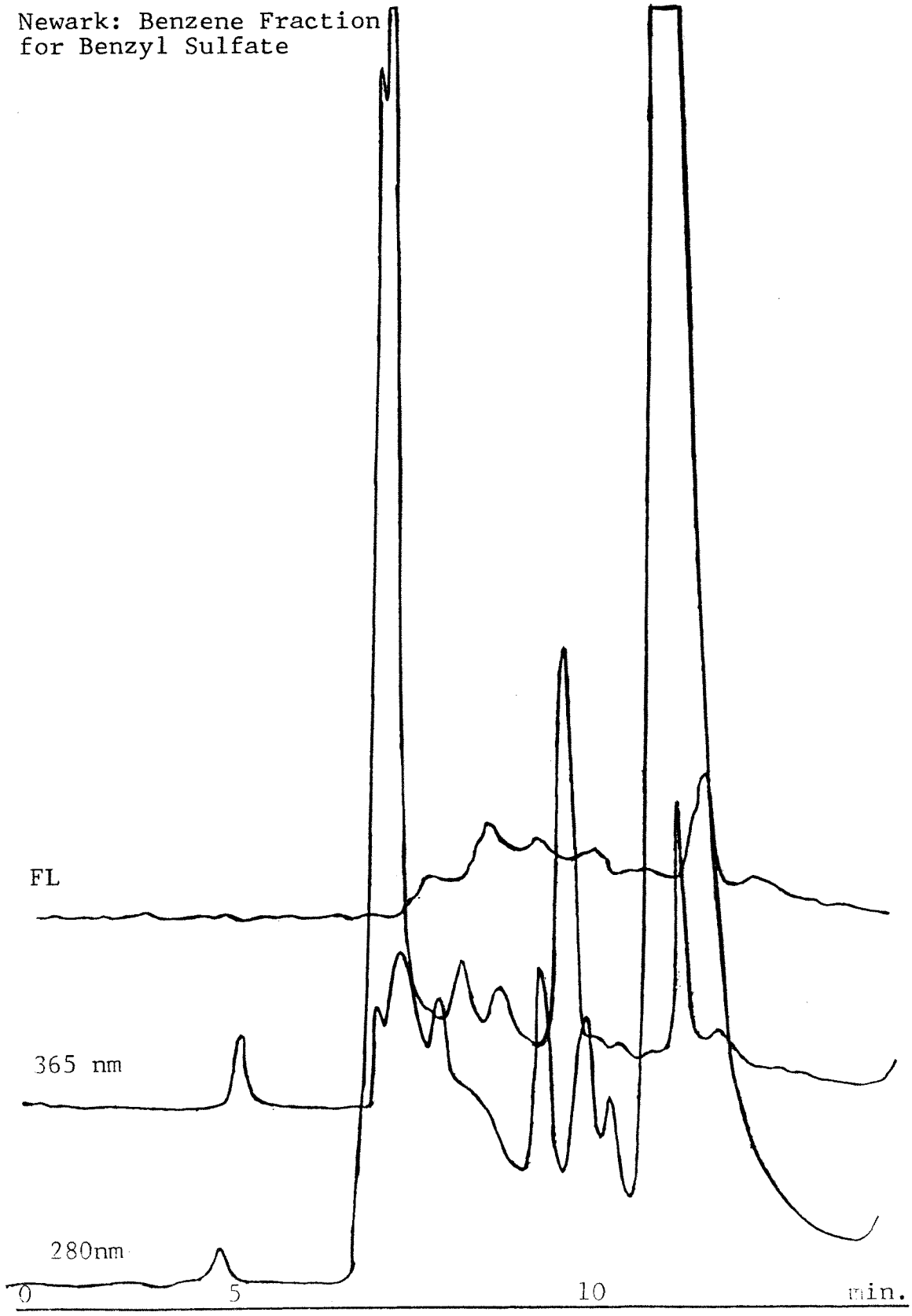
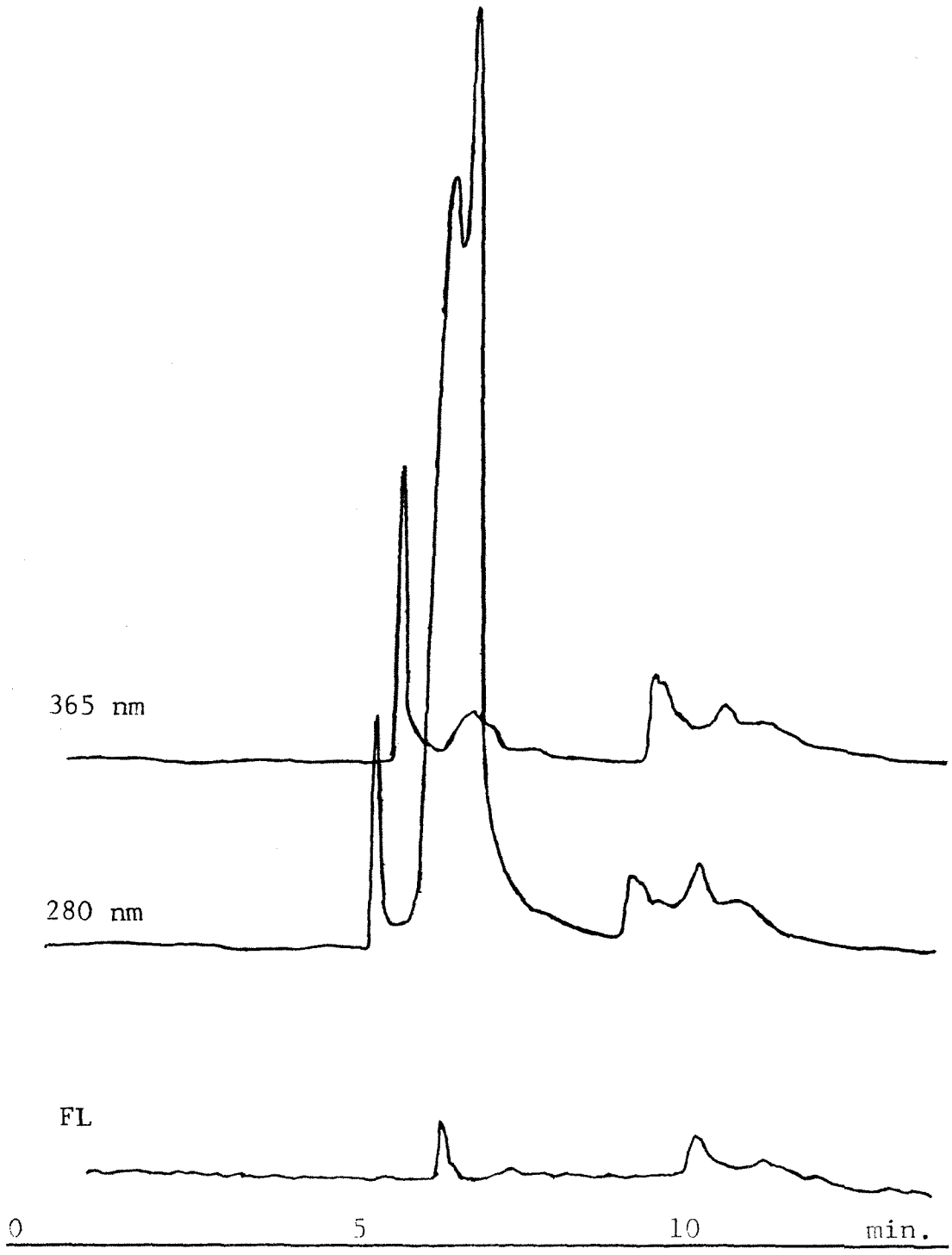

Newark: Benzene Fraction
for Benzyl Sulfate
FL
365 nm
280nm
0
5
10
min.

Figure XVII

Newark: Acetone Fraction for Benzyl Sulfate



## CONCLUSIONS

The first investigation on H/D exchange on the surface of fly ash shows that adsorptive matrices do play a part in the reaction of PAHs adsorbed on its surface. Further characterization of the fly ash particles is necessary to explain how the fly ash influences reactions between the adsorbed PAHs.

The second investigation on the quantitation of CcdP and BghiF agrees with previous studies that CcdP is a major constituent of automobile exhaust and is normally present in much greater amounts than BghiF. CcdP's concentrations decrease rapidly when exposed to light and long periods of storage because of its photochemical decomposition and relative instabilty. BghiF is a more stable compound exhibiting some mutagenic properties. However, further research is needed to determine its contributions with CcdP to the total carcinogenic/mutagenic properties of automobile emissions.

The last investigation involves the identification of benzyl sulfate on air particulates. The results are not conclusive in determining the presence of the sulfate ester of benzyl alcohol. A definite answer to this question is desirable since the sulfate ester of one arylmethanol 7-HMBA has been converted to an active metabolite in laboratory test rats.

Studying PAHs on airborne partculates covers many areas of research. Any additional information that research-

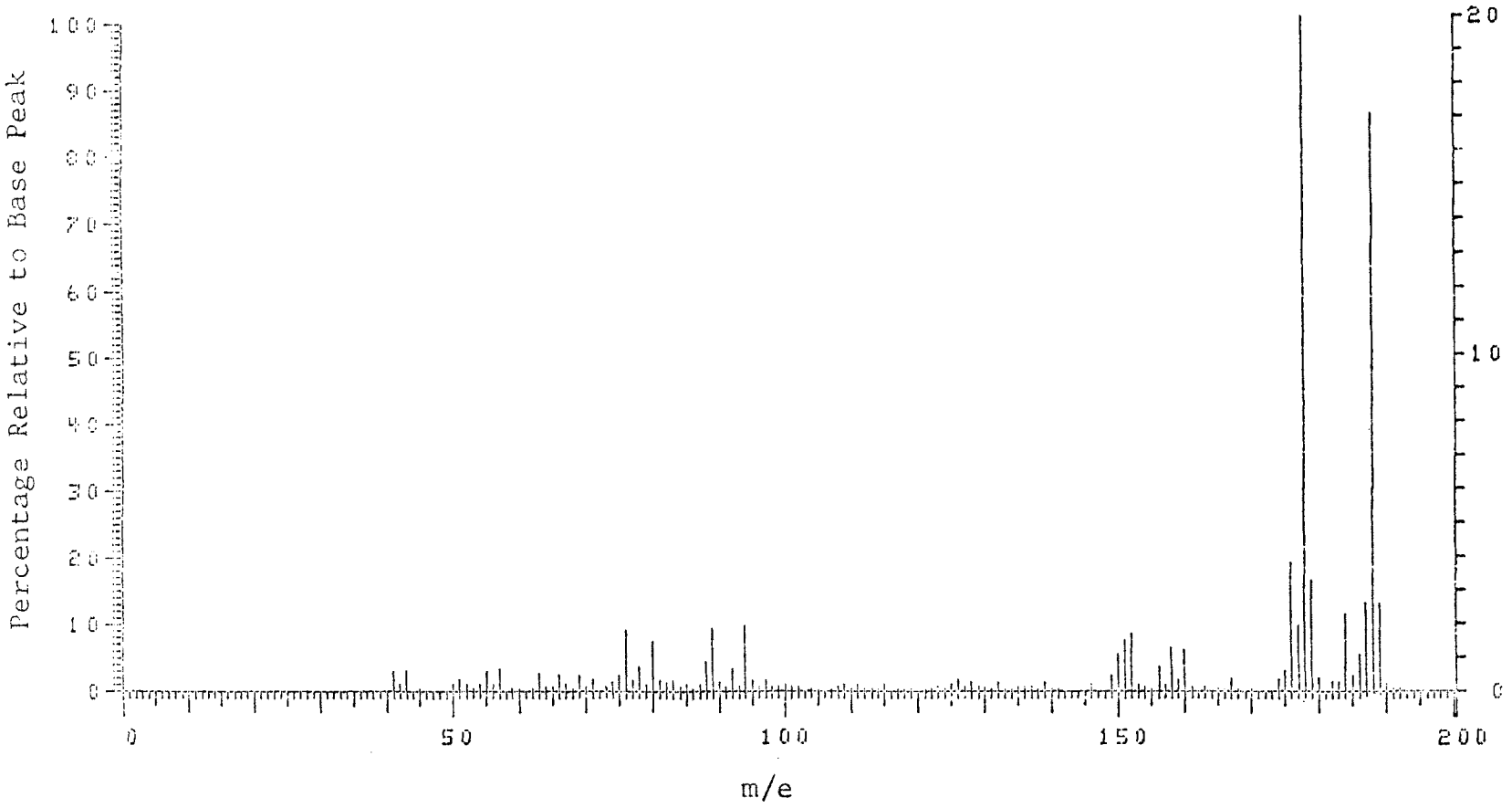
ers can determine on the abundance, carcinogenic and or mutagenic properties, derivatives, interactions, etc. of PAHs can only assist in the environmental control of these compounds.

# APPENDIX A

This appendix consists of thirteen mass spectrograms obtained during our investigation on the activity of fly ash samples. Spectrograms I-XII are the fly ash samples and Spectrogram XIII is the control sample.

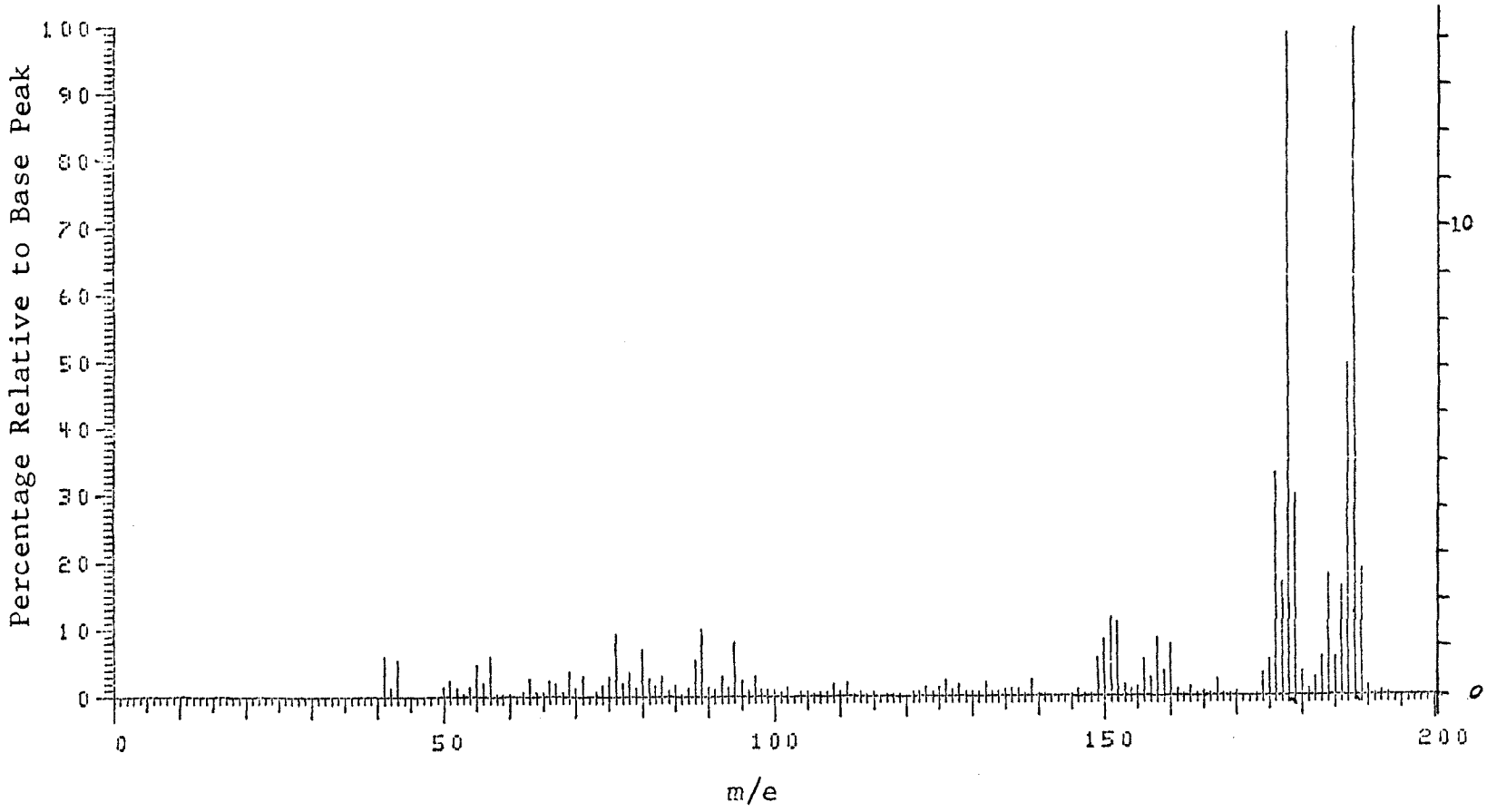
Mass Spectrogram I

Nora Fly Ash: One Day Incubation, Run 1



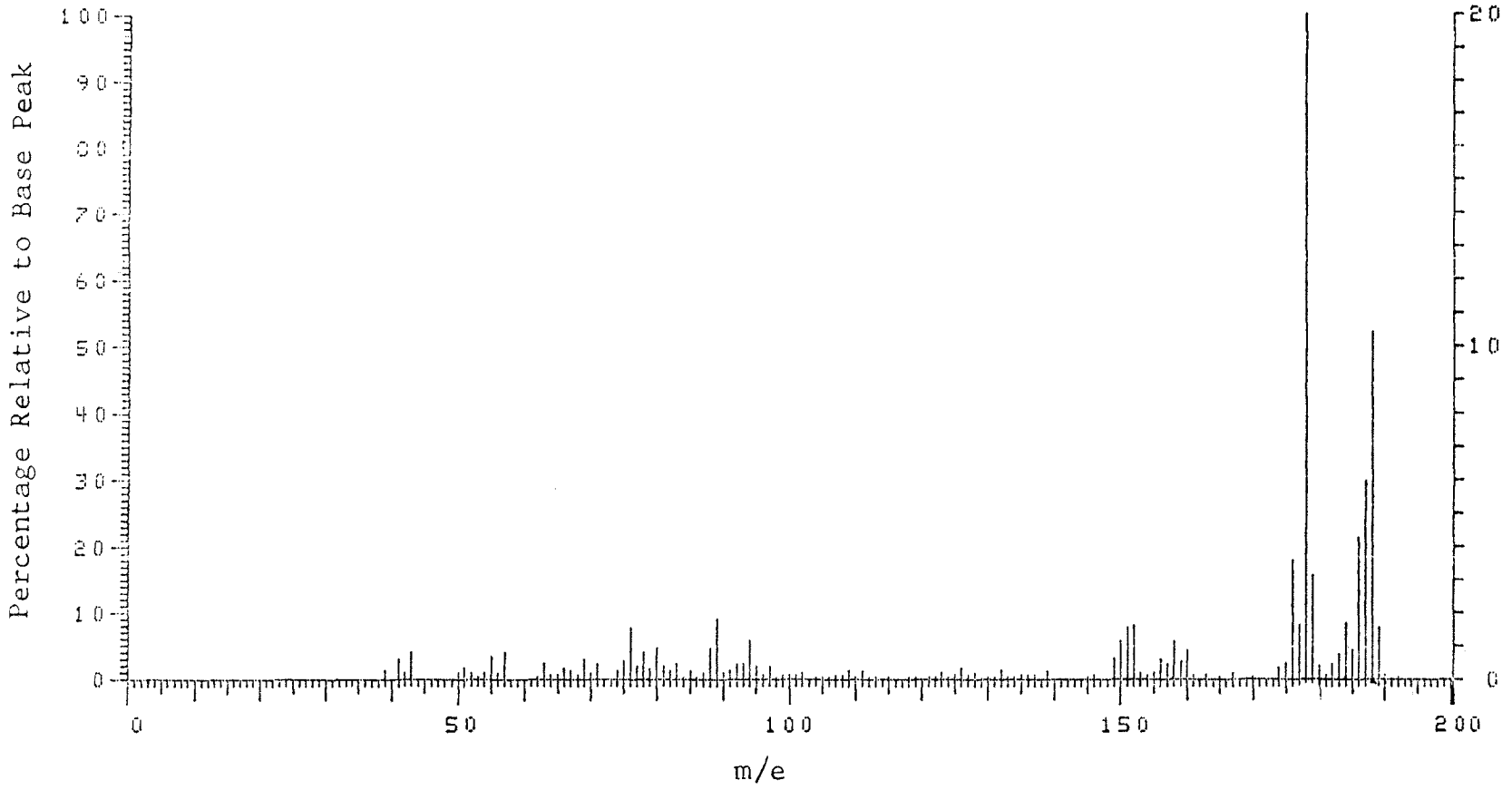
Mass Spectrogram II

Nora Fly Ash: One Day Incubation, Run 2



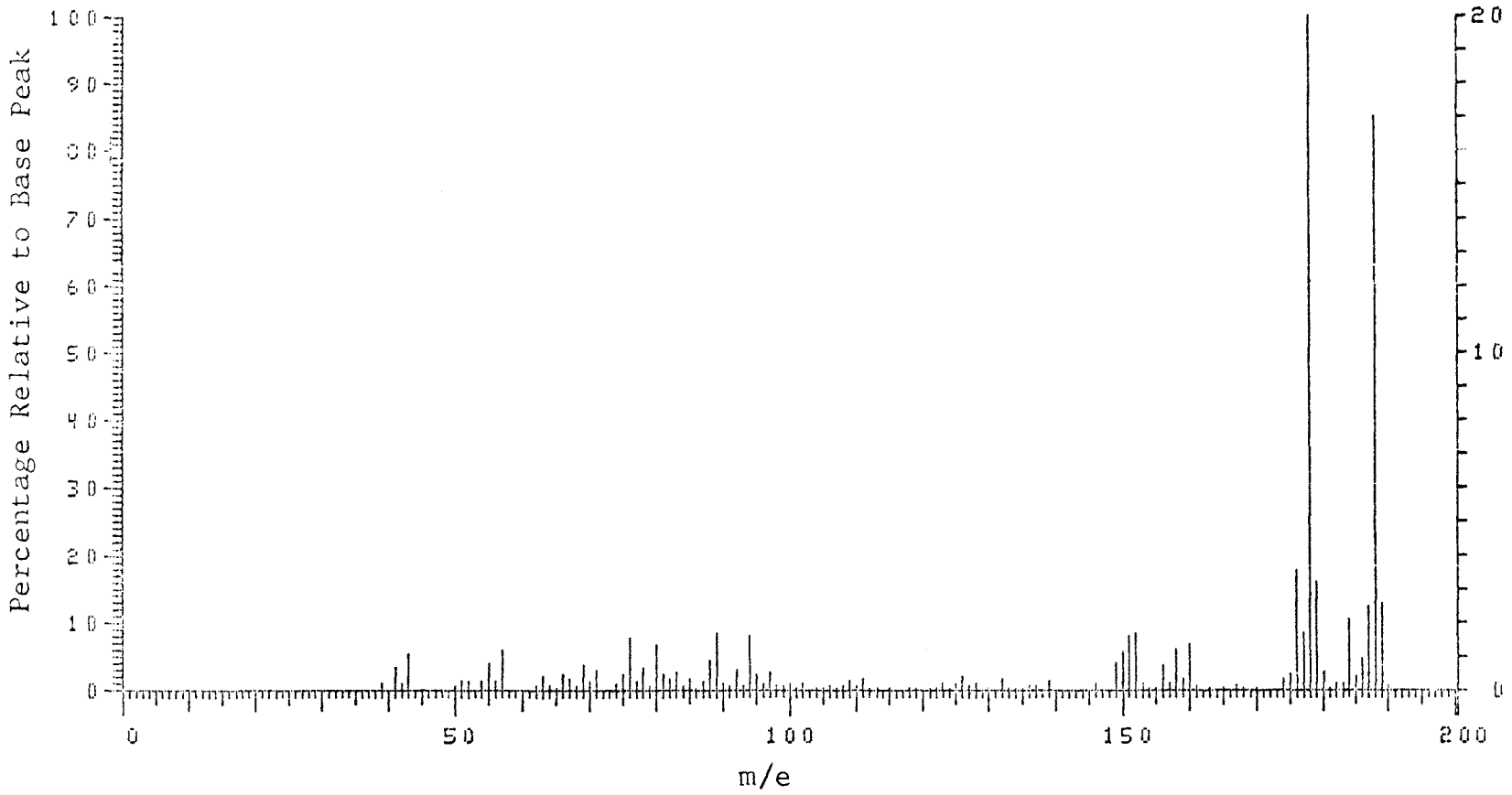
Mass Spectrogram III

Nora Fly Ash: One Day Incubation, Run 3



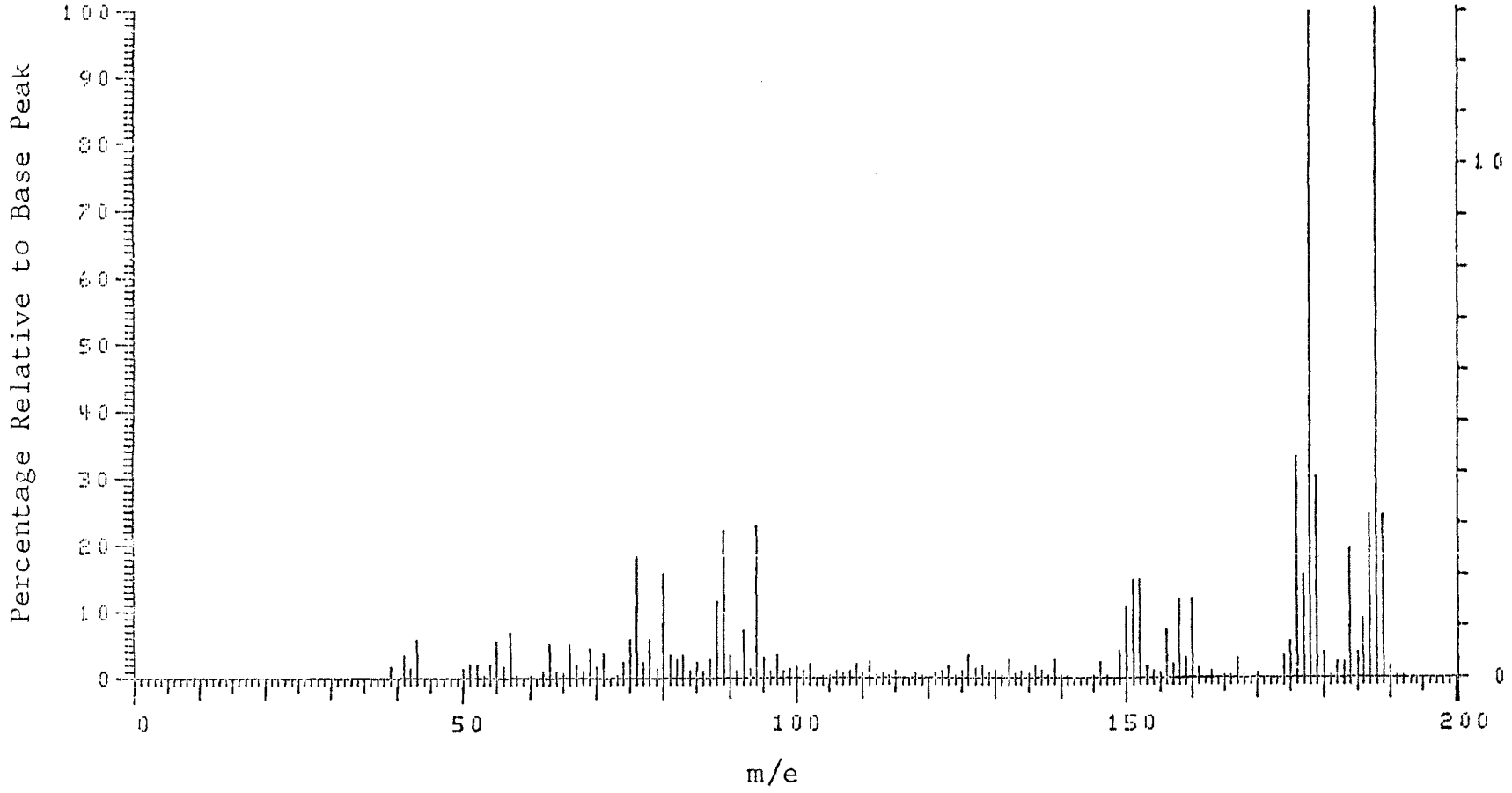
Mass Spectrogram IV

Nora Fly Ash: Five Days Incubation, Run 1



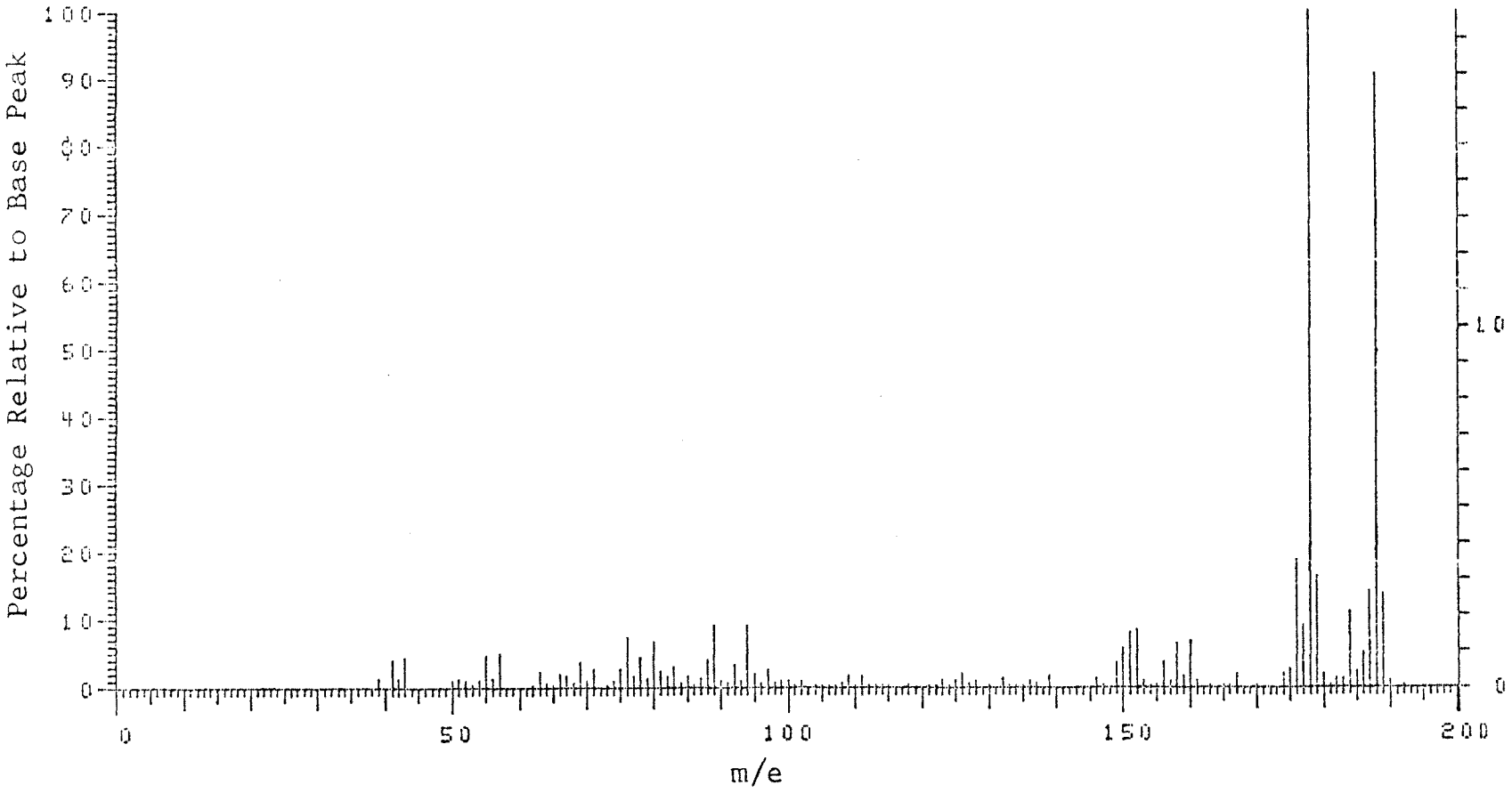
Mass Spectrogram V

Badger Fly Ash: One Day Incubation, Run 1



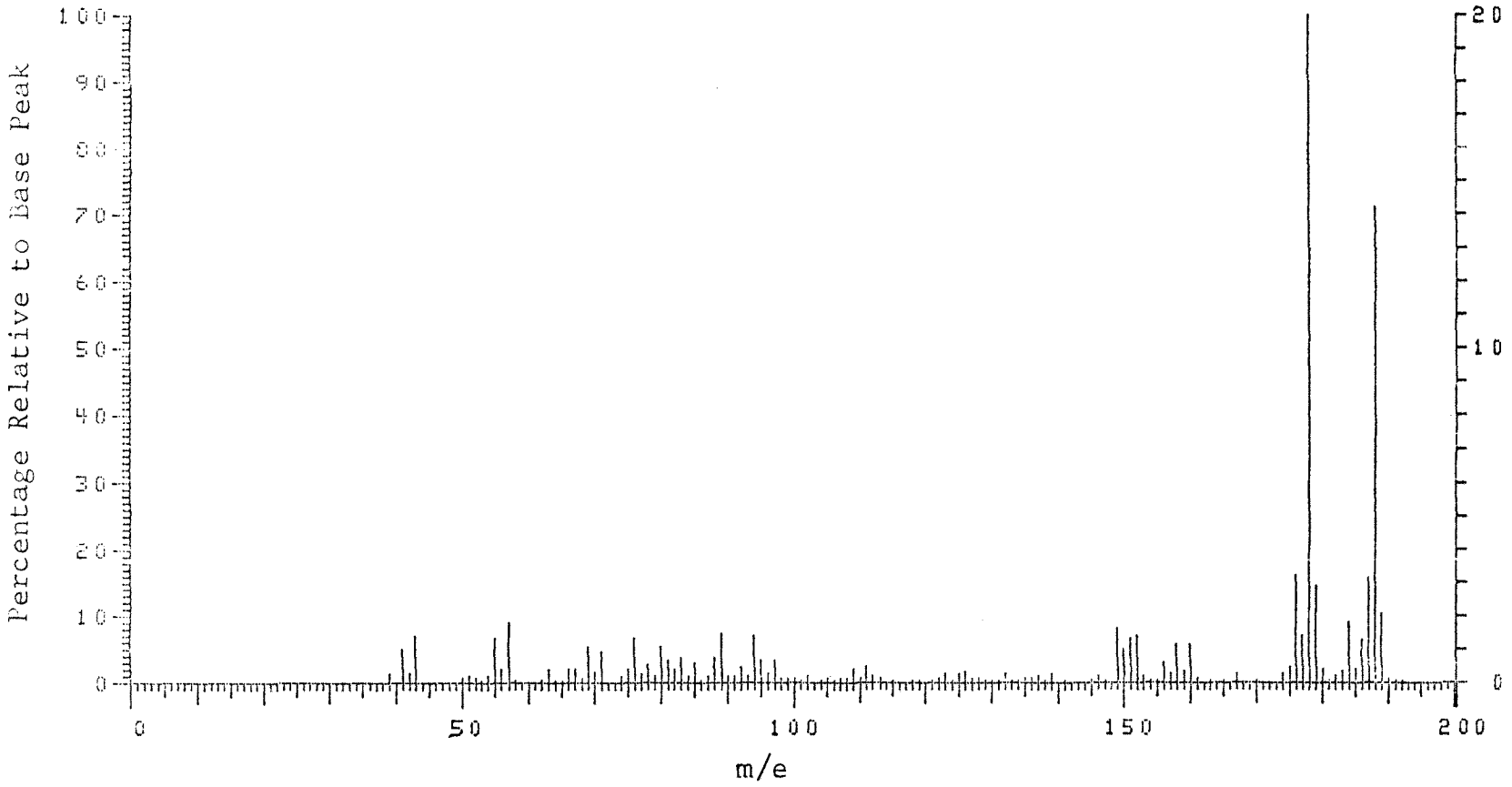
Mass Spectrogram VI

Badger Fly Ash: One Day Incubation, Run 2



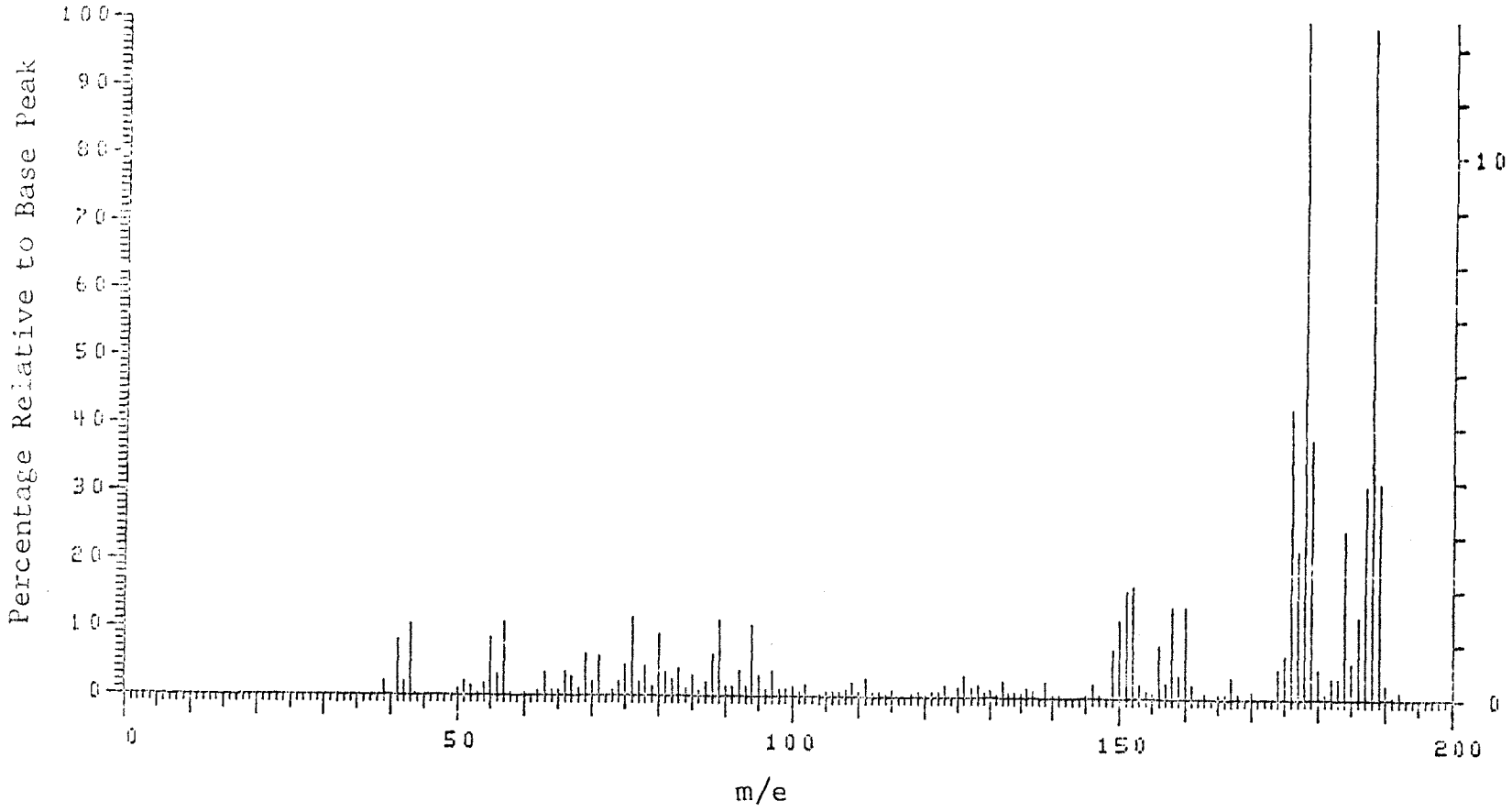
Mass Spectrogram VII

Badger Fly Ash: One Day Incubation, Run 3



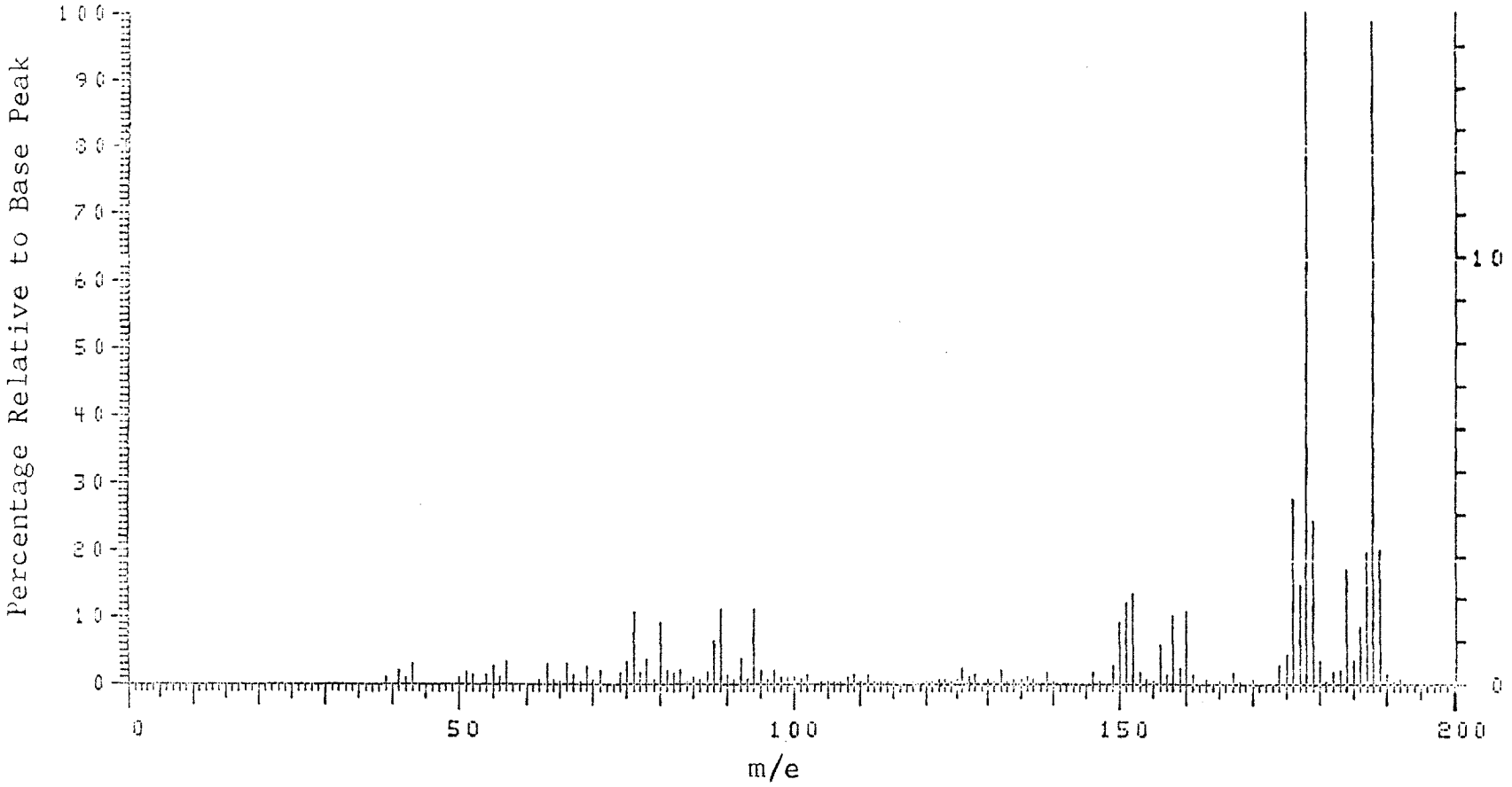
Mass Spectrogram VIII

Badger Fly Ash: Five Days Incubation, Run 1



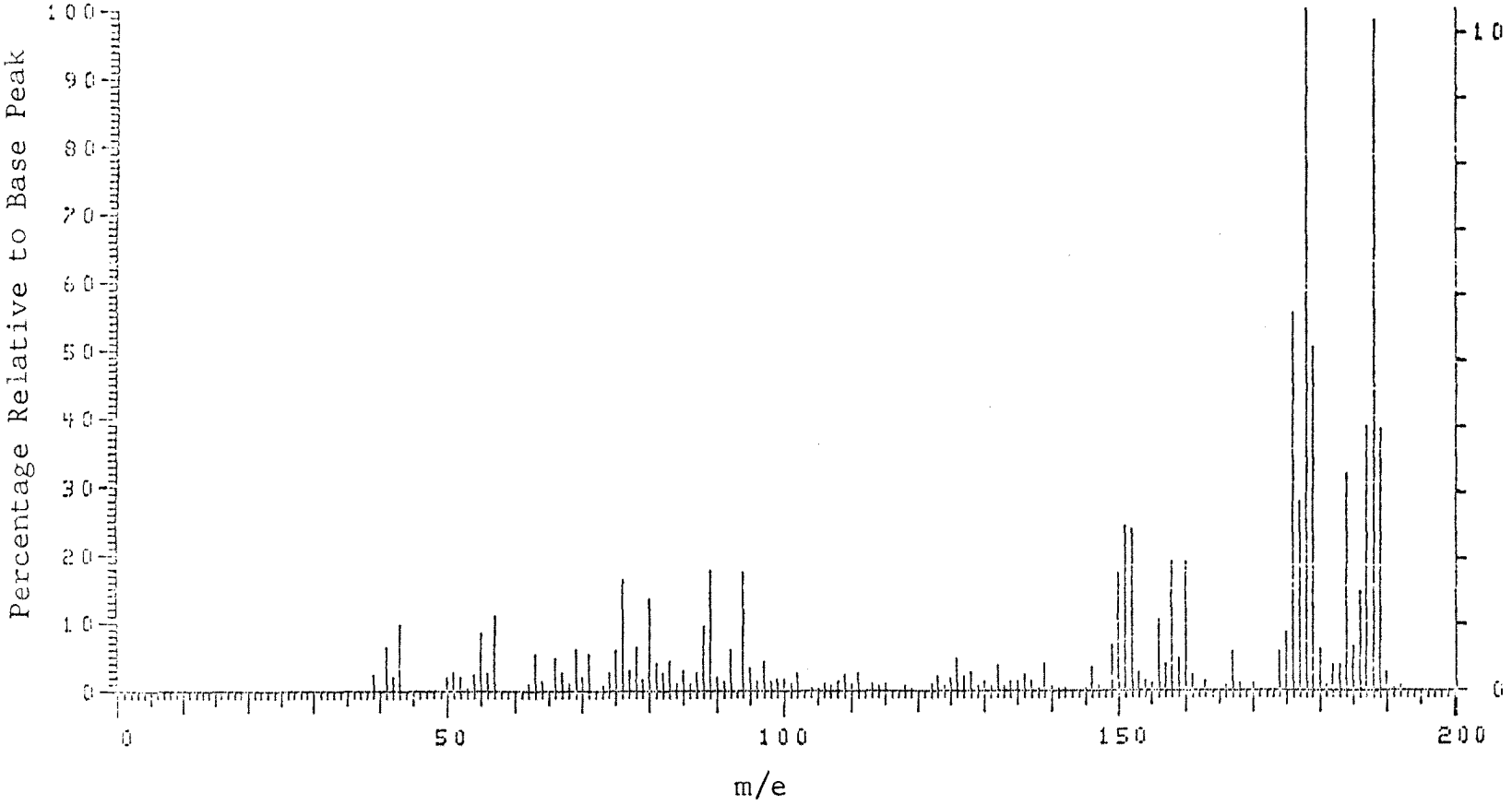
Mass Spectrogram IX

Badger Fly Ash: Seven Days Incubation, Run 1



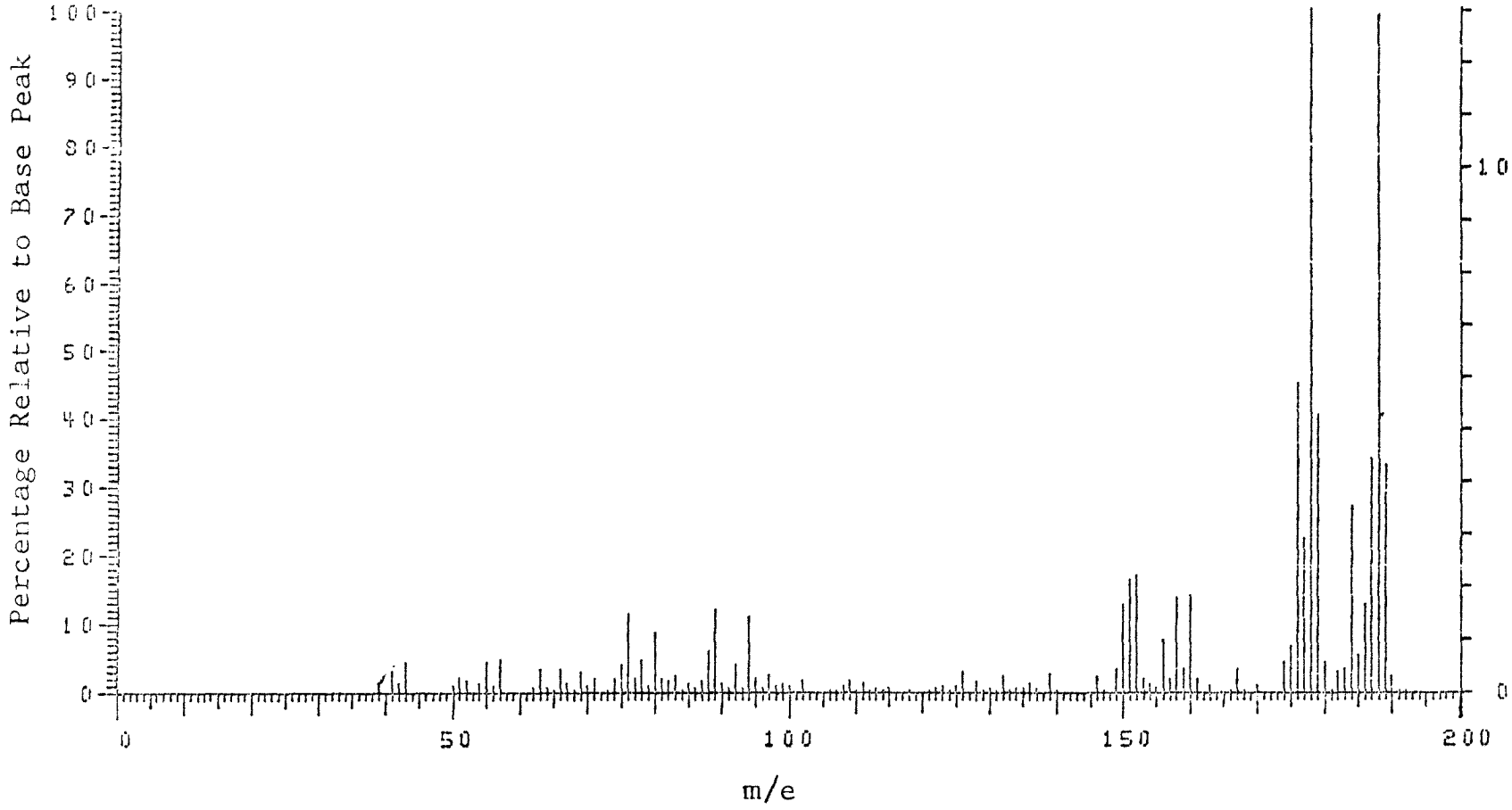
Mass Spectrogram X

Upshur Fly Ash: Five Days Incubation, Run 1



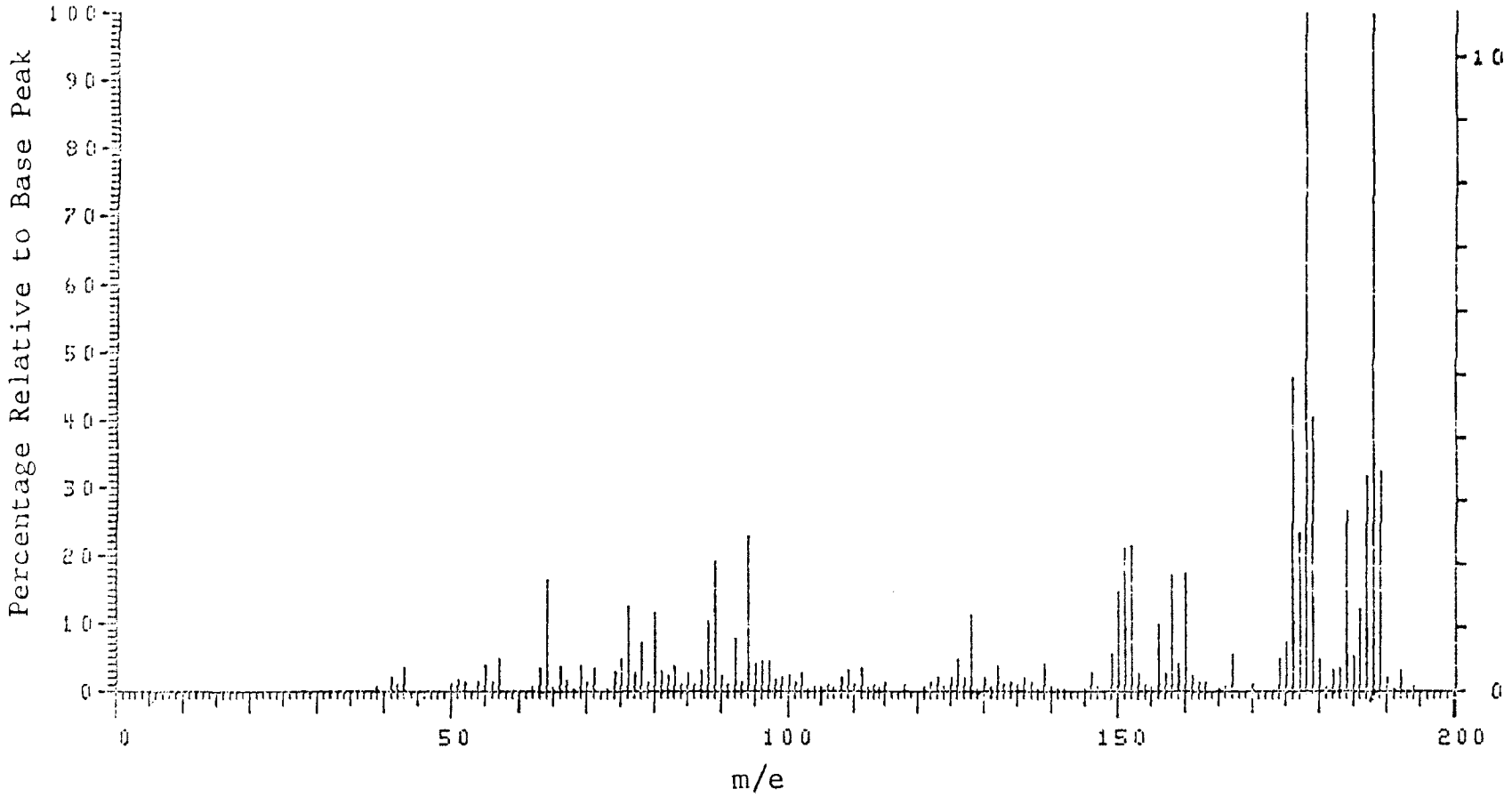
Mass Spectrogram XI

Upshur Fly Ash: Heat Pretreatment,
One Day Incubation, Run 1



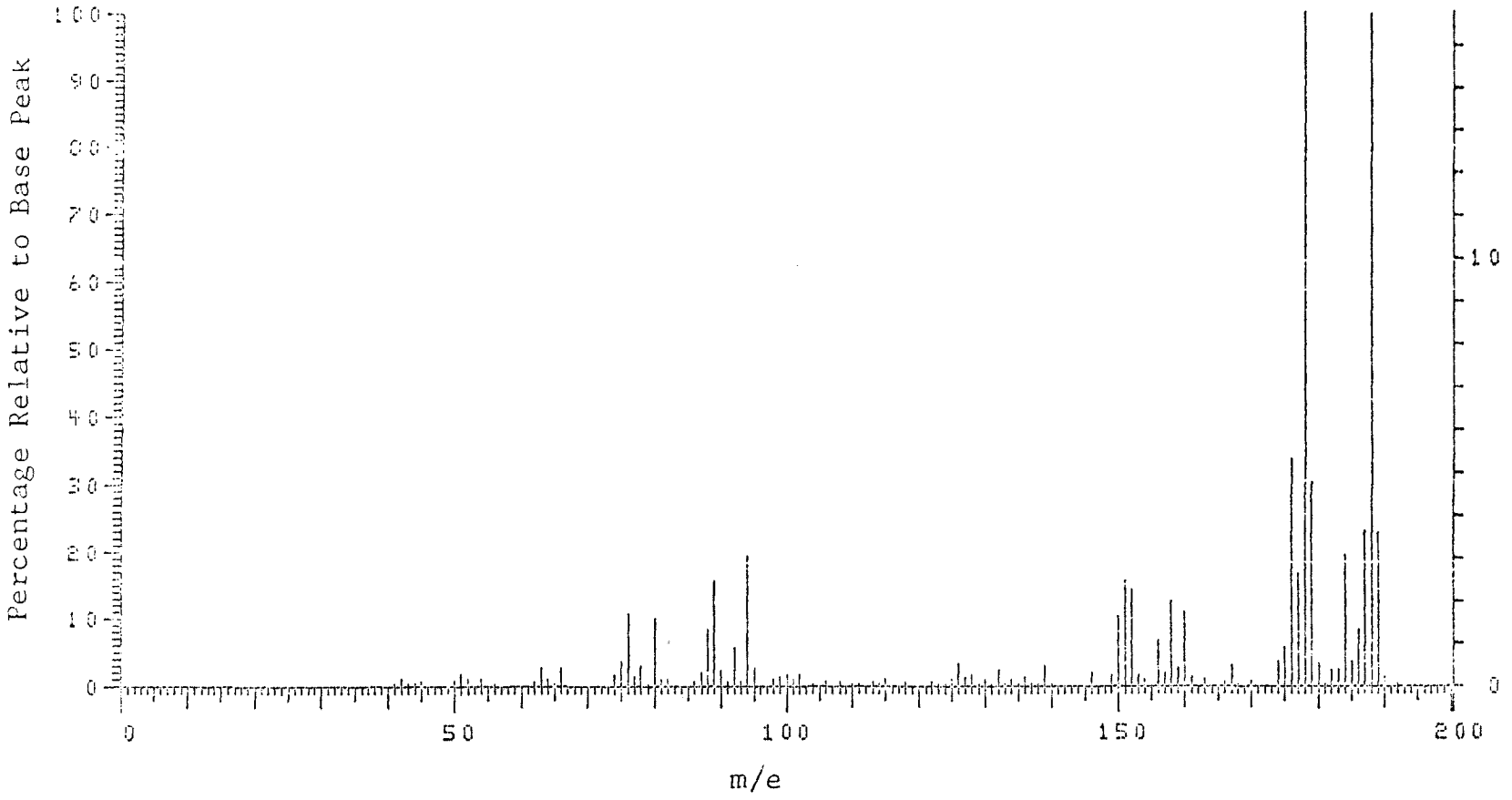
Mass Spectrogram XII

Upshur Fly Ash: Methanol Pretreatment,
One Day Incubation, Run 1



Mass Spectrogram XIII

Control Sample



## APPENDIX B

This appendix consists of eight figures. The figures are sections of the chromatograms obtained during our investigation of cyclopenta[cd]pyrene (CcdP) and benzo[ghi]-fluoranthene (BghiF) in the tunnel automobile exhaust condensates. Figure XVIII is the standard of CcdP and BghiF and Figures XIX-XV are the tunnel samples.

Figure XVIII

Standard of CcdP and BghiF

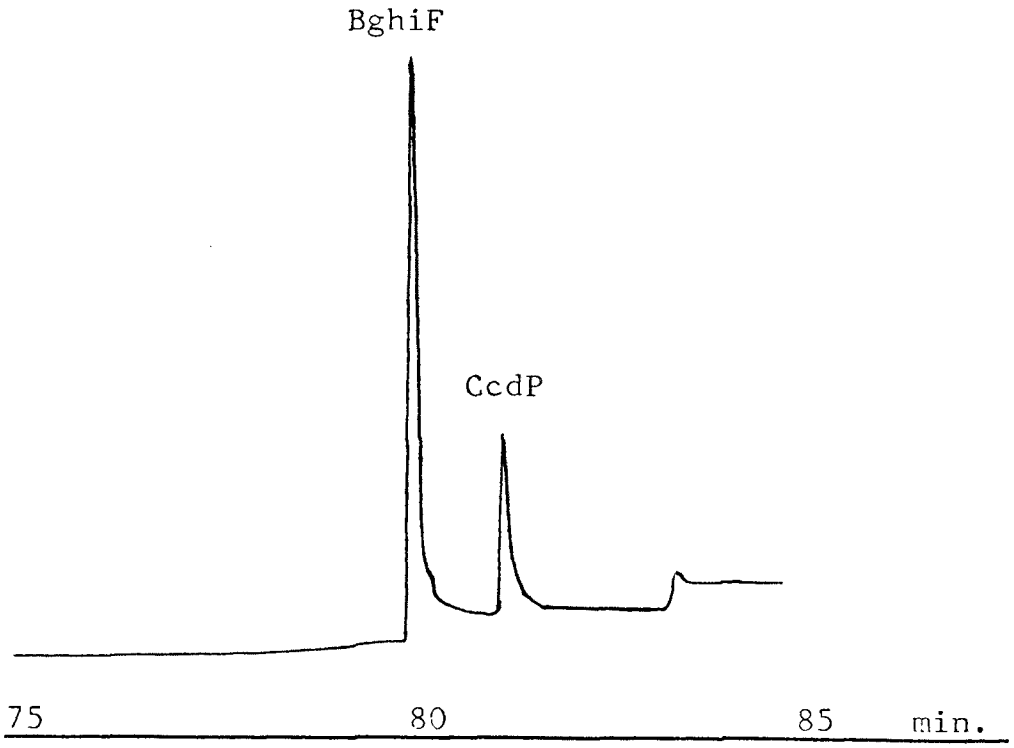


Figure XIX

Lincoln Tunnel Sample 11/24/81, 11:53am-12:38pm

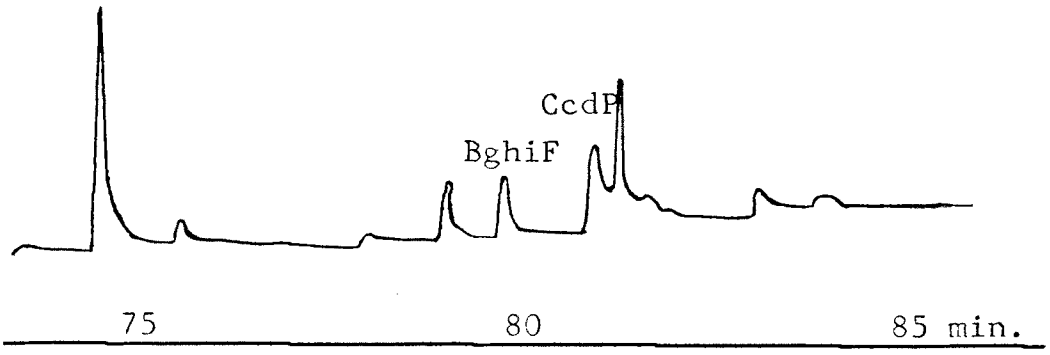


Figure XX

Lincoln Tunnel Sample 11/24/81, 4:08pm-4:40pm

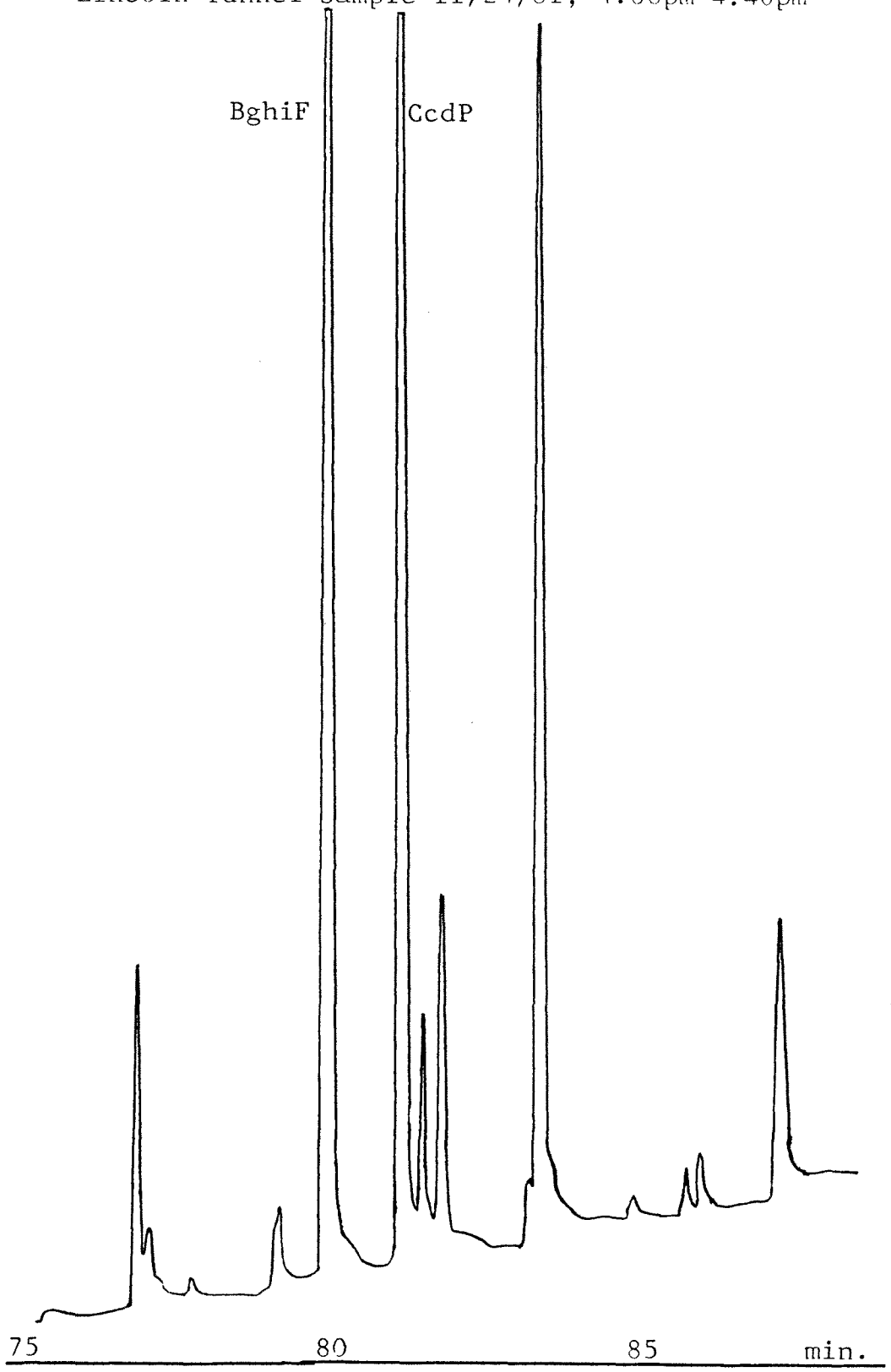


Figure XXI

Lincoln Tunnel Sample 11/25/81, 10:30am-11:03am

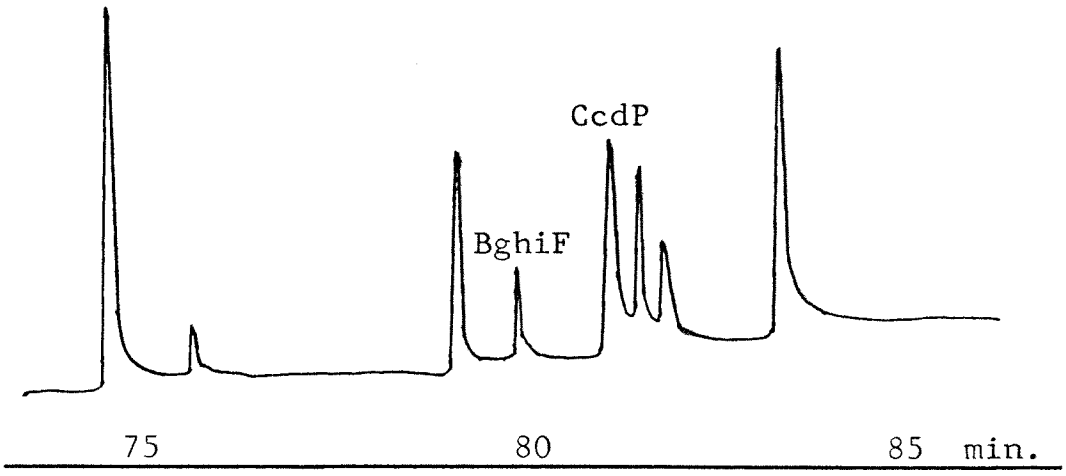


Figure XXII

Lincoln Tunnel Sample 11/25/81,
1:52pm-2:24pm

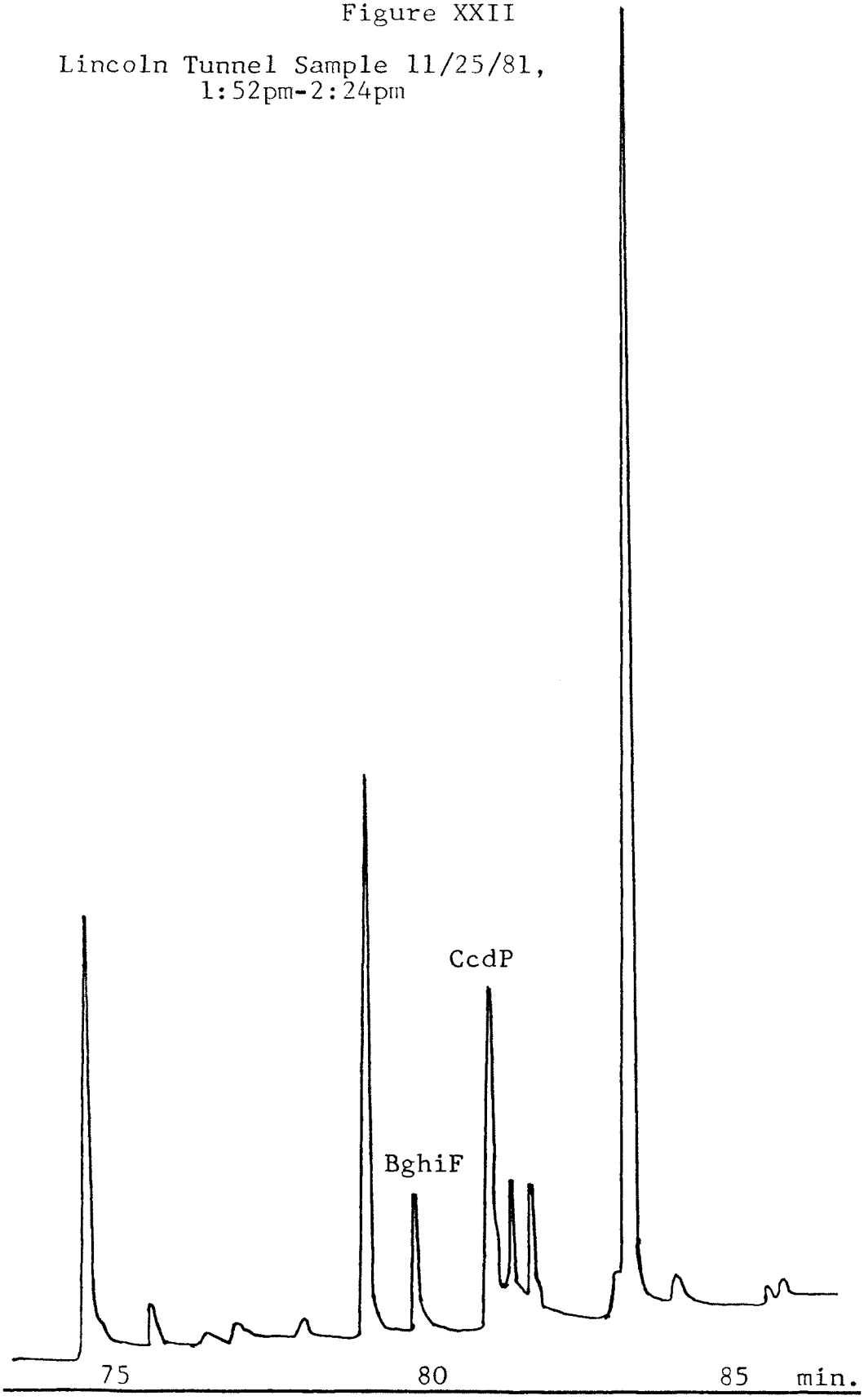


Figure XXIII

Holland Tunnel Sample 11/24/81,
3:10pm-3:45pm

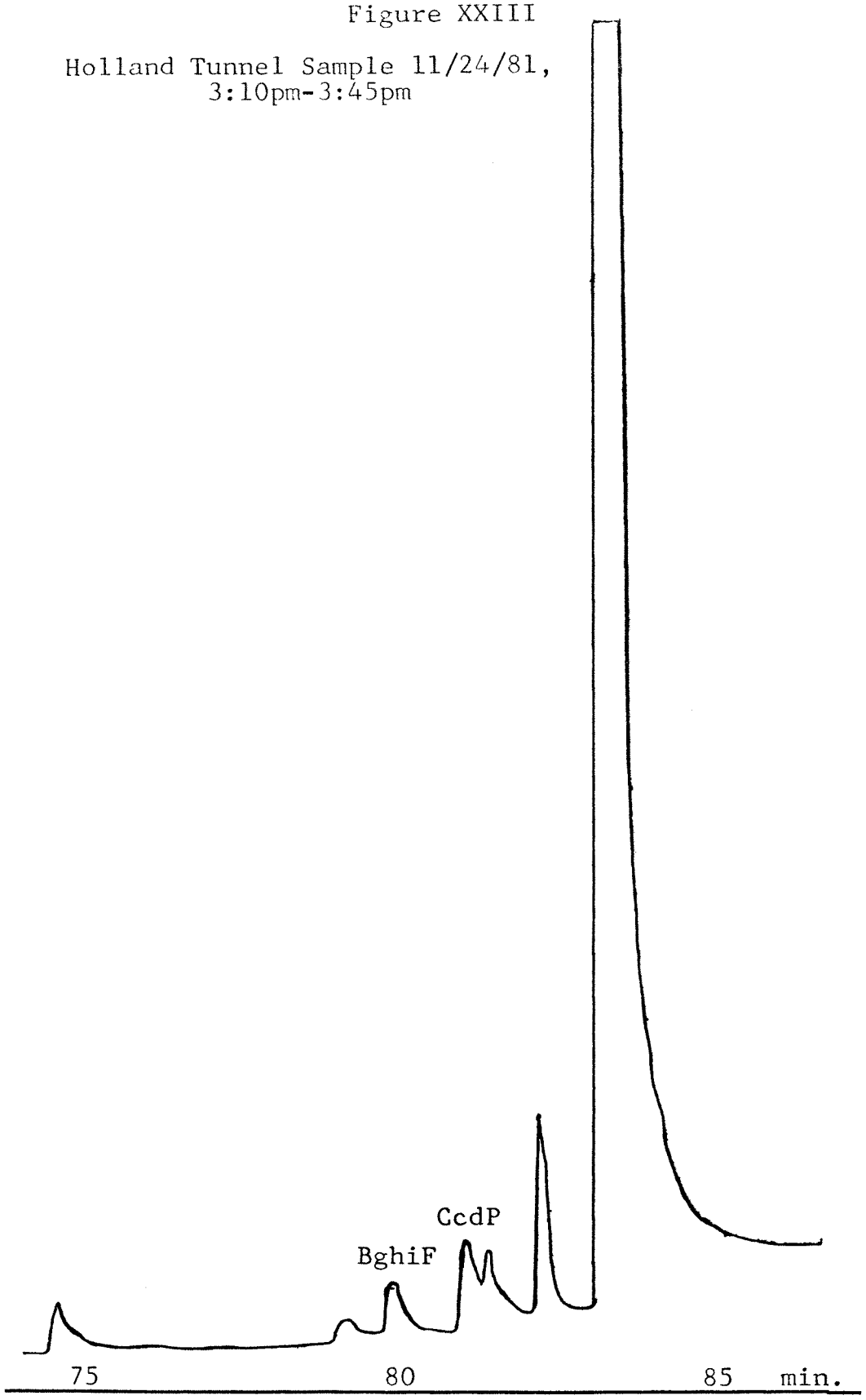


Figure XXIV

Holland Tunnel Sample 11/25/81, 12:05pm-12:45pm

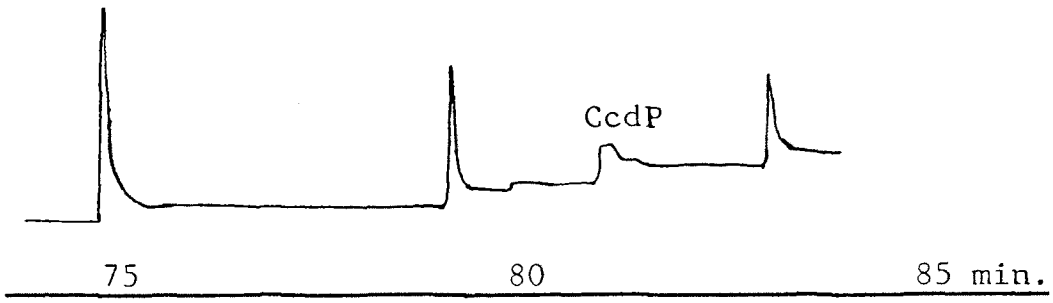
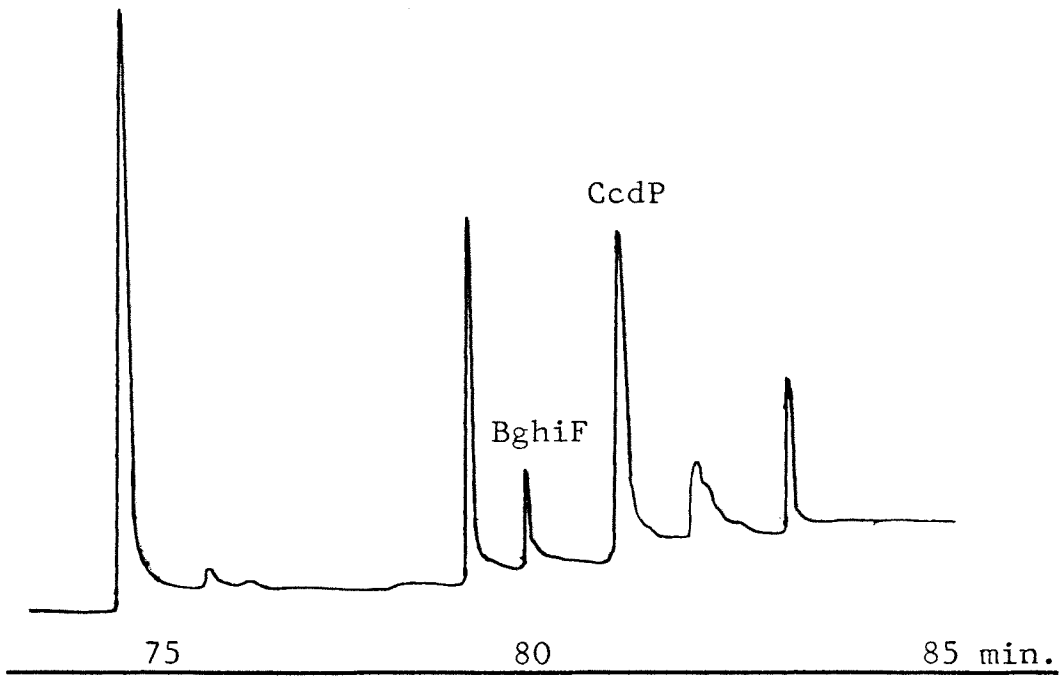


Figure XXV

Holland Tunnel Sample 11/25/81, 12:50pm-1:35pm



## REFERENCES


1. Davidson, R. J., Natusch, D. F. S., Wallace, J. R., and Evans, C. A., Jr., "Trace Elements in Fly Ash," Environmental Science and Technology, vol. 8, December, 1974, pp. 1107-1113.

2. Jager, J., "Behavior of Polycyclic Aromatic Hydrocarbons Adsorbed on Solid Carriers. Part I. Extraction of Polycyclic Aromatic Hydrocarbons from Solid Carriers," Ceskoslav. Hyg., vol. 14, 1969, pp. 135-142.

3. Griest, W. H., Yeatts, L. B., Jr., and Caton, J. E., "Recovery of Polycyclic Aromatic Hydrocarbons Sorbed on Fly Ash for Quantitative Determination," Analytical Chemistry, vol. 52, January, 1980, pp. 199-201.

4. Griest, W. H., Caton, J. E., Guerin, M. R., Yeatts, L. B., Jr., and Higgins, C. E., "Extraction and Recovery of Polycyclic Aromatic Hydrocarbons from Highly Sorptive Matrices Such as Fly Ash," Polynuclear Aromatic Hydrocarbons, Ann-Arbor Science Publishers, 1980, pp. 819-828.

5. Griest, W. H., and Caton, J. E., "Studies of the Interaction of Polycyclic Aromatic Hydrocarbons with Fly Ash," Chemical, Analytical, and Biological Fate: Polynuclear Aromatic Hydrocarbons, International Symposium, 5th 1980, Batelle Press, 1981, pp. 719-730.

6. Watabe, T., Ishizuka, T., Isohoe, M., and Ozawa, N., "7-Hydroxymethyl Sulfate Ester as an Active Metabolite of 7,12-Dimethylbenz[a]anthracene," Science, vol. 215, January, 1982, pp. 403-405.

7. Korfmacher, W. A., Natusch, D. F. S., Taylor, D. R., Mamantov, G., and Wehry, E. L., "Oxidative Transformations of Polycyclic Aromatic Hydrocarbons Adsorbed on Coal Fly Ash," Science, vol. 207, February, 1980.

8. Cavalieri, E., Rogan, E., Toth, B., and Munhall, A., "Carcinogenicity of the Environmental Pollutants Cyclopenteno[cd]pyrene and Cyclopentano[cd]pyrene in Mouse Skin," Carcinogenesis, vol. 2, No. 4, 1981, pp. 277-281.

9. Grimmer, G., "Analysis of Automobile Exhaust Condensates," Air Pollution and Cancer in Man, 1977, pp. 29-39.

10. Grimmer, G., Naujack, K. W., and Schneider, D., "Changes of PAH-Profiles in Different Areas of a City During The Year," Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Batelle Press, 1980, pp. 107-125.

11. Cavalieri, E., Munhall, A., Rogan, E., Salmasi, S., and Patil, K., "Syncarcinogenic Effect of the Environmental Pollutants Cyclopenteno[cd]pyrene and Benzo[a]pyrene in Mouse Skin," Carcinogenesis, vol. 4, No. 4, 1983, pp. 393-397.

12. Grimmer, G., Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons: Chemistry, Occurence, Biochemistry, Carcinogenicity, CRC Press, Inc., 1983, pp. 20-21.

13. Kebbekus, B., Greenberg, A., Horgan, L., Bozzelli, J., Darack, F., Eveleens, C., and Strangeland, L., "Concentrations of Selected Vapor and Particulate-Phase Substances in the Lincoln and Holland Tunnels," Journal of the Air Pollution Control Association, vol. 33, April, 1983, pp. 328-330.

14. Alsberg, T., Rannug, U., and Sundvall, A., "Evaluation of Extraction Methods For Carbon Black; POM Analysis and Mutagenicity Assay," Polynuclear Aromatic Hydrocarbons Physical and Biological Chemistry, Batelle Press, 1982, pp. 73-82.

15. Novotny, M., and Farlow, R., "A Simple Method for Concentrating Dilute High-Boiling Samples for Capillary Gas Chromatography," Journal of Chromatogrphy, vol. 103, 1975, pp. 1-6.

16. Grimmer, G., Bohnke, H., and Hidebrandt, A., "Packed High-Performance GC-Columns (about 50000 HETP) for Profile Analysis of Carcinogenic Polycyclic Aromatic Hydrocarbons in Foods, Mineral Oil Products, Vehicle Exhaust- and Cigarette Smoke Condensates etc.," Fresenius Zeitschrift Fuer Analytische Chemie, vol. 279, 1976, pp. 139-140.

17. Nikolaou, K., Masclet, P., and Mouvier, G., "Sources and Chemical Reactivity of Polynuclear Aromatic Hydrocarbons in the Atmosphere- A Critical Review," The Science of the Total Environment, vol. 32, 1984, pp. 103-132.

18. Polycyclic Aromatic Hydrocarbons: Evaluation on Sources and Effects, Committee on Pyrene and Selected Analogues, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, and National Research Council, National Academy Press,

Washington D.C., 1983, p. 3-30.

19. Brune, H. F. K., "Experimental Results With Percutaneous Applications of Automobile Exhaust Condensates in Mice," Air Pollution and Cancer in Man, 1977, pp. 41-47.

20. Butler, J. D., and Crossley, P., "Reactivity of Polycyclic Aromatic Hydrocarbons Adsorbed on Soot Particles," Atmospheric Environment, vol. 15, 1981, pp. 91-94.

21. Dong, M., Locke, D. C., and Ferrand, E., "High Pressure Liquid Chromatographic Methods for Routine Analysis of Major Polycyclic Aromatic Hydrocarbons in Suspended Air Particulate Matter," Analytical Chemistry, vol. 48, February, 1976, pp. 368-371.

22. Eisenberg, W. C., "Fractionation of Organic Material Extracted from Suspended Air Particulate Matter Using High Pressure Liquid Chromatography," Journal of Chromatographic Science, vol. 16, April, 1978, pp. 145-151.

23. Fox, M. A., and Staley, S. W., "Determination of Polycyclic Aromatic Hydrocarbons in Atmospheric Particulate Matter by High Pressure Liquid Chromatography Coupled with Fluorescence Techniques," Analytical Chemistry, vol. 48, June, 1976, pp. 992-998.

24. Giger, W., and Schaffner, C., "Determination of Polycyclic Aromatic Hydrocabons in the Environment by Glass Capillary Gas Chromatography," Analytical Chemistry, vol. 50, February, 1978, pp. 243-249.

25. Greenberg, A., Yokoyama, R., Giorgio, P., and Cannova, F., "Analysis of Polynuclear Aromatic Hydrocarbons on the Airborne Particulates of Urban New Jersey," Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Batelle Press, 1980, pp. 193-198.

26. Lee, M. L., Novotny, M., Bartle, K. D., "Gas Chromatography/Mass Spectrometric and Nuclear Magnetic Resonance Determination of Polycyclic Aromatic Hydrocarbons in Airborne Particulates," Analytical Chemistry, vol. 48, September, 1976, pp. 1566-1572.

27. Lee, M. L., and Hites, R. A., "Characterization of Sulfur Containing Polycyclic Aromatic Hydrocarbons in Carbon Blacks," Analytical Chemistry, vol. 48, November, 1976, pp. 1890-1893.

28. Boyland, E., and Sims, P., "Metabolism of Polycyclic Compounds," Biochemistry Journal, vol. 95, 1965, pp. 780-787.

29. Flesher, J. W., and Sydnor, K. L., "Carcinogenicity of Derivatives of 7,12-Dimethylbenz[a]anthracene," Cancer Research, vol. 31, December, 1971, pp.1951-1954.

30. Cavalieri, E., Roth, R., and Rogan, E., "Hydroxylation and Conjugation at the Benzylic Carbon Atom: A Possible Mechanism of Carcinogenic Activation For Some Methyl-Substituted Aromatic Hydrocarbons," Polynuclear Aromatic Hydrocarbons, Ann-Arbor Science Publishers, 1979, pp. 517-527.

31. Fox, M. A., and Olive, S., "Photooxidation of Anthracene on Atmospheric Particulate Matter," Science, vol. 205, August, 1979, pp. 582-583.

32. Kong, R. C., Lee, M. L., Tominaga, Y., Pratap, R., Iwao, M., and Castle, R. N., "Mesogenic Polysiloxane Stationary Phase for High-Resolution Gas Chromatography of Isomeric Polycyclic Aromatic Hydrocarbons," Analytical Chemistry, vol. 54, September, 1982, pp. 1802-1806.

33. Korfmacher, W. A., Wehry, E. L., Mamantov, G., and Natusch, D. F. S., "Resistance to Photochemical Decomposition of Polycyclic Aromatic Hydrocarbons Vapor-Adsorbed on Coal Fly Ash," Environmental Science and Technology, vol. 14, September, 1980, pp. 1094-1099.

34. Lee, M. L., Vassilaros, D. L., White, C. M., and Novotny, M., "Retention Indices for Programmed-Temperature Capillary-Column Gas Chromatography of Polycyclic Aromatic Hydrocarbons," Analytical Chemistry, vol. 51, May, 1979, pp. 768-773.

35. Lee, M. L., and Wright, B. W., "Capillary Column Gas Chromatography of Polycyclic Aromatic Compounds: A Review," Journal of Chromatographic Science, vol. 18, August, 1980, pp. 345-358.

36. Maskarinec, M. P., and Olerich, G., "Thermally Stable Dexsil-400 Glass Capillary Columns," Analytical Chemistry, vol. 52, March, 1980, pp. 588-591.

37. Natusch, D. F. S., and Tomkins, B. A., "Theoretical Considerations of the Adsorption of Polynuclear Aromatic Hydrocarbon Vapor onto Fly Ash in a Coal-Fired Power Plant," Carcinogenesis, Vol. 3:

Polynuclear Aromatic Hydrocarbons, Raven Press, 1978, pp. 145-153.

38. Novotny, M., Lee, M. L., and Bartle, K. D., "The Methods for Fractionation, Analytical Separation and Identification of Polynuclear Aromatic Hydrocarbons in Complex Mixtures," Journal of Chromatographic Science, vol. 12, October, 1974, pp.606-612.

39. Weisberger, J. H., Yamamoto, R. S., William, G. M., Grantham, P. H., Matsushima, T., and Weisberger, E. K., "On the Sulfate Ester of N-Hydroxy-N-2-fluoranylacetamide as a Key Ultimate Hepatocarcinogen in the Rat," Cancer Research, vol. 32, March, 1972, pp. 491-499.

40. Zlatkis, A., Wang, F. -S., and Shanfield, H., "Trace Gas Chromatographic Analysis by Use of Large Sample On-Column Injection with Bonded Phase Capillary Columns," Analytical Chemistry, vol. 54, December, 1982, pp. 2466-2469.