

POLYCYCLIC AROMATIC HYDROCARBONS IN STILL CREEK SEDIMENTS:
DISTRIBUTIONS, CONCENTRATIONS AND POSSIBLE SOURCES

By

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ABSTRACT

The extent of polycyclic aromatic hydrocarbon (PAH) contamination was determined in the stream sediments of an urban watershed, and some possible sources were investigated.

Analytical methodology was adapted from the literature for determination of polycyclic aromatic hydrocarbons in sediments. Alkaline digestion of samples was followed by solvent-solvent partition and Florisil chromatography. An alumina column clean-up effected preliminary separation of compounds before determination using high pressure liquid chromatography (HPLC). Some extracts were also analyzed on a capillary gas chromatography system and by capillary gas chromatography-mass spectrometry (GCMS) to provide additional qualitative information as a result of the much improved resolution.

Samples of stream sediments were obtained from Still Creek in the Brunette River watershed. Analysis for PAH in sediments from five sampling sites (sampled two consecutive years) revealed that all sediments contained a wide range of PAH. While the profile of the extracts (by HPLC) was remarkably similar from site to site, and from the first to second year, the amounts of PAH present varied by two orders of magnitude among sites. Levels of individual compounds quantitated ranged from mid part per billion (55 ppb) to mid part per million (38.5 ppm).

PAH identified by HPLC analysis (eleven compounds) were mainly non-alkylated parent compounds, but GCMS data indicated that many alkyl-substituted or heteroatom polycyclics were also present.

Preliminary investigations of PAH levels found in oligochaetes

indicated bioconcentrations of some compounds, and suggested a possible competition for PAH among high organic content sediments and the live oligochaetes present.

Investigation of possible sources of polycyclic aromatic hydrocarbons to stream sediments included street surface contaminants and crankcase oil. Street sediments exhibited HPLC elution profiles strikingly similar to those of stream sediments. Levels of PAH found were slightly lower than, but comparable to, concentrations in stream sediments, and the same individual compounds were quantitated in each type of sample. Thus the street sediments were determined to be an important source of PAH to stream sediments, with transport occurring through urban street runoff.

Crankcase oil demonstrated a linear buildup of PAH with mileage over approximately the first 2000 km, then a leveling off occurred. Based on HPLC profiles, used crankcase oil contributes to street surface contamination.

Regression analysis of total PAH levels in various samples failed to provide significant correlations with location of sampling point (on stream), traffic volume at sampling point, land use (for street sediment samples) or sediment characteristics. However, sediment characteristics (particle size, organic content) were identified as being the most important of the factors considered.

Implications of the presence of a PAH burden in the stream sediments (comparable to other older, larger urban watersheds) were discussed with relation to natural systems. Recommendations were made for study to further elucidate PAH sources and transport to stream sediments.

Research Supervisor

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TABLE OF CONTENTS

	<u>Page</u>
Abstract.	ii
Acknowledgements.	iv
Table of Contents	v
List of Figures	vii
List of Tables.	xi
Chapter I - Introduction, Purpose and Scope of Thesis	1
A. Introduction.	1
B. Purpose and Scope of Thesis	2
Chapter II - Literature Review.	6
A. Physical and Chemical Properties of PAH	6
B. PAH in the Environment.	7
1. Sources to the Environment	7
2. PAH in Air and Water	9
3. PAH in Food.	17
C. PAH in Sediments.	21
1. Determination of PAH Sources	21
2. Natural PAH Levels	23
3. Point Sources.	28
4. Non-Point Sources.	29
5. Summary.	36
D. Assessment of PAH Exposure.	37
E. Analysis of PAH	43
1. Introduction	43
2. Extraction Procedures.	43
3. Partition and Clean-up	45
4. High Pressure Liquid Chromatography (HPLC)	47
5. Gas Chromatography (GC).	56
6. Gas Chromatography Mass Spectrometry (GCMS).	62
Chapter III - Experimental Techniques	66
A. Outline of Analytical System.	66
1. Scope.	66
2. Analytical Scheme.	66
3. Instrumental Analysis.	67
4. Data Analysis.	68
B. Sampling.	68
1. Sediments.	68
2. Street Surface Contaminants.	68
3. Oligochaetes	68
4. Crankcase Oil.	71

	<u>Page</u>
C. General Procedures.	71
1. Glassware.	71
2. Solvents	71
3. Other Chemicals and Supplies	72
4. Determination of Particle Size and Organic Content	72
5. Standards.	73
D. Extraction and Clean-up Procedures.	73
1. Sediments and Street Surface Contaminants.	73
2. Oligochaetes	77
3. Crankcase Oil.	77
E. Recovery Studies.	77
F. Instrumental Analysis	79
1. Liquid Scintillation Counting.	79
2. HPLC	79
3. UV Spectrophotometry	79
4. GC	81
5. GCMSDS	81
G. Statistics Treatment.	82
Chapter IV - Results and Discussion	84
A. Development of Analytical Scheme.	84
1. Introduction	84
2. Choice of Target Standards	84
3. Choice of Analytical Method.	85
4. Development of LC System	86
5. Recoveries	90
6. Sampling Rationale	93
7. Comments on Analytical Methodology	96
B. Analysis of Stream Sediments.	98
1. Sediment Characteristics	98
2. HPLC Analysis.	99
3. Capillary GC Analysis.	113
4. GCMS Analysis.	127
5. Statistics Treatment	142
6. Summary.	145
C. Street Sediments.	152
1. Sediment Characteristics	152
2. HPLC Analysis.	155
3. Statistics Treatment	155
4. Discussion	165
D. Oligochaetes.	167
E. Crankcase Oil	169
F. Summary of Results: Sources and Fates of PAH.	175
Chapter V - Summation, Implications and Recommendations	178
A. Summation	178
B. Implications.	180
C. Recommendations	180
References.	182

LIST OF FIGURES

		<u>Page</u>
Figure 1-1	Possible Pathways for Movement of PAH in an Urban Watershed	3
1-2	Relationships Investigated in this Thesis	5
3-1	Sampling Locations	69
3-2	Analysis Scheme	74
3-3	Alumina Separation of Polycyclic Extract	76
3-4	Aliquots Taken for Liquid Scintillation Counting	78
4-1	Linearity of UV Detector, 280 nm	89
4-2	Naphthalene Recoveries, 3 Trials	92
4-3	B(a)P Recoveries - No Alumina Preparation Procedures, 3 Trials	92
4-4	B(a)P Recoveries - Alumina Deactivation Problems, 3 Trials	94
4-5	Particle Size Distribution and Organic Matter, D(S)('78) and D(N)('79)	101
4-6	Particle Size Distribution and Organic Matter, D(N)('78) and D(N)('79)	101
4-7	Particle Size Distribution and Organic Matter, L('78) and L('79)	102
4-8	Particle Size Distribution and Organic Matter, W('78) and L('79)	102
4-9	Particle Size Distribution and Organic Matter, G('78) and G('79)	103
4-10	% Organic Matter (OM) vs. % Silt and Clay, (d)	106
4-11	Chromatogram of Douglas (N) Extract (a) ('78) III (b) ('79) III	107
4-12	Chromatogram of Willingdon Extract (a) ('78) III (b) ('79) III	

		<u>Page</u>
4-13	Chromatogram of Douglas (N) Extract (a) ('78) IV (b) ('79) IV	108
4-14	Chromatogram of Lougheed Extract (a) ('78) IV (b) ('79) IV	109
4-15	Chromatogram of Douglas (N) Extract (a) ('78) V (b) ('79) V	110
4-16	Chromatogram of Lougheed Extract (a) ('78) V (b) ('79) V	111
4-17	Lougheed Extract at Two Mobile Phase Concentra- tions (a) 60%, L('79) III (b) 70%, L('79) III	112
4-18	Capillary GC Chromatogram, D(S)('79) (a) III 2 μ l (b) III 0.2 μ l (c) IV (d) V	121
4-19	Capillary GC Chromatogram, D(S)('78) (a) III (b) IV (c) V	123
4-20	Capillary GC Chromatogram, W('78) (a) III (b) IV (c) V	125
4-21	GCMS of D(S)('78) III (a) TI chromatogram (b) TI chromatogram, scan #1080 to #1440 (c) spectrum, scan #1406 (d) TI chromatogram, scan #1800 to #2060 (e) spectrum, scan #1818	133
4-22	GCMS of D(S)('79) III (a) TI chromatogram (b) screen for masses 178, 202, 228, 166, 216 and 279	136

		<u>Page</u>
4-23	GCMS of D(S)('79) IV (a) TI chromatogram (b) TI chromatogram, scan #920 to #1160 (c) spectrum, scan #955 (d) TI chromatogram, scan #1200 to #1440 (e) spectrum, scan #1308	138
4-24	GCMS of D(S)('79) V (a) TI chromatogram	141
4-25	Distribution of Levels of Individual PAH in Sediments	146
4-26	Distribution of Levels of Equivalent B(a)P in Sediments	146
4-27	Particle Size Distribution and Organic Matter, C ₁ and C ₂	153
4-28	Particle Size Distribution and Organic Matter, Gr ₁ and Gr ₂	153
4-29	Particle Size Distribution and Organic Matter, I ₄ and I ₅	154
4-30	Particle Size Distribution and Organic Matter, R ₁ and R ₂	154
4-31	HPLC Analysis of Street Sediment R ₂ (a) III (b) IV (c) V	157
4-32	HPLC Analysis of C ₂ (a) III (b) IV (c) V	159
4-33	HPLC Analysis of I ₄ (a) III (b) IV (c) V	161
4-34	HPLC Analysis of Gr ₂ (a) III (b) IV (c) V	163

	<u>Page</u>
4-35 Crankcase Oil Sample DD	170
(a) accumulated distance 874 km	
(b) accumulated distance 1665 km	
(c) accumulated distance 2623 km	
4-36 Crankcase Oil Sample RM	172
(a) accumulated distance 895 km	
(b) accumulated distance 1943 km	
(c) accumulated distance 2545 km	
4-37 Eq B(a)P vs accumulated distance - DD	174
4-38 Eq B(a)P vs accumulated distance - RM	174

LIST OF TABLES

		<u>Page</u>
Table 2-1	PAH in Petroleum and Petroleum Products	8
2-2	PAH Levels in Air	10
2-3	PAH in Water	11
2-4	PAH in Aquatic Organisms	13
2-5	PAH in Mussels	15
2-6	PAH in Various Plant Products ($\mu\text{g}/\text{kg}$)	18
2-7	PAH in Field Crops Grown in Controlled Climate, Greenhouse or Open Fields ($\mu\text{g}/\text{kg}$)	18
2-8	PAH in Smoked Foods ($\mu\text{g}/\text{kg}$)	20
2-9	PAH in Sediments ($\mu\text{g}/\text{kg}$)	24
2-10	PAH in Sediments, Boston as a Point Source	33
2-11	Estimation of B(a)P Intake (per person/year) in Hungary	38
2-12	Estimates of B(a)P Intake, From an Occupational Viewpoint	40
2-13	PAH Levels in Foods Used to Calculate Human Exposure Due to Food Consumption	41
2-14	Estimates of Allowable Daily Intake	42
2-15	Column Chromatography Systems	46
2-16	HPLC Systems Used for PAH Analysis	49
2-17	Column Packings Used in GC Analysis of PAH	57
2-18	Capillary Columns Used for PAH Analysis	60
2-19	Examples of GCMS Systems Used for PAH Analysis	63
3-1	Sampling Locations	70
3-2	HPLC Conditions	80
3-3	Capillary GC Conditions	80
4-1	Variation in Retention Time and Peak Area	87

		<u>Page</u>
4-2	Limit of Detection for PAH Standards with UV Detector	91
4-3	Elution Pattern for B(a)P From Alumina	95
4-4	Particle Size Ranges	100
4-5	PAH Quantitated in Stream Sediments	105
4-6	Comparison of Conditions for Determination of PAH Retention Indices	114
4-7	Retention Indices for Target PAH	116
4-8	Tentative Identification of Peaks by Retention Index (I), D(S)('79)	117
4-9	Tentative Identification of Peaks by Retention Index (I), D(S)('78)	118
4-10	Tentative Identification of Peaks by Retention Index (I), D(S)('78)	119
4-11	Scope of GCMS Analysis	128
4-12	Results of GCMS Analysis - D(S)('78) III	130
4-13	Results of GCMS Analysis - D(S)('79)	131
4-14	Values of Variables Examined in Regression Analysis; "eq B(a)P", "km", and "travol"	144
4-15	PAH Levels and Equivalent B(a)P for Street Sediments	156

CHAPTER I

INTRODUCTION, PURPOSE AND SCOPE OF THESIS

A. INTRODUCTION

Throughout this thesis "polycyclic aromatic hydrocarbons" refers to compounds of two or more six membered aromatic rings, and is abbreviated PAH, or shortened as polycyclics. Abbreviations for the many polycyclics are contained, along with their structures, in Appendix 1.

Polycyclics are of concern due to their usefulness as indicators of wide ranging contamination of environments. Many of the PAH are carcinogens, varying widely in their carcinogenic strength (Appendix 1), and may act in combination. Alkylated or hetero-substituted polycyclics are found in conjunction with non-substituted PAH and have potential to be even stronger carcinogens.

Sources of polycyclics to the environment include natural inputs (oil seeps, microbial transformation and perhaps forest fires) and the far greater anthropogenic sources (oil spills, combustion of fuels, production of coke). Emissions of benzo(a)pyrene (B(a)P), a potent carcinogen, were estimated worldwide at over 5000 tons per year (Suess, 1976).

Polycyclics can be deposited in aquatic sediments through sedimentation of atmospheric particulates, runoff from catchment areas, and (in urban watersheds) runoff from streets and curbs. Sediments near urbanized areas are potentially the "sink" for PAH resulting from fuel combustion (heating, power generation, vehicular exhaust), used lubricating oils, asphalt wear particulates, or tire wear products, either

directly or resulting from street run-off (see Figure 1-1).

Due to the potential of stream sediments for acting as the contaminant "sink" for a watershed, and for providing an averaging of polycyclic input, levels and distributions of PAH in sediments can provide useful information on source and importance of inputs.

The build-up of carcinogenic compounds in aquatic systems (including sediments) is being studied due to the possible detrimental effects on aquatic ecosystems (bacteria, benthic invertebrates, fish, and species dependent on fish). These effects directly impact on human systems (exposure to carcinogens through ingestion or skin contact) and indirectly by threatening the diversity and strength of natural systems providing food.

In a study by Hall et al. (1976) the water quality of an urban tributary (Brunette River System) was investigated in order to assess its contribution to water quality in the lower Fraser River. Concentrations and distributions of trace metals and of selected organochlorine compounds were determined throughout the Brunette watershed. The researchers utilized analysis of sediments (lake, river and stream), street surface deposits, and storm waters to elucidate inputs to the Brunette system. Impact of various land uses on levels of contaminants was examined for street deposits and stream sediments.

B. PURPOSE AND SCOPE OF THESIS

The objectives of this thesis shall be to:

- 1) adapt or develop analytical methodology appropriate for the type of sample encountered;
- 2) determine the extent and nature of the PAH contamination in,

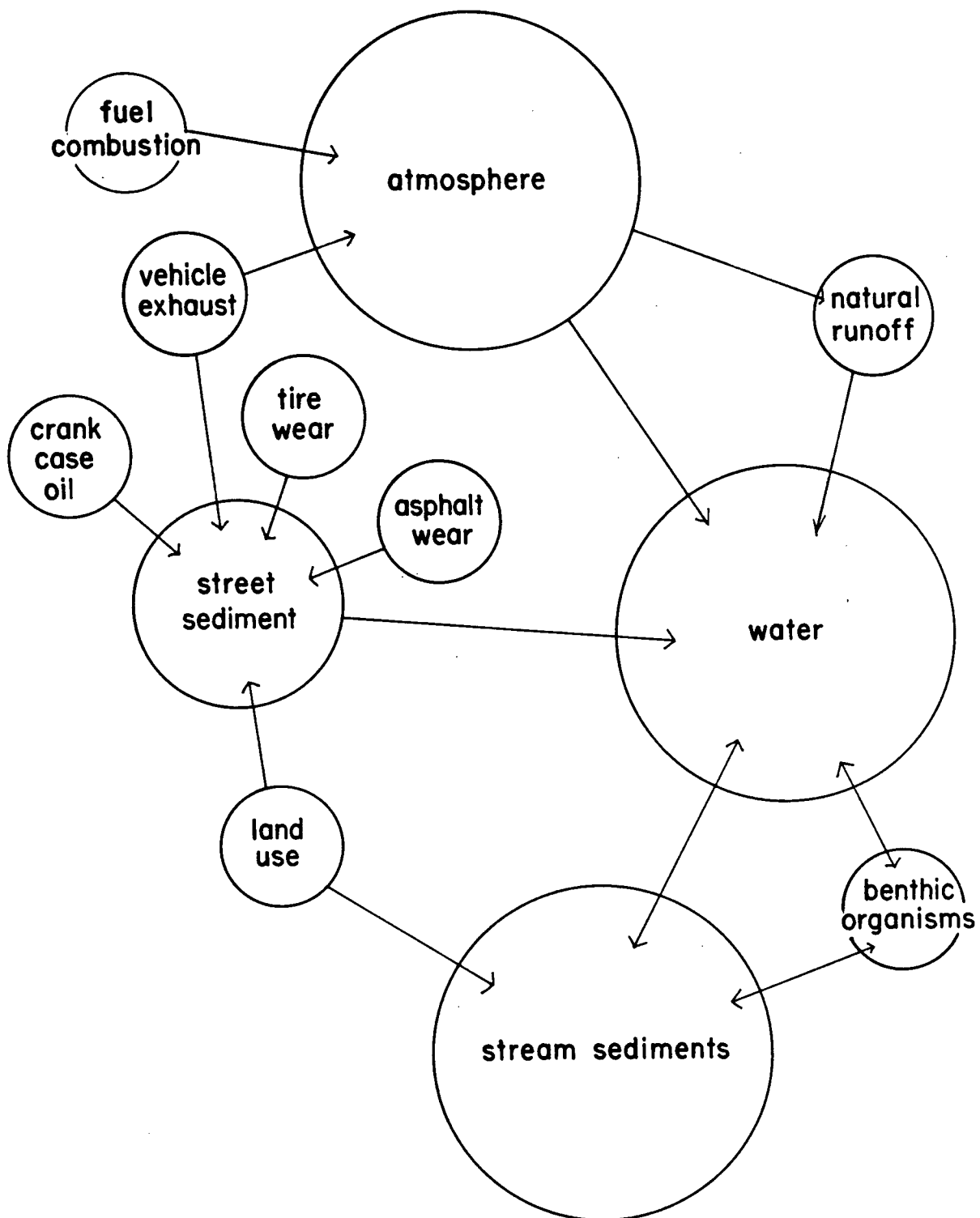


Figure 1-1. Possible pathways for movement of PAH in an urban watershed

primarily, selected Still Creek sediments, (and associated benthic organisms);

- 3) examine PAH contamination in street sediments and crankcase oil to investigate potential sources of polycyclics to Still Creek stream sediments, and (by investigating physical characteristics of sediments) attempt to identify environmental factors which regulate PAH distribution.

The study will be designed to complement the work by Hall et al. in the Brunette watershed but shall be restricted to the Still Creek section. Relationships investigated are depicted in Figure 1-2.

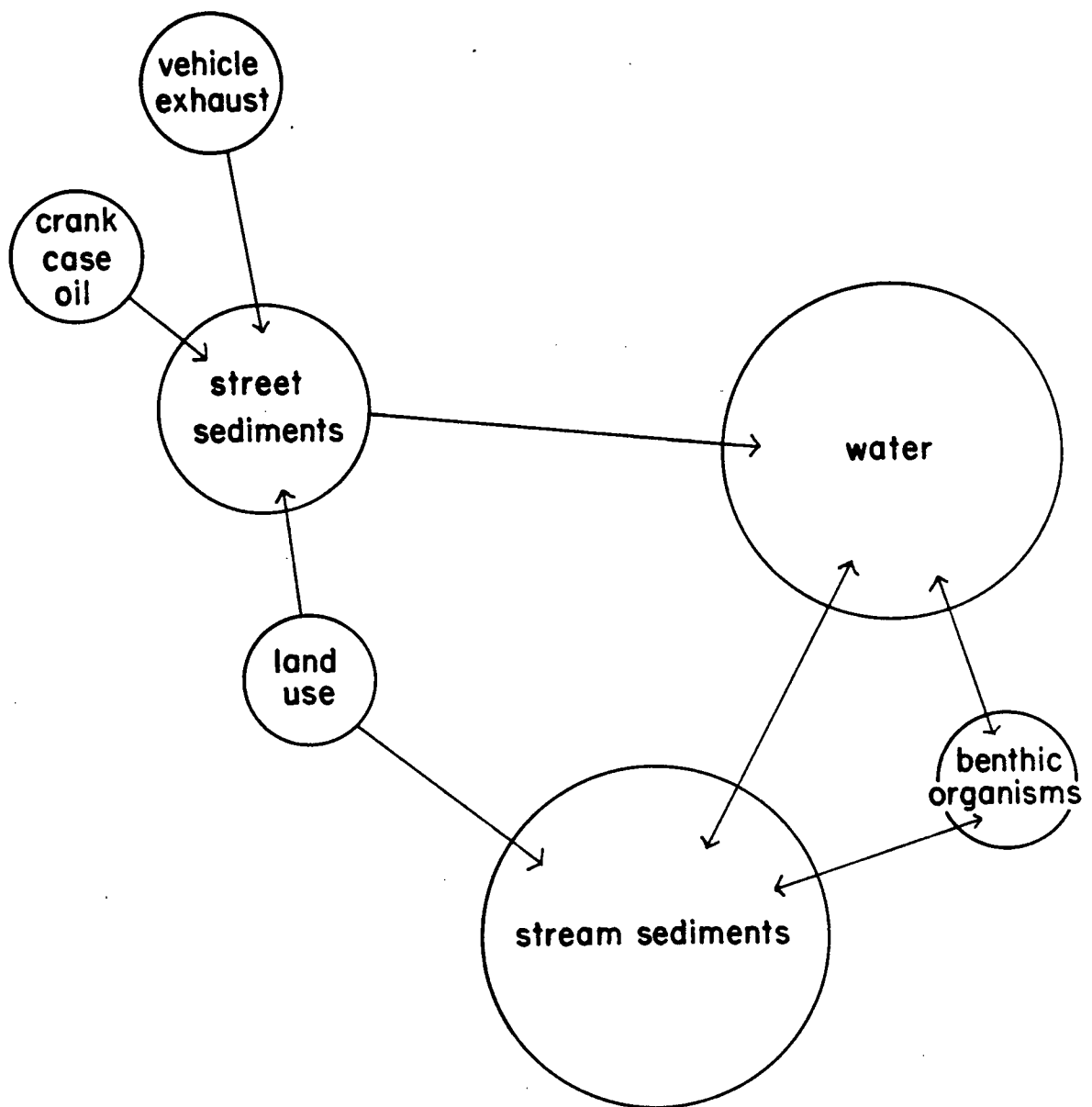


Figure 1-2. Relationships investigated in this thesis.

CHAPTER II

LITERATURE REVIEW

A. PHYSICAL AND CHEMICAL PROPERTIES OF PAH

Polycyclic aromatic hydrocarbons (PAH) are organic compounds containing two or more fused benzene rings with two or more carbon atoms being common to two or more rings. Non-aromatic rings may be included in the structure, for example fluorene, and there may be substituted groups (alkyl, nitro, hydroxyl) on one or more rings. Appendix 1 lists some of the major polycyclics, including their structures and carcinogenic potentials. Rules for naming these compounds have been adopted by IUPAC and are contained in The Ring Index (Patterson et al., 1960).

A summary of these naming and numbering rules are given in Appendix 1. Abbreviations for PAH names which are used in this thesis are also included in Appendix 1. In many references to PAH in the literature, benzo(a)pyrene (B(a)P) is given as representative of the PAH class. B(a)P's history as the first isolated polycyclic carcinogen and its strength as a carcinogen have resulted in many researchers concentrating their analysis on this one compound. In quantitative terms, however, B(a)P is often a very minor component of the polycyclic fraction in environmental samples.

Physical properties of various PAH are tabulated in Appendix 2 (melting and boiling points and molecular weights). The aromatic character of these fused rings results in relatively stable compounds (and corresponding extended lifetimes in some environmental systems). PAH tend to undergo substitution rather than addition. It is suggested that addition in the 'K' region (9, 10 bond of phenanthrene) may be

important in carcinogenesis. For a complete review of polycyclic hydrocarbon chemistry, see Clar (1964). For later work see Bjørseth and Dennis (1980). PAH undergo photo-oxidation in certain atmospheric conditions, resulting in potential interactions with urban air pollutants (reviewed in N.A.S., 1972).

B. PAH IN THE ENVIRONMENT

1. Sources to the Environment

The load of PAH in the environment includes material from both natural and anthropogenic sources. Petroleum products and coal contain polycyclics, sometimes to substantial levels (see Table 2-1). The levels of B(a)P and of total PAH vary considerably from crude oils to highly refined oils (Hermann et al., 1980; Tomkins et al., 1980). Even automobile fuels contain widely different amounts of B(a)P and aromatics (Newhall et al., 1973; Zaghini et al., 1973; Pederson et al., 1980). In general, products having higher average molecular weights contain higher PAH levels. Additional natural sources of PAH include forest fires (Youngblood and Blumer, 1975), and perhaps volcanic action.

The most significant mechanisms for introducing PAH into the environment are the combustion of fossil fuels, production of coke, and the catalytic cracking of petroleum products. Combustion products from heat and power generation are then deposited in aquatic or atmospheric systems. Suess (1976) estimated the emissions of B(a)P to the atmosphere, based on 1966-1969 data, as a staggering total emission of 5044 tons per year. On a world-wide basis, heating and power generation accounted for 52% of the B(a)P load while industrial processes account

Table 2-1. PAH in petroleum and petroleum products.

Material	Aromatic Content	B(a)P (μg/kg)	PAH (μg/g)	Reference
crude oil, from coal tar distillation		2×10^5	~700	Hermann <u>et al.</u> , 1980
petroleum distillate				
A		840	78	
B		<10	54	
crankcase oil				
new		< 5	nd	
old		6×10^4	15	
white oil, medicinal		< 5	0.64	
crude oil, from shale		2.5×10^4		Tomkins <u>et al.</u> , 1980
crude oil		2.6×10^3		
diesel fuel, from shale		38		
unleaded fuel				
A	25 %	nd		Newhall <u>et al.</u> , 1973
B	38	800		
unleaded fuel				
A	48	70		Zaghini <u>et al.</u> , 1973
B	48 (C ₉ -C ₁₀)	900		
C	48 (benzene)	nd		
leaded fuel				
A	40 (mixture)	120		Pedersen <u>et al.</u> , 1980
B	40 (benzene)	4		
	40 (O-xylene)	1		

nd = not detected

for 21%. Vehicular emissions, although important in urban areas, only contribute about 1% to the estimated total.

2. PAH in Air and Water

Numerous studies have been carried out to determine the extent of PAH contamination in the environment. Results of a few of the more recent studies are summarized in Tables 2-2 and 2-3.

In their study of aerosols over Lake Michigan, Strand and Andren (1980) sampled on 12 cruises over 26 months. By examining the size of the aerosol particulates it was determined that the major PAH source is anthropogenic combustion. These particles are deposited in the water micro-layer (top 300 μm), awaiting eventual adsorption and sedimentation in the Lake.

Greenburg et al. (1980) sampled air over urban New Jersey and the PAH found in a sample from Newark are listed in Table 2-2. The PAH levels were found to vary throughout the year, being lower in summer. This may be due to greater volatilization, more photochemical decomposition of PAH and smaller amounts of fuel used in residential heating. The presence of cyclopenta(c,d)pyrene is of note due to its mutagenic properties and relatively high concentrations in carbon black (Neal and Trieff, 1972; Wallcave et al., 1975).

The concentrations of PAH in drinking water are generally low (ng/l) but are still monitored closely due to possible risk from continuous exposure to carcinogens. Olufsen (1980) identified about 30 PAH in Norwegian drinking water ranging from < 0.05 ng/l to 3.1 ng/l Ph (see Table 2-3).

After having investigated PAH levels in Ottawa tap water, Benoit

Table 2-2. PAH levels in air.

PAH	level (ng/m ³) ^a	range (times found)	level (ng/m ³) ^b
Fl	0.8	0-2.2 (9)	
Ph	0.4	0-1.0 (8)	
A	0.4	0-1.0 (8)	
F	0.9	0-1.7 (10)	
2,3BF	1.0	0-4.1 (8)	
P	0.9	0-4.2 (10)	
B(a)A	0.5	0-2.5 (8)	0.7
Per	0.1	0-1.7 (3)	
Tri	0.1	0-0.5 (6)	
B(a)P	0.2	0-1.8 (2)	1.5
Ind(1,23-cd)P	0.1	0-0.9 (1)	
B(ghi)Per	0.3	0-2.0 (2)	3.3
B(k)F			1.3
Cor			1.2
Cyclopenta(cd)-pyrene			0.5

a - Strand and Andren, 1980.

b - Greenburg et al., 1980.

Table 2-3. PAH in water

PAH	level (ng/l) ^a	level A (ng/l) ^b	level B (ng/l)
N	2.9	5.8	0.9
2-meN	1.4	}	}
1-meN	1.1		
acenaphthene	0.82		
Fl	0.72	0.4	0.5
Ph	3.1	}	}
A	0.35		
2-meA	0.06		
4,5 dimePh	0.30		
1-mePh	0.37	3.0	0.6
F	2.6	0.8	0.3
P	1.1	0.4	0.07
B(a)Fl	<0.05		
B(b)Fl	<0.05		
4-meP	<0.05		
1-meP	<0.05		
B(a)A	0.49	}	}
Chr/Tri	0.79		
B(b)F	0.21		
B(j)F/B(k)F	0.07		
B(e)P	0.20		
B(a)P	<0.05		

a - Norweigan tap water; Olufsen, 1980.

b - average of two treated drinking water samples, Eastern Ontario

A - heavy population and industry

B - low population and industry

- Benoit et al., 1979.

et al. (1979) sampled drinking water at six municipal water treatment plants in Eastern Ontario. The PAH levels varied considerably in the raw water but less so in treated waters. The land use and population density in the water basins seem to affect the PAH concentrations (see Table 2-3). A basin with a large population and significant industrial activity (basin A) shows higher PAH levels than basin B, with small population and little industrial development.

Levels of polycyclics in aquatic organisms have been studied for shell fish and finfish around the world. Table 2-4 lists a few examples of concentrations in marine tissue samples found off Eastern United States and in a freshwater system in Ontario.

Kalas et al. (1980) investigated the incidence of PAH in the marsh snail Cipangopaludina chinensis and found polycyclics present up to the low ppm level (Table 2-4, col. I). The marsh environment for this snail was in a heavily populated area near major intercity highways, and the snail is considered to be pollution tolerant. In contrast, Brown and Pancirov (1979; Table 2-4, col. III-VI) were unable to detect many of their target polycyclics in bottom-feeding fish from Baltimore Canyon. Those PAH quantitated occurred in the low ppb range. Similar levels were determined in a previous study of shellfish and finfish off the Eastern US coast (Pancirov and Brown, 1977). In addition to the polycyclic levels found in clams as listed in Table 2-4, col. VII-IX, the researchers also analyzed a lake trout from northern Canada, in which they detected no PAH.

The majority of studies of polycyclics in shellfish deal with the levels of B(a)P in mussels. Dunn and Stich (1975) proposed that mussels should be used to monitor contamination of marine environments,

Table 2-4. PAH in Aquatic Organisms

PAH	ref. a		ref. b				ref. c		
	Col. I	II	III	IV	V	VI	VII	VIII	IX
P	37.00	2.50	2	2	4.1	nd	1.0	12	nd
meP	nd	1.00	1	nd	2.7	nd	nd	2.5	nd
B(a)P	25.10	nd	nd	nd	nd	nd	0.3	nd	nd
B(a)A			nd	nd	1.1	0.3	0.3	1	nd
N	nd	2.00							
meN	nd	0.50							
A, Ph	134.80	3.00							
meA, mePh	60.80	0.70							
F	97.50	3.00							
B(a)Fl	216.00	nd							
B(b)Fl	217.50	nd							
Chr	302.80	3.00							
binaphthyl	181.90	nd							
Per	25.90	nd							
Cor	110.90	nd							

ref. a Kalas et al. (1980), $\mu\text{g/kg}$, dry wt.
 col. I - levels in marsh snail
 II - marsh sediment

ref. b Brown and Pancirov (1979), $\mu\text{g/kg}$, wet wt.
 col. III - summer flounder
 IV - scup
 V - sea scallops
 VI - red hake

ref. c Pancirov and Brown (1977) $\mu\text{g/kg}$ wet wt.
 col. VII - clam
 VIII - clam
 IX - clam

using B(a)P as an indicator. In their sampling of mussels from various sites, a correlation was found between industrial activity, urban development, or presence of creosote, and B(a)P levels. In some areas creosoted pilings seemed to contribute significantly to B(a)P levels, while untreated sewage/urban runoff were possible sources in other areas (Dunn and Stich, 1976a).

The B(a)P levels tabulated in Table 2-5 demonstrate the variability encountered. It appears that the background concentration of B(a)P is 0.1 to 1.0 ppb, with mussels from highly contaminated areas containing over 2 ppm (Bjørseth et al., 1979).

Mackie et al. (1980) examined PAH and aliphatic hydrocarbon levels in mussels from 27 sampling sites around Scotland. They concluded that, due to the slow depuration of larger molecular weight PAH from mussels, the mussel watch was of limited value in monitoring current marine contamination. Dunn and Stich (1976b) found that in naturally contaminated mussels, B(a)P had a half-life of approximately two weeks in clean water. This is much longer than the one to three days generally allowed for elimination of bacterial contamination before marketing.

Pathobiology of aquatic organisms (briefly reviewed by Sonstegard and Leatherland, 1980) serves as an indication of potential pollution problems in the aquatic environment, and can provide a monitoring system for the presence of a carcinogen. Examination of thousands of fish (those caught currently and museum specimens from the 1950's) have provided information on several types of tumours and related disorders. Gonadal tumours in goldfish and carp suggest induction of tumours by a pollution source, as do increased incidences of lip tumours in bottom-

Table 2-5. B(a)P in mussels

Location	B(a)P $\mu\text{g/kg}$ (wet wt.)	Comments/Reference
Norway	74	15 km from ferro-alloy smelter/ Bjorseth <u>et al.</u> , (1979)
	2707	next to smelter
Vancouver, Canada	0.1	open Pacific / Dunn and Stich (1975)
	2	outer harbour, Vancouver, B.C.
	18	wharf and dock areas
	42	poorly flushed inlet, False Creek, Vancouver, B.C.
California, U.S.	0.1-8.2	sites from isolated areas to heavily populated harbours/ Dunn and Young (1976)
Mass., U.S.	<0.5	unpolluted area / Pancirov and Brown (1977)
	0.5	polluted harbour
Scotland	50	average of 29 sites, range 1-329/Mackie <u>et al.</u> , (1980)

feeding white suckers found around an industrial complex in Lake Ontario.

Fish tumour pathology and industrial pollution were investigated in the outflow area of the Buffalo River into Lake Erie (Black et al., 1980). Applying the Ames test for mutagens in the sediment pinpointed a source of mutagens at the outflow of the local dye industry. Sediments collected at this point contained twenty PAH, including B(a)P and B(a)A. HPLC traces of extracts from sediments, invertebrates and fish demonstrate strong similarities, especially once extracts were acid washed (interferences perhaps due to basic aniline dyes). Examination of some of the fish able to survive in the highly contaminated outflow area revealed gonadal abnormalities, lip tumours and some highly malignant dermal tumours.

Black et al. (1980) determined that PAH were concentrated by six times in tubifex, and twenty times in carp, with respect to the sediment levels. This bioaccumulation of polycyclics was also noted in mussels by Dunn and Stich (1976a), and by Mackie et al. (1980) as discussed above. Kalas et al. (1980) quantitated specific PAH in both sediments and snails (see Table 2-4, col. I-II). Calculated on dry weight of mollusc soft parts, concentrations of PAH were up to 200 times higher than in sediments. Exceptions to this were found with naphthalene (also meN and dimeN), biphenyl and meP, where none were detected in the snail.

Bioaccumulation of polycyclics can be predicted by their lipophilicity, expressed as their octanol:water partitioning factor. Southworth et al. (1980) investigated the predicted and actual bioaccumulation of diB(a,h)ac, B(a)ac, acridine and quinoline in fathead

minnows. Actual bioaccumulation of the higher molecular weight compounds never reached the predicted levels but evened out within 24 hours due to formation of metabolites. Exposure of Daphnia pulex to the same four compounds resulted in concentrations very closely matching those predicted. Obviously each organism reacts in a different way to various compounds.

While Southworth et al. (1980) dealt with bioaccumulation of compounds from water the same concentration effect has been investigated between sediments and organisms. Bindra and Hall (1979) analyzed the distribution of several trace metals in waters, organisms and sediments. Metal levels in benthic organisms were generally found to be related to sediment concentrations, however, organism contamination was lower than expected in some heavily contaminated sediments, and higher in "clean" sediments. Bindra and Hall postulated that total lead, for example, experienced competitive adsorption between live organisms and fine organic matter. Sediments with lower amounts of fines, and lower organic content would have higher lead concentrations in associated organisms (which offer organic adsorption sites).

3. PAH in Food

There has been considerable question as to whether bio-synthesis is a source of the global PAH background. Levels of polycyclics in plants seem to depend on the extent of urban/industrial development in the immediate area, but even very rural regions have a PAH background. When studying the levels of eight PAH in Hungarian grain Soós, (1974) found that concentrations were higher in grain from industrial areas than from rural areas (Table 2-6).

Table 2-6. PAH in Various Plant Products ($\mu\text{g/kg}$)

Food/Reference	F	P	Chr	B(a)P	B(e)P	diB(a,h)A	1,2BPer	B(a)A
Fruits Vegetables /Soós, 1980				1.5 3.0				
Wheat industrial rural	1.1 0.6	2.1 0.2	0.23 0.08	0.29 0.15	0.18 0.15	0.03 nd	0.03 nd	0.40 0.22
Barley industrial rural				1.3 0.3				
Rye rural /Soós, 1974				0.15				

Table 2-7. PAH in Field Crops Grown in Controlled Climate, Greenhouse or Open Fields, $\mu\text{g/kg}$.

Food ^a	B(a)P	B(e)P	Per	Anthran- threne	B(ghi)Per	DiB(a,h)A	Cor
Lettuce seeds	nd	nd	nd	nd	nd	nd	nd
controlled - climate	"	"	"	"	"	"	"
greenhouse	4.2	3.7	0.4	0.2	2.5	0.4	0.6
field	4.3	4.2	0.4	0.2	2.2	0.6	0.7
Rye							
controlled - climate	nd	nd	nd	nd	nd	nd	nd
greenhouse	3.4	1.6	0.9	0.1	0.9	0.1	0.1
field	1.2	0.64	0.10	0.06	0.34	0.1	0.1
Soybeans							
controlled - climate	nd	nd	nd	nd	nd	nd	nd
greenhouse	4.3	3.1	0.3	"	1.5	0.1	0.4

a - Grimmer and Duvel, 1972.

Some investigators have grown plants under controlled conditions to determine whether PAH bio-synthesis takes place. As Schamp and van Wassenhove (1972) point out, great care has to be taken to avoid false positives. Grimmer and Duvel (1972) grew various crops (tobacco, lettuce, rye, and soybeans) in open fields, greenhouse, and controlled climate chambers. The controlled climate chamber had an elaborate filter system for its air supply and was used with an airlock and special clothes for any personnel entering the room. As indicated in Table 2-7, crops grown in open fields or greenhouse showed similar, low levels of seven polycyclics while those grown in the special chambers contained none of those PAH.

Smoked meats and fish have been extensively investigated with respect to B(a)P levels and, more recently, levels of other polycyclics. Table 2-8 lists the results obtained from some of the later surveys of smoked foods.

Levels of PAH in smoked foods can vary considerably. Joe et al. (1979) found no samples with B(a)P and the highest total PAH was five ppb in a sample of frankfurters. In contrast to those results, Larsson (1982) analyzed samples of "home-smoked" herring (Table 2-8 lists commercially smoked herring) for B(a)P and found 128 ppb in the skin and 11.3 ppb in the flesh of one sample. The total of all quantitated PAH for that sample was 1,100 ppb. Commercial smoking procedures result in much lower but still significant polycyclic levels. Different types of fish or meat seem to develop varying concentrations of polycyclics on smoking.

In a survey of Canadian foods, Panalaks (1976) determined B(a)P levels ranging from 0 to 15 ppb. The other most frequently found PAH

Table 2-8. PAH in Smoked Foods (ug/kg)

Food	Ph	A	F	P	B(a)A	Chr/Tr1	B(b)F	B(j)F	B(k)F	B(e)P	B(a)P	Per	Ind(1,2,3cd)P	B(ghi)Per	diB(a,h)P	diB(a,j)A	Cor	7,12diMeB(a)A
Herring ^a	138	29	30	26	3.7	4.7	0.7	1.1	0.5	1.2	0.2	0.3	0.1					
Meckeral	112	22	25	2.8	2.8	3.9	0.7	1.3	0.6	1.0	0.2	0.4	0.5					
Eel	108	20	19	16	2.0	2.0	0.4	0.5	0.3	0.6	0.1	0.9	0.3					
Salmon	23	5.5	3.3	12	1.0	1.0	0.2	0.2	0.1	0.4	0.2	nd	nd					
Bologna ^b					nd		nd		50	2.0	nd	nd	nd	0.5	nd	nd	nd	
Frankfurters					nd		-		nd	2.0	-	-	-	nd	-	-	2.0	-
Ham					0.2		-		0.2	2.0	-	0.5	-	1.0	-	-	nd	-
Bacon					0.5		-		nd	0.5	-	nd	-	1.0	0.6	4.0	-	-
Beef, Smoked					8.0		-		-	nd	-	0.5	-	nd	nd	nd	-	-
Port, -					nd		-		-	0.2-0.3	-	nd	-	-	-	-	-	-
Herring, -				20			-		20	15	-	0.2-90	-	-	-	-	8.0	-
Fish, -					1.0		-		1.5	0.5	-	nd	-	5.0	-	-	10.0	-
Gouda Cheese					nd		-		nd	0.5	-	-	-	nd	-	-	nd	-
Oysters				30			-		1.0	nd	-	2.0	-	-	-	-	-	25.0
Oysters, Smoked				15-30			25		1.6	20	20.0	5.0	4.0	-	-	1.0	-	38.0
Bacon ^c			7.8				0.30		0.05		0.05	2.5	nd					
Kippers			2.4				0.35		0.10		0.10	2.7	3					
Cheese			4.2				0.30		0.15		0.20	nd	nd					

^a - Larson, 1982, Sweden^b - Panalaks, 1976, Canada^c - Crosby et al. 1981, Britain.

were B(a)A (0-30 ppb) and B(e)P (0-16 ppb). Smoked oysters had high levels of B(b)F, B(a)A, B(e)P, 7,12-dimeB(a)A and Per.

In studies of PAH levels resulting from charcoal-broiling, investigators examined effects of percent fat in the food, length of time food is broiled and distance between heat source and the food. Lijinsky and Ross (1967) determined that the polycyclics produced increased as meat percent fat increased. Analysis of hamburger broiled in a gas or electric oven resulted in no PAH formation while charcoal broiling in a no-drip pan resulted in only traces of a few PAH and no B(a)P. Bories (1979) reports that the lowest B(a)P (0.5 ppb) in charcoal broiled steak is obtained by lightly braising the steak at 10 cm from the charcoal.

In general, the more heavily smoked a food is, the higher the PAH level. External smoke generation (smoke produced in a separate chamber from food) results in lower polycyclic concentrations. The control over smoking conditions in commercial smoking establishments results in much lower PAH formation when compared to most home-smoked foods. Charcoal broiling of high-fat foods at close range results in elevated PAH concentrations.

C. PAH IN SEDIMENTS

1. Determination of PAH Sources

Concentrations and distributions of polycyclics in marine or freshwater sediments are of considerable interest as indicators of natural PAH levels in non-contaminated areas, or as records of polycyclic input to aquatic systems in more developed areas.

Several approaches have been used to determine the important

sources of PAH in a given sediment sample. The techniques include geological surveys, comparisons of alkyl homologue abundances, and an analysis of sedimentary cores.

It is often possible to determine input of PAH to sediments from a point or semi-point source by correlating polycyclic levels with distance of sampling points from given source(s). Also, samples taken near or far from urban centres should indicate the PAH loads. Similarly, samples taken in south or north hemispheres should reflect the greater population and industrial development in the northern hemisphere.

The PAH composition of sediments is often very similar from one site to another. However, the relative abundances of non-alkylated polycyclics and the various alkyl homologues are distinctive for particular PAH sources. It has been found that the polycyclics from petrogenic sources (coal, petroleum oils, shales) commonly have three- or four- carbon side chains with little or no non-alkylated PAH. (See LaFlamme and Hites (1978) for a review of methods to determine the sources of PAH in recent sediments.) Polycyclics derived from combustion processes have very similar qualitative distribution regardless of the fuel. However, differences in relative abundances of alkylated and non-alkylated PAH are seen from fuel to fuel and with differences in combustion temperature. Low combustion temperatures (eg. cigarettes) result in an abundance of alkyl polycyclics. (As mentioned above, petrogenic polycyclics derived through low temperatures over very long periods of time are almost entirely alkyl-substituted.) Combustion carried out at very high temperatures, as in carbon black furnaces, results in non-alkylated PAH.

The degree of alkylation encountered in the PAH fraction of a

sample is often indicated through alkyl homologue plots, where the relative abundance of each homologue is arranged by increasing total carbon number of the substitution. Various isomers of a single molecular weight parent may be included in a given plot, and distinction between single or multiple substitution of the parent isn't generally made (Blumer and Youngblood, 1975; or Giger and Blumer, 1974). Also, Blumer et al. (1977) examined aza-arene homologue series in Massachusetts sediments.

As an additional tool to help develop understanding of PAH deposition in recent sediments, sediment cores are analyzed and the layers dated to correlate PAH levels with estimated inputs. A great deal of information can be gained from sediment cores, especially if there has been minimal mixing.

2. Natural PAH Levels

Considerable effort has been made to determine the "natural" levels of PAH in sediments. Investigations have been carried out on numerous surface sediments and on several sets of sediment cores.

Analysis of water and sediments of a northern Ontario wilderness lake was carried out by Brown and Starnes (1978). Low levels (ppb) of nineteen PAH were determined in the sediment (see Table 2-9, column I). From the low relative abundance of methyl-substituted polycyclics in comparison to the parent compound, petroleum contamination was ruled out. Several other possible sources of PAH to this remote, undeveloped lake were considered; atmospheric fall-out of contamination from distant urban-industrial centres, biogenic generation in-situ, and polycyclics from forest fires. Of these, the biogenic formation and

Table 2-9. PAH in Sediments (µg/kg)

	ref.a	ref.b						ref.c		ref.d		ref.e				ref.f			ref.g			ref.h			
Polycyclic	Col. I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XIV	
Ph		5000	43	7	nd	2	6	300	10	2175	150	230	140	1880	260				53	42	8	340	210	3500	
F	38	15000	120	1	nd	0.6	3	950	2	2135	15	870	1260	3000	650	420	172	23.5	130	130	11	420	390	10000	
B(e)P	13							90	nd	6974	14	610	530	1730	280	420	149	33.2				210	150	3900	
B(a)P	28							250	nd	6313	16	340	350	1610	270	443	99	30.2				160	190	270	
B(b)F	25									5587	22														
B(k)F	25	33000	200	1	nd	0.6	500	450	nd	2540	7	1270	1140	3520	580	693	260	38.3	340	380	26				
B(j)F	8																								
Per	20							35	750	1658	nd	130	150	450	150	151	45	4.0				40	30	660	
B(a)Fl								60	nd	30	nd	190	210	920	150	112	38	26.1							
B(b/c)Fl								80	nd			120	150	610	90	88	22	32.2							
B(ghi)F	14											70	100	230	50	153	35	15.3							
Tri	10							300	nd	416	29														
Chr	23											510	1140	2200	330	356	143	24.4	160	200	12				
B(a)A	7	21000	80	2	nd	1	13	90	nd	2160	21														
B(c)Ph										39	nd														
P	23	13000	100	1	nd	0.6	3	950	2	1517	6	650	610	2860	430	383	138	20.3	120	120	7	380	330	7200	
IndF												230	240	550	120	80	50	54.5							
B(ghi)Per	28									8703	nd	640	730	1660	360	335	154	54.5							
Anthanthrene								25	nd			40	100	280	70	nd	nd	----							
Cor	6							50	nd			190	260	400	110	323	76	----							
A								40	nd	728	nd	30	30	340	70							30	20	450	
N																									
me-N																									
Acenaphthene										32	nd														
Fl								150	nd																
mePh/MeA								490*	25*	769	16	150*	90*	820*	160*										
meP	<2									157	nd	650	650	1690	360										
Ind(1,2,3-cd)P								700	nd																
diB(a,c/a,h)A								65	nd	1970	nd														

*mePh

ref.

- a - Brown and Starnes, (1978); Wilderness Lake.
- b - LaFlamme and Hites, (1978); dry wt. basis
 - col. II Charles River (Boston, MA)
 - III Gulf of Maine
 - IV Yosemite soil (wooded)
 - V Nevada soil (desert)
 - VI Alaska sediment (intertidal)
 - VII Rio Ica, Amazon River system
- c - Wakeham et al., (1980a); Greifensee Lake
 - col. VIII 0-3 cm, approximate values
 - IX 63-68 cm, approximate values
- d - Bjørseth et al., (1979); Saudafjord sediments, dry wt.
 - col. X 0-2 cm, next to smelter
 - XI 0-2 cm, 15 km from smelter
- e - Grimmer and Bohnke, (1975b); Grosser Ploner Sea, dry wt.
 - col. XII 5-7 cm, undeveloped shore
 - XIII 54-56 cm, undeveloped shore
 - XIV 4-6 cm, developed shore
 - XV 60-63 cm, developed shore
- f - Muller et al., (1977); Lake Constance
 - col. XVI 0-1 cm
 - XVII 14-15 cm
 - XVIII Enrichment factor, see text.
- g - Hites et al., (1977); Buzzards Bay, dry wt.
 - col. XIX 0-4 cm, 1970
 - XX 20-24 cm, 1900
 - XXI 38-42 cm, 1850
- h - Giger and Schaffner, (1978); dry wt.
 - col. XXII Lake sediment
 - XXIII River sediment
 - XXIV Street dust

forest fires were indicated as the most probable sources. Significant forest fire haze was observed in the region of the lake.

LaFlamme and Hites (1978) carried out a survey of PAH in recent sediments on a global basis to determine the baseline levels of polycyclics. Sediments sampled ranged from the highly contaminated Charles River (mid ppm range PAH, see Table 2-9, column II) to near pristine sediments in Alaska (low ppb range PAH, column VI). In all samples the qualitative pattern of PAH distribution was found to be very similar. The composition of the polycyclic fraction was usually Ph (12%); F(16%); P(15%); molecular weight 228 including B(a)A, Chr, Tri and B(c)Ph, (23%); and molecular weight 252 including BF, BP and Per (35%). This distribution of compounds in the polycyclic fraction (and the abundances of alkyl homologues) indicate combustion as an important PAH source. Increasing polycyclic levels with increasing proximity to industrialized areas indicate mainly anthropogenic sources. Two sets of compounds form distinct exceptions to the general pattern; alkyl phenanthrenes and Per. The alkyl phenanthrenes are largely comprised of 1-methyl-7-isopropyl-phenanthrene (retene) and also 1,7-dimethyl phenanthrene (pimanthrene). These compounds are perhaps derived from abietic or pimaric acid, both components of pine rosin. Samples from several sites with nearby pine forest all contained these alkylphenanthrenes and also other rosin components.

Work by Wakeham et al. (1980b) on sediments of four lakes indicate the presence of retene and pimanthrene, as mentioned above by LaFlamme and Hites (1978), as well as other phenanthrene homologues. Wakeham et al. propose that the phenanthrene series may be formed by diagenesis in recent sediments by microbial action (perhaps dehydrogenation of

steroids) on naturally occurring compounds. In the cores studied, Ph was always present at much higher levels than any homologues, but the importance of the alkyl compounds increased with increasing depth. The presence of retene and pimanthrene specifically was related to the extent of coniferous cover in the surrounding area. Lake Washington sediments (Seattle area) demonstrated much higher levels of these two polycyclics than the sediments of three Swiss lakes, where conifers have been relatively scarce.

Wakeham et al. (1980b) also tentatively identified a series of PAH (three to five aromatic rings) in Lake Washington sediments which they suggest could be derived from pentacyclic triterpenes. Successive dehydrogenation (again possibly microbial mediation) of these compounds has been demonstrated to occur and would account for the variety of polycyclics present in these very recent sediments.

The presence of perylene in the vast majority of sediments studied by LaFlamme and Hites (1978) was in accordance with work done by Aizenshtat (1973) on sediments, shale and peat. Aizenshtat found that, in order for Per to be formed and preserved in sediment, a source of material is required (perhaps terrigenous extended quinone pigments), then rapid conversion under reducing conditions, and preservation of Per, probably by π -bonding with transition metals.

In their survey of baseline PAH concentrations, LaFlamme and Hites (1978) found that the group of $C_{20}H_{12}$ isomers was generally composed of mainly benzofluoranthenes and benzopyrenes. However in several samples they found very high levels of perylene, up to 97% of the total PAH fraction. Samples containing such high levels of Per were often found in the Amazon River system (Table 2-9, column VII) although Per has

been found in most other sediments around the world. LaFlamme and Hites sampled additional non-reducing sediments in the Amazon River region (flood plains) and found no significant amounts of perylene. This gives support to Aizenshtat's proposals of diagenetic transformation of terrigenous pigments under reducing conditions.

3. Point Sources

In some cases it has been possible to determine a point source for the input of polycyclics to an aquatic system by quantitating PAH levels in the sediments at various sampling sites.

Dunn and Stich (1976a) sampled sediments near the Iona sewage treatment plant near Vancouver, B.C. As the sample sites were moved further from the sewage outfall, the level of B(a)P in the sediments fell sharply; 121 µg/kg dry weight next to the outfall compared to 0.7 µg/kg 5 km from the outfall.

Bjørseth et al. (1979) determined levels of over twenty PAH in sediments at various points along Saudafjord in Norway (Table 2-9, column X, XI). A ferroalloy smelter has been in operation at the head of the inlet since 1923, discharging waste from scrubbers into the fjord without treatment. It was found that, for most of the compounds, concentrations fell off steeply as sampling sites were moved from the smelter, so that "uncontaminated" sediments had only 1% of the PAH levels determined for sediments next to the smelter. Relative abundances of Ph, however, increased in uncontaminated sediments as was observed by LaFlamme and Hites (1978) and Wakeham et al. (1980b).

As another example of studies of suspected point sources of polycyclic contamination, Gump et al. (1977), sampled water and sediment

near a refinery tanker jetty. Water samples indicated a very slight increase in hydrocarbon load compared to that of uncontaminated sites. The sediments, however, demonstrated a significant increase in hydrocarbons, including approximately 800 ppb B(a)P.

The contribution of recent coal mining to polycyclic input to aquatic systems was investigated by John et al. (1979). The system chosen was the Severn Drainage in Wales which includes several rivers running through coalfields and exposed oil shales, as well as receiving effluent from heavy industry (chemicals and steel). By determining the polycyclic levels (9 compounds) in sediments from various sites in the system, it was found that levels were highest in areas which had had recent coal mining activity. This is of some concern since there may be a significant swing to the use of coal as fuel rather than oil, possibly resulting in higher inputs of PAH to the environment.

4. Non-Point Sources

Sediment cores were studied by Grimmer and Bohnke (1975b) to compare PAH levels encountered near an undeveloped forested lakeshore, and near a well built-up lakeshore (including highway and railway) on Grosser Ploner See. The cores taken extended back to approximately 1910, and were quantitated for PAH, elemental analysis and metal concentrations. It was found that the polycyclic levels have remained constant over the sixty-five year span covered by the core from the undeveloped southern shore. PAH levels increased five times over the same time in the core from the more highly developed area (Table 2-9, column XII-XV). The authors dismiss vehicle emissions as the main source for these increased levels since B(ghi)Per and Cor were present

at very low concentrations, and ratios of these two PAH to B(a)P were very different from that found in car exhaust. Similarly, petroleum wasn't considered an important polycyclic source due to lower than expected levels of S-containing PAH. Grimmer and Bohnke concluded that local coal combustion was mainly responsible for higher levels. This conclusion was supported by a decrease in polycyclic concentrations in surface layers of cores from the developed shore, coinciding with the recent replacement of coal as fuel for the local railway engines.

As a type of follow-up study, Muller et al. (1977) examined concentrations of heavy metals and PAH in sediment cores from Lake Constance. Cores of lake sediment (mainly derived from the Rhine River) were taken to a depth of 40 cm, with 25 cm corresponding to approximately 1900. In order to compare the increases in concentration among the polycyclics and the heavy metals, an enrichment factor was defined. The enrichment factor is the ratio of the component's highest level found since 1900 to its concentration in the 1900 layer. Some of the metals (K, Li, Fe, Cu, Cr, Co, Ni) showed no change in their concentrations over the span of the cores, having enrichment factors close to 1. Four other metals (Zn, Cd, Hg, Pb) however, showed significantly increased concentrations having enrichment factors ranging from 2.96 for Zn to 4.35 for Pb. On examination of PAH levels over the same time it became evident that their enrichment factors were much higher (Table 2-9, column XVI-XVIII). The lowest increase was noted for Per (enrichment factor 4.0) which as discussed previously, is often found in significant levels in uncontaminated lake sediments. B(ghi)F was another PAH with somewhat lower enrichment factor (15.3). All other eleven polycyclics quantitated showed enrichment factors between twenty

(P, 20.3) and nearly sixty (IndP, 56.9). Since there is such a strong correlation between metal levels (Cd, Hg, Pb, Zn) and PAH, the authors propose a common source; the combustion of coal. Coal ash has been shown to contain high levels of Pb, Cd, Zn, Cu, and Hg, as well as a wide range of polycyclics. Muller et al. set forth two possible explanations for the much higher enrichment factors found for PAH:

- 1) Polycyclics and metals are released in similar proportions and deposited in soils, but PAH are more easily "leached" than metals, thus resulting in much greater sediment levels.
- 2) Polycyclics are also produced as vehicle emissions.

The second proposal was discounted on the basis of the presence of benzonaphthothiophene (enrichment factor 25.6) in sediments and its absence in vehicle exhaust. Also, the ratio B(a)P: B(e)P is inappropriate for a significant petroleum hydrocarbon input. Thus the increase in levels of both metals and PAH is attributed largely to the combustion of coal.

Hites et al. (1977) examined sediments in Buzzards Bay, Massachusetts at different depths (Table 2-9, column XIX-XXI). The total PAH found in the sediment layer reflects the production of PAH by fuel combustion. As can be seen, the PAH concentrations rose sharply from 1850 to 1900 but stayed relatively constant till 1970. This levelling off during the last seventy years parallels a change from burning wood and coal (producing greater amounts of PAH) to oil and gas (which result in lower PAH emissions).

The soils of Nova Scotia and sediments of the Gulf of Maine were sampled by Hites et al. (1980). With the Boston area in mind as a semi-point source, samples were taken downwind as far as northern Nova

Scotia, including transects across the Gulf of Maine and Massachusetts Bay. Some deep ocean sediments were also taken. The results are summarized in Table 2-10. As can be seen, total PAH levels in Nova Scotia soils and the deep ocean sediments are low in contrast to the very high levels in Charles River sediments. PAH concentrations increased with increasing proximity to the Boston area in the transect across Massachusetts Bay and in deep ocean samples.

Hites and co-workers suggest that the major source of polycyclics to the study are in the particulate matter from various fuels burned in the Boston area. Larger particulates will be deposited near the combustion source, being washed off buildings and streets into local "sinks", such as the Charles River and Massachusetts Bay. These heavily contaminated sediments may be slowly transported to nearby basins (possibly explaining the variable PAH levels in the Gulf of Maine). PAH associated with small particles may be transported much further, accounting for the fall-off in PAH concentrations in deep ocean samples and low values in Nova Scotia soils.

In a study of application of capillary GC to PAH analysis, Giger and Schaffner (1978) included analysis of lake and river sediments, air and river particulates and street dust (Table 2-9, column XXII-XXIV). The distribution of polycyclics in each of these samples was generally similar. The air particulates lacked many of the lower molecular weight PAH, however this may be a result of the sampling technique. In considering the quantitative data, the authors consider PAH from street dust to be the important factor in sediments which receive urban runoff.

Wakeham et al. (1980a) continued this investigation of the

Table 2-10. PAH in Sediments, Boston as a Point Source

Sample Site	Total PAH ($\mu\text{g}/\text{kg}$, dry wt.) ^a	Comments
Nova Scotia	50	median of 10 soil samples
Gulf of Maine	540	
	500	
	870	
	200	
Massachusetts Bay	160	increasing proximity to Boston ↓
	830	
	3400	
	8500	
Charles R., Boston	120000	increasing proximity to Boston ↓
Deep Ocean	18	
	97	
	160	↓

a - Hites et al., (1980).

possible sources of polycyclics to sediments, looking at cores from three Swiss Lakes and Lake Washington. They found, as expected, higher PAH concentrations in sediments near urban areas than in sediments with input from rural areas. Alkylated PAH were very common and the abundance of the homologues decreased rapidly with an increase in carbon number in the side chain. Also, linear polycyclics such as anthracene were very much less common in comparison to non-linear, or angular, isomers such as Ph.

The enrichment of PAH found by Wakeham et al. (1980a) over approximately the last hundred years ranged from five times (Lake Lucerne) to forty times (Lake Zurich), indicating that, although atmospheric transport of polycyclics adsorbed to air particulates could account for qualitative similarities, other factors must contribute to quantitative differences. (See Table 2-9, column VIII-IX, for PAH levels in Lake Greifensee core.)

Street dust may contain PAH derived from asphalt particles, tire wear particles, atmospheric fall-out, vehicular exhaust particulates, motor oil or lubricating grease. Wakeham et al. (1980a) found strong qualitative correlation between the polycyclic fraction in street dust and river or lake sediments, similar molecular weight range and alkyl substitution pattern, and preponderance of unsubstituted PAH. In comparisons between street dust samples taken from asphalt roads and those from cement roads the qualitative distribution of polycyclics was the same, but asphalt roads consistently carried several times the PAH loading of cement roads. Street dust was shown to comprise particles up to 500 μm in diameter while vehicle exhaust particulates were generally smaller than 50 μm in diameter. The authors concluded from the

above study that the PAH input to river or lake sediments from street dusts (especially from asphalt surface) was of major importance in urban areas.

High levels of contamination in street surface dusts were reported in an extensive study of the subject by Sartor et al. (1974). Sediments from 12 American cities were analyzed for oxygen demand (chemical and biochemical), algal nutrients, heavy metals, pesticides and PCB's and coliform bacteria. PCB's were found in all sediments at levels approximately twenty times those of the organochlorine pesticides. Most of the contaminants were associated with the small fraction of organic fines (less than 10% of the total sediment weight). In a comparison of pollutant loadings on asphalt and concrete surfaces, asphalt surfaces were consistently about 80% more heavily loaded than concrete street surfaces. This may offer a different perspective on the high PAH content of street dust from asphalt streets as mentioned in Wakeham et al. (1980a).

The incidence of polycyclics in used crankcase oils has been of concern due to the carcinogenicity of some isomers, and the huge volumes of used crankcase oil which are released to the environment each year. Payne et al. (1980) tested used oil for mutagenicity with the Ames test. Contrary to their expectations, the major fraction responsible for mutagenicity was not that containing B(a)P or B(a)A, the classic carcinogens which had been found in used oils.

The factors affecting the buildup of PAH in crankcase oil were investigated by Handa et al. (1979), concentrating on car mileage and oil mileage. It was found that each car demonstrated a different rate of B(a)P accumulation in oil, but that the accumulation is directly

proportional to oil mileage. Handa and co-workers were able to relate the B(a)P accumulation to engine age; an equation was eventually developed incorporating a linear function of car mileage and a quadratic function of oil mileage.

MacKenzie and Hunter (1979) used sulfur-specific detection for GC analysis of urban stormwater runoff, crankcase oils (used and unused), fuel oils, and river sediments. Profiles were determined for each of these, and the used crankcase oil profile correlated most closely with that of river sediment. Their investigations indicated that the lighter diaromatics (eg. benzthiophene) were lost due to natural weathering but that the larger molecular-weight sulfur compounds (associated with particulates) could readily be transported in urban runoff.

5. Summary

In summary, the levels of polycyclic aromatic hydrocarbons in sediments predating the Industrial Revolution are extremely low (low ng/g range). In these early sediments there are several groups of PAH (Per, Ph and its alkyl homologues, and pentacyclics from triterpenes) which appear to be of diagenetic origin. It is possible that forest fires also contributed to these low levels.

Recent sediments, however, generally reflect the increase in industrial activity since approximately 1800. Whether these elevated levels of polycyclics are due to increased petroleum input, urban and industrial combustion fallout or increased vehicular traffic, is a matter of some question. The methods for determining the source(s) for a PAH residue have been reviewed, and examples given. In sampling areas

where a point or semi-point source (such as creosoted pilings, urban sewage effluent, smelter scrubber wastewater, coal mining or exposed oil shales) isn't identified, atmospheric transport of PAH adsorbed to particulates appears to be the main polycyclic source. Systems receiving urban runoff may get the largest PAH load from street dust (especially from asphalt roads).

D. ASSESSMENT OF PAH EXPOSURE

The complexity of the PAH component in environmental samples is well appreciated and becoming more evident with each improvement in analytical technique. In addition to compositional complexity, the effects of the PAH (individually, in combination, or with other components) are extremely difficult to determine. Efforts have been made, however, to assess (to the best of our knowledge) the risks to human health posed by polycyclics.

Following extensive surveys of PAH levels in Hungarian foodstuffs, Soós (1980) estimated the dietary B(a)P load in Hungary. The calculations are summarized in Table 2-11. It can be seen that, if the polycyclic level in fruits or vegetables are representative, the vast majority of the B(a)P in diets is attributable to fruits and vegetables. Smoked meats and meat products contribute only one percent to the estimated B(a)P load.

Considering mainly B(a)P exposure through air pollution, Bridbord et al. (1976) assess the potential exposure for several occupations and lifestyles. A person smoking a pack of cigarettes each day would be receiving a B(a)P load under half of that encountered by a worker in a restaurant or an airplane (where recirculated air becomes "enriched"

Table 2-11. Estimation of B(a)P Intake (per person/year) in Hungary^a

Food	Average B(a)P level ($\mu\text{g/kg}$)/range	Average B(a)P intake ($\mu\text{g/person/year}$)	% of Estimated Total Intake
Fruits	1.5 (0.92-1.73)	101.5 (62.3-117.1)	21
Vegetables	3.0 (1.30-4.40)	258 (118.8-378.4)	54
Edible oils	4.8	17.3	4
Margarines	4.7	9.4	2
Bread	0.66	62.2	13
Baker's ware	0.70	8.2	2
Floury products	0.87	3.0	1
of sweets industry			
Smoked Meats and meat products	0.67 (0.60-0.74)	6.3 (5.6-6.9)	1
Smoked cheese	0.85	~0.1	-
Grilled meat	<0.05	-	-
'Pure' coffee extract	0.4	9.6	2
Total		475.6 (290.2-612.2)	100

a - Soós, 1980.

with cigarette PAH). Workers in coke factories, or using coal tar pitch, experience by far the greatest risk (see Table 2-12).

A more comprehensive attempt at quantitating PAH exposure is made by Santodonato et al. (1980). Urban PAH concentrations in ambient air are used to calculate a figure for daily intake of B(a)P and total PAH from air. Likewise, levels of PAH contamination of drinking water sources are used to calculate the water-related daily load.

Assessment of dietary B(a)P (or PAH) intake is complicated by the differences in food origin, method of preparation and relative amounts of food consumed. See Table 2-13 for a summary of the polycyclic concentrations used by the authors to calculate intake. The estimated polycyclic load for air, water and food are combined into a total daily intake.

Calculation of an allowable daily intake (ADI), relies on findings from animal carcinogenicity tests (see Table 2-14). Assumptions are made with respect to dose response relationships for PAH, activation of carcinogenic polycyclics and applicability of animal tests in order to assess the potential for cancer in humans.

In Table 2-14, the calculated allowable daily intake has been listed for B(a)P, diB(a,h)A and two PAH mixtures. In comparison to estimated daily B(a)P load, it can be seen that the food contribution may exceed the ADI by three to 30 times. Smoking (one pack per day) exceeds the ADI by approximately eight times. These assessments indicate that dietary intake may be the most important source of PAH for a non-smoker. The daily dietary exposure calculated by Soós (1980) is in agreement with the upper range of the daily dietary exposure estimated by the Santodonato assessment.

Table 2-12. Estimates of B(a)P Intake, From an Occupational Viewpoint^a

	B(a)P intake μg/day	Cigarette equivalents packs/day
Smoking, 1 pack/day	0.4	1
Coke oven workers		
Topside exposures	180	450
Side & bench exposures	70	175
Coal tar pitch worker	750	1875
Airplane pilots		
Transatlantic flights	0.93	2.3
Domestic cross country	1.38	3.5
Employee in restaurant	0.8	2
Person living near expressway		
24 hr/day adverse meteorology	0.02	0.05
Commuter on expressway		
2 hr/day adverse meteorology	0.04	0.10
Exposure to ambient B(a)P levels 8 hrs/day	0.02	0.05

a - Bridbord et al., 1976.

Table 2-13. PAH Levels in Foods Used to Calculate Human Exposure
Due to Food Consumption^a

Food	B(a)P	Total PAH
vegetable oils and margarine	0.2 - 6.8	2.1 - 136
fish and other aquatic foods		
smoked	trace - 6.6	5.2 - 162
non-smoked	0	1.8 - 3.2
smoked meats and meat products	trace - 3.6	1.5 - 150
cooked meat		
charcoal broiled		
hamburger	0 - 2.6	0.3 - 43.9
steak	4.4 - 50.4	70 - 183.7
barbequed	3.5 - 10.5	37.5 - 186.1
fruits	nd - 29.7	
grains and cereal products	0.1 - 60	
sugar and adjuncts	0.2 - 72.0	
vegetables	nd - 24.3	
beverage	nd - 21.3	

a - Santodonato et al., 1980.

Table 2-14. Estimate of Allowable Daily Intake^aa. Estimated B(a)P, Total PAH exposures ($\mu\text{g/day}$)

Source	B(a)P	Total PAH
air	0.0095 - 0.0435	0.207
water	0.0011	0.029
food	0.16 - 1.6	1.6 - 16
(smoking)(1 pack/day)	0.4	

b. Allowable Daily Intake (ng/day)

Compound	Route	ADI (ng/day)
B(a)P	Dietary	47
B(a)P	Intratracheal	48
B(a)P	Dermal	4.7
PAH mixture	Oral	43
PAH mixture	Dermal	4.2
DiB(ah)A	Oral	108

c. Exposure in Relation to ADI

	B(a)P (ng)	Proportion
ADI	48	1.0
Water	1.1	0.02
Air	9.3 - 43.5	0.02 - 0.91
Food	160 - 1600	3.3 - 33
Smoking	400	8.3

a - Santodonata et al., 1980.

E. ANALYSIS OF PAH

1. Introduction

Due to the considerable complexity of the polycyclic fractions in sediments, analysis methods have generally been long and complex procedures. Since early instrumental methods involved very low selectivity (thin layer chromatography or paper chromatography), the clean-up procedures were required to remove most of the interfering compounds. With the greater resolving power of HPLC and GC, analysis schemes have been simplified to a large extent. The improved resolution, however, often reveals a much greater complexity in environmental samples than was indicated by low resolution methods. Thus, work on analysis schemes aims to 1) stream-line sample workup and 2) achieve adequate resolution of important polycyclics once interferences have been eliminated. The usual approach to analysis of PAH in sediments involves extraction of the analytes from the sample; separation of the analytes from any aqueous phase; clean-up by column chromatography; and perhaps gross separation by additional column chromatography before final separation by instrumental methods.

2. Extraction Procedures

Several of the older methods are adapted from methods used for the analysis of PAH in foods. The work of Grimmer and Bohnke (1979) described a method based on a IUPAC recommended method for polycyclics in meat. Dried sewage sludge or soil samples are extracted by refluxing with acetone. Another example of the adaptation of food-related methods is the procedure used by Dunn (1976). Samples (tissue or sediments) are refluxed with alkaline ethanol in order to extract the

organics and to saponify lipids. Alkaline methanol has also been used to saponify extracts (Bieri et al., 1978).

Soxhlet extraction of sediments is widely used; the extraction solvent and the reflux time vary widely. Giger and Blumer (1974) developed a very thorough procedure to prepare sediment samples for analysis by UV-Vis spectrophotometry and MS. The sediment is Soxhlet extracted for 24 hours with benzene then methanol is added and the mixture Soxhlet refluxed for a further 24 hours. La Flamme and Hites (1978) also used this procedure. Keizer et al. (1978) combined the benzene and methanol, and reduced the time to a total of 18 hours. Brown and Starnes (1978) used a 6 hour Soxhlet using benzene only. In efforts to simplify the Giger and Blumer (1974) procedure to be used with high resolution capillary GC, Giger and Schaffner (1978) used dichloromethane to Soxhlet extract the dry sediments and reduced the time to 6 hours.

MacLeod et al. (1982) reported on interlaboratory comparisons of determinations of trace hydrocarbons in sediments, conducted by the Northwest and Alaska Fisheries Centre for the National Oceanic and Atmospheric Administration. Two sets of samples were sent to participating labs; for the first set of determinations the extraction methods included Soxhlet, reflux, sonication, and tumbler-shaker extraction. In an effort to improve the comparability of results from different labs, Soxhlet or tumbler-shaker extraction were specified for the second set of determinations. Partly due to these requirements, results from the second round of analyses showed improved reproducibility. It was shown that Soxhlet extraction of dried sediments with dichloromethane is equivalent to dewatering the sediment using

methanol, then extracting with methanol-dichloromethane in a tumbler shaker.

3. Partition and Clean-Up

After the polycyclics have been extracted from the sediment the extract is usually cleaned up with some type of solvent-solvent partition. If the extract still contains water, the PAH are extracted into iso-octane (Dunn, 1976; Brown and Starnes, 1978), pentane (Giger and Blumer, 1974; Keizer et al., 1978) or hexane (LaFlamme and Hites, 1978; Bieri et al., 1978). Interfering compounds are left behind in the hydrocarbon solvent when it is partitioned with dimethylsulfoxide (DMSO) (Natush and Tomkins, 1978; Dunn, 1976), nitromethane (LaFlamme and Hites, 1978) or aqueous N,N-dimethyl-formamide (DMF) as recommended by Wallcave et al. (1971). Usually water is added to these fractions to allow the PAH to be back-extracted into hydrocarbon solvent.

Some of the column clean-up methods used are listed in Table 2-15. Florisil is generally used to separate aliphatics from aromatics (Dunn, 1976) while silica or alumina are used as clean-up and/or separation columns. Silica columns were used for clean-up by LaFlamme and Hites (1978) with hexane as eluent. Bieri et al. (1978) also used silica but eluted the polycyclics with benzene. Brown and Starnes (1978) cleaned up the PAH fraction on alumina using a cyclohexane-benzene-methanol system. An alumina system used by Sorrell and Reding (1979) results in three fractions, separating B(a)P/B(e)P, Chr/Tri, and B(b)F, B(k)F from B(a)P.

Several procedures for isolating PAH exploit the differing properties of the adsorbants in order to obtain clean polycyclic residues.

Table 2-15. Column Chromatography Systems

Column Type	Elution System	Function	Reference
Alumina	hexane-benzene-chloroform	separation of PASH, PAH, and PANH	Vassiliaros <u>et al.</u> , 1982a
Bio-beads	dichloromethane	clean-up	as above
Silica	pet.ether-pet.ether acetonitrile	clean-up	Guerrero <u>et al.</u> , 1976
Alumina	cyclohexane-benzene-methanol	clean-up	Pancirov & Brown, 1977
Alumina	pentane through to dichloromethane	separation of some PAH (three fractions) and clean-up	Sorrell & Reding, 1979
Silica Sephadex	cyclohexane <u>iso</u> -propanol	clean-up " "	Grimmer & Bohnke, 1975
Florisil	<u>iso</u> -octane-benzene	separation of aliphatics, aromatics	Dunn, 1976, and Howard, 1979

LaFlamme and Hites (1978) use Sephadex LH-20 gel permeation, then silica/alumina in a single column, charge transfer complex formation followed by further cleanup on a column of silica by itself, and concluding with fractionation of the PAH on alumina. With the use of high resolution chromatographic methods (HPLC or capillary GC) several of these steps can be dropped. Giger and Schaffner (1978) and Grimmer and Bohnke (1979) both use silica and Sephadex for clean-up, although in different orders. Giger and Schaffner (1978) tentatively identified the interferences (those that pass through silica but are removed with Sephadex LH-20) as long chain wax esters. MacLeod et al. (1982) recommend following silica with Sephadex in order to eliminate aliphatics and also highly alkylated PAH. As long as analysis of only parent polycyclics or mono- to tri-alkyl PAH are required, needless interferences in the capillary separations are avoided.

4. High Pressure Liquid Chromatography (HPLC)

Several techniques have been applied to the identification and quantitation of PAH in environmental samples. The various techniques have to be able to deal with the large numbers of different PAH present in most samples, the part-per-billion levels which must be detected, and the separation of carcinogenic and non-carcinogenic isomers.

HPLC has been applied to the analysis of PAH in samples in the last few years. A persistent problem has been the separation of isomers such as Ph/A, B(a)A/Chr/Tri, B(e)P/B(b)F/B(k)F/B(a)P and B(ghi)Per/Ind(1,2,3,c,d)P. Wise et al. (1980) examined the properties of nine HPLC columns (eight C₁₈, one amino column). Seventeen PAH (fourteen of the EPA priority pollutants) were run on these columns.

The amino column generally separates by number of rings while C_{18} columns are more selective. The C_{18} columns tested were Lichrosorb RP-18, Micropak CH-10, Micropak MCH-10, Nucleosil 10 C_{18} , Partisil 5 ODS, Radial PAK A, Vydac 201 TP reverse phase and Zorbax ODS. The Vydac column demonstrated quite different selectivity to the PAH than any of the other columns and will separate 18 PAH very well, including the more difficult PAH. The authors recommend using the amino column for separations by ring size and then taking fractions for analysis on a reverse phase system (preferably Vydac or Zorbax).

The use of gradient elution can result in improved peak shape and better resolution. Ogan et al. (1979) used an acetonitrile-water system to optimize separation of a group of 16 PAH.

A chemically bonded stationary phase for HPLC, phthalimido-propylsilane (PPS), was prepared for PAH separations (Hunt et al., 1977). It is able to separate B(k)F and Per, and B(e)P/B(cd)F, but not B(e)P/3-me Col.

The vast majority of PAH HPLC analyses are done on C_{18} columns. Some representative studies are summarized in Table 2-16. Acetonitrile water and methanol-water are the most widely used solvent systems (methanol-acetonitrile-water is also used). The number of PAH determined varies from two (Guerrero et al., 1976) to 17 (Crosby et al., 1981). It is interesting to note that only one group is using the Vydac columns recommended by Wise et al. (1980). In many cases only a few PAH are analyzed, avoiding the problem of quantitating isomers. If LC retention time is the only basis used for peak identification, results may not be accurate due to co-eluting polycyclics.

The study by Wise et al. (1980) didn't consider C_8 reversed phase

Table 2-16. HPLC Systems Used for PAH Analysis.

Sample Type	Column	Solvent System	Detection System	Comments	Reference
Oils & Fats	LiChrosorb SRP18	CH ₃ OH:H ₂ O 95:5	fluorescence: ex365,em410.	•detect 5 PAH, some interference from sample	Van Heddeghem <u>et al.</u> , (1980)
Smoke concentrate	Partisil 10 ODS II	CH ₃ OH:CH ₃ CN:H ₂ O 35 : 35 : 30	fluorescence: ex280,em390.	•considerable interferences from samples with earlier PAH	Silvester (1980)
Beer	RP-18, 5 μ m	CH ₃ OH:CH ₃ CN:H ₂ O 47.5: 47.5: 5	UV at 287, fluorescence: ex287,em370.	•4 PAH studied, fluorescence has much less interference	Joe <u>et al.</u> , (1981)
Shellfish	μ Bondapak-C ₁₈	CH ₃ OH:H ₂ O 75 : 25	UV at 383.	•no chromatograms included •B(a)P, B(ghi)P only	Guerrero <u>et al.</u> , (1976)
Smoked, charcoal broiled meats	Vydac ODS	CH ₃ OH:H ₂ O 87 : 13	UV at 254, fluorescence: ex320-400, em400-700.	•selectivity improved by use of two detectors •9 PAH detected but not all well-resolved	Panalaks (1976)
Meats, smoked	LiChrosorb ODS	CH ₃ OH:THF:H ₂ O 80 : 8 : 12	fluorescence: ex290,em430, ex282,em457.	•17 PAH detected •marked decrease in interferences with fluorescence detector	Crosby <u>et al.</u> , (1981)
	Partisil PPS	" " "	" "	•meB()A interfere, also B(b)F/Per •interference of B(a)P/diB(a,h)A, also B(b)F/B(ghi)F/7-meB(a)A	
Sediments, oysters	LiChrosorb RP-18, 5 μ m	CH ₃ :H ₂ O 70: 30	fluorescence, various excitation, emission wavelengths	•analyzed for P,A, B(a)P, B(a)A, B(k)F, B(b)F, B(ghi)Per	Obana <u>et al.</u> , (1981)

columns, or smaller particle size C_{18} columns. The C_8 columns can have better hydrocarbon coverage on the silica backbone, giving a more hydrophobic surface. This may result in different selectivity for PAH. Smaller particle sizes can result in better chromatography, again giving potential for improved analysis of polycyclics.

In efforts to improve resolution on HPLC columns various researchers have investigated very small diameter columns, following the same move as in capillary columns for GC. Since the resistance to mass transfer is about 10^4 times greater in liquid phase than in gas phase, the columns should be even smaller interior diameter than GC columns. Several approaches to this problem have been taken, including open columns with chromatographic phase coated on the interior walls, and very small particles of packing materials in micro-bore columns. In either case the capillary HPLC columns requires low dead volume sample introduction, connections, and detector cells. Scott and Kucera (1979) compared peak shapes for various detector cell volumes and the reduction of band width with lower cell volumes can be easily demonstrated. One benefit of such low volume system is the reduction in mobile phase requirements; flow rates are typically $\mu\text{l}/\text{min}$ as compared to ml/min in a more conventional HPLC system. Although sample capacity is very low, much higher resolution can be obtained, usually at the expense of analysis times.

Tsuda et al. (1978) coated the walls of a capillary tube (0.06 mm) with the reverse phase of octadecylsilane. A mixture of 7 PAH standards was separated on a column with about 600 plates/metre. In isocratic elution, it required 90 minutes to elute pyrene at $1 \mu\text{l}/\text{min}$. This could be reduced to 20 minutes by the use of gradient elution.

The authors suggest that a monolayer of stationary phase may improve mass transfer. Also they note that k' is generally under 2 while it should be 10 or greater for adequate separation of complex mixtures.

Hirata and Novotny (1979) used a packed micro-capillary column to separate PAH in a coal tar extract. The column was 55 m long and 0.070 mm interior diameter, packed with 0.03 mm basic alumina-octadecylsilane. Using a stepwise gradient from 80% methanol in water, through methanol to 3% dichloromethane in methanol, separation of the coal tar was achieved in about 17 hours. Sixteen different polycyclics were identified out of the fifty to hundred peaks present.

Scott and Kucera (1979) also applied microbore packed columns for the separation of polycyclics in coal tar. The column was packed with Partisil ODS-2 and was used with an isocratic acetonitrile-water system at 5 μ l/min. The coal tar sample was run for over twenty hours, resulting in over 120 peaks in the chromatogram. The last peak to be eluted had a k' of 60 and was still well separated from preceding peaks.

Continuing research in capillary LC columns may result in important separation capabilities for complex polycyclic mixtures.

The column and the solvent system are used to manipulate the elution of analytes but temperature control is often ignored. Chmielowiec and Sawatzky (1979) studied the effects of temperature control on PAH separations. The control of the system temperature can result in changing elution orders for some polycyclic pairs. Thus, peaks which co-elute at one temperature (3,4-BF and 9,10-dimeB(a)A at 25°C) will be resolved at a different temperature (45°C or partial resolution at 15°). Temperature gradients during a run will result in

improved peak shape for later-eluting peaks, and may change elution orders.

HPLC is used for PAH analysis because elution times for some larger polycyclics can be much shorter than for packed-column GC, some isomer pairs are better resolved than by GC analysis and the detection systems offer extra sensitivity and selectivity.

A comparison was made by Christiansen and May (1978) of ultra-violet and fluorescence detectors for HPLC analysis of PAH. They noted that both systems have good sensitivity for PAH, and fluorescence detectors can be used to increase sensitivity. At 254 nm a UV detector's sensitivity to PAH would range from 0.16 ng injected for N to 0.025 ng injected for Ph. Fixed wavelength detectors can provide an order of magnitude greater sensitivity at a given wavelength than do variable wavelength detectors. Since polycyclics absorb strongly at 254 nm, a fixed wavelength detector at 254 nm can provide comparable sensitivity to the fluorescence detector, although less selectivity.

It has been found that UV detectors in series can be used to aid in identification of peaks and in the selective detection of various polycyclics. Sorrell and Reding (1979) used three UV detectors in series enabling them to selectively monitor a given polycyclic in a co-eluting PAH peak, or in a poorly resolved peak. Even for peaks that weren't subject to interferences (polycyclic or other) the choice of wavelength allowed quantitation at the optimum wavelength, lowering the detection limits in some cases. The detection limit for the individual polycyclics varied from 0.25 to 1 ng/l.

Additional information to aid in the identification of specific polycyclics is the UV spectrum of the compound obtained from a selected

fraction for spectrophotometry. The spectra for PAH can, however, be quite similar for isomers and alkylated PAH.

UV spectra of peaks in a sample can be obtained by collecting fractions for spectrophotometry; stopping the mobile phase at the top of a peak and using a scanning UV detector to determine the spectrum while the peak is still in the flow cell; or performing rapid scanning of a wide wavelength range during the entire run. The last approach is the easiest operationally and can yield the greatest amount of information.

Rapid scanning UV detectors generally use either a diffraction grating to produce scans or a photodiode array to monitor many different wavelengths at once. With appropriate data processing, these systems can provide UV spectra of the LC eluent at any given time or, in pseudo three-dimensional plots, spectra over several hundred wavelengths for the entire LC run (absorbance vs wavelength vs time). This allows the separated peaks to be identified by their spectra. Resolution of co-eluting peaks can be improved by choosing to monitor absorbance at the λ_{max} of the analytes of interest. Selectivity can also be improved by monitoring the first derivative of the absorbance and setting dA/dt at zero for interferences. Being able to monitor at optimized wavelengths can also result in improved selectivity.

All the possible manipulations of the enormous amounts of the data allow much more information to be obtained, especially in the separation of complex mixtures. Denton et al. (1976) used an oscillating mirror-diffraction grating to split the incident beam into the various wavelengths to be scanned before going to flow cell. They were able to obtain uniform resolution over the whole range (200-930 nm) with a full

spectrum of the chosen range each second.

An array of 256 solid state diodes was used by Milano et al. (1976) as an HPLC detector. Half of these (128) were used to determine spectra to store data over longer LC runs. With the data processing used (on tape) the spectra were collected at an effective rate of 1 per 2 seconds; this could be changed with other data processing systems. Milano et al. (1976) found that the use of the first derivative of the LC trace was very useful for deconvoluting peaks.

Dessy et al. (1976) developed an LC detector based on a photodiode array similar to that used by Milano et al. (1976) but with a xenon source rather than deuterium. Fibre optics were also employed, to reduce transmission and reflection losses. The system is capable of 20 spectra/sec., and was used for detection of low levels of carcinogen metabolites in mouse urine. The UV spectra of metabolites can aid in their identification.

Selectivity is obtained with the fluorescence detector by choosing optimum excitation and emission wavelengths for selected compounds. Thus, by using an excitation at 271 nm Ph can be detected when it is only one-tenth the concentration of anthracene present (Christiansen and May, 1978). In order to quantitate anthracene in the presence of ten times as much Ph, excitation at 325 nm is used. Under these conditions the detector can "tune out" the more concentrated compound and detect the trace component. When dealing with environmental samples, many compounds will absorb UV radiation, but fewer will fluoresce. This results in cleaner chromatograms, allowing the quantitation of PAH in the presence of other UV absorbers.

An example of the selectivity of fluorescence detection was

provided by Wheals et al. (1975) in their analysis of five PAH at various excitation/emission wavelength combinations. In a mixture of A,N,F,P and Per, conditions can be set, for example, to detect only N (ex 275, em 320), or A, F and P (ex 335 em 385), or Per only (ex 410, em 470). This type of selectivity was applied to analysis of engine oils for polycyclics.

Time-resolved fluorescence detection was investigated for selectivity and sensitivity in HPLC separation of PAH mixtures by Richardson et al. (1980). Laser-induced fluorescence was monitored at 0 to 45 nanosecond (ns) delays, resulting in selectivity towards those polycyclics with long fluorescence lifetimes (eg. F, P, or B(11,12)Per). Detection limits of approximately 20 pg were obtained for F and P. This highly sensitive and highly selective method was used for analysis of F in a coal gasification fraction. At 0 ns delay F is lost in surrounding fluorescence peaks, but at 45 ns delay, the F peak can be clearly seen.

Fluorescence spectra of PAH can be of considerable value in identifying the individual polycyclics. Fox and Staley (1976) collected peaks for determination of fluorescence spectra. First and second derivatives of the fluorescence spectra can aid in detecting trace components and potential interferences.

Certain precautions are necessary for the quantitative fluorescent detection of polycyclics (Fox and Staley, 1976). Analytical standards are required in order to construct calibration curves since quantitation may be affected by solvent composition, the system of filters used, fluorescence efficiency and lifetime, and extinction coefficient of the sample. Quenching by oxygen may also be a problem.

Combining the advantages of both UV and fluorescence detectors, Christiansen and May (1978) recommend a UV fixed wavelength detector at 254 nm followed by a fluorescence detector to provide selectivity.

HPLC analysis of PAH offers the advantages of separation of some troublesome isomer pairs, sensitive detection, possible identification by recording UV or fluorescence spectra and elution of higher molecular weight polycyclics. Disadvantages include some persistent isomer separation problems, ability to separate a relatively low number of compounds, and some operational problems with detectors. Research is continuing on greater selectivity and separation power for HPLC columns.

It is anticipated that improved detector systems (greater sensitivity and selectivity, more spectral information from a single run) will strengthen the whole HPLC system for PAH analysis.

5. Gas Chromatography (GC)

GC is used extensively for the separation and detection of PAH. The separation of isomers on GC columns (packed, and to a much smaller extent, capillary) is a significant problem. See Table 2-17 for some examples.

In their method for analysis of polycyclics, Grimmer and Bohnke (1975a) use a 5% OV101 column with flame ionization detector. This system has been used by many investigators. The OV101 systems will not separate B(a)A/Chr/Tri or the benzofluoranthenes. The authors state that OV17 packing will provide the B(a)A/Chr separation and capillary column systems are necessary for the benzofluoranthenes.

Several of the Dexil series of packings have been evaluated for

Table 2-17. Column Packings Used in GC Analysis of PAH

TYPE OF SAMPLE	COLUMN TYPE	CONDITIONS	INTERFERENCES	REFERENCES
High-protein foods, oils, fats	57% OV101	2 & 3 rings; 120° 240° @ 1°/min 4-7 rings, 250° iso-thermal	B(a)A/Chr/Tri, B(b)F/B(k)F/ B(ghi)F	Grimmer & Bohnke, (1975)
Chlorinated water samples	1.95% SP2401, 1.5% SP2250	182° iso-thermal photo-ionization detection	not mentioned	Oyler et al., 1978
Smoked and charbroiled food	5% OV101	260° iso-thermal	B(a)P/Per, partly resolved	Panalaks, 1976
Atmospheric samples	Dexil 300, " 400, " 410. OV101 for GC-MS	165° - 295° at 4°/min	Dexil 300 Ph/A, B(a)A/ Chr/Tri, B(a)P/ B(e)P, B(ghi)Per/ anthanthrene Dexil 400,410 Ph/A, Chr/Tri, B(k)F/B(b)F, B(ghi)Per/anth- anthrene	Lao et al., 1975
Standards only	Liquid crystal BPhBT (see text)	270° to 290° iso-thermal		Janini et al., 1976
Sediments	2% Dexil 300	100° to 300°	Chr/B(a)A	Cretney et al., 1980

polycyclic analysis by Lao et al. (1975). The greater thermal stability of these phases makes them logical candidates for chromatographing the higher molecular weight PAH. As can be seen in Table 2-17, there are several groups of polycyclics which are unresolved on both Dexil 300 and Dexil 400. Dexil 400 can separate, for example, B(a)P from Chr or Tri and B(a)P from B(e)P. Since this packing can achieve the same separations as Dexil 410, but with shorter analysis times, Dexil 400 was the packing of choice for Lao and co-workers.

Since so many conventional packing materials have trouble separating important polycyclics, considerable interest has been shown in liquid crystals as stationary phases. Janini et al. (1976) reported using the liquid crystal N,N'-bis(p-phenylbenzylidene) α,α' -bi-p-toluidine (BPhBT) for PAH analysis. Separation of isomers depends on their length-to-breadth ratio; molecules having a larger ratio (rod-like molecules) are retained longer. For example, diB(a,c)A, B(ghi)Per, diB(a,h)A, picene and pentacene were all baseline resolved at 270°C. There are some serious limitations to the use of liquid crystals, including a narrow dynamic range (225° to 270° with optimum at 270°C), relatively low column efficiencies, some problems with column bleed, short lifetimes and no data on alkylated PAH. These problems vary among liquid crystals and new compounds may be developed to minimize these difficulties.

Grob and Grob (1975) compared a packed column and a capillary column, both OV1, for PAH analysis. The greater efficiency of the capillary column is immediately evident; the packed column indicated that a water sample contained 118 PAH compounds, while the capillary column separated 490 components.

The great increase in resolution in going from packed to capillary columns reduces the separation problems but it can be seen from Table 2-18 that there are still interferences. It is sometimes difficult to assess separation due to lack of information provided by various authors. Also, no single system will be able to separate all the PAH present in a complex sample, but should separate the important polycyclics (e.g. Chr/Tri/B(a)A, or B(e)P/B(a)P). It is often the alkylated PAH that are extremely difficult to separate (Vassilaros et al., 1982a; Lee et al., 1979a; Bjørseth et al., 1979).

In studying the effect of pyrolysis temperature on the production of methylated A and methylated Ph, Adams et al. (1982) used both the non-polar OV101 and the polar Carbowax 20M to separate the methylated PAH. They weren't able to use the OV101 for separation of 1-meA/9-mePh, or 4-mePh/4,5dimePh.

One of the problems with using capillary columns for PAH is that, with so many peaks resolved, they then have to be identified. The identified peak also requires confirmation, preferably by capillary GC-MS. Lee et al. (1979a) developed a system of retention indices for PAH on a capillary SE52 column. A retention index system based on four polycyclics (N, Ph, Chr, picene) shows better reproducibility (and less sensitivity to changes in conditions) than the system based on n-alkanes. The indices were insensitive to column bore size, film thickness or the temperature program used. Over two hundred PAH were included in the retention index, including some alkylated or nitrogen substituted polycyclics.

The retention index system was used in the capillary G.C. analysis of PAH in fish tissue (Vassilaros et al., 1982a). Up to 17 compounds

Table 2-18. Capillary Columns Used for PAH Analysis

TYPE OF SAMPLE	COLUMN TYPE	CONDITIONS	SOME POSSIBLE INTERFERENCES	REFERENCES
Mussels	50 m SE 54	120° - 250°	mePh/meA, Chr/Tri, B(j)F/B(k)F, diB(a)A/diB(ah)A	Bjørseth <u>et al.</u> , 1979
Smoked meats	55 m SE 54	165° - 225°	Chr/Tri, B(j)F/B(k)F	Larsson, 1982
Sediments	20 m SE 52	60° - 250°	Chr/Tri	Giger & Shaffner 1978
Fish tissue	13-20m SE 52 fused silica	50° - 250°	Ind-P/diBA, alkylated PAH	Vassilaros <u>et al.</u> , 1982a
Atmospheric samples	40 m SE 30	190° - 290°	meChr/B(a)A/Tri, mePh/meA, meB()F/B()P	Choudhury and Bush, 1981
Maize	50 m SE 54	180° - 250°	None mentioned for quantitated PAH	Winkler <u>et al.</u> , 1977
Air samples	19 m SE 52	70° - 250°	mePh/meA, meBP/meBF	Lee <u>et al.</u> , 1977
Standards only	12 m SE 52	50° - 250°	dimeN, B(b)F1/meP, B(a)A/B(a)acridine Chr/Tri	Lee <u>et al.</u> , 1979
Water samples	35 m → 60 m OV1	25° - 175° 25° - 250°	None mentioned	Grob and Grob, 1975
Pyrolysis products	OV101, 50 m and 25 m Carbowax 20 M, fused silica	150° - 230° for OV101 100° - 200° for carbowax	mePh, meA	Adams <u>et al.</u> , 1982
Air samples	OV 17	100° - 260°	None mentioned	Grimmer <u>et al.</u> , 1980

were identified by cap GC and confirmation. A BASIC program (written for Hewlett-Packard 5880) was employed to provide tentative identification through the retention index system. Fish extracts were chromatographed on fused silica columns and the effluent split (to FPD and FID) to enable analysis of sulfur heterocycles among the PAH. A nitrogen-phosphorus detector was also used to identify nitrogen heterocycles.

Vassilaros and co-workers (1982a) report a detection limit of < 0.2 ppb (wet wt) for PAH in fish. Winkler et al. (1977) reported a sensitivity of 0.25 ng B(a)P by capillary GC while Christiansen and May (1978) report detection limits of 0.007 to 0.21 ng for HPLC fluorescence or UV detectors. Capillary GC's much greater resolution allows small peaks (possibly shoulders of much larger peaks by HPLC) to be identified and quantitated. In general, capillary GC systems allow detection in the low part per billion range (Giger and Schaffner, 1978 and Bjørseth et al., 1979). Futoma et al., (1981) concluded that 0.1 to 30 ng of individual PAH can be detected by FID, the most common detector for polycyclics.

The use of fused silica columns should result in better chromatography of polycyclics due to fewer active sites. Columns with bonded phases should provide further chromatographic improvements, as well as extended lifetimes due to resistance to stripping liquid phase by injection solvents.

The FID and mass spectrometer are by far the most widely used GC detectors for PAH. The FID is sensitive to organics but gives little information for peak identification. As mentioned above, NPD and FPD are also employed for selective detection of heterocycles (Vassilaros et al., 1982a). The photoionization detector (PID) has been applied to

polycyclic analysis (Oyler et al., 1978) with ten to forty times the sensitivity to PAH. Since this detector has little sensitivity to solvents such as acetonitrile or water, fractions from LC analysis were injected directly. The authors found variations in detector linearity and reproducibility with prolonged use. These drawbacks may be reduced with improved detector design.

6. Gas Chromatography Mass Spectrometry (GCMS)

The GC MS combination has been widely used to provide identification of polycyclics. Table 2-19 lists some of the applications of GCMS to various sample types. Under conditions of electron impact ionization, PAH have very simple spectra, with little fragmentation (Seversen et al., 1976; Hase et al., 1976). The M^+ ion is very stable and quite intense, with a much less intense $(M-1)^+$. A double charged ion at $(M-2)/2e^-$ is often large and in substituted PAH there may be peaks at $(M-C_2H_2)^{2+}$. The lack of fragmentation under electron impact ionization makes it very difficult to distinguish isomers. Thus for isomers that differ in their carcinogenic potential, the best possible separation by capillary G.C. is recommended.

Lane et al. (1980) experimented with a quadrupole system with chemical ionization carried out at atmospheric pressure. When using charge transfer conditions (benzene as chemical ionization reagent) some isomer pairs could be distinguished on the basis of different $(M+1)/M$ ratios. N, Fl, 9-fluorenone, Ph, A, F, and P were studied. Shushan et al. (1979) investigated linked-scan monitoring of metastable ions to distinguish isomers of molecular weight 228 (Chr, Tri, B(a)A, B(b)A). In this study B and E are linked (B/E is constant) to transmit

Table 2-19. Example of GCMS Systems Used for PAH Analysis.

Type of Sample	Type of Column	MS System	Comments	Reference
water samples	11m SE54	Finnigan 4023, EI	used as evaluation of EPA protocol	Nowicki <u>et al.</u> , 1980
standards only	-	TAGA 2000 APCI-M, CI	mobile, real time sampling	Lane <u>et al.</u> , 1980
fly ash	30m SE54	HP 5985, SIM	complements HPLC with fluorescence	Zelensky <u>et al.</u> , 1980
combustion effluents	Dexil 300	Finnigan 3200, CI, methane	confirmation of PAH	Strup <u>et al.</u> , 1976
air samples	30m SE52	HP 5982A	mixed charge exchange - chemical ionization to determine isomeric PAH	Lee <u>et al.</u> , 1979b
sediments	13-20m SE52	HP 5985	confirmation of PAH	Vassilaros <u>et al.</u> , 1982a.

ions with $m/e = 228$. An integrating ion monitor was set to detect $m/e = 228$ peak as well as any resulting ions (daughter ions). After examining the relative intensities of the daughter ions, only M, (M-1) and (M-2) were eventually monitored. It was found that the ratios of relative intensities (M-2)/(M-1) and (M-1)/M are distinctive for each isomer and these ratios can be related to the number of hydrogen atoms in each molecule which are capable of benzo isomers (B(b)A,0; B(a)A,1; Chr,2; Tri,3). Thus the four interactions could be identified on the basis of the two ratios (M-2)/(M-1) and (M-1)/M.

Using mixed charge exchange-chemical ionization MS of peaks from capillary GC, Lee et al. (1979b) were able to distinguish between isomers of unsubstituted PAH in meA/mePh and meF regions. Using a chemical ionization reagent consisting of 5-10% methane in argon, quite different $(M+1)^+/M$ ratios are obtained for A and Ph, as examples. In methylated polycyclics, the PAH isomer can be determined but the position of the methyl group on the backbone structure cannot be identified. Ten nanograms per PAH is required for a spectrum under these conditions (compare to less than one nanogram required for most fluorescence HPLC detectors). Single Ion Monitoring (SIM) as in Lane et al. (1978) can detect as low as one nanogram of compound.

Zelensky et al. (1980) compared results obtained by HPLC with fluorescence to those obtained with SIM capillary GCMS. It was found that the fluorescence system was more sensitive but resulted in some mis-identifications. They recommend HPLC-fluorescence for screening and GCMS for confirmation.

A great number of studies on PAH use GC with GCMS as confirmation. The GCMS is usually carried out in the electron impact mode. Despite

somewhat lower sensitivity to PAH, the vast amount of structural information provided by capillary GCMS makes it the method of choice at present (Futoma et al., 1981; Lee and Wright, 1980). Even the most efficient capillary GC systems will not be able to separate all the PAH present in environmental samples but the important isomer pairs should be separable.

CHAPTER III

EXPERIMENTAL TECHNIQUES

A. OUTLINE OF ANALYTICAL SCHEME

1. Scope

In order to quantitate the extent of PAH residues in the sediments of the Still Creek system, samples of stream sediments were taken at different points along the creek, once in 1978 and again in 1979. Preliminary investigations into polycyclic levels incorporated into the sediment organisms was also carried out. Since street run-off was suspected as a source of contamination to the Still Creek system, street surface contaminants were sampled in the second year of sampling. And finally the contribution of crankcase oil to street contaminations was investigated through sampling crankcase oil at various mileages. As additional data, the percent organic content and the particle size distribution of both stream sediments and street deposits were determined.

A set of twenty PAH were chosen as target compounds in the analytical scheme, these polycyclics were reported in environmental studies by other researchers.

2. Analytical Scheme

Stream sediments, street surface deposits and sediment organisms (mainly oligochaetes) were all extracted according to the scheme developed for sediments and tissues by Dunn (1976). This procedure involves a basic hydrolysis (in ethanol) of the sediment, partition of polycyclics into iso-octane, and clean-up of the extract with Florisil

chromatography and a DMSO iso-octane partition. A preliminary separation of the PAH was carried out on an alumina column (Sorrell et al., 1977) before analysis by HPLC.

Crankcase oil samples were analyzed using an abbreviated form of the extraction procedure for sediments.

3. Instrumental Analysis

Sediment extracts were analyzed by reversed-phase HPLC with UV detection. Retention times of the extract peaks relative to retention times of standards (injected either before or after the sample) were determined at two mobile phase compositions. These retention times, combined with comparisons of absorbance ratios (absorbance₂₅₄: absorbance₂₈₀) and separation into three fractions by alumina chromatography, allowed tentative identification of sample peaks to be made. The peaks were collected (with a larger injection size) during HPLC separations for determination of their UV spectra. Thus peak identification was based on retention times, UV absorbance ratios and UV spectra.

Several sediments were analyzed by capillary GC with a flame ionization detector. Standard solutions were injected to act as markers and some of the peaks were tentatively identified using the retention index developed by Lee et al. (1979a).

GCMSDS (DS = data system) analysis (with capillary column) was carried out on a few samples to screen for the twenty target compounds or, in one case, to examine all major peaks for the presence of PAH.

4. Data Analysis

Correlations were examined between levels of specific polycyclics (or also total quantitated PAH), watershed characteristics and various sediment parameters. The total area of the LC chromatograms (considered as equivalent B(a)P) was also examined with respect to correlations with watershed and sediment characteristics. The levels of PAH in street deposits and in organisms were treated similarly.

Crankcase oil profiles were compared to those of sediments for similarities and differences.

B. SAMPLING

1. Sediments

Stream sediments were sampled at five different sites, using some of the locations sampled by researchers Hall, Yesaki and Chan (1976). The sampling locations are listed in Table 3-1 and indicated in Figure 3-1. Samples were obtained with a metal scoop and stored in double plastic bags. Sediments were frozen until required for analysis and after thawing and mixing, a weighed subsample taken for extraction.

2. Street Surface Contaminants

Sampling locations for street surface contaminants are also listed in Table 3-1 and indicated in Figure 3-1. Samples were obtained in September 1979 using a metal spoon and were frozen in plastic bags. After thawing and mixing, a weighed subsample was taken for analysis.

3. Oligochaetes

Samples of stream sediments for oligochaetes were taken at the

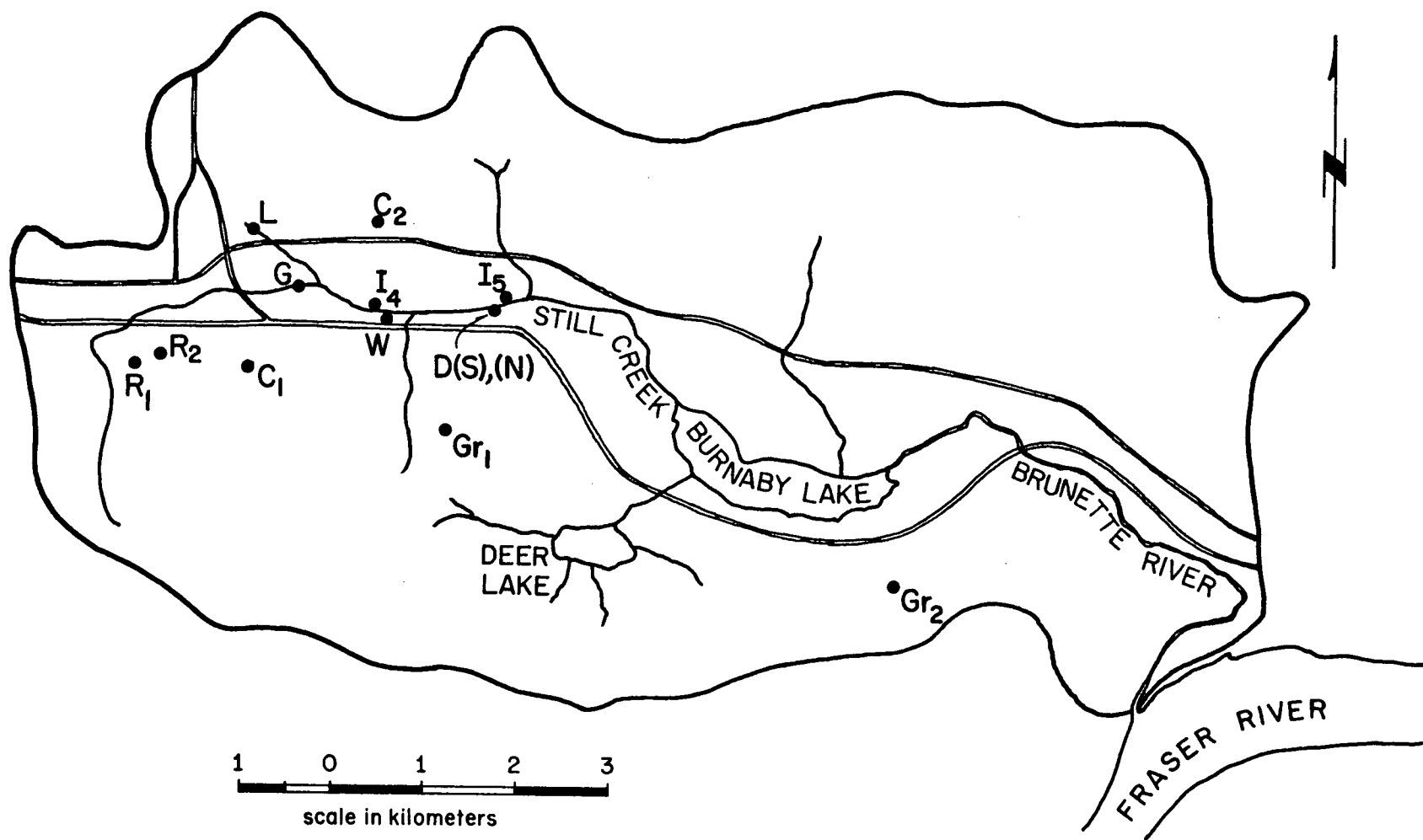


Figure 3-I Sampling Locations

Table 3-1. Sampling Locations

Location/ Land Use	Abbreviation	Description	Equivalent ^a	
			Metals	Organics
Sediments				
Lougheed	L('78)	49°15'57"N 123°01'05"W	32	21
	L('79)			
Gilmore	G('78)	49°15'40"N 123°00'48"W	31	18
	G('79)			
Willingdon	W('78)	49°15'33"N 123°00'13"W	30	17
	W('79)			
Douglas	D(N)('78)	49°15'38"N 122°58'59"W	35	12
	D(N)('79)			
	D(S)('78)			
	D(S)('79)			
Street Surface				
Industrial	I ₄	Willingdon north of 401 interchange	I ₄	I ₄
	I ₅	Douglas Road at Still Creek	I ₅	I ₅
Commercial	C ₁	Canada Way at Boundary Road	C ₁	C ₁
	C ₂	Willingdon at Lougheed (Brentwood Shopping Centre)	C ₂	C ₂
Residential	R ₁	E.14 Ave.(Blk.E. of Renfrew)	R ₁	R ₁
	R ₂	E.16 Ave. (between Renfrew and Rupert Street)		
Green Space	Gr ₁	Forest Lawn Cemetary	G ₁	G ₁
	Gr ₂	Robert Burnaby Park	G ₂	G ₂

^a Hall *et al.* 1976. Equivalent sampling sites for metals and for chlorinated hydrocarbons.

same time as the 1979 stream sediments. Organisms and coarse organic detritus were removed from the sediment with a $0.61\ \mu$ sieve. The oligochaetes were preserved with small amounts of formaldehyde, and refrigerated.

4. Crankcase Oil

Crankcase oil was sampled unused and then at intervals of approximately 500 miles (800 km) for up to 5000 miles (8000 km). Samples were collected through Teflon tubing under a slight vacuum, and stored (frozen) in glass vials until required for analysis.

C. GENERAL PROCEDURES

1. Glassware

Glassware was solvent rinsed after use and washed with detergent and distilled water. Each piece was rinsed in a chromic acid bath, and after thorough rinsing with tap water and distilled water, heated overnight at 150°C .

Normal lab glassware was used with the exception of the column for alumina chromatography, which was constructed with a water-cooling jacket. The column was 1 cm i.d. by 25 cm length for packing, with a reservoir and a Teflon stopcock.

Florisil chromatography was carried out using a 4 cm i.d. glass column of 40 cm length, also with a Teflon stopcock.

2. Solvents

Benzene and ethanol were analytical reagent grade while dimethylsulphoxide (DMSO), dichloromethane and pentane were spectral quality.

Iso-octane was distilled in glass over sodium. The methanol or acetonitrile for HPLC were Fisher HPLC grade and were filtered (0.5 μ filters) before use. Water for HPLC mobile phase was distilled and filtered (0.5 μ filter).

3. Chemicals and Supplies

Potassium hydroxide and granular anhydrous sodium sulfate were analytical reagent grade.

Alumina was supplied by BDH (Merck, neutral alumina for column chromatography) and was prepared by heating each portion (20 g) at 150°C for 16 hours. After being removed from the oven, alumina was stored in an evacuated dessicator until use.

Florisil (100-200 mesh) was prepared according to Dunn (1976), by separating out the fines and washing with water to remove any sodium sulfate. After removing water by rinsing with methanol and drying overnight, the Florisil was activated at 250°C for 18 hours and cooled before being deactivated to 2% water. Florisil was prepared in batches of about 250 g.

The fluor used for liquid scintillation counting in recovery studies was Bray's solution, chosen for its compatibility with both organic and aqueous phases. Bray's solution is comprised mainly of dioxane with PPO (2,5-diphenyl-oxazole, 0.4%) as the primary solute. Secondary solute is M₂POPOP (2,2,1(1,4-phenylene)bis[4-methyl-15-phenyloxazole], 20%); ethylene glycol (20%), methanol (10%) and naphthalene (6%) are also added.

4. Determination of Particle Size and Organic Content

Wet sediments and street surface contaminants were heated at 110°C

to dry thoroughly, and this weight was used in dry weight calculations. The dried sample was disaggregated in a mortar and sieved, resulting in four fractions; coarse sand and larger (>0.589 mm), medium sand ($0.589-0.250$ mm), fine or very fine sand ($<0.250-0.074$ mm) and silt or clay (<0.074 mm). Weight of sediment in each fraction was determined.

Percent organic matter was calculated by determining the portion of the dry sediment sample which was lost on ashing at high temperature (600°C).

5. Standards

Analytical standards were obtained for approximately fifty polycyclics. The majority of these were donated through the kind generosity of Bruce Dunn, of Cancer Research. The remaining PAH were ordered from K and K Fine and Rare Chemicals. Solutions of the polycyclics (singly or as mixture) for HPLC analysis were dissolved in acetonitrile (HPLC grade).

D. EXTRACTION AND CLEAN-UP PROCEDURES

1. Sediments and Street Surface Contaminants

The procedure for extraction of polycyclics from sediment was taken from Dunn (1976), with some minor modifications (Figure 3-2). A weighed sample was refluxed with KOH (5 g), ethanol (100 ml) and boiling chips for 1.5 hours. Liquid extract was decanted off the solid material and filtered through a glass wool plug into a 2 litre separatory funnel. Sediment was washed with two further portions of ethanol (2×50 ml), which were added to funnel. After addition of water (150 ml), the extract was partitioned with iso-octane (3×200 ml). These

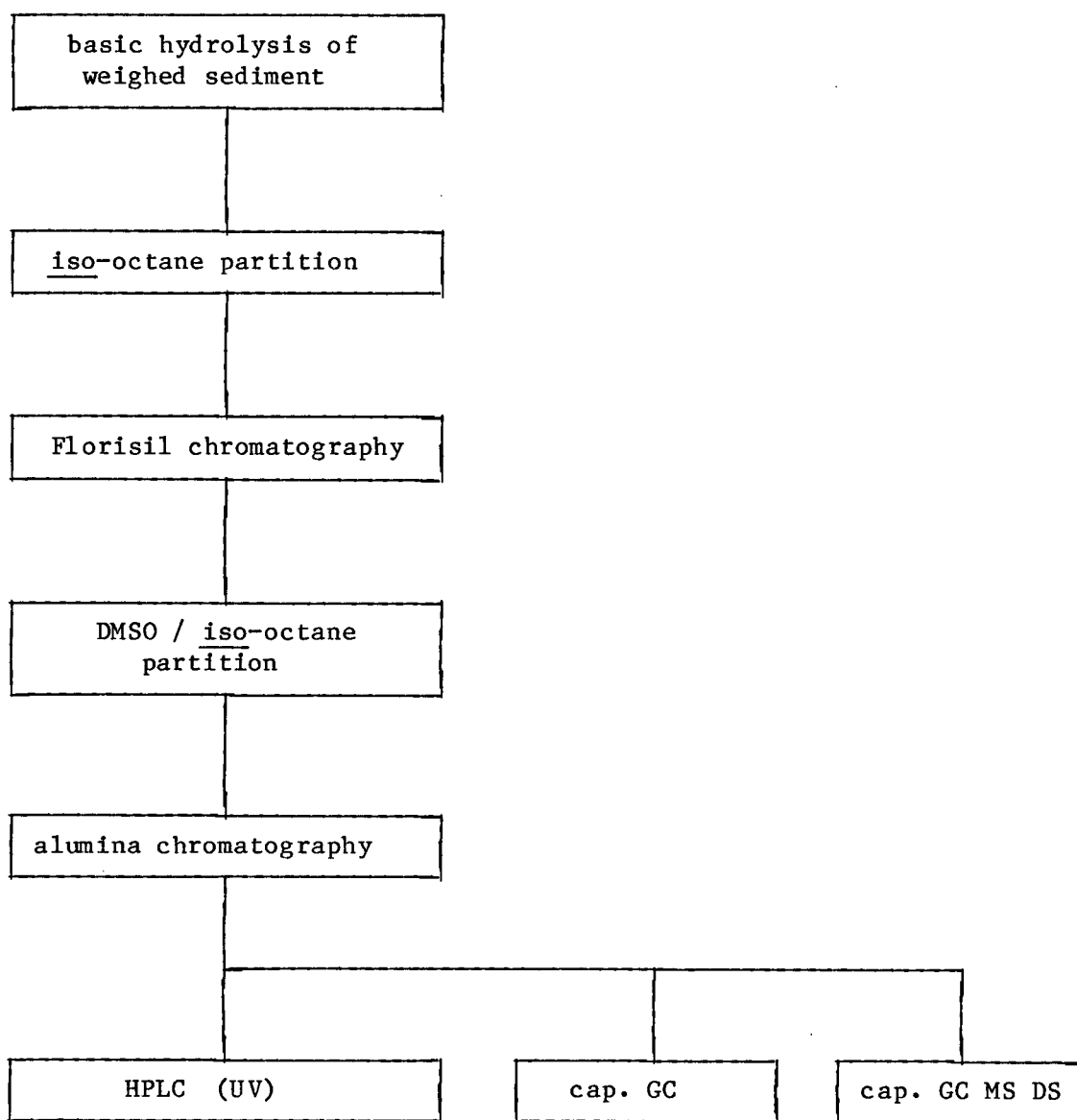


Figure 3-2 Analysis Scheme

combined organic portions were washed with another four volumes of water (4 x 200 ml). A Florisil column (30 g Florisil topped with 60 g Na_2SO_4) was prepared and the iso-octane fraction passed through the column, then the aliphatics were washed off the column with further iso-octane (2 x 100 ml). Polycyclics were eluted from Florisil with benzene (3 x 100 ml) and the benzene was removed from the extract by evaporating on a rotary evaporator to near dryness (5 ml), addition of iso-octane (50 ml) and reducing the volume once again to 5 ml.

The iso-octane PAH extract was partitioned with DMSO (3 x 5 ml) in order to eliminate some fluorescent interferences. PAH were back-extracted into iso-octane by addition of water (30 ml) to the combined DMSO portions and partitioning with fresh iso-octane (2 x 10 ml). Any residual DMSO was removed with water washes (3 x 20 ml) and extract dried through a bed of sodium sulphate (10 g). Extract volume was reduced to approximately 0.5 ml for alumina chromatography.

At this point an alumina column was included as a preliminary separation of the polycyclics (see Sorrel et al., 1977). The alumina column was prepared by wetting very lightly packed alumina (16 ml) with pentane (15 ml), and topping with Na_2SO_4 (2 cm). After rinsing with dichloromethane (20 ml), the column was reconditioned with pentane (20 ml). The polycyclic extract was added to top of column followed by pentane from two small rinses of the flask. Pentane from the first elution (I, 30 ml - see Figure 3-3) was used to rinse the flask. The first and second fractions (II, 25% CH_2Cl_2 in pentane, 20 ml) were discarded, while the third, fourth and fifth fractions (III 50% CH_2Cl_2 in pentane, 25 ml; IV 50% CH_2Cl_2 , 5 ml then 75% CH_2Cl_2 in pentane, 30 ml; V CH_2Cl_2 , 60 ml) were collected and the solvent evaporated to near

alumina column (16 ml) with extract (0.5 ml)				
elute				
I	II	III	IV	V
pentane (30 ml)	25% CH ₂ Cl ₂ in pentane (20 ml)	50% CH ₂ Cl ₂ in pentane (25 ml)	50% CH ₂ Cl ₂ in pentane (5 ml)	CH ₂ Cl ₂ (60 ml)
- discarded	-discarded	-Ph -A -P -F -B(e)P -Fl -2-mePh -3,6-dimePh -9,10-dimeA -Tri -B(a)Fl -B(a,j)acridine -9-meA	then 75% CH ₂ Cl ₂ in pentane (30 ml)	- B(b)F - B(k)F
			-B(a)P -Chr -B(a)A -Per -B(ghi)Per	

Figure 3-3 Alumina Separation of Polycyclic Extract

dryness on a rotary evaporator. Extracts were made up in 1.0 ml CH_3CN for HPLC analysis.

2. Oligochaetes

Once the oligochaetes were removed from the sediment with tweezers, they were washed with ethanol to remove water and then dried at 110°C overnight. Dried oligochaetes were ground carefully with mortar and pestle to a fine powder. The extraction of ground organisms continued as for sediments.

3. Crankcase Oil

A subsample (1 ml) of the crankcase oil was partitioned with DMSO (3 x 1 ml) then water (5 ml) was added to the combined DMSO fractions. PAH were back-extracted into iso-octane (2 x 5 ml) which was dried through Na_2SO_4 before being replaced with acetonitrile (1 ml) for HPLC.

E. RECOVERY STUDIES

A sediment sample was spiked with 0.5 μCi (0.5 ml) as $[7,10\text{-}^{14}\text{C}]$ B(a)P or as $[1(4,5,8)\text{-}^{14}\text{C}]$ N and extracted as per regular extraction procedure sediments. Sub-samples of extracts from different steps in the procedure were dissolved in Bray's solution and counted for radioactivity on a LS8000 Liquid Scintillation Counter (Figure 3-4). To determine recoveries, it was necessary to make corrections for the radioactivity removed at each step in the procedure.

Recovery studies indicated problems with preparation of both Florisil and alumina. Standardized procedures for preparing the adsorbents (see General Procedures C-3) resulted in reproducible chromatography.

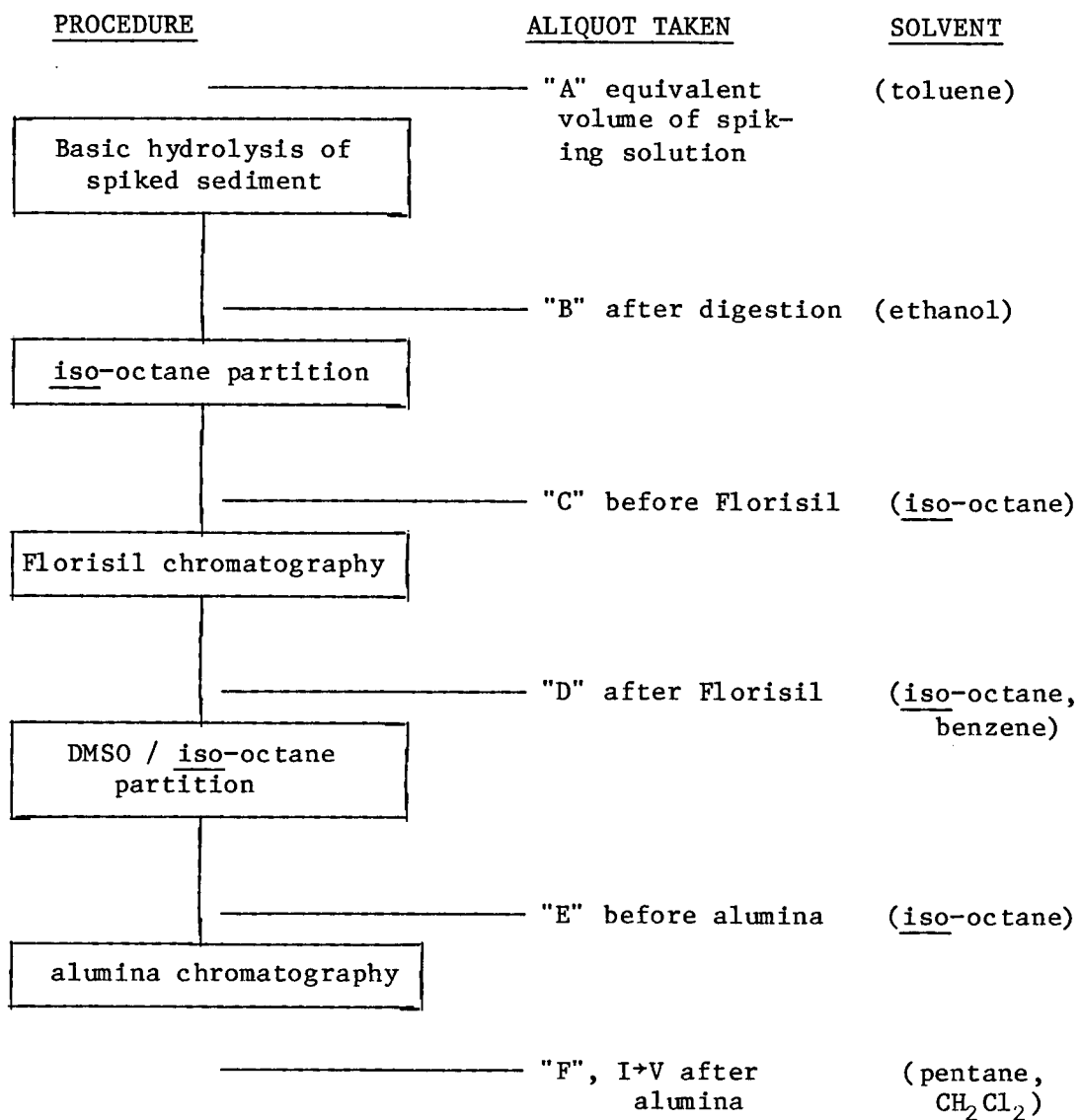


Figure 3-4 Aliquots Taken for Liquid Scintillation Counting

F. INSTRUMENTAL ANALYSIS

1. Liquid Scintillation Counting

Liquid scintillation counting was carried out using a Beckman LS8000 series counter with a Silent 700 data printer. A series of seven standards and one background sample were incorporated into the program in order to construct a quench curve. Counting efficiency was determined for each sample and was indicated by the appropriate "H-number". This allowed automatic quench compensation to be applied to the samples. The preset counting time was 10.00 min. and data reported as normalized disintegrations per minute.

2. HPLC

HPLC analyses were carried out on a Waters LC system. Model 6000A pumps provided mobile phase flow and were controlled by a model 720 System Controller. Injections were made on the U6K injector. Peaks were detected at 280 nm and 254 nm, with the 440 UV detector. The signal from 280 nm detector cell was integrated by the printer-plotter, model 730 Data Module. Detector parameters were selected manually and wavelengths changed by selecting appropriate filters.

An analytical μ -Bondapak- C_{18} column (10 μ , 30 cm x 5 mm i.d.) was preceded by a Corasil- C_{18} guard column (40 μ , 4 cm x 7 mm i.d.).

Elution was isocratic.

Conditions used are listed in Table 3-2.

3. UV Spectrophotometer

UV spectra were obtained for all the PAH standards available

Table 3-2. HPLC Conditions

Mobile phase:	60% CH ₃ CN or 70% CH ₃ CN	40% H ₂ O 30% H ₂ O
Flow:	1.0 ml/min	
Detector:	280 nm 254 nm	
AUFS:	0.02 or as required	
Chartspeed:	0.5 cm/min	
Area rejection:	100	
Noise rejection:	50.000	

Table 3-3 Capillary GC Conditions

Inlet temperature:	225°C
Detector temperature:	325°C
Oven temperature:	- program 40°C for 2.00 min 2.00°C/min to 250°C hold 20.00 min post run at 300°C for 5.00 min
Chart speed:	- program 0 to 4.00 min: 0.5 cm/min 4.00 to 60.00 min: 0.25 cm/min 60.00 to 88.00 min: 2.00 cm/min 88.00 to 130.00 min: 0.5 cm/min
Attenuation:	2+0
Threshold:	0
Peak width:	0.02

(approximately fifty compounds). Spectra were also used to aid in identifying peaks collected from HPLC fractions.

The samples were run on a Cary 15 instrument using a 10 mm path-length. Fractions collected from HPLC runs were placed in small volume (approximately 0.7 ml) 10 mm cells.

Generally standards or collected fractions were scanned 400 nm to 220 nm at 5 mm/sec. Sensitivity was set at "4" and spectra run at either 1 or 0.1 AUFS, as required.

4. Capillary GC

Several of the samples run on HPLC were also run on a capillary GC system. A Hewlett-Packard 5880A GC with a capillary splitless injection system was used with a flame ionization detector. The fused silica capillary column was a Hewlett Packard methyl silicone (SE54), 0.31 mm i.d. x 20 m. Samples were injected in CHCl_3 .

Conditions used are listed in Table 3-3. A post-run heat up was included to prevent interference by late-eluting peaks in subsequent runs.

5. GCMSDS

GCMSDS analysis was carried out on a few of the sediment extracts. An HP 5840 GC with a capillary column was used in an HP 5985 GCMSDS. The splitless injection technique was used on an HP SE54 column 30 m x 0.31 mm i.d. The system was calibrated for mass with an injection of perfluorotributylamine. Decafluorotriphenylphosphine (200 ng) was used as internal standard. The GC was programmed from 60°C (held for one minute) to 290°C at 8° per minute.

G. STATISTICS TREATMENT

Data from HPLC analysis of samples was collected and analyzed for possible correlations, using the Triangular Regression Package computer program (Le & Tenisci, 1977). This package (revised Oct., 1978) permits application of regression analysis to various sets of data.

Three routines were used to treat the data:

- 1) INMSDC - treatment of raw data to produce means, standard deviations and correlations,
- 2) SIMREG - provides simple linear regression equations,
- 3) STPREG - step wise regression - performs simple and multiple regression, also step wise backwards and forwards regression analysis.

The final regression equation is in the form

$$y = b_0 + b_1y_1 + \dots + b_my_m$$

where b = constant and m = number of variables.

Output from the STPREG routine includes several parameters which allow assessment of the accuracy of regression equation:

- 1) R^2 - squared multiple regression coefficient - R^2 takes values between 0 and 1, fit of equation to data improves as R^2 approaches 1,
- 2) F-probability - probability of obtaining an R^2 value greater than or equal to the value calculated, if the F-probability is less than 0.05, indicates that R^2 is significantly different from zero.

Missing data was flagged by use of the code "-99" in the raw data. In most cases, names were assigned to variables used in regression

analysis.

Data sets requiring STPREG were generally run using backwards stepwise regression, i.e. forcing all variables into the equation at the first step and allowing the insignificant variables to be eliminated one at a time.

Files were created for required data under the names STREAM, STREET, BUGS, and OILS to apply to the four types of samples analyzed.

CHAPTER IV

RESULTS AND DISCUSSION

A. DEVELOPMENT OF ANALYTICAL SCHEME

1. Introduction

In this chapter the results of the experiments outlined in Chapter III are presented and discussed. Chromatograms (HPLC or capillary GC) are included as required to illustrate results or as typical examples. Conditions for each figure may be found in Appendix 3.

2. Choice of Target Standards

Twenty target PAH were chosen such that they satisfied the following requirements: 1) standard material was available; 2) compounds chromatograph on HPLC system used in experiments; 3) peaks were separable on LC column; 4) peaks detectable at both 280 nm and 254 nm; 5) compound eluted from alumina; 6) compound not significantly split between alumina fractions; 7) values for absorbance ratios available; 8) PAH had been found in environmental samples previously; 9) some of the important isomers included; 10) EPA priority pollutants included.

Of the compounds targeted 11 are on the EPA priority pollutant list. The twenty compounds eventually selected (see Figure 3-3 for distribution of the target compounds among the three alumina fractions) were separable at a mobile phase of 60% CH₃CN (with three pairs of interferences at higher ambient temperatures) but some co-eluted at 70% CH₃CN. See Appendix 3. As well, meA and mePh absorb very weakly at 280 nm, making the ratio of absorbance difficult to determine.

3. Choice of Analytical Method

Prior to selection of basic hydrolysis/digestion of sediments as the analytical approach, other methods were investigated.

Samples were originally prepared using the Soxhlet extractions described by Giger and Blumer (1974), involving 24-hour extraction with methanol and then the same with benzene. This method was time-consuming and required large volumes of solvent. Also the HPLC chromatograms of crude extracts were very complex, so various column clean-up methods were investigated.

Florisil was used with iso-octane to apply crude sediment extract to column while PAH were eluted with benzene. Polycyclics were successfully eluted, however very little fractionation was obtained. Alumina systems were also explored, starting with the system described by Giger and Blumer (1974). PAH were eluted from an alumina column (packed in pentane) with increasing strength of dichloromethane in pentane. The majority of the polycyclics encountered in sediment extracts eluted very quickly, resulting in little improvement in HPLC chromatograms.

Various alumina systems were devised using petroleum ether to pack the alumina and then increasing percentages of benzene to elute polycyclics. It was found that the elution patterns were very sensitive to the activity of the alumina.

Taking into account the difficulties encountered with the extraction system and the alumina clean-up, a combination of the extraction and preliminary clean-up steps from Dunn (1976) and the alumina column chromatography from Sorrell et al. (1977) was adopted. This allowed extraction of the sediments in 1.5 hours rather than 48 hours, and with

smaller volumes of solvent. The alumina system developed by Sorrell et al. allowed separation into three fractions several of the polycyclics which would otherwise interfere during HPLC analysis (i.e.

B(a)P/B(e)P/B(k)F/B(b)F).

4. Development of LC System

Original LC instrumentation comprised a single M-6000 pump, syringe injector, a 254 nm detector and a strip-chart recorder. As updated modules were made available a system was finally assembled with two pumps and a controller, loop injector, multi-wavelength detector and a data processor (see Section III F-1, Table 3-2).

During the time the LC system was limited to one pump, gradients were developed by adding the stronger eluent through a dropping funnel to the stirred mobile phase. This approach lacked reproducibility since flow from the dropping funnel was difficult to adjust accurately. A more reproducible result was obtained by drawing the stronger eluent into the mobile phase (in a sealed flask) from an unsealed flask. As mixed mobile phase was pumped out, the resultant vacuum drew the stronger eluent into the stirred mobile phase. It was found that this gave reproducible gradients, with a slightly convex profile.

When two pumps and a system controller were installed, gradients of various shapes were easily obtained, with each pump supplying a different eluent. Eluents investigated include CH_3OH or CH_3CN in combination with H_2O . A small percentage of iso-propanol was used with CH_3OH gradients in order to sharpen up later-eluting peaks. Eventually isocratic elution was chosen in order to exploit greater retention time reproducibility and to eliminate turn-around time between samples.

Table 4-1. Variability in Retention Time and Peak Area

Sample	Retention Time	Standard Deviation/(%)	Peak Area	Standard Deviation/(%)
Ph	8.20			0.6 (7%)
	8.20		8.71	
	8.20		9.85	
	8.20		8.92	
Fl	8.06	0.04 (0.5%)	4.69	0.73 (13%)
	7.93		6.10	
	7.93		5.71	
D(N)('79)III	8.20	0.04 (0.5%)		
	8.23			
	8.16			
	8.43	0.04 (0.5%)		
	8.43			
	8.36			
	10.53	0.07 (0.6%)		
	10.46			
	10.40			
	18.91	0.10 (0.5%)		
	18.80			
	18.71			
run #127	7.95	---	1.02	-9%*
	#130 7.95		1.13	
#127	8.65	---	5.71	-8%*
	#130 8.65		6.19	
#127	9.42	---	5.48	+6%*
	#130 9.42		5.17	

*percent difference wrt #130

Other parameters investigated include column temperature, stop-scan chromatography, recycle, flow rate and injection size. Increased column temperature (achieved with a heating tape in a glass tube) resulted in shorter retention times and sharper later peaks but, since a proper column heater wasn't available, temperature control was not continued due to repeatability problems.

Stop-scan chromatography was applied to a sediment extract (Perkin Elmer HPLC) but no fine detail in UV spectra was obtained.

Recycle chromatography (where two interfering peaks are eluted through the LC column several times) was investigated but was not very useful for sediment extracts since only early-eluting peaks can be treated in this way.

The LC conditions finally adopted incorporated isocratic elution at two different mobile phase concentrations ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$), monitored at two wavelengths. Flow rate (1.0 ml/min) and injection size (1.0 to 10 μl) were optimized for the sample extracts.

Detector linearity was determined for both wavelengths and a log-log plot of response versus amount injected is presented in Figure 4-1. Response on 280 nm detector is expressed as peak height in Figure 4-1; response at 254 nm demonstrates similar behaviour.

Repeatability of retention time was good for a series of injections, with standard deviation in the range 0.5%. (See Table 4-1 for examples.) Variations in retention times were experienced with changes in ambient temperature, hence the use of a mixed standard injected before or after each injection of an extract. Response (peak area quantitated at 280 nm) demonstrated greater variability (standard deviation about 10%) as shown in Table 4-1.

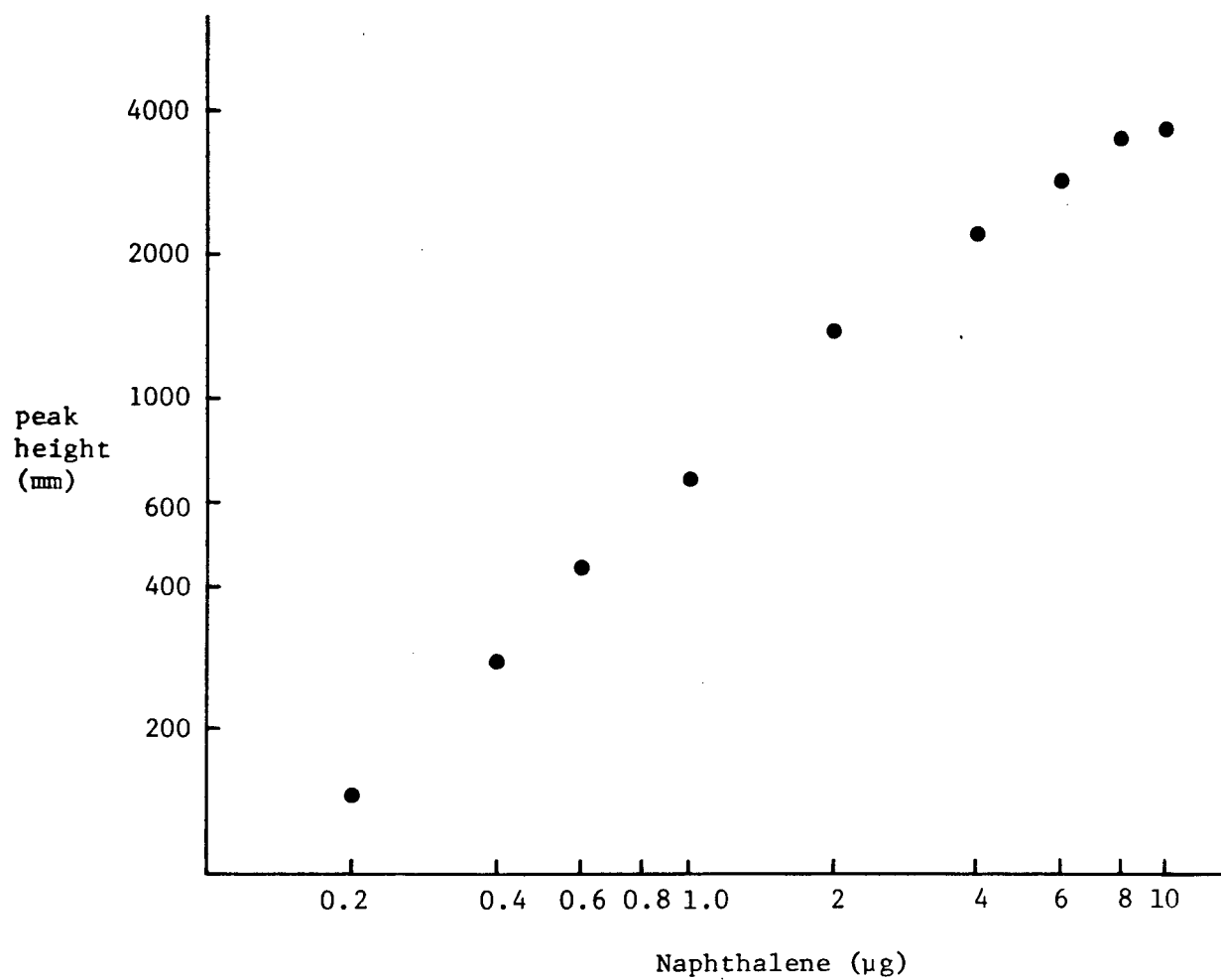


Figure 4-1. Linearity of UV Detector, 280 nm.

The limit of detection (LOD) for the twenty target PAH vary according to their UV characteristics. In general however, 1 to 2 ng PAH is required to obtain a 1% full scale deflection at 0.02 AUFS. (At this attenuation noise is less than 0.5% full scale.) See Table 4-2 for LOD at 280 nm or 254 nm. These LOD reflect a detectable polycyclic level in sediments from the low to middle part per billion range (wet weight).

5. Recoveries

Sediment samples were spiked with ^{14}C B(a)P and ^{14}C N as described in section III-E. The spiking level was 0.29 $\mu\text{g/g}$ to 0.58 $\mu\text{g/g}$ (wet weight). The amounts of labeled polycyclic were counted at six points through the procedure (see Figure 3-4). It was found that counting efficiency varied from point to point, largely as a result of high coloration or particulate matter in, most seriously, subsample B (after digestion). Although both polycyclics originally demonstrated variable recovery through Florisil, N recovery consistently dropped to less than 10% at point D (after Florisil). See Figure 4-2 for N recoveries over three trials. Investigations of the problem indicated that heavy loss of N was occurring during rotary evaporation of Florisil column eluent. No changes were made to the procedure but limitations to the scope of the procedure were taken into account.

B(a)P recoveries through Florisil were highly variable (ranging from 4% to 90%) until a standardized procedure was instituted for preparation of the adsorbent (activation overnight at 250°C, deactivation 2%, see Section III C-3). Once chromatography on Florisil was made consistent, B(A)P demonstrated overall recoveries of 62% (standard

Table 4-2. Limit of Detection for PAH Standards With UV Detector

PAH	LOD (280) ^a	LOD (254) ^b	
Ph	1.8		a-ng PAH required for a 1% response (AUFS 0.020) at 280 nm.
A	1.1		
P	2.3		
Chr	1.8		b-ng PAH required for a 1% response (AUFS 0.020) at 254 nm.
F	0.67		
B(a)A	0.59		
B(a)P	1.1		
B(e)P	0.98		
B(b)F	1.4		
B(k)F	1.5		
Per	2.8		
B(ghi)Per	2.4		
Fl	2.4		
2-mePh	27	0.027	
3,6-dimePh	2.4		
9,10-dimeA	50	0.29	
9-meA	7.4	0.40	
Tri	2.2		
B(a)Fl	1.8		
diB(a,j)Ac	0.43		

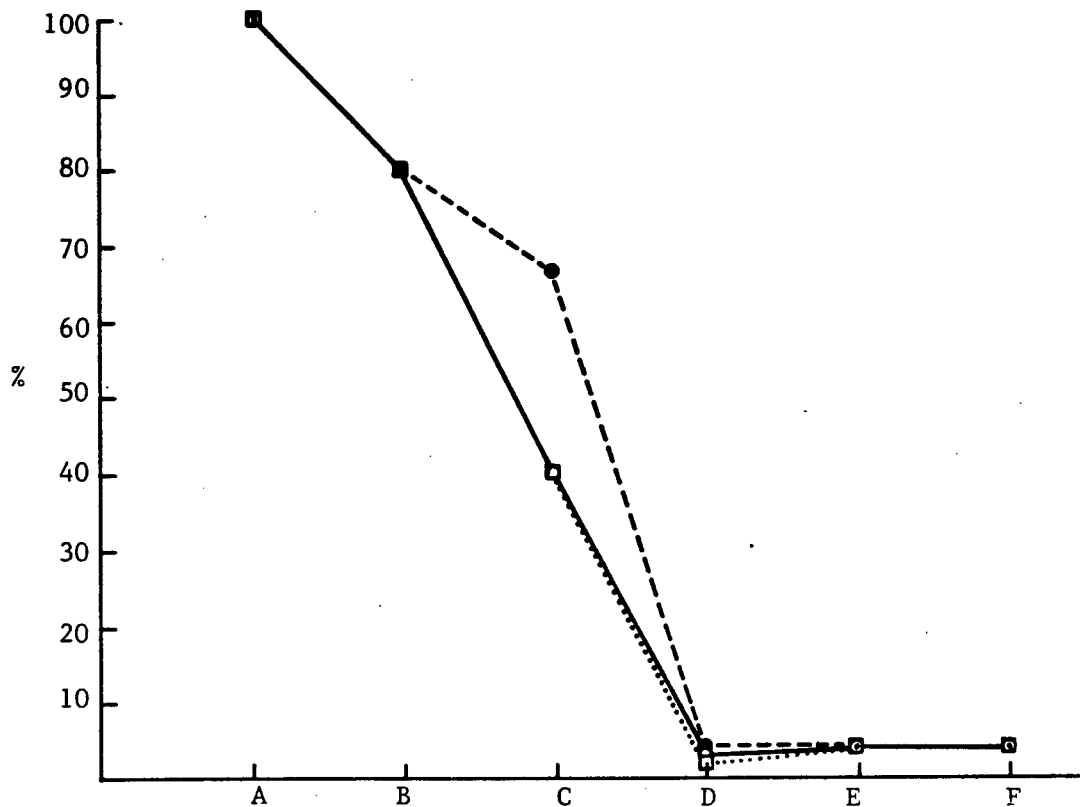


Figure 4-2. Naphthalene Recoveries, 3 Trials.

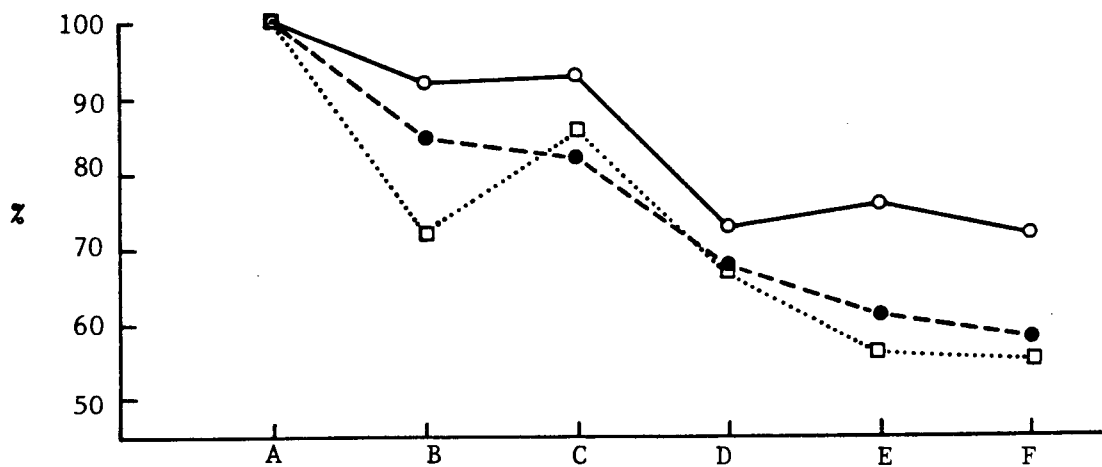


Figure 4-3. B(a)P Recoveries - No Alumina Preparation Procedure, 3 Trials

A before digestion
 B after digestion
 C before Florisil
 D after Florisil
 E before alumina
 F after alumina

deviation 13%) (see Figure 4-3). However problems subsequently developed with control over the activity of the alumina, resulting in the recovery profiles in Figure 4-4. B(a)P was being eluted in fraction II, and therefore not collected for counting (nor analysis in non-spiked sediments). Proper activation procedures for alumina (section III C-3) finally produced reproducible chromatography and high recoveries (96% average, see Table 4-3). With systems in place for preparation of both adsorbents, B(a)P recovery through the entire procedure averaged 84% over three trials.¹

This overall recovery of 84% for B(a)P is in good agreement with B(a)P recoveries reported by Obana et al. (1981) at 81-91%, or Giger and Blumer (1974) at 88%. Dunn and Stich (1976a), using basically the same procedure as in this thesis work, had 60-80% recoveries. Giger and Blumer (1974) with their very thorough fractionation procedure (see Section II E-2, II E-3) reported recoveries of Ph as only 39%, and various other PAH exhibiting recoveries between that and the 88% for B(a)P.

In general, data on repeatability is not often provided in the literature.

6. Sampling Rationale

Stream sediments were sampled to provide a profile of polycyclic concentrations in the "sink" of the watershed. The sampling locations were chosen to correspond to those used by Hall et al., 1976 (Section III A-1). Hall and co-workers studied trace metals and chlorinated hydrocarbons in the Brunette River watershed (of which Still Creek is the largest tributary) since it drains a typical urban area, with some

¹A reagent blank was carried through the procedure, including the alumina chromatography. HPLC analysis of this blank resulted in a few small early peaks, eluting before any peaks of interest.

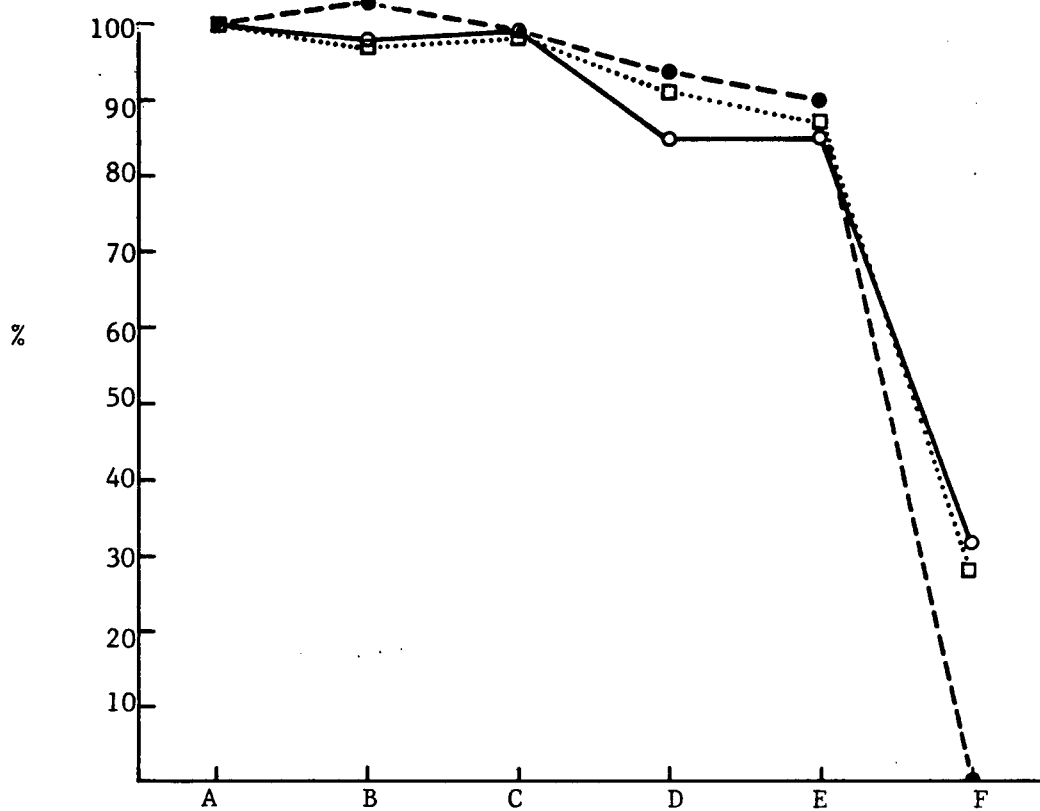


Figure 4-4. B(a)P Recoveries - Alumina Deactivation Problems, 3 Trials

Table 4-3. Elution Pattern of B(a)P From Alumina

		% B(a)P				
		I	II	III	IV	V
Trial 1					100	
2				10	85	
3					100	
4					100	
Standard Deviation					7.5	

strong industrial input. Still Creek drains areas of all four major land-use types; commercial, residential, industrial and greenspace.

Five sampling points were chosen for stream sediments from those used by Hall and co-workers. The stream was sampled at three points along its main branch and at one point on a secondary branch. The sampling point furthest downstream was sampled on both shores of the creek. Each stream sampling point was sampled in two consecutive years in order to investigate annual variation.

Sample sites for street sediments (also selected from those established by Hall et al., 1976) were chosen to be representative of the land use types encountered in the Brunette River (and to a smaller extent in Still Creek) drainage (Table 3-1).

Oligochaetes present in samples were analyzed to investigate possible "competition" relationships between PAH levels in sediments and levels in organisms.

Samples of crankcase oil were analyzed to examine the possibility of used engine oil acting as a source of polycyclics to the Still Creek system. Oil samples were taken from the engine crankcase after various mileages to provide profiles of PAH build-up over time.

7. Comments on Analytical Methodology

Choice of HPLC as the main instrumental method determined the sample preparation procedures and the specific PAH studied (section IV A-2). The decision to use HPLC followed consideration of 1) instrumentation availability, 2) instrumental sensitivity to PAH, 3) chromatographic resolving power. Instrumentation available consisted of packed column GC and HPLC with UV detection. Sensitivity of UV detectors to

polycyclics is generally 0.1 to 1 ng injected (see Table 4-2) while FID response for PAH would range from 1 to 30 ng required per compound (section II E-5). HPLC - UV is thus the better choice with respect to sensitivity. The third consideration, resolution, is important in polycyclic analysis since so many isomers exist for the higher molecular weight compounds. Separation abilities of HPLC and GC are discussed in section II E-4 to II E-6, but HPLC has the important ability to resolve some isomers that would co-elute in GC analysis. (Alumina chromatography relieves some of HPLC's remaining interference problems.)

Capillary columns (GC) allow so much improvement in resolution of PAH in comparison to HPLC or packed GC that, had the instrumentation been available at the start of this study, it probably would have been used routinely. Retention indices and confirmation of identification by GCMS result in a powerful method.

Considerations of safety would result in changes in several aspects of the sample preparation when further work with polycyclics is planned. The use of benzene (a human carcinogen) would be reduced if possible, substituting toluene or dichloromethane for elution of PAH from florisil (section II E-3). Also DMSO will readily transport PAH through the skin, providing a possible route for ingestion of the carcinogenic polycyclics. Special precautions should be taken to prevent skin contact with DMSO solutions of PAH.

In comparison with literature methods, the analytical methodology described for this thesis utilizes available facilities to provide a reproducible, efficient sample workup. The extraction procedure (reflux with basic ethanol) as adopted from Dunn (1976) allows preparation

of a wet sediment in only 1.5 hours, using small solvent volumes. Procedures used by many researchers (Giger and Blumer, 1974; LaFlamme and Hites, 1978; Keizer et al., 1978) involved highly time-consuming Soxhlet extractions, which may be required for older compacted sediments but don't appear necessary for loosely packed stream or street sediments.

Solvent-solvent partition and then clean-up of extract by running through florisil eliminates the need to reduce the iso-octane volume, saving time. The combination of florisil and then alumina column chromatography results in an extract suitable for HPLC analysis. The alumina system (adopted from Sorrell and Reding, 1979) is designed for use with HPLC detection on reverse-phase column, separating B(a)P from B(e)P, B(k)F, B(b)F and separating Chr from Tri. A size-exclusion clean-up was not required for the system used in this thesis work but was used, for example, by LaFlamme and Hites (1978), Vassilaros et al. (1982a), or Giger and Schaffner (1978), all of whom used GC analysis systems with less selective flame ionization detection.

The HPLC column used was not Zorbax or Vydac, as recommended by Wise et al. (1980), but the lower μ -Bondapak C₁₈ resolution was partially overcome by use of the alumina separation. PAH extracts were eluted isocratically, as with the majority of studies using HPLC instrumentation (see Table 2-16).

Recoveries of B(a)P through the analytical procedure (as discussed above) are reproducible and quite acceptable for environmental samples.

B. ANALYSIS OF STREAM SEDIMENTS

1. Sediment Characteristics

Subsamples of each stream sediment were taken for determination of

organic content and the particle size distribution. The particle size ranges used are presented in Table 4-4 (along with abbreviations for particle size fractions) while profiles of size distribution (and percent organic matter) are graphed in Figures 4-5 to 4-8. Samples from the same sites for 1978 and 1979 are compared in each figure. With the exception of samples from Loughheed, sediment characteristics were remarkably similar in the two years' samples. Sediments from Still Creek at Douglas Avenue were taken at the north and south ends of a floating weir. Figures 4-5 and 4-6 show the greater percentage of fine sand and clay in the south end sediments where the water current is somewhat slower. Willingdon samples (Figure 4-8) were composed mainly of silts, while Gilmore sediments were generally coarse sand or small gravel. As expected, sediment organic matter correlates with percent silt and clay (% d in Figure 4-10).

2. HPLC Analysis

Sediment extracts were analyzed on reversed phase columns with acetonitrile-water mobile phase as described in III A-3. Chromatograms of sediments are very similar when comparing sediments from the same sampling site. Figure 4-11 shows chromatograms of fraction III of Douglas Avenue samples taken two consecutive years. Similarities are also found in the last two alumina fractions for Douglas Avenue sediments (Figures 4-13 and 4-15). The differences found are in relative intensities of the peaks and in the amounts of polycyclic matter present. On examination of UV spectra of peaks that matched retention times of standards, differences are found from 1978 to 1979. For a peak with the same relative retention time (RRT) maxima of UV spectra

Table 4-4. Particle Size Ranges

range (mm)	> 0.589	0.589-0.250	0.250-0.074	< 0.074
soil type	coarse sand	medium sand	fine & very fine sand	silt and clay
designation	a	b	c	d

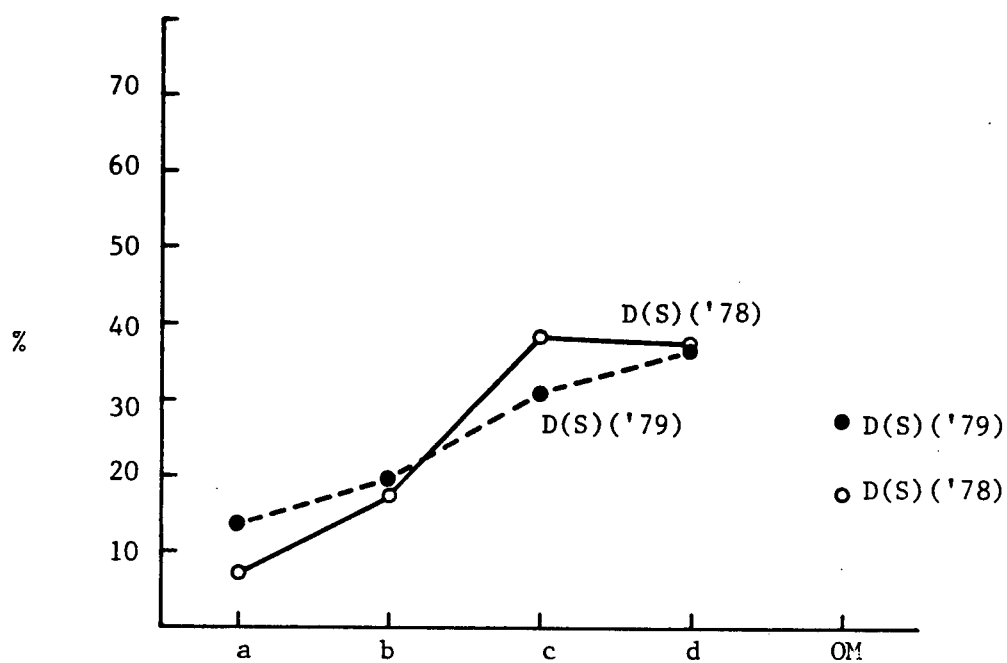


Figure 4-5. Particle Size Distribution and Organic Matter.
D(S)('78) and D(S)('79)

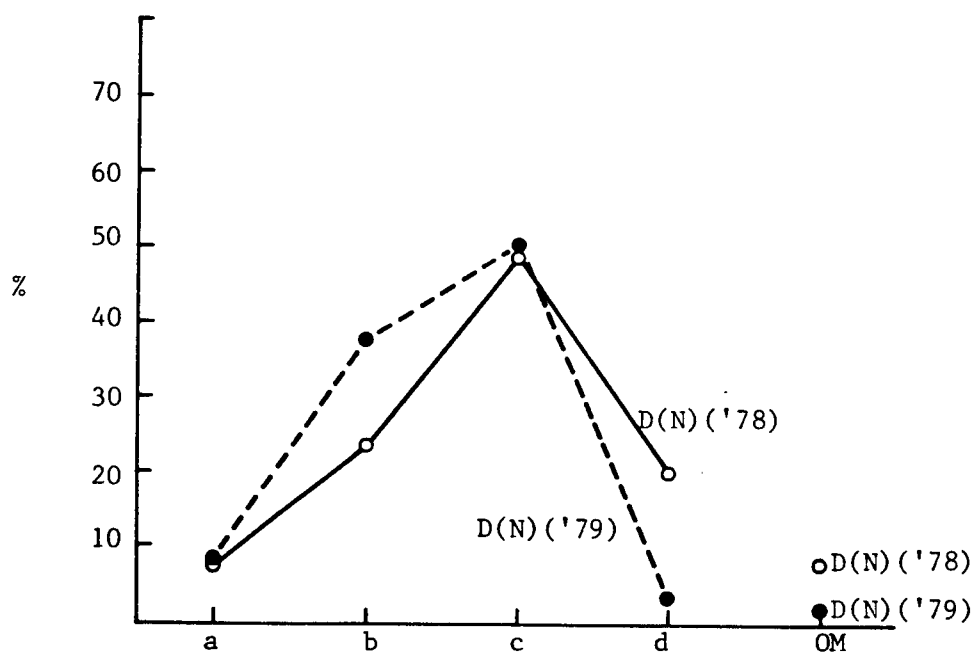


Figure 4-6. Particle Size Distribution and Organic Matter.
D(N)('78) and D(N)('79)

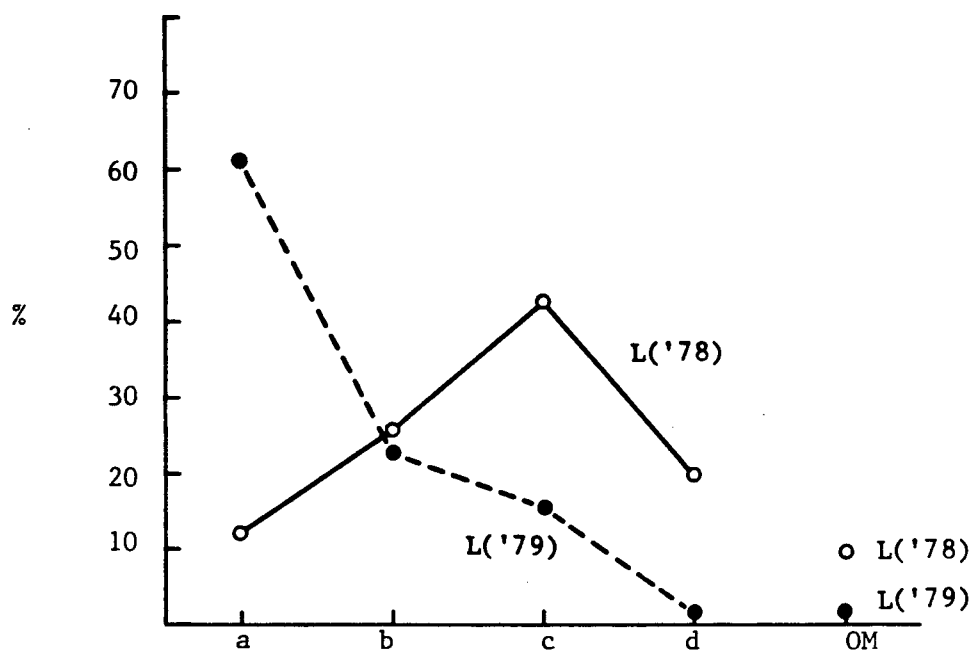


Figure 4-7. Particle Size Distribution and Organic Matter.
L('78) and L('79)

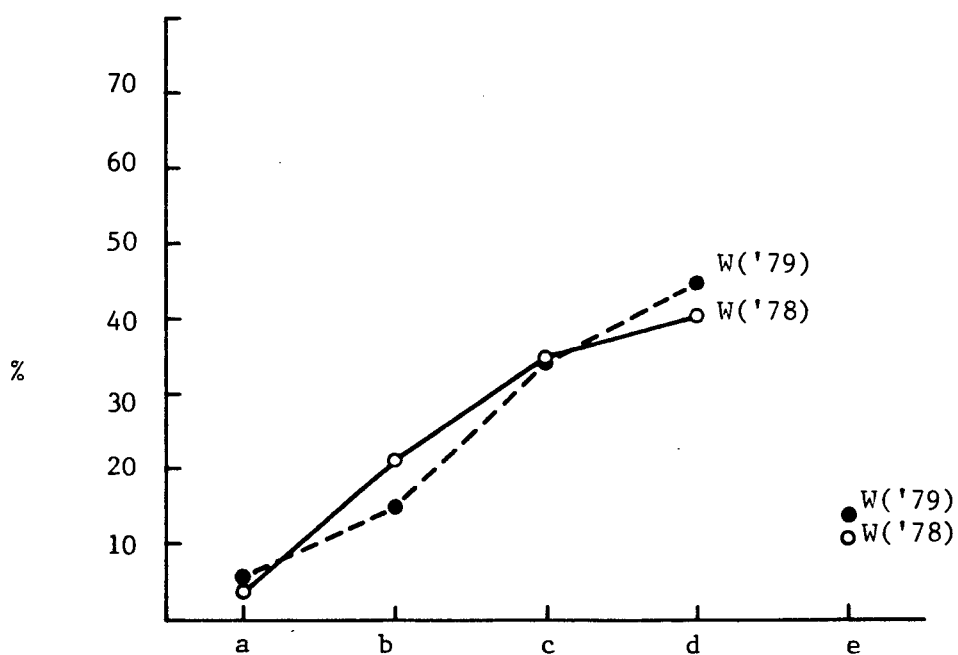


Figure 4-8. Particle Size Distribution and Organic Matter.
W('78) and W('79)

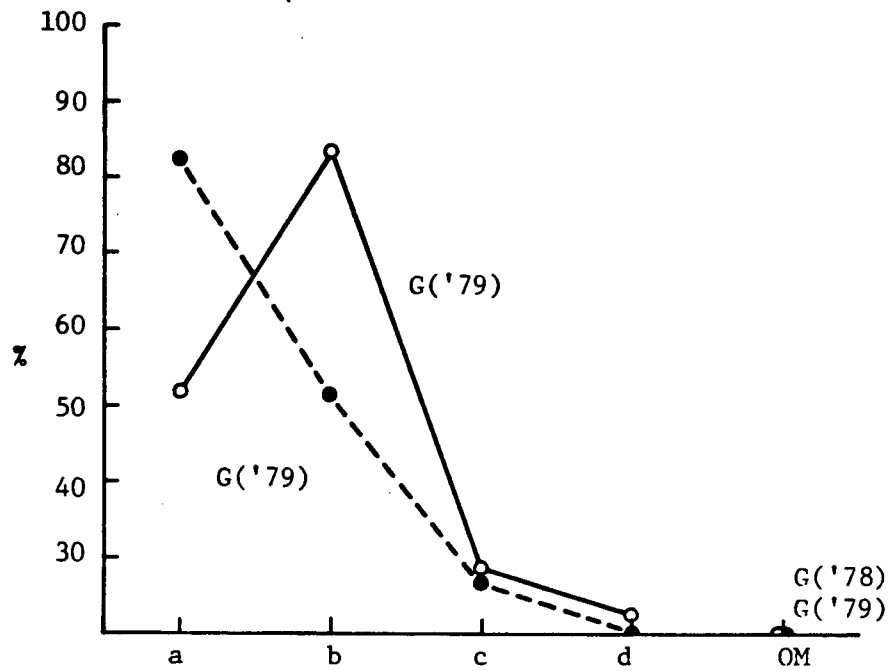


Figure 4-9. Particle Size Distribution and Organic Matter.
G('78) and G('79)

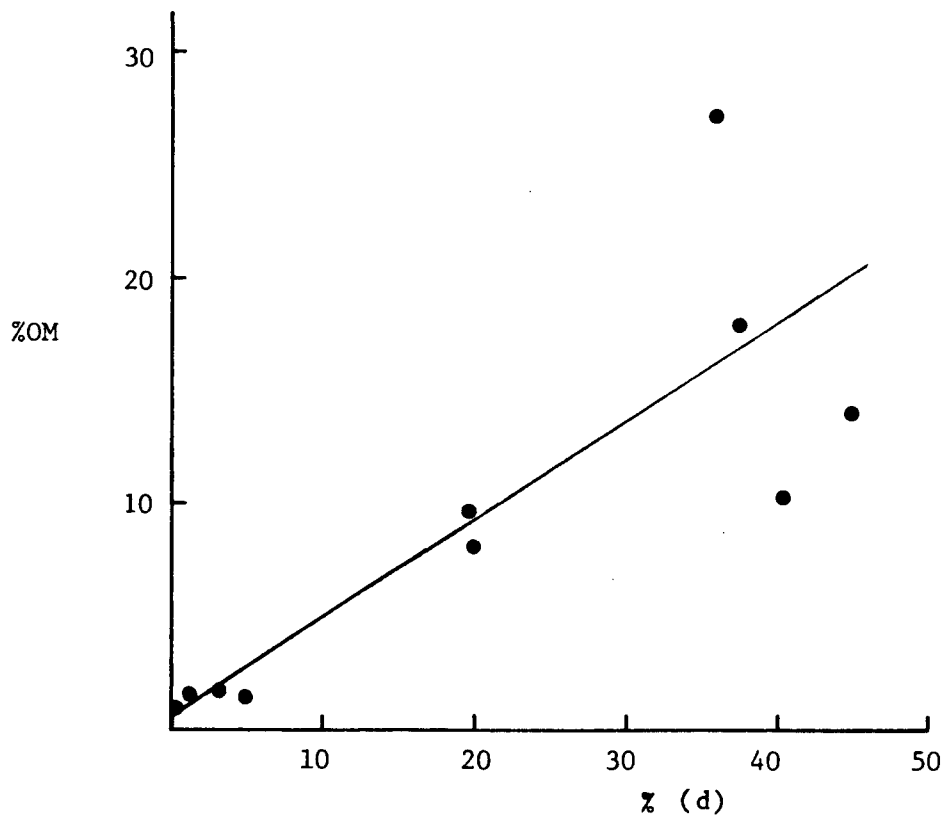


Figure 4-10. % Organic Matter (OM) vs. % Silt and Clay (d)

were often slightly shifted, perhaps due to differences in degree of alkylation. No consistent pattern in UV spectra changes from 1978 to 1979 was observed.

Extracts from different sampling sites showed some similarities in HPLC traces. This can be demonstrated by comparing alumina fraction III from several sites. See traces in Figures 4-11, 4-12 and the 70% trace in Figure 4-17.

By far the largest differences among the sediments was in the amount of PAH matter present. If the response to UV detection for each sediment is normalized per gram of dry weight, this range of response can be easily demonstrated. Comparing the Douglas 1979 extract to Gilmore extract for the same year, Douglas gives a response over two orders of magnitude greater than Gilmore.

Following procedure outlined in III A-3 (Instrumental Analysis) the sediment extracts were screened for the twenty target PAH. It was found that in a significant number of cases, the peaks showed appropriate chromatography for identification as a target polycyclic, but the UV spectra indicated the presence of other UV absorbers. This problem was encountered with the more highly contaminated sediments such as Douglas (S) and Willingdon, while "cleaner" extracts (eg. Gilmore) exhibited few such interferences.

PAH quantitated in the ten sediment extracts are listed in Table 4-5. Some of the polycyclics quantitated were not included in the original set of 20 compounds but have been identified through relative retention times, UV absorbance ratios and UV spectras of standard and sample.

Table 4-5. PAH Quantitated in Stream Sediments

Sediment	PAH	ppm (dry wt)
D(S)('78)	Chr	0.29
	B(a)P	1.79
	B(b)F	0.47
G('78)	B(ghi)F	0.080
	P	1.06
	B(ghi)Per	0.21
	B(k)F	0.099
	diB(ai)Ph	0.017
W('78)	Chr	3.99
	B(e)P	0.85
	B(ghi)Per	1.08
	diB(a,i)Ph	0.055
L('78)	P	8.22
	B(e)P	3.40
D(N)('79)	B(ghi)Per	0.26
D(S)('79)	Tri	37.6
	B(ghi)Per	38.5
L('79)	2-mePh	3.18
	B(b)F	0.057
W('79)	B(a)P	3.14
	B(ghi)Per	1.49

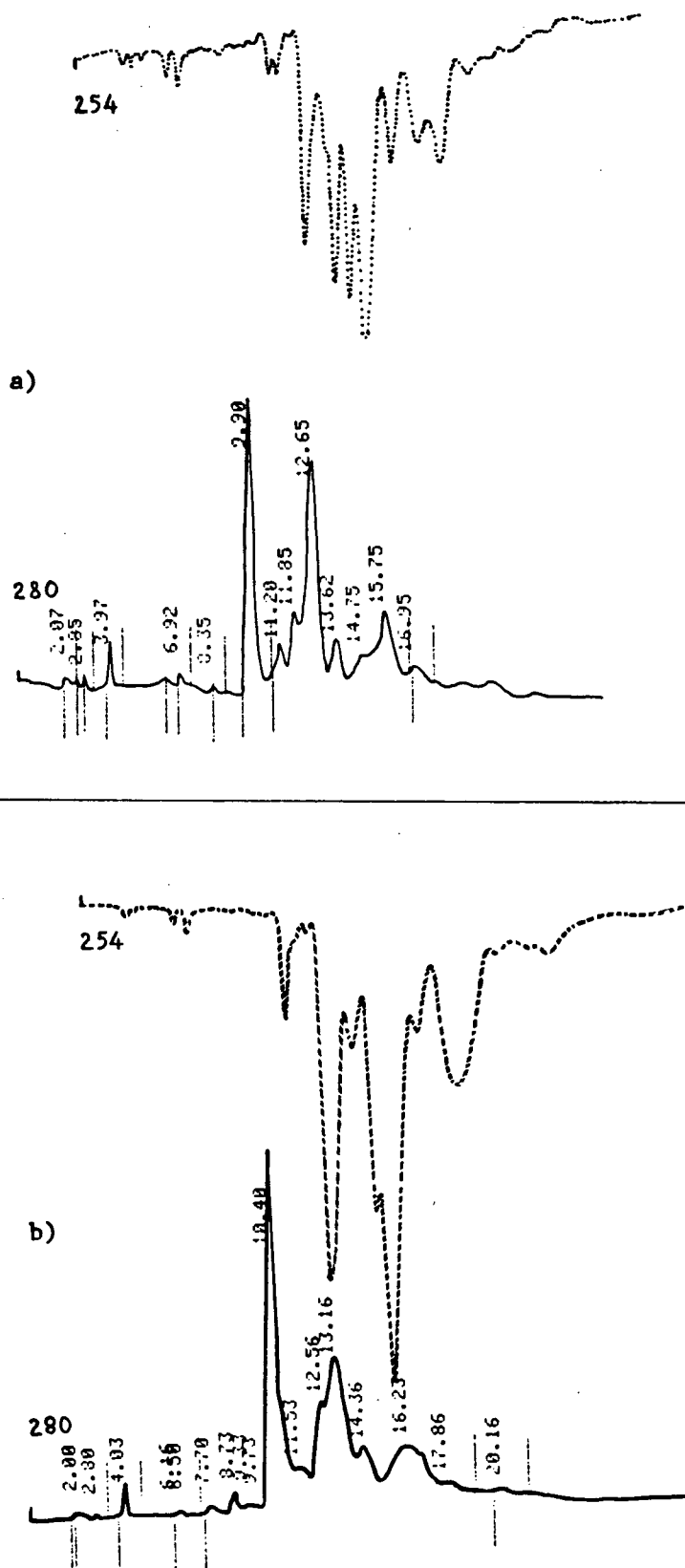


Figure 4-11. Chromatogram of Douglas (N) Extract

- a) ('78)III
- b) ('79)III

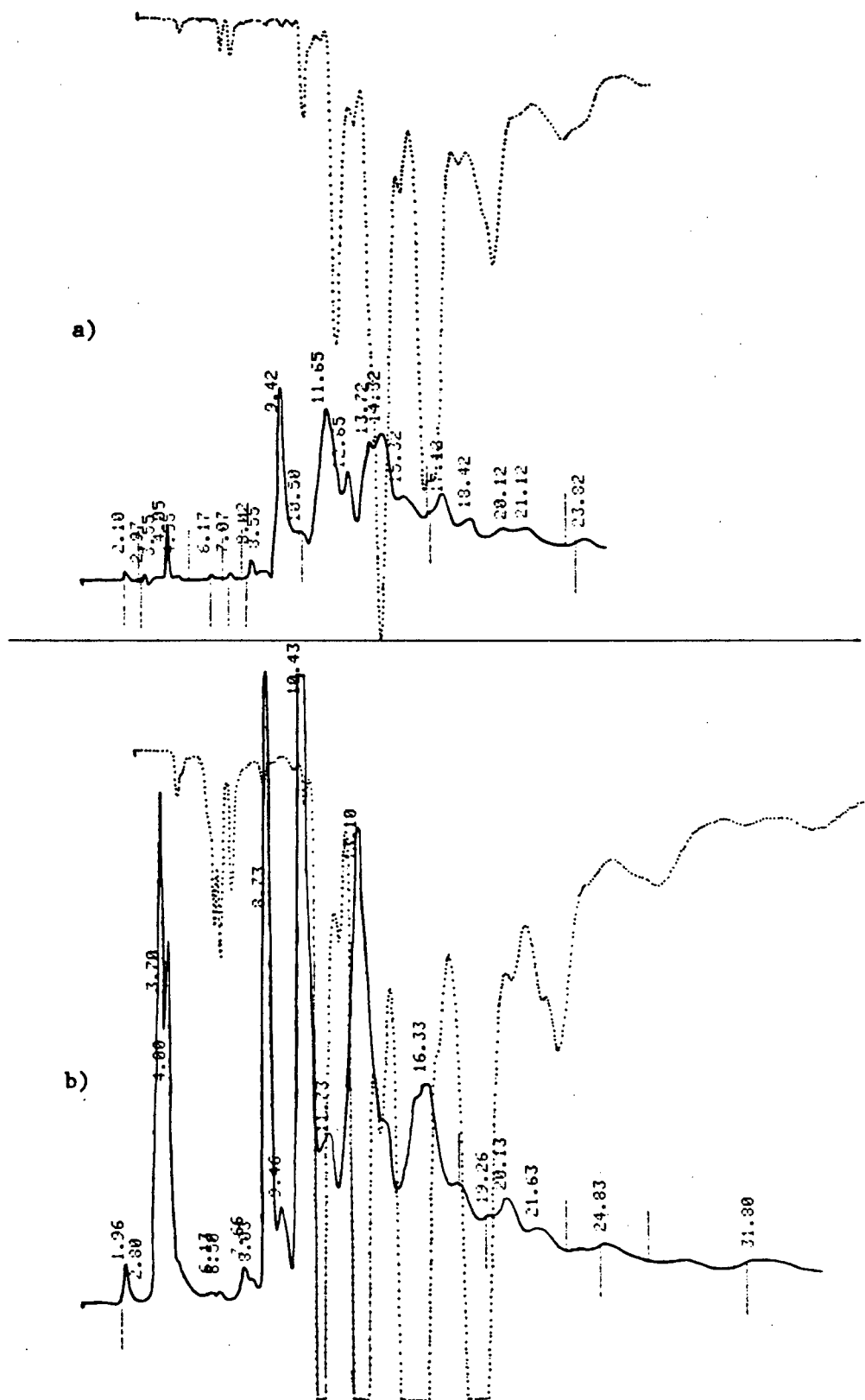


Figure 4-12. Chromatogram of Willingdon Extract

a) ('78)III
b) ('79)III

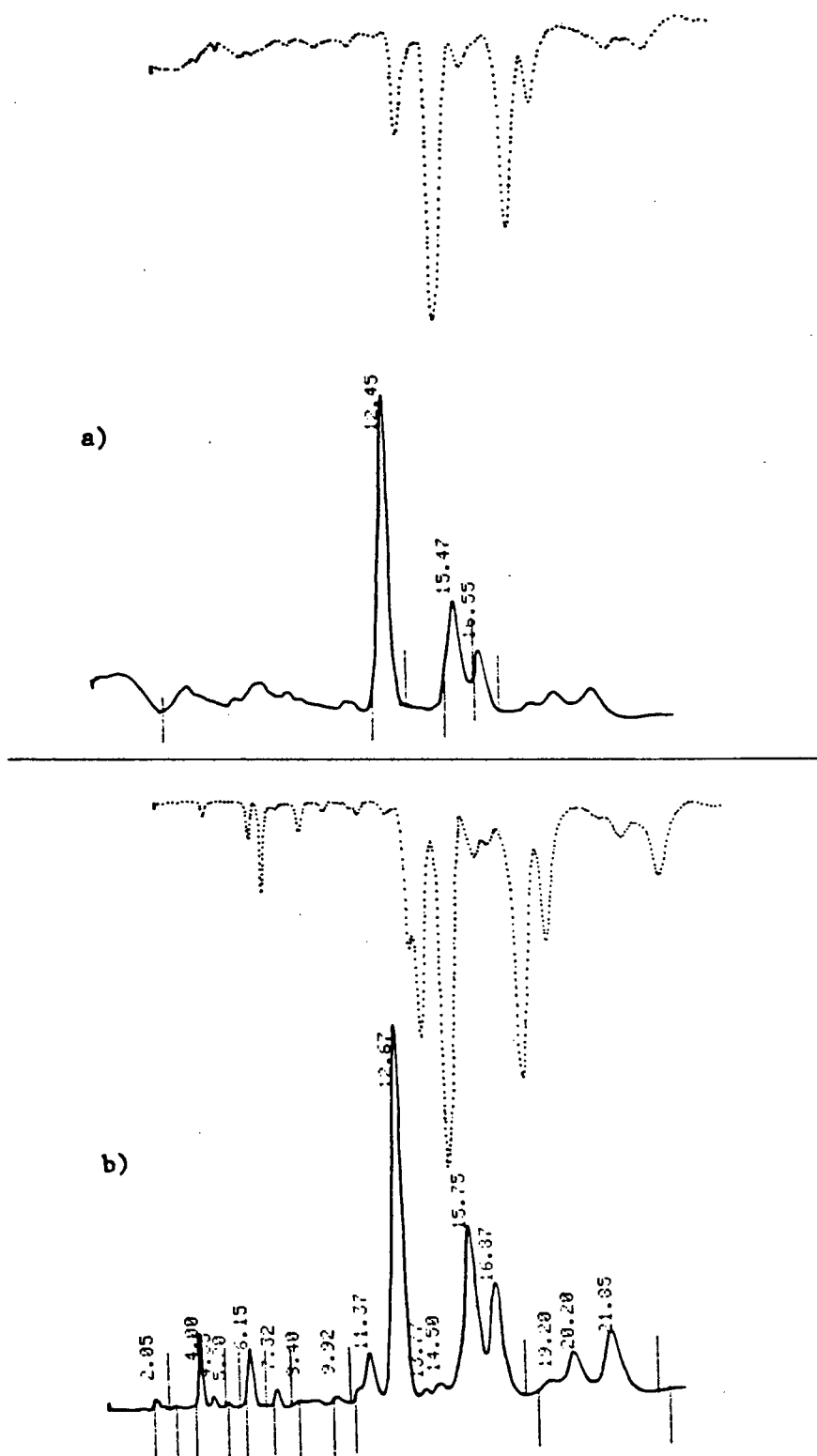


Figure 4-13. Chromatogram of Douglas (N) Extract

a) ('78)IV
b) ('79)IV

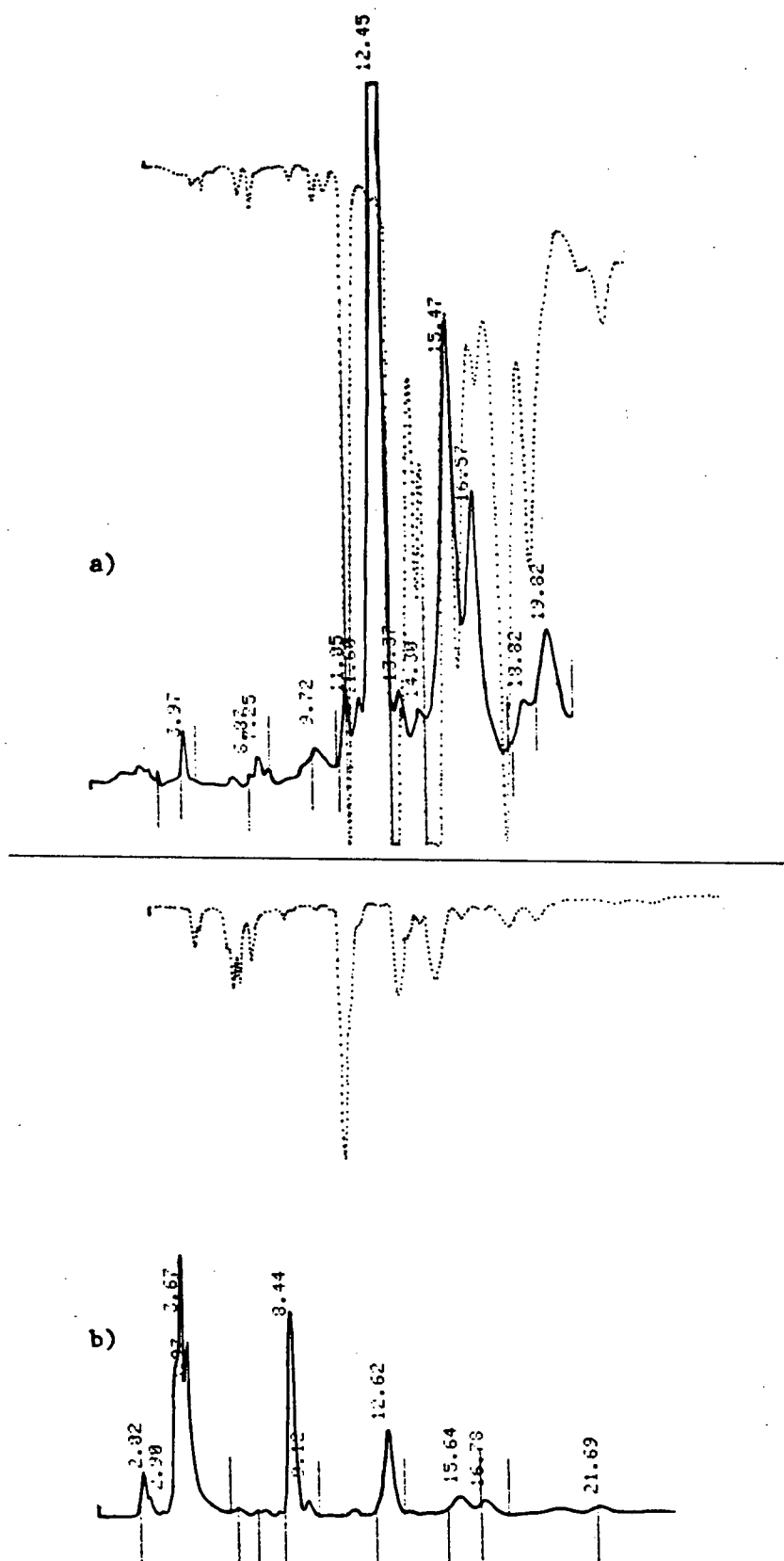


Figure 4-14. Chromatogram of Loughheed Extract

- a) ('78)IV
b) ('79)IV

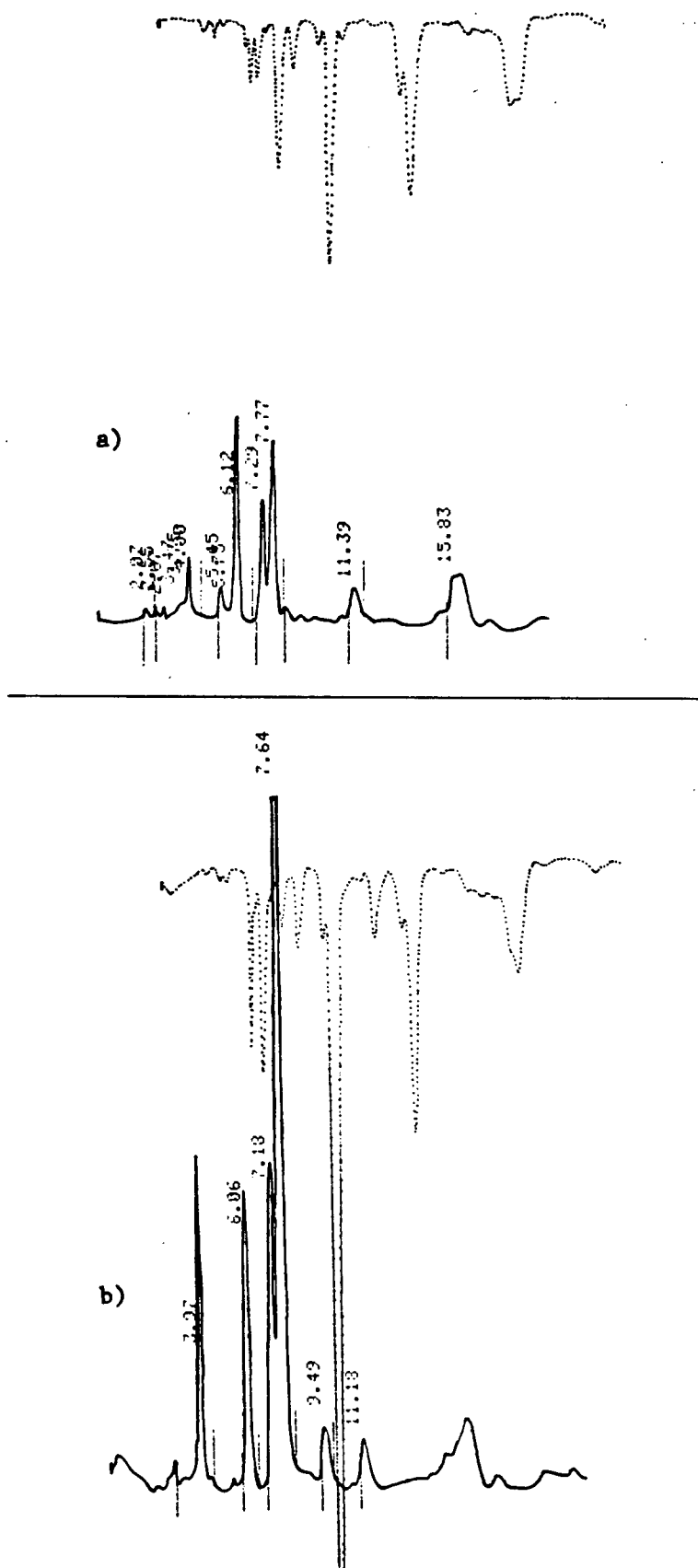


Figure 4-15. Chromatogram of Douglas (N) Extract

a) ('78)V
b) ('79)V

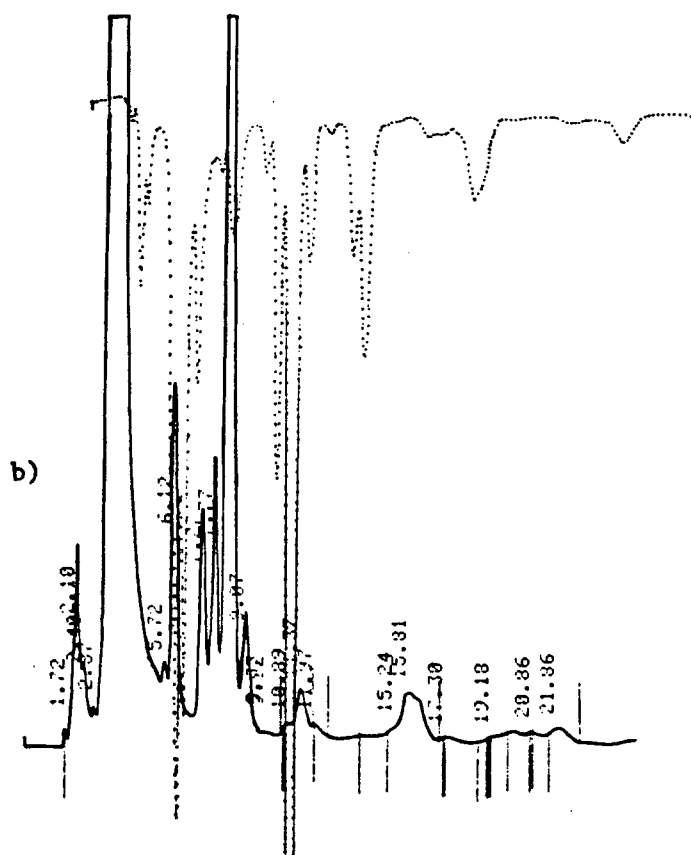
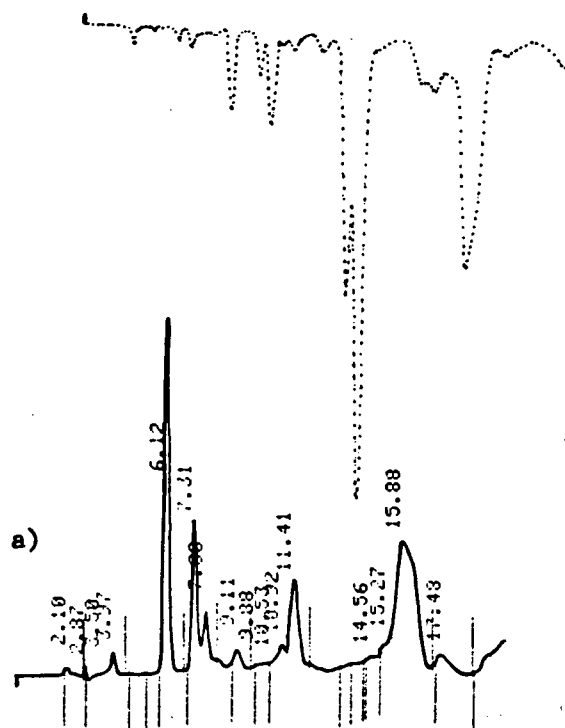


Figure 4-16. Chromatogram of Loughheed Extract

- a) ('78)V
b) ('79)V

3. Capillary GC Analysis

Extracts of several sediments were analyzed on a capillary GC with FID in order to exploit the confirmatory advantages of a different detector, and most importantly, to exploit the considerable resolving powers of the fused silica capillary column.

The extracts Douglas (S) ('78), Douglas (S)('79) and Willingdon ('78) were run on the capillary system described in Section III F-4. Standards were also run in order to provide retention time information. Peaks in the mixed standards were identified by the use of a retention index system designed for use with polycyclics.

Lee et al. (1979a) investigated various retention index systems for the capillary analysis of PAH but found reproducibility problems with the system in use for other compounds. Lee and co-workers developed a set of standard polycyclics to define their retention index (see II E-5) and Vassilaros et al. (1982b) evaluated performance under a wide variety of conditions. The polycyclics N, Ph, Chr, and picene are used as markers for the retention index system under specified temperature-programmed elution conditions. The time between these standards is divided into 100 units and elution times of other PAH calculated to 0.01 unit. Vassilaros et al. (1982b) examined the effects of column internal diameter, temperature program, and initial temperature, on the indices. The relative retention times maintained good reproducibility despite variations. See Table 4-6 for a comparison of the standard conditions used by Vassilaros and co-workers and those used in this study. It was found that the different programming rate and, more importantly, the different column coating, resulting in small differences between the retention indices published and those

Table 4-6. Comparison of Conditions for Determination of PAH Retention Indices.

Parameter	Vassilaros <u>et al.</u> (1982b)	This Study
initial temp (°C)	40	40
initial time (min)	2	2
program rate (°C/min)	4	2
final temp (°C)	265	250
final time (min)	--	20
column type	SE-52	SE-54
column dimensions	20m x 0.31mm	20m x 0.31mm
standards used	N I = 200.00 Ph 300.00 Chr 400.00 picene 500.00	Ph I = 300.00 Chr 400.00 Tri 400.00 B(ghi)Per 501.32
carrier gas	H ₂	He

calculated on the SE-54 column.

Table 4-7 lists the retention indices for the standards eluted in alumina fraction III and fraction IV. (See Appendix 3 for chromatograms.) As can be seen from the peak identification in III, retention data isn't available for all polycyclics (especially isomers of alkylated PAH). The differences in I_{ref} values (a,b) listed are due to a 2 minute iso-thermal period incorporated at the beginning of the program in ref b (Vassilaros et al., 1982b). Correlation between I_{ref} and retention indices for standard mixtures for this study is good (0.01% to 1.2% for ST III) although matching is not perfect. Terminating the oven temperature program at 250°C (rather than continuing to 265°C) makes the retention index system inapplicable to calculating I values for peaks beyond that point (eg. B(ghi)Per in ST IV), although elution orders will be the same. As pointed out by Vassilaros et al. (1982b) absolute agreement in I values from lab to lab cannot be expected, however the system is extremely useful in identifying polycyclics by elution order, especially important for the various isomers.

In Table 4-8 through 4-10, retention indices (I) are listed for the samples analyzed by capillary GCFID. Since peaks with tentative I-value match are listed, often only a small fraction of the peaks in a sample is represented. (For example, in sample W('78)IV, I-values were calculated for 41 peaks while only 16 peaks appear in Table 4-10). For compounds included in the set of 20 target PAH, it was known in which alumina fraction to expect the peak, however that information wasn't available for the other polycyclics with retention indices, hence a given PAH may be identified in two fractions of a sample.

GCMS analysis of sediment extracts will be discussed more fully in

Table 4-7. Retention Indices for Target PAH

PAH Standard	Retention Time (min)	I(calc'd)	I _{ref} ^a	I _{ref} ^b	Peak Identification	
III	52.11	265.66	268.17	269.955	Fl	
	62.91	300.00	300.00	300.000	Ph	
	63.79	302.79	301.69	301.364	A	
	72.79	331.41	329.13		9-meA	
	73.91	334.98				^a Lee <u>et al.</u> , 1979a
	74.70	337.49				
	76.98	344.74	344.01	344.471	F	^b Vassilaros <u>et al.</u> , 1982b
	78.47	349.48			P	
	79.32	352.18	351.22	351.484	B(a)Fl	^c trace amount
	83.91	366.77	366.74			
	84.72 ^c	369.35				
	94.36	400.00	400.00		Tri	
	99.06	414.94				
	108.71	445.63	450.73		B(e)P	
			490.66		diB(a,j)Ac	
IV					2-mePh	
					3,6-dimeA	
					9,10-dimePh	
	92.58	398.13	398.50		B(a)A	
	93.21	400.00	400.00		Chr	
	107.99	447.57	453.44		B(a)P	
	108.77	450.08	456.22		Per	
			501.32		B(ghi)Per	

Table 4-8. Tentative Identification of Peaks by Retention Index (I), D(S)('79) 117.

Fraction	Retention Time	I(calc'd)	I _{ref} ^a	Tentative Identification	MS Correlation
III	57.68	287.91	287.69	1,2,3,4,5,6,7,8-octa-hydroanthracene	Ph 3-mePh 1-mePh MS at 354.75, Mass 202, P
	61.52	300.16	300.00	Ph	
	64.11	308.42	308.79	phenanthridine	
	66.20	315.09	315.19	1-phenylnaphthalene	
	66.53	316.14	316.37	1,2,3,10b-tetrahydro F	
	67.57	319.46	319.46	3-mePh	
	67.89	320.48	320.17	2-mePh	
	69.05	324.18	323.90	1-mePh	
	70.60	329.12	329.13	9-meA	
	71.67	332.54	332.59	2-phenylnaphthalene	
	73.85	339.49	339.38	1,2,3,6,7,8-hexahdropyrene	
	74.82	342.59	342.45	6-phenylquinoline	
	78.80	355.28	355.49	9,10di-meA	
	80.19	359.72	359.91	9-me-10-ethyl Ph	
	80.70	361.34	361.38	benzo[kl]xanthene	
	83.30	369.64	369.39	B(b)Fl	
	86.91	381.15	381.56	5,12-dihydronaphthacene	
	87.06	381.63	381.85	9,10-dimethyl-3-ethylphenanthrene	
	88.14	385.08	385.35	1-ethylP	
	90.39	392.25	392.50	B[c]acridine	
	92.85	400.10	400.00	Tri	
	94.39	405.01	405.35	1,2'-binaphthyl	
	98.88	419.34	419.39	12-meB(a)A	
IV	53.14	269.31	269.67	trans-1,2,3,4,4a,9a-hexahydrodibenzothiophene	(C ₁₃ H ₁₀ N ₂) Chr Mass 243, 10-meB(a)acridin (C ₁₄ H ₁₀ O ₅) 1-me Chr
	54.26	272.89	272.57	2,3,5-trimethylindole	
	68.04	316.84	216.37	1,2,3,10b-tetrahydro-F	
	70.14	323.53	323.33	1-meA	
	72.96	332.54	332.59	2-phenylnaphthalene	
	84.27	368.62	368.62	1-methyl-7-isopropyl-Ph	
	84.56	369.54	369.39	B(b)Fl	
	84.98	370.88	370.86	4,5,6-trihydroB(de)A	
	89.72	386.00	386.36	B(a)A	
	94.08	399.91	400.00	Chr	
	96.13	406.45	406.54	7-B(de)anthrene	
	96.81	408.62	408.30	naphthacene	
	98.45	413.85	413.78	2-meB(a)A	
	99.55	417.36	417.16	9-me-10-phenylPh	
	100.70	421.03	421.12	2,2'-biquinoline	
	101.28	422.88	422.87	1-me Chr	
	105.67	436.88	436.82	1,12dimeB(a)A	
V	47.44	255.24	255.48	2,3-dimeindole	
	52.46	271.26	271.39	cis-1,2,3,4,4a,9a-hexahydrodibenzothiophene	
	62.12	302.07	302.22	benz(h)quinoline	
	66.37	315.63	315.19	1-phenylnaphthalene	
	69.00	324.02	324.46	2-methylacridine	
	77.21	350.21	350.30	9-n-propylPh	
	80.37	360.29	360.73	m-terphenyl	
	82.14	365.94	366.10	p-terphenyl	
	94.79	406.29	406.54	7-benz(de)A	
	102.99	432.45	432.32	1,3-dimeTri	

a - Lee et al., 1979a.

Table 4-9. Tentative Identification of Peaks by Retention Index (I), D(S)('78)

Fraction	Retention Time	I(calc'd)	I _{ref} ^a	Tentative Identification	MS Correlation
III	62.86	299.84	300.00	Ph	Ph
	65.00	306.67	306.76	1,2,3,4-tetrahydrocarbazole	
	68.95	319.21	319.46	3-mePh	me-Ph
	70.38	323.75	323.90	1-mePh	me-Ph
	71.93	328.68	328.99	9-n-butylfluorene	
	73.02	332.15	332.59	2-phenylnaphthalene	
	76.13	342.04	342.45	6-phenylquinoline	phenylquinoline
	76.86	344.36	344.01	F	F
	79.17	351.71	351.22	P	P
	81.22	358.23	358.53	B[1mn]phenanthridine	
	82.04	360.83	360.73	m-terphenyl	
	82.28	361.60	361.38	B[kl]xanthene	
	85.00	370.25	370.15	2-mePh	
	99.08	415.02	414.87	1-n-butylP	
IV	53.34	269.95	269.67	trans-1,2,3,4,4a,9a-hexahydrodibenzothiophene	-no MS run
	56.47	280.80	280.48	xanthene	
	62.78	300.06	300.00	Ph	
	66.54	312.06	312.13	carbazole	
	68.04	316.84	316.37	1,2,3,10b-tetrahydro-carbazole	
	72.14	329.92	329.69	4,5,9,10-tetrahydroP	
	78.69	350.82	350.30	9-n-propylPh	
	83.32	365.59	366.10	p-terphenyl	
	84.95	370.79	370.86	4,5,6-trihydroB(de)A	
	88.52	382.17	382.09	9-phenylcarbazole	
	89.35	384.82	385.35	1-ethylP	
	89.72	386.00	386.34	2,7-dimeP	
	90.37	388.08	388.38	1,1'-binaphthyl	
	93.90	399.34	400.00	Chr	
	95.68	405.01	405.35	1,2'-binaphthyl	
	96.72	408.33	408.30	naphthacene	
	98.47	413.91	413.78	2-meB(a)A	
	98.84	415.10	414.87	1-n-butylpyrene	
	99.50	417.20	417.16	9-me-10-phenyl Ph	
	99.84	418.29	418.10	3-me Chr	
	100.68	420.96	420.83	4-me Chr	
	104.22	432.26	432.32	1,3-dime Tri	
V	52.60	271.55	271.39	cis-1,2,3,4,4a,9a-hexahydrodibenzothiophene	-no MS run
	53.52	274.48	274.59	4,4'-dimethylbiphenyl	
	65.86	313.84	313.97	9-ethylcarbazole	
	69.20	324.50	324.46	2-methylacridine	
	75.82	345.62	345.78	9-isopropylPh	
	76.87	348.81	348.54	9-n-hexylFl	
	83.71	370.79	370.86	4,5,6-trihydroB(de)A	
	87.34	382.37	382.09	9-phenylcarbazole	
	93.39	401.67	401.81	benz(a)carbazole	
	104.51	437.14	436.82	1,12-dimeB(a)A	

a - Lee et al., 1979a.

Table 4-10. Tentative Identification of Peaks by Retention Index (I), W('78).

Fraction	Retention Time	I(calc'd)	I _{ref} ^a	Tentative Identification
III	57.64	287.78	278.69	1,2,3,4,5,6,7,8-octahydroA
	61.46	299.97	300.00	Ph
	64.33	309.12	309.25	benzo(f)quinoline
	67.84	320.32	320.17	2-mePh
	68.76	323.26	323.33	1-meA
	69.00	324.02	323.90	1-mePh
	70.57	329.03	328.99	9-n-butylFl
	71.63	332.41	332.59	2-phenylnaphthalene
	73.82	339.40	339.38	1,2,3,6,7,8-hexahydroP
	74.77	342.43	342.45	6-phenylquinoline
	80.18	359.68	359.91	9-methyl-10-ethylPh
	80.70	361.34	361.38	benzo[kl]xanthene
	81.50	363.90	364.22	4H-benzo[def]carbazole
	82.15	365.97	366.10	p-terphenyl
	82.49	367.05	367.04	11-meB(a)Fl
IV	68.02	316.78	316.37	1,2,3,10tetrahydroF
	77.54	347.15	347.47	2-phenylindole
	78.67	350.75	350.30	9-n-propylPh
	84.09	368.04	367.97	9,10-diethylPh
	84.34	368.84	368.67	1-me-7-isopropylPh
	84.95	370.79	370.86	4,5,6-trihydroB[de]A
	88.49	382.08	382.09	9-phenylcarbazole
	94.03	399.75	400.00	Chr
	95.70	405.08	405.35	1,2'-binaphthyl
	96.77	408.49	408.30	naphthacene
	98.54	414.14	414.37	1-meB(a)A
	99.57	417.42	417.56	8-meB(a)A
	99.92	418.52	418.72	5-meB(a)A
	100.71	421.06	421.12	2,2'-biquinoline
	104.28	432.45	432.32	1,3-dimeTri
	105.65	436.82	436.82	1,12-dimeB(a)A
V	54.26	272.89	272.57	2,3,5-trimethylindole
	64.73	306.28	306.78	1,2,3,4-tetrahydrocarbazole
	66.53	312.03	312.12	carbazole
	67.44	314.93	315.19	1-phenylnaphthalene
	68.53	318.41	318.01	9-n-propylFl
	77.84	348.11	348.54	9-n-hexylFl
	84.05	367.92	367.97	9,10 diethylPh
	84.35	368.87	368.67	1-me-7-isopropylPh
	84.59	369.64	369.64	4-meP
	93.11	396.82	396.54	cyclopenta[cd]pyrene
	98.52	414.07	414.37	1-meB(a)A
	101.28	422.88	422.87	1-me Chr

a - Lee et al., 1979a.

Section IV B-4, but its utility for confirming GCFID identifications is demonstrated in Tables 4-8 and 4-9. The D(S)('79) extract was screened at masses of the 13 target compounds eluted in alumina fraction III, confirming the assignment of a peak at $I = 355.28$ (9,10-dimeA versus P). Fraction IV was screened for polycyclics through the whole range of masses. Many PAH compounds identified through GCMS weren't included in the table of retention indices so correlations don't appear in Table 4-7. GCMS screening of the alumina fraction V was again restricted to the two target compounds, B(k)F and B(b)F. The only other sediment fraction analyzed by GCMS is D(S)('78)III, allowing confirmation of Ph, (and me-Ph) F,P, and phenyl quinoline (Table 4-9).

Chromatograms (capillary GC) are presented in Figures 4-18, 4-19 and 4-20. The number of peaks resolved on the capillary column is many times greater than that resolved by HPLC. For example, for W('78)III 54 peaks are integrated on the capillary system while 14 peaks are detected by HPLC. The possible co-elution of polycyclics on HPLC was indicated by the number of peaks which had appropriate retention times but their UV spectra didn't match those of the standard PAH.

The traces from each of the three sediments were compared, fraction by fraction, for similarities in GC elution patterns. Alumina fraction III demonstrates strong similarities between D(S)('79) and W('78) (see Figures 4-18 and 4-20) with many peaks having the same retention indices, while the fraction III for D(S)('78) shares only a few similar I values with the other two. The fourth fraction is highly consistent across all the sediment extracts, with at least ten large peaks being common to all chromatograms. Fraction V, however, is variable, with no observable correlation among the extracts. The fractions

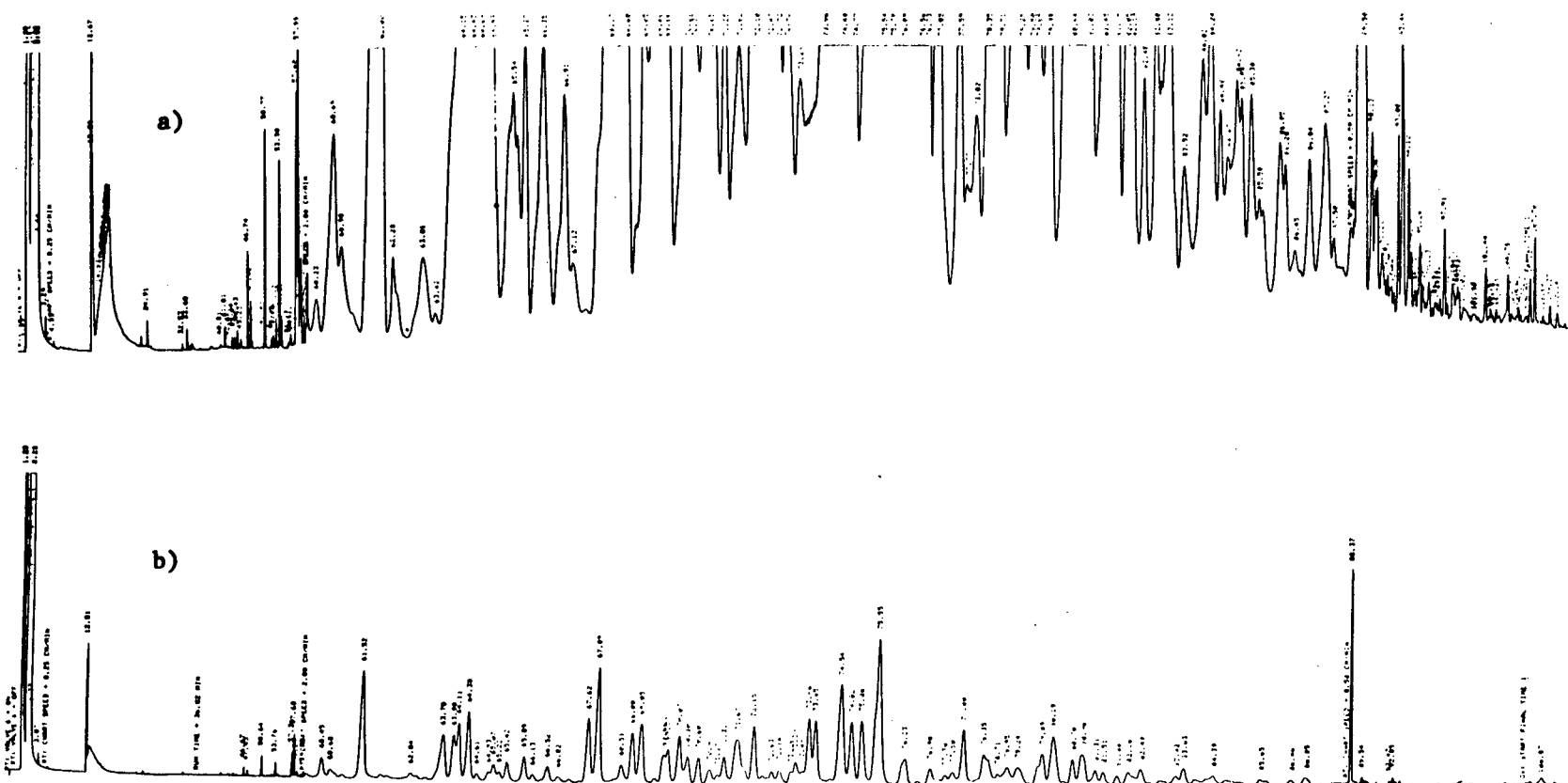
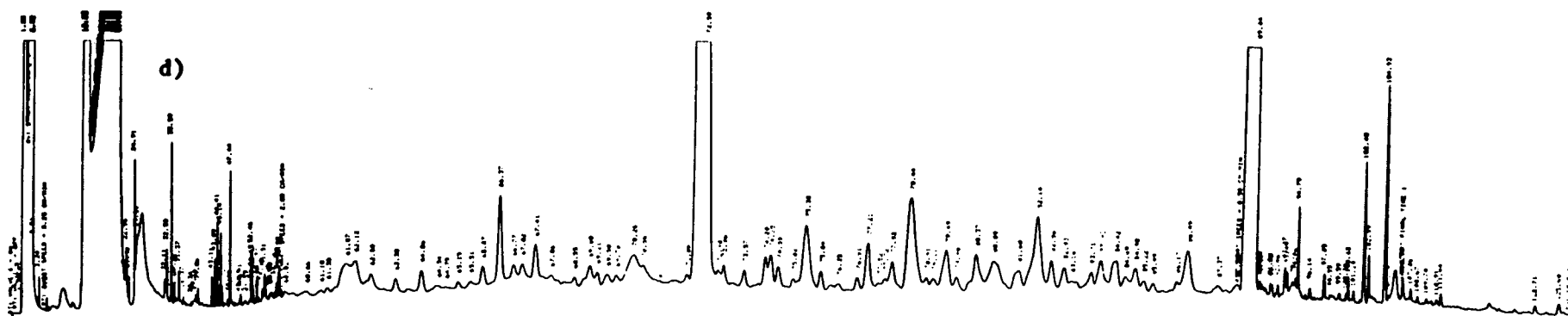
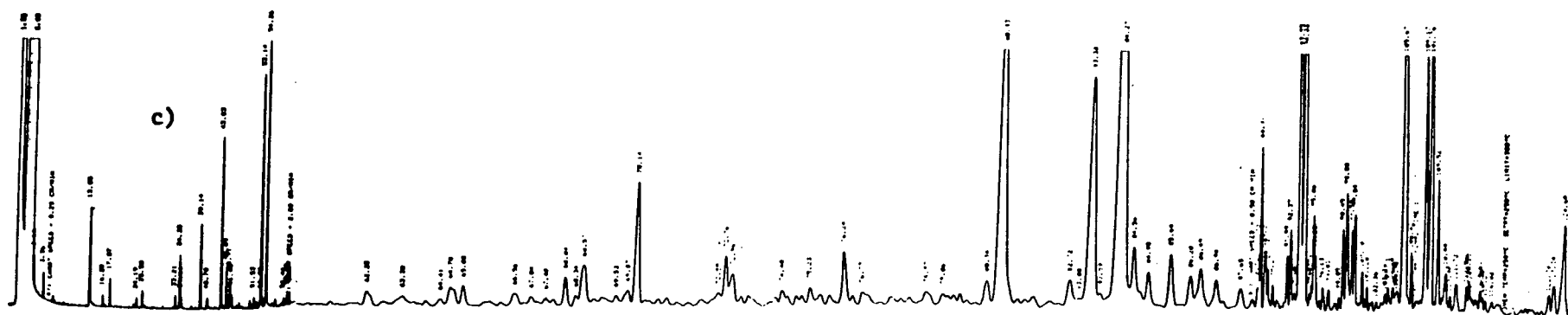


Figure 4-18. Capillary GC Chromatogram, D(S)('79)

- a) III, 2µl
- b) III, 0.2 µl



4-18 Capillary GC Chromatogram, D(S)('79) Continued

c) IV
d) V

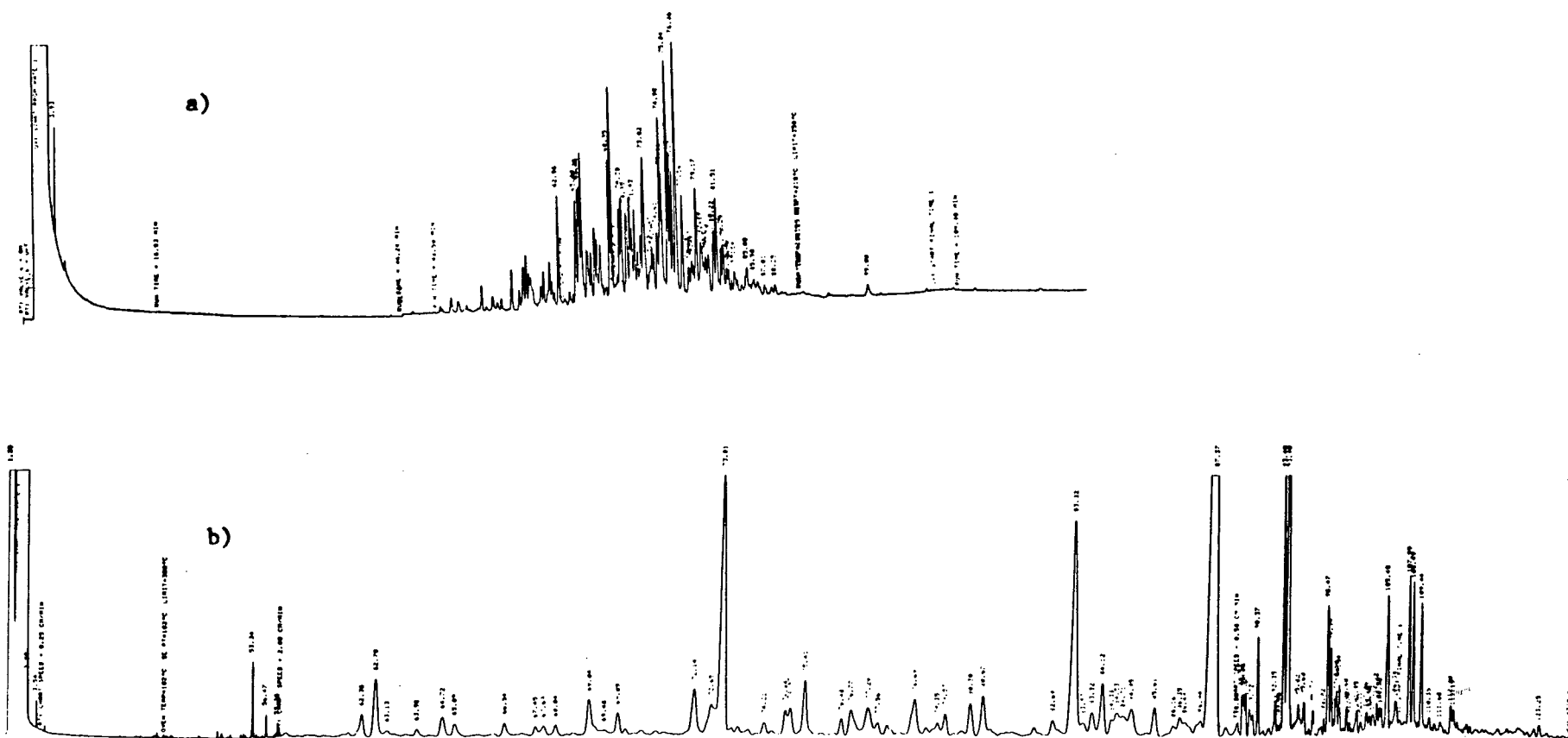
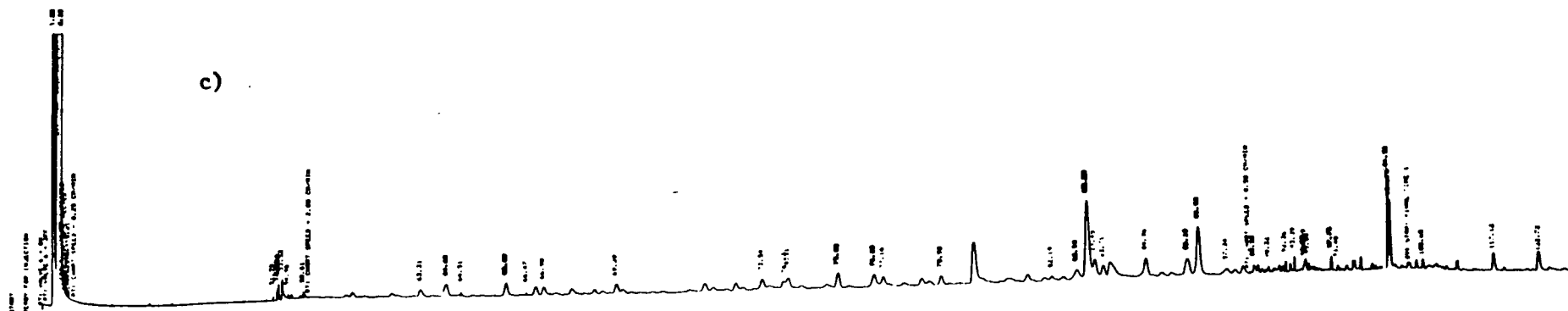


Figure 4-19. Capillary GC Chromatogram, D(S)('78)

- a) III
- b) IV



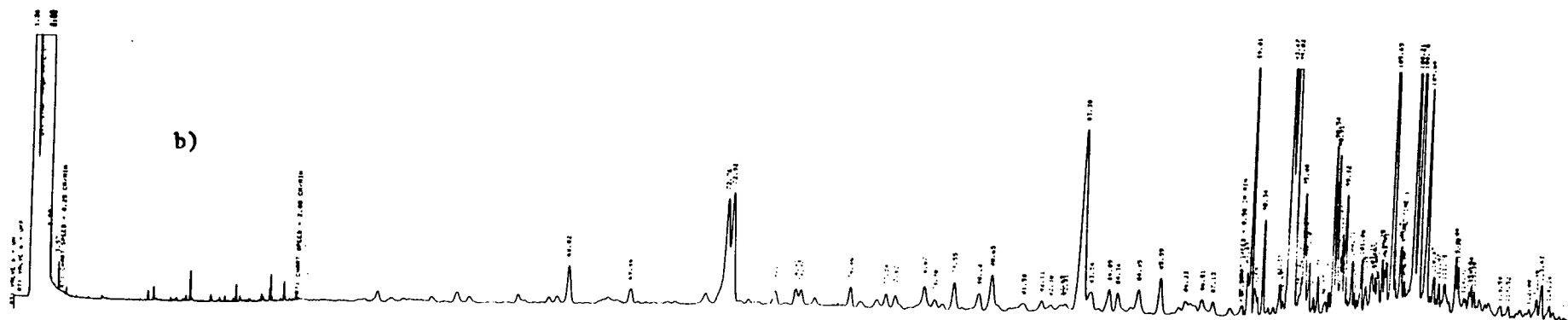
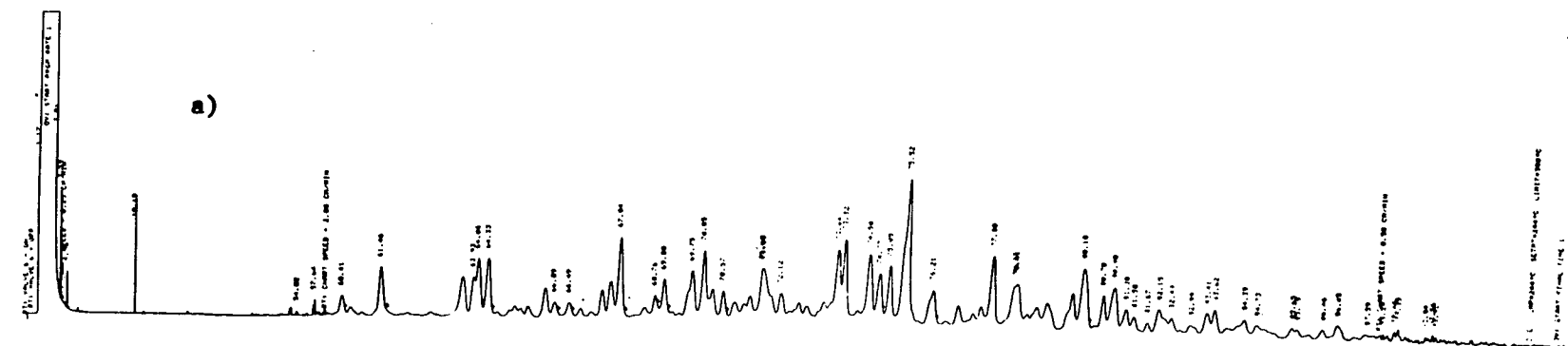
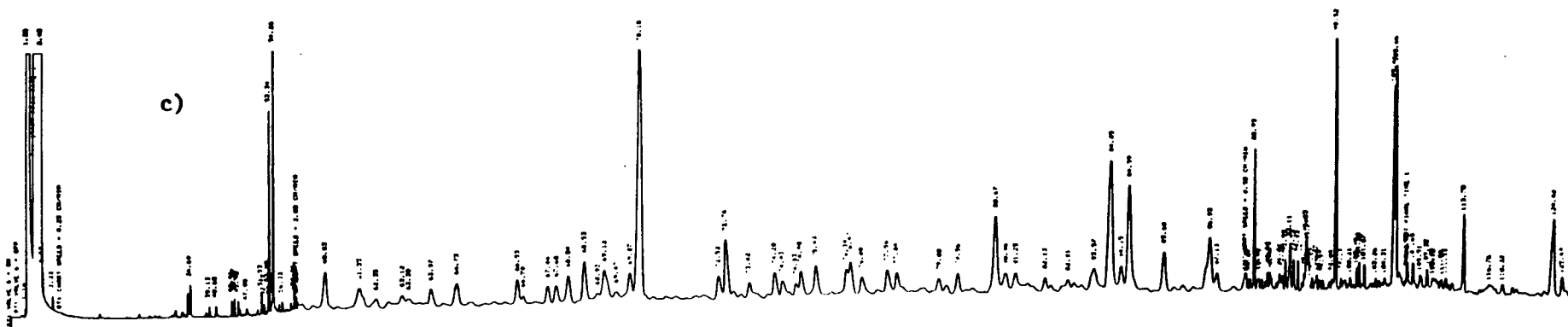


Figure 4-20. Capillary GC Chromatogram, W('78)

- a) III
- b) IV



4-20 Capillary GC Chromatogram, W('78) Continued

c) V

showing similar profiles indicate a common source for the polycyclics, as expected from HPLC traces, and as indicated by workers examining PAH profiles in an urban environment (Wakeham et al., 1980a). The differences in profiles demonstrated by the V fraction extracts, or by D(S)III from one year to the next, are more difficult to explain. Perhaps the polycyclics eluted in fraction V are indicative of the presence or absence of a particular small polycyclic source. Build-up time may also be a determining factor in the development of PAH profiles, resulting in differences in the various extracts. Stream flow characteristics could also have an effect on PAH fractions since flood conditions may scour sediments and result in redistribution of polycyclics along the stream.

It should be pointed out that the capillary GC chromatograms cannot be compared quantitatively to HPLC results due to changes in total extract volume.

4. GCMS Analysis

Four sediment fractions were analyzed by GCMS (see section III F-5). D(S)('79) was screened for target PAH in fractions III and V while fraction IV was examined in detail for a wide range of polycyclics. Also, D(S)('78)III was screened for the masses of the target PAH. Details are given in Table 4-11. Injections were made in chloroform and masses scanned from 30 to 500.

In some cases hydroxy- or alkyl-substituted PAH were picked up in the screen for target PAH since the parent ion mass occurred in their spectra. An example of this is seen in Table 4-12, where isomers of hydroxy Ph or hydroxy A were identified in D(S)('78)III.

Table 4-11. Scope of GCMS Analysis.

Sample	Fraction	Mass	PAH
D(S)('78)	III	166	Fl
		178	Ph,A
		192	9-meA, 2-mePh
		202	P,F
		206	3,6-dimePh, 9,10-dimeA
		216	B(a)Fl
		228	Tri
		252	B(e)P
		279	diB(a,j)acridine
D(S)('79)	III	-as for D(S)('78)	-as for D(S)('78)
	IV	142-302	-screened for polycyclics in EPA/NIH mass spectral data base
	V	252	B(k)F, B(b)F

Retention indices were calculated for the MS peaks by identifying Ph at mass 178 and Chr or Tri at mass 228, then comparing scan numbers with those of the peaks in chromatograms of standards III and IV. Many PAH were found (including polycyclics containing oxygen, sulfur or nitrogen) for which no I-value was available. For those peaks which were identified by GCMS and a retention index, assignment of isomers was possible. It is this confirmation of basic structure as well as the identification of distinct PAH isomers which makes the retention index system so useful.

Although generally fewer peaks were identified when GCMS was used to screen for target PAH, as compared to peaks identified using capillary GC results and the retention system, the detailed screen for polycyclics (D(S)('79)IV) resulted in identifying nearly three times as many peaks as the GCFID system. Many of the peaks identified weren't included in the retention index table. Some of the identifications made by capillary GC-FID (and I value) were not confirmed by GCMS analysis. The possible reasons for this include; 1) several peaks having the same or similar I values; 2) the compounds indicated by the retention index are not necessarily found in the EPA/NIH mass spectral data base; 3) the I values are not exactly transferable due to differences in capillary columns, and oven temperature programming.

Almost half (40%) of the peaks found in the detailed search of fraction IV are alkyl-substituted polycyclics while 20% contain a hetero atom, most often oxygen (in hydroxy groups). The alkyl substitutions are normally methyl, occasionally ethyl.

HPLC analysis of D(S)('78) identified only three PAH, all in fractions IV or V, allowing no comparison between GCMS and HPLC results.

Table 4-12. Results of GCMS Analysis. D(S)('78)III.

Mass	GCMS Identification	I _{MS}	I _{ref} ^a	Compound ^b
178	Ph,A	300	300	Ph
194	hydroxy-Ph, hydroxy-A	303.55		
194	hydroxy-Ph, hydroxy-A	303.95		
210	dihydroxy-A	307.13		
192	mePh, meA	315.60	319.46	3-mePh
192	mePh, meA	319.88	320.17	2-mePh
179	phenanthridine	320.55		
208	mixture of	320.63		
	1) phenanthridine			
	2) 3'-(trimethylsiloxy)-acetophenone			
	3) methoxyA			
	4) methoxyPh			
206	dimePh, dimeA	335.40		
206	dimePh, dimeA	336.83		
206	dimePh, dimeA	337.13		3,6-dimePh
206	dimePh, dimeA	337.43		2,7-dimePh
206	dimePh, dimeA	340.13		
206	dimePh, dimeA	341.25		
206	dimePh, dimeA	342.23		
206	dimePh, dimeA	342.53		
202	P,F	343.95	344.01	F
226	B(ghi)F,1-(1,1-dimethylethyl)-4-phenoxybenzene, cyclopenta[cd]pyrene	347.33		
202	P,F	353.55	351.22	P
216	me-P, B(a)F1, B(b)F1	383.25		
216	as above	389.55		

a - Lee et al., 1979a

b - as confirmed by I value from ref. a

Table 4-13. Results of GCMS Analysis. D(S)('79).

Fraction	Mass	GCMS Identification	I _{MS}	I _{ref} ^a	Compound
III	166	Fl	273.80	268.17	Fl
	178		289.97		
	178	Ph,A	300.00	300.00	Ph
	202	P,F	317.46		
	192	mePh, meA	318.88	319.46	3-mePh
	192	mePh, meA	324.43	323.90	1-mePh
	202	P,F	340.71		
	206	dimePh, dimeA	340.83	339.23	2,7-dimePh
	202	P,F	347.20	344.01	F
	202	P,F	354.75	351.22	P
	228	Tri, Chr, B(c)Ph	389.21	391.39	B(c)Ph
	228	Tri, Chr, B(c)Ph	400.00	400.00	Tri
	228	Tri, Chr, B(c)Ph	454.23		
	228	Tri, Chr, B(c)Ph	454.93		
	228	Tri, Chr, B(c)Ph	462.96		
IV	142	meN	237.66		
	142	meN	238.94		
	168	3-mebiphenyl	257.12	254.81	3-mebiphenyl
	168	4-mebiphenyl	258.25		
	180	9-meFl	286.94	288.21	2-meFl
	178	Ph,A	300.00	300.00	Ph
	178	Ph,A	301.42	301.62	A
	194	dimeFl	305.68		
	194	dimeFl	312.78		
	192	mePh	314.34		
	211	4-(1-methylethyl)-N-phenylbenzamine	318.03		
	208	methoxyPh	319.74		
	208	methoxyPh	320.31		
	194	2-phenyl-1H-pyrolo-[2,3-6]pyridine	323.57		
	206	dimePh, dimeA	337.63	337.83	3,6-dimePh
	206	dimePh, dimeA	339.62	339.23	2,7-dimeA
	206	dimePh, dimeA	340.33		
	206	dimePh, dimeA	341.32		
	202	P,F	343.03	343.01	F
	202	P,F	350.13	351.22	P
	230	p-terphenyl	363.05	366.01	p-terphenyl
	216	B(b)Fl	365.88	366.74	B(a)Fl
	244	1,3,8-trihydroxy-9H-xanthen-9-one	379.24		
	216	meP	370.43	370.15	2-meP
	234	B(b)naphtho[2,1-d]-thiophene	395.14	389.37	B(b)naphtho[2,3-d]-thiophene
	228	B(a)A, Chr, Tri	400.00	400.00	Chr
	243	meB(a)acridine	406.50		
	258	1,3,6-trihydroxy-8-methyl-9H-xanthen-9-one	408.91		

Continued...

Table 4-13. Continued.

Fraction	Mass	GCMS Identification	I _{MS}	I _{ref} ^a	Compound
IV cont'd	248	meB(b)naphtho[2,3-d]-thiophene	413.03	412.08	7-meB(b)naphtho [2,1-d]-thiophene
	242	meChr, meTri, meB(a)A	416.01	416.32	1-meTri
	258	9,10-dimethyldiB[b,h]-[1,6] naphthyridine	419.71		
	242	meChr, meTri, meB(a)A	421.69	422.87	1-meChr
	254	2,2'-binaphthyl	423.11	423.91	2,2'-binaphthyl
	256	dimeB(c)Ph, dimeB(a)A, ethylChr	431.49		
	256	as above	432.63	432.32	1,3-dimeTri
	252	B()F, B()P, Per	439.30	440.92	B(j)F
	252	B()F, B()P, Per	443.99	443.99	B(k)F
	252	B()F, B()P, Per	451.09	450.73	B(e)P
	252	B()F, B()P, Per	455.06	453.44	B(a)P
	266	meB(j)aceanthrylene	456.63		
	266	meB(j)aceanthrylene	459.47		
	266	meB(j)aceanthrylene	462.73		
	276	B(ghi)Per, Ind[1,2,3-cd]P, diB(def, mno)Chr	480.34	481.87	Ind(1,2,3-cd)P
	276	isomer of above	475.51		
	276	isomer of above	483.04		
	278	B()Chr, diB()A, B(a)naphthacene, pentacene, pentaphene, diB()Ph	483.89	486.81	Pentacene
	276	B(ghi)Per, Ind[1,2,3-cd]P, diB(def,mno)Chr	489.00		
	276	as above	490.92		
	276	as above	491.56		
	302	diB()Chr, B()Per, naphtho[1,2-e]P	515.70		
	300	Cor	525.92		
V	252	B()F, B()P, Per	3 peaks		

a - Lee et al., 1979a.

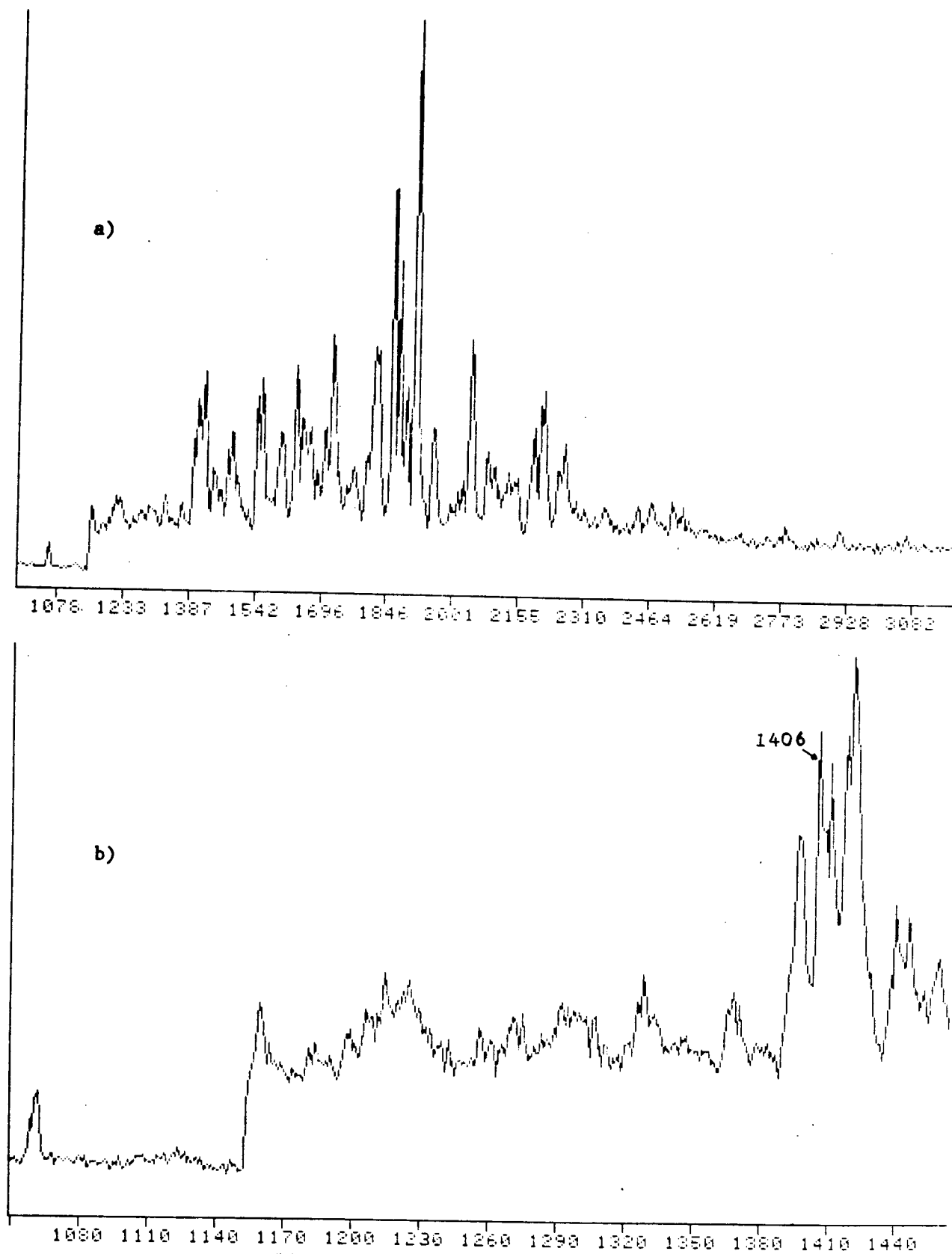
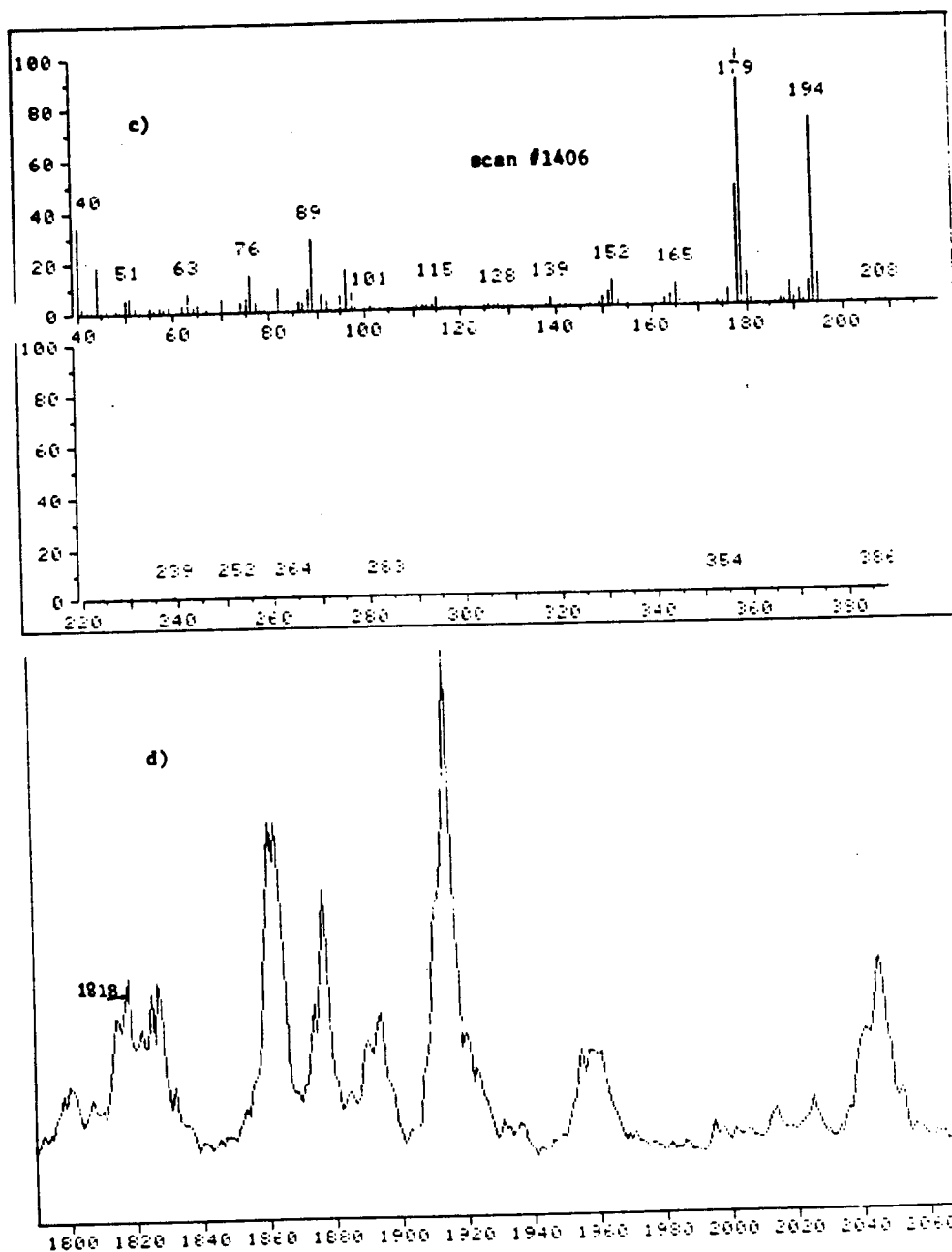


Figure 4-21. GCMS of D(S)('78)III

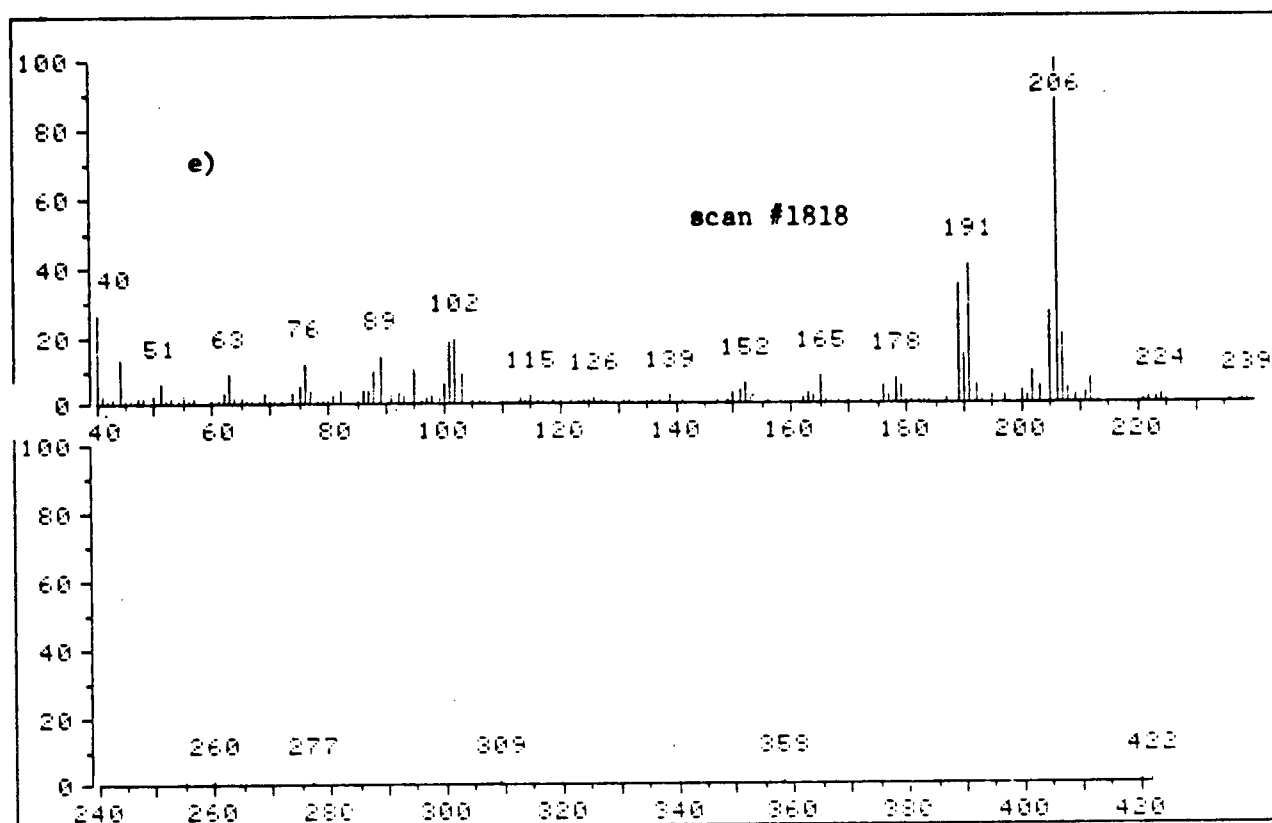
- a) TI chromatogram
- b) TI chromatogram, scan #1080 to #1440



4-21 GCMS of D(S)('78) III Continued

(c) spectrum, scan #1406

(d) TI chromatogram, scan #1800 to #2060



4-21 GCMS of D(S)('78) III Continued
e) spectrum, scan #1818

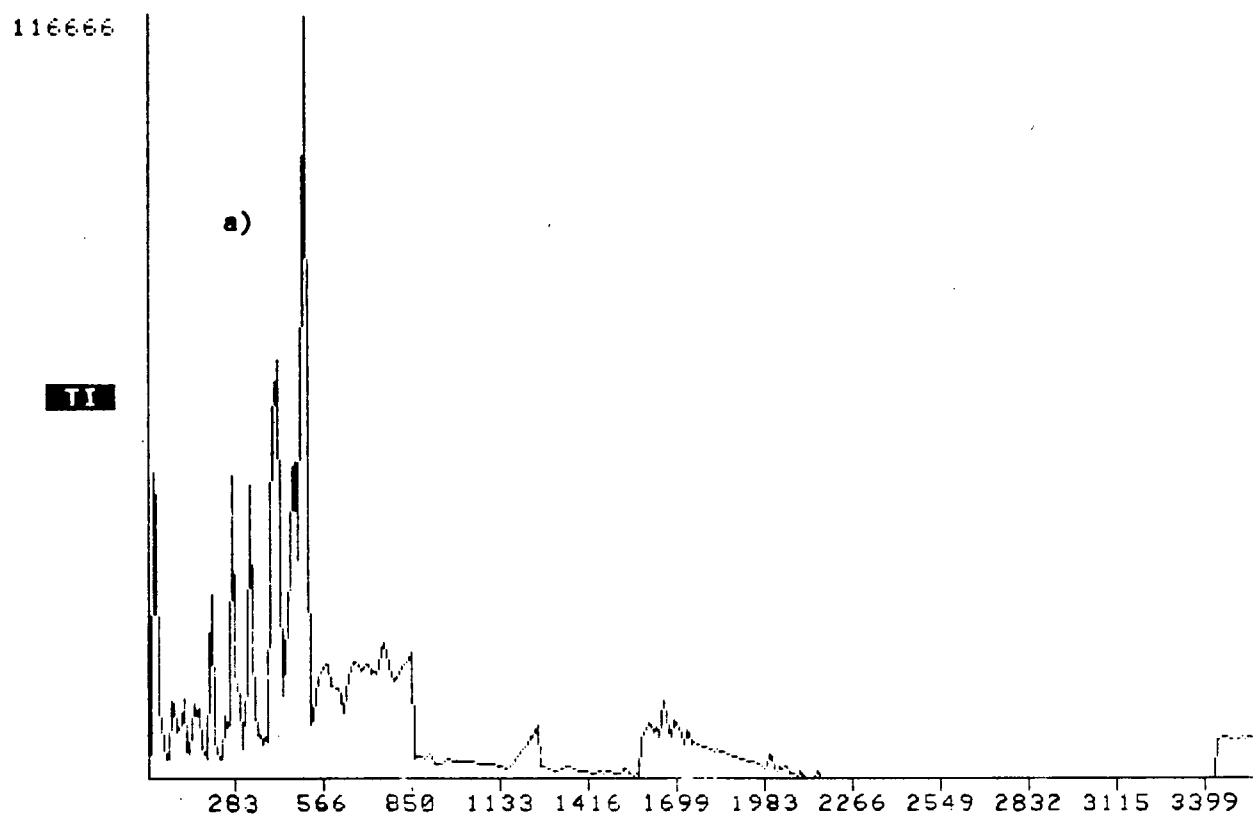
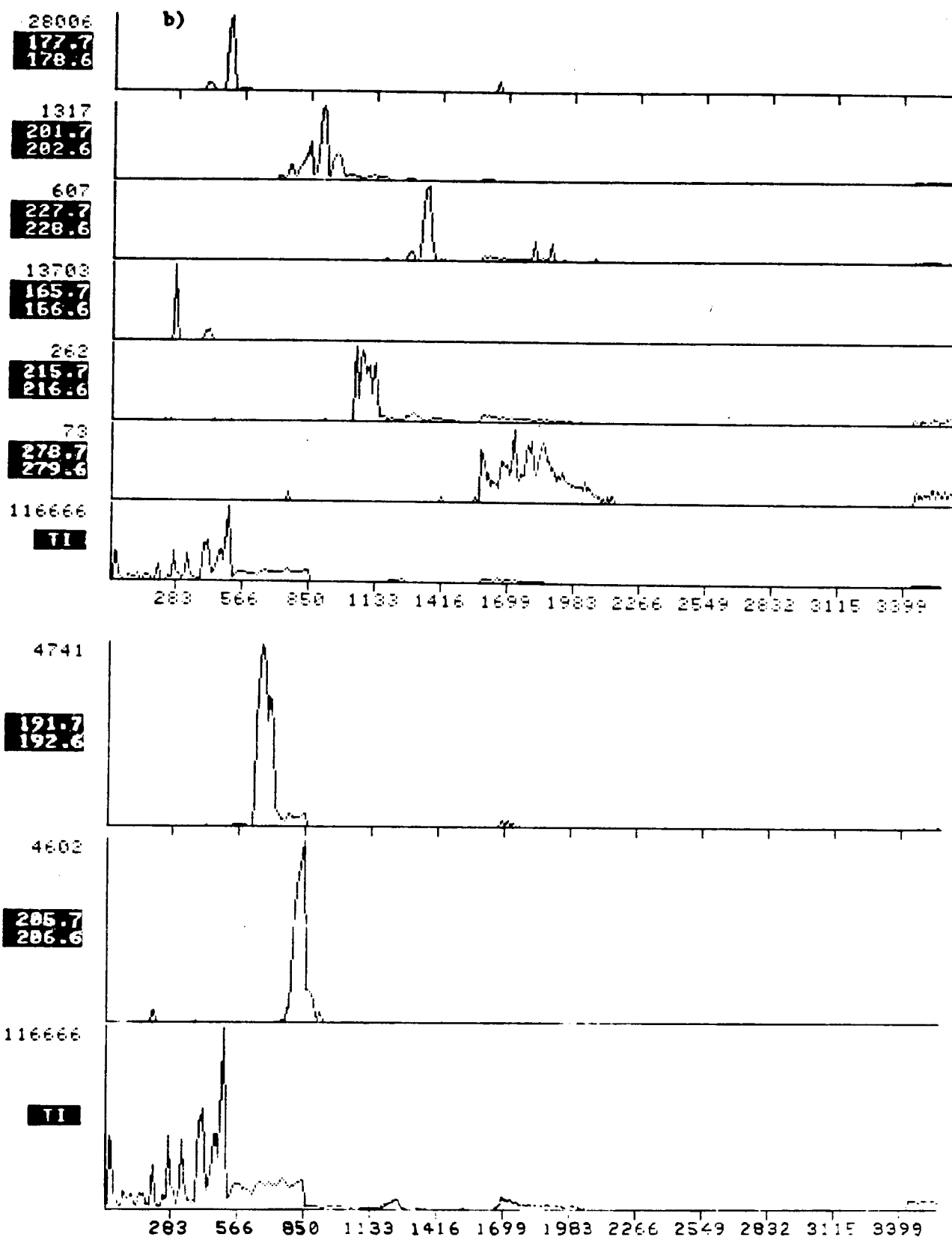


Figure 4-22. GCMS of D(S)('79)III

a) TI chromatogram



4-22 GCMS of D(S)('79) III Continued

b) screen for masses 178, 202, 228, 166, 216, and 279

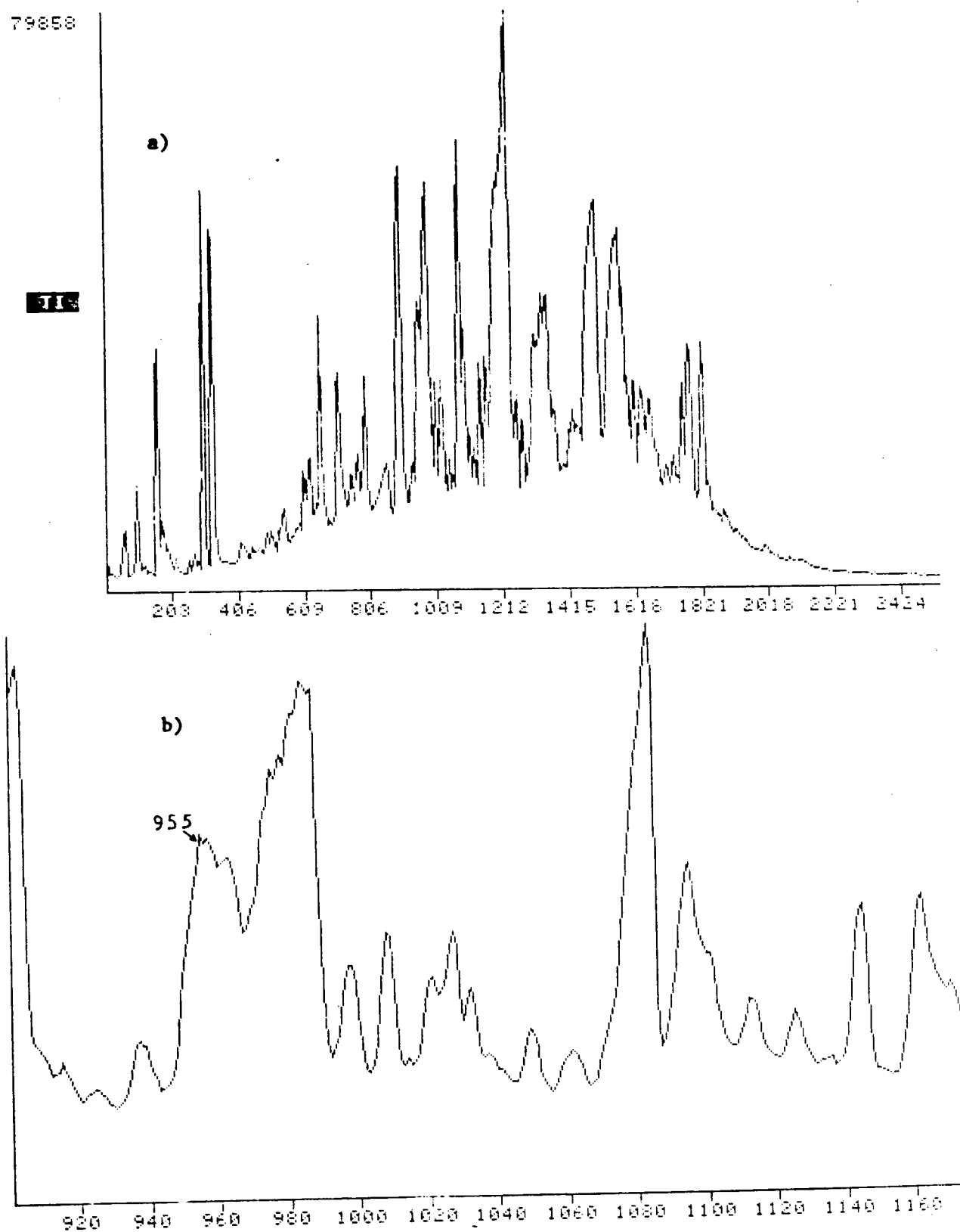
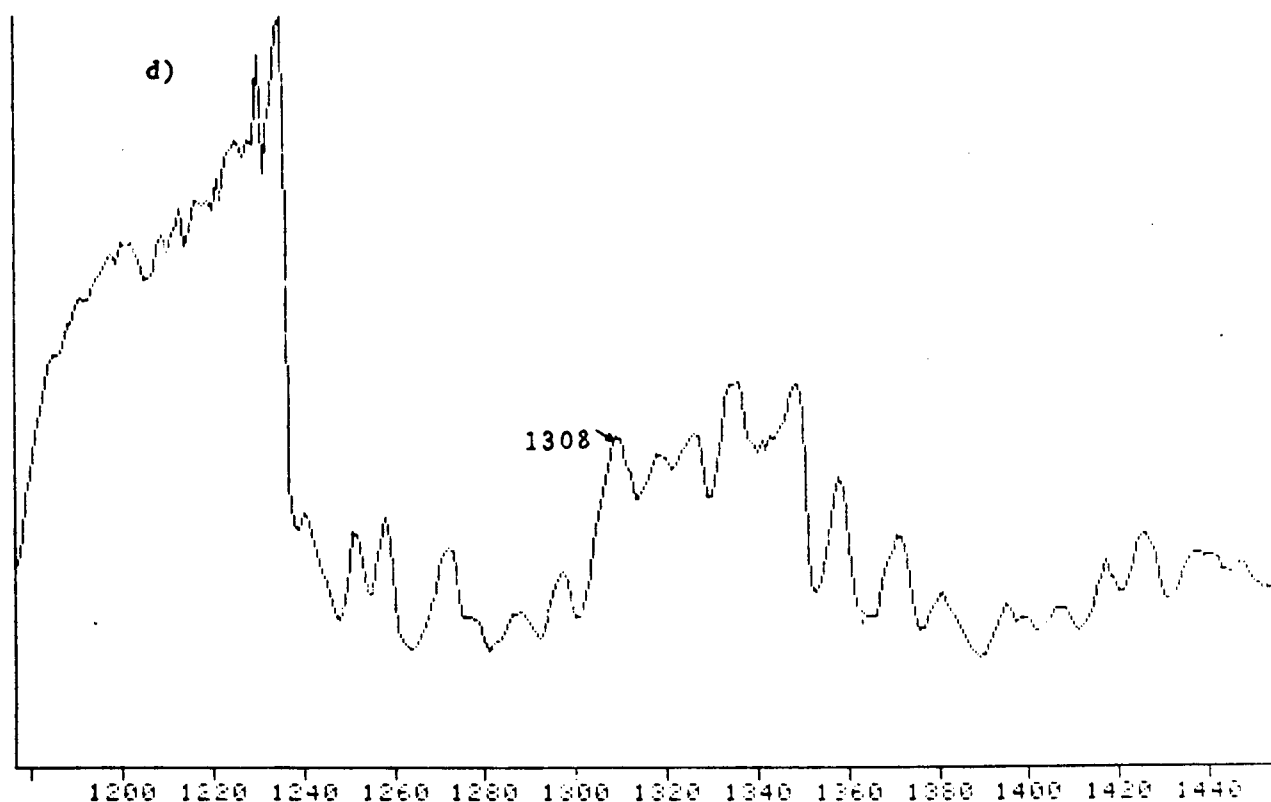
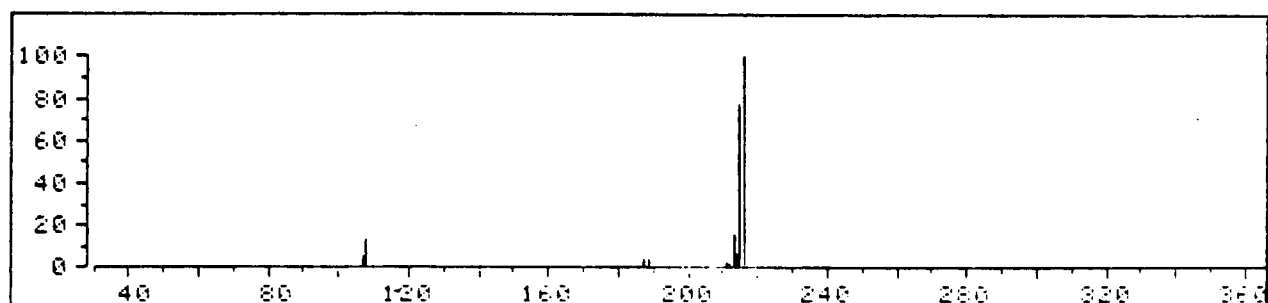
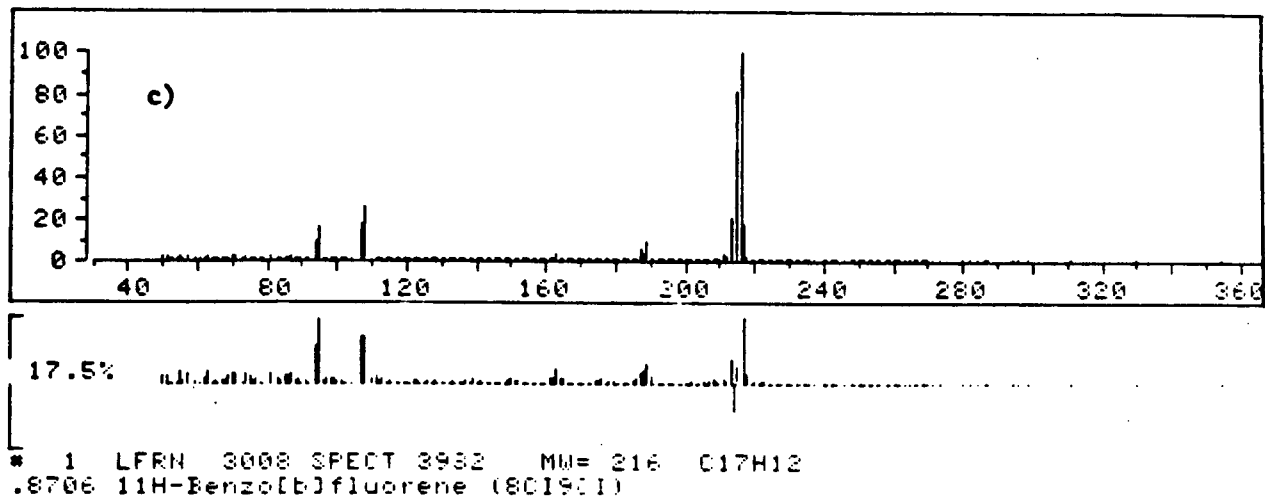


Figure 4-23. GCMS of D(S)('79)IV

- a) TIC chromatogram
- b) TIC chromatogram, scan #920 to #1160

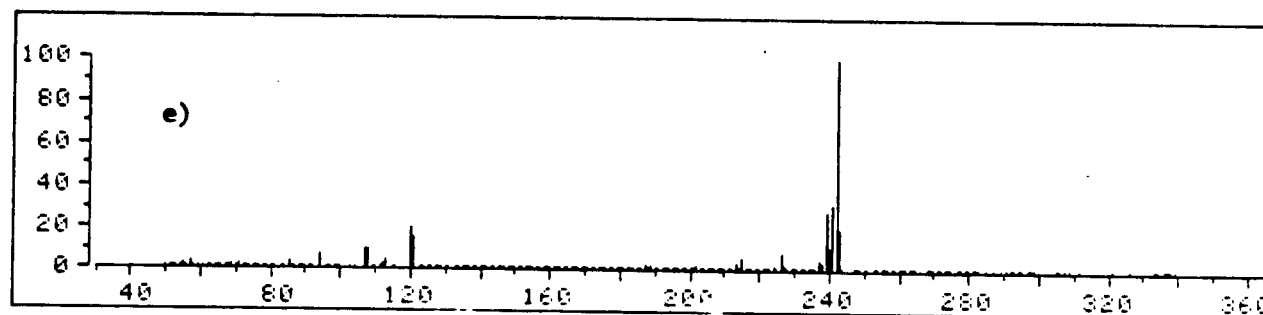


4-23 GCMS of D(S)('79) IV Continued

c) spectrum, scan #955

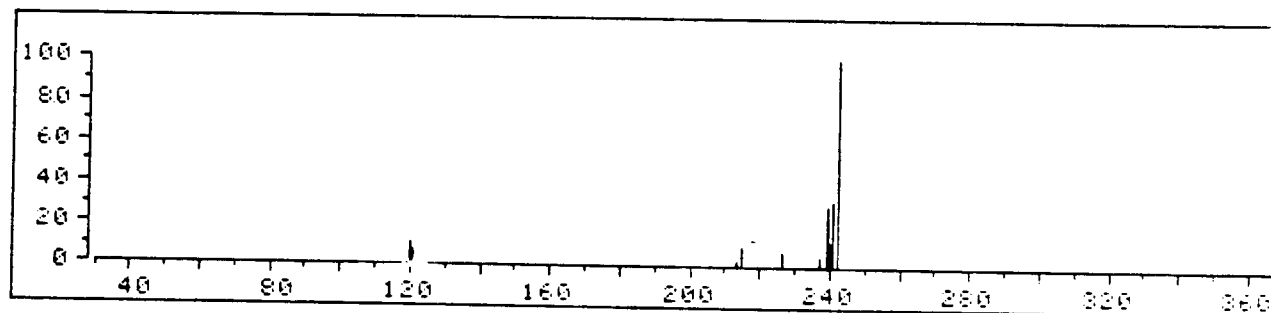
d) TI chromatogram, scan #1200 to 1440

10 HITS: AVERAGED SPECTRUM (FRN 11300) +1308



20.0%

* 1 LFRN 3011 SPECT 1680 MM= 242 C19H14
.9818 Chrysene, 5-methyl- (801901)



4-23 GCMS of D(S)('79) IV Continued

e) spectrum, scan #1308

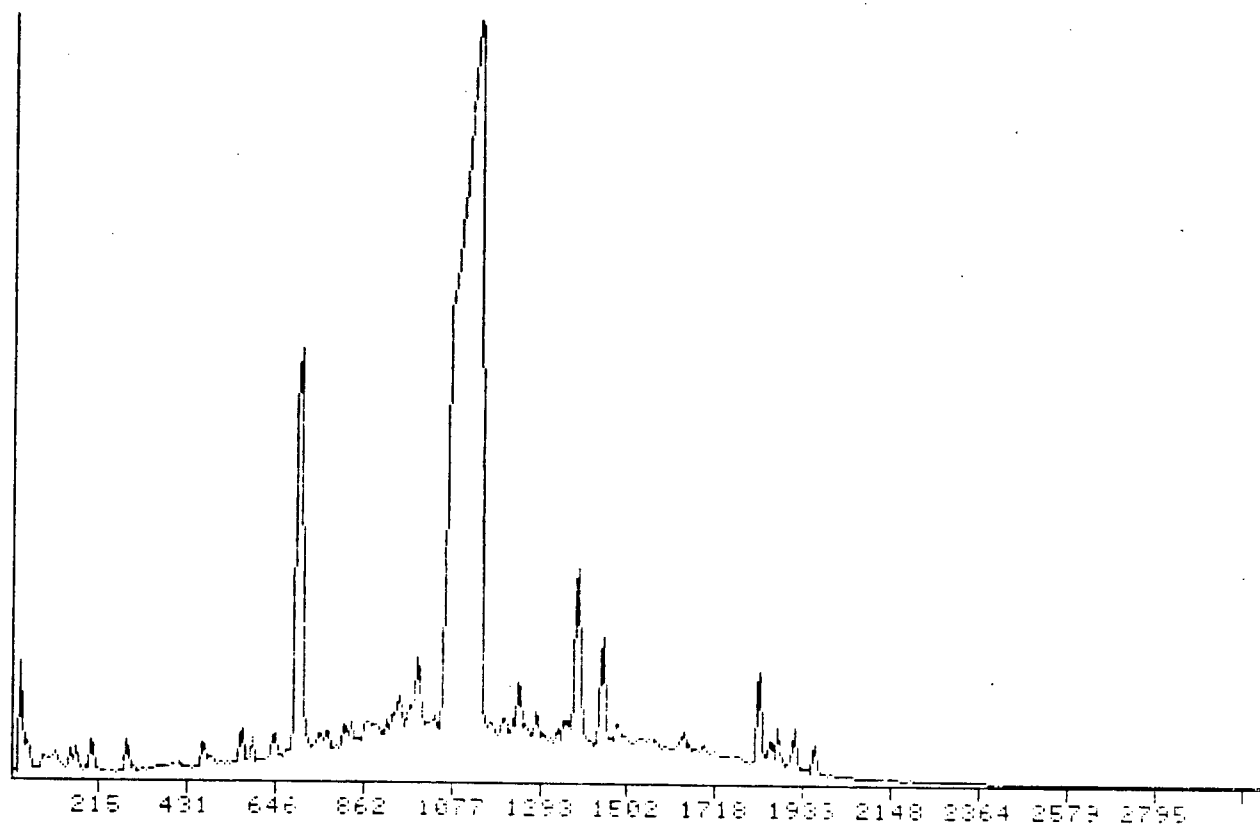


Figure 24. GCMS of D(S)('79)V

a) TI chromatogram

The two polycyclics quantitated by HPLC in D(S)('79) are also found in GCMS analysis although I values don't match as expected.

Total ion (TI) currents traces for the four extracts are presented in Figures 4-21 through 4-24. For D(S)('78)III the TI is shown in Figure 4-21 a) a section of the chromatogram is selected in b), then the spectrum (in c) of scan #1406 (I = 303.95) identifies the peak as hydroxy A or hydroxy Ph. From a different section of the TI chromatogram in d) the spectrum of peak at scan #1818 (I = 336.83) indicates dimeA or dimePh. Selected ions were monitored as indicated in Figure 4-22-b) to pick up the target PAH in D(S)('79)III. The detailed examination of peaks in D(S)('79)IV is illustrated by the examples in Figure 4-23. TI chromatograms and spectra are presented for B(a)Fl and 1 me-Tri.

5. Statistics Treatment

Data on stream sediments was analyzed using the Triangular Regression Package program (see section III-G) to examine possible correlations between various sample characteristics and levels of polycyclics found. The file STREAM contained data for the variables set out below for the ten sediment samples.

- 1) "eqB(a)P" - equivalent B(a)P ppm - integrated area under HPLC chromatogram expressed as B(a)P. Normalized to a 1 μ l injection of a 1 ml extract, 1 ppm B(a)P dry wt corresponds to 85.5 area units. Hence the total integrated area (normalized to area/g dry wt) over 85.5 equals eqB(a)P. It is acknowledged that this expression of "area under the curve" doesn't take into account different response factors (area response/ng injected) for

different polycyclics; contributions to the total area by non-PAH components; or any affects of chromatography on response factors. Nonetheless it provides a useful technique for quantitating an approximate "total PAH" in a sample. (See Table 4-14.)

- 2) "sum PAH" - the sum of PAH (in ppm) quantitated for that sample.
- 3) to 13) - concentrations (in ppm) of the individual polycyclics;
B(ghi)Per, P, Tri, B(e)P, B(k)F, B(a)P, B(b)F, B(ai)Ph, Chr, 2-mePh, B(ghi)F.
- 14) "% OM" - percent organic matter.
- 15) to 18) - sediment size distribution; % a, % b, % c, and % d.
- 19) "year" - 1978 or 1979, assigned a value of 1 or 2 respectively.
- 20) "km" - distance in kilometres of the sampling point upstream from the entrance to Burnaby Lake (see Table 4-14).
- 21) "travol" - traffic volume - volume (vehicles per 24 hour period) at the nearest junction to sampling point. Volumes were assumed to have been increasing at a rate of 5% per year (as is documented for bridge traffic 1977 to 1978). See Table 4-14.

Values for the three variables which are discussed for the first time in this section ("eqB(a)P", "km", and "travol") are listed in Table 4-14.

The first application of the TRP set as the independent variables those numbered 14 to 21 above; the sediment characteristics (organic matter, size distribution), the year the sediment was collected, the position of the sampling point on the stream, and the daily traffic volume. The dependent variables were set as "eqB(a)P", "sum PAH", as well as the seven quantitated polycyclics and each was examined for correlation separately. For these runs both forward and backward

Table 4-14. Values of Variables Examined in Regression Analysis;
 "eqB(a)P", "km", and "travol".

Sample	"eqB(a)P" in ppm	"km"	"travol"		
			Source	Sample Point	Volume
L ('78)	19.72	5.83	BC ^a	Lougheed @ Boundary	25,983
G ('78)	1.48	3.98	Burnaby ^b	Gilmore @ Lougheed	7,330
W ('78)	4.50	3.28	"	Willingdon S. of Juneau	19,830
D(S)('78)	23.46	1.61	"	Douglas @ Still Creek	11,647
D(N)('78)	2.68	1.61	"	Douglas @ Still Creek	11,647
L ('79)	12.59	5.83			27,350
G ('79)	0.77	3.98			7,716
W ('79)	26.23	3.28			20,874
D(S)('79)	88.12	1.61			12,260
D(N)('79)	3.07	1.61			12,260

a - BC Ministry of Transport, Communications and Highways

b - Municipality of Burnaby, Highways Department.

regression was used. In all cases, regression analysis equations collapsed to a constant. In other words no significant correlation among the variables exists.

By examining the steps of the regression analysis, it was found that the last variables to be forced out of the equations included "% d" and "km". A subset of independent variables was constructed; "% OM", "% d", "km", and "travol". Analysis was run against these with first "eqB(a)P" and then "sum PAH" as the dependent variables. Again, only constants were produced as the regression equations. "% d" (percent fine silt or clay) was consistently one of last variables forced out. Since the amount of fine particulates is generally related to a sediments organic content ("% OM"), it isn't surprising that it should have some importance to the PAH load in the sample.

The lack of significant correlations may be due to inadequate numbers of sampling sites, or to considering too few variables, or not including important variables. Perhaps quantitation of specific compounds over all the samples (as compared to at most five) would yield more meaningful results.

6. Summary

Quantitative results of HPLC analysis have been set out in Table 4-5 for specific PAH, and are listed in Table 4-14 expressed as equivalent B(a)P. The values for specific compounds range from 55 ppb to a high of 38.5 ppm (dry wt basis). The average of all 21 values is 5.04 ppm but two-thirds of the quantitations fall between 55 ppb and 1.79 ppm. See frequency chart, Figure 4-25. When considering values of eqB(a)P, much the same pattern emerges, with 50% of the sediments

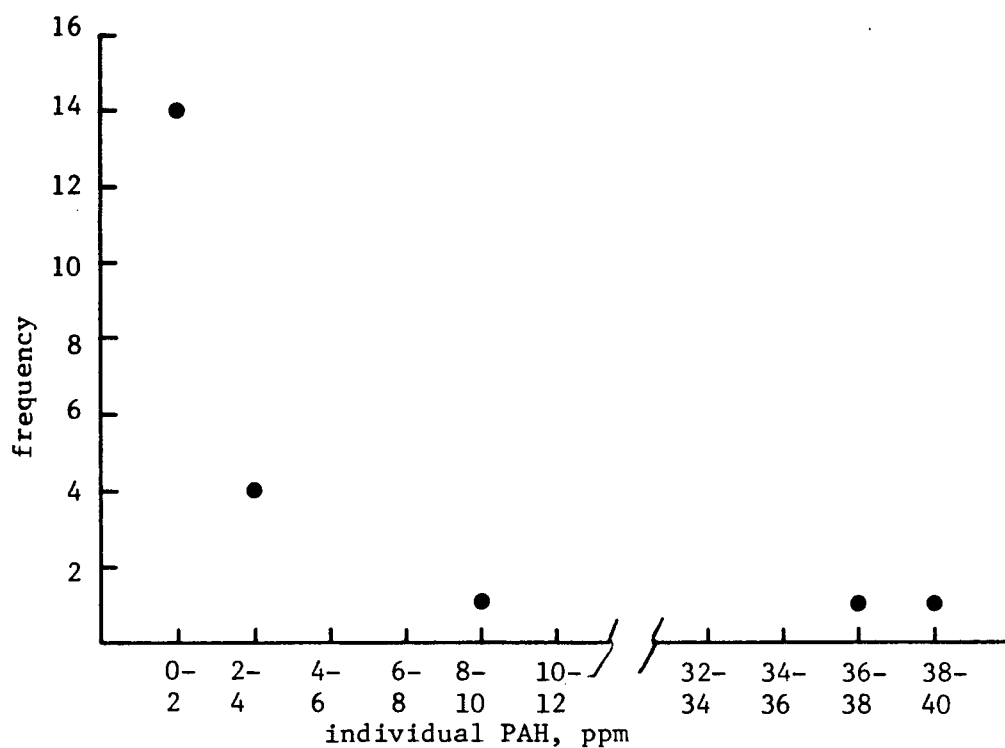


Figure 4-25. Distribution of Levels of Individual PAH in Sediments.

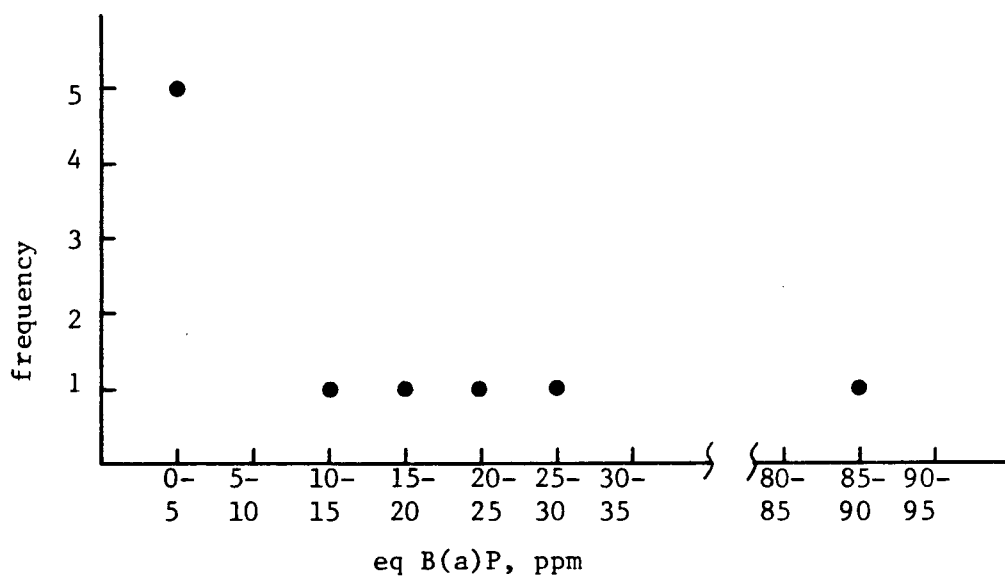


Figure 4-26. Distribution of Levels of Equivalent B(a)P in Sediments.

eqB(a)P values falling in the range 0.77 ppm to 4.50 ppm, with a single high value of 88.12 for D(S)('79) (as expected from high levels of Tri and B(ghi)Per). Figure 4-26 graphs this distribution.

In comparing results obtained in this study to those obtained by other researchers, sampling areas resembling the urban Still Creek watershed were chosen if possible. As discussed in detail in section II C, Table 2-9, sediment PAH levels were determined in the Charles River of Boston (LaFlamme and Hites, 1978), the developed shore of a European lake (Grimmer and Bohnke, 1975b), Buzzards Bay, Mass. (Hites et al., 1977), and river sediment (Giger and Schaffner, 1978). The values for B(ghi)Per and Tri in D(S)('79) sediment (each is approximately 38 ppm, dry wt) are higher than any other individual reported concentrations, although levels of 15 ppm (F) and 13 ppm (P) are found in the Charles River (LaFlamme and Hites, 1978). Reported concentrations for the other polycyclics quantitated in Still Creek sediments are in the same range as this study's results, but are generally slightly lower. The 38 ppm concentrations of PAH in D(S)('79) are possibly due to the combination of a floating weir across the creek immediately downstream to the sampling point and the slower current on the south shore, allowing significant amounts of contamination to settle out at this point. Collection of sediments samples at the Douglas weir caused small "slicks" of oil to rise to the creek surface at every move of the sample shovel.

LaFlamme and Hites (1978) examined the distribution of polycyclics in sediments from around the world as discussed in Section II C-2. The majority of the sediments contained polycyclics in the relative abundance (by molecular weight, MW; Ph (MW178) 12%, F (MW202) 16%, P (MW202)

15%, while MW228 isomers comprised 23%, and the remaining 35% was made up of MW252 isomers. Similar interpretation of data for Still Creek is difficult since complete molecular weight distributions aren't available, but several estimations are possible.

Quantitative stream sediment data (as a result of HPLC analysis) indicates that the most frequently quantitated MW fractions are MW252 (30% of all quantitated PAH) while MW276 accounts for 24% of the determinations, and a further 10% are of MW228 isomers. Summing the concentrations of these fractions across all stream sediments, MW228 accounts for the greatest amount, MW276 isomers contribute nearly as much, while MW252 compounds are quantitated at total concentrations approximately one-quarter those of MW228 or MW276 PAH. It should be noted that there are many peaks present in HPLC chromatograms which could not be quantitated, often due to co-elution of peaks, or no standard available for matching.

Molecular weight distribution data is most extensive for the sample D(S)('79) where all three fractions underwent some form of GCMS analysis. The frequency of occurrence of each molecular weight was calculated for D(S)('79). The MW252 isomers accounted for 10% of all peaks while MW228, MW276, and MW206 compounds were each responsible for 9% of the peaks detected. The fraction MW202 was 7% of all peaks. The remaining peaks spanned molecular weights from MW142 to MW302. Since alumina fractions III and V were only screened for specific masses (Table 4-11) the molecular weight profiles will not be completely accurate.

In comparison with data presented by LaFlamme and Hites (1978) the Still Creek analyses confirm the importance of fractions with molecular

weight 252 (eg. BP, BF, Per) and molecular weight 228 (eg. Tri, Chr, B(a)A), and to lesser extent MW202(F,P). In addition, isomers of MW276 (eg. B(ghi)Per, Ind(1,2,3-cd)P) and MW206 (eg. dimeA, DimePh) made significant contributions to Still Creek sediments. Incidence of Ph (MW178) was not nearly as high for Still Creek as for LaFlamme and Hites data. Similarities between the two studies support the basic conclusion of LaFlamme and Hites, that the major PAH source to sediments is combustion. The lack of quantitative GCMS data may explain the decreased importance of MW178 or MW202 fractions to the Still Creek system. The high levels of polycyclic contamination in Still Creek, especially the D(S)('79) sample, may provide a greater range of PAH (hence the importance of the MW276 fraction). Finally, the different MW profiles may indicate a slightly different combination of combustion sources than found in areas sampled by LaFlamme and Hites (1978).

Giger and Schaffner (1978) reported that P and F are the most abundant compounds in the river or lake sediment, or street dust which they sampled. As well, B(a)P and B(e)P were generally present in approximately a one-to-one ratio. Still Creek sediment quantitations (HPLC data) do not confirm P,F as most abundant, perhaps due to peak coeluting problems preventing all compounds being identified. The only quantitative data available on B(a)P, B(e)P levels indicates that each of these two isomers are present in similar concentration ranges (0.5 to 4.0 $\mu\text{g/g}$). This concentration ranges compares to the upper end of B()P data presented by Giger and Schaffner (see Table 2-9, col. XXII-XXIV).

Several qualitative aspects of the PAH distributions found in this work can be compared to the reports discussed in section IIC. The

degree of alkylation has been used to indicate the source of polycyclic contamination, since petrogenic sources result in highly alkylated compounds with three to four carbons in side-chains, while high temperature combustion (e.g. carbon black furnaces) produces virtually no alkyl polycyclics). Examination of GC and GCMS data for this study indicates a wide variety of alkyl PAH present, but mainly methyl- or dimethyl- compounds. This distribution rules out either petrogenics or high-temperature combustion as the main sources to the Still Creek sediments.

Discussion by Grimmer and Bohnke (1975b) of sediment polycyclic concentrations encompasses several factors that can be addressed with reference to Still Creek results. Grimmer and Bohnke compared sediment levels of B(ghi)Per and Cor to those found in vehicular exhaust, which contains significant amounts of these two compounds. As well, the ratio of B(a)P to the levels of B(ghi)Per and Cor is very high in automobile emissions. The third distinguishing feature was the absence of benzo [b]naphtho(2,1-d)thiophene, generally derived from lubrication oils rather than car exhausts.

Applying these criteria, the high level of B(ghi)Per supports vehicular traffic as a source although the lack of quantitative data for Cor make assessment of ratios among it, B(a)P and B(ghi)Per impossible. The identification of benznaphthothiophene isomers (GCMS data), however, indicates lubricating oil contamination of sediments. It is reasonable to assume that both factors (vehicular emission and lubrication oils) contribute to PAH levels.

Investigations by Dunn and Stich (1976) into B(a)P levels around creosote pilings, and in the outflow area of Iona sewage treatment

plant, were able identify point sources of B(a)P contamination. No such isolated source was found in Still Creek sediments. Equivalent B(a)P levels didn't correlate with the sampling point on the stream ("km"), and land use data for points upstream is not extensive enough to allow determinations of point sources. The highly similar profiles among sediment extracts is indicative of a general polycyclics source.

The study of Brunette River watershed (including Still Creek) by Hall et al. (1976) analyzed sediments for trace metals and organo-chlorine compounds. The results for lead and PCB's were compared to eqB(a)P levels for equivalent sample sites. Sediment lead levels tended to cluster around 400 ppm except for the Douglas site, where lead was present at 840 ppm. Thus lead levels showed no direct correlation with eqB(a)P concentrations, however, annual variation in contaminant levels is great enough that simultaneous sampling would be valuable. Values for PCB concentration demonstrated considerable scatter. Hall and co-workers report that the middle reach of Still Creek (where sampling points for this thesis work are situated) is the most heavily contaminated section of the Brunette system. Certainly the range of polycyclic quantitations is comparable to values reported in other highly urbanized areas (e.g. Charles River, LaFlamme and Hites, 1978).

The polycyclics identified include a number of carcinogens, including B(a)P, B(k)F, B(b)F, B(ghi)Per, and cyclopenta(cd)pyrene (see listing of carcinogenic potentials in Appendix 1). Quantitation of carcinogen load to environment isn't possible with the data available here, but potential for additional human exposure to these compounds is demonstrated by their presence in the sediments. Estimations of

carcinogenic impact are further complicated by the possible interaction of the various PAH and also their action combined with other cancer causes.

The application of statistics programs to the quantitative data resulted in no specific correlations among sediment sample characteristics and polycyclic levels. Other factors which could have been investigated include:

- a) site characteristics - flow/current parameters, and the extent of "competition" for xenobiotics by sediment organisms;
- b) stream characteristics - extent of most recent rainfall and build-up time since that rainfall, effects of the changes in season, and length of curb (or street side) drained by that section of stream;
- c) watershed characteristics - patterns of fuel use in the watershed, auto exhaust input to watershed, and a quantitation of industrial or commercial landuse, especially upstream of specific sampling sites.

C. STREET SEDIMENTS

1. Sediment Characteristics

Particle size distribution and organic matter content were determined for street sediment samples. Size ranges used are the same as described in Section IV B-1 (Table 4-4). Distributions are plotted in Figures 4-27 to Figure 4-30 and it can be seen that the sediments have very similar profiles, with roughly equivalent amounts of the three larger fractions and approximately ten percent fine silt and clay. Percent organic matter closely correlates with % d (fine silt and

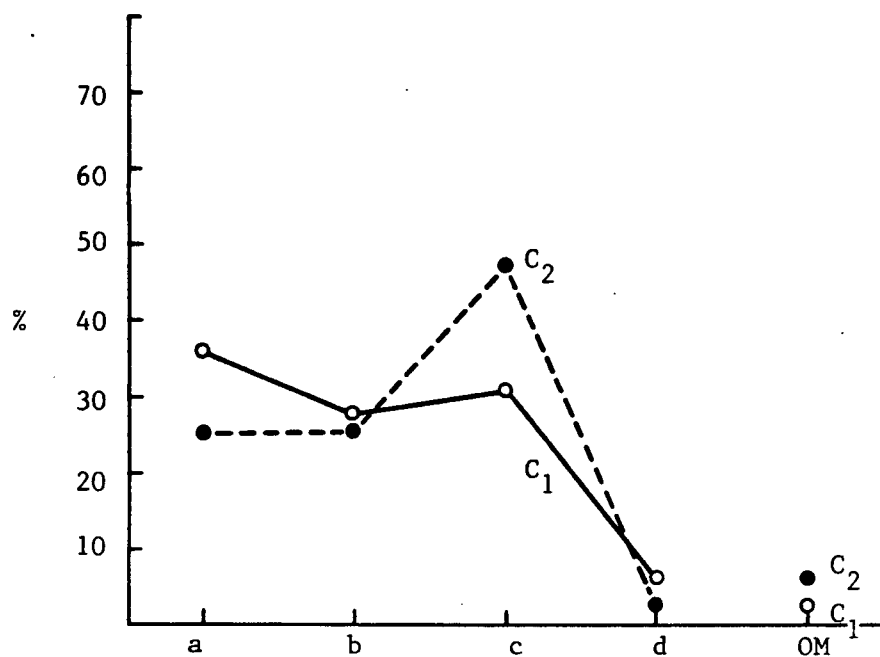


Figure 4-27. Particle Size Distribution and Organic Matter, C₁ and C₂.

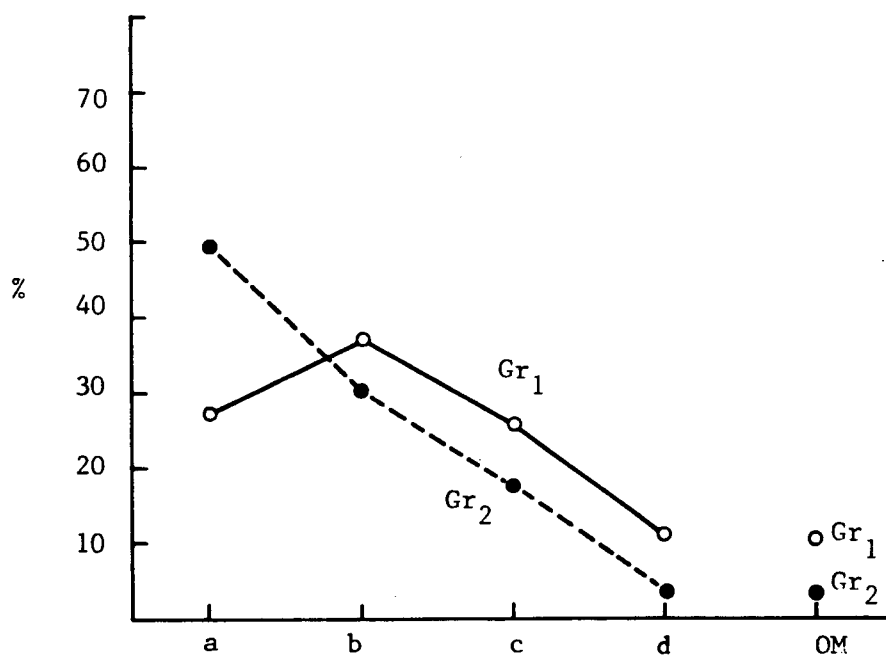


Figure 4-28. Particle Size Distribution and Organic Matter, Gr₁ and Gr₂.

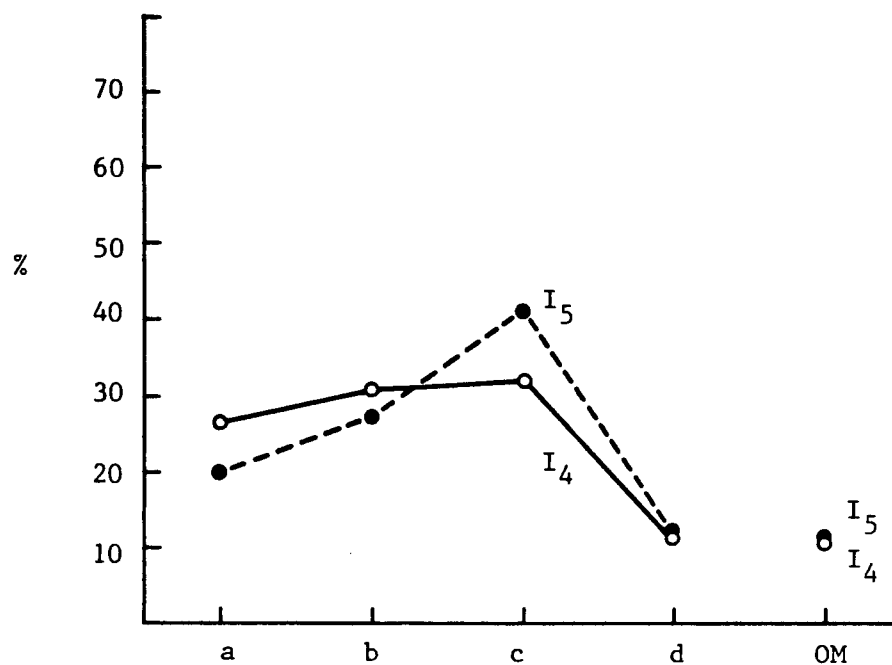


Figure 4-29. Particle Size Distribution and Organic Matter, I_4 and I_5 .

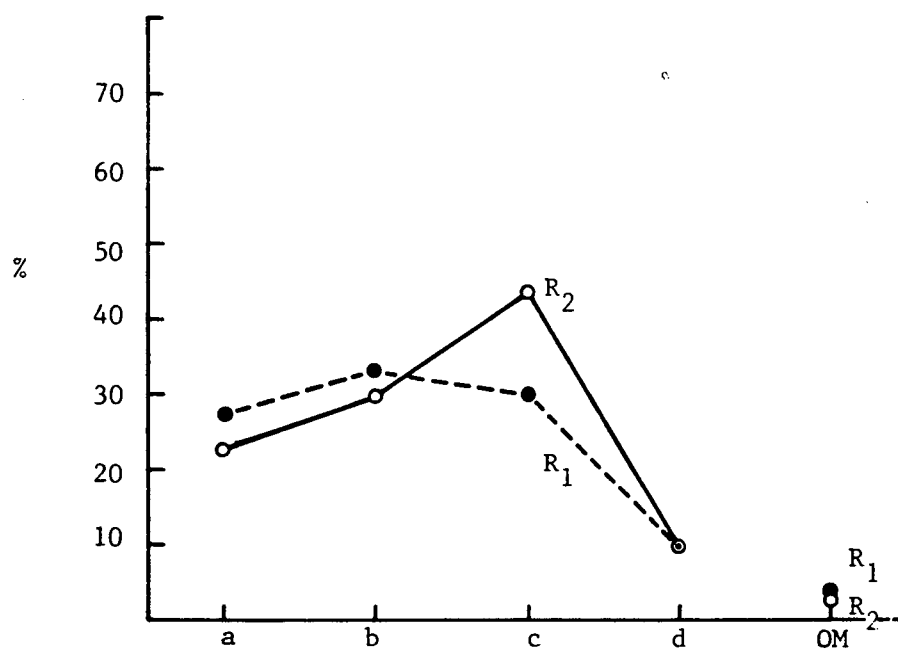


Figure 4-30. Particle Size Distribution and Organic Matter, R_1 and R_2 .

clay). Street sediments demonstrate much less variation sample to sample than is found in Still Creek sediments, and organic matter is generally much lower.

2. HPLC Analysis

Extracts for the eight street sediments (three fractions each) were analyzed by HPLC as described in Section III F-2. Extract injections were either preceded or followed by injections of mixed standards.

The levels of quantitated PAH are set out in Table 4-15, as well as the calculated "equivalent B(a)P" for each sediment. In comparison to levels in stream sediments, street samples have somewhat lower but comparable PAH levels, and fewer PAH were quantitated. Wakeham et al. (1980a) reported that material on asphalt-paved streets consistently contained several times the PAH load of material collected on concrete street surfaces. Unfortunately, street samples in this study weren't chosen to allow consideration of this factor.

Examples of HPLC traces of street samples can be found in Figures 4-31 through 4-34. A qualitative comparison of these profiles to those of stream sediments (Figures 4-11 through 4-16) indicate that the profiles have many similarities. The same peaks are present in the majority of the extracts, and most differences are due to varying peak heights. Street and stream extracts, fraction IV, have highly similar profiles.

3. Statistics Treatment

Regression analysis (as described in Section II-G) was applied to

Table 4-15. PAH Levels and equivalent B(a)P for Street Sediments.

Sediment	PAH	Level (ppm)	eqB(a)P (ppm)
R ₁	B(k)F	0.056	5.81
R ₂	P	0.70	2.31
	9,10-dimeA	6.50	
	B(ghi)Per	0.32	
C ₁	B(ghi)Per	0.63	10.11
C ₂	P	0.45	4.11
	B(e)P	0.65	
	B(ghi)Per	0.78	
I ₄	Tri	0.25	3.64
	B(ghi)Per	0.20	
	P	5.98	
I ₅			15.61
Gr ₁			0.62
Gr ₂	P	3.52	6.92
	Tri	1.16	
	B(k)F	0.16	

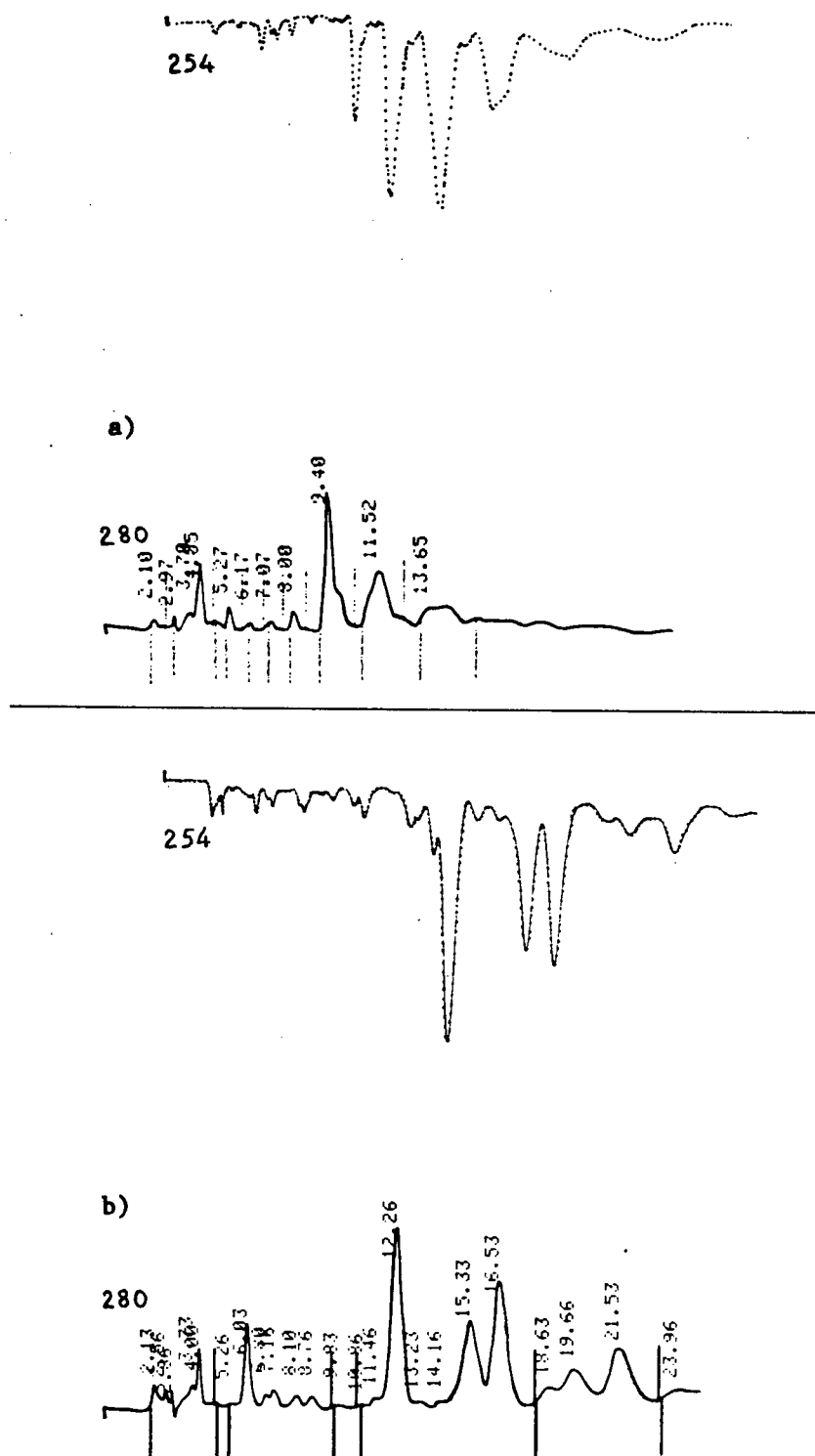
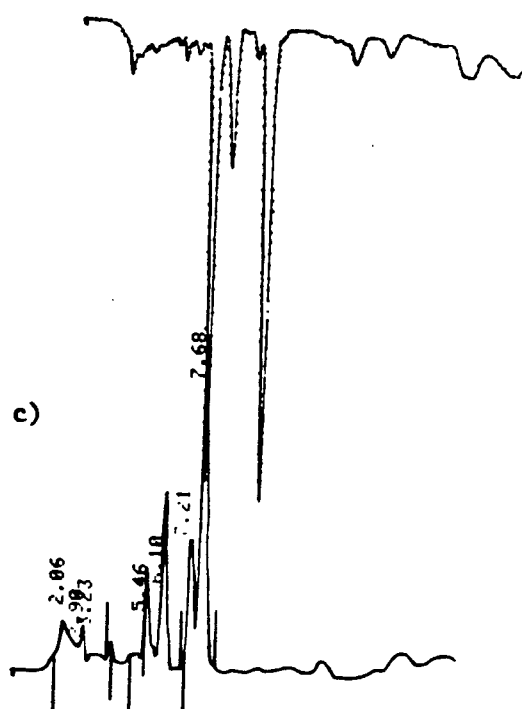


Figure 4-31. HPLC Analysis of Street Sediment R₂.

a) III
b) IV



4-31 HPLC Analysis of Street Sediment R₂ Continued

c) v

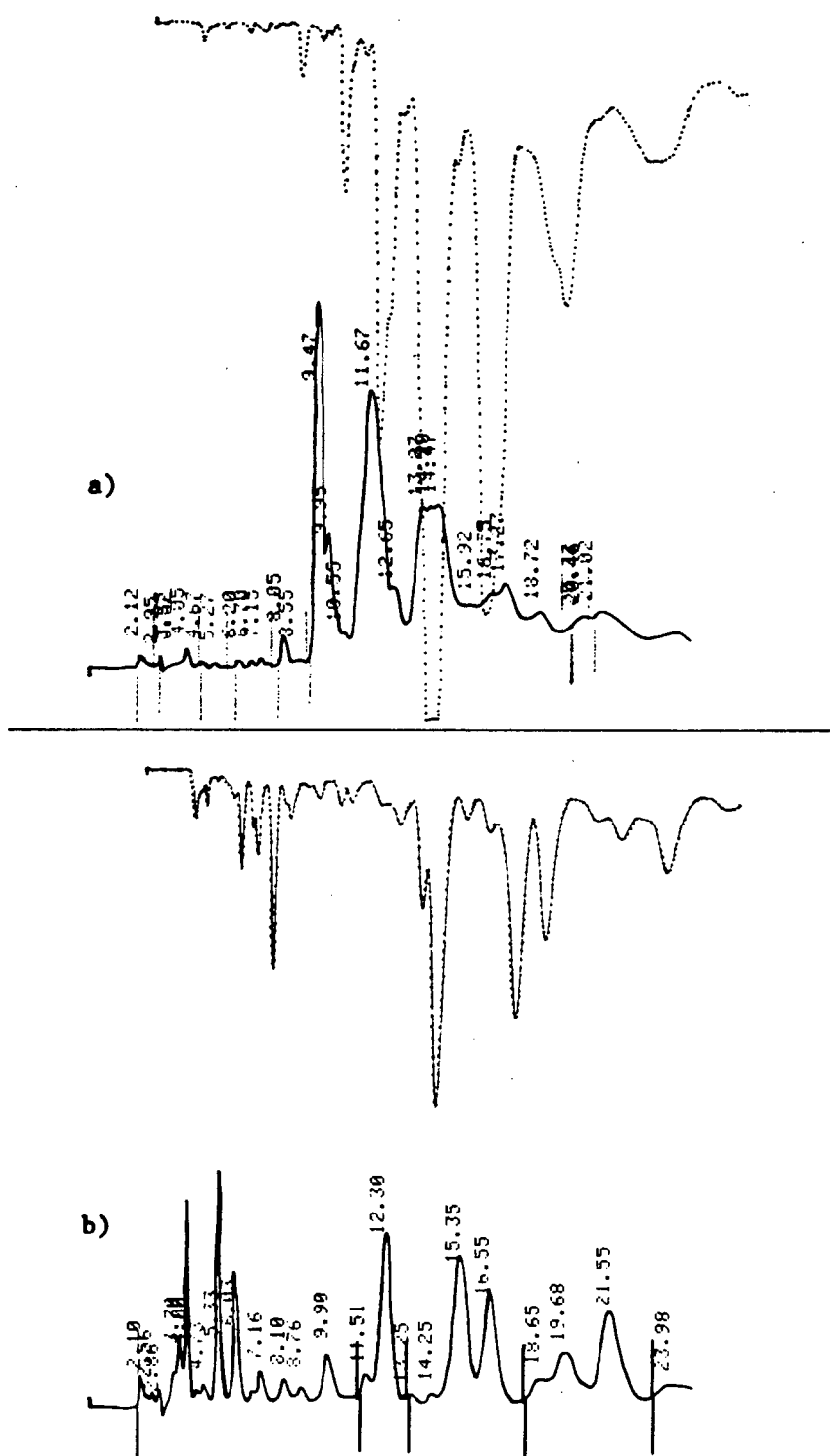
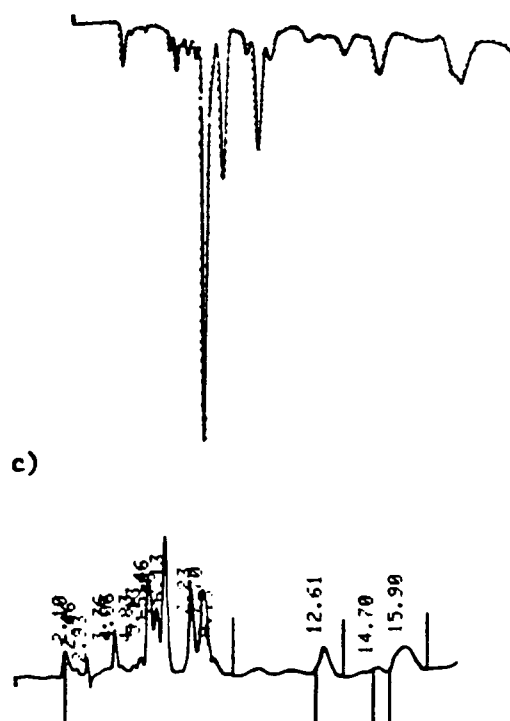
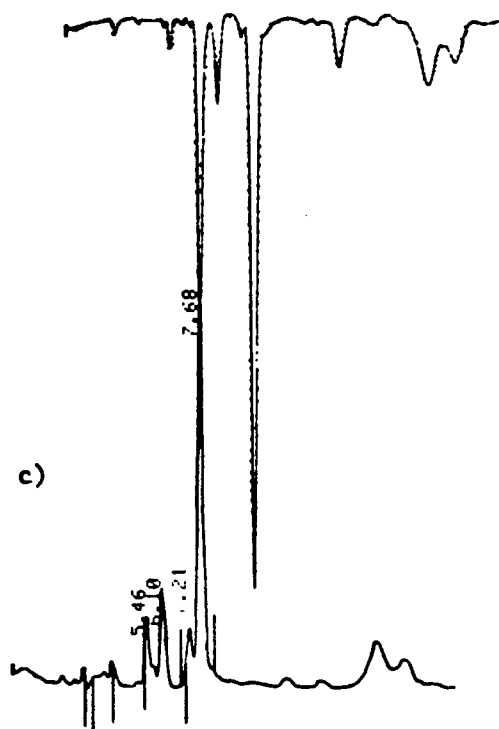


Figure 4-32. HPLC Analysis of C_2 .

- a) III
b) IV

4-32 HPLC Analysis of C₂ Continued

c) v



4-33 HPLC Analysis of I₄ Continued

c) V

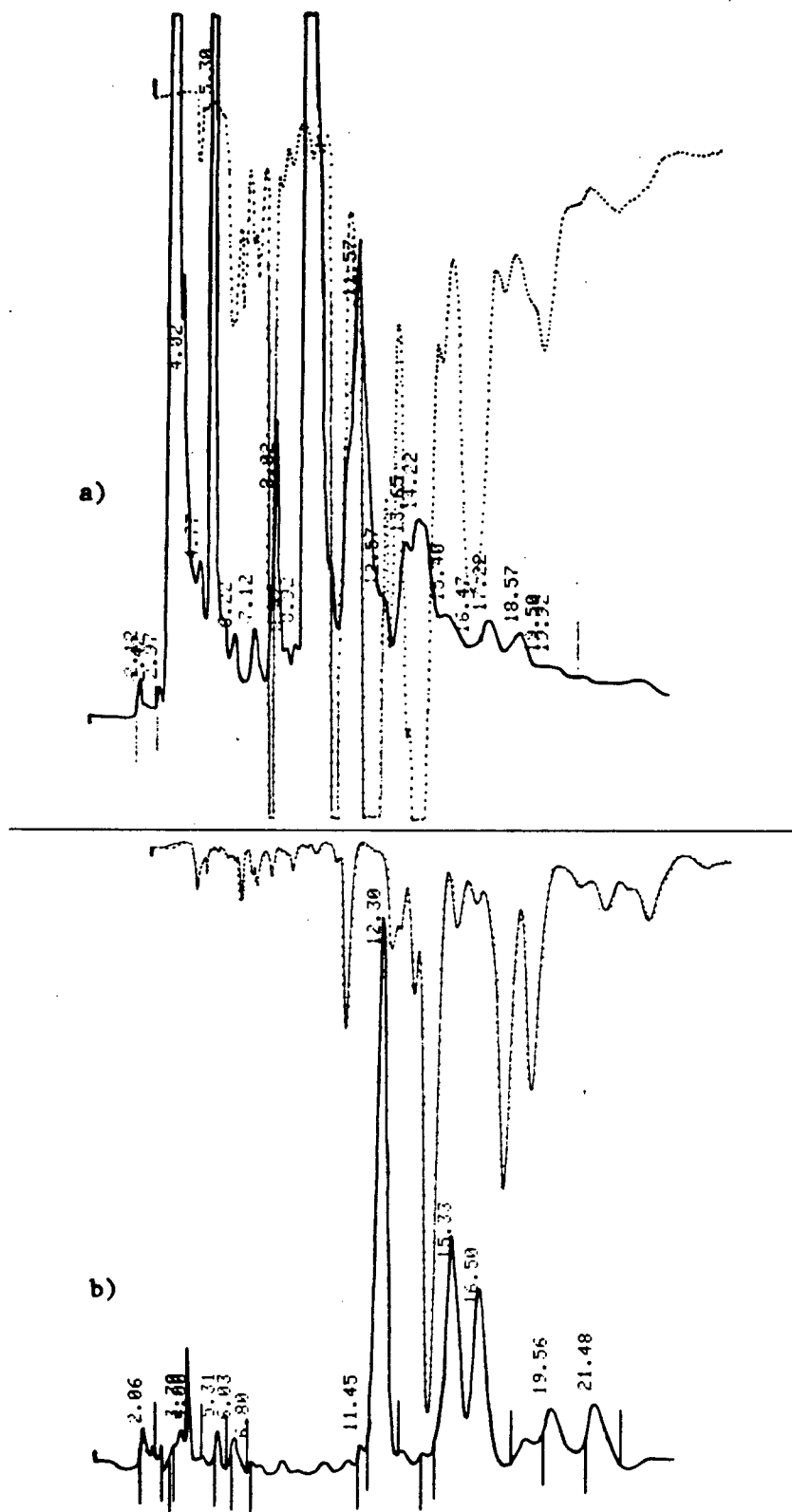
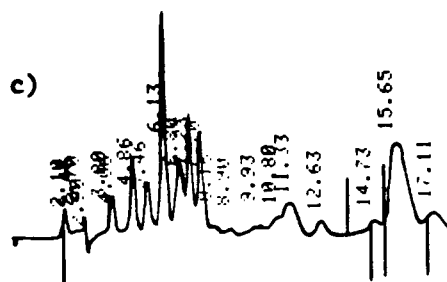
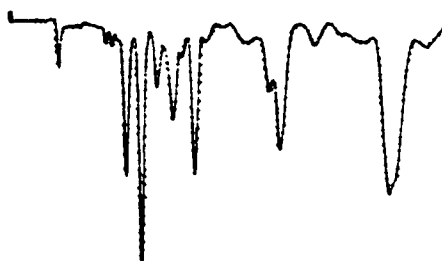


Figure 4-34. HPLC Analysis of Gr₂.

a) III
b) IV

4-34 HPLC Analysis of Gr₂ Continued.

c) v

the street sediment results to investigate possible correlations.

Parameters investigated are listed below.

- 1) "eqB(a)P",
- 2) "sum PAH" - total concentration of quantitated PAH,
- 3) to 8) - concentrations of the individual polycyclics,
- 9) "Land use" - four land uses (Gr, R, C, I) assigned values 1 to 4 respectively,
- 10) "% OM" - percent organic matter,
- 11) to 14) - sediment size distribution, % a to % d,
- 15) "travol" - traffic volume - vehicles per 24 hour period at nearest monitored junction to sampling points.

Traffic volumes may be slightly low due to differences in location of sampling point and the traffic monitor, but relative volumes from one sampling site to another should be accurate.

Both forward and backward stepwise regression were run on these parameters with each of the following being set as the dependent variables in turn; eqB(a)P, sum PAH, and the individual polycyclic values. Most trials collapsed to a constant (i.e. no significant correlation) but sum PAH was expressed in terms of % b with an R^2 value of 0.89. Percent organic matter was eliminated in the penultimate step, indicating its strong contribution. Since both % b and % OM are physical sediment characteristics, indications are that the polycyclic load is at least to some extent determined by the composition of the sediment.

4. Discussion

Comparison of street extract profiles to those of sediment

extracts indicated a significant qualitative similarity. Quantitations of polycyclics were in the same range as for sediments. Contribution of street sediments PAH to stream deposits appears likely in view of qualitative similarity and loading level. Wakeham et al. (1980a) reported that street dust had the same molecular weight range and similar alkyl homologue plots to river sediments, thus leading them to conclude that street run-off forms an important input to river sediments. The street sediments sampled by Wakeham et al. (1980a) demonstrated a preponderance of non-alkylated PAH. The quantitated polycyclics in Still Creek watershed street sediments (see Table 4-15) also tend to be the parent compound, confirming this observation. Alkylated polycyclics were identified in GCMS analysis of stream sediments but no comparable data is available for street sediments.

In a study by Zurcher et al. (1980), which investigated the total hydrocarbon levels in highway run-off and in secondary effluent from a sewage treatment plant, an important contribution of highway run-off material to the effluent was established. Correlations of hydrocarbon content with lead levels also implicated vehicular exhaust.

Asphalt road surfaces (studied by Wakeham et al., 1980a) consistently carried greater polycyclic loads than cement roads. Other contaminants (pesticides and PCB's) were also in higher concentrations on asphalt surfaces than on cement surfaces in an extensive survey of road deposits by Sartor et al. (1974). Street surface samples in the Still Creek study were collected without regard to whether the surface was asphalt or concrete, or the age of the asphalt. However, one sample of asphalt was extracted (Soxhlet extraction) to investigate this possible PAH source. HPLC analysis resulted in only a single

peak, in contradiction to complexity of asphalt sample analyzed by Wakeham et al. (1980a).

Other factors which could have been assessed in analysis of street deposits include lead (or metal) content of sediments, asphalt contribution, build-up time prior to sampling, traffic volumes for specific sites sampled, and a more precise expression of land use.

Levels of eqB(a)P for street deposits collected in this study, and concentrations of lead and PCB's from the previous study by Hall and co-workers (1976) were compared to determine any correlations. For either contaminant (Pb or PCB), levels in greenspace samples were lower than for any other land use, but otherwise the results showed no significant correlation to polycyclic levels determined in this study.

D. OLIGOCHAETES

Samples for oligochaetes (benthic invertebrates) were obtained at the same time (and the same sites) as the sediment samples in 1979. The oligochaetes were separated from the rest of the sediment and analyzed for polycyclics. Results of these analyses are only preliminary due to deactivation problems with alumina chromatography. B(a)P (used to monitor alumina elution behaviour) was eluted too quickly, so some polycyclics in oligochaete extracts (and in corresponding sediment extracts) were inadvertently discarded in alumina fractions I and II. Comparisons between obligochaetes and the sediments are therefore limited to those peaks which were collected. No attempt was made to account for the oligochaete contribution to sediment polycyclic levels for this comparison.

Qualitative comparisons of HPLC traces indicates that only some of

the peaks present in the sediments are found in the oligochaetes at that sampling point. This observation points to the possibility of selective uptake of PAH by oligochaetes. Sediments with higher PAH levels tend to share earlier peaks with oligochaetes while in "cleaner" samples the later-eluting peaks were in common in both sediments and oligochaetes.

Equivalent B(a)P is much higher for the oligochaetes than for the sediments, perhaps a reflection of association of polycyclics with organic matter. Also, the eqB(a)P for oligochaetes from Gilmore and Douglas (N) are higher than for the more heavily contaminated sediments.

As discussed in Section II B-2, levels of polycyclics in aquatic organisms are dependent on the PAH contamination in the environment, and on the organism itself. Fish caught in the ocean depths generally have PAH levels below detection levels, while pollution tolerant snails contained polycyclics at mid ppm concentrations (see Table 2-4). Bioconcentration of PAH from the surrounding environment varies from an average concentration factor of 6 for tubifex, through 20 for carp (Black et al., 1980). Each compound is accumulated to a different level.

Assessment of bioconcentration by oligochaetes in Still Creek sediments can only be preliminary, yet yields some interesting results. Area under the HPLC trace (normalized to gram dry material) (eqB(a)P) was calculated for each oligochaete extract and the corresponding sediment extract. The ratio of area (oligochaete) to area (sediment) varied tremendously from 1.8 to 127. The highest ratios were obtained from Gilmore (very low organic content) and Douglas (N) (also low organic

content). This observation could be explained by the competition for PAH occurring between sediment organic matter and live organisms, as postulated by Bindra and Hall (1979) for explanation of lead distributions in sediments.

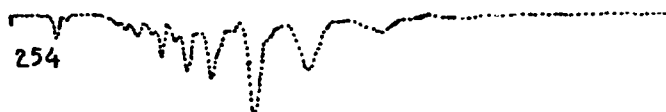
E. CRANKCASE OILS

Study of polycyclics in crankcase oils involved taking samples (at approximately 800 km intervals) from two different vehicles. Oil from a four cylinder Toyota car was sampled over 8000 km (RM), while a V-8 Mustang (newly reconditioned engine) was driven 6200 km (DD) during the sampling period.

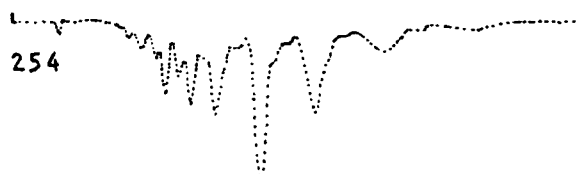
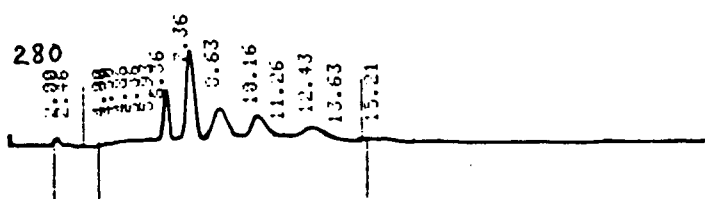
As can be seen from Figures 4-35 and 4-36, the same peaks are present in each subsample, and the oils from both vehicles have the same profile. Equivalent B(a)P for sample DD was consistently greater than that for RM extracts by a factor of approximately five. Research in the automotive industry indicates that fuel composition, engine condition, and combustion temperatures (and to a lesser extent, oil composition) effect the quantities of PAH produced.

The build-up of polycyclics in oil (DD) is clear in Figure 4-37 over the first section of highway driving. Once freeway driving began, however, the accumulation dropped off and some scatter of results is evident. The lower PAH levels may be due to higher engine temperatures during constant highway driving. Inadvertent sampling of sludge may explain the one high value in the freeway driving section.

In the RM sample series (Figure 4-38) it is also clear that the build-up is occurring. It can be seen that a change in oil but not the filter will reduce oil polycyclic levels considerably but not eliminate



a)



b)

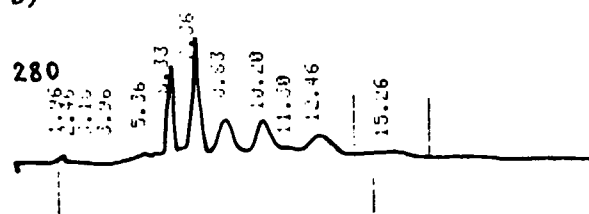
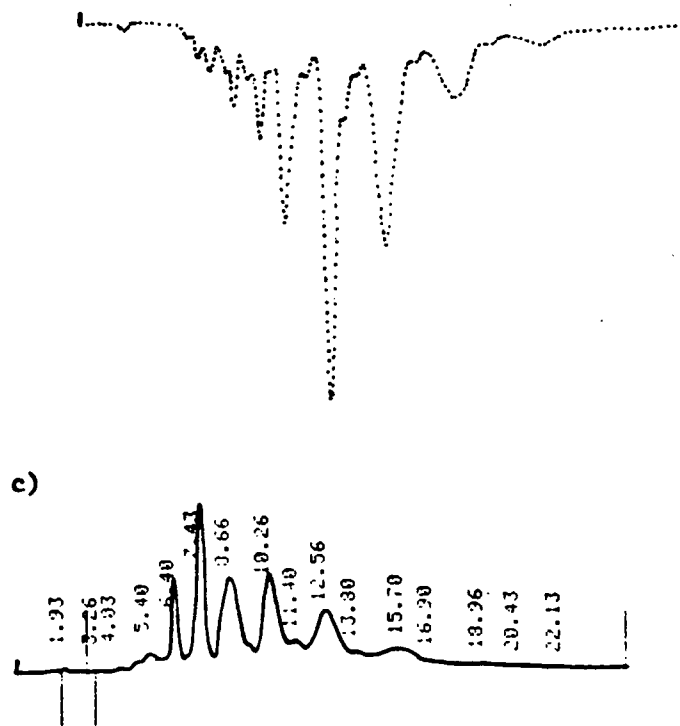


Figure 4-35. Crankcase Oil Sample DD.

- a) accumulated distance 874 km
 b) accumulated distance 1665 km



4-35 Crankcase Oil Sample DD Continued
c) accumulated distance 2623 km

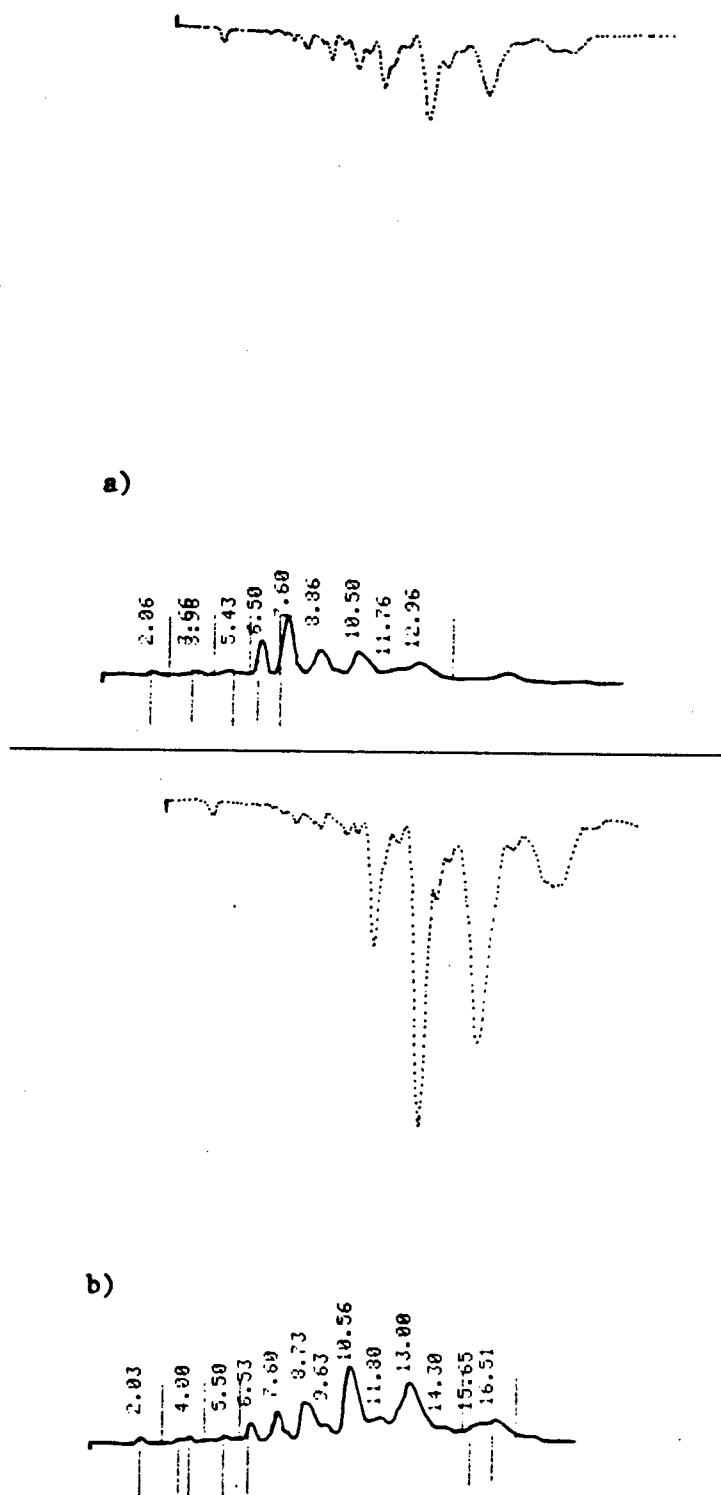
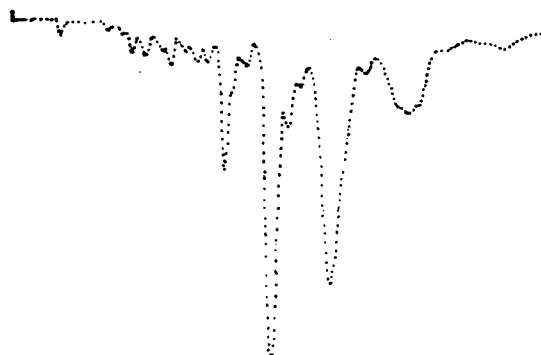
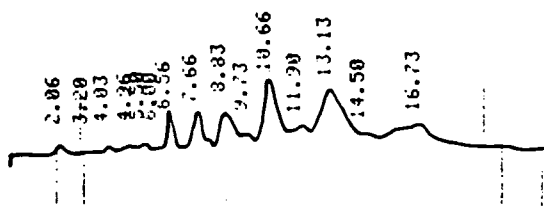


Figure 4-36. Crankcase Oil Sample RM.

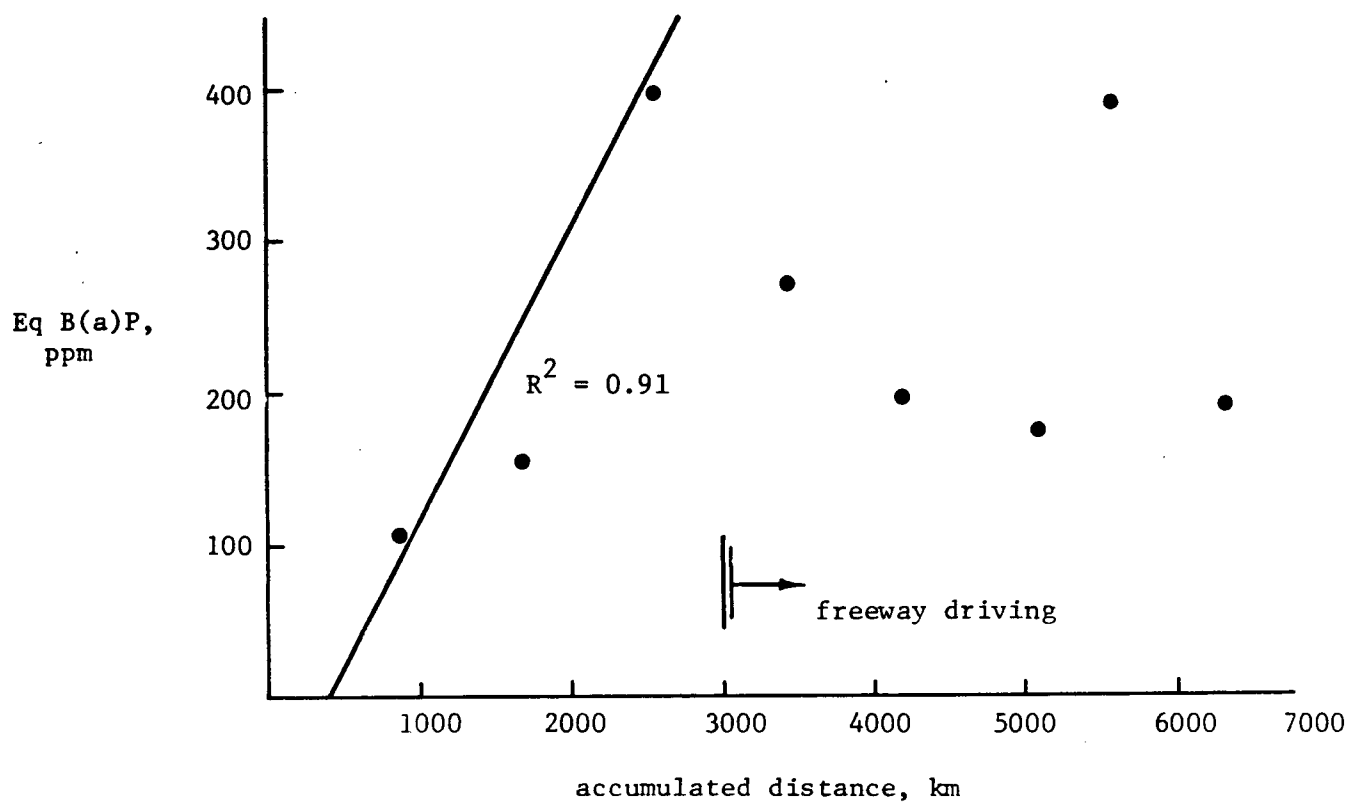
- a) accumulated distance 895 km
- b) accumulated distance 1943 km



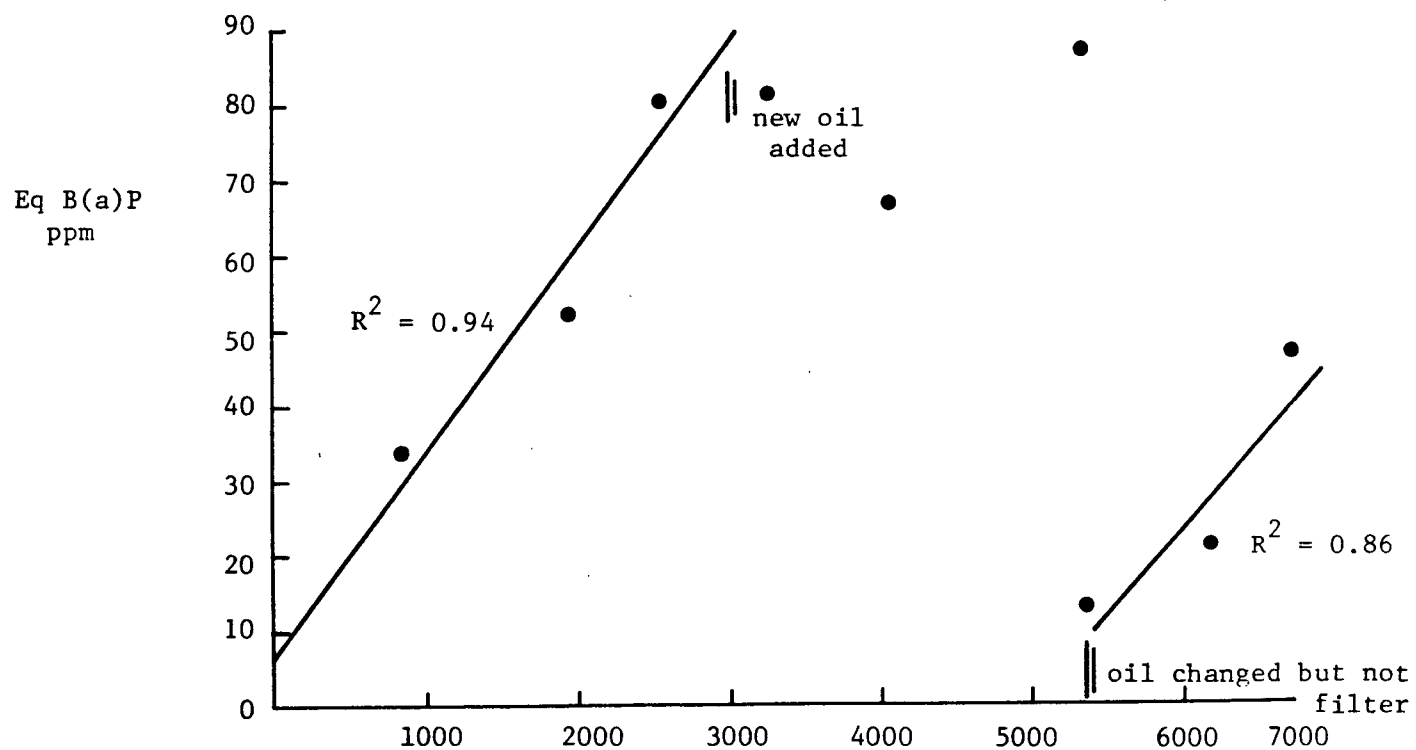
c)



4-36 Crankcase Oil Sample RM Continued
c) accumulated distance 2545 km



4-37 Eq B(a)P vs accumulated distance - DD



4-38 Eq B(a)P vs accumulated distance - RM

them. (Unused oil contained no UV-detectable peaks.)

Some peaks concentrated in oils are also present in sediment samples indicating possible contribution to sediment polycyclic levels. Peaks in oils tend to be earlier eluting than the bulk of the sediment extract peaks, suggesting that other sources are also significant for sediments.

Comparison of results discussed here and those reported by Handa et al. (1979) in section II C-4 gives agreement with the increase in PAH due to increased oil mileage. Also, the different accumulation rates found between the two tests run for this thesis is expected from work done by Handa and co-workers. However, if car (engine) mileage is important, RM samples would be expected to contain the higher polycyclic levels, rather than DD samples. EqB(a)P for oil samples was found to increase as a linear function of oil mileage as compared to a quadratic function as determined by Handa et al.. Disagreement notwithstanding, the dependence of polycyclic levels on oil mileage was confirmed.

One aspect of oil sample profiles which wasn't explored in this thesis work was the analysis of extracts with the sulfur-specific flame photometric detector. This approach could provide more easily characterized profiles, aiding in determining fate of crankcase oil in the urban watershed, as described by MacKenzie and Hunter (1979).

F. SUMMARY OF RESULTS, SOURCES AND FATES OF PAH

Data and interpretation from previous sections will be summarized here, and the thesis work as a whole will be discussed briefly.

Investigations into levels and distribution of polycyclics in

Still Creek stream sediments demonstrated that the PAH fraction is very similar from one sediment to another. The most striking difference among stream sediments is the range of concentration of polycyclics to be found (eqB(a)P varies by two orders of magnitude). PAH concentrations are comparable to those found in other urban watersheds, although the highest quantitations are greater than any others reported.

Possible sources of this polycyclic fraction include street sediments, atmospheric particulate fall-out, vehicle exhaust, lubricating oils, and crankcase oils. Examination of stream sediment and street sediment HPLC elution profiles indicates a very strong similarity between the two types of samples. Quantitated PAH are similar in identity and in level for the two sediment types, although the maximum levels in stream sediments are not matched by street sediments. Street surface deposits thus appear to be one of the main polycyclic sources to stream sediments. The higher concentrations determined for the stream may be due to the stream acting as a "sink" for the watershed, or the stream may also receive direct PAH input from atmospheric particulate fall-out or dumping of lubrication (crankcase) oils.

Crankcase oil extracts demonstrated profiles similar to street surface samples, but were less well related to stream sediments. This fact implicates crankcase oil as a source of PAH to street sediments, but indicates that both street and stream sediments receive additional inputs or are modified by microbial and chemical processes during transport to streams.

The indicated transport of polycyclics from streets to stream systems is supported by Giger and Schaffner (1978), and Wakeham et al. (1980a), since both groups have determined strong similarities between

street surface material and watershed sediments. These researchers contend that urban watersheds receive a major portion of their PAH input from street surfaces.

Assessment of sources to street sediments in the Brunette River watershed is more difficult, since emphasis was placed on analysis of Still Creek sediments. The presence of B(ghi)Per (often quantitated in Still Creek) is indicative of vehicle exhaust while benzonaphthothio-
phene isomers are related to lubricating oils residues (Grimmer and Bohnke, 1975b).

Degree of alkylation (and abundance of alkylated polycyclics) in stream sediments implicates low to medium temperature combustion as a major source to street materials, and hence to stream sediments.

Preliminary analysis of oligochaetes was of interest due to the evidence of bioconcentration of certain compounds. Degree of concentration varied by over 100 times, and may be controlled by a competition for polycyclics between sediment organic matter and live organisms.

CHAPTER V

SUMMATION, IMPLICATIONS AND RECOMMENDATIONS

A. SUMMATION

This thesis work investigated the extent of PAH contamination in selected stream sediments of an urban watershed, Still Creek, and examined some of the possible sources of polycyclics to sediments.

The first section of the thesis concerned the analytical methodology necessary for determining PAH in sediments. An extraction system was adapted from Dunn (1976) and the resulting extract was cleaned-up using alumina chromatography from Sorrell et al. (1977). Samples were analyzed by HPLC, and several of the extracts were also analyzed by GC and GCMS for additional qualitative information.

In the second part, samples were obtained of stream sediments from Still Creek, (including one set of oligochaete samples), street sediments from the Brunette River watershed, and crankcase oil samples. In stream sediments, PAH residues were found in each sample with levels of individual compounds varying from mid part per billion (55 ppb) to mid part per million (38.5 ppm). Although the majority of the eleven compounds identified by HPLC were not alkyl-substituted, GCMS analysis indicated that many alkyl- and hetero-substituted polycyclics were present. Alkyl substitution was generally methyl or ethyl. The composition of the PAH fraction was very similar from site to site, with the greatest variation occurring in the concentrations found. Application of regression analysis to the data (eqB(a)P, individual PAH, sediment characteristics, traffic volumes, point on stream) did not result in any statistically significant correlations but indicated that sediment

characteristics were important.

Analysis of street sediments revealed PAH present at slightly lower levels than in stream sediments. Profiles of street sediments were similar to those of the stream sediments, indicating that street surface runoff is one of the main source of polycyclics to stream sediments. Average levels of B(a)P in each land use increased as generally expected (lowest levels in greenspace, highest in industrial area) although regression analysis pointed to sediment characteristics as being important.

Investigations of polycyclic buildup in crankcase oils revealed a linear increase of eqB(a)P with mileage at first and then a leveling off. Profiles of oil extracts corresponded more closely to those of street surface materials than to stream sediments.

Preliminary results of analysis of oligochaetes indicated that bioconcentration was occurring (accumulations of up to two hundred times in comparison to sediments) and that concentration of PAH in organisms may be controlled by competition between organisms and sediment organic matter.

In summary, PAH were found to be present in all samples taken in Brunette River watershed, at levels comparable with other highly urbanized areas. Street surface materials were determined to be an important source of polycyclics to the Still Creek sediments. Used crankcase oil probably contributes significantly to street contamination, while other potential sources include vehicle exhaust, wear particulates from asphalt or tires and atmospheric particulates from combustion of organic materials.

B. IMPLICATIONS

Levels of PAH in sediments are comparable to those found in other urban watersheds, often those with a much longer history of development. The potential load of contaminants deposited in sediments from a large metropolitan area can provide a significant input to aquatic systems. Since such pollutants include carcinogens (eg. PAH) and heavy metals, there is considerable concern for the health of such productive systems as streams and deltas. Potential effects include increased tumour incidence, lowered feeding or reproductive success, or reduced mobility.

In order to maintain productivity and diversity of these "sink" areas, expensive management strategies may be required to reduce contaminant loads. With respect to input of polycyclics, it may be necessary to reduce PAH in vehicle exhaust, maintain close control on waste crankcase oils, or reduce emissions of polycyclics (generally associated with particulates) resulting from incineration, heating or power generation.

C. RECOMMENDATIONS

Results from this thesis elucidate some aspects of PAH contamination of Still Creek, and provide a data base for further investigations.

- a) Analytical methodology could be simplified for determination of polycyclics in sediments. Extraction of dried sediments with dichloromethane (Vassilaros et al., 1982a) should be examined. Analysis of extracts by capillary GC, using GCMS as confirmation) would provide improved resolution and, with GCMS, additional

structural confirmation.

- b) Further refinement to the understanding of PAH inputs to stream sediments could be obtained through quantitating the street surface contribution to streams. This could be done by monitoring PAH loads in urban stormwater, correlating results to size of catchment areas and curb lengths. Quantitation of land-use would aid in these investigations as well as for street surface materials.
- c) Bioconcentration of PAH in benthic organisms should be researched, including elucidation of the apparent selectivity of oligochaetes in concentrating specific compounds. The indicated competition between organisms and sediments requires further research.
- d) Perhaps most importantly, sources of polycyclics to street sediments need to be determined more clearly in order to take effective steps toward reducing PAH inputs to aquatic systems. Potential sources to be examined in more detail include:
 - i) crankcase oil
 - ii) vehicle exhaust
 - iii) asphalt wear particulates
 - iv) tire wear particulates
 - v) atmospheric particulate "fallout" resulting from combustion of fuels

Consideration of microbial modification of PAH once they enter the environment should be included.

REFERENCES

- Adams, J.D., LaVoie, E.J. and Hoffmann, D., (1982). "Analysis of Methylated Polynuclear Aromatic Hydrocarbons by Capillary G.C.: Influence of Temperature on the Pyrosynthesis of Anthracene, Phenanthrene and Their Methylated Derivatives", *J. Chromatogr. Sci.*, 20, p. 274-277.
- Aizenshtat, Z., (1973). "Perylene and Its Geochemical Significance", *Geochim. Cosmochim. Acta*, 37, p. 559-567.
- Benoit, F.M., LeBel, G.L., and Williams, D.T., (1979). "Polycyclic Aromatic Hydrocarbon Levels in Eastern Ontario Drinking Waters, 1978", *Bull. Environ. Contam. Toxicol.*, 23, p. 774-778.
- Bieri, R.H., Cueman, M.K., Smith, C.L., and Su, C.-W., (1978). "Polynuclear Aromatic and Polycyclic Aliphatic Hydrocarbons in Sediments from the Atlantic Outer Continental Shelf", *Intern. J. Environ. Anal. Chem.*, 5, p. 293-310.
- Bindra, K.S. and Hall, K.J., (1979). "Geochemistry of Selected Sediments and Factors Affecting Organism Concentration", *Proceedings: Heavy Metals in the Environment*, London, September 1979, C.E.P. Consultants, Edinburgh.
- Bjørseth, A., Knutzen, J., and Skei, J., (1979). "Determination of Polycyclic Aromatic Hydrocarbons in Sediments and Mussels from Saudafjord, W. Norway, by Glass Capillary Gas Chromatography", *Sci. Total Environ.*, 13, p. 71-86.
- Bjørseth, A., and Dennis, A.J. (eds.), (1980). Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio.
- Black, J.J., Holmes, M., Dymerski, P.P. and Zapisek, W.F., (1980). "Fish Tumour Pathology and Aromatic Hydrocarbon Pollution in a Great Lakes Estuary", in B.K. Afghan and D. Mackay (eds.), Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Plenum Press, New York, p. 559-565.
- Blumer, M., Dorsey, T., and Sass, J., (1977). "Azaarenes in Recent Marine Sediments", *Science*, 195, p. 283-285.
- Blumer, M., and Youngblood, W.W., (1975). "Polycyclic Aromatic Hydrocarbons in Soils and Recent Sediments", *Science*, 188, p. 53-55.
- Bories, G., (1979). "Le Probleme de la Contamination des Denrees Alimentaires par les Hydrocarbures Aromatiques Polycycliques", *Méd. et Nut.*, 15, p. 177-184.
- Bridbord, K., Finklea, J.F., Wagoner, J.K., Moran, J.B. and Caplan, P., (1976). "Human Exposure to Polynuclear Aromatic Hydrocarbons", in

Carcinogenesis, Vol. 1, Polynuclear Aromatic Hydrocarbons:
Chemistry, Metabolism and Carcinogenesis, p. 319-324.

- Brown, R.A. and Pancirov, R.J., (1979). "Polynuclear Aromatic Hydrocarbons in Baltimore Canyon Fish", *Environ. Sci. Technol.*, 13, p. 878-879.
- Brown, R.A. and Starnes, P.K., (1978). "Hydrocarbons in the Water and Sediment of Wilderness Lake II", *Marine Pollution Bulletin*, 9, p. 162-165.
- Budde, W.L. and Eichelberger, J.W., (1979). "Organics in the Environment - Report", *Anal. Chem.*, 51, p. 567A.
- Chmielowiec, J. and Sawatzky, H., (1979). "Entropy Dominated High Performance Liquid Chromatographic Separations of Polynuclear Aromatic Hydrocarbons. Temperature as a Separation Parameter." *J. Chromatogr. Sci.*, 17, p. 245-252.
- Choudhury, D.R. and Bush, B., (1981). "Chromatographic-Spectrometric Identification of Airborne Polynuclear Aromatic Hydrocarbons", *Anal. Chem.*, 53, p. 1351-1356.
- Christiansen, R.G. and May, W.E., (1978). "Detectors for Liquid Chromatographic Analysis for Polynuclear Aromatic Hydrocarbons", *J. Liq. Chromatogr.*, 1, p. 385-399.
- Clar, E., (1964). Polycyclic Hydrocarbons. Academic Press, N.Y.
- Cretney, W.J., Christensen, P.A., McIntyre, B.W. and Fowler, B.R., (1980). "Quantification of Polycyclic Aromatic Hydrocarbons in Marine Environmental Samples", in B.F. Afghan and D. Mackay (eds.), Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Plenum Press, New York, p. 315-336.
- Crosby, N.T., Hunt, D.C., Philp, L.A. and Patel, I., (1981). "Determination of Polynuclear Aromatic Hydrocarbons in Food, Water and Smoke Using High-Performance Liquid Chromatography", *Analyst*, 106, p. 135-145.
- Denton, M.S., DeAngelis, T.P., Yacynych, A.M., Heineman, W.R. and Gilbert, T.W., (1976). "Oscillating Mirror Rapid Scanning Ultraviolet-Visible Spectrometer as a Detector for Liquid Chromatography". *Anal. Chem.*, 48, p. 20-24.
- Dessy, R., Reynolds, W.D., Nunn, W.G., Titus, C.A. and Moler, G.P., (1976). "New Mini-Computer Automated Linear Photodiode Array Spectrometer System for High-Resolution Liquid Chromatography", *J. Chromatogr.*, 126, p. 347-368.
- Dunn, B.P., (1976). "Techniques for Determination of Benzo(a)pyrene in Marine Organisms and Sediments", *Env. Sci. Technol.*, 10, p. 1018-1021.

- Dunn, B.P. and Stich, H.F., (1975). "The Use of Mussels in Estimating Benzo(a)pyrene Contamination of the Marine Environment", Proc. Soc. Exp. Bio. Med., 150, p. 49-51.
- Dunn, B.P. and Stich, H.F., (1976a). "Monitoring procedures for Chemical Carcinogens in Coastal Waters", J. Fish. Res. Board Can., 33, p. 2040-2046.
- Dunn, B.P., and Stich, H.F., (1976b). "Release of the Carcinogen Benzo(a)pyrene from Environmentally Contaminated Mussels", Bull. Environ. Contam. Toxicol., 15, p. 398-401.
- Dunn, B.P. and Young, D.R., (1976). "Baseline Levels of Benzo(a)pyrene in Southern California Mussels", Mar. Poll'n. Bull., 7, p. 231-234.
- Emerole, G.O., (1980). "Carcinogenic Polycyclic Aromatic Hydrocarbons in Some Nigerian Foods", Bull. Environ. Contam. Toxicol., 24, p. 641-646.
- Environmental Health Directorate, Health Protection Branch (1979). Polycyclic Aromatic Hydrocarbons: Environmental Health Criteria Document. National Health and Welfare, Ottawa.
- Finnigan, R.E., Hoyt, D.W. and Smith, D.E., (1979). "Priority Pollutants - II - Cost Effective Analysis", Environ. Sci. Tech., 13, p. 534-541.
- Fox, M.A. and Staley, S.W., (1976). "Determination of Polycyclic Aromatic Hydrocarbons in Atmospheric Particulate Matter by High Pressure Liquid Chromatography Coupled with Fluorescence Techniques", Anal. Chem., 48, p. 992-998.
- Futoma, D.J., Smith, S.R., Tanaka, J. and Smith, T.E., (1981). "Chromatographic Methods for the Analysis of Polycyclic Aromatic Hydrocarbons in Water Systems", C.R.C. Critical Reviews in Analytical Chemistry, 12, p. 79-149.
- Giger, W. and Blumer, M., (1974). "Polycyclic Aromatic Hydrocarbons in the Environment: Isolation and Characterization by Chromatography, Visible, Ultra-violet, and Mass Spectrometry", Anal. Chem., 46, p. 1663-1671.
- Giger, W.R. and Schaffner, C., (1978). "Determination of Polycyclic Aromatic Hydrocarbons in the Environment by Glass Capillary Gas Chromatography", Anal. Chem., 50, p. 243-249.
- Greenberg, A., Yokoyama, R., Giorgio, P. and Cannova, F., (1980). "Analysis of Polynuclear Aromatic Hydrocarbons on the Airborne Particulates of Urban New Jersey", in A. Bjørseth and A.J. Dennis, (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 193-198.

- Grimmer, G. and Bohnke, H., (1975a). "Polycyclic Aromatic Hydrocarbon Profile Analysis of High-Protein Foods, Oils, and Fats by Gas Chromatography", J. Assoc. Off. Anal. Chem., 58, p. 725-733.
- Grimmer, G. and Bohnke, H., (1975b). "Profile Analysis of Polycyclic Aromatic Hydrocarbons and Metal Content in Sediment Layers of a Lake", Cancer Letters, 1, p. 75-84.
- Grimmer, G. and Bohnke, H., (1979). "Gas Chromatographic Profile Analysis of Polycyclic Aromatic Hydrocarbons in (I) High Protein Foods, (II) Fats and Vegetable Oils and (III) Plants, Soils and Sewage Sludge", I.A.R.C. Publ. 29, p. 163-173.
- Grimmer, G. and Duvel, D., (1972). "Investigation of Biosynthetic Formation of Polycyclic Hydrocarbons in Higher Plants", Z. Naturforsch., 25, p. 1171-1175.
- Grimmer, G., Naujack, K.-W. and Schneider, D., (1980). "Changes in PAH-Profiles in Different Areas of a City During the Year", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 107-125.
- Grob, K. and Grob, G., (1975). "Glass Capillary Gas Chromatography in Water Analysis: How to Initiate Use of the Method", in H.L. Keith (ed.), Identification and Analysis of Organic Pollutants in Water, Ann Arbor Science Publishers, Ann Arbor, Mich., p. 75-85.
- Guerrero, H., Biehl, E.R., Kenner, C.T., (1976). "High Pressure Liquid Chromatography of Benzo(a)pyrene and Benzo(ghi)perylene in Oil-Contaminated Shellfish", J. Assoc. Off. Anal. Chem., 59, p. 989-992.
- Gump, B.H., Hertz, H.S., May, W.E. and Chesler, S.N., (1977). "The Hydrocarbon Burden in the Marine Environment Surrounding a Refinery Tanker Jetty", J. Environ. Sci. Health, A12, p. 105-113.
- Haenni, E.O., (1978). "Recommended Methods for the Determination of Polycyclic Aromatic Hydrocarbons in Foods", Pure and Appl. Chem., 50, p. 1763-1773.
- Hall, K.J., Yesaki, I. and Chan, J., (1976). "Trace Metals and Chlorinated Hydrocarbons in the Sediments of a Metropolitan Watershed", Technical Report No. 10, Westwater Research Centre, University of British Columbia, Vancouver, B.C., 74 p.
- Handa, T., Yamamura, T., Kato, Y., Saito, S. and Ishii, T., (1979). "Factor Analysis and Derivation of an Experimental Equation on Polynuclear Aromatic Hydrocarbon Emissions from Automobiles", Environ. Sci. Technol., 13, p. 1077-1081.
- Hase, A., Lin, P.H. and Hites, R.A., (1976). "Analysis of Complex Polycyclic Aromatic Hydrocarbon Mixtures by Computerized GC-MS", in R.I. Freudenthal and P. Jones (eds.), Carcinogenesis - A

Comprehensive Survey: Polynuclear Aromatic Hydrocarbons -
Chemistry, Metabolism and Carcinogenesis, 1, p. 435-442.

- Hermann, M., Durand, J.P., Charpentier, J.M., Chaudé, O., Hofnung, M., Pétróff, N., Vandecasteele, J.-P. and Weill, N., (1980). "Correlations of Mutagenic Activity with Polynuclear Aromatic Hydrocarbon Content of Various Mineral Oils", in A. Bjørseth and A.J. Dennis, (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 899-916.
- Hirata, Y. and Novotny, M., (1979). "Techniques of Capillary Liquid Chromatography", J. Chromatogr., 186, p. 521-528.
- Hirsch, D.E., Hopkins, R.L., Coleman, H.J., Cotton, F.O. and Thompson, C.J., (1972). "Separation of High-Boiling Petroleum Distillates Using Gradient Elution Through Dual-Packed (Silica Gel-Alumina Gel) Adsorption Columns", Anal. Chem., 44, p. 915-919.
- Hites, R.A., LaFlamme, R.E. and Farrington, J.W., (1977). "Sedimentary Polycyclic Aromatic Hydrocarbons: The Historical Record", Science, 198, p. 828-831.
- Hites, R.A., LaFlamme, R.E. and Windsor, J.G., Jr., (1980). "Polycyclic Aromatic Hydrocarbons in the Marine Environment: Gulf of Maine Sediments and Nova Scotia Soils", in B.K. Afghan and D. Mackay (eds.), Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Plenum Press, New York, p. 397-403.
- Howard, J.W., (1979). "Analysis of Benzo(a)pyrene and Other Polycyclic Aromatic Hydrocarbons in Foods", I.A.R.C. Publ., 29, p. 175-191.
- Howard, J.W. and Fazio, T., (1980). "Review of Polycyclic Aromatic Hydrocarbons in Foods: Analytical Methodology and Reported Findings of Polycyclic Aromatic Hydrocarbons in Foods", J. Assoc. Off. Anal. Chem., 63, p. 1077-1104.
- Hunt, D.C., Wild, P.J. and Crosby, N.T., (1977). "Phthalimidopropylsilane - a New Chemically Bonded Stationary Phase for the Determination of Polynuclear Aromatic Hydrocarbons by High Pressure Liquid Chromatography", J. Chromatogr., 130, p. 320-323.
- Janini, G.M., Muschik, G.M., Schroer, J.A. and Zielinski, W.L., (1978). "Gas-Liquid Chromatographic Evaluation and Gas-Chromatography/Mass Spectrometric Application of New High Temperature Liquid Crystal Stationary Phases for Polycyclic Aromatic Hydrocarbon Separations", Anal. Chem., 48, p. 1879-1883.
- Joe, F.L., Jr., Roseboro, E.L. and Fazio, T., (1979). "Survey of Some Market Basket Commodities for Polynuclear Aromatic Hydrocarbon Content", J. Assoc. Off. Anal. Chem., 62, p. 615-620.
- Joe, F.L., Jr., Roseboro, E.L. and Fazio, T., (1981). "High Pressure Liquid Chromatographic Method for Determination of Polynuclear Aromatic Hydrocarbons in Beer", J. Assoc. Off. Anal. Chem., 64, p.

641-646.

- John, E.D., Cooke, M. and Nickless, G., (1979). "Polycyclic Aromatic Hydrocarbons in Sediments Taken from the Severn Estuary Drainage System", *Bull. Environ. Contam. Toxicol.*, 22, p. 653-659.
- Kalas, L., Murdoch, A. and Onuska, F.I., (1980). "Bioaccumulation of Arene and Organochlorine Pollutants by Cipangopaludina Chinensis (Gray, 1834) (Mollusca: Gastropoda) from Ponds in the Royal Botanical Gardens, Hamilton, Ontario", in B.K. Afghan and D. Mackay (eds.), Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Plenum Press, New York, p. 567-576.
- Keizer, P.D., Dale, J. and Gordon, D.C., Jr., (1978). "Hydrocarbons in Surficial Sediments from the Scotian Shelf", *Geochim. Cosmochim. Acta.*, 42, p. 165-175.
- Kolarovic, L. and Traitler, H., (1982). "Determination of Polycyclic Aromatic Hydrocarbons in Vegetables Oils by Caffeine Complexation and Glass Capillary Gas Chromatography", *J. Chromatogr.*, 237, p. 263-272.
- LaFlamme, R.E. and Hites, R.A., (1978). "The Global Distribution of Polycyclic Aromatic Hydrocarbons in Recent Sediments", *Geochim. Cosmochim. Acta*, 42, p. 289-303.
- Lane, D.A., Sakuma, T. and Quan, E.S.K., (1980). "Real-Time Analysis of Gas Phase Polycyclic Aromatic Hydrocarbons Using a Mobile Atmospheric Pressure Chemical Ionization Mass Spectrometer System", in A. Bjørseth and A.J. Dennis (eds.), Polycyclic Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 199-214.
- Lao, R.C., Thomas, R.S. and Monkman, J.L., (1975). "Computerized Gas Chromatographic-Spectrometric Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples", *J. Chromatogr.*, 112, p. 681-700.
- Larsson, B.K., (1982). "Polycyclic Aromatic Hydrocarbons in Smoked Fish", *Z. Lebensm. Unters. Forsch.*, 174, p. 101-107.
- Le, C. and Tenisci, T., (1977). Triangular Regression Package, UBC Computing Centre, Vancouver, B.C., Revised October 1978.
- Lee, M.L., Novotny, M.V., Bartle, K.D., (1981). Analytical Chemistry of Polycyclic Aromatic Compounds. Academic Press, N.Y., p. 441-449.
- Lee, M.L., Prado, G.P., Howard, J.B. and Hites, R.A., (1977). "Source Identification of Urban Airborne Polycyclic Aromatic Hydrocarbons by Gas Chromatographic Mass Spectrometry and High Resolution Mass Spectrometry", *Biomedical Mass Spectrometry*, 4, 182-186.

- Lee, M.L., Vassilaros, D.L., White, C.M. and Novotny, M., (1979a). "Retention Indices for Programmed-Temperature Capillary-Column Gas Chromatography of Polycyclic Aromatics", Anal. Chem., 51, p. 768-773.
- Lee, M.L., Vassilaros, D.L., Pipkin, W.S. and Sorensen, W.L., (1979b). "Combined Glass Capillary-Column Gas Chromatography and Mixed Charge Exchange - Chemical Ionization Mass Spectrometry of Isomeric Polycyclic Aromatic Hydrocarbons", in Trace Organic Analysis, NBS Special Publication 519, US Gov't Printing Office, Washington, D.C., p. 731-738.
- Lee, M.L. and Wright, B.W., (1980). "Capillary Column Gas Chromatography of Polycyclic Aromatic Compounds: A Review", J. Chromatogr. Sci., 18, p. 345-358.
- Lijinsky, W. and Ross, A.E., (1967). "Production of Carcinogenic Polynuclear Hydrocarbons in the Cooking of Food", Fd. Cosmet. Toxicol., 5, p. 343-347.
- Lo, M.T. and Sandi, E., (1978). "Polycyclic Aromatic Hydrocarbons (Polynuclears) in Foods", Residue Rev., 69, p. 35-86.
- McGuire, J.M., Carter, M.H. and Alford, A.L., (1975). "Development of Computerized GC/MS Techniques Within the US EPA", in H.L. Keith (ed.), Identification and Analysis of Organic Pollutants in Water. Ann Arbor Science Publishers, Ann Arbor, Mich., p. 177-183.
- Mackenzie, M.J. and Hunter, J.V., (1979). "Sources and Fates of Aromatic Compounds in Urban Stormwater Runoff", Environ. Sci. Technol., 13, p. 179-183.
- Mackie, P.R., Hardy, R., Whittle, K.J., Bruce, C. and McGill, A.S., (1980). "The Tissue Hydrocarbon Burden of Mussels from Various Sites Around the Scottish Coast", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 379-393.
- MacLeod, W.D., Jr., Prohaska, P.G., Gennero, D.D. and Brown, D.W., (1982). "Interlaboratory Comparisons of Selected Trace Hydrocarbons from Marine Sediments", Anal. Chem., 54, p. 386-392.
- Milano, M.J., Lam, S. and Grushka, E., (1976). "Rapid Scanning Diode Array as a Multi-Wavelength Detector in Liquid Chromatography", J. Chromatogr., 125, p. 315-326.
- Muller, G., Grimmer, G. and Bohnke, H., (1977). "Sedimentary Record of Heavy Metals and Polycyclic Aromatic Hydrocarbons in Lake Constance", Naturwissenschaften, 64, p. 427-431.
- National Academy of Sciences, (1972). Particulate Polycyclic Organic Matter, N.A.S., Washington, D.C.

- Natusch, D.F.S. and Tomkins, B.A., (1978). "Isolation of Polycyclic Organic Compounds by Solvent Extraction with Dimethylsulfoxide", *Anal. Chem.*, 50, p. 1429-1434.
- Neal, J. and Trieff, N.M., (1972). "Isolation of an Unknown Carcinogenic Polycyclic Hydrocarbon from Carbon Blacks", *Health Lab. Sci.*, 9, p. 32-38.
- Newhall, H.K., Jentoft, R.E. and Ballinger, P.R., (1973). "The Effect of Unleaded Fuel Composition on Polynuclear Aromatic Hydrocarbon Emissions", in National Combined Farm, Construction and Industrial Machinery and Fuels and Lubricants Meetings, Society of Automotive Engineers, Paper #730834, 12 pages.
- Nowicki, H.G., Kieda, C.A. and Bassett, D.O., (1980). "Quantitative Evaluation of Priority Pollutant Polycyclic Aromatic Hydrocarbons at One Part per Billion Using EPA Recommended Priority Pollutant Protocol", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 75-87.
- Obana, H., Hori, S. and Kashimoto, T., (1981). "Determination of Polycyclic Aromatic Hydrocarbons in Marine Samples by High Performance Liquid Chromatography", *Bull. Environ. Contam. Toxicol.*, 26, p. 613-620.
- Ogan, K., Katz, E. and Slavin, W., (1979). "Determination of Polycyclic Aromatic Hydrocarbons in Aqueous Samples by Reversed-Phase Liquid Chromatography", *Anal. Chem.*, 51, p. 1315-1320.
- Olufsen, B., (1980). "Polynuclear Aromatic Hydrocarbons in Norwegian Drinking Water Resources", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 333-343.
- Oyler, A.R., Bodenner, D.L., Welsh, K.J., Luikkonen, R.J., Carlson, R.M., Kopperman, H.L. and Caple, R., (1978). "Determination of Aqueous Chlorination Reaction Products of Polynuclear Aromatic Hydrocarbons by Reversed Phase High Performance Liquid Chromatography - Gas Chromatography", *Anal. Chem.*, 50, p. 837-842.
- Panalaks, T., (1976). "Determination and Identification of Polycyclic Aromatic Hydrocarbons in Smoked and Charcoal-Broiled Food Products by High Pressure Liquid Chromatography", *J. Environ. Sci. Health*, series B., 11, p. 299-315.
- Pancirov, R.J. and Brown R.A., (1977). "Polynuclear Aromatic Hydrocarbons in Marine Tissues", *Environ. Sci. Technol.*, 11, p. 989-992.
- Patterson, A.M., Capell, L.T. and Walker, D.F., (1960). The Ring Index. A List of The Ring Systems Used in Organic Chemistry, 2nd edition, McGregor and Werner, Washington, D.C.

- Payne, J.F., Martins, I. and Rahimtula, A., (1980). "Crankcase Oils: Are They a Major Mutagenic Burden in the Aquatic Environment?", *Science*, 200, p. 329-330.
- Pedersen, P.S., Ingwersen, J., Nielsen, T. and Larsen, E., (1980). "Effects of Fuel, Lubricant, and Engine Operating Parameters on the Emission of Polycyclic Aromatic Hydrocarbons", *Environ. Sci. Technol.*, 14, p. 71-79.
- Richardson, J.H., Larson, K.M. Haugen, G.R., Johnson, D.C. and Clarkson, J.E., (1980). "Time Resolved Laser-Induced Fluorescence with High-Pressure Liquid Chromatography for Analysis of Polycyclic Aromatic Hydrocarbon Mixtures", *Anal. Chim. Acta.*, 116, p. 407-411.
- Santadonato, J., Basu, D. and Howard, P.H., (1980). "Multimedia Human Exposure and Carcinogenic Risk Assessment for Environmental PAH", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 435-453.
- Sartor, J.D., Boyd, G.B. and Agardy, F.J., (1974). "Water Pollution Aspects of Street Surface Contaminants", *J. Wat. Poll. Contr. Fed.*, 46, p. 458-467.
- Schamp, N., and van Wassenhove, F., (1972). "Determination of Benzo(a)pyrene in Bitumen and Plants", *J. Chromatogr.*, 69, p. 421-425.
- Scott, R.P.W. and Kucera, P., (1979). "Mode of Operation and Performance Characteristics of Microbore Columns for Use in Liquid Chromatography", *J. Chromatogr.*, 169, p. 51-72.
- Seversen, R.F., Snook, M.E., Higman, H.C., Chrotyk, O.T. and Akin, F.J., (1976). "Isolation, Identification, and Quantitation of the Polynuclear Aromatic Hydrocarbons in Tobacco Smoke", in R.I. Freudenthal and P. Jones (eds.) "Carcinogenesis - A Comprehensive Survey; Polynuclear Aromatic Hydrocarbons - Chemistry, Metabolism and Carcinogenesis", 1, p. 253-270.
- Shushan, B., Safe, S.H. and Boyd, R.K., (1979). "Mass Spectrometry of Polycyclic Aromatic Hydrocarbons by Linked-Scan Studies of Metastable Ions", *Anal. Chem.*, 51, p. 156-158.
- Silvester, D.J., (1980). "Determination of 3,4-Benzopyrene and Benzanthracene (PAH) in Phenolic Smoke Concentrates", *J. Fd. Technol.*, 15, p. 413-420.
- Sonstegard, R.A. and Leatherland, J.F., (1980). "Aquatic Organism Pathobiology as a Sentinal System to Monitor Environmental Carcinogens" in B.K. Afghan and D. Mackay (eds.), Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Plenum Press, New York, p. 513-520.

- Soós, K., (1974). "Content of Carcinogenic Polynuclear Hydrocarbons in Hungarian Grain", *Z. Lebensm. Unters. Forsch.*, 156, p. 344-346.
- Soós, K., (1980). "The Occurrence of Carcinogenic Polycyclic Hydrocarbons in Foodstuffs in Hungary", in *Further Studies in the Assessment of Toxic Actions*, *Arch. Toxicol. Suppl.*, 4, p. 446-448.
- Sorrell, R.K., Dressman, R.C. and McFarren, E.F., (1977, prepublication copy). "High Pressure Liquid Chromatography for the Measurement of Polynuclear Aromatic Hydrocarbons in Water", presented at Water Quality Technology Conference, Kansas City, Missouri, Dec., 5-6, 1977.
- Sorrell, R.K. and Reding, R., (1979). "Analysis of Polynuclear Aromatic Hydrocarbons in Environmental Waters by High-Pressure Liquid Chromatography", *J. Chromatogr.*, 185, p. 655-670.
- Southworth, G.R., Keffer, C.C. and Beauchamp, J.J., (1980). "Potential and Realized Bioconcentration. A Comparison of Observed and Predicted Bioconcentration of Azaarenes in the Fathead Minnow (*Pimephales promelas*)". *J. Environ. Sci. Technol.*, 14, p. 1529-1531.
- Strand, J.W. and Andren, A.W., (1980). "Polyaromatic Hydrocarbons in Aerosols over Lake Michigan, Fluxes to the Lake", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 127-137.
- Strup, P.E., Giammar, R.D., Stanford, T.B. and Jones, P.W., (1976). "Improved Measurement Techniques for Polycyclic Aromatic Hydrocarbons from Combustion Effluents", in R.I. Freudenthal and P. Jones (eds.), "Carcinogenesis - A Comprehensive Survey; Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis", 1, p. 241-251.
- Suess, M.J., (1976). "The Environmental Load and Cycle of Polycyclic Aromatic Hydrocarbons", *Sci. Total Environ.*, 6, p. 239-250.
- Swallow, W.H., (1976). "Survey of Polycyclic Aromatic Hydrocarbons in Selected Foods and Food Additives Available in New Zealand", *N.Z. J. Sci.*, 19, p. 407-412.
- Tomkins, B.A., Kubota, H., Griest, W.H., Caton, J.E., Clark, B.R. and Guerin, M.R., (1980). "Determination of Benzo(a)pyrene in Petroleum Substitutes", *Anal. Chem.*, 52, p. 1331-1334.
- Tsuda, T., Hibi, K., Nakanishi, T., Takeuchi, T. and Ishii, D., (1978). "Studies of Open-Tubular Micro-Capillary Liquid Chromatography II Chemically Bonded Octadecylsilane Stationary Phase", *J. Chromatogr.*, 158, p. 227-232.

- Van Heddeghem, A., Hugghebaert, A. and DeMoor, H., (1980). "Determination of Polycyclic Aromatic Hydrocarbons in Fat Products by High Pressure Liquid Chromatography", *Z. Lebensm. Unters. Forsch.*, 171, p. 9-13.
- Vassilaros, D.L., Stoker, P.W., Booth, G.M. and Lee, M.L., (1982a). "Capillary Gas Chromatographic Determination of Polycyclic Aromatic Compounds in Vertebrate Fish Tissue", *Anal. Chem.*, 54, p. 106-112.
- Vassilaros, D.L., Kong, R.C., Later, D.W. and Lee, M.L., (1982b). "Linear Retention Index System for Polycyclic Aromatic Compounds: Critical Evaluation and Additional Indices", *J. Chromatogr.*, 252, p. 1-20.
- Wakeham, S.G., Schaffner, C. and Giger, W., (1980a). "Polycyclic Aromatic Hydrocarbons in Recent Lake Sediments - I. Compounds Having Anthropogenic Origins", *Geochim. Cosmochim. Acta.*, 44, 403-413.
- Wakeham, S.G., Schaffner, C. and Giger, W., (1980b). "Polycyclic Aromatic Hydrocarbons in Recent Lake Sediments - II. Compounds Derived from Biogenic Precursors During Early Diagenesis", *Geochim. Cosmochim. Acta.*, 44, p. 415-429.
- Walker, E.A., (1977). "Some Facts and Legislation Concerning Polycyclic Aromatic Hydrocarbons in Smoked Foods", *Pure and Appl. Chem.*, 49, p. 1673-1686.
- Wallcave, L., Garcia, H., Feldman, R., Lijinsky, W. and Shubik, P., (1971). "Skin Tumorigenesis in Mice in Petroleum Asphalts and Coal-Tar Pitches of Known Polynuclear Aromatic Hydrocarbon Content", *Toxicol. Appl. Pharmacol.*, 18, p. 41-52.
- Wallcave, L., Nagel, D.L., Smith, J.W. and Waniska, R.D., (1975). "Two Pyrene Derivatives of Widespread Environmental Distribution: Cyclopenta(cd)pyrene and Acepyrene", *Environ. Sci., Technol.*, 9, p. 143-145.
- Weast, R.C., (ed.), (1975). Handbook of Chemistry and Physics, CRC Press, Cleveland, Ohio.
- Wheals, B.B., Vaughan, C.G. and Whitehouse, M.J., (1975). "Use of Chemically Modified Microparticulate Silica and Selective Fluorometric Detection for the Analysis of Polynuclear Hydrocarbons by High-Pressure Liquid Chromatography", *J. Chromatogr.*, 106, p. 109-118.
- Winkler, E., Buchele, A. and Muller, O., (1977). "Method for the Determination of Polycyclic Aromatic Hydrocarbons in Maize by Capillary-Column Gas-Liquid Chromatography", *J. Chromatogr.*, 138, p. 151-162.
- Wise, S.A., Bonnett, W.J. and May, W.E., (1980). "Normal- and Reverse-Phase Liquid Chromatographic Separations of Polycyclic Aromatic

- Hydrocarbons", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 791-806.
- Youngblood, W.W. and Blumer, M., (1975). "Polycyclic Aromatic Hydrocarbons in the Environment: Homologous Series in Soils and Recent Marine Sediments", Geochem. Cosmochim. Acta, 39, p. 1303-1314.
- Zaghini, N., Mangolini, S., Arteconi, M. and Sezzi, F., (1973). "Polynuclear Aromatic Hydrocarbons in Vehicle Exhaust Gas", in National Combined Farm, Construction and Industrial Machinery and Fuels and Lubricants Meetings, Society of Automotive Engineers, Paper # 730836, 12 pages.
- Zelenski, S.G., Hunt, G.T. and Pangaro, N., (1980). "Comparison of SIM GC/MS and HPLC for the Detection of Polynuclear Aromatic Hydrocarbons in Fly Ash Collected From Stationary Combustion Sources", in A. Bjørseth and A.J. Dennis (eds.), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, p. 589-597.
- Zurcher, F., Thuer, M. and Davis, J.A., (1980). "Importance of Particulate Matter on the Load of Hydrocarbons of Motorway Runoff and Secondary Effluents", in Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, Environmental Science Research, Volume 16, Plenum Press, New York, p. 373-385.

APPENDIX 1 - Abbreviations, naming and numbering rules, structures and carcinogenic activity.

This appendix contains a tabulation of major polycyclics discussed in the thesis, including abbreviations used in the text and in tables; names and structures; as well as an indication of carcinogenic activity.

A summary of naming and numbering rules is provided, condensed from The Ring Index (Patterson et al., 1960).

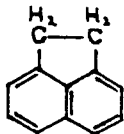
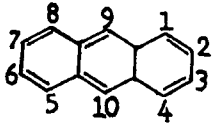
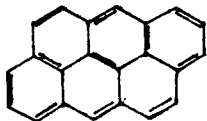
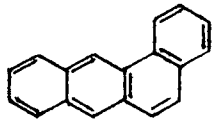
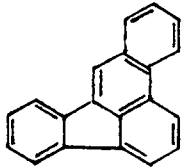
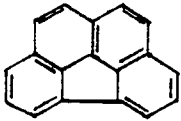
1. The names of polycyclic hydrocarbons with maximum number of non-cumulative double bonds end in "ene". Examples include anthracene, chrysene, fluoranthene, fluorene, naphthalene, phenanthrene, pyrene and triphenylene.
2. The names of hydrocarbons containing five or more fused benzene rings in a straight linear arrangement are formed in a straight linear arrangement are formed from a numerical prefix followed by "acene". Examples: pentacene, hexacene.
3. For purposes of numbering, the structures are oriented so that the greatest number of rings are in a horizontal row, and the maximum number of rings is in the upper righthand quadrant. Numbering starts with the carbon atom (not common to 2 or more rings) in the most counter-clockwise position in the upper right quadrant. Numbers are assigned in a clockwise direction and carbon atoms common to two or more rings are omitted. As examples see the numbering of pyrene or triphenylene. Anthracene and phenanthrene have non-standard numbering systems.

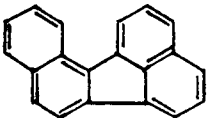
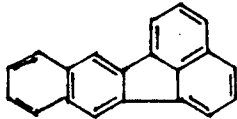
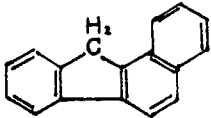
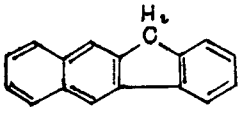
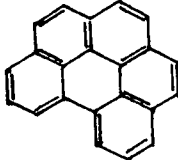
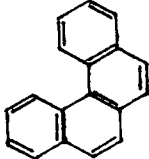
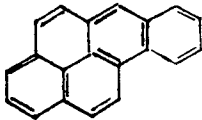
4. Compounds which are comprised of additional rings (with five or more members) are named by affixing "benzo" (one six-membered ring) or "naphtho" (two fused rings) to "ene" compounds listed in rule 1. The largest "ene" compound possible should be chosen, with the simplest prefix possible. For example, dibenzoanthracene (diB(a,c)A) rather than naphthophenanthrene.
5. Isomers of "ene" compounds with additional rings are distinguished by assigning letters to the sides of the original compound. Letters begin with the "1-2" side and continue around the periphery of the structure, assigning a letter to each side available for fusion. When several naming options exist, the combination for the lowest number and the earliest alphabetic letter shall be used.

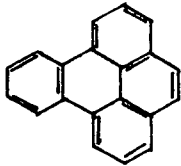
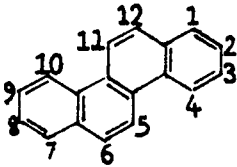
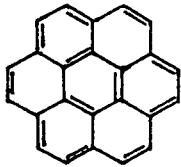
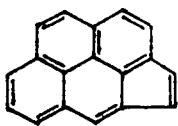
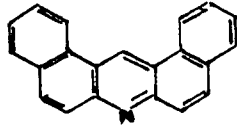
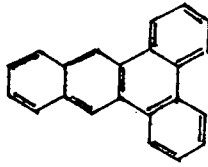
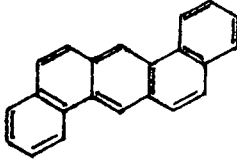
Data on carcinogenic activity of various polycyclics is taken from a compilation by Lee et al. (1981). Although studies are not completely comparable due to differences in animal species, age, diet, dose range and method of administration, the information provides a useful guideline to PAH carcinogenicity. Major parent polycyclics have been included in this appendix as well as some alkyl-substituted PAH.

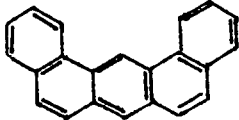
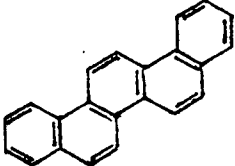
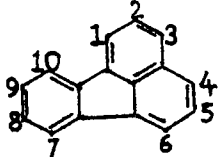
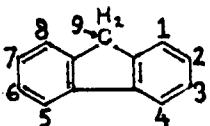
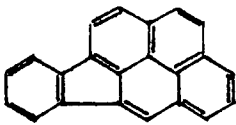
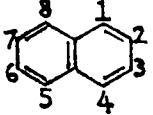
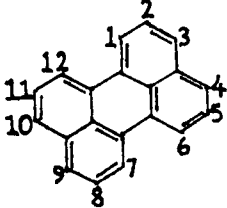
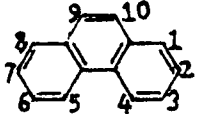
Carcinogenicity is indicated by

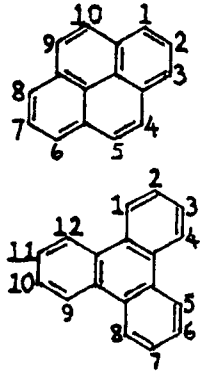
- "o" in studies where none of the test animals developed tumours;
- "+" in cases where up to 33% tumour incidence was found (weakly carcinogenic);
- "++" for studies in which over 33% of the animals developed tumours (strongly carcinogenic).

ABBREVIATION	NAME	CARCINOGENIC ACTIVITY	STRUCTURE
-	acenaphthene	o	
A	anthracene	o	
meA	methylanthracene	-	
9-meA	9-methylanthracene	o	
dimeA	dimethylanthracene	-	
9,10dimeA	9,10-dimethylanthracene	o/+	
-	anthranthene	-	
B(a)A	benzo(a)anthracene	+	
4-meB(a)A	4-methylbenzo(a)anthracene	o/+	
6-meB(a)A	6-methylbenzo(a)anthracene	++	
7-meB(a)A	7-methylbenzo(a)anthracene	++	
5,12-dimeB(a)A	5,12-dimethylbenzo(a)anthracene	o	
B(b)F	benzo(b)fluoranthene	++	
B(ghi)F	benzo(ghi)fluoranthene	o	

ABBREVIATION	NAME	CARCINOGENIC ACTIVITY	STRUCTURE
B(j)F	benzo(j)fluoranthene	++	
B(k)F	benzo(k)fluoranthene	++	
B(a)Fl	benzo(a)fluorene	o	
B(b)Fl	benzo(b)fluorene	-	
B(ghi)Per	benzo(ghi)perylene	+	
B(a)Ph	benzo(a)phenanthrene	+	
B(a)P	benzo(a)pyrene	++	
4-meB(a)P	4-methylbenzo(a)pyrene	++	
5-meB(a)P	5-methylbenzo(a)pyrene	+	
8-meB(a)P	8-methylbenzo(a)pyrene	o	
1,3-dimeB(a)P	1,3-dimethylbenzo(a)- pyrene	++	

ABBREVIATION	NAME	CARCINOGENIC ACTIVITY	STRUCTURE
B(e)P	benzo(e)pyrene	o/+	
Chr	chrysene	+	
1-meChr	1-methylchrysene	o/+	
5-meChr	5-methylchrysene	++	
6-meChr	6-methylchrysene	+	
Col	cholanthrene	++	
Cor	coronene	o/+	
-	cyclopenta[c,d] pyrene	+	
diB(a,j)Ac	dibenzo(a,j)acridine	+	
diB(a,c)A	dibenzo(a,c)anthracene	+	
diB(a,h)A	dibenzo(a,h)anthracene	+	
7-mediB(a,h)A	7-methyldibenzo(a,h)-anthracene	++	

ABBREVIATION	NAME	CARCINOGENIC ACTIVITY	STRUCTURE
diB(a,j)A	dibenzo(a,j)anthracene	+	
diB(a,i)Ph	dibenzo(a,i)phen- anthrene (picene)	o	
F	fluoranthene	o	
2-meF IndF Fl	2-methylfluoranthene indeno[1,2,3-cd]fluorene	+ - o	
Ind[1,2,3-cd]P	indeno[1,2,3-cd]pyrene	+	
N	naphthalene	o	
Per	perylene	o	
Ph	phenanthrene	o	

ABBREVIATION	NAME	CARCINOGENIC ACTIVITY	STRUCTURE
2-mePh	2-methylphenanthrene	-	
3,6-dimePh	3,6-dimethyl- phenanthrene	-	
P	pyrene	o	
1-meP	1-methylpyrene	o	
Tri	triphenylene	o	
1-meTri	1-methyl triphenylene	o	

APPENDIX 2 - Physical Properties of PAH

ABBREVIATION	MOLECULAR WEIGHT	MELTING ^a POINT	BOILING ^a POINT
A	178.24	216.2	340
B(b)F	252.32	168	-
B(j)F	252.32	166	-
B(k)F	252.32	217	480
B(a)Fl	216.29	189	413
B(a)P	252.32	255	448
B(c)Ph	228.30	68	-
diB(a,c)A	278.36	205	-
diB(a,h)A	278.36	269	-
diB(a,i)A	278.36	263	-
diB(a,j)A	267.36	197	-
diB(b,h)Ph	267.36	257	-
Fl	166.23	116	294
F	202.26	111	375
N	128.19	80.55	218
Per	252.32	278	-
Ph	178.24	101	340
P	202.26	156	393
Picene	278.36	367	518
Tri	228.30	119	425

a - CRC Handbook of Chemistry and Physics, 1975.

APPENDIX 3 - Chromatography Conditions, Chromatograms of

Mixed Standards

1. HPLC traces - Chromatography conditions are set out in section III F-2 and in Table 3-2. Additional details are provided (listed by figure number) in Tables A and C below.

Absorbance at 254 nm is the upper trace (dotted or dashed line) in each figure. 254 nm absorbance increased towards the bottom of the page, and is offset slightly horizontally from the 280 nm trace. (AUFS = 0.020).

280 nm absorbance (solid line) is integrated, and integration marks and retention times (in minutes) are included in figures. (AUFS = 0.020).

2. Capillary GC traces - Chromatography conditions are found in III F-3 and in Table 3-3. Additional details are listed by figure number in Table B below.

Table A - HPLC Conditions Figure 4-11 to 4-17

FIGURE NUMBER	SAMPLE	INJECTION SIZE (μ l)	%CH ₃ CN
4-11 a)	D(N)('78)III	5	70
b)	D(N)('79)III	3	"
4-12 a)	W('78)III	5	"
b)	W('79)III	3	"
4-13 a)	D(N)('78)IV	10	"
b)	D(N)('79)IV	1	"
4-14 a)	L('78)IV	5	"
b)	L('79)IV	1	"
4-15 a)	D(N)('78)V	10	"
b)	D(N)('79)V	5	"
4-16 a)	L('78)V	5	"
b)	L('79)V	5	"
4-17 a)	L('78)III	3	60
b)	L('79)III	3	70

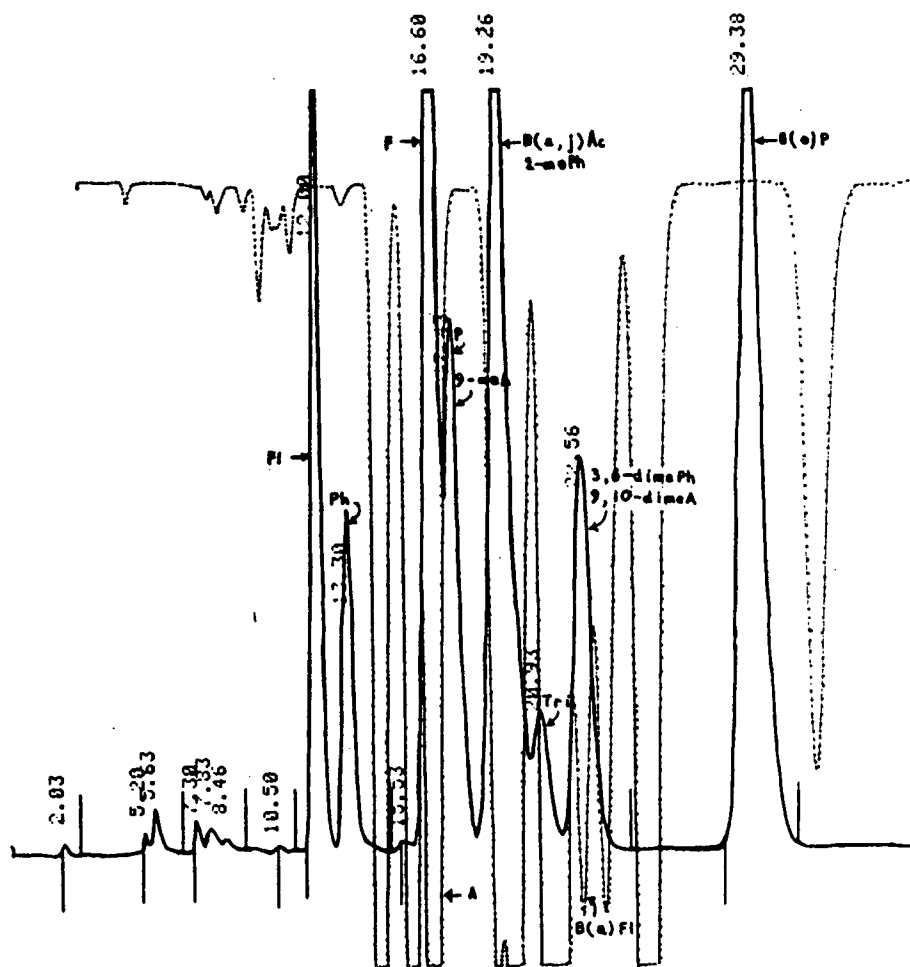
Table B - Capillary GC Conditions Figure 4-14 to 4-20.

FIGURE NUMBER	SAMPLE	INJECTION SIZE (μ l)	COMMENTS
4-18 a)	D(S)('79)III	2	chart speed 0.5 cm/min
b)	III	0.2	
c)	IV	2	
d)	V	2	
4-19 a)	D(S)('78)III	2	
b)	IV	2	
c)	V	2	
4-20 a)	W('78)III	2	
b)	IV	2	
c)	V	2	

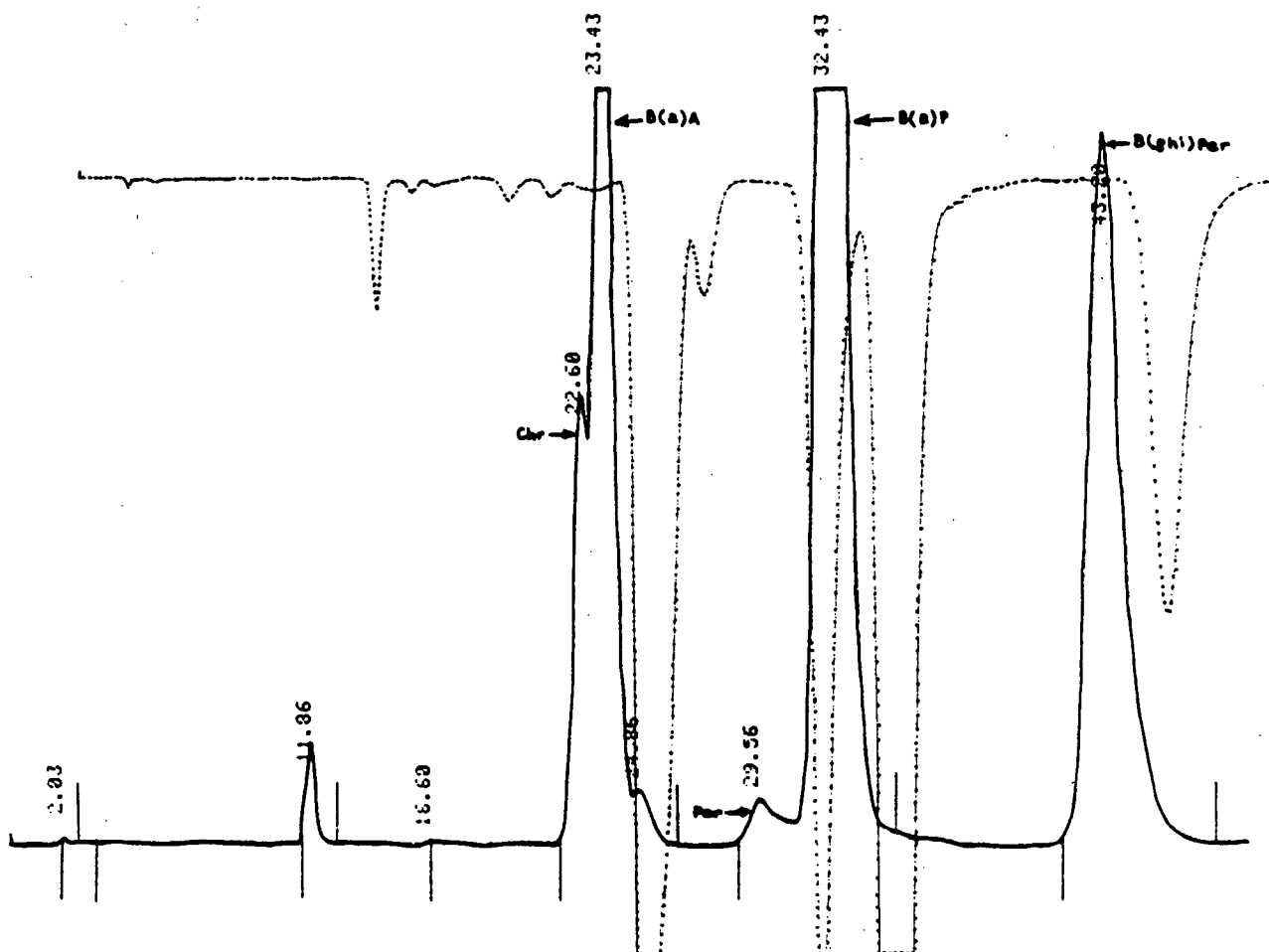
Table C - HPLC Conditions Figure 4-31 to 4-36.

FIGURE NUMBER	SAMPLE	INJECTION SIZE (μ l)	% CH ₃ CN	AUFS 254 nm	AUFS 280 nm
4-31 a)	R ₂ III	5	70	0.02	0.02
b)	IV	5	"	"	"
c)	V	5	"	"	"
4-32 a)	C ₂ III	5	"	"	"
b)	IV	5	"	"	"
c)	V	5	"	"	"
4-33 a)	I ₄ III	5	"	"	"
b)	IV	5	"	"	"
c)	V	5	"	"	"
4-34 a)	Gr ₂ III	10	"	"	"
b)	IV	5	"	"	"
c)	V	5	"	"	"
4-35 a)	DD 874km	1	"	0.200	0.200
b)	1665km	1	"	"	"
c)	2623km	1	"	"	"
4-36 a)	RM 895km	1	"	0.050	0.050
b)	1943km	1	"	"	"
c)	2545km	1	"	"	"

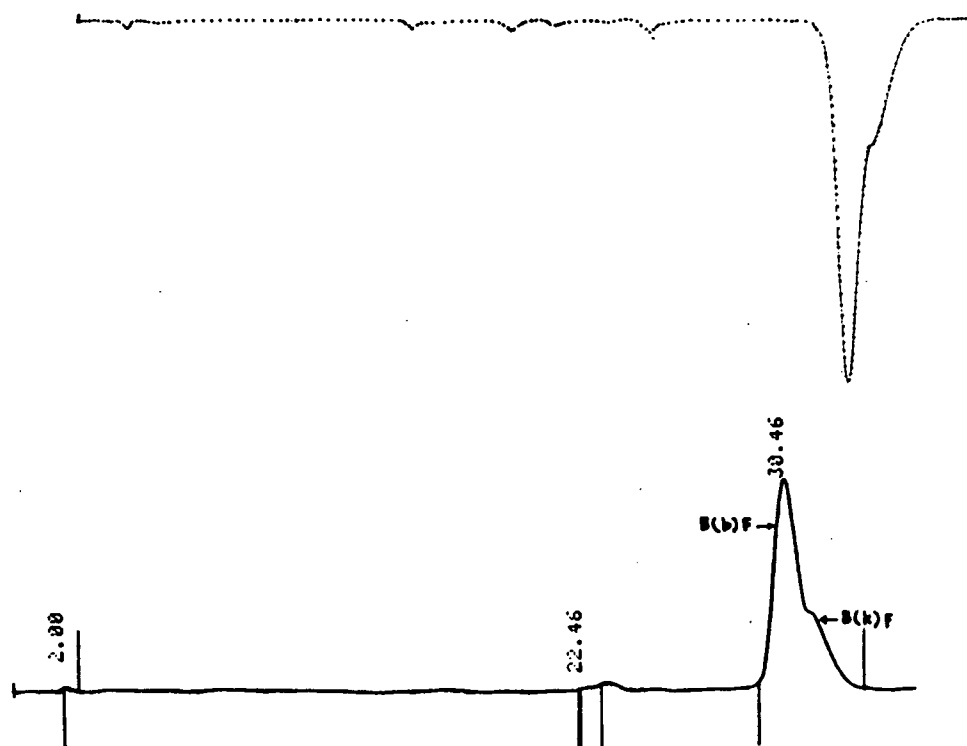
3. Mixed Standards, HPLC-Mixed standards were run for each of the three alumina fractions. Chromatograms are included in this appendix for each standard at 60% CH₃CN and at 70% CH₃CN.
4. Mixed Standards, capillary GC - Chromatograms of mixed standards corresponding to each alumina fraction are included. Conditions set out in section III F-3 and Table 3-3.



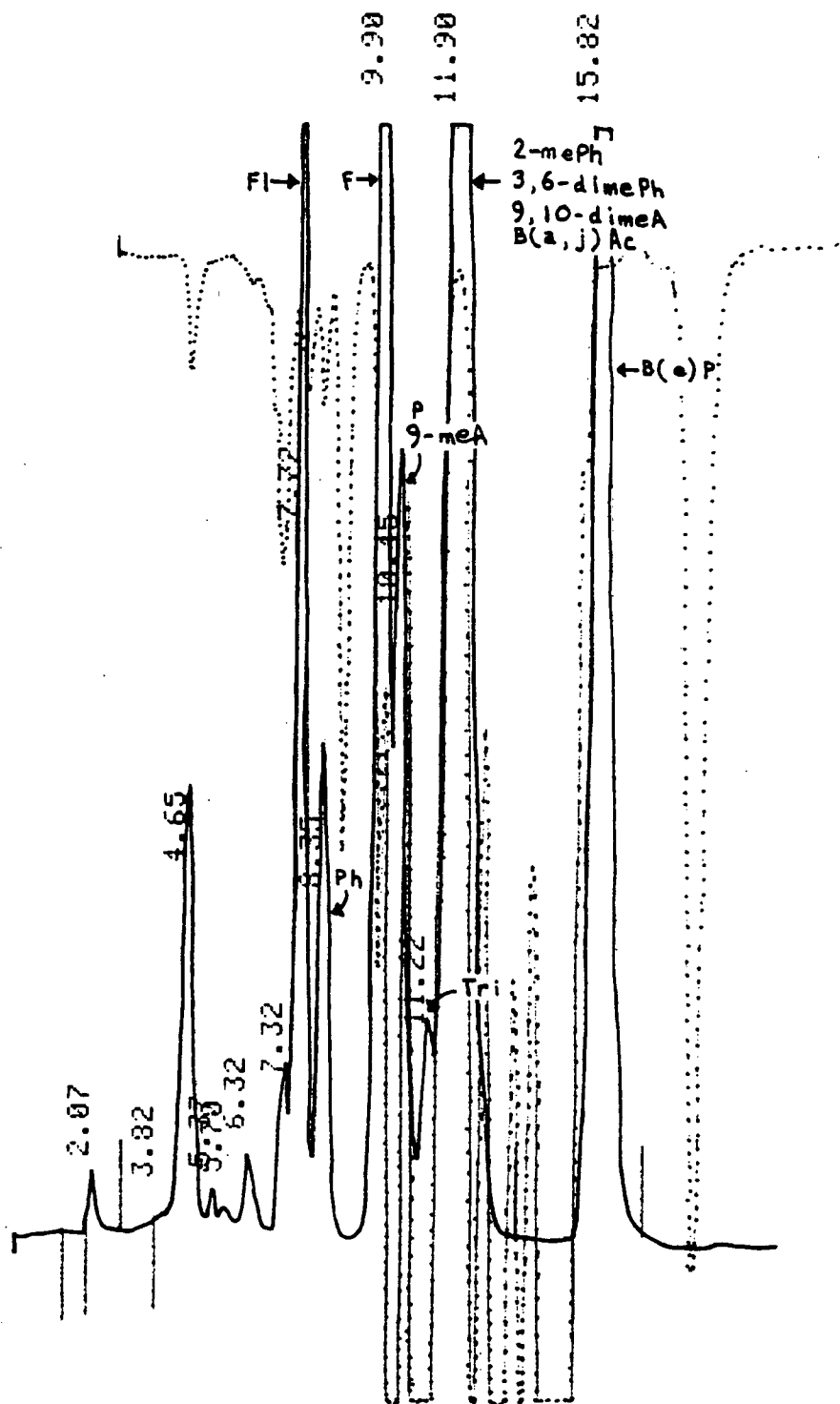
Standards Eluted in Alumina Fraction III, 60%, HPLC



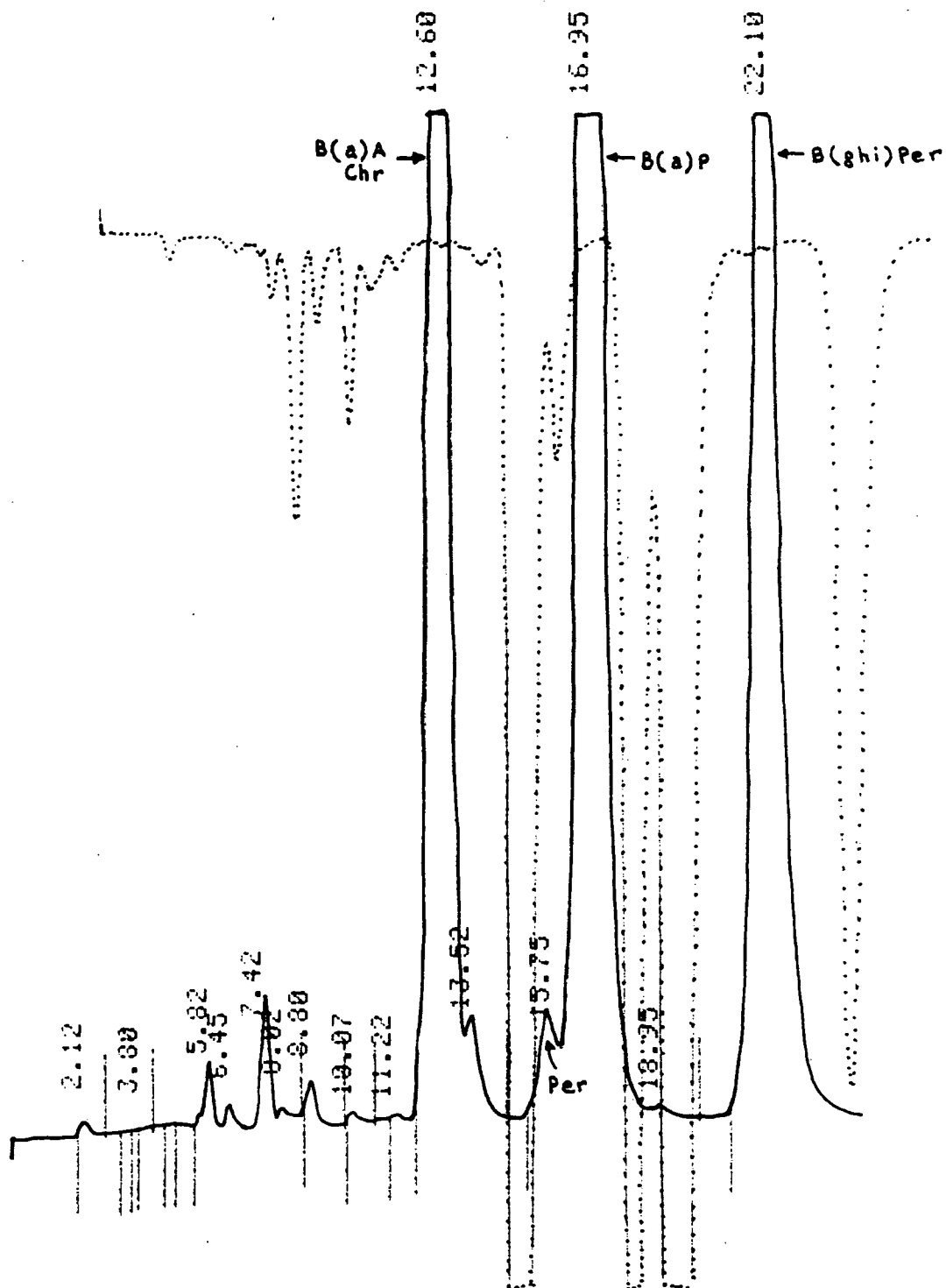
Standards Eluted in Alumina Fraction IV, 60% HPLC



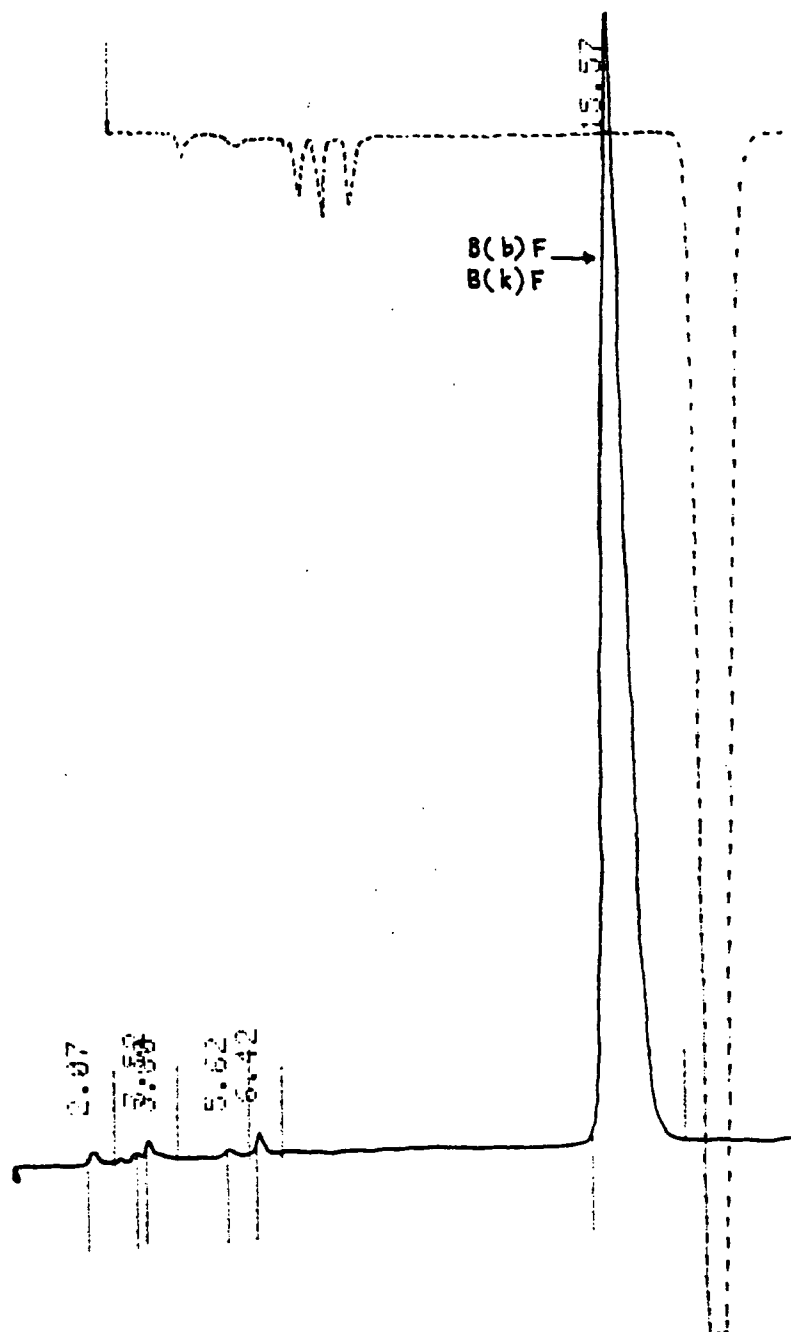
Standards Eluted in Alumina Fraction V, 60%, HPLC



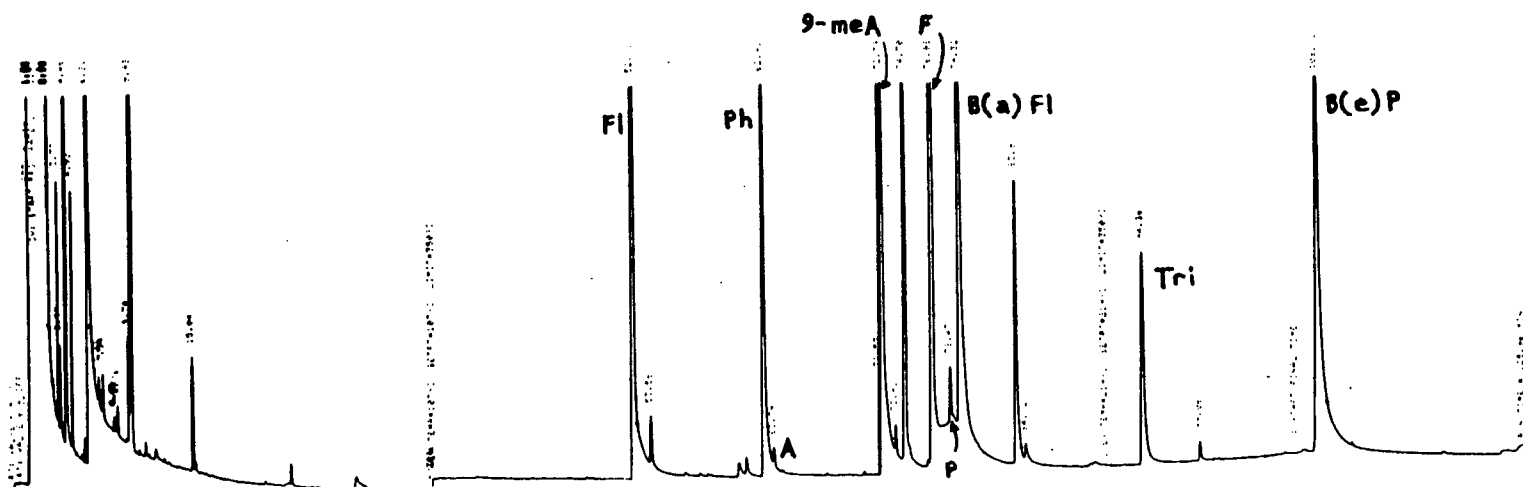
Standards Eluted in Alumina Fraction III, 70%, HPLC



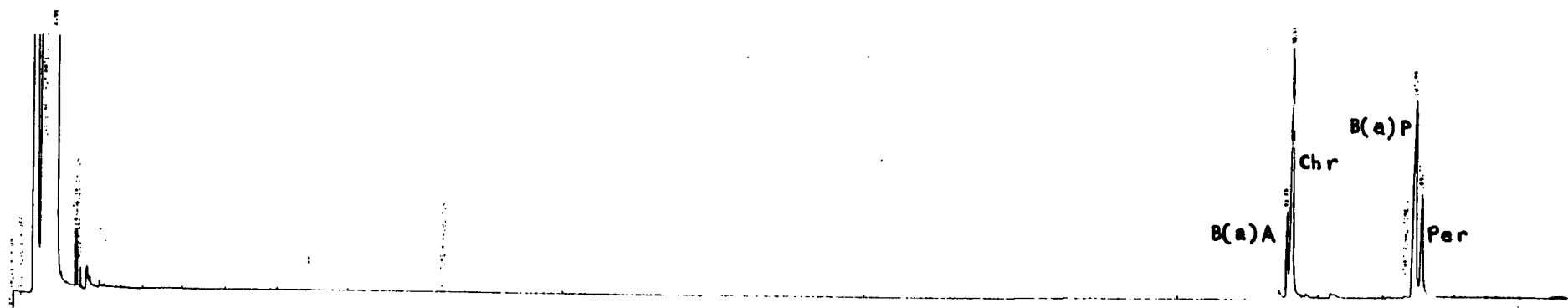
Standards Eluted in Alumina Fraction IV, 70%, HPLC



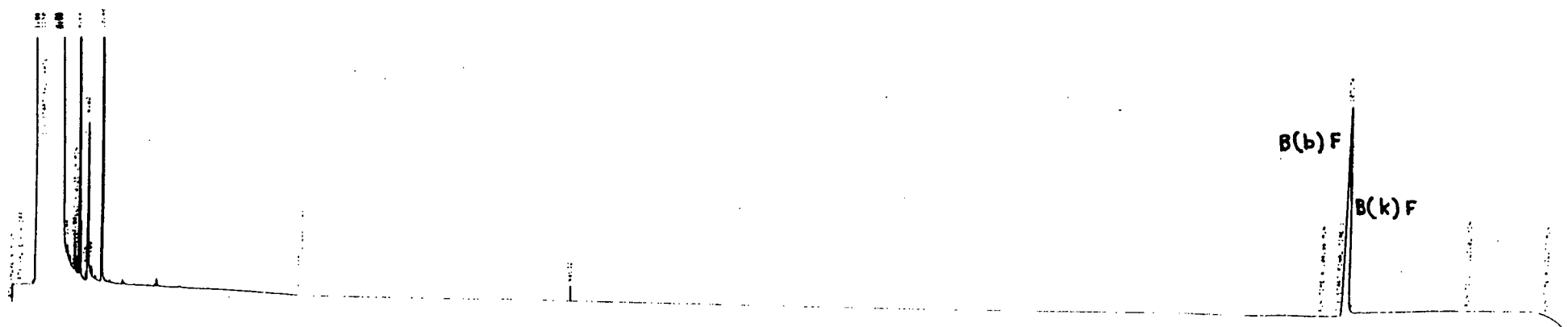
Standards Eluted in Alumina Fraction V, 70%, HPLC



Standards Eluted in Fraction III, capillary GC



Standards Eluted in Fraction IV, capillary GC



Standards Eluted in Fraction V, capillary GC