CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF SOUTHERN ONTARIO URBAN AIR PARTICULATE

by

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CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF SOUTHERN ONTARIO URBAN AIR PARTICULATE

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PREFACE

Parts of the text, figures and tables in this thesis have been previously published in the following journal articles:

Legzdins, A.E.; McCarry, B.E.; Bryant, D.W. *Polycyclic Aromatic Compounds* **1994**, *5*, 157-165.

Legzdins, A.E.; McCarry, B.E.; Marvin, C.H.; Bryant, D.W. *International Journal of Environmental Analytical Chemistry* **1995**, *60*, 79-94.

Morris, W.A.; Versteeg, J.K.; Bryant, D.W.; Legzdins, A.E.; McCarry, B.E.; Marvin, C.H. *Atmospheric Environment* **1995**, *29*, 3441-3450.

ABSTRACT

Ambient concentrations of polycyclic aromatic compounds [PAC], other air pollutants and the mutagenic potency of respirable air particulate extracts were used to study air quality in Hamilton from May of 1990 to June of 1991. Concentrations of polycyclic aromatic hydrocarbons [PAH], thiaPAH, oxygenated PAC and in some cases nitroPAH as well as the mutagenic activity caused by these compounds were determined in 68 samples of air particulate, collected over a number of days with widely varying air pollutant concentrations and atmospheric conditions.

Chemical analyses of the non-polar aromatic fractions of the air particulate extracts showed a 550-fold range in PAC concentrations, from 0.31 to 170 ng/cubic metre air, while biological assays showed a 140-fold range of mutagenic potencies. The non-polar aromatic fraction represented about three-quarters of the sum of the mutagenic responses in the polar and non-polar aromatic fractions. The mutagenicity and PAC concentrations of air particulate collected in Hamilton are low to average compared to air particulate samples collected in other cities around the world.

Relationships between mutagenic potencies, PAC concentrations and atmospheric conditions were examined using principal component analysis, which revealed good positive correlation between the mutagenic potencies of the non-polar aromatic fractions, nitrogen dioxide and sulfur dioxide concentrations. Average windspeed and ozone concentrations were inversely correlated with mutagenicity. Principal component analysis also showed that springtime thermal inversion episodes correlated with conditions

favoring the atmospheric transformation of PAH to mutagenic nitroPAH. The mutagenic potency of the non-polar aromatic fraction of air particulate extract rose in direct proportion to nitroPAH concentrations arising from atmospheric transformation.

Enhanced-sensitivity strains of *Salmonella typhimurium* were used in all bioassays of air particulate extract mutagenicity, thereby making more extract available for extensive chemical analyses. For example, bioassay analyses of a pooled sample of air particulate extract were carried out using six different strains of *S. typhimurium*. Strains containing increased levels of enzymes responsible for metabolizing compounds such as nitroPAH showed 7- to 10-fold enhanced mutagenic responses compared to standard tester strains. Normal-phase HPLC separation of the non-polar aromatic fraction of the pooled air particulate extract was combined with bioassays utilizing three different enhanced-sensitivity *S. typhimurium* strains, allowing quantitation of individual mutagens through analytical chemical methods as well as accurate determination of the mutagenic activity arising from each compound.

Normal-phase HPLC separation was also used in the bioassay-directed fractionation of an extremely mutagenic air particulate sample collected at the side of Highway 404 in Toronto, Ontario. NitroPAH arising from combustion sources were determined to be responsible for virtually all of the mutagenic activity detected in this particular sample, from which was isolated a potent mutagen not previously described in air particulate extracts - 3,8-dinitrofluoranthene.

ACKNOWLEDGEMENTS

The author wishes to express his thanks to Drs. B.E. McCarry and D.W. Bryant for the time and effort they have contributed towards the supervision of the research described herein. The author also wishes to thank the third member of the supervisory committee, Dr. A.D. Bain, for much advice and guidance, especially in the use of advanced statistics.

Sample collection in Hamilton was made possible through the assistance of the West Central Region Office of the Ontario Ministry of Environment and Energy. Atmospheric data was made available through the efforts of the MOEE's Air Resources Branch, Rexdale, as well as the staff of the Mt. Hope weather station. Dr. W.A. Morris of McMaster's Geology Dept. is especially thanked for his principal component and vector directional analysis of data collected in Hamilton during the 1990-1991 sampling season. Dr. W. Vance's gift of authentic dinitrofluoranthene isomers was also greatly appreciated.

Thanks also to Leah and Laurie Allan, Denny Lin, Dr. Chris Marvin, Rick Pauloski, Faj Ramalen, Dr. Richard Smith, J. Ken Versteeg and John Villella for technical support. The financial assistance of the Great Lakes University Research Fund, the McMaster EcoResearch Program and McMaster's Department of Chemistry is gratefully acknowledged.

Last but not least, I would like to thank my parents for their considerable moral and material support through almost seven long years of what was at times extremely challenging work.

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LIST OF ABBREVIATIONS

A_{254}	absorbance at 254 nanometres
amu	atomic mass units
avg	average
BaP	benzo[a]pyrene
CCIW	Canada Centre for Inland Waters
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNF	dinitrofluoranthene
DNP	dinitropyrene
EI	electron impact ionization
FID	flame ionization detection
GC-MS	gas chromatography-mass spectrometry
g	gram
HPLC	high performance liquid chromatography
IS	internal standard
M^+	molecular ion
mg	milligram
MOEE	Ontario Ministry of Environment and Energy
MTO	Ontario Ministry of Transport
m/z	mass to charge ratio
NBS	United States National Bureau of Standards
NFA	nitrofluoranthene
ng	nanogram
NICI	negative ion chemical ionization
NIST	United States National Institute of Standards and Technology
NP	nitropyrene
NPD	nitrogen-phosphorous detector
NPLC	normal-phase liquid chromatography
NQ	not quantified
NR	no response
PAH	polycyclic aromatic hydrocarbons
PAC	polycyclic aromatic compounds
pg	picogram
PICI	positive ion chemical ionization
revs	revertants
RPLC	reversed phase liquid chromatography
S9	rat liver supernatant
SD	standard deviation
SIM	selected ion monitoring
SRM	standard reference material
TEA	thermal energy analysis
TIC	total ion chromatogram
TNF	trinitrofluoranthene
UV	ultraviolet
µg	microgram
µl	microlitre

I. INTRODUCTION

I.1 Overview

The development of methods for the chemical and biological analyses of polycyclic aromatic compounds (PAC) extracted from air particulate is the theme of this thesis. The significance of PAC and their atmospheric transformation products in urban air particulate will be reviewed in this chapter. Chemical and biological analyses of PAC will be discussed separately and both concepts will be united in an introduction to the bioassay-directed fractionation of complex mixtures. The application of both chemical and biological analyses of PAC in air particulate extracts will then form the basis for pursuing the objectives of this study.

I.2 Urban Air Particulate

Urban air particulate is a complex mixture of inorganic and organic materials arising principally from: combustion processes and the combined influence of wind-borne terrestrial dust, sea spray, biogenic and volcanic processes (1). Combustion can generate particulate of a variety of sizes or mean aerodynamic diameters. That portion of air particulate with a mean aerodynamic diameter of 10 μm or less is referred to as respirable air particulate or PM-10. When inhaled, PM-10 is capable of depositing in the deepest regions of the lung or alveolae from where it cannot be removed by ordinary involuntary responses such as coughing or expectoration. Human health concerns regarding PM-10 are associated with its composition and the direct contact with areas of gas exchange

in the lung (2).

Respirable air particulate can be separated from larger particulate on the basis of differing momenta. This principle has been used to develop the PM-10 air sampler (Figure 1), which collects only respirable particulate with mean aerodynamic diameters of less than or equal to 10 μm. A motor in the PM-10 sampler pulls air and particulate of all sizes into the inlet cover and through multiple circular impactor nozzles, where larger particles are caught on an impaction surface between the nozzles. Only respirable particulate which stays in suspension can be drawn through the nozzles to collect on a Teflon glass fibre filter.

Respirable particulate has been collected to study particulate size distribution (1,4) as well as the chemicals which are adsorbed onto the particulate surfaces. Organic extracts of these adsorbed compounds have been known to be genotoxic since the early 1940s (5). Hundreds of biological and chemical experiments have been carried out since then [see (1) and (6) for reviews] which have identified polycyclic aromatic compounds as the chemicals responsible for the majority of this genotoxicity.

I.3 Polycyclic Aromatic Compounds

Polycyclic aromatic compounds (PAC) are multibenzenoid ring systems which may contain oxygen, nitrogen or sulfur heteroatoms (7); some examples appear in Figure 2. PAC include the polycyclic aromatic hydrocarbons (PAH), which were the first pure compounds to be identified as carcinogens (8). Various substituents, such as nitro, amino or alkyl groups, may be attached to the rings of PAH to afford PAC.

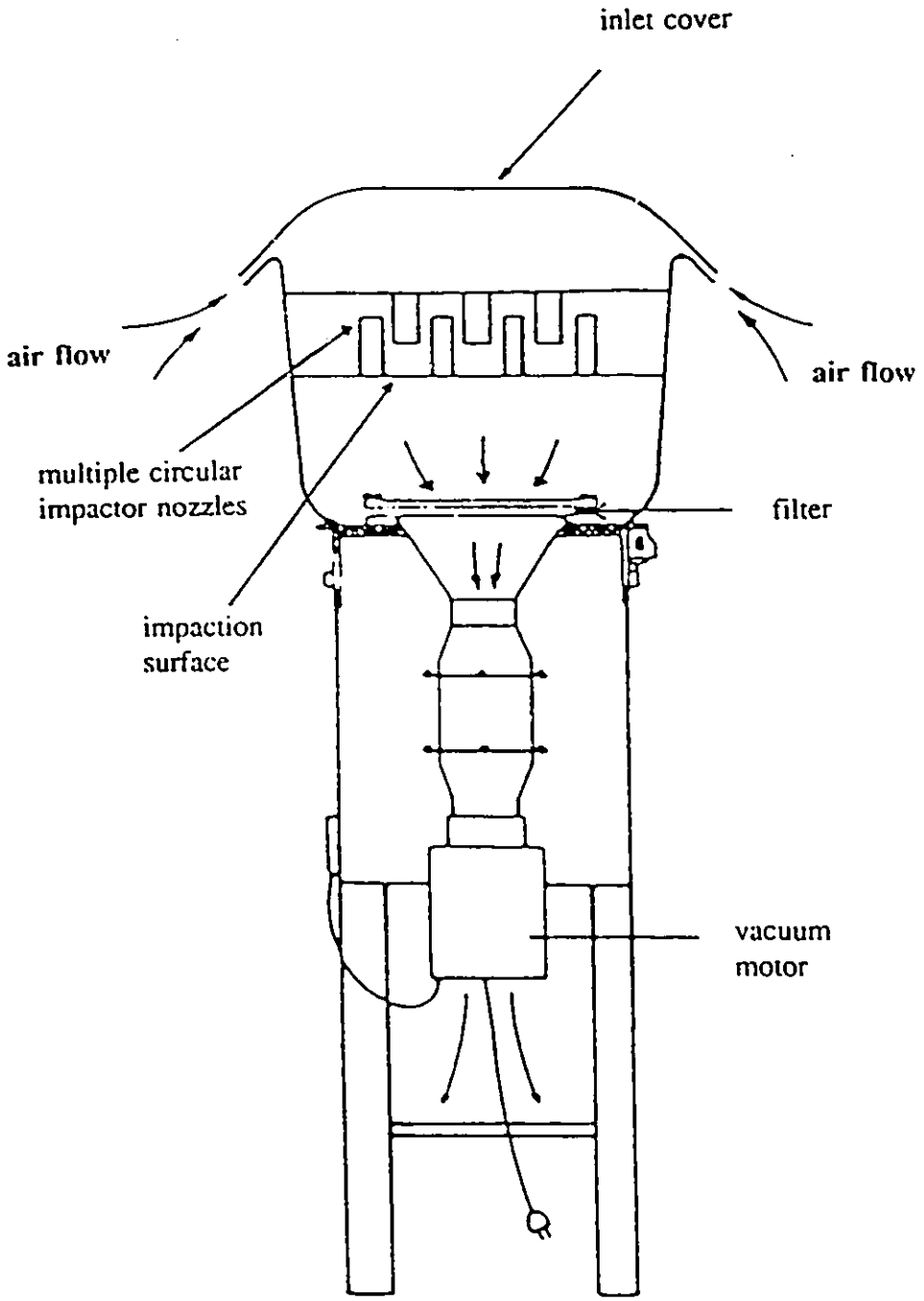


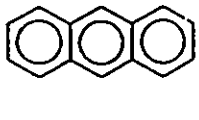
Figure 1. Cross-section of the PM-10 sampler. Reproduced from (3).

Figure 2. Structures and molecular weights of various polycyclic aromatic compounds detected in urban air particulate.

A. Polycyclic aromatic hydrocarbons (PAH).

B.ThiaPAH, ketoPAH, PAH quinones and nitroPAH.

A. PAH



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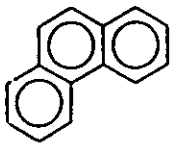

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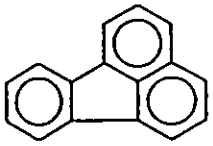

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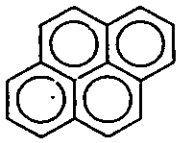

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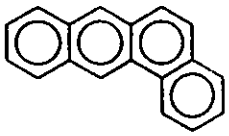
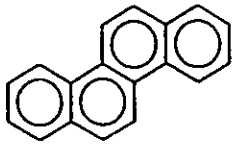


benzo[a]anthracene (228)



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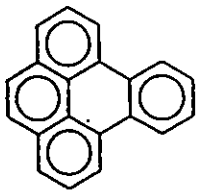

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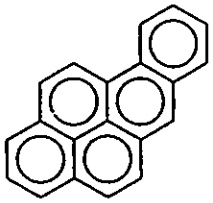

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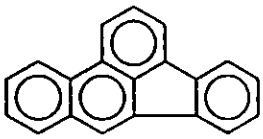

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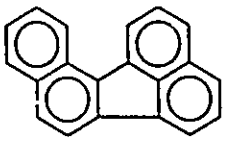

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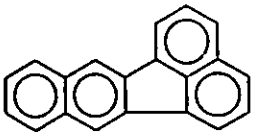

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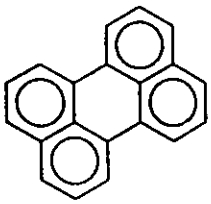

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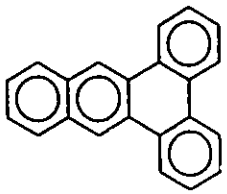
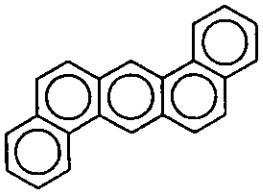
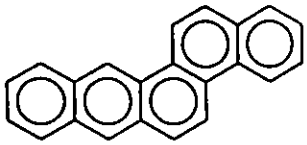


dibenzo[a,c]anthracene (278)



dibenzo[a,h]anthracene (278)



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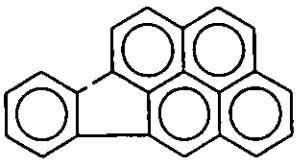
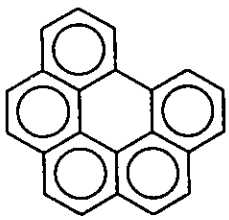


indeno[cd]pyrene (276)



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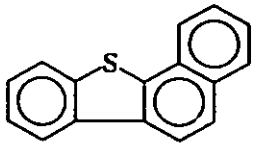
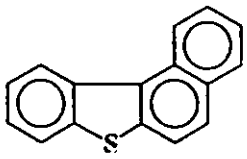
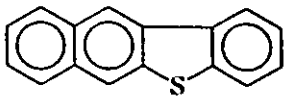


benzo[b]naphtho[2,1-d]thiophene
(234)

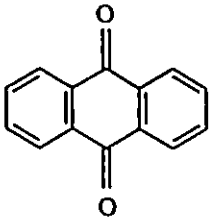


benzo[b]naphtho[1,2-d]thiophene
(234)

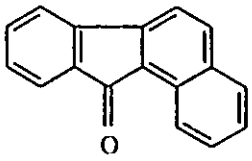


benzo[b]naphtho[2,3-d]thiophene
(234)

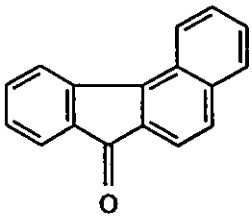
B. ThiaPAH, ketoPAH, PAH quinones and nitroPAH



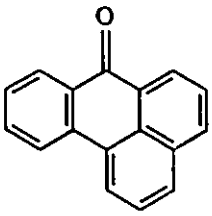
anthraquinone
(208)



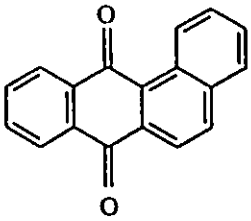
11H benzo[a]fluoren-11-one
(230)



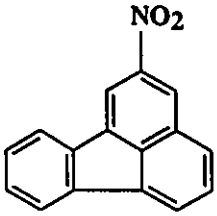
7H-benzo[c]fluoren-7-one
(230)



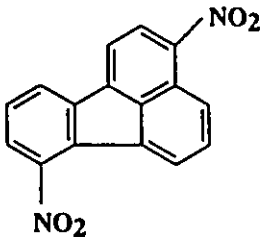
benzanthrone
(230)



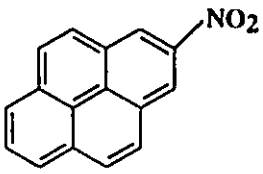
benz[a]anthracene-7,12-dione
(258)



2-nitrofluoranthene
(247)



3,7-dinitrofluoranthene
(292)



2-nitropyrene
(247)

PAC can occur in the vapour phase in ambient air or can be found adsorbed to particulate. Their abundance in either phase is a function of ambient temperature and their vapour pressure. Recently, Subramanyam *et al.* (9) and Chuang *et al.* (10) have reviewed various aspects of PAC partitioning between gaseous and particulate-bound phases in the atmosphere.

Due to the high vapour pressures of naphthalene, biphenyl, fluorene and dibenzothiophene, these compounds are found almost entirely in the vapour phase. Compounds with slightly lower vapour pressures, such as carbazole, phenanthrene and anthracene, are partitioned roughly 20:80 between particulate and vapour phases at ambient temperatures. For both fluoranthene and pyrene, the particulate to vapour phase ratio can range from 40:60 to 60:40, depending on the ambient temperature. The particulate to vapour phase ratio for compounds with molecular weights greater than 202 is almost always 80:20 or higher (11).

The partitioning of PAH between the vapour and particulate phases is an important factor in reactions with atmospheric gases or the hot gas streams released from combustion. Vapour phase naphthalene, anthracene, phenanthrene, fluoranthene and pyrene react readily with combustion gases, while PAH adsorbed to particulate are far less reactive (12).

I.4 Sources of PAC

The various classes of PAC which are found adsorbed to air particulate arise due to four processes: (1) pyrosynthetic reactions during combustion, (2) the release of PAC already present in unburned fuel, (3) the formation of PAC in exhaust gases immediately after combustion and (4) atmospheric transformation. PAC artifacts may also be formed during air particulate sampling.

I.4.1 Pyrosynthesis of PAC During Combustion

PAC formation during combustion of organic fuel is believed to begin with the pyrolysis of small molecules to form unstable radical fragments. The radicals recombine to give relatively stable polycyclic compounds shown in Figure 3. Biosyntheses of PAC are also believed to exist but these do not contribute significantly to the PAC seen in urban air particulate (13).

I.4.2 The Release of PAC in Unburned Fuel

PAC occur in crude oil as a result of biomass degradation during fossil fuel formation (14). The production of gasoline and diesel fuel does not involve the removal of PAC from crude oil during refining, which led to the hypothesis that PAC detected in diesel exhaust particulate arose mostly from PAC occuring in the diesel fuel before combustion (15). This hypothesis was recently confirmed through the addition of radiolabelled benzo[a]pyrene to diesel fuel, which was burned in an experimental engine.

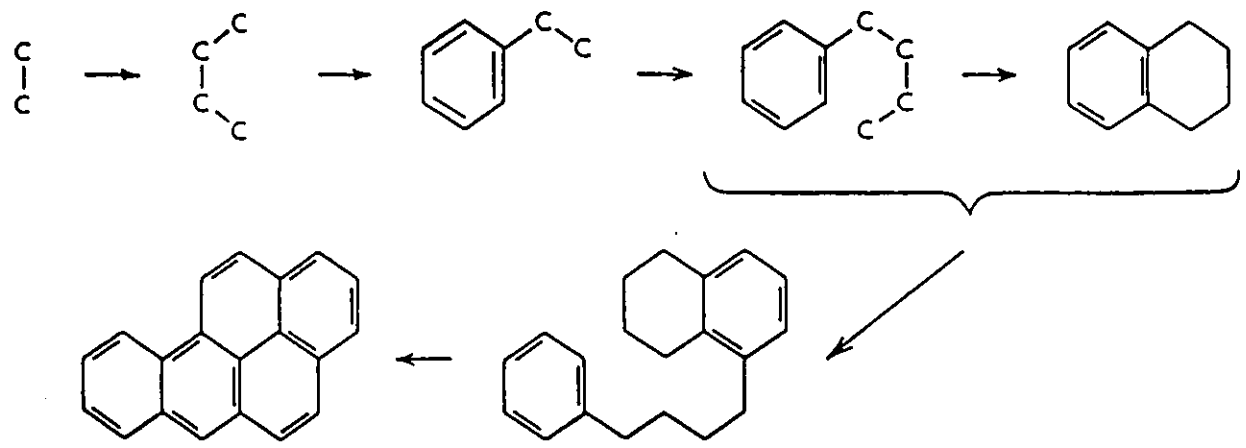


Figure 3. Proposed stepwise synthesis of benzo[a]pyrene. Reproduced from (17).

Chemical analysis determined that 80% of the benzo[a]pyrene detected in the exhaust particulate was derived from labelled benzo[a]pyrene in the fuel. The remaining 20% of benzo[a]pyrene in the exhaust was proposed to arise from pyrosynthetic routes (16). Although this experiment demonstrated the significance of PAH in fuel for the first time, the study was only performed with a single experimental engine. Estimates have not been made as to the amount of PAC released per volume of fuel burned in everyday use Hence, the relevance of these results to PAC concentrations in urban air particulate has not been determined.

I.4.3 Formation of PAC in Exhaust Gases Immediately After Combustion

As combustion gases cool, PAH in the gas phase condense onto the surfaces of the carbonaceous particulate matter that constitutes smoke. Oxygen and oxides of nitrogen combine with PAH immediately after combustion to afford various classes of PAC, including PAH ketones, quinones, nitroPAH and dinitroPAH; for a review, see (18) and (19).

Combustion-derived nitration of PAH is believed to take place through an electrophilic reaction, since the abundance and substitution patterns of nitroPAH isomers formed through combustion and electrophilic nitration are very similar. For example, nitration of fluoranthene with nitric acid and acetic anhydride gives 1-, 3-, 7- and 8-nitrofluoranthene, with 3-nitrofluoranthene being most abundant (20). Analysis of nitroPAH in diesel exhaust shows the same four isomers in the same order of abundance

(21). The ratio of combustion-derived nitroPAH to PAH is very low in urban air particulate (about 1:1000) but is about 100-fold higher in diesel exhaust particulate (21).

I.4.4 Atmospheric Transformation

This introduction to the atmospheric tranformation of PAC will give two examples of the reaction of PAH with atmospheric gases to generate nitroPAC. A description of the atmospheric transformation of fluoranthene to nitrofluoranthene will be followed by the transformation of phenanthrene to nitrodibenzopyranone. Non-anthropogenic, genotoxic compounds are produced from non-genotoxic combustion products in both cases. The photolysis of 9-nitroanthracene will give an example of PAC atmospheric decomposition.

I.4.4.1 Atmospheric Nitration of Gas-Phase PAH

Gas-phase nitration of ambient PAH depends on the presence of hydroxyl radicals, which are generated from ozone in the presence of sunlight and water according to the following reactions (1):

$$O_3 + h\upsilon\ (\lambda < 320\ nm) \longrightarrow O_2(^1\Delta_g) + O\ (^1D)$$

$$O(^1D) + H_2O \longrightarrow 2OH$$

Singlet atomic oxygen reacts with water to form hydroxyl radicals which participate in thousands of atmospheric reactions [for reviews, see (22,23)]. The reactions of OH with gas-phase fluoranthene and pyrene in the presence of nitrogen dioxide are

important since they produce the most abundant nitroPAH found in urban air: 2-nitrofluoranthene and 2-nitropyrene. These two genotoxic nitroPAH isomers are not produced through combustion and occur in particulate at concentrations at least ten times higher than the corresponding combustion-derived nitroPAH isomers (e.g., 1-, 3-, 7- and 8-nitrofluoranthene and 1-nitropyrene) (21). Figure 4 shows the transformation of gas-phase fluoranthene into 2-nitrofluoranthene, which condenses onto particulate once formed and which is found in urban and rural air particulate collected around the world (24).

Transformation of fluoranthene to 2-nitrofluoranthene can also take place through reaction with N_2O_5 or the nitrate radical, but the contribution of these reactions to ambient levels of 2-nitrofluoranthene is thought to be of little significance (12).

A second example of the atmospheric nitration of PAH involves the production of compounds more polar and less abundant than nitroPAH. The reaction of phenanthrene with hydroxyl radicals, nitrogen oxides and oxygen produces at least two isomers of nitrodibenzopyranone, genotoxins which have been detected in ambient air particulate (25). A reaction scheme for nitrodibenzopyranone formation is shown in Figure 5 and is reproduced from the report of Helmig and Harger (25). This reaction scheme is one example of the often convoluted pathways for atmospheric transformation, which generate a complex mixture of products from an already complex mixture of starting materials.

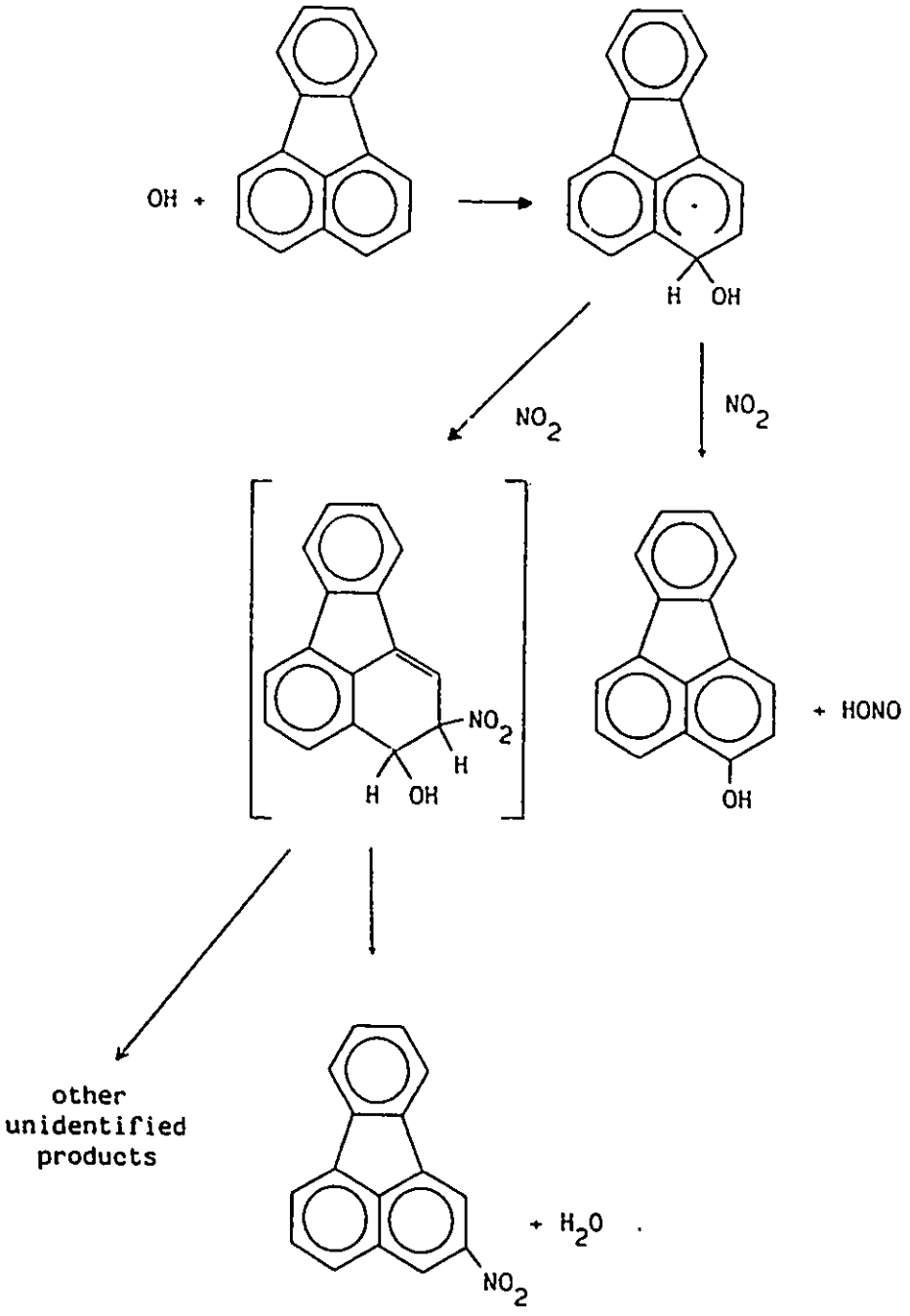


Figure 4. The reaction of hydroxyl radical with fluoranthene in the presence of nitrogen dioxide. Reproduced from (12).

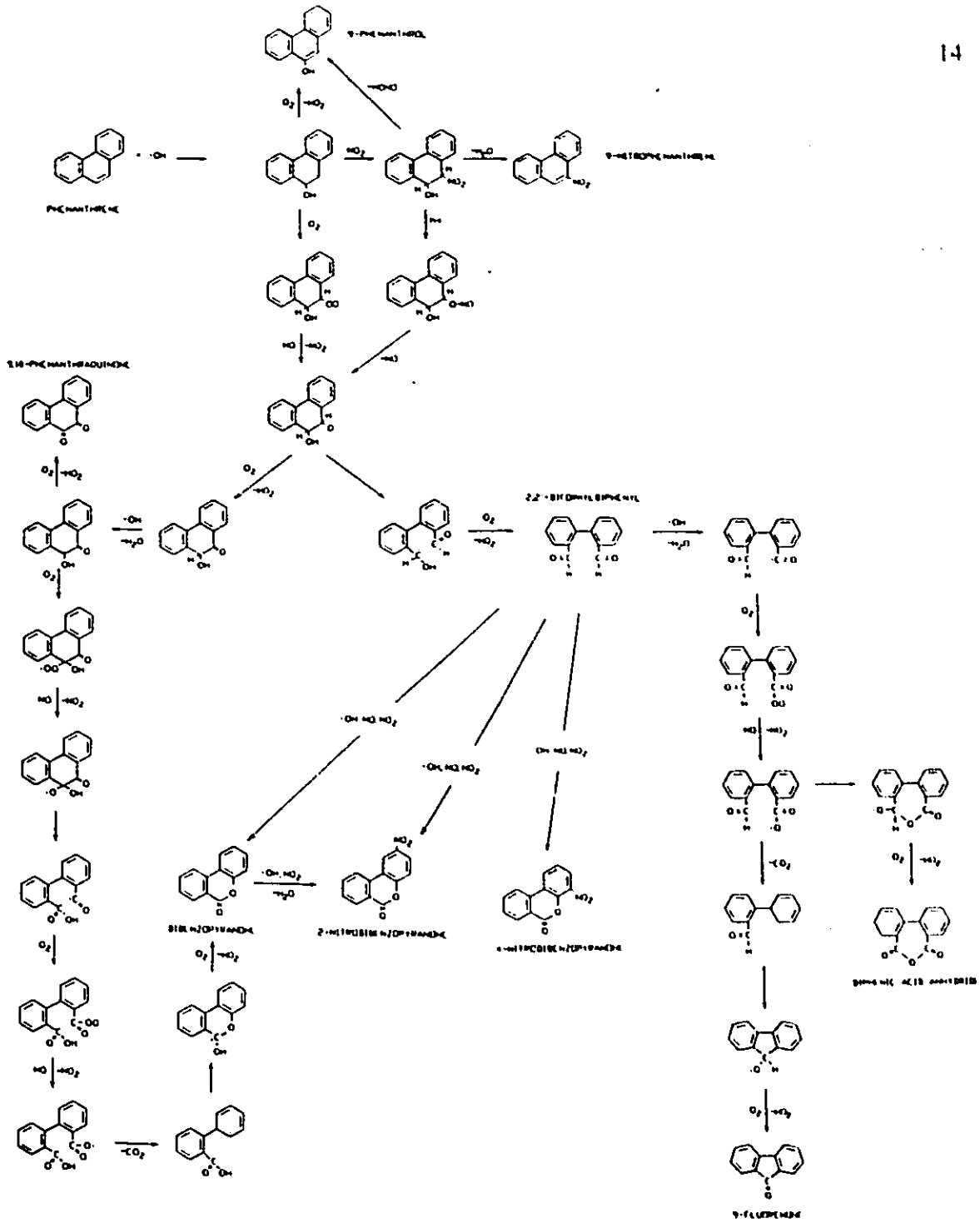


Figure 5. Reaction scheme for the atmospheric transformation of phenanthrene to nitrodibenzopyranone. Adapted from (25).

1.4.4.2 Atmospheric Reactions of NitroPAH

NitroPAH are themselves subject to atmospheric transformation. For example, 9-nitroanthracene reacts with sunlight and oxygen to give anthraquinone which is shown in Figure 6. The decomposition of 9-nitroanthracene is significant, since it creates a non-genotoxic PAH quinone which is also produced from combustion and the atmospheric transformation of anthracene by singlet oxygen or ozone (26). Thus, anthraquinone in air particulate can arise from at least three different sources.

While the decomposition of 9-nitroanthracene yields a non-genotoxic product, some nitroPAH decomposition products are genotoxic. Photolysis of 1-nitropyrene produces mutagenic hydroxynitropyrenes (27).

1.4.5 Reactions of PAH During the Collection of Air Particulate

Air particulate, with its adsorbed PAC, is collected on various types of filters continuously over periods ranging from hours to days. Ambient air is pulled past the particulate that has already collected on the filter. This continual drawing of air over collected particulate has been suspected of creating sampling artifacts through the nitration or oxidation of PAH; these hypotheses have not been confirmed (29). The formation of artifacts is minimized through the use of Teflon-coated glass fibre filters (30).

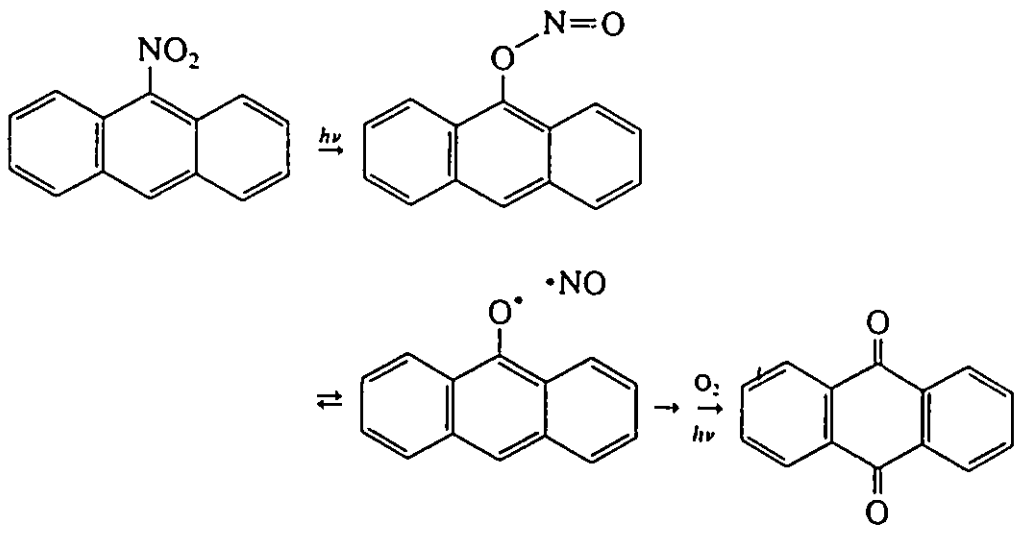


Figure 6. Decomposition of 9-nitroanthracene in the presence of sunlight and oxygen. Adapted from (1) and (28).

I.5 Preparation of Air Particulate Samples for Chemical Analysis

Preparation of air particulate for chemical analysis of aromatic compounds involves two distinct steps: (1) the extraction of organic compounds from the particulate to give a crude organic extract and (2) the separation of unwanted organic and inorganic compounds to prepare a PAC-enriched fraction through the use of a chromatographic cleanup of the crude organic extract.

I.5.1 Extraction of Air Particulate with Organic Solvents

Air particulate contains a great variety of aliphatic, aromatic and phenolic compounds, as well as elemental carbon, metals and inorganic compounds (31). Organic compounds adsorbed to the surface of air particulate can be removed by extraction using care to choose the proper organic solvents for efficient extraction of PAC.

Non-polar solvents such as benzene or cyclohexane extract PAH from particulate most efficiently. A dimethylformamide wash removes traces of any polar PAH derivatives, allowing identification and quantitation of PAH in the purified extract by GC-MS (32, 33). This approach of using non-polar solvents selectively enriches PAH target compounds and may not extract polar, genotoxic PAC such as aza-aromatics (34) or nitrodibenzopyranones (25). Such compounds can be quantitatively recovered by applying a combination of polar and non-polar solvents to extract both polar and non-polar PAC from air particulate. Many different solvent combinations have been evaluated using

standard preparations of air particulate or diesel soot, which both contain PAH and polar PAC (19, 31, 35, 36). Direct comparison of several extraction solvent regimes showed dichloromethane followed by methanol produced the highest yield of organic extractable material from both diesel soot and air particulate (31). This solvent combination has been used routinely to extract PAC from a wide variety of environmental matrices (37-39).

1.5.2 Preparation of Non-Polar Aromatic Fractions from Crude Organic Extracts of Air Particulate

The crude organic extracts obtained with a combination of non-polar and polar solvents are not amenable to gas chromatographic analysis without additional cleanup, owing to the presence of non-volatile compounds, e.g., extremely polar compounds (40) and compounds with molecular masses in excess of 300 amu (41). Certain abundant aliphatic compounds (such as docosane and hexacosane) found in crude extracts of air particulate can interfere with GC analyses, due to co-elution with PAH (42).

Methods which remove polar organic acids and bases from PAC by extraction with aqueous acid or base destroy some genotoxic PAC present in the organic extract (43) and may lead to the formation of tars or stable emulsions (44). Open-column chromatography of the organic extract over silica has been used extensively to remove aliphatics and extremely polar compounds from PAC, but silica has been implicated in the decomposition of nitroPAH (45) and in the polymerization of carbonyl-containing compounds (46). The activity of the adsorbent (a function of the amount of water

adsorbed to the adsorbent) may contribute to analyte destruction in such cases (6).

Open-column chromatography on neutral alumina offers improvement over the use of silica gel. Rapid analyte elution and reproducible compound class separation have resulted in negligible losses of target analytes through irreversible adsorption. A simple alumina methodology has been developed by Later *et al.* (47) which we have adapted to our needs. PAC mixtures were applied to alumina as solutions in organic solvent and the solvent was removed through evaporation at reduced pressure; subsequent elution of the alumina with hexane removed the aliphatics while PAC remained adsorbed to the column. Neutral PAC were then eluted from the column with benzene, which gave a non-polar aromatic fraction. Polar organic solvents such as chloroform eluted polar azaPAC. Methanol and methanol-water eluted even more polar PAC. This methodology has been successfully applied in the separation of various PAC classes found in fuels (47), crude oil (48), steel foundry dust (37), harbour sediments (38,39,49) and urban air particulate extracts (50,51).

I.6 Quantitation of PAH in Non-Polar Aromatic Fractions

PAH-rich non-polar aromatic fractions are suitable for analysis using high-performance liquid chromatography (HPLC) with diode-array detection (52) and/or fluorescence detection (53) to quantify PAH. Alternatively, gas chromatographic methods such as GC-MS and GC-FID may be employed. Wise *et al.* (54), who have compared HPLC and GC methodologies, found detection of PAH using either method equally

sensitive, owing to the excellent fluorescence of PAH and the high concentration of PAH relative to other PAC present in organic extracts of air particulate.

A comparison of the maximum concentrations of PAH and other PAC determined in air particulate extracts from around the world is presented in Table 1. There is a very large range between the concentrations of PAH and the concentrations of trace species such as nitroPAH and dinitroPAH. Experimental strategies to characterize particulate-associated PAC have either involved quantitation of the more abundant PAH from a large number of samples or quantitation of selected target PAC in a small number of samples. The latter case is likely due to the extra separation steps necessary to remove PAH interferences prior to analysis for trace species (6).

I.7 Separation of PAH from Trace PAC in Non-Polar Aromatic Fractions

Separation of PAH from PAC is most effectively achieved by application of normal-phase liquid chromatography [NPLC] (226). Elution of analyte from a stationary normal phase depends on the use of successively more polar solvents, beginning with hexane and progressing to a more polar solvent such as dichloromethane. Under these conditions, PAH elute first, followed by nitroPAH and polar PAC such as polycyclic aromatic ketones and quinones. A final application of a very polar organic solvent (such as ethanol or acetonitrile) removes any remaining polar compounds (65). This approach has been used to characterize PAC from air particulate

Table 1.
Maximum Concentrations for Various Classes of PAC
Detected in Urban Air Particulate.

Compound Class	*Compound*	*Typical Maximum Conc. ($pg \cdot m^3$)*	*Location*	*Ref.*
PAH	benzo[a]pyrene	42000	Katowice, Poland	(55)
OxyPAH	benzanthrone	4000	Duisburg, Germany	(56)
ThiaPAH	benzo[b]naphtho-(2,1-d)thiophene	1000	Essen, Germany	(57)
AzaPAH	isoquinoline	1000	Los Angeles, California	(58)
Oxy-nitroPAH	2-nitrodibenzo-pyranone	400	Los Angeles, California	(59)
NitroPAH	2-nitrofluoranthene	300	Los Angeles, California	(60)
DinitroPAH	1,8-dinitropyrene	10	Kanazawa, Japan	(61)

including nitroPAH, dinitroPAH, aminoPAH and azaPAH (60, 62-64) as well as hydroxynitroPAH, polycyclic aromatic ketones and quinones (65). Although normal-phase liquid chromatography separates classes of polar PAC with greater resolution than reversed-phase methods, NPLC can suffer from poorly reproducible retetention times (64). Identification and quantitation of individual PAC isomers requires efficient separation such as that achieved on capillary GC columns, which have substantially higher numbers of theoretical plates compared to normal- or reversed-phase HPLC columns. For example, capillary GC has been used to separate seven different isomeric nitroPAH of molecular weight 247: 1-, 2-, 3-, 7- and 8-nitrofluoranthene, as well as 1- and 2-nitropyrene (66), while normal-phase or reversed-phase HPLC methods fail to do so (42). On the other hand, NPLC has been used successfully to separate synthetic nitration products of PAH (67, 68).

I.8 Detection of Trace PAC in Gas Chromatographic Analyses

PAC can be detected in gas chromatographic analyses by various methods, which include: flame ionization detection, nitrogen-phosphorus detection, electron-capture detection, thermionic ionization detection, photoionization detection, thermal energy analysis, atomic emission detection and mass spectrometry. These techniques have been critically reviewed elsewhere (69,70) but only mass spectrometry can provide information which makes tentative structural identification possible without the use of an authentic standard, which may or may not be commercially available (71).

Mass spectrometry when combined with gas chromatography (GC-MS) most often relies on two types of ionization: electron impact ionization (EI^+) and chemical ionization (CI). PAH often yield only the molecular ion in the EI^+ mode, but additional information may be gathered from mass spectral data to identify individual isomers on the basis of fragment ion abundances and GC retention indices. For example, nitroPAH produce four major ions corresponding to the molecular ion, M^+, and 3 characteristic fragment ions: $(M\text{-}NO)^+$, $(M\text{-}NO_2)^+$ and $(M\text{-}CNO_2)^+$. Polycyclic aromatic quinones produce molecular ions $(M)^+$ as well as the fragment ions $(M\text{-}CO)^+$ and $(M\text{-}2CO)^+$. Individual PAC isomers can be identified by relative ion abundance but electron-impact mass spectra of certain PAC may give very low abundances for the molecular ions, making identification difficult (72).

PAC have been identified using both positive ion chemical ionization (PICI) and negative ion chemical ionization (NICI), which have been reviewed by Hites *et al.* (73,74). PICI with methane as the reagent gas depends on the formation of CH_5^+ from methane. Protonated methane, CH_5^+, can donate a proton to an analyte, giving an $(M+H)^+$ ion (73). On the other hand, NICI with methane as the reagent gas uses methane as a 'buffer' gas (B) to remove excess energy from the negative molecular ion as it is formed, thereby reducing the probability of fragmentation (75):

$$M + e^- + B \longrightarrow M^- + B$$

NICI or PICI have the advantage of increasing the abundance of the molecular ion when compared to EI^+ methods. NICI is about 15-fold more sensitive to nitroPAH than

PICI (76) and therefore is the method of choice for nitroPAH analyses. There are some disadvantages to CI methods, however. Compared to EI', CI methods may not provide fragment ion data and higher pressures encountered in the ion source may increase routine maintenance (75). EI', NICI and PICI have been extensively compared and contrasted (73,74).

I.9 Determination of Genotoxicity through Biological Assays

Genotoxicity tests are used to characterize the mutagenic potential of chemicals by detecting their ability to interact with the genetic material, DNA. Exposure of DNA to certain PAC metabolites produces adducts *in vitro* and exposure of cells to mutagens *in vivo* can result in mutations or chromosomal abberations. The production of DNA adducts, mutations and aberrations in biological material may lead to cancer in animals and man (77).

Mutagens have been detected in unfractionated complex mixtures through the use of short-term bioassays (78). Mutagenicity assays have been used as a semi-quantitative detector of genotoxic activity in chromatographic fractions prepared from the crude extract of environmental samples such as air particulate (79).

I.9.1 Bacterial Tests for Mutagenicity

The most common bacterial strains utilized in mutagenicity assays include *Salmonella typhimurium* and *Eschericia coli.* The *S. typhimurium* (or Ames) assay offers

advantages over the *E. coli* assay, including speed and simplicity (80). The *S. typhimurium* assay measures the ability of chemicals to produce mutations at a specific locus in the genome of *S. typhimurium:* either *his*D3052 or *his*G48 (77,81). A positive response is defined as an increase (over background) in histidine protrophs in the bacterial population resulting from exposure to a specific chemical. A large amount of data on the mutagenic effect of chemicals or complex mixtures is available in the published literature using the *S. typhimurium* assay.

The basis of the *S. typhimurium* bioassay is the detection of a small number of bacteria which grow unaided in the absence of exogenous histidine, in the midst of a large population which is unable to synthesize histidine. The application of a mutagen alters or causes reversion of the gene for histidine biosynthesis, allowing a bacterium which is mutated at a specific genetic site or locus to grow without exogenous histidine and thus be detected. When transferred to a solid support which supplies minimal essential nutrients for growth, mutated bacteria grow into colonies which become visible in two or three days. The number of such revertant colonies is proportional to the mutagenic potency and the amount of the chemical which was administered (82,83).

Bacteria such as *S. typhimurium* are often incapable of metabolizing certain chemicals and require an external source of oxidative metabolic activity to generate mutagenic intermediates. In the *Salmonella*/microsome assay, a rat liver homogenate (known as 'S9') provides this metabolic capability. The S9 extract contains mixed function monooxygenases (MFO) which may be detected by their content of cytochrome P450. Rat

liver MFO (S9) normally detoxify xenobiotics *in vivo* and are capable of oxidizing aromatic hydrocarbons to oxidized analogues *in vitro*. The method used in the *S. typhimurium* assay for mutagenicity is represented schematically in Figure 7. The first assay which uses *S. typhimurium* bacteria only is a measure of the mutagenicity of the 'direct-acting' mutagens (i.e., those compounds which undergo reductive metabolism or which are mutagenic when mixed with the bacteria directly). Examples of compounds which are 'direct-acting' mutagens include many alkylating agents and nitroPAH. The second type of assay measures mutagenic potency in *S. typhimurium* in the presence of MFO provided by S9, in order to detect 'indirect-acting' mutagens, which are defined as chemicals which require oxidative metabolism to produce a mutagenic intermediate. Examples of compounds which are 'indirect-acting' mutagens include PAH and azaPAH.

Typically, the organic extract from air particulate matter is mixed with *S. typhimurium* bacteria in molten agar, with or without S9. The mixture is transferred to a Petri dish, where it is allowed to incubate for 48 to 72 hours and the number of revertant colonies are counted.

There are a number of ways by which the detection of mutagenic activity of chemicals has been enhanced in *Salmonella*. These include (1) alteration of the target in histidine biosynthesis (TA98 versus TA100) and (2) alteration in metabolic capability of the bacteria to enhance production of mutagenically active intermediates. Strains described in the second case will be referred to later.

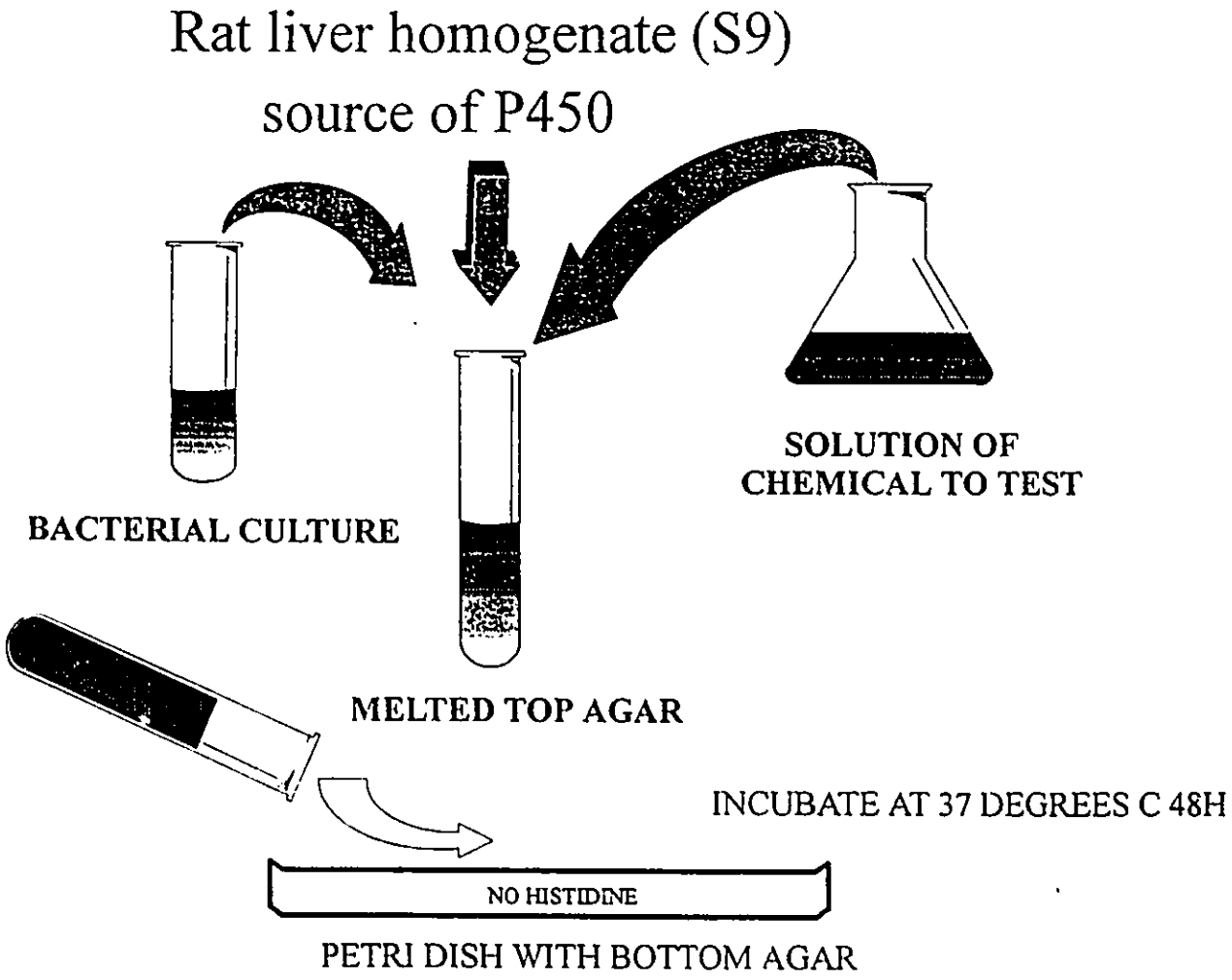


Figure 7. Schematic diagram of the *Salmonella typhimurium* assay for mutagenicity.

Strains referred to as 'TA98-like' detect frameshift mutagens primarily, which are caused by DNA base deletions or additions and result in transcriptional errors. The 'TA100-like' strains sense base-pair substitution mutagens primarily, which arise due to the selection of an incorrect base during DNA replication and which result in altered base-pairing (82,88).

Mutagenic activity may be described quantitatively. For example, the mutagenicity of a series of nitropyrenes and nitrofluoranthenes in *S. typhimurium* strain TA98 (-S9) has been reported (84). These nitroPAH have been quantified in Los Angeles air particulate extracts and the mutagenicity of these extracts was assessed. Using analytical chemical and biological data, the mutagenic potency of the air particulate extract can be expressed in terms of the number of bacterial revertants per cubic metre of air sampled or as a function of chemical concentration (85). If the mutagenicity of 1-nitropyrene (expressed in revertants/ng) is multiplied by the concentration of 1-nitropyrene in air particulate (expressed in ng/m^3 air sampled), the *'calculated'* mutagenicity (in revertants/m^3) can be compared to the number of revertants/m^3 determined for the organic extract of air particulate in which the 1-nitropyrene was quantified. One can make the assumption that direct-acting mutagenic activity for individual chemicals in a mixture is additive. Thus, 1-nitropyrene can be reported as being responsible for a certain percentage of the mutagenic activity of the organic extract. This relationship between *'calculated'* and experimentally determined mutagenicity is not well characterized in the literature (85-87). Bacterial tests for mutagenicity of Canadian urban air particulate extracts have not

appeared in the literature to date, except for two reports from this group submitted to the Ontario Ministry of Environment and Energy (50,51).

Strain TA98 has been used in bioassays of air particulate to determine the direct contribution to mutagenic potency of combustion emissions from nitroPAH, including nitropyrenes and nitrofluoranthenes (85). On the other hand, the atmospheric transformation product 2-nitrofluoranthene (which is not found in combustion emissions) was responsible for up to 8% of the mutagenic potency (determined using strain TA98) of extracts of air particulate collected during air pollution episodes in Los Angeles (85). This was among the first reports to link the mutagenicity of air particulate extracts to meteorological conditions favoring the production of atmospheric transformation products. The correlation between PAC content, the mutagenicity of air particulate extracts and the relationship to atmospheric conditions has yet to be explored in any city over a prolonged sampling time or over a range of atmospheric conditions. Organic extracts of air particulate have also been reported to cause base-pair substitution mutations, which are detected with *S. typhimurium* strain TA100 +S9 (90). The high mutagenic potencies of unfunctionalized PAH such as benzo[a]pyrene and indeno(1,2,3-cd)pyrene were believed to be responsible for this activity (91,92).

The second type of enhancement for detection of chemical mutagens relies on modification of the metabolic activity of the bacterium. Bacterial metabolism of certain PAC depends on enzymes such as nitroreductases and O-acetyltransferases (93). For example, nitroPAH are readily converted into electrophilic (nitrenium ion) intermediates

by nitroreductases; these intermediates can react with DNA and form DNA adducts (84). Dinitropyrenes, on the other hand, also need O-acetyltransferases to complete their activation and lead to DNA adduct formation. Extra copies of the genes coding for nitroreductase or O-acetyltransferase have been introduced into the genome of *S. typhimurium* TA98, yielding strains YG1021 and YG1024, respectively (93). When the mutagenicity of 1-nitropyrene was evaluated in strains YG1021 (-S9) and YG1024 (-S9), the increase in sensitivity over strain TA98 was determined to be 24-fold and 7-fold, respectively (93). However, the response to 1,8-dinitropyrene was shown to have increased by 1.1-fold and 30-fold, respectively.

The increased sensitivity of these *S. typhimurium* strains led our research group to conclude that these strains might be sensitive enough for the determination of mutagenic potencies of extracts prepared from single 24-hour air particulate collections. Strain YG1024 (-S9) had already been used to determine the mutagenic potencies of air particulate extracts (94) but strain YG1021 had not been used. The sensitivity of strain TA100 had also been enhanced (93,95) through the introduction of extra copies of nitroreductase and O-acetyltransferase to give strains YG1026 and YG1029, respectively, which had been used to assay air particulate in only one report (94).

I.10 The Combination of Analytical Chemistry and Bioassays: Bioassay-Directed Fractionation

Bioassay-directed fractionation is the coupling of chromatographic separations with bioassays in order to associate a specific compound or class of compounds in a complex mixture with a biological indicator such as mutation. This approach has been successfully exploited in the analysis of a variety of complex mixtures extracted from environmental matrices (79).

In any bioassay-directed fractionation method, the purpose of the chromatographic separation is to isolate the biologically active compounds from interferences to quantitation and bioassay in as few steps as possible. Chemical interferences to the identification and isolation of analytes include aliphatic compounds, while interferences to the biological assay (such as the *S. typhimurium* assay for mutagenicity) include toxins and antimutagens.

In order to decrease the chemical complexity of isolated chromatographic fractions, the number of chromatographic steps usually increases. The ideal separation system produces individual compounds and/or isomers, which can be identified through the techniques of analytical chemistry. Successive fractionation steps in a bioassay-directed fractionation scheme should exploit a different mechanism of separation or stationary chromatographic phase to increase the probability of separation of the analytes of interest from other compounds. For example, open-column chromatography might be followed by normal-phase and/or reversed-phase high performance liquid chromatography and then by

high resolution gas chromatography.

Bioassay-directed fractionation is especially useful in the isolation and quantitation of trace components present in complex mixtures; a table listing the successes and failures of bioassay-directed fractionation is given in the Appendix. Compounds of interest such as nitroPAH occur in air at the pg/m^3 level, at a concentration often three orders of magnitude lower than PAH (ng/m^3).

Methods using bioassay-directed fractionation of environmental samples have been only partly successful [see Alfheim *et al.* (6), Schuetzle *et al.* (79) and the Appendix of this thesis for critical reviews]. A methodology developed by our research group, originally reported by McCalla *et al.* (37) and later improved by Marvin *et al.* (96) has been successfully applied to a wide variety of complex samples including steel foundry dust (37), harbour sediments (38,49,96,97) and even Zebra mussel tissue (39). The method described by Marvin *et al.* (96) used a solvent extraction followed by the preparation of a non-polar aromatic fraction using alumina chromatography and removal of residual aliphatics by Sephadex LH20 chromatography. A portion of the crudely fractionated material was then further separated by NPLC into PAH and nitroPAH fractions. A careful choice of solvent gradient in the NPLC method can separate the PAH by molecular mass. Reversed-phase HPLC and/or GC-MS have been used to quantify the PAC in these fractions. TA100(+S9) or similar strains have been used to detect PAH and TA98(-S9) and related strains have been used to detect combustion emissions.

I.11 Hamilton, Ontario and Previous Analyses of Hamilton Air Particulate

Hamilton, a city of approximately 300,000 inhabitants, is the site of Canada's two largest steel mills and many other industries and is thus the most heavily industrialized city in Canada. In 1980, PAH concentrations in Hamilton air were reported to be as much as ten-fold greater than PAH levels determined in the air of other Canadian cities (98,99). Coke oven emissions from the steel mills, heavy vehicular traffic and geographical features contribute to the high PAH values (98,99). The urban area of Hamilton and the heavy industry its population supports are located in an area surrounded by a 300 foot escarpment. This geological feature tends to trap pollutants, particularly during certain meteorological conditions such as thermal inversions which occur during the spring and summer months (100).

Ambient air particulate samples contain PAH and PAH derivative classes (1). PAH arise from all manner of combustion processes. Some crude source apportionment may be possible by analysis for thiaPAH, which are products of coal combustion and coking processes, as well as diesel engines (14). Oxygenated PAH derivatives (oxyPAC) which include ketones and quinones are produced by combustion sources and by atmospheric transformation. Some nitroPAH are products of atmospheric transformation while other nitroPAH are found in diesel engine emissions. The methodologies at hand may be utilized for the quantitation of some of these compounds in Hamilton air particulate, for purposes of source apportionment and to show the relative contributions of various

sources.

The high particulate concentrations observed in Hamilton (191) have been related to the incidence of asthma in this city (192). Another measure of the biological effect of respirable air particulate is the determination of mutagenic activity. Hamilton's atmospheric conditions are the most studied in Canada (105-108), hence an examination of the relationship between atmospheric conditions, PAC concentrations and particulate extract mutagenicity in this city is a logical choice.

I.12 Research Objectives

PAC adsorbed to respirable air particulate are products of combustion processes and atmospheric transformation. The PAC can be isolated, then identified and quantified by analytical chemical means, while biological assays may be used to determine their genotoxic potential. It is the objective of this thesis to bring together both biological and chemical methods in order to characterize PAC arising from both atmospheric and combustion sources in an urban/industrial area of southern Ontario.

Combined biological and chemical methods to characterize PAC in air particulate extracts can be used to identify new mutagens through the process of bioassay-directed fractionation. Combined chemical separation techniques and biological methods can apportion the mutagenic activity of a given air particulate extract to a specific compound, group of compounds or compound class in a complex mixture. Bioassay-directed fractionation and the apportionment of mutagenicity to specific compounds would not be possible through either biological assays or quantitation of PAC alone.

Most investigations of air particulate PAC concentrations and/or air particulate extract mutagenicity have been conducted over short time periods of several days or weeks, usually during episodes of severe air pollution (1, 12, 26). However, an extended sampling period of several months would allow a more complete picture of air particulate PAC concentrations, mutagenic activity and the correlation between these two variables, since a long sampling timeframe would include periods of low and intermediate as well as high air pollution. Thus, **an examination of the relationship between mutagenic activity and PAC concentrations in air particulate extracts over an extended sampling period is an objective of this thesis.**

The atmospheric transformation of PAH with little or no mutagenic activity [such as fluoranthene and pyrene (225)] to mutagenic PAC [such as 2-nitrofluoranthene and 2-nitropyrene] demonstrates the importance of atmospheric gases to the determination of both air particulate PAC concentration and extract mutagenicity. 2-Nitrofluoranthene and 2-nitropyrene would not be produced in the absence of nitrogen dioxide and hydroxyl radicals arising from the degradation of ambient ozone. The monitoring of atmospheric transformation product concentration in air particulate is time- and labour-intensive and is therefore not routinely carried out. The concentrations of atmospheric gases responsible for atmospheric transformation are continually monitored by a network of stations throughout Ontario. If the concentrations of certain atmospheric gases were found to correlate well with the concentration of certain atmospheric transformation products, these gases could be used as surrogates to estimate the concentration of

atmospheric transformation products in air particulate. An extended sampling period of several months would again give the most information on a range of atmospheric conditions. Thus, **an examination of the relationship between PAC concentrations in air particulate and continuously monitored air pollutants over an extended sampling period is an objective of this thesis.** This objective will be reached by quantifying PAC in air particulate extracts using GC-MS and comparing the results to air pollutant concentrations monitored by the MOEE.

Industry has been demonstrated to have a significant impact on air quality in Hamilton (100,103,105). PAC concentrations and the genotoxic potential of PAC are two ways to evaluate the contribution of industrial emissions on general urban air quality. However, Hamilton's industrial area is located within its urban area and PAC arising from industrial emissions are therefore difficult to distinguish from PAC arising from urban activities. To compare air particulate extract mutagenicity and PAC concentrations in a primarily industrial area to those observed in a primarily urban area, a sampling site remote from Hamilton is required. A sampling site at the side of a highway in an area containing only light industry and homes would therefore make a suitable comparison to a site close to Hamilton's industry. Thus, **one objective of the thesis will be to determine if there is a relationship between industrial emissions and urban emissions, through the analysis of [a] PAC concentrations collected at both types of sites and [b] the mutagenic activity arising from the PAC.**

Most studies of PAC and their genotoxic potential in both urban and industrial areas have centered on the sixteen PAH described as 'priority pollutants' by the U.S.

Environmental Protection Agency (1). Fewer studies have attempted to gauge the concentrations and genotoxic potential of PAC derivatives. PAH can account for less than half of the mutagenic activity observed in most samples of air particulate extract (1). Clearly, all of the mutagens occuring in air particulate have not yet been identified; previously undescribed (or 'new') mutagens such as nitrodibenzopyranones (25) have only recently been isolated through bioassay-directed fractionation. Attempts to identify new mutagens could conceivably result in the discovery of compounds which could account for a significant amount of the mutagenic activity of air particulate collected. Investigations into bioassay-directed fractionation could also lead to improved separation of chromatographic fractions prepared from air particulate extracts, thereby making PAC quantitation and bioassay more facile. Therefore **the identification and quantitation of new mutagens in air particulate extracts through bioassay-directed fractionation is an objective of this thesis.**

Bioassay-directed fractionation utilizes bioassays such as the *S. typhimurium* assay for mutagenicity. Typically TA98-type strains are used to detect direct-acting mutagens such as nitroPAH, while TA100-type strains are used to detect PAH. However, more recently developed strains of *S. typhimurium* have not been extensively tested on air particulate extracts. If these new strains (e.g., YG1021 and YG1024) were determined to be more sensitive than the traditional TA98-type strain, less air particulate extract would be required for bioassay and more extract could be used to identify new mutagens. Thus, **it is an objective of this thesis to compare and contrast the sensitivity and utility of a variety of *S. typhimurium* strains in the bioassay of air particulate extracts.**

II. COLLECTION OF AIR PARTICULATE & ATMOSPHERIC DATA; SAMPLE SELECTION

II.1 Overview

This chapter will describe the collection of air particulate and atmospheric data at two different sampling sites in Hamilton. A total of 330 filters were collected over a 13-month period at the two sites. Atmospheric data from the entire sampling period was then tabulated in order to look for any trends or patterns. Sixty-eight filters (consisting of 64 filters from one site plus 4 filters from the second site) were selected for extraction based on the atmospheric conditions on the day of particulate collection. Filters were selected in order to obtain data for conditions which spanned the full range of all atmospheric parameters, from very clear days to thermal inversion events.

II.2 Air Particulate Collection

A large number of air particulate samples were collected over a 13-month period in Hamilton from May, 1990 until June, 1991. Respirable air particulate was collected using PM-10 high volume air samplers, operating at a flow rate of 40 ft^3/min (1630 m^3/24 h), from midnight to midnight. Sampling was carried out every second day at two sites operated by the Ontario Ministry of Environment and Energy (MOEE) in Hamilton, except for a few days such as holiday periods or in the event of instrument breakdown or maintenance.

We are grateful to the MOEE for allowing us to set up our samplers on the roofs of their buildings and for allowing us 24-hour access to these sampling sites. Both of the

sampling locations were equipped with air quality monitoring equipment that recorded hourly readings of the concentrations of a number of gaseous pollutants.

The downtown Hamilton sampling site (site A) was located at the corner of Kelly and Elgin Streets, just east of downtown Hamilton and *ca.* 2-5 km southwest of Hamilton's heavy industry. The West Hamilton sampling site (site B, in Westdale) was located near Main Street West and the on-ramp to Highway 403, *ca.* 6-8 km west of the industries. The location of both sites is shown on the map (Figure 8).

Samples of suspended air particulate were collected on Teflon-coated glass fibre filters. One-hundred and sixty-five filters were collected at each site to give a total of 330 filters for the 1990-1991 sampling period. Once filters had been collected, they were weighed, sealed in water-proof bags and stored at -20°C for later use. Selection of filters for extraction and analysis began with an evaluation of air particulate loading and particulate concentration over time.

The average mass of particulate collected at the downtown Hamilton site was 59.4 mg (± 36.1 mg S.D.), while the average mass of particulate collected at the West Hamilton site was 54.4 mg (± 39.4 mg S.D.). These amounts can also be reported as respirable particulate concentrations by expressing particulate mass in micrograms and dividing by the volume of air sampled (1630 m^3). The average respirable particulate concentration at the downtown Hamilton site was 36.4 $\mu g/m^3$ (± 22.1 $\mu g/m^3$ S.D.), while the average respirable particulate concentration collected at the West Hamilton site was 33.4 $\mu g/m^3$ (± 24.1 $\mu g/m^3$ S.D.). There was no significant difference in average particulate

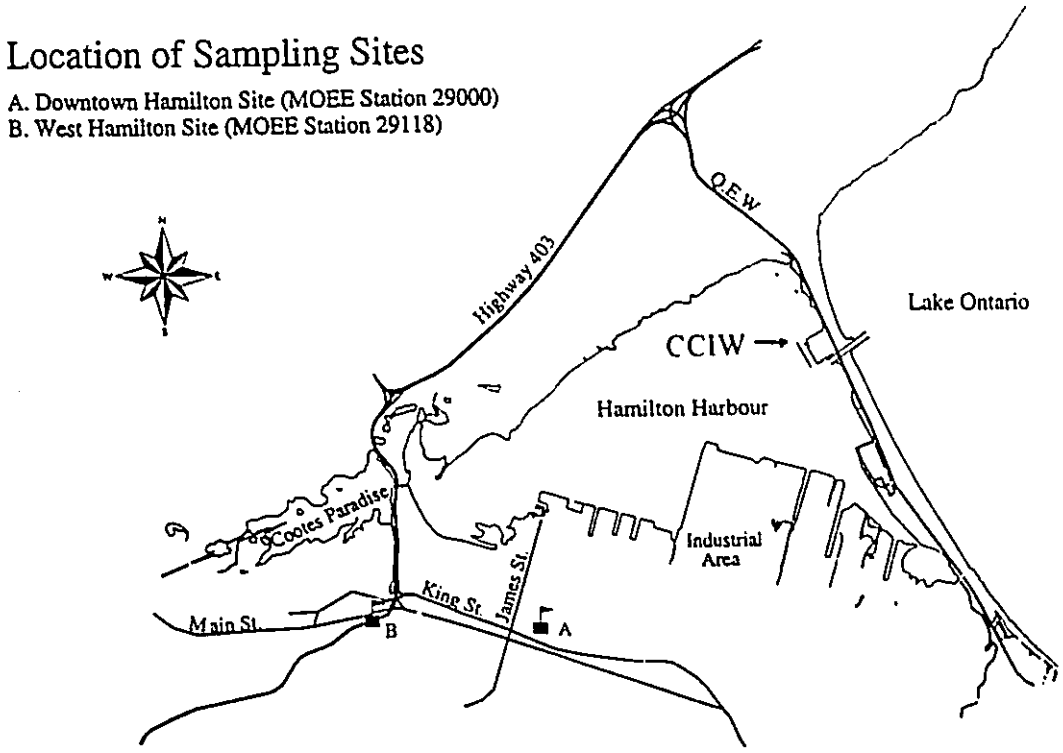


Figure 8. Map of Hamilton showing sites for air particulate collection.

mass or concentration between the two sites over the sampling period.

Figure 9 shows how particulate concentrations varied over the sampling period. Particulate concentrations at the downtown Hamilton station exceeded 100 $\mu g/m^3$ on four occasions (Figure 9A) while particulate concentrations only approached 100 $\mu g/m^3$ once at the West Hamilton site (Figure 9B). A particulate concentration of 100 $\mu g/m^3$ or greater is an internationally recognized definition of severely polluted air and is of concern to human health (102). Particulate concentrations were highest in the spring of both 1990 and 1991, but were generally low in mid-winter. The occasionally very high particulate concentration at the downtown Hamilton site is not reflected in the averages for the entire 13-month sampling period, which are virtually identical. Since sample loadings were greater at the downtown Hamilton site and since air particulate samples had been collected at this site for many years (50,51,100), it was decided to select filters collected only at the downtown Hamilton site. This decision cut the number of filters to be selected for extraction from 330 to 165. Further selection of filters depended on the analysis of atmospheric conditions on the day of collection at the downtown Hamilton site.

II.3 Atmospheric Data Collection

The MOEE provided hourly data for all atmospheric parameters collected at these two sites for 1990 and 1991. These included values for the concentrations of a number of gases including SO_2, CH_4, NO_2, NO, O_3 and for coefficient of haze, a dimensionless unit which measures light absorbance by respirable particulate (103). Non-methane hydrocarbon data were only monitored at the downtown Hamilton station.

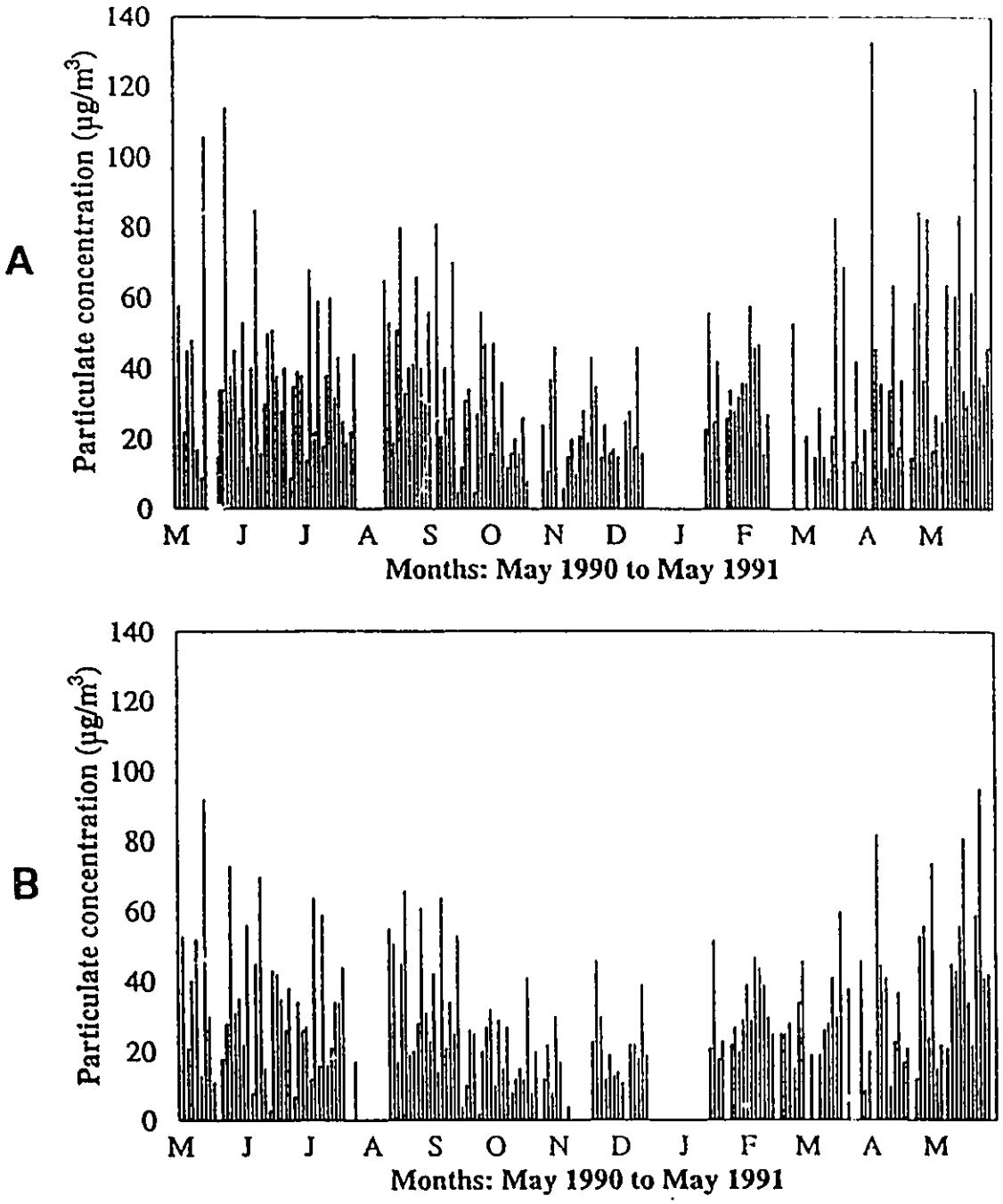


Figure 9. Respirable particulate concentration versus time, as determined at the (A) downtown Hamilton and (B) West Hamilton sites, during the 1990-1991 sampling season.

Hourly data for wind speed, wind direction and air temperature were also supplied by the MOEE; these data were collected at two heights from the tower at the Hamilton Sewage Treatment Plant. Relative humidity data was supplied by Environment Canada from data collected at Hamilton airport, Mt. Hope.

Hourly data for the sampling dates were processed and 24-hour averages and standard deviations were calculated for all parameters. Next, monthly averages and standard deviations were calculated for sulfur dioxide, nitrogen dioxide, nitric oxide, ozone and non-methane hydrocarbon concentrations, wind speed, wind direction, relative humidity and air temperature. The monthly averages for these parameters at the Kelly site (downtown Hamilton, site A) are summarized in Figures 10 though 12.

Monthly averages were calculated in order to look for any trends that might assist in the selection of filters for extraction. With the exceptions of ozone (Figure 10C) and temperature (Figure 12), no major differences in the atmospheric parameters were noted during the course of the sampling period, due to the large overlap of the standard deviations. Overlap was greatest for the monthly average coefficient of haze, total reduced sulfur, methane and carbon monoxide.

For example, the monthly average coefficient of haze for 1990-1991 ranged between 3 and 5 units, with a standard deviation ranging from 2 to 4 units. Monthly average total reduced sulfur ranged between 0 and 1 ppm, with a standard deviation ranging between 1 and 2 ppm. Thus, monthly averages could not be used to make a selection of filters for extraction.

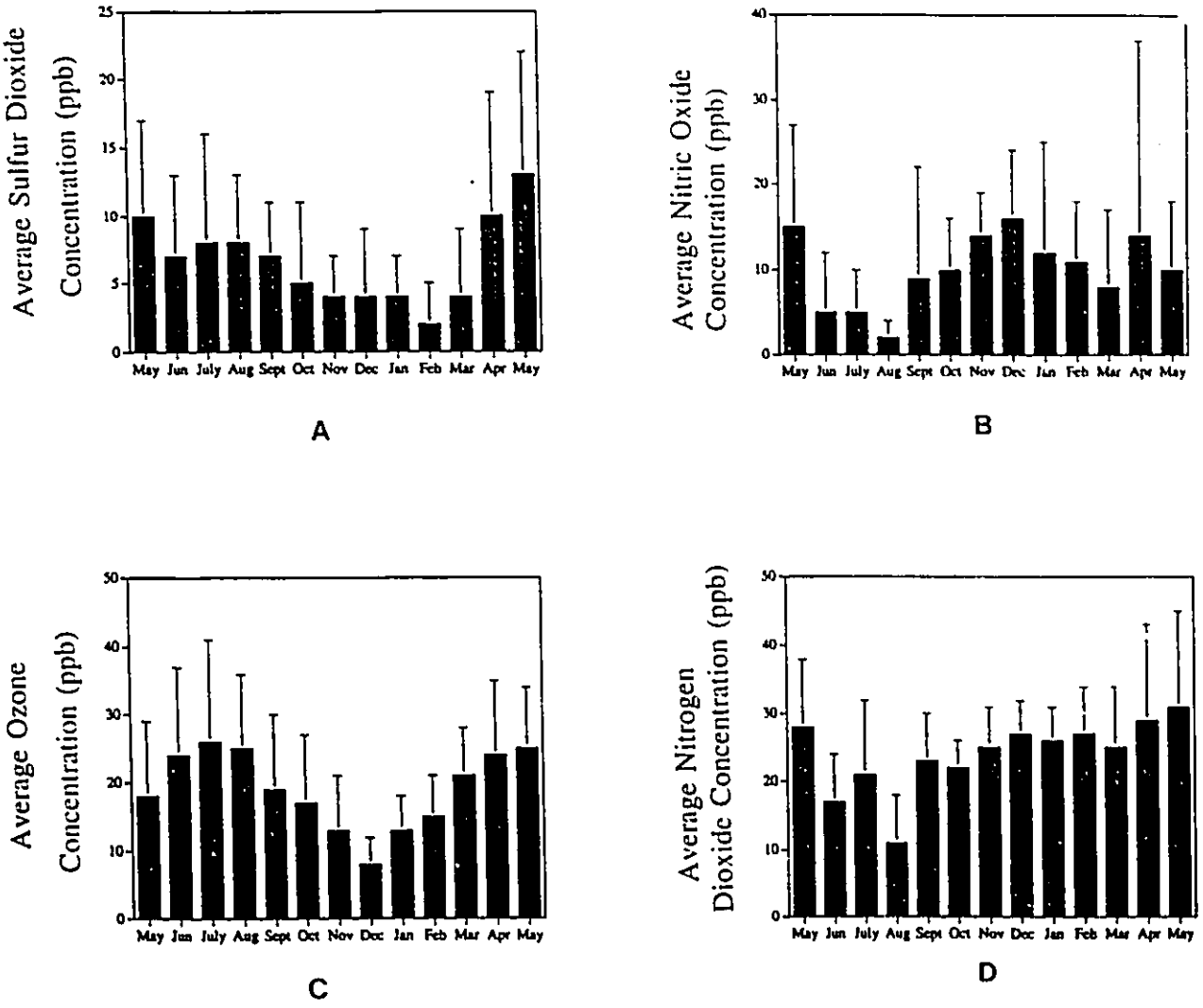


Figure 10. Monthly average concentration versus month of sampling (May 1990 to May 1991). Solid bars represent average and error bars represent standard deviation, for (A) sulfur dioxide, (B) nitric oxide, (C) ozone and (D) nitrogen dioxide.

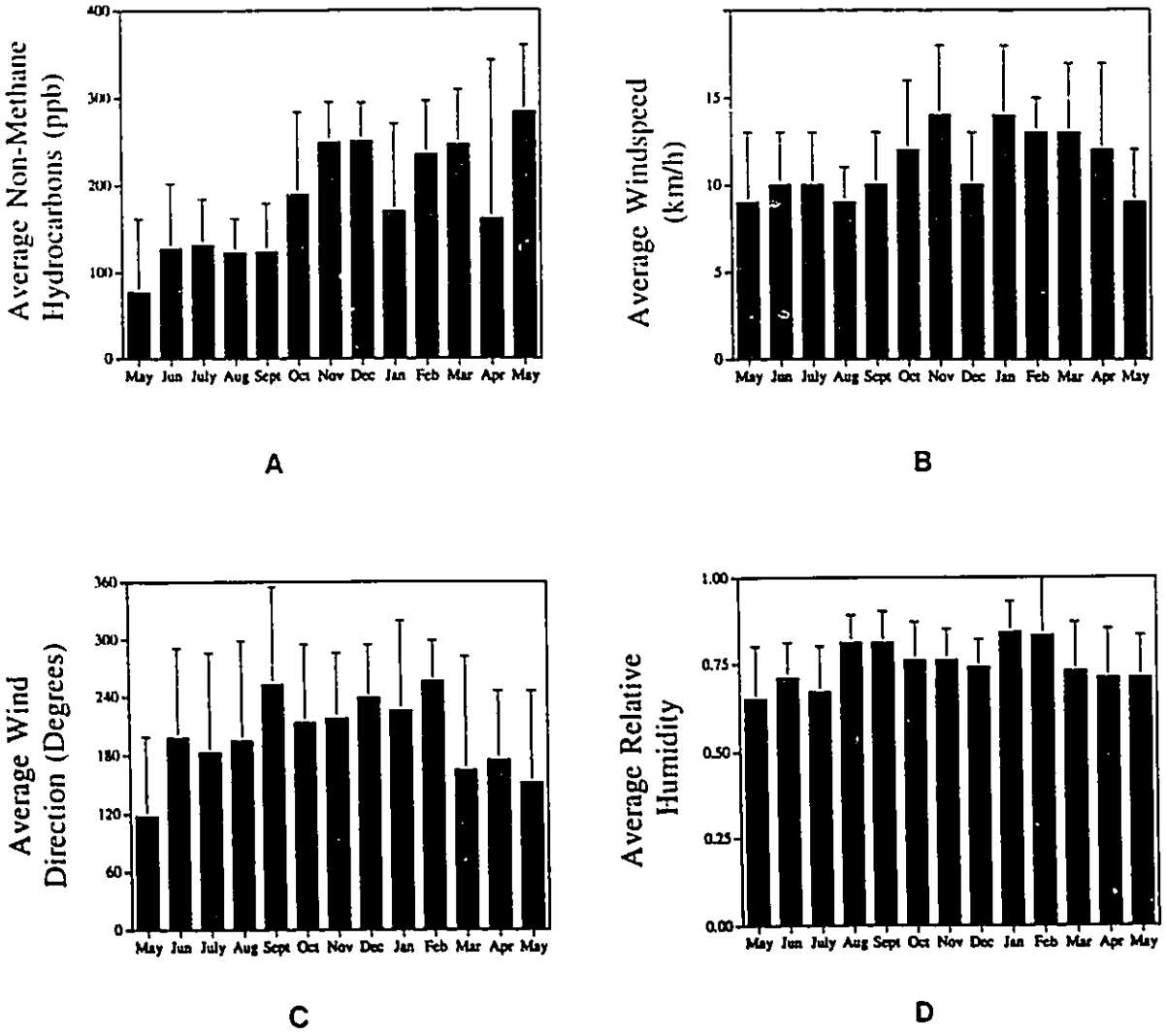


Figure 11. Monthly average versus month of sampling (May 1990 to May 1991). Solid bars represent average and error bars represent standard deviation, for (A) non-methane hydrocarbon concentration, (B) windspeed, (C) wind direction and (D) relative humidity.

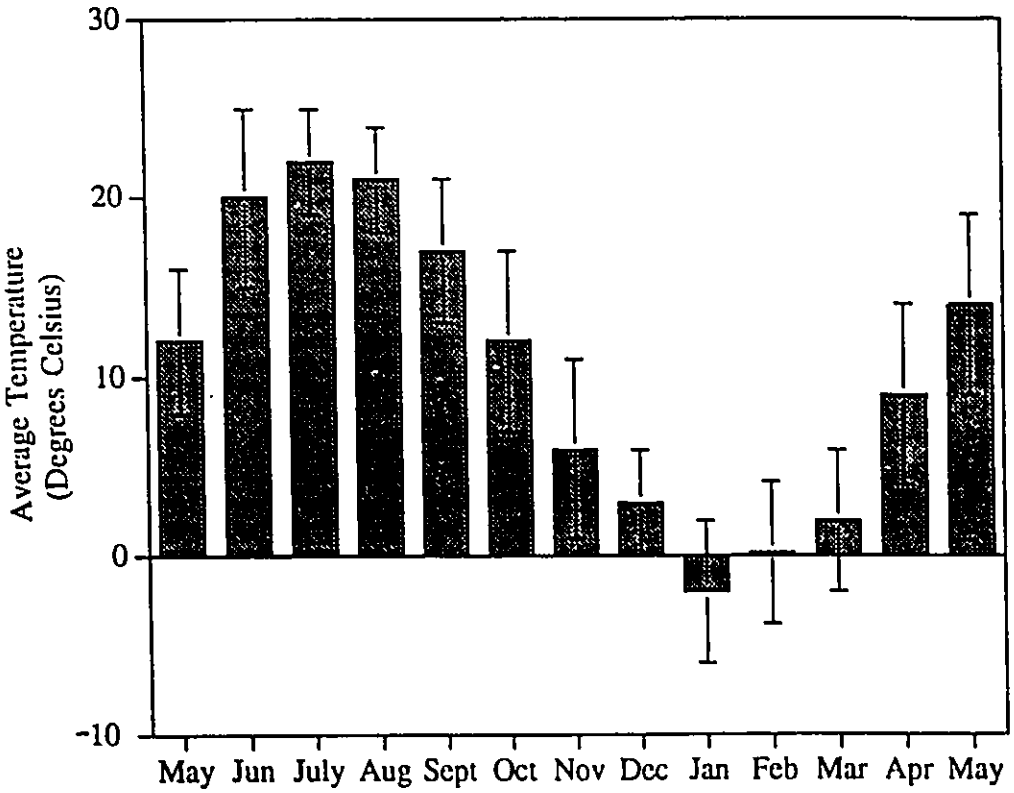


Figure 12. Monthly average air temperature versus month of sampling (May 1990 to May 1991). Solid bars represent monthly average and error bars represent standard deviation.

Monthly averages did reveal a springtime peak in sulfur dioxide, nitrogen dioxide, nitric oxide and non-methane hydrocarbon concentrations, however (see Figures 10A, 10B, 10D and 11A). Respirable particulate concentrations were highest in the spring of 1990 and 1991 (Figure 9). These springtime episodes of increased atmospheric pollution corresponded to thermal inversion events, which occur when a bubble of cold air over a city is trapped by a large blanket of warm air above it. Pollutants build up in this bubble and cannot be swept away by winds until the inversion collapses (104).

Thermal inversions are often seen in cities such as Los Angeles (1). Thermal inversions in Hamilton can occur due to the 'lake breeze effect.' During the day (especially during warm spring days in April, May and early June) air warmed over land surrounding Lake Ontario rises vertically; this draws a lake breeze of cool air off the lake, which can become trapped over the city underneath the warm air mass. The situation is further aggravated by bright sunlight, which generates photochemical smog resulting in a brown haze over the city. A polluted air mass over Hamilton is also trapped by the 300 foot Niagara Escarpment.

Thermal inversions have been documented in Hamilton for many years. The number of thermal inversions observed during the 1990-1991 sampling season is typical of the conditions seen in Hamilton in previous years (100,105-107). Average monthly temperatures were not altered significantly from their seasonal values since thermal inversions only lasted one or two days.

In summary, monthly average pollutant concentrations and monthly average atmospheric parameters could not in themselves assist in making a selection of filters for extraction. Only filters collected at the downtown sampling station would be selected, owing to high particulate concentrations and previous published work on particulate collected at this site.

II.4 Selection of Filters for Extraction

Filters were selected such that the atmospheric conditions on the days of collection covered the entire range of values observed, including maximal, minimal and in-between values for all parameters monitored. Filters collected on days that were declared 'pollution events' were of particular interest, since it was felt that such filters should afford larger amounts of extract for extensive chemical and biological analyses.

'Pollution events' are defined by the MOEE as days when the Air Quality Index (or AQI) exceeds 32 units. These days are typified by high SO_2 concentrations and are caused by a warm southerly air mass creating an inversion over Hamilton. 'Pollution events' occured on May 15th, May 24-25th, June 8th, November 21st and December 21st, 1990, as well as April 4th, May 15-16th and May 23rd, 1991 (108). All of the filters that had been collected at the downtown site during these 'pollution events' were selected for extraction. No sample was collected on December 21st, 1990.

Many filters were available for days with low or average pollutant concentrations, but relatively few filters were available from days with high pollutant concentration. In

order to compensate for this distribution, a higher percentage of filters collected on more highly polluted days were selected while a lower percentage of filters collected on relatively unpolluted or moderately polluted days were selected for extraction. Thus, sixty-four of the 165 filters collected at the downtown sampling site were selected for extraction, chemical and biological analysis.

Four filters were selected from the West Hamilton sampling site in addition to the 64 filters selected from the downtown Hamilton sampling site. These four filters were chosen in order to investigate any differences between the two sites on these four days. The sampling dates of these filters corresponded to the dates of 4 filters already selected as part of the downtown Hamilton set.

Figures 13, 14 & 15 summarize and compare the atmospheric data for all filters (grey bars) and for the selected filters (black bars). At least two filters were selected from each month during the 13-month sampling campaign; Figure 16 shows the number of filters collected and selected from each month.

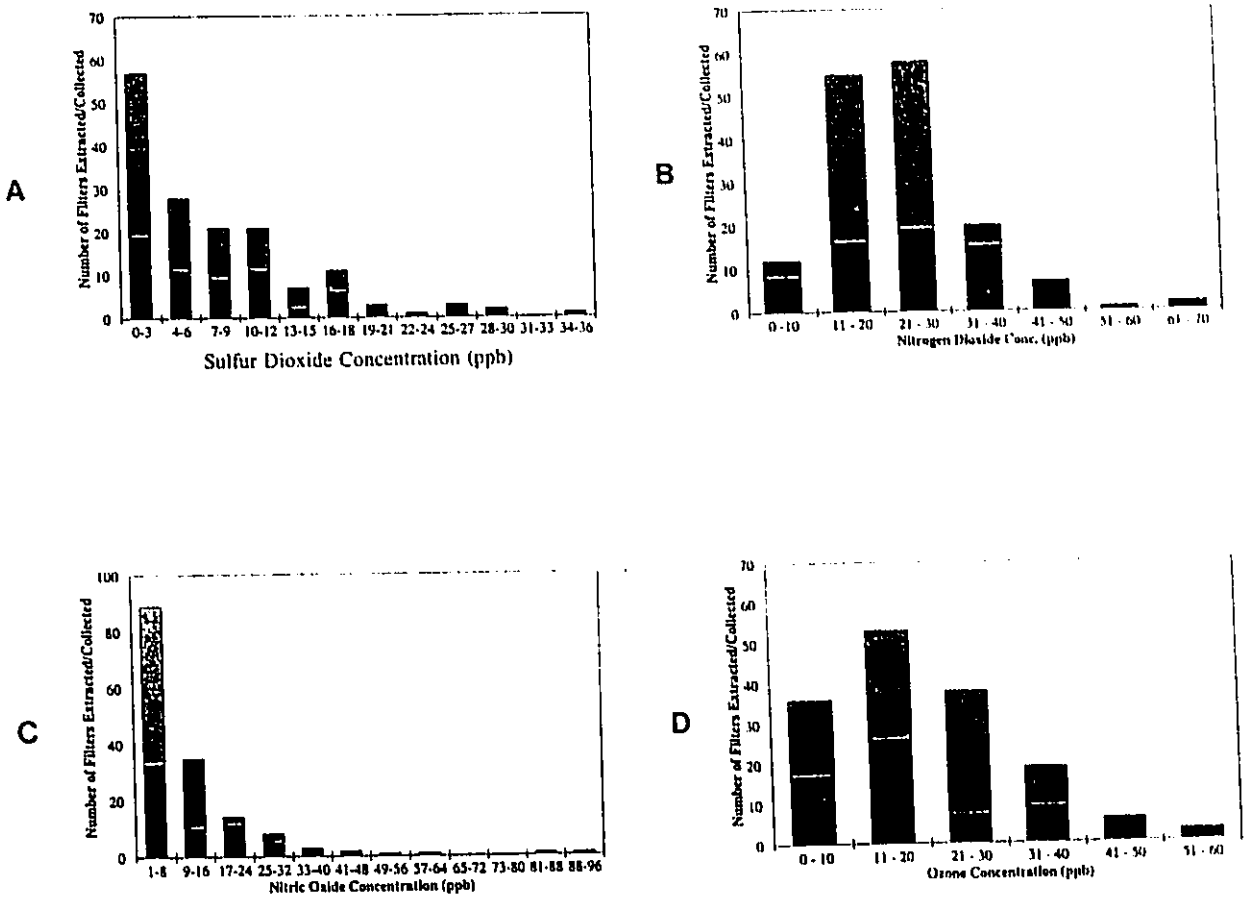


Figure 13. Number of filters collected (grey bars) versus number of filters extracted (black bars) for different concentrations of (A) sulfur dioxide, (B) nitrogen dioxide, (C) nitric oxide and (D)ozone.

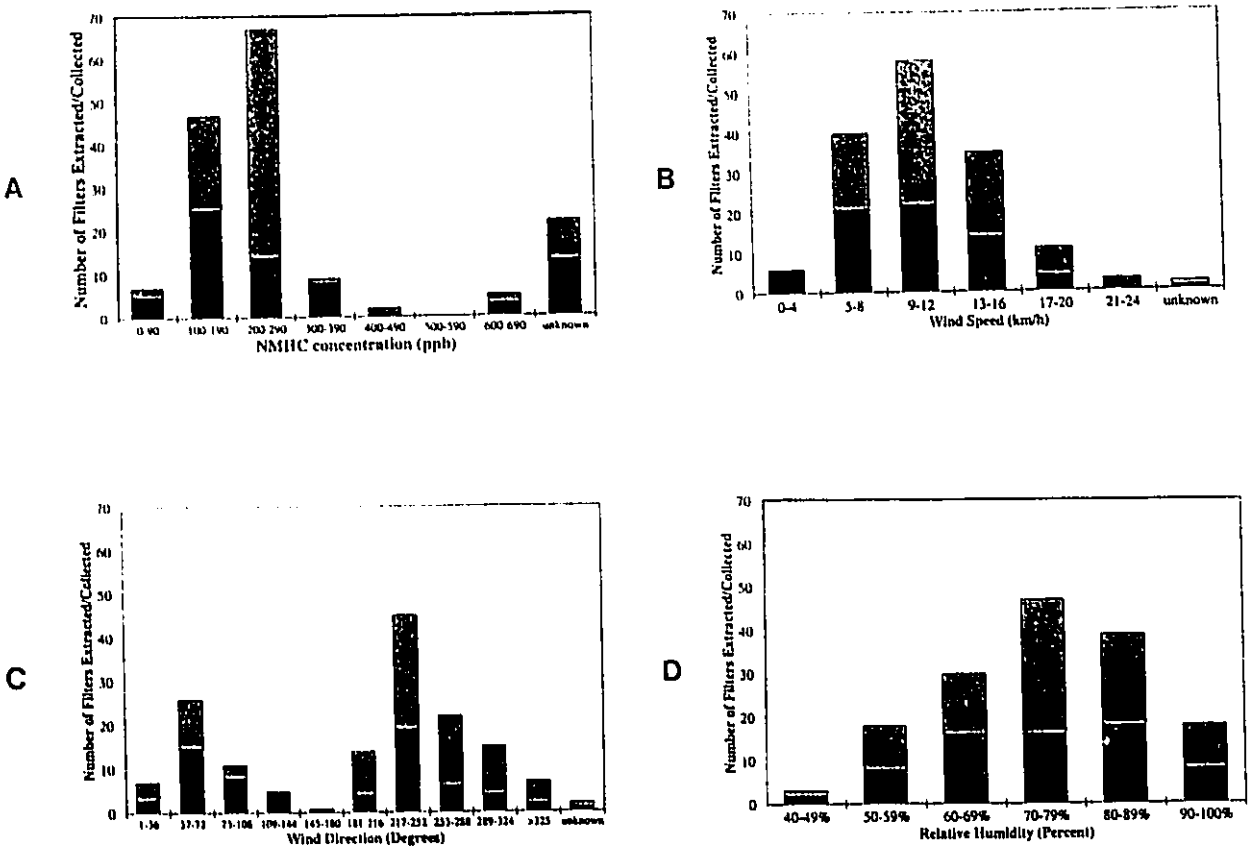
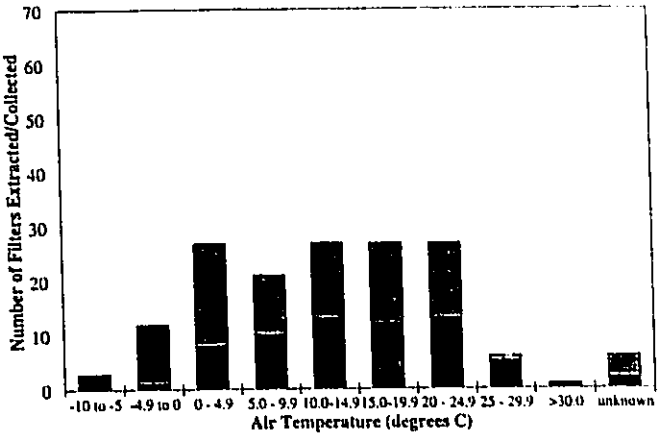


Figure 14. Number of filters collected (grey bars) versus number of filters extracted (black bars) for (A) different concentrations of non-methane hydrocarbons, (B) windspeed, (C) wind direction and (D) relative humidity.

A



B

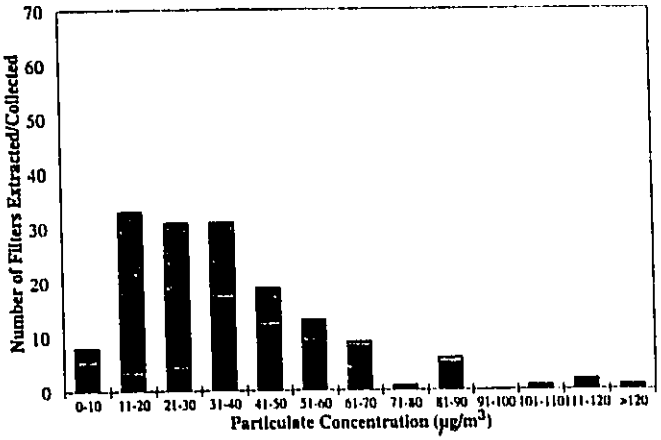


Figure 15. Number of filters collected (grey bars) versus number of filters extracted (black bars) for (A) different average air temperatures and (B) different respirable particulate concentrations.

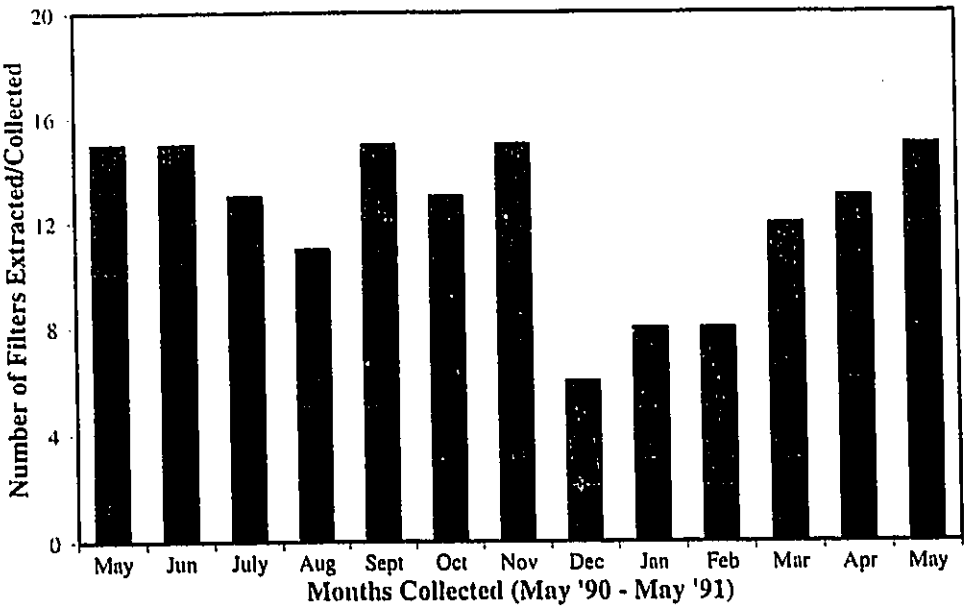


Figure 16. Number of filters collected collected (grey bars) versus number of filters extracted (black bars) for different months of the 1990-1991 sampling period.

III. METHODOLOGY

III.1 Overview

The purpose of this chapter is to introduce the various methods for extraction, chromatographic cleanup and bioassay to be used in this thesis and to show the validation of each method. Each step of the chemical and biological method for analysis of air particulate extracts, from extraction to PAC quantitation and the determination of mutagenicity, will be evaluated using either air particulate, chemical standards or standard reference materials.

III.2 Introduction

The protocol for extraction, cleanup and fractionation of air particulate as practised by this research group is shown in Figure 17. Air filters were initially extracted for 24 hours with dichloromethane using a Soxhlet apparatus; this was followed by a second extraction with methanol for 24 hours. The crude organic extracts from both extractions were combined, concentrated and applied to a neutral alumina column. Aliphatic material was removed from the column by elution with hexane. The non-polar PAC were obtained by sequential elution with benzene, then with dichloromethane:ethanol (99:1) to give fractions A2 and A3, which were combined to give fraction A23. Polar PAC were eluted from the alumina column by sequential elution with methanol, followed by methanol: water (80:20), to give fractions A4 and A5, which were combined to give fraction A45.

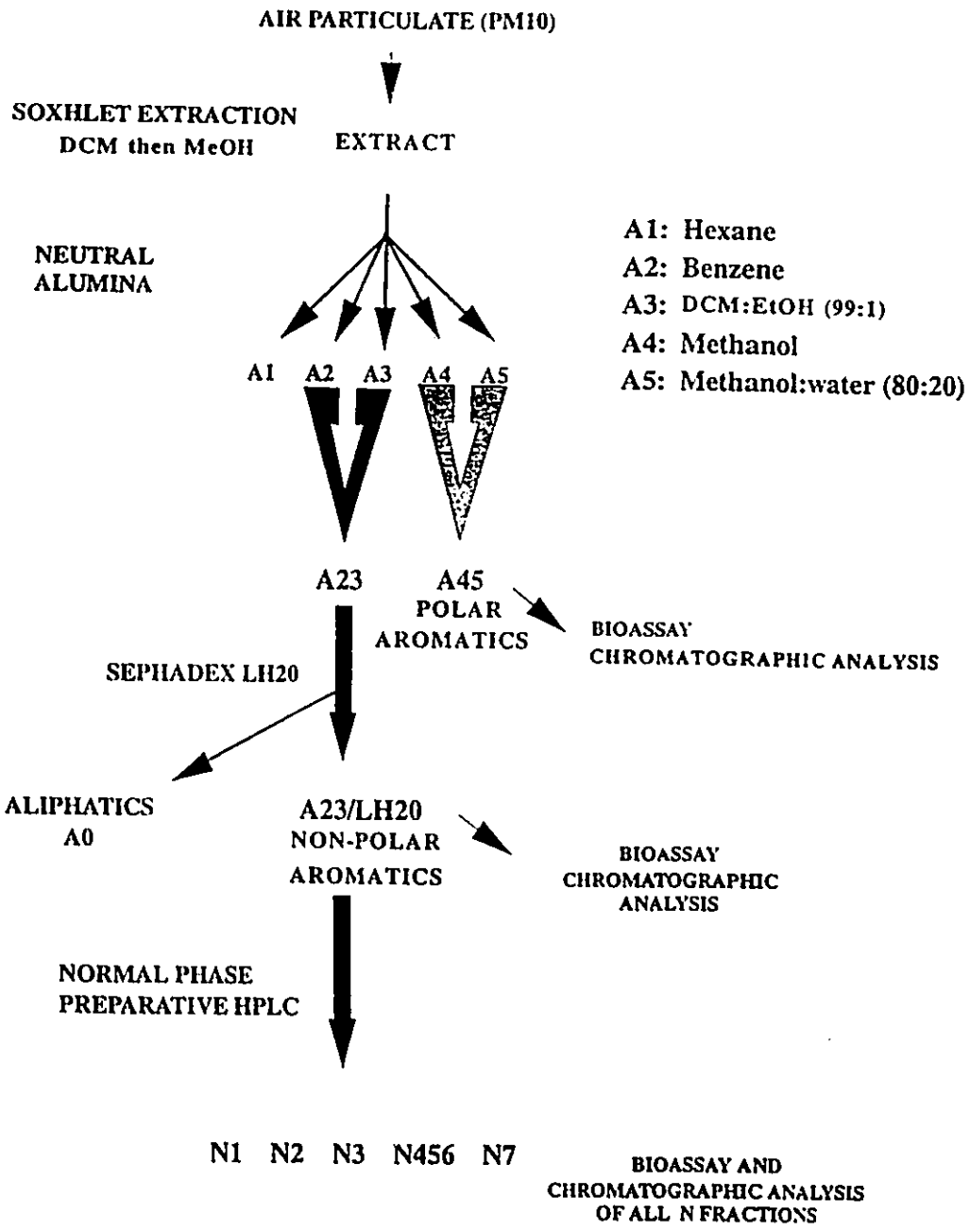


Figure 17. Schematic of sample cleanup and fractionation scheme. The procedure is decribed in detail in the experimental section.

The non-polar PAC fraction (A23) was chromatographed on a Sephadex LH20 column to remove any remaining aliphatic components. The resultant non-polar aromatic fraction (A23/LH20) was then used in bioassays or analyzed by GC-MS. Fractionation of this non-polar aromatic fraction by normal-phase preparative HPLC generated five fractions of increasing polarity. Fraction N1 contained PAH up to 278 amu; Fraction N2 contained higher mass PAH and mononitroPAH. Fraction N3 contained dinitroPAH as well as anthraquinone, while fraction N456 contained compounds such as PAH ketones, quinones and nitrodibenzopyranones. Fraction N7 contained polar PAC which were washed from the column by acetonitrile.

This fractionation scheme has been successfully applied to a wide variety of sample matrices, as was described in section I.10. Although this method for the extraction and cleanup of PAC from complex environmental matrices has been used for some time, extensive testing of the methodology had not been carried out. For this reason, each step of the fractionation scheme was re-evaluated, using chemical standards or standard reference materials.

III.3 Testing of Soxhlet Extraction Efficiency

Different research groups have used various solvents and solvent combinations for the extraction of PAC from air particulate; the time required for Soxhlet extractions has varied between 6 hours with a single solvent (33) to 48 hours with two solvents (65). A 24-hour period for Soxhlet extraction is most commonly used (6). The following

experiment was carried out in order to determine the time required to extract the PAC from air particulate using dichloromethane followed by methanol as extraction solvents.

Two air particulate filters collected in Hamilton were placed in a Soxhlet apparatus and extracted with dichloromethane at a rate of approximately 6 cycles per hour. The extraction solvent was replaced with fresh dichloromethane after one hour. The extraction was continued and the solvent changed after 5, 9, 19 and 24 hours of extraction. Following 24 hours of extraction with dichloromethane, the extraction was continued with methanol. Methanol extracts were collected after 12, 18, 24 and 27 hours of extraction. Each extract was analyzed by reversed phase HPLC using UV detection. The total area under all peaks measured at 254 nm (the traditional wavelength for PAH detection) was then determined.

The results of these experiments appear in Tables 2 and 3. The vast majority of material with UV-absorbance (such as PAC) was extracted within the first hour of dichloromethane extraction and within the first twelve hours of methanol extraction. This concurs with observations made in the extraction of diesel particulate (19) which showed that 99% of the organic extractable material was extracted within 8 hours but an additional 16 hours were required to extract the remaining 1% of organic extractable material. As a result of these experiments, the protocol chosen for extraction of Hamilton air particulate was sequential Soxhlet extraction with dichloromethane for 24 hours and with methanol for 24 hours.

Table 2.
UV Absorbance of Dichloromethane Extracts of Air Particulate Versus Extraction Time.

Duration of Extraction (hours)	*Integrated Area of All Peaks Detected at 254 nm in the Reversed Phase HPLC Analysis of the Extract**	*Percentage*	*Accumulated Percentage of Total Sample*
1	2250	92.7 %	52.8 %
5	90	3.7	54.9
9	37	1.5	55.8
19	44	1.8	56.8
24	7	0.3	57.0
	2428 units		

Table 3.
UV Absorbance of Methanol Extracts of Air Particulate Versus Extraction Time.

Duration of Extraction (hours)	*Integrated Area of All Peaks Detected at 254 nm in the Reversed Phase HPLC Analysis of the Extract**	*Percentage*	*Accumulated Percentage of Total Sample*
12	1780	97.1 %	98.7 %
18	33	1.8	99.5
24	17	0.9	99.9
27	4	0.2	100.0
	1834 units		

* arbitrary units of the HPLC's integration software

III.4 Evalution of Alumina Chromatography

Crude organic extracts of air particulate contain a vast array of organic compounds, including aliphatics, phenols, PAC and highly polar, water-soluble organic compounds such as simple organic acids (109). The distribution of various PAC and phenols in the chromatographic fractions from the alumina column was determined using a mixture of 12 compounds which were representative of some of the compound classes found in air particulate extracts. Fractions were collected according to the scheme shown at the top of Figure 17 and these fractions were analyzed by reversed-phase HPLC. The distribution of the standard compounds among the fractions eluted from the alumina column is shown in Table 4. Very small amounts of non-polar PAC eluted in fraction A1. An aza-aromatic, an amine and two carbonyl derivatives eluted in fraction A3 while three phenols eluted in fractions A4 and A5. Fractions A2 and A3 contained all of the non-polar PAC applied to the column. In the cleanup scheme shown in Figure 17, fractions A2 and A3 are combined and this experiment confirmed that fraction A23 does contain the non-polar aromatic compounds.

III.5 Testing of Sephadex LH20 Chromatography

Alumina chromatography of the crude extract of air particulate removed most of the aliphatics but some non-aromatic compounds and aliphatics are known to remain in fraction A23. Thus, a Sephadex LH20 column which removed aromatics by selectively adsorbing PAC was introduced into the separation scheme; this type of separation has

Table 4.
PAC Distribution (by Percent) in Various Alumina Fractions.

	Fraction				
Compound	*A1*	*A2*	*A3*	*A4*	*A5*
Dibenzofuran	<0.1	100.0	<0.1	<0.1	<0.1
Dibenzothiophene	0.4	99.6	<0.1	<0.1	<0.1
Pyrene	0.6	99.4	<0.1	<0.1	<0.1
1,5-Dinitronaphthalene	0.6	83.9	15.5	<0.1	<0.1
Dibenzo[a,i]carbazole	0.4	<0.1	99.6	<0.1	<0.1
3-Aminofluoranthene	0.4	<0.1	99.6	<0.1	<0.1
Xanthone	0.4	<0.1	99.6	<0.1	<0.1
Carbazole	0.5	<0.1	99.5	<0.1	<0.1
Anthraquinone	0.3	<0.1	99.7	<0.1	<0.1
3,5-Dimethylphenol	<0.1	<0.1	<0.1	100.0	<0.1
α-Naphthol	<0.1	<0.1	<0.1	69.4	30.6
p-Nitrophenol	<0.1	<0.1	<0.1	94.2	5.8

Method detection limit: 0.1% or 60 ng for most PAC

been used by other research groups (55, 112-114). These compounds need to be removed because some aliphatic hydrocarbons have molecular weights similar to those of ambient PAH and thus can complicate PAH quantitation in air particulate extracts (42, 110). In addition, some non-aromatic compounds may have biological effects which could interfere with the Ames assays (111).

An efficient separation of aliphatic hydrocarbons from PAH was achieved using a Sephadex LH20 column. A standard mixture of six aliphatic hydrocarbons (n-$C_{12}H_{26}$ to n-$C_{26}H_{54}$) and four PAH (naphthalene, anthracene, pyrene and benzo[a]pyrene) was prepared and the mixture was analyzed by GC-FID (Figure 18A). A solution of naphthalene which was applied to the Sephadex LH20 column acted as an indicator to show when aromatic compounds first eluted from the column. The eluate from the time of sample application up to the beginning of the naphthalene peak was called the aliphatic fraction, while all compounds eluting with or after naphthalene were called the aromatic fraction. The solvent volumes were reduced by evaporation under reduced pressure and their contents were analyzed. The GC-FID chromatograms of the aliphatic and aromatic fractions appear in Figures 18B and 18C, respectively. The aliphatic fraction (Figure 18B) contained only aliphatic hydrocarbons from the standard mixture while the aromatic fraction contained all the PAH along with trace levels of aliphatics (Table 5). The low recovery of naphthalene was probably due to evaporative losses during the removal of LH20 solvent.

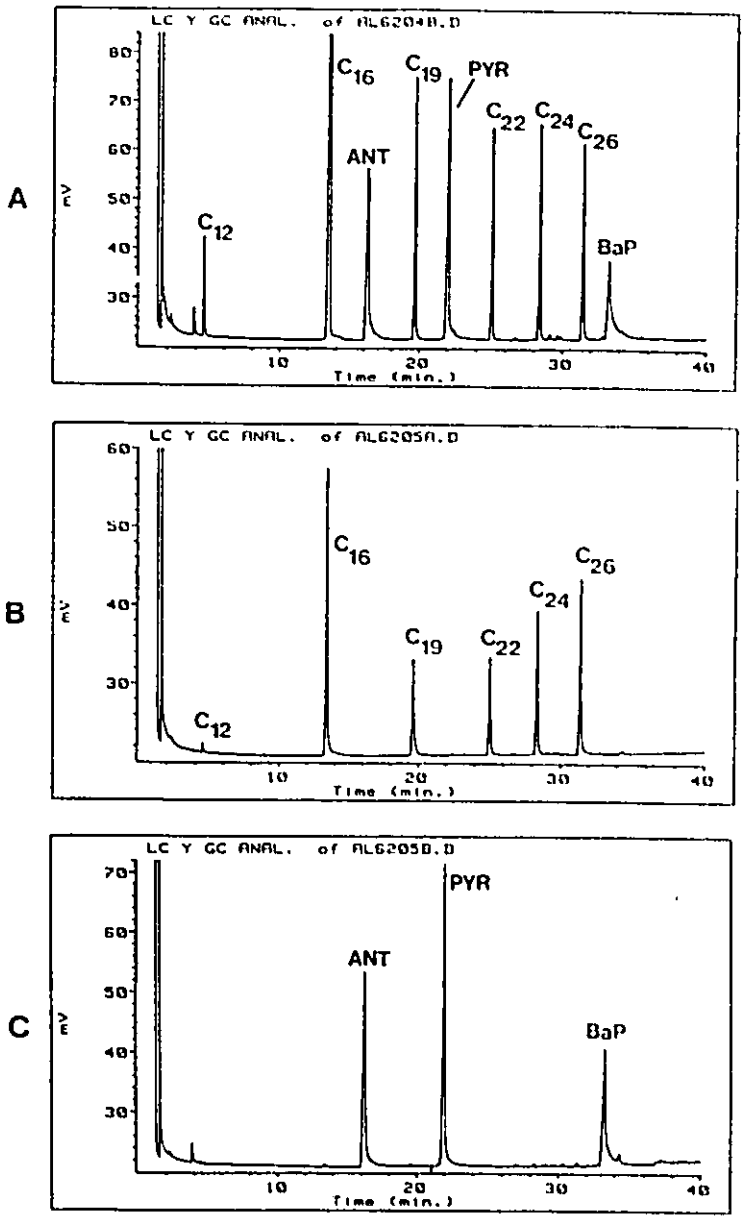


Figure 18. GC-FID chromatograms of (A) a mixture of aliphatic hydrocarbons and PAH, before separation; (B) the aliphatic fraction from the Sephadex LH20 column and (C) the aromatic fraction from the Sephadex LH20 column. Abbreviations for the various analytes are explained in Table 5.

Table 5.
Percent Recovery of Various Compounds in the Aromatic Fraction after Sephadex LH20 Chromatography.

Compound and Abbreviation	*Percent recovery**
Naphthalene (NAP)	77 %
n-Dodecane (C_{12})	2
n-Hexadecane (C_{16})	1
Anthracene (ANT)	100
n-Nonadecane (C_{19})	1
Pyrene (PYR)	100
n-Docosane (C_{22})	1
n-Tetracosane (C_{24})	2
n-Hexacosane (C_{26})	2
Benzo[a]pyrene (BaP)	100

*Normalized with respect to pyrene. Detection limit: 0.5%.

III.6 Method Validation Using a Standard Reference Material

Standard Reference Materials (SRMs) are valuable tools for analytical method validation. The concentrations of 'certified' analytes in these complex mixtures have been determined by a variety of documented analytical methods; analytes quantified by only one method are referred to as 'non-certified' (115,116). The U.S. National Institute of Standards and Technology (NIST) is the source for a variety of such standards.

Standard reference material SRM1650 which was derived from diesel exhaust particulate contains certified amounts of PAH and 1-nitropyrene. SRM1650 was selected to validate the analytical method used throughout this research since quantitation of both PAH and nitroPAH was routinely carried out for the air particulate samples collected in Hamilton. Briefly, the method validation consisted of the following steps which evaluated an extract of SRM1650, subjected to the chromatographic cleanup shown in Figure 17. (1) The mass of the crude organic extract was determined and compared to previously published results. (2) The crude organic extract was put through the cleanup procedure and separated by normal-phase HPLC into PAH and nitroPAH fractions. PAH and 1-nitropyrene were quantified by GC-MS and these concentrations were compared to certified values published by the NIST in order to determine the recovery of the certified analytes through the entire extraction and fractionation scheme.

III.6.1 The Determination of Mass of Crude Organic Extract

A sample of SRM1650 (172.3 mg) was extracted in a Soxhlet apparatus with dichloromethane for 24 hours followed by methanol extraction for 24 hours. The dichloromethane and methanol extracts were combined and an aliquot representing 1/30th of the total crude extract was withdrawn and applied carefully to a weighing planchette; the weighing planchette was dried in a desiccator over P_2O_5 and the mass was determined. A crude organic extract of 51.5 mg was obtained, which corresponds to 30% organic extractable material and compares well with the 32% organic extractables reported in previous studies using the same extraction protocol (31).

The crude extract was then subjected to chromatography with alumina and Sephadex LH20 to give a non-polar aromatic fraction, also known as fraction A23/LH20.

III.6.2 Normal-Phase Chromatography of the Non-Polar Aromatic Fraction

PAH occur in air particulate at concentrations typically ranging from 1 to 20 ng/m^3 (1,9,11,30,33) while nitroPAH are found at levels between 1 and 1000 pg/m^3 (12,117). Thus, PAH can be significant interferences in nitroPAH analyses. Normal phase HPLC methods have been used by our group to separate PAH from nitroPAH. The retention times for a selection of PAH, nitroPAH and other PAC standards were determined using normal-phase HPLC (see Table 6 and Figure 19A). This is an important step to determine the effectiveness and reproducibility of the chromatographic protocol.

Table 6.
Normal-Phase Retention Time Data for PAH and PAH Derivatives

Compound	*M.W.*	*Abbreviation*	*Average Retention time**	*Absolute S.D.**
Dibenzothiophene	184 amu	DBT	9.72 min.	0.07 min.
Anthracene	178	ANT	11.12	0.09
Fluoranthene	202	FLUR	13.71	0.13
Pyrene	202	PYR	15.61	0.13
Benzo[b]naphtho[2,3-d]-thiophene	234	BN23T	16.59	0.28
Chrysene	228	CHRY	19.57	0.09
Benzo[a]pyrene	252	BaP	21.45	0.09
Indeno(1,2,3-cd)pyrene	276	IcdP	24.25	0.13
1-Nitronaphthalene	173	1-NN	24.53	0.11
6-Nitrobenzo[a]pyrene	297	6-NBaP	30.65	0.17
2-Nitrofluoranthene	247	2-NFA	31.36	0.23
Benz[a]anthracene-7,12-dione	258	BaAD	38.78	0.64
9-Fluorenone	180	FONE	40.38	0.73
Anthraquinone	208	ANTQ	42.31	0.30
Carbazole	167	CBZ	43.49	0.11
Naphthacene-5,12-quinone	258	NAPQ	47.08	0.13
Benzanthrone	230	BZONE	52.35	0.20
Dibenzo[ai]carbazole	267	DBCBZ	56.16	0.05

*average and standard deviation calculated from three consecutive injections

Column: 5 µm Whatman analytical polyaminocyano using gradient A (section IX.12).

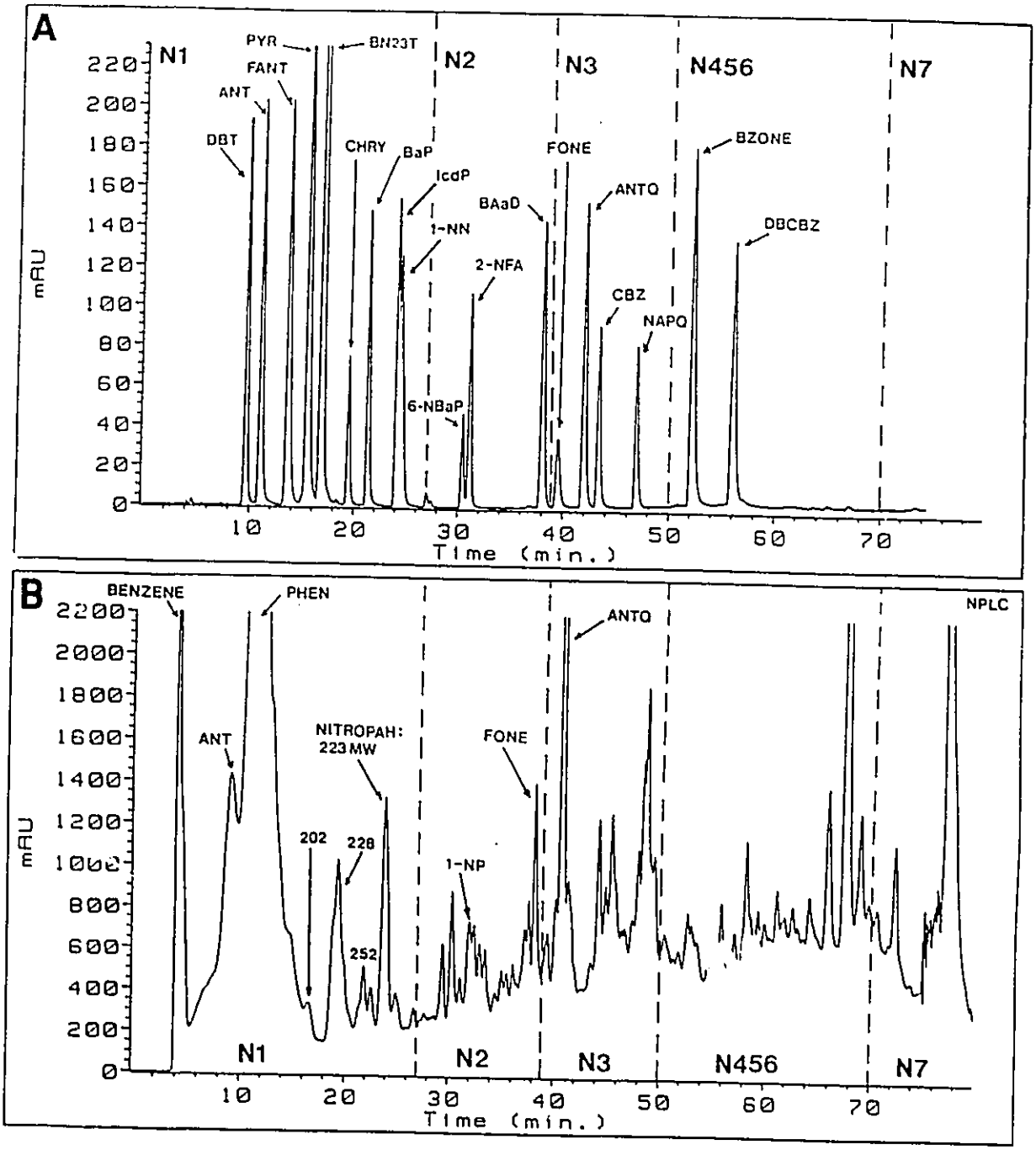


Figure 19. Normal-phase HPLC chromatogram of (A) various PAH, nitroPAH and polar PAC and (B) the non-polar aromatic fraction obtained from SRM1650, separated on a 5 µm Whatman polyaminocyano column. Dashed lines indicate zones of collection for PAH (N1), nitroPAH(N2) and other N fractions.

Collection times for the PAH and nitroPAH fractions (N1 and N2, respectively) were based on the elution times of PAH and nitroPAH standards, respectively.

The NPLC retention times for the various standards showed that PAH eluted from the column in order of increasing molecular weight. The eluate caught from the HPLC column between 0 and 27 minutes contained PAH up to molecular weight 278, as well as thiaPAH and nitroPAH with less than four rings; this first normal phase fraction was called N1 (see Figure 19B).

The PAH in fraction N1 were followed by increasingly polar compounds as the mobile phase polarity increased. Eluate collected between 27 and 39 minutes (fraction N2) contained PAH with a molecular masses above 278, 4- and 5-ring nitroPAH as well as fluorenone. Fraction N3 which contained anthraquinone was collected between 39 and 50 minutes, while fraction N456 (collected between 50 and 70 minutes) contained carbazoles, benzanthrone and similar compounds. DinitroPAH are known to elute in fraction N3 so this method is useful for the separation of mononitroPAH from dinitroPAH. Finally, acetonitrile was used to elute any remaining compounds as fraction N7. NPLC retention time data for the various PAC standards (Table 6) showed remarkably low standard deviations for a normal phase method, provided the column was throughly re-equilibrated after exposure to acetonitrile. The non-polar aromatic fraction obtained from SRM1650 was separated into the five different N fractions (Figure 19B), based on the compound-class elution of standards seen in Figure 19A. Certain peaks (such as 1-nitropyrene) were identified through UV spectral library matches.

III.6.3 GC-MS Analysis of PAH & NitroPAH Fractions Obtained Through NPLC Cleanup

The PAH and nitroPAH-containing fractions (N1 and N2, respectively) obtained from the SRM1650 extract were analyzed by GC-MS in the selected ion monitoring mode using electron-impact ionization. PAC concentration ranges determined by the NIST were then compared to concentrations determined in this laboratory; both sets of values appear in Table 7. Concentrations determined in this laboratory generally fell within the certified range reported by the NIST (115). Since the PAC concentrations (on a μg/g basis) in diesel soot and air particulate are similar, these experiments have demonstrated that our methodology is applicable and appropriate to the analysis of PAH and nitroPAH in Hamilton air particulate.

III.7 Testing of Methodology for the *S. typhimurium* Assay of Mutagenicity

While the Ames assay (using *Salmonella typhimurium* strains TA98 or TA100) has been used extensively to examine air particulate extracts and standard mutagens (118-121), strain YG1021 has been used in the bioassay of unfractionated air particulate extracts less frequently (122).

S. typhimurium strain YG1021 was chosen for routine bioassay analysis in this study, based on the reports of Hagiwara *et al.* (93, 123) which showed that strain YG1021 (-S9) was between 24 and 37 times more sensitive to 1-nitropyrene than was TA98 (-S9), the strain traditionally used to bioassay air particulate extracts.

Table 7.
Recoveries of PAH and NitroPAH from SRM1650 (Diesel Soot)

Compound	*Concentration Range (as reported by NIST)*		*Concentration Range Determined in this Laboratory*
Fluoranthene	47 - 55 µg/g	(certified)	42 ± 4 µg/g*
Pyrene	44 - 52	(certified)	40 ± 4
Benz[a]anthracene	5.4 - 7.6	(certified)	4.6 ± 0.2
Benzo[a]pyrene	0.9 - 1.5	(certified)	0.92 ± 0.04
Benzo[ghi]perylene	1.8 - 3.0	(certified)	3.1 ± 0.09
1-Nitropyrene	17 - 21	(certified)	22 ± 0.1
Phenanthrene	71	(non-certified)	68 ± 10
Chrysene	22	(non-certified)	12 ± 0.4
Benzo[e]pyrene	9.6	(non-certified)	5.2 ± 0.2
Perylene	0.13	(non-certified)	0.10 ± 0.005
Indeno[cd]pyrene	2.3	(non-certified)	4.1 ± 0.1

*Error in concentration range derived from standard deviation of injection (section IX.15).

For purposes of detection of nitroPAH using *S. typhimurium*, strain YG1021(-S9) offered clear advantages over strain TA98 including the requirement for greatly reduced volumes of extract and a substantial improvement in sensitivity. The use of strain TA98 (-S9) would have required the consumption of the majority of a 24-hour air particulate extract sample, leaving very little material for chemical analyses and no opportunity for repeat bioassay experiments in the event of a failed or equivocal result.

S. typhimurium YG1021 (-S9) was tested first on 2-nitrofluoranthene, a known mutagen in *S. typhimurium* TA98 (-S9) and the most abundant nitroPAH in some air particulate extracts (124). If *S.typhimurium* YG1021(-S9) could detect nanogram amounts of 2-nitrofluoranthene, it was believed that this strain could be used on less than half of a single day's collection of air particulate in Hamilton.

Exact concentrations of 2-nitrofluoranthene were prepared from ethanol solutions using the compound's UV extinction coefficient (125). Five dosages were prepared in duplicate, tested with strain YG1021 and revertant colonies were counted after three days. The least squares linear fit of this data afforded a slope which is a measure of the mutagenic activity of this compound which is reported in units of revertants per nanomole (revs/nmol). The mutagenic activities of 1-nitropyrene and 1,8-dinitropyrene were also determined and compared to literature values (Table 8).

These experiments showed that nanogram amounts of 2-nitrofluoranthene could be detected using *S. typhimurium* YG1021 (-S9). The mutagenicity of 7900 revs/nmol corresponds to a value of 30 revs/ng. The experiments with common mutagens such as

1-nitropyrene and 1,8-dinitropyrene showed that our results were consistent with literature values. Thus, 2-nitrofluoranthene was chosen as the positive control for all bioassay experiments using *S. typhimurium* YG1021 (-S9). A mid-range dose (40 ng) on the linear portion of the dose-response curve was selected and this dose was routinely tested in duplicate whenever YG1021 (-S9) was used.

Table 8.
Mutagenicities of NitroPAH in
S. typhimurium strain YG1021 (-S9).

Compound	*Mutagenicity determined in this lab*	*Lit. Value*	*Ref.*
1-Nitropyrene	10,100 revs/nmol	12,172 revs/nmol 16,805	(93) (123)
2-Nitro-fluoranthene	7,900	not reported	
1,8-Dinitro-pyrene	2,552,000	2,850,000	(95)

IV. CHEMICAL AND BIOLOGICAL ANALYSES OF FILTERS COLLECTED IN HAMILTON, 1990-1991

IV.1 Overview

This chapter will describe the extraction, cleanup, bioassay and GC-MS analysis of 68 individual samples of air particulate collected in Hamilton over a 13-month period between 1990 and 1991. The presentation of results of chemical and biological analyses of the non-polar aromatic fraction from Hamilton air particulate extract will be followed by a discussion of the relationship between PAC concentrations, atmospheric conditions and mutagenicity.

IV.2 Extraction of Air Filters and the Determination of Masses of Crude Organic Extracts

Individual filters were extracted by sequential exposures to dichloromethane then methanol for 24 hours each in a Soxhlet apparatus. The mass of each crude extract was obtained by carefully weighing 1/30th of the crude extract. The relationship between total particulate mass collected on the filters and the mass of extractable material is shown in Figure 20. The line of best fit (obtained *via* linear regression) had a correlation coefficient (or R value) of 0.63; the percentage of organic extractable material averaged 33 $\pm$ 17%. A value of 33% is above average when compared to other studies conducted elsewhere (33,126,127). For example, the percent benzene extractables from respirable air particulate collected in Athens and Santiago ranged between 2% and 17% (33, 127).

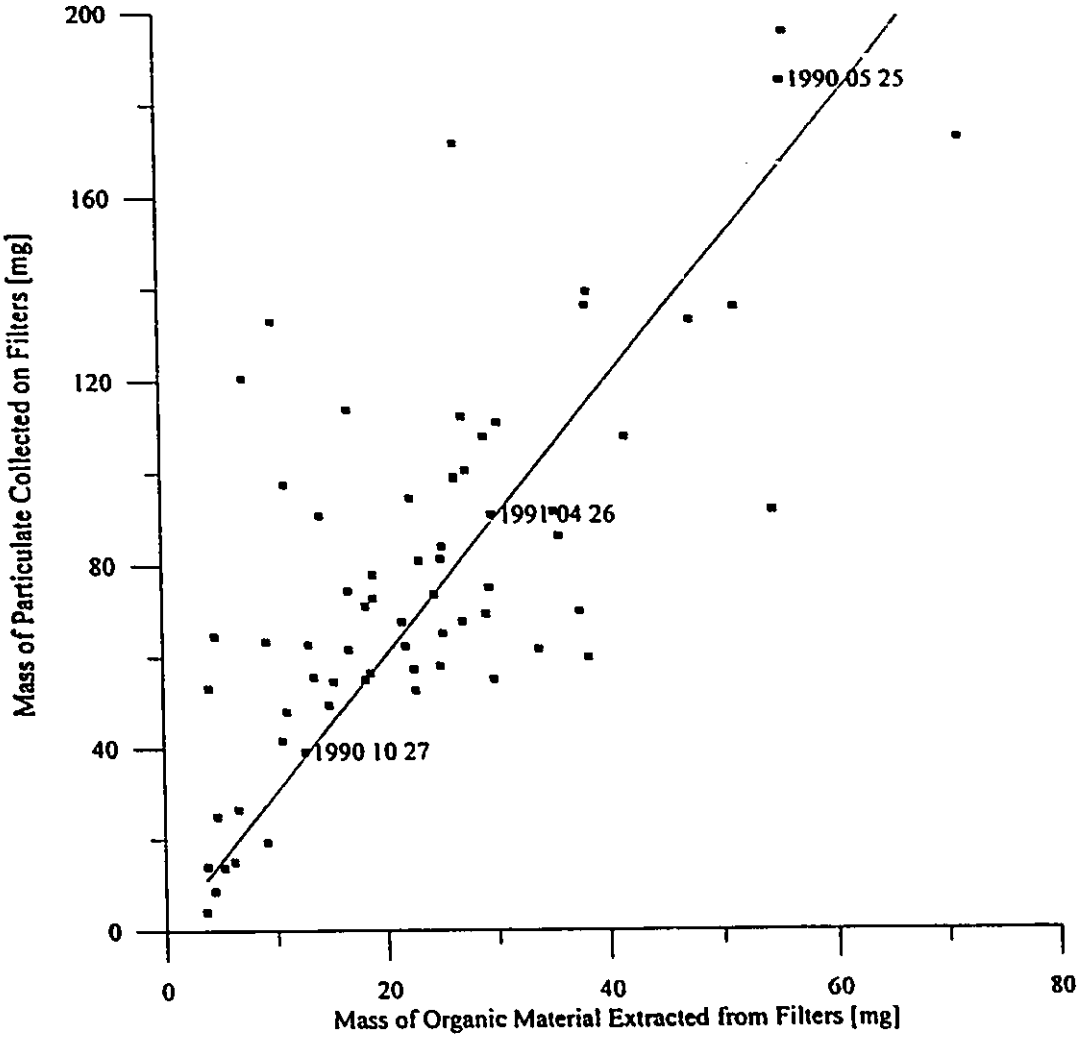


Figure 20. Mass of particulate collected on filters versus mass of organic material extracted from filters with line of best fit. Reconstructed ion chromatograms for the 3 labelled data points (indicating date of collection) appear in Figure 21.

The difference between the 33% organic extractables seen in Hamilton and the 2% and 17% extractables seen in Athens and Santiago may reflect the nature of the solvents: dichloromethane-methanol (polar and very polar) as used in this study versus benzene (non-polar). Previous studies of Hamilton air particulate (50,51,101) did not give any data on percent organic extractables. The masses of organic extracts (820 μg ± 560 μg) were about 80-fold greater than the uncertainty (± 10 μg) in the mass measurement, which leads to the conclusion that the data in this study is accurate.

Filters were weighed within one hour of collection without equilibration under constant humidity conditions. Recently, Kamens *et al.* (128,129) reported that the mass of water associated with particulate increases in direct proportion to the relative humidity, with an increase of up to 12% in mass at a relative humidity of 90%. In light of this data, 16 filters which had been weighed previously without equilibration were placed in a dessicator for four days and reweighed. On average, the filters showed a 14% decrease in mass after drying, with an S.D. of 8%; the decrease in mass ranged from 3% to 27%.

This decrease in mass was directly proportional to the relative humidity on the day of collection and was consistent with the observations of Kamens *et al.* (128,129). However, an adjustment to the data shown in Figure 20 to compensate for the effect of humidity would not change significantly the nature of the relationship between particulate mass collected and mass of organic extract. Clearly, particulate filters should be dried before and after collection, in order to minimize the effect of adsorbed humidity on the masses of material collected. More recent work in this group has followed this protocol.

IV.3 Preparation of Non-Polar & Polar Aromatic Fractions and Determination of Masses

Each crude extract was subjected to alumina chromatography to afford aliphatic, non-polar aromatic and polar aromatic fractions, as described in the previous chapter. Residual aliphatics were removed from the non-polar aromatic fraction (A23) using Sephadex LH20 chromatography to yield fraction A23/LH20. The masses of each non-polar aromatic (A23/LH20) fraction and polar aromatic (A45) fraction were determined gravimetrically. All samples were dried in a dessicator over P_2O_5 prior to weighing.

The average mass of a non-polar aromatic fraction was 5.0% (± 3.5% S.D.) of the mass of the crude organic extract with a range from 0.4% to 20.5%. The non-polar material represented only 1.4% (± 0.7 % S.D.) of the mass of particulate material collected, with a range from 0.2% to 4.6%. Errors in the measurement of the masses were typically on the order of 1% and never exceeded 10%.

The masses of the polar aromatic fractions were considerably greater than the masses of the corresponding non-polar aromatic fractions. The polar material (fraction A45) represented 50% of the mass of the crude extract (± 23% S.D.) with a range from 2% to 97%. The polar fractions corresponded, on average, to 16% of the total mass of particulate collected. This type of data is rarely reported in any study of air particulate; see Greenberg *et al.* (43) for an exception.

IV.4 GC-MS Analysis of Non-Polar Aromatic Fractions

The analysis of the 68 non-polar aromatic fractions, using GC-MS with electron impact ionization in the selected-ion monitoring mode, was carried out in order to quantify a variety of PAC. From the outset, it was decided to quantify 25 different PAC routinely, representing three different classes of compounds: PAH, thiaPAH and oxyPAC. A list of these PAC appears in Table 9, together with data on the concentrations of these compounds determined in Hamilton air particulate extracts.

The PAH to be quantified were selected from the list of priority PAH (Table 10) as defined by the United States Environmental Protection Agency (130). Naphthalene, acenaphthylene, acenaphthene and fluorene were not quantified because they have high vapour pressures and are found almost exclusively in the vapour phase, rather than in association with air particulate (11,131). Four PAH which are not on the EPA list were added to our list of compounds; benzo[j]fluoranthene, dibenz[ac]anthracene, perylene and benzo[b]chrysene have low vapour pressures and are ubiquitous in air particulate extracts (132).

Three benzonaphthothiophenes which have not been routinely quantified in previous studies of air particulate extracts (14) were also added to the list. One of these compounds, benzo[b]naphtho[2,3-d]thiophene, has been suggested as a marker for diesel engine emissions (155). PAH quinones and ketones, previously determined to be ubiquitous in urban air particulate (56,133,134) and combustion emissions (135) made up the final members of the list of compounds monitored.

Table 9
PAC Concentrations (pg/m^3) in 68 Samples of Hamilton Air Particulate, 1990-1991

Compound (abbreviation)	Average	S.D.	Min.	Max.
Phenanthrene (PHEN)	950	1200	12	7200
Anthracene (ANT)	91	140	2	660
Fluoranthene (FLUR)	2100	2800	16	19000
Pyrene (PYR)	1800	2200	16	15000
Benz[a]anthracene (BaA)	2400	2900	5	13000
Chrysene (CHRY)	3100	3600	31	17000
Benz[b,j&k]fluoranthenes (BbjkF)	7800	8800	40	34000
Benzo[e]pyrene (BeP)	3400	3900	17	16000
Benzo[a]pyrene (BaP)	2600	3100	8	13000
Perylene (PERY)	740	890	5	3900
Dibenz[ac&ah]anthracenes (dBAs)	910	1100	3	5700
Benzo[b]chrysene (BbC)	340	400	1	1600
Indeno[cd]pyrene (IcdP)	4200	4700	26	21000
Benzo[ghi]perylene (BghiP)	4800	4700	66	25000
Benzo[b]naphtho[2,1-d]thiophene (BN21T)	300	400	3	2200
Benzo[b]naphtho[1,2-d]thiophene (BN12T)	80	100	2	550
Benzo[b]naphtho[2,3-d]thiophene (BN23T)	220	280	1	1400
Anthraquinone (ANTQ)	680	830	11	5500
11H-Benzo[a]fluoren-11-one (BaFONE)	900	930	24	5200
7H-Benzo[c]fluoren-7-one (BcFONE)	780	710	36	3400
Benzanthrone (BZONE)	1000	960	43	4600
Benz[a]anthracene-7,12-dione (BaAD)	840	900	24	5200
Total PAH	35000	38000	310	170000
Total thiaPAH	610	760	6	4200
Total oxyPAC	420	4200	11	24000

Table 10.
Priority PAH and Their Vapour Pressures at 25°C*.

Compound	*Vapour Pressure (mm Hg)*
Naphthalene	7.8×10^{-2}
Acenaphthylene	6.7×10^{-3}
Acenaphthene	2.2×10^{-3}
Fluorene	6.0×10^{-4}
Phenanthrene	1.2×10^{-4}
Anthracene	6.0×10^{-6}
Fluoranthene	9.2×10^{-6}
Pyrene	4.5×10^{-6}
Benz[a]anthracene	2.1×10^{-7}
Chrysene	6.4×10^{-9}
Benzo[b]fluoranthene	not described
Benzo[k]fluoranthene	9.6×10^{-11}
Benzo[a]pyrene	5.6×10^{-9}
Indeno[cd]pyrene	not described
Dibenzo[ah]anthracene	not described
Benzo[ghi]perylene	1.1×10^{-10}

*Priority PAH as defined by the U.S. Environmental Protection Agency. Vapour pressures are taken from the review of Lee and Huang (131) and the apparent discrepancies in the vapour pressures of similar compounds [e.g., benz[a]anthracene and chrysene] have been critically reviewed by Vo-Dinh (7).

The amount of each non-polar aromatic fraction injected onto the GC column was diluted such that roughly constant amounts of PAC were injected each time. A convenient 'rule of thumb' for determining the dilution factor arose from observations made during the course of Sephadex LH20 chromatography separations.

The UV absorbance of the eluate from the Sephadex LH20 column was monitored at 254 nm and recorded using a chart recorder (1600 mAU full scale). The aromatic compounds eluted as a broad peak of absorbance which caused the recorder pen to go off-scale. The width of the off-scale portion of this peak was found to be directly proportional to the dilution required to obtain sufficient signal for trace PAC; the most abundant PAH never exceeded 40 ng per injection. Thus, as the width of the peak increased, the dilution factor increased. The amounts injected ranged from the equivalent of 2 m^3 to 180 m^3 of air sampled; more details can be found in section IX.15.

Three samples representive of high, medium and low mass filters were highlighted in the plot of particulate mass versus organic extract mass (Figure 20). The non-polar aromatic fraction obtained from the sample with the highest mass (collected May 25th, 1990) required the greatest dilution prior to GC-MS analysis; the equivalent of 2 m^3 of air was injected. The medium and low mass samples required lesser dilutions; the equivalent of 5 m^3 and 10 m^3 were injected, respectively. The reconstructed ion chromatograms of all three samples are shown in Figure 21. The 3 chromatograms in Figure 21 are comparable since they all have a similar number of major peaks and the peak area ratios for some PAH (the benzofluoranthenes, benzopyrenes, indeno[cd]pyrene,

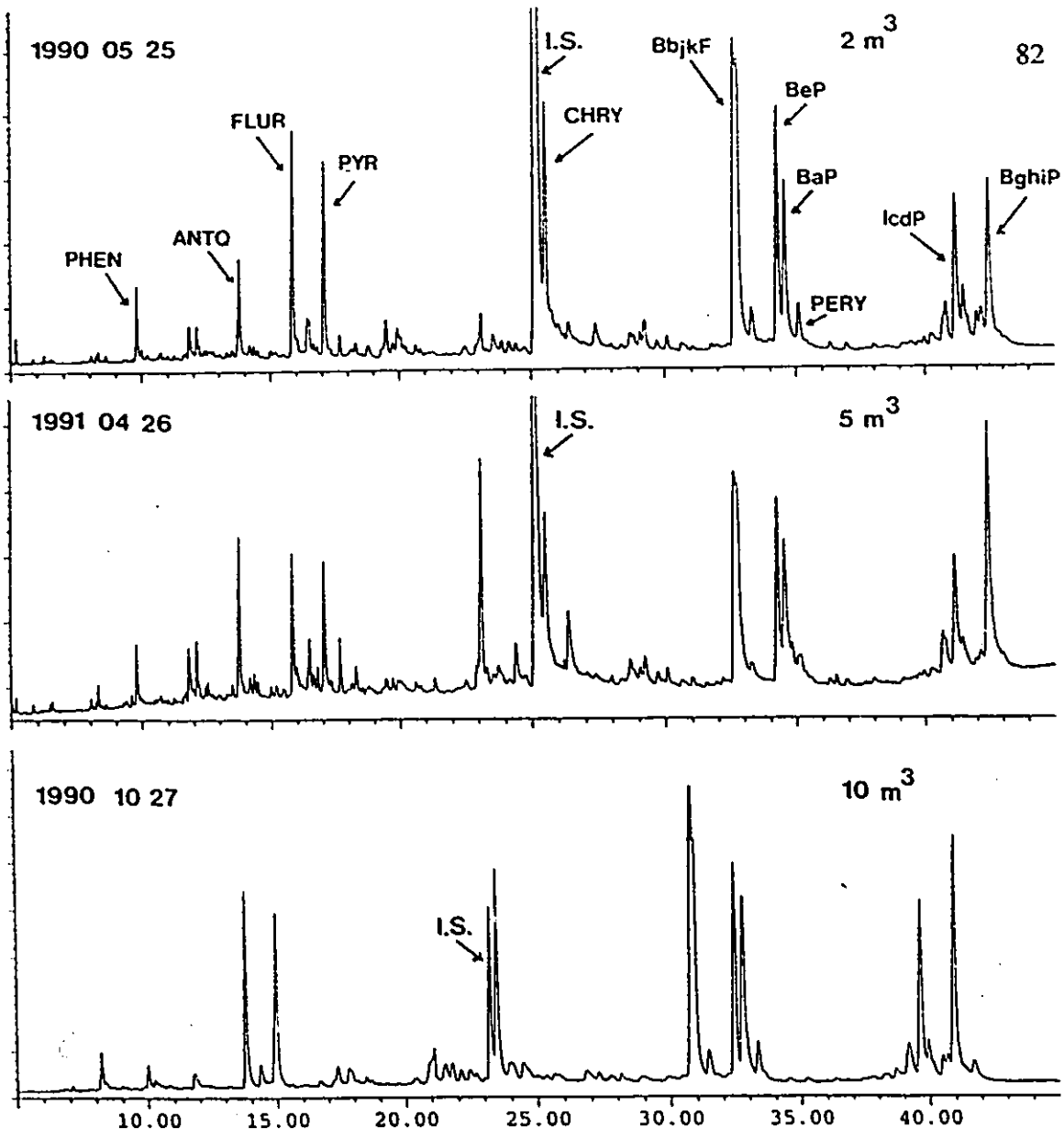


Figure 21. Reconstructed ion chromatograms of 3 representative non-polar aromatic fractions prepared from air samples collected in Hamilton, 1990-1991. Date of sample collection is indicated on the left side of each chromatogram while the amount of sample extract injected onto the GC column (in cubic metres air equivalent) is indicated at the top right of each chromatogram. The bottom chromatogram was acquired one year before the top two chromatograms; retention times differ owing to the slightly different column lengths. Chromatographic conditions are described in the method (IX.15). I.S. stands for internal standard (benz[a]anthracene-d_{12}).

benzo[ghi]perylene) are identical. Although the benzofluoranthenes were not resolved by this particular GC method, these compounds can be resolved by RPLC (181,182). Recent work in this laboratory has shown that analysis on a DB-17ht column affords resolution of the three benzofluoranthene isomers. In general, PAH, thiaPAH and oxyPAC concentrations were observed to rise and fall in unison. Similar trends have been noted in studies recently carried out in England (136).

The 'reconstructed ion chromatograms' (Figure 21) represent the sum of the mass chromatograms for all ions monitored. PAC were quantified by integrating areas under selected peaks in specific mass chromatograms (e.g., Figures 22 and 23). One requirement for PAC quantitation was that the signal-to-noise (S/N) ratio for thiaPAH and oxyPAC which occured at concentrations 60- to 90-fold lower than PAH, had to equal or exceed 200:1. Analyses were repeated to obtain this ratio when required. Conversely, PAH were so abundant in all samples analyzed that over-injection of these compounds (i.e., >50 ng/PAH) had to be avoided. If the amounts of certain PAH (e.g., benzo[ghi]perylene) were >50 ng per injection, the sample was diluted and re-injected. The detection limit for a typical analysis [defined (180) as an S/N ratio of 2.5:1] ranged from 30 pg to 100 pg injected, depending on the compound and the condition of the mass spectrometer. This resulted in method detection limits between 0.30 and 30 pg/m^3, depending on the number of cubic metres air equivalent injected. Typically, the amount of PAH injected onto the column was hundreds and occasionally thousands of times above the detection limit. PAH were quantified in all 68 extracts; thiaPAH and oxyPAC were below the detection limit in seven samples which had been almost totally used up in bioassays.

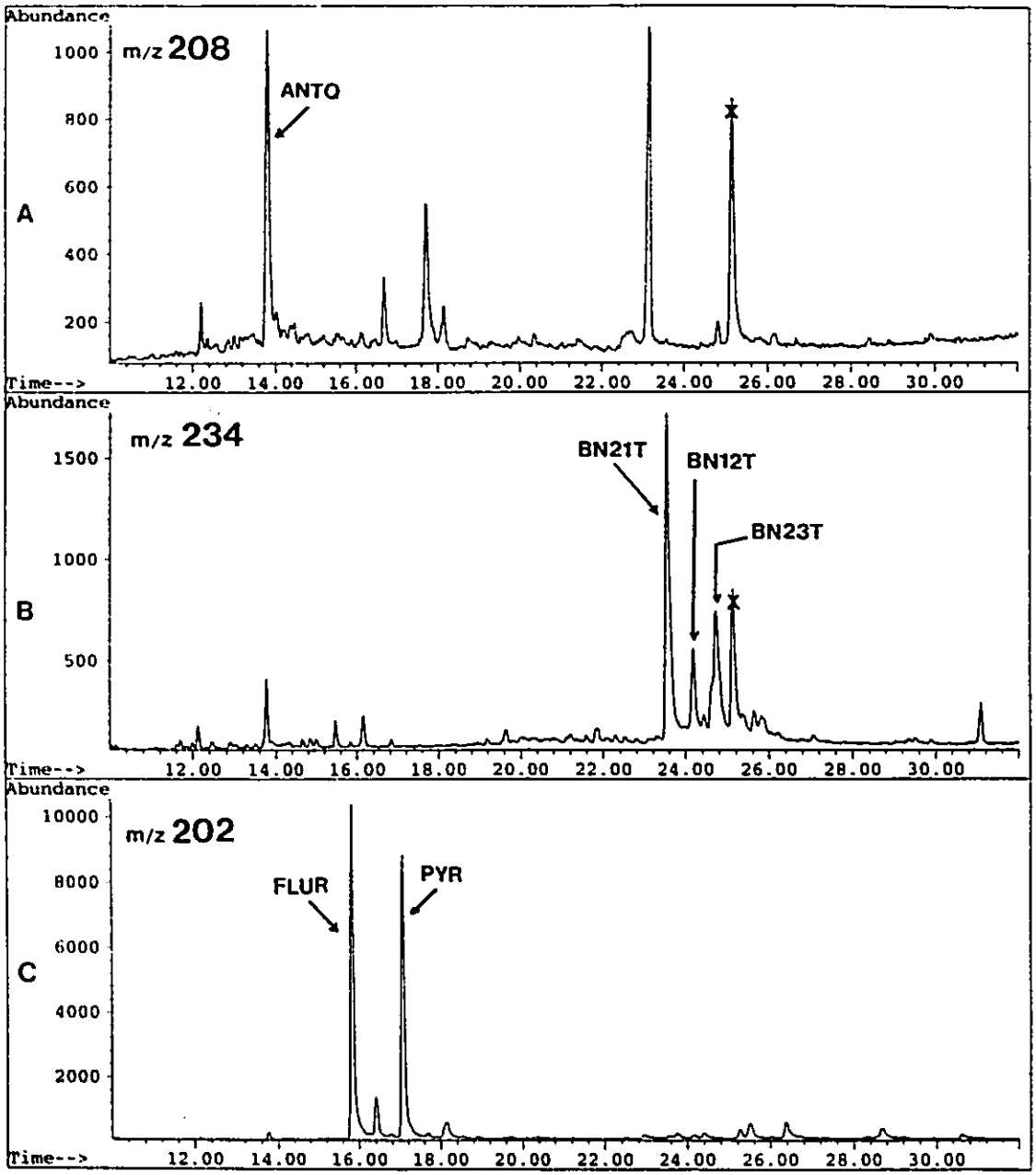


Figure 22. Mass chromatograms for (A) m/z 208, showing anthraquinone, (B) m/z 234, showing benzo[b]naphtho(2,1-d)thiophene (BN21T), benzo[b]naphtho(1,2-d)thiophene(BN12T) and benzo[b]naphtho(2,3-d)thiophene (BN23T) and (C) m/z 202, showing fluoranthene and pyrene. Mass chromatograms are from the analysis of a non-polar aromatic fraction prepared from particulate collected at the downtown Hamilton samping site, 1991 05 17. The 'x' indicates ions from the internal standard.

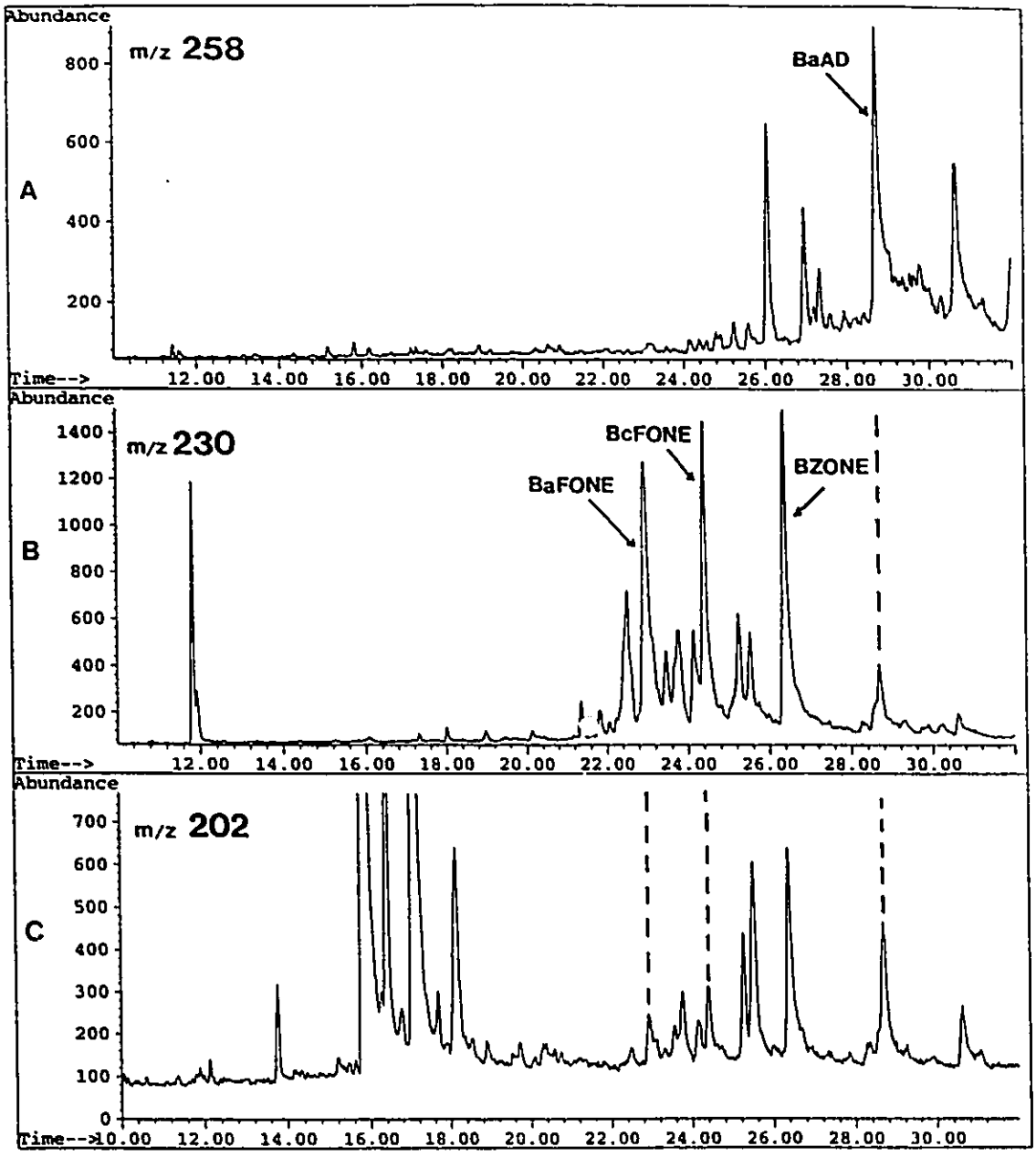


Figure 23. Mass chromatograms for (A) m/z 258, (B) m/z 230 and (C) m/z 202, showing 11H-benzo[a]fluoren-11-one (BaFONE), 7H-benzo[c]fluoren-7-one (BcFONE), benzanthrone (BZONE) and benzo[a]anthracene-7,12-dione (BaAD). Mass chromatograms are from the analysis of a non-polar aromatic fraction prepared from particulate collected at the downtown Hamilton sampling site, 1991 05 17.

GC-MS system performance was evaluated routinely by the injection of a mixture of 20 PAC standards, all of known concentration and each between 10 and 40 ng/μL. Acceptable performance criteria were met when the mass spectrometer showed good sensitivity (as defined by the detection limits described previously) and when the peaks of the standards had maximal widths at half-height of 0.15 minutes. Substandard instrument sensitivity or peak width was usually corrected by removal of the first 30 cm of the retention gap or of the column. A table of standards and their performance appears in Table 53, section IX.15.

IV.5 Bioassays of Non-Polar Aromatic Fractions in *S. typhimurium* YG1021 (-S9)

IV.5.1 Introduction

Bioassays of individual non-polar aromatic (A23/LH20) fractions were carried out to determine their mutagenic potencies. *Salmonella typhimurium* strain YG1021 in the absence of S9 was selected for routine bioassays analyses due to its elevated sensitivity to nitroPAH such as the atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene. Strain YG1021(-S9) had also been shown to give a response that was about 2.4 times greater than TA98(-S9) in the bioassay analysis of a complex mixture, the urban dust standard reference material SRM1649 (122).

The bioassays were performed using the procedure of Maron and Ames (83). Five dosages of each fraction were bioassayed in duplicate. Positive controls (40 ng of 2-nitrofluoranthene dissolved in 50 μL of dimethyl sulfoxide) as well as negative controls (50 μL of dimethyl sulphoxide) were included with every set of bioassays. After 72 hours incubation at 37°C (as required for strain YG1021) colonies were counted with a colony counter and linear dose-responses were obtained. Spontaneous reversion rates were 42 ± 20 revertants per plate and positive control values (2-nitrofluoranthene) were 1340 ± 350 revertants per plate.

Obtaining good quality bioassay data was dependant on the selection of a proper dosage range for each extract. The highest dose of each sample had to be adjusted carefully. With too high a dose, more colonies would be formed than could be counted accurately (>1500 revertants). High doses could also lead to bacterial toxicity, resulting in curved rather than linear dose-response data. Thus, it was important to find a dose range for each sample which gave linear dose responses without toxicity.

The dose range for each sample was estimated from observations made during Sephadex LH20 chromatography in a manner similar to that described in section IV.4. The width of the off-scale portion of the peak (descibed on page 81 of this thesis) was found to be inversely proportional to the highest dose needed to obtain a linear dose response in *S. typhimurium*. Thus, as the width of the UV absorbance peak increased, the top dose applied to the bacteria decreased; details of this protocol are described in section IX.18. The top dose averaged 35 m^3 (S.D., 38 m^3) and ranged from 4 m^3 to 163 m^3.

This method for the estimation of dose range worked extremely well and cut down the time needed to obtain good bioassay results dramatically. Optimal dose ranges are usually determined by trial and error which often requires two, three or more sets of bioassays with the concommitant consumption of a considerable portion of the sample. Occasionally, the number of revertants did not double over the dose range, in which case the dosage was boosted; in other cases, the number of revertants exceeded 1500 for most doses, in which case dosages were decreased the next time the bioassay was carried out.

IV.5.2 Results

The mutagenic potency of non-polar aromatic fractions displayed a broad range of responses, from 1 to 140 revertants per cubic metre air (revs/m^3) with an average of 28 ± 31 revertants per cubic metre. Representative dose-response data, obtained from the analysis of five of these fractions are shown in Figure 24. All 68 fractions gave linear responses over the dose ranges tested. The correlation coefficients for all dose-response curves were 0.95 or higher. The most mutagenic sample (140 revs/m^3) was tested over a dose range of 1 to 6 m^3 equivalents (in order to avoid toxicity above 6 m^3), while the sample showing the lowest mutagenic activity (1 revertant/m^3) had to be tested from 40 m^3 to 163 m^3 in order to observe a doubling of colonies over the spontaneous (also known as background) reversion rate.

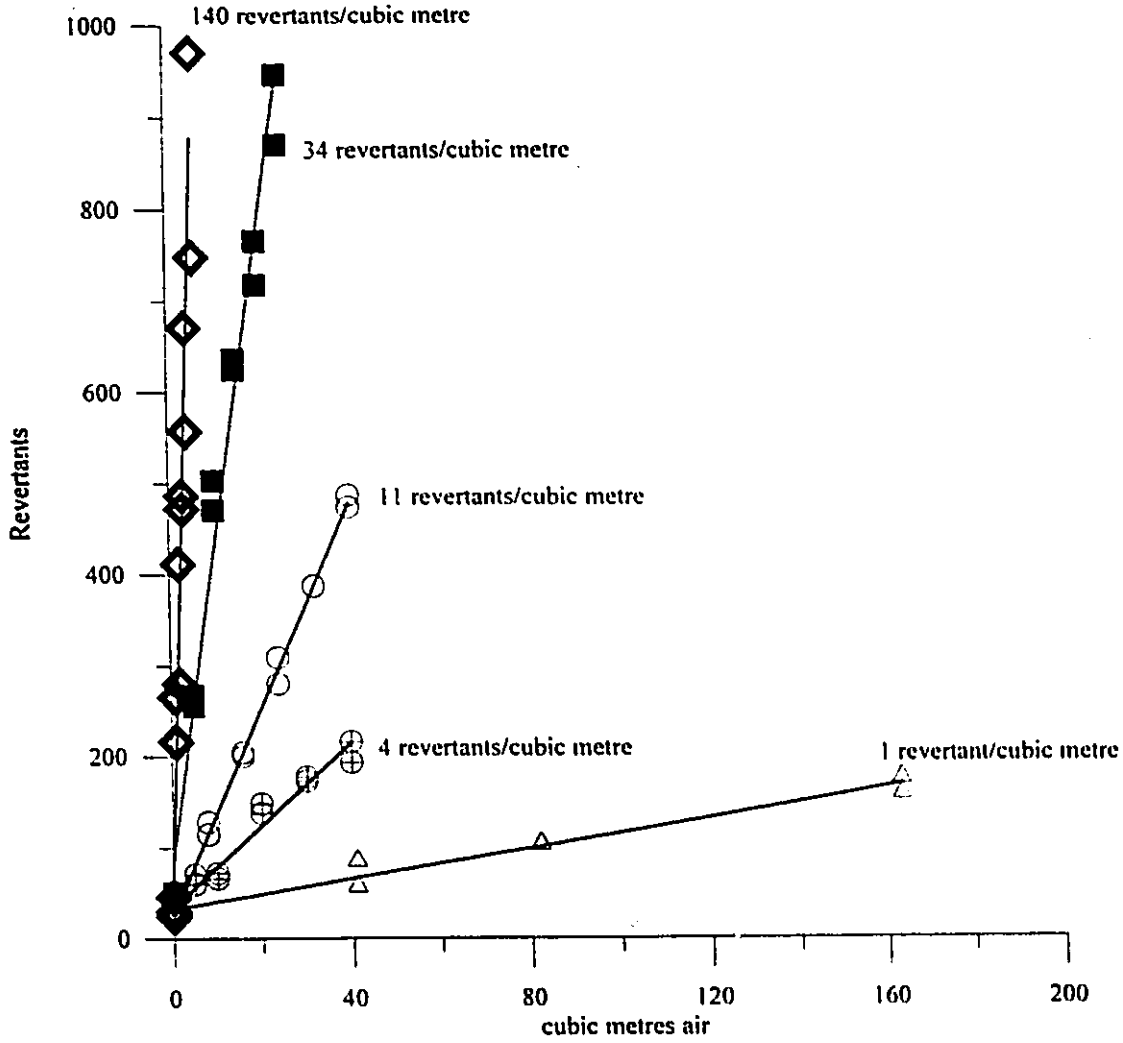


Figure 24. Dose-response data for 5 representative non-polar aromatic fractions using *S. typhimurium* YG1021(-S9).

The sample with a mutagenic activity of 1 revertant/m^3 (Figure 24) can be used to calculate an approximate detection limit for this bioassay. For this sample, the negative control (i.e., background) gave 28 spontaneous revertants while the top dose of 163 m^3 equivalent produced 158 revertants per plate, leading to a value of 130 net revertants. The criterion (185) for a minimal response in this assay is a doubling of the background reversion rate (i.e., about 2 X 28 = 56 revertants). In this case the response at the highest dose was 130/28 or about 4.6 times greater than the minimum response. Therefore the detection limit of this method is approximately (28/130) X 1 revertant/m^3 or 0.22 revertants/m^3. The linear dynamic range for *S. typhimurium* YG1021 is about 50-fold, i.e., from a background of 30 revertants to a maximum of 1500 revertants.

The mutagenic potencies of the 68 fractions are plotted chronologically over the sampling period (May 1990 - May 1991) in Figure 25. The salient features of this figure include the higher mutagenicity values seen in the spring months of 1990 and 1991 as well as the lower mutagenic potencies through the winter, with the exception of one day in December, 1990. The filter collected on this day was selected for extraction because of above average sulfur dioxide concentrations. Overall, the mutagenic potencies of these extracts showed a wide range of values throughout the year.

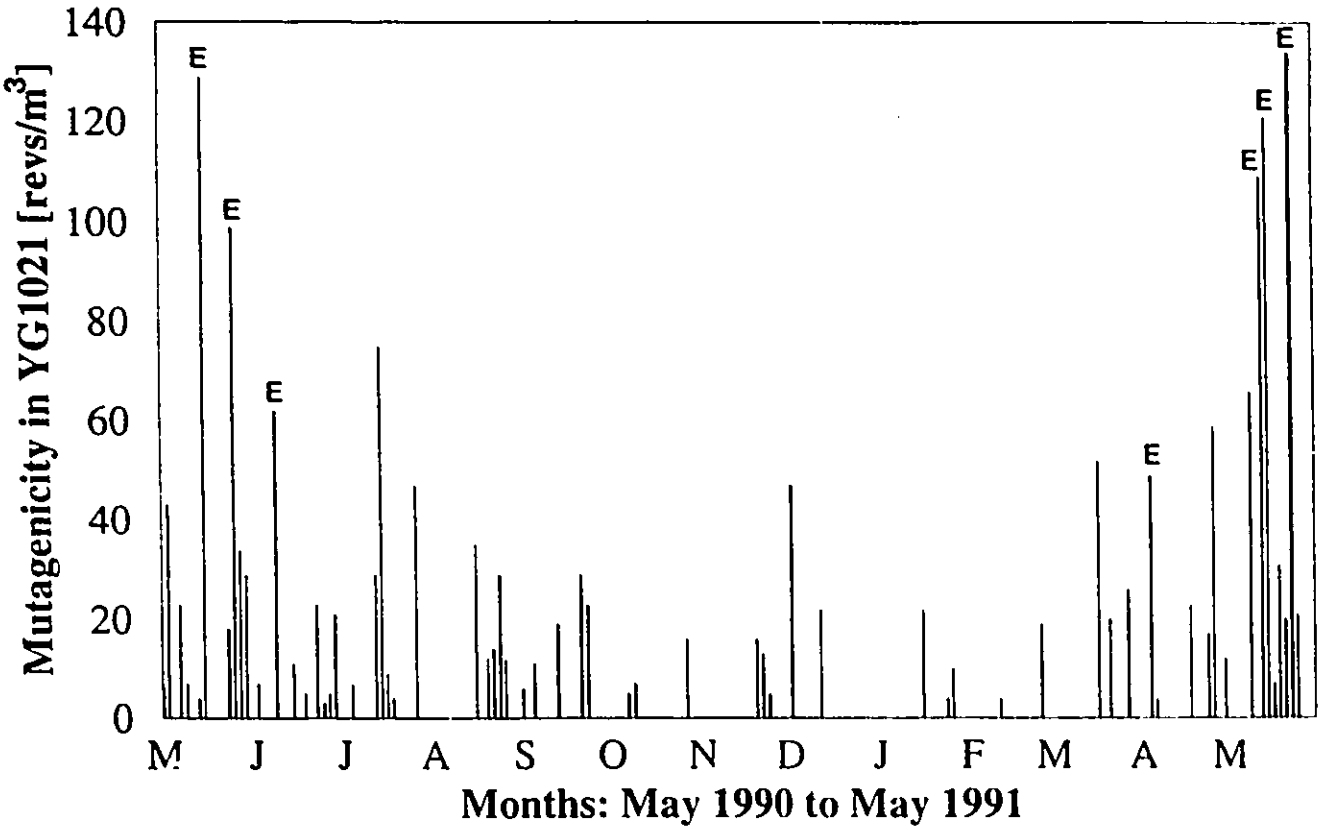


Figure 25. Mutagenicity of non-polar aromatic fractions as a function of time during the 1990-1991 sampling season. 'E' signifies days which were identified as pollution events by the Ontario Ministry of Environment and Energy.

Air pollution events are identified in Figure 25 with an 'E.' Pollution events are declared when the MOEE's Air Quality Index (AQI) exceeds 32 and are typified by high particulate levels and high sulfur dioxide concentrations (see section II.3). The 1990/1991 sampling season was fairly typical of atmospheric conditions in Hamilton over the past 8 years (100,103,105-108) and the high mutagenic potencies seen in the spring of 1990 and 1991 were also observed in the spring of 1993 (137) and the spring of 1989 (51). A discussion of these results and their relationship with PAC levels and atmospheric conditions appears in section IV.7.4.

IV.5.3 Stability of Mutagenic Potency of Extracts

All non-polar aromatic fractions were stored in glass vials at 4°C in the dark. Concerns were raised that the mutagens in these extracts might degrade, i.e., the mutagenicity of the non-polar aromatic fraction could decrease with time. To address this issue, two non-polar aromatic fractions were stored under these conditions and were bioassayed repeatedly.

First, a non-polar aromatic fraction of relatively low mutagenicity was bioassayed repeatedly over 2 months; later, another fraction of higher mutagenic potency was bioassayed several times over a 22-month period. The results (Tables 11 and 12) clearly demonstrate that non-polar aromatic fractions maintained a constant mutagenic potency for up to two years after extraction under the storage conditions used in this study. Thus, it is unlikely that the other samples would have undergone any significant losses of mutagenic potencies under similar storage conditions.

Table 11.
Mutagenicity of a Non-Polar Aromatic Fraction over a Two-Month Period*

Time Since Extraction of Sample	*Mutagenicity (revs m^3)*
16 days	21
26 days	18
39 days	16
46 days	15
61 days	16
Average:	17 revertants/m^3
Absolute standard deviation:	$\pm$ 2 revertants/m^3
Relative standard deviation:	$\pm$ 14%

* Mutagenicity determined using *S. typhimurium* YG1021 (-S9).

Table 12.
Mutagenicity of a Non-Polar Aromatic Fraction Over a Twenty-Two Month Period*.

Time Since Extraction of Sample	*Mutagenicity (revs m^3)*
0.3 months	120
2.0	97
3.0	100
6.5	98
8.7	93
12.0	110
20.2	100
22.3	110
Average:	104 revertants/m^3
Absolute Standard Deviation:	$\pm$ 9 revertants/m^3
Relative Standard Deviation:	$\pm$ 9 %

* Mutagenicity determined using *S. typhimurium* YG1021 (-S9).

IV.6 Bioassays of Selected Polar Aromatic Fractions in *S. typhimurium* YG1021(-S9)

Ten polar aromatic (A45) fractions were selected and subjected to the bioassay for mutagenicity using *S. typhimurium* YG1021 (-S9) with a dose range identical to that used for the corresponding non-polar aromatic fraction. Mutagenic potencies of the polar aromatic fractions ranged from 3 to 53 revertants/m^3 with an average of 18 revertants/m^3 and a standard deviation of 16 revertants/m^3. The mutagenicity of a given polar aromatic fraction was substantially lower than the mutagenicity of the corresponding non-polar aromatic fraction in all but one case. The ratio of the mutagenicities of the non-polar aromatic and polar aromatic fractions averaged about 75:25 (Table 13). Six of the selected samples corresponded to pollution events.

The relationship between mutagenic potencies of the non-polar aromatic (A23/LH20) fractions and the polar aromatic (A45) fractions is explored in Figures 26A and 26B. First, Figure 26B shows that the mutagenic potencies of both fractions increase in a fairly linear fashion when plotted against the sum of these values. Thus, overall the mutagenicity of the non-polar aromatic fractions increases about three units for each unit of increase in the mutagenic potency of the polar fractions.

The data in Table 13 showed a range of ratios of the mutagenic potencies of the non-polar versus the polar aromatic fractions. However, the highest ratios were observed on the days which had the highest mutagenicity values. Figure 26A shows a plot of these individual mutagenicity values versus this ratio. Overall, the mutagenic potencies of the

Table 13.

Mutagenicities of Selected Non-Polar Aromatic (A23/LH20) and Polar Aromatic (A45) Fractions in *S. typhimurium* YG1021(-S9).

Date* of Collection	A23/LH20 Mutagenicity	A45 Mutagenicity	Ratio of A23/LH20:A45 Activity
1990 05 15†	130 revs/m^3	12 revs/m^3	92: 8 (12:1)
1990 05 25†	100	19	84:16 (5.2:1)
1990 06 08†	62	8	88:12 (7.3:1)
1990 06 24	3	NR**	-
1990 07 18	4	3	57:43 (1.3:1)
1990 11 20	16	6	73:27 (2.7:1)
1991 04 04†	49	13	79:21 (3.8:1)
1991 04 24	17	35	33:67 (0.5:1)
1991 05 15†	120	11	92:8 (12:1)
1991 05 23†	140	53	72:28 (2.6:1)
Avg. ± S.D.	64 ± 54	18 ± 16	74 ± 19: 25 ± 19

*listed chronologically

**NR = no response, i.e., no doubling of revertant colonies with a top dose of 160 m^3 equivalent and below the detection limit of the bioassay (nq2 revs/m^3).

† = Pollution event/thermal inversion

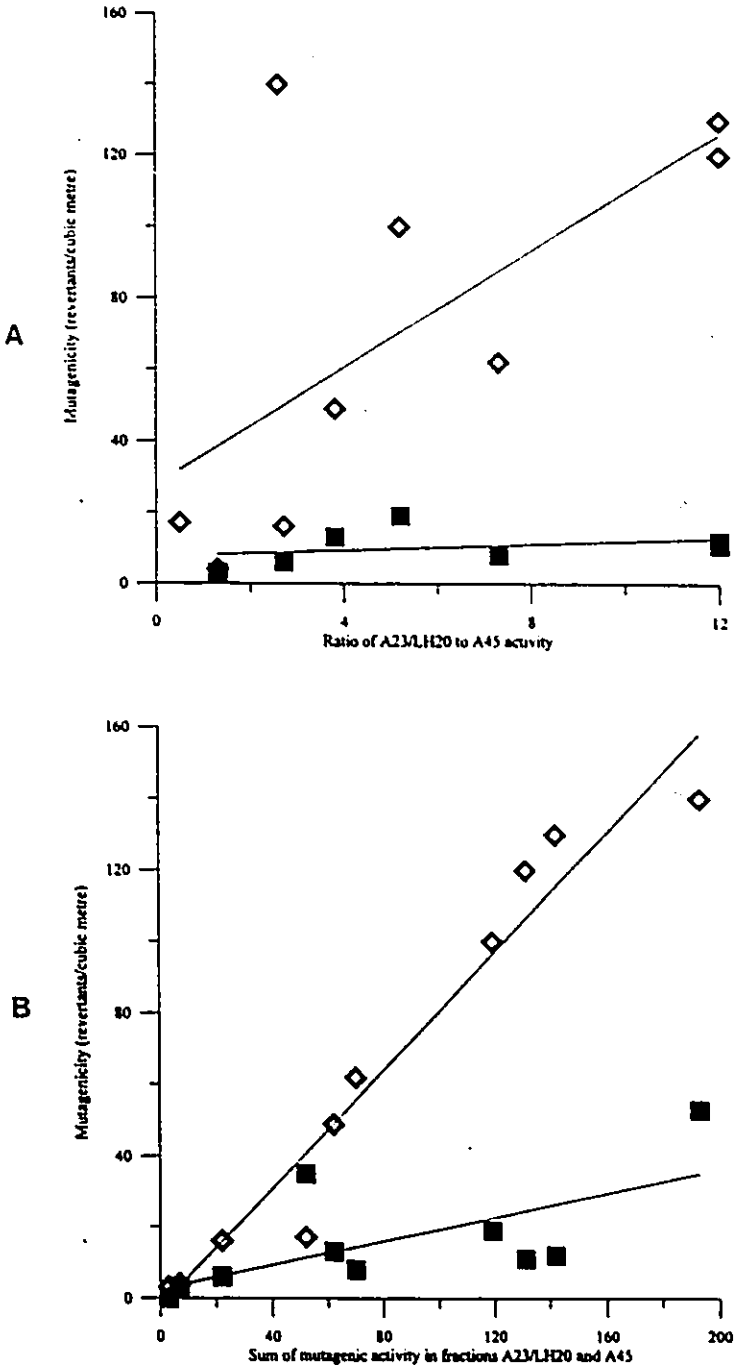


Figure 26. Mutagenicities of the non-polar aromatic fractions (A23/LH20, ◇) and polar aromatic fractions (A45, ■) plotted against: (A) the ratio of A23/LH20 to A45 activitities and (B) the sum of the mutagenic activities of A23/LH20 and A45, with lines of best fit. Mutagenicity was determined using *S. typhimurium* YG1021 (-S9).

non-polar aromatic fractions (A23/LH20) increase as the ratio increases whereas the polar fractions (A45) show a relatively constant trend. Thus, as the mutagenic potencies of the extracts increased the relative contribution of the non-polar aromatic fraction increased much more rapidly than did the contribution from the polar fraction. Clearly, the chemical components in the non-polar aromatic fraction responsible for these changes are of greater concern than the components in the polar fraction.

The polar aromatic fractions were not amenable to gas chromatographic analyses (138) and they were not analyzed further. Speculation as to the nature of the compounds responsible for the mutagenic activity in the polar aromatic fraction is therefore not possible.

IV.7 Discussion

IV.7.1 Overview

The first part of this section will compare mutagenicity data from this study with data reported from other cities. The second part will examine the relationships between the mutagenicity data, PAC data and atmospheric conditions; next, a more advanced examination of these relationships using principal component analysis and vector directional plots will be presented. The discussion will conclude with a comparison between samples collected on the same day at the two different sampling sites in Hamilton.

IV.7.2 Mutagenicity of Air Particulate Extracts in Hamilton & Elsewhere

A variety of chromatographic methods as reviewed by Alfheim *et al.* (6) have been used to prepare crude organic extracts of air particulate for biological analyses. The *S. typhimurium* strain most commonly used to examine the resultant extracts has been strain TA98 (-S9) although other strains and conditions have also been used in a few cases.

In our laboratory, crude extracts of two standard reference materials, diesel exhaust particulate (SRM 1650) and urban air particulate (SRM 1649), were found to be approximately 5.6 times more mutagenic in strain YG1021 (-S9) than in strain TA98 (-S9) (122). This result suggested that by using this ratio the mutagenicity data obtained in our laboratory using YG1021 (-S9) could be compared to data obtained elsewhere using strain TA98 (-S9); see Table 14. The quality of the data in Table 14 is variable, since only about one-half of the studies reported mutagenicity values derived from proper dose-response experiments using single or duplicate assays; these studies are noted in Table 14. The samples bioassayed in Table 14 were prepared in different ways using different extraction solvents, which would probably afford different mixtures of PAC.

The comparison of mutagenic responses for air particulate from different locations is instructive for a number of reasons. First, the average and standard deviation of mutagenicity for Hamilton air particulate extracts is relatively low compared to most other cities in the table. High standard deviations were observed for all studies indicating a wide range of mutagenicity values were seen in all of these cities. Air particulate collections for the studies shown in Table 14 were carried out over periods

Table 14.
Mutagenicity of Non-Polar Aromatic Fractions Collected in Hamilton and Elsewhere.

Location	*n**	*Date*	*Average (± S.D.) Mutagenicity (revs m³)***		*Range (revs m³)***
Los Angeles, CA† (140)	8	Fall 1980	104	± 59	50 - 180
Katowice, Poland† (141)	7	Winter 1985	58	± 56	18 - 180
Torrance, CA† (85)	8	Winter 1986	57	± 42	15 - 120
Santiago, Chile† (127)	105	Spring 1990	41	± 32	3 - 106
Stockholm, Sweden (142)	15	Winter 1981	23	± 15	2 - 55
Delft, Holland (139)	70	Summer 1982	22	± 7	5 - 33
Athens, Greece (33)	172	Spring 1984	20	± 10	5 - 35
Pisa, Italy (143)	52	Winter 1987	10	± 10	1 - 32
Oslo, Norway (144)	19	Winter 1979	8	± 4	3 - 14
Fukuoka, Japan† (90)	13	Winter 1978	6	± 4	1 - 14
Hamilton, ON† (this study)	68	1990-1991	5***	± 5	0.2 - 24
Detroit, MI (145)	40	Summer 1985	2	± 2	0 - 8

* number of samples subjected to bioassay
** as determined using *S. typhimurium* strain TA98 (-S9)
*** estimate; calculated by dividing the mutagenicity in strain YG1021 (-S9) by 5.6.
† studies which used dose-response curves to determine extract mutagenicities

between 8 days and 6 months, with sample sizes ranging between 8 and 172 samples. In most cases, atmospheric and meteorological conditions at the time of sample collection were not reported. The number of samples subjected to bioassay in this study is exceeded only by the number collected in Delft (139), Santiago (127) and Athens (33), where 70, 105 and 172 samples were bioassayed, respectively.

IV.7.3 PAC Concentrations in Air Particulate from Hamilton & Elsewhere

The literature on PAH concentrations in the air of various cities around the world is extensive, especially for priority PAH. While these studies have always quantified PAH, little or no data has been reported for thiaPAH or oxyPAC concentrations; see the report of Rogge *et al.* (58) for an exception. Conversely, studies undertaken to quantify either thiaPAH or oxyPAC have ignored ambient PAH concentrations (56). Thus, the correlation between PAH, thiaPAH and oxyPAC concentrations in ambient air has not been evaluated.

The concentrations of PAH in respirable particulate in Hamilton are relatively low compared to other cities (Table 15). Benzo[a]pyrene is a useful compound to use for comparison with other studies since it is routinely quantified in even the simplest experiments; in some cases this may involve quantitation of only benzo[a]pyrene by measuring the fluorescence of the crude particulate extract (146). In the studies described in Table 15, samples were usually collected and analyzed during episodes of atmospheric pollution. In the present study benzo[a]pyrene concentrations

Table 15.
Benzo[a]pyrene Concentrations in Air Particulate Collected in Hamilton & Elsewhere.

Location	*n**	*Method*	*Date*	*Average (± S.D.) BaP Conc. (ng/m³)*		*Range (ng/m³)*	*Quality***
Jonquière, PQ (149)	not given	GC-MS	Fall 1982	58	(S.D. not given)	1 - 545	A
Ruda Slaska, Poland (150)	110	GC-MS	Wint. 1988	46	± 42	19 - 61	B
Magnito-gorsk, USSR (146)	8	fluorescence of crude extract	1985-1987	37	± 27	10 - 75	B
Trenton, NJ (227)	468	RPLC	1984-1985	6	(S.D. not given)	0.6 - 20	A
Hamilton, ON (99)	90	TLC, fluorescence	1977-1978	3.6	± 0.9	2.5 - 4.7	A
Hamilton (this study)	68	GC-MS	1990-1991	2.6	± 3.1	0.01 - 12.9	A
Hamilton, ON(98)	25	TLC, fluorescence	1975-1976	2.3	± 0.9	1.4 - 3.5	A
London, U.K. (152)	86	RPLC	1989-1990	1.5	± 0.6	0.7 - 2.5	A
Rotterdam, Holland (153)	48	RPLC	not given	1.3	± 0.8	0.4 - 2.6	B
Toronto, ON (98)	25	TLC, fluorescence	1975-1976	0.8	± 0.3	0.7 - 1.7	A
San Francisco (154)	81	GC-MS	1979-1988	0.4	± 0.2	0.1 - 2.5	A

* number of samples analyzed
** quality of method: A = precision and accuracy of quantitation method stated explicitly;
B = no data for precision or accuracy of quantitation method given.

were determined for a range of conditions including highly polluted and lightly polluted days, as well as for a range of conditions in-between.

ThiaPAH have been identified occasionally in PAC extracted from air particulate (147,148) and individual thiaPAH isomers such as benzo[b]naphtho(2,1-d)thiophene have been quantified rarely (3,57). Benzo[b]naphtho(1,2-d)thiophene and benzo[b]naphtho(2,3-d)thiophene have not been quantified previously and certain oxyPAC have been quantified in only one study (56). A comparison between the reported concentrations of benzo[b]naphtho(2,1-d)thiophene for several studies appears in Table 16A. Table 16B affords a comparison of the concentrations of four selected oxyPAC with data obtained in a study conducted in Germany (56).

Source apportionment studies often compare the concentrations of various PAC to the concentration of benzo[e]pyrene in the same extract. Benzo[e]pyrene is ubiquitous, relatively unreactive when compared to benzo[a]pyrene and is found almost exclusively in the particulate phase (132). The ratio of benzo[e]pyrene to other PAC was examined by determining the correlation coefficients between these variables and the mutagenic potencies of an extract (Table 17). Correlation coefficients between benzo[e]pyrene and most of the monitored PAC were quite high. Lower values were generally observed for the more volatile components such as anthracene. Overall the correlation coefficients averaged to 0.83 $\pm$ 0.09. The correlation between benzo[e]pyrene and the concentration of other PAC has been previously reported in other studies (136,155). Total PAH and the concentrations of monitored thiaPAH and oxyPAC are well correlated.

Table 16.
Concentrations of Benzo[b]naphtho(2,1-d)thiophene and Four OxyPAC in Air Particulate Collected in Hamilton and Elsewhere.

A. Benzo[b]naphtho(2,1-d)thiophene

Location	*Date*	*Average (± S.D.) Conc. (ng/m³)*	*Number of Samples Analyzed*	*Ref.*
Hamilton	1990-91	0.08 ± 0.10 (min. 0.005, max. 0.55)	68	this study
Roskilde, Denmark	1982	0.11 ± 0.05	20	(63)
Kokkola, Finland	1985	not detected	11	(3)
Essen, Germany	1979	50 (for one sample, where BaP conc. was 208 ng/m³)	1	(57)

B. OxyPAC

Compound	*Concentration Range (ng/m³)* Hamilton '90-91*	*Duisburg, Winter 1982*
Benzanthrone	0.04 - 4.61	0.46 - 3.66
Anthraquinone	0.01 - 5.48	0.22 - 1.89
Benzo[a]anthracene-7,12-dione	0.02 - 5.22	0.29 - 1.05
11H-Benzo[a]fluoren-11-one	0.04 - 5.15	0.24 - 2.58
Number of samples analyzed:	68	12

*No average or standard deviation was given or could be calculated for the Duisburg study (56)

Table 17.

Correlation Coefficients Between the PAC Quantified in Sixty-Eight Samples of Hamilton Air Particulate, Benzo[e]pyrene and the Mutagenicity of the Non-Polar Aromatic Fractions

Polycyclic Aromatic Compound	*Correlation (R Value) with B[e]P Concentration*	*Mutagenicity*[•]
PAH		
Phenanthrene	0.79	0.36
Anthracene	0.56	0.29
Fluoranthene	0.76	0.39
Pyrene	0.80	0.37
Benzo[a]anthracene	0.89	0.43
Chrysene and Triphenylene	0.95	0.35
Benzo[b,j & k]fluoranthenes	0.96	0.41
Benzo[e]pyrene	(1.00)	0.32
Benzo[a]pyrene	0.95	0.40
Perylene	0.95	0.35
Dibenzo[ac&ah]anthracenes	0.88	0.45
Benzo[b]chrysene	0.89	0.33
Indeno(1,2,3-cd)pyrene	0.85	0.40
Benzo[ghi]perylene	0.76	0.53
ThiaPAH		
Benzo[b]naphtho(2,1-d)thiophene	0.78	0.32
Benzo[b]naphtho(1,2-d)thiophene	0.76	0.34
Benzo[b]naphtho(2,3-d)thiophene	0.88	0.37
OxyPAC		
Anthraquinone	0.73	0.33
Benzanthrone	0.82	0.35
11H-Benzo[a]fluoren-11-one	0.82	0.34
7H-Benzo[c]fluoren-7-one	0.76	0.35
Benzo[a]anthracene-7,12-dione	0.84	0.34
Average ± standard deviation	0.83 ± 0.09	0.37 ± 0.05

[•]Mutagenicity determined using *S. typhimurium* YG1021 (-S9).

Other measures of content of the organic extracts such as mutagenic potency were rather poorly correlated (average correlation coefficient 0.37 $\pm$ 0.05). This low correlation indicates that the PAC in Table 17 are unlikely to be responsible for the mutagenic activity in *S. typhimurium* YG1021 (-S9). This is not surprising since PAH produce only a weak response in this strain under these conditions (93).

Relatively few studies have involved both bioassays and the quantitation of PAC in air particulate (3, 43, 60, 86, 124). Still fewer studies have linked these two factors with the atmospheric conditions on the day of sample collection. Variations in the mutagenicity of non-polar aromatic fractions prepared from particulate collected in Pisa, Italy, show a negative correlation with increased ambient temperature (156). Increased temperature was believed to result in more rapid photochemical inactivation of mutagens. Thus, ambient temperature could have been used as a surrogate for the determination of particulate extract mutagenicity.

Previous studies of Hamilton air particulate (50,51) have briefly explored the possibility of using atmospheric data as a surrogate for the determination of particulate extract mutagenicity or PAC concentrations. In this study, four variables of meteorological conditions and the concentrations of atmospheric pollutant gases were examined as potential surrogates for PAC concentrations and extract mutagenicity. These variables were: the coefficient of haze (COH), air pollution index (API), total reduced sulfur (TRS) and the difference in air temperature at two heights (ΔT).

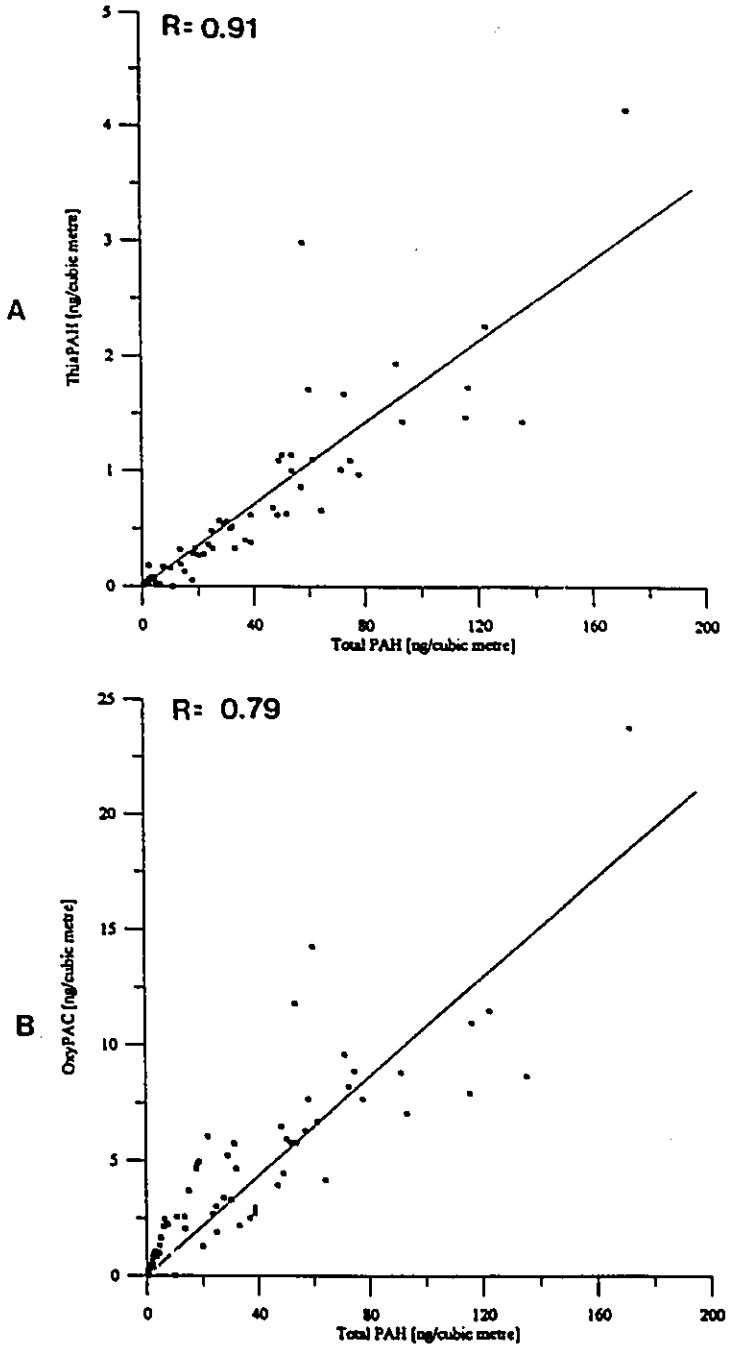


Figure 27. (A) Sum of concentrations of three thiaPAH and (B) sum of concentrations of five oxyPAC versus total PAH determined in 68 non-polar aromatic fractions prepared from air particulate collected in Hamilton, 1990-1991. Correlation coefficients (R) are shown at the top of each plot.

COH is a dimensionless measure of the amount of total suspended particulate in air. The air pollution index (API) in Hamilton is related to COH and SO_2 levels and is calculated by the MOEE using the formula (103):

$$API = 1.47\ (16.4*COH + 122.9*SO_2)$$

When the API reaches 32, major industries in the city are notified and asked to curtail certain operations. The API system is not used outside Ontario (103). Total reduced sulfur (TRS) is the term used by the MOEE to define total ambient concentrations of H_2S or other gaseous compounds with sulfhydryl groups (103).

The value of ΔT is the difference between air temperature measured by sensors fixed on a tower at the Hamilton Sewage Treatment Plant at heights of 33 and 300 feet. Under normal atmospheric conditions, air temperature decreases with increasing height and the difference between the upper and lower sensors is negative. During a thermal inversion, the values for ΔT are positive.

Correlation coefficients were calculated between atmospheric conditions and [a] mutagenicity of the non-polar aromatic fraction determined using *S. typhimurium* YG1021 (-S9) and [b] total concentration of monitored PAC (Table 18); correlation coefficients ranged from 0.72 to -0.58. Reasonable correlations (R= 0.72 and 0.70) were observed between non-polar aromatic fraction mutagenicity and daily average coefficient of haze (COH) and nitrogen dioxide concentrations, respectively (Figures 28A and 28B). The mutagenicity data also correlated fairly well with sulfur dioxide (R=0.66, Figure 28C). The conclusion is that as COH, NO_2 and SO_2 all increase during thermal inversions,

Table 18.

Correlation Coefficients Between Mutagenicity*, Total Concentration of Monitored PAC** and Various Atmospheric Parameters

Parameter	Correlation Coefficient (R) with Mutagenicity*	Correlation Coefficient (R) with Total PAC**
Coefficient of Haze	0.72	0.64
Nitrogen dioxide	0.70	0.52
Sulfur dioxide	0.66	0.56
Total reduced sulfur	0.61	0.69
Air Pollution Index	0.58	0.46
ΔT***	0.52	0.46
Non-methane hydrocarbons	0.45	0.46
Nitric oxide****	0.36	0.44
Air temperature at 33 feet	-0.04	-0.17
Relative humidity	-0.15	-0.09
Ozone	-0.35	-0.41
Windspeed	-0.58	-0.52

*Mutagenicity of the non-polar aromatic (A23/LH20) fraction using *S. typhimurium* YG1021 (-S9).

**Total concentration of all PAC quantified in the non-polar aromatic fraction (Table 9).

***Difference in air temperature at heights of 33 and 300 feet, as measured at the tower at the Hamilton sewage treatment plant.

****Not measured directly. Obtained by subtracting NO_2 concentration from 'oxides of nitrogen' concentration.

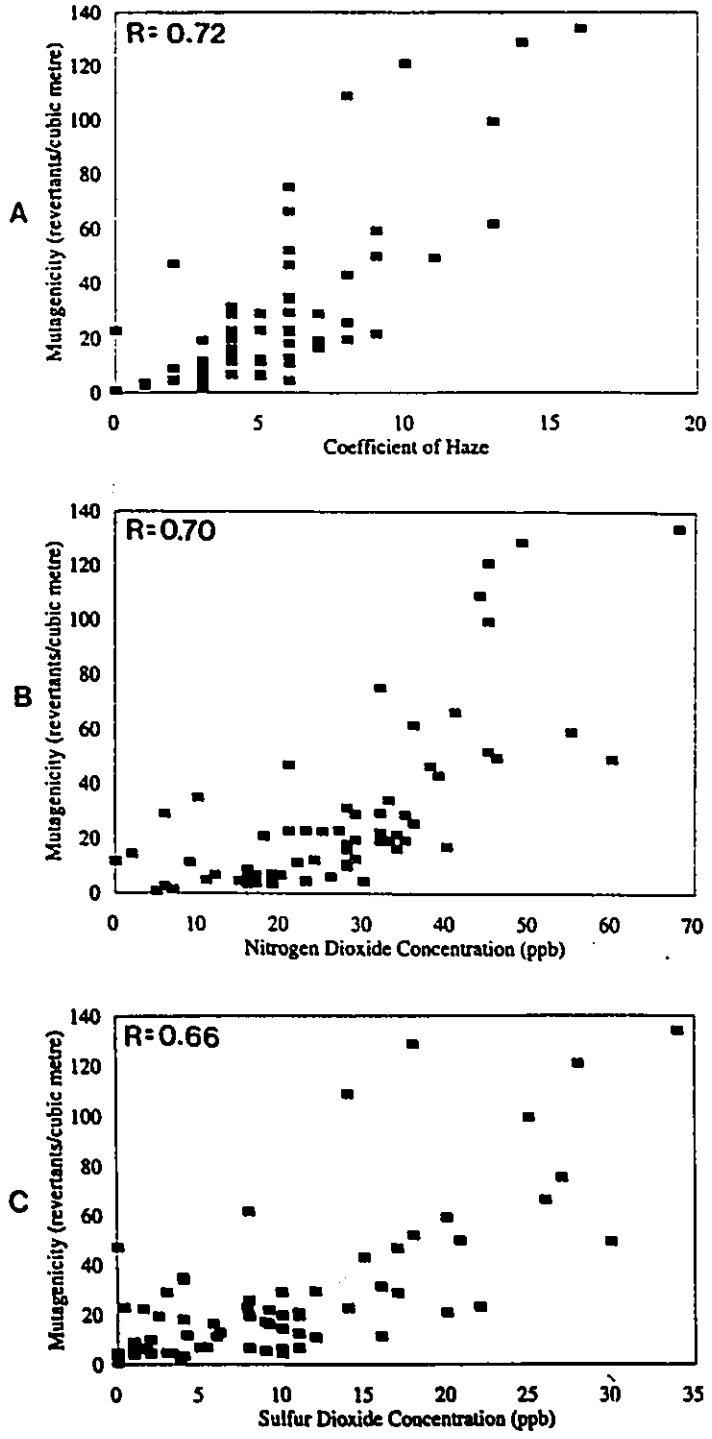


Figure 28. Plots of mutagenicity of the non-polar aromatic fraction of air particulate extracts versus (A) coefficient of haze and concentrations of (B) nitrogen dioxide and (C) sulfur dioxide. Mutagenicity was determined using *S. typhimurium* YG1021 (-S9). Correlation coefficients are shown at the top of each plot.

concentrations of mutagenic PAC also increase. On the other hand, the mutagenicity decreased with both increasing ozone concentration (Figure 29A) and increasing windspeed (Figure 29B), as evidenced by correlation coefficients of -0.35 and -0.58, respectively. Increased ozone concentrations may oxidize potentially mutagenic compounds to form non-mutagenic products. Increased windspeed tends to sweep air particulate out of an airshed, reducing total particulate loading at the sampling sites.

A correlation between total monitored PAC and total reduced sulfur yielded a correlation coefficient of 0.69 (Figure 30). Other atmospheric variables showed poorer correlations. Concentrations of carbon monoxide and methane were not included in these correlations, since the data quality for these two variables was low (Figures 31A and 31B) compared to other parameters. The detection limit set by the MOEE instrumentation was too high to provide reliable data.

IV.7.4 Principal Component Analysis of PAC Concentrations, Mutagenicity and Atmospheric Data

With the wealth of chemical, biological and atmospheric data that had been collected in this study, we felt it would be appropriate to determine what correlations existed between multiple variables in this data set, instead of merely examining correlation coefficients between two variables at a time. An effective statistical tool for this type of study is principal component analysis (or PCA), which transforms a set of intercorrelated variables into a set of uncorrelated variables that are linear combinations of the original variables (158).

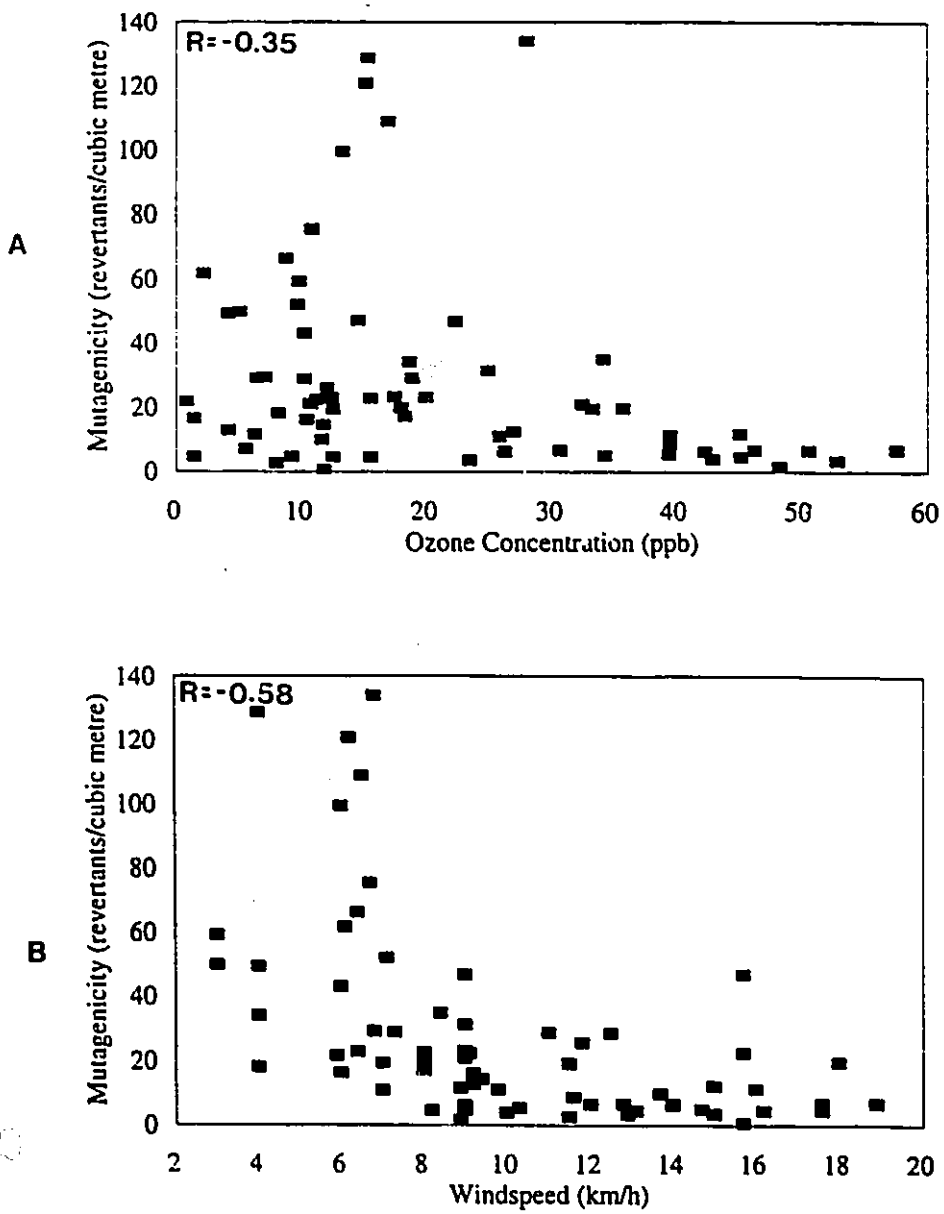


Figure 29. Plot of mutagenicity of the non-polar aromatic fraction of air particulate extracts versus (A) ozone concentration and (B) windspeed. Mutagenicity was determined using *S. typhimurium* YG1021 (-S9). The correlation coefficients (R) are shown in the top left corner of each plot.

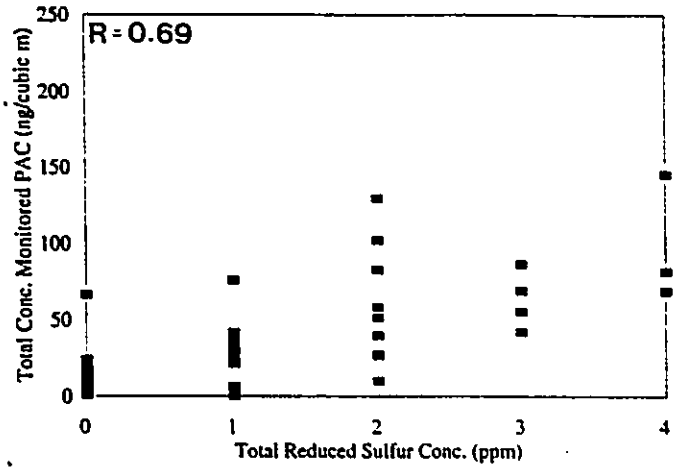


Figure 30. Total concentration of monitored PAC versus total reduced sulfur concentration for samples collected in Hamilton, 1990-1991.

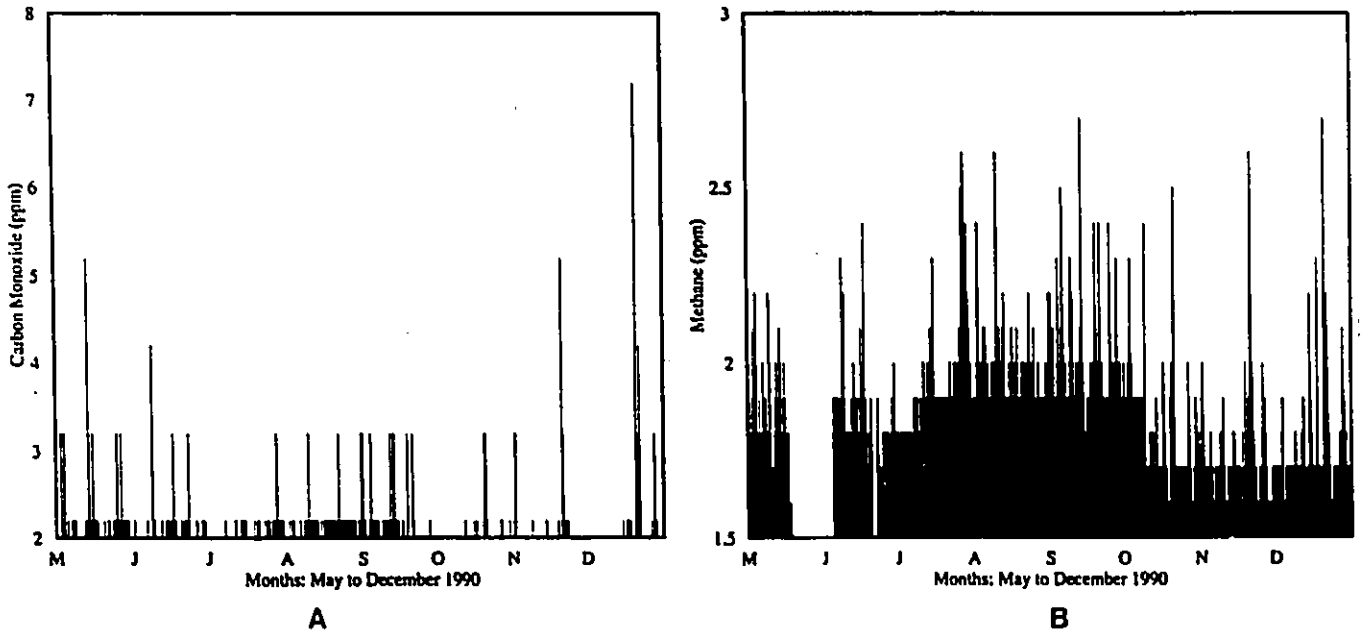


Figure 31. Hourly values for concentrations of (A) carbon monoxide and (B) methane for the period May 1st - December 31st, 1990.

Principal component analysis began with the calculation of correlation coefficients between the variables of interest to this study. These variables included: concentrations of all monitored PAH (ΣPAH), thiaPAH (ΣtPAH) and oxyPAC (ΣoPAC) for any given day; the air pollution index (API) and coefficient of haze (COH); concentrations of total reduced sulfur (TRS), SO_2, NO_2, NO and O_3; the logarithm of mutagenicity (lmut); concentrations of non-methane hydrocarbons (NMHC), ambient temperature at 33 feet above ground level (T); the difference between air temperature at 33 and 300 feet (ΔT); the logarithm of the mass of particulate collected (lmass); relative humidity (rh) and windspeed (wspd). The correlation coefficients between these variables are shown in a correlation matrix (Table 19). Correlation coefficients which equal or exceed 0.64 are highlighted in the table. The value of 0.64 was selected arbitrarily, since no formal definition of excellent or poor correlation exists (226).

In addition to the good correlations between [a] PAH, thiaPAH and oxyPAC concentrations, [b] extract mutagenicity and COH, NO_2 and SO_2 concentrations and [c] concentrations of total reduced sulfur and total PAC already noted, many other correlations became apparent from the correlation matrix. Most of the higher correlations were anticipated based on the previous analyses; others were new. For example, the modest correlation (R= 0.64) between temperature and ozone reflects summertime episodes of high ozone concentrations arriving from the Ohio Valley and the good correlation between total reduced sulfur and SO_2 concentrations (R=0.72) is believed to arise from emissions of both types of compounds from Hamilton's steel industries (103).

Table 19.
Correlation Matrix of 17 Variables Monitored for Hamilton Air Particulate Extracts.

Variable*	ΣPAH	ΣtPAH	ΣoPAC	API	COH	TRS	SO_2	NO_2	NO	O_3	lmut	NMHC	T(°C)	ΔT	lmass	rh	wspd
ΣPAH	1.00																
ΣtPAH	0.91	1.00															
ΣoPAC	0.79	0.88	1.00														
API	0.46	0.41	0.36	1.00													
COH	0.64	0.55	0.50	0.84	1.00												
TRS	0.69	0.62	0.41	0.53	0.67	1.00											
SO_2	0.56	0.54	0.32	0.55	0.61	0.72	1.00										
NO_2	0.52	0.50	0.55	0.60	0.72	0.60	0.61	1.00									
NO	0.44	0.35	0.42	0.46	0.53	0.31	0.31	0.62	1.00								
O_3	-0.41	-0.34	-0.40	-0.11	-0.22	-0.28	-0.24	-0.40	-0.50	1.00							
lmut	0.64	0.60	0.56	0.58	0.72	0.61	0.66	0.70	0.36	-0.35	1.00						
NMHC	0.46	0.42	0.50	0.62	0.50	0.33	0.32	0.65	0.64	-0.35	0.45	1.00					
T(°C)	-0.17	-0.19	-0.38	0.14	0.08	0.03	0.11	-0.31	-0.30	0.64	-0.04	-0.36	1.00				
ΔT	0.46	0.45	0.36	0.51	0.59	0.51	0.41	0.54	0.37	-0.27	0.52	0.51	-0.23	1.00			
lmass	0.48	0.46	0.48	0.61	0.61	0.36	0.45	0.42	0.10	0.26	0.58	0.37	0.22	0.36	1.00		
rh	-0.09	-0.13	-0.08	-0.14	-0.06	-0.13	-0.18	-0.18	0.01	-0.35	-0.15	-0.26	-0.15	-0.14	-0.30	1.00	
wspd	-0.52	-0.45	-0.46	-0.56	-0.71	-0.45	-0.54	-0.56	-0.51	0.21	-0.58	-0.42	-0.09	-0.37	-0.46	0.04	1.00

*Abbreviations are explained on page 114. Correlation coefficients greater than or equal to 0.64 are highlighted.

The coefficient of haze is used to calculate the air pollution index and so a high correlation (R=0.84) between COH and API was expected; the lower correlation between SO_2 and API (R=0.55) was somewhat surprising. Good positive correlations between the coefficient of haze with total reduced sulfur (R=0.67) and with NO_2 (R=0.72), together with the negative correlation between the coefficient of haze and windspeed (R= -0.71) are all indicative of atmospheric conditions observed during a thermal inversion.

The correlation between PAH concentrations and extract mutagenicity is modest (R= 0.64) but surprising since the correlation coefficients between individual PAH and mutagenicity were lower by a factor of two (Table 17) and since the PAH monitored had no mutagenic activity in *S. typhimurium* strain YG1021 (-S9).

The analysis of relationships between more than two variables at a time required further data processing through the determination of eigenvectors and eigenvalues for the correlation matrix, which gave the factor matrix (Table 20). The values in the factor matrix were used to construct a three-dimensional factor loading plot (Figure 32), *i.e.*, a graph of the factors or principal components, which are linear, algebraic combinations of the experimental variables analyzed. Clustering of the points representing different variables indicates a close correlation between those variables, while points which are widely separated show little or no correlation.

The factor loading plot is a three-dimensional representation of a four dimensional factor analysis. Thus, the view in Figure 32, while judged to be the best view of this plot, has some juxtapositions of variables which are circumstantial. The correlation coefficients

Table 20.

Factor Matrix of 17 Variables Monitored for Hamilton Air Particulate Extracts.

Variable	*Factor 1*	*Factor 2*	*Factor 3*	*Factor 4*
COH	0.87			
NO_2	0.83			
ΣPAH	0.83		0.44	
lmut	0.82			
ΣtPAH	0.79		0.54	
API	0.76			
TRS	0.75			
ΣoPAC	0.74		0.44	
SO_2	0.72			
windspeed	-0.71			-0.43
nmhc	0.69			
ΔT	0.66			
NO	0.62		-0.43	
lmass	0.61	0.55		
T (°C)		0.84		
O_3	-0.42	0.79		
rh		-0.43		0.71

*Abbreviations: COH, coefficient of haze; ΣPAH, total monitored PAH; lmut, logarithm of mutagenicity of the non-polar aromatic fraction; ΣtPAH, total monitored thiaPAH; API, air pollution index; TRS, total reduced sulfur; ΣoPAC, total monitored oxyPAC; nmhc, non-methane hydrocarbons; ΔT, difference in air temperature at heights of 33 and 300 feet; lmass, logarithm of the mass of particulate collected, T (°C), air temperature at a height of 33 feet; rh, relative humidity.

Factors of less than or equal to 0.40 are not shown in this Table or the factor plot (Figure 32).

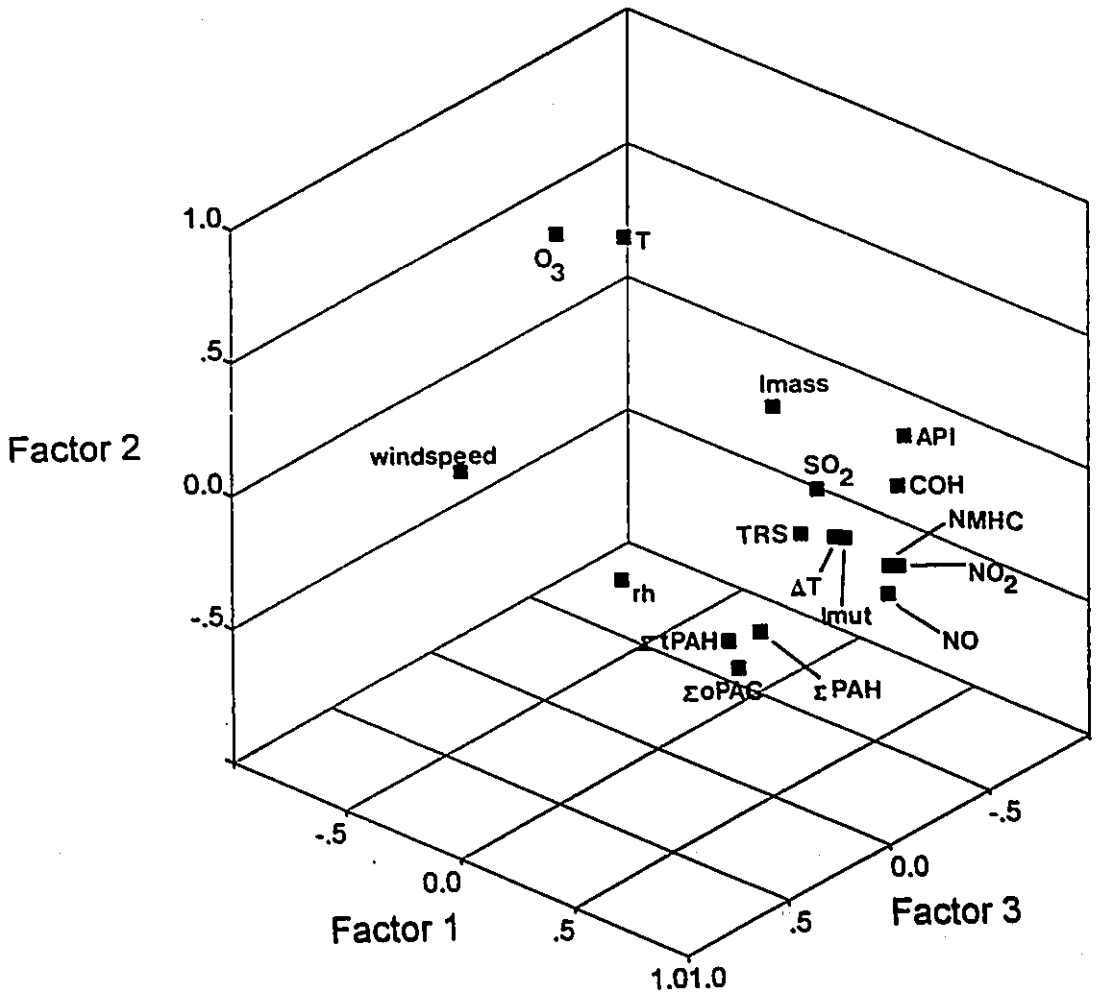


Figure 32. Three-dimensional factor loading plot derived from the principal component analysis of meteorological, mutagenicity and PAC data. Abbreviations are explained in Table 20. Clustering of the data points indicates close correlation, but absolute distance between the data points shown is not directly proportional to correlation coefficient in this particular projection. E.g., points labelled lmut, ΔT, NMHC and NO_2 appear to be overlapping due to the perspective of the plot but are actually separated in three-dimensional space.

(Table 19) provide the best indicators of relationships. Nonetheless factors which are poorly correlated or anti-correlated will appear to be far apart in this factor plot.

Windspeed, ozone concentration, ambient temperature and relative humidity had no relationship with any other parameters measured, as evidenced by the isolation of the points representing these variables (Figure 32). However, the very close correlation between concentrations of total monitored PAH, oxyPAC and thiaPAH is seen by the clustering of points representing these variables. All of the parameters which rise and fall in unison due to the trapping of pollutants during thermal inversions (mass of particulate collected, API, SO_2 concentration, COH, NMHC, NO and NO_2, TRS and ΔT) are loosely correlated with mutagenicity. The apparent overlap between points representing ΔT and mutagenicity is an illusion of perspective resulting from the projection of a four-dimensional matrix into two dimensions as described above. These points which appear to overlap are actually fairly well separated and therefore weakly correlated (Table 20). Thus, principal component analysis has identified the variables which are totally uncorrelated, loosely correlated or intimately correlated with PAC concentrations, mutagenicity and atmospheric conditions.

IV.7.5 Chronology of PAC Concentrations, Mutagenicity and Atmospheric Data

Principal component analysis indicated which variables were related or unrelated with each other but gave no sense of the variability of these parameters over time. Thus,

variables which showed the highest correlation coefficients and are therefore of greatest interest to this study (mutagenicity, NO_2 and SO_2 concentrations, particulate mass and total PAC concentrations) were plotted for the 68 days studied between May 1990 and June, 1991 (Figure 33A-E). Mutagenicity and PAC concentrations were both at their highest and lowest values in the spring and winter, respectively. Mutagenicity values were especially high during 'pollution episodes' arising from thermal inversions (Figure 33A). Sulfur dioxide concentrations (Figure 33C) and particulate mass values (Figure 33D) also followed this trend, but the differences between high and low values were not as dramatic. Nitrogen dioxide concentrations (Figure 33B) did not show a seasonal trend at all, apart from the high values observed in April and May of 1991. All variables (except for NO_2) showed large differences for consecutive sampling days.

The high and low values in the chronological plots (Figure 33) may simply reflect the effect of springtime thermal inversions and cold winter temperatures, respectively. Mutagenicity (Figure 33A) may have depended on the atmospheric transformation of PAH to nitroPAH, which was maximized on warm spring days during thermal inversions, when fluoranthene and pyrene were abundant in the gas phase and ozone and sunlight were readily available. Thus, atmospheric nitration of fluoranthene and pyrene to produce 2-nitrofluoranthene (Figure 4) and 2-nitropyrene might be thought to increase the mutagenicity of the non-polar aromatic fraction. Cold winter temperatures may have discouraged inversions and decreased the amount of gas-phase PAH available for nitration.

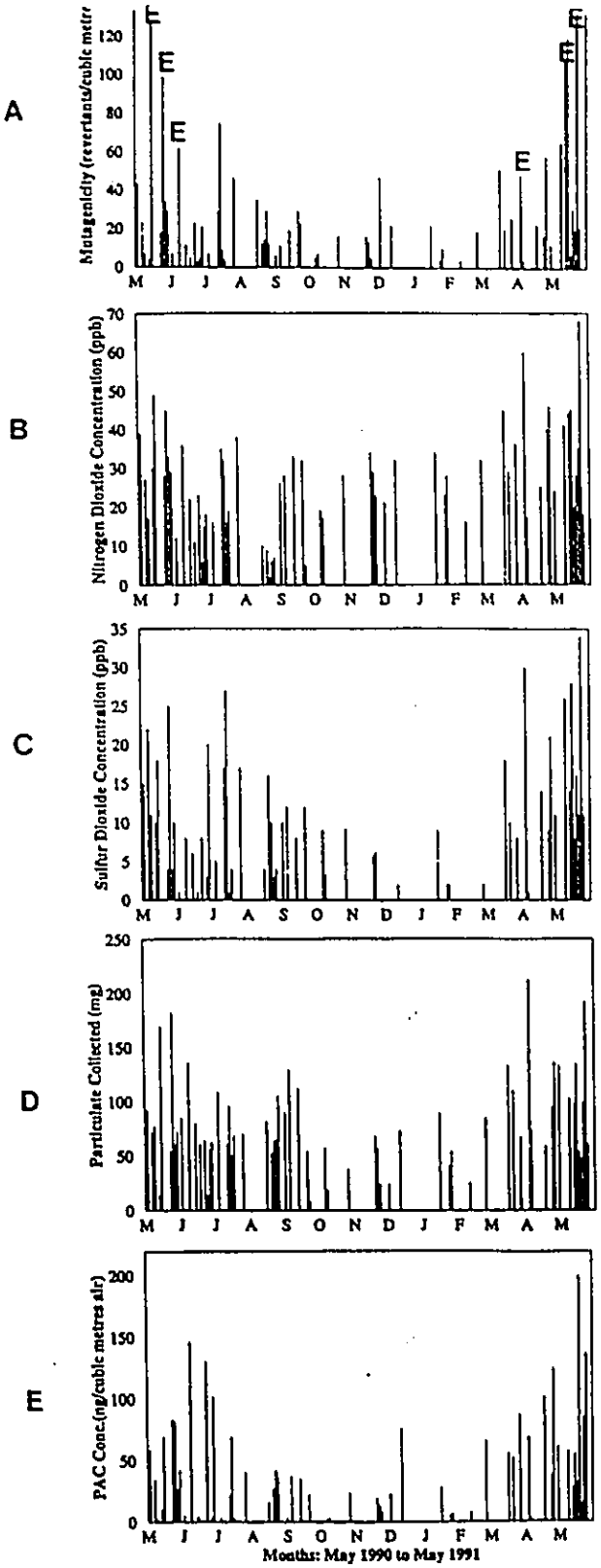


Figure 33. Plots of (A) mutagenicity, (B) nitrogen dioxide, (C) sulfur dioxide, (D) mass of particulate collected and (E) total PAC over the sampling period in Hamilton, May 1990 to May 1991. Pollution events, as defined by the MOEE, are signified by an 'E'.

Nitrogen dioxide concentrations (Figure 33B) were fairly constant all year long, since nitrogen dioxide arose primarily from oxidation of combustion-derived nitric oxide (1). High nitrogen dioxide concentrations did not assure high nitroPAH concentrations, however; excess cloud cover may have led to decreased hydroxyl radical formation and precipitation (not monitored in this study) may have washed particulate and adsorbed nitroPAH out of the air.

Sulfur dioxide concentrations reached their highest values in the spring during thermal inversions (Figure 33C). However, PAH do not react with ambient concentrations of sulfur dioxide (26). Good correlation between sulfur dioxide concentrations, PAC and mutagenicity over time may have been the result of simultaneously generated PAC and sulfur dioxide emissions from steel plants, which are responsible for most of Hamilton's sulfur dioxide (105-108).

Particulate masses were highest during springtime thermal inversions, but factors other than atmospheric conditions may have increased particulate mass (e.g., local auto traffic, wind-blown dust), hence correlation with atmospheric conditions was poor (Figure 33D).

IV.7.6 Correlations Between Wind Direction, Atmospheric Conditions and Mutagenicity

The relationships between wind direction, atmospheric conditions and mutagenicity were examined using spherical coordinate plots; wind direction was measured from 0° to

360°. These experiments were carried out to see if increased mutagenicity or particulate loading could be correlated to particulate sources, such as Hamilton's steel industry. Plots were prepared by Dr. Bill Morris, to whom we are most grateful.

Spherical coordinate plots show the relationship between wind direction and the concentrations of SO_2, NO_2, and O_3 on the day of collection (Figures 34A, 34B and 34C). The position of each data point with respect to the centre of the plot represents the average direction from which the wind was blowing over the 24-hour collection period. Each quadrant of the plot is identified using a number between 1 and 4. The distance of each data point from the centre of the graph is directly proportional to the amount of that pollutant measured. The number in square boxes near the concentric rings represent the average pollutant concentration in ppb.

Figures 34A and 34C show that high concentrations of SO_2 and low concentrations of O_3 are observed in quadrants 1 and 2, i.e., in winds arising to the east of the downtown Hamilton sampling site, possibly from Hamilton's industrial area. Quadrants 3 and 4 show lower levels of NO_2 and SO_2 combined with higher levels of O_3, with the highest O_3 concentrations observed in winds arising from the southwest. Previous studies have indicated that high concentrations of ozone arrive in Hamilton from the Ohio Valley, which is southwest of Hamilton (100).

Spherical coordinate plots were also prepared showing wind direction versus mass of particulate collected and mutagenicity of the non-polar aromatic fraction obtained from the particulate extract. These plots appear in Figures 35A and 35B, where windspeed is

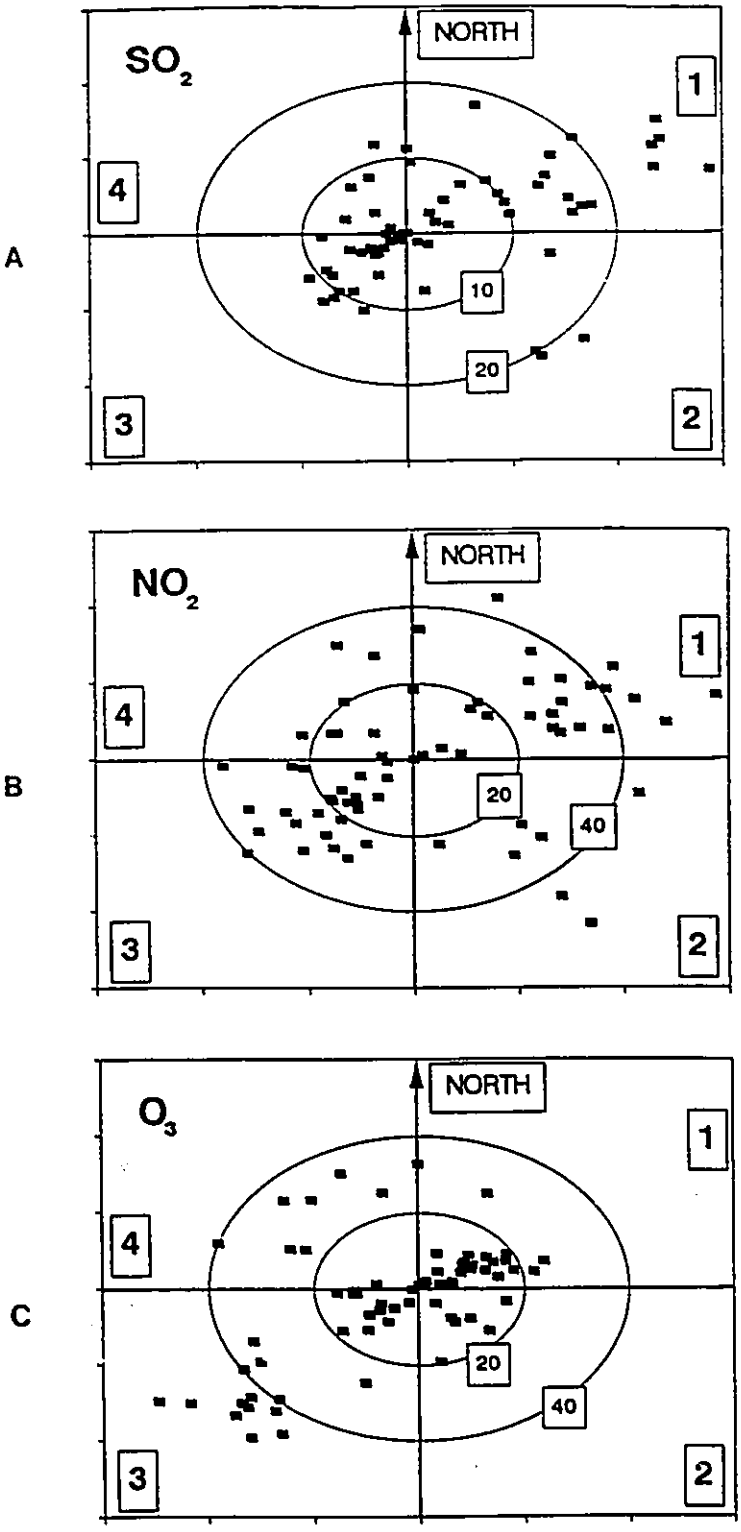


Figure 34. Spherical coordinate plots of wind direction versus average (A) SO_2, (B) NO_2 and (C) O_3 concentrations. Direction and distance from the centre of the plot represent wind direction and gas concentration, respectively. Reproduced, with permission, from the report by Morris *et al.* (161).

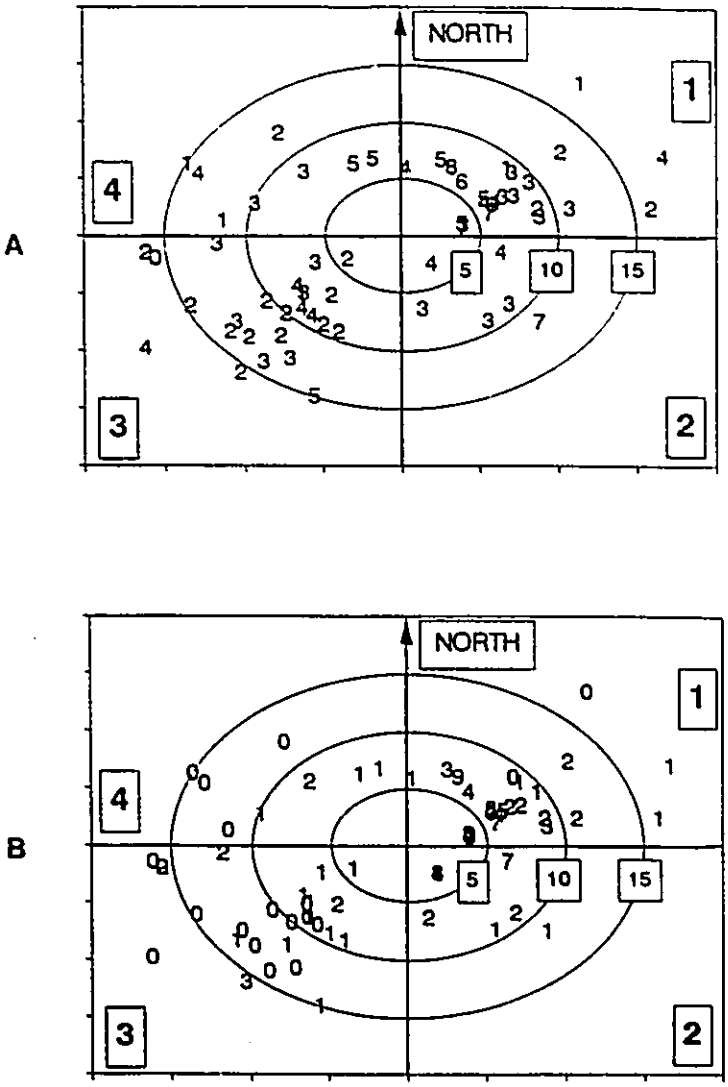


Figure 35. Spherical coordinate plots of wind direction versus (A) mass of particulate collected and (B) mutagenicity of the non-polar aromatic fraction of the particulate extract. Direction from the centre of the plot represents wind direction and distance from the centre of the plot represents the windspeed. The number symbolizing each data point represents the subdivision of (A) particulate mass and (B) mutagenicity into linearly increasing bins. Reproduced, with permission, from the report of Morris *et al.* (161).

indicated by the distance from the centre of the plot and the angle shows direction. Each data point in these plots is represented by a number; these numbers increase in value with increasing particulate mass (Figure 35A) or increasing mutagenicity (Figure 35B). A data point symbolized by a '7' has roughly seven times more particulate mass or mutagenicity than a data point labelled '1'. The highest particulate loadings are observed during low wind speeds, while wind speeds >8 km/h result in decreased particulate loading. Some filters collected on days with high windspeeds had high particulate loading, but the non-polar aromatic fractions of the extracts of these filters did not show high mutagenicity. A possible cause of the high particulate mass in these cases is wind-entrained soil. The representation of the mutagenicity data is even more dramatic. Figure 35B shows that the highest mutagenicity is associated with slow winds from the east. Hamilton's foundries and other heavy industries are about 1.5 - 4 km northeast of the sampling site and slow winds are typical of pollution episodes or thermal inversions.

The relationship between mutagenicity and wind direction was further explored through the extraction, cleanup and bioassay of 4 filters collected at the Westdale sampling site on days when samples were also collected in downtown Hamilton. Data for the 8 filters collected on these four days (Table 21) show that PAC concentrations at both sites on the same days were very similar. However, the mutagenicity values differed by an order of magnitude when the wind blew from the west or southwest. When the wind blew from the opposite direction and during a pollution event, the mutagenicities at the two sites were similar. More data is needed to draw any firm conclusions.

Table 21.

Mutagenicity and PAC Concentrations for
Air Particulate Collected on Identical Dates at Two Different
Sampling Sites in Hamilton.

Date	*Wind Direction & Speed*	*Sampling Site*	*Particulate Mass (mg)*	*Total PAC Conc. (ng·m^3)*	*Mutagenicity of Non-Polar Fraction* ** *(revs·m^3)*
1990 08 26	SW, 9 km/h	W.Hamilton	99.1	1.27	2
		Downtown	107.1	1.01	12
1990 09 23	W, 16 km/h	W.Hamilton	4.1	0.48	1
		Downtown	8.5	0.53	23
1990 10 09	NE, 18 km/h	W.Hamilton	13.6	3.21	5
		Downtown	19.3	4.30	7
1991 04 26*	SE, 3 km/h	W.Hamilton	90.8	125.12	50
		Downtown	107.9	64.32	59

* Pollution event (thermal inversion).

** Mutagenicity of fraction A23/LH20 determined using *S. typhimurium* YG1021 (-S9).

IV.7.7 Conclusions

On average, particulate-phase PAC concentrations in Hamilton were below the levels reported in other cities around the world. The range of PAC concentrations observed in all samples was over 500-fold and day-to-day fluctuations were as large as 130-fold. The levels of three thiaPAH and four oxyPAC rose and fell in concert with the PAH concentrations. Heavy industry in the east end of Hamilton was a significant contributor to PAC levels, as indicated by the analysis of wind direction data. During thermal inversions, the concentrations of pollutant gases, particulate material and PAC increased dramatically.

The mutagenic potency of these extracts showed a 140-fold range. The ratio of the responses in the non-polar aromatic and polar aromatic fractions was about 3:1; as the mutagenic potency of extracts increased, the proportion of the mutagenicity associated with the non-polar aromatic fraction increased to about 10:1. Thus, during pollution events the non-polar aromatic fraction is of greatest genotoxic concern. Further work in this thesis will focus on the non-polar aromatic fraction.

Principal component analysis showed that the mutagenic potency of the non-polar aromatic fraction was strongly correlated with increased levels of nitrogen dioxide and sulfur dioxide. This analysis also showed that mutagenic potency was [a] not directly correlated with PAH concentrations and [b] inversely correlated with wind speed and ozone concentrations. High nitrogen dioxide concentrations were shown to be associated with increased levels of airborne mutagens and may result in increased atmospheric

transformation of non-mutagenic compounds to mutagens. On the other hand, high ozone concentrations were shown to be associated with decreased levels of airborne mutagens; atmospheric reactions between ozone and PAC may produce less mutagenic compounds.

Most importantly, PAC concentrations in respirable air particulate have been extensively correlated with routinely measured atmospheric parameters and with the genotoxicity of air particulate extract for the first time.

V. QUANTITATION AND BIOASSAY OF NITROPAH IN HAMILTON AIR PARTICULATE, 1990-1991

V.1 Overview

This chapter will focus on the chromatographic separation of non-polar aromatic fractions into subfractions containing different classes of PAC for the purpose of bioassay analyses and the quantitation of nitroPAH by GC-MS. An analysis of the relationship between mutagenicity, atmospheric conditions and nitroPAH concentrations, as well as attempts to identify and quantify other mutagenic compounds, will also be discussed.

V.2 Introduction

The atmospheric transformation of PAH into mutagenic nitroPAH during episodes of high concentrations of oxides of nitrogen (NO and NO_2) has been shown to be responsible for a substantial portion of the mutagenicity of non-polar aromatic fractions obtained from the extracts of air particulate collected in Los Angeles (12,162). However, the relationship between extract mutagenicity in *S. typhimurium* TA98 (-S9), concentrations of nitroPAH and oxides of nitrogen was not explored in detail by these authors.

In the present work, increased nitrogen dioxide concentrations coincided with increased mutagenicity of non-polar aromatic fractions prepared from Hamilton air particulate using *S. typhimurium* YG1021 (-S9); this strain which contains extra copies of the gene for nitroreductase is expected to be sensitive to atmospherically-produced

nitroPAH (Chapter IV). Hence, nitroPAH were suspected of being responsible for at least some of the mutagenicity of Hamilton air particulate extracts. A detailed examination of the relationship between nitroPAH, the mutagenic activity caused by these compounds and the atmospheric conditions responsible for nitroPAH formation necessitated the quantitation of nitroPAH in some of these samples.

The main chemical interference to nitroPAH quantitation is the presence of PAH at approximately 100-fold higher concentrations; dinitroPAH may be responsible for biological responses that might be attributed to nitroPAH. While dinitroPAH have been found at levels approximately 30-50 times lower than nitroPAH in air particulate (Table 1), their mutagenic potencies are orders of magnitude greater than nitroPAH (Table 8). The normal phase HPLC protocol described in Chapter III has been used to separate PAH, nitroPAH and dinitroPAH in extracts of steel foundry dust (37) and extracts of Hamilton air particulate (50). Reversed-phase HPLC methods have not proven to be as effective as normal-phase HPLC in the separation of compound classes although RPLC can be used to separate individual compounds in chromatographic fractions generated by normal-phase HPLC.

Thus, the normal-phase chromatography of non-polar aromatic fractions of air particulate extracts was used to prepare PAH, nitroPAH and dinitroPAH subfractions as well as other subfractions containing polycyclic aromatic ketones and azaPAC. Bioassays were used to determine the mutagenic potencies of these subfractions and GC-MS analyses were used to identify and quantify individual compounds within each subfraction.

Previous studies (50,51) of Hamilton air particulate showed that the sum of mutagenic potencies of the NPLC subfractions was approximately equal to the potency of the non-polar aromatic fraction. The generality of this observation will be investigated through the preparation of NPLC subfractions from twenty different non-polar aromatic fractions, collected over a wide variety of atmospheric conditions.

V.3 Chemical & Biological Analyses of NPLC Fractions Prepared from Individual Non-Polar Aromatic Fractions

V.3.1 Preliminary Experiments

Before a set of non-polar aromatic fractions were selected for normal-phase cleanup, bioassays and nitroPAH quantitation, the experimental methodology was applied to three selected non-polar aromatic fractions of low, medium and high mutagenic potencies. These experiments were designed to test the sensitivity of the instrumental and mutagenic responses. Non-polar aromatic fractions were prepared from [a] particulate of high mutagenicity (130 revs/m^3) collected during a thermal inversion/pollution event on May 23rd, 1991; [b] particulate of moderate mutagenicity (17 revs/m^3), collected April 24th, 1991 and [c] two particulate samples with low mutagenicities (3 and 4 revs/m^3) collected June 24th, 1990 and July 18th, 1990, respectively. The last two samples were combined to supply a sufficient amount of nitroPAH for both chemical and biological analyses.

Previous chemical and biological analyses of these non-polar aromatic fractions (Chapter IV) had left less than half of the original samples available for further use. For

the sample collected on May 23rd, 1991, only 800 m^3 equivalent of the original 1630 m^3 remained, all of which was subjected to NPLC fractionation. Similarly, 800 m^3 equivalent of the sample collected April 24th, 1991 and the combined sample of June 24th/July 18th were fractionated by NPLC. The solvent gradient used in these experiments (gradient C, section IX.12) successfully separated each non-polar aromatic fraction into PAH, nitroPAH and dinitroPAH subfractions, referred to as N1, N2 and N3, respectively (Figure 36). Two polar subfractions were also prepared: N456, which contained polar PAC such as benzanthrone and 2-nitrodibenzopyranone, and N7, which contained highly polar PAC. These experiments were performed using a solvent gradient which did not separate the PAH on the basis of molecular mass, unlike the subsequently developed method described previously (gradient A, section IX.12, which was used in the NPLC fractionation of SRM1650, section III.6.2).

Figure 36 shows the chromatographic profiles of each of the three NPLC analyses and zones of collection of the N fractions. Similarities in the chromatographic profiles and the reproducibility of retention times were observed for the three analyses. The UV absorbance was highest for the most mutagenic sample and lowest for the least mutagenic sample, although the amount injected (800 m^3 equivalent) for all 3 samples was identical.

Bioassays of the N fractions were carried out using *S. typhimurium* YG1021 (-S9); the results (Table 22) indicated that at least 60% of the non-polar aromatic fraction's mutagenicity arose from compounds present in fraction N2, the nitroPAH-containing fraction. Fractions N1 and N3 had mutagenic activities close to or below the level of

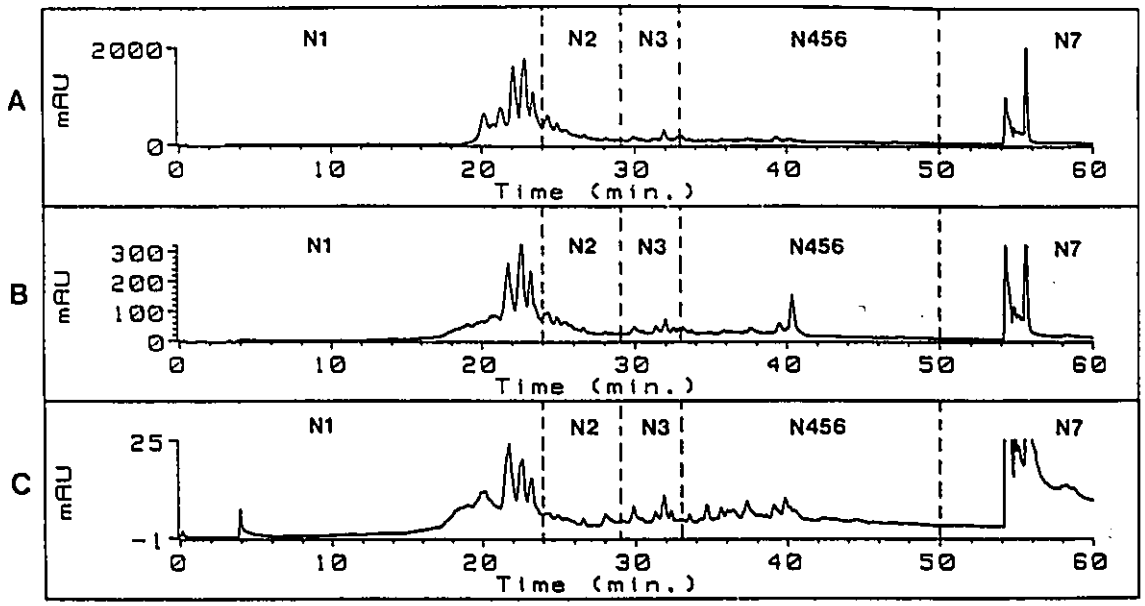


Figure 36. Normal-phase HPLC chromatograms of non-polar aromatic fractions prepared from particulate collected (A) 1991 05 23 and (B) 1991 04 24. The NPLC chromatogram of a non-polar aromatic fraction prepared from the combined extract of particulate collected on 1990 06 24 and 1990 07 18 appears in (C). Separation was carried out using gradient C. Dashed lines indicate zones of collection for PAH (N1), nitroPAH (N2), dinitroPAH (N3) and other, more polar fractions.

Table 22.
Mutagenic Activities of N Fractions Prepared From Three
Non-Polar Aromatic Fractions of Hamilton Air Particulate Extract.

Date of Sample Collection	*Mutagenicity of Fraction A23/LH20 Before NPLC Fractionation*	*Mutagenicity of N Fractions (Revertants m^3)*						*Mutagenicity of Reconstituted N Fractions*[****]
		N1	*N2*	*N3*	*N456*	*N7*	*Σ*[***]	
1991 05 23	130 revs/m^3	nr[**]	100	nr	35	6	141	130
1991 04 24	17	nr	16	1	8	2	27	not determined
1990 06 24+ 1990 07 18[*]	4	nr	2	nr	1	nr	3	not determined

[*] combined samples

[**] nr signifies 'no response', i.e., no doubling of revertant colonies with a top dose of 60 m^3 equivalent of each fraction, using *S. typhimurium* strain YG1021 (-S9). Detection limit for this set of bioassays: 0.5 revertants/m^3

[***] Mathematical sum of N-fraction mutagenic activities

[****] Equal aliquots of fractions N1 through N7 were mixed and the resulting mixture was subjected to bioassay. There was insufficient material remaining to perform analyses of the lower activity samples.

detection for this set of bioassays. The low activity associated with fraction N3 showed that the contribution of dinitroPAH to the mutagenicity of these samples was insignificant.

The recombination of aliquots of the N fractions from the most mutagenic sample afforded a reconstituted non-polar aromatic fraction whose mutagenicity was equal (140 revertants/m^3) to that of the unfractionated non-polar aromatic fraction (130 revertants/m^3). This result showed that there was no evidence for inhibitory or synergistic effects in this sample. The absence of inhibitory and synergistic effects in the bioassays of chromatographic fractions of air particulate extracts has been observed before in this research group (50) and in other laboratories (163).

Fraction N2 was subjected to further chromatographic separations to identify the compounds responsible for the biological activity, using reversed-phase HPLC (two 10 µm Vydac 201TP columns in series utilizing gradient C, section IX.13). The retention times of various standards using this method (Table 23) showed that more polar PAC eluted first and the non-polar PAC eluted later. Furthermore, while this RPLC method separated PAH from nitroPAH, it could not separate the five nitrofluoranthene and two nitropyrene isomers (data not shown). Retention time precision was good, although standard deviations increased for compounds with longer retention times.

The reversed-phase HPLC chromatogram of fraction N2 from the most mutagenic sample (collected May 23rd, 1991, Figure 37A) shows a peak with a retention time of 15.83 minutes, which was identified on the basis of its UV-visible spectrum and RPLC retention time as 2-nitrofluoranthene (Figure 37B). The peaks between 30 and 50 minutes

Table 23.

Reversed Phase HPLC Retention Times and Standard Deviations for Various PAC.

Compound	*Retention time (min.)*	*Absolute Standard Deviation (min.)*
1,2-Dihydroxybenzene	6.22	0.01
p-Aminophenol	6.53	
p-Nitrophenol	6.67	0.11
α-Naphthoic acid	6.74	0.01
3,5-Dimethylphenol	7.38	0.02
Quinoline	8.36	
1,5-Dinitronaphthalene	8.77	0.16
Carbazole	9.16	0.02
1-Nitropyrene	9.46	
1-Aminoanthracene	9.69	0.01
Fluorenone	9.81	0.03
Xanthone	9.88	
Anthraquinone	10.22	0.03
2-Aminopyrene	11.10	
9-Acetylphenanthrene	11.36	
Isoquinoline	11.50	0.01
Dibenzofuran	12.39	0.01
5,6-Benzoquinoline	12.76	
Benzanthrone	13.56	
Dibenzothiophene	13.94	0.03
Phenanthrene	14.12	0.09
Anthracene	15.01	0.11
Acridine	15.02	0.02
Dibenzo[a,i]carbazole	15.74	0.01
2-Nitrofluoranthene	15.83	
3-Nitrofluoranthene	15.91	
Fluoranthene	16.39	0.13
Pyrene	17.47	0.14
Benzo[a]anthracene	20.43	0.15
Chrysene	20.78	0.23
Benzo[j]fluoranthene	23.26	0.27
Benzo[k]fluoranthene	24.94	0.32
Benzo[a]pyrene	26.48	0.45
Picene	31.96	0.52

Columns: two Vydac 201TP columns in series & solvent gradient C (section IX.13).
S.Ds are for 3 consecutive injections; if no S.D. is given, standards were injected once.

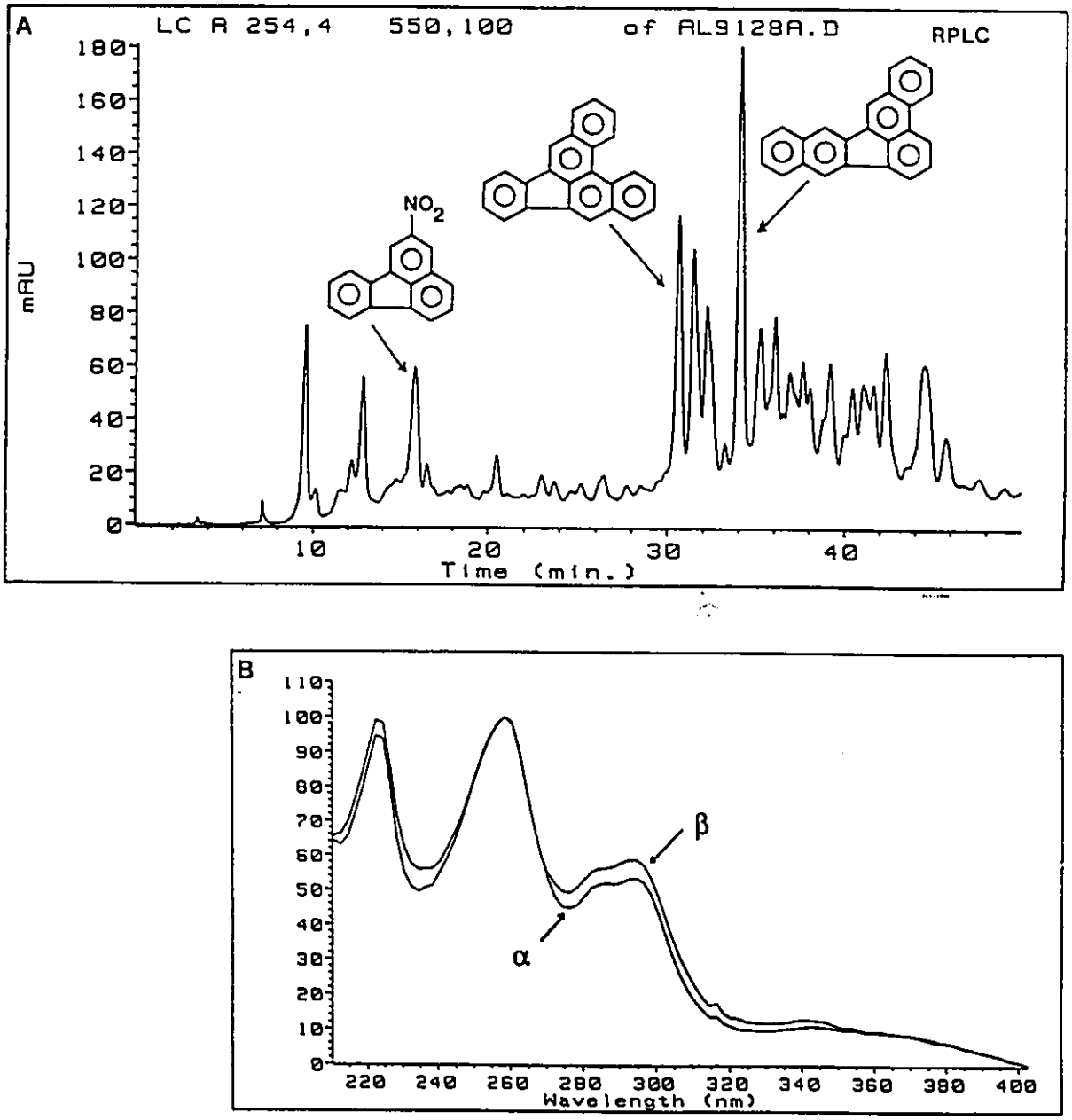


Figure 37. (A) Reversed-phase HPLC chromatogram (using gradient D) of the N2 fraction obtained from particulate collected 1991 05 23. Three peaks were identified on the basis of UV spectra as (from left to right): 2-nitrofluoranthene, dibenzo[b,e]fluoranthene and dibenzo[b,k]fluoranthene. (B) UV spectra of α, authentic 2-nitrofluoranthene and β, the peak in Figure 37A with a retention time of 15.83 minutes.

elution time in Figure 37A correspond to high molecular mass PAH, which are well separated from the nitroPAH. UV spectral matches were made for 302 amu dibenzo[b,e]fluoranthene and dibenzo[b,k]fluoranthene, compounds often associated with air particulate (41,164).

The chromatographic eluate from the RPLC analysis (Figure 37A) was collected in one-minute subfractions and one quarter of each subfraction was bioassayed in duplicate using *S. typhimurium* strain YG1021 (-S9). The resulting data was plotted to yield a mutation chromatogram or mutachromatogram (Figure 38). One particularly potent fraction (labelled 'b') with a retention time of between 16 and 17 minutes, corresponded to the peak identified as 2-nitrofluoranthene.

A portion of each fraction (labelled 'a' to 'd', Figure 38) was analyzed by GC-MS in the selected-ion monitoring mode. The atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene were identified and quantified in these three fractions (Figure 39 and Table 24) on the basis of their GC retention times and fragmentation patterns, which matched those of authentic standards. Except for 2-nitrofluoranthene and 2-nitropyrene, other nitroPAH and methylnitroPAH derived from PAH of molecular masses 202, 228 and 252 were below the detection limit in all three N2 fractions. The GC-MS data presented in Table 24 was only preliminary, however; low sensitivity of the GC-MS instrument used for these initial experiments often resulted in concentrations of 1-nitropyrene and 2-nitropyrene below the detection limit of these experiments.

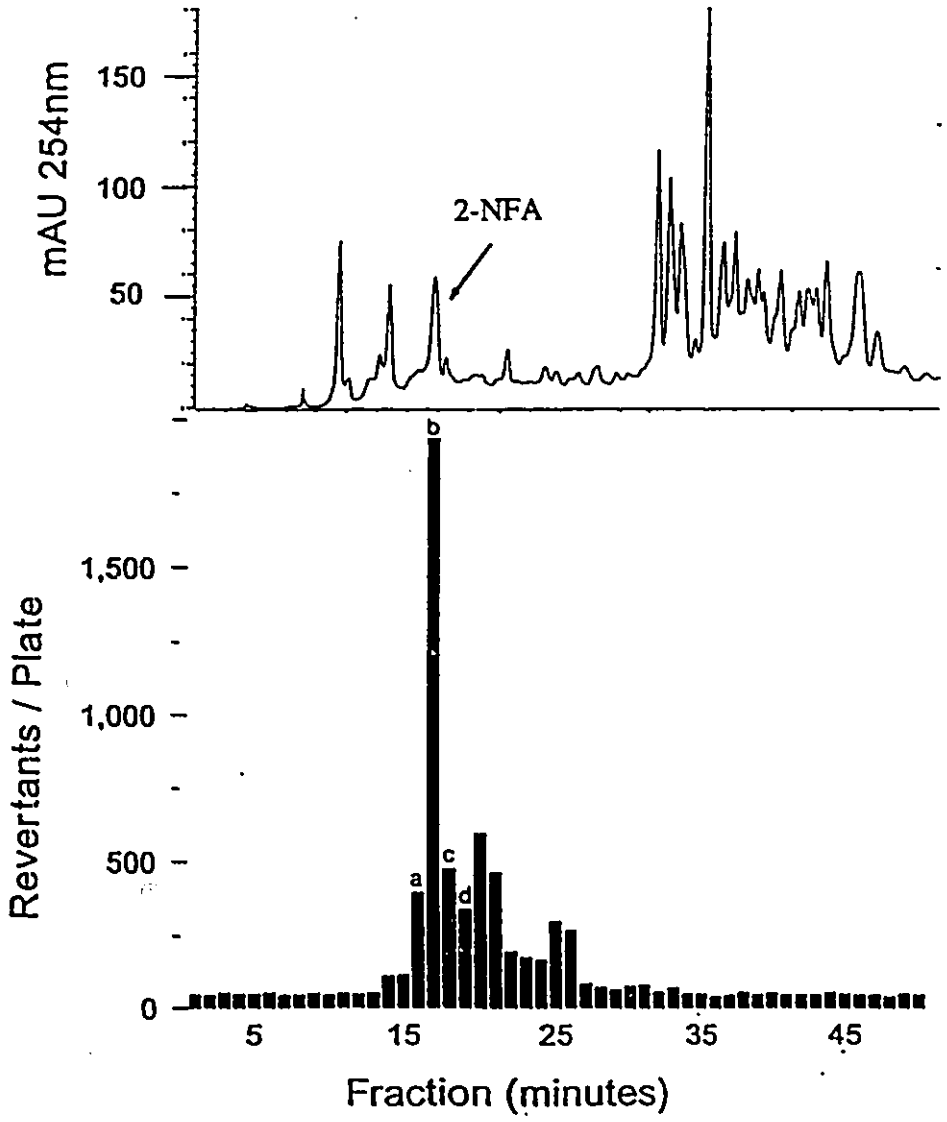


Figure 38. Mutachromatogram of an N2 fraction obtained from air particulate collected 1991 05 23. An amount of extract equivalent to 400 m^3 of air was analyzed by reversed phase HPLC (see Figure 37) and separated into one-minute fractions. One-fourth of each fraction was tested in duplicate with *S.typhimurium* YG1021(-S9); the average of the two experiments is shown above. The results of GC-MS analyses of fractions a, b, c and d appear in Figure 39.

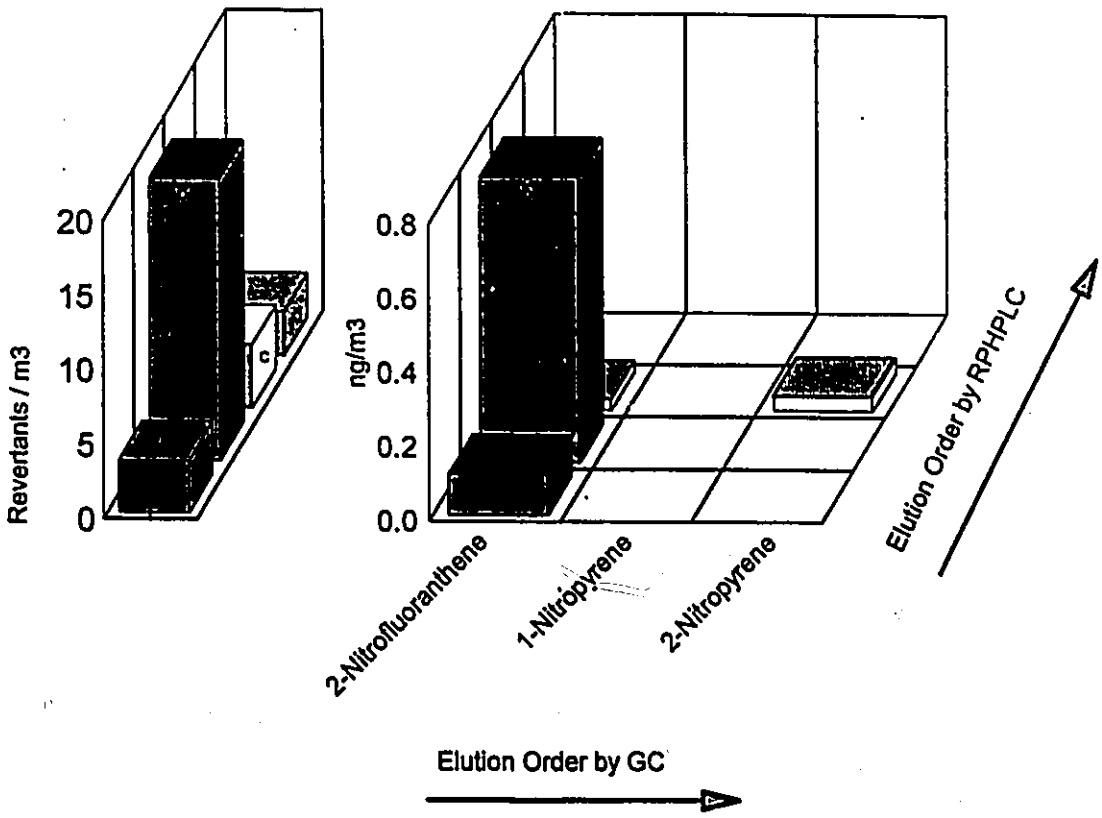


Figure 39. Mutagenicity and nitroPAH concentrations determined by GC-MS from individual one-minute fractions, prepared from the N2 fraction of particulate collected 1991 05 23.

Table 24
A. Mutagenic Activities of Selected RPLC Fractions,
Obtained From Particulate Collected 1991 05 23.

RPLC Retention time (min.)	*Conc. of Analytes (pg/m³)** 2-NFA	1-NP	2-NP	*Mutagenic Activity (revs / plate)***	*Calculated Mutagenicity of Analytes (revs/plate)*	*Percentage of Mutagenicity Accounted for By Analytes*
15.00-15.99	110	nq	nq	353	352	100%
16.00-16.99	770	nq	nq	1890***	2460	130%
17.00-17.99	30	nq	40	434	304	70%
18.00-18.99	nq	nq	nq	296	0	0

B. NitroPAH Concentrations Determined in N2 Fractions.

Date of Sample Collection	*Conc. of Analytes (pg/m³)* 2-NFA	1-NP	2-NP	*Mutagenic Activity (revs/m³)* Determined	Calculated	*Percentage of Mutagenicity Accounted for By Analytes*
1991 05 23	910	nq	40	3	0.30	10%
1991 04 24	240	nq	20	17	9.2	55
1990 06 24+ 1990 07 18†	10	nq*	nq	130	32	25

*75 m^3 equivalent injected per sample. 'nq' signifies concentrations below the method detection limit of 10 pg/m^3

**100 m^3 applied to each plate, in duplicate. Number of *S. typhimurium* YG1021 (-S9) revertants is the average of two determinations.

***Probably an underestimate due to bacterial toxicity.
† Combined sample

Concentrations of two nitroarene products (2-nitrofluoranthene and 2-nitropyrene, Table 24) are only two-fold higher than values reported elsewhere during pollution episodes (162,165). The concentration of 2-nitropyrene is somewhat lower than levels reported in Europe during typical sampling conditions (64,166). NitroPAH concentrations are compared with literature values in greater detail later, when more samples have been analyzed.

The mutagenicity of authentic 2-nitropyrene (18,800 revs/nmol) and 2-nitrofluoranthene (7,900 revs/nmol) in *S. typhimurium* YG1021 (-S9) as determined in this laboratory accounted for between 10% and 55% of the mutagenic activity of the non-polar aromatic fraction (Table 24B).

Bioassay-directed fractionation of a fairly mutagenic sample of air particulate extract confirmed the hypothesis that atmospheric transformation products appeared to be largely responsible for the mutagenic potency of some air particulate samples collected in Hamilton. Concentrations determined for nitroPAH fell within the range described in the literature and 24-hour collections of particulate in Hamilton could be used to provide both bioassay and quantitative GC-MS data for nitroPAH. Intensive exploitation of a single day's collection of air particulate extract in this manner has not been reported previously in the literature.

NPLC cleanup was sufficient to remove the PAH in fraction N1 and the dinitroPAH in fraction N3, affording fraction N2 with the atmospheric transformation products. GC-MS, using electron impact ionization and detection in the selected-ion

monitoring mode could therefore be used to analyze fraction N2 for the targeted nitroPAH. Routine RPLC separation of high molecular mass PAH from nitroPAH for subsequent samples was not considered necessary.

V.3.2 Selection of Additional Non-Polar Aromatic Fractions for NitroPAH Quantitation and Determination of N-Fraction Mutagenicity

Seventeen additional non-polar aromatic fractions were selected for bioassay and the quantitation of nitroPAH (Table 25). Normal-phase HPLC was used to prepare N fractions from each of the selected samples. Fractions N1 to N7 were bioassayed with *S. typhimurium* YG 1021(-S9) and nitroPAH were quantified in fraction N2 by GC-MS. The GC-MS protocol was altered to increase the sensitivity for detection of nitroPAH. The non-polar aromatic fractions selected for NPLC fractionation were chosen to include samples of various mutagenic potencies prepared from air particulate collected over a wide range of atmospheric conditions over the entire sampling period.

V.3.3 Normal-Phase HPLC Analyeis of Seventeen Non-Polar Aromatic Fractions

The 17 non-polar fractions selected for nitroPAH quantitation were subjected to the same type of NPLC cleanup method (gradient B, section IX.12) as was used for SRM1650 (section III.6.2) with a 5 μm Whatman polyaminocyano column.

Figure 40 shows a representative NPLC chromatogram with the zones of collection for fractions N1 and N2. Occasionally, fraction N3 was collected together with fraction N456. Several N7 fractions were often allowed to accumulate on the normal phase column

Table 25.
Dates of Collection and Mutagenicities of Non-Polar Aromatic Fractions Selected for N-Fraction Bioassays and NitroPAH Quantitation.

Date of Sample Collection	*Mutagenicity (S. typhimurium YG1021-S9)*
1991 02 11	4 revertants/m^3
1990 10 07	5
1990 08 22	14
1990 11 20	16
1990 06 22	23
1991 04 18	23
1991 05 07	23
1991 05 19	31
1990 12 02	47
1991 04 04	49
1991 04 26	59
1990 06 08	64
1990 05 09	66
1990 07 14	76
1991 05 13	110
1991 05 15	120
1990 05 15	130

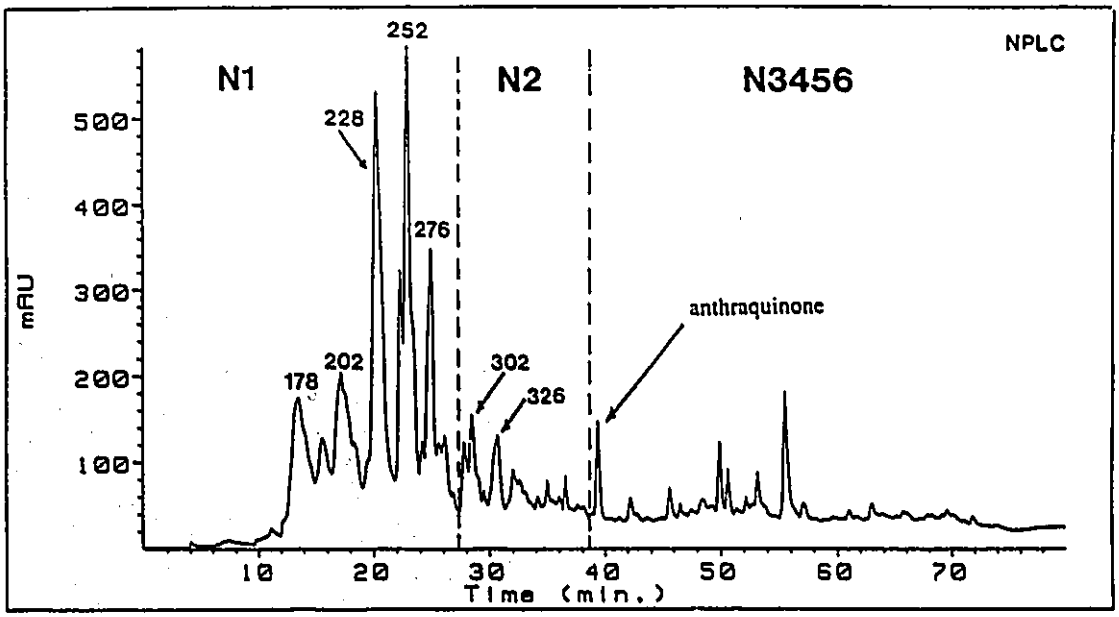


Figure 40. Representative normal-phase HPLC chromatogram of a non-polar aromatic fraction from Hamilton air particulate collected 1990 05 07, showing zones of collection for PAH (N1), nitroPAH (N2) and other fractions. Peak labels indicate PAH molecular weights, as determined by comparison of UV-visible spectra with authentic standards.

and then eluted as a single, pooled N7 fraction.

Results of the bioassays carried out on the various N fractions prepared from 17 different extracts of air particulate appear in Table 26. Fractions N1 and N7 revealed consistently low mutagenic activities in strain YG1021 (-S9) in all 17 samples. Fractions N2 and N3456 contained almost all of the direct acting mutagenic activity with the majority of the response normally in fraction N2, regardless of the mutagenic potency of the sample. The arithmetic sum of N fraction mutagenicities was approximately equal to the mutagenicity of the unfractionated non-polar aromatic fraction (within a factor of two). The arithmetic sum averaged to 97% of the unfractionated value. This data is clear evidence that there is minimal inhibition or synergy between compounds in the non-polar aromatic fraction.

From the data in Table 26 it appeared that as the mutagenic potency of the non-polar aromatic fracton increased, the relative proportion of fraction N2 also increased. To explore this relationship this data was plotted as Figure 41. As the mutagenicity of the non-polar aromatic (A23/LH20) fraction increased, the mutagenic activity of fraction N2 increased dramatically while the activity in fraction N3456 increased at a much slower rate. The ratio of these slopes is about 4:1, reflecting their relative contributions. Thus, fraction N2 contains, on average, 75% of the mutagenic compounds in the non-polar aromatic fraction. Since the non-polar aromatic fraction corresponds to about 75% of the mutagenicity of the crude extract, fraction N2 represents about 55% of the mutagenic activity of the crude extract.

Table 26.
Mutagenicity of N Fractions Prepared from Hamilton Air Particulate
Using *S.typhimurium* YG1021 (-S9).

A. Mutagenicity of Non-Polar Aromatic Fractions Separated into Fractions N1, N2, N3, N456 and N7 Fractions.

Date of Sample Collection	*Mutagenicity (revertants·m³)* N1	N2	N3	N456	N7	*Mutagenicity of Non-Polar Aromatic Fraction*	*Arithmetic Sum of N-Fraction Mutagenicities***	
1990 06 08	nr*	52	nr	12	8	64	72	(112%)
1990 11 20	nr	9	1	4	2	16	16	(100%)
1991 04 04	1	40	7	13	3	49	64	(131%)

B. Mutagenicity of Non-Polar Aromatic Fractions Separated into N1, N2, N3456 and Pooled N7 Fractions.

Date of Sample Collection	*Mutagenicity (revertants·m³)* N1	N2	N3456	N7	*Mutagenicity of Non-Polar Aromatic Fraction*	*Arithmetic Sum of N-Fraction Mutagenicities***	
1990 05 07	nr	10	10		23	20	(87%)
1990 05 15	25	42	18		129	85	(66%)
1990 06 22	5	12	7		23	24	(104%)
1990 08 22	nr	6	7	1	14	13	(93%)
1990 10 07	nr	3	5		5	8	(160%)
1991 02 11	nr	1	8		4	9	(225%)
1991 04 18	nr	12	13		23	25	(109%)
1991 05 13	nr	32	30		109	62	(57%)
1990 07 14	5	22	10		75	37	(49%)
1990 12 02	nr	26	18		47	44	(94%)
1991 04 26	1	23	8	2	59	32	(54%)
1991 05 09	nr	27	8		66	35	(53%)
1991 05 15	nr	38	53		121	91	(75%)
1991 05 19	nr	16	9		31	25	(81%)
						avg:	97% ± 44%

*nr signifies 'no response', *i.e.*, the detection limit for bioassays is 30 revertants above a background of 30 revertants at a top dose of 150 m^3 equivalent or 30 revs/150 m^3 = 0.2 revertants per cubic metre air sampled. Experimental error in the number of revs/m^3: ± 14%

**bracketed value shows the arithmetic sum of the N-fraction mutagenicities as a percentage of the experimentally determined mutagenicity of the non-polar aromatic fraction

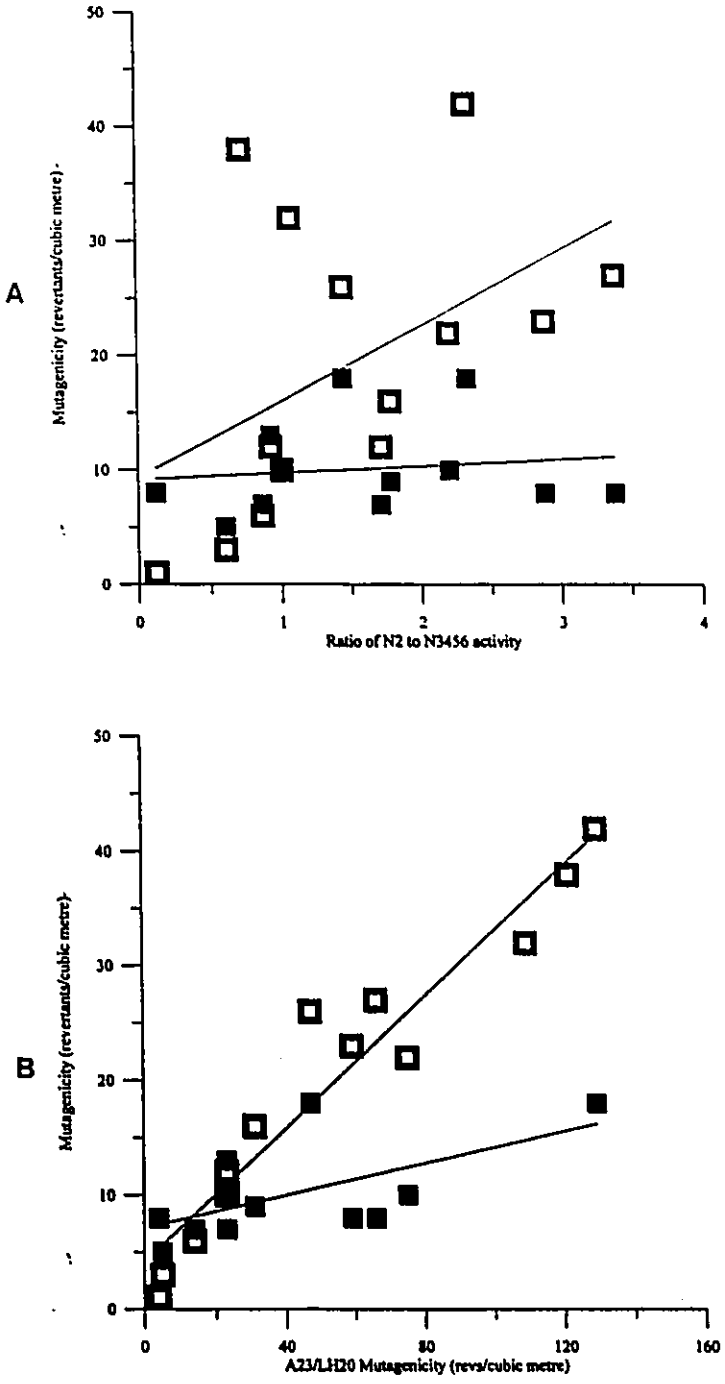


Figure 41. Mutagenic activity of fraction N2 (◘) and N3456 (■) versus (A) the ratio of N2 to N3456 activity and (B) mutagenic activity of fraction A23/LH20, with lines of best fit. Mutagenic activity determined in *S. typhimurium* YG1021 (-S9).

V.3.4 GC-MS Analysis of N2 Fractions for NitroPAH

Fifteen of the seventeen N2 fractions described previously were analyzed by GC-MS; two sets of N fractions were used up completely in bioassays. Portions of dry N2 fractions were taken up in toluene containing an internal standard, usually benzo[a]anthracene-d_{12} or sometimes 1-nitropyrene-d_9. A 30 m DB-17ht GC column with a selected-ion monitoring program for nitroPAH and electron impact ionization (EI^+) were used in the analyses (see section IX.15). The 30 m DB-17ht was shown to separate all nitrofluoranthene and nitropyrene isomers to baseline (Figure 42B) which other GC columns (e.g., DB-5) could not (21,64,66). Figure 42 shows the series of ion chromatograms used to identify the nitroPAH target compounds; ion chromatograms of a representative N2 fraction appear in Figure 43.

In all of the N2 fractions analyzed by GC-MS, 2-nitrofluoranthene was the predominant nitroPAH and was on average 10-fold ($\pm$4.4) more abundant than the other atmospherically-derived nitroPAH (Table 27). Small amounts of 1-nitropyrene were detected in most samples while 7- and 8-nitrofluoranthene were detected only occasionally. Curiously, 3-nitrofluoranthene was below the method detection limit (either 0.30 or 3.0 pg/m^3) in all analyses (Table 27). In previous reports, 3-nitrofluoranthene was identified together with 7- and 8-nitrofluoranthene in atmospheric samples (21,42,167), which is indicative of combustion sources. Electrophilic nitration of fluoranthene also produces 3-nitrofluoranthene as the most abundant isomer (168). While atmospherically-derived nitroPAH usually predominate over combustion-derived nitroPAH (24), the very

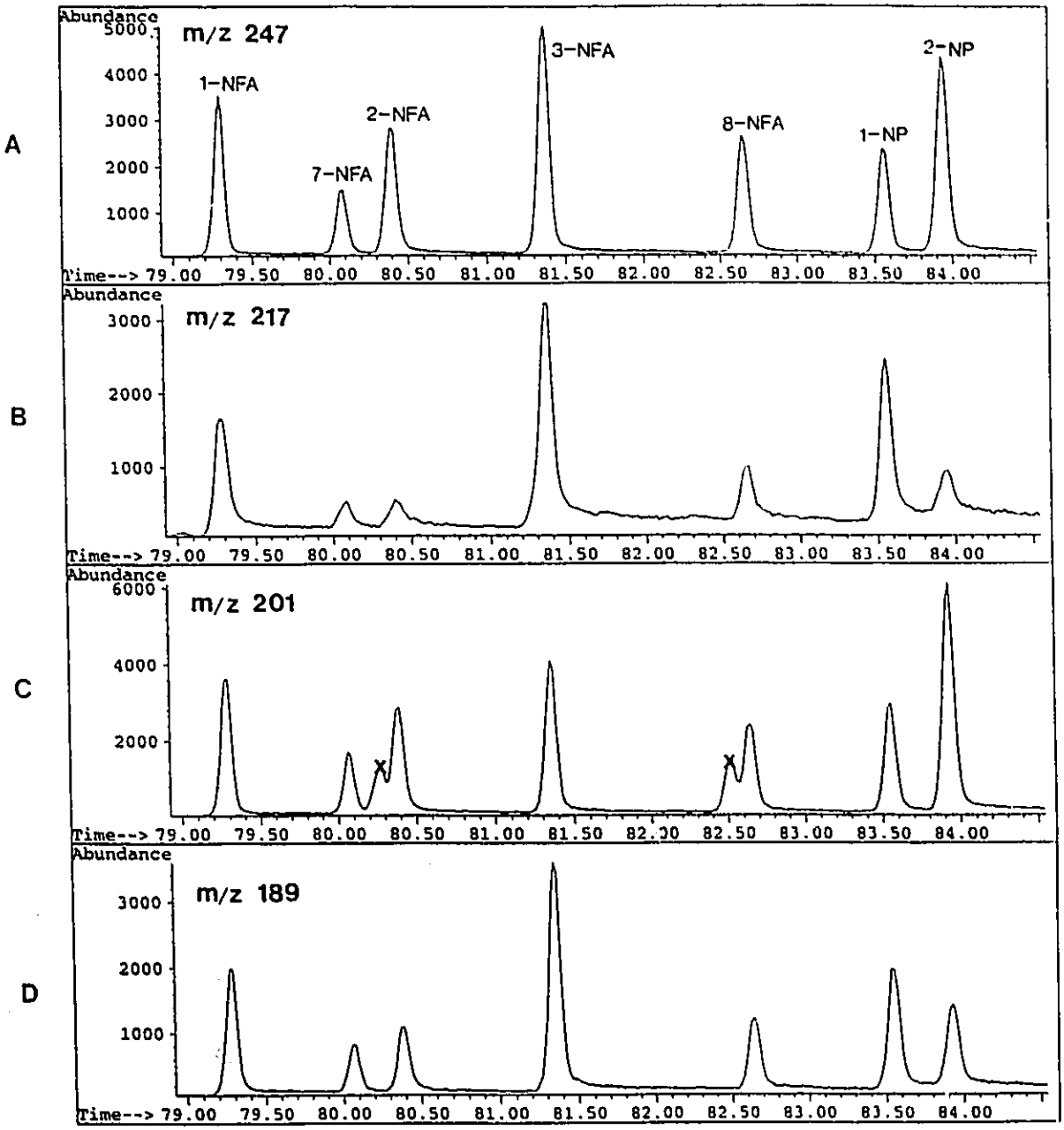


Figure 42. Ion chromatograms for a mixture of standard nitroPAH, monitored at (A) m/z 247, (B) m/z 217, (C) m/z 201 and (D) m/z 189. NFA and NP represent nitrofluoranthene and nitropyrene, respectively. Peaks marked with an X in the ion chromatogram for m/z 201 are fragments from benzanthrone and benzo[a]anthracene-7,12-dione, which were also injected together with the 7 nitroPAH shown. GC column: 30 m DB-17ht, using the method described in section IX.19.

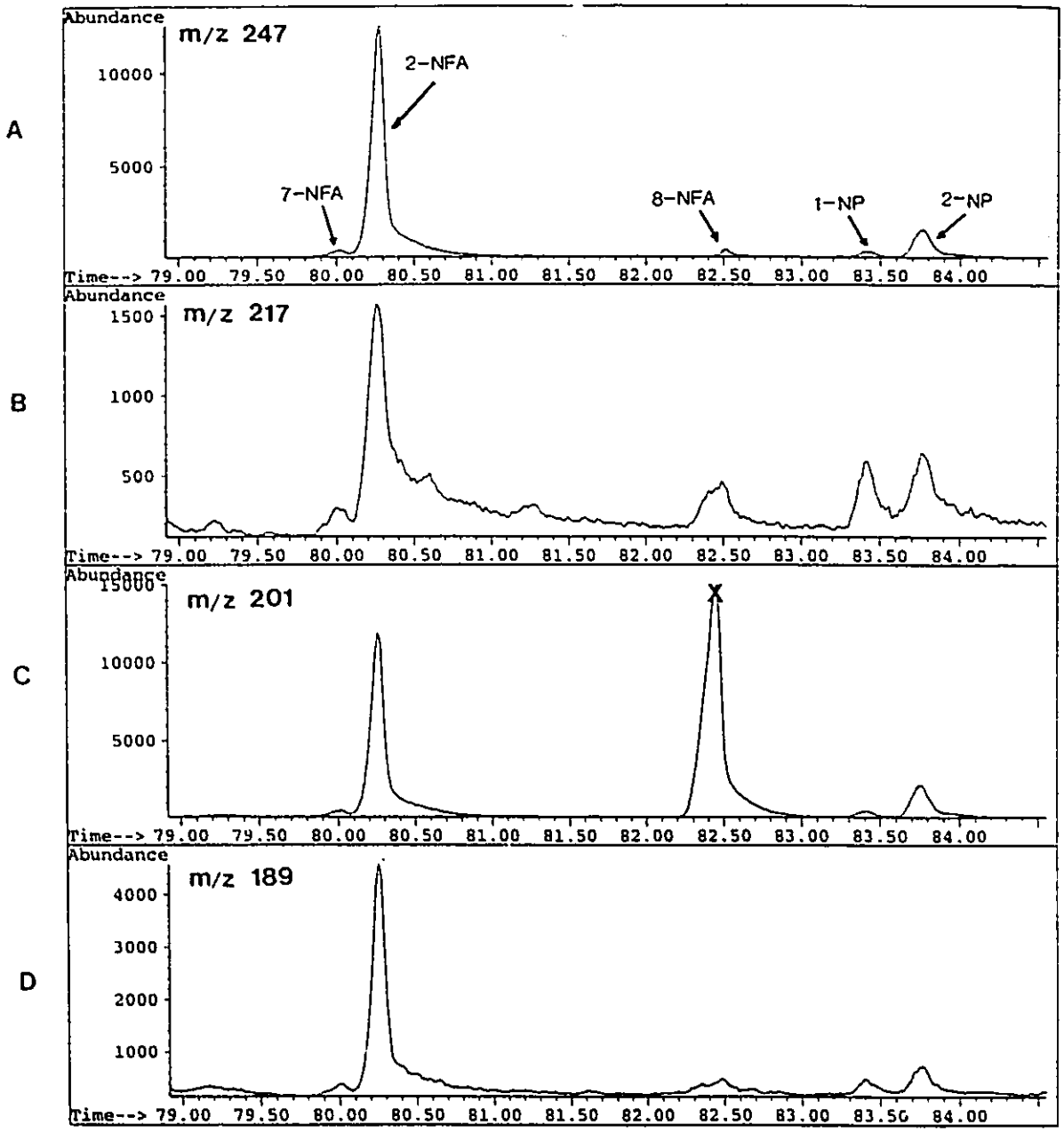


Figure 43. Ion chromatograms for (A) m/z 247, (B) m/z 217, (C) m/z 201 and (D) m/z 189, for an N2 fraction of Hamilton air particulate collected 1991 05 15. NFA and NP represent nitrofluoranthene and nitropyrene, respectively. The peak marked with an X in the ion chromatogram for m/z 201 represents an ion from a polycyclic aromatic ketone. GC column: 30 m DB-17ht, using the method described in section IX.19.

Table 27.
NitroPAH Quantified in N2 Fractions Prepared from
Hamilton Air Particulate, 1990-1991.

Date of Sample	*Concentrations in pg m³:*				
Collection	*[2-NFA]*	*[1-NP]*	*[2-NP]*	*[7-NFA]*	*[8-NFA]****
1990 05 07	200	25	15	<0.3*	<0.3
1990 05 15	460	20	93	<3.0**	<3.0
1990 06 08	61	6.3	12	<0.3	<0.3
1990 06 22	68	4.7	7.1	<0.3	<0.3
1990 07 14	56	5.1	13	<0.3	1
1990 08 22	39	2.0	3.6	<0.3	<0.3
1990 10 07	5.4	0.7	0.4	<0.3	<0.3
1990 12 02	300	45	23	9.1	<3.0
1991 02 11	14	3.8	1.3	<0.3	<0.3
1991 04 18	45	3.8	3.6	<0.3	<0.3
1991 04 24	240	<3.0**	20	<3.0**	<3.0
1991 04 26	120	8.0	19	0.52	1.3
1991 05 09	100	9.1	16	<0.3	0.8
1991 05 13	220	3.9	22	<0.3	<0.3
1991 05 15	260	13	37	3.9	<0.3
1991 05 19	35	1.6	2.9	<0.3	<0.3
1991 05 23	900	<3.0**	40	<3.0**	<3.0
1990 06 24 & 1990 07 18†	10	<3.0**	<3.0	<3.0**	<3.0

Detection limit: 80 pg for all nitroPAH.

*If a sufficient amount of sample remained after bioassays, 265 m^3 of fraction N2 was injected, giving a method detection limit of 0.3 pg/m^3.

**If most of the sample had been destroyed through bioassays, 27 m^3 of fraction N2 was injected, giving a method detection limit of 3.0 pg/m^3.

***3-NFA was below the detection limit in all N2 fractions analyzed. No nitroPAH or methylnitroPAH corresponding to parent PAH masses of 202, 226, 228 and 252 were detected except for those listed above. For further details see methods section IX.19.

†combined sample

low abundance of 3-nitrofluoranthene in the samples in this study is unusual. The complete absence of 1-nitrofluoranthene in these analyses may arise from the low yield (11%) for this particular isomer in the electrophilic nitration of fluoranthene (168).

The results of the present study are considerably different from the analysis of a single sample of air particulate collected at the downtown Hamilton site at an unspecified date as described by Chiu of Environment Canada (177), who reported detecting approximately 10 pg/m^3 3-nitrofluoranthene, 80 pg/m^3 1-nitropyrene and low but unspecified amounts of 7-nitrobenzo[a]anthracene and 6-nitrochrysene. However, both the 3-nitrofluoranthene and 1-nitropyrene results in Chiu's report are suspect since he used a 30 m DB-5 column for the GC analyses. In our hands, this column and temperature program cannot resolve 2-nitrofluoranthene from 3-nitrofluoranthene or 1-nitropyrene from 2-nitropyrene. We feel that he was probably detecting 2-nitrofluoranthene and 2-nitropyrene. Chiu used negative ion chemical ionization to detect the nitroPAH, resulting in detection limits of approximately 2-10 pg per nitroPAH which is between 8- and 40-fold more sensitive than the $EI^{\cdot}$ techniques used in this study. Thus, 7-nitrobenzo[a]anthracene and 6-nitrochrysene may have been present in the samples collected in 1990-1991 but were at levels below the detection limit of the present study (80 pg).

NitroPAH concentrations in Hamilton air particulate are compared to concentrations found in other cities (Table 28). As was the case with benzo[a]pyrene (Table 15), nitroPAH concentrations in Hamilton air particulate are moderate, relative to

Table 28.

NitroPAH Concentrations in Air Particulate Collected in Hamilton and Elsewhere.

			Concentration range (pg/m³)			
Location	*n**	*Date*	*2-NFA*	*8-NFA*	*1-NP*	*2NP*
Vienna, Austria (64)	4	1989	300-1800	not detected**	90-600	60-600
Claremont, CA (162)	12	Spring 1986	30-1700	1-50	3-60	1-60
Columbus, OH (165)	8	Winter 1986	210-1200	not detected	120-360	not detected
Hamilton, ON (this study)	18	1990-1991	5-900	0.8-1.3	1-45	0.4-93
Torrance, CA (60)	4	Spring 1986	40-400	not detected	30-40	30-40
Milan, Italy (166)	14	Fall 1990	50-250	not detected	5-50	15-60

* Number of samples collected and analyzed
**Detection limit not given

other locations. Due to the extra effort needed to perform nitroPAH analyses, the number of samples (between 4 and 18) analyzed in all studies is much lower than one normally observes in a PAH analysis paper. The variety of different cleanup methods used in nitroPAH analyses is summarized in Table 29.

NitroPAH concentrations may be related to the total concentrations of NO and NO_2. High nitroPAH concentrations observed in Los Angeles (Table 28) were observed on days when concentrations exceeded 500 ppb (60, 162); however, NO_x concentrations in Hamilton never exceeded 150 ppb during the 1990-1991 sampling period. Relationships between oxides of nitrogen, mutagenicity and nitroPAH concentrations in this study will be further investigated in section V.4.

The method described in this thesis for the extraction, cleanup and quantitation of nitroPAH in air particulate involves more steps than any other published method but at the same time has allowed nitroPAH quantitation at concentrations at least two-fold lower than those described elsewhere. The good detection limits are presumably the result of the cleanliness of our N2 fractions; other methods yield nitroPAH fractions with a variety of interferences to quantitation.

V.3.5 Contribution of NitroPAH Towards the Mutagenicity of Chromatographic Fractions of Hamilton Air Particulate Extracts

This section will describe the mutagenicity of nitroPAH and the contribution of these individual compounds towards the mutagenicity of various chromatographic

Table 29.

A Comparison of Various Methods for the Quantitation of NitroPAH in Ambient Air Particulate.

Criterion	*This method*	*Arey (60)*	*Kamens (169)*	*Liberti (170)*
Number of steps (including extraction)	4	2	2	3
Removal of aliphatics	yes	no	no	no
Separation of polars from PAC before HPLC	yes	no	no	yes
HPLC method used	NPLC	NPLC	NPLC	NPLC
Gas chromatographic detection method	EI^+	EI^+	EI^+	EI^+

Criterion	*Nielsen(62)*	*Niles (171)*	*Oehme (172)*	*Ramdahl (173)*
Number of steps (including extraction)	2	3	2	3
Removal of aliphatics	no	yes	no	no
Separation of polars from PAC before HPLC	no	no	no	no
HPLC method used	NPLC	NPLC	NPLC	NPLC
Gas chromatographic detection method	NPD	TEA	NICI	EI^+

Table 29 (Continued)
A Comparison of Various Methods for the Quantitation of NitroPAH in Ambient Air Particulate.

Criterion	*Schil-habel(174)*	*Schneider (64)*	*Schuetzle (175)*	*Tokiwa (176)*
Number of steps (including extraction)	2	3	3	3
Removal of aliphatics	no	yes	yes	yes
Separation of polars from PAC before HPLC	no	no	no	yes
HPLC method used	NPLC	NPLC	NPLC	RPLC
Gas chromatographic detection method	NICI	EI^+	NPD	EI^+

Abbreviations:

NPLC normal-phase liquid chromatography
RPLC reversed-phase liquid chromatography
EI^+ electron impact ionization
NPD nitrogen-phosphorous detector
TEA thermal energy analyzer

fractions of air particulate extract. By multiplying the mutagenic potencies of individual nitroPAH by the concentrations of those nitroPAH in air particulate, we can calculate the number of revertants per cubic metre due to that nitroPAH isomer. The values for the various nitroPAH can be summed and compared to the number of revertants/m^3 determined experimentally for the non-polar aromatic fraction or the N2 fraction.

Salmonella typhimurium strains YG1020 and TA98 are identical except for the inclusion of plasmid pBR 322 in strain YG1020, which makes no difference in determinations of mutagenicity (D.W. Bryant, unpublished observation). The mutagenicity of the nitropyrene and nitrofluoranthene isomers were determined in YG1021 (-S9) and YG1020 (-S9), the latter in order to compare values obtained in this lab with values reported elsewhere using strain TA98 (-S9) (Table 30). The mutagenicity of the nitropyrenes and nitrofluoranthenes in strains YG1020 (-S9) were equal to literature values within a factor of two; the mutagenic potencies in YG1021 (-S9) were found to be 3.6- to 16.6-fold greater than in strain YG1020 (-S9).

Values obtained for the mutagenicity of 1-nitropyrene, which has been assayed using strain YG1021 (-S9), were in good agreement with literature values (Table 30). Note that the ranking of nitroPAH for mutagenic potency in strain YG1021 matches the order for these compounds in strain TA98 (84). Accurate mutagenicity values in any strain are critically dependant on nitroPAH purity. Traces of 1,8-dinitropyrene sometimes found in 1-nitropyrene can lead to major errors in the determination of 1-nitropyrene mutagenicity (84). All nitroPAH were purified by HPLC and checked for dinitroPAH by

Table 30.

Mutagenicity of NitroPAH Determined in this Laboratory and Elsewhere.

Mutagenicity in S. typhimurium (revs/nmol)

Compound	*YG1020 (-S9) (this study)*	*TA98 (-S9) (literature)*	*YG1021 (-S9) (this study)*	*YG1021 (-S9) (literature)*	*Refs.*
1-NFA	680 ± 15%	500	3640 ± 15%		(93)
2-NFA	1230	1030	7900		(124)
3-NFA	14300	7700	56300		(124)
7-NFA	82	47	950		(124)
8-NFA	24900	18200	199000		(124)
1-NP	610	543	10100	24700 12172 16805	(95, 178) (93) (123)
2-NP	5200	2200	18800		(84)

Abbreviations: NFA, nitrofluoranthene
NP, nitropyrene

GC-MS before bioassays were carried out in this laboratory.

With the concentrations and mutagenic potencies of individual nitroPAH determined, the mutagenic activity corresponding to these compounds was calculated and compared to the mutagenic responses determined in the N2 fraction and in the non-polar aromatic fraction (Table 31). Thus, the nitropyrenes and nitrofluoranthenes accounted for 32% ± 21% of the mutagenic activity of fraction N2, with a range of 7% to 86%. Between 4% and 54% of the mutagenic potency of the non-polar aromatic fraction (average 15% ± 13%) was ascribed to these compounds. In the only other report to study the contribution of nitroPAH to extract mutagenicity, Arey *et al.* found that nitropyrenes and nitrofluoranthenes were responsible for 1% to 8% of the mutagenic activity of the non-polar aromatic fraction from Los Angeles air particulate, using *S. typhimurium* TA98(-S9) (85). The higher percentages seen in Hamilton presumably reflect the increased sensitivity of strain YG1021 to nitroPAH.

Not all mutagens in air particulate extracts are nitroPAH. The range of percentages of mutagenic activity attributable to nitroPAH for three samples with a common A23/LH20 mutagenicity of 23 revertants/m^3 (8, 13 and 37%) and in two A23/LH20 fractions with a mutagenic activity of 130 revs/m^3 (18 and 25%) in Table 31 is noteworthy in this regard. Clearly, a certain proportion of the mutagenic potency of the non-polar aromatic fraction was not derived from nitroPAH.

The mutagenic potencies of fraction N2 and the non-polar aromatic fraction were plotted as a function of the concentration of 2-nitrofluoranthene (Figures 44A and 44B).

Table 31.
Mutagenicity in *S. typhimurium* YG1021(-S9) Due to NitroPAH in N2 and Non-Polar Aromatic (A23/LH20) Fractions of Hamilton Air Particulate.

Date Collected	*Mutagenicity Calculated from conc. of Monitored NitroPAH*	*Mutagenicity of Fraction N2*	*Percentage N2 Mutagenicity Attributed to NitroPAH*	*Mutagenicity of A23/LH20*	*Percentage A23/LH20 Mutagenicity Attributed to NitroPAH*
1990 05 07	8.6 revs/m^3	10 revs/m^3	86 %	23 revs/m^3	37 %
1990 05 15	23	42	55	130	18
1990 06 08	3.1	29	11	64	5
1990 06 22	2.9	12	24	23	13
1990 07 14	3.0	22	14	76	4
1990 08 22	1.6	6.4	25	14	11
1990 10 07	0.2	2.7	7	5.4	4
1990 12 02	13	26	50	47	28
1991 02 11	0.7	1.2	58	3.5	20
1991 04 18	1.9	12	16	23	8
1991 04 24	9.2	16	58	17	54
1991 04 26	5.6	23	24	59	10
1991 05 09	4.8	26	18	66	7
1991 05 13	8.9	32	28	110	8
1991 05 15	12	38	32	120	10
1991 05 19	1.4	16	9	31	4
1991 05 23	32	100	32	130	25
1990 06 24+ 1990 07 18	0.3	1.5	30	3.0	10
Average:	7.3 revs/m^3	23 revs/m^3	32 %	52 revs/m^3	15%
Std. Dev.:	± 8.5	± 23	± 21	± 44	± 13
Minimum:	0.3	1.2	7	3.0	4
Maximum:	32	100	86	130	54

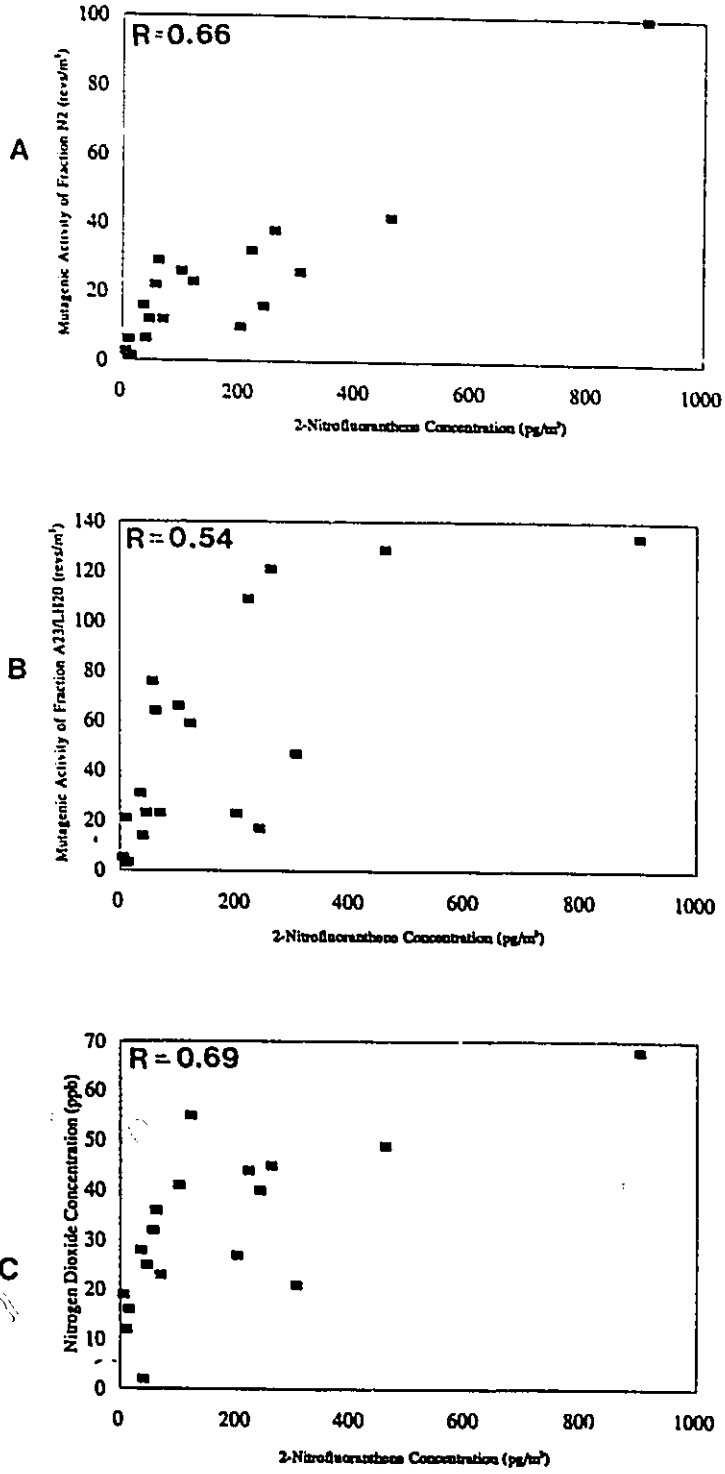


Figure 44. Plots of (A) mutagenic activity of fraction N2, (B) mutagenic activity of the non-polar or A23/LH20 fraction and (C) average nitrogen dioxide concentration on the day of collection versus 2-nitrofluoranthene concentration. Mutagenic activity was determined using *S. typhimurium* YG1021 (-S9).

The concentrations of NO_2 on the days of collection were also plotted against the concentration of 2-nitrofluoranthene. The correlation coefficients are included with each graph (Figure 44).

The plot of mutagenic activity in fraction N2 versus 2-nitrofluoranthene concentration (Figure 44A) shows two populations of data: a set of points from 0-150 pg/m^3 2-NFA (with a steeper slope) and a second set of points for 2-NFA concentrations between 150 and 1000 pg/m^3, with a shallower slope. The data points in each set were carefully examined for any relationships with atmospheric conditions and thermal inversion events, but no correlations were found. Student's *t* test (180) was applied to see if the difference between the two data sets was significant. The test did indicate a significant difference (at the 99% confidence interval) but the reason for this difference could not be established. A more detailed statistical study of nitroPAH concentrations, mutagenicity data and atmospheric conditions in the form of a principal component analysis will be presented in the next section.

V.4. Principal Component Analysis of NitroPAH Concentrations, Mutagenicity and Atmospheric Conditions

Principal component analysis of the data presented in this chapter began with the calculation of correlation coefficients between 18 variables of interest to this study. These variables included the concentrations of: all monitored PAH (ΣPAH), thiaPAH (ΣtPAH), oxyPAC (ΣoPAC), 2-nitrofluoranthene (2-NFA), 2-nitropyrene (2-NP) and 1-nitropyrene (1-NP); the coefficient of haze (COH); concentrations of NO_2, NO, O_3 and non-methane

hydrocarbons (NMHC); the logarithms of the mutagenicity of fractions A23/LH20 (lmA23) and N2 (lmN2); the logarithm of the mass of particulate collected (lmass); air temperature at a height of 33 feet (T) and the difference between this temperature and the temperature at a height of 300 feet (ΔT); windspeed (wspd) and relative humidity (rh). Correlation coefficients are shown in a correlation matrix (Table 32). The values in this table are somewhat different from the values reported in Table 19 because the number of filters in this data set is only 20, compared to 68 in the data set discussed previously (Chapter IV).

Correlation coefficients with a value of R=0.64 or greater are ranked in Table 33. The value of R=0.64 was selected arbitrarily for the reasons described previously (section IV.7.4). The high correlation coefficients between oxyPAC, thiaPAH and PAH were previously noted (Table 19), as were the correlations between coefficient of haze and NO_2, mutagenicity of fraction A23/LH20 and COH or NO_2 or ΔT and the mass of particulate collected and windspeed. All pollutant concentrations rose during a temperature inversion, as windspeed fell and the mass of particulate increased and extract mutagenic activity increased. The corresponding correlation coefficients from Table 19 are included as a column in Table 33 for comparison.

Table 32.
Correlation Matrix for PAH, ThiaPAH, OxyPAC and NitroPAH,
Atmospheric Conditions and Mutagenicity*

Variable	ΣPAH	ΣtPAH	ΣoPAC	2-NFA	2-NP	1-NP	COH	NO_2	NO	O_3	NMHC	lmA23	lmN2	lmass	T(°C)	ΔT	wspd	rh
ΣPAH	1.00																	
ΣtPAH	0.89	1.00																
ΣoPAC	0.85	0.96	1.00															
2-NFA	-0.17	-0.14	-0.04	1.00														
2-NP	-0.15	-0.11	-0.11	0.66	1.00													
1-NP	-0.34	-0.27	-0.24	0.15	0.31	1.00												
COH	0.23	0.07	0.06	0.68	0.69	-0.15	1.00											
NO_2	0.16	0.12	0.11	0.69	0.58	-0.12	0.82	1.00										
NO	0.24	0.05	-0.01	0.06	0.16	-0.05	0.38	0.57	1.00									
O_3	-0.04	0.21	0.31	0.42	0.02	-0.20	-0.10	0.09	-0.36	1.00								
NMHC	0.36	0.28	0.23	-0.01	0.11	-0.18	0.28	0.60	0.89	-0.20	1.00							
lmA23	0.19	0.15	0.08	0.54	0.62	0.16	0.74	0.74	0.32	-0.23	0.31	1.00						
lmN2	0.24	0.20	0.19	0.66	0.58	0.13	0.76	0.78	0.29	-0.11	0.31	0.94	1.00					
lmass	0.21	0.04	-0.03	0.60	0.64	-0.10	0.93	0.77	0.38	-0.25	0.28	0.85	0.85	1.00				
T (°C)	0.23	0.14	0.07	0.01	0.12	-0.28	0.31	0.09	-0.02	-0.18	0.05	0.45	0.41	0.47	1.00			
ΔT	0.14	0.14	0.04	0.25	0.70	-0.02	0.66	0.61	0.40	-0.23	0.44	0.68	0.55	0.66	0.33	1.00		
wspd	-0.44	-0.29	-0.16	-0.12	-0.37	0.40	-0.68	-0.61	-0.54	0.30	-0.53	-0.60	-0.55	-0.78	-0.64	-0.67	1.00	
rh	-0.10	-0.29	-0.30	-0.45	-0.16	-0.16	-0.11	-0.39	-0.22	-0.64	-0.26	-0.17	-0.19	0.01	0.33	-0.13	-0.16	1.00

*determined in 20 samples of Hamilton air particulate extract

Table 33.

Selected Correlation Coefficients Obtained from the NitroPAH Analysis of Twenty Samples of Hamilton Air Particulate Extract.

Variables	*Correlation Coefficient (R)*	*Corresponding Values from Table 19*[*]
OxyPAC and thiaPAH	0.96	0.88
Mutagenicity of fractions N2 and A23/LH20	0.94	---
Coefficient of haze and mass of particulate collected	0.93	0.61
ThiaPAH and PAH	0.89	0.91
Non-methane hydrocarbons and NO	0.89	0.64
OxyPAC and PAH	0.85	0.79
Mass of particulate and N2 or A23/LH20 mutagenicity	0.85	0.58
Coefficient of haze and NO_2	0.82	0.72
Mutagenicity of fraction N2 and COH or NO_2	0.77	---
NO_2 and mass of particulate collected	0.77	0.42
Mutagenicity of A23/LH20 and COH or NO_2 conc.	0.74	0.71
Difference in temperature and 2-nitropyrene conc.	0.70	---
NO_2 and 2-nitrofluoranthene	0.69	---
COH and 2-nitropyrene or 2-nitrofluoranthene conc.	0.68	---
Difference in temperature & mutagenicity of A23/LH20	0.68	0.52
Concentrations of 2-nitropyrene & 2-nitrofluoranthene	0.66	---
Mutagenicity of N2 and 2-nitrofluoranthene conc.	0.66	---
Difference in temperature & COH or particulate mass	0.66	0.60
Mass of particulate collected and 2-nitropyrene conc.	0.64	---
Windspeed and coefficient of haze	-0.68	-0.71
Mass of particulate collected and windspeed	-0.78	-0.46

[*] 68 filters were included in this analysis

The very high correlation between the mutagenicity of fractions N2 and A23/LH20 (R=0.94) was previously noted (Figure 41B) and can be explained on the basis of other correlations (Table 33) as follows. The concentrations of 2-nitrofluoranthene and 2-nitropyrene showed much better correlations with nitrogen dioxide concentrations (R=0.69 and R=0.58, respectively) than did 1-nitropyrene (R=-0.12), presumably due to the atmospheric nitration of fluoranthene and pyrene to give the former nitroPAH. The good correlation between 2-nitrofluoranthene and 2-nitropyrene (R=0.66) implies that both compounds were formed by similar processes, while the low correlations of these compounds with 1-nitropyrene (R=0.15 and 0.31, respectively) indicates that different processes are involved in 1-nitropyrene formation. Similarly, the low correlations (R=-0.04 to -0.17) between the atmospheric transformation products and combustion products (PAH, thiaPAH, oxyPAC) suggest that 2-NFA and 2-NP are not formed through combustion.

As the concentrations of 2-nitrofluoranthene and 2-nitropyrene increased, the mutagenic activity of fraction N2 increased in direct proportion to these compounds (R=0.66) and the correlation between 2-nitrofluoranthene concentration and A23/LH20 mutagenicity was only slightly lower (R=0.54). The relationship between 2-nitrofluoranthene concentration, generic NO_x concentrations and mutagenic activity has been noted previously (60,162) but the current study appears to be the first observation of [a] good correlation between NO_2 and 2-nitrofluoranthene (R=0.69) and [b] the lack of correlation between 2-nitrofluoranthene and NO (R=0.06).

The principal component process continued with the determination of eigenvectors and eigenvalues of the correlation matrix which gave the factor matrix (Table 34). The values in the factor matrix were used to construct a three-dimensional factor loading plot (Figure 45). Clustering of the points representing different variables indicates a close correlation between those variables, while points which are widely separated show little or no correlation.

The isolation of points representing ozone, windspeed, 1-nitropyrene and relative humidity indicates that these variables are unrelated to any other measured parameter (Figure 45). The points representing total monitored PAH, thiaPAH and oxyPAC are clustered together, which indicates a common source of these compounds. However, PAH, thiaPAH and oxyPAC are well removed from any other points in the plot, indicating little, if any, correlation with other parameters. Points representing 2-nitrofluoranthene and 2-nitropyrene are closer to each other than to the point representing 1-nitropyrene, once again indicating that 1-NP is not an atmospheric transformation product. Points representing variables affected by temperature inversions (mass of particulate collected, ΔT, COH, NMHC, NO, NO_2) are clustered together with the points representing the mutagenicity of fractions N2 and A23/LH20.

Table 34.
Factor Matrix for PAH, ThiaPAH, OxyPAC and NitroPAH Concentrations, Atmospheric Conditions and Mutagenicity.

Variable	*Factor 1*	*Factor 2*	*Factor 3*	*Factor 4*
lmass	0.92			
COH	0.90			
NO_2	0.88			
lmA23	0.88			
lmN2	0.88			
wspd	-0.80		0.43	
ΔT	0.78			
2-NP	0.68	-0.46		
ΣtPAH		0.88		
ΣPAH		0.87		
ΣoPAC		0.85		
1-NP		-0.51		
O_3			0.83	
rh			-0.82	
2-NFA	0.58	-0.44	-0.59	
NO	0.54			-0.76
NMHC	0.54			-0.69
T (°C)	0.41		-0.46	0.54

Abbreviations: lmass, logarithm of the mass of particulate collected; COH, coefficient of haze; lmA23, logarithm of the mutagenicity of fraction A23/LH20; lmN2, logarithm of the mutagenicity of fraction N2; wspd, windspeed; ΔT, difference in air temperature at heights of 33 and 300 feet; 2-NP, concentration of 2-nitropyrene; ΣtPAH, total monitored thiaPAH; ΣPAH, total monitored PAH; ΣoPAC, total monitored oxyPAC; 1-NP, concentration of 1-nitropyrene; rh, relative humidity; 2-NFA, concentration of 2-nitrofluoranthene; NMHC, non-methane hydrocarbons; T (°C), air temperature at a height of 33 feet.

Factors of less than or equal to 0.40 are not shown in this Table or the factor plot (Figure 45).

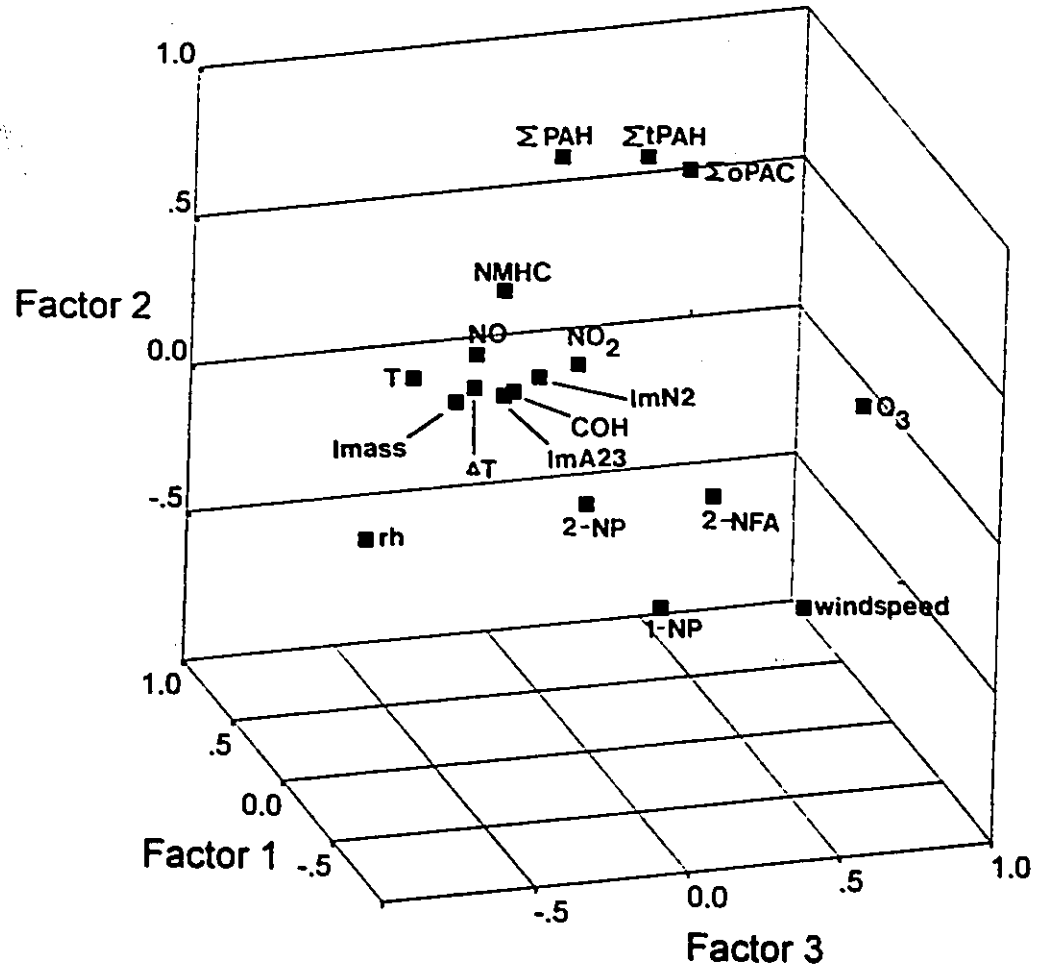


Figure 45. Three-dimensional factor loading plot derived from the principal component analysis of meteorological, mutagenicity, PAC and nitroPAH data. Abbreviations are explained in Table 34. Clustering of data points indicates close correlation, but absolute distance between data points is not directly proportional to correlation coefficient in this particular projection. The overlap between points representing lmA23 (mutagenicity of the A23/LH20 fraction) and COH (coefficient of haze) reflects the high correlation between these two variables (Table 32).

Thus, the data shown in the factor plot (Figure 45) confirmed the results of the correlation matrix (Table 32): as pollutant concentrations increase (especially during thermal inversions), increased atmospheric transformation produces more 2-nitrofluoranthene and 2-nitropyrene, with resultant increases in the mutagenic activity of fractions N2 and A23/LH20.

V.5 Attempted Bioassay-Directed Fractionation of Fraction N456

Although the mutagenicity of N2 fractions has been linked to nitroPAH (Table 32), the compounds responsible for mutagenic activity in N456 fractions were still unidentified. Towards this end, fraction N456 from the sample collected on May 23rd, 1991, was subjected to a reversed-phase HPLC separation (Figure 46), using gradient D, section IX.13. One-minute subfractions were collected and subjected to bioassays in *S. typhimurium* YG1021 (-S9). The resulting mutation chromatogram (Figure 46B) shows that a single one-minute subfraction was responsible for at least 90% of the mutagenic activity of the sample. This fraction eluted early in the gradient, indicating that this compound or compounds was fairly polar. Almost all of fraction N456 was used up in order to generate the mutation chromatogram which precluded GC-MS analyses of the one-minute subfractions.

To facilitate multiple bioassays and GC-MS analyses, an extract of air particulate was needed which contained far more PAC than a single day's collection. Thus, a pooled sample of Hamilton air particulate extract was prepared from a large number of filters;

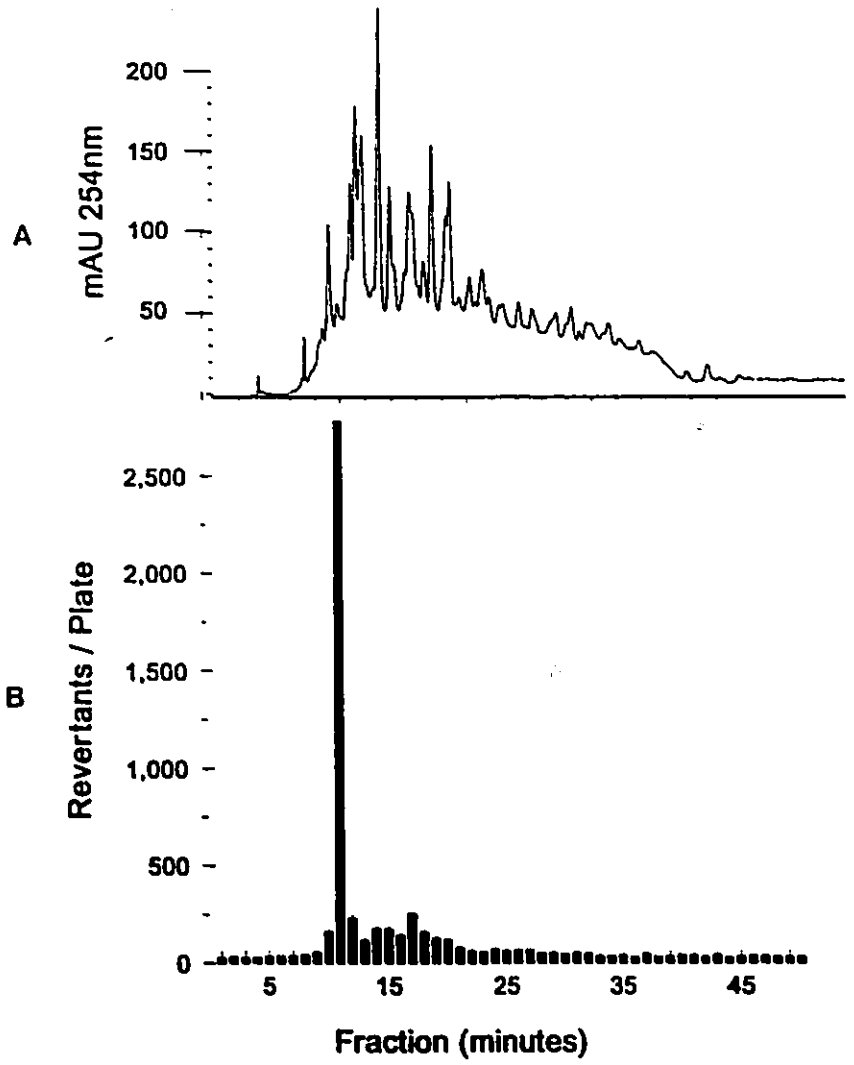


Figure 46. Reversed-phase mutation chromatogram of fraction N456, prepared from particulate collected in Hamilton, 1991 05 23. (A) Chromatogram of fraction N456 on dual Vydac columns (gradient D, section IX.13) and (B) results of bioassays carried out using *S. typhimurium* YG1021 (-S9).

the chemical and bioassay analyses of this 'pooled' sample are described in the following chapter.

V.6 Conclusions

The results presented in this chapter have demonstrated that fractions prepared by normal-phase HPLC from non-polar aromatic fractions of air particulate showed a range of mutagenic responses. The sum of the responses of these fractions was approximately equal to the mutagenic potency of the original non-polar aromatic fraction within a factor of two. Further separation of N fractions through the use of reversed-phase HPLC showed the approach of using multi-dimensional chromatography (in this case, NPLC followed by RPLC) to be useful in identifying mutagens, particularly when coupled with GC-MS analyses.

Fractions N1, N3 and N7 showed relatively low mutagenic activities for all 20 samples subjected to direct-acting bioassays with a nitroreductase-rich strain of *S. typhimurium* YG1021. Conversely, fraction N2 was the most mutagenic N-fraction and corresponded to 75% of the activity in YG1021 in the non-polar aromatic fraction. Fraction N456 accounted for the remainder of the bioassay response. Furthermore, as the mutagenicity of the non-polar aromatic fraction increased, the proportion of the mutagenic activity attributed to fraction N2 also increased. Thus, for air particulate extracts of high mutagenic activity, fraction N2 is of greatest concern.

Within fraction N2, the most abundant nitroPAH were the atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene. These two compounds were detected in virtually all samples analyzed, together with the combustion product 1-nitropyrene. Two other combustion products, 7-nitrofluoranthene and 8-nitrofluoranthene, were detected at low levels in a few samples, but both 1-nitrofluoranthene and 3-nitrofluoranthene were below the detection limit in all samples analyzed. Similarly, methylnitroPAH corresponding to PAH of molecular mass 202 and both methylnitroPAH and nitroPAH corresponding to PAH of molecular masses 226, 228 and 252 were also below the detection limit.

The concentrations of the nitropyrenes and nitrofluoranthenes in Hamilton air particulate are moderate relative to concentrations determined elsewhere. The three nitropyrenes and nitrofluoranthenes accounted for 32% $\pm$ 21% (range: 7% to 86%) of the mutagenicity of the N2 fraction and 15% $\pm$ 13% (range: 4% to 54%) of the mutagenicity of the non-polar aromatic fraction.

Principal component analysis of the nitroPAH, PAC, mutagenicity and atmospheric data revealed that nitrogen dioxide (but not nitric oxide) concentrations correlated well with 2-nitrofluoranthene and 2-nitropyrene but not 1-nitropyrene levels. The concentrations of the two atmospheric transformation products (2-nitrofluoranthene and 2-nitropyrene) correlated well with mutagenicities of fraction N2 and the non-polar aromatic fraction.

Finally, this combination of NPLC, RPLC and GC-MS allowed the unequivocal identification of the three principal mutagens in fraction N2. This approach was also applied to a more polar fraction (N456) but there was insufficient material for GC-MS analyses. However, this method appears to be viable for general application to extracts from complex environmental mixtures.

VI. ANALYSIS OF POOLED FRACTIONS FROM HAMILTON AIR PARTICULATE EXTRACTS

VI.1 Introduction and Overview

Extracts prepared from 24-hour collections of Hamilton air particulate may not contain enough material to permit the isolation and characterization of new mutagens or to permit examination of these samples using more than one strain of *S. typhimurium*. A pooled fraction representing the extract from several grams of respirable air particulate material and tens of thousands of cubic metres of air was prepared. Part of this non-polar aromatic fraction was separated into various PAC classes which were then subjected to bioassays using a number of *S. typhimurium* strains. Finally, normal-phase mutation chromatographic analyses were performed and extensive efforts were made to identify new mutagens in two subfractions.

VI.2 Preparation of a Pooled Non-Polar Aromatic Fraction

The pooled sample was prepared in the following manner. The non-polar aromatic (A23/LH20) fractions prepared previously from Hamilton air particulate (Chapter IV) were ranked on the basis of their mutagenicity in *S. typhimurium* YG1021 (-S9); fractions with mutagenic potencies of at least 20 revs/m^3 were selected and aliquots representing about 1000 m^3 were transferred to a clean vessel and combined (Table 35A). In addition, non-polar aromatic fractions prepared from particulate collected at the Canada Centre for Inland Waters (CCIW, Figure 8) during the fall and winter of 1991 and 1992 (Table 35B)

Table 35.
Non-Polar Aromatic Fractions Used to Create a Pooled Non-Polar Aromatic Fraction.

A. Samples Collected in Downtown Hamilton

Date of Sample Collection	*Cubic metres air used for pooled fraction*
1990 05 03	1050
1990 05 07	1050
1990 05 15	1130
1990 05 25	880
1990 05 27	1080
1990 05 29	1190
1990 07 12	1130
1990 07 14	1130
1990 07 26	1190
1990 08 16	930
1990 08 24	950
1990 09 21	1050
1991 01 16	950
1991 03 17	1180
1991 03 21	1190
1991 04 18	1190
1991 04 26	1130
1991 05 09	1130
1991 05 13	1130
1991 05 15	1130
Total* volume air sampled:	21790 m^3

*Note: only 20610 m^3 was contributed towards the pooled non-polar aromatic sample.

B. Samples Collected at the Canada Centre for Inland Waters

Date of sample collection	*Cubic metres air sampled*
1991 09 14	1630
1991 09 20	1630
1991 09 27	1630
1991 10 04	1630
1991 10 10	1630
1991 10 17	3260
1991 10 31	3260
1991 11 12	1630
1991 11 22	3260
1991 12 06	1630
1991 12 13	1630
1991 12 20	4890
1992 02 14	6520
1991 04 14	19570
Total** volume air sampled:	53800 m^3

**Note: 30580 m^3 equivalent of this sample was added to the samples listed in Table 35A.

were added to this vessel. Dr. C. Marvin kindly prepared the A23/LH20 fraction from CCIW particulate.

Thus, a non-polar aromatic fraction and a polar aromatic fraction were prepared which corresponded to the extracts from twenty filters collected in Hamilton, representing 1298 mg of particulate obtained from the sampling of 20,610 m^3 of air and eighteen filters collected at the CCIW site, representing 679 mg of particulate obtained from the sampling of 30,580 m^3 of air.

VI.3 Chemical Analysis of the Pooled Non-Polar Aromatic Fraction

The GC-MS chromatogram of the pooled non-polar aromatic fraction appears in Figure 47 and the PAC concentrations determined through this analysis appear in Table 36. PAC concentrations in this sample were about two-fold lower than the average of all 68 Hamilton air samples. For example, the concentrations of three representative compounds, benzo[a]pyrene, benzo[b]naphtho(2,1-d)thiophene and benzanthrone, were 2.3-fold, 2.7-fold and 2.6-fold lower than average concentrations for Hamilton air particulate. Thus, from a chemical analysis perspective, this pooled sample is representative of an 'average' Hamilton air sample.

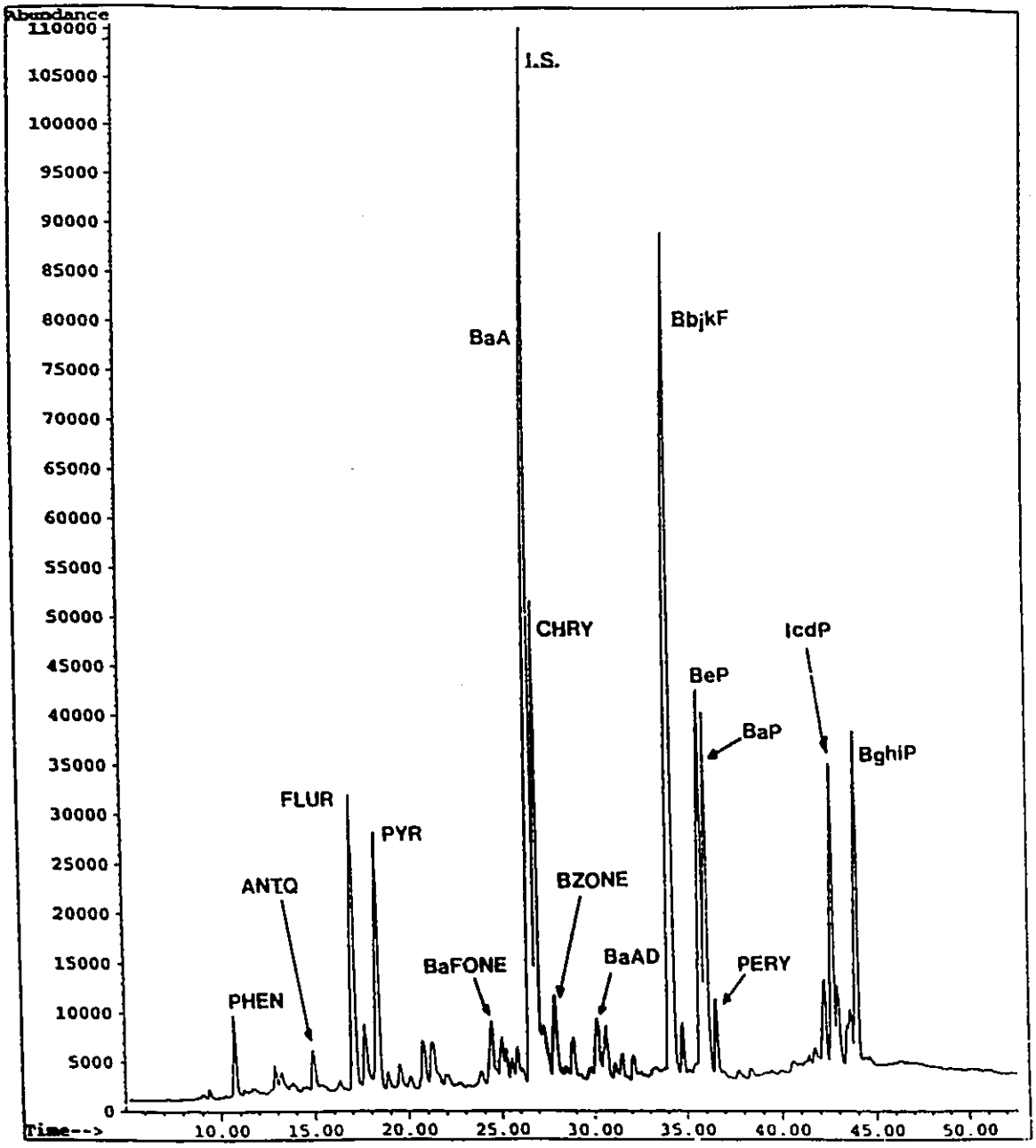


Figure 47. Reconstructed ion chromatogram of the pooled non-polar aromatic fraction from Hamilton air. Benzo[a]anthracene-d_{12} was used as an internal standard and is labelled I.S. Abbreviations are listed in Table 36.

Table 36.
PAC Concentrations in the Pooled Non-Polar Aromatic Fraction.

Abbreviation	*Polycyclic Aromatic Compound*	*Concentration (ng m^3)* *Pooled Sample*	*Hamilton Average***
PHEN	Phenanthrene	0.19	0.95
ANT	Anthracene	<0.0003*	0.091
FLUR	Fluoranthene	0.68	2.10
PYR	Pyrene	0.63	1.80
BaA	Benzo[a]anthracene	1.01	2.40
CHRY	Chrysene/Triphenylene	1.71	3.10
BbjkF	Benzo[b,j&k]fluoranthenes	4.06	7.80
BeP	Benzo[e]pyrene	1.69	3.40
BaP	Benzo[a]pyrene	1.15	2.60
PERY	Perylene	0.32	0.74
DBAs	Dibenzo[ac&ah]anthracenes	0.10	0.91
BbC	Benzo[b]chrysene	0.14	0.34
IcdP	Indeno[cd]pyrene	2.72	4.20
BghiP	Benzo[ghi]perylene	2.67	4.80
BN21T	Benzo[b]naphtho(2,1-d)thiophene	0.11	0.30
BN12T	Benzo[b]naphtho(1,2-d)thiophene	0.02	0.08
BN23T	Benzo[b]naphtho(2,3-d)thiophene	0.05	0.22
ANTQ	Anthraquinone	0.14	0.68
BZONE	Benzanthrone	0.39	1.00
BaFONE	11H-Benzo[a]fluoren-11-one	0.32	0.90
BcFONE	7H-Benzo[c]fluoren-7-one	0.27	0.78
BaAD	Benzo[a]anthracene-7,12-dione	0.26	0.84

* Method detection limit for PAH, thiaPAH and oxyPAH: 0.0003 ng/m^3

Concentrations determined by GC-MS on a 30 m DB-5 column, with a temperature program and selected-ion monitoring as described in section IX.15.

**Data from Table 19.

VI.4 Bioassays of the Pooled Non-Polar and Polar Aromatic Fractions

Bioassays of both the pooled non-polar aromatic and polar aromatic fractions were carried out using a variety of *S. typhimurium* strains, with and without S9. The purpose of these experiments was to determine the relative responses of these new strains to a complex environmental sample. The O-acetyltransferase rich strains (YG1024 and YG1029) and the nitroreductase-rich strains (YG1021 and YG1026) which were engineered to contain multiple copies of the genes that encode for proteins responsible for the metabolism and biological activation of some urban air pollutants were of particular interest. Strains YG1021 and YG1024, which contain the hisD3052 allele and resemble the original Ames strain TA98, are particularly sensitive to compounds which induce frameshift mutations (e.g., nitroPAH). Strains YG1026 and YG1029, on the other hand, contain the hisG36 allele (which resembles Ames strain TA100) and are much more sensitive to compounds which cause base-pair substitution type mutations (e.g., PAH).

While all of the above strains had been used in bioassays of urban air particulate (SRM1649) and diesel particulate standard reference materials (SRM1650; unpublished work by Bryant, 122), the relative responses of these strains to an extract of urban air particulate were unknown. The strains with the highest responses would be used in bioassay-directed fractionation experiments. The aliphatic fractions from the alumina and the Sephadex LH20 chromatographic steps (Figure 17) were not tested, since they have never shown any response in a bioassay (51).

The data for the polar and non-polar fractions are listed in Table 37 and are shown in Figure 48. The *Salmonella* strains containing extra copies of the nitroreductase gene (YG1021 and YG1026) or extra copies of the O-acetyltransferase gene (YG1024 and YG1029) showed marked increases in their mutagenic responses compared to the standard tester strains YG1020 and YG1025, respectively (Table 37). In the absence of S9, the increase in response in the TA98 and TA100 type strains varied between 7- and 10-fold. The increased sensitivities of these strains to mutagens in air particulate extracts is both dramatic and useful; about 7-fold to 10-fold less extract would be consumed in each set of bioassay analyses using these strains. In the presence of S9 the increase in response was less dramatic, only 2.5- to 3-fold.

The strains YG1021 (-S9), YG1024 (-S9), YG1024 (+S9) and YG1029 (+S9) had shown the greatest increases in response to the broad range of mutagenic compounds present in the pooled non-polar aromatic fraction (Table 37) and would therefore prove useful in the subsequent bioassay-directed fractionation of this sample. The contribution of the non-polar aromatic fraction toward the mutagenic activity in the organic extract averaged 77% (±15% S.D.) in all of the strains and conditions tested (Table 37). The large contribution of the non-polar aromatic fraction is not surprising, since this fraction contains both PAH and nitroPAH, which are known to respond well in TA100-type and TA98-type strains of *S. typhimurium*, respectively. The non-polar aromatic fraction represented only 3% of the mass of the crude extract. For the 68 samples collected in Hamilton during 1990-1991 (section IV.6), the non-polar aromatic fraction accounted for

Table 37.

Mutagenic Responses of the Pooled Non-Polar Aromatic (A23/LH20) and Polar Aromatic (A45) Fractions in Various Strains of *S. typhimurium*

Salmonella strain		*A23 LH20 mutagenicity*	*A45 mutagenicity*	*Percent total activity in A23 LH20 fraction*
TA98-like strains:				
YG1020	-S9	3 rev/m^3 ± 14%*	1 rev/m^3 ± 14%*	75% ± 20%
	+S9	10	2	83
YG1021	-S9	21	4	84
	+S9	32	5	86
YG1024	-S9	30	8	79
	+S9	24	5	83
TA100-like strains:				
YG1025	-S9	1	2	33**
	+S9	13	2	87
YG1026	-S9	11	3	78
	+S9	38	5	88
YG1029	-S9	12	6	67
	+S9	29	7	80
			average (±S.D.) -S9	69% (± 19%)
			+S9	84% (± 3%)
			overall average(±S.D.)	77% (± 15%)

*The ± 14% error is derived from section IV.5.3.
** Verified by the results of two separate bioassays
Detection limit for bioassays: 0.3 revertants/m^3 (see section IV.5.2).

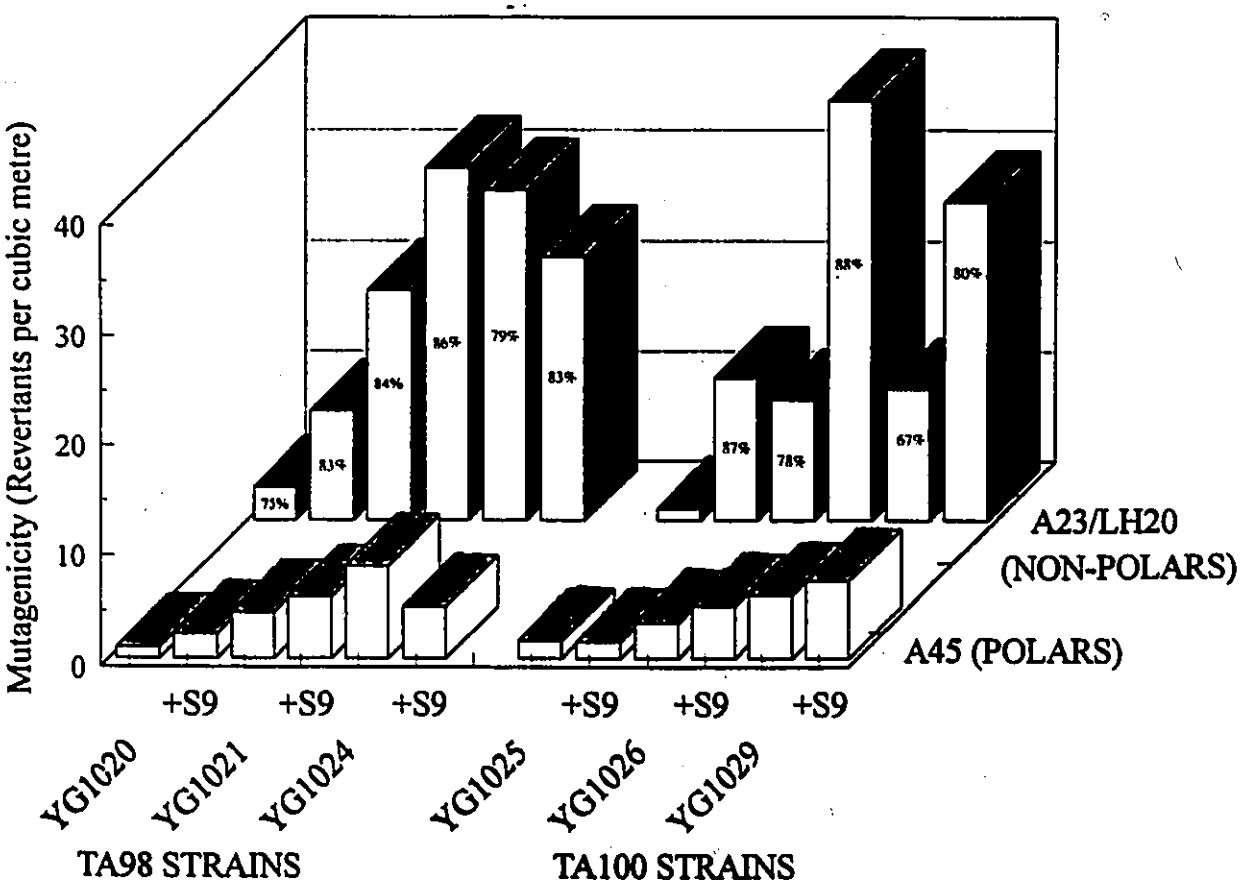


Figure 48. Histograms of mutagenic activity of the pooled polar and non-polar aromatic fractions of Hamilton air particulate in various strains of *S.typhimurium*, with and without S9. Percentages on the histograms represent the percentage of the total activity in the non-polar aromatic fraction.

an identical amount of the mutagenic burden within experimental error [74 ± 19%, using strain YG1021 (-S9)]; this fraction also represented an identical amount of the mass of the crude extract (5% ± 4%). Thus, from a bioassay analysis perspective this pooled sample is representative of an 'average' Hamilton air sample.

The polar aromatic fraction (A45) showed low mutagenic activities in the *S. typhimurium* strains tested. In strain YG1021 (-S9), the polar aromatic fraction represented only 16% of the total mutagenic burden of the air particulate extract, which is slightly below average (25 ± 19%) but within the range of values seen for ten individual A45 fractions.

VI.5 Normal-Phase Chromatography of the Pooled Non-Polar Aromatic Fraction

Experiments using various strains of *S. typhimurium* showed that strains YG1021 (-S9) and YG1024 (-S9) were the most sensitive for the detection of direct-acting mutagens, while strains YG1029 (+S9) and YG1024 (+S9) were choices for the detection of mutagens requiring metabolic activation. Thus, these four tester conditions were selected for subsequent experiments.

A normal phase gradient was developed with a view to providing a greater separation of PAC into compound classes and PAH into molecular mass classes and was based on a separation of PAH using an amino column reported by Wise *et al.* (181,182). The conditions reported by Wise required a two-hour elution time to elute only PAH.

Using our gradient, PAH are separated into molecular mass classes and more polar PAC elute from the column in a reproducible fashion. The reproducibility of this method (Table 38) was critically dependant on equilibration of the column (see section IX.12).

Approximately two-thirds of the remaining pooled non-polar aromatic fraction (representing the equivalent of 34,200 m^3 of air) was applied to a semi-preparative normal-phase HPLC column using gradient B. Due to the large amount of PAC in this sample, three separate injections were needed to collect fractions N1 through N456. The polar compounds (N7 material) were allowed to accumulate on the NPLC column until the third injection of the non-polar aromatic fraction, when the column was flushed with acetonitrile (gradient A, section IX.12) to yield a combined fraction N7. The chromatogram of this third injection appears in Figure 49. The large peak at 75 minutes elution time represents N7 material from 34,200 m^3 of air, while the chromatographic profile for fractions N1 - N456 represents the material from only a few thousand cubic metres equivalent. Prior to this work, NPLC column performance was evaluated through the injection of a mixture of standards. Actual samples of air particulate extract were only injected if the standards eluted with the retention times described in Table 38. Elution of the column with acetonitrile (to collect fraction N7) increased retention times slightly for more polar PAC, but this made virtually no difference to the composition of the various N fractions or to the reproducibility of the methods.

Table 38.

Normal-Phase HPLC Retention Time Data for PAH and PAH Derivatives on a Whatman 10 μm Polyaminocyano (PAC) Column.

Compound (amu)	*Method A Average* retention time*	*std.dev.*	*Method B Average* retention time*	*std.dev.*
Dibenzothiophene (184)	9.51min.	0.20 min.	9.15 min.	0.04 min.
Anthracene(178)	10.93	0.21	10.49	0.06
Fluoranthene (202)	13.80	0.21	13.17	0.09
Pyrene (202)	15.65	0.16	15.04	0.10
Benzo[b]naphtho(2,3-d)thiophene (234)	17.00	0.13	16.45	0.10
Chrysene (228)	19.44	0.10	18.94	0.09
Benzo[a]pyrene (252)	21.52	0.08	21.04	0.08
Indeno(1,2,3-cd)pyrene (276)	24.43	0.06	23.98	0.06
9-Nitroanthracene (223)	25.43	0.06	24.99	0.08
1-Nitronaphthalene (173)	26.45	0.06	25.91	0.12
Dibenzo[a,i]pyrene (302)	27.42	0.04	27.03	0.05
Dibenzo[j,p]chrysene (328)	**	**	28.56	0.06
6-Nitrobenzo[a]pyrene (297)	31.58	0.03	31.22	0.07
2-Nitrofluoranthene (247)	33.08	0.03	32.66	0.11
Carbazole (167)	41.98	0.03	41.25	0.13
Benz[a]anthracene-7,12-dione (258)	41.98	0.03	41.75	0.09
9-Fluorenone (180)	45.35	0.02	44.87	0.22
Anthraquinone (208)	45.84	0.03	45.54	0.08
Naphthacene-5,12-quinone (258)	50.30	0.02	49.54	0.09
Benzanthrone (230)	54.34	0.02	53.42	0.13
Dibenzo[ai]carbazole (267)	55.59	0.03	54.39	0.18
2-Nitrodibenzopyranone (241)	59.06	0.02	57.88	0.19
Quinoline (129)	75***	**		
Acridine (179)	75***	**		

*average of three determinations
** not determined
***eluted with 100% acetonitrile

Method A exposes the column to acetonitrile at the end of the gradient whereas method B does not (see section IX.12 for details). Bracketed values are the molecular masses of the standards in amu.

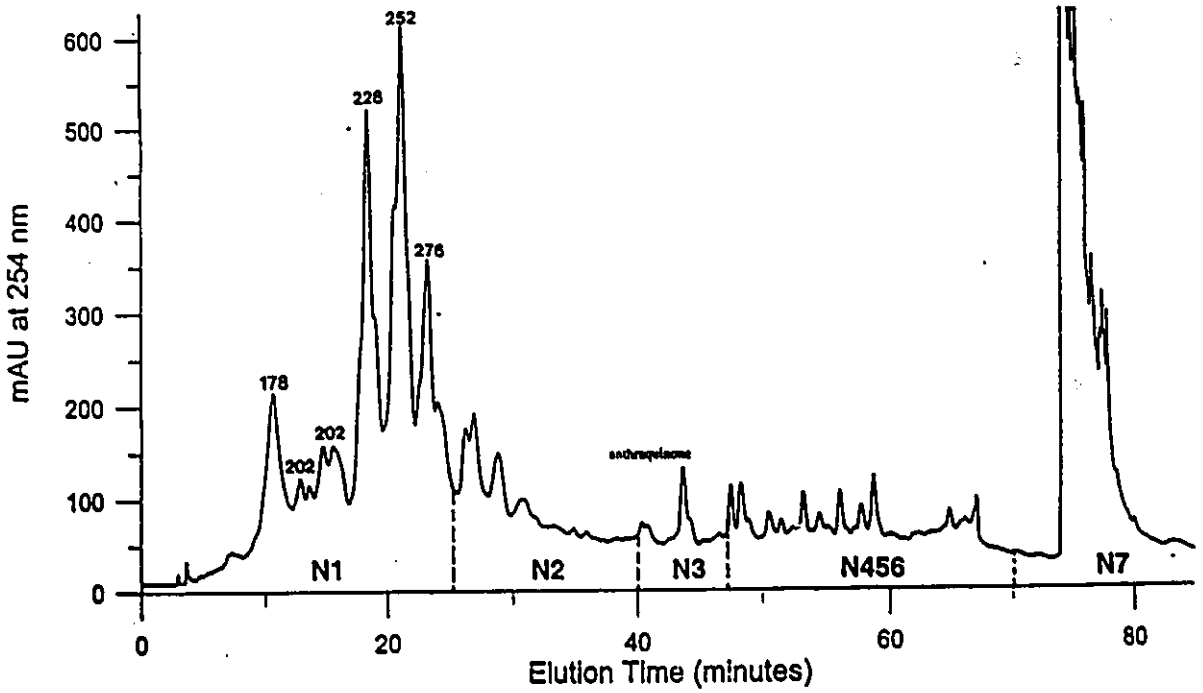


Figure 49. Normal-phase HPLC chromatogram (plotted at 254 nm) of the third preparative injection of several thousand cubic metres of fractions N1 through N456 and 34,200 m^3 of fraction N7 from the pooled non-polar aromatic fraction of Hamilton air particulate extract, showing cut points used in the collection of the various N fractions. Labels above the chromatographic peaks show the molecular masses (in amu) of the predominant constituents.

Once the N fractions had been collected (Figure 49), the mutagenic potency of each fraction was determined using *S. typhimurium* strains YG1021-S9, YG1024-S9, YG1024+S9 and YG1029+S9. Results of these bioassays (Table 39) revealed that fraction N2 was the most mutagenic fraction using strain YG1021 (-S9) while fraction N456 was the most mutagenic fraction in strain YG1024, with or without S9. Fraction N1, which contained PAH up to molecular weight 278, showed the greatest activity in strain YG1029+S9. The sensitivity of YG1029+S9 was not restricted to PAH, as indicated by the substantial responses to fractions N2 and N456. Fractions N3 and N7 showed little mutagenic response in any of the strains tested, indicating that dinitroPAH levels in fraction N3 must be very low and that the contributions of highly polar PAC in N7 to the total mutagenic activity of the sample was also low. The recovery of mutagenicity, calculated by taking the sum of the mutagenic activities of the N fractions and dividing by the mutagenic activity of the unfractionated non-polar aromatic fraction, was approximately 55% in all strains tested. Recovery of mutagenicity to within a factor of two is fairly typical for experiments of this type.

Full-scan GC-MS analyses of these N-fractions (data not shown) revealed that each N-fraction contained hundreds of different PAC, any number of which might be responsible for the observed biological activity. These fractions are very complex mixtures in their own right. The pooled non-polar aromatic fraction therefore had to be separated into a large number of subfractions (each containing only a few compounds), if there was to be any chance of ascribing the mutagenic responses to individual PAC.

Table 39.
Mutagenicity of N Fractions Obtained by NPLC Fractionation of Pooled Fraction A23/LH20.

	Mutagenicity (revertants m^3)			
Fraction	*YG1021(-S9)*	*YG1024(-S9)*	*YG1024(+S9)*	*YG1029(+S9)*
A23/LH20	21± 14%*	30 ± 14%*	24 ± 14%	29 ± 14%*
N1	2.0	1.9	4.5	5.8
N2	4.3	4.2	0.7	2.4
N3	0.5	3.7	0.4	0.6
N456	3.8	6.6	4.4	5.0
N7	0.6	1.0	2.6	1.7
ΣN**	11	17	13	16
ΣN X 100 / A23/LH20	52%***	57%***	54%***	55%***

*Experimental error in the determination of mutagenicity (section IV.5.3).

**Arithmetic sum of N fraction mutagenic activities

***Recovery of mutagenic activity

Detection limit of bioassays: 0.2 revertants/m^3

VI.6 Normal-Phase Mutation Chromatograms of the Pooled Non-Polar Aromatic Fraction

The following experiment was carried out to attempt to identify the PAC classes responsible for the differing responses in the four *S. typhimurium* strains (Table 39). A 1.3 mg sample of the non-polar aromatic fraction (corresponding to 132 mg of air particulate material collected from 4000 m^3 of air) was chromatographed in a single injection under normal-phase HPLC conditions with a polyaminocyano column using gradient A. The chromatographic eluate was collected in one-minute subfractions and each subfraction was then divided into five equal portions. Four portions were assayed individually using the four *S. typhimurium* tester conditions in Table 39; the fifth portion was used for GC-MS analysis. Unfortunately, the bioassay experiment using strain YG1024 (+S9) failed to give any meaningful data. Failures using this strain in mutation chromatogram experiments have been observed previously in this laboratory (88); the reasons for these failures are not clear.

The net mutagenic responses of the one-minute subfractions in the tester strains YG1021 (-S9), YG1024 (-S9) and YG1029 (+S9) were plotted together with the UV absorption profile at 254 nm to afford three mutation chromatograms (Figure 50). The resulting profiles of mutagenic activity showed similarities and differences which illustrate the utility of this NPLC/multiple bioassay approach. All three *S. typhimurium* assay conditions showed the presence of mutagens in the 31-33 minute and the 75-76 minute elution ranges (Figure 50B-D). Strain YG1024 (-S9) was the only bioassay condition to

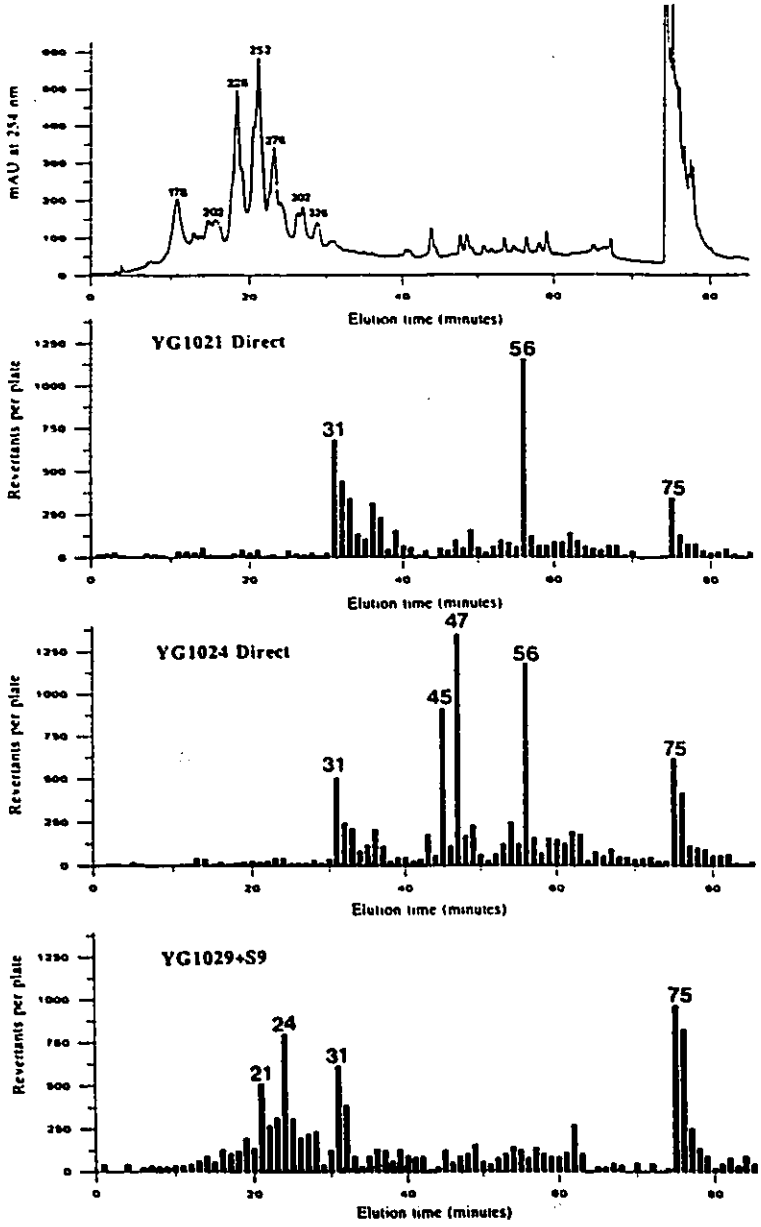


Figure 50. Mutation chromatogram obtained from bioassay analyses of one-minute subfractions collected during semi-preparative HPLC analysis of the pooled non-polar aromatic fraction. The UV absorption profile (A) was plotted at 254 nm; labels above the chromatographic peaks show the molecular weights (in amu) of the predominant constituents, as determined by GC-MS analyses. Subfractions (each equivalent to 800 m^3 of air) were bioassayed with strain YG1021(-S9) (B), strain YG1024 (-S9) (C) and strain YG1029 (+S9) (D). The mutagenic potencies of the subfractions have been plotted as net revertants.

respond to mutagens in the 45-47 minute range (Figure 50C). Similarly, strains YG1021 and YG1024 (-S9) responded to a mutagen in the 56 minute range (Figures 50B and 50C) while strain YG1029+S9 did not (Figure 50D). The PAH eluted between 15 and 30 minutes and showed mutagenic responses only with strain YG1029+S9 (Figure 50D).

The four subfractions which eluted from 30-33 minutes (Figure 50A) were analyzed by GC-MS in the selected-ion monitoring mode to detect nitroPAH. The 31 and 32 minute subfractions contained 2-nitrofluoranthene, 1-nitropyrene and 2-nitropyrene at concentrations of 6, 12 and 4 pg/m^3, respectively. Identification of these compounds was confirmed by comparison of their mass spectral ion ratios and their retention index values (66) with those of authentic standards under the same chromatographic conditions. However, these three nitroPAH could only account for about one-quarter of the mutagenicity (1 rev/m^3) detected in fraction N2 (4.3 revs/m^3, Table 39) using *S. typhimurium* YG1021 (-S9). This observation led to the experiments described in section VI.7, designed to identify the other mutagens in fraction N2.

The YG1029 (+S9) mutachromatogram (Figure 50D) exhibited mutagenic responses between 21 and 28 minutes, a range which corresponds to the retention times of higher molecular mass PAH. Two fractions (21 and 24 minutes) showed substantial responses. In the 21-minute subfraction the comparison of retention times (Table 38) and UV-visible spectra (210 nm to 400 nm) of standards (183) resulted in the identification of 252 amu PAH, specifically the benzofluoranthenes and benzo[a]pyrene. PAH of molecular mass 276 (indeno[1,2,3-cd]pyrene and benzo[ghi]perylene) were identified

similarly in the 24- minute subfraction.

GC-MS analyses of each subfraction from 16 to 28 minutes showed that all of the 252 molecular mass PAH were contained in the 21 and 22 minute subfractions, corresponding to the peak marked '252' (Figure 50A). The earlier eluting peak (18-19 minutes) was shown to contain only 223 amu PAH, while the later eluting peak at 24 minutes was shown to contain only 276 amu PAH. The relevant peaks in Figure 50A have been annotated to indicate the molecular masses of the PAH in those peaks. This normal-phase chromatographic method has greatly simplified the isolation and identification of higher molecular mass PAH that had previously been unresolved under other HPLC analysis conditions (97).

The shoulder on the front side of the '252' peak was identified as benzo[a]pyrene from its UV spectrum; all of the benzo[a]pyrene in the non-polar aromatic fraction was found in the 21-minute subfraction. Of the 252 MW PAH identified in this sample, benzo[a]pyrene is by far the most potent mutagen; in TA100 (+S9) the benzofluoranthenes (184), benzo[e]pyrene and perylene (185) exhibited mutagenic potencies ranging from no response to about 4% of the mutagenic response obtained with benzo[a]pyrene (91). Thus, most of the mutagenic activity in the 21-min. subfraction was ascribed to benzo[a]pyrene.

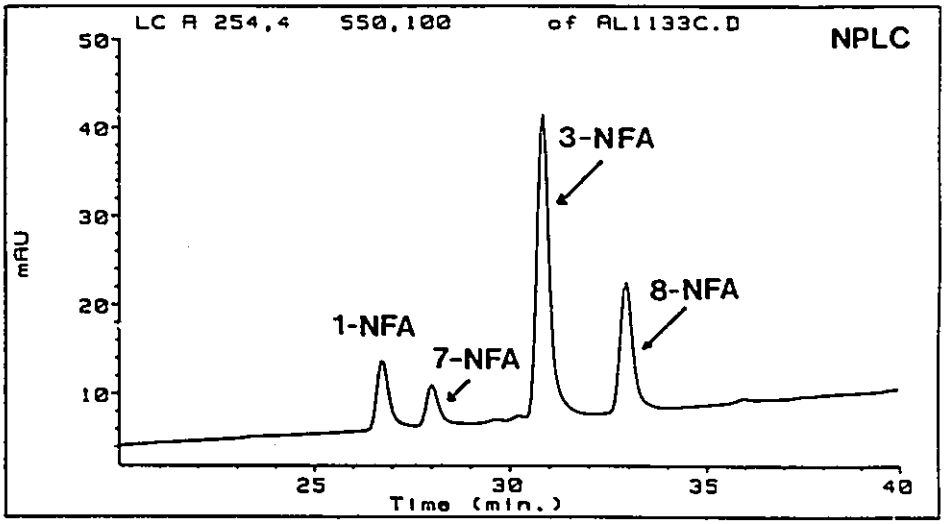
The GC-MS analysis of the 24-minute subfraction showed that it contained two compounds, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene. Neither of these compounds was detected in the GC-MS analyses of the 23- or 25-min. subfractions. These two compounds which were present in almost equal amounts (Table 36) have different

mutagenic responses in *S. typhimurium*; indeno[1,2,3-cd]pyrene is about ten times more mutagenic than benzo[ghi]perylene (91,92). Thus, the principal mutagen in the 24-minute subfraction was identified as indeno[1,2,3-cd]pyrene. Since this work was carried out, compounds of 302 amu (which were not sought out in the current study) have been identified as potent mutagens in TA100-type strains of *S. typhimurium* (228) and may therefore be responsible for some of the mutagenic activity in the mutation chromatogram in the zone between 276 amu PAH and 2-nitrofluoranthene. Efforts to identify the mutagens responsible for the biological activities in the 45, 47, 56 and 75-minute subfractions were unsuccessful.

VI.7 Bioassay-Directed Fractionation of Pooled Fraction N2

The normal-phase mutation chromatogram approach (Figure 50) facilitated the identification of the PAH responsible for the mutagenic activity detected using strain YG1029+S9 and the nitroPAH in fractions 31 and 32 responsible for the mutagenic activity detected using strains YG1021 (-S9) and YG1024 (-S9). However, the three nitroPAH detected in these fractions only accounted for about 25% of the mutagenicity of pooled fraction N2 in *S. typhimurium* YG1021 (-S9). The normal-phase mutation chromatogram approach was therefore applied to this fraction. NPLC was selected as the chromatographic method to prepare the mutachromatogram of pooled fraction N2, since N2 fractions contain nitroPAH mixtures and NPLC was observed to separate nitroPAH isomers with greater resolution than RPLC. For example, a mixture of nitrofluoranthenes was easily resolved by NPLC (Figure 51A) whereas the RPLC method failed (Figure 51B).

A



B

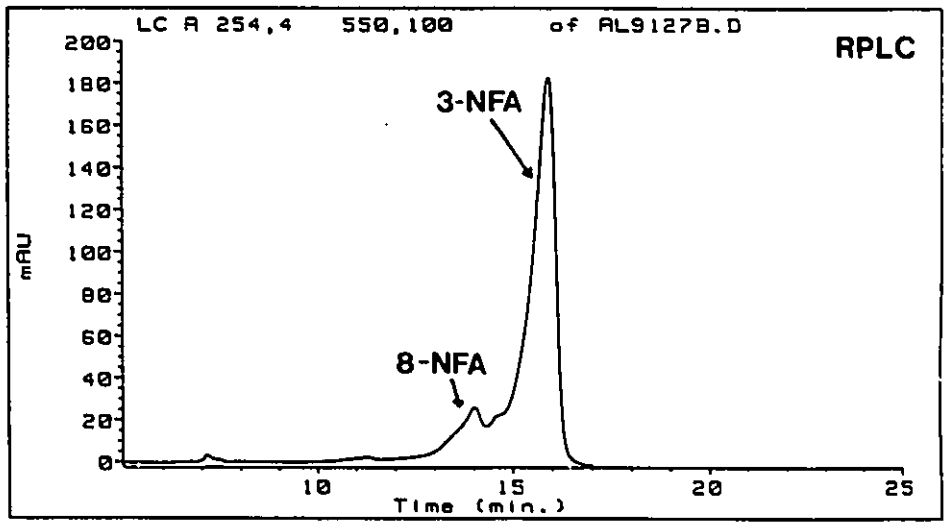


Figure 51. HPLC chromatograms of a synthetic mixture of 1-, 3-, 7- and 8-nitrofluoranthene using (A) normal-phase HPLC and (B) reversed-phase HPLC columns. A Whatman 10 µm polyaminocyano column was used for NPLC, while two Vydac 201TP columns in series were used for RPLC. Gradient A (section IX.12) was used for the NPLC analysis, while gradient D (section IX.13) was used for the RPLC analysis.

Pooled fraction N2, equivalent to 10,000 m^3 of air particulate, was applied to the NPLC column using the method (gradient A, section IX.12) described previously. The chromatographic eluate was collected in 30-second fractions. Single bioassays using *S. typhimurium* YG1021 (-S9) were carried out at two doses for each fraction. In the first experiment, the equivalent of 1000 m^3 (1/10th of the sample) was applied to each bioassay plate; in the second, the equivalent of 2000 m^3 (1/5th of the sample) was applied. The resultant NPLC mutation chromatogram and the mutation response data are shown in Figure 52A-C.

Each 30-second chromatographic fraction between 30 and 36 minutes was analyzed by GC-MS in both full scan and selected-ion monitoring modes. Ions were selected to monitor nitroPAH with molecular masses of 223, 237, 247, 261, 273 and 297 amu; the molecular ion (M^+) and four characteristic fragment ions (M-30, M-46, M-58 and M-73) were selected for each parent mass value.The total number of unique compounds detected in each 30-second fraction are plotted in Figure 52D. As few as 4 and as many as 70 unique compounds were observed in a single individual 30-second fraction. Thus, even the 30-second LC fractions contain a large number of chemical substances; these mixtures are truly complex.

The atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene and the combustion product 1-nitropyrene were identified and quantified in the 31 and 31.5 minute fractions. These nitroPAH accounted for 52% and 67% of the mutagenic activity seen in these fractions. The mutagenic activity in other fractions could not be attributed

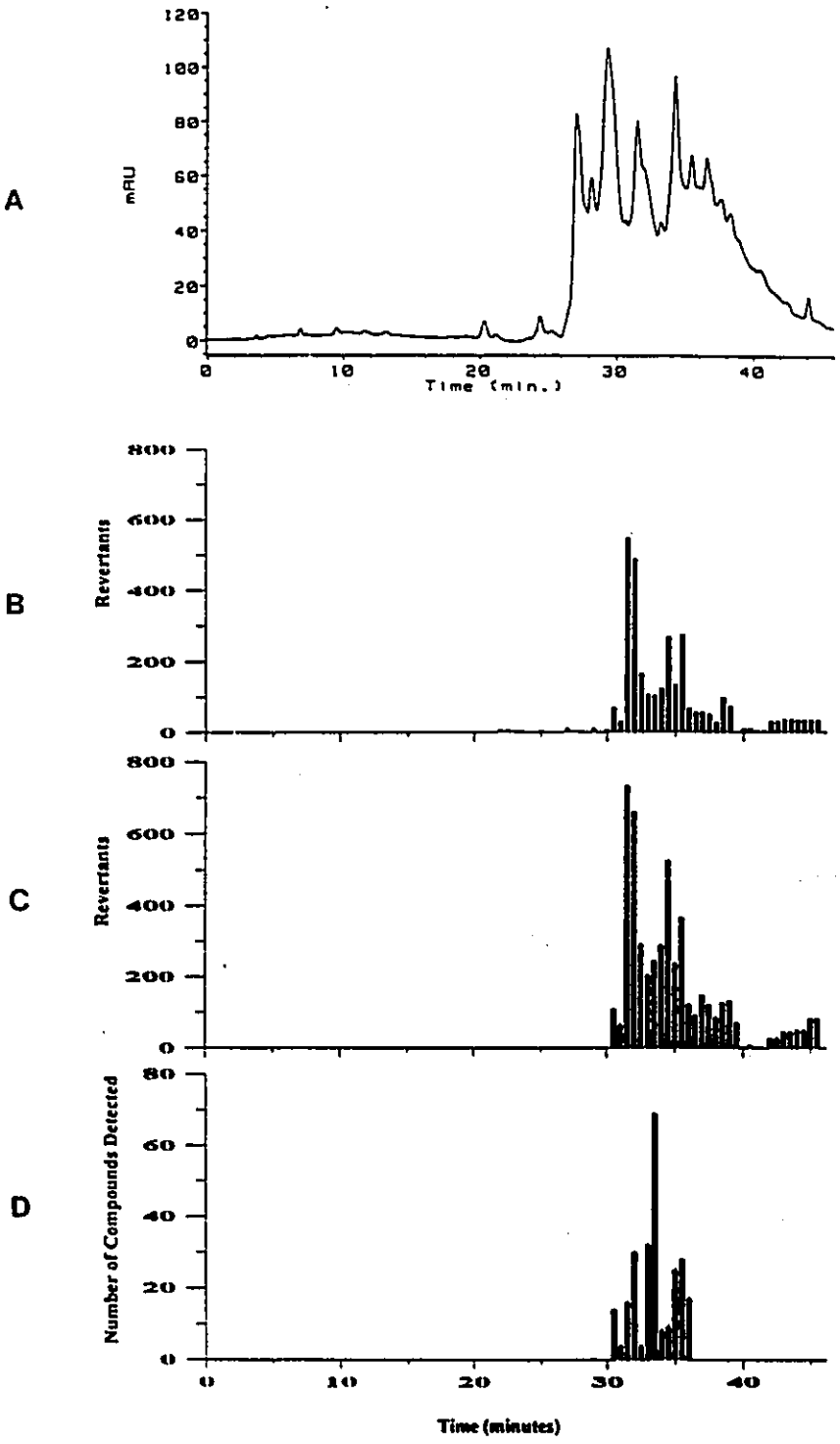


Figure 52. (A) Normal-phase HPLC chromatogram of pooled fraction N2 and mutagenicity of the equivalent of (B) 1000 m^3 and (C) 2000 m^3 applied to each bioassay plate, using *S. typhimurium* YG1021 (-S9); (D) shows the number of unique chemical compounds detected in each fraction by GC-MS. Gradient A (section IX.12) was used for the NPLC analysis.

to other nitropyrene or nitrofluoranthene isomers since these isomers were not present above the detection limit of 330 pg or 0.10 pg/m^3 corresponding to an injection of 3000 m^3 equivalent. NitroPAH with molecular masses of 223, 237, 261, 273 and 297 amu were not observed in any chromatographic fraction above the detection limit, either. Most compounds detected in full scan could not be identified, except for a few polycyclic aromatic ketones and aldehydes derived from PAH of 216 amu. These compounds have little or no mutagenic activity in *S.typhimurium* (186).

Pooled fraction N2 was used up completely in these chemical and biological analyses, thereby precluding any further work. The search for new mutagens in pooled fraction N2 had failed due to the sample's relatively low mutagenic potency and the very complex nature of these samples.

VI.8 Bioassay-Directed Fractionation of Pooled Fraction N456

Pooled fraction N456 which showed the greatest mutagenic response with *S. typhimurium* YG1024 (-S9)(Table 39) was subjected to a normal-phase mutation chromatographic analysis using strain YG1024 (-S9), owing to the sensitivity of this strain to compounds in this fraction. The eluate was collected every 30 seconds and an aliquot of each fraction was bioassayed. Fractions which gave mutagenic responses over 1500 revertants/plate were re-assayed using a series of doses. Dose-response data was used to prepare a normal-phase mutation chromatogram (Figure 53B).

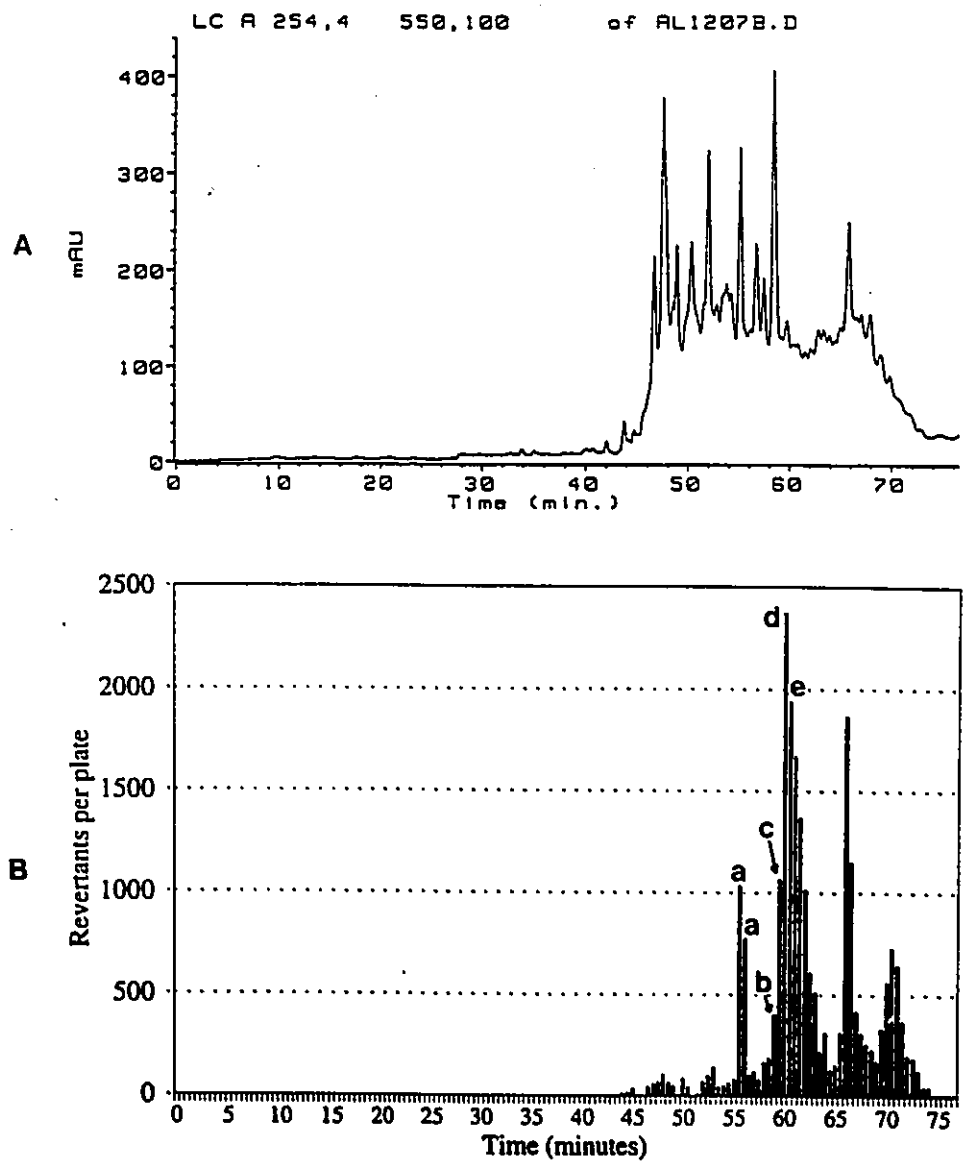


Figure 53. (A) Normal-phase HPLC chromatogram of pooled fraction N456 and (B) mutagenicity of the equivalent of 720 m^3 applied to each bioassay plate, using *S. typhimurium* YG1024 (-S9). Dose-respose curves were constructed for all chromatographic fractions with mutagenic activity greater than 1500 revertants per plate. Fraction **a** was collected between 55.5-56.5 minutes elution time, while fractions **b,c,d** and **e** were collected between 58.5-59.0, 59.0-59.5, 59.5-60.0 and 60.0-60.5 minutes elution time, respectively. Fractions **a,b,c,d** and **e** were used to prepare reversed-phase mutation chromatograms; see Figures 54-58.

UV-absorbing material began to elute from the column at 44 minutes and finally dropped off at about 72 minutes. The eluate between 44 and 54 minutes showed very little mutagenic response. Four peaks of mutagenic activity were observed at retention times around 55, 59-62, 66-67 and 71 minutes. The UV spectra of the chromatographic peaks in these zones could not be correlated with any standards. The peaks of mutagenic activity labelled **a,b,c,d** and **e** in Figure 53B were applied individually to a reversed-phase HPLC column and the eluate was collected in 60 1-minute cuts, which were bioassayed using *S.typhimurium* YG1024 (-S9).

The reversed-phase mutation chromatograms are presented in Figures 54 to 58. The mutagens detected in fraction **a** (Figure 54) eluted earlier and are more polar than the mutagens in fractions **b** through **e** (Figures 55-58). The mutation chromatograms of fractions **b** through **e** were surprisingly similar and showed two different zones of activity between 33-35 minutes and 42-43 minutes. Each of the RPLC chromatograms contained many peaks which eluted over a 40-minute span from 15 to 55 minutes. These chromatograms were further proof of the extraordinary complexity of each of these 30-second NPLC fractions.

Aliquots of the mutagenic fractions from Figures 54 - 58 were analyzed by GC-MS in the full scan mode, but the compounds in these fractions were either below the detection limit of the instrument or did not elute from the GC column. However, an estimate of the mutagenic potency of these unknown compounds could be made in the following manner. The zone of mutagenic activity at 42 minutes (Figure 57) corresponded

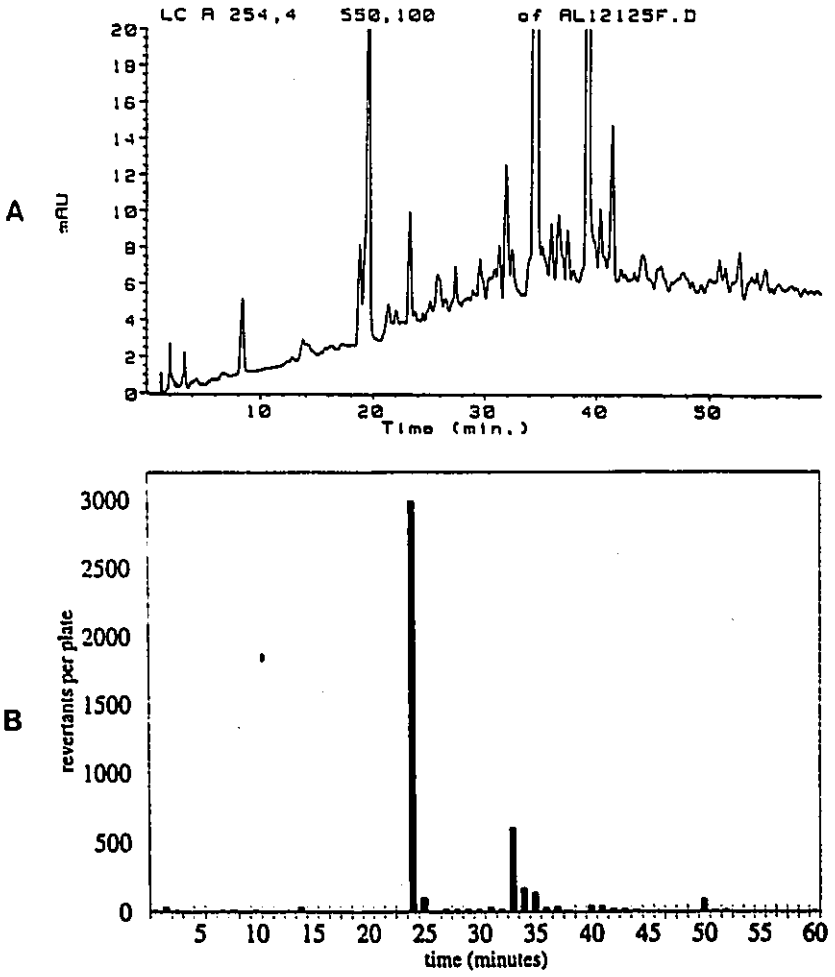


Figure 54. (A) Reversed-phase HPLC chromatogram of fraction a shown in Figure 53 and (B) mutagenicity of the equivalent of 7000 m^3 applied to each bioassay plate, using *S. typhimurium* YG1024 (-S9). The mutagenic activity of 3000 revs/plate for the fraction eluting between 23.00 and 23.99 minutes is based on the results of a dose-response curve analysis of this fraction.

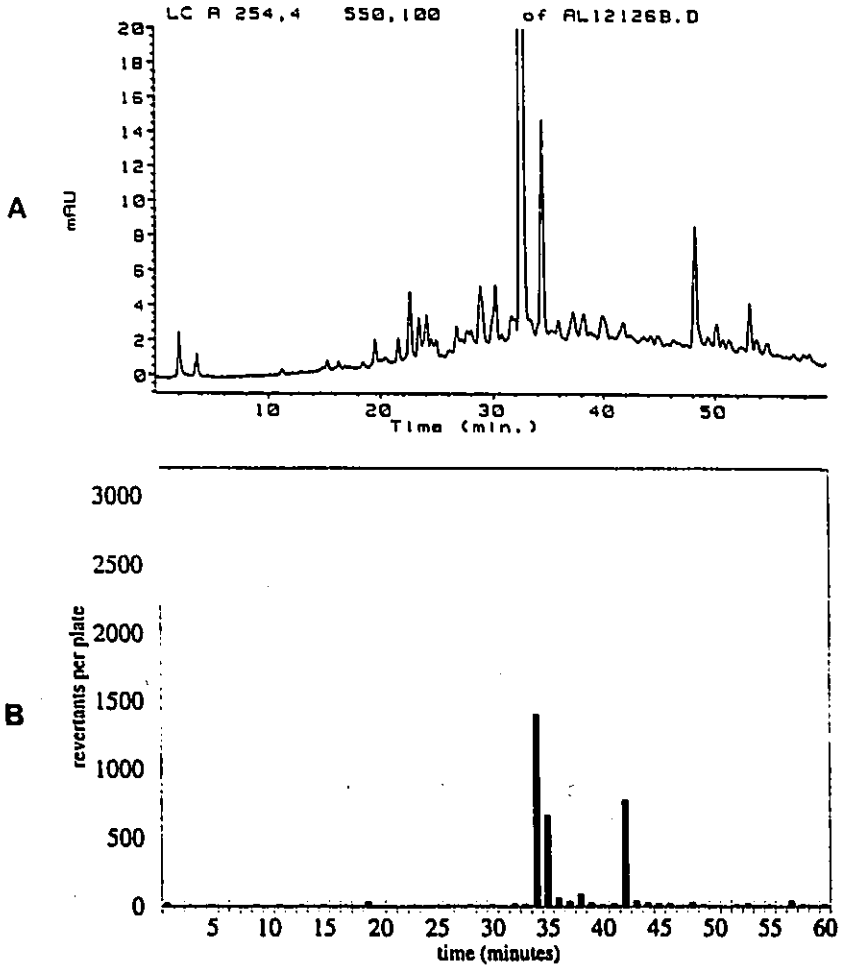


Figure 55. (A) Reversed-phase HPLC chromatogram of fraction b shown in Figure 53 and (B) mutagenicity of the equivalent of 4600 m^3 applied to each bioassay plate, using *S.typhimurium* YG1024 (-S9).

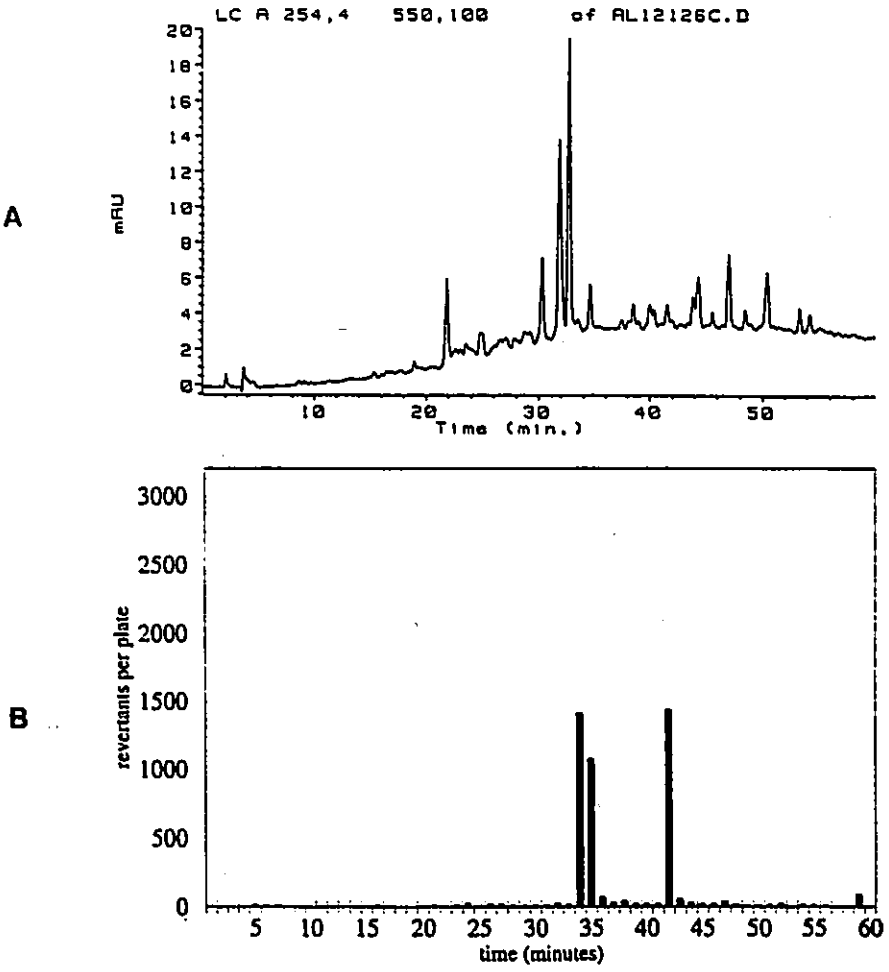


Figure 56. (A) Reversed-phase HPLC chromatogram of fraction c shown in Figure 53 and (B) mutagenicity of the equivalent of 4600 m^3 applied to each bioassay plate, using *S. typhimurium* YG1021 (-S9).

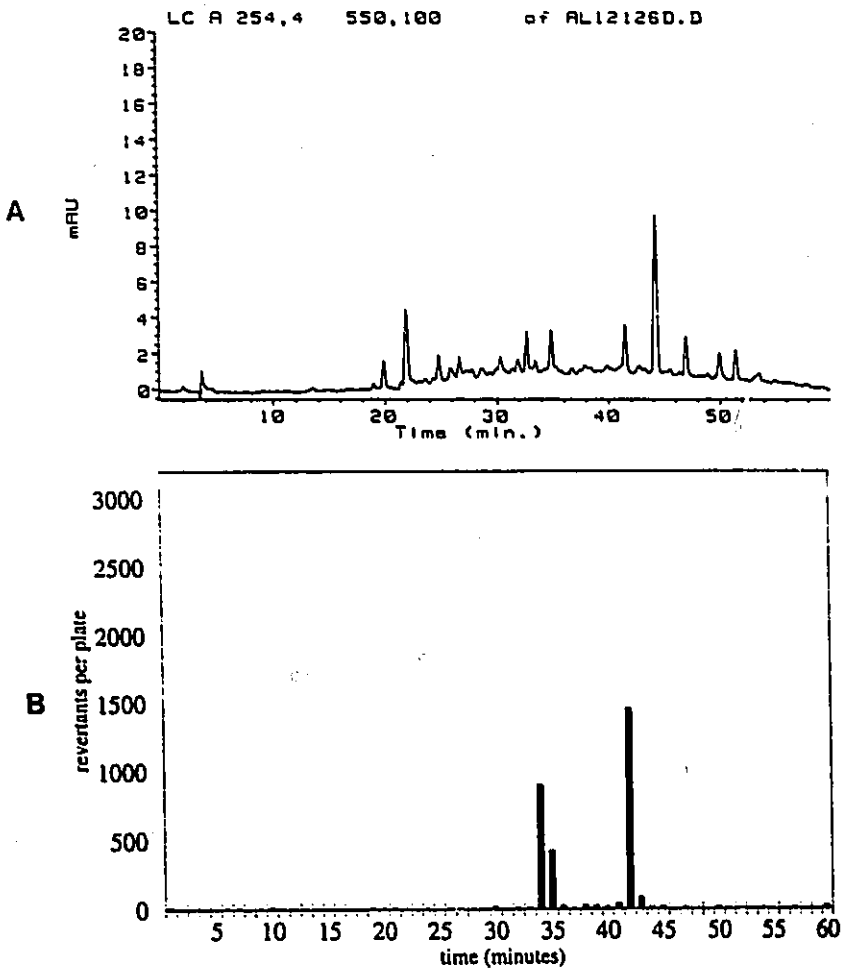


Figure 57. (A) Reversed-phase HPLC chromatogram of fraction d shown in Figure 53 and (B) mutagenicity of the equivalent of 4600 m^3 applied to each bioassay plate, using *S.typhimurium* YG1024 (-S9).

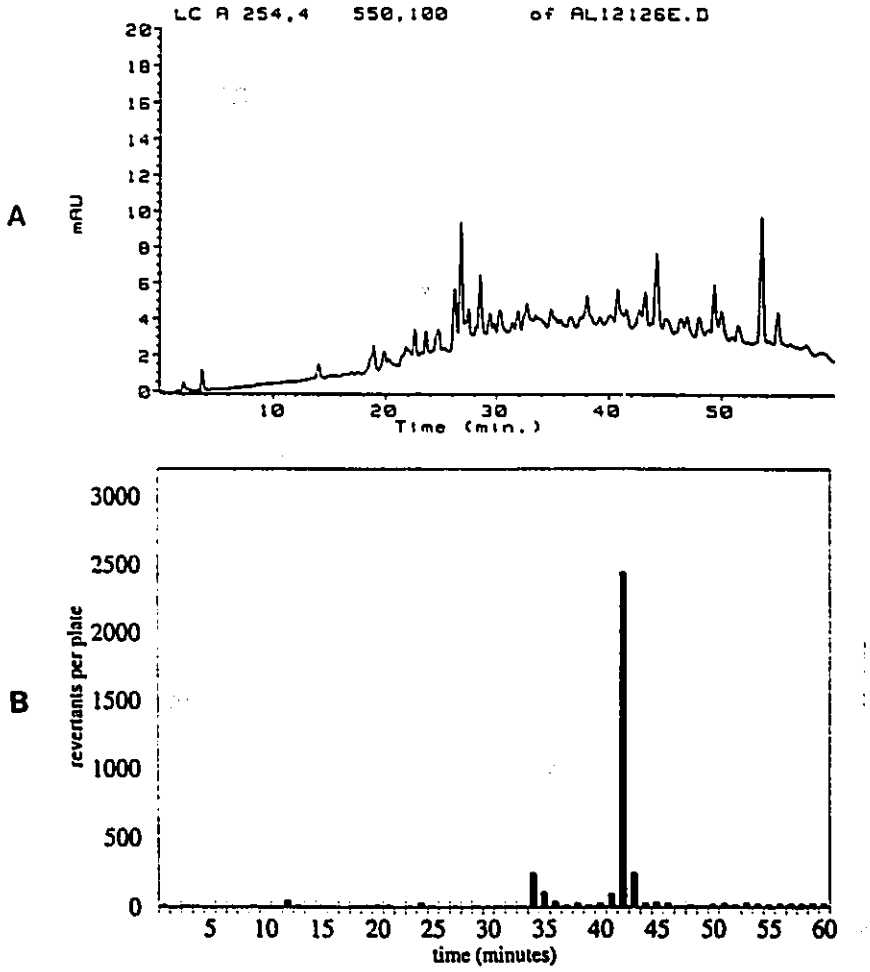


Figure 58. (A) Reversed-phase HPLC chromatogram of fraction e shown in Figure 53 and (B) mutagenicity of the equivalent of 4600 m^3 applied to each bioassay plate, using *S.typhimurium* YG1024 (-S9). The fraction with a mutagenic activity of 2400 revs/plate for the fraction eluting 41.00 and 41.99 minutes is based on the results of a dose-response analysis of this fraction.

to an RPLC peak with a height of 3 mAU, which corresponds to approximately 0.2 μg of a PAC, assuming an extinction coefficient of 20,000 at 254 nm. In this example the unknown compound (0.2 μg) produced *ca.* 1460 revertants. Assuming a molecular mass of 200 amu/mole (or 0.2 μg/nmole) the mutagenicity of this unknown compound calculated to 1500 revs/nanomole, which is approximately two-fold lower than the activity of 1-nitropyrene in *S. typhimurium* YG1024 (-S9) (123). Thus, the compounds causing the mutagenic response in Figures 54 to 58 appear to possess moderate mutagenic potencies.

The subfractions shown in the RPLC mutation chromatograms were used up in unsuccessful GC-MS analyses precluding further analyses. However, application of a three-dimensional approach to the identification of mutagens (*i.e.*, preparation of a non-polar aromatic fraction, followed by NPLC- then RPLC-mutation chromatograms) succeeded in the deconvolution of an extremely complex mixture. The mutagens detected by *S. typhimurium* YG1024 (-S9) in Figures 54 through 58 could have been detected by GC-MS if a larger amount of air particulate had been available.

VI.9 Conclusions

Bioassay analyses of a large sample of air particulate extract were carried out using six different *Salmonella* strains both with and without S9, which permitted a comparison of the sensitivities of the different strains and assay conditions. Four bioassay conditions showed substantially increased responses compared to the standard tester strains and conditions. The *Salmonella* strain TA98 variants containing extra copies of the

nitroreductase gene (YG1021) and acetyltransferase gene (YG1024) showed 7-fold and 10-fold enhanced responses compared to the standard tester strain (TA98 or YG1020).

The selectivity of different strains was demonstrated when these assay conditions were used in parallel to analyze the eluate from an NPLC separation. The coupling of a reproducible chromatographic method with a suite of complementary genotoxicity assays provided a series of mutation chromatograms that afforded an extraordinary profile of a complex mixture. Strain YG1029+S9 is the strain of choice for PAH detection, while strains YG1021 (-S9) and YG1024 (-S9) did not respond to PAH at all. Strains YG1021 (-S9) and YG1024 (-S9) showed many similar responses in this mutation chromatogram; only strain YG1024 (-S9) detected mutagens which eluted in the 45-47 minute region of the chromatogram. For this reason, extracts of air particulate should be bioassayed using YG1024 (-S9) and YG1029 (+S9), if a complete picture of the mutagenic activity of the non-polar aromatic fraction is desired.

The dose-response bioassays of the N-fractions and the multiple normal-phase mutation chromatograms of the non-polar aromatic fraction are complementary experiments. For example, the pooled N3 fraction showed 7-fold more activity in strain YG1024 (-S9) than strain YG1021 (-S9) and this increase is the result of mutagens eluting at 45 and 47 minutes (Figure 50) which were detected only by strain YG1024 (-S9). The compounds eluting at 45 and 47 minutes (Figure 50) may have been dinitroPAH, based on the relative responses of dinitroPAH standards in strains YG1021 (-S9) and YG1024 (-S9) as well as the NPLC retention times of these standards (see Chapter VII).

The mutagenic activity observed in all three strains at 75 minutes (Figure 50) may

have been due to hydroxynitroPAH. Schuetzle *et al.* (65) reported that hydroxynitroPAH eluted from a silica NPLC column with methanol only after the PAH, nitroPAH and nitro-oxyPAC had already eluted with hexane. In the present study, the mutagens eluting at 75 minutes (Figure 50) also eluted after the PAH, nitroPAH and nitro-oxyPAC [e.g., nitrodibenzopyranone] had eluted with hexane/dichloromethane. The current study did not involve the synthesis, chromatography or bioassay of hydroxynitroPAH and the contribution of these compounds towards the mutagenic activity of Hamilton air particulate was therefore not quantified.

Nishioka *et al.* (65) have indicated that hydroxynitroPAH may be contributors to urban air particulate extract mutagenicity in *S. typhimurium* strain TA98 (-S9). However, Schuetzle *et al.* (221) have speculated that hydroxynitroPAH may arise from the atmospheric transformation of PAH and that their concentration may vary depending on atmospheric conditions. Thus, the contribution of hydroxynitroPAH to the mutagenicity of urban air particulate may be important at times, since the concentration of these compounds could vary widely and may be determined by many variables.

This work has shown that the multiple bioassay mutation chromatogram approach was the methodology which provided the greatest amount of information about the pooled sample. The experimental effort to obtain this data was equal to or less than the effort expended using the N-fraction/dose-response bioassay approach. In our opinion the multiple bioassay mutation chromatogram methodology is a very powerful approach to profiling complex mixtures and identifies regions of a chromatogram which could benefit from application of additional chromatographic separation methodologies.

VII. CHEMICAL AND BIOLOGICAL ANALYSIS OF AIR PARTICULATE COLLECTED IN TORONTO, 1994

VII.1 Overview and Introduction

This chapter will describe the chemical and biological analyses of extracts prepared from 22 respirable air particulate samples collected on both sides of Highway 404 in Toronto, Ontario, during the summer of 1994. This collection provided our research group with a set of samples which contained material primarily of vehicular origin; the analytical results from these samples will be compared with data from samples collected in Hamilton which contained material from both vehicular and industrial emissions.

Highway 404 is a four-lane expressway which runs north and south as an extension of the Don Valley Parkway north of Highway 401 in Toronto (Figure 59). Studies by the Ontario Ministry of Transport (MTO) have shown that Highway 404 is used mostly by gasoline-powered vehicles (B. Snodgrass, personal communication). Air particulate sampling was carried out for the MTO as part of an environmental assessment for a proposed widening of this highway. Our research group lent air samplers for this study and was contracted to provide information on the air particulate collected on both sides of the Highway over a 4-month period.

Air particulate was extracted, non-polar aromatic fractions were prepared and subjected to GC-MS analyses by our laboratory group. Bioassays and the processing of GC-MS data, as well as bioassay-directed fractionation were carried out by the author.

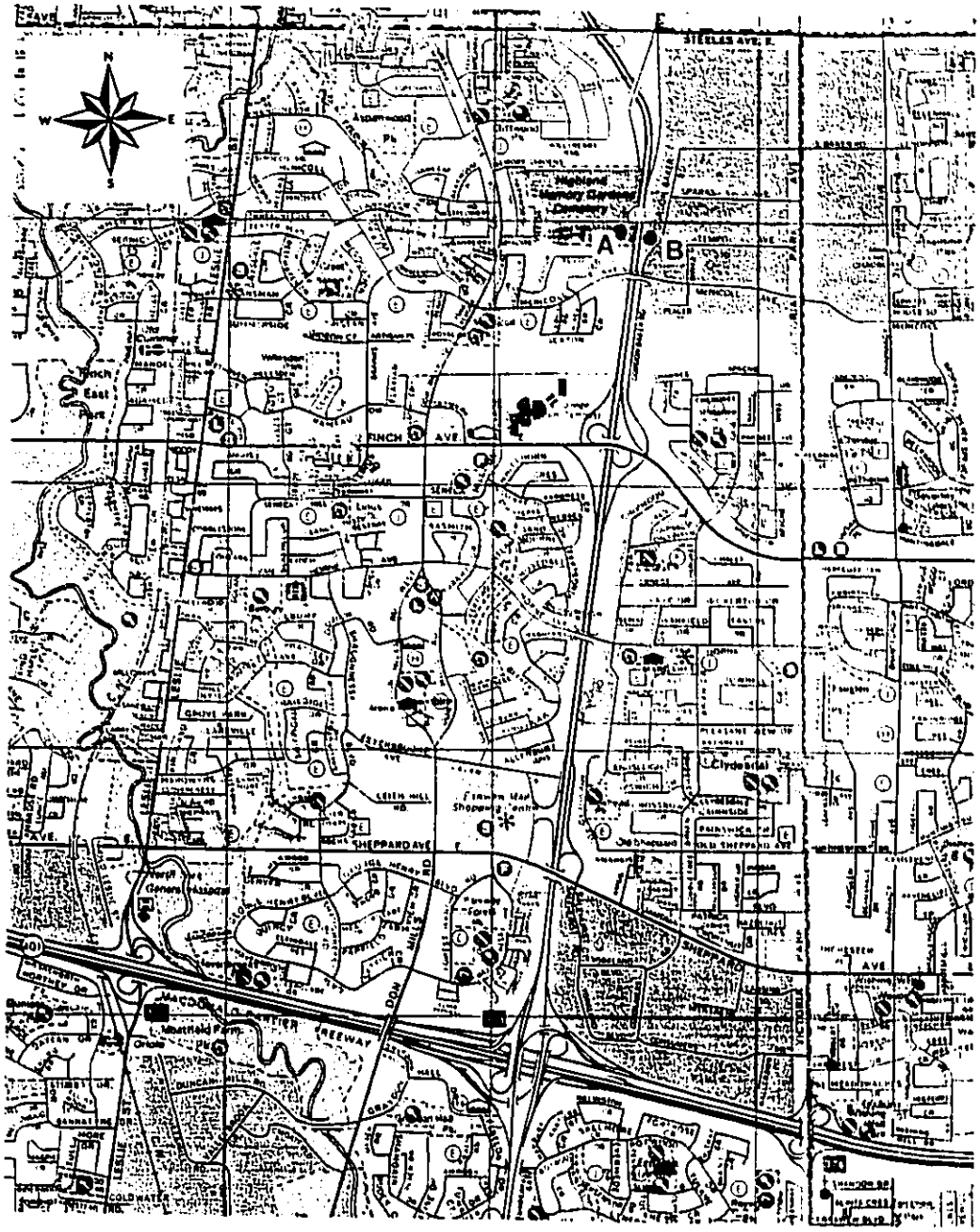


Figure 59. Map of Toronto showing sites for air particulate collection. Site (A) was on the grounds of Highland Memorial Gardens Cemetery, while site (B) was directly across Highway 404 from site (A), between Gordon Baker Road and the east side of Highway 404.

The results of GC-MS analyses for PAH and simple bioassays were included in [a] an unpublished report by Bryant (187) submitted to the Ontario Ministry of Transport and [b] an undergraduate thesis by K. Hamilton of the McMaster Geology Department (188), which also included magnetic susceptibility data. The bioassay-directed fractionation of a particularly mutagenic sample is reported here for the first time.

VII.2 Air Particulate Collection and Selection of Filters for Extraction

Two hundred and ten air particulate samples were collected during two sampling periods in 1994: from April 13th to May 10th and from May 26th to August 20th. Air particulate was collected every day, from midnight to midnight, using two PM-10 high volume air samplers at two sites on either side of Highway 404 (Figure 59). Samples were collected by employees of an engineering consulting firm. One sampler was located at the fence on the west side of the highway, on the grounds of Highland Memorial Gardens Cemetery; the other sampler was located at the fence on the east side of the highway in a light industrial area near Gordon Baker Road.

Data for particulate mass was converted to particulate concentration data (stated in terms of $\mu g/m^3$; see section II.2). Plots of particulate concentrations for the west side and east side samplers between May 25th and August 7th are shown in Figures 60A and 60B, respectively. The patterns are somewhat similar; higher particulate concentrations were generally observed on weekdays while lower concentrations were observed on

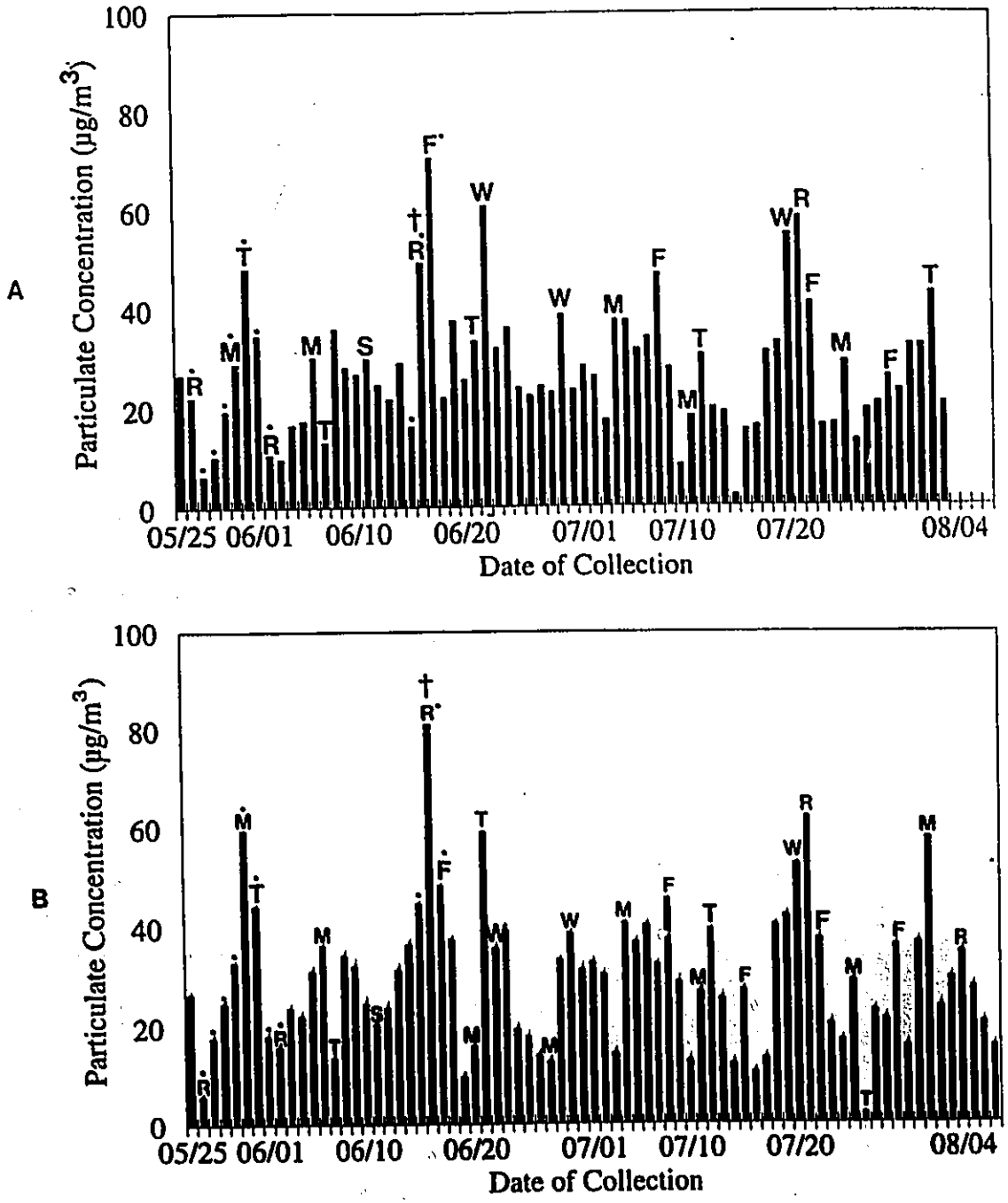


Figure 60. Particulate concentration versus date at (A) Highland Memorial Gardens [site A, west side of Highway 404] and (B) at Gordon Baker Road [site B, east side], between May 25th and August 7th, 1994. Labels indicate day of particulate collection: M=Monday, T=Tuesday, W=Wednesday, R=Thursday, F=Friday and S=Saturday. No samples were collected on the west side between August 4th and 7th. Samples selected for chemical and biological analyses are indicated with an asterisk (*) and the thermal inversion of June 16th is indicated with a cross (†).

weekends. The higher values on weekdays co-incided with higher vehicular traffic, based on data from the MTO.

The similarity of particulate concentrations on both sides of the highway is apparent in averages for the 146 samples collected between May 25th and August 7th. Average particulate concentrations for the west side (28 ± 12 $\mu g/m^3$) versus the east side (30 ± 14 $\mu g/m^3$) are identical (within experimental error) not only to each other but also to average concentrations observed for the 330 samples collected in downtown Hamilton (36 ± 22 $\mu g/m^3$) and West Hamilton (33 ± 24 $\mu g/m^3$) from May, 1990 to May, 1991. Thus, average particulate loadings in Toronto and Hamilton at these sites were indistinguishable.

Our research group wished to study the PAC concentrations and mutagenicity of extracts prepared from particulate collected before, during and after episodes of above average particulate concentration. Two specific episodes caught our attention early in the sampling period and those days are noted with asterisks in Figures 60A and 60B. Particulate concentrations on the east side of the highway (Figure 60B) rose from a low value of 6 $\mu g/m^3$ (May 26th) to a high value of 60 $\mu g/m^3$ (May 30th) and then fell to a low value (16 $\mu g/m^3$) on June 2nd. Thus, 8 filters each from both the east and west sides of the highway collected between May 26th and June 2nd were selected for extraction - a total of 16 filters.

A further selection was made from filters collected before, during and after a thermal inversion which occured on June 16th. Particulate concentrations on the east

side rose from 45 $\mu g/m^3$ on June 15th to 81 $\mu g/m^3$ on June 16th and decreased to 48 $\mu g/m^3$ on June 17th. The filters collected on both sides of the highway on these three days gave an additional 6 filters for a grand total of 22 filters.

VII.3 Extraction of Air Filters and the Determination of Masses of Crude Organic Extracts

Organic extracts from each of the 22 filters were prepared and the mass of each extract was determined as described in section IV.2. The average percentage of extractable material (34% $\pm$ 15%) was identical to the value (33% $\pm$ 17%) obtained in the Hamilton study of 1990-1991. Non-polar aromatic fractions were prepared from the crude organic extracts as described in section IV.3 and subjected to GC-MS analyses, followed by *S. typhimurium* bioassays for mutagenicity.

VII.4 GC-MS Analyses of Non-Polar Aromatic Fractions

Analysis by GC-MS with electron impact ionization in the selected-ion monitoring mode was used to quantify 37 different PAC in the non-polar aromatic fraction. The number of target compounds (Table 40) had been increased in this work to correspond more closely to the list of target compounds in the MOEE analytical methods (189). The concentrations of vapour-phase PAH (e.g., acenaphthylene and other compounds of molecular weight 152 or less) were usually below the detection limit. Total monitored PAC concentrations on the west and east sides of the highway are shown in Figures 61A and 61B, respectively.

Table 40.
PAC Quantified in Air Particulate Samples Collected on Both Sides of Highway 404, May-June 1994.

Polycyclic Aromatic Compound	*Concentration (pg/m³)* Average	Std.Dev.	Range (pg/m³)	*Average Conc. in Hamilton* (pg/m³)
PAH				
Naphthalene	1	5	nq* - 23	
1- & 2-Methylnaphthalene	nq*			
Biphenyl & Acenaphthene	nq*			
Acenaphthylene	nq*			
Fluorene	2	2	nq* - 6	
Phenanthrene	65	15	8 - 146	950
Anthracene	1	1	nq* - 11	91
o-Terphenyl	8	8	1 - 29	
1-Methylphenanthrene	13	13	nq* - 61	
Fluoranthene	79	45	8 - 176	2100
Pyrene	105	63	11 - 314	1800
9,10-Dimethylanthracene	nq*			
m-Terphenyl	4	2	1 - 8	
p-Terphenyl	2	1	nq - 6	
Benzo[a]fluorene	16	11	nq - 42	
Benzo[b]fluorene	7	7	nq - 25	
Benz[a]anthracene	54	43	2 - 155	2400
Chrysene	130	91	9 - 390	3100
Benzo[b&j]fluoranthenes	252	197	16 -780	7800
Benzo[k]fluoranthene	65	72	6 - 301	
Benzo[e]pyrene	130	90	9 - 41	3400
Benzo[a]pyrene	47	39	1 - 140	2600
Perylene	21	36	nq -135	740
3-Methylcholanthrene	nq			
Indeno[1,2,3-cd]pyrene	175	130	11 - 510	4200
Dibenz[ac&ah]anthracenes	17	16	1 - 65	910
Picene	27	25	1 - 88	
Benzo[ghi]perylene	265	182	18 - 750	4800
ThiaPAH				
Dibenzothiophene	7	5	1 - 19	
Benzo[b]naphtho(2,1-d)thiophene	20	11	nq - 41	300
Benzo[b]naphtho(2,3-d)thiophene	8	4	nq -16	220
OxyPAC				
Anthraquinone	104	56	13 - 242	680
Benzanthrone	106	62	8 - 232	1000
Benz[a]anthracene-7,12-dione	66	45	4 - 203	840
NitroPAH				
2-Nitrofluoranthene	34	79	nq - 385	

* Below method detection limit of 0.2 pg/m³ for PAH and 1.0 pg/m³ for nitroPAH.

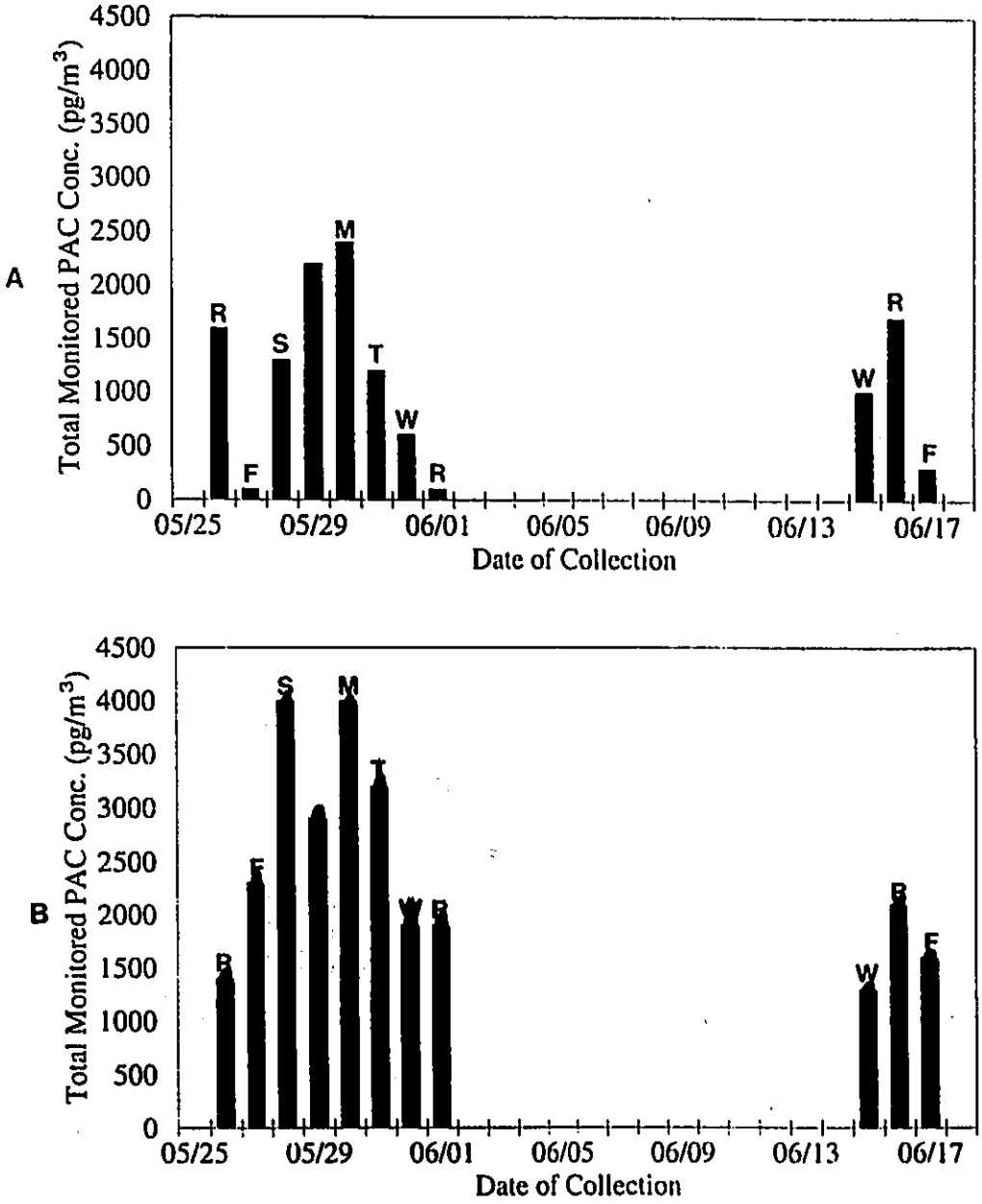


Figure 61. Total monitored PAC concentrations on the (A) west side and (B) east side of Highway 404, May 26th-June 1st and June 15th-17th, 1994. PAC concentrations were not determined between June 2nd and June 14th inclusive. Labels indicate day of particulate collection as described in Figure 60.

The pattern of total monitored PAC concentrations on the west side of the highway was considerably different from that observed on the east side from May 26th to June 1st (Figure 61); average PAC concentrations on the west side (1140 ± 770 pg/m^3) were about two-fold lower than average concentrations on the east side (2420 ± 930 pg/m^3). K. Hamilton (188) observed that PAC concentrations were not correlated to wind direction and offered the suggestion that the higher concentrations on the east side may be a result of local light industrial activity and the absence of surrounding trees, which sheltered the sampler on the west side. Similar PAC concentrations on both sides between June 15th-17th were probably the result of the low windspeeds (0-5 km/h) typically associated with thermal inversions.

The PAC concentrations in these 22 fractions were unremarkable and are near the lower end of PAC concentrations reported around the world (cf. Tables 15 and 16). These PAC concentrations were also far lower than those observed in 68 samples prepared from Hamilton air particulate. For example, the average benzo[a]pyrene concentration for the Hamilton samples was 2600 pg/m^3 (± 3100 pg/m^3 S.D., Table 9) while the average benzo[a]pyrene concentration on both sides of Highway 404 was 55-fold lower, only 47 ± 39 pg/m^3 (Table 40). Thus, heavy industry may be responsible for the high PAC concentrations seen in Hamilton, as has been previously hypothesized (100,103).

Calculation of the four PAH ratios traditionally calculated in source apportionment studies (155) did not provide particular insight (Table 41). First, the Hamilton

Table 41.

Ratios of PAH Observed in Particulate at Highway 404, in Hamilton and in Various Source Apportionment Studies.

Ratio*	Engine Soot: Gas	Engine Soot: Diesel	Coking Plant Soot	SRM 1649	SRM 1650	Hamilton Air Particulate (1990-91)	Highway 404 Particulate (May-June 1994)
BaA/BeP	2.9	4 - 9	1.5 - 4.8	0.7	0.7	0.7 ± 0.4	0.4 ± 0.3
BaP/BeP	1.0	1 - 5	1.5 - 2.3	0.9	0.1	0.7 ± 0.2	0.3 ± 0.2
BghiP/BeP	2.9	0.5-1.8	0.5 - 1.2	1.4	0.2	2.0 ± 1.0	2.0 ± 0.4
IcdP/BeP	1.24	**	0.6	1.0	0.2	1.4 ± 0.5	1.3 ± 0.3

*Abbreviations: BaA, benzo[a]anthracene; BeP, benzo[e]pyrene; BaP, benzo[a]pyrene; BghiP, benzo[ghi]perylene, IcdP, indeno[1,2,3-cd]pyrene.

**not described

Ratios for gasoline and diesel engine soot, as well as coking plant soot, are from the report of Daisey *et al.* (155). Ratios for SRM1649 and SRM1650 are from the reports of Uriano (190) and Rasberry (115), respectively. Ratios for Hamilton and Highway 404 particulate are the averages for 68 and 22 samples analyzed, respectively.

and Toronto values were identical for three of the four PAH ratios; only the BaP/BeP ratio was slightly different. Second, the values for both cities were close to the values for SRM1649, the urban dust reference material, but different from values reported for SRM1650, the diesel engine particulate.

VII.5 Bioassays of Non-Polar Aromatic Fractions

Bioassays of the Highway 404 non-polar aromatic fractions were carried out using three strains of *S. typhimurium* [YG1021 (-S9), YG1024 (-S9) and YG1024 (+S9)]. The number of bioassays was increased for this study due to observations made with the pooled air particulate extract from Hamilton (section VI.8). Strain YG1024 (-S9) was shown to detect mutagens not detected by strain YG1021 (-S9). The mutagenicity data for these 22 samples, including the ratio of YG1024 (-S9) to YG1021 (-S9) activities, are listed in Table 42. The mutagenicity values for all samples were higher than in the extracts from Hamilton. Indeed, two days (May 27th and June 16th) showed responses in strain YG1021 (-S9) [240 and 500 revs/m^3] that were 2-fold and 4-fold higher than any samples from Hamilton; June 16th corresponded to a thermal inversion event. The corresponding YG1024 (-S9) values were even higher: 280 and 2300 revs/m^3. The data in Table 42 also indicated that on the west side of the highway, the ratio of activities in strain YG1024 (-S9) to strain YG1021 (-S9) was fairly constant except for three days: May 28th, June 1st and June 16th. The June 16th sample showed the highest mutagenic response of any air particulate sample analyzed by our research group.

Table 42.
Mutagenic Activities of Samples Collected From Both Sides of Highway 404.

A. Samples collected on the west side (on the grounds of Highland Memorial Gardens).

Collection	*Mutagenic Activity (revertants m³)*			*Ratio of YG1024(-S9):*
Date	*YG1024 (-S9)*	*YG1024 (+S9)*	*YG1021 (-S9)*	*YG1021(-S9)*
1994 05 26	30	2.6	14	2.1
1994 05 27	280	1.7	240	1.2
1994 05 28	160	2.5	27	5.9
1994 05 29	39	4.9	16	2.4
1994 05 30	240	5.8	99	2.4
1994 05 31	30	2.1	9.5	3.2
1994 06 01	41	0.5	7.2	5.7
1994 06 02	22	1.1	10	2.2
1994 06 15	20	2.3	14	1.4
1994 06 16	2300	72	500	4.6
1994 06 17	8.8	0.6	9.6	0.9
avg ± s.d.	290 ± 670	8.7 ± 21	86 ± 154	2.9 ± 1.7

B. Samples collected on the east side (between Gordon Baker Road and Highway 404).

Collection	*Mutagenic Activity (revertants/m³)*			*Ratio of YG1024(-S9):*
Date	*YG1024 (-S9)*	*YG1024 (+S9)*	*YG1021 (-S9)*	*YG1021(-S9)*
1994 05 26	45	3.1	7.5	6.0
1994 05 27	49	8.8	10	4.9
1994 05 28	56	7.9	15	3.7
1994 05 29	61	13	13	4.7
1994 05 30	51	10	18	2.8
1994 05 31	43	11	13	3.3
1994 06 01	76	4.7	11	6.9
1994 06 02	85	8.9	7.8	11
1994 06 15	55	4.4	15	3.7
1994 06 16	28	4.4	15	1.9
1994 06 17	18	2.9	9.1	2.0
avg ± s.d.	52 ± 19	7.2 ± 3.4	12 ± 3.3	4.6 ± 2.6
avg ± s.d. (both sites)	170 ± 480 revs/m³	8.0 ± 15 revs/m³	49 ± 110 revs/m³	3.8 ± 2.3
range	8.8-2300	0.5-72	7.2-500	0.9-10.9

The mutagenicity data in Table 42 was plotted chronologically (Figure 62A) together with the ratio of mutagenic potencies in strains YG1024 (-S9) to YG1021 (-S9) (Figure 62B). The peak responses on May 27, May 30 and June 16 were particularly interesting (Figure 62A). No pattern was observed in the YG1024 (-S9)/YG1021 (-S9) ratios in these samples. The extremely mutagenic nature of the three most potent samples caught us by surprise. These samples were so mutagenic that the bioassay experiments were repeated because we thought that the extraordinary number of revertants at every dose was due to contamination. Thus the May 27 and May 30 samples were completely used up in mutagenicity testing. The use of three bioassay conditions [YG1021 (-S9) and YG1024 (+ and -S9)] resulted in samples being consumed three times faster than in previous work. Following GC-MS analyses and bioassays of the non-polar aromatic fraction, only one-third of the June 16th sample remained for any bioassay-directed fractionation experiments.

We wished to compare the mutagenic potency of the June 16th sample to the mutagenicities of other samples reported in the literature. Since all studies conducted elsewhere had used *S. typhimurium* strain TA98 (-S9), this sample was bioassayed using strain YG1020 (-S9), which is identical to strain TA98 except for the pBR322 plasmid. Thus, the mutagenicity of the June 16th sample was found to be 272 revs/m^3, a value which makes this sample the most mutagenic air particulate extract ever reported. This result is compared to literature values in Table 43.

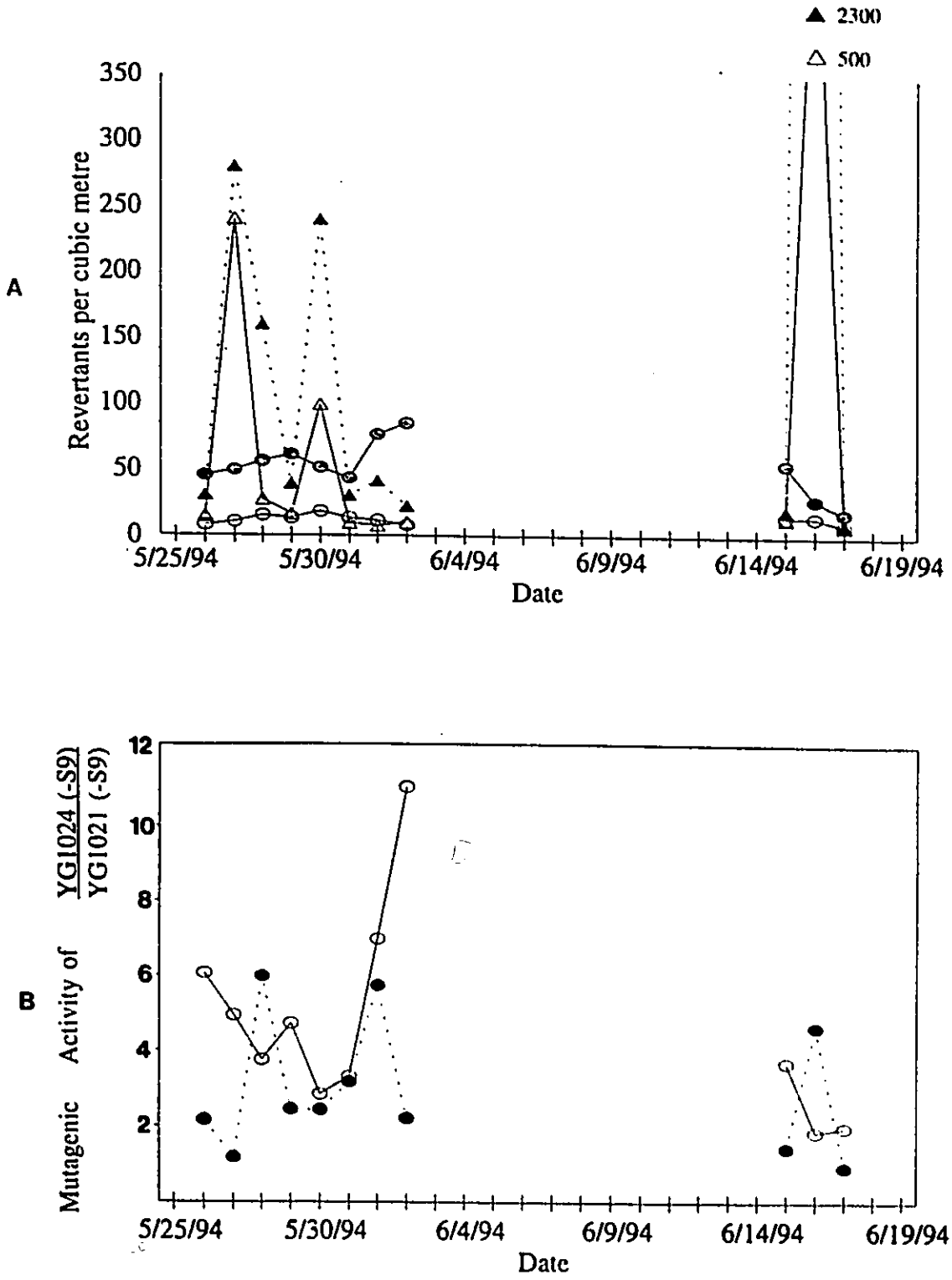


Figure 62. (A) Mutagenic activity of non-polar aromatic fractions collected on the east and west sides of Highway 404 versus date of collection. Legend: ▲, strain YG1024 (-S9), west side; △ strain YG1021 (-S9), west side; ⊙, strain YG1024 (-S9), east side; ○ strain YG1021 (-S9), east side. (B) Ratio of mutagenic activities in strain YG1024 (-S9) to strain YG1021 (-S9). Legend: ●, west side ratio; ○, east side ratio.

Table 43A.
Mutagenic Activity of Non-Polar Aromatic Fractions
in *S. typhimurium* YG1021 (-S9).

Sampling Campaign	*Number of Samples Bioassayed*	*Mutagenic Activity (revertants/m³)* Average	S.D.	Range
Hamilton, 1990-1991	68	28	± 31	1 - 130
Hamilton, spring 1993	8	34	± 65	3.3 - 190
Highway 404, summer 1994	22	71	± 210	7.2 - 504

Table 43B.
Mutagenic Activity of Non-Polar Aromatic Fractions in *S. typhimurium* TA98 (-S9).

Sampling Campaign	*Number of Samples Bioassayed*	*Mutagenic Activity (revertants/m³)* Average	S.D.	Range	Reference
Hamilton, 1990-1991	68	5*	± 5*	1 - 24*	this study
Highway 404 Summer 1994	22	13*	± 38*	0 - 272**	this study
Oslo, Norway Winter 1979	19	8	± 4	3 - 14	(144)
Athens, Greece Spring 1984	172	20	± 10	5 - 35	(33)
Santiago, Chile Spring 1990	105	41	± 32	3 - 106	(127)
Katowice, Poland Winter 1985	7	58	± 56	18 - 180	(141)
Los Angeles, CA Fall 1980	8	104	± 59	50 - 180	(140)

*estimated by dividing the YG1021 response by a factor of 5.6 (see section IV.7.2)
** the value of 272 revs/m³ (for the June 16th sample) was determined using strain YG1020 (-S9).

VII.6 Normal-Phase Mutation Chromatogram of an Extremely Mutagenic Non-Polar Aromatic Fraction

A normal-phase bioassay-directed fractionation experiment was carried out in an attempt to identify and quantify the compounds responsible for the very high mutagenicity in the June 16th sample. It was decided to use two *S. typhimurium* strains to evaluate the response of each sub-fraction: YG1021 (-S9) and YG1024 (-S9). The response in strain YG1024 (+S9) had always been low (Table 42). The high mutagenic activities of nitroPAH and particularly of dinitroPAH (Table 8) led to the hypothesis that the high mutagenic activity of the June 16th sample may be caused by the PAC derivatives (193). Experience with normal-phase HPLC separation of nitroPAH (Figure 51) indicated that nitropyrenes and nitrofluoranthenes eluted between 27 and 35 minutes. DinitroPAH eluted somewhat later. To increase resolution of individual nitroPAH in the mutation chromatogram, eluate was collected every 30 seconds between 27 and 35 minutes. For the zones between 0 to 26 minutes and from 36 minutes until the end of the NPLC gradient, fractions were collected every minute.

The equivalent of 526 m^3 (or 1/3rd of the June 16th sample) of the non-polar aromatic fraction was injected onto the normal-phase HPLC column in a single injection. The resultant NPLC chromatogram (Figure 63A) showed four prominent peaks eluting between 27 and 35 minutes; these peaks (at retention times of 27.81, 31.27, 32.03 and 34.08 minutes) were identified as 1-, 2-, 3- and 8-nitrofluoranthene, respectively, based on comparisons with the NPLC retention times and UV-visible spectra of authentic

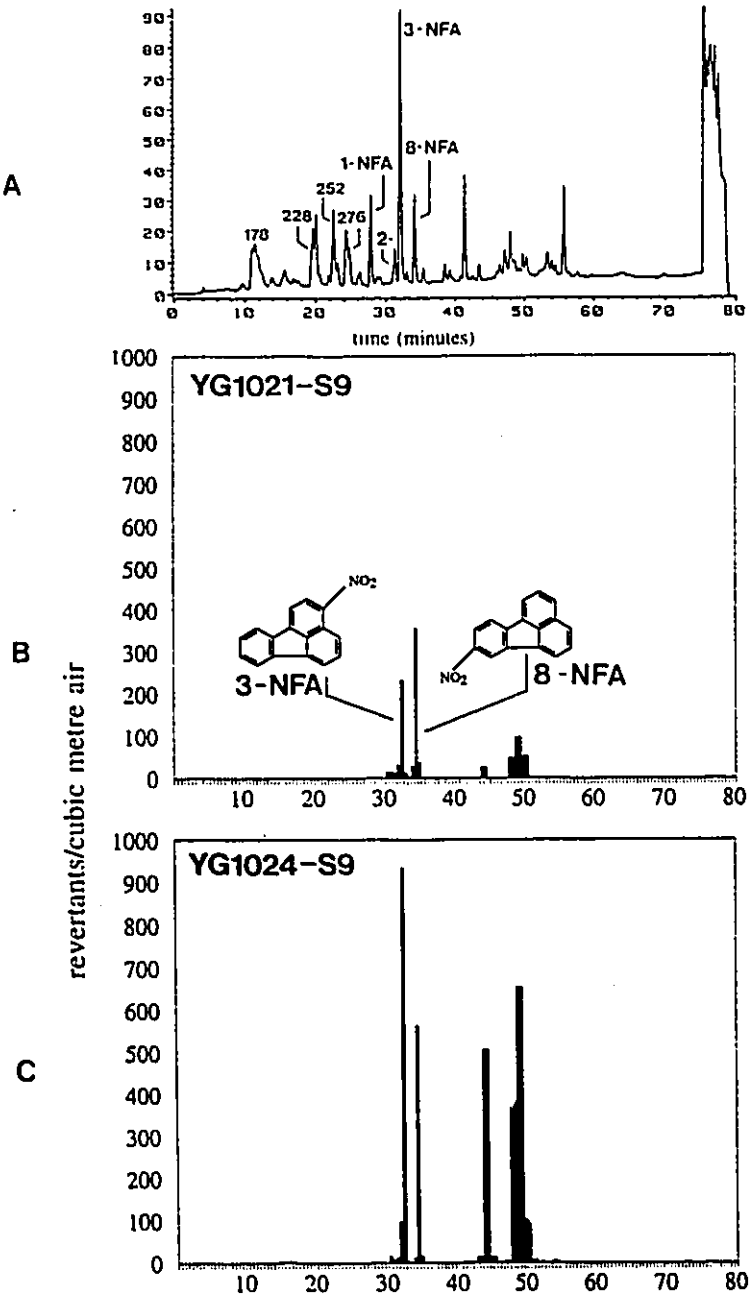


Figure 63. Mutation chromatograms obtained from bioassay analyses of one-minute and 30-second subfractions prepared from the normal-phase HPLC analysis of the non-polar aromatic fraction of particulate collected on the west side of Highway 404 on June 16th, 1994. The UV absorption profile (**A**) was plotted at 254 nm; labels above the chromatographic peaks show the molecular masses (in amu) of the predominant constituents. Subfractions were bioassayed using strain YG1021(-S9) (**B**) and YG1024 (-S9) (**C**). The mutagenic potencies of the subfractions have been plotted as net revertants/m^3; mutagenic fractions in the 4 peaks of activity were reassayed with multiple doses in order to obtain proper dose-response relationships.

standards (see section IX.20). The UV-visible spectra of these peaks also matched the spectra of nitrofluoranthenes published elsewhere (67,125). The identities of these peaks were confirmed by GC-MS analyses (see below).

The mutation chromatograms (Figures 63B and 63C) showed that strain YG1024 (-S9) gave a better mutagenic response than strain YG1021 (-S9). This data was obtained by applying a single dose of each sub-fraction to bioassay plates. Those sub-fractions which showed a reasonable response were re-assayed at three single doses to afford dose-response data. In this example four peaks of activity were observed. The quality of the bioassay data allowed the calculation of ratios of these two strains.

The mutagenic activities of authentic nitrofluoranthene and nitropyrene isomers were determined in both YG1021 (-S9) and YG1024 (-S9) (Table 44). The activities of 1-nitrofluoranthene and 2-nitrofluoranthene were low, which explains the low bioassay response to these compounds in the mutation chromatogram. Conversely, the high mutagenic response of peaks corresponding to 3-nitrofluoranthene and 8-nitrofluoranthene (Figure 63) is accounted for by the high mutagenic activity of these two comounds. Most importantly, the ratios of activity in strain YG1024 (-S9) to strain YG1021 (-S9) for these two peaks (4.0 and 1.6) were identical to the ratios for pure 3-nitrofluoranthene and 8-nitrofluoranthene (3.8 and 1.6), respectively. The ratios for the other nitrofluoranthenes and nitropyrenes were equal to or less than one.

Table 44.
Mutagenic Activity of Nitrofluoranthenes and Nitropyrenes in *S. typhimurium* strains YG1024 (-S9) and YG1021 (-S9).

Compound*	*Mutagenic Activity (revs/nmol)*** *YG 1024 (-S9)*	*YG 1021 (-S9)*	*Ratio of 1024 (-S9): 1021 (-S9)*	*Mutagenic Activity Described in the Literature*
1-NFA	2,960	3,630	0.82	not described
2-NFA	6,180	7,900	0.78	3,000 (YG1024 - S9) (194)
3-NFA	213,000	56,300	3.8	47,000 (YG1024 - S9) (194)
7-NFA	330	950	0.35	not described
8-NFA	313,000	200,000	1.6	not described
1-NP	3,700	10,100	0.37	3700 (YG1024 - S9) (123)
2-NP	20,500	18,800	1.1	not described

* Abbreviations: NFA, nitrofluoranthene; NP, nitropyrene

** as determined in this laboratory

The mutagenicity data in Table 44 can also be reported in revertants per picogram, e.g., 3-nitrofluoranthene and 8-nitrofluoranthene have mutagenic activities of 0.86 and 1.3 revertants/pg in strain YG1024 (-S9), respectively. By dividing the mutagenic activity (in revertants/m^3) of peaks corresponding to 3- and 8-nitrofluoranthene by the mutagenic activity of the respective compounds (in revs/pg), the result (in pg/m^3) is an estimate of the concentration of these two compounds in the sample. Thus, the YG1021 (-S9) mutation chromatogram gave an estimated concentration of 260 pg/m^3 3-nitrofluoranthene and 250 pg/m^3 8-nitrofluoranthene, while the YG1024 (-S9) mutation chromatogram gave an estimated concentration of 4200 pg/m^3 3-nitrofluoranthene and 630 pg/m^3 8-nitrofluoranthene.

Quantitation of the nitrofluoranthenes by integration of NPLC peak areas and comparison with areas for known amounts of authentic standards gave concentrations of 1600 and 240 pg/m^3 of 3-nitrofluoranthene and 8-nitrofluoranthene, respectively. A more accurate quantitation was carried out by GC-MS analysis of each 30-second fraction which eluted between 27 and 35 minutes. Small amounts of 7-nitrofluoranthene, 1-nitropyrene and 2-nitropyrene were also identified and quantified. The results of the GC-MS analyses appear in Table 45 and indicate that quantitation by integration of NPLC peak areas was more accurate than quantitation through the use of mutagenic responses. The determination of nitroPAH concentrations allowed the calculation of the mutagenicities corresponding to observed amounts of mutagens in each fraction; this data is also shown in Table 45.

Table 45.
Mutagenic Activities of Fractions from an
NPLC Mutation Chromatogram of a Sample Collected
June 16th, 1994.

NPLC Elution Time of Fraction (min.)	*Compounds Detected and Concentration (pg/m³)*		*Mutagenic Activity (revertants/m³) YG1024(-S9)* Calc.	Actual	*YG1021 (-S9)* Calc.	Actual
26-27	no nitroPAH detected*		0	0	0	0
27-28	54	1-NFA	1	1	1	3
28-29	9	1-NFA	0	1	0	1
29-30	1	1-NFA				
	8	7-NFA	0	0	0	0
30-30.5	1	7-NFA	0	16	0	15
30.5-31	no nitroPAH detected*		0	8	0	15
31-31.5	55	2-NFA				
	23	2-NP	4	13	4	13
31.5-32	2	2-NFA				
	100	3-NFA	86	99	23	32
32-32.5	1510	3-NFA	1300	935	344	233
32.5-33	2	3-NFA				
	2	1-NP	2	5	1	14
33-33.5	2	8-NFA	0	2	0	5
33.5-34	232	8-NFA	295	565	187	357
34-35	3	8-NFA	4	18	2	38
35-36	no nitroPAH detected*		0	5	0	2
Totals:	64 pg/m³	1-NFA	1695	1682	564	757
	57	2-NFA				
	1610	3-NFA				
	9	7-NFA				
	237	8-NFA				
	2	1-NP				
	23	2-NP				

*above a method detection limit of 0.30 pg/m³

Perhaps the most unusual feature of Table 45 is the high concentrations of combustion-derived nitroPAH as opposed to products of atmospheric transformation; the concentrations of 1-, 3- and 8-nitrofluoranthene were much higher than the concentrations of 2-nitrofluoranthene and 2-nitropyrene. There was also excellent agreement between total calculated mutagenicity and total actual (experimentally determined) mutagenicity in both bacterial strains.

The NPLC method used in this current study resolved 1-, 3-, 7- and 8-nitrofluoranthene from each other but did not separate the nitropyrenes from the nitrofluoranthenes. Nonetheless, the quality of the separation of the nitrofluoranthenes by NPLC made the excellent results of the mutation chromatogram (Figure 63) possible. The final analysis by GC-MS on a DB-17ht GC column separated all of the nitrofluoranthene and nitropyrene isomers to baseline.

A three-dimensional plot (Figure 64) shows the mutagenic responses of the nitroPAH together with the separation of these compounds both by NPLC and GC. The high mutagenic responses in strain YG1024 (-S9) at 32 and 34 minutes NPLC elution time correspond to fractions containing 3-nitrofluoranthene and 8-nitrofluoranthene, respectively. Although this particular sample was extensively fractionated, nitroPAH quantitation could have been carried out in a single analysis of fraction N2, prepared in the usual manner from the non-polar aromatic fraction (Chapter V).

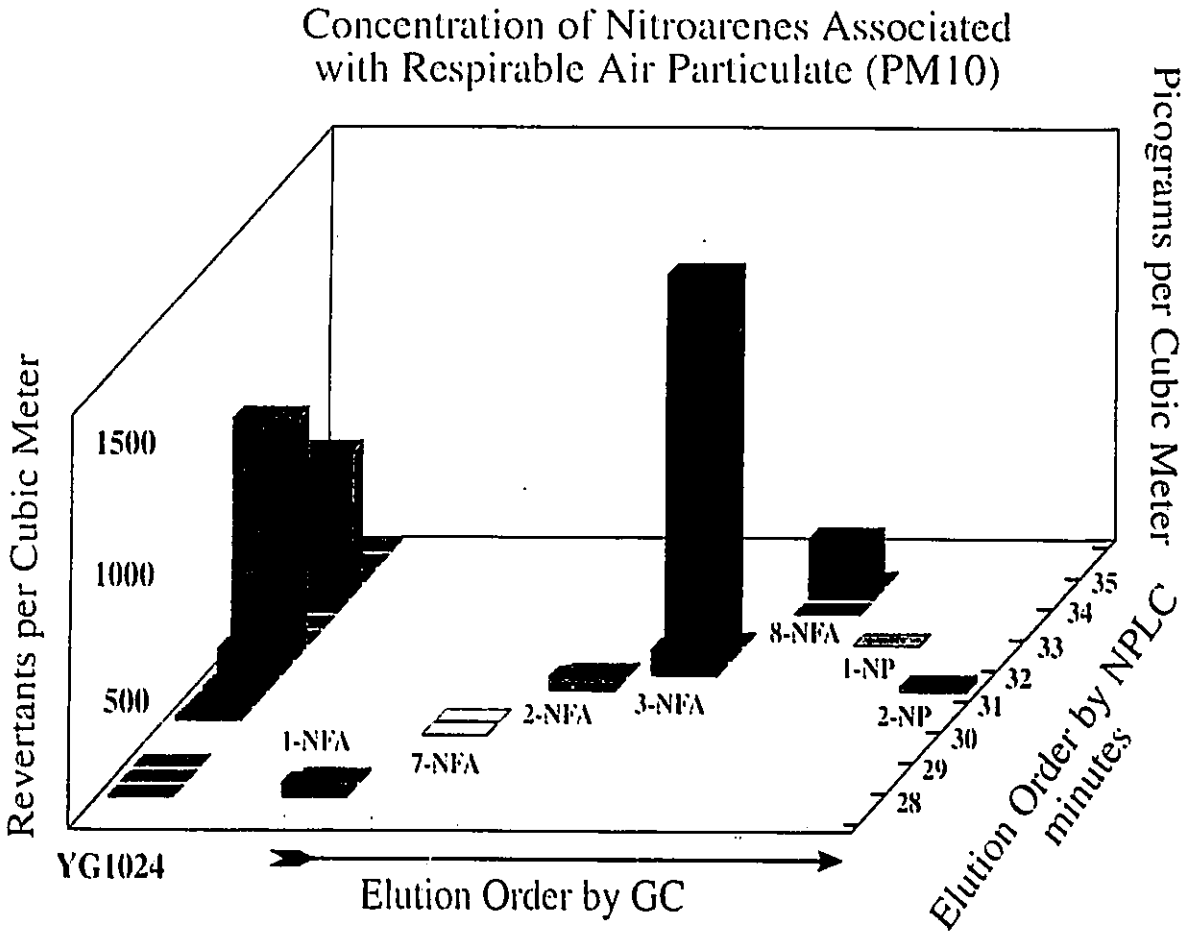


Figure 64. Combination of two separate three-dimensional plots for the non-polar aromatic fraction prepared from particulate collected June 16th, 1994. The left-hand column shows the mutagenic response in *S. typhimurium* strain YG1024 (-S9) as a function of elution time by NPLC (a replotting of Figure 63C). The remainder of the three-dimensional plot shows the relationship between the elution of nitroarenes on GC versus NPLC columns, together with the quantitation of each compound in each fraction (in pg/m^3). Atmospheric transformation products are represented by magenta-coloured bars.

Quantitation of nitroPAH in the June 16th sample allowed a comparison with nitroPAH concentrations determined elsewhere. NitroPAH concentrations observed in Hamilton and at Highway 404 are compared with other studies in Table 46. The atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene and the combustion products 1-nitropyrene and 7-nitrofluoranthene occur in the June 16th sample at concentrations which are typical for Hamilton, Rome or suburban Los Angeles. However, the concentrations of 3-nitrofluoranthene and 8-nitrofluoranthene are far higher than any previously reported concentrations in either urban air or diesel soot (Table 46) while the concentration of 1-nitropyrene in the June 16th sample is very low. Thus, the June 16th sample does not resemble diesel soot, based on nitroPAH concentrations. The reason for the high concentrations of certain nitroPAH is unclear.

The pattern of nitrofluoranthene isomers in the June 16th sample was similar to that seen previously for the electrophilic nitration product mixture of fluoranthene (Figure 51). Accidental contamination of this sample with the synthetic nitrofluoranthene mixture was possible. However, the relatively high abundance of 1-nitrofluoranthene and the low abundance of 7-nitrofluoranthene in the June 16th sample (Table 45) were considerably different than the abundances of these two compounds in the synthetic mixture; accidental contamination was therefore ruled out.

The compounds responsible for the high mutagenic activity in the area between 44 and 51 minutes NPLC retention time (Figure 63) still remained to be identified. From the outset, the high concentration of nitrofluoranthenes suggested that

Table 46.
NitroPAH Concentrations (in μg/g) from Various Sources

Compound*	*Hwy 404, 1994 06 16*	*Diesel Engine Soot (21)*	*Claremont, CA (162)*	*Air Particulate Rome (21)*	*Hamilton, ON 1990-91(this study)*
1-NFA	0.9	0.02 - 0.03	not detected	not detected	not detected
2-NFA	0.8	not detected	0.4 - 10	0.05 - 0.41	0.01 - 10
3-NFA	23	0.02 - 0.20	0.03 - 0.50	0.05 - 0.41	not detected
7-NFA	0.1	0.03 - 0.04	not detected	0.02 - 0.20	0.01 - 0.31
8-NFA	3.4	0.02 - 0.14	0.02 - 0.40	0.05 - 0.20	0.01 - 0.02
1-NP	0.03	0.34 - 7.0	0.03 - 0.60	0.15 - 1.00	0.01 - 1.5
2-NP	0.33	not detected	0.01 - 0.40	0.20 - 1.10	0.01 - 0.88

*Abbreviations: NFA, nitrofluoranthene; NP, nitropyrene

dinitrofluoranthenes might be responsible, since 3,4-, 3,7- and 3,9-dinitrofluoranthene possess high mutagenic activity in *S. typhimurium* strains YG1021 (-S9) and YG1024 (-S9) (195). Alternatively, dinitropyrenes might also be responsible for the peaks of mutagenic activity between 40 and 50 minutes in the NPLC mutation chromatogram (Figure 63) and the chromatographic behaviour of dinitropyrenes was therefore examined more closely.

Authentic standards of 1,3-, 1,6- and 1,8-dinitropyrene were shown to have NPLC retention times of 43.07, 45.31 and 47.18 minutes, respectively. However, dinitropyrenes were ruled out as the compounds responsible for the mutagenic activity since dinitropyrene UV spectra did not match the spectra of peaks in the zones between 43-44 and 47-50 minutes. Dinitropyrenes arise from the nitration of nitropyrenes during combustion (61) and dinitrofluoranthenes are synthesized through the nitration of nitrofluoranthenes (197). Thus, the high concentrations of 3-nitrofluoranthene and 8-nitrofluoranthene may have acted as starting materials for the formation of dinitrofluoranthenes in the June 16th sample and dinitrofluoranthenes were therefore studied.

The individual dinitrofluoranthene isomers required for this study are not commercially available and were isolated from a crude nitration mixture of fluoranthene containing nitrofluoranthenes, dinitrofluoranthenes and trinitrofluoranthenes (see section IX.21). Isolation of individual isomers involved considerable effort, since there are 25 possible dinitrofluoranthene isomers (198). The crude nitration mixture was subjected to

NPLC analysis using the same conditions used for the NPLC mutation chromatogram (Figure 63). A peak in the nitration mixture later identified as 3,7-dinitrofluoranthene eluted with an identical NPLC retention time and UV-visible spectrum to the peak of mutagenic activity at 44 minutes in the mutation chromatogram. Figure 65 shows a stacked plot of the region between 43 and 51 minutes NPLC elution time for (A) the June 16th sample, (B) the crude mixture of mono-, di- and tri-nitrofluoranthenes and (C) the mutagenic response in *S. typhimurium* YG1024 (-S9). The chromatograms were plotted at 382 nm, since this wavelength is an absorbance maximum for most dinitrofluoranthenes (197). The peaks corresponding to 3,7-dinitrofluoranthene in chromatograms of the June 16th sample (Figure 65A) and the crude nitration mixture of fluoranthene (Figure 65B) elute with the same retention time. The spectral match between authentic 3,7-dinitrofluoranthene and the peak at 44 minutes in the NPLC mutation chromatogram appears in Figure 66. The fraction eluting at 44 minutes was also analyzed by GC-MS; the mass spectral fragmentation pattern and retention index of the single peak detected in this fraction and authentic 3,7-dinitrofluoranthene were identical.

Compounds responsible for the mutagenic activity from 47-50 minutes elution time remained to be identified. A mixture of 3,8- and 3,9-dinitrofluoranthene (obtained as a gift from Dr. W. Vance) was analyzed by NPLC and these two compounds eluted with retention times and UV-visible spectra identical to the peaks eluting at 47 and 48 minutes in the NPLC mutation chromatogram. Spectra of 3,8- and 3,9-dinitrofluoranthene are compared to the spectra of peaks in the June 16th sample (Figure 67).

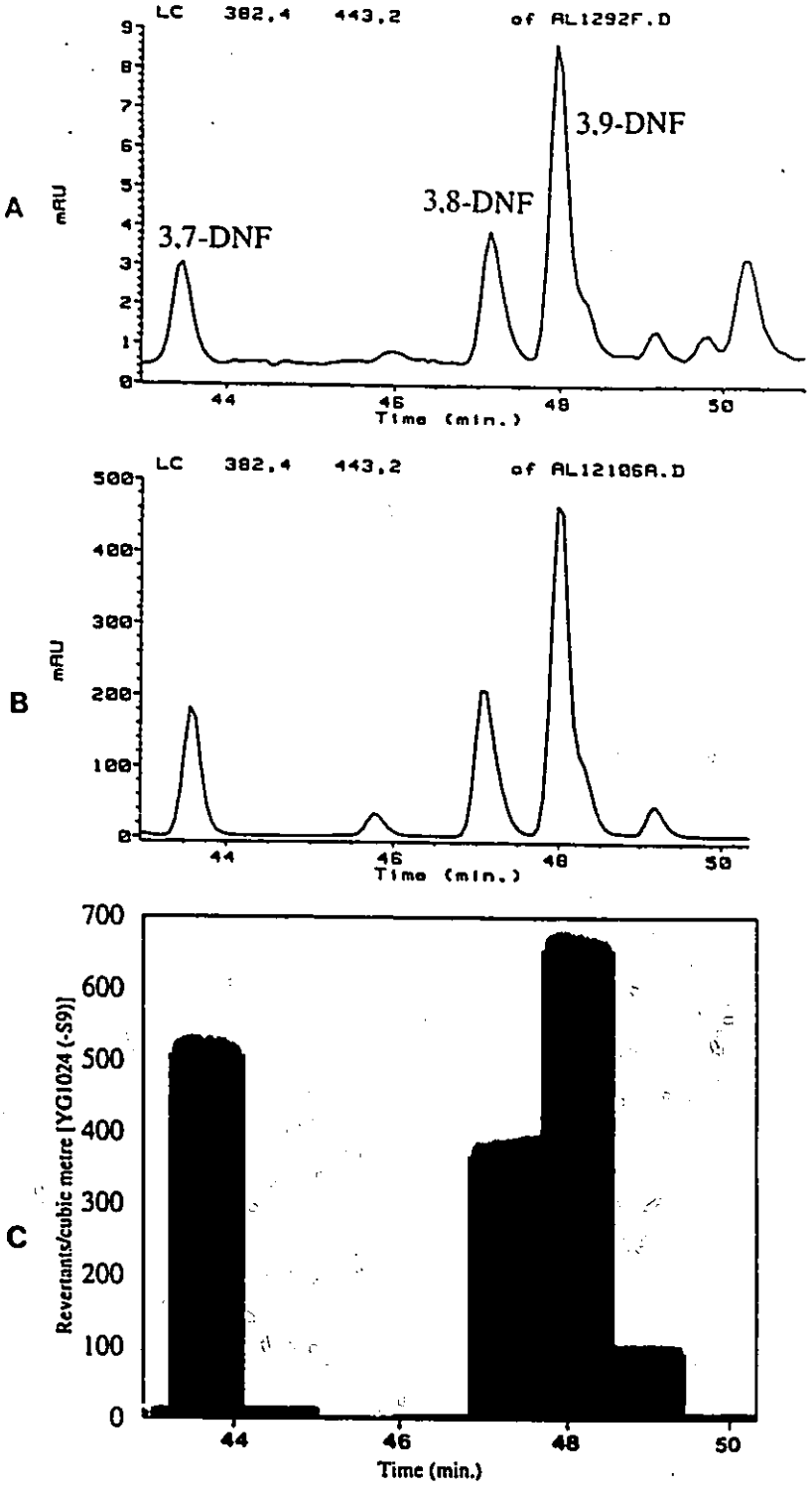


Figure 65. UV absorption profiles at 382 nm of the NPLC analysis of (A) the June 16th sample: this is a replotting of Figure 63A between 43 and 50 minutes and (B) a crude nitration mixture of fluoranthene. (C) Mutagenicity of the fractions obtained from the June 16th sample; bioassays were carried out using *S. typhimurium* YG1024 (-S9). The abbreviation DNF represents dinitrofluoranthene.

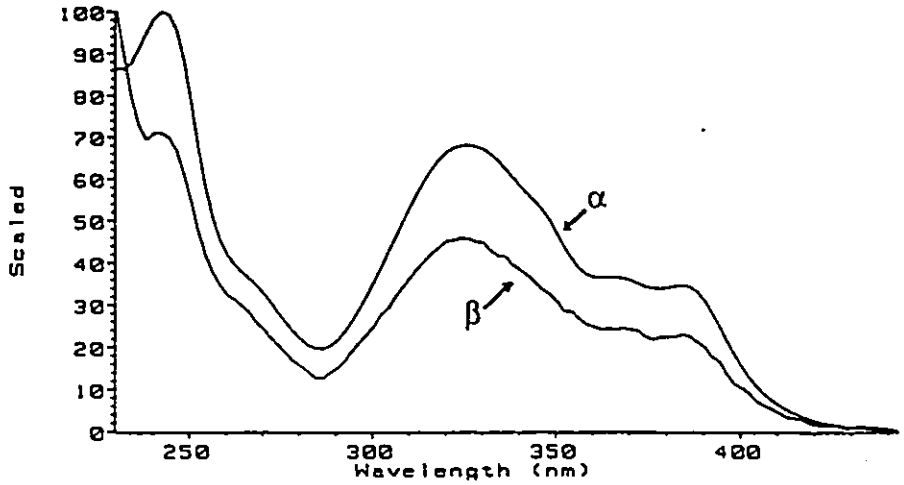


Figure 66. UV spectra of α, authentic 3,7-dinitrofluoranthene and β, the peak in Figures 65A and 65B with a retention time of 43.5 minutes.

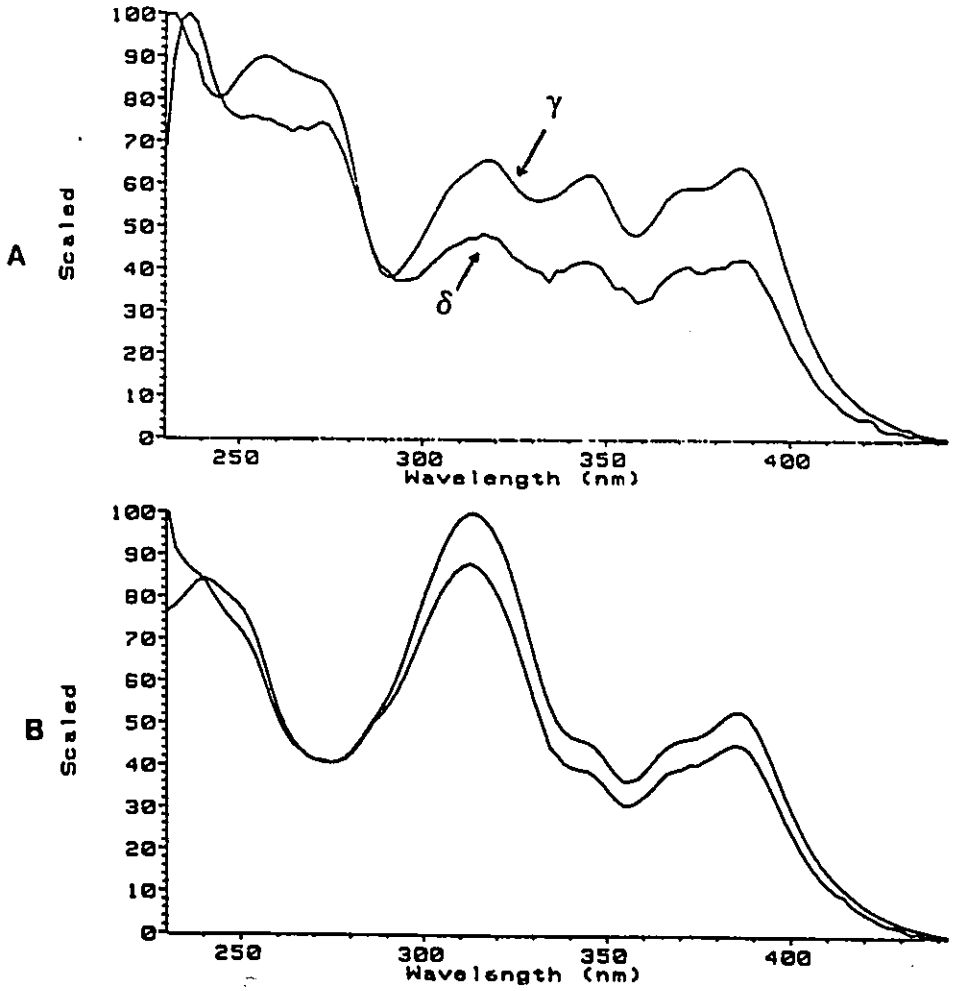


Figure 67. (A) UV spectra of γ, authentic 3,8-dinitrofluoranthene and δ, the peak in Figures 65A and 65B with a retention time of 47.2 minutes. (B) UV spectra of authentic 3,9-dinitrofluoranthene and the peak in Figures 65A and 65B with a retention time of 48.0 minutes.

The mutagenicity which could be ascribed to the dinitrofluoranthenes was of interest and 3,4- and 3,7-dinitrofluoranthene were therefore isolated from the fluoranthene nitration mixture. Since 3,8- and 3,9-dinitrofluoranthene were particularly difficult to isolate from this mixture, these two compounds (supplied unseparated by W. Vance) were isolated by NPLC. Subsequent to GC-MS analyses for purity, each isomer was subjected to bioassay in *S. typhimurium* YG1021 and YG1024 (both -S9); results were then compared with the literature (Table 47). This study appears to be the first to report the mutagenic activity of 3,8-dinitrofluoranthene.

The YG1024 (-S9)/YG1021 (-S9) ratio of mutagenic activities for the authentic dinitrofluoranthenes can be compared to the responses in the mutation chromatogram for fractions believed to contain dinitrofluoranthenes. The YG1024 (-S9)/YG1021 (-S9) ratio for 3,7-dinitrofluoranthene is 18 and the same ratio for the peak believed to contain this compound at 44 minutes in the mutation chromatogram is 19. Agreement between ratios for other compounds is complicated by the lack of resolution in the mutation chromatogram for peaks eluting between 47 and 48 minutes. The YG1024 (-S9)/YG1021 (-S9) ratios for 3,8-and 3,9-dinitrofluoranthene are 5.0 and 9.0, but the same ratios for the peaks suspected to contain these compounds in the mutation chromatogram are 7.6 and 6.7, respectively. Thus, the current study has identified dinitrofluoranthenes in the June 16th sample on the basis of NPLC retention time, UV-visible spectrum, GC retention index, mass spectral fragmentation pattern and, at least in the case of 3,7-dinitrofluoranthene, the ratio of mutagenic activities in YG1024 (-S9) to YG1021 (-S9).

Table 47.
Mutagenic Activities of DinitroPAH as Determined in this Laboratory and Elsewhere.

Compound	*Mutagenicity (revs/nmol)* YG1021(-S9)	YG1024 (-S9)	*Ratio* YG1024(-S9) / YG1021(-S9)	*Mutagenicity determined elsewhere (revs/nmol)*
3,4-DNF	8,410	59,100	7.0	8,200 (YG1021 -S9) 52,400 (YG1024-S9) (195)
3,7-DNF	117,000	2,100,000	18	157,000 (YG1021-S9) 6,600,000 (YG1024-S9) (195)
3,8-DNF	232,000	1,160,000	5.0	not reported
3,9-DNF	319,000	2,900,000	9.0	151,000 (YG1021-S9) 1,910,000 (YG1024-S9) (195)
1,8-DNP	2,550,000	3,980,000	1.6	2,850,000 (YG1021-S9) 4,780,000 (YG1024-S9) (95,123)

Quantitation of the dinitroPAH by GC-MS analyses was then desired, but this technique is fraught with problems owing to the low abundance of dinitrofluoranthnes in the environment and the poor GC-MS detection limits for these compounds, arising from poor ionization and extensive fragmentation when using EI' (199). Nevertheless, attempts at dinitrofluoranthene quantitation in the June 16th sample were made and 3,7-dinitrofluoranthene was successfully quantified (Table 48). However, attempts at further resolution of 3,8 and 3,9-dinitrofluoranthene in the June 16th sample ended in failure when the NPLC fractions collected between 47 and 50 minutes in the mutation chromatogram became contaminated with small amounts of the fluroanthene nitration mixture.

To estimate the concentration of 3,8- and 3,9-dinitrofluoranthene in the sample, a cut-and-weigh technique was used, following the injection of known amounts of both compounds onto the NPLC; results appear in Table 48, which also shows attempts to ascribe the mutagenic activity of selected fractions of the NPLC mutation chromatogram to individual dinitrofluoranthenes. Agreement between theoretical and actual mutagenicity for the fraction containing 3,7-dinitrofluoranthene is good, but poor resolution in the mutation chromatogram between 3,8- and 3,9-dinitrofluoranthene results in major differences between calculated and actual mutagenic activities. Calculation of mutagenicity for the peak corresponding to 3,9-dinitrofluoranthene is complicated by the co-elution of this compound with another unknown analyte in both the samples of air particulate and the fluoranthene nitration mixture (see Figures 65A and 65B).

Table 48.
Concentrations of Dinitrofluoranthenes Determined in the Sample of June 16th, 1994.

		Mutagenicity in YG1021 (-S9)		*in YG1024 (-S9)*	
Compound	*Concentration*	*Calculated*	*Actual*	*Calculated*	*Actual*
3,7-DNF	70 pg/m³*	28 revs/m³	27 revs/m³	500 revs/m³	508 revs/m³
3,8-DNF	90**	71	48	360	368
3,9-DNF	190**	210	98	1890	655

*This concentration was determined through GC-MS and NPLC analysis, as well as the mutagenic response in YG1021 (-S9) and YG1024 (-S9).

**Determined by cut-and-weigh techniques: GC-MS analyses not possible due to insufficient sample.

Table 49.
DinitroPAH Concentrations for Samples Collected at Highway 404 and Elsewhere.

Compound	*Source of Particulate and Date of Collection*	*Concentration (μg/g)*	*Reference*
3,4-DNF	not detected in this study***	0.01	(176)
3,7-DNF	Tokyo air particulate, 1990	0.01	(176)
	kerosene heater soot, Japan	0.14	(176)
	June 16th, 1994 sample at Highway 404	0.87	this study
3,8-DNF	June 16th, 1994 sample at Highway 404	1.1	this study
3,9-DNF	Tokyo air particulate, 1990	0.01	(176)
	June 16th , 1994 sample at Highway 404	2.7	

***above a detection limit of 20 pg/m³

Note: concentrations reported by Tokiwa *et al.* (176) are for single samples of air particulate and heater soot.

The concentrations of 1-, 3- and 8-nitrofluoranthene as well as 3,9-dinitrofluoranthene in the June 16th sample exceed any previously reported concentrations in either air particulate or diesel soot (see Tables 46 and 49). Most importantly, the June 16th sample contains an amount of 1-nitropyrene more typical for air particulate samples, not diesel soot. Thus, the ultimate source of the nitroPAH and dinitroPAH in the June 16th sample remains a mystery. Since diesel emissions are an unlikely source, perhaps a nearby industry released nitroPAH or dinitroPAH directly into the air, as has been observed in Italy (21). Additional analyses of other samples collected at the Highway 404 site may produce additional data and confirmation of the results described here.

VII.7 Conclusions

The results described in this chapter provide a useful basis for comparison of particulate collected in Hamilton or Toronto, as well as between samplers on either side of Highway 404. Average particulate concentrations and percent organic extractables were identical for both sides of the highway and proved similar to Hamilton samples, despite the heavy vehicular and industrial influences at the respective sites.

Although the percent organic extractables did not differ, PAC concentrations on the east side of Highway 404 were slightly higher than those observed on the west side of the highway. Shielding of the west side sampler by surrounding trees and light industrial activity on the east side have been put forward as possible explanations for this difference (187,188). PAC concentrations at both highway sites were substantially lower

than those seen in Hamilton, where heavy industry is a major contributor to higher PAC levels.

Bioassays of non-polar aromatic fractions prepared from particulate collected at the Highway 404 sites between May 25th and June 2nd indicated a 100-fold range of mutagenic potencies with the higher mutagenicities observed on the west side of the highway. The mutagenic activity of samples from both sides of the highway was as much as 4-fold higher than the activities seen for particulate collected in Hamilton. The mutagenic response of the Highway 404 samples almost always followed the pattern YG1024 (-S9)> YG1021 (-S9)> YG1024 (+S9), which was also observed in the bioassay responses of nitroPAH and dinitroPAH standards.

NitroPAH and dinitrofluoranthenes were found to be responsible for virtually all of the mutagenic activity detected in the non-polar aromatic fraction of particulate collected on the west side of the highway on June 16th during a thermal inversion event. A normal-phase mutation chromatogram allowed separation of almost all of the nitroPAH and some of the dinitroPAH, while high-resolution GC-MS facilitated the identification and quantitation of individual isomers. Thus, the power of the normal-phase mutation chromatogram approach was demonstrated once again.

Dinitropyrenes were not detected in any of the samples analyzed in this thesis. The separation of the most abundant dinitrofluoranthene and dinitropyrene isomers with the GC methods used in this chapter was demonstrated.

The dinitrofluoranthenes described in this chapter may account for the peaks of mutagenic activity observed at 45 and 47 minutes in the normal-phase mutation chromatogram (Figure 50) as well as the mutagenic activity of pooled fractions N3 and N456 (Table 39), since these fractions were collected over elution time zones which included the zones of elution of the dinitrofluoranthenes. The ratio of activity in YG1024 (-S9)/YG1021 (-S9) for pooled fraction N3 was equal to 7.4, which is slightly higher than the same ratio for the Highway 404 samples (Table 42) and which is within the range of ratios observed for authentic dinitrofluoranthenes (Table 47). The YG1024 (-S9)/YG1021 (-S9) ratio can be used to rule 1,8-dinitropyrene as a cause for the 45 or 47 minute peak of mutagenic activity in the normal-phase mutation chromatogram of the pooled non-polar aromatic fraction (Figure 50), since this particular compound would give an equal response (within a factor of two) in both strains.

Dinitrofluoranthenes may well have been responsible for the mutagenic activity in Hamilton N3 and/or N3456 fractions as well (Chapter V), since the less polar dinitrofluoranthenes (e.g., 3,7-DNF, 3,8-DNF) could have eluted in fraction N3 while the more polar species (e.g., 3,4-DNF) could have eluted in fraction N456. However, dinitrofluoranthenes were probably not responsible for the mutagenic activity in pooled fraction N456 (Chapter VI), since mutagenic compounds more polar than the dinitrofluoranthenes only began to elute from the NPLC column after 55 minutes.

VIII. CONCLUSIONS AND FUTURE WORK

This chapter will address each objective and bring together previously stated conclusions in an overall synthesis of how air quality can be examined in urban and industrial areas of southern Ontario. The importance of the current study with regards to the literature on PAC, air pollutant data and studies of mutagenicity will also be discussed.

The introduction to this thesis described how atmospheric researchers such as Pitts (1), Atkinson (12) and Lane (26) have conducted their studies of air particulate over short time intervals of days or weeks. These short sample collection periods are usually carried out during episodes of severe air pollution and therefore do not examine the relationship between continuously monitored air pollutants, PAC concentrations and air particulate extract mutagenicity during periods of low or moderate air pollution. The current study addressed this problem through the collection of air particulate and air pollutant data over a 13-month period which included episodes of light, moderate and severe air pollution. Samples were selected for analysis in order to best represent each of these three conditions and the mutagenicity and PAC content of the non-polar aromatic fractions of the air particulate extracts were determined. Principal component analysis was then used to examine the relationship between [a] the concentration of PAC and the mutagenic activity of particulate extract and [b] the daily variation in PAC concentrations and the concentration of continuously monitored pollutants in urban air - the first and second objectives of this thesis, respectively.

From the results presented in the current study, it may be concluded that nitroPAH are the most important mutagenic compounds in Hamilton air particulate extracts, as measured using *S. typhimurium* strains YG1021 (-S9) and YG1024 (-S9). Compounds more polar than nitroPAH contribute to the mutagenic activity of Hamilton air particulate to a lesser extent, while PAH and thiaPAH appear to contribute very little to the mutagenicity of Hamilton air particulate in these two strains.

During air pollution episodes, nitroPAH are the most important contributors to the mutagenic activity of air particulate in the Los Angeles area (12, 24, 60, 66, 124, 167) while polycyclic aromatic compounds slightly more polar than nitroPAH have been observed to contribute to air particulate extract mutagenicity to a lesser extent (25). Thus, the relative order of contribution of nitroPAH and more polar PAC to air particulate extract mutagenicity may be similar in Hamilton and Los Angeles, even though air pollutant concentrations in Los Angeles can greatly exceed the concentrations observed in Hamilton (1, 105-108). The relatively low contribution of PAH and thiaPAH to air particulate extact mutagenicity is not surprising, since strains YG1021 and YG1024 are usually unresponsive to these compounds in the absence of metabolic activation (92-94).

The atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene were determined to be the most abundant nitroPAH in Hamilton air particulate extracts. The combustion products 1-nitropyrene as well as 1-, 7- and 8-nitrofluoranthene were also quantified in some samples of Hamilton air particulate extract, though at

concentrations close to the method detection limit of 0.30 pg/cubic metre air. The range of nitroPAH concentrations observed in Hamilton is similar to the range observed in Los Angeles (162), Italy (166) and elsewhere around the world (64,117,165). However, the current study is the first to use an experimental method which not only allows PAH and nitroPAH quantitation in particulate collected over a single 24 hour period, but also leaves enough sample remaining [after chemical analyses] for the collection of reliable bioassay data through the use of a dose-response curve.

In twenty Hamilton air particulate samples analyzed for nitroPAH, the atmospheric transformation products 2-nitrofluoranthene and 2-nitropyrene were responsible for about one-sixth of the mutagenic activity found in the non-polar aromatic fraction. Principal component analysis revealed that 2-nitrofluoranthene concentrations correlated well with the mutagenic activity of the non-polar aromatic fraction (R=0.66). Furthermore, the extended sampling period of this study indicates that atmospheric nitration of fluoranthene and pyrene was highest during springtime thermal inversion episodes in both 1990 and 1991. Thus, it would appear that the current study is the first to identify an annual pattern to increased nitroPAH concentrations.

The current study also appears to be the first to examine PAC concentrations, mutagenicity and air pollutant concentration data using principal component analysis; previous studies using this technique (158, 179) have examined only two of these three variables. The application of principal component analysis to the relationship between

mutagenicity, PAC concentrations and atmospheric conditions in this thesis yielded a variety of useful correlations, but none of these relationships were completely unexpected. Factor matrices and three-dimensional factor loading plots did not add much information to that already provided by the correlation matrices.

The second objective of this thesis was an examination of the relationship between daily variation in PAC concentrations and the concentration of continuously monitored pollutants in urban air over an extended sampling period. From the current study, it may be concluded that most indicators of thermal inversion events [such as increased sulfur dioxide and nitrogen dioxide concentrations] are positively correlated with the concentration of total monitored PAC, since combustion-derived PAC and sulfur dioxide both arise from common sources and nitrogen dioxide can arise from the atmospheric oxidation of combustion-derived nitric oxide. However, the correlation coefficients between these variables were fair to poor (R=0.56 or less). The relationship between total monitored PAC, total reduced sulfur (R=0.69) and the coefficient of haze (R=0.64) showed better correlation.

Correlation between air pollutants and only those PAC which are atmospheric transformation products was generally good, since the concentration of nitroPAH arising from atmospheric transformation was critically dependant on the concentration of continuously monitored pollutants such as nitrogen dioxide. For example, 2-nitrofluoranthene concentrations correlated well with nitrogen dioxide concentrations

(R=0.69) and the concentrations of both pollutants rose together with the coefficient of haze during thermal inversion events. Thus, the correlation coefficient between 2-nitrofluoranthene and the coefficient of haze was good (R=0.68). The importance of nitrogen dioxide concentrations has been observed before, both in Los Angeles and in other cities (117). The current study appears to be the first to correlate the coefficient of haze with atmospheric transformation concentration and is also the first to determine atmospheric transformation product concentrations in Hamilton - previous investigations studied only particulate extract mutagenicity (50, 51), PAH concentrations (98,99) or the conditions responsible for thermal inversions (105-107) without quantifying nitroPAH.

Previous studies of PAH in Hamilton could not determine if this city's heavy industry was responsible for increased ambient PAH concentrations (98,99). To estimate the impact of industrial emissions on air quality in Hamilton [another objective of this thesis], samples were collected in areas well removed from heavy industry - for example, at the side of Highway 404 in Toronto. It is concluded that industrial emissions increase PAH concentrations in particulate blown from the direction of Hamilton's industrial area, based on the results presented in the current study.

Vector directional analysis was used to study concentrations of combustion-derived PAC, mutagenicity of the non-polar aromatic fraction and wind direction for all samples collected in Hamilton. Winds blowing across the industrial area of Hamilton were observed to have increased PAC concentrations and mutagenicity over particulate blown into the city from other directions. Conversely, the Highway 404 site was located

in an area where industry has a low impact but mobile emissions from vehicles predominate. Despite particulate concentrations, percentage of organic extractables and PAH ratios which were identical both in downtown Hamilton and at the Highway 404 site, PAH concentrations at Highway 404 were approximately 55-fold lower than those seen in Hamilton. However, the mutagenic potency of air particulate extracts collected at Highway 404 were two-fold higher than those seen in downtown Hamilton. Thus, it appears that high PAH concentrations in Hamilton are the result of heavy industry, not vehicular emissions. PAH concentrations and particulate extract mutagenicity in Hamilton are low to average compared to other cities around the world (139-145).

Collection of air particulate at the Highway 404 site not only assisted in making a comparison with Hamilton but also facilitated the search for mutagenic PAC not previously described in the literature. An objective of this thesis involved the identification and quantitation of new mutagens in air particulate extracts through the use of bioassay-directed fractionation. It may be concluded that the fractionation method described in the current study resulted in the tentative identification of 3,8-dinitrofluoranthene in air particulate for the very first time. This particular dinitroPAH was isolated from air particulate collected in Toronto through the use of a normal-phase HPLC mutation chromatogram.

It should be noted that the normal-phase mutation chromatogram is but one example of a technique known as multidimensional chromatography, which has been found to be especially useful in the analysis of complex mixtures in general (79). Each

'dimension' in multidimensional chromatography supplies a wealth of experimental results while simultaneously reducing the number of analytes in a complex mixture. The preparation of the non-polar aromatic fraction constituted the first 'chromatographic dimension'. At this level, PAH, thiaPAH and oxyPAC could be quantified and bioassays could be carried out on the polar and non-polar aromatic fractions.

The separation of the non-polar aromatic fraction into further subfractions (or N-fractions) required a second 'chromatographic dimension', namely normal-phase HPLC. NPLC separated [a] PAH from nitroPAH and other PAC classes and [b] separated PAH on the basis of molecular mass. To separate compounds which co-eluted by NPLC, a third 'chromatographic dimension' was required in the form of RPLC. The most successful application of the third dimension was the separation of mutagens in fraction N456, obtained from a pooled non-polar aromatic fraction of Hamilton air particulate extract.

Other experiments carried out in this laboratory (37, 38, 49, 96, 97) have demonstrated that this multi-dimensional approach can be successfully applied to a diverse array of complex mixtures, such as foundry dust, harbour sediments and Zebra mussel tissue. The results presented in the current study demonstrate that air particulate extracts can be added to the list of matrices whose complexity can be successfully de-convoluted using the technique of multi-dimensional chromatography.

This thesis has demonstrated that within the second or third dimension, the degree of resolution in mutation chromatograms can be adjusted to meet the requirements of the

experiment. In the normal-phase HPLC separation of the non-polar aromatic fraction, preparation of N-fractions gave samples of sufficient purity to allow quantitation of nitroPAH without the interference of PAH in GC-MS analyses and the interference of dinitroPAH in biological assays. The normal-phase mutation chromatogram of the pooled Hamilton air particulate sample was prepared through the collection of subfractions every minute, which provided mutagenicity information unobtainable through the collection of N-fractions. Finally, collection of subfractions every 30 seconds allowed identification and quantitation of virtually all of the mutagens collected at the Highway 404 site on June 16th, 1994. Failure of the normal-phase mutation chromatogram approach with pooled fraction N2 resulted from an inadequate amount of sample available and did not result from problems with the multi-dimensional chromatographic approach.

Given the relatively small amount of air particulate collected over a 24 hour period and the relatively large amount of sample required for both bioassays and GC-MS analyses of trace mutagens, the insensitivity of traditional *S. typhimurium* tester strains (e.g., TA98) for use in the mutagenicity assay presented a problem. To compare and contrast the sensitivity and usefulness of a variety of newly developed bacterial strains in the bioassay of air particulate extract [the final objective of this thesis], six different bacterial strains were used in the bioassay of a pooled sample of Hamilton air particulate extract. It is concluded that strains with extra copies of genes which metabolize nitroPAH showed greatly enhanced mutagenic responses to extracts of respirable air particulate.

Bacterial strains with extra copies of the nitroreductase gene (YG1021) and the acetyltransferase gene (YG1024) showed 7-fold and 10-fold enhanced responses to mutagens found in the non-polar aromatic fraction, when compared to the mutagenic response of the standard tester strain (TA98 or YG1020). Strain YG1024 appears to be the most useful of the two strains, owing to dramatically increased sensitivity to dinitroPAH not observed in strain YG1021. Since *S. typhimurium* YG1024 (-S9) was demonstrated to be highly sensitive to both nitroPAH and dinitroPAH in samples of air particulate extracts and strain YG1029 (+S9) showed good sensitivity to combustion-derived PAH, future bioassays of Hamilton air particulate extracts should use both strains YG1024 (-S9) and YG1029 (+S9) to gauge the mutagenic activity of direct-acting mutagens and compounds requiring metabolic activation, respectively.

Future research may involve quantitation of 2-nitrofluoranthene in a variety of samples to investigate the possible use of this compound as a surrogate for the determination of air particulate extract mutagenicity or the amount of atmospheric transformation taking place. 2-Nitrofluoranthene quantitation in unfractionated air particulate extracts using negative-ion chemical ionization has been recently reported (10) and may be more facile than the method of quantitation described herein.

Renewed attempts to isolate previously uncharacterized mutagens from Hamilton air particulate could be attempted once more. A normal-phase mutation chromatogram, possibly followed by reversed-phase mutation chromatograms, would probably prove more successful than the preparation and analyses of pooled N-fractions. Larger samples

of particulate extract or high sensitivity GC-MS systems such as ion traps (201-203) might allow more successful GC-MS analyses.

This thesis has shown that Hamilton's greatest problem with air quality arises from its industry. Winds from the city's industrial area carry increased levels of combustion-derived PAC, especially PAH. Each spring, thermal inversion episodes increase atmospheric transformation of PAH to nitroPAH, which in turn results in increased particulate extract mutagenicity. The current study has shown how these problems of air quality can be examined and quantified, not only in Canada's most heavily industrialized area but also at the side of a highway in its largest city. This thesis also describes a highly effective method of bioassay-directed fractionation to determine exactly which compounds are responsible for increased mutagenic activity.

The conditions favoring the establishment of the annual springtime thermal inversions in Hamilton- namely the lake breeze effect and the trapping of pollutants by the Niagara Escarpment - cannot realistically be altered by anthropogenic means. However, if Hamilton's industries were to dramatically decrease the release of respirable particulate and its adsorbed PAC into the air [e.g., from coking operations and from diesel engines], less PAC would be available for atmospheric transformation and the mutagenic potency of air particulate extract would correspondingly decrease. Less combustion-derived particulate might even decrease the incidence of asthma in Hamilton, which is highest in the most heavily industrialized areas of the city (191,192).

IX. METHODS AND MATERIALS

IX.1 Locations of Air Particulate Collection Sites

IX.1.1 The Downtown Hamilton Sampling Site

This site (MOEE air monitoring station No. 29000) is located at the corner of Kelly and Elgin Streets, just east of downtown Hamilton at a latitude of 43°15' and a longtitude of 79°52'. This location is shown on the map in Figure 8. The following parameters were monitored by the MOEE automatically and continuously at this site: sulfur dioxide, coefficient of haze, carbon monoxide, ozone, nitric oxide, nitrogen dioxide, methane, non-methane hydrocarbons and total reduced sulfur. The instruments used by the MOEE for these purposes have been described elsewhere (103). Atmospheric data was made available as hourly averages.

Sampling of air particulate was carried out for 24 hour periods every second day from May, 1990 to June, 1991, with a few exceptions for holidays and instrument maintenance. A total of 155 filters were collected at this site.

IX.1.2 The West Hamilton Sampling Site (Westdale)

This site (MOEE air monitoring station No. 29118) was located approximately 30 m from the on-ramp from Main Street West to Highway 403 at a latitude of 43°14' and a longtitude of 79°54'. This location is shown on the map in Figure 8. All of the pollutants monitored at station 29000 were also monitored at this site, except for non-methane hydrocarbons. Sampling dates and sampling methods were carried out as

described for the downtown Hamilton sampling site. A total of 155 filters were collected at this site.

IX.1.3 The CCIW Sampling Site

This site was located at the dock of the Canada Centre for Inland Waters (CCIW) in Burlington, Ontario. This location is shown on the map in Figure 8. Facilities at this site did not include monitors for air pollutants or meteorological conditions. The author thanks Dr. C.H. Marvin for collection of air particulate samples at this site. Sampling was carried out for 24 hour periods, about once a week on a somewhat irregular basis from September, 1991 to April, 1992. A total of 14 filters were collected.

IX.1.4 The Highway 404 Sampling Sites

Sampling was carried out at the boundary fences on both the east and west sides of Highway 404 in North York, Ontario. These locations are shown on the map in Figure 59. One site was on the grounds of Highland Memorial Gardens Cemetery (on the west side of Highway 404) and the other site was on the east side on Gordon Baker Road in a light industrial area. Samples were collected by workers from Rowan, Williams, Davies and Irwin, who were subcontracted by the Ontario Ministry of Transport.

IX.2 Air Particulate Collection at the Sampling Sites

Respirable airborne particulate was collected using a PM10 high volume air

sampler (General Metal Works Ltd., Village of Cleves, OH) operating at a flow rate of 40 ft^3/min. or 1630 m^3/24 h. Air sampling was controlled with a digital timer, a flow controller and a pressure transducer and was generally carried out for a 24-hour period from midnight to midnight. All suspended air particulate was collected on Teflon-coated glass fibre filters (Pallflex 8 X 10 inch, type TX40H120WW, Pall Corp., Putnam, Connecticut). Samplers were sited about four metres above ground level at the locations described above. A schematic cut-away of a PM10 sampler appears in Figure 1.

Filters were weighed on an analytical balance and stored at .oom temperature in an opaque envelope until mounted in the PM10 air sampler. Following air particulate collection, the filters were weighed and the difference in mass recorded. No correction was made for humidity effects which have been previously reported to affect the weighing of air particulate due to its inherently hygroscopic nature (128,129). Following weighing, filters were placed in opaque envelopes, sealed in polyester pouches (type 55416-1681, Kapak Corp., Minneapolis, MN) and stored at -80°C until extraction, as has been recommended elsewhere (205).

IX.3 Collection and Processing of Atmospheric and Meteorological Data

Atmospheric data (hour by hour values for coefficent of haze, total reduced sulfur and the concentrations of a number of gases including SO_2, CH_4, CO, NO_2, NO, O_3 and non-methane hydrocarbons) were monitored at both the downtown Hamilton sampling site and the west Hamilton sampling site during the entire sampling campaign. Data was

supplied in ASCII format by Phil Kiely of the MOEE, Rexdale. Hourly data for wind speed and wind direction were also supplied by the MOEE but was measured at the Hamilton Sewage Treatment Plant at Woodward and Glow Streets, Hamilton.

The ASCII data files, which ranged in size from 130 kilobytes to 1.24 megabytes, were converted to smaller files of 69 kilobytes each using the 'Split 4.96' computer program (M.Dingacci, Public Domain Program, 1989). The 69 kilobyte files were then put through a 'delimiter' program (supplied by Carl Otta, MOEE, Rexdale) which parsed the large blocks of data into 19 fields with delineations at positions 13, 15, 17, 19, 21, 25, 31, 35, 39, 43, 47, 51, 55, 59, 63, 67, 71 and 75. The parsed data was then imported into Quattro Pro for Windows (Novell Inc., Orem, Utah). Data was transposed from MOEE format (lateral rows of 12 hourly values) to the format used in the Quattro Pro spreadsheets (continuous vertical columns of hourly data) using a Quattro Pro macro supplied by Dawn Berwick, Computer Resource and Training Centre, McMaster University.

IX.4 Gases

High purity helium and nitrogen, 'ultrazero' air and ultra-high purity hydrogen were purchased from Canadian Liquid Air Limited (Burlington, Ontario).

IX.5 Solvents

All solvents were either HPLC grade or were distilled in glass in the laboratory.

Hexane (HPLC grade, BDH Inc., Toronto, Ontario) and acetonitrile (HPLC grade, Caledon Laboratories Ltd., Georgetown, Ontario) were used as delivered. Dichloromethane (distilled in glass grade) and methanol (HPLC grade) were purchased from Caledon Laboratories and were distilled before use. Benzene (Fisher Scientific, Fairlawn, New Jersey), ethanol (Caledon Labs) and toluene (Fisher Scientific) were obtained as reagent grade and were distilled before use. Distillations were carried out using a 60 cm Vigreux column and 3 L stillpot at a distillation rate of about one to two mL/minute; the first 70 mL of distillate were discarded and the remainder of the distillate was collected and stored in glass bottles in the dark.

A Milli-Q purification system (Waters Associates, Millford, Massachusetts) was used to further purify departmental distilled water.

IX.6 Chemicals

Standards were used as received. The following standards were purchased from Aldrich Chemical Company Inc. (Milwaukee, WI): acenaphthene, acenaphthylene, acridine, 3-aminofluoranthene, 2-aminofluorene, anthracene, anthraquinone, benzo[a]anthracene, benzo[a]anthracene-7,12-dione, benzanthrone, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, benzo[e]pyrene, biphenyl, carbazole, chrysene, coronene, dibenzo[a,c]anthracene, dibenzo[a,h]anthracene, dibenzo[a,i]carbazole, dibenzofuran, dibenzothiophene, 9,10-dimethylanthracene, 3,5-dimethylphenol, 1,5-dinitronaphthalene, n-docosane, n-dodecane,

fluoranthene, fluorene, fluorenone, n-hexadecane, n-hexacosane, 1-methylnaphthalene, 2-methylnaphthalene, 1-methylphenanthrene, naphthacene-(5,12)-quinone, naphthalene, alpha-naphthol, 9-nitroanthracene, 2-nitrodibenzopyranone, 1-nitronaphthalene, p-nitrophenol, 1-nitropyrene, n-nonadecane, perylene, phenanthrene, pyrene, quinoline, o-terphenyl, m-terphenyl, p-terphenyl, n-tetracosane and xanthone.

The following compounds were purchased: 2-nitropyrene, 2-nitrofluoranthene, 3-nitrofluoranthene (Chemsyn Ltd., Lenexa, KS); indeno[1,2,3-cd]pyrene and 6-nitrobenzo[a]pyrene (Analabs Inc., North Haven, CT); pyrene-d_{10} and benzo[a]anthracene-d_{12} (Cambridge Isotope Labs Ltd., Woburn, MA); anthanthrene and dibenzo[a,i]pyrene (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium); benzo[a]fluorene and benzo[b]fluorene (Fluka AG, Buchs, Switzerland); 3-methylcholanthrene (Research Biochemicals Incorporated, Natick, MA); dibenzo[j,p]chrysene (ChemService, West Chester, PA) and picene (Dr. Ehrenstorfer GmbH, Augsburg, Germany).

The following compounds were received as gifts: benzo[b]naphtho(1,2-d)thiophene, benzo[b]naphtho(2,1-d)thiophene and benzo[b]naphtho(2,3-d)thiophene (Dr. John Fetzer, Chevron Corporation, Richmond, CA); a mixture of 3,8- and 3,9-dinitrofluoranthene (Dr. William Vance, California Environmental Protection Agency, Sacramento, CA) and a mixture of various di- and trinitrofluoranthenes (Ken Hoo, McMaster University). The separation of individual isomers from this mixture is described below. A mixture of three dinitropyrenes (1,3-, 1,6- and 1,8-) was a gift from LC Services, Woburn, Massachusetts.

IX.7 Instrumentation

Reversed and normal phase HPLC were carried out on a Hewlett-Packard model 1090 liquid chromatograph with built-in diode-array detector and Chemstation data system (Hewlett-Packard Co., Mississauga, Ontario). Gas chromatography-mass spectrometry experiments were performed using one of three GC-MS instruments. Most analyses were carried out using on-column injection on a Hewlett-Packard model 5890 Series II gas chromatograph with electronic pressure control, equipped with an HP5971 mass selective detector; a few analyses were carried out using an HP5971A mass selective detector combined with splitless injection on an HP5890 Series II gas chromatograph.

For the first three determinations of nitroPAH in air particulate extracts, analyses were carried out using cool on-column injection on an HP 5890 gas chromatograph interfaced to a Finnigan MAT model 4023 quadrupole mass spectrometer with an Incos data system (Finnigan Corp., San Jose, CA).

Gas chromatography with flame ionization detection was performed using an HP5890 series I gas chromatograph equipped with an on-column injector. A Beckman model 110A HPLC pump combined with a Beckman model 153 ultraviolet absorption detector (Beckman Instruments, Fullerton, CA) was used in the Sephadex LH20 clean-up procedure. Data from the Sephadex LH20 runs was recorded using a Kipp and Zonen BD41 strip chart recorder.

IX.8 Sample Handling and Storage

All glassware, except for sample vials, was scrupulously washed with Sparkleen detergent (Fisher Scientific) and rinsed at least five times with distilled water before use.

All glass sample vials were rinsed with dichloromethane before use and were fitted with Teflon liners, cut from sheet Teflon (0.015" thickness, Johnson Industrial Plastics Ltd., Toronto). All vials were stored in the dark at 4°C, as has been recommended elsewhere (205).

IX.9 Extraction Method

The following extraction method is adapted from that of Schuetzle *et al.* (65) and McCalla *et al.* (37). Individual Teflon-coated glass fibre filters were folded in half and manipulated with clean tongs to fit inside a clean, glass Soxhlet apparatus. The Soxhlet extraction vessels (approx. 80 mL volume) were fitted with 250 mL round-bottom flasks and filled with 180 mL distilled dichloromethane and a few boiling chips. The flasks were wrapped with tin foil and heated (at a 'medium' dial setting) so that the solvent cycled at an approximate rate of one cycle every ten minutes for a period of 24 hours.

After 24 hours, solvent reflux was stopped by removing the heating source The 250 mL flask containing the dichloromethane extract was replaced by a second 250 mL round-bottom flask containing 180 mL of distilled HPLC-grade methanol, the foil replaced and refluxing resumed (at a 'high' dial setting) for an additional 24 hours. The solvent cycling rate for methanol was approximately one cycle every ten minutes.

The volumes of organic solvents were reduced to 2-3 mL under reduced pressure using a rotary evaporator. The extract concentrates were transferred using a Pasteur pipette to a 2-dram vial, the flasks washed with 1-2 mL of fresh solvent and the remainder transferred to the same vial. The vial cap was fitted with a Teflon liner for storage. The combined dichloromethane and methanol extracts were reduced to a volume less than 5 mL before being transferred to a 5.00 mL volumetric flask and made up to 5.00 mL using dichloromethane.

The volumetric flask was allowed to stand in the dark at room temperature for about two hours, until insoluble materials (probably sand and elemental carbon) had settled out. Then, a 200 µL aliquot was withdrawn by syringe from the volumetric flask without disturbing the sediment and transferred to a clean, pre-weighed scintillation counting planchette for weighing. Alternatively, small pieces of aluminum foil were used in place of planchettes. Planchettes had been dried over P_2O_5 in a dessicator and tared before samples were dried onto them. The solvent was allowed to evaporate and the planchette was stored in a dessicator over P_2O_5 overnight. In all cases, the weight of the planchette was determined immediately after removal from the dessicator, since crude extracts were found to be hygroscopic.

IX.10 Alumina Clean-Up of the Crude Organic Extract

The alumina chromatography procedure, first described by Lee *et al.* (47), has been modified somewhat by our research group for separation of PAC from various

complex environmental samples (38,39,49). The following procedure was adapted and scaled down for the smaller samples encountered in the present work.

Neutral alumina (Fisher, Brockman activity I, 80-200 mesh), which had been heated for at least 48 hours at 170°C, was allowed to cool to room temperature immediately before being weighed for use in the following protocol.

The entire crude extract from one air particulate filter, dissolved in approximately 5 mL of dichloromethane/methanol, was added to a sample of cooled, neutral alumina (1 g). The solvent was removed by evaporation under reduced pressure and the resulting alumina was poured on top of 2 g of cooled alumina which had been dry-packed in a glass column (1 cm diameter X 25 cm). Elution of the column with 20 mL of hexane afforded fraction A1, which contained primarily aliphatic material; sequential elution with 20 mL benzene, then with 25 mL of dichloromethane/ethanol (99:1, v/v) afforded fraction A23 which contained non-polar PAC. Sequential elution with 20 mL of methanol followed by 20 mL of methanol/water (4:1, v/v) afforded fraction A45 (polar PAC). A final fraction (A6) was obtained by elution with 20 mL of water. All A23 fractions were subjected to Sephadex LH20 clean-up. The A1 fractions were usually discarded, while some A45 fractions were evaluated using the chromatographic and bioassay procedures described below.

IX.11 Sephadex LH20 Clean-Up of the Non-Polar Aromatic (A23) Fraction

The use of Sephadex LH20 gel to separate PAH was first described thirty years

ago (206) and this procedure has been modified to separate aliphatics from aromatics in organic complex mixtures (55). The following method is a scaled-down version of a procedure which had already been published by our group (49).

The solvent used for LH20 chromatography was a mixture of 6 volumes hexane, 4 volumes methanol and 3 volumes dichloromethane, hereafter referred to as LH20 solvent. Sephadex LH20 gel was allowed to soak overnight in LH20 solvent; the swollen gel was degassed under vacuum before it was poured into a steel column (10 cm X 0.94 cm diameter, Niagara Valve and Fittings Ltd., Hamilton, Ont.) fitted with 1/2" OD X 1/32" thick, 2 micron stainless steel frits (Mandel Scientific, Guelph, Ont.). After allowing the excess solvent to drain, more slurry was immediately added to the moist gel until the column was filled.

The loosely packed gel was compacted by pumping LH20 solvent through the column at a rate of 0.6 mL/min.; extra gel was added as required until the column was filled with packed gel. Degassed LH20 solvent was then pumped through the column at a rate of 0.4 mL/min for at least 1 hour before the column performance was tested. The column eluate was run through a Beckman model 153 analytical UV detector at 254 nm; the output signal was plotted on a strip-chart recorder with a full-scale signal of 1600 mAU. Flow cell pathlength was 5 mm, recorder span was 10 mV and strip chart recorder speed was 2 mm/min.

Whenever the column had not been used for more than several hours or after it had been freshly packed, column performance was evaluated by the injection of a

naphthalene standard. Approximately 1 mg of naphthalene was dissolved in 50 µL dichloromethane and 950 µL of LH20 solvent. An aliquot (250 µL) of the naphthalene solution was injected onto the column using a 250 µL loop and the time at which the naphthalene first began to elute from the column was noted. Typically, naphthalene retention times ranged between 18 and 19 minutes.

When injecting A23 fractions prepared from air particulate extracts, material eluting prior to the naphthalene elution time was discarded, while material eluting after this time was retained as a fraction designated 'A23/LH20'. For air particulate extracts, the A23 fraction obtained from alumina chromatography was concentrated by rotary evaporation at reduced pressure to an oil (with a volume of approximately 10 µL) in a 10 mL round-bottomed flask. To this residue was added 10 µL of pure dichloromethane followed by 190 µL of LH20 solvent. The A23/LH20 fraction was collected from the start of the naphthalene elution until the UV detector signal returned to baseline. The total volume collected was typically about 20 mL. Often, coloured material eluted prior to naphthalene. A23/LH20 fractions were carefully evaporated to dryness under a gentle stream of nitrogen without heating and stored in the dark in Teflon-capped glas vials at 4°C.

IX.12 Normal Phase HPLC Analysis

Whatman Partisil M9 PAC columns (Whatman, Clifton, NJ) and an amino precolumn (Brownlee Labs, Santa Clara, CA, 1.5 cm X 3.2 mm i.d.) were used for all

normal-phase HPLC analyses. The HPLC solvent gradients as described below were used for both analytical and preparative applications. Differences between the analytical and preparative parameters were as follows: loop size (20 µL analytical and 100 µL preparative), flow rates (1.0 mL/min. analytical and 4.2 mL/min. preparative) as well as column dimensions (5 µm particles, 4.6 mm X 25 cm for analytical applications compared to 10 µm particles, 9.4 mm X 25 cm for preparative work).

The HPLC operating conditions were as follows: diode array UV absorption detection over the wavelength range from 230 to 440 nm; column temperature 40°C. Gradients A and B used the same hexane/dichloromethane gradient profile; however, in gradient A the column was eluted with acetonitrile at the end of the gradient in order to elute residual polar compounds from the column such as quinolines, acridines and other polar compounds. Gradient C, developed several years before gradients A and B, was a more primitive solvent program and was only used in the NPLC analyses shown in Figure 36.

Gradient A: (elapsed time, composition of mobile phase): initial, 95% hexane and 5% dichloromethane; 10 min., 95% hexane and 5% dichloromethane; 35 min., 70% hexane and 30% dichloromethane; 55 min., 30% hexane and 70% dichloromethane; 65 min., 100% dichloromethane; 70 min., 100% dichloromethane; 75 min., 100% acetonitrile; 80 min., 100% acetonitrile, 85 min., 100% dichloromethane, 100 min., 95% hexane, 5% dichloromethane; 130 min., 95% hexane, 5% dichloromethane.

Gradient B is a modification of Gradient A with the following changes: 70 min., 100% dichloromethane; 75 min., 95% hexane, 5% dichloromethane; 110 min., 95% hexane, 5% dichloromethane. In cases which required a series of repetitive injections to prepare sufficient material, Gradient B was used for all of the injections except for the last injection which was made using Gradient A. Thus, all of the polar material from the previous injections eluted in the acetonitrile wash at the end of the last injection.

Gradient C is the primitive system no longer used by our research group and is as follows (elapsed time, composition of mobile phase): initial, 100% hexane; 5 min., 100% hexane; 10 min., 99% hexane and 1% dichloromethane; 15 min., 95% hexane and 5% dichloromethane; 40 min., 100% dichloromethane; 50 min., 100% dichloromethane; 60 min., 100% acetonitrile; 65 min., 100% acetonitrile.

IX.13 Reversed Phase HPLC Analysis

In reversed phase HPLC, a number of gradient elution methods were used depending on the degree of separation required. *Gradient D* is a general purpose method, which can be applied to all types of polar and non-polar mixtures. *Gradient E* was designed for the separation of relatively intractable mixtures, such as normal-phase cuts from fraction N456 which are to be further separated by RPLC.

Gradient D was carried out using two 10 micron 25 cm X 4.6 mm i.d. Vydac 201TP104 reversed phase analytical columns linked in series (Separations Group, Hesperia, CA). The solvent program was as follows (elapsed time, concentration of

mobile phase): initial, 40% water and 60% acetonitrile; 30 min., 0% water and 100% acetonitrile.

Gradient E was carried out using a single 5 micron 25 cm X 4.6 mm i.d. Vydac 201TP54 reversed phase analytical column, with a solvent program as follows (elapsed time, concentration of mobile phase): initial, 80% water and 20% acetonitrile; 60 min., 0% water and 100% acetonitrile.

IX.14 GC-FID Analyses

Gas chromatography with flame ionization detection was carried out using a 25 m DB5 column with a helium head pressure of 10 psi and a flow rate of 25 cm/second. For routine analyses of Hamilton air A23/LH20 fractions, sample volumes of 1 µL were injected using a cold on-column injector with the following temperature program: 80°C to 160°C at 20°C/min.; 160°C to 290°C at 3°C/min.; hold at 290°C for 10 minutes. Total analysis time was 58 minutes.

IX.15 GC-MS Analyses

Regardless of the GC column used, samples were always dissolved in distilled toluene and the injection volume was always 1 µL. Quadrupole electron multiplier voltage was automatically set by the MSD during tuning and ranged between 1700-2200 EMV, while the ion source temperature was maintained at 300°C. For full scan analyses, scan parameters were set to range between m/z 50 and m/z 550 with a threshold of 100 units.

Solvent delays, oven temperature programs, carrier gas electronic pressure control and selected-ion monitoring programs depended on the GC column used; see Table 50.

Table 50. GC-MS Analysis Protocols

Column	Analysis	Solvent Delay	Oven Temp. Program	Helium Flow Rate	Initial Helium Linear Velocity	SIM Program	Std. Dev. of Method
30 m X 0.25 mm i.d.DB5, 0.25 µm film thickness	68 Hamilton air particulate extracts	5.0 min.	Injection at 100°C, 100°-160° at 20°/min 160°-290° at 3°/min., hold at 290° for 10 min.	0.8 mL/min.	33.3 cm/s	Table 52	Table 53
60 m X 0.25 mm i.d. DB5ms, 0.25 µm film thickness	22 Toronto air particulate extracts	10.0 min.	Injection at 90°C,hold at 90° for 2 min, 90°-290° at 4°/min., hold at 290° for 50 min.	0.9 mL/min.	24.7 cm/s	Table 54	Table 55
60 m X 0.25 mm i.d. DB5ms, 0.25 µm film thickness	general purpose method/ analysis of nitroarom-atics	10.0 min.	Injection at 130°C, 130°-300° at 1.6°/min., hold at 300° for 20 min.	1.0 mL/min.	26.3 cm/s	Table 56	Table 57
30 m X 0.25 mm i.d. DB17ht, 0.10 µm film thickness	analysis of nitro-aromatics with resol-ution of all isomers	15.0	Injection at 40°C, 40°-300° at 2.5°C/min, hold at 300° for 16 min.	1.0 mL/min.	36.3 cm/s	Table 58	Table 59

All GC columns were manufactured by J&W (Folsom, California) and purchased through Chromatographic Specialties (Brockville, Ontario). Both the 60m DB5ms and the 30m DB17ht columns were fitted with J&W 5 m retention gaps (0.530 mm i.d.) with J&W universal 2-way glass unions. Thirty centimetre sections were cut from the front of the retention gap when required; see section IX.16.

Non-polar aromatic (A23/LH20) fractions were diluted for GC-MS analysis based on observations made during the Sephadex LH20 chromatography step (section IX.11). The UV absorbance of the eluate from the Sephadex LH20 column was monitored at 254 nm and recorded using a strip chart recorder (1600 mAU full scale). The aromatic compounds eluted as a broad peak of absorbance which caused the recorder pen to go off-scale. The width of this off-scale portion of the peak was then used to calculate the number of cubic metres equivalent of air to be injected onto the GC column as shown in Table 51.

Selected-ion monitoring programs appear in Tables 52 through 59. Selected ion monitoring programs varied depending on the type of chromatographic fraction analyzed, column type and column length.

Table 51.
UV Peak Width from Sephadex LH20 Chromatography Versus
Amount of A23/LH20 Fraction Injected for GC-MS Analyses.

Width of UV Peak at 1600 mAU	*Amount Injected for GC-MS Analyses (m^3 equivalent)*
0 - 2.49 cm	60 m^3
2.50 - 3.00	40
3.00 - 3.49	10
3.50 - 3.60	5
>3.60	2

Table 52.
Selected-ion Monitoring Program for Hamilton Air Particulate A23/LH20 Fractions.

PAH ions	**ThiaPAH ions**	**OxyPAC ions**	**internal standard**
178, 192 202, 216 228, 242 252, 266 276 278	184, 198, 212 234, 248	208 230 258	212 (pyrene-d_{10}) or 240 (B[a]A-d_{12})

The following ions were selected in order to monitor the following compounds: 178 (phenanthrene/anthracene), 184 (dibenzothiophenes), 192 (C_1-178 PAH), 198 (C_1-dibenzothiophenes), 202 (fluoranthene/pyrene), 208 (anthraquinone and thiaPAH), 212 (C_2-dibenzothiophenes and the internal standard pyrene-d_{10}), 216 (C_1-202-PAH and benzofluorenes), 228 (benzo[a]anthracene and chrysene), 230 (benzanthrone and benzofluorenones), 234 (benzonaphthothiophenes), 240 (benzo[a]anthracene-d_{12}, internal standard), 242 (C_1-228 PAH), 248 (C_1-benzonaphthothiophenes), 252 (benzopyrenes & benzofluoranthenes), 258 (benzo[a]anthracenedione), 266 (C_1-252 PAH), 276 (indeno[cd]pyrene & benzo[ghi]perylene, 278 (dibenzoanthracenes, picene).

Table 53
Standard Deviation of the Method for Hamilton Air Particulate Analyses on the 30 m DB5 column

PAC	retention time (min.)	R.I.*	Relative Standard Deviation (%) of retention time	of peak area
phenanthrene	9.02	300.00	0.11	5.1
anthraquinone	13.03	330.31	0.10	5.9
fluoranthene	15.07	343.46	0.10	10.7
pyrene-d_{10}	16.23	350.59	0.10	0.0
pyrene	16.34	351.26	0.10	10.1
benzo[b]naphtho-(2,3-d)thiophene	24.30	395.79	0.11	3.9
benzo[a]anthracene	24.91	398.68	0.14	8.8
chrysene	25.23	400.00	0.14	5.4
benzo[e]pyrene	34.41	453.42	0.11	6.6
perylene	35.23	458.41	0.11	7.4
indeno[cd]pyrene	41.68	494.62	0.10	7.7
benzo[ghi]perylene	43.07	502.50	0.12	9.7

*Retention index
Data obtained from 4 consecutive injections.

Table 54.
Selected-ion Monitoring Program for Toronto Air Particulate A23/LH20 Fractions.

Time (minutes)	PAH ions	ThiaPAH ions	OxyPAC ions	AzaPAH and NitroPAH ions
10.0-30.5	128, 127 142, 141 152, 151 154, 153 166, 165			129, 115
30.5-39.7	152 178 192, 191 230	184, 192 198 212 208	180 208 230, 202	179 229
39.7-46.0	202 228 230 216, 215 206, 205	212	230 202	191, 203 231
46.0-54.0	228, 226, 113 231, 230, 202 240 (I.S.)	234, 117 248 262	258, 236, 230, 202	247, 217, 201, 189
54.0-64.0	202, 230 252, 126 268, 266	262	258 230 202	231
64.0-77.0	276, 138 278 292, 290			
77.0-110.0	292, 290 300, 150 302, 151			

Table 55.
Standard Deviation of the Method for Analysis of Toronto Air Particulate A23/LH20 Fractions

PAC	retention time (min.)	retention index	Relative Standard Deviation (%) of retention time	of peak area
naphthalene	15.61	200.00	0.18	24.8
1-methylnaphthalene	19.59	236.93	0.12	24.3
acenaphthylene	24.02	246.64	0.09	23.7
fluorene	28.03	269.22	0.08	17.2
dibenzothiophene	32.89	295.08	0.07	14.8
phenanthrene	33.64	300.00	0.07	14.3
anthraquinone	38.44	330.31	0.06	10.4
pyrene	41.98	351.48	0.07	10.4
benzo[b]fluorene	44.44	368.96	0.06	9.0
benzo[a]anthracene	49.10	398.72	0.06	3.8
2-nitrofluoranthene	50.83	407.81	0.06	1.5
1-nitropyrene	52.04	413.99	0.06	0.6
perylene	58.06	458.43	0.08	4.9
indeno[cd]pyrene	67.02	494.42	0.11	3.7
benzo[ghi]perylene	69.81	502.25	0.12	2.6

Column: 60 m DB5ms.
Data obtained from 4 consecutive injections.

Table 56.
Selected-ion Monitoring Program for NitroPAH in Hamilton Air Particulate.

Time (minutes)	PAH ions	NitroPAH ions*
17.5-45.5		173, 143, 127, 115
45.5-65.5		211, 181, 165, 153 223, 193, 177, 165 225, 195, 179, 167 237, 207, 191, 179
65.5-75.0	228, 240 (I.S.)	223, 193, 177, 165 237, 207, 191, 179 247, 217, 201, 189 261, 231, 215, 203
75.0-83.0		247, 217, 201, 189 261, 231, 215, 203
83.0-97.0		247, 217, 201, 189 261, 231, 215, 203 273, 243, 227, 215 287, 257, 241, 229
97.0-100		273, 243, 227, 215 287, 257, 241, 229 297, 267, 251, 239
100-120	276, 278, 300, 302, 314, 316, 328, 330	297, 267, 251, 239

* The four ions selected correspond to the molecular ion of the nitrocompound [M^+] and the three fragments arising from the molecular ion: [M-NO]$^+$, [M-NO_2]$^+$ and [M-NO-CO]$^+$.

Column: 60 m DB5ms.

Table 57.
Standard Deviation of the Method
for Analysis of NitroPAH in Hamilton Air Particulate.

PAC	retention time (min.)	Retention Index	Relative Standard Deviation (%) of retention time & area	
1-nitronaphthalene	26.26	272.38	0.26	12.6
anthraquinone	46.81	329.63	0.13	2.6
7-nitrofluoranthene	74.48	406.42	0.07	5.8
2-nitropyrene	80.20	424.09	0.09	7.3
6-nitrochrysene	91.65	459.69	0.10	7.3
indeno[cd]pyrene	102.78	494.32	0.09	3.1
picene	104.66	500.00	0.12	2.1
benzo[ghi]perylene	105.33	502.20	0.09	1.9

Column: 60 m DB5ms. Data obtained from 8 consecutive injections.

Table 58.
Selected-ion Monitoring Program
for Analysis of NitroPAH in Hamilton Air Particulate
(Alternate Method).

PAH ions	NitroPAH ions	Type of Nitrocompound
228, 278, 240 (I.S.)	247, 217, 201, 189	nitro-202 PAH
	261, 231, 215, 203	methylnitro-202 PAH
	271, 241, 225, 213	nitro-226 PAH
	273, 243, 227, 215	nitro-228 PAH
	297, 267, 251, 239	nitro-252 PAH

Ions monitored throughout entire run. Program is for use with the 30 m DB17ht column.

Table 59.
Standard Deviation of the Method for Analyses with the 30 m DB17ht column.

PAC	retention time (min.)	retention index	Relative Standard Deviation (%) of retention time & peak area	
chrysene	77.76	400.00	0.16	8.4
1-nitrofluoranthene	79.09	406.08	0.20	8.3
7-nitrofluoranthene	79.89	409.74	0.19	5.3
2-nitrofluoranthene	80.12	410.79	0.24	9.3
3-nitrofluoranthene	81.13	415.41	0.22	7.0
2-nitropyrene	83.67	427.02	0.23	9.2
6-nitrochrysene	90.91	460.13	0.19	2.9
picene	99.63	500.00	0.17	4.0

Data obtained from 4 consecutive injections.

IX.16 Monitoring and Maintenance of GC-MS Column Performance

Column chromatographic performance was monitored routinely by the injection of a toluene solution of PAH, oxyPAH, thiaPAH and nitroPAH. Amounts injected onto the column ranged from 4 to 20 ng of each component in 1 μL of toluene. Separation of compounds at m/z 276 (benzo[ghi]perylene, indeno[cd]pyrene) and m/z 278 (picene and dibenz[a,c]anthracene) was closely monitored. As intractable material accumulated on the retention gap, peak tailing increased to the point where the peaks began to run together.

Once the m/z 276 and m/z 278 peaks began to run together (without baseline separation),approximately 30 cm was cut from the front of the retention gap. The same standards were then re-injected to check performance a second time. Approximately 5 A23/LH20 samples could be injected onto the DB5ms or DB17ht columns before retention gap cutting was required, although this was dependant on PAC concentrations in the samples and the amount of sample injected. Approximately 5 N2 fractions could be injected on the DB17ht column before retention gap cutting was required.

IX.17 Quantitation of Analytes Using GC-MS Methods

An internal standard method was used for quantitation (benzo[a]anthracene-d_{12} for PAH analyses and, occasionally, 1-nitropyrene-d_9 for nitro-PAH analyses). The synthesis of 1-nitropyrene-d_9 is described below, in section IX.19.1.

IX.18 Bioassays

The *Salmonella typhimurium* bacterial strains used in this study were strains YG1020, YG1021, YG1024, YG1025, YG1026 and YG1029 (93,95) received as gifts from Dr. M. Watanabe, National Institute of Hygienic Sciences, Tokyo, Japan. Strains YG1020, YG1021 and YG1024 are TA98 type strains that are auxotrophic for histidine and contain plasmid pKM101. Strain YG1020 is equivalent to strain TA98, while strains YG1021 and YG1024 are variants of strain YG1020 which have been modified by the addition of plasmid pBR322; this multi-copy plasmid was engineered to contain either the gene for the enzyme nitroreductase (about 50 copies per bacterium, YG1021) or the gene for the enzyme O-acetyltransferase (YG1024). Strains YG1025, YG1026 and YG1029 are the TA100 type counterparts of strains YG1020, YG1021 and YG1024, respectively.

The protocol used for the bioassays was modified from Ames (83). Bacteria were grown for 10 hours at 37°C in Oxoid Nutrient Broth #2 (15 mL) with ampicillin (50 μg/mL) and tetracycline (6.25 μg/mL). A dose range of five concentrations of organic extracts (dissolved in 50 μL DMSO) was tested in duplicate. Some assay conditions included the addition of a preparation of rat liver microsomes (S9) as a source of metabolic activation (4% Aroclor 1254-induced rat liver S9), which was prepared by Dr. D.W. Bryant. After a 48-hour incubation period at 37°C (or 72 hours in the case of strain YG1021), the number of revertant colonies (histidine independant) were determined using a Biotran II colony counter (New Brunswick Scientific, Edison, NJ). Mutagenic potency was calculated from the linear segments of the dose response curves at lower doses. For

the mutation chromatograms, individual assays of the one minute subfractions of HPLC eluate were performed either in the absence (YG1021 and YG1024) or in the presence (YG1029) of 4% S9.

Dosage for bioassays of the non-polar aromatic (A23/LH20) fractions was estimated from the observations made during Sephadex LH20 chromatography (see section IX.15). Fraction A23/LH20 eluted from the Sephadex LH20 column as a broad peak which was off-scale at 1600 mAU. The width of the off-scale portion of the peak was used to set the highest dose for bioassays (Table 60). Non-polar aromatic fractions were bioassayed using 5 doses in duplicate. Typically, the second highest dose was four-fifths of the top dose, the third highest dose was three-fifths of the top dose, the fourth highest dose was two-fifths of the top dose and the lowest dose was one-fifth of the top dose.

IX.19 Nitropyrenes

IX.19.1 Synthesis and Isolation of 1-Nitropyrene-d_9

The following synthesis was adapted from the method of Radner (207). To acetic anhydride (1875 μL) cooled in an ice bath was added slowly with stirring, concentrated nitric acid (125 μL) to afford the nitration reagent. To a solution of pyrene-d_{10} (10 mg, 0.047 mmole, 98 atom% D, Cambridge Isotope Laboratories, Woburn, MA) in warm acetic anhydride (250 μL) was added with stirring, the above nitrating reagent (100 μL) all at once. The solution of pyrene became yellow and after three minutes a precipitate began to appear. Thirty minutes after the addition of the nitrating reagent, the reaction was quenched by the addition of distilled water (1 mL) and allowed to stir for 30 minutes.

Table 60.
UV Peak Width from Sephadex LH20 Chromatography Versus
Highest Dose for Bioassays using *S. typhimurium* YG1021 (-S9).

Width of UV Peak at 1600 mAU	*Top Dose for Dose-Response Curve (m^3)*
0 - 2.49 cm	60 m^3
2.50-3.00	40
3.00-3.49	10
3.50-3.60	5
>3.60	2

The resulting yellow mixture was extracted three times with dichloromethane and the organic layer was washed twice with water. The dichloromethane layer was reduced in volume by evaporation under reduced pressure to give a yellow solid which was dried *in vacuo* for two hours at 0.4 mm Hg. GC-FID analysis failed to detect any unreacted pyrene and RPLC analysis failed to detect any 1,8-dinitropyrene below a level of 60 ng per 70 μg of pyrene nitrated, using a Beckman ODS Ultrasphere 5 μm (4.6 mm X 25 cm) column combined with reversed-phase gradient (gradient C) described above. The mass spectrum of the final product was as follows, m/z (relative intensity): 256 (100), 226 (61), 210 (94), 209 (78), 198 (70).

IX.19.2 Physical Properties of 1- and 2-Nitropyrene

Mass spectra for the two nitropyrenes were as follows, m/z (relative intensity); 1-nitropyrene, 247(76), 217(26), 201(100), 200(81), 189(45); lit. value (66) 247(58), 217(39), 201(100), 189(56). The mass spectrum for 2-nitropyrene was as follows: 247(55), 217(4), 201(100), 200(46), 189(14); lit. value (66) 247(49), 217(8), 201(100), 189(19). UV spectra for the nitropyrenes matched previously described data (207-210) and is as follows, where the extinction coefficient is stated as $\log_{10}\varepsilon$: 1-NP, 398 nm (4.06), 374 nm (4.07); 2-NP, 290 nm (4.62).

IX.20 Nitrofluoranthenes

IX.20.1 Synthesis and Isolation of Individual Nitrofluoranthene Isomers

The method for the nitration of pyrene (as described above in section IX.19.1) was carried out using 10 mg of fluoranthene. The four mononitrofluoranthene isomers (1-, 3-, 7- and 8-) were isolated using semi-preparative NPLC (Whatman 10 micron Partisil M9 PAC column, 25 cm X 9.4 mm i.d.) under the following conditions (elapsed time, composition of mobile phase): initial, 10% dichloromethane, 90% hexane; 22 min., 30% dichloromethane, 70% hexane; 26 min., 80% dichloromethane, 20% hexane; 28 min., 80% dichloromethane, 20% hexane; 30 min., 10% dichloromethane, 90% hexane. Individual isomers were identified by comparison with authentic UV-spectra (67,68) and GC-MS retention index values and fragmentation patterns (66). UV data for the unequivocally identified dinitrofluroanthenes are as follows, where the extinction coefficient is stated as $\log_{10}\varepsilon$: 3,4-DNF, 384 nm (4.15); 3,7-DNF, 381 nm (3.96), 321 nm (4.24), 237 nm (4.42); 3,9-DNF, 382 nm (4.15), 308 nm (4.41) and 236 nm (4.35) (200). GC-MS retention index values and fragmentation patterns for mono- and dinitrofluoranthenes are listed in Tables 62 and 63.

IX.21 Isolation of Dinitrofluoranthene Isomers

A crude mixture of mono-, di and tri- nitrofluoranthenes had been prepared some years ago by Ken Hoo, who had nitrated several grams of fluoranthene. Small amounts of the crude nitration mixture were dissolved in dichloromethane and applied to a

Whatman Partisil 5 polyaminocyano column (column dimensions: 24 cm X 0.25 cm i.d.) using Gradient B, as described in section IX.12. The HPLC UV chromatogram of the mono-, di- and tri-nitrofluoranthene mixture appears in Figure 68. Chromatographic peaks were collected as they eluted from the HPLC. Solvent was evaporated under a gentle stream of nitrogen, and peaks were identified on the basis of UV spectra [in the case of 3,4-, 3,7- and 3,9-dinitrofluoranthene, (197)] or on the basis of mass spectral fragmentation patterns (199) and retention index values (198). Normal-phase HPLC (Gradient B) was used to separate a mixture of 3,8- and 3,9-dinitrofluoranthenes obtained from Dr. W. Vance (California Environmental Protection Agency, Sacramento). Peak identities in Figure 68 are listed in Table 62, based on mass spectral data and retention index data (Table 63).

Table 61.
Mass Spectral Fragmentation Patterns of the Nitrofluoranthenes

Compound	Exptally. determined	Literature Reference (66)
1-nitrofluoranthene	247 (76) 217 (9) 201 (100) 200 (64) 189 (30)	247 (57) 217 (11) 201 (100) 200 (70) 189 (34)
2-nitrofluoranthene	247 (88) 217 (2) 201 (98) 200 (100) 189 (34)	247 (66) 217 (4) 201 (100) 200 (62) 189 (27)
3-nitrofluoranthene	247 (100) 217 (32) 201 (92) 200 (95) 189 (59)	247 (81) 217 (46) 201 (91) 200 (100) 189 (67)
7-nitrofluoranthene	247 (78) 217 (5) 201 (100) 200 (63) 189 (27)	247 (65) 217 (8) 201 (100) 200 (65) 189 (34)
8-nitrofluoranthene	247 (88) 217 (10) 201 (100) 200 (86) 189 (31)	247 (78) 217 (22) 201 (100) 200 (82) 189 (37)

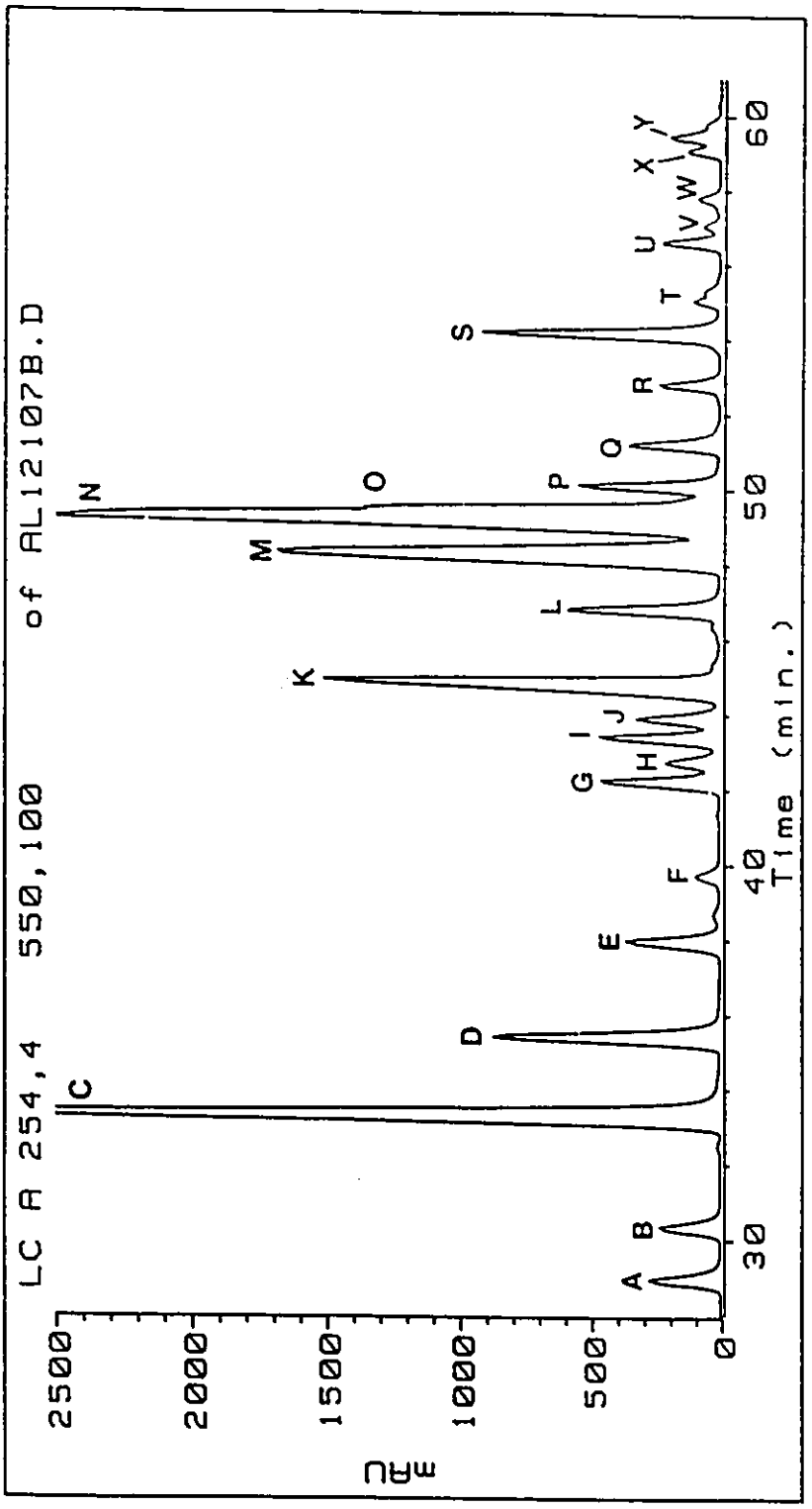


Figure 68. Normal-phase HPLC analysis of a crude nitration mixture of fluoranthene. Peak labels are defined in Table 62.

Table 62.
Mass Spectra of Nitrofluoranthenes in a Fluoranthene Nitration Mixture.

Peak label	Tentative identification*	Mass Spectrum	Lit. Mass Spectrum from (66) & (199)
A	1-NFA	247(76)217(9)201(100)189(30)	247(57)217(11)201(100)189(34)
B	7-NFA	247(78)217(5)201(100)189(27)	247(65)217(8)201(100)189(34)
C	3-NFA	247(100)217(32)201(92)289(59)	247(81)217(46)201(91)189(67)
D	8-NFA	247(88)217(10)201(100)189(31)	247(78)217(22)201(100)189(37)
E	1,7-DNF	292(100)246(18)200(64)188(39)	292(100)246(15)200(45)188(32)
F	2,3-DNF	292(100)204(8)200(82)188(39)	292(100)204(42)200(92)188(40)
G	1,2-DNF	292(100)200(56)188(25)187(25)	292(100)200(86)188(63)187(48)
H	1,4- or 1,5-DNF	not determined	292(100)246(12)200(75)188(39) 292(100)246(42)200(94)188(23)
I	3,10-DNF	292(100)246(11)200(39)187(35)	292(100)246(15)200(61)187(54)
J	1,8- 2,5- & 2,7-DNF	292(100)246(22)200(44)187(27) 292(100)246(35)200(64)188(20) 292(100)246(40)200(72)188(24)	292(100)246(29)200(55)188(34) 292(100)246(44)200(83)188(21) 292(100)246(35)200(88)188(20)
K	3,7-DNF	292(100)262(28)200(59)188(24)	292(100)262(30)200(96)188(39)
L	2,4-or 2,10-DNF	not determined	292(100)262(26)200(59)188(40) 292(100)246(45)200(80)188(38)
M	3,8-DNF	292(100)246(21)216(12)200(78)	292(100)246(22)216(19)200(82)
N	3,9-DNF	292(100)262(48)246(32)200(83)	292(100)262(30)246(20)200(68)
O	2,8-DNF	292(100)246(28)200(67)187(42)	292(100)246(35)200(80)187(20)
P	2,9-DNF	292(100)246(34)200(62)188(20)	292(100)246(42)200(90)288(27)
Q & R	1,9-DNF or TNFs	not determined	292(100)246(22)200(52)188(40)
S	3,4-DNF	292(58)246(47)216(56)188(100)	292(40)246(43)216(52)188(100)
T-Y	TNFs	not determined	not described

*Abbreviations: NFA: nitrofluoranthene, DNF: dinitrofluoranthene, TNF: trinitrofluoranthene.

For retention indices, see Table 63. For NPLC and GC-MS retention times, see Table 64.

Table 63.
Retention Index Values for Products from the Nitration of Fluoranthene.

Peak Label	Tentative ID	RI determined (60 m DB5ms)	Literature RI (60 m DB5)	Lit.RI-Determined RI
A	1-NFA	404.3	405.4	1.1
B	7-NFA	406.4	407.5	1.1
C	3-NFA	412.8	413.3	0.5
D	8-NFA	420.2	420.1	-0.1
E	1,7-DNF	455.7	455.8	
F	2,3-DNF	459.2	459.8	
G	1,2-DNF	462.7	461.7	-1.0
H	1,4- or 1,5-DNF	not determined	464.1 464.9	
I	3,10-DNF	469.7	470.4	0.7
J	1,8-DNF 2,5-DNF 2,7-DNF	474.6 471.6 471.0	476.0 472.8 472.5	1.4 1.2 1.5
K	3,7-DNF	472.2	474.3	2.1
L	2,4 or 2,10-DNF	not determined	478.6 483.2	
M	3,8-DNF	485.8	486.3	0.5
N	3,9-DNF	487.0	487.6	0.6
O	2,8-DNF	486.5	487.5	1.0
P	2,9-DNF	490.9	490.9	0
Q & R	1,9-DNF or TNFs	not determined	487.9	
S	3,4-DNF	491.8	494.3	2.5
T-Y	TNFs	not determined	not described	

Literature retention index values are from references (66) and (198).

Table 64.
NPLC and GC-MS Retention Times for Nitrofluoranthenes.

*Peak Label**	*Compound*	*NPLC Retention Time*	*GC Retention Time***
A	1-NFA	28.93 minutes	73.64 minutes
B	7-NFA	30.33	74.31
C	3-NFA	33.33	76.36
D	8-NFA	35.41	78.73
E	1,7-DNF	37.97	90.40
F	2,3-DNF	39.71	91.52
G	1,2-DNF	42.23	92.63
H	1,4- or 1,5-DNF	42.74	not determined
I	3,10-DNF	43.41	94.89
J	1,8-DNF	43.91	96.41
	2,5-DNF		95.44
	2,7-DNF		95.26
K	3,7-DNF	44.88	95.54
L	2,4 or 2,10-DNF	46.80	not determined
M	3,8-DNF	48.27	100.22
N	3,9-DNF	49.19	100.69
O	2,8-DNF	49.47	100.53
P	2,9-DNF	50.10	101.69
Q	1,9-DNF or TNFs	51.20	not determined
R	1,9-DNF or TNFs	52.80	not determined
S	3,4-DNF	54.16	101.89
T	TNFs	55.05	not determined
U	TNFs	56.60	not determined
V	TNFs	57.05	not determined
W	TNFs	57.79	not determined
X	TNFs	59.06	not determined
Y	TNFs	59.43	not determined

*From Figure 68.
**60 m DB5ms column, with the temperature program described in Tables 56 and 57.

IX.22 Solvent Blanks

Distillation of solvents for use in extraction of air particulate or sediments has occasionally been described (38,163), but the analysis of the resulting distilled solvents has not usually been reported. Since the extraction and work-up of air particulate described in section IX.9 concentrates solutes in the organic solvents about 9000 fold (between Soxhlet extraction and GC-MS analysis), solvents were occasionally assessed for purity by concentrating both distilled and undistilled solvents. The experimental method to analyze solvent blanks is described below.

A 180 mL volume of each solvent was evaporated nearly to dryness under reduced pressure and the remainder of the solvent was transferred to a Reacti-vial and blown down to dryness using a stream of argon gas. The residue was taken up in 20 μL of distilled toluene and 1 μL was injected onto a 5 m DB5 column with FID detector. Generally, contaminants up to a level of 50 ng/L of distilled solvent were considered tolerable for use. Solvents containing contaminants above 100 ng/L were deemed unacceptable for use in extraction or chromatography.

IX.23 Time-Course Study of Soxhlet Extraction

In order to determine optimal extraction times with each solvent, the method described above in section IX.9 was modified. Two air particulate filters (containing a total of 170.4 mg of material) were placed in a Soxhlet apparatus and extracted with 180 mL dichloromethane. After 1 hour, the reflux flask was replaced by a second flask

containing fresh dichloromethane and the refluxing continued. In a similar fashion, a new round-bottom flask containing fresh dichloromethane was changed after 5, 9, 19 and 24 hours. Following 24 hours extraction with dichloromethane, the flask was changed to one containing 180 mL of methanol, and the extraction was continued. Fresh flasks with fresh methanol were replaced after 12, 18, 24 and 27 hours of cycling. The solvent cycling times were every 10 minutes.

Each extract was reduced to a volume of approximately 10 μL by rotary evaporation at reduced pressure. Dichloromethane extracts were diluted with acetonitrile and methanol extracts were diluted with methanol. RPLC analysis of diluted extracts was carried out using gradient C and the area underneath all peaks seen in the RPLC run was integrated. The integrated total area was recorded as a function of extraction time and these results appear in Tables 3 and 4.

IX.24 Investigation of Analyte Distribution in Alumina Chromatography

A mixture of alpha-naphthol (2.3 mg), pyrene (0.3 mg), 3-aminofluoranthene (0.3 mg), p-nitrophenol (4.2 mg), 3,5-dimethylphenol (12.1 mg), 1,5-dinitronaphthalene (1.2 mg), carbazole (0.2 mg), xanthone (0.2 mg), anthraquinone (0.9 mg), dibenzofuran (1.1 mg), dibenzothiophene (0.2 mg) and dibenzocarbazole (0.3 mg) was dissolved in a mixture of dichloromethane and methanol (10 mL). One-tenth of this mixture was applied to the alumina column as described above and six individual fractions (A1 through A6) were collected and reduced in volume by rotary evaporation to approximately 100 μL.

Each fraction was blown down to a volume of approximately 5-10 µL and diluted with acetonitrile (for A1, A2 and A3 fractions) or methanol (for A4 and A5 fractions) to approximately 200 µL. Aliquots (20 µL) were analyzed by reversed-phase HPLC using gradient C. Results appear in Table 5.

IX.25 Investigation of Analyte Distribution in Sephadex LH20 Chromatography

A mixture of naphthalene (1.8 mg), anthracene (2.0 mg), pyrene (1.9 mg), benzo[a]pyrene (1.5 mg), n-hexacosane (1.6 mg), n-tetradecane (1.4 mg), n-docosane (1.1 mg), n-nonadecane (1.3 mg), n-dodecane (1.0 mg) and n-hexadecane (1.0 mg) was dissolved in dichloromethane (10 mL). An aliquot of this solution (50 µL) was diluted with LH20 solvent (200 µL) and injected onto the Sephadex LH20 column as described in Section IX.11. The eluate from the time of injection up to, but not including the elution time of naphthalene was retained as an aliphatic fraction, while all compounds eluting with or after naphthalene were kept as an aromatic fraction. Each fraction was reduced in volume by rotary evaporation at reduced pressure to approximately 500-1000 µL. Volume was further reduced to about 10-20 µL under a gentle stream of nitrogen. Finally, the volume of each fraction was adjusted to 1000 µL with toluene and 1 µL of each toluene solution was analyzed by GC-FID, as described in section IX.14. Results appear in Table 6.

X. BIBLIOGRAPHY

1. Finlayson-Pitts, B.J.; Pitts, J.N. *Atmospheric Chemistry: Fundamentals and Experimental Techniques*; John Wiley and Sons, New York, 1986, pp. 870-960.

2. Scheepers, P.T.J.; Bos, R.P. *Int. Arch. Occup. Environ. Health* **1992**, *64*, 163-177.

3. Pyysalo, H.; Tuominen, J.; Wickstrom, K.; Skytta, E.; Tikkanen, L.; Salomaa, S.; Sorsa, M.; Nurmela, T.; Mattila, T.; Pohjola, V. *Atmos. Environ.* **1987**, *21*, 1167-1180.

4. Owen, M.K.; Ensor, D.S.; Sparks, L.E. *Atmos. Environ.***1992**, *26A*, 2149-2162.

5. Leiter, J.; Shimkin, M.B.; Shear, M.J. *J.N.C.I.* **1942**, *3*, 155-165.

6. Alfheim, I.; Bjorseth, A.; Moller, M. *C.R.C. Crit. Rev. Environ. Control* **1984**, *14*, 91-150.

7. Vo-Dinh, T. *Chemical Analysis of Polycyclic Aromatic Compounds*; Wiley, New York, 1988, pp. 1-30.

8. Cook, J.W.; Hewett, C.L.; Hieger, I. *J. Chem. Soc.* **1933**, 395-398.

9. Subramanyam, V.; Valsaraj, K.T.; Thibodeaux, L.J.; Reible, D.D. *Atmos. Environ.* **1994**, *28*, 3083-3091.

10. Wilson, N.K.; McCurdy, T.R.; Chuang, J.C. *Atmos. Environ.* **1995**, *29*, 2575-2584.

11. Thrane, K.E.; Mikalsen, A. *Atmos. Environ.* **1981**, *15*, 909-918.

12. Atkinson, R.; Arey, J. *Environ. Health Perspect.* **1994**, *102*, *Supplement 4*, 117-126.

13. Lee, M.L.; Novotny, M.V.; Bartle, K.D. *Analytical Chemistry of Polycyclic Aromatic Compounds*; Academic Press: New York, 1981.

14. Jacob, J. *Sulfur Analogues of Polycyclic Aromatic Hydrocarbons (Thiaarenes)*; Cambridge University Press, Cambridge, 1990.

15 Crebelli, R.; Conti, L.; Crochi, B.; Carere, A.; Bertoli, C.; Del Giacomo, N. *Mutat. Res.* **1995**, *346*, 167-172.

16. Tancell, P.J.; Rhead, M.M.; Trier, C.J.; Bell, M.A.; Fussey, D.E. *Sci. Total Environ.* **1995**, *162*, 179-186.

17. Badger, G.M.; Buttery, R.G.; Kimber, R.W.L.; Lewis, G.E.; Moritz, A.G.; Napier, I.M. *J. Chem. Soc.* **1958**, 2449-2452.

18. Scheepers, P.T.J.; Bos, R.P. *Int. Arch. Occup. Environ. Health* **1992**, *64*, 149-161.

19. Levsen, K. *Fresenius Z. Anal. Chem.* **1988**, *331*, 467-478.

20. Streitwieser, A.; Fahey, R. *J. Org. Chem.* **1962**, *27*, 2352-2355.

21. Ciccioli, P.; Cecinato, A.; Brancaleoni, E.; Draisci, R.; Liberti, A. *Aerosol Sci. Technol.* **1989**, *10*, 296-310.

22. Thompson, A. *Israel J. Chem.* **1994**, *34*, 277-288.

23. Gershenzon, Y.M.; Zvenigorodskii, S.G.; Rozenshtein, V.B. *Russ. Chem. Revs.* **1990**, *59*, 928-944.

24. Ramdahl, T.; Zielinska, B.; Arey, J.; Atkinson, R.; Winer, A.M.; Pitts, J.N. *Nature* **1986**, *321*, 425-426.

25. Helmig, D.; Harger, W.P. *Sci. Total Environ.* **1994**, *148*, 11-21.

26. Lane, D.A. in *Chemical Analysis of Polycyclic Aromatic Compounds*; Vo-Dinh, T., Ed.; John Wiley and Sons, New York, 1989; pp. 31-58.

27. Lofroth, G.; Nilsson, L.; Agurell, E.; Yasuhara, A. *Z. Naturforsch.* **1983**, *39C*, 193-195.

28. Chapman, O.L.; Heckert, D.C.; Reasoner, J.W.; Thackaberry, S.P. *J. Am. Chem. Soc.* **1966**, *88*, 5550-5554.

29. Brorstrom, E.; Grennfelt, P.; Lindskog, A. *Atmos. Environ.* **1983**, *17*, 601-605.

30. De Raat, W.K.; Bakker, G.L.; De Meijere, F.A. *Atmos. Environ.* **1990**, *24A*, 2875-2887.

31. May, W.E.; Benner, B.A.; Wise, S.A.; Schuetzle, D.; Lewtas, J. *Mutat. Res.* **1992**, *276*, 11-22.

32. Bjorseth, A. *Anal. Chim. Acta* **1977**, *94*, 21-27.

33. Viras, L.G.; Anthanasiou, K.; Siskos, P.A. *Atmos. Environ.* **1990**, *24B*, 267-274.

34. Warshawsky, D. *Environ. Carcino. & Ecotox. Revs.* **1992**, *C10*, 1-71.

35. Lee, H.; Law, S.M.; Lin, S.T. *Toxicol. Lett.* **1991**, *58*, 59-67.

36. Montreuil, C.N.; Ball, J.C.; Gorse, R.A.; Young, W.C. *Mutat. Res.* **1992**, *282*, 89-92.

37. McCalla, D.R.; Quilliam, M.A.; Kaiser-Farrell, C.; Tashiro, C.; Hoo, K.; Gibson, E.S.; Lockington, N.J.; Kerr, A.A.; Sheldrake, C. in *Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes*; King, C.M., Romano, L.J., Schuetzle, D., Eds.; Elsevier Science Publishing Co., Inc.: New York, 1988; pp. 47-63.

38. Marvin, C.H.; Allan, L.; McCarry, B.E.; Bryant, D.W. *Int. J. Environ. Anal. Chem.* **1992**, *49*, 221-230.

39. Marvin, C.H.; McCarry, B.E.; Bryant, D.W. *J. Great Lakes Res.* **1994**, *20*, 523-530.

40. Edye, L.A.; Richards, G.N. *Environ. Sci. Technol.* **1991**, *25*, 1133-1137.

41. Wise, S.A.; Benner, B.A.; Liu, H.; Byrd, G.D.; Colmsjo, A. *Anal. Chem.* **1988**, *60*, 630-637.

42. Ciccioli, P.; Brancaleoni, E.; Cecinato, A.; Di Palo, C.; Buttini, P.; Liberti, A. *J. Chrom.* **1986**, *351*, 451-464.

43. Greenberg, A.; Lwo, J.; Atherholt, T.B.; Rosen, R.; Hartman, R.; Butler, J.; Louis, J. *Atmos. Environ.* **1993**, *27A*, 1609-1626.

44. Clark, B.R.; Ho, C.H.; Jones, A.R., *A.C.S. Advances in Chemistry Series*, **1978**, No. 170, 282-294.

45. Sicherer-Roetman, A.; Ramlal, M.; Voogd, C.E.; Bloemen, H.J.T. *Atmos. Environ.* **1988**, 22, 2803-2808.

46. Chriswell, C.D.; Glatz, B.A.; Fritz, J.S.; Svec, H.J. In *Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures*; Waters, M.D., Nesnow, S., Huisingh, J.L., Sandhu, S.S., Claxton, L., Eds.; Plenum Press: New York, 1978.

47. Later, D.W.; Lee, M.L.; Bartle, K.D.; Kong, R.C.; Vassilaros, D.L. *Anal. Chem.* **1981**, *53*, 1612-1620.

48. Fung, R.L.C., *The Determination of Nitrogen-Containing Compounds in Heavy Syncrude Oils*, M.Sc. Thesis, McMaster University, 1989.

49. Marvin, C.H.; Allan, L.; McCarry, B.E.; Bryant, D.W. *Environ. Mol. Mutagen.* **1993**, 22, 61-70.

50. Bryant, D.W.; McCalla, D.R.; McCarry, B.E. *Classical PAH Form a Minor Portion of Mutagens Detected in Hamilton Air Particulates*. Interim Report for the Ontario Ministry of the Environment, Project 386G, 1986.

51. McCarry, B.E.; Bryant, D.W.; McCalla, D.R. In *Proceedings. Environmental Research: 1989 Technology Transfer Conference. November 20 & 21, 1989*; Research and Technology Branch, Environment Ontario: Toronto, 1989, Vol. 1: Feature Presentations, Air Quality Research, Water Quality Research, pp. 138-147.

52. Dong, M.W.; Greenberg, A. *J. Liquid Chrom.* **1988**, *11*, 1887-1905.

53. Mahanama, K.R.R.; Gundel, L.A.; Daisey, J.M. *Int. J. Environ. Anal. Chem.* **1994**, *56*, 289-309.

54. Wise, S.A.; Hilpert, L.R.; Byrd, G.D.; May, W.E. *Polycyclic Arom. Compounds* **1990**, *1*, 81-98.

55. Bodzek, D.; Luks-Betlej, K.; Warzecha, L. *Atmos. Environ.* **1993**, *27A*, 759-764.

56. Konig, J.; Balfanz, E.; Funcke, W.; Romanowski, T. *Anal. Chem.* **1983**, *55*, 599-603.

57. Grimmer, G.; Naujack, K.W.; Schneider, D. In *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*; Bjorseth, A., Dennis, A.J., Eds.; Battelle Press: Columbus, Ohio, 1980; pp. 107-125.

58. Rogge, W.F.; Mazurek, M.A.; Hildemann, L.M.; Cass, G.R.; Simoneit, B.R.T. *Atmos. Environ.* **1993**, *27A*, 1309-1330.

59. Helmig, D.; Lopez-Cancio, J.; Arey, J.; Harger, W.P.; Atkinson, R. *Environ. Sci. Technol.* **1992**, *26*, 2207-2213.

60. Arey, J.; Zielinska, B.; Atkinson, R.; Winer, A.M. *Atmos. Environ.* **1987**, *21*, 1437-1444.

61. Hayakawa, K.; Murahashi, T.; Butoh, M.; Miyazaki, M. *Environ. Sci. Technol.* **1995**, *29*, 928-932.

62. Nielsen, T. *Anal. Chem.* **1983**, *55*, 286-290.

63. Nielsen, T.; Seitz, B.; Ramdahl, T. *Atmos. Environ.* **1984**, *18*, 2159-2165.

64. Schneider, E.; Krenmayr, P.; Varmuza, K. *Monatsh. Chem.* **1990**, *121*, 393-401.

65. Schuetzle, D.; Jensen, T.E.; Ball, J.C. *Environ. Int.* **1985**, *11*, 169-181.

66. Ramdahl, T.; Sweetman, J.A.; Zielinska, B.; Atkinson, R.; Winer, A.M.; Pitts, J.N. *JHRC & CC* **1985**, *8*, 849-852.

67. Svendsen, H.; Ronningsen, H.P.; Syndnes, L.K.; Greibokk, T. *Acta Chem. Scand.* **1983**, *B37*, 833-844.

68. Greibokk, T.; Iversen, B.; Johansen, E.J.; Ronningsen, H.P.; Svendsen, H. *JHRC & CC* **1984**, *7*, 671-678.

69. Tomkins, B.A. In *Nitrated Polycyclic Aromatic Hydrocarbons*; White, C.M., Ed.; Alfred Huethig Verlag: Heidelberg, 1985, pp. 87-120.

70. Quimby, B.D.; Sullivan, J.J. *Anal. Chem.* **1990**, *62*, 1027-1034.

71. White, C.M. In *Nitrated Polycyclic Aromatic Hydrocarbons*; White, C.M., Ed.; Alfred Huethig Verlag: Heidelberg, 1985, pp. 1-86.

72. Schuetzle, D.; Jensen, T.E. In *Nitrated Polycyclic Aromatic Hydrocarbons*; White, C.M., Ed.; Alfred Huethig Verlag: Heidelberg, 1985, pp. 121-168.

73. Hites, R.A. In *Chemical Analysis of Polycyclic Aromatic Compounds*; Vo-Dinh, T., Ed.; John Wiley and Sons: New York, 1989, pp. 219-261.

74. Ong, V.S.; Hites, R.A. *Mass Spec. Revs.* **1994**, *13*, 259-283.

75. Rose, M.E.; Johnstone, R.A.W. *Mass Spectrometry for Chemists and Biochemists*; Cambridge University Press, Cambridge, 1982.

76. Low, G.K.C.; Batley, G.E.; Lidgard, R.O.; Duffield, A.M. *Biomed. Environ. Mass Spec.* **1986**, *13*, 95-104.

77. Shirname-More, L. In *Diesel Exhaust : A Critical Analysis of Emissions, Exposure and Health Effects*, Health Effects Institute: Cambridge, MA, 1995, p. 294.

78. Lewtas, J. In *Genetic Toxicology of Complex Mixtures*; Waters, M.D., Ed.; Plenum Press: New York, 1990, pp. 353-361.

79. Schuetzle, D.; Lewtas, J. *Anal. Chem.* **1986**, *58*, 1060A-1075A.

80. Klaassen, C.D. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*; Klaassen, C.D., Amdur, M.O., Doull, J., Eds.; Collier Macmillan Canada Inc., Toronto, 1986, p. 28.

81. Flessel, P.; Wang, Y.Y.; Chang, K.I.; Wesolowski, J.J. *J. Chem. Ed.* **1987**, *64*, 391-395.

82. Ames, B.N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347-364.

83. Maron, D.M.; Ames, B.N. *Mutat. Res.* **1983**, *113*, 173-215.

84. Tokiwa, H.; Ohnishi, Y. *C.R.C. Crit. Rev. Toxicol.* **1986**, *17*, 23-60.

85. Arey, J.; Zielinska, B.; Harger, W.P.; Atkinson, R.; Winer, A.M. *Mutat. Res.* **1988**, *207*, 45-51.

86. Siak, J.; Chan, T.L.; Gibson, T.L.; Wolff, G.T. *Atmos. Environ.* **1985**, *19*, 369-376.

87. De Raat, W.K.; Boers, J.P.; Bakker, G.L.; De Meijere, F.A.; Hooimeijer, A.; Lohman, P.H.M.; Mohn, G.R. *Sci. Total Environ.* **1994**, *153*, 7-28.

88. Marvin, C.H., *A Multi-Media Bioassay-Directed Investigation of the Hamilton Harbour Area of Western Lake Ontario*, Ph.D. Thesis, McMaster University, 1994.

89. Commoner, B. *J. Toxicol. Environ. Health* **1978**, *4*, 59-69.

90. Tokiwa, H.; Kitamori, S.; Takahashi, K.; Ohnishi, Y. *Mutat. Res.* **1980**, *77*, 99-108.

91. Sakai, M.; Yoshida, D.; Mizusaki, S. *Mutat. Res.* **1985**, *156*, 61-67.

92. Rice, J.E.; Coleman, D.T.; Hosted, T.J.; LaVoie, E.J.; McCausland, D.J.; Wiley, J.C. *Cancer Res.* **1985**, *45*, 5421-5435.

93. Einisto, P.; Watanabe, M.; Ishidate, M.; Nohmi, T. *Mutat. Res.* **1991**, *259*, 95-102.

94. Houk, V.S.; Goto, S.; Endo, O.; Claxton, L.D.; Lewtas, J.; Matsushita, H. *Environ. Mol. Mutagen.* **1992**, *20*, 19-28.

95. Watanabe, M.; Ishidate, M.; Nohmi, T. *Mutat. Res.* **1989**, *216*, 211-220.

96. Marvin, C.H.; Tessaro, M.; McCarry, B.E.; Bryant, D.W. *Sci. Total Environ.* **1994**, *156*, 119-131.

97. Marvin, C.H.; Lundrigan, J.A.; McCarry, B.E.; Bryant, D.W. In *Coastal Zone Canada 94, Cooperation in the Coastal Zone: Conference Proceedings*, Bedford Institute of Oceanography: Dartmouth, Nova Scotia, 1994; Vol. 3, pp. 1260-1272.

98. Katz, M.; Sakuma, T.; Ho, A. *Environ. Sci. Technol.* **1978**, *12*, 909-915.

99. Katz, M.; Chan, C. *Environ. Sci. Technol.* **1980**, *14*, 838-843.

100. Dobroff, F. *1991-1992 Air Quality Data Summary, Regional Municipality of Hamilton-Wentworth*; Queen's Printer for Ontario, Toronto, 1993.

101. Bryant, D.W.; McCarry, B.E., *Genotoxicity Testing of Polycyclic Aromatic Compounds Associated with Respirable Air Particulate*, unpublished manuscript **1991**.

102. Nauss, K.M.; Busby, W.F.; Cohen, A.J.; Green, G.M.; Higgins, M.W.P.; McClellan, R.O.; Rosenkranz, H.S.; Sawyer, R.F.; Upton, A.; Watson, A.Y.; Watts, W.F.; Winer, A.M. In *Diesel Exhaust: A Critical Analysis of Emissions, Exposure and Health Effects*; Nauss, K.M., Ed.; Health Effects Institute: Cambridge MA, 1995; pp. 11-61.

103. Air Quality and Meteorology Section, Ontario Ministry of Environment and Energy. *Air Quality in Ontario 1991*; Queen's Printer for Ontario, Toronto, 1992.

104. Brimblecombe, P. *Air Composition & Chemistry*; Cambridge University Press, Cambridge, 1986.

105. Dobroff, F. *1987 Hamilton-Wentworth Air Quality*; Queen's Printer for Ontario, Toronto, 1989.

106. Dobroff, F. *1988 Hamilton-Wentworth Air Quality*; Queen's Printer for Ontario, Toronto, 1991.

107. Dobroff, F. *1990 Air Quality Data Summary, Regional Municipality of Hamilton-Wentworth*; Queen's Printer for Ontario, Toronto, 1992.

108. Dobroff, F., Personal communication, May 31, 1993.

109. Graedel, T.E. *Chemical Compounds in the Atmosphere*; Academic Press, New York, 1978.

110. Rogge, W.F.; Hildemann, L.M.; Mazurek, M.A.; Cass, G.R.; Simoneit, B.R.T. *Environ. Sci. Technol.* **1993**, *27*, 636-651.

111. Iwado, H.; Naito, M.; Hayatsu, H. *Mutat. Res.* **1991**, *246*, 93-102.

112. Tan, Y.L. *Anal. Letts.* **1988**, *21*, 553-562.

113. Takada, H.; Onda, T.; Ogura, N. *Environ. Sci. Technol.* **1990**, *24*, 1179-1186.

114. *Sephadex LH-20 Chromatography in Organic Solvents*; Pharmacia Fine Chemicals AB, Uppsala, 1980.

115. Rasberry, S.D. *National Bureau of Standards Certificate of Analysis-SRM 1650*, **1987**.

116. Wise, S.A.; Schantz, M.M.; Benner, B.A.; Parris, R.M.; Rebbert, R.E.; Sander, L.C.; Koster, B.J., Chesler, S.N.; May, W.E. *Fres. Z. Anal. Chem.* **1993**, *345*, 325-329.

117. Ciccioli, P.; Cecinato, A.; Brancaleoni, E.; Frattoni, M.; Zacchei, P.; DeCastro-Vasconcellos, P. *Ann. Chim.* **1995**, *85*, 455-468.

118. Krewski, D.; Leroux, B.G.; Creason, J.; Claxton, L. *Mutat. Res.* **1992**, *276*, 33-59.

119. Claxton, L.D.; Douglas, G.; Krewski, D.; Lewtas, J.; Matsushita, H.; Rosenkranz, H. *Mutat. Res.* **1992**, *276*, 61-80.

120. Nielsen, P.A. *Mutat. Res.* **1992**, *276*, 117-123.

121. Claxton, L.D.; Creason, J.; Leroux, B.; Agurell, E.; Bagley, S.; Bryant, D.; Courtois, Y.A.; Douglas, G.; Clare, C.B.; Goto, S.; Quillardet, P.; Jagannath, D.R.; Kataoka, K.; Mohn, G.; Nielsen, P.A.; Ong, T.; Pederson, T.C.; Shimizu, H.; Nylund, L.; Tokiwa, H.; Vink, G.J.; Wang, Y.; Warshawsky, D. *Mutat. Res.* **1992**, *276*, 23-32.

122. Bryant, D.W., Personal communication to L. Claxton, March 8, 1991.

123. Hagiwara, P.; Watanabe, M.; Oda, Y.; Sofuni, T.; Nohmi, T. *Mutat. Res.* **1993**, *291*, 171-180.

124. Zielinska, B.; Arey, J.; Harger, W.P.; Lee, R.W.K. *Mutat. Res.* **1988**, *206*, 131-140.

125. Chemsyn Science Laboratories, *2-Nitrofluoranthene*, Product Information, 1992.

126. Nishioka, M.G.; Chuang, C.C.; Petersen, B.A.; Austin, A.; Lewtas, J. *Environ. Int.* **1985**, *11*, 137-146.

127. Adonis, M.; Gil, L. *Mutat. Res.* **1993**, *292*, 51-61.

128. Vartiainen, M.; McDow, S.R.; Kamens, R.M. *Chemosphere* **1994**, *29*, 1661-1669.

129. McDow, S.R.; Vartiainen, M.; Sun, Q.; Hong, Y.; Yao, Y.; Kamens, R. *Atmos. Environ.* **1995**, *29*, 791-797.

130. United States Environmental Protection Agency, Method 610. Code of Federal Regulations, Chapter I, Part 136, Appendix A, 1989.

131. Lee, W.M.G.; Huang, Y.F. *J. Environ. Sci. Health* **1992**, *A27*, 2051-2074

132. Harvey, R.G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*; Cambridge University Press, Cambridge, 1991.

133. Ramdahl, T. *Environ. Sci. Technol.* **1983**, *17*, 666-670.

134. Ramdahl, T. *Environ. Int.* **1985**, *11*, 197-203.

135. Spitzer, T.; Kuwatsuka, S. *Environ. Poll.* **1989**, *62*, 63-71.

136. Lewis, A.C.; Kupiszewska, D.; Bartle, K.D.; Pilling, M.J. *Atmos. Environ.* **1995**, *29*, 1531-1542.

137. Kosuta, S., *Characterization and Mutagenicity of Hamilton Airborne Particulate; Correlation of Results with Meteorological Data*, B.Sc. Thesis, McMaster University, 1994.

138. Martens, R., *The Analysis of a Mutagenic Fraction Derived from Hamilton Air Particulate*, B.Sc. Thesis, McMaster University, Hamilton, 1990.

139. De Raat, W.K.; De Meijere, F.A. *Sci. Total Environ.* **1988**, *73*, 159-179.

140. Pitts, J.N.; Harger, W.; Lokensgard, D.M.; Fitz, D.R.; Scorziell, G.M.; Mejia, V. *Mutat. Res.* **1982**, *104*, 35-41

141. Motykiewicz, G.; Szeliga, J.; Cimander, B.; Chorazy, M. *Mutat. Res.* **1989**, *223*, 243-251.

142. Alfheim, I.; Lofroth, G.; Moller, M. *Environ. Health Perspect.* **1983**, *47*, 227-238.

143. Barale, R.; Zucconi, D.; Giorgelli, F.; Carducci, A.L.; Tonelli, M.; Loprieno, N. *Environ. Mol. Mutagen.* **1989**, *13*, 227-233.

144. Moller, M.; Alfheim, I.; Larssen, S.; Mikalsen, A. *Environ. Sci. Technol.* **1982**, *16*, 221-225.

145. Chan, T.L.; Siak, J.; Korsog, P.E.; Wolff, G.T. *Aerosol Sci. Technol.* **1989**, *11*, 26-38.

146. Bezuglaya, E.; Shchutskaya, A.B.; Smirnova, I.V. *Atmos. Environ.* **1993**, *27A*, 773-779.

147. Lee, M.L.; Vassilaros, D.L.; Later, D.W. *Int. J. Environ. Anal. Chem.* **1982**, *11*, 251-262.

148. Spitzer, T.; Dannecker, W. *JHRC & CC* **1984**, *7*, 301-305.

149. Roussel, R.; Allaire, M.; Friar, R.S. *J. Air Waste Manage. Assoc.* **1992**, *42*, 1609-1613.

150. Motykiewicz, G.; Cimander, B.; Szeliga, J.; Tkocz, A.; Chorazy, M. In *Complex Mixtures and Cancer Risk*; Vainio, H., Sorsa, M., McMichael, A.J., Eds.; International Agency for Research on Cancer: Lyon, 1990; pp. 261-268.

151. Masclet, P.; Mouvier, G.; Nikolaou, K. *Atmos. Environ.* **1986**, *20*, 439-446.

152. Back, S.O.; Goldstone, M.E.; Kirk, P.W.W.; Lester, J.N.; Perry, R. *Environ. Technol.* **1991**, *12*, 107-129.

153. De Raat, W.K.; Kooijman, S.A.; Gielen, J.W.J. *Sci. Total Environ.* **1987**, *66*, 95-114.

154. Flessel, P.; Wang, Y.Y.; Chang, K.L.; Wesolowski, J.J.; Guiguis, G.N.; Kim, I.S.; Levaggi, D.; Siu, W. *J. Air Waste Manage. Assoc.* **1991**, *41*, 276-281.

155. Daisey, J.M.; Cheney, J.L.; Lioy, P.J. *JAPCA* **1986**, *36*, 17-33.

156. Scarpato, R.; DiMarino, F.; Strano, A.; Curti, A.; Campagna, R.; Loprieno, N.; Barrai, I.; Barale, R. *Mutat. Res.* **1993**, *319*, 293-301.

157. Dobroff, F., personal communication, December 7th, 1995.

158. Wolff, G.T.; Siak, J.S.; Chan, T.L.; Korsog, P.E. *Atmos. Environ.* **1986**, *20*, 2231-2241.

159. Harman, H.H., *Modern Factor Analysis*, 2nd Edition. University of Chicago Press, Chicago, 1967.

160. Henry, R.C.; Hidy, G.M. *Atmos. Environ.* **1979**, *13*, 1581-1596.

161. Morris, W.A.; Versteeg, J.K.; Bryant, D.W.; Legzdins, A.E.; McCarry, B.E.; Marvin, C.H. *Atmos. Environ.* **1995**, *29*, 3441-3450.

162. Zielinska, B.; Arey, J.; Atkinson, R.; Winer, A.M. *Atmos. Environ.* **1989**, *23*, 223-229.

163. Lewtas, J.; Chuang, J.; Nishioka, M.; Petersen, B. *Int. J. Environ. Anal. Chem.* **1990**, *39*, 245-256.

164. Schmidt, W.; Grimmer, G.; Jacob, J.; Dettbarn, G.; Naujack, K.W. *Fresenius Z. Anal. Chem.* **1987**, *326*, 401-413.

165. Chuang, J.C.; Mack, G.A.; Kuhlman, M.R. *Atmos. Environ.* **1991**, *25B*, 369-380.

166. Ciccioli, P.; Cecinato, A.; Cabella, R.; Brancaleoni, E.; Buttini, P. *Atmos. Environ.* **1993**, *27A*, 1261-1270.

167. Arey, J.; Zielinska, B.; Atkinson, R.; Winer, A.M.; Ramdahl, T. *Atmos. Environ.* **1986**, *20*, 2339-2345.

168. Ruehle, P.; Bosch, L.C.; Duncan, W.P. In *Nitrated Polycyclic Aromatic Hydrocarbons*; White, C.M., Ed.; Alfred Huethig Verlag: Heidelberg, 1985; pp. 169-230.

169. Kamens, R.M.; Zhi-Hua, F.; Yao, Y.; Chen, D.; Chen, S.; Vartiainen, M. *Chemosphere* **1994**, *28*, 1623-1632.

170. Liberti, A.; Ciccioli, P. *JHRC & CC* **1986**, *9*, 492-501.

171. Niles, R.; Tan, Y.L. *Anal. Chim. Acta* **1989**, *221*, 53-63.

172. Oehme, M. *Chemosphere* **1985**, *14*, 1285-1297.

173. Ramdahl, T., Becher, G.; Bjorseth, A. *Environ. Sci. Technol.* **1982**, *16*, 861-865.

174. Schilhabel, J.; Levsen, K. *Fresenius Z. Anal. Chem.* **1989**, *333*, 800-805.

175. Schuetzle, D.; Lee, F.S.C.; Prater, T.J. *Int. J. Environ. Anal. Chem.***1981**, *9*, 93-144.

176. Tokiwa, H.; Sera, N.; Kai, M.; Horikawa, K.; Ohnishi, Y. in *Genetic Toxicology of Complex Mixtures;* Waters, M.D., Ed.; Plenum Press, New York, 1990, pp. 165-172.

177. Chiu, C. An Improved Method for NitroPAH Analysis. *Polycyclic Arom. Compounds* **1996**, in press.

178. Jung, H.; Shaikh, A.U.; Heflich, R.H.; Fu, P.P. *Env.Mol. Mut.* **1991**, *17*, 169-180.

179. Librando, V. *Polycyclic Arom. Compounds* **1994**, *5*, 175-183.

180. Harris, D.C. *Quantitative Chemical Analysis;* W.H. Freeman and Co., New York, 1982.

181. Wise, S.A.; Deissler, A.; Sander, L.C. *Polycyclic Arom. Compounds* **1993**, *3*, 169-184.

182. Wise, S.A.; Chesler, S.N.; Hertz, H.S.; Hilpert, L.R.; May, W.E. *Anal. Chem.* **1977**, *49*, 2306-2310.

183. Karcher, W. *Spectral Atlas of Polycyclic Aromatic Compounds*; D. Reidel Publishers, Dordrecht, 1985.

184. Hecht, S.S.; LaVoie, E.J.; Amin, S.; Bedenko, V.; Hoffman, D. In *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*; Bjorseth, A.J., Dennis, A.J., Eds.; Battelle: Columbus, Ohio, 1980, pp. 417-433.

185. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T. *Environ. Mol. Mutagen.* **1992**, *19, Supplement 21*, 2-141.

186. Moller, M.; Hagen, I.; Ramdahl, T. *Mutat. Res.* **1985**, *157*, 149-156.

187. Bryant, D.W., *Final Report*, March 26, 1995. Personal communication to W.J. Snodgrass.

188. Hamilton, K., *Comparisons of Mutagenicity and Magnetic Susceptibility for Respirable Airborne Particulate at Highway 404*, B.Sc. Thesis, McMaster University, Dept. of Geology.

189. Environment Ontario, Laboratory Services Branch, Quality Management Office, *The Determination of Polycyclic Aromatic Hydrocarbons in Ambient Air by GC/MS*, VOCAIR-E3314A, February 10th, 1993.

190. Uriano, G.A. *National Bureau of Standards Certificate of Analysis - SRM1649*, 1982.

191. Pengelly, L.D.; Goldsmith, C.H.; Kerigan, A.T.; Furlong, W.; Toplack, S.; *JAPCA* **1987**,*37*, 1421-1428.

192. Pengelly, L.D.; Goldsmith, C.H.; Kerigan, A.T.; Furlong, W.; Toplack, S. in *Aerosols;* Lee, S.D.; Schneider, T.; Grant, L.D.; Verkerk, P.J., Eds., Lewis Publishers, Chelsea, Michigan, 1986.

193. Lee, H.; Lin, T.L.; Shieh, R.L.; Bian, S.S. *Mutat. Res.* **1994**, *324*, 77-84.

194. Ball, L.M.; Rosser-Duncan, P.; Boucher, M.N. *Polycyclic Arom. Compounds* **1994**, *7*, 35-42.

195. Tokiwa, H.; Horikawa, K.; Ohnishi, Y. *Mutat. Res.* **1993**, *297*, 181-195.

196. Hagiwara, Y.; Watanabe, M.; Oda, Y.; Sofuni, T.; Nohmi, T. *Mutat. Res.* **1993**, *291*,171-180.

197. Nakagawa, R.; Horikawa, K.; Sera, N.; Kodera, Y.; Tokiwa, H. *Mutat. Res.* **1987**, *191*, 85-91.

198. Ramdahl, T.; Arey, J.; Atkinson, R.; Winer, A.M. *JHRC & CC* **1986**, *9*, 515-517.

199. Ramdahl, T.; Zielinska, B.; Arey, J.; Kondrat, R.W. *Biomed. Environ. Mass Spec.* **1988**, *17*, 55-62.

200. D'Agostino, P. *The Analysis of Nitropolycyclic Aromatic Hydrocarbons in Diesel Exhaust and Urban Airborne Particulate Samples*, Ph.D. Thesis, McMaster University, 1983.

201. Creaser, C.S.; Mitchell, D.S.; O'Neill, K.E. *Int.J.Mass Spec. Ion Process.* **1991**, *106*, 21-31.

202. McLuckey, S.A.; Glish, G.L.; Asano, K.G. *Anal. Chim. Acta* **1989**, *225*, 25-35.

203. Lewis, A.C.; Askey, S.A.; Robinson, R.C.; Bartle, K.D.; Pilling, M.J. *Anal. Proc.* **1995**, 297-300.

204. Sera, N.; Fukuhara, K.; Miyata, N.; Tokiwa, H. *Mutagenesis* **1994**, *9*, 47-52.

205. Sverdrup, G.M.; Buxton, B.E.; Chuang, J.C.; Casuccio, G.S. *Environ. Sci. Technol.* **1990**, *24*, 1186-1195.

206. Wilk, M.; Rochlitz, J.; Bende, H. *J. Chrom.* **1966**, *24*, 414-416.

207. Radner, F. *Acta Chem. Scand.* **1983**, *B37*, 65-67.

208. Paputa-Peck, M.C.; Marano, R.S.; Schuetzle, D.; Riley, T.L.; Hampton, C.V.; Prater, T.J.; Skewes, L.M.; Jensen, T.E.; Ruehle, P.; Bosch, L.C.; Duncan, W.P. *Anal. Chem.* **1983**, *55*, 1946-1954.

209. Wolfbeis, O.S.; Posch, W.; Gubitz, G.; Tritthart, P. *Anal. Chim. Acta* **1983**, *147*, 405-410.

210. Chemsyn Science Laboratories, *2-Nitropyrene*, Product Information, 1992.

211. Cook, J.W.; Hewett, C.L.; Hieger, I. *J. Chem. Soc.* **1933**, 395-398.

212. Wynder, E.L.; Hoffman, D. *JAPCA* **1965**,*15*, 155-159.

213. Rosenkranz, H.; McCoy, E.C.; Sanders, D.R.; Butler, M.; Kiriazides, D.K., Mermelstein, R. *Science* **1980**, *209*, 1039-1043.

214. Schuetzle, D.; Lee, F.S.C.; Prater, T.J. *Int. J. Environ. Anal. Chem.* **1981**, *9*, 93-144.

215. Rappaport, S.M.; Wang, Y.Y.; Wei, E.T.; Sawyer, R.; Watkins, B.E.; Rappaport, H. *Environ. Sci. Technol.* **1980**, *14*, 1505-1509.

216. Pitts, J.N.; Lokensgard, D.M.; Harger, W.; Fisher, T.S.; Mejia, V.; Schuler, J.J.; Scorziell, G.M.; Katzenstein, Y.A. *Mutat. Res.* **1982**, *103*, 241-249.

217. Manabe, Y.; Kinouchi, T.; Ohnishi, Y. *Mutat. Res.* **1985**, *158*, 3-18.

218. Harris, W.R.; Chess, E.K.; Okamoto, D.; Remsen, J.F.; Later, D.W. *Environ. Mutagen.* **1984**, *6*, 131-144.

219. Harris, W.R.; Remsen, J.F.; Chess, E.K.; Later, D.W. *J. Toxicol. Environ. Health* **1987**, *20*, 81-103.

220. Matsumoto, H.; Inoue, K. *Arch. Environ. Contam. Toxicol.* **1987**, *16*, 409-416.

221. Nishioka, M.G.; Howard, C.C.; Contos, D.A. *Environ. Sci. Technol.* **1988**, *22*, 908-915.

222. Sera, N.; Kai, M.; Horikawa, K.; Fukuhara, K.; Miyata, N.; Tokiwa, H. *Mutat. Res.* **1991**, *263*, 27-32.

223. Ball, L.M.; King, L.C.; *Environ. Int.* **1985**, *11*, 335-362.

224. Lewtas, J.; King, L.C.; Williams, K.; Ball, L.M.; DeMarini, D. *Mutagenesis* **1990**, *5*, 481-489.

225. Ball, L.M.; Stocking, L.M.; Kohan, M.J.; Warren, S.H.; Lewtas, J. *Mutagenesis* **1995**, *10*, 497-504.

226. Archdeacon, T.J. *Correlation and Regression Analysis: A Historian's Guide*. University of Wisconsin Press, Madison, 1994.

227. Harkov, R.; Lioy, P.J.; Daisey, J.M.; Greenberg, A.; Darack, F.; Bozzelli, J.W.; Kebbekus, B. *J. Air Pollution Control Assoc.* **1986**, *36*, 388-392.

228. Cavalieri, E.; Higginbotham, S.; Rogan, E.G. *Polycyclic Arom. Compounds* **1994**, *6*, 177-183.

Appendix. A Critical Review of Bioassay-Directed Fractionation in Environmental Research.

It would appear that bioassay-directed fractionation was first used in the late 1920s in order to isolate murine carcinogens known to exist in coal tar pitch (211). The fractionation method consisted of fractional distillation, ethanol extraction and recrystallization, while mouse skin-painting experiments were used as a bioassay for the various fractions prepared. Despite the primitive chromatographic and analytical methods available, benzo[a]pyrene was unequivocally identified as the principal murine carcinogen in pitch. Thus, for the first time, a previously unknown compound in a complex mixture was determined to be a carcinogen through the use of a bioassay-directed fractionation protocol. Other milestones in bioassay-directed fractionation of complex environmental mixtures are summarized in Table 65.

Table 65.
Milestones in the bioassay-directed fractionation of complex environmental mixtures.

Research Group	*Matrix*	*Method*	*Mutagen(s) Isolated*	*Comments*
Cook *et al.*, 1933 (211)	coal tar pitch	distillation, EtOH extraction, recrytallization, bioassays with mice	benzo[a]pyrene	first isolation of a new mutagen despite primitive methods
Leiter *et al.*, 1942 (5)	air particulate	benzene extraction, bioassays with mice	none	among the first bioassays of air particulate; no chromatography meant no mutagens isolated
Wynder & Hoffman, 1965 (212)	air particulate	benzene extraction, acid/base/neutral fractionation, alumina chromatography, bioassays with mice & UV/VIS spectroscopy	benzo[a]anthracene & benzo[a]pyrene	primitive compound-class fractionation allowed PAH quantitation for the first time
Rosenkranz *et al.*, 1980 (213)	xerographic toner	toluene extraction, RPLC(C_{18} SepPak), bioassays with *S. typhimurium* TA98(-S9), RPLC(cyano column), probe MS	nitropyrene & dinitropyrenes	first use of multi-step fractionation to isolate new mutagens
Schuetzle *et al.*, 1981 (214)	diesel exhaust particulate	dichloromethane extraction, 2 NPLC steps, *S. typhimurium* TA98 (-S9)	1-nitropyrene, 3-nitrofluoranthene, 8-nitrofluoranthene	first isolation of nitroPAH from diesel soot, first major use of GC-MS

Table 65 (Continued)

Research Group	*Matrix*	*Method*	*Mutagen(s) Isolated*	*Comments*
Rappaport, 1980 & Pitts *et al.* 1982 (215,216)	diesel exhaust particulate	solvent extraction, NPLC on silica column, *S.typhimurium* TA98 (-S9)	pyrene dicarboxylic acid anhydride & phenanthropyranone	successful isolation of oxyPAC with low inherent mutagenicity; Rappaport's group failed to detect highly mutagenic nitro- & dinitroPAH, an example of the possible errors in bioassay directed fractionation
Schuetzle *et al.*, 1985 (65)	diesel exhaust particulate	solvent extraction, fractionation by silica column, followed by 2 NPLC steps; *S.typhimurium* TA98 (-S9), GC-MS	hydroxynitropyrenes, hydroxynitrofluorenes	first indication of mutagenicity of hydroxynitroPAH, but no identification of specific isomers; a common problem with bioassay-directed fractionaction
Manabe *et al.*, 1985 (217)	diesel exhaust particulate	solvent extraction, acid-base-neutral fractionation, RPLC with octadecylsilane column; *S. typhimurium* TA98 (-S9), GC-MS	nitroacetoxypyrenes	first use of RPLC to give a mutation chromatogram. New class of mutagens found, but these compounds haven't been detected in any other reports

Table 65 (Continued)

Research Group	*Matrix*	*Method*	*Mutagen(s) Isolated*	*Comments*
Harris *et al.*, 1984 & 1987 (218,219)	coal fly ash	solvent extraction, NPLC on cyano column, *S.typhimurium* TA98 (-S9), GC-MS	alkylnitro-phenanthrenes	first use of GC retention indices to identify new mutagens
Matsumoto *et al.*, 1987 (220)	air particulate	solvent extraction, fractionation by silica gel, RPLC with octadecylsilane column, *S.typhimurium* TA98(-S9), GC-MS	none	no new compounds; RPLC chromatography too crude to give individual mutagens; mutation chromatograms must be high-resolution to isolate new mutagens
McCalla *et al.*, 1988 (37)	steel foundry dust	solvent extraction, fractionation with Al_2O_3 chromatography, followed by NPLC, RPLC; *S.typhimurium* TA98 (-S9), probe MS	1-nitropyrene & dinitropyrenes	NPLC combined with RPLC gave high resolution mutation chromatograms; first isolation of nitroPAH from foundry dust
Nishioka *et al.*, 1988 (221)	air particulate	as above, except for fractionation with silica and GC-MS instead of probe MS	hydroxynitroPAH	high-resolution GC-MS allowed identification of many trace species, not possible by probe MS

Table 65 (Continued)

Research Group	*Matrix*	*Method*	*Mutagen(s) Isolated*	*Comments*
Tokiwa *et al.*, 1990-91 (176,222)	air particulate	extraction with dichloromethane, silica gel chromatography, RPLC, & *S.typhimurium* TA98 (-S9); GC-MS	dinitrobenzo-[a]pyrene & dinitrofluoranthenes	isolation of new mutagens made possible only through high resol'n RPLC; this can sometimes replace the NPLC cleanup step
Ball & Lewtas, 1985 & 1990 (223,224)	rat & mouse organs	as above	hydroxy-nitropyrenes	as above; showed that bioassay directed fractionation could easily be applied to complex metabolic mixtures
Marvin *et al.*, 1993 (49)	harbour sediment	extraction with dichloromethane, fractionation by alumina, isolation by NPLC and RPLC; *S. typhimurium* TA100 -type strains (+S9), GC-MS	high molecular wt. PAH	among the first methods to separate PAH on basis of molecular weight by HPLC