Mutagenic Particulate Matter in Air Pollutant Source Emissions and in Ambient Air

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Acknowledgments

I came with visions of the Lorax, "to speak for the trees,"

And seven years later I leave on a wonderful Rocky mountain breeze,

Still yearning to have a voice for those trees.

Much work has been done,

And looking back, some even fun.

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One last word for those who read on,

Make sure your chair is comfy, but try not to yawn.

Abstract

Procedures are developed and demonstrated that can be used to investigate biological changes caused by fine particulate air pollution exposure, and to focus attention on the origin of those chemical components in the ambient fine particle mixture that are capable of causing genetic damage.

Ambient fine particulate matter samples were collected throughout 1993 at four urban sites in Southern California, and at an upwind background site on San Nicolas Island. No systematic seasonal or spatial variation in mutagenic potency was observed in the urban ambient air samples. This suggests that the mutagenicity of the ambient particulate matter is due to ubiquitous primary emissions sources and that if atmospheric chemical reactions contribute important atmospheric mutagens then these reactions must proceed in the winter as well as in the photochemically more active summer season. The mutagen density in Los Angeles urban air was 1 order of magnitude greater that at the background site, showing that the city is indeed a source of mutagenic organic chemicals.

The human cell mutagenicity of the 1993 Los Angeles area particulate matter was examined by bioassay-directed chemical analysis. A composite consisting of a portion of all the urban filter samples was created and separated into fractions of varying polarity. Subfractions containing the ordinary polycyclic aromatic hydrocarbons (PAH) were responsible for a large portion of the ambient human cell mutagenic potency. Six ordinary PAH (cyclopenta[*cd*]pyrene, benzo[*a*]pyrene, benzo[*ghi*]perylene, benzo[*b*]fluoranthene, indeno[1,2,3-*cd*]pyrene, and benzo[*k*]fluoranthene)

accounted for most of the mutagenic potency assigned to specific compounds. Important semipolar mutagens that were identified include 2-nitrofluorathene and 6*H*-benzo[*cd*]pyren-6-one.

The contribution of specific particle emissions sources to the 1993 Los Angeles area airborne fine particle organic compound mass concentration was determined using a chemical mass balance receptor model based on organic compound tracers. These source contributions are used with measurements of the mutagenic potencies of the primary particles emitted from sources in the Los Angeles area to predict the mutagenic potency that would prevail if those primary particle emissions were transported without further chemical reaction. The predicted mutagenicity of the calculated source mixture was statistically indistinguishable from that of the actual atmospheric sample modeled.

Table of Contents

Chapter 1

Introduction 1

Motivation 1

Research Objectives and Approach 4

Outline of the Thesis Research 5

References 10

Chapter 2

Bacterial Mutagenicity of Urban Organic Aerosol Sources in Comparison to Atmospheric Samples 14

Introduction 14

Experimental Section 16

Source Samples 16

Ambient Samples 23

Extraction and Concentration 24

Bacterial Bioassay 27

Results 29

Mutagenicity of Source Samples in Bacterial Assay 29

Mutagenicity of Ambient Samples in Bacterial Assay 39

Comparison of Source and Ambient Aerosol Mutagenicity 42

Conclusions 46

References 48

Chapter 3

Seasonal and Spatial Variation of the Bacterial Mutagenicity of the Fine Organic Aerosol in Southern California 56

Introduction 56

Experimental Section 58

Airborne Particle Samples 58

Extraction and Concentration 63

Bacterial Bioassay 64

Results and Discussion 65

Organic Carbon Measurements 65

Mutagenicity of Ambient Samples 68

Summary and Conclusions 80

References 83

Chapter 4

Seasonal and Spatial Variation of the Human Cell Mutagenicity of the Fine Organic Aerosol in Southern California 91

Introduction 91

Experimental Section 94

Sample Collection 94

Extraction and Concentration 98

Human Cell Mutation Assay 99

Results and Discussion 100

Fine Particulate Matter Measurements 100

Mutagenicity of Ambient Samples in the Human Cell Assay . . . 104

References 118

Chapter 5

Bioassay-Directed Chemical Analysis of Los Angeles Airborne Particulate Matter Using a Human Cell Mutagenicity Assay **125**

Introduction 125
Experimental Section 129
Sample Collection 129
Extract Preparation 131
Human Cell Mutation Assay 132
Fractionation 134
Chemical Analysis 139
Human Cell Assay Results 141
Unfractionated (Whole) Sample Extract 141
Fractionated Sample Extracts 142
Subfractionated Sample Extracts 145
Chemical Analysis Results 149
Aromatic Nonpolar 1 149
Aromatic Nonpolar 2 157
Aromatic Semipolar Subfractions 160
Aliphatic Semipolar Subfraction 162
Aromatic Polar Subfraction 163
Aliphatic Polar Subfraction 163
Discussion 164
References 167

Chapter 6

Source Contributions to the Human Cell Mutagenicity of Urban Particulate Air Pollution 172

Introduction 172

Experimental Section 175

Ambient Samples 175

Source Samples 177

Extract Preparation 180

Human Cell Mutation Assay 181

Bacterial Mutation Assay 182

Chemical Analysis 183

Chemical Mass Balance Model 184

Results 188

Apportionment of Fine Particulate Organic Mass 188

Apportionment of Ambient Particulate Mutagenicity 200

Conclusions 204

References 207

Chapter 7

Summary and Conclusions 214

Summary of Results 214

Recommendations for Future Research 220

List of Tables

2.1 Fine aerosol emissions within the area surrounding Los Angeles for 1982 as used to produce emissions-weighted average values of specific mutagenicity of composite primary sources . . . 17

2.2 Bacterial mutation results for the source effluents and ambient aerosol samples 30

3.1 Summary of bacterial mutation assay dose-response curve characterization 72

5.1 Organic compounds quantified in the mutagenic subfractions and individual compound mutagenic potency contribution to whole sample determined 150

5.2 Targeted semipolar compounds sought in SIM mode that are either found in subfractions other than the mutagenic subfractions shown in Table 5.1, or that are not found at all 158

6.1 Summary of bioassay results, both human cell and bacterial, for the particle emission sources 178

6.2 Summary of source contributions to fine ambient organic aerosol annual average mass concentration and mutagen density in the Los Angeles area during 1993 197

List of Figures

2.1 Bacterial mutagenicity dose-reponse curves for vehicle exhaust source samples 31

2.2 Bacterial mutagenicity dose-reponse curves for stationary combustion source samples 33

2.3 Bacterial mutagenicity dose-reponse curves for fireplace wood combustion source samples 35

2.4 Bacterial mutagenicity dose-reponse curves for fugitive dust and other noncombustion source samples 38

2.5 Bacterial mutagenicity dose-reponse curves for ambient aerosol quarterly composite samples in relation to emissions-weighted source sample composites 41

3.1 The 1993 particulate air pollution sampling network locations and the annual average fine particulate organic carbon (OC) concentration observed at each location 59

3.2 The seasonal variation of fine particulate OC concentration at each sampling site 67

3.3 The spatial variation of fine particulate OC concentration during the summer and winter 69

3.4 Bacterial mutagenicity dose-response curves for each ambient sample studied here 71

3.5 Seasonal variation, at each sampling location, of the bacterial mutagenic potency (mutagenicity per mass of airborne particulate OC given to the assay) 74

3.6 Spatial variation of the 1993 annual average bacterial mutagenic potency and bacterial mutagenic density 76

3.7 Seasonal variation, at each sampling location, of the bacterial mutagenic density (mutagenicity per mass of airborne particulate OC given to the assay) 78

4.1 The 1993 particulate air pollution sampling network locations and the annual average fine particulate organic carbon (OC), elemental carbon (EC), and total mass concentrations observed at each location 95

4.2 The seasonal variation of fine particulate OC, EC, and total mass concentrations at each sampling site 103

4.3 Human cell mutagenicity dose-response curves for each ambient urban sample studied here 106

4.4 Seasonal variation, at each sampling location, of the human cell mutagenic potency (mutagenicity per mass of airborne particulate OC given to the assay) 108

4.5 Spatial variation of the 1993 annual average human cell mutagenic potency and human cell mutagenic density . . . 111

4.6 Human cell mutagenicity dose-response curve for the San Nicolas Island, background air monitoring station, sample 115

4.7 Seasonal variation, at each sampling location, of the human cell mutagenic density (mutagenicity per mass of airborne particulate OC given to the assay) 117

5.1 HPLC solvent program used in the normal phase column fractionation procedure along side the elution cutpoints that define the various sample fractions and subfractions studied. Also shown is the typical compounds found in each of the 4 fractions . . 135

5.2 Flow chart for the bioassay-directed chemical analysis . . 138

5 3 Human cell mutagenicity dose-response curves for the whole sample extract, each of the 4 fractions created at the first separation step, and each of the subfractions created at the second fractionation step 143

5.4 Demonstration of the reduction of interfering background compounds obtained through sample fractionation at various levels 148

6.1 Source profiles used in the chemical mass balance model for gasoline-powered vehicle exhaust and natural gas combustion particles 189

6.2 Source profiles used in the chemical mass balance model for diesel vehicle exhaust and wood combustion particles . . 190

6.3 Source profiles used in the chemical mass balance model for paved road dust and vegetative detritus 191

6.4 Source profiles used in the chemical mass balance model for tire wear debris and cigarette smoke 192

6.5 Source profile used in the chemical mass balance model for meat cooking aerosol 193

6.6 Comparison of model predictions to measured ambient concentrations for the chemical mass balance compounds . 195

6.7 Relative source contribution to modeled ambient concentrations of organic compounds in the mass balance model 201

Chapter 1

Introduction

Motivation

The smoking-adjusted risk of lung cancer among urbanites has been found to be up to 1.5 times that of people who live in rural areas *(1)*. This is but one of the many types of human health effects that are thought to be related to exposure to airborne fine particulate matter; other associated health problems include increases in mortality from cardiovascular or respiratory failure on days of high fine particle levels, as well as increases in asthma and bronchitis rates *(2)*. While knowledge of how the complex human system deals with air pollutant exposure is still incomplete, it is becoming clear that serious health risks are present and that fine particulate air pollution is somehow involved.

At present, ambient air quality standards in the United States limit airborne particle mass concentrations in sizes smaller than 10 μm diameter (PM10). The U.S. government recently has announced an intention to restrict airborne particle mass concentration in sizes smaller than 2.5 μm diameter as well, referred to as PM2.5 or fine particles. In both the cases of the PM10 air quality standard and the forthcoming PM2.5 standard, compliance is measured by weighing airborne particle filter samples before and after collection. No consideration is given to the chemical composition of the particle samples.

Urban particulate air pollution contains literally thousands of different organic compounds, many of which are present in trace amounts, and all of which are mixed in the atmosphere with a much larger amount of likely

harmless but bulky material from other sources (e.g., airborne soil dust, sea salt). It is entirely likely that by focusing attention on particle mass concentration, or even the fine particle mass concentration, those chemical components of the particulate air pollution mixture that are actually capable of inducing the sort of biological changes that could affect health will be missed entirely.

A number of studies have been undertaken in the past to identify chemical components of the particulate air pollution mixture that cause the types of biological change that could lead to the lung cancer excess observed by Hemminki et al. *(1)*. In the course of that work, organic extracts of airborne particle samples have been shown to be mutagenic to bacteria *(3-6)*, and carcinogenic to test animals *(7, 8)*. Using bacterial mutagenicity as the biological endpoint, several studies have attempted to find the specific chemical species that cause that biological effect: Wise et al. *(6)* found that a significant fraction of the direct-acting (i.e., assay performed without additional enzymatic activation) bacterial mutagenicity of airborne particle samples is due to nitro-polycyclic aromatic hydrocarbons (nitroPAH), Arey et al. *(9)* found that nitroPAH account for 1-8% of the direct-acting bacterial mutagenicity of atmospheric particle samples collected in Southern California, and Helmig et al. *(10)* concluded that a nitro-polycyclic aromatic compound (nitroPAC) accounted for ~45% of the direct-acting bacterial mutagenicity of airborne particle samples collected in Southern California. To further search for the cause of the observed mutagenicity of particulate air pollution samples, several particulate matter emission sources also have been studied. When measuring diesel engine exhaust, Nishioka et al. *(11)* found that nitroPAC accounted for 20-25%

of the direct-acting bacterial mutagenicity, while Salmeen et al. *(12)* found that mono- and di-nitroPAH accounted for 30-40% of the direct-acting bacterial mutagenicity. Grimmer et al. *(13-15)* studied several emission sources using carcinogenic effects in mice as a biological endpoint and found that ordinary PAH with more than 3 rings (not nitroPAH) accounted for the total activity. Using human cell mutation assays, Skopek et al. *(16)* found that 8% of the observed mutagenicity of kerosene soot was due to a single PAH - cyclopenta[*cd*]pyrene, and Barfknecht et al. *(17)* report that a significant fraction of the mutagenicity of diesel engine exhaust is due to another PAH - fluoranthene.

Determining the effect of specific air pollution sources on the biological activity of ambient air pollution samples based on the existing scientific literature is difficult at best. Several biological endpoints have been used, not to mention that large differences exist in the procedures used by different laboratories to collect and analyze air pollution samples. That these differences between laboratories can have a significant effect on the outcome was shown in a study by the International Programme on Chemical Safety (IPCS) *(18-20)*, which found that 55-95% of the variability seen in the bacterial mutagenicity of three environmental mixtures tested could be accounted for by between-laboratory variations in procedures rather than by actual sample differences. In addition, few air pollution emission source types have been analyzed through bioassay systems to date with much of the work being focused on diesel engine exhaust.

In order to better understand the biological activity of the emissions from specific air pollution sources in relationship to atmospheric samples, additional research is required. Such a study must apply both biological testing and

chemical analysis procedures to a comprehensive set of urban particulate emission source samples and a comprehensive set of urban particulate air pollution (receptor) samples. These source and atmospheric samples must be collected by comparable methods and analyzed in the same laboratories in order to avoid changes in analytical procedures between laboratories. New methods must be found for comparing source and atmospheric samples through development and application of air quality modeling methods that connect source emissions to atmospheric concentrations and effects.

Research Objectives and Approach

The main objective of the present research was to determine how major urban air pollution source types contribute to the mutagenicity of atmospheric fine particulate matter. There were three major stages to this work. First, large quantities of representative urban ambient particulate matter were acquired for subsequent biological testing and chemical analysis along with comparable samples from the major urban emission sources of fine particulate air pollution. The sampling strategy was based on a case study of the Los Angeles area. Second, the mutagenicity of these source and ambient samples were measured and chemical analyses were conducted to identify those organic compounds or compound classes that are associated with the mutagenicity observed in the samples. In this work, both bacterial mutation assays and novel human cell mutation assays were used such that the differences in the information gained from these two assay types could be compared. Finally, air quality models were developed that relate the emission sources of urban particulate air pollution to ambient pollutant concentrations in a way that those sources that contribute the

compounds or compound classes determined in step 2 to be associated with the mutagenicity of atmospheric particles could be identified.

Accomplishment of these objectives advanced the understanding of the origins of the mutagenicity of ambient particulate air pollution. The importance of chemical species emitted directly from individual particulate air pollution sources versus those chemical substances that are formed via atmospheric transformations were clarified in relation to both the bacterial and human cell mutation potential of the entire airborne fine particle mixture. This study further provided for measurements and data analyses (including determination of source/receptor relationships) which could be used in the future to estimate the effect on atmospheric mutagenicity levels of certain control strategies involving reduction in the emissions from specific primary particle emissions source types. As newer and more sophisticated biological assays are developed, the present study could serve as a guide to the procedures that could be followed to determine the effect of air pollution source emissions on other similar biological endpoints.

Outline of the Thesis Research

The purpose of this section is to outline the material found in the remaining chapters of this thesis. In Chapter 2, a preliminary study was undertaken to assess the bacterial mutagenicity of fine particulate emission source samples in comparison to urban airborne particle samples. This study was considered preliminary because the results were used to focus the remaining work. Both the emission source and ambient samples examined in Chapter 2 were withdrawn from sample archives collected as part of prior

sampling programs *(21, 22)*. The set of source samples used represented emission source types that account for approximately 80% of the fine particulate organic compound emissions to the Los Angeles area *(22)*, including catalyst-equipped automobile exhaust, noncatalyst automobile exhaust, heavy duty diesel truck exhaust, fuel oil-fired boiler emissions, natural gas home appliance emissions, fireplace combustion of pine, oak, and synthetic logs, cigarette smoke, charbroiled burger smoke, roofing tar pot emissions, paved road dust, tire wear particles, brake lining wear debris, and urban vegetative detritus. Results from the bacterial mutagenicity testing of these source samples were used to dictate which sources are studied in the remaining work, as only source samples which contain bacterial mutagens at or above a specific dose level were selected as the focus of the later work. In addition, limited ambient sample material allowed only an initial investigation of any seasonal (4 seasons) or spatial (2 locations) patterns observable in the bacterial mutagenicity data. The mutagenic potency (mutagenicity per mass of airborne particulate organic carbon) of the ambient samples was compared to a weighted average of the mutagenic potency of the source samples assembled in proportion to their emission rates in the Los Angeles area. The results of this comparison showed the feasibility of continuing to investigate the source/receptor relationships that govern the mutagenicity of the airborne particle mixture.

The next step in this research involved the collection of a new set of ambient fine particulate air pollution samples during a year-long sampling campaign at four urban locations in the greater Los Angeles area and at one background offshore island location upwind of that city. This year-long

sampling effort took place during 1993 and entailed both organic and inorganic chemical characterization. These samples were utilized in the remaining work.

In Chapter 3, the seasonal and spatial variation of the bacterial mutagenicity of fine particulate air pollution in the Los Angeles area was investigated. Sufficient fine particle mass was collected during the 1993 ambient sampling campaign to allow seasonal comparison at the level of 6 bimonthly composite samples at each of the four urban sampling sites. For the 1993 sampling campaign, the four urban air monitoring sites as well as the background site were selected to represent 5 locations with exposure to characteristically different air pollution source types. Therefore, an investigation of the seasonal and spatial variation of the bacterial mutagenicity can be used to search for evidence of the importance, if any, of primary particle emission sources (e.g. wood smoke) whose effect on air quality generally peaks in the winter months in Los Angeles, versus the products of atmospheric transformation reactions that occur predominantly during the summer photochemical smog season. Since some of the air monitoring stations were located in high traffic neighborhoods while others are in industrial areas or were located at photochemical smog receptor sites, study of the spatial distribution of the aerosol mutagenicity likewise could be used to search for clues as to the relative importance of the various source types.

A study of the seasonal and spatial variation of the human cell mutagenicity of fine particulate air pollution in the Los Angeles area was reported in Chapter 4. This research attempted to accomplish the same objectives as those described in Chapter 3 but with the use of a different biological endpoint, i.e., the generation of human cell mutations instead of

bacterial mutations. As such, this work represented the first use of a human cell mutation assay to study atmospheric samples from a network of community air monitoring stations. A comparison of the results generated from these two bioassay systems when applied to the same atmospheric samples also was reported here.

In order to determine the chemical compounds or compound classes that were responsible for the mutagenicity seen in fine particulate air pollution samples, bioassay-directed chemical analysis of a year-long composite Los Angeles area fine particulate air pollution sample was undertaken. Bioassay-directed chemical analysis is a technique which involves chemical separation (or fractionation) of a sample by a coherent scheme that results in the isolation of a portion of the organic compounds from the original complex sample within a fraction of that original sample containing a, hopefully, small group of compounds of similar polarity or chemical structure. The resultant fractions are tested for mutagenic potency in a bioassay. Then further chemical analysis is focused on only those fractions in which the mutagenic organic compounds have been isolated. The bioassay-directed chemical analysis of the 1993 Los Angeles composite fine particulate air pollution sample using a human cell mutation assay was reported in Chapter 5. This study presented the general chemical character of the fractions in which the mutagenicity was isolated and then proceeded to identify and quantify as many of the suspected mutagenic organic compounds as is practical by gas chromatography with mass spectrometry.

In Chapter 6, a previously developed atmospheric modeling technique which uses organic compounds as tracers *(23)* was used to determine the

primary particle emission source contributions to the ambient fine particle mass concentration in the Los Angeles area atmosphere during calendar year 1993. These emission source contributions were used along with measurements of the mutagenic potencies of the primary particles emitted from relevant emissions sources in the Los Angeles area to predict the mutagenic potency that would prevail if those primary particle emissions were transported without further chemical reaction. Both the human cell and bacterial mutation assays were employed in this analysis such that the results for these two biological endpoints could be compared.

Finally, in Chapter 7, the major accomplishments and important results of this study were summarized. Areas that warrant future research were discussed.

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Chapter 2

Bacterial Mutagenicity of Urban Organic Aerosol Sources in Comparison to Atmospheric Samples

Introduction

Particulate organic compounds are emitted to the urban atmosphere from a wide variety of air pollution sources. There are fossil fuel combustion sources, both stationary and mobile, including industrial boilers, home heaters, and gasoline- and diesel-powered vehicles. Their effluents are mixed in the atmosphere with fugitive dusts that contain organic compounds, including paved road dust, tire wear debris, and brake lining wear particles. Domestic activities such as food cooking operations (e.g., charbroiling of meat), fireplace combustion of wood, and even cigarette smoke add aerosol carbon emissions to the atmosphere.

The resulting atmospheric mixture of directly emitted organic aerosol thus consists of small contributions from a large number of sources. Each of these source effluents in turn consists of a complex mixture of organic compounds. Many of the individual chemical compounds, of greatest interest because of their possible mutagenic or carcinogenic potential, are present in small quantities such that their identification is made very difficult by the presence of

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Reference: Hannigan, MP; Cass, GR; Lafleur, AL; Longwell, JP; Thilly, WG *Environ Sci Technol*, 1994, 28, 2014-2024.

the complicated chemical matrix consisting of hundreds of more abundant but less hazardous substances that are found in most environmental samples.

Biological assay procedures have been developed for use as screening tools that help to focus further investigation on those contributors to complex environmental samples that are capable of producing measurable biological changes. Bacterial mutagenicity assays *(1, 2)* often are used at an early stage in such a screening program and can be followed by mutagenicity studies conducted in human cells *(3)* and in test animals *(4)*. Through such procedures it has been shown that particulate matter filtered from ambient air is a bacterial mutagen *(5-7)* and that certain direct emission sources of organic aerosol, including diesel engine exhaust *(8)*, wood smoke *(9-11)*, and cigarette smoke *(12, 13)*, likewise contain mutagenic compounds. Bioassay-directed chemical analysis procedures have been developed that use bacterial mutagenicity assays in conjunction with chemical separation and analysis procedures to identify the chemical compounds within complex samples that are responsible for the observed mutagenic response *(14-19)*. Such bioassay-directed chemical analysis also has been developed as a tool for studying the atmospheric transformations that create or destroy mutagenic compounds due to atmospheric reactions *(20)*.

Comparison of the relative mutagenicity of air pollution sources and ambient samples based on the present scientific literature is difficult because differences in the procedures used by different laboratories can mask actual differences between different pollutant source effluents. A recent collaborative study by the International Programme on Chemical Safety (IPCS) *(21-23)*, a subgroup of the World Health Organization (WHO), found 55-95% of the

variability seen in the mutagenicity of three environmental mixtures tested could be accounted for by between-laboratory variations in procedures rather than by actual differences in the samples. The ability to compare source and ambient aerosol mutagenicity results reported in the literature is further limited by the fact that no uniformity exists in methods for sample collection, storage, and extraction.

In this paper, we report a study of the bacterial mutagenicity of a comprehensive set of urban particulate air pollution source samples. Fifteen major source types are examined that directly account for approximately 70% of the primary organic aerosol emissions to the Los Angeles area as described by Hildemann et al. *(24)*. Comparison is made to the mutagenicity of ambient particulate samples collected by Gray et al. *(25)* by methods similar to the source sampling procedures. Through careful matching of source and ambient sampling methods and by subjecting all samples simultaneously to identical extraction and bioassay procedures in the same laboratory, variations in test results due to changes in methods have been minimized. The bacterial mutation assay used in this study is a version of the *Salmonella typhimurium* forward mutation assay developed by Skopek et al. *(2)*.

Experimental Section

Source Samples

The aerosol source samples used in the present study were collected by Hildemann et al. *(24)* from 15 sources types that collectively represent about 70% of the fine organic aerosol emissions to the atmosphere in the Los Angeles area. The source types tested are listed in Table 2.1. A portable dilution source

Table 2.1. Contribution of Sources to Fine Aerosol Organic Carbon Emissions within the 80 x 80 km Area Surrounding Los Angeles for 1982 As Used To Produce Emissions-Weighted Average Values of Specific Mutagenicity of Composite Primary Sources.

source type	% of OC emitted[a]	profile no. of source tested in this study	profile assigned to estimate specific mutagenicity of sources not tested
(1) meat-cooking operations			
charbroiling	16.6	1	
(2) paved road dust	15.9	2	
(3) fireplaces			
pine wood	11.2	3	
oak wood	2.8	4	
synthetic logs	nk[j]	5	
(4) noncatalyst gasoline vehicles			
automobiles	7.0	6	
other vehicles[b]	3.7		6
(5) diesel vehicles			
heavy-duty trucks	4.2	7	
other vehicles[c]	2.1		7
(6) surface coating	4.8		
(7) forest fires	2.9		
(8) cigarettes	2.7	8	
(9) catalyst-equipped gasoline vehicles			
automobiles	2.6	9	
other vehicles[d]	0.3		9
(10) organic chemical processes	2.3		
(11) brake lining dust	2.3	10	
(12) roofing tar pots	1.9	11	
(13) tire wear	1.4	12	
(14) misc. industrial point sources	1.3		
(15) natural gas combustion			
residential/commercial	0.1	13	
other sources[e]	0.9		13
(16) misc. petroleum industry processes	1.0		
(17) primary metallurgical processes	0.8		
(18) railroad (diesel oil)	0.7		7
(19) residual oil stationary sources[f]	0.7		14
(20) refinery gas combustion	0.7		13
(21) secondary metallurgical processes	0.6		
(22) mineral industrial processes	0.5		
(23) other organic solvent use	0.4		
(24) jet aircraft	0.3		
(25) asphalt roofing manufacturing	0.3		11
(26) coal burning	0.3		
(27) wood processing	0.2		

Table 2.1 (continued)

source type	% of OC emitted[a]	profile no. of source tested in this study	profile assigned to estimate specific mutagenicity of sources not tested
(28) residual oil-fired ships	0.2		
(29) structural fires[g]	0.2		3
(30) distillate oil stationary sources			
industrial	0.04	14	
other[h]	0.08		14
(31) vegetative detritus	nk	15	
(32) other sources[i]	0.8		6,7,13,14

[a] Total OC emitted equals 29822 kg/day; for more detailed description of the procedures used to construct the emission inventory, see Hildemann et al. *(24)*. [b] Noncatalyst light trucks, medium trucks, heavy-duty trucks, off-road gasoline vehicles, and motorcycles. [c]Diesel autos, diesel light trucks, and off-road diesel vehicles. [d] Catalyst-equipped light and medium trucks. [e] Electric utility boilers NG, electric utility turbines NG, refineries NG, industrial boilers NG; NG = natural gas used. [f] Electric utility boilers burning residual oil, refineries burning residual oil, industrial boilers burning residual oil, residential/commercial combustion of residual oil. [g] Structural fires are assumed to show a similar organic profile as found for pine wood combustion in residential fireplaces. [h] Residential/commercial distillate oil combustion. [i] Other sources include: diesel powered ships, electric utilities burning distillate oil, stationary source LPG combustion, industrial internal combustion engines using gasoline. [j] nk = not known.

sampling system designed by Hildemann et al.*(26)* was used. In a dilution sampler, hot exhaust emissions are mixed with purified dilution air in order to cool the sample to ambient conditions, thus allowing certain vapor-phase organics found in the exhaust of hot combustion sources to condense onto existing aerosol within the sampling system as would normally occur in the atmospheric plume downwind of the source.

A diagram of the dilution source sampler used here is presented by Hildemann et al. *(26, 24)*. Exhaust emissions are withdrawn from the source through a cyclone separator that removes coarse particles that are greater than 10 μm in diameter. The source effluent then flows into the dilution sampler through a heated Teflon inlet line. The source emissions next are diluted nominally 50-fold with purified dilution air in the stainless steel dilution tunnel. The purified dilution air is slightly cooled so that the mixture of the hot emissions plus dilution air can be brought to ambient temperature. The dilution air is purified by passage through an activated carbon bed and a HEPA filter. Approximately 10% of the cooled and diluted emissions then are withdrawn into a stainless steel residence time chamber where condensable organics in the exhaust gases are given sufficient time to condense into the aerosol phase. Samples are then drawn from the residence time chamber through AIHL-design cyclone separators *(27)*, which remove particles with aerodynamic diameters greater than 2 μm. There are six of these cyclone separators connected in parallel, each drawing air at a rate of 27.9 ± 0.3 L/min. Three parallel filter holders are located downstream of each cyclone. Of these 18 filter holders, two contain Teflon filters used for bulk chemical analysis while the other 16 filter holders contain quartz fiber filters (47-mm diameter, Pallflex Tissuquartz 2500

QAO). One of these quartz filters during each source test was used to determine the organic carbon (OC) and elemental carbon (EC) mass emission rate from the source by the thermal evolution and combustion method of Huntzicker and co-workers *(28, 29)*. The other 15 quartz fiber filters were used for detailed organic chemical analysis.

The remaining 90% of the source effluent that does not enter the residence time chamber of the source sampling system is drawn through a high-volume filter holder at the end of the sampling train. That filter holder contained a 25.4 cm by 20.3 cm quartz fiber filter (Pallflex Tissuquartz 2500 QAO). Samples collected on this high-volume filter were used for the bioassay program described in the present paper. While the aerosol collected on the high-volume filter nominally represents a sub-10-μm size cut established by the in-stack cyclone, size distribution measurements made in the dilution tunnel *(30)* show that the aerosol from the combustion sources tested is in fact submicron in size. Therefore, the high-volume filter collection can be compared closely to other measurements of fine aerosol properties taken from the residence time chamber in the sampler.

All quartz fiber filters were prebaked for at least 2 h prior to use at 750 °C to lower their carbon blank. Field blanks also were taken to ensure that there was no contamination of the source sampling system. Source samples were collected during the period 1986-1989. The filters were stored within 2 h of collection in annealed glass jars with solvent-washed Teflon lid liners at –25 °C in the dark until later analysis. The filters were extracted in 1991, the extracts stored at –80 °C in the dark, and bacterial assays were completed in less than 1 month following filter extraction.

The four nonexhaust samples (brake lining wear particles, tire wear debris, paved road dust, and urban vegetative detritus) were collected by a grab sampling technique *(24)*. This technique involves resuspension of bulk aerosol material within a Teflon bag. The aerosol then is withdrawn through 2-µm size cut cyclone separators followed by the series of 47-mm diameter filters described previously. Cigarette smoke and roofing tar pot effluents were sampled using abbreviated versions of the dilution sampling system as described by Hildemann et al. *(24)*. In these six cases, a composite of samples collected on several 47-mm diameter prebaked quartz fiber filters was used for the bioassays reported here.

The source sampling system program is described in detail by Hildemann et al. *(26, 24)*; only a brief description of the sources tested will be given here. The sampling strategy focuses on obtaining representative samples that show major differences that exist between sources of different types. Variability between sources of the same type is addressed by compositing samples from several sources (e.g., may different motor vehicles, different plant species, different cigarette brands) before extraction and bioassay. The fuel oil-fired boiler was a Babcock and Wilcox dual FM-type industrial scale watertube boiler burning no. 2 fuel oil and operating at 60% capacity in steady-state mode. The natural gas home appliances consisted of a space heater (Western Gravity Model 8G100) and a water heater (American Standard Model G-531-H) connected to a common exhaust duct. Fireplace smoke samples were taken while burning wood in a residential undampered brick fireplace. Smokes from the combustion of three different woods [seasoned pine, seasoned oak, and a synthetic log (Pine Mountain Brand)]

were collected while sampling from the chimney of this fireplace. The catalyst-equipped automobile exhaust sample consisted of a composite of the exhaust aerosol from seven automobiles. Each was sampled while undergoing the cold start Federal Test Procedure (FTP) urban driving cycle. The noncatalyst automobile exhaust sample was a composite of the exhaust aerosol from six automobiles that were tested over the same driving cycle as the catalyst-equipped automobile. Particulate matter collected from two 1987 model heavy-duty diesel trucks operated on a chassis dynamometer was composited to form the heavy-duty diesel truck sample. Organometalic brake lining wear particles were obtained from the dust of the rear brake drums of a light-duty truck. The tire wear debris sample came from particles shed by a 195/60R15 Toyo tire that was operated on a rolling resistance test stand. Paved road dust was obtained by sweeping Pasadena, CA, area streets using a vacuum sweeper truck. The roofing tar pot sampled contained petroleum-based builtup asphalt, GAF brand. The cigarette smoke sample consisted of the sidestream and exhaled smoke from several different types of cigarettes composited in proportion to their market shares. The charbroiled meat smoke sample was taken while cooking regular (21% fat) hamburger meat over a natural gas charbroiler in a commercial scale kitchen. The urban vegetative detritus sample consisted of plant fragments shed when agitating the leaves obtained from a composite of 64 species of plants that represent the relative abundances of vegetation in the Los Angeles basin *(31, 32)*. Quartz fiber filters were cut or combined to bring the source material supplied to the extraction step of the present study into the range of 1.2-4.7 mg of organic carbon per sample, as determined by combustion analysis of parallel filter samples. Equivalent organic carbon (EOC)

will be defined as this amount of organic carbon present in the source sample prior to extraction as determined by thermal evolution and combustion analysis, a quantity which provides a direct connection back to the carbonaceous aerosol emission rate from each source through the work reported by Hildemann et al. *(24)*.

Ambient Samples

The ambient fine organic aerosol samples used in this study were acquired by Gray et al. *(25)*. A fine particle air-monitoring network was operated at 10 sites in the Los Angeles basin during the year 1982. Ambient samples were taken every 24 h every sixth day for the entire year. In the present study, aerosol samples collected at two of these sites were selected, Long Beach and Azusa. Use of these monitoring stations permits comparisons to be made between an upwind coastal site near an industrial area that is dominated by primary pollutants, Long Beach, and a downwind site that is known for its high levels of photochemical smog, Azusa.

A schematic diagram of the fine aerosol ambient sampler used here is presented by Gray et al. *(25)*. Ambient air is drawn through an AIHL-design cyclone separator, which removes particles with aerodynamic diameters greater than 2.1 μm. Next, the airflow is divided between four parallel filter holders. These four filter holders contain (1) a Teflon filter from which trace metals concentrations are determined by X-ray fluorescence analysis, (2) a nuclepore filter from which ionic pollutant concentrations are determined by ion chromatography, (3) two sequential quartz fiber filters: one for determination of OC and EC mass and one for estimation of possible sampling artifacts, and (4)

another quartz fiber filter for organic chemical analysis and bioassays. The preparation of the quartz fiber filters is the same as was described for the source sampling system. Flow rates through the quartz fiber filters were 10 L/min. OC and EC mass concentrations were determined by the thermal evolution and combustion method of Johnson et al. *(28)* and Huntzicker et al. *(29)* as was used for the source samples. For further details of the ambient sampling program, consult Gray et al. *(25)*.

Ambient filter samples placed in dark storage at –25 °C in 1982 were withdrawn for bioassay evaluation in 1991 as was described previously for the source samples. Half of each of the ambient aerosol filters collected on the fourth filter holder assembly at Azusa and Long Beach were grouped for solvent extraction into four quarter-year composites at each site. These quarterly composites on average contained 0.63 mg of organic carbon per composite as determined from the combustion analysis data of Gray et al. *(25)*, with a range from 0.39 to 1.15 mg of organic carbon per composite.

Extraction and Concentration

The objective of this study is to examine the bacterial mutagenic potential of the samples described previously. Therefore, the sample extraction and concentration procedures used should maximize the transfer of mutagenic organic compounds from the filters to the solvent used in the bioassay. In a study conducted by Jungers and Lewtas *(33)*, two extraction methods (sonication and soxhlet extraction) and six solvents (cyclohexane, dichloromethane, acetone, methanol, toluene, and dimethyl sulfoxide) were tested to determine the effect of solvent extraction procedures on mutagenic

yield. Results of that study indicate that soxhlet extraction in dichloromethane (DCM) will effectively transfer mutagenic organics from filters to solvent.

The extraction and concentration procedure developed for use in the present study was as follows. All glassware was washed sequentially with purified water, glass-distilled methanol, and DCM. In addition, the empty soxhlets were extracted for a minimum of 2 h to reduce their organic contaminant blank. Filter extraction in DCM was conducted for at least 16 h. Each soxhlet contained 40 mL of DCM. Soxhlet extracts were combined so that each extract corresponded to a single source or ambient sample. Concentration of the DCM source extracts down to 1 mL was accomplished in a vacuum centrifuge. The bioassay procedure requires that extracts be exchanged into dimethyl sulfoxide (DMSO). The transfer of the extract from DCM to DMSO can be accomplished in the vacuum centrifuge because DCM will more readily evaporate than DMSO. A total of 100 μL of DMSO was added to the 1-mL DCM extract that contained each sample, and concentration continued in the vacuum centrifuge until the volume of extract was 100 μL. To ensure that the DCM had been entirely removed, a gentle stream of dry N_2 was blown over the extract for 10 min.

The bioassay investigation, which will be discussed later in this paper, showed that certain wood smoke samples were toxic to the bacteria. Much of the toxicity is thought to be due to the presence of certain polar compounds in these smoke samples. Therefore, an aliquot of each fireplace wood smoke sample DCM extract also was fractionated using a gravity-flow cyano column to remove polar organic compounds from the total sample. These gravity-flow columns are based on 6-mL polypropylene syringe bodies, fitted with

polyethylene filters and packed with 1 g of cyanopropyl-bonded packing material. Columns were obtained from Analytichem Int., Harbor City, CA. These columns were cleaned and conditioned by washing with 10-mL volumes of methanol and dichloromethane *(18, 34)*. This allows comparisons to be made between the bioassay results for whole wood smoke extract versus the bioassay results for the nonpolar wood smoke extract.

In addition to the ambient and source samples, blank and control samples were also prepared. These samples included blank filters and filters spiked with the following PAH: (1) benzo[*a*]pyrene and (2) a polynuclear aromatic hydrocarbon mix which consisted of acenaphthene, acenaphthylene, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene. The blank samples were subjected to the bioassay to control for possible contamination, and the samples of the known mutagen benzo[*a*]pyrene were used in the bioassay to confirm the efficiency of the procedures used. All sample extracts were stored at -80 °C between the time of extraction and testing.

The DCM used was distilled in glass from Caledon Laboratories Ltd., Lot No. 01063B. The soxhlets were 50-mL flask type from Reliance Glass Works. The thimbles used in the soxhlets were from Kontes Scientific Glassware, No. 292100-000. The vacuum centrifuge used was a Savant environmental speed vac, Model ESC2000.

Bacterial Bioassay

The bacterial bioassay used in this study is a miniaturized version of the forward mutation assay in *S. typhimurium*, strain TM677, using resistance to 8-azaguanine as developed by Skopek et al. *(2)*. Under normal conditions, *S. typhimurium* strain TM677 will not survive in the presence of 8-azaguanine; however, certain mutations to the genetic code enable the bacteria to develop a resistance to 8-azaguanine. Therefore, if an organic extract given to the bacteria, in the presence of 8-azaguanine, results in the development of colonies in significantly greater number than the number of colonies associated with background mutations, then the extract is considered to be mutagenic, and this mutagenicity can be quantified by colony counts. This bioassay is run under two conditions: with and without further enzymatic activation obtained by adding a post-mitochondrial supernatant (PMS, also referred to elsewhere as S9) preparation containing rat liver enzymes to the bioassay procedure. These enzymes carry out a series of oxidation and conjugation reactions on a wide variety of substrates including PAH and other environmental pollutants. The mutagenicity of many compounds depends on such metabolic 'activation' steps. These two assay procedures will be referred to as +PMS and –PMS, respectively.

Detailed descriptions of the bacterial assay protocols have been given previously *(2, 35)*. To summarize, *S. typhimurium* were suspended in medium in the presence of the sample for 2 h. Three different dilutions of each sample, providing organic contaminant concentrations ranging from 2.4 to 473 μg/mL, were exposed to the bacteria both with and without the presence of 5% (v/v) Aroclor-induced PMS. In light of the small amount of organic material available

from the aerosol samples, a miniaturized version of this procedure was used. The culture volume was reduced from 1 mL to 100 µL *(16)*. Cultures containing PMS had a NADPH-generating system. After 2 h, the reaction was quenched, and aliquots were plated in the presence and in the absence of the 8-azaguanine (at 50 µg/mL). The results from two independent cultures, each plated in triplicate, were averaged to estimate toxicity and mutagenicity of each treatment condition. Colonies were counted after 48 h, and the mutant fraction was determined as the number of colonies formed in the presence of 8-azaguanine divided by the number of colonies formed in its absence multiplied by the dilution factor. If this ratio is greater than that found for untreated concurrent control cultures such that 99% confidence intervals about the assay mean value and its concurrent control do not overlap and if that ratio also exceeds the 95% upper confidence limit of the mutant fraction for the cumulative historical negative control, the test was considered positive. Occasionally the observed variation among replicate assays is less than expected given the inherent numerical variation of the assay *(36)*. In such a case, the higher expected confidence limits are used in determining significance.

Both positive and negative concurrent control assays are performed in parallel with each source sample. The negative concurrent control consists of a dose of 1 µL of pure DMSO. The positive concurrent negative control consists of a dose of 1 µL of 4-nitroquinoline *N*-oxide (10 µg/mL) in the absence of PMS and 1 µL of benzo[*a*]pyrene (2 mg/mL) in the presence of PMS. The mean ± SD of the concurrent negative control mutant fraction in this series of experiments was $(9.0 \times 10^{-5}) \pm (2.5 \times 10^{-5})$, yielding a 95% upper confidence limit very close to the 95% upper confidence limit for the historical negative control (14×10^{-5}).

The mean ± SD of the concurrent positive control mutant fraction in this series of experiments was (390×10^{-5}) ± (210×10^{-5}) in the absence of PMS and (59×10^{-5}) ± (20×10^{-5}) in the presence of PMS. It is also known that the original strain TM677 used in the present bioassay does not lack nitroreductase; 1-nitropyrene (1-NP), 4-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, 1,8-DNP are all potent –PMS mutagens in TM677, which was constructed in our laboratory from one of Ames' deep rough mutants *(2, 35)*.

Results

Mutagenicity of Source Samples in Bacterial Assay

The source samples listed in Table 2.1 were tested in the *S. typhimurium* forward mutation assay. The results are summarized in Table 2.2. Entries indicated by a plus sign are considered to be mutagenic within the context of this assay, having produced a mutant fraction greater than the concurrent negative control such that 99% confidence intervals on both the sample mean response and its concurrent negative control do not overlap and having produced a mutant fraction that exceeds the 95% confidence limits of the historical negative controls (14×10^{-5}). These criteria are sufficiently stringent so that there is great confidence that false positive test results are excluded.

Each of the combustion source samples tested is mutagenic in this bioassay. The relationship between increasing mutant fraction and increasing equivalent organic carbon dose provided to the bioassay for the case of the motor vehicle exhaust samples is shown in Figure 2.1. The mutagenic response of cigarette smoke aerosol also is shown in that figure for reference

Table 2.2 Summary of Bioassay Results, Showing Those Source Effluents and Ambient Aerosol Samples That Tested Positive (+) or Negative (–)[a] in *S. typhimurium* Mutation Assay, with or without PMS.

source samples	without PMS	with PMS
catalyst-equipped automobile exhaust	+	+
noncatalyst automobile exhaust	+	+
heavy-duty diesel truck exhaust	+	+
fuel oil-fired boiler	(+)[b]	(+)
natural gas home appliances	+	+
fireplace, pine smoke	+	(+)
fireplace, oak smoke	+	(+)
fireplace, synthetic log smoke	+	+
cigarette smoke	+	(+)
roofing tar pot	(+)	–
charbroiled burger smoke	–	–
paved road dust	–	–
brake wear dust	–	–
tire wear dust	–	–
urban vegetative detritus	–	–

ambient samples	without PMS	with PMS
Azusa: Jan, Feb, Mar	+	+
Azusa: Apr, May, Jun	+	+
Azusa: Jul, Aug, Sep	+	–
Azusa: Oct, Nov, Dec	+	–
Long Beach: Jan, Feb, Mar	+	+
Long Beach: Apr, May, Jun	+	–
Long Beach: Jul, Aug, Sep	NC[c]	+
Long Beach: Oct, Nov, Dec	+	+

[a] A negative result does not mean that the sample is not mutagenic, but rather that the sample is not mutagenic at the doses tested in this study. [b] A test result in parentheses indicates that that test meets the statistical criteria for a positive response but that due to sample toxicity an absolute increase in the number of mutant colonies does not occur as EOC dose is increased. [c] Test was inconclusive; mutagenicity is not interpreted, and the result is designated no call (NC).

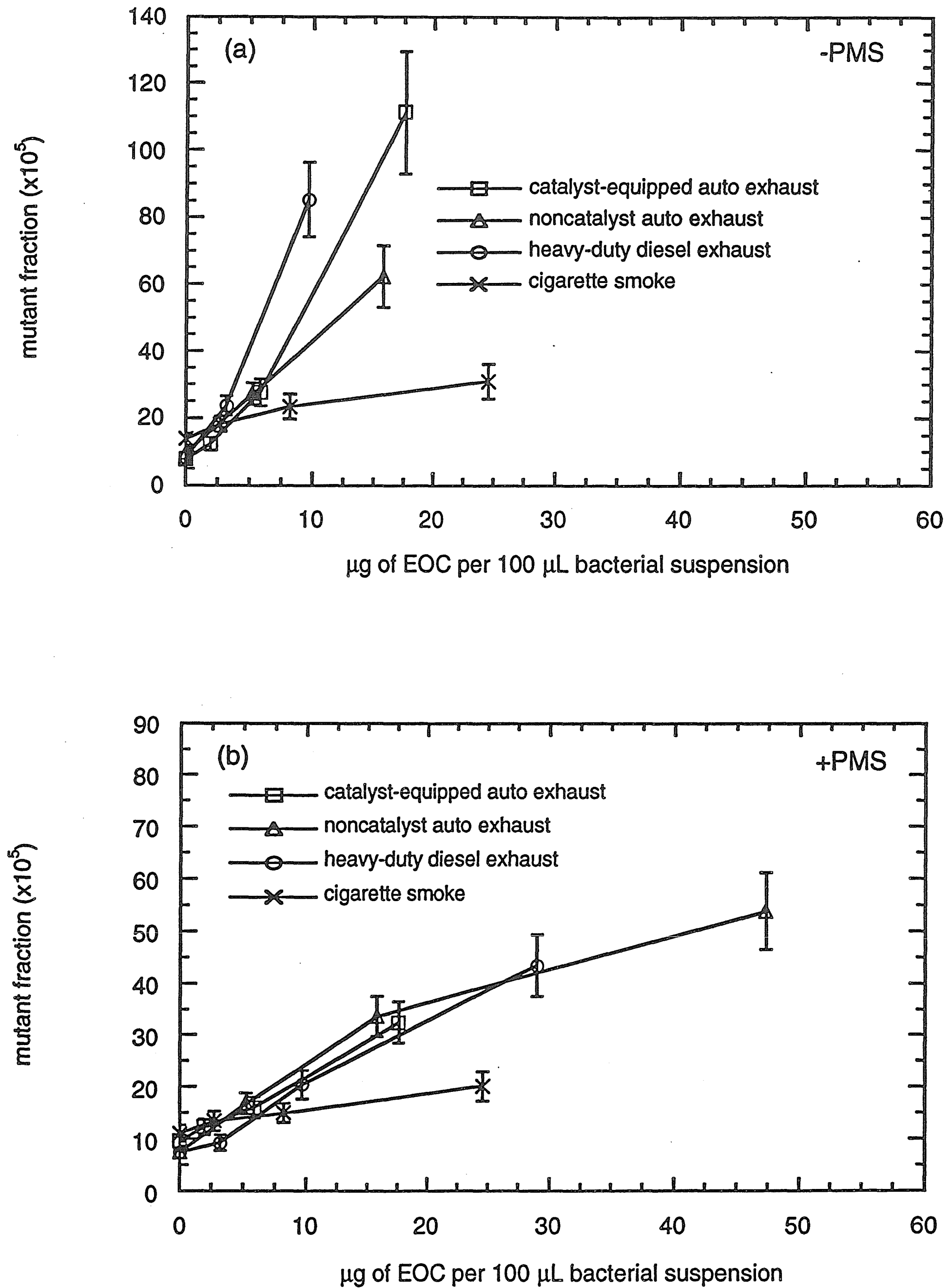

Figure 2.1. Vehicle exhaust sources: (a) dose-response curves without PMS; (b) dose-reponse curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

purposes. The vehicle exhaust samples all are more mutagenic than cigarette smoke per unit of equivalent organic carbon.

Gasoline engine and diesel engine exhaust samples when tested in the Ames assay with strains TA98 and TA100 generally are found to be mutagenic both in the presence and in the absence of PMS *(37-43)* There is considerable variation between studies. The present work suggests that all three vehicle types emit mutagens of comparable activity per mass of organic carbon (OC) emitted. OC emission rates per kilometer driven are higher for the diesel trucks tested in the present study (132.9 mg of OC/km) than for the noncatalyst autos (38.9 mg of OC/km) or for the catalyst-equipped cars (9.0 mg of OC/km). On the basis of mutagenic activity per kilometer driven, the emissions from the diesel trucks would exceed that of the noncatalyst cars which in turn would exceed that of the catalyst-equipped autos.

Stationary fossil fuel combustion sources tested included a distillate oil-fired industrial scale boiler and natural gas-fired home heaters (a space heater plus a water heater). The natural gas home appliance aerosol showed the highest mutagenic response per microgram of EOC of all the sources tested. As seen in Figure 2.2, its specific mutagenicity was about 30×10^{-5} (mutant fraction)/μg of EOC supplied to 100 μL of bacterial suspension –PMS and about 20×10^{-5} (mutant fraction) +PMS. The fuel oil-fired boiler shows a –PMS mutagenicity per microgram of EOC between that of the natural gas aerosol and cigarette smoke and a +PMS mutagenicity per microgram of EOC about equal to that of cigarette smoke, as seen in Figure 2.2. Hildemann et al. *(24)* found that the natural gas-fired home appliances tested emitted 0.0389 μg of OC/kJ of fuel burned while the fuel oil-fired boiler tested emitted 0.450 μg of OC/kJ. Even

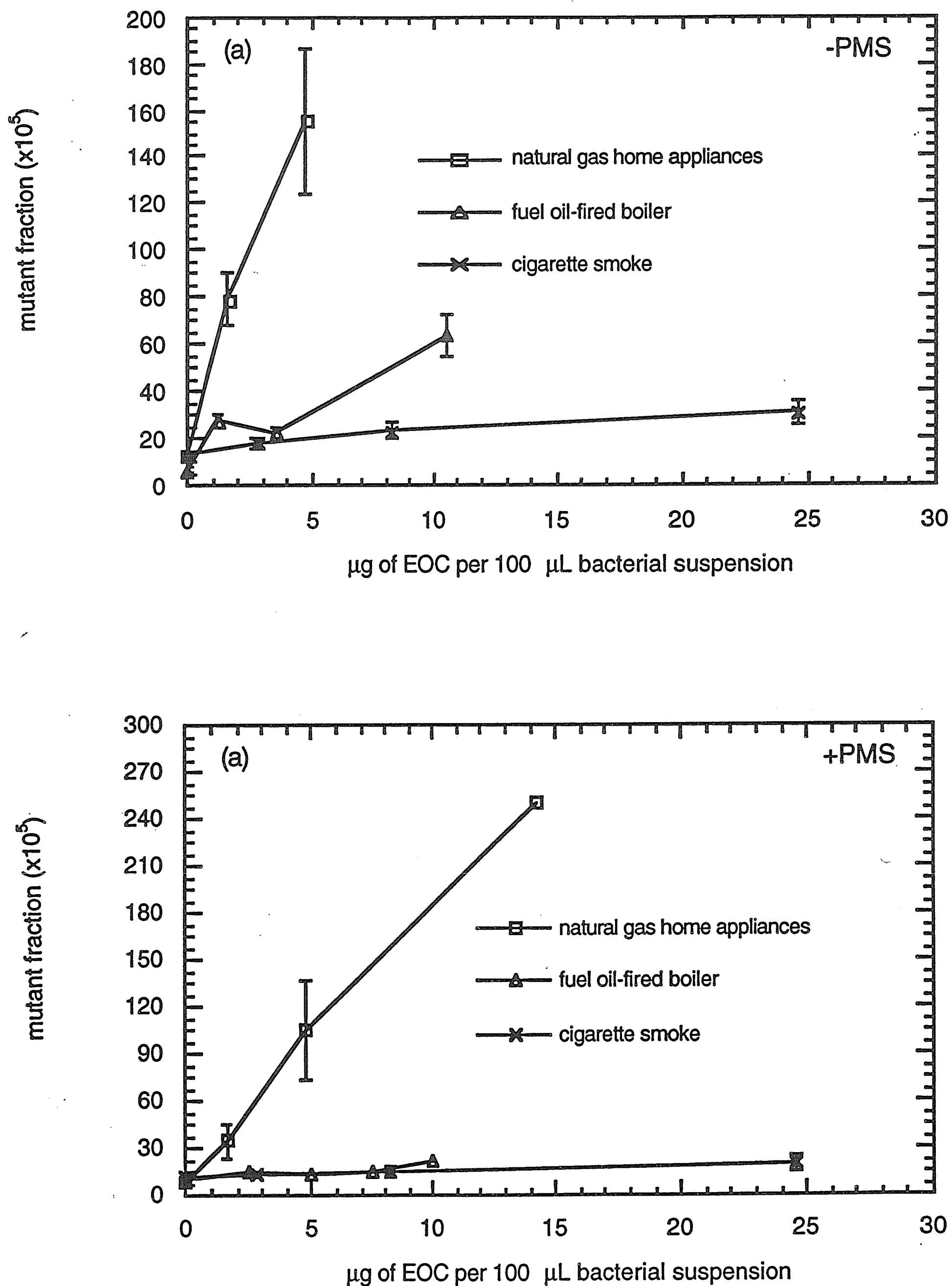

Figure 2.2. Stationary combustion sources: (a) dose-response curves without PMS; (b) dose-reponse curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

though the OC emission rate from natural gas combustors tested is more than an order of magnitude smaller than from the fuel oil-fired boiler, the mutagenicity per unit mass of OC emitted by the natural gas appliances is nearly an order of magnitude higher than from the fuel oil-fired boiler. Thus, it appears that the natural gas home appliances cannot be neglected relative to the oil combustion source even though natural gas combustors are often thought to be particularly "clean" sources.

The mutagenic response of the wood smoke samples tested is shown in Figure 2.3. Each of the whole wood smoke samples is mutagenic in the present bioassay. At the highest levels of EOC supplied, the whole wood smoke samples typically were toxic to the bacteria used in the bioassay. In an attempt to examine a less toxic fraction of the wood smoke, a nonpolar fraction of each source sample was prepared, as described earlier in this text. The nonpolar fractions proved to be less toxic but also less mutagenic than the whole wood smoke samples, seen in Figure 2.3.

Previous studies of the mutagenicity of wood smoke in the Ames assay have been reported by Dasch *(9)*, Kamens et al. *(11)*, Bell et al. *(44)*, McCrillis et al. *(45)*, and Nielsen et al. *(46)*. McCrillis et al. *(45)* compared oak and pine smokes in the Ames assay, strain TA98, and found as we did that oak smoke shows a higher response per microgram of EOC than pine smoke in the absence of PMS, but a lower response in the presence of PMS. Using potassium and lead as source markers for wood smoke and vehicle exhaust, respectively, Lewis et al. *(47)* estimated from ambient air samples that the mutagenic potency of vehicle exhaust was about three times that of wood smoke in the Ames assay, strain TA98 with PMS. In the present study, a direct

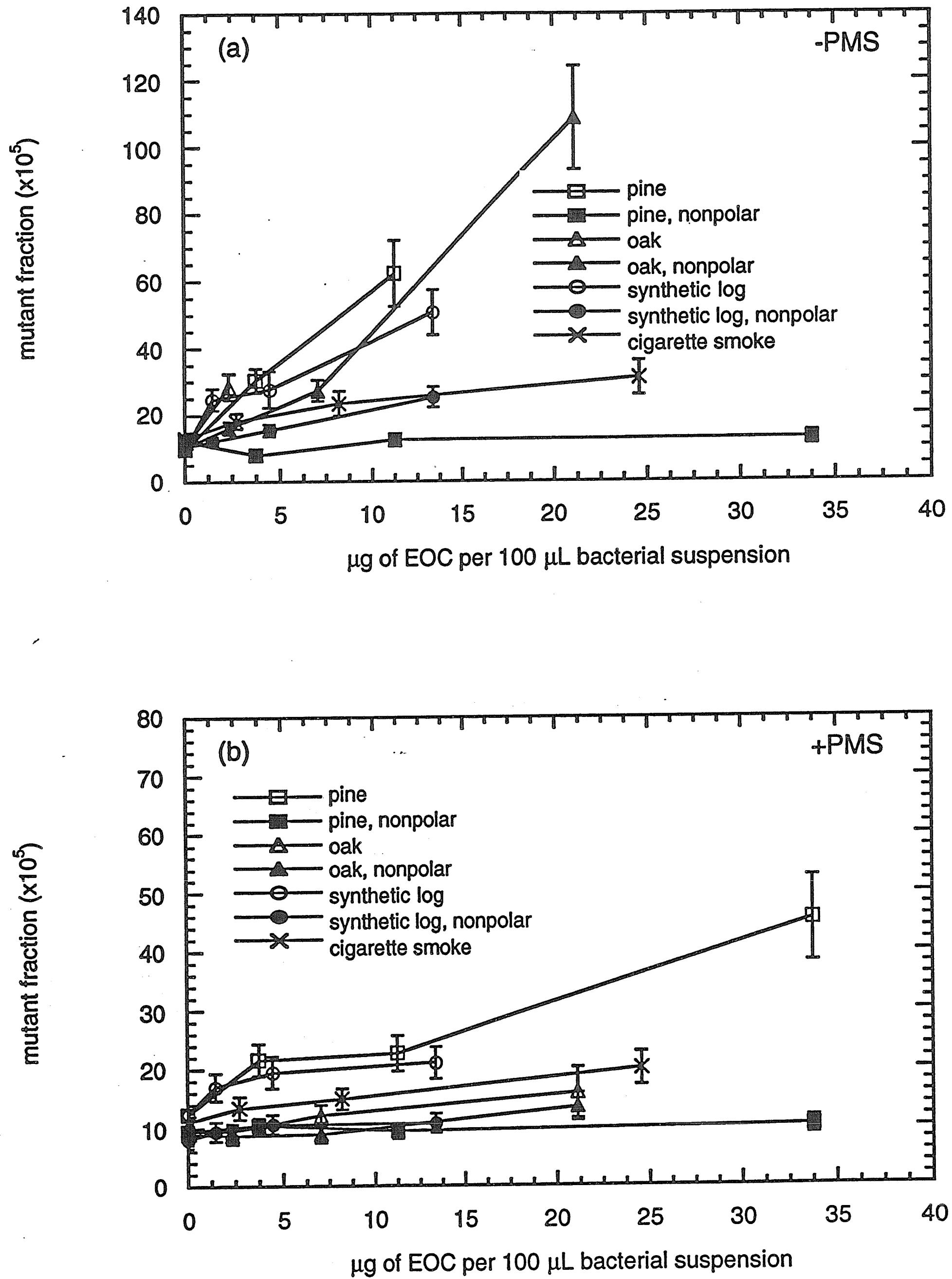

Figure 2.3. Fireplace combustion of wood: (a) dose-response curves without PMS; (b) dose-reponse curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

comparison of wood smoke to motor vehicle exhaust aerosol samples is possible. The pine and synthetic log smokes show mutagenic activity per mass of EOC emitted that is similar to the vehicle exhausts both with and without PMS, while oak smoke shows higher –PMS mutagenicity and lower +PMS mutagenicity than is the case for vehicle exhaust aerosol samples.

Cigarette smoke is a known carcinogen *(48)*. Kier et al. *(12)* and Mizusaki et al. *(13)* have found that cigarette smoke is mutagenic in the presence of PMS using Ames assay, strains TA1538 and TA1535. Albert et al. *(37)*, Claxton et al. *(49)*, and Asita et al. *(50)* have found that cigarette smoke is mutagenic in the Ames assay, strain TA98. The present study also indicates that it is mutagenic in the *S. typhimurium* forward mutation assay both with and without PMS. It is interesting to compare the mutagenic activity of cigarette smoke to that of the other combustion sources which have been discussed previously. Notice in Figures 2.1-2.3 that the dose-response curve for cigarette smoke has been added as a reference. The cigarette smoke shows weaker mutagenic response per mass of EOC than all the vehicle exhaust aerosols, both with and without PMS, and all the whole wood smokes except oak, both with and without PMS. This agrees with previous work. Albert et al. *(37)* found that cigarette smoke is less mutagenic than diesel engine exhaust and noncatalyst automobile exhaust, and Asita et al. *(50)* found that various wood smokes are as mutagenic or more mutagenic than cigarette smoke.

The results of the present forward mutation assay can be compared quantitatively to those of the Ames reverse mutation assay provided that certain differences between the assay procedures are understood. One major difference between the two assays arises from the way that sample toxicity is

addressed. As the dose of a toxic sample supplied to the bacteria increases, the number of surviving bacteria within the assay decreases. This means that fewer surviving mutants will be seen than if the sample was not toxic. The *S. typhimurium* forward mutation assay corrects for this problem by also plating for toxicity and then reporting results as a mutant fraction, i.e., mutants divided by surviving bacteria. Typical Ames reversion assay results are not corrected for survival, and therefore, a typical dose-response curve for an Ames assay of a mutagenic sample starts with a monotonic rising dose-response curve at low doses but then begins to level off as the sample dose becomes toxic. This difference makes a comparison of relative sensitivities of the two assays difficult. Skopek et al. *(51)* compared the two assays by performing both assays with toxicity plates and calculating mutant fractions. In that study, 16 mutagens were tested and a set of five reversion strains were compared to the forward mutation strain TM677. The results was that the forward mutation assay was equisensitive with the most sensitive of any of the set of five Ames tester strains.

Turning to the noncombustion source samples tested, it was found that those samples generally were not mutagenic in the present bioassay. As seen in Figure 2.4, organic extracts of tire wear debris, brake lining wear particles, and paved road dust all show mutagenic responses that fall below the upper confidence limit on the historical negative control (i.e., below a mutant fraction of 14×10^{-5}) both +PMS and –PMS. The asphalt roofing tar pot aerosol sample shows a barely detectable –PMS mutagenic response at the highest EOC dose examined. Albert et al. *(37)* studied roofing tar pot aerosols in the Ames assay, strain TA98, and found them to be about as mutagenic as cigarette smoke. This result is similar to the results obtained in the current study. The charbroiled

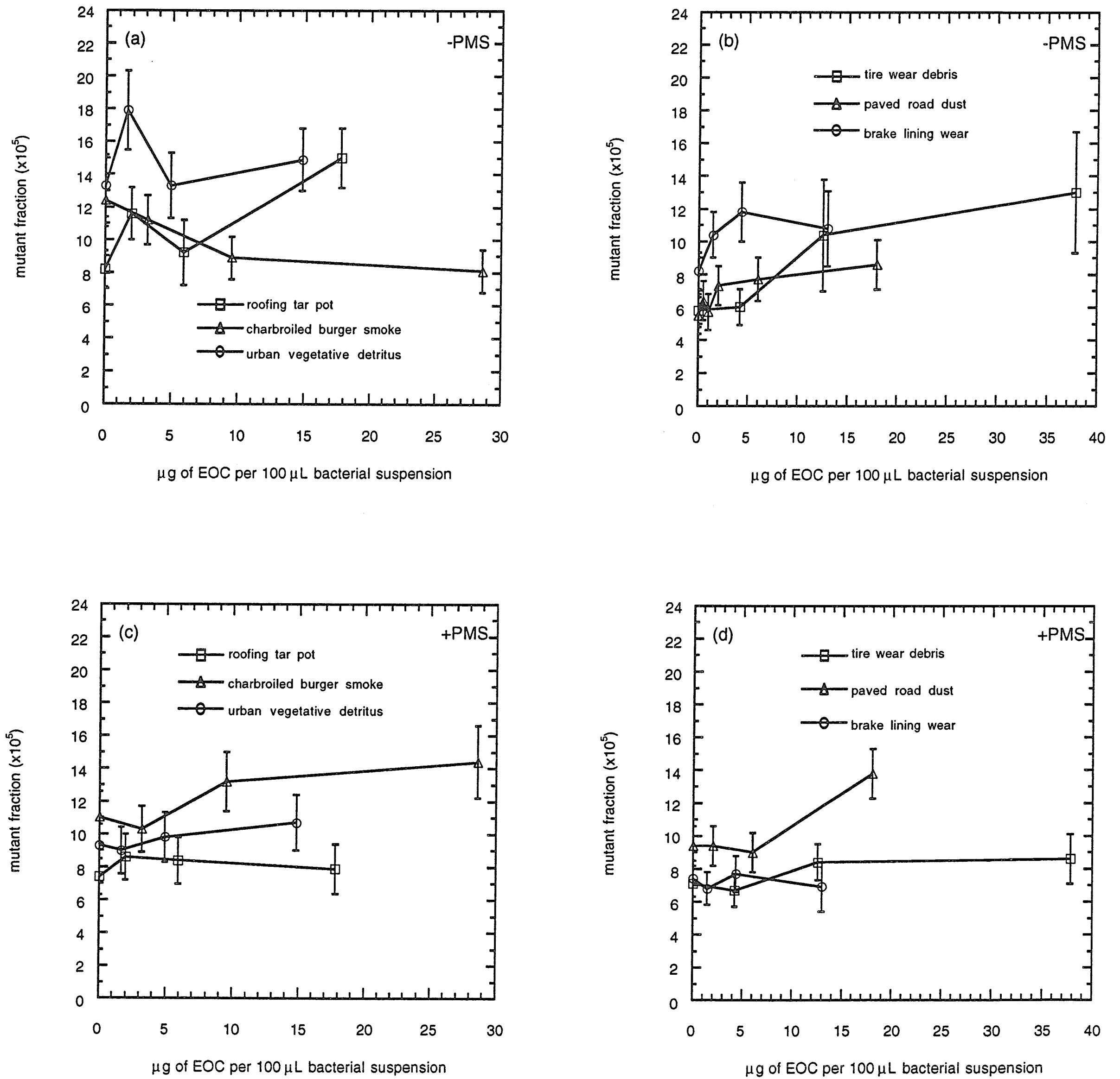

Figure 2.4. Fugitive dust sources and other noncombustion sources: (a) and (b) dose-response curves without PMS; (c) and (d) dose-response curves with PMS. Error bars represent 99% confidence limits.

burger smoke and paved road dust samples both contain measurable amounts of polycyclic aromatic hydrocarbons (PAHs) *(52, 53)*, yet they do not produce a positive mutagenic response in the present assays. This raises the question, "Were the PAH levels in those samples too low to produce a clear mutagenic response?" To answer that question, the quantity of the individual PAH present in the hamburger smoke and road dust samples as well as in all other source samples was computed based on the chemical analysis of these samples reported by Rogge *(54)*. The individual ordinary PAH present in all of the source samples analyzed here were at least an order of magnitude lower than the doses required to produce a mutagenic response (as judged by the administration of pure compounds like benzo[*a*]pyrene (BaP) to the assay). Therefore compounds present in the combustion source effluents in addition to the routinely monitored ordinary PAH would appear to be responsible for most of the mutagenicity of the samples in the present assays. PAH such as BaP indeed are mutagenic in the present assay if one supplies enough BaP to the assay (see the earlier discussion of the concurrent positive control tests). Therefore, if higher EOC doses were supplied to the bioassay, the charbroiled meat smoke and road dust source samples would be expected to be mutagenic. All of the noncombustion sources are clearly less mutagenic than the combustion sources per unit of EOC used in the bioassay.

Mutagenicity of Ambient Samples in Bacterial Assay

Ambient fine aerosol samples from Azusa and Long Beach, CA, aggregated into 3-months duration were tested in the *S. typhimurium* forward mutation assay, and the results are summarized in Table 2.2. All eight of the

ambient sample composites showed some mutagenic activity in the absence of PMS; however, due to sample toxicity, only seven samples met the statistical criteria for declaration of a positive mutagenic response for the –PMS test condition. In the presence of PMS, the ambient samples showed less mutagenic activity, with only five of the eight samples meeting the statistical criteria for declaration of a positive mutagenic response. Dose-response curves for the ambient aerosol samples taken at Azusa and Long Beach are shown in Figure 2.5.

Most of the ambient aerosol samples tested here showed high –PMS mutagenic activity. Many past studies also have found that ambient air samples are mutagenic in the Ames assay, strain TA98. Tokiwa et al. *(7)* detected Ames assay, strain TA98, mutagens in both industrial and residential ambient air samples in Japan. Dehnen et al. *(5)* found mutagens in the airborne particulate matter in industrialized Germany using the Ames assay, strains TA98 and TA1537.

Our study also indicates that there is a seasonal variation in the ambient particulate mutagenicity. At both Azusa and Long Beach, the samples aggregated from January to March and from April to June show the highest mutant fractions. Alfheim et al. *(55)* found a peak seasonal direct-acting mutagenicity from December through March in 1978 in Stockholm using the Ames assay, strain TA98. Flessel et al. *(56)* investigated the mutagenicity of four month composite samples from 1979 to 1988 in the San Francisco Bay area using the Ames assay, strain TA98, with and without PMS, and also found the winter composites to have the greatest mutagenicity. The seasonal variability of the ambient aerosol samples from Azusa and Long Beach also

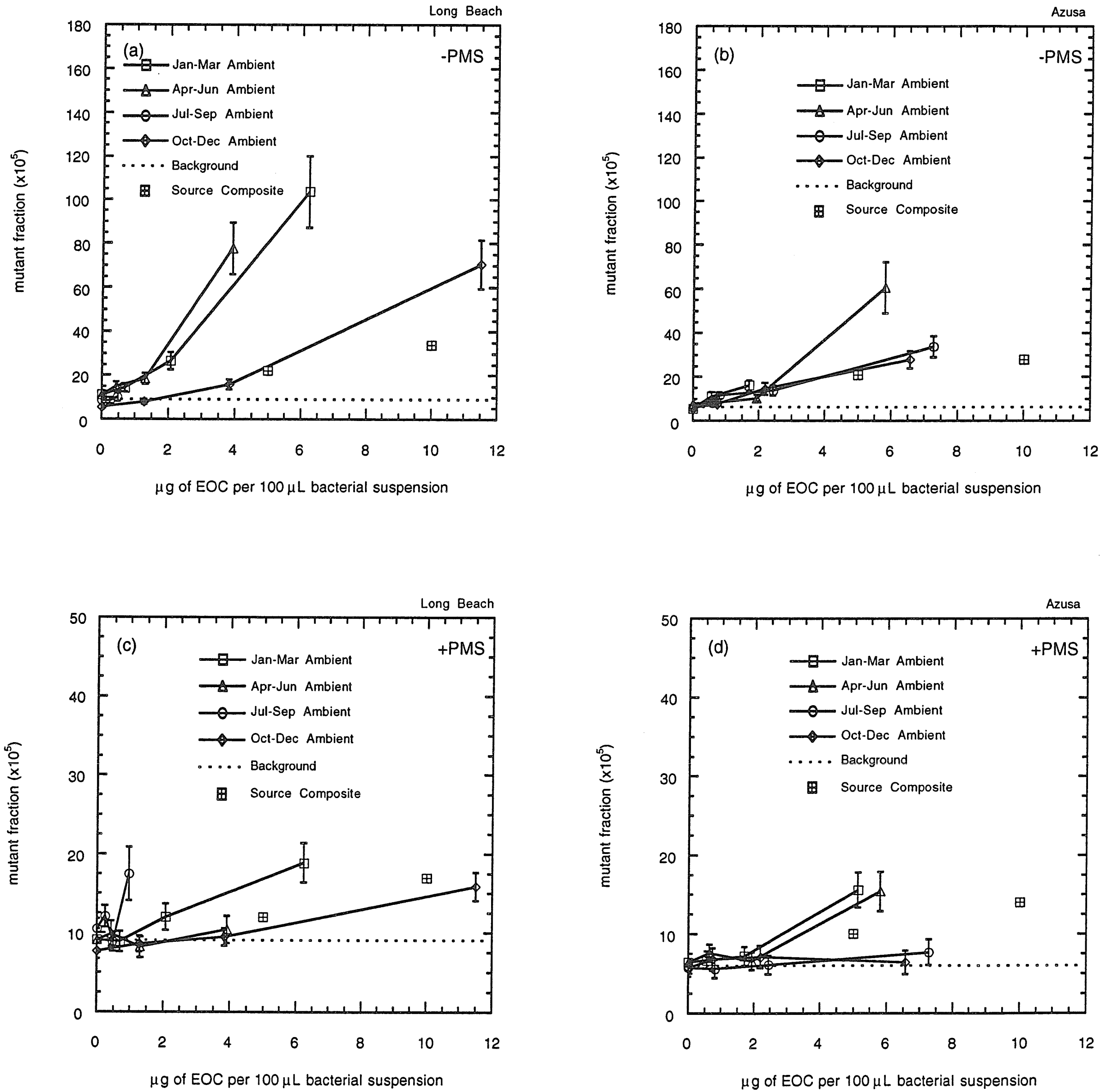

Figure 2.5. Ambient aerosol quarterly composites: (a) and (b) dose-response curves at Long Beach and Azusa –PMS; (c) and (d) dose-response curves at Long Beach and Azusa +PMS. Error bars represent 99% confidence limits.

indicate a greater mutagenicity during colder months than during the summer. The apparently negative results obtained during the summer months in some cases in the present study may be due to the small amount of ambient aerosol available for testing. The recommended OC dose to the bacterial mini-assay should be at least 10 μg/100 μL of bacterial suspension before a negative result is considered to be conclusive; less than 10 μg of OC/100 μL of bacterial suspension was available in some of the ambient samples examined. Very high toxicity also was found at low EOC levels in the July-September sample at Long Beach, thereby preventing that dose-response curve from being extended to higher EOC levels.

Comparison of Source and Ambient Aerosol Mutagenicity

Fine organic aerosol emissions within an 80 km by 80 km study area centered over Los Angeles have been reported by Gray *(57)* and by Hildemann et al. *(24)*. That study area, shown in Figure 2.5 of Rogge et al. *(52)*, contains both the Long Beach and Azusa monitoring sites examined here. While more than 70 different source types contribute organic aerosol emissions in that area, the 15 source types studied here account for nearly 70% of the direct organic particle emissions from sources and can be used to represent approximately 80% of the primary emissions if sources similar to those tested are included by analogy (e.g., by assumption that medium-duty noncatalyst gasoline vehicle exhaust behaves like light-duty noncatalyst gasoline vehicle exhaust; see Table 1).

The specific mutagenicity (mutant fraction per microgram of EOC supplied to each 100 μL of bacterial suspension) was examined separately for

each source sample, both –PMS and +PMS. The source samples tested were first divided into two groups: (1) those that are mutagenic at the EOC levels tested here and (2) those that are not. The nonmutagenic samples do not necessarily show a linear dose-response relationship, instead their response may reflect the noise contributed by the background mutation rate of the bioassay. This does not matter because those sources do not contribute to the comparison of source samples to ambient sample mutagenicity. Those samples that are mutagenic in the present assay generally show quite linear trends, with increasing mutant fraction as the quantity of EOC supplied to the test is increased when adjusting for toxicity as described earlier. A linear least squares regression equation describing mutant fraction as a function of EOC supplied to the bioassay was fit to each of the source sample data sets for those samples listed as positive in Table 2.2 and having survival greater than 10%. Of the 19 regression equations fit to source samples shown as positive in Table 2.2 (both +PMS and –PMS), 12 data sets fit a straight line so closely that the correlation coefficient is greater than 0.98; 4 more show correlation coefficients in the range of 0.95-0.98, 1 in the range 0.91-0.92, and 2 in the range 0.82-0.85. The data sets with correlation coefficients in the range 0.82-0.85 are from synthetic log smoke (+PMS) and the roofing tar pot (–PMS) experiments. The roofing tar pot effluent is barely positive in the present assay, and being close to the level of the historical negative control mutation rate, it is expected that that sample set will show greater variability due to the influence of the background mutation rate variability within the bioassay. The synthetic log smoke sample does not account for significant emissions to the atmosphere and will not be

used in our forthcoming analysis as shown in Table 2.1; any nonlinearities in that data set are unimportant.

Graphs showing the least squares fit between mutant fraction and equivalent organic carbon dose were entered at a dose of 5 μg of EOC/100 μL of bacterial suspension. Next, the mutant fraction attributable to background mutations was removed from each mutagenicity value by subtracting the mutant fraction observed in the concurrent negative control sample during each assay. The background-subtracted mutant fraction values then were converted to specific mutagenicities in terms of mutant fraction per microgram of EOC supplied to each 100 μL of bacterial suspension. A weighted average of these background-subtracted specific mutagenicity values was calculated separately for the −PMS and +PMS data sets, weighted in proportion to the percentage contribution of each of the primary source types to the overall primary inventory of organic carbon aerosol emissions to the study area atmosphere in 1982, as shown in Table 2.1. The emissions-weighted average specific mutagenicities were scaled to the equivalent use of 5 and 10 μg of EOC/100 μL of bacterial suspension and then added to the average of the background mutant fraction values observed during the ambient aerosol bioassays. The results are plotted as the source composite shown in Figure 2.5. The resultant values simulate the effect of a 100 μL bioassay conducted on a sample of size 5 and 10 μg of EOC, respectively, assembled by pooling the source samples in proportion to their emission rates to the local atmosphere, under the hypothesis of a linearly additive bioassay response at the low levels of EOC used *(14)*. The purpose here is not to suggest that the atmospheric aerosol is in fact a purely linear combination of the sources or that the bioassay is precisely linear and additive.

Indeed, the formation or destruction of mutagenic organics by atmospheric chemical reactions is to be expected. The purpose is, however, to gain a rough insight into whether or not the atmospheric aerosol is grossly more or less mutagenic than the aerosol mixture contributed by the sources.

From examination of Figure 2.5, it is seen that the weighted average +PMS mutagenic response of the primary source samples is comparable to that of the ambient samples. The +PMS activity of both the ambient samples and the source composites is lower than the –PMS activity of the same sample sets. The principal departure from the rough equivalence between the source composites and the ambient samples occurs at Long Beach, where the –PMS mutant fraction found for the ambient samples taken in the first half of the calendar year is much higher than can be explained by an emission-weighted average of the primary sources studied here. The –PMS mutagenicity of the April-June ambient aerosol at Azusa also noticeably exceeds that of the source composite. Additional months at Long Beach and Azusa also might have shown such a result if larger amounts of ambient organic aerosol had been available for use in the bioassay. This excess –PMS mutagenicity is likely due to atmospheric transformations. Past studies *(58, 20)* have demonstrated that atmospheric chemical reactions involving PAH will create potent –PMS bacterial mutagens. Further field experiments designed to systematically collect larger amounts of atmospheric aerosol in order to extend the graphs in Figure 2.5 to higher EOC levels are suggested.

Conclusions

Mutagens affecting *S. typhimurium* are present in the particle samples from each of the urban combustion sources tested, including catalyst-equipped auto exhaust, noncatalyst auto exhaust, diesel truck exhaust, wood smoke, distillate fuel oil combustion aerosol, and natural gas combustion aerosol. These urban combustion source effluents generally are more active in the bioassay used here per microgram of organic carbon supplied to the test than is the case for cigarette smoke. Noncombustion sources including paved road dust, motor vehicle brake dust, tire dust, and meat cooking aerosol generally are not mutagenic at organic carbon doses comparable to those that produced a positive response in the combustion source effluent assays. PAH are known to be present at low levels in the road dust, meat smoke, and in dry deposits on urban leaf surfaces, so a mutagenic response might be expected from those sources if higher organic loadings are employed in future bioassays.

Analysis of atmospheric fine particle samples from Azusa and Long Beach, CA, demonstrates in most cases that airborne fine particles also are mutagenic in the *S. typhimurium* forward mutation assay. The specific mutagenicity of the ambient aerosol samples (mutant fraction per microgram of organic carbon supplied to the bacterial assay) can be compared to a weighted average of the specific mutagenicities of the source samples, assembled in proportion to their emission rates in the Los Angeles area. That comparison, given in Figure 2.5, shows that the +PMS activity of the ambient samples and the source composites is comparable. The magnitude of the –PMS mutagenic activity of the Long Beach ambient aerosol in the first half of the calendar year and at Azusa during the April-June period is much greater than can be

explained by the direct emissions from the sources studied here. This may indicate atmospheric transformation of some effluents to form more potent bacterial mutagens. Further chemical analysis of subfractions of source and ambient aerosol samples should be conducted to confirm whether these similarities and differences persist as samples are subdivided in a way that may help to identify the particular compounds or compound classes responsible for the observed mutagenic response.

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Chapter 3

Seasonal and Spatial Variation of the Bacterial Mutagenicity of Fine Organic Aerosol in Southern California

Introduction

Particulate matter that is directly emitted to the atmosphere from combustion sources contains organic compounds, such as polycyclic aromatic hydrocarbons (PAH) and nitro-PAH *(1-5)* , that are both mutagenic *(6-10)* and carcinogenic *(11-14)*. These direct emissions from sources mix in the atmosphere and are transported downwind. During transport, atmospheric chemical reactions can act to deplete the directly emitted organic compounds *(15, 16)* while new compounds can be added to the aerosol, for example, via production of nitro-PAH and oxy-PAH as OH and NO_3 radicals attack vapor phase PAH *(17-24)*. Using bacterial mutagenicity assays *(25, 26)*, organic particulate matter filtered from ambient air has repeatedly been shown to be mutagenic *(27-30)*. Much less is known about the interrelationship between the emissions, meteorological conditions and atmospheric chemical reactions that lead to this result.

The three key processes: emission source characteristics, atmospheric mixing, and chemical alteration, are each highly dependent on location and meteorology. Since different locations and different weather patterns create

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Reference: Hannigan, MP; Cass, GR; Lafleur, AL; Busby, WF, Jr; Thilly, WG *Environ Health Perspect*, 1996, 104, 428-436.

different ambient organic particulate mixtures, clues that point to the origin of mutagenic chemicals in the atmosphere can be obtained by measuring the bacterial mutagenicity differences at various locations during different weather patterns. Tokiwa et al. *(29)* showed that the aerosol in industrial locations in Japan exhibits greater bacterial mutagenicity than samples taken at residential sites. Other investigators *(31, 32)* have since shown that the bacterial mutagenicity of the ambient aerosol (per cubic meter of air sampled) differs between urban sites. Several investigators also have found bacterial mutagenicity to vary with season in Scandinavia *(33)* , in the San Francisco Bay area *(34)* , in northwest Italy *(35)* , and in Newark, NJ *(36)* . In a previous pilot study, we showed that the bacterial mutagenicity of atmospheric fine particulate samples taken in Southern California (per unit organic carbon supplied to the bioassay) is about equal in mutagenic activity to an emissions-weighted average of the mutagenic activity of primary organic aerosol samples from the most important local emissions sources *(37)*. There were indications that the mutagenicity of the ambient aerosol may exceed that of the primary emissions from sources at a few times and locations. However, the small atmospheric sample sizes available during that prior study required that the samples be composited quarterly , which obscured the ability to examine seasonal trends between receptor air monitoring sites, and the fact that samples from only two sites were available eliminated the possibility of examining spatial trends.

In the present paper we report a study of the bacterial mutagenicity of a comprehensive set of urban fine organic aerosol samples taken at 5 sites in Southern California during the entire year of 1993. The purposes are: (1) to document and evaluate the spatial and seasonal dependence of the bacterial

mutagenicity of the atmospheric organic aerosol in relationship to source locations, transport patterns and periods of high photochemical reactivity, and (2) to acquire large atmospheric samples that can later be used to support bioassay-directed chemical analysis of the identity of the mutagenic compounds that are present. The 5 atmospheric sampling sites consist of 1 background station located on an upwind offshore island, and 4 urban stations, each in a location with carefully chosen relationship to surrounding or upwind emission sources. Bimonthly composites of filters were assembled that enable seasonal trends to be observed. To further ensure the validity of spatial and seasonal comparisons, all samples were collected, stored, extracted, and tested under identical conditions. The bacterial mutation assay used in the present study is a version of the *Salmonella typhimurium* forward mutation assay developed by Skopek et al. *(26)*, which thus permits comparison against earlier reports of the mutagenicity of particulate matter emitted directly from Los Angeles area air pollutant emission sources *(37)*.

Experimental Section

Airborne Particle Samples

The ambient air particulate matter samples used here were collected from 5 sites in Southern California; including 1 background site at an offshore island, as shown in Figure 3.1. The 4 on-land sites were chosen because each has a different proximity to major types of air pollution sources. The Long Beach atmosphere is influenced by direct emissions from industrial sources (e.g.. many petroleum refineries and steam-electric power plants) plus shipping activities in Long Beach Harbor. Central Los Angeles is a focal point of the

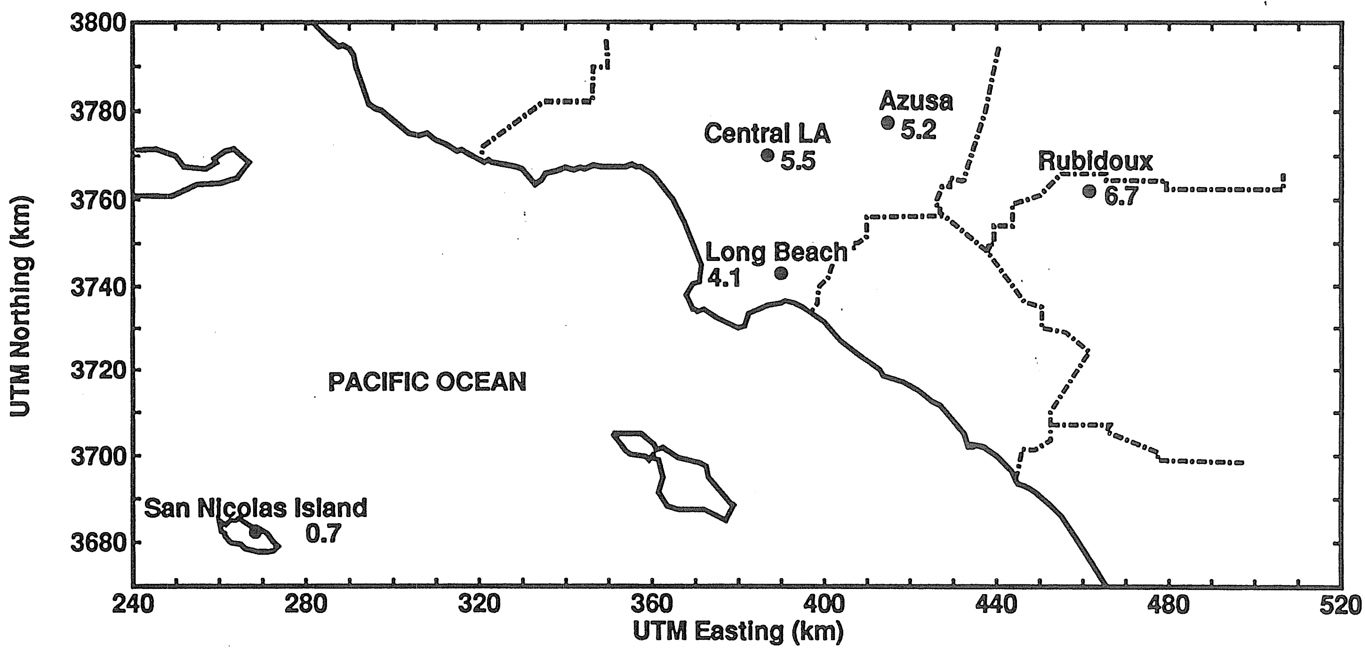

Figure 3.1. Southern California, showing locations of the ambient monitoring stations. The 1993 annual average fine particulate organic carbon concentration in micrograms per cubic meter is given adjacent to each site.

local highway system and is exposed to the direct emissions from dense motor vehicle traffic. Azusa, which is generally downwind of central Los Angeles, is known for experiencing very high ozone levels and receives atmospherically transformed, or secondary, aerosol transported from upwind. Rubidoux is farther downwind, and generally receives even more secondary aerosol, including some of the highest secondary nitrate and secondary organic aerosol concentrations in the nation *(38, 39)*. All 4 of these urban sites were located at South Coast Air Quality Management District (SCAQMD) air monitoring stations. San Nicolas Island is a background site located off the coast (upwind) of Los Angeles; samples taken there are intended to assess the mutagenicity of the aerosol before air masses enter the Los Angeles area.

Ambient particulate matter samples were taken for 24 h every sixth day for the entire year of 1993 at all the urban sites. This every sixth day sampling calendar was identical to that of the SCAQMD and national particulate matter sampling networks. Eighteen 24 h samples were taken at San Nicolas Island at times when access to the island could be obtained.

The sampling systems employed at each site were identical and consisted of a high volume dichotomous virtual impactor and a separate low volume aerosol sampler. The high volume dichotomous virtual impactor is described in detail by Solomon et al. *(40)*. This sampler was chosen because it can collect a large quantity of size-separated material within a single 24 h period. Briefly, the high volume virtual impactor works by accelerating ambient air at a flow rate of nominally 300 L min^{-1} through a converging nozzle, after which the flow is divided. 90% of the air flow through the nozzle is caused to make an abrupt 90° turn; the fine particles smaller than 3-4 μm aerodynamic

diameter follow the fluid streamlines and in that way 90% of the fine particles are collected on a fine particle filter at a flow rate of nominally 270 L min^{-1}. The coarse particles larger than 3-4 μm aerodynamic diameter cannot follow this turn due to their greater inertia. These coarse particles are concentrated into the remaining 30 L min^{-1} of inlet air and along with the 10% of fine particles remaining in that 10% of inlet air are collected on a second filter that is located downstream of the diverging nozzle that is aligned with the major axis of the inlet of the sampler. The filters used for both collecting coarse and fine particulate matter samples are 102 mm diameter quartz fiber filters (Pallflex Tissuquartz 2500 QAO).

The low volume aerosol samplers used here are similar to those described in detail by Salmon et al. *(41)*. This system consists of two parallel sampling trains, one for collection of total ambient particulate matter and the other for collection of fine ambient particulate matter. Fine particle samples are collected by drawing ambient air through an AIHL-design cyclone separator, which removes particles with aerodynamic diameter > 2 μm *(42)*. The airflow then is divided between four parallel 47 mm filter holders. These four filter holders contain; (1) a Teflon filter (Gelman 47 mm Teflo with 2 μm and 1 μm pore size) operated at a flow rate of 3 L min^{-1} from which aerosol mass concentration is determined gravimetrically and trace metals concentrations are determined by X-ray fluorescence analysis, (2) another Teflon filter operated at a flow rate of 5 L min^{-1} from which ionic compound concentrations are determined by ion chromatography, (3) a quartz fiber filter (Pallflex, 2500 QAO) operated at 10 L min^{-1} used for determination of organic carbon (OC) and elemental carbon (EC) concentrations, and (4) another quartz fiber filter

operated at 10 L min^{-1} which is reserved for future studies of organic compound concentrations by gas chromatography/mass spectrometry.

All quartz fiber filters were baked for at least 6 h prior to use at 750 °C to lower their carbon blank. Field and laboratory blanks were also taken to ensure that there was minimal contamination of the ambient sampling system. Each filter was loaded the day prior to sampling, and unloaded on the day after sampling. The 102 mm diameter quartz fiber filters were transported to the sampling sites in baked aluminum foil packages, and brought back to the laboratory in annealed glass jars with solvent-washed Teflon-lined lids. The smaller quartz fiber filters were transported in sealed baked aluminum foil lined petri dishes. The Teflon filters were transported in petri dishes sealed with Teflon tape. Upon return to the laboratory all filters were immediately transferred to a freezer at Caltech maintained at -21 °C, where they remained until the end of the field experiment. The frozen samples were then transported to the laboratories at MIT where the samples were first stored in a sub-zero freezer, next extracted, and the extracts subjected to the intended bioassays.

The respirable fine particulate matter samples from the high volume dichotomous virtual impactors were used for both the organic carbon concentration measurements and the bioassays reported here. Each of these quartz fiber filters was cut into wedge-shaped segments which were allocated as follows: one twelfth of each filter was used to determine the organic carbon (OC) and elemental carbon (EC) mass concentration by the thermal evolution and combustion method of Huntzicker and co-workers *(43, 44)*, segments equal to three fourths of each filter were used for bioassay and chemical analysis, and one sixth of each filter was placed in storage. Bioassay and our intended later

chemical analysis for individual organic compounds require large quantities of sample, and individual fine particulate matter samples were not large enough for such analyses. In order to assemble enough sample for both biological and chemical analyses, and yet still maintain the seasonal variation inherent in these samples, the filter portions were pooled to create bimonthly composites at each urban site. Due to the very low pollutant concentrations at the offshore island background site, filter portions taken there were composited to create a winter and a separate summer sample. When organic particulate matter is supplied to the bioassays in the present paper the quantities are reported as equivalent organic carbon (EOC), which is defined as the amount of organic carbon present in a particular sample prior to extraction as determined by thermal evolution and combustion analysis of the quartz fiber filter sections cut from the same filters that were extracted for use in the bioassay. This measure provides a direct connection from the bioassay results back to the ambient concentration of organic aerosol.

Extraction and Concentration

A description of sample extraction and concentration procedures is given in detail by Hannigan et al. *(37)*. Briefly, all filters used in this study were soxhlet-extracted with dichloromethane (DCM) for at least 16 h. DCM extracts were concentrated down to approximately 1 mL in a vacuum centrifuge. The sample portion designated for the bacterial assay was then exchanged into dimethyl sulfoxide (DMSO) by adding DMSO to the DCM extract and then concentrating the extract to the volume of DMSO added under a gentle stream of dry N_2. On average, the extracted mass from an ambient particulate matter

sample, as measured by a microscale evaporation method *(45)* , was 0.93 mg of extracted mass per mg of EOC.

Bacterial Bioassay

The bacterial bioassay used in this study is a miniaturized version of the forward mutation assay in *S. typhimurium*, strain TM677, using resistance to 8-azaguanine as developed by Skopek et al. *(26)*. This bioassay is run under two conditions: with and without further enzymatic activation obtained by adding a post-mitochondrial supernatant (PMS, also referred to elsewhere as S9) preparation containing rat liver enzymes to the bioassay procedures. These two assay procedures will be referred to as +PMS and –PMS, respectively.

Detailed descriptions of the bacterial assay protocols have been given previously *(26, 9)*. To summarize, *S. typhimurium* were suspended in medium in the presence of the sample for 2 h. Three to five different dilutions of each sample were exposed to the bacteria both with and without the presence of 5% (v/v) Aroclor-induced PMS. In the miniaturized version of the procedure, the culture volume was reduced from 1 mL to 100 μL *(46)*. Cultures containing PMS had a NADPH-generating system. After 2 h, the reaction was quenched, and aliquots were plated in the presence and absence of 50 μg/mL of 8-azaguanine. The results from two to four independent cultures, each plated in triplicate, were averaged to estimate toxicity and mutagenicity at each of the three sample dilutions. Colonies were counted after 48 h, and the mutant fraction was determined as the number of colonies formed in the presence of 8-azaguanine divided by the number of colonies formed in its absence, multiplied by a dilution factor. Both positive and negative concurrent controls are

processed in parallel with each sample. The negative concurrent control consists of a dose of 1 µL of pure DMSO. The positive concurrent control consists of a dose of 1 µL of 4-nitroquinoline N-oxide (2.5 µg/mL) in the absence of PMS and 1 µL of benzo[a]pyrene (2 mg/mL) in the presence of PMS. The mean ± SD of the negative concurrent control mutant fraction in this series of experiments was (5.3×10^{-5}) ± (1.4×10^{-5}). For an extract to be deemed mutagenic in this assay, a mutant fraction must be greater than the concurrent negative control (CC) such that 2.3 times the standard deviation (the 99% confidence limit) of the concurrent negative control plus the mean value of the control does not overlap the sample mean value minus 2.3 times its standard deviation (the 99% confidence limit), and that mutant fraction must exceed the 95% upper confidence limit of the historical negative control (HC), which is 8.5×10^{-5} for this series of experiments. It is also known that the TM677 strain used in this study does not lack nitroreductase; 1-nitropyrene (1-NP), 4-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, and 1,8-DNP are all potent –PMS mutagens in TM677.

Results and Discussion

Organic Carbon Measurements

In order to discuss the significance of the seasonal and spatial variations of bacterial mutagenicity, it is important to understand how season and location affect the ambient particulate matter mixture. The annual average fine particulate organic carbon (OC) concentration in the Los Angeles area for the year 1993 is given adjacent to the monitoring site locations in Figure 3.1. Ambient fine particulate OC concentrations over the ocean are very low,

averaging only 0.7 $\mu g\ m^{-3}$ over the year 1993. Moving onshore, it is seen that fine particle OC concentrations are much higher, ranging from 4.1 $\mu g\ m^{-3}$ near the coast at Long Beach to a high of 7.0 $\mu g\ m^{-3}$ at the farthest inland site at Rubidoux. As shown in Figure 3.2, the seasonal variation in ambient fine particle OC concentrations at San Nicolas Island is slight, varying by about 0.5 $\mu g\ m^{-3}$ over the course of the year. In contrast, the fine particle OC concentrations at Long Beach vary substantially from month to month with a maximum in the winter (November-December) and a minimum in the late spring/early summer (May-June). Central Los Angeles also shows a strong seasonal variation in fine particle OC concentration, and like Long Beach, the maximum is during the winter and the minimum is during the summer. Moving inland to the Azusa and Rubidoux sites, the relative seasonal variation in fine particle OC concentrations is different; instead of winter maxima and summer minima, the OC concentrations at Azusa and Rubidoux peak in September-October which coincides with the peak photochemical smog season during 1993.

The seasonal patterns at these four sites agree with previous measurements by Gray et al. *(47)* taken during 1982. Air quality modeling studies applied to explain the results of those 1982 experiments *(48)* show that the spatial differences in the seasonal variation of ambient OC in the Los Angeles area that we also observed during 1993 result from seasonal changes in wind speed, wind direction, mixing depth and secondary aerosol formation rates. During the summer months, the wind blows from the ocean toward the land most of the day. As air masses move over the city, primary organic aerosol emissions accumulate and are transported downwind (eastward). Secondary

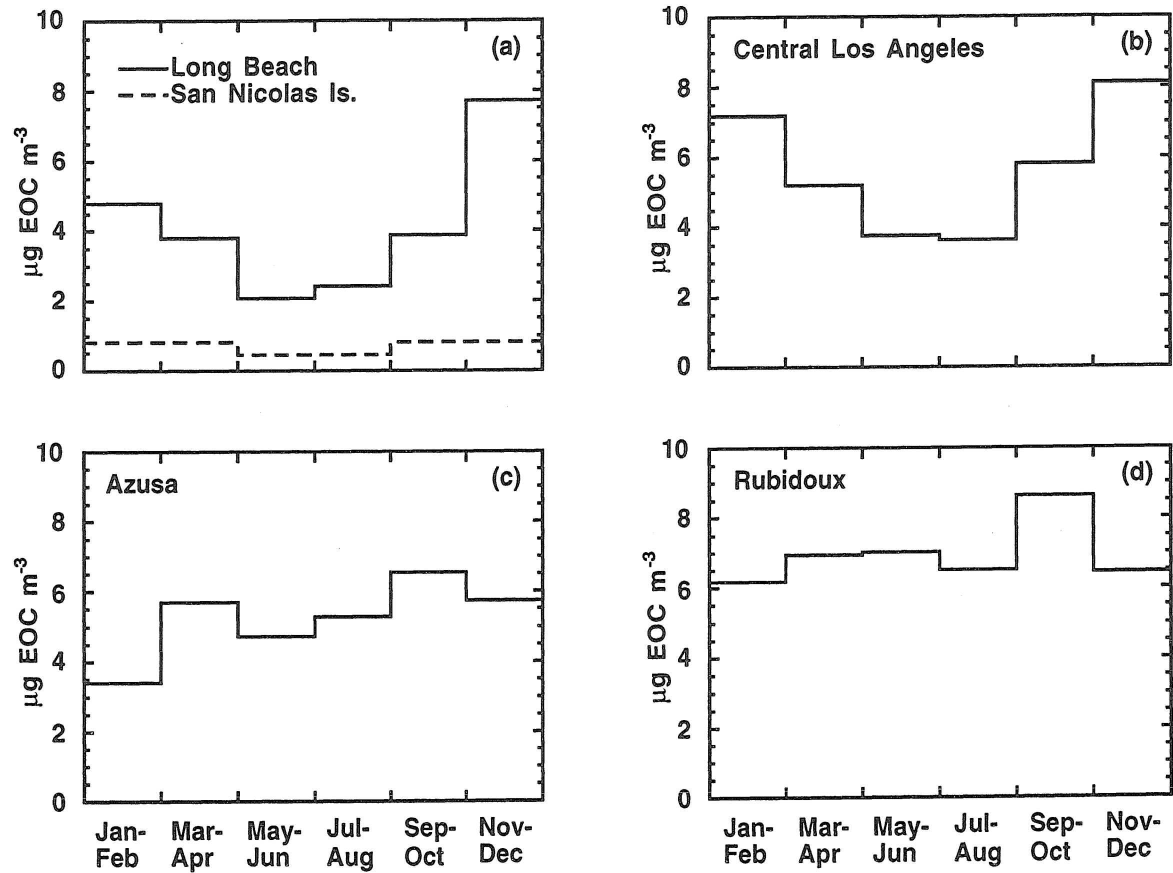

Figure 3.2. Seasonal variation in fine particulate organic carbon concentration in the South Coast Air Basin during 1993 at (a) Long Beach and San Nicolas, (b) Central Los Angeles, (c) Azusa, and (d) Rubidoux.

organic aerosol formation during the summer photochemical smog season acts to further increase fine particle OC concentrations with distance downwind. This secondary organic aerosol formation process occurs as gas phase chemical reactions involving certain higher molecular weight alkanes, olefins, and aromatics produce low vapor pressure reaction products that subsequently condense onto existing particles in the atmosphere *(49, 50)* . The result is that fine particle OC concentrations increase with downwind distance over the metropolitan area in the summer (see Figure 3.3a). During the winter months, mixing depths are lower, resultant wind speeds are slower and the resultant wind direction often is from the land toward the sea. The highest average fine particle OC concentrations during the winter months are observed close to the areas of highest source emission density on the west side of the air basin for this reason (see Figure 3.3b). Given the highest OC concentrations in the air basin during the winter and the lowest during the summer, sites on the western side of the air basin display considerable seasonal variation in ambient OC concentrations as is seen at Long Beach and Los Angeles in Figure 3.2. Sites in the eastern area of the air basin are upwind of the city during the stagnant winter months and downwind of the city during the summer; given lower winter concentrations and higher summer concentrations the sites in the eastern area of the air basin display much less seasonal variation in OC concentrations.

Mutagenicity of Ambient Samples

Organic extracts from fine particulate matter samples were combined to form bimonthly composites at each of the four urban sites in the Los Angeles area and semiannual composites at the background site on San Nicolas Island,

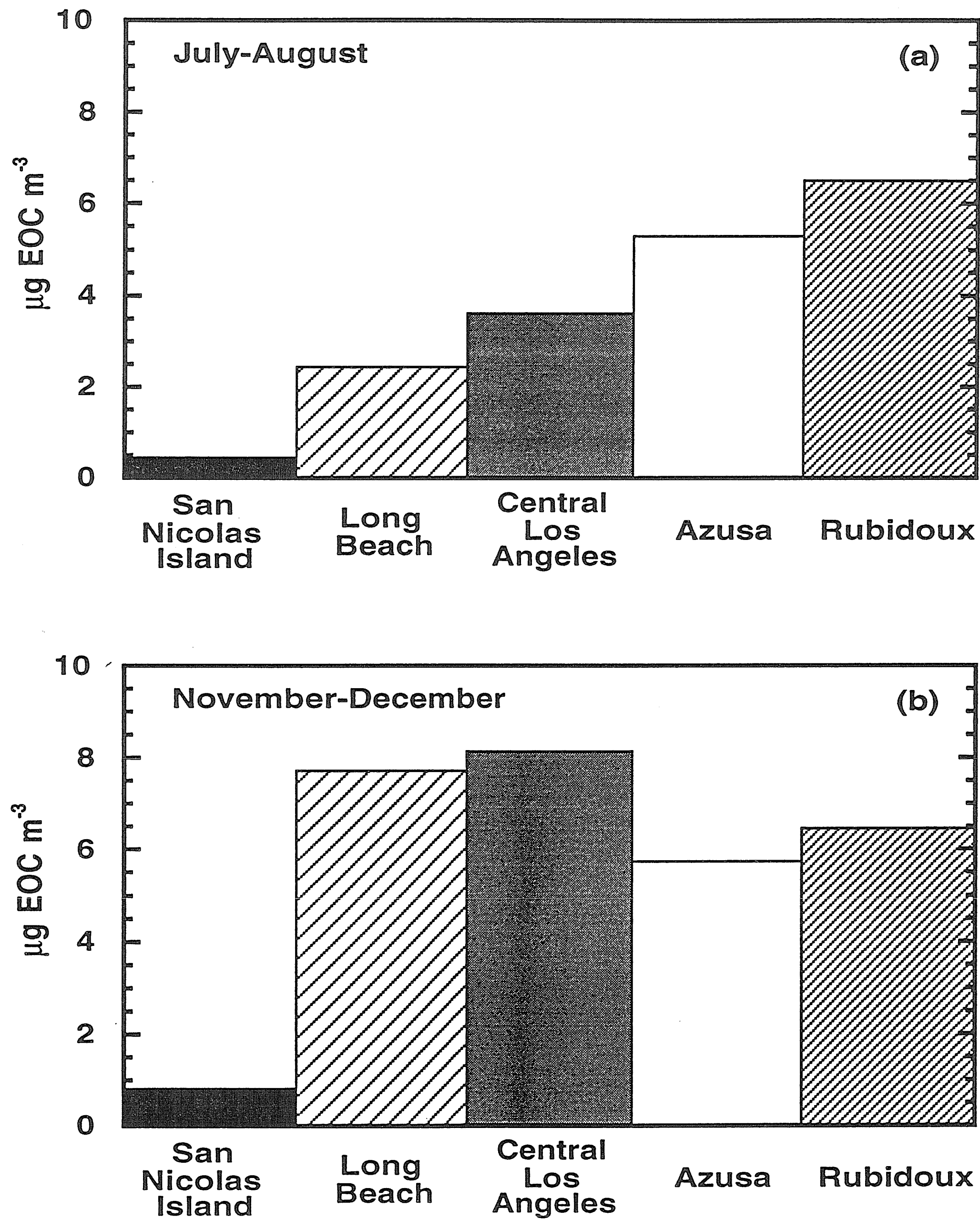

Figure 3.3. Spatial comparison of the average fine particulate organic carbon concentration across the South Coast Air Basin during (a) July-August, 1993, and (b) November- December, 1993.

and were tested for mutagenicity in the absence and presence of PMS. Each of the ambient aerosol extracts tested met the statistical criteria (described previously) to be considered mutagenic except for the Azusa September-October composite (with PMS) and the San Nicolas Island summer composite (with PMS). Both of those extracts would likely be mutagenic at higher doses, as they show an increase in response with increasing dose.

Dose-response curves were generated for each composite under both assay conditions and are plotted in Figure 3.4, showing mutant fraction (mutant colony counts corrected for sample toxicity) versus μg of EOC supplied to the 100 μL bacterial suspension. As seen by comparing the data at San Nicolas Island to the samples taken at onshore sites in the urban area, the mutagenicity of the aerosol in the city is generally much greater than that upwind of the city. At most sites and times, the –PMS mutagenicity of these samples is greater than the +PMS mutagenicity; the ratio of –PMS mutagenicity to +PMS mutagenicity ranges from 2 to 9 (with one exception, the Sep-Oct period at Rubidoux has a higher +PMS than –PMS mutagenicity), with an average value of 3.

The mutagenic potency for each sample tested is defined as the slope of a linear least-squares fit to the dose-response curve (Figure 3.4) which gives an estimate of the increase in mutant fraction ($x10^5$) per μg of EOC supplied to the assay. These mutagenic potency values, shown in Table 3.1, were determined by a weighted linear fit to the dose-response curves (generalized least squares estimate, GLS). The weighted linear fit technique was preferred to an ordinary least squares (OLS) linear fit because the GLS weighting procedure normalizes the data according to the standard deviation of the mean mutant fraction at each dose, and therefore will be less influenced by high dose-response values that

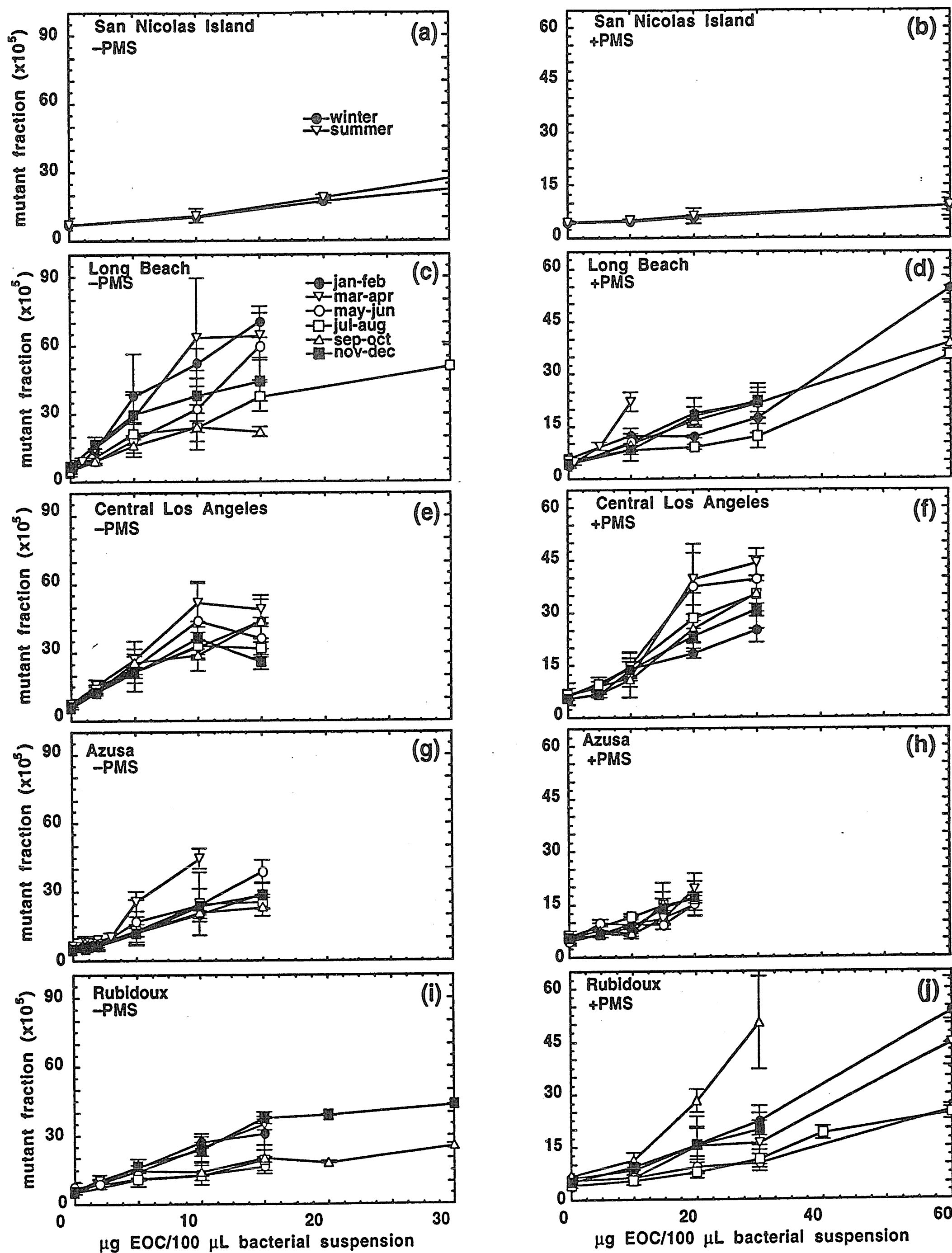

Figure 3.4. Average dose-response curves for San Nicolas Is., (a) −PMS, (b) +PMS; Long Beach, (c) −PMS, (d) +PMS; Central LA, (e) −PMS, (f) +PMS; Azusa, (g) −PMS, (h) +PMS; Rubidoux, (i) −PMS, (j) +PMS. Error bars = 1 SD.

Table 3.1: Summary of dose-response curve characterization.

bimonthly composite	mutagenic potency[a] (mean mutant fraction ($x10^5$) per μg of EOC) −PMS[b]	+PMS[b]
	Long Beach	
Jan-Feb	4.4±0.32	0.47±0.04
Mar-Apr	5.1±0.22	1.29±0.21
May-Jun	2.6±0.54	0.63±0.08
Jul-Aug	2.0±0.24	0.49±0.03
Sep-Oct	1.2±0.15	0.62±0.02
Nov-Dec	2.9±0.52	0.63±0.11
Annual Mean	3.0±0.15	0.69±0.04
	Central Los Angeles	
Jan-Feb	2.4±0.11	0.62±0.08
Mar-Apr	3.2±0.36	1.0±0.00
May-Jun	2.3±0.32	1.2±0.06
Jul-Aug	2.0±0.25	1.0±0.18
Sep-Oct	3.0±0.36	0.94±0.18
Nov-Dec	1.8±0.20	0.92±0.07
Annual Mean	2.5±0.12	0.95±0.05
	Azusa	
Jan-Feb	1.6±0.10	0.39±0.08
Mar-Apr	3.3±0.41	0.50±0.11
May-Jun	2.1±0.32	0.41±0.07
Jul-Aug	1.4±0.22	0.54±0.07
Sep-Oct	1.5±0.12	0.16±0.10
Nov-Dec	1.9±0.05	0.62±0.09
Annual Mean	2.0±0.10	0.44±0.04
	Rubidoux	
Jan-Feb	2.0±0.15	0.35±0.06
Mar-Apr	2.0±0.14	0.43±0.09
May-Jun	0.59±0.23	0.36±0.02
Jul-Aug	0.61±0.06	0.29±0.03
Sep-Oct	0.40±0.03	0.87±0.12
Nov-Dec	0.94±0.03	0.46±0.10
Annual Mean	1.1±0.05	0.46±0.03
	San Nicolas Island[c]	
Winter	0.47±0.03	0.07±0.03
Summer	0.62±0.04	0.07±0.04
Annual Mean	0.52±0.03	0.07±0.03

[a] Slope of a straight line fit by generalized least squares to the dose-response curve ± the standard error. [b] Tested in the absence (−PMS) or presence (+PMS) of postmitochondrial supernatant. [c] Due to the limited sample mass available for the background site, only two composite samples were generated.

are accompanied by large uncertainties. Using the weighted linear fit, 46 of the 50 data sets fit a straight line very closely and produced correlation coefficients (r) greater than 0.95.

Figure 3.5 shows the mutagenic potency for each of the samples in the same format as the OC concentrations were shown. The most obvious point is that nearly all samples have a higher response –PMS than +PMS. We observed this previously for both source and ambient samples in Los Angeles using the same assay procedures *(37)*. The lower mutagenic potency of the aerosol at the background site is also very noticeable. Pitts et al. *(28)* and Alfheim et al. *(33)* have also observed greatly reduced or no mutagenicity at background air monitoring sites. Systematic seasonal variations in mutagenicity per unit organic carbon supplied to each test are not as readily observable as was the case for the seasonal variations in organic carbon concentrations discussed earlier. The Long Beach samples show the highest –PMS potency during the winter-like months with the greatest air stagnation (Jan-Feb, Mar-Apr, and Nov-Dec) while –PMS mutagenic potency begins to approach background levels during the Sept-Oct period with the most sustained onshore air flow. This suggests either that –PMS mutagenicity at Long Beach is contributed by sources whose influence can be reduced during periods of better ventilation of that site, or that the compounds responsible for the aerosol mutagenicity at Long Beach are being destroyed by atmospheric chemical reactions in the late summer months. An increased potency in the Mar-Apr composite +PMS is observed at Long Beach for reasons that are not yet understood. Mutagenic potency at central Los Angeles exhibits no systematic seasonal variation. All but one –PMS data point lies within $\pm 2\sigma$ of the annual

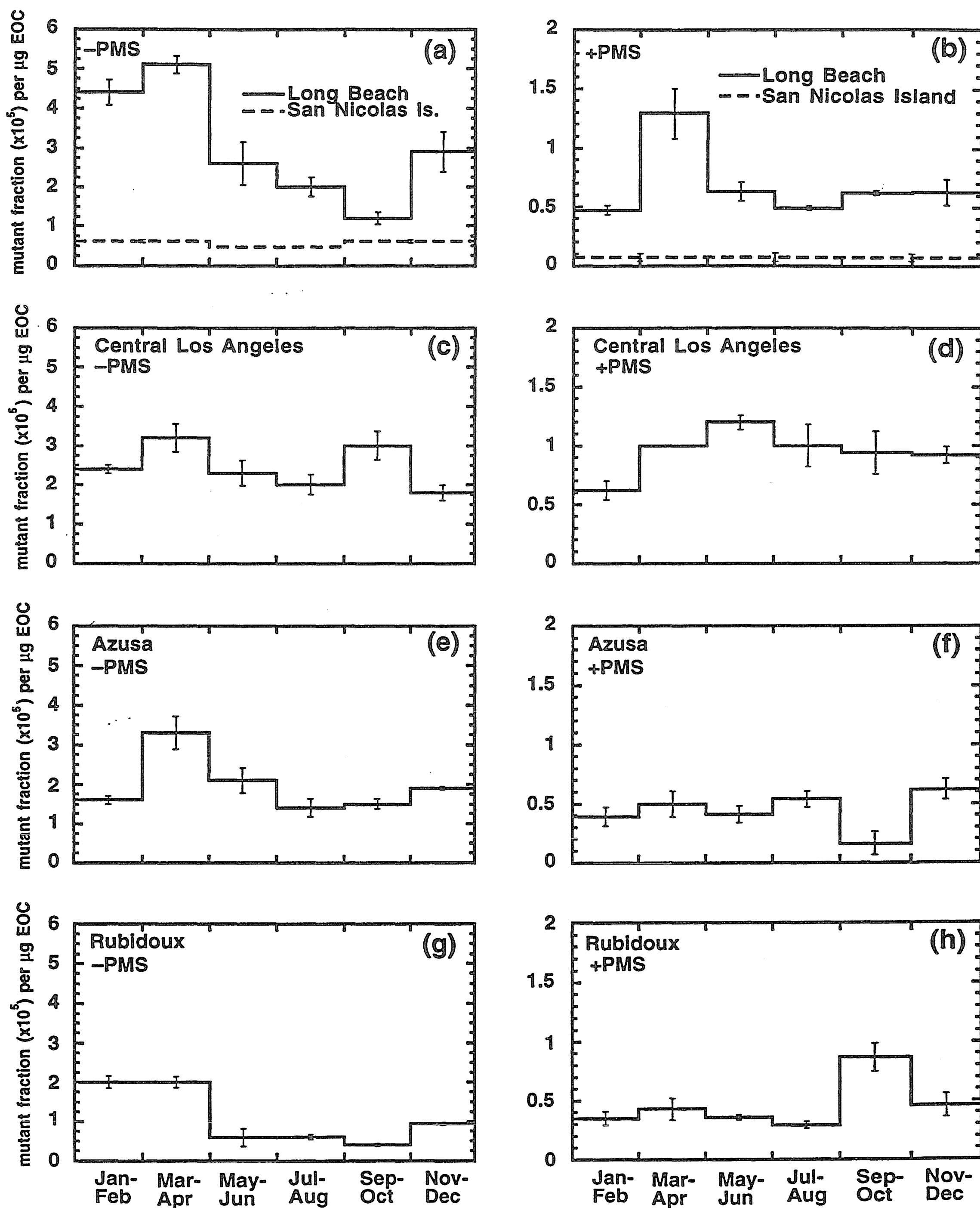

Figure 3.5. Mutagenic potency of the fine aerosol for 1993 at Long Beach and San Nicolas Is., (a) −PMS, (b) +PMS; Central LA, (c) −PMS, (d) +PMS; Azusa, (e) −PMS, (f) +PMS; Rubidoux, (g) −PMS, (h) +PMS. Error bars = 1 SD.

mean value. The +PMS mutagenic potency values at central Los Angeles also fall within $\pm 2\sigma$ of the annual mean value except during Jan-Feb at that site. Azusa shows only occasional mutagenic potency differences between bimonthly composites; the Mar-Apr composite seems to be more potent than the other composites –PMS, and the Sep-Oct composite is less potent than the others +PMS. Rubidoux, the farthest downwind smog receptor site, shows the most interesting seasonal variations. Like Long Beach which is more or less directly upwind in the summer, the –PMS mutagenic potency at Rubidoux is highest during the colder months and lowest in the summer, but unlike the other sites the late summer composite which occurs during the peak in the photochemical smog season in September of this year is more potent than the other +PMS composites at Rubidoux. The two seasonal samples available at San Nicolas Island show mutagenic potencies that are very similar to each other, and both are very low compared to samples taken within the urban area.

While the mutagenic potency data do not reveal seasonal trends that are common to all sites there are significant spatial differences in the annual average mutagenic potency between sites. This can be seen most clearly by looking at the annual mean mutagenic potency values given in Figure 3.6ab. In the absence of PMS, mutagenic potency decreases as one moves inland from Long Beach (3.0 ± 0.15 mutant fraction x10^5/µg EOC) and central Los Angeles (2.5 ± 0.12) to Azusa (2.0 ± 0.10) and Rubidoux (1.1 ± 0.05). In the presence of PMS, the aerosol at central Los Angeles has the highest mutagenic potency (0.95 ± 0.05 mutant fraction x10^5/µg EOC), followed by Long Beach (0.69 ± 0.04), with Azusa and Rubidoux at a lower level (0.44 ± 0.04 and 0.46 ± 0.03). This variation in mutagenic potency from site to site can be compared with

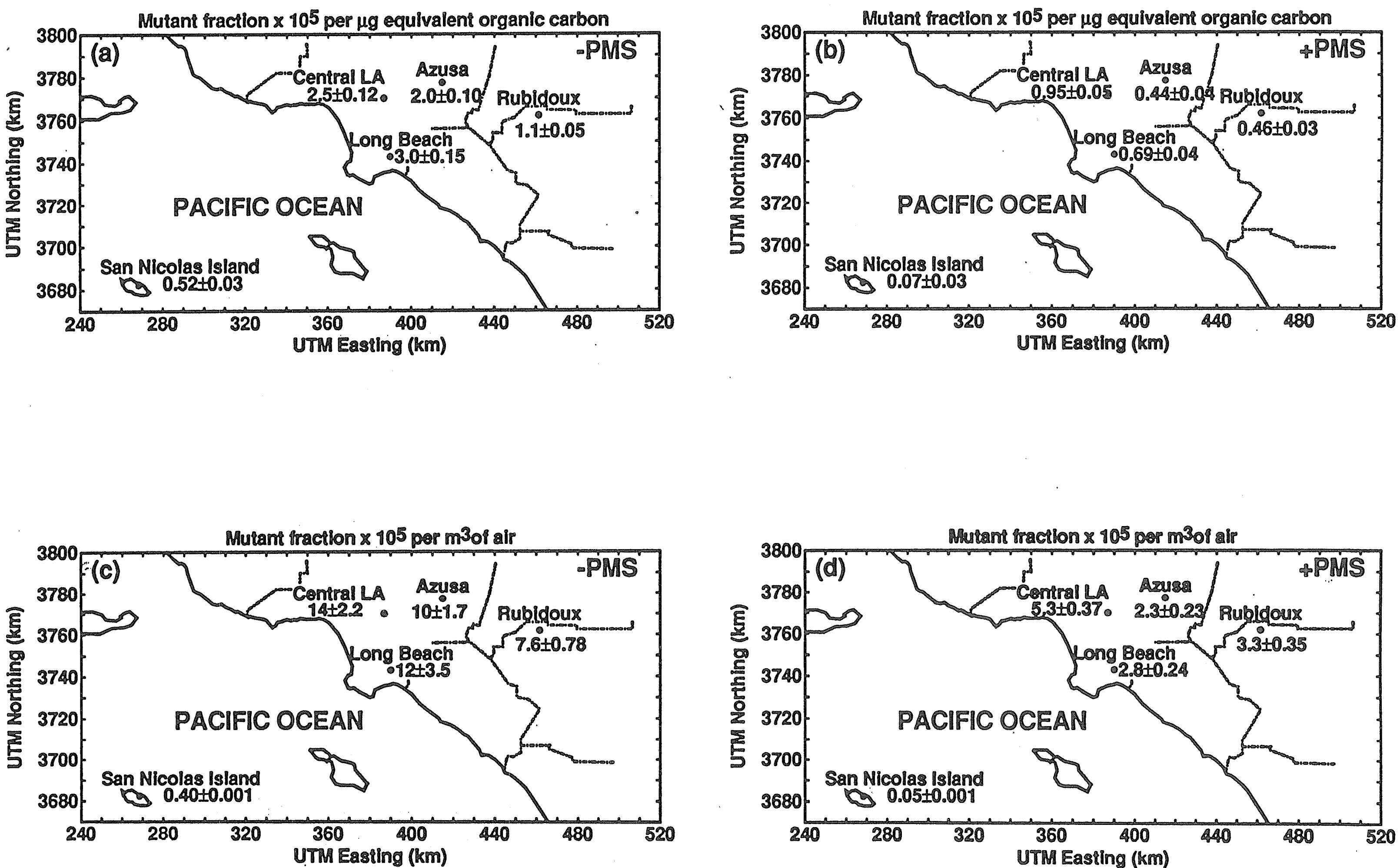

Figure 3.6. The spatial variation of 1993 annual average mutagenic potency across the South Coast Air Basin - mutagenicity per mass of organic carbon, a. -PMS and b. +PMS. The spatial variation of 1993 annual average mutagenic density across the South Coast Air Basin - mutagenicity per volume of air, c. -PMS and d. +PMS. Error given represents one standard deviation.

previous studies. Tokiwa et al. *(29)* found greater mutagenic potency at industrial sites versus residential sites in Japan. Butler et al. *(32)* measured mutagenic potencies at 5 different urban sites, New York City, Elizabeth, N.J., Mexico City, Beijing, and Philadelphia and showed that those potencies differed between cities. While Pitts and co-workers *(28, 51)* measured mutagenic potencies at various sites in the Los Angeles area in 1976 and in 1980; and Atkinson et al. *(52)* measured mutagenic potencies in several locations in California during 1986-1987, no direct comparison between the present work and the previous Los Angeles basin studies is possible because the previous studies were conducted over a shorter period of time and sampling rotated between different sites at different times; thus, any variation observed between sites in the previous studies could have been caused by short-term meteorological events.

Figure 3.7 combines the organic aerosol concentration data of Figure 3.2 with the mutagenic potency (mutagenicity per μg EOC) values given in Figure 3.5, and shows mutagenicity in units of mutant fraction($x10^5$) m^{-3} of ambient air. The term mutagenic density will be used to describe this measure of mutagenicity per unit air volume sampled. Atmospheric samples taken at both Long Beach and central Los Angeles show a strong seasonal variation in –PMS mutagenic density; the aerosol at both sites has a significantly greater –PMS mutagenic density value in the winter than in the summer months. These seasonal differences in mutagenic density seen at Long Beach and central Los Angeles occur because of the influence of seasonal variations in the OC concentrations. Several previous investigators also have measured higher mutagenic density in the winter months; Alfheim et al. *(33)* at various sites in

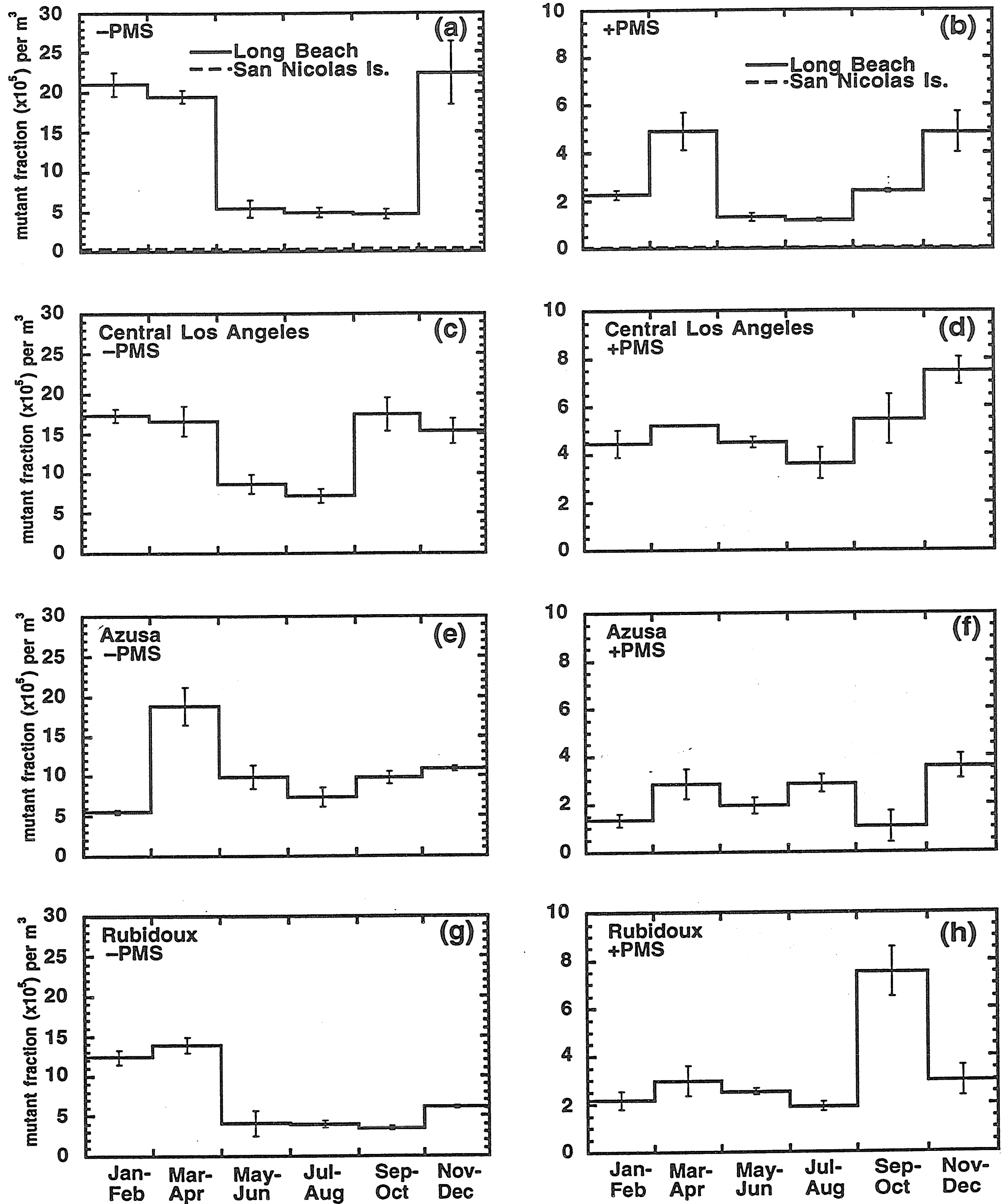

Figure 3.7. Mutagenic density of the fine aerosol for 1993 at Long Beach and San Nicolas Is., (a) –PMS, (b) +PMS; Central LA, (c) –PMS, (d) +PMS; Azusa, (e) –PMS, (f) +PMS; Rubidoux, (g) –PMS, (h) +PMS. Error bars = 1 SD.

Scandinavia, Flessel et al. *(34)* in the San Francisco Bay area, and Scarpato et al. *(35)* in northwestern Italy. The aerosol at Azusa and Rubidoux does not show any pronounced seasonal trend in mutagenic density; however, each of those sites has one bimonthly composite with an unusually high mutagenic density value. At Azusa the –PMS assay during Mar-Apr shows a high mutagen density value while at Rubidoux an increase in mutagenic density +PMS occurs during Sep-Oct. The annual average mutagenic densities, shown in Figure 6cd, are highest at central Los Angeles and decline as one moves away from the center of the city in a manner not unlike local traffic and population density.

The measures of mutagenic potency shown in Figure 3.6 relate to mutagenicity adjusted to comparable quantity of organic aerosol mass, not air volume. The higher relative potency at the more congested and industrialized sites at Long Beach and central Los Angeles is not a function of aerosol mass concentration in the atmosphere but rather is a function of an aerosol chemical composition which causes an increase in mutation frequency per μg of organics supplied to the assay. The lower relative potency at the background site on San Nicolas Island likewise does not represent small aerosol concentrations, but an ambient compound mixture which causes fewer mutants per μg of organic carbon supplied to the assay. This suggests that proximity to direct pollutant emission sources has a significant effect on the potency of the ambient aerosol. Several past studies have suggested that direct emissions are the main contributor to ambient mutagenicity. Pitts *(51)* showed a positive correlation between ambient mutagenicity and the concentrations of primary pollutants CO and NO, while seeing an anticorrelation with secondary photochemically generated pollutants such as ozone and peroxyacetyl nitrate

(PAN). Barale et al. *(53)* correlated mutagenicity with Pb, and found no spatial variation in ambient mutagenic potency normalized relative to Pb levels, leading to the conclusion that a ubiquitous emitter like automobile exhaust was the main source of mutagens. The only strong indication of an increase in mutagenic potency during the late summer photochemical smog season occurs at Rubidoux during September-October. That sample in particular should be examined to see if it contains unusual quantities of mutagenic organics that could have been formed by atmospheric chemical reactions. At the other sites, if there are important mutagen-forming atmospheric reactions, then they must occur during cold as well as warm seasons. This has been hypothesized to be the case by Greenberg et al. *(36)* based on their study of the interseasonal variation of mutagen levels and organic compound mixtures in Newark, NJ.

Summary and Conclusions

Organic carbon concentrations and bacterial mutagens present in a set of 1993 bimonthly composited urban fine particulate air pollution samples from sites in the greater Los Angeles area have been measured. Organic aerosol concentrations were quantified by thermal evolution and combustion analysis while bacterial mutagens were determined by the *Salmonella typhimurium* TM677 forward mutation assay. Ambient fine particulate samples were collected for 24 hours every sixth day for the entire year of 1993 at four urban sites, including Long Beach, central Los Angeles, Azusa, and Rubidoux, and at an upwind background site on San Nicolas Island. Samples were collected using a high volume dichotomous virtual impactor. These fine particulate samples were composited bimonthly at the urban sites so that seasonal

variations could be observed. Long Beach and central Los Angeles are source-dominated urban areas that are expected to experience high concentrations of primary aerosol emitted directly from industry and from motor vehicles, while Azusa and Rubidoux are located farther downwind of the most densely populated areas and receive both transported primary air pollutant emissions plus the transformation products of atmospheric chemical reactions. San Nicolas Island is located off the coast of southern California and acts as a background air monitoring site.

Seasonal trends in organic particulate matter concentrations are observed that show a progressive increase in OC concentrations with downwind transport distance over the city in the summer accompanied by high winter concentrations during November-December at all sites within the urban area. This seasonal aerosol OC concentration trend can be explained by meteorological patterns that concentrate primary pollutants near their source in the western portion of the air basin in the winter combined with the increased secondary aerosol production that occurs during transport toward the downwind sites during the summer. The two sampling sites located in the western portion of the Los Angeles basin at Long Beach and central Los Angeles show a pronounced seasonal variation of –PMS bacterial mutagenicity per m^3 of air sampled with peak values in the winter and minimum values in the summer. The downwind site Rubidoux shows a high value of +PMS mutagenicity per m^3 of air sampled during the September-October peak photochemical smog period, which reflects both an elevated organic aerosol mass concentration and an elevated mutagenicity per μg of organic compounds during that 2-month period.

Significant spatial variations in the organic aerosol and mutagenicity data are apparent. Both the organic aerosol concentration and the mutagenicity of the aerosol per µg of organic carbon was much lower at the background site on San Nicolas Island than within the urban area. As a result, the bacterial mutagenicity per m^3 of air sampled at the background site on San Nicolas Island was more than an order of magnitude less than was observed at the urban locations, demonstrating that the city is indeed a source of mutagenic aerosol emissions. Within the urban area, average organic particulate matter concentrations during 1993 were highest at the most inland site at Rubidoux, but the mutagenicity per µg of organic carbon in the aerosol was highest at those monitoring sites closest to the major primary air pollution sources at Long Beach and at central Los Angeles. As a result, the highest values for mutagenicity per m^3 of air sampled were observed at central Los Angeles both ±PMS. These findings seem to stress the importance of the direct emissions of bacterial mutagens from the major primary sources in the most heavily populated and industrial areas in the western portion of the Los Angeles area. Since mutagenicity per unit aerosol mass is not obviously higher at most sites during the summer photochemical smog season, the results imply that if important mutagen-forming atmospheric reactions occur then they must occur in the cold seasons as well as the warm seasons. Further chemical analysis of subfractions of these ambient samples is planned in order help to identify the specific chemical compounds or groups of compounds present in these samples that produce the mutagenic response quantified here.

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Chapter 4

Seasonal and Spatial Variation of the Human Cell Mutagenicity of Fine Organic Aerosol in Southern California

Introduction

It has been known for many years that the death rate due to lung cancer is higher among residents in urban areas than among those living in the countryside *(1, 2)*. Recent surveys of epidemiological data place the smoking-adjusted risk of lung cancer among urban populations at up to 1.5 times that of rural residents *(3)*. The extent to which this excess lung cancer incidence may be caused by exposure to urban air pollution remains uncertain, but it is clear that chemical mutagens and known human carcinogens, particularly PAH, plus oxy-PAH and nitro-PAH are present in urban air *(4)*.

Short-term bioassays can be used to detect the presence of chemical mutagens in atmospheric particulate matter samples and to quantify the relative potency of chemical substances isolated from those samples. Extracts of organic particulate matter filtered from ambient air repeatedly have been shown to be mutagenic when presented to bacterial mutation assays *(5-8)*. Extrapolation of the observed effects from bacteria to human cells is problematic and continually leads to questions about the relevance of bacterial assays. In response to such concerns new mutation assay procedures are under development that seek to

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Reference: Hannigan, MP; Cass, GR; Penman, BW; Crespi, CL; Lafleur, AL; Busby, WF, Jr; Thilly, WG *Environ Sci Technol*, 1997, 31, 438-447.

use cultured human cell lines as the means for exploring the mutagenic activity of organic chemical substances. In the present work we use one such human cell mutation assay to explore the mutagenic activity of respirable airborne fine particulate matter collected from air monitoring sites in Southern California.

Ambient organic particulate matter is a complex mixture of the emissions from many different air pollution sources which is altered chemically through numerous pathways following its release to the atmosphere. Three key factors that govern particle concentration and chemical composition include emission source characteristics, atmospheric mixing, and chemical reaction pathways; each of these processes are highly dependent on location or meteorology. Since different locations and different weather patterns create different ambient organic particulate mixtures, investigation of the mutagenicity differences that occur between various locations during different weather patterns may indicate the nature and location of the most important factors governing mutagen concentrations in the atmosphere. Several researchers have used bacterial assays to probe the seasonal and spatial character of airborne mutagen concentrations *(9, 10)*. It has been shown that the ambient particulate matter collected at different urban sites exhibits different levels of bacterial mutagenicity. Tokiwa et al. *(7)* found that organic extracts from aerosol samples collected near industrial sites were roughly an order-of-magnitude (per volume of air sampled) more effective at inducing bacterial mutations than aerosol samples from residential sites in Japan. Lewtas et al. *(11)* found interurban differences in the mutagenicity of aerosol samples collected from two urban sites in the United States. Butler et al. *(12)* discovered aerosol mutagenicity differences between cities in 6 different countries. Studies have also found bacterial mutagenicity to

vary with season, in Scandinavia *(13)*, in the San Francisco Bay area *(14)*, in northwest Italy *(15)*, and in Newark, NJ *(16)*. In our previous work *(17)*, little seasonal variation was seen in the bacterial mutagenic potency (mutagenicity per µg organic material supplied to the assay) of 1993 fine urban organic aerosol samples from the Los Angeles area; however, definite spatial variation was observed with the highest bacterial mutagen concentrations found in the immediate vicinity of the densest collections of emission sources, leading us to conclude that direct emissions of mutagenic organics from primary emission sources are very important to the bacterial mutagenicity of the ambient aerosol. Human cell assays *(18-22)* have been used both to investigate the mutagenicity of organic compounds *(23-25)* and to detect mutagens in some environmental samples such as diesel soot *(26)* , freshwater pond sediments *(27, 28)* , and National Institute of Standards and Technology (NIST) urban dust standard reference material (SRM 1649)*(29)*. There has yet to be a comparative study of the human cell mutagenicity of ambient aerosol samples collected by community air monitoring networks.

The purpose of the present paper is to report a study of the human cell mutagenicity of a comprehensive set of 1993 urban fine organic aerosol samples. Five air monitoring sites were studied, including 1 background station located on an offshore island upwind of the metropolitan Los Angeles area and 4 urban stations within Southern California, each chosen for either its proximity to major primary air pollutant emission sources or for its location as a downwind photochemical smog receptor site. A time series of bimonthly composites of filter samples was assembled at each urban site to enable a comparison of seasonal and spatial trends in the human cell mutagenicity of the aerosol samples. To

further enable spatial and seasonal comparison, all samples were collected, stored, extracted, and tested under identical conditions. The human cell mutation assay used in this study *(22)* tests mutagenic activity at the thymidine kinase locus in h1A1v2 cells using a 72 hr exposure. h1A1v2 cells are AHH-1 TK+/- cells bearing the plasmid pHSRAA. The plasmid pHSRAA contains two copies of the human CYP1A1 cDNA and confers resistance to 1-histidinol. The entire metabolic content of human lung epithelial cells is not completely resolved at present and we make no claim that this assay exactly replicates the response of human lung tissue. However, CYP1A1 is the P450 with the highest known capability to activate PAH, which leads to a sensitive human cell assay. On the basis of studies with pure compounds *(25)* which showed that several polycyclic aromatic hydrocarbons (PAH) and nitroPAH will induce mutations of the h1A1v2 cells, plus studies on environmental mixtures *(29)*, we believe this assay to be a useful screening tool for the detection of a wide range of compounds that are mutagenic to human cells.

Experimental Section

Sample Collection

Airborne particulate matter samples were collected from 4 urban sites in the Los Angeles area and from 1 background site located off the coast of Southern California, as shown in Figure 4.1. The background air monitoring station was placed on San Nicolas Island, upwind of Los Angeles. The 4 urban sites were chosen because each has a different characteristic exposure to ambient aerosol sources. The central Los Angeles air monitoring site is located within a ring of freeways and experiences high concentrations of the pollutants

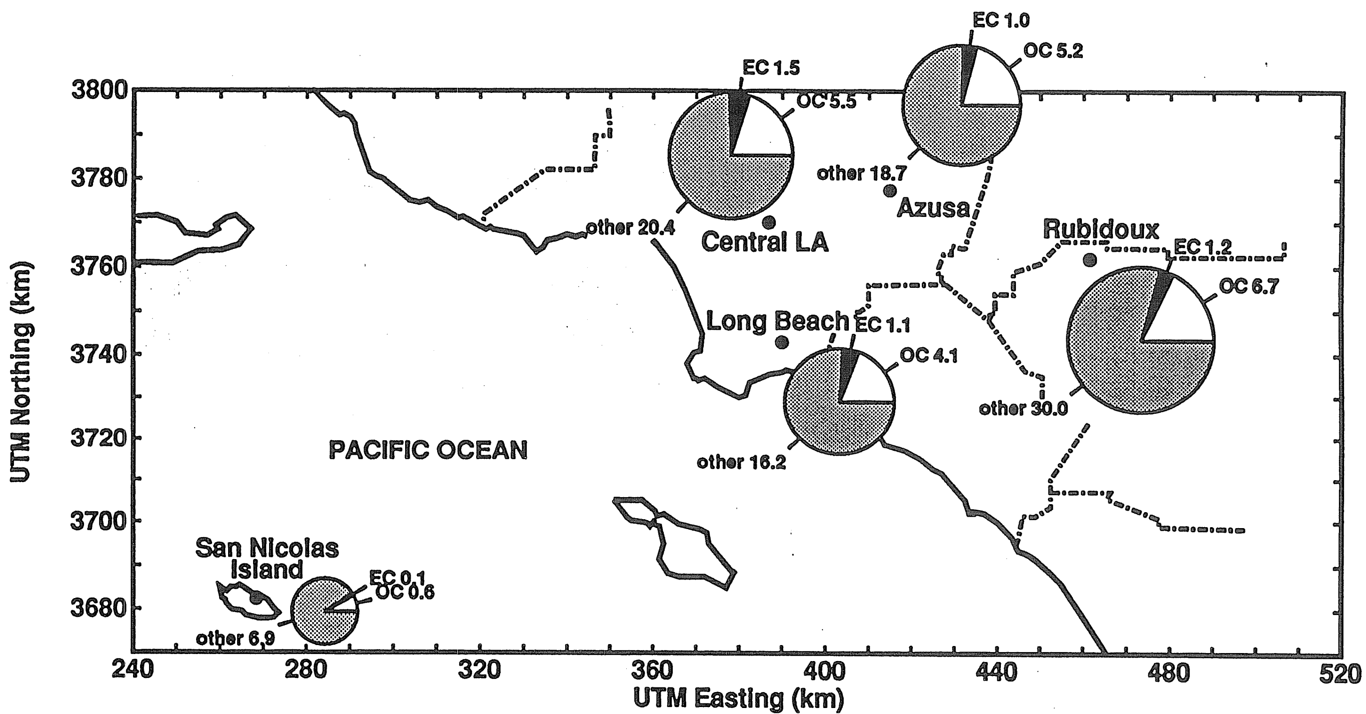

Figure 4.1. Southern California, showing the locations of the ambient monitoring stations. Pie graphs adjacent to each site show the 1993 annual average fine particulate organic carbon concentration and elemental carbon concentration and their relative contribution to the total fine aerosol mass concentration. Units are in microgram per cubic meter, and the area of the pie chart is in proportion to the fine mass concentration.

emitted directly from motor vehicles. Long Beach is located in close proximity to the direct emissions from industrial sources in Southern California, which include many power plants, petroleum refineries, and the Los Angeles - Long Beach harbor complex. Azusa is known as a receptor site for the high ozone levels that occur downwind of central Los Angeles and is characterized by relatively high levels of atmospherically transformed, or secondary, aerosol. Rubidoux is located farther downwind, and generally receives even more secondary aerosol than Azusa. At all 4 of the urban sites, air monitoring equipment used in the present experiments was located at the South Coast Air Quality Management District (SCAQMD) air monitoring stations in the communities named.

Ambient particulate matter samples were taken for 24 h every sixth day for the entire year of 1993 at the four urban sites. This every sixth day sampling calendar coincided with the SCAQMD and national particulate matter sampling schedule. Eighteen samples were collected throughout the year at the background site when access to San Nicolas Island was possible; these samples were spaced at least seven days apart.

The sampling equipment at each air monitoring station incorporated a high volume dichotomous virtual impactor and a separate low volume aerosol sampler. The low volume sampler is described in detail elsewhere *(30, 17)*. Fine particle mass concentration data reported in the present paper are obtained by gravimetric determination of the aerosol mass collected by the low volume sampler on 47 mm diameter Teflon filters (Gelman Teflo) located downstream of an AIHL cyclone separator that removes particles with aerodynamic diameter greater than 2 μm. The high volume dichotomous virtual impactor is described in detail by Solomon et al. *(31)* . This sampler can collect a large quantity of size-

separated organic aerosol over the course of a single day. In the high volume virtual impactor, ambient air at a flow rate of nominally 300 lpm is accelerated through a converging nozzle. 90% of the flow is forced to make an abrupt 90° turn; the fine particles smaller than 3-4 μm aerodynamic diameter follow the fluid streamlines and in that way 90% of the fine particles are collected on a fine particle filter at a flow rate of nominally 270 lpm. The coarse particles larger than 3-4 μm aerodynamic diameter do not follow the turn but instead are concentrated into the remaining 30 lpm of the inlet air flow and along with 10% of the fine particles are collected on a coarse particle filter which is located downstream of a diverging nozzle that is aligned with the major axis of the inlet of the sampler.

Quartz fiber filters (102 mm diameter Pallflex Tissuquartz 2500 QAO) were used within the high volume dichotomous sampler for both coarse and fine particulate matter collection. All quartz fiber filters were prebaked for at least 6 h prior to use at 750 °C to lower their carbon blank. Each filter was loaded the day prior to sampling, and unloaded on the day after sampling. Field and laboratory blanks were also taken to ensure that there was minimal contamination of the ambient sampling system. The 102 mm diameter quartz fiber filters were transported to the sampling sites in prebaked aluminum foil, and brought back to the laboratory in annealed glass jars with solvent-washed Teflon-lined lids. Upon return to the laboratory all filters were stored in a freezer at -21 °C.

The respirable fine particulate matter samples from the high volume dichotomous virtual impactor were allocated as follows: one twelfth of each filter was used to measure the atmospheric particulate organic carbon (OC) and elemental carbon (EC) mass concentration by thermal evolution and combustion analysis *(32, 33)*, one quarter of each filter was used for organic chemical

analysis, one quarter of each filter was used for bacterial mutagenicity assays, one twelfth of each filter was used for the human cell mutagenicity assays reported here, one sixth of each filter was used to make a 1993 urban composite for later bioassay-directed chemical analysis, and one sixth of each filter was placed in storage. The human cell mutagenicity assay used here requires approximately 500 µg of organic carbon per test, and generally, duplicate tests are performed. Individual fine particulate matter samples were not large enough for such analyses. In order to assemble enough sample mass for use in the bioassays while still retaining samples that can track the seasonal variation of aerosol properties, filter portions were pooled to create bimonthly composites at each urban air monitoring site. Due to the very low pollutant concentrations at the San Nicolas Island background sampling station, filter sections were combined to create a winter and a summer composite sample at that site. All sample organic aerosol mass is reported in units of µg of equivalent organic carbon (EOC), which is defined as the amount of organic carbon present on the filter composites prior to extraction as determined by thermal evolution and combustion analysis of sections of the same quartz fiber filters. This measure provides a direct connection from the human cell bioassays back to the ambient carbonaceous aerosol concentrations.

Extraction and Concentration

A detailed description of sample extraction and concentration procedures is given by Hannigan et al. *(34)*. The extraction procedure chosen for this study involved soxhlet extraction with dichloromethane (DCM) for at least 16 h. A vacuum centrifuge was used to concentrate all DCM extracts down to a volume

of approximately 1 mL. The sample portion designated for the human cell assay was then exchanged into dimethyl sulfoxide (DMSO) by adding DMSO to the DCM extract and then blowing a gentle stream of N_2 over the extract until the volume was reduced to the volume of DMSO added. To enable comparison between traditional measures of extracted mass as an indication of the quantity of organic compounds present versus our measure of organic carbon (EOC) as determined by thermal evolution and combustion analysis prior to sample extraction, the extracted mass from an ambient particulate matter test sample was measured both by the thermal evolution and combustion method and by a microscale evaporation method *(35)*. The results of this test showed 0.93 µg of extracted mass per µg of EOC.

Human Cell Mutation Assay

Procedures for the routine use of the h1A1v2 cell line for mutagenicity testing at the thymidine kinase locus have been described in detail elsewhere *(22)*. Testing was performed by exposing duplicate 12 mL cultures of 1.8×10^6 exponentially growing cells to aliquots of sample extract for 72 hrs. All extracts were in DMSO. Each entire culture was then centrifuged and resuspended in fresh media (30 mL) and the cultures were counted. One day after termination of the exposure, the cultures were counted and diluted to 80 mL at 2×10^5 cells mL^{-1}. After the 3 day phenotypic expression period, cultures were plated in the presence of trifluorothymidine (3, 96 well microtiter plates, 20,000 cells per well) and in the absence of selective conditions (2, 96 well microtiter plates, 2 cells per well). The positive control was 1.0 µg mL^{-1} benzo[*a*]pyrene, and DMSO was used as the negative control. Mutant fractions and their associated confidence

intervals (standard deviations) were calculated using methods described elsewhere *(36)*. Each sample was quantitatively analyzed for mutagenic response by pooling the results from each experiment done on that sample. To permit the pooling of all replicate experiments on each sample despite varying background mutation rates (i.e. variations in the concurrent negative controls from one experiment to another), the results from all experiments were converted to induced mutant fraction by subtracting the mean mutant fraction of the concurrent negative control from the mean mutant fraction observed for the filter sample extracts. The mutagenic potency of each sample (mutant fraction per μg EOC supplied to the test) was estimated quantitatively by determining the slope of the induced mutant fraction vs. dose relationship. That slope was estimated by pooling all experimental points for each sample and then computing the slope of the dose/response relationship using a least-squares fit to the data that was forced through the origin (because at zero dose, there is by definition zero induced mutant fraction).

Results and Discussion

Fine Particulate Matter Measurements

Figure 4.1 shows the 1993 annual average fine particulate (black) elemental carbon (EC) concentrations and organic carbon (OC) concentrations in relation to the total fine particulate mass concentrations at the Southern California air sampling sites. The total area of each circular graph in Figure 4.1 is proportional to the annual average fine particle mass concentration and the relative fraction of the total fine particle burden that is contributed by carbonaceous aerosol is indicated by the size of the pie shaped wedges. Annual

averages are computed as the arithmetic mean of 6 bimonthly concentration values. The bimonthly concentration values are based on the arithmetic average of all 24-hr filter samples that were composited for the purposes of the bioassays associated with each bimonthly period. At the background site, San Nicolas Island, which represents the aerosol present in air masses before they reach the coast of Southern California, EC, OC, and fine aerosol mass concentrations are all very low, averaging only 0.1, 0.6, and 7.6 $\mu g\ m^{-3}$, respectively, over the year. Aerosol carbon (EC+OC) makes up 9% of the fine particle mass concentration at San Nicolas Island, and the OC/fine aerosol ratio is 0.08. The EC, OC, and fine particle concentrations all increase as the air masses move onshore. At each of the urban air monitoring sites, aerosol carbon accounts for very close to one quarter of the total fine particle mass concentration. The highest black EC concentrations occur at central Los Angeles near the location of the highest density of diesel-powered vehicle traffic. The highest fine particle mass concentrations and highest organic carbon concentrations occur at the farthest downwind receptor site at Rubidoux. Organic carbon as a fraction of the fine organic aerosol mass is fairly constant over the urban area, accounting for 21%, 19%, 20%, and 18% of the fine particle mass concentrations at Long Beach, central Los Angeles, Azusa, and Rubidoux, respectively. Organic compounds present in the aerosol contain hydrogen, oxygen, and nitrogen in addition to organic carbon, and the Los Angeles area aerosol is rich in oxygenated organics due to the secondary atmospheric chemical reactions that occur in the photochemical smog *(37, 38)*. Organic compound mass concentrations in the Los Angeles air are estimated to equal 1.2-1.4 times the mass of organic carbon

present *(39)*. Thus approximately 22-29% of the fine aerosol mass in the Los Angeles area in 1993 consisted of organic compounds.

Figure 4.2 shows the seasonal variation in EC and OC concentrations in relation to fine particle mass concentrations at all sampling sites. The Long Beach aerosol shows a substantial seasonal variation in EC, OC, and fine particle mass concentrations, with maxima in the winter and a minimum in the late spring and early summer. The aerosol measured at central Los Angeles shows a seasonal variation in EC and OC concentrations similar to that at Long Beach; the maximum is during November-December and the minimum is during the summer. The relative seasonal variation in EC, OC, and fine particle mass concentrations is different at the farthest inland sites: the inland sites do not show a pronounced wintertime maxima and summertime minima. Instead, little variation in OC levels is seen with season, while fine particle mass concentrations at both Azusa and Rubidoux show a relative peak during September-October (which was the peak photochemical smog season in 1993), while Rubidoux also experiences high fine particle mass concentrations during March-April of 1993.

A discussion of the causes of the observed seasonal and spatial patterns present in the fine particle OC concentration data base for the Los Angeles area is given by Hannigan et al. *(17)*. The seasonal and spatial EC and fine OC concentration variations observed during 1993 are explained by the seasonal changes in wind speed, wind direction, mixing depth and secondary aerosol formation rates. For purposes of the present discussion, it is important to note that changes in EC concentrations over time are a good measure of changes in the degree of dilution of primary particles emitted directly from area-wide

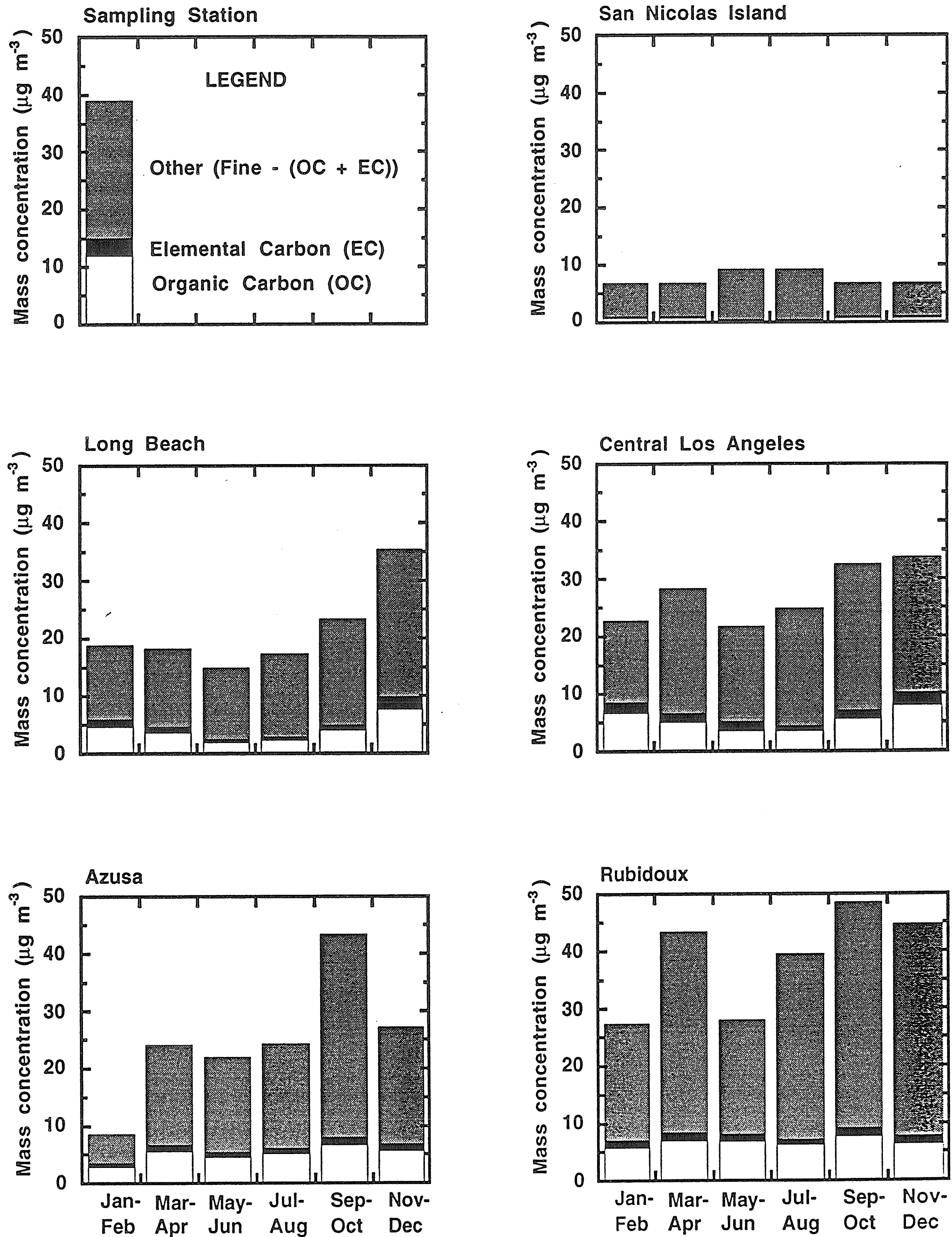

Figure 4.2. Seasonal variation of the fine particulate organic carbon, elemental carbon, and fine particle mass concentrations during 1993 at San Nicolas Is., Long Beach, central Los Angeles, Azusa, and Rubidoux.

sources, because EC particles are chemically stable, they are too small to settle out of the atmosphere rapidly, and in Los Angeles their concentration is dominated by area-wide emissions from diesel engines that do not show a pronounced seasonal variation in emission rate *(40)*. In Long Beach and central Los Angeles, the ratio of the annual average EC to the annual average OC is 0.26. The ratio of the annual average EC to the annual average OC in Azusa and Rubidoux are at a lower value, 0.20 at Azusa and 0.18 at Rubidoux. The lower EC/OC ratio at these two sites would indicate either a slightly different ratio of diesel exhaust to primary organic aerosol emission rate for the sources located near Azusa and Rubidoux when compared to Long Beach and central Los Angeles or a persistent enrichment at Azusa and Rubidoux in secondary organic aerosol formed from the products of atmospheric chemical reactions involving vapor-phase organics.

Mutagenicity of Ambient Samples in the Human Cell Assay

The fine particulate matter samples were extracted, and the extracts were combined to form bimonthly composites at the four urban sites and semiannual composites at the background site on San Nicolas Island. The bimonthly composites at each urban site, and one of the semiannual composites from the background site (only the wintertime composite contained enough organic carbon), were tested for mutagenicity at the tk locus of h1A1v2 cells using a 72 hr exposure. All mutation assays performed on each bimonthly composite sample were pooled, and the mutagenic potency (mutant fraction per μg EOC supplied to the assay) of each sample was estimated by a least squares fit to the dose/response data, as described earlier. Dose-response curves estimated for

the samples taken at the urban air monitoring sites appear in Figure 4.3. When there are two points, with error bars, at an assay dose of 120 μg EOC per 12 mL assay, for example, these two points show that two separate assays were done on separate days at that dose with each assay consisting of a pair of duplicate tests. The heavy solid lines in each plot represent a least-squares linear fit to the data (forced through the origin as discussed earlier); the slope of that line provides the mutagenic potency estimate for each bimonthly aerosol composite. A measure of the reproducibility of this assay when applied to the organics from airborne particles can be obtained by determining the slopes (i.e. mutagenic potencies) of the dose/response curves from individual independent tests of single samples and then comparing these slopes. This has been done for the five samples taken at Azusa for which slope determinations are available from three individual independent multipoint assays of each sample. The standard deviation of the three independent slope determinations ranges from ±7% to ±51% (average ±32%) of the slope obtained when all data for a single sample are pooled prior to analysis as has been done throughout this paper. A measure of the sensitivity of this assay when applied to the complex mixture of organics from airborne particles can be described by determining the minimum detectable mutagen concentration (MDMC) which is the minimum quantity of the atmospheric organic mixture needed to produce a positive mutagenic activity that exceeds the 95% confidence limit of the concurrent negative control sample and also exceeds the 99% confidence limit of the historical negative control sample *(23, 25)*. The MDMC for a typical 1993 Los Angeles area sample is 25 μg EOC supplied to each 12 mL assay which can typically be obtained from 5 m^3 of ambient air in Los Angeles. A procedural blank sample was carried through all

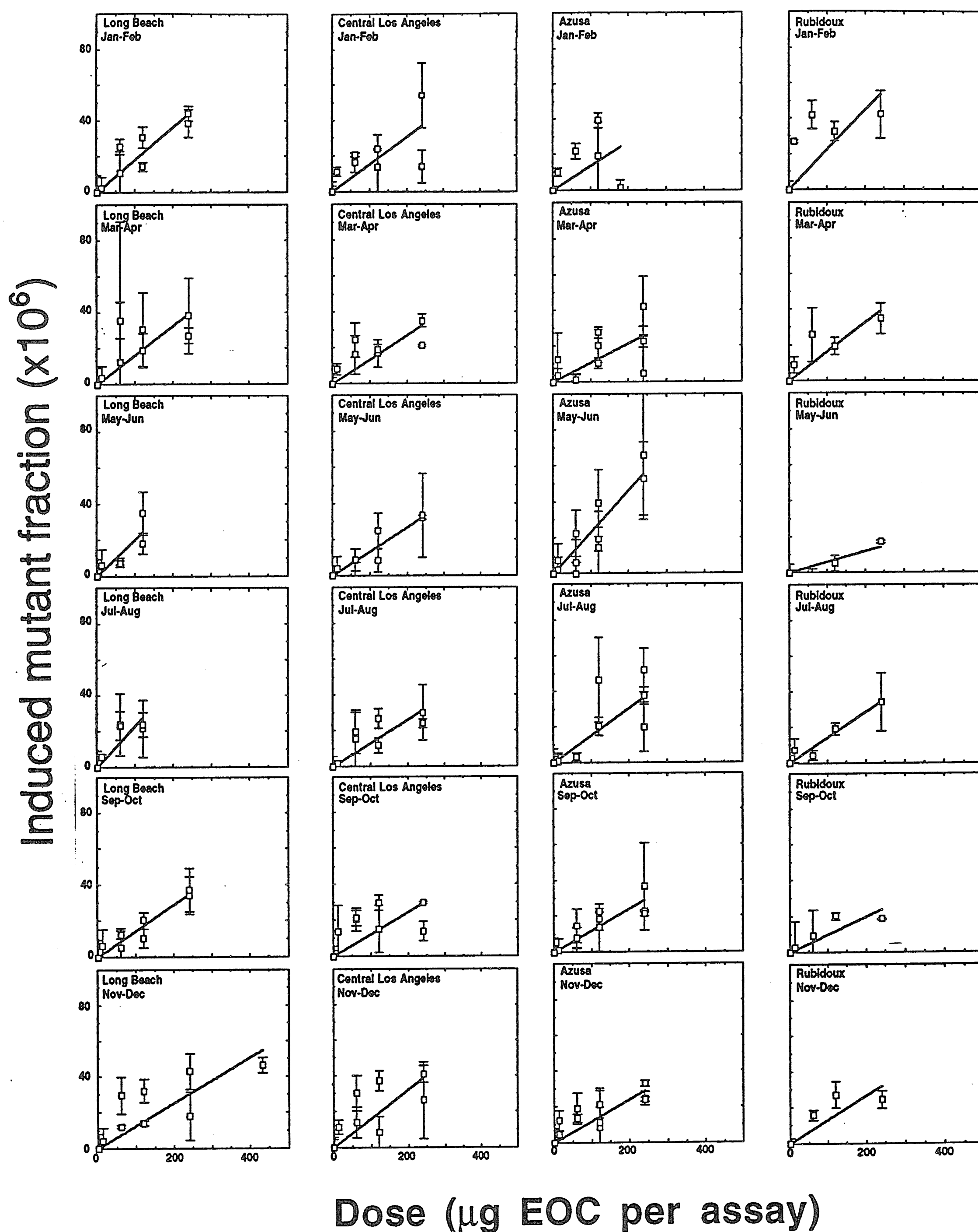

Figure 4.3. Dose-respone curves for each sample studied. Multiple points at a given dose represent independent experiments at that dose, with error bars = 1 SD. Least squares linear fit (forced thru origin) is shown.

phases of field sampling and laboratory analysis except for drawing urban air through the filter. The extract of the procedural blank sample produced a dose/response curve with a slope that is insignificantly different from zero.

All samples tested during this study met the statistical criteria for a positive response at at least one dose tested and are considered to be mutagenic. Figure 4.4 shows the time series of the mutagenic potency estimates at each of the urban sampling sites. Error bars represent ± one standard error of the slope parameter estimate resulting from the least-squares fit. There is no significant seasonal variation in the mutagenic potency estimates derived from the time series of samples taken at Long Beach; all bimonthly composite mutagenic potency values are within $\pm 2\sigma$ of the annual average mutagenic potency. Figure 4.4b shows the seasonal variation of mutagenic potency at central Los Angeles. Again, no statistically significant seasonal variation is observed. Figure 4.4c shows the seasonal variation of mutagenic potency at Azusa. With the exception of the May-Jun sample, no statistically significant seasonal variation is observed. Figure 4.4d shows the time series of aerosol extract mutagenic potency at Rubidoux. The Jan-Feb sample shows a high mutagenic potency, but large error bars also, which indicates that the dose-response curve for this particular sample does not conform well to the linear slope/zero intercept hypothesis built into the induced mutant fraction calculation. We have also evaluated the winter samples at Azusa and Rubidoux in this study using the non-linear MDMC model *(23, 25)* and the Rubidoux Jan-Feb sample is the only sample where a significantly higher mutagenic potency might be discovered by more testing at lower doses. Even if further analysis were to show that this single sample is an outlier, that one outlier would not change the broad based conclusions of this study. This sample would

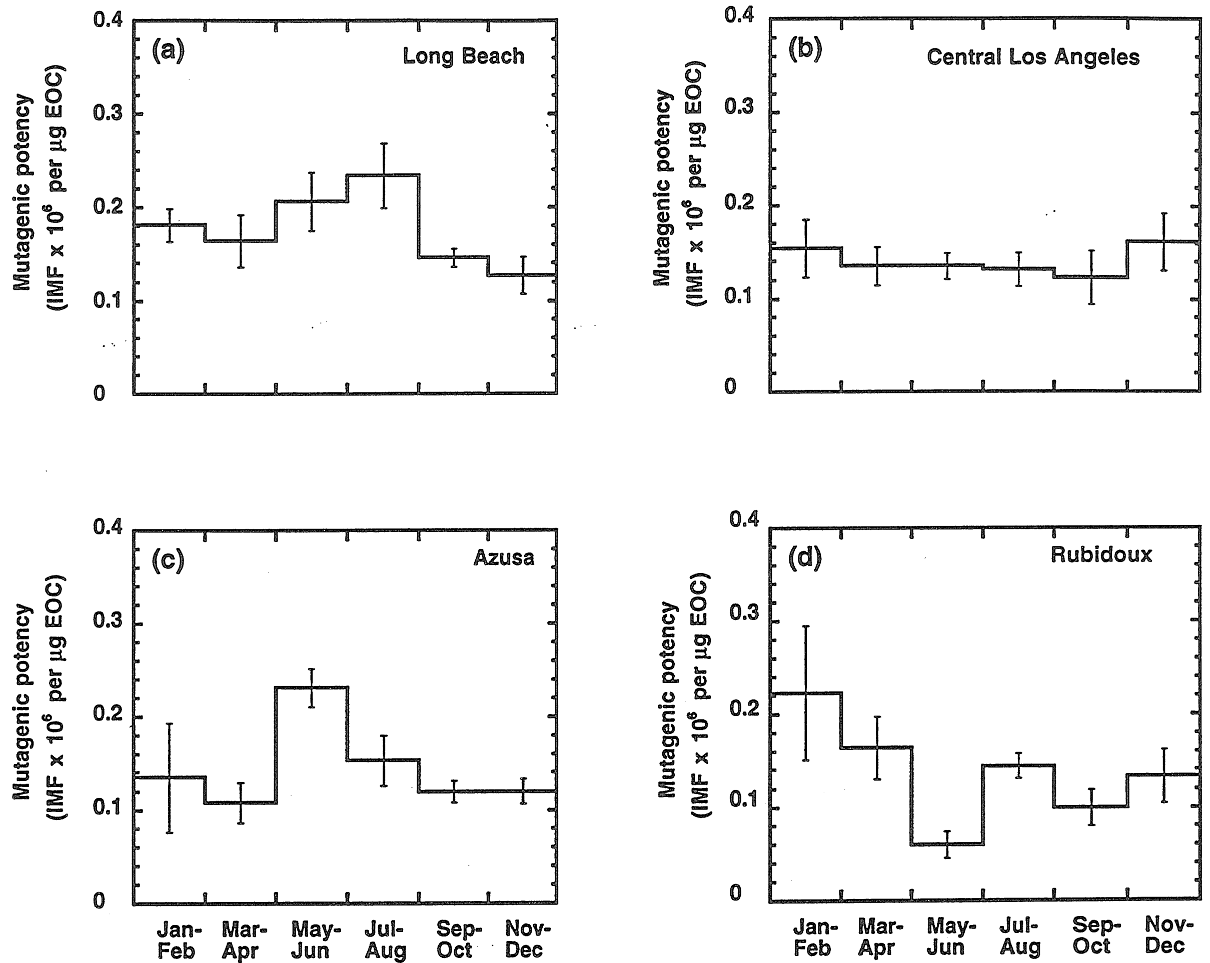

Figure 4.4. Mutagenic potency of the fine particulate matter in the South Coast Air Basin during 1993 at Long Beach, central Los Angeles, Azusa, and Rubidoux. Error bars = 1 SD.

be of interest for further study. The May-Jun sample at Rubidoux exhibits a lower mutagenic potency than the rest of the samples tested, which otherwise exhibit no statistically significant departure from the annual mean mutagenic potency.

Within the data sets at each of the monitoring sites, the mutagenic potencies of each bimonthly composite fall within $\pm 2\sigma$ of the annual average at that site with the exception of the May-June samples at the inland sites at Azusa and Rubidoux. The May-June samples at Azusa and Rubidoux depart from typical values, but in opposite directions: that sample at Azusa is higher than usual, while at Rubidoux the mutagenic potency of the May-June sample is unusually low. Therefore, we conclude that there is no systematic seasonal pattern of changes in mutagenicity per unit dose of EOC mass supplied to the test that can be observed across these sites, within the variability of the assay. We observed a similar lack of seasonal variation in mutagenic potency for the same samples tested in a bacterial assay (a *Salmonella typhimurium* TM677 forward mutation assay) *(17)*. This lack of a pronounced seasonal variation in the mutagenic potency of the aerosol samples is an interesting result, as it suggests that the proportion of mutagenic compounds within the fine organic aerosol mass may not change significantly with season at these sites. This further suggests that the most important human cell mutagens in the Los Angeles airborne particulate matter are not dominated by emission sources that show substantial seasonal variations (e.g. wood combustion), nor is the mutagenic potency apparently changed greatly by the secondary aerosol production reactions which show a pronounced effect on the overall fine particle and OC concentrations at the downwind sites, Azusa and Rubidoux, during the September-October, 1993, photochemical smog season. Therefore, the

important human cell mutagens in the Los Angeles airborne particulate matter likely are released as part of the primary particulate emissions from sources which operate on a year-round basis, and if the atmospheric transformation products of photochemical reactions are important then these reactions must occur in the winter as well as during the summer photochemical smog season.

Another important result can be observed in Figure 4.5a. The annual average mutagenic potencies of the organic aerosol measured at central Los Angeles, Azusa, and Rubidoux are virtually identical. The average mutagenic potency of the aerosol at Long Beach is significantly greater (with 95% confidence) than that at the other sites in a statistical sense, but as a practical matter the mutagenic potency values average only about 25% greater than those elsewhere in the air basin. This similarity of mutagenic potency values across widely separated monitoring sites suggests that the human cell mutagenicity of the aerosol is due largely to ubiquitous emission sources (e.g. motor vehicle traffic or stationary source fuel combustion) rather than to proximity to isolated point sources of unusual mutagenic organic compounds. Particle phase probable human carcinogens and known animal carcinogens as classified by IARC include benzo[*a*]pyrene, benzo[*b*]fluoranthene, and indeno[*1,2,3-cd*]pyrene. Schauer et al. (41) show that the ambient concentrations of these compounds in the Los Angeles atmosphere are primarily due to automobile exhaust and residential natural gas combustion. Thus it is known from prior work that the atmospheric concentrations of some of the particulate animal carcinogens and probable human carcinogens are governed by such ubiquitous emission sources; the present work extends that observation to human cell mutagens as well.

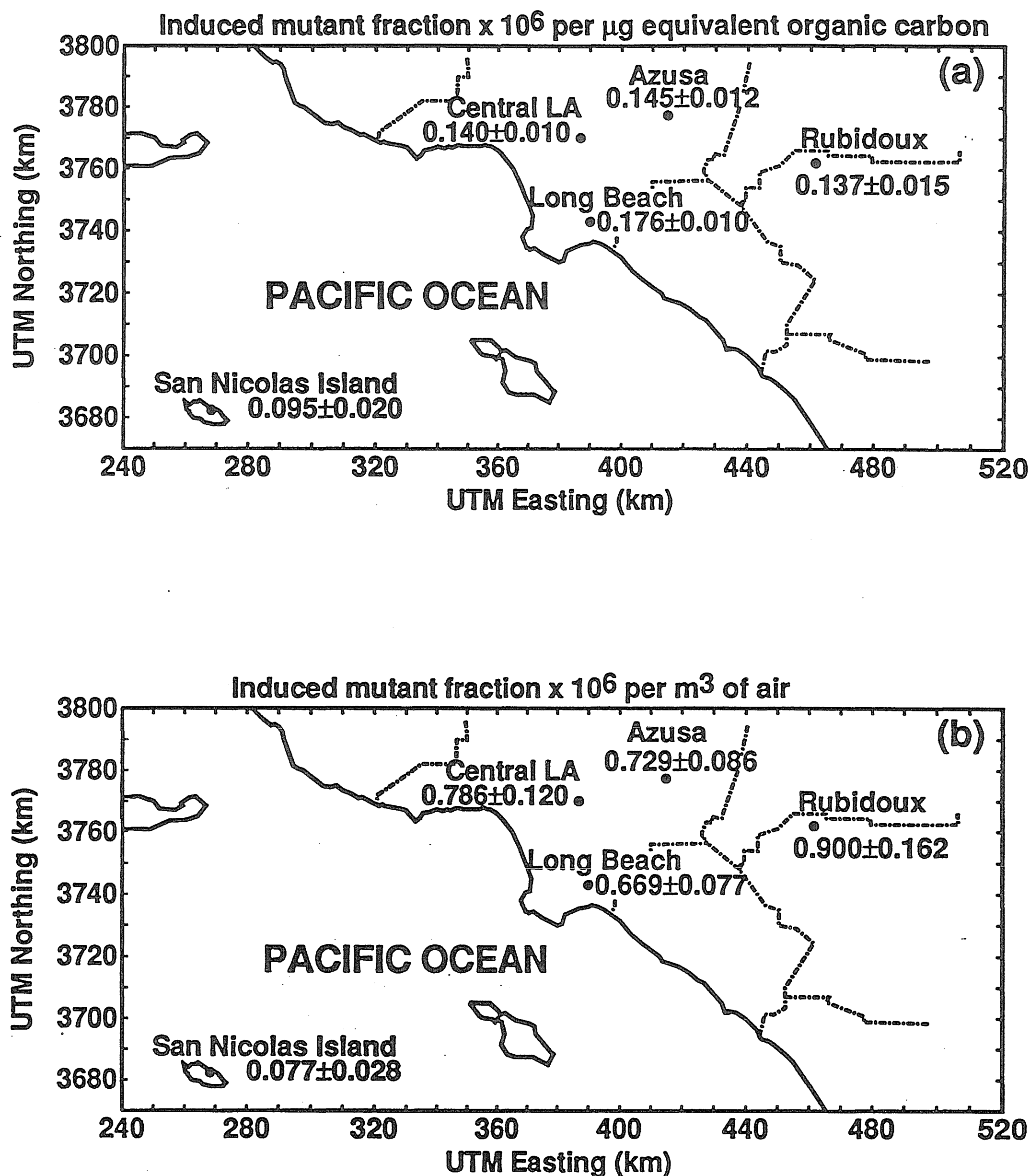

Figure 4.5. a. Spatial variation of 1993 annual average mutagenic potency across the South Coast Air Basin. b. Spatial variation of 1993 annual average mutagen density across the South Coast Air Basin.

There are two ways that the results from the h1A1v2 can be compared to the results obtained from previous bacterial assay systems. First, pure compounds can be tested in both assays. Second, both assays can be applied to evaluate the same atmospheric complex mixtures of organics. Both approaches are described here.

The mutagenic potencies of the nitro-PAH tested to date fall entirely within the range of the potencies of the ordinary PAH in the h1A1v2 human cell assay. For example, the minimum mutagenic compound concentrations (nmol/mL) in liquid suspension within the assay needed to produce a positive response in the assay is in the range: 21 for 3-nitrofluoranthene; 17 for 9-nitroanthracene; 2.2 to negative for the mono-nitropyrenes; 1.1 to 21 for the nitrofluoranthenes, and 0.2×10^{-2} to 1.8 for the dinitropyrenes *(25)*. The nitro-PAH act as promutagens in this assay *(24)*. The minimum concentration needed to produce a response for the ordinary PAH in the same human cell assay spans the range from 0.8×10^{-2} for cyclopenta[*cd*]pyrene to 5.5×10^{-2} for benzo[*a*]pyrene, 0.48 for benzo[*k*]fluoranthene, to 3.3 for chrysene, to no mutagenic response for fluoranthene and pyrene for example *(25)*. In contrast, some nitro-PAH are extraordinarily potent in mutating the *S. typhimurium* used in bacterial assays when compared to the ordinary PAH. For example, the minimum mutagenic compound concentration in liquid suspension within the *S. typhimurium* forward mutation assay of Skopek et al. *(10, 42)* needed to produce a positive result (–PMS) for 1,8-dinitropyrene is only 5×10^{-4} nmol/mL, much lower than for ordinary PAH like benzo[*a*]pyrene which requires 5.8 nmol/mL in that bacterial assay (+PMS) in order to produce a positive mutagenic response *(43, 24)*. Since nitro-PAH are present at much lower concentration in the atmosphere than the

ordinary PAH, when considering both potency and concentration the contribution of the nitro-PAH relative to the ordinary PAH in h1A1v2 human cell mutagenicity assays of urban air samples, is expected to be small.

The annual average human cell mutagenic potencies of the organic aerosol at the 5 monitoring stations in the present study can be compared to the mutagenic potency values generated using the bacterial assay system described by Skopek et al. *(10, 42)* applied to exactly the same atmospheric samples (17). The ratios of h1A1v2 assay mutagenic potencies (induced mutant fraction x 10^6 per μg EOC per 12 mL assay) to bacterial assay mutagenic potencies (mutant fraction x 10^5 per μg EOC per 100 μL assay) are as follows: (–PMS) Long Beach, 0.60; central Los Angeles, 0.56; Azusa, 0.75; Rubidoux, 1.27; San Nicolas Island, 1.92; (+PMS) Long Beach, 2.6; central Los Angeles, 1.5; Azusa, 3.4; Rubidoux, 3.0; San Nicolas Island, 14. Since little spatial variation of mutagenic potency is observed with the h1A1v2 assay, the spatial variation in the ratios of the two assays shows that in the bacterial assay system there is a decrease in the potency of the aerosol as one moves away from the urban center. Certain nitro-PAH that can be formed by atmospheric chemical reactions are very potent bacterial mutagens, suggesting that the secondary aerosol formed by atmospheric chemical reactions may be an important contributor to the mutagenicity of airborne particulate matter *(44-46)*. In that case, one would logically expect higher than average mutagenic potencies at the downwind photochemical smog receptor sites (Azusa and Rubidoux) during the summer photochemical smog season. Given that the h1A1v2 human cells are not extraordinarily sensitive to nitro-PAH, it is not surprising that there is little difference in human cell mutagenic potency seen in the present study between

monitoring sites with high vs. low potential for secondary organic aerosols. However, bacterial assays, which are quite sensitive to such compounds, when applied to the same Southern California samples also fail to show a pronounced increase in mutagenic potency at downwind photochemical smog receptor sites during the summer (17); instead the highest potencies are found near the primary particle sources in the winter as discussed above. For that reason we have previously concluded (17) that if mutagens associated with secondary organic aerosols are of major importance to bacterial assays then those secondary mutagens must be distributed throughout the air basin (not just in the downwind areas) and must be present both in winter as well as during the summer months. The same conclusion now can be drawn based on the h1A1v2 human cell assay.

Pitts *et al. (47)*, Alfheim *et al. (13)*, and our previous study *(17)* observed significantly reduced or no mutagenic potency at background air monitoring sites as compared to urban air monitoring sites when measured using bacterial assays. The dose-response curve for the wintertime composite taken at San Nicolas Island is shown in Figure 4.6, and the mutagenic potency for this sample (not the annual average) is shown in Figure 4.5a. Data from the upwind background site on San Nicolas Island shows a wintertime mutagenic potency value in Figure 4.5a based on the human cell assay which is lower than the grand annual average over all the urban sites, but not by as much as one might expect from previous experiments performed at background sites using bacterial assay systems. The OC concentrations at the background site, as seen in Figures 4.1 and 4.2, are much lower than those at the urban sites, but OC is still present in the aerosol at San Nicolas Island. In previous work by Rogge et al. *(38)*, the fine particulate organic aerosol at San Nicolas Island was investigated.

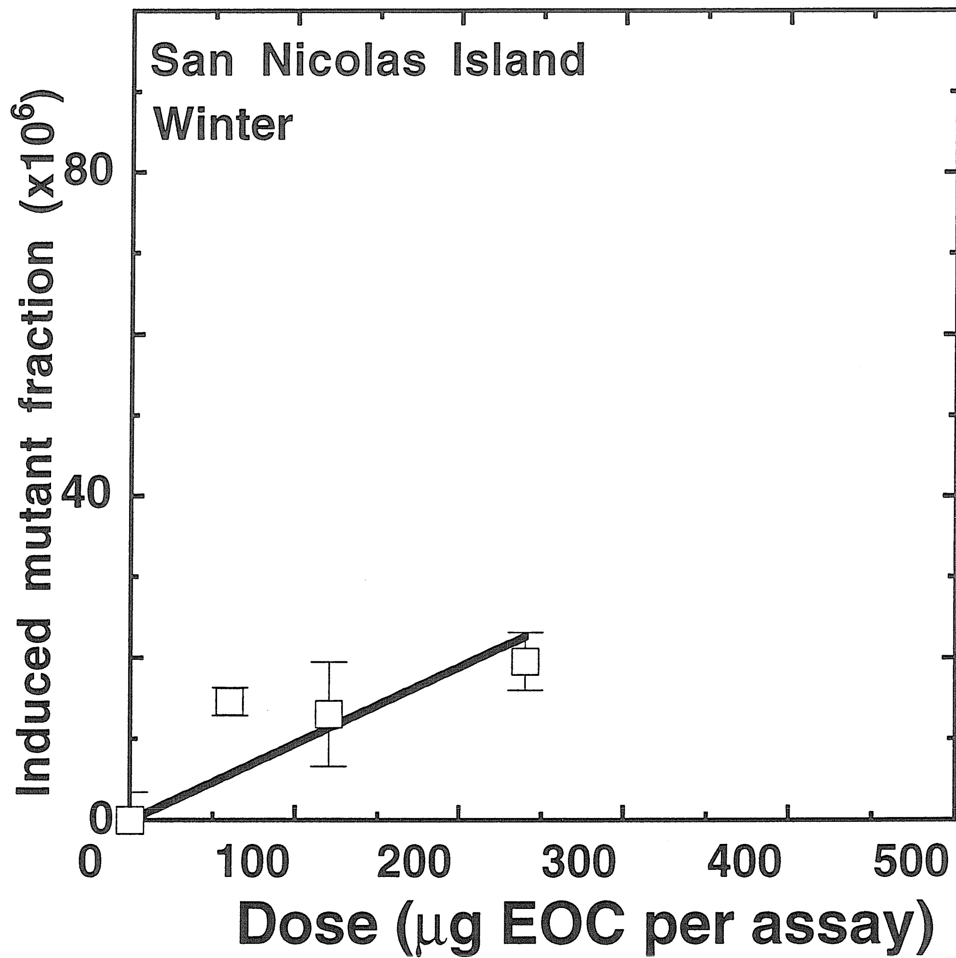

Figure 4.6. Dose-response curve for the wintertime San Nicolas Island sample.

Several PAH and other combustion by-products were identified; the OC at San Nicolas Island is a "regional background" aerosol, not solely a "natural background" aerosol, and it contains some combustion-generated OC.

The picture emerging from this analysis is that the human cell mutagenic potency of the fine aerosol (i.e. the mutagenic strength of a unit quantity of organic aerosol) in Southern California is largely independent of season and spatial location. The dose of particle phase mutagens inhaled by residents does however vary by location in proportion to the spatial and temporal variation in ambient organics mass concentration. The measure of mutagenic potency (mutagenicity per unit airborne fine organic particulate matter mass) can be converted to a measure that we will call mutagen density (mutagenicity per unit volume of air) by combining the airborne fine organic particulate matter mass concentrations shown in Figure 4.2 with the mutagenic potency values shown in Figures 4.4. These mutagen density values for each bimonthly period at each urban monitoring site are shown in Figure 4.7, and the annual average values are presented in Figure 4.5b. As expected since there is no pronounced seasonal variation of the mutagenic potency of a unit quantity of ambient fine organic aerosol but there is a characteristic seasonal pattern for the ambient OC concentration changes at Long Beach and central Los Angeles, mutagen density (mutagenicity per unit air volume) exhibits the same trends that ambient OC concentrations exhibit. Figure 4.5b further shows that when weighted according to the quantity of OC present, urban air contains much higher absolute concentrations of human cell mutagens (by an order of magnitude) than background air in Southern California.

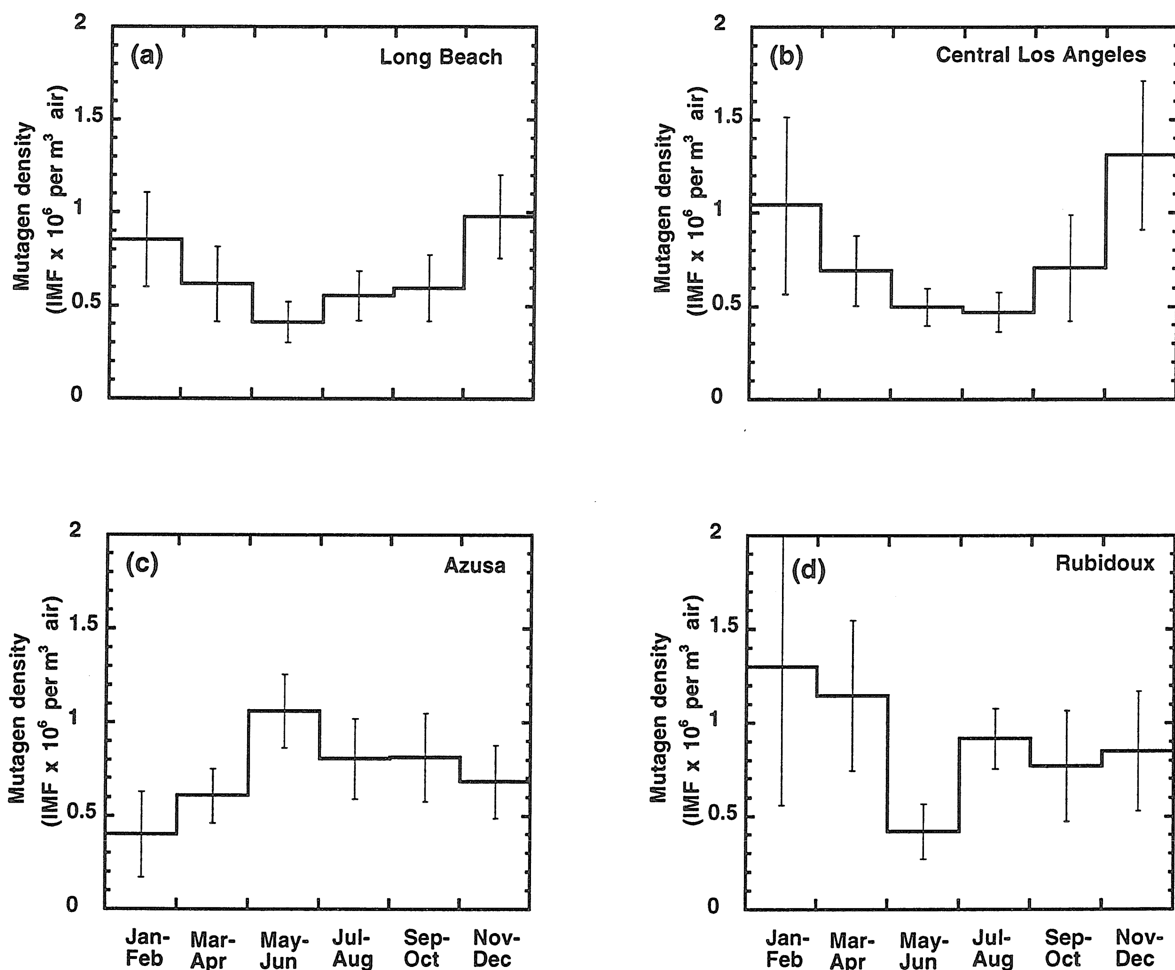

Figure 4.7. Mutagen density of the fine particulate matter in the South Coast Air Basin during 1993 at Long Beach, central Los Angeles, Azusa, and Rubidoux. Error bars = 1 SD.

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Chapter 5

Bioassay-Directed Chemical Analysis of Los Angeles Airborne Particulate Matter Using a Human Cell Mutagenicity Assay

Introduction

Organic particulate matter filtered from ambient air has repeatedly been shown to be mutagenic to bacteria *(1-4)*, mutagenic to human cells *(5)*, and carcinogenic *(6, 7)* . Hemminki et al. *(8)* report an increase in the smoking-adjusted risk of lung cancer among urbanites of up to 1.5 times that of rural residents which adds to the concern that urban air may contain significant concentrations of mutagens and carcinogens.

Particulate air pollution is a complex mixture of thousands of chemical species. It is virtually impossible to identify every chemical species and then determine if that compound is a mutagen. However, much can be done to gauge the general character of the important mutagens in an atmospheric particulate matter sample. In an attempt to isolate the important chemical mutagens in an environmental sample, previous researchers *(9-20)* have developed and refined a technique aptly named bioassay-directed chemical analysis. Bioassay-directed chemical analysis involves separating a sample into coherent pieces, called fractions, that contain organic chemicals of similar functionality and polarity. The fractions are tested in a bioassay to determine their mutagenic potency, and the bioassay results are used to direct attention to detailed chemical analysis of those fractions in which the most important

mutagens have been isolated. This process of separating and testing can be repeated until the chemical complexity of each mutagenic fraction has been reduced to the point where a fairly comprehensive chemical analysis of the mutagenic fractions can be completed.

Researchers have attempted a myriad of variations on the theme of chemical separation within the context of bioassay-directed chemical analysis, but the primary bioassay used in the past has been a bacterial mutation assay. When studying diesel engine exhaust, Nishioka et al. *(21)* found that nitro polycyclic aromatic compounds (nitroPAC) accounted for 20-25% of the bacterial mutagenic activity observed without further enzymatic activation of the assay (i.e. absent the addition of post mitochondrial supernatant preparation, a test condition called –PMS or –S9). Salmeen et al. *(22)* found that mono- and di-nitro polycyclic aromatic hydrocarbons (mono- and di-nitroPAH) account for 30-40% of the bacterial mutagenicity (–S9) of diesel engine exhaust particles. The hunt for important mutagens then moved from emission sources to ambient air pollution as Wise et al. *(4)* found significant bacterial mutagenic activity (–S9) due to nitroPAH in ambient air, Arey et al. *(23)* found that nitroPAH accounted for 1-8% of the bacterial mutagenic activity (–S9), and Helmig et al. *(24)* concluded that a specific nitroPAC (2-nitro-6*H*-dibenzo[*b,d*]pyran-6-one) accounted for ~45% of the bacterial mutagenic activity of their ambient samples (–S9). Abundant literature exists detailing the significance of nitroPAC as bacterial mutagens.

Bioassay-directed chemical analysis for mutagens has seldom been attempted using biological endpoints other than bacterial assays, although a few studies do exist. Grimmer et al. *(25, 10, 26)* studied extracts of the

particulate matter from several air pollutant emission sources using carcinogenic effects in rats as an endpoint and found ordinary PAH with more than 3 rings to account for the total carcinogenic activity. Using a human cell forward mutation assay in which rat liver provided the catalyst for xeno metabolism (the TK-6 cell line), Skopek et al. *(27)* found that 8% of the observed activity of kerosene soot was due to cyclopenta[*cd*]pyrene (CPP). Using the same assay, Barfknecht et al. *(28)* report that a significant fraction of the activity of diesel engine exhaust when viewed through human cell assays based on the TK6 cell line is due to fluoranthene. Durant et al. *(20)* found that benzo[*a*]pyrene (BaP) accounted for as much as 50% of the activity of the organics extracted from urban pond sediment in the MCL-5 human cell line which expresses human xeno metabolizing enzymes.

When comparing the results of studies using bacterial mutation assays to the few studies conducted to data on various air pollution source effluents using either cultured human cells or whole mammalian animals (e.g. mice), a pattern seems to emerge that deserves further investigation. The bacterial assays appear to be reacting to the nitroPAC content of the samples while the mammalian cells including human cells seem to be most affected by the ordinary PAH content of the samples. Even though nitroPAC are mutagenic in the human cell assays *(29)*, their concentration-weighted activity in typical complex mixtures may be lower than that of the concentration-weighted activity of the ordinary PAH in a human cell assay because nitroPAC are present at trace levels by comparison to the ordinary PAH. The outcome of the bioassay-directed chemical analysis of complex mixtures seems to be extremely dependent on the biological endpoint used. The above hints that the most

important mutagens affecting human cells may be different than previously inferred from bacterial assays are obtained from a few studies of air pollutant source materials and not from an examination of actual ambient air samples. The ambient air is more complex than the source material because it also contains nitroPAC and oxyPAC that are formed by atmospheric reactions *(30)*. To date, the character of the most important human cell mutagens in the urban atmosphere remains to be determined, as no bioassay-directed chemical analyses have been published to date based on samples taken at community routine air monitoring stations.

The present chapter seeks to characterize the most important mutagens present in urban airborne particulate matter using bioassay-directed chemical analysis that is based on a recently developed human cell assay for gene mutation. A fine particulate matter sample representative of long-term exposure conditions in Southern California is created by compositing a portion of every filter sample collected during a 1993 air monitoring campaign which consisted of 24 hr sampling every sixth day for the entire year at four urban locations in southern California. The human cell mutation assay used in this study *(31)* tests mutagenic activity at the thymidine kinase locus in h1A1v2 cells using a 72 hr exposure. The h1A1v2 cells are AHH-1 TK+/- cells bearing the plasmid pHSRAA. The plasmid pHSRAA contains two copies of the human CYP1A1 cDNA and confers resistance to 1-histidinol. This cell line has been shown to sensitive to both PAH and nitroPAC *(32)*, and has been used previously to investigate the seasonal and spatial variation of the human cell mutagenicity of fine organic aerosol in southern California *(5)*. To the best of our knowledge, this study reports the first bioassay-directed chemical analysis of airborne

particulate matter samples taken at community air monitoring stations using a human cell assay.

Experimental Section

Sample Collection.

An airborne fine particulate matter sample is created by compositing a portion of every urban fine particulate filter sample collected during a 1993 southern California air monitoring campaign. This air monitoring campaign is described briefly below, and a more detailed description can be found elsewhere *(33, 5)*. The air sampling network consists of 4 urban sites which were selected because each has a different characteristic exposure to ambient aerosol sources, plus a background site located on an offshore island upwind of Los Angeles. Samples taken at the background site are not included in the urban aerosol composite examined in the present chapter. The 4 urban sites included central Los Angeles, which is surrounded by freeways and experiences high concentrations of motor vehicle emissions; Long Beach, which is located in close proximity to the direct emissions from industrial sources such as power plants, petroleum refineries, and the Los Angeles - Long Beach harbor complex; Azusa, which is generally downwind of central Los Angeles and is characterized by relatively high levels of ozone and secondary aerosol that is formed by atmospheric chemical reactions; and Rubidoux, which is located farther downwind and generally receives even more secondary aerosol than Azusa. All air monitoring equipment was located at the South Coast Air Quality Management District (SCAQMD) air monitoring stations in the communities named. The ambient particulate matter samples were taken for 24

h every sixth day for the entire year of 1993 at these four urban air monitoring stations. Quartz fiber filters (102 mm diameter Pallflex Tissuquartz 2500 QAO) were used for the particulate matter collection. All quartz fiber filters were prebaked for at least 6 h prior to use at 750 °C to lower their carbon blank. Each filter was loaded the day prior to sampling, and unloaded on the day after sampling. Field and laboratory blanks were also taken to ensure that there was minimal contamination of the ambient sampling system. The filters were transported to the sampling sites in prebaked aluminum foil, and returned to the laboratory in annealed glass jars with solvent-washed Teflon-lined lids. All filters were stored in a freezer at -21 °C.

A high volume dichotomous virtual impactor, described in detail by Solomon et al. *(34)*, was used at each site to collect the fine particulate matter samples used for this study. This sampler has the ability to gather a large quantity of size-separated organic aerosol in a 24-h period. Fine particles (those particles with an aerodynamic diameter smaller than 3-4 μm) are separated from coarse particles according to the higher inertia associated with the larger, coarse particles traveling at high velocity. 90% of the incoming ambient air flow (~300 lpm) is forced to make a 90° turn; the fine particles follow the fluid streamlines and in that way 90% of the fine particles are collected on a fine particle filter at a flow rate of nominally 270 lpm. The coarse particles do not follow the turn but instead are concentrated into the remaining 30 lpm of the inlet air flow and are collected on a second filter along with the remaining 10% of the fine particles. One sixth of each fine particle filter was used to make a 1993 urban composite that physically represents the annual average aerosol concentration and composition averaged over all four urban sites. Filter

allocation is described in detail elsewhere *(5)*. The human cell mutagenicity assay used here requires approximately 500 μg of organic carbon per test, and generally, duplicate tests are performed. All sample organic aerosol mass is reported in units of μg of equivalent organic carbon (EOC), which is defined as the amount of organic carbon present in the filter composite prior to extraction as determined by thermal evolution and combustion analysis of separate sections cut from the same quartz fiber filters *(5)*. The 1993 urban composite assembled for bioassay-directed chemical analysis contained 84 mg of EOC, thus providing enough organic material to permit multiple level separation procedures designed to isolate small groups of similar mutagenic compounds within small fractions of the original sample extract. Measurements reported relative to the EOC content of the composite provides a direct connection from the human cell bioassays back to the ambient carbonaceous aerosol concentrations, which amounted to 5.38 μg m^{-3} of fine particle organic carbon averaged over the four sites studied during 1993 when each bimonthly composite sample that forms a part of the annual composite is weighted equally *(5)* or 5.23 μg m^{-3} of fine particle EOC if each individual filter within the annual composite is weighted equally.

Extract Preparation

A brief description of sample extraction and concentration procedures appears below; for a more detailed description see Hannigan et al. *(35)*. All fine particulate matter filters used for this study were extracted in a soxhlet apparatus with dichloromethane (DCM) for at least 16 h. These DCM extracts were then concentrated in a vacuum centrifuge down to a volume of

approximately 1 mL. Extracts from all filter portions were then pooled to create a single extract. Sample portions designated for the human cell assay were exchanged into dimethyl sulfoxide (DMSO) by adding DMSO to the DCM extract and then blowing a gentle stream of high purity N_2 over the extract until the volume was reduced to the volume of DMSO added. Sample portions designated for chemical fractionation were reduced to a volume of just less than 100 μL by evaporation under a gentle stream of high purity N_2. To enable comparison between our measure of organic carbon (EOC) as determined by thermal evolution and combustion analysis prior to sample extraction and the traditional measures of extracted mass as an indication of the quantity of organic compounds present, the extracted mass from a portion of this sample was measured both by the thermal evolution and combustion method and by a microscale evaporation method *(36)*. The results of this test showed 0.93 μg of extracted mass per μg of EOC.

Human Cell Mutation Assay

A detailed description of the procedures for the routine use of the h1A1v2 cell line for mutagenicity testing at the thymidine kinase (*tk*) locus have been described in detail elsewhere *(29, 31)*. Testing of aliquots of sample DMSO extract was performed by exposing duplicate 12 mL cultures of 1.8×10^6 exponentially growing cells for 72 hrs. Exposure was terminated by centrifuging and resuspending the cells in fresh media (30 mL). One day after termination of the exposure, the cultures were counted and diluted to 80 mL at 2×10^5 cells mL^{-1}. After the 3 day phenotypic expression period, cultures were plated in 96-well microtiter plates in the presence of the selective agent to determine

mutagenicity (n=3 with 20,000 cells per well) and in the absence of the selective agent to determine plating efficiency (n=2 with 2 cells per well). Trifluorothymidine is the selective agent used for this forward mutation assay. After an additional 13 day incubation period the plates were scored for the presence of a colony in each well. The positive control was 1.0 $\mu g\ mL^{-1}$ benzo[*a*]pyrene, and DMSO was used as the negative control.

Plating efficiencies, mutant fractions, and their associated confidence intervals (standard deviations) were calculated using methods developed by Furth et al. *(37)* The whole sample extract and each sample fraction was tested in two independent assays to assure test reproducibility. The results from these experiments were converted to induced mutant fraction (IMF) by subtracting the mean mutant fraction of the concurrent negative control from the mean mutant fraction observed for the filter sample extracts. Then the results of the independent assays performed on each sample were pooled to allow for quantitative evaluation of the mutagenicity of each sample fraction. The mutagenicity of a sample fraction will be described in terms of its mutagenic potency, which is defined here as the IMF per unit mass of EOC present in the whole sample prior to extraction and fractionation. As fractionation proceeds, the amount of EOC present in the original sample does not change. Thus the mutagenic potencies of each sample fraction can be compared to each other as being parts of the whole sample. The mutagenic potency of a sample was estimated by pooling all experimental points for each sample fraction and then computing the initial slope of the dose/response relationship observed at low doses using a least-squares fit to the data that was forced through the origin (because at zero dose, there is by definition zero IMF). This technique has

been used with success previously *(5)* to evaluate the seasonal and spatial variation of human cell mutagenicity of the same southern California air pollution samples that make up the composite sample tested here.

Fractionation

A successful fractionation procedure should not only isolate mutagens into smaller, less complex mixtures but also must be efficient at transmitting mutagens through the separation procedures with as little loss as possible. At the same time, losses will occur during separation, and these losses have been monitored, resulting in a range of concentrations for the targeted compounds that declines as additional processing takes place. Lafleur et al. *(16)* investigated four types of chromatographic materials to determine the degree of recovery of mutagens during column chromatography. Of the four (silica, alumina, Florisil, and cyanopropyl-bonded silica), the cyanopropyl material proved to be the most efficient material for mutagen recovery. For this reason our sample separation sequence was initiated with a normal-phase cyanopropyl bonded (CN) HPLC fractionation technique.

The primary fractionation procedure developed for the present study is shown in detail in Figure 5.1. A Varian 5000 HPLC coupled to a Hewlett-Packard 8450A UV/VIS spectrophotometer was employed. The column used had a length of 25 cm, an internal diameter of 10 mm, packed with 10 μm Alltech CN material, and the guard column was a 7 μm cyano column (Brownlee newguard column). The solvent program consisted of a 20 minute hold at 95% hexane and 5% dichloromethane (DCM), a 10 minute ramp to 100% DCM, a 10 minute hold at 100% DCM, a 10 minute ramp to 100% isopropanol, and finally a 10 minute hold at 100% isopropanol. A flow rate of 4

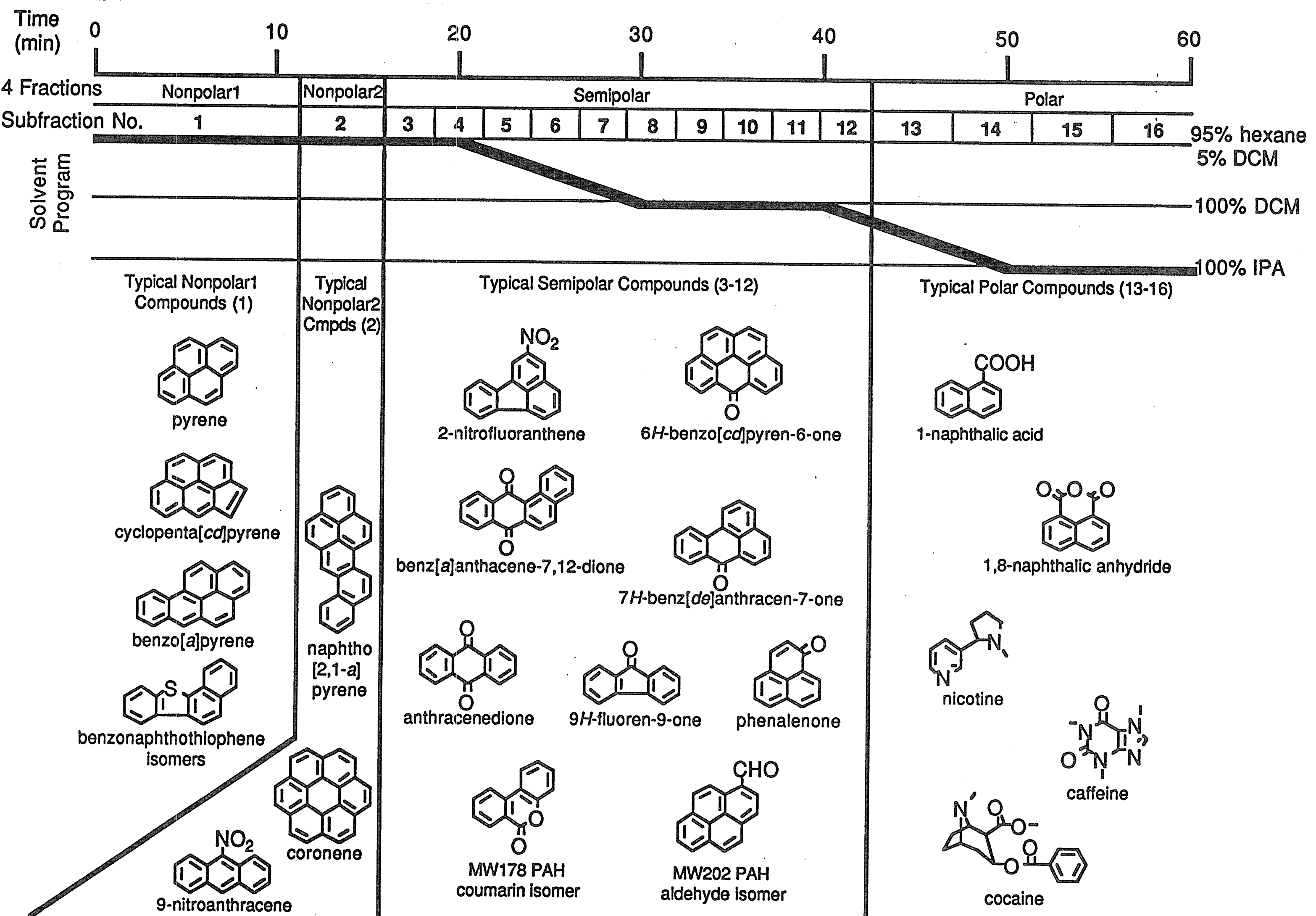


Figure 5.1. HPLC solvent program used in the normal phase column fractionation procedure along side the elution cutpoints that define the various sample fractions and subfractions studied. Also shown is the typical compounds found in each of the 4 fractions (see Table 5.1 for more examples).

mL min^{-1} was maintained. As shown in Figure 5.1, four fractions were created initially. The elution points that divide these four fractions from each other were chosen based on standard runs and these standards were selected in order to isolate targeted chemical classes: ordinary PAH in the first and second fractions, nitroPAH in the second and third fractions, moderately polar organic compounds in the third fraction, and polar organic compounds in the fourth fraction. These four fractions are designated Nonpolar 1, which primarily includes alkanes and PAH; Nonpolar 2, which includes high molecular weight PAH and some lower molecular weight nitroPAH such as 9-nitroanthracene; Semipolar, which includes numerous moderately polar compounds including higher molecular weight nitroPAH, polycyclic aromatic ketones, polycyclic aromatic quinones, and some aldehydes; and Polar, which includes some aldehydes, alcohols, and acids. Fractionation system blanks were monitored to ensure that interference with targeted potential mutagens was negligible.

Additional fractionation steps were applied to further isolate the mutagens. The additional fractionation procedure used the primary normal phase HPLC fractionation procedure described above as a template, with some modifications. An initial fractionation step was added to remove inactive aliphatic compounds from the bioactive aromatic compounds. This initial fractionation step involved the same HPLC described above but fitted with a size exclusion column. The size exclusion column used was 50 cm in length, 1.0 cm in diameter, and packed with 500-Å JordiGel poly(divinylbenzene) material (Jordi Associates, Inc., Bellingham, MA). The mobile phase used was DCM at a flow rate of 1.5 mL min^{-1}. This size exclusion fractionation procedure has been used previously to isolate mutagens from pond sediments *(20)*, and is

described by Lafleur et al. *(38)*. The sample was split into two fractions, designated Aliphatic and Aromatic (although as expected with any fractionation procedure there is some overlap as there are some aromatic compounds in the Aliphatic fraction and vice versa). The cutoff point for this separation was determined by standard runs, and the cutoff was chosen to be at the elution point of 1,6-dinitropyrene, with this compound being part of the Aromatics fraction. A preliminary test using this procedure found that the chosen cut point effectively isolates bacterial mutagens in the Aromatics fraction. The two fractions created by separation over the size exclusion column (Aromatics and Aliphatics) were then subfractionated using the primary normal phase HPLC fractionation procedure described above with modifications in the fraction cut points needed to greatly expand the number of subfractions into which the semipolar and polar compounds are separated according to the flow diagrams shown in Figures 5.1 and 5.2. The Aromatics fraction was divided using the same cutpoints as in the primary fractionation procedure for Nonpolar 1 and Nonpolar 2 subfractions, however the Semipolar aromatics fraction was split into 10 subfractions (designated subfractions 3a-12a) and the Polar fraction was split into 4 subfractions (designated subfractions 13a-16a). The Aliphatic Nonpolar 1 and Nonpolar 2 fractions were combined for further testing as neither aliphatic nonpolar fraction contained significant human cell mutagen concentrations. One purpose of removing the aliphatic material from the aromatics is to facilitate chemical analysis of the aromatics by removing much of the background petroleum alkanes from the sample therefore making the bioactive PAH-type compounds easier to detect and measure. Authentic standards are available for dozens of ordinary PAH so it is possible to quantify

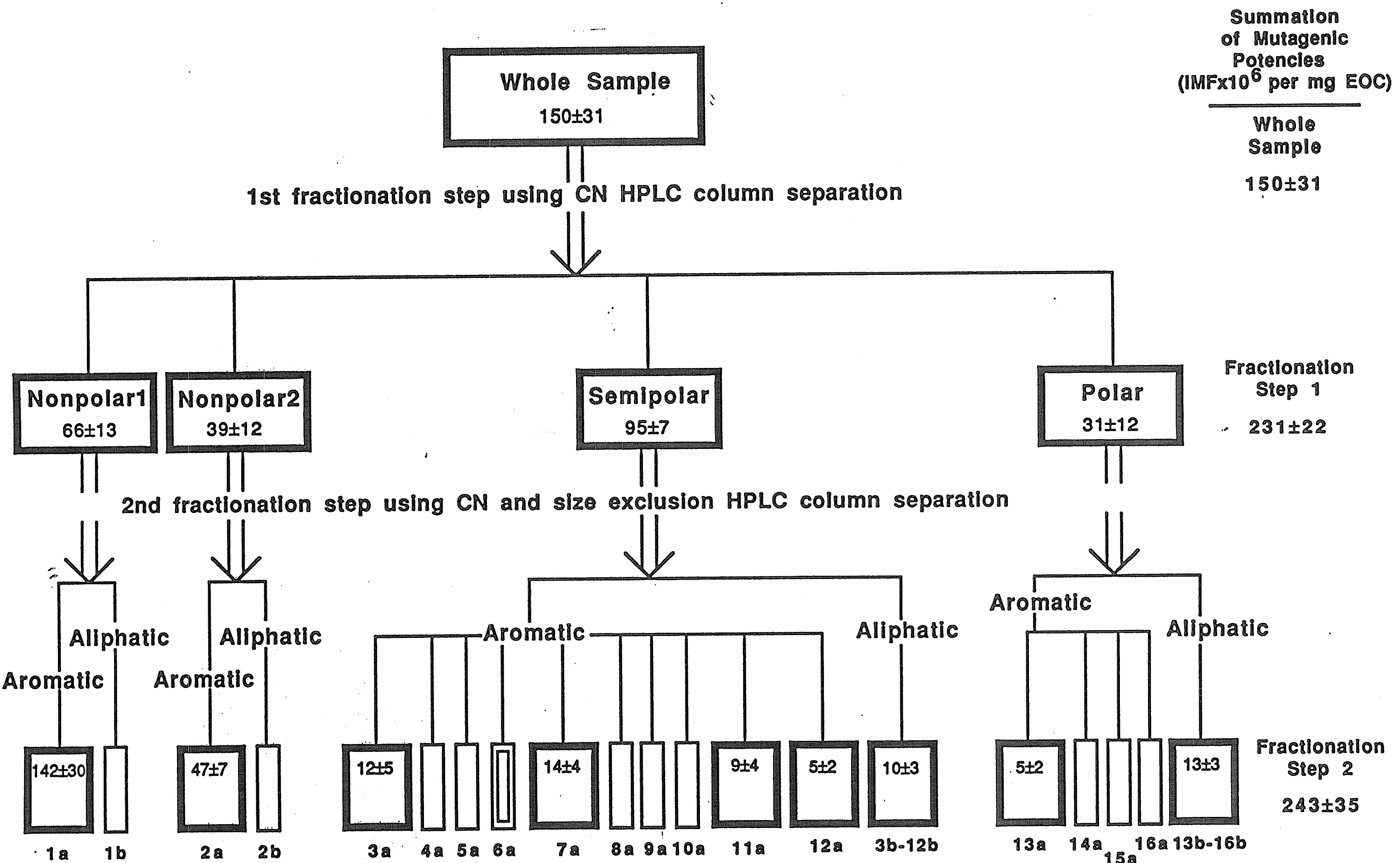


Figure 5.2. Flow chart for the bioassay-directed chemical analysis. Mutagenic fractions and subfractions are indicated in bold outline and the mutagenic potencies (±1 SD) of those fractions and subfractions are given inside the boldly outlined boxes. The subfraction which is double boxed (subfraction 6a) has a mutagenic potency of 16±9 IMF ($x10^6$) per mg EOC, which is not significantly different from zero in a statistical sense, but the best estimate of the mutagenic potency is high enough that this subfraction warrants continued investigation.

many PAH within a single non-polar fraction. Very few oxyPAH are available as pure compounds to be used through bioassays of single compounds to identify the most important mutagens in a complex mixture of semipolar organics. The purpose of extensively subdividing the semipolar and polar extracts was to see if the mutagens in those categories could be isolated in a few small subfractions since it was unlikely that pure compounds could be obtained to aid interpretation of very complex mixtures. By extensively separating the semipolar and polar fractions at least it can be learned whether the mutagens in these fractions are closely grouped by polarity or whether the mutagens are distributed broadly throughout these fractions.

Chemical Analysis

Analysis of mutagenic subfractions was performed using gas chromatographic separation with mass-selective detection. The system used was an HP model 5890II gas chromatograph (GC) coupled with an HP model 5972 mass-selective detector (MSD). The GC was equipped with a DB1701 fused silica capillary column (bonded 86% dimethyl- 14% (cyanopropyl) phenylpolysiloxane) that had a length of 30 m, an inside diameter of 0.25 mm, and a 0.25 μm film thickness. The GC was operated as follows: split/splitless injection, constant flow of 1 mL of He min^{-1}, injector temperature of 275 °C, GC-MSD interface temperature of 275 °C, and an oven program which consisted of a 10 min hold at 65 °C, a ramp of 10 °C min^{-1} for 21 min, and a 45 min hold at 275 °C. The MSD was operated in selected ion monitoring (SIM) and full scan (i.e. total ion mode) modes for the fractions Nonpolar 1, Nonpolar 2, and the Semipolar fractions, and in full scan mode for the Polar fractions.

SIM methods were developed for each of the three types of fractions studied by that approach. Each SIM method was developed by creating a list of target compounds that might appear in that fraction. Target compounds are defined as any organic compound which falls into one of the following categories: (1) compound has been tested in the h1A1v2 assay and is known to be a human cell mutagen (a list of these compounds is given by Durant et al. *(32)*); or (2) compound is a known bacterial mutagen (based on extensive literature review). The key ions of the target compounds were entered into a specific fraction's SIM method following method development work performed in full scan mode conducted on a Long Beach winter atmospheric aerosol test sample. Key ions were selected by a combination of standard runs, literature review if no standard existed, and in light of the observed signal-to-noise ratio for those key ions observed during full scan mode runs performed on the Long Beach winter test sample. Due to the extremely limited information on the mutagenicity of polar compounds, the development of a SIM method that targeted single compounds that were suspected in advance to be mutagenic within the Polar fractions was not pursued. Instead the Polar fractions were searched in the full scan mode to identify the prominent compound peaks that were present. Polar fractions were run under two different conditions; (1) as-is, and (2) derivatized through the addition of diazomethane to convert labile organic acids to their methyl ester analogues.

Compounds were identified by comparison with authentic standards where available, with the Wiley (5th edition) and NIST mass spectral library, and published mass spectra from the literature. Quantification was achieved through standard runs. A relative response factor (RRF) which relates the

compound key ion area counts to the compound mass through the use of a co-injection standard (1-phenyl dodecane) was developed for each compound through injection of standards. Certain standards exhibited different RRF for low concentration versus high concentration analyses, and for these compounds a second (low concentration) RRF was determined. Compound identification and quantification was categorized as follows: (a) *positive*, when the sample spectrum and retention time matches that of an authentic standard, (b) *probable*, when sample spectrum matches the NIST mass spectral library, sample relative retention time matches published values, and RRF for a compound with similar molecular weight (MW) and functional group was used, (c) *possible*, when sample spectrum matches the NIST mass spectral library or sample spectrum and relative retention time matches published values, and RRF for a compound with similar structure was used, (d) *tentative*, when sample spectrum contains additional mass fragments from one or more coeluting compounds (noise) as compared to the NIST mass spectral library and/or published values, and RRF for a compound with similar structure was used. A mixture of standards was run multiple times during the time period in which samples were being run to check GC/MS performance.

Human Cell Assay Results

Unfractionated (Whole) Sample Extract

The human cell mutagenicity of a characteristic southern California fine particulate air pollution sample was examined. The sample was a composite of a portion of every sample collected during a 1993 air monitoring campaign which consisted of 24 hr sampling every sixth day for the entire year at four

urban locations; Long Beach, central Los Angeles, Azusa, and Rubidoux. This composite extract was tested for mutagenicity at the *tk* locus of h1A1v2 cells using a 72 hr exposure in two independent experiments. The dose-response curve generated from this testing as well as the least squares linear fit used to obtain a mutagenic potency value are shown in Figure 5.3. The resulting mutagenic potency (i.e. induced mutant fraction per mass of fine particulate organic carbon present in the ambient sample composite prior to extraction) was determined to be 150±31 IMF ($x10^6$) per mg EOC. In a previous study *(5)* in which the human cell mutagenicity of bimonthly composites of the same air pollution samples analyzed separately at each urban site was investigated, annual average mutagenic potency values (IMF ($x10^6$) per mg EOC) were calculated for each site: Long Beach, 176, central Los Angeles, 140, Azusa, 145, and Rubidoux, 137. The similarity of the mutagenic potency of the annual urban composite extract used for the present study versus the arithmetic average of the bimonthly composites is an indication that the earlier assay results are reproducible.

Fractionated Sample Extracts

The urban annual average composite extract was fractionated into four fractions using normal phase HPLC. The four fractions were created in an attempt to isolate specific compound classes: PAH; low molecular weight nitroPAH; moderately polar organics (e.g.. nitroPAC; oxyPAH); and polar organics (e.g. acids and alcohols). Figure 5.1 shows examples of compounds that elute in each of these fractions. This fractionation procedure separates organics primarily based on polarity, thus the fractions were named to represent

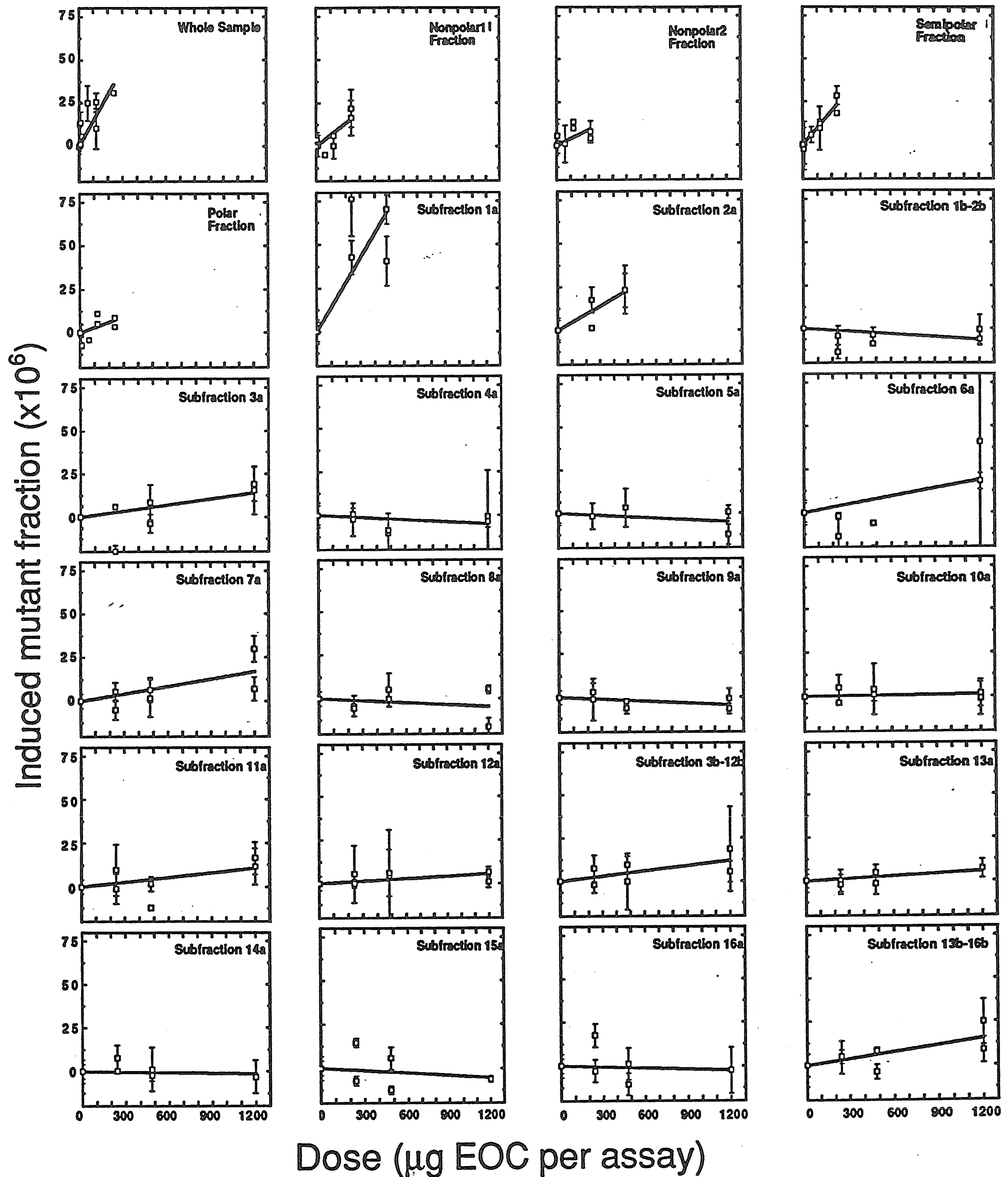


Figure 5.3. Dose-response plots for the whole sample extract, each of the 4 fractions created at the first separation step, and each of the subfractions created at the second fractionation step. Multiple points at a given dose represent independent experiments at that dose, and error bars represent ±1 SD about the experimental mean. Least-squares linear fit to the data (forced through the origin) is shown for each sample.

the types of compounds contained; Nonpolar 1, which contains PAH; Nonpolar 2, which contains the higher molecular weight PAH and some nitroPAH; Semipolar, which contains some nitroPAH, polycyclic aromatic ketones, polycyclic aromatic quinones, and some aldehydes; and Polar, which contains some aldehydes, acids and alcohols. These four fractions were tested for mutagenicity at the *tk* locus of h1A1v2 cells using a 72 hr exposure in two independent experiments. Similar extract dose levels were used to those used for testing of the unfractionated extract. The dose-response curves along with the least squares linear fit used to obtain mutagenic potency values for each fraction are shown in Figure 5.3.

The mutagenic potency values determined from this experiment appear in Figure 5.2. Fractions having a mutagenic potency significantly greater than zero are presented in bold outline in that figure, and the mutagenic potency values for the active fractions are given inside each boldly outlined box in units of IMF ($x10^6$) per mg EOC in the whole sample originally supplied to the extraction and fractionation process. At the first 4-way separation step, all fractions exhibit some mutagenicity, with Nonpolar 1 producing a potency of 66±13 IMF ($x10^6$) per mg EOC, Nonpolar 2 accounting for a potency of 39±12 IMF ($x10^6$) per mg EOC, the Semipolar fraction contributing a potency increment of 95±7 IMF ($x10^6$) per mg EOC, and the Polar fraction accounting for a potency increment of 31±12 IMF ($x10^6$) per mg EOC. The sum of the mutagenic potency increments from the four fractions totals 231±22 IMF ($x10^6$) per mg EOC or 138% of that of the unfractionated sample extract. Such a gain in mutagenic potency upon fractionation has been observed in other studies *(20)* and could well be due to reduced interference among compounds when

the bioassays are performed on the less complex mixtures present in the fractionated samples. This fractionation procedure isolated most of the mutagenicity into two fractions, Nonpolar 1 and Semipolar. The Nonpolar 1 fraction, besides containing PAH, also contains numerous other nonpolar compounds, mostly aliphatic in nature, and is thus still an extremely complex mixture. The Semipolar fraction, as seen in Figure 5.1, contains a wide range of compound classes, and isolating the activity into this fraction gives little clue as to mutagen identity. Therefore, refinement of the fractionation procedure was undertaken.

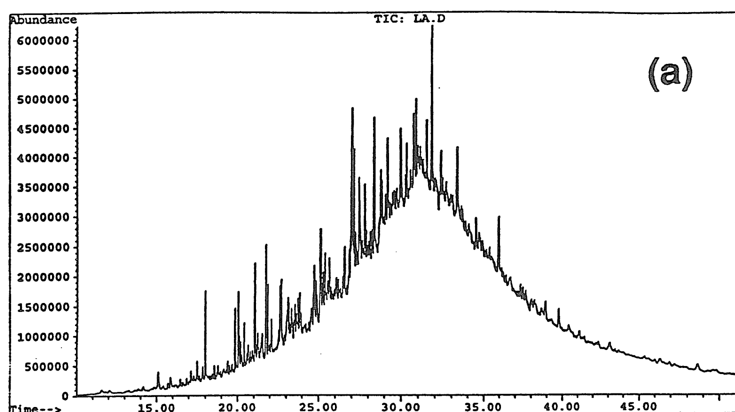
Subfractionated Sample Extracts

The annual urban composite extract was further separated into many subfractions described in Figures 5.2 and 5.3 in order to further isolate the important mutagens from each other. The subfractionation procedure involved an initial step which attempted to isolate the aromatic compounds from the aliphatic compounds. Beginning with a fresh example of the whole sample extract, two fractions, named Aliphatics and Aromatics, were created using size exclusion HPLC, as described previously. This naming convention indicates characteristic compounds found in each of these two samples and should not be thought of as an absolute separation of all aromatic compounds from all aliphatic compounds; as with any chromatographic separation there will be some overlap, with a portion of the aromatic compounds appearing in the Aliphatics sample for example. These two fractions were tested for mutagenicity at the *tk* locus of h1A1v2 cells using a 72 hr exposure in two independent experiments. Similar extract dose levels were used to those used for testing of

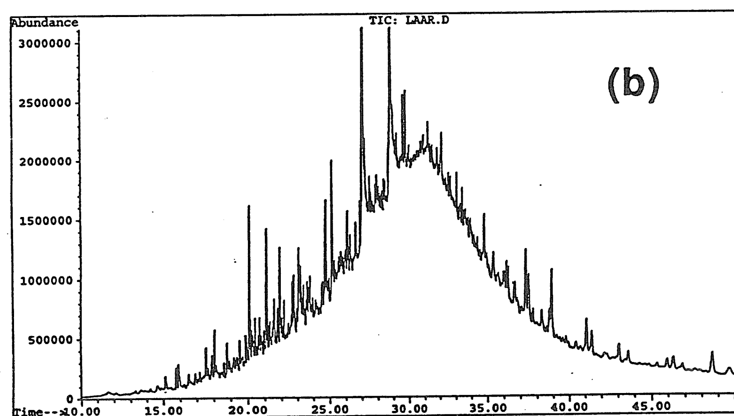
the unfractionated whole extract. The absolute mutagenic potency values of the two extracts tested (whole Aliphatic and whole Aromatic) summed to 163±34 IMF ($x10^6$) per mg EOC which is statistically indistinguishable from the 150±31 IMF ($x10^6$) per mg EOC value determined for the original unfractionated whole sample extract. The whole Aromatics fraction contained 83% of the mutagenic potency and the whole Aliphatics fraction contained 17% of the mutagenic potency. Use of this procedure based on the size exclusion column confirms as expected that most of the mutagenicity exists within the Aromatics fraction.

These two fractions, Aromatics and Aliphatics, were then further separated using the same normal-phase HPLC procedure used to create the four fractions (Nonpolar 1, Nonpolar 2, Semipolar, Polar) discussed previously. The Aromatics extract was split such that 10 additional subfractions were created within the Semipolar group and 4 additional subfractions were created within the Polar group. The elution times at which the subfractions are divided are shown near the top of Figure 5.1; i.e. subfractions 1-16. The Aliphatics extract was split into the four subfractions defined earlier, Aliphatic Nonpolar 1, Aliphatic Nonpolar 2, Aliphatic Semipolar, and Aliphatic Polar. All subfractions were tested for mutagenicity at the *tk* locus of h1A1v2 cells using a 72 hr exposure in two independent experiments; the Aliphatics Nonpolar 1 and 2 were combined during testing as no mutagens were found in either fraction. Low extract dose levels were used that were similar to those used for testing of the unfractionated extract, and additional high dose bioassays were added in order to see smaller increments of mutagenic potency that might be contained within the least potent extract subfractions.

The results of these bioassays are shown in Figure 5.2. The mutagenicity seen in the Nonpolar fractions has been isolated into the aromatic subfractions. The mutagenic potency value for the Aromatic Nonpolar 1 subfraction is significantly larger than was observed in the Nonpolar 1 fraction; 142±30 IMF (x10^6) per mg EOC vs. 66±13 IMF (x10^6) per mg EOC, while the potency of the Aromatic Nonpolar 2 fraction is very similar to that of the unseparated Nonpolar 2 fraction (39±12 versus 47±7 IMF (x10^6) per mg EOC). The mutagenicity seen in the Semipolar fraction has been isolated into 4 of the 10 Aromatic Semipolar subfractions and in the Aliphatic Semipolar subfraction. If we sum the mutagenic potency values observed in these subfractions we get a total for the Semipolar subfractions of 50±13 IMF (x10^6) per mg EOC. This is a decrease from what was observed in the Semipolar fraction, which exhibited a mutagenic potency value of 95±7 IMF (x10^6) per mg EOC, before it was subdivided. The mutagenicity observed in the Polar fraction has been isolated into 1 of the 4 Aromatic Polar subfractions and in the Aliphatic Polar subfraction. The sum of the absolute values of the subfraction mutagenic potency values totals 243±35 IMF (x10^6) per mg EOC, or 162% of that of the unfractionated whole sample extract and 105% of that of the total of the mutagenic potencies measured at the four-fraction level. This subfractionation procedure successfully isolated most of the overall sample mutagenicity within a few subfractions that are chemically less complex then the total sample. An example of the reduction in chemical complexity can be seen in Figure 5.4 which shows the full scan mode GC chromatograms for the whole sample extract, the whole Aromatic fraction, followed by the Aromatic Semipolar subfraction 6a. The results of the targeted chemical analysis of suspected



Whole Sample Extract



Whole Aromatic Fraction

Semipolar Aromatics Subfraction 6a

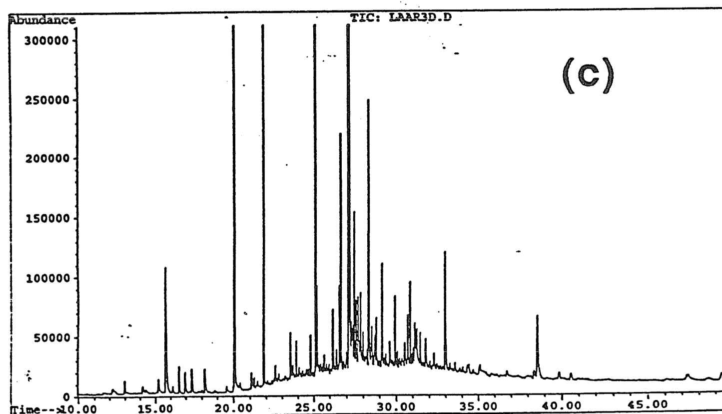


Figure 5.4. Demonstration of the reduction of interfering background compounds obtained through sample fractionation at various levels: (a) full scan mode chromatogram of the Whole sample extract; (b) full scan mode chromatogram of the whole Aromatic fraction, and (c) full scan mode chromatogram of the Aromatic Semipolar subfraction 6a.

mutagens in the subfractions next will be given for those subfractions which exhibited significant mutagenic potency.

Chemical Analysis Results

Aromatics Nonpolar 1

The organic compounds quantified in this subfraction are shown in Table 5.1. Mass concentrations are shown in units of ng of a specific compound per mg EOC (organic carbon on the atmospheric filter samples as determined by thermal evolution and combustion analysis prior to sample extraction). As can be seen from this table, 43 specific PAH and an additional 37 PAH that are partially characterized have been quantified in this subfraction, accounting for 1690-455 ng of PAH per mg EOC. The range of concentrations shown in Table 5.1 reflects the range of concentrations observed for each compound when quantified in the whole sample extract and in the various fractionated and subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. PAH and n-alkanes were quantified in SIM mode. Other compounds identified in the full scan mode in this subfraction include cholestanes, hopanes, and benzonaphthothiophene isomers intended for use as source tracers *(39)* that will be described in a later paper on that subject.

Also shown in Table 5.1 is the range of estimated mutagenic potency contributions from specific compounds. The mutagenic potency contribution values are calculated by multiplying the mass concentration range of a specific compound (ng/mg EOC) by the mutagenic potency (IMF ($x10^6$)/ng) value for that specific compound if it is known from prior bioassays applied to that pure

Table 5.1. Organic compounds quantified in the mutagenic subfractions.

compound	composition	concentration range (ng per mg EOC)[a]	single compound mutagenic potency (IMF ($\times10^6$) per ng)[b]	contribution to potency of ambient mixture (IMF ($\times10^6$) per mg EOC)	ID[c]
		Aromatic Nonpolar 1 Subfraction (1a)			
Polycyclic Aromatic Hydrocarbons (PAH)					
phenanthrene	$C_{14}H_{10}$	24.3 – 13.9	0.00	0.00	a
anthracene	$C_{14}H_{10}$	1.6 – 0.8	u	u	a
3-methylphenanthrene	$C_{15}H_{12}$	8.5 – 3.9	u	u	b
2-methylphenanthrene	$C_{15}H_{12}$	10.4 – 5.8	u	u	b
2-methylanthracene	$C_{15}H_{12}$	1.5 – 0.6	u	u	a
9-methylphenanthrene	$C_{15}H_{12}$	4.5 – 2.3	u	u	b
1-methylphenanthrene	$C_{15}H_{12}$	3.7 – 2.6	$1.5\text{x}10^{-4}$	5.6 - 3.9($\text{x}10^{-4}$)	a
total methyl 178μ PAH	$C_{15}H_{12}$	31.3 – 17.3			
total C_2H_6 178μ PAH	$C_{16}H_{14}$	63.0 – 24.4			
retene	$C_{18}H_{18}$	10.0 – 6.3	u	u	a
fluoranthene	$C_{16}H_{10}$	45.0 – 21.4	0.00	0.00	a
acephenanthrylene	$C_{16}H_{10}$	6.7 – 5.3	u	u	b
pyrene	$C_{16}H_{10}$	52.5– 25.8	0.00	0.00	a
2-methylfluoranthene	$C_{17}H_{12}$	4.9 – 3.2	u	u	a
total methyl 202μ PAH	$C_{17}H_{12}$	48.7 – 24.3			b
total C_2H_6 202μ PAH	$C_{18}H_{14}$	31.5 – 17.8			b
benzo[*a*]fluorene	$C_{17}H_{12}$	2.8 – 0.7	0.00	0.00	b
benzo[*b*]fluorene	$C_{17}H_{12}$	6.0 – 3.4	0.00	0.00	a
benzo[*c*]phenanthrene	$C_{18}H_{12}$	5.7 – 2.8	0.0073	0.04 – 0.02	a
benzo[*ghi*]fluoranthene	$C_{18}H_{10}$	34.7 – 16.2	0.00	0.00	b
cyclopent[*hi*]acephenanthrylene	$C_{18}H_{10}$	1.2[d]	u	u	b
cyclopent[*hi*]aceanthrylene	$C_{18}H_{10}$	ND	u	u	b
benz[*a*]anthracene	$C_{18}H_{12}$	23.9 – 12.2	$4.0\text{x}10^{-4}$	9.6 - 4.8($\text{x}10^{-3}$)	a
cyclopenta[*cd*]pyrene	$C_{18}H_{10}$	20.3 – 8.3	0.64	12.93 – 5.31	a
chrysene & triphenylene	$C_{18}H_{12}$	47.2 – 24.8	$4.0\text{x}10^{-4}$	0.02 – 0.01	a
total methyl 226μ PAH	$C_{19}H_{12}$	34.9 – 7.6			b
total methyl 228μ PAH	$C_{19}H_{14}$	80.6 – 25.8			b
total C_2H_6 228μ PAH	$C_{19}H_{16}$	38.7 – 7.2			b
benzo[*k*]fluoranthene	$C_{20}H_{12}$	76.7 – 17.0	0.015	1.17 – 0.26	a
benzo[*b*]fluoranthene	$C_{20}H_{12}$	71.5 – 17.7	0.029	2.06 – 0.51	a
benzo[*j*]fluoranthene	$C_{20}H_{12}$	15.3 – 3.9	0.013	0.20 – 0.05	a
benzo[*e*]pyrene	$C_{20}H_{12}$	84.5 – 17.6[e]	$2.1\text{x}10^{-4}$	18 - 3.7($\text{x}10^{-3}$)	a
benzo[*a*]pyrene	$C_{20}H_{12}$	33.9 – 7.9	0.11	3.72 – 0.87	a
perylene	$C_{20}H_{12}$	21.6 – 4.8	$1.3\text{x}10^{-4}$	2.9 - 0.6($\text{x}10^{-3}$)	a
total methyl 252μ PAH	$C_{21}H_{14}$	237 – 34.3			b
276μ PAH isomer	$C_{22}H_{12}$	29.6 – 3.5	u	u	b
276μ PAH isomer	$C_{22}H_{12}$	1.3[d]	u	u	b
276μ PAH isomer	$C_{22}H_{12}$	32.1 – 4.2	u	u	b
276μ PAH isomer	$C_{22}H_{12}$	3.3[d]	u	u	b
indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	87.2 – 11.8[e]	0.020	1.73 – 0.24	a
276μ PAH isomer	$C_{22}H_{12}$	1.3[d]	u	u	b
benzo[*ghi*]perylene	$C_{22}H_{12}$	190 – 27.9[e]	0.013	2.43 – 0.36	a
276μ PAH isomer	$C_{22}H_{12}$	0.8[d]	u	u	b
anthanthrene	$C_{22}H_{12}$	6.0[d]	u	u	a

Table 5.1. (continued)

compound	composition	concentration range (ng per mg EOC)[a]	single compound mutagenic potency (IMF (x10^6) per ng)[b]	contribution to potency of ambient mixture (IMF (x10^6) per mg EOC)	ID[c]
278μ PAH isomer	$C_{22}H_{14}$	1.5[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.1[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.0[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	3.0[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.9[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.2[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	0.6[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.2[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	1.7[d]	u	u	b
278μ PAH isomer	$C_{22}H_{14}$	2.9[d]	u	u	b
dibenzo[*a,h*]anthracene	$C_{22}H_{14}$	41.9 – 3.7	0.020	0.82 – 0.07	a
benzo[*b*]chrysene	$C_{22}H_{14}$	9.4[d]	u	u	a
278μ PAH isomer	$C_{22}H_{14}$	13.6 – 1.0	u	u	b
picene	$C_{22}H_{14}$	21.5 – 3.6	$1.4x10^{-4}$	3.0 - 0.5($x10^{-3}$)	a
300μ PAH isomer	$C_{24}H_{12}$	1.0[d]	u	u	b
300μ PAH isomer	$C_{24}H_{12}$	0.6[d]	u	u	b
300μ PAH isomer	$C_{24}H_{12}$	3.7[d]	u	u	b
300μ PAH isomer	$C_{24}H_{12}$	1.1[d]	u	u	b
coronene	$C_{24}H_{12}$	28.8[d]	$1.5x10^{-4}$	$4.3x10^{-3}$	a
302μ PAH isomer	$C_{24}H_{14}$	0.2[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.1[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.1[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.3[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.2[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.5[d]	u	u	b
302μ PAH isomer	$C_{24}H_{14}$	0.5[d]	u	u	b
naphtho[2,3-*j*]fluoranthene	$C_{24}H_{14}$	0.7[d]	$6.5x10^{-4}$	$4.6x10^{-4}$	a
naphtho[1,2-*k*]fluoranthene	$C_{24}H_{14}$	0.7[d]	0.0081	0.01	a
naphtho[2,3-*b*]fluoranthene	$C_{24}H_{14}$	ND	0.0083	0.00	a
dibenzo[*b,k*]fluoranthene	$C_{24}H_{14}$	ND	0.020	0.00	a
dibenzo[*a,l*]pyrene	$C_{24}H_{14}$	ND	1.73	0.00	a
dibenzo[*a,k*]fluoranthene	$C_{24}H_{14}$	0.4[d]	0.023	0.01	a
naphtho[2,3-*k*]fluoranthene	$C_{24}H_{14}$	0.3[d]	$3.4x10^{-4}$	$1.0x10^{-4}$	a
dibenzo[*a,e*]pyrene	$C_{24}H_{14}$	ND	0.24	0.00	a
naphtho[2,1-*a*]pyrene	$C_{24}H_{14}$	ND	0.024	0.00	a
total 302μ PAH	$C_{24}H_{14}$	4.0[d]			
Total PAH		*1690 – 455*		*25.1 – 7.72*	
		Aromatic Nonpolar 2 Subfraction (2a)			
PAH					
benzo[*e*]pyrene	$C_{20}H_{12}$	0.5 – 0.1[e]	$2.1x10^{-4}$	1.0 - 0.2($x10^{-4}$)	a
indeno[1,2,3-*cd*]pyrene	$C_{22}H_{12}$	0.7 – 0.1[e]	0.020	0.01 – 0.00	a
benzo[*ghi*]perylene	$C_{22}H_{12}$	3.4 – 0.5[e]	0.013	0.04 – 0.01	a
coronene	$C_{24}H_{12}$	2.8[d]	$1.5x10^{-4}$	$4.2x10^{-4}$	a

Table 5.1. (continued)

compound	composition	concentration range (ng per mg EOC)[a]	single compound mutagenic potency (IMF (x10^6) per ng)[b]	contribution to potency of ambient mixture (IMF (x10^6) per mg EOC)	ID[c]
naphtho[2,3-*b*]fluoranthene	$C_{24}H_{14}$	1.4[d]	0.0083	0.01	a
dibenzo[*b,k*]fluoranthene	$C_{24}H_{14}$	1.4[d]	0.020	0.03	a
dibenzo[*a,k*]fluoranthene	$C_{24}H_{14}$	1.2[d]	0.023	0.03	a
naphtho[2,3-*k*]fluoranthene	$C_{24}H_{14}$	1.2[d]	$3.4x10^{-4}$	$4.1x10^{-4}$	a
dibenzo[*a,e*]pyrene	$C_{24}H_{14}$	0.6[d]	0.24	0.14	a
naphtho[2,1-*a*]pyrene	$C_{24}H_{14}$	0.4[d]	0.024	0.01	a
Total PAH		*13.6 --9.7*		*0.27 – 0.23*	
Nitro-PAH					
9-nitroanthracene	$C_{14}H_9NO_2$	3.8[d]	$3.9x10^{-4}$	$1.5x10^{-3}$	a
Aromatic Semipolar Subfraction (3a)					
Polycyclic Aromatic Ketones (PAK) and Quinones (PAQ)					
fluorenone	$C_{13}H_8O$	49.2 – 10.5[e]	u	u	a
phenanthrenone or isomer	$C_{14}H_{10}O$	3.2[d]	u	u	c
4*H*-cyclopenta[*def*]phenanthren-4-one	$C_{15}H_8O$	10.6 – 3.8[e]	0.00	0.00	a
1-methylanthracene-9,10-dione	$C_{15}H_{10}O_2$	11.7 – 2.4	u	u	b
dimethylanthracene-9,10-dione	$C_{16}H_{12}O_2$	5.6[d]	u	u	c
11*H*-benzo[*a*]fluoren-11-one	$C_{17}H_{10}O$	30.4 – 16.2[e]	u	u	b
methylbenzathrone or isomer	$C_{18}H_{12}O$	13.1[d]	u	u	c
benz[*a*]anthracen-7,12-dione	$C_{18}H_{10}O_2$	38.2 – 13.4[e]	0.00	0.00	a
Total PAK and PAQ		*162 – 68.2*		*0.00*	
Nitro-PAH					
2-nitrofluoranthene	$C_{16}H_9NO_2$	28.4 – 6.4	0.019	1.27 – 0.12	a
1-nitropyrene	$C_{16}H_9NO_2$	0.2[d]	0.0013	$2.6x10^{-4}$	a
2-nitropyrene	$C_{16}H_9NO_2$	2.3[d]	0.00	0.00	b
Total Nitro-PAH		*30.9 – 8.9*		*1.27 – 0.12*	
Aromatic Semipolar Subfraction (6a)					
PAK and PAQ					
7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$	81.3 – 49.9[e]	$4.0x10^{-4}$	0.03 – 0.02	a
11 *H*-benzo[*b*]fluoren-11-one	$C_{17}H_{10}O$	3.5 – 2.0[e]	u	u	b
methylbenzanthrone or isomer	$C_{18}H_{12}O$	18.8[d]	u	u	c
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	90.7 – 44.5[e]	0.018	1.63 – 0.80	a
naphthacene-5,12-dione	$C_{18}H_{10}O_2$	13.2 – 4.8	0.00	0.00	b
Total PAK and PAQ		*208 – 120*		*1.66 – 0.82*	
Other Polycyclic Aromatic Carbonyls (PAC)					
pyrene aldehyde or isomers	$C_{16}H_{10}O$	10.0 – 9.7[e]	u	u	b
3,4-benzocoumarin or isomer	$C_{13}H_8O_2$	25.3 – 9.7[e]	u	u	b
3,4,4a,5-naphthocoumarin or isomer	$C_{15}H_8O_2$	15.8 – 11.1[e]	u	u	b
3,4-naphthocoumarin or isomer	$C_{17}H_{10}O_2$	2.4[d]	u	u	c

Table 5.1. (continued)

compound	composition	concentration range (ng per mg EOC)[a]	single compound mutagenic potency (IMF (x10^6) per ng)[b]	contribution to potency of ambient mixture (IMF (x10^6) per mg EOC)	ID[c]
xanthone	$C_{13}H_8O_2$	8.2 – 1.8	u	u	a
phthalic anhydride	$C_8H_4O_3$	5.9[d]	u	u	c
Total PAC		*67.6 – 40.6*		*0.00*	
Aromatic Semipolar Subfraction (7a)					
PAK and PAQ					
7*H*-benz[*de*]anthracen-7-one	$C_{17}H_{10}O$	1.1 – 0.7[e]	4.0x10^{-4}	4.4 - 2.8(x10^{-4})	a
cyclopenta[*cd*]pyren-3(4*H*)-one	$C_{18}H_{10}O$	2.4 – 2.2	6.6x10^{-4}	1.6 - 1.5(x10^{-3})	a
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	39.7 – 19.5[e]	0.018	0.72 – 0.35	a
cyclopenta[*cd*]pyrenedione or isomer	$C_{18}H_8O_2$	5.4[d]	u	u	c
Total PAK and PAQ		*48.6 – 27.8*		*0.72 – 0.35*	
PAC					
3,4-naphthocoumarin or isomer	$C_{17}H_{10}O_2$	3.1[d]	u	u	c
dimethylphthalide isomers	$C_{10}H_{10}O_2$	6.8[d]	u	u	d
methylphthalide isomers	$C_9H_8O_2$	3.1[d]	u	u	d
Nitro-PAC					
hydroxynitrofluorenone isomer	$C_{13}H_7NO_4$	2.8[d]	u	u	d
Aromatic Semipolar Subfractions (11a & 12a)					
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	1.1[d]	0.00	0.00	a
1,8-naphthalic amide	$C_{12}H_7NO_2$	5.0[d]	u	u	c
2,3-naphthalic amide	$C_{12}H_7NO_2$	1.8[d]	u	u	c
phthalamide isomer	$C_8H_5NO_2$	45.9[d]	u	u	c
dimethylazanaphthalene isomers	$C_{11}H_{11}N$	109[d]	u	u	d
azanaphthalenedione isomer	$C_9H_7NO_2$	15.6[d]	u	u	d
Total PAC		*178*		*0.00*	
Nitro-PAC					
nitrobenzocoumarin isomer	$C_{13}H_7NO_4$	3.3[d]	u	u	d
nitroindole isomer	$C_8H_6N_2O_2$	78.2[d]	u	u	d
Total Nitro-PAC		*81.5*		*0.00*	
Aliphatic Semipolar Subfraction (3b - 12b)					
PAC					
6*H*-benzo[*cd*]pyren-6-one	$C_{19}H_{10}O$	1.6 – 0.8[e]	0.018	0.03 – 0.01	a
cyclopenta[*ghi*]perylenone or isomer	$C_{21}H_{10}O$	1.3[d]	u	u	b
1,8-naphthalic anhydride	$C_{12}H_6O_3$	5.0[d]	0.00	0.00	a
Total PAC		*7.9 – 7.1*		*0.03 – 0.01*	

Table 5.1. (continued)

compound	composition	concentration range (ng per mg EOC)[a]	single compound mutagenic potency (IMF (x10^6) per ng)[b]	contribution to potency of ambient mixture (IMF (x10^6) per mg EOC)	ID[c]
		Aromatic Polar Subfraction (13a)			
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	86.7[d]	0.00	0.00	a
1,2-naphthalic anhydride	$C_{12}H_6O_3$	3.1[d]	0.00	0.00	a
1,10-phenanthrene dicarboxylic acid anhydride or isomer	$C_{16}H_8O_3$	7.5[d]	u	u	b
naphthoic acid isomer	$C_{11}H_8O_2$	17.6[d]	u	u	b
vanillin	$C_8H_8O_3$	14.6[d]	u	u	d
Total PAC		*130*		*0.00*	
Other Compounds					
nonanal	$C_9H_{18}O$	164 – 72.7[e]	u	u	a
decanal	$C_{10}H_{20}O$	21.5[d]	u	u	b
caffeine	$C_8H_{10}N_4O_2$	25.4[d]	u	u	a
cocaine	$C_{17}H_{21}NO_4$	11.4 – 5.2[e]	u	u	a
Total		*222 – 125*		*0.00*	
		Aliphatic Polar Subfraction (13b - 16b)			
PAC					
1,8-naphthalic anhydride	$C_{12}H_6O_3$	1.4[d]	0.00	0.00	a
1,2-naphthalic anhydride	$C_{12}H_6O_3$	0.5[d]	0.00	0.00	a
Total PAC		*1.9*		*0.00*	
Other Compounds					
nonanal	$C_9H_{18}O$	83.4 – 36.9[e]	u	u	a
decanal	$C_{10}H_{20}O$	9.4[d]	u	u	b
nicotine	$C_{10}H_{14}N_2$	12.9[d]	u	u	a
caffeine	$C_8H_{10}N_4O_2$	4.6[d]	u	u	a
cocaine	$C_{17}H_{21}NO_4$	8.3 – 3.8[e]	u	u	a
Total		*119 – 67.6*		*0.00*	

[a] Range of concentrations shown reflect the range of concentrations measured for each compound when quantified in the whole sample extract and in the various subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. Compound concentrations are stated relative to the quantity obtained when processing one mg of organic carbon (EOC) contained within the whole ambient composite filter samples prior to extraction. The Los Angeles area 1993 ambient annual average composite airborne fine particle organic carbon concentration was 5.38 µg per m^3 EOC.

[b] Single compound mutagenic potency in the h1A1v2 assay as determined by regression analysis applied to the data collected by Durant and coworkers *(32)*. "u" is given for compounds which have not yet been tested in that assay, and therefore the mutagenic potency is unknown. Units are induced mutant fraction multiplied by 10^6 per ng of compound applied to the standard 12 mL h1A1v2 assay *(39)*.

Table 5.1. (continued)

[c] Compounds have been identified and quantified at different levels of confidence; a=positive, b=probable, c=possible, d=tentative (see criteria in text).

[d] Compound measurable only in subfractions; not measured in whole sample.

[e] Compound also measured in another subfraction, so the concentration reported as quantified from the whole sample extract was determined by multiplying the total concentration quantified in the whole extract by the percentage of the compound concentration occurring in this specific subfraction.

compound *(32)*. If a "u" (i.e. unknown) appears in this column then this compound has not been tested in the h1A1v2 assay, and therefore no mutagenic potency value can be calculated.. The overall mutagenic potency of the aromatics in the Nonpolar 1 subfraction is 66±13 to 142±30 IMF ($x10^6$)/mg EOC based on the results shown in Figure 5.2. The sum of the mutagenic potency contributions of the 31 compounds identified in this subfraction for which pure compound bioassays have been conducted to date is 25.1 to 7.7 IMF ($x10^6$)/mg EOC, roughly 38 to 5% of this subfraction's total activity. The largest single contributor to the activity among known compounds is due to cyclopenta[*cd*]pyrene, which accounts for 12.9/25.1 to 5.3/7.7 = 51 to 69% of the activity that can be assigned to a specific compound. Other contributing compounds include benzo[*a*]pyrene at 3.72 to 0.87 IMF ($x10^6$)/mg EOC, benzo[*ghi*]perylene at 2.43 to 0.36 IMF ($x10^6$)/mg EOC, benzo[*b*]fluoranthene at 2.06 to 0.51 IMF ($x10^6$)/mg EOC, indeno[1,2,3-*cd*]pyrene at 1.73 to 0.24 IMF ($x10^6$)/mg EOC, and benzo[*k*]fluoranthene at 1.17 to 0.26 IMF ($x10^6$)/mg EOC.

Ten additional PAH that can be named specifically remain untested in the h1A1v2 assay; their contribution to the overall mutagenicity of this fraction can be computed in the future if such bioassays are performed. Table 5.1 shows that numerous heavy PAH isomers exist in the ambient sample composite which have not been identified specifically because standards do not exist. These heavy PAH are potentially important mutagens as is evidenced by the fact that three of the four most mutagenic compounds tested in the h1A1v2 assay are 302µ PAH (i.e. PAH with molecular weight of 302 amu) *(32)*. Further notice that significant quantities of methyl PAH isomers also exist in this sample subfraction. These methyl isomers are potentially important mutagens as all of

the methyl isomers tested at present in the h1A1v2 assay are more potent than the parent PAH *(32)*. Thus, conceivably, the methyl PAH isomers and the unidentified heavy (5+ rings) PAH may account for the remaining portion of this subfraction's activity. It is worth noting that a single PAH, cyclopenta[*cd*]pyrene accounts for most of the mutagenic activity identified in this sample to date (potentially up to 12.9 of the 150 IMF ($x10^6$)/mg EOC found in the whole sample); it may not take more than a few additional very active compounds to account for the remaining activity of the Aromatic Nonpolar 1 sample subfraction.

Aromatics Nonpolar 2

The organic compounds found in this subfraction are shown in Table 5.1. Notice that some of the heavier PAH are contained within this subfraction. Other compounds that were sought in the SIM mode include a full suite of nitro-PAH, aromatic ketones and quinones, a detailed list appears in Table 5.2. Of these compounds only 9-nitroanthracene was identified in this subfraction. 13 other peaks are visible in the TIC mode, and from these peaks, quinoline and squalene were identified as being present.

The mutagenic potency of the aromatics in this subfraction is in the range 39±12 to 47±7 IMF ($x10^6$)/mg EOC based on the results shown in Figure 5.2. The sum of the mutagenic potency contributions of the compounds identified in this subfraction is 0.27 to 0.23 IMF ($x10^6$)/mg EOC. Thus, an insignificant amount of this subfraction's activity can be accounted for by those compounds identified in this study. Again, the further identification of the heavy PAH

Table 5.2. Targeted semipolar compounds sought in SIM mode that are either found in subfractions other than the mutagenic subfractions shown in Table 5.1, or that are not found at all.

Targeted compounds found in non-mutagenic subfractions	
compound	concentration range (ng per mg EOC)[a]
Aromatic Semipolar Subfraction (4a)	
anthracene-9,10-dione	6.4-2.6[b]
2-methylanthracene-9,10-dione	5.9-2.3[b]
benzanthrone or isomer	22.9-11.1[b]
methylbenzanthrone or isomer	2.6-2.0[b]
benz[*a*]anthracene-7,12-dione	1.7-0.6[b]
phenanthrene aldehyde or isomer	2.4[c]
Aromatic Semipolar Subfraction (5a)	
anthracene-9,10-dione	25.5-10.3[b]
2-methylanthracene-9,10-dione	7.9-3.1[b]
7*H*-benz[*de*]anthracen-7-one	2.4-1.5[b]
11*H*-benzo[*b*]fluoren-11-one	57.8-33.5[b]
methylbenanthrone or isomer	0.8-0.4[b]
cyclopenta[*ghi*]perylenone or isomers	6.7[c]
phenanthrene aldehyde or isomer	5.0[c]
pyrene aldehyde or isomers	10.0[c]
Aromatic Semipolar Subfraction (8a)	
phenalenone	182-93.0[b]
7*H*-benz[*de*]anthracen-7-one	0.8-0.5[b]
11*H*-benzo[*a*]fluoren-11-one	1.5-0.8[b]
11*H*-benzo[*b*]fluoren-11-one	0.5-0.3[b]
benzanthrone or isomers	22.2-19.4
6*H*-benzo[*cd*]pyren-6-one	2.2-1.1[b]
3,4,4a,5-naphthocoumarin or isomer	0.4-0.3[b]
1,8-naphthalic anhydride	99.4[c]
1,10-phenanthrene dicarboxylic acid anhydride or isomer	32.3[c]
Aromatic Semipolar Subfraction (9a)	
flourenone	8.4-1.8[b]
phenalenone	1.6-0.8[b]
7*H*-benz[*de*]anthracen-7-one	1.8-1.1[b]
11*H*-benzo[*b*]fluoren-11-one	0.9-0.5[b]
1,8-naphthalic anhydride	1.5[c]
1,10-phenanthrene dicarboxylic acid anhydride or isomer	0.2[c]
Aromatic Semipolar Subfraction (10a)	
1,8-naphthalic anhydride	0.4[c]

Table 5.2. (cont.)

Targeted compounds not found in any semipolar subfraction	
nitronaphthalene isomers	dinitrofluorene isomers
nitrofluorene isomers	dinitropyrene isomers
other nitropyrene isomers	dinitrobenzo[*a*]pyrene isomers
methyl nitropyrene isomers	hydroxynitropyrene isomers
nitrochrysene isomers	nitrofluorenone isomers
nitrobenzo[*a*]pyrene isomers	thioxanthone
	dibenzothiophene isomers

a Range of concentrations shown reflect the range of concentrations measured for each compound when quantified in the whole sample extract and in the various subfractionated extracts. Generally, the highest values are those seen in the whole extract as it has been subjected to the least opportunity for losses during separations and processing. Compound concentrations are stated relative to the quantity obtained when processing one mg of organic carbon (EOC) contained within the whole ambient composite filter samples prior to extraction. The Los Angeles area 1993 ambient annual average composite airborne fine particle organic carbon concentration was 5.38 μg EOC per m^3 air.

b Compound also measured in another subfraction, so the concentration reported as quantified from the whole sample extract was determined by multiplying the total concentration quantified in the whole extract by the percentage of the compound concentration occurring in this specific subfraction.

c Compound measurable only in subfractions; not measured in whole sample.

isomers could be an important next step. The single nitro-PAH identified accounted for essentially none of the activity.

Aromatic Semipolar Subfractions

The organic compounds identified in the Aromatic Semipolar subfractions that contain significant mutagenic activity are shown in Table 5.1. In addition, chemical analysis of another Aromatic Semipolar subfraction (6a), which exhibited a high nominal mutagenic potency value (16 IMF ($x10^6$)/mg EOC) but with a large uncertainty (±9 IMF ($x10^6$)/mg EOC), also appears in Table 5.1 and will be discussed. The compounds which were on the list of targeted semipolar organics that were sought by the SIM method but that were not found in a mutagenic subfraction appear in Table 5.2. This list includes numerous oxygenated and nitrated aromatic compounds.

The targeted compounds identified in subfraction 3a consist primarily of aromatic ketones, aromatic quinones, and nitro-aromatics. This subfraction was also processed in full scan mode and 10 additional peaks were found (i.e. 10 more peaks than were quantified using the list of targeted compounds that were sought in SIM mode). These 10 peaks include the following compounds: benzanthrone or isomer, dimethylanthracene-9,10-dione, methylbenzanthrone or isomer, and 4 partially aromatic ketones. These additional compounds are entered in Table 5.1 with the exception of the 4 partially aromatic ketones for which we have no corresponding similar compounds from which to estimate a response factor. This subfraction's mutagenic potency is 12±5 IMF ($x10^6$)/mg EOC. The lone significant contributing compound identified in this subfraction is

2-nitrofluoranthene, which accounts for 1.27 to 0.12 IMF (x10^6)/mg EOC. Additional testing of pure compounds is recommended.

Subfraction 6a consists mostly of oxygenated aromatic compounds, including ketones, quinones, coumarins, and aldehydes. A full scan mode analysis of this sample found 23 additional peaks in this subfraction, of which 10 can be tentatively identified as methylbenzanthrone or isomers, 3,4-naphthocoumarin or isomer, phthalic anhydride, diphenyl methyl pentene isomers, and 2,4-bis(dimethyl)6-t-butylphenol. Those identified aromatic compounds found by full scan mode analysis have been entered in Table 5.1 as well. This subfraction's mutagenic potency is 16±9 IMF (x10^6)/mg EOC. One compound identified in this subfraction has been found to be mutagenic at significantly low enough doses to be a contributing mutagen: 6*H*-benzo[*cd*]pyren-6-one contributes 1.63 to 0.80 IMF (x10^6)/mg EOC or 10 to 5% of the best estimate of the mutagenic potency of this subfraction.

Subfraction 7a also contains mostly oxygenated aromatic compounds. From the targeted list of semipolar compounds sought in the SIM mode four compounds were identified: 7*H*-benz[*de*]anthracen-7-one, cyclopenta[*cd*]pyren-3(4*H*)-one, 6*H*-benzo[*cd*]pyren-6-one, and tentatively a hydroxynitrofluorenone isomer. In the full scan mode analysis, 24 additional peaks are visible of which 6 can be tentatively identified as 3,4-naphthocoumarin or isomer, cyclopenta[*cd*]pyrenedione or isomer, 2 methylphthalide isomers, and a dimethyl phthalide isomer. The mutagenic potency of this subfraction is 14±4 IMF (x10^6)/mg EOC, and again 6*H*-benzo[*cd*]pyren-6-one was found to contribute 0.72 to 0.35 IMF (x10^6)/mg EOC or 5 to 2.5% to the mutagenic potency of this subfraction.

Subfractions 11a and 12a contain only 2 compounds on the list of targeted semipolar compounds, 1,8-naphthalic anhydride and a nitrobenzocoumarin isomer. Neither of these two compounds contribute significantly to the mutagenic potency of these subfractions, which is 9±4 IMF ($x10^6$)/mg EOC for subfraction 11a and 5±2 IMF ($x10^6$)/mg EOC for subfraction 12a. Other compounds identified in these subfractions using full scan mode analysis include 1,8-naphthalic amide, 2,3-naphthalic amide, a phthalamide isomer, an azanaphthalenedione isomer, dimethylazanaphthalene isomers, a nitroindole isomer, carvone, hydroxycarvone, hexadecanamide, heptadecanamide, octadecanamide, and (Z)-9-octadecenamide. Those compounds for which response factors could be estimated were entered in Table 5.1.

Aliphatic Semipolar Subfraction

As explained previously, the separation of aliphatic compounds from aromatic compounds by the size exclusion column is not 100% effective, and thus a few polycyclic aromatic compounds (PAC) can be expected to be found in the "aliphatic" fractions. Three targeted PACs identified in this subfraction (3b-12b) are shown in Table 5.1. There were 35 peaks visible in the full scan chromatogram, and of these peaks the following compounds have been tentatively identified: nonanal, decanal, dodecanal, hexyl butanoate, dioctyl hexadecane-dioate, tributyl phosphate, hexadecanol, octadecanol, dodecanol, (3β,24S)-stigmast-5-en-3β-ol, and 5α-stigmastan-3-one. Very few of these compounds have been tested in the h1A1v2 assay system, and thus an insignificant portion of the subfraction's activity (10±3 IMF ($x10^6$)/mg EOC) can

be attributable to specific compounds identified to date. Since there is some of overlap of a few aromatic compounds into this fraction, further identification and pure compound bioassay testing of these compounds may help to explain the source of this subfraction's activity.

Aromatic Polar Subfraction

The organic compounds identified in the mutagenic Aromatic Polar subfraction (13a) are shown in Table 5.1, and include naphthalic anhydride isomers, a pyrene anhydride or isomer, naphthalic acid isomers, vanillin, as well as aldehydes such as nonanal and decanal. The cyclic compounds caffeine and cocaine are found in this subfraction, indicating their presence at measurable levels in Los Angeles outdoor air. In addition to those compounds quantified in Table 5.1 another 25 peaks were observed in the full scan chromatogram, and those tentatively identified include C_{12} to C_{18} alkanoic acids, 2-(2-butoxyethoxy)-ethanol, caprolactam, (1S,2S,3R,5S)-(+)-pinanediol, and triphenyl phosphine oxide. Again, no significant amount of this subfraction's activity (5±2 IMF ($x10^6$)/mg EOC) can be assigned to identified compounds; however, only 2 of the compounds identified have been tested in the h1A1v2 human cell mutation assay to date.

Aliphatic Polar Subfraction

The organic compounds identified in the Aliphatic Polar subfraction are shown in Table 5.1, and include nonanal, decanal, and naphthalic anhydride isomers. Nicotine, caffeine, and cocaine also are detected in this fraction. In addition to those compounds quantified in Table 5.1 another 25 peaks were

observed in the full scan chromatogram, and those tentatively identified include phthalamide, 2-(2-butoxyethoxy)-ethanol, 2-[2-(2-methoxyethoxy) ethoxy]-ethanol, 2-[2-(2-ethoxyethoxy)ethoxy]-ethanol, 2-[2-(2-butoxyethoxy) ethoxy]-ethanol, nonanamide, palmitamide, oleamide, glyceryl monopalmitate, glyceryl monostearate, and 2-butoxy-ethanol phosphate(3:1). The mutagenic potency of this subfraction was 13±3 IMF ($x10^6$)/mg EOC, and none of this activity can be attributed to specific compounds found within this subfraction as there has been very limited testing of polar compounds in the h1A1v2 assay to date. The size exclusion column produces a less well-defined separation between aromatic and aliphatic compounds in this polarity range, as many of the same compounds appear to some extent in both fractions. Therefore, the observation that there is significantly more mutagenic potency in the Aliphatic Polar subfraction than there is in the Aromatic Polar subfractions does not necessarily mean that the polar mutagens are aliphatic in nature.

Discussion

The Aromatic Nonpolar 1 and Nonpolar 2 subfractions that contain the ordinary PAH contribute a large portion of the mutagenicity of the whole atmospheric aerosol sample. This is consistent with previous results reported for assays involving mammalian cells and whole mammalian animals *(9, 27, 25, 10, 26, 20)*. Six specific PAH that have been tested as pure compounds in the h1A1v2 human cell assay are among the most important mutagens identified to date in the atmospheric sample. Under the assumption of additive effects, these 6 compounds together could account for an induced mutant fraction per unit of organic aerosol supplied to the h1A1v2 assay of about 24 to 7.6 IMF ($x10^6$) per

mg EOC. That can be compared to the whole sample mutagenic potency of 150 IMF (x10^6) per mg EOC or to the summation of the potencies of the fractionated samples which is in the range of 231-243 IMF (x10^6) per mg EOC. Among these PAH, cyclopenta[*cd*]pyrene (12.9 to 5.31 IMF (x10^6) per mg EOC) is the most important contributor to the mutagenic potency of the sample, followed by benzo[*a*]pyrene (3.72 to 0.87 IMF (x10^6) per mg EOC), benzo[*ghi*]perylene (2.43 to 0.36 IMF (x10^6) per mg EOC), benzo[*b*]fluoranthene (2.06 to 0.51 IMF (x10^6) per mg EOC), indeno[1,2,3-*cd*]pyrene (1.73 to 0.23 IMF (x10^6) per mg EOC), and benzo[*k*]fluoranthene (1.17 to 0.26 IMF (x10^6) per mg EOC). Cyclopenta[*cd*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene historically have been emitted largely from non-catalyst equipped gasoline-powered motor vehicles in Los Angeles, while emissions of benzo[*b*]fluoranthene and benzo[*k*]fluoranthene have been dominated by vehicle exhaust plus natural gas combustion aerosol *(39)*. Many other ordinary PAH and substituted PAH that have yet to be tested in the h1A1v2 human cell assay are present in the atmospheric samples. If these compounds are further characterized by testing in the human cell assay, there is the prospect that much more of the total mutagenic activity of the Los Angeles area atmospheric aerosol can be explained. This is particularly true since the substituted PAH tested to date are generally more potent mutagens than their unsubstituted relatives, and many of the substituted PAH remain to be tested in the human cell assay.

Important mutagens also are found in the fractions beyond those containing mainly ordinary PAH. The 4-way separation of the whole sample shows that more than half of the mutagenicity may reside within the semipolar

and polar compound groups. Further subfractionation of the semipolar and polar fractions shows that these mutagens are distributed widely across 8 of the 16 semipolar and polar subfractions studied. The identity of the semipolar and polar mutagenic compounds is particularly hard to establish. Few pure compound standards are available for semipolar and polar aromatics, yet such compounds are needed for both positive chemical identification and pure compound testing in the h1A1v2 assay. Nevertheless, a few important semipolar mutagens have been identified and quantified in the atmospheric samples, including 2-nitrofluoranthene (1.27 to 0.12 IMF ($x10^6$) per mg EOC), and 6*H*-benzo[*cd*]pyren-6-one (summed potency over subfractions 6a & 7a of 2.35 to 1.15 IMF ($x10^6$) per mg EOC). 2-nitrofluoranthene is a product of atmospheric chemical reactions in Los Angeles *(30)* , while 6*H*-benzo[*cd*]pyren-6-one historically has been emitted largely by non-catalyst gasoline-powered motor vehicles in Los Angeles *(39)*.

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Chapter 6

Source Contributions to the Human Cell Mutagenicity of Urban Particulate Air Pollution

Introduction

A major aim of most air pollution control programs is to limit the atmospheric concentrations of pollutants that could cause adverse effects on human health. Careful design of an emission control program requires knowledge about the connections between pollutant source emissions, atmospheric transport and chemical transformation, and the ambient pollutant concentrations to which people are exposed. Such source-to-receptor relationships often are determined through computer-based air quality models by solving continuity equations for individual compounds or compound classes, and often the pollutant species being tracked is essentially a simplified index for the presence of a much more complex pollutant mixture. In the case of particulate air pollution, the presently regulated index of pollutant exposure in the United States is defined in terms of the airborne particle mass concentration contributed by particles with a diameter smaller than 10 μm.

Urban particulate air pollution contains an extremely complex mixture of thousands of different organic compounds, many present in only trace amounts, along with acid aerosols, trace metals, plus a much larger amount of likely harmless but bulky material from other sources such as airborne soil dust and sea salt. By focusing attention on particle mass concentration, or even the fine particle mass concentration, it is entirely likely that those chemical components

of the particulate air pollution mixture that are actually capable of inducing the sort of biological changes that could affect health will be missed entirely.

Certain chemical components of the particulate air pollution mixture are capable of inducing genetic damage. Particulate air pollution has been shown to mutagenic to bacteria *(1-4)*, mutagenic to human cells *(5, 6)*, and carcinogenic *(7, 8)*. Using bacterial mutagenicity as the biological endpoint, several studies have attempted to identify the specific chemical species present in atmospheric particles that can cause genetic damage using bioassay-directed chemical analysis *(4, 9, 10)*. In a program of bioassay-directed chemical analysis, the organic extracts of atmospheric particle samples are separated chemically, analyzed chemically to identify the mutagenic compounds present, and the compounds found are subjected to bioassays until the most important mutagens in the complex atmospheric sample have been identified. Particulate matter emissions from sources such as diesel engine exhaust also have been studied using bioassay-directed chemical analysis *(11, 12)*. The effluents from automobile exhaust, coal-fired furnaces, and kerosene flames have been examined using different biological endpoints, such as carcinogenic effect in mice *(13-15)* and human cell mutagenicity *(16, 17)*.

Determining the effect of air pollution sources on the mutagenic potency of atmospheric particulate matter based on the existing scientific literature is difficult at best. Several biological endpoints have been used to create the existing data base on emission source characteristics. Furthermore, important differences exist in the procedures used by different laboratories to collect and analyze air pollution samples even when the bioassay procedures to be applied to the samples are nominally similar *(18-20)*. These differences

between laboratories can significantly obscure comparisons between the properties of source samples and atmospheric samples that were analyzed by different research groups. In addition, the list of air pollutant emission sources studied to determine their mutagenic potency often is quite short, with much of the previous work being focused on one source, diesel engine exhaust.

The present study seeks to establish methods for determining the contributions of specific fine particle emissions sources to the human cell mutagenicity of ambient particulate air pollution. Methods developed will be tested in the Los Angeles area using data on the organic chemical composition and human cell mutagenicity of a comprehensive set of atmospheric fine particle samples and a comprehensive set of urban organic aerosol source samples, both collected specifically for use in this study. The analysis proceeds by first determining the contribution of specific fine particle emission sources to the ambient fine particle organic compound mass concentration using a previously developed chemical mass balance receptor modeling technique that uses organic compounds as tracers *(21)*. The calculated source contributions will be used along with the human cell mutagenic potency values (mutant fraction per μg of fine particulate organic carbon emitted from the source) for each emission source to determine the mutagenic density (mutant fraction per m^3 of air) of the ambient aerosol that would exist if atmospheric chemical reactions were unimportant to transformation of the ambient mixture. This calculated mutagenic potency of the ambient fine particle mixture then can be compared to the measured value and the importance of specific sources and/or secondary chemical reactions can be discussed.

Filter samples collected throughout the course of the year 1993 at four air monitoring sites in Southern California will be used here to create a composite fine particulate air pollution sample that is representative of long-term exposure conditions in Southern California. A comprehensive set of urban fine particle emission source samples, which represent approximately 80% of the fine organic particulate matter emissions in the Los Angeles area *(22)*, is analyzed in the present study in order to describe the character of primary source emissions. The human cell mutation assay *(23)* used in this study tests for mutagenic activity at the thymidine kinase locus in the h1A1v2 cell line (AHH-1 TK± cells bearing the plasmid pHSRAA, which contains two copies of the human CYP1A1 cDNA). For purposes of comparison against earlier studies, the bacterial mutagenicity of the source and ambient samples also will be explored. The bacterial mutation assay used is a version of the *S. typhimurium* TM677 forward mutation assay developed by Skopek et al. *(24)* which measures resistance to 8-azaguanine.

Experimental Section

Ambient Samples

An urban particulate air pollution sample representative of exposure conditions in the greater Los Angeles area was created by compositing a portion of every urban fine particulate filter sample collected during a 1993 Southern California air monitoring campaign. This air monitoring campaign is described in more detail elsewhere *(25, 5)*. Individual urban particulate air pollution samples were taken for 24 h every sixth day for the entire year of 1993 at four urban sites, and at a background site located on an offshore island

upwind of Los Angeles. Samples taken at the background site were not included in the urban particulate air pollution composite used in this study. The 4 urban sites were chosen in an attempt to capture the variety of typical particulate matter exposures seen in the South Coast air basin: a highly industrialized area, Long Beach; a location that experiences dense freeway traffic, central Los Angeles; a downwind residential area with high ozone and secondary aerosol concentrations, Azusa; and a site even farther downwind with even more secondary aerosol, Rubidoux.

In order to collect a large volume of size-separated organic aerosol in a 24 h period, a high volume dichotomous virtual impactor was employed at each site. Details of the design of this sampler are given by Solomon et al. *(26)* and a description of its use for this sampling campaign can be found elsewhere *(25, 5)*. When operated at a nominal flow rate of 300 L min^{-1}, this sampler produces a size cut between fine and coarse particles at approximately 3-4 μm aerodynamic diameter. Quartz fiber filters (102 mm diameter Pallflex Tissuquartz 2500 QAO) were used for the particulate matter collection, and details of the filter handling procedures are given by Hannigan et al. *(25)* One sixth of each fine particle filter was extracted to form the composite sample used here. This composite physically represents the annual average ambient fine particulate matter concentration averaged over all four urban sites. All sample and organic aerosol extract mass is reported in units of μg of equivalent organic carbon (EOC), which is defined as the amount of organic carbon present on the filter sections prior to extraction as determined by thermal evolution and combustion analysis of separate sections cut from the same quartz fiber filters.

The EOC measure provides a link from the assay results to the ambient organic aerosol concentrations.

In addition to the high volume dichotomous sampler, a low volume fine particle sampler employing an AIHL-design cyclone separator was located at each sampling site during the 1993 field measurement campaign. For the collection of fine particulate matter (diameter less than 2 μm), the sampler was equipped with 2 quartz fiber filters (47 mm diameter, Pallflex 2500QAO) each operated at a flow rate of 10 L min^{-1} and 2 polytetrafluoroethylene (PFTE) filters (46 mm diameter, Gelman Teflo, 0.5 μm pore size) operated at flow rates of 3 and 5 L min^{-1}. Samples collected on the PFTE filters were used for inorganic chemical analysis as well as for the determination of the total fine particle mass concentration. Details of the operation of this sampler can be found elsewhere *(25)*.

Source Samples

The aerosol source samples used in the present study were collected by Hildemann et al. *(27, 22)*. The sampling strategy focuses on obtaining representative samples from 15 source types that collectively account for about 80% of the fine organic aerosol emissions to the atmosphere in the Los Angeles area. All 15 source types are shown in Table 6.1. A portable dilution source sampling system designed by Hildemann et al. *(27)* was used. In a dilution sampler, hot exhaust emissions are mixed with purified dilution air in order to cool the sample to ambient conditions, thus allowing vapor-phase semi-volatile organics found in the exhaust of hot combustion sources to condense onto existing aerosol within the sampling system as normally would occur in the

Table 6.1. Summary of bioassay results for the particle emission sources.

Source Sample	Human Cell Mutagenic Potency[a] IMF (x10^6) per μg EOC	Bacterial Assay Mutagenic Potency[b] −PMS MF (x10^5) per μg EOC	Bacterial Assay Mutagenic Potency[b] +PMS MF (x10^5) per μg EOC	Lumped Source Type[c]
Catalyst-Equipped Automobiles	0.11±0.04	2.8±0.95	0.37±0.03	1
Noncatalyst Automobiles	0.40±0.07	2.3±0.10	0.83±0.07	1
Heavy-Duty Diesel Trucks	0.18±0.04[d]	5.1±2.9	0.76±0.08	2
Fuel Oil-Fired Boiler	0.07±0.02	1.0±0.61	0.70±0.27	
Natural Gas Home Appliances	4.1±1.4	13±4.7	18±2.9	3
Fireplace, Pine Combustion	0.08±0.03	1.4±0.15	0.15±0.04	4
Fireplace, Oak Combustion	0.08±0.08	2.9±0.43	0.11±0.10	4
Fireplace, Synthetic Log	0.00±0.01	0.39±0.07	0.00±0.09	
Cigarette Smoke	0.01±0.01	0.10±0.05	0.07±0.02	5
Roofing Tar Pot	0.10±0.07	0.07±0.01	0.07±0.05	
Meat Charbroiling	nd	–	–	6
Paved Road Dust	nd	–	–	7
Brake Wear Dust	nd	–	–	
Tire Wear Debris	nd	–	–	8
Urban Vegetative Detritus	nd	–	–	9

[a] The human cell mutagenic potency value represents an estimate of the initial slope of the dose/response relationship obtained using a least-squares linear fit

Table 6 footnote [a] (continued)

to the data which was forced through the origin (zero dose by definition yields zero induced mutant fraction). Only those source samples which tested positive in the bacterial assay were tested in the human cell assay; the human cell mutagenic potency for the other source samples is labeled not determined, or nd. [b] The bacterial mutagenic potency was estimated under two assay conditions, with the addition of PMS (referred to elsewhere as S9) and in the absence of PMS. The potency value is determined by calculating the initial slope of the dose/response curve using a least-squares linear fit to the data. A "–" value indicates that the sample was not mutagenic at the dose levels tested. [c] Lumped source type numbers indicate which source sample chemical fingerprints were combined to form a single lumped source type for use in the chemical mass balance model. No number indicates that these sources were not used in the chemical mass balance model. [d] Human cell mutagenic potency value for Heavy-Duty Diesel Trucks is based on testing extract of the NBS SRM 1650 diesel exhaust particulate matter sample.

atmospheric plume downwind of the source. A diagram of the dilution source sampler and a detailed description of its operation is presented by Hildemann et al. *(27, 22)* . All samples were collected on quartz fiber filters, which were prepared and stored by the same procedures as those used for ambient samples. Variability between sources of the same type is addressed by compositing samples from several sources (e.g., a small fleet of different motor vehicles) before extraction and bioassay. In a previous study using bacterial mutation assays, 5 non-combustion sources from among the 15 source types studied here were determined to be nonmutagenic at dose levels where the other 10 were active *(28)* . Further biological analysis using the human cell assay in the present study was conducted only for that group of 10 mutagenic source types. During the course of the human cell bioassays, the diesel sample of Hildemann et al. *(22)* was fully consumed and was replaced by extract prepared from the SRM 1650 diesel exhaust aerosol sample distributed by the National Institute of Standards and Technology (NIST). Concurrently with the execution of the new human cell assays of these source samples, sample extracts were retested repeatedly in the bacterial assay system in order to obtain further direct comparison of the mutagenic potency of the source and ambient samples in both the bacterial and human cell systems.

Extract Preparation

All source and ambient particulate matter filter samples used in this study were extracted in soxhlets with dichloromethane (DCM) for at least 16 h. Extracts were concentrated in a vacuum centrifuge. Further concentration or solvent exchange into dimethyl sulfoxide (DMSO) for assay testing was

accomplished by blowing a gentle stream of dry ultra-pure N_2 over the sample. The extracted mass from a portion of the composited ambient aerosol sample was measured by a microscale evaporation method *(29)* and was compared to the mass of organic carbon (EOC) originally present on the filter samples prior to extraction as determined by thermal evolution and combustion analysis *(30, 31)*. The results of this test showed 0.93 μg of extracted mass per μg of EOC. For a more detailed description of the filter extraction procedure, see Hannigan et al. *(28)*.

Human Cell Mutation Assay

The procedures for the routine use of the h1A1v2 cell line for mutagenicity testing at the thymidine kinase (*tk*) locus are given by Penman et al. *(23)* and Busby et al. *(32)*. Aliquots of sample extract are tested by exposing duplicate 12 mL cultures of 1.8×10^6 exponentially growing cells for 72 hrs. Exposure is terminated by centrifuging and resuspending the cells in fresh media (30 mL). One day after the exposure is terminated, the cultures are counted and diluted to 80 mL at 2×10^5 cells mL^{-1}. After the 3 day phenotypic expression period, cultures were plated in 96-well microtiter plates in the presence of the selective agent to determine mutagenicity (n=3 with 20,000 cells per well) and in the absence of the selective agent to determine plating efficiency (n=2 with 2 cells per well). The selective agent used for this forward mutation assay is trifluorothymidine. After an additional 13 day incubation period the plates are scored for the presence of a colony in each well. The positive control sample consists of 1.0 μg mL^{-1} benzo[*a*]pyrene (BaP), and DMSO is used as the negative control.

Plating efficiencies, mutant fractions, and their associated confidence intervals (standard deviations) are calculated using methods described by Furth et al. *(33)* Each sample is tested in a minimum of two independent assays to ensure test reproducibility. Independent assay results are pooled to allow for quantitative evaluation of mutagenicity. To control for varying background mutation rates (i.e. variations in the concurrent negative controls from one experiment to another), the results from all experiments are converted to induced mutant fraction (IMF) by subtracting the mean mutant fraction of the concurrent negative control from the mean mutant fraction observed for the sample extracts. The mutagenic potency of a sample is defined as the IMF per μg EOC supplied to the test. The mutagenic potency of each sample is estimated by pooling all experimental points for each sample and then computing the initial slope of the dose/response relationship using a least-squares fit to the data that is forced through the origin (because at zero dose, there is by definition zero IMF). This technique has been used previously to evaluate the seasonal and spatial variation of the human cell mutagenicity of Southern California airborne fine particle samples *(5)* .

Bacterial Mutation Assay

A detailed description of the procedures for the routine use of the *S. typhimurium* forward mutation assay can be found elsewhere *(24, 34)*. Briefly, the suspended bacteria undergo a 2 h exposure to several dilutions of the sample. The exposure is done under two conditions, with or without the presence of 5% (v/v) Aroclor induced post mitochondrial supernatant preparation (PMS, also refer to elsewhere as S9). After the exposure period,

the reaction is quenched and aliquots are plated in the presence and absence of 50 µg/mL of 8-azaguanine. The results from a minimum of four independent cultures, each plated in triplicate, are averaged to estimate toxicity and mutagenicity at each sample dilution. Colonies are counted after 48 h. The mutant fraction (i.e., mutagenicity) is determined as the number of colonies formed in the presence of 8-azaguanine divided by the number of colonies formed in its absence, multiplied by a dilution factor. Positive concurrent control samples (BaP for +PMS and 4-nitroquinoline oxide for –PMS) and negative concurrent control samples (DMSO, both + and –PMS) are processed in parallel with each sample. The bacterial mutagenic potency is determined by calculating the initial slope of the dose-response curve using a least squares linear fit to the data. It is known that the TM677 strain used for this study does not lack nitroreductase; 1-nitropyrene (1-NP), 4-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, and 1,8-DNP are all potent –PMS mutagens in our strain of TM677 *(34)*.

Chemical Analysis

Organic chemical analysis by gas chromatography/mass spectrometry (GC/MS) was conducted in order to quantify both mutagenic compounds in the source and ambient samples as well as to quantify the concentration of organic compounds that act as tracers for the presence of the effluent from specific sources when found in an ambient sample *(21)*. Procedures used for GC/MS analysis in the present study are given in detail elsewhere *(35)*. The system employed is a Hewlett-Packard GC (model 5890) equipped with a DB1701 fused silica capillary column (J&W Scientific) connected to a Hewlett-Packard

mass sensitive detector (model 5972). All GC/MS analyses used in the source apportionment phase of this study were done in full scan mode using coinjection of a known amount of 1-phenyl dodecane as a measure of instrument response. For quantification of each organic compound, relative response factors (RRF) were obtained from multiple injections of an authentic standard or from multiple injections of a compound with a similar molecular weight (MW) and functionality. A 45 component standard mixture was injected periodically between sample injections to ensure that instrument performance was stable throughout the long series of analyses.

The concentration of the trace elements Al and Si required for use by the source apportionment technique *(21)*, were determined by X-ray fluorescence analysis *(36)* applied to samples collected on 47 mm diameter PFTE filters collected in parallel with each of the quartz fiber filters used for source and ambient measurements. Elemental carbon (EC) and organic carbon (OC) concentrations present on portions of each 102 mm diameter filter that went into the ambient composite as well as on sections cut from the source sample filters were determined by the thermal evolution and combustion method described by Huntzicker and co-workers *(30, 31)*, as modified by Birch and Cary *(37)*.

Chemical Mass Balance Model

A previously published chemical mass balance model that uses organic compounds as tracers for the presence in the atmosphere of the emissions from specific sources *(21)* is used in this study. Details of this source apportionment technique are given by Schauer et al. *(21)*. Briefly, the concentration of a specific compound in the ambient sample is described as a linear combination

of emissions of that specific compound from the various emission sources. Mathematically the model is expressed as follows:

$$c_i = \sum f_{ij} a_{ij} s_j \qquad \text{for } j = 1,2,\ldots m \qquad (1)$$

where c_i is the concentration of chemical species i measured in an atmospheric sample, f_{ij} is the fractional loss or gain of species i emitted from source j before that species reaches the atmospheric monitoring site, a_{ij} is the fractional concentration of species i in the emissions from source j, and s_j is the increment to the entire fine particle organic mass concentration at the receptor site due to emission source j. The value of f_{ij} is not easily determined for highly reactive organic compounds and therefore for the purposes of this study we will use only those compounds that are stable enough in the atmosphere that the values of f_{ij} are empirically observed to be near unity. From our GC/MS measurements of ambient and source sample composition, respectively, values for c_i and a_{ij} have been obtained. When the number of chemical species emitted exceeds the number of source types considered, the over-determined set of equations (1) can be solved to obtain values of the s_j using the effective-variance weighted least squares fitting technique of Watson et al. *(38, 39)*. Thus the relative importance of the various emissions sources that contribute to the overall fine organic compound concentration in the atmospheric sample can be estimated.

The decision to use only chemical species with f_{ij} values that are near unity makes the selection of chemical species to be used in this analysis extremely important. If a chemical species is destroyed rapidly by atmospheric

chemical reactions then a linear combination of the chemical composition profiles measured at the sources will produce predictions for that compound that greatly exceed the observed ambient concentration. If a particulate chemical species is created during atmospheric transformation reactions (i.e. via secondary aerosol formation from gas-phase precursors) then a linear combination of source composition profiles that contain that compound will underestimate the observed ambient concentration. Using strict chemical species selection criteria, Schauer et al. *(21)* developed a list of organic chemical species plus EC, Al and Si that were used successfully to apportion the fine organic aerosol concentration and fine particle mass concentration in the Los Angeles area atmosphere between nine of our contributing sources. This compound list was used as a starting point for the present study.

The selection of emission source types also is important. If two emission source types have similar chemical fingerprints, then they will be indistinguishable in this analysis. Therefore, some grouping of similar source types must occur for the model to generate useful results. Schauer et al. *(21)* describe in detail the procedure for determining which source profiles must be combined. For the Los Angeles area source samples used here, the pine and oak wood combustion source profiles are combined to produce an emissions-weighted average source profile for softwood plus hardwood smoke and the catalyst plus noncatalyst gasoline-powered motor vehicle exhaust profiles are combined to produce an emissions-weighted average profile for gasoline-powered vehicles. The composite woodsmoke profile is assembled exactly as described by Schauer et al. *(21)*. The gasoline-powered motor vehicle exhaust composite profile is recalculated as a weighted average of the catalyst and

noncatalyst engine exhaust samples assembled to reflect a 1993 mixture of emissions from catalyst versus noncatalyst vehicles according to the 1993 vehicle mileage tables published by the South Coast Air Quality Management District *(40)* plus estimates of off -road gasoline engine use.

Diesel engine emissions are distinguished from gasoline-powered vehicle engine emissions in part by the larger fraction of black elemental carbon in diesel exhaust. In recent years, diesel engine emission control technology has improved greatly, creating a vehicle fleet in current use that represents a mixture of old and newer technology engines with varying emissions ratios of elemental carbon to organic compounds. In the present study, a fleet average ratio of elemental carbon to particulate organic compounds from on-road diesel vehicles first was computed from the literature review of diesel engine emissions characteristics as a function of production era reported by Sawyer and Johnson *(41)* combined with estimates of relative annual mileage accumulation by trucks and autos of different ages *(42, 43)*. The analysis suggests that diesel engine emissions in the South Coast Air Basin that surround Los Angeles in 1993 should consist of approximately 33 % elemental carbon and 31 % organic compounds by mass, plus trace amounts of Si and Al as measured in diesel exhaust by Hildemann et al. *(22)*. The combined diesel engine profile used in the present study thus consists of EC, Al, Si, and total organics in those proportions, with the relative composition of the organic compounds taken from our GC/MS analysis of filter samples taken during the source tests of Hildemann et al. *(22)*.

The completed source profiles for the nine emissions source types showing the relative concentration of tracer compounds that will be supplied to

the chemical mass balance model are given in Figures 6.1-6.5. All chemical compound concentrations in the source samples supplied to the human cell assay in this study are based on new GC/MS analyses of high volume filter samples collected by Hildemann et al. *(27, 22, 44)* since those high volume filters were the source of the organic material used in the new human cell bioassays and since the ambient samples used here were taken with high volume dichotomous samplers. Analysis of the 5 source samples which were found to be nonmutagenic in our previous work consisted solely of quantification of tracer compounds used in the source apportionment calculations, and therefore previously generated extracts of these samples were used. A description of the sample extraction and concentration protocol used for the 5 nonmutagenic source samples can be found in Mazurek et al. *(45)*. Elimination of variation in analytical conditions and techniques makes it necessary to quantify the organic compound concentrations in all source and ambient samples used in the chemical mass balance model on the same instrument, with the same protocol, over a short period of time. By processing both source and ambient samples by GC/MS simultaneously, any biases due to chemical analysis procedures are equalized between the source and ambient samples, which in turn should aid the matching of source profiles to the ambient samples.

Results

Apportionment of Fine Particulate Organic Mass

The chemical mass balance model constructed previously by Schauer et al. *(21)* was used to apportion the fine organic particulate matter in the Los

Figure 6.1. Source profiles for gasoline-powered vehicle exhaust aerosol and natural gas combustion particles.

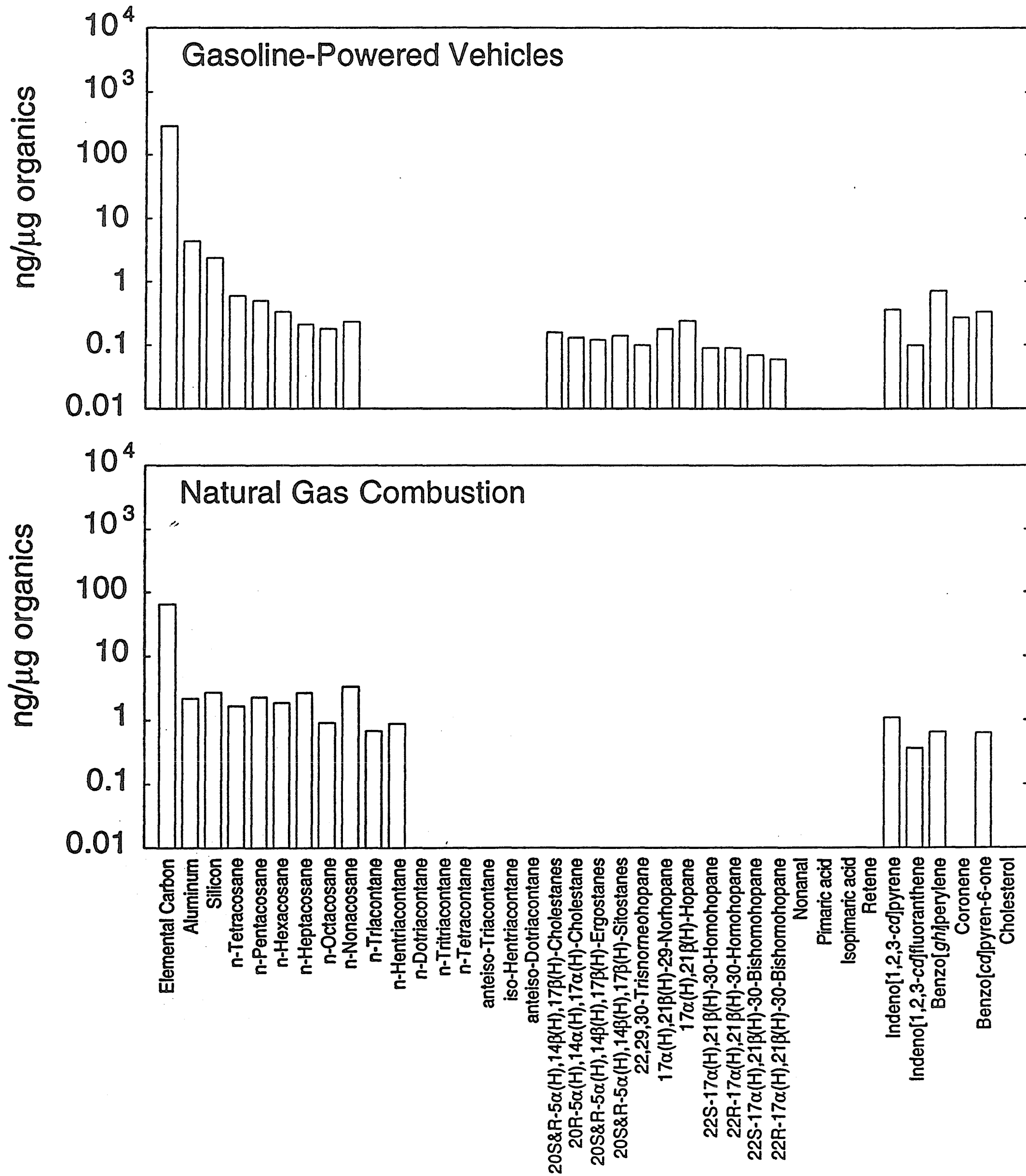


Figure 6.2. Source profiles for diesel-powered vehicle exhaust aerosol and wood combustion particles.

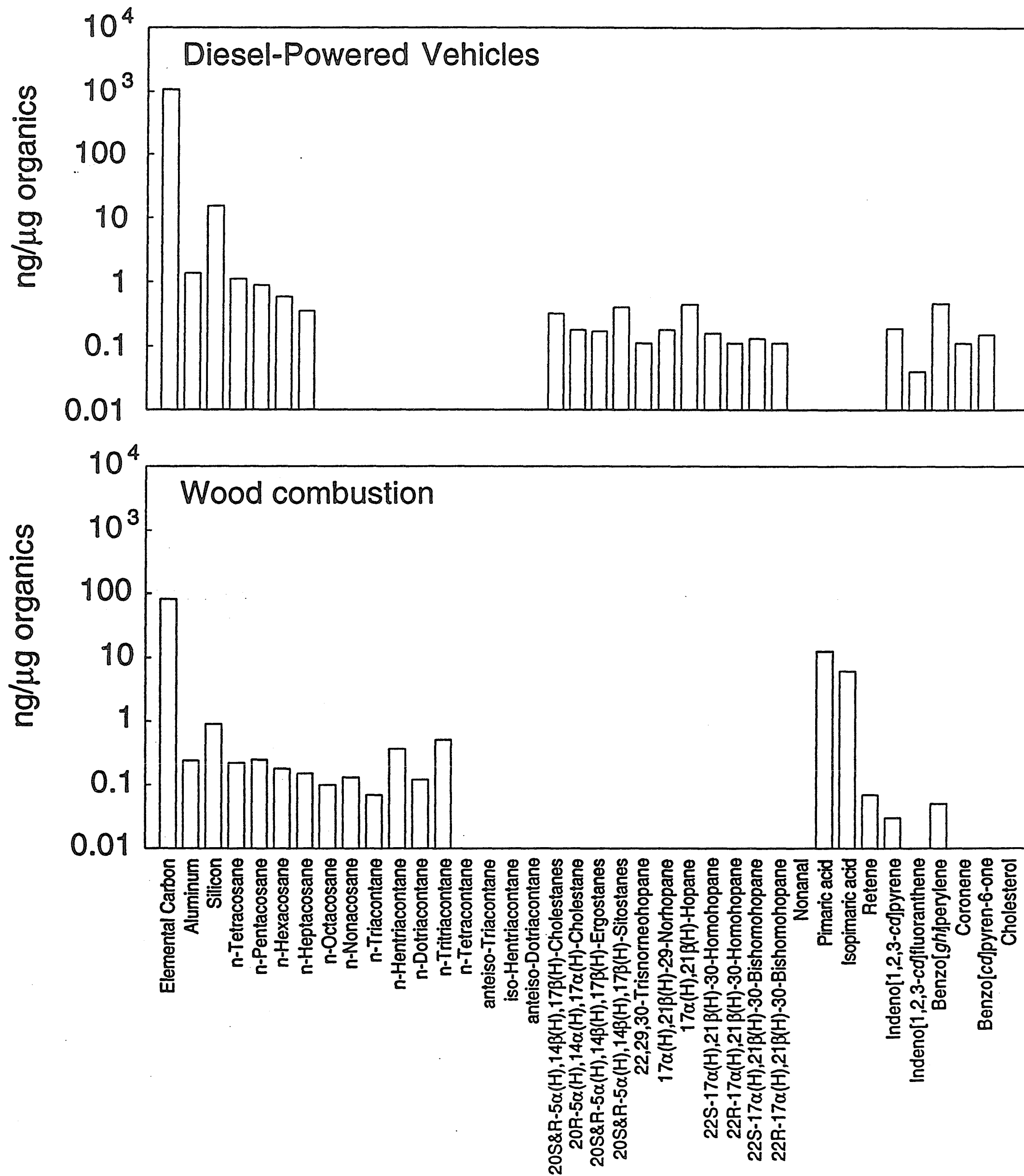


Figure 6.3. Source profiles for paved road dust and vegetative detritus.

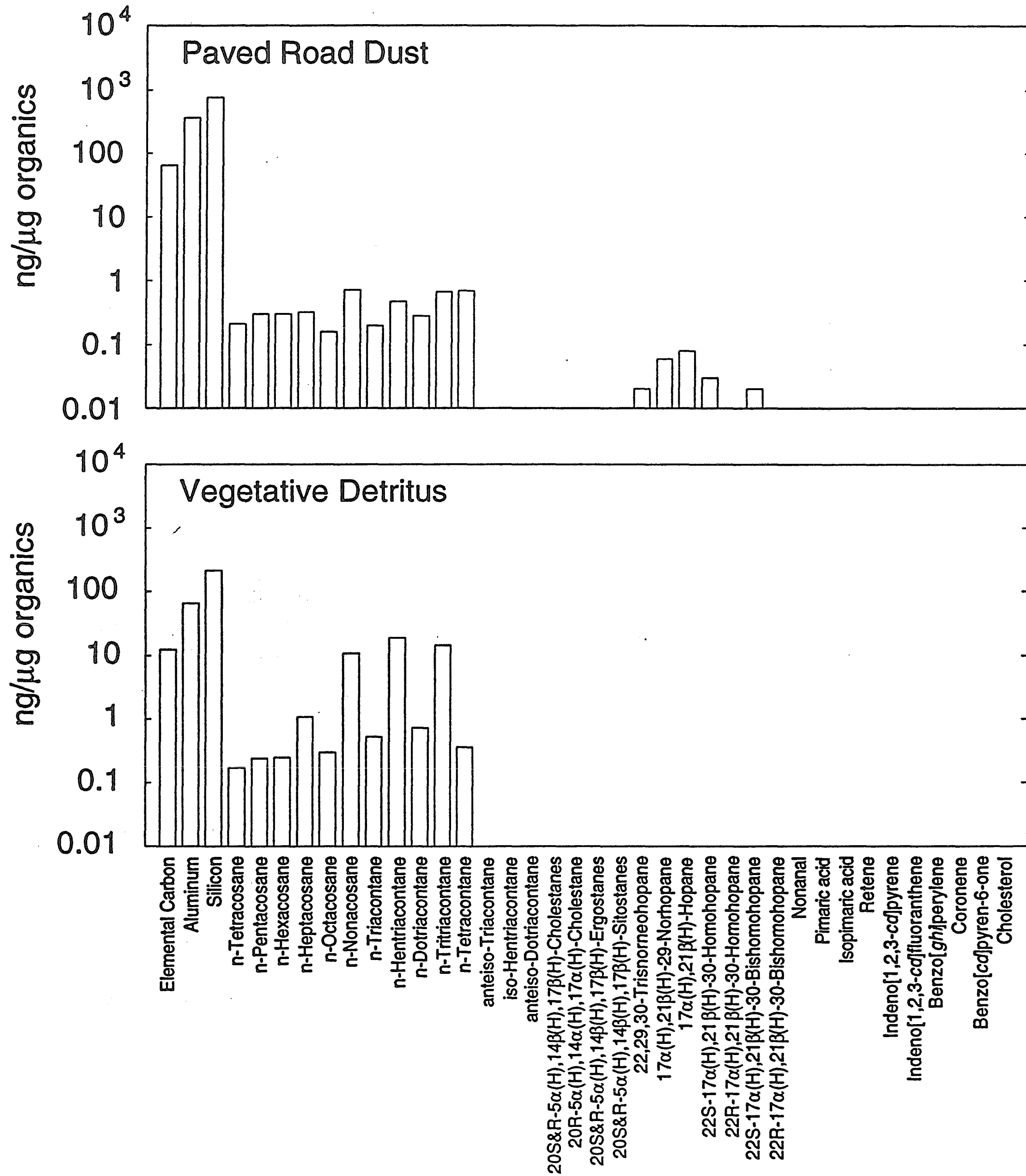


Figure 6.4. Source profiles for tire wear debris and cigarette smoke.

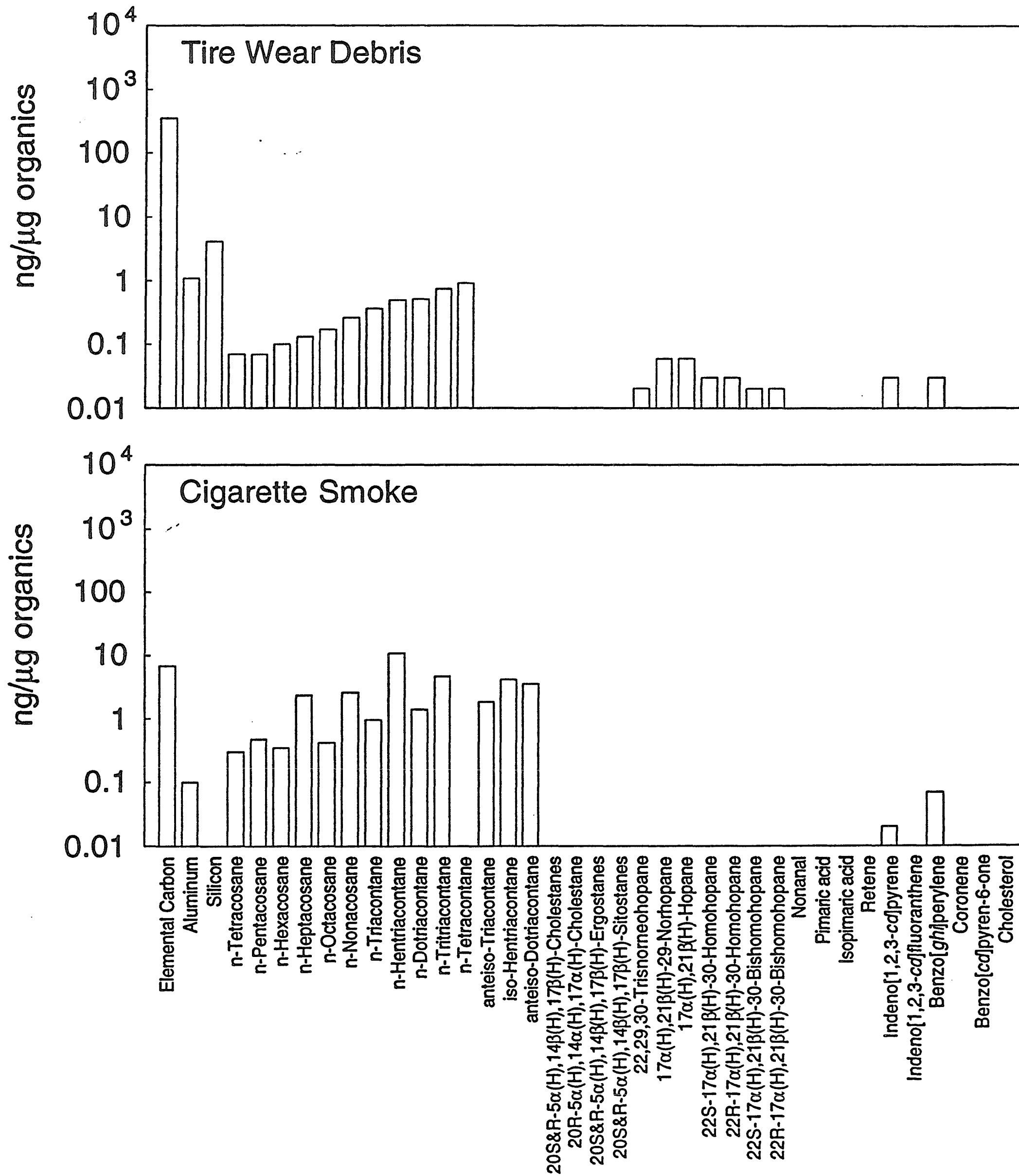
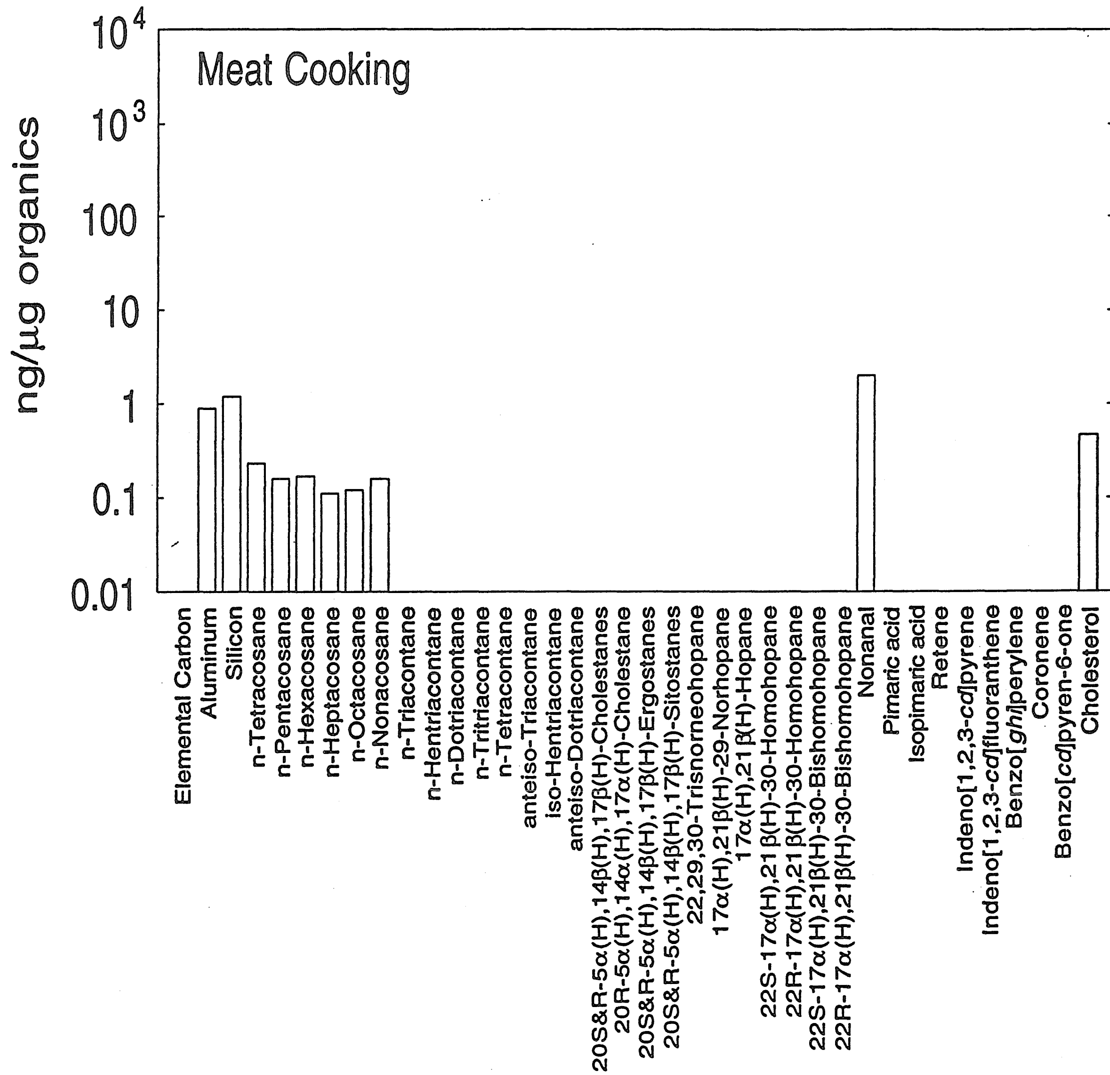
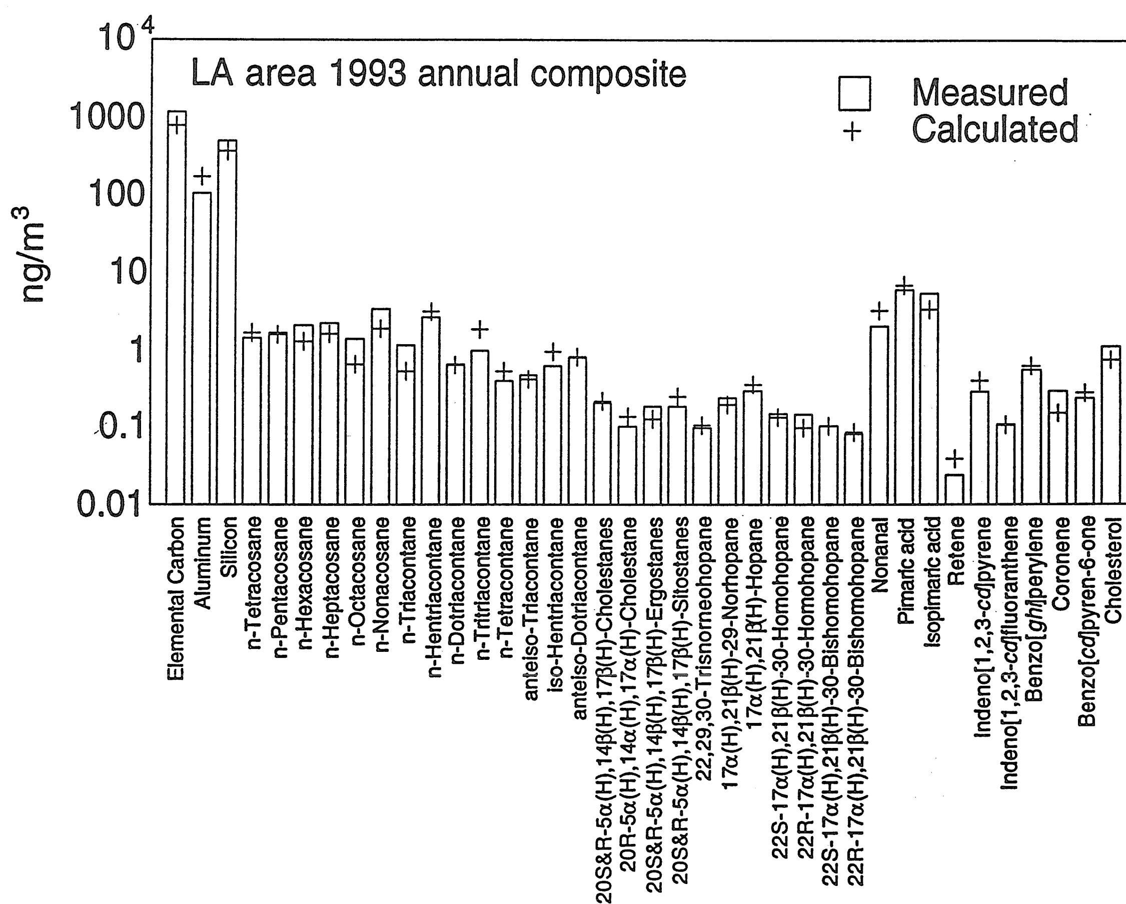


Figure 6.5. Source profiles for meat cooking aerosol.



Angeles area 1993 annual composite sample between 9 source types. These source types include diesel vehicles, gasoline-powered vehicles, paved road dust, tire wear debris, wood combustion, cigarette smoke, meat cooking, urban vegetative detritus, and natural gas combustion. Improvements were made to the model prescribed by Schauer et al. *(21)* based on the more extensive set of chemical species available from the present series of chemical analyses. The most volatile PAH and oxy-PAH used by Schauer et al. *(21)*, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, 7H-benzo[*de*]anthracen-7-one, and benz[*a*]anthracen-7,12-dione, were removed from the model and replaced by a very non-volatile PAH, coronene. The most volatile n-alkane used by Schauer et al. *(21)*, n-tricosane, also was removed from the calculation because it has been determined to partition significantly into the vapor phase *(46)*. The potentially reactive olefinic acid, oleic acid, previously used as part of the mass balance for detecting meat smoke was replaced by a better meat smoke tracer, cholesterol, that is now quantifiable in the ambient samples. Base case (i.e. use of the chemical mass balance as prescribed by Schauer et al. *(21)* with the above improvements) mass balance calculations yielded a result in which one of the minor source types, urban vegetative detritus, did not contribute a statistically significant portion of fine particle organic mass. Therefore, the chemical mass balance model was reconstructed in such a way that any compound in the mass balance calculation that could be coming in significant amounts from vegetative detritus is not used in fitting the model to the ambient data. The model was then fit to the eight remaining source types.

Figure 6.6. Comparison of model predictions to measured ambient concentrations for the chemical mass balance compounds.



A comparison of the calculated and measured ambient concentrations for each of the mass balance chemical species is shown in Figure 6.6. There is generally good agreement between the calculated and measured values across the 34 diverse compounds and 3 chemical elements used in the chemical mass balance. The mean (± st dev of the population) of the ratio of calculated to measured concentrations for each chemically distinct tracer group is 0.84 (± 0.07) for the n-alkanes, 1.15 (±0.19) for the iso- and anteiso-alkanes, 1.01 (± 0.09) for the steranes and hopanes, 1.14 (± 0.78) for the conifer resin acids and their thermal alteration products, and 1.01 (± 0.13) for the PAH.

The calculated source contributions to the Los Angeles area 1993 annual average particulate organics mass concentration are given in Table 6.2. The largest contributors to the fine particulate organics mass are meat cooking, wood combustion, diesel engine exhaust, and paved road dust. These results generally agree with the previous work by Schauer et al. *(21)*, which apportioned the fine particulate organic mass in the Los Angeles area atmosphere during 1982. In the Los Angeles area during 1982, these researchers found fine particle tire wear to account for 0.13 to 0.00 μg organics m^{-3} (versus 0.23±0.13 μg m^{-3} in the present study), fine particle paved road dust to account for 0.89 to 0.49 μg organics m^{-3} (versus 0.47±0.09 μg m^{-3} in the present study), cigarette smoke to account for 0.19 to 0.13 μg organics m^{-3} (versus 0.23±0.03 μg m^{-3} in the present study), meat cooking to account for 1.69 to 1.22 μg organics m^{-3} (versus 1.61±0.31 μg m^{-3} in the present study), wood combustion to account for 1.57 to 0.31 μg organics m^{-3} (versus 0.56±0.25 μg m^{-3} in the present study), gasoline-powered vehicles to account for 1.56 to 0.25 μg organics m^{-3} (versus 0.37±0.17 μg m^{-3} in the present study), and

Table 6.2. Summary of source contributions to fine ambient organic aerosol annual average mass concentration and mutagen density in Los Angeles study area during 1993.

Source Type	Source Type Contribution µg organics per m^3	Human Cell Mutagen Density Contribution[a] IMF ($x10^6$) per m^3	Bacterial Assay Mutagen Density Contribution[b] –PMS MF ($x10^5$) per m^3	Bacterial Assay Mutagen Density Contribution[b] +PMS MF ($x10^5$) per m^3
Diesel-Powered Vehicles	0.49±0.12	0.09±0.03	0.37±0.10	2.50±1.54
Tire Wear Debris	0.23±0.13	nd	0	0
Paved Road Dust	0.47±0.090	nd	0	0
Urban Vegetative Detritus[c]	0	nd	0	0
Natural Gas Combustion	0.14±0.059	0.56±0.31	2.74±1.25	1.78±1.00
Cigarette Smoke	0.23±0.034	0.002±0.002	0.02±0.01	0.02±0.01
Meat Cooking	1.61±0.31	nd	0	0
Gasoline-Powered Vehicles	0.37±0.17	0.07±0.04	0.19±0.09	0.97±0.51
Wood Combustion	0.56±0.25	0.04±0.03	0.07±0.04	0.95±0.43
Sum	4.09±0.48	0.77±0.31	3.40±1.25	6.23±1.96
Measured	6.28±0.58	0.94±0.21	1.57±0.24	8.28±2.68
Ratio	0.65±0.10	0.82±0.38	2.16±0.86	0.75±0.34

Footnote to Table 6.2

[a] The human cell mutagen density contribution is calculated by multiplying the relative source contribution (μg EOC per m^3 air) by the source's human cell mutagenic potency (IMF($x10^6$) per μg EOC). Only those source samples which tested positive in the bacterial assay were tested in the human cell assay, therefore the human cell mutagenic potency contribution for the other source samples is labeled not determined, or nd, even though it is expected to be close to zero. [b] The bacterial mutagen density contribution is calculated by multiplying the relative source contribution (μg EOC per m^3 air) by the source's bacterial mutagenic potency. A "–" value indicates that the sample was not mutagenic at the dose levels tested. [c] The contribution of this source to the ambient sample fine particle organic compound concentration in 1993 is not significantly different from zero, therefore this source was removed from the model calculations.

diesel vehicles to account for 2.72 to 1.02 μg organics m^{-3} (versus 0.49±0.12 μg m^{-3} in the present study). The main difference between the 1982 and 1993 results is the reduction in the contribution from diesel vehicles, which also has been documented by Christoforou et al. *(47)* who illustrate the decline in black elemental carbon concentration in the Los Angeles atmosphere over the period 1982-1993. That decline is consistent with the introduction of cleaner diesel engine technologies and cleaner diesel fuels over the period 1982-1993 *(41)*.

Each of the source contributions calculated in the 1993 study is significantly different from zero with 95 % confidence, except for the tire dust contribution which is positive with greater than 90% confidence. Tire dust and paved road dust form a similarity cluster in which the model has difficulty resolving these two sources separately but can resolve them very clearly as a group. This occurs perhaps because there is tire dust present in the paved road dust sample *(48)*.

The sum of the calculated source contributions to ambient fine particulate organics mass can be compared to the measured ambient fine particulate organics mass concentration. The observed 1993 annual average fine organics mass concentration measured by high volume sampling is 6.28 ± 0.58 μg m^{-3} (calculated by multipying the 1993 annual average fine OC concentration by 1.2 to compensate for the mass due to elements other than carbon), while the sum of the calculated source contributions is 4.09 ± 0.48 μg m^{-3}, or 65 ± 10 % of the measured value. The remaining 35 % places an upper limit on the amount of secondary organic particulate matter that could be present due to gas-to-particle conversion processes in the atmosphere. Schauer et al. *(21)* found this upper limit on secondary organic aerosol to range

from 31 to 13 % in the Los Angeles area in 1982. Since the sources used in the mass balance are expected to account for only 80 % of the primary organic aerosol emissions in the Los Angeles area *(22)*, the best estimate would be that secondary organic aerosol concentrations are lower, in the vicinity of 20 % of the fine organic aerosol on an annual basis in 1993.

The relative contribution of the source types to the concentration of the individual chemical species also can be calculated from the mass balance model. The percentages of the calculated concentrations of each chemical species in the model that are due to the individual source types are shown in Figure 6.7.

Apportionment of Ambient Particulate Mutagenicity

Each of the 15 source samples shown in Table 6.1 were tested previously *(28)* in a bacterial mutagenicity assay (the *S. typhimurium* forward mutation assay of Skopek et al. *(24)*). These source samples represent emission source types that accounted for approximately 80% of the fine particle emissions in the Los Angeles area in 1982. Of these 15 source samples, 10 were found to be mutagenic at the doses tested, and further biological analyses using both bacterial *(24)* and human cell *(23)* mutation assays were performed on the 10 mutagenic source samples. The mutagenic potency values shown in Table 6.1 represent the mutagenic strength (mutagenicity per mass of organics emitted) of a source sample in the mutation assay systems studied. In all three assay systems, the natural gas home appliance emissions sample showed the highest mutagenic potency. In the human cell mutation assay, the source type showing the second highest mutagenic potency was noncatalyst automobile

Figure 6.7. Relative source contribution to modeled ambient concentrations of organic compounds in the mass balance.

exhaust aerosol followed by diesel engine exhaust aerosol and then followed by several other samples having approximately the same mutagenic potency: catalyst-equipped automobile exhaust, fuel oil-fired boiler emissions, smoke from fireplace combustion of pine wood, smoke from fireplace combustion of oak wood, and roofing tar pot emissions. In the bacterial assay system with addition of PMS, noncatalyst auto exhaust aerosol, heavy-duty diesel truck exhaust particles, and fuel oil-fired boiler emissions exhibited approximately equal mutagenic potency, followed by catalyst-equipped auto exhaust particles, smoke from fireplace combustion of pine and oak wood as well as cigarette smoke and roofing tar pot emissions. In the bacterial assay system without addition of PMS, the source sample with the second highest mutagenic potency is heavy-duty diesel truck exhaust, followed in order of potency by smoke from fireplace combustion of oak wood, catalyst and noncatalyst-equipped automobile exhaust aerosol, smoke from fireplace combustion of pine wood, fuel oil-fired boiler emissions, smoke from fireplace combustion of synthetic logs, and then cigarette smoke and roofing tar pot emissions.

The product of the mutagenic potency of an emission source times the contribution of that source type to the ambient fine organics mass concentration provides an estimate of the contribution of that source type to the mutagenic potency of the ambient aerosol composite under the assumption of linearly additive contributions to the atmospheric sample and under the hypothesis that the mutagenic potency of the source effluents is not transformed by atmospheric chemical reactions. This multiplication of mutagenic potency (mutagenicity per unit organics mass) times the quantity of organics contributed from the source yields a quantity that we have previously defined as the mutagen density, which

is the induced mutant fraction per m^3 ambient air sampled in order to supply organics to the standard bioassays. These source contributions to mutagen density are shown in Table 6.2. The mutagenic potency of the gasoline-powered vehicle composite sample is estimated as a linear combination of the potencies of the catalyst and noncatalyst gasoline-powered engine exhaust fine particle samples assembled in proportion to their contribution to fine particle organics mass emissions from the 1993 Los Angeles fleet as calculated earlier. The sum of the mutagen density contributions from each source can be compared to the mutagen density observed in the ambient sample.

In the human cell assay, the sum of the mutagen density contributions from the source types used in this analysis accounts for an IMF of 0.77×10^{-6} per m^3 air, or 82 ± 38 % of the mutagen density of the composite ambient sample. The overall contribution of the primary source effluents is statistically indistinguishable from the total human cell mutagenicity of the ambient sample. This is consistent with previous inferences drawn from the seasonal and spatial patterns of the mutagenic potency of ambient samples taken in the Los Angeles area during 1993, which also suggest that mutagenic potency values do not vary seasonally and do not increase with downwind transport away from the primary source areas as might be expected if the most important human cell mutagens were being created by atmospheric chemical reactions *(5)*. The natural gas home appliance emissions estimate is by far the largest predicted primary source contributor to the mutagen density of the atmospheric sample even though the mass concentration of the natural gas source material is extraordinarily low. This occurs because the mutagenic potency of the natural gas combustion aerosol is very high. The PAH in the natural gas sample are

not accompanied by a large amount of particle-phase hydrocarbons from unburned fuel that contribute to the mass concentration but not to the mutagenicity of many of the other samples *(49, 50)*

In a previous study *(28)*, we weighted the bacterial mutagenicity of different extracts from these same source samples by their relative Los Angeles area emissions for 1982, and compared the mutagenicity of this emissions-weighted source effluent composite to the bacterial mutagenicity measured in 1982 ambient fine particle samples taken in Los Angeles. When the bacterial mutagenicity measured in the ambient sample was compared to the bacterial mutagenicity of the emissions-weighted source composite we found that this simple approximation generated rough agreement between the mutagenicity of the two types of samples.

Given the new ambient samples from 1993 and the newly calculated 1993 source contributions it is now possible to repeat that earlier study for a more recent year. The sum of the mutagen density contributions in the bacterial assay from the source types used in this analysis accounts for 216 ± 86 % of the ambient mutagen density in the presence of PMS, and 75 ± 34 % in the absence of PMS. Again, the bacterial mutagenicity of a source contribution-weighted composite of the primary source samples is about large enough to account for nearly all of the bacterial mutagenicity of the ambient samples.

Conclusions

The primary fine particle organic mass concentration in the Los Angeles area atmosphere in 1993 has been apportioned between 8 emission source types. The largest primary contributors to organic aerosol mass are meat

cooking, wood combustion, diesel vehicles, and paved road dust. This result agrees with a previous analysis performed by Schauer et al. *(21)*, using ambient samples taken during 1982. The major difference between the two studies is a decrease in the source contribution from diesel vehicles. That decrease in diesel engine exhaust aerosol concentration is believed to be consistent with introduction of cleaner diesel engines and cleaner burning diesel fuels between 1982 and 1993.

The fine particle mutagenicity of the Los Angeles area atmospheric aerosol in 1993 also has been apportioned between the same 8 source types using both a human cell assay system as well as a bacterial assay system. In both the human cell and bacterial assay systems, a linear combination of the mutagenicity values of the primary source samples assembled in proportion to their calculated contribution to the atmospheric sample composite is statistically indistinguishable from the measured mutagenicity of the atmospheric sample itself. In both the human cell assay system and bacterial assay system (–PMS), the largest source of the predicted contribution to the mutagenicity of the atmospheric sample is due to natural gas combustion in residential appliances, with smaller contributions from diesel exhaust, gasoline-powered vehicle exhaust and woodsmoke. In the bacterial assay system (+PMS) diesel exhaust is the largest contributor, followed by natural gas appliances, gasoline-powered vehicles and woodsmoke.

Known primary emissions sources are clearly capable of emitting mutagenic organic matter to the urban atmosphere in amounts sufficient to account for the observed mutagenicity of the ambient samples. The error bounds on this analysis, however, are wide enough to admit to the possible

presence of additional mutagenic organics that are formed by atmospheric chemical reaction (e.g. 2-nitrofluoranthene that has been identified as one of the more important single human cell mutagens in the atmospheric composite studied here, accounting for about 1 % of the total sample mutagenic potency *(35)*).

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Chapter 7

Summary and Conclusions

Summary of Results

As discussed in Chapter 1, the main objective of this thesis was to determine how major urban air pollution source types contribute to the mutagenicity of atmospheric fine particulate matter. Three steps have been accomplished in order to meet this objective. First, large quantities of Los Angeles area airborne fine particulate matter were collected for later biological and chemical characterization through construction of a 5 station air monitoring network in Southern California. Large quantities of the particulate material that is emitted directly from the major urban emission sources in the Los Angeles area had been collected previously and also was available for use in this research. Second, the mutagenicity of these source and ambient samples was characterized both in older bacterial assay systems as well as in new human cell assay systems, and chemical analyses were conducted to determine to the extent possible the specific organic compounds or compound classes that are associated with the observed human cell mutagenicity. Finally, a chemical mass balance model was used to relate the emission sources of urban particulate matter to airborne fine organic particulate matter concentrations, with particular attention to determining the origin of the source effluents that contribute mutagenic airborne particulate matter to the atmospheric samples. A detailed description of the results follows.

A preliminary study was undertaken to assess the bacterial mutagenicity of urban organic aerosol sources in comparison to atmospheric samples. The bacterial mutagenicity of a comprehensive set of urban particulate air pollution source samples was examined using the *Salmonella typhimurium* forward mutation assay. Each of the combustion source samples examined, including catalyst-equipped vehicles, noncatalyst vehicles, heavy-duty diesel trucks, natural gas combustion, distillate oil combustion, and wood combustion, was mutagenic in this assay, with a response per μg of organic carbon in these samples generally greater than that of cigarette smoke. The noncombustion source samples tested generally were not mutagenic at the levels examined. The mutagenic potency (mutant fraction per μg of organic carbon) of the ambient aerosol samples collected at two sites in Southern California in 1982 was compared to a weighted average of the mutagenic potencies of the primary source samples assembled in proportion to their emission rates in the Los Angeles area. In most cases where a comparison could be made, the mutagenic potencies of the source composites and the ambient samples were of similar magnitude, with the exception that the –PMS mutagenicity of the aerosol at Long Beach, CA, during the first half of the calendar year 1982 and at Azusa, CA, during the April-June 1982 period was much higher than can be explained by direct emissions from the sources studied here. This study showed the feasibility of continuing to investigate the relationship between the mutagenicity of particulate air pollution source effluents and the mutagenicity of airborne particulate matter.

The next step in this research involved the collection of a new set of ambient fine particulate air pollution samples. Ambient fine particulate samples

were collected for 24 hours every sixth day for the entire year of 1993 at four urban sites, including Long Beach, central Los Angeles, Azusa, and Rubidoux, CA, and at an upwind background site on San Nicolas Island. Long Beach and central Los Angeles are congested urban areas where air quality is dominated by fresh emissions from air pollution sources, while Azusa and Rubidoux are located farther downwind and receive transported air pollutants plus increased quantities of the products of atmospheric chemical reactions. This year-long sampling effort entailed both organic and inorganic chemical characterization. These samples were utilized in the remaining work.

The bacterial mutagenicity of this new set of 1993 urban particulate air pollution samples was examined using the *Salmonella typhimurium* TM677 forward mutation assay. Fine aerosol samples from Long Beach and Los Angeles showed a pronounced seasonal variation in bacterial mutagenicity per m^3 of ambient air, with maxima in the winter and a minimum in the summer. While the downwind smog receptor site at Rubidoux shows peak +PMS mutagenicity (but no peak –PMS) during the September-October periods when direct transport from upwind sources can be expected, at most sites the mutagenicity per μg of organic carbon (mutagenic potency) from the aerosol was not obviously higher during the summer photochemical smog period than during the colder months. Significant spatial variation in bacterial mutagenicity is observed: mutagenicity per m^3 of ambient air on average was more than an order of magnitude lower at San Nicolas Island than within the urban area. The highest mutagenic potencies were found at the most congested urban sites at central Los Angeles and Long Beach. The highest annual average values of mutagenicity per m^3 air sampled occurred at central Los Angeles. These

findings stress the importance of proximity to sources of direct emissions of bacterial mutagens, and imply that if important mutagen-forming atmospheric reactions occur then they likely occur in the winter and spring seasons as well as during the photochemically more active summer and early fall periods.

Next, the human cell mutagenicity of the same Los Angeles area 1993 particulate air pollution samples was measured. The human cell mutation assay used in this study tests mutagenic activity at the thymidine kinase locus in h1A1v2 cells using a 72 hr exposure. No systematic seasonal variation of the mutagenic potency was observed at any of the urban sites. This suggests that if the atmospheric transformation products of photochemical air pollution are involved then these reactions must occur during the winter as well as during the summer photochemical smog season. No significant spatial variation of the annual average mutagenic potency of the aerosol was observed between 3 of the 4 urban sites; while the average mutagenic potency of the Long Beach aerosol was slightly higher than elsewhere in the air basin. This similarity of mutagenic potency values across widely separated monitoring sites suggests that the mutagenicity of the aerosol is due largely to ubiquitous emission sources (e.g. motor vehicle traffic or stationary source fuel combustion) rather than to proximity to isolated point sources of unusual mutagenic organics. The average mutagen concentration per m^3 of ambient air was computed by weighting the mutagenic potency values of the aerosol according to the mass concentration of organics present in each bimonthly composite sample at each air monitoring site. The human cell mutagen concentration in Los Angeles urban air was found to be an order of magnitude greater than at the background

site studied upwind of the city, showing that the city is indeed a source of human cell mutagens.

In order to determine the most important organic compounds or compound classes responsible for the mutagenicity observed in the Los Angeles airborne fine particulate matter, bioassay-directed chemical analysis was undertaken in which the human cell mutation assay described previously was used to identify subfractions of the ambient aerosol extract that contained the strongest mutagens. For this study a 1993 average urban fine particle air pollution sample was created by compositing a portion of every fine particle filter collected at the 4 urban sites throughout the entire year. This sample was separated via liquid chromatography into fractions containing organic compounds of varying polarity. Samples were analyzed by the h1A1v2 human cell mutagenicity assay to identify those fractions that contain human cell mutagens and by GC/MS to identify the chemical character of those mutagens. Those subfractions that contain the ordinary PAH were responsible for a considerable portion of the mutagenic potency of the whole atmospheric sample. Six ordinary PAH (cyclopenta[*cd*]pyrene, benzo[*a*]pyrene, benzo[*ghi*]perylene, benzo[*b*]fluoranthene, indeno[1,2,3-*cd*]pyrene, and benzo[*k*]fluoranthene) account for most of the mutagenic potency that could be assigned to specific compounds within the atmospheric samples. Important semipolar mutagens that were quantified include 2-nitrofluoranthene and 6*H*-benzo[*cd*]pyren-6-one. A large number of other aromatic organics were identified as candidates for future testing as pure compounds in the human cell assay, at which time it should be possible to account for more of the mutagenic potency of the atmospheric samples.

Finally, a chemical mass balance model which uses organic compounds as tracers was employed to investigate the relationship between the mutagenicity of the urban organic aerosol sources and the mutagenicity of the atmospheric samples. The fine particle organic mass concentration present in the 1993 annual average Los Angeles area composite atmospheric aerosol sample was apportioned between 8 emission source types. The largest source contributions to fine particulate organic compound mass concentration were identified as smoke from meat cooking, diesel-powered vehicle exhaust, wood smoke, and paved road dust. This result agreed with previous work by Schauer et al. published in Atmospheric Environment in 1996, who did a similar analysis using airborne fine particle samples taken in the Los Angeles area during 1982. Comparison of these two studies showed that the contribution of diesel vehicle exhaust to airborne organic aerosol concentrations in Los Angeles declined between 1982 and 1993 over a period of time when cleaner diesel engines and cleaner diesel fuels were introduced into the vehicle fleet.

Emission source contributions to atmospheric mutagen concentrations were determined under the hypothesis of linearly additive source contributions and for the case where the primary particle source effluents are transported without net gain or loss of mutagenicity due to atmospheric chemical transformations. As viewed by the human cell assay system and by the bacterial assay system (+PMS), natural gas-fired home appliances were the largest contributor to the mutagenicity of the ambient sample, followed by diesel exhaust, gasoline-powered vehicle exhaust, and wood combustion. In the bacterial assay system (–PMS), the largest source contributions to airborne mutagen concentrations were due to diesel vehicle exhaust and natural gas

combustion followed by gasoline-powered motor vehicle exhaust, and wood combustion. In both the human cell and bacterial assay systems, the combined mutagenicity of the composite of primary source effluents predicted to be present in the atmosphere was statistically indistinguishable from the mutagenicity of the actual atmospheric sample composite modeled. Known primary emissions sources are clearly capable of emitting mutagenic organic matter to the urban atmosphere in amounts sufficient to account for the observed mutagenicity of the ambient samples. The error bounds on this analysis, however, are wide enough to admit to the possible presence of additional mutagenic organics that are formed by atmospheric chemical reaction (e.g. 2-nitrofluoranthene that has been identified as one of the more important single human cell mutagens in the atmospheric composite studied here, accounting for about 1 % of the total sample mutagenic potency).

Recommendations for Future Research

One result of this research is an established protocol that can be used to investigate the origins of the mutagenicity of airborne particle samples. The specific case studied involved 1993 Los Angeles area fine particulate air pollution. Further work should be done using particulate air pollution samples from other locations and/or time periods to help clarify the origin of the mutagenic organic compounds present in other urban areas.

In addition, only about 15 % of the mutagenicity of the air pollution samples studied was attributable to compounds that were both identified in the atmospheric samples and tested as pure compounds in the human cell mutagenicity assay. Further mutagenicity testing of pure compounds as well as

improved identification of polar and semipolar organic compounds will lead to identification of specific compounds that are responsible for a larger portion of the mutagenicity of atmospheric particulate matter samples. Organic chemical analysis techniques exist that can be applied to nonpolar and semipolar organic compounds; their use is currently limited only by acquisition of authentic standards of the pure compounds. However, chemical analysis techniques for the quantification of the most polar organic compounds are still being developed, and much future research can be done in this area.

The biological endpoint used in this study focused on determining the mutagenic potency of both source samples, atmospheric samples and certain pure compounds. Two different assay systems (human cell and bacterial) were used to measure mutagenic potency. The research protocol developed here could be applied to study other biological endpoints (e.g. carcinogenicity, irritant potency, etc.)

Additionally, this research investigated only the particle-phase portion of the air pollution mixture. New research has recently shown that there is a great deal of information to be learned about vapor-phase semi-volatile organic compounds which include many PAH that also could be potent human cell mutagens. Further studies should be done to investigate these additional segments of the air pollution mixture.