IDENTIFICATION OF PARTICULATE MUTAGENS IN SOUTHERN CALIFORNIA'S ATMOSPHERE

Final Report
Contract Number A1-155-32
California Air Resources Board
March 1984

Principal Investigator

Dr. James N. Pitts, Jr.

Co-investigators

Dr. Arthur M. Winer
Dr. David M. Lokensgard

Program Manager

Dr. Janet Arey Sweetman

Contributing Staff

Mr. Travis M. Dinoff
Ms Margaret C. Dodd
Mr. Dennis R. Fitz
Mr. William P. Harger
Ms Victoria Mejia
Dr. Hanns-R. Paur
Mr. Phillip C. Pelzel
Ms Gina Scorziell
Dr. Barbara Zielinska

STATEWIDE AIR POLLUTION RESEARCH CENTER
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CA 92521

ABSTRACT

We report results from a one-year element of our CARB/UC-supported research program to obtain laboratory and field data on the atmospheric levels, sources and sinks and mutagenicities of compounds present in respirable ambient particles collected at selected sites across California's South Coast Air Basin. These data are essential inputs into risk assessment evaluations and development of cost-effective strategies, if these are deemed necessary, for the protection of public health.

In field studies designed to investigate the levels of ambient mutagens to which populations are exposed, and their diurnal variations, we found average 3-hr mutagen densities (revertants m^{-3} air sampled) on Salmonella strain TA98 (-S9) at a West Los Angeles (WLA) site ranged from a minimum of 32 revertants m^{-3} between midnight and 0300 hr to a maximum of 150 revertants m^{-3} between 0600 and 0900 hr. The mutagenicity profiles were comparable to those we found earlier at an East Los Angeles (ELA) site and were generally higher than those reported from other major urban airsheds throughout the world. Additionally, offshore (east to west) air flows which drain the air basin between midnight and 0600 were shown to result in elevated mutagen density levels at the western edge of the Los Angeles Basin (e.g., ~72 revertants m^{-3} at 0300-0600 hr). During the period from 1200 to 2400 on March 9, 1983, concurrent measurements of particulate mutagen densities at sites upwind and downwind of the San Diego Freeway (I-405) took place under wind conditions favorable for demonstrating that the incremental burden of direct mutagens in respirable POM attributable to freeway traffic reached 50 rev m^{-3}. Finally, diminished response on the nitroreductase-deficient strain TA98NR vs. TA98 suggested that nitroarenes contributed significantly to the direct mutagenicity of ambient POM collected at the WLA sites. As with our ELA study, over a 24-hr period highs and lows in mutagen densities occurred over short time intervals (several hours) probably because of changes in emissions, mixing heights and wind speeds. These short-term peak mutagen densities clearly can be much higher than 24- or 12-hr averages typically reported in the literature.

In our initial study of the feasibility of determining changes in mutagenic POM during west-to-east transport across the basin, we found no significant difference in specific activity (rev μg^{-1} extract) between the POM

collected in Redlands and that collected later in the day at Whitewater, a site farther downwind and approximately 60 km to the east of Redlands. As would be expected from dilution of the polluted air mass during transport, the mutagen densities of POM collected in Whitewater were less than that of Redlands. However, when the mutagen data were normalized on the basis of elemental carbon to account for dilution, the activity for Whitewater was greater than for Redlands. This suggests chemical transformations and/or injection of emissions from additional sources during air parcel transport. In an attempt to simulate the effects of "transport and transformation," we also developed and tested a protocol for measuring changes in the mutagenic activity of diesel POM exposed to conditions simulating atmospheric transport in our 40,000-ℓ SAPRC outdoor environmental chamber.

In a comparison study between samples collected with the SAPRC ultrahigh volume sampler ("megasampler") and samples collected simultaneously with standard hi-vol instruments, we found no significant difference in the mutagen densities toward Salmonella strains TA98, TA98NR, and TA98/1,8-DNP_6 for these two collection methods. The significantly lower response observed for both collection methods on the nitroreductase-deficient strain TA98NR relative to TA98 was indicative of the presence of mutagenic mononitroarenes while the lower response on strain TA98/1,8-DNP_6 relative to the other two strains suggested that dinitropyrenes may also have been present.

In chemical characterization studies, we found that less than 50% of the activity of the base/neutral portion of an ambient POM extract was in the chromatographic fractions in which mono- and polynitro-PAH or nitroalkyl-PAH would elute and that the ambient POM samples were enriched in polar mutagens, relative to a diesel POM sample. The diminished response on strain TA98NR indicated some of polar mutagens may have been substituted mononitro-PAH.

Finally, in collaboration with a team from the Brookhaven National Laboratory who were experts on the use of the Tradescantia stamen hair assay for gas phase mutagens, we found statistically significant mutagenic activities for a surrogate smog mixture and showed they were consistent with the sum of the mutagenicities of PAN, O_3 and NO_2 determined separately in laboratory tests. The mutagenicity of ambient air determined in a 10-day Tradescantia exposure could also be accounted for by the sum of the mutagenic activities of PAN, O_3, and NO_2. To our knowledge this is the first time the mutagenic activity of gaseous PAN has been observed under atmospherically relevant conditions.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

ACKNOWLEDGMENTS

We express our appreciation to Drs. John R. Holmes and Jack K. Suder of the California Air Resources Board staff for their valuable technical and administrative advice.

We thank Professor Bruce N. Ames for the Salmonella strain TA98 and Professor Herbert S. Rosenkranz for Salmonella strains TA98NR and TA98-1,8-DNP_6. We also thank Mr. Robert Giauque at the Lawrence Berkeley Laboratory for the lead analyses, Dr. James Huntzicker of the Oregon Graduate Center for the carbon analyses and Ms Minn P. Poe of our technical staff for statistical analyses of the data.

We acknowledge the generous assistance of Mr. Al Wheelock of the California Department of Transportation in making available traffic count data for the I-405 freeway and Mr. Charles Benjamin and Mr. Robert Joe for their permission to use the Westwood CALTRANS facility as one site for our study. We also thank Sgt. James Moad for permission to use the West Los Angeles Police Station repair garage as the other ambient air monitoring site employed in this investigation.

The excellent assistance of our laboratory technicians, Gregory Butters and Carl Nicholson was appreciated. We also wish to acknowledge the assistance in preparing this report given by I. M. Minnich, Christy J. LaClaire and Neva G. Friesen.

This report was submitted in partial fulfillment of Contract No. A1-155-32, "Identification of Particulate Mutagens in Southern California's Atmosphere," by the Statewide Air Pollution Research Center, University of California, Riverside, under the sponsorship of the California Air Resources Board. Work was completed as of March 30, 1984.

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

FIGURES

FIGURES (continued)

FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

I. PROJECT SUMMARY

A. Introduction and Statement of the Problem

In addition to degrading visibility, fine particles collected from ambient urban air and primary emission sources such as diesel engines are in the respirable size range and their organic extracts contain chemicals which are strong mutagens in bacterial and other short-term biological assay systems. Whether or not exposure to such particles constitutes a health hazard to the general population is not known; however, it is currently a subject of great scientific and societal interest.

This year's research program was an element of our CARB/UC supported effort to obtain critical source emission and population exposure data required for a reliable risk assessment of the possible health impacts of these respirable airborne mutagens and, if deemed necessary by the CARB, cost-effective measures for their control. The program has two overall objectives:

- To determine, using the Ames Salmonella bacterial assay system, the ambient levels of airborne particulate mutagens encountered during various seasons and under various meteorological conditions at a variety of urban/suburban/rural sites across the South Coast Air Basin, and
- To isolate and chemically characterize those major pollutants present in extracts of samples of ambient particulate organic matter (POM) which are responsible for their strong, direct mutagenicities in bacterial and other short-term assay systems.

We emphasize that, as chemists, we do not attempt to link the bacterial mutagenicities of POM samples with possible genotoxic and carcinogenic effects on animals or humans. Instead we utilize the Ames test in our studies because today it is widely used throughout the world in industry, government and universities as a convenient, straightforward and cost-effective means for screening both individual compounds and complex environmental mixtures for their mutagenic activities. Additionally, the Salmonella typhimurium and associated nitroreductase deficient bacterial systems, are essential components of our "activity directed," integrated chemical/microbiological procedures for the isolation and identification of major direct acting chemical mutagens in respirable POM.

B. Background

The realization that chemicals which are mutagenic in bacterial assays and/or carcinogenic in animal tests are contained in extracts of respirable particulate organic matter (POM) collected in urban environments has raised concern over the effects of inhalation of this material by the general population. For example, it is now established that respirable sub-micron particles collected from ambient air, as well as from primary combustion-generated sources such as diesel exhaust, contain compounds which are strong direct mutagens (-S9) in the Ames Salmonella typhimurium bacterial reversion assay. This behavior is in contrast to that of certain "classical" animal and human carcinogens including certain polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene (BaP). These latter chemicals have been known for over three decades to be present in ambient POM; however they require mammalian activation (+S9) to be mutagenic in the Ames bacterial assay (they are referred to as promutagens). Unfortunately, at this time the specific chemical identities of most of the direct mutagens and many of the promutagens in ambient POM are unknown.

Currently, international attention has been focussed on the nitropolycyclic aromatic hydrocarbons (NO_2-PAH), recently identified in both primary and ambient POM, because certain members of this group of chemicals are strong direct mutagens and certain of them are animal carcinogens (for a review see Rosenkranz and Mermelstein 1983). Indeed, results from our present and previous California Air Resources Board (CARB) sponsored research, as well as from other laboratories, suggest that a significant portion of the total mutagenicity of diesel POM can be explained by the amounts and specific mutagenicities of the mono- and dinitro-PAH's present in the material. However, the contribution that NO_2-PAH makes to the total mutagenicity of ambient POM is subject to some controversy.

Our previous CARB-supported studies of the diurnal variations in the direct and activatable mutagenicity of airborne POM in California's South Coast Air Basin (CSCAB) have suggested strongly that mobile source primary emissions were important contributors to the high mutagen levels we observed (Pitts et al. 1981, 1982a). Additionally, Flessel and co-workers at the Air Industrial Hygiene Laboratory recently reported that during

smog episodes in Contra Costa County in August and October 1981, vehicular transportation sources were the predominate mutagenic contributors (Flessel et al. 1983). Finally, at least one epidemiological study has suggested a correlation with highway traffic and cancer incidence (Blumer et al. 1977); however, subsequently this study in Switzerland has been challenged (Polissar and Warner 1981).

Several million commuters, as well as residents living near freeways, in the CSCAB undergo exposure to primary vehicle emissions daily. The incremental exposure to the mutagen burden associated with such heavily travelled freeways in Los Angeles, above that associated with the "background" ambient POM, has not been previously investigated. A major task during this contract period, therefore, was to establish the magnitude of such a "freeway mutagen increment."

Further questions about population exposure levels to mutagenic POM concern the chemical fate(s) of particulate mutagens during long-range transport, for example, across the South Coast Air Basin. Fine particles are known to remain aloft for periods up to a week, thus allowing time for chemicals adsorbed on the surface of the POM (e.g., PAH's) to undergo chemical reactions with gaseous co-pollutants. Indeed, such chemical reactions, which can enhance or diminish the biological activities of certain PAH in POM (for recent reviews see Nielsen et al. 1983 and Pitts 1983) have been demonstrated under controlled conditions in simulated atmospheres in this and other laboratories. The magnitude of such chemical transformations in urban air has not as yet been well established, in part because of the concern over the possible importance of artifacts occurring during sampling on hi-vol filters (Pitts et al. 1978, Pitts 1979, Lee et al. 1980, Brorström et al. 1983, Grosjean 1983, Grosjean et al. 1983, Fitz et al. 1984). Nevertheless, one result of transport may be that long-range downwind receptor sites in the South Coast Air Basin may be impacted by POM which is qualitatively and quantitatively different than that which is initially released to the atmosphere in or near downtown Los Angeles (DTLA).

This research is an element of a continuing effort which addresses two major aspects of this overall problem (1) assessment of the ambient levels of airborne particulate mutagens (i.e., mutagen densities in revertants m^{-3} of air) encountered during various seasons and under

various meteorological conditions at a variety of urban/suburban/rural sites across the South Coast Air Basin and (2) the isolation and chemical characterization of the major chemical species present in extracts of these POM samples and responsible for their strong, direct mutagenicities in bacteria, and other short-term assay systems (Lewtas 1983).

A brief description of the specific tasks, their objectives and our results and conclusions follows.

C. Evaluation of the Levels of Mutagenicity of Respirable Ambient Particles in the Western Portion of the South Coast Air Basin, and the Impact of a Major Freeway on These Levels

The major objectives of this field study were:

- To determine if the diurnal variations and "background" levels in the mutagenicity of samples of ambient air collected at two sites in West Los Angeles (WLA) (and expressed as mutagen densities, i.e., rev m^{-3} of air) were similar to those we previously observed in our CARB-supported studies just east of downtown Los Angeles (ELA).
- To assess the incremental contribution of a heavily travelled freeway to the mutagenic burden of respirable ambient particles.
- To estimate the contribution of nitroarenes to the direct mutagenicities of extracts of the ambient particulate matter by comparing the activities of these extracts toward Ames strain TA98 to that of the nitroreductase-deficient strain TA98NR.

Our approach was to measure the mutagenicity of samples of ambient POM collected for consecutive 3-hr time intervals over a 27-hr period during March 1983 at two sampling sites on opposite sides of the heavily travelled San Diego Freeway (I-405) near Wilshire Blvd. in West Los Angeles. Under the typical wind pattern of onshore winds during the day and offshore winds at night, one site was generally upwind and the other downwind from the freeway. This allowed the contribution of the freeway traffic to the mutagenicity of the ambient POM collected near the freeway to be estimated. It also permitted evaluation of the effect of offshore air flow (i.e., east-to-west) which generally drains the air basin at night, on mutagen densities at the WLA sites.

Results and Conclusions. At the two sites on opposite sides of the I-405 freeway, diurnal variations in the direct mutagenic burden of

airborne particulates were similar to those we previously observed at a site just east of downtown Los Angeles near the intersection of I-10 and I-605 freeways. Furthermore, the particulate mutagenicity levels observed at the WLA sites were generally comparable to those found at the ELA site and, consistent with our earlier findings, were generally higher than those reported from other major urban airsheds throughout the world. For example, the "background" mutagen densities we measured, i.e., those observed upwind of the freeway, ranged from 30 to 100 rev m^{-3}.

Additionally, we found that offshore air flows which generally drain the air basin between midnight and 0600 by an east to west movement can result in high mutagen density levels at the western edge of the Los Angeles Basin.

During the period from 1200 to 2400 on March 9, 1983, concurrent measurements of particulate mutagen densities at the upwind and downwind sites took place under wind conditions favorable for distinguishing the effects of the freeway. The incremental burden of direct mutagens in respirable POM attributable to freeway traffic reached 50 rev m^{-3} during this period.

Consistent with the results from our previous ELA study, we found significantly diminished response on the nitroreductase-deficient strain TA98NR vs. TA98. This suggests that nitroarenes contributed significantly to the direct mutagenicity of ambient POM collected at the WLA sites.

As in the case of our ELA study, we have found that over a 24-hr period, maxima and minima in mutagen densities can occur over relatively short time intervals (several hours) due to changes in emissions, mixing heights and wind speeds. Furthermore, these short-term peak mutagen densities clearly can be much higher than 24- or 12-hr averages typically reported in the literature. The results of this study will be presented at the Air Pollution Control Association (APCA) meeting in San Francisco, June 1984, and subsequently submitted for publication in JAPCA.

D. Characterization of Chemical Mutagens in Ambient Particulate Organic Matter

1. Mutagenicities of Diesel Exhaust and Ambient Particulate Extracts

The major objectives of this research element were:

- To develop and utilize a method for chromatographic separation of mutagenic substances from the nonmutagenic sample components in a form suitable for their further analysis.
- To carry out semi-preparative chromatographic separations of the base/neutral (B/N) fractions from diesel and ambient particulate extracts.
- To assay the mutagenicities of the chromatographic fractions on Salmonella strains TA98 and the nitroreductase-deficient strain TA98NR, thus allowing comparison of the resulting profiles between diesel and ambient particulate extracts.
- To compare the extracts of ambient POM obtained by Soxhlet extraction with dichloromethane (DCM) with that obtained by ultrasonic agitation in a 1:1:1 mixture of DCM, methanol and toluene.

In order to obtain, in a reasonable time, samples of ambient POM adequate for chemical characterization of the mutagenic constituents we employed the ultra-high volume sampler ("megasampler") developed at SAPRC. The megasampler has an inlet with a 50% cut point of 20 μm limiting the particulate collection to the respirable range. The same face velocity as a standard hi-vol apparatus is maintained, while the four 16 in. x 20 in. filters provide sixteen times the collection capacity (640 cfm vs. 40 cfm).

The collected particulate was then extracted, separated into base/neutral (B/N) and acid fractions, and further fractionated using High Performance Liquid Chromatography (HPLC). Mutagenicity tests of the HPLC fractions were then performed on Salmonella strains TA98 and TA98NR. The resulting mutagenicity-HPLC fraction profiles were compared to similar profiles determined for diesel exhaust POM extracts.

Results and Conclusions. Less than 50% of the activity of the B/N portion of an ambient POM extract was found in the HPLC fractions in which mono- and polynitro-PAH or nitroalkyl-PAH would elute. The ambient POM samples were enriched in polar mutagens, relative to a diesel POM sample. Indeed, the majority of the activity of these ambient samples was

in the polar HPLC fractions. Furthermore, the observed difference in response on strains TA98 and TA98NR indicates that some of the polar mutagens present in the ambient POM may be substituted NO_2-PAH.

There was good agreement for the mutagen distributions in an ambient POM sample between the B/N HPLC fractions of the DCM extract and the 1:1:1 mixture of DCM, methanol and toluene extracts. The DCM Soxhlet extraction was, however, more efficient for extracting mutagenicity than ultrasonic agitation with the solvent mixture.

2. Filter and Sampler Comparison Study

In preparation for a future study in which POM from two locations will be compared (sampling at one location with the mega-sampler and at the other with hi-vols), our objectives were:

- To compare the mutagen densities (revertants per m^3 of air sampled), mutagen loadings (revertants per mg total particulate collected) and specific activities (revertants per μg extract) of ambient particles collected using the SAPRC megasampler with those collected simultaneously with a standard hi-vol apparatus and a hi-vol apparatus with a 10 μm size cut-off inlet. The latter have been used for years by the EPA, CARB, etc., as instruments for a routine collection of ambient particulate matter.

- To compare the mutagen densities, mutagen loadings and specific activities of ambient samples collected with Pallflex T60A20 Teflon impregnated glass fiber (TIGF) filters with those of samples collected simultaneously utilizing the more efficient TX40HI20 TIGF filters.

- To utilize three Salmonella strains (TA98, TA98NR and TA98/1,8-DNP_6) for determining direct mutagenic activity in order to examine the contributions of mono- and dinitroarenes to the ambient POM extract activity.

On August 3, 1983, the megasampler and eight hi-vol samplers located at El Monte were run in parallel for 24 hours. The four time intervals chosen for the planned 1983-1984 studies of ambient particulate at a central and downwind receptor site were used: 0600-1000, 1000-1500, 1500-2100 and 2100-0600. The megasampler was operated with T60A20 TIGF filters, as were four standard hi-vols, two with and two without 10 μm size selective inlets (General Metal Works GMW-9000). Four additional hi-vols (two with and two without inlets) were operated with TX40HI20 TIGF

filters which have a higher collection efficiency for particles <1 μm than have the T60A20 filters.

Results and Conclusions. Hi-vols with and without size selective inlets (10 μm cut-off) gave equivalent mutagen densities and specific activities confirming that the mutagenic material is associated with the smaller, predominately sub-μm particles.

The megasampler gave mutagen densities and specific activities for ambient samples that were, within experimental error of ~±10% (Belser et al. 1981) equivalent to those sampled with the standard hi-vol instrument. Furthermore, no significant differences were found for mutagen densities or specific activities between samples collected on T60A20 or TX40HI20 TIGF filters.

The significantly reduced activities of the ambient samples on strains TA98NR and TA98/1,8-DNP_6 indicated the presence of nitroarenes and dinitroarenes, respectively, in these ambient samples collected at El Monte, as has been previously observed for TA98NR in DTLA, Claremont, Riverside, and in this study of West Los Angeles.

E. Exploratory Studies in Real and Simulated Atmospheres of the Transformations of Chemical Mutagens Adsorbed on Ambient POM

1. Mutagenicity of Ambient POM at Redlands, CA and Whitewater, CA

The overall objective of this study was to investigate the feasibility of field studies to determine if there are changes in the mutagenicity of respirable ambient particles during west-to-east transport out of the Los Angeles air basin. The specific objectives were:

- To measure and compare the mutagenicity of ambient particulate matter associated with the Los Angeles urban plume sampled downwind at Redlands, CA and at a longer-range receptor site, Whitewater, CA.
- To compare the measured mutagen densities with those observed at other sites in the Los Angeles basin.

In order to investigate the feasibility of studies to determine the change in the mutagenicity of POM during transit, ambient particulate matter was collected as it left the basin (Redlands) and at the east end of the San Gorgonio pass (Whitewater) 60 km downwind. Levels of the particulate elemental carbon and the mutagenicity of the corresponding extracted POM were determined in order to normalize the mutagenicity to

the observed elemental carbon (revertants per μg elemental C). This normalized mutagenicity takes into account both the physical dilution of the air mass and the possible dilution of mutagenic POM by chemical secondary aerosol formation, both processes occurring during transport. Collection at Whitewater was initiated upon arrival of the polluted air mass, as determined by substantial increases in β_{scat}, NO_2 and ozone levels.

Results and Conclusions. There was no significant difference in specific activity (rev μg^{-1} extract) between the POM collected in Redlands and that collected later in Whitewater approximately 60 km to the east. As would be expected to result from dilution of a transported polluted air mass, the mutagen densities of POM collected in Whitewater were less than that of Redlands. However, if one normalized the mutagen data on the basis of elemental carbon, to account for dilution, the activity for Whitewater was greater than for Redlands. Possible explanations for this interesting result include one or more of the following: transformation reactions in the particulate phase that result in compounds of increased mutagenicity, gas to particle conversion to mutagenic secondary aerosols, injection of fresh POM during transport from Redlands to Whitewater and differing Los Angeles plume trajectories prior to impacting the two receptor areas.

Mutagen densities in Redlands and Whitewater were similar in magnitude to those observed in Riverside in 1980 and 1981 (Pitts et al. 1981). However, these mutagen densities are factors of 2-3 lower than typical 24-hr values observed in East Los Angeles in 1980 and 1981 (Pitts et al. 1981, 1982a) and factors of 5-10 lower than peak 3-hr mutagen densities recorded in West Los Angeles in 1983 (see Section II-D).

2. Initial Studies of Chemical Transformations and Associated Changes in the Mutagenicity of Diesel Particles Exposed to Simulated Atmospheres in an Outdoor Environmental Chamber

The objective of this limited exploratory study was to examine the feasibility of using the SAPRC dynamometer/outdoor chamber facility in a future effort to evaluate possible chemical transformations of diesel POM by monitoring changes in the mutagenicity of diesel exhaust exposed to photochemical smog or its major gaseous constituents in large outdoor environmental chambers. Diesel exhaust was diluted with filtered ambient

air, flowed through a diffusive denuder to reduce gaseous pollutants and passed into a 40,000-ℓ outdoor Teflon chamber. Samples as small as 20 m^3 were taken for mutagenicity testing on Salmonella strains TA98 and TA98NR.

Results and Conclusions. It was found that only a small fraction of the chamber volume need be sampled to allow determinations of mutagen densities on Salmonella strains TA98 and TA98NR. Therefore, future studies simulating the effect of transport on the mutagenicity of particulate emissions are possible under realistic atmospheric conditions in our 40,000-ℓ SAPRC outdoor environmental chamber. Such studies would give highly useful information on chemical transformations on the surface of diesel particles under various conditions of simulated smog and its major gaseous components, e.g., NO_2, O_3, HNO_3 and PAN in simulated atmospheres.

F. Exploratory Testing for Gas Phase Mutagens Using the Tradescantia Stamen Hair Assay

The Tradescantia stamen hair bioassay has been used extensively in studies of mutation for the past 25 years (Swanson 1957). Early radiobiological studies led to the extension of the use of the plant Tradescantia in studies of chemical mutagenesis (Underbrink et al. 1973). Laboratory studies with chemicals demonstrated (Sparrow et al. 1974) that this system was highly sensitive to gaseous mutagens and should be able to respond to ambient levels of gas phase pollutants. Indeed, with EPA support, researchers at the Brookhaven National Laboratory (BNL) designed, constructed and tested a mobile laboratory for use of this plant hair bioassay system as a test of gas phase mutagens at a variety of urban, industrial, suburban and rural sites throughout the U. S. (Schairer et al. 1982). The highest responses were seen in regions of major industrial pollution (e.g., at Elizabeth, New Jersey), but despite a major effort the gaseous pollutants causing the mutagenic response were not identified.

The Tradescantia test system uses an interspecific hybrid which is the cross between pink- and blue-flowering parents. The visible marker of mutation is a phenotypic change from blue to pink in mature flowers. Mutation is induced by exposing young developing flowers to the test gas; genetic damage is expressed 5 to 18 days later as isolated pink cells or groups of pink cells in the stamen hairs of mature flowers. The flowers are analyzed under a dissecting microscope each day as they bloom for up

to two weeks after treatment. Induced mutation is defined as the ratio of pink (mutational) events to total number of stamen hairs (Schairer et al. 1982).

The specific objectives of the Tradescantia studies we conducted in cooperation with Dr. Lloyd Schairer and Mr. Neil Tempel of the BNL were:

- To bioassay specific air pollutants generated and tested under controlled laboratory conditions.
- To bioassay photochemical smog generated synthetically in a large SAPRC outdoor chamber.
- To bioassay ambient air pollution in Riverside, CA.

Controlled laboratory exposures of Tradescantia cuttings to individual pollutants (e.g., O_3, PAN and NO_2) over a concentration range from 1 to 100 ppm were carried in 12-ℓ glass chambers brought from BNL. The SAPRC 50,000-ℓ outdoor smog chamber was employed to create a highly polluted simulated atmosphere to which the Tradescantia cuttings were also exposed. In addition, a 10-day exposure to ambient air was carried out at Riverside.

Results and Conclusions. The statistically significant mutagenic activities obtained with the surrogate smog mixture demonstrated the usefulness of simulated atmospheres in smog chamber experiments for the study of the ambient levels of gas phase mutagens and their chemical characterization. Specifically, the joint BNL/SAPRC team found:

- The mutagenicity of surrogate smog in the outdoor chamber was consistent with the sum of the mutagenicities of PAN, O_3 and NO_2 determined separately in laboratory tests.
- The mutagenicity of ambient air could also be accounted for by the sum of the mutagenic activities of PAN, O_3 and NO_2.

Interestingly, to our knowledge this is the first time the mutagenic activity of PAN has been observed in atmospherically relevant systems.

G. Recommendations for Future Research

o Research should be continued toward characterizing the chemical identities of the unknown polar mutagens which we have shown contribute significantly to the overall mutagenic burden of respirable ambient particles.

- Changes in the mutagenicity and chemical composition of ambient POM resulting from long exposures to gaseous co-pollutants should be investigated.
- Efforts should be directed towards adapting the Ames Salmonella mutagenicity assay for the determination of the mutagenicity of gas phase pollutants.
- Attempts should be made to correlate observed diurnal variations in mutagenicity of ambient POM with the time-concentration profiles of reactive gaseous pollutants and radical intermediates, as well as indicators of primary emissions such as lead and elemental carbon.
- Further studies of changes in chemical identity and biological activity of diesel POM due to exposure to gaseous pollutants should be carried out under carefully controlled simulated atmospheric conditions.

II. EVALUATION OF THE LEVELS OF MUTAGENICITY OF AMBIENT RESPIRABLE PARTICLES IN THE WESTERN PORTION OF THE SOUTH COAST AIR BASIN, AND THE IMPACT OF A MAJOR FREEWAY ON THESE LEVELS

A. Introduction and Statement of the Problem

Extracts of airborne particulate organic matter (POM) have been found to display direct mutagenicity (not requiring S9 metabolic activation) towards Ames Salmonella strain TA98 (Pitts et al. 1975, Pitts et al. 1977, Talcott and Wei 1977, Pitts 1983). This activity predominates in respirable sub-micron particles (Pitts et al. 1978, Talcott and Harger 1980, Löfroth 1981). Extracts of POM from both diesel exhaust and gasoline engine exhaust have also been found to be directly mutagenic (Huisingh et al. 1978, Löfroth 1981, Lewtas 1982, Pierson et al. 1983). In addition, the direct activity towards S. typhimurium of motor vehicle POM emissions, both from gasoline and diesel engines, has been shown to correlate highly with mammalian cell mutagenesis and skin tumor initiation assays (Lewtas 1983).

Our previous study, supported by the California Air Resources Board, of the diurnal variations in the direct and activatable mutagenicity of airborne POM in California's South Coast Air Basin (CSCAB) suggested strongly that mobile source primary emissions were important contributors to the high mutagen levels we observed (Pitts et al. 1981, Pitts et al. 1982a). Recently, Flessel and co-workers at the Air Industrial Hygiene Laboratory reported that during smog episodes in Contra Costa County in August and October 1981, vehicular transportation sources were the predominate mutagenic contributors (Flessel et al. 1983). At least one epidemiological study has suggested a correlation with highway traffic and cancer incidence (Blumer et al. 1977) although this has subsequently been challenged (Polissar and Warner 1981).

Several million commuters, as well as residents near freeways, in the CSCAB undergo exposure to primary vehicle emissions daily. We undertook to examine, on a day of light photochemical pollution with typical wind pattern conditions prevailing, the mutagen levels in the western end of the CSCAB and the incremental mutagenic burden of ambient particulate contributed by a heavily travelled freeway in West Los Angeles.

B. Research Objectives

The specific objectives of the study were:

(1) To determine if the diurnal variations and "background" levels of mutagen densities in West Los Angeles (WLA) were similar to those we previously observed just east of downtown LA (ELA).

(2) To assess the incremental contribution of a heavily travelled freeway to the mutagenic burden of respirable ambient particulate in the vicinity of the freeway.

(3) To estimate the contribution of nitroarenes to the direct mutagenicity of extracts of those ambient particulate by comparing the response of Ames strain TA98 to that of the nitroreductase-deficient strain TA98NR.

C. Experimental Methods

Particulate Collection. To meet these objectives, the mutagenicity of ambient POM was measured for consecutive 3-hr time intervals over a 27-hr period from 0600 PST on March 9 until 0900 PST on March 10, 1983 at two sampling sites on opposite sides of the heavily travelled San Diego Freeway (I-405) in West Los Angeles (see Figure II-1).

We chose days of light to moderate pollution so that the "freeway effect" would not be overshadowed by generally high pollutant levels. The typical wind pattern of West Los Angeles is onshore winds during the day and offshore winds at night. For sampling sites on opposite sides of the freeway, since the I-405 freeway generally runs north-to-south, at any given time one site would generally be upwind and the other downwind of the freeway. A California Department of Transportation (CALTRANS) facility east of the intersection of Wilshire and Sepulveda, near a busy interchange on I-405, was chosen for the "downwind" site during the day, because it is heavily impacted by vehicle emissions from the daily commuter freeway-traffic. Because onshore winds prevail during the day, a site west of the freeway would be "upwind" and receive minimal impact from freeway emissions during the daylight hours. A West Los Angeles police department (WLAPD) repair garage west of I-405 met our security and power requirements, so it was chosen as the "upwind" site. During the hours from approximately midnight to sunrise when an offshore flow occurred, the WLAPD site was downwind and the CALTRANS site upwind of the freeway.

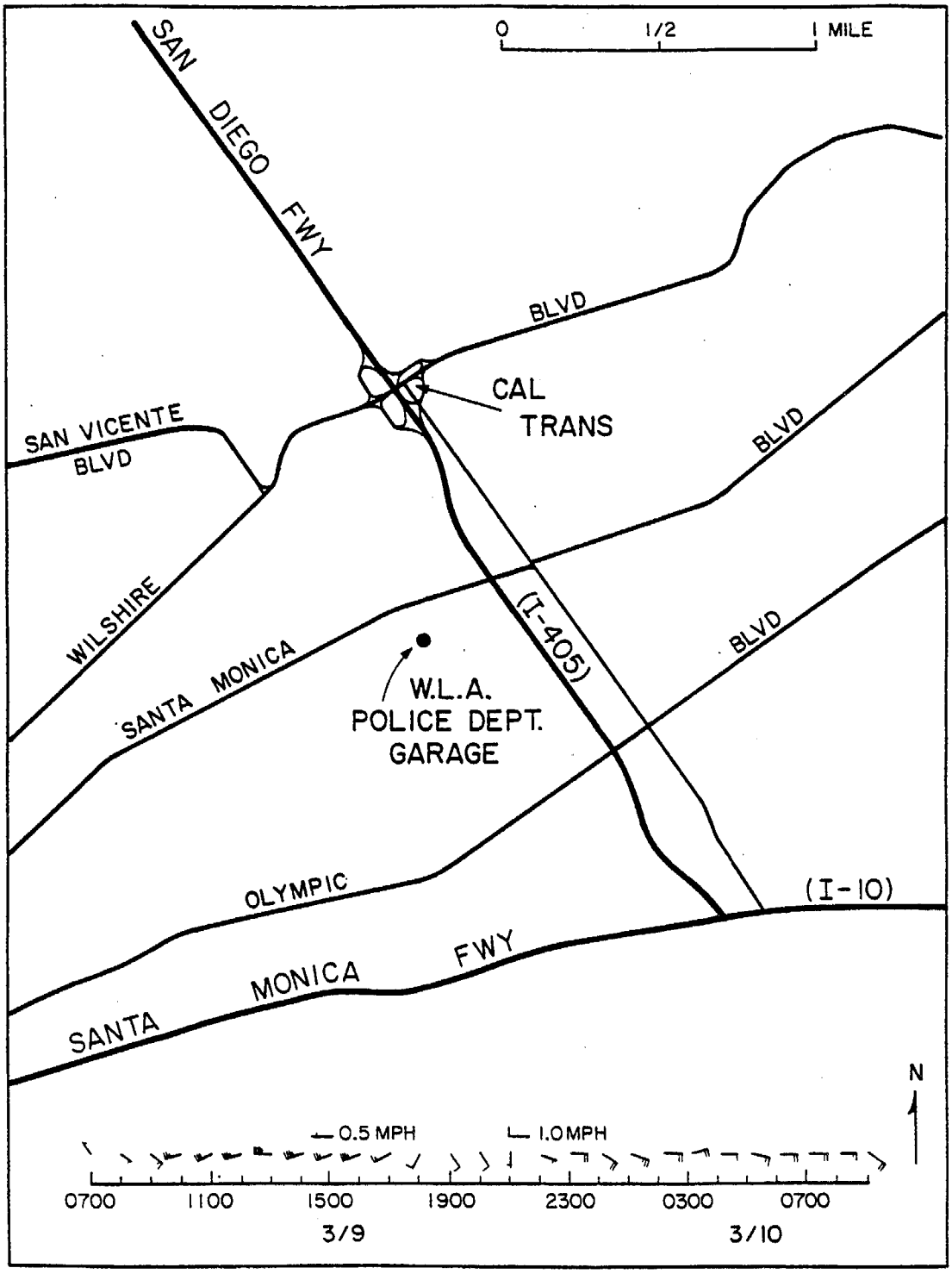

Figure II-1. Location of sampling sites in West Los Angeles. Hourly average wind speeds and directions for 0600 March 9 to 0900 PST March 10, 1983 are designated at the bottom of the map.

Particulate collections were made at 3-hr intervals using standard hi-vol samplers (Sierra Instruments Model 305-2000) equipped with 10 micrometer-cutoff inlets and mass flow controllers. Pre-cleaned (Soxhlet extracted with dichloromethane and methanol for 24-hr each) Pallflex TX40H120-WW Teflon-coated filters were employed and were equilibrated to 50% R.H. and weighed before and after collection to determine particulate loading. Two hi-vols were employed at the CALTRANS site and three at the WLAPD site to collect sufficient material for mutagenicity assays. At each site, two lo-vol samplers were operated with the same sampling intervals as the hi-vols. One lo-vol was equipped with pre-cleaned quartz fiber filters for carbon analysis and the second was equipped with polycarbonate Nuclepore filters for lead analysis.

Carbon monoxide was monitored continuously at the CALTRANS site with a Byron Model 401 analyzer which was calibrated prior to use with a certified gas mixture [7.3 ppm CO and 3.6 ppm CH_4 (Scott Marrin CC12041)]. Other air quality data obtained at both sites included NO, NO_x concentrations (Beckman Model 952 at CALTRANS, Teco Model 14B at WLAPD) calibrated before use by a gas phase titration versus an NBS cylinder which had 97.4 ppm NO (#8381 SRM 1684A), O_3 concentrations (Dasibi Model 1003AH compared to a calibrated Model 1003 AH) and for light scattering aerosols, β_{scat}, (MRI Model 1550). Wind speed and direction and relative humidity (wet bulb-dry bulb method) were measured at the WLAPD site. The hourly average wind speeds and directions are indicated on the bottom of Figure II-1. The cross marks on the wind direction vectors give the wind speed (a long mark representing 1 mile hr^{-1} and a short mark 0.5 mile hr^{-1}). The cross marks are placed at the end of the arrow from which the wind originates. The averaged hourly values of wind speed and direction are shown above the ending time of the hourly interval.

Particulate Analyses. To prevent photochemical degradation of the collected particulate samples, dark room conditions were employed during the sample preparation and bioassay. Each particulate sample (two or three 3-hr filters) was Soxhlet extracted for 18-hr with dichloromethane followed by extraction with acetonitrile. The extracts were combined, filtered and reduced in volume under vacuum, evaporated to constant weight under a stream of dry nitrogen and taken up in dimethyl sulfoxide for the

Ames assay. A blank consisting of three pre-cleaned filters was extracted in the same manner as the samples.

The lo-vol Nuclepore filters were analyzed for lead with an X-ray fluorescence technique by Robert Giauque at the UC Lawrence Berkeley Laboratory. The lo-vol quartz filters were analyzed for organic and elemental carbon with a thermal-optical method by James Huntzicker at the Oregon Graduate Center (Huntzicker et al. 1982).

Mutagenicity Testing. All samples were tested on Ames Salmonella strain TA98 (Ames et al. 1975) and TA98NR, a nitroreductase-deficient strain (Rosenkranz and Poirier 1979, Rosenkranz et al. 1981) according to our standard protocol (Belser et al. 1981) without S9, and on TA98 with S9 (2% v/v mix). Strain TA98 was used because it has been found to be the most sensitive strain to airborne frameshift mutagens. Strain TA98NR is an isolate of TA98 which is deficient in the "classical" bacterial nitroreductase--the enzyme which catalyzes the bioactivation of most mononitroarenes to mutagenic metabolites. Thus, a lower response on this strain relative to TA98 indicates the probable presence of mononitroarenes in the sample. However, TA98NR is still sensitive to the potent mutagens 1,8-dinitropyrene and 1,6-dinitropyrene because these compounds are activated by a second "nonclassical" reductase.

Cultures were grown for 16-hr in L-broth and were diluted with sterile medium until the turbidity (measured as absorbance at 550 nm) reached a previously determined value corresponding to a concentration of 10^9 cells ml^{-1}. In each test, the strains were checked for the following genetic markers: (1) ampicillin sensitivity, (2) ultraviolet sensitivity, (3) crystal violet sensitivity, (4) standard spontaneous reversion and (5) mutagenic response to the standard mutagens benzo(a)pyrene, 2-nitrofluorene and quercetin.

After dilution, the cultures were kept on ice to ensure that the population remained the same. Exact titers were determined by dilution and plating on histidine-supplemented minimal medium. After a 63-hr incubation period, the colonies on the plates were counted and the titer determined. No attempt was made to adjust mutagenicity values for slight differences in cell population because the exact relationship between titer and response is not currently known; in our experiments the effect was small.

The mammalian-metabolic activation system (S9) made from liver homogenate from Aroclor 1254-induced rats was purchased from Litton Bionetics. Because protein present in the S9 mix can bind the highly reactive metabolites of nitroarenes (Wang et al. 1981), a low concentration of S9 (2% v/v) was employed in these tests. With our current S9 preparation, this corresponded to a protein concentration of 0.83 mg $plate^{-1}$ and benzo(a)pyrene hydroxylase activity of 0.12 moles hydroxy-BaP min^{-1} $plate^{-1}$ (manufacturer's specifications).

Eight doses were tested in triplicate and the mean of the three responses was used to determine the dose-response curve. Positive controls were 2-nitrofluorene (360 rev μg^{-1} TA98; 41 rev μg^{-1} TA98NR) and quercetin (8.2 rev μg^{-1} TA98; 9.2 rev μg^{-1} TA98NR) for TA98 and TA98NR, and benzo(a)pyrene (450 rev μg^{-1}) for TA98 with 2% S9.

D. Results and Discussion

Measurements were begun at 0600 PST on March 9, 1983. The wind speed and directions indicated at the bottom of Figure II-1 show the typical pattern of onshore winds during the day. The WLAPD site, therefore, was not impacted by emissions from the I-405 freeway until midnight when the wind direction reversed. The study occurred during a period of light photochemical pollution with an ozone maximum during the study period of 100 ppb measured at 1400 hours on March 9 at the South Coast Air Quality Management District Station in west Los Angeles. Temperature soundings (reported by the National Weather Service) on the UCLA campus at 0600 on March 9 showed a ground-based inversion extending to 2250 ft. The 78° breaking temperature of the inversion was reached between 1200-1300 on that day. On March 10 the inversion base at 0600 was at 1400 ft and extended to 3200 ft.

Figures II-2 and II-3 are the diurnal plots of mutagen density (revertants per cubic meter of air sampled) on Salmonella strains TA98 (-S9), TA98NR (-S9) and TA98 (+S9) for the CALTRANS and WLAPD sites, respectively. These mutagen densities are comparable to those we previously measured in ELA (Pitts et al. 1982a). The observed decreased response by the nitroreductase deficient strain TA98NR relative to TA98 indicates the presence of nitroarenes (Mermelstein et al. 1981, Rosenkranz et al. 1981). This is consistent with nitroarenes having been identified in POM

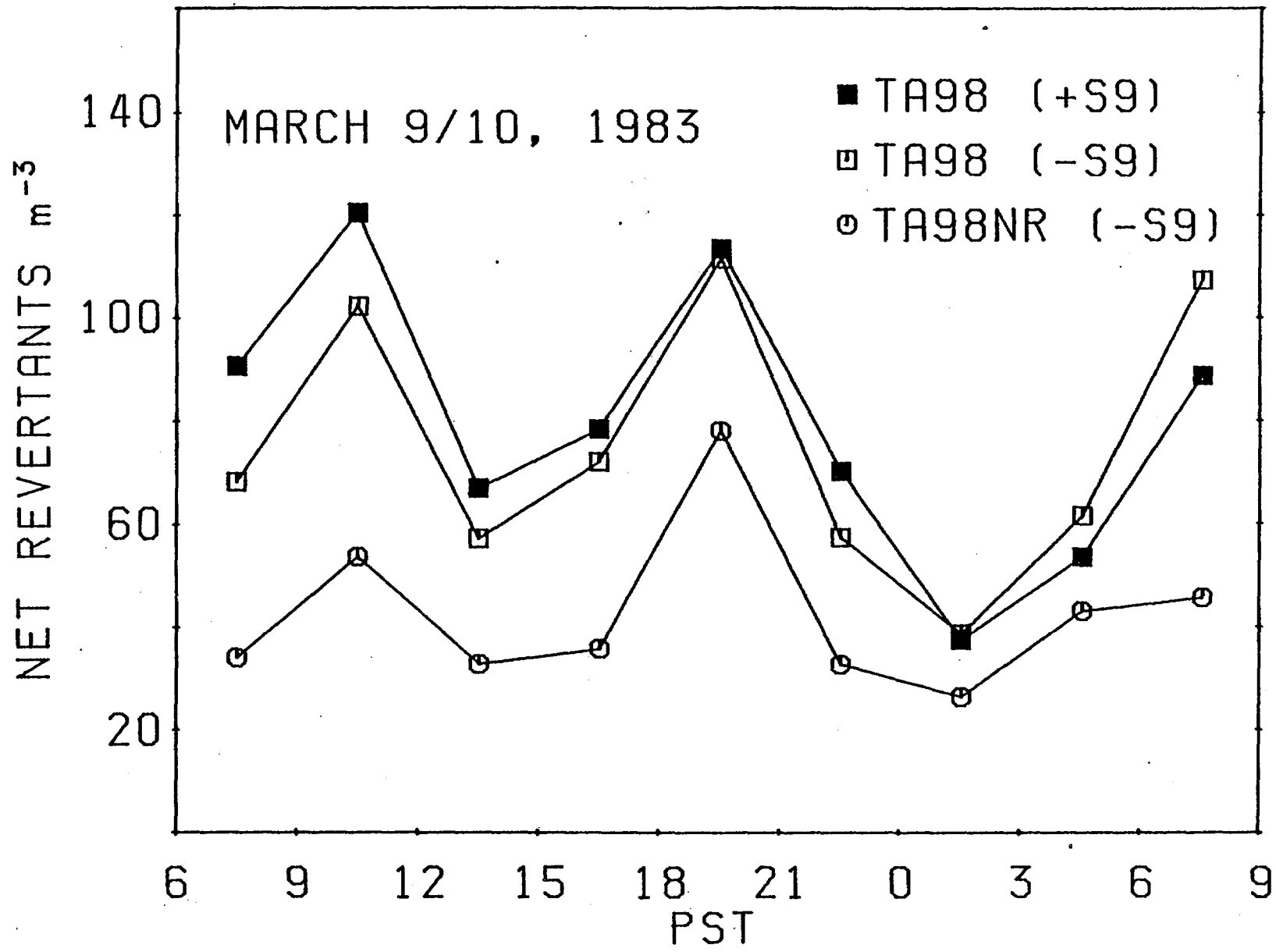

Figure II-2. Diurnal variation in mutagen densities (revertants m^{-3} of sampled air) of ambient POM collected at CALTRANS I-405 freeway site on March 9-10, 1983.

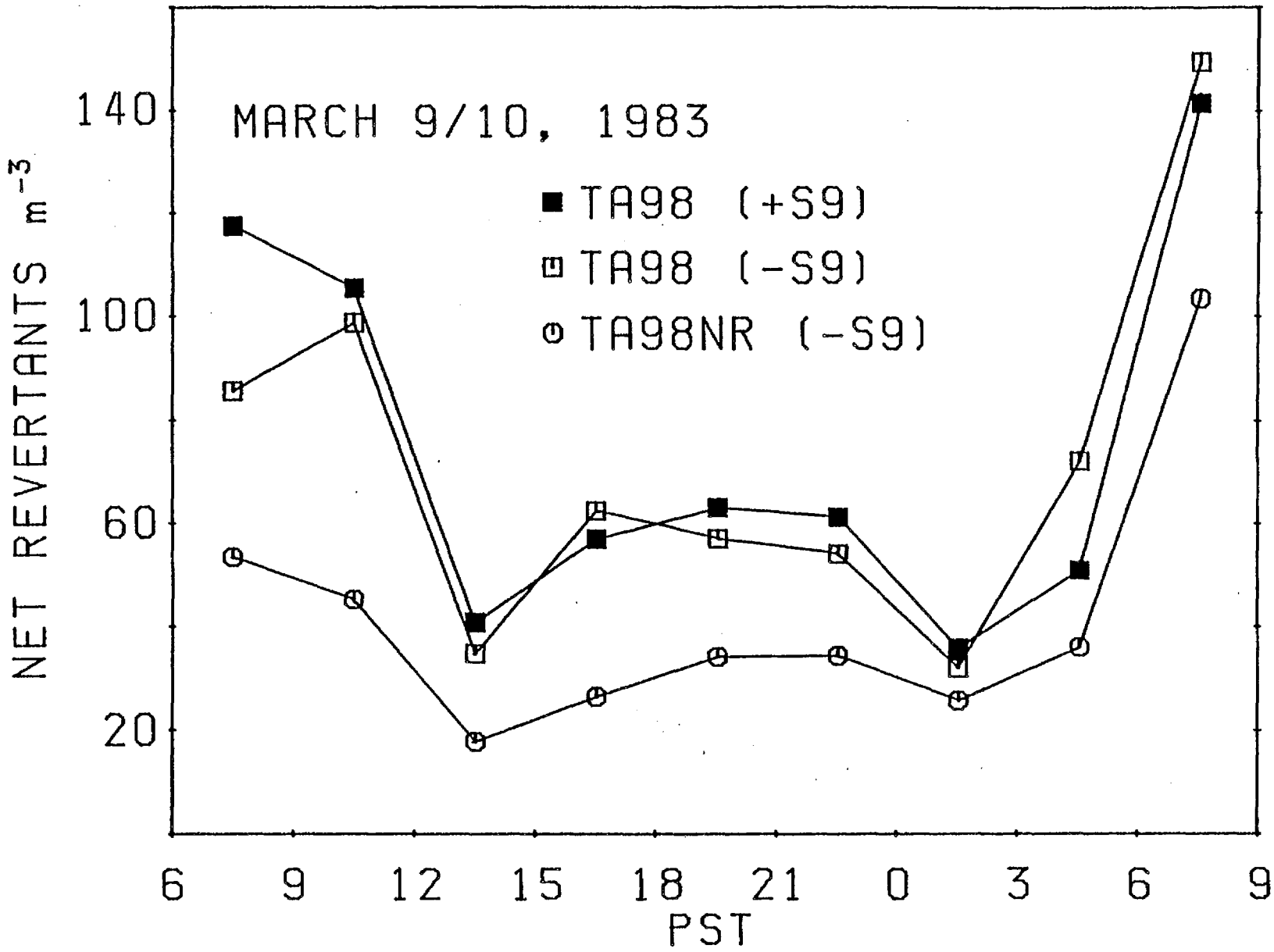

Figure II-3. Diurnal variation in mutagen densities (revertants m^{-3} of sampled air) of ambient POM collected at WLAPD site just west of I-405 freeway on March 9-10, 1983.

from both diesel (Pitts et al. 1982b, Schuetzle et al. 1982) and gasoline vehicles (Gibson 1983). The decrease in response towards TA98NR relative to TA98 was ~50%, comparable to what we found previously in our ELA study (Pitts et al. 1982a).

The mutagen densities, specific activities (revertants per microgram of extract) and mutagen loadings (revertants per milligram of particulate collected) at each site were highly correlated and showed very similar diurnal profiles (see Figures II-4 and II-5 for the specific activities and Figures II-6 and II-7 for the mutagen loadings at the CALTRANS and WLAPD sites, respectively). The high correlation of mutagen densities with specific activities strongly suggests that meteorological factors alone were not responsible for the observed variations in mutagen densities because, for example, increased mutagen densities due to decreased atmospheric mixing or lower inversion heights would not be expected to cause increases in specific activity. As was the case in our ELA study, over the 3-hr sampling intervals employed, daily highs and lows in mutagen density occurred due to changes in emissions, mixing heights and wind speeds. These short-term peak mutagen densities clearly can be much higher than 24- or 12-hr averages reported in the literature.

Particulate lead is often used as a tracer for automobile exhaust, although ambient levels are decreasing with the increasing use of unleaded and limited lead gasolines. In the CSCAB, in addition to lead, CO and NO_x are largely attributed to motor vehicle traffic with over 85% of the CO emissions being derived from motor vehicles and 58% of the NO_x emissions attributed to on-road vehicles (South Coast Air Quality Management District and Southern California Association of Governments 1982). The maximum lead concentration observed in this study was 1.9 $\mu g\ m^{-3}$ compared to a measurement of 3.5 $\mu g\ m^{-3}$ obtained in downtown Los Angeles in September 1980 (Pitts et al. 1982a). The CO values coincident with these lead maxima were both about 9 ppm, indicating a true decrease in lead emissions rather than a dilution effect.

Figure II-8 shows the diurnal variation in mutagen density (TA98, -S9) at the CALTRANS site plotted along with particulate lead and the 3-hr averages of the gaseous co-pollutants CO and NO_x. The coincidences of the maxima of the CO, NO_x and the particulate lead are to be expected where the ambient POM is largely due to primary vehicle emissions. These

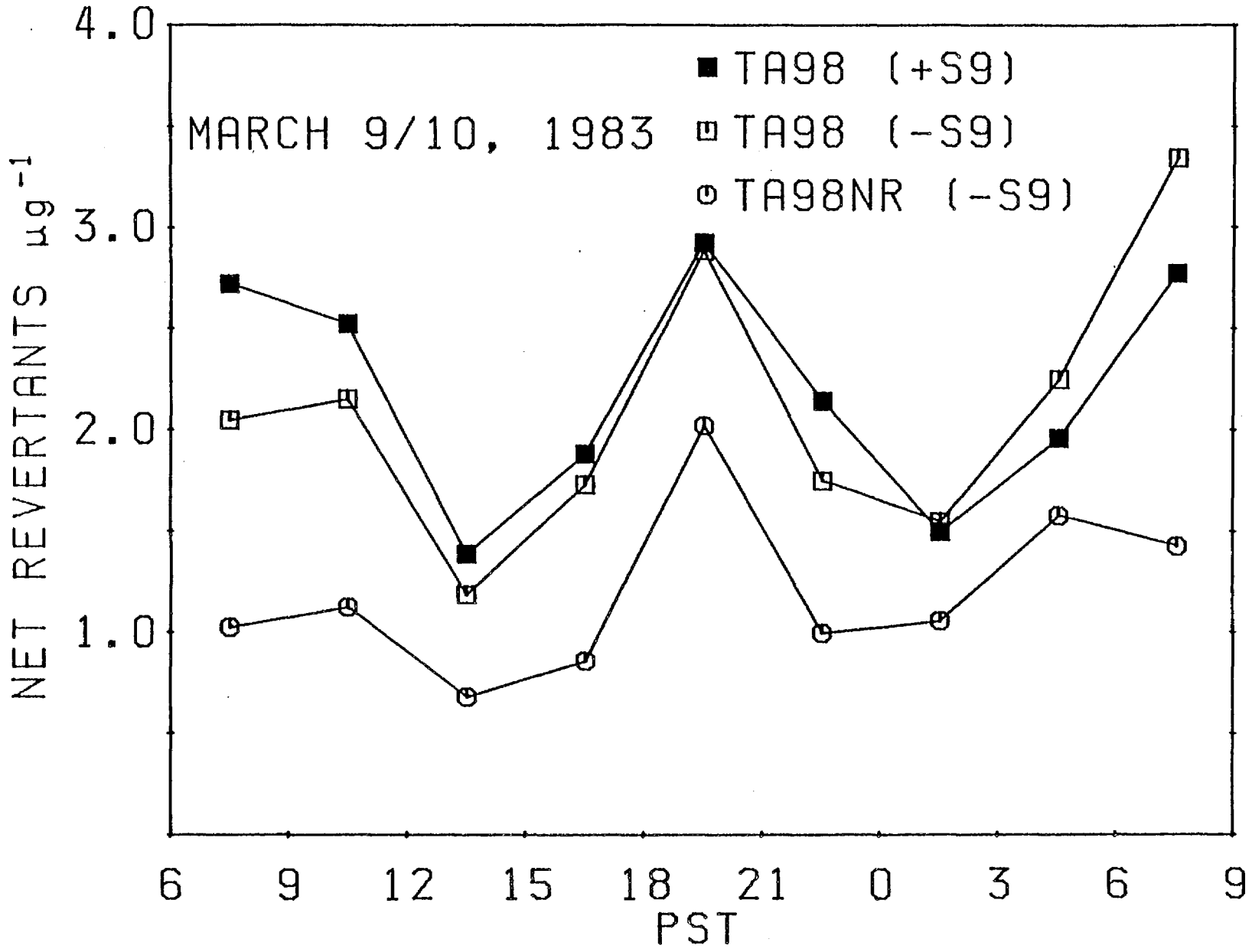

Figure II-4. Diurnal variation in specific activities (revertants μg^{-1} of extract) of ambient POM collected at CALTRANS site on March 9-10, 1983.

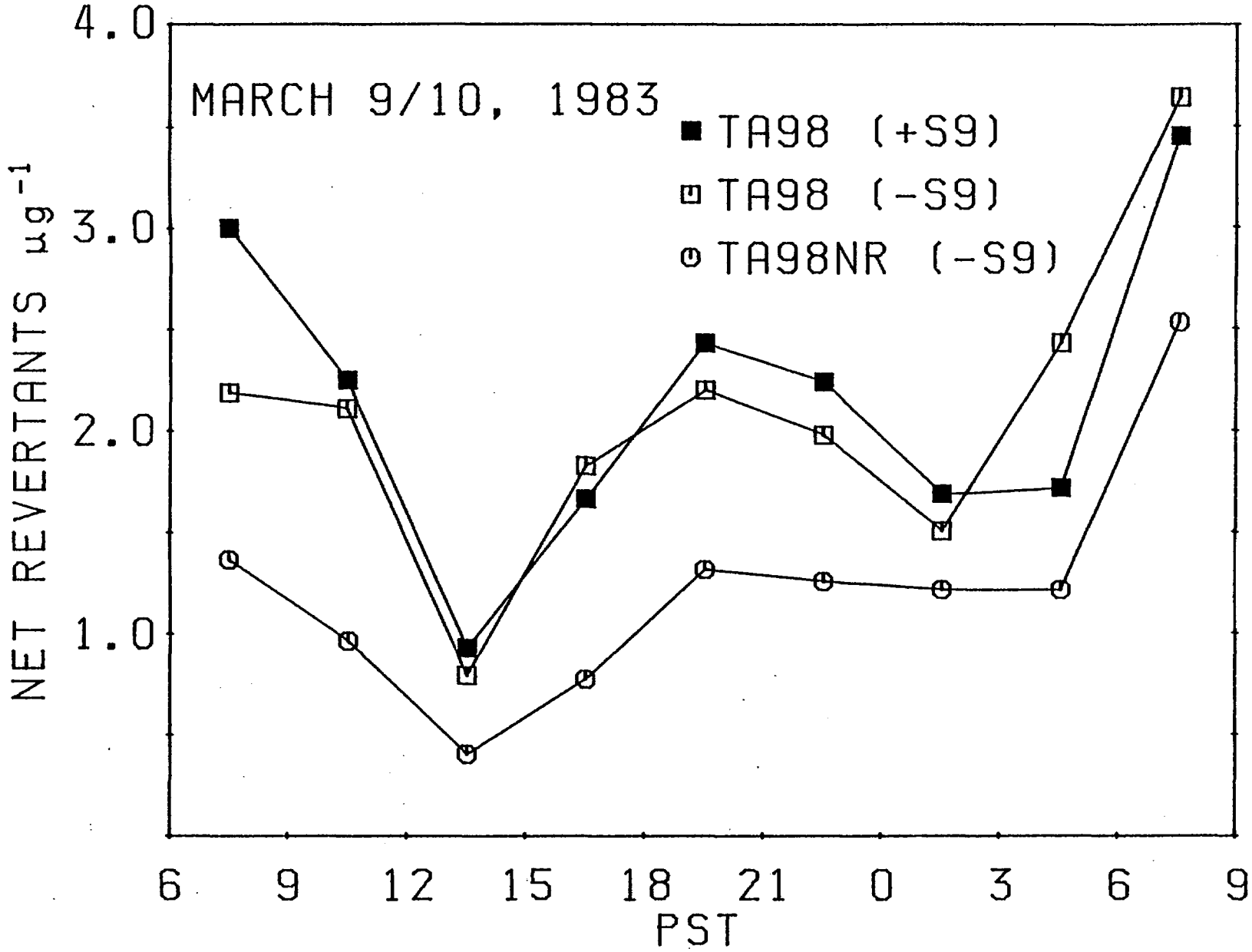

Figure II-5. Diurnal variation in specific activities (revertants μg^{-1} of extract) of ambient POM collected at WLAPD site on March 9-10, 1983.

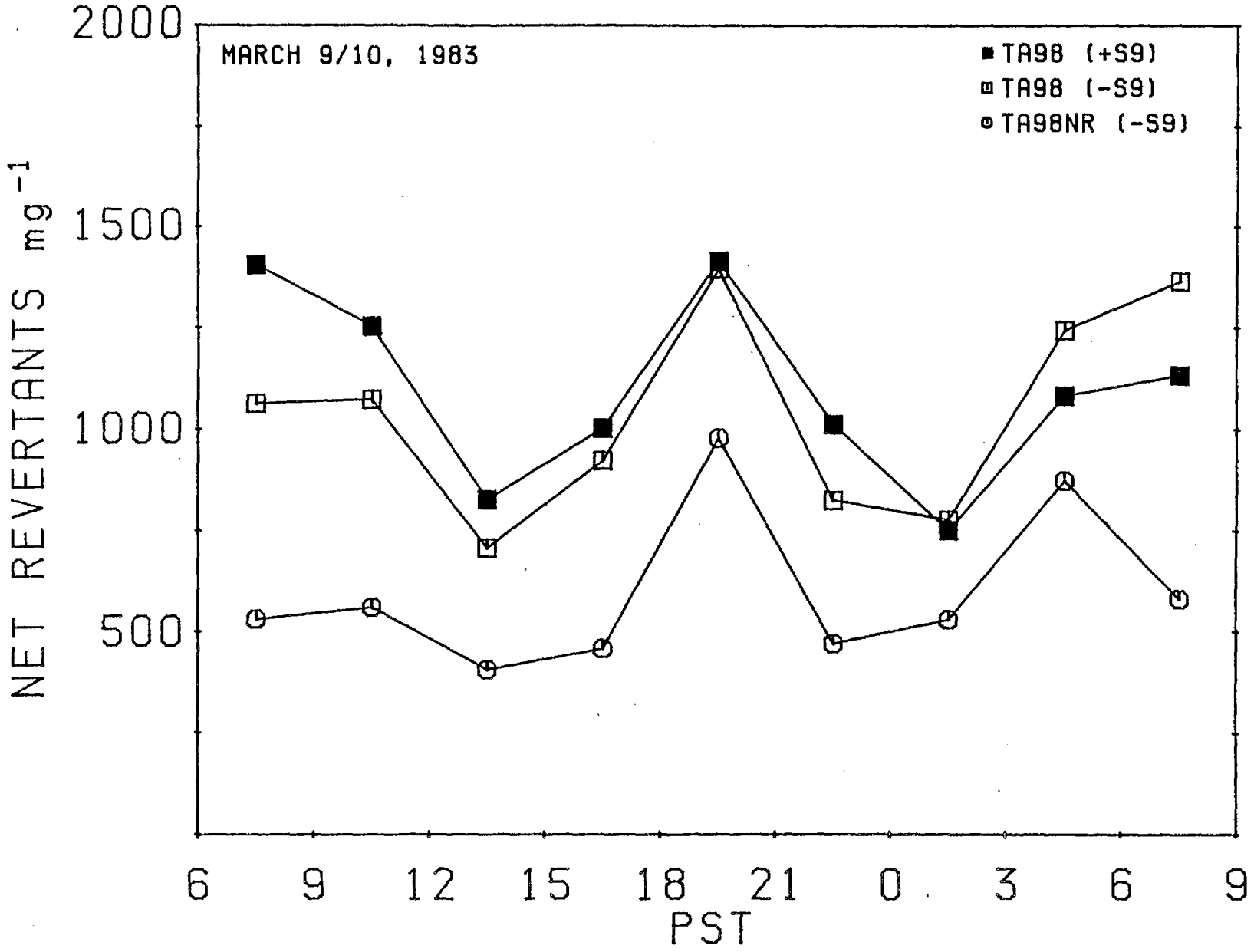

Figure II-6. Diurnal variation in mutagen loadings (revertants mg^{-1} of particulate) of ambient POM collected at CALTRANS site on March 9-10, 1983.

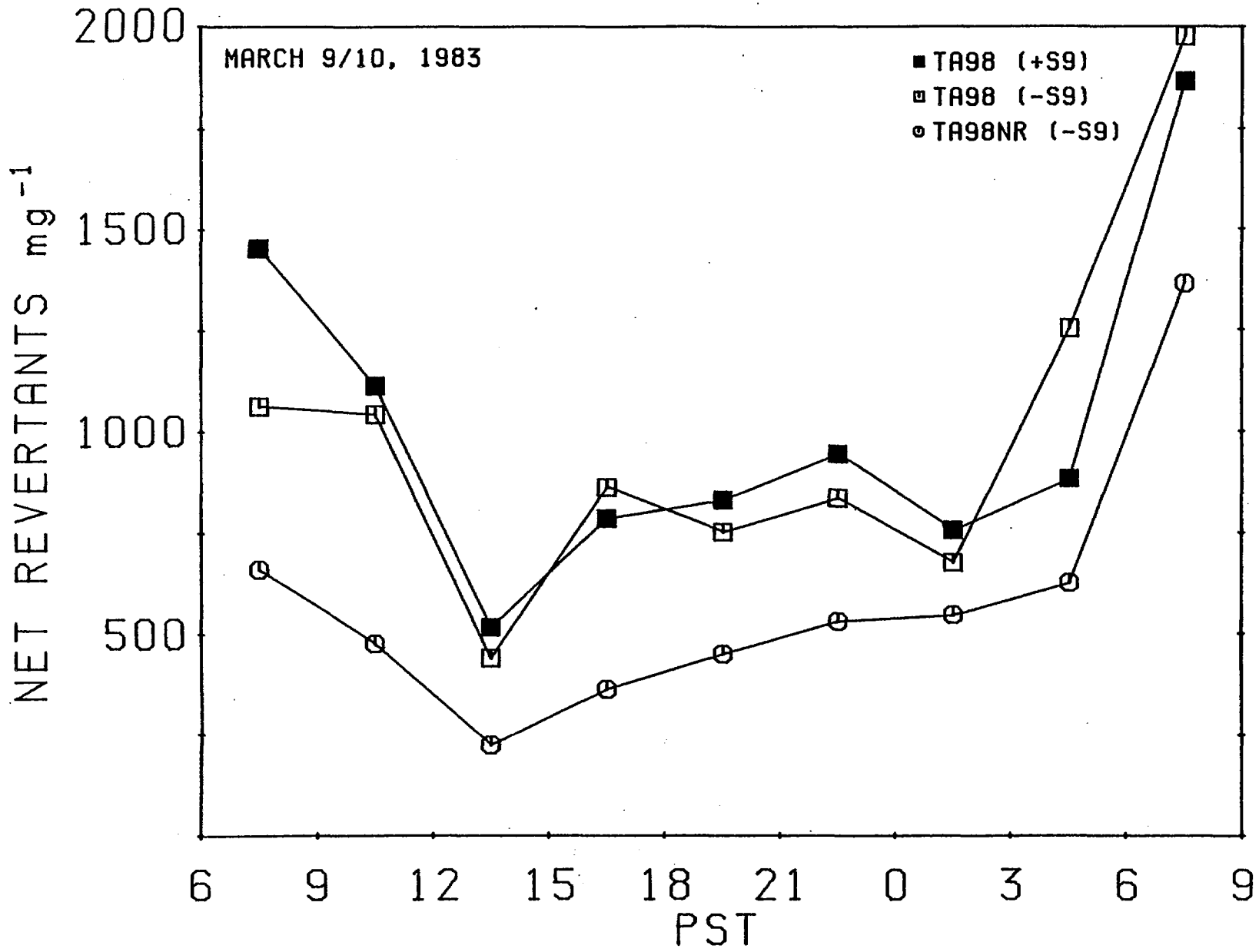

Figure II-7. Diurnal variation in mutagen loadings (revertants mg^{-1} of particulate) of ambient POM collected at WLAPD site on March 9-10, 1983.

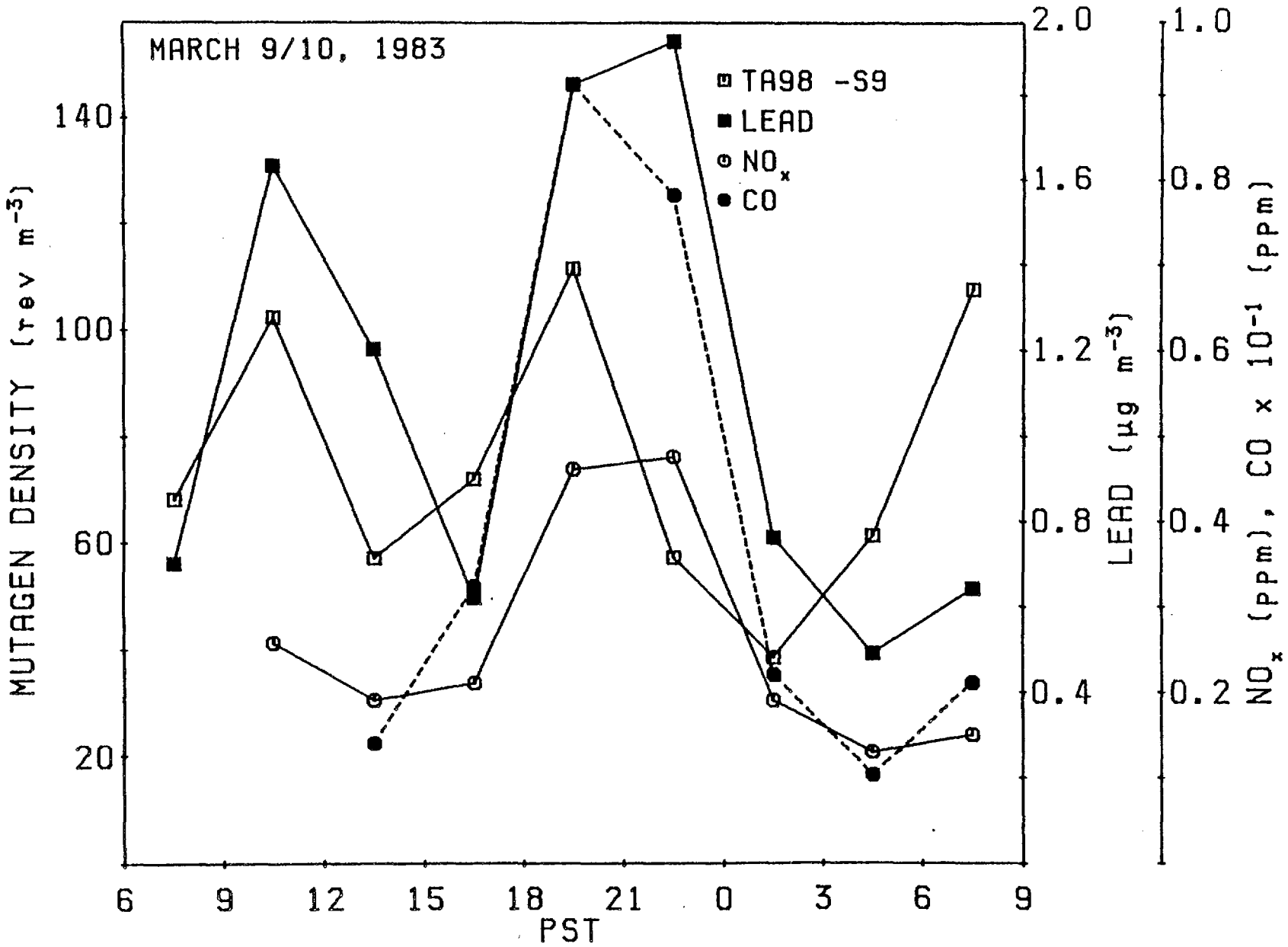

Figure II-8. Diurnal variations in mutagen density (TA98, -S9), lead, NO_x and CO at the CALTRANS site on March 9-10, 1983.

coincidences, however, do not rule out the presence of additional sources of POM. Figure II-9 shows the diurnal variation in mutagen density (TA98, -S9) at the WLAPD site along with particulate lead and 3-hr averages of NO_x. As at the CALTRANS site, the lead and the NO_x were well correlated.

Table II-1 indicates, for each sampling interval, which site was downwind of the freeway and gives the differences in lead, NO_x and mutagen density (TA98, -S9) for the downwind site minus the upwind site. The "background" mutagenicity, that is, the mutagenicity of the upwind site is also given. Generally, the site downwind of the freeway had a higher mutagen density than the upwind site. However, the differences in mutagen density across the freeway did not correlate with the differences in the vehicle tracer lead. This lack of correlation could be the result of the following factors or a combination of them: (1) a varying distribution of vehicles using unleaded fuel, (2) the presence of a mutagen source other than primary POM from vehicle traffic, and/or (3) the rapid atmospheric transformation of the mutagens on vehicular POM.

Freeway I-405 is a heavily travelled route averaging, on a typical day: 14,000 cars hr^{-1} from 0600-1800; 12,000 cars hr^{-1} from 1800-2100; 8,000 cars hr^{-1} from 2100-0000 and 2,000 cars hr^{-1} from 0000-0600 (Wheelock 1983). This freeway is, therefore, a good source of primary vehicular POM. At 0600 on March 9 the inversion in Los Angeles was ground-based and the influx of primary pollutants from the freeway would be expected to have had a large effect. Between 0600-0900 on that morning the wind was light and variable. Lead values at the WLAPD site were not measured, but the mutagen density at this time was higher at the WLAPD site. Lead and mutagen density both show a rise for the 0900-1200 sampling interval on this day at each site indicating an accumulation of POM. From 0900 until 1800 the wind was generally from the west, and the CALTRANS site, downwind of the freeway, showed higher mutagen densities and lead and NO_x levels than the upwind WLAPD site. The 1200-1500 minimum in lead and mutagen density at both sites was probably due to the breaking up of the inversion allowing increased vertical mixing.

From 1800-2400 the wind was very light and generally from the south. As can be seen from the map on Figure II-1, the CALTRANS site was still impacted by POM from the traffic on I-405 during this time while the WLAPD site was up-wind. The impact of vehicular emissions was again

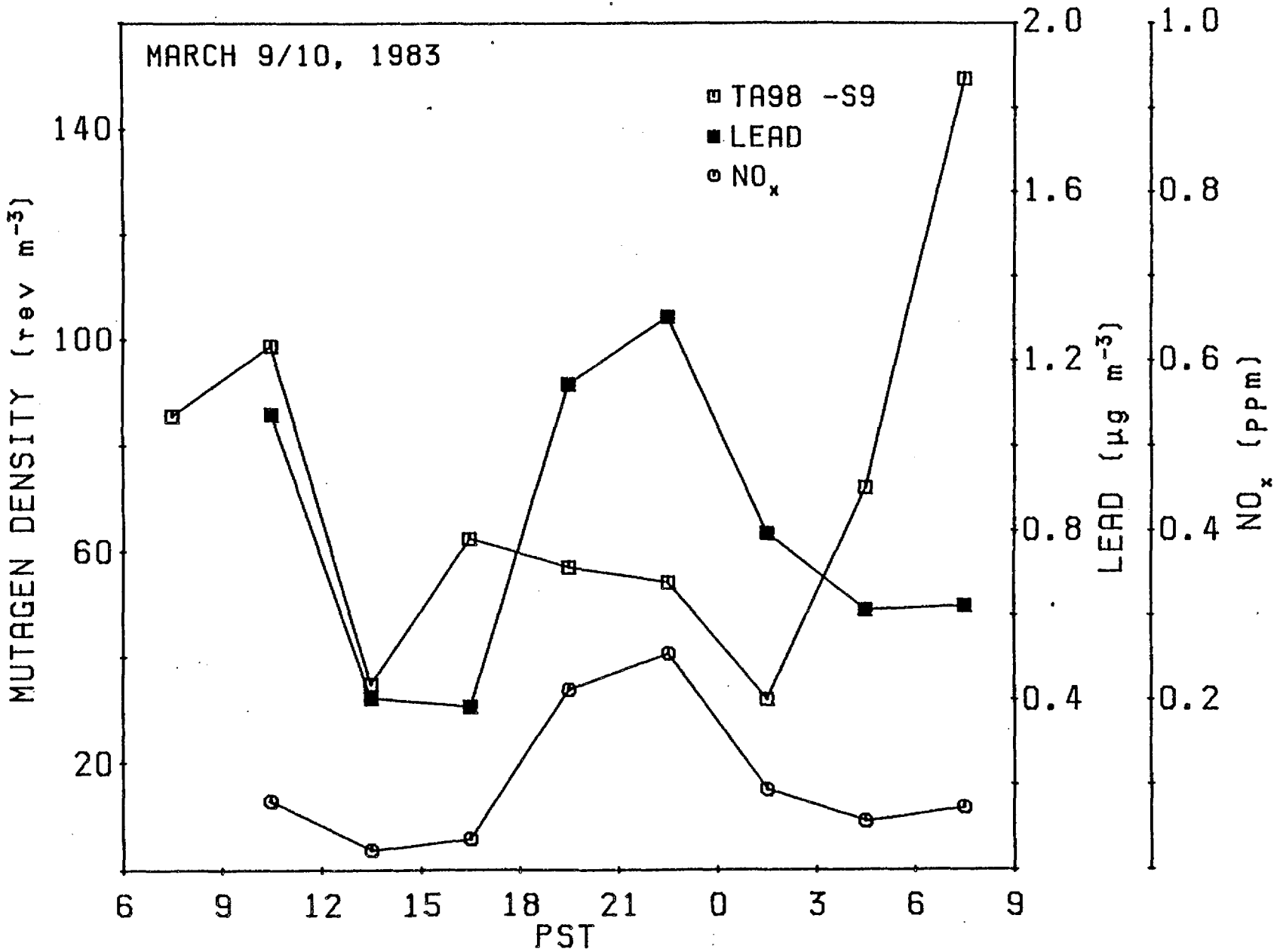

Figure II-9. Diurnal variations in mutagen density (TA98, -S9), lead and NO_x at the WLAPD site on March 9-10, 1983.

Table II-1. Differences in Mutagen Density (TA98, -S9), Lead and NO_x Between the Sites Downwind and Upwind of Freeway I-405

Date and Sampling Interval (PST)	Downwind Site	ΔMutagenicity[a] TA98, -S9 (rev m^{-3})	ΔPb[a] ($\mu g\ m^{-3}$)	ΔNO_x[a] (ppm)	Mutagenicity at Upwind Site TA98, -S9 (rev m^{-3})
March 9, 1983					
0600-0900	WLAPD[b]	17	-[c]	-	68
0900-1200	CALTRANS	4	0.6	0.2	98
1200-1500	CALTRANS	22	0.8	0.2	35
1500-1800	CALTRANS	10	0.2	0.2	62
1800-2100	CALTRANS[b]	54	0.7	0.3	57
March 10, 1983					
2100-0000	CALTRANS[b]	3	0.6	0.2	54
0000-0300	WLAPD	-6	0.03	-0.1	38
0300-0600	WLAPD	11	0.1	-0.1	61
0600-0900	WLAPD	42	-0.02	-0.1	107

[a]Difference between values observed at downwind and upwind sites.
[b]Wind direction variable and light.
[c](-) no data.

reflected in higher mutagen densities, lead and NO_x at this site, with the incremental mutagen dose reaching 50 rev m^{-3} during this interval.

The sharp increases in lead and mutagen densities at the CALTRANS site for the 0900-1200 and 1800-2100 sampling intervals on March 9 were followed by decreases in mutagen density for the next sampling period which were not reflected in corresponding decreases in lead values. Destruction of labile mutagenic compounds present on the fresh vehicular POM may account for the observed decreases in mutagen density and also in specific activity. Also, the apparent "lag time" in the decrease in lead concentrations may be emphasized by the integration of the values over 3-hr intervals.

From midnight to approximately 0600 on March 10, an offshore air flow drained the air basin from east to west and both sites were impacted by

the same general air mass. The increasing mutagenicity at both sites from 0300-0900 without corresponding increases in lead, NO_x and CO could be due to inland sources of mutagenic POM, either stationary sources or diesel vehicles, or possibly to transformations of the adsorbed organics on the vehicular POM to more potent mutagenic compounds (Pitts 1983).

On March 10 the 0600 inversion base was at 1400 ft and primary emissions would be expected to be diluted more than those of the morning of March 9 due to additional vertical mixing. In accord with the reported mixing height effects, the lowest lead and NO_x values at the CALTRANS site and the smallest differences in these values between upwind and downwind of the freeway were observed on the morning of March 10. Although essentially no differences in lead and NO_x values were observed between the upwind CALTRANS and the downwind WLAPD sites on the morning of March 10, large differences were observed in mutagen densities particularly for the 0600-0900 sampling interval. The carbon analyses at the two sites provide some possible explanations for these results. Tables II-2 and II-3 show the results of the total carbon (TC) and elemental carbon (EC) analyses for the CALTRANS and WLAPD sites, respectively. Cass and co-workers developed a carbon and lead emissions inventory in 1980 for the Los Angeles basin (Cass et al. 1982). The calculated highway signature for TC/EC/lead was 9.2/2.8/1 and for fully mixed basin air the TC/EC/lead ratio was 17.0/5.3/1. The average TC/EC/lead ratio for our study (18 pts) was 52/12/1 again showing the decreasing lead concentrations occurring in recent years.

The ratio of elemental carbon to organic carbon for the CALTRANS and WLAPD sites are given in Table II-2 and II-3, respectively. At WLAPD the ratio shows a large increase for 0600-0900 on March 10, while the same ratio at CALTRANS does not show this rise. During the 0300-0900 sampling intervals on March 10, the particulate lead at the two sites was very similar while the mutagen density difference increased to over 40 rev m^{-3}, with the WLAPD site downwind of I-405, higher in mutagen density. Since diesel sources show the highest ratio of elemental carbon to organic carbon in Cass' emission inventory for the Los Angeles basin (Cass et al. 1982) and diesel fuel is unleaded, a high mutagen density at the WLAPD site coinciding with a high ratio of elemental carbon to organic carbon

Table II-2. Carbon Analyses of Particulate Collected at the CALTRANS Site

Date and Sampling Interval (PST)	Total Carbon ($\mu g\ m^{-3}$)	Elemental Carbon ($\mu g\ m^{-3}$)	Ratio of Elemental Carbon to Organic Carbon[a]
March 9, 1983			
0600-0900	62	17	0.37
0900-1200	105	18	0.20
1200-1500	46	9	0.25
1500-1800	48	11	0.29
1800-2100	57	16	0.38
March 10, 1983			
2100-0000	70	20	0.40
0000-0300	132	25	0.24
0300-0600	23	6	0.35
0600-0900	32	9	0.40

[a]Organic carbon = total carbon-elemental carbon.

Table II-3. Carbon Analyses of Particulate Collected at the WLAPD Site

Date and Sampling Interval (PST)	Total Carbon ($\mu g\ m^{-3}$)	Elemental Carbon ($\mu g\ m^{-3}$)	Ratio of Elemental Carbon to Organic Carbon[a]
March 9, 1983			
0600-0900	73	24	0.48
0900-1200	36	9	0.33
1200-1500	51	5	0.11
1500-1800	24	5	0.24
1800-2100	32	9	0.37
March 10, 1983			
2100-0000	33	10	0.42
0000-0300	19	5	0.40
0300-0600	20	6	0.40
0600-0900	28	10	0.61

[a]Organic carbon = total carbon-elemental carbon.

would be consistent with the presence of increased diesel traffic on the freeway during the 0300-0900 period.

In a 1979 study at the Allegheny Mountain Tunnel on the Pennsylvania Turnpike, it was found that per kilometer of travel, heavy-duty diesels emitted POM which exhibited six times the mutagenicity of that emitted from spark-ignition vehicles (Pierson et al. 1983). In the present study, heavy-duty diesels would be expected to have a proportionately higher influence because of California's strict emission controls for vehicles equipped with spark-ignition engines. Therefore, diurnal variations in the number of diesel trucks on the freeway may be responsible for the lack of correlation between the lead and mutagen density differences upwind and downwind of the freeway.

Although collection of samples took place under conditions of light photochemical air pollution, the differences in mutagen density observed between the sites upwind and downwind of the freeway were often small in comparison to the "background" mutagen density. However, a maximum of 50 revertants m^{-3} greater mutagen density downwind of the freeway was observed. The magnitude of this freeway effect becomes more apparent when compared to typical literature values of mutagen densities in other urban areas (although we are aware that there are inter-laboratory variations in mutagenicity determinations) (Grafe et al. 1981). For example, for both New York City (Daisey et al. 1980) and Contra Costa County, CA (Flessel et al. 1981), the reported seasonally composited averaged mutagen densities (TA98, -S9) were less than 20 rev m^{-3}. On the other hand, values as high as 55 rev m^{-3} (TA98, -S9) have been measured at a narrow street with heavy traffic in Oslo, Norway (Møller et al. 1982), confirming the influence of traffic on observed mutagen densities.

E. Conclusions

- Results of this study at two sites on opposite sides of the I-405 freeway in the western end of the Los Angeles air basin revealed diurnal variations in the direct mutagenic burden of airborne particulates similar to those we previously obtained at a site just east of downtown Los Angeles near the intersection of the I-10 and I-605 freeways. Furthermore, the particulate mutagenicity levels observed at the WLA sites were

generally comparable to those found at the ELA site and, consistent with our earlier findings, were generally higher than those reported from other major urban airsheds throughout the world. For example, the "background" mutagen densities (i.e., those observed upwind of the freeway) measured during this study ranged from 30 to 100 rev m^{-3}.

- Our results also demonstrated that offshore air flows which generally drain the air basin between midnight and 0600 by an east to west movement can result in substantially elevated mutagen density levels at the western edge of the Los Angeles Basin.

- A major objective of this study was achieved when, during the period from 1200 to 2400 on March 9, 1983, concurrent measurements of particulate mutagen densities at the upwind and downwind sites took place under wind conditions favorable for distinguishing the effects of the freeway. The incremental burden of direct mutagens in respirable POM attributable to freeway traffic reached 50 rev m^{-3} during this period.

- Again consistent with our results for our previous ELA study, the significantly diminished response on the nitroreductase-deficient strain TA98NR vs. TA98 suggested that nitroarenes contributed significantly to the direct mutagenicity of POM collected at the WLA sites.

- As in the case of our ELA study, it was shown that over a 24-hr period, highs and lows in mutagen densities occurred over short time intervals (several hours) due to changes in emissions, mixing heights and wind speeds. These short-term peak mutagen densities clearly can be much higher than 24- or 12-hr averages reported in the literature.

- The results of this study will be presented at the Air Pollution Control Association (APCA) meeting in San Francisco, June 1984, and are presently being prepared for submission for publication in JAPCA.

III. CHARACTERIZATION OF CHEMICAL MUTAGENS IN AMBIENT PARTICULATE ORGANIC MATTER

A. Mutagenicities of Diesel Exhaust and Ambient Particulate Extracts

1. Introduction and Statement of the Problem

Extracts of both ambient (Pitts et al. 1975, Pitts et al. 1977, Talcott and Wei 1977) and diesel POM (Huisingh et al. 1978, Lewtas 1982) display direct mutagenicity towards Ames Salmonella strain TA98. Many of the compounds identified in diesel exhaust particulate, including oxygenated PAH derivatives and nitro-PAH are also present on respirable ambient air particles. Summaries from the literature concerning PAH derivatives identified in extracts of vehicle exhaust POM and in extracts of ambient POM, as well as the portion of the direct mutagenicity that has been attributed to these compounds, are given in Tables III-1 and III-2, respectively. While it is clear that nitro-PAH are important contributors to the mutagenicity of diesel particulate extracts, the significance of the contribution of nitro-PAH to the direct activity of ambient particulate extracts is not well established.

In our study of diurnal variations in mutagenicity of ambient POM in the CSCAB (Pitts et al. 1982a), ambient extracts showed mutagenic activities toward the nitroreductase-deficient Salmonella strain TA98NR that were 50-60% lower than observed with strain TA98. This was indirect evidence for the presence of nitroarenes (Rosenkranz and Poirier 1979, Mermelstein et al. 1981, Rosenkranz et al. 1981). In the present study, we utilized chromatographic separation techniques in conjunction with mutagenicity assays on TA98 and TA98NR to gain information on the identities of the direct mutagens in ambient POM extracts.

2. Research Objectives

The objectives of this research element were:

(1) To develop and utilize a method for chromatographic separation of mutagenic substances from the nonmutagenic sample components in a form suitable for further analysis.

(2) To carry out semi-preparative chromatographic separations of the base/neutral (B/N) fractions from diesel and ambient particulate extracts.

Table III-1. PAH Derivatives Identified in Vehicle Exhaust POM Extracts

Sample	Compounds Identified	% Mutagenicity of Extract Attributed to Compound	Reference
Diesel	Ketone, quinone, carboxaldehyde, hydroxy-PAH derivatives	a	Choudhury 1982
Diesel	1-Nitropyrene	2-8%	Gibson 1983
Gasoline	1-Nitropyrene	0.3-0.7%	
Diesel	1-Nitropyrene, 3-nitrofluoranthene	a	Nakagawa et al. 1983
	1,6- and 1,8-Dinitropyrene	43%	
Diesel	21-nitro-PAH including: ketone, dinitro and alkylnitro derivatives	19-25%	Nishioka et al. 1982
	1-Nitropyrene	3-15%	
Gasoline	1-Nitropyrene	0.2%	
Diesel	Nitro-PAH	33% of sample mutagenicity in this HPLC fraction	Pederson and Siak 1981
	1-Nitropyrene	(<5%)	
Diesel	6-Nitrobenzo(a)pyrene, 9-nitroanthracene	0	
	1-Nitropyrene	5	Pitts et al. 1982[b]
	Pyrene lactone	0	
Diesel	Pyrene-3,4-dicarboxylic acid anhydride	a	Rappaport et al. 1980
Diesel	1-Nitropyrene	16-31%	Salmeen et al. 1982
	Dinitro-pyrenes	21%	Schuetzle 1983
	Nitrohydroxypyrenes	3-29%	
Diesel	1-Nitropyrene	24%	Schuetzle et al. 1981
	Oxygenated PAH including: ketones, quinones, carboxaldehydes, acid anhydrides	a	Schuetzle et al. 1982
Diesel	50 nitro-PAH including: mononitro-PAH, alkyl nitro-PAH, dinitro-PAH, oxygenated nitro-PAH	a	Xu et al. 1982

[a]No mutagenicity data. [b]Updated on the basis of new mutagenicity data.

Table III-2. PAH Derivatives Identified in Ambient POM Extracts

Sample Site	Compounds Identified	% Mutagenicity of Extract Attributed to Compound	Reference
Urban & suburban (MI)	1-Nitropyrene, 6-nitrobenzo(a)pyrene	0.1-.0.7%	Gibson 1982, 1983
Industrial	1-Nitropyrene	8%	Gibson 1983
	Dinitropyrenes	20%	Gibson 1983
Prague, Czechoslovakia	3-Nitrofluoranthene, 6-nitrobenzo[a]pyrene	a	Jäger 1978
Duisburg, Germany	38 PAH derivatives including: ketones, quinones, anhydrides, coumarins and aldehydes	a	König et al. 1983
Rural (Denmark)	9-Nitroanthracene, 1-nitropyrene, 10-nitro-benz[a]anthracene	a	Nielsen 1983
St. Louis, MO	Nitronapthalenes, 9-nitroanthracene, 3-nitro-fluoranthene, 1-nitropyrene, arenecarbonitriles, ketone, quinone and anhydride PAH derivatives	a	Ramdahl et al. 1982
Elverum, Norway	PAH ketones and quinones	a	Ramdahl 1983
Santiago, Chile	1-Nitropyrene	<1%	Tokiwa et al. 1983
	Dinitropyrenes	b	

[a]No mutagenicity data.
[b]Although the % mutagenicity attributed to dinitropyrenes was not determined, it may have been substantial.

(3) To determine the mutagenicities of the chromatographic fractions on Salmonella strain TA98 and the nitroreductase-deficient strain TA98NR, thus allowing comparison between the resulting profiles for diesel and ambient particulate extracts.

(4) To compare the extracts of ambient POM obtained by Soxhlet extraction with dichloromethane (DCM) with that obtained by ultrasonic agitation in a 1:1:1 mixture of DCM, methanol and toluene.

3. Experimental Methods

The samples analyzed included the base/neutral (B/N) fractions of a dichloromethane (DCM) extract of ambient POM collected with the SAPRC megasampler (Fitz et al. 1983) located at the ARB Haagen-Smit Laboratory in El Monte on April 19-20, 1982 and a "supersolvent" (1:1:1, DCM:methanol:toluene) extract from the same sample. These were compared with a DCM extract of diesel particulate obtained from the U. S. EPA. Because the chromatographic behavior of the unknown mutagens in the samples provided useful qualitative information about their chemical structure and physical properties, an effort was made to determine quantitatively the recovery of both mass and direct-acting mutagenicity in the separated fractions.

a. Collection of Ambient Samples

Four identical ambient samples were collected simultaneously on 16-in. x 20-in. Pallflex T60A20 Teflon-impregnated glass fiber (TlGF) filters using the SAPRC megasampler (see Figure III-1) which has an inlet with a 50% cut point of 20 μm, limiting the particulate collection to the respirable range (Fitz et al. 1983). As detailed below, two of the filters from the April 19-20, 1982 POM collection were Soxhlet extracted with dichloromethane and two filters were extracted by ultrasonic agitation with supersolvent.

b. Extraction Procedures

DCM-B/N Fractionation. After Soxhlet extraction of the particle-laden filters for 24-hr with DCM, the extract was fractionated into Base-neutral (B/N) and acid fractions. To remove the acids present in the extract, each sample was extracted in a separatory funnel with five 25-mℓ portions of fresh 1N KOH, followed by a 15-mℓ portion of distilled water. The acids were recovered from the combined aqueous extracts by acidification to pH 2 with ~10 mℓ of concentrated H_3PO_4. The acidified

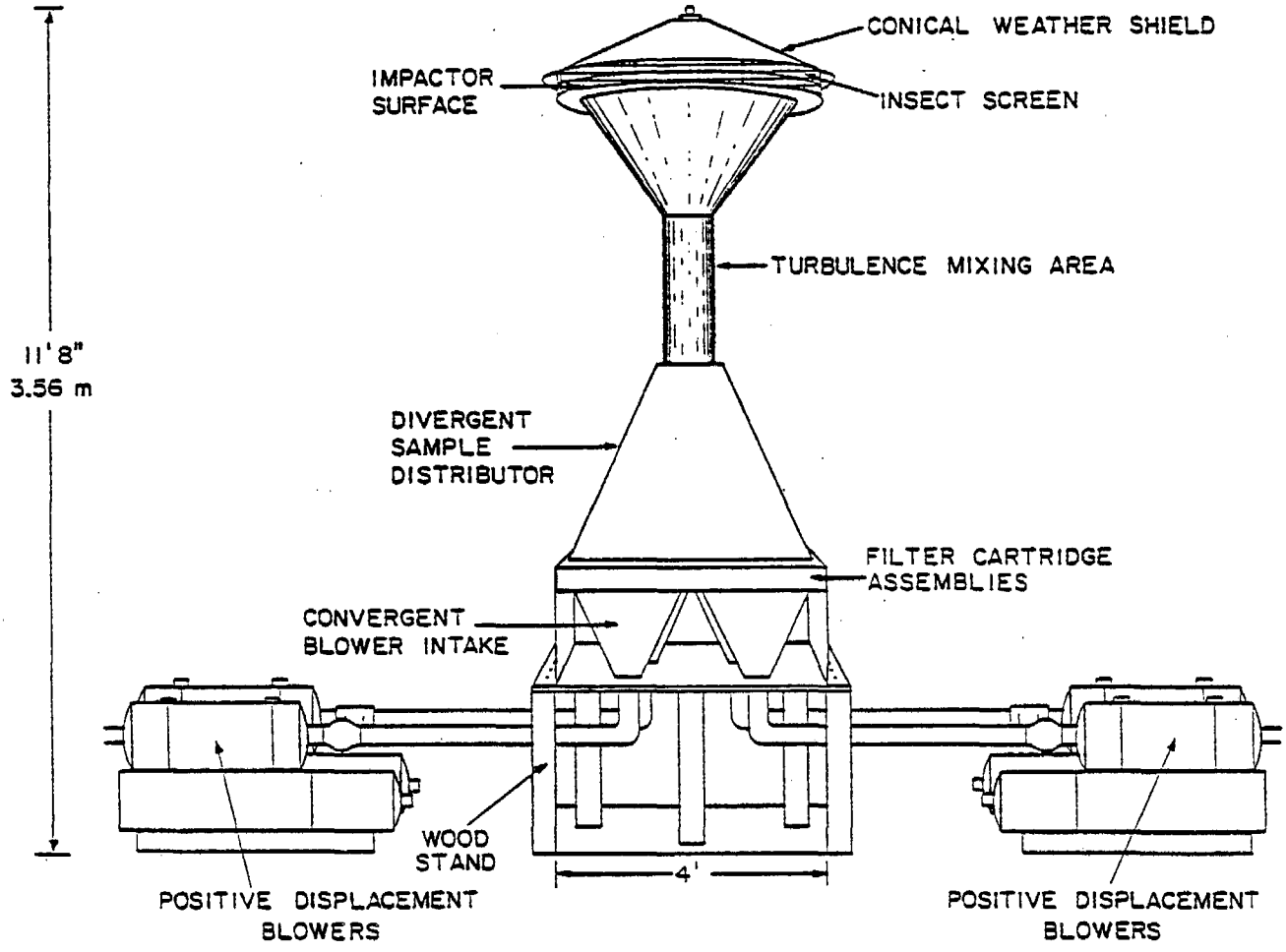

Figure III-1. The SAPRC ultra-high volume megasampler. Total flow 640 SCFM through four 16-in. x 20-in. filters.

solution was then extracted with 5-25 mℓ portions of DCM. The organic layer was finally extracted with 15 mℓ of distilled water. Water was removed from the B/N and acid fractions with 2 g each of Na_2SO_4. The extracts were subsequently filtered through 0.5 μm millipore filters, taken to dryness under a stream of pure nitrogen and weighed.

Supersolvent B/N Fractionation. The filters were extracted by ultra-sonic agitation with three 200 mℓ aliquots of "supersolvent" (1:1:1, DCM:methanol:toluene). The combined extracts were reduced in volume to ~5-10 mℓ by rotary evaporation under reduced pressure. The extracts were redissolved in 100 mℓ of water-saturated ether which had been treated with dimethyl sulfide to remove peroxides and 30 mℓ 1N KOH. The ether was extracted twice with 10 mℓ portions of KOH. The organic layer was finally extracted with 15 mℓ of distilled water. The acids were recovered as described above. Water was removed from the B/N and acid fractions, and the extracts were filtered, dried and weighed as detailed above.

c. High Performance Liquid Chromatography

The solid residues from the B/N fraction were dissolved in DCM and injected onto a 10 x 250 mm Altex Ultrasphere-Si column in 100-500 μℓ portions. The main column was protected by a guard column packed with Perisorb A. The separations were performed using a system composed of a Spectra-Physics Model 8100 liquid chromatograph, Model 4100 computing integrator, Model 8400 variable-wavelength absorbance detector, and an ISCO Model 2200 fraction collector.

The mobile phase program during elution is shown in Table III-3 at a flow rate of 3.0 mℓ min^{-1}. Fourteen 2.5-min fractions were collected after each injection. The system void volume (injector to fraction collector outlet) was about 8 mℓ, so that unretained material was collected in fraction 2 and the DCM and acetonitrile (ACN) solvent fronts were collected in fractions 7 and 11, respectively. The column was flushed with DCM and re-equilibrated with the initial mobile phase composition after each collection. A chromatogram was recorded during each run using a detection wavelength of 450 nm, in order to avoid photochemical degradation of the sample components while verifying proper system operation.

After collection, 20% of each fraction was transferred to an amber vial, evaporated to dryness and submitted for Ames assay by our

Table III-3. Mobile Phase Composition for POM Base/Neutral Fractions

Minutes	Solvent Composition at Injection Valve, %		
	n-Hexane	Dichloromethane	Acetonitrile
0.0	95	5	0
12.6	95	5	0
12.7	0	100	0
22.6	0	100	0
22.7	0	0	100
32.7	0	0	100

microbiological group. An additional 10% of each fraction was removed and recombined to form a composite sample. The remaining 70% of each fraction was evaporated to dryness, weighed and stored for further analysis. The total fraction mass and the weights of the samples sent to the microbiology laboratory were generally calculated from the 70% values. Some of the fraction masses were very small, and thus were not highly accurate. This does not affect the total activity figures, however.

d. Mutagenicity Bioassay

The Ames strain TA98 (Ames et al. 1975) and the nitroreductase-deficient strain TA98NR (Rosenkranz and Porier 1979, Rosenkranz et al. 1981) were used in these assays. Strain TA98 was used because it has been found to be most sensitive to airborne frameshift mutagens. Strain TA98NR is an isolate of TA98 which is deficient in the "classical" bacterial nitroreductase--the enzyme which catalyzes the bioactivation of most mononitroarenes to mutagenic metabolites. Thus, a lower response on this strain relative to TA98 indicates the probable presence of mononitroarenes in the sample. However, TA98NR is still sensitive to the extremely potent mutagens 1,8-dinitropyrene and 1,6-dinitropyrene because these compounds are activated by a second "nonclassical" reductase.

The mutagenicity bioassay procedures are described in more detail in Section II-C. Each sample was tested at a minimum of six different concentrations chosen to give a linear region of dose-response; when the sample size was not limiting, three replicates were run at each concentration and the average of the three responses was used to determine the

dose-response curve. The mutagenic potency (specific activity in revertants μg^{-1} of sample) was defined as the slope of the line determined by a least-squares regression on the linear region of dose-response.

4. Results and Discussion

Ambient Sample. The samples collected in El Monte on April 19-20, 1982 were used to compare the efficiencies of two different solvents and extraction methods in recovering mutagenic material from the respirable POM. The two methods were (a) sequential Soxhlet extraction with DCM and then ACN, and (b) ultrasonic agitation in a 1:1:1 mixture of DCM, methanol and toluene ("supersolvent"). The DCM and supersolvent B/N fractions were fractionated by HPLC in order to allow comparison of the distribution of activity in the two types of extracts and also to allow comparison with the diesel POM extract sample.

DCM Extract. A 26.57 mg aliquot of the B/N portion of the DCM extract was separated by HPLC as described in the experimental section above, and 19.41 mg were recovered in the separated HPLC fractions. The specific activities (-S9) of the DCM B/N fraction on TA98 and TA98NR were 6.4 and 3.8 revertants μg^{-1}, respectively, leading to calculated total activities of 1.7×10^5 and 1.0×10^5 revertants, respectively. The separated HPLC fractions were assayed only on TA98. Table III-4 gives the mass and activity data for each fraction.

The specific activity of the composited sample (which consisted of 10% of each HPLC fraction combined before Ames assay) toward TA98 (-S9) was 9.1 rev μg^{-1}. The sample masses listed for the composited samples in Table III-4 and the tables which follow have been normalized to 100% of the sample. The calculated total activity of the sample based on the composited samples' specific activity on TA98 is 1.8×10^5 revertants, in good agreement with the starting samples activity given above (1.7×10^5). In comparison, the sum of the total activity of the fourteen individual fractions was 2.1×10^5 revertants. All of this difference can be accounted for by the unexpectedly high total activity seen in fraction 2. The PAH expected to elute in this fraction, with the mobile phase program (Table III-3) employed in these separations, are not direct mutagens. Therefore, the activity in fraction 2 may have been the result of contamination.

Table III-4. April 19-20 Megasampler DCM-B/N HPLC Fractionation

Fraction Number	Sample Mass (μg)	Specific Activity (rev μg^{-1}) TA98 (-S9)	Total Activity x 10^{-3} (rev) TA98 (-S9)
1	161	0.29	0.05
2	8,416	3.8	32
3	47	190	9.1
4	213	23	4.9
5	211	11	2.2
6	281	13	3.7
7	83	268	22
8	410	27	11
9	324	10	3.3
10	1,211	9.6	12
11	5,170	12	60
12	1,493	13	20
13	827	20	17
14	563	19	10
Total, 1-14	-	-	207
Composite	19,410[a]	9.1	180
Unfractionated	26,570	6.4	170

[a]For the purpose of totaling figures for mass balances, sums in this table and those which follow have not been rounded to the appropriate number of significant figures.

Fraction 11 displayed the highest total direct activity. A second highly active fraction was fraction 7 which, under the HPLC conditions used, contained the nitro-PAH species. The total activity of fractions 3-14 are depicted in Figure III-2.

Supersolvent Extract. Two filters from the April 19-20 megasampler collection were extracted by ultrasonic agitation with "supersolvent" as described above. The total direct activity of the B/N fraction of the supersolvent extract on TA98 was 1.2 x 10^5 revertants, as compared to 1.7 x 10^5 revertants for the DCM-B/N extract. The DCM extraction, therefore, was more efficient for base/neutral mutagenic compounds than the supersolvent extraction.

A 36.12 mg aliquot of the B/N fraction of the supersolvent extract (an amount of extract derived from the same volume of air as the 26.57 mg

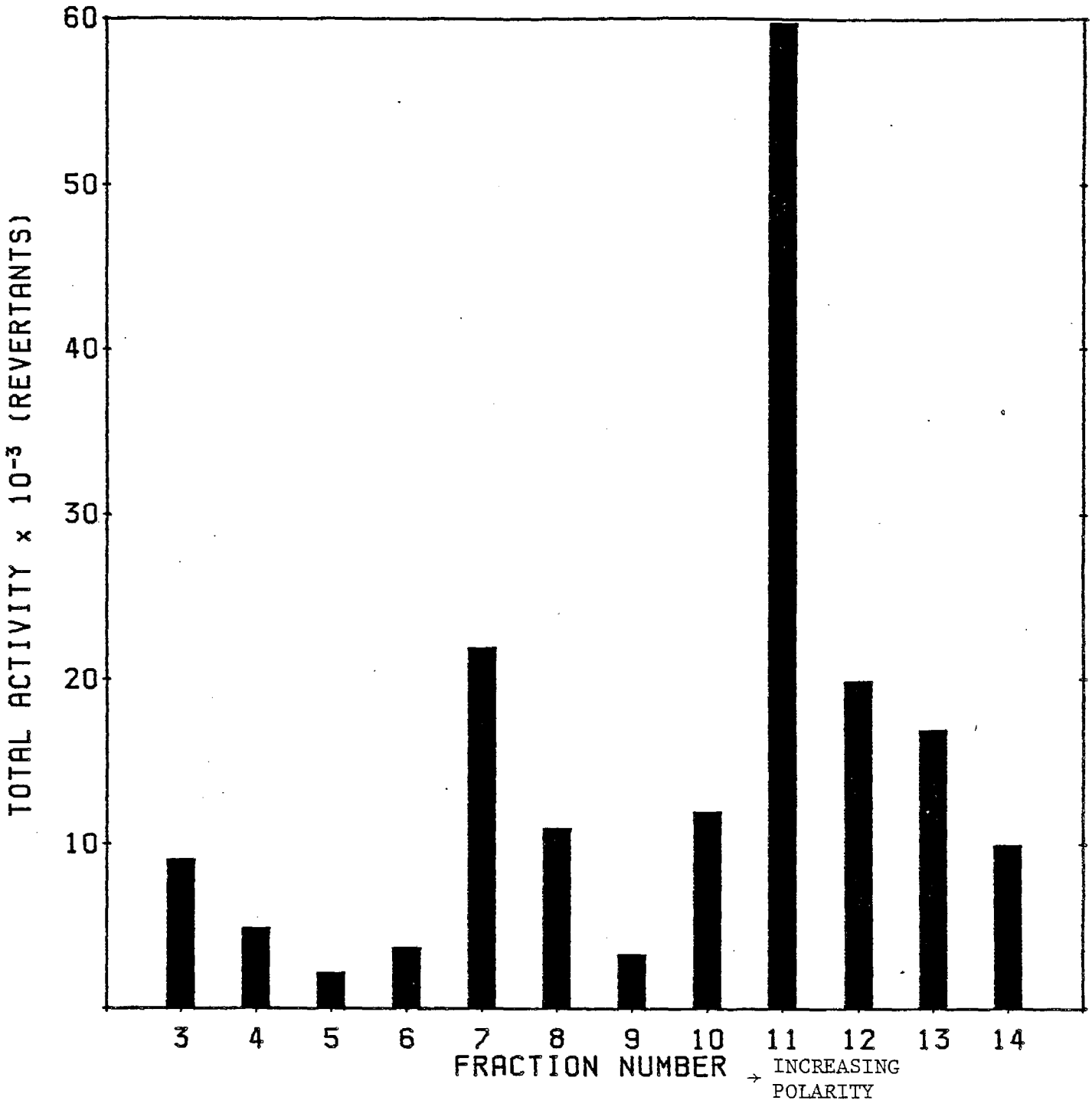

Figure III-2. Mutagenic activities (TA98, -S9) of HPLC fractions from April 19-20 megasampler ambient POM collection in El Monte, CA. Dichloromethane extract-HPLC fractionation of base/neutrals.

of DCM extract described above) was separated with HPLC as described in the experimental section. In this HPLC fractionation, the samples dedicated to the Ames test (20% of each HPLC fraction) were weighed directly. Because of the small mass of the weighed samples, some of the fraction masses were reported as zero, and the recovered mass could not be precisely calculated. A slow leak in the injector may also have contributed to the recovery of only about one-third of the starting mass. The total activities given in Table III-5 are calculated from the percent of the sample assayed (i.e., 20%).

The sums of the total activities of the fourteen HPLC fractions of 9.1×10^4 and 5.5×10^4 revertants on TA98 and TA98NR, respectively, were in excellent agreement with the corresponding values for the composite sample of 9.6×10^4 and 4.8×10^4 revertants, respectively. The data for individual fractions are given in Table III-5. The distribution of activity among the fractions is displayed in Figure III-3 and should not be affected by sample losses or unknown fraction masses. As was seen for the DCM extract, fraction 11 is again the most active fraction.

Figure III-3 shows that most fractions exhibited a diminished response on the nitroreductase-deficient strain, the maximum differential occurring for fraction 7, in which nitroarenes are expected to elute. Fraction 11 also showed a large difference in response to this pair of Salmonella test strains, which suggests that nitrosubstituted mutagens more polar than simple nitroarenes may be present.

For both the DCM and supersolvent extracts of the April 19-20 ambient samples, the combined activities of HPLC fractions 7-10, which contain any nitro-PAH, nitroalkyl-PAH or polynitro-PAH present (Pitts et al. 1982b) in the samples, was <50% of the total activity of the sample. The majority of the activity of these samples was in the polar fractions 11-14. The identities of these substances are unknown, but the observed differential response on TA98 and TA98NR suggest that nitroreduction may play a role in their activation to mutagenic species.

Diesel DCM Extract. The diesel exhaust particulate from which this extract was obtained was collected at the EPA Cincinnati Exposure Facility. The generation and collection system consisted of a Nissan six-cylinder diesel engine discharging through a muffled exhaust system into a dilution tunnel, which in turn discharged into a larger mixing chamber.

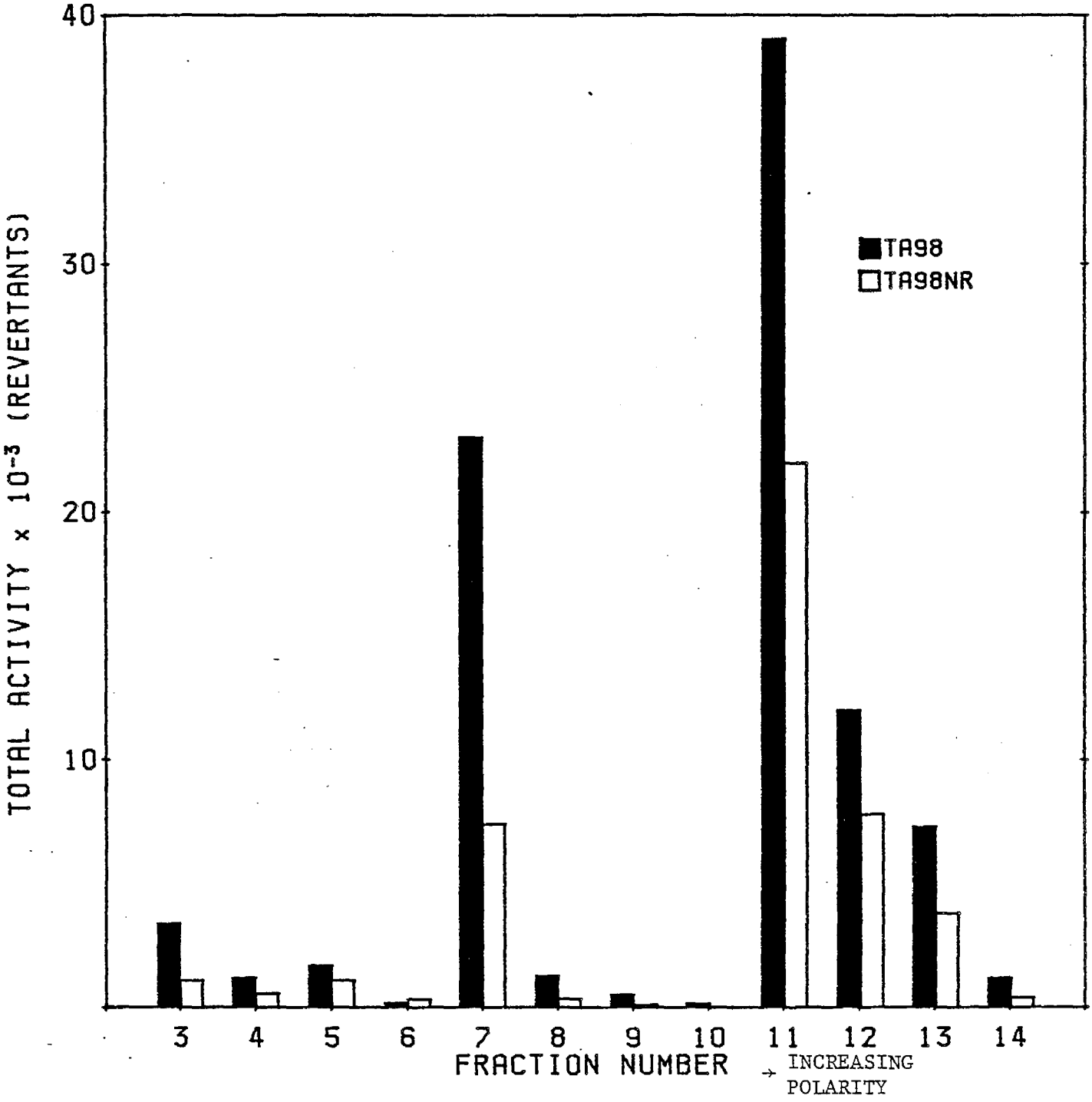

Figure III-3. Mutagenic activities (TA98, -S9 and TA98NR, -S9) of HPLC fractions from April 19-20 megasampler ambient POM collection in El Monte, CA. Supersolvent extract-HPLC fractionation of base/neutrals.

Table III-5. April 19-20 Megasampler Supersolvent-B/N HPLC Fractionation

Fraction Number	Sample Mass (μg)	Specific Activity (rev μg^{-1})		Total Activity x 10^{-3} (rev)	
		TA98 (-S9)	TA98NR (-S9)	TA98 (-S9)	TA98NR (-S9)
1	14	0	0	0	0
2	5,450	0.99	0.54	5.4	2.9
3	*	*	*	3.4	1.1
4	*	*	*	1.2	0.55
5	677	2.6	1.7	1.7	1.1
6	*	*	*	0.18	0.33
7	676	34	11	23	7.4
8	*	*	*	1.3	0.34
9	*	*	*	0.52	0.12
10	*	*	*	0.16	0
11	6,250	6.2	3.6	39	22
12	1,291	9.2	6.1	12	7.8
13	424	17	8.9	7.3	3.8
14	*	*	*	1.2	0.40
Total, 1-14	-	-	-	96	48
Composite	14,782	-	-	91	55
Unfractionated	36,120	3.4	2.4	120	86

*Mass unknown; total activity based on fraction of sample submitted for Ames assay.

In subsequent studies of a range of diesel engines, this Nissan engine was shown to be a particularly "dirty" engine. The hi-vol samples, collected on Teflon filters, were taken from the mixing chamber (Pitts et al. 1982c).

The B/N fraction was chromatographed under the conditions described above. A 37.17 mg sample was used, which had previously shown specific activities of 4.3 and 3.3 revertants μg^{-1} on TA98 and TA98NR, respectively. The total activities before chromatography were thus 1.6×10^5 TA98 revertants and 1.2×10^5 TA98NR revertants. The total sample mass recovered after chromatographic separation was 28.77 mg.

The dose-response curve of fraction 7 was discontinuous, with a value at the highest dose tested (20 μg $plate^{-1}$) which was more than twice as high as expected on the basis of the data from lower doses. Taking the total activity of this fraction from the slope of the response at low

doses, the recovered activity in the fractions was 1.7×10^5 revertants on TA98 and 9.1×10^4 revertants on TA98NR. The corresponding total activities for the composite sample were 2.7×10^5 and 1.3×10^5 revertants. The dose response curve for the composite sample was concave upward, as was the case for fraction 7. The data for this separation are presented in Table III-6 and the distribution of total activity in the fractions is given in Figure III-4.

Because the mutagenicity value for the composite sample of the diesel POM extract was somewhat higher than that for the total activity of the unfractionated sample, the separation procedure was repeated. The fraction weights and mutagenicity data are given in Table III-7. The activities of the first nine fractions, which include the nitro-PAH, were in good agreement with the values previously found for the individual fractions. Specific activities for the original and replicate unfractionated samples were also in good agreement (5.6 and 4.3 rev μg^{-1} on TA98, -S9) indicating that refrigerated storage of the diesel sample had not degraded its mutagenicity. The activity of the composite sample was lower than previously found, agreeing more closely with the unfractionated sample activity.

For this separation, the total activity of the fourteen HPLC fractions agreed very well with the composite activity. The more polar fractions (11-14) contained less activity than previously found. A comparison of the profiles of the fraction activities for the two diesel sample fractionations (Figures III-4 and III-5) clearly shows the dominance of fraction 7 in which the nitroarenes are found.

After the usual solvent cleaning of the HPLC column, a typical gradient was run and the solvent collected and submitted for Ames assay. Three fractions were collected corresponding to the three solvents employed. The hexane and dichloromethane solvent "blanks" were nonmutagenic, but the acetonitrile fraction contained mutagenic material. Apparently the polar mutagenic compounds were not quantitatively eluted from the column under the conditions employed. This explains the discrepancies observed in the polar mutagenic activity values for the two fractionations of the diesel sample. However, these differences did not appear to significantly affect the relative ranking of the fractions with the highest activity.

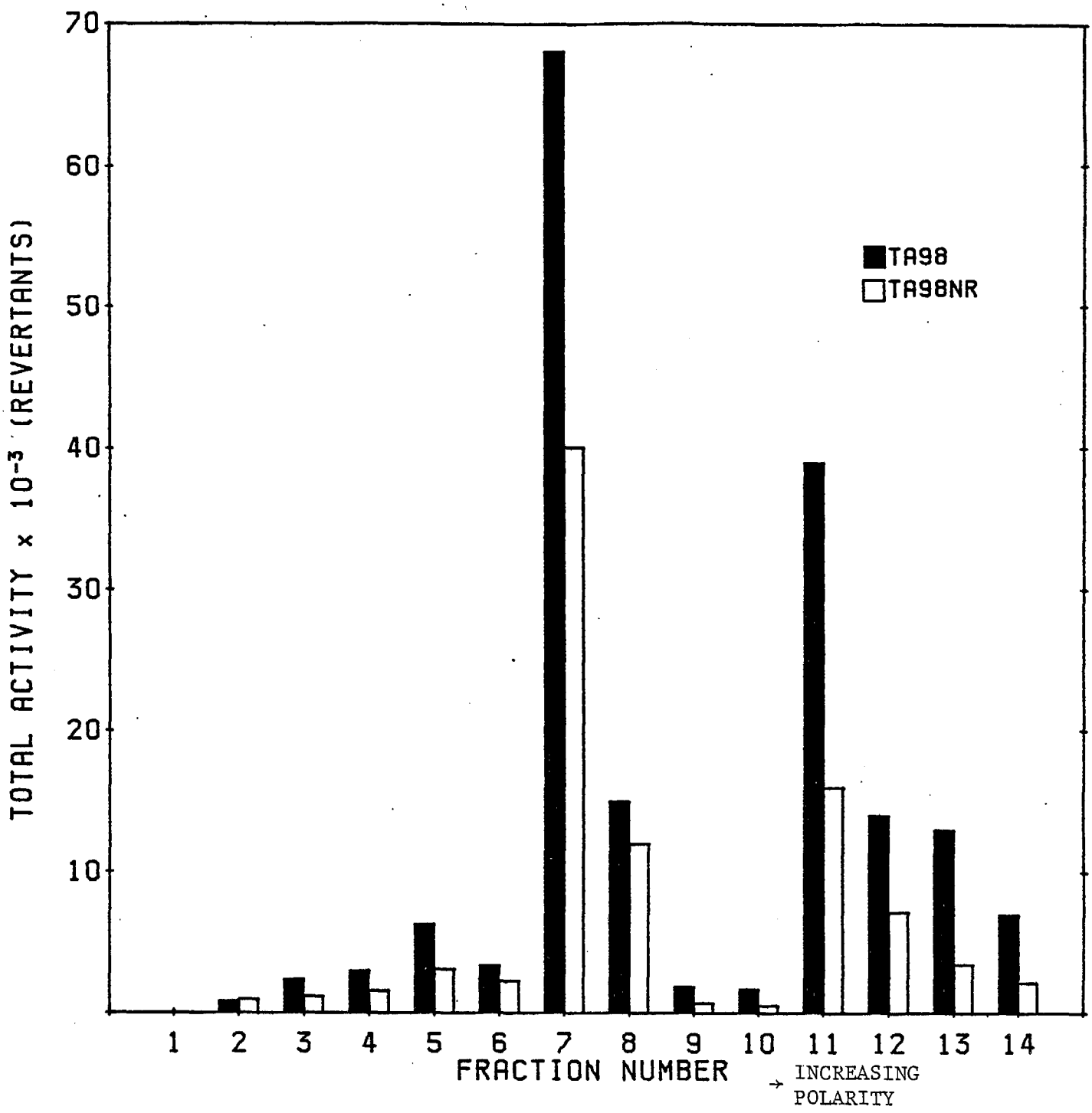

Figure III-4. Mutagenic activities (TA98, -S9 and TA98NR, -S9) of HPLC fractions of diesel POM extract. Dichloromethane extract-HPLC fractionation of base/neutrals.

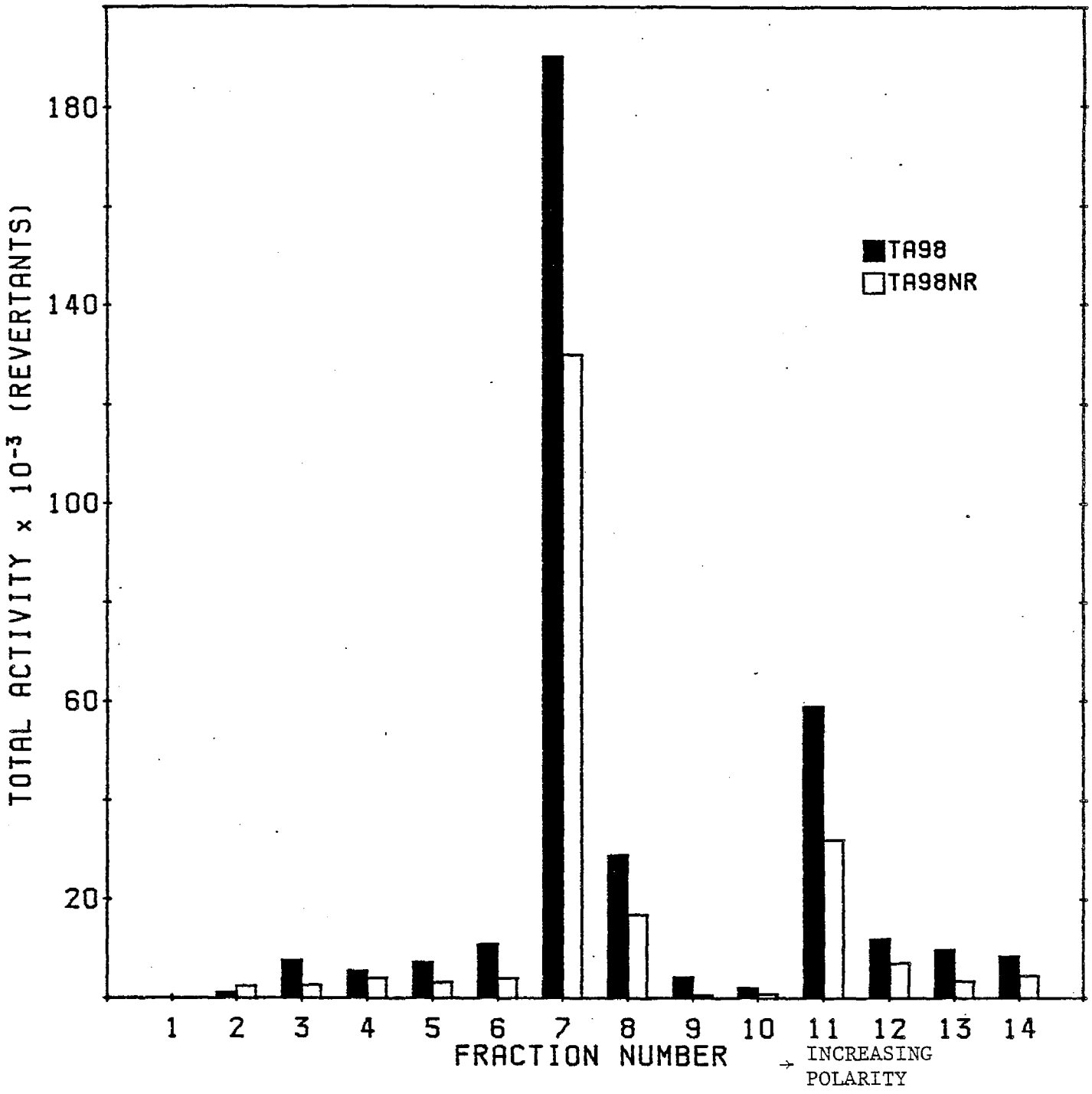

Figure III-5. Mutagenic activities (TA98, -S9 and TA98NR, -S9) of HPLC fractions of diesel POM extract. Dichloromethane extract-replicate HPLC fractionation of base/neutrals.

Table III-6. Diesel Exhaust Particulate DCM-B/N HPLC Fractionation

Fraction Number	Sample Mass (μg)	Specific Activity (rev μg^{-1})		Total Activity x 10^{-3} (rev)	
		TA98 (-S9)	TA98NR (-S9)	TA98 (-S9)	TA98NR (-S9)
1	7	0	0	0	0
2	22,297	0.038	0.044	0.85	0.98
3	494	4.8	2.4	2.4	1.2
4	139	21	12	3.0	1.6
5	221	28	14	6.3	3.1
6	220	16	11	3.4	2.3
7	1,085	63	37	68	40
8	380	39	31	15	12
9	191	10	3.8	1.9	0.72
10	1,291	0.83	0.44	1.7	0.57
11	1,757	22	9.3	39	16
12	226	61	32	14	7.2
13	239	55	15	13	3.5
14	224	31	10	7.0	22
Total, 1-14	-	-	-	170	91
Composite	28,771	9.5	4.5	270	130
Unfractionated	37,168	4.3	3.3	160	120

By comparing Figures III-2 and III-3 with III-4 and III-5 it can be seen that the distributions of mutagenic activity for the diesel and ambient samples were markedly different. For the diesel sample, fraction 7 which contained the nitroarenes was the most active fraction, while for the ambient sample fraction 11 was most active. Thus the ambient samples appear to be enriched in more polar mutagens relative to the diesel sample. It is interesting to note that this difference between diesel and ambient samples would be expected if atmospheric transformations play a role in changing the composition of direct mutagens in POM through oxidative or photooxidative reactions, leading to an increase in the proportion of polar mutagens, or to a decrease in the proportion of unsubstituted nitroarenes. However, the result can also be interpreted to mean that sources <u>other</u> than diesel emissions contribute to the predominance of polar mutagens in the ambient samples examined.

Table III-7. Diesel Exhaust Particulate DCM-B/N HPLC Replicate Fractionation

Fraction Number	Sample Mass (μ)	Specific Activity (rev μg^{-1})		Total Activity x 10^{-3} (rev)	
		TA98 (-S9)	TA98NR (-S9)	TA98 (-S9)	TA98NR (-S9)
1	197	0	0	0	0
2	60,976	0.02	0.04	1.2	2.4
3	1,279	6	2	7.7	2.6
4	235	24	17	5.6	4.0
5	265	28	12	7.4	3.2
6	296	39	14	11	4.1
7	3,540	55	36	190	130
8	752	38	23	29	17
9	314	14	2	4.4	0.63
10	310	7	3	2.2	0.93
11	4,533	13	7	59	32
12	1,215	10	6	12	7.3
13	708	14	5	9.9	3.5
14	573	15	8	8.6	4.6
Total, 1-14	-	-	-	350	210
Composite	75,193	4.6	4.1	350	310
Unfractionated	74,000	5.6	4.0	410	300
Total, 1-14*	-	-	-	170	91
Composite*	28,800	9.5	4.5	270	130
Unfractionated*	37,200	4.3	3.3	160	120

*Values from previous separation (Table III-6).

5. Conclusions

- There was good agreement for the mutagen distributions in an ambient POM sample between the base/neutral HPLC fractions of the DCM extract and the "supersolvent" extracts although the DCM was a more efficient solvent for extracting mutagenic compounds.
- Less than 50% of the activity of the base/neutral portion of an ambient POM extract was found in the HPLC fractions in which mono- and polynitro-PAH or nitroalkyl-PAH would elute.

- The ambient POM samples were enriched in polar mutagens relative to a diesel POM sample.
- The observed difference in response on TA98 and TA98NR indicates that some of the polar mutagens present in the ambient POM may be substituted NO_2-PAH.

B. Filter and Sampler Comparison Study

1. Introduction and Statement of the Problem

Because chemical analyses for nitroarenes and other mutagenic compounds require large quantities of particulate to provide sufficient sample, we previously constructed an ultra-high volume sampler ("megasampler") (Fitz et al. 1983). This device has an inlet with a 50% cut point of 20 μm limiting the particulate collection to the respirable range. The total flow is 640 SCFM through four 16 in. x 20 in. filters (see Figure III-1). The same face velocity as a standard hi-vol is maintained while the sampling rate is 16 times larger (640 cfm vs. 40 cfm). Since a proposed CARB-supported field study would involve using the megasampler to collect ambient particulate in El Monte for comparison with particulate collected in Riverside employing standard hi-vol apparatus, a study was done to ensure the equivalency of these two methods of particulate collection with respect to determining mutagen densities (revertants per cubic meter of air sampled).

Teflon-impregnated glass fiber (TIGF) filters were used to minimize sample degradation (Lee et al. 1980). Two types of TIGF filters have been used in previous studies, Pallflex T60A20 and Pallflex TX40HI20. The manufacturer-reported collection efficiencies based on dioctylphthalate tests are given in Table III-8 (the standard face velocity employed in our collections was 36 cm sec^{-1}). The mutagenic activity of ambient POM extracts has been found to predominate in the respirable sub-micron particles (Talcott and Harger 1980). Therefore, we operated hi-vols using both these types of filters to see if the better efficiency of the TX40HI20 filters for smaller particles would result in higher mutagen densities.

During this study, in addition to measuring direct activity on strains TA98 and TA98NR, a third strain TA98/1,8-DNP_6, was employed. Strain TA98NR is an isolate of TA98 which is deficient in the "classical" bacteria nitroreductase, the enzyme which catalyzes the bioactivation of

Table III-8. Collection Efficiencies of Pallflex T60A20 and TX40HI20 TIGF Filters Based on Dioctylphthalate Tests

T60A20 Filter

V (cm sec^{-1})	29.0	40.6
D_p (μm)	Collection Efficiency, Percent	
0.035	85.3	70
0.10	81.6	62
0.30	97.8	93.6
1.0	99.8	98.1

TX40HI20 Filter

V (cm sec^{-1})	27.1	91.6
D_p (μm)	Collection Efficiency, Percent	
0.035	99.7	98.9
0.10	99.8	99.9
0.30	>99.99	>99.99
1.0	>99.99	>99.99

most mono-nitroarenes to mutagenic metabolites. Thus, a lower response on this strain relative to TA98 indicates the probable presence of mononitroarenes in the sample. However, TA98NR is still sensitive to the potent mutagens 1,8-dinitropyrene and 1,6-dinitropyrene because these compounds are activated by a second "nonclassical" reductase. Strain TA98/1,8-DNP_6 is deficient in this enzyme and consequently is less sensitive to these dinitropyrenes. Thus, a lower response for TA98/1,8-DNP_6 relative to TA98NR and TA98 may indicate the presence of dinitropyrenes in the sample (Rosenkranz et al. 1981, 1982).

2. Research Objectives

The objectives of this study were:

(1) To compare the mutagen densities, mutagen loadings and specific activities of ambient samples collected with the SAPRC mega-

sampler with those of samples collected simultaneously with a standard hi-vol apparatus and a hi-vol apparatus with a 10 μm size cut-off inlet.

(2) To compare the mutagen densities, mutagen loadings and specific activities of ambient samples collected with T60A20 TIGF filters with those of samples collected simultaneously utilizing TX40HI20 TIGF filters.

(3) To utilize three Salmonella strains (TA98, TA98NR and TA98/1,8-DNP_6) for determining direct mutagenic activity in order to examine the contributions of mono- and dinitroarenes to the ambient POM extract activity.

3. Experimental Methods

On August 3, 1983, the megasampler and eight hi-vol samplers located at El Monte were run in parallel for 24 hours. The four time intervals chosen for the planned 1983-1984 studies of ambient particulate at a central and downwind receptor site were used: 0600-1000, 1000-1500, 1500-2100 and 2100-0600 (see Table III-9). These intervals were selected based on earlier SAPRC-ARB time-resolved studies of the variations in particulate mutagenicities and concentrations of gaseous co-pollutants carried out simultaneously in downtown Los Angeles and Riverside in September 1980 (Pitts et al. 1982b).

The megasampler was operated with T60A20 TIGF filters, as were four standard hi-vols, two with and two without 10 μm size selective inlets (General Metal Works GMW-9000). Four additional hi-vols (two with and two without inlets) were operated with TX40HI20 TIGF filters which, as described above, have a higher collection efficiency for particles <1 μm than have the T60A20 filters. All filters were prewashed by Soxhlet extraction with dichloromethane and methanol (24 hours each solvent).

The particulate weights were determined after equilibration at 50% RH and 70°F. The duplicate hi-vol collections were combined before extraction, and a single quadrant (equivalent to four standard hi-vols) of the megasampler was extracted for each of the four sampling intervals. The residues from 24-hr Soxhlet extractions with dichloromethane followed by acetonitrile were combined and assayed for mutagenicity. The mutagen assay procedures are given in Section II-C.

Table III-9. Sampling Schedule

Time Interval, PST	Reason for Interval
0600-1000	Maximum emission of primary pollutants in El Monte and Riverside and period of peak mutagenicities of POM
1000-1500	Local photochemical formation of O_3 and other secondary pollutants in El Monte and Riverside; minima in particle mutagenicities
1500-2100	Influx of "aged" air mass containing O_3 and other secondary pollutants in Riverside; second maxima in primary pollutant concentrations and mutagen densities
2100-0600	Night sample of primary pollutants in El Monte and Riverside

4. Results and Discussion

The results are presented in Table III-10. As expected, the equivalent loadings (mg per 1000 m^3 sample) for the hi-vols without size selective inlets were consistently higher than for the hi-vols with 10 μm inlets or for the megasampler with an effective 20 μm inlet (the single exception is the megasampler equivalent loading for the 2135-0600 sampling interval). The lack of any consistent trend in the mutagen densities (revertants m^{-3}) between the hi-vols with and without size selective inlets indicates that the mutagenic material is associated with the sub-μm particles, as expected (Talcott and Harger 1980). The specific mutagenicities (revertants per μg extract) also were similar for hi-vols with and without size selective inlets and the megasampler, indicating that the extractable organic material is also associated with sub-μm particles and that the material collected by the megasampler has equivalent mutagenicity to that collected with standard hi-vols.

The equivalent loadings of the TX40HI20 filters were consistently slightly higher (by up to 8%) than for the corresponding samples using the T60A20 filters. The lack of a similar trend in the mutagen densities and

Table III-10. Comparison of Mutagenicities of Samples Collected with Standard Hi-Vol Samplers vs. the SAPRC Megasampler for Two Types of Filters and With and Without Size Selection

A. Sampling Interval: 0600-1000

	T60A20			TX40HI20	
	No Inlet	10 μm Inlet	Mega-sampler (20 μm Inlet)	No Inlet	10 μm Inlet
Sample Code	A-85	A-87	A-89	A-86	A-88
Hi-Vol #'s	7,2	5,6	Mega	15,10	X,11
Mg loading	73.3	57.0	113 ± 10	76.8	59.3
Equivalent loading[a]	139	105	103 ± 10	143	110
Mg extracted	21.1	18.2	31.4	28.6	24.7
Equivalent extracted[a]	40	34	b	53	46
% Extractable	29	32	b	37	42
TA98					
Rev m^{-3}	140	130	b	170	150
Rev μg^{-1} extract	3.6	3.9	3.4	3.3	3.2
Rev mg^{-1} loading	1,000	1,200	b	1,200	1,300
Controls	**2-Nitrofluorene**		**Quercetin**		**1,8-Dinitropyrene**
Rev μg^{-1}	400		8.6		930,000
TA98NR					
Rev m^{-3}	74	62	b	85	72
Rev μg^{-1} extract	1.9	1.9	1.6	1.6	1.6
Rev mg^{-1} loading	530	590	b	590	660
Controls	**2-Nitrofluorene**		**Quercetin**		**1,8-Dinitropyrene**
Rev μg^{-1}	47		11		1,100,000
TA98/1,8-DNP$_6$					
Rev m^{-3}	25	19	b	27	22
Rev μg^{-1} extract	0.62	0.56	0.51	0.50	0.47
Rev mg^{-1} loading	180	180	b	190	200
Controls	**2-Nitrofluorene**		**Quercetin**		**1,8-Dinitropyrene**
Rev μg^{-1}	62		7.3		18,000

(continued)

Table III-10. (continued) - 2

B. Sampling Interval: 1035-1500

	T60A20			TX40HI20	
	No Inlet	10 μm Inlet	Mega-sampler (20 μm Inlet)	No Inlet	10 μm Inlet
Sample Code	A-90	A-92	A-94	A-91	A-93
Hi-Vol #'s	10,15	11,X	Mega	7,2	6,5
Mg loading	116.8	89.3	164.0	121.2	97.4
Equivalent loading[a]	197	150	138	209	162
Mg extracted	35.9	43.2	57.8	44.9	b
Equivalent extracted[a]	61	72	48	77	b
% Extractable	31	48	35	37	b
TA98					
Rev m^{-3}	89	71	53	75	62
Rev μg^{-1} extract	1.5	1.0	1.1	1.0	b
Rev mg^{-1} loading	450	470	390	360	380
Controls	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	440	14	1,200,000		
TA98NR					
Rev m^{-3}	38	24	19	26	25
Rev μg^{-1} extract	0.63	0.33	0.40	0.33	b
Rev mg^{-1} loading	190	160	140	120	150
Control	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	70	9.8	1,400,000		
TA98/1,8-DNP_6					
Rev m^{-3}	7.9	5.0	7.3	12	8.3
Rev μg^{-1} extract	0.13	0.07	0.15	0.15	b
Rev mg^{-1} loading	40	34	53	56	51
Controls	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	84	10	18,000		

(continued)

Table III-10. (continued) - 3

C. Sampling Interval: 1535-2100

	T60A20			TX40HI20	
	No Inlet	10 μm Inlet	Mega-sampler (20 μm Inlet)	No Inlet	10 μm Inlet
Sample Code	A-95	A-97	A-99	A-96	A-98
Hi-Vol #'s	7,2	6,5	Mega	10,15	11,X
Mg loading	76.0	64.1	116.1	83.6	67.2
Equivalent loading[a]	106	87	79	115	92
Mg extracted	21.1	19.1	32.3	25.1	21.7
Equivalent extracted[a]	30	26	22	35	30
% Extractable	28	30	28	30	32
TA98					
Rev m^{-3}	71	86	72	88	69
Rev μg^{-1} extract	2.4	3.3	3.3	2.6	2.3
Rev mg^{-1} loading	660	990	910	770	750
Controls	**2-Nitrofluorene**	**Quercetin**	**1,8-Dinitropyrene**		
Rev μg^{-1}	411	12.3	917,000		
TA98NR					
Rev m^{-3}	27	33	29	35	31
Rev μg^{-1} extract	0.91	1.3	1.3	1.0	1.0
Rev mg^{-1} loading	250	380	370	310	340
Controls	**2-Nitrofluorene**	**Quercetin**	**1,8-Dinitropyrene**		
Rev μg^{-1}	77	12	1,100,000		
TA98/1,8-DNP$_6$					
Rev m^{-3}	11	9.6	7.9	9.7	10.4
Rev μg^{-1} extract	0.37	0.37	0.36	0.28	0.35
Rev mg^{-1} loading	100	110	100	84	110
Controls	**2-Nitrofluorene**	**Quercetin**	**1,8-Dinitropyrene**		
Rev μg^{-1}	78	6.6	15,000		

(continued)

Table III-10. (continued) - 4

D. Sampling Interval: 2135-0600

	T60A20			TX40HI20	
	No Inlet	10 μm Inlet	Mega-sampler (20 μm Inlet)	No Inlet	10 μm Inlet
Sample Code	A-100	A-102	A-104	A-101	A-103
Hi-Vol #'s	15,10	11,X	Mega	7,2	6,5
Mg loading	108.7	94.2	247.2	109.5	102.3
Equivalent loading[a]	96	83	107	99	89
Mg Extracted	44.0	40.8	68.1	46.8	44.1
Equivalent extracted[a]	39	36	29	42	39
% Extractable	40	43	28	43	43
TA98					
Rev m^{-3}	74	71	65	99	94
Rev μg^{-1} extract	1.9	2.0	2.2	2.3	2.4
Rev mg^{-1} loading	770	850	610	1000	1000
Controls	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	410	12	920,000		
TA98NR					
Rev m^{-3}	42	36	31	50	45
Rev μg^{-1} extract	1.1	1.0	1.1	1.2	1.2
Rev mg^{-1} loading	440	430	290	510	500
Controls	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	77	12	1,100,000		
TA98/1,8-DNP_6					
Rev m^{-3}	15	13	12	19	20
Rev μg^{-1} extract	0.38	0.35	0.41	0.48	0.53
Rev mg^{-1} loading	150	150	110	190	230
Controls	2-Nitrofluorene	Quercetin	1,8-Dinitropyrene		
Rev μg^{-1}	78	6.6	15,000		

[a]For a 1000 m^3 sample.
[b]No data.

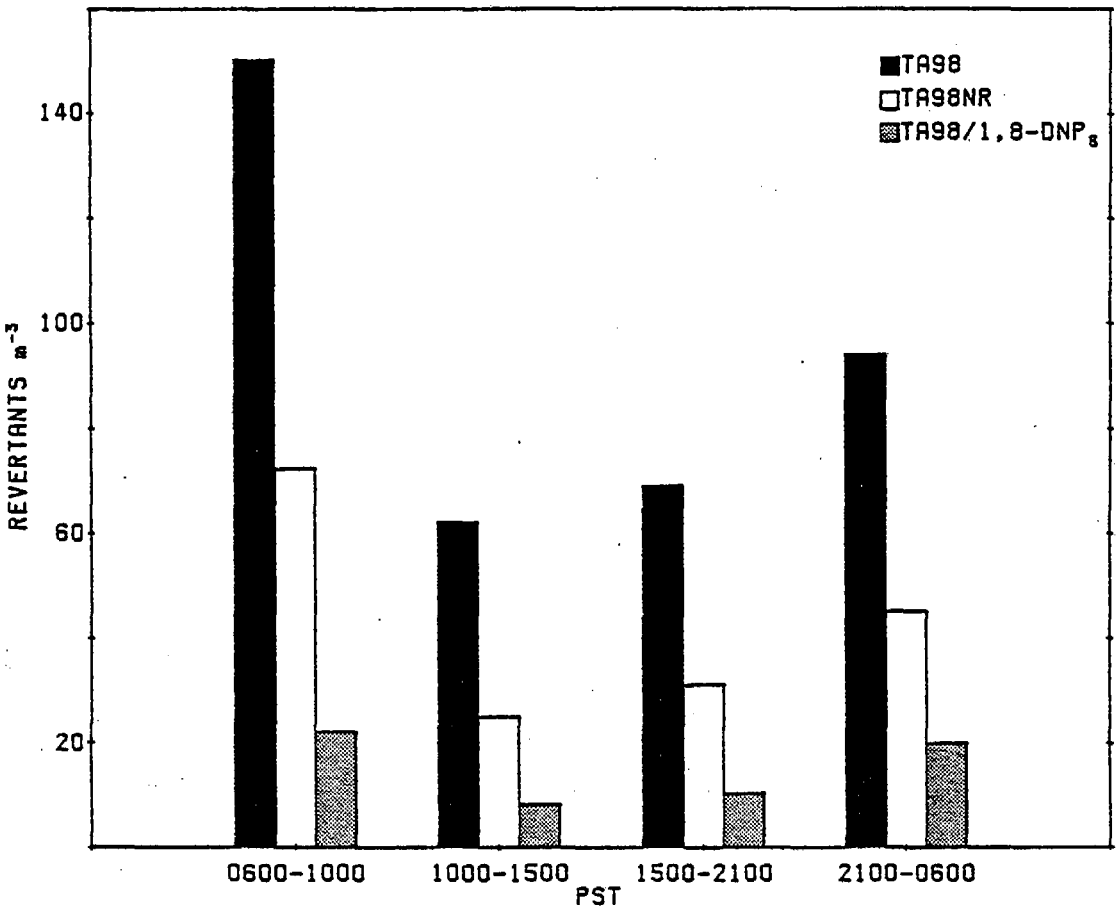

Figure III-6. Mutagen densities of respirable particles collected on Teflon-impregnated glass fiber (TIGF) filters at El Monte, CA, August 3, 1983, for four consecutive time periods (24 hr) with two hi-vol samplers. Responses are to strains TA98, TA98NR and TA98/1,8-DNP_6.

specific mutagenicities can be attributed to the variability inherent in the mutagenicity assay. The more efficient TX40HI20 filters will be used for future particulate collections.

The reduced activity of the ambient samples on strains TA98NR relative to TA98 indicated the presence of nitroarenes in these samples (see Figure III-6). The reduced activity of the ambient samples on strain TA98/1,8-DNP_6 relative to TA98 and TA98NR indicated that dinitropyrenes may have been present in these ambient samples. Dinitro-PAH could be important contributors to the observed mutagen densities; for example, note the high activity (shown in Table III-10) of the 1,8-dinitropyrene used as a positive control in these assays.

5. Conclusions

- Hi-vols with and without size selective inlets (10 μm cut-off) gave equivalent mutagen densities and specific activities confirming that the mutagenic material is associated with the sub-μm particles.
- Ambient samples collected with the SAPRC megasampler exhibit mutagen densities and specific activities that are equivalent to those for samples collected with a standard hi-vol sampler.
- No significant differences were found for mutagen densities or specific activities between samples collected on T60A20 or TX40HI20 TIGF filters.
- The reduced activity of the ambient samples on strains TA98NR relative to strain TA98 indicated the presence of nitroarenes in these ambient samples.
- The reduced activity of the ambient samples on strain TA98/1,8-DNP_6 relative to TA98 and TA98NR indicated that dinitropyrenes may have been present in these ambient samples.

IV. EXPLORATORY STUDIES IN REAL AND SIMULATED ATMOSPHERES OF THE TRANSFORMATIONS OF CHEMICAL MUTAGENS ADSORBED ON AMBIENT POM

A. Mutagenicity of Ambient POM at Redlands, CA and Whitewater, CA

1. Introduction and Statement of the Problem

Since particulate emissions are known to remain aloft for periods up to a week, there is ample time for POM to undergo chemical reactions with gaseous co-pollutants. Thus a possible result of transport of particulate matter may be that long-range downwind receptor sites are impacted by POM which is qualitatively and quantitatively different in chemical composition and bioactivity than that of the initially formed aerosol.

Indeed, related studies in our laboratory have shown that PAH adsorbed on hi-vol filters and exposed to air containing ambient levels of gaseous pollutants such as NO_2 (with traces of nitric acid) and O_3 readily form directly mutagenic compounds (Pitts et al. 1978, 1980). Two recent reviews summarize our present state of knowledge concerning transformations of PAH present in ambient POM (Pitts 1983, Nielsen et al. 1983), and discuss the phenomenon of artifactual formation of mutagens during sampling (Pitts et al. 1978, Pitts 1979, Pitts et al. 1980, Lee et al. 1980, Brorström et al. 1983, Grosjean 1983, Grosjean et al. 1983, Fitz et al. 1984).

2. Research Objectives

The overall objective of this study was to investigate the feasibility of field studies designed to determine the changes in mutagenic POM during west-to-east transport through the Los Angeles air basin. Specific objectives were:

(1) To measure and compare the mutagenicity of ambient POM associated with the Los Angeles urban plume at Redlands, CA and at a long-range receptor site, Whitewater, CA.

(2) To compare these observed mutagen densities with those observed previously at other sites in the basin.

3. Experimental Methods

Two sites east and west of the San Gorgonio pass were selected for this study. In this area, during periods of westerly winds the basin is ventilated to the east and the urban air mass is constrained to follow the pass between two high mountain ranges. Because there are relatively few stationary emission sources of POM along this trajectory, the

potential for acquiring significant amounts of primary aerosol during transit is minimized.

Sampling was conducted at the South Coast Air Quality Management District (SCAQMD) station on the University of Redlands campus, and at the Southern California Edison (SCE) wind power experimental station near Whitewater, these sampling points being separated by ~60 km (Figure IV-1). The Redlands SCAQMD station is located south of the University maintenance yard in a large open field, and is about 1 km north of Interstate 10 (I-10). The SCE facility in Whitewater is 1 km east of State Highway 64 and 3 km north of I-10. Local sources of particulate emissions between the two sites include I-10, the cities of Banning and Beaumont and a portion of the city of Redlands.

Sampling was carried out on October 6, 1982, a day of clear skies and moderate temperatures. The air pollution parameters monitored at each site were β_{scat}, ozone and nitrogen oxides. An instrumented van at the wind power station provided all air quality data for the Whitewater site. A nephelometer and a chemiluminescence nitrogen oxides analyzer were added to the instrumentation at the SCAQMD station in Redlands. Ozone levels were obtained from the SCAQMD.

Two hi-vol (1.1 m^3 min^{-1}) particulate samplers were installed at each site, at heights above the ground of 5 m and 2 m at Redlands and Whitewater, respectively. One hi-vol at each site was fitted with a pre-extracted (CH_2Cl_2) Teflon-impregnated glass fiber filter, while the other sampler was fitted with a pre-fired (450°C for 13 hr) quartz fiber filter for elemental carbon analyses (carried out by Dr. James Huntzicker at the Oregon Graduate Center). All hi-vols were operated with size-selective inlet systems (General Metal Works, East Cleveland, OH) designed to exclude nonrespirable particulate matter (mean effective aerodynamic diameter of greater than 10 micrometers).

4. Results and Discussion

The air quality data obtained at the two sampling sites during the collection periods are given in Table IV-1. At both sites NO concentrations were always below the detection limits of 5 ppb during these sampling periods. As seen from the O_3 and/or β_{scat} data, polluted air masses reached Redlands and Whitewater at approximately 1500 PST and 1700 PST, respectively.

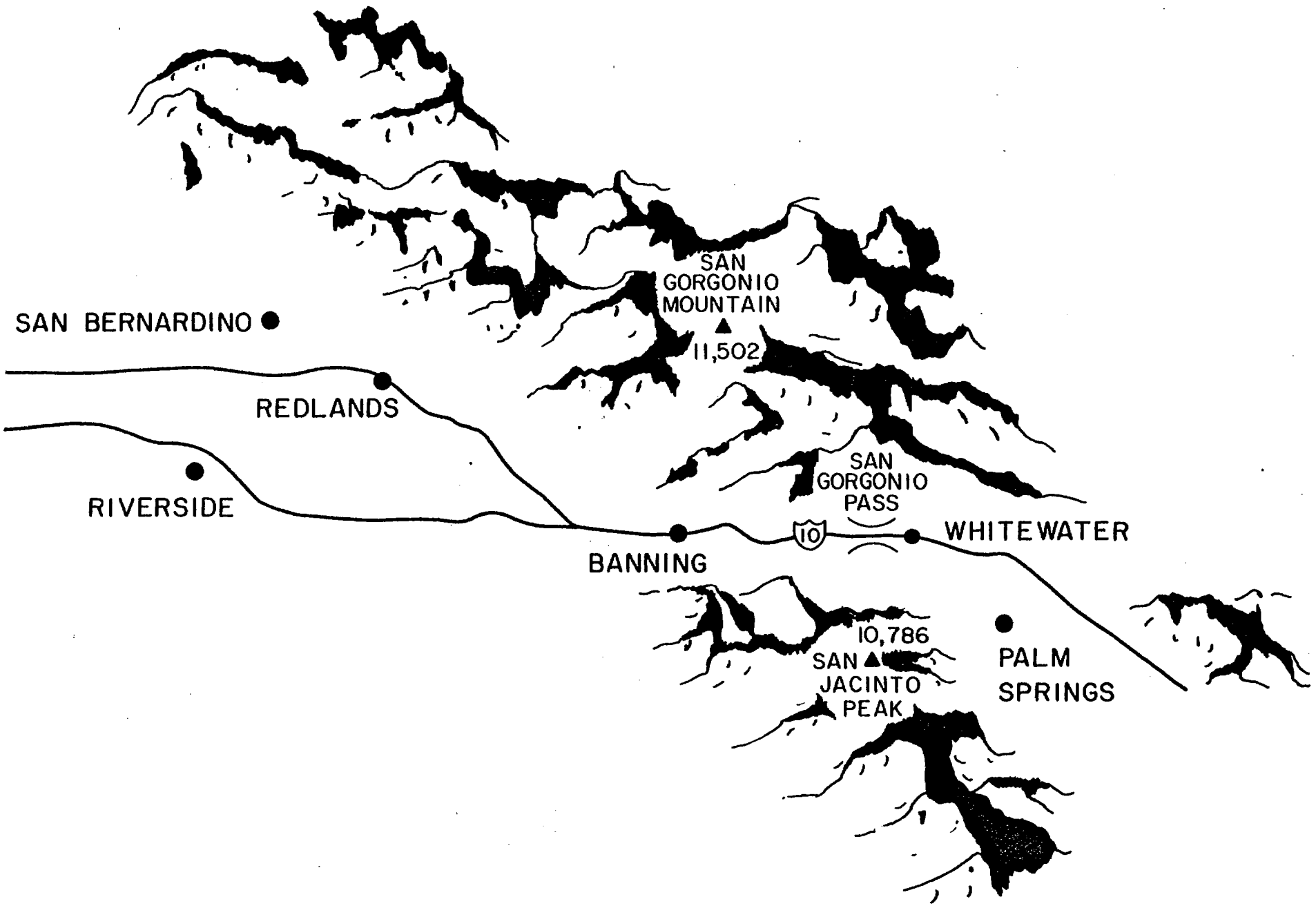

Figure IV-1. Location of the sampling sites Redlands, CA and Whitewater, CA. Redlands is 64 miles east of downtown Los Angeles.

Table IV-1. Air Quality Data for Particulate Sampling Period, October 6, 1982

Time (PST)	Whitewater NO_2 (ppb)	Whitewater O_3 (ppb)	Whitewater β_{scat} x 10^{-4} m^{-1}	Redlands NO_2 (ppb)	Redlands O_3 (ppb)	Redlands β_{scat} x 10^{-4} m^{-1}
0800-0900	a	a	a	5	40	b
0900-1000	a	a	a	20	40	0.2
1000-1100	a	a	a	15	60	0.2
1100-1200	a	a	a	15	70	0.2
1200-1300	15	17	0.2	15	70	0.3
1300-1400	15	17	0.2	10	80	0.3
1400-1500	15	22	0.2	10	70	0.3
1500-1600	15	34	0.3	35	150	5.6
1600-1700	20	31	0.6	45	150	4.3
1700-1800	b	20	1.4	55	140	5.6
1800-1900	b	17	2.0	85	60	a
1900-2000	b	24	b	a	40	a
2000-2100	40	42	b	a	a	a

[a]No data taken.
[b]No data available.

The particulate loading and mutagenicity data from the Teflon-impregnated filters and elemental carbon values (from the quartz fiber filters) are given in Table IV-2. At a given site, very similar particulate loadings were collected on the Teflon-impregnated and quartz fiber filters (54.5 mg and 56.2 mg, respectively, at Redlands and 24.1 mg and 26.1 mg, respectively, at Whitewater).

The TA98 mutagen densities of 32 rev m^{-3} and 17 rev m^{-3} for Redlands and Whitewater, respectively (Table IV-2), are in the range of to those obtained in Riverside in 1980 and 1981 (Pitts et al. 1981). However, these mutagen densities are factors of 2-3 lower than typical 24-hr values observed in East Los Angeles in 1980 and 1981 (Pitts et al. 1982a) and factors of 5-10 lower than peak 3-hr mutagen densities recorded in West Los Angeles in 1983 (see Section II-D).

As may be expected from the fact that Whitewater is more distant than is Redlands from the Los Angeles source area, the direct-acting mutagen density [i.e., for strain TA98 (-S9)] was less, by a factor of ~2, in Whitewater than in Redlands. This was also the case (Table IV-2) for the

Table IV-2. Particulate Loading and Mutagenicity Data for Transport Study Sites, October 6, 1982

	Whitewater	Redlands
Sampling time (8-1/2 hr)	1045-1913 (PST)	0853-1730 (PST)
Particulate loading	24 mg	54 mg
Total carbon	3.9 mg	13 mg
Elemental carbon	0.21 mg	1.8 mg
Organic carbon	3.8 mg	11 mg
Extracted weight	10 mg	17 mg
Percent extractable	44	31
$\frac{\text{Total carbon}}{\text{Particulate loading}}$	16%	24%
	Mutagen Density (rev m^{-3})	Mutagen Density (rev m^{-3})
TA98 (-S9)	17	32
TA98NR (-S9)	5.4	14
TA98/1,8-DNP_6 (-S9)	3.0	4.9
TA98 (+S9)[a]	13	15
	Specific Activity (rev μg^{-1})	Specific Activity (rev μg^{-1})
TA98 (-S9)	1.0	1.1
TA98NR (-S9)	0.31	0.50
TA98/1,8-DNP_6 (-S9)	0.17	0.17
TA98 (+S9)[a]	0.73	0.51
	Rev μg^{-1} Elemental C	Rev μg^{-1} Elemental C
TA98 (-S9)	48	10
TA98NR (-S9)	15	5
TA98/1,8-DNP_6 (-S9)	10	2
TA98 (+S9)[a]	34	5
	Rev μg^{-1} Organic C	Rev μg^{-1} Organic C
TA98 (-S9)	2.6	1.7
TA98NR (-S9)	0.82	0.79
TA98/1,8-DNP_6 (-S9)	0.45	0.27
TA98 (+S9)[a]	1.9	0.80

[a]2% S9 (v/v).

mutagen densities obtained using stains TA98NR (-S9) and TA98/1,8-DNP_6 (-S9).

Since elemental carbon is a primary emission from stationary and mobile sources, it can be used to normalize the mutagen density data to attempt to account for dilution of the air mass and the possible dilution of mutagenic POM by secondary aerosol formation. Table IV-2 shows that the mutagen density normalized to elemental carbon (revertants per μg elemental C) for Ames strain TA98 (-S9) was significantly higher, by a factor of ~5, at Whitewater than at Redlands. The mutagen densities normalized to elemental carbon were also higher at Whitewater for strains TA98NR (-S9), TA98/1,8-DNP_6 (-S9) and TA98 (+S9). A similar effect was also seen for the mutagen densities normalized to organic carbon (revertants per μg organic carbon) (Table IV-2).

These increases could be explained by the formation of mutagenic secondary aerosols from gas-to-particle conversion and/or transformation reactions in the particulate phase, resulting in compounds of increased mutagenicity, or to different air parcels with different sources of POM reaching the two sites. The observation that the specific activity at Whitewater is not greater than at Redlands could be due to a higher contribution of inorganic material to the extract weight. This is consistent with the fact that the total carbon at Whitewater was 17% of the particulate loading, while at Redlands it was 24% of the particulate loading.

These data show that on this date pollutants reaching Redlands and Whitewater were significantly different in mutagen densities (with Whitewater having lower values than Redlands), and in mutagen densities normalized to elemental carbon and organic carbon (with Whitewater having significantly higher values of these quantities than Redlands). The reasons for these pronounced differences in ambient POM mutagenicities are not known, but could be due to differing Los Angeles plume trajectories prior to impacting the two receptor areas investigated, or to passage over different emission sources.

B. Exploratory Chamber Study

Although the feasibility of using ambient sampling to study the atmospheric transformation of POM was demonstrated by the project discussed above, two disadvantages of this approach were noted. First, this

approach is dependent on favorable meteorological conditions throughout the sampling interval. Second, verification that the upwind and downwind samples were taken from the same air parcel would require a costly tracer study. However, with the recent installation of a chassis dynamometer at the SAPRC facility, a controlled method for studying aerosol aging became possible.

In this pilot study, our dynamometer/outdoor chamber facility was used to evaluate outdoor smog chambers as a means of further understanding particulate matter transformations in an air mass during simulated transport. A Chevette diesel automobile was run on the SAPRC dynamometer and the exhaust was diluted, passed through the diffusive denuder to further reduce the concentration of gas phase components, and used to fill the large outdoor chamber. An initial sample of the particulate matter was drawn from the chamber and the remaining portion allowed to age either in the dark or under irradiation. Particulate samples were collected with a hi-vol sampler from less than 20 m^3 of chamber air and extracted for mutagenicity testing.

1. Experimental Methods

Diluent air was drawn from the atmosphere by a hi-vol motor and passed through a glass fiber filter to remove any ambient particulate matter. This filtered air was then directed through a venturi and added to the exhaust. The exhaust flow was controlled by a 1/2-in. pipe nipple as a bypass and a 2-in. gate valve to control back pressure. Once the exhaust was diluted, it was directed from the venturi through the diffusive denuder and then into the outdoor chamber.

Direct measurements of CO and HC in the exhaust were made with our Sun Engine Analyzer using the 1/2-in. nipple as an inlet port. The parameters measured were total hydrocarbons (as methane, THC), O_3, NO, NO_2, CO, CH_4, dew point (DP) and temperature.

The experimental conditions and measured gaseous pollutant levels for a diesel exhaust sample aged in the dark are given in Table IV-3. The experimental conditions and measured gaseous pollutant levels for a diesel exhaust sample aged under irradiation are given in Table IV-4. Propene was added to the exhaust to promote ozone formation.

Table IV-3. Experimental Conditions and Gaseous Pollutant Concentrations for Diesel Exhaust Aged in an Outdoor Environmental Chamber

Run 008: Dark Aging of Diesel Particulate
Auto: Diesel Chevette, 21,411 mi
Mode: 50 mph, 10 hp
Chamber: #28 covered entire run
Exhaust: CO ~0.05%, THC ~50 ppm
Dilution: 4 min with ambient air

	Initial Conditions	Final Conditions (3-hr dark)
THC	3.6 ppm	3.6 ppm
CH_4	2.0 ppm	1.6 ppm
O_3	0.000 ppm	0.000 ppm
NO	1.24 ppm	1.25 ppm
NO_2	1.30 ppm	-[a]
CO	5.2 ppm	5.6 ppm
DP	11°C	-
Temperature	26°C	32°C

[a]Not determined.

Table IV-4. Experimental Conditions and Gaseous Pollutant Concentrations for Diesel Exhaust Irradiated in an Outdoor Environmental Chamber

Run 010: Irradiation of Diesel Particulate
Auto: Diesel Chevette
Mode: 50 mph, 10 hp
Chamber: #28 covered at fill
Fill time: 10 min
Exhaust: CO <0.05%, THC ~50 ppm
Dilution: 3 min with ambient air
HC added: 230 ml of propylene

	Results	
	Initial Conditions	Final Conditions (3-hr irradiation)
THC	8.2 ppm	6.0 ppm
CH_4	2.0 ppm	2.0 ppm
O_3	0.002 ppm	0.378 ppm (0.487 peak)
NO	0.10 ppm	0.000 ppm
NO_2	0.48 ppm	0.50 ppm
CO	1.5 ppm	2.6 ppm
DP	12°C	11°C
Temperature	28°C	40°C

2. Results

These exploratory experiments indicated a decrease in mutagenic activity with the age of the POM. In one run in which the particulate matter was aged in the dark for three hours there was a significant decrease both in specific mutagenicity (rev per μg extract) and in mutagen density (rev m^{-3}) (Table IV-5). In a second run, particulate aged three hours under solar irradiation also showed decreased specific mutagenicity and mutagen density. The ratio of the response on Salmonella strain TA98NR to TA98 remained constant in the dark but increased in the irradiated exposure. This change with irradiation could indicate a change in nitroarene composition of the POM. However, additional detailed experiments under various environmental conditions are necessary before definitive conclusions can be drawn about the atmospheric transformations of POM.

Table IV-5. Mutagen Testing Results of Diesel Particulate Matter Aged in an Outdoor Environmental Chamber

Experiment Number	Extract Weight (mg)	Volume Sampled (m^3)	TA98 (-S9) Specific Mutagenicity (rev μg^{-1})	TA98 (-S9) Mutagen Density (rev m^{-3})	TA98NR (-S9) Specific Mutagenicity (rev μg^{-1})	TA98NR (-S9) Mutagen Density (rev m^{-3})	TA98NR / TA98
008 initial	2.97	12.7	2.7	630	1.6	0.37	0.59
008 3-hr dark	2.94	14.6	1.8	360	1.0	0.20	0.56
010 initial	2.73	12.7	0.98	210	0.35	0.075	0.36
010 3-hr light	2.81	12.7	0.56	120	0.42	0.093	0.78

V. GAS PHASE MUTAGEN TESTING USING TRADESCANTIA STAMEN HAIR ASSAY

A. Introduction and Statement of the Problem

The Tradescantia stamen hair bioassay has been used extensively in studies of mutation for the past 25 years (Swanson 1957). Early radiobiological studies led to the extension of the use of the plant Tradescantia in studies of chemical mutagenisis (Underbrink et al. 1973). Laboratory studies with chemicals demonstrated (Sparrow et al. 1974) that this system was highly sensitive to gaseous mutagens and should be able to respond to ambient levels of gas phase pollutants.

Under EPA sponsorship and direction by researchers at the Brookhaven National Laboratory (BNL), a mobile monitoring laboratory equipped for exposures of the test organism to ambient air was constructed and, over a four-year period, 18 sites in the United States were monitored (Schairer et al. 1982). Instruments on board the laboratory continuously monitored SO_2, NO_2, NO, NO_x, O_3, CO and total hydrocarbons. Organic vapors were collected on Tenax-GC cartridges for later analysis by GC-MS. Although mutagenic activity was consistently associated with sites downwind of petroleum refineries, no specific compound or group of compounds could be correlated with mutagenic activity (Schairer et al. 1982).

Our study took place at UCR in the summer of 1982 and was a collaborative effort between researchers from SAPRC and BNL. We sought to determine if gas phase pollutants such as PAN, which are too reactive to be retained by Tenax trapping, might be responsible for a significant part of the mutagenic activity previously observed by the Brookhaven team in other areas of the U. S.

B. Research Objectives

The specific objectives of the Tradescantia studies were:

(1) To bioassay specific compounds generated and tested under controlled laboratory conditions.

(2) To bioassay photochemical smog generated synthetically in a large SAPRC outdoor chamber.

(3) To bioassay ambient air pollution in Riverside, CA.

C. Experimental Methods

The Tradescantia test system uses an interspecific hybrid which is the cross between pink- and blue-flowering parents. The visible marker of mutation is a phenotypic change from blue to pink in mature flowers. Mutation is induced by exposing young developing flowers to the test gas; genetic damage is expressed 5 to 18 days later as isolated pink cells or groups of pink cells in the stamen hairs of mature flowers. The flowers are analyzed under a dissecting microscope each day as they bloom for up to two weeks after treatment. Induced mutation is defined as the ratio of pink (mutational) events to total number of stamen hairs (Schairer et al. 1982).

Controlled laboratory exposures were made using 12-ℓ glass chambers brought from BNL. Each chamber has a capacity of 60 cuttings in a container with Hoagland's nutrient solution, uses a standard flow rate of 2 ℓ min^{-1} and has a fan to ensure thorough mixing of the gas during exposure. Input and exhaust ports are available for gas sampling.

Ozone. Four 12-ℓ exposure chambers were set up in the CHAMP fixed site building at SAPRC. A Welsbach O_3 generator was used and concentrations of 1, 10 and 100 ppm were obtained by dilution with air from an Aadco pure air generator. The 1 and 10 ppm ozone exposures were monitored with a Dasibi instrument, while the ~100 ppm ozone concentrations were calculated from the dilutions. The fourth chamber was used for a concurrent control. In each exposure, 60 cuttings of Tradescantia clone 4430 were inserted into holders within glass dishes filled with Hoagland's nutrient solution. Exposures lasted for six hours with temperature, relative humidity and O_3 being measured hourly.

PAN and NO_2. Three exposure chambers were set up as described for the O_3 experiments. PAN concentrations of 1, 10 and 100 ppm were obtained by dilution from a stock tank containing about 580 ppm PAN as measured by IR. NO_2 was tested at 1, 5 and 20 ppm.

Surrogate Smog. Our 50,000-ℓ outdoor smog chamber was filled with clean air and a concentrated primary pollutant "surrogate mixture" consisting of 10 ppm HC + 0.5 ppm NO was injected. The chamber was filled in the dark and then exposed to sunlight. Two 12-ℓ Tradescantia exposure chambers and one control were connected directly with 1-in. x 4-ft glass tubes. The glass tubes were wrapped with wet cheesecloth to cool the air

stream entering the exposure chamber during the 6-hr exposure. A 2-ℓ min^{-1} flow rate was obtained by pulling that amount from the exhaust port, resulting in the gradual deflation of the bag. Ozone, NO_2 and PAN were monitored continuously; other organics were monitored at hourly intervals by gas chromatography.

Ambient Air: Chronic Exposure July 20-30, 1982. The three growth chambers on the BNL Mobile Monitoring Vehicle were used for this 10-day exposure to ambient air. The mutagenicity of the ambient air was monitored independently in two separate chambers with the third chamber serving as the filtered air control. Air was drawn into two chambers through a 4-in. glass duct at a rate of 18 cfm and was conditioned to 20°/18°C day/night temperatures with 75% relative humidity. The control chamber received the same conditioning features and flow rate, but the air was scrubbed by passing through charcoal, purafil and HEPA filters. Each chamber contained three dishes of cuttings (60 each). The cuttings for all exposures described here were shipped by air from New York City to Los Angeles via American Airlines. The cuttings were packed in wet paper toweling in plastic bags and hand carried to and from designated flights. At the end of the exposure, the cuttings were returned to BNL for analysis.

D. Results and Discussion

Upon the return of the cuttings to BNL, they were placed in fresh Hoagland's solution and the flowers were analyzed daily as they bloomed for over a week. In all samples, flower production was good; no adverse stress due to shipment and handling of the cuttings was indicated. The cuttings were stable genetically as indicated by consistently low background mutation frequencies for the various control samples. The overall control average for the Riverside study was 0.00318 ± 0.00014 which is in excellent agreement with the baseline rates of 0.00335 ± 0.00009 and 0.00310 ± 0.00011, for the Grand Canyon and Pittsboro studies, respectively.

PAN, O_3 and NO_2. All exposures of Tradescantia to PAN resulted in positive mutagenic responses (Table V-1). The mutation response increased with increasing PAN concentrations (from 1 to 100 ppm), although the physiological injury was pronounced within a day after the 100 ppm

Table V-1. Mutagenicity of Individual Compounds Following 6-Hr Exposures Under Laboratory Conditions

Treatment	No. of Flowers	No. of Hairs	Pink Events	(Events Hair^{-1}) x 10^3 ±SE Minus Control*	Stat. Sig.
Peroxyacetyl Nitrate (PAN)					
1 ppm	147	60,769	271	1.20±0.31	1%
10 ppm	161	64,867	309	1.50±0.33	1%
100 ppm	152	60,876	299	1.65±0.34	1%
*Control	264	107,237	350	3.26±0.17	
Ozone (O_3)					
1 ppm	138	56,056	238	0.98±0.32	1%
10 ppm	132	52,747	228	1.06±0.36	1%
100 ppm	100	40,590	129	-0.9±0.30	NS
*Control	264	107,237	350	3.26±0.17	
Nitrogen Dioxide (NO_2)					
1 ppm	82	37,761	181	0.36±0.44	NS
5 ppm	83	37,300	154	-0.30±0.44	NS
20 ppm	78	36,082	216	1.56±0.57	1%
*Control	80	38,592	171	4.43±0.32	

exposure. The induced mutation increment for 1 ppm of PAN was a 37% increase over background, which is large enough to suggest that the stamen hair test system would be sensitive enough to respond to PAN at ambient levels of 5-10 ppb over a 10-day exposure period.

Ozone was weakly mutagenic at the 1 and 10 ppm levels; the 100 ppm treatment produced physiological damage such as leaf tip burning and flower bud blasting, but no increase in mutagenicity. NO_2 was weakly mutagenic at the 20 ppm level.

Surrogate Smog. Tradescantia exposures made during the 6-hr exposure to the surrogate smog mixture showed highly significant mutation increases of 0.00209 ± 0.00038 and 0.00173 ± 0.00035 above background (Table V-2). From the data obtained for the individual pollutant exposures, this result

Table V-2. Summary of Somatic Mutation Results Following Tradescantia Exposures to Specific Compounds, Surrogate Smog and Ambient Air at SAPRC

Treatment	Concentration	Total Dose	(Events Hair^{-1})x10^3 ±SE Minus Control
PAN	6 hr at 1 ppm	6 ppm-hrs	1.20 ± 0.31
Ozone	6 hr at 1 ppm	6 ppm-hrs	0.98 ± 0.32
NO_2	6 hr at 1 ppm	6 ppm-hrs	0.36 ± 0.44
			Sum 2.54 ± 0.75
Smog Chamber	6-hr exposure		
PAN	0.001-0.7 ppm	1.65 ppm-hrs	
Ozone	0.01-1.1 ppm	3.6 ppm-hrs	1.90 ± 0.30
NO_2	1.5 ppm	9 ppm-hrs	
Ambient Air	10-day exposure		
PAN	1-8 ppb	0.5 ppm-hrs	
Ozone	0.06 ppm	14.4 ppm-hrs	0.90 ± 0.15
NO_2	0.045 ppm	10.8 ppm-hrs	

is consistent with the high levels of PAN and ozone observed in this experiment. Ozone increased from about 0.01 ppm to a maximum of 1.1 ppm while PAN increased from 1 to 700 ppb over the same time period. An average of the response from both exposed populations gave a 64% increase over background mutation frequency which may be attributed to either additive or perhaps synergistic effects of primarily O_3 and PAN.

Ambient Air. Cuttings from each of the two ambient air chambers gave positive mutagenic responses statistically significant at the 1% level (Table V-2). The pooled results from both ambient chambers reflected the observation of 969 flowers or 380,781 stamen hairs giving 1442 mutant events as compared to the control 533 flowers, 216,345 hairs and 625 mutant events. The results are summarized in Table V-2. Although the ambient air-induced mutation frequency is only about 30% above background, the large stamen hair population analyzed gives validity to the effect (Underbrink et al. 1973).

When working with chemical compounds which are highly reactive with light, UV, humidity, etc., it is difficult to establish the absolute dosage received during either acute or chronic exposures. Earlier experiments on the effects of 1,2-dibromoethane on Tradescantia have

demonstrated that mutation response is directly proportional to the integrated chemical mutagen dose for periods up to about three weeks (Schairer et al. 1982). From our individual Tradescantia exposures to 1 ppm levels of O_3, PAN and NO_2 for 6 hr, mutagenic responses of 0.0012, 0.00098 and 0.00036 mutations $hair^{-1}$, respectively, were obtained. These values were used to calculate an expected response (mutations $hair^{-1}$ per ppm-hr of exposure) for each gaseous species.

During the 6-hr smog chamber exposure, the PAN levels ranged from 0.001 to 0.7 ppm, O_3 ranged from 0.01 to 1.1 ppm and NO_2 averaged about 1.5 ppm with a net mutagenic response of 0.0019 events per hair and total doses of 1.65, 3.6 and 9 ppm-hrs, respectively. The expected responses from the total doses of O_3, PAN and NO_2 present during the smog chamber exposure were 0.00033, 0.00059 and 0.00054 mutations $hair^{-1}$, respectively. The sum of the expected responses, 0.0015 mutations $hair^{-1}$, is in good agreement with the overall response observed for the smog chamber experiments of 0.0019 mutations $hair^{-1}$.

For the 10-day ambient air exposure, the estimated 0.5, 14.4 and 10.8 ppm-hr doses of PAN, O_3 and NO_2 would produce expected responses of 0.0001, 0.0023 and 0.0006 mutations $hair^{-1}$, respectively which, when summed, more than account for the observed response of 0.0009 mutations $hair^{-1}$. Because of the uncertainty in the expected O_3 response, it is reasonable to suggest that PAN, O_3 and NO_2 are the major mutagens detected by Tradescantia in ambient air.

E. Conclusions

- The statistically significant mutagenic activities obtained for the surrogate smog mixture demonstrated the potential usefulness of smog chamber experiments in studying the mutagenic activity of gas phase mutagens.
- The mutagenicity of surrogate smog in the outdoor chamber was consistent with the sum of the mutagenicities of PAN, O_3 and NO_2 determined separately in laboratory tests.
- The mutagenicity of ambient air could also be accounted for by the sum of the mutagenic activities of PAN, O_3, and NO_2.
- To our knowledge this is the first time the mutagenic activity of PAN has been characterized.

VI. REFERENCES

Ames, B. N., McCann, J. and Yamasaki, E. (1975): Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res., 31:347-364.

Belser, W. L., Jr., Shaffer, S. D., Bliss, R. D., Hynds, P. M., Yamamoto, L., Pitts, J. N., Jr. and Winer, J. A. (1981): A standardized procedure for quantification of the Ames Salmonella/mammalian-microsome mutagenicity test. Environ. Mut., 3:123-139.

Blumer, M., Blumer, W. and Reich, T. (1977): Polycyclic aromatic hydrocarbons in soils of a mountain valley: Correlation with highway traffic and cancer incidence. Environ. Sci. Technol., 11:1082-1084.

Brorström, E., Grennfelt, P. and Lindskog, A. (1983): The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere. Atmos. Environ., 17:601-605.

Cass, G. R., Boone, P. M. and Macias, E. S. (1982): Particulate Carbon: Atmospheric Lifecycle, Plenum Press, NY, pp. 207-243.

Choudhury, D. R. (1982): Characterization of polycyclic ketones and quinones in diesel emission particulates by gas chromatography/mass spectrometry. Environ. Sci. Technol., 16:102-106.

Daisey, J. M., Kneip, T. J., Hawryluk, I. and Makai, F. (1980): Seasonal variations in the bacterial mutagenicity of airborne particulate organic matter in New York City. Environ. Sci. Technol., 14:1487-1490.

Fitz, D. R., Doyle, G. J. and Pitts, J. N., Jr. (1983): An ultrahigh volume sampler for the multiple filter collection of respirable particulate matter. APCA Journal, 33:877-879.

Fitz, D. R., Lokensgard, D. M. and Doyle, G. J. (1984): Investigation of filtration artifacts when sampling ambient particulate matter for mutagen assay. Atmos. Environ., 18:205-213.

Flessel, C. P., Wesolowski, J. J., Twiss, S., Cheng, J., Ondo, J., Monto, N. and Chan, R. (1981): Integration of the Ames bioassay and chemical analyses in an epidemiological cancer incidence study. In: Short-Term Bioassays in the Analysis of Complex Environmental Mixtures II, M. D. Waters, S. S. Sandhu, J. L. Huisingh, L. Claxton and S. Nesnow (Eds.), Plenum Publishing, pp. 67-83.

Flessel, P., Guirguis, G., Cheng, J., Chang, K., Hahn, E., Chan, R., Ondo, J., Fenske, R., Twiss, S., Vance, W., Wesolowski, J. and Kado, N. (1983): Monitoring of mutagens and carcinogens in community air. Final Report, California Air Resources Board Contract No. A1-029-32.

Gibson, T. L. (1982): Nitroderivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter. Atmos. Environ., 16:2037-2040.

Gibson, T. L. (1983): Sources of direct-acting nitroarene mutagens in airborne particulate matter. Mutat. Res., 122:115-121.

Grafe, A., Mathern, I. E. and Green, M. (1981): A European collaborative study of the Ames assay. I. Results and general interpretation. Mutat. Res., 85:391-410.

Grosjean, D. (1983): The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere. Atmos. Environ. 17:2112-2114.

Grosjean, D., Fung, K. and Harrison, J. (1983): Interactions of polycyclic aromatic hydrocarbons with atmospheric pollutants. Environ. Sci. Technol. 17:673-679.

Huisingh, J., Bradow, R., Jungers, R., Claxton, L., Zweidinger, R., Tejada, S., Bumgarner, J., Duffield, F., Waters, M., Simmon, V. F., Hare,C., Rodriquez, C. and Snow, L. (1978): Application of bioassay to characterization of diesel particle emissions. Presented at Symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, VA, February 21-23, 1978. In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, M. D. Waters, S. Nesnow, J. L. Huisingh, S. S. Sandhu and L. Claxton (Eds.), Plenum Press, NY, pp. 381-418.

Huntzicker, J. J., Johnson, R. L., Shah, J. J. and Cary, R. A. (1982): Analysis of organic and elemental carbons in ambient aerosols by a thermal-optical method. In: Particulate Carbon-Atmospheric Life Cycle, G. T. Wolff and R. L. Klimisch (Eds.), Plenum Press, NY, pp. 79-85.

Jäger, J. (1978): Detection and characterization of nitro derivatives of some polycyclic aromatic hydrocarbons by fluorescence quenching after thin-layer chromatography: Application to air pollution analysis. J. Chromatog., 152:575-578.

König, J., Balfanz, E., Funcke, W. and Romanowski, T. (1983): Determination of oxygenated polycyclic aromatic hydrocarbons in airborne particulate matter by capillary GC and GC-MS. Anal. Chem., 55:599-603.

Lee, F. S.-C., Pierson, W. R. and Ezike, J. (1980): The problem of PAH degradation during filter collection of airborne particulates: An evaluation of several commonly used filter media. Reprinted from Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, A. Bjørseth and A. J. Dennis (Eds.), Battelle Press, Columbus, OH, pp. 543-563.

Lewtas, J. (1982): Toxicological Effects of Emissions from Diesel Engines, Vol. 10, Elsevier Biomedical, NY.

Lewtas, J. (1983): Evaluation of the mutagenicity and potential carcinogenicity of combustion emissions using short-term bioassays. Environ. Health Perspec., 47:141-152.

Löfroth, G. (1981): Comparison of the mutagenic activity in carbon particulate matter and diesel and gasoline engine exhaust. In: Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures, II, M. D. Waters, S. S. Sandhu, J. L. Huisingh, L. Claxton and S. Nesnow (Eds.), Environmental Science Research, Vol. 22, Plenum, NY, pp. 319-336.

Mermelstein, R., Kiriazides, D. K., Butler, M., McCoy, E. C. and Rosenkranz, H. S. (1981): The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat. Res., 89:187-196.

Møller, M., Alfheim, I., Larssen, S. and Mikalsen, A. (1982): Mutagenicity of airborne particles in relation to traffic and air pollution parameters. Environ. Sci. Technol., 16:221-225.

Nakagawa, R., Kitamori, S., Horkiawa, K., Nakashima, K. and Tokiwa, H. (1983): Identification of dinitropyrenes in diesel exhaust particles. Their probable presence as the major mutagens. Mutat. Res. 124:201-211.

Nielsen, T. (1983): Isolation of polycyclic aromatic hydrocarbons and nitro derivatives in complex mixtures by liquid chromatography. Anal. Chem., 55:286-290.

Nielsen, T., Ramdahl, T. and Bjørseth, A. (1983): The fate of airborne polycyclic organic matter. Environ. Health Perspec., 47:103-114.

Nishioka, M. G., Petersen, B. A. and Lewtas, J. (1982): Comparison of nitro-aromatic content and direct-acting mutagenicity of diesel emissions. In: Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry, M. Cooke, A. J. Dennis and G. L. Fisher (Eds.), Battelle Press, pp. 603-613.

Pederson, T. C. and Siak, J.-S. (1981): The role of nitroaromatic compounds in the direct-acting mutagenicity of diesel particle extracts. J. Appl. Tox., 1:54-60.

Pierson, W. R., Gorse, R. A., Szkarlat, A. C., Brachaczek, W. W., Japer, S. M., Lee, F. S.-C., Zweidinger, R. B. and Claxton, L. D. (1983): Mutagenicity and chemical characteristics of carbonaceous particulate matter from vehicles on the road. Environ. Sci. Technol., 17:31-44.

Pitts, J. N., Jr., Doyle, G. J., Lloyd, A. C. and Winer, A. M. (1975): Chemical transformations in photochemical smog and their application to air pollution control strategies. Second Annual Progress Report, National Science Foundation-Research Applied to National Needs Grant No. AEN73-02904 A02, p. V-8.

Pitts, J. N., Jr., Grosjean, D., Mischke, T. M., Simmon, V. F. and Poole, D. (1977): Mutagenic activity of airborne particulate organic pollutants. Toxicol. Lett., 1:65-70.

Pitts, J. N., Jr., Van Cauwenberghe, K. A., Grosjean, D., Schmid, J. P., Fitz, D. R., Belser, W. L., Jr., Knudson, G. B. and Hynds, P. M. (1978): Atmospheric reactions of polycyclic aromatic hydrocarbons: Facile formation of mutagenic nitro derivatives. Science, 202:515-519.

Pitts, J. N., Jr. (1979): Photochemical and biological implications of the atmospheric reaction of amines and benzo(a)pyrene. Phil. Trans. Royal Soc. (London), A290:551-576.

Pitts, J. N., Jr., Lokensgard, D. M., Ripley, P. S., Van Cauwenberghe, K. A., Van Vaeck, L., Shaffer, S. D., Thill, A. J. and Belser, W. L., Jr. (1980): "Atmospheric" epoxidation of benzo(a)pyrene by ozone: Formation of the metabolite benzo(a)pyrene-4,5-oxide. Science, 210:1347-1349.

Pitts, J. N., Jr., Fitz, D. R., Harger, W., Lokensgard, D. M. and Katzenstein, Y. A. (1981): Geographical and Temporal Distribution of Atmospheric Mutagens in California. Final Report to California Air Resources Board, Contract No. A9-077-31.

Pitts, J. N., Jr., Harger, W., Lokensgard, D. M., Fitz, D. R., Scorziell, G. M. and Mejia, V. (1982a): Diurnal variations in the mutagenicity of airborne particulate organic matter in California's South Coast Air Basin. Mutat. Res., 104:35-41.

Pitts, J. N., Jr., Lokensgard, D. M. and Fitz, D. R. (1982b): Chemical nature of particulate atmospheric mutagens in California's South Coast Air Basin. Final Report to California Air Resources Board, Contract No. A0-139-32, December.

Pitts, J. N., Jr., Lokensgard, D. M., Harger, W., Fisher, T. S., Mejia, V., Schuler, J. J., Scorziell, G. M. and Katzenstein, Y. A. (1982c): Mutagens in diesel exhaust particulate: Identification and direct activities of 6-nitrobenzo(a)pyrene, 9-nitroanthracene, 1-nitropyrene and 5H-phenanthro(4,5-bcd)pyran-5-one. Mutat. Res., 103:241-249.

Pitts, J. N., Jr. (1983): Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres. Environ. Health Perspec., 47:115-140.

Polissar, L. and Warner, H., Jr. (1981): Automobile traffic and lung cancer. An update on Blumer's Report. Environ. Sci. Technol., 15:713-714.

Ramdahl, T., Becher, G. and Bjørseth, A. (1982): Nitrated polycyclic aromatic hydrocarbons in urban air particles. Environ. Sci. Technol., 16:861-865.

Ramdahl, T. (1983): Polycyclic aromatic ketones in environmental samples. Environ. Sci. Technol. 17:666-670.

Rappaport, S. M., Wang, Y. Y., Wei, E. T., Sawyer, R., Watkins, B. E. and Rappaport, H. (1980): Isolation and identification of a direct-acting mutagen in diesel-exhaust particulates. Environ. Sci. Technol., 14:1505-1509.

Rosenkranz, H. S. and Poirier, L. A. (1979): Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. J. Natl. Cancer Inst., 62:873-891.

Rosenkranz, H. S. McCoy, E. C., Mermelstein, R. and Speck, W. T. (1981): A cautionary note on the use of nitroreductase-deficient strains of Salmonella typhimurium for the detection of nitroarenes as mutagens in complex mixtures including diesel exhausts. Mutat. Res., 91:103-105.

Rosenkranz, E. J., McCoy, E. C., Mermelstein, R. and Rosenkranz, H. S. (1982): Evidence for existence of distinct nitroreductases in Salmonella typhimurium: Roles in mutagenesis. Carcinogenesis, 3:121-123.

Rosenkranz, H. S. and Mermelstein, R. (1983): Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. Mutat. Res., 114:217-267.

Salmeen, I., Durisin, A. M., Prater, T. J., Riley, T. and Schuetzle, D. (1982): Contribution of 1-nitropyrene to direct-acting Ames assay mutagenicities of diesel particulate extracts. Mutat. Res., 104:17-23.

Schairer, L. A., Sautkulis, R. C. and Tempel, N. R. (1982): Monitoring ambient air for mutagenicity using the higher plant Tradescantia. In: Genotoxic Effects of Airborne Agents, R. R. Tice, D. L. Costa, K. M. Schalch (Eds.,), Plenum Press, NY, pp. 123-140.

Schuetzle, D., Lee, F. S.-C., Prater, T. J. and Tejada, S. B. (1981): The identification of polynuclear aromatic hydrocarbon (PAH) derivatives in mutagenic fractions of diesel particulate extracts. Int. J. Environ. Anal. Chem., 9:93-144.

Schuetzle, D., Riley, T. L., Prater,T. J., Harvey, T. M. and Hunt, D. F. (1982): Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulates. Anal. Chem., 54:265-271.

Schuetzle, D. (1983): Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspec., 47:65-80.

South Coast Air Quality Management District and Southern California Association of Governments (1982): Final Air Quality Management Plan Revision.

Sparrow, A. H., Schairer, L. A. and Villalobos-Pietrini, R. (1974): Comparison of somatic mutation rates induced in Tradescantia by chemical and physical mutagens. Mutat. Res., 26:265-276.

Swanson, C. P. (1957): Cytology and Cytogenetics. Prentice-Hall, Englewood Cliffs, NJ.

Talcott, R. and Wei, E. (1977): Airborne mutagens bioassayed in Salmonella typhimurium. J. Natl. Cancer Inst., 58:449-451.

Talcott, R. and Harger, W. (1980): Airborne mutagens extracted from particles of respirable size. Mutat. Res., 79:177-180.

Tokiwa, H., Kitamori, S., Nakagawa, R., Horikawa, K. and Matamala, L. (1983): Demonstration of a powerful mutagenic dinitropyrene in airborne particulate matter. Mutat. Res., 121:107-116.

Underbrink, A. G., Schairer, L. A. and Sparrow, A. H. (1973): Tradescantia stamen hairs: A radiobiological test system applicable to chemical mutagenesis. In: Chemical Mutagens: Principles and Methods for Their Detection, Vol. 3, A. Hollaender (Ed.), Plenum Press, NY, pp. 171-207.

Wang, Y. Y., Talcott, R. E., Seid, D. A. and Wei, E. T. (1981): Antimutagenic properties of liver homogenates, proteins and glutathione on diesel exhaust particulates. Cancer Lett., 11:265-275.

Wheelock, A. (1983): personal communication.

Xu, X. B., Nachtman, J. P., Jin, Z. L., Wei, E. T., Rappaport, S. M. and Burlingame, A. L. (1982): Isolation and identification of mutagenic nitro-PAH in diesel-exhaust particulates. Anal. Chim. Acta, 136:163-174.