

EXECUTIVE SUMMARY
TO
IDENTIFICATION OF PARTICULATE MUTAGENS IN
SOUTHERN CALIFORNIA'S ATMOSPHERE

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EXECUTIVE 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 nitro-polycyclic aromatic hydrocarbons (NO₂-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 NO₂-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, 1982). 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 NO₂-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 m³ 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-DNP₆) 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 $<1 \mu\text{m}$ than have the T60A20 filters.

Results and Conclusions. Hi-vols with and without size selective inlets ($10 \mu\text{m}$ 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 $\sim\pm 10\%$ (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-DNP₆ 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 ($\text{rev } \mu\text{g}^{-1}$ 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, 1982) and factors of 5-10 lower than peak 3-hr mutagen densities recorded in West Los Angeles in 1983.

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 m³ 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., NO₂, O₃, HNO₃ 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 higher plant 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., O₃, PAN and NO₂) 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, O₃ and NO₂ determined separately in laboratory tests.

- The mutagenicity of ambient air could also be accounted for by the sum of the mutagenic activities of PAN, O₃ and NO₂.

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

- 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.

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