

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

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The bacterial mutagenicity of a set of 1993 urban particulate air pollution samples is examined using the *Salmonella typhimurium* TM677 forward mutation assay. Ambient fine particulate samples were collected for 24 hr every sixth day throughout 1993 at four urban sites, including Long Beach, central Los Angeles, Azusa, and Rubidoux, California, and at an upwind background site on San Nicolas Island. Long Beach and central Los Angeles are congested urban areas where air quality is dominated by fresh emissions from air pollution sources; Azusa and Rubidoux are located farther downwind and receive transported air pollutants plus increased quantities of the products of atmospheric chemical reactions. Fine aerosol samples from Long Beach and Los Angeles show a pronounced seasonal variation in bacterial mutagenicity per cubic meter of ambient air, with maximum in the winter and a minimum in the summer. The downwind smog receptor site at Rubidoux shows peak mutagenicity (with postmitochondrial supernatant but no peak without postmitochondrial supernatant) during the September–October periods when direct transport from upwind sources can be expected. At most sites the mutagenicity per microgram of organic carbon from the aerosol is not obviously higher during the summer photochemical smog period than during the colder months. Significant spatial variation in bacterial mutagenicity is observed: mutagenicity per cubic meter of ambient air, on average, is more than an order of magnitude lower at San Nicolas Island than within the urban area. The highest mutagenicity values per microgram of organics supplied to the assay are found at the most congested urban sites at central Los Angeles and Long Beach. The highest annual average values of mutagenicity per cubic meter of air sampled occur at central Los Angeles. These findings stress the importance of proximity to sources of direct emissions of bacterial mutagens and imply that if important mutagen-forming atmospheric reactions occur, they likely occur in the winter and spring seasons as well as the photochemically more active summer and early fall periods. **Key words:** ambient particulate matter, bacterial mutagenicity, organic air pollutants, primary particle emissions, *Salmonella typhimurium* TM677, seasonal comparison, spatial comparison. *Environ Health Perspect* 104:428–436 (1996)

Particulate matter directly emitted to the atmosphere from combustion sources contains organic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs (1–5), that are both mutagenic (6–10) and carcinogenic (11–14). These direct emissions from sources mix in the atmosphere and are transported downwind. During transport, atmospheric chemical reactions can act to deplete the directly emitted organic compounds (15,16), while new compounds can be added to the aerosol, for example, via production of nitro-PAH and oxy-PAH as OH and NO₃ radicals attack vapor-phase PAH (17–24). Using bacterial mutagenicity assays (25,26), organic particulate matter filtered from ambient air has repeatedly been shown to be mutagenic (27–30). Much less is known about the interrelationship between the emissions, meteorological conditions, and atmospheric chemical reactions that lead to this result.

The three key processes, emission source characteristics, atmospheric mixing, and chemical alteration, are each highly dependent on location and meteorology. Since

different locations and different weather patterns create different ambient organic particulate mixtures, clues that point to the origin of mutagenic chemicals in the atmosphere can be obtained by measuring the bacterial mutagenicity differences at various locations during different weather patterns. Tokiwa et al. (29) showed that the aerosol in industrial locations in Japan exhibits greater bacterial mutagenicity than samples taken at residential sites. Other investigators (31,32) have since shown that the bacterial mutagenicity of the ambient aerosol (per cubic meter of air sampled) differs between urban sites. Several investigators also have found bacterial mutagenicity to vary with season in Scandinavia (33), in the San Francisco Bay area (34), in northwest Italy (35), and in Newark, NJ (36). In a previous pilot study, we showed that the bacterial mutagenicity of atmospheric fine particulate samples taken in southern California (per unit organic carbon supplied to the bioassay) is about equal in mutagenic activity to an emissions-weighted average of the mutagenic activity of primary organic aerosol samples from

the most important local emissions sources (37). There were indications that the mutagenicity of the ambient aerosol may exceed that of the primary emissions from sources at a few times and locations. However, the small atmospheric sample sizes available during that prior study required that the samples be composited quarterly, which obscured the ability to examine seasonal trends between receptor air monitoring sites, and the fact that samples from only two sites were available eliminated the possibility of examining spatial trends.

In the present paper we report a study of the bacterial mutagenicity of a comprehensive set of urban fine organic aerosol samples taken at five sites in southern California throughout 1993. The purposes are to document and evaluate the spatial and seasonal dependence of the bacterial mutagenicity of the atmospheric organic aerosol in relation to source locations, transport patterns, and periods of high photochemical reactivity, and to acquire large atmospheric samples that can later be used to support bioassay-directed chemical analysis of the identity of the mutagenic compounds that are present. The five atmospheric sampling sites consist of one background station located on an upwind offshore island, and four urban stations, each in a location with carefully chosen relationship to surrounding or upwind emission sources. Bimonthly composites of filters that enable seasonal trends to be

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Received 14 September 1995; accepted 5 December 1995.

observed were assembled. To further ensure the validity of spatial and seasonal comparisons, all samples were collected, stored, extracted, and tested under identical conditions. The bacterial mutation assay used in the present study is a version of the *Salmonella typhimurium* forward mutation assay developed by Skopek et al. (26), which permits comparison against earlier reports of the mutagenicity of particulate matter emitted directly from Los Angeles area air pollutant emission sources (37).

Methods

Airborne particle samples. The ambient air particulate matter samples used here were collected from five sites in southern California including one background site at an offshore island, as shown in Figure 1. The four on-land sites were chosen because each has a different proximity to major types of air pollution sources. The Long Beach atmosphere is influenced by direct emissions from industrial sources (e.g., many petroleum refineries and steam-electric power plants) plus shipping activities in Long Beach Harbor. Central Los Angeles is a focal point of the local highway system and is exposed to the direct emissions from dense motor vehicle traffic. Azusa, which is generally downwind of central Los Angeles, is known for experiencing very high ozone levels and receives atmospherically transformed, or secondary, aerosol transported from upwind. Rubidoux is farther downwind and generally receives even more secondary aerosol, including some of the highest secondary nitrate and secondary organic aerosol concentrations in the nation (38,39). All four urban sites were located at South Coast Air Quality Management District (SCAQMD) air monitoring stations. San Nicolas Island is a background site located off the coast (upwind) of Los Angeles; samples taken there are intended to assess the mutagenicity of the aerosol before air masses enter the Los Angeles area.

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

The sampling systems employed at each site were identical and consisted of a high volume dichotomous virtual impactor and a separate low volume aerosol sampler. The high volume dichotomous virtual impactor is described in detail by Solomon et al. (40). This sampler was chosen because it

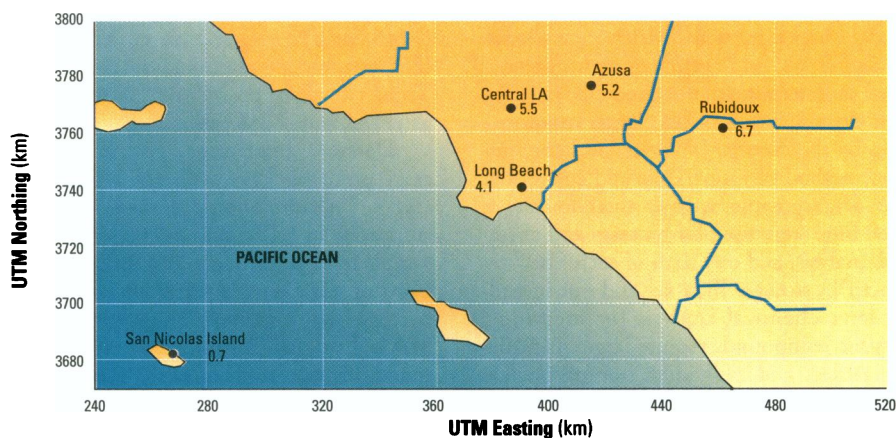


Figure 1. Southern California, showing the locations of the ambient monitoring stations. The 1993 annual average fine particulate organic carbon concentration in micrograms per cubic meter is given adjacent to each site.

can collect a large quantity of size-separated material within a single 24 hr period. The high volume virtual impactor works by accelerating ambient air at a flow rate of nominally 300 l/min through a converging nozzle, after which the flow is divided. Ninety percent of the air flow through the nozzle is caused to make an abrupt 90° turn; the fine particles smaller than 3–4 μm aerodynamic diameter follow the fluid streamlines collecting 90% of the fine particles on a fine particle filter at a flow rate of nominally 270 l/min. The coarse particles larger than 3–4 μm aerodynamic diameter cannot follow this turn due to their greater inertia. These coarse particles are concentrated into the remaining 30 l/min of inlet air and, along with the 10% of fine particles remaining in that 10% of inlet air, are collected on a second filter located downstream of the diverging nozzle that is aligned with the major axis of the inlet of the sampler. The filters used for both collecting coarse and fine particulate matter samples are 102 mm diameter quartz fiber filters (Tissuquartz 2500 QAO, Pallflex Corp., Putnam, Connecticut).

The low volume aerosol samplers used here are similar to those described in detail by Salmon et al. (41). This system consists of two parallel sampling trains, one for collecting total ambient particulate matter and the other for collecting fine ambient particulate matter. Fine particle samples are collected by drawing ambient air through an AIHL-design cyclone separator, which removes particles with aerodynamic diameter $>2 \mu\text{m}$ (42). The airflow then is divided between 4 parallel 47 mm filter holders. These 4 filter holders contain: 1) a Teflon filter (Gelman 47 mm Teflo with 2 μm and 1 μm pore size) operated at a flow rate of 3 l/min from trace metals concentrations are determined by X-ray fluorescence analysis,

2) another Teflon filter operated at a flow rate of 5 l/min from which aerosol mass concentration is determined gravimetrically and ionic compound concentrations are determined by ion chromatography, 3) a quartz fiber filter (Pallflex, 2500 QAO) operated at 10 l/min used for determination of organic carbon (OC) and elemental carbon (EC) concentrations, and 4) another quartz fiber filter operated at 10 l/min which is reserved for future studies of organic compound concentrations by gas chromatography/mass spectrometry.

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

The respirable fine particulate matter samples from the high volume dichotomous virtual impactors were used for both the organic carbon concentration measurements and the bioassays reported here. Each

of these quartz fiber filters was cut into wedge-shaped segments which were allocated as follows: one twelfth of each filter was used to determine the organic carbon (OC) and elemental carbon (EC) mass concentration by the thermal evolution and combustion method of Huntzicker and co-workers (43,44), segments equal to three-fourths of each filter were used for bioassay and chemical analysis, and one sixth of each filter was placed in storage. Bioassay and our intended later chemical analysis for individual organic compounds require large quantities of sample, and individual fine particulate matter samples were not large enough for such analyses. To assemble enough sample for both biological and chemical analyses and yet still maintain the seasonal variation inherent in these samples, the filter portions were pooled to create bimonthly composites at each urban site. Due to the very low pollutant concentrations at the offshore island background site, filter portions taken there were composited to create a winter and a separate summer sample. When organic particulate matter is supplied to the bioassays the quantities are reported in this paper as equivalent organic carbon (EOC), which is defined as the amount of organic carbon present in a particular sample prior to extraction as determined by thermal evolution and combustion analysis of the quartz fiber filter sections cut from the same filters that were extracted for use in the bioassay. This measure provides a direct connection from the bioassay results back to the ambient concentration of organic aerosol.

Extraction and concentration. A description of sample extraction and concentration procedures is given in detail by Hannigan et al. (37). Briefly, all filters used in this study were Soxhlet-extracted with dichloromethane (DCM) for at least 16 hr. DCM extracts were concentrated down to approximately 1 ml in a vacuum centrifuge. The sample portion designated for the bacterial assay was then exchanged into dimethyl sulfoxide (DMSO) by adding DMSO to the DCM extract and then concentrating the extract to the volume of DMSO added under a gentle stream of dry N₂. On average, the extracted mass from an ambient particulate matter sample, as measured by a microscale evaporation method (45), was 0.93 mg extracted mass/mg EOC.

Bacterial bioassay. The bacterial bioassay used in this study is a miniaturized version of the forward mutation assay in *S. typhimurium*, strain TM677, using resistance to 8-azaguanine as developed by Skopek et al. (26). This bioassay is run under two conditions: with and without further enzymatic activation obtained by

adding a postmitochondrial supernatant (PMS, or S9) preparation containing rat liver enzymes to the bioassay procedures. These two assay procedures are referred to as +PMS and -PMS, respectively.

Detailed descriptions of the bacterial assay protocols have been given previously (9,26). To summarize, *S. typhimurium* were suspended in medium in the presence of the sample for 2 hr. Three to five different dilutions of each sample were exposed to the bacteria both with and without the presence of 5% (v/v) Aroclor-induced PMS. In the miniaturized version of the procedure, the culture volume was reduced from 1 ml to 100 μ l (46). Cultures containing PMS had a NADPH-generating system. After 2 hr, the reaction was quenched, and aliquots were plated in the presence and absence of 50 μ g/ml of 8-azaguanine. The results from two to four independent cultures, each plated in triplicate, were averaged to estimate toxicity and mutagenicity at each of the three sample dilutions. Colonies were counted after 48 hr, and the mutant fraction was determined as the number of colonies formed in the presence of 8-azaguanine divided by the number of colonies formed in its absence, multiplied by a dilution factor. Both positive and negative concurrent controls are processed in parallel with each sample. The negative concurrent control consists of a dose of 1 μ l of pure DMSO. The positive concurrent control consists of a dose of 1 μ l of 4-nitroquinoline *N*-oxide (2.5 μ g/ml) in the absence of PMS and 1 μ l of benzo[a]pyrene (2 mg/ml) in the presence of PMS. The mean \pm SD of the negative concurrent control mutant fraction in this series of experiments was $(5.3 \times 10^{-5}) \pm (1.4 \times 10^{-5})$. For an extract to be deemed mutagenic in this assay, a mutant fraction must be greater than the concurrent negative control (CC) such that 2.3 times the SD (the 99% confidence limit) of the concurrent negative control plus the mean value of the control does not overlap the sample mean value minus 2.3 times its SD (the 99% confidence limit), and that mutant fraction must exceed the 95% upper confidence limit of the historical negative control (HC), which is 8.5×10^{-5} for this series of experiments. It is also known that the TM677 strain used in this study does not lack nitroreductase; 1-nitropyrene (1-NP), 4-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, and 1,8-DNP are all potent -PMS mutagens in TM677.

Results and Discussion

Organic Carbon Measurements

In order to discuss the significance of the seasonal and spatial variations of bacterial

mutagenicity, it is important to understand how season and location affect the ambient particulate matter mixture. The annual average fine particulate organic carbon (OC) concentration in the Los Angeles area for 1993 is given adjacent to the monitoring site locations in Figure 1. Ambient fine particulate OC concentrations over the ocean are very low, averaging only 0.7 μ g/m³ for 1993. Fine particle OC concentrations are much higher onshore, ranging from 4.1 μ g/m³ near the coast at Long Beach to a high of 6.7 μ g/m³ at the farthest inland site at Rubidoux. As shown in Figure 2, the seasonal variation in ambient fine particle OC concentrations at San Nicolas Island is slight, varying by about 0.5 μ g/m³ over the course of the year. In contrast, the fine particle OC concentrations at Long Beach vary substantially from month to month with a maximum in the winter (November–December) and a minimum in the late spring/early summer (May–June). Central Los Angeles also shows a strong seasonal variation in fine particle OC concentration, and like Long Beach, the maximum is during the winter and the minimum is during the summer. Moving inland to the Azusa and Rubidoux sites, the relative seasonal variation in fine particle OC concentrations is different; instead of winter maxima and summer minima, the OC concentrations at Azusa and Rubidoux peak in September–October which coincides with the peak photochemical smog season during 1993.

The seasonal patterns at these four sites agree with previous measurements by Gray et al. (47) taken during 1982. Air quality modeling studies applied to explain the results of those 1982 experiments (Gray and Cass, submitted) show that the spatial differences in the seasonal variation of ambient OC in the Los Angeles area that we also observed during 1993 result from seasonal changes in wind speed, wind direction, mixing depth, and secondary aerosol formation rates. During the summer months, the wind blows from the ocean toward the land most of the day. As air masses move over the city, primary organic aerosol emissions accumulate and are transported downwind (eastward). Secondary organic aerosol formation during the summer photochemical smog season acts to further increase fine particle OC concentrations with distance downwind. This secondary organic aerosol formation process occurs as gas phase chemical reactions involving certain higher molecular weight alkanes, olefins, and aromatics produce low vapor pressure reaction products that subsequently condense onto existing particles in the atmosphere (48,49). The

result is that fine particle OC concentrations increase with downwind distance over the metropolitan area in the summer (Fig. 3a). During the winter months, mixing depths are lower, resultant wind speeds are slower and the resultant wind direction often is from the land toward the sea. The highest average fine particle OC concentrations during the winter months are observed close to the areas of highest source emission density on the west side of the air basin for this reason (Fig. 3b). Given the highest OC concentrations in the air basin during the winter and the lowest during the summer, sites on the western side of the air basin display considerable seasonal variation in ambient OC concentrations, as is seen at Long Beach and Los Angeles in Figure 2. Sites in the eastern area of the air basin are upwind of the city during the stagnant winter months and downwind of the city during the summer; given lower winter concentrations and higher summer concentrations, the sites in the eastern area of the air basin display much less seasonal variation in OC concentrations.

Mutagenicity of Ambient Samples

Organic extracts from fine particulate matter samples were combined to form bimonthly composites at each of the four urban sites in the Los Angeles area and semiannual composites at the background site on San Nicolas Island, and were tested for mutagenicity in the absence and presence of PMS. Each of the ambient aerosol extracts tested met the statistical criteria (described previously) to be considered mutagenic except for the Azusa September–October

composite (with PMS) and the San Nicolas Island summer composite (with PMS). Both of those extracts would likely be mutagenic at higher doses, as they show an increase in response with increasing dose.

Dose–response curves were generated for each composite under both assay conditions and are plotted in Figure 4, showing mutant fraction (mutant colony counts corrected for sample toxicity) versus microgram of EOC supplied to the 100 μ l bacterial suspension. As seen by comparing the data at San Nicolas Island to the samples taken at onshore sites in the urban area, the mutagenicity of the aerosol in the city is generally much greater than that upwind of the city. At most sites and times, the -PMS mutagenicity of these samples is greater than the +PMS mutagenicity; the ratio of -PMS mutagenicity to +PMS mutagenicity ranges from 2 to 9 (with one exception, the September–October period at Rubidoux has a higher +PMS than -PMS mutagenicity), with an average value of 3.

The mutagenic potency for each sample tested is defined as the slope of a linear least-squares fit to the dose–response curve (Fig. 4) which gives an estimate of the increase in mutant fraction ($\times 10^5$) per μ g of EOC supplied to the 100- μ l assay. These mutagenic potency values, shown in Table 1, were determined by a weighted linear fit to the dose–response curves (generalized least squares estimate, GLS). The weighted linear fit technique was preferred to an ordinary least squares (OLS) linear fit because the GLS weighting procedure normalizes the data according to the standard deviation of the mean mutant fraction at

each dose, and therefore will be less influenced by high dose–response values that are accompanied by large uncertainties. Using the weighted linear fit, 46 of the 50 data sets fit a straight line very closely and produced correlation coefficients (r) greater than 0.95.

Figure 5 shows the mutagenic potency for each of the samples in the same format as the OC concentrations were shown. The most obvious point is that nearly all samples have a higher response -PMS than +PMS. We observed this previously for both source and ambient samples in Los Angeles using the same assay procedures (37). The lower mutagenic potency of the aerosol at the background site is also very noticeable. Pitts et al. (28) and Alheim et al. (33) have also observed greatly reduced or no mutagenicity at background air monitoring sites. Systematic seasonal variations in mutagenicity per unit organic carbon supplied to each test are not as readily observable as was the case for the seasonal variations in organic carbon concentrations discussed earlier. The Long Beach samples show the highest -PMS potency during the winterlike months with the greatest air stagnation (January–February, March–April, and November–December) while -PMS mutagenic potency begins to approach background levels during the September–October period with the most sustained onshore air flow. This suggests either that -PMS mutagenicity at Long Beach is contributed to by sources whose influence can be reduced during periods of

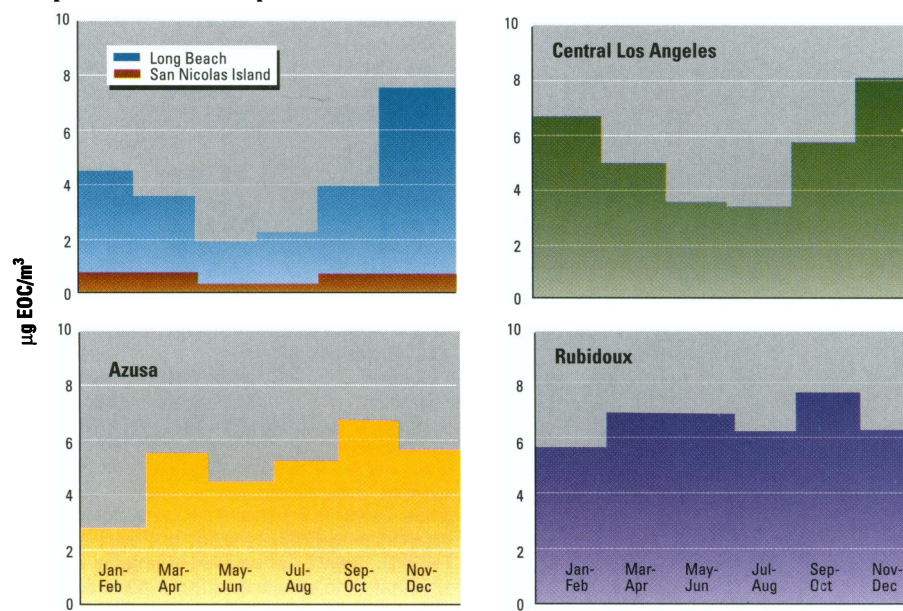


Figure 2. Seasonal variation in fine particulate equivalent organic carbon (EOC) concentration in the South Coast Air Basin during 1993 at Long Beach and San Nicolas Island, Central Los Angeles, Azusa, and Rubidoux.

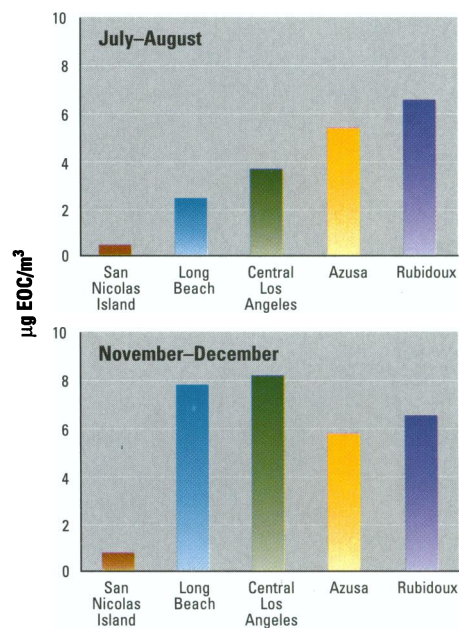


Figure 3. Spatial comparison of the average fine particulate equivalent organic carbon (EOC) concentration across the South Coast Air Basin during July–August 1993, and November–December 1993.

better ventilation of that site, or that the compounds responsible for the aerosol mutagenicity at Long Beach are being destroyed by atmospheric chemical reactions in the late summer months. An increased potency in the March–April composite +PMS is observed at Long Beach for reasons that are not yet understood. Mutagenic potency at central Los

Angeles exhibits no systematic seasonal variation. All but one -PMS data point lies within $\pm 2\sigma$ of the annual mean value. The +PMS mutagenic potency values at central Los Angeles also fall within $\pm 2\sigma$ of the annual mean value except during January–February at that site. Azusa shows only occasional mutagenic potency differences between bimonthly composites; the

March–April composite seems to be more potent than the other composites -PMS, and the September–October composite is less potent than the others +PMS. Rubidoux, the farthest downwind smog receptor site, shows the most interesting seasonal variations. Like Long Beach, which is more or less directly upwind in the summer, the -PMS mutagenic potency at Rubidoux is highest during the colder months and lowest in the summer, but unlike the other sites, the late summer composite which occurs during the peak in the photochemical smog season in

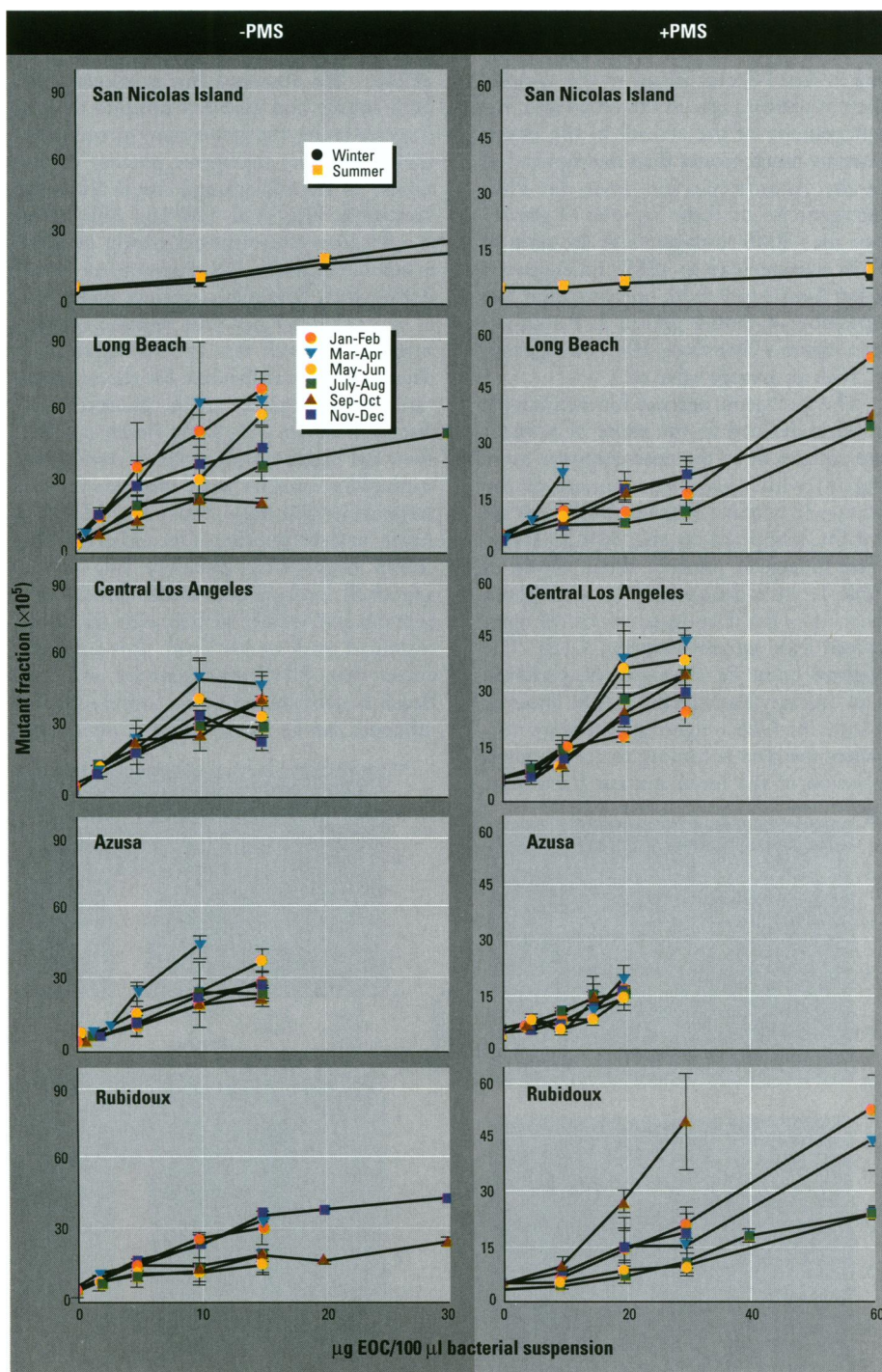


Figure 4. Average dose–response curves for San Nicolas Island, Long Beach, central Los Angeles, Azusa, and Rubidoux, with and without postmitochondrial supernatant (+PMS, -PMS, respectively). Error bars represent 1 SD.

Table 1. Summary of dose–response curve characterization

Bimonthly composite	Mutagenic potency ^a (mean mutant fraction $\times 10^5/\mu\text{g}$ of EOC)	
	-PMS	+PMS
Long Beach		
Jan–Feb	4.4 \pm 0.32	0.47 \pm 0.04
Mar–Apr	5.1 \pm 0.22	1.29 \pm 0.21
May–Jun	2.6 \pm 0.54	0.63 \pm 0.08
Jul–Aug	2.0 \pm 0.24	0.49 \pm 0.03
Sep–Oct	1.2 \pm 0.15	0.62 \pm 0.02
Nov–Dec	2.9 \pm 0.52	0.63 \pm 0.11
Annual mean	3.0 \pm 0.15	0.69 \pm 0.04
Central Los Angeles		
Jan–Feb	2.4 \pm 0.11	0.62 \pm 0.08
Mar–Apr	3.2 \pm 0.36	1.0 \pm 0.00
May–Jun	2.3 \pm 0.32	1.2 \pm 0.06
Jul–Aug	2.0 \pm 0.25	1.0 \pm 0.18
Sep–Oct	3.0 \pm 0.36	0.94 \pm 0.18
Nov–Dec	1.8 \pm 0.20	0.92 \pm 0.07
Annual mean	2.5 \pm 0.12	0.95 \pm 0.05
Azusa		
Jan–Feb	1.6 \pm 0.10	0.39 \pm 0.08
Mar–Apr	3.3 \pm 0.41	0.50 \pm 0.11
May–Jun	2.1 \pm 0.32	0.41 \pm 0.07
Jul–Aug	1.4 \pm 0.22	0.54 \pm 0.07
Sep–Oct	1.5 \pm 0.12	0.16 \pm 0.10
Nov–Dec	1.9 \pm 0.05	0.62 \pm 0.09
Annual mean	2.0 \pm 0.10	0.44 \pm 0.04
Rubidoux		
Jan–Feb	2.0 \pm 0.15	0.35 \pm 0.06
Mar–Apr	2.0 \pm 0.14	0.43 \pm 0.09
May–Jun	0.59 \pm 0.23	0.36 \pm 0.02
Jul–Aug	0.61 \pm 0.06	0.29 \pm 0.03
Sep–Oct	0.40 \pm 0.03	0.87 \pm 0.12
Nov–Dec	0.94 \pm 0.03	0.46 \pm 0.10
Annual mean	1.1 \pm 0.05	0.46 \pm 0.03
San Nicolas Island^b		
Winter	0.47 \pm 0.03	0.07 \pm 0.03
Summer	0.62 \pm 0.04	0.07 \pm 0.04
Annual mean	0.52 \pm 0.03	0.07 \pm 0.03

^aSlope of a straight line fit by generalized least squares to the dose–response curve \pm SE of the slope. Tested in the absence of postmitochondrial supernatant (-PMS) or in the presence of PMS (+PMS). Quantity of equivalent organic carbon (EOC) refers to amount used in the 100- μl assay.

^bDue to the limited sample mass available for the background site, only two composite samples were generated.

September 1993 is more potent than the other +PMS composites at Rubidoux. The two seasonal samples available at San Nicolas Island show mutagenic potencies that are very similar to each other, and both are very low compared to samples taken within the urban area.

While the mutagenic potency data do not reveal seasonal trends that are common to all sites there are significant spatial differences in the annual average mutagenic potency between sites. This can be seen

most clearly by looking at the annual mean mutagenic potency values given in Table 2. In the absence of PMS, mutagenic potency decreases as one moves inland from Long Beach (3.0 ± 0.15 mutant fraction $\times 10^5/\mu\text{g}$ EOC) and central Los Angeles (2.5 ± 0.12) to Azusa (2.0 ± 0.10) and Rubidoux (1.1 ± 0.05). In the presence of PMS, the aerosol at central Los Angeles has the highest mutagenic potency (0.95 ± 0.05 mutant fraction $\times 10^5/\mu\text{g}$ EOC), followed by Long Beach (0.69 ± 0.04), with Azusa and

Rubidoux at a lower level (0.44 ± 0.04 and 0.46 ± 0.03). This variation in mutagenic potency from site to site can be compared with previous studies. Tokiwa et al. (29) found greater mutagenic potency at industrial sites versus residential sites in Japan. Butler et al. (32) measured mutagenic potencies at five different urban sites, New York City, Elizabeth, New Jersey, Mexico City, Beijing, and Philadelphia and showed that those potencies differed between cities. While Pitts and co-workers (28,50) measured mutagenic potencies at various sites in the Los Angeles area in 1976 and in 1980, and Atkinson et al. (51) measured mutagenic potencies in several locations in California during 1986–1987, no direct comparison between the present work and the previous Los Angeles basin studies is possible because the previous studies were conducted over a shorter period of time and sampling rotated between different sites at different times; thus, any variation observed between sites in the previous studies could have been caused by short-term meteorological events.

Figure 6 combines the organic aerosol concentration data of Figure 2 with the mutagenic potency (mutagenicity per μg EOC) values given in Figure 5, and shows mutagenicity in units of mutant fraction ($\times 10^5$)/ m^3 of ambient air. The term mutagenic density will be used to describe this measure of mutagenicity per unit air volume sampled. Atmospheric samples taken at both Long Beach and central Los Angeles show a strong seasonal variation in -PMS mutagenic density; the aerosol at both sites has a significantly greater -PMS mutagenic density value in the winter than in the summer months. These seasonal differences in mutagenic density seen at Long Beach and central Los Angeles occur because of the influence of seasonal variations in the OC concentrations. Several previous investigators also have measured higher mutagenic density in the winter months, including Alheim et al. (33) at various sites in Scandinavia, Flessel et al. (34) in the San Francisco Bay area, and Scarpato et al. (35) in northwestern Italy. The aerosol at Azusa and Rubidoux does not show pronounced seasonal trend in mutagenic density; however, each of those sites has one bimonthly composite with an unusually high mutagenic density value. At Azusa the -PMS assay during March–April shows a high mutagen density value while an increase in mutagenic density +PMS occurs at Rubidoux during September–October. The annual average mutagenic densities, shown in Table 2, are highest at central Los Angeles and decline as one moves away from the center of the city in a

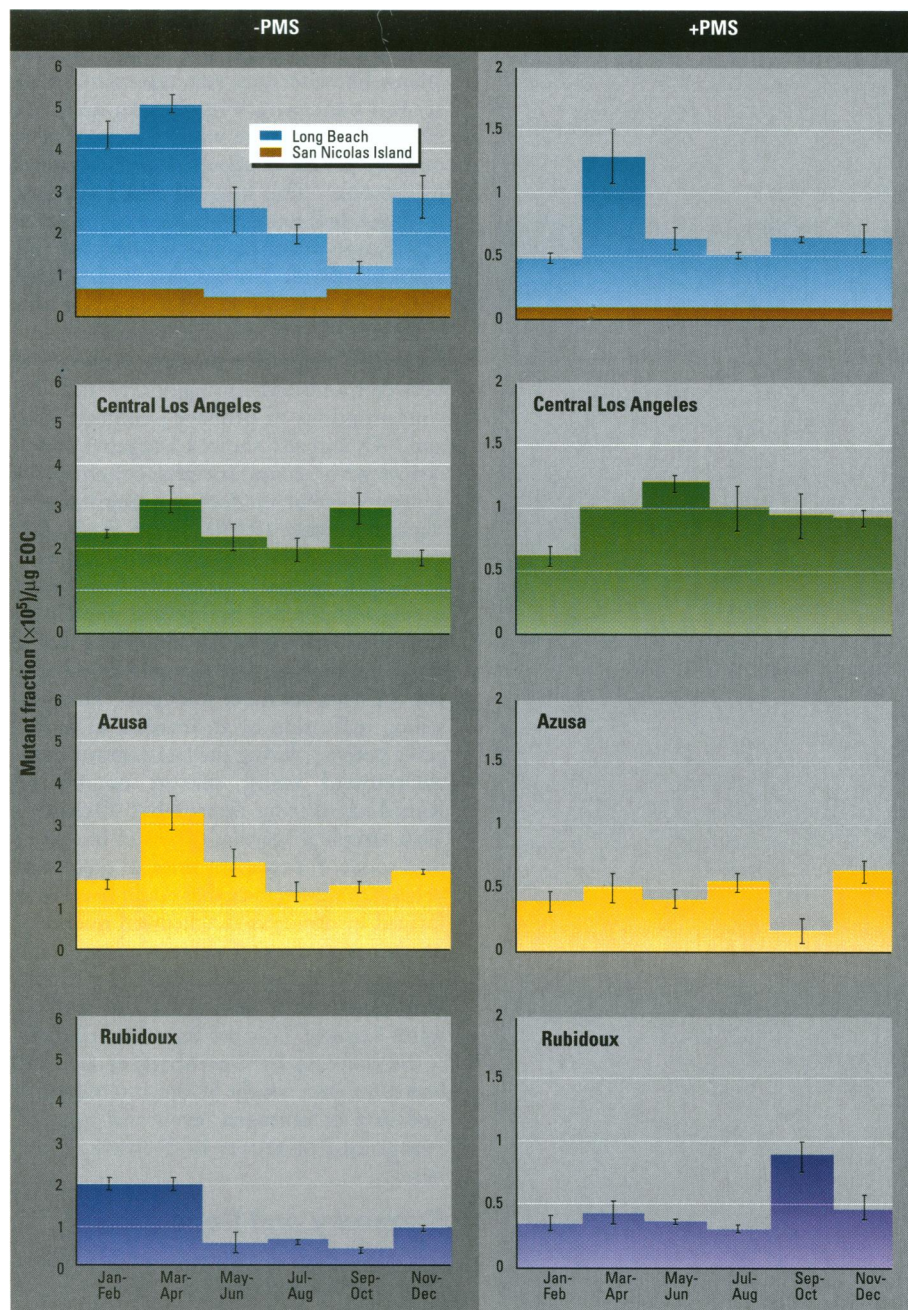
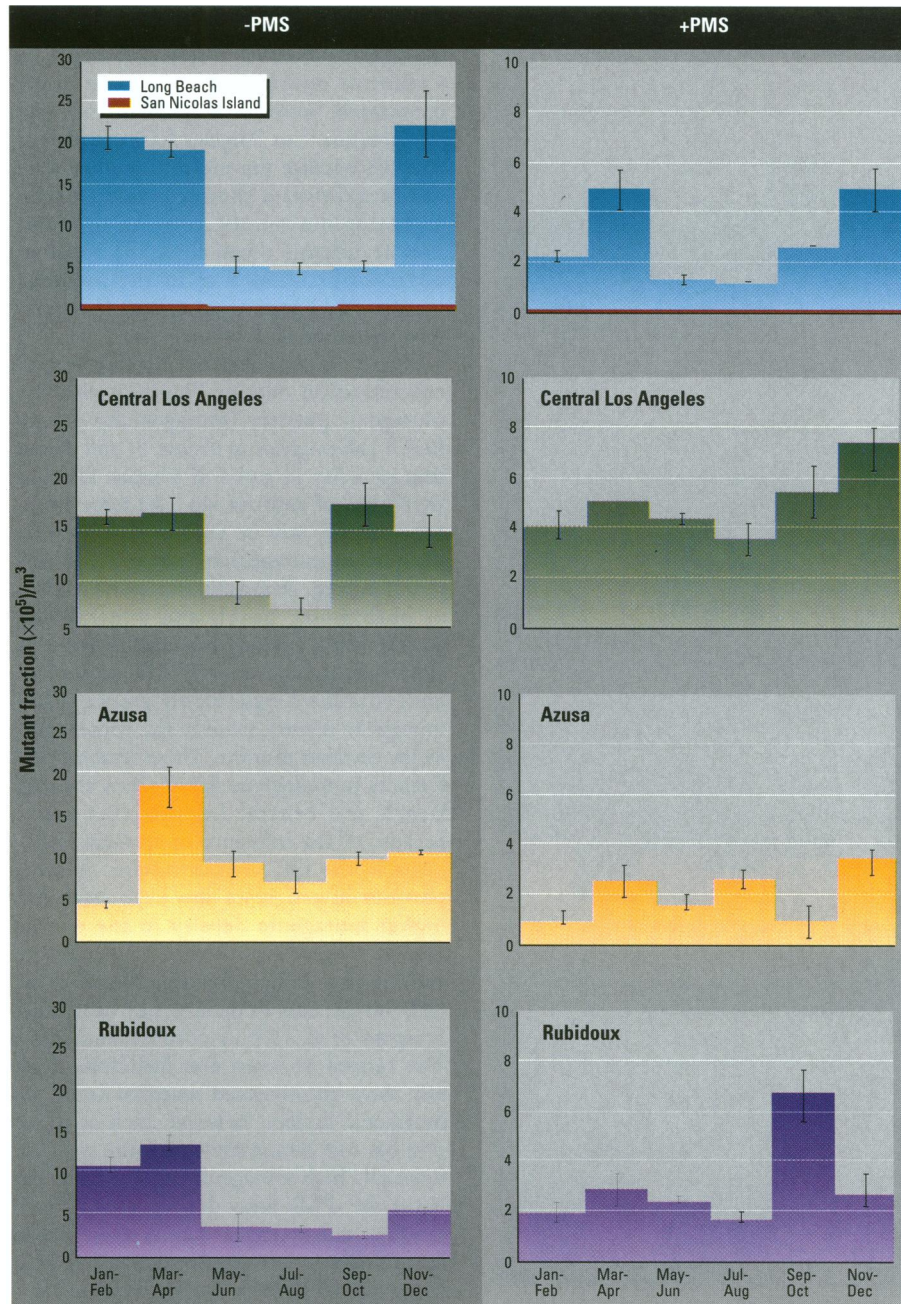


Figure 5. Mutagenic potency (average mutant fraction per mass of equivalent organic carbon, EOC) of the fine particulate matter in the South Coast Air Basin for 1993 at Long Beach and San Nicolas Island, central Los Angeles, Azusa, and Rubidoux, with and without postmitochondrial supernatant (+PMS, -PMS, respectively). Error bars represent 1 SD.

Table 2. The spatial variation of 1993 annual average mutagenic properties across the South Coast Air Basin

	Mutagenic potency (mutant fraction $\times 10^5/\mu\text{g EOC}$)		Mutagenic density (mutant fraction $\times 10^5/\text{m}^3$ air)	
	-PMS	+PMS	-PMS	+PMS
Long Beach	3.0 ± 0.15	0.69 ± 0.04	12.8 ± 0.8	2.8 ± 0.20
Central Los Angeles	2.5 ± 0.12	0.95 ± 0.05	13.3 ± 0.6	5.0 ± 0.24
Azusa	2.0 ± 0.10	0.44 ± 0.04	10.2 ± 0.5	2.2 ± 0.20
Rubidoux	1.1 ± 0.05	0.46 ± 0.03	7.1 ± 0.4	3.2 ± 0.23
San Nicolas Island	0.52 ± 0.03	0.07 ± 0.03	0.4 ± 0.01	0.1 ± 0.01

Abbreviations: EOC, equivalent organic carbon; PMS, postmitochondrial supernatant.

**Figure 6.** Mutagenic density (average mutant fraction per volume of ambient air) in the South Coast Air Basin in 1993 at Long Beach and San Nicolas Island, Central Los Angeles, Azusa, and Rubidoux, with and without postmitochondrial supernatant (+PMS, -PMS, respectively). Error bars represent 1 SD.

manner not unlike local traffic and population density.

The measures of mutagenic potency shown in Table 2 relate to mutagenicity adjusted to comparable quantity of organic aerosol mass, not air volume. The higher relative potency at the more congested and industrialized sites at Long Beach and central Los Angeles is not a function of aerosol mass concentration in the atmosphere but rather is a function of an aerosol chemical composition which causes an increase in mutation frequency per μg of organics supplied to the assay. The lower relative potency at the background site on San Nicolas Island likewise does not represent small aerosol concentrations, but an ambient compound mixture which causes fewer mutants per μg of organic carbon supplied to the assay. This suggests that proximity to direct pollutant emission sources has a significant effect on the potency of the ambient aerosol. Several past studies have suggested that direct emissions are the main contributor to ambient mutagenicity. Pitts (50) showed a positive correlation between ambient mutagenicity and the concentrations of primary pollutants CO and NO, and also showed a negative correlation with secondary photochemically generated pollutants such as ozone and peroxyacetyl nitrate (PAN). Barale et al. (52) correlated mutagenicity with lead and found no spatial variation in ambient mutagenic potency normalized relative to lead levels, leading to the conclusion that a ubiquitous emitter like automobile exhaust was the main source of mutagens. The only strong indication of an increase in mutagenic potency during the late summer photochemical smog season occurs at Rubidoux during September–October. That sample in particular should be examined to see if it contains unusual quantities of mutagenic organics that could have been formed by atmospheric chemical reactions. At the other sites, if there are important mutagen-forming atmospheric reactions, they must occur during cold as well as warm seasons. This has been hypothesized to be the case by Greenberg et al. (36) based on their study of the interseasonal variation of mutagen levels and organic compound mixtures in Newark, New Jersey.

Summary and Conclusions

Organic carbon concentrations and bacterial mutagens present in a set of 1993 bimonthly composited urban fine particulate air pollution samples from sites in the greater Los Angeles area have been measured. Organic aerosol concentrations were quantified by thermal evolution and com-

bustion analysis while bacterial mutagens were determined by the *Salmonella typhimurium* TM677 forward mutation assay. Ambient fine particulate samples were collected for 24 hr every sixth day throughout 1993 at four urban sites, including Long Beach, central Los Angeles, Azusa, Rubidoux, and at an upwind background site on San Nicolas Island. Samples were collected using a high volume dichotomous virtual impactor. These fine particulate samples were composited bimonthly at the urban sites so that seasonal variations could be observed. Long Beach and central Los Angeles are source-dominated urban areas that are expected to experience high concentrations of primary aerosol emitted directly from industry and from motor vehicles, while Azusa and Rubidoux are located farther downwind of the most densely populated areas and receive both transported primary air pollutant emissions plus the transformation products of atmospheric chemical reactions. San Nicolas Island is located off the coast of southern California and acts as a background air monitoring site.

Seasonal trends in organic particulate matter concentrations are observed that show a progressive increase in OC concentrations with downwind transport distance over the city in the summer accompanied by high winter concentrations during November–December at all sites within the urban area. This seasonal aerosol OC concentration trend can be explained by meteorological patterns that concentrate primary pollutants near their source in the western portion of the air basin in the winter combined with the increased secondary aerosol production that occurs during transport toward the downwind sites during the summer. The two sampling sites located in the western portion of the Los Angeles basin at Long Beach and central Los Angeles show a pronounced seasonal variation of -PMS bacterial mutagenicity/m³ of air sampled with peak values in the winter and minimum values in the summer. The downwind site Rubidoux shows a high value of +PMS mutagenicity/m³ of air sampled during the September–October peak photochemical smog period, which reflects both an elevated organic aerosol mass concentration and an elevated mutagenicity per µg of organic compounds during that two-month period.

Significant spatial variations in the organic aerosol and mutagenicity data are apparent. Both the organic aerosol concentration and the mutagenicity of the aerosol per microgram of organic carbon was much lower at the background site on San Nicolas Island than within the urban area.

As a result, the bacterial mutagenicity/m³ of air sampled at the background site on San Nicolas Island was more than an order of magnitude less than was observed at the urban locations, demonstrating that the city is indeed a source of mutagenic aerosol emissions. Within the urban area, average organic particulate matter concentrations during 1993 were highest at the most inland site at Rubidoux, but the mutagenicity per microgram of organic carbon in the aerosol was highest at those monitoring sites closest to the major primary air pollution sources at Long Beach and at central Los Angeles. As a result, the highest values for mutagenicity/m³ of air sampled were observed at central Los Angeles both ±PMS. These findings seem to stress the importance of the direct emissions of bacterial mutagens from the major primary sources in the most heavily populated and industrial areas in the western portion of the Los Angeles area. Since mutagenicity per unit aerosol mass is not obviously higher at most sites during the summer photochemical smog season, the results imply that if important mutagen-forming atmospheric reactions occur, they must occur in the cold seasons as well as the warm seasons. Further chemical analysis of subfractions of these ambient samples is planned in order help to identify the specific chemical compounds or groups of compounds present in these samples that produce the mutagenic response quantified here.

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American Society of Tropical Medicine and Hygiene 45th Annual Meeting

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