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

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The bacterial mutagenicity of a comprehensive set of urban particulate air pollution source samples is examined using the *Salmonella typhimurium* forward mutation assay. Each of the combustion source samples examined, including the exhaust from catalyst-equipped autos, noncatalyst autos, heavy-duty diesel trucks, plus natural gas, distillate oil, and wood combustion sources, is mutagenic in this assay, with a response per microgram of organic carbon in these samples generally greater than that of cigarette smoke aerosol. The noncombustion source samples tested generally are not mutagenic at the levels examined. The specific mutagenicity (mutant fraction per microgram of organic carbon) of ambient aerosol samples collected in southern California is compared to a weighted average of the specific mutagenicity of the primary source samples assembled in proportion to their emission rates in the Los Angeles area. In most cases where a comparison can be made, the specific mutagenicity of the source composites and the ambient samples are of similar magnitude, with the exception that the -PMS mutagenicity of the aerosol at Long Beach, CA, during the first half of the calendar year 1982 and at Azusa, CA, during the April-June 1982 period is much higher than can be explained by direct emissions from the sources studied here.

Introduction

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

The resulting atmospheric mixture of directly emitted organic aerosol thus consists of small contributions from a large number of sources. Each of these source effluents in turn consists of a complex mixture of organic compounds. Many of the individual chemical compounds, of greatest interest because of their possible mutagenic or carcinogenic potential, are present in small quantities such that their identification is made very difficult by the presence of the complicated chemical matrix consisting of hundreds of more abundant but less hazardous substances that are found in most environmental samples.

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

Comparison of the relative mutagenicity of air pollution sources and ambient samples based on the present scientific literature is difficult because differences in the procedures used by different laboratories can mask actual differences between different pollutant source effluents. A recent collaborative study by the International Programme on Chemical Safety (IPCS) (*21-23*), a subgroup of the World Health Organization (WHO), found that 55-95% of the variability seen in the mutagenicity of three environmental mixtures tested could be accounted for by between-laboratory variations in procedures rather than by actual differences in the samples. The ability to compare source and ambient aerosol mutagenicity results reported in the literature is further limited by the fact that no uniformity exists in methods for sample collection, storage, and extraction.

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

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Table 1. Contribution of Sources to Fine Aerosol Organic Carbon Emissions within the 80 × 80 km Area Surrounding Los Angeles for 1982 As Used To Produce Emissions-Weighted Average Values of Specific Mutagenicity of Composite of Primary Sources

source type	% of OC emitted[a]	profile no. of source tested in this study	*profile assigned to* estimate specific mutagenicity of sources not tested
(1) meat-cooking operations			
charbroiling	16.6	1	
(2) paved road dust	15.9	2	
(3) fireplaces			
pine wood	11.2	3	
oak wood	2.8	4	
synthetic logs	nk[j]	5	
(4) noncatalyst gasoline vehicles			
automobiles	7.0	6	
other vehicles[b]	3.7		6
(5) diesel vehicles			
heavy-duty trucks	4.2	7	
other vehicles[c]	2.1		7
(6) surface coating	4.8		
(7) forest fires	2.9		
(8) cigarettes	2.7	8	
(9) catalyst-equipped gasoline vehicles			
automobiles	2.6	9	
other vehicles[d]	0.3		9
(10) organic chemical processes	2.3		
(11) brake lining dust	2.3	10	
(12) roofing tar pots	1.9	11	
(13) tire wear	1.4	12	
(14) misc. industrial point sources	1.3		
(15) natural gas combustion			
residential/commercial	0.1	13	
other sources[e]	0.9		13
(16) misc. petroleum industry processes	1.0		
(17) primary metallurgical processes	0.8		
(18) railroad (diesel oil)	0.7		7
(19) residual oil stationary sources[f]	0.7		14
(20) refinery gas combustion	0.7		13
(21) secondary metallurgical processes	0.6		
(22) mineral industrial processes	0.5		
(23) other organic solvent use	0.4		
(24) jet aircraft	0.3		
(25) asphalt roofing manufacturing	0.3		11
(26) coal burning	0.3		
(27) wood processing	0.2		
(28) residual oil-fired ships	0.2		
(29) structural fires[g]	0.2		3
(30) distillate oil stationary sources			
industrial	0.04	14	
other[h]	0.08		14
(31) vegetative detritus	nk	15	
(32) other sources[i]	0.8		6, 7, 13, 14

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

Experimental Section

Source Samples. The aerosol source samples used in the present study were collected by Hildemann *et al.* (*24*) from 15 source types that collectively represent about 70% of the fine organic aerosol emissions to the atmosphere in the Los Angeles area. The source types tested are listed in Table 1. A portable dilution source sampling system designed by Hildemann *et al.* (*26*) was used. In a dilution sampler, hot exhaust emissions are mixed with purified dilution air in order to cool the sample to ambient conditions, thus allowing certain vapor-phase organics found in the exhaust of hot combustion sources to condense onto existing aerosol within the sampling system as would normally occur in the atmospheric plume downwind of the source.

A diagram of the dilution source sampler used here is presented by Hildemann *et al.* (*24*, *26*). Exhaust emissions are withdrawn from the source through a cyclone separator that removes coarse particles that are greater than 10 μm in diameter. The source effluent then flows into the dilution sampler through a heated Teflon inlet line. The source emissions next are diluted nominally 50-fold with purified dilution air in the stainless steel dilution tunnel. The purified dilution air is slightly cooled so that the

mixture of the hot emissions plus dilution air can be brought to ambient temperature. The dilution air is purified by passage through an activated carbon bed and an HEPA filter. Approximately 10% of the cooled and diluted emissions then are withdrawn into a stainless steel residence time chamber where condensable organics in the exhaust gases are given sufficient time to condense into the aerosol phase. Samples are then drawn from the residence time chamber through AIHL-design cyclone separators (*27*), which remove particles with aerodynamic diameters greater than 2 μm. There are six of these cyclone separators connected in parallel, each drawing air at a rate of 27.9 ± 0.3 L/min. Three parallel filter holders are located downstream of each cyclone. Of these 18 filter holders, two contain Teflon filters used for bulk chemical analysis while the other 16 filter holders contain quartz fiber filters (47-mm diameter, Pallflex Tissuquartz 2500 QAO). One of these quartz fiber filters during each source test was used to determine the organic carbon (OC) and elemental carbon (EC) mass emission rate from the source by the thermal evolution and combustion method of Huntzicker and co-workers (*28, 29*). The other 15 quartz fiber filters were used for detailed organic chemical analysis.

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

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

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

The source sampling program is described in detail by Hildemann *et al.* (*24, 26*); only a brief description of the sources tested will be given here. The sampling strategy focuses on obtaining representative samples that show major differences that exist between sources of different types. Variability between sources of the same type is addressed by compositing samples from several sources (e.g., many different motor vehicles, different plant species, different cigarette brands) before extraction and bioassay. The fuel oil-fired boiler was a Babcock and Wilcox dual FM-type industrial scale watertube boiler burning no. 2 fuel oil and operating at 60% capacity in steady-state mode. The natural gas home appliances consisted of a space heater (Western Gravity Heat Model 8G100) and a water heater (American Standard Model G-531-H) connected to a common exhaust duct. Fireplace smoke samples were taken while burning wood in a residential undampered brick fireplace. Smokes from the combustion of three different woods [seasoned pine, seasoned oak, and a synthetic log (Pine Mountain Brand)] were collected while sampling from the chimney of this fireplace. The catalyst-equipped automobile exhaust sample consisted of a composite of the exhaust aerosol from seven automobiles. Each was sampled while undergoing the cold start Federal Test Procedure (FTP) urban driving cycle. The non-catalyst automobile exhaust sample was a composite of the exhaust aerosol from six automobiles that were tested over the same driving cycle as the catalyst-equipped automobiles. Particulate matter collected from two 1987 model heavy-duty diesel trucks operated on a chasis dynamometer was composited to form the heavy-duty diesel truck sample. Organometalic brake lining wear particles were obtained from the dust of the rear brake drums of a light-duty truck. The tire wear debris sample came from particles shed by a 195/60R15 Toyo tire that was operated on a rolling resistance test stand. Paved road dust was obtained by sweeping Pasadena, CA, area streets using a vacuum sweeper truck. The roofing tar pot sampled contained petroleum-based builtup asphalt, GAF brand. The cigarette smoke sample consisted of the sidestream and exhaled smoke from several different types of cigarettes composited in proportion to their market shares. The charbroiled meat smoke sample was taken while cooking regular (21% fat) hamburger meat over a natural gas charbroiler in a commercial scale kitchen. The urban vegetative detritus sample consisted of plant fragments shed when agitating the leaves obtained from a composite of 64 species of plants that represent the relative abundances of vegetation in the Los Angeles basin (*31, 32*). Quartz fiber filters were cut or combined to bring the source material supplied to the extraction step of the present study into the range of 1.2–4.7 mg of organic carbon per sample, as determined by combustion analysis of parallel filter samples. Equivalent organic carbon (EOC) will be defined as this amount of organic carbon present in the source sample prior to extraction as determined by thermal evolution and combustion analysis, a quantity which provides a direct connection back to the carbonaceous aerosol emission rate from each source through the work reported by Hildemann *et al.* (*24*).

Ambient Samples. The ambient fine organic aerosol samples used in this study were acquired by Gray *et al.* (*25*). A fine particle air-monitoring network was operated at 10 sites in the Los Angeles basin during the year 1982. Ambient samples were taken for 24 h every sixth day for

the entire year. In the present study, aerosol samples collected at two of these sites were selected, Long Beach and Azusa. Use of these monitoring stations permits comparisons to be made between an upwind coastal site near an industrial area that is dominated by primary pollutants, Long Beach, and a downwind site that is known for its high levels of photochemical smog, Azusa.

A schematic diagram of the fine aerosol ambient sampler used here is presented by Gray et al. (*25*). Ambient air is drawn through an AIHL-design cyclone separator, which removes particles with aerodynamic diameters greater than 2.1 μm. Next, the airflow is divided between four parallel filter holders. These four filter holders contain (1) a Teflon filter from which trace metals concentrations are determined by X-ray fluorescence analysis, (2) a nuclepore filter from which ionic pollutant concentrations are determined by ion chromatography, (3) two sequential quartz fiber filters: one for determination of OC and EC mass and one for estimation of possible sampling artifacts, and (4) another quartz fiber filter for organic chemical analysis and bioassays. The preparation of the quartz fiber filters is the same as was described for the source sampling system. Flow rates through the quartz fiber filters were 10 L/min. OC and EC mass concentrations were determined by the thermal evolution and combustion method of Johnson *et al.* (*28*) and Huntzicker *et al.* (*29*) as was used for the source samples. For further details of the ambient sampling program, consult Gray *et al.* (*25*).

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

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

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

The bioassay investigation, which will be discussed later in this paper, showed that certain wood smoke samples were toxic to the bacteria. Much of the toxicity is thought to be due to the presence of certain polar compounds in these smoke samples. Therefore, an aliquot of each fireplace wood smoke sample DCM extract also was fractionated using a gravity-flow cyano column to remove polar organic compounds from the total sample. These gravity-flow columns are based on 6-mL polypropylene syringe bodies, fitted with polyethylene filters and packed with 1 g of cyanopropyl-bonded packing material. Columns were obtained from Analytichem Int., Harbor City, CA. These columns were cleaned and conditioned by washing with 10-mL volumes of methanol and dichloromethane (*19*, *34*). This allows comparisons to be made between the bioassay results for whole wood smoke extract versus the bioassay results for nonpolar wood smoke extract.

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

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

Bacterial Bioassay. The bacterial bioassay used in this study is a miniaturized version of the forward mutation assay in *S. typhimurium*, strain TM677, using resistance to 8-azaguanine as developed by Skopek *et al.* (*2*). Under normal conditions, *S. typhimurium* strain TM677 will not survive in the presence of 8-azaguanine; however, certain mutations to the genetic code enable the bacteria to develop a resistance to 8-azaguanine. Therefore, if an organic extract given to the bacteria, in the presence of 8-azaguanine, results in the development of colonies in significantly greater number than the number of colonies associated with background mutations, then the extract is considered to be mutagenic, and this mutagenicity can be quantified by colony counts. This bioassay is run under two conditions: with and without further enzymatic activation obtained by adding a post-mitochondrial su-

pernatant (PMS, also referred to elsewhere as S9) preparation containing rat liver enzymes to the bioassay procedure. These enzymes carry out a series of oxidation and conjugation reactions on a wide variety of substrates including PAH and other environmental pollutants. The mutagenicity of many compounds depends on such metabolic 'activation' steps. These two assay procedures will be referred to as +PMS and -PMS, respectively.

Detailed descriptions of the bacterial assay protocols have been given previously (*2*, *35*). To summarize, *S. typhimurium* were suspended in medium in the presence of the sample for 2 h. Three different dilutions of each sample, providing organic contaminant concentrations ranging from 2.4 to 473 μg/mL, were exposed to the bacteria both with and without the presence of 5% (v/v) Aroclor-induced PMS. In light of the small amount of organic material available from the aerosol samples, a miniaturized version of this procedure was used. The culture volume was reduced from 1 mL to 100 μL (*16*). Cultures containing PMS had a NADPH-generating system. After 2 h, the reaction was quenched, and aliquots were plated in the presence and in the absence of the 8-azaguanine (at 50 μg/mL). The results from two independent cultures, each plated in triplicate, were averaged to estimate the toxicity and mutagenicity of each treatment condition. Colonies were counted after 48 h, and the mutant fraction was determined as the number of colonies formed in the presence of 8-azaguanine divided by the number of colonies formed in its absence multiplied by the dilution factor. If this ratio is greater than that found for untreated concurrent control cultures such that 99% confidence intervals about the assay mean value and its concurrent control do not overlap and if that ratio also exceeded the 95% upper confidence limit of the mutant fraction for the cumulative historical negative control, the test was considered positive. Occasionally the observed variation among replicate assays is less than expected given the inherent numerical variation of the assay (*36*). In such a case, the higher expected confidence limits are used in determining significance.

Both positive and negative concurrent control assays are performed in parallel with each source sample. The negative concurrent control consists of a dose of 1 μL of pure DMSO. The positive concurrent control consists of a dose of 1 μL of 4-nitroquinoline *N*-oxide (10 μg/mL) in the absence of PMS and 1 μL of benzo[*a*]pyrene (2 mg/mL) in the presence of PMS. The mean ± SD of the concurrent negative control mutant fraction in this series of experiments was $(9.0 \times 10^{-5}) \pm (2.5 \pm 10^{-5})$, yielding a 95% upper confidence limit very close to the 95% upper confidence limit for the historical negative control of 14×10^{-5}. The mean ± SD of the concurrent positive control mutant fraction in this series of experiments was $(390 \times 10^{-5}) \pm (210 \times 10^{-5})$ in the absence of PMS and $(59 \times 10^{-5}) \pm (20 \times 10^{-5})$ in the presence of PMS. It is also known that the original strain TM677 used in the present bioassay does not lack nitroreductase; 1-nitropyrene (1-NP), 4-NP, 1,3-dinitropyrene (1,3-DNP), 1,6-DNP, and 1,8-DNP are all potent -PMS mutagens in TM677, which was constructed in our laboratory from one of Ames' deep rough mutants (*2*, *35*).

Results

Mutagenicity of Source Samples in Bacterial Assay. The source samples listed in Table 1 were tested in the *S. typhimurium* forward mutation assay. The results are summarized in Table 2. Entries indicated by a plus sign are considered to be mutagenic within the context of this assay, having produced a mutant fraction greater than the concurrent negative control such that 99% confidence intervals on both the sample mean response and its concurrent negative control do not overlap and having produced a mutant fraction that exceeds the 95% confidence limits of the historical negative controls (14×10^{-5}). These criteria are sufficiently stringent so that there is great confidence that false positive test results are excluded. The mutant fraction and relative survival for each source sample at the various equivalent organic carbon levels (μg of EOC/100 μL of bacterial suspension) tested are shown in Table A in the supplementary material.

Each of the combustion source samples tested is mutagenic in this bioassay. The relationship between increasing mutant fraction and increasing equivalent organic carbon dose provided to the bioassay for the case of the motor vehicle exhaust samples is shown in Figure 1a,b. The mutagenic response of cigarette smoke aerosol also is shown in that figure for reference purposes. The vehicle exhaust samples all are more mutagenic than cigarette smoke per unit of equivalent organic carbon.

Gasoline engine and diesel engine exhaust samples when tested in the Ames assay with strains TA98 and TA100 generally are found to be mutagenic both in the presence and in the absence of PMS (*37–43*). There is considerable variation between studies. The present work suggests that all three vehicle types emit mutagens of comparable activity per mass of organic carbon (OC) emitted. OC

Table 2. Summary of Bioassay Results, Showing Those Source Effluents and Ambients Aerosol Samples That Tested Positive (+) or Negative (-)[a] in *S. typhimurium* Forward Mutation Assay, with or without PMS

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

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

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

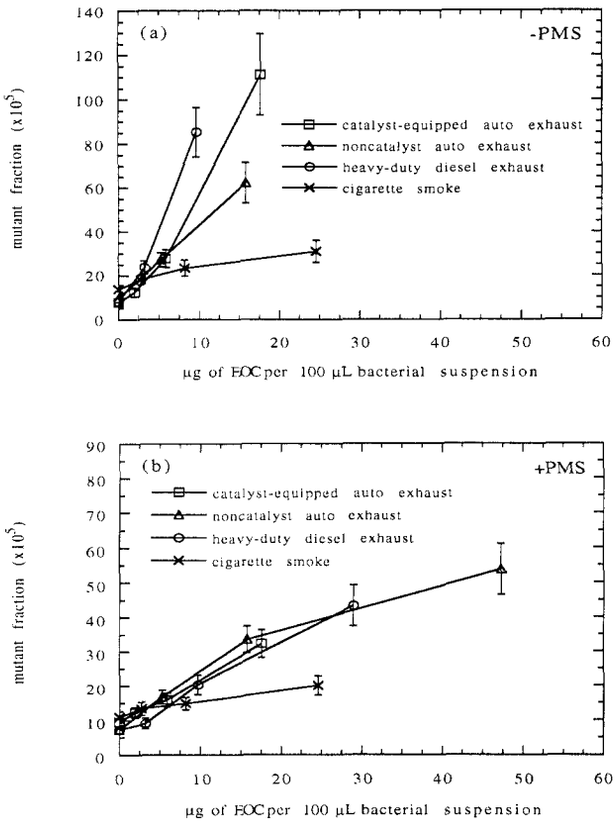

Figure 1. Vehicle exhaust sources: (a) dose–response curves without PMS; (b) dose–response curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

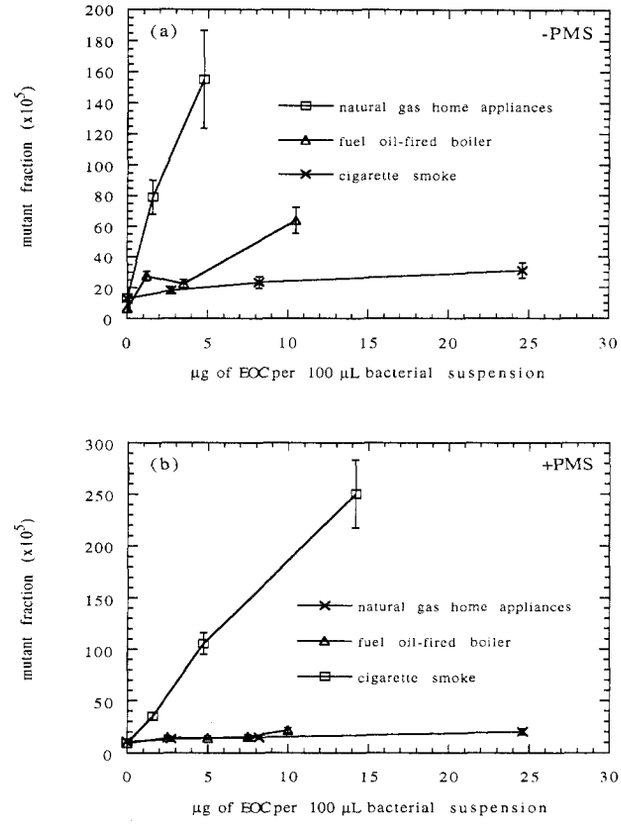

Figure 2. Stationary combustion sources: (a) dose–response curves without PMS; (b) dose–response curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

emission rates per kilometer driven are higher for the diesel trucks tested in the present study (132.9 mg of OC/km) than for the noncatalyst autos (38.9 mg of OC/km) or for the catalyst-equipped cars (9.0 mg of OC/km). On the basis of mutagenic activity per kilometer driven, the emissions from the diesel trucks would exceed that of the noncatalyst cars which in turn would exceed that of the catalyst-equipped autos.

Stationary fossil fuel combustion sources tested included a distillate oil-fired industrial scale boiler and natural gas-fired home heaters (a space heater plus a water heater). The natural gas home appliance aerosol showed the highest mutagenic response per microgram of EOC of all the sources tested. As seen in Figure 2a,b, its specific mutagenicity was about 30×10^{-5} (mutant fraction)/μg of EOC supplied to 100 μL of bacterial suspension –PMS and about 20×10^{-5} (mutant fraction) +PMS. The fuel oil-fired boiler shows a –PMS mutagenicity per microgram of EOC between that of natural gas aerosol and cigarette smoke and a +PMS mutagenicity per microgram of EOC about equal to that of cigarette smoke, as seen in Figure 2a,b. Hildemann *et al.* (*24*) found that the natural gas-fired home appliances tested emitted 0.0389 μg of OC/kJ of fuel burned while the fuel oil-fired boiler tested emitted 0.450 μg of OC/kJ. Even though the OC emission rate from the natural gas combustors tested is more than an order of magnitude smaller than from the fuel oil-fired boiler, the mutagenicity per unit mass of OC emitted by the natural gas appliances is nearly an order of magnitude higher than from the fuel oil-fired boiler. Thus, it appears that the natural gas home appliances cannot be neglected relative to the oil combustion source even though natural gas combustors are often thought to be particularly "clean" sources.

The mutagenic response of the wood smoke samples tested is shown in Figure 3a,b. Each of the whole wood smoke samples is mutagenic in the present bioassay. At the highest levels of EOC supplied, the whole wood smoke samples typically were toxic to the bacteria used in the bioassay, as evidenced by the low survival rates at high EOC doses shown in Table A in the supplementary material. In an attempt to examine a less toxic fraction of the wood smoke, a nonpolar fraction of each source sample was prepared, as described earlier in this text. The nonpolar fractions proved to be less toxic but also less mutagenic than the whole wood smoke samples, as seen in Table A in the supplementary material and in Figure 3.

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

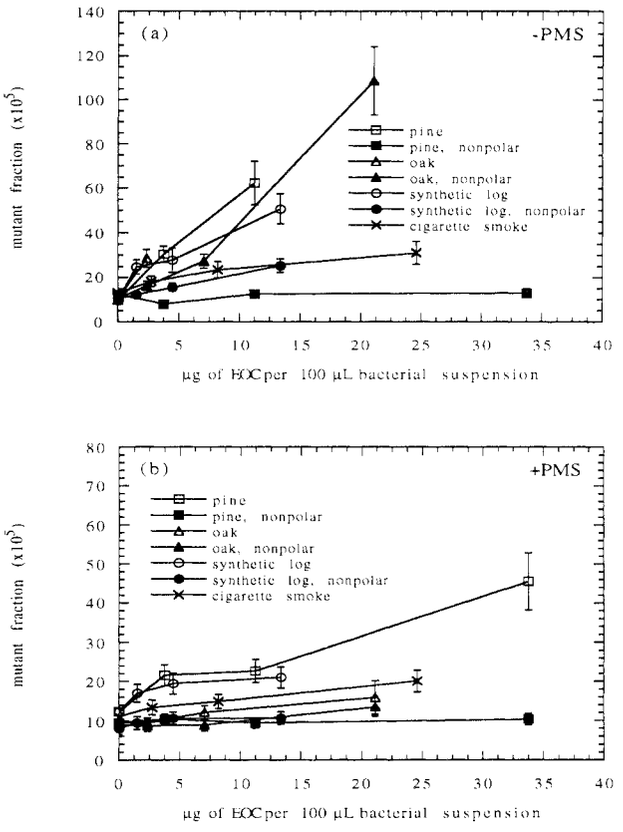

Figure 3. Fireplace combustion of wood: (a) dose–response curves without PMS; (b) dose–response curves with PMS. Cigarette smoke shown for purposes of comparison. Error bars represent 99% confidence limits.

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

Cigarette smoke is a known carcinogen (*48*). Kier *et al.* (*12*) and Mizusaki *et al.* (*13*) have found that cigarette smoke is mutagenic in the presence of PMS using the Ames assay, strains TA1538 and TA1535. Albert *et al.* (*43*), Claxton *et al.* (*49*), and Asita *et al.* (*50*) have found that cigarette smoke is mutagenic in the Ames assay, strain TA98. The present study also indicates that it is mutagenic in the *S. typhimurium* forward mutation assay both with and without PMS. It is interesting to compare the mutagenic activity of cigarette smoke to that of the other combustion sources which have been discussed previously. Notice in Figures 1–3 that the dose-response curve for cigarette smoke has been added as a reference. The cigarette smoke shows weaker mutagenic response per mass of EOC than all of the vehicle exhaust aerosols, both with and without PMS, and all the whole wood smokes except oak, both with and without PMS. This agrees with previous work. Albert (*43*) found that cigarette smoke is less mutagenic than diesel engine exhaust and noncatalyst automobile exhaust, and Asita *et al.* (*50*) found that various wood smokes are as mutagenic or more mutagenic than cigarette smoke.

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

Turning to the noncombustion source samples tested, it was found that those samples generally were not mutagenic in the present bioassay. As seen in Figure 4, organic extracts of tire wear debris, brake lining wear particles, and paved road dust all show mutagenic responses that fall below the upper confidence limit on the historical negative control (i.e., below a mutant fraction of 14×10^{-5}) both +PMS and –PMS. The asphalt roofing tar pot aerosol sample shows a barely detectable –PMS mutagenic response at the highest EOC dose examined. Albert (*43*) studied roofing tar pot aerosols in the Ames assay, strain TA98, and found them to be about as mutagenic as cigarette smoke. This result is similar to the results obtained in the current study. The charbroiled burger smoke and paved road dust samples both contain measurable amounts of polycyclic aromatic hydrocarbons (PAHs) (*55*, *56*), yet they do not produce a positive mutagenic response in the present assays. This raises the question, "Were the PAH levels in those samples too low to produce a clear mutagenic response?" To answer that question, the quantity of individual PAH present in the hamburger smoke and road dust samples as well as in all other source samples was computed based the chemical analysis of these samples reported by Rogge (*57*). The individual PAH contained in the hamburger smoke and road dust samples were determined to be insufficient to produce a mutagenic response at the level supplied to the present assay. Indeed, the individual ordinary PAH present in *all* of the source samples analyzed here were at least an order of magnitude lower than the doses required to produce a mutagenic response (as judged by the administration of pure compounds like benzo[*a*]pyrene (BaP) to the assay). *Therefore compounds present in the combustion source effluents in addition to the routinely monitored ordinary PAH would appear to be responsible for most of the mutagenicity of the samples in the present assays.* PAH such as BaP indeed are mutagenic in the present assay if one supplies enough BaP to the assay (see the earlier discussion of the concurrent positive control tests). Therefore, if higher EOC doses were supplied to the bioassay, the charbroiled meat smoke and road dust source samples would be expected to be mutagenic. All of the noncombustion sources are clearly less mutagenic than the combustion sources per unit of

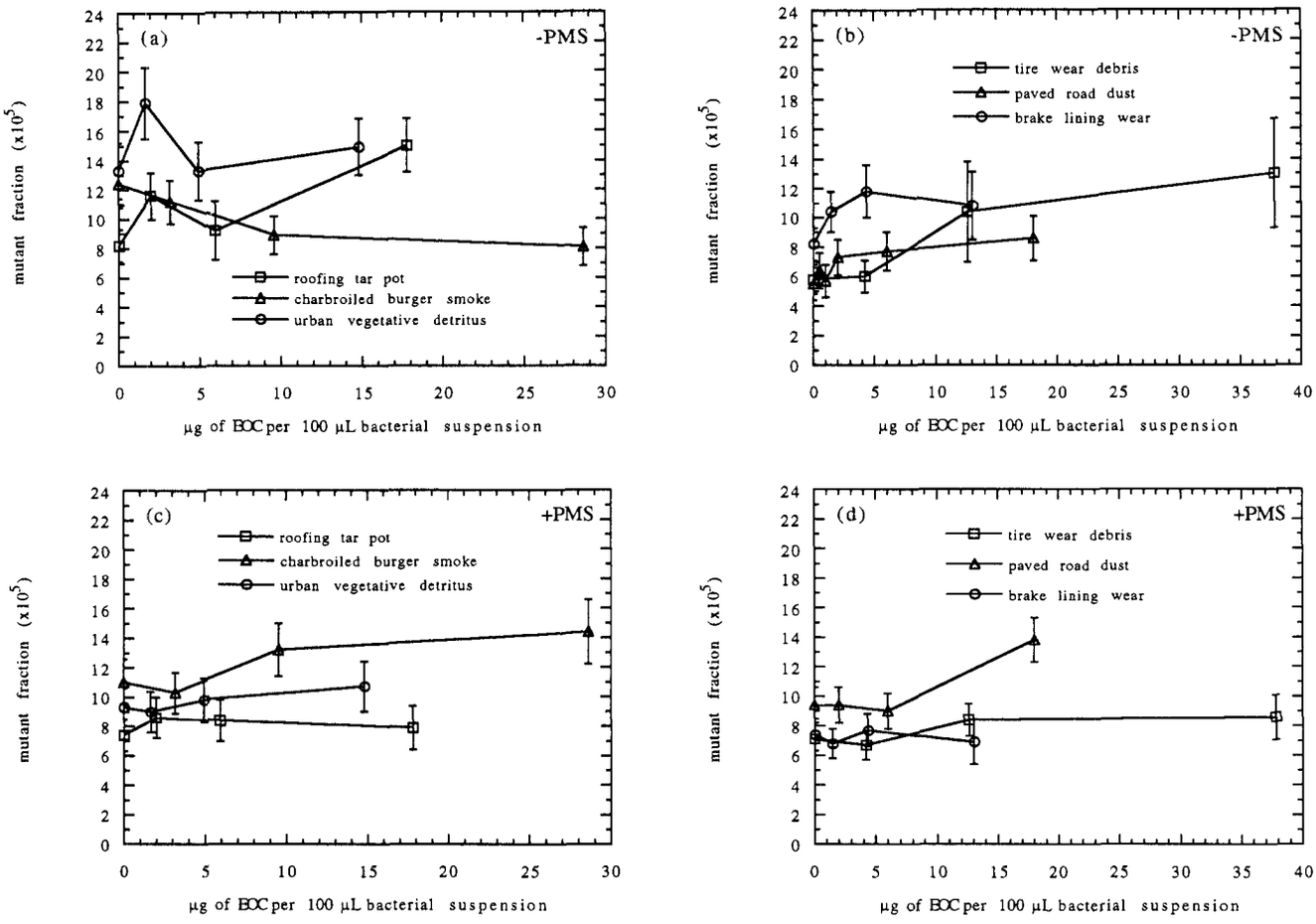

Figure 4. Fugitive dust sources and other noncombustion sources: (a) and (b) dose–response curves without PMS; (c) and (d) dose–response curves with PMS. Error bars represent 99% confidence limits.

EOC used in the bioassay.

Mutagenicity of Ambient Samples in Bacterial Assay. Ambient fine aerosol samples from Azusa and Long Beach, CA, aggregated into composites of 3-months duration were tested in the *S. typhimurium* forward mutation assay, and the results are summarized in Table 2. All eight of the ambient sample composites showed some mutagenic activity in the absence of PMS; however, due to sample toxicity, only seven samples met the statistical criteria for declaration of a positive mutagenic response for the -PMS test condition. In the presence of PMS, the ambient samples showed less mutagenic activity, with only five of the eight samples meeting the statistical criteria for declaration of a positive mutagenic response. The mutant fraction and relative survival for the bacteria exposed to each ambient sample at the EOC levels tested are shown in Table B in the supplementary material. Dose–response curves for the ambient aerosol samples taken at Azusa and Long Beach are shown in Figure 5.

Most of the ambient aerosol samples tested here showed high -PMS mutagenic activity. Many past studies also have found that ambient air samples are mutagenic in the Ames assay, strain TA98. Tokiwa *et al.* (*6*) detected Ames assay, strain TA98, mutagens in both industrial and residential ambient air samples in Japan. Dehnen *et al.* (*7*) found mutagens in the airborne particulate matter in industrialized Germany using the Ames assay, strains TA98 and TA1537.

Our study also indicates that there is a seasonal variation in the ambient particulate mutagenicity. At both Azusa and Long Beach, the samples aggregated from January to March and from April to June show the highest mutant fractions. Alfheim *et al.* (*58*) found a peak seasonal direct-acting mutagenicity from December through March in 1978 in Stockholm using the Ames assay, strain TA98. Flessel *et al.* (*59*) investigated the mutagenicity of four month composite samples from 1979 to 1988 in the San Francisco Bay area using the Ames assay, strain TA98, with and without PMS, and also found the winter composites to have the greatest mutagenicity. The seasonal variability of the ambient aerosol samples from Azusa and Long Beach also indicate a greater mutagenicity during the colder months than during the summer. The apparently negative results obtained during the summer months in some cases in the present study may be due to the small amount of ambient aerosol available for testing. The recommended OC dose to the bacterial mini-assay should be at least 10 μg/100 μL of bacterial suspension before a negative result is considered to be conclusive; less than 10 μg of OC/100 μL of bacterial suspension was available in some of the ambient samples examined. Very high toxicity also was found at low EOC levels in the July–September sample at Long Beach, thereby preventing that dose response curve from being extended to higher EOC levels.

Comparison of Source and Ambient Aerosol Mutagenicity

Fine organic aerosol emissions within an 80 km by 80 km study area centered over Los Angeles have been reported for the year 1982 by Gray (*60*) and by Hildemann *et al.* (*24*). That study area, shown in Figure 5 of ref 55,

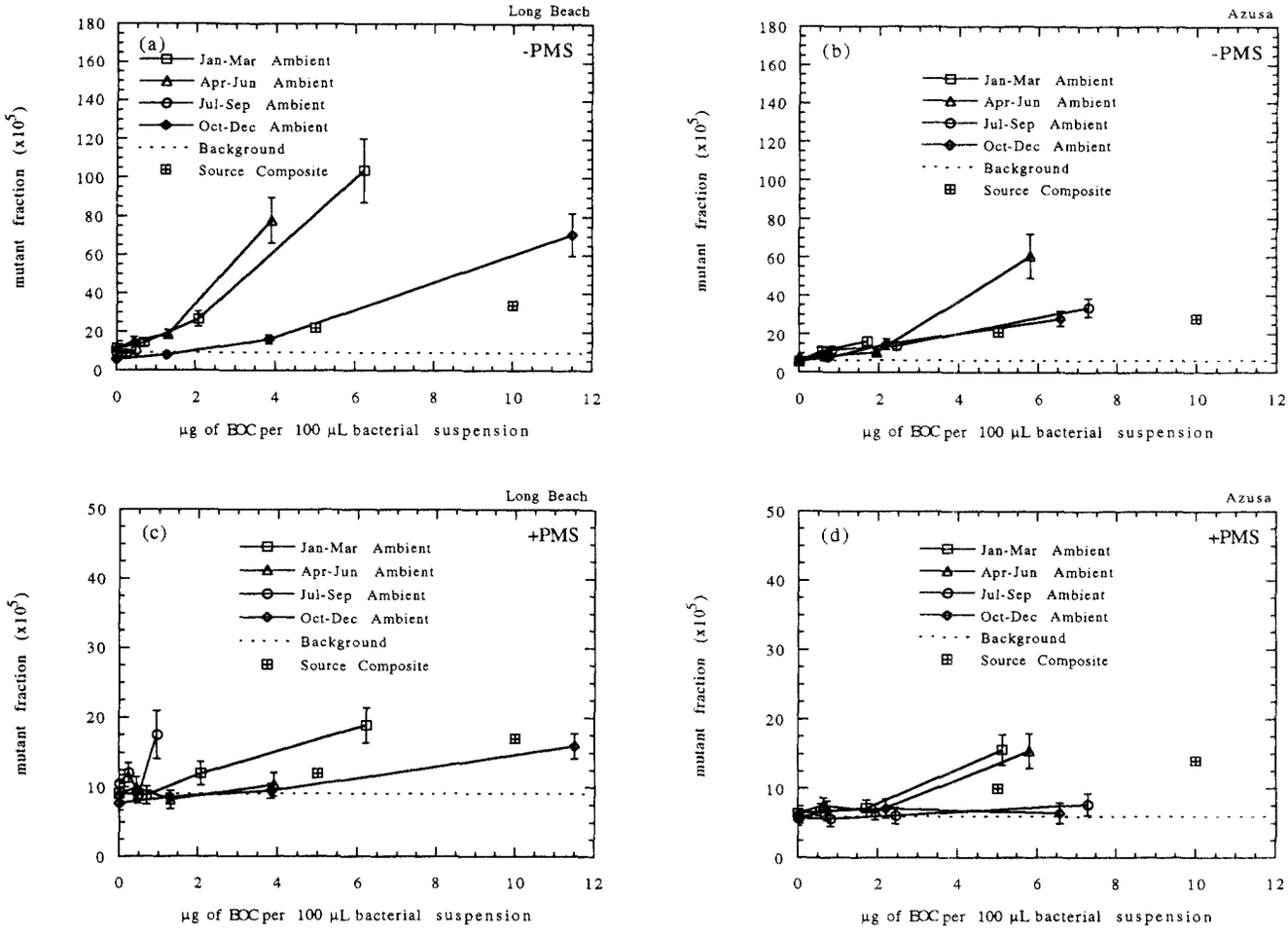

Figure 5. Ambient aerosol quarterly composites: (a) dose–response curves at Long Beach without PMS; (b) dose–response curves at Azusa without PMS; (c) dose–response curves at Long Beach with PMS; (d) dose–response curves at Azusa with PMS. Error bars represent 99% confidence limits.

contains both the Long Beach and Azusa monitoring sites examined here. While more than 70 different source types contribute organic aerosol emissions in that area, the 15 source types studied here account for nearly 70% of the direct organic particle emissions from sources and can be used to represent approximately 80% of the primary emissions if sources similar to those tested are included by analogy (e.g., by the assumption that medium duty noncatalyst gasoline vehicle exhaust behaves like light-duty noncatalyst gasoline vehicle exhaust; see Table 1).

The specific mutagenicity (mutant fraction per microgram of EOC supplied to each 100 μL of bacterial suspension) was examined separately for each source sample, both –PMS and +PMS. The source samples tested were first divided into two groups: (1) those that are mutagenic at the EOC levels tested here and (2) those that are not. The nonmutagenic samples do not necessarily show a linear dose–response relationship, instead their response may reflect the noise contributed by the background mutation rate of the bioassay. This does not matter because those sources do not contribute to the comparison of source sample to ambient sample mutagenicity. Those samples that are mutagenic in the present assay generally show quite linear trends, with increasing mutant fraction as the quantity of EOC supplied to the test is increased when adjusted for toxicity as discussed earlier. A linear least squares regression equation describing mutant fraction as a function of EOC supplied to the bioassay was fit to each of the source sample data sets for those samples listed as positive in Table 2 and having survival greater than 10%. Of the 19 regression equations fit to source samples shown as positive in Table 2 (both +PMS and –PMS), 12 data sets fit a straight line so closely that the correlation coefficient is greater than 0.98; four more show correlation coefficients in the range 0.95–0.98, one in the range 0.91–0.92, and two in the range 0.82–0.85. The data sets with correlation coefficients in the range 0.82–0.85 are from the synthetic log smoke (+PMS) and the roofing tar pot (–PMS) experiments. The roofing tar pot effluent is barely positive in the present assay, and being close to the level of the historical negative control mutation rate, it is expected that that sample set will show greater variability due to the influence of the background mutation rate variability within the bioassay. The synthetic log smoke sample does not account for significant emissions to the atmosphere and will not be used in our forthcoming analysis as shown in Table 1; any nonlinearities in that data set are unimportant.

Graphs showing the least squares fit between mutant fraction and equivalent organic carbon dose were entered at a dose of 5 μg of EOC/100 μL of bacterial suspension. Next, the mutant fraction attributable to background mutations was removed from each mutagenicity value by subtracting the mutant fraction observed in the concurrent negative control sample during each assay. The background-subtracted mutant fraction values then were converted to specific mutagenicities in terms of mutant fraction per microgram of EOC supplied to each 100 μL

of bacterial suspension. A weighted average of these background-subtracted specific mutagenicity values was calculated separately for the –PMS and +PMS data sets, weighted in proportion to the percentage contribution of each of the primary source types to the overall primary inventory of organic carbon aerosol emissions to the study area atmosphere in 1982, as shown in Table 1. The emissions-weighted average specific mutagenicities were scaled to the equivalent use of 5 and 10 μg of EOC/100 μL of bacterial suspension and then added to the average of the background mutant fraction values observed during the ambient aerosol bioassays. The results are plotted as the source composite shown in Figure 5a–d. The resultant values simulate the effect of a 100-μL bioassay conducted on a sample of size 5 and 10 μg of EOC, respectively, assembled by pooling the source samples in proportion to their emission rates to the local atmosphere, under the hypothesis of a linearly additive bioassay response at the low levels of EOC used (*14*). The purpose here is not to suggest that the atmospheric aerosol is in fact a purely linear combination of the sources or that the bioassay is precisely linear and additive. Indeed, the formation or destruction of mutagenic organics by atmospheric chemical reactions is to be expected. The purpose is, however, to gain a rough insight into whether or not the atmospheric aerosol is grossly more or less mutagenic than the aerosol mixture contributed by the sources.

From examination of Figure 5, it is seen that the weighted average +PMS mutagenic response of the primary source samples is comparable to that of the ambient samples. The +PMS activity of both the ambient samples and the source composites is lower than the –PMS activity of the same sample sets. The principal departure from the rough equivalence between the source composites and the ambient samples occurs at Long Beach, where the –PMS mutant fraction found for the ambient samples taken in the first half of the calendar year is much higher than can be explained by an emissions-weighted average of the primary sources studied here. The –PMS mutagenicity of the April–June ambient aerosol at Azusa also noticeably exceeds that of the source composite. Additional months at Long Beach or Azusa also might have shown such a result if larger amounts of ambient organic aerosol had been available for use in the bioassay. This excess –PMS mutagenicity is likely due to atmospheric transformations. Past studies (*20, 61*) have demonstrated that atmospheric chemical reactions involving PAH will create potent –PMS bacterial mutagens. Further field experiments designed to systematically collect much larger amounts of atmospheric aerosol in order to extend the graphs in Figure 5 to higher EOC levels are suggested.

Conclusions

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

Analysis of atmospheric fine particle samples from Azusa and Long Beach, CA, demonstrates in most cases that airborne fine particles also are mutagenic in the *S. typhimurium* forward mutation assay. The specific mutagenicity of the ambient aerosol samples (mutant fraction per microgram of organic carbon supplied to the bacterial assay) can be compared to a weighted average of the specific mutagenicities of the source samples, assembled in proportion to their emission rates in the Los Angeles area. That comparison, given in Figure 5, shows that the +PMS activity of the ambient samples and the source composites is comparable. The magnitude of the –PMS mutagenic activity of the Long Beach ambient aerosol in the first half of the calendar year and at Azusa during the April–June period is much greater than can be explained by the direct emissions from the sources studied here. This may indicate atmospheric transformation of some source effluents to form more potent bacterial mutagens. Further chemical analysis of subfractions of source and ambient aerosol samples should be conducted to confirm whether these similarities and differences persist as samples are subdivided in a way that may help to identify the particular compounds or compound classes responsible for the observed mutagenic response.

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Supplementary Material Available

Two tables showing the data for each experiment in terms of organic carbon supplied to the assay, resulting mutant fraction, and survival (5 pages) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms Office, American Chemical Society, 1155 16th St. NW, Washington, DC 20036. Full bibliographic citation (journal, title of article, names of authors, inclusive pagination, volume number, and issue number) and prepayment, check or money order for $15.00 for photocopy ($17.00 foreign) or $12.00 for microfiche ($13.00 foreign), are required. Canadian residents should add 7% GST.

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