Wood Smoke: Measurement of the mutagenic activities of its gas- and particulate-phase photooxidation products

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · MAY 1986

Impact Factor: 5.33 · DOI: 10.1021/es00147a009 · Source: PubMed

Environ. Sci. Technol. 1986, 20, 493–501

Wood Smoke: Measurement of the Mutagenic Activities of Its Gas- and Particulate-Phase Photooxidation Products

Tadeusz E. Kleindienst,* Paul B. Shepson, and Edward O. Edney

Northrop Services, Inc.—Environmental Sciences, Research Triangle Park, North Carolina 27709

Larry D. Claxton

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

Larry T. Cupitt

Atmospheric Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

■ Dilute mixtures of wood combustion emissions (with and without additional NO_x) were irradiated in a 22.7-m^3 Teflon smog chamber. The effluent was tested for mutagenic activity by exposing *Salmonella typhimurium*, strains TA100 and TA98, to the filtered gas-phase components. The particulate matter was tested by using the plate incorporation procedure. Without added NO_x, irradiated dilute wood smoke showed a measurable increase in mutagenic activity for gas-phase products only. Additional NO_x was added in other irradiations to enhance the formation of gas- and particulate-phase products. Although only lower and upper limits were obtainable, the gas-phase products showed considerably more activity (1.1–8.2 revertants/μg) in TA100 exposures than did the particulate product extracts. With TA98 the activities of both phases were comparable on a mass basis. Since the total quantity of gas-phase components was greater than the particulate-phase components, the mutagenic activity on a volume basis was greater for the gas phase.

Introduction

Recently, there has been increased concern that human exposure to wood stove and fireplace emissions may provide a public health concern. In a study of the Denver "brown cloud", Wolff et al. (*1*) determined that approximately one-third of the organic fine particulate matter (<2.5 μm) from Denver aerosol could be attributed to wood smoke emissions. Several studies have recently appeared (*2–4*) in which particulate extracts from wood smoke have been tested for mutagenic activity using the *Salmonella*/mammalian microsome reversion assay (*5*). These studies have generally shown low direct-acting mutagenic activity with *Salmonella typhimurium* strain TA98, although Dasch (*2*) reports substantial increases with the addition of S9 metabolic activation.

Kamens et al. (*6*) have reported substantial increases in the direct-acting mutagenic activity (TA98) of wood smoke when it was allowed to react in the dark with NO_2 and O_3. This study (as well as those in ref 3 and 4) focused on the particulate matter only, with emphasis on the mutagenic activity of the polycyclic aromatic hydrocarbons (PAHs). More recently, Kamens et al. (*7*) have determined that the PAH fraction of the particulate extract accounts for only a small fraction of the total mutagenic activity in both the reacted and unreacted wood smoke. The majority of the mutagenic activity resided in the most polar fractions of the extracts.

Two potentially important aspects of the health impact of wood smoke have not yet been addressed fully: (1) what is the effect of photooxidation reactions on the mutagenic activity of wood smoke, and (2) what is the relative mutagenic activity of the gas-phase reactants and products compared to those of the particulate phase?

We have previously undertaken a series of studies to examine the mutagenic activity of product mixtures formed in the NO_x photooxidations of several hydrocarbons. We have found that the gas-phase photooxidation products from an ethylene (C_2H_4)/NO_x irradiation are nonmutagenic to *S. typhimurium* strains TA98 and TA100 except for a small contribution in TA100 due to formaldehyde (HCHO) (*8*). Simple gas-phase hydrocarbons, however, such as propylene (C_3H_6) and toluene (C_7H_8) are converted to gas-phase photooxidation products that exhibit strong mutagenic activity with strains TA98 (*9*) and TA100 (*9*, *10*). In each of these systems, a large fraction of the total mutagenic response could not be accounted for on the basis of the mutagenic activities of individual products. In another study (*11*), strains TA98 and TA100 were exposed to irradiated mixtures of ethylene/aniline/NO_x. In this case, the gas-phase products showed no activity in either strain, but a sizable response was observed in TA98 from extracts of particulate matter formed during the irradiation.

Since there are significant amounts of toluene and propylene in the gas-phase wood smoke emissions (*3*), it would seem likely that the gas-phase photooxidation products of wood smoke would show some activity and are thus worthy of consideration. In this paper, we report the results of several experiments in which the total mutagenic response in TA98 and TA100 was measured for dilute mixtures of wood smoke irradiated in the presence and absence of added NO_x. The mutagenic activities of the reactants and products were determined by using a modified version of the Ames test (*9*) for gas-phase species and the standard plate incorporation test (*5*) for particulate-phase species. The data are analyzed to allow a comparison to be made between the relative amounts of mutagens in the gas and particulate phases for both reacted and unreacted wood smoke mixtures.

Experimental Section

A schematic diagram of the wood stove dilution system, reaction chamber, and exposure chamber (i.e., biochamber) system is presented in Figure 1. Wood combustion for these experiments was conducted by using an Ashley Model 7150B wood stove. The wood used was a mixture of locally obtained varieties of oak. Since a major objective of this work was to examine the relative mutagenic activity of the gas- and particulate-phase components of wood smoke, no attempt was made to investigate the dependence of the absolute mutagenic activity on burn conditions. This subject has been treated to some extent elsewhere (*3*). The wood stove was operated under conditions of relatively fast burn rate (with vents fully open) for all experiments.

0013-936X/86/0920-0493$01.50/0

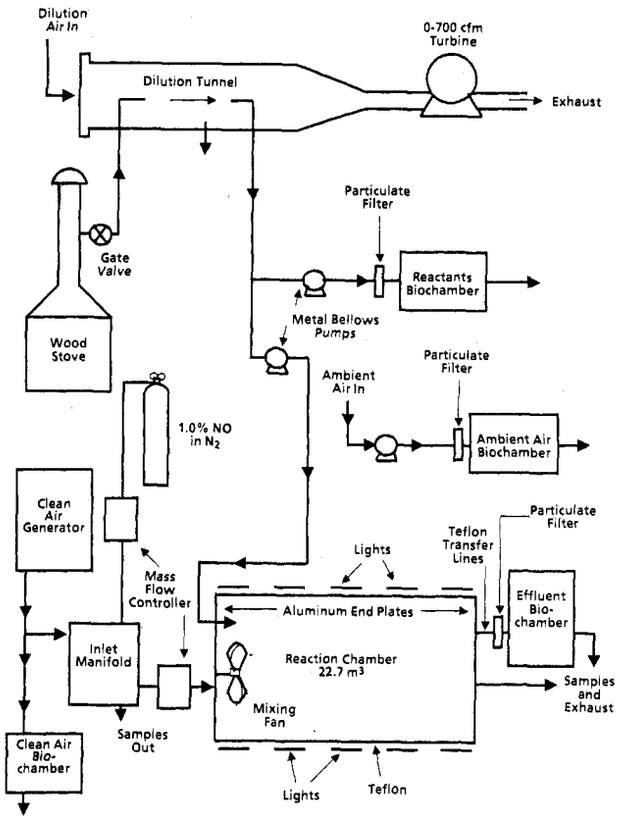

Figure 1. Experimental schematic of the wood stove, reaction chamber, and exposure apparatus.

To load the reaction chamber, a portion of the wood smoke from the chimney was continuously drawn through an 8-cm steel pipe to a dilution tunnel where the wood smoke was diluted with ambient air. The unfiltered ambient air was drawn through the dilution tunnel by using a 0–20 m^3/min turbine. The dilute wood smoke could then be added to the reaction chamber by using a Metal Bellows Corp. Model MB-151 metal bellows pump.

The reaction chamber is a 22.7-m^3 cylindrical vessel constructed of 0.13-mm Teflon that is sealed to aluminum end plates coated with fluorocarbon paint. The chamber is surrounded longitudinally with a combination of sun lamps and ultraviolet (UV) blacklights. Additional details of the reaction chamber are presented elsewhere (*9*). For some of these experiments, additional NO was added to the chamber from a 1% mixture of NO in N_2 (MG Scientific) by metering this mixture through a mass flow controller and allowing it to mix with clean dilution air in the 150-L stainless steel inlet manifold, as shown in Figure 1.

Four 190-L rectangular biochambers were employed for the exposure of *S. typhimurium* to the gas-phase mixtures: a clean air biochamber, a reactants biochamber, an ambient air biochamber, and the reaction chamber effluent biochamber. The air masses were transferred to each of the biochambers through 9.5-mm Teflon tubing at a flow rate of 14 L/min maintained and measured with a needle valve and rotameter. Teflon-impregnated glass-fiber filters (T60A20 Pallflex 13.34 cm) were used to remove particulate matter from the reactants, effluent, and ambient biochamber air masses, as shown in Figure 1. The particulate matter collected on these filters was then used for bioassay of the particulate phase mutagens. Filter loadings from the reactant and effluent air streams ranged from 1 to 10 mg per filter depending on particle concentration and sampling time.

Sample collection for gas-phase and particulate-phase species was done at the end plate opposite the inlet manifold. The analytical methods employed for measurement of NO_x, O_3, peroxyacetyl nitrate (PAN), and the aldehydes were as previously described. Particulate matter concentrations were measured by using a condensation nuclei counter (CNC) (*9*).

Methane, acetylene, ethylene, and ethane were measured with a Varian 1400 gas chromatograph (GC) using a 3.2 mm × 2 m stainless steel column packed with 60/80 Carbosieve G, operated isothermally at 150 °C. Chloromethane, propylene, propane, and 1-butene were measured with a Varian 1200 GC using a 6.4 mm × 2 m stainless steel column packed with 80/100 Porapack QS, operated isothermally at 130 °C. Isoprene, furan, and 2-methylfuran were measured with a Hewlett-Packard Model 5840A GC using a 2 mm × 2 m glass column packed with 0.1% SP-1000 on 80/100 Carbopack C, temperature programmed from 40 to 200 °C at 20 °C/min. Sample injection for these three chromatographs was performed with Seizcor gas-sampling valves with 5-mL sample loops. Calibrations were conducted by diluting samples of the pure compound with zero air in 100-L Teflon bags to concentrations in the low parts per million range. Benzene, toluene, xylenes, benzaldehyde, styrene, and *m*-methylstyrene were measured by pumping 25-L samples through Pyrex tubes packed with Tenax GC. The Tenax-filled tubes were then thermally desorbed at 275 °C with a Nutech 320 thermal desorption unit. The desorbed samples were analyzed with a Hewlett-Packard 5985 GC/MS containing a 2 mm × 2 m glass column packed with 10% SP-1000 on 80/100 Supelcoport. The column was temperature programmed from 50 to 225 °C at 20 °C/min. Calibrations were performed by desorption of Tenax tubes containing samples of the pure compounds at the 1-nmol level. CO was measured on a Bendix Model 8501-5CA infrared CO analyzer. SO_2 was measured on a Meloy Labs Model SA285 sulfur analyzer. Total hydrocarbon (HC) content was measured on a Beckman Model 400 HC analyzer. The HC analyzer was calibrated in units of ppm of carbon (ppm of C) using pure samples of propane. Aerosol size distributions (0.01–1 μm) were measured with a Thermo Systems, Inc., Model 3030 electrical aerosol analyzer (EAA).

The particulate-phase filter samples were Soxhlet extracted with 250 mL of pesticide-grade methylene chloride (Fisher Scientific) for 6 h. The extracts were then concentrated under a stream of prepurified N_2 (MG Scientific) and analyzed for PAH concentration by using the GC/MS by injection onto a 2 mm × 2 m glass column packed with 3% SP-2250 on 80/100 Supelcoport. The column was programmed from 200 to 300 °C at 10 °C/min. For the compounds measured the GC/MS peak areas were measured relative to pyrene and quantified as pyrene. (The reported concentrations are, therefore, only accurate to within a factor of 2. However, this degree of accuracy was deemed adequate for the purpose of this study.) The extracts were prepared for bioassay by solvent exchanging a portion of the methylene chloride extract with 2-mL samples of dimethyl sulfoxide (Burdick & Jackson Laboratories, Inc.). Blank filters were also extracted and served as both chemical and bioassay controls.

The bacteria *S. typhimurium*, strains TA100 and TA98, were used as the biological assay for this study. (Strain TA100 mutates by a base-pair substitution mechanism and TA98 by a frameshift mechanism.) The plates were prepared by adding 0.1 mL of the *S. typhimurium* culture to 3 mL of an agar overlay at 45 °C, with or without 0.5 mL

of S9 mix. This mixture was then poured onto ~45 mL of plate agar in a Pyrex glass petri plate. The *S. typhimurium* tester strains were provided by Dr. Bruce Ames (University of California, Berkeley, CA). Colony counting was done with an Artec 880 automatic colony counter using previously published guidelines (*12*). The test procedures used were those of Ames et al. (*5*), except for the following modifications: (1) glass Petri dishes were used, (2) 45 mL of base agar per plate was used, (3) minimal histidine at the same final total concentration was placed in the bottom agar rather than the top agar, and (4) 3 mL of overlay agar with ~1 × 10^8 bacteria was used. The rat liver homogenate (S9) fraction was from male Charles River CD-1 rats (Wilmington, MO) induced with Aroclor 1254 (*5*).

The particulate extract bioassays were performed by using the standard plate incorporation test. Bioassay of the gas-phase species was conducted by exposing uncovered test plates to the air masses that flow through the biochambers. Plate dosage occurs as a result of gas-phase species (particularly those that are oxygenated or polar) dissolving into the agar during the exposure, as described in detail in a previous publication (*10*).

The experiments were conducted by first loading the reaction chamber to a total HC level of ~18 ppm of C. The residence time of the air mass in the reaction chamber was ~6 h during loading, and therefore, the final air mass represents an integration of wood stove burn conditions over this time scale. Prior to irradiation in Experiments B and C, NO was slowly added to the chamber until the desired initial concentration of ~0.6 ppm of NO_x was reached. Experiments with added NO were conducted both as a means of slowing the reaction (to facilitate analytical measurements) and of controlling the extent of HC conversion. Once the desired initial conditions were reached, the chamber lights were turned on, thus starting the photochemical reactions. Dilution air was added at ~10 L/min to compensate for an equivalent sampling rate during the irradiation. The irradiations were conducted until the PAN concentration leveled off, indicating that all NO_2 in the system was removed. (This corresponded roughly to the O_3 maximum.) The lights were then turned off, and the final reaction chamber samples were acquired. The exposure of the biological assay to the product mixture was then conducted by flushing the reaction chamber contents through the exposure chamber at a known flow rate. The reaction chamber species concentrations were, therefore, continuously being diluted at this rate. As a consequence of the extreme difficulty in maintaining constant combustion output, this "static" exposure technique was employed, in contrast to the dynamic exposure which has been used in previous work (*9*, *10*). Three other biochambers were employed as controls. They were used to measure the possible mutagenic activity of the clean air, ambient air, and initial (wood combustion) reactants. Once the biochambers were adequately flushed with the appropriate exposure air, the plate covers were removed, effectively starting the exposure. A flow of 14 L/min was maintained through each of the biochambers during the exposure. Groups of plates were covered at various times during the exposure (0–10 h) so that dose–response curves could be constructed. Each biochamber was loaded with 24 test plates and 6 survivor plates for each strain. The survivor plates were prepared by diluting the 3-mL culture so that the bacteria concentration was ~500/plate. Additional histidine was added to the survivor plates so that all bacteria grew to form colonies in the absence of toxic effects. The survivor counts therefore reflect the degree to which the test plates may experience toxic effects.

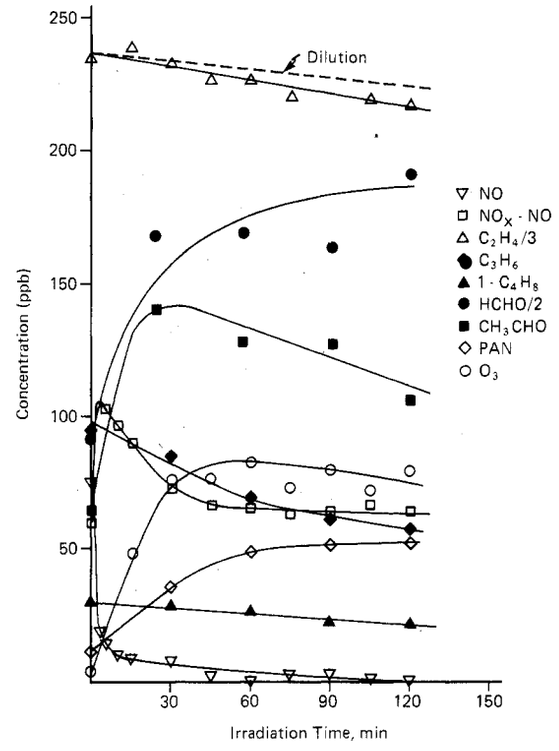

Figure 2. Wood smoke static irradiation. Selected reactant and product profiles.

Dilution air at 20 L/min was added to the reaction chamber during the exposure to account for an equivalent sampling rate for chemical analysis and the effluent feed for the biochamber. The air mass for the reactant biochamber was prepared by maintaining the gas and particulate reactants (in the dilution tunnel) at the same concentration as those in the reaction chamber at the start of the irradiation. This mixture flowed continuously from the dilution tunnel to the reactants biochamber during the course of the exposure. At the end of the exposure, the plates were then covered, removed from the biochambers, and incubated at 37 °C under sterile conditions for 48 h, and the number of revertant colonies per plate was counted. The filters used to remove particulate matter from the biochamber air masses were removed and extracted for the bioassay.

The results for three gas-phase exposures are reported here. In experiment A (April 18), the exposure was conducted with combustion NO_x only at an initial HC and NO_x level of 20 ppm of C and 0.13 ppm, respectively. Experiments B and C (March 7 and March 28, respectively) were performed as duplicates of each other and conducted at initial HC and NO_x levels of ~17 ppm of C and 0.6 ppm, respectively. Several additional static runs were performed, mainly for chemical analysis, for which particulate-phase biological assay data were also obtained.

Results

Wood Smoke Irradiation (Combustion NO_x Only). Figure 2 shows the reactant and product profiles for a number of major species present in the wood smoke, gas-phase system. Since the only source of NO_x was from the wood combustion, the HC/NO_x ratio was extremely high (~100/1). The photooxidation proceeded very rapidly, but the extent of reaction was severely limited by the lack of NO_x. The light intensity corresponded to a photodissociation rate for NO_2 of approximately 0.3 min^{-1} during the 2-h irradiation. (A reasonable solar zenith angle under wintertime conditions of approximately 60° during

Table I. Individual Gas-Phase Inorganic and Hydrocarbon Concentrations[a] (ppb)

species	A (April 18) initial	A (April 18) final	B (March 7) initial	B (March 7) final	C (March 28) initial	C (March 28) final
nitric oxide	75	0	454	0	461	0
NO_x	135	64	657	252	576	259
ozone	0	79	0	467	0	696
carbon monoxide, ppm	33.5	32.0	38.0	33.4	38.7	35.5
methane	–[b]	–	5060	4500	4480	3920
ethane	–	–	146	135	–	–
propane	–	–	9	9	–	–
ethylene	702	652	537	313	847	439
propylene	92	56	126	8	100	7
1-butene	30	22	58	3	39	0
isoprene	7	0	–	–	7	0
acetylene	–	–	177	155	–	–
benzene	68	62	62	50	102	68
toluene	27	16	62	15	24	10
m- + *p*-xylene	14	7	40	19	12	3
o-xylene	5	3	13	4	2	0
styrene	10	2	22	0	7	0
m-methylstyrene[c]	5	–	9	0	3	0
furan	49	21	71	0	59	0
2-methylfuran	80	23	61	0	64	0
formaldehyde	325[d]	381	269	365[e]	229	383
acetaldehyde	140[d]	106	88	109[e]	57	75
acetone	50[d]	41	–	–	–	–
acrolein	35[d]	22	–	–	–	–
benzaldehyde	–	–	15	12	–	–
2-furaldehyde	48[d]	19	31	2[e]	22	6
glyoxal	24[d]	16	41	45[e]	26	24
methylglyoxal	–	–	41	36[e]	13	16
biacetyl	22[d]	25	–	–	7	5
PAN	0	52	0	174	0	232
chloromethane	–	–	18	18	–	–
HC,[f] ppm of C	20.6	19.1	16.4	13.2	17.2	15.0

[a] Other gas-phase species identified: phenol, butadiene, methyl acetate, 2-methyl vinylacetylene, methyl vinyl ketone, methyl ethyl ketone, and benzofuran. [b] Not measured. [c] Tentatively identified based on mass spectrum. [d] At 0.4-h irradiation time. [e] At 1.5-h irradiation time. [f] Corrected or dilution.

Table II. Particulate Extract Data (ng/mg of Particulate)[a]

species	A (April 18) initial	A (April 18) final	B (March 7) initial	B (March 7) final	C (March 28) initial	C (March 28) final
fluoranthene	647	47	721	70	611	203
pyrene	602	57	672	50	560	218
anthracene + phenanthrene	256	50	457	100	132	89
acenaphthene	454	37	371	30	103	≤100
fluorene	261	33	214	20	224	≤100
chrysene + triphenylene	272	76			100	≤100
CNC ($\times 10^9$ particles/m^3)	18	15	33	28	13	10
EAA (nL/m^3)	137	232	286	591	72	265

[a] Other particulate-phase species identified: 4-hydroxy-3,5-dimethoxybenzaldehyde; 4-hydroxy-3-methylbenzoate; 4-hydroxy-5-methoxybenzoic acid; benzo[*a*]pyrene.

midday would correspond to an atmospheric photolysis rate for NO_2 of about 0.38 min^{-1} (*13*). In general for these experiments the integrated radiation with respect to NO_2 photolysis is less than that expected for an average clear winter day.) As observed in Figure 2, NO_x–NO reached its maximum concentration in less than 5 min, and the O_3 maximum occurred within an hour.

The first two columns in Table I (experiment A) give the concentration of many of the reactants and for some of the products immediately before and after irradiation. In molar quantities, the major reactive HCs included alkenes (ethylene, propylene, and 1-butene), aromatics (benzene, toluene, and xylenes), and oxygen atom heterocycles (furan, 2-furaldehyde, and 2-methylfuran). A large component of the wood stove, gas-phase emissions was aldehydes, the two main species being HCHO and acetaldehyde (CH_3CHO). These two compounds existed as both reactants and products in this system. For example, the oxidation of propylene leads to the production of HCHO and CH_3CHO. The photooxidation of aromatics (toluene and xylenes) has been shown to yield glyoxal ($(CHO)_2$), methylglyoxal (CH_3COCHO), and biacetyl ($(CH_3CO)_2$) (*14*), all of which were also present as reactants.

Physical and chemical characterization of the particulate phase was obtained both before and following irradiation. The number distribution of the aerosols, although not shown, was similar to that observed by other researchers (*2, 6*); i.e., the vast majority of unreacted particulates were less than 1 μm in size with the maximum of the number distribution occuring at approximately 0.1 μm. In experiment A, the maximum in the number distribution shifted to 0.2 μm after irradiation. The volume distribution of the particulate reactants and products as measured by the EAA is given in Figure 3. Although the absolute number of particles following irradiation was smaller than before (Table II), the contribution to the volume (and thus

Table III. Measured Mutagenic Activities of the Gas and Particulate Phases of Irradiated Wood Smoke[a]

	gas (revertants/h)		particulate (revertants/μg)	
	TA100	TA98	TA100	TA98
combustion NO_x				
reactants	−5.9 ± 2.4[b]	(−0.42 ± 0.86)[c]	0.29 ± 0.12	0.20 ± 0.09
products	35.8 ± 5.5	3.37 ± 0.90	(0.10 ± 0.23)	0.26 ± 0.10
added NO_x				
reactants	(1.6 ± 2.7)	(−0.4 ± 1.2)	0.30 ± 0.10	0.22 ± 0.06
products	174 ± 16	30.3 ± 3.7	0.27 ± 0.15	0.94 ± 0.30

[a] Values represent slopes from the dose–response curves (cf. Figures 4 and 6). The uncertainty given is the 95% confidence interval. [b] Survivor data indicate no toxicity. Slight negative response probably a reflection of the large relative uncertainty with a small response. [c] Values in parentheses are zero within the uncertainty.

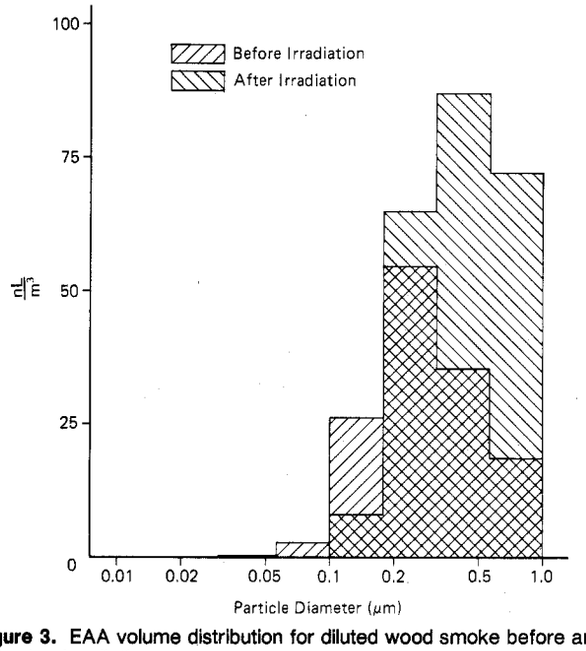

Figure 3. EAA volume distribution for diluted wood smoke before and following irradiation.

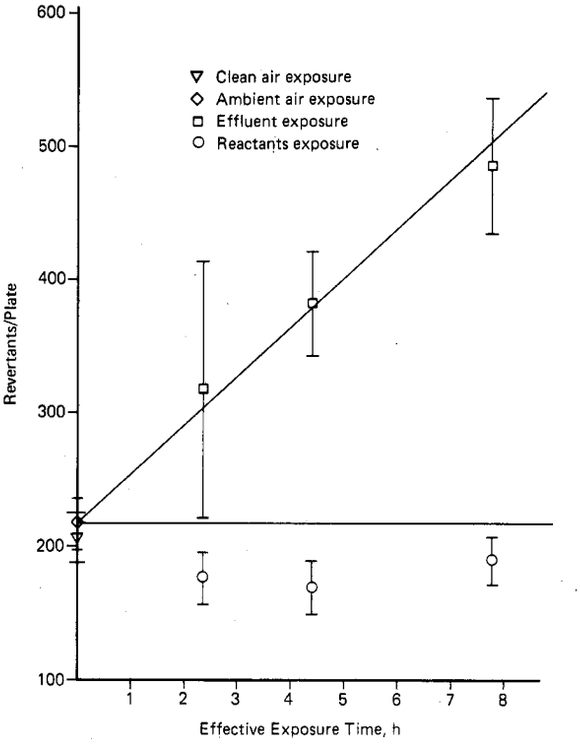

Figure 4. Dose–response curve for the mutagenic activity (TA100) of the gas-phase components of wood smoke before and following irradiation.

the mass) of the particulate matter increases as r^3, giving the volume distribution for products shown in Figure 3 with a peak at 0.4 μm. This increase in volume following irradiation has been observed in all our photooxidation experiments with wood smoke. It would seem likely that the increase in the total volume of particulate matter was the result of polar gas-phase photooxidation products adsorbing onto the particulate matter already present. In the absence of irradiation, neither the number or volume distribution maximum of the particles changed with time while they were in the chamber.

Some particulate-phase organic species have been quantified from analysis of the filter extracts. The results for several PAHs that have been identified are given on a mass per extractable mass basis in Table II. For each compound presented, significant degradation was observed as result of the irradiation. Part of the apparent degradation occurs solely as a result of the increase in particulate mass (due to adsorption from the gas phase) as discussed previously. However, there does appear to be some reaction of the PAHs although no photolysis or reaction products from these compounds were observed.

The bioassay results for the exposure of TA100 to the gas-phase products of experiment A are shown in Figure 4. (As implied by Figure 2, the exposure was conducted for the product distribution at an extent of reaction 2 h). The mutagenic activity of clean air and ambient air are within the experimental error of the background spontaneous levels (188 ± 16). Each dose for the reactants and effluent represents an average from eight plates randomly distributed within the exposure chambers.

For this experiment, the real exposure times were 2.5, 5, and 10 h. However, since there is significant dilution during the course of the exposure (0.055 h^{-1}), the chamber concentrations were continuously reduced exponentially. The exposure times have been corrected to account for these continuously decreasing concentrations. The effective exposure time, t_{eff}, is given as

$$t_{eff} = \int_0^{t_{ex}} \exp(-kt)\, dt \qquad (1)$$

where t_{ex} is the exposure time in real time and k is the dilution rate constant. The longest effective exposure time for a real time of 10 h is 7.78 h. From the slope of the curve in Figure 4, we observe a linear reversion rate with exposure time and calculate a slope of 36 revertants $plate^{-1}$ h^{-1}. The data for all gas phase exposures are presented in Table III.

In Table III we also present the results for the exposure of TA98 to the gas-phase effluent for experiment A. Again for this exposure, the clean air, ambient air, and reactant chamber revertant levels per plate were within the ex-

perimental uncertainty of the spontaneous revertant level (41 ± 6 revertants/plate). The exposure times for the reactant and effluent were the same as for TA100. For both TA100 and TA98, the survivor plates at each exposure time showed no sign of toxicity.

Table III also indicates the mutagenic activity (revertants/μg) of the particulate phase reactants and products for TA100 and TA98, respectively. A filter upstream of the ambient air exposure chamber was also extracted and tested. In no case did the ambient air extracts shows a mutagenic activity above the spontaneous reversion level for either strain, although the total mass collected on the ambient air filters tended to be very small (<100 μg). Each value in the table is based on from four to six extract doses. The particulate extract data for TA100 show no increase in the mutagenic activity of the products over the reactants. For TA98, the products show a slight increase in activity, but it is less than the experimental uncertainty (~0.1 revertants/μg).

Wood Smoke Irradiation with Added NO_x. The extent of reaction in experiment A was so limited as to make an accurate comparison between the gas- and particulate-phase product mutagenicity difficult. Experiments B and C were conducted with additional NO added to reduce the HC/NO_x ratio to approximately 25. Urban ratios usually range from 5 to 15 (*15*). The addition of NO served to convert HO_2 to OH during irradiation, and the oxidation of reactive HCs under the conditions with added NO tended to be more extensive. Product formation was significantly increased since OH was the primary oxidizing species in this system. (The NO_2 produced substantially increased O_3 formation.) This addition of NO_x also had the effect of slowing down the initial reaction rate. Chloromethane was measured since it is one of the few chlorinated compounds generated in wood combustion (*16*), and its potential as an atmospheric tracer has been recognized.

The initial conditions for the experiments with added NO_x are given in Table I under the columns for experiments B and C. The degree of reproducibility of adding wood smoke reactants to this system is indicated by comparing the initial conditions for these experiments. The time profiles of the major reactants and products for experiment B are seen in Figure 5. The increased degree of oxidation of the major alkenes, ethylene, propylene, and 1-butene, is apparent. The formation of O_3 and PAN is extended in time with the concentration maxima occurring past 2 h. The formation of all major products is significantly greater than in the case without added NO_x.

The particulate extract PAH data for these experiments are tabulated in the last two sets of columns in Table II. The upper limits appearing in the experiment C data set reflect the relatively low quantity of particulate matter collected during the exposure (the maximum exposure time was 6 h for this experiment). For these compounds, the GC/MS detection limit was ~10 ng.

The particle size and volume distributions from the EAA exhibited the same characteristics as in the experiments without added NO_x (see Figure 3). For these experiments, the maximum in the volume distribution increased from 0.2 μm before irradiation to 0.4 μm following irradiation. The increase in the total volume of particulate matter as seen in Table II is 2–3 times greater (cf. experiment A), a reflection of the larger extent of product formation with added NO_x.

The mutagenic activity exhibited in strains TA100 and TA98 from the exposure to the gas-phase species is given in the lower half of Table III. In all cases, the clean air

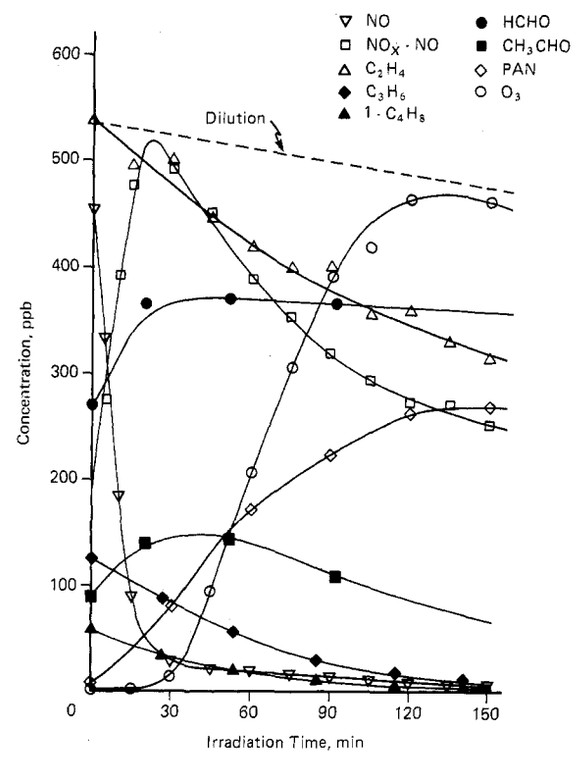

Figure 5. Wood smoke static irradiation with 500 ppb of additional NO_x. Selected producted and reactant profiles.

and ambient air revertant levels were (within experimental error) identical with the spontaneous laboratory controls (TA100:202; TA98:36). The exposure time for each plot has been corrected as previously described. In experiment B, data for an exposure time of 10 h (uncorrected) were also obtained. However, the irradiated mixture was toxic as indicated by depressed revertant levels in the survivor plates. For all other exposure times, no other toxic effects were observed. Systematically higher revertant levels were observed for experiment B. Part of the reason can be attributed to the relatively greater extent of HC conversion for this experiment, as seen in Table I.

The mutagenic activity exhibited with strains TA100 and TA98 for the particulate organic phase is also presented in Table III. As in the case without added NO_x, the extracts from the products show no increased activity over that of the reactants in TA100. Only in TA98 do the product extracts show significantly higher mutagenicity than do the reactants (approximately a factor of 3) as shown in Figure 6. These particulate data are a compilation of filter extracts for all wood smoke/NO_x (added NO_x) irradiations performed during this study. The straight lines drawn through the data represent a least squares fit to all the data.

We also performed exposures of the gas-phase reactants and products and conducted plate incorporation tests for extracts using TA100 and TA98 with metabolic activation (S9). For exposures up to 10 h and extract doses up to 850 μg/plate, no significant difference was observed for the addition of S9 for either reactants or products in both strains when compared to the data without S9. In the interest of presenting results in the most succinct fashion, these data have not been presented.

Discussion

It is apparent from the results of this work that irradiation of wood smoke/NO_x mixtures dramatically increases the mutagenic activity over that of the initial reaction mixture, particularly for gas-phase species. The

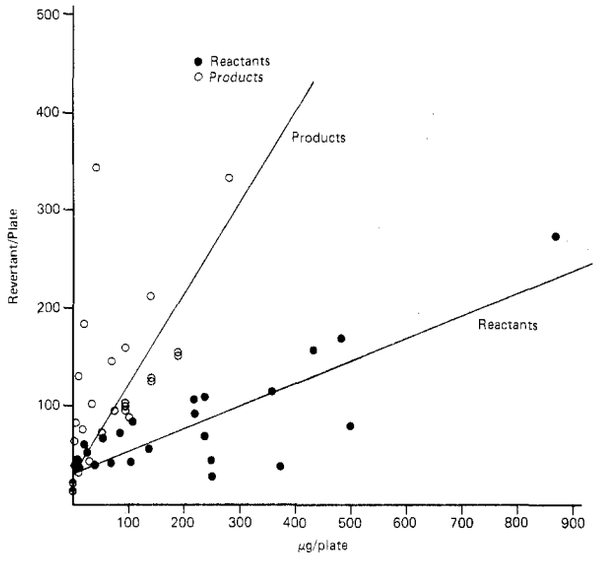

Figure 6. Dose–reponse curve for the mutagenic activity (TA98) of the particulate extracts of wood smoke (+500 ppb of NO_x) before and following irradiation.

wood smoke gas-phase mixture consists of a wide variety of reactive species including alkenes, aromatics, aldehydes, and oxygen heterocycles. From examination of Figures 2 and 5 and Table I, it is clear that this is a highly reactive system. For experiment B, from the HCs that we were able to identify and quantify, we can see that at least 2.4 ppm of C of reactants were converted to products. In addition, from the observed decrease in the particulate-phase species concentrations (Table II), it appears that there is considerable particulate-phase reaction occurring. From the data in Table I, it can be seen that although we have identified a large number of gas-phase reactants, we have not accounted for ~50% of the measured total HC level. A detailed analysis of the total observed mutagenic activity would require an extensive analysis of minor products and measurement of their individual mutagenic activities. We have found, for example, that the mutagenic activity of irradiated toluene/NO_x and propylene/NO_x mixtures is apparently due largely to the presence of minor products (*9, 10*). In addition, although detailed reaction mechanisms have been formulated (and summarized by Atkinson and Lloyd (*17*)) for the photooxidations of a wide variety of HCs, large uncertainties exist, particularly for aromatic species such as toluene. Considering that the well-known aromatic ring fragmentation products glyoxal and methylglyoxal (which are very reactive to OH) either increase or do not significantly decrease during the irradiation, it would appear that there is a significant degree of aromatics photooxidation occurring. It therefore is apparent that an analysis of these results, on the basis of mutagenic contributions from individual products, is not feasible for this study.

In this discussion, we will focus on comparing the relative mutagenic activities of the sum of all gas-phase reactants and products vs. the particulate-phase reactants and products. To achieve this, both the mutagenic activity and corresponding dose for each phase must be determined. The particulate-phase dose using the plate incorporation test is completely straightforward (i.e., a known weight of the extract is added to the plates). To determine the gas-phase dose, the total quantity of gas-phase species that deposits into the test medium during the exposure must be measured. In previous studies involving propylene and toluene, we have found that the deposition for pure HCs (containing only carbon and hydrogen) is extremely low (<1% of the total gas-phase mass). On the other hand, polar compounds such as formaldehyde are generally very soluble. Thus, the more polar products tend to dissolve into the medium whereas reactant HCs do not. We have measured the total HC concentration in the effluent (product) biochamber both before and after the plates were opened using the total HC analyzer. (Wall loss of gas-phase species in the exposure chambers is negligible as determined from the total HC.) The decrease in the total gas-phase HC concentration can be related to the total mass of material deposited into the plates. We have made measurements of the HC decrease for the two experiments with added NO_x and have obtained an average value of 1.36 ppm of C. For the experiment with combustion NO_x only, the amount of conversion of reactants to gas-phase products was considerably less, and an accurate measurement of the decrease in HC when the plates were opened was not attainable. In addition, since the revertant responses were much greater in the experiments with added NO_x, their measurement can be made with greater accuracy. Therefore, the balance of this discussion will focus on the results from the experiments with added NO_x.

With these data and the use of two assumptions, we can estimate the mass of gas-phase material deposited into each plate. The method (and equations) is analogous to those previously described (*10*), except that this calculation is performed for the sum total of products. Given 1.36 ppm of C measured deposition into the plates and assuming the average total HC analyzer sensitivity to the deposited products is the same as for acetone (71% that of C_3H_8), we calculate that 1.11 μmol of carbon has deposited into each plate per hour of exposure. (The sensitivity of the THC analyzer to acetone was measured in a separate experiment using a known gas-phase concentration of acetone. The response was 71% of that obtained when measuring the same concentration of propane. Acetone was selected since its average molecular weight per carbon atom, 19.3 g/mol, is nearly the same as the average molecular weight per carbon of the measured gas-phase product constituents in Table II, 18.5 g/mol.) From the products columns in experiments B and C of Table I, an average mass of 18.5 g associated with each mole of carbon is calculated. When this value is used, a deposition rate of 20.5 $\mu g\ plate^{-1}\ h^{-1}$ is obtained for gas-phase products. We are now able to estimate the mutagenic activity of the gas-phase species on a per mass basis. Table III shows mutagenic activities of 174 revertants $plate^{-1}\ h^{-1}$ for TA100 and 30.3 revertants $plate^{-1}\ h^{-1}$ for TA98 (as averages for the two experiments). When these values are divided by the calculated deposition for the gas-phase products, activities of 8.5 and 1.5 revertants/μg are obtained for TA100 and TA98, respectively. These values represent a reasonable estimate of the mutagenic activity of the species that are actually transported into the test medium. If it were assumed that the undissolved materials were also equally mutagenic, then these values would represent a reasonable estimate of the mutagenic activity per microgram of the total gas-phase product mixture. The last assumption is probably not valid, since the highly polar products are more likely to dissolve into the medium, and these components also tend to be more mutagenic (*8–10*). It is more reasonable, therefore, to consider the activities of 8.5 and 1.5 revertants/μg as estimates of the upper limit of the mutagenic activity of the total gas-phase effluent mixture.

A lower limit for the mutagenic activity per microgram can be calculated by assuming that all the undissolved gaseous materials are completely nonmutagenic. The

Table IV. Comparison of the Gas- and Particulate-Phase Mutagenic Activity (revertants/μg) for Wood Smoke and Irradiated Wood Smoke, Strains TA100 and TA98 without Metabolic Activation

	TA100		TA98	
	gas	particulate	gas	particulate
reactants	0	0.3	0	0.2
products	1.2–8.5	0.3	0.2–1.5	0.9

Table V. Comparison of the Gas- and Particulate-Phase Total Mutagenic Activity (revertants/m^3) for Wood Smoke and Irradiated Wood Smoke, Strains TA100 and TA98 without Metabolic Activation

	TA100		TA98	
	gas	particulate	gas	particulate
reactants	0	100	0	80
products	12300–90600	180	2130–16000	730

dissolved and undissolved components taken together represent the total gas-phase HCs shown in Table II. (Since the majority of the total effluent HC is probably unreacted saturated HCs, an instrumental sensitivity equivalent to that for propane has been used in this calculation.) By use of the average value of 14.1 ppm of C from experiments B and C, lower limit values of 1.2 and 0.20 revertants/μg are calculated for TA100 and TA98, respectively.

The true mutagenic activity is probably intermediate between the values cited above. Nonpolar compounds are probably not completely nonmutagenic nor is it reasonable to assume that all of the polar, gas-phase mutagenic materials are removed under these experimental conditions. We have shown that PAN, which is both polar and mutagenic, is only 15% deposited under these experimental conditions (*9*). The undissolved products are, therefore, likely to have some mutagenic potential, although presently there is no best estimate for their contribution to the mutagenic activity without invoking severe assumptions. The calculated range of values is given in Table IV.

The values for the gas-phase reactants are somewhat more uncertain since the deposition into the plates was not measured. For both strains, the mutagenic activity (revertants h^{-1}) is zero within the experiment error. Since the reactants do contain appreciable quantities of polar species such as HCHO, the deposition, though lower than 1.36 ppm of C, is probably significant. Therefore, it is reasonable to assume the gas-phase reactant mutagenic activities to be 0 excess revertants/μg for both strains.

The data for the mutagenic activity on a per mass basis for both phases have been summarized in Table IV. The particulate-phase activity is obtained directly from the data in Table III for both reactants and products. For TA98, the particulate-phase and gas-phase reactants show low activity, whereas the products in both phases show significant mutagenic activity. As we have stated, the volume (and thus mass) of the particulate matter increases considerably during irradiation, probably due to adsorption of gas-phase reaction products. Since the particulate-phase mutagenicity increases for TA98, these gas-phase condensibles may be significantly mutagenic, at least for this strain. For TA100, the gas-phase products, only, show substantial mutagenic activity.

It should be noted that the slopes of the dose–response curves (Table III) for experiment A (combustion NO_x only) are several times smaller (factors of 5 and 9 for TA100 and TA98, respectively) than those for the experiments with added NO_x. In experiment A, the conversion of gas-phase HCs is approximately a factor of 2 less, and therefore the total deposition and observed mutagenic activity might be expected to be lower by roughly this amount. If, however, some of the mutagens present are nitrated species, then the relative mutagenic activity of the gas-phase products may be lower in experiment A since less NO_x is available for the production of nitrates.

The mutagenic activity of the particulate phase with TA98 is generally consistent with the data of Kamens et al. (*6*). In their work, the reactant particulate matter consistently yielded mutagenic activities of 0.1–0.3 revertants/μg (–S9). For the dark oxidation of wood smoke particulate matter by O_3 and NO_2, mutagenic activities ranging from 0.4 to 2.3 revertants/μg were observed for a wide variety of reaction conditions. This activity is on the same order (within a factor of 2) of the response observed in this study when wood smoke was irradiated in the presence of added NO_x. In addition, they did present one set of experiments in which an irradiation was performed with combustion NO_x (50 ppb) only. The mutagenic activity of the particulate-phase products rose only slightly, from 0.18 to 0.23 revertants/μg. These results are very similar to our results for the experiment with combustion NO_x only, in which the particulate-phase mutagenic activity (TA98) rose from 0.20 to 0.26 revertants/μg following irradiation, although this increase is within the stated uncertainties.

The analysis up to this point gives only the mutagenic activities (revertants/μg) and does not take into account the relative amounts of material present in the two phases. However, it is clear that for TA100 the only significant source of mutagenic activity arises from gas-phase products. It should be noted that, in general, TA100 is believed to be relatively more sensitive to low molecular weight polar mutagens, and TA98 is more sensitive to high molecular weight species such as PAHs.

An alternative means for summarizing these results is to calculate the total mutagenic activity for the two phases in terms of revertants per cubic meter. In this way, the values obtained will also reflect the relative amounts of material present in each phase as well as its inherent mutagenic activity. It should be noted that the relative quanity of particulate- and gas-phase emissions are a complex function of burn conditions and wood type. Ramdahl et al. (*3*) indicated that burn conditions appear to be the major factor determining emissions, far outweighing the dependence on wood type. Dasch (*2*) has found that under fast burn conditions there is relatively less particulate mass than under slow burn conditions. In preparing the reactant system during this study, we introduced wood smoke into the reaction chamber over an extended period (12 h) to integrate transient effects of burn conditions. The total mutagenic activity (revertants/m^3) for the gas and particulate phases is calculated by multiplying the mutagenic activity (revertants/μg) by the gas- and particulate-phase concentrations (μg/m^3). The results of these calculations are shown in Table V. The ranges for the gas-phase products correspond to the upper and lower limits given in Table IV.

The particulate- (and gas-) phase activity for the products could conceivably be low (in terms of revertant/m^3) due to wall loss during the irradiation. The wall loss rate for the particulate phase has been measured in a separate experiment in which the wall loss rate for reactants was determined by using the EAA. Determining the wall loss rate using the EAA is valid assuming the particles are in the range 0–1 μm and that the density of the particles remains constant with time (the EAA measures the total particulate volume, nL/m^3). In the absence of any pho-

tochemical reaction, this second assumption is expected to hold. Gravimetric analysis was not used because its precision is not as good as the EAA unless very large samples are acquired, which would tend to limit severely the number of available samples. The wall loss experiment indicated an overall rate of disappearance for particles (0–1 μm) of 4–5% h^{-1}, which translates to a 10% loss of particulate matter to the walls during a 2.5-h irradiation. The particulate-phase wall loss after the irradiation (during sample collection) would have no effect on the calculation of mutagenic activity in terms of revertants per cubic meter, since the result is obtained by multiplying the particulate mass concentration ($\mu g/m^3$) at the end of the irradiation by the mutagenic activity in revertants per microgram. The only conceivable impact would be if particles of a particular diameter and mutagenic activity significantly different from the average were preferentially lost to the walls. It seems reasonable to assume for these calculations that this is not the case. Thus, a correction to account for the wall loss is not expected to increase significantly the total mutagenic activity of the particulate phase.

Wall losses for gas-phase products could also be important, especially for polar compounds, e.g., organic hydroperoxides or nitrates. Such a wall loss would decrease the mutagenic activity with time. However, if gas-phase losses were important, negative curvature would be seen in Figure 4, for example. A wall loss experiment using the total HC analyzer indicated that wall losses over the time scale of these exposures would not be significant. In addition, although dark gas-phase reactions could produce or remove mutagenic compounds during the course of the exposure, the linear dose–response curves argue against this occurrence.

Conclusions

We have demonstrated in this study that whereas the gas- and particulate-phase components of wood combustion show little direct-acting mutagenic activity, the mutagenic activities of the gas-phase (as measured using TA100 and TA98) and particulate-phase (with TA98) species increased significantly upon irradiation in the presence of NO_x. In addition, when measured in terms of revertants per microgram the gas-phase products are $\sim$ 3–30 times more mutagenic than the particulate-phase products as measured with strain TA100, and the mutagenic activities are comparable for the two phases with TA98. When compared on a per cubic meter basis, the gas-phase products are far more mutagenic than either the starting materials or the particulate products. In light of the great diversity and number of studies of the mutagenicity of complex air mixtures in which the particulate phase has been the focus of attention, this study clearly indicates that gas-phase species should be considered as well and that they could be very important.

Acknowledgments

We thank G. R. Namie and J. H. Pittman, Northrop Services, Inc.—Environmental Sciences, for their assistance in conducting the experiments, and E. Perry, Environmental Health and Research Testing, for her assistance in conducting the biotesting. We also thank R. M. Kamens for his helpful discussions.

Registry No. PAN, 2278-22-0; NO, 10102-43-9; N_2O, 10024-97-2; O_3, 10028-15-6; CO, 630-08-0; CH_4, 74-82-8; CH_3CH_3, 74-84-0; $CH_3CH_2CH_3$, 74-98-6; $CH_2{=}CH_2$, 74-85-1; $CH_2{=}CHCH_2$, 115-07-1; $CH_2{=}CHEt$, 106-98-9; $CH_2{=}C(Me)CH{=}CH_2$, 78-79-5; CH≡CH, 74-86-2; PhH, 71-43-2; PhMe, 108-88-3; *p*-MeC_6H_4Me, 106-42-3; *o*-MeC_6H_4Me, 95-47-6; *m*-MeC_6H_4Me, 108-38-3; $PhCH{=}CH_2$, 100-42-5; *m*-$MeC_6H_4CH{=}CH_2$, 100-80-1; NO_2, 10102-44-0; HCHO, 50-00-0; AcH, 75-07-0; AcMe, 67-64-1; PhCHO, 100-52-7; $ClCH_3$, 74-87-3; furan, 110-00-9; 2-methylfuran, 534-22-5; acrolein, 107-02-8; 2-furaldehyde, 98-01-1; glyoxal, 107-22-2; methylglyoxal, 78-98-8; biacetyl, 431-03-8; fluoranthene, 206-44-0; pyrene, 129-00-0; anthracene, 120-12-7; phenanthrene, 85-01-8; acenaphthene, 83-32-9; fluorene, 86-73-7; chrysene, 218-01-9; triphenylene, 217-59-4.

Literature Cited

(1) Wolff, G. T.; Countess, R. J.; Groblicki, P. J.; Ferman, M. A.; Cadle, S. H.; Muhlbaier, J. L. *Atmos. Environ.* **1981**, *15*, 2485–2502.
(2) Dasch, J. M. *Environ. Sci. Technol.* **1982**, *16*, 639–645.
(3) Ramdahl, T.; Alfheim, I.; Rustad, S.; Olsen, T. *Chemosphere* **1982**, *11*, 601–611.
(4) Alfheim, I.; Ramdahl, T. *Environ. Mutagen.* **1984**, *6*, 121–130.
(5) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347–364.
(6) Kamens, R. M.; Rives, G. D.; Perry, J. M.; Bell, D. A.; Paylor, R. F., Jr.; Goodman, R. G.; Claxton, L. D. *Environ. Sci. Technol.* **1984**, *18*, 523–530.
(7) Kamens, R.; Bell, D.; Dietrich, A.; Perry, J.; Goodman, R.; Claxton, L.; Tejada, S. *Environ. Sci. Technol.* **1985**, *19*, 63–69.
(8) Kleindienst, T. E.; Edney, E. O.; Namie, G. R.; Claxton, L. D. *Atmos. Environ.*, in press.
(9) Shepson, P. B.; Kleindienst, T. E.; Edney, E. O.; Namie, G. R.; Pittman, J. H.; Cupitt, L. T.; Claxton, L. D. *Environ. Sci. Technol.* **1985**, *19*, 249–255.
(10) Kleindienst, T. E.; Shepson, P. B.; Edney, E. O.; Cupitt, L. T.; Claxton, L. D. *Environ. Sci. Technol.* **1985**, *19*, 620–627.
(11) Shepson, P. B.; Kleindienst, T. E.; Edney, E. O.; Namie, G. R.; Pittman, J. H.; Claxton, L. D.; Cupitt, L. T.; Perry, E. J. *Environ. Health, Part A*, **1985**, *A20* (5), 503–519.
(12) Claxton, L. D.; Toney, S.; Perry, E.; King, L. *Environ. Mutagen.* **1984**, *6*, 331–342.
(13) Parrish, D. D.; Murphy, P. C.; Albritton, D. L.; Fehsenfeld, F. C. *Atmos. Environ.* **1983**, *17*, 1365–1379.
(14) Shepson, P. B.; Edney, E. O.; Corse, E. W. *J. Phys. Chem.* **1984**, *88*, 4122–4126.
(15) "Air Quality Criteria for Ozone and other Photochemical Oxidants" 1984, EPA-600/8-84-020.
(16) Khalil, M. A. K.; Rasmussen, R. A. *Chemosphere* **1981**, *10*, 1019–1023.
(17) Atkinson, R.; Lloyd, A. C. *J. Phys. Chem. Ref. Data* **1984**, *13*, 315–444.

Received for review July 25, 1985. Revised manuscript received December 16, 1985. Accepted January 26, 1986. Although the research described in this article has been funded wholly or in part by the U.S. Environmental Protection Agency through Contract 68-02-4033 to Northrop Services, Inc.—Environmental Sciences, it has not been subject to the Agency's peer and administrative review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.