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Mutagenic Transformations of Dilute Wood Smoke Systems in the Presence of Ozone and Nitrogen Dioxide. Analysis of Selected High-Pressure Liquid Chromatography Fractions from Wood Smoke Particle Extracts

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■ Dilute wood smoke from a residential wood stove was added to 25-m^3 outdoor Teflon chambers and then reacted in the dark with sub-ppm levels of O_3 and NO_2. Chemical fractionation of unreacted and O_3 and NO_2 reacted wood smoke indicated that most of the extracted mass as well as the mutagenicity was contained within the most polar fractions. The PAH fraction contributed 12–17% of the total TA98+S9 mutagenicity of unreacted wood smoke. After reaction with O_3 and NO_2, the mutagenic contribution of the PAH fraction declined substantially. One of the moderately polar fractions that contained compounds with the polarity of aromatic ketones contributed 4% to the total direct-acting mutagenicity in unreacted samples. After reaction with O_3 + NO_2 wood soot extract in this fraction made up 16–30% of the total direct-acting mutagenicity. Analysis of this reacted fraction tentatively indicated the formation of odd nitrogen organic compounds and other oxygenated species.

Introduction

Laboratory studies have suggested (*1–3*) the potential for atmospheric reactions of particle-bound polycyclic organic compounds, but actual documentation of the extent to which these reactions and subsequent mutagenic changes take place is much more limited. Recently, workers (*4–6*) at the University of North Carolina outdoor chamber facility have shown that dilute wood smoke systems can react with sub-ppm levels of O_3 and NO_2 and cause a manyfold increase in the direct-acting TA98 mutagenicity of wood smoke particle extracts.

Given the large mutagenic increases that O_3 and NO_2 mixtures impart to wood smoke, a chamber study was undertaken to generate unreacted and O_3 + NO_2 reacted wood smoke for chemical fractionation. The purpose was to determine the fractions in which mutagenic changes were occurring and to isolate associated chemical transformations which gave rise to some of these changes in mutagenicity. The preliminary results of that study are presented in this paper.

Experimental Section

Sample Generation and Preparation. Wood smoke was added to two 25-m^3 outdoor Teflon film chambers directly from the chimney of a residential woodstove (free standing intermediate size Buck woodstove, Smoky Mountain Enterprises, Asheville, NC). After the addition of wood smoke to the chambers, ~30–60 mg of wood smoke particles was collected on 13.34-cm Teflon-impregnated glass fiber filters (Pallflex). This left, depending on the experiment, 2000–2500 $\mu g/m^3$ smoke particles in the chambers. UV-generated O_3 and NO_2 (in nitrogen) from a commercially prepared high concentration cylinder were then added and immediately diluted into the chambers. The dilute wood smoke and NO_2 + O_3 mixture were permitted to react in the dark for periods of up to 4 h at which time another 13.34-cm filter sample was taken. A more detailed description of the chambers, injection of wood smoke, associated instrumentation, sampling apparatus, and aerosol behavior is given in previous papers published elsewhere (*4–6*).

Table I. Step Gradient Program for Fractionation of Wood Smoke[a]

time, min	% hexane	% $MeCl_2$	% ACN
0–12	100	0	0
12–17	100–95	0–5	0
17–25	95	5	0
25–26	95–80	5–20	0
26–35	80	20	0
35–36	80–40	20–60	0
36–48	40	60	0
48–49	40–0	60–100	0
49–70	0	100	0
70–72	0	100–0	0–100
72–90	0	0	100
90–92	0	0–100	100–0
92–102	0	100	0
102–104	0–100	100–0	0
104–120	100	0	0

[a] Fractions collected at the following times to optimize separation: A fraction, 0–12 min; B fraction, 12–28.5 min; C fraction, 28.5–37.5 min; D fraction, 37.5–50.5 min; E fraction, 50.5–72.5 min; F fraction, 72.5–93.0 min. Column flow rate 2.0 mL/min.

Wood smoke particulate filter samples were immediately Soxhlet extracted with 100 mL of methylene chloride ($MeCl_2$) for 16 h and concentrated to ~10 mL by rotary evaporation. Gravimetric determinations on the extracted mass were made by evaporating 100-μL aliquots of the extract on preweighed 47-mm Teflon-impregnated glass fiber filters (Pallflex). Extracts were concentrated with a dry nitrogen stream to 50–200 μL. Most of the extract was then fractionated for subsequent chemical and bioassay analysis on the basis of polarity, with a normal-phase liquid chromatographic technique (*7*).

Fractionation was conducted with a ternary Spectra-Physics Model 8700 high-pressure liquid chromatographic (HPLC) pump and a 5 mm × 30 cm Bio-sil A, 20–44 μm, (Bio-Rad Laboratories, Richmond, California) column using a multistep gradient program (as shown in Table I). Five milligrams of concentrated extract was injected onto the column, and six fractions were collected at the detector outlet according to the time windows shown in Table I. The fractionation was monitored with a Varian Fluorichrom (340–380-nm excitation and 470–530-nm emission) fluorescence detector. A chromatographic trace of a typical fractionation is shown in Figure 1. Characterization of this system has shown the recoveries of selected aliphatic, aromatic, nitroaromatic, and aromatic carbonyl compounds to be greater than 80% (*3*).

Individual fractions were then concentrated to 1 mL, and gravimetric determinations of the mass were made in the

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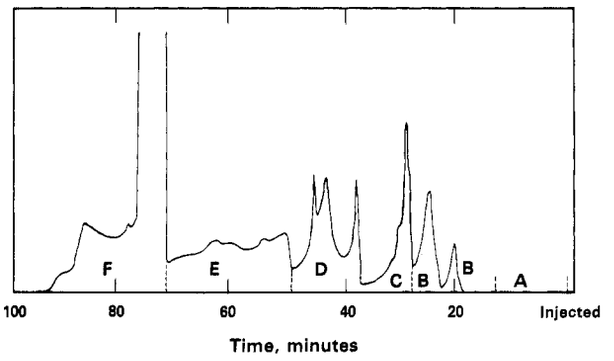

Figure 1. Fluorescence chromatogram of normal phase fractionation of O_3 + NO_2 chamber reacted dilute wood smoke extract. Excitation wavelength 340–380 nm and emission wavelength 470–530. 5 mm × 30 cm Bio-Sil A, 20–44 μm column, 2 mL/min; mobile phase and fraction windows given in Table I.

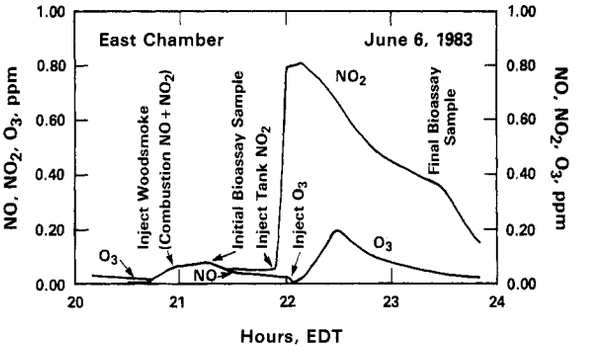

Figure 2. NO_x and O_3 time vs. concentration profiles for dilute wood smoke experiment on June 6, 1983.

same manner described above. Chemical analysis was routinely conducted on a Carlo Erba Model 4130 gas chromatograph with a 30-m DB-5 fused silica column or a Spectra Physics 8700 HPLC with UV or fluorescence detectors and a reverse-phase C18 column. Selected fractions were analyzed on a VG-Micromass 7070F (double-focusing, magnetic sector) mass spectrometer interfaced to a Hewlett-Packard 5710A capillary gas chromatograph. Analysis conditions are presented elsewhere (*8*). These fractions were also analyzed for the presence of selected nitroaromatic compounds by using the reverse-phase HPLC technique developed by Tejada and coworkers (*9*). The method takes advantage of the high sensitivity at which aminoarenes can be detected by fluorescence and the fact that nitro-PAH generally are difficult to measure with fluorescence at sub-ppb levels. Conversion of nitro-PAH to their complementary amino-PAH compounds was achieved catalytically within the HPLC system.

Mutagenicity Assay. The *Salmonella typhimurium* plate incorporation assay was performed as described by Ames et al. (*10*) with minor modifications (*11*). Tester strain TA98 was used with and without rat liver microsomal activation (S9) at five to six doses using triplicate plates. When sample mass was low, duplicate or single plates were used. To conserve sample, the dose range was compressed to assure a maximum number of points in the linear range of the dose/response curve.

Slope values from mutagenicity dose/response curves were reported as revertants per microgram of extract. All data points were used unless toxicity to the bacteria was observed. The positive controls were 2-nitrofluorene and 2-aminoanthracene (+S9), and tester strain monitoring and maintenance procedures were followed to assure reproducibility (*11*). Samples from an experiment were assayed on the same day by the same worker at the Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC.

Results and Discussion

Three wood smoke O_3 + NO_2 experiments were conducted in the dark to generate both unreacted and reacted wood smoke particulate extracts for fractionation followed by chemical and bioassay analysis. The experiments on June 6 and April 24, 1983, will be described in detail, and additional information from the other experiment (Dec 4, 1982) will be used to address similarities and variations that occurred in these experiments.

The June 6, 1983, experiment started with ~2500 μg/m^3 wood smoke particles, 0.79 ppm of NO_2, and 0.20 ppm of O_3. The April 24, 1983, experiment began with ~2000 μg/m^3 wood smoke particles, 0.76 ppm of NO_2, and 0.31 ppm of O_3. Both experiments exhibited similar O_3 and NO_2 behavior, and for illustrative purposes the NO_x and O_3 time–concentration profiles for the June 6 experiment are shown in Figure 2. Note the appearance of combustion NO and NO_2 as wood smoke was added to the chambers and the reaction of both O_3 and NO_2 soon after they were injected into the chambers.

Table II. Mass Contribution of Each Fraction to the Unfractionated $MeCl_2$ Wood Soot Particle Extract

	June 6, 1983[a]		April 24, 1983[b]	
fraction	unreacted, %	reacted, %	unreacted, %	reacted, %
A	0.89	0.23	1.60	0.34
B	0.62	0.11	0.94	0.29
C	0.39	0.16	0.56	0.29
D	2.82	1.35	2.69	2.80
E	6.03	4.06	8.31	3.27
F	80.51	90.43	83.86	86.28
A–F	91.26	96.34	97.96	93.27
wood smoke particle mass, mg	80.39	90.53	38.71	58.13
unfractionated extracted mass, mg	61.02	56.13	31.90	38.19

[a] Red oak (6 cm × 0.5 m logs) used as the fuel and stove operated at a constant burn rate of 4.3 kg/h. [b] Same fuel with stove burn rate of 4.8 kg/h.

The percent mass determined in each fraction from both experiments is shown in Table II. Ninety-one to ninety-eight percent of the original wood soot extract that was fractionated was recovered through this system. Only 11–14% of the unreacted filter mass extract appeared in the first five neutral or semipolar fractions, and 81–84% eluted in the sixth or very polar fraction. This agrees with the original findings of Hubble et al. (*12*), who reported that most of the particle-bound organics in wood smoke were very polar in nature. After reaction with O_3 + NO_2, the particle extract became more polar. Only 6–7% of the mass appeared in the first five fractions, and more than 86–90% appeared in the final most polar fraction.

The TA98±S9 dose/response curves for selected fractions from the June 6, 1983, experiment, both before and after reaction, are shown in Figure 3. As can be seen, large increases in the mutagenic response for the C–E fractions were observed when unreacted and reacted samples were compared. These curves usually had linear regression correlation coefficients greater than or equal to 0.9 for fractions having mutagenicities greater than 0.3 revertant/μg. At the doses tested (1–100 μg), slope values for nonmutagenic and marginally mutagenic fractions (i.e.,

Table III. Estimation of the Mutagenic Contribution of Each Fraction to the Total Sample for June 6, 1983, Dilute Wood Smoke Experiment: Initial Wood Smoke Particle Concentration = 2500 $\mu g/m^3$, Ozone = 0.20, and NO_x = 0.79 ppm

	TA98–S9						TA98+S9					
	unreacted			reacted			unreacted			reacted		
	slope,[a] rev/μg	rev/mg[b]	%[c]	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%
unfractionated sample	0.22	167.0		2.08	1289.6	100.0	0.37	280.8	100.0	0.99	613.8	100.0
fraction												
A	−0.03	0.0	0	−0.11	0.0	0.0	1.05	7.1	2.5	−0.05	0.0	0.0
B	0.91	4.3	2.6	NT[d]			7.27	34.2	12.2	1.58	1.1	0.2
C	0.40	1.2	0.7	9.67	9.6	0.7	2.22	6.6	2.4	NT		
D	0.31	6.6	4.0	25.07	209.8	16.3	1.47	31.5	11.2	13.40	112.2	18.3
E	0.28	12.8	7.7	6.09	153.3	11.9	0.71	32.5	11.6	5.56	140.0	22.8
F	0.09	55.0	32.9	1.04	583.1	45.2	0.30	183.3	65.3	0.56	314.0	51.2
∑A–F		79.9	47.9		955.8	74.1		295.2	105.2		567.3	92.5

[a] Mutagenic slope from linear portion of dose/response curves in revertants per microgram (rev/μg) of extract. [b] rev/mg of wood smoke particles = slope[a] in rev/mg × mg of extract in fraction ÷ mg of particles, i.e., 80.39 mg for unreacted sample and 90.53 mg for reacted sample. [c] Percent mutagenic contribution to unfractionated sample. [d] Not tested.

Table IV. Estimation of the Mutagenic Contribution of Each Fraction to the Total Sample for April 24, 1983, Dilue Wood smoke Experiment: Initial Wood Smoke Particle Concentration = 1985 $\mu g/m^3$, Ozone = 0.31 ppm, and NO_2 = 0.76 ppm

	TA98–S9						TA98+S9					
	unreacted			reacted			unreacted			reacted		
	slope,[a] rev/μg	rev/mg[b]	%[c]	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%	slope, rev/μg	rev/mg[b]	%
unfractionated sample	0.20	164.8	100	1.16	762.1	100	0.42	346.1	100	0.96	630.7	100.0
fraction												
A	0.20	2.6	1.6	−0.28	0.0	0.0	1.77	23.3	6.7	NT[d]		
B	0.07	0.5	0.3	−0.38	0.0	0.0	7.75	60.0	17.3	1.29	2.5	0.4
C	0.09	0.4	0.2	5.28	10.1	1.3	1.50	6.9	2.0	NT		
D	0.29	6.4	3.9	12.86	236.6	31.0	1.98	43.9	12.7	11.93	219.5	34.8
E	0.88	60.3	36.6	5.26	113.0	14.8	1.23	84.2	24.3	4.78	102.7	16.3
F	0.10	69.1	41.9	0.43	243.7	32.0	0.08	55.3	16.0	0.44	249.4	39.5
∑A–F		139.3	84.5		603.4	79.1		273.6	79.0		574.1	91.0
reconstituted ∑A–F	0.16	131.9	80.0	0.85	525.6	68.4						

[a] Estimated slope from linear portion of dose/response curve in revertants per microgram (rev/μg) of extract. [b] rev/mg of wood smoke particles = slope[a] in rev/mg × mg of extract in fraction ÷ mg of particles, i.e., 38.71 mg of wood soot particles for unreacted sample and 58.13 mg for reacted sample. [c] Percent mutagenic contribution to unfractionated sample. [d] Not tested.

<0.1 revertants/μg) had correlation coefficients equal to or less than 0.7. This lower correlation was primarily a function of plate count scatter at or near the spontaneous revertant level.

In order to qualitatively assess each fraction's mutagenic contribution to the whole sample, a weighted estimate was calculated by taking the product of the mass in a given fraction and the mutagenic slope value determined from that fraction's dose/response curve and dividing this by the product of the mass and the slope of the unfractionated sample. Results of these calculations along with the number of revertants per milligram of wood smoke particles which appears in each fraction are displayed in Tables III and IV. In addition, in the April 24 experiment aliquots of the appropriate mass portion from the −S9 fractions were recombined (reconstituted sample), bioassayed, and compared to the unfractionated sample. Approximately 80% of the mutagenicity was accounted for by reconstituting the unreacted fractions and 68% by reconstituting the reacted fractions.

Nonpolar Fractions. The most nonpolar fraction, A, was eluted with 100% hexane and contributed approximately 1% to unreacted, extracted particle mass. Gas chromatography/mass spectrometry (GC/MS) analysis indicated that this fraction was initially dominated by a mixture of alkanes and alkenes, primarily in the C_{16}–C_{30} range. After reaction with O_3 + NO_2, the percentage of mass in this fraction declined substantially. Analysis of the O_3 + NO_2 reacted A fraction did not show detectable levels of olefinic compounds. Presumably these alkenes had reacted with O_3 to form carbonyl and acid derivatives. These product compounds would appear in the more polar fractions of the final or reacted extract. All of our experimental data have shown that the aliphatic fraction did not contribute substantially to the direct-acting mutagenicity before or after reaction with O_3 + NO_2. In the April 24 experiment we did observe ~7% of the indirect-acting mutagenicity in the unreacted fraction. On this day, however, the fractionating column became slightly deactivated after the fifth pass through the system, and some two- and three-ring PAH did elute into the A fraction.

The PAH fraction, fraction B, was eluted by using a mobile phase of 95% hexane and 5% $MeCl_2$. It was found that this fraction contributed less than 1% of the mass to the total unfractionated extract. After reaction with O_3 + NO_2 the mass in this fraction decreased by 70–90%. The PAH compounds that have been identified by GC/MS and gas chromatography/flame ionization detection (GC/FID) retention time data were similar to those previously reported by Ramdahl et al. (*13*, *14*). The distribution of PAH compounds that were typically observed in unreacted

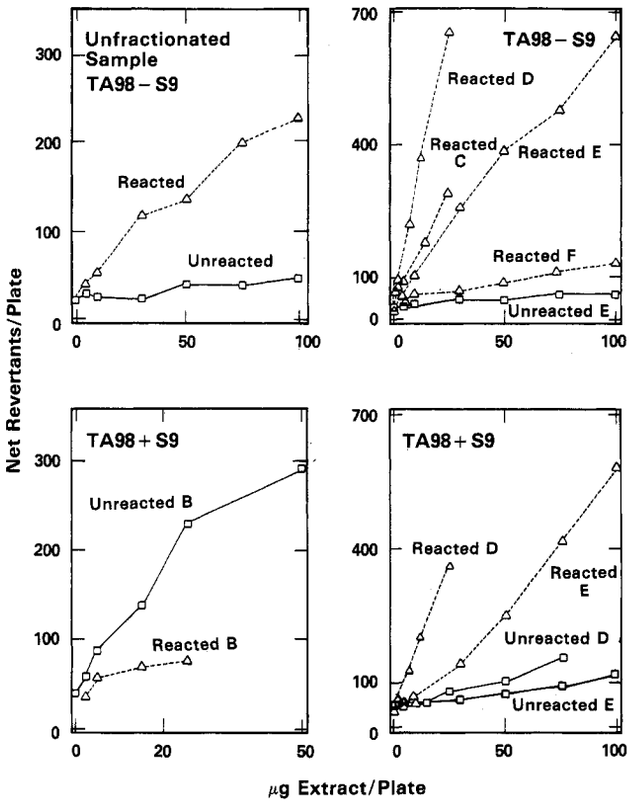

Figure 3. Selected TA98 ± S9 dose/response curves from June 6, 1983, dilute wood smoke + O_3 + NO_2 experiment.

chamber wood smoke samples is shown in Figure 4.

In certain experiments, the concentrations of selected PAH were monitored over the course of the O_3 + NO_2 reaction. This was done by analyzing the extracts from 47-mm filter samples which were taken hourly during the experiment. As shown in Figure 5, PAH disappeared with time. By comparison (Figure 5), wood smoke aged in the dark in the presence of 45 ppb of combustion NO_x showed comparative stability of particle-bound PAH.

Mutagenicity testing of the PAH fractions showed a small (<3%) contribution to the direct-acting mutagenicity of unreacted samples and no apparent contribution to reacted samples. Given, however, that PAH require metabolic activation for full expression in Ames bacterial assays, it was not surprising that the PAH fraction contributed 12–17% to the total unreacted indirect-acting mutagenicity. After reaction with O_3 + NO_2, almost no indirect-acting mutagenicity was observed in this fraction. This loss of indirect-acting mutagenicity between unreacted and reacted samples was also consistent with the above-mentioned decay of PAH concentrations over time (Figure 5) and the dramatic loss of mass in this fraction.

Semipolar and Moderately Polar Fractions. A 20% $MeCl_2$ and 80% hexane mobile phase was used to elute the first of the semipolar and moderately polar fractions. This fraction, fraction C, generally comprised less than 0.6% of the unreacted filter extract. After reaction the percent mass of this fraction declined by 50%.

Analysis of the unreacted C fraction for nitroarenes by conversion to amino-PAH did not suggest the significant presence of nitroaromatic compounds. After reaction with O_3 + NO_2, however, the possible formation of some unidentified nitroarenes was indicated.

The initial TA98–S9 slope of the dose/response curve for the C fraction was in the 0.3–0.4 revertant/μg range. After reaction with O_3 + NO_2 the slope of the direct-acting dose/response curve increased to 5–10 revertants/μg, depending on the experiment. Although this increase in slope was rather significant, the final mass in the fraction was comparatively small. Thus, when compared to the net increase in the final unfractionated sample, the increase in the C fraction only made up a small percentage of the total direct-acting mutagenicity.

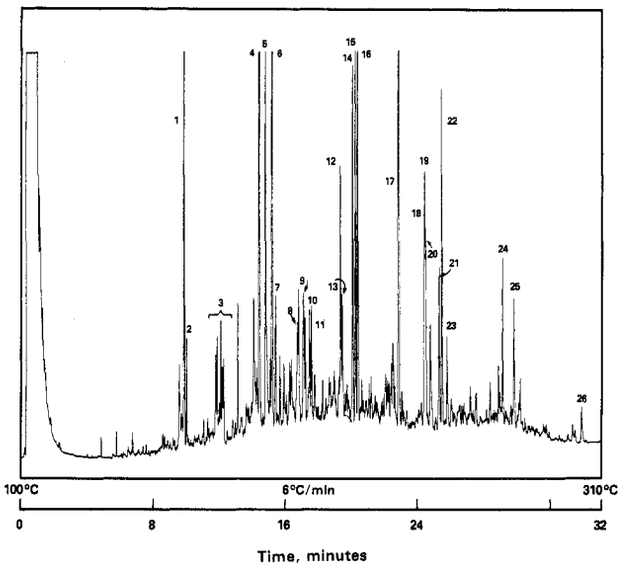

Figure 4. Chromatogram of the PAH fraction. DB-5 30 m fused silica column, H2 carrier. Identifications based on retention time of knowns (denoted by superscript a), comparison to GC/MS reconstructed ion chromatograms and MS reference spectra (denoted by superscript b), and comparison to Ramdahl chromatograms (10, 11) (denoted by superscript c): (1) phenanthrene[a,b], (2) anthracene[a,b], (3) order of elution according to Ramdahl[c,b], 3-methylphenanthrene[b], 2-methylphenanthrene[b], 4,5-methylenephenanthrene[b], 4- and/or 9-methylphenanthrene[b], 1-methylphenanthrene[b], (4) fluoranthene[a,b], (5) acephenanthrylene[b,c], (6) pyrene[a,b], (7) ethylmethylenephenanthrene[b,c], (8) benzo[*a*]fluorene[b,c], (9) benzo[*b*]fluorene/4-methylpyrene[b,c], (10) 2-methylpyrene and/or methylfluoranthene[b,c], (11) 1-methylpyrene[b,c], (12) benzo[*ghi*]fluoranthene[b,c], (13) benzo[*c*]phenanthrene[b,c], (14) cyclopenta(*cd*]pyrene[a,b], (15) benzo[*a*]anthracene[a,b], (16) chrysene and triphenylene[a,b,c], (17) internal standard β,β′-binaphthyl, (18) benzo[*b*]fluoranthene[a,b], (19) benzo[*j*]fluoranthene[b,c], (20) benzo[*k*]-fluoranthene[a,b], (21) benzo[*e*]pyrene[a,b], (22) benzo[*a*]pyrene[a,b], (23) perylene[a,b], (24) indeno[1,2,3-*cd*]pyrene[a,b], (25) benzo[*ghi*]perylene[a,b], and (26) coronene[b,c].

The D fraction was eluted with a 60% $MeCl_2$ and 40% hexane solvent mixture and was characterized by compounds with the polarity of aromatic ketones. Four-ring nitroaromatics also eluted in this fraction. As will be seen, the D fraction was the most interesting fraction and thus had the greatest amount of analysis directed toward it.

This fraction consistently showed the largest direct-acting mutagenicity slope increase between unreacted and reacted samples. The unreacted slope (~0.3 revertant/μg) when combined with the initial mass in the fraction accounted for 4% of the initial direct-acting mutagenicity. After reaction, slope values increased to 12–25 revertants/μg. Thus, even though less than 3% of the reacted mass remained in this fraction, it contributed ~16–30% of the total direct-acting mutagenicity (Tables III and IV). Similar changes in indirect-acting mutagenicity (+S9) were also observed.

The most prominent aromatic carbonyl compounds in this fraction which were confirmed by GC/MS with authentic standards and retention time included fluoren-9-one, 9,10-anthraquinone, and 7*H*-benz[*de*]anthracen-7-one (benzanthrone). These compounds along with many other aromatic ketones have been tentatively identified in wood smoke by Ramdahl (*15*). Very little is known about the mutagenic or carcinogenic qualities of aromatic carbonyl

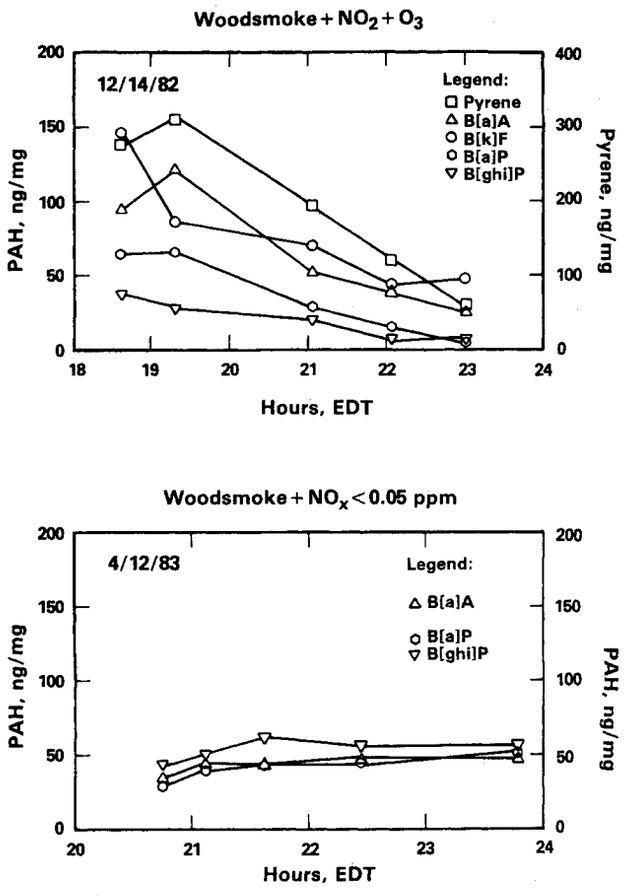

Figure 5. Stability of particle-bound wood smoke PAH in the presence of (1) 0.63 ppm of NO_2 + 0.43 ppm of O_3 (particle concentration = 3850 $\mu g/m^3$) and (2) NO_x below 0.05 ppm + 0.00 O_3 (particle concentration = 3702 $\mu g/m^3$).

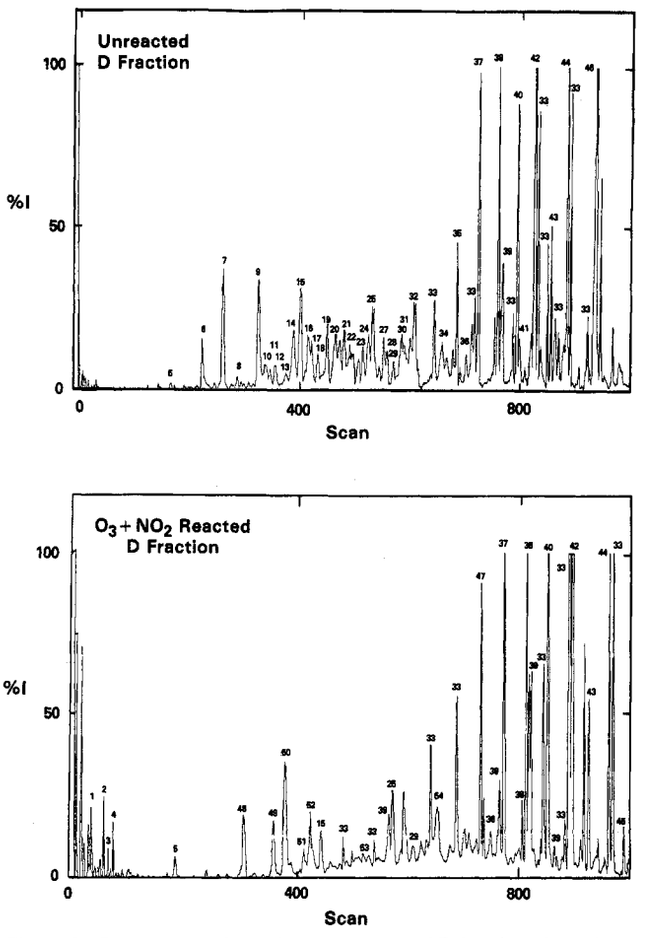

Figure 6. Reconstructed ion chromatograms of D fractions from June 6, 1984, wood smoke analysis. GC conditions: DB5-30M (J&W); 1 μL on column at room temperature, programmed 100–300°C at 8 °C/min. Italicized compounds have been identified on the basis of the comparison of mass spectral and retention time data to those of an authentic standard. Other compounds have been tentatively identified on the basis of mass spectral interpretation and library matching. The presence of xanthone (M_r 196), anthrone (M_r 194) and naphthaquinone (M_r 158) have been ruled out on the basis of retention times of authentic samples. (1) M_r 120 acetophenone, (2) M_r 122 hydroxybenzaldehyde, (3) M_r 120 methylbenzaldehyde, (4) M_r 136 ethylbenzaldehyde, (5) M_r 148, (6) M_r 164 methoxypropenylphenol, (7) M_r 144 1-hydroxynaphthalene, (8) M_r 176 alkyl-substituted benzophenone, (9) M_r 158 hydroxymethyl-1-hydroxynaphthalene, (10) M_r 158 hydroxymethylnaphthalene, (11) M_r 190 dihydroxymethoxynaphthalene, (12) M_r 174 hydroxymethoxynaphthalene, (13) M_r 204, (14) M_r 172 dimethylhydroxynaphthalene, (15) M_r 180 *9-fluorenone*, (16) M_r 184 hydroxydibenzofuran or dibenzo-*p*-dioxane, (17) M_r 188 hydroxymethoxymethylnaphthalene, (18) M_r 184, (19) M_r 200 methoxymethylnaphthaldehyde, (20) M_r 184, (21) M_r 198, (22) M_r 182 phenylbenzaldehyde, (23) M_r 214, (24) M_r 198, (25) M_r 208 *9,10 anthraquinone*, (26) M_r 212, (27) M_r 196 methoxyfluorene, (28) M_r 194, (29) M_r 204 cyclopenta[*def*]phenanthrone, (30) M_r 270 chlorinated compound, (31) M_r 224, (32) M_r 194 hydroxyanthracene or phenanthrane, (33) alkyl derivative, (34) M_r 208 isomers, (35) M_r 236 alkyl derivative, (36) M_r 230 *benzanthrone*, (37) M_r 340 alkyl derivative, (38) M_r 354 alkyl derivative, (39) phthalate ester, (40) M_r 368 alkyl derivative, (41) M_r 218, (42) M_r 382 alkyl derivative, (43) M_r 396 alkyl derivative, (44) M_r 410 alkyl derivative, (45) M_r 424 alkyl derivative, (46) M_r 438 alkyl derivative, (47) M_r 326 alkyl derivative, (48) M_r 183 C_2-oxynitrophenol, (49) M_r 197 C_3-oxynitrophenol, (50) M_r 167 C_2-oxynitrophenol, (51) M_r 211, (52) M_r 181 C_3-nitrophenol, (53) M_r 194, and (54) M_r 245.

compounds. A few compounds like fluoren-9-one have been tested and found to be nonmutagenic or toxic in Ames type assays. Leary et al. (*16*) using the forward mutation 8-azaguanine assay obtained a very strong mutagenic response from 1*H*-phenalen-1-one and almost no response from fluoren-9-one. 1*H*-Phenalen-1-one has been tentatively identified by Ramdahl (*15*) in urban air samples. The mutagenicity of larger molecular weight aromatic ketones with a phenalen-1-one molecular sub-unit is unknown.

Compounds that have been confirmed and tentatively identified in the D fractions are listed in Figure 6. Both fractions contained the aromatic ketones 9-fluorenone, 9,10-anthraquinone, and benzanthrone. Additionally, there was a homologous series of high molecular weight alkyl compounds which was common to the reacted and unreacted fraction. Through interpretation of EI spectra and the use of library search techniques, a series of substituted naphthalenes and hydroxyaromatics have tentatively been identified in the unreacted D fraction. On the basis of the interpretation of molecular weight data, there was no evidence of odd nitrogen-containing compounds. In contrast, on the basis of molecular weight there were six odd N-containing compounds in the O_3 + NO_2 reacted D fraction; four of these have been tentatively identified as substituted nitrophenols and nitrobenzene compounds. The formation of nitrophenol possibly resulted from an NO_3/NO_2 type oxidation. This mechanism has been proposed (*2, 3*) for the oxidation of hydroxyaromatic compounds to hydroxynitro-PAH. Presumably in this scheme, NO_3 produced from the reaction of O_3 + NO_2 abstracts a phenolic hydrogen. NO_2 then adds to one of the intermediate species, and the OH group is regenerated.

As in fraction C, we selectively looked for certain nitro-PAH in both the unreacted and reacted D fraction using the method of Tejada et al. (*9*). No appreciable amino-PAH enhancement was observed when the un-

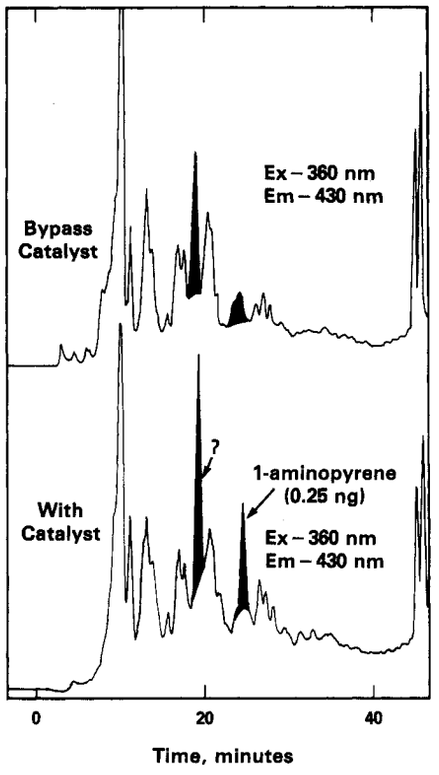

Figure 7. Fluorescence detection of 1-aminopyrene using Tejada et al. (*9*) HPLC technique. 15 cm × 5 mm Zorbax ODS column, mobile phase 25:75 H_2O/MeOH for 30 min, 15:85 For 30–40 min, flow = 1 mL/min, platinum–rhodium catalyst at 70 °C, 10-μL sample.

reacted D fraction was analyzed. As shown in Figure 7, however, the reacted D fraction had two peaks which are significantly enhanced after passage through the platinum–rhodium catalyst. The latter eluting peak was tentatively identified as 1-nitropyrene. This was done by optimizing the sensitivity of the fluorescence detector to the 360- and 430-nm excitation and emission maxima for 1-aminopyrene. When another C_{18} reverse-phase column was placed after the catalyst, additional chromatographic confirmation of 1-aminopyrene was made. The first peak noted in Figure 7 had an elution time that did not exactly correspond to that of 1,6-dinitropyrene. When samples were rerun with optimum excitation and emission maxima of 369 and 442 nm for 1,6-diaminopyrene, a decrease as opposed to an increase in response was observed. Hence, a positive identification of 1,6-dinitropyrene could not be made. No enhancement was observed in the region where 1,8-dinitropyrene elutes.

The observed concentration of the 1-nitropyrene peak was used to estimate the total amount of 1-nitropyrene in the reacted fraction. For the April 24 experiment this translated into 0.7 ng/mg of reacted wood soot particles. In this same system, a comparison between unreacted and reacted filter samples suggested that 84 ng of pyrene/mg of soot had reacted. This did not include pyrene, which potentially existed and reacted in the vapor phase, and/or pyrene lost during filter sampling. It may also be presumed that more 1-nitropyrene mass actually proceeded through this reaction but that some of the formed nitropyrene was additionally reacted with chamber O_3. In either event, it appeared that only a small portion of the reacted pyrene was ultimately observed in the form of 1-nitropyrene.

The mutagenic contribution of the observed 1-nitropyrene to the direct-acting mutagenicity in the reacted D fraction in the April 24 experiment was computed from its observed mass and its reported direct-acting mutagenicity of 2000 revertants/μg (*17*). This was then compared to the number of revertants in this fraction (237 revertants/mg, Table IV) and indicated that 1-nitropyrene contributed ~0.6% to the total direct-acting mutagenicity in the final D fraction and thus less than 0.1% to the total mutagenicity of the sample. Although these calculations are qualitative in nature, they suggest that the formation of 1-nitropyrene had little impact on the observed mutagenic increases resulting from the reaction of O_3 + NO_2 with dilute wood smoke. If 1,6- and 1,8-dinitropyrene formed, however, at trace levels just below our detection limits (i.e., 0.03 and 0.10 ng), then these compounds, given their large individual mutagenicity, would substantially contribute to the observed mutagenicity in this fraction. It is also possible that nitro derivatives of other PAH like fluoranthene, benz[*a*]anthracene, and benz[*a*]pyrene formed. Since the detection of these amino analogues is also not as sensitive as for 1-aminopyrene, these may have been present but below our detection limit as well.

The next fraction of higher polarity, fraction E, was eluted with 100% $MeCl_2$. It initially comprised 6–8% of the total fractionated mass, and after reaction it was reduced to 3–4%. Compounds that were tentatively identified in the unreacted E fraction by GC/MS (by comparison to library spectra) included substituted hydroxyaromatics such as a phenolic specie at M_r 154, a vanillic acid at M_r 168, napthols (M_r 144), and substituted dibenzofurans at M_r 182 and 196. In addition, a homologous series of C_{16}–C_{20} aliphatic alcohols appeared to be present. As in the D fraction, the reacted E fraction contained nitrogen-substituted compounds. Compounds that have been tentatively identified are *p*-nitrophenol (M_r 139) and *p*-nitroanisole (M_r 153). Benzaldehyde (M_r 106), hydroxybenzaldehyde (M_r 122), and naphthoic anhydride (M_r 198) were also identified.

The E fraction underwent large changes in mutagenicity after reaction with NO_2 + O_3. Its direct-acting slope showed an 8–20-fold increase and indirect mutagenicity increased from 4- to 8-fold. The change in percent mutagenic contribution of this fraction was not directionally consistent between experiments.

Polar Fraction (F). The most polar fraction, fraction F, was eluted with 100% ACN. As indicated before more than 80% of the mass appeared in this fraction both before and after reaction. The very polar nature of this fraction has proved to be a major obstacle for analytical work. This fraction has not been particularly amenable to mutagenicity analysis either, due to occasional toxicity to the tester bacteria. Because of the large percentage of mass appearing in this fraction, it contributes proportionally large percentages to the mutagenicity of wood smoke particles. In unreacted samples, although the slope values were relatively low compared to those of some of the moderately polar fractions, this fraction made the largest contribution to the direct-acting mutagenicity of the sample. After reaction with NO_2 + O_3, a large proportion (30–70%) of the direct- and indirect-acting mutagenicity was still observed in this fraction.

Summary and Conclusions

Chemical fractionation from unreacted and O_3 + NO_2 reacted wood smoke indicated that most of the extracted mass as well as the mutagenicity was contained within the most polar fractions. The PAH fraction generally made up less than 1% of extracted wood sample mass and contributed 12–17% of the total indirect-acting TA98 mutagenicity to fresh or unreacted chamber wood smoke. After reaction with O_3 + NO_2, the mutagenic contribution of the

PAH fraction declined substantially, and this was consistent with the observed loss in particle-bound PAH which were monitored over the course of the reaction. One of the moderately polar fractions, which contained compounds with the polarity of aromatic ketones contributed ~4% to the total direct-acting mutagenicity in unreacted samples. After reaction with $O_3 + NO_2$, this fraction, which only contained 2–3% of the extracted mass, made up 16–30% of the total direct-acting mutagenicity. GC/MS analysis of this reacted fraction tentatively indicated the formation of odd nitrogen organic hydroxy compounds and other oxygenated species. 1-Nitropyrene was found to contribute less than ~0.1% to the overall reacted mutagenicity.

It may be assumed that nitro-PAH analogues of all of the reacted PAH originally present in wood smoke could form to some degree in the $O_3 + NO_2$ system. Since many of these compounds were below the detection limit of our analytical systems, the mutagenic contribution of this class of compounds could not be evaluated.

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