Mutagenic Changes in Dilute Wood Smoke as it Ages and Reacts with Ozone and Nitrogen Dioxide: An Outdoor Chamber Study

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ever, the *variance* from one site to another could be very high compared with that for a random sampling scheme, because any particular set of samples is likely to detect too few or too many pockets of waste (Figure 3, top). Such waste distribution may at first seem unlikely, but at least two mechanisms might produce it. The first could occur if one load a day (or week etc.) of the toxic wastes were dumped at a municipal landfill with a fairly uniform filling rate. Or, a trash hauler might set a pattern of dumping a more or less fixed distance from his previous load (to protect his tires, or to stay away from smelly materials, or whatever).

It would probably be rare for a regularly spaced sample grid to "just miss" a regular spacing of wastes, but it would also be quite undesirable. (This problem of regular sampling of regular grids is similar to the "aliasing" problem in periodic sampling of periodic phenomena (*1*) and to problems in stereological morphometry (*2*).)

A randomized sampling scheme, for example, one based on a random number table as in Figure 3 (bottom), would be a better choice for regularly spaced wastes. Figure 3 (bottom) shows a stratified random sampling using 25 wells (the same number as in Figure 3 (top)) on 32 triangles. First, 25 of the 32 triangles were selected at random to be sampled. For each of those, three uniformly distributed random numbers (x_1, x_2, x_3) were selected from a table. Then $y_i = x_i/(x_i + x_2 + x_3)$ was calculated for $i = 1, 2, 3$; only two of these are independent because their sum is unity. Finally, the three y's form the coordinates of the sampling point in a triangular coordinate system similar to that used for describing soil texture as percentages of clay, silt, and sand (*3*).

The randomizing scheme just described was stratified across triangles. However, with random location of samples, there is no longer any advantage to triangles, and the samples could more easily be stratified on a square or rectangular grid. A randomized grid would be harder to lay out, but its advantages ought at least to be considered for some applications. It is worth reiterating that if the wastes themselves are located at random in the dump, then a regularly spaced grid will work as well as random samples.

A further consideration is that the goal in sampling a dump containing toxic wastes may not be to obtain an unbiased estimate of the density of waste clusters but rather to find *all* such clusters. In this case, the sampling network will need to be very dense, and a regular triangular grid will then be best.

Finally, the ideas above are very general, and should apply to many other area-based sampling problems. Indeed, one anonymous reviewer stated that triangular grids are commonly used in mineral exploration, but I have not been able to corroborate that statement.

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Mutagenic Changes in Dilute Wood Smoke as It Ages and Reacts with Ozone and Nitrogen Dioxide: An Outdoor Chamber Study

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■ Dilute wood smoke from a residential wood stove was added to two 25-m^3 outdoor Teflon film chambers. The smoke was permitted to age by itself or react with sub-ppm levels of NO_2, $NO_2 + O_3$, or O_3 in the presence and absence of natural sunlight. Most wood smoke particles fell into the 0.07–0.23 μm size range. The shape of the particle size distributions did not subsequently change during a 4-h reaction period. After reaction with $O_3 + NO_2$, the direct-acting bacterial mutagenicity (TA98–S9) of wood smoke extracts increased 2–10-fold. These changes occurred very rapidly. Increases were also observed when wood smoke was exposed to NO_2 alone, but these increases were not as great as those resulting from combined effects of $O_3 + NO_2$. Preliminary experiments with wood smoke aged in the dark or in the light in the presence of low levels of NO_2 and O_3 (i.e., <0.06 ppm) did not show increases in bacterial mutagenicity.

Introduction

Over the past decade many researchers have attempted to document the impact that the increased use of residential wood combustion could have on both the indoor and outdoor environment. Various investigators have found that during the winter months, emissions from wood combustion can contribute between 60 and 80% of the ambient fine particle loading observed over some communities (*1*, *2*). Since the organic extractable mass of wood smoke particles is very high, it is not surprising that other estimates (*3*) have shown that residential wood combustion accounts for more of the annual emission of polycyclic organic compounds (POC) than any other source. This is further supported by Lewtas (*4*), whose calculations show that, in the U.S. on an annual basis, more than 30% of the mutagenic material emitted into the atmosphere comes from wood combustion.

Little is known about the extent to which these POC react once they are emitted into the atmosphere. There is some preliminary and indirect evidence to indicate that changes do occur. In 1978, Pitts and co-workers (*5*) reported that the bacterial mutagenicity of selected polyaromatic hydrocarbons (PAH) adsorbed on filters was strongly enhanced by drawing sub-ppm concentrations of nitrogen dioxide (NO_2) across the filters. Trace levels of nitric acid ($HONO_2$) in the NO_2 gas stream were thought to be primarily responsible for promoting the observed

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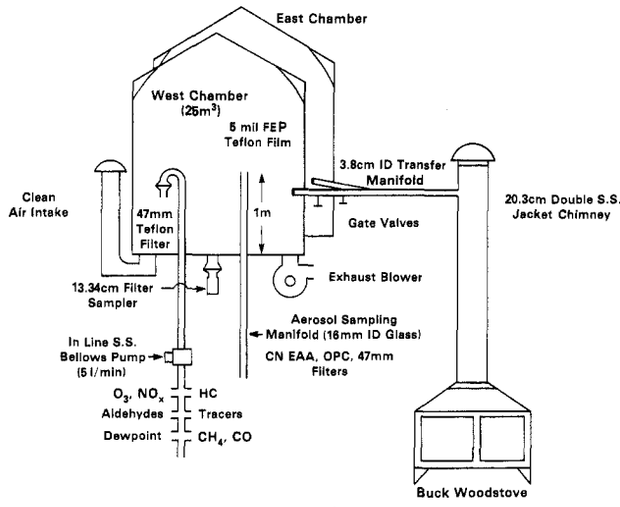

Figure 1. Illustration of UNC 25-m³ outdoor Teflon film chambers, sampling manifolds, and associated wood stove (not to scale).

increase in nitroaromatic product formation (*6*, *7*) and corresponding increases in mutagenicity. This underscores the potential importance of nitric acid–organic reactions because nitric acid ($HONO_2$) can be formed in the atmosphere from the reaction of NO_2 and O_3. In this reaction sequence, O_3 and NO_2 react to produce NO_3 which can further react with NO_2 to give N_2O_5. The significance of this reaction is apparent when one considers that the reaction of N_2O_5 and water to yield $HONO_2$ could take place on soot particle surfaces. This would provide ample opportunity for the reaction of $HONO_2$ with surface-adsorbed or condensed POC.

It could be also speculated (*8*) that NO_3 from the reaction of O_3 and NO_2 may react with the phenolic group of hydroxy-PAH and form hydroxynitro-PAH. This mechanism was originally proposed by others (*9*) for the smog formation of hydroxynitrotoluenes from cresols and NO_3 and could possibly be extended to higher molecular weight aromatic species. In addition to nitrogen species–POC reactions, reactions of PAH with light, O_3, and O_2 could potentially lead to the production of mutagenic compounds. A review of these and other related atmospheric reactions has been presented by Pitts (*8*).

To provide more direct evidence on possible atmospheric mutagenic and chemical transformations of particle-bound POC, an outdoor chamber study was undertaken. This paper describes some of the mutagenic changes that dilute wood smoke can undergo as it ages in the dark or the light and/or reacts with sub-ppm levels of O_3 and NO_2.

Experimental Approach and Procedures

Chambers and Injection of Wood Smoke. The two 25-m³ outdoor Teflon film chambers (referred to as the east chamber and west chamber) in this study were constructed at the University of North Carolina Ambient Air Research Facility (*10*) and have been described in detail elsewhere (*11*). A two-chamber system was used so that the effect of two different species on the same wood smoke could be observed under the same outdoor conditions. The chamber system, wood stove injection manifold, and sampling apparatus are illustrated in Figure 1.

Combustion aerosol was added to the chambers from a free-standing, medium size Buck wood stove (Smoky Mt. Enterprises, Asheville, NC). A 4.5 m × 3.81 cm i.d. iron pipe connected the chamber with the stove chimney. During injection, a slight vacuum (~0.7 cm of H_2O) was applied to the chambers with the main chamber exhaust blower. This provided impetus for the smoke to move from the chimney to the chambers. With an average flow through the pipe of ~50 L/min, smoke particles spent 6–8 s in the transfer manifold.

Seasoned red oak was used as the combustion wood. It was split into two sizes which were approximately 6 cm or 12 cm in diameter and 0.5 m in length. Fires were usually started with 0.25 kg of oak kindling, with the split logs being added soon thereafter. This fire was permitted to burn with the stove vents open for 15 min. If a low burn rate was desired (i.e., <2 kg/h), the stove was loaded with ~8 kg of the 12-cm wood. After the initial 15-min burn period, the air intakes to the stove were completely closed, and another 15–20 min of burn was permitted. In the high burn rate case (4–8 kg/h), 6-cm split wood was used to load the firebox with 8–16 kg of wood, and the air intakes were opened. Crude estimates of the burn rate were made by removing and reweighing the wood from the stove after the chamber injection process was completed. In a few experiments dried North Carolina peat was burned in the stove and peat smoke injected into the chambers.

During injection of smoke into one or both chambers, the total aerosol concentration was monitored with either an Environment/One condensation nuclei (CN) counter or a Thermo Systems, Inc., Model 3030 electrical aerosol analyzer (EAA). This facilitated the injection of desired soot concentrations into both chambers at the same time. At the start of an experiment, background conditions were determined. A high concentration of wood smoke was then injected into both chambers. After the smoke was permitted to equilibrate in the chambers for 15–30 min, wood smoke particles were collected on T60A20 Pallflex 13.34-cm Teflon-impregnated glass fiber filters. Approximately 5–40 mg of wood soot was collected over a 10-min period at a flow rate of 0.9 m³/min. These initial samples were used to establish the base-line bacterial mutagenicity of the unreacted chamber wood smoke. After this sample was taken, O_3, NO_2, or both were added from an electrical discharge ozone generator or high concentration NO_2 (in nitrogen) tank. At the conclusion of the experiment, another 13.34-cm filter sample was taken. Since aerosol mass was lost from the chambers over the course of an experiment, these final or reacted filter samples were taken for 30–40 min.

Instrumentation. During most wood soot experiments, chamber gas-phase concentrations of NO, NO_2, and O_3 were monitored with Bendix Model 8101-B and 8002 analyzers. Vapor-phase C_2–C_{10} individual hydrocarbons were monitored with an automated Carle 211 gas chromatograph; a Beckman Model 6800 gas chromatograph was used to monitor CO and CH_4. In selected experiments, C_1–C_8 aldehydes were traced with a modified 2,4-dinitrophenylhydrazine–HPLC technique (*12*).

Aerosol characterization data were derived from three different aerosol instruments. A condensation nuclei counter, which is not size discriminatory, measured particles in the 0.01–0.3-μm range. Particles in the 0.013–0.750-μm range were counted and sized by an electrical aerosol analyzer (EAA) while a Climet Model CI-208 optical particle counter (OPC) was used for particles in the optical range (0.3–5 μm). Teflon-impregnated 47-mm filter samples (Pallflex) were also taken to monitor the particle mass concentration. A flow rate of 0.07 m³/min and a sample time of 10 min were used.

Sample Workup and Mutagenicity Testing. The 13.34-cm filter samples for bioassay analysis were immediately Soxhlet extracted in the dark for 16 h with 100 mL of methylene chloride ($MeCl_2$). The soot extract was then

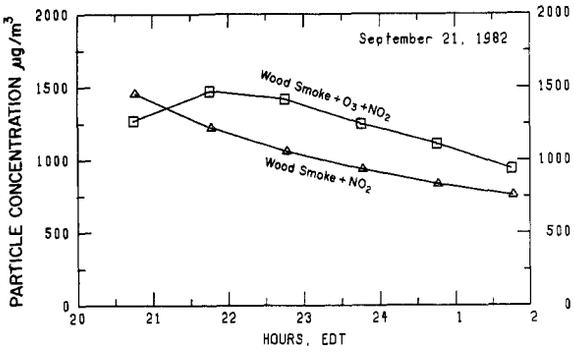

Figure 2. Wood smoke particle concentrations vs. time as determined with 47-mm Teflon-impregnated glass fiber filters (T60A20, Pallflex).

concentrated by rotary evaporation to 5 mL. Extraction of the 47-mm filter samples was performed in 25-mL micro Soxhlets for 16 h, followed by concentration to 2 mL with micro Snyder columns (Supelco). Mass determination of the extracts was made by adding small aliquots of the extract onto 47-mm Teflon-impregnated glass fiber filters, evaporating the solvent, and determining the gain in mass. In general 65–80% of the unreacted particle mass was observed in the solvent extract. After reaction the wood soot particle extracts declined to 50–65%. Extract samples were prepared for bioassay by solvent exchange with dimethyl sulfoxide.

The *Salmonella typhimurium* plate incorporation assay was performed as described by Ames et al. (*13*) with minor modifications (*14*). All assays were conducted at the Environmental Protection Agency laboratories, Research Triangle Park, NC. The bioassays were performed by using TA98 with triplicate plates at five to six doses with and without metabolic activation (±S9). When sample extracts were low in mass (<1.0 mg), only duplicate plates were run. Overall quality control of the tester strains was monitored and maintained on a weekly basis, and TA98 was evaluated during each experiment for response to 2-anthramine (+S9) and 2-nitrofluorene (–S9).

Results and Discussion

Wood Smoke Particle Behavior in the Chambers. Filter mass data from over 20 experiments have shown that approximately 40–50% of the chamber particle mass disappeared in 3.5–5 h. An illustration of this loss is plotted in Figure 2. Chamber leaks together with gas and aerosol sampling accounted for approximately 10–15% of this loss. Particle losses were presumed to be a function of agglomeration, settling, and electrostatic processes (*15*). On a number of occasions, a slight initial increase in particle mass concentration was observed after the addition of NO_2 and O_3 (Figure 2). A possible explanation is that O_3 and NO_2 immediately reacted with vapor-phase species forming polar compounds which had lower vapor pressures than the original reactants. These products then condensed on existing particle surfaces giving rise to an increased initial aerosol mass.

Typical EAA number and volume vs. particle diameter distributions for wood smoke immediately after injection into the chambers and after 4 h of aging are shown in Figure 3. These distributions are similar in shape to those reported by Dasch (*16*) for fireplace emissions. A decline in the number of particles in the 0.076- and 0.133-μm range occurred over the 4-h period. During this period a slight shift to larger particle sizes was observed for both the number and volume distributions. Nevertheless, number and volume distributions did not significantly change. Generally, particles in the 0.1–0.3-μm range made the

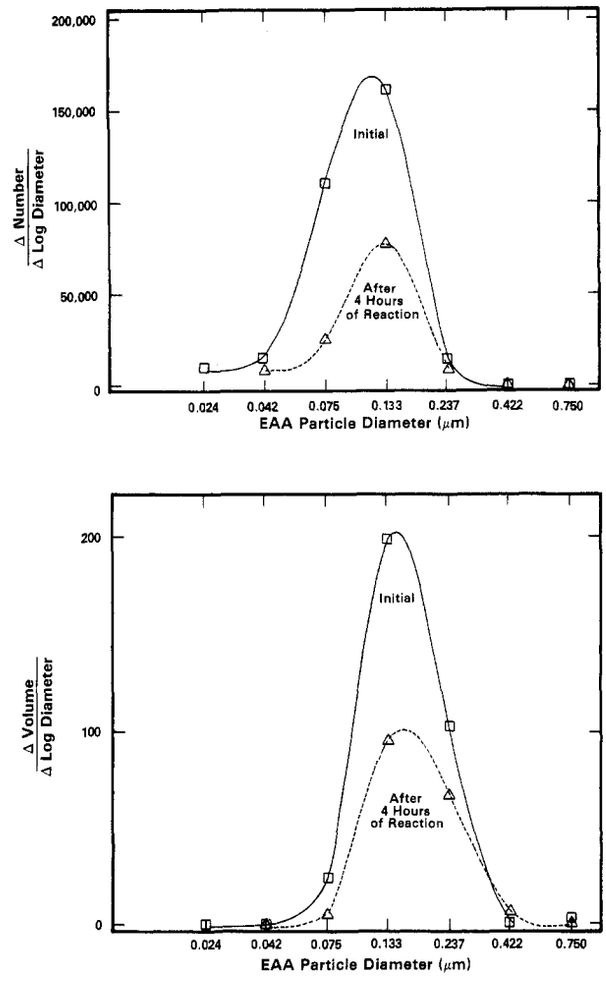

Figure 3. EAA number and volume particle size distributions for wood smoke in UNC 25-m^3 chambers.

largest contribution to the volume and, hence, the mass in the EAA size range. Optical particle counter measurements showed that most of the particles in the optical range were below 0.5 μm in diameter while almost no particles were larger than 1 μm in optical diameter (*11*). In addition, no shift in particle size distribution in this range was observed over time. It is important to note that the OPC and EAA were used primarily for qualitative size distribution analysis. Previously stated limitations on the use of these instruments apply here (*15*). Nevertheless, it was felt that the observed trends in both the EAA and OPC data do approximate the overall particle behavior in the system.

Mutagenic Behavior of Dilute Wood Smoke Systems without Metabolic Activation. As mentioned previously, there is evidence that NO_2 or $HONO_2$ can react with polycyclic organic compounds on airborne soot and increase the mutagenicity of the soot particle extracts. Since nitric acid can be formed in the atmosphere from the reaction of O_3 with NO_2, $O_3 + NO_2$ systems were among the first tested. Data characterizing the conditions for all of the experiments presented are given in Table I. Representative vapor-phase concentrations that appeared with dilute wood smoke in the chamber are shown in Table II.

An example of the NO_2, NO, and O_3 concentration vs. time plots for a typical dual chamber wood smoke run is shown in Figure 4. The purpose of this experiment was to compare the effects of NO_2 alone to those of $O_3 + NO_2$ on dilute wood smoke. Note the presence of combustion NO and NO_2 as wood smoke was simultaneously added to

Table I. Summary Data from Dilute Wood Smoke Experiments

run date	Rate chamber	burn rate, kg/h	reaction time, h	particle concn, $\mu g/m^3$	initial concn, ppm			final concn, ppm			slope[b] from dose-response curves: TA98–S9, rev/μg		TA98+S9, rev/μg	
					NO[a]	NO_2[a]	O_3	NO	NO_2	O_3	initial	final	initial	final
09-07-82	east	ND[c]	dark/3.5	1286	0.00	0.86	0.53	0.00	0.23	0.05	0.11	1.36	0.51	0.79
	west	ND	dark/3.5	1436	0.11	0.05	0.00	0.14		0.00	0.07	0.11	0.23	0.18
09-21-82	east	ND	dark/4.0	1271	0.00	0.32	0.22	0.01	0.12	0.00	0.24	0.83	0.27	0.34
	west	ND	dark/4.0	1457	0.02	0.31	0.00	0.10	0.22	0.00	0.09	0.21		
12-02-82	east		dark/0.8	16	0.00	0.39	0.27	0.00	0.23	0.17		0.18		
	west	4.2	dark/0.8	2549	0.00	0.27	0.27	0.00	0.16	0.15	0.18	0.41		
12-07-82	east	6.2	dark/3.0	1535	0.00	0.55	0.37	0.00	0.15	0.06	0.12	2.32		
	west	6.2	dark/3.0	1402	0.00	0.54	0.00	0.01	0.46	0.00	0.17	0.69	0.73	1.10
12-14-82	east	1.8	dark/4.0	3850	0.00	0.62	0.42	0.00	0.11	0.05	0.17	1.7		
	west	1.8	dark/3.0	1509	0.00	0.05	0.57	0.00	0.02	0.42	0.14	0.62		
02-15-83	east[d]	0.6	dark to midday/19.0	2597	0.00	0.48	0.32	0.00	0.06	0.23	0.06	0.61	0.24	0.45[f]
	west	4.8	dark to midday/19.0	7717	0.00	0.47	0.26	0.00	0.12	0.09	0.34	1.04	0.98	0.83
03-16-83	west	3.1	morning to next afternoon/27.0	2345	0.02	0.03	0.00	0.00	0.01	0.00	0.03	0.02	0.12	ND
04-12-83	east	3.3	night/3.0	4813	0.00	0.54	0.24	0.00	0.17	0.06	0.08	~0.8		
05-25-83	west	>4	days/5.0	1998	0.01	0.05	0.00	0.00	0.03	0.01[e]	0.18	0.23	0.87	0.43

[a] Chemiluminescent NO and NO_2. [b] Estimated linear slope from dose–response curves. Points up to the 300-μg dose range were used unless toxicity resulted, and then doses up to 200 μg were used. [c] Not determined. [d] Peat smoke. [e] Photochemically generated O_3. [f] Mutagenicity after 4 h.

Table II. Initial Conditions after Initial Bioassay Sample in Both Chambers for Sept 21, 1982, Dilute Wood Soot Run

	east chamber	west chamber
particle concn, $\mu g/m^3$	1270	1456
NMHC, ppm of C	3.1	3.3
CO, ppm	11.5	11.5
CH_4, ppm	3.24	3.53
ethane, ppm of C	0.04	0.05
ethylene, ppm of C	0.10	0.14
propane, ppm of C	0.04	0.05
propylene, ppm of C	0.07	0.08
C_6–C_8, ppm of C	1.48	1.62
formaldehyde, ppm	0.12	0.18
acetaldehyde, ppm (volume)	0.08	0.11
propionaldehyde, ppm (volume)	0.10	0.13
acetone, ppm (volume)	0.05	0.06
C_4 carbonyls, ppm (volume)	~0.06	~0.06
dew point, °F	60	62
temperature, °F	65	65

both chambers and the decline in NO and NO_2 concentrations when the initial unreacted wood smoke filter samples were taken. As O_3 from the high concentration O_3 generator was added to the east chamber, an immediate titration of combustion NO to NO_2 occurred. Continued O_3 injection resulted in a concentration of 0.37 ppm of O_3. NO_2 was then added from a cylinder to a concentration of 0.55 ppm. In the west chamber a small injection of O_3 was made to titrate existing combustion NO to NO_2. This was followed by a 0.54-ppm injection of NO_2. Note that after 40 min, only a slight loss in NO_2 concentration was observed in the west chamber (wood smoke + NO_2). However, in the east chamber (wood smoke + NO_2 + O_3), both O_3 and NO_2 disappeared rapidly. These two observations suggest that in the east chamber much of the NO_2 loss was due to reaction with O_3 to form NO_3. This was followed by the subsequent production of N_2O_5, and ultimately nitric acid.

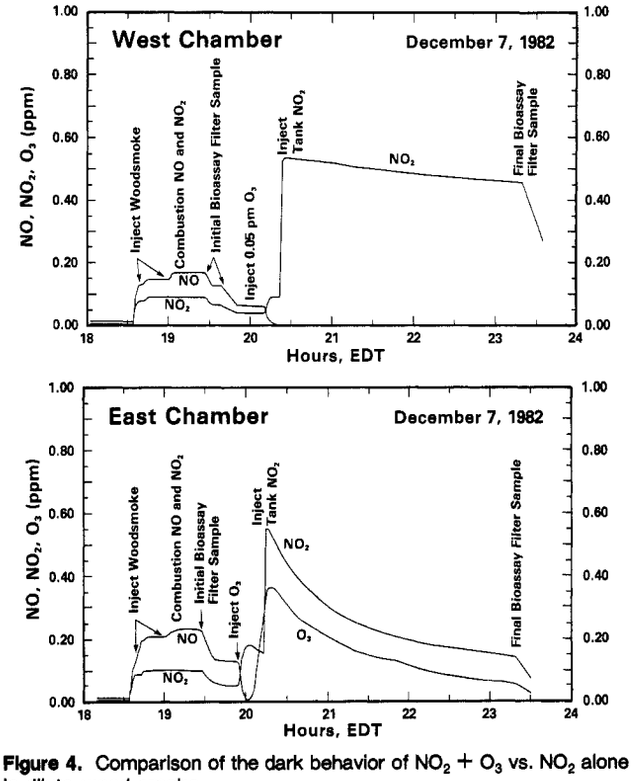

Figure 4. Comparison of the dark behavior of NO_2 + O_3 vs. NO_2 alone in dilute wood smoke.

The relative mutagenic dose–response (TA98–S9) curves for the wood smoke systems described in Figure 4 are shown in Figure 5. The initial wood soot samples from both chambers had very similar dose–response curves. We have found this to be generally true if wood smoke injections were made into both chambers simultaneously and if the bioassay analysis of the wood soot extracts were conducted on the same day. Both NO_2 and NO_2 + O_3 greatly increased the mutagenicity of dilute wood soot. However, the enhancement due to O_3 + NO_2 was much

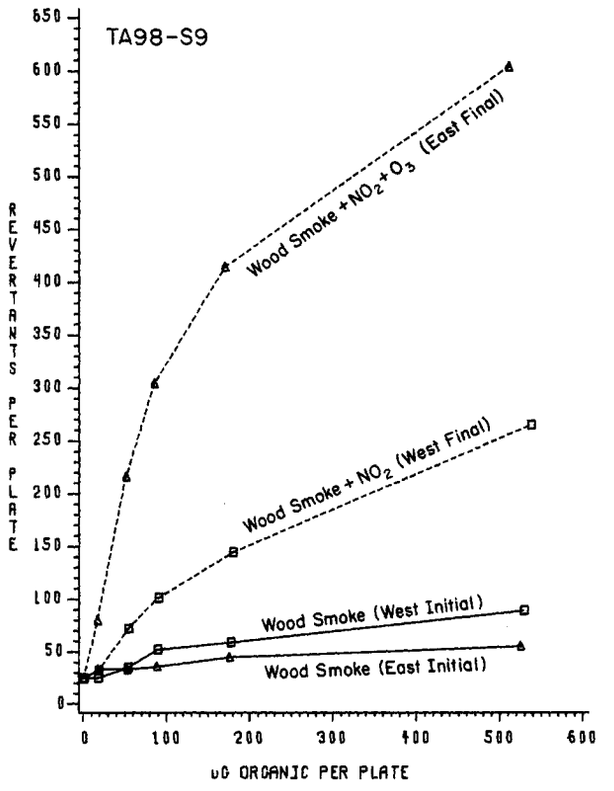

Figure 5. TA98–S9 mutagenic dose–response curves for the Dec 7, 1982, dilute wood smoke experiment.

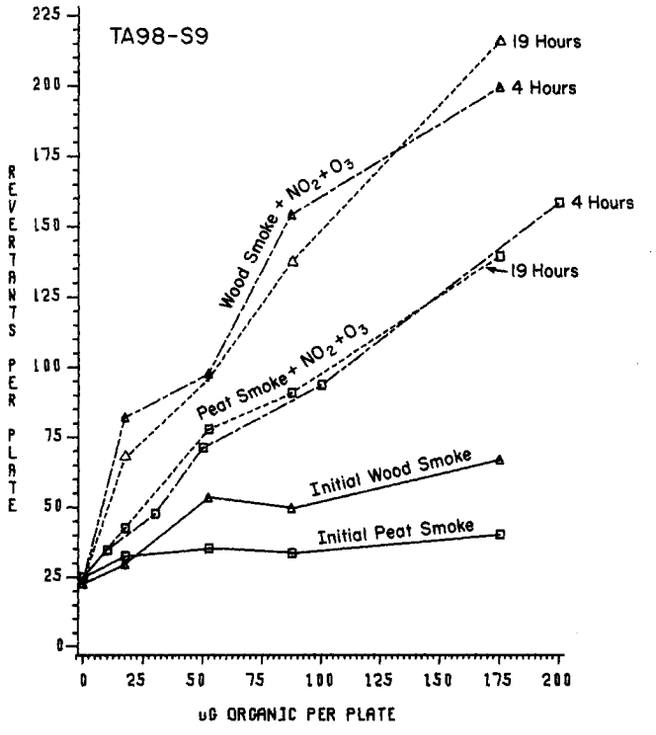

Figure 6. TA98–S9 mutagenic dose–response curves from a dual experiment on Feb 15, 1983, with O_3 + NO_2 + dilute peat smoke in one chamber and O_3 + NO_2 + wood smoke in the other chamber.

larger than that of NO_2 alone. Other experiments illustrating this effect are shown in Table I.

We have also observed that the above-mentioned NO_2 and O_3 effect is not unique to wood soot emissions. This is illustrated in Figure 6 in which both dilute wood smoke and dilute peat emissions were reacted in the dark with O_3 + NO_2. After 4 h of dark reaction both wood and peat soot showed an increase in –S9 mutagenicity. After 19 h

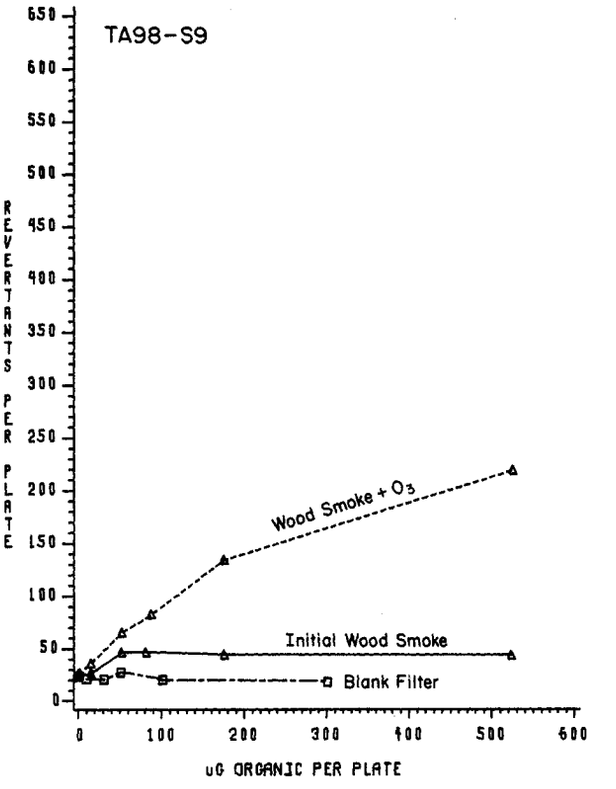

Figure 7. TA98–S9 mutagenic dose–response curve from a dilute wood smoke + O_3 experiment on Dec 14, 1982.

of reaction, most of which occurred during the daylight hours, the original 4-h (TA98–S9) mutagenic enhancements were unchanged. An increase in indirect-acting mutagenicity occurred, however, between the 4-h and 19-h exposure period.

It is important to mention that contaminating, low ppb levels of nitric acid (as in the Pitts et al. (5) PAH-filter experiments) were possibly added to the chambers during the NO_2 (cylinder) injections. The extent to which this promoted some of the observed mutagenic increase in NO_2–wood smoke systems is unknown. To determine the effects of O_3 alone on dilute wood smoke was also a difficult task. Ideally, these experiments should have been conducted so that wood soot particles appeared in the chambers in the complete absence of combustion NO_x. This would preclude the production of nitric acid when O_3 was added. Although this was not possible, attempts were made to minimize the amount of combustion NO_x injected by using low wood burn rates. In one such experiment, which occurred on Dec 14, 1982, 0.05 ppm of NO_x (~92 $\mu g/m^3$ as NO_2) and 1523 $\mu g/m^3$ wood soot particles were present in the chamber after the initial bioassay filter sample was taken. Ozone was then injected to a level of 0.57 ppm. As shown in Figure 7, an increase in direct-acting mutagenicity resulted. Given, however, that more than half of the 92 $\mu g/m^3$ NO_2 disappeared in the O_3 + wood smoke experiment, it is probable that nitric acid formed. Thus, as in the NO_2–wood smoke experiments, the $HONO_2$ contribution to the observed mutagenic increases in the O_3–wood smoke experiment could not be estimated.

Concurrent with the previously described experiments, an evaluation of wood smoke aged in the dark was undertaken. Dilute wood smoke was injected into one chamber and compared to the second chamber containing wood smoke reacted with O_3 + NO_2. The dose–response curves for both chambers from this experiment are shown in Figure 8. Note that, as before, the chamber (east) which

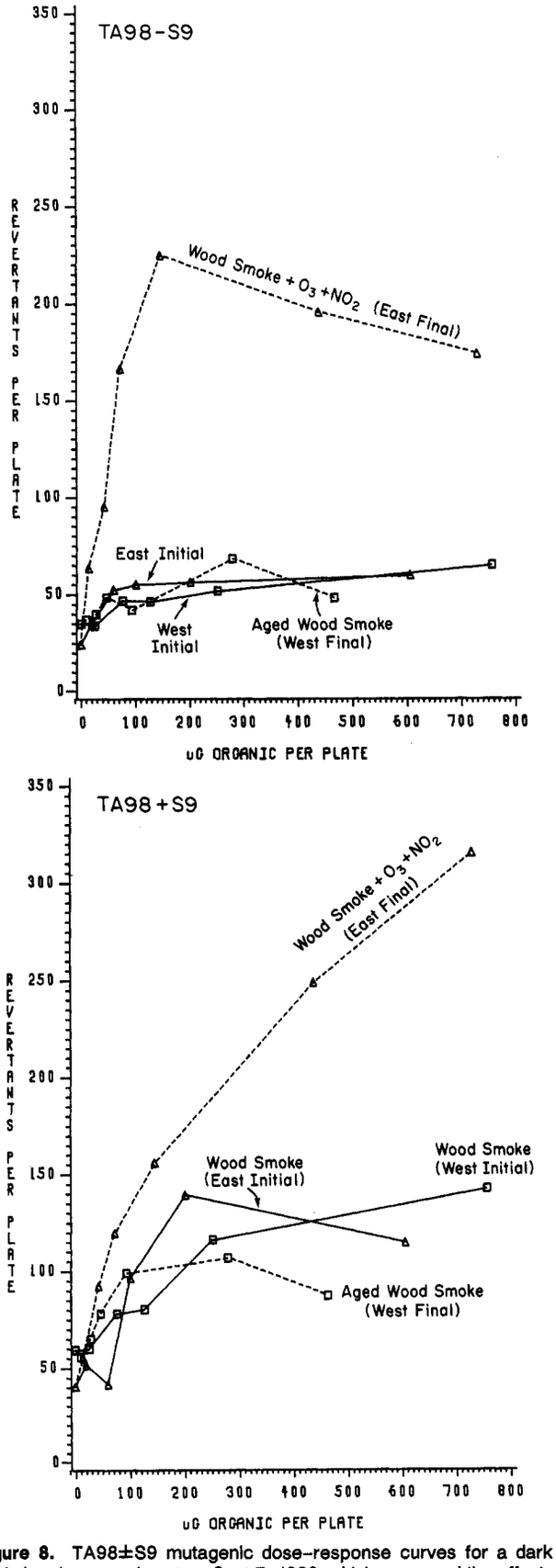

Figure 8. TA98±S9 mutagenic dose–response curves for a dark dual-chamber experiment on Sept 7, 1982, which compared the effects of aging dilute wood smoke without additional O_3 or NO_2 vs. the effects of adding O_3 + NO_2.

received both O_3 and NO_2 showed a sharp increase in direct (–S9) mutagenic activity. The west chamber, containing only dilute wood smoke, did not show any mutagenic

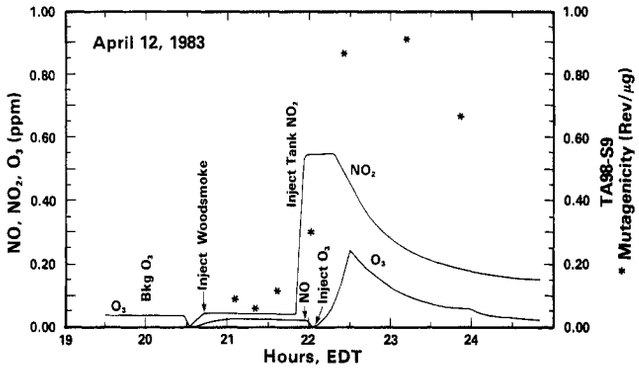

Figure 9. Rate of TA98–S9 mutagenic increase of dilute wood smoke after exposure to O_3 + NO_2.

difference between initial and final samples.

Preliminary wood soot experiments were also conducted in the sunlight (Table I, March 16 and May 25, 1983, experiments). Low burn rate conditions were used to limit the amount of combustion NO_x injected into the chambers to less than 0.06 ppm. This created a NO_x-limited photochemical mixture (HC/NO_x = ~50/1 ppm of C/ppm) due to the high gas-phase volatile hydrocarbon concentrations which were similar to those shown in Table II. The high HC/NO_x ratio and possible O_3 losses to the wood soot particle surfaces tended to limit the levels of photochemically generated O_3 which appeared in these systems. Hence, it was not surprising that less than 0.01 ppm of O_3 was measured in these daytime experiments. Under these conditions, no *increase* in either direct-acting or indirect-acting mutagenicity was observed.

Mutagenicity of Wood Smoke Systems with Metabolic Activation. Bioassays of the particle extracts from more than 20 initial dilute wood smoke chamber samples (*17*) showed that the mutagenicity with S9 activation was always higher than without S9 activation. Lewtas (*18*) reported mutagenic activity of wood stove emissions using a nonlinear model (*19*) to determine the slope of the dose–response curve. When oak was burned, a slope of 0.9 revertant/μg (rev/μg) of organic extract was observed with the addition of S9 and 0.15 revertant/μg without metabolic activation. To provide a comparison, the slope values in this study were also calculated by using the same nonlinear model (*19*). This gave dilute wood smoke slope values similar to those reported by Lewtas (*18*). The average nonlinear model slope was 0.83 revertant/μg in an activated system and 0.28 revertant/μg without S9 activation.

For wood smoke exposed to O_3 + NO_2, indirect-acting (+S9) mutagenicity showed a less dramatic increase than direct-acting (–S9) mutagenicity (Figure 8). This suggests a greater production of direct-acting mutagens than of indirect-acting mutagens from NO_2 + O_3 systems.

Rate of Mutagenic Increase and Sampling Artifacts. In order to assess the rate at which the mutagenic nature of wood smoke was increased by O_3 + NO_2, a separate experiment was conducted. The 47-mm filter samples, which were typically used to monitor particle concentrations, were extracted and bioassayed with TA98–S9. This permitted much better time resolution of the mutagenic changes. The mutagenic results of this experiment are plotted in Figure 9 along with the NO_2 and O_3 behavior of the system. The mutagenicity of each filter sample reported in Figure 9 was calculated from the linear portion of the dose–response curve. Note that three filter samples were taken soon after wood smoke was injected into the chambers. This established the base-line mutagenicity of the dilute wood smoke soon after it was injected and before NO_2 and O_3 were added. Ten minutes after NO_2 was

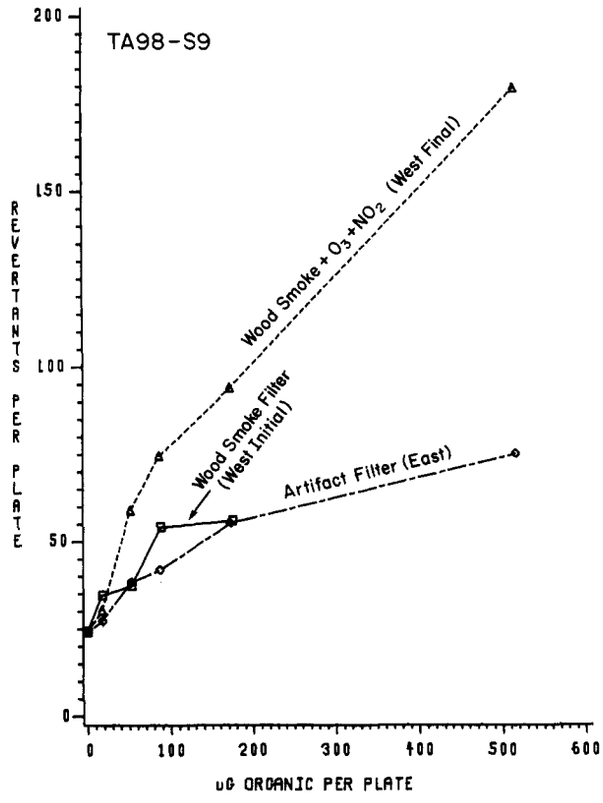

Figure 10. Wood smoke mutagenicity filter artifact experiment with O_3 + NO_2.

injected another filter sample was taken and the -S9 mutagenicity of the particle extract had increased from approximately 0.1 to 0.3 rev/μg. After O_3 was added, the mutagenicity increase was even more striking. These results suggest that mutagenic increases from wood soot exposure to O_3 and NO_2 occur very rapidly.

There has been much concern about the potential for confounding mutagenic artifact formation during the filter sampling process (*6*, *7*, *20*, *21*). For this reason, experiments were designed to distinguish between mutagenic changes which occur while particles were in the airborne state and possible mutagenic artifact processes which occur during sampling.

These experiments differed from others in that wood smoke was injected into only one of the chambers. The other chamber was closed to the surrounding atmosphere and the wood stove injection manifold. Hence, it contained only rural background air. Two filter samples were taken from the chamber that had just received the wood smoke injection. One of these wood soot filter samples was placed in the sampling apparatus of the background air chamber. This sample was designated as the artifact filter. The other wood soot sample was used to establish the initial base-line wood soot mutagenicity of the unreacted chamber wood smoke. Ozone and NO_2 were then added to *both* chambers. After ~1 h, air from the background air chamber (with O_3 + NO_2) was drawn across the artifact filter. Simultaneously, a final sample was collected from the chamber which contained dilute wood smoke, O_3, NO_2, and other reaction products.

The results for an experiment conducted on Dec 2, 1982, are illustrated by the dose–response curves shown in Figure 10. The NO_2 and O_3 concentrations in the chambers both before and after reaction are reported in Table I. Note that higher concentrations of O_3 and NO_2 were drawn over the wood smoke artifact filter than those which were present in the wood smoke + O_3 + NO_2 chamber (when the final bioassay filter sample was taken). As expected, the O_3 + NO_2 reacted wood smoke sample was more mutagenic (TA98–S9) than the initial unreacted sample. Most important, however, was that little difference could be discerned between the initial, unreacted, and artifact filter samples. These types of experiments suggest that, at the sampling rates and times used and at the O_3 and NO_2 concentrations typically present in the chambers when final filter samples were taken, significant artifact effects on direct-acting TA98 mutagenicity did not result.

Summary

In this study dilute wood smoke at particle concentrations of 1300–8000 $\mu g/m^3$ was aged in the dark or in sunlight for several hours in outdoor Teflon film environmental exposure chambers. The shapes of the particle size distributions of wood soot in the chambers did not appreciably change over a period of 4 h. Aging dilute wood smoke in the presence of low levels of combustion-generated NO_2, either in the sunlight or in the dark, did not increase the TA98 bacterial mutagenicity of dilute wood smoke. The addition of sub-ppm mixtures of O_3 + NO_2 (in the 0.3–0.9 ppm range) to dilute wood smoke substantially increased the direct-acting mutagenicity of the particle extracts. This enhancement in direct mutagenic activity occurred within a period of minutes. The indirect-acting or promutagenic activity of wood smoke particle extract was also increased by NO_2 + O_3 mixtures, but to a lesser extent. When NO_2 alone was reacted with wood smoke, the increase in direct mutagenic activity was not as great as from combined NO_2 + O_3 systems. Systems containing NO_2 + O_3 most probably have a high potential for heterogeneous nitric acid formation on the surface of soot particles. The observed increased bacterial mutagenicity of the wood soot extracts in such systems, therefore, may be caused by the direct reaction of this surface-generated nitric acid with particle-bound organics.

Preliminary results also indicate that emissions from peat combustion react with O_3 and NO_2 in a manner similar to wood combustion products. This result suggests that particulate emissions from other combustion fuels such as coal, oil, diesel, or gasoline may also be subject to atmospheric transformation. It is our contention that as air pollution research advances and research findings are translated into regulatory policy, there will be a need to go beyond the characterization of stack or tailpipe emissions, to an approach which factors in the potential "atmospheric mutagenicity" resulting from atmospheric processes. However, a realistic assessment of these atmospheric changes, and their possible health implications, will require a substantial increase in the study of both real and simulated atmospheres.

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Registry No. Ozone, 10028-15-6; nitrogen dioxide, 10102-44-0; ethane, 74-84-0; ethylene, 74-85-1; propane, 74-98-6; propylene, 115-07-1; formaldehyde, 50-00-0; acetaldehyde, 75-07-0; propionaldehyde, 123-38-6; acetone, 67-64-1; carbon monoxide, 630-08-0; methane, 74-82-8.

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Effect of Censoring Trace-Level Water-Quality Data on Trend-Detection Capability

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■ Monte Carlo experiments were used to evaluate whether trace-level water-quality data that are routinely censored (not reported) contain valuable information for trend detection. Measurements are commonly censored if they fall below a level associated with some minimum acceptable level of reliability (detection limit). Trace-level organic data were simulated with best- and worst-case estimates of measurement uncertainty, various concentrations and degrees of linear trend, and different censoring rules. The resulting classes of data were subjected to a nonparametric statistical test for trend. For all classes of data evaluated, trends were most effectively detected in uncensored data as compared to censored data even when the data censored were highly unreliable. Thus, censoring data at any concentration level may eliminate valuable information. Whether or not valuable information for trend analysis is, in fact, eliminated by censoring of actual rather than simulated data depends on whether the analytical process is in statistical control and bias is predictable for a particular type of chemical analyses.

Introduction

Certain key types of water pollutants, principally synthetic organic compounds and some metals, often occur only in trace-level concentrations and yet are environmentally important at trace levels. Trace-level concentrations are herein defined as being so low that their detection and identification based on a single analytical determination is reasonably in doubt—the probability of incorrectly deciding that the compound is present is greater than about 5%. Both imprecision and the potential for systematic error in quantitative measurements of trace levels are generally high and poorly understood. The combination of uncertain detection and poor reliability of trace-level measurements has led laboratories to adopt rules that specify concentration levels, usually referred to as detection limits but called data-reporting limits in this report, below which measurements are not reported to data users. From the standpoint of statistical data analysis, this practice results in "censored" data sets. The term censored is used strictly in the statistical sense and is not intended to imply that laboratories are intentionally withholding valuable information or have anything but the data users' best interest in mind. Measurements that are censored are usually reported as "not detected" or as "less than" the reporting limit.

Many typical statistical analyses such as comparisons of means or variances and regression analyses are difficult or impossible to apply to censored data sets (*1*). The reliability of statistical analyses that can be applied to censored data generally decreases as the degree of censoring increases. The adverse effects of censoring are particularly acute for data on trace-level contaminants of surface waters because dilution is commonly great, resulting in low concentrations and high degrees of censoring. For example, quaterly data collected for 5 years at more than 150 stations as part of the National Pesticide Monitoring Network for Rivers indicate that more than 95% of measured concentrations for 25 pesticides were censored. Such degrees of censoring and resulting difficulties with statistical analysis may not be important for chemicals that have environmental effects only at high concentrations. But, for many of the 25 pesticides, for example, data-reporting limits were near or above water-quality criteria.

Censoring according to data reporting limits that are above concentrations that may have adverse effects on

