United States
Environmental Protection
Agency

Atmospheric Sciences
Research Laboratory
Research Triangle Park NC 27711

Research and Development

EPA/600/S3-86/049 Dec. 1986

EPA

Project Summary

Mutagenic Activities of Wood Smoke Photooxidation Products

T. E. Kleindienst, P. B. Shepson, and E. O. Edney

The full report presents the results of experiments designed to evaluate the mutagenic potential of wood smoke in the environment. The experiments were conducted by injecting emissions from a wood stove into a Teflon smog chamber and irradiating the diluted mixture. The mutagenic activity of the gas phase component was tested by exposing the bacteria *Salmonella typhimurium*, strains TA98 and TA100, to the filtered effluent after the irradiation. The particulate phase was tested for mutagenic activity using the plate incorporation procedure on the filter extracts. The data show that the irradiated mixture is more mutagenic than the direct wood smoke emissions for TA100 in the gas phase and for TA98 in both the gas and particulate phases. Comparison of the mutagenic activities of the gas and particulate phase components indicated that the activity of the gas phase transformation products was much greater than the particulate phase when expressed in revertants per cubic meter of air.

This Project Summary was developed by EPA's Atmospheric Sciences Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Recently, there has been increased concern that human exposure to wood stove and fireplace emissions may provide a public health concern. Several published reports have indicated substantial increases in the mutagenic activity of the particulate phase of wood smoke after reaction in the dark with O_3 and NO_2 and that the most polar components of the extract gave rise to the majority of the mutagenic activity.

There has been little study of the mutagenic activity of the gas phase emissions from wood stoves. Recent investigations have shown that, whereas low molecular weight alkenes and aromatics themselves show relatively low mutagenic activity, the photolytically induced reaction products can show significant activity. Since there are significant amounts of low molecular weight reactive hydrocarbons in wood smoke, the photooxidation products might be expected to show substantial mutagenic activity. In this study we report the results of several experiments in which the total mutagenic response in *Salmonella typhimurium* strains TA98 and TA100 was measured for dilute mixtures of wood smoke irradiated in the presence of NO_x.

Procedures

The experimental apparatus consisted of three major components: (1) the wood stove and dilution tunnel, (2) the reaction chamber, and (3) the exposure chambers. The wood stove/dilution tunnel combination allowed dilute mixtures of wood smoke to be injected into the reaction chamber. The chamber was loaded such that the initial total hydrocarbon concentration was about 18 ppmC.

The reaction chamber was a 22.7-m^3 cylindrical vessel of 0.13-mm Teflon sealed to fluorocarbon-coated, aluminum end plates. The chamber was surrounded longitudinally with a com-

bination of sun lamps and ultraviolet black light. In some experiments, nitric oxide was added to the dilute wood smoke in the reaction chamber such that the total NO_x concentration was about 600 ppb. NO was added to increase the hydrocarbon conversion during the irradiation such that a sufficient quantity of reacted material would be available for the exposure. Experiments were also performed in which the only source of NO_x was from the combustion.

The experiments were conducted by loading the reaction chamber with wood smoke to the desired HC level for several hours. For the experiments with additional NO_x, NO was added immediately before the start of irradiation. When the reaction mixture had been sufficiently mixed, the lights were turned on, starting the photochemical conversion. The irradiation continued until the ozone and peroxyacetyl nitrate (PAN) that formed in the system reached constant levels. The chamber lights were then turned off, and the effluent was filtered and allowed to flow into the exposure chamber, which contained the biological assay. After exposure, the filter was solvent extracted, and the extract was tested for mutagenic activity. Three exposure chambers used as controls allowed measurement of the gas phase mutagenic activity of the clean air, the ambient air used in the dilution tunnel, and the initial reactant mixture. Filters were also collected and extracted for the ambient air and reactant controls.

The mutagenic activity of the particulate phase was measured using the standard plate incorporation test with the *S. typhimurium* strains TA98 and TA100. The gas phase exposures were performed using petri dishes containing the same strains of bacteria seeded in base agar, which were placed in an exposure chamber that was connected to the reaction chamber. The air from the reaction chamber was filtered and passed over the bioassay plates in the exposure chamber. During the exposure, gas components in the air mass that were soluble dissolved into the agar medium, thus allowing contact with the biological assay.

Inorganic and organic gas phase species were monitored by continuous gas analyzers and gas chromatography. Aldehyde concentrations were measured by derivatization in a solution of 2,4-dinitrophenylhydrazine and subsequent analysis of the formed hydrazones by high performance liquid chromatography (HPLC). The total hydrocarbon (HC) concentration was measured using a total hydrocarbon analyzer with flame ionization detection.

The size distribution of the particulate matter in the range 0.01 to 1 μm was determined using an electrical aerosol analyzer, and the total particle concentration was measured using a condensation nuclei counter. The particles collected on the glass fiber filters were extracted and analyzed for polynuclear aromatic hydrocarbon (PAH) concentration by GC/MS.

Results

Four gas phase exposure experiments were performed. In one experiment, dilute wood smoke alone was irradiated and the effluent used in the exposure. In the other three experiments, 0.5 ppm of NO was added to the diluted wood smoke in the reaction chamber. In one of the experiments with added NO_x the exposure was performed using *S. typhimurium* with metabolic activation (S9).

For the irradiation with wood smoke only, 135 ppb of NO_x was available (as a result of the combustion), giving an HC/$NO_x > 100$. As a result, the photolytic conversion of reactants to products was extremely rapid, but the extent of conversion was limited by the low concentration of NO_x. The major reactive gas phase components for wood smoke included low molecular weight alkenes, aromatic compounds, oxygen atom heterocyclic compounds, and aldehydes. The aldehydes were present not only as reactants in the initial mixture but also as products formed during photooxidation.

The initial particulate distribution was in the range of 0.01 to 1 μm, with the maximum in the number distribution occurring at about 0.1 μm. After irradiation, the total number of particles in this range decreased, and the maximum in the number distribution shifted to 0.2 μm. The volume distribution of the particles initially present had a maximum at 0.2 μm. After irradiation, the total volume of particles in the range 0.01 to 1 μm increased, with the maximum in the volume distribution occurring at 0.4 μm. This increase probably resulted from the absorption of gas phase photooxidation products onto the surface of particles already present. The particulate matter which was collected and extracted indicated the presence of several PAHs (e.g., pyrene, fluoranthene, anthracene, fluorene, chrysene). The PAHs showed significant degradation after irradiation, some of which appears to result from chemical reactions.

In the experiment where wood smoke alone was irradiated, the gas phase component of the product mix showed mutagenic activities of 36 ± 6 and 3.4 ± 0.9 revertants/h for TA100 and TA98, respectively. The gas phase starting materials showed no mutagenic activity within the experimental error. The mutagenic activities for the particulate phase reactants, as well as the irradiated mixture, were weak (0.2 to 0.3 revertants/μg) for both strains.

Three of the four irradiations, however, contained additional NO. Since NO converts HO_2 to OH during irradiation, greater conversion of reactants to products was achieved in these experiments. The gas phase reactant mixtures (with 0.5 ppm NO) showed no mutagenic activity above the control levels for either strain. However, the mutagenic activity of the irradiated gas phase products increased to an average of 174 ± 16 revertants/h for TA100 and 30 ± 4 revertants/h for TA98. The mutagenic activity of all particulate phase extracts remained at a level of 0.2 to 0.3 revertants/μg except that the extract resulting from the product mixture gave a mutagenic activity of 0.9 ± 0.3 revertants/μg withTA98.

An experiment was also performed to determine whether wood smoke emissions (with 0.5 ppm additional NO) or the irradiated mixture showed substantially different mutagenic activities when S9 metabolic activation was added to the assay. Within the experiment error the data showed that adding S9 did not change the previously observed mutagenic activities (without S9).

Discussion

The results of these experiments indicate that irradiation of wood smoke and wood smoke/NO_x mixtures can increase their mutagenic activity over that of the reactant mixture. The effect is most apparent for the gas phase component. However, identifying the species which give rise to the obsefved mutagenic activity is impractical for a number of reasons. Wood smoke emissions represent a complex mixture; for example, the gas phase species identified account for only half of the available carbon. For most of the reactant compounds, reliable photooxidation reaction mecha-

nisms have not been elucidated. Finally, even for an irradiated system in which the mechanism has been fairly well elucidated and which shows a mutagenic response (e.g., propylene/NO_x), the identity of the products, giving rise to a large fraction of the mutagenic activity, has not been established.

Instead, the data have been analyzed in a way to evaluate the relative contribution of each phase to the total mutagenic activity for each strain. The primary difficulty in this approach is the calculation of the mass of material depositing the assay during the gas phase exposure. By measuring the total HC concentration in the exposure chamber before and after deposition into the assay occurs, an estimate of the loading rate of the gas phase species can be made.

For the experiments with added NO_x about 10% of the gas phase product concentration appeared to deposit in the exposure chamber. We estimate an average molecular weight of 18.5 g associated with each mole carbon which deposits, based on the average molecular weight (per carbon atom) for the species which have been identified in the product mixture. Thus a deposition rate of 20 $\mu g\ plate^{-1}\ h^{-1}$ was determined for the gas phase products. Combining this number with the mutagenic activity (revertants/h), the mutagenic activity of the soluble gas phase products was calculated on a mass basis. This calculated value is an upper limit, however, since there is evidence that the mutagenic activity of the nonsoluble component is less than that of the soluble component. A lower limit to the mutagenic activity of the gas phase species is obtained by assuming that the mutagenic activity of the nonsoluble component is zero. On the other hand, the mutagenic activity of the particulate phase is straightforward since a known dose of the extract can be added to the biological assay. Table 1 summarizes the mutagenic activities for the two phases.

A comparison of the mutagenic activities on a volume basis is perhaps more pertinent since this calculation includes the relative quantities of material present in each phase. The results for this comparison are shown in Table 2, with the range for the gas phase products corresponding to the lower and upper limits given in Table 1.

In summary, the study indicates that the photooxidation products of wood smoke show far greater mutagenic activity (for TA100 and TA98) than the emissions. In addition, the contnribution to the mutagenic activity of the gas phase products are at least as great, if not significantly greater, than that of the particulate phase products for both strains.

Table 1. *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*

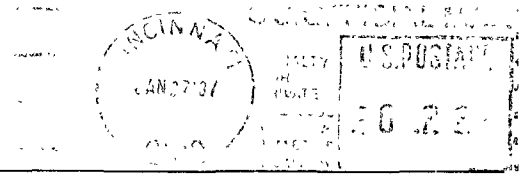
Table 2. *Comparison of the Gas and Particulate Phase Total Mutagenic (Revertants/m^3) for Wood Smoke and Irradiated Wood Smoke, Strains TA100 and TA98 Without Metabolic Activation*

	TA100		*TA98*	
	Gas	*Particulate*	*Gas*	*Particulate*
Reactants	*0*	*100*	*0*	*80*
Products	*12,300-90,600*	*180*	*2,130-16,000*	*730*

T. E. Kleindienst, P. B. Shepson, and E. O. Edney are with Northrop Services, Inc.—Environmental Sciences, Research Triangle Park, NC 27709.
***L. T. Cupitt** is the EPA Project Officer (see below).*
The complete report, entitled "Mutagenic Activities of Wood Smoke Photooxidation Products," (Order No. PB 86-239 837/AS; Cost: $11.95, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Atmospheric Sciences Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268



Official Business
Penalty for Private Use $300
EPA/600/S3-86/049