The mutagenic activity products of propylene photooxidation

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affect the overall variability in ash composition. Extrapolations concerning sampling designs might be made to other coal-fired plants, provided extreme differences do not exist in the plant operations. For example, a highly variable coal source might result in time variabilities in ash composition greater than those measured in these studies. The basic approach and equations can be used in sampling designs for other waste discharges from point sources, provided the time variability can be described by a normal distribution.

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The Mutagenic Activity of the Products of Propylene Photooxidation

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■ The reactants and products in irradiated propylene/NO_x mixtures were brought to a steady-state distribution in a Teflon smog chamber operated in a dynamic mode. The effluent from the chamber was then tested for total mutagenic activity by exposing *Salmonella typhimurium* strain TA100 to it. The data show an increased mutagenic activity for the products when compared with the reactants and controls. In addition, the mutagenic activity at long reaction times is substantially greater than at short reaction times. To examine a subset of the propylene/NO_x photooxidation products, an exposure of strain TA100 to the products of the propylene/N_2O_5 dark reaction was conducted. Although a small mutagenic activity was observed for this mixture, a number of mutagenic organic nitrates were identified. The results for the irradiated propylene/NO_x mixture were analyzed in terms of the mutagenic activities of the individual products. The major products (carbon monoxide, ozone, formaldehyde, acetaldehyde, nitric acid, and peroxyacetyl nitrate) account for no more than 20% of the observed mutagenic response, assuming additivity.

Introduction

There has been a long-standing interest in possible adverse health effects from chemicals emitted into the atmosphere. Over the past decade, toxicologists have developed many short-term biological assays to screen compounds rapidly for possible adverse health effects (*1*). The testing of compounds emitted or formed in the atmosphere has now begun by using these techniques (*2*) in both laboratory and field studies.

We have recently undertaken a program to examine the total mutagenic activity of products from irradiated hydrocarbon/NO_x mixtures using a photochemical reaction chamber operated in a dynamic mode. This reactor has the advantage of maintaining the reactant and product distribution once a steady state is reached. This situation is required in controlled mutagen assays since the sampling time is long compared to the change in product distribution that would occur in a static reaction system.

Recently we reported the results of a study in which irradiated mixtures of toluene/NO_x were tested for mutagenic activity using the *Salmonella*/mammalian microsome reversion assay (*3*). In this study, the bacterial strains TA100 and TA98, both with and without S9 metabolic activation, were used. The results showed an increased mutagenic activity for the product mixture when compared to the reactant mixture in both strains. A higher response was also observed for product distributions at longer reaction times. However, the observed response could not be accounted for by summing the responses for the observed products. Although there has been renewed interest in the mechanism of this system (*4-6*), major uncertainties exist, and a large fraction of the reacted HC remains unaccounted for.

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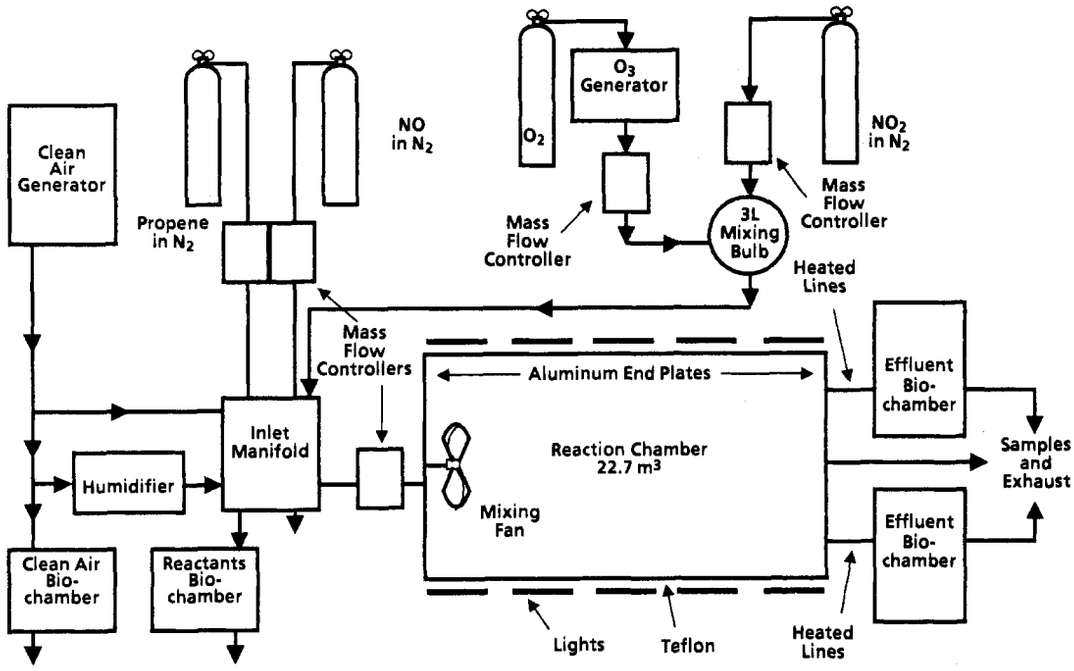

Figure 1. Schematic diagram of the reaction chamber apparatus.

The present study has been undertaken to investigate a system in which the mechanism has been more fully elucidated. The propylene (C_3H_6)/NO_x system has been studied by several laboratories (*7, 8*). All of the major products and a number of the minor products have been quantified, and the time dependence of the major products has been established. There has recently been increased interest in reactions of the NO_3 radical with C_3H_6 (*9*), in part due to the toxicity of one of the reaction products, propylene glycol dinitrate (PGDN) (*10, 11*). Since nitrated products become increasingly significant as the reaction proceeds, it is possible that some of the mutagens present at long reaction times in irradiated HC/NO_x systems may be organic nitrates. To focus on this group of products formed in the irradiated C_3H_6/NO_x system, we also examined a C_3H_6/N_2O_5 dark reaction mixture.

In this paper, we present the results of two irradiated C_3H_6/NO_x exposures at two different extents of reaction and the results of a C_3H_6/N_2O_5 exposure. The data are analyzed in terms of individual responses of the component products.

Experimental Section

Most of the experimental apparatus and analytical techniques have been described previously (*3*), and only an abbreviated description is presented here. 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 sunlamps and UV blacklights. The reactants, C_3H_6, NO, and NO_2 (and O_3 for the C_3H_6/N_2O_5 exposure), are metered through mass flow controllers and mixed with clean air and added humidity (except in the C_3H_6/N_2O_5 exposure) in an inlet manifold. The manifold is connected directly to the reaction chamber with 13-mm Teflon tubing. The input flow is measured by using a 150-Lpm (liters per minute) mass flowmeter. This configuration is required to sustain a constant, well-mixed reactant flow.

For the C_3H_6/N_2O_5 exposure, the N_2O_5 was produced by mixing O_3 and NO_2 (at ~1% concentration in N_2) in a 3-L Pyrex mixing bulb. The O_3 was produced at ~1% with a Welsbach Model T-408 O_3 generator supplied with zero-grade O_2. The O_3 reacts with NO_2 in the mixing bulb to produce NO_3, which is in equilibrium with N_2O_5, as shown in reactions 1 and 2. The resultant N_2O_5/NO_2

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \quad (1)$$

$$NO_3 + NO_2 \rightleftharpoons N_2O_5 \quad (2, -2)$$

mixture was then diluted with clean dry air in the inlet manifold at 140 L/min. When either the NO_2 or the O_3 was allowed to flow separately into the inlet manifold, the concentrations were ~1.8 and ~1.5 ppm, respectively. However, because of the possibility of NO_3 reacting with itself at the relatively high mixing bulb concentrations, and because it is unknown how the NO_x monitors respond to N_2O_5, the actual inlet manifold N_2O_5 concentration was uncertain. The inlet manifold O_3 concentration was determined to be zero throughout the exposure, and the inlet C_3H_6 concentration was ~1.2 ppm.

The product effluent was sampled at the end plate opposite the input flow. Chemical analyses generally required 3–13 Lpm, whereas the biological assay consistently required 28 Lpm. The walls of the rectangular biological exposure chambers (which are 190 L volume) are coated with fluorocarbon paint. A schematic of the experimental apparatus is shown in Figure 1.

The sampling and analytical techniques for the measurement of NO, NO_x, O_3, peroxyacetyl nitrate (PAN), the aldehydes, the relative humidity, and the physical parameters were conducted as previously described (*3*). Propylene was measured on a Hewlett-Packard Model 5840A gas chromatograph (GC) employing a 6.4 mm × 2 m stainless steel column packed with 80/100 Porapak QS operated isothermally at 130 °C. Injection was accomplished by using a Seizcor six-port gas sampling valve with a 5-mL sample loop. Propylene standards were prepared by diluting a 100-μL sample of pure C_3H_6 with 100 L of pure air in a Teflon bag. Organic nitrates were measured on a Varian 1200 GC employing a 6.4 mm × 2 m glass column packed with 10% SP-1000 on 80/100 Supelcoport operated isothermally at 155 °C. Detection was achieved on a Valco Model 140B electron capture detector. Samples were injected with a 5-mL glass and Teflon syringe.

Products were identified under the same conditions using a Hewlett-Packard Model 5985 GC/MS. Details of the product identification and the calibration procedure are in a separate publication (*12*).

Nitric acid (HNO_3) was collected on 47-mm nylon filters by using a Teflon filter holder. The filters were extracted in 10^{-5} M perchloric acid, and HNO_3 was measured as nitrate ion by ion chromatography (IC) using a CO_3^{2-}/HCO_3^- eluent. Weak acids (e.g., HCOOH) that might be formed in this system were collected by bubbling the effluent through a 1 mM NaOH solution. Ion chromatography employing a 2.5 mM $B_4O_9^{2-}$ eluent was used for separation and detection of the weak acid anions. Calibrations were made with solutions prepared from the alkali salts.

The biological assay used in this work employs the bacteria *Salmonella typhimurium*, strain TA100. 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 ~40 mL of plate agar in a glass Petri plate. The *S. typhimurium* tester strain TA100 was 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 (*13*). The test procedures used were those of Ames et al. (*14*), 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 $\sim 1 \times 10^8$ bacteria was used. The rat-liver homogenate (S9) fraction was prepared from male Charles River CD-1 rats (Wilmington, MO) induced with Aroclor 1254 (*14*). Surrogate plates representing the biological assay were also placed in the exposure chambers. One set of surrogates contained water buffered to the same level as the assay (i.e., pH 7.4); another set contained deionized water only. These were analyzed for formaldehyde (HCHO) by chromatropic acid and nitrate, nitrite, formate, and acetate by using IC. Mutagenic activities for pure compounds were performed by using the standard plate incorporation test (*14*). The synthesis and quantitation for compounds first observed experimentally in this system are presented elsewhere (*12*). In each case, the compounds were diluted in a suitable solvent (sterile water, dimethyl sulfoxide (Me_2SO), and methanol) at the nanomole to micromole level, and the response was measured at six concentrations. For some compounds that are unstable or exist in the vapor phase, a second technique, the single-component, gas-phase exposure, was performed. This technique was used to determine the mutagenic activity of PAN (*15*).

The procedures employed for performing the photooxidation experiments are similar to those previously described (*3*). To establish the desired extent of reaction for the dynamic experiments, a static C_3H_6/NO_x experiment was performed. For this experiment the reactor was operated as a conventional smog chamber. The reactants were added through the mixing manifold to the desired initial concentrations at a relative humidity of approximately 50%. In the dynamic mode, the reactor provided a continuous stream of products with a constant distribution. The extent of reaction giving rise to the distribution was established from the average residence time, τ, of the gases in the reactor (τ = volume of reactor/flow rate). For the C_3H_6/NO_x irradiations, the reaction chamber was operated at flows of 50 and 140 Lpm, yielding average residence times of 7.5 and 2.7 h, respectively. In the C_3H_6/N_2O_5 exposure, the average residence time was 2.7 h.

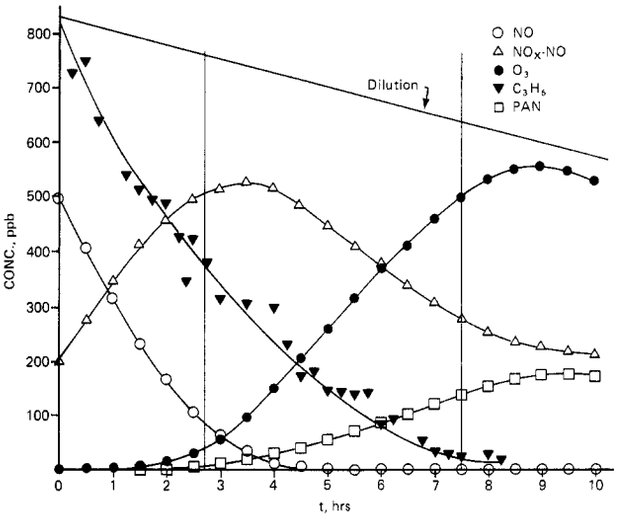

Figure 2. Time profiles for the major products in the C_3H_6/NO_x irradiation (static mode). The concentrations intersecting the vertical lines at 2.7 and 7.5 h represent the nominal product distributions for the two residence times.

For the C_3H_6/NO_x irradiations, the effluent from the reactor flowed through two exposure chambers in parallel. One chamber contained plates with TA100 and the other with TA100 plus S9. Surrogate plates for chemical analyses were placed in both chambers. The flow through the exposure chambers was 14 Lpm, yielding an exposure chamber residence time of 13.5 min. The exposures were conducted at 25 °C for 20 h, and the total gas volume through each chamber was 17 m^3. Two additional exposure chambers were employed. Clean air was passed through one chamber and the reactant mixture through the second. Each chamber contained TA100, with and without metabolic activation, and two sets of surrogate plates.

For the C_3H_6/N_2O_5 exposure, the two effluent exposure chambers contained 25 plates each of TA100 with and without S9 mix. To one of these exposure chambers we added 0.7 ppm of NO (continuously) to test for the presence of mutagenic peroxynitrates (which are removed due to the presence of NO). For the C_3H_6/N_2O_5 exposure, a reactants exposure chamber was not employed since N_2O_5 would react with the water evaporated from the plates to produce gas-phase HNO_3, which is toxic to the bacteria.

Each of the exposures was conducted by adjusting the reactant concentrations to the desired levels in the inlet manifold and allowing the product distribution to reach a steady state in the chamber. Once it was determined that this was the case, the exposure chambers were loaded with the covered test plates. The exposure chambers were then resealed, and the product concentrations in the exposure chambers were brought back to their steady-state levels. At this point, the plates were uncovered, effectively starting the exposures.

Results

C_3H_6/NO_x Irradiations. Figure 2 shows the time profiles of O_3, NO, NO_x–NO, C_3H_6, and PAN measured in the C_3H_6/NO_x static run, which had the same initial conditions as employed in the dynamic experiments. The NO_2 photolysis rate constant under these conditions was ~0.3 min^{-1}, and the dilution rate was 0.037 h^{-1}. The relative humidity was maintained at ~50%. In Figure 2, the vertical lines at 2.7 and 7.5 h indicate the product distributions predicted for dynamic experiments with

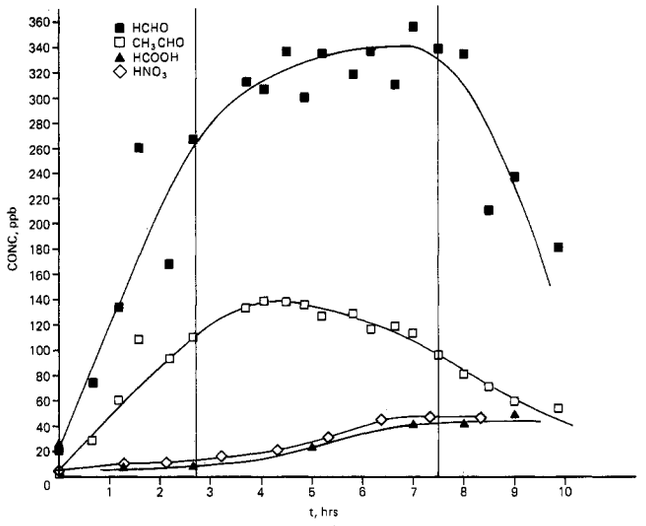

Figure 3. Time profiles for the aldehydes and nitric and formic acids in the C_3H_6/NO_x irradiation.

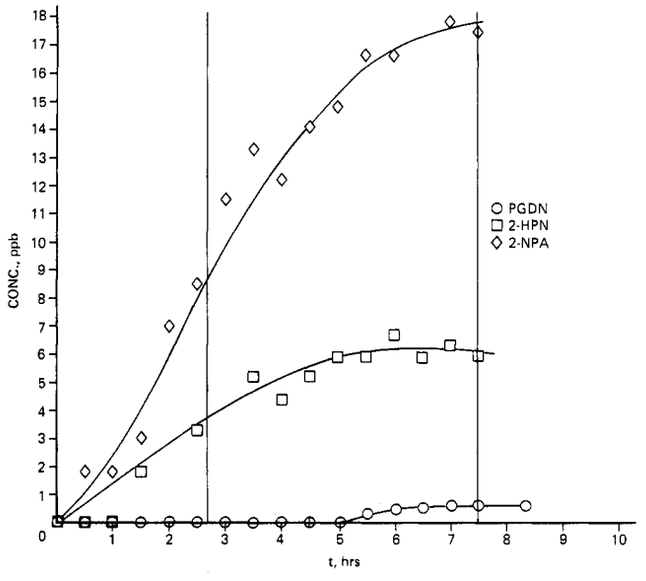

Figure 4. Time profiles for nitrates in the C_3H_6/NO_x irradiation.

residence times of 2.7 and 7.5 h, respectively, and performed under the same initial conditions as the static experiment. At 2.7 h, the reaction is still at a relatively early stage since there is approximately 20% (100 ppb) of the initial NO remaining. At 7.5 h, the reaction is near the O_3 and PAN maxima.

The time profiles of HCHO, acetaldehyde (CH_3CHO), NO_3^-, and formate are given in Figure 3. The aldehydic concentration difference between the two residence times is minimal. Nitrate extracted from nylon filters is generally identified with HNO_3. In this experiment, the measured formate is associated with formic acid (HCOOH). For conditions under which the sampling was performed, the detection limit for HCOOH is 15 ppb. Figure 4 shows the formation of the following nitrates produced during the photooxidation: PGDN, 2-hydroxypropyl nitrate (2-HPN), and 2-nitratopropyl alcohol (2-NPA). The reaction of OH with C_3H_6 in the presence of O_2 and NO forms 2-HPN and 2-NPA as stable products.

Dynamic experiments conducted at the two residence times had the same initial concentrations as the static experiment within experimental uncertainty. The inlet concentrations, total flow, and irradiation intensity remained constant throughout the dynamic experiments and

Table I. Average Reactant and Product Concentrations (ppb) for C_3H_6/NO_x Irradiation (Dynamic Mode)

compd	input concn	effluent concn (τ = 2.7 h)	effluent concn (τ = 7.5 h)
C_3H_6	826 ± 33	548 ± 24	100 ± 13
NO	505 ± 24	155 ± 29	BDL[b]
NO_x–NO	200 ± 39	521 ± 38	347 ± 21
O_3		18 ± 7	451 ± 29
HCHO		219 ± 32	247 ± 71
CH_3CHO		118 ± 8	90 ± 4
PAN		6 ± 3	181 ± 12
HNO_3		40 ± 10	73 ± 17
CH_3ONO_2		1.2 ± 0.2	1.4 ± 0.2
PGDN		BDL	0.8 ± 0.2
2-HPN		1.8 ± 0.4	3.0 ± 1.0
2-NPA		5.6 ± 1.1	10.4 ± 2.6
CO	81	163	
RH[a] (%)		52	65
HCOOH		BDL	40

[a] RH, relative humidity in units of percent. [b] BDL, below detection limit.

Table II. C_3H_6/NO_x Irradiations: Concentrations of Species Detected from Exposure Chamber Decrease (eq I) and/or Appearance in the Surrogate Plates (Concentration given as Micromoles per Plate)

	τ = 2.7 h		τ = 7.5 h	
compd	chamber decrease	surrogate plates	chamber decrease	surrogate plates
HCHO	1.62	1.15	2.28	1.65
PAN	0.048	–	0.89	1.48[b]
HNO_3	–[a]	0.085	0.78	0.83
CH_3ONO_2	0.001	–	0.004	–
PGDN	0	–	0.0024	–
2-HPN	0.017	–	0.034	–
2-NPA	0.047	–	0.10	–
$HCOO^-$	–	0.21	–	1.29

[a] Not measured. [b] From nitrite measurement.

yielded steady-state product distributions. The average values for the observed product concentrations in the reaction chamber for each of the two dynamic experiments are given in Table I. These concentrations can be compared with those of the static experiment by examining Figures 2–4 at the appropriate extent of reaction. A nominal correspondence between the two can be seen.

Chemical sampling of gas-phase products was also performed from the exposure chambers before and during the exposure of the biological assay. These values are used as one means of estimating the amount of material deposited into each plate. The net deposition of material into the plates during the exposure is tabulated in Table II under the heading "chamber decrease". These values are calculated from the relationship given in eq I where

$$X_i\left(\frac{\mu\text{mol}}{\text{plate}}\right) = \left(\frac{P_i(\text{R}) - P_i(\text{E})}{RT}\right)\left(\frac{V_\text{E}}{N_\text{P}}\right) \qquad \text{(I)}$$

X_i is the plate concentration of species i, P_i(R) and P_i(E) are the partial pressures (μatm) of species i in the reaction and exposure chambers, respectively, V_E is the volume of effluent passing through the exposure chamber, and N_P is the total number of plates. The amounts of material presented in Table II represent upper limits since some of the material may be lost to the exposure chamber walls.

A second method for determining the amount of material deposited into the biological assay is from the analysis of the surrogate plates. The results for each measured compound are given in Table II. A comparison of the

Table III. Measured Mutagenic Activity for Exposure of TA100 to the Experimental Gas Streams in Revertants per Plate for the C_3H_6/NO_x Irradiations

exposure condition	clean air irradiation		τ = 2.7 h		τ = 7.5 h	
	w/o S9	w/S9	w/o S9	w/S9	w/o S9	w/S9
spontaneous	164 ± 12	178 ± 11	142 ± 10	131 ± 8	184 ± 29	131 ± 18
15.4 nmol of sodium azide	909 ± 73		919		1388	
2.6 nmol of 2-aminoanthracene		1525		421		536
clean air chamber[a]	361 ± 60	379 ± 70	194 ± 15	225 ± 32	278 ± 92	290 ± 58
reactant chamber[a]	356 ± 48	363 ± 67	261 ± 41	293 ± 54	294 ± 58	268 ± 44
effluent chamber[b]	374 ± 59	404 ± 69	356 ± 69	373 ± 48	903 ± 69	968 ± 101

[a] Numbers are averages for ~25 plates. [b] Numbers are averages for ~50 plates.

results for the two methods can be made for HCHO, PAN, and HNO_3. In each case, the agreement is within 50%. This enhances the confidence for a species that can be measured by using only one of the techniques.

The mutagenic activities observed for the C_3H_6/NO_x photooxidation mixture are presented in Table III. Three sets of experiments are presented, each with its own set of laboratory controls and background measurements. The first three rows in Table III represent laboratory measurements of the characteristics of the particular assay used in the experiment. The "spontaneous" plates measure the natural reversion rate observed under sterile conditions in the laboratory. These serve as a check to establish the viability of the bacteria. The strain sensitivity is determined by adding 1.0 and 0.5 μg, respectively, of known mutagens, sodium azide and 2-aminoanthracene, to TA100 and TA100+S9, respectively. A clean air control chamber was employed to measure the background reversion rate in the ambient environment under which the photochemical experiments take place. In addition, a reactants exposure chamber, which samples the reactant gases directly from the inlet manifold, was used to check for mutagenic activity of the reactants. The clean air irradiation (Table III) is a separate experiment that serves as a check to ensure that the observed revertant level is due to the photochemical effluent rather than a chamber artifact. The revertant levels at both residence times show increased values over the clean air control. However, the mutagenic activity for the long residence time is substantially greater than that for the short residence time. It is also observed that the addition of S9 metabolic activation does not statistically increase the mutagenic activity, relative to those plates without S9 mix, indicating that the mutagens present are direct acting.

C_3H_6/N_2O_5 Exposure. To determine the extent to which NO_3 reaction with C_3H_6 may lead to mutagenic products that could account for the large mutagenic activity observed in the irradiated C_3H_6/NO_x system (at τ = 7.5 h), we conducted a C_3H_6/N_2O_5 exposure.

As mentioned previously, to one effluent stream we added NO at ~0.7 ppm. It has been reported (*9*) that the reaction of C_3H_6 with NO_3 leads to the production of large yields of nitroxyperoxypropyl nitrate (NPPN). Since we have shown that PAN (a peroxy nitrate) is a mutagen with TA100 (*15*), it is possible that NPPN or other peroxy nitrates formed may be mutagenic as well. Since peroxy nitrates such as PAN are in equilibrium with their respective peroxy radical and NO_2 (*16*), they can be removed via NO addition as shown in reactions 3 and 4 where R

$$RO_2NO_2 \rightleftharpoons RO_2 + NO_2 \qquad (3, -3)$$

$$RO_2 + NO \rightarrow RO + NO_2 \qquad (4)$$

is an organic group. The resultant alkoxy radical would then be removed by reaction with O_2 or NO_2 or by unimolecular decomposition. The presence of mutagenic peroxy nitrates can therefore be checked by comparison of the results for the two exposure chambers, since these compounds will not be present in the exposure chamber with added NO.

Table IV. Average Reactant and Product Steady-State Concentrations for the C_3H_6/N_2O_5 Exposure

parameter measured[a]	inlet	effluent
inlet reactant flow, L/min	140 ± 0.1	
NO_x	1.76 ± 0.20	1.94 ± 0.19
C_3H_6	1.26 ± 0.04	1.00 ± 0.04
HCHO		0.017 ± 0.004
CH_3CHO		0.023 ± 0.007
α-nitratoacetone		0.032 ± 0.011
PGDN		0.002 ± 0.001
2-HPN		0.008 ± 0.002
2-NPA		0.002 ± 0.001
PAN		0.020

[a] Concentrations in parts per million.

The average inlet and effluent reactant concentrations, chamber parameters, and steady-state concentrations for the products measured in the C_3H_6/N_2O_5 exposure are presented in Table IV. This experiment was conducted without added humidity.

Since the reactants C_3H_6 and N_2O_5 are diluted and mixed in the inlet manifold, they can react there as shown in reactions −2 and 5. In a separate C_3H_6/N_2O_5 static

$$NO_3 + C_3H_6 \rightarrow \text{products} \qquad (5)$$

experiment under similar conditions (*12*), we observed a value of $\Delta N_2O_5/\Delta C_3H_6$ of roughly 2/1. Since ΔC_3H_6 in this experiment is 0.26 ppm, the assumption can be made that the initial N_2O_5 concentration was ~0.5 ppm. This then leads to an initial NO_2 concentration of ~0.8 ppm, given the total NO_x value of 1.8 ppm listed in Table IV. By use of the values for k_2 and k_{-2} of 4.6×10^{-12} cm^3 molecule^{-1} s^{-1} and 3.7×10^{-1} s^{-1}, respectively (*17*), an equilibrium NO_3 concentration of 2.2 ppb is obtained. Using the value $k_5 = 4.2 \times 10^{-15}$ cm^3 molecule^{-1} s^{-1} (*18*), and a residence time in the inlet manifold of 1 min, leads to ≤1% C_3H_6 reacted in this time. Therefore, the inlet manifold concentrations can be accurately taken as those before the reaction begins.

The mechanisms for formation of the products listed in Table I have been presented elsewhere (*9*, *12*). A mass balance of the product concentrations from Table IV indicates that we cannot account for a large part of the reacted C_3H_6. It may be that much of it is present as NPPN, which we could not detect. The PAN concentration was measured in a separate experiment under equivalent conditions and was found to be present at 0.020 ppm.

The results of the bioassays performed are presented in Table V, along with the laboratory controls. The final plate concentrations for each product of the C_3H_6/N_2O_5 reaction are presented in Table VI, as calculated from eq I.

Table V. Observed Mutagenic Activities in Revertants per Plate for the C_3H_6/N_2O_5 Exposure

exposure condition	TA100	TA100+S9
spontaneous	133 ± 13	122 ± 21
15.4 nmol of sodium azide	668	
2.6 nmol of 2-aminoanthracene		590
clean air chamber[a]	180 ± 31	169 ± 33
effluent chamber[a]	375 ± 122	351 ± 102
effluent (+NO) chamber[a]	323 ± 81	369 ± 112

[a] Numbers are averages for ~25 plates.

Table VI. Concentrations of Products (C_3H_6/N_2O_5 Exposure) Detected in Micromoles per Plate As Calculated from Equation I

product	μmol/plate (max, eq I)	product	μmol/plate (max, eq I)
HCHO	0.2	PGDN	0.02
HNO_3	0.6	CH_3ONO_2	
PAN	0.2	HCOOH	
2-HPN	0.1	$CH_3C(O)CH_2ONO_2$	0.4
2-NPA	0.02		

In trying to account for an observed mutagenic response in a complex mixture, summing the mutagenic activities of the individual species is the most straightforward approach, assuming the individual responses are additive. These responses are clearly a function of concentration (*19*). (Note, although CH_3CHO is a major product in this photooxidation, it is not listed in Tables II and VI, since it appears that *S. typhimurium* bacteria produce CH_3CHO in the course of their metabolism. This was evidenced by the fact that CH_3CHO was observed in the clean air exposure chamber when the plates were opened.)

The final step in our analysis is the compilation of mutagenic activities for major and minor products formed in the oxidation system. These are used to determine a calculated total response, R_T, from a summation of the response of the individual components according to eq II

$$R_T = \sum_i \beta_i X_i \qquad \text{(II)}$$

where β_i is the mutagenic activity for species i, and X_i is its concentration per plate. The mutagenic activities are determined from plate incorporation experiments and the single-component, gas-phase exposures. The majority of compounds has been tested by using the plate incorporation procedure. However, O_3, HCHO, H_2O_2, and PAN were measured by gas-phase exposure. Table VII gives mutagenic activities in terms of revertants per micromole per plate. The experimental data found in Tables II, VI, and VII are used in conjunction with eq II to calculate R_T. The values are then compared with the data in Tables III and V.

Discussion

C_3H_6/NO_x Irradiations. For the purposes of this discussion, we will consider the results for TA100 without S9 only, since the response observed is essentially the same in the two cases and since the mutagenic activities for the mutagenic products we have detected are not dependent on the presence of S9 mix.

As can be seen from the data in Table III, the clean air and reactant exposure chambers exhibit revertant levels somewhat higher than those of the laboratory spontaneous plates. We attribute this to a brief exposure of the plates to ultraviolet light during handling in preparation for the exposure. Therefore, the data for the effluent exposure chambers should be compared with that of the clean air

Table VII. Mutagenic Activities for Products of the C_3H_6/NO_x Irradiation

compd	mutagenic activity, revertants/μmol per plate: TA100	TA100 + S9
O_3	BDL[a,b]	BDL[a]
HCHO	12[a]	12[a]
CH_3CHO	BDL	BDL
PAN	41[a]	50[a]
HNO_3	BDL	BDL
CO	BDL	BDL
2-HPN	3	3
2-NPA	3	3
PGDN	BDL	BDL
CH_3ONO_2	BDL	BDL
$CH_3C(O)CH_2ONO_2$	55	55
HCOOH	BDL	BDL
H_2O_2	10[a]	10[a]
$(CH_3)_3COOH$	100	284
$CH_3C(O)OOH$	BDL	BDL

[a] Single component, gas-phase exposure. BDL, below detection limit, 2 revertants/μmol.

or reactant chamber. The number of clean air chamber revertants per plate can be subtracted from the effluent chamber revertants per plate to obtain the number of excess revertants. The data presented in Table III show that there is essentially no increase in the bioactivity (revertant level for TA100 without S9) of the reactant biochambers above that found in the clean air biochamber; there is a small response (~160 excess revertants/plate) found at a residence time of 2.7 h, and there is a large excess (~625 revertants/plate) in the effluent biochambers at a residence time of 7.5 h.

It is reasonable to attempt to account for the excess revertants found in the effluent biochambers by summing the contributions of the individual reaction products (even though we have no evidence of additivity in such a complex mixture). As shown in eq II, the total response can be taken as the product of the mutagenic activity times the amount of each component deposited in a plate. Therefore, a very mutagenic minor product can be just as or more important than weakly mutagenic major products. Studies have shown that mutagenic activities as large as 100 000 revertants/μmol for TA100 exist (*19*). Because of this, even though a large number of reaction products have been determined for an irradiated C_3H_6/NO_x mixture, it may be that a chemical whose yield is small is responsible for the increased mutagenic activity. However, as a first step in trying to account for the response, the contributions of the major products should be considered.

The major products formed during the photooxidation are O_3, HCHO, CH_3CHO, CO, CO_2, HNO_3, NO_2, and PAN. The steady-state concentrations at 2.7 and 7.5 h are shown in Table I. In the table, NO_x–NO is approximately equal to the sum of NO_2 and PAN. Carbon dioxide concentrations were not determined since a background level of 320 ppm was present in the diluent air. The amounts of HCHO, PAN, and HNO_3 in the surrogate plates are shown in Table II.

Table VII shows that, of the major products listed, only HCHO and PAN have mutagenic activities above the detection limit. A numerical value for the mutagenic activity of NO_2 has not been given, but the fact that the revertant level in the reactant chamber that was exposed to 200 ppb of NO_2 is similar to that found in clean air demonstrates that NO_2 does not contribute to the revertant levels found at 2.7 and 7.5 h. Calculations using eq

II shows that HCHO accounts for 19 revertants/plate at 2.7 h and 27 revertants/plate at 7.5 h. Similary, PAN accounts for 2 revertants/plate at 2.7 h and 36 revertants/plate at the longer residence time. Therefore, the major products account for only a small percentage of the measured mutagenicity; at 2.7 h HCHO and PAN contribute 21 revertants/plate total, or 13% of the total revertant level, and at 7.5 h the total contribution of HCHO and PAN is 63 revertants/plate or 10% of the total.

The minor products identified in the system were CH_3ONO_2, PGDN, 2-HPN, 2-NPA, and HCOOH. On the basis of the amounts of material deposited into the plates and the mutagenic activities, the total contributions of the minor products for both 2.7 and 7.5 h are found to be less than 1 revertant/plate, assuming additivity.

It therefore appears that the bioactivity found in the effluent exposure chambers cannot be accounted for by species identified and quantified during the experiment. However, it is well-known (*9*) that other minor products are formed during the irradiation. These include H_2O_2, CH_3OOH, and $CH_3C(O)OOH$. Organic peroxides have been found to be strong mutagens in the strain TA102 (*20*). Plate incorporation tests using TA100 were conducted for H_2O_2, $CH_3C(O)OOH$, and *tert*-butyl hydroperoxide, which served as a model for CH_3OOH. Their mutagenic activities are included in Table VII. A reasonable upper limit for the concentrations of these species at 7.5 h is 50 ppb. Assuming such a contribution and that all the material goes into the plates, 0.60 μmol/plate of each would be deposited during these experiments. The mutagenic contributions for the chemicals would be the following: H_2O_2, 6 revertants/plate; $(CH_3)_3COOH$ (CH_3OOH), 60 revertants/plate. The mutagenic activity of $CH_3C(O)OOH$ was below the detectable limit. The calculation is presented only for 7.5 h because at 2.7 h there still remains some NO that prevents the RO_2–HO_2 reactions necessary to generate the hydroperoxides. If the 66 additional revertants contributed by the peroxides are added to the 7.5 h contributions due to PAN and HCHO, 20% of the measured excess revertants can be accounted for.

C_3H_6/N_2O_5 Exposure. Since it has been shown that NO_3 reaction with C_3H_6 produces products that are toxic (*9–11*), and because a large response was observed at long residence time where NO_3 would be expected to be present, the C_3H_6/N_2O_5 exposure seemed a reasonable means for attempting to determine to what extent the C_3H_6/NO_3 reactions may be the cause of this large response.

As shown in Table V, the two sets of effluent chamber plates exhibit revertant levels of approximately 180 revertants/plate above the clean air values. There seems to be no significant difference between those with and those without metabolic activation, indicating that the mutagens present are direct acting. In addition, there is no significant difference in the results for the two effluent exposure chambers. Although this may indicate that the peroxy nitrates present are not mutagenic, their transfer efficiencies to the exposure chambers may be low, or they may be deposited on the reaction chamber walls during the 2.7-h average residence time. Bandow et al. (*9*) have observed that NPPN has a significant wall loss rate in their reaction chamber.

Using the data presented in Tables VI and VII, we can estimate to what extent each of the products of the C_3H_6/N_2O_5 reaction that we measured could account for the total observed response. Although nitric acid (HNO_3) is listed in Table VI, since it was undoubtedly present, we were unable to measure its concentration accurately due to an interference caused by the presence of N_2O_5. As indicated in Table VII, several of the organic nitrates are mutagenic but would be classified as weak mutagens (*21*). The compound that contributes the most, although very little relative to the total, is α-nitratoacetone. HCHO, PAN, and α-nitratoacetone could contribute 2, 8, and 22 revertants/plate, respectively. Each of the two hydroxy nitrates (2-HPN and 2-NPA) could account for no more than 1 revertant/plate. It should be noted that organic hydroperoxides such as 2-HPPN may be present and would be produced as shown in reactions 6 and 7. These

$$C_3H_6 + NO_3 \xrightarrow{O_2} CH_3CH(OO)CH_2ONO_2 \quad (6)$$

$$CH_3CH(OO)CH_2ONO_2 + HO_2 \rightarrow CH_3CH(OOH)CH_2ONO_2 \quad (7)$$

organic hydroperoxides may be, like *tert*-butyl hydroperoxide, significantly mutagenic.

It is clear, however, that for the C_3H_6/N_2O_5 exposure we have accounted for only a small fraction of the total observed response (~19%) with known reaction products. Although there are several possible reasons for this, the most likely is that we have not detected all the mutagenic products in this system. As stated previously, although a presumably major product for which we have no biotesting data is NPPN, if this compound were very mutagenic there would likely have been significant differences in the results for the two effluent exposure chambers. It would appear then that there may be other unidentified stable products that are mutagenic such as an organic hydroperoxide. Since we have found several organic nitrates that are mutagens, and we know that organic hydroperoxides (e.g., $(CH_3)_3COOH$) are mutagens, a species such as 2-HPPN is likely.

In this experiment, we have shown that chemical mutagens are produced from the reaction of NO_3 with C_3H_6. However, a computer modeling study that we have conducted of the C_3H_6/NO_x irradiated system indicates that NO_3 reaction with C_3H_6 would have occurred only to the extent of ~0.010 ppm at 7.5 h. Since ΔC_3H_6 due to NO_3 reaction in the C_3H_6/N_2O_5 exposure was 0.26 ppm, we can estimate that NO_3 reactions would have contributed on the order of only 10 revertants/plate in the irradiated C_3H_6/NO_x system at 7.5 h.

It is therefore clear from the above analysis that the observed mutagenic response in the irradiated C_3H_6/NO_x system cannot be accounted for, although there are a number of possible explanations. It is conceivable that there exist small yields of a very potent mutagen. If the remaining ~500 excess revertants found at 7.5 h were due to the presence of a single unknown mutagen present at 10 ppb, it would have a mutagenic activity of ~4000 revertants/μmol. This would certainly be classified as a strong mutagen (*19*).

It is also possible that the mutagen responsible may be produced from a reaction that occurs in the test medium. However, to test this possibility we conducted a 20-h exposure of an irradiated C_2H_4/NO_x mixture under similar conditions. This system yields, as products, HCHO, O_3, NO_2, HNO_3, H_2O_2, and HCOOH, which represent (as seen in Table I) a subset of the C_3H_6/NO_x photooxidation products. In this experiment at long residence time, no detectable mutagenic response was observed over the clean air control. This, then, to a limited extent, argues against the possibility of reactions in the medium playing a significant role. In addition, further studies are required to compare the results obtained from the gas-phase exposures and the standard plate incorporation tests. Unless special precautions are taken, the possibility of losses of material when the plate incorporation test is performed for volatile

compounds exists. It is also possible that there are other systematic differences between results obtained with these two tests. Finally, the possibility that mutation processes exist that require the presence of two (or more) chemicals simultaneously has not been eliminated. (Experiments are currently being planned for testing various binary and tertiary mixtures of known mutagens.) However, such investigations should take place in conjunction with reasonable attempts for an explanation by single chemical components.

Conclusion

In this work, we have demonstrated that the photooxidation products of C_3H_6 are mutagenic, as determined with the Ames test. This is a fairly significant result given the fact that C_3H_6 is a simple hydrocarbon that is an important reactive constituent for urban air. Although the total observed response could not be accounted for, HCHO, PAN, and several organic nitrates were found to contribute significantly. It is possible that the organic nitrates that are products of NO_3/alkene reactions may be an important source of the chemical mutagens present at night in urban air. Much more work is necessary to better determine the photooxidation products of sample atmospheric hydrocarbons that represent a human health hazard.

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Registry No. PAN, 2278-22-0; PGDN, 6423-43-4; 2-HPN, 20266-65-3; 2-NPA, 20266-74-4; C_3H_6, 115-07-1; NO_x, 11104-93-1; N_2O_5, 10102-03-1; NO, 10102-43-9; O_3, 10028-15-6; HNO_3, 7697-37-2; CO, 630-08-0; CH_3ONO_2, 598-58-3; H_2O_2, 7722-84-1; HCOOH, 64-18-6; $CH_3C(O)CH_2ONO_2$, 6745-71-7; $CH_3C(O)OOH$, 79-21-0; $(CH_3)_3COOH$, 75-91-2; CH_3CHO, 75-07-0; HCHO, 50-00-0.

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