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ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · DECEMBER 1988

Impact Factor: 5.33 · DOI: 10.1021/es00177a017

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Received for review February 17, 1988. Accepted June 1, 1988. Funding for this research was provided by the National Oceanic and Atmospheric Administration Grant 50-DGNC-5-00262 (Status and Trends Mussel Watch Project) and Texas A&M University Sea Grant (R/ES-18).

MS/MS Analysis of the Products of Toluene Photooxidation and Measurement of Their Mutagenic Activity

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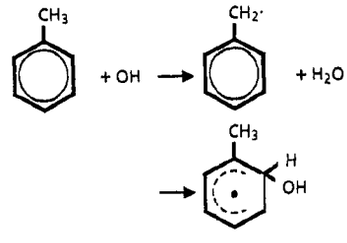
■ Products of the photooxidation of toluene from an irradiated 5.1 ppm toluene/0.9 ppm NO_x mixture were identified by use of a triple-quadrupole MS/MS operated in an atmospheric pressure ionization mode. The reaction was carried out in a flow-mode 22.7-m^3 Telfon smog chamber. The steady-state reactant and product mixture was continuously transferred to the mass spectrometer inlet at 144 L/min. By using structurally similar standards, semiquantitative MS/MS analyses for many of the ring fragmentation products were conducted. Quantitative analyses by chromatographic methods and semiquantitative analyses by MS/MS were conducted for a variety of ring fragmentation products. The following products were found with yields of 1% (C/C) or greater: methylglyoxal, glyoxal, benzaldehyde, methylbutenedial, hydroxymethylbutenedial, peroxyacetyl nitrate, oxoheptadienal, CH_3COOH, HCHO, hexadienal, and hydroxyoxoheptadienal. The sum of the yields of all products measured was ~60%, assuming a total cresol yield of 16%. Several ring fragmentation products that have not been previously detected were identified in this study. Mechanisms are described for some major product pathways. The mutagenic activity of the steady-state product mixture was measured by using the Ames assay *Salmonella typhimurium* strain TA 100. The mutagenic activity data are discussed relative to our earlier findings (*10*) that resulted from different reaction conditions.

Introduction

Toluene is present in polluted urban atmospheres with concentrations typically ranging from 10 to 30 ppb (*1*). A comlete understanding of photochemical smog necessarily requires an understanding of the mechanism for the photooxidation of toluene, since toluene is one of the most important reactive atmospheric hydrocarbons. The photooxidation of toluene in the presence of NO_x occurs entirely through reaction with the OH radical (*2*).

The reaction of OH with toluene proceeds via either addition to the ring or abstraction, as shown in reaction 1, where $k_{1a}/(k_{1a} + k_{1b}) \simeq 0.1$ (*3*). The abstraction pathway yields mainly benzaldehyde with smaller yields of benzyl nitrate (*3*).



Since the observation was reported that biacetyl is produced from the reaction of OH with *o*-xylene (*4*), it has been clear that ring fragmentation of the initially formed OH adduct (reaction 1b) does occur for aromatic hydrocarbons. For toluene, glyoxal and methylglyoxal have been shown to be important ring fragmentation products. However, the yields of these two products have been reported to be only 11 and 15%, respectively (5).

The production of glyoxal and methylglyoxal implies the possibility of simultaneous production of other and perhaps larger ring fragments; the production of butenedial and 4-oxo-2-pentenal has recently been reported (*6*, *7*). These products, along with the products glyoxal and methylglyoxal, imply reactions 2–7 as a possible mechanism, although other mechanisms have been formulated (*8*). In addition, Dumdei and O'Brien (*7*) have observed the seven-carbon ring-opening product 6-oxo-2,4-heptadienal, which would be produced according to reactions 8 and 9.

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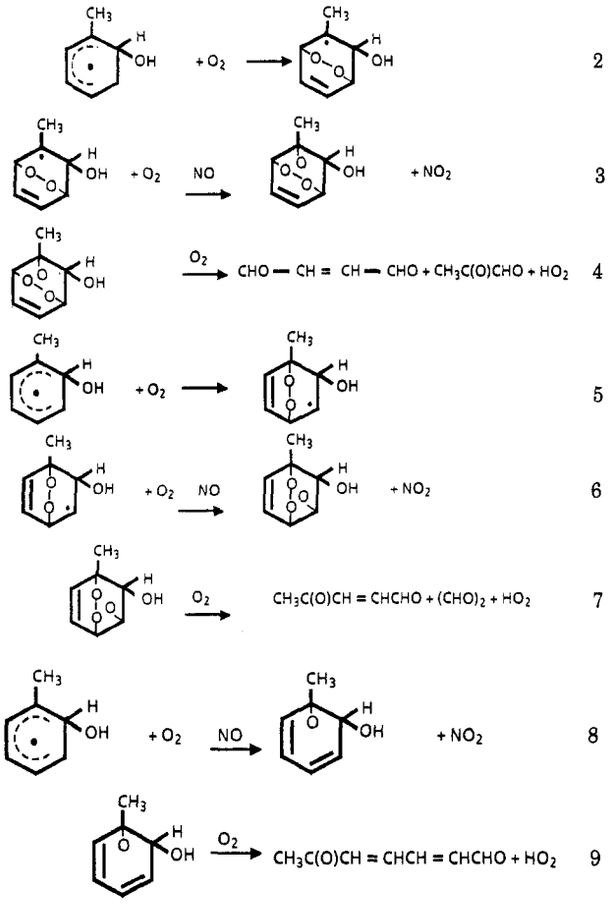


2 3 4 5 6 7 8 9

Because of the variety of addition sites for OH and for reactions involving O_2 addition to the ring, the number of possible ring fragmentation products is large (*7*). Unfortunately, these products, which are polar and present in small yields, are difficult to detect (particularly chromatographically) and, since standards are not available, are difficult to quantify in terms of yields.

The advent of MS/MS as an analytical tool provides the opportunity for selective detection and identification of species present in complex mixtures. This technique is applicable to all gas-phase species that can be ionized in the mass spectrometer ion source. If a sufficiently large flow rate of sample is available, the ability to operate the MS/MS in an atmospheric pressure chemical ionization mode leads to sensitivities enabling detection of minor products (*9*). In this paper we present the results of a study aimed at quantitative identification of the photooxidation products of toluene, in which a 5 ppm toluene/0.9 ppm NO_x mixture was irradiated in a 22.7-m^3 Teflon smog chamber operated in a dynamic mode. The residence time of gases in the chamber was 2.6 h, corresponding to a flow rate of 144 L/min, all of which was available to the MS/MS system for analysis. In this paper we will provide identification of several previously unknown photooxidation products, along with possible mechanisms for their production. The products identified (including cresols and benzaldehyde) can account for ~60% of the total reacted toluene (see Discussion).

It has recently been demonstrated that the photooxidation of toluene leads to mutagenic products (*10*). In addition, the products of toluene photooxidation have been shown to increase the frequency of sister chromatid exchanges in cultured Chinese hamster cells and to cause growth inhibition in these cells (*11*). These observations thus provide further impetus for identification of the photooxidation pathways and resultant products. Our previous study (*10*) showed that the products present at long extent of reaction (i.e., near the ozone maximum) are significantly mutagenic as determined by using Ames assay strain TA100. However, under these conditions, secondary reactions could produce the mutagenic products; for example, many of the ring fragmentation products can undergo photolysis (*7*) or reaction with ozone. It is therefore desirable to measure the mutagenic activity under conditions where fewer secondary reactions could be occurring. In addition, in the experiments of Shepson et al. (*10*), the mutagenic activity was measured at only one dose, and survivor plate data were not obtained; it is therefore unclear whether those measurements were influenced by toxicity effects. In the present study, an exposure of *Salmonella typhimurium* strain TA100 to the effluent from the irradiated toluene/NO_x mixture was conducted where doses were obtained corresponding to exposure times of 2, 4, 6, 8, 10, and 20 h, enabling construction of a dose–response curve. The experimental conditions were such that the extent of reaction was considerably shorter.

Experimental Section

A 5.1 ppm toluene/0.94 ppm NO_x mixture was irradiated by using a 22.7-m^3 cylindrical Telfon smog chamber. The chamber is equipped with a combination of sunlamps and black lights. Additional details of the reaction chamber are provided elsewhere (*10*). The reaction chamber was operated with the input reactant mixture flowing at 144 L/min, so that the residence time of gases in the chamber was 2.6 h. Toluene was added to the chamber through a dilution manifold by bubbling N_2 through pure toluene maintained at 0 °C. The reactant NO_x was added by using an NO/N_2 mixture metered into the inlet manifold using a mass flow controller. The light intensity corresponded to an NO_2 photolysis rate constant of ~0.09 min^{-1}.

Once the reactant and product concentration distribution in the reaction chamber reached steady state, the following species were periodically measured in the inlet and effluent air streams: toluene, NO, NO_x, O_3, HNO_3, PAN, CH_3COOH, HCHO, CH_3CHO, C_6H_5CHO, $(CHO)_2$, and $CH_3(O)CHO$. The aldehydes were measured by a DNPH/HPLC technique as previously described (*10*). Nitric acid was collected on 47-mm nylon fibers in a Teflon filter holder and extracted with 10^{-5} M $HClO_4$. Acetic acid was collected by bubbling air through a small volume of water at 0 °C. Nitrate and acetate ions were then quantified by ion chromatography. The inorganic gases NO, NO_x, and O_3 were measured by use of continuous monitors, and the toluene and PAN were measured by gas chromatography as previously described (10). The gas chromatograph used for PAN measurements was calibrated by bubbling calibration samples of PAN in air through pH 12 water, followed by ion chromatographic analysis of the acetate ion.

For the MS/MS analysis of the chamber effluent, the air from the chamber was transferred to the inlet system of the MS/MS through 1.5-cm-i.d. Teflon tubing at ~140 L/min. Background samples were also obtained from the reaction chamber inlet manifold. All MS/MS analyses were conducted with a direct sampling TAGA 6000 triple-quadrupole mass spectrometer (Sciex, Inc., Thornhill, Ontario). The TAGA 6000 can be used as a tandem mass spectrometer to produce atmospheric pressure chemical ionization (CI) spectra or as a triple-stage quadrupole mass spectrometer to produce collisionally activated dissociation (CAD) spectra. For these experiments, the atmospheric pressure chemical ionization (APCI) source was used. In APCI, the ionization process is initiated by electrons

Table I. Reactant and Product Concentrations (ppb) and Product Yields [% (C/C)]

species	inlet	effluent (method) chromat	effluent (method) MS/MS	% yield (C/C)	MS/MS surrogate
toluene	5145 (±310)	4403 (±144)			
NO	896	2[a]			
NO_x	942	394[a]			
O_3		153[a]			
HNO_3		160			
$CH_3C(O)CHO$		134	80	7.7[b]	*g*
$(CHO)_2$		150	120	5.8[b]	*g*
methylbutenedial[h]			60	5.8	*f*
C_6H_5CHO		37		5.0	
hydroxymethylbutenedial[h]			35	3.4	*e*
peroxyacetyl nitrate		85		3.3	
oxoheptadienal[h]			20	2.7	*f*
CH_3COOH		47	30	1.8[c]	*g*
HCHO		50		1.0	
hexadienal[h]			9	1.0	*f*
hydroxyoxoheptadienal[h]			9	1.0	*f*
hydroxydioxohexenal[h]			7	0.8	*f*
dioxohexenal[h]			6	0.7	*e*
hydroxyoxohexenal[d]			5	0.6	*e*
hydroxyhexadienal[h]			5	0.6	*f*
methyl vinyl ketone			6	0.5	*e*
methlfuran[d]			5	0.5	*f*
hydroxyoxobutanal[d]			7	0.5	*g*
hexadienedial[d]			3	0.3	*f*
hydroxymethyl vinyl ketone[h]			4	0.3	*e*
butenedial			4	0.3	*f*
hydroxybutenedial[h]			3	0.2	*f*
CH_3CHO			6	0.2	*g*
pentadienal[d]			1	0.1	*f*
acrolein			1	0.06	*e*
pyruvic acid[d]			1	0.06	*e*
total				44.2	

[a] By continuous monitors. [b] By the DNPH method. [c] By ion chromatography. [d] Tentative MS/MS identification. [e] 4-Hexen-3-one used as surrogate at 70 900 ion counts s^{-1} ppm^{-1}. [f] 2,4-Hexadienal used as surrogate at 60 700 ion counts s^{-1} ppm^{-1}. [g] Butanal used as surrogate at 42 000 ion counts s^{-1} ppm^{-1}. [h] Or isomers.

created in a corona discharge. The high-energy electrons remove electrons from N_2 to form N_2^+. This ionization begins a sequence of reactions that eventually results in a population of reagent ions with the formula $(H_2O)H_3O^+$. The reagent ions then protonate analyte molecules that are present to form parent molecular ions. Details of operation are given elsewhere (*9*, *12*, *13*). Sample flow from the smong chamber is passed directly into the ionization region of the mass spectrometer with no prior collection, concentration, or separation. This minimizes any possible degradation or contamination of the sample.

The two tandem mass analyzers also act to reduce the possibility of interferences between components of a complex matrix such as the one being studied (*14*). Many of the previously identified and proposed reaction products are not commercially available. Work is currently under way to synthesize all the products. To conduct semiquantitative analysis of the CAD-identified products, response factors obtained from structurally similar standards were utilized, as discussed below. Although response factors in APCI MS/MS can vary over several orders of magnitude for compounds with different functionalities, response factors are approximately equal for structurally similar compounds (*15*).

The mutagenic activity of the effluent product mixture was measured, as previously described (*10*), by flowing the effluent (at 14 L/min) through a 190-L exposure chamber loaded with the Ames assay test plates. The plates were dosed by uncovering groups of seven for 2, 4, 6, 8, 10, and 20 h. As described previously (*10*, *16*), species that are soluble in the plate agar will deposit into the plates as the air mass flows through the exposure chamber. A second exposure chamber was used as a 20-h control exposure of TA100 to clean air.

The test plates were prepared by adding 0.1 mL of the *S. typhimurium* culture to 3 mL of an agar overlay at 45 °C. This mixture was then poured onto ~45 mL of plate agar in a Pyrex glass Petri plate. Colonies were counted with an Artek 880 automatic colony counter, by following previously published guidelines (*17*). The test procedures used were those of Ames et al. (*18*) 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. After the exposures, the plates were incubated at 37 °C for 48 h, and the numbers of revertants/plate were counted.

Results

The inlet (reactant) and effluent (reactant and product) concentrations measured during the MS/MS analysis and Ames assay exposures are presented in Table I. The absolute uncertainty in measurement of species by ion chromatography or HPLC was ~10%. Also listed in Table I is the yield (percent of reacted carbon) for each of the carbon-containing products measured. It can be seen in the table that under the conditions of this experiment 742 ppb toluene (or 5.2 ppm C) had reacted. It should be noted that the indicated uncertainty for the inlet toluene concentration in Table I is a reflection of the fact that the toluene input at the inlet fluctuated by ±6% during the course of the experiment. The analytical precision of the toluene measurements was ±2%. Consequently, we es-

timate the uncertainty in the averaged Δ toluene to be ±15%. Under these conditions, the extent of reaction is just at the beginning of the increase in ozone, which is considerably earlier than for our previous exposure of TA100 conducted near the ozone maximum. The relatively short extent of reaction and low light intensity should diminish the extent of secondary reaction for the products produced.

For the products measured by APCI/MS/MS, identifications were made by computer search of library CAD spectra, where possible. For those compounds not in the computer spectral libratory, the CAD spectra were interpreted and compared to previous MS/MS identifications (*7*, *9*). For those compounds listed in Table I as tentatively identified, the CAD spectra are consistent with the compounds proposed, but no standard spectra or previous CAD data were available for verification. Identifications made by MS/MS do not rule out the presence of various isomers that differ in the position of the methyl and/or hydroxyl groups. For example, the name "methylbutanedial" implies other possible isomers including 4-oxo-2-pentenal, which may be the predominant isomer on the basis of isotopically labeled toluene experiments (*7*) and of mechanistic inference (*6*).

The calculated concentrations for species semiquantitatively determined by MS/MS are based on calibration factors measured for 4-hexen-3-one, 2,4-hexadienal, and butanal. The measured response factors for these compounds were all within a factor of 2 of each other. The individual reference compound most structurally similar to the identified compound was used in all cases. Table I identifies which of these surrogates was used to semiquantify each of the identified products and presents the response factors used for each surrogate standard. We estimate that the response factors utilized for each species result in an uncertainty of a factor of 2 in the MS/MS determinations. Only the hydroxy ring fragmentation products were expected to deviate significantly from the values used. The hydroxy functionality generally increases the APCI sensitivity; therefore, concentrations reported for these products are expected to be maximum values. Other products observed by MS/MS but not quantified are cresols, nitrotoluene, nitrobenzaldehyde, nitrocresols, and benzoic acid (or hydroxybenzaldehyde). The data in Table I indicate that the measured ring fragmentation products account for ~38% of the reacted carbon. Note, however, that since the MS/MS determined yields are uncertain to a factor of ±2, and that the total yield of fragments determined by MS/MS is 20%, then the total yield of ring fragments is uncertain by +20 to −10% C/C. It is quite likely, however, that there were species undetected by the techniques used and that many of these products, which are expected to be very reactive with respect to OH radicals, have undergone significant secondary decay. Although many of the species presented in Table I have been identified previously (*6*, *7*, *9*), the yields of most of the ring fragments have not been reported previously.

The results of the exposure of TA100 to the effluent from the chamber are presented in Figure 1. The point at $t = 0$ indicates the result for a 20-h exposure of TA100 to clean air. As indicated in this figure, there is a very linear dose–response curve up through a 10-h exposure time, but there is some indication of toxicity effects at the 20-h exposure. This was confirmed by a slightly lower number of survivor counts for survivor plates exposed for 20 h.

Discussion

In addition to the ring fragmentation products listed in Table I, the cresols are known to be major products of OH reaction with toluene, the sum of the yields of the three cresol isomers being 16% (*3*). Therefore, the sum of the ring fragmentation product yields plus the benzaldehyde and the cresols yields is ~60%. Although this implies that there are as yet unidentified products, there is considerable uncertainty (a factor of ±2) in the ring fragment yields as determined by MS/MS. We note, however, that for the three species (i.e., glyoxal, methylglyoxal, and acetic acid) measured by both MS/MS and by chromatographic methods, the agreement is within the expected factor of 2 (i.e., −20, −40, and −36%, respectively). Our yields for glyoxal and methylglyoxal are somewhat larger than those reported by Tuazon et al. (*5*), but our benzaldehyde yields are consistent with those previously reported (*3*). We previously reported yields for the ring fragments butenedial, methylfuran, and methyl vinyl ketone of 0.6, 0.3, and 0.1% C/C, respectively (*6*), as compared with the values 0.3, 0.5, and 0.5% reported here. There are a variety of factors that could account for the differences, including the fact that the GC/MS in our previous study was calibrated only for toluene, and the sampling procedures used in that study could have been a source of sample loss. In addition, the extent of secondary reaction in the two cases would be different. It is worthwhile to note that the average yield for the 25 ring fragmentation products listed in Table I is ~1.5%, even though in sum they account for nearly 40% of the reacted toluene. Thus, it is clear at this point why there have been difficulties in the past with regard to obtaining reasonable carbon balances by more conventional (i.e., chromatographic) analytical techniques. It should be noted that MS/MS does not discriminate well between possible isomers, so our reported concentrations for some identified products may likely contain contributions from several positional isomers. For example, hydroxymethylbutenedial has seven possible isomeric forms. However, on the basis of mechanistic considerations (cf. reactions 10–13), we assume that 3-hydroxy-4-oxopentenal is the most likely isomer. The possibility of a variety of isomeric forms (in low concentrations) would exacerbate the problem of detection by chromatographic techniques.

Although the semiquantitative yields are approximate, the measured ratio of "methylbutenedial" to butenadial is over 10:1. On the basis of the mechanism presented in reactions 2–7, one would expect this ratio to be similar to that of glyoxal to methylglyoxal, i.e., close to 1:1. However,

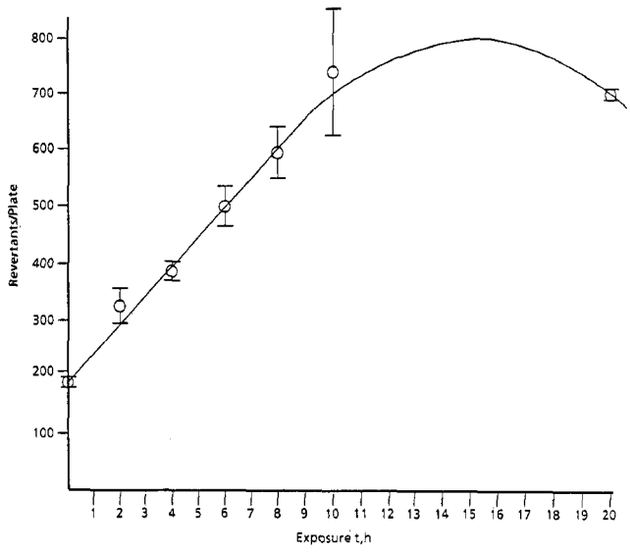


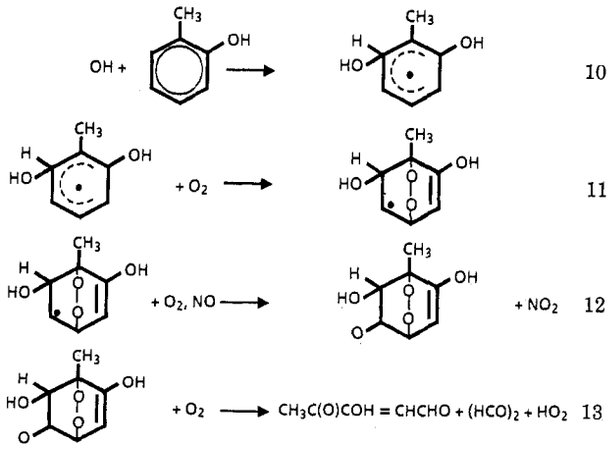
Figure 1. TA100 dose–response curve for toluene photooxidation products.

it is important to keep in mind that there are possible important secondary formation and loss processes for all four of these species, as discussed in detail below, and that it is difficult to apply these yields to any particular mechanistic step due to the complexity of the chemistry in this system. More specifically, the rate constant for reaction of OH with toluene is 6.2×10^{-12} cm^3 molecule^{-1} s^{-1}, and for products such as 4-oxo-2-pentenal, it is likely to be roughly a factor of 10 higher [e.g., the OH rate constant for *cis*-hexene-2,5-dione is $(6.3 \pm 0.6) \times 10^{-11}$ cm^3 molecule (*19*)]. Thus, for products with very small yields, it is likely that the measured yields are impacted by various secondary formation and/or removal processes.

In addition to the cresols and benzaldehyde, there are other aromatic products that could be present in significant yields. For example, Gery et al. (*20*) recently reported large yields of nitrotoluene and nitrocresols in their study of the mechanism of OH reaction with toluene. However, the NO_2 concentrations used in their experiments were high (5–20 ppm), and these products would be expected to have considerably smaller yields under the conditions of the experiment reported here.

It is also possible that there was some secondary reaction occurring that could account for some of the missing carbon. For example, in their study of toluene photooxidation, Bandow et al. (*8*) observed 4% yields of maleic anhydride, which is presumably a product of OH reaction with butenedial. O'Brien et al. (*21*) have found significant yields of CO and CO_2, which can be formed through photolysis of, or OH reaction with, the aldehydic products. Therefore, some of the missing carbon may be present as CO and CO_2.

Mechanisms have been suggested for production of the major products methylglyoxal, glyoxal, methylbutenedial, and oxoheptadienal (*6–8, 22*). In addition, a mechanism for production of methylfuran, tentatively identified here as a minor product, has been suggested by Shepson et al. (*6*). Possible mechanisms for production of many of the various hydroxy-substituted products have not been identified as yet. Although a reasonable mechanism has been proposed by Dumdei et al. (*7*) for production of hydroxyoxoheptadienal, the mechanism for production of products such as "hydroxymethylbutenedial" is not clear. The most likely possibility is that hydroxymethylbutenedial (i.e., 3-hydroxy-4-oxo-2-pentenal) is produced from OH addition to the product *o*-cresol, as shown in reactions 10–13. Although the yield of *o*-cresol is only ~13%, the



rate constant for OH reaction with *o*-cresol is ~6.5 times greater than that with toluene. Therefore, many of the hydroxy-substituted products may be secondary products involving OH reaction with *o*-cresol. For example, "hydroxyoxoheptadienal" is likely 6-oxo-5-hydroxy-2,4-heptadienal, produced as a seven-carbon ring-opening product of OH reaction with *o*-cresol, analogous to reactions 8 and 9. To determine if this is the case, these products could be monitored during a static-mode smog chamber run to determine if they exhibit an induction period. Although it is tempting to think of these hydroxy-substituted products as being products of OH reaction with some of the primary ring fragmentation products, OH addition to these species should produce aldehydic products as opposed to hydroxy-substituted products. It is interesting to note that there are terminal vinyl products such as pentadienal present as well. O'Brien et al. (*7*) suggested that H atom shift reactions involving the OH adduct could explain the existence of these products.

PAN is produced with a molar yield of 11%, whereas the yield of CH_3CHO (a possible PAN precursor) is only 0.8%. It has been reported by Plum et al. (*23*) that methylglyoxal photolyses rapidly to yield (at least part of the time) acetyl radicals. Thus, methylglyoxal is likely an important source of PAN in this system. However, since the primary methylglyoxal yield (i.e., in the absence of secondary reactions) is 15% (*5*), the ratio ([PAN] + [methylglyoxal])/Δ toluene should not exceed 15% if methylglyoxal is the only source of PAN. As shown in Table I, the observed ratio is 30%, using the HPLC methylglyoxal data. This seems to imply that there may be other sources of the acetyl radical. However, there are likely to be a variety of secondary sources of methylglyoxal. For example, as stated above, the OH rate constants for products such as 6-oxo-2,4-heptadienal are expected to be relatively large, and thus this compound could react with OH to yield methylglyoxal (and butenedial) as indicated in reactions 14–16.

$$CH_3C(O)CH{=}CHCH{=}CHCHO + OH \rightarrow CH_3C(O)CH(OO)CHOHCH{=}CHCHO \quad (14)$$

$$CH_3C(O)CH(OO)CHOHCH{=}CHCHO + NO \rightarrow CH_3C(O)CH(O)CHOHCH{=}CHCHO + NO_2 \quad (15)$$

$$CH_3C(O)CH(O)CHOHCH{=}CHCHO \rightarrow CH_3C(O)CHO + CHOCH{=}CHCHO + HO_2 \quad (16)$$

Therefore, it remains a possibility that methylglyoxal photolysis is a principal source of PAN. Acetyl radicals could also be produced from photolysis of some of the other ring fragments, particularly those that are α,β-dicarbonyl compounds. For example, Shepson et al. (*6*) reported the product 3,4-dioxopentene, which could be formed through H atom shift reactions and could photolyze to yield acetyl radicals. It should be noted, however, that the light intensity was rather low (estimated to be k_{NO_2} = 0.09 min^{-1}), and thus if acetyl radicals are produced photolytically, the photolysis rate constants would have to be large. It is conceivable that acetyl radicals could be produced as a direct ring fragmentation product, but this is awkward mechanistically. To address these questions requires, in part, that the ring fragmentation products be synthesized and that their photolysis and OH reaction rates and mechanisms be studied carefully.

In our previous studies of the mutagenic activity of the photooxidation products of toluene (*10*), we conducted exposures of TA100 to the products present at long extent of reaction, for a 20-h exposure time, only. Given that the extent of toluene reaction (600 ppb consumed) was nearly as great as for this experiment, it is likely that toxicity effects were present in the previous experiments. Therefore, the observed mutagenic activity for the previous experiments may have been lower than it would be in the

absence of toxicity effects. The observed response in this experiment corresponds to 0.07 revertants h^{-1} ppb^{-1} toluene consumed, compared with 0.05 revertants h^{-1} ppb^{-1} for the previous study (these responses are actually the same within the limits of uncertainity of the bioassay). The large response under these conditions is significant since the product mixture is probably simpler because of a lesser extent of secondary reaction. In addition, the light intensity was roughly a factor of 4 lower here than in the previous experiment, which would lead to fewer photolysis products involving carbonyl compounds (7).

We have recently found (24) that exposures of TA100 to PAN at concentrations ranging from ~100 to 500 ppb yields a reversion rate of ~14 revertants/h, independent of PAN concentration, making PAN a moderately strong mutagen (24). Assuming that this value applies under the conditions of this exposure, then PAN accounts for ~27% of the measured reversion rate for the products of the photooxidation of toluene of 52 revertants/h. Given that the bioassay data are generally reproducible to within, at worst, ±50%, it would appear that some of the other photooxidation products (most likely the ring fragments) are significantly mutagenic. We have previously found the cresols and benzaldehyde to be nonmutagenic as determined using strain TA100.

Conclusions

In this study we have conducted quantitative and semiquantitative analyses of a variety of ring fragmentation products of OH reaction with toluene. The yields for most of these products have not been previously reported. The sum of the yields of the ring fragmentation products, benzaldehyde, and the cresols is ~60%. The remaining reacted toluene probably was not accounted for due to secondary reactions leading to a variety of products with very small yields. When attempting to measure yields of products of the photooxidation of toluene and other aromatics, it is important to carefully consider the influence of secondary reactions. It was found in this study that a large number of ring fragmentation products (and thus mechanistic routes) account for the reacted toluene. To better determine the yields of various products for OH reaction with toluene, measurements must be taken at very short extents of reaction to minimize the extent of secondary reactions, thus requiring utilization of very sensitive analytical techniques.

It was demonstrated here that the products of the photooxidation of toluene are significantly mutagenic, even at relatively short extent of reaction. Although PAN was shown to account for ~27% of the observed mutagenic activity, there remain unidentified mutagenic products. More work is necessary to fully understand the various mechanisms for conversion of toluene to products under atmospheric reaction conditions and to determine the nature of the mutagenic products.

Acknowledgments

We thank E. Perry and G. Harris of Environmental Health Research and Testing, Inc., for their assistance with the bioassay work. Bruce Ames (University of California, Berkeley, CA) provided the *S. typhimurium* tester strain TA100.

Registry No. NO, 10102-43-9; NO_x, 11104-93-1; O_3, 10028-15-6; HNO_3, 7697-37-2; $CH_3C(O)CHO$, 78-98-8; $(CHO)_2$, 107-22-2; C_6H_5CHO, 100-52-7; CH_3CO_2H, 64-19-7; HCHO, 50-00-0; CH_3CHO, 75-07-0; toluene, 108-88-3; peroxyacetyl nitrate, 2278-22-0; methyl vinyl ketone, 78-94-4; butenedial, 2363-83-9; acrolein, 107-02-8.

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Received for review May 26, 1987. Revised manuscript received May 2, 1988. Accepted June 21, 1988. Although the research described in this article was funded wholly or in part by the U.S. Environmental Protection Agency through Contracts 68-02-4033 and 68-02-4443 to Northrop Services, Inc.—Environmental Sciences, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.