Alkyl hydroperoxides can be found in remote atmospheres at concentrations approaching 1 part per billion. However, very little is known about the impact of these alkyl hydroperoxides on human health. It is conceivable, based on the chemical reactivity of hydroperoxides, that short-chain alkyl hydroperoxides can oxidize DNA bases, resulting in genetic damage. In this study we investigate the mutagenic properties of a series of short-chain alkyl hydroperoxides. Methyl, ethyl, n-propyl, and n-butyl hydroperoxide were synthesized using conventional methods and tested for direct-acting mutagenicity (i.e., without the addition of rat liver S9) in the Ames assay using Salmonella typhimurium strains TA98, TA100, and TA102. All four compounds are mutagenic in strain TA102 and weakly mutagenic in strain TA100. In strain TA100, methyl and n-butyl hydroperoxide are toxic at concentrations that are mutagenic for ethyl and n-propyl hydroperoxides. None of these compounds are mutagenic in strain TA98. These results suggest that alkyl hydroperoxides, formed as the result of atmospheric processes, are genotoxic in bacteria. The effect of these hydroperoxides on human health is difficult to assess because of their low concentrations in the environment and their unknown toxicological properties in humans.

Introduction

The chemistry of anthropogenic and natural hydrocarbons in the atmosphere is complex (1). Most hydrocarbons are oxidized by OH radicals to yield alkyl radicals, which react very rapidly with oxygen to form alkyl peroxo radicals. For example

\[
\text{CH}_3 + \cdot \text{OH} \rightarrow \cdot \text{CH}_3 + \text{H}_2\text{O} \quad (1)
\]

\[
\cdot \text{CH}_3 + \text{O}_2 \rightarrow \cdot \text{CH}_2\text{OO}^* \quad (2)
\]

The fate of alkyl peroxo radicals is determined, to a large degree, by the amount of nitrogen oxides (NOx) in the atmosphere. In urban areas, where NOx levels are high, alkyl peroxo radicals react with NO to form alkyl nitrates, which then react with O3 to form aldehydes (2). In rural and remote areas, where NOx levels are low, alkyl peroxo radicals can react with other alkyl peroxo radicals or with HO2 to form a variety of products (2, 3). Reaction of the simplest alkyl peroxo radicals, methyl peroxo and ethyl peroxo radicals, with the hydroperoxy radical, \( \cdot \text{HO}_2 \), has been shown to lead exclusively to the corresponding hydroperoxides (4, 5)

\[
\text{CH}_3\text{OO}_2^+ + \cdot \text{HO}_2 \rightarrow \cdot \text{CH}_3\text{OOOH} + \text{O}_2 \quad (3)
\]

\[
\text{C}_2\text{H}_5\text{OO}_2^+ + \cdot \text{HO}_2 \rightarrow \cdot \text{C}_2\text{H}_5\text{OOOH} + \text{O}_2 \quad (4)
\]

Methyl hydroperoxide has been detected in ambient air at levels up to 0.8 parts per billion (ppb) and in rainwater (6, 7). While hydroperoxides can be formed from the oxidation of the respective alkanes, which are emitted into the atmosphere from man-made and natural sources (6–10), simple peroxo radicals can also be formed during the oxidation of complex hydrocarbons, generally through C–C bond scission of alkyl radicals formed during the oxidation process (1). A modeling study, incorporating detailed peroxo radical chemistry (2), suggests that total hydroperoxide concentrations could be 1 ppb in remote atmospheres.

Despite the ubiquitous nature of hydroperoxides in the atmosphere, very little is known about the effect of alkyl hydroperoxides on human health. It is conceivable, based on the chemical reactivity of hydroperoxides (11), that short-chain alkyl hydroperoxides can oxidize DNA bases, resulting in genetic damage. It is possible that these genotoxic lesions could lead to mutations and, possibly, cancer. To the best of our knowledge, there has been only one study of a short-chain alkyl hydroperoxide, tert-butyl hydroperoxide, which showed this compound to be mutagenic in a bacteria strain (TA102) sensitive for oxidative mutagens (12). As an integral part of an experimental effort in our laboratory to define the environmental impact of the release of hydrocarbons into the atmosphere, we have carried out the first study of the direct-acting mutagenicity of a series of alkyl hydroperoxides. Herein we report that methyl, ethyl, n-propyl, and n-butyl hydroperoxides are mutagenic in Salmonella typhimurium strain TA102 and weakly mutagenic in strain TA100. Methyl and n-butyl hydroperoxides are toxic at concentrations where ethyl and n-propyl hydroperoxide are mutagenic. None of these alkyl hydroperoxides are mutagenic in strain TA98.

Materials and Methods

Ames Assay Mutagenicity. Ames assays were carried out using Salmonella typhimurium strains TA98 and TA100 (Bruce Ames, Berkeley, CA) without the addition of rat liver S9 extract, as described previously (13–15). Concentration-dependent mutagenicity curves for 2-nitrofluorene were determined as positive controls for Ames assays using strain TA98 (27 ± 4 revertants/mmol, \( n = 5 \)); concentration-dependent curves for methyl methanesulfonate were determined as positive controls for experiments using strain TA100 (149 ± 17 revertants/μmol, \( n = 7 \)). It is possible that n-alkyl hydroperoxides can react with DMSO, the typical solvent used to prepare stock solutions of mutagens. The effect of DMSO on the mutagenicity of n-alkyl hydroperoxides was checked by using deionized, distilled water as a solvent for methyl hydroperoxide; there were no differences in the mutagenicity of this compound using DMSO or water solvents. To minimize the reaction of DMSO with any of the compounds tested, the solutions containing the compounds were prepared the morning of the experiment and were used immediately.

Mutation experiments with strain TA102 were carried out using a slightly different procedure because it was difficult to obtain a reproducible level of mutagenicity with tert-butyl hydroperoxide, a reference mutagen for this strain (12). A 20-μL aliquot from a frozen culture was grown up overnight in Oxoid No. 2 broth. This culture was diluted with 0.02 M sodium phosphate, pH 7.4, buffer and plated onto agar plates that contained Oxoid No. 2 broth supplemented with 10 μg/mL tetracycline to obtain single colonies. The plate was allowed to incubate for 3 days and a large, single colony was used to inoculate 20 mL of Oxoid No. 2 broth containing no tetracycline. This overnight culture (14 h) was used for the mutation experiments. The mutagenicity of the released hydroperoxides could be 1 ppb in remote atmospheres.
Figure 1. Mutagenicity of alkyl hydroperoxides in TA100: (A) methyl hydroperoxide, ○; ethyl hydroperoxide, ◼; n-propyl hydroperoxide, □; n-butyl hydroperoxide, ■. Error bars represent the range of duplicate determinations.

Figure 2. Mutagenicity of alkyl hydroperoxides in TA102: (A) methyl hydroperoxide, ○; ethyl hydroperoxide, ◼; n-propyl hydroperoxide, □; n-butyl hydroperoxide, ■. Error bars represent the range of duplicate determinations.

of tert-butyl hydroperoxide was determined to be 2000 ± 200 revertants/μmol (n = 5), in good agreement with the value reported by Levin et al. (12) (2300 revertants/μmol). tert-Butyl hydroperoxide (Aldrich Chemical Co.) was assayed for peroxide content using an iodometric titration and was 87% pure, with the impurities being about equal amounts of water and tert-butyl alcohol. The mutagenicity reported above for tert-butyl hydroperoxide in strain TA102 has been corrected for purity. Photomicrographs of the background lawn were taken to assess cell killing (16).

Independent Ames assays using strains TA98, TA100, and TA102 were repeated for each compound at least once. The concentration-dependent mutagenicity curves were essentially identical, and only one experiment is shown in Figures 1 and 2 for clarity. Mutagenicities are determined in duplicate for each concentration tested, and the error bars shown in Figures 1 and 2 represent the range of the duplicate determinations.

Synthesis of Alkyl Hydroperoxides. Methyl hydroperoxide was synthesized from the reaction of dimethyl sulfate with alkaline hydrogen peroxide (4). Methyl hydroperoxide was purified by repeated vacuum distillation and impurities were less than 6% as determined by GC-MS. GC-MS analysis was carried out on a Hewlett-Packard Model 5970 coupled to a 60-m DB-1 column. Compounds were eluted using a temperature program, with the oven being held at 40 °C for 6.8 min followed by ramping the temperature to 280 °C at 15 °C/min. Mass spectra were collected before and after elution of the solvent (methylene chloride). Impurities detected in the methyl hydroperoxide were methanol (4%), benzene (2%), and trace amounts of diethyl ether and ethanol (<0.2%).

The remaining three n-alkyl hydroperoxides were synthesized by the reaction of their respective n-alkyl methanesulfonates and alkaline hydrogen peroxide (17). Ethyl hydroperoxide was 84% pure, with the impurities being ethanol (12%) and benzene (4%). n-Propyl hydroperoxide was 95% pure and was contaminated with 1-propanol (4%) and hydrogen peroxide (1%). n-Butyl hydroperoxide was 90% pure, with the contaminants being benzene (8%), 1-butanol (1%), and 1-butanal (1%). Benzene was present as a contaminant because it was used as a solvent to extract the reaction mixtures. The corresponding alcohols were impurities due to the route of synthesis and decomposition of the alkyl hydroperoxides. The amount of hydrogen peroxide present as a contaminant in the n-propyl hydroperoxide preparation was too low to give rise to a mutagenic response in any of the bacteria strains tested. For example, at the highest n-propyl hydroperoxide concentration tested (100 μg), 1 μg of hydrogen peroxide would be present. The mutagenicity of 1 μg of hydrogen peroxide is about 7-8 revertants above the spontaneous revertant level in strain TA102, a mutagenic response too low to be detected (12). None of the other impurities are mutagenic in strain TA102.

Results and Discussion

The mutagenicities of methyl and ethyl hydroperoxide in strain TA100 are shown in panel A of Figure 1. The mutagenicity of ethyl hydroperoxide peaks at ~4 μmol/plate, about twice the level of the spontaneous revertants. Methyl hydroperoxide has the same initial slope as that of ethyl hydroperoxide but does not reach the same level of mutagenicity due to toxicity of this hydroperoxide. Panel B of Figure 1 shows the mutagenicity of n-propyl
The mutagenicities of these n-alkyl hydroperoxides in TA102 demonstrate that short-chain normal alkyl hydroperoxides can cause genetic damage in bacteria. Methyl hydroperoxide has been positively identified in the atmosphere (6, 7), and other alkyl hydroperoxides have been postulated to form in the atmosphere (2, 20), especially in environments with low NO\textsubscript{x} concentrations. Although these results suggest that short-chain alkyl hydroperoxides may be genotoxic, the effect of these hydroperoxides on human health is difficult to assess because of their low concentrations in the environment and their unknown toxicological properties in humans. Further studies to determine the extent of formation and fate of these alkyl hydroperoxides in polluted urban air are ongoing.

**Registry No.** MeOOH, 3031-73-0; BuOOH, 3031-74-1; PrOOH, 8068-96-8; BuOOH, 4813-50-7.

### Literature Cited


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**Table 1. Mutagenicity of Methyl, Ethyl, n-Propyl, n-Butyl, and tert-Butyl Hydroperoxide in TA102**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Revertants/μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl hydroperoxide</td>
<td>300</td>
</tr>
<tr>
<td>Ethyl hydroperoxide</td>
<td>310</td>
</tr>
<tr>
<td>n-Propyl hydroperoxide</td>
<td>360</td>
</tr>
<tr>
<td>n-Butyl hydroperoxide</td>
<td>1200</td>
</tr>
<tr>
<td>tert-Butyl hydroperoxide</td>
<td>2000</td>
</tr>
</tbody>
</table>

(positive control)