Sensory Irritation of Acetic Acid, Hydrogen Peroxide, Peroxyacetic acid and their Mixture in Mice

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The expiratory bradypnoea indicative of upper airway irritation in mice was evaluated during a period of 60 min of oronasal exposure to acetic acid, hydrogen peroxide and peroxyacetic acid vapours. The airborne concentration resulting in a 50% decrease in the respiratory rate of mice (RD50) was calculated for each chemical. The concentration–response curves of acetic acid, hydrogen peroxide and peroxyacetic acid had similar slopes. The results did however show that the three chemicals had different irritant potencies. The RD50 values of acetic acid, hydrogen peroxide and peroxyacetic acid were 227, 113 and 5.4 p.p.m. respectively. Moreover, a mixture containing 53% acetic acid, 11% hydrogen peroxide and 36% peroxyacetic acid had an RD50 of 10.6 ppm, 3.8 ppm being peroxyacetic acid, which is 1.4 times lower than the theoretical value estimated from the fractional concentrations and the respective RD50s of the individual components. On the basis of a TLV-STEL (threshold limit value for short-term exposure limit) equal to 0.1 RD50, the TLV-STELs for acetic acid, hydrogen peroxide and peroxyacetic acid should not exceed 20, 10 and 0.5 p.p.m. respectively. On the basis of a TLV-TWA (time-weighted average) equal to 0.03 RD50, the TLV-TWAs for these same chemicals should not exceed 5, 3 and 0.2 p.p.m. respectively. Finally, these values and existing TLVs in Europe and the USA are compared.

Keywords: peroxyacetic acid, hydrogen peroxide, sensory irritation, mice

INTRODUCTION

Peroxyacetic acid, the peroxide of acetic acid, is a disinfectant that has the desirable properties of hydrogen peroxide, i.e. broad-spectrum activity against microorganisms, lack of harmful decomposition products, and infinite water solubility. Peroxyacetic acid has greater lipid solubility than hydrogen peroxide and is free from deactivation by catalase and peroxidase enzymes (Block, 1992). Peroxyacetic acid is a more powerful antimicrobial agent than hydrogen peroxide and most disinfectants (Baldry, 1983; Dychdala, 1988; Alasri et al., 1993). Peroxyacetic acid also has excellent sporocidal activities (Alasri et al., 1993; Samrakandi et al., 1994). Aqueous solutions are composed of the acid in combination with hydrogen peroxide, acetic acid, water and a stabilizing agent. The strong antimicrobial activity of peroxyacetic acid makes it valuable in maintaining sterile conditions in the production of germ-free animals. It is used in hospitals to sterilize the surfaces of medical instruments and may be found in laboratories, central supply and patient care units. It has been accepted worldwide in the food processing and beverage industries as being ideal for clean-in-place systems. It is also used as a sanitizer in the pharmaceutical and cosmetic industries (Dychdala, 1988; ECETOC, 2001). Peroxyacetic acid is recognized as a strong irritant and a lacrimator, and could be responsible for the irritation felt by workers employed in premises where it is used as a disinfectant. However, there have been no formal concentration–response studies of the respiratory irritation caused by peroxyacetic acid. In this study, the irritating power of ‘pure’ peroxyacetic acid and commercially available peroxyacetic acid (a mixture of peroxyacetic acid, acetic acid and hydrogen peroxide) was evaluated and compared to that of acetic acid and hydrogen peroxide using a quantitative methodology initially developed to test the sensory irritation properties of airborne chemicals in mice. This method is based on observations showing that irritants stimulate the
trigeminal nerve-endings in the nasal mucosa of oro-nasally exposed mice, thereby causing a characteristic reflexively induced decrease in respiratory rate. This decrease in respiratory rate is related to concentration. The concentration responsible for a 50% decrease in the respiratory rate (RD50) is used to compare the irritant potencies of chemicals and to establish acceptable levels of exposure (Kane et al., 1979, 1980; Alarie, 1981a,b; Schaper, 1993).

MATERIALS AND METHODS

Chemicals

Acetic acid (Normapur, 100% pure) and hydrogen peroxide (Normapur, 30%) were supplied by Prolabo, Fontenay sous Bois, France. Peroxyacetic acid (a mixture of 39% peroxyacetic acid, 45% acetic acid and 6% hydrogen peroxide) was purchased from Fluka, Buchs, Switzerland.

Animals

Male OF1 mice (IFFA Credo, Domaine des Oncins, Saint-Germain-sur-l’Arbresle, France), weighing 20–25 g, were housed for 7 days in polycarbonate cages (37.5 cm long × 21.6 cm wide × 14.7 cm tall, each holding 10 mice), with hardwood-chip bedding, under controlled environmental conditions before the study period. Room temperature (22°C), humidity (55 ± 5%) and light cycle (07:00–19:00 h) were controlled automatically. Filtered tap water (pore size 0.3 µm) and food (UAR-Alimentation, Villenoissson, Epinay-sur-Orge, France; sterilized with γ-rays) were available ad libitum except during exposure periods.

Generation, sampling and analysis of test atmospheres

Exposures were conducted in a 2.5 l inhalation chamber equipped with four plethysmographs. The conditions of generating, sampling and analysing test atmospheres of acetic acid, hydrogen peroxide, pure peracetic acid and commercially available peroxyacetic acid are described in detail in the accompanying paper (Hecht and Hery, 2002). Briefly, acetic acid, hydrogen peroxide and commercially available peroxyacetic acid vapours were generated by using a peristaltic pump to feed a vaporization chamber with the products purchased from Fluka and Prolabo as indicated above. The pure peroxyacetic acid vapour was generated by buffering the commercially peroxyacetic acid at pH 7 extemporaneously with a phosphate buffer. The different pKₐ values of acetic and peroxyacetic acids and the different vapour pressures of peroxyacetic acid and hydrogen peroxide allowed us to generate peroxyacetic acid vapours alone. Feeding the vaporization chamber with constantly renewed solutions of buffer and commercially available peroxyacetic acid regulated the emission of gaseous peroxyacetic acid without impoverishing the reaction medium.

Experimental design and conduct

Breathing frequency was used as an index of upper respiratory tract irritation. Stimulation of the trigeminal nerves in the eyes and the upper respiratory tract causes sensory irritation. In mice, it causes an elongation of the period from the end of the inspiration until the start of the expiration. Thus, sensory irritation pattern is characterized by a ‘break’ in the respiration. Stimulation of the vagal nerves in the lungs may cause pulmonary irritation. In mice, pulmonary irritation may increase the time from the end of the expiration to the initiation of the following inspiration. The reflex pattern of pulmonary irritation is thus characterized by a ‘pause’ in the respiration (Vijayaraghavan et al., 1993). The method for measuring the respiratory rate in oronasally exposed mice has already been described in detail (De Ceaurriz et al., 1981; ASTM, 1984). Briefly, the mice were restrained in body plethysmographs, while the head was enclosed in the inhalation chamber. The breathing frequency was monitored with a pressure transducer (Validyne DP 45) before and during the 60 min exposure period, and throughout the recovery period. For each concentration, the maximum decrease in respiratory rate occurring during the exposure period was recorded and the RD₅₀ calculated. The effects of six or seven different exposure concentrations were tested for each chemical. Eight previously unexposed mice were used for testing each concentration.

Statistical analysis

Differences in the respiratory rates before and during exposure to hydrogen peroxide, acetic acid and peroxyacetic acid were analysed statistically by Student’s t-test for paired data. The level of significance was set at P < 0.05. The concentration–response curves were analysed by least-squares linear regression and the RD₅₀ values calculated with their 95% confidence intervals.

RESULTS

Acetic acid

An example of the time–effect curves for mice exposed to four different concentrations of acetic acid is presented in Fig. 1. The onset of the response was rapid. The response almost reached a maximum within the first 2 min. There was no drop-off in this response. For each concentration, the response was characteristic of sensory irritation. The respiratory break was observed at the start of the expiratory phase of respiration. There was no trace of pulmonary irritation, i.e. a characteristic pause present at the end of expiration. Recovery was rapid after exposure. After 15 min of recovery, the respiratory rate
reached 86–98% of that before exposure. The different exposure concentrations produced a broad range of effects and the exposure concentration–response relationship was used to calculate the linear regression equation, the RD_{50} and its corresponding 95% confidence interval (Table 1). The RD_{50} was calculated to be 227 p.p.m. (560 mg/m^3).

**Hydrogen peroxide**

The time–effect curves for mice exposed to four different concentrations of hydrogen peroxide are presented in Fig. 2. The onset of the response was slightly less faster than that of acetic acid, particularly at the low concentrations. There was no fall-off in the response, the effect being steady throughout the 60 min exposure. After exposure, recovery was rapid and concentration dependent. After 15 min of recovery, the respiratory rate returned to between 74 and 98% of that before exposure. The linear regression equation, the RD_{50} and its corresponding 95% confidence interval are presented in Table 1. The RD_{50} was calculated to be 113 p.p.m. (157 mg/m^3).

**Peroxyacetic acid**

The time–effect curves for mice exposed to four different concentrations of peroxyacetic acid are presented in Fig. 3. As with acetic acid and hydrogen peroxide, the onset of the response was rapid, particularly at the two highest concentrations where the maximal response was reached within the first 4 min. The effect was steady during the rest of the exposure except for the lowest concentration (1.8 p.p.m.) where there was a slight increase in the effect from the 30th to the 60th min of exposure. After 60 min of exposure, recovery was rapid and concentration dependent. After 15 min of recovery, the respiratory rate returned to between 66 and 97% of that before exposure. The linear regression equation, the RD_{50} and its corresponding 95% confidence interval are presented in Table 1. The RD_{50} was calculated to be 5.4 p.p.m. (17 mg/m^3).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Range of exposure concentrations (p.p.m.)</th>
<th>Linear regression equation</th>
<th>Correlation coefficient</th>
<th>RD_{50} (p.p.m.)</th>
<th>95% confidence interval (p.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>57–389</td>
<td>( y = 18.89 \ln(x) - 52.5 )</td>
<td>0.977 (7)</td>
<td>227</td>
<td>198–269</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>25–212</td>
<td>( y = 17.95 \ln(x) - 34.9 )</td>
<td>0.978 (6)</td>
<td>113</td>
<td>95–141</td>
</tr>
<tr>
<td>Peroxyacetic acid</td>
<td>1.8–24</td>
<td>( y = 18.11 \ln(x) + 19.5 )</td>
<td>0.996 (7)</td>
<td>5.4</td>
<td>5.0–5.8</td>
</tr>
<tr>
<td>Peroxyacetic acid (mixture)</td>
<td>1.6–11.6</td>
<td>( y = 20.13 \ln(x) + 23.1 )</td>
<td>0.970 (7)</td>
<td>3.8</td>
<td>3.2–4.4</td>
</tr>
</tbody>
</table>
Peroxyacetic acid (mixture)

The time–effect curves for mice exposed to four different concentrations of peroxyacetic acid are presented in Figs 4 and 5. The profile of the time–effect curves was essentially the same as that of pure peroxyacetic acid. After 15 min of recovery, the respiratory rate returned to between 80 and 97% of that before exposure. The linear regression equation, the RD50, and its corresponding 95% confidence interval are presented in Table 1. The RD50 was calculated to be 3.8 p.p.m. (12 mg/m³).

The concentration–response curves of mice exposed to acetic acid, hydrogen peroxide, peroxyacetic acid and peroxyacetic acid 'mixture', are summarized in Fig. 5.

DISCUSSION

The results of this study show that peroxyacetic acid, acetic acid and hydrogen peroxide possess sensory-irritating properties. With the bioassay used in this study, clear differences emerged in the biological potency of the three chemicals. From the RD50 calculated in our study, the relative potency of peroxyacetic acid compared to acetic acid and hydrogen peroxide was 42 and 21 respectively.

The RD50 of 227 p.p.m. for acetic acid is close to the 163 p.p.m. previously found by De Ceaurriz et al. (De Ceaurriz et al., 1981).

To the best of our knowledge, no RD50 has been published for hydrogen peroxide. The RD50 of 123 p.p.m. found for this chemical makes it a rather strong irritant, comparable in irritant potency to amines such as propylamine, n-butylamine or n-pentylamine (Nielsen and Vinggaard, 1988; Vinggaard et al., 1989; Gagnaire et al., 1989, 1993).

The present study showed peroxyacetic acid to be an even stronger sensory irritant. With an RD50 of 5.4 p.p.m., its irritant potency is similar to that of other powerful irritants such as formaldehyde (Kane and Alarie, 1977; De Ceaurriz et al., 1981; Steinhaugen and Barrow, 1984), allylic compounds (Nielsen et al., 1984; Gagnaire et al., 1987, 1989), chloropicrine (Kane et al., 1979), and chlorine or nitrogen trichloride (Gagnaire et al., 1994).

The results also show that the mixture of peroxyacetic acid, acetic acid and hydrogen peroxide has a strong irritant potency. It would appear from Table 2 that the fractional concentration of each component was relatively constant throughout the seven experiments. The fractional concentrations of peroxyacetic acid, acetic acid and hydrogen peroxide averaged 36, 53 and 11% respectively. A theoretical estimation of the irritant potency of the mixture may be made if the additivity rule is assumed. This is generally accepted as a basic approach when the effects of low concentrations of airborne chemicals are investigated: $[C_{(component 1)}/RD_{50_{(component 1)}}] + [C_{(component 2)}/RD_{50_{(component 2)}}] + ... + [C_{(component n)}/RD_{50_{(component n)}}] = 1/RD_{50_{(mixture)}}$ (Schaper...
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and Detwiler-Okabayashi, 1995). Thus, for the mixture studied in the present study, the theoretical RD$_{50}$ can be estimated at 14.3 p.p.m., with 5.1 p.p.m. being due to peroxyacetic acid, which is close to the experimental value of 3.8 p.p.m. Although the contribution of hydrogen peroxide and/or acetic acid to the effect observed cannot be excluded, it is unlikely because the concentrations of these two chemicals are relatively low in terms of their irritant potency (Table 2). From the rectilinear log-concentration–response relationships, the threshold concentrations for acetic acid and hydrogen peroxide are 16.1 and 7.0 p.p.m. Thus, only acetic acid could have had a slight effect on the mixture at the highest concentration studied, where its concentration reached 17.2 p.p.m.

Previous studies with several chemicals have shown that the RD$_{50}$ values can be used successfully to predict safe industrial exposures if sensory irritation is the most sensitive endpoint (Schaper, 1993). In this case, the RD$_{50}$ values could be used as a basis to determine threshold limit values (TLVs) to prevent any unpleasant sensation such as piquancy, stinging or itching. At 0.1 RD$_{50}$, humans would experience a slight discomfort and this should be the highest level permitted in industry. Furthermore, 0.03 RD$_{50}$ has been shown to correlate well with threshold limit values for a wide range of chemicals (Schaper, 1993). Existing TLVs of acetic acid and hydrogen peroxide and suggested standards on the basis of 0.03 RD$_{50}$ are given below. If the TLV-STEL (short-term exposure limit) is equal to 0.1 RD$_{50}$ and the TLV-TWA (time-weighted average) is 0.03 RD$_{50}$, then the equivalent values for acetic acid will be 20 and 7 p.p.m. respectively. This latter value is close to the present TLV-TWA of 10 p.p.m. These values would be 10 and 3 p.p.m. respectively for hydrogen peroxide. The present TLV-TWA for hydrogen peroxide is 1 p.p.m., and the present standard for hydrogen peroxide therefore seems appropriate to protect workers from the irritancy caused by this substance. Currently, no standards exist for regulating exposure to peroxyacetic acid. The predicted safe level for this compound to prevent upper airway sensory irritation (TLV-STEL) should not exceed 0.5 p.p.m., and a TLV-TWA is estimated to be 0.2 p.p.m. Data on

Table 2. Concentrations (p.p.m.) and percentages of peroxyacetic acid, acetic acid, and hydrogen peroxide obtained in the exposure chamber when generating the ‘peracetic acid mixture’ vapours

<table>
<thead>
<tr>
<th>Peroxyacetic acid</th>
<th>Peroxyacetic acid mixture</th>
<th>H$_2$O$_2$</th>
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<tbody>
<tr>
<td>1.6 (33%)</td>
<td>2.6 (53%)</td>
<td>0.7 (14%)</td>
</tr>
<tr>
<td>3.0 (37%)</td>
<td>4.3 (52%)</td>
<td>0.9 (11%)</td>
</tr>
<tr>
<td>3.4 (33%)</td>
<td>5.6 (53%)</td>
<td>1.5 (14%)</td>
</tr>
<tr>
<td>5.6 (37%)</td>
<td>8.0 (53%)</td>
<td>1.6 (10%)</td>
</tr>
<tr>
<td>7.1 (38%)</td>
<td>10.2 (54%)</td>
<td>1.6 (8%)</td>
</tr>
<tr>
<td>8.2 (39%)</td>
<td>11.1 (53%)</td>
<td>1.8 (8%)</td>
</tr>
<tr>
<td>11.6 (36%)</td>
<td>17.2 (53%)</td>
<td>3.5 (11%)</td>
</tr>
</tbody>
</table>

Fig. 5. Concentration–response curves of mice exposed to acetic acid, hydrogen peroxide, peroxyacetic acid and peroxyacetic acid ‘mixture’.
concentrations of peroxyacetic acid encountered in workplaces are scarce. The values proposed in this paper can be exceeded in workplaces where concentrations of peroxyacetic acid were reported to vary from 0.005 mg/m$^3$ (0.002 p.p.m.) to 1.84 mg/m$^3$ (0.6 p.p.m.) in a university hospital over a period of 8 h (Schaffernicht and Muller, 1998).

REFERENCES


