Assessing various carbon dioxide flow rates to minimize distress during laboratory mouse euthanasia

Carly Moody, Beverly Chua, I. Joanna Makowska, Daniel M. Weary
Faculty of Land and Food Systems, University of British Columbia

Introduction

- Laboratory rodents are commonly euthanized by exposure to carbon dioxide (CO₂) gas using the gradual-fill method
- Current recommended flow rates (20-30% chamber vol/min) result in rodents experiencing dyspnea, a distressing sensation of breathlessness
- The aim of this study was to determine the flow rate that minimizes the duration of dyspnea

Methods

- Five flow rates were tested (20, 30, 40, 50, 60%) using female albino C57BL/6 mice
- Using an oxygen analyzer and a gas holding technique, CO₂ concentrations within the apparatus did not reach levels associated with pain (> 40%)
- A surgical glove was projected into the apparatus to allow one hand to be placed into the box during each trial
- Dyspnea onset was recorded when the mouse first showed labored breathing
- Upon the appearance of recumbency we tested loss of the righting reflex by placing the animal on its back
- Pedal withdrawal reflex was then tested every 10 seconds using a hemostat to pinch alternating hind paw interdigital webbing, loss of reflex was recorded when the animal failed to respond to 3 consecutive pinches

Results

<table>
<thead>
<tr>
<th>BEHAVIORAL PARAMETER</th>
<th>CO₂, FLOW RATES</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>Dyspnea onset</td>
<td>19 ± 2</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Loss of righting reflex</td>
<td>71 ± 3</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Loss of pedal reflex</td>
<td>118 ± 9</td>
<td>106 ± 9</td>
</tr>
<tr>
<td>Dyspnea onset - loss of righting reflex</td>
<td>46 ± 9</td>
<td>42 ± 9</td>
</tr>
<tr>
<td>Dyspnea onset - loss of pedal reflex</td>
<td>100 ± 9</td>
<td>90 ± 9</td>
</tr>
</tbody>
</table>

Conclusions

- Dyspnea onset and loss of righting reflex occurred earlier with increasing flow rates, but loss of the pedal reflex did not vary with flow rate.
- Mice may suffer during the onset of dyspnea to loss of consciousness. Two different intervals assessed this potential - dyspnea onset to loss of righting reflex and dyspnea onset to loss of pedal reflex.
- The interval between dyspnea and loss of righting reflex was numerically lowest at the 30% flow rate, but neither interval showed a significant difference in relation to flow rate.