Steps for CO2 Euthanasia of Rodents

You must be trained before you perform this procedure

If you have questions, contact: CMP at 979-845-7433; ARU: at (214) 828-8149; PAR: at (713) 677-7471; PRF: at (361) 221-0770.

For instruction on the safe use of compressed gases, contact EHS: 979-845-2132.

1) Read TAMU-G-028 and check your setup before starting:

<table>
<thead>
<tr>
<th>DO</th>
<th>DON'T</th>
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<tbody>
<tr>
<td>Use a clear chamber (home cage is best)</td>
<td>Leave animals unattended</td>
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<tr>
<td>Use a different euthanasia method for neonates</td>
<td>Overcrowd</td>
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<tr>
<td>Use a flow meter and pressure-reducing regulator</td>
<td>Mix species or incompatible animals in the chamber</td>
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<tr>
<td>Follow your approved animal use protocol</td>
<td>Prefill the chamber with CO2</td>
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2) Look up or calculate the correct flow rate for the specific size of your euthanasia chamber: For a humane death, the CO2 flow rate must equal 10-30% of chamber volume per minute.

$$\text{CO}_2 \text{ flow rate in L/min} = \frac{\text{height (cm)} \times \text{width (cm)} \times \text{length (cm)}}{1000} \times \frac{0.2}{\min}$$

Mark the rate on the flow meter and record it here:

Chamber type: ________________ CO2 flow rate: ____ L/min

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3) Place the rodents into the chamber and close it. Alternatively, attach the tubing from the flow meter to the home-cage inlet.

4) Open the cylinder valve to release CO2 into the chamber and adjust the flow to the correct flow rate.

5) Keep the chamber closed and the CO2 flowing until you see that respiration has ceased in all animals. Continue for at least 1 additional minute before closing the value or flow meter to stop the delivery of CO2.

6) Ensure that all animals are dead by using a secondary method of euthanasia as approved in your animal use protocol.

7) Place the carcass(s) in the appropriate container in the necropsy cooler, or in your facility’s designated carcass disposal location and complete any necessary euthanasia/necropsy logs.

8) Before the next group of animals, refill the chamber with room air by turning it on its side to let the heavier CO2 flow out. Clean the chamber, as needed.

9) After the last group of animals, close the cylinder valve. Also, clean and disinfect the chamber and any instruments used (e.g. guillotine), per laboratory SOP (See TAMU-G-026).

10) If the gas cylinder is (almost) empty, make sure that the appropriate personnel have been notified.