Instructions: Humane Euthanasia of Rodents using Carbon Dioxide (CO₂)

1. Place rodent(s) (> 15 days of age) in a non-precharged chamber. Do not overcrowd: animal(s) must be able to make normal postural adjustments.

2. Introduce CO₂ into chamber at a calculated flow rate (10-30% of chamber volume per minute*) until animal(s) becomes unconscious. CO₂ flow should be maintained for at least 1 minute after respiratory arrest.

<table>
<thead>
<tr>
<th>Chamber Type</th>
<th>Flow Rate (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0 Liter Mouse Cage</td>
<td>0.7 – 2.1</td>
</tr>
<tr>
<td>25 Liter Rat Cage</td>
<td>2.5 – 7.5</td>
</tr>
</tbody>
</table>

3. **Verify clinical death** via a secondary method of euthanasia (e.g., exsanguination, cervical dislocation) or by palpating to ensure cardiac arrest.

4. Turn off gas flow and close tank valve when finished. Clean chamber thoroughly.

*For instructions on calculating the correct Flow Rate for your specific chamber, See RARC SOP: Calculating Flow Rate for CO₂ Chambers (next page)

See the UW-Madison All Campus ACUC Policy 2003-018 titled Euthanasia of Rodents using Carbon Dioxide for further information: www.rarc.wisc.edu/policy/2003-018.html
Title: Calculating Flow Rate for CO₂ Chambers

Location(s): University of Wisconsin-Madison Research Animal Facilities

Safety: Wear a minimum of lab coat and gloves when handling animals

Responsibility: Approved UW-Madison Animal Users that have been properly trained in euthanasia technique, equipment and agents.

I. Purpose: To outline proper calculation of a CO₂ flow rate at 10-20% of chamber volume per minute, for use in rodent euthanasia.

II. Materials Needed: Compressed CO₂ gas in cylinder (properly secured), regulator and flow meter (Liters per minute), euthanasia chamber, tubing to connect tank to chamber.


IV. Safety:
   a. Wear a minimum of lab coat and gloves when handling animals. Always follow any additional facility, room, and/or study specific PPE requirements.

V. Procedure – CO₂ Flow rate must be calculated to 10-30% of chamber volume per minute.
   a. To calculate your flow rate first measure your chamber:
      i. Length x Width x Height= ___ Cubic Inches
   b. Then convert cubic inches to liters
      i. 1 liter = 61.02 cubic inches
      ii. Take ___cubic inches/ 61.02 liters
   c. Last multiply by 10 % then 30 % to figure out your range of flow.

VI. Example:
   a. Your chamber is 19” Long x 10.5” wide x 8” high
   b. Your flow rate should be 10-30% of the chambers volume
      i. 19x10.5x8= 1596 cubic inches
      ii. 1596 cubic inches / 61.02 cubic inches/ liter = 26.16 liters
      iii. 26.16 x 0.1 = 2.6 liters per minute (lpm)
      iv. 26.16 x 0.3 = 7.8 lpm
   c. Your flow rate should be set between 2.6 lpm and 7.3 lpm.
RARC Guidelines:
Gas Flowmeters for use in Rodent CO₂ Euthanasia Systems

To measure appropriate gas flow though a euthanasia chamber that is similar in size to standard mouse or rat cage, a flowmeter that delivers 1 – 12 liters per minute in increments ≤ 0.5 liters per minute is a good choice.

Regulator + Flowmeter combination units are available:
RC Medical Inc  Product M1-320-12FM for Large CO₂ Cylinders
~ $215  Product M1-940-12FM for Small CO₂ Cylinders

Inline Flowmeters are available if a regulator is already used with your cylinder:
RC Medical Inc  Product FME801 (with no adapter)
~ $95  Product FME802 or FME803 (with Male or Female adapter)

Using a Flowmeter Calibrated for a Different Gas

If you have a flowmeter calibrated for air or another gas, the readings cannot be directly applied to CO₂, as the meters are calibrated to a specific density. A conversion will allow you to estimate the flowrate of CO₂ from a meter calibrated for another gas:

\[ F_2 = F_1 \times \left(\frac{D_1}{D_2}\right)^{0.5} \]

Where:

- \( F_1 \) = Flowrate indicated by device for gas 1
- \( F_2 \) = Actual flow rate for desired gas 2
- \( S_1 \) = Density of gas 1 that flow meter is calibrated for
- \( S_2 \) = Density of desired gas 2 going through meter

For example: you read 100 L/min on your meter which is calibrated for air (gas 1). Density of dry air at 25°C 1.184 kg/m³. Density of dry CO₂ at 25°C is 1.799 kg/m³. The actual flow of CO₂ (gas 2) is 81.1 L/min.

Caution:
The flow meter is calibrated for air at a specific temperature and pressure. The above calculation assumes the meter is calibrated for standard conditions and that similar conditions apply when using gas 1 and gas 2.