Proper Operation of the ACP
Approved Carbon Dioxide Regulator
for Euthanasia of Rodents
• ACP approved CO\textsubscript{2} Regulator

• Instructions posted on the wall
• This regulator is coupled with a flow restrictor (shown) to deliver carbon dioxide at a constant rate of approximately 6 liters per minute.

• When used with the 20 liter chamber, the system meets the new [UCSD policy 13.03](#) requirements for delivery of carbon dioxide gas used to euthanize rodents.

• This requirement specifies that CO$_2$ is delivered at a rate of between 10 and 30% of the chamber volume per minute.
• ACP approved
20 liter chamber

Do not substitute for a larger or smaller volume chamber
• Step 1

Assure that the line valve is in the off position (perpendicular to the line)
• Step 2

Turn tank valve on (counter clockwise)
• Step 3

Determine that the tank pressure is sufficient to supply the system
• Step 4

Line pressure gauge (shown) MUST read between 40-50 psi

If it does not, please contact ACP for assistance
• Do not adjust the line pressure

If you feel it is not working correctly
Please contact ACP for assistance
• **Step 5**

Place compatible rodents directly into the chamber, or within a mouse cage WITH the lid removed.
The chamber lid MUST be replaced so that the original 20 liter volume is maintained.
• Step 6

Turn the line valve on (parallel with the line)

Note that the line pressure will drop to between 40-45 psi
• Step 7

Adult rodents will typically be unconscious within a minute

Monitor continuously until the rodents are no longer moving. Then wait approximately 1 minute more before closing the line valve and removing the lid.
• Step 8

• Turn off the line valve
• Step 9

Empty the chamber between each use, by turning it over \((\text{CO}_2 \text{ is heavier than air})\)

Clean the chamber after use
• Step 10

• Turn off the tank valve (clockwise) when finished
Background

- **UCSD IACUC Policy 13.03**
  
  When using carbon dioxide gas as a method of euthanasia,
  
  a. Investigator personnel must use an ACP approved apparatus (see additional information).

  b. If using alternative equipment or methods, it is the investigator’s responsibility to document that they meet the specifications of the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition:
     
     i. A “flow rate for CO2 euthanasia systems should displace 10% to 30% of the chamber or cage volume/min.”
     
     ii. A “commercially supplied cylinder or tank; an appropriate pressure reducing regulator and flow meter or equivalent equipment must be used.”

  c. The chamber may not be pre-filled with carbon dioxide.

  d. The chambers must not be overcrowded. Incompatible animals must not be mixed together in the same chamber/box.

  e. Dry ice may not be used to produce CO2 for euthanasia.
Carbon dioxide, with or without premedication with inhaled anesthetics, is acceptable with conditions for euthanasia of small rodents.

- Compressed CO₂ gas in cylinders is the recommended source of CO₂ because gas inflow to the chamber can be precisely regulated.
- An optimal flow rate for CO₂ euthanasia systems should displace 10% to 30% of the chamber or cage volume/min.
- Prefilled chambers are unacceptable.
- If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses.
- It is important to verify that an animal is dead after exposure to CO₂. Death may be confirmed by physical examination, ensured by an adjunctive physical method, or obviated by calibration and validation of the euthanasia chamber and process.
- If an animal is not dead, CO₂ narcosis must be followed with another method of euthanasia.
Contacts

- ACP Veterinary Services – for hardware or training information
  ACP-vetservices@ucsd.edu

- UCSD IACUC – for policy questions
  IACUC@ucsd.edu
Proper Use of the Carbon Dioxide (CO2) Systems for Euthanasia of Rodents

For systems with a **CO2 tank and pressure regulator:**

1. Turn Tank Valve on (*counter-clockwise*)
2. Verify there is pressure in the tank.
3. Verify that the regulator pressure is 50 psi or less (fixed pressure regulators are pre-set to 50 psi)

For systems with a **building supplied CO2 valve:**

1. Verify that the regulator pressure is less than 50 psi. *(20 liter volume is essential for proper operation)*
2. Place single mouse cage as described in below for **both systems**
3. Turn line valve ON (*parallel with the tubing*)

For both systems:

1. Place rodent(s) either directly *(or within a mouse cage with the cage lid removed)* into the chamber. The lid of the chamber is replaced. ***(Note that there is no need for additional ventilation holes in the container as the container lid is not an airtight seal and will provide sufficient ventilation.)*
2. Carbon dioxide is flowing slowly into the chamber at a rate of less than 30% of the total volume per minute *(approximately 6 liters per minute)*.
3. Monitor the rodents while being euthanized. The slow introduction of carbon dioxide is essential for achieving appropriate anesthesia prior to euthanasia.
4. Allow the animals to remain in sealed chamber for a full minute after they have stopped breathing.
5. Perform secondary physical method of euthanasia to assure death per UCSD policy 13.03.
6. **IMPORTANT** - Empty the chamber of Carbon Dioxide by turning it over between each use.

Reference: Changed federal regulatory requirements for rodent CO2 euthanasia have led to revision of the UCSD Euthanasia Policy 13.03