

Colorado State University
Institutional Animal Care and Use Committee
Directions for CO₂ Euthanasia of Rodents

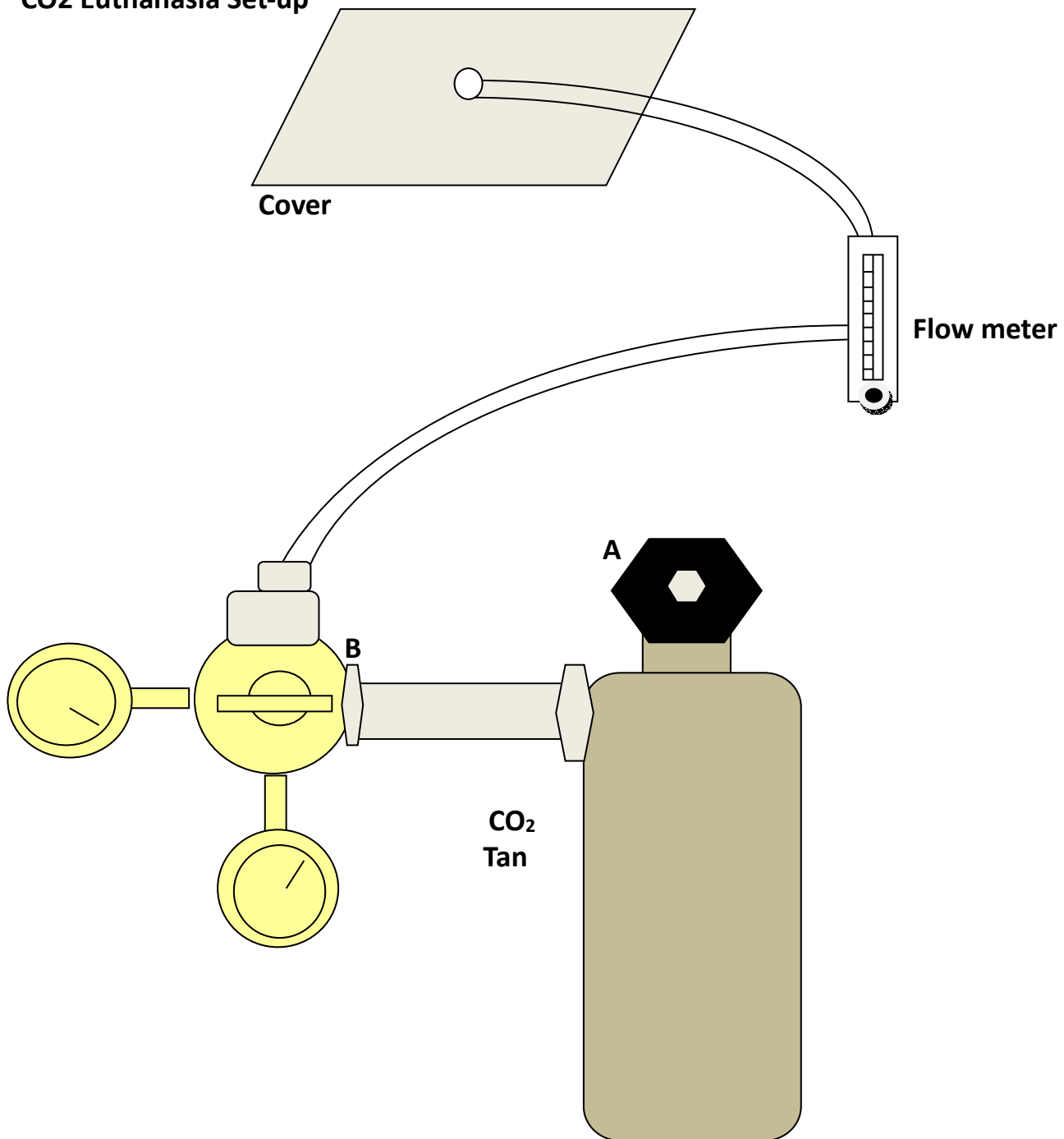
1. Secure a euthanasia chamber, ideally using the home cage. Increasing animal density and mixing of different animals from separate cages in the euthanasia chamber should be minimized to decrease pre-euthanasia anxiety.
2. The volume of the euthanasia chamber (in liters) should be calculated and then divided by 2 (3.33-1.43) in order to determine the appropriate CO₂ flow rate of 50% (30-70%) . If using one of the LAR standard cages the volumes are as follows:
 - #1 Mouse Thoren cage: 5.8L, flow rate of **3.0 (1.7-4) L/min**
 - #9 Mouse Thoren cage: 6.5L, flow rate of **3.5 (1.9-4.5) L/min**
 - OptiMice cages: 6.2L, flow rate of **3.0 (1.8-4.3) L/min**
 - #2 Rat cage: 10.4L, flow rate of **5.0 (3.1-7.3) L/min**
 - Techniplast (TP) Greenline Rat Cage: 19.8L, flow rate of **10 (5.9-13.8) L/min**
 - TP Mouse Greenline/Blueline cages: 7.6L, flow rate of **4.0 (2.3-5.3) L/min**
 - TP Mouse IsoCage/Orange Line cages: 7.1L, flow rate of **3.5 (2.1-5.0) L/min**
 - Ancare R20 deep rat cage: 26.2L, flow rate of **13.5 (7.8-18.3) L/min**
 - Allentown Purple mouse cage: **6.0L, flow rate of 3.0 (1.8-4.2)**

** If your current flowmeter does not measure high enough for the cage you have, you may either set the meter on the highest measured flow rate, or you may move the animals into a clean smaller cage to use a lower calculated flow rate.

3. Verify the flow meter is in the off position. Place the cover over the euthanasia chamber/cage. Turn the CO₂ canister valve (A, below diagram) to the on position. The pressure gauge closest to the tank should register pressure. Turn on regulator gauge (B, below diagram). After both valves have been turned on, the flow meter can be adjusted to the appropriate flow rate (liters/min) to gradually introduce 100% CO₂ to result in the replacement of 50% of the cage volume per minute. This flow rate has been shown to produce a loss of consciousness without apparent distress (see attached report, Appendix 1).
4. Following the induction of unconsciousness (this will take approximately 1-3 minutes), the CO₂ flow rate can be raised to 3-4 times the initial flow rate to accelerate the process. Following apparent clinical death of the animal, gas flow should be maintained for at least one minute.
5. Following death, the flow meter and two gauges should all be turned to the off position.
6. Death must be confirmed and a secondary method of confirmation can be performed to assure the animal will not regain consciousness. Biohazardous or otherwise hazardous carcasses should be discarded using procedures established for the study in question.

NOTE: Neonate rodents (“pinkies”) will become anesthetized with CO₂. However, due to their high levels of fetal hemoglobin, they are resistant to death via CO₂. Cervical dislocation or decapitation (following CO₂ narcotization) is the best method of euthanasia for these neonates.

CO₂ Euthanasia Set-up



Reference:

[AVMA Guidelines on Euthanasia](#) (2020)

[Guide for the Care and Use of Laboratory Animals](#) (2011)

Original and Revisions: 7/21/2009, 12/16/2014, 5/15/2018, 2/25/2020, 5/23/2023, 5/26/2026