



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:

DO	DON'T
<i>Use a clear chamber (home cage is best)</i>	Leave animals unattended
<i>Use a different euthanasia method for neonates</i>	Overcrowd
<i>Use a flow meter and pressure-reducing regulator</i>	Mix species or incompatible animals in the chamber
<i>Follow your approved animal use protocol</i>	Prefill the chamber with CO ₂

- 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.

$$CO_2 \text{ flow rate in L/min} = \frac{\text{height (cm)} \times \text{width (cm)} \times \text{length (cm)}}{1000} \times \frac{0.2}{\text{min}}$$

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 valve 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.