



A. PURPOSE

To provide investigators with guidance on use of CO₂ for euthanasia of rodents.

B. GUIDELINES

Compressed CO₂ gas in cylinders is the only approved source of CO₂ because the flow to the euthanasia chamber can be regulated. CO₂ generated by other methods such as from dry ice, fire extinguishers, or chemical means is unacceptable.

The euthanasia chamber must be clear or have a clear window or lid for observation of the animals. The lid must be able to be secured and the euthanasia chamber should vent at the top, so that the lighter air is displaced by the heavier CO₂. Animals should be euthanized in their original cage bottoms with microisolator lids removed when possible to reduce distress. This can be accomplished using a Euthanex lid or a DIY euthanasia chamber as described below.

Rats and mice must never be euthanized in the same chamber. The IACUC strongly discourages mixing rodents from different cages just prior to CO₂ euthanasia, as this alone can induce distress, especially the mixing of adults which may cause fighting. Euthanasia chambers must not be overcrowded. There must be sufficient room in the chamber for normal postural movements for all the animals.

To further reduce distress, euthanasia should be performed out of direct sight of other animals. In addition, euthanasia chambers must be kept clean of urine and feces and should be wiped between uses to remove odors such as pheromones that can cause distress to the next group of animals.

Do not prefill the euthanasia chamber, as high concentrations of CO₂ cause pain and distress. Instead, place the animal(s) into the chamber with room air, cover, and fill the chamber with CO₂ at a flow rate that displaces 30-70% of the chamber volume per minute (*fig. 1*) utilizing a pressure-reducing regulator and flow meter combination, or equivalent (*figs. 2 & 3*). The following flow rates are for use with a **CO₂-specific** flow meter only – if you are using any other type of meter, contact UAC veterinarians for guidance. If you have questions regarding the size of your chamber volume and the Liters per minute contact UAC veterinarians for guidance.

Figure 1

Chamber volume	Flow Rate	
	Liters per minute	Cubic feet per hour
6 quart (shoe box)	1.8-4.2	3.8-8.9
15 quart (4 gallon)	4.5-10.5	9.5-22.2

This flow rate achieves a balanced gas mixture resulting in rapid unconsciousness with minimal distress to the animals. The euthanasia chamber does not have to vent into a fume hood. Ensure the CO₂ cylinder is appropriately secured to the wall or in an approved cylinder stand.

Under these conditions, the CO₂ renders the animal unconscious in about 2-3 minutes. Continued exposure causes death, usually within 2 minutes after the animal loses consciousness. Immature animals need to be exposed for an extended period of time to ensure death. If the animal has a faded eye color and a lack of respiration (chest movement) and other spontaneous movements have ceased, the CO₂ should be left flowing into the chamber for at least 1 minute after apparent clinical death.



As CO₂ anesthesia is reversible, it is important to verify that an animal is dead. Death must be confirmed by someone specifically trained to recognize cessation of vital signs in rodents e.g., faded eye color, lack of respiration and heartbeat.

Note that unintended recovery of animals after apparent death from CO₂ constitutes serious non-compliance with PHS Policy which is reportable to the Office of Laboratory Animal Welfare (OLAW). Such occurrences must be reported to the IACUC.

A secondary method of death must be employed following CO₂ euthanasia. Methods such as exsanguination, bilateral thoracotomy, vital organ harvest, decapitation or cervical dislocation are acceptable. A less preferred alternative is to leave the animal in the euthanasia chamber with CO₂ flowing for an extended period of time after the clinical signs of death. This period should be at least 50% of the time that it took for death to occur. For example, if unconsciousness to death occurred in 2 minutes, then the animal must remain under CO₂ for an additional 1 minute and preferably longer, to ensure death has occurred.

Some animals are less susceptible to CO₂ as a method of euthanasia. Notably, fetal and neonatal rats and mice are particularly resistant to CO₂ narcosis. Please see IACUC Guidance 202 for acceptable methods to euthanize fetal and neonatal rats and mice.

C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION

DIY CO₂ euthanasia chamber

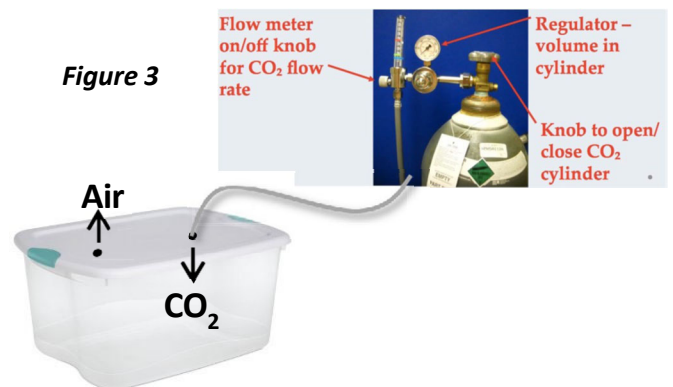
The chamber must be clear or have a clear window so that the animal can be easily observed. There must be a lid that latches in some way. The lid does not have to be completely airtight, but it must fasten so that it cannot be easily dislodged.

A simple chamber can be constructed from a ~6-15 quart plastic storage box with a snap lid. A 6 quart box is approximately the same size as a shoe box and is suitable for mice. The 15 quart box should be used for rats. These storage boxes are commonly available at Walmart, Home Depot, etc.

Figure 2: Flow meter



Figure 3



Two ¼-½” holes are made in the lid. The first hole is for introduction of CO₂. The second hole allows the ambient air to be displaced as the CO₂ flows into the chamber, see figure #3. If the hole is made small enough, the CO₂ hose will stay in place. Alternatively, a plastic or metal hose barb can be wedged into the hole, and it can be secured with glue or caulk, if desired.



References:

- AVMA Guidelines for the Euthanasia of Animals, 2020 Edition:
<https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>
- Euthanex: <https://www.ezsystemsinc.com/product-category/cage-level-systems/>
- CO2 Flow Meter: <https://www.dakotainstruments.com/dakota/dakota-rotameters/acrylic-rotameters/carbon-dioxide-flow-meters/carbon-dioxide-flow-meters-acrylic-brass-fittings-valve-included>
- [I-IC-GU-202 Euthanasia of Mouse and Rat Fetuses and Neonates](#) – Link to Guidance & SOPs page