

**Excerpt from the University Standard for Rat and Mouse Euthanasia (Formerly IACUC Rodent Euthanasia Policy):**

**How to use the Carbon Dioxide (CO<sub>2</sub>) Euthanasia Chamber (non-automated systems):**

The 2020 AVMA Guidelines for the Euthanasia of Animals recommends that the gradual displacement rate of CO<sub>2</sub> into the euthanasia chamber should be 30-70% to minimize pain and distress.

1. Remove each animal from the housing chamber and place into the euthanasia chamber. Never place the housing chamber into the euthanasia chamber. Never pre-charge the chamber. Do not place different animal species in the chamber at the same time. Do not overcrowd the chamber. Each animal should have enough floor space available to lie down.
2. Place the stainless steel lid over the euthanasia chamber. The lid should be connected to a CO<sub>2</sub> tank via a plastic hose.
  - a) Make sure the two holes on the top of the lid are not blocked, as these holes allow air to be pushed out by the heavier CO<sub>2</sub>.
  - b) Make sure the euthanasia chamber does not have an automatic watering opening.
3. Turn on the valve located on top of the CO<sub>2</sub> tank. Next, set the flow of CO<sub>2</sub> entering the chamber by adjusting the regulator valve on the left side of the flow meter:
  - a) **Standard DCM Shoebox style RAT cage: 12.0 liters per minute (lpm)**
  - b) **Standard DCM Shoebox style MOUSE cage: 2.8 liters per minute (lpm)**
  - c) Other CO<sub>2</sub> Chambers: Use the following formula to calculate the appropriate flow rate:  
$$\frac{\text{Height} \times \text{width} \times \text{length}}{61} = \text{liters} \times .45 = \text{flow rate/minute (units = inches)}$$
4. Continue to allow CO<sub>2</sub> to flow into the chamber for one minute after breathing stops (approximately 6 minutes for mice and 8 minutes for rats). Young animals, certain strains of mice, and sick animals may require more time to become deeply anesthetized.
5. Once animals are fully anesthetized, **immediately perform a physical method of euthanasia** (i.e. cervical dislocation, thoracotomy, major organ harvest, or decapitation) to confirm death. Refer to the full Standard for more details on acceptable physical methods.
6. Note: If a terminal procedure (i.e. cardiac puncture, tissue collection) must be performed before the secondary physical method, ensure that animals remain deeply anesthetized and that a physical method of euthanasia is performed following the terminal procedure.
7. Place dead animals into a non-PVC containing bag. DCM provides these bags in a variety of sizes. Label the bag with the IACUC protocol #. Seal the bag securely. Place the bag with dead animal(s) into the DCM carcass freezer available in each animal facility. Please see the University Standard on Disposal of Rodent Carcasses for more information.  
<https://unc.policystat.com/policy/6503203/latest/>
8. Disinfect the euthanasia chamber bottom after each use.

This posting is an excerpt from the University Standard on Rat and Mouse Euthanasia – to be posted at ALL CO<sub>2</sub> Euthanasia stations (both DCM and laboratory maintained).

For further information, please reference the Standard in its entirety at: <https://unc.policystat.com/policy/6820937/latest/>

2020 AVMA Guidelines for the Euthanasia of Animals: <https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>