

## Use of Flowmeters for Mouse and Rat CO2 Euthanasia for SOM Researchers

**Background:** Flow meters allow gradual fill CO2 delivery for euthanasia of adult mice and rats. Flow rates should be between 30 and 70 percent of container volume per minute per the AVMA 2020 Euthanasia Guidelines. Ex: A 10 liter container would need between 3 and 7 liter per minute.

Ideally, rodents should be euthanized in home cages, although it is allowable to use a clean, dedicated chamber. If a dedicated chamber is used, it must allow for visualization of the subjects.

Rodents from different cages must not be mixed.

As the euthanasia of neonatal rodents may be even more time-consuming and complicated, please consult with a DAR veterinarian or training coordinator to develop the procedures best for your circumstance.

**CO2 Euthanasia Training (in person) is required for all staff performing CO2 Euthanasia at SOM-DAR. Contact DAR Training (dartrn@emory.edu) to schedule within 30 days of protocol approval. Please list approved personnel and the date the training was completed with EU DAR.**

Researcher Name	EU DAR CO2 euthanasia training date

**Please use the templates below as instructions for CO2 Euthanasia in your lab space**

**CO<sub>2</sub> Euthanasia of Adult Mice in a Dedicated Chamber** by the [insert PI name here] Lab

1. Place the mice or mice in the chamber. Do not combine mice from multiple cages in the same chamber.
2. Open the valve on the regulator, ensure CO<sub>2</sub> is flowing.
3. Adjust the CO<sub>2</sub> flow rate to \_\_\_\_\_. *[Note: this is where you will need to know your chamber volume and provide specific instruction to ensure that the gas displacement is between 30% -70% of the chamber volume. For example, for a 1 liter chamber, the rate in the SOP should be stipulated to be 300-700 ml/minute]. [See below for flow rate calculation examples].*
4. Expose mice to flowing CO<sub>2</sub> for at least 5 minutes.
5. Expose rats to flowing CO<sub>2</sub> for at least 10 minutes.
  - a. Death must be ensured either by: Using cervical dislocation or other physical method (approved in your protocol) once the animal is recumbent, not breathing and non-responsive to toe pinch.
6. After each use, invert the container so as to evacuate any residual CO<sub>2</sub>.
7. Clean the chamber after each use.
8. This SOP may not be used for neonatal rodents.

~Contact the DAR Vet Staff (7-3248) with any questions or DAR Training (dartrn@emory.edu)~

Date Implemented: [Insert the date implemented by the lab, group or site here]

**Instructions for CO<sub>2</sub> Flow rate calculations:**

1. Measure the internal dimensions (length, width, and height) of a rectangular euthanasia container in inches.
  - a. Length x width x height = total cubic inches.
  - b. 1 Liter = 61.02 cubic inches
  - c. Divide total cubic inches by 61.02 = liters
2. Flow rate needs to be 30-70% so multiple Liters x 0.3 OR 0.7 to get min and max flow rates.
3. For non-rectangular euthanasia containers, the volume of the container will need to be calculated.
  - a. You can use this website to calculate total cubic inches.
  - b. <https://www.calculator.net/volume-calculator.html> Follow steps 1b – 1d (above) to calculate min and max flow rates.

**Flow rates for standard euthanasia containers:**

Container	Volume	30% Flow Rate (min)	70% Flow Rate (max)
Standard DAR mouse cage	6 L	2 L/min	4 L/min
Standard DAR rat cage (rectangle)	20 L	6 L/min	14 L/min
Thoren DAR rat cage (square)	15 L	5 L/min	11 L/min