

## Carbon Dioxide Euthanasia Procedures for Rodents

<b>SCOPE</b>	Applies to routine rodent euthanasia by carbon dioxide (CO <sup>2</sup> ) narcosis performed by Howard University (HU) personnel authorized to work with animals
<b>PURPOSE</b>	Describes appropriate techniques and equipment approved to be used by qualified staff in order to ensure a painless death for rodents used in research at HU
<b>EQUIPMENT</b>	CO <sup>2</sup> gas cylinder, CO <sup>2</sup> delivery bottle, scissors, scalpel, plastic bag
<b>REFERENCES</b>	American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: Edition 2013 (AVMA Euthanasia Guidelines)

### I. Responsibility

- A. The Veterinary Services Operations Manager (VSOM) will: Provide training on the use of equipment used to perform CO<sup>2</sup> euthanasia as described in this SOP.
- B. Oversee VS and research personnel to ensure that euthanasia procedures are performed and equipment is used in accordance with this approved SOPs and other applicable policies and regulations.
- C. Research Staff Performing CO<sup>2</sup> euthanasia using comparable equipment in HU laboratories outside of the VS facility will comply with the AVMA Euthanasia Guidelines.

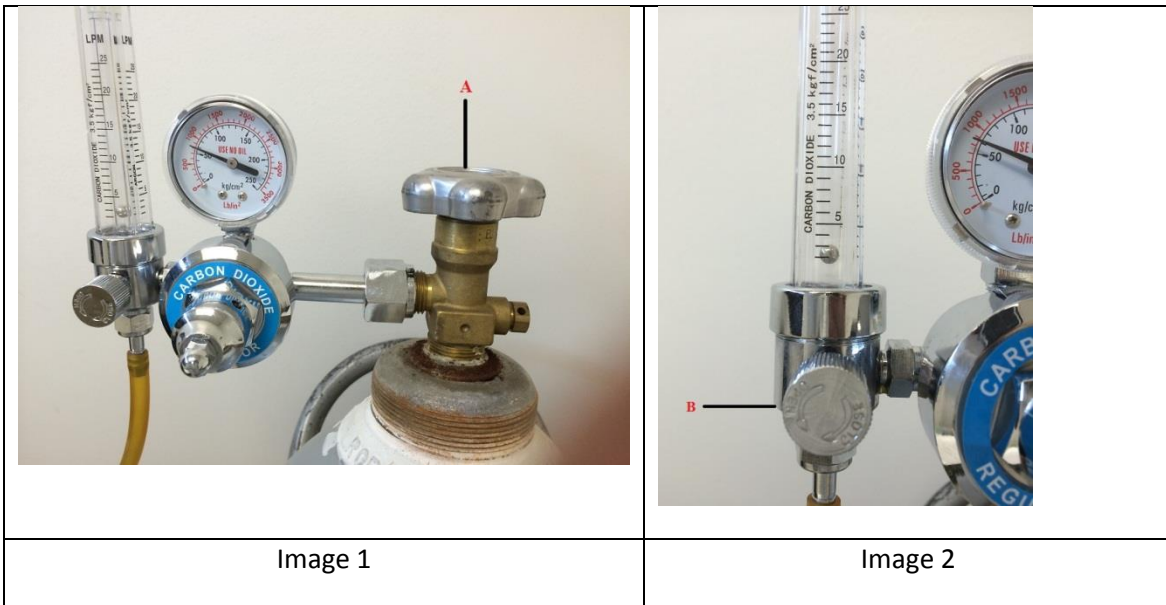
### II. Safety

<b>PERSONAL PROTECTIVE EQUIPMENT</b>	<ul style="list-style-type: none"> <li>• Don disposable gloves, mask, bouffant cap, and gown or lab coat before performing this procedure.</li> </ul>
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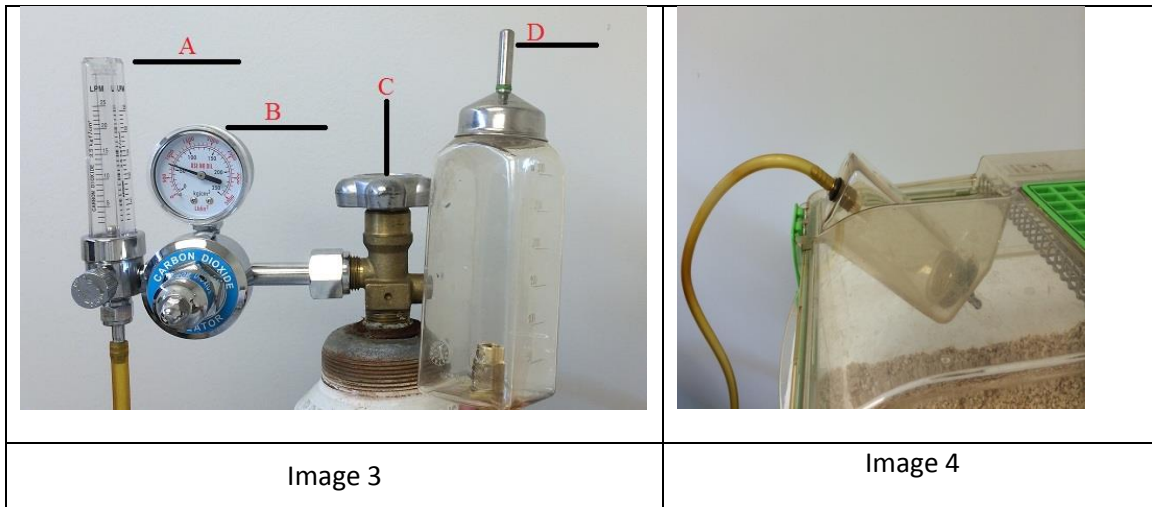
### III. Procedure:

- A. **Carbon Dioxide Narcosis Procedures**

- \* Neonatal rodents may take up to 1 hour of CO<sup>2</sup> exposure to ensure death. Therefore, this method is not recommended for neonates up to 10 days of age.
  - \* It is better to fill the chamber from the top than from the bottom in order to avoid creating a layering effect; with animals trying to avoid CO<sup>2</sup> accumulating at the cage bottom.
  - \* Do not pre-load cages/chambers with CO<sub>2</sub> prior to placing animals in cage/chamber
  - \* Do not overcrowd cage/chamber; animals should not occupy more than 75% of floor space and be able to assume normal positions and be able to turn around in the cage.
  - \* Do not leave animals unattended during this procedure
1. Rodents will be euthanized in a room isolated from all other animals. If all rodents from a single cage are not to be euthanized, leave only the rodents to be euthanized in the home cage and transfer all remaining rodents to a new cage. **DO NOT** place any cages containing animals on the floor.



2. Take the “home cage” containing mice to Room 5107, take the “home cage” containing rats to Room 5103.
3. Open valve A on the CO<sup>2</sup> cylinder (Image 1).
4. Close valve B on flow meter (Image 2).
5. Remove the water bottle from the home cage containing animal(s) to be euthanized.



6. Place CO<sup>2</sup> delivery bottle D into water bottle slot on the home cage lid (Images 3 and 4).
7. Start delivery of CO<sup>2</sup> by turning knob B on the CO<sup>2</sup> flow meter (Image 2).
8. Adjust flow of CO<sup>2</sup> to displace 10% to 30% of chamber (home cage) volume per minute (Image 2).
  - a) The GR900 rat cage CO<sup>2</sup> flow rate should be approximately 7 liters per minute to displace 20% of the chamber volume per minute.
  - b) The GM500 mouse cage CO<sup>2</sup> flow rate should be approximately 3 liters per minute to displace 20% of chamber volume per minute.
  - c) Use the following formula to compute the CO<sup>2</sup> flow rate for chambers (home cages) of different sizes. In some instances home cages may have to be placed into a larger chamber to create a closed system; and the larger chamber dimensions will be used to compute the CO<sup>2</sup> flow rate (from Veterans Affairs IACUC Training Exercise #2-2014).:
    - (1) Determine volume of euthanasia chamber: Length x width x height of euthanasia chamber in inches = chamber volume in cubic inches;
    - (2) 1 liter = 61.02 cubic inches, so divide the total cubic inches by 61.02 cubic inches/liter to get the volume of the chamber in liters.
  - d) Calculate flow rate needed for 10-30% displacement of chamber volume per minute. Multiply chamber volume in liters by displacement rate (0.1 to 0.3) to get the needed regulator flow rate (See practice problem at the end of this SOP).
  - e) Maintain CO<sup>2</sup> flow until **1 minute after** all signs of movement and breathing have ceased.
  - f) Turn off CO<sup>2</sup> flow at flow meter (Image 2).
  - g) Remove animal(s) from cage and perform a secondary method of euthanasia

- h) Repeat steps III.A.3 to III.A.10 for any additional rodents to be euthanized
  - i) Place euthanized animals in bag and place in freezer, located in corridor 5100, for disposal
  - j) Record number of animal(s) and date euthanized on appropriate cage card(s)
  - k) Close valve on CO<sup>2</sup> cylinder
9. Place empty soiled cage(s) on dirty cage cart located next to freezer in corridor 5100

Practice problem: An IACUC approves a 30% displacement rate for rodent carbon dioxide euthanasia. The euthanasia chamber is in the shape of a cube, with each side measuring 20 inches. The flow regulator on the carbon dioxide tank is in liters per minute. To what value should the flow meter be set for this chamber to provide 30% displacement per minute by carbon dioxide gas?

Practice problem answer: Volume of chamber= 20.0 in x 20.0 in x 20.0 in= 8,000 cubic inches; 8,000 cubic inches/ 61.02 cubic inches/liter= 131 liters

131 liters x 0.3 displacement/minute= 39.3 liters/minute

Answer- the flow regulator should be set to about 39 liters per minute.

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Revision approved