In compliance with PHS Policy on adoption of 2013 AVMA Guidelines on Euthanasia, the following procedure must be followed when sacrificing small groups of rodents.

- Mice and rats are euthanized in the euthanasia chamber in Procedure Room 2 or BL2 room.
- A regulated flow valve restricts the flow of the gas to this chamber to 20% of chamber volume per minute. It is installed in the supply side and preset based on the volume of the euthanasia chamber. Opening the main valve will supply carbon dioxide to the correct level and no additional steps are needed. It will take a few minutes for the CO2 to reach an anesthetic concentration and death may take several minutes longer.
- **Prefilling of the euthanasia chamber with carbon dioxide is not allowed.**
- When possible mice should be left in their home cage, and the entire cage, without lid, placed into the euthanasia chamber. Alternatively, if mice are to be euthanized one by one, place a clean cage in the euthanasia chamber and place mice inside the cage. This saves having to clean the chamber every time.
- Rats should be euthanized one by one in a clean mouse cage without lid.
- A maximum of five to eight mice per cage may be euthanized at one time. No overcrowded cages allowed.
- Procedure:
  1. Place the mice (in their home cage if possible) into the chamber.
  2. Open the main valve and allow gas to flow (do not prefill the chamber). Observe the animals for complete cessation of breathing.
  3. **Gas flow MUST continue for at least one minute post apparent death.**
  4. The staff must be physically present during the entire time of euthanasia.
  5. Neonates (up to 10 days of age) and fetal mice >15 days pc are resistant to carbon dioxide asphyxiation and must be humanely euthanized after deep narcosis, either by decapitation or opening of the chest.
  6. Euthanized animals are taken to the freezer by the staff for collection and disposal.