CO_2 Euthanasia BYU Standard Operating Procedure

Updated 04/01/2020

General:

- All CO_2 euthanasia systems/set-ups must be evaluated by ORCA's veterinarian, the vivarium manager, or Risk Management and Safety prior to use to ensure humane euthanasia.
- The following statement is recommended by IACUC for inclusion in an Animal Use Application for any species where CO_2 euthanasia is to be used:

  "Animals will be euthanized by a displacement of chamber air with compressed CO_2 at a rate of 30%-70%. Following [unconsciousness/death] the animals will be subject to [cervical dislocation/decapitation/thoracotomy] as a secondary means to ensure death."

- Please refer to the *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* (page 61) and *Guide for the Care and Use of Laboratory Animals, 8th Edition* (123-124) for more information.

Before Euthanizing:

1. Cylinders of compressed CO_2 gas are the only recommended CO_2 source. Cylinders should be secured to the wall using a strap at the top and bottom.
2. The cage in which the animal will be euthanized must allow visibility. Typically, animals will be euthanized in their home cage.
3. Place the euthanasia cage under a fume hood while CO_2 is in use. If a fume hood is not available, place the cage near the laboratory exhaust vent to better dissipate residual gas.
4. Do not overcrowd the cage. All animals in the cage should be able to make normal postural adjustments. Follow recommendations for cage density as listed in *The Guide*. Cage capacity guidelines are on the IACUC website.
5. Do not leave the room or become distracted while euthanizing animals. You must be present and observe the euthanasia process.

Euthanasia Procedure:

1. Find the hose attached to the gas tank. Attach the end of the hose to the air intake ports on the back of the cage.
2. Low gas flow is essential for humane euthanasia. A two stage CO_2 regulator is required to control the rate of gas. **Gently** turn the knob on the flow regulator (a clear cylinder with a metal ball inside) until the ball hovers at 4 liters/minute for Mice and 9 liters/minute for Rats in their home cage. (If this does not work, use the knob attached to the gas tank to make sure that gas is flowing). Watch animals continuously; if they are jumping or distressed, adjust the flow of gas. **Do not attempt to hasten euthanasia by increasing the gas flow to the cage. This is inhumane and unnecessary.**
3. For weanling and adult animals, 60 seconds should be adequate time to fill the cage with CO_2. Carefully use the flow regulator to turn off the flow of gas. **Do no leave cages unattended.**
4. Leave the hose attached for at least 90 seconds after turning off the gas to complete euthanasia. Do not remove the cage lid. Note: neonatal animals do not respond well to CO_2 euthanasia. See SOP 'Isoflurane Euthanasia' for information on euthanizing neonates.
5. Leave animals in cage until clinical death has been ensured. Unintended recovery must be prevented by the use of appropriate CO_2 concentrations and the use of a secondary means to ensure death. The secondary methods may include decapitation, cervical dislocation, thoracotomy, etc.
6. If an animal is not dead following CO_2 exposure, another approved method of euthanasia (e.g. decapitation) must be added while the animal is under CO_2 narcosis to assure death. Please refer to *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* for more information.
7. Place euthanized animals in labeled bags in the chest freezer.