Georgia State University
Institutional Animal Care and Use Committee (IACUC)

It is the responsibility of the Georgia State University (GSU) Institutional Animal Care and Use Committee (IACUC) to ensure judicious and humane use of animals used in its teaching and research programs that is consistent with federal requirements.*

Policy on Carbon Dioxide Euthanasia

Carbon dioxide inhalation is an effective method of euthanasia in appropriate species. Bottled, compressed carbon dioxide is the only recommended source of the gas because the rate of inflow into the euthanasia chamber can be regulated precisely. Important points:

- Do not perform euthanasia or any other procedure on an animal until a person experienced with the procedure has trained you and you feel confident performing the technique.

- Use of carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g. antacids) is unacceptable.

- Carbon dioxide is not recommended for euthanasia on larger animals such as rabbits, dogs, cats, and swine because it appears to induce greater distress in these species.

- Only one species at a time should be placed into a chamber, and the chamber must not be overcrowded. When placed into the chamber, all animals must have floor space. Euthanasia should always be done in cohorts (live animals should not be placed in the chamber with dead animals). Chambers should be kept clean to minimize odors that might distress animals prior to euthanasia. Animals must not be euthanized in animal housing rooms, except under special circumstances such as during quarantine for infectious disease agents.

- Because inspiration of high concentrations of CO₂ is both aversive and painful, in lieu of pre-charging the chamber, the animals should first be placed into the chamber, followed by the addition of CO₂ at a low flow rate (e.g. a rate sufficient to displace approximately 30% of the chamber volume per minute) to complete the process.

- As it is easy to mistake a deeply anesthetized animal for a dead animal, additional methods should be employed to ensure that death has occurred (please refer to the physical method specified in your protocol). Accordingly, gas flow should be maintained for at least 1 minute after apparent clinical death (e.g. at least one minute after the animal has quit breathing). Upon removal of the animal from the chamber, unintended recovery must be obviated by the use of a physical method of euthanasia (e.g. thoracotomy, cervical dislocation, decapitation, or aortic transection).

As neonatal rodents are resistant to high CO₂ levels, rodents under 11 days old should not be euthanized by carbon dioxide inhalation. Instead, rodent pups should be swiftly decapitated with a sharp pair of scissors.
SOP on Carbon Dioxide Euthanasia

**AMOUNT IN TANK**

**TURN ON FIRST**
(COUNTER-CLOCKWISE)

**TURN OFF LAST**
(CLOCKWISE)

**TURN TO SET AIRFLOW**

**Cage Type** | **Airflow setting**
---|---
OptiMICE ACS | 2.0 LPM
OptiRAT ACS | 6.0 LPM
Conventional Rat/Hamster | 5.0 LPM
Small Conventional Hamster | 1.5 LPM

**DO NOT TOUCH**
**SOP for CO₂ Euthanasia of Rodents**

1. Secure a euthanasia chamber, ideally using the home cage. Animal density and mixing of different animals from separate cages in the euthanasia chamber should be minimized to decrease pre-euthanasia anxiety.

2. The volume of the euthanasia chamber (in liters) should be calculated and then divided by 5 in order to determine the appropriate CO₂ flow rate (30%).

   - If using one of the DAR standard cages the volumes are as follows:
     - OptiMICE ACS cage: 6.1 L volume, flow rate of **2.0 L/min**
     - OptiRAT ACS cage: 21.0 L volume, flow rate of **6.0 L/min**
     - Conventional Rat/Hamster cage: 17.3 L volume, flow rate of **5.0 L/min**
     - Conventional Siberian shoebox cage: 5.2 L volume, flow rate of **1.5 L/min**
     - Allentown BCU cage: 5.5 L volume, flow rate of **1.6 L/min**

3. The cover should be placed over the euthanasia chamber/cage, and the flow meter should be checked to ensure it is in the off position. The CO₂ canister valve is then turned on (A) and the pressure gauge closest to the tank should register a pressure. The second gauge (B) should then be turned on. After both valves have been turned on, the flow meter can be adjusted to the appropriate flow rate (liters/min) in order gradually introduce 100% CO₂ to result in the replacement of 20% of the cage volume per minute. This flow rate has been shown to produce a loss of consciousness without apparent distress.

4. Following the induction of unconsciousness (this will take approximately 3-4 minutes), the CO₂ flow rate can be raised to 3-4 times the initial flow rate to accelerate the process. Following apparent clinical death of the animal, gas flow should be maintained for at least one minute.

5. Following death the flow meter and two gauges should all be turned to the off position.

6. Cervical dislocation, decapitation, or bilateral thoracotomy should be performed to assure the animal will not regain consciousness. Euthanized animals should be placed in a carcass bag, the bag labeled with a carcass sticker, and placed in the carcass freezer unless there is some other need for the animals or their tissues.

**Pertinent Regulations**

- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- Public Health Service Policy
- Guide for the Care and Use of Laboratory Animals
- Animal Welfare Act (AWA) and AWA Regulations

**IACUC Approval Date:** 6/4/2015

**Signature IACUC Chair:**

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