

Research Animal Standard Operating Procedures (SOP) must meet the following criteria:

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

Name of Procedure	Euthanasia using Carbon Dioxide Gas	
Species	Mouse, Rat, Guinea Pig, Small birds	
ACEC	Reference	SOP#74 – Jun23 - Euthanasia using Carbon Dioxide Gas- Rodents and small birds
	Author	Jenny Smart
	Version	1.3
	Date approved	23 June 2023
	Date for review	23 June 2026
	Procedure classification	2
Ethical considerations	<ol style="list-style-type: none"> 1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent. 	

Details

1. Purpose

To describe the correct method of euthanasia of small rodents and birds using inhalation of carbon dioxide gas.

2. Description of procedure

EQUIPMENT

- Carbon dioxide must be supplied from either a cylinder of medical grade carbon dioxide gas or plumbed building carbon dioxide gas distribution systems equipped with an appropriate pressure reducing regulator and flow meter combination.
- Regulator and flow meter for gas cylinder
- Plastic or silicone tubing attached to carbon dioxide flow meter, or sufficient length to reach the bottom of euthanasia chamber
- Euthanasia chamber. Ideally this should be of sufficient size to fit the animal's home cage into the chamber.

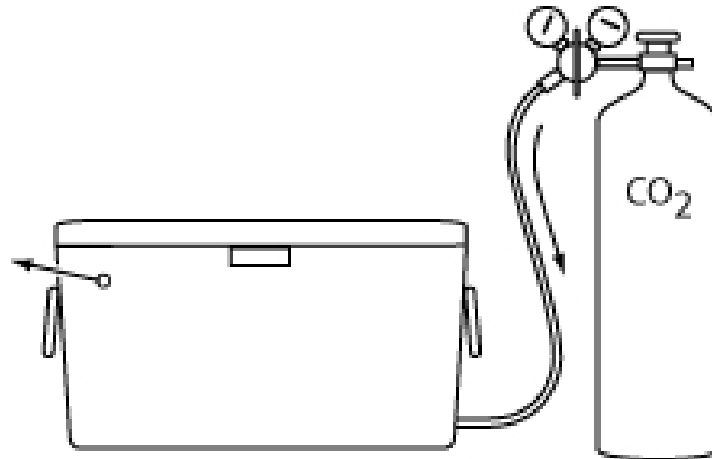
PROCEDURE

- To reduce stress, rodents should be euthanased in their home cage wherever possible.
- For open top cages, place animal cage in clean euthanasia chamber. Ideally the whole cage should be placed into the chamber to reduce stress associated with a change in environment. If the cage is too large for the chamber, the animal can be placed directly into the chamber, in the company of others from the same cage.
- Small birds should be confined in a calico bag or similar before placing in the chamber.
- Place the tubing from the carbon dioxide cylinder or wall outlet into the euthanasia chamber. Place the tubing into the animals' cage if the cage is placed into the chamber. For animals in an IVC cage, carbon dioxide can be delivered via the air supply port of the IVC.
- Turn on the carbon dioxide gas and start filling the chamber at a rate that displaces 30-70% of the chamber volume per minute.
- Maintain gas flow for at least 1 minute after apparent death of the animal (cessation of breathing, pale eyes in albino animals).
- Remove the animal from the chamber and confirm death via lack of a heartbeat. A secondary method of euthanasia may be employed such as cervical dislocation, decapitation or thoracotomy.
- Empty the carbon dioxide from the euthanasia chamber and clean out the chamber between animals to remove faeces, urine and pheromones.

NOTES

- Carbon dioxide is heavier than air so the euthanasia chamber will fill from the bottom and carbon dioxide will remain in the chamber after removal of the animal. The chamber must be emptied of carbon dioxide before placing the next animal into the chamber by inverting the chamber.
- Carbon dioxide has rapid depressant, analgesic and anaesthetic effects. Carbon dioxide has been shown to have no effect on corticosterone concentrations in rats and to not distort murine cholinergic markers.
- Neonatal rats and mice (less than 10 days old) are resistant to the effects of carbon dioxide and alternate methods of euthanasia should be used.

Carbon Dioxide euthanasia chamber setup



References

2020 AVMA Guidelines for the Euthanasia of animals.

https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx?utm_source=prettyurl&utm_medium=web&utm_campaign=redirect&utm_term=issues-animal_welfare-euthanasia-pdf

ACEC Chair

