

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats Institutional author: UQ Biological Resources AEC Reviewed & Approved: September 2025 SOP Expiry: September 2028	Version #4.2 Page 1 of 4
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LAB_008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats (Expiry: Sept 2028)

NOTE

- When citing this document, note that *LAB_100 Euthanasia Methods in Rats and Mice* also forms part of this SOP.
- The use of (*) indicates this statement is dependent on the facility procedures
- The use of (**) indicates this statement is dependent on AEC Approvals

I. OBJECTIVE

To describe the standard, safe and humane use of carbon dioxide for euthanasia of rodents and used across UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR.

II. DEFINITIONS – Refer to LAB_100 Euthanasia Methods in Mice and Rats

III. COMMENTS / RECOMMENDATIONS– Refer to LAB_100 Euthanasia Methods in Mice and Rats

IV. SAFETY AND COMPLIANCE

- Refer to LAB_100 Euthanasia Methods in Mice and Rats
- CO₂ euthanasia stations without a regulator must not be used.

V. TRAINING CONSIDERATIONS – Refer to LAB_100 Euthanasia Methods in Mice and Rats

VI. EQUIPMENT

- PPE(*)
- Home cage enclosure (*)
- Paper towel
- CO₂ delivery chamber:
 - This may be the home cage sometimes using a modified lid (that permits CO₂ delivery)
 - A specific euthanasia chamber (of a defined volume)
- CO₂ supply/outlet unit fitted with timed automatic CO₂ delivery and flow regulator
- Cadaver bag

VII. PREPARATION – Refer to LAB_100 Euthanasia Methods in Mice and Rats

VIII. PROCEDURE

1. Place rodent cage onto the bench or move rodents to the euthanasia chamber.
2. Connect carbon dioxide (CO₂) delivery hose to either:
 - The home cages
 - Tecniplast cages may place the hose into the water bottle opening
 - Optimice cages may place the hose into the modified Optimice lid inlet
 - The euthanasia chamber

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
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3. Press the start button on the UQBR CO₂ timer, the light should illuminate.
4. Turn on CO₂ gas and introduce into the chamber/cage at rate of 30% of the euthanasia chamber / home cage's volume per minute
 - Reticulated CO₂ – turn nozzle on at wall to start flow
 - CO₂ cylinders – turn on cylinder valve

Assess the flow regulator to ensure gas is flowing at the appropriate pre-determined rate. As an example, when using a 7.7L container this means the flow rate of CO₂ entering the chamber should be set to 2.3L/min (i.e. 7.7L x 0.3/min = 2.3L/min).

Note only those strains or models that have an identified abnormal response to 30% flow rate may apply the range of 20-40% flow rate as advised by a UQBR Veterinarian.

Information relative to CO ₂ flow rates in rodents.		
Type of carbon dioxide delivery chamber used		CO ₂ flow rate to ensure 30% chamber volume displacement/ minute
Tecniplast Greenline Mouse IVC GM500		Volume 7.7L at 30% Displacement = 2.3L/minute
OptiMICE cage		Volume 6.83L at 30% Displacement = 2L/minute
OptiRAT cage		Volume 28.8L at 30% Displacement = 8.6L/minute
Tecniplast Greenline Rat IVC GR900		Volume 18.9L at 30% Displacement = 5.7L/minute
Tecniplast Greenline Rat IVC GR1800		Volume 47L at 30% Displacement = 14.1L/minute

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5. Monitor the animals while the chamber / home cage is filling for normal behavioural responses. These behavioural responses should occur within 5 minutes of CO₂ supply.

It is essential to ensure adequate training has been provided to assess these behaviours which will vary with different individuals and situations. If behaviour is shown that is not expected a more experienced person should be consulted immediately and no further euthanasias performed until there is confidence that the procedure is being performed correctly and humanely.

Table 1. Signs of CO ₂ asphyxia	
Expected behavioural responses (in succession)	Abnormal behavioural responses
Unremarkable behaviour	Erratic behaviour
Excitability (increased activity),	Jumping,
Elevated respiratory rate	Severe excitability
Ataxia (staggers)	Nasal or mouth discharge
ventral recumbence with elevated respiratory rate	Report when distress is observed.
unconsciousness (unresponsive to stimuli)	
Recumbence with reduced respiratory rate,	
erratic respiratory rate (agonal breathing)	
loss of spontaneous respiration	

Table 1. **Signs of CO₂ asphyxia**. The left column lists the progression of signs displayed by mice in response to CO₂. The right column describes signs that are considered abnormal.

6. Animals should be kept in the chamber for at least 5 minutes and until they appear to have died.
Timed automatic CO₂ delivery units are preset before automatically shutting of gas supply to the unit box as a safety control.
7. Leave the chamber closed for a further 1 to 2 minutes then open to confirm death (absence of eye reflexes, absence of spontaneous breathing, absence of a rhythmic heartbeat) and perform a secondary method of euthanasia if necessary, as per LAB_100 Euthanasia Methods in Mice and Rats.
If there is any doubt in confirmation of death, repeat the CO₂ euthanasia steps as described above or immediately implement an alternative humane method of killing. e.g. LAB_007 Euthanasia - Cervical Dislocation in Mice and Rats; LAB_011 Euthanasia - Lethal Injection in Mice and Rats.

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8. Once death is confirmed, ensure CO₂ has been turned off, place the cadaver(s) into the cadaver bags and dispose of them as appropriate (*)
 - *Turn off both the valve or nozzle to CO₂ and power to the timed automatic CO₂ delivery units*
 - *In case of pregnant females, the pups do not need to be separately euthanised as they remain in the uterus/amniotic sac and remain unconscious and do not respond to hypoxia.*
9. Discard the home cage for cleaning, as appropriate (*). If euthanasia was performed using a specific euthanasia chamber, ensure the chamber is cleaned and that any potential remaining waste gas has been removed prior to reuse.

Carbon dioxide is heavier than the ambient air and will pool in undisturbed, deep spaces. This must be considered when cleaning a specific euthanasia chamber to ensure all waste gas is removed.

Neonatal rodents

Neonate procedures are to be followed as per adult mice, however, there are few notable differences:

- Sealed bags may be used as an alternative option to the home cage or specific euthanasia chamber
- Neonates must be left undisturbed within the high concentration gas chamber for a prolonged period relative to adults as represented below.

Table 2. Minimum time of 100% carbon dioxide exposure to produce death in mice and rats

AGE	MICE	RATS
Non-haired pups 0–6 days	60 minutes	40 minutes
Haired pups, eyes closed 7–14 days	20 minutes	20 minutes
Haired pups, eyes open, pre-weaning 15–20 days	10 minutes	10 minutes
Weanlings and adults 21+ days	5 minutes	5 minutes

IX. BIBLIOGRAPHY - Refer to LAB_100 Euthanasia Methods in Mice and Rats

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Outcome
4.1	Laboratory Biomedicine AEC	July 2025	Approved with comment
4.1	Molecular Biosciences AEC	July 2025	Deferred Approval- minor clarifications required
4.1	Health Sciences and Anatomical Biosciences AECs	July 2025	Approved
4.2	Molecular Biosciences and Laboratory Medicine AECs	September 2025	Approved

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