



# Guidelines on the use of carbon dioxide as a method of euthanasia for laboratory mice and rats

## Background

### **Australian code for the care and use of animals for scientific purposes 8<sup>th</sup> edition (2013)<sup>1</sup>**

Investigators and animal carers must use humane procedures for killing an animal that are appropriate to the species and circumstances (Clauses 2.4.22 and 2.5.8).

The method and procedures used for killing an animal must be humane and:

- (i) avoid pain or distress and produce rapid loss of consciousness until death occurs
- (ii) be compatible with the purpose and aims of the project or activity
- (iii) be appropriate to the species, age, developmental stage and health of the animal
- (iv) require minimum restraint of the animal
- (v) be reliable, reproducible and irreversible
- (vi) ensure that animals are killed in a quiet, clean environment away from other animals
- (vii) ensure that death is established before disposal of the carcass, fetuses, embryos and fertilised eggs (Clause 3.3.45).

Dependent offspring of animals to be killed must be cared for or humanely killed (Clause 3.3.46).

## Recommendation

Carbon dioxide (CO<sub>2</sub>) can be used as a primary method of euthanasia in small laboratory animals particularly in rodents<sup>2-7</sup>.

Potential impacts of CO<sub>2</sub> euthanasia on the wellbeing of animals include distress due to pain (formation of carbonic acid on respiratory and ocular membranes), air hunger or breathlessness, or direct stimulation of ion channels within the amygdala associated with the fear response<sup>3</sup>. The physiological effects of CO<sub>2</sub> should also be considered as this may have an impact on experimental outcomes, (e.g. increased serum potassium level in mice<sup>8</sup>, pulmonary and nasal haemorrhage<sup>9</sup>). As there is still no consensus on whether the use of gaseous anaesthesia prior to CO<sub>2</sub> euthanasia is more humane and has less impact on animal wellbeing<sup>10-23</sup>, the Committee recommend CO<sub>2</sub> exposure using a gradual-fill method as a primary form of euthanasia for adult and neonate mice and rats (more than 10 days of age).

Alternative methods of euthanasia are recommended for neonate animals (up to 10 days old) as they are resistant to the effects of CO<sub>2</sub> and inhalation anaesthetics<sup>24-26</sup>. However, narcosis in neonatal animals can be induced using CO<sub>2</sub> or inhalation anaesthetics, prior to a physical form of euthanasia (e.g. decapitation).

The Committee recommend that the following steps should be taken to minimise or prevent any pain and distress arising from this procedure.

1. All personnel performing CO<sub>2</sub> euthanasia must be appropriately trained and deemed competent.
2. The only recommended source of CO<sub>2</sub> for euthanasia purposes is commercially supplied compressed CO<sub>2</sub> in cylinders or tanks fitted with a gas flow meter to allow controlled gas inflow into the induction chamber.
3. Animal(s) should be placed in chambers that contain room air and gradually introduce 100% CO<sub>2</sub> at a displacement rate of 30% to 70% of the chamber volume/minute<sup>3, 27, 28</sup> (for example, a flow rate of 3 to 7 litres per minute in a 10-liter volume chamber). Flow rates can be increased once the animals have lost consciousness. CO<sub>2</sub> flow should be maintained for at least 1 minute after respiratory arrest<sup>5</sup>.
4. Where applicable, rodents should be euthanased in their home cages to prevent stress. This can be achieved by using custom built or commercially available lids or CO<sub>2</sub> delivery systems to achieve the desired CO<sub>2</sub> displacement rate and final concentration (e.g. Euthanex<sup>®</sup>, Quietek<sup>™</sup>).
5. Cages or alternative chambers used for CO<sub>2</sub> euthanasia should allow for visualisation of the animal(s) to confirm loss of consciousness.
6. Batch euthanasia may be performed; however, mixing or combining of incompatible or unfamiliar animals should be avoided. Chambers should not be crowded and should allow for each animal to stand with all four feet, have sufficient space to turn around and allow display of normal postural movements.
7. Chambers should be cleaned after each batch of animals to remove residual CO<sub>2</sub><sup>28</sup> and minimise odours from faeces, urine, pheromones that might distress animals that will subsequently be euthanased.
8. Chambers should not be pre-filled with CO<sub>2</sub> as inhalation of CO<sub>2</sub> at high concentrations has been shown to be aversive and painful to rodents<sup>3,11,14,18,19</sup>.
9. Death of the animal(s) must be confirmed prior to disposal. The following are signs to indicate death<sup>5</sup>
  - glazing or loss of eye colour
  - absence of cardio-respiratory activity
  - loss of colour in mucous membrane
  - loss of corneal and palpebral reflex.
10. Unintentional recovery of animal(s) must be avoided by using the appropriate CO<sub>2</sub> concentration and exposure times, and by performing a secondary form of euthanasia, e.g. cervical dislocation, exsanguination.
11. Any method of humane killing or euthanasia should be performed away from other animals.

## References

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