Background

Australian code for the care and use of animals for scientific purposes
8th edition (2013)\(^1\)

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\(_2\)) can be used as a primary method of euthanasia in small laboratory animals particularly in rodents\(^6,7\). The researcher should take into consideration the potential impact of CO\(_2\) euthanasia on the wellbeing of animals, and should take steps to minimise or prevent any pain and distress arising from this procedure. The physiological effects of CO\(_2\) should also be considered as this may have an impact on experimental outcomes, e.g. increased serum potassium level in mice\(^8\), pulmonary and nasal haemorrhage\(^9\). As there is currently no general consensus if the use of gaseous anaesthesia prior to CO\(_2\) euthanasia is more humane and has less impact on animal wellbeing\(^10-23\), the Committee is recommending the use of CO\(_2\) as a primary euthanasia agent.

Alternative methods of euthanasia are recommended for neonate animals (up to 10 days old) as they are resistant to the effects of carbon dioxide and also inhalation anaesthetics\(^24,26\). However, narcosis in neonatal animals can be induced using CO\(_2\) or inhalation anaesthetics, prior to a physical form of euthanasia e.g. decapitation.

1. All personnel performing CO\(_2\) euthanasia must be appropriately trained and deemed competent.
2. The only recommended source of CO2 for euthanasia purposes is compressed CO2 in cylinders 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% CO2 at a fill rate of 30% of the chamber volume/minute (for example, use a flow rate of >3 litres per minute for a 10-liter volume chamber)\textsuperscript{3,27,28}. Flow rates can be increased once the animals have lost consciousness. Animals should be left inside the chamber with continuing flow of CO2 until death has been established.

4. Where applicable, rodents should be euthanased in their home cages to prevent stress. This can be achieved by using appropriate lids (custom-built or commercially-available e.g., Euthanex\textsuperscript{®}, Quietek\textsuperscript{™}) or commercially-available automatic CO2 delivery systems to achieve the desired CO2 concentration.

5. Cages or alternative chambers used for CO2 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 CO2\textsuperscript{28} and minimise odours from faeces, urine, pheromones that might distress animals that will subsequently be euthanased.

8. Chambers should not be pre-filled with CO2 as inhalation of high concentration of CO2 has been shown to be aversive and painful to rodents\textsuperscript{11,14,18,19}.

9. Death of the animal(s) must be confirmed prior to disposal. The following are signs to indicate death\textsuperscript{5}:
   - 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 CO2 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

1. Australian code for the care and use of animals for scientific purposes. 8th edition. Australian National Health and Medical Research Council. 2013


4. CCAC Guidelines on Euthanasia of Animals Used in Science; Canadian Council on Animal Care (CCAC): Ottawa, ON, Canada, 2010; pp. 1–32.

5. Guidelines to promote the wellbeing of animals used for scientific purposes the assessment and alleviation of pain and distress in research animals. 2008. Australian National Health and Medical Research Council.


