Humane Killing of Animals used for Scientific Purposes Guidelines

These guidelines should be read in conjunction with the Humane killing of animals used for scientific purposes policy and the Australian code for the care and use of animals for scientific purposes. The aim of these guidelines is to assist investigators to choose a method of humane killing of animals used for scientific purposes that will be acceptable to the Animal Ethics Committee, compliant with regulatory requirements and that will meet the expectations of the public. It is recognised that when animals are used for other purposes different, often lower, standards are accepted however animals used for scientific purposes command special treatment that is aligned with current veterinary practice and minimise any negative effects the method may have on the animals involved.

The methods described in these guidelines meet current acceptable practice as outlined in the veterinary, animal care and welfare and medical research literature. They outline acceptable methods for animals at all stages of life and for a range of species. Methods not listed in these guidelines will be considered by the AEC on a case-by-case basis if the committee is provided with adequate justification for the use of the method and references that support its use in the proposed circumstances.

Acceptable methods of euthanasia of adult animals

Laboratory animals

Animal Type	Recommended	Acceptable
Mice and rats	• Overdose of inhalational anaesthetic • Carbon dioxide inhalation • Pentobarbitone IP • Cervical dislocation (under 150g only) • Decapitation	• followed by a secondary method (cervical dislocation, decapitation, exsanguination) • Stunning and exsanguination • Focused beam microwave radiation (commercial machine and if justified only)
Guinea pigs	• Carbon dioxide inhalation • Pentobarbitone IP (IV not acceptable)	• Inhalation anaesthetics overdose • Stunning and exsanguination • Overdose of anaesthetic followed by a secondary method (cervical dislocation, decapitation, exsanguination)
Rabbits	• Pentobarbitone IP or IV	• Inhalation anaesthetics overdose • Stunning and exsanguination • Cervical dislocation or decapitation • Captive bolt (penetrative) • Overdose of anaesthetic followed by a secondary method (cervical dislocation, decapitation, exsanguination)

Ferrets and related species	• Pentobarbitone IV with local anaesthetic	• Overdose of anaesthetic followed by a secondary method, • Pentobarbitone IP
Dogs and cats	• Pentobarbitone IV	• Overdose of anaesthetic followed by a secondary method
Non-human primates	• Pentobarbitone IV	• Overdose of anaesthetic followed by a secondary method

IV – intravenous, IP - intraperitoneal

Livestock

Animal Type	Recommended	Acceptable
Sheep and goats	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination • Gunshot • Overdose of anaesthetic +/- a secondary method
Cattle	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination • Gunshot • Overdose of anaesthetic +/- a secondary method
Deer, antelope and related species	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination • Gunshot • Overdose of anaesthetic +/- a secondary method
Horse, donkeys and related species	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination • Gunshot • Overdose of anaesthetic +/- a secondary method
Camelids	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination or pithing • Gunshot • Overdose of anaesthetic +/- a

		secondary method
Pigs	• Pentobarbitone IV	• Captive bolt followed by exsanguination or pithing • Electrical stunning followed by exsanguination • Gunshot • Overdose of anaesthetic followed by exsanguination or pithing
Poultry	• Pentobarbitone IV or IH • Cervical dislocation • Decapitation • Carbon dioxide	• Electrical stunning and exsanguination • Overdose of anaesthetic followed by a secondary method

IV – intravenous, IH – Intrahepatic, IP - intraperitoneal

Wildlife

Animal Type	Recommended	Acceptable
Crustaceans and molluscs	• MS-222 • CO_2 bubbled in water • Clove oil (AQUI-S)	• Chilling followed by a secondary method
Cephalopods	• MS-222 • Clove oil (AQUI-S) • Magnesium salts immersion	• Pithing
Amphibians	• MS-222 (solution or IP) • Benzocaine (after neutralising solution) • Clove oil (AQUI-S) • Pentobarbitone IP	• Overdose of anaesthetic followed by pithing • Stunning followed by decapitation • Pithing • Hypothermia (warm water species) followed by pithing
Reptiles	• Pentobarbitone IP or MS-222 IP	• Overdose of anaesthetic followed by pithing • Stunning followed by decapitation or pithing • Pentobarbitone IP
Turtles and tortoises	• Captive bolt (large turtles) • MS-222 IP (small turtles and tortoises)	• Stunning followed by secondary method • Pentobarbitone IP
Fish	• MS-222 (solution or IP) • Benzocaine immersion (after neutralising solution) • Clove oil (AQUI-S) • Hypothermia (warm water species) followed by pithing • 2-Phenoxyethanol	• Stunning and brain destruction • Cervical dislocation • Stunning followed by pithing • Pentobarbitone IP

Birds – small (wren, finch, small parrots)	• Pentobarbitone IH or PO • Carbon dioxide inhalation	• Inhalation anaesthetics overdose • Cervical dislocation • Decapitation
Birds – medium (cockatoos, large parrots, magpie, ducks)	• Pentobarbitone IV or IH • Carbon dioxide inhalation	• Gunshot • Inhalation anaesthetics overdose • Cervical dislocation • Decapitation
Birds (Emu, ostrich, geese, pelican, stork)	• Pentobarbitone IV • Injectable anaesthesia overdose followed by secondary method	• Gunshot • Inhalation anaesthetics overdose
Bats	• Carbon dioxide inhalation • Pentobarbitone IP	• Cervical dislocation (under 150g only) • Decapitation • Stunning and exsanguination • Inhalational anaesthetics followed by a secondary method
Mammals under 300g (mice, rats, small dasyurids, small possums, small bats)	• Carbon dioxide inhalation • Pentobarbitone IV, IP or IH	• Cervical dislocation (under 150g only) • Decapitation • Stunning and exsanguination • Inhalational anaesthetics followed by a secondary method
Mammals over 300g under 5kg (larger possums, quoll, mala, small macropods, echidna)	• Pentobarbitone IV, IP or IH	• Injectable anaesthesia overdose followed by secondary method • Gunshot
Mammals over 5kg (kangaroos, wallabies, wombats, koala)	• Pentobarbitone IV	• Injectable anaesthesia overdose followed by secondary method • Gunshot • Stunning followed by exsanguination
Marine mammals	• Pentobarbitone IV or IP • Captive bolt • Gunshot	• Inhalational anaesthesia overdose followed by secondary method

IV – intravenous, IH – Intrahepatic, IP – intraperitoneal, PO – per os (orally) (NB the humane provision of pentobarbitone is facilitated by the provision of an anaesthetic or sedative)

Acceptable methods of early life stages of animals

Animal Type	Recommended	Acceptable

Laboratory rodents		
Embryos and foetuses <15 days gestation	Euthanasia of mother and/or removal of foetus and decapitation	
Foetuses >15 days gestation	• Pentobarbitone IP • Decapitation	• Hypothermia followed by secondary method (including fixation if furless) • Anaesthesia followed by secondary method including fixation (if fur is present)
Neonates <10 days	• Pentobarbitone IP • Decapitation	• Hypothermia followed by secondary method (<7 days only) • Stunning and cervical dislocation or decapitation • Anaesthetic overdose followed by secondary method
Rabbits and guinea pigs		
Embryos and foetuses <50% of gestation	• Euthanasia of mother and/or removal of foetus	
Foetuses >50% of gestation	• Pentobarbitone • Decapitation	• Cervical dislocation
Neonates <10 days	• Pentobarbitone	• Decapitation • Cervical dislocation
Larger mammals		
Embryos and foetuses <50% gestation	Euthanasia of mother and/or removal of foetus	
Foetuses >50% gestation	• Pentobarbitone IP	• Stunning followed by secondary method
Neonates (pre-weaning)	• Pentobarbitone IP	• Stunning followed by secondary method
Marsupials		
Pouch young <50g (no fur)	• Pentobarbitone	• Decapitation
Amphibians and fish		
Eggs	• MS-222 • Destroy viability of the egg (freeze, puncture, hypochlorite solution)	• Cooling (4^{o}C, 20 minutes) followed by fixation or rapid freezing (liquid N_2)
Tadpoles/larvae/fry	• MS-222 • Benzocaine (after neutralising solution)	• Decapitation
Reptiles and Birds		
Eggs/embryos <50% gestation	• Destroy viability of the egg (freeze, shake, puncture, inject pentobarbitone)	
Eggs/embryos >50% gestation and hatchlings	• Pentobarbitone • Decapitation (depending on size) • Cervical dislocation	• Pithing if foetus is large enough Carbon dioxide inhalation • Inhalational anaesthetic

	(depending on size)	overdose followed by secondary method

Unacceptable methods

The following methods are not acceptable as a primary method of humane killing:
• Air embolism (injection of air intravenously)
• The use of chloral hydrate, chloroform or ether (usually by inhalation)
• Drowning
• Hypothermia (adult exothermic animals)
• Exsanguination (without preceding loss of consciousness)
• Formaldehyde immersion or injection
• Non-penetrative captive bolt
• Stunning alone
• Intravenous potassium chloride, magnesium sulphate or neuromuscular blockers
• Smothering or strangulation
• Freezing
• Toxins or poisons
• Thoracic compression

Additional Information to Note

Pentobarbitone IP

Commercially available euthanasia formulations of pentobarbitone are intended for IV use in domestic and large animals and contain between 200-325mg/mL depending on the brand. At this level of concentration the pentobarbitone solution has a pH of between 8.5 and 11. Injecting solutions at this concentration and pH into tissue or body cavities can be extremely damaging and painful. As such IP injection of pentobarbitone should only be used in emergency situations or when an animal is already anaesthetised or unconscious. IM or SC injection routes should never be used.

Where an IP or IH injection of a pentobarbitone solution is the likely route of administration in a project (eg turtles and some small birds, reptiles and mammals) the solution must be diluted to a concentration of between 50-60mg/mL with isotonic saline to reduce the pH and tonicity to a more acceptable level.

Intracardiac injections

Intracardiac injections of pentobarbitone or potassium chloride are acceptable in emergency situations where the animal is anaesthetised, unconscious (no reflexes or pain response) or moribund and where other routes are not accessible.

Use of hypothermia, chilling or freezing

Hypothermia is an acceptable method of killing for some animals and at certain stages of life. When it is used the animals should not be able to come into contact the chilling agent or any precooled solid surface. This includes ice or the surfaces of the chiller.

Emergency euthanasia of animals at field sites

It may be necessary to carry out the euthanasia of an animal in the absence of appropriate drugs or equipment where it is not feasible to transport the animal to a veterinarian.

In these instances a method of euthanasia may need to be used that would be considered unacceptable in other circumstances. Investigators must make an ethical decision weighing up the likely suffering the animal may experience by using the unacceptable method against the suffering the animal may experience if no action is taken (keeping the safety of personnel and other animals in mind).

If an animal is killed by gunshot, a headshot should be used if possible, however, if the operator is unskilled or unable to keep the animal still a chest shot (preferably a double-shot) at the level of the heart should be used.

For smaller animals and wildlife, stunning from a blow to the back of the skull at the level of the skull-C1 joint is effective at causing death by cervical dislocation, but should preferably be done by experienced personnel.

In field situations pharmaceutical agents such as pentobarbitone should only be used if there is means of disposing of the animal's carcase. Pentobarbitone can remain in the animal's tissues in high enough concentrations to anaesthetise and/or kill any scavengers.

Procedure for the humane use of carbon dioxide for rodents

(1) When humanely killing rodents with CO_2 the following guidelines should be followed:

- (a) Room and environment:
  - (i) The area where the equipment is housed should have some sort of scavenger system or a way to ensure that excess gas does not accumulate in the room and pose a risk to personnel.
  - (ii) Must be a designated area that is away from other animals, the general circulation of people, is clean and quiet.
- (b) Source of CO_2:
  - (i) Cylinders of medical or industrial compressed CO_2 (not CO_2 from dry ice or other unreliable generation methods).
- (c) Euthanasia chamber:
  - (i) Use a transparent container.

(ii) The rodent's normal cage is preferable as it is the most familiar environment for the animals and will reduce any undue stress associated with handling or a change of environment. If this an IVC or microisolator (filter-top) cage the CO_2 hose can be fed directly into the cage and filled.

(iii) Alternatively a purpose built euthanasia chamber must be used, in which case the chamber must be emptied of CO_2 and disinfected between uses.

(d) Animal criteria:

(i) Animals from different cages or different species of animals should not be combined into the chamber

(e) Procedure for filling the chamber:

(i) CO_2 is an irritant to the membranes of the mouth, nose and eyes at concentrations over 30% meaning conscious animals feel extreme discomfort when exposed to higher concentrations. The procedure for filling the chamber should ensure that CO_2 levels do not exceed 30% before the animal loses consciousness.

(ii) The chamber should be filled slowly at a rate not exceeding 30% of the chamber volume per minute until the animals are seen to have lost consciousness.

Flow rate for humane killing using CO_2 (L/min) < Volume of the chamber (L) x 0.3 per minute (min)

(iii) The flow rate may be increased after all animals have been assessed to be unconscious.

(f) Animals must remain undisturbed in the chamber for about 5 minutes (mice) and 8-10 minutes (rats) after respiratory movement has ceased in all animals.

(g) After this time, the CO_2 can be turned off, the exhaust system can be activated and the chamber can be opened.

(h) Every animal must be assessed individually and death confirmed before carcase disposal.

(i) If the chamber is to be reused, all CO_2 must be evacuated and cleaned. Evacuation of CO_2 can be done by placing the chamber upside down.

(j) Many facilities have developed procedures for CO_2 that are specific to the size of the available chambers.

Confirmation of Death

Death is defined as having an absence of circulation and/or destruction of the brain. Animals must be assessed individually and shown to have an absence of:

- respiratory movement
- heartbeat
- pulse
- corneal reflex

In order for a definitive confirmation of death to be made, several observations should be made over 2 to 5 minutes and if necessary a secondary method can be employed.

In fish, amphibians, crustaceans and early life stages of animals it may be difficult to determine when death has occurred. Whenever possible, a secondary method should be employed including decapitation, pithing or another physical method.

References

1. American Veterinary Medical Association Guidelines for the euthanasia of animals: 2013 Edition.
2. NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes.
3. NHMRC: A guide to the care and use of native Australian mammals in research and teaching.
4. NSW Office of Environment and Heritage Code of practice for injured, sick and orphaned fauna.
5. University of Texas: Guideline for the humane euthanasia of laboratory animals.
6. Neiffer, D.L., Stamper, M.A. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. ILAR Journal, 50(4), 343-360
7. South Australian Museum Wildlife Ethics Committee 2013. Euthanasia of research animals in the field policy.

Administration

Approval Details

Policy Sponsor	Animal Welfare Officer
Approval Authority	AEC
Date for next review	[The policy review should be scheduled 3 years from the approval date]

[Approval date - the date the approval authority approved the establishment, minor or major amendment or disestablishment]

[Implementation Date - the date the policy was published in the Policy Library and is the date the policy takes effect]

Version	Approval date	Implementation date	Details	Author
1.0	<dd/mm/yyyy>	<dd/mm/yyyy>	<Policy established>	<Name and role of policy author>

Keywords	Animal Ethics, Animal Research, Humane Killing, Euthanasia