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

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)

Laboratory animals

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 CO₂ 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 methodPentobarbitone 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) Birds – medium	 Pentobarbitone IH or PO Carbon dioxide inhalation Pentobarbitone IV or IH 	 Inhalation anaesthetics overdose Cervical dislocation Decapitation Gunshot 	
(cockatoos, large parrots, magpie, ducks)	Carbon dioxide inhalation	 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 IPCaptive boltGunshot	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

Reonates <10 days • Pentobarbitone IP • Anaesthesia followed by secondary method including fixation (if fur is present) Neonates <10 days • Pentobarbitone IP • Hypothermia followed by secondary method (<7 days only) Rabbits and guinea pigs • Decapitation • Anaesthetic overdose followed by secondary method (<7 days only) Rabbits and guinea pigs • Euthanasia of mother and/or removal of foetus • Anaesthetic overdose followed by secondary method Foetuses >50% of gestation • Pentobarbitone • Cervical dislocation Neonates <10 days • Pentobarbitone • Cervical dislocation Neonates <10 days • Pentobarbitone • Decapitation Neonates <10 days • Pentobarbitone IP • Stunning followed by secondary method Rabbits and foetuses • Pentobarbitone IP • Stunning followed by secondary method Neonates (pre-weaning) • Pentobarbitone IP • Stunning followed by secondary method Marsupials • Decapitation • Stunning followed by secondary method Pouch young <50g (no fur) <td< th=""><th>Laboratory rodents</th><th></th><th></th></td<>	Laboratory rodents		
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<50% of gestation	Rabbits and guinea pigs		
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Neonates <10 days Pentobarbitone Decapitation Larger mammals Euthanasia of mother and/or removal of foetus Cervical dislocation Embryos and foetuses <50% gestation			Cervical dislocation
Embryos and foetuses Euthanasia of mother and/or removal of foetus <50% gestation	-		
<50% gestation	Larger mammals		
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Neonates (pre-weaning)• Pentobarbitone IP• Stunning followed by secondary methodMarsupials•Pentobarbitone• DecapitationPouch young <50g (no fur)• Pentobarbitone• DecapitationAmphibians and fish••Cooling (4°C, 20 minutes) followed by fixation or rapid freeze, puncture, hypochlorite solution)Tadpoles/larvae/fry• MS-222 • Benzocaine (after neutralising solution)• DecapitationReptiles and Birds••Destroy viability of the egg (freeze, shake, puncture, inject pentobarbitone)•Eggs/embryos >50% gestation and hatchlings•Pentobarbitone • Decapitation (depending•	Foetuses >50% gestation	Pentobarbitone IP	
Pouch young <50g (no fur)	Neonates (pre-weaning)	Pentobarbitone IP	 Stunning followed by
Amphibians and fish • MS-222 • Cooling (4°C, 20 minutes) Eggs • Destroy viability of the egg (freeze, puncture, hypochlorite solution) • Cooling (4°C, 20 minutes) Tadpoles/larvae/fry • MS-222 • Decapitation Tadpoles/larvae/fry • MS-222 • Decapitation Reptiles and Birds • Destroy viability of the egg (freeze, shake, puncture, inject pentobarbitone) • Decapitation Eggs/embryos >50% gestation and hatchlings • Pentobarbitone • Pithing if foetus is large enough Carbon dioxide	Marsupials		
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• Destroy viability of the egg (freeze, puncture, hypochlorite solution)followed by fixation or rapid freezing (liquid N2)Tadpoles/larvae/fry• MS-222 • Benzocaine (after neutralising solution)• DecapitationReptiles and Birds• Destroy viability of the egg (freeze, shake, puncture, inject pentobarbitone)• Pithing if foetus is large enough Carbon dioxide	Amphibians and fish		
• Benzocaine (after neutralising solution) Reptiles and Birds Eggs/embryos <50% gestation	Eggs	• Destroy viability of the egg (freeze, puncture,	followed by fixation or rapid
Reptiles and Birds • Destroy viability of the egg (freeze, shake, puncture, inject pentobarbitone) Eggs/embryos >50% gestation and hatchlings • Pentobarbitone • Decapitation (depending • Decapitation (dependin	Tadpoles/larvae/fry	MS-222Benzocaine (after	Decapitation
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gestation and hatchlings	gestation	(freeze, shake, puncture, inject pentobarbitone)	
on size) Inhalation Cervical dislocation Inhalational anaesthetic		• Decapitation (depending on size)	enough Carbon dioxide inhalation

	overdose followed by secondary method

Unacceptable methods

ne fo	llowing methods are not acceptable as a primary method of humane killing:
٠	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
٠	Stunning alone
٠	Intravenous potassium chloride, magnesium sulphate or neuromuscular blocker
•	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 CO₂ 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 CO₂:
 - (i) Cylinders of medical or industrial compressed CO₂ (not CO₂ 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 CO₂ 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 CO₂ 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) CO₂ 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 CO₂ 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 CO₂ (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 CO₂ 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 CO₂ must be evacuated and cleaned. Evacuation of CO₂ can be done by placing the chamber upside down.
- (j) Many facilities have developed procedures for CO₂ 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. <u>American Veterinary Medical Association Guidelines for the euthanasia of animals: 2013 Edition</u>.
- 2. <u>NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes.</u>
- 3. <u>NHMRC: A guide to the care and use of native Australian mammals in research and teaching.</u>
- 4. <u>NSW Office of Environment and Heritage Code of practice for injured, sick and orphaned fauna.</u>
- 5. University of Texas: Guideline for the humane euthanasia of laboratory animals.
- 6. <u>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</u>
- 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]

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