

## **Guidelines for Euthanasia of Animals for Scientific Purposes**

### **Revised from**

- **ANZCCART 2001 Edited by J.S. Reilly**
- **AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**
- **Recommended On-farm Euthanasia Practices by Jennifer Woods, Jan K Shearer and Jeff Hill, Chapter 10 in Improving Animal Welfare: a Practical Approach edited by Temple Grandin**

### **General considerations**

- The anxiety, fear, pain, and distress must be minimized for the animal(s) to be euthanised.
- All personnel involved in the process should have the relevant skills and competency for the method of euthanasia, and where required, appropriate legal approvals, for example a firearms licence or veterinary approval to use to certain veterinary medicines.
- For some species restraint is recommended, and this should occur for the shortest time possible and use the least stressful method available.
- The most appropriate method of euthanasia should be selected, and considerations include:
  - human safety
  - the welfare of the animals
  - the species of animal
  - the number of animals to be euthanised
  - the location (for example laboratory compared to farm or other outdoor location)
  - the ability to restrain the animal(s)
  - methods available at the time (for example in remote locations)
  - the competency of the operator
  - carcass disposal considerations
  - whether tissue samples are to be collected for diagnostic or research purposes.
- For all methods of euthanasia, the animal must be checked after the procedure to ensure it is dead. This should occur immediately following the procedure and again a few minutes later.
- Performing euthanasia is an unpleasant task and due consideration needs to be given to the personnel involved for level of comfort with the method being used, the number of times euthanasia is being performed, and support that they may require following the procedures.

### **Use of carbon dioxide for euthanasia**

Institutions currently using carbon dioxide for euthanasia are encouraged to consider alternatives to this method. However, it is appreciated that the choice of euthanasia method depends on a balance of animal welfare, staff safety and what is practical, and currently there is no similar alternative to carbon dioxide, especially in situations where large numbers of mice are used.

## **Commentary on the use of carbon dioxide for euthanasia from the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition**

### ***Advantages***

1. The rapid depressant, analgesic, and anaesthetic effects of CO<sub>2</sub> are well established.
2. Carbon dioxide is readily available in compressed gas cylinders.
3. Carbon dioxide is inexpensive, non-flammable, and nonexplosive and poses minimal hazard to personnel when used with appropriate equipment.

### ***Disadvantages***

1. Substantial and conflicting differences in response to CO<sub>2</sub> inhalation exist.
2. CO<sub>2</sub> is aversive in some species.
3. Because CO<sub>2</sub> is heavier than air, layering of gas or incomplete filling of a chamber may permit animals to climb or raise their heads above the effective concentrations and avoid exposure.
4. Immature individuals and some aquatic and burrowing species may have extraordinary tolerance for CO<sub>2</sub>.
5. Reptiles and amphibians may breathe too slowly for the use of CO<sub>2</sub>.
6. Euthanasia by exposure to CO<sub>2</sub> with O<sub>2</sub> supplementation may take longer than euthanasia by other means.
7. Induction of loss of consciousness at concentrations < 80% may produce post-mortem pulmonary and upper respiratory tract lesions.
8. Dry ice and liquid CO<sub>2</sub> are potential sources of distress or injury if permitted to directly contact animals.
9. If animals are anesthetized with inhaled agents prior to completing the euthanasia process with CO<sub>2</sub>, sufficient time should be allowed to prevent rapid recovery during the wash-in of CO<sub>2</sub> and the subsequent wash-out of inhaled agent.

### ***General Recommendations***

1. Carbon dioxide is acceptable with conditions for euthanasia in those species where aversion or distress can be minimized.
2. Carbon dioxide exposure using a gradual-fill method is less likely to cause pain due to nociceptor activation by carbonic acid prior to onset of unconsciousness; a displacement rate from 30% to 70% of the chamber volume/min is recommended for rodents.

### Rats and Mice

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b> <b>Inhalant</b>	None	Halothane	*Carbon dioxide Ether Hydrogen cyanide Carbon monoxide Nitrogen Chloroform
<b>Injectable</b>	Pentobarbitone sodium i.p.  Ethanol i.p.		
<b>Physical</b>	None	Cervical dislocation in animals >150g if stunned or anaesthetized first.  Decapitation with training.  Stunning and exsanguination with training.	Microwave irradiation Decompression Asphyxia Rapid freezing

\* A number of studies have concluded that CO<sub>2</sub> causes pain and distress in animals. This indicates a need for careful reconsideration of its use. Reviewed in <https://doi.org/10.1258/0023677053739747>.

### Guinea Pigs

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b> <b>Inhalant</b>	None	Halothane Nitrous oxide used with other inhalants	*Carbon dioxide Ether Hydrogen cyanide Carbon monoxide Chloroform
<b>Injectable</b>	Pentobarbitone sodium i.p.		No intravenous agents are acceptable.
<b>Physical</b>	None	Cervical dislocation with training.  Stunning and exsanguination with training.	

\* A number of studies have concluded that CO<sub>2</sub> causes pain and distress in animals. This indicates a need for careful reconsideration of its use. Reviewed in <https://doi.org/10.1258/0023677053739747>.

### Laboratory Rabbits

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b> <b>Inhalant</b>	None	Halothane	*Carbon dioxide Ether Hydrogen cyanide Carbon monoxide Chloroform
<b>Injectable</b>	Pentobarbitone sodium i.p. or i.v.		
<b>Physical</b>	None	Cervical dislocation with training.  Stunning and exsanguination with training.	

\* A number of studies have concluded that CO<sub>2</sub> causes pain and distress in animals. This indicates a need for careful reconsideration of its use. Reviewed in <https://doi.org/10.1258/0023677053739747>.

## Cattle

Code of Welfare MS20:

- Dairy Cattle Code of Welfare - dairy cattle must be rendered immediately insensible and remain in that state until death is confirmed.
- Sheep and Beef Cattle Code of Welfare - beef cattle must be rapidly rendered insensible and remain in that state until death.

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b>			
<b>Inhalant</b>	None	None	
<b>Injectable</b>	None	Pentobarbitone sodium – vet only /under vet supervision (Sedation prior to procedure recommended)	Magnesium sulphate alone Potassium chloride alone Guaiphenesin (cough expectorant) alone Mephenesin (muscle relaxant) alone Succinyl choline alone
<b>Physical</b>	Penetrating captive bolt, followed by exsanguination or pithing, with training	Shooting (free bullet head shot) with training Electrical stunning followed by exsanguination (requires specialist facilities)	Blunt force trauma to head Shooting to any part of the body other than the head Use of non-penetrating captive bolt

### Calves

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b>			
<b>Inhalant</b>	None	None	
<b>Injectable</b>	None	Pentobarbitone sodium – vet only / under vet supervision (Sedation prior to procedure recommended)	Magnesium sulphate alone Potassium chloride alone Guaiphenesin (cough expectorant) alone Mephenesin (muscle relaxant) alone Succinyl choline alone
<b>Physical</b>	Penetrating captive bolt, followed by exsanguination or pithing, with training	Non-penetrating captive bolt, followed by exsanguination, with training Shooting (free bullet head shot) with training Electrical stunning followed by exsanguination (requires specialist facilities)	Manually applied blunt force trauma to head (Prohibited by regulation under AW Act) Shooting to any part of the body other than the head Exsanguination alone

## Sheep and Goats

Note:

- Sheep and Beef Cattle Code of Welfare recommended best practice is that sheep should be rapidly rendered insensible and remain in that state until death
- Goat Code of Welfare - goats must be rapidly rendered insensible and remain in that state until death, MS 19

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b>			
<b>Inhalant</b>	None		
<b>Injectable</b>	Pentobarbitone sodium - vet only /under vet supervision		
<b>Physical</b>	Penetrating captive bolt, followed by exsanguination or pithing, with training	Non-penetrating captive bolt, followed by exsanguination, with training Shooting (free bullet head shot) with training Electrical stunning followed by exsanguination (requires specialist facilities)	Exsanguination alone



**Lambs and Kid Goats**

<b>Techniques</b>	<b>Recommended</b>	<b>Acceptable with reservations</b>	<b>Not acceptable</b>
<b>Chemical</b>			
<b>Inhalant</b>	None		
<b>Injectable</b>	Pentobarbitone sodium – vet only /under vet supervision		
<b>Physical</b>	Penetrating or non-penetrating captive bolt, followed by exsanguination or pithing, with training	Shooting (free bullet head shot) with training Electrical stunning followed by exsanguination (requires specialist facilities)	Exsanguination alone Manually applied blunt force trauma to the head

## Pigs

Code of Welfare MS19:

- Pigs must be rapidly rendered insensible and remain in that state, until death.
- Animals rendered insensible by a blow or shot to the brain must be bled out immediately to ensure death occurs before recovery from stunning.

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b>			
<b>Inhalant</b>	None		Carbon dioxide
<b>Injectable</b>	Pentobarbitone sodium – vet only / under vet supervision		
<b>Physical</b>	Pigs up to weaning: a blow to the frontal region of the skull, sufficient to fracture the skull, followed by bleeding out	<p>Pigs up to weaning: Captive bolt, followed by exsanguination, with training Shooting with training Electrical stunning and exsanguination (use of tong stunners)</p> <p>Grower, finisher, adult pigs: Shooting, with training Electrical stunning, either as a 2-step or single step process (requires specialist equipment)</p>	<p>Exsanguination without prior stunning.</p> <p>Note – large pigs have extensive frontal sinuses, and any physical slaughter method must take this into account</p>

## Horses

Code of Welfare MS15:

- Horses must be rapidly rendered insensible and remain in that state, until death.
- Horses must not be shot in the back of the head.
- The spinal cord must not be severed or broken in any horse.
- Animals rendered insensible by a blow or shot to the brain must be bled out immediately to ensure that death occurs before recovery from stunning

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b> <b>Inhalant</b>  <b>Injectable</b>	None  Pentobarbitone sodium – vet only /under vet supervision (sedation prior to procedure recommended)		Chloral hydrate
<b>Physical</b>		Captive bolt, followed by exsanguination, with training Shooting, followed by exsanguination, with training	Exsanguination without prior stunning.

**Poultry (including chickens, turkeys, quail, pheasants, ducks, geese)**

Meat Chicken Code of Welfare MS15, Layer Hen Code of Welfare MS17:

- The method(s) used for the humane destruction of meat chickens and layer hens, including unhatched eggs in the last half of incubation and day-old chicks, must ensure rapid death, which is confirmed by inspection
- Maceration equipment used for humane destruction must be designed to cause very rapid and complete fragmentation of the material into small particles
- When using gas, the procedure must ensure the collapse of every chicken/ hen within 35 seconds of exposure to the gas. Birds must remain in the gas for at least a further two minutes following collapse and be inspected to ensure that they are dead upon removal from the gas.
- Acceptable methods for emergency humane destruction:
  - Electrical stunning followed by neck dislocation and exsanguination
  - Neck dislocation alone
  - Gas using a mixture of inert gases and carbon dioxide
  - Immediate fragmentation/maceration for unhatched eggs and day-old chicks

<b>Techniques</b>	<b>Recommended</b>	<b>Acceptable with reservations</b>	<b>Not acceptable</b>
<b>Chemical</b>			
<b>Inhalant</b>	A mixture of inert gases with a low concentration of carbon dioxide (i.e. up to 30%) to produce an atmosphere with less than 2% oxygen by volume (RBP Code of Welfare)	Carbon dioxide Nitrogen or argon	
<b>Injectable</b>	Overdose of injectable anaesthetics		
<b>Physical</b>		Cervical dislocation (with training) Manually applied blunt force trauma (with training) Electrocution followed by neck dislocation and exsanguination	Exsanguination without prior stunning, or full anaesthesia.

		<p>Captive bolt, both penetrating and non-penetrating (with training)</p> <p>Gunshot for free ranging birds when capture or restraint would be highly stressful, or dangerous for personnel</p> <p>Immediate maceration/fragmentation for unhatched eggs and day-old chicks</p>	
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### Non-native, unprotected birds caught in leg-hold traps

- Native animals should be released if you are confident they are uninjured, or injuries are minor and the animal is likely to survive and exhibit normal behaviour. If the animal is not likely to survive even if treated it will need to be killed. If the animal requires treatment (to survive or address suffering) then it may be appropriate to seek care for the animal following DOC wildlife health management guidance, which can be accessed at [www.doc.govt.nz/our-work/wildlife-health](http://www.doc.govt.nz/our-work/wildlife-health). Non-threatened native animals that have significant injury and are not a priority for treatment should be dispatched.

Techniques	Recommended	Acceptable with reservations	Not acceptable
Chemical			
Inhalant			
Injectable			
Physical	Blunt force trauma to the head followed by dislocation of the head from the body (with training)		

**Non-native reptiles and amphibians (small lizards, salamanders or frogs) caught in live traps**

- Blunt force trauma may not be appropriate for dispatching large numbers of small animals or if they must be kept intact for analytical studies. For amphibians immersion in an anaesthetic agent may be a viable option. Inhaled anaesthetic agents may be appropriate for euthanasia of lizards. Seek advice from a veterinarian to determine an appropriate method, agent and procedure for the species concerned, and potentially the life cycle stage, as you need to.

<b>Techniques</b>	<b>Recommended</b>	<b>Acceptable with reservations</b>	<b>Not acceptable</b>
<b>Chemical</b>  <b>Inhalant</b>  <b>Injectable</b>			
<b>Physical</b>	Blunt force trauma to the head (with training) Followed by <i>“complete destruction of the brain (severely compressed/macerated), using repeat strikes if necessary to achieve this.”</i>		

### Feral dogs caught in live traps

- Where feral dogs are targeted (by cage trapping) carry a firearm. If trapping in a remote location, you should plan to carry a firearm as it will be necessary or most practical to shoot the animal at the trap site.

<b>Techniques</b>	<b>Recommended</b>	<b>Acceptable with reservations</b>	<b>Not acceptable</b>
<b>Chemical</b> <b>Inhalant</b> <b>Injectable</b>			
<b>Physical</b>	Shooting (free bullet head shot) with training.		



### Feral cats caught in live traps

- Two options – choose the best one that suits the situation. Shooting is preferred for cats caught in cage traps.
- A live feral cat in a trap can be difficult and dangerous to handle. Feral cats should be approached confidently and quietly. It is a good idea to get gear organised out of the cat’s line-of-sight first. This will minimise disturbing the cat to prevent it pulling out of the trap. Trappers must be experienced and capable of using the chosen technique.

Techniques	Recommended	Acceptable with reservations	Not acceptable
<p><b>Chemical</b></p> <p><b>Inhalant</b></p> <p><b>Injectable</b></p>			
<p><b>Physical</b></p>	<p><b>Option 1.</b> Shooting (free bullet head shot) with training.</p> <p><b>Option 2.</b> Blunt force trauma to the head (with training)</p> <p><i>Followed by “additional blows to the head until the skull compressed” then “lift the animal and check it bleeds in a continuous stream from the skull and/or ear; or bend the head back to expose the throat and cut across the throat to sever all major blood vessels” to confirm death</i></p>		

**Possums, mustelids, rabbits, hares, rodents, wallabies caught in live traps**

- Two options – choose the best one that suits the situation

Techniques	Recommended	Acceptable with reservations	Not acceptable
<p><b>Chemical</b></p> <p><b>Inhalant</b></p> <p><b>Injectable</b></p>			
<p><b>Physical</b></p>	<p><b>Option 1.</b> Blunt force trauma to the head (with training)</p> <p><i>Followed by “additional blows to the head until the skull compressed” then “lift the animal and check it bleeds in a continuous stream from the skull and/or ear; or bend the head back to expose the throat and cut across the throat to sever all major blood vessels” to confirm death</i></p> <p><b>Option 2.</b> Shooting (free bullet head shot) with training.</p>		

### Hedgehogs caught in live traps

- It will usually be necessary to uncurl the hedgehog to expose the head. This can be done by positioning the animal on its side, then placing a foot on and pressing firmly downward on the spines and skin on the upper half of the body, forcing it to uncurl.

Techniques	Recommended	Acceptable with reservations	Not acceptable
<b>Chemical</b> <b>Inhalant</b> <b>Injectable</b>			
<b>Physical</b>	Blunt force trauma to the head (top of the cranium) (with training)  Followed by <i>“additional blows to the head until the skull is compressed”</i>		

**Fish**

<b>Techniques</b>	<b>Recommended</b>	<b>Acceptable with reservations</b>	<b>Not acceptable</b>
<b>Chemical</b>  <b>Inhalant</b> <b>Injectable</b>	Anaesthetic overdose (made up at double strength, immerse fish for at least 10 minutes)		
<b>Physical</b>		Stunning and cerebral pithing, with training	Electrosedation