Euthanasia Guidelines

1. Introduction

- Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the following guidelines.
- All individuals performing euthanasia must be trained and competent and have completed the AALAS learning library group course 'Euthanasia of Laboratory Animals'. They must be skilled at confirming death by recognising the cessation of vital signs of the species being euthanised.
- The use of "cervical dislocation" as a sole method of euthanasia of un-anaesthetised rodents is only allowed in mice and rats <200g with justifications and approved by CULATR (Committee for the Use of Laboratory Animals in Teaching and Research), and must only be performed by competent staff.
- In some cases, vocalisation and release of pheromones occur during induction of unconsciousness. For that reason, other animals should ideally not be present when euthanasia is performed.
- The act of euthanasia should be performed away from the presence of other animals to avoid any potential vocalisation, fearful behaviour, or release of odours or pheromones from the euthanised animal causing distress to other animals. This can be achieved by:
    - Ensuring no direct visual line of sight between waiting animals and the euthanasia procedure being performed.
    - Using the least stressful method available. e.g. anaesthetic overdose
    - Ideally performing the euthanasia in a separate room to where animals are awaiting euthanasia. If this cannot be achieved, clearly delineating an area for temporary holding of animals which is separated from the euthanasia area.
    - Performing the euthanasia in a hood (such as fume hood or BSC).
    - Never performing euthanasia in an animal holding room unless there is a BSC or fume hood present

2. Mice, rats and hamsters

- **Use diluted pharmaceutical grade Dorminal** 5% solution (pentobarbital sodium 50 mg/ml) (1ml Dorminal® + 3ml water for injection) at a dosage of 150 mg/kg (1.5mg/10g) intraperitoneally (ip). Diluted pentobarbital (Dorminal) has the same pH as undiluted pentobarbital, but to ensure

sterility and stability must be used within the same day as prepared. Undiluted pentobarbital at higher doses may be used in cases where tissue collection or analysis is not needed.

Body Weight (**g**)	Pentobarbital Sodium required (**mg**)	Dorminal® 5% solution (50mg/ml) Calculated volume (**mL**)
< 10	2	0.04
50	10	0.2
100	20	0.4
200	40	0.8
300	70	1.4
400	90	1.8
500	100	2.0
600	120	2.4
700	140	2.8
800	160	3.2
900	180	2.7
1000	200	3.0

- **Overdose of anaesthetic** (such as Ketamine and Xylazine) by IP injection is an alternative to pentobarbital. The overdose used should be at least 3 times the anaesthetic dose.
- **Overdose of isoflurane.** Rodents can be exposed to an overdose of isoflurane vapours either by:
    - Anaesthetic machine with vaporiser, or
    - Using a 'open-drop' method (where liquid isoflurane is placed onto an absorbent material such as cotton wool, which is then placed in the bottom of a container, with a partition that physically separates the animal from the soaked isoflurane). Using this method should only be performed in a location which has adequate scavenging of isoflurane (such as fumehood).
- Following administration of euthanasia agent and before disposal of carcass, confirmation of death should be performed by ensuring one or more of the following:
    - Cessation of respiration and heartbeat
    - Lack of corneal reflex
    - Presence of rigor mortis
- Alternatively, instead of confirmation of death, a secondary method of euthanasia can be performed such as:
    - Thoracotomy (making incision into the chest cavity)
    - Exsanguination (such as blood sampling by cardiac puncture)
    - Cervical dislocation (while the animal is deeply anaesthetized)

3. Pigs, goats, rabbits, and other large animals

Use undiluted Dorminal® 20% solution (pentobarbital sodium 200 mg/ml) at a dosage of 100-150 mg/kg (all species) given intravenously (iv). Intracardiac injection can only be performed on fully anaesthetized animals.

Animal Species	Body Weight (**kg**)	Pentobarbital Sodium required (**mg**)	Dorminal® 20% solution (200mg/ml)	
			Calculated volume (**mL**) at 100 mg/kg	Calculated volume (**mL**) at 150 mg/kg
Rabbit	3	300-450	1.5	2.3
	4	400-600	2.0	3.0
	5	500-750	2.5	3.8
Pig and Goat	10	1000-1500	5.0	7.5
	20	2000-3000	10.0	15.0
	30	3000-4500	15.0	22.5
	40	4000-6000	20.0	30.0
	50	5000-7500	25.0	37.5

4. Rodent Foetuses and neonates

Follow AVMA Guidelines for the euthanasia of animals, important points are extracted from the guideline <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf >:

- No need to remove mammalian foetuses for euthanasia after the dam is euthanized
- Non-flammable volatile anaesthetic agents are effective for in utero foetuses. Neonatal rodents may take up to several hours to die from CO_2 exposure). Validation of the time required for euthanasia must be performed, or otherwise an adjunctive method (e.g., cervical dislocation, or decapitation) must be performed after a neonate is non-responsive to painful stimuli
- Cervical dislocation by pinching and disrupting the spinal cord in the high cervical region is acceptable with conditions for foetal and neonatal mice and rats.

5. Zebrafish

Follow AVMA Guidelines section S2.5 laboratory fish, and S6 Fish and Aquatic Invertebrates. Important points are as follows:

Ad**ult Zebrafish (>14dpf)**

- Rapid chilling (2° to 4°C) until loss of orientation and operculum movements is acceptable for zebrafish. Prepare a tank containing 5 parts ice and 1 part water to achieve a temperature of 2 - 4°C. Use a spawning barrier to prevent the fish from coming into direct contact with the ice. Submerge the adult fish until loss of orientation and operculum

movement, and then leave for a minimum of 10 additional minutes following loss of operculum movement to ensure death.

- Immersion of adult zebrafish in buffered Tricaine methanesulfate (MS 222) at a concentration of 250 to 500mg/L (or 5-10 times the anaesthetic dosage) is acceptable for euthanasia.
    - For a 1 step process, immersion should be for 30 minutes following loss of rhythmic opercular movements, (subsequent secondary physical method is optional).
    - For a 2 step process, immersion should render the fish unconscious, followed by a secondary (physical) method of euthanasia to ensure death, such as decapitation, pithing or freezing.
- Immersion of adult zebrafish in Eugenol/clove oil is acceptable as long as products with standardized, known concentrations are used so that accurate dosing is possible. Use a dose of >100 ppm. Fish should be left in the anaesthetic solution for a minimum of 10 minutes after cessation of opercular movement. Please note isoeugenol is a potential carcinogen.
- **Fry (4-14 dpf)**Rapid chilling (2° to 4°C) for a minimum of 20 minutes following loss of opercular movement. **Embryos (<7dpf)** Immersion into diluted sodium or calcium hypochlorite solution at concentration of 500mg/L for at least 5 minutes.

6. Xenopus laevis

- Immersion of *Xenopus laevis* in buffered MS 222 at a concentration of 5g/L will result in deep anaesthesia within 4 minutes, and euthanasia after 1 hour of immersion. If immersing at a concentration <5 g/L or duration shorter than 1 hour, a secondary euthanasia method should be used, such as exsanguination, vital tissue harvest, or decapitation with pithing.

7. Birds

Follow AVMA Guidelines section S5 Avians. Important points are as follows:

- Manual restraint for the purpose of administering pre-euthanasia or euthanasia drugs is possible for the commonly used bird species held in CCMR (*Gallus gallus and Agapornis roseicollis*).
- Following manual restraint, intravenous injection of injectable euthanasia agent (such as pentobarbitone at 150mg/kg) can be performed. Wild, fearful, or excited birds may require sedative or anaesthesia before IV injection can be performed.
- If IV injection is impossible, injectable euthanasia agents can be administered via intracoelomic, intracardiac, or intraosseous routes only if a bird is unconscious or anaesthetized.

8. CO_2 Euthanasia (Rodents only)

- All individuals performing CO_2 Euthanasia on rodents must undergo assessment of training and competency, signed off by a CCMR Veterinarian or a delegated Technician that has been previously assessed to be trained and competent.
- The SOP of the specific euthanasia chamber, which is displayed in close proximity to each chamber must be followed.
- The displacement rate of manually operated flow meters must be in accordance with AVMA 2020 Guidelines (currently 30-70% displacement/ minute).
- Death must be confirmed by recognising the cessation of vital signs or via exsanguination in manually operated chambers, whereas automated chambers must be periodically calibrated to ensure effectiveness.
- Euthanasia should be performed with animals in their home cage whenever possible. If animals need to be euthanised directly in the chamber then the chamber should be cleaned with 70% ethanol or other suitable disinfectant after each use