NEW_002 Euthanasia in Fat-tailed Dunnarts (Expiry: July 2028)

NOTE

- **If using anaesthesia, you must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)**
- **The use of (*) indicates this statement is dependent on the facility procedures**
- **The use of (**) indicates this statement is dependent on AEC Approvals**

I. OBJECTIVE

To describe the standard, safe and humane killing of Fat-tailed Dunnarts via intraperitoneal injection of sodium pentobarbital used across UQ research projects, also reflecting the procedure used to train workers across UQ within UQBR.

II. DEFINITIONS

Competent - "the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments."[1]

The following two definitions are similar and either can be used in most situations.

Euthanasia: - "The humane killing of an animal in the interest of its own welfare, to alleviate pain and distress".

Humane Killing: - "The killing of an animal using a humane method". For example, for culling or as part of a scientific investigation.

In animal ethics, the term **cull** generally means to remove animals from studies because they could not be used. For example, they were a sex or genotype that could not be used in the experiment. The term "cull" is also used when applying for animal ethics and reporting on outcomes. Ideally, the term "Cull" should not be used to mean "Euthanise" or "Humanely Kill".

As per the NHMRC Guidelines to promote the wellbeing of animals used for scientific purposes (2008), the key difference between humane killing and euthanasia is the reason that the animal is being killed. Humane killing is used at the end of studies to provide tissues for scientific purposes, when animals are no longer used for breeding and when stock are not required (e.g. unsuitable for particular research purpose).
Euthanasia refers to circumstances where pain, distress or suffering are likely to exceed humane end points and must be alleviated promptly. However, using the terms "euthanasia" and "humane killing" interchangeably is reasonable. The term "cull" should be reserved for describing animals that could not be used. These animals would be "humanely killed".

III. COMMENTS / RECOMMENDATIONS

- The humane killing of dunnarts MUST:
  - Result in a rapid loss of consciousness
  - Not allow recovery
  - Inflict minimal pain or distress
  - Be appropriate to the development stage of the animal

- If performed incorrectly, attempts to euthanase can fail to kill the animal and cause pain and distress.
- In many protocols experimental animals are anaesthetised in order to collect samples, perfuse tissues or perform recordings before euthanasia is performed. Euthanising animals while under anaesthesia (without recovery) is the preferred method of euthanasia whenever practical.
- Using the restraint method that produces the least distress should always be used. Anaesthetising animals before performing euthanasia should be considered whenever possible.
- Death must be unequivocally established before disposal of the carcase.

Environment conditions
Animals must be humanely killed or euthanised with efforts made to isolate them from potential stressful auditory, visual or olfactory stimuli that may be perceived by other animals. Therefore, animals are killed:

- In a quiet environment
- Separate from other housed animals.
- In a location that can be cleaned between animals.

IV. SAFETY AND COMPLIANCE

- Refer to UQBR-OM-09 Management of Dunnart Husbandry across UQBR.

V. TRAINING CONSIDERATIONS

Any training on live animals must be part of an approved process or have the training activity approved in an animal ethics project.

- All animal euthanasia and humane killing MUST:
  - Be performed by appropriately trained personnel
  - who have been deemed to be competent in the procedure
  - and are confident in completing the procedure
  - OR be under the direct supervision of a person who is competent
- Training in euthanasia MUST:
  - Be undertaken on cadaver animals until the trainee is deemed competent by the trainer
  - Further training should be undertaken on animals under general anaesthesia
  - Training should include additional methods for confirming death such as the use of a stethoscope or direct observation/palpation of the heart *

- During training, and in methods performed under deep anaesthesia, the thorax may be opened after the relevant humane killing technique to directly observe cessation of a heartbeat and assist in confirming death.
- Workers trained by UQBR must complete:
  - Pre-requisite training such as handling and restraint
  - Relevant online learning prior to receiving training in euthanasia procedures

VI. EQUIPMENT

- PPE* *Although PPE is facility dependent, minimum expectations include disposable gloves, clean long-sleeved laboratory gown, hair bonnet, eye protection, face mask.*
- Anaesthetic machine (Isoflurane and induction chamber
- 1mL syringe
- 25g needle
- Sharps container
- Paper towel
- Facility disinfectant – 70% ethanol or 1% F10
- Clinical waste bag for cadaver.

VII. PREPARATION

- Check Animal Ethics Committee approvals to ensure that the correct procedure and personnel are approved for the planned work
- Check cage cards, animal records and identification to ensure the correct animals are euthanised.

Dunnart sodium pentobarbitone euthanasia solution: Final volume for injection relative to weight				
Weight range	**Volume of commercially available sodium pentobarbital (325mg/mL)**	**Volume of water for injection (or normal saline)**	**Final concentration**	**Volume to be injected IP per animal**
<20g body weight	0.05mL	0.05mL	162.5mg/mL	0.05mL (Only half of made-up volume used)
> 20g body weight	0.05mL	0.05mL	162.5mg/mL	0.1mL
> 50g body weight	If the animal's body weight is in excess of specified parameters (e.g. a 60g dunnart), it is advised that you consult a UQBR veterinarian to reduce the volume of water for injection (or normal saline) used to make the dilution.			

VIII. PROCEDURE

PREPARATION

1. Weigh the fat-tailed dunnart using a digital scale (accurate to 0.1 g) to determine body weight for accurate dosing calculations.

2. If the fat-tailed dunnart is individually housed, return the dunnart to its home cage. If group housed, place the animal in a clean, empty holding cage.

3. Calculate the required dose of pentobarbital sodium (325 mg/mL), using the appropriate dilution rate and body weight following the table provided above.

4. Transport the fat-tailed dunnart to the procedure room using a trolley to move to the procedure room, minimising handling stress.

5. Prepare the syringe by draw up the calculated volume of diluted pentobarbital into a 1 mL syringe, ensuring there are no air bubbles.

6. Attach the needle securely using 25-gauge needle to the syringe.

PROCEDURE

7. Carefully remove the dunnart from its cage, applying minimal pressure to avoid restricting respiration. Place it into the induction chamber/container.

   Note: If the animal is in severe distress, proceed directly to euthanasia without anaesthesia.

8. Follow LAB_003 Fat-tailed Dunnart Anaesthesia – Isoflurane to anaesthetise the dunnart.

9. Once anaesthetised, remove the dunnart from the induction chamber and gently restrain following UQBR-WIN-070 Handling and restraint in dunnarts.

10. Insert the 25-gauge needle at a 45-degree angle into the lower right quadrant of the abdomen (between the right hip and the midline). Advance 0.25–0.5 cm depending on body size, taking care to avoid internal organs.

11. Slowly inject the pre-calculated volume of diluted pentobarbital into the peritoneal cavity.

12. Remove the needle and place back into the cage for observation.

13. Immediately dispose of the used syringe and needle in a designated sharps container.

14. Monitor for signs of death, remain in the procedure room to observe the dunnart and intervene if necessary.

15. Confirm death (After 5 Minutes) by verifying the absence of all the following:
    - Eye reflexes
    - Spontaneous, rhythmic breathing
    - Detectable heartbeat (palpation or stethoscope)

16. If the dunnart is unconscious but shows signs of life (e.g., shallow breathing or weak heartbeat), administer a second intraperitoneal injection of the same dose. Reassess after 3 minutes.

17. If death remains unconfirmed after 8 minutes, apply an approved secondary method (e.g., CO_2 asphyxiation or cervical dislocation). Note: Only personnel trained and assessed as competent may perform these procedures.

CONCLUSION

18. Place the cadaver in a labelled bag including:
    - Date
    - Ethics number
    - Chief Investigator (CI)
    - Animal ID
    - A note if it needs to be held for necropsy

19. Store the cadaver in a -20°C freezer if clinical waste collection is pending. Disposal must occur via the UQ-approved contractor (ACE Waste). If the carcase is required for necropsy, consult with the vet as to the best way to store it (fridge or freezer).

20. Clean and disinfect surfaces and equipment (e.g., scales, induction chamber, procedure area) using an approved disinfectant such as:
    - 70% ethanol
    - 1% F10
    - 1% bleach solution

IX. BIBLIOGRAPHY - Refer to UQBR-OM-09 Management of Dunnart Husbandry across UQBR

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Outcome
1.0	NEWMA AEC	July 2025	Approved with admin changes (v1.1)