

## NEW\_001 Euthanasia - Cane Toads

### I. OBJECTIVE

To describe humane methods of killing of cane toads (*Rhinella marina*) under field and laboratory conditions.

### II. COMMENTS & RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: the method(s) that will be used, any intended variation to this SOP.
- Methods considered **ACCEPTABLE**: see Part A-D, below.
- Methods considered **UNACCEPTABLE**: Freezing without prior sedation, AQUI-S®, Benzocaine, Dettol® (1-3)
- Other methods may be considered **CONDITIONALLY ACCEPTABLE**; however, these are reviewed by the AEC on an individual basis. Consider consulting with the Veterinary Officer prior to AEC application.
- Death must be confirmed at the end of all euthanasia procedures (see Table 1, XIV. Reference Information)
- This procedure has the potential to present stressful stimuli to other animals (auditory, olfactory, and visual). Reasonable efforts must be made to accommodate isolation of these potential stressors: e.g. isolation from other animals, adequate ventilation, appropriate cleaning prior to, and between animals.
- The methods described in this SOP are generally appropriate for many other spp. of amphibian, however, species-specific variations exist (e.g. 8°C is inappropriate for sedation in cold-adapted frog spp.). If you wish to use this SOP for other spp. first review current literature and consult with the Veterinary Officer.
- Human health and safety risks must be considered e.g. chemical exposure and intoxication. Ensure risk is appropriately assessed and managed e.g. review of SDSs, use of appropriate PPE, animal carcass/tissue disposal as per building/site requirements - for specific advise contact a UQ biosafety advisor.
- Repeated exposure of staff to humane killing of animals carries a potential increased risk of psychological distress, and associated manifestations, e.g. compassion fatigue. This is likely to be a particular risk for physical methods such as stun & decapitate, and double pithing. Efforts should be made to manage staff exposure.
- Required training in any of these techniques must be sought from within your individual school or research group.

PART	Method	Specific comments & recommendations	References
A	0.5% MS-222	Hyperactivity may be observed prior to sedation. MS-222 is acidic in solution and must be buffered prior to use.	(3-6)
B	Stun & decapitate	Notable investment in staff training is required to ensure consistent humane death and safety of animal technician.	(1, 3, 7)
C	Double pithing	As above, notable investment in staff training is required. Prior to pithing cane toads must be rendered senseless.	(1-3, 7)
D	Cool & freeze	Hyperactivity may be observed prior to sedation. Cooling must occur for a sufficient period to ensure the toad is rendered senseless before freezing can occur.	(1, 2, 4, 8, 9)

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#### **PART A – 0.5% MS-222**

- *Considering MS-222 is acidic in solution it is important that sodium bicarbonate is included within the anaesthetic solution to buffer the pH*
- *Ensure appropriate disposal of any excess MS-222, as restrictions may apply (6)*

#### **III. EQUIPMENT (0.5% MS-222)**

- *10g MS-222 (powder)*
- *2L Non-chlorinated water (at approximately 25°C)*
- *10g Sodium bicarbonate (powder)*
- *Mixing jug (≥2L capacity)*
- *pH indicator (e.g. single use pH indicator strips or digital pH meter)*
- *Euthanasia chamber (approximately 30 x 30 x 20cm) non-porous, with a secure ventilated lid [e.g. modified large ice-cream container – cleaned and dried, with small air holes cut out of the lid]*
- *PPE (eye protection, laboratory coat/overall/apron, enclosed shoes, nitrile non-powdered disposable gloves)*
- *First aid kit, eye rinsing products or facilities and hand washing facilities.*
- *Stethoscope or sound doppler (optional)*

#### **IV. PROCEDURE (0.5% MS-222), as per (5)**

1. *Apply PPE.*
2. *Mix 10g of MS-222 with 2L of non-chlorinated water within the mixing jug. This will make an anaesthetic concentration of 0.5% MS-222.*
3. *With the pH indicator at hand, add sodium bicarbonate to the solution, titrating to achieve a pH between 7.0 and 7.4.*
4. *Pour the titrated anaesthetic solution into the euthanasia chamber, filling to a depth of 1-2cm.*  
*The solution should not be so deep that it would cover the cane toad's nostrils once recumbent (i.e. mitigating the risk of airway aspiration and drowning – which in a partially anaesthetised animal is stressful).*
5. *Place the euthanasia chamber in a quiet, dark area, before proceeding.*
6. *Place a cane toad into the euthanasia chamber. Toads may elicit a short period of hyperexcitability, as well as erythema (blushing) of the ventral skin, prior to anaesthetic induction (and then death).*
7. *The cane toad should remain in the solution until death is confirmed (see Table 1, XIII Reference information).*

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## **PART B – Stun & Decapitate**

- *This is a relatively technical procedure. Notable investment in training and competency assessment is required to ensure staff can consistently perform this procedure in a humane and safe manner.*
- *Take care with handling during this procedure: the incision site runs through the region of the parotid gland, which contains the toad's toxins. Appropriate technique and use of PPE is important.*
- *Please note: decapitation alone is not considered a humane method of killing Cane toads.*

## **V. EQUIPMENT (Stun & Decapitate)**

- *A large hammer with a flat metal head for the stunning process (face diameter ~2cm), (note: rubber mallets are not a suitable).*
- *Chopping board (rigid wood or plastic).*
- *A large very sharp knife or cleaver (minimum 15cm blade length).*
- *PPE (face shield, laboratory coat/overall/apron, enclosed shoes, nitrile non-powdered disposable gloves. Also consider: chainmail glove)*
- *First aid kit, eye rinsing products or facilities and hand washing facilities.*

## **VI. PROCEDURE (Stun & Decapitate)**

1. *Apply PPE.*
2. *Place the chopping board on a stable surface*
3. *Restrain the cane toad by firmly holding the hind limbs and place onto the chopping board. Apply gentle pressure to the body so that the head and neck are secured.*
4. *Stun the animal: using the hammer deliver a forceful blow to the middle of the head, just behind the eyes (see Figure 1, XIII Reference information).*  
*Stunning or hitting any other part of the body is not acceptable.*
5. *Decapitate the animal: using the using the knife/cleaver, decapitate the head just caudal to the termination of the skull and jaw bones (see Figure 1, XIII Reference information). This should be performed immediately following stunning.*
6. *Confirm death has occurred (see table 1, XIII Reference information).*

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## PART C – Double Pithing

- **Prior to pithing cane toads must be rendered senseless.** This may be performed by stunning (see part B), cooling (see part D), anaesthesia (see part A) or sedation (eugenol, as described in this part).
- Alternative anaesthetics, prescribed by a registered veterinarian, may also be used (e.g. alfaxalone)
- Anaesthetics are known to alter physiology – this needs to be considered for any ex vivo tissue use. For example, eugenol may cause moderate reduction in heart rates (10-50% of baseline), making it a potentially viable for sedation prior to pithing and tissue harvest (10).

## VII. EQUIPMENT (Sedate & Double Pith)

- Eugenol (e.g. Croaked®: Cane Toad Control)
- Animal holding container
- Pithing equipment: specimen tray, sharp pithing probe (vanadium Kirschner wire or sharpened straight probe of 1-2mm diameter), sharpening stone, paper towel (*please note: the pithing probe must be sharp*)
- PPE (eye protection, laboratory coat/overall/apron, enclosed shoes, nitrile non-powdered disposable gloves)
- First aid kit, eye rinsing products or facilities and hand washing facilities.
- Stethoscope or sound doppler (optional)

## VIII. PROCEDURE (Sedate & Double Pith)

1. Apply PPE.
2. Place the animal holding container in a quiet, dark area, before proceeding.
3. Place a cane toad into the holding container and coat the animals dorsal surface in a thin layer of eugenol (e.g. 2 sprays of Croaked).  
*This anaesthetic is readily absorbed through the cane toad's skin and the average adult cane toad should be sufficiently anaesthetised within one or two minutes. Note: gastric prolapse is a known complication that is expected to occur at a low frequency (e.g. <5%).*
4. Once non-responsive, gently remove the toad from the holding container, securing the animal with your non-dominant hand.
5. Focusing on the base of the skull, ventri- and dorsiflex the head to identify the non-bony gap between the first cervical vertebra and the base of the skull.
6. Holding the pithing rod in the dominant hand, forcefully insert the pithing rod through the non-bony gap at the base of the skull, into the brain. To achieve this the rod is inserted in a cranial direction, through the foramen magnum, rotating the rod as it is inserted.
7. Once in the brain, pivot the handle of the rod so that the rod's tip within the brain moves left and right within the calvarium: the aim being to cause severe damage to the brain tissue.  
*Typically, some reflex actions of rigidity will be experienced.*
8. In a controlled manner withdraw the pithing rod until just the tip of the rod remains within the neural (spinal) canal. Then rotate the rod caudally so that the tip can be advanced in the caudal direction down the spinal canal. Proceed to advance the rod caudally down the spinal canal (this action is termed 'spinalisation').  
*Take care for toxic parotoid gland excretion which may occur during this step. Successful completion will usually result in the flaccid paralysis of all limbs.*
9. Withdraw the pithing rod and confirm death has occurred (see table 1, XIII Reference information).

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## PART D – Cool & Freeze

- *This method is variably supported in literature as appropriate for use in cane toads. There have been some recent compelling arguments in support of this technique (8, 9)*
- *It is important that sufficient hypothermia occurs, such that the animal is rendered senseless, prior to commencing of freezing – this is because the freezing process is expected to be painful, and many amphibians will retain consciousness in all but severe hypothermia.*
- *This method is unlikely to be appropriate for use in physiologically “cold-adapted” amphibians. If you wish to use this procedure in other amphibian spp. first review current literature and consult with the Veterinary Officer, prior to applying to the AEC.*

## IX. EQUIPMENT (Cool & Freeze)

- *Animal holding container (approximately 30 x 30 x 20cm), with a secure ventilated lid (ideally use the toad’s routine housing container)*
- *Insulating material (such as leaf matter or a clean chemical free damp towel)*
- *A shelter or hutch (such as empty pipe, cardboard boxes, or pots on their side)*
- *4°C fridge*
- *-20°C freezer*
- *Rectal thermometer*
- *PPE (eye protection, laboratory coat/overall/apron, enclosed shoes, nitrile non-powdered disposable gloves)*
- *First aid kit, eye rinsing products or facilities and hand washing facilities.*
- *Stethoscope or sound doppler (optional)*

## X. PROCEDURE (Cool & Freeze)

1. *Apply PPE.*
2. *Ensure the animal holding containers have some form of insulating material (preventing direct skin contact with hard cold surfaces), and a shelter or hutch (to accommodate natural behaviours)*  
*Such actions are believed to help reduce stress.*
3. *If the animal holding container being used for this procedure is not the toad’s routine home container gently transfer the cane toad(s) and apply the secure ventilated lid.*
4. *Place the animal holding container, containing the toad(s), into the refrigerator.*  
*Avoid using a noisy refrigerator or one that may experience frequent traffic.*
5. *Leave the toads within the refrigerator until they are unconscious, and core body temperature has reached  $\leq 8^{\circ}\text{C}$ . To ensure sufficient hypothermia, overnight refrigeration should be considered a safe minimum.*  
*Among other factors, the volume of toad biomass (size and number of toads) will influence the required refrigeration time.*
6. *Using the rectal thermometer, confirm body temperature has reached  $\leq 8^{\circ}\text{C}$ , and from pinching the digits, ensure there is no response to pain (withdrawal reflex responses). If the cane toad is  $> 8^{\circ}\text{C}$ , or responsive to pain, repeat step 5.*
7. *Transfer the cane toads from the fridge into the freezer. The anaesthetised bodies may be transferred into containment bags for this step, instead of the animal holding container.*
8. *Keep bodies within the freezer for 48 – 72 hours minimum (depending on the size and number of cane toads). The toads’ body temperature will continue to drop, and they will die without regaining consciousness.*
9. *Confirm death has occurred (see table 1, XIII Reference information).*

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## XI. REFERENCE INFORMATION

### 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.” (as per, Australian code for the care and use of animals for scientific purposes, 2013)*

**Euthanasia** – *a form of humane killing that is performed for the interests of the animal’s own welfare, to alleviate pain and distress. It is most often used with terminally unwell or injured animals, where the prognosis is considered hopeless and which case it is considered a therapy, performed for the benefit of the subject. For the purposes of this SOP, “euthanasia” and “humane killing” should be considered interchangeable (despite the notable differences in the traditional meaning of these words).*

**Humane killing** – *“the act of inducing death using a method appropriate to the species that results in a rapid loss of consciousness without recovery and minimum pain and/or distress to the animal.” (as per, Australian code for the care and use of animals for scientific purposes, 2013) Humane killing may be performed as an elective procedure (e.g. humane killing of animals at an abattoir) or as an emergency procedure (e.g. euthanasia following rapid disease progression). For the purposes of this SOP, “euthanasia” and “humane killing” should be considered interchangeable (despite the notable differences in the traditional meaning of these words).*

**Rendered senseless** – *made to be void of conscious perceptions of pain, distress and any other form of general discomfort. In cane toads this may be performed by anaesthesia (see part A), stunning (see part B), sedation (see part C), or cooling (see part D).*

### Table 1. Signs for confirmation of death.

*Following humane killing it is a requirement to confirm signs of death. Do not assume a toad is dead just because it is not moving or does not have obvious signs of rhythmic breathing. If death cannot be confirmed, the operator should repeat the same, or perform an alternative, euthanasia procedure.*

<b>Test</b>	<b>Expected confirmation signs of death</b>
<b>Loss of righting reflex</b>	<i>The toad is placed onto its dorsal surface (i.e. onto its back) and it is unable to right itself back onto its ventral surface.</i>
<b>Loss of withdrawal reflex</b>	<i>There is no response to a light squeeze to the skin in-between the digits.</i>
<b>Loss of deep pain reflex</b>	<i>There is no response to moderate pressure applied to a digital bone.</i>
<b>Absence of respiratory movement</b>	<i>Cessation of the rhythmic throat movements that indicate lung breathing.</i>
<b>Absence of heart contractions</b>	<i>Cessation of heartbeat as determined by observation of the chest (looking for visual signs of cardiac impulse beneath the skin), by palpation of the chest, by listening with a stethoscope, or by the use of a Doppler heart monitor.</i>

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**Figure 1. Landmarks for stunning and decapitation** Image source: [Queensland Government](#).

[Red circle, blue outline: location of impact with a hammer for stunning. Red line: location of cutting to perform decapitation].



## XII. REFERENCES

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