Curtin University Standard Operating Procedure

EUTHANASIA OF NON-RODENT SPECIES

Number: TEC 04
Version: 1.2
Date: 01/10/2008

Aim/Purpose: Whilst considerations as to euthanasia methods will need to be decided on an individual species basis, this SOP outlines some methods of euthanasia in non-rodent species housed currently within the facility.

Procedure:
1. Larger mammal species e.g. native marsupials
   - Overdose of an anaesthetic agent by use of pentobarbital is likely to be only method available.
     - Pentobarbital should be obtained and stored in accordance with the Poisons Act (S8 drug).
     - Inject by an acceptable intravenous route. Dosage: 110mg/kg.
     - As such animals may not be tame, administration of a sedative may be necessary prior to intravenous injection. A veterinary surgeon or other individual with experience of the use of anaesthesia in the species concerned should be consulted as to the choice of sedative drug(s).
     - It is recommended to dilute the pentobarbitone 1:1 with PBS or sterile saline to minimise pain on injection

2. Amphibia e.g. cane toads
   a. Non-physical methods- should be followed by pithing to confirm death
      - MS222- (Tricaine Methane Sulphonate)
        - This is a commonly used anaesthetic agent in fish and amphibia. Concentrations of 250mg/l in the tank water are used for euthanasia.
        - Note that MS222 is an acidic solution so concentrations of greater than 500 mg/l/water should be buffered with sodium bicarbonate to prevent distress to animals.
      - Benzocaine Hydrochloride
        - This is another anaesthetic agent added to tank water. Concentrations of greater than 250 mg/l should be used.
• **Pentobarbital**
  
  i. Inject intra-peritoneally at a dose of 100mg/kg bodyweight.

  ii. Note that this can take 30 minutes to take effect due to the slow metabolic rate.

  iii. It is recommended to dilute the pentobarbitone 1:1 with PBS or sterile saline to minimise pain on injection.

All of the above methods should be applied until the animal appears dead (absence of respiratory movement) and death should be confirmed by use of pithing.

b. **Pithing -destruction of the brain- (required by Dept of Agriculture before removal from toad room- performed after use of another euthanasia method)**

- The pithing rod needs to be of stainless steel or other metal of similar rigidity (orthopaedic Kirschner wires are ideal). The diameter must be narrower than that of the neural arches through which it will pass i.e. about 2mm.

- The animal is held firmly in the left hand (if right handed) and the head gently flexed downwards with the forefinger. A non-bony gap is thus created between the first vertebra and occipital region of the cranium.

- The pithing rod should be held near the end of the shaft and inserted forcefully into the brain by rotating it over the forefinger.

- Withdraw the pithing rod to a position where it can be rotated caudally and pushed backwards through the spinal arch (spinalisation). As soon as the rod has entered the posterior spinal canal there can be massive discharge of the parotid glands releasing their toxic contacts. Safety goggles should be worn to protect the eyes.

---

**Author:** Dr Tara Pike  
**Date of Approval:** 01/10/2008  
**Reviewed:**

<table>
<thead>
<tr>
<th>DATE</th>
<th>2009-2013</th>
<th>15/04/2014</th>
<th>8/5/2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>REVIEWER</td>
<td>Reviewed</td>
<td>Dr Tara</td>
<td>Dr Tara</td>
</tr>
<tr>
<td></td>
<td>Annually by Dr Beng Chua</td>
<td>Pike</td>
<td>Pike</td>
</tr>
</tbody>
</table>

Please Check Online for the Latest Version