Curtin University Standard Operating Procedure

EUTHANASIA RODENTS

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Aim/Purpose: To document methods of humane killing in the rodent species. Any deviations from the SOP required by an investigator should be detailed in the proposals submitted to the AEC. Investigators should have received training on methods of euthanasia and be competent to perform the techniques before using them. Euthanasia should take place in an area out of sight or smell of other animals.

Euthanasia means literally "a gentle and easy death". Numerous methods are available. The following are those recommended by this institution for rodents. Choice of method may depend on the experimental protocol or personal preference. It should be noted that there may be different and more suitable methods available dependant on life stage of the animal e.g. neonate or foetal and embryonic forms. Following use of all methods, death should be confirmed by one of the methods listed below.

ALL ANIMALS MUST BE HANDLED HUMANELY i.e. carefully and kindly in accordance with the relevant SOP'S.

Chemical Methods

1. Overdose of an anaesthetic agent

a) By use of pentobarbital

Species suitable: All species.

Comments: Pentobarbital should be obtained and stored in accordance with the Poisons Act (S4 drug).

Procedure:

- Inject by an acceptable intravenous route or intraperitoneally (See relevant SOP). Dosage: 110mg/kg.
- Note that intraperitoneal injection has a slow duration of effect (minutes).
- For intraperitoneal injections it is recommended to dilute the pentobarbitone 1:1 with PBS or sterile saline or local anaesthetic (Svendsen et al, 2007) to minimise pain on injection

b) By use of volatile inhalational anaesthetic agents (Isoflurane)

Species suitable: All species.
Comments: Anaesthetic agents are only available under veterinary prescription.

Procedure:

- Place the animals in the anaesthetic chamber. Turn on the carrier gas (oxygen) and the inhalational agent (isoflurane). The oxygen remains at a fixed flow rate- suitable for the species (see anaesthesia SOP) the flow rate of the volatile anaesthetic agent should be increased gradually to the maximum flow rate.

- Animals will gradually lose the ability to right themselves and respiratory rates will decrease.

- Animals may appear distressed- research has shown that use such agents are aversive to animals but are unlikely to cause irritation to the mucous membranes.

- Time to onset of death using this method is slow due to the wide safety margin of such agents.

- Confirm the animal is dead by one of the methods listed below.

N.B Use of the above method to anaesthetise animals and then transfer to a pre-filled CO_2 chamber to kill the animals is acceptable and may reduce distress potentially caused by irritation to the mucous membranes of rising CO_2 concentrations. At present however, CO_2 is not available in our facility.

2. Carbon Dioxide- Only to be used after the animal is anaesthetised using an approved method by the AEC. At present not available in our facility.

Species suitable: Mice, rats, small guinea pigs, hamsters, gerbils- **not** suitable for neonates.

Comments: There is currently much debate on the humaneness of CO_2 as a method of euthanasia. Whilst research is continuing it seems acceptable to use this method however:

a. The animals should be anaesthetised first with another agent such as isoflurane, then the CO_2 administered into the chamber being used. This can be at high flow rates and once the animal's respiration has ceased, the animal must be observed until all muscle activity and signs of life have been absent for at least 30 seconds. Eyes should be

dilated, and mucus membranes will no longer be pink. After removal from the container, check again to confirm respiratory arrest. Confirm the animal is dead using one of the physical methods below.

b. Pre-fill of the chamber should **NOT** be used on conscious animals.

c. CO_2 must be of the highest quality, food grade level

d. Dry Ice or cold CO_2 is not acceptable.

Physical Methods

All physical methods may be repugnant to the operator; no person should be coerced into using such a method if they do not wish to do so. Methods should be practiced on cadavers before use on live animals.

1. Cervical dislocation – Non preferred Method (Needs justification and approval by the AEC).

Species suitable: Rodents under 150g

Procedure:

- Grasp the animal by the base of the tail and place it on a firm surface.
- Press a rod across the neck at the base of the skull (Atlanto-occipital joint) or alternatively use fingers.
- With the other hand grasp either the base of the tail or the hind limbs.
- Quickly pull back and slightly up on the animal whilst resisting this motion with the hand holding the rod, or with the fingers.
- Check that separation of the joint has occurred by palpation of the neck.
- Confirm the animal is dead by one of the methods listed below.

[May be acceptable if the animal is anaesthetised first.]

2. Exsanguination

This is a form of a terminal blood collection- i.e. the blood is collected via a needle directly into the heart, and the blood is aspirated out. The collection of blood causes the heart to stop, and the mouse to die. **Anaesthesia is required for all exsanguination techniques.**

Procedure:

- The animal is anaesthetised, and a deep level of anaesthesia is ensured by making sure there are no reflexes present.

- For a mouse, use a 25-26 G needle and 1ml syringe. For a rat use a 23 g needle and a 3ml syringe.

- Approach the heart from either the left lateral thoracic wall near the point of the flexed elbow, or from under the sternum.

- Once the needle penetrates into the heart, stabilise the hub of the needle and syringe and gently apply negative pressure to the plunger.

- Do not repeatedly probe with the needle.

- Ensure the heart has stopped bleeding once all the blood has been collected.

- A secondary method of confirmation is recommended in this situation, such as cervical dislocation

Methods suitable for foetal and embryonic forms (once removed from the mother) and for neonates up to 5 days of age.

Decapitation

This procedure may be permissible in neonates of less than 5 days of age. However AEC approval will need to be given with justification for use of the method. The procedure involves decapitation at the level of the 1st-2nd cervical vertebra with **sharpened** scissors.

Procedure:

- Place the foetus/embryo on a firm surface and using a suitable sized, sharp surgical blade sever the head at the highest possible level (C1-C2). **Sharp** scissors may also be used.

Methods suitable neonates up until 2 weeks of age:

1) **Cervical Dislocation**

See above for techniques

Methods of confirmation of death

1. Onset of rigor mortis (rigidity) of the body.

2. Confirmation of cessation of the circulation- no palpable heart beat felt on placing fingers over the chest wall.

3. Exsanguination by severing the vessels of the neck using surgical blade or scissors (carotid artery severance).

Table H1 Methods of humane killing and euthanasia in rats and mice

Recommended	Acceptable with reservations	Not acceptable
Chemical		
• Inhalant: – carbon dioxide[e] • Injectable: – pentobarbitone sodium IP	• Inhalant: – isoflurane[bde]	• Inhalants: – ether[bc] – hydrogen cyanide[bf] – carbon monoxide[b] – nitrogen[f] – chloroform[b]
Physical		
• None recommended	• Cervical dislocation[a] (acceptable; possibly inhumane in animals heavier than 150 g without prior stunning or anaesthesia) • Decapitation[aef] • Stunning and exsanguination[af]	• Microwave irradiation (not yet proven to be humane)[ce] • Decompression[ef] • Asphyxia[cef] • Rapid freezing[ce]

IP = intraperitoneal

[a] Training required
[b] Occupational health and safety issues
[c] Inhumane
[d] Expensive
[e] Requires specialised equipment
[f] Aesthetically unpleasant

Taken from the NHMRC Guidelines to Promote the Wellbeing of Animals used for Scientific Purposes.

References

1. Svendsen O[1], Kok L, Lauritzen B. 2007 Nociception after intraperitoneal injection of a sodium pentobarbitone formulation with and without lidocaine in rats quantified by expression of neuronal c-fos in the spinal cord--a preliminary study. 2007 Apr;41(2):197-203.

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