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Euthanasia of laboratory animals

SCOPE

The humane killing of mice and rats for culling or part of a scientific investigation must avoid pain and distress, be reliable and produce rapid loss of consciousness until death occurs. Animals must be killed in a quiet environment that is away from other animals wherever possible. Death must be established before disposal of carcase.
Methods of killing must be appropriate to the developmental stage of the animal.
This SOP applies to mice, rats, guinea pigs and rabbits.
In many protocols experimental animals are anaesthetised in order to collect samples, perfuse tissues or perform recordings before euthanasia is performed. This method of anaesthesia without recovery is the preferred method of euthanasia whenever practical.

AUTHORISATION TO UNDERTAKE PROCEDURE

Animal technicians and investigators approved by the AEC and/or have undertaken BRC training and assessed as competent to perform procedure, or under the supervision of technician approved to assess procedure.

SPECIAL REQUIREMENTS/SAFETY

Anaesthetic drugs are scheduled substances and must be stored, used and recorded in accordance with current 'Poisons Control Plan'.

PROCEDURE

1. **Foetal euthanasia**

- Mouse and rat foetus <15 days gestation and guinea pig <34 days gestation
  Death of the mother results in death of foetus as heart and vascular system is not sufficiently developed
- Mouse and rat foetus>15days gestation to birth and guinea pig >24days gestation to birth
  The literature suggests there is a possibility of pain perception from this stage and newborn pups or piglets are resistant to oxygen deprivation thus require special techniques

Methods of foetal euthanasia

1. Carbon dioxide asphyxiation
   Place foetus removed from uterus in a carbon dioxide chamber and keep in the chamber for several minutes after they are cyanotic and all signs of life absent. This could be up to 10 minutes. This should be immediately followed with decapitation or hypothermia

2. Decapitation with sharp scissors, surgical blade or guillotine
3. When chemical fixation of the foetus is required for histology, the foetus should be anaesthetized with monitored hypothermia or deep anaesthesia of the mother with barbiturate (Pentobarbital, Lethabarb 200mg/kg), prior to removal of the foetus.
4. Hypothermia is achieved by placing pups on a non-stick surface in a freezer for several minutes or immersion in slushy, crushed ice.

2. Mouse, rat, guinea pig and rabbit neonates (up to 5 days of age)

Methods of neonate euthanasia

1. Anaesthesia by carbon dioxide inhalation, or immersion in crushed ice, or placed on tissues in container in freezer for 10 minutes, followed by decapitation with sharp scissors is the most effective method of euthanasia
2. Overdose of anaesthetic agent by intraperitoneal injection is also acceptable
    - Pentobarbital (Lethabarb, 200mg/kg)
    - Ketamine (300mg/kg) + Xylazine (30mg/kg)
3. Cervical dislocation using thumb and forefinger squeeze to break neck can be used if options 1 and 2 above are not available.

3. Mice, rats, rabbits and guinea pigs (> 5days to adult)

1. Overdose of anaesthetic agent by intraperitoneal injection of pentobarbital [Lethabarb]
   As some barbiturate euthanasia solutions can be irritant to tissues the solutions are diluted prior to injection. This is not necessary if the animal is anaesthetised prior to euthanasia.

   Dose rate for euthanasia solution of sodium pentobarbital sodium [Lethabarb] diluted 1 in 2 with water or saline.

   The following volumes administer approximately 350mg/kg

Mouse	<20g	0.05ml i/p
	20-30g	0.1ml i/p
Rat	250g	0.25ml i/p
	500g	0.5ml i/p or i/v
Guinea pig	500g	0.5ml i/p
Rabbit		1ml/kg i/p or i/v

2. Ketamine:Xylazine mixture

Administered at three times the anaesthetic dose- approx. 300mg/kg Ketamine and 30mg/kg Xylazine

Mouse	20g	0.06ml K and 0.03ml X i/p or s/c
Rat	/100g	0.3ml K and 0.15ml X i/p or s/c
Guinea pig	/100g	0.3ml K and 0.15ml X i/p or s/c
Rabbit	/kg	1.0 ml K and 0.5ml X i/p or s/c

3. Cervical dislocation if less than 100g

4. Carbon dioxide inhalation is efficient method of euthanasia for mice and rats.

Mice
In the BRC using the euthanasia cabinet place the box of mice in the cabinet in their home cage and fill with carbon dioxide for 1 minute [BRC SOP 60 *Use of Euthanasia Cabinet*]

Rats
In EMSU fill clear container with CO^2 gas on full flow for 2 minutes, place rats in their home cage in container and run gas for 1minute, then leave on low flow for 5 minutes. Leave in CO^2 atmosphere for another few minutes until death is confirmed.

5. Decapitation after deep surgical anaesthesia, using a guillotine, is acceptable for particular tissue preservation techniques.

MONITORING REQUIREMENTS

Animal should be observed until death has occurred confirmed by no breathing, absence of heart rate and no reflexes.

ASSOCIATED PROCEDURE/S

AEC SOP 52 *Intraperitoneal injection in mice*
BRC SOP 60 *Use of the CO_2 Euthanasia Cabinet*

REFERENCES

Australian Code for Practice for the Care and Use of Animals for Scientific Purposes (8th Edition 2013)

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Date Approved by AEC: 20 November 2013	**Review Due:** November 2016