

**THE UNIVERSITY OF MELBOURNE  
ANIMAL WELFARE COMMITTEE**

**GUIDELINES ON HUMANE KILLING  
AND EUTHANASIA OF ANIMALS**

**Introduction**

'Humane killing' is the process of killing an animal with minimum pain and distress. In laboratory animal science it is applicable whenever:

- animal numbers exceed requirements or animals are not suitable for project requirements eg inappropriate sex or genotype
- it is a requirement of the project itself eg when fresh tissues are required for analysis
- a predetermined experimental endpoint is reached
- an animal has come to the end of its breeding or experimental life.

'Euthanasia' is the humane killing of an animal, in the interests of its own welfare, to alleviate pain and distress. In laboratory animal science it is applicable whenever:

- a predetermined humane endpoint is reached
- an animal has become sick or moribund or is suffering pain which cannot be alleviated.

**Policy**

All scientific procedures carried out on animals must comply with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004), which states:

- 3.3.18 When it is necessary to kill an animal, humane procedures must be used. These procedures must avoid pain or distress, be reliable and produce rapid loss of consciousness until death occurs. The procedures should also be compatible with the scientific or educational aims.
- 3.3.19 The procedures must be performed only by personnel approved as competent by the AEC or under the direct supervision of a competent person.
- 3.3.20 Animals should be killed in a quiet, clean environment, that is away from other animals where possible. Death must be established before disposal of the carcass occurs.
- 3.3.21 Where practicable, tissue from animals being killed should be shared among investigators and teachers in line with the principle of Reduction [see 2.2.16 (vii)].

3.3.22 Dependent offspring of animals being killed must also be killed or appropriate provision made for their care.

3.3.23 Methods of killing must be appropriate to the developmental stage of the animal. Disposal of fertilised eggs, fetuses and embryos must not occur until death is assured.

### **Choice of Method**

Humane killing and euthanasia may be carried out using either physical or chemical means.

Factors which influence choice of method are:

- sensitivity, training and experience of staff
- species, size, and numbers of animals to be killed
- cost and availability of agents/equipment required, as well as any hazards involved in their use
- the reason for killing and the data to be obtained.

### **Methods used for humane killing or anaesthesia**

The methods described in these guidelines are listed as either 'acceptable' or as 'acceptable with reservations'. The reservations may arise because the method requires specialised equipment and/or training, is aesthetically unpleasant, or has occupational health and safety issues. Some methods will only be acceptable where alternative, more acceptable methods, will interfere with scientific outcomes.

### ***Methods of Humane Killing and Euthanasia of Small Laboratory Animals Rats, Mice, Guinea Pigs, and Rabbits***

Reference: *The Victorian Code of Practice for the Housing and Care of Laboratory Mice, Rats, Guinea Pigs and Rabbits.*

<b>Species</b>	<b>Acceptable</b>	<b>Acceptable with reservations</b>
<b>Mice and Rats</b>		
Post-neonatal	Carbon dioxide (slow-fill method*) Cervical dislocation (animals <150g). Pentobarbitone sodium i.p. (or i.c. after sedation) Xylazine/Ketamine	Halothane Isoflurane Methoxyflurane Decapitation Stunning and exsanguination
Neonatal (birth to 10 days)	Decapitation Cervical dislocation Overdose with injectable anaesthetic	

Foetal (E15 to birth)	Decapitation Cervical dislocation Overdose with injectable anaesthetic	
<b>Guinea Pigs</b>		
Post-neonatal	Carbon dioxide (animals <600g, slow-fill method*) Cervical dislocation (animals <150g) Pentobarbitone sodium i.p. (or i.c. after sedation)	Halothane Isoflurane Methoxyflurane Nitrous oxide (must be used with other inhalants) Stunning and exsanguination
Neonatal	Overdose with injectable anaesthetic Cervical dislocation (animals <150g)	
Foetal (from 60% gestation) guinea pigs	Decapitation (preferably with a guillotine)	
<b>Rabbits</b>		
Post-neonatal	Pentobarbitone sodium i.v. or i.p.	Halothane Isoflurane Methoxyflurane Nitrous oxide (must be used with other inhalants) Ketamine with a premedicant Stunning Captive bolt Cervical dislocation (animals must be anaesthetised first) Decapitation (animals must be anaesthetised first)
Neonatal (birth to 10 days)	Overdose with injectable anaesthetic Cervical dislocation (animals <100g)	
Foetal (from 60% gestation)	Decapitation (preferably with a guillotine)	

i.c. = intracardiac, i.p. = intraperitoneal, i.v. = intravenous.

\*carbon dioxide administered at a rate of 20% chamber volume per minute

***Methods of Humane Killing and Euthanasia of Cold Blooded Vertebrates, Birds, and Large Laboratory Animals***

References: *American Veterinary Medical Association Guidelines on Euthanasia 2007*  
*ANZCCART 2001 Euthanasia of Animals Used for Scientific Purposes*

<b>Species</b>	<b>Acceptable</b>	<b>Acceptable with reservations</b>
<b>Toads and Frogs</b>	Trichaine methane sulphonate (MS 222, 0.4% in water neutralised to pH 7.0)	Stunning and decapitation Stunning followed by pithing
<b>Fish and Tadpoles</b>	Trichaine methane sulphonate (MS 222, 0.1% in water neutralised to pH 7.0)	
<b>Birds</b>		

Small/medium	Carbon dioxide* Cervical dislocation Pentobarbitone sodium i.p., i.v. (or i.c. after sedation)	Halothane Isoflurane
Large	Pentobarbitone sodium i.p. (or i.c. after sedation)	
Embryos (from 50% incubation) to 3 days old	Decapitation	
<b>Large Animals</b>	Overdose with injectable anaesthetic	Captive bolt followed by exsanguination

i.c. = intracardiac, i.p. = intraperitoneal, i.v. = intravenous.

\*carbon dioxide administered at a rate of 20% chamber volume per minute