

## **Laboratory Animal Euthanasia**

## **Humane Euthanasia of Animals in Research**

### **Introduction**

The killing of animals used for scientific purposes is a very sensitive issue and requires special consideration to ensure that animal anxiety and fear is reduced to a minimum.

Euthanasia is defined as killing painlessly. Animals are euthanased in laboratories or breeding establishments for the following reasons:

- At the termination of studies, to provide tissues for scientific purposes.
- When pain, distress or suffering are likely to exceed designated levels.
- Where the health or welfare of animals are grounds for concern.
- When animals are no longer used for breeding.
- When stock are not required for certain reasons, such as sex preference utilisation.

### **Objectives of Euthanasia**

To meet the objectives of euthanasia procedures must:

- Avoid distress and produce rapid loss of consciousness until death occurs.
- Be reliable, reproducible and irreversible.
- Be appropriate for age, species, and health of the animal.
- Require minimum restraint.
- Be compatible with the objectives of the study.
- Be simple to administer.
- Be safe for the operator.
- Be aesthetically acceptable to the operator, where at all possible.

### **Personnel Training**

Personnel must have training and be skilled in how to handle the species involved, how to apply the euthanasia method being used in a way to minimise stress in animals, and to be able to recognise and confirm death in the species they are working with.

### **Recognition and Confirmation of Death**

Recognition of death may be made by cessation of heartbeat and respiration, absence of reflexes, including corneal and palpebral reflexes, loss of colour in mucous membranes, and glazing of eyes. If any doubt occurs concerning the fact that death has occurred a second euthanasia technique should be used to ensure the death of the animal.

### **Euthanasia of the Foetus and Newborn Animal**

There are two factors that must be taken into account when choosing a euthanasia method for the foetal or newborn animal. They are resistant to hypoxia and they metabolise drugs more slowly. It is therefore considered that two methods acceptable for the species should be combined and death confirmed by accurate observation of the above signs.

### **Scientific Considerations**

Methods of euthanasia can impact on data variability either directly or indirectly. As a general principle, animals are killed by the most humane method for that species but this might be over-ruled when it is established to the satisfaction of the Animal Ethics Committee that the effects of the euthanasia method on data would compromise the scientific validity of the research proposal. If, because of the demonstrated influence on scientific data of any method recommended for the species it is considered justified to use any method which is acceptable with reservations, then all necessary precautions must be taken to minimise any impact on the welfare of the animal. If there is a reason to believe a recommended method may influence data but there is no supportive evidence then the need to use an alternate method should be validated in a pilot study.

## **Location of Euthanasia and Disposal of Carcasses**

Animals should be killed in a clean quiet environment, away from other animals where possible. Death must be established before disposal of the carcass. Records should be kept. Where practicable, tissue from animals being killed should be shared among investigators and teachers. Dependent neonates of animals being killed must also be killed or provision made for their care. If immediate disposal is not available carcasses should be frozen. Where infectious organisms are involved appropriate disposal methods and disinfection practices prescribed by Australian Standards must be strictly adhered to. OGTR requirements must be met in their entirety.

## **General Comments on Techniques of Euthanasia**

The techniques listed here fall within two general categories:

1. Those methods that are recommended.
2. Those methods that are acceptable with reservations. The reservations may be on aesthetic grounds, the need for special equipment, or pose some possible human safety hazard. These might be used where, for example, the recommended method may impact negatively on the science, or as a second method to ensure that death has occurred.

Techniques can be further divided into:

1. Chemical, which is further subdivided into: Inhalant, and injectable
2. Physical

Carbon Dioxide, used as a euthanasia agent on its own, carries with it some attendant ethical and physiological reservations. Appropriate alternatives are being investigated. At its January 2007 meeting the AEEC felt that the administration of CO<sub>2</sub> is best preceded by an inhalation anaesthetic, such as Isoflurane. In the absence of an appropriate alternative to CO<sub>2</sub> the AEEC will make mandatory the use of Isoflurane before CO<sub>2</sub> administration on 31 December 2008.

Euthanasia Methods by Species (Taken from Euthanasia of Animals Used for Scientific Purposes- ANZCCART 2001)

### **Animals**

- Rats and Mice
- Guinea Pigs
- Rabbits
- Dogs and Cats
- Non-Human Primates
- Sheep and Goats
- Pigs
- Amphibia
- Reptiles
- Birds

## Rats and Mice

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"> <li>• <b>Inhalant:</b> Carbon dioxide preceded by Isoflurane</li> <li>• <b>Injectable:</b> Pentobarbitone sodium i/p</li> </ul>	
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"> <li>• Cervical dislocation (Rats heavier than 150g to be anaesthetised first)</li> <li>• Decapitation (may be required under special experimental design - Aesthetically unpleasant)</li> <li>• Stunning and exsanguination (Aesthetically unpleasant)</li> </ul>

### Recommended Techniques

#### Carbon Dioxide:

Carbon dioxide, passed through a reduction valve, can be piped into plastic bags or deep containers at an optimal flow rate that displaces 20% of the chamber volume per minute. Considerable debate has occurred relative to three methods of administration of carbon dioxide for euthanasia of rats and mice:

- Placing them into a container pre-filled with carbon dioxide
- Placing them in air and then rapidly filling the container with carbon dioxide
- Using a carbon dioxide/oxygen mixture eg 70%CO<sub>2</sub>/30%O<sub>2</sub>

There are publications supporting each of the three methods but the European Commission, DGXI-Working Party(1996) believed that all three methods were acceptable until there is more conclusive information available. Generally speaking there is disquiet about the use of CO<sub>2</sub> as a euthanasia agent by itself. **The AEEC prefers that CO<sub>2</sub> is preceded by isoflurane**

#### Pentobarbitone sodium injection:

Pentobarbitone sodium at a concentration of 60mg/ml given i/p at a dose rate of (200mg/Kg) produces quiet induction and death. Animals euthanised by this method should not be fed to animals or birds because of the likelihood of residues of the barbiturate being present.

It should be noted that the high concentration veterinary euthanasia pentobarbitone solutions (Greater than 300mgs/ml) may produce peritoneal irritation with pain and therefore a fast acting local anaesthetic solution should be added to the solution.

### Techniques that are Acceptable with Reservations

#### Cervical Dislocation:

This involves holding the animal prostrate with the operators thumb and fore-finger firmly squeezing the neck, with the free hand pulling the quarters caudally. This method has traditionally been used in rodents but may be aesthetically unpleasant for the operator and if this is the case animals should be anaesthetised beforehand.

#### Decapitation:

May be considered an acceptable method of choice for some neuroscience studies where general anaesthesia may interfere with study parameters although it is generally regarded as not being aesthetic and on those grounds and where the science allows it cervical dislocation is generally more aesthetically acceptable.

## Guinea Pigs

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"> <li>• <b>Inhalant:</b> Carbon dioxide</li> <li>• <b>Injectable:</b> Pentobarbitone sodium i/p</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Inhalant:</b> Halothane</li> </ul>
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"> <li>• Cervical dislocation (Rats heavier than 150g to be anaesthetised first)</li> </ul>

### Recommended Techniques

#### Carbon Dioxide

To minimise the period of breathlessness guinea pigs should be placed in a container pre-filled with carbon dioxide, rather than filling the container with CO<sub>2</sub> once the guinea pigs have been placed inside. The 70%CO<sub>2</sub>/30% O<sub>2</sub> method described in rats and mice is also recommended by some authorities.

#### Pentobarbitone sodium

Pentobarbitone sodium formulated at a concentration of 60 mgs per ml produces quiet induction and death when administered intraperitoneally at a dose rate of 90 mg/Kg. Again it should be noted that the veterinary euthanasia pentobarbitone sodium solutions formulated at or greater than 300mgs/ ml may irritate the peritoneum and therefore fast acting local anaesthetic should be added to the mixture.

### Technique Acceptable with Reservations

#### Halothane

A concentration of 4% halothane can produce cardiac arrest in 90 seconds, however because of the human health implications of halothane this must be done in a fume cupboard and carbon dioxide preceded by isoflurane is the preferred agent of choice.

#### Cervical Dislocation

This technique must only be performed by skilled operators and a strong recommendation is that it is only used on young guinea pigs. It may be learnt by unskilled operators by practicing on dead or anaesthetised animals. It is performed by holding the animal with the fingers of one hand around the back of the neck with the elbow flexed while restraining the hindquarters with the other. The elbow at the back of the neck is extended and the wrist flicked backwards as the elbow becomes straight. There are aesthetic reservations regarding this technique.

## Rabbits

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> None recommended</li><li>• <b>Injectable:</b> Pentobarbitone sodium i/v or i/p</li></ul>	<ul style="list-style-type: none"><li>• <b>Inhalant:</b> Halothane</li></ul>
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"><li>• Captive bolt</li><li>• Neck dislocation or decapitation following anaesthesia</li></ul>

### Recommended Techniques

#### Pentobarbitone sodium

Pentobarbitone sodium given at a concentration of 60 mgs/ml at a dose rate of 60mgs/kg produces quiet induction and death even when given by the i/p route although the i/v route is preferred. Again the high concentration sodium pentobarbitone “veterinary euthanasia solutions” may be associated with irritation of the peritoneum and therefore if these are used they should be used with fast acting local anaesthetic added.

### Technique Acceptable with reservations

#### Halothane

Again human safety concerns dictate that this must only be used in a fume cupboard.

#### Captive bolt

Use of a captive bolt may be an option when the use of an anaesthetic is not acceptable within the scientific aims of the study. The AEEC may approve this method but proposals requesting this need to thoroughly document their reasons for requesting this method. Operators need training in this technique. The captive bolt is applied to the head between the ears and aimed downwards so as to parallel the vertical plane of the head. Performed correctly it results in immediate unconsciousness and immediate loss of reflexes and respiration.

## Dogs and Cats

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> None recommended</li><li>• <b>Injectable:</b> Pentobarbitone sodium i/v or i/p</li></ul>	<ul style="list-style-type: none"><li>• <b>Inhalant:</b> Halothane, Carbon monoxide</li></ul>
PHYSICAL	
Please note there are no recommended physical procedures	

### Recommended technique

#### Pentobarbitone sodium

By far the most acceptable technique for euthanasia of dogs and cats is the intra-venous administration of sodium pentobarbitone at a dose rate of 150 mg/Kg with almost immediate unconsciousness followed by respiratory depression. The intraperitoneal administrative route may take up to 15 minutes and aesthetically is considered less acceptable. A concentration of 60mg/ml is not associated with side effects, but evidence exists that shows veterinary euthanasia pentobarbitone sodium solutions formulated at greater than 300 mg/ml may be associated with peritoneal irritation and resulting pain and therefore fast acting local anaesthetic should be added to the solution prior to i/p administration.

### Technique Acceptable with Reservations

#### Halothane

Halothane is placed in the acceptable with reservations category because of OH&S considerations at concentrations of greater than 2ppm and because high concentrations are irritant.

#### Carbon monoxide

Carbon monoxide is a very dangerous gas in so much as it odourless and colourless but lethal at 3% and therefore should be regarded as hazardous to personnel and only used with extreme caution. It has been used extensively for euthanasia of unwanted dogs and cats and unconsciousness and death occurs quickly without pain or appreciable discomfort.

## Non-Human Primates

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"> <li>• <b>Inhalant:</b> Carbon dioxide preceded by isoflurane (Marmosets only)</li> <li>• <b>Injectable:</b> Pentobarbitone sodium</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Inhalant:</b> Halothane, Carbon monoxide</li> </ul>
PHYSICAL	
Please note there are no recommended physical procedures	

### Recommended technique

#### Pentobarbitone sodium

This is given intravenously, following physical or chemical restraint, at a dose rate of 60mg/Kg. Non-human primates are associated with a number of human safety concerns and therefore euthanasia must only be performed by trained, experienced personnel with at least one other to assist. Physical restraint includes squeeze cages for larger primates and holding with appropriately gloved hands for smaller species. Appropriate chemical restraint is provided by ketamine hydrochloride given by the intra-muscular route at approximately 20 mg/Kg. For marmosets only alphaxalone/alphadalone may be administered i/m into the large muscles of the hind leg at a dose rate of 18mg/Kg.

#### Carbon dioxide preceded by isoflurane

This is only appropriate for marmosets where it may be piped into a dark plastic bag to produce rapid unconsciousness with minimal distress. This is definitely not recommended for larger primates where the time to unconsciousness is slow

## Sheep and Goats

Recommended	Acceptable with Reservations
CHEMICALS	
• <b>Injectable:</b> Pentobarbitone sodium i/v	
PHYSICAL	
Please note there are no recommended physical procedures	• Captive bolt

### Recommended technique

#### Pentobarbitone sodium

This is normally given by the i/v route using the jugular vein at a dose rate of 60mg/Kg with the animal restrained in a sitting position and the head laterally inclined to make the jugular apparent. In sheep wool may need to be removed to enable the vein to be seen. Care should be taken in disposing of the carcass after euthanasia with this technique to ensure that it is not consumed by animals or humans.

### Technique Acceptable with reservation

#### Captive bolt

This method is acceptable in the hands of a trained operator but reflex movements following euthanasia may be aesthetically objectionable. Experience would indicate with horned and hornless animals the best site of placement is 1cm behind the nuchal crest and aimed vertically downward towards the gullet.

## Pigs

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> None</li><li>• <b>Injectable:</b> Pentobarbitone sodium i/v</li></ul>	
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"><li>• Captive bolt</li></ul>

### Recommended technique

#### Pentobarbitone sodium

This is administered i/v via the ear vein at a dose rate of 150mgs/Kg. Dilation of the ear vein to facilitate administration may be achieved by warming the ear.

### Technique Acceptable with reservations

#### Captive bolt

The captive bolt should be placed at the intersection of lines drawn from each eye to the opposite ear. The operator must ensure that the captive bolt is of an appropriate size with appropriate sized cartridges. A cattle captive bolt pistol is appropriate for larger pigs.

## Amphibia

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> None</li><li>• <b>Injectable:</b> Pentobarbitone sodium</li><li>• <b>Skin absorption:</b> MS-222, benzocaine, chloral hydrate</li></ul>	
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"><li>• Stunning and decapitation</li><li>• Stunning followed by pithing</li></ul>

### Recommended technique

#### Pentobarbitone sodium

Effective at a dose rate of 60mg/Kg. The intravenous route is recommended where possible especially when using concentrated euthanasia solutions, otherwise the i/p route may be used using the 60mg/ml solution usually used for anaesthetic purposes.

### Skin absorption

#### Chloral hydrate

For frogs and toads place the animal in a container holding a 2–3 mm layer of 3% solution of chloral hydrate. This is absorbed through the ventral skin and the animal dies in a relaxed state within a few minutes.

#### MS-222, benzocaine

Tricaine methane sulphonate (Buffered MS-222) or benzocaine dissolved in water can be used similarly to chloral hydrate but in both cases, because of reduced pH both solutions should be neutralised with bicarbonate to reduce possible skin irritation.

### Acceptable with Reservations

Stunning and decapitation, and pithing following stunning are acceptable but operators must be well trained and experienced in the methods.

### Technique Acceptable with Reservations

## Reptiles

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> None</li><li>• <b>Injectable:</b> Pentobarbitone sodium</li></ul>	
PHYSICAL	
Please note there are no recommended physical procedures	

### Recommended Technique

#### Pentobarbitone sodium

A humane method at a dose rate of 60mg/Kg. The i/v route is recommended where possible particularly when using the high concentrate (Greater than 300mg/ml) solutions. 60mg/ml solutions may be used i/p.

## Birds

Recommended	Acceptable with Reservations
CHEMICALS	
<ul style="list-style-type: none"><li>• <b>Inhalant:</b> Carbon dioxide (Chicks)</li><li>• <b>Injectable:</b> Pentobarbitone sodium</li></ul>	<ul style="list-style-type: none"><li>• <b>Inhalant:</b> Carbon dioxide (adult birds)</li></ul>
PHYSICAL	
Please note there are no recommended physical procedures	<ul style="list-style-type: none"><li>• Cervical dislocation (Chicks, small and medium birds only)</li><li>• Shooting</li></ul>

### Recommended Technique

#### Pentobarbitone sodium

Use a dose rate of at least 80mg/kg given i/p using a 60mg/ml anaesthetic solution.

#### Carbon dioxide

Recommended for chicks only. Chicks should remain in the CO<sub>2</sub> chamber for at least ten minutes.

Acceptable with reservations with older birds but chamber should be filled with carbon dioxide before putting birds in. Excessive flapping with older birds following loss of consciousness may be aesthetically unpleasant.