

# Charles Darwin University Animal Ethics Committee

## Standard Operating Procedure:

### GSOP 07.2022 Euthanasia of research animals in the field

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#### NOTE:

- For cane toad euthanasia, please refer to GSOP 02.2019 Cane Toad Euthanasia



## WILDLIFE ETHICS COMMITTEE

### Euthanasia of research animals in the field policy

The euthanasia of any animal must be achieved in the shortest time possible, with the minimum of distress. Intravenous (into a vein), intracardiac (into the heart – only suitable in a previously sedated animal) or intrahepatic (into the liver) injection of specially formulated euthanasia solutions offers almost instantaneous death. Where these routes are not available, suitable alternatives may be considered. These include intraperitoneal (into the abdominal cavity) injection if a non-irritant solution is used, except in birds.

The recommendations in this policy are intended for remote or field situations. Where full veterinary facilities are available, there may be more appropriate alternatives (such as inhalant anaesthetics), which should be used in preference to the methods outlined here.

Any person involved in euthanising animals must be familiar with the anatomical aspects associated with the particular species with which they are dealing. All operators need to have sufficient familiarity with the properties and required doses of the drugs with which they are dealing, and an ability to accurately estimate body weights to ensure suitable doses administered.

#### **1. BARBITURATE EUTHANASIA – GENERAL COMMENTS**

Barbiturate euthanasia is the most commonly employed method for euthanasia of research animals in the field, such as for the acquisition of voucher specimens. Sodium pentobarbitone is the most commonly used barbiturate for euthanasia. Pentobarbitone is licensed for the euthanasia of a number of domestic animals. Recommendations for appropriate use of pentobarbitone in wildlife species are provided below.

##### **1.1 Pentobarbitone formulations and dilution**

Lethabarb® (Virbac (Australia)) is a 325 mg/mL solution of pentobarbitone intended for intravenous use. It is not appropriate to administer pentobarbitone at this concentration via any other route of administration in a conscious animal as it is very irritant to tissues when injected outside a vein.

Pentobarbitone is also available commercially as a 60 mg/mL solution (e.g. Ilium Pentobarbitone), licensed for use as an anaesthetic agent in a number of domestic animals. Note that the dosages provided on the label of such products are for anaesthesia, not euthanasia.

Extra-vascular administration of pentobarbitone (injection into the abdomen, liver etc.) should only be done using dilutions of 60 mg/mL or less to avoid unacceptable tissue irritation and pain.

Diluting Lethabarb down to 60 mg/mL with water or saline prior to injection is acceptable. For intra-abdominal injection, care should be taken to avoid injection directly into the stomach or intestines as this slows the absorption and subsequent effect of the drug.

Muscle spasms may result from injection with pentobarbitone at 60 mg/mL in small reptiles. It is recommended that 6 mg/mL pentobarbitone be used for such animals.

### **1.2 Pentobarbitone dosage (dose rate) for euthanasia**

The recommended dosage of sodium pentobarbitone for euthanasia of wildlife is 150 mg/kg bodyweight.

### **1.3 Dose calculations**

The dose is the amount of drug that will be administered. For pentobarbitone this is generally expressed in milligrams (mg). The volume of drug to be injected (mL) depends on both the dose required (mg) and the concentration of the drug in solution (mg/mL).

The following formula may be useful:

**Volume (in ml) = dose rate (in mg/kg) x bodyweight (in kg) / concentration (in mg/ml).**

### **1.4 Sedation**

In medium to large mammals, birds and reptiles, sedation prior to euthanasia is strongly recommended. This reduces stress to both animal and operator, and facilitates easy access to the preferred route for injection. Sedation requires specific veterinary expertise.

### **1.5 Operator responsibilities**

Euthanasia solutions are not selective in their actions and operators carry a public health responsibility to ensure the correct use, safe storage and disposal of all material in their possession. A licence from SA Health is required to possess controlled substances. Suitable logbooks should be maintained to monitor drug usage, in accordance with SA Health requirements.

Great care should be taken to avoid exposing the operator, assistants or the environment to **any** euthanasia solutions, as they will be absorbed orally, through broken skin or mucus membranes (such as the eye). Less than 1 mL of barbiturate can result in symptoms of narcosis (drowsiness) in an adult person. Appropriate protection methods (eye protection; face mask; nitrile gloves) should be used where necessary

## **2. RECOMMENDATIONS FOR EUTHANASIA OF SPECIFIC TAXA**

### **2.1 Small birds** (e.g. wren, finch, miner, myna, starling)

Intravenous (or intrahepatic) injection of a non-irritant pentobarbitone solution is recommended. An intracoelomic route is not recommended for all but skilled operators due to the presence of large air sacs from which drugs are poorly absorbed. Use a fine gauge needle (25-27G). Carbon dioxide in a sealed environment is suitable for small birds (up to 300 grams).

### **2.2 Medium-sized birds** (e.g. galah, kookaburra, duck, ibis)

Intravenous or intrahepatic injection of a non-irritant pentobarbitone solution is recommended (see Wildlife Ethics Committee (WEC) Policy on Collection of Blood for recommended veins). In a

previously sedated or a moribund animal, the intracardiac route can also be used. Use a 23-27G appropriate to the size of the bird. Carbon dioxide used in a sealed environment is acceptable where large numbers of birds are involved and where disposal of contaminated carcasses would present a hazard; refer to the relevant Code of Practice (COP) listed below. A firearm is acceptable where injectable anaesthetics are not possible; refer to the relevant COP listed below for appropriate firearms and shot sizes.

### **2.3 Large birds** (e.g. goose, emu, pelican)

In a previously sedated or moribund animal, an intravenous or intracardiac injection of pentobarbitone can be used (see WEC Policy on Collection of Blood for recommended veins). Use a 23-27G appropriate to the size of the bird. A captive bolt or firearm is acceptable where injectable anaesthetics are not possible; refer to the relevant COP listed below for appropriate firearms and shot sizes.

### **2.4 Small mammals** (e.g. mouse, rat, dunnart, insectivorous bat)

Intra-abdominal (peritoneal) or intrahepatic injection of a non-irritant pentobarbitone solution is recommended. Use a fine gauge needle (25-27G). Carbon dioxide used in a sealed environment is acceptable for mammals up to 300 grams.

### **2.5 Medium-sized mammals** (e.g. possum, cat, fox, rock wallaby, echidna, koala)

Intravenous injection of pentobarbitone, or intrahepatic or intra-abdominal injection of a non-irritant pentobarbitone solution, is recommended. In a previously sedated or moribund animal, an intracardiac route can also be used (see WEC Policy on Collection of Blood for recommended veins). Use a 21-25G needle appropriate to the size of the animal. A captive bolt or firearm is acceptable in the absence of injectable anaesthetics, or where use of a firearm will avoid the distress of capture and handling; refer to the relevant COP listed below.

### **2.6 Large mammals** (e.g. wombat, dingo, kangaroo, pig)

In a previously sedated or moribund animal, an intravenous or intracardiac injection of pentobarbitone solution can be used (see WEC Policy on Collection of Blood for recommended veins). Use a 19-24G needle appropriate to the size of the animal. A captive bolt or firearm is acceptable where injectable anaesthetics are not possible; refer to the relevant COP listed below for appropriate firearms, shot sizes and point of aim.

### **2.7 Reptiles**

Intracoelomic or intrahepatic injection of a non-irritant pentobarbitone solution is recommended. For small reptiles, the 60 mg/mL solution of pentobarbitone can result in muscle spasms that distort a specimen for vouchering purposes. Therefore a tenfold dilution, to make a 6 mg/mL solution of pentobarbitone, is recommended; note that the recommended dosage (150 mg/kg) still applies. Use a 23-30G needle depending on the size of the animal. For very small reptiles, where even small gauge needles may cause undue pain, suitably diluted pentobarbitone may be administered orally.

### **2.8 Frogs and tadpoles**

Intracoelomic or intrahepatic injection of a non-irritant pentobarbitone solution is recommended. In a previously sedated or moribund animal, an intracardiac or intravenous route can be used (see WEC Policy on Collection of Blood for recommended veins). Use a 26-30G needle depending on the size of

the animal. Tricaine methane sulphonate (MS-222) in an appropriately buffered solution is also a suitable euthanasia agent for adult amphibians or tadpoles, either via intracoelomic injection or via prolonged immersion as for fish. Application of a 20% benzocaine over-the-counter oral gel to the ventral skin of amphibians is acceptable. Placing a frog in a shallow bath of a 3% solution of chloral hydrate is another acceptable method. Tadpoles may be euthanised with clove oil as for fish.

## **2.9 Fish**

Clove Oil (available from pharmacies) may be used via prolonged immersion at a dose rate of 10 drops per litre of water (0.65 mL/L); dissolving the clove oil in a small volume of ethanol improves its solubility in water. Tricaine Methane Sulfonate (MS-222) is very effective but must be neutralised with buffering agents to prevent irritation. Dose rates for MS-222 vary with species, but generally 250 mg/L is appropriate. The fish should be left in the clove oil or MS-222 solution for at least 10 minutes following the last observed movement. Wherever possible, this should be followed by decapitation, cervical dislocation or exsanguination (e.g. by severing gill arches) to ensure that death has occurred. If a physical technique of euthanasia is used, it should entail the physical destruction of brain tissue by pithing or crushing the brain.

## **2.10 Humane killing of pest animals**

Most feral animals have a high reproductive capability, and killing a few individuals will have no significant impact on local populations, and thus no conservation benefit. The use of euthanasia drugs results in problems disposing of carcasses, which need to be incinerated or deeply buried. Carbon dioxide may be impractical or hazardous to use in the field. For these reasons, the position of the Department for Environment and Water is that the routine killing of feral animals is not required. However, if the killing is to prevent the establishment of feral animals in a region where they do not currently occur, or it will have a direct conservation benefit to native species, or it will result in useful knowledge (e.g. from stomach contents) AND it can be carried out humanely by suitably skilled operators, then it can be considered in WEC applications. Researchers should also be aware that under the *Natural Resources Management Act 2004* it is an offence to release non-native animals unless an exemption has been provided.

## **3. EMERGENCY SITUATIONS**

It may be necessary to euthanise an animal in the absence of appropriate drugs. For example, in remote areas, or where an animal is suffering acutely and cannot be immediately transported to a veterinarian. In these instances, a method should be employed that achieves rapid loss of consciousness and death in the shortest time as is possible, with the minimum of distress. If a firearm is available, then the animal should be killed with a head shot, although a chest (heart) shot may be used if a head shot is not possible. For smaller animals, a blow to the rear of the skull delivered with sufficient force to immediately destroy the brain may be used. Whatever method is employed, the euthanasia must be carried out by a suitably skilled and experienced operator.

#### **4. UNACCEPTABLE METHODS OF EUTHANASIA**

- It is not appropriate to use Lethabarb or similarly concentrated solutions directly into the abdomen due to their irritant nature unless suitably diluted. For this route the non-irritant anaesthetic-grade solutions should be used.
- Intrathoracic (into the chest) injections are not a suitable route for euthanasia. Fluids of any nature given into lung tissue are likely to cause the animal distress as drowning and anoxia will occur.
- Ether and Chloroform are both irritant and not considered appropriate as inhalant anaesthetics. Their volatile nature makes them unsafe.
- Exsanguination (bleeding) is only suitable with an already anaesthetised animal.
- The use of car exhaust fumes is not generally an acceptable method of euthanasia. While car exhaust does contain carbon monoxide, the concentration of this gas is not usually adequate to cause a rapid death. In addition, car exhaust is hot and contains other gases which are highly irritating to the respiratory tract. Cooled exhaust from four-stroke petrol engines may be appropriate for some species.
- The euthanasia of any animal, including reptiles, amphibians and fish, via freezing is unacceptable (except where the animal has first been anaesthetised). A review of scientific literature has failed to provide adequate evidence that reptiles, amphibians or fish become insensitive to pain during the cooling/freezing process, and the formation of ice crystals within the body is likely to cause pain.
- Carbon dioxide or other inhalants are not appropriate for use in reptiles or amphibians due to their relatively low respiratory rates, ability to breath-hold and resistance to the effects of hypoxia.

#### **5. REFERENCES**

Guidelines for Euthanasia of Nondomestic Animals. Charlotte Kirk Baer (Ed). American Association of Zoo Veterinarians (AAZV). 2006

Euthanasia of Animals Used for Scientific Purposes. J.S. Reilly (Ed) Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCAART) 2<sup>nd</sup> Edition 2001 (Currently under Revision)

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Material Safety Data Sheet, Lethabarb Euthanasia Injection. Virbac (Australia) Pty. Ltd.

Wildlife Ethics Committee Collection of Blood from Wildlife Policy.

Relevant codes of practice for the humane destruction of wildlife can be found on the Department for Environment and Water website:

[http://www.environment.sa.gov.au/managing-natural-resources/Plants Animals/Animal welfare/Codes of practice/Codes of practice for the humane destruction of wildlife](http://www.environment.sa.gov.au/managing-natural-resources/Plants%20Animals/Animal%20welfare/Codes%20of%20practice/Codes%20of%20practice%20for%20the%20humane%20destruction%20of%20wildlife)

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