

Charles Darwin University Animal Ethics Committee

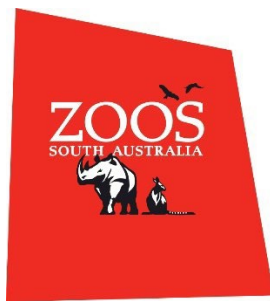
Standard Operating Procedure:

Euthanasia of research animals in the field policy (SA DEW)

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NOTE: The CDU AEC approves the following SOP with the below amendments/conditions:

- This SOP is primarily intended for voucher specimen collection and is most applicable for practitioners skilled in injectable methods
- Please refer to alternative SOPs for other non-injectable field euthanasia techniques where necessary (for example Euthanasia of Animals under Field Conditions (WA DBCA) and Cane Toad Euthanasia (Sharp, Lothian, Munn & Saunders)).



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 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, care must be taken in birds to avoid intracoelomic air sacs.

Most wildlife research projects do not require death as end point. The most likely situation when this would be necessary is for the collection of voucher specimens, therefore morphological preservation of the carcase is required and injectable methods are most appropriate. Unexpected events may also arise where a decision is made to euthanise an animal to prevent unreasonable or unnecessary suffering.

The recommendations in this policy are intended for remote or field situations. Where full veterinary facilities are available, there may be more appropriate alternatives (with the assistance of 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. They should also be able to confirm that death has occurred.

1. BARBITURATE EUTHANASIA – GENERAL COMMENTS

Barbiturate euthanasia is the most commonly employed method for injectable 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

Letharb® (Virbac (Australia)) is a 325 mg/mL solution of pentobarbitone intended for intravenous use only. 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 due to its high pH.

A non-irritant Pentobarbitone solution is also available at a 60 mg/mL (e.g. Ilium Pentobarbitone), which is licensed for use as an anaesthetic agent in several domestic animals, although production is intermittent due to limited commercial demand. NB the dose rates provided on the label of such products are for anaesthesia, not euthanasia and should therefore be disregarded.

Extra-vascular (outside the circulation) administration of pentobarbitone (injection into the abdomen, liver etc.) in conscious animals should only be done using non-irritant solutions (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 as a substitute if the commercial non-irritant pentobarbitone solution is not available. 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 dilution to 6 mg/mL pentobarbitone be used for such animals.

1.2 Pentobarbitone dosage (dose rate) for euthanasia

The recommended minimum dose rate of sodium pentobarbitone to affect euthanasia of wildlife is **150 mg/kg** bwt (bodyweight) in all situations. The delivery of higher dose rates achieves the same outcome (death) but lower dose rates may be inadequate.

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 total dose required (mg) and the concentration of the drug in solution (mg/mL).

The following formula may be useful:

Volume (in ml) = dose rate (150 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 by chemical means often requires specific veterinary expertise. Sedation will also be observed as the euthanasia solution is being absorbed.

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.

1.6 Confirmation of Death

It is imperative that death is confirmed before the carcass is left unattended or disposed of. The lack of reflexes, and continuous cardiac (lack of heartbeat) and respiratory (lack of breathing) arrest should be observed. In any doubt further doses of euthanasia solution can be given via more direct routes in the now sedated animal or secondary physical measures such as brain tissue destruction (per emergency situations below), decapitation, cervical dislocation or exsanguination will ensure that death has occurred. The processing of a carcass for vouchering purposes will enact secondary physical measures.

2. RECOMMENDATIONS FOR EUTHANASIA OF SPECIFIC TAXA

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

If intravenous routes are not available then an 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 (see Wildlife Ethics Committee (WEC) Policy on Collection of Blood for recommended veins) injection of pentobarbitone or intrahepatic injection of a non-irritant pentobarbitone solution, is recommended. In a previously sedated or a moribund animal, the intracardiac route can also be attempted with a needle of sufficient length. 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 from a distance 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 voucherizing 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, oral delivery of diluted pentobarbitone (6mg/ml dilution) is recommended.

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 gill 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.

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) injection is 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 fourstroke 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

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[Wildlife Ethics Committee collection of Blood from Wildlife Policy](#)

[Humane destruction of wildlife Department for Environment and Water](#)

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