# Standard Operating Procedure

# SC24-11 EUTHANASIA OF ANIMALS UNDER FIELD CONDITIONS

Animal welfare is the responsibility of all personnel involved in the care and use of animals for scientific purposes.

Personnel involved in an Animal Ethics Committee approved project should read and understand their obligations under the *Australian code for the care and use of animals for scientific purposes*.

Version 1.6 August 2024



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1.6	07/08/2024	Amendment to Table 1b regarding crocodile hatchlings.	A. Robey	J. Richards	August 2024

<sup>\*</sup>Previously titled Humane Killing of Animals under Field Conditions in Wildlife Management.

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## 1 Acknowledgements

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## 2 Purpose

This Standard Operating Procedure (SOP) provides direction on the euthanasia of animals under field conditions for Department of Biodiversity, Conservation and Attractions (DBCA) personnel conducting biological surveys, fauna monitoring and research programs, translocations, relocations, introduced pest animal control, and handling sick, injured, orphaned and seized fauna.

DBCA personnel may be required to euthanase animals:

- 1. as a welfare action for animals with untreatable injuries, illness or disease;
- 2. as a welfare action for orphaned young that cannot be brought into care;
- 3. as a requirement of a research procedure approved by an Animal Ethics Committee (AEC);
- 4. for vouchering purposes approved by an AEC;
- 5. as a requirement for live-captured animals where release is prohibited (e.g., declared pests);
- 6. for the purposes of diagnostics and disease screening.

The word "euthanasia" is derived from the Greek and translates directly as "good death". Some texts use the term "humane killing" to refer to euthanasia conducted for purposes other than welfare (such as points 3-6 above). However, in the context of this document, the term "euthanasia" will be used to cover all the activities described above and is defined as the act of inducing a humane death, by using species-appropriate techniques that result in a rapid and irreversible loss of consciousness and minimum pain and distress to the animal.

## 3 Scope

This SOP applies to all animal activities under field conditions that are undertaken across Western Australia by DBCA (hereafter department) personnel. It has been written for both scientific and animal management purposes and is endorsed by the department's Animal Ethics Committee (AEC). All department personnel involved in animal research, and all personnel involved in the taking of fauna under licences other than research, should be familiar with the content of this document.

Projects involving fauna may require a licence/authorisation under the *Biodiversity Conservation Act 2016*. Personnel should consult the department's Wildlife Licensing Section and Animal Ethics Committee Executive Officer for further guidance.

This SOP may also be used as a guide by Natural Resource Management groups, consultants, researchers and other individuals or organisations undertaking field activities that may require euthanasia of animals.

## 4 Animal Welfare Considerations

Recommendations for euthanasia of free-ranging animals draw extensively on published euthanasia guidelines, which are often based on observations of animals in relatively controlled environments. While these guidelines are a very important resource for driving ethical and humane decision-making for wildlife euthanasia, it is also important to recognise that they may be a poor fit for some field scenarios. The management of free-ranging wildlife may justify certain decisions and euthanasia methods, which would not necessarily meet the expectations for what is considered humane in more controlled circumstances. The field operator is therefore encouraged to think carefully about the considerations of the specific circumstances in order to make the most appropriate decisions about when and how to undertake euthanasia. This section gives some guidance on the important considerations.

### 4.1 Making a decision to euthanase

The decision to undertake euthanasia should not be made lightly, regardless of why it is being undertaken. Any decision to euthanase should include due consideration of the following factors:

#### 1. Animal welfare

What is the animal's current and future welfare? Is the animal experiencing continuous suffering? Will euthanasia result in unacceptable levels of additional stress and pain for this individual and/or for other animals within perception range? Are there options other than euthanasia, which will address welfare needs equally or better than euthanasia?

#### 2. Human safety

Will my actions create unacceptable human safety risks?

#### 3. Logistics

Am I adequately informed, trained, equipped and resourced to carry out euthanasia? Consider pre-euthanasia arrangements, animal handling, euthanasia methods and agents, confirmation of death and post-euthanasia disposal.

#### 4. Personal, emotional and professional integrity

Am I comfortable with my decision? If there is something that troubles me, what is it? Am I motivated by the right reasons? Is euthanasia warranted morally or scientifically? Are my actions aligned with DBCA policy and principles?

#### 5. Overall outcomes

Consider Points 1-4 above as a whole. Does euthanasia produce the best possible balance of benefits over harms in this situation?

#### 4.2 Use of euthanasia as a welfare tool

The decision to euthanase an animal to alleviate suffering must be based on weighing up the perceived degree of current and (likely) future suffering and the chances of recovery for return to the wild, against the prospective suffering caused by the euthanasia process. Guidance criteria for decision making are provided in Figure 1. This process is aligned with the Biodiversity Conservation Regulations (2018); further information on decision making in relation to rehabilitation can be found in the department *Code of Practice for Wildlife Rehabilitation in Western Australia March 2020*.

If an animal to be euthanased has dependent offspring, then the dependent offspring must also be euthanased unless alternative care is appropriate and available (see Figure 1). Rehabilitation of dependent offspring must only be considered if consistent with the department guidelines *Making Decisions on the Fate of Rehabilitated Fauna* and the department *Code of Practice for Wildlife Rehabilitation in Western Australia*. See also department SOP for *Care of Evicted Pouch Young*.

For a list of Threatened species in Western Australia, refer to the department's Threatened Species and Communities webpage: (<a href="https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities">https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities</a>).

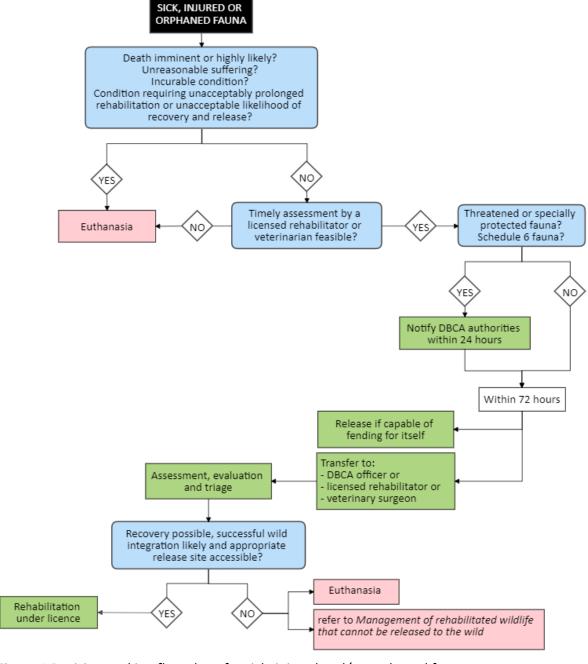


Figure 1 Decision-making flow chart for sick, injured and/or orphaned fauna

#### 4.3 Documentation and authorisation to conduct euthanasia

In Western Australia, any person using animals for scientific purposes must also be covered by a licence issued under the *Animal Welfare Act 2002*. This SOP complements the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (The Code). The Code contains an introduction to the ethical use of animals in wildlife studies and should be referred to for broader issues. A copy of the code is available on the National Health and Medical Research Council website (http://www.nhmrc.gov.au).

Department monitoring and research projects involving animals must have a documented Euthanasia Action Plan, which is identified in the AEC application and endorsed by the AEC. This action plan should identify and address all potential situations where euthanasia may be required as part of the tasks and duties to be undertaken by personnel and the protocol assigned to manage individual situations. Personnel with adequate training and experience in the proposed euthanasia technique/s, as documented in the plan, must be present or accessible when carrying out field-based duties. If external support and assistance is planned (e.g., veterinary assistance), this should be pre-arranged and documented. All personnel must be aware of the agreed action plan prior to undertaking field work. Where unforeseen situations occur that are not designated under an action plan, general guidance for decision making should be taken from this SOP.

For projects approved by the department's AEC, all adverse events, including unplanned euthanasia, must be reported in writing to the AEC Executive Officer immediately following the incident, or at the soonest opportunity (as per 2.2.28 of The Code) by completing an *Adverse Events Form* available on the department's AEC Intranet webpage.

All animal deaths (including euthanasia) and injuries should be recorded and communicated to the Chief Investigator of the project. Chief Investigators must provide statistics of all animal deaths (including euthanasia) and injuries in annual reports submitted at the end of the year and at the completion of a project.

ANIMAL WELFARE: To minimise the animal welfare impacts of handling stress, animals being assessed or restrained for euthanasia should be handled with competence and care, and for the minimum time possible. Improper restraint can lead to major physiological disturbances (hyperthermia, stress, shock, myopathy), injury, and other negative impacts on welfare. Any negative welfare impacts on the animal must be taken into consideration when selecting an appropriate euthanasia method, as the need for prolonged or stressful handling may outweigh the benefits of the proposed euthanasia technique. Refer to the department SOPs for Animal Handling and Restraint using Soft Containment and Hand Restraint of Wildlife, and Section 6 of this SOP, for further guidance.

## 5 Equipment

The following equipment is required to conduct euthanasia:

 euthanasia tool/tools specific to the method to be used as outlined in Section 6 (e.g., firearm; blunt trauma object; euthanasia chemicals). Consideration should also be given to the equipment required to provide options for secondary euthanasia method/s (e.g., pithing needle);

- personal protective equipment specific to the euthanasia method (see Section 12);
- first aid kit;
- depending on the species, it may be useful to have equipment to assist in the confirmation of death (e.g., pen light to check pupillary responses, stethoscope).

## 6 Selecting a euthanasia method

To bring about "a good death", a euthanasia method should:

- induce loss of consciousness and death with a minimum of pain and distress;
- induce a rapid, irreversible loss of consciousness;
- produce a reliable outcome when used appropriately.

When deciding on a euthanasia method, consider the following factors:

<u>Animal biology, physiology and health status</u>: Is the selected method acceptable for this species, at its current age and stage of development (see Table 1a and 1b)? Does the animal's health status preclude or promote the use of a particular method?

<u>Potential for further suffering</u>: Is the selected method likely to cause an unacceptable level of pain, discomfort or stress to the animal prior to euthanasia (consider requirements for transport and handling)? Are animals within perception range likely to be adversely affected? If the method requires external expertise (e.g., veterinary involvement), can this be sourced in a timely way?

<u>Human safety</u>: Is the proposed method safe to administer in the specific situation? Can the chosen method be safely and effectively administered by the available personnel?

<u>Authorisation</u>: Is the proposed euthanasia method approved by this SOP? Are available personnel authorised for its use in the specific situation at hand?

<u>Logistics</u>: Are available personnel adequately informed, trained, equipped and resourced to carry out the selected method of euthanasia? Consider pre-euthanasia arrangements, palliative care, animal handling, euthanasia agents, confirmation of death and posteuthanasia disposal.

<u>Personal, emotional and professional integrity</u>: Does the chosen method induce unacceptable anxiety or discomfort in the operator, other personnel or bystanders? Has the level of understanding and perceptions of those witnessing the euthanasia (both staff and bystanders) been considered, and/or their exposure to the process mitigated? Are the proposed actions aligned with DBCA policy and principles?

<u>Post-euthanasia disposal</u>: Does the proposed disposal method create unacceptable environmental or safety risks? (e.g., risk of harmful residues from euthanasia chemicals to non-target scavengers; heavy lifting; zoonosis).

In addition to following a Euthanasia Action Plan, expert judgement may be required to determine the most appropriate means to proceed. The following experts, in order of priority, can be contacted to assist in decision making:

 a veterinarian experienced in the management of the species in question. It is preferable to have a source of veterinary expertise pre-selected and documented in the euthanasia plan;

- a department Wildlife Officer;
- the DBCA Animal Ethics Office

Euthanasia must be performed by a person competent in, and qualified for, the method to be used, or under the supervision of a competent, authorised person.

## 7 Acceptable euthanasia methods

The methods recommended for the euthanasia of animals in various field situations are described in this section.

Euthanasia methods not described in this SOP are generally considered either unsuitable for field conditions in the context of this document or are not acceptable from a welfare perspective for field euthanasia of animals. However, there are situations in which methods not described or recommended in this SOP may be valid, subject to approval by department's AEC. For example, as knowledge and experience with their use grows, captive bolt devices may be approved as suitable for use in an expanded range of circumstances or species.

Table 1a and 1b summarise methods of euthanasia for various vertebrate taxa. Methods which are evaluated as "acceptable with conditions" are those techniques that may require certain conditions to be met to consistently produce humane death, may have greater potential for operator error or safety hazard, are not well documented in the scientific literature, or may require a secondary method to ensure death. Methods acceptable with conditions are equivalent to acceptable methods when all criteria for application of a method (as outlined in this SOP) can be met.

Table 2 is a qualitative comparison of each method.

Note: These tables must always be used in conjunction with the explanatory information that follows.

## 7.1 Firearms (shooting)

Shooting is a quick and effective means of euthanasing larger animals in field situations, particularly where animals cannot be safely handled and restrained. Shooters can euthanase an animal from a distance, and with the correct placement of an accurate shot, it produces an instant or rapid death.

Shooting is considered an acceptable means of euthanasia for medium and large size animals of most taxa. In smaller animals, other methods are likely to be more reliable and present better safety outcomes for humans.

DBCA SOP 002 Use of Firearms for the Humane Destruction of Animals outlines the instructions related to the use of firearms for euthanasia. SOP 002 Appendix 3 Guide for the Humane Destruction of Animals Using Firearms provides diagrams illustrating points of aim for commonly encountered species, as well as a guide to the recommended firearms, calibre and shot specifications for different sizes and taxa of animals.

Shooting requires specialised equipment and must only be carried out by personnel who have completed training recognised by the department, who have been issued a nominated persons authorisation by Western Australian Police and who are listed on the department's Corporate Firearms Licence.

Firearm users must strictly observe all relevant safety guidelines relating to firearm ownership, possession and use as outlined in DBCA Corporate Policy Statement 20 Departmental Use of Firearms, DBCA Corporate Guideline No. 42 Departmental Use of Firearms 2020 and associated SOPs. These documents are located on the Corporate Firearms page of the Regional and Fire Management Services (RFMS) Intranet.

Firearms are not appropriate for the euthanasia of cetaceans >7 m long (see department SOP *Euthanasia of small cetaceans using firearms*). The appropriateness of firearms for whales 6-7 m long should be considered on a case-by-case basis with the advice of staff experienced in the euthanasia of whales of this size. Under specific circumstances, experienced staff may consider the use of explosive charge methods to be the most appropriate euthanasia method for whales over 6 m long. This method must only be undertaken by department personnel specifically trained in the technique. See Coughran *et al.* (2012) for a description of this technique.

### 7.2 Captive bolt device

Captive bolt devices (CBDs) are designed for the euthanasia of animals. Their mode of action is to cause concussion and trauma to the brain and brainstem, leading to immediate loss of consciousness and subsequent death by disruption of central brain functions.

CBDs may be a useful alternative to firearms in situations where firearms cannot be lawfully or safely used. Their use requires firm restraint of the animal, which may increase the stress for the individual.

CBDs can be either penetrative (PCBD: the bolt penetrates the skull) or non-penetrative (NPCBD: the bolt produces unconsciousness without entering the skull). Currently, two models of PCBD are approved for DBCA use; no NPCBD models are currently approved.

The department SOP for *Euthanasia of animals using captive bolt devices* contains all the details of the authorisations, approvals and techniques required for the safe and effective use of approved CBD models. Note: for all species where CBD use is approved, the minimum skull diameter requirements for the specific model of CBD must be met. See SOP *Euthanasia of animals using captive bolt devices* for details.

#### 7.3 Blunt force trauma

This technique involves delivering a hard, sharp blow to the base of the skull. It is an acceptable form of euthanasia if a single sharp blow is delivered with sufficient force to produce immediate loss of consciousness through depression of the central nervous system and destruction of brain tissue.

The blow may be delivered with a heavy, blunt instrument such as a broad hammer or tyre iron. The animal should be sufficiently restrained to ensure accuracy of delivery of the blow and safety of the operator. Devices such as blankets, towels, bags or sacks may assist with immobilisation, reducing animal awareness and quietening animal activity. For very small animals (<200 g), an alternative technique is to hold the animal firmly by the hindquarters (around the top of the back legs and base of tail) and then swing firmly and quickly in an arc so that the rear of the animal's head is hit against a large solid surface that will not move or compress during the impact.

Blunt force trauma is inexpensive, and is effective when delivered by experienced operators. It is recognised that in remote field situations, it may be the only practical means of euthanasia for small animals. However, it can be unpleasant to carry out and should only be used where the animal can be sufficiently restrained to ensure the operator carries out the method quickly and effectively. Its use in the context of this SOP is restricted to small to medium-sized individuals in certain taxa (Table 1a and 1b). A secondary means of euthanasia should be available whenever blunt force trauma is the intended primary method (see Section 8).

#### 7.4 Cervical dislocation

Cervical dislocation leads to separation of the skull and the brain from the spinal cord by traction applied behind the base of the skull. This damages the brain stem, leading to respiratory and cardiac arrest. When performed by well-trained individuals on the appropriate animals, this method is humane and does not require specialised equipment. However, it requires significant technical skill; the operator must be confident of performing the technique quickly and effectively and should have a secondary means of euthanasia planned. This method should not be used where the animal cannot be sufficiently restrained.

Cervical dislocation without chemical restraint is an acceptable method of euthanasia for small birds and rodents that are easily handled (up to 200 g).

The technique varies depending on the species. For rodents, the animal is held prostrate on a solid surface, with the thumb and forefinger of the operator firmly squeezing the neck behind the head of the animal. The hindquarters are grasped firmly with the free hand and pulled caudally away from the head. An instrument such as scissors or a steel rod can be used in place of the thumb and forefinger. For birds, the legs are taken in one hand and the head held between the first two fingers of the other hand with the thumb under the beak. A sharp jerk with each hand, pulling in directly opposite directions, will break the spinal cord and carotid arteries.

### 7.5 Decapitation

Decapitation causes direct loss of brain function by cutting off oxygen to the brain and depriving the body of central regulatory functions. It requires some training but minimal specialised equipment when used on animals in approved taxa below a certain size (<200 g).

Decapitation may be a useful alternative to cervical dislocation under field conditions as there is clear evidence of a successful procedure; it may also be a useful secondary means of euthanasia in some cases (Table 1a and 1b).

Decapitation alone is not acceptable in fish, amphibians and reptiles because their nervous systems are tolerant to deoxygenation. Rapid loss of brain function in these species requires that decapitation be followed by a method such as pithing (see Table 1b).

Decapitation must be undertaken with scissors or sharp blades, of an appropriate size for the animal in question. The instrument and technique must ensure rapid and unobstructed severing of the head from the neck. Tools used for the purpose must be maintained in good working order.

### 7.6 Pithing

Pithing (also called "spiking") refers to a physical method of increasing destruction of brain and spinal cord tissue. In the scope of this SOP, pithing is not acceptable as a primary euthanasia method except in fish, amphibians and marine turtles. It can be used as a second-step euthanasia method in unconscious animals as outlined in Tables 1a and 1b.

In all cases, pithing requires the use of an appropriately sharp and strong penetrating implement. In smaller animals, a long, sharp needle or inflexible sharpened wire will be appropriate. Custom designed "ike jime" spikes used for pithing fish are commercially available. For sea turtles, a stout chisel with a sharp point and handguard collar will be required (Figure 4).

In fish, the insertion spot is behind and above the eye, aiming for a point of entry halfway between the eye and the top of the gill plate, and inserting downward at a 45-degree angle towards the midline (Figure 2). The implement is then moved back and forth to destroy the brain tissue. The site of insertion needs to be adapted slightly based on the anatomy of the fish head (Figure 2C).

In amphibians the most common pithing site is the foramen magnum, the hole where the spinal cord emerges from the skull. The foremen magnum is identified by a slight midline skin depression at the base of the skull when the neck is flexed (Figure 3). The pithing implement is inserted through the foramen magnum directed toward the brain, then moved about rapidly to destroy brain and spinal cord tissue. The foramen magnum site can be used for pithing as a secondary euthanasia method for the species identified in Table 1.

In turtles, a heavy (>5 kg) sledgehammer is used as shown in Figure 4 to drive the chisel into the brain from above. The same equipment can be used for all sizes of sea turtle; in younger animals this technique will typically split the skull down the middle because the seams of the skull bones are not fully fused. It is critical that the head is placed on a firm surface such as a cement block, concrete or a flat rock, rather than sand or dirt. This ensures that the entire force of the hammer blow to the chisel translates to penetration of the skull and is not dissipated in loose substrate. For details of this technique, see Work and Balasz (2013).

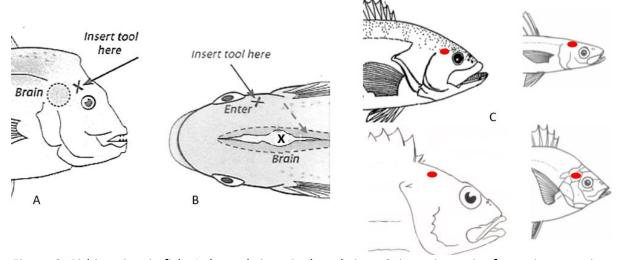


Figure 2: Pithing sites in fish. A: lateral view; B: dorsal view; C: insertion point for various species

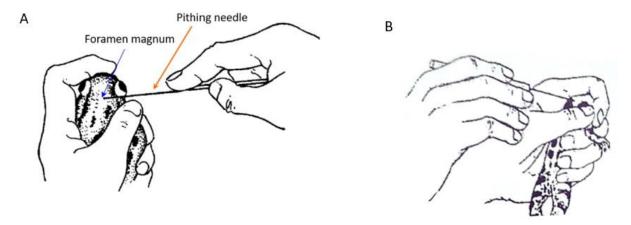


Figure 3: Pithing site in frogs. A: dorsal view; B: lateral view. Adapted from Li Jingxin Physiological Department <a href="https://slideplayer.com/slide/17447048/">https://slideplayer.com/slide/17447048/</a>

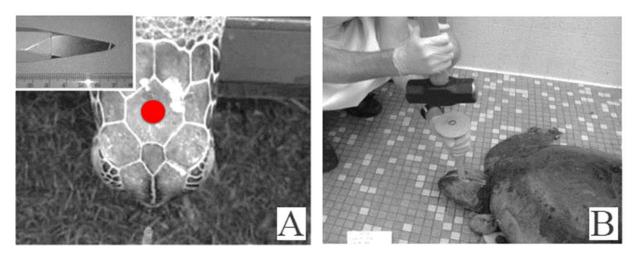


Figure 4: Pithing site and technique for sea turtles (Work and Balasz 2013)

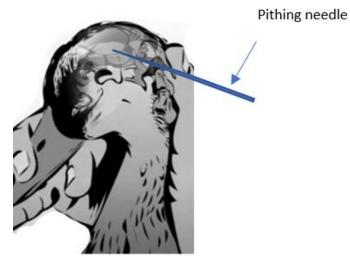


Figure 5: pithing site in birds. (after Game Management Authority <a href="https://www.youtube.com/watch?v=CdsjulrFKWq">https://www.youtube.com/watch?v=CdsjulrFKWq</a>)

### 7.7 Injectable barbiturate (pentobarbitone) overdose

This technique involves administration of sodium pentobarbitone, a barbiturate anaesthetic, by intravenous (IV) or non-intravenous injection. Barbiturates depress the central nervous system; high doses lead to death following cessation of breathing and heart function.

Injectable sodium pentobarbitone can be used for euthanasia of most vertebrates and is a rapid and effective means of euthanasia when used appropriately. Administration of pentobarbitone requires considerable training, appropriate animal restraint, and specific legal authorisations. In Western Australia (WA), registered veterinarians are authorised to use sedatives and anaesthetics and euthanasia solutions in animals. Sodium pentobarbitone can only be kept by non-veterinary personnel who have satisfied the applicable permit requirements of the WA Department of Health. In addition to obtaining the appropriate authorisations, non-veterinary personnel seeking to use this method must be trained in the correct dose rates and injection techniques, e.g. by attending the DBCA pentobarbitone training course (see Section 11).

The IV route of administration achieves the most rapid loss of consciousness. Injection by non intravenous routes (e.g. intrahepatic, intraperitoneal, intracoelomic) is sometimes considered less technically difficult but studies in rats and mice have demonstrated that mis-injection occurs commonly. All methods of barbiturate administration require significant training, effective animal restraint and careful attention to dose rates if they are to be used humanely.

As with all chemical agents, operators using barbiturates, sedatives and anaesthetics must ensure accurate dosing by adopting a documented dosing regime specific to the purpose, and using products with a known, standardised concentration of the active ingredient. Operators wishing to use pentobarbitone must attend specific training in the use of this euthanasia technique. The primary way of achieving this is through the DBCA Pentobarbitone use course. The course includes training in dose calculation, route of administration, injection technique and recording requirements. The AEC can consider any applications for a different competency recognition on a case by case basis.

Personnel using pentobarbitone, who are operating under a DBCA AEC approval, must record the details and outcomes of this method using the Pentobarbitone Administration Log issued during training. These records should be submitted to the AEC Executive Officer on request, to enable further refinement of the use of pentobarbitone in the scope of this SOP.

The conditions for the administration for pentobarbitone for various taxa are summarised in Table 1a and 1b. The administration of sedation or anaesthetic before undertaking euthanasia is often referred to as "two-step" euthanasia. Two-step euthanasia is a condition for the use of barbiturates for euthanasia in certain situations (Table 1a and 1b).

Further information on the use of pentobarbitone euthanasia is provided during training.

#### 7.8 Other chemical euthanasia methods

Several other chemicals can be used in various species to achieve euthanasia. The chemicals covered by this SOP are benzocaine gel (Orajel®) and eugenol (also known as clove oil).

As with all chemical agents, the operator must ensure accurate dosing by adopting a documented dosing regime specific to the purpose, and using products with a known, standardised concentration of the active ingredient.

#### 7.8.1 Benzocaine gel (Orajel®)

Benzocaine hydrochloride gel is an anaesthetic agent, which acts to depress the nervous and cardiac systems. At appropriate doses it can be an effective euthanasia agent for fish and amphibians, although a secondary euthanasia method is recommended for amphibians. It is well suited for field euthanasia use as it is low in toxicity, is not a controlled substance and poses little environmental risk. However, it is not an appropriate euthanasia option for animals intended for consumption so this must be considered when disposing of carcases. Products with a known, standardised concentration of the active ingredient should be used so that accurate dosing occurs.

#### 7.8.2 Eugenol (clove oil)

Clove oil is thought to act like a local anaesthetic and may also induce paralysis. It is well suited for field euthanasia use as it is low in toxicity, is not a controlled substance and is relatively inexpensive. However, its environmental impacts are not well studied and it is not an appropriate euthanasia option for animals intended for consumption, so this must be considered when disposing of carcasses. Clove oil is an acceptable agent for the euthanasia of fish. Products with a known, standardised concentration of the active ingredient should be used so that accurate dosing occurs.

#### Euthanasia techniques for field use for DBCA projects and personnel

**Important note:** These recommendations consider the circumstances of the department's field projects and department personnel working in the field, and may not reflect recommendations for euthanasia under other circumstances or by other personnel.

**KEY**: ✓ acceptable <u>if undertaken as outlined in this SOP</u>; □ acceptable <u>with conditions as outlined in this table</u>; **X** not acceptable as a standard method – propose to AEC before proceeding;  $\Leftrightarrow$  preferred technique for this taxon;  $\bigstar$  acceptable but not preferred if other acceptable techniques available

Table 1a: - Mammals

Taxon	Shooting	CBD <sup>a</sup>	Blunt Trauma	Cervical Dislocation	Decapitation	Pithing	Pentobarbitone Injection <sup>c</sup>	Other chemical methods
Mammals < 300g (e.g., micro bats, mice, rats)	<b>✓★</b>	х	✓	□ rodents < 200g	□ ☆ <200g; >200g secondary method only	secondary method only	✓ ☆	Х
Macropods <sup>b</sup> (PY = pouch young; YAF= young at foot)	<b>✓</b> ☆	□ ☆ adults, young at foot  X PY	□★ two-step process recommended for adults □☆ PY; YAF <5kg	□★ unfurred PY <5cm including tail	□☆ unfurred PY <5cm including tail; secondary method only for PY >5cm	□ ☆ secondary method only	✓ ☆ >300g: two step process. >2kg: IV route only	х
Dingoes/dogs, foxes, cats	<b>√</b> ☆	<b>✓</b> ☆	two-step process recommended for adults	□★ juveniles < 200g	х	Х	✓ ☆ >300g: two step process. >2kg: IV route only	х
Other mammals 300g  – 3kg (e.g. chuditch, quenda, rabbits)	<b>✓</b> ☆	Х	✓ ☆	□ ★ juveniles < 200g	х	□ ☆ secondary method only	✓ ☆ >300g: two step process. >2kg: IV route only	Х
Cetaceans <sup>c</sup> , sirenians and pinnipeds	<ul> <li>✓ ☆ cetaceans ≤ 6m long;</li> <li>□ ☆ cetaceans 6-7m long assessed by experienced operator</li> <li>✓ ☆ sirenians &amp; pinnipeds</li> </ul>	Х	Х	Х	Х	х	✓ ☆ two-step process only	х
Domestic hoofstock	<b>✓</b> ☆	Х	Х	Х	Х	х	✓ ☆ two-step process only	Х

- a only device models approved by DBCA in SOP 2021-2 Use of captive bolt devices for the euthanasia of fauna.
- b based on AgriFutures Australia 2020 National Code of Practice for the Humane Shooting of Kangaroos and Wallabies for Commercial Purposes
- c see Pentobarbitone Administration Log for further details on requirements for pentobarbitone euthanasia.
- d see notes on firearms use for details of conditions relating to cetaceans > 6m.

Table 1b: Other vertebrates

Taxon	Shooting	CBD a	Blunt Trauma	Cervical Dislocation	Decapitation	Pithing	Pentobarbitone Injection <sup>b</sup>	Other chemical methods
Birds		Х	<b>√</b> ☆	□☆<200g	☐ ☆<200g; >200g secondary method only	□ ☆ secondary method only	✓ >300g:	x
Lizards and snakes	<b>√</b> ★	Х		х	☐ ☆ followed by or used as a secondary method			^
Freshwater turtles		Х		Х	□ ★ <200g; >200g two step process or secondary method		✓ ☆ >300g: IV	
Marine turtles		<b>✓</b> ☆	☐ ☆ juveniles < 300g only; follow by secondary method	Х	only	<b>√</b> ☆	only, two step process.	
Crocodiles	<b>√</b> ☆ <sup>c</sup>	□☆<2m total length	Х	Х	□ ☆ hatchlings: pithing follow decapitation; <2m total length by cervical spinal cord severance	: pithing, followed	□ ☆ >300g: IV only, two step process	х
Amphibians	Х	Х	✓ ☆	х	✓ ☆ followed by or used as a secondary method	✓ ☆	<b>✓</b> ☆	□☆ benzocaine gel followed by secondary method
Fish	Х	Х		Х		✓ ☆	✓ ☆	✓☆ benzocaine gel; ✓☆ eugenol (clove oil)

a only device models approved by DBCA in SOP SC21-02 Use of captive bolt devices for the euthanasia of fauna.

b see *Pentobarbitone Administration Log* for further details on requirements for pentobarbitone euthanasia.

c refer to NRMMC 2009 *Code of Practice on the humane treatment of wild and farmed Australian crocodiles* for detailed description of technique.

### Table 2 Qualitative comparison of acceptable euthanasia methods

Important note: Comparisons assume effective, correct use of the method by suitably authorised and qualified personnel consistent with recommendations in Table 1.

Factors	Shooting	CBD	Blunt Trauma	Cervical Dislocation	Decapitation	Pithing	Pentobarbitone Injection	Benzocaine, Eugenol
Requirement for certification and training	High	High	Low	Medium	Medium	Medium	High	Medium
Technical difficulty	Medium	Medium	Medium	Medium	Low	Medium	High	Low
Specialised equipment and costs	Medium	Medium	Low	Low	Low	Low	High	Low
Relative time to loss of consciousness <sup>a</sup>	Instantaneous	Instantaneous	Rapid	Rapid	Rapid	Secondary method only	Rapid	1-2 minutes
Relative time to death <sup>a</sup>	Very rapid	Rapid	Variable - may require secondary euthanasia method	Rapid	Variable – some taxa will require secondary method	Rapid	Rapid	Minutes
Stress to animal before or during euthanasia	Low		h handling stress two-step process	•	Moderate handling stress unless a secondary method	Not to be used as a primary euthanasia method	Low if two-step process; potentially high handling stress otherwise	Low
Level of risk/hazard to personnel health and safety	Moderate	Moderate	Low	Low	Low	Low	Moderate	Low
Level of amenity and distress to personnel and the public	Moderate	Moderate	High if primary method	Moderate if primary method	Moderate	Moderate	Low	Low
Potential for environmental contamination or scavenger/predator risk?	No	No	No	No	No	No	Yes	Benzocaine: no Eugenol: unknown

a. Does not include time taken for restraint in the case of methods requiring animal restrain

### 8 Confirmation of death

After euthanasia by any method, it is essential to establish that the animal is dead.

Methods used to confirm death must be appropriate to the species being euthanased, and all animals should be monitored over at least five minutes to ensure death has occurred. If there is any doubt about confirmation of death, a secondary euthanasia method must be used to ensure the animal is dead (Section 8).

At least three of the criteria below must be used to establish that death has occurred:

- absence of a visible, palpable and/or audible (if stethoscope used) heartbeat on two separate checks by a competent examiner at least five minutes apart;
- lack of palpable pulse (femoral or jugular) on two separate checks by an experienced examiner at least five minutes apart;
- absence of respiratory movement when monitored continuously for a minimum of one minute;
- no blink response or eye-protection reflex (corneal reflex) elicited when the corner of the eye is touched gently;
- mammals only: pupils fixed and dilated and do not constrict when a light is shone on them;
- loss of response to noxious stimulus (e.g., no limb withdrawal in response to a firm toe pinch);
- change of mucous membrane colour (white or grey/blue rather than rosy pink) in non-pigmented areas;
- absence of capillary refill in gums: press firmly with a finger on the gum until it goes
  white, then watch to make sure it does not return to its previous colour. This
  demonstrates absence of capillary blood flow (a normal healthy animal has a
  capillary refill of 1-3 seconds)
- rigor mortis (onset after several hours).

Additional care must be taken to ensure death following euthanasia in ectothermic vertebrates, i.e., fish, reptiles and amphibians. These may normally exhibit very low heart rates and can hold their breath for an hour or more (particularly large aquatic species such as crocodiles and adult sea turtles). Absence of heartbeat and/or breathing will not necessarily provide confirmation of death in these animals and they must be monitored closely; if in doubt, a secondary method of euthanasia should be used.

It should also be noted that in reptiles, the heart may continue beating for several minutes after euthanasia. Operators should be prepared to employ a secondary euthanasia method.

## 9 Secondary methods of euthanasia

When a euthanasia method causes unconsciousness but does not cause death, a second step may be necessary to quickly and efficiently ensure death. The operator should always plan for how to achieve euthanasia if the primary method is unsuccessful.

Signs of consciousness, which may indicate that death has not occurred, include:

- natural spontaneous blinking of the eyes;
- eyelid closure in response to lightly touching the eyeball;
- rhythmic breathing (ribs moving in an out);
- lifting the head or trying to stand up;
- vocalising.

Euthanasia methods recommended for secondary euthanasia are not necessarily recommended for stand-alone euthanasia in conscious animals, but in conjunction with other methods (in already unconscious or dying animals) can be used to rapidly bring about death. The suitability of various euthanasia techniques as a secondary method are described in Section 6. Note that it may be appropriate to repeat the primary euthanasia method if the first attempt is unsuccessful, but the suitability of this approach should be considered in the planning phase.

## 10 Preservation and/or disposal of dead animals

Disposal of carcasses is the responsibility of the land-owner/manager and must be done in accordance with local government by laws.

Carcasses euthanased via barbiturate overdose represent an environmental toxicity hazard, and post-euthanasia disposal must prevent predation and follow conditions of Department of Health permit.

Local councils/shires and Main Roads WA (138 138) are responsible for disposal of carcasses on roads and verges. However, as good neighbour policy, if practicable (and if barbiturate contamination is not a concern), carcasses may be disposed of as follows:

- Towns or domestic locations: transport to a waste disposal site.
- Field locations: carcasses should be disposed of
  - at least 20 m from a roadside (to reduce the danger to road users and other animals);
  - o at least 50 m from a water point or the centre line of a watercourse;
  - at least 200 m from a recreation site;
  - out of a public water catchment area (refer to DWE Regulation Water quality protection note 96 PDWSA for more information on carcass disposal requirements in water catchment areas).
  - o carcasses may be left in situ, provided they meet the above criteria.

For marine animals, refer to DBCA SOP *Marine Animal Carcass Management* December 2019. Refer to the department SOP for *Vouchering Vertebrate Fauna Specimens* and *Tissue Sample Collection and Storage for Mammals* for information on the regulatory and technical requirements for preserving, storing and transporting biological specimens.

If bodies or samples are not required for diagnostic or scientific purposes, euthanased animals should be offered to the Western Australian Museum.

## 11 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to capture and handle animals successfully, and appropriately manage adverse events as required. Department personnel, and other external parties covered by the department's Animal Ethics Committee, undertaking fauna-related activities require approval from the committee and will need to satisfy the competency requirements detailed in Table 3. Other groups, organisations or individuals using this SOP to guide their field euthanasia activities are encouraged to also meet these competency requirements as well as their animal welfare legislative obligations.

Table 3 Competency requirements for Animal Handlers of projects involving euthanasia of animals under field conditions

Euthanasia Technique	Competency requirement	Competency assessment		
Shooting	Suitable training, authorisation and documentation	- Completion of DBCA firearms course - Corporate Firearms Licence issued		
CBD	Suitable training, authorisation and documentation	<ul> <li>training and written endorsement of competency in specified procedures by a trainer approved by DBCA's AEC</li> <li>written approval by Regional Manager, renewed every 3 years</li> </ul>		
Blunt trauma	At minimum, shown how to perform the described method by an	- successfully demonstrating the specified technique for the specified taxon to the satisfaction of a person		
Cervical	experienced person.	experienced in the technique.		
dislocation	General skills/experience for manual techniques	- in emergency cases: at least some form of training and direction in the specific technique by a person trained in		
Decapitation	Experience under an experienced supervisor/Chief Investigator for	the technique  - DBCA staff may require a letter of competency specifying		
Pithing	each described technique.	the range of techniques and species to which their experience extends.		
		- completion of DBCA euthanasia training course		
Pentobarbitone	Authorisation for drug use, storage and disposal.	- completion of DBCA pentobarbitone use training course; or		
	Demonstrated ability to calculate	- training and written endorsement of competency in		
	and administer appropriate dose for	specified procedures by a veterinarian on a case by case		
	the taxon and size of animal.	basis		
	Correct use of the DBCA			
Danasaina asl	Pentobarbitone Administration Log.			
Benzocaine gel	Demonstration of the technique by	- successfully demonstrating the specified technique,		
or Eugenol (clove oil)	an experienced supervisor/Chief Investigator.	including dose calculation, for the specified taxon to the satisfaction of a person experienced in the technique		
(clove oii)	Demonstrated ability to calculate	satisfaction of a person experienced in the technique		
	and administer appropriate dose			

## 12 Approvals

A licence or authorisation may be required under the Biodiversity Conservation Act 2016

(examples below). Contact the department's Wildlife Licensing Section for more information. It is your responsibility to ensure you comply with the requirements of all applicable legislation.

- Fauna taking (scientific or other purposes) licence (Reg 25)
- Fauna taking (biological assessment) licence (Reg 27)
- Fauna taking (relocation) licence (Reg 28)
- Section 40 Ministerial Authorisation to take or disturb threatened species.

## 13 Occupational Health and Safety

A job safety analysis is recommended prior to undertaking any fauna-related activities. The following departmental SOPs are relevant to occupational health and safety:

- SOP Managing Disease Risk in Wildlife Management
- SOP Hand Restraint of Wildlife

Bystanders can be a major hazard to the safety of the animal, personnel and themselves. Where possible, and without increasing the stress of the animal, euthanasia of an animal should be conducted away from bystanders. Only personnel who need to be directly involved should remain in the immediate vicinity.

Departmental personnel, contractors and volunteers have duties and responsibilities under the Occupational Safety and Health Act 1984 and Occupational Safety and Health Regulations 1996 to ensure the health and safety of all involved. Fieldwork is to be undertaken in line with the department's corporate guidelines, policies and standard operating procedures, including but not limited to, risk management and job safety analyses. Further information can be found at

https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/SOPs.aspx

If department personnel or volunteers are injured, please refer to the departmental Employee Relations and Safety Section's 'Reporting Hazards, Near-misses and Incidents' intranet page, which can be found at <a href="https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx">https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx</a>

## 13.1 Manual handling

Manual handling of animals is often a necessary prelude to euthanasia and the risk of injury to the animal and personnel is increased where an animal is stressed and/or in pain. Personnel must have experience when carrying out techniques such as cervical dislocation and blunt force trauma to euthanase an animal. Personnel must also be trained in the appropriate handling techniques for the manual restraint of animals. Always use PPE appropriate to the species being handled (e.g., leather gloves) consistent with the departmental SOP for *Hand restraint of wildlife*.

#### 13.2 Firearms

All personnel using firearms must have completed training recognised by the Department,

must have been issued a nominated persons authorisation by WA Police and must be registered on the department's Corporate Firearms Licence.

Firearm users must strictly observe all relevant safety guidelines relating to firearm ownership, possession and use as outlined in DBCA Corporate Policy Statement 20 Departmental Use of Firearms, DBCA Corporate Guideline No. 42 Departmental Use of Firearms 2020 and associated SOPs. These documents are located on the Corporate Firearms page of the RFMS Intranet.

### 13.3 Chemicals and restricted drugs

Personnel must be extremely careful of their own safety when administering lethal injection or other restricted drugs such as sedatives and anaesthetics. Seek immediate medical attention in the event of accidental self-injection or needle-stick injuries. Only personnel trained and authorised to use pentobarbitone should be present in the immediate vicinity of the animal.

Refer to Material Safety Data Sheets (MSDS) relevant to any hazardous substances being used, including preservatives such as formalin and ethanol. Refer to the departmental SOP for *Vouchering Vertebrate Fauna Specimens* and *Tissue Sample Collection and Storage for Mammals* for information on the regulatory and technical requirements for storing and transporting preserved specimens.

### 13.4 Mental health and welfare

Wildlife biologists, wildlife managers, and wildlife health professionals may experience distress and anxiety in undertaking euthanasia, particularly if their actions are subject to external scrutiny, or pressure to save animals rather than destroy them. Operators and staff are strongly encouraged to consider and discuss their personal and professional opinions about euthanasia and euthanasia methods as part of the planning for a euthanasia event (see Section 3.1). Operators and staff who are undertaking euthanasia and experience distress or anxiety after the event are encouraged to document their concerns as part of the feedback for the event, to discuss this with their supervisor and/or to contact Peer Support (http://intranet/csd/People\_Services/rm/Pages/PeerSupport.aspx).

## 14 Further Reading

The following SOPs have been mentioned in this advice and it is recommended that they are consulted when planning and undertaking fauna-related activities involving euthanasia:

- Department Code of Practice for Wildlife Rehabilitation in Western Australia
- Department Corporate Policy Statement 20 Departmental use of firearms
- Department Corporate Guideline No. 42 *Departmental Use of Firearms 2020* and associated SOPs

•	Department SOP	Euthanasia of small stranded cetaceans using firearms
•	Department SOP	Use of captive bolt devices for euthanasia of fauna
•	Department SOP	Use of firearms for the humane destruction of animals
•	Department SOP	Vouchering vertebrate fauna specimens
•	Department SOP	Animal Handling and Restraint using Soft Containment
•	Department SOP	Care of Evicted Pouch Young

Department SOP First Aid for Animals
 Department SOP Hand Restraint of Wildlife

• Department SOP Managing Disease Risk in Wildlife Management

Department SOP Marine animal carcass management

• Department SOP Tissue Sample Collection and Storage for Mammals

Department SOP Transport and temporary holding of wildlife

#### For further advice refer also to:

National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes*, 8th edition. Canberra: National Health and Medical Research Council.

Australian and New Zealand Council for the Care of Animals in Research and Teaching resources: https://anzccart.org.nz/anzccart-resources/;

National Health and Medical Research Council animal ethics page: <a href="https://www.nhmrc.gov.au/research-policy/ethics/animal-ethics">https://www.nhmrc.gov.au/research-policy/ethics/animal-ethics</a>;

WA Health -Public Health: Licences and permits for medicines and poisons <a href="https://ww2.health.wa.gov.au/Articles/J">https://ww2.health.wa.gov.au/Articles/J</a> M/Licences-and-permits

## 15 References

AgriFutures Australia. (2020). *National Code of Practice for the Humane Shooting of Kangaroos and Wallabies for Commercial Purposes*. AgriFutures Australia Publication No. 20-126.

American Veterinary Medical Association. (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. Retrieved from: <a href="https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf">https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf</a>

Animal Health Australia. (2015). Operational manual: Destruction of animals (Version 3.2). Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3, Agriculture Ministers' Forum, Canberra, ACT.

Coughran, D. K., Stiles, I. and Mawson, P. R. (2012). Euthanasia of beached humpback whales using explosives. *Journal of Cetacean Research and Management* 12(1): 137-144.

Kells, N. J., Beausoleil, N. J., Sutherland, M. A., & Johnson, C. B. (2018). Electroencephalographic responses of anaesthetised pigs to intraperitoneal injection of sodium pentobarbital. *Animal Welfare*, 27(3): 205-214.

Laferriere CA and Pang DSJ. (2019). Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents. *Journal of the American Association for Laboratory Animal Science* 59 (3): 254-263.

Laferriere, C. A., Leung, V. S., & Pang, D. S. (2020). Evaluating intrahepatic and intraperitoneal sodium pentobarbital or ethanol for mouse euthanasia. *Journal of the American Association for Laboratory Animal Science*, 59(3), 264-268.

Natural Resource Management Ministerial Council (NRMMC). (2009). *Code of Practice on the Humane Treatment of Captive and Wild Crocodiles*. Commonwealth of Australia.

NRMMC. (2008). *National Code of Practice for the Shooting of Kangaroos and Wallabies for Non-commercial Purposes*. Commonwealth of Australia.

NRMMC. (2009). *Code of practice on the humane treatment of wild and farmed Australian crocodiles*. Commonwealth of Australia.

Museum Victoria Animal Ethics Committee. (2010). Manual of Standard Operating Procedures (SOPs) for the Ethical Use of Live Aquatic Animals. Retrieved from: https://museumvictoria.com.au/about/animal-ethics/

Reilly, J.S. (Ed.). (2001). Euthanasia of Animals Used for Scientific Purposes. ANZCCART.

Rose, K. (2005). Wildlife Health Investigation Manual. The Australian Registry of Wildlife Health.

Sharp, J. and Saunders, G. (2004). *GEN001 Methods of euthanasia*. NSW Department of Primary Industries.

Sikes, R.S., Gannon, W.L., and American Society of Mammalogist. (2016). Guidelines for the use of wild mammals in research. *Journal of Mammalogy* 97 (3): 663-688.

University of Notre Dame. (2007). Institutional Animal Care and Use Committee Standard Operating Procedure for Euthanasia.

Work TM and Balasz GH. (2013). A simple humane method to euthanize a sea turtle. *Marine Turtle Newsletter* 136: 5-6.

Zatroch, K. K., Knight, C. G., Reimer, J. N., & Pang, D. S. (2016). Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (Rattus norvegicus). *BMC veterinary research*, 13(1), 1-7.