

ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

HUMANE KILLING OF REPTILES

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

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1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:

- Working with reptiles

2. SUMMARY

- 2.1** Reptiles are a large group of vertebrates comprising 4 classes and several thousand species, including lizards, snakes, crocodiles, turtles and tuatara. They range in size from tiny geckos weighing a few grams to Salt Water Crocodiles at over a tonne, presenting a number of logistical challenges to those who work with them.
- 2.2** Research involving reptiles may be conducted to better understand their biology, behaviour or reproduction under different conditions. This data can be used to make evidence-based management decisions for the species, and to greatly enhance the effectiveness of conservation programs.
- 2.3** Taking into account the unique anatomy and physiology of these animals is essential to developing a humane method of euthanasia. Investigators in laboratory and field settings may be required to euthanize animals for any number of reasons and need to be appropriately trained to carry this out, ensuring the welfare of the reptile is maintained.
- 2.4** The current literature advocates a two-stage euthanasia process for reptiles; general anaesthesia followed by humane euthanasia, except where the pain and distress of handling or anaesthesia is greater than with direct administration of barbiturates. Following euthanasia of any animal it is critical to confirm death, which is often more difficult in reptiles compared to mammals.
- 2.5** This Standard deals only with the use of anaesthetic drugs given prior to humane killing or euthanasia (i.e. non-recovery situations). Where anaesthesia or sedation is required for procedures with recovery, investigators should seek specific advice from the Animal Welfare Officer (AWO) or relevant literature.
- 2.6** In this document, references are made to both 'humane killing' and 'euthanasia'. These terms denote a terminal procedure that results in the death of an animal which occur by the same technical process, though the reason for the death vary. 'Humane killing' occurs when an animal is identified as meeting project specific criteria on an AEC approved Intervention Criteria Sheet, while 'euthanasia' may be performed for any animal deemed to be suffering.

3. BENEFITS & RISKS

- 3.1 Maintaining animal welfare is of paramount importance when they are utilized for research purposes, and this includes ensuring their death is as free of distress as it can be. A two-step euthanasia process allows the animal to be anaesthetized and lose consciousness prior to the administration of euthanasia solution, or other physical means to cause death.
- 3.2 This standard aims to provide a number of acceptable options for euthanasia in laboratory and field settings, allowing selection of the most appropriate method for the size and species of reptile. This is critical to ensure no undue suffering of these animals following their use in research and teaching activities.
- 3.3 It can be exceptionally difficult to identify pain in reptilian species as they often do not display the same overt signs as would be seen in more familiar mammals. A reptile in pain may be unusually quiet or inactive, have a hunched or flattened posture and may become anorectic. By ensuring a loss of consciousness prior to humane killing methods it is hoped that minimal or no pain will be experienced by the animal.
- 3.4 Where a euthanasia procedure is not carried out by an approved method, or if the procedure is performed incorrectly, there is an unacceptable risk of causing pain and distress to the reptile in its final moments.

4. PROCEDURE/PROTOCOL

4.1 Two-step euthanasia

- 4.1.1 A two-stage euthanasia process requires administration of an anaesthetic agent (injectable or inhalant) followed by a primary method of euthanasia (see section 4.3).
- 4.1.2 Death must be verified after the euthanasia procedure by ensuring cessation of heart beat, respiration and brain function. For methods of euthanasia where death cannot be assured, a secondary guaranteed lethal procedure should be performed to ensure death.
- 4.1.3 Dose rates listed in this document are a starting point only. Investigators are advised to consult multiple references and the AWO to identify the most appropriate methods and drug doses for their given species and set of circumstances.

4.2 Step 1: Anaesthesia

- 4.2.1 Reptiles should be kept at their preferred optimal body temperature when any drugs are administered, including anaesthetic agents and euthanasia drugs, as they need to be at their ideal body temperature in order to metabolise drugs effectively. This can be achieved by placing them on a heat mat (calibrated to a maximum of 27°C) or returning them to their usual enclosure after injectable agents are given, provided they can still be monitored.
- 4.2.2 Investigators may cool the ambient temperature for a short period prior to administering injectable anaesthetic agents to reduce the distress associated with handling, however the animal should then be returned to its preferred optimal temperature zone to allow for more effective metabolism of the drugs. Following administration of an anaesthetic agent, sufficient time must be allowed for the agent to take full effect and reflexes to diminish. For example, Zoletil (tiletamine/zolazepam) typically requires 15-30 minutes to induce deep anaesthesia but this may vary with dose, species and individual animals.
- 4.2.3 The depth of anaesthesia should be monitored by assessment of the pedal reflex, righting reflex and overall muscle tone (See section 5.1). Step 2 may only take place once these reflexes are absent and the muscles are fully relaxed, indicating deep anaesthesia and loss of consciousness.
- 4.2.4 Some reptile species are very interactive with humans and respond to handling, while others may be more timid and prefer to hide. Following anaesthesia, these alert reptiles should become relaxed, quiet and cease to interact with handlers.
- 4.2.5 **Drug selection and doses**
 - 4.2.5.1 Pre-euthanasia agents must be given at a dose suitable for deep anaesthesia and not at doses listed for light anaesthesia or sedation.
 - 4.2.5.2 Tiletamine/zolazepam (Zoletil®) delivered by intramuscular injection is the preferred pre-euthanasia option for all reptiles. The animal should be placed at its preferred temperature before the injection and investigators should allow 15-30 minutes (or more if required) for it to

be fully effective before administering euthanasia drugs or employing other methods of euthanasia.

4.2.5.3 **Table 1. Pre-euthanasia anaesthetic agents guidelines**

Product	Drug	Concentration	Species	Dosage/ Route
Zoletil	Tiletamine/Zolazepam	100 mg/ml	Lizards, chelonians Snakes	15-30 mg/kg IM, SC 5-10 mg/kg IM
Alfaxan	Alfaxalone	10 mg/ml	Lizards, chelonians	20-30 mg/kg IM
	Isoflurane		All reptiles	2-5% in oxygen, in chamber or via mask if practical
MS222	Tricaine Methanesulfonate		All reptiles	ICo injection of pH neutralized solution

4.2.5.4 Anaesthetic drugs may be given by intramuscular injection (IM) or subcutaneous injection (SC) in most species, and in some cases intravenously (IV), if venous access is easily achieved.

4.2.5.5 Intracoelomic injection (ICo) of pH neutralised MS222 may be used as the first step in a euthanasia protocol, however it does not eliminate deep pain stimulus and should be used with caution. Further details on its use are detailed in section 4.4.5.

4.2.5.6 Appropriate sites for IM injection in reptiles include the proximal hind limbs (thigh area), proximal forelimbs or paralumbar muscles, approximately 5-10 mm either side of the spine. Subcutaneous injections can be given anywhere along the dorsum or flank where there is loose skin. Where a species is covered in scales the needle will need to be aimed between scales to ensure it can pass through to the skin or muscle layers underneath.

4.2.5.7 Induction of anaesthesia may be greatly prolonged when inhaled agents are used as many reptiles, particularly aquatic or diving species, have a tendency to hold their breath. Injectable agents are preferred for this reason.

4.3 Step 2: Euthanasia

4.3.1 Following anaesthesia by an injectable or inhaled agent and confirmation of anaesthetic depth, euthanasia may be carried out by one of the methods listed in section 4.4.

4.3.2 Where the method of euthanasia cannot be assured to result in death, a second, guaranteed lethal procedure (e.g. decapitation) must be performed to ensure death.

4.3.3 Sodium pentobarbitone administered intravenously is the preferred method of euthanasia in reptiles, ideally following anaesthesia with Zoletil.

4.4 Acceptable methods

4.4.1 Sodium pentobarbitone

4.4.1.1 Reptiles should be maintained at their preferred body temperature when giving injectable agents to ensure optimal metabolism and efficiency in drug uptake.

4.4.1.2 Sodium pentobarbitone (100-150 mg/kg) should preferably be administered intravenously, but can be given via the intracoelomic (ICo) route where this is not possible, or the benefits are outweighed by the distress of additional restraint or pain caused by alternate methods. The intra-cardiac (ICa) route is also acceptable following anaesthesia in most reptiles.

4.4.1.3 A lower dose of sodium pentobarbitone is likely to be required where animals have first been anaesthetised.

4.4.1.4 Intravenous administration of sodium pentobarbitone is permitted without prior anaesthesia where investigators have undergone sufficient training in this type of injection for the species under consideration.

4.4.1.5 In general, IV access for lizards and snakes is via the ventral coccygeal vein (tail vein), but this may be difficult to achieve in very small animals. IV access in many chelonian species is via the jugular veins. Investigators should familiarise themselves with the anatomy of their specific species prior to attempting any injections.

- 4.4.1.6 A competent and trained assistant is required to help restrain reptiles for IV injections if anaesthesia is not being given beforehand.
- 4.4.1.7 Where it is difficult or dangerous to physically restrain an animal for venous access, anaesthesia must be given beforehand to limit the risk of the drug being delivered outside the vein, causing pain and irritation to the reptile.
- 4.4.1.8 Sodium pentobarbitone may only be given by intra-cardiac injection following anaesthesia and loss of consciousness.
- 4.4.1.9 If sodium pentobarbitone is administered by IV or ICa routes, or where Doppler is available to confirm cessation of heartbeat, then a secondary method of euthanasia may not be required. If it is given by it ICo route, then a secondary method such as pithing must be used.
- 4.4.1.10 Administration of sodium pentobarbitone must be by approved routes only. It must not be given by any other route (e.g. subcutaneous, oral) due to the variable absorption rates and questionable efficacy of these methods. A summary is found in Table 2.

Table 2. Sodium pentobarbitone use in reptiles

Delivery method	Conditions	Secondary method recommended
Intravenous (IV)	-Minimal distress with restraint to access vein -Normal ambient temperature -Conscious or anaesthetized animal -Competency assessed investigator -Knowledge of species anatomy	-Not necessary but can be used
Intracoelomic (ICo)	-Minimal distress with restraint -Normal or cool ambient temperature. -Conscious or anaesthetized animal	-Yes, always (e.g. pithing)
Intra-cardiac (ICa)	-Anaesthetised animal only -Normal ambient temperature -Competency assessed investigator -Knowledge of species anatomy	-Not necessary but can be used
Oral (PO)	-Not permitted	N/A
Subcutaneous (SC)	-Not permitted	N/A
Intramuscular (IM)	-Not permitted	N/A

4.4.2 Liquid Nitrogen

- 4.4.2.1 Following induction of deep anaesthesia, small reptiles (<4 g) may be humanely euthanized by immersion in liquid nitrogen, which results in rapid freezing and death.
- 4.4.2.2 This technique must never be used on species that have adapted freeze tolerance strategies, conscious reptiles or animals weighing over 4 g.

4.4.3 Tricaine Methanesulfonate (TMS, MS-222)

- 4.4.3.1 TMS is a water soluble chemical that may be mixed with water for euthanasia of fish and amphibians by immersion; however it is not suitable for euthanasia of reptiles in this manner.
- 4.4.3.2 Anaesthesia in reptiles can be achieved using 250-500 mg/kg of 0.7-1% sodium bicarbonate buffered MS-222 solution by ICo injection.
- 4.4.3.3 Euthanasia may be achieved following MS-222 anaesthesia using a second ICo injection of 0.1-1.0 ml unbuffered 50% (v/v) MS-222 solution.
- 4.4.3.4 MS-222 does not eliminate responses to deep pain stimuli when used as an anaesthetic agent. As such, only physical methods that result in immediate brain destruction may be used for euthanasia following anaesthesia with TMS.

4.4.4 Inhaled agents: Carbon Dioxide (CO₂) and Isoflurane

- 4.4.4.1 The unique physiology of many reptiles, especially chelonians, means that they can breath-hold and survive extended periods without oxygen. Inhalant agents are not recommended for these species.
- 4.4.4.2 An ideal inhaled agent would cause an initial induction phase resulting in anaesthesia, and after continued exposure to the agent, death would occur. Anaesthetic induction time may vary with different species and for different agents.
- 4.4.4.3 Isoflurane may be used for anaesthesia in many snakes and lizards, as these species do not commonly breath-hold. Prolonged exposure to the gaseous agent is unlikely to result in death, so isoflurane should not be used as a sole agent for euthanasia.
- 4.4.4.4 Carbon dioxide inhalation may be used effectively for anaesthesia and euthanasia, but only in the more active reptilian species. It results in rapid loss of consciousness, though the lengthy exposure time required to achieve death may render its use impractical. For this reason, it is not a preferred method of euthanasia, and death by this method should be followed up by a secondary physical procedure.
- 4.4.4.5 The use of bell jars or chambers, where the animal is placed inside the vessel with a cotton ball soaked in isoflurane or similar, is not permitted. The unreliable respiration of reptiles combined with the uncontrolled and unknown inhaled dosage of anaesthetic gas by this method means it is not sufficient to guarantee a deep plane of anaesthesia will be achieved.
- 4.4.4.6 Death must be verified prior to terminating the use of the inhaled agent, or a second, guaranteed lethal procedure (e.g., decapitation and pithing) should be performed to ensure death.

4.5 Conditionally acceptable physical methods

4.5.1 Decapitation

- 4.5.1.1 Reptiles have a unique physiology which enables them to survive extended periods with reduced availability of oxygen to the brain tissue. As a result, decapitation alone does not cause rapid unconsciousness when the head is severed from the body.
- 4.5.1.2 Where it is used as a method of humane killing, decapitation must be part of a 3-step euthanasia protocol; injectable or inhaled anaesthetic, decapitation, pithing.
- 4.5.1.3 Deeply anaesthetized animals may be decapitated using a guillotine or sharp scissors, whichever is appropriate for the size of the reptile, followed by pithing of the brain and/or spinal cord.
- 4.5.1.4 Decapitation of conscious reptiles is not permitted.

4.5.2 Firearms or penetrating captive bolt

- 4.5.2.1 Use of firearms as a mechanism for euthanasia should be limited to large reptiles, and only when other options are not practical due to animal or human safety concerns (e.g. adult salt water crocodiles, some chelonians).
- 4.5.2.2 Euthanasia is achieved by delivery of a penetrating captive bolt or gunshot to the brain, delivered at a location specific to the species (diagrams can be obtained from the AWO).
- 4.5.2.3 Where firearms or captive bolt are used as the primary method of humane killing, a secondary method is not required.
- 4.5.2.4 The procedure should only be performed outdoors after the safety of staff, the public and other animals nearby have been considered. The emotional impact of carrying out euthanasia in this manner may be considerably higher than for some other methods and personnel should be counselled appropriately.
- 4.5.2.5 Shooting must only be performed by highly skilled personnel who have received appropriate training, and are in possession of a valid firearms licence.
- 4.5.2.6 Further technical details regarding the ballistics for this type of euthanasia can be obtained from the AWO if required.

4.6 Destruction of embryos and eggs

- 4.6.1 Where embryos/eggs are <50% gestation, prior to neural tube formation, the viability of the egg may be destroyed by freezing (<4°C for 4 hours) or crushing.
- 4.6.2 Embryos/eggs >50% gestation should be euthanised using an anaesthetic agent with secondary physical method to ensure death, as listed in sections 4.4.1 to 4.5.1.

4.7 Unacceptable methods of euthanasia

4.7.1 Hypothermia or cooling

- 4.7.1.1 Freezing or cooling of reptiles is not permitted as a sole method of euthanasia.
- 4.7.1.2 Immobilisation of reptiles by rapid or extreme chilling or freezing is considered painful and inhumane, even if combined with other methods of euthanasia. Freezing enables the formation of ice crystals in the cells of the skin and viscera that in turn produces pain, making it an inappropriate choice.
- 4.7.1.3 Lowering the ambient temperature to facilitate handling is acceptable, provided the temperature range is within what the species would normally encounter in the wild.
- 4.7.1.4 The animal's perception of pain remains unchanged following gentle cooling; however their ability to respond to stimuli is slowed. This means that although reptiles may be more amenable to handling and minor procedures, a reduced behavioural or physiological reaction should not be viewed as the absence of pain.

4.8 Step 3: Secondary method of euthanasia

- 4.8.1 Following anaesthesia and a primary method of euthanasia, pithing or decapitation with pithing, may be performed to verify death due to the guaranteed lethal outcome of the procedure. It must never be performed on a conscious animal.

4.8.2 Decapitation with pithing

- 4.8.2.1 Decapitation requires rapid severance of the head and brain from the spinal cord, generally performed at the level of the neck. The precise location may vary depending on the species.
- 4.8.2.2 Decapitation must always be followed by pithing (decapitation with pithing) or another method to destroy brain tissue.

4.8.3 Pithing

- 4.8.3.1 Destruction of brain tissue (pithing) or destruction of the brain and spinal cord (double-pithing) must take place following deep anaesthesia and may or may not be preceded by decapitation.
- 4.8.3.2 Pithing is performed by inserting a rigid rod or probe into the skull cavity via a species appropriate entry point (e.g. the opening (foramen magnum) at the base of skull) to cause permanent destruction of the brain tissue. The same procedure may be applied via the vertebral canal in order to destroy the spinal cord tissue.

4.9 Considerations for field work

4.9.1 Training

Methods of anaesthesia and humane euthanasia must be supplied for review by the AEC prior to any field work being conducted. It is the responsibility of investigators to ensure they have a good understanding of the anatomy, physiology and methods of euthanasia for their species of interest and to arrange training in these methods with a competent supervisor or the AWO.

4.9.2 Handling and storage of drugs

Any scheduled drugs supplied to investigators under permit are to be stored according to the appropriate regulations and legislation, and must only be used in the manner approved by the AEC. Particular care should be taken to ensure pentobarbitone is locked away securely when not in use due to the potential for misuse or abuse in humans.

4.9.3 Emergency euthanasia in the field

- 4.9.3.1 The nature of field work means that on rare occasions, a reptile may be encountered with severe injuries or illness that requires euthanasia on humane grounds. Investigators are

required to have a method of euthanasia to ensure these animals do not suffer and provide this information to the AEC in their project applications.

- 4.9.3.2 Direct pithing or blunt force trauma are acceptable methods of euthanasia in the field situation, where access to the usual drugs and laboratory equipment are not practical and the time to obtain such things would ultimately result in more suffering. In the interest of the animal's welfare, exceptions to the usual procedures may be permitted at the discretion of the AEC.
- 4.9.3.3 Where alternate methods of euthanasia are proposed for use in the field, the AWO and/or AEC may request to assess competency or view training logs prior to approving the procedure.

4.9.4 Disposal of remains

- 4.9.4.1 Large reptile species including crocodiles and sea turtles should not be euthanised using sodium pentobarbitone in the field due to the difficulty associated with carcass removal. Physical methods such as firearms or penetrating captive bolt may be considered in these cases.
- 4.9.4.2 The bodies of animals euthanised with pentobarbitone sodium should be wrapped and secured in a plastic bag, and then should be preferably incinerated or disposed of via the medical waste service at the university. Where these options are not appropriate, bodies may be buried at a depth greater than one metre provided the relevant permits are obtained first.
- 4.9.4.3 If using MS-222 for euthanasia of reptiles in the field, investigators must ensure that the agent does not contaminate surface water and that any contaminated water or tissues containing drug residue are removed from the field site and disposed of appropriately.

5. MONITORING & INTERVENTION

5.1 Monitoring depth of anaesthesia

- 5.1.1 Reptiles anaesthetised by the methods discussed in section 4.2 will require investigators to monitor their depth of anaesthetic. A deep plane of anaesthesia is required prior to commencing euthanasia (see section 4.3).
- 5.1.2 Initially animals should become quiet, still and minimally responsive to handling when they are lightly anaesthetised. The pedal and righting reflex will become absent and muscle, along with muscle tone should be monitored to determine
 - 5.1.2.1 **Pedal reflex:** Squeeze the toe or foot of the reptile; if the limb is retracted toward the body, then the animal is too light. This reflex will disappear when deeply anaesthetised.
 - 5.1.2.2 **Righting reflex:** Gently attempt to move the reptile's body position from laying on its ventrum (upright and normal) to lying on its back. This can be done by directly touching the animal, or carefully tilting the holding container. A normal animal will try to correct itself into an upright position, but after anaesthesia this reflex should be absent.
 - 5.1.2.3 **Muscle tone:** Observe the reptile's posture and movements, and feel the body to assess for muscle tone. As muscle tone is diminished they will become more still, posture is low to the ground and the body becomes soft and flaccid instead of firm and freely moving. This is most useful in snakes where the pedal reflex is not an option.
 - 5.1.2.4 Note the palpebral reflex that is commonly used in mammals is not reliable in reptiles, and not present at all in species such as geckoes that lack eyelids. It is not a suitable parameter for monitoring anaesthesia.

5.2 Confirmation of death

- 5.2.1 Confirmation or verification of death is necessary to ensure that the euthanasia procedure has been carried out correctly and that death has occurred. Death of the animal is the point at which brain activity, heart beat and respiration ceases.
- 5.2.2 The ability for reptilian tissues to cope with low oxygen conditions caused by reduced blood flow (hypoxia) poses two main issues for euthanasia. Firstly, decapitation does not result in immediate loss of consciousness or brain death; secondly, it is possible for the heart to continue beating after brain

death. The brain, nervous system and cardiac muscle all have an innate ability to survive after their blood supply is terminated.

- 5.2.3 For methods of euthanasia where death cannot be assured, it must be confirmed by physical intervention using a guaranteed lethal procedure such as pithing, double-pithing and/or decapitation with pithing.
- 5.2.4 The presence or absence of a heartbeat cannot be reliably used to verify death. If the heartbeat has ceased, then secondary destruction of brain tissue may still be needed to confirm death. The necessity to perform a secondary lethal procedure will depend on the initial method of euthanasia used (i.e. Pentobarbitone sodium given intravenously vs inhaled agents).
- 5.2.5 Assessment of heart sounds is difficult using a stethoscope and is best achieved using Doppler ultrasonography. The Doppler probe is placed externally over the heart with contact gel between the probe and scales/skin. The heart rate may be dramatically slower following anaesthesia or at low environmental temperatures.
- 5.2.6 Where the brain and/or spinal cord tissues are specifically required as part of an investigation, methods of euthanasia with greater assurance of death (e.g. IV or ICA sodium pentobarbitone) should be selected. Exceptions may be granted on an individual basis at the discretion of the AEC.

6. ADDITIONAL INFORMATION

- N/A

7. ENFORCEABLE REQUIREMENTS

- 7.1 Strict adherence to methods of euthanasia listed as acceptable in this standard.
- 7.2 Sodium pentobarbitone must only be administered by approved routes, with IV delivery being the preferred route where practical. Oral, subcutaneous or intramuscular administration is not permitted.
- 7.3 Where anaesthesia is administered, reptiles must be fully anaesthetized prior to humane killing.
- 7.4 Animals over 4 g must never be euthanized by immersion in liquid nitrogen and those <4 g must be fully anaesthetized prior to it.
- 7.5 Freezing or cooling must not be used as a sole method of humane killing.
- 7.6 Decapitation must not be performed on conscious animals and must always be followed by pithing or double-pithing.
- 7.7 A secondary method to confirm death is required after every primary method of humane killing, unless an exception is specified by this Standard or approved by an AEC.
- 7.8 All projects that involve reptiles and ultimately their killing must outline what method of humane euthanasia will be used. Only procedures that have been approved by the AEC are permitted.
- 7.9 Only appropriately trained individuals or those under direct supervision are permitted to perform humane killing of reptiles.
- 7.10 Remains of euthanized animals must be disposed of safely and in accordance with relevant legislation.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.

9. UNEXPECTED ADVERSE INCIDENTS

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University's AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

10. GLOSSARY

Scientific Term	Lay Description
Distal	Away from the body (e.g. The foot is distal to the body)
Euthanasia	A humane method of killing that results in a painless death
Pithing	The act of destroying brain and/or spinal cord tissue using a rod or probe to ensure death. This must only be performed on deeply anaesthetised animals or immediately after decapitation of a deeply anaesthetised animal.
Poikilotherm	An animal that cannot regulate its own body temperature, except by behavioural means such as basking or burrowing, and is heavily influenced by the temperature of its environment (e.g. Reptiles and fish)
Proximal	Towards or closest to the body (e.g. The hip is proximal to the body)
v/v	"Volume/volume" denotes concentration as percentage; volume of chemical per 100ml volume of solution.
Ventrum	Underside of the animal; the skin on the abdominal area is the ventral surface
Viscera	Catch-all term for internal organs e.g. Stomach, kidneys, liver, heart etc

11. REFERENCES & RESOURCES

The following source material contributed to the development of this Standard:

- Ascher, J.M., Bates, W., Ng, J., Messing, S. and Wyatt J. (2012). Assessment of xylazine for euthanasia of Anoles (*Anolis carolinensis* and *Anolis distichus*). *J. Amer. Assoc. for Lab. Anim. Sci.*, 51: 83-87.
- Conroy, C.J., Papenfuss, T., Parker, J. and Hahn, N.E. (2009). Use of Tricaine Methanesulfonate (MS222) for euthanasia of reptiles. *J. Amer. Assoc. Lab. Anim. Sci.*, 48: 28-32.
- Close, B., Banister, K., Baumans, V., Bernoth, E., Bromage, N., Bunyan, J., Ehart, W., Flecknell, P., Gregory, N., Hackbarth, H., Morton, D. and Warwick, C. (1997). Recommendations for euthanasia of experimental animals: Part 2. *Lab. Anim.*, 31: 1-32.
- Reilly, J.S. (Ed.) (2001). Euthanasia of Animals Used for Scientific Purposes. ANZCCART (Australian and New Zealand Council for the Care of Animals in Research and Teaching), Second Edition, Adelaide University, Adelaide, SA.
- Warren, K. (2014). Reptile Euthanasia – No Easy Solution? *Pacific Conservation Biology*, 20(1): 25-27.

The following resources may provide additional or supplementary information:

- AVMA (American Veterinary Medical Association), 2013. Guidelines for the Euthanasia of Animals: 2013 Edition. Available from http://www.avma.org/issues/animal_welfare/euthanasia.pdf
- Mader, D.R. (2006). Euthanasia. In: Mader, D.R. (Ed.) *Reptile Medicine and Surgery*. Elsevier. Philadelphia, Pennsylvania. Pp. 564-568.