This document has been developed by The Australian National University's (ANU) Research Ethics Office. It has been endorsed by the ANU Animal Ethics Committee (AEC). It is designed to provide guidance regarding current best practice to institutional animal users and carers on the care and use of animals for scientific purposes. It has been prepared in consultation with the Australian code for the care and use of animals for scientific purposes 8th edition 2013.

Document_016_Guideline_Field Euthanasia of Wildlife V1.1

Background

The selected method of euthanasia must have prior approval from the AEC. In an emergency situation the ANU Veterinarian may provide on the spot approval for particular methods to be followed.

The purpose of this document is to provide guidance on humane killing methods for research or legislative purposes and where necessary guide appropriate euthanasia in emergencies and alleviate welfare concerns. The term euthanasia has been used throughout the document but could be interchanged with 'humane killing' depending on the situation/purpose.

The method must be appropriate for the species in question; must be carried out with the intention to minimise pain and distress; and must be performed in the shortest possible time.

As per the Code Section 3.3.45: "*The method and procedure used for killing an animal must be humane and:*

- *i) avoid pain or distress and produce rapid loss of consciousness until death occurs*
- *ii) be compatible with the purpose and aims of the project or activity*
- *iii) be appropriate to the species, age, developmental stage and health of the animal*
- *iv) require minimum restraint of the animal*
- *v) be reliable, reproducible and irreversible*
- *vi) ensure the animals are killed in a quiet, clean environment away from other animals*
- *vii) ensure that death is established before disposal of the carcass, foetuses, embryos and fertilised eggs. Dependant offspring of animals to be killed must be cared for or humanely killed".*

The method of euthanasia must be undertaken in accordance with current best practice.

Definitions

Blunt force trauma: the use of a blunt instrument to create an impact against the body in a defined area (usually the head) so as to cause death or incapacitation.

Code: The NHMRC's Australian code for the care and use of animals for scientific purposes 8th Edition 2013.

Euthanasia: the act of inducing death using a method appropriate to the species that results in a rapid loss of consciousness without recovery and minimum pain and/or distress to the animal.

Intracardiac: within or administered into the heart

Intracoelomic: within or administered into coelom (body cavity present in birds, fish, reptiles and amphibians

Intrahepatic: within or administered into the liver

Intraperitoneal: within or administered into the peritoneal cavity (abdominal cavity of mammals).

Intravenous: within or administered into a vein

Operator: the person undertaking the euthanasia.

Pithing: piercing or severing of the brain or spinal cord to cause death.

Sedation: the calming of mental excitement or abatement of physiological function, especially by the administration of a drug.

Stunning: the act of rendering an animal unconscious through different means. In itself not an acceptable method of euthanasia but may be used rarely in conjunction with a secondary method. Different methods are acceptable depending on species and these should be established prior to the start of the project.

Unexpected adverse event: an event that may have a negative impact on the wellbeing of any animals and was not foreshadowed in the approved project or activity.

Vertebrate pest animals: vertebrate animals, including native and introduced species that are generally regarded, or have been declared under state or territory legislation, as a 'pest species'.

Wildlife: free-living animals of native or introduced species, including those that are captive bred and those captured from free living populations.

General Information and considerations

Access to Veterinary Support and Training Requirements

Where the humane killing of animals is part of a planned component of an animal ethics protocol, those undertaking these practices must be trained and deemed competent in the procedure(s) to be utilised.

Where humane killing is not part of the planned protocol, it is not expected that research groups have the ability or training to euthanase all species that may be at risk in emergency situations, however, appropriate options must be in place relevant to the location of the work and potential impact on wildlife.

A veterinary coverage plan, that covers suitable treatment of animals that may be directly impacted as a result of the research activities, must be included in an approved wildlife animal ethics protocol. Such plans should consider the availability of a veterinarian, the competency and knowledge of the field workers, the likelihood of adverse events and how they may impact animal welfare. The plan must include who may be involved in performing euthanasia should this be necessary, their training, and a communication plan surrounding this requirement. In all situations, where an injured or ill animal is identified, then veterinary advice must be sought immediately. Any variations to these requirements must be clearly identified in an approved animal ethics protocol.

Protocols that are remote may incorporate the need to train individuals undertaking field work in at least one humane method of euthanasia as outlined in this protocol.

Protocols that are within reasonable distance of a veterinary clinic may nominate to make a written agreement with a veterinary clinic and submit this agreement with their application. The ANU Veterinary Services Team must also be kept up to date with any events and treatment or actions undertaken and are also available for advice and discussion where required.

For protocols without a written agreement, the ANU vets must be contacted as soon as possible in the event of any animal welfare emergency and an action plan will be determined based on location and availability of resources. In some situations, physical support from the ANU vet may not be suitable and advice may be provided to attend a local veterinary clinic for assessment, diagnosis and potential euthanasia.

ANU Training and Support for Field Euthanasia of Wildlife

The ANU Veterinary Services Team, are available to provide some training and advice in the most appropriate methods of euthanasia.

You are encouraged to liaise with the ANU vets in developing a veterinary coverage plan and your animal ethics protocol.

Operator Responsibility

It is the responsibility of the Primary Investigator to ensure that all operators are fully trained and skilled in the appropriate methods of euthanasia approved under the protocols they are working under. The operator must keep up to date with current best practice and undertake regular refresher training. For skills that are used regularly, this may be every 3 years. Refresher training is required if there are any gaps in their skills as identified through Unexpected Adverse Events or senior members of the group, or confidence has been lost, or there has been a period of time between the uses of the technique.

Persons involved with the euthanasia of animals must be familiar with the anatomy and physiology relevant to euthanasia, as well as appropriate dosing, restraint, and confirmation of death. The guidelines described here are intended for field conditions; fully-equipped veterinary hospitals and personnel should be utilised whenever available.

Firearms

The operator must have the relevant skill and licensing to operate a firearm, which must be stored in accordance with local legislation. 'Relevant skill' includes the appropriate selection of firearm type, position and ammunition necessary to produce instantaneous or near instantaneous loss of consciousness and death. A copy of the operator's current firearms licence must be provided with the ethics application or amendment to the AEC.

Drug Use and Record Keeping

The operator also has a responsibility to ensure the correct use, safe storage, and disposal of all material used to undertake euthanasia; for example, appropriate licensing and record keeping for substances used as part of the euthanasia procedure. Records must be up to date and easily produced if requested by veterinarians or via audits undertaken by the relevant Government authority, with the amount of time records are kept dependant on the materials used (for example, Schedule 8 drugs and sodium pentobarbitone records must be kept for a minimum of two years).

Personal Safety

ANU WH&S requirements must be adhered to at all times. This includes the completion of risk assessments. Where a risk assessment has identified a requirement for personal protective equipment, it must be available, used, and disposed of appropriately by the operators.

The principal investigator and operator should be aware of potential zoonotic disease risks, and where appropriate expert medical advice should be obtained – for example, rabies vaccinations for those working with bats.

Contaminated Carcass and Instrument Disposal

The operator should be aware, and be skilled in, appropriate disposal of animal carcasses and potential hazardous materials specific to the methods and species they are working with. Activities must be undertaken in accordance with approved standard operating procedures and risk assessments. Appropriate measures must be taken to ensure that carcasses are disposed of in a manner that prevents environmental contamination, or exposure of other animals or humans to the contaminated material/s.

Unacceptable Methods of Euthanasia

Sodium pentobarbitone should not be injected directly into the abdomen or coelom unless appropriately diluted with sterile water for injection to prevent irritation. Intrathoracic injections are not suitable for euthanasia. The following methods are not acceptable for the euthanasia of wildlife:

- Freezing
- Burning
- Suffocation via chest compression, drowning or strangulation
- Carbon dioxide and carbon monoxide inhalation
- Hypothermia (adult exothermic animals)
- Intravenous potassium chloride, magnesium sulphate or neuromuscular blockers
- Air embolism (injection of air intravenously)
- Toxins or poisoning
- Exsanguination without prior anaesthesia
- Electrocution
- Neuromuscular blocking agents
- Chloroform, chloral hydrate or ether
- Formaldehyde immersion or injection
- Non-penetrative captive bolt without a secondary method
- Stunning without a secondary method

Blunt force trauma may only be carried out by an operator skilled and experienced in the method, and only in the absence of another more reliable and humane method.

Sedation

Sedation is strongly recommended for large birds, mammals and reptiles prior to euthanasia. This reduces the stress to both the operator and the animal, provides additional restraint, and minimises the risk of error. Sedation must be approved by the ANU veterinarians and clear

guidelines for its use outlined in the approved animal ethics protocol. The operator must have the appropriate licence, storage, and record keeping system in place prior to use.

Euthanasia Recommendations for Specific Taxa

Small Birds (e.g. wren, small parrots, finches, mynas)

The preferred method for euthanasia of small birds is intrahepatic injection of sodium pentobarbitone solution diluted 1:4 with sterile water for injection to ensure the solution is non-irritant.

Intracoelomic Injection should only be performed by highly skilled operators due to the risk of injecting into large air sacs present in the body.

For any injection routes other than intravenous, sodium pentobarbitone must be diluted 1:4 and a small gauge needle used. If the above methods are not available, cervical dislocation or decapitation may be utilised, but this should only be performed by an operator highly skilled in these techniques.

Medium Birds (e.g magpie, galah, cockatoo, duck)

The preferred method for medium birds is either intravenous or intrahepatic injection of sodium pentobarbitone diluted 1:4 to ensure it is non-irritant. Use a needle appropriate for the size of the bird. If these methods are not available, cervical dislocation is permitted only by an operator highly skilled in this technique.

Large Birds (e.g emu, goose, pelican)

The preferred method for large birds is intravenous injection of sodium pentobarbitone using an appropriate size needle. Intracardiac injection can be used in a sedated animal. If these methods are not available, the use of a firearm or a penetrative captive bolt is permitted by an operator highly skilled in these techniques.

Small Mammals (e.g mouse, microbats, antechinus, unfurred pouch young)

For any mammal under 300g, the preferred method is intraperitoneal, intrahepatic, or intravenous (if possible) injection of a non-irritant solution of sodium pentobarbitone using a small gauge needle. For intraperitoneal and intrahepatic injections, the sodium pentobarbitone must be diluted 1:4 with sterile water for injection. If these methods are not available, cervical dislocation (under 150g only) or decapitation may be performed by an operator highly skilled in these techniques.

Medium Mammals (e.g brushtail possum, quoll, bettong, echidna)

The preferred method for mammals between 300g and 5kg is euthanasia with sodium pentobarbitone injected intravenously, intracoelomically or intrahepatically using an appropriately sized needle. The sodium pentobarbitone should be diluted 1:4 with sterile water for injection for intraperitoneal and injections. Intracardiac injection of sodium pentobarbitone can be used in a sedated animal without dilution. If the methods above are not available, euthanasia may be delivered by the use of a firearm by a skilled and licensed operator if the conditions are deemed suitable.

Large Mammals (e.g kangaroo, dingo, wombat)

Given the danger and risk of injury to the operator and the likelihood of stress to the animal, animals over 5kg must first be sedated, followed by an intravenous injection of sodium pentobarbitone using a needle appopriate to the size of the animal. If this method is not available, a firearm or penetrative captive bolt gun may be used by a skilled and licenced operator in a safe environment.

Reptiles

Reptiles should be euthanised with intravenous, intrahepatic, (or intracoelomic sodium pentobarbitone solution using a needle appropriate to the size of the animal. The sodium pentobarbitone should be diluted 1:4 with sterile water for injection. If these methods are not available, the animal can be anaesthetised with alfaxalone intravenously, intracoelomically, or intrahepatically then decapitated or pithed.

Amphibians

Amphibians should be euthanised either with sodium pentobarbitone intracoelomically (diluted 1:4 with sterile water for injection) or Intravenously with a needle appropriate to the size of the animal, or through prolonged immersion in a buffered tricaine methane sulfonate (MS-222) solution. Tadpoles may be euthanised with formulated isoeugenol solution (Aqui-S) as for fish. If these are not available, the animal may be anaesthetised and then pithed or decapitated.

Fish

Fish should be euthanised with an appropriate dose of formulated isoeugenol solution (Aqui-S) or via a prolonged immersion of buffered tricaine, methane sulfonate (MS-222) solution until 10 minutes after the last opercular (gill) movements cease. For small bodied (<5cm) tropical fish (both marine and freshwater) hypothermal shock (rapid chilling) through immersion into an ice slurry of 2-4^{o}C is an acceptable method of euthanasia. This method is not suitable for fish above 5cm in size or for cold tolerant species.

If the methods discussed above are not available, stunning followed by pithing or severing of gill arches before consciousness is regained may be used.

Emergency or Urgent Situations

There may be situations where an animal must be euthanised and the appropriate drug/s are not available e.g. remote area where an animal is suffering and cannot be immediately or practically transported to a veterinarian. In these situations, if possible, the ANU Veterinary team should be contacted by phone (available 24/7) on 02 6125 1130 and their directions followed with the aim to achieve minimal distress to the animal. The euthanasia must be carried out by an operator skilled and experienced in the method of euthanasia. Investigators are encouraged to ensure all co-investigators working in the field are competent in undertaking more than one method of euthanasia per target species. Nominating back up options for veterinary services that are available close to the specific field sites is also encouraged.

Vertebrate Pest Animals

The euthanasia of vertebrate pest animals (as declared under state and territory legislation) while undertaking research activities may only be carried out if approved under an animal ethics protocol. As with other animals covered by the Code, the method, dosage and drugs must be appropriate to the taxa and size of the animal in question. Consideration should also be given to

bycatch species where animals are being trapped and that some states or territories may prohibit the release of some species back into the wild. Provision needs to be made for this possibility in the ethics application and a plan provided.

Activities undertaken by third party organisations to directly manage pest species e.g. fox baiting by local Government, often serve a secondary purpose in supporting research activities. Where the pest animal management activity is undertaken by a third party as part of their usual pest animal management practices (i.e. not primarily for the purposes of research and not by ANU staff/students), this guidance document will not apply to the pest animal.

Monitoring, Intervention and Reporting

Any euthanasia that is undertaken in the field that is not part of the planned and approved ethics protocol must be reported as an Unexpected Adverse Event to the AEC as per the ANU Procedure for Managing and Reporting Unexpected Adverse Events. There may also be additional reporting requirements to local jurisdictions depending on the requirements of any licences and local legislation. It is a requirement that all people those working on a particular protocol are familiar with these requirements.

Minimum Requirements

Minimum requirements that align with the Code:

- Records must be kept for all training and competency assessment of investigators and operators.
- All drug use, associated record keeping and storage must meet local legislative requirements and ANU procedures.
- All planned activities must be undertaken as per the approved protocol and approved methods of euthanasia must be utilised. Any use of non-approved methods must be reported as an Unexpected Adverse Event.
- Methods that are listed as “unacceptable methods of euthanasia” as per this Guideline are not to be utilised unless explicit approval has been provided under the approved animal ethics protocol.

References and Resources

American Medical Association (2020) The AVMA Guidelines for the Euthanasia of Animals.

Department of Parks and Wildlife – Government of Western Australia (2013) Standard Operating Procedure – Humane killing of animals under field conditions in wildlife management. (accessed on 07 February 2020)

James Cook University. Humane Killing of Animals used for Scientific Purposes Guidelines (2013) (accessed on 02 February 2020)

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 (accessed on 29 January 2020)

National Health and Medical Research Council (2014) A guide to the care and use of Australian native mammals in research and teaching, Canberra: National Health and Medical Research Council. (accessed 29 January 2020)

Procedure for Managing & Reporting Unexpected Adverse Events

Tidemann, C.R. & King, D.H. (2009) Practicality and humaneness of euthanasia of pest birds with compressed carbon dioxide (CO2) and carbon monoxide (CO) from petrol engine exhaust. Wildlife Research, 36, 522–527 (accessed 02 February 2020)