

Standard Operating Procedure GEN001: Methods of euthanasia

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Background

The word euthanasia means an easy death and should be regarded as an act of humane killing with the minimum of pain, fear and distress.

Euthanasia of a range of animal species is often necessary during pest animal control programs and occasionally in research involving the capture or restraint of pest animals. Therefore, all researchers and personnel involved with pest animal control must be familiar with the approved euthanasia methods for the range of species encountered (both target and non-target) and have appropriate equipment or possibly drugs on hand so that euthanasia can be performed effectively and quickly.

Many recommended methods of euthanasia for captive animals are not feasible under field conditions; however the challenges presented by field conditions should not lessen the ethical obligation of the operator to reduce pain and distress to the greatest extent possible during euthanasia.

This standard operating procedure (SOP) is a guide only; it does not replace or override the legislation that applies in the relevant state or territory jurisdiction. The SOP should only be used subject to the applicable legal requirements (including OH&S) operating in the relevant jurisdiction.

Application

- This document is a summary of the current best practices for providing a humane death for a range of species that may be encountered when working with pest animals. Euthanasia of animals may be required for a number of reasons including:

- Emergency euthanasia of animals with untreatable injuries incurred during capture, handling or transit
- Cases of untreatable disease where an animal is suffering
- Collection of voucher specimens
- Requirement of a research procedure, eg when fresh tissues are required for analysis
- Destruction of live-captured declared pest animals (where release is prohibited).
- The document is not aimed at the shooting of ‘free-ranging’ pest animals or wildlife. It is also not designed to provide specific guidance on the killing of pest animals as part of control programs. Species-specific procedures for the humane control of pest animals are available for this purpose.
- Factors to consider when choosing an appropriate method of euthanasia are species, size, safety, location of the animal and expertise and preference of the operator. The most appropriate method for species and age and size of animal should always be used.
- Euthanasia procedures must be performed by persons competent in and qualified for the methods to be used, or under the direct supervision of a competent person. Some methods require considerable training and experience to be used appropriately. Training should include:
 - familiarity with the normal behaviour of the species being euthanased
 - an appreciation of how handling and restraint affect behaviour





GEN001: Methods of euthanasia

- an understanding of the mechanisms by which the selected technique induces loss of consciousness and death
- recognition of signs of pain and distress.
- The acquisition, care and use of animals for scientific purposes in Australia must be in accord with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and with Commonwealth, State and Territory legislation. All animal research must be approved by an Animal Ethics Committee (AEC) and covered by a valid animal research authority issued by an AEC.
- Schedule 4 drugs (eg barbiturates, anaesthetics and tranquillisers) can only be administered by a veterinarian as an act of veterinary science (or an authorised person under their direction) or, under certain circumstances, by an investigator (or an authorised person under their direction) under a valid research authority. As there is some variation in the statutory requirements of each state for the supply, possession, use and storage of these drugs, operators must consult the relevant legislation before use.

Animal welfare considerations

Impact on target animals

- When it is necessary to kill an animal, humane procedures must always be used. These procedures must avoid distress, be reliable, and produce rapid loss of consciousness without pain until death occurs.
- Many methods of euthanasia require that animals be physically restrained. Proper handling and restraint is essential to minimise pain, fear, distress and anxiety experienced by the animal and also for the safety of the operator.
- Where capture or restraint may cause significant pain, injury or anxiety to the animal or pose a danger to the operator, the prior use of sedative and/or immobilising drugs may be necessary.
- It is important to recognise that some physical methods of euthanasia (eg stunning followed by exsanguination) which cannot be made aesthetically pleasant may, nonetheless, be humane in that it ensures immediate insensitivity to pain. The choice of technique should be made based on the sensibilities of the animal to be killed rather than the sensitivities of the observer or operator.
- If possible, any dependent young of animals being euthanased should also be killed or provision made for their care.

Health and safety considerations

Animal handling and restraint

- Many animal species are capable of inflicting serious injury to persons handling them. Appropriate handling and restraint techniques should be used, and training in their application should be provided to avoid injury to both animals and humans.
- Protective clothing, footwear and gloves may reduce the chances of injury when handling wild animals.
- When working in the field, personnel should work in teams of at least two people especially when involved in the physical or chemical restraint and handling of animals.
- After stunning larger animals (eg deer, horses), always approach the animal from the dorsal (or spinal) side to prevent injury from the involuntary paddling of legs.
- Some animal carcasses can be heavy (eg feral pigs can be >100 kg), so care must be taken when lifting/dragging.

Firearm safety

- Firearms are potentially hazardous. All people should stand well behind the shooter when an animal is being shot. The line of fire must be chosen to prevent accidents or injury from stray bullets or ricochets.
- Firearm users must strictly observe all relevant safety guidelines relating to firearm ownership, possession and use.
- When not in use, firearms must be securely stored in a compartment that meets state legal requirements. Ammunition must be stored in a locked container separate from firearms.
- Adequate hearing protection should be worn by the shooter and others in the immediate vicinity of the shooter. Repeated exposure to firearm noise can cause irreversible hearing damage.
- Safety glasses are recommended to protect the eyes from gases, metal fragments and other particles.

Zoonotic hazards

- Care must be taken when handling live animals and carcasses as they may carry diseases that can affect humans and other animals eg hydatidosis, dermatophytosis, sarcoptic mange, leptospirosis, Q fever, brucellosis, sparganosis, melioidosis, tuberculosis etc. Routinely wash hands after handling all animals and carcasses.
- Australian bat lyssavirus (ABL) and rabies vaccination is recommended for people who come into regular contact with bats (both flying foxes and microbats). Operators should avoid bites and scratches and use protective

equipment when handling all bats. All wounds inflicted by bats or flying foxes should be washed thoroughly with soap and water as soon as possible. Operators should always seek medical advice regarding post-exposure treatment whenever a bite, scratch or mucous membrane exposure to saliva from any Australian bat has occurred. Where the bat is available it should be tested for the presence of ABL.

- Q fever can be transmitted to humans during contact with infected animals, or with infected uterine or placental tissue. A variety of animals may be infected including kangaroos, wallabies, dogs, cats, cattle, sheep and goats. Vaccination is recommended for people who come into regular contact with potentially infected animals. Skin or blood testing of personnel is recommended to assess previous exposure, followed by vaccination for susceptible individuals

Chemical hazards

Use of anaesthetic gases

- There is evidence that chronic exposure to inhalation of anaesthetic agents may be associated with psychomotor, hepatic and renal dysfunction.
- They should only be used in well-ventilated areas.

Use of carbon monoxide

- Carbon monoxide is extremely hazardous to humans as it is highly toxic and difficult to detect. Exposure from inhalation of carbon monoxide fumes can cause fatal poisoning. Non-fatal poisoning may result in permanent nervous system damage.
- If carbon monoxide fumes are inhaled, remove patient from contaminated area. Lay patient down and keep warm and rested. Early signs of toxicosis are headache, dizziness and weakness. If patient is not breathing, apply artificial respiration and perform cardiopulmonary resuscitation (CPR) if necessary. Transport patient to a hospital or doctor without delay.

Use of carbon dioxide

- Carbon dioxide should be used in a well ventilated place.
- Carbon dioxide is non-flammable, non-explosive and poses minimal risk to personnel when used with properly designed equipment. However, inhalation of significant concentrations of CO₂ can cause narcosis and/or asphyxia.
- If CO₂ is inhaled, remove patient from the contaminated area to allow them to breathe fresh air. Early signs of exposure are headache and shortness of breath. If patient is not breathing, make sure airway is clear and apply artificial resuscitation. Keep warm. Oxygen may be given but only under the supervision of a trained person.

- Although prolonged exposure to low levels of CO₂ (up to 1.5 % in inhaled air) are well tolerated, chronic health effects can result.
- For further information refer to the Material Safety Data Sheet (MSDS), available from the supplier.

Methods of euthanasia

While no ideal method of euthanasia exists, the procedure of choice should approach as closely as possible the following criteria:

- Is painless
- Produces rapid loss of consciousness and death
- Interrupts consciousness and reflexes simultaneously
- Requires minimum restraint
- Avoids excitement and causes minimal psychological stress to the animals
- Is appropriate for the age of the animal
- Exhibits consistent and predictable action
- Is easily and safely administered by properly trained personnel
- Causes minimal emotional effects to operator and observers
- Is not subject to abuse by humans
- Is not a disease or environmental problem
- Does not result in tissue changes that would affect a post-mortem diagnosis
- Is economical and readily available
- Does not leave carcasses that, if consumed, will result in secondary poisoning.

Methods of euthanasia fall into two broad categories - chemical and physical. These methods may cause death by three basic mechanisms:

- Hypoxia, direct or indirect
- Direct depression of neurons vital for life functions
- Physical destruction of brain activity and destruction of neurons vital for life.

Below is a brief description of some of the recommended methods of euthanasia.

Physical methods

Shooting

- Shooting is a quick and effective means of humanely destroying animals and in most situations is the only practical method available for use in the field.
- Head shots are preferred when euthanasing animals that are immobilised by injury or physical restraint. Correctly placed head shots cause brain function to cease and result in an instantaneous loss of consciousness.



GEN001: Methods of euthanasia

- Shots must be aimed so that the projectile enters the brain, causing instant loss of consciousness. This must take into account differences in brain position and skull conformation between species as well as the energy requirement for skull bone and sinus penetration. Accurate targeting for a shot to the head in various species has been described below.
- In some situations chest shots may be required where an accurate head shot cannot be achieved (eg injured free-ranging animals or animals that cannot be restrained) or where a shot to the brain may prevent collection of samples for research or disease diagnosis. Death from a shot to the chest is due to massive tissue damage and haemorrhage from major blood vessels. Insensibility will occur after an interval ranging from a few seconds to a minute or more. If a shot stops the heart functioning, the animal will lose consciousness rapidly.
- Shooting should only be performed by skilled operators who have the necessary experience with firearms and who hold the appropriate licences and accreditation.
- Storage and transportation of firearms and ammunition must comply with relevant legislative requirements.
- There may be legal restrictions on discharging a firearm in certain areas. Police permission may be necessary.
- The accuracy and precision of firearms should be tested against inanimate targets prior to the commencement of any shooting operation.
- Non-essential personnel should keep away from the area to allow the animal to become less agitated. The shooter should approach the animal in a calm and quiet manner.
- To maximise the impact of the shot and to minimise the risk of misdirection the range should be as short as possible, eg 5–20 cm from the head if using a rifle, 1–2 m if using a shotgun. The barrel should never be touching the animal's head.
- Never fire when the animal is moving its head; be patient and wait until the animal is motionless before shooting. Accuracy is important to achieve a humane death. One shot should ensure instantaneous loss of consciousness and rapid death without resumption of consciousness.

Rifles

- Firearms no smaller than a .17 calibre rimfire are adequate for euthanasia of most species of animals at short range (< 5 m), as long as the shot is correctly positioned. Soft-nose or hollow point ammunition which expands on impact should be used.

Shotguns

- 12 gauge shotguns with shot sizes of BB or AAA may also be used.

Handguns

- Handguns are class H firearms and their use is tightly controlled in all jurisdictions.
- Rifles are preferred to handguns because the longer barrel length ensures better control by the shooter and places the shooter further away from the animal.
- Where handguns have to be used for euthanasia, the shooter must be especially careful as the shorter sight radius and barrel length greatly increases the risk of accidental injury to bystanders.

Penetrating Captive-Bolt Pistols

- A penetrating captive-bolt pistol uses a blank cartridge to fire a bolt into the brain of the animal. This causes concussion and trauma to the cerebral hemispheres and brainstem which renders the animal instantaneously unconscious. A correctly stunned animal will collapse immediately often with muscular contractions and involuntary kicking.
- A penetrating captive bolt can cause varying degrees of brain injury depending on the placement and length of the bolt and the force with which it is projected into the brain. In some cases the animal may not die quickly, therefore, it is recommended that immediately after the procedure, death of the animal is ensured by a second procedure such as exsanguination or pithing.
- Is an acceptable method of euthanasia in larger animals ie horses, pigs and ruminants (sheep, cattle, goats etc).
- Suitable restraint of the animal and exact placement of the bolt is essential. Captive-bolt pistols must be firmly held against the head when firing.
- The manufacturers recommendations should be followed on the most appropriate blank cartridges for each species.
- Captive-bolt pistols are not regarded as firearms, however, operators must be properly trained in their use.

Stunning by a blow to the head

- Stunning the animal by a blow to the head may be acceptable in small or young animals with a soft skull (eg neonatal pigs <3 weeks old, rats, mice, kittens, newborn pups, birds, reptiles, amphibians, fish).
- A single, sharp blow should be delivered to the central skull bones. This can be achieved with a hard and heavy,

blunt instrument (e.g. metal pipe, wooden club etc.). Alternatively, small animals can be held by the hind quarters and swung in an arc so that the back of the head is struck on the edge of a hard object.

- Stunning usually only renders an animal unconscious; therefore it must be immediately followed by a second method that ensures death (e.g. exsanguination, cervical dislocation, pithing)
- When properly performed with sufficient force, immediate depression of the central nervous system and destruction of brain tissue occurs. Loss of consciousness is rapid.
- It must be properly applied to be effective and humane therefore, training and skill of operator is essential. If not performed correctly, various degrees of consciousness with accompanying pain can occur.

Cervical dislocation

- Acceptable for small animals which are easily handled eg small to medium sized birds, rodents and, rabbits (weighing <1 kg).
- The operator must be confident of performing this technique quickly and effectively. It requires mastering of technical skills to ensure that loss of consciousness is rapidly induced.
- This method involves separation of the skull and the brain from the spinal cord by pressure applied posterior to the base of the skull. The brain stem - which controls respiration and heart activity - is consequently damaged, stopping breathing and reducing blood flow to the brain, leading to death. Studies in rats have shown that electrical activity in the brain persists for around 13 seconds following cervical dislocation. This may represent a period of remaining consciousness.
- Violent muscular contraction can occur after cervical dislocation.

Decapitation

- Decapitation involves the rapid severing of the head from the body using a guillotine or sharp blade.
- Loss of consciousness is thought to develop rapidly in warm-blooded animals.
- Violent muscular contraction can occur after decapitation.
- This method is acceptable method for very small mammals (eg very, small unfurred kangaroo pouch young) providing that appropriate and well-maintained equipment is used.
- In cold-blooded vertebrates, the animals must be stunned or rendered insensible prior to decapitation as they are very tolerant of anoxia.

- This method is not acceptable for birds as they may be conscious for up to 30 seconds after decapitation.

Exsanguination

- Exsanguination or 'bleeding out' of the carcass is achieved by cutting the major blood vessels in the neck (ie the carotid arteries and jugular veins).
- Exsanguination must not be used as a sole method of euthanasia as extreme hypovolaemia is associated with anxiety. Pain is also associated with cutting the deeper blood vessels.
- This method is not acceptable for birds because of the tendency for the blood to clot relatively quickly and thus result in incomplete bleeding-out. It is also not acceptable for reptiles and other cold-blooded vertebrates because of their slow metabolic rate and tolerance to hypoxia.
- Should only be used on unconscious animals i.e. those that have been stunned or anaesthetised.
- Often used in the field after prior stunning to ensure death.

Pithing

- Pithing is sometimes used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means (e.g. stunning).
- It is often more acceptable than exsanguination as little blood is spilled.
- It is carried out by inserting a sharp needle or probe through the foramen magnum into the base of the brain to ensure brain destruction. Pithing can also be performed by inserting a probe through the hole made by a captive bolt.
- This method is used for some fish, amphibians and reptiles.

Chemical methods

Inhalant agents

- Euthanasia with inhaled gases is slow due to the requirement for any gas being inhaled to reach the required concentration in the tissues before taking effect. A closed chamber for holding the gas is needed and personnel safety must be considered in order to avoid exposure to the toxic gas.
- Euthanasia by inhalation of toxic gases is not suitable for animals that hold their breath (eg diving or burrowing birds and mammals) or breathe at low frequency (eg amphibians and reptiles).
- Inhalant methods are also not ideal for neonatal animals as they are relatively resistant to hypoxia. Neonates have



GEN001: Methods of euthanasia

physiological mechanisms to protect the animal from cerebral damage when oxygen is limited in the uterus and during birth. The tolerance to hypoxia gradually decreases with increasing age. Thus, inhalant agents that cause hypoxia, for example, carbon dioxide, may take longer to cause unconsciousness and death in neonatal animals compared with adult animals. Therefore, it is recommended that inhalant agents not be used as a sole method of euthanasia in neonatal animals less than 16 weeks old.

Carbon dioxide (CO₂)

- Carbon dioxide used in a sealed environment is suitable for animals up to 3 kg and is mostly used for small birds and rodents.
- There is less risk to the operator when using CO₂ compared to anaesthetic gases or carbon monoxide.
- Carbon dioxide is available in cylinders as a compressed gas (food, medical or industrial grade). The gas can be piped via a pressure reducing valve into either a plastic bag that encloses a cage or into a deep container with lid.
- Another source of CO₂ is dry ice (solidified CO₂). Chips of dry ice are placed into a beaker of water inside the container where the animals are to be killed. The beaker needs to be covered so that the animals do not come into contact with the dry ice. 1kg of dry ice will produce approximately 0.5m³ of CO₂ gas.
- When animals are placed into a chamber containing up to 70% CO₂ they lose consciousness very quickly due to the narcotic effect of the high intake of CO₂ on the brain without causing hypoxia. Death is caused by direct depression of CNS, respiratory and cardiac functions. One hundred percent CO₂ can cause severe dyspnoea (difficulty in breathing) and distress in conscious animals but this higher concentration is recommended for immature and neonatal animals as they are more tolerant of hypoxia and hypercarbia.
- Compressed CO₂ gas in cylinders should be used so the inflow to the chamber can be regulated precisely.
- Animals can either be: (1) removed from the trap and placed into a container pre-filled with CO₂, or (2) remain in holding cages, which are then enclosed within an impervious container or plastic sack.
- A continuous inflow of CO₂ should then be allowed to flow into the sack. A constant level of CO₂ should be maintained for at least 3 minutes and anaesthesia will occur within 60 seconds.

- With animals inside the chamber, an optimal flow rate should displace at least 20% of the chamber volume per minute.
- Carbon dioxide is heavier than air so incomplete filling of a chamber may permit some animals to climb, fly up or raise their heads above the higher concentrations to avoid exposure to the gas.
- Care must be taken to limit the number of animals in a chamber at any one time so as to maintain a constant CO₂ concentration.
- Each animal must be verified as dead before removing it from the chamber. If uncertain whether the animal is dead CO₂ narcosis must be followed by another euthanasia method eg cervical dislocation.

Carbon monoxide (CO)

- When inhaled, carbon monoxide binds to haemoglobin in the red blood cells, with an affinity 250 times that of oxygen. This results in reduced oxygen-carrying capacity and altered delivery of oxygen to cells. Hypoxia - the reduction of oxygen supply to the tissues - eventually leads to unconsciousness and death.
- Death occurs rapidly at CO concentrations of 4 to 6%. Carbon monoxide concentrations greater than 2% are sufficient to cause loss of consciousness within minutes. Failure of the respiratory centre then occurs followed by death from cardiac arrest.
- Carbon monoxide is extremely hazardous to humans as it is highly toxic and difficult to detect. Exposure from inhalation of combustion products can cause fatal poisoning. Non-fatal poisoning may result in permanent nervous system damage.
- Source of carbon monoxide is compressed CO in cylinders. Is also available as combustible cartridges, but these are only registered for use in natal fox dens. Euthanasia of trapped pest birds is sometimes carried out with cooled and scrubbed exhaust from non-vehicular petrol engines without a catalytic converter.
- Carbon monoxide sourced from the cooled exhaust of vehicular petrol engines with a catalytic converter (ie from cars less than approximately 10 years old) is not acceptable on the basis of all current information. For example, research has shown that the levels of carbon monoxide drop off very quickly after the engine has started, leaving only a small window where concentration is adequate for a rapid death (i.e. for up to approx 60 seconds after a car has been cold started). It is also likely that the level of potential irritants (eg carbon), are highest during this short time.

Anaesthetic gases

- Halothane and the halogenated ethers, for example, isoflurane, methoxyflurane can be used with small animals (< 7kg).
- The animal can be placed in a sealed chamber containing cotton or gauze soaked with an appropriate amount of an anaesthetic or the anaesthetic vapour can be introduced from a vaporiser into the container or a plastic bag that has been placed around a cage containing the animal. The animal will become unconscious quickly and quietly (some voluntary, then involuntary resistance behaviour usually occurs).

Using soaked gauze

- Anaesthetic vapour from the soaked cotton or gauze can reach toxic concentrations within the chamber leading to death. However, lethal concentrations cannot be guaranteed so the animal should be removed from the cage once unconscious and euthanased with a secondary method of euthanasia for example, an intravenous or intraperitoneal injection of pentobarbitone sodium. Note that if intraperitoneal pentobarbitone is used, the animal must be returned to the gas-filled chamber until it has died.

Using a vaporiser

- When vapour is introduced using a vaporiser, lethal concentrations are unlikely to be achieved and the animal must be removed from the cage once unconscious and euthanased using a secondary method of euthanasia for example, an intravenous or intraperitoneal injection of pentobarbitone sodium. Note that if intraperitoneal pentobarbitone is used, the animal must be returned to the gas-filled chamber until it has died.
- When using a vaporiser, the addition of an appropriate concentration of nitrous oxide will produce a more rapid loss of consciousness
- This method is less hazardous to the operator as the gases can be vented or scavenged thus lowering exposure.
- These methods are not used in large animals because of high cost and difficulty in administration.
- The use of chloroform is *not acceptable* due to OH&S risks (due to hepatotoxicity, renotoxicity and suspect carcinogenicity)
- The use of ether is *not acceptable* for both OH&S and animal welfare reasons (high explosive risk, the induction of anaesthesia is slow, causes irritation to the skin and mucous membranes, causes marked excitement in some species).

- Anaesthetic gases are restricted substances, listed as Schedule 4 under the *Standard for the Uniform Scheduling of Drugs and Poisons* (SUSDP). This listing requires that they be restricted to medical, dental or veterinary prescription or supply, or to approved use in research. Refer to the relevant state and territory legislation for specific details on the supply, possession, use and storage of these drugs.

Injectable agents

Barbiturates

- One of the most humane methods of euthanasia is the administration of a barbiturate overdose either by the intravenous, intraperitoneal or intracardiac routes.
- Injections by the intraperitoneal route work much more slowly than the intravenous route, but are easier for an operator working alone. The intraperitoneal route is used when an intravenous injection would result in stressful handling or be dangerous to the animal or the operator and when there are no easily accessible veins.
- When using the intraperitoneal route, a non-irritant barbiturate solution should be used ie a normal anaesthetic solution of barbiturate, eg sodium pentobarbitone (Nembutal®). The more concentrated commercially prepared ‘euthanasia solutions’ (e.g. Euthatal® or Lethabarb®) are very alkaline and are thought to cause irritation of the peritoneum and pain prior to unconsciousness. Large volumes would have to be used in anything other than a very small animal as these solutions are approximately five times more concentrated than the same barbiturates designed for anaesthesia.
- Because of the difficulty and unpredictability in performing the injection accurately, intracardiac injection should only be used if the animal is heavily sedated, unconscious or anaesthetised. In some situations, if the operator is skilled and experienced in the technique, intracardiac injection may be used on neonates or small animals without causing distress.
- Animals need to be well restrained. If animals cannot be handled they may need to be immobilised in a restraint cage or sack or sedative drugs/anaesthetic gases administered prior to injection of barbiturate.
- Barbiturates are restricted substances, listed as Schedule 4 under the *Standard for the Uniform Scheduling of Drugs and Poisons* (SUSDP). This listing requires that they be restricted to medical, dental or veterinary prescription or supply, or to approved use in research. Refer to the relevant state and territory legislation for specific details on the supply, possession, use and storage of these drugs.



GEN001: Methods of euthanasia

Sedative drugs

- A review of sedative, tranquillising or anaesthetic drugs (eg ketamine) for wildlife is beyond the scope of this document. Extensive information on these substances, methods of application and their use in the field is available throughout the scientific literature. Recommendations on appropriate drugs (including combinations thereof), dose rates and methods of application can vary dramatically between species. Users of sedative drugs should refer to the most recent, species specific literature and manufacturer recommendations before proceeding to field application.
- Most of these drugs are restricted substances, listed as Schedule 4 or Schedule 8 under the *Standard for the Uniform Scheduling of Drugs and Poisons* (SUSDP). This listing requires that they be restricted to medical, dental or veterinary prescription or supply, or to approved use in research. In addition, Schedule 8 drugs are subject to stringent storage and record-keeping requirements. Refer to the relevant state and territory legislation for specific details on the supply, possession, use and storage of these drugs.

Disposal of carcasses

- The carcasses of animals euthanased by barbiturate overdose or other chemical agents may contain potentially harmful residues. They should be disposed in a manner that will prevent them from being consumed by humans or predatory/scavenger animal species. Toxicosis, sedation and death have occurred in pets and wildlife that ingest portions of carcasses euthanased by some chemical agents.
- Carbon dioxide is the only chemical used for euthanasia that does not result in tissue residues.

Signs of death

- After the application of a euthanasia procedure, it is essential that the death of the animal be confirmed. Signs that should be used to indicate death are listed below. This information has been extracted from the ANZCCART publication, *Euthanasia of Animals Used for Scientific Purposes* (2001).
 - *Absence of respiratory movement*: this sign alone is not sufficient as the heart may continue to beat for some time.
 - *Absence of heart beat*: determined by using a stethoscope or by palpation of the chest.
 - *Absence of pulse*: The femoral pulse can be palpated

in the proximal medial aspect of the hind limb in the live animal, and is lost after death. This technique is of most use in larger species, as it can be impossible to discern in small species.

- *Loss of colour in mucous membranes*: mucous membranes become pale and mottled, there is no refill after pressure is applied and they become dry and sticky. More useful in larger species where the mucous membranes in the mouth are easily accessible.
- *Corneal and palpebral reflexes are lost*: the corneal reflex is elicited when the eyeball is touched and the palpebral reflex is elicited when the eyelids are stroked. The eye should remain open and the lid should not move.
- *Glazing of eyes*: this will occur rapidly after death. The cornea loses its clear moist appearance and becomes opaque, dry and somewhat wrinkled.
- Always check for these signs and do not assume an animal is dead just because it is not moving or apparently not breathing. If death cannot be confirmed, the operator should repeat the same or an alternative procedure.
- If the animal is unconscious, the major blood vessels in the neck can be cut with a sharp knife so that the animal dies of blood loss (ie exsanguination).

Disposal of carcasses

- Carcasses should only be discarded once death has been established.
- Carcasses should be disposed of properly and in accordance with acceptable practices as required by local councils and applicable State or Commonwealth regulations.
- If the carcass presents a risk to wildlife (e.g. it contains infectious disease agents or residues of euthanasia chemicals) it should be disposed of by incineration or by burying in a deep hole after covering with lime.
- If large numbers of animals are to be killed, provisions should be made to dispose of carcasses in an appropriate manner. Numerous guidelines are available which describe disposal methods eg Burton 1999, *AUSVETPLAN Operational Procedures Manual: Disposal* (1996), NSW EPA (2001) *Guidelines for disposal of dead stock*.

Recommended Methods of Euthanasia for a Range of Species

It is recognised that exceptions to the recommended practice may be required under certain conditions. In those circumstances considerations of common sense for both animal and human welfare should prevail.

Amphibians (including cane toads)

Stunning followed by decapitation

- Stunning is done by restraining the animal and applying a forceful blow to the middle of the head just behind the eyes.
- Immediately after stunning, the animal must be decapitated using a knife.
- In cane toads, stunning methods may cause the release of poison from the parotid skin glands. Precautions against operator contamination are necessary.

Gassing with carbon dioxide for >4 hours

- This method has been used for the euthanasia of large numbers of cane toads.
- Toads must be confirmed as dead prior to disposal.

Trans-dermal (skin) absorption of chloral hydrate, tricaine methane sulphonate (MS-222) or benzocaine

- Solution is absorbed through the skin.
- These methods are considered to be quick, non-irritant and humane methods of killing amphibians.

Overdose of barbiturate

- Intravenous or intraperitoneal route.

Refer to *CAN001 Standard Operating Procedure for the Euthanasia of Cane Toads*.



Image: Leonie Jacobs

Bats and flying foxes

Inhalation of anaesthetic gases or CO₂

Overdose of barbiturate

- Intraperitoneal route.
- May need prior sedation (eg intramuscular ketamine)

Birds (large size) eg emu, ibis

Shooting

- A shot to the brain, using a 12 gauge shotgun, is preferred when the bird is in close range (<20 m). If the bird is >20m from the shooter, a chest shot using a large calibre centrefire rifle (eg .243) should be used.

Overdose of barbiturate

- Intravenous or intraperitoneal route

Birds (small to medium size) eg starlings, mynas, cockatoos, galahs

Neck (cervical) dislocation

- Dislocate the neck by taking the birds legs in the left hand (if right handed) and the head between the first two fingers of the right hand with the thumb under the beak. A sharp jerk with each hand, pulling the head backward over the neck aims to herniate the brain stem and may break the spinal cord and carotid arteries.
- Cervical dislocation is not suitable for birds larger than 3 kg as it is difficult to pull the neck quickly. Average weights for some species are starlings (50–80 g), sulphur crested cockatoos (1 kg), corellas (565 g), galahs (330 g), ibis (2.5 kg), ducks (1–2 kg).

Overdose of barbiturate

- Intraperitoneal route

Inhalation of anaesthetic gases or CO₂

- Carbon dioxide suitable for adult birds
- Anaesthetic gases suitable for chicks, and small/medium adult birds

Shooting (with shotgun)

- Shot to the brain

Cats

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm is aimed at the centre of the head slightly below a line drawn midway between the ears.



GEN001: Methods of euthanasia

- *Behind the ear (side view)*: Shoot from the side aiming behind the ear so that the shot will pass through the brain towards the opposite eye.

Overdose of barbiturate

- May need prior sedation.
- Intravenous route preferred, intraperitoneal route if necessary.

Inhalation of anaesthetic gases or CO₂

- Suitable for feral cats in cage traps that are difficult to sedate with an injection.
- Anaesthetic gas or carbon dioxide is introduced into a plastic bag that has been placed around the cage. The animal will become unconscious quickly and quietly, followed by death after a period depending on the gas used and its concentration. If preferred, once the animal is unconscious, it may be removed from the cage and euthanased with an intravenous or intraperitoneal injection of pentobarbitone sodium.

Cattle/Buffalo

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: Aim at the point of intersection of lines taken from the base of each ear to the opposite eye.
 - *Temporal position (side view)*: Aim from the side of the head at a point midway between the eye and the base of the ear.
 - *Rear of the head*: Aim just behind the poll in the direction of the animal's muzzle.

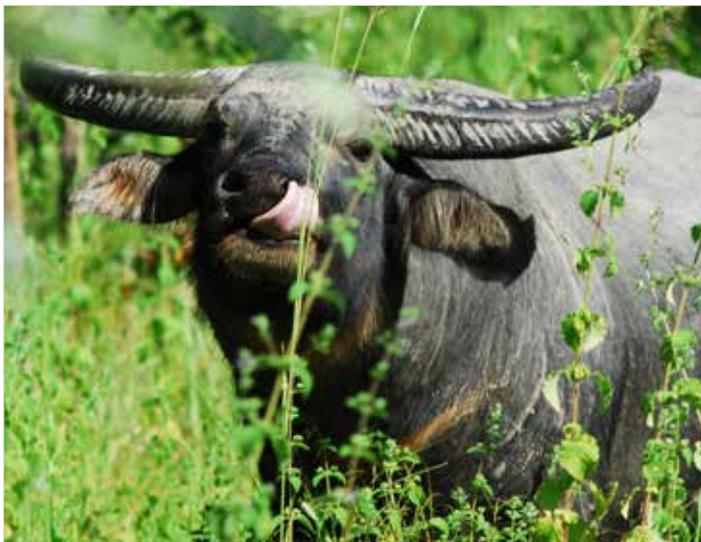


Image: James Wright

Captive bolt stunning

- The frontal position should be used. Note that camel bulls in rut develop thick glands on the top of their head that prevent the effective use of the captive bolt by the frontal method.
- Follow immediately with second method (eg exsanguination).

Overdose of barbiturate

- Intravenous route
- Large volumes of euthanasia solution are required for adult animals.

Crocodiles

Shooting

- Shot to the brain.
- Point of aim is the cranial platform.

Spinal severance followed by pithing

Overdose of barbiturate

- Intraperitoneal route
- Use in small crocodiles only.

Refer to the DEWHA (2009) *Code of Practice on the Humane Treatment of Wild and Farmed Australian Crocodiles* for further details.

Deer

Shooting

- Shot to the brain.
- Points of aim:
 - *Frontal position (front view)*: This is the preferred method for fawns/calves. The firearm is aimed at the middle of the forehead at the crossing point of two imaginary lines drawn from the eyes to the tops of the opposite ears. In stags this point is found between, and sometimes just behind, the antlers.
 - *Temporal position (side view)*: This method is preferred for mature/older animals. The firearm should be aimed at the side of the head so that the bullet enters the skull at a point midway between the eye and the base of the ear on the same side of the head.
 - *Rear of the head (rear view)*: This method is preferred for mature/older animals that cannot be approached from the side. The firearm should be aimed at the back of the head at a point just behind the base of the antlers and directed towards the animals' muzzle.

Overdose of barbiturate

- May need prior sedation
- Intravenous route

Dogs

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm is aimed at a point midway between the level of the eyes and the base of the ears, but slightly off to one side so as to miss the bony ridge that runs down the middle of the skull. The aim should be slightly across the centreline of the skull and towards the spine.
 - *Temporal position (side view)*: The firearm is aimed at the side of the head at a point midway between the eye and the base of the ear.

Overdose of barbiturate

- May need prior sedation
- Intravenous route

Echidna

Anaesthetic chamber supplied with halothane, methoxyflurane or carbon dioxide

Overdose of barbiturate

- There are no easily accessible veins.
- Intraperitoneal route
- May need prior sedation

Shooting

- Shot to the brain

Foxes

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm is aimed at a point midway between the level of the eyes and the base of the ears, but slightly off to one side so as to miss the bony ridge that runs down the middle of the skull. The aim should be slightly across the centreline of the skull and towards the spine.
 - *Temporal position (side view)*: The firearm is aimed at the side of the head at a point midway between the eye and the base of the ear.

Overdose of barbiturate

- May need prior sedation
- Intravenous route



Image: Jason Wishart

Goats

Shooting

- Shot to the brain
- The horn structures on adult goats make the temporal (side-on) or rear head shots the preferred points of aim. Shots to the front of the head can be used on kids, however this method is not recommended for mature goats as the brain is located well back in the skull.
- Points of aim:
 - *Temporal position (side view)*: The firearm is aimed from the side of the head so that the bullet enters the skull at a point midway between the eye and the base of the ear on the same side of the head. The bullet should be directed horizontally into the skull.
 - *Rear of the head*: The firearm should be aimed at the back of the head at a point between the base of the horns and directed towards the mouth.

Captive bolt stunning

- Follow immediately with a second method (eg exsanguination).

Overdose of barbiturate

- Intravenous route (jugular vein)

Horses/Donkeys

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm should be directed at the point of intersection of diagonal lines



GEN001: Methods of euthanasia



Image: Craig Fardell

taken from the base of each ear to the opposite eye.

- *Temporal position (side view)*: The horse is shot from the side so that the bullet enters the skull midway between the eye and the base of the ear.

Overdose of barbiturate

- Intravenous route
- Administration of barbiturate with either xylazine or succinyl choline (a neuromuscular blocking agent) is recommended. Sodium pentobarbitone alone may result in unacceptable excitement prior to unconsciousness.
- Should only be attempted by well-trained personnel.
- Large volumes of euthanasia solution are required for adult animals.
- May need prior sedation.

Captive bolt stunning

- Follow immediately with a second method eg exsanguination.

Macropods

Shooting

- Shot to the brain
- Most humane method for adult animals.
- Points of aim:

- *Temporal position*: This method is the preferred head shot as it is a larger target area. The firearm should be aimed at the side of the head so that the bullet enters the skull at a point midway between the eye and the base of the ear on the same side of the head.

- *Frontal position*: The firearm is aimed at the middle of the forehead so that the bullet enters the skull at a point midway between the level of the eyes and the base of the ears.
- *Rear of the head*: This method is sometimes used on animals that cannot be approached from the side. The firearm should be aimed at the back of the head at a point midway between the base of the ears and directed towards the animal's muzzle.

Stunning by a blow to the head

- Suitable for larger pouch young.
- If damage to the brain is not sufficient to cause immediate death stunning must be followed by a secondary method eg exsanguination.

Decapitation with a sharp instrument

- Suitable only for very small hairless pouch young.

Refer to the DEWHA (2008) *National Code of Practice for the Humane Shooting of Kangaroos and Wallabies for Non-commercial Purposes* for further details.

Pigs

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: For smaller pigs only. The firearm should be aimed at a point midway across the forehead and about 2cm above the level of the eyes.
 - *Temporal position (side view)*. For larger pigs. This method is preferred for adult pigs due to the heavier bone structure of the front of the skull. The firearm is aimed from the side of the head so that the bullet enters the skull at a point midway between the eye and the base of the ear on the same side of the head.

Overdose of barbiturate

- May need prior sedation especially in large pigs.
- Intravenous route - usually ear vein but not easy to use without prior training.

Captive bolt stunning

- Follow immediately with second method (eg exsanguination).
- Satisfactory for immature pigs. Problems may occur in adult animals due to large frontal sinuses.

Stunning by a blow to the head followed by exsanguination

- For young piglets < 3 weeks old only

Platypus

Overdose of barbiturate

- There are no easily accessible veins; therefore the intraperitoneal route should be used. Can be picked up by the tail but beware of venomous spurs on the hindlegs of males.
- May need prior sedation if animal cannot be handled readily.

Shooting

- Shot to the brain

Possums (brush-tail)

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm is aimed at the centre of the head slightly below a line drawn midway between the ears.
 - *Behind the ear (side view)*: Shoot from the side aiming behind the ear so that the shot will pass through the brain towards the opposite eye.

Overdose of barbiturate

- May need prior sedation.
- Intraperitoneal route.

Inhalation of anaesthetic gases or CO₂

- There are two approaches that can be taken:
 - Place the animal in a hessian sack or cloth bag and place this in an anaesthesia chamber OR
 - Introduce the gas into a plastic bag that has been placed around a cage containing the possum.
- The animal will become unconscious quickly and quietly, followed by death after a period depending on the gas used and its concentration. If preferred, once the animal is unconscious, it may be removed from the cage and euthanased with an intravenous or intraperitoneal injection of pentobarbitone sodium.

Rabbits/Hares

Cervical dislocation

- The recommended technique is either (1) cervical dislocation, or (2) stunning, by a sharp blow to the back of the head, followed by neck dislocation:
 - *Neck (cervical) dislocation*: This technique should only be used on smaller rabbits (<1 kg). In larger rabbits, greater muscle mass in the neck region makes manual cervical dislocation physically more difficult.

Accordingly, it should be performed only by individuals who have demonstrated proficiency in euthanasing heavier animals or preferably, after the rabbit has been stunned by a blow to the head (see below). Hold the rabbit head downwards by grasping the hind legs in one hand; turn the palm of the other hand towards the rabbit head and take the neck between the thumb and index finger or between the index and middle fingers. Push down so that the neck is stretched and the head moves backwards, until dislocation is felt.

- *Stunning followed by neck (cervical) dislocation*. This technique should be used on larger rabbits (>1 kg). Suspend the rabbit by the hind legs, grasping around both hocks with the left hand. Deliver a single, heavy, sharp blow to the back of the skull, behind the ears, with a cudgel. Alternatively, if no implement is available, the rabbit can be picked up by the hind legs and swung so that the back of its head hits a hard surface such as a rock or post. Dislocate the neck using the technique described above.

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: The firearm is aimed at the centre of the head between the eyes.
 - *Temporal position (side view)*: The firearm is aimed at a point between the eye and the base of the ear directed towards the opposite eye.

Overdose of barbiturate

- Intravenous or intraperitoneal route



Image: Neil Schultz



GEN001: Methods of euthanasia

Inhalation of anaesthetic gases or CO₂

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 - Introduce the gas into a plastic bag that has been placed around a cage containing the possum.
- The animal will become unconscious quickly and quietly, followed by death after a period depending on the gas used and its concentration. If preferred, once the animal is unconscious, it may be removed from the cage and euthanased with an intravenous or intraperitoneal injection of pentobarbitone sodium.

Rodents

Do not handle native rodents (mice) by the tail as it will often break off.

Cervical dislocation

- This technique should not be used on conscious animals heavier than 150 grams (i.e. adult rats)

Stunning plus exsanguination or cervical dislocation

- Mice (non-native) can be held by the tail and swung in an arc so that the back of the head only contacts a hard, solid object.
- Rats are held by the hindquarters and brought downwards quickly so as to strike the back of the head on a hard, solid object.

Inhalation of anaesthetic gases or CO₂

- Involves minimal handling of the animals and larger numbers can be killed simultaneously.

Overdose of barbiturate

- Intraperitoneal route

Reptiles - snakes and lizards

Stunning/decapitation/destruction of the brain

- A sharp blow just behind the head followed by decapitation and destruction of the brain by pithing.

Shooting

- Shot to the brain

Reptiles - tortoises and turtles

Stunning/decapitation/destruction of the brain

- A sharp blow just behind the head followed by decapitation and destruction of the brain by pithing.

Shooting

- Shot to the brain

Overdose of barbiturate

- Intravenous route- injection into the venous sinus in the dorsal midline of the tail.
- Intraperitoneal route - enter the abdominal cavity anterior to a hind leg.
- May need prior sedation (eg with an injection of Ketamine or alfaxalone into the quadriceps muscle, or in terrestrial species, by inhalation of an anaesthetic gas).

Sheep

Shooting

- Shot to the brain
- Points of aim:
 - *Frontal position (front view)*: Aim at a point in the middle of the face just above the level of the eyes while aiming along the neck.
 - *Temporal position (side view)*: Aim from the side of the head at a point midway between the eye and the base of the ear.
 - *Rear of the head*: Aim just behind the poll in the direction of the animal's muzzle.
- Frontal shots should be used on horned sheep and rams.

Captive bolt stunning

- The frontal position should be used.
- Follow immediately with a second method (eg exsanguination).

Overdose of barbiturate

- By the intravenous route

Exsanguination (or bleeding out)

- Prior stunning is required (eg using a captive bolt pistol, .22 rifle, heavy hammer or iron bar)
- A long (minimum length 14cm) sharp knife is essential
- Make a transverse cut high up on the neck ensuring that the carotid arteries on both sides have been severed. Successful severance of the se arteries, as opposed to the jugular veins which are closer to the surface of the neck, can be recognised by obvious pulsatile bleeding. Both sides need to be cut to ensure that onset of insensibility is rapid.
- Do not attempt to sever the spinal cord or dislocate the neck.
- This method can be inhumane if the proper technique is not used.

Wombats

Shooting

- Shot to the brain

Overdose of barbiturate

- It is likely that prior sedation will be required. Wombats are strong animals that can inflict serious bites and scratches.
- Intravenous route preferred (via radial vein on medial aspect of forearm, or cephalic vein). Intraperitoneal route can be used after sedation.

Further information

Contact the relevant federal, state or territory government agency from the following list of websites:

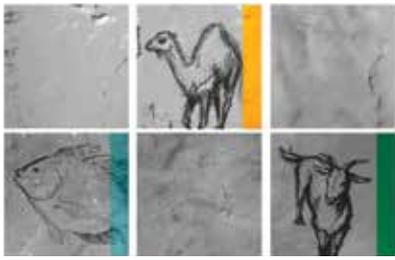
- Australian Department of Sustainability, Environment, Water, Population and Communities
<http://www.environment.gov.au/>
- Australian Department of Agriculture, Fisheries and Forestry
<http://www.daff.gov.au>
- ACT Territory and Municipal Services Directorate
<http://www.tams.act.gov.au/live/environment>
- NSW Department of Primary Industries
<http://www.dpi.nsw.gov.au/>
- NT Department of Land Resource Management
<http://lrm.nt.gov.au/>
- Qld Department of Agriculture, Fisheries and Forestry
<http://www.daff.qld.gov.au/>
- Biosecurity SA, SA Department of Primary Industries and Regions
<http://www.pir.sa.gov.au/biosecuritysa>
- Tas Department of Primary Industries, Parks, Water and Environment
<http://www.dpiw.tas.gov.au/>
- Vic Department of Primary Industries
<http://www.dpi.vic.gov.au/>
- WA Department of Agriculture and Food
<http://www.agric.wa.gov.au>

Also refer to:

Invasive Animals Cooperative Research Centre
<http://www.invasiveanimals.com/>
or <http://www.feral.org.au>

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GEN001: Methods of euthanasia

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