STANDARD OPERATING PROCEDURE



# **RES002** restraint and handling of **pest animals** used in research

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## Background

Research involving pest animals may require the live capture, restraint and handling of individual animals. Wild animals may try to avoid capture, handling and restraint during which they are capable of inflicting damage to themselves and their potential captors. When physical contact is necessary, the safety of animals and operators should be the primary consideration.

Restraint and handling techniques must be appropriate for the species and minimise distress and the risk of injury to the animal. Inappropriate techniques may lead to major and possibly fatal physiological disturbances.

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 OHS) operating in the relevant jurisdiction.

## Application

- This document provides guidelines for research involving pest animals. It aims to ensure that restraint and handling procedures are performed humanely and effectively.
- The acquisition, care and use of animals for scientific purposes in Australia must be in accordance 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.
- Personnel handling animals should be thoroughly trained in the planned procedure as well as in contingency methods of capture and restraint that may be required.
- The restraint and handling of animals usually requires that the animal be captured. Proper capture techniques are essential to minimise pain, fear, distress and anxiety experienced by the animal and also for the safety of the operator. For more information on capture techniques refer to ***RES001 Live capture of pest animals used in research***.

- The objectives of many wildlife studies depend upon the reliable identification of individual animals and therefore require some form of identifying mark be applied to the animal while it is restrained. For more information on marking techniques refer to ***RES004 Marking of Pest Animals used in Research***.
- For many situations physical restraint, rather than chemical will be the most appropriate. Procedures that cause more than momentary or slight distress to the animal should be performed with appropriate sedation, analgesia or anaesthesia.
- Consultation and/or participation of veterinarians with wildlife experience should be sought in projects involving chemical restraint or medical/surgical procedures or where there are concerns regarding animal health.
- Schedule 4 drugs (e.g. 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.

## Terminology

***Restraint*** – restriction of an animal's movement.

***Physical restraint*** – restriction of an animal's movement by physical rather than chemical means.

***Chemical restraint*** – restricting the ability an animal to move by the use of drugs; these may be anaesthetics or immobilising agents.

***Anaesthesia*** – drug-induced state of unconsciousness that is characterised by depression of the central nervous system and varying degrees of analgesia.

***Immobilisation*** – chemical restraint of an animal without anaesthesia or analgesia.

***Neuromuscular blocking agents*** – drugs which cause paralysis or paresis of skeletal muscle by their effect on the neuromuscular junction.

***Analgesia*** – reduction or the absence of the sensation of pain.

***Sedation*** – moderate suppression of the central nervous system inducing a sleep-like state from which the animal can be roused.

***Tranquillisation*** – a state of behavioural change, wherein anxiety is relieved, behavioural responses suppressed and the animal is relaxed but aware of its surroundings. Spinal and other reflexes are not affected.

## Animal Welfare Considerations

- Evidence from behavioural and physiological studies indicates that restraint and handling are significant stressors for wild animals which also present a great risk of injury. Handling and restraint techniques must therefore aim to minimise the stress on the animal and maximise the safety of the animal and also the operator.

- Capture, handling and restraint should be avoided in animals already compromised by pre-existing stressors (such as pregnancy, lactation, lack of food and/or water, social factors or extremes of temperature) as they have a decreased ability to deal with more stress. Whenever possible, avoid capture during late pregnancy, birthing periods or when females have dependent young at foot, unless capture of these animals is a necessary and approved part of the research protocol.
- To understand and potentially reduce the impact of restraint and handling procedures on animals, the researchers must have a thorough knowledge of the habits and behaviours of the species under study and be experienced in handling the species. For example, details of social structure, defensive capabilities and reaction to stress and pain.
- Operators must anticipate and be prepared to deal with the range of conditions that may cause undue stress and/or injury to the animals. If an animal is injured during restraint and handling procedures it must receive appropriate treatment. Animals that are suffering intractable pain and/or distress should be euthanased using a technique that is suitable for the species. For more information on euthanasia techniques refer to ***GEN001 Methods of Euthanasia.***
- Precautions must be taken to prevent the spread of infectious disease from one animal to another. Contaminated equipment should be disinfected between animals.

## Physical Restraint

- The potential welfare implications of physical restraint include:
  - Physical injury, pain, discomfort-and occasionally death-resulting directly from restraint
  - Capture myopathy in some species
  - Distress
- Handling and restraint times should always be kept to a minimum. For some highly excitable species (e.g. deer, some macropods), prolonged physical restraint without some chemical sedation may result in self-inflicted injury, physiological disturbances or occasionally death.
- Every effort must be made to avoid physical restraint procedures that result in cardiogenic shock, capture myopathy and other stress-related causes of mortality. Stress-related damage may not be immediately apparent but may lead to debility or death after the animal has been released.
- Appropriate chemical restraint should be used if the period of handling is likely to cause pain or significant distress to the animal or endanger the safety of operators.
- Excessive noise and sudden movements should be minimised. For many species, covering the animals' eyes, or placing it in a darkened environment, may help to reduce external stimuli and prevent arousal.
- An adequate number of sufficiently trained and equipped operators must be available to complete the task safely.

- At the completion of handling, and when the animal has recovered from the short-term effects of restraint, the animal must be released as close to the point of capture as possible. If possible, the timing of release should be consistent with the animal's normal daily rhythms of activity.

### Chemical Restraint

- Chemical methods of restraint include sedatives, tranquillisers and anaesthetics. Neuromuscular blocking agents (e.g. vecuronium, pancuronium, succinylcholine) should not be used as the sole method of chemical restraint and their use even with anaesthetics is not recommended for invasive procedures as they mask many of the signs used in monitoring anaesthesia.
- Animals that have been chemically restrained require an appropriate recovery environment that is quiet, dark, and free from physical hazards and disturbance but still able to be monitored.
- The potential welfare implications of chemical restraint include:
  - Overdose and death
  - Physical injury from the use of remote drug administration equipment
  - Prolonged sedation and hypo/hyperthermia and/or dehydration
  - Under dosing – resulting in injury to self, injury to operator and premature recovery
  - Injury during recovery
  - Depressive effects on the foetus in pregnant animals
  - Adverse drug effects
  - Adverse effects due to pre-existing disease
  - Complications such as regurgitation and consequent aspiration
  - Release before full recovery increasing risk of predation
  - Infection at injection sites
  - Inadequate monitoring
  - Apparent insensibility masking pain indicators
  - Masking of signs of poor welfare, especially health and behaviour
  - Conflict when re-establishing social status on return to social groups.

## Health and Safety Considerations

### Animal handling

- Operators need to be wary of the potential for serious injury when handling wild animals. Some species can be aggressive and may attack e.g. feral pigs.
- When working in the field, personnel should work in teams of at least two people.
- Protective clothing, footwear and gloves may reduce the chances of injury when handling wild animals. However, the use of heavy gloves decreases sensitivity and dexterity and may increase the risk of handling injuries to small species.

### Chemical restraint

- Drugs used for chemical restraint are potentially toxic to humans and should be used and stored with appropriate safety precautions. Operators should be familiar with the specific risks associated with each agent being used.

### Zoonotic hazards

- Care must be taken when handling live animals and carcasses as they may carry diseases that can affect humans and other animals e.g. hydatidosis, sarcoptic mange, leptospirosis, Q fever, brucellosis, melioidosis, tuberculosis, psittacosis (Chlamydiosis/Chlamydophilosis) etc.
- Routinely wash hands and other skin surfaces after handling all animals and also carcasses or bodily fluids.
- Operators must be protected by tetanus immunisation in case of infection of wounds.
- Bite wounds from some animals (e.g. feral cats, foxes, wild dogs) can result in serious infections and should be treated by a doctor.
- 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. Blood testing of personnel is recommended to assess previous exposure, followed by vaccination for susceptible individuals.
- Zoonotic risks from birds include psittacosis (chlamydiosis), aspergillosis, erysipelas, yersiniosis and salmonellosis. Face masks, are recommended to reduce the risk of contracting disease.
- Some bird species can deliver painful bites and scratches. For example, parrots (e.g. cockatoos, galahs, corellas) have large, heavy beaks and strong jaws that are capable of inflicting serious injury. Protective gloves can be used if required for handling large birds, although these may hinder dexterity. A towel is useful to place over the birds head.

## Methods of Restraint and Handling

### Physical

- Physical restraint is appropriate for simple procedures such as physical examination, injections, venipuncture etc.
- Physical restraint should only be performed by operators who are familiar with the normal behaviour patterns of the species that is to be restrained.
- The duration of the procedure should be kept to a minimum, with prolonged stressful restraint unacceptable.
- Appropriate restraining devices (e.g. bag, rope, restraint board, catch-pole, squeeze cage or crush) may be required to avoid injury to the animal or researcher.
- Personnel should approach the target animal in a calm and quiet manner. Unnecessary people should keep away from the area to avoid making the animal more agitated.
- For many species, it is important to cover the animal's head (e.g. with a blindfold, bag, blanket etc.) to impair its view of the environment and reduce stress. Excessive noise and sudden movement should be kept to a minimum.

## Chemical

- Chemical restraint may be appropriate in the following situations:
  - When the period of handling is likely to cause undue stress to the animal;
  - With aggressive or nervous species that are unable to be physically restrained because they are likely to cause serious injury to the handler and/or themselves (e.g. adult feral pigs, feral deer);
  - When the procedure to be performed is likely to cause significant pain or discomfort. Analgesia should also be used in these cases.
- Chemical immobilisation of animals can result in significant morbidity and mortality.
- To ensure that chemical restraint is performed both humanely and effectively, personnel must be experienced with all aspects of drug use and assessment/ monitoring of anaesthesia. They must also be familiar with the target species and have knowledge of the best techniques available.
- While there is no perfect drug for immobilisation, researchers should endeavour to meet the following criteria as closely as possible when selecting an anaesthetic. The drug used for immobilising an animal should be:
  - stable in solution over a large range of temperatures;
  - be able to be delivered at an effective concentration in a small dose (e.g. one dart or syringe);
  - be suitable for intramuscular injection;
  - result in sufficient immobilisation rapidly and smoothly. In the case of invasive, painful or otherwise very stressful procedures, the drug should render the animal unconscious and leave it unaware of manipulations being performed;
  - have a wide therapeutic index. An accurate weight and condition status of the target animal is rarely known prior to immobilisation and as such, drugs with a wide therapeutic index (or 'safety margin') should be used;
  - have minimal adverse effects and;
  - be short acting and /or reversible, resulting in rapid and smooth recovery from immobilisation (this decreases recovery time and allows the animal to defend itself or escape predators more readily).
- It is important to choose a drug delivery system that will deliver the appropriate volume of drug with the minimum amount of physical trauma to the animal. There are two drug delivery systems available:
  - **Hand-held injection**

    This requires the animal to be physically restrained before administration;
  - **Remote drug administration system (RDAS)**

    There are four basic types:

    *i.* ***Pole syringes*** are cheap, safe and quiet, but are only useful when the animal is physically restrained or is in a trap. Care must be taken to ensure that needles do not break off (e.g. when administering large volumes or using a long needle) and placement of the needle in large muscle masses needs to be accurate as pole syringes may harm the animal if incorrectly placed;

ii. ***Blow pipes*** are the cheapest and safest system. However they only have a range of 10–15 m and experience is required for them to be used effectively;

iii. ***Dart pistols*** are more accurate and have a range of approximately 20 m, but their greater power represents an increase in the risk of danger to the animal; and

iv. ***High-velocity dart rifles*** have the greatest range (up to 50 m) and accuracy; however they also present the greatest risk of injury to the animal. They are generally much less accurate than conventional firearms and should only be used by experienced operators. Accidental death of the animal can occur from using excessive velocity to propel the dart and/or missing the target area, causing penetration of a vital organ, body cavity, and/or broken bones.

- It is critical to hit a proper injection site, especially when using RDASs. As large skeletal muscle masses are usually the most appropriate sites, regular practice with RDASs and knowledge of the anatomy of the target species is required to ensure consistent hitting of the appropriate injection site.
- Anaesthetised animals require close monitoring of their cardio-vascular, respiratory and thermo-regulatory systems. Constant monitoring is necessary, with operators trained to recognise and alleviate potential complications. The depth of anaesthesia should also be closely monitored.
- It is essential to provide a quiet recovery place.
- Where appropriate, reversing agents can be used to facilitate recovery.

## Methods of Restraint and Handling for a Range of Species

***Recommendations on appropriate drugs (including combinations thereof), dose rates and methods of application can vary greatly between species. Dose rates depend on species, age, behaviour, health status, intended procedure and environment and should be determined by reference to recent authoritative species-specific literature and manufacturer recommendations.***

### Rabbits

**Physical restraint**

- These animals may kick out and scratch the handler with the claws of their hind feet. Long sleeved, heavy duty overalls and long trousers should be worn to protect the operator from being scratched during handling. Protective gloves may be used if required, although these may hinder dexterity.
- Rabbits are prone to spinal injury as a result of struggling during inappropriate or prolonged handling. Sudden kicking with the hind legs while restrained can result in fractures of the vertebrae and even paralysis.
- These animals should not be lifted by the ears, neck or hind legs. Doing so may result in injury to the operator and/or animal.
- Keep the head/eyes covered as much as possible.
- There are two approaches to handling which should be used as appropriate:

  *Scruffing*

  Grasp the loose skin on the scruff of the neck and press down on a flat surface initially (preferably until the animal relaxes).

Pick up with one hand over the ears and nape of the neck, holding the scruff, the other hand supporting the rump; bring the rabbit in so that it is supported against the handler's body.

The animal should be cradled in one arm with the head and eyes tucked beneath the upper arm.

***Bagging***

A strong cotton bag (e.g. calico bag, pillowcase) is placed over the rabbit whilst in a trap or whilst it is 'stunned'. The bag securely holds the rabbit without risk of suffocation. It will often rest quietly because its eyes are covered. Use only one rabbit per bag. Expose the back legs, whilst leaving the head covered, during injection procedures. Wash new calico bags at least twice before use to ensure adequate ventilation.

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of xylazine and ketamine.
- Injection sites are the large muscle masses in the hind limbs.

## Foxes, wild dogs and cats

**Physical restraint**

- Trapped wild dogs, foxes and feral cats can be dangerous to handle. They will be nervous and aggressive and can inflict serious injuries with teeth and claws.
- Zoonotic risks include hydatidosis, sarcoptic mange, toxoplasmosis, ringworm and sarcosporidiosis. An animal with obvious mange should only be handled while wearing gloves.
- Personnel should wear thick, gloves when handling these animals to protect from scratching, however this may interfere with scruffing.

  ***Animal caught in a leg-hold trap***

  - Pin the animal's neck to the ground with a soft-headed broom and place a dark blanket or other cloth over the head of the animal ensuring that the eyes are completely covered. Alternatively a pole snare may be used to pin the animal.
  - While one person maintains pressure with the broom or pole snare, another person approaches the animal and grasps the scruff of the neck, with the second hand applying pressure to the rump.
  - The broom or pole snare may be released once the scruff is firmly held.
  - A muzzle (e.g. bandage muzzle) may facilitate a brief examination of a physically restrained fox or dog.
  - When chemical restraint is not used for wild dogs (i.e. very brief examination such as removing a radio collar) a muzzle (e.g. bandage muzzle) and restraint board should be used. Restraint boards consist of a wooden board larger than the animal with three restraint straps to keep the animal immobilised (neck, chest and lower abdomen).
  - Slide the board under the animal that is restrained by scruff and pole snare.
  - Slip the restraint straps around the animal and tighten so that there is enough tension to hold the animal without causing it injury.
  - Cats should be chemically immobilised as soon as possible.

*Animal caught in a cage trap*

- Animals captured in cage traps will often push themselves into a corner where it is possible to immobilise them with an anaesthetic injection. In the case of the animal not being in a corner, a stick poked through the cage (though not contacting the animal) will force the animal into a corner.

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:

  *Foxes*

  - Tiletamine and zolazepam;
  - Ketamine and xylazine – this combination can be reversed with yohimbine given 45–60 minutes after the last ketamine administration;
  - Ketamine and promazine; or
  - Ketamine and midazolam

  *Dogs*

  - Tiletamine-zolazepam (combination = 'Zoletil');
  - Tiletamine-zolazepam and medetomidine. Medetomidine can be reversed with atipamezole at 3–4 times the medetomidine dose but not less than 20 minutes after initial administration as this uses a low dose of Zoletil.

  *Cats*

  - Ketamine and xylazine;
  - Ketamine and diazepam;
  - Ketamine and acetylpromazine;
  - Ketamine and medetomidine; or
  - Tiletamine-zolazepam and medetomidine

- In the case of dogs and foxes, dose-dependent sedation (i.e. very light to profound) can be achieved by the use of medetomidine or xylazine alone. When these drugs are used alone, premature recovery may occur in response to noise. These agents can be reversed with atipamezole and yohimbine, respectively, preferably by the intramuscular route.
- Injection sites are the large muscle masses in the hind limbs, paravertebral muscles or muscles of the neck.

## Pigs

**Physical restraint**

- Zoonotic risks include leptospirosis, Q fever, brucellosis, melioidosis and tuberculosis.
- Feral pigs can react quickly and unpredictably and are capable of causing significant injury. Adult pigs have sharp teeth and strong jaws and can inflict serious injury on the unwary. Boars also have tusks that are used for goring. Sows with litters are aggressive animals and should be approached with caution. They can initiate an attack especially when cornered away from their litter.
- Adult pigs will need to be chemically restrained to reduce stress to the animal and risk of injury to the handler.
- Piglets can be handled without chemical restraint only after the sow has been immobilised. They should be held by supporting the body under the chest.

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:
  - Tiletamine – zolazepam and xylazine. Induction occurs within 5 minutes and lasts for 50–60 minutes;
  - Fentaz® (a combination of fentanyl citrate and azaperone). Induction occurs within 5 minutes and is reversible with of Lethidrone® (nalorphine hydrochloride);
- Injection sites are the large muscle masses in the hind limbs or side of the neck and should be administered using a pole syringe, blow pipe or dart gun.

## Goats

**Physical restraint**

- Individual goats can be singled out in yards and caught by hand.
- Straddle the goat and if horned, hold the animal's head by the base of the horns (not at the tip as this may cause the horn to break)
- Covering the goat's eyes while handling may calm the animal. Also, raising one of its back legs prevents the risk of injury to both handlers and the goat because it cannot suddenly struggle to escape.
- Zoonotic risks include Q fever and scabby mouth (orf).

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:
  - Tiletamine and zolazepam;
  - Ketamine and xylazine – this combination can be reversed with yohimbine given 45–60 minutes after the last ketamine administration;
  - Ketamine and promazine; or
  - Ketamine and midazolam.
- Injection sites are the large muscle masses in the hind limbs.

## Deer

**Physical restraint**

- Trapped feral deer will be very nervous and flighty and can inflict serious injuries with their legs if allowed to thrash around. Physical restraint should be kept to a minimum.
- Particular care must be taken when handling deer with antlers in velvet as they are easily broken and can result in significant blood loss.
- Dogs should not be used during handling as they cause severe stress to deer.
- Zoonotic risks include salmonellosis and yersiniosis.

  ***Deer caught in trap yard with race***
  - It is important to move slowly and quietly when approaching deer as they are extremely sensitive to unusual sound and movement.
  - Slowly and carefully move the animals into the race.
  - Blindfold the animal.

*Deer caught in trap yard/cage trap without restraining facilities*

- Immobilise with an appropriate intramuscular injection using a blow pipe, pole syringe or dart gun.

*Deer caught in netting*

- Rapid immobilisation is required for animals caught in nets as they may struggle forcibly enough to break a limb or neck.
- Place a dark blanket or other cloth over the head of the deer ensuring that the eyes are completely covered.
- One person approaches the animal and holds the hind quarters while another person secures the head/neck.
- Immobilise with an appropriate intramuscular injection.

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:
  - Fentaz® – (combination of fentanyl citrate and azaperone);
  - Ketamine and xylazine;
  - Ketamine and detomidine;
  - Ketamine and medetomidine; or
  - Tiletamine and zolazepam and xylazine.
- Dose dependent sedation can be achieved by the use of xylazine or detomidine alone.
- Injection sites are the large muscle masses in the hind limbs or side of the neck. A handheld syringe can be used in a crush or race while projectile syringes are used on deer in yards.

## Birds

**Physical restraint**

- Birds are fragile and must be handled carefully. Control the bird's head by grasping at the nape. The handhold used will depend on the size or strength of the bird:
  - *Small birds* – place the thumb and forefinger on either side of the lower mandible (do not completely encircle the neck). It is important when handling very small birds that the cup of the hand does not completely surround the sternum and impede respiration.
  - *Large birds* – as for small birds but place thumb beneath the lower mandible and exert upward pressure. With the other hand hold the body and legs.
- Approach the bird from behind to grasp the head and the body, and hold the legs together. Larger birds may be approached with an outstretched towel thrown over it and then picked up wrapped in the towel.
- Taping the beak shut may prevent bite injuries to the handler. The tape should not obstruct the nares.

**Chemical restraint**

- Anaesthesia can be achieved by inhalation with a face mask or preferably an induction chamber using 4–5% isofluorane (then 1–2% for maintenance) in oxygen.
- Intramuscular injection of ketamine may also be used.

- Birds may be wrapped in newspaper to prevent them from flapping wings or falling over as they recover.

## Rodents

**Physical restraint**

- These animals have sharp incisors and may try to bite.
- Remove the animal from the trap by gently tipping directly into a calico catching bag.
  - ***Hand hold***

    While still in the bag, hold the animal's head down with one hand and with the other hand grab it over the shoulders so that the head is held securely between the index and middle fingers. Release the head and use this hand to support the rump of the animal when it is picked up (for larger rodents).
  - ***Scruffing***

    While still in the bag, hold the animal's head down with one hand and with the other hand grasp the loose skin on the scruff of the neck using the thumb and forefinger. Release the head and use this hand to support the rump of the animal when it is picked up (for larger rodents).

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:
  - ***Rats:*** Ketamine and xylazine
  - ***Mice:*** Ketamine and xylazine.
- Injection sites are the large muscle masses in the hind limbs.
- Inhalational anaesthesia (halothane or isofluorane) may be used. Animals should be placed in an induction chamber at 3–5% in oxygen at 1 L/min, and then maintained at 1–2% by face mask.

## Horses/Donkeys

**Physical restraint**

- Zoonotic risks include melioidosis, ringworm and dermatophilosis.
- Beware of animals kicking directly backward with either or both hind feet. Horses can also strike, bite and crush people against fences.
- Electric prods and dogs must NOT be used to assist in the handling of feral horses/donkeys.
- Feral horses/donkeys should be handled quietly without force to avoid panic and trampling.

  ***Horse/donkey caught in trap yard with race***
  - Move the animals into the race.
  - Blindfold the animal.

  ***Horse/donkey caught in trap yard without restraining facilities***
  - Immobilise with an appropriate intramuscular injection using a blow pipe, or dart gun.

**Chemical restraint**

- Anaesthesia can be achieved by an intramuscular injection of the following:
  - A combination of etorphine, xylazine, acetylpromazine and atropine delivered intramuscularly using a RDAS, with diazepam administered intravenously to reduce muscular rigidity and tremors. Reversal of this anaesthetic can be achieved with diprenorphine and yohimbine;
  - Etorphine is hazardous to humans even in very small doses. Operators should be experienced with its use, have the reversing agent diprenorphine or naloxone on hand and have an assistant available to assist in the case of inadvertent self-administration.
  - Intravenous injection of xylazine or detomidine or a combination of xylazine, acetylpromazine and methadone achieves short acting sedation with the horse still standing.
  - A combination of guaifenesin, ketamine and xylazine;
  - Ketamine;
  - Xylazine and tiletamine – zolazepam. T-Z cases rhythmic muscular movements but these are reduced by the xylazine.
- Intramuscular injection sites are the gluteal muscles or muscles of the side of the neck. Intravenous injections should be given via the jugular vein.
- The chemical agent and dose used will determine whether the animal will become recumbent or remain standing during sedation.

## Macropods

**Physical restraint**

- The hind legs of macropods are very powerful and can cause serious injury to operators.
- After capture, the animal should be transferred to a sack (e.g. Hessian sack or a wool bale for larger macropods). This is done by grasping the animal by the base of the tail, lifting it off the ground and quickly placing it in the sack head first. Letting the animal's front feet touch the ground may sometimes assist with transfer. A second person is required to open the entrance to the sack and assist with gentle but firm restraint until the animal can be sedated.
- Larger macropods (> 50 kg) may require darting with a sedative before being transferred into a sack.
- If the head is kept covered, it is possible to pull various parts of the body (tail, hind limb etc.) out of a sack for examination or injection.
- Sedation of macropods is usually required to reduce stress during handling (see below).

**Chemical restraint**

- Sedation can be achieved for 1–3 hours by an intramuscular injection of diazepam. Injection sites are the thigh muscles. An intravenous injection of diazepam provides a more rapid effect with injection sites in the coccygeal vein near the base of the tail or the cephalic or medial saphenous veins.
- Anaesthesia can be achieved by an intramuscular injection of Zoletil® – a combination of tiletamine and zolazepam. Injection sites are the thigh muscles. Recovery may be slow (1–5 hours) and violent.

- Inhalation anaesthesia may also be used with a face mask (3–5% isoflurane in oxygen, then 1–3% for maintenance). Prior sedation is necessary to prevent stress and struggling. Induction is rapid and smooth, and endotracheal intubation is often not needed unless performing extended procedures or examination of the face or mouth.
- During recovery the animal should be isolated in a small dark enclosure that is free of obstacles. If this is not possible then the head must be covered with a towel.

## Cane Toads

**Physical restraint**

- Cane toads secrete a toxin from the parotid glands situated behind the eardrums. Although gentle handling does not normally elicit secretion of this milky substance, hands should always be washed thoroughly after any handling activity.
- The hands should always be clean, moistened and soap/detergent free before handling a toad. Alternatively, unpowdered plastic/vinyl gloves may be worn.
- Toads may be gently gripped around the body (more suitable for smaller toads) or picked up and held by the hind legs, with support of the body.
- Damp gauze may be wrapped around the legs to assist with control.
- To restrain a toad, hold the ventral side down with the hind legs held firmly together with one hand. The thumb and forefinger of the other hand is used to pin the toad's elbows down at 90° to the body, with the palm of the hand cupped over the toad's eyes.

**Chemical restraint**

- The skin of amphibians is sufficiently permeable to allow absorption of some anaesthetic chemicals, including:

  ***Tricaine methanesulfonate ethyl 14-aminobenzoate (MS-222):***

  - Prepare an aqueous solution of 2% MS222 (2 g/L) and pour into a clean glass container that is large enough to comfortably fit the animal. The depth of solution should not exceed 5 mm.
  - Place the toad in the prepared container and place a cover over to prevent the toad from escaping.
  - The depth of anaesthesia can be determined by the presence or absence of a withdrawal response to compression at an inter-phalangeal joint.
  - The duration of induction will vary with the size, body mass and skin thickness of the animal, combined with the body and water temperature.
  - The animal should be kept moistened throughout anaesthesia.
  - Reversal of the anaesthetic is brought about by exposing the toad to a stream of cold water beneath a tap.

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