RES004 MARKING OF PEST ANIMALS USED IN RESEARCH

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Background

Research involving pest animals may require the reliable identification of individual animals. Where possible, researchers should make use of natural identifying marks. However, the majority of species require the application of some form of identifying mark or tag. A variety of techniques is available and includes temporary, semi-permanent and permanent markers. The type of marker used should be selected to minimise distress and cause the least interference with the normal functioning of the animal.

Restraint and handling techniques must be appropriate for the species and minimise distress and the risk of injury to the animal. Improper restraint 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 OH&S) operating in the relevant jurisdiction.

Application

- This document provides guidelines for research involving pest animals. They aim to ensure that marking and identification 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 majority of marking methods require that animals be captured and/or restrained. Proper capture techniques are 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. For more information on capture, handling and restraint techniques refer to ***RES001 Live Capture of***

Pest Animals used in Research and ***RES002 Restraint and Handling 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.

Animal Welfare Considerations

- To understand and potentially reduce the impact of marking 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 marking 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.
- The potential animal welfare implications of marking include:
    - Pain (short or long-term) and injury from application of tags, tattoos, etc.
    - Infections arising from skin damage from tags and collars.
    - Chafing or constriction from collars/harnesses.
    - Toxicity from paints, dyes and other markers.
    - Increased predation by other animals because of increased visibility.
    - Decreased ability to capture prey because prey animals may be more aware of the animal's presence.
    - Inhibition of normal behaviours.
    - Catching of tags, collars, etc on branches or similar resulting in injury or immobilisation.
    - Sampling of excessive amounts of hair, resulting in hypothermia / sunburn
    - Interference with ability to fly, glide, burrow, etc.

- Marking procedures must be performed by persons competent in the methods to be used. Some methods require considerable training and experience to be used appropriately.
- The mark should be quick and easy to apply, with minimal pain or distress.
- The mark should be readily visible, coloured and/or numbered for ease of identification.
- The mark should be detected with minimal or no handling required.

- The animal should experience no immediate or long-term adverse effects on its normal behaviour or health.
- The mark should persist on the animal until all research objectives using that individual are fulfilled.
- Telemetry transmitters must be fitted correctly. They should be a weight and size appropriate for the species and should not cause irritation.
- Techniques such as toe clipping or ear notching cause considerable pain and distress and should not be used as superior methods are available

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 eg. 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 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 eg. 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 (eg. 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 (eg. 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 Marking

Temporary Methods

Paints and Dyes

- Paint and dye can be easily applied directly to the hair or fur of many animals and is a highly visible form of marking.
- Non-toxic paints and dyes should be used.
- Application most often involves painting the mark directly onto the animal by hand using stencils and brushes, or more remotely with brush-tipped poles. Some studies have used paint filled balloons or a paintball gun to mark animals from a distance, but this method is not recommended as there is a potential for injury.
- Paint should not be used on animals with thick fur, as grooming will cause ingestion of the paint and even if the mark persists, fur matting may cause fur loss or skin irritation.
- Paint should not be applied to amphibians (other than the carapace of turtles) due to their moist and highly absorbent skin which plays an important role in gas and water exchange.
- Paint should not be applied to the wings of birds as it may affect the aerodynamic properties of the wing.
- Paint or dye markings may alter the animal's behaviour (eg. increased time spent grooming) and increase its visibility to predators or prey.
- The advantage of using paints or dyes is that handling and restraint time of the animal can be kept to a minimum.
- The longevity of this type of marking is a few weeks to several months and depends on hair shedding (moulting), wear (rubbing) and fade (eg. dyes).

- *Method*
    - Restrain the animal.
    - Apply paint or dye to the area of the animal that will be most visible to the observer (eg. both flanks for ground observation and the back for aerial observation). Use stencils if it is a requirement of the study to identify individual animals. If individual identification is not required then more remote methods may be used (eg. balloon filled with paint) although marking of both sides may not be possible.
    - Allow the mark to dry before releasing the animal.

Fur Removal

- This method of marking can only be used on animals with sufficient hair/fur.
- Fur can be clipped or shorn from sections of the animal's body that will be easily visible to the observer, whilst minimising the visibility of the animal to predators or prey. The use of powered clippers should be minimised as the

noise can be an additional stressor. For larger animals, unique marks (eg. numbers) may be applied to allow/improve individual animal identification.
- Removal of extensive amounts of fur must be avoided to reduce the possibility of sunburn or hypothermia.
- The longevity of this type of marking is a few weeks to several months and depends on when the next moulting occurs. As such, this form of marking is only appropriate for short term studies.

- *Method*
    - Restrain the animal.
    - Use blunt-ended, curved blade scissors to clip hair from the area of the animal that will be most visible to the observer and most appropriate for the purposes of the study. Electric shears may be used on quieter animals to hasten the procedure.
    - Ensure the mark is easily identifiable but does not involve excessive fur removal.

Semi-Permanent Methods

Semi-permanent markers are those that are required to last for months to years. Most marks are lost during the lifetime of the animal or are removed after recapture.

Tags

- Care must be taken to ensure that the tag is of an appropriate size, shape and colour to permit normal behaviour of the animal marked. There is generally a trade-off between tag visibility and tag size and, therefore, the negative effects on the animal (eg. snagging on vegetation or grooming activity). Appropriate ear tags and attachment tools for most animals are available from rural suppliers and come with instructions for correct attachment.
- Tags are most often attached to the ears of the study species and are either self-piercing or inserted through a punched hole. Attachment locations are usually the lower, inner region of the ear where there is heavier cartilage and the tag is better protected. Also this placement reduces the chance of ear tearing or the tag being pulled out.
- The person attaching the ear tag must understand the ear anatomy of the study animal to avoid blood vessels and select optimum attachment points.
- The application site must be treated appropriately to prevent infection and ensure healing.

- *Method (Ear tags)*
    - Restrain the animal so that it cannot move its head.
    - Cleanse the site of application with povidone iodine or chlorhexidine.
    - Use the tagging tool appropriate for the type of tag being used. Most of the tools used to insert ear tags through the ear are sharp and can cause injury to the animal and handler.
    - The tag is attached, quickly and without hesitation, to the lower, proximal region of the pinna, avoiding blood vessels.
    - The site of insertion is treated with an antiseptic agent to minimise the risk of infection.
    - It must be ensured that the tag is attached correctly and will not impair the behaviour of the animal.

Bands

- Leg bands are most often used for birds.
- Handling and restraint of the animal is required at application and subsequent identification.
- The Australian Bird and Bat Banding Scheme (ABBBS) supplies numbered metal bands to banders. A list of approved band sizes for birds is available from the following webpage: http://www.deh.gov.au/biodiversity/science/abbbs/pubs/band-size-list.pdf
- If males and females of that species take different band sizes, determine the sex of the animal (if this cannot be done with confidence then release the animal unbanded).
- Extreme care must be taken when attaching bands onto animals, especially if they are juveniles and still growing. If bands are too tight, there may be skin damage, infection, impaired circulation and limb / digit loss. Loose bands can chafe or snag on such things as vegetation.
- Bands that are constricting limbs should be referred to a veterinarian for removal.

- *Method (Leg bands)*
    - Restrain the bird.
    - Before a bird is banded ensure that the band to be used is the correct size and metal type for the species, size and sex.
    - Select the correct string of bands and use the next band in sequence. Use the appropriate ABBBS pliers to attach the band. There are two types of pliers available for closing standard bands:
        -Small, 5-hole pliers: all size 1-8 bands; size 23-25 parrot bands; and size 16 shearwater bands
        -Large, 2-hole pliers: all size 9-15 bands; 20-22 bands; and 27-28 bands
    - Fit the band in the appropriate hole of the banding pliers with the gap of the band aligned with the open jaws.
    - Close the band around the bird's lower leg by gently squeezing the handles of the pliers so that the two ends are brought together. Bands should never be closed with the fingers as this could cause the bands to close unevenly.
    - Once the initial closure of the band is completed, the pliers should be positioned around the band with the gap aligned at 90° to the open jaws. The band should become fully closed and completely circularised by applying increasing pressure with the pliers.
    - Ensure that the band is fully closed and that no sharp edges are protruding.
    - Release the bird at the point of capture.

External Radio-Telemetry Transmitters

- Radio-telemetry is expensive and time consuming. Radio-telemetry transmitters (RTTs) should only be attached when project funding guarantees

the ability to monitor a tagged animal for the life-span of the transmitter and the removal of the RTT when no longer required.

- The basic system includes a transmitter, power supply, antenna, material to protect the electronic components and a collar, harness or adhesive to attach the transmitter to the animal.
- Transmitter packages should be as light in weight as possible. The total weight (ie. collar, transmitter, battery, aerial and bonding material) should ideally be no more than 5% of the animals' bodyweight.
- Transmitter profile may be more important than weight for some animals. Investigators need to consider the behaviour of the animals under study.
- Reliable RTTs with the longest battery life possible (ie. around 5 years for larger animals) should be used. It is preferable that they be fitted with mortality sensors.
- There are a number of modes of attachment including harnesses, glue, tail clips, collars and implantation. There is no one best method: it depends on the animal and its habits. The guiding principle is to minimise the impact on the natural movement of the animal and to avoid short-term and long-term injury resulting from the transmitters.
- For some species, sedation or anaesthesia may be required during the attachment procedure.
- There are numerous harness formats available for birds, with the most common being backpacks. The RTT is held on the centre of the birds back with the antenna trailing down the back and tail. Various harness materials such as Teflon ribbon, bungee cord, PVC tubing or other elastic materials are used to loop under the wings and then around the base of the neck or around the abdomen.
- Harnesses or other attachment materials should be designed so that they have a weak link, ensuring that the transmitter is detached if it becomes entangled.
- Expandable collars/harnesses should be used on growing animals.
- Transmitters attached by adhesive will usually eventually detach. This method is preferred if it is unlikely that the animal can be recaptured.
- Except where attachment devices are designed to eventually detach, the animal should be recaptured before the battery is depleted in order to remove the transmitter. As the RTT may fail or the animal may move out of range, making recapture unlikely, RTTs should be designed so that long-term attachment will not result in injury.
- Fit should be checked by looking for signs of irritation or hair loss under the collar/harness when recaptured.
- Collars should be made of materials which are durable; comfortable and safe for the animal; can withstand extreme environmental conditions; do not absorb moisture; and maintain their flexibility in low temperatures. Common materials used include flat nylon webbing, butyl or urethane belting, PVC plastic and tubular materials. The collar is closed with a buckle or clamps.
- Whip antennae should be incorporated into the collar wherever possible to prevent snagging on vegetation
- RTTs should always be tested before and after attachment to the animal (before release) to ensure they are functioning correctly.

- It may be advantageous to attach (or implant) the transmitter on an individual in captivity and observe the animal for signs of impaired movement, irritation or rubbing caused by the transmitter over an appropriate period before release.

- *Method (Glue-on tags)*
    - The animal should be restrained.
    - In birds, the RTT should be attached to the skin by separating the feathers on the dorsal midline between-or cranial to-the base of the wings. The attachment area should be cleaned and dried and a smear of fast-setting cyanoacrylate adhesive applied to the radio tag which is then held firmly to the site of attachment for five minutes. The long axis of the RTT should be aligned with the animal's dorsal axis.
    - For other species, the fur should be clipped as close to the skin as possible, to allow adhesion to the skin rather than the fur.
    - Cotton gauze may be attached to the radio tag using cyanoacrylate before applying to the skin and trimmed to overlap the tag by 1mm. This has been shown to enhance the adhesion to the animal.
    - Allow an additional 10 minutes for the adhesive to strengthen. During this time, the animal should be placed in a dark, quiet area.
    - Release the animal after checking that the RTT is operating correctly and that the attachment is effective.

- *Method (Collars)*
    - The animal should be restrained by one handler while the other attaches the collar.
    - The collar should be fitted snugly on the neck to ensure that no irritating movement or rubbing occurs. However, enough space should be left to allow the animal to behave normally and for it not to experience any discomfort while moving or feeding.
    - To reduce the risk of irritation on the neck, the collar should be fastened at the side and any metal fitting should be covered or at least smoothed on the inside.
    - Before releasing the animal, the magnet (battery stop) is removed or the collar turned on if it is fitted with a magnetic switch and transmitter operation and frequency is checked.
    - Once the collar has been attached, and before release, the animal should be observed for any unusual behaviour that could indicate that the collar may cause a problem (eg. affecting balance, impeding movement or causing irritation to the skin)

- *Method (Harness – Birds)*
    - The animal should be restrained.
    - The harness is attached by holding the bird's legs parallel with the tail in order to pass both through the body loop.
    - The loops should be adjusted to ensure that they fit the bird correctly. It is recommended that inexperienced operators release the harnessed bird into a small enclosure to observe the effects of the harness and ensure that it is not inhibiting the flight of the bird.

- *Method (Tail clips – Birds)*
    - The animal should be restrained by one handler while the other attaches the clip.
    - The tail feathers are fanned out and the transmitter placed on top of the feathers at the base of the tail. The tail clip is attached to the central six feathers of the tail closest to the upper tail coverts (the body). The middle catch is inserted between the central two tail feathers and the end catch and flip wire inserted between the two tail feathers on each side.

Permanent Methods

Permanent marks are those that are unlikely to be lost during the life of the animal.

Tattooing

- Tattooing is considered to be a permanent method for marking wildlife, but the longevity of this type of marking can depend on the species and age of the marked animal, and the quality and location of the mark.
- Animals of all sizes can be tattooed, with the advantages being that they add no weight to the animal; do not alter behaviour or make the animal more conspicuous to predators.
- However, tattooing requires prolonged restraint during application, the process is painful, may result in infection and the animal requires restraint again when the tattoo is read. Tattoos can also fade or become otherwise illegible.
- Best results are achieved by tattooing any lightly pigmented area that is clean and relatively hairless. The most commonly tattooed area is the inside surface of the pinna.
- There are two tattooing methods:
    - Forceps, pliers or hammer instruments are used to pierce the skin in the required pattern, and a highly contrasting dye, ink or paste (eg. red, green or black for non-pigmented skin) is then rubbed into the puncture wounds. The ink must be rubbed in well to make sure of a permanent mark. Forceps or pliers pierce the skin in patterns of letters and numbers when squeezed together, while hammer systems are 'slapped' onto larger animals to create a pattern of pinpricks.
    - Electro-vibrator systems or needles that both pierce the skin and inject the dye can be used to simply 'write' an identifying code into the skin.
- Certain sites are particularly painful to tattoo (such as foot pads and any areas that closely overlie bone) and should be avoided.

- *Method*
    - Restrain the animal so that it cannot move its head.
    - Short acting (inhalational) anaesthesia may be appropriate.
    - The tattoo site is cleaned with povidone iodine or chlorhexidine to minimise infection risk.
    - If using tattoo pliers: before applying the tattoo to the ear check that the pliers have been loaded properly (eg. numbers are placed in reverse to normal viewing). A piece of cardboard is a good aid for checking this.

- Ensure that tattoo numbers and letters are sharp and clean so that they will pierce the skin deeply enough to allow for the absorption of the tattoo ink.
- Rub ink onto the area of the ear that will be tattooed and clamp the tattoo pliers on it tightly to puncture the ear avoiding where possible, hair, blood vessels or ribs in the ear. Unclamp and remove the pliers and promptly rub plenty of ink well into the ear with a cotton-tipped applicator. Rub ink well into the holes.
- If using an electro-vibrator, hold the ear firmly and scribe the desired mark.
- Wipe off the excess ink and clean the ear.

Passive Integrated Transponders

- Passive Integrated Transponder (PIT) tags are small, durable microchips that have no power supply. A hand-held scanner passed over the PIT ('scanning') generates a low energy radio signal that energises the PIT tag to transmit a unique number.
- PITs are quick to apply, long lasting, reliable and provide unequivocal identification. Their small size and weight do not normally alter the behaviour or appearance of the animals.
- Application is briefly painful, but for a shorter period than tattooing. Poor technique may result in prolonged pain and/or infection.
- Animals must usually be recaptured to identify marked individuals although they require only minimal short-term restraint. In some cases, scanning may occur passively as the animal walks past or through a scanner, minimising the need for handling.
- Appropriate restraint, anaesthetic, asepsis and analgesia must be undertaken.
- All PIT tag marking must be conducted using sterile injectors and tags.
- They are most often implanted subcutaneously or intra-abdominally using a large bore syringe. The tags should be implanted in areas of low movement such as the body cavity (only for small animals), around the ears or between the shoulder blades. Whatever site is chosen, it should be used consistently to ensure ease of location at reading.
- As PIT tags may migrate, the whole animal may need to be scanned.
- PIT weight should not exceed 5% of the body weight in very small animals.
- Disadvantages of this technique are the expense of the equipment required and that the tags may migrate if applied subcutaneously, making them more difficult to read in larger species.

- *Method – Subcutaneous application (except cane toads – see below)*
  - The animal must be restrained.
  - The entire animal is scanned to ensure it has not already been implanted with a PIT.
  - The PIT (within its single-use needle) is scanned to ensure that it is functioning and that it is indicating the number shown on the packaging.
  - The skin is cleaned with povidone iodine or chlorhexidine at the point where the needle will be inserted. Brief application may have little effect on reducing bacterial numbers so the solution should be in

contact with skin for at least a minute if this does not require excessive handling. The point of insertion will be a couple of cm behind where the PIT will be deposited.
    - The skin is lifted at the point of insertion. The needle is then pushed under the skin and the plunger depressed then the needle removed all in a single rapid but smooth action. Ensure that all parts of the applicator and needle are removed.
    - The insertion site is then scanned to ensure the PIT has been applied.

- *Method – Cane toads.*
    - The method is as above with the following variations:
    - The PIT tag is inserted parallel to the urostyle due to the large parotid gland preventing insertion between the scapulae.
    - The site is swabbed with 0.1% iodine solution.
    - The needle is inserted subcutaneously just posterior to the axilla facing toward the vent to a depth of 2-3 cm.
    - The injection wound edges are held together while a drop of medical grade cyanoacrylate (eg. Vetbond®, 3M Australia) is applied to seal the opening.
    - The animal should be placed in a calico bag for 5 minutes to allow it to recover.

Recommended Methods of Marking for a Range of Species

Species / Group	Recommended Method	Comments
Rabbits	Ear Tags	o One third of ear length from base of ear
	Fur clipping	
	RTT	
	Tattooing	
	PIT	o Between shoulder blades
Foxes	PIT	
	RTT	o Should be able to slip one finger between the animals' neck and the collar.
Pigs	Ear tags	o Numbered or telemetric
	RTT	o Should be able to slip two fingers between the animals' neck and the collar.
Dogs	RTT	
	PIT	
Cats	RTT	
	PIT	
Goats	Paints & Dyes	
	Fur removal	
	Ear tags	
	RTT	
	Tattooing	
Deer	Paints & Dyes	
	Fur removal	
	Ear tags	
	RTT	
	Tattooing	
Birds	Leg bands	
	RTT	o Adhesive, tail-clip or harness
	PIT	o Between wings at base of neck
		o Where possible, enclose the bird's head in a hood (eg. draw-string bag of appropriate size made from black, open-weave cotton) in order to keep the animal as calm as possible.
Rodents	PIT	
Horses	Paints & Dyes	
	Fur removal	
	Ear tags	
	RTT	
	Tattooing	
Macropods	Fur removal	
	Ear tags	
	RTT	
	PIT	o Between shoulder blades - chemical restraint may be required
Cane toads	PIT	

RTT =radio telemetry transmittter PIT = passive integrated transponder

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