CAN001 Methods for the field euthanasia of cane toads

Prepared by Trudy Sharp[1], Andrew Lothian[2], Adam Munn[2] & Glen Saunders[1]

[1]Vertebrate Pest Research Unit, Industry & Investment NSW

[2] School of Biological Sciences, University of Wollongong

CAN001 – August 2011

Background

The cane toad (*Bufo marinus*) is native to northern South America, parts of Central America and Southern Texas. It was deliberately imported from Hawaii in 1935 and introduced into Australia's tropical north-east in an unsuccessful attempt to control the cane beetle, a damaging insect pest of sugarcane crops. The toads quickly established in the new environment and began to spread. Today, they inhabit most of the Australian tropics and sub-tropics and have reached Western Australia. Their great expansion can be attributed to the combination of being highly adaptable to a range of environmental and climatic conditions, high fecundity and also being highly unpalatable.

Cane toads release potent toxins from their parotid glands as a defence strategy and predators who attempt to consume toads can be killed by ingestion of these toxins. Cane toad eggs also contain high levels of toxins and are also a danger to vertebrate predators. The direct impact of cane toads in Australia has been extensively studied and a review of this research has revealed that it is the lethal toxic ingestion of toads by frog-eating predators that is the major single mechanism of impact (Shine 2010). Although the cane toad has not been responsible for the extinction of any native species, some populations of predator species (varanid and scincid lizards, elapid snakes, freshwater crocodiles, and dasyurid marsupials) may be vulnerable, especially when toads first appear in a new area. However this negative impact can be variable and some of the taxa severely impacted by toad invasion recover within a few decades, via aversion learning and longer-term adaptive changes. The indirect impacts of toads such as food-web mediated effects are less understood and research is continuing in this area.

The control of cane toads is challenging because of their wide-spread distribution, large population numbers, high breeding capacity, small size and burrowing behaviour. Years of investigation into potential biocontrol agents have not yet been successful and currently there is no effective tool for broad-scale reduction of toad populations. Therefore, in the short term, management focuses on frontline surveys and removal of toads. Removal involves the intensive collection of toads by hand, sometimes aided by traps and/or barrier/deflection fencing.

This Standard Operating Procedure (SOP) contains current best practice for the euthanasia (or humane killing) of cane toads. The recommendations are based on information in the literature as well as behavioural observations and time to death recorded in a project to examine the welfare impact on cane toads of a range of euthanasia techniques. The research was conducted at the School of Biological Sciences, University of Wollongong (UOW): 'Evaluating the humaneness of known-to-be-lethal euthanasia techniques for cane toads that are used by community groups' (Munn and Lothian, 2010, unpublished). As new information becomes available the appropriateness of euthanasia methods for cane toads will be reviewed.

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

Application

- The aim of this SOP is to promote the use of acceptable methods for killing and disposing of cane toads. It outlines recommended humane procedures and explains why some methods are considered unacceptable and should not be used. It is aimed at community action 'toad-busting' groups, residential communities as well as land management agencies and other government employees and researchers.
- The methods described herein are for use on adult cane toads only. Methods suitable for use on tadpole and juvenile cane toads are yet to be evaluated.
- Euthanasia procedures must be performed by persons competent in or 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 cane toads;
    - an appreciation of how handling and restraint affect behaviour;
    - an understanding of the mechanisms by which the selected technique induces loss of consciousness and death; and
    - recognition of signs of pain and distress.
- Methods used for killing cane toads should comply with all relevant Federal and State/Territory legislation, policy and guidelines relating to animal welfare.
- Storage, use and transportation of knives must comply with relevant legislative requirements.

General Animal Welfare Considerations

- Published scientific studies investigating general signs of pain or distress in amphibians are lacking, therefore determining negative welfare impacts on these animals can be challenging. Compared to mammals, amphibians predominantly lack facial expressions, show less spontaneous movement than mammals and have minimal vocalisations. Reported behavioural responses to pain include colour change, rapid respiration, and immobility/lethargy/closed eyes and there can either be a decrease or increase in activity (Alworth and Harvey, 2007). Pain that arises from the threat of tissue damage is likely to result in vigorous activity, whereas the response to damage that may have already occurred may be inactivity (Machin, 1999). Cane toads will secrete toxin from their parotid glands when they feel threatened or stressed and they have also been reported to evert their stomach after ingesting noxious substances (Lafortune et al. 2001). An excerpt from the Cornell University College of Veterinary Medicine webpage (http://www.vet.cornell.edu/pain/recognize.htm) lists possible behavioural signs of pain in amphibians as:
    - anorexia
    - hunched posture
    - stillness or immobility

- aggression in normally passive animals
- stinting or guarding on palpation or touch
- blepharospasm – spasm of the muscle of the eyelids, causing the eyes to shut tightly
- aeorphagia – abnormal spasmodic swallowing of air
- dysphagia – difficulty in swallowing
- elevated head and/or extended neck position
- colour changes
- constant dull colour
- avoidance and withdrawal reactions
- biting at affected areas
- lethargy
- isolation
- lameness
- ataxia – loss of ability to coordinate muscular movement
- absence of normal behaviours
- foot or digit flicking
- rapid respirations

- When killing toads, humane procedures must always be used. These procedures must avoid distress, be reliable, and produce rapid loss of consciousness without pain until death occurs.
- Some methods of euthanasia require that toads 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.
- It is important to recognise that some physical methods of euthanasia (e.g. stunning followed by decapitation) which cannot be made aesthetically pleasant may nonetheless be humane in that they ensure 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, although the latter should not be disregarded.
- After the application of a euthanasia procedure, it is essential that the death of the animal be confirmed. Determining death can be more challenging with amphibians compared to other animals. Heart rate can be difficult to detect and respiration can occur through the skin as well as the lungs. Therefore, it is important that a number of criteria are met before confirming that a toad is dead. These are
    - Loss of righting reflex – the toad will not right itself onto its ventral surface when turned onto its back;
    - Loss of withdrawal reflex - there will be no response to a light squeeze to the skin in-between the digits;
    - Loss of deep pain reflex – there will be no response to moderate pressure applied to a digit bone;
    - Absence of respiratory movement - cessation of the throat movements that indicate breathing; and

- Absence of heart contractions – cessation of heart beat as determined by observing the chest for a visual cardiac impulse beneath the skin, and/or by palpation of the chest and/or by listening with a stethoscope.

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 euthanasia procedure. If the animal is unconscious i.e. it has no withdrawal, deep pain or righting reflexes but has a heartbeat, the toad should be euthanased using stunning followed by decapitation (see below).

Collection of cane toads

- Cane toads should be collected and held in containers that are closed, adequately ventilated, constructed of non-toxic materials, and insulated to protect the animals against temperature variations. The cane toads must be euthanased as soon as possible and not held for long periods of time. Only amphibians identified as cane toads should be collected and euthanased. Native frogs must be released at the same location where they were captured.

Impacts on non-target animals

- The euthanasia techniques outlined here are target specific and will not impact on other species, unless native amphibian species are misidentified as cane toads. Thus, it is essential that animals are identified as cane toads before they are killed. The WA Department of Environment and Conservation (2009) have produced a fact sheet titled "*Is it a cane toad? Identifying toads*" (http://www.dec.wa.gov.au/index.php?option=com_docman&task=doc_download&gid=3374&Itemid=1) which describes the important features used to identify adult cane toads. These are:
  - Adult toads are heavily built and are typically around 100 to 150 mm in length.
  - The skin is dry. Adult males have rough skin on the dorsal surface that feels a little like sandpaper whereas females have smoother skin.
  - The skin on top of the adults ranges from dull brown to yellowish or blackish. The underside of adults is a dirty cream colour while juveniles have a grey and cream marbled pattern that is lost with age. They are NEVER a bright green colour.
  - They DO NOT have suckers or enlarged toe pads on their digits.
  - They have a very distinctive bony 'm'-shaped ridge over their nose. These ridges meet in the middle.
  - Cane toads have large bumps, called parotid glands, at the top of each shoulder which secrete a milky substance when the toad feels threatened. This substance is poisonous and is harmful to humans, pets and wildlife.
  - The call of the male toad is a guttural trill sustained for around 30 seconds (http://www.environment.nsw.gov.au/resources/pestsweeds/canetoad.mp3). It is very different to most native frog calls.
- Fact sheets to assist with the identification of cane toad are also available from:

Biosecurity SA at
http://www.pir.sa.gov.au/biosecuritysa/nrm_biosecurity/sa_alert_pests/sa_alert_pest_animals/alert_pest_animal_fact_sheets

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/Territory or Commonwealth regulations.
- If the carcasses present a risk to humans, pets or wildlife they should be disposed of by burying in a deep hole or incinerating. If buried, the carcasses must be deep enough (at least 30cm) so they will not be dug up by other animals.
- If large numbers of cane toads are to be killed, provisions should be made to dispose of carcasses in an appropriate manner.

General Health and Safety Considerations

- The potentially hazardous nature of handling poisonous animals requires that safety protocols be strictly followed. Personnel applying euthanasia methods must be aware of and trained in all aspects of safely handling cane toads.
- Personnel instructing or assisting with euthanasia procedures or disposal of carcasses must also wear the appropriate personal protective equipment as the toxin can spray a considerable distance.
- PVC or nitrile gloves should be worn when handling cane toads to prevent contact of toxins with the skin.
- Hands should be washed after handling cane toads. Clothes contaminated with toxin should also be washed.
- See also the ‘Specific health and safety considerations’ listed for each of the methods below.

Acceptable methods of euthanasia of cane toads in the field

1) Stunning followed by decapitation

***<u>This method is considered CONDITIONALLY ACCEPTABLE for the euthanasia of small numbers of cane toads when</u>*:**

- **it is carried out by experienced and skilled persons;**
- **the animal is held by the back legs against a solid surface;**
- **a large headed hammer is used for stunning;**
- **correct stun placement and stun force is used; and**
- **the toad is promptly decapitated with a sharp knife or cleaver.**

Introduction

- Stunning (blunt trauma or cranial concussion) followed by decapitation is an effective and humane method that is recommended for the euthanasia of amphibians (AVMA, 2007). When performed correctly, stunning is sufficient to render the animal insensible (Close et al. 1996). Decapitation, which involves severing the neck of the animal close to the head using a sharp instrument, is then used to ensure death of the stunned animal.
- Animals must always be stunned or rendered insensible prior to decapitation. Decapitation on its own is NOT an acceptable euthanasia technique since cold-blooded vertebrates are very tolerant to hypoxic and hypotensive conditions and time to insensibility and death may be prolonged (Close et al. 1996; AVMA, 2007).
- Operators using this method must be skilled in its use and confident enough to deliver a blow of sufficient force and accuracy to render the animal immediately insensible.
- Stunning must be conducted so that the cane toad is securely held against a solid surface that will not move or compress during the stun impact (e.g. a chopping board on a table or the tray of a utility vehicle). If conducted against a softer surface (e.g. on the ground) the animal may not be rendered immediately insensible.
- Stunning followed by decapitation can also be used as a secondary killing method in the event that another method fails to kill the animal. Animals should always be checked to confirm death (see above) before being disposed of, and equipment to perform stunning and decapitation should be on hand for such use.
- Although it is a relatively simple method, with very little outlay for equipment, stunning followed by decapitation may not be cost-effective for killing large numbers of toads if there are only one or two operators. It can be time-consuming and labour intensive, taking 1 to 2 minutes to kill each animal and operators may become fatigued.

Results from the UOW trials

- In the UOW trials, a number of toads were dissected following stunning to inspect the brain. In all cases, stunning resulted in complete destruction of the brain. Death was considered to occur very quickly. Although stunning by itself typically resulted in a rapid death for a toad, death is ensured by following a stun with decapitation.

Impacts on target animals

- Humaneness of stunning followed by decapitation as a control technique depends on the skill and judgement of the person conducting the stun. If properly carried out, it can be a humane method of destroying cane toads, as death occurs very quickly with minimal distress. On the other hand, if inexpertly carried out for example with insufficient force, stunning can result in wounding which may cause considerable pain and suffering.
- Toads will experience some distress when they are being handled and restrained by the back legs prior to stunning.
- Stunning must be conducted in a manner which maximises its effect thus causing rapid death. This requires the use of appropriate stun placement (to the region of the head between and just behind the eyes) and appropriate tools. A large headed metal hammer should be used to stun.
- Only head (brain) stuns must be used. Stunning or hitting any other parts of the body is NOT acceptable. Likewise for decapitation, only the head must be removed.
- The stun and decapitation of single animals must occur before moving on to the next animal. It is NOT acceptable to stun multiple animals first and then decapitate them later. Toads will suffer if they have been stunned but not killed and they regain consciousness before being decapitated.

Specific health and safety considerations

- When toads are stunned with a blow to the head, the toxin glands (situated just behind the head) may rupture and spray toxin. Operators should use a full face mask/visor to avoid getting toxins in their eyes, mouth and nose.
- Care must be taken when stunning toads with a hammer as there is a risk that operators may strike a thumb or fingers.
- Working with sharp knives can be dangerous. Operators should use a chainmail glove to protect the hand that is restraining the toad.

Equipment required

Stunning equipment

- A large hammer with a flat, metal head, or equivalent should be used for stunning. The face of the hammer head should be at least 2.0cm in diameter. Rubber mallets are not acceptable as they may absorb the impact.
- A rigid wooden or plastic chopping board (approximately 30 x 20 x 2.5 cm), or equivalent.

Decapitation equipment

- A large, sharp knife or cleaver, with a blade at least 15cm long, should be used for decapitation. The knife should be sharpened regularly.

Other equipment:

- Personal protective equipment including gloves and appropriate eye/face protection.
- Chainmail glove

- First-aid kit

Procedures

- Whilst wearing appropriate personal protective equipment, restrain the cane toad by firmly holding the back legs.
- Gently press the belly of the cane toad onto a chopping board that is on a stable and solid surface.
- Using a hammer, apply a forceful blow to the middle of the head just behind the eyes (see diagram in Appendix).
- Immediately after delivering the stun, use the knife to decapitate the head. Cut in a line just behind the skull and lower jaw bones (see diagram in Appendix). These can be felt by following back along the jaw line until the bone turns inwards. Caution must be taken as this line runs through the toxin glands and toxin may be forced out during cutting (especially if the knife is not sharp).
- Once death has been established, dispose of carcass by deep burial or other approved method.

2) Gassing with carbon dioxide for >4 hours

<u>This method is considered CONDITIONALLY ACCEPTABLE for the field euthanasia of cane toads when:</u>

- **carbon dioxide concentration is greater than 90%;**
- **exposure time is four hours or longer;**
- **the maximum number of toads per bag (approx. 56 L capacity) is 20;**
- **a warming coil and/or plastic tube is used to pre-warm the carbon dioxide; and**
- **toads are confirmed as dead prior to disposal.**

Introduction

Carbon dioxide (CO_2) is used by community groups to kill large numbers of cane toads (e.g. see Kimberley Toad Busters (undated) Fact Sheet Number 4: CO_2 Euthanasing Adults Cane Toads in the Field). However, the use of CO_2 has been controversial. The ANZCCART (2001) euthanasia guidelines state that there are no recommended inhaled agents for amphibians. Wright (2001) states that CO_2 is not a satisfactory method of euthanasia since many amphibians are tolerant to hypercarbic conditions and the time to death will be prolonged. On the other hand, the AVMA (2007) Guidelines on Euthanasia recommend the inhalation of CO_2 as a euthanasia method for amphibians, but state that although loss of consciousness develops rapidly, the exposure time required for death will need to be prolonged. The length of exposure time is a significant issue since community groups have typically left the toads exposed to CO_2 for only an hour, but a pilot study conducted in 2005 by WA CALM indicated that CO_2 gassing is ineffective at causing death at an exposure time of 80 minutes (provided by W. Kay, unpublished data, 2005). The trials conducted at UOW found that an exposure time of 60 minutes was NOT effective in causing death and that a prolonged exposure time of at least 240 minutes (four hours) is required.

Results from the UOW trials

All of the trials at UOW were conducted with CO_2 concentrations of greater than 90% (as measured by a GFM430 Landfill Gas Detector, Air-Met Scientific, North Sydney, Australia). The main findings from the trials conducted at UOW were:

Treatment of individual toads in chambers

- Carbon dioxide DID NOT reliably cause death after 60 minutes exposure. Toads recovered with no ill-effects within 18 minutes of being removed from the 60-minute exposure.
- Carbon dioxide DID NOT reliably cause death after 120 minutes of exposure. Around half of the toads survived (i.e. they had an eye reflex and were breathing and had a heartbeat approximately two hours after removal from the 120-minute exposure to carbon dioxide).

- Carbon dioxide DID NOT reliably cause death after 180 minutes exposure. One toad had a righting reflex immediately following exposure, whilst another still had a heartbeat many hours post-treatment.
- Carbon dioxide DID reliably cause death after 240 minutes exposure, with no toads exhibiting any reflexes or heartbeat after treatment.

Treatment of groups of toads in plastic bags

- To determine the effectiveness of CO_2 as applied to multiple animals, toads were placed in large plastic bags (either 5 or 20 animals) and the air removed. CO_2 directly from a cylinder was then used to inflate bags, which were tied off and left for 240 minutes. No signs of aggression were observed between toads under either the 5 or 20 toads/bag treatments.
- In all of the group trials, toads did not have eye, deep pain or righting reflexes post-treatment, however 7% of toads from the 20/bag trials had a detectable heartbeat for a number of hours post-treatment. All of these toads remained unresponsive to external stimuli and the heart ceased beating within 24 hours. Two toads from a single 20/bag treatment also excreted toxin from the parotid glands during exposure. Thus each animal MUST be checked for a heartbeat prior to disposing of the carcass.
- To avoid gas diffusing through the wall of the plastic bag only thick plastic bags were used. It is possible that the CO_2 could slowly diffuse through thin-walled plastic shopping bags, reducing the concentration of gas inside. To ensure carbon dioxide remains above 90% for four hours, heavy duty plastic garbage bags (approximately 56 L capacity) are recommended as a minimum standard.
- Carbon dioxide is very cold when released from the compressed gas cylinder (i.e. the temperature ranges from < 0°C to -30°C or lower depending on release rate). Therefore, it is important that the gas is passed through an air-warming coil (copper) and/or a length of tubing (approximately 3 metres in length and 8mm inner diameter) to minimise cold shock to the toads.

Impacts on target animals

- Carbon dioxide is commonly used to euthanase laboratory animals, especially rodents, however its use remains controversial as animals may experience significant pain and distress prior to unconsciousness (Hawkins et al., 2006). Although a number of studies have concluded that inhalation of CO_2 is aversive to a range of different species (Conlee et al., 2005) there is little information in the literature describing the effects of carbon dioxide on amphibians. Many euthanasia guidelines do not recommend CO_2 as a euthanasia agent because amphibians can tolerate low levels of oxygen.
- In the UOW trials, toads gassed with CO_2 exhibited mild signs of distress. During introduction of gas to the bag, some toads hopped around a bit and some animals exhibited aerophagia, blepharospasm and limb flicking. During the first 5 minutes of exposure, most urinated, some bloated and the ventral skin turned pink in some animals however, there was no aggression, vocalisation or gastric eversion. As the CO_2 took effect, most animals closed their eyes, and laid with their chin down, some lost coordination and fell on their side or back. Also, a low number of toads secreted toxin during treatment. There was a decrease in heart rate after exposure to CO_2 and in animals where it was recorded, the respiration rate dropped to zero (although the heart remained beating for a number of hours after).
- If the toads are NOT exposed to 90% CO_2 for four hours it is possible that they will not be killed. To ensure that toads are not buried (or otherwise disposed of) while still alive they MUST be treated for the specified time at the correct concentration of gas. Death MUST also be confirmed in each animal prior to disposal of the carcass.

- As the compressed CO_2 coming out of a cylinder expands it draws in a lot of heat. Thus it is extremely cold as it comes out; cold enough to cause burning of the skin. For this reason, the gas must be passed through sufficient length of tubing (or a warming coil) to warm it to the surrounding air temperature.
- If only a small number of toads are to be euthanased (i.e. less than 20 per bag), a four hour exposure to 90% CO_2 is still necessary.

Specific health and safety considerations

- Carbon dioxide cylinders can be very heavy so care must be taken when lifting and transporting cylinders.
- 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 carbon dioxide can cause narcosis and/or asphyxia, therefore carbon dioxide should always be used in a well-ventilated place.
- If carbon dioxide is inhaled, remove patient from the contaminated area to allow them to breathe in fresh air. Early signs of exposure are headache and shortness of breath. If patient is not breathing, make sure the 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 carbon dioxide (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.

Equipment required

Carbon dioxide equipment

- Compressed carbon dioxide in cylinders.
- Carbon dioxide gas regulator.
- Air warming coil and/or 3 metres of tubing to go from regulator to the gassing bag or container.
- Bags or container for cane toads to be gassed in.
    - Heavy duty plastic garbage bags (approx. 56 L capacity and about 50 microns thickness) are recommended. Thicker bags become difficult to tie off. Thinner bags become prone to puncture or diffusion of gas.
    - Alternatively an approved, sealable, flow-through container can be used.

Other equipment:

- Personal protective equipment including gloves and appropriate eye/face protection.
- First aid kit

Procedures

- Place cane toads in a large heavy duty plastic garbage bag (approx. capacity 56 L). A maximum of twenty cane toads (approximately 100g in weight, and 100mm in length) should be placed in each bag. If bags are less than 56L capacity, only five cane toads can be gassed at a time.
- Once toads are in the bag, remove as much air as possible from the bag and pinch off at the top of the bag.

- Whilst preventing entry of air into the bag, insert the tubing from the regulator into the top of the bag, making sure it goes right down inside the bag.
- Turn on the flow of carbon dioxide at a maximum rate of 10L/min until the bag is full. Compressed carbon dioxide gas in cylinders with a regulator should be used so the inflow to the chamber can be regulated precisely.
- Turn off gas and remove tubing from bag without letting any air into the bag.
- Tie off bag with a tight, secure knot and check that there are no leaks.
- Make sure cane toads are spread out over the bottom of the bag. If piled on top of each other they may not receive the required exposure.
- Place bag in the shade on smooth ground, ensuring it does not puncture.
- Label the bag with the time filled with CO_2 and leave for a minimum of four hours, ensuring the bags remain in the shade for the entire time.
- After four hours, open bags in a well ventilated area. Remove each cane toad and check for signs of death (see above). If any toads are not dead they must either be re-treated with CO_2 or stunned and decapitated. All toads must be confirmed as dead prior to disposal of carcasses.
- Dispose of dead cane toads by deep burial or other approved method.

3) Spraying with Hopstop®

This method is considered CONDITIONALLY ACCEPTABLE for the field euthanasia of cane toads when:

- **The toad is treated with sufficient spray to anaesthetise and then kill them quickly and effectively, therefore two sprays are recommended for all animals; and**
- **Toads are confirmed as dead prior to disposal.**

Introduction

Hopstop® is an aerosol spray specifically developed by Pestat Pty. Ltd. for the euthanasia of cane toads. It contains 4% chloroxylenol and 67% ethanol, as well as isopropanol, citral and alkanes which act as a propellant. (U.S. Patent Application No. 12/312,500, Publication No. 2010/0069506, 2010; Pestat Pty. Ltd., 2008). Chloroxylenol (which is also the active ingredient in Dettol®) acts as the pesticidal or toad killing agent in the spray and the ethanol is used as an anaesthetising agent. The isopropanol is used as a carrier for the chloroxylenol. Hopstop has achieved registration with the Australian Pesticides & Veterinary Medicines Authority.

Results from the UOW trials

In the UOW trials, after being sprayed according to the manufacturers instructions, toads exhibited a range of behaviours consistent with distress. These included limb-flicking, urination and blepharospasm and avoidance of the spray which included crawling and hopping movements and attempted burrowing in the corner of the container. After a few minutes some toads developed ataxia, and most stopped moving and lay with their chins down until death. The ventral skin also turned red and the average time to death was 19 minutes (range from 5 to 36 minutes). In contrast to treatment with Dettol®, there was no toxin secretion or gastric eversion. Also, the heart rate *slowed* to below baseline 5-10 minutes post-treatment with Hopstop®, whereas the heart rate of toads treated with Dettol® *increased* from baseline 5-10 minutes post-treatment. A decrease in heart rate would be expected to occur in amphibians that have been sedated or anaesthetised (Lafortune et al., 2001).

Impacts on target animals

- Ethanol is used as an anaesthetic for amphibians but it is only recommended for terminal procedures due to long-term physiological consequences that can occur (Wright, 2001). In the initial trials to determine a suitable lethal agent for cane toads the manufacturers of Hopstop® found that ethanol reliably and rapidly induced loss of coordination and general stupefaction of toads, as indicated by loss of the righting reflex, however it was not effective in killing them (U.S. Patent Application No. 12/312,500, Publication No. 2010/0069506, 2010).
- Chloroxylenol, a phenolic compound, is effective at killing toads but its actual mode of action is unclear.
- Toads are likely to experience some distress for the period after the spray is applied but before the ethanol has taken effect to sedate or anaesthetise them. In the initial trials of Hopstop®, most toads stopped hopping in less than 2 minutes after spraying. The time to death, as indicated by cessation of heartbeat, ranged from 70 to 120 minutes (U.S. Patent Application

No. 12/312,500, Publication No. 2010/0069506, 2010). In the UOW trials, toads appeared to be distressed for a period of about 2-3 minutes before they stopped moving. Death then occurred around 20 minutes later. Further detailed research to determine if the cane toads are insensible to pain after they stop moving or if they are just heavily sedated would be useful in assessing the humaneness of this method further.

Specific health and safety considerations

- Users must read the Material Safety Data Sheet (MSDS) for Hopstop® which can be obtained from the supplier at: http://www.pestat.com.au/extras/HopStop-MSDS.pdf.
- Do not puncture or incinerate the can even once it is empty. Keep the product out of the reach of children. Deliberately concentrating and/or inhaling the contents could be harmful or fatal.
- HopStop® should only be used outdoors. Do not use it inside buildings or other enclosed areas, and do not inhale the spray. Do not spray it towards yourself, other people or animals, and avoid direct application to plant foliage. Do not spray it into or over dams or fishponds. Chloroxylenol is toxic to aquatic animals and should not be applied where it could contaminate water bodies.
- HopStop® is supplied in a pressurised dispenser, and should be treated like any other aerosol product. The contents of the can are highly flammable and the product should not be stored or used near any sources of ignition, naked flames or any incandescent material. Do not smoke while using HopStop®. Store the can in a cool place away from direct sunlight.
- Avoid contact of HopStop® with your skin, eyes and clothes. Wear suitable clothing, and wear gloves when using the product or handling toads. In case of accidental skin contact, wash the affected area immediately with soap and water. In case of eye contact, wash eyes immediately with water.
- If poisoning occurs, contact a doctor or the Poisons Information Centre (Tel: 131 126).

Equipment required

Can of Hopstop®

Other equipment:

- Personal protective equipment including gloves and appropriate eye/face protection.
- First aid kit

Procedures

The following instructions include directions for use from the manufacturer available at: http://www.pestat.com.au/html/products.htm.

Full directions for use are also provided on the label of each can and include:

- Identify cane toads. Only amphibians identified as cane toads should euthanased. Native frogs must not be sprayed.
- Shake the can of Hopstop® briefly and gently before use.
- Hold the can upright about 20-30cm from the toad and spray the toad on the back for 2-3 seconds. The toad will hop for a short time, then stop moving and flatten into a ‘hunched’ position. If you spray the toad where it can move towards a barrier such as a wall, fence or rock, it will then stop and settle down. Do not chase the toad as this is likely to alarm it and cause it to panic and try to hop further and faster. Once the toad has stopped moving apply a second spray.

- Always watch where the toad hops to and then leave it undisturbed until it dies. After 2 hours check each toad for signs of death (see above). Do not directly handle toads, always wear gloves. If any toads are not dead they must either be re-treated with Hopstop® or stunned and decapitated. All toads must be confirmed as dead prior to disposal of carcasses.
- Dispose of dead cane toads by deep burial or other approved method.

Methods that are NOT considered acceptable for field euthanasia of cane toads

Rapid Freezing or Cooling Followed by Freezing

Rapid freezing is another method used extensively in the field to kill cane toads, however its use is controversial. Published guidelines on euthanasia do not recommend the use of hypothermia for killing amphibians nor do they recommended it for sedating or anesthetising amphibians (AVA, 2007; AVMA, 2007; ANZCART, 2001; Close et al. 1996). The guidelines state that placing conscious animals in very cold temperatures is considered inhumane since it may cause pain or distress due to the formation of ice crystals on the skin and in tissues. Therefore, only the quick freezing of *deeply anaesthetised or unconscious animals* is recommended as a euthanasia method

It has been previously argued that a period of cooling at 4-6°C prior to freezing would anaesthetise amphibians and thus reduce their perception of pain. Based on this assumption, the NSW Animal Welfare Advisory Council (2004) approved the use of freezing (when preceded by cooling to 4°C) as the most practical and humane option for killing cane toads. This was despite a review by Martin (1995) which concluded that current studies "do not support hypothermia as a clinically efficacious method of anaesthesia". Cooling is known to slow the metabolism and reduce activity of amphibians, and a study by Suckow et al. (1999) demonstrated that it can have a local anaesthesia effect, but it remains uncertain whether we can extrapolate localised cooling with subsequent pain relief to whole of body cooling and insensibility to pain.

The UOW trials of this method did not produce conclusive results. Although the freezing of pre-cooled toads was effective in causing their death, some toads were still moving and others still had corneal, deep and superficial pain reflexes (but had lost the righting reflex) after a period of 1 hour in the fridge at 6^{o}C. So when placed in the freezer it is possible that they were still capable of perceiving pain.

Thus, currently there is limited scientific knowledge on the effectiveness of hypothermia for anaesthesia of amphibians and further detailed physiological research is required to establish if cane toads are insensible to pain when cooled.

Rapid freezing (without prior cooling) is NOT appropriate for field euthanasia of cane toads.

Cooling followed by freezing is also NOT appropriate for field euthanasia of cane toads since the efficacy of cooling as a means of inducing anaesthesia is unclear.

Benzocaine gel

Benzocaine has previously been reported to be an effective topical anesthetising and euthanasing agent for amphibians, for example see Chen and Coombs (1999) and AVMA guidelines (2001).

A gel containing 7.5% benzocaine was tested as a euthanasia method for cane toads in the UOW trials, however it showed mixed results with regards to efficacy. Initial trials resulted in the quick death of cane toads with minimal stress. But, the efficacy of the benzocaine gel appeared to be greatly reduced approximately one month after it was prepared. In these later trials, only 9 out of 13 animals died following the application of a large amount of benzocaine gel and they took longer to

die (average of 24 minutes). Further testing would be required to determine if this reduction in effectiveness was due to deterioration of the active ingredient in the gel or to other factors.

Access to this method is difficult since over-the-counter preparations of benzocaine gel (e.g. OraGel®) are no longer available in Australia, therefore it must be prepared by a compound chemist and only with a veterinary prescription. It is also relatively expensive at approximately $2.50 per toad (at a dose rate of 2.0g per toad). Xylocaine is now used as the active ingredient in most over-the-counter anaesthetic gels. A gel containing 5% xylocaine was also trialled as a euthanasia agent, but was found to be ineffective.

Based on variable effectiveness, limited availability and high cost, benzocaine gel is NOT appropriate for the field euthanasia of cane toads.

Clove oil solution

Clove oil, which contains the active constituent, eugenol, is an effective anaesthetic and euthanasing agent in fish (Ross and Ross, 2009). It is also used as an anaesthetic in some amphibian species (Mitchell, 2009), however there is the potential for the undesirable side effect of gastric prolapse to occur (Gentz, 2007).

In the UOW trials, clove oil solution was tested as a method for cane toad euthanasia and found to be non-lethal at over three times the dose recommended for anaesthesia in other amphibian species (Lafortune et al. 2001 and Guenette et al. 2007). Deep anaesthesia was induced in toads immersed in a bath of clove oil (1.0mL clove oil in 1.0L of water) but some animals recovered without any ill-effects.

Suitable trials to determine the optimum concentration of clove oil to cause death in cane toads have not been performed, therefore this method is NOT appropriate for field euthanasia.

Dettol®

Dettol® is a household disinfectant containing chloroxylenol (4.8% w/v), isopropyl alcohol (10-30%) and pine oil (<10 %). Some community groups have used diluted and undiluted Dettol® to kill cane toads, for example one method is to place toads into a sealed plastic bag with approximately 100mL of undiluted Dettol and they reportedly die within minutes (Kimberley Toad Busters, 2006). Although it is known to be effective in killing cane toads, the welfare impact of applying Dettol to the skin of toads is often disputed. On one hand anecdotal reports claim that it causes pain and suffering whereas others maintain that it appears to be as humane as other methods such as carbon dioxide .

In the UOW trials, cane toads placed in a shallow bath of undiluted Dettol® exhibited signs of distress. On contact with the skin, the toads showed increased activity and avoidance of the solution, blepharospasm, limb flicking, gastric eversion, dysphagia and some secreted toxin from their parotid glands. Around 5-10 minutes after exposure the heart rate of toads had increased from baseline. The ventral skin also turned a pink or red colour and the average time to death was 15 minutes (range 8 to 30 minutes).

Although it is effective at killing cane toads, due to the degree of suffering caused by the application of this chemical, this method is NOT appropriate for the field euthanasia of cane toads.

AQUI-S®

AQUI-S®, containing the active ingredient isoeugenol (concentration of ~50%), is a commonly used fish anaesthetic, developed by AQUI-S New Zealand Ltd.

In the UOW trials, a solution of 20mL AQUI-S® in 1.0L of demineralised water was tested as a euthanasia agent. AQUI-S® was effective in causing the death of all toads tested with the average time to death being 19 minutes. However, prior to death animals showed signs of distress including gastric eversion, gulping and gagging, blepharospasm, dysphagia, aerophagia and limb flicking.

Although it is effective at killing cane toads, due to the degree of suffering caused by the application of this chemical, this method is NOT appropriate for the field euthanasia of cane toads.

Table1: Humaneness, Efficacy, Cost-effectiveness and Target Specificity of Cane Toad Euthanasia Methods

Euthanasia Technique	**Acceptability of technique with regard to humaneness***	**Efficacy**	**Cost-effectiveness**	**Target Specificity**	**Comments**
Stunning followed by decapitation	Conditionally acceptable	Effective	Cost-effective	Target-specific when only cane toads are targeted	Impractical for large scale application. Best used for individuals or low numbers of animals. Must be done against solid surface with large head hammer and sharp knife. Humane and effective when performed by confident and skilled operators using the correct equipment and technique.
Carbon dioxide	Conditionally acceptable	Effective	Cost-effective	Target-specific when only cane toads are targeted	Practical and cost-effective for large scale use. Exposure to CO_2 must be for minimum of 4 hours exposure to 90% (or greater) concentration of CO_2 and no more than 20 animals per bag. All animals must be confirmed dead prior to disposal of carcasses.
Hopstop®	Conditionally acceptable	Effective	Relatively expensive	Target-specific when only cane toads are targeted	Behavioural observations of treated toads indicate that there may be a period of suffering prior to death however this is likely to be only for a short period (2-3 minutes) until the toads becomes sedated or anaesthetised. Best used for individuals or low numbers of animals. Has the potential to be practical for large scale use, however may not be cost-effective.

Euthanasia Technique	Acceptability of technique with regard to humaneness*	Efficacy	Cost-effectiveness	Target Specificity	Comments
Benzocaine 7.5% gel or cream	Not acceptable at this dose. Further research is required to establish optimum dose for euthanasia	Potentially effective at higher dose	Expensive	Target-specific when only cane toads are targeted	Not cost-effective for use on large numbers of toads. May be effective at higher concentration for small scale use.
Clove oil (at 1.0mL/L)	Not acceptable at this dose. Further research is required to establish optimum dose for euthanasia.	May be effective at higher dose.	Unknown as optimum dose has not been determined for euthanasia.	Target-specific when only cane toads are targeted.	Effective at causing deep anaesthesia, however side effects such as gastric eversion or prolapse may occur at the higher doses required for euthanasia
Rapid freezing	Not acceptable	Effective	Cost-effective	Target-specific when only cane toads are targeted	Rapid freezing is only acceptable as a euthanasia method when it is done on *deeply anaesthetised* animals. Cooling has not been demonstrated to anaesthetise amphibians.
Cooling followed by freezing	Not acceptable	Effective	Cost-effective	Target-specific when only cane toads are targeted	Potentially practical and cost-effective for large scale use, however since toads may suffer prior to becoming insensible this method is not acceptable.

Euthanasia Technique	Acceptability of technique with regard to humaneness*	Efficacy	Cost-effectiveness	Target Specificity	Comments
AQUI-S®	Not acceptable	Effective	Expensive	Target-specific when only cane toads are targeted	Effective at causing deep anaesthesia, Behavioural observations of treated toads indicate that there is a period of significant suffering prior to death.
Dettol®	Not acceptable	Effective	Cost-effective	Target-specific when only cane toads are targeted	Behavioural observations of treated toads indicate that there is a period of significant suffering prior to death.

Further Information

Contact the relevant Commonwealth, State or Territory government agency from the following list of websites:

Commonwealth Department of Sustainability, Environment, Water, Population and Communities
http://www.environment.gov.au/

ACT Department of Territory and Municipal Services
http://www.tams.act.gov.au/live/environment

NSW Industry & Investment NSW
www.industry.nsw.gov.au

NT Natural Resources, Environment, The Arts and Sport
http://www.nt.gov.au/nreta

QLD Department of Primary Industries and Fisheries
http://www.dpi.qld.gov.au

QLD Department of Environment Protection Agency
www.epa.qld.gov.au

SA Biosecurity SA
http://www.pir.sa.gov.au/biosecurity

TAS Department of Primary Industries and Water
http://www.dpiw.tas.gov.au/

VIC Department of Primary Industries, Agriculture & Food
www.dpi.vic.gov.au

WA Department of Environment and Conservation
www.dec.wa.gov.au

Also refer to:

Invasive animals Cooperative Research Centre
http://www.invasiveanimals.com/index.php

References

Alworth L.C. and Harvey S.B. (2007). IACUC issues associated with amphibian research. *ILAR Journal* **48**, 278-289.

American Veterinary Medicine Association (AVMA)(2007). The American Veterinary Medicine Association Guidelines on Euthanasia. Available at: http://www.avma.org/issues/animal_welfare/euthanasia.pdf

Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART (2001). *Euthanasia of Animals Used for Scientific Purposes*, 2nd edition, Reilly J.S. (ed.), ANZCCART, Adelaide.

Australian Veterinary Association (AVA)Draft Policies and Position Papers (2007) Collection, euthanasia and disposal of the cane toad (*Bufo marinus*).

Chen, M. H. and Combs, C.A. 1999. An alternative anesthesia for amphibians: ventral application of benzocaine. *Herpetological Review* **30**, 34.

Conlee, K.M., Stephens, M. L., Rowan, A. N. and King, L.A. (2005). Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Laboratory Animals, **39**, 137-161.

Close, B. et al. (1996). Recommendations for euthanasia of experimental animals: Part 1. DGXI of the European Commission. *Laboratory Animals* **30**, 293-316

Gentz, E. J. (2007). Medicine and surgery of amphibians. *ILAR Journal* **48**, 255-259.

Guénette S.A., Hélie P., Beaudry F., and Vachon P. (2007). Eugenol for anaesthesia of African clawed frogs (*Xenopus leavis)*. *Veterinary Anaesthesia and Analgesia* **34**, 164–170.

Hawkins, P. et al. (2006). Newcastle consensus meeting on carbon dioxide euthanasia of laboratory animals. Report of a meeting held at University of Newcastle upon Tyne, UK, from 27-28 February 2006. Available at: http://www.nc3rs.org.uk/downloaddoc.asp?id=416&page=292&skin=0

Kimberley Toad Busters (undated) Fact Sheet Number 4: CO_2 Euthanasing Adults Cane Toads in the Field). Available at: http://www.canetoads.com.au/datasheet4.htm

Kimberley Toad Busters (2006). *Kimberley TAFE Ranger Program Toad Bust, Wednesday 7th & Thursday 8th September 2006.* KTB Field Report. Available at: http://www.canetoads.com.au/fieldrep2006.htm#9

Lafortune M., Mitchell M.A, Smith J.A. *(*2001*)* Evaluation of medetomidine, clove oil and propofol for anesthesia of leopard frogs (*Rana pipiens). Journal of Herpetelogical Medicine and Surgery* **11**, 13–18.

Machin, K.L. (1999). Amphibian pain and analgesia. *Journal of Zoo Wildlife Medicine*, **30**, 2-10.

Martin, B. (1995). Evaluation of hypothermia for anesthesia in reptiles and amphibians. *ILAR Journal* **37**, 186–190.

Mitchell, M.A. (2009). Anesthetic considerations for amphibians. *Journal of Exotic Pet Medicine*, **18**, pp. 40-49.

Munn, A. and Lothian, A. (2010, unpublished). *Evaluating the humaneness of known-to-be-lethal euthanasia techniques for cane toads that are used by community groups*. School of Biological Sciences, University of Wollongong.

NSW Animal Welfare Advisory Council (2004). *Euthanasia of cane toads*. AWAC 164. Monday 5 April 2004.

Pestat Pty. Ltd. (2008). MSDS for Hopstop®. Available at: http://www.pestat.com.au/extras/HopStop-MSDS.pdf.

Ross, L. G. and Ross, B. (2009). *Anaesthetic and Sedative Techniques for Aquatic Animals*, 3rd ed. Blackwell Publishing, Oxford.

Shine, R. 2010. The ecological impact of invasive cane toads (Bufo marinus) in Australia. *Quarterly Review of Biology* **85**, 253-291.

Suckow, M. A., Terril, L. A., Grigdesby, C. F. & March, P.A. (1999) Evaluation of hypothermia-induced analgesia and influence of opioid antagonists in leopard frogs (*Rana pipiens*). *Pharmacology, Biochemistry and Behavior*, **63**, 39-43.

U.S. Patent Application No. 12/312,500, Publication No. 2010/0069506 (published Mar. 18, 2010)(David Dall, Joan Dawes, applicants). Available at: http://www.freepatentsonline.com/y2010/0069506.html

WA Department of Environment and Conservation (2009). *Is it a cane toad? Identifying toads*. Available at: http://www.dec.wa.gov.au/content/view/4982/2172/

Wright, K.M. (2001). Restraint techniques and euthanasia. In: Amphibian Medicine and Captive Husbandry, Wright K.M. and Whitaker B.R. (eds.), Krieger, Malabar, Florida, p121.

Australian Government
Department of Sustainability, Environment, Water, Population and Communities

NSW GOVERNMENT Industry & Investment

University of Wollongong

Appendix

Stunning and decapitation of cane toads: point of aim for stunning and line of cut for decapitation