

21 August 2023

Cane toad euthanasia – relative humaneness of current methods

This document outlines what is currently known about the relative humaneness of cane toad euthanasia methods based on objective scientific research.

Background

Cane toads are a highly invasive species and are regarded as a major environmental pest in Australia. The invasion and establishment of cane toads in NSW has been identified as a key threatening process under the *Biodiversity Conservation Act 2016*. When ingested, cane toad toxin can be lethal to domestic pets, livestock and threatened species. Cane toads also pose a threat to Aboriginal cultural heritage and ecotourism. Management of cane toads aims to minimise or eliminate the impacts of cane toads and prevent their spread.

While there is universal agreement over the need to control cane toads, there is significant debate over what is the most humane method to use. Although there has been some research into the impact of killing methods on toads, it is difficult to measure pain and distress in amphibians, and experimental results can be hard to interpret and are often not clear-cut.

Two studies have been conducted to specifically examine the animal welfare impacts of a range of cane toad euthanasia methods. The most recent at the **University of Western Australia (UWA)** in 2021 and prior to that at the **University of Wollongong (UOW)** in 2010. The UOW study mostly observed behavioural responses to euthanasia methods whereas the UWA measured both behavioural and physiological responses via: (electroencephalogram (EEG) – a signal that varies over time generated by the electrical activity of the brain; and electrocardiogram (ECG) – a signal that varies over time generated by the electrical activity of the heart; and body temperature). Although some of the data from the UWA research is still being analysed and interpreted, this document has been produced to summarise what we know so far from these two studies. The aim is to assist with decision-making regarding the most appropriate method to use and to complement the existing 2011 Standard Operating Procedure (SOP), CAN001 Methods for the field euthanasia of cane toads.

Once the experimental data from the UWA trials has been analysed, an updated SOP for the euthanasia of cane toads will be prepared. It is envisaged that this will be published within the next 12 months.

Euthanasia methods

Hopstop® spray – CONDITIONALLY ACCEPTABLE

A commercially available aerosol spray specifically developed for killing cane toads.

It contains 4% chloroxylenol and 67% ethanol, as well as isopropanol, citral and alkanes which act as propellants. Chloroxylenol acts as the pesticidal or toad killing agent in the spray and the ethanol is included as an anaesthetising agent. The isopropanol is used as a carrier for the chloroxylenol¹.

In the UOW trials, after being sprayed according to the manufacturer's instructions, toads exhibited a range of behaviours consistent with distress. These included limb-flicking, urination and blepharospasm (eyelid movements) 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 the UWA trials, there was physiological and behavioural evidence of pain and distress for a period after spraying but before the toads stopped moving and died. For some toads this period was up to 45 mins, while for others it was around 15 minutes. All toads died within 2 hours of spraying. Toads euthanased with Hopstop® exhibited the *fewest* and *least severe* changes in behaviour compared with the other chemical methods tested (i.e., Croaked® and Lethabarb®).

Hopstop® spray is therefore considered acceptable as long as:

- the toad is treated with sufficient spray to anaesthetise and then kill them quickly and effectively; thus two sprays are recommended for all animals; and
- toads are confirmed as dead prior to disposal; for example, placed in the freezer post treatment with Hopstop®.

Croaked® spray – NOT RECOMMENDED

A commercially available trigger spray specifically developed for killing cane toads.

It is made using the fish anaesthetic AQUI-S® which contains the active ingredient eugenol. The manufacturer claims that the spray kills toads within 8 minutes without irritation, pain or suffering.

The UOW study revealed physiological and behavioural evidence of severe pain and distress after application of the spray. Testing was therefore discontinued after spraying only 3 toads. None of these toads died within 8 minutes and so they were euthanased using double-pithing.

Toads euthanased with Croaked® exhibited the most severe changes in behaviour compared with the other chemical methods tested (i.e., Hopstop® and Lethabarb®).

In the UOW trials, a solution of AQUI-S® (20mL 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 (eyelid movements), dysphagia, aerophagia and limb flicking.

¹ 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>

Given that both the UOW and UWA trials found that eugenol causes a high degree of distress and suffering in cane toads, and that Croaked® did not kill toads within the time stated on the label, the use of Croaked® spray is **not recommended**.

Lethabarb® - FURTHER RESEARCH REQUIRED

A solution of anaesthetic agent used widely by veterinarians to euthanase animals (not available for the general public to administer).

In the UWA study, toads were injected into the body cavity with a standard dose of sodium pentobarbital (81 mg.kg⁻¹) using a 23G needle.

The standard solution (Lethabarb®) of sodium pentobarbital (325 mg/ml) is very alkaline (pH = 11.0) and can cause pain when injected in sites other than intravenously. Therefore, the standard solution was diluted to 80 mg.ml⁻¹ by diluting it in sterile saline (0.25 ml of euthanasia solution added to 0.75 ml of saline). That solution was injected at 1 ml/kg. The recommended standard overdose for amphibians is 60 – 100 mg/kg, thus the dose selected was in the middle of this range (80 mg/kg).

In the UWA study, toads exhibited behavioural and physiological signs of distress during positioning and injection of the solution into the coelomic cavity, but movement quickly ceased and breathing slowed after injection. Although 8 toads were injected, only one died (within 5 minutes). Thus, to be effective a much higher dose than 80mg/kg is needed and even then, time to death could be variable and, in some cases, prolonged. Carcasses of toads killed by sodium pentobarbital must also be disposed by incineration or deep burial.

Given these findings and current recommendations in euthanasia guidelines (such as the 2020 AVMA Guidelines for the Euthanasia of Animals), the optimum dose rate needs to be determined for cane toads before recommendations can be made.

Cooling followed by freezing – CONDITIONALLY ACCEPTABLE

In the UWA trials, toads were cooled (at 4°C, either in ice bath OR fridge) for 1 hour and then placed in a freezer (at -20°C) for 3 hours. The toads did not exhibit any obvious stressful behaviour, they were mostly stationary with occasional movement. In addition, preliminary analysis suggests that they did not show signs of distress or pain on EEG. However, 3 of the 12 toads regained consciousness after thawing (i.e., rinsed with warm water and allowed to defrost for 1 hour). Further analysis of data obtained during the cooling and freezing treatments is being conducted and we will be in a better position to understand the effects of hypothermia on cane toad consciousness and pain perception once this has been completed.

The UOW trials of cooling followed by freezing 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°C. So, when placed in the freezer it is possible that they were still capable of perceiving pain.

Cooling followed by freezing is therefore tentatively recommended with the following conditions:

- toads are held in a suitable container in a refrigerator (at 4°C) for minimum of 8-12 hours;
- the container is then transferred to a freezer (at -20°C) and held for a further 24 hours as a minimum;
- there must be sufficient cool air around each individual toad to allow for uniform cooling and freezing (e.g. toads placed individually in take-away containers); and
- toads are confirmed as dead prior to disposal.

Freezing only – NOT RECOMMENDED

Freezing directly (at -20°C) for 3 hours *without* prior cooling is **not recommended**. Direct freezing is likely to be more aversive than cooling followed by freezing. In addition, frozen toads have regained consciousness after thawing (i.e., rinsed with warm water and allowed to defrost for 1 hour).

Carbon dioxide – CONDITIONALLY ACCEPTABLE

In the UOW trials, toads gassed with carbon dioxide exhibited 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 gas 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 small number of toads secreted toxin during treatment. There was a decrease in heart rate after exposure to carbon dioxide and, in toads where breathing was recorded, the respiration rate dropped to zero (although the heart remained beating for a number of hours after breathing had stopped).

In the UWA study, toads gassed with carbon dioxide also showed behavioural and physiological signs of distress prior to a reduction of brain activity, heart rate and movement over time. Toads actively tried to escape the testing chamber when first exposed to the gas indicating that it was aversive. All toads tested died within 3 hours.

The use of carbon dioxide gas is recommended for the euthanasia of cane toads when:

- carbon dioxide concentration is greater than 90%;
- exposure time is 4 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.

Stunning and decapitation – CONDITIONALLY ACCEPTABLE

Stunning (blunt trauma to the head or cranial concussion) followed by decapitation is an effective and humane method for the euthanasia of amphibians when the following conditions are observed:

- 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.

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 and 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.

More Information

- ANZCAART Guidelines for the Humane Killing of Cane Toads Vol 29, Number 2
<https://anzccart.adelaide.edu.au/system/files/media/documents/2019-07/an29022016.pdf>
- Australian Veterinary Association Collection, Euthanasia and disposal of the cane toad *Rhinella marina*.
<https://www.ava.com.au/policy-advocacy/policies/euthanasia/collection-euthanasia-and-disposal-of-the-cane-toad-rhinella-marina/>
- The Department of Climate Change, Energy, the Environment and Water (DCCEEW)
<https://www.dcceew.gov.au/environment/invasive-species/publications/can001-methods-field-euthanasia-cane-toads>

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