

ANZCCART Guidelines for the Humane Killing of Cane Toads

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Inside this issue:

ANZCCART Guidelines for the Humane Killing of Cane Toads	1
Updated Principles And Guidelines for the Care and Use of Non-Human Primates for Scientific Purposes	7
Victorian Animal Welfare Under Review	7
ANZCCART is on the move	7
Recent Articles of Interest	8

Introduction

These guidelines aim to provide an interim update on the humane killing of cane toads (*Rhinella marina*) that will aid those whose work in research or teaching involves the humane killing of these animals while the ANZCCART Guidelines on Euthanasia are being revised. As this document is specifically prepared for use by those involved in research and teaching with animals, it is assumed that all workers are appropriately trained and adequately equipped to undertake procedures at the standards required by institutional Animal Ethics Committees operating under the *Australian Code for the Care and Use of Animals for Scientific Purposes* (8th Edition, 2013) (The Code). However, we are aware that ANZCCART documents are consulted more broadly particularly with respect to controlling the spread of feral species such as the cane toad. Therefore, this document acknowledges that people using it may not have access to the equipment and agents that would normally be assumed as being standard in the research laboratory setting.

Background

While cane toads are regarded as a feral pest species in Australia, they are sentience animals and are therefore entitled to be treated as such.

ANZCCART considers ill-informed suggestions that cane toads can be subject to mistreatment or the subject of "sport" as seriously inappropriate. However, we recognise that as a pest species cane toads, when caught, should be humanely killed. It is imperative that cane toads are adequately identified before being killed as a number of native species can be mistakenly confused with *Rhinella marina*. There are a number of guides to the identification of cane toads and they should be used for this identification. See:

<http://www.environment.nsw.gov.au/pestsweeds/IdentifyingACaneToad.htm>

<https://nt.gov.au/environment/animals/feral-animals/cane-toads/introduction>

<http://www.feralscan.org.au/docs/Is%20it%20a%20cane%20toad%20Identifying%20toads.pdf>

https://www.frogsafe.org.au/cane_toads/juv_adults.shtml

<http://www.qldfrogs.asn.au/be-toadally-sure-campaign/>

There are different reasons for the humane killing of cane toads such as participation in control / eradication programmes or collection of specimens for use in dissection classes at senior secondary or tertiary levels of education. In both situations, those charged with the responsibility for catching and humanely killing the toads need to be either closely supervised or thoroughly trained in the identification of cane toads so that native species of frogs are not taken by mistake and also trained in the humane killing of the species. We do not consider it appropriate for school aged children to be responsible for killing toads.

One of the unusual characteristics of cane toads when compared with mammals for example, is that even though they are quite sensitive to dehydration, they rarely drink water. Cane toads need constant access to moisture to survive. Instead of drinking, they absorb water through the skin on their belly – from dew, moist sand, damp grass, or any other moist material. Ironically, if forced to remain in flooded conditions, cane toads can absorb too much water and die, just as they can die from water loss during dry conditions.

This rather unusual method for maintaining hydration opens up a number of possibilities when it comes to controlling cane toads and their continual migration across Australia. Firstly from a basic biological perspective, it means that the potential range of cane toads is naturally limited as they are not able to migrate freely across the drier regions of Australia. It also raises some interesting questions about the possible effects of the Wet Season on toad numbers in parts of Northern Australia that are particularly susceptible to flooding. However, potentially one of the most interesting aspects of these attributes is that they provide a relatively simple way to administer soluble anaesthetics and / or other control agents via the absorbent areas of skin.

While the costs associated with the humane killing of animals such as cane toads will be a consideration for some, it must never be used as an excuse to use an inhumane method.

Safety precautions

All parts of the cane toad (including their eggs and tadpoles) should be considered toxic and should not be handled without adequate protection. At the very least, this should include wearing latex gloves and eye protection. Large quantities of the toxin are produced in the parotoid glands, which are located behind their eyes, but toxin can be present anywhere on the toad's skin. Where adult toads are

concerned, individuals handling them should also wear a full-face shield, as stressed toads can actually squirt toxin from their parotoid glands.

If people handling toads do come into contact with the toxin (particularly if it is squirted onto the skin or eyes), appropriate first aid measures should be undertaken immediately. First aid treatment includes flushing the affected area thoroughly with water. Seek medical attention if any symptoms occur. If toxin is squirted into the mouth, immediately flush the mouth with water and seek emergency medical advice.

If swallowed, the toxin may affect the heart, blood pressure and breathing. It may cause paralysis, salivation, twitching and vomiting, with death through cardiac arrest being possible in severe cases, sometimes within 15 minutes.

For more information on cane toad poisoning, contact the Poisons Information Line on 13 11 26 anywhere in Australia 24 hours a day.

Potential Methods for Humane Killing of Cane Toads

Commercial Sprays (Acceptable with reservations)

There is now a commercially available product for the humane killing of cane toads that is designed for use by untrained personnel. "HopStop" is available in an aerosol can and can be purchased at most hardware stores in affected areas. While the price of such products may be an impediment to their large scale use, they do offer a reasonable alternative which does not require handling live toads. The manufacturers recommend that each toad should receive at least one 2-3 second blast of spray; however, there are reports of some (particularly larger – i.e 10⁺cm long) toads becoming quite distressed after such treatment. This may indicate a problem with under-dosing, so users should administer two or more doses of spray (as required) to ensure larger toads in particular do not suffer. Hopstop spray is made up of a combination of alcohols (ethanol and isopropanol) which serve as both solvents for the active euthanasia agent and initial intoxicants leading to immobilization while the active (lethal) ingredient (chloroxylenol) takes effect. Toads should lose consciousness in 1 minute or less and then die while unconscious. This usually takes around 45 minutes, although may take longer so the manufacturers recommend leaving the sprayed toad(s) for at least 1 - 2 hours before disposal.

Refrigeration followed by freezing (Acceptable with reservations)

Unlike most mammals and birds whose metabolism rises to maintain body temperature, reptiles and amphibians are not able to do this. When a cane toad's core temperature falls below 20°C there is a substantial drop in heart rate and when it gets down to around 8°C its natural response is to fall asleep. Once the toad's core body temperature has reached 8°C or below it can then be placed in the freezer where its body temperature will continue to drop and it will die without regaining consciousness.

The size of the toad will influence the time required for body temperature to fall below 8°C as will the number of toads put together in one container. To ensure the toad is given sufficient time to become unconscious, the container with the toad(s) inside must be kept in the refrigerator for an extended period (at least overnight as a minimum). The toads should be checked to confirm they are (a) deeply unconscious and (b) totally unresponsive, before the container is transferred to a freezer for at least a further 48 - 72 hours (minimum depending on size and number of toads), to ensure death. If the toads are responsive to any extent the container must be placed back in the refrigerator for several more hours.

This method may cause technical issues associated with cell damage during the freezing process that may render it impractical for some users. This would particularly be the case where recovery of live or intact cells from any tissue or organ is associated with the use of toads.

Use of clove oil then freezing (Acceptable with reservations)

An alternative method for ensuring the toad is asleep before moving it into the freezer is to place one or two drops of clove bud oil on to the toad's skin. The natural anaesthetic present in clove bud oil is easily absorbed through the toad's skin and an average sized toad will fall asleep within one or two minutes. However, once again, it is essential that you confirm toads are deeply unconscious by testing withdrawal reflex to a toe pinch (or similar) before placing in the freezer. It is essential that toads remain in the freezer long enough to ensure death. They should remain at -20°C for at least 48 - 72 hours depending on the size and number of toads present.

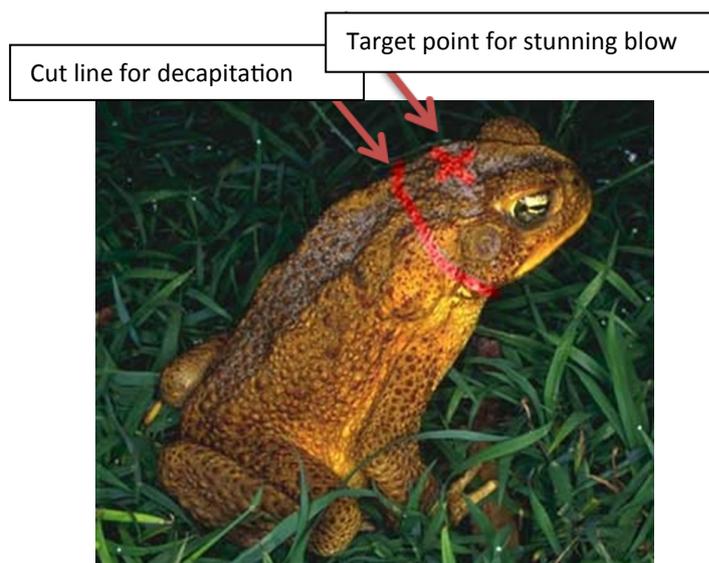
This method may cause technical issues associated with cell damage during the freezing process that may render it impractical for some users. This would

particularly be the case where recovery of live or intact cells from any tissue or organ is associated with the use of toads.

Stunning and decapitation (Acceptable with reservations)

While this method may be one of the quickest and most humane ways to kill cane toads, it is only acceptable after a required level of training. If not carried out in an effective and appropriate way, there are serious risks that the toad will not die immediately and will suffer, and the operator be exposed to toxins. It must also be acknowledged that this method is aesthetically challenging and should never be carried out in the presence of children or in situations where children may be exposed to the bodies of toads killed in this way (for example, class dissection exercises) as the animal will be grossly disfigured and there is high likelihood that toxin could be released as a direct result of the physical blow employed as a part of this method.

This method should only be employed by experienced, skilled and confident operators. It is best carried out while holding the toad by the back legs and against a solid surface using a hammer with a large head. The stunning blow should be confidently administered between and slightly behind the eyes (see diagram below) with enough force to ensure that the animal is rendered insensible before it is promptly decapitated (along the line also indicated below) using a sharp knife or cleaver.



CO₂ and Deep Burial (Acceptable with reservations)

This method has been developed by members of community action groups such as the Kimberly Cane Toad Busters and is based on their specific need to be able to humanely kill large to very large numbers of toads without the use of any physical or pharmacological agents.

The caught toads are brought to a central point where they are individually identified as cane toads and placed into a large plastic (garbage) bag contained within a bin. When there are enough toads in the bag, the neck of the bag is closed off and the bag is gently squeezed to remove most of the air in it. A hose connected via a pressure regulator to a cylinder of pure CO₂ is then pushed through the neck of the bag and it is filled with gas. Once the bag is filled, the neck is tied off and the bag is left in a quiet, shaded spot for several hours (usually at least overnight).

The bag is opened and toads checked for signs of life (the above process being repeated if there are any signs of life detected). All air is gently squeezed out of the bag and the bag tied off. This bag is then secured inside a second plastic rubbish bag before being buried under at least 1 metre of soil and covered in rocks to prevent access by predators or scavengers.

Freezing (Unacceptable)

Killing toads by freezing alone is not considered an acceptable method as there is a high likelihood of severe pain being associated with the formation of ice crystals, particularly at the extremities, which will reach temperatures low enough for this to be a problem well before core temperatures fall below 8°C - the point where toads lose consciousness.

Cane toad eradication methods

There are a number of community and conservation groups that have devoted themselves to capturing and eliminating as many cane toads as possible. Realistically, the likelihood of these groups being able to significantly reduce the number of cane toads in Australia or even in any part of Australia is very low at best. This is simply a result of the huge weight of numbers of toads, their amazing breeding efficiency and the remote nature of the lands through which they are spreading. These groups are also often hamstrung by the diverse nature of their members and frequently, their lack of technical expertise when it comes to species identification, animal handling

and more technical skills. It is therefore almost impossible to consider any of the physical methods of humane killing as being appropriate as they require not only the technical skills but also the confidence to carry them out proficiently. There are also some fundamental psychological issues that must be considered when young people are involved, so the social implications of employing physical methods of humane killing must also be borne in mind.

Working through all these considerations leaves us with a potentially surprising option that is potentially the most effective way of limiting the expansion of numbers and the simplest and least offensive method. This would be the harvesting of cane toad spawn and leaving it out in the sun to desiccate. Bearing in mind that each breeding can produce up to 30,000 plus tadpoles and eventually toads, removing the strands of toad spawn from waterways (see photo below) can seriously limit their ability to proliferate at such a rapid rate.



Cane toad spawn

Eggs of the cane toad are laid in jelly strands and, for this reason, are easily distinguished from the eggs of native frog species. Removing toad eggs from the water and leaving them to dry in the sun can help reduce toad numbers, provided this is done on a regular basis.

Remember cane toad eggs are also poisonous so please take care to observe all safety precautions below.

There are a number of ways to control toads in your own backyard or neighbourhood:

1. Cane toad spawn is found in long strands. Remove eggs if you find them. This is best done during the day. Toad eggs are laid in strings of jelly and are easily distinguished from the eggs of native frogs which are laid in globular blobs (not strings). As Australia has no native toads, it's a safe bet that eggs laid in long strings like those shown in the photograph here are cane toad eggs. Gently lift the strands of eggs out of the water and lay them in the sun to dry. If this is done regularly after summer rains it can help to keep toad numbers down. Always wear gloves and appropriate skin protection when

Table 1: Summary of potential methods for humane killing of cane toads

Users /	HopStop	Cool & Freeze	CO ₂	Clove Oil & freezing	Clove Oil	Stun &	Aqui-S	5% MS-222	Pentobarbitone	Benzocaine
Schools	Yes	Yes	No	Yes	Potentially, but would require secondary measures	No	No	Potentially, but requires veterinary prescription	No	Now requires Veterinary prescription
Community Groups	Acceptable but scale of operation and expense may make it impractical	Acceptable but impractical	Acceptable with reservations	Acceptable but possibly impractical	Potentially, but would require secondary measures	No	No	Requires veterinary prescription	No	Now requires Veterinary prescription
Researchers	Yes	Yes, subject to application as the recovery of viable cells is unlikely.	Potentially, but would require secondary measures to confirm death	Yes, subject to application as the recovery of viable cells is unlikely.	Potentially, but would require secondary measures	Acceptable with reservations	Not acceptable at this stage. Properly constructed dose – response studies would be required to determine if there is a safe and effective way to use this agent	Yes	No longer commercially available in the anaesthetic form that might be acceptable	Now requires Veterinary prescription

Freshly prepared Benzocaine (at least 7.5%) gel appears to be humane but is no longer available over the counter. The alternatives such as Xylocaine appear to be much less effective, so Xylocaine does not seem to be a practical solution.

to keep toad numbers down. Always wear gloves and appropriate skin protection when handling toad eggs as cane toads are toxic at all stages of their life cycle: eggs, young toadlets and adults.

2. Young and adult toads can be collected and humanely euthanised by first sending to sleep (in the fridge or via the use of clove oil) and then placing them in the freezer for 48 hours. Details of this method are described above. The best time to catch toads is in the evening or at night. While children may enjoy the fun of searching after dark with flashlights, they must be supervised at all times by a responsible adult as cane toads are toxic.

Methods not considered humane

Spraying with Dettol or placing in a Dettol solution appears to cause significant pain / distress as the toads become agitated, have an increased heart rate, exhibit avoidance of the solution, flick limbs, and may display gastric eversion, secretion of toxin and redness of the skin.

Aqui-S, although an anaesthetic agent, appears to cause distress similar to that associated with Dettol. However, no well-constructed dose response studies have been reported to date.

Methods not considered reliable enough to kill toads

Clove oil alone is not effective enough on all toads even at high concentrations. Toads will generally recover consciousness unless secondary measures are adopted to ensure a humane death.

Ethanol (methylated spirits) does work as an anaesthetic in some amphibian species but trials with cane toads show that it does not reliably kill them. So again, if the use of ethanol is being considered, it is essential that secondary measures are also employed to ensure a humane death.

Areas that clearly require further research

There are disparate views reported in the literature on the use of some standard aquatic anaesthetic agents and their effects on and / or efficacy in cane toads. Unfortunately this includes agents such as Aqui-S (which is often cited as the preferred agent to use in a wide variety of aquatic species) as there have been reports describing its use as both

effective in cane toads and a source of clear distress and gastric evulsion. The biggest problem when it comes to the logical evaluation of such reports is the serious lack of important detail contained in many of them. Essential details such as dose rate and/or concentration of agent administered need to be considered as part of comparative evaluation, so if they are not provided, the report is essentially useless. We would therefore suggest that the testing of such agents be undertaken as a matter of priority and include properly constructed dose response trials.

Another agent that really should be trialled in properly constructed dose response tests would be clove oil. Again, this has been reported to be a very safe and extremely effective anaesthetic agent in cane toads, although there seems to be a dearth of information about the effective dose(s) employed. Equally, there are serious concerns raised about its use as a humane killing agent by other investigators who have suggested that it is unlikely to actually kill toads. This shortage of detail available regarding the appropriateness of clove oil as a euthanasia agent rather than just as an anaesthetic means that we cannot recommend its use as a sole agent in this context. We would be particularly keen to see these trials conducted as soon as possible as the ready 'over-the-counter' availability of clove oil and the ease of its use, safety and lack of environmental concerns, means that it could be an ideal agent for broader use. Equally, if these tests show that it is not suitable for use as a humane killing agent for cane toads, then that would also be a valuable outcome.

As this article will be of special interest to those working with cane toads, it will be published by ANZCCART as a separate Fact Sheet, available from the ANZCCART website:

<https://www.adelaide.edu.au/ANZCCART/publications/fact-sheets/>

Updated Principles and Guidelines for the Care and Use of Non-Human Primates for Scientific Purposes

The NHMRC have just released their newly revised and updated guidelines document *Principles and Guidelines for the Care and Use of Non-Human Primates for Scientific Purposes* which provide up-to-date principles and best practice guidance on the care and use of non-human primates, and are intended for use by investigators, animal carers and members of institutional animal ethics committees.

In announcing this release, the CEO of NHMRC Professor Anne Kelso AO said she was very pleased to be able to do so because the use of non-human primates for scientific purposes raises special ethical and welfare issues, and the updated principles and guidelines issue specific guidance on their care and use.

The Guidelines are a revision of NHMRC's earlier document: *Policy on the care and use of non-human primates for scientific purposes (2003)*. The Guidelines are part of the strong framework that exists in Australia for the regulation and oversight of the care and use of non-human primates for scientific purposes. This framework includes controls through Commonwealth, and state and territory legislation, and the requirement for approval from an institutional animal ethics committee before any work begins. The Guidelines support the implementation of the *Australian code for the care and use of animals for scientific purposes* (the Code), which is adopted in all state and territory legislation.

The development of the Guidelines was overseen by NHMRC's Animal Welfare Committee, with advice from a working committee comprising members with expertise in veterinary science, ethics, animal welfare and the care of non-human primates, as well as community representatives.

A copy of the Guidelines is available from the [NHMRC website](#).

Victorian Animal Welfare Under Review

The Victorian Government is currently developing a five-year action plan for animal welfare in the State that they hope will help them revise their animal welfare legislation and engage the public in the process through consultation.

They have identified three priority action areas to improve animal welfare in Victoria. These are:

1. To develop new, contemporary laws for animal welfare in consultation with Victorian stakeholders and the community.
2. To develop collaborative approaches that underpin knowledge, commitment and investment in animal welfare.
3. To ensure compliance and enforcement is efficient and effective.

Having now produced a draft five-year action plan (2016 – 2021), the Victorian government is now asking for comment and the plan can be found at:

<https://animalwelfarevic.economicdevelopment.vic.gov.au/about-the-action-plan>

ANZCCART is on the move

ANZCCART has been hosted by the University of Adelaide since ACCART (as it was then) moved from the offices of CSIRO in Canberra in 1992. The decision to move to the University of Adelaide was a result of a competitive tender process with the University of Adelaide putting forward the most compelling case to host ACCART and we have enjoyed what has hopefully been a mutually beneficial and close working relationship with the University ever since.

When the move to Adelaide occurred, ACCART set up offices at the Waite campus of the University in the Davies Building, which at that stage was also the home of the University's Department of Animal Science and we remained co-located with that Department until they moved to the Roseworthy Campus of the University in June 2000. At the same time ANZCCART also moved, but we moved into offices within the Department of Environmental

Biology, located within the Darling building on the University's main North Terrace Campus and we remained there until the Department grew to a point where they required the space that ANZCCART was occupying in 2003 and so ANZCCART moved into the basement of the Mitchell Building in March 2003, where we were co-located with the University's Animal Welfare Officer. However, changes in University governance and the system of reporting research output as a part of receiving government funding meant that four years later, we were on the move again.

We have been located in Building One - The Office Block at the University's Research Park - Thebarton Campus since March 2007, but the University's decision to sell the Thebarton Campus will mean that we will need to move prior to the finalization of that sale at the end of November.

Thanks to the generosity of the University of Adelaide and their ongoing support, ANZCCART will be returning to our 'ancestral home' in Adelaide with a move back to the Waite Campus. Current plans are for this move to take place in mid-October and we will put a note on the web site to let everyone know about our new offices in the Hannaford Building.

The current plans are that our email address, mailing address (C/o The University of Adelaide, South Australia, 5005) and telephone numbers will all remain the same, so the move should be fairly seamless. However there will obviously be some down time associated with the move that will mean slower than usual response times and possibly a day or two where we cannot be reached by phone while the necessary changes are made. Please accept our apologies for any inconvenience that may result from this move, but with a lot of help from the University, we are endeavouring to make it as quick and efficient as possible.

Recent Articles of Interest

John Hopkins Ends Use of Live Animals to Train Med Students

The John Hopkins School of Medicine in Baltimore will no longer use live animals to train its medical students. The controversial course which involved students operating on live anaesthetized pigs was well attended and popular with students. After reviewing their medical course, the School found that the procedure was not essential and it was time for a change. Read the full article at:

<http://www.nbcnews.com/news/education/johns-hopkins-end-use-live-animals-train-med-students-n576481>

Animal-based antibodies: Obsolete

Animal-Friendly Affinity reagents (AFAs) are antibodies which are generated through technology in viruses or yeasts and it is thanks to these AFAs that animals are no longer required for their antibody production. As well as the animal welfare implications, AFAs have wide-ranging applicability, are commercially available and researchers have greater control over the anti-body properties, generation time and cost. Despite the increased techniques in producing these AFAs, the production of antibodies using animals still continues. Read about the actions proposed to put an end to this needless animal use.

http://science.sciencemag.org/content/353/6298/452.2?utm_campaign=toc_sci-mag_2016-07-28&et rid=33788102&et cid=671354

Spain Joins the Bandwagon for "Openness" About Animal Research

The Confederation of Spanish Scientific Societies (COSCE) recently launched a set of standards on how research organisations in Spain should be more transparent with the public about their use of laboratory animals. The scientific community has joined the growing movement for "openness" across Europe with ninety research centres, universities, scientific societies and companies in Spain now adopting these standards.

Read more at:

<http://www.sciencemag.org/news/2016/09/spain-joins-bandwagon-openness-about-animal-research>

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The opinions expressed in *ANZCCART NEWS* are not necessarily those held by ANZCCART.

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