

THE UNIVERSITY OF NEW ENGLAND ANIMAL ETHICS COMMITTEE (AEC)

STANDARD OPERATING PROCEDURES FORM (For Native Fauna/Wildlife & Laboratory Animals)

Title of Procedure: Euthanasia with Benzocaine – W22

Objective:

This document outlines the procedure related to the ethical euthanasia of amphibians using the chemical benzocaine.

Details of Procedures:

1. Place a 182 mg/kg benzocaine through application of a 2 cm × 1 mm strip (wet weight, 100 mg) of 20% benzocaine gel on the ventral abdomen of each animal (Figure 1). Observed until death results and no reflex is observed. Reflex of amphibians includes response to corneal stimuli (lightly touching eye and observing a blink) and toe pinch. In tadpole, reflexes can occur from touching the side of their abdomen and observing movement.
2. Verify the animal is dead by performing a physical method of euthanasia (pith, decapitate) or by assuring the heart has stopped beating.
3. Safely discard all solutions after use.

This procedure takes a maximum of ten minutes.



Figure 1. An adult female South African clawed frog (*Xenopus laevis*) with 1 cm × 1 mm ventral application of 20% benzocaine gel.

Drug, Chemicals or Biological Agents:

- Read full MSDS for benzocaine before purchasing or using
- **Benzocaine cannot be washed down the drain.** When making considerations for the purchase of benzocaine, be aware that waste solutions and empty benzocaine containers need to be disposed of through a chemical disposal company.
- Benzocaine is considered an irritant to humans
- Nitrile gloves and a lab coat should be worn when handling benzocaine or animals exposed to benzocaine.

Care of Animals after the Procedure:

Amphibians being euthanized must be treated with the same care and diligence as amphibians not being euthanized. If possible, conducting the euthanasia procedure in their enclosure or natural habitat is best, to keep animal calm, and within a comfortable temperature range.

Investigators must make an ethical decision weighing up the likely suffering the animal may experience by euthanasia against the suffering the animal would experience if no action is taken.

Qualifications, Experience, Skills or Training Necessary to Perform this Procedure:

Persons carrying out procedure must be trained in the handling and care of the animal species in question.

To be deemed competent to carry out procedure, they must first be shown the procedure by a capable person, and then observed conducting the procedure themselves.

They must also be shown how to assess when death has been reached by an amphibian, and be confident to do so.

The procedure will result in death. Therefore, euthanasia needs to be done for a valid reason. *Animals being used for scientific purposes may need to be killed humanely for the following reasons:*

- *The experimental endpoint is reached and the animals cannot be rehomed/rehoused, returned to normal husbandry conditions, released or reused.*
- *Vital tissues must be collected for analysis or post-mortem analyses require.*
- *An animal has come to the end of its breeding or experimental life and cannot be rehomed or released.*
- *Animals are bred in surplus to requirements or are not suitable for use in projects and cannot be rehomed or released.*
- *An adverse event has occurred or humane endpoint reached and an animal's health and wellbeing is challenged to an extent that it is not in the animal's best interest to be treated and kept alive.*
- *The animal is classed as a pest or feral animal species and so if captured must not be released back into the environment.*
- *It is a condition of a permit issued by an external agency that the animal is not released into wild and the animal cannot continue to be held or rehomed."*

Effects of Procedure on Wellbeing of Animals:

The procedure is used for anaesthetic in low doses and euthanasia in high doses. A rare side effect, benzocaine can cause methemoglobinemia in several laboratory species when used topically or systemically.

Pain Relief Measures:

Animals should be handled with care and in a mild temperature (5°C – 25°C), not exposed to excessive sunlight. Benzocaine is an anaesthetic and therefore no pain should be associated with this procedure. Death is indicated by a lack of reflex. Reflex of amphibians includes response to corneal stimuli (lightly touching eye and observing a blink) and toe pinch.

References:

Torreilles, Stéphanie L., Diane E. McClure, and Sherril L. Green. "Evaluation and refinement of euthanasia methods for *Xenopus laevis*." *Journal of the American Association for Laboratory Animal Science* 48.5 (2009): 512-516.

Prepared by: Deborah Bower July 2019

Reviewed by: Rebecca Webb July 2019

Approved by: UNE AEC July 2019