THE UNIVERSITY OF NEW ENGLAND ANIMAL ETHICS COMMITTEE (AEC)

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

Title of Procedure: Euthanasia with MS-222 – W23

Objective:

This document outlines the procedure related to the ethical euthanasia of amphibians using the chemical MS-222 (Tricaine Methanosulfonate).

Details of Procedures:

1. Create a solution of MS-222 at a concentration of 3g/L in tapwater. MS-222 is acidic in solution and must be buffered by adding an equal weight of sodium bicarbonate or titrating to pH=7.0-7.5 P (Conroy 2009)
2. Place the animal in enough buffered solution that the ventral surface of the amphibian is covered. Immerse until death results and no reflex is observed. Reflex of amphibians includes response to cornial 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.
3. Verify the animal is dead by performing a physical method of euthanasia (pith, decapitate or freeze) or by assuring the heart has stopped beating.
4. Safely discard all solutions after use. If discarding amphibian body, take into account the method of carcass disposal to ensure that the MS-222 used doesn't enter the food chain or affect the environment. A wet lab waste bin should be identified prior to disposal.

This procedure takes a maximum of ten minutes.

Drug, Chemicals or Biological Agents:

- Read full SDS for MS-222 before purchasing or using
- **MS-222 cannot be washed down the drain.** When making considerations for the purchase of MS-222, be aware that waste solutions and empty MS-222 containers need to be disposed of through a chemical disposal company.
- MS-222 is considered an irritant to humans – all handling of MS-222 in powder form should take place in a fume hood (alternatively, a dusk mask and safety goggles can be used).
- Nitrile gloves and a lab coat should be worn when handling MS-222 powder/solution or animals exposed to MS-222.

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.
When carrying out emergency euthanasia in the field, investigators may need to use a method of euthanasia that would be considered unacceptable in

other circumstances. 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 a amphibian, and be confident to do so.

Effects of Procedure on Wellbeing of Animals:
The procedure will result in death. Therefore, euthanasia needs to be done for a valid reason. Animals b*eing 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."*

Pain Relief Measures:
Animals should be handled with care and in a mild temperature (5°C – 25°C), not exposed to excessive sunlight. MS-222 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:
Conroy, C. J., Papenfuss, T., Parker, J., & Hahn, N. E. (2009). Use of tricaine methanesulfonate (MS222) for euthanasia of reptiles. *Journal of the American Association for Laboratory Animal Science*, *48*(1), 28-32.

Cornell University, 2017 "Fish and Amphibian Euthanasia", Document ACUP306.02.

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