

**Research Animal Standard Operating Procedures (SOP) must meet the following criteria:**

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

<b>Name of Procedure</b>	Euthanasia of frogs and tadpoles	
<b>Species</b>	All species of frogs and tadpoles	
<b>ACEC</b>	<b>Reference</b>	SOP#92 – Sep23 - Euthanasia of frogs and tadpoles
	<b>Author</b>	Michael Mahony
	<b>Version</b>	1.2
	<b>Date approved</b>	10 September 2023
	<b>Date for review</b>	10 September 2026
	<b>Procedure classification</b> 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	2
<b>Ethical considerations</b>	1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.	

## Purpose

It may be necessary to euthanase a frog or tadpole for a number of reasons; some are for an experimental procedure; for the collection of a voucher specimen; at the end of an experiment; in the case of a sick or injured individual.

## Description of procedure

This SOP outlines methods for the euthanasia of frogs and tadpoles.

- the frog or tadpole is transferred into an aqueous solution of MS222 0.4% solution; 4g/L), buffered with 0.4 g NaHCO<sub>3</sub> per 100 ml (0.4%).

- for a frog the MS222 solution should be placed in a suitably sized sealable plastic container that prevents the frog from moving about. The solution should cover the bottom of the container and be deep enough to come up to the elbow of the frog when sitting. It should not be deeper than the nostril, although there is no reason to believe that if the fluid was to enter the mouth or nostril that it would cause adverse effects.

- Place a transparent lid on the container.

- observe the frog continuously during the process of sitting in the solution.

- After a period of two minutes test the frog to see whether its eyelid reflexes are being affected (tap gently with the blunt end of a pencil on the upper skin above the eye. If the anaesthetic is taking effect the nictitating membrane will not move up and the eye will be retracted as is normal).

- Repeat the above eyelid reflex procedure every two minutes.

- The frog should be euthanized after about 10 minutes, but there is no reason to remove it from the solution until 20 minutes. At this time when the frog is removed test again for eyelid reflex.

For a Tadpole the MS222 solution should be placed in a glass beaker and be deep enough for the tadpole to swim around.

- observe the tadpole continuously while it is in the solution.

- After a period of two minutes test the tadpole to see whether its reflexes are being affected (inability to move)

- The tadpole should be tested for loss of movement every two minutes.

- The tadpole should be euthanized after about 10 minutes, but there is no reason to remove it from the solution until 20 minutes. At this time turn the tadpole to observe the ventral pectoral surface and under a binocular microscope observe the heart to see that it is not beating.

**MS 222** (synonyms: Tricaine mesilate, tricaine methanesulfonate, m-Aminobenzoic acid ethyl ester methansulfonate)

Molecular formula C<sub>9</sub>H<sub>11</sub>O<sub>2</sub>N + CH<sub>3</sub>SO<sub>3</sub>H

Molecular weight 261.3

Appearance: Fine, white, odourless crystalline powder

Solubility: Freely soluble in water (20°C) dissolving equally readily in sea water, spring water and tap water.

Free from Chlorides, sulphates, alkaloids and heavy metals.

Storage Store in a dry place, below 25°C and protect from light.

Stability The dry powder is stable for up to 5 years. A stock solution (10g MS 222/litre water) can be made if desired. It is stable for at least a month when stored cool in a dark or opaque bottle.

### Pharmacology

MS 222 is rapidly absorbed through the gills. Its mode of action is by preventing the generation and conduction of nerve impulses. It has direct actions on the central nervous system, cardiovascular system, neuromuscular junctions and ganglion synapses.

MS 222 is rapidly metabolised primarily by the liver but also in the kidneys, blood and muscle. Conjugation and hydrolysis are the main metabolic pathways. The primary metabolites are the acetyl-conjugates of ethyl *m*-aminobenzoate (non polar) and *m*-benzoic acid (polar). When MS 222 dissolves in fresh water the sulphonate radical hydrolyses and can reduce the pH of the anaesthesia system to a limited degree.

MS 222 and its non-polar metabolites are excreted through the gills in fish. Polar metabolites are excreted through the kidneys.

MS 222 like all fish and amniote anaesthetic agents has a dose dependent effect which varies with

species as well as individual variations.

Lower doses of MS 222 induce tranquillisation and sedation (useful for minor procedures) while higher doses lead to full general anaesthesia levels (used for example with surgical interventions). Higher doses are used for euthanasia.

### References

Ross L.G. and Ross B.R. (1999) Anaesthetic and Sedative Techniques for Aquatic Animals  
Blackwell Science. Oxford.

AVMA Guidelines for the Euthanasia of Animals 2020.

### ACEC Chair

