

STANDARD OPERATING PROCEDURES**BUACC-SOP-01 Fish Anaesthetic & Euthanasia**

The present SOP has been developed to outline proper anaesthetic and euthanasia technique for fish species.

1. Materials

1. Eugenol (98%, ex. Sigma-Aldrich, preferred); Water, MS-222™

A. Considerations

1. The use of eugenol should be the first option considered in the anaesthesia and euthanasia of fish, as it is far less toxic than MS-222. Its use is very similar to the use of MS-222. All individuals manipulating eugenol or fish exposed to eugenol should wear protective clothing, gloves, and goggles. Concentrated stock solution should be prepared inside a fume hood, and work areas should be protected from spills by placing an absorbent pad with absorbent material facing up. Stock solution may be diluted as required.
2. Tricane methanesulphonate (MS-222TM) is one of the few anaesthetic registered for veterinary use on fish in Canada and has a demonstrated records as an effective fish anaesthetic. MS-222TM will be the only anaesthetic used;
3. MS-222 TM is produced as a white powder that easily dissolves in water and is absorbed through the fish's gills;
4. MS-222 TM tends to lower the pH of the receiving water therefore an equal mass of sodium bicarbonate should be added to the water to buffer this effect;
5. The long-term health effects of MS-222TM on humans are not well understood. Thus, all personnel working with this anaesthetic will wear protective gear (e.g., nitrile gloves) and avoid skin contact;
6. MS-222TM should not be disposed of in the field. It should be capped and returned to the laboratory for disposal.

B. Dosage

1. Clove oil (eugenol) – 98% solution (ex. Sigma-Aldrich) dissolved 1:9 in 95% ethanol for 100mg/mL stock solution. Dose: 55mg/L clove oil (eugenol) in water (dose ranges from 40-100mg/L depending on the fish species, water temperature and anesthetic depth required).
2. The concentration of MS-222TM required to achieve a desired effect varies with the species and size of fish, and the temperature and chemistry of the water;
3. Dosage guidelines for a 1 kg rainbow trout at 15 °C are as follows:
 - I. 60 mg L⁻¹ to induce deep anesthesia;
 - II. 30 mg L⁻¹ for maintenance anesthesia during surgery;
 - III. 250 mg L⁻¹ for euthanasia.

C. Water

1. All water tanks, aquaria, or holding tanks used during transporting, anesthesia and recovery should be well aerated and/or circulated;
2. Water for holding tanks or anaesthetic chambers should be drawn from the environment where the fish most recently lived prior to being used in the experiment;
3. If water from another source must be used, water quality parameters (e.g., buffered, temperature, pH, ammonia) should be modified to approximate those of the fish's natural environment;
4. Water temperature should be maintained at the species normal optimum during both anesthesia and recovery;
5. Anaesthetic and recovery chambers should be of adequate volume to accommodate the fish size of interest.

2. Anesthesia and Euthanasia Procedures

A. Fasting

1. Whenever possible, fish should be fasted for 12-24 hours prior to anaesthesia to reduce the risk of regurgitation of food that may lodge in the gills or foul the water;
2. Fasting also decreases fecal contamination of water used for anaesthesia and recovery.

B. Anesthesia - The following criteria should be used to assess the effect of the anaesthetic

1. When a fish reaches the depth of anaesthesia sufficient to perform surgery, there is a total loss of equilibrium and muscle tone, decreased respiratory rate (as determined by the rate of opercular movement) and no response to stimuli;
2. A firm squeeze at the base of the caudal fin should elicit no response;
3. Gill color should be dark pink to light red;
4. If respirations become extremely slow or stop, the fish may be placed in anaesthetic-free recovery water until respirations resume.

C. Euthanasia

1. The fish will be placed in a container of adequate volume to accommodate it;
2. Water for the holding tank will be drawn from fish's point of origin;
3. With clove oil (eugenol): Mix 1-3 mL clove oil in 10 mL ethanol; mix 10mL of this solution to 1L water; immerse fish until loss of equilibrium and gill vents stop; follow by a physical method to cause brain death (see below);
4. With MS-222: water quality parameters will be modified to approximate those of the fish's natural environment, with MS-222 this involves adding sodium bicarbonate to the water bath to buffer water to a pH 7-7.5;
5. The appropriate Personal Protective Equipment (PPE) will be worn while handling MS-222, the fish, and any waste produced:
 - I. Nitrile gloves

- II. Chemical resistant apron
- III. Safety glasses
- 6. MS-222 will be added to the water, amount varies with size and species of fish but guidelines for euthanasia of a 1kg rainbow trout at 15 °C are 250 mg L⁻¹;
- 7. The fish will be placed in the anaesthetic solution until overdose endpoints are observed. These will be determined by waiting ten minutes following loss of rhythmic operculum movements;
- 8. For fish too large to immerse in the solution, concentrated MS-222 will be poured over the fishes operculum until endpoints are reached;
- 9. The fish will be decapitated after endpoints have been reached;
- 10. The carcass will be disposed / preserved of through federal, provincial, or departmental guidelines when applicable;
- 11. MS-222 waste will be transported back to Bishop's University via a sealable watertight container and disposed of via the departments chemical disposal system;
- 12. In areas or situations where the use of MS-222 is not safe a viable option physical methods may be deemed appropriate by the Principal Investigator.

D. Euthanasia – physical methods

- 1. The fish will be detained by an experienced assistant with the head facing away from the assistant;
- 2. The euthanasiast will strike the fish, using a priest/club, on top of the head just behind the eyes;
- 3. They will then create an incision between the skull and first vertebra, exposing the spinal cord;
- 4. Then inserting a sharp probe into the brain they will move it in a circular motion to destroy the brain tissue;
- 5. And push the sharp probe down the spinal column to destroy the nerve tissue;
- 6. The carcass will be disposed of through federal, provincial, or departmental guidelines when applicable and the instruments sterilized using the departmental autoclave.

3. Safety

A person who is or may be pregnant or who may have a compromised immune system should contact Human Resources **prior** to handling animals, cleaning habitat/aquaria, and/or participating in field studies where they may come into direct contact with animals. A person who is or may be pregnant or who may be immuno compromised will **not** be required to handle animals or come into contact with untreated animal waste/habitat bedding.

4. SOP Revision History

These Standard Operating Procedures were last revised and approved by Bishop's University's Animal Care Committee on January 14, 2019.