McGill

STANDARD OPERATING PROCEDURE #303

FISH AND AMPHIBIAN EUTHANASIA

1. PURPOSE

This Standard Operating Procedure (SOP) describes acceptable procedures for fish and amphibian euthanasia. It ensures that animals are euthanized in the most humane way possible.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff, veterinary care staff.

3. MATERIALS

3.1. Chemical euthanasia agent (e.g. sodium pentobarbital, eugenol (clove oil), tricaine methanesulfonate, benzocaine hydrochloride, or 2-phenoxyethanol)

3.2. Mechanical euthanasia tool (e.g. freezer or pithing tool)

4. PROCEDURES FOR FISH

4.1. Non-physical methods:

4.1.1. Injectable agent (sodium pentobarbital):

4.1.1.1. Inject sodium pentobarbital intravenously at a dose of 60 to 100 mg/kg body weight.

4.1.1.2. Verify the animal is dead before disposing of the carcass by monitoring for opercular movement and lack of response to sharp tail pressure. Time to effect may vary, with death occurring in up to 30 minutes.

4.1.1.3. If sodium pentobarbital does not produce death, follow the injection with a physical method of euthanasia to ensure death as per section 4.2.

4.1.2. External or topical agents:

4.1.2.1. Eugenol (clove oil):

4.1.2.1.1. Mix 1-3 ml Clove Oil in 10 ml of Ethanol

4.1.2.1.2. Mix 10 ml of this solution to 1L of water

4.1.2.1.3. Immerse fish until loss of equilibrium and gill vents stop

4.1.2.1.4. Follow by a physical method to cause brain death.

4.1.2.2. Tricaine methanesulfonate (TMS/MS222):

4.1.2.2.1. MS222 is acidic and in concentrations >500 mg/L, it should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5.

4.1.2.2.2. Tank method:

- Place fish in a solution of MS222 dissolved in water (minimum concentration of 250 mg/L) until death is achieved.
- Verify the animal is dead before disposing of the carcass by monitoring absence of opercular movement for at least 3 minutes.
- Follow by a physical method to cause brain death.

4.1.2.2.3. Alternative method.

- Remove fish from water and flush gills with a concentrated solution of MS222 (>250 mg/L).
- Follow by a physical method to cause brain death.

4.1.2.3. Benzocaine hydrochloride:

4.1.2.3.1. Place fish into a bath of benzocaine hydrochloride solution of >250 mg/L.

4.1.2.4. 2-phenoxyethanol:

4.1.2.4.1. Place fish into a bath of 2-phenoxyethanol solution at a concentration of 0.5 to 0.6mL/L or 0.3 to 0.4mg/L.

4.1.2.4.2. Follow by a physical method to cause brain death.

4.1.2.5. Rapid cooling (hypothermia):

4.1.2.5.1. This method can only be used for small (<3cm) tropical fish.

4.1.2.5.2. Prepare a tank containing approximately equal amounts of crushed ice and tank water to achieve a temperature of 2 to 4 °C.

4.1.2.5.3. Use a spawning barrier to prevent the fish from coming into direct contact with the ice.

4.1.2.5.4. Submerge the fish until opercular movement ceases. Leave the fish in the ice water bath for an additional 2 minutes minimum.

4.1.2.5.5. Follow by a physical method of euthanasia.

4.1.3. Inhalant agents:

4.1.3.1. All inhalant agents require long exposure times to achieve death.

4.1.3.2. Follow with a physical method of euthanasia after loss of consciousness.

4.1.3.3. Inhalant methods cannot be used for amphibians due to their ability to hold their breath while surviving for long periods of anoxia

4.1.3.4. Inhalant anesthesia:

4.1.3.4.1. Euthanize fish by extended induction of inhalant anesthesia.

4.2. Physical Methods:

4.2.1. Anesthesia or sedation must be applied prior to the use of physical techniques unless approved by the Facility Animal Care Committee (FACC).

4.2.2. Decapitation:

4.2.2.1. Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is quickly separated from the body rapidly and completely.

4.2.2.2. Follow decapitation with pithing.

4.2.3. Pithing:

4.2.3.1. Insert a rigid metal rod into the foramen magnum which is identified by the slight midline skin depression posterior to the eyes when the neck is flexed. Ensure that both the brain and the proximal end of the spinal cord are destroyed.

4.2.3.2. Follow pithing with decapitation.

5. PROCEDURES FOR AMPHIBIANS

5.1. Non-physical methods:

5.1.1. Injectable agent (sodium pentobarbital):

5.1.1.3. Inject sodium pentobarbital 1100 mg/kg and 141 mg/kg sodium phenytoin intracoelomically.

5.1.1.4. Verify the animal is dead before disposing of the carcass by monitoring for respiratory movement and lack of response to stimuli. Time to effect may vary, with death occurring in up to 1 hour.

5.1.1.5. If sodium pentobarbital does not produce death, follow the injection with a physical method of euthanasia to ensure death as per section 5.2.

5.1.2. External or topical agents:

5.1.2.1. Tricaine methanesulfonate (TMS/MS222):

5.1.2.1.1. MS222 is acidic and in concentrations >500 mg/L, it should be buffered with sodium bicarbonate to saturation resulting in a solution pH of 7.0 to 7.5.

5.1.2.1.2. Tank method:

- Place amphibian in a 1-5g/L solution of MS222 dissolved in water until death is achieved.
- Verify the animal is dead before disposing of the carcass by monitoring absence of respiratory movement for at least 3 minutes.
- Follow by a physical method to cause brain death.

5.1.2.2. Benzocaine hydrochloride (cutaneous application):

5.1.2.2.1. Apply a 2cm × 1mm strip of 20% benzocaine gel directly from the tube on the ventral abdomen. No special preparation of the skin is required.

5.1.2.2.2. Euthanasia may take up to 3-5 hours

5.1.2.2.3. Verify the animal is dead before disposing of the carcass by monitoring absence of respiratory movement.

5.1.2.2.4. Follow by a physical method to cause brain death.

5.2. Physical Methods:

5.2.1. Anesthesia or sedation must be applied prior to the use of physical techniques unless approved by the Facility Animal Care Committee (FACC).

5.2.2. Decapitation:

5.2.2.3. Use sharp equipment of the appropriate size for the species to be euthanized to ensure that the head is quickly separated from the body rapidly and completely.

5.2.2.4. Follow decapitation with pithing.

5.2.3. Pithing:

5.2.3.5. Insert a rigid metal rod into the foramen magnum which is identified by the slight midline skin depression posterior to the eyes when the neck is flexed. Ensure that both the brain and the proximal end of the spinal cord are destroyed.

5.2.3.6. Follow pithing with decapitation.

6. SAFETY PRACTICES

6.1. MS-222:

6.1.1. Wear protective clothing, gloves and goggles when handling the MS-222 powder.

6.1.2. Wear gloves to handle animals exposed to MS-222

6.1.3. Making MS-222 solutions:

6.1.3.1. Work inside a fume hood to prepare a concentrated stock solution by mixing an appropriate amount of MS-222 powder in a small volume of water.

6.1.3.2. Dilute the stock solution further as required.

6.1.3.3. Wear gloves and use a utensil to stir until all powder is dissolved.

6.1.4. Disposal of MS-222 waste:

6.1.4.1. MS-222 should be collected and disposed of as chemical waste. Contact the Waste Management department for details.

6.1.4.2. Do not discard MS-222 directly into sinks, drains, surface water, storm water conveyances or catch basins.

6.2. Eugenol:

6.2.1. Wear protective clothing, gloves and goggles when handling eugenol.

6.2.2. Wear gloves to handle animals exposed to eugenol.

6.2.3. Making eugenol solutions:

6.2.3.1. Work inside a fume hood to prepare a concentrated stock solution.

6.2.3.2. Work areas should be protected from spills by placing an absorbent pad with absorbent material facing up.

6.2.3.3. Dilute the stock solution further as required.

6.2.4. Disposal of eugenol waste:

6.2.4.1. Eugenol should be collected and disposed of as chemical waste. Contact the Waste Management department for details.

6.2.4.2. Do not discard eugenol directly into sinks, drains, surface water, storm water conveyances or catch basins.

6.2.5. Thoroughly wash hands after handling or administering eugenol.

7. REFERENCES

7.1. Wilson JM, Bunte RM, Carty AJ. Evaluation of Rapid Cooling and Tricaine Methanesulfonate (MS222) as Methods of Euthanasia in Zebrafish (Danio rerio). *Journal of the American Association for Laboratory Animal Science* : JAALAS 2009;48(6):785-789.

7.2. Torreilles SL, McClure DE, Green SL. Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*. *Journal of the American Association for Laboratory Animal Science : JAALAS*. 2009;48(5):512-516.

SOP REVISION HISTORY

DATE	PREVIOUS VERSION	NEW VERSION
2015.11.26	4.1.2.5 (NO TEXT)	4.1.2.5. **Rapid cooling (hypothermia):** **4.1.2.5.1 This method can only be used for small (<3cm) tropical fish.** **4.1.2.5.2. Prepare a tank containing approximately equal amounts of crushed ice and tank water to achieve a temperature of 2 to 4 °C.** **4.1.2.5.3. Use a spawning barrier to prevent the fish from coming into direct contact with the ice.** **4.1.2.5.4. Submerge the fish until opercular movement ceases. Leave the fish in the ice water bath for an additional 2 minutes minimum.**
2016.03.16	4. PROCEDURES	4. PROCEDURES **FOR FISH** 5. **PROCEDURES FOR AMPHIBIANS**
2016.03.16	6.1.4 (NO TEXT)	6.1.4. **Disposal of MS-222 waste:** **6.1.4.1. MS-222 should be collected and disposed of as chemical waste. Contact the Waste Management department for details.** **6.1.4.2. Do not discard MS-222 directly into sinks, drains, surface water, storm water conveyances or catch basins.**
2016.03.16		6.2.4. **Disposal of eugenol waste:** **6.2.4.1. Eugenol should be collected and disposed of as chemical waste. Contact the Waste Management department for details.** **6.2.4.2. Do not discard eugenol directly into sinks, drains, surface water, storm water conveyances or catch basins.**
2017.12.08	4.1.2.5.5. (NO TEXT)	4.1.2.5.5. **Follow by a physical method of euthanasia.**