STANDARD OPERATING PROCEDURE #303

FISH AND AQUATIC AMPHIBIAN EUTHANASIA

1.

5.3.

5.3.1. pH 7.0 to 7.5 hydrochloride solution of >250 mg/L.

5.3.3.

5.3.4.

5.4.

5.4.1. phenoxyethanol solution at a concentration of 0.5 to 0.6 mL/L or 0.3 to 0.5 mg/L.

5.4.2.

5.4.3.

5.5.

5.5.1.

5.5.2.

5.5.3.

5.6.

5.6.1.

5.6.2. at a dose of 5 to 20 ml/L

5.6.3.

5.6.4.

5.7.

5.7.1.

5.7.2.

5.7.2.1.

5.7.2.2.

5.7.3.

5.7.3.1.

5.7.4.

5.7.4.1.

6. PROCEDURES FOR AQUATIC AMPHIBIANS

6.1.

6.1.1.

6.1.2.

7.2.4.

7.2.5.

7.3.

7.3.1.

7.3.2.

7.3.3.

7.3.4.

8. REFERENCES

8.1.

JAALAS 2009;48(6):785

8.2. [illegible]
[illegible]

2021.09.02	5.1. Tricaine methanesulfonate (MS222): 5.1.1. MS222 is acidi~~c and in concentrations >500 mg/L, it should be buffered~~ and causes an aversive reaction in unanesthetized fish. Buffer with sodium bicarbonate to saturation resulting in a solution of pH 7.0 to 7.5. ~~Tank method~~: 5.1.2. Place fish in a solution of MS222 dissolved in w~~ater (minimum concentration of 250 mg/L)~~ at a concentration of 250-500 mg/L ~~until death is achieve~~d. 5.1.3. ~~Verify the animal is dead by monitoring absence of opercular movement for at least 3 minutes.~~ Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. 5.1.4. Follow by ~~a physical method to cause brain death~~ an adjunctive method of euthanasia such as decapitation or pithing to complete euthanasia. ~~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 de~~ath.
2021.09.02	5.2. Eugenol, isoeugenol, (clove oil): 5.2.1. Use products with standardized, known concentrations of essential oils to ensure accurate dosing. 5.2.2. ~~Mix~~ Prepare a stock solution by mixing 1-3 ml eugenol, isoeugenol, or clove oil in 10 ml of ethanol. 5.2.3. Mix 10 ml of this solution to 1L of water. 5.2.4. Immerse the fish ~~until loss of equilibrium and gill vents stop~~ for at least 10 minutes after opercular movement ceases 5.2.5. Follow by ~~a physical method to cause brain death~~ an adjunctive method of euthanasia such as decapitation, pithing, or freezing to complete euthanasia
2021.09.02	5.3. Benzocaine hydrochloride: 5.3.1. Buffer benzocaine hydrochloride solutions to a pH 7.0 to 7.5 to avoid tissue irritation. 5.3.2. ~~Place~~ Immerse fish into a bath of benzocaine hydrochloride solution of >250 mg/L. 5.3.3. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. 5.3.4. Follow by an adjunctive method of euthanasia such as decapitation, pithing, or freezing to complete euthanasia.
2021.09.02	5.4. Rapid cooling (hypothermia): 5.4.1. This method can only be used for small (<3cm) tropical fish. 5.4.2. Prepare a tank or insulated cooler containing ~~equal amounts of~~ approximately 5 parts crushed ice to 1 part tank water to achieve a temperature of 2 to 4 °C. 5.4.3. Fish should not be in direct contact with the ice in the water. Use a spawning barrier or create a depression in the ice slurry to expose the entire surface of the fish only to the chilled water, not the ice ~~to prevent the fish from coming into direct contact with the~~ ice 5.4.4. ~~Submerge~~ Immerse the fish ~~until opercular movement ceases~~ for at least 10 minutes after opercular movement ceases~~. Leave the fish in the ice water~~ bath ~~for an additional 2 minutes minimum~~. 5.4.5. Where it is difficult to visualize opercular movement, fish should be left in the ice water for at least 20 minutes after cessation of all movement to ensure death by hypoxia. 5.4.6. Follow by ~~a physical~~ an adjunctive method of euthanasia such as decapitation, pithing, or freezing to complete euthanasia.
2021.09.02	5.5. 2-phenoxyethanol: 5.5.1. Place fish into a bath of 2-phenoxyethanol solution at a concentration of 0.5 to 0.6 mL/L or 0.3 to 0.5 mg/L. 5.5.2. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. 5.5.3. Follow by ~~a physical~~ an adjunctive method ~~to cause brain death~~ of euthanasia such as decapitation, pithing, or freezing to complete euthanasia
2021.09.02	5.6. ~~Injectable agent~~ Sodium pentobarbital injection: 5.6.1. Inject sodium pentobarbital intravenously at a dose of 60 to 100 mg/kg body weight. 5.6.2. Verify the animal is dead 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. 5.6.3. Follow ~~the injection with a physical~~ by an adjunctive method of euthanasia ~~to ensure death as per section 2.4.~~ such as decapitation, pithing, or freezing to complete euthanasia.
2021.09.02	5.7. ~~Inhalant~~ agents Liquid anesthetics (isoflurane, sevoflurane) ~~All inhalant agents require long exposure times to achieve d~~eath. 5.7.1. Prepare a tank or container for euthanasia. 5.7.2. Add liquid anesthetic to the water at a dose of 5 to 20 ml/L using a syringe and needle to facilitate dispersal into the water. 5.7.3. Immerse the fish for at least 10 minutes after opercular movement ceases. 5.7.4. Follow by a physic 38Td ( )Tj -0.3 1 Tf -0.002 Tc 6.924 0 Td [(F)-8 (n)0.5 (e)--8 (n)-.5 (l)-j.4 (6)0.5 (i)-16.7 (to).2 (i)-TJ -0.003 T8 0.003 T8 [(a p14.6 3934.5- (e)