

FLSC Standard Operating Procedure for the Storage of Immersion Euthanasia Solutions

Purpose

Immersion euthanasia solutions are used to euthanize aquatic species in the animal facility. These solutions are often retained for extended periods. This SOP is to ensure the efficacy of the solutions and the safety of personnel working with them.

Procedure

1. All containers for storage of euthanasia solutions for immersion must be labeled with: name of euthanasia substance, concentration, date mixed, preparer's name or initials.
2. All containers for storage of euthanasia solutions for immersion must have a lid that seals.
3. Euthanasia solutions stored refrigerated can be retained for 6 months unless the solution becomes cloudy, yellow, or contains debris or mold. Any solutions that develop these characteristics must be discarded.
4. Diluted benzocaine must be discarded after use.
5. Euthanasia solutions stored at room temperature may be retained for 3 months unless the solution becomes cloudy, yellow, contains debris or mold. Any solutions that develop these characteristics must be discarded.
6. When large numbers of animals are to be euthanized, it is suggested that fresh solution be mixed to induce rapid death.
7. To discard used or expired solutions it is acceptable to pour into a sanitary sewer and flush with an excess of water. If in a remote location where a sewer may not be readily available, further dilute the solution with water and dump wastes on land, in a location away from water. Do not discard solutions directly into surface water, storm water conveyances or catch basins.

Precautions

1. Personal Protective Equipment (PPE) is required when handling immersion euthanasia solutions or animals that have been euthanized in the solutions. PPE required includes: nitrile gloves, safety glasses or safety glasses with a face shield, and a lab coat.
2. A face shield with safety glasses must be worn when pouring euthanasia solutions.
3. Tricaine Methane Sulfonate also known as MS-222, Tricaine, and TMS is mixed for euthanasia at a minimum concentration of 100 mg/ liter of tap water for zebrafish and up to 500 mg/liter of tap water for amphibians and larger fish.
4. MS222 is acidic should be buffered with sodium bicarbonate to a pH of 7.0–7.5.
5. 2-phenoxyethanol also known as 1-hydroxy-2-phenoxyethane and 2-PE is an aromatic oily liquid soluble in water. The lethal dose is as low as 500 µL/L, which leaves little margin for safety when using for anesthesia. Because there is a narrow margin between inductive and lethal doses, and the potential for toxic side effects and significant impact on the cardiovascular system, it is best for use with fish in non-survival procedures or for euthanasia. Avoid contact with the skin and eyes.
6. Benzocaine stock solution (10 g dissolved in 100 ml ethanol) is used at 2 – 4 ml /liter tap water for euthanasia. The stock solution is prepared according to the FLSC SOP Benzocaine Immersion Anesthesia for Frogs. The stock solution is stable indefinitely. The tap water will turn cloudy when mixed with the benzocaine stock solution and some precipitate may form on the surface. Stirring will eliminate this problem.
7. Benzocaine solution must be mixed fresh and cannot be retained after use for future euthanasia.

References

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