



University Animal Care Committee Standard Operating Procedure		
Document No: 15.3	Subject: Anesthesia and Euthanasia of Zebrafish	
Date Issued: April 7, 2016	Revision: 1	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the standard procedure for anesthesia and the humane euthanasia of zebrafish at various life stages.

1) Introduction and Definitions: The term anesthesia refers to an artificially induced insensitivity to pain. In zebrafish, a surgical plane of anesthesia is achieved when an animal has no voluntary locomotor activity (i.e. lost its righting reflex, shallow rate of opercular movement, no reflex responses such as a tail fin pinch). The term euthanasia is used to describe ending the life of an individual animal in a way that minimizes or eliminates pain and distress.

- Dpf: days post fertilization
- Zebrafish embryo: ≤ 3 dpf
- Zebrafish fry: 4-14 dpf
- Adult zebrafish: ≥ 14 dpf

2) Materials:

- Tank, or similar container, large enough to hold at least 1L of water (referred to as anaesthesia/euthanasia tank)
- System water, or water of similar water quality to that of the environment from which the fish originated
- Tricaine methanesulfonate (MS-222)
- Sodium bicarbonate (baking soda)
- Bleach solution (sodium hypochlorite 6.15%)
- Lidocaine 20 %
- Ice
- Stirring utensil
- Personal Protective Equipment (PPE)
- Body bags
- Freezer
- Small fish tank net

3) Procedures:

Anesthesia of zebrafish adults:

- Add 1L of system water to an empty anesthesia tank.
 - Add 150 mg of MS-222 and 300 mg baking soda (1:2) to anesthesia tank and mix thoroughly. Check that the pH of the solution is between 6-7.
 - Prepare recovery tank by adding 1L of system water to a second empty tank (recovery tank).
 - Use a disinfected, dry fish tank net to remove zebrafish from the home tank and place directly into anesthesia tank.
 - Monitor for loss of ability to right itself and slowing of opercular movement (typically 1-2 minutes). Pinch tail fin. Once there is no reflex response to tail fin pinch, a surgical plane of anesthesia has been achieved.
 - Recover fish by placing in recovery tank. Monitor for return of physiology and behavior. Once recovered, animal can be returned to home tank.
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Euthanasia of zebrafish embryos:

- Mix 1-part bleach to 5-parts system water in empty euthanasia tank.
- Mix with stirring utensil.
- Remove zebrafish embryos from home tank and place in euthanasia tank for at least 5 minutes.
- Collect euthanized embryos and place in plastic bag, then place plastic bag in freezer until disposal.

Euthanasia of zebrafish fry:

- Add ice and system water to empty euthanasia tank, to form a slushy (~ 5-parts ice to 1-part water, 0-4°C).
- Use a disinfected, dry fish tank net to remove zebrafish fry from home tank and place in euthanasia tank for at least 20 minutes. Check temperature of solution periodically to ensure temperature remains between 0-48°C.
- Collect euthanized fry and place in a body bag, then place the bag in freezer until disposal.

Euthanasia of zebrafish adults:

- Add 1L of system water to empty euthanasia tank.
- Add 300mg of MS-222 and 600mg baking soda (1:2) to euthanasia tank and mix thoroughly.
- Use a disinfected, dry fish tank net to remove adult zebrafish from home tank and place in euthanasia tank.
- Observe for cessation of opercular movement. 10 minutes after cessation of opercular movement, remove zebrafish from euthanasia tank, and transect the spinal cord with a scalpel blade. Place the zebrafish in a body bag and place the bag in the freezer until disposal.
- Alternatively, add 40mg of lidocaine (20mg/ml) to 100ml of system water in a small glass beaker. Place the zebrafish to be euthanized into the beaker and leave for at least 15 minutes monitoring for cessation of opercular movement. Five minutes after cessation of opercular movement, remove the zebrafish from the beaker and transect the spinal cord with a scalpel blade. Place the zebrafish in a body bag and place the bag in the freezer until disposal.

References:

- 1) Dr. Nicole Compo DVM
- 2) The Laboratory Zebrafish, Claudia Harper & Christian Lawrence, CRC Press, Boca Raton FL, 2011
- 3) Zebrafish Husbandry Course 5th International, Leading Advances in Zebrafish Husbandry, September 27-30, 2016, Bugaggiate (VA), Italy
- 4) Lidocaine Hydrochloride Compared with MS-222 for the Euthanasia of Zebrafish (*Danio rerio*), Chereen Collymore, E. Kate Banks, Patricia V. Turner, JAALAS 55 (6) 816-820, November 2016
- 5) <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3966278/>

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