

IACUC Guidelines for Zebrafish Euthanasia

Last Review: 4/2022

Revision History: 9/2020

Zebrafish ≥ 8 dpf the following methods are acceptable for euthanasia:

- Immersion in solutions of buffered MS222, buffered benzocaine, lidocaine, and quinaldine sulfate.
 - Immersion for 30 minutes after cessation of opercular movement in solutions of buffered MS222
- Rapid chilling in water of 2° to 4° C with a minimum of 10 minutes as long as the transfer from acclimatized temperatures to water associated with a 2° to 4° C ice slurry occurs rapidly with as little transfer of warmer water as possible
- Maceration
- Blunt force trauma or decapitation followed by pithing or exsanguination

Zebrafish Embryos and Larvae:

- Fry 4 to 7 dpf
 - Rapid chilling for at least 20 minutes following loss of operculum movement
- Less than 3 dpf
 - Rapid chilling in water of 2° to 4° C followed by immersion in a dilute sodium hypochlorite or calcium hypochlorite solution of 500 mg/L
 - Immersion in buffered MS222 solutions: need higher concentrations and/or longer exposure time (adjunctive methods may be needed to guarantee death)

Adjunctive Methods

- Decapitation
- Pithing
- Exsanguination
- Freezing
- Other physical or chemical methods after the fish have been rendered unconscious with an acceptable method

Unacceptable Methods

- Slow chilling or freezing of unanesthetized animals
- Anoxia
- Dessication
- Exposure to caustic chemicals
- Prolonged traumatic injury

Ensuring death

- Loss of movement, loss of reactivity to any stimulus, initial flaccidity
- More useful:
 - Cessation of rhythmic opercular activity for a minimum of 30 minutes
 - Loss of eye-roll- (vestibulo-ocular reflex, the movement of the eye when the fish is rocked from side to side)

MS222 Usage

MS222 in solution should be frozen or refrigerated in between use and should be disposed of appropriately if it discolors or is not effective. The expiration date on the label of the powder form should be followed.