IACUC Guideline
RAPID COOLING TO INDUCE EUTHANASIA OF ZEBRAFISH

The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has developed the following guideline to help the research community with appropriate euthanasia of zebrafish (Danio spp.). All investigators, laboratories, and facilities performing euthanasia of zebrafish are expected to adhere to the minimum standards addressed in this IACUC guideline. Deviations from or modifications to the policy must be requested of, and approved by, the IACUC.

The American Veterinary Medical Association (AVMA) Guidelines for Euthanasia\(^1\) state that rapid chilling (hypothermic shock) for small bodied (3.8 cm long or smaller) tropical and sub-tropical species is an acceptable method of euthanasia.

The following must be performed when using rapid-cooling to induce euthanasia of zebrafish:

1. Ice that is used to cool the euthanasia tank must not come in direct contact with the zebrafish. Direct contact may induce pain or distress. **There must be a physical barrier between the zebrafish and the ice.**
2. The water temperature in the euthanasia tank must range between 2°C to 4°C.\(^3\)
3. Zebrafish must be transferred between the holding tank and the euthanasia tank by net or other device to minimize the amount of “warm” water transferred to the chilled tank.
4. Stocking density of zebrafish in the chilled euthanasia tank may not exceed a total of 40 zebrafish per 1 L of chilled water.
5. Death must be confirmed by decapitation or by maintaining the zebrafish in the chilled water for at least 10 minutes following loss of opercular movement.
6. Euthanasia of juvenile zebrafish (4-30 days post-fertilization) requires a two-step method: (1) placement in chilled water as described above, with an additional 20 minutes after cessation of movement; followed by (2) addition of a chemical agent (5.75% Sodium Hypochlorite at a ratio of 1 part Sodium Hypochlorite to 5 parts water) to ensure death.\(^2\)

The University of Pennsylvania IACUC approves rapid cooling as a form of euthanasia for zebrafish (Danio spp.). The IACUC also continues to acknowledge and accept other methods to euthanize fish as described in the AVMA Guidelines on Euthanasia (2013).

References

APPROVED
2/9/2016