IACUC POLICY
RAPID COOLING TO INDUCE EUTHANASIA OF ZEBRAFISH

The American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (2007) states,

Cooling—It has been suggested that, when using physical methods of euthanasia in ectothermic species, cooling to 4°C will decrease metabolism and facilitate handling, but there is no evidence that whole body cooling reduces pain or is clinically efficacious...This method is not recommended [for euthanasia]. Formation of ice crystals on the skin and in tissues of an animal may cause pain or distress. Quick freezing of deeply anesthetized animals is acceptable.

The 2007 AVMA Guidelines on Euthanasia conclude that cooling to 4°C will decrease metabolism and facilitate handling of ectothermic species. This method by itself should only be used to facilitate handling, and it does not constitute an acceptable method of euthanasia since there is no evidence that it is clinically efficacious.

However, the AVMA Guidelines do not discuss cooling to 4°C as a method for euthanasia specifically for tropical species. Zebrafish (Danio rerio.) have minimal to no physiologic adaptation mechanism for acclimating to cold (4°C) water, cooling to below 4°C should be considered an acceptable method of euthanasia. Rapid cooling (immediately decreasing the temperature from approximately 26°C to 4°C) induces rapid loss of consciousness and is lethal to zebrafish.

The Principal Investigators, in consultation with a ULAR Veterinarian, may devise a strategy to best accomplish their needs considering the specific research model. This strategy must include the following considerations:

1. If ice is used to cool the chilled euthanasia tank, the zebrafish must not come in direct contact with ice. 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 1°C and 4°C.
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 forty (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 5 minutes.

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 (2007).

References

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