UNIVERSITY OF COLORADO DENVER (CU DENVER)

Subject: Zebrafish Housing and Euthanasia

Source: Institutional Animal Care and Use Committee (IACUC)
Effective Date: 10/12/2020
Replaces: N/A
Applies to: Zebrafish research/teaching/training personnel and care staff
Reference: PHS Policy on Humane Care & Use of Laboratory Animals; Guide for the Care & Use of Laboratory Animals

IACUC 10/12/2020

Introduction

The Institutional Animal Care and Use Committee (IACUC) maintains oversight of federally mandated rules and regulations of animal research for the University of Colorado Denver. Zebrafish are an important research model with unique housing and care requirements compared to terrestrial animals.

Policy Statement

This policy provides information on the housing and care of zebrafish at CU Denver. Per guidance from the National Institutes of Health (NIH), Office of Laboratory Animal Welfare (OLAW), zebrafish are regulated research animals once they have hatched, approximately 3 days post-fertilization (dpf).[1,2,3] Therefore, zebrafish are overseen by the IACUC beginning at 3 dpf. Zebrafish embryos, larvae, and adults are maintained in a centralized facility. Research labs may also house zebrafish embryos and larvae in IACUC-approved satellite facilities. The Office of Laboratory Animal Resources (OLAR) works closely with laboratory staff to ensure excellent care is provided for zebrafish at all life stages.

Zebrafish numbers: IACUC protocols must describe the use of zebrafish and justify the number of fish needed to achieve research objectives. To provide a complete narrative of animal work, experiments using zebrafish embryos < 3 dpf should be briefly summarized in the IACUC protocol, but should not be factored into the animal numbers calculations. Good faith estimations of fish ≥ 3 dpf used in research activities, including breeding, are reported to the IACUC as requested.

Satellite zebrafish facilities:

- Prior to housing ≥ 3 dpf zebrafish outside the central facility for ≥ 24 hours, researchers must submit a satellite facility application to the IACUC Office and arrange inspection of proposed housing space.
- To establish a satellite, labs must:
  - Provide adequate justification, to be reviewed and approved by the IACUC
  - Provide a temperature-controlled environment with a diurnal light cycle and all needed animal care
  - Conduct and document daily health and facility checks while ≥ 3 dpf zebrafish are present (including weekends/holidays)
- OLAR veterinarians arrange regular visits to ensure standard of care for fish ≥ 3 dpf in satellites.

Housing density: Zebrafish are a social species, and live in groups of varying sizes in the wild. As per the Guide for the Care and Use of Laboratory Animals, zebrafish should be housed in social groups.[4] Aggression between zebrafish increases with small group sizes, therefore, it is recommended to house zebrafish in groups of at least three fish.[5,6] Zebrafish can be bred in pairs or in small groups. Maximal group sizes for housing zebrafish are defined by the Zebrafish Overcrowding Policy.

Tricaine methanesulfonate (MS-222) preparation, storage, and disposal:

- Tricaine methanesulfonate (MS-222) is available as a pharmaceutical-grade compound for fish anesthesia and euthanasia. As for other species, the use of non-pharmaceutical-grade anesthetics must be justified in the IACUC protocol.
- MS-222 in its powdered form is a respiratory irritant. Researchers working with this agent should take precautions, including use of a certified fume hood, respirator, or other methods recommended by Environmental Health and Safety (EHS).
- Stock solutions of MS-222 must be appropriately labeled with the date created, and may be stored frozen for up to six months.
- Working solutions of MS-222 must be buffered to neutral pH to prevent irritation when fish are immersed in the solution. Working solutions should be stored protected from light for a maximum of 30 days. Discard and replace working solutions if precipitate or color change occurs. See the Use of Non-pharmaceutical Grade Chemicals and Compounded Pharmaceutical Grade Drugs Policy for general guidance on labeling

and storage.
- MS-222 is considered a hazardous chemical, and must not be disposed via sink. All research labs using MS-222 should collect it in a registered satellite accumulation area and dispose of all MS-222, of any concentration, through the EHS chemical waste management system.

Euthanasia:

- Acceptable methods of euthanasia are described in the AVMA Guidelines on Euthanasia[7] and must be included in all IACUC protocols.
- Adult fish euthanized by immersion methods must be monitored for spontaneous movement (e.g. opercular movement, "gilling"). Euthanasia of adults is considered complete 5-10 minutes after the last movement.[7,8,9] Carcasses of adult fish should be frozen and disposed through OLAR.
- Fish embryos and larvae are more tolerant of chemical or temperature changes and therefore require longer exposures and a secondary method to ensure euthanasia. Primary methods may include 20-30 minute immersion in high-dose buffered MS-222 or ice water bath, followed by freezing or immersion in a 1:5 bleach solution as a secondary method. Euthanized embryos and larvae must be collected for proper disposal through OLAR or EHS red bin waste. Removal of euthanized zebrafish embryos and larvae from immersion baths is facilitated by use of a fine-mesh tea strainer lined with a kimwipe or paper towel to be disposed with the carcasses through OLAR or EHS.

Exceptions to this policy will be considered by the IACUC on a case by case basis on presentation of adequate scientific justification.

Per regulatory requirements, failure to comply with this policy may result in notification of your funding agency (e.g. NIH) and regulatory agencies (e.g. USDA) that your research has violated federal and/or local policies regarding the humane use of animals. This notification may affect continuous funding of your animal-related research. Further, depending on the violation, you may be required to take additional training and/or your privilege to conduct animal research at UC Denver might be temporarily suspended or even completely revoked.

References:

1. National Institutes of Health, Office of Laboratory Animal Welfare: Public Health Service Policy on Humane Care and Use of Laboratory Animals, Frequently Asked Questions. https://olaw.nih.gov/guidance/faqs

2. Bartlett DH and Silk SB. 2016. Office of Laboratory Animal Welfare Comments. Zebrafish, vol 13(6)

3. National Institutes of Health, Office of Animal Care and Use Animal Research Advisory Committee: Guidelines for Use of Zebrafish in the NIH Intramural Research Program. Revised 2016.

4. National Research Council of the National Academies: Guide for the Care and Use of Laboratory Animals, 8th ed. 2011. The National Academies Press, Washington, DC.

5. Harper C and Lawrence C. 2011. The Laboratory Zebrafish. CRC Press.

6. Keck VA, Edgerton DS, et al. 2015. Effects of Habitat Complexity on Pair-Housed Zebrafish. J. A. Assoc Lab Anim Sci, vol 54(4)

7. Leary S, et al. 2013. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. American Veterinary Medical Association. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

8. Wilson JM, Bunte RM, and Carty AJ. 2009. Evaluation of Rapid Cooling and Tricaine Methanesulfonate (MS-222) as Methods of Euthanasia in Zebrafish (Danio rerio). J.A. Assoc Lab Anim Sci, vol 48(6)

9. Wallace CK, et al. 2018. Effectiveness of Rapid Cooling as a Method of Euthanasia for Young Zebrafish (Danio rerio). J. A. Assoc Lab Anim Sci, vol 57(1)