

POLICY REGARDING THE USE OF ZEBRAFISH

PURPOSE:

The purpose of this policy is to establish definitions of what constitutes a live, vertebrate animal when conducting research or teaching activities with zebrafish (*Danio*). Further, this policy is designed to ensure compliance with the Animal Welfare Act, Public Health Service Policy, and established best practices for zebrafish care and management.

POLICY:

DEFINITIONS:

The following definitions¹ shall be enforced when referring to the stages of zebrafish growth and development:

- Embryos
 - ≤72 hours post-fertilization; prior to hatching
- Larvae
 - 3-29 days post-fertilization
- Juveniles
 - 30-89 days post-fertilization
- Adults
 - ≥90 days post-fertilization

IACUC protocols are not required under the following circumstances:

Research or teaching protocols that explicitly and solely utilize zebrafish embryos that will not survive beyond 72 hours. In cases where an embryo unintentionally survives beyond 72 hours, the investigator must adhere to the below guidance on “resolution of unanticipated hatching.”

The IACUC must approve the use of zebrafish embryos if those animals are expected to survive beyond 72 hours. Zebrafish embryos that are manipulated before the 72-hour mark and which are appropriately euthanized before that time are not regulated by the IACUC. All euthanasia methods must be performed in accordance with current AVMA Guidelines.

IACUC protocols are required under the following circumstances:

The Liberty University IACUC must evaluate and approve all research or teaching activities in which zebrafish are allowed to develop beyond 72 hours.

- Zebrafish larvae (3-29 days post-fertilization)
- Zebrafish juveniles (30-89 days post-fertilization)
- Zebrafish adults (≥90 days post-fertilization)

RESOLUTION OF UNANTICIPATED HATCHING/DEVELOPMENT

On protocols where investigators are using zebrafish embryos, the possibility exists whereby eggs may unintentionally hatch or survive past 72 hours. In these instances, the IACUC has established the following procedures to ensure compliance with the regulations:

OPTION A: Immediate Transfer to Approved Protocol

- Should zebrafish hatch or survive past 72 hours on a protocol where such work is not approved, the larvae must be immediately transferred to an IACUC-approved protocol that covers zebrafish use beyond the embryonic stage. If no such protocol exists, the zebrafish must be euthanized (Option B).

OPTION B: Euthanasia

- Should zebrafish hatch or survive past 72 hours on a protocol where such work is not approved, and option A was not possible, the zebrafish larvae must be immediately euthanized using an AVMA approved method. These methods may include² immersion in solutions of buffered tricaine methanesulfonate (MS-222), buffered benzocaine, quinaldine sulfate, and 2-phenoxyethanol OR rapid chilling as long as transfer from acclimatized temperatures to water associated with 2 degree to 4 degree ice slurry occurs rapidly with as little transfer of warmer water as possible.

OCCUPATIONAL HEALTH CONSIDERATIONS

Working with Zebrafish may result in exposure to certain risks.

REFERENCES

1. Kimmel et al., 1995. Developmental Dynamics 203:253-310.
2. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition