

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**  
**Policy #23**  
**USE OF ZEBRAFISH FOR RESEARCH AND TEACHING**

In concurrence with  
TTUHSC El Paso Assurance #D19-01056  
and Federal Regulations and Guidelines

**Purpose**

This document is intended to assist researchers/instructors working with zebrafish in determining when Institutional Animal Care and Use Committee (IACUC) review is required. These guidelines were adopted from several sources, including the *Guidelines for Use of Zebrafish in the NIH Intramural Research Program*.

**General Information and Definitions:**<sup>3</sup>

Embryos - Fish at 0-3 days post-fertilization (dpf).

Hatchlings - The NIH Office of Laboratory Animal Welfare (OLAW) considers fish species to be "live vertebrate animals" at "hatching". Although this is an imprecise stage in zebrafish, OLAW considers zebrafish hatching to occur at 72 hours or 3 (dpf).<sup>4</sup>

Early Larval Stages - The larval stage begins after hatching and proceeds until Day 30. There is no evidence to suggest the presence of higher order cognition in zebrafish during the first week of development<sup>1</sup>. While the capacity for suffering is the primary criterion for establishing a threshold for 8 dpf for euthanasia in zebrafish, the criterion of independent feeding (and not feeding from the yolk sac, which continues through 7 dpf) also supports this age.

Older fish (larvae, juveniles and adults) ( $\geq 8$  dpf) - From Days 30-90 the fish are called "juveniles," and from Day 90 onward, they are considered as adult fish<sup>3</sup>.

Many studies have demonstrated that older zebrafish show evidence of higher order cognition, including learning to avoid aversive stimuli. Still, the ability of older fish to experience suffering remains controversial in the scientific literature. However, there is sufficient evidence to take a cautious approach in older zebrafish by instituting guidelines that ensure rapid euthanasia.

For the purposes of animal studies that need IACUC approval, the most important developmental stages are the embryo, 4–7 dpf, and 8 dpf and beyond.

**3. Protocol Procedures**

Zebrafish embryos are not considered live vertebrate animals and do not need to be included in your IACUC protocol. However, a description of their use may be necessary as part of a complete description of experimental protocols involving adult breeding zebrafish. In addition, guidelines on euthanasia must be followed for both embryos and adults.

Zebrafish early larvae ( $> 3$  dpf) are considered live vertebrate animals and must be included in the IACUC protocol, with their numbers justified. Because early stage animals (3–7 dpf) do not feel pain or distress, the researcher/instructor may check "No" for the relevant pain and distress section of the protocol application. For the IACUC approval process, applications using these early stage animals may designate the protocol as a "Category C". The pain and distress categorization of the  $\geq 8$  dpf fish should be determined according to the specific procedures performed as described in the protocol.

**4. Euthanasia Guidelines**<sup>2</sup>

A. For embryos  $\leq 3$  dpf (i.e., before hatching), development should be terminated using bleach as follows:

Addition of bleach solution (sodium hypochlorite 1 - 10%) to the culture system water at 1 part bleach to 5 parts water. The embryos should remain in this solution for at least five minutes prior to disposal to ensure death. As detailed above in the scientific background section, pain perception has not developed at this early stage so this is not considered a painful procedure.

B. For zebrafish 4–7 dpf the following methods are acceptable for euthanasia:

- a. Immobilization by submersion in ice water (5 parts ice/1 part water, 0–4° C) for at least 20 minutes following the loss of opercular movements to ensure death by hypoxia.

Euthanasia may also be accomplished using bleach as described above in item 4.A.

C. For zebrafish  $\geq 8$  dpf the following methods are acceptable for euthanasia:

Immobilization by submersion in ice water (5 parts ice/1 part water, 2°–4° C) for at least 10 minutes until loss of orientation and following cessation of opercular (i.e., gill) movement. In any fish where it is difficult to visualize opercular movement, fish should be left in the ice water for at least 20 minutes after cessation of all movement to ensure death by hypoxia.

Overdose of tricaine methane sulfonate (also known as MS 222 [CAS no. 886-86-2], an FDA-approved fish anesthetic, 200–300 mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement. 30 minutes is recommended as a precautionary measure until research is available to demonstrate immersion times needed to reliably cause irreversible death in zebrafish.

Zebrafish show some signs of stress when immersed in MS 222, and a secondary (physical) method of euthanasia is recommended to ensure death. MS 222 alone is not effective for euthanasia of zebrafish eggs, embryos, or larvae (< 14 days old), and other methods should be used for these life stages.

Anesthesia with MS 222 at 168 mg/l, followed by rapid chilling by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes.

Decapitation with a sharp blade by a trained individual when its use is required by the experimental design and approved by the IACUC. Otherwise, decapitation may ONLY be used as a second step of a 2-step procedure when fish have been rendered unconscious prior to their application by an acceptable or acceptable-with-conditions, first-step method.

D. Zebrafish carcasses should be disposed of according to standard LARC procedures.

## **Related policies**

Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

IACUC policy #0: Introduction to the IACUC policies and Procedures

IACUC policy #02: Veterinary Care

IACUC policy #04: Pain Categories for Experimental Protocols

IACUC policy #07: Designated Reviews

IACUC policy #13: Protocol Approval, Implementation, Review and Amendment Process.

IACUC policy #24: Expired Drugs

## **References**

1. Guidelines for use of zebrafish in the NIH intramural research program. Bethesda, MD: NIH, 2009. Available at: <https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/zebrafish.pdf>. Rev 06/22/2016
2. National Institutes of Health (2009) Final Report to OLAW on Euthanasia of Zebrafish.
3. Westerfield, M. 2000. The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish Danio (Brachydanio) rerio. 5th ed, Univ. of Oregon Press, Eugene. [http://zfin.org/zf\\_info/zfbook/zfbk.html](http://zfin.org/zf_info/zfbook/zfbk.html)
4. Zebrafish 2016; 13(6):1-2, OLAW Comments. [https://grants.nih.gov/grants/olaw/references/zeb\\_2016\\_1344.pdf](https://grants.nih.gov/grants/olaw/references/zeb_2016_1344.pdf)
5. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition\*