CMRC/IACUC Guidelines for Zebrafish Users
(Approved by the IACUC on August 22, 2006)

Purpose

To help provide guidance and answers to frequently asked questions related to the care and use of zebrafish. More details on standard procedures related to zebrafish may be found in *The Zebrafish Book,* also available at http://www.zfin.org.

Description of the proposed use of animal

At 7 days post fertilization, the zebrafish yolk sac is considered to be completely depleted and the organism requires additional care such as feeding to maintain its health and welfare. Based on this, all manipulations on 7 day or older fish must be fully described and approved by IACUC.

Investigators should assign fish to pain categories based on procedures performed on the fish

The following Pain Categories are suggested by the IACUC.

B – Breeding, only to maintain colony
C – Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals (e.g. collecting zebrafish embryos).
D – Procedures or tests involving the administration of appropriate anesthetic, analgesic or tranquilizer drugs to avoid pain or distress (e.g. fin clips, MS222-tricaine on adults from which sperm and eggs are squeezed)*
E – Procedures or tests that for scientific validity are performed involving pain or distress without the administration of an appropriate anesthetic, analgesic, or tranquilizer drug (e.g., chemical mutagenesis of adults: ENU)**

* In practice, MS222-tricaine anesthesia is sometimes used to facilitate the capture and handling of the fish at any stage after the embryos become motile, even though the procedures produce no or minimal discomfort. Even invasive procedures done with embryos could not produce discomfort because the neural centers are still undeveloped.

** Please note that for "E" category the investigator must provide a written justification for the procedure.

Anesthesia

MS222- tricaine is added to the water (100-200mg/l) to anesthetize the fish. Anesthesia is observed as slow gill movement and loss of equilibrium. It is the best anesthesia available for lower (aquatic "cold-blooded") vertebrates. The dosage is age dependent. Immersing the fish in the anesthetic solution will induce anesthesia and once the fish are pulled from the MS222 treated water and placed in fresh water they will fully

recover in less than 10 minutes. To maintain the fish under MS222 anesthesia for longer periods of time, the dose should be 15-100mg/l.

Standard method of euthanasia

Zebrafish should be euthanized by methods consistent with the 2000 Report of the AVMA Panel on Euthanasia.

1) Immobilization by submersion in ice water immediately followed by decapitation.
2) Overdose with MS-222 (200-300 mg/l) by prolonged immersion. Fish should be left in the solution at least 10 minutes following cessation of opercular movement. Death should be assured by decapitation.
3) Anesthesia with tricaine (MS-222 168 mg/l) followed by rapid freezing in liquid nitrogen.