



## Institutional Animal Care and Use Committee

February 20, 2008

### GUIDELINES FOR ZEBRAFISH USE

Federal animal welfare regulations apply to vertebrate animals but do not cover embryos. In the case of zebrafish, the IACUC has established 7 days post fertilization as the point at which they transition from the embryonic stage to a free-living organism. At that time the yolk sac is completely depleted and the small hatchlings become free feeding. Once feeding is necessary to maintain the health and welfare of the fish and they are considered “fully hatched” by the IACUC. Thus, an animal protocol is required for zebrafish older than 7 days post-fertilization, but not for zebrafish embryos or larva younger than this. An animal protocol is always needed for adult fish that will be used for breeding, tissue harvesting or experimental manipulations. Any areas outside the vivarium in which the investigator intends to house zebrafish are subject to approval as satellite housing areas and will require IACUC inspection.

**Pain Categories:** Investigators should assign fish to pain categories based on the type of procedures that will be performed:

- B - Breeding, only to maintain colony and produce embryos.
- C - Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals.
- D - Procedures involving the administration of appropriate anesthetic/analgesic drugs to avoid pain or distress (e.g. fin clips, surgery)
- E - Procedures involving pain or distress without the administration of an appropriate anesthetic/analgesic drug (e.g., chemical mutagenesis of adult animals ENU). *Please note that for "E" category the investigator must provide scientific justification.*

**ANESTHESIA:**

The dosage of all anesthetics is age dependent. If using a new anesthesia regimen, PI should anesthetize a few (2-5) fish and follow them through to full recovery to ensure an adequate drug dosage that is safe and provides sufficient anesthetic depth for intended procedures. If respirations become extremely slow or stop, place the fish in anesthetic-free recovery water until respirations resume. Adequate oxygenation of all water chambers should be maintained during anesthesia and recovery. Water taken from the original holding tank is optimal to use for transport, anesthetic and recovery chambers. If using another water source, duplicate the water quality parameters and temperature of the home tank as much as possible.

Agent	Dose	Comments
MS-222 (tricaine methanesulfonate)	75–125 mg/L (induction) and 50–75 mg/L (maintenance)	Sodium bicarbonate should be added to stock solution to maintain neutral pH. Only FDA approved anesthetic for fish
Benzocaine hydrochloride	25–100 mg/L	Very small margin of safety between effective and lethal doses. Sodium bicarbonate should be added to stock solution to maintain neutral pH.
2 - phenoxyethanol	1/1000 dilution (~0.3–0.4 mg/L)	



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### **Stages of anesthesia:**

<b>Stage 1: Deep sedation</b>	<b>Stage 2: Deep narcosis</b>	<b>Surgical Anesthesia</b>
Cessation of voluntary swimming; decreased response to stimuli.	Decreased muscle tone; equilibrium loss; appropriate level for fin and gill biopsies.	Slow respiration and heart rate; total loss of reactivity to stimuli.

### **EUTHANASIA:**

Zebrafish should be euthanized by methods consistent with the 2000 Report of the AVMA Panel on Euthanasia. However, the choice of method depends on the intended use of the fish after euthanasia. The following are acceptable:

1) *Chemical Methods:* A slightly more concentrated solution of an anesthetic agent is typically used for this purpose. Fish should be left in the solution for 5-10 min following the cessation of opercular movement. Investigator should verify the animal is dead before disposing of carcass.

- MS-222 (tricaine methanesulfonate) at 200-500 mg/liter (buffered with sodium bicarbonate to maintain neutral pH).
- Benzocaine hydrochloride at 250-500 mg/liter (buffered with sodium bicarbonate to maintain neutral pH).
- 2-phenoxyethanol typically 1:500 dilution (~0.6–0.8 mg/L).

2) *Physical Methods:* **Important:** Anesthesia/sedation must be applied prior to use of a physical technique unless otherwise approved by the IACUC.

- Immobilization by submersion in ice water followed by decapitation.
- Anesthesia (e.g. using MS-222) followed by rapid freezing in liquid nitrogen.