Zebralfish (*Danio rerio*)
Anesthesia, Euthanasia, Interventions

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**MS222** (tricaine methanesulfonate, Ethyl 3-aminobenzoate)

- sulfonated analog of benzocaine (makes it more hydrophilic)
- inhibits sodium ion channels
- acts systemically in fish
- analgesic, sedative and paralytic activity
  

- widely used in aquaculture, large safety margin
  (EC50 up to 50 times higher than dose for anesthesia, depends on species)

- is rapidly taken up via gills

- only approved anesthetic in USA and Germany

- use at 0.02%

- in egg or adult fish water (E3 usually for embryos)

- can reduce pH > adjust with NaOH to 7
  
  (Weidinger: 25x Tricaine stock in 20mM Tris pH 7)
Alternatives to MS-222

propofol/lidocaine combination could be used as alternative to MS-222

Anesthesia

Induction

• add fish to beaker or petridish containing MS222

• level 1: light sedation
  reaction to visual and tactile stimuli reduced

• level 2: deep sedation
  no reaction to visual and tactile stimuli, reduced opercular movement

• level 3: partial loss of equilibrium
  erratic swimming, increased opercular movement, still reaction to pressure

• level 4: loss of equilibrium
  no movement, reduced opercular movement, no reflexes
  stage for surgical interventions

• level 5: shallow opercular movement, decreased heart rate

• level 6: no opercular movement > will soon lead to death

Anesthesia

Maintenance
• it’s OK if adult fish reach stage 5 for a few minutes > all recover
• for prolonged anesthesia: perfusion (water flow through mouth over gills)

• short interventions (< 1min): fish on glass or plastic surface
• longer interventions: put fish on damp sponge

Recovery
• transfer fish to large volume of embryo or fish water
• monitor: if adult fish has not recovered (begun to swim) within 3 minutes > use transfer pipette to blow water over gills
Common surgical interventions

**Partial fin amputation**
- caudal fin is amputated at 50% of its length with scalpel
- bleeding stops within seconds
- fish behavior (swimming, feeding, mating) is not impaired
- fin regenerates within 2-3 weeks

**Intraperitoneal & retroorbital injection**

**Heart injury**
- ventricular resection
- cryoinjury

**Common features**
- wounds are small > no wound care necessary
- infections are extremely rare > no sterile environment necessary
- **Precautions:** Isolation, addition of methylene blue (suppresses fungal and bacterial growth), addition of STRESS COAT®, which forms synthetic slime coating
Humane killing

**MS222 overdose**
- 0.2% in buffered fish water
- dead when operculum movement has stopped for > 5 minutes

**Rapid cooling**
- icewater (no chunks of ice which could burn skin)
- shown to be faster & less stressful (fewer signs of distress) than MS222
  
  (J Am Assoc Lab Anim Sci. 2009 Nov;48(6):785-9.)
- illegal in EU
Resources

Zebrafish International Resource Center (ZIRC), University of Oregon
protocols for husbandry, pathology services, source for wild-type and transgenic / mutant fish lines

European Zebrafish Resource Center, Karlsruhe Institute for Technology, ezrc.kit.edu
European repository for fish lines, screening facility

Zebrafish model organism database (ZFIN). zfin.org
Info on fish lines (transgenic, mutant), research reagents (antibodies, morpholinos), genome annotation

Zebrafish husbandry organisation. zhaonline.org
Non-profit, promotes husbandry standards through education & research

European Society for Fish Models in Biology and Medicine (EuFishBioMed)
promotes collaboration and exchange between fish labs