Zebrafish (*Danio rerio*)
Anesthesia, Euthanesia, Interventions

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Anesthesia & Analgesia

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

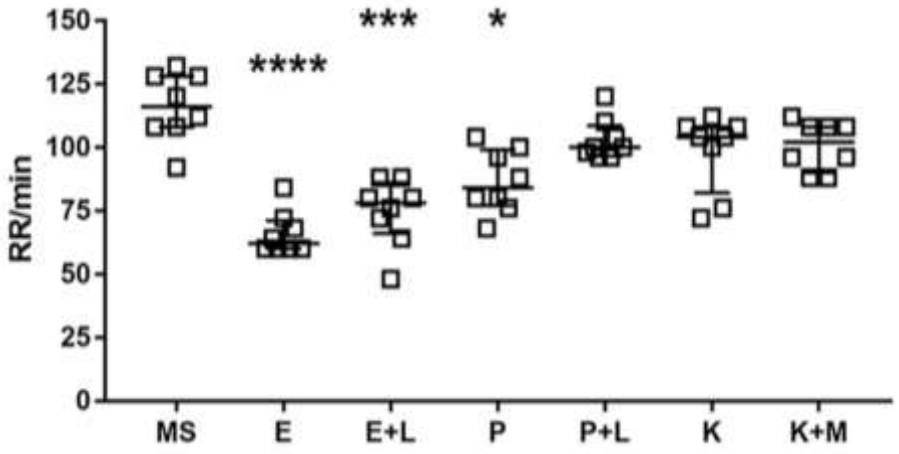
- sulfonated analog of benzocaine (makes it more hydrophilic)
- inhibits sodium ion channels
- acts systemically in fish
- analgesic, sedative and paralytic activity
  shown to block motor and sensory neurons in *Xenopus* explants. Ramlochansingh et al. PLoS One. 2014; 9(7): e101606.
- widely used in 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

Table 1. Anaesthetic protocols.

Single anaesthetic agent	Combination of anaesthetic agents
100 μg/mL MS-222 (MS)	2 μg/mL E + 100 μg/mL Lidocaine (L)
2 μg/mL Etomidate (E)	1.25 μg/mL P + 100 μg/mL L
1.25 μg/mL Propofol (P)	100 μg/mL K + 1.25 μg/mL Medetomidine (M)
100 μg/mL Ketamine (K)	100 μg/mL K + 1.25 μg/mL M / 3.125 μg/mL Atipamezole (A)



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

Martins et al. (2018) PLoS ONE 13(5): e0197846.

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

Matthews, M. & Varga, Z. M. Anesthesia and euthanasia in zebrafish. *ILAR journal / National Research Council, Institute of Laboratory Animal Resources* **53**, 192-204, (2012).

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

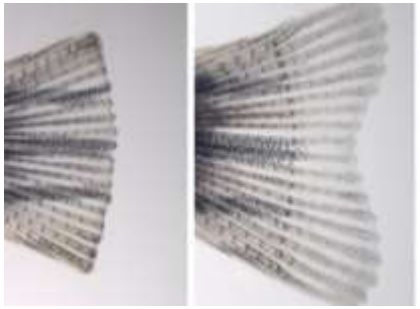
Recovery

- transfer fish to large volume of embryo or fish water
- monitor: if adult fish has not recovered (begun to swim) within 3 minutes > use tranfer 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