

## STANDARD OPERATING PROCEDURES

**SOP No:** W-3

**SUBJECT:** Euthanasia of Fish.

**POLICY:**

1. The procedure should be compatible with the scientific or educational aims of the activity<sup>1</sup>.
2. Practices must ensure rapid loss of consciousness<sup>1</sup>.
3. A two step euthanasia process should be used, especially for temperate/cold or low oxygen adapted species<sup>2,3</sup>.
4. Chilling alone is not acceptable for large and cold-adapted species<sup>3</sup>.
5. Zebrafish and other small-bodied fish (e.g. < 4 cm total length) may be euthanised by rapid chilling followed by an adjunctive method<sup>2,3</sup>.
6. Captive bolt stunning, manual blunt force trauma and pithing require specialist equipment and training<sup>2</sup>.

**PRECAUTIONS:** Operator safety.

**EQUIPMENT:** Recognised treatments (ice slurry), or fish narcotics (e.g. Clove oil, Aqui-S (isoeugenol), MS222 (tricaine methane sulfonate), lidocaine or benzocaine), phenoxyethanol, that rapidly induce euthanasia (2,3) Lidocaine may be least aversive to zebrafish<sup>7</sup>. Holding container (as appropriate for fish size- as proposed in Ethics Application)

**PROCEDURE:** **Primary euthanasia Step:**

1. Ice Slurry – For small bodied temperate and tropical species such as Zebrafish. The procedure involves immersing the fish in ice water at a temperature of 4°C or less. (Adult zebrafish should be exposed for a minimum of 10 additional minutes following the loss of opercular movements. Zebrafish fry 4 to 7 days after fertilization (dpf) should be exposed for at least 20 additional minutes following loss of opercular movements)<sup>5</sup>.

2. Narcotics – the particular narcotic mixture can be made into a holding container as per recommended by the manufacturer <sup>4</sup> and fish introduced. Alternatively for highly water miscible chemicals such as Aqui-S the stock solution can be dripped into the fish holding tank (after capture) until the required dosage is reached. The dosage rate will vary among species and ages within species, and this will need to be accounted for. Fish behaviour should be monitored as per 1.
3. For larger fish, sharks and rays, a combination of ketamine, at dosages of 1 to 2 mg/kg, with medetomidine, at dosages of 0.05 to 0.1 mg/kg may be administered via IM injection followed by a lethal dose of pentobarbital IV<sup>2</sup>.
4. Captive bolt stunning or manual blunt force trauma to the head. This method should not be used for small-bodied fishes <sup>3</sup>.


**Secondary euthanasia Step:**

1. Decapitation or cervical dislocation <sup>2</sup>
2. Exsanguination by severing of the gill arches <sup>2,6</sup>
3. Lethal dose of pentobarbital <sup>2</sup>
4. If captive bolt stunning or blunt force is used as a primary euthanasia method, the procedure must be followed by pithing (destruction of the brain tissue by spiking) <sup>2,3,6</sup>

**RECOMMENDATIONS:**

**APPROVED:** 2024

**TO BE REVISED:** 2026

<b>APPROVED DOCUMENT ON INTRANET ONLY – UNCONTROLLED DOCUMENT WHEN PRINTED.</b>						
Version No:	Description	Approving Authority	Signature:	Contact Details	Date	Review Date
V02	Euthanasia of Fish	Animal Ethics Committee		#56618	2016	2018
V03	Euthanasia of Fish	Animal Ethics Committee		#56618	2021	2023
V04	Euthanasia of Fish	Animal Ethics Committee		#56618	2024	2026

## REFERENCES

1. NHMRC (2013) *Australian code for the care and use of animals for scientific purposes, 8th edition*. Canberra: National Health and Medical Research Council.
2. American Veterinary Medical Association (2020). *Guidelines for the Euthanasia of Animals: 2020 Edition*. <https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>.
3. Canadian Council on Animal Care (2023) (Draft) *CCAC Guideline: Fish*. <https://ccac.ca/en/about/news-and-media/2023/10/10/contribute-to-the-review-of-the-ccacs-fish-guidelines/>
4. Barker D., Allan G.L., Rowland S.J. & Pickles J.M. (2017) *A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research 4<sup>th</sup> Edition*. Port Stephens, NSW: NSW Fisheries Animal Care and Ethics Committee, Port Stephens Fisheries Centre, New South Wales.
5. Canadian Council on Animal Care (2020) CCAC guidelines: *Zebrafish and other small, warm-water laboratory fish*. [https://ccac.ca/Documents/Standards/Guidelines/CCAC\\_Guidelines-Zebrafish\\_and\\_other\\_small\\_warm-water\\_laboratory\\_fish.pdf](https://ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines-Zebrafish_and_other_small_warm-water_laboratory_fish.pdf)
6. Government of South Australia Department of Environment and Water (2017). *Euthanasia of research animals in the field policy*. <https://cdn.environment.sa.gov.au/environment/docs/wec-euthanasia-of-research-animals-policy-gen.pdf>
7. Von Krogh K, Higgins J, Saavedra Torres Y et al. (2021) *Screening of Anaesthetics in Adult Zebrafish (Danio rerio) for the Induction of Euthanasia by Overdose. Biology*. <https://www.mdpi.com/2079-7737/10/11/1133>