1. ***Purpose:***   To provide instructions for delivering immersion analgesia to zebrafish and to meet or exceed the standards as set out in *CCAC Guidelines – Zebrafish and other small warm-water laboratory fish.*

2. ***Responsibilities:***   Animal care staff, veterinarians, and trained individuals listed on an approved Animal Utilization Protocols (AUPs). All animal users performing procedures in animals must have successfully completed relevant training courses and mentor-facilitated training.

3. *Introduction:*

CCAC Guidelines – Zebrafish and other small warm-water laboratory fish states that "fish should be provided with analgesia for procedures that are likely to be painful, based on the best available scientific evidence". Due to their small size and aquatic habitat, analgesics are most easily administered to zebrafish using a medicated bath (immersion) with the drug lidocaine. Other methods of providing analgesia exist; the choice of method should factor in logistical constraints of the experiment and procedure(s) to be performed, with the overarching goal of minimizing pain and distress.

In this context, lidocaine provides analgesia, not anesthesia. Anesthesia refers to the loss of consciousness and sensation but does not necessarily entail the loss of sensitivity to painful stimuli while anesthetized or the prevention of post-operative pain. Analgesia should be provided with anaesthesia for procedures that are likely to be painful. At higher doses, lidocaine can be used as an anesthetic agent, but the duration of exposure required to provide sustained analgesia may be dangerous to the fish at these anesthetic doses.

Empirical evidence is lacking for when the effectiveness of lidocaine begins and when it wears off. Until additional evidence is available, the lidocaine immersion protocol for a particular experiment should aim to minimize fish handling and maximize the potential analgesic effect.

Any procedures in zebrafish must be outlined in an approved Animal Utilization Protocol (AUP). Deviations from this policy must be detailed in the protocol and approved by the Animal Care Committee.

4. *Procedures*

There is limited evidence to define a minimum treatment time for lidocaine immersion, and studies to date have restricted assessment to fin clipping. Based on a compilation of methods from several studies,

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and until additional evidence is available, zebrafish should be exposed to lidocaine for a minimum total time of 45 min. To date, this method has only been evaluated in adult zebrafish (>3 months old). ACS veterinarians should be consulted if procedures will be performed in juvenile zebrafish (<3 months old). Lidocaine can be acquired from the Ontario Veterinary College Pharmacy (ovcpharm@uoguelph.ca) by completing the drug request form found at:
https://www.uoguelph.ca/research/system/files/Drug%20Request%20June%202016.pdf

The procedure described below includes both a pre- and post-immersion in lidocaine for 30 and 15 min, respectively. If only pre- or post-immersion is used, timing should be adjusted to 45 min. Studies in other species have shown that preventative analgesia, where pain control is provided before pain circuits are activated, is preferable.

I. Fill a designated container with system water to a minimum depth of 10-15 cm and note volume. Add a gently bubbling air stone. The container size and final volume should be adjusted to accommodate the number of fish requiring analgesic treatment and stocking density should not exceed CCAC recommendations (5-8 fish/L).

II. Dilute 20 mg/mL lidocaine to 5 mg/mL by mixing 1 part lidocaine with 3 parts sterile saline (e.g., 1 mL of 20 mg/mL lidocaine mixed with 3 mL sterile saline for a total of 4 mL of 5 mg/mL lidocaine). Label the solution (date, dilution strength, initials) and protect it from light either with an amber bottle or by wrapping the bottle in tinfoil. Refrigerate the solution.

III. Add 1 mL of solution for every L of tank water to achieve a dose of 5 mg/L. The extra mLs being added to the tank can be treated as negligible (i.e., you do not need to remove a mL of tank water for every mL of lidocaine added). Stir the water in the tank.

IV. Using a clean net, gently transfer experimental fish to the lidocaine analgesic bath. Cover the container to prevent escape. Limit any visual disturbance of the fish during treatment.

V. After 30 min, gently transfer fish to the anesthetic bath and complete the required procedure as described in the AUP.

VI. Once complete, return the fish to the lidocaine bath for 15 min and monitor recovery.

VII. Once recovered, return the fish to the home tank.

VIII. Discard the dilution at 30 days.

Immersion analgesia can also take place in the home tank to avoid additional handling, but this approach requires that the tank be disconnected from the recirculating system prior to the addition of lidocaine and that the home tank water be replaced before it is reconnected. This approach works best if all fish in the tank require analgesic treatment.

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5. *References*

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

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