

## Lidocaine Hydrochloride vs MS222 for the Euthanasia of Zebrafish

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MS222 is the most commonly used chemical agent for euthanasia of zebrafish despite its disadvantages. Fish exposed to it demonstrate aversive behaviour; in powder form it is hazardous to personnel; and stock solutions must be stored appropriately to maintain effectiveness. Lidocaine hydrochloride was recently evaluated as a chemical anesthetic for zebrafish. It elicits less aversive behaviour; is not hazardous to personnel; and does not require special storage. Although lidocaine hydrochloride has several advantages over MS222, its effectiveness as a euthanasia agent for zebrafish is unknown. To examine this question, lidocaine hydrochloride was compared to MS222 for the euthanasia of adult zebrafish; fish ( $n = 10/\text{group}$ ) were exposed to 250 mg/L of MS222 and 400, 500, and 600 mg/L of lidocaine hydrochloride. Once opercular movement ceased, fish remained in the euthanasia solution for 10 min prior to placement in a recovery tank for a 30-min observation period. Time to loss of righting reflex, time to cessation of opercular movement, aversive behaviour, and recovery were evaluated. Time until loss of righting reflex was similar between the MS222 group and all lidocaine hydrochloride groups. Opercular movement ceased faster in all lidocaine hydrochloride groups compared with the MS222 group. Fewer fish in the lidocaine hydrochloride groups displayed aversive behaviour (e.g., piping, erratic swimming) compared with the MS222 group. No fish in the lidocaine hydrochloride groups recovered from euthanasia, whereas one fish in the MS222 group recovered. Similarly, larval zebrafish at 9-16 days post-fertilization ( $n=10/\text{group}$ ) were exposed to 250 mg/L MS222 and 400, 500, 600, 700, 800, 900 or 1000 mg/L lidocaine hydrochloride and observed for loss of heartbeat. None of the larval fish lost their heartbeats at any dose or with either drug. Our results suggest lidocaine hydrochloride may be an effective alternative chemical euthanasia agent to MS222 for adult but not larval zebrafish.