



U.S. Fish &amp; Wildlife Service

## Aquatic Animal Drug Approval Partnership

**DRUG RESEARCH INFORMATION BULLETIN**

## Efficacy of Chloramine-T to Control Mortality in Bluegill *Lepomis macrochirus* Naturally Infected with External Columnaris Disease

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Columnaris disease (causative agent, *Flavobacterium columnare*) is an acute-to-chronic bacterial infection with a worldwide distribution capable of infecting most freshwater fishes (Noga 2000). The disease most commonly occurs as an external infection; however, it can also occur as a systemic infection with no visible external signs (Plumb 1999). *Flavobacterium columnare* is more pathogenic at temperatures >15°C, and both mortality and acuteness of disease increase with temperature (Noga 2000). Typically, an external columnaris outbreak requires intervention (e.g., improving fish culture conditions and/or using chemotherapeutants) to reduce the bacterial load on fish. Several chemotherapeutants have historically been used to control mortality caused by external columnaris, and chloramine-T (CLT) is generally regarded as one of the most effective. To support U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine approval of CLT for use in aquaculture, data are required that demonstrate its effectiveness and safety.

In this bulletin, we summarize the results of a trial conducted to demonstrate the effectiveness of CLT to control mortality in bluegill (BLG) *Lepomis macrochirus* fingerlings naturally infected with external columnaris disease.

**Methods**

The trial was conducted in 2008 at the State of Florida Bass Conservation Center's Richloam Fish Hatchery (RFH) in Webster, Florida, USA. Test fish were BLG fingerlings (mean length, 8.8 cm). A single production tank of BLG fingerlings was used as the reference population.

After reference population fish were presumptively diagnosed with external columnaris, completely randomized design procedures were used to assign fish and treatment conditions (treated vs. nontreated control) to test tanks. Rectangular, fiberglass test tanks (rearing volume, 379 L) were stocked with fish impartially collected from the reference population. Each treatment condition was replicated four times ( $n = 8$  test tanks at 200 fish/tank). Each trial comprised a 1-d acclimation period, 5-d treatment period, and 14-d posttreatment observation period. During the treatment period, CLT was administered to the four treated tanks at a target concentration of 20 mg/L in a static bath for 60 min per day on three alternate days, and the four control tanks received a hatchery water sham treatment under static-bath conditions. Mortality, general fish behavior, feeding behavior (i.e., non-aggressive, semi-aggressive, aggressive), water temperature, and dissolved oxygen concentration data were collected daily throughout the trial. Water samples were collected for CLT dose verification from test tank approximately 45 min into each 60-min treatment. Analytical dose verification was conducted with a HACH DR/890 colorimeter (HACH Co., Loveland, CO).

The SAS PROC GLIMMIX (logit link) procedure was used to compare mean cumulative mortality in control tanks to that in treated tanks. Treatment levels were judged statistically significant if  $P < 0.05$ .

**Results**

At the end of the trial (Figure 1), mean percent cumulative mortality in treated tanks,  $12.9 \pm 2.349$  (mean  $\pm$  SD), was significantly less ( $P = 0.0304$ ) than mean percent cumulative mortality in control tanks  $26.9 \pm 10.313$  (mean  $\pm$  SD).

Based on dose verification samples collected (n = 4 treated; n = 2 control), the overall mean CLT concentration administered to treated tanks was 22.1 mg/L. The doses administered were with-in FDA-acceptable limits. Chloramine-T was not detected in control tanks.

Mean water temperature (22.2 °C) and mean dissolved oxygen concentration (11.9 mg/L) during the trial were suitable for rearing healthy BLG. General fish behavior was considered normal, and no substantial differences were seen between treated and control tanks. During the treatment period, all fish were characterized as feeding non-aggressively. During the posttreatment period, feeding characterization ranged from non-aggressive to aggressive for all fish.

## Discussion

In this trial, CLT administered at 20 mg/L for 60 min daily in a static bath on three alternate days was effective in controlling mortality caused by external columnaris in fingerling BLG for a period up to 14 d posttreatment. Results were accepted by FDA to support the approved use of CLT to control mortality in a variety of freshwater finfish due to external columnaris disease associated with *Flavobacterium columnare*. The final study report can be found at: <http://www.fws.gov/fisheries/aadap/studiesChloramineT.htm>.

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## References

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**Figure 1. Mean percent cumulative mortality of BLG in treated and control tanks during the trial (error bars =  $\pm 1$ SD). Test tanks were treated on trial days 1, 3, and 5 (Trt<sub>1</sub>, Trt<sub>2</sub> and Trt<sub>3</sub>, respectively).**

