



U.S. Fish &amp; Wildlife Service

## Aquatic Animal Drug Approval Partnership

# DRUG RESEARCH INFORMATION BULLETIN

## Efficacy of 35% PEROX-AID® (35% Hydrogen Peroxide) to Control Mortality in Fingerling Bluegill Diagnosed with External Columnaris Disease

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Columnaris (causative agent, *Flavobacterium columnare*) is an acute-to-chronic external or systemic bacterial disease affecting freshwater-reared finfish worldwide. Generally, outbreaks occur when water temperatures exceed 15°C (Noga 2000). In external infections, *Flavobacterium columnare* attacks a wide variety of external tissues, resulting in the appearance of circular lesions on the head, opercula, gills, fins, or body (Post 1987). During external columnaris outbreaks, fish culturists can often minimize mortality by improving environmental rearing conditions, administering chemotherapeutic bath treatments, or both.

A number of external sanitizing agents, including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), have historically been used and tested for the control of mortality in freshwater-reared finfish populations diagnosed with external columnaris. However, it was not until January 2007, when 35% PEROX-AID® (35% active H<sub>2</sub>O<sub>2</sub>; Eka Chemicals, Inc., Marietta, Georgia USA) was approved by the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) for use in certain freshwater-reared finfish species to control mortality caused by external columnaris, that an FDA-approved product was available for use. Currently, 35% PEROX-AID® is approved for use to control mortality caused by external columnaris in all freshwater-reared, coolwater finfish and channel catfish *Ictalurus punctatus* (a commonly reared warmwater finfish). The approved treatment regimen is as follows:

*Use 35% PEROX-AID® at 50 – 75 mg/L H<sub>2</sub>O<sub>2</sub> as a static bath for 60 min/d on three alternate days.*

Ongoing efforts by the U.S. Fish and Wildlife Service have been directed toward expanding the current approval of 35% PEROX-AID® for control of mortality caused by external columnaris to include all freshwater-reared, warmwater finfish. As such, and with concurrence from FDA/CVM, we designed and coordinated a controlled field trial to evaluate the efficacy of 35% PEROX-AID® to control mortality in bluegill (BLG) *Lepomis macrochirus* diagnosed with external columnaris disease. This bulletin summarizes the results of that trial.

### Methods

The trial was conducted August–September 2008 at the Florida Bass Conservation Center's Richloam Fish Hatchery, Webster, Florida USA. The test article was 35% PEROX-AID® (35% active H<sub>2</sub>O<sub>2</sub>). Test fish were BLG fingerlings (mean length, 9.7 cm; mean weight, 16.5 g) impartially collected from a reference population presumptively diagnosed with external columnaris. Completely randomized design procedures were used to allocate test fish and treatment conditions among 12 test tanks (N = 6 treated tanks; N = 6 nontreated control tanks; N = 105 fish/tank). Test-tank rearing volume was 379 L. Thus, loading density in each tank was 4.58 g/L.

The trial comprised a 1-d acclimation period, 5-d treatment period, and 14-d posttreatment period. During the treatment period, 35% PEROX-AID® was administered to treated tanks as a static bath at a target concentration of 50 mg H<sub>2</sub>O<sub>2</sub>/L for 60 min/d on three alternate days. Concurrently, a sham treatment of pure hatchery water was administered to nontreated control tanks. Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily during the acclimation, treatment, and post-treatment periods. Hardness, alkalinity, and pH of source water were measured twice during the trial. Water samples for dose verification were collected from each test tank approximately 45 min into each treatment. The titrimetric method described by Jeffery et al. (1989) was used to verify H<sub>2</sub>O<sub>2</sub> concentrations in the water samples.

The SAS PROC GLIMMIX procedure was used to statistically compare mean cumulative mortality in control tanks to that

in treated tanks on each day of the treatment and posttreatment periods. Treatment differences were judged significant if  $P < 0.05$ .

## Results and Discussion

On the last day of the trial, mean cumulative mortality in treated tanks (10.3%; range, 5.8 – 15.3%) was significantly ( $P = 0.005$ ) less than mean cumulative mortality in control tanks (19.0%; range, 13.4 – 24.6%; Figure 1). General fish behavior was considered normal in both treated and nontreated control tanks, and all fish were characterized as feeding aggressively throughout the trial.

The mean  $H_2O_2$  concentration administered to treated tanks was 48.6 mg/L (range, 46.3 to 51.4 mg/L). Hydrogen peroxide was not detected in the control tanks. Mean water temperature was  $24.9 \pm 0.03^\circ\text{C}$ , and mean dissolved oxygen concentration was  $15.2 \pm 0.65$  mg/L. Mean source water hardness, alkalinity, and pH were 345 mg/L as  $\text{CaCO}_3$ , 370 mg/L as  $\text{CaCO}_3$ , and 7.6, respectively. All water quality parameters were considered suitable for rearing healthy BLG.

Based on these results, we concluded that 35% PEROX-AID® administered at a target concentration of 50 mg  $H_2O_2$ /L as a static bath for 60 min/d on three alternate days was effective in controlling mortality in BLG fingerlings diagnosed with external columnaris. Mean cumulative mortality at the end of the trial was significantly less in treated tanks than in control tanks. Results have been submitted to FDA/CVM and will be used to support expanding the current use of 35% PEROX-AID® to include all freshwater-reared, warmwater finfish.

## Acknowledgments

Michael Matthews and staff at Richloam Fish Hatchery conducted the trial. Mark Gaikowski, U.S. Geological Survey Upper Midwest Environmental Sciences Center, analyzed the mortality data. Tom Bell and Dave Erdahl, USFWS AADAP, critically reviewed this bulletin.

## References

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**Figure 1. Mean percent cumulative mortality in  $H_2O_2$  treated and nontreated control tanks during the trial (error bar =  $\pm 1\text{SD}$ ).**

