



APR 14 2008

I-009321-P-0093-EF

U.S. Department of the Interior
Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Attention: David Erdahl, Ph.D.
Branch Chief, AADAP
4050 Bridger Canyon Road
Bozeman, MT 59715

Re: Effectiveness technical section for chloramine-T in largemouth bass

Dear Dr. Erdahl:

Your Effectiveness technical section for the use of chloramine-T to control mortality in largemouth bass due to external columnaris disease is incomplete. In your submission dated September 25, 2007, as amended October 5, 2007, you submitted to the Investigational New Animal Drug (INAD) file 009321 a final study report (Study Number CHLT-07-EFF.1-01) entitled "The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*" in support of the Effectiveness technical section for chloramine-T in largemouth bass. You also included draft Freedom of Information (FOI) Summary language. The proposed indication for chloramine-T when administered at a dose of 20 mg/L in a continuous flow water supply or as a static bath for 60 minutes once daily on consecutive days for three treatments is to control mortality in largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.

We have the following comments:

GENERAL COMMENTS

1. The data and study results for Study Number CHLT-07-EFF.1-01 are acceptable as pivotal evidence of effectiveness for the use of chloramine-T when administered at a dose of 20 mg/L for 60 minutes on consecutive days for three treatments to control mortality in largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*. In order to complete the Effectiveness technical section for the proposed indication, at least one additional supportive study with equivalent or better results will be needed. Therefore, the Effectiveness technical section remains incomplete.

2. The final study report does not include a report from the Study Monitor. The Study Monitor should have conducted on-site inspections or been in contact with the Investigator to determine whether study procedures were being conducted according to the study protocol. Information about the Study Monitor contacts with the Investigator should have been recorded in the form of a report, and this report included with the raw data. Please refer to Section 5 of Guidance for Industry Number 85 – Good Clinical Practice, available on the CVM website.
3. When submitting future studies to complete the Effectiveness technical section, please include a draft effectiveness section for the FOI Summary, draft label language, and information to address All Other Information. Please include draft language for the dose characterization section of the effectiveness section of the FOI Summary.

BIOMETRICS COMMENT

Regarding the masking procedure for the dose verification study, we note that for the first treatment day the masked study participant assigned the letters to the sample bottles in sequence; that is, the letter A was assigned to the bottle for tank 7, the letter B to the bottle for tank 8, the letter C to the bottle for tank 9, etc. This assignment method is not random. In future studies, please ensure that randomization techniques are done according to the protocol. We note that for the second and third treatment days, the letters were assigned to the sample bottles randomly, as described in the protocol.

FOI SUMMARY COMMENT

A copy of the draft effectiveness section of the FOI Summary is enclosed. Please review the FOI Summary for accuracy and notify us if you find any errors. CVM will prepare the final version of the FOI Summary and will provide you with a copy when the technical section is complete.

If you submit correspondence relating to your submission to the investigational file, you should reference the date and the principal submission identifier found at the top of this letter. If you have any questions, please contact me at 240-276-8341 or Dr. Donald Prater, Leader, Aquaculture Drugs Team, at 240-276-8343.

Sincerely,

Handwritten signature in cursive script, appearing to read "MSAull for".

Cindy L. Burnsteel, DVM
Acting Director, Division of Therapeutic
Drugs for Food Animals
Office of New Animal Drug Evaluation
Center for Veterinary Medicine

Enclosure: Draft section of the FOI Summary

DRAFT SECTION OF FREEDOM OF INFORMATION (FOI) SUMMARY

II. EFFECTIVENESS

B. Substantial Evidence:

Substantial evidence of effectiveness was demonstrated in a clinical field trial for the control of mortality in largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.

1. Clinical Field Effectiveness Study - Study CHLT-07-EFF.1-01

Title: The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*.

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Michael Matthews
Florida Bass Conservation Commission
Richloam Fish Hatchery
Webster, FL

Study Location: Richloam Fish Hatchery
Webster, FL

General Design of the Study:

- a. Objective: To evaluate the effectiveness of chloramine-T as a static bath at a concentration of 20 mg/L for 60 minutes every day for three consecutive days to control mortality in fingerling largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.
- b. Test Animals: 3,630 fingerling largemouth bass
Mean body weight 4.7 g, length 6.9 cm
- c. Test Article/Controls: The test article was chloramine-T (H & S Chemical Co.). The control fish were unmedicated.

- d. **Experimental Design:** The study was conducted with naturally infected largemouth bass. A total of 3,630 fingerlings were randomly assigned to twelve tanks (approximately 300/tank). The study had a completely randomized design with six treatment tanks and six control tanks. Ten fish were collected from the reference population for disease diagnosis. All external and internal features appeared normal with the exception of skin lesions (“saddleback” and lesions on fish body surface and tail) characteristic of external columnaris disease. Disease diagnosis was made by observation of distinct strands or “haystacks” of bacteria on wet mounts and stained samples from skin scrapes. Fish were fed feed specifically formulated for bass. Water flow rate to the test tanks was checked and adjusted daily.

Sixty-minute chloramine-T treatments of 20 mg/L were administered as a static bath every day for three treatments. Tanks of infected, unmedicated fish served as controls (0 mg/L). The treatment period was three days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 17 days.

- e. **Measurements and Observations:** Mortality was the primary variable and was recorded daily. Water samples were collected from all test tanks during each of the three treatments. Water was analyzed for chloramine-T concentration. Water temperature and dissolved oxygen were measured twice daily. Water hardness, alkalinity and pH were also measured.

The effectiveness of chloramine-T immersion to control mortality in largemouth bass due to external columnaris disease was evaluated by comparing the proportion of mortalities in the chloramine-T treatment groups to the untreated control groups.

Statistical Analysis: A generalized linear model was used to test for a group effect of the test article on cumulative mortality. The ratios of dead/total were analyzed at a 0.05 level of significance.

Results: Mortality results are summarized in Table 1.

Table 1. Mortality results for a field effectiveness study in largemouth bass with a 3-day treatment period and a 14-day post-treatment period.

Chloramine-T Dose (mg/L)	Percent Cumulative Mortality 14 days post-treatment
0	35.3 (642/1817)
20	26.9 (487/1813)

The treated and untreated control groups differ significantly in the cumulative percent mortality ($p = 0.010$) after 14 days post-treatment.

The overall mean daily chloramine-T concentration administered was 19.5 mg/L. The average water temperature was 25.2 °C. The total hardness (as CaCO₃) and alkalinity of the water were 245 mg/L (as CaCO₃) and 373 mg/L (as CaCO₃), respectively. The average pH and dissolved oxygen of the water were 8.05 and 15.8 mg/L, respectively.

Conclusions: The results of this study demonstrate the effectiveness of chloramine-T at 20 mg/L for 60 minutes a day for three consecutive treatments to control mortality in largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.



United States Department of the Interior



U.S. FISH & WILDLIFE SERVICE
AQUATIC ANIMAL DRUG APPROVAL PARTNERSHIP PROGRAM
4050 BRIDGER CANYON ROAD
BOZEMAN, MT 59715
PHONE 406-994-9904/FAX 406-582-0242

October 5, 2007

Dr. Joan Gotthardt
Director, Division of Therapeutic Drugs
for Food Animals
Document Control Unit, HFV-199
Center for Veterinary Medicine
7500 Standish Place, MPN-2
Rockville, MD 20855

Dear Dr. Gotthardt:

The purpose of this submission is to request that the enclosed revised information be amended to the Final Study Report (FSR) titled "The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*." The FSR, which is identified by Study Number CHLT-07-EFF.1-01, was submitted to CVM by AADAP on September 27, 2007. The enclosed revised information comprises only the contents of Appendix L: Water Temperature and Dissolved Oxygen Concentration. The only revisions made to the contents of Appendix L were changes made to (1) the mean water temperature during the 14-d post-treatment period (i.e., from 24.1°C to 25.4°C), and (2) the overall mean maximum water temperature (i.e., from 26.2°C to 26.4°C). We refer to your INAD file number I-9321 E-0086 dated July 26, 2007.

The current sponsor of INAD #9321 is Dr. David Erdahl, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, U.S. Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, MT. We would like to thank you in advance for your time and consideration with respect to the above-described request. If you have any questions, please contact Dr. Erdahl at (406) 994-9904.

Sincerely,

Dr. David Erdahl
Branch Chief, AADAP Program

Enclosure: 3 copies of revised Appendix L (FSR Number CHLT-07-EFF-01)



United States Department of the Interior

U.S. FISH & WILDLIFE SERVICE
AQUATIC ANIMAL DRUG APPROVAL PARTNERSHIP PROGRAM
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September 25, 2007

Dr. Joan Gotthardt
Director, Division of Therapeutic Drugs
for Food Animals
Document Control Unit, HFV-199
Center for Veterinary Medicine
7500 Standish Place, MPN-2
Rockville, MD 20855

Dear Dr. Gotthardt:

The purpose of this submission is to request a formal review of the enclosed Final Study Report (FSR) titled "The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*." The FSR is identified by Study Number CHLT-07-EFF.1-01. Please note that we also request that the FSR be included in the chloramine-T efficacy technical section in support of a New Animal Drug Approval for chloramine-T, and that the FSR be filed in the Service's Investigational New Animal Drug (INAD) file #9321. We refer to your INAD file number I-9321 E-0086 dated July 26, 2007.

The enclosed FSR, including the Freedom of Information (FOI) Summary, demonstrate that 20 mg/L chloramine-T administered as a static bath for 60 min/d on three consecutive days was effective in controlling mortality in fingerling largemouth bass caused by external columnaris. At the end of the 14-d post-treatment period, mean percent cumulative mortality in treated tanks (26.8%) was significantly different ($P = 0.010$) than that in control tanks (35.3%). Analytical verification of water samples collected during treatments confirmed chloramine-T treatment concentrations were within $\pm 25\%$ of the target concentration. Over the course of the study, fish behavior and appetite were considered normal and environmental and culture conditions were characterized as adequate for rearing healthy largemouth bass.

The current sponsor of INAD #9321 is Dr. David Erdahl, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, U.S. Fish and Wildlife Service, 4050 Bridger Canyon Road, Bozeman, MT. We would like to thank you in advance for your time and consideration with respect to the above-described request. If you have any questions, please contact Dr. Erdahl at (406) 994-9904.

Sincerely,



ACTING FOR

Dr. David Erdahl
Branch Chief, AADAP Program

Enclosure: 3 copies of FSR CHLT-07-EFF.1-01 (including the FOI Summary)
1 copy of Study Protocol CHLT-07-EFF.1