DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

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I-009321-P-0091-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

Dear Dr. Erdahl:

Based on the information in your submission dated August 14, 2007, and the information contained in the Investigational New Animal Drug (INAD) files I-004000, I-009321, and I-010974, the Division of Therapeutic Drugs for Food Animals considers the effectiveness technical section to be complete for the use of chloramine-T when administered at a dose of 10 to 20 mg/L in a continuous flow water supply or as a static bath for 60 minutes once daily on alternate or consecutive days for 3 treatments to control mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*.

FOI SUMMARY COMMENTS:

During our review of the cumulative mortality data, we found that there was statistical significance between the mortality in the treated versus control tanks through Day 5 of the post-treatment period. This is different than what was presented in the final study report which stated that statistical significance of mortality was seen through post-treatment Day 4. The Freedom of Information (FOI) Summary has been modified accordingly.

A copy of the FOI Summary for the effectiveness technical section is enclosed. Please review the FOI Summary for accuracy and notify us if you find any errors. If you request changes other than the correction of errors, CVM may re-open the effectiveness technical section. CVM will prepare the final version of the FOI Summary and will provide you a copy when the last technical section is complete.

We will make a final decision on whether we can approve your application after we have reviewed all of the data for all applicable technical sections submitted in support of an Administrative New Animal Drug Application (NADA), NADA, or supplemental NADA,

and any other information available to us, as a whole, and determined whether the requirements for approval set forth in the Federal Food, Drug, and Cosmetic Act have been met.

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,

Cindy L. Burnsteel DVM

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

Enclosure:
Freedom of Information Summary

2. *EFFECTIVENESS*

a. *Dosage Characterization:*

The primary effect of chloramine-T results from localized action at the topical site of administration. The concentration of the active drug at the topical site is a function of the administered concentration, exposure period, and water conditions. These three conditions and the sensitivity of the pathogen to the drug are considered the primary determinants of effectiveness.

Initial doses for the treatment of external columnaris disease on walleye were selected based on published literature and the experience of managers of state and federal fish hatcheries. An effective dose range of 10 to 20 mg/L administered as a static bath every other day for three treatments was determined to be effective in pilot testing. A dose determination study at the lower and upper end of the dose range was conducted as described below.

To demonstrate the ability to accurately dose and maintain concentrations of the drug in a flow-through system, a special study was conducted as described below.

b. *Substantial Evidence:*

Substantial evidence of effectiveness was demonstrated in two clinical field trials for the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*. Additionally, a special study to measure concentrations of the drug in a flow-through system was conducted in a common concrete raceway.

1. Clinical Field Effectiveness Study - Study CAP-02-CLT-06

Title: Efficacy of Chloramine-T to Control Mortality Associated with External Columnaris on Walleye *(Sander vitreus)* Fingerlings.

Study Investigator: Jeffrey J. Rach
US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

Study Location: Rathbun Fish Hatchery
Moravia, IA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of chloramine-T as a static bath at concentrations of 0, 10, and 20 mg/L for 60 minutes every other day for three treatments to control mortality due to external columnaris disease associated with *Flavobacterium columnare* on walleye fingerlings *(Sander vitreus)*.

b. Animals: 306 walleye fingerlings
Mean body weight 12 g, Length 10-13 cm

c. Test Article/Controls: The test article was chloramine-T (Akzo Nobel Chemicals, Inc.). The control fish were unmedicated.

d. Study Design: The study was conducted with naturally infected walleye. A total of 306 fingerlings were randomly assigned to nine tanks (34/tank). The study had a completely randomized design with three treatment groups and three replicates of each treatment. Three samples of four fish each were collected from the reference population for disease diagnosis. Fish with yellow, mucoid lesions predominately on the tail and in the areas of the dorsal, pelvic, and pectoral fins were selected. Disease diagnosis was made by observation of distinct strands or "haystacks" of bacteria on wet mounts and stained samples from skin scrapes and gill smears. Sixty-minute chloramine-T treatments of 10 mg/L and 20 mg/L were administered as a static bath every other day for three treatments. Tanks of infected, unmedicated fish served as controls (0 mg/L). The duration of the treatment period was five days and was followed by a post-treatment observation period of 10 days resulting in a total study duration of 15 days. Fish were fed a standard commercial walleye grower feed. Fish were not fed on treatment days. Water was supplied to the tanks from a rearing tank by using a submersible pump. Water flow rate to the test tanks was checked and adjusted daily.

e. Variables Measured: Mortality was the primary variable and was recorded daily with the first count made 24 hours after the first treatment. Water samples were collected from one replicate of each treatment group during each of the three treatments. Water was analyzed for chloramine-T concentration. Dissolved oxygen, water hardness, total alkalinity, temperature, and pH were recorded daily for each test tank during the treatment period.

Statistical Analysis: A generalized linear model was used to test for a group effect of the test article on cumulative mortality. The ratio of dead/total was analyzed at $\alpha = 0.05$.

Results: Mortality results are summarized in Table 1.

Table 1. Cumulative and percent mortality of walleye fingerlings at Day 10 post-treatment.

Chloramine-T (mg/L)	Cumulative Mortality	Percent Mortality
0	90/103	87.4
10	44/102	43.1 ($p = 0.022$)
20	67/102	65.7 ($p = 0.044$)

The probability of survival of walleye treated with chloramine-T was significantly greater than the untreated control fish. In all groups, mortality was greatest during the treatment period. In two of the control tanks, all of the fish died by Day 4. Cumulative mortality in the 10 mg/L treatment group (44 fish) was lower than in the 20 mg/L group (67 fish). In the 10 mg/L group mortality was higher in one tank (28 fish) than the other two tanks (6 and 10 fish). Mortality was similar among the 20 mg/L tanks (19, 21, and 27 fish). Post-mortem examinations of several fish showed the presence of the typical "haystacks" of bacteria associated with columnaris infection. Chloramine-T concentrations were calculated to be within ±15% of the dose. The average water temperature was 25.2 °C. The total hardness (as $CaCO_3$) and alkalinity of the water was 90 mg/L and 75 mg/L, respectively. The average pH and dissolved oxygen of the water was 7.58 and 7.4 mg/L, respectively.

Conclusions: The results of this study demonstrate the effectiveness of chloramine-T at 10 mg/L and 20 mg/L for the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*.

2. Clinical Field Effectiveness Study - Study CHLT-96-EFF-07

Title: The Efficacy of Chloramine-T to Control Mortality of Walleye *Sander vitreus* Caused by Columnaris, Causative Agent *Flavobacterium columnare*.

Study Director: James D. Bowker
US Fish and Wildlife Service
Bozeman, MT

Study Investigator: Alan Johnson
Rathbun Fish Culture and Fisheries Research Facility
Moravia, IA.

Study Location: Iowa Department of Natural Resources
Rathbun Fish Culture and Fisheries Research Facility
Moravia, IA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of chloramine-T administered at 20 mg/L in a static bath for 60 minutes on alternate days for three treatments to control mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*.

b. Animals: 1,736 fingerling walleye (*Sander vitreus*)
Mean body weight 0.83 g, Mean length 4.6 cm

c. Test article/controls: The test article was chloramine-T (Axcentive SARL/International Specialty Chemicals, Inc. Tarrytown, NY, Lot No. 0299510520572). The control fish were unmedicated.

d. Study Design: The study was conducted with naturally infected walleye. One thousand seven hundred thirty six (1,736) walleye from a reference population were randomly allocated into each of eight rectangular fiberglass test tanks (217/tank). Five fish from the reference population were evaluated pre-treatment for clinical signs of disease. The body surface was examined and external columnaris disease was confirmed by preparing and identifying the etiologic agent using microscopic examination of wet mounts of skin scrapes to detect the presence of the typical "haystacks" of bacteria associated with external columnaris infection. The tanks were randomly assigned to treatments according to a randomized complete block design. There were four treated tanks and four control tanks. Chloramine-T was administered at a dose of 20 mg/L to the treated tanks as a static bath every other day for three treatments. Tanks of infected, unmedicated fish served as controls (0 mg/L). Water inflow to each test tank was set at 2 L/min. The duration of the treatment period was five days and was followed by a post-treatment observation period of 11 days resulting in a total study duration of 16 days. Fish were fed a commercial walleye feed. Fish were not fed on treatment days. One moribund or recently dead fish was sampled from three treated and one control tank at the end of the treatment period to reconfirm disease diagnosis. Microscopic wet mounts confirmed the initial disease diagnosis of external columnaris disease. No concomitant therapies were administered to the test fish during the study.

e. Variables measured: Cumulative mortality was the primary variable and was recorded daily starting on the first day of the treatment period. Chloramine-T concentrations in the test tanks were measured using a colorimetric method. Temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity, and pH were measured twice daily.

Statistical Analysis: A generalized linear mixed model was used to test for a group effect on cumulative mortality at post-treatment day 5 and at the end of the study (post-treatment day 11). The ratio of dead/total was analyzed at $\alpha = 0.05$.

Results: Mortality results are summarized in Table 2.

Table 2. Cumulative and percent mortality of walleye fingerlings at Day 5 and Day 11 post-treatment.

Chloramine-T (mg/L)	Cumulative Mortality	Percent Mortality
0	713/868	82.1
20	659/868	75.9 ($p = 0.043$)*
0	721/868	83.1
20	686/868	79.0 ($p = 0.186$)**

*Post-treatment Day 5 **Post-Treatment Day 11

Post-treatment microscopic examinations of wet mount skin scrapes from four fish showed the presence of the typical "haystacks" of bacteria associated with columnaris infection. No parasites were seen microscopically. Mean hardness, alkalinity, and pH were 154 mg/L $CaCO_3$, 110 mg/L $CaCO_3$, and 7.7, respectively. Water temperatures ranged from 20.5 to 25.5 °C. Dissolved oxygen concentrations ranged from 5.7 to 9.6 mg/L.

Conclusions: The results of this study are supportive of the effectiveness of chloramine-T when administered at a dose of 20 mg/L to control mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*.

3. Flow-through Treatment Method

Title: Analytical Verification of Chloramine-T to Confirm Target Dosage in a Bath Solution Administered Using a Flow-Through Treatment Method.

Study Director: James D. Bowker
US Fish and Wildlife Service
Bozeman, MT

Study Location: Bozeman Fish Technology Center
Bozeman, MT

General Design of the Study:

a. Purpose: To determine if concentrations of chloramine-T measured in water samples taken from various locations within a raceway at various times during a one-hour treatment are within 25% of the target dose of 12 mg/L.

b. Animals: No fish were used in the study.

c. Test Article: The test article was chloramine-T (Akzo Nobel Chemicals Inc., Denver, CO).

d. Study Design: Dosing and collection of water samples were done in two 58 x 6 x 3.7 ft outdoor raceways. The water volume of each raceway was approximately 580 feet^3. Each raceway was dosed in duplicate for a total of four dosing experiments. The raceway water was sampled in three locations prior to the addition of drug to verify that the source water contained no chloramine-T. The amount of chloramine-T used was calculated using the following formula:

   Chloramine-T (g) = Target dose (mg/L) x water volume (gallons) x 0.00378

   A stock solution was prepared and added to the raceways using a poultry commercial water dosing machine to maintain the target dose of 12 mg/L.

e. Variables Measured: Chloramine-T concentration of the water samples was the only variable measured. From each raceway water samples were collected in 27 locations at 0 (after charging), 30, and 60 minutes during the treatment period using a centrally aligned square grid systematic sampling scheme. Samples were collected from the raceway at the head-end, middle, and tail-end; the surface, mid-depth, and bottom; and along the left-hand side, mid-line, and right-hand side.

Statistical Analysis: One sample t-test analysis of the data was used to test the hypotheses that the overall mean concentration of chloramine-T for all experiments, mean concentration for each individual experiment, and overall mean concentrations of samples collected for all four dosing experiments at 0, 30, and 60 minutes were significantly greater than the lower limit (9 mg/L) and were significantly lower than the upper limit (15 mg/L).

Results: The mean overall chloramine-T concentration from the four experiments was 10.8 mg/L. The mean concentrations at 0, 30, and 60 minutes were 11.7, 10.5, and 10.2 mg/L, respectively. The mean concentrations from all four experiments at the head-end, middle, and tail-end of the raceway were 10.8, 10.6, and 11.1 mg/L, respectively. The mean concentrations from all four experiments at the surface, middle, and bottom of the raceway were 10.7, 11, and 10.7 mg/L, respectively. The mean concentrations from all four trials at the right hand-side, midline, and left-hand side of the raceway were 10.7, 10.9, and 10.8 mg/L, respectively.

Conclusions: The results of this study demonstrate the ability to adequately deliver a target concentration of chloramine-T in a flow through system.

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 406-994-9905/FAX 406-582-0242

August 14, 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 Walleye *Sander vitreus* Caused by Columnaris, Causative Agent *Flavobacterium columnare.*" The FSR is identified by Study Number CHLT-96-EFF-07. Please note that we also request that the FSR be included in the chloramine-T (CLT) efficacy technical section in support of a New Animal Drug Approval for CLT, and that the FSR be filed in the Service's Investigational New Animal Drug (INAD) file #9321. We refer to your file number INAD I-009321-E-0083 dated May April 20, 2007.

The enclosed FSR, including the Freedom of Information (FOI) Summary, supports the claim that CLT is effective in controlling mortality in walleye caused by external columnaris. The CLT efficacy study was conducted under research study protocol #4000-1 at the Iowa Department of Natural Resource's Rathbun Fish Culture and Research Facility (Moravia, IA) during June 6 - 22, 2006. The FSR summarizes results from a study in which mean cumulative mortality in walleye fingerling naturally infected with *Flavobacterium columnare* (causative agent, Columnaris disease) was lower in tanks treated with 20 mg/L chloramine for 60 min daily on three alternate days than in untreated tanks. Although mean cumulative mortality at the end of the study was not significantly different between treated and untreated tanks, a significant difference was detected at earlier times during the 11-d post-treatment period. We request that if you concur that the FSR supports the above-mentioned claim, that the technical section for this claim be considered complete.

AADAP
Aquatic Animal Drug Approval
Partnership Program

The current sponsor for INAD #9321 is Dr. David Erdahl, U.S. Fish and Wildlife Service, AADAP Program – Branch Chief, 4050 Bridger Canyon Road, Bozeman, MT 59715. We would like to thank you in advance for your time and consideration with respect to the above described request. If you have questions, please contact Dr. Erdahl at (406)-994-9904.

Sincerely,

Dr. Tom Bell
Branch Chief – Acting, AADAP Program

Enclosure: Three copies of the FSR, including the FOI Summary; one copy of the research study protocol