



INAD 010697 P 0032

David A. Erdahl
National INAD Coordinator
USFWS, National INAD Office
Bozeman Fish Technology Center
4050 Bridger Canyon Rd.
Bozeman, MT 59715

AUG 15 2003

Dear Dr. Erdahl:

We refer to your submission dated February 10, 2003, to your investigational new animal drug (INAD) file for the use of florfenicol (Aquaflor™) as an oral antibacterial in fish. You requested our review of a study completed to demonstrate the effectiveness of florfenicol to control mortality in hybrid striped bass, *Morone americana* x *M. saxatilis*, due to streptococcal septicemia caused by *Streptococcus iniae*.

We have completed our review and have the following comments.

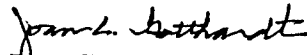
1. The results of this study support the effectiveness of florfenicol administered at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in hybrid striped bass, *Morone americana* x *M. saxatilis*, due to streptococcal septicemia caused by *Streptococcus iniae*. This study should be included in the Freedom of Information Summary. For substantial evidence and to complete the effectiveness technical section for this claim, at least one additional study will be needed.
2. The raw data includes forms that appear to be the study facility's forms rather than the forms included with the study protocol. While many facilities have forms for recording husbandry and health information, when a study is conducted under a protocol that indicates that particular forms will be used, those forms should be used to ensure that all the necessary data are collected.
3. A health or pathology report is not complete when it merely states that clinical signs consistent with a particular disease were observed. In the future, please ensure that the observed clinical signs are recorded and included in the final study report.
4. Using a production tank containing fish, especially diseased fish to which medications are being administered, to provide water to study tanks makes evaluating the study data more difficult. The effects of exposure of the fish in the study tanks to pathogens shed by the fish in the production tank and to additional concentrations of antimicrobials, regardless of the concentration, are not known. Using a production tank containing no fish to supply water to the study tanks would reduce the confounding factors when evaluating the study data.

5. Because of the amoxicillin administration to the fish in the production tank supplying water to the study tanks, the study in this submission should have ended on Day 21. Exposure to even minimal concentrations of amoxicillin may have provided an additional or synergistic effect on the streptococcal septicemia in the study fish.

Future correspondence regarding this submission to your INAD file should be identified by the date of this letter and our file number, INAD 010697 P 0032, and should be submitted to the Document Control Unit, HFV-199. Please include only one request per submission, clearly stating the request in the first paragraph of the submission.

If you have any questions regarding this letter, please contact Dr. Donald Prater, Leader, Aquaculture Drugs Team, at 301-827-7567.

Sincerely yours,



Joan C. Gotthardt, D.V.M.
Director, Division of Therapeutic
Drugs for Food Animals
Office of New Animal Drug Evaluation
Center for Veterinary Medicine



United States Department of the Interior

FISH AND WILDLIFE SERVICE

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(406) 587-9265/FTS 585-4900



February 10, 2003

Dr. Joan Gotthardt
Director, Division of Therapeutic 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 florfenicol-medicated feed to control mortality of fingerling hybrid striped bass caused by streptococcal septicemia, causative agent *Streptococcus iniae*." The FSR is identified by Study Number FLOR-01-EFF-02.b. Please note the also request that the FSR be included in the florfenicol-medicated feed efficacy technical section in support of New Animal Drug Approval for florfenicol, and that the FSR be filed in the U. S. Fish and Wildlife Service's Investigational New Animal Drug (INAD) file #10-697. We refer to your file number INAD 10-697 P-0016, dated October 4, 2002.

The enclosed FSR demonstrates the efficacy of florfenicol-medicated feed to control mortality in hybrid striped bass caused by streptococcal septicemia when administered at a dosage of 10 mg florfenicol/kg of fish/day for 10 days. The study was conducted under research study protocol FLOR-01-EFF (dated 7-18-01) at the Kent SeaTech Corporation in Mecca, CA. There was a significant difference between total mortality in treated and untreated tanks at day 7 of the post-treatment period. However, due to a reinfection of streptococcal septicemia, differences were not significant on the remaining days of the study (day 8 to day 14). It should be noted however that although differences were not significant, at the end of the 24-d study total mortality in treated tanks was lower than in untreated tanks. In addition, although florfenicol-medicated feed was administered to fish in a production tank that supplied water to the test tanks, water soluble florfenicol that potentially could have been delivered to the test tanks would not have affected the outcome of the study due to the following:

1. Worst-case scenario of the hypothetical concentration of florfenicol in water delivered to test tanks (i.e. assuming no feed was consumed by production fish and that all top-coated florfenicol was completely dissolved in water) was approximately 1.6 ppm per

day using the following equations: $(40,000 \text{ gal} \times 0.00378) / 24 \text{ g Aquaflor} = 3.2 \text{ ppm Aquaflor}$; $3.2 \text{ ppm} / 2 = 1.6 \text{ ppm florfenicol}$.

(Note: production tank volume was 40,000 gal; fish were fed twice daily; 24 g Aquaflor was administered to the tank each day in the form of medicated feed; Aquaflor contains 50% florfenicol).

As a result of the twice daily feeding, worst-case scenario of the hypothetical concentration of florfenicol in the water delivered to test tanks after each feeding would have been 0.8 ppm.

Production tank water inflow consisted of 75% re-circulated water and 25% "new" well water. Therefore, the hypothetical amount of florfenicol dissolved in production tank water would have been continuously diluted by 25% resulting in extremely low levels after a short period. As a result, water pumped to the test tanks would have contained less than the hypothetical 0.8 ppm dissolved florfenicol.

2. Water exchange rate in the test tanks was nearly 11 times per hour (55 gal test tanks and water flow of 10 gpm to each test tank). Based on the above-described scenario, hypothetical florfenicol concentrations in the water would have been highest immediately after feeding and would have decreased as clean well water was added to the production tank. Consequently, each exchange of water in the test tanks would have been with water containing lower hypothetical concentrations of florfenicol until the hypothetical florfenicol present in the production tank water was depleted.
3. Fish in the production tank were fed at a rate of 1% of their body weight. Consequently, fish were observed actively feed at the surface when medicated-feed was hand delivered indicating that fish appetite was good and that nearly all feed was consumed.
4. Mortality in the production tank increased over a 3-d period at the start of the INAD trial from 0.2% to 1.7% per day. Starting on day 4 of the treatment period, mortality began to decrease to near-zero levels by the end of the treatment period. Reduction in mortality was a positive indicator that fish were very likely consuming their full ration of medicated feed (see attachment I).
5. Mortality in the production tank increased on day 7 of the post-treatment period (same time as in the treated test tanks) indicating a reinfection of streptococcal septicemia. Reinfection would indicate that control of mortality during the treatment period was due to florfenicol and not from spontaneous recovery (see attachment I).

In summary, we acknowledge that: 1) no significant difference existed in total mortality between treated and untreated tanks at the end of the study; and 2) as a result of study design small

Dr. Joan Gotthardt - 3

concentrations of florfenicol dissolved from medicated feed administered to the production tank may have been delivered to test tanks. However, we believe the data indicated that florfenicol-medicated feed treatment was efficacious in controlling mortality in hybrid striped bass caused by streptococcal septicemia until the time treated test fish were naturally reinfected with the pathogen. We also believe that although very small concentrations of dissolved florfenicol may have existed in the treatment water, these levels would have been sub-therapeutic and would not have affected study results. Hence, in spite of these events, we believe this study demonstrated efficacy of florfenicol-medicated feed to control mortality in hybrid striped bass caused by streptococcal septicemia, and these data should be considered pivotal or supportive by CVM. Therefore, we request that these data be included in the "body of evidence" that is considered in support of a New Animal Drug Approval for florfenicol.

The current sponsor of INAD #10-697 is William Knapp, Deputy Assistant Director - Fisheries, U. S. Fish and Wildlife Service, 4401 N. Fairfax Dr., Arlington, VA 22203. 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. David Erdahl, National INAD Office, Bozeman, MT at (406) 587-9265, ext. 125.

Sincerely,



Dr. David Erdahl
National INAD Coordinator

enclosure