Freedom of Information Summary (Effectiveness section)

II. EFFECTIVENESS

A. Dose Characterization

Florfenicol has been shown to have a spectrum of activity against both Gram-positive and Gram-negative bacteria. The pharmacokinetic profile of florfenicol in various species of finfish has been reported in the published literature. Additionally, the published literature reports the use of florfenicol to treat a variety of bacterial diseases in finfish using a dose of 10 mg florfenicol/kg of fish/day for 10 days.

Dosage characterization trials were conducted under field conditions to evaluate florfenicol to control mortality due to septicemia in hybrid striped bass associated with *Streptococcus iniae*. Florfenicol was administered at a dose of 10 mg/kg of fish/day for 10 consecutive days. The trials did not include another treatment group for comparison. During most trials, mortality in the treated fish decreased significantly once treatment began, decreased to low levels by the end of the treatment period, and remained low following treatment.

B. Substantial Evidence

The data summarized in this section are publicly available and contained in Investigational New Animal Drug file 010697. The data were compiled by the U.S. Department of the Interior, Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program.

1. Field Study

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fingerling Hybrid Striped Bass Caused by Bacterial Streptococcal Septicemia, Causative Agent *Streptococcus iniae*" (Study Number FLOR-01-EFF-02.b)

b. Investigator: Vaughn E. Ostland
Kent SeaTech Corporation
Mecca, CA

c. Study Design:

1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality due to streptococcal septicemia associated with *S. iniae* in hybrid striped bass.

2) Study Animals: Approximately 600 fingerling hybrid striped bass (*M. chrysops* x *M. saxatilis*)

3) Treatment Groups: The study included two treatment groups with three replicates of each treatment. Each replicate was a tank of fish. Treatments were assigned to tanks using a completely randomized study design.

4) Drug Administration: Florfenicol was administered in a commercial salmonid feed at a dose of either 0 or 10 mg/kg of fish daily. Study feeds were fed for 10 consecutive days.

5) Measurement and Observations: *S. iniae* was identified on cultures of brain and kidney tissue collected from two fish seven days before the start of the treatment period. The clinical signs observed were consistent with streptococcal septicemia. Approximately 100 fish were randomly transferred to each study tank. The study included a 10-day treatment period and a 7-day post-treatment period. Dead fish (mortalities) were counted and recorded twice daily. Two apparently healthy fish were collected for examination from each tank on Days 11 and 17. A low-grade, subclinical infection with *Ambiphyra* spp. and *Trichodina* spp. parasites were found on the gills and skin on both fish on both days. Fish behavior and appetite were observed throughout the study, but were not recorded. Medicated feed samples were collected on Days 1, 4, and 10 of the study for analysis of florfenicol concentration. Water temperature, pH, and dissolved oxygen concentration were measured and recorded once or twice daily. Water hardness, pH, total ammonia, and alkalinity were measured in samples from one of the study tanks on Days 2, 4, and 10.

6) Statistical Analysis: Mortality was analyzed using a mixed model with treatment group, day, and the interaction between treatment group and day as fixed effects, and tank within treatment as a random effect.

d. Results: Mortality results are included in the following table.

Table 1. Mortality results for a field effectiveness study in hybrid striped bass with a 10-day treatment period and 7-day post-treatment period.

Florfenicol Dose (mg/kg of fish)	Percent Cumulative Mortality 7 days post-treatment
0	36.7 (110/300)
10	29.3 (88/300)

The treated and untreated control groups differ significantly in the cumulative percent mortality (P=0.0039) after seven days post-treatment.

The mean florfenicol dose was 9.8 mg/kg of fish/day. Mean water hardness, alkalinity, pH, and ammonia were 89 mg/L, 153 mg/L, 7.1 and 5.72 mg/L, respectively. The mean water temperature was 30.4°C. The mean dissolved oxygen concentration was 12.6 mg/L.

e. Adverse Reactions: No adverse reactions were reported in this study.

f. Conclusion: Results of this study demonstrate the effectiveness of florfenicol for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in hybrid striped bass (*M. chrysops* x *M. saxatilis*) when administered at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days.

2. Field Study

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Hybrid Striped Bass *Morone chrysops* x *M. saxatilis* Caused by Bacterial Strep, Causative Agent *Streptococcus iniae*" (FLOR-01-EFF.3-19)

b. Investigator: Vaughn E. Ostland
Kent SeaTech Corporation
Mecca, CA

c. Study Design:

1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality due to streptococcal septicemia, associated with *S. iniae* in hybrid striped bass.

2) Study Animals: Approximately 300 subadult hybrid striped bass (*M. chrysops* x *M. saxatilis*)

3) Treatment Groups: The study included two treatment groups with three replicates of each treatment. Each replicate was a tank of fish. Treatments were assigned to tanks using a completely randomized study design.

4) Drug Administration: Florfenicol was administered in a commercial salmonid feed at a dose of either 0 or 10 mg/kg of fish daily. Study feeds were fed for 10 consecutive days.

5) Measurement and Observations: Four moribund fish from the reference population were examined. Based on gross observations, bacteriology, and histopathology, streptococcal septicemia was diagnosed. Test fish were randomly transferred into each of 6 test tanks. The study included a 10-day treatment period and a 7-day post-treatment period. Dead fish (mortalities) were counted and recorded twice daily. Post-treatment mortality was recorded for 7 days. One fish was collected for examination from each tank on the last day of the treatment period. The medicated feed was assayed to confirm the florfenicol concentration. Water temperature and dissolved oxygen concentration were measured and recorded at least once daily. Medicated feed samples were collected on Days 1, 5, and 10 of the treatment period for analysis of florfenicol concentration. Water hardness, pH, and alkalinity were measured on Days 4 and 11.

6) Statistical Analysis: Mortality was analyzed using a mixed model with treatment group, day, and the interaction between treatment group and day as fixed effects, and tank within treatment as a random effect.

d. Results: Mortality results are included in the following table.

Table 2. Mortality results for a field effectiveness study in hybrid striped bass with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg of fish)	**Percent Cumulative Mortality 14 days post-treatment**
0	52 (78/150)
10	19.3 (29/150)

The treated and untreated control groups differ significantly in the cumulative percent mortality (P=0.0001) after 14 days post-treatment.

The mean florfenicol concentration in the feed samples was 830 mg/kg and the mean florfenicol dose was 8.3 mg/kg of fish/day. Mean water hardness, alkalinity, and pH were 66 mg/L, 92 mg/L, and 6.7, respectively. The mean water temperature was 26.6 °C. The mean dissolved oxygen concentration was 13.3 mg/L.

e. Adverse Reactions: No adverse reactions were reported in this study.

f. Conclusion: Results of this study demonstrate the effectiveness of florfenicol for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in hybrid striped bass (*M. chrysops* x *M. saxatilis*) when administered at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days.