Date of Approval: April 4, 2012

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-246

AQUAFLOR

florfenicol
Type A medicated article
Freshwater-reared warmwater finfish
Freshwater-reared finfish

"To increase the maximum florfenicol dose for the existing enteric septicemia indication for catfish, to add an indication for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish, and to add an indication for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish."

Sponsored by:

Intervet Inc.

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number: NADA 141-246

B. Sponsor:

Intervet, Inc.
556 Morris Ave.
Summit, NJ 07901

Drug Labeler Code: 000061

C. Proprietary Name: AQUAFLOR

D. Established Name: Florfenicol

E. Pharmacological Category: Antimicrobial

F. Dosage Form: Type A medicated article

G. Amount of Active Ingredient: 500 g florfenicol/kg (227.27 g/lb)

H. How Supplied:

2 kg foil laminate foil pouch (12 x 16 in)

16 kg fiber board drum (8 x 2 kg pouches)

I. How Dispensed: VFD

J. Dosages:

Freshwater-reared warmwater finfish: 10 – 15 mg/kg of fish/day for 10 consecutive days

Other freshwater-reared finfish: 10 mg/kg of fish/day for 10 consecutive days

K. Route of Administration: Oral

L. Species/Classes:

Freshwater-reared warmwater finfish

Freshwater-reared finfish

M. Indications:

Freshwater-reared warmwater finfish: For the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae*

Freshwater-reared finfish: For the control of mortality due to columnaris disease associated with *Flavobacterium columnare*

N. Effects of Supplement: This supplement provides for an increase in the maximum florfenicol dose for the existing enteric septicemia indication for catfish, the addition of new species/classes, and the addition of indications for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish and for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish.

II. EFFECTIVENESS:

A. Dosage Characterization:

Columnaris disease: The supplemental approval for the columnaris disease indication does not change the previously approved dosage. The Freedom of Information (FOI) Summaries for the original approval of NADA 141-246 dated October 24, 2005, and for the supplemental approval of NADA 141-246 dated March 19, 2007, contain dosage characterization information for the 10 mg/kg of fish/day dose.

Streptococcal Septicemia: 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.

Hybrid Striped Bass: 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 body weight/day for 10 consecutive days. 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.

Tilapia: Two laboratory studies using a challenge model infection were conducted. Florfenicol was administered at doses of 5, 10, and 15 mg/kg body weight/day for 10 consecutive days. The studies included at least six replicates of each dose group and each replicate was a tank of 20 fish. Fish mortality was recorded during the 10-day treatment period and a 14-day post-treatment period. The results of both studies showed all florfenicol doses reduced mortality. The 15 mg/kg body weight/day dose was selected for field trials.

B. Substantial Evidence:

The effectiveness for florfenicol for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish was demonstrated by an experimentally induced infection model study in channel catfish and natural outbreak field studies in largemouth bass, bluegill, coho salmon, and rainbow trout.

1. Induced Infection Model Study

   Title: A tank study using florfenicol in channel catfish (*Ictalurus punctatus*) for control of mortality associated with *Flavobacterium columnare* (Study No. X07-136-01) July – August 2007

   Study Director: Patricia S. Gaunt, DVM, PhD
   Mississippi State University
   Thad Cochran National Warmwater Aquaculture Center
   College of Veterinary Medicine

Study Location: Mississippi State University
Thad Cochran National Warmwater Aquaculture Center
Stoneville, MS

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in fingerling channel catfish.

b. Animals: Approximately 600 fingerling channel catfish

c. Test article/controls: Study feeds were prepared with a commercial feed to deliver 10 and 0 mg florfenicol/kg body weight daily.

d. Study Design: The study fish came from a population that was antibody negative for *Edwardsiella ictaluri* and culture negative for *F. columnare*. After a 7-day acclimation period, the fish were challenged on Day 0 by a 4-hour immersion in six tanks, each with 100 fish, containing 8.75×10^5 *F. columnare* per mL. The isolate used was obtained from a natural outbreak in channel catfish with typical clinical signs of columnaris disease in Mississippi in 2007. The fish were indiscriminately netted from the challenge water in groups of 20 and placed in the study tanks. Each treatment group included 15 replicate tanks. Tanks were randomly assigned to treatments. The experimental unit was a tank of fish. Starting on Day 1, fish received study feeds for 10 consecutive days. All fish were monitored for mortality and feeding behavior during acclimation, the treatment period, and a 14-day post-treatment period. At the end of the post-treatment period, all surviving fish were euthanized, examined by gross necropsy, and evaluated for the presence of *F. columnare* by bacterial culture.

e. Parameters Measured: Mortality and water quality.

f. Statistical Analysis: A generalized linear mixed effects model was used to test for a group effect of florfenicol on cumulative mortality. The model contained a fixed treatment effect, a random block effect, and was adjusted for possible over-dispersion among tanks. The difference in the proportion of dead fish between the treated and untreated groups was analyzed at a two-sided significance level of $\alpha = 0.05$.

Results: Mortality results are included in the following table.

Table 1. Mortality results for an infection challenge model study with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg body weight)	Percent Cumulative Mortality
0	54.2% (162/299)
10	8.0% (24/300) ($p<0.0001$)

The water hardness, alkalinity, and pH ranges were 274 to 359 mg/L, 188 to 308 mg/L, and 8.7 to 9.4, respectively. Water temperature in the study tanks ranged from 25.4 to 31.6 °C. The nitrite, ammonia, and chloride ranges were 0.000 to 0.924 mg/L, 0.5 to 0.8 mg/L, and 15 to 38 mg/L, respectively.

The mean florfenicol concentration in the medicated feed was 0.49 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 9.8 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus*.

2. Field Study

Title: "The Efficacy of AQUAFLOR-Medicated Feed to Control Mortality of Bluegill *Lepomis macrochirus* Caused by Systemic Columnaris Disease, Causative Agent *Flavobacterium columnare"* (AQFLR-09-EFF-01)
November – December 2009

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

Investigator: Michael Matthews
Florida Fish and Wildlife Conservation Commission
Florida Bass Conservation Center

Study Location: Florida Fish and Wildlife Conservation Commission
Florida Bass Conservation Center
Richloam Fish Hatchery
Webster, FL

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in fingerling bluegill.

b. Animals: Approximately 800 fingerling bluegill *Lepomis macrochirus*

c. Test article/controls: Trial feeds were prepared with a commercial feed to deliver 10 and 0 mg florfenicol/kg body weight daily.

d. Study Design: The fish were contained in a single tank prior to the start of the study. To determine the average fish weight, the total weight of three groups of fish was measured and the number of fish in each sample was counted. The total weight of each sample was divided by the number of fish in that sample. The average weight of the three samples was averaged to estimate a single average weight. To determine the average fish length,

twenty fish were measured and the results used to calculate an average length. The experimental unit was a tank of fish. Prior to allocating fish to the study tanks, ten fish were collected from the reference population for examination. *F. columnare* was recovered from cultures of kidney tissue. There were four replicates of each treatment. Approximately 100 fish were allocated to each study tank. The study included a one-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration. At the end of the study, the number of fish in each tank was counted and added to the number of mortalities removed from each tank. This total number of fish was used to calculate the percent cumulative mortality.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted once daily during the treatment and post-treatment periods. Fish behavior and appetite were observed and recorded once daily. Temperature and dissolved oxygen were measured once daily in all tanks. Water hardness, alkalinity, and pH were measured once during the study.

f. Statistical Analysis: A generalized linear mixed effects model was used to test for a group effect of florfenicol on cumulative mortality. The model contained a fixed treatment effect and adjusts for possible over-dispersion among tanks. The difference in the odds ratios of dead to total fish between the treated and untreated groups was analyzed at two-sided significance level of $\alpha = 0.05$.

Results: Mortality results are included in the following table.

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

Florfenicol Dose (mg/kg body weight)	Percent Cumulative Mortality
0	38.4% (150/391)
10	18.7% (75/395) (p=0.0098)

The water hardness, alkalinity, and pH were 365 mg/L, 340 mg/L, and 7.9 – 8.0, respectively. Water temperature in the study tanks averaged 22.3 °C (20.3 – 23.8 °C). The dissolved oxygen concentration averaged 10.5 mg/L (9.1 – 11.1 mg/L).

The mean florfenicol concentration in the medicated feed was 0.68 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 10.2 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in bluegill, *Lepomis macrochirus*.

3. Field Study

Title: "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fingerling Coho Salmon, *Oncorhynchus kisutch*, Caused by Columnaris,

Causative Agent *Flavobacterium columnare*"
(Study Number FLOR-01-EFF-08) July – August 2002

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

Investigator: Mike Muller
Bellingham State Fish Hatchery

Study Location: Bellingham State Fish Hatchery
Washington Department of Fish and Wildlife
Bellingham, WA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in fingerling coho salmon.

b. Animals: Approximately 45,000 fingerling coho salmon

c. Test article/controls: Trial feeds were prepared with a commercial feed to deliver 10 and 0 mg florfenicol/kg body weight daily.

d. Study Design: The fish were randomly placed in six raceways just prior to the start of the study. The average weight of the fish was estimated using a length-weight conversion table. Four fish were collected from each of the raceways for examination. *F. columnare* was recovered on cultures of skin, gill, and kidney tissue. There were three replicates of each treatment. The study included a 4-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration. At the end of the study the number of fish in each raceway was counted and added to the number of mortalities removed from each raceway. This total number of fish was used for data analysis.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily during the treatment and post-treatment periods. Fish behavior and appetite were observed throughout the study, but were not recorded. Temperature and dissolved oxygen were measured once or twice daily in all tanks. Water hardness, alkalinity, and pH were measured twice during the study.

f. 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 the tanks within treatment as a random effect. The difference in percent cumulative mortality between the treated and untreated groups was analyzed at a two-sided significance level of $\alpha=0.05$.

Results: Mortality results are included in the following table.

Table 3. Mortality results for a field effectiveness study in coho salmon with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg body weight)	Percent Cumulative Mortality
0	67.0 (14,167/21,407)
10	47.0 (11,720/24,786) (p=0.0388)

The mean water hardness, alkalinity and pH were 20 mg/L, 17 mg/L, and 8.06, respectively. The mean water temperature was 20.8°C. The mean dissolved oxygen concentration was 7.9 mg/L.

The mean florfenicol concentration in the medicated fed was 0.68 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 10.2 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in coho salmon, *Oncorhynchus kisutch*.

4. Field Study

Title: "The Efficacy of AQUAFLOR-Medicated Feed to Control Mortality of Rainbow Trout *Oncorhynchus mykiss* Caused by Columnaris Disease, Causative Agent *Flavobacterium columnare"*
(Study Number FLOR-01-EFF.3-33) July 2008

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

Investigator: Kevin Clark
Bellingham State Fish Hatchery

Study Location: Bellingham State Fish Hatchery
Washington Department of Fish and Wildlife
Bellingham, WA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in fingerling rainbow trout.

b. Animals: Approximately 50,000 fingerling rainbow trout *Oncorhynchus mykiss*

c. Test article/controls: Trial feeds were prepared with a commercial feed to deliver 10 and 0 mg florfenicol/kg body weight daily.

d. Study Design: The fish were randomly placed in six raceways just prior to the start of the study. To determine the average fish weight, the total weight of a group of fish was measured and the number of fish in each sample was counted. The total weight of the sample was divided by the number of fish in the sample to estimate the average fish weight. Two fish were collected from each of the raceways for examination. Bacteria characteristic of *F. columnare* were observed in samples collected from the skin, gill, and kidney. There were three replicates of each treatment. The study included a 5-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration. At the end of the study the number of fish in each raceway was counted and added to the number of mortalities removed from each raceway. This total number of fish was used for data analysis.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily during the treatment and post-treatment periods. Fish behavior and appetite were observed and recorded daily throughout the study. Temperature and dissolved oxygen were measured once daily in all tanks. Water hardness, alkalinity, and pH were measured twice during the study.

f. Statistical Analysis: A generalized linear model was used to test for a group effect of the test article on cumulative mortality. The ratios dead/total were analyzed as such with significance level $\alpha = 0.05$.

Results: Mortality results are included in the following table.

Table 4. Mortality results for a field effectiveness study in rainbow trout with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg body weight)	Percent Cumulative Mortality
0	30.4 (7,584/24,927)
10	18.2 (4,566/25,137) (p=0.0551)

The water hardness, alkalinity, and pH were 32 mg/L, 22 mg/L, and 7.0, respectively. Water temperature in the study tanks averaged 20.0 °C. The dissolved oxygen concentration averaged 8.1 mg/L, and was greater than or equal to 6.0 mg/L throughout the study.

The mean florfenicol concentration in the medicated feed was 0.47 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 9.4 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in rainbow trout, *Oncorhynchus mykiss*.

5. Field Study

Title: "The Efficacy of AQUAFLOR-Medicated Feed to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by Systemic Columnaris Disease, Causative Agent *Flavobacterium columnare*"
(Study Number FLOR-01-EFF.3-35) May – June 2009

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

Investigator: Michael Matthews
Florida Fish and Wildlife Conservation Commission
Florida Bass Conservation Center

Study Location: Florida Fish and Wildlife Conservation Commission
Florida Bass Conservation Center
Richloam Fish Hatchery
Webster, FL

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to columnaris disease associated with *Flavobacterium columnare* in fingerling largemouth bass.

b. Animals: Approximately 5,000 fingerling largemouth bass *Micropterus salmoides*

c. Test article/controls: Trial feeds were prepared with a commercial feed to deliver 10 and 0 mg florfenicol/kg body weight daily.

d. Study Design: The fish were contained in a single tank prior to the start of the study. To determine the average fish weight, the total weight of three groups of fish was measured and the number of fish in each sample was counted. The total weight of each sample was divided by the number of fish in that sample. The average weight of the three samples was averaged to estimate a single average weight. To determine the average fish length, twenty fish were measured and the results used to calculate an average length. Prior to allocating fish to the study tanks, six fish were collected from the reference population for examination. *F. columnare* was recovered from cultures of kidney tissue. There were five replicates of each treatment. The study included a one-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration. At the end of the study, the number of fish in each tank was counted and added to the number of mortalities removed from each tank. This total number of fish was used to calculate the percent cumulative mortality.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily during the treatment and post-treatment periods. Fish behavior and appetite were observed and recorded once daily.

Temperature and dissolved oxygen were measured once daily in all tanks. Water hardness, alkalinity, and pH were measured once during the study.

f. Statistical Analysis: The design of this study precluded statistical analysis.

Results: Mortality results are included in the following table.

Table 5. Mortality results for a field effectiveness study in largemouth bass with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg body weight)	Percent Cumulative Mortality
0	11.74 (290/2,470)
10	5.83 (132/2,263)

The water hardness, alkalinity, and pH were 353 mg/L, 380 mg/L, and 7.7 to 7.8, respectively. Water temperature in the study tanks averaged 23.9 °C. The dissolved oxygen concentration averaged 13.7 mg/L.

The mean florfenicol concentration in the medicated feed was 0.21 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 10.5 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study showed a clinically apparent reduction in mortality in the florfenicol-treated fish compared to the untreated control fish. Therefore, the results from this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in largemouth bass, *Micropterus salmoides*.

The effectiveness for florfenicol for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish was demonstrated by natural outbreak field studies in hybrid striped bass and tilapia.

6. Clinical Field Trial

Title: "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) August - September 2001

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

Investigator: Vaughn E. Ostland
Kent SeaTech Corporation

Location: Kent SeaTech Corporation
Mecca, CA

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of body weight/day for 10 consecutive days to control mortality due to streptococcal septicemia associated with *Streptococcus iniae* in hybrid striped bass.

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

c. Test Article/Control: Trial feeds were prepared with commercial salmonid feed to deliver 10 or 0 mg florfenicol/kg of body weight daily.

d. Study Design: The fish were contained in a single tank (reference population) prior to the start of the study. Two fish from the reference population were examined seven days prior to the start of the study. The clinical signs observed were consistent with streptococcal septicemia. *S. iniae* was identified on cultures from brain and kidney tissue collected from the two fish. There were three replicates of each treatment. The study included a one-day acclimation period, a 10-day treatment period, and a 7-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily. Fish behavior and appetite were observed throughout the study, but were not recorded. Temperature, pH, and dissolved oxygen concentration were measured and recorded once or twice daily. Water hardness, pH, total ammonia, and alkalinity were measured three times during the study.

f. 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. The difference in percent cumulative mortality between the treated and untreated groups was analyzed at a two-sided significance level of $\alpha=0.05$.

Results: Mortality results are included in the following table.

Table 6. 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
0	36.7 (110/300)
10	29.3 (88/300) (p=0.0039)

The 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.

The mean florfenicol concentration in the medicated feed was 0.98 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 9.8 mg/kg of fish/day.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control mortality due to streptococcal septicemia associated with *Streptococcus iniae* in hybrid striped bass, *Morone chrysops* x *M. saxatilis*.

7. Clinical Field Trial

Title: "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)
September - October 2003

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

Investigator: Vaughn E. Ostland
Kent SeaTech Corporation

Location: Kent SeaTech Corporation
Mecca, CA

General Design of the Study:

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

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

c. Test Article/Control: Trial feeds were prepared with commercial salmonid feed to deliver 10 or 0 mg florfenicol/kg of body weight daily.

d. Study Design: The fish were contained in a single tank (reference population) prior to the start of the study. Four fish from the reference population were examined one day prior to the start of the study. *S. iniae* was identified on cultures from brain and kidney tissue collected from the fish. There were three replicates of each treatment. The study included a one-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily. Fish behavior and appetite were observed throughout the study, but were not recorded. Temperature and dissolved oxygen concentration were measured and recorded once daily. Water hardness, pH, total ammonia, and alkalinity were measured twice during the study.

f. 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. The difference in percent cumulative mortality between the treated and untreated groups was analyzed at a two-sided significance level of $\alpha=0.05$.

Results: Mortality results are included in the following table.

Table 7. 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
0	52 (78/150)
10	19.3 (29/150) (p=0.0001)

The 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.

The mean florfenicol concentration in the feed samples was 0.83 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 8.3 mg/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of body weight/day for 10 consecutive days to control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in hybrid striped bass, *Morone chrysops* x *M. saxatilis*.

8. Clinical Field Trial

Title: "Field Effectiveness of AQUAFLOR (50% Type A Medicated Article), Florfenicol – SCH25298 administration in feed to control mortality associated with *Streptococcus iniae* in tilapia (*Oreochromis* spp.)"
(Study Number C05-082-01) August – September 2007

Study Director: Mark P. Gaikowski, MA
USGS Biologic Resources Division
Upper Midwest Environmental Sciences Center

Location: MinAqua Fisheries
Rennville, MN

General Design of the Study:

a. Purpose: To evaluate the effectiveness of florfenicol administered in feed at a dose of 15 mg/kg of body weight/day for 10 consecutive days to control mortality due to streptococcal septicemia associated with *S. iniae* in tilapia (*Oreochromis* spp.).

b. Animals: Approximately 1,720 juvenile tilapia of two strains, *O. niloticus* x *O. niloticus* and *O. niloticus* x *O. aureus*

c. Test Article/Control: Study feeds were prepared with a commercial tilapia feed to deliver 15 or 0 mg florfenicol/kg of body weight daily.

d. Study Design: The fish were contained in a single tank (reference population) prior to the start of the study. Sixty fish from the reference population were examined on the first day of the treatment period of the study. The average fish weight was calculated from the weights of the fish examined. The clinical signs observed were consistent with streptococcal septicemia. *S. iniae* was identified on cultures from brain, liver, and kidney tissue. There were ten replicates of each treatment. The study included a 10-day treatment period and a 14-day post-treatment period. The medicated feed was assayed to confirm the florfenicol concentration.

e. Parameters Measured: Mortality, fish behavior, and water quality. Mortalities were counted twice daily. Feed consumption was observed and recorded once daily. Dissolved oxygen, temperature, and pH were measured and recorded once daily. Ammonia, nitrite, and alkalinity were measured twice during the study. Nitrate and carbon dioxide were measured once during the study.

f. Statistical Analysis: The ratios dead/total were analyzed using a generalized linear model, and the treatment effect on cumulative mortality was test (α = 0.05) at post-treatment day 14.

Results: Mortality results are included in the following table.

Table 8. Mortality results for a field effectiveness study in tilapia with a 10-day treatment period and 14-day post-treatment period.

Florfenicol Dose (mg/kg of fish)	Percent Cumulative Mortality
0	14.7 (119/812)
15	11.3 (93/820) (p=0.0218)

Dissolved oxygen ranged from 8.8 to 10.7 mg/L, pH ranged from 7.3 to 7.4, temperature ranged from 27.2 to 29.3 °C, and water flow ranged from 624 to 644 ml/min. Alkalinity ranged from 115 to 209 mg/L, ammonia ranged from 0.1 to 0.6 mg/L, and nitrite ranged from 0.03 to 0.17 mg/L.

The mean florfenicol concentration in the medicated feed was 0.27 g/kg of feed. The calculated dose of florfenicol administered to fish in treated tanks was 13.5 g/kg of fish/day. No florfenicol was detected in the control feed.

Conclusion: The results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 15 mg florfenicol/kg of fish/day for 10 consecutive days to control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in tilapia.

III. TARGET ANIMAL SAFETY:

Target animal safety was determined by considering the studies summarized in this section, as well as the data provided in the effectiveness trials. The target animal safety studies conducted in catfish and rainbow-trout demonstrated a margin of safety for a florfenicol dose up to 15 mg/kg body weight/day for 10 consecutive days. In order to demonstrate the safety of the 15 mg/kg body weight/day dose for freshwater-reared finfish, studies were conducted in sunshine bass, yellow perch, and tilapia. When considered together, the data demonstrate that florfenicol is safe when administered to freshwater-reared finfish at a dose up to 15 mg florfenicol/kg body weight/day for 10 consecutive days.

A. Margin of Safety Studies – Catfish and Freshwater-reared salmonids

The FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, contains a summary of target animal safety data for catfish. The FOI Summary for the supplemental approval of NADA 141-246 dated March 19, 2007, contains a summary of target animal safety data for freshwater-reared salmonids. These data are acceptable to demonstrate the safety of florfenicol when administered to catfish and freshwater-reared salmonids at a dose up to 15 mg florfenicol/kg body weight/day for 10 consecutive days.

B. Margin of Safety Study – Sunshine Bass

1. Title: "The Safety of AQUAFLOR (50% Florfenicol; Type A Medicated Article) Administered in Feed to Sunshine Bass Female White Bass *Morone chrysops* x Male Striped Bass *M. saxatilis*" (Study No. FLOR-08-TAS-SSB-01) March to April 2009

2. Study Director: James D. Bowker
U.S. Department of the Interior, Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program

3. Alternate Study Director: David L. Straus
U.S. Department of Agriculture
Agricultural Research Service
Stuttgart National Aquaculture Research Center

4. Study Facility: U.S. Department of Agriculture, Agricultural Research Service
Harry K. Dupree – Stuttgart National Aquaculture Research Center
Stuttgart, AR

5. General Design of the Study:

a.Purpose: To estimate a margin of safety associated with administering florfenicol-medicated feed to sunshine bass fingerlings at 0X, 1X, 3X, or 5X the proposed maximum therapeutic dose of 15 mg florfenicol/kg of fish/day for 2X the proposed therapeutic treatment duration of 10 consecutive days. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

b. Animals: Approximately 670 sunshine bass (female white bass *M. chrysops* x male striped bass *M. saxatilis*). The mean length and weight of the fish at the start of the study were 11.2 cm and 13.7 g, respectively.

c.Test article/controls: Study feeds were prepared with a commercial feed to deliver 0, 15, 45, and 75 mg florfenicol/kg body weight daily, which is 0, 1X, 3X, and 5X, respectively, of the maximum therapeutic dose of 15 mg florfenicol/kg of fish/day.

d. Study Design: The study included a 10-day acclimation period and a 20-day treatment period. There were 3 replicates of each treatment. The experimental unit was a tank containing 20 fish. Three additional tanks (growth tanks), each containing 20 fish, were used to estimate fish weight and adjust the amount of feed delivered. Adjustment of feed amounts for exposure groups was based on the weights of the growth tank fish measured on Days 1, 8, and 15. The reference population fish were held in two tanks prior to the start of the acclimation period. On Day -2, reference population fish were allocated to the 15 study tanks, which included 3 growth tanks and 12 study tanks. Feed samples were collected on Days -1, 1, 7, 14, and 21.

a.On Day 0, 30 fish were collected from a reference population tank for gross examination and tissue collection for histopathology. Ten fish were randomly selected to undergo gross examination and the 20 remaining fish were collected for histological examination. On Day 21, all surviving fish in the study tanks were euthanized and measured for total length and weight. Ten fish were randomly selected from each study tank for histopathological examination and the 10 remaining fish underwent necropsy. From the 10 fish selected for histopathology from each study tank and the reference population fish, two were randomly selected for examination of gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine. Gill, liver, anterior kidney, and posterior kidney were examined in the remaining eight fish. Tissues from the reference population fish and fish

in the 0 and 5X groups were examined first. Because a greater prevalence of moderate kidney lesions were detected in the 5X group, kidney tissue from the fish in the 3X group was also examined.

e.Parameters Measured: Mortality, fish behavior, feeding behavior, histopathology of selected tissues, and water quality. Water temperature and dissolved oxygen concentration were measured daily in all test tanks. Water quality parameters measured weekly during the study included hardness, alkalinity, and pH.

f. Statistical Analysis: No statistical analyses were performed.

6. Results:

Behavior/Mortality: Fish behavior was normal for all test fish throughout the study. Fish consumed all feed offered. No fish died or exhibited signs of morbidity during the study.

Fish Length/Weight: At the end of the study, the mean length and weight of the fish were 12.6 cm and 21.0 g respectively.

Water Quality: Overall mean water temperature was 21.9 °C (21.1 – 22.6 °C). Overall mean dissolved oxygen concentration was 6.1 mg/L (4.7 – 8.9 mg/L). The overall mean water hardness (114 mg/L as $CaCO_3$; 113-115 mg/L), alkalinity (195 mg/L as $CaCO_3$; 194 – 195 mg/L), and pH (7.5; 7.4 – 7.7) were acceptable for rearing healthy sunshine bass.

Pathology: For the fish collected on Day 0, all external and internal tissues examined appeared normal. No histological lesions were observed in the heart, liver, skin, or muscle. Lesions observed in the brain, spleen, pyloric intestine, rectal intestine, gill, anterior kidney, and posterior kidney were considered normal for sunshine bass reared under culture conditions. Similar lesions were observed in the tissues of fish collected on Day 21. No test article related lesions were observed in the tissues examined.

Florfenicol Concentration: The mean florfenicol concentration in the study feeds were 0, 0.73, 2.27, and 3.73 g/kg of feed and the nominal florfenicol concentration in the feeds were 0, 0.75, 2.25, and 3.75 g/kg of feed, respectively.

7. Conclusion: The study demonstrated that florfenicol medicated feed is safe when administered in feed to sunshine bass at the maximum therapeutic dose of 15 mg/kg of fish/day for 10 consecutive days.

C. Margin of Safety Study – Yellow Perch

1. Title: "The Safety of AQUAFLOR (50% florfenicol; Type A Medicated Article) Administered in Feed to Yellow Perch *Perca flavescens*"
(Study No. FLOR-08-TAS-YP.1-01) February to March 2010

2. Study Director: James D. Bowker
U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program

3. Alternate Study Director: Daniel Carty
U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program

4. Study Facility: U.S. Fish & Wildlife Service Bozeman Fish Technology Center
Bozeman, MT

5. General Study Design:

   a. Purpose: To estimate the margin of safety associated with administering florfenicol-medicated feed to yellow perch fingerlings at 0, 1X, 3X, or 5X the maximum therapeutic dose of 15 mg florfenicol/kg of fish/day for 2X the proposed therapeutic duration of 10 consecutive days. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

   b. Animals: 225 yellow perch (*Perca flavescens*). The mean total length and weight of test fish at the start of the study were 1.6 cm and 3.4 g, respectively.

   c. Test article/controls: Study feeds were prepared to deliver 0, 15, 45, and 75 mg florfenicol/kg body weight daily.

   d. Study Design: The study included a 6-day acclimation period and a 20-day exposure period. There were 3 replicates of each treatment. The experimental unit was a tank containing 15 fish. Three additional tanks (growth tanks), each containing 15 fish, were used to estimate fish weight and adjust the amount of feed delivered. Adjustment of feed amounts for exposure groups was based on the weights of the growth tank fish measured on Days 1, 8, and 15. The reference population fish were held in one tank prior to the start of the acclimation period. On Day -6, reference population fish were allocated to the 15 study tanks, which included 3 growth tanks and 12 study tanks. Feed samples were collected on Days -5, 2, 7, 14, and 20.

      Two days prior to the start of the acclimation period, 20 reference population fish were collected for gross examination and tissue collection for histopathology. Gross necropsies were performed on all 20 fish; sampling for histological examination was performed on 10 randomly selected fish. On Day 21, all surviving fish from the 12 test tanks were euthanized, measured for total length and weight, and examined grossly. Ten randomly selected fish from each study tank were processed for histological examination. Gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric intestine, and rectal intestine were collected from all 10 fish. All tissues were examined microscopically in two of the ten fish. Gill, liver, anterior kidney, and posterior kidney only were examined microscopically in the remaining eight fish. Tissues from the reference population fish, and fish from the control and 5X groups were examined.

   e. Parameters Measured: Mortality, fish behavior, feeding behavior, length and weight, histopathology of selected tissues, and water quality. Temperature and dissolved oxygen were measured daily in all tanks. Alkalinity and hardness were measured on Days 2, 9, and 16.

   f. Statistical Analysis: Fish mortality and proportion of fish with histological changes were analyzed using a generalized linear model procedure, assuming

a binomial distribution and logit link. Tank was the experimental unit for the analysis. The statistical model included treatment as the only fixed effect and the treatment effect was tested at two-sided α=0.10. For fish mortality, the dependent variable was cumulative proportion of dead fish in each tank at Day 20 and the observations were weighted according to number of evaluable fish in each tank. For the analysis of histological changes, a separate analysis was done for each tissue and pathology.

6. Results:

General Behavior/Feeding Behavior: General fish behavior and feeding behavior were normal in all test tanks.

Mortality: Mortalities occurred in the control, 1X, and 3X exposure groups and are summarized in Table 1.

Table 9. Mortality results for a target animal safety study in yellow perch treated for 20 days with florfenicol-medicated feed.

Florfenicol Dose (mg/kg)	Percent Cumulative Mortality at Treatment Day 20
0	6.7
15	2.2
45	10
75	0

There was no significant difference in cumulative mortality between any of the exposure groups.

Fish Length/Weight: At the end of the study, the mean length and weight of the fish were 8.5 cm and 7.2 g. There were no significant differences in mean length and mean weight among the four exposure groups.

Water Quality: Overall mean water temperature in test tanks was 23.3 ºC (22.6 - 23.3 ºC). Overall mean dissolved oxygen concentration in test tanks was 6.0 mg/L (5.7 - 6.4 mg/L). The overall mean water hardness (276 mg/L as $CaCO_3$; 252 - 306 mg/L), alkalinity (168 mg/L as $CaCO_3$; 164 - 170 mg/L), and pH (7.9; 7.5 - 8.0) were acceptable for rearing yellow perch.

Pathology: Histological findings were dichotomized into pathological (biologically significant) and non-pathological. There were no statistically significant differences between the control and 5X treatment groups with respect to proportion of fish with histological changes in tissues collected at Day 21. No lesions or background histological changes were observed in the heart, brain, muscle, skin, pyloric intestine, rectal intestine, and gill in the control and 5X groups. Lesions observed in the anterior kidney, posterior kidney, and liver were considered normal for yellow perch reared under culture conditions. No test article related lesions were observed in the tissues examined.

Florfenicol Concentration: The mean florfenicol concentrations in the study feeds were 0, 1.54, 4.56, and 7.7 g/kg of feed and the nominal florfenicol concentration in the feeds were 0, 1.5, 4.5 and 7.5 g/kg of feed, respectively.

7. Conclusion: This study demonstrates that florfenicol medicated feed is safe for use in yellow perch when administered in feed at the maximum therapeutic dose of 15 mg/kg body weight/day for 10 consecutive days.

D. Margin of Safety Study – Tilapia

1. Title: "Safety of AQUAFLOR (50% Type A Medicated Article), Florfenicol – SCH25298 administration in feed to tilapia (*Oreochromis* sp.)" (Study No. N09-083-01) December 2009

2. Study Director: Mark Gaikowski, MA
U.S. Geological Survey
Upper Midwest Environmental Sciences Center

3. Study Facility: Upper Midwest Environmental Science Center
La Crosse, WI

4. General Design of the Study:

a.Purpose: To estimate a margin of safety associated with administering florfenicol-medicated feed to tilapia at 0X, 1X, 3X, or 5X the proposed maximum therapeutic dose of 15 mg florfenicol/kg of fish/day for 2X the proposed therapeutic treatment duration of 10 consecutive days. This study was conducted in accordance with the Good Laboratory Practice regulations (21 CFR 58).

b. Animals: Approximately 300 Nile tilapia (*Oreochromis niloticus*) and hybrid tilapia (*O. niloticus* X *O. aureus*). The mean length and weight of the fish at the start of the study was 12.6 cm and 48.5 g, respectively. Fish within two standard deviations of the calculated mean weight were used in the study (24.9 to 66.8 g). Fish were used regardless of gender or strain.

c.Test article/controls: Study feeds were prepared with a commercial feed to deliver 0, 15, 45, and 75 mg florfenicol/kg body weight daily, which is 0, 1X, 3X, and 5X, respectively, of the maximum therapeutic dose of 15 mg florfenicol/kg of fish/day.

d. Study Design: The study included an 8-day acclimation period and a 20-day treatment period. There were 3 replicates of each treatment. The experimental unit was a tank containing 20 fish. Three additional tanks (growth tanks), each containing 20 fish, were used to estimate fish weight and adjust the amount of feed delivered. Feed amounts were adjusted on Days 0, 5, 10, and 15. Feed amount was also adjusted for mortality during the treatment period. The reference population fish were held in two tanks prior to the start of the acclimation period. On Day -8, fish were allocated from the reference population to the 15 tanks, which included 3 growth tanks and 12 study tanks. Feed samples were collected upon receipt at the study site, at the start of the treatment period, and at the end of the treatment period.

On Day 20, all surviving fish in the study tanks were euthanized and measured for total length and weight. Ten fish were randomly selected from each study tank for gross necropsy and histopathological examination. The remaining fish in each study tank underwent gross external examination. Gill, liver, anterior kidney, posterior kidney, brain, heart, muscle, skin, spleen, pyloric

intestine, and rectal intestine were collected from all 10 fish. All tissues were examined microscopically in two of the ten fish. Gill, liver, anterior kidney, and posterior kidney only were examined microscopically in the remaining eight fish. Tissues from the fish in the 0 and 5X groups were examined first. Because a greater prevalence of kidney, liver, and gill lesions were detected in the 0 and 5X group, these tissues were also examined in the 1X and 3X groups.

e.Parameters Measured: Mortality, fish behavior, feeding behavior, uneaten feed, histopathology of selected tissues, and water quality. Water temperature, pH, and dissolved oxygen concentration were measured daily in all test tanks. Alkalinity and hardness were measured on Days 3, 9, and 17.

f. Statistical Analysis: Mortality was summarized but not statistically analyzed. Feed consumption was analyzed by repeated measures analysis (MIXED procedure in SAS). Body weight gain and total fish length were analyzed by analysis of variance.

5. Results:

General Behavior/Feeding Behavior: Normal behavior responses were observed during the acclimation and treatment period. Subdued responses were noted in the florfenicol-treated groups intermittently during Days 0 to 9 of the treatment period, which increased in a dose-related manner during Days 10 to 19 of the treatment period. There were no significant differences in feed consumption between the treatment groups during the first 10 days of the treatment period. During the second 10 days of the treatment period, there was a statistically significant decrease in feed consumption in the 3X and 5X treatment groups compared with the control group. Fish in the control and 1X treatment groups consumed >99.8% of the feed offered during the treatment period. Fish in the 3X and 5X treatment groups consumed 79.2% and 75.1% of the feed offered during the treatment period, respectively.

Mortality: There were no mortalities during the acclimation period. There were three mortalities during the treatment period, one in each florfenicol treatment group. None of the mortalities was attributed to the test article.

Fish Length/Weight: The body weight in the control and 1X treatment group at the end of the study was similar. The weight gain in the control group (34.5 g) was significantly greater than that of the 1X (29.6 g), 3X (20.7 g), and 5X (17.6 g) treatment groups. Mean total fish length in the control group (15.4 cm) was significantly greater than that of the 1X (15.1 cm), 3X (14.5 cm), and 5X (14.2 cm) treatment groups.

Water Quality: The water temperature ranged from 27.4 to 29.0 °C. Dissolved oxygen concentration ranged from 4.4 to 6.1 mg/L. The water hardness (117-125 mg $CaCO_3$/L), alkalinity (108 - 167 mg $CaCO_3$/L), and pH (7.4 – 8.2) were acceptable for rearing tilapia.

Pathology: An increase in moderate lamellar epithelial hyperplasia in the gill was seen in the 3X and 5X treatment groups. A minimal to mild decrease in lymphoid tissue in the anterior kidney, an increase in individual cell necrosis, and an increase in tubular epithelial degeneration and mineralization in the posterior kidney were seen all treated groups. The differences are summarized in the following table.

Table 10. Incidence and severity of histopathologic findings in tilapia administered florfenicol for 20 days.

Histological Finding	0 mg/kg	15 mg/kg	45 mg/kg	75 mg/kg
Gill				
Lamellar epithelial hyperplasia				
Minimal	5	0	0	0
Mild	20	13	7	4
Moderate	5	17	24	26
Anterior Kidney				
Lymphoid tissue decrease	8	20	26	29
Cell necrosis	0	10	8	9
Posterior Kidney				
Tubular epithelial degeneration	1	10	14	14
Mineralization	9	16	15	19
Liver – Hepatocellular vacuolation				
Glycogen-type				
Minimal	11	6	4	5
Mild	15	17	18	10
Moderate	4	7	8	15
Lipid-type				
Minimal	9	9	11	3
Mild	11	17	15	17
Moderate	5	4	3	10

The differences were small and did not clinically impact the fish.

Florfenicol Concentration: The mean florfenicol concentration in the study feeds were 0, 0.62, 2.04, and 3.44 g/kg of feed and the nominal florfenicol concentration in the feeds were 0, 0.75, 2.25, and 3.75 g/kg of feed, respectively.

6. Conclusion: The study demonstrated that florfenicol medicated feed is safe when administered in feed to tilapia at the maximum therapeutic dose of 15 mg/kg of fish/day for 10 consecutive days.

IV. HUMAN FOOD SAFETY:

A. Microbial Food Safety (Antimicrobial Resistance):

CVM evaluated microbial food safety (antimicrobial resistance) information for AQUAFLOR (florfenicol) for the control of mortality in all finfish due to coldwater disease associated with *Flavobacterium psychrophilum*, furunculosis associated with *Aeromonas salmonicida*, septicemia associated with *Edwardsiella ictaluri*, streptococcal septicemia associated with *Streptococcus iniae*, and columnaris disease associated with *Flavobacterium columnare* using a qualitative risk assessment procedure. This risk assessment procedure involved conducting 1) a *release assessment* to describe the probability that florfenicol and its use in finfish will result in the emergence and dissemination of phenicol-resistant bacteria or

phenicol resistance determinants in finfish under the proposed conditions of use, 2) an *exposure assessment* to describe the likelihood of human exposure to the phenicol-resistant bacteria or phenicol resistance determinants through consumption of edible products from treated finfish, and 3) a *consequence assessment* to describe the potential human health consequences of exposure to finfish-associated, phenicol-resistant bacteria or phenicol resistance determinants by considering the human medical importance of phenicols in the treatment of human infectious diseases.

The outcome of the *release assessment* was determined to be **medium**. The outcome of the *exposure assessment* was determined to be **low**, and the outcome of the *consequence assessment* was determined to be **medium**. These outcomes were integrated into an overall risk estimation of **medium** for florfenicol under the proposed conditions of use.

Decision Statement: Risk management strategies associated with an overall risk estimation of **medium** are compatible with the proposed use of florfenicol, and include a low to medium extent of use in a targeted group of freshwater-reared warmwater finfish, and veterinary oversight of prescription and medication *via* a veterinary feed directive (VFD).

B. Impact of Residues on Human Intestinal Flora:

1. Determination of the need for establishing a microbiological ADI

i. Step 1: Are residues of florfenicol and (or) its metabolites microbiologically active against representative human colonic bacteria?

Yes, the compound is active against relevant human colonic bacteria. This conclusion is substantiated by data derived from a study titled, "Activity of florfenicol against bacterial strains representing the normal human intestinal microbiota: determination of minimum inhibitory concentration," which is summarized below.

Activity of florfenicol against bacterial strains representing normal human intestinal microbiota: determination of minimum inhibitory concentration.
Study No.: 052/06
Report Date: February 16, 2010
Study Director: Andrew Pridmore B.Sc.; Ph.D.
Study Location: Don Whitley Scientific Limited, 14 Otley Road, Shipley, West Yorkshire BD17 7SE United Kingdom

Summary: The study included two phases, where isolates were collected in 2004 and susceptibility testing was performed in 2007. The minimum inhibitory concentration (MIC) of florfenicol against 100 bacterial strains (10 isolates from each of 10 bacterial groups) representing normal human intestinal flora was studied. All bacterial strains were collected from fecal microbiota of healthy, unmedicated human volunteers. The testing system was a standardized agar dilution method as described in Clinical and Laboratory Standards Institute (CLSI) guidelines. For each strain used in the MIC testing, the standardized inoculum was enumerated to demonstrate compliance with CLSI standards.

Results and Conclusions: Florfenicol is active against all bacterial groups tested, with slightly higher MICs for *E. coli* and *Lactobacillus* spp. (see table 11 below). The MIC_{50} of all 10 groups was at or below 8 µg/ml, with half of the groups at 2 µg/ml. The lowest MIC_{50} was for *Fusobacterium* group, at 0.5 µg/ml.

Table 11. Susceptibility of florfenicol against representative bacterial groups from healthy human subjects

Bacterial Group	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**	**MIC Range (µg/ml)**
Bacteroides fragilis group	2	2	All 2
Bacteroides, other species	2	2	1-2
Bifidobacterium spp.	2	2	1-4
Clostridium spp.	2	4	1-4
Enterococcus spp.	2	2	All 2
Escherichia coli	8	8	4-16
Eubacterium spp.	1	2	1-2
Fusobacterium spp.	0.5	2	0.25-4
Lactobacillus spp.	4	8	4-8
Peptostreptococcus spp.	1	2	0.5-4
All isolates (total # of 100)	2	8	0.25-16

Note: MIC_{50} and MIC_{90} data indicate the concentration that inhibited 50% and 90% of the isolates tested, respectively.

Based on susceptibility data, and findings that each bacterial group tested has an MIC_{50} at or below 8 µg/ml, all 10 groups are used in the calculation MIC_{calc}, which will be subsequently used for determination of a mADI.

ii. Step 2: Do florfenicol residues enter the human colon?

Yes, florfenicol and its metabolites enter the human colon. This conclusion was reached based on previously reviewed and accepted study data outlined in the Freedom of Information Summary for florfenicol (NADA 141-206). It was found that recovery of radioactivity in the feces of swine fed radio-labeled florfenicol was in a range of 17 to 22%. In the absence of human data, swine data are considered most closely reflect to humans; thus, it is concluded that about 22% of florfenicol or florfenicol-related metabolites enter the human colon.

iii. Step 3: Do florfenicol residues entering the human colon remain microbiologically active?

Yes, the firm was able to demonstrate through experimentation that about 72% of residues entering the human colon remain biologically active. This conclusion was supported by the following two studies:

1) Study titled, "SCH 25298 (Florfenicol): A Total Residue Depletion Study in Swine Following Oral Administration of 14C-SCH 25298" (Study No. 96618, Report No. P-6853). Description and conclusions of this study are published in the Freedom of Information Summary for florfenicol (NADA 141-206). Results demonstrated that 91.9% of total residues in pooled swine feces can be extracted by methanol. This would yield the most possible "unbound" residues in the feces. In the same study, it was shown that florfenicol amine, florfenicol oxamic acid, and florfenicol alcohol accounted for a minimum of 21.5% of all extractable residues.

2) Study titled, "Determination of microbial inhibition growth."
Study numbers: SLI 1560-0890-6155-790, SLI 1560-0890-6128-790, SLI 1560-0890-6127-790, and SLI 1560-0890-6129-790
Report Date: 12-16-1991
Study Director: Paul H. Fackler, PhD
Study Location: Springborn Laboratories, Inc., Environmental Sciences Division, 790 Main Street, Wareham, MA 02571

Study Summary: Inhibition of microbial growth by florfenicol and three metabolites - metabolites tested included florfenicol amine (SCH 40458), florfenicol oxamic acid (SCH 48057), and florfenicol alcohol (SCH 45705). Bacterial strains tested included *Clostridium perfringens* (ATCC 27058) and *Bacillus subtilis* (ATCC 23059). Florfenicol and its metabolites were serially diluted in an agar for the purpose of determination of MICs.

Results and Conclusions: Of the compounds tested, parent florfenicol had the most potent activity against the two standard strains. There is a significant reduction in activity for the three metabolites tested, with a minimum of 40- to 100-fold lower activity than parent florfenicol for anaerobic and aerobic strains, respectively. MICs of the compounds tested are listed in Table 12. In conjunction with the study above, florfenicol amine, florfenicol oxamic acid, and florfenicol alcohol represent 21.5% of all extractable residues in feces which are not active as compared with the MIC_{calc}, while assuming that the rest of residues are biologically active.

Table 12. MICs (µg/ml) of florfenicol and metabolites

Testing Strain	**Florfenicol (SCH 25298)** **Under SLI Report # 91-2-3675**	**Florfenicol amine (SCH 40458)** **Under SLI Report # 91-1-3612**	**Florfenicol oxamic acid (SCH 40457)** **Under SLI Report # 91-2-3642**	**Florfenicol alcohol (SCH 45705)** **Under SLI Report # 91-1-3615**
Clostridium perfringens (ATCC 27058)	1.0	80	>1,000	40
Bacillus subtilis (ATCC 23059)	0.4	40	>1,000	40

In summary, the projected percentage of residues entering the human colon that are biologically active are 91.9% (extractable portion) X 78.5% (assumed active against reference strains of bacteria), yielding 72%.

iv. **Step 4**: Is there any scientific justification to eliminate testing for either one or both endpoints of concern?

Yes, a determination of a mADI for the endpoint *change in resistant population* is unnecessary at this time. Furthermore, the firm made an assessment that resistance selection and dissemination through human exposure to the relatively low concentration of florfenicol residues in edible animal tissues is minimal; however, the firm proposed that a determination based on the concern over *disruption of colonization barrier* is needed.

2. Determination of the final Microbiological ADI

The mADI for disruption of the colonization barrier of the human colon is determined based on the *in vitro* MIC data and by applying the following formula:

$$ADI = \frac{MIC_{calc} \times \text{Mass of Colon Content (220 g/day)}}{\text{Fraction of oral dose available to microorganisms} \times \text{60 kg person}}$$

Where, parameters are described in the table 13 below.

Table 13. Parameters for the calculation of florfenicol microbiological ADI

MIC_{cacl} (see Step 1)	1.34 µg/ml
Human fecal content	220 grams
Weight of human subject	60 kg
Fraction of oral dose available, a function of the following three factors (See Steps 2 and 3)	0.157
Proportion of florfenicol-related residues entering the colon = 0.218 Proportion of extractable florfenicol-related residues = 0.919 Proportion of microbiologically active extractable residues = 0.785	

In summary, the microbiological ADI is rounded to **31 µg/kg bw/day or 1.9 mg/person/day**.

C. Toxicology:

CVM did not require toxicology studies for this supplemental approval. As described in the FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, a summary of all toxicology studies are contained in the FOI Summary for the original approval of NADA 141-063, NUFLOR injectable solution for cattle, dated May 31, 1996.

D. Assignment of the Final ADI:

Because the toxicological ADI of 10 micrograms per kilogram of bodyweight per day is lower than the calculated microbiological ADI of 31 micrograms/kilogram of bodyweight per day, the toxicological ADI (10 micrograms/kg bw/day) remains the final ADI for total florfenicol residues.

E. Safe Concentrations for Total Residues:

No reassessment of the safe concentrations for total residues was needed for this supplemental approval because the final ADI is not changed. The FOI Summary for the original approval of NADA 141-063, NUFLOR injectable solution for cattle, dated May 31, 1996, contains a summary of all toxicology studies. The FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, contains an assessment of the impact of residues on human intestinal flora.

F. Residue Chemistry

1. Summary of Residue Chemistry Studies

i. Total Residue and Metabolism Study

CVM did not require a total residue and metabolism study for this supplemental approval. The FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, contains a summary of the total residue and metabolism study completed in salmonids.

ii. Comparative Metabolism Study

Comparative metabolism of florfenicol in the rat (the animal used in the toxicity tests) and in salmon was satisfactorily demonstrated by data in the original approval of NADA 141-063 (florfenicol in cattle, FOI Summary dated May 31, 1996) and in studies conducted in salmonids (FOI Summary for the original approval of NADA 141-246 dated October 24, 2005). In addition, the determinative assay for residues uses an acid-catalyzed hydrolysis step to convert parent florfenicol and florfenicol metabolites to a common marker, florfenicol amine.

iii. Residue Depletion Information

Florfenicol residue data from depletion studies conducted to provide data for the NADA file and from articles in the public literature allow withdrawal periods to be established for all freshwater-reared warmwater finfish (maximum dose of 15 mg/kg/day for 10 days) and for all other freshwater-reared finfish (maximum dose of 10 mg/kg/day for 10 days). Residue depletion data in tilapia held in both recirculating systems or in a flow-through tank are provided.

Residue Depletion Study in Tilapia

Depletion of florfenicol amine in tilapia (*Oreochromis* sp.) maintained in a recirculating aquaculture system following Aquaflor-medicated feed therapy (20 mg/kg bodyweight/day for 10 days)

Study No.: N-08-243-01

Study Dates: April 15, 2009-November 5, 2009

Study Director: Mark Gaikowski, M.A.
U.S. Geological Survey
Upper Midwest Environmental Science Center (UMESC)
La Crosse, WI

In-Life Facility: UMESC, La Crosse, WI

Analytical Facility for Tissue Samples: MPI Research, Inc., State College, PA

Analytical Facility for Feed Samples: Eurofins AvTech Laboratories

Analytical Facility for Analysis of Water for Florfenicol and for Contaminants – Intervet Inc., Lafayette, NJ

The study was conducted according to Good Laboratory Practices (21 CFR 58). Two hundred eighty-two tilapia (equally mixed *O. niloticus* x *O. niloticus* and *O. niloticus* x *O. aureus*) with an average bodyweight of 447 grams were held in a recirculating aquaculture system in 27 ºC water. The fish were fed florfenicol-medicated feed at a nominal rate of 0.75 % bodyweight/day for 10 consecutive days. The mean dose consumed was 19.6 mg florfenicol/kg of body weight/day. Samples of muscle with attached skin collected from the tilapia before and after the dosing period were analyzed with the determinative method for florfenicol amine.

Table 14. Florfenicol amine concentrations in muscle/skin of tilapia fed florfenicol in feed at a nominal dose of 20 mg/kg of body weight/day for 10 days

Sampling timepoint (hours)	**Mean ± Std Deviation n=20**
1	13.77±5.21
12	13.45±4.39
24	7.67±3.27
36	5.74±2.58
48	4.97±1.33
72	2.84±1.66
96	2.16±0.78
120	1.38±0.53
240	0.39±0.16

Residue depletion study in rainbow trout

Depletion of Florocol® (50% florfenicol) residues in the tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum) at warm temperature (approx. 15°C)

Study No.: X00-239-01

Study Dates: April 4, 2001-February 28, 2002

Study Directors: Marianne D. Pearson, B.V.M.&S., M.Sc., PhD
William J. Roy, PhD
Institute of Aquaculture
University of Stirling
Stirling, United Kingdom

In-Life Testing Facility: Aquatic Research Facility, Institute of Aquaculture, University of Stirling, Stirling, United Kingdom

Analytical Facilities – ABC Laboratories Europe, Coleraine, Northern Ireland

The study was conducted according to Good Laboratory Practices (21 CFR 58). One hundred thirty-eight rainbow trout (approximately 300 grams bodyweight) were held in flow-through tanks in 15°C water. The fish were fed florfenicol-medicated feed at a rate of 0.4% biomass/day for 10 days. Feed consumption was 100% of target for fish receiving unmedicated feed and 97.8% of target for fish receiving medicated feed. Samples of muscle with attached skin collected from the rainbow trout before and after the dosing period were analyzed with the determinative method for florfenicol amine. Feeding hierarchies among the fish resulted in highly variable residue concentration at each sampling timepoint.

Table 15. Florfenicol residues measured as florfenicol amine (ppm) in muscle/skin of rainbow trout fed 10 mg florfenicol/kg body weight/day for 10 days at 15°C

Sampling Time (Days)	No. of fish per sampling time	Number of fish with residues < LOD of 0.019 ppm	Florfenicol amine (ppm) Mean ± Standard Deviation
1	15	11	5.2 ± 6.6
2	15	11	1.7 ± 1.9
4	15	7	0.87 ± 0.27
7	15	4	0.40 ± 0.12
10	15	10	0.25 ± 0.14
14	15	7	0.17 ± 0.05
21	15	7	0.16 ± 0.03
28	12	4	0.15 ± 0.03

Selected Public Literature References for Florfenicol Residue Data in Fish

Bowser, PR, *et al*. 2009. Florfenicol residues in Nile tilapia after 10-d oral dosing in feed: Effect of fish size. *J Aquatic Animal Health* **21**:14-17. Study funded in part by NRSP-7.

Feng, JB, *et al*. 2008. Tissue distribution and elimination of florfenicol in tilapia (*Oreochromis niloticus* x *O. aureus*) after a single oral administration in freshwater and seawater at 28°C. *Aquaculture* **276**:29-35.

Horsberg, *et al*. 1994. The disposition of ^{14}C-florfenicol in Atlantic salmon (*Salmo salar*). *Aquaculture* **122**:97-106.

Kosoff, RE, *et al*. 2009. Florfenicol residues in three species of fish after 10-day oral dosing in feed. *J Aquatic Animal Health* **21**:8-13. Study funded in part by NRSP-7.

Pinault, L, Millot, L., and P. Sanders. 1997. Absolute oral bioavailability and residues of florfenicol in the rainbow trout. *J. Veterinary Pharm. and Ther.* **20**:294-295.

Wang, W, *et al*. 2009. Tissue distribution and elimination of florfenicol in topmouth culter (*Culter alburnus*) after oral administration. *Czech J of Food Science* **27**:216-221.

Wrzesinski, C, *et al*. 2006. Florfenicol residue deletion in channel catfish, *Ictalurus punctatus*. *Aquaculture* **253**:309-316.

These public literature data provide supporting information about the residue depletion of florfenicol in fish species in various water temperatures and housing conditions. The data support assignment of a 15-day withdrawal for all freshwater-reared warmwater finfish dosed with florfenicol up to 15 mg/kg/day for 10 days and a 15-day withdrawal period for all other freshwater-reared finfish dosed with florfenicol at 10 mg/kg/day for 10 days.

2. Target Tissue and Marker Residue Assignment

   For fish, the target tissue is muscle with adhering skin except for species such as catfish where the skin is not typically consumed by humans.

   Florfenicol amine is assigned as the marker residue because the determinative method converts parent and all metabolites to that compound.

3. Tolerance Assignment

   The tolerance for florfenicol amine is 1 ppm as described in the original approval of NADA 141-246 dated October 24, 2005.

4. Withdrawal Period

   A 15-day withdrawal period is assigned for freshwater-reared warmwater finfish (maximum dose of 15 mg/kg/day for 10 days) and for all other freshwater-reared finfish (maximum dose of 10 mg/kg/day for 10 days).

G. Analytical Method for Residues:

1. Description of the Analytical Method

   The FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, contains the analytical method summaries for florfenicol in catfish. The FOI Summary for the supplemental approval of NADA 141-246 dated March 19, 2007, contains the analytical method summaries for florfenicol in salmonids. The HPLC-UV analytical assays for florfenicol in catfish and salmonids have been combined into one procedure that also includes tilapia.

2. Availability of the Method

   The validated regulatory method for detection and confirmation of residues of florfenicol is available from the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to AQUAFLOR:

"Avoid inhalation, oral exposure, and direct contact with skin or eyes. Operators mixing and handling AQUAFLOR (florfenicol) should use protective clothing, gloves,

goggles and NIOSH-approved dust mask. Wash thoroughly with soap and water after handling. If accidental eye contact occurs, immediately rinse thoroughly with water. If irritation persists, seek medical attention. Not for human consumption. Keep out of reach of children. The Material Safety Data Sheet (MSDS) contains more detailed occupational safety information. For more information or to report adverse effects, call 1-800-224-5318. For customer service, call 1-800-521-5767. For a copy of MSDS sheet, call 1-800-770-8878."

CVM examined the material safety data sheet to conclude that user safety concerns have been appropriately addressed in the labeling.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that AQUAFLOR, when used according to the label, is safe and effective for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish and for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish. Additionally, data demonstrate that residues in food products derived from freshwater-reared finfish treated with AQUAFLOR will not represent a public health concern when the product is used according to the label. The single VFD order form for florfenicol includes multiple freshwater-reared finfish indications because each comprises multiple species and is approved in each for use under similar directions and conditions of use.

Commercially available feeds are commonly used to prepare medicated feeds for use in freshwater-reared finfish species. Consequently, in current practice, feeds having the same or similar composition as salmonid or catfish feeds are often used to deliver an approved new animal drug to non-salmonid and non-catfish freshwater-reared finfish species while meeting the nutritional requirements of these finfish species. Because the nutritional composition of feed may affect characteristics such as the mixing, stability, and accuracy of assay of drugs they contain, an evaluation of these characteristics was performed. The evaluation of florfenicol for use in freshwater-reared finfish was performed on catfish and salmonid feeds in which some common nutritional parameters such as lipids and protein ranged from 5 to 25% and from 28.5 to 55%, respectively.

A. Marketing Status:

A valid veterinary feed directive (VFD) is required to dispense this drug. Any animal feed bearing or containing this drug will be fed to animals only by or on a lawful veterinary feed directive issued by a licensed veterinarian in the course of their professional practice. In addition, the veterinary feed directives issued for this drug are not refillable.

Labeling restricts this drug to use by or on the order of a licensed veterinarian. The decision to restrict this drug to use by or on the order of a licensed veterinarian was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product and (b) restricting this drug to use by or on the order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues. Because the drug will be administered in feed, the drug will be marketed as a VFD drug.

B. Exclusivity:

AQUAFLOR, for the following intended uses, for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish except catfish and for the control of mortality in freshwater-reared warmwater finfish due to streptococcal septicemia associated with *Streptococcus iniae*, qualifies for SEVEN years of exclusive marketing rights beginning as of the date on the approval letter. This new animal drug qualifies for exclusive marketing rights under section 573(c) of the Federal Food, Drug, and Cosmetic Act (the act) because it has been declared a designated new animal drug by FDA under section 573(a) of the act. Except as provided in section 573(c)(2) of the act, we may not approve or conditionally approve another application submitted for such new animal drug with the same intended use.

This supplemental approval for AQUAFLOR qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act because the supplemental approval included safety and effectiveness studies. This exclusivity begins as of the date on the approval letter and only applies to the increase in the maximum dose for the existing enteric septicemia indication for catfish, the new indication for the control of mortality due to columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish, and the new indication for the control of mortality due to streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish.

The exclusive marketing rights and exclusivity for this drug run concurrently.

C. Supplemental Applications:

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.