Date of Approval: March 19, 2007

# FREEDOM OF INFORMATION SUMMARY

## SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-246

AQUAFLOR

Florfenicol
Type A medicated article
Freshwater-reared salmonids

"for the control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*"

Sponsored by:

Schering-Plough Animal Health Corp.

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## I. GENERAL INFORMATION:

| | |
|---|---|
| **A. File Number:** | NADA 141-246 |
| **B. Sponsor:** | Schering-Plough Animal Health Corp.<br>556 Morris Ave.<br>Summit, NJ 07901<br><br>Drug Labeler Code: 000061 |
| **C. Proprietary Name(s):** | AQUAFLOR |
| **D. Established Name(s):** | Florfenicol |
| **E. Pharmacological Category:** | Antimicrobial |
| **F. Dosage Form(s):** | Type A medicated article |
| **G. Amount of Active Ingredient(s):** | 500 g of florfenicol per kg (227.27 g per lb) |
| **H. How Supplied:** | 2-kg foil laminate foil pouches (12 x 16 inches)<br>16-kg fiber board drum (8 x 2-kg pouches) |
| **I. How Dispensed:** | VFD |
| **J. Dosage(s):** | 10 mg florfenicol/kg of fish/day for<br>10 consecutive days |
| **K. Route(s) of Administration:** | Oral |
| **L. Species/Class(es):** | Freshwater-reared salmonids |
| **M. Indication(s):** | For the control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*. |
| **N. Effect(s) of Supplement:** | This supplement provides for the addition of the indication for the control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*. |

## II. EFFECTIVENESS:

### A. Dosage Characterization:

*Flavobacterium psychrophilum* is the bacterial pathogen associated with coldwater disease in freshwater-reared salmonids. This disease is frequently present as a systemic disease that may result in high levels of mortality in affected fish. *F. psychrophilum* is a Gram-negative filamentous rod, and is considered nutritionally fastidious when grown in culture.

The florfenicol dose selected for the effectiveness trial was based on the *F. psychrophilum* minimum inhibitory concentration of florfenicol and the pharmacokinetic profile of florfenicol in various species of fish available in the published literature.

Based on that information, florfenicol administered at a dose of 10 mg per kg of fish daily had the potential to control outbreaks of coldwater disease associated with *F. psychrophilum* in freshwater-reared salmonids.

### B. Substantial Evidence:

The results of the four following studies, when considered together, demonstrate that florfenicol is effective when administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days for the control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum.*

#### 1. Field Study

a. "The Efficacy of Florfenicol-medicated Feed to Control Mortality of Fingerling Steelhead Trout *Oncorhynchus mykiss* Caused by Bacterial Coldwater Disease, Causative Agent *Flavobacterium psychrophilum*" (Study Number FLOR-01-EFF-06)

b. Investigator: Al Jensen
Makah National Fish Hatchery
U. S. Fish and Wildlife Service
Neah Bay, WA

c. Study Design:

1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality due to coldwater disease associated with *F. psychrophilum* in steelhead trout.

2) Study Animals: Approximately 2,420 fingerling steelhead trout

3) Treatment Groups: The study included two treatment groups with six replicates in the untreated control group and five replicates in the florfenicol-treated group. Each replicate was a tank of fish. One florfenicol-treated tank was removed from the study because of an interruption in water flow. 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 florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days.

5) Measurements and Observations: Before fish were transferred to the study tanks, 15 moribund fish with clinical signs characteristic of coldwater disease were collected from the reference population for health evaluation. Fish were examined (body surface, fins, gills, and internal organs) and skin lesion, kidney, and brain tissues were collected for culture. An equivalent number of fish were randomly transferred to the study tanks in four rounds, for a total of approximately 220 fish per tank. The study included a one day acclimation period, a 10-day treatment period, and a 13-day post-treatment period. Dead fish (mortalities) were counted once daily throughout the study. Moribund fish were collected during the treatment period (7 fish) and the post-treatment period (1 fish). Kidney tissue was collected from each fish for culture. Identification of *F. psychrophilum* was confirmed by fluorescent antibody technique. Fish behavior and appetite were evaluated once daily throughout the study.

6) Statistical Methods: 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 for the treatment period, the post-treatment period, and the total study period.

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

**Table 1.** Mortality results for a field effectiveness study in steelhead trout with a 10-day treatment period and 13-day post-treatment period

| Florfenicol Dose (mg/kg of fish) | Percent Cumulative Mortality |
|---|---|
| 0 | 5.0 (66/1,320) |
| 10 | 2.0 (22/1,100) |

There was a statistically significant decrease in mortality in the treated group as compared to the untreated control group for both the treatment period (P=0.0500) and for the total study period (P=0.0332).

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

f. Conclusion: Results from this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day

for 10 days to control mortality due to coldwater disease associated with *F. psychrophilum* in steelhead trout.

2. **Field Study**

   a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Westslope Cutthroat Trout Fry *Oncorhynchus clarki lewisi* Caused by Bacterial Coldwater Disease, Causative Agent *Flavobacterium psychrophilum*" (Study Number FLOR-01-EFF-12)

   b. Investigator: Jay Pravecek
      Washoe Park State Fish Hatchery
      Montana Fish, Wildlife, and Parks
      Anaconda, MT

   c. Study Design:

      1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality due to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

      2) Study Animals: Approximately 8,000 westslope cutthroat trout fry

      3) Treatment Groups: The study included two treatment groups with six 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 florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days.

      5) Measurements and Observations: Before fish were transferred to the study tanks, five moribund fish with clinical signs characteristic of coldwater disease were collected from the reference population for fish health evaluation. Fish were examined (body surface, fins, gills, and internal organs). Brain and collective internal organ tissues were collected for culture. Cultures presumptively identified as *F. psychrophilum* were confirmed using polymerase chain reaction. An equivalent number of fish were randomly transferred to the study tanks in two rounds, for a total of approximately 680 fish per tank. The study included a one-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. Dead fish (mortalities) were counted once daily throughout the study. Moribund fish were collected during the treatment period (36 fish) and the post-treatment period (37 fish). Fish were examined (body surface, fins, gills and internal organs) and skin, gill, brain, and internal organ tissues were collected for culture. Post-treatment mortality was observed for

14 days. Fish behavior and appetite were evaluated once daily throughout the study.

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 the tanks 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 westslope cutthroat trout with a 10-day treatment and 14-day post-treatment periods

| Florfenicol Dose (mg/kg of fish) | Percent Cumulative Mortality |
|---|---|
| 0 | 94.0 (3,632/4,127) |
| 10 | 75.0 (3,090/3,881) |

There was a statistically significant decrease in mortality in the treated group as compared to the untreated control group for the treatment period ($P<0.0001$), the post-treatment period ($P=0.0105$), and the total study period ($P<0.0001$).

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

f. Conclusion: Results from this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 days to control mortality due to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

**3. Field Study**

a. "The Efficacy of Florfenicol-medicated Feed to Control Mortality of Fingerling Westslope Cutthroat Trout *Oncorhynchus clarki* Caused by Bacterial Coldwater Disease, Causative Agent *Flavobacterium psychrophilum*" (Study Number FLOR-01-EFF-04)

b. Investigator: Jim Schreiber
Murray Springs Trout Hatchery
Montana Fish, Wildlife and Parks
Eureka, MT

d. Study Design:

1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality due to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

2) Study Animals: Approximately 30,000 fingerling westslope cutthroat trout

3) Treatment Groups: The study included two treatment groups with four 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 florfenicol/kg of fish daily. Study feeds were fed for 10 consecutive days.

5) Measurements and Observations: The test fish were housed in eight tanks which had each been stocked with an equivalent number fish 24 days prior to the start of the treatment period. On Day 1, three to seven moribund fish showing signs characteristic of coldwater disease were collected from each study tank for fish health evaluation. Fish were examined (body surface, fins, gills, and internal organs), and splenic tissue was collected from each for culture. Cultures presumptively identified as *F. psychrophilum* were confirmed using polymerase chain reaction. On Day 1, the fish in each tank were weighed to estimate the number of fish in each tank. Treatments were then randomly assigned to the test tanks. Dead fish (mortalities) were counted once daily during the 10-day treatment period and during the 14-day post-treatment period. Fish behavior and appetite were observed periodically throughout the study, but data were not recorded.

6) Statistical Methods: The design of this study precluded statistical analysis.

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

**Table 3.** Mortality results for a field effectiveness study in westslope cutthroat trout with a 10-day treatment and 14-day post-treatment periods

| Florfenicol Dose (mg/kg of fish) | Percent Cumulative Mortality |
|---|---|
| 0 | 2.8 (467/16,579) |
| 10 | 1.8 (230/12,977) |

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

f. Conclusion: The level of mortality among the different test tanks was low and exhibited significant variability at the start of the study. This observation indicated that the level of infection in the study tanks may have also varied significantly. However, the results showed a clinically apparent reduction in mortality in the florfenicol-treated fish compared to the untreated control fish. Therefore, results from this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality due

to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

4. **Field Study**

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fingerling Westslope Cutthroat Trout, *Oncorhynchus clarki*, Caused by Bacterial Coldwater Disease, Causative Agent *Flavobacterium psychrophilum*" (Study Number FLOR-01-EFF-03)

b. Investigator: Mark Sweeney
Washoe Park State Fish Hatchery
Montana Fish, Wildlife, and Parks
Anaconda, MT

c. Study Design:

1) Objective: To evaluate the effectiveness of florfenicol administered in feed at a dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days to control mortality due to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

2) Study Animals: Approximately 14,330 fingerling westslope cutthroat trout

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

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

5) Measurements and Observations: Fish with clinical signs characteristic of coldwater disease were collected 2 days prior to the start of the acclimation period for fish health evaluation. Bacteria, characteristic of *F. psychrophilum*, were seen on tissue imprints from external lesions and splenic tissue. *F. psychrophilum* was recovered from cultures of splenic tissue samples. The fish in all four study tanks were combined for weight and size estimates. Equivalent numbers of fish were then randomly transferred back to each of the four study tanks in four rounds, for a total of approximately 3,580 fish per tank. The study included a 7-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. Treatments were randomly assigned on the first day of the treatment period. Mortalities were counted once daily during the treatment period and the post-treatment period.

6) Statistical Methods: The design of the studies precluded statistical

analysis.

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

**Table 4.** Mortality results for a field effectiveness study in westslope cutthroat trout with a 10-day treatment and 14-day post-treatment periods

| Florfenicol Dose (mg/kg of fish) | Percent Cumulative Mortality |
|---|---|
| 0 | 7.3 (440/7,168) |
| 10 | 4.6 (316/7,162) |

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

f. Conclusion: The results showed a clinically apparent reduction in mortality in the florfenicol-treated fish compared to the untreated control fish. Therefore, results from this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 days to control mortality due to coldwater disease associated with *F. psychrophilum* in westslope cutthroat trout.

## 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. When considered together, the data demonstrate that florfenicol is safe when administered to freshwater-reared salmonids at the label dose of 10 mg florfenicol/kg of fish/day for 10 consecutive days.

### A. Margin of Safety Study

1. "Target Animal Safety (TAS) Study on Florfenicol Administered in Feed to Rainbow Trout (*Oncorhynchus mykiss* Walbaum)" (Study No. X00-241-01)

2. Study Director: Marianne Pearson, B.V.M&S, M.Sc., Ph.D. and
William J. Roy, B.Sc., M.Sc., Ph.D.
University of Stirling, Institute of Aquaculture
Stirling, Scotland

3. Study Design:

a. Objective: To assess the safety of florfenicol, administered in feed to rainbow trout (*Oncorhynchus mykiss* Walbaum) for 20 consecutive days (2X the recommended treatment duration) at 1X, 3X, and 5X the recommended dose of 10 mg florfenicol/kg of fish body weight. This study was conducted in accordance with United Kingdom Principles of Good Laboratory Practice which appear comparable to the US FDA Good Laboratory Practice Regulations (21 CFR 58).

b. Study Animals: 240 rainbow trout (*Oncorhynchus mykiss* Walbaum),

approximately one year of age and ranging in weight from 45 to 75 g

c. Treatment Groups: The study included four treatment groups. Each treatment group included three replicates. Each replicate was a tank of fish. Treatments were assigned using a randomized block design.

d. Drug Administration: Florfenicol was administered at 0, 10, 30, and 50 mg florfenicol/kg of fish/day, or 0, 1X, 3X, and 5X, respectively, of the recommended dose of 10 mg florfenicol/kg of fish/day. Florfenicol was administered in commercial trout feed for 20 days.

e. Measurements and Observations: The study included a 17-day acclimation period and a 20-day treatment period. Mortality, fish behavior, feed consumption, and fish appearance were observed throughout the study. After the treatment period, all surviving fish were euthanized and necropsied. Gill, liver, anterior kidney, and posterior kidney were collected from 8 randomly selected fish per tank. In addition, eye, brain, gill, liver, heart, spleen, stomach, anterior and posterior kidney, small intestine, colon, and muscle with attached skin were collected from 2 randomly selected fish per tank. Tissues from the untreated control and 5X treatment groups were examined by a pathologist.

4. Results: Fish in each group consumed >99% of feed that was offered. No mortality or signs of morbidity were observed over the course of the study. No clinically observable changes were detected in fish behavior among the treated fish relative to the controls. No gross abnormalities of the internal organs were observed on necropsy. No morphological differences were observed between the untreated control and 5X groups during the histopathological examination of collected tissues. Therefore, the 1X and 3X treatment group tissues samples were not examined.

5. Conclusion: This study demonstrated that AQUAFLOR (florfenicol) Type A medicated article can be safely administered in feed to rainbow trout at the label dose and duration of 10 mg florfenicol/kg of fish/day for 10 consecutive days.

## B. Margin of Safety Study

1. "SCH 25298: Florfenicol in Atlantic salmon parr: Safety and Efficacy against furunculosis" (Study No. V-079)

2. Study Director: P.H. Richards
   University of Scotland
   Institute of Aquaculture
   Stirling, Scotland

3. Study Design:

   a. Objective: To evaluate the safety of florfenicol administered in feed to Atlantic salmon parr at doses of 10, 50, and 100 mg florfenicol/kg of fish/day. This study was not conducted in accordance with Good Laboratory Practice Regulations (21 CFR 58). A complete copy of the raw data for the safety portion of the study was not available, and study participants, other than the histopathologist, were not masked with regard to treatment assignments.

   b. Study Animals: 100 Atlantic salmon parr with an average weight of approximately 11 g

   c. Treatment Groups: The study included 4 treatment groups. Each treatment group included one replicate. Each replicate was a tank of fish. Two untreated, control groups were included, one held for 10 days and one held for 60 days.

   d. Drug Administration: Florfenicol was administered to fish in one tank at a dose of 100 mg florfenicol/kg of fish/day for 10 consecutive days. Florfenicol was administered to one tank each of fish at doses of 50 mg florfenicol/kg of fish/day for 10 days and 10 mg florfenicol/kg of fish/day for 10 days, followed by 10 days on a normal diet, with this cycle repeated a total of three times.

   e. Measurements and Observations: Feeding and behavior were observed daily. All fish were weighed following the completion of the treatment period. Following the completion of treatments, at either 10 or 60 days, the fish were euthanized, and samples of gill, kidney, and liver were collected for histopathological examination from 10 fish per tank.

4. Results: All medicated diets were consumed well and the fish behaved normally in all the groups. No significant histological abnormalities were observed in any study fish except for high levels of melanin in the kidney samples from all fish. This suggested a period of under feeding, starvation, or inappetence. No specific pathology was associated with the increase in melanin in the kidneys.

5. Conclusion: This study supports the safety of AQUAFLOR (florfenicol) Type A medicated article administered in feed to Atlantic salmon at the label dose and duration of 10 mg florfenicol/kg of fish/day for 10 consecutive days.

## IV. HUMAN FOOD SAFETY:

### A. Toxicology:

CVM did not require toxicology studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-063 (florfenicol in cattle) dated May 31, 1996, contains a summary of all toxicology studies. The ADI for florfenicol is 10 micrograms per kilogram body weight per day. The safe concentration of total drug-related residues is 2 ppm in salmonid muscle/skin.

An assessment was presented on the effects of florfenicol residues present in edible tissues of freshwater-reared salmonids on human intestinal flora. It was concluded that the amount of active florfenicol residues reaching the human colon following a 15-day withdrawal period for freshwater-reared salmonids is probably too low to produce any adverse effect on the human intestinal flora.

### B. Residue Chemistry:

#### 1. Summary of Residue Chemistry Studies

##### a. Total Residue and Metabolism Study

SCH 25298 (Florfenicol): Total residue depletion of $^{14}$C-SCH25298 following a multiple (10-day) oral dose regimen in Atlantic salmon (*Salmo salar)* maintained at 5 °C

Study No. 93702

In-Life Facility – Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island

Analytical Labs – Total residue and metabolism work was conducted at Schering-Plough Research Institute, Lafayette, New Jersey. The determinative assay was performed at Hazelton Wisconsin, Inc., Madison, WI.

The study was conducted according to Good Laboratory Practices (21 CFR 58). Fifty-eight Atlantic salmon (25 months of age, weight range 488 to 793 grams) were used. Fifty-four fish (30 male, 24 female) were test fish. Four fish (2 male, 2 female) were controls. The fish were acclimated for two weeks in 5 ± 0.5 °C seawater. The test fish were fed feed containing 2.7 g florfenicol/kg of feed to obtain an approximate dose of 10 mg florfenicol/kg of fish/day for 9 consecutive days. On Day 10, the test fish were dosed once by oral gavage with 10 mg $^{14}$C-florfenicol/kg body weight. Control fish received nonmedicated feed throughout the study. Six fish were sampled at 3 hours, 12 hours, 1 day, 3 days, 7 days, 15 days, 30 days, 45 days,

and 60 days post-dose. Liver, plasma, kidney, muscle, skin, bone, bile, and retained gut contents were collected from each fish. Tissues were analyzed for total radioactivity by combustion and liquid scintillation counting. The radioactive components of pooled samples of plasma, bile, liver, kidneys, muscle, and skin were extracted and characterized by high pressure liquid chromatography (HPLC). Liver, muscle, and skin samples were analyzed using the determinative method for florfenicol amine.

**Table 5.** Total radioactive residues (μg $^{14}$C-florfenicol equivalents/g) in muscle and in skin of Atlantic salmon maintained at 5 °C and dosed first for 9 days with medicated feed containing 10 mg florfenicol/kg of fish/day and then for 1 day by oral gavage with 10 mg $^{14}$C-florfenicol/kg body weight.

| Withdrawal Time | Muscle (mean ± standard deviation) | Skin (mean ± standard deviation) |
|---|---|---|
| 3 hours | 0.327 ± 0.3254 | 0.453 ± 0.4797 |
| 12 hours | 0.414 ± 1.507 | 4.20 ± 1.801 |
| 1 day | 5.85 ± 3.074 | 5.51 ± 2.843 |
| 3 days | 1.17 ± 0.321 | 1.65 ± 0.527 |
| 7 days | 0.097 ± 0.0155 | 0.506 ± 0.0736 |
| 15 days | 0.027 ± 0.0134 | 0.217 ± 0.1272 |
| 30 days | 0.016 ± 0.0118 | 0.156 ± 0.1395 |
| 45 days | 0.030 ± 0.0216 | 0.247 ± 0.1603 |
| 60 days | 0.008 ± 0.0071 | 0.090 ± 0.067 |

**Table 6.** Florfenicol amine residues (μg florfenicol equivalents/g) in muscle and in skin of Atlantic salmon maintained at 5 °C and dosed first for 9 days with medicated feed containing 10 mg florfenicol/kg of fish/day and then for 1 day by oral gavage with 10 mg $^{14}$C-florfenicol/kg body weight.

| Withdrawal Time | Muscle (mean ± standard deviation) | Skin (mean ± standard deviation) |
|---|---|---|
| 3 hours | 12.5 ± 2.42 | 10.7 ± 3.48 |
| 12 hours | 16.6 ± 6.33 | 15.9 ± 3.10 |
| 1 day | 14.8 ± 5.06 | 17.2 ± 6.51 |
| 3 days | 4.22 ± 1.57 | 6.91 ± 3.90 |
| 7 days | 0.436 ± 0.066 | 1.37 ± 0.485 |
| 15 days | <0.3 | 1.19 ± 0.461 |
| 30 days | <0.3 | 0.416 ± 0.106 |
| 45 days | <0.3 | 0.534 ± 0.156 |
| 60 days | <0.3 | 0.371 ± 0.024 |

**Table 7.** Total radioactive residues (μg $^{14}$C-florfenicol equivalents/g) and florfenicol amine (μg florfenicol amine/g) concentrations in muscle/skin of Atlantic salmon maintained at 5°C and dosed first for 9 days with medicated feed containing 10 mg florfenicol/kg of fish/day and then for 1 day by oral gavage with 10 mg $^{14}$C-florfenicol/kg body weight. Muscle/skin concentrations were calculated using values of 90% muscle and 10% skin as an edible portion.

| Withdrawal Time | Total Residues (ppm) | Florfenicol Amine (ppm) |
|---|---|---|
| 3 hours | 0.339 | 12.34 |
| 12 hours | 0.793 | 16.54 |
| 1 day | 5.816 | 15.02 |
| 3 days | 1.22 | 4.46 |
| 7 days | 0.138 | 0.52 |
| 15 days | 0.046 | 0.38 |
| 30 days | 0.03 | 0.31 |
| 45 days | 0.052 | 0.32 |
| 60 days | 0.016 | 0.30 |

Parent drug and the metabolites, florfenicol amine, florfenicol alcohol, florfenicol oxamic acid, and monochloroflorfenicol, were found in all tissues. The major radioactive residue found in muscle and skin at three hours

withdrawal was parent. By three days withdrawal, the major radioactive residue in muscle was florfenicol amine.

**b. Comparative Metabolism Study**

Comparative metabolism of florfenicol in the rat (the animal used in the toxicity tests) and in salmon has been satisfactorily demonstrated by data in NADA 141-063 (florfenicol in cattle) and in this NADA for freshwater-reared salmonids. 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.

**c. Residue Depletion Studies**

1. Depletion of FLOROCOL (50% florfenicol) residues in the tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum) at low temperature (≈8 °C).

Study No X00-240-01

Principal Investigators: Marianne D. Pearson, Ph.D. and William J. Roy, Ph.D., University of Stirling, Stirling, Scotland

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

Analytical Facilities: ABC Laboratories Europe, Coleraine, Northern Ireland

The in-life portion of the study was conducted following United Kingdom Principles of Good Laboratory Practices. The analytical portion of the study was conducted following OECD Principles of Good Laboratory Practice and United Kingdom Principles of Good Laboratory Practice.

One hundred eighty rainbow trout (presumed to be all female, approximately 200 grams body weight) were used. Thirty fish were controls and 150 were test fish. The fish were acclimated for 81 days during which time the water temperature was adjusted to 8 ± 1 °C. The test fish were fed medicated feed containing 10 mg florfenicol/kg of fish/day for 10 days. The control fish were fed a standard feed. Test fish were sampled at 1, 3, 7, 10, 14, 21, 28, and 35 days after dosing. Residues of florfenicol were measured in muscle/skin using the determinative HPLC method for the marker residue, florfenicol amine.

**Table 8.** Florfenicol residues measured as florfenicol amine (ppm) in muscle/skin of rainbow trout fed 10 mg florfenicol/kg of fish/day for 10 days at 8 °C.

| Withdrawal Time(days) | Number of fish sampled (Number of fish with residues <LOQ) | Mean[1] ± standard deviation |
|---|---|---|
| 1 | 18 (10) | 8.22 ± 6.25 |
| 3 | 18 (8) | 4.05 ± 2.46 |
| 7 | 18 (10) | 0.87 ± 0.432 |
| 10 | 18 (10) | 0.42 ± 0.221 |
| 14 | 18 (2) | 0.34 ± 0.137 |
| 21 | 18 (9) | 0.26 ± 0.100 |
| 28 | 20 (11) | 0.19 ± 0.080 |
| 35 | 21 (10) | 0.20 ± 0.061 |

[1]excludes values below the LOQ of 0.1 ppm

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

Study No. X00-239-01, Report No. 42329

Principle Investigators: Marianne D. Pearson, Ph.D. and William J. Roy, Ph.D., University of Sterling, Stirling, Scotland

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

Analytical Facilities: ABC Laboratories Europe, Coleraine, Northern Ireland

The in-life portion of the study was conducted following United Kingdom Principles of Good Laboratory Practices. The analytical portion of the study was conducted following OECD Principles of Good Laboratory Practice and United Kingdom Principles of Good Laboratory Practice.

One hundred twenty-six rainbow trout (presumed to be all female, approximately 300 grams body weight) were used. Six fish were controls and 120 were test fish. The fish were acclimated for 61 days during which time the water temperatures were adjusted to 15 ± 1 °C. The test fish were fed medicated feed containing 10 mg florfenicol/kg of fish/day for 10 days. The control fish were fed standard feed. The test fish were sampled on days 1, 2, 4, 7, 10, 14, 21, and 28 after dosing. Residues of florfenicol were measured in muscle/skin using the determinative HPLC method for the marker residue, florfenicol amine.

**Table 9.** Florfenicol residues measured as florfenicol amine (ppm) in muscle/skin of rainbow trout fed 10 mg florfenicol/kg of fish/day for 10 days at 15 °C.

| Withdrawal Time (days) | Number of fish sampled (Number of fish with residues <LOQ) | Mean[1] ± 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) | 0.31 ± 0.18 |
| 14 | 15 (7) | 0.17 ± 0.05 |
| 21 | 15 (7) | 0.16 ± 0.03 |
| 28 | 12 (4) | 0.15 ± 0.03 |

[1]excludes values below the LOQ of 0.1 ppm

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. Therefore, the target tissue for freshwater-reared salmonids is muscle/skin.

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

3. **Tolerance Assignments**

Data were not available on the portion of total residues measured by the marker, florfenicol amine, in salmon muscle/skin. The level of marker residues could not be determined when the total residues are 2 ppm (the safe concentration). Therefore, data from a major species were used to establish a tolerance of 1 ppm for florfenicol measured as florfenicol amine in salmon muscle/skin.

4. **Withdrawal Time**

A 15-day withdrawal time was calculated using 99% statistical tolerance and 95% confidence with the residue depletion data in Study No. X00-240-01 (8 °C) and a tolerance of 1 ppm.

## C. Microbial Food Safety:

The Agency assessed available microbial food safety information on the use of florfenicol in freshwater-reared salmonids. This assessment characterized the hazard associated with the use of florfenicol under the proposed conditions of use in

freshwater-reared salmonids. The hazard is defined as human illness, caused by antimicrobial-resistant bacteria, attributable to an animal-derived food commodity (fish), and treated with the human antimicrobial drug of interest (chloramphenicol).

It was determined that the magnitude of the hazard associated with the use of florfenicol in freshwater-reared salmonids was compatible with the proposed conditions of use as a Type A Medicated Article: 10 mg florfenicol/kg of fish/day for 10 consecutive days for the control of mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*.

**D. Analytical Method for Residues:**

**1. Determinative Method**

The HPLC determinative procedure approved under NADA 141-063 for bovine tissues was successfully validated according to the Agency's guidelines for the quantitation of florfenicol amine (marker residue) residues in the edible tissues of salmonids (muscle/skin) receiving AQUAFLOR.

The determinative assay for the marker residue, florfenicol amine, in the edible tissues, is an HPLC method that provides acceptable sensitivity, specificity, accuracy, and precision for the routine monitoring of florfenicol residues in salmonids. Florfenicol residues (and those of related metabolites) are converted to the marker residue, florfenicol amine, by acid-catalyzed hydrolysis. The determinative procedure was successfully validated at 1 ppm in an independent laboratory.

**2. Confirmatory Method**

The FOI Summary for the original approval of NADA 141-246 dated October 24, 2005, contains the confirmatory method summary for florfenicol in salmonids.

**3. Availability of 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 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 the MSDS sheet, call 1-800-770-8878."

We looked at 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 in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*. Additionally, the data demonstrate that residues in food products derived from freshwater-reared salmonids 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 both catfish and freshwater-reared salmonid indications because each comprises multiple species and is approved in each for use under similar directions and conditions of use.

### A. Marketing Status:

This drug may be dispensed only under a valid Veterinary Feed Directive (VFD). Any animal feed bearing or containing this VFD drug will be fed to animals only by or on a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice. In addition, 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, (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, and (c) the rate of emergence of florfenicol-resistant organisms may be reduced by the involvement of veterinarians in product use. Because the drug will be administered in feed, the drug will be marketed as a VFD drug.

### B. Exclusivity:

Under section 573(c) of the Federal Food, Drug, and Cosmetic Act (the Act), this approval qualifies for SEVEN years exclusive marketing rights beginning on the date of approval because the new animal drug has been declared a designated new animal drug by FDA under section 573(a) of the Act.

### 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:

The sponsor did not submit any patent information with this application.

## VII. ATTACHMENTS:

Facsimile Labeling:

AQUAFLOR (florfenicol) Type A medicated article label 2 kg
AQUAFLOR (florfenicol) Type A medicated article label 8 x 2 kg
AQUAFLOR (florfenicol) Type C medicated feed label for fresh-water reared Salmonids
AQUAFLOR (florfenicol) VFD Form