Date of Approval: October 26, 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 furunculosis associated with *Aeromonas salmonicida*"

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. 556 Morris Ave. Summit, NJ 07901 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) 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 10 consecutive days
K. Route(s) of Administration:	Oral
L. Species/Class(es):	Freshwater-reared salmonids
M. Indication(s):	For the control of mortality due to furunculosis associated with *Aeromonas salmonicida*.
N. Effect(s) of Supplement:	This supplement provides for the addition of an indication for the control of mortality in freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida*.

II. EFFECTIVENESS:

A. Dosage Characterization:

Aeromonas salmonicida is the bacterial pathogen associated with furunculosis in freshwater-reared salmonids. This disease is frequently present as a systemic disease that may result in high levels of mortality in affected fish.

The florfenicol dose selected for the effectiveness trials was based on the *A. salmonicida* minimum inhibitory concentration of florfenicol, the pharmacokinetic profile of florfenicol in salmonids, and the results of field studies for saltwater-reared salmonids available in the published literature.

Based on that information, florfenicol administered at a dose of 10 mg/kg of fish daily had the potential to control furunculosis outbreaks associated with *A. salmonicida* in freshwater-reared salmonids.

B. Substantial Evidence:

The results of the three 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 furunculosis associated with *Aeromonas salmonicida*.

1. Field Study

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fingerling Coho Salmon, *Oncorhynchus kisutch*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*" (Study Number FLOR-01-EFF-01)

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 in fingerling coho salmon due to furunculosis associated with *A. salmonicida*.

2) Study Animals: Approximately 2,400 fingerling coho salmon

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. One

florfenicol-treated tank was removed from the study because of an interruption in water flow.

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) Measurement and Observations: *A. salmonicida* was identified on cultures of spleen and kidney tissue collected 5 days before the start of the treatment period (60 fish), and on the first day of the treatment period (54 fish). The clinical signs observed were consistent with a furunculosis infection. Approximately 220 fish were randomly transferred to the study tanks. The study included a one-day acclimation period, a 10-day treatment period, and a 7-day post-treatment period. Dead fish (mortalities) were counted and recorded twice daily. On Day 18, six fish were collected from each tank for examination and collection of samples for culture. Fish examinations on Day 18 revealed an infection with the external parasite, *Trichodina* sp. The study was ended and treatment for the parasite infestation was initiated.

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

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

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

Florfenicol Dose (mg/kg of fish)	**Percent Cumulative Mortality**
0	30.3 (331/1,092)
10	11.1 (145/1,306)

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

f. Conclusion: Results of this study support the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in coho salmon, *Oncorhynchus kisutch*, due to furunculosis associated with *Aeromonas salmonicida*.

2. Field Study

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Fall Chinook Salmon, *Oncorhynchus tshawytscha*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*" (Study No. FLOR-01-EFF.3-22)

b. Investigator: Randy Rickert
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 in fall chinook salmon fingerlings due to furunculosis associated with *A. salmonicida*.

2) Study Animals: Approximately 1,812 fall chinook salmon fingerlings

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 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. The trial feed was assayed to confirm the florfenicol concentration.

5) Measurement and Observations: Fish were held in one raceway prior to allocation to study tanks. Dead, moribund, and healthy fish were collected 5 days prior to the start of the acclimation period for examination. *A. salmonicida* was identified on cultures and imprints of kidney tissue. Equivalent numbers of fish were transferred to each study tank in five rounds, for a total of approximately 151 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 or twice daily. Dead fish were collected during the treatment period (31 fish) and the post-treatment period (10 fish). Kidney tissue was collected from each fish for culture. *A. salmonicida* was identified via cultures of kidney tissue collected from the fish. Fish behavior and appetite were observed daily.

6) Statistical Analysis: Arcsine-transformed Kaplan Meier estimates of survival were calculated for each tank. Means of treated and control tanks were then compared using a t-test. Significant differences were seen between treated and control groups immediately after treatment, after treatment plus a 10-day holding period, and after treatment plus a 14-day holding period. Mixed model repeated measures analyses using a binomial distribution with canonical logistic link showed that most of the differences in cumulative survival were due to reduced daily mortality rates among treated individuals from 4 to 18 days after the start of florfenicol administration.

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

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

Florfenicol Dose (mg/kg of fish)	Percent Cumulative Mortality (Cumulative Mortality)
0	94.4 (808)
10	16.2 (141)

The treated and untreated control groups differ significantly in the cumulative percent mortality ($p<0.001$).

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

f. Conclusion: Results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in fingerling fall chinook salmon, *Oncorhynchus tshawytscha*, due to furunculosis associated with *A. salmonicida*.

3. Field Study

a. "The Efficacy of Florfenicol-Medicated Feed to Control Mortality of Coho Salmon, *Oncorhynchus kisutch*, Caused by Furunculosis, Causative Agent *Aeromonas salmonicida*" (Study No. FLOR-01-EFF.3-27)

b. Investigator: Randy Rickert
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 in coho salmon fingerlings due to furunculosis associated with *A. salmonicida*.

2) Study Animals: Approximately 1,920 coho salmon fingerlings

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 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. The trial feed was assayed to confirm the florfenicol concentration.

5) Measurement and Observation: Fish were held in one raceway prior to allocation to experimental tanks. Dead and moribund fish were collected

2 days prior to the start of the acclimation period for examination and tissue collection for culture. *A. salmonicida* was identified on cultures of kidney tissue. Equivalent numbers of fish were randomly transferred to each study tank in four rounds, for a total of approximately 160 fish per tank. The study included a 2-day acclimation period, a 10-day treatment period, and a 14-day post-treatment period. Dead fish (mortalities) were counted once or twice daily. Moribund fish were collected during the treatment period (3 fish) and the post-treatment period (2 fish). Kidney tissue was collected from each fish for culture. *A. salmonicida* was identified via cultures of kidney tissue collected from control group fish. Fish behavior and appetite were observed daily.

6) Statistical Analysis: Arcsine-transformed proportion survival was calculated for each tank. Means of treated and control tanks were then compared using a t-test. Significant differences were seen between treated and control groups immediately after treatment, after treatment plus a 10-day holding period, and after treatment plus a 14-day holding period. Mixed model repeated measures analyses on arcsine transformed daily mortality rates showed that most of the differences in cumulative survival were due to reduced daily mortality rates among treated individuals from 8 to 19 days after the start of florfenicol administration.

d. 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 7-day post-treatment period.

Florfenicol Dose (mg/kg of fish)	**Percent Cumulative Mortality (Cumulative Mortality)**
0	29.5 (282/956)
10	16.0 (151/946)

The treated and untreated control groups differ significantly in the cumulative percent mortality ($p<0.005$).

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

f. Conclusion: Results of this study demonstrate the effectiveness of florfenicol administered in feed at a dose of 10 mg/kg of fish/day for 10 consecutive days to control mortality in fingerling coho salmon, *Oncorhynchus kisutch*, due to furunculosis associated with *A. salmonicida*.

III. TARGET ANIMAL SAFETY:

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-246 dated March 19, 2007,

contains a summary of target animal safety studies for the use of florfenicol at a dose of 10 mg/kg of fish/day for 10 consecutive days in freshwater-reared salmonids.

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, and for the supplemental approval of NADA 141-246 dated March 19, 2007, contain summaries 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.

B. Residue Chemistry:

CVM did not require residue chemistry studies for this supplemental approval. The FOI Summary for the supplemental approval of NADA 141-246 dated March 19, 2007, contains a summary of residue chemistry studies for freshwater-reared salmonids.

C. Microbial Food Safety:

CVM considered the impact of adding a claim for the control of mortality due to furunculosis to the approved label for florfenicol for freshwater-reared salmonids on microbial food safety, and determined that additional microbial food safety information for this supplemental approval was not required. The FOI Summary for the supplemental approval of NADA 141-246, dated March 19, 2007, contains microbial food safety information regarding the use of florfenicol in freshwater-reared salmonids.

D. Analytical Method for Residues:

The FOI Summary for the supplemental approval of NADA 141-246 dated March 19, 2007, contains the analytical method summaries for florfenicol in salmonids.

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 a copy of the MSDS sheet, call 1-800-770-8878. For more information or to report adverse effects, call 1-800-211-3573."

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 furunculosis associated with *Aeromonas salmonicida*. Additionally, 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.

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:

AQUAFLOR in the dosage form and for the intended uses approved by FDA, for the control of mortality in freshwater-reared salmonids due to furunculosis associated with *Aeromonas salmonicida*, qualifies for seven years of exclusive marketing rights beginning as of the date of approval. 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.

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