Date of Approval: OCT 2 4 2005

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-246

AQUAFLOR Type A Medicated Article (florfenicol),
An Antibiotic

For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

Sponsored by:

Schering-Plough Animal Health Corporation

1. GENERAL INFORMATION

a. File Number:	NADA 141-246
b. Sponsor:	Schering-Plough Animal Health Corporation 1095 Morris Ave. Union, NJ 07083 Drug Labeler Code: 000061
c. Established Name:	Florfenicol
d. Proprietary Name:	AQUAFLOR Type A Medicated Article (florfenicol), An Antibiotic
e. Dosage Form:	Medicated feed
f. How Supplied:	2-kg foil laminate foil pouches (12 x 16 inches) 16-kg fiber board drum (8 x 2-kg pouches)
g. How Dispensed:	Veterinary Feed Directive
h. Amount of Active Ingredients:	500 g of florfenicol per kg
i. Route of Administration:	Oral via feed
j. Species/Class:	Catfish
k. Recommended Dosage:	10 mg of florfenicol per kg of body weight for 10 consecutive days
l. Pharmacological Category:	Antimicrobial
m. Indications:	For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

2. *EFFECTIVENESS:*

a. *Dosage Characterization:*

The effectiveness of florfenicol was evaluated for the control of mortality associated with enteric septicemia of catfish (ESC) during a range-finding and dose titration study. The florfenicol formulation used was the commercial formulation and was administered in feed. For both studies, the ESC infection was induced by immersion challenge with *Edwardsiella ictaluri*, the pathogen associated with ESC.

The range-finding study included five treatment groups: 1) not challenged with *E. ictaluri* and fed unmedicated feed, 2) challenged with *E. ictaluri* and fed unmedicated feed, 3) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 10 mg/kg/day for 5 days, 4) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 20 mg/kg/day for 5 days, 5) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 40 mg/kg/day for 5 days. Each treatment group included four tanks with 20 fish per tank (400 total fish). The fish were challenged on Day 0, florfenicol was administered on Days 1 through 5, and monitored for 17 days following treatment. Morbidity and mortality were monitored during the treatment and post-treatment period. Following the post-treatment period all surviving fish were euthanized, examined by gross necropsy and histopathology, and evaluated for the presence of *E. ictaluri*.

A 5-day regimen of 10, 20, or 40 mg florfenicol/kg body weight/day resulted in 0, 1.25, and 1.25% cumulative mortality, respectively. The cumulative mortality for untreated, challenged fish was 57.5% and for untreated, unchallenged fish was 2.5%.

The dose titration study included five treatment groups: 1) not challenged with *E. ictaluri* and fed unmedicated feed, 2) challenged with *E. ictaluri* and fed unmedicated feed, 3) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 5 mg/kg/day for 10 days, 4) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 10 mg/kg/day for 10 days, 5) challenged with *E. ictaluri* and fed medicated feed at a dose rate of 15 mg/kg/day for 10 days. Each treatment group included six tanks with 20 fish per tank (600 total fish). The fish were challenged on Day 0, florfenicol was administered on Days 1 through 10, and monitored for 14 days following treatment. Morbidity and mortality were monitored during the treatment and post-treatment period. All dead fish were assessed microbiologically for the presence of *E. ictaluri*. Following the post-treatment period all surviving fish were euthanized, examined by gross necropsy and histopathology, and evaluated for the presence of *E. ictaluri*.

A 10-day regimen of 5, 10, or 15 mg florfenicol/kg body weight/day resulted in 4.2, 0.8, and 2.5% cumulative mortality, respectively. The unchallenged, untreated group had no mortalities and the challenged, untreated group had 60.0% mortalities.

A dose rate of 10 mg/kg/day administered for 10 consecutive days was selected for the dose confirmation study due to anticipated variability in individual fish responses under field conditions and the potential that fish infected with *E. ictaluri* may not consume medicated feed as readily as required to deliver an effective dosage of florfenicol.

b. *Substantial Evidence*

1. Dose Confirmation Study, Study No. X00-088-01, Report No. 39963

Title: Florfenicol use in channel catfish (*Ictalurus punctatus*) for treatment of *Edwardsiella ictaluri*: a dose confirmation study

Study Director: Patricia A. Gaunt, D.V.M., Ph.D.

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

General Design of the Study:

a. Purpose: To confirm the appropriate dose rate and duration of administration of florfenicol for the control of mortality associated with enteric septicemia of catfish caused by *E. ictaluri* in channel catfish.

b. Animals: Channel catfish, *Ictalurus punctatus,* fingerlings that ranged in weight from 6.0 to 10.6 g and in age from 150 to 180 days were used. A total of 600 fingerlings were stocked into 30 fish tanks (20 fish/tank). Tanks were supplied with freshwater from wells at temperatures ranging from 25 to 28 °C. Water temperature, pH, chloride, nitrite, ammonia, hardness, and alkalinity levels were recorded.

c. Test article/controls: Florfenicol was incorporated into catfish feed pellets. The test rations were prepared to contain 0 and 400 milligrams florfenicol per kg of basal diet to supply 0 and 10 mg/kg body weight daily respectively when fed at 2.5% of body weight.

d. Study Design: The dose confirmation study was conducted with laboratory-reared channel catfish fingerlings held in 80 L tanks. Fish were evaluated by a modified agglutination assay to determine their immunological status relative to *E. ictaluri* prior to inclusion in the study. Fifteen (15) tanks of fish were assigned to each of the two treatment groups: 1) challenged with *E. ictaluri* and treated with florfenicol or 2) challenged with *E. ictaluri* and not treated. After a 21-day acclimation period, fish were exposed to *E. ictaluri* in water on Day 0 and fed unmedicated feed through Day 1. Starting on Day 2, treated fish received florfenicol-medicated feed for 10 consecutive days (Days 2-11), and untreated fish received unmedicated feed. All fish were monitored for morbidity/mortality during acclimation, during the 10-day dose administration period, and during the 14-day post-

treatment observation period. After the observation period, all surviving fish were euthanized, examined by gross necropsy, and evaluated for the presence of *E. ictaluri* by bacterial culture (isolation and determination of the minimum inhibitory concentration). One florfenicol-treated tank was excluded from the study and the statistical analysis due to inadequate evidence of infection by *E. ictaluri* as the cause of mortality.

e. Parameters measured: Mortality, feeding activity, and water quality parameters were noted throughout the trial. All dead fish were assessed microbiologically for the presence of *E. ictaluri*.

Results: Mortality results are included in the following table.

Table 1. Cumulative mortality for the 10-day treatment period and 14-day post-treatment period for a dose confirmation study in channel catfish.

Florfenicol Dose (mg/kg)	Cumulative Mortality	Mean Tank Percent Cumulative Mortality
0	262	87.3
10	27	9.5

The MIC (minimum inhibitory concentration) of florfenicol for this strain of *E. ictaluri* was 0.25 µg/mL in all 26 fish that were assayed. The mean Kirby-Bauer zone of inhibition for 285 of the 286 isolates was 34.5 mm (range: 32 to 41 mm) from all fish from which *E. ictaluri* was isolated.

Statistical Analysis: Data were analyzed by Logistic Regression using a General Linear Mixed Model with fish nested within tank and tank nested within treatment. The mortality in the dose group treated with florfenicol was significantly ($p<0.001$) lower than the control group.

Conclusion: Florfenicol administered to channel catfish, *Ictalurus punctatus*, at a dose of 10 mg/kg body weight per day for 10 consecutive days is effective for the control of mortality due to enteric septicemia of catfish associated with *E. ictaluri*.

2. Clinical Field Effectiveness Trial, Study No. X01-027-01, Report No. 40128

Title: Florfenicol use in channel catfish (*Ictalurus punctatus*) for control of mortality associated with *Edwardsiella ictaluri*: a pond study

Study Director: Patricia A. Gaunt, D.V.M., Ph.D.

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

General Design:

a. Purpose: To confirm the appropriate dose rate and duration of

administration of florfenicol for the control of mortality associated with enteric septicemia of catfish caused by *E. ictaluri* in channel catfish under field conditions in small ponds.

b. Animals: Approximately 154,000 channel catfish fingerlings 150 to 180 days of age that weighed 6.6 to 7.8 g were used in the study. Fish were allocated to each of fourteen 0.1-acre ponds at the rate of approximately 11,000 fish/pond.

c. Test article: Florfenicol was incorporated into catfish feed pellets. The two trial rations were prepared to contain 0 and 400 milligrams florfenicol per kg of basal diet to supply 0 and 10 mg/kg body weight daily respectively when fed at 2.5% of body weight.

d. Study Design: The pond study was conducted with channel catfish fingerlings held in ponds. Fish were from an ESC-free facility. Ponds were assigned to 2 treatment groups, one group received florfenicol-medicated feed and one group received unmedicated feed. The ponds were challenged with *E. ictaluri* either naturally or by exposure to fish challenged with *E. ictaluri* cultured from a naturally occurring outbreak added to the water. Ponds were observed until the cumulative morbidity/mortality rate attributable to ESC based on clinical signs and/or lesions reached 0.3% per pond. Ponds received the assigned test ration for 10 consecutive days and were monitored for morbidity/mortality. Throughout the study moribund fish were counted as mortalities. After the treatment period, ponds were observed for a 14-day post-treatment observation period during which dead and/or moribund fish were collected, examined by gross necropsy and the presence of *E. ictaluri* was determined microbiologically. A maximum of 5 moribund/dead fish that were not degraded by autolysis which could interfere with bacterial isolation were cultured per week from each pond for isolation of *E. ictaluri*. At the end of the post-treatment observation period, the ponds were harvested, the fish harvested were counted and euthanized, and 20 fish from each pond were examined by gross necropsy and evaluated for the presence of *E. ictaluri* by bacterial culture. MICs were determined on a maximum of 15 fish from each pond. Two of the florfenicol-treated ponds were excluded from the study because florfenicol-medicated feed was administered following the 10-day treatment period.

e. Parameters Measured: Mortality, feed consumption, and microbiological assessments to confirm the presence of *E. ictaluri* in morbid/dead fish and the sensitivity of *E. ictaluri* isolates to florfenicol. Water quality parameters were monitored.

Results: Mortality and harvest results are included in the following table.

Table 2. Cumulative mortality and harvest results for the 10-day treatment period and 14-day post-treatment period for a field study in channel catfish.

Florfenicol Dose-mg/kg (Number of Ponds)	Percent Cumulative Mortality* (Cumulative Mortality)	Percent Recovery at Harvest* (Number)	Percent Missing after Harvest* (Number)
0 (7)	3.0 (2300)	59.4 (45,579)	37.6 (28,891)
10 (5)	2.3 (1256)	64.9 (35,563)	32.8 (17,996)

*Based on the number of fish allocated minus pre-treatment mortality to the ponds.

The missing fish can be attributed to deaths due to handling during stocking, bird parasitism, autolysis, and cannibalism.

The MIC (minimum inhibitory concentration) of florfenicol for this strain of *E. ictaluri* was 0.25 µg/mL in all fish that were assayed. The mean Kirby-Bauer zone of inhibition was 36.8 mm (range: 32 to 50 mm) from all fish from which *E. ictaluri* was isolated.

Conclusion: Florfenicol administered to channel catfish, *Ictalurus punctatus*, at a dose of 10 mg/kg/day for 10 consecutive days is effective for the control of mortality due to enteric septicemia of catfish associated with *E. ictaluri*.

c. ***Microbiology***

1. **Dose Selection Study, Study No. 97-049, Report No. 44210**

Title: Preliminary Assessment of Florfenicol for Use in Channel Catfish (*Ictalurus punctatus*) for Treatment of *Edwardsiella ictaluri*: a Range Finding Study

Study Director: Patricia A. Gaunt, D.V.M., Ph.D.

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

General Design of the Study:

a. Purpose: To determine the *in vitro* minimum inhibitory concentration (MIC) of florfenicol against *E. ictaluri* and to determine the susceptibility of *E. ictaluri* to florfenicol by the Kirby-Bauer technique using florfenicol impregnated discs.

b. Procedures: Twelve isolates of *E. ictaluri* obtained from infected channel catfish in Mississippi (1994, 1996, and 1997) were characterized to determine susceptibility to florfenicol in terms of the minimum inhibitory concentration and the zone of inhibition by the Kirby-Bauer method.

Minimum Inhibitory Concentration: Florfenicol was serially diluted in agar at concentrations of 0, 0.002, 0.004, 0.008, 0.016, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 µg/mL and poured into two plates at each concentration. Plates were inoculated with an *E. ictaluri* strain, cultured at 27 °C for 2 days and observed to determine which concentration completely inhibited growth of *E. ictaluri.*

Zone of Inhibition (Kirby Bauer technique): Plates containing Mueller-Hinton medium with 5% sheep blood were inoculated with *E. ictaluri.* A disc impregnated with 30 µg florfenicol was placed on each plate. Plates were cultured for 2 days at 27 °C and the zone of bacterial growth inhibition was measured (mm) in accordance with the current NCCLS guidelines.

Results: The MIC of florfenicol for 12 isolates of *E. ictaluri* was 0.25 µg/mL. The mean zone of inhibition for the 12 *E. ictaluri* isolates by the Kirby-Bauer technique was 46.8 mm (range: 41 to 51 mm).

Conclusion: The 12 field isolates of *E. ictaluri* obtained from channel catfish in Mississippi during 1994, 1996, and 1997 appear to be susceptible to florfenicol *in vitro.*

2. Minimum Inhibitory Concentrations (MIC) Data

In vitro investigations of certain bacterial fish pathogens have demonstrated florfenicol's activity range. These findings are summarized in **Table 3.**

Table 3. Minimum inhibitory concentrations of florfenicol against selected fish pathogens

Organism	No. of Isolates	MIC (µg/mL)	Year
Edwardsiella ictaluri	12	0.25	1998
Edwardsiella ictaluri	1	0.25	1998
Edwardsiella ictaluri	16	0.25	2000
Edwardsiella ictaluri	26	0.25	2000
Edwardsiella ictaluri	40	0.25	2001

3. TARGET ANIMAL SAFETY:

a. Study No. 97-049, Report No. 44211

This non-GLP study was conducted to determine the appropriate dose rate of florfenicol administered in feed to catfish for the control of mortality associated with enteric septicemia caused by infection with *E. ictaluri.* As part of the study all fish were necropsied at the end of the study and findings from gross pathological and histopathological examinations were recorded. The study methods are summarized in the effectiveness section of this document.

The fish had external and internal lesions compatible with published lesions for enteric septicemia. No lesions indicative of any concurrent diseases were observed. An increased degree of inflammatory cell infiltrate occurred in the liver, heart, gills, anterior kidney, and spleen of the untreated/challenged fish compared to the unchallenged and florfenicol-treated fish.

No significant changes attributable to treatment with florfenicol were observed upon gross necropsy of the skin, fins, mouth, gills, eyes, and viscera. No significant changes attributable to treatment with florfenicol were observed upon histopathological examination of the anterior kidney, posterior kidney, brain, gill, heart, liver, or spleen.

Conclusions: Florfenicol administered in feed to channel catfish, *Ictalurus punctatus*, at dose rates of 10, 20, and 40 mg/kg/day for 5 consecutive days caused no significant histopathological changes in the anterior kidney, posterior kidney, brain, gill, heart, liver, or spleen attributable to treatment.

b. Tolerance Study in Catfish, Study No. 97-049, Report No. 44214

Title: Preliminary Assessment of Florfenicol for Use in Channel Catfish (*Ictalurus punctatus*) for Treatment of *Edwardsiella ictaluri*: A Tolerance Study.

Study Director: Patricia A. Gaunt, D.V.M., Ph.D.

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

General Design of the Study:

a. Purpose: To determine the tolerance of channel catfish for florfenicol when assessed by toxicological and histological methods.

b. Animals: Channel catfish fingerlings in the weight range of 36.9 to 48.5 g were used. A total of 400 fingerlings were stocked into 20 tanks (20 fish/tank). Tanks were supplied with fresh water from wells at temperatures ranging from 21 to 27 °C. Water temperature, pH, chloride, nitrite, ammonia, hardness, and alkalinity levels were recorded.

c. Test article/controls: Florfenicol was incorporated into catfish feed pellets. Trial rations were prepared to contain 0, 400, 800, 1,600, and 4,000 milligrams florfenicol per kg of basal diet to supply 0, 10, 20, 40, and 100 mg/kg body weight daily respectively when fed at 2.5% of body weight.

d. Study Design: The study was conducted with laboratory-reared channel catfish fingerlings held in 120 L tanks. Four tanks were assigned to each of five treatment groups: 1) fed unmedicated feed, 2) fed 10 mg florfenicol/kg body weight (bw), 3) fed 20 mg florfenicol/kg bw, 4) fed 40 mg florfenicol/kg bw, and 5) fed 100 mg florfenicol/kg bw. After the acclimation period, fish

were fed either unmedicated feed or medicated feed for 10 consecutive days. All fish were monitored for feeding activity, mortality, and morbidity for the 10-day treatment period. After the treatment period, all surviving fish were euthanized, necropsied and examined by histopathology.

e. Parameters Measured: Mortality and feeding activity were noted throughout the trial as were water quality parameters. In addition, all fish were necropsied at the end of the study and findings from gross pathological and histopathological examinations were recorded.

GLP Compliant: No

Results: No mortality occurred during the study. The feeding activity of the control fish was vigorous throughout the study. The feeding activity of the treated groups was slightly decreased during the first three days of the treatment period, but was vigorous for the remainder of the treatment period. A 10-day regimen of 0, 10, 20, 40, or 100 mg florfenicol/kg body weight/day resulted in a 16.25%, 15.25%, 20.0%, 14.0%, and 14.5% weight gain respectively. No significant changes attributable to treatment with florfenicol were observed upon gross necropsy of the skin, fins, mouth, gills, eyes, and viscera. No significant changes attributable to treatment with florfenicol were observed upon histopathological examination of the anterior kidney, posterior kidney, brain, gill, heart, liver, or spleen.

Conclusions: No significant changes attributable to treatment with florfenicol were observed upon gross necropsy or histopathological examination of the experimental fish.

c. Safety Study in Catfish, Study No. X00-242-01, Report No. 45485

Title: Target Animal Safety Study of Aquaflor (50% Type A Medicated Article), Florfenicol – SCH25298, Administered in Feed to Channel Catfish, *Ictalurus punctatus*.

Study Director: Mark P. Gaikowski, M.A.

Study Location: U.S.G.S. Biological Resources Division
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of the Study:

a. Purpose: To determine the safety of Aquaflor (50% Type A Medicated Article), administered in feed to channel catfish, *Ictalurus punctatus*, at doses of 1X, 3X, and 5X the recommended dose rate of 10 mg/kg body weight for twice the recommended treatment duration of 10 consecutive days.

b. Animals: Channel catfish fingerlings with a mean fish weight per tank of 19.1 to 22.3 g were used. A total of 240 fingerlings were stocked into 12 tanks (20 fish/tank). Tanks were supplied with fresh water from wells at temperatures ranging from 26.3 to 29 °C.

c. Test article/controls: Florfenicol was incorporated into catfish feed pellets. Trial rations were prepared to contain 0, 500, 1,500, and 2,500 mg florfenicol per kg of basal diet to supply 0, 10, 30, and 50 mg/kg body weight daily respectively when fed at 2.0% of body weight.

d. Study Design: The study was conducted with laboratory-reared channel catfish fingerlings held in 80 L tanks. Three tanks were assigned to each of four treatment groups: 1) fed unmedicated feed, 2) fed 10 mg florfenicol/kg (body weight) bw, 3) fed 30 mg florfenicol/kg bw, and 4) fed 50 mg florfenicol/kg bw. After the acclimation period, fish were fed either unmedicated feed or medicated feed for 20 consecutive days. Fish were monitored for feeding activity, mortality, and morbidity. After the treatment period, fish were necropsied and examined histopathologically.

e. Parameters Measured: Mortality, feeding activity, and water quality parameters were noted throughout the trial. In addition, all fish were necropsied at the end of the study and findings from gross pathological and histopathological examinations were recorded.

GLP Compliant: Yes

Statistical Methods: No fish died during treatment, and no fish morbidity was observed during the treatment period. No analysis of mortality or morbidity was conducted. For the histopathological results, the proportion of fish in a tank with a particular pathological lesion was analyzed by logistic regression in a general linear mixed model, using fish (observation unit) nested within tank (experimental unit). Block and tank were random variables.

Results: 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. Although feed consumption significantly declined through the latter part of the dosing period at the 30 and 50 mg/kg dose rates, there were no significant differences in the fish size at the end of the study. The gross pathology findings during the study were determined to be due to confinement, such as blunted fins and punctate, epidermal erosions. A microscopically evident minimal to mild dose-dependent decrease in hematopoietic/lymphopoietic tissue was observed within the anterior kidneys, posterior kidneys, and spleens of fish that received florfenicol. The incidence of H/L decrease in each organ in each dose group is included in the following table.

Table 4. Incidence and severity of decreased hematopoietic/lymphopoietic tissue in florfenicol-treated channel catfish

Dose Group	Number of Tissues Examined	Tissue*	Decreased Hematopoietic/Lymphopoietic Tissue		
			Minimal	Mild	Total**
Control	30	AK	3	0	3
	30	PK	2	0	2
	30	SP	1	0	1
10 mg/kg	30	AK	9	0	9 (p=0.0685)
	30	PK	8	3	11(p=0.0130)
	30	SP	11	1	12 (p=0.0163)
30 mg/kg	30	AK	14	4	18 (p=0.0004)
	30	PK	12	12	24 (p<0.0001)
	30	SP	13	9	22 (p=0.0009)
50 mg/kg	30	AK	15	8	23 (p<0.0001)
	31	PK	14	12	26 (p<0.0001)
	28	SP	11	8	19 (p=0.0013)

*AK=anterior kidney PK=posterior kidney SP=spleen
**p<0.10 significant

No other histopathological changes were noted in the muscle, skin, brain, gill, heart, or liver.

Conclusions: No significant changes attributable to treatment with florfenicol were observed upon gross necropsy and minor dose-related histopathological changes were observed in the kidney and spleen of fish that received florfenicol. Since the duration of treatment in this study was twice the 10-day recommended treatment duration, florfenicol is safe to administer to catfish at a dose of 10 mg/kg body weight/day for 10 consecutive days.

4. HUMAN FOOD SAFETY:

a. Toxicology:

Summaries of toxicology studies supporting the human food safety of AQUAFLOR 50% Type A Medicated Article are contained in the FOI Summary dated May 31, 1996, for the original approval of NADA 141-063, NUFLOR injectable solution for cattle. For the current approval, an assessment was presented on the effects of florfenicol residues present in edible tissues of catfish on human intestinal flora. It was concluded that the amount of active florfenicol residues reaching the human colon following a 12-day withdrawal period for catfish is probably too low to produce any adverse effect on the human intestinal flora.

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 catfish muscle.

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, Wisconsin.

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 body weight/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 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 body weight/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 body weight/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 body weight/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

Table 8. Percent distribution of ^{14}C-SCH 25298 metabolites in Atlantic salmon muscle and skin salmon maintained at 5°C and dosed first for 9 days with medicated feed containing 10 mg florfenicol/kg body weight/day and then for 1 day by oral gavage with 10 mg ^{14}C-florfenicol/kg body weight.

^{14}C-component	3 hour		12 hour		1 day		3 day		7 day		15 day		30 day	
	muscle	skin	muscle	skin	muscle	skin	muscle	skin	muscle	skin	muscle	skin	muscle	skin
florfenicol amine	9.84	14.51	15.74	NA	36.51	NA	60.77	NA	44.67	37.32	25.63	NA	17.80	14.66
unknown 1	---	---	1.34	NA	1.15	NA	---	NA	2.18	---	---	NA	---	---
florfenicol oxamic acid	0.14	1.19	---	NA	1.25	NA	2.25	NA	1.04	8.36	26.65	NA	20.76	3.08
unknown 2	---	---	---	NA	---	NA	---	NA	---	---	---	NA	---	---
florfenicol alcohol	1.07	2.33	1.14	NA	3.11	NA	8.14	NA	7.67	8.40	---	NA	---	1.79
unknown 3	---	---	---	NA	---	NA	---	NA	1.04	---	---	NA	---	---
unknown 4	---	---	---	NA	---	NA	---	NA	---	---	---	NA	---	---
other unknown(s)	---	---	---	NA	---	NA	---	NA	2.24	---	---	NA	---	---
monochloro-florfenicol	---	---	---	NA	---	NA	---	NA	---	---	1.80	NA	0.65	---
florfenicol	76.41	70.23	70.24	NA	45.10	NA	14.30	NA	1.42	2.84	2.29	NA	0.59	0.38

NA = not analyzed

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 studies conducted with florfenicol in salmon, Study No. 93702. All of the major metabolites of florfenicol seen in salmon tissues were also seen in rat tissues or excreta. Individual unknown metabolites in salmon were not greater than 2% of tissue total radioactivity. A metabolism study in catfish was not completed since the metabolism of florfenicol in catfish is anticipated to be similar to that in salmon. Also, 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 Study

SCH 25298 (Florfenicol): A Final Residue Depletion Study in Channel Catfish Following Administration in Feed

Study No. 00214, Report No. 00214

Study Director: Christopher L. Wrzesinski, Schering-Plough Research Institute, Lafayette, NJ

Investigator: Patricia S. Gaunt, Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS

In-Life Testing Facility: Delta Western Research Center, Indianola, MS

Tissue Collection Facility: Thad Cochran National Warmwater Aquaculture Center, Stoneville, MS

Analytical Facility: Schering-Plough Research Institute, Lafayette, NJ

The in-life portion of the study was conducted in a non-GLP facility. Deviations from GLP were provided. The analytical phase of the study was conducted following GLPs (21 CFR 58).

Male and female catfish (2 pounds average bodyweight at the beginning of acclimation) were used. The fish were held in a 0.1 acre pond with a stocking density of 7,000 fish/acre. Water temperature remained <25 °C at all times over the course of medication and withdrawal with an average daily high and low water temperature during treatment of 21.9 °C and 19.4 °C, respectively and an average daily high and low water temperature during withdrawal of 19.1 °C and 16.6 °C, respectively. The fish were acclimated for 44 days prior to dosing. Control fish were collected during acclimation and prior to dosing. The test fish were fed medicated pelleted fish feed for 12 days at a target dose of 10 mg florfenicol/kg

bodyweight/day. The average dose over the 12-day dosing period was 8.1 mg florfenicol/kg bodyweight/day. Groups of twenty-five fish were sampled at 1, 2, 4, 7, 14, and 21 days after treatment ended. Residues of florfenicol were measured in muscle of twenty fish per time point using the determinative HPLC method for the marker residue, florfenicol amine.

Table 9. Mean florfenicol amine residues in muscle of catfish fed 8.1 mg florfenicol/kg body weight/day for 12 days.

Withdrawal Time (days)	Mean (ppm) ± standard deviation
1	5.378 ± 7.014
2	2.303± 2.959
4	0.876 ± 0.537
7	0.232 ± 0.109
14	0.157 ± 0.059
21	0.169 ± 0.050

Individual values below the lowest point on the standard curve (0.075 ppm) were not used to calculate the means.

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 catfish is muscle.

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

3. Tolerance Assignment

Data were not available on the portion of total residues measured by the marker, florfenicol amine, in catfish muscle. 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 catfish muscle.

4. Withdrawal Times

A 12-day withdrawal time was calculated using 99% statistical tolerance and 95% confidence with the residue depletion data in Study No. 00214 and a tolerance of 1 ppm.

c. Microbial Food Safety

CVM evaluated microbial food safety information for florfenicol for the control of mortality in channel catfish associated with infection by *Edwardsiella ictaluri* using a qualitative risk assessment procedure. This risk assessment procedure

involved conducting 1) a release assessment to describe the probability that the antimicrobial new animal drug and its use in animals will result in the emergence and dissemination of resistant bacteria or resistant determinants in the food animal under proposed conditions of use, 2) an exposure assessment to describe the likelihood of human exposure to the resistant bacteria or resistance determinants through consumption of edible products from treated animals, and 3) a consequence assessment to describe the potential human health consequences of exposure to the defined resistant bacteria or resistance determinants by considering the human medical importance of florfenicol in the treatment of human infectious disease.

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 (10 mg/kg body weight per day in feed for 10 consecutive days) in catfish. Risk management strategies associated with an overall risk estimation of **medium** are compatible with the proposed use of florfenicol in catfish.

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 catfish (muscle) receiving AQUAFLOR Type A Medicated Article, An Antibiotic.

The determinative assay for the marker residue, florfenicol amine, in the edible tissues, is a high performance liquid chromatography (HPLC) method that provides acceptable sensitivity, specificity, accuracy and precision for the routine monitoring of florfenicol residues in catfish. 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 summary for the confirmatory method for AQUAFLOR Type A Medicated Article, An Antibiotic is contained in NADA 141-063.

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.

5. *USER SAFETY:*

Human warnings are provided on the product labeling as follows:

"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 to 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."

6. *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 of the implementing regulations. The data demonstrate that AQUAFLOR Type A Medicated Article (florfenicol), An Antibiotic when administered at a dose of 10 mg florfenicol/kg of body weight daily for 10 consecutive days, is safe and effective for the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision 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 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 Veterinary Feed Directive drug.

A tolerance of 1 ppm in muscle tissue in catfish was established using data from a major species. A 12-day withdrawal time was calculated. Microbial food safety (generation or selection of antimicrobial-resistant bacteria of public health concern and subsequent impact on human therapy) associated with the use of florfenicol in catfish as described in this document was assessed. An overall risk estimation for florfenicol in catfish under the proposed conditions (10 mg/kg body weight per day in feed for 10 consecutive days) was determined to be medium. Risk management strategies associated with the proposed conditions of use of florfenicol in catfish are compatible with an overall risk estimation of medium.

Under section 573(c) of the Federal Food, Drug, and Cosmetic Act (the Act), this approval qualifies for SEVEN years of 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.

7. *ATTACHMENTS:*

Facsimile labeling is attached as indicated below.

AQUAFLOR Type A Medicated Article (florfenicol), An Antibiotic Label 2 kg
AQUAFLOR Type A Medicated Article (florfenicol), An Antibiotic Label 8 x 2 kg
AQUAFLOR Type C Catfish Medicated Feed Label
AQUAFLOR Type A Medicated Article (florfenicol), An Antibiotic VFD Form

PANTONE COLORS
BLACK
PMS 201
PMS 298

PACKAGING COMPONENT APPROVAL

SUBMISSION # 3

EXAMINE BLACK & WHITE COPY AND ACCOMPANYING CYAN LINE ART

	APPROVED	NOT APPROVED	DATE
DESIGNER			
COPY EDITOR			
MEDICAL			
TRADEMARKS			
PATENTS			
PACKAGING			
SPECIFICATIONS			
MARKETING			
REGULATORY			

ART DUE DATE: _____ INV. LOCATION: _____

SDA: _____

APPROVAL VERIFIED: _____ DATE: _____

SCHERING-PLOUGH CORPORATION
PACKAGING ARTWORK PRE-PRESS INFO

DATE: 6/16/05

DESIGNER: AF

PRODUCT/COMPONENT: AQUAFLOR FRONT LABEL

QUANTITY/STRENGTH: 2.0 KG (4.4 LB) / 500 KG

RIC NUMBER: 28395205

DIMENSIONS: 253mm X 152mm

AFFECTED RIC: N/A

OUTPUT RESOLUTION 100%

LTS#: 40170

BARCODE TYPE (NDC): N/A

BARCODE SIZE/X DIMENSION _____

BAR CODES: Supplier responsible for supplying actual LIVE bar code represented by "FPO" with magnification factor based on process requirements in accordance with UCC standards. Minimum acceptance criteria is grade "C" based on ANSI X3.182 "Bar Code Print Quality Guideline."

INK DRAWDOWNS: (Light, Target & Dark Limits) Five sets must be provided to Schering-Plough incoming inspection for approval. Color Standards must be approved by Schering-Plough.

PROOFS: Must be provided to the Labeling Control Analyst for approval. Proofs must be approved by Schering-Plough prior to printing. The supplier is not allowed to make any changes without written approval by Schering-Plough.

HUMAN READABLE REQUIRED: YES _____ NO _____

FULL NUMBER *(SYS. CHARACTER + NDC)*:

3-

NOTE: For GTIN - Package Level Indicator set at 0 (zero).

FOR CARTONS: INTERLEAVED 2 OF 5 (RIC NUMBER) BAR CODE NEEDED ON TUCK FLAPS OR GLUE ENDS, AS PER APPROVED ART.

NDC 0061-1355-01

2.0 kg (4.4 lb)

Aquaflor Type A Medicated Article

(Florfenicol), An Antibiotic

For Use in Catfish Feeds Only **Do Not Feed Undiluted**

CAUTION: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this veterinary feed directive drug shall be fed to animals only by or upon a lawful veterinary feed directive (VFD) issued by a licensed veterinarian in the course of the veterinarian's professional practice.

Active Drug Ingredient: Florfenicol 500 g per kg (227.27 g per lb)
Inert ingredients: Lactose and Povidone.

Description: Each kg of Aquaflor® Type A Medicated Article contains 500 grams (1.1 lb) of the antibiotic florfenicol in a palatable base.

Activity: *In vitro* and *in vivo* investigations in catfish have established florfenicol's activity against *Edwardsiella ictaluri* (Table 1).

Table 1: Minimum inhibitory concentration (MIC) of florfenicol against *Edwardsiella ictaluri* isolated from channel catfish, between 1998-2001.

Organism	No. of Isolates	MIC_{50} (µg/mL)	MIC_{90} Range (µg/mL)
Edwardsiella ictaluri	95	0.25	0.25

Indication: For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

Caution: The effects of florfenicol on reproductive performance have not been determined.

A dose-related decrease in hematopoietic/lymphopoietic tissue may occur. The time required for the hematopoietic/lymphopoietic tissues to regenerate was not evaluated.

WARNING: Feeds containing florfenicol must be withdrawn 12 days prior to slaughter.

IMPORTANT: Must be thoroughly mixed in feeds before use.

Mixing Instructions: For making Aquaflor® -Type C Medicated Feed for catfish: a) Aquaflor® is added to other feed ingredients in the mixer prior to extrusion, b) the medicated feed is mixed thoroughly to insure homogeneity, c) the mixture is extruded and pellets are dried, d) the pellets are dry-mixed/coated with a predetermined amount of fish or vegetable oil, and e) at the completion of mixing, the product is transferred to a storage tank for packaging or transport.

Recommended Aquaflor® Type A Medicated Article Inclusion Rates for Preparation of Type C Medicated Feed

Feeding Rate	Florfenicol Concentration in Feed	Amount of Aquaflor® - Type A Medicated Article per Ton	Biomass of Fish Medicated per Ton of Feed per 10-day Treatment Period
% Biomass	Grams/ton	lbs	lbs
0.5	1,815	8.00	40,000
1.0	908	4.00	20,000
2.0	454	2.00	10,000
3.0	300	1.32	6,666
5.0	182	0.80	4,000

Feeding Directions: Feed as the sole ration for 10 consecutive days. Aquaflor medicated feed should only be administered once disease associated with *Edwardsiella ictaluri* has been appropriately diagnosed. Feeding fish at a percent of biomass and corresponding florfenicol concentration included in the table above will deliver 10 mg florfenicol per kg of fish.

Caution: Feed containing Aquaflor® (florfenicol) shall not be fed to catfish for more than 10 days. Following administration, fish should be reexamined by a licensed veterinarian before reinitiating further course of therapy. The expiration date for VFD for Aquaflor® (florfenicol) must not exceed 15 days from the date of issuance. VFD for Aquaflor® (florfenicol) shall not be refilled.

WARNING: 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 MSDS sheet, call 1-800-770-8878.

STORAGE CONDITIONS: Store at 2-30°C (36-86°F).

NADA #141-245, Approved by FDA.

Aquaflor is a registered trademark of Schering-Plough Veterinary Corporation.

Schering-Plough Animal Health
Aquaculture

XXXXXXXX X/XX

TAKE TIME OBSERVE LABEL DIRECTIONS

0061-1355-01

PANTONE COLORS
BLACK
PMS 201
PMS 201 15%

SCHERING-PLOUGH CORPORATION
PACKAGING ARTWORK PRE-PRESS INFO

DATE: 8/10/05 DESIGNER: AF

PRODUCT/COMPONENT: AQUAFLOR BACK LABEL QUANTITY/STRENGTH: 2.0 KG (4.4 LB) / 500 KG

RIC NUMBER: XXXXXXXX DIMENSIONS: 253mm X 152mm

AFFECTED RIC: XXXXXXXX OUTPUT RESOLUTION 100%

LTB#: XXXXXX BARCODE TYPE (NDC): N/A

BAR CODES: Supplier responsible for supplying actual LIVE bar code represented by "XXX" with magnification factor based on process requirements in accordance with UCC standards. Minimum acceptance criteria is grade "C" based on ANSI X3.182 "Bar Code Print Quality Guideline."

BEE DRAWINGS/ART: (Light, Target & Dark Limits) Fine spec must be provided to Schering-Plough incoming inspection for approval. Color Standards must be approved by Schering-Plough.

PROOFS: Must be provided to the Labeling Control Analyst for approval. Proofs must be approved by Schering-Plough prior to printing. The supplier is not allowed to make any changes without written approval by Schering-Plough.

BARCODE SIZE/X DIMENSION

HUMAN READABLE REQUIRED: YES NO

FULL NUMBER (SYS. CHARACTER + NDC): 3-

NOTE: For GTIN - Package Level Indicator set at 0 (zero).

FOR CARTONS WITH BELLBAND 3 COPIES OF UPC NUMBER BAR CODE REQUIRED ON TUCK FLAPS OR GLUE ENDS, AS PER APPROVED ART.

PACKAGING COMPONENT APPROVAL

SUBMISSION # 0

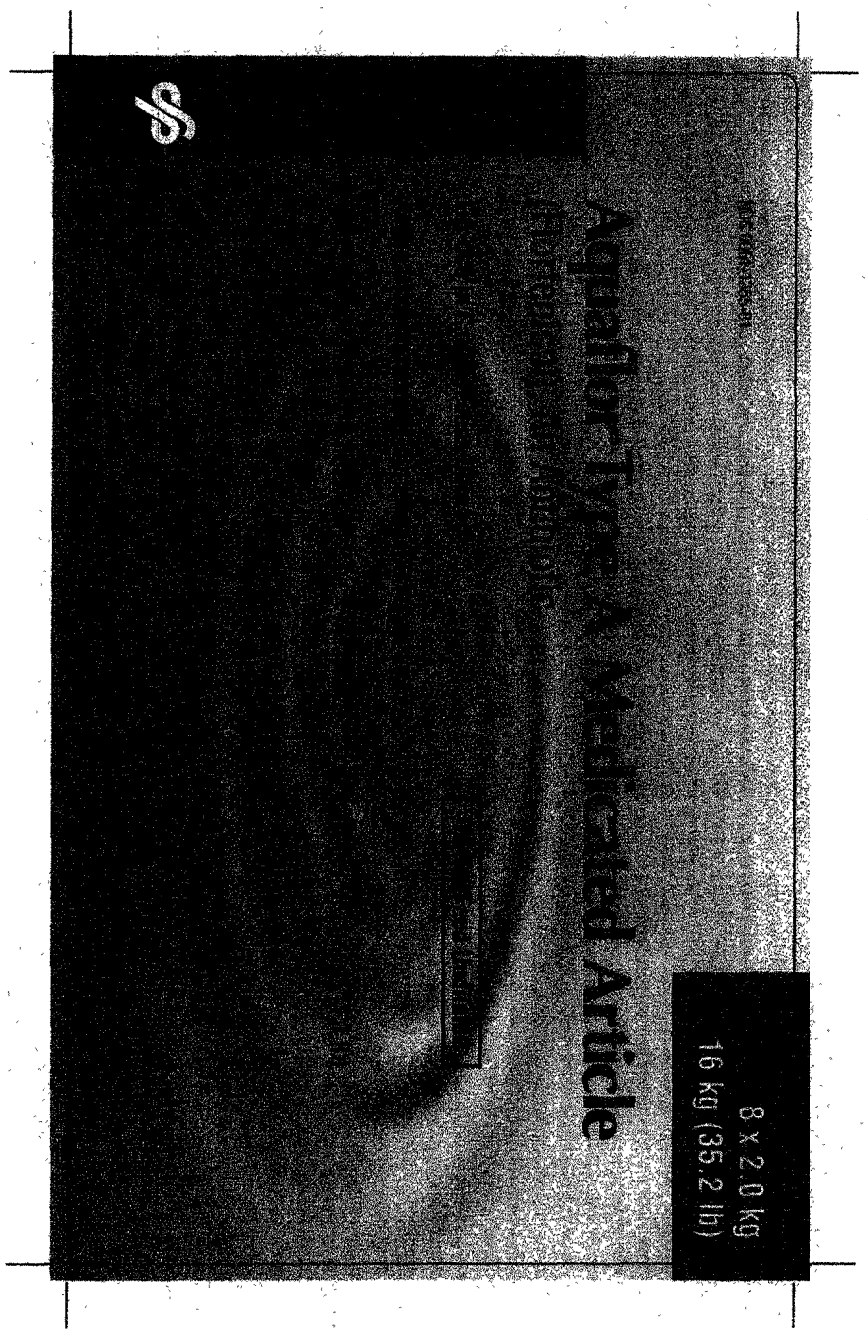
EXAMINE BLACK & WHITE COPY AND ACCOMPANYING COLOR BREAK.

	APPROVED	NOT APPROVED	DATE
DESIGNER	☐	☐	
COPY EDITOR	☐	☐	
MEDICAL	☐	☐	
TRADEMARKS	☐	☐	
PATENTS	☐	☐	
PACKAGING	☐	☐	
SPECIFICATIONS	☐	☐	
MARKETING	☐	☐	
REGULATORY	☐	☐	

ART DUE DATE: _____ INV. LOCATION: _____

SQA:
APPROVAL VERIFIED: _____ DATE: _____

033



PANTONE COLORS
BLACK
PMS 201
PMS 298

SCHERING-PLOUGH CORPORATION
PACKAGING ARTWORK PRE-PRESS INFO

DATE: 6/16/05
DESIGNER: AF
PRODUCT/COMPONENT: AQUAFLOR FRONT LABEL
QUANTITY/STRENGTH: 8 x 2.0 KG (35.2 LB) / 500 KG
RIC NUMBER: 28395906
DIMENSIONS: 253mm X 152mm
AFFECTED RIC: N/A
OUTPUT RESOLUTION 100%
LTS#: 40172
BARCODE TYPE (NDC): N/A
BARCODE SIZE/X DIMENSION
HUMAN READABLE REQUIRED: YES NO

BAR CODES: Supplier responsible for supplying actual LIVE bar code represented by "PPO" with magnification factor based on process requirements in accordance with UCC standards. Minimum acceptance criteria is grade "C" based on ANSI X3.182 "Bar Code Print Quality Guidelines."
INK DRAWDOWNS: (Light, Target & Dark Limits) Five sets must be provided to Schering-Plough Incoming Inspection for approval. Color Standards must be approved by Schering-Plough.
PROOFS: Must be provided to the Labeling Control Analyst for approval. Proofs must be approved by Schering-Plough prior to printing. The supplier is not allowed to make any changes without written approval by Schering-Plough.

FULL NUMBER *(SYS. CHARACTER + NDC)*:
3-
NOTE: For GTIN - Package Level Indicator set at 0 (zero).
FOR CARTONS: INTERLEAVED 2 OF 5 (RIC NUMBER) BAR CODE NEEDED ON TUCK FLAPS OR GLUE ENDS, AS PER APPROVED ART.

PACKAGING COMPONENT APPROVAL
SUBMISSION # 3
EXAMINE BLACK & WHITE COPY AND ACCOMPANYING COLOR BREAK.

	APPROVED	NOT APPROVED	DATE
DESIGNER			
COPY EDITOR			
MEDICAL			
TRADEMARKS			
PATENTS			
PACKAGING			
SPECIFICATIONS			
MARKETING			
REGULATORY			

ART DUE DATE: INV. LOCATION:
SQA:
APPROVAL VERIFIED: DATE:

NDC 0061-1355-01

8 x 2.0 kg
16 kg (35.2 lb)

Aquaflor Type A Medicated Article

(florfenicol), An Antibiotic

For Use in Catfish Feeds Only **Do Not Feed Undiluted**

CAUTION: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this veterinary feed directive drug shall be fed to animals only by or upon a lawful veterinary feed directive (VFD) issued by a licensed veterinarian in the course of the veterinarian's professional practice.

Active Drug Ingredient: Florfenicol 500 g per kg (227.27 g per lb)
Inert Ingredients: Lactose and Povidone.

Description: Each kg of Aquaflor® Type A Medicated Article contains 500 grams (1.1 lb) of the antibiotic florfenicol in a palatable base.

Activity: *In vitro* and *in vivo* investigations in catfish have established florfenicol's activity against *Edwardsiella ictaluri* (Table 1).

Table 1: Minimum inhibitory concentration (MIC) of florfenicol against *Edwardsiella ictaluri* isolated from channel catfish, between 1998-2001.

Organism	No. of Isolates	MIC_{50} (mcg/mL)	MIC Range (mcg/mL)
Edwardsiella ictaluri	95	0.25	0.25

Indication: For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

Caution: The effects of florfenicol on reproductive performance have not been determined.

A dose-related decrease in hematopoietic/lymphopoietic tissue may occur. The time required for the hematopoietic/lymphopoietic tissues to regenerate was not evaluated.

WARNING: Feeds containing florfenicol must be withdrawn 12 days prior to slaughter.

IMPORTANT: Must be thoroughly mixed in feeds before use.

Mixing Instructions: For making Aquaflor® - Type C Medicated Feed for catfish: a) Aquaflor® is added to other feed ingredients in the mixer prior to extrusion, b) the medicated feed is mixed thoroughly to insure homogeneity, c) the mixture is extruded and pellets are dried, d) the pellets are dry-mixed/coated with a predetermined amount of fish or vegetable oil, and e) at the completion of mixing, the product is transferred to a storage tank for packaging or transport.

Recommended Aquaflor® Type A Medicated Article Inclusion Rates for Preparation of Type C Medicated Feed

Feeding Rate	Florfenicol Concentration in Feed	Amount of Aquaflor® - Type A Medicated Article per Ton	Biomass of Fish Medicated per Ton of Feed per 10-day Treatment Period
% Biomass	Grams/ton	lbs	lbs
0.5	1,816	8.00	40,000
1.0	908	4.00	20,000
2.0	454	2.00	10,000
3.0	300	1.32	6,666
5.0	182	0.80	4,000

Feeding Directions: Feed as the sole ration for 10 consecutive days. Aquaflor medicated feed should only be administered once disease associated with *Edwardsiella ictaluri* has been appropriately diagnosed. Feeding fish at a percent of biomass and corresponding florfenicol concentration included in the table above will deliver 10 mg florfenicol per kg of fish.

Caution: Feed containing Aquaflor® (florfenicol) shall not be fed to catfish for more than 10 days. Following administration, fish should be reevaluated by a licensed veterinarian before reinitiating further course of therapy. The expiration date for VFD for Aquaflor® (florfenicol) must not exceed 15 days from the date of issuance. VFD for Aquaflor® (florfenicol) shall not be refilled.

WARNING: Avoid inhalation, oral exposure, and direct contact with skin or eyes. Operators mixing and handling Aquaflor® should use protective clothing, gloves, goggles and MOSH-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.

STORAGE CONDITIONS: Store at 2-30°C (36-86°F).

NADA #141-246, Approved by FDA.

Aquaflor is a registered trademark of Schering-Plough Veterinary Corporation.

Schering-Plough Animal Health
Aquaculture

Made in Ireland. XXXXXXXX XXX

TAKE TIME OBSERVE LABEL DIRECTIONS

3 0061-1355-01 X

SCHERING-PLOUGH CORPORATION
PACKAGING ARTWORK PRE-PRESS INFO

DATE: 8/10/05 DESIGNER: AF

PRODUCT/COMPONENT: AQUAFLOR BACK LABEL QUANTITY/STRENGTH: 8 x 2.0 KG (35.2 LB) / 500 KG

RIC NUMBER: XXXXXXXX DIMENSIONS: 253mm X 152mm

AFFECTED RIC: XXXXXXXX OUTPUT RESOLUTION 100%

LTS#: XXXXX BARCODE TYPE (NDC): N/A

BARCODE SIZE/X DIMENSION

BAR HEIGHT: Supplier responsible for supplying actual LIVE bar code represented by "PMS" with magnification factor based on process requirements in accordance with UCC standards. Minimum acceptance criteria is grade "C" based on ANSI X3.182 "Bar Code Print Quality Guideline."
BAR BEARERBARS: (Light Target & Dark Limit) Film sets must be provided to Schering-Plough receiving inspection for approval. Color Standards must be approved by Schering-Plough.
PROOFS: Must be provided to the Labelling Control Analyst for approval. Proofs must be approved by Schering-Plough prior to printing. The supplier is not allowed to make any changes without written approval by Schering-Plough.

HUMAN READABLE REQUIRED: YES NO

FULL NUMBER (97% CHARACTER + NDC): 3-

NOTE: For GTIN - Package Level Indicator set at 0 (zero).

FOR CARTONS: INTERLEAVED 2 OF 5 (ITC) NUMBER/BAR CODE NEEDED ON TUCK FLAPS OR GLUE ENDS, AS PER APPROVED ART.

PACKAGING COMPONENT APPROVAL

SUBMISSION # 0

EXAMINE BLACK & WHITE COPY AND ACCOMPANYING COLOR BREAK.

	APPROVED	NOT APPROVED	DATE
DESIGNER	☐	☐	
COPY EDITOR	☐	☐	
MEDICAL	☐	☐	
TRADEMARKS	☐	☐	
PATENTS	☐	☐	
PACKAGING	☐	☐	
SPECIFICATIONS	☐	☐	
MARKETING	☐	☐	
REGULATORY	☐	☐	

ART DUE DATE: _____ INV. LOCATION: _____

SQA:
APPROVAL VERIFIED: _____ DATE: _____

PANTONE COLORS
BLACK
PMS 201
PMS 201 15%

0375

BLUEBIRD FEED COMPANY
BLUEBIRD CATFISH FEED
Medicated Type C Feed
FOR USE IN CATFISH ONLY

Indication

For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri.*

Active Drug Ingredient

Florfenicol..... 182 to 1816 g/ton*

Guaranteed Analysis

Crude Protein (Min).....%
Crude Fat (Min).....%
Crude Fiber (Max).....%
Phosphorus (Min).....%

Ingredients

Each ingredient must be named in accordance with the names and definitions adopted by the Association of American Feed Control Officials (AAFCO).

Feeding Directions

"Feed as a sole ration at a rate of ___% biomass daily for 10 consecutive days. Feeding at this rate will deliver 10 mg florfenicol per kg of fish"**.

Caution

Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this veterinary feed directive (VFD) drug shall be fed to animals only by or upon a lawful veterinary feed directive issued by a licensed veterinarian in the course of the veterinarian's professional practice.

Feed containing Aquaflor (florfenicol) shall not be fed to catfish for more than 10 days. Following 10 days administration, fish should be reevaluated by a licensed veterinarian before reinitiating a further course of therapy. The expiration date for VFD for Aquaflor (florfenicol) must not exceed 15 days from the date of issuance. VFD for Aquaflor (florfenicol) shall not be refilled.

A dose related decrease in hematopoietic/lymphopoietic tissue may occur. The time required for the hematopoietic/lymphopoietic tissues to regenerate was not evaluated.

The effects of florfenicol on reproductive performance have not been determined.

WARNING: Feeds containing florfenicol must be withdrawn 12 days prior to slaughter.

Storage Conditions: Store in a cool, dry place. Avoid excessive moisture and heat.
Manufactured By: Bluebird Feed Mill, Robin, IN 46813
NET WEIGHT 50 lbs (22.7 kg)

Expiration Date: _____
Lot No._____

*Final printed label must bear a single drug concentration.
**Feed according to the veterinarian instructions on the VFD.

6/05

035 A

Aquaflor® Type A Medicated Article (Florfenicol), An Antibiotic Veterinary Feed Directive

Schering-Plough Animal Health
Aquaculture

Client: _____ **Veterinarian:** _____

Address: _____ **Address:** _____

_____ _____

_____ _____

Phone: _____ **Phone:** _____

Fax: _____ **Fax:** _____

E-mail: _____ **E-mail:** _____

Catfish to be Treated: Number, Total Weight (Biomass):
Farm Location: Farm Address, Pond Identification (Pond Number, etc.):

Indication: For the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

Mix into Type C Medicated Feed to Provide: Check one: ☐ **182 g/ton** ☐ **300 g/ton** ☐ **454 g/ton** ☐ **908 g/ton** ☐ **1816 g/ton**

Amount of Final (Type C) Feed: _____ (Pounds or Tons)

Feeding Directions: Feed as the sole ration for 10 consecutive days. Feeding at this rate will deliver 10 mg florfenicol per kg of fish.

Feeding Rate	Florfenicol Concentration in Feed	Amount of Aquaflor® - Type A Medicated Article per Ton	Biomass of Fish Medicated per Ton of Feed per 10-day Treatment Period
% Biomass	Grams/ton	lbs	lbs
0.5	1,816	8.00	40,000
1.0	908	4.00	20,000
2.0	454	2.00	10,000
3.0	300	1.32	6,666
5.0	182	0.80	4,000

Feeding Rate: _____ % Biomass

Special Instructions

Date of Treatment: _____ (Month/Day/Year)

Expiration Date: _____ Month/Day/Year (Not to exceed 15 days from date of issuance.)

Veterinarian's Signature: _____ Date: _____

License Number and State: _____

Caution: Federal law limits this drug to use under the professional supervision of a licensed veterinarian. Animal feed bearing or containing this veterinary feed directive drug shall be fed to animals only by or upon a lawful veterinary feed directive (VFD) issued by a licensed veterinarian in the course of the veterinarian's professional practice. Extra-label use (i.e., use of this VFD feed in a manner other than as provided by the VFD drug approval) is strictly prohibited.

Feed containing Aquaflor (florfenicol) shall not be fed to catfish for more than 10 days. Following 10 days administration, fish should be re-evaluated by a licensed veterinarian before initiating a further course of therapy. The expiration date for VFD for Aquaflor (florfenicol) must not exceed 15 days from the date of issuance. VFD for Aquaflor (florfenicol) shall not be refilled. A dose related decrease in hematopoietic/lymphopoietic tissue may occur. The time required for the hematopoietic/lymphopoietic tissues to regenerate was not evaluated. The effects of florfenicol on reproductive performance have not been determined.

WARNING: Feeds containing florfenicol must be withdrawn 12 days prior to slaughter.

Warning: 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. To obtain 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, call 1-800-770-8878.

NADA 141-246, Approved by FDA.
Aquaflor® is a registered trademark of Schering-Plough Veterinary Corporation.

TAKE TIME
OBSERVE LABEL DIRECTIONS

White Copy- Supplier Canary Copy- Client Pink Copy- Veterinarian