Schering-Plough Animal Health Corporation would like to thank: The Aquatic Animal Drug Approval Partnership Program (AADAP); Thad Cochran National Warmwater Aquaculture Center, Mississippi State University College of Veterinary Medicine; The National Coordinator for Aquaculture New Animal Drug Applications; The United States Fish and Wildlife Service; and The United States Geological Survey for their contributions to the development and registration of the Aquaflor® Type A Medicated Article in the United States.

Schering-Plough Animal Health Corporation would also like to thank The Catfish Institute for use of their photos in this publication.
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Introduction

Bacterial diseases represent some of the most significant challenges facing fish farming worldwide. According to the 2000 United States Department of Agriculture National Animal Health Monitoring System (USDA-NAHMS) report, bacterial diseases account for approximately 70% of all diseases affecting catfish (*Ictalurus punctatus*) in the southeastern US.¹

The most important bacterial disease in commercial catfish aquaculture is enteric septicemia of catfish (ESC), a highly fatal systemic disease caused by infection with *Edwardsiella ictaluri*.²,³ Signs of *E. ictaluri* infection include inflammation through the sutra fontanel of the skull (“hole in the head”); hemorrhages on the skin, fins, and/or gills; white spots or nodules on the skin and/or fins; oral hemorrhages; abdominal distention (ascites); and exophthalmia. Mortality rates in susceptible catfish can reach 100%; and in surviving fish, growth rates and weight gains may be significantly affected.⁴,⁵

Outbreaks of ESC typically occur in the spring and fall months, when water temperatures are 20° – 28°C and other conditions are optimal for growth of *E. ictaluri*. Husbandry and environmental stress play significant roles in determining the clinical and economic impact of *E. ictaluri* infections. Nevertheless, this organism is considered a primary pathogen and is capable of causing substantial losses even on well-managed farms.

For successful treatment during an outbreak of ESC, the diagnosis must be made and treatment with an effective antibiotic initiated while the majority of fish are still feeding.³ *E. ictaluri* is susceptible to a variety of antibiotics,⁶ but until recently only 2 antibacterial agents were approved for use in food fish: sulfadimethoxine-ormetoprim (Romet 30®)⁷ and oxytetracycline (Terramycin®).

Resistance to both of these antibiotics has been reported in fish.⁸⁻¹⁰ Inadequate intake of medicated feed, whether from poor palatability (Romet)⁷ or inappropriate pellet type (oxytetracycline),³ has also contributed to the limited efficacy of these products. Clearly, a more palatable and efficacious oral antibiotic is needed for the treatment of ESC in catfish.
Aquaflor® 50% Type A Medicated Article is a feed premix containing florfenicol, a broad-spectrum antibiotic. Florfenicol (FFC) has activity against a wide range of fish pathogens in vitro and in vivo, including *E. ictaluri*. Treatment with Aquaflor®, incorporated into floating catfish feed prior to pelleting and administered at a dose rate of 10 mg FFC/kg body weight daily for 10 consecutive days, results in a rapid decline in mortality rates due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*.

**Key Characteristics of Aquaflor®:**

- Highly palatable
- Administered in floating feed
- Well tolerated by catfish
- Highly effective against *E. ictaluri* infection
- Effective in a range of water temperatures favored by *E. ictaluri*
- Effective against multiple antibiotic-resistant organisms
- Minimal environmental effect
- Antibiotic developed exclusively for veterinary medicine

Florfenicol is a synthetically produced antibacterial agent that has been specifically developed for veterinary use. It is a fluorinated analogue of thiamphenicol, a chloramphenicol analogue, and these structural modifications confer advantages in activity, particularly against bacteria resistant to thiamphenicol and chloramphenicol. Florfenicol is chemically different from chloramphenicol and lacks the functional group responsible for chloramphenicol’s human toxicity concerns (bone marrow suppression and aplastic anemia).

Studies with florfenicol indicated potent activity against a number of bacterial fish pathogens in vitro and in a variety of fish species in vivo. Experimental efficacy has been demonstrated against *E. ictaluri* in channel catfish, *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*) in yellowtail, *Edwardsiella tarda* in eels, *Vibrio anguillarum* in goldfish, *Aeromonas salmonicida*, *Vibrio salmonicida* in Atlantic salmon, and *Yersinia ruckeri* and *Flavobacterium psychrophilum* in trout (data on file).

There is a limited number of products approved for treatment of bacterial disease in fish, and antibacterial resistance to some of these products has already become widespread. Oral administration of antimicrobials is the preferred route of chemotherapy in finfish aquaculture due to the ease of use and lack of any additional stress to the fish during treatment. Reduction in appetite due to clinical disease can be addressed by higher feed inclusion rates of the medication.
Florfenicol was first approved for aquaculture use in Japan in 1990 for treatment of susceptible bacterial diseases, including pasteurellosis and streptococcosis in yellowtail, red sea bream, coho salmon, horse mackerel, rainbow trout, sweetfish, tilapia and eel. Since then it has been approved for use in Atlantic salmon in Norway, Chile, Canada and in the U.K. Most recently Aquaflor® has been approved in the U.S. for control of mortality in catfish due to enteric septicemia of catfish associated with Edwardsiella ictaluri.

Aquaflor® was developed by the Research Division of Schering-Plough Animal Health Corporation specifically to provide fish producers with a product that combines highly effective control of susceptible bacterial diseases with safety for fish, palatability ease of administration and a different mode of action from other antibiotics.

**CHEMISTRY**

The following data describe the active ingredient.

Florfenicol is the active ingredient in Aquaflor®. It is a monofluorinated derivative of thiamphenicol, a chloramphenicol analogue in which the p-nitro group on the aromatic ring is substituted with a sulphonylmethyl group.

Chemical Structure:

![Chemical Structure of Florfenicol]

**Scientific Name:** ([R-(R*,S*)]-2,2-dichloro-N-[[fluoromethyl]-hydroxy-[[4-(methylsulfonyl) phenyl]ethyl]-acetamide

**Generic Name:** florfenicol

**Molecular Formula:** C_{12}H_{14}Cl_{2}FNO_{4}S

**Molecular Weight:** 358.21
MODE OF ACTION

Florfenicol is a synthetic, broad-spectrum antibiotic active against many Gram-negative and Gram-positive bacteria. Florfenicol acts by binding to the 50S ribosomal subunit, thereby preventing bacterial protein synthesis.11

In vitro activity has been demonstrated against commonly isolated bacterial fish pathogens including Edwardsiella ictaluri, Aeromonas salmonicida, Edwardsiella tarda, Flavobacterium psychrophilum, Photobacterium damselae subsp. piscicida, Vibrio anguillarum, V. salmonicida, other Vibrio spp., and Yersinia ruckeri (see Table 1-1).

Table 1-1  In vitro activity (Minimum Inhibitory Concentrations [MIC]) of florfenicol against some common fish pathogens

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of Isolates</th>
<th>MIC Range (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas salmonicida</td>
<td>98</td>
<td>0.25 – 1.6</td>
</tr>
<tr>
<td>Edwardsiella ictaluri</td>
<td>779</td>
<td>0.25</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>52</td>
<td>0.4 – 1.6</td>
</tr>
<tr>
<td>Flavobacterium psychrophilum</td>
<td>48</td>
<td>0.001 – 16</td>
</tr>
<tr>
<td>Photobacterium damselae</td>
<td>225</td>
<td>0.004 – 0.6</td>
</tr>
<tr>
<td>(formerly Pasteurella piscicida)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio anguillarum</td>
<td>151</td>
<td>0.2 – 0.8</td>
</tr>
<tr>
<td>Vibrio salmonicida</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td>3</td>
<td>1.25</td>
</tr>
<tr>
<td>Yersinia ruckeri</td>
<td>5</td>
<td>0.6 – 10</td>
</tr>
</tbody>
</table>

Bacteria resistant to chloramphenicol, through chloramphenicol acetyltransferase production, are sensitive to florfenicol.11 In vitro tests with fish pathogens and a range of chemotherapeutants, including chloramphenicol, streptomycin, tetracycline, ampicillin, trimethoprim, furazolidone, kanamycin, naladixic acid, amoxycillin, oxolinic acid and florfenicol, demonstrated that florfenicol had greater antibacterial activity than the other compounds tested.13,15–17
**DOSAGE FORM**

Premix: Aquaflor® is a 50% (w/w) Type A Medicated Article for inclusion into fish feed. The composition is 50% florfenicol and 50% inert carriers. Aquaflor® is:

- Supplied in 2-kg (4.4 lb) foil packages, with eight 2-kg packages in a fiber drum
- Has a shelf-life of 3 years

Medicated Feed Production (see label for explanation):

- The product should be mixed in unmedicated fish feed prior to pelleting
- It should be administered in feed to deliver 10 mg florfenicol per kg body weight daily (See Table 1-2 for recommended Aquaflor® feed inclusion rate).

Dose Rate:

- Recommended dose rate is 10 mg of florfenicol/kg body weight/day for 10 consecutive days

<table>
<thead>
<tr>
<th>Feeding Rate (% of biomass)</th>
<th>Florfenicol Concentration in Feed (grams/ton)</th>
<th>Amount of Aquaflor® Type A Medicated Article per Ton of Feed (lbs)</th>
<th>Biomass of Fish Medicated per 10-day Treatment Period (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>1,816</td>
<td>8.00</td>
<td>40,000</td>
</tr>
<tr>
<td>1.0%</td>
<td>908</td>
<td>4.00</td>
<td>20,000</td>
</tr>
<tr>
<td>2.0%</td>
<td>454</td>
<td>2.00</td>
<td>10,000</td>
</tr>
<tr>
<td>3.0%</td>
<td>300</td>
<td>1.32</td>
<td>6,666</td>
</tr>
<tr>
<td>5.0%</td>
<td>182</td>
<td>0.80</td>
<td>4,000</td>
</tr>
</tbody>
</table>

Feeding Directions: Aquaflor® Type C medicated feed should only be administered once the 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.
VETERINARY FEED DIRECTIVE DRUG

Aquaflo® Type A Medicated Article is a Veterinary Feed Directive (VFD) drug. Please note the following cautionary information for sale and use of Aquaflo® Type A Medicated Article: 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 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 for the VFD drug approval) is strictly prohibited.

Feed containing Aquaflo® (florfenicol) should not be fed to catfish for more than 10 days. Following administration, fish should be re-evaluated by a licensed veterinarian before initiating further therapy. The expiration date for a VFD for Aquaflo® (florfenicol) must not exceed 15 days from the date of issuance. VFDs for Aquaflo® (florfenicol) should not be refilled.
Nearly 80% of catfish eggs brought to hatcheries survive. Typically, eggs hatch after 7 days.

Fingerlings infected with enteric septicemia of catfish (ESC) caused by *E. ictaluri*.

Photomicrograph of bacterial pathogen *Edwardsiella* spp.
While the pharmacokinetics of florfenicol have not been studied in catfish, the absorption, distribution, metabolism and excretion of florfenicol have been studied in numerous other species, including cattle, pigs, Muscovy ducks, broiler chickens, horses, rainbow trout and Atlantic salmon (in freshwater and seawater). Conclusions from these studies were consistent for all species: florfenicol is well absorbed, and excreted in bile, feces and urine.

**STUDIES IN FISH**

Atlantic salmon (*Salmo salar*) were used in a number of studies to determine the fate of florfenicol in fish; one study was a whole body autoradiography study with salmon in seawater at 8° – 11°C; other studies were radiolabeled residue depletion studies conducted with salmon in seawater at 5°C and 10°C. Results from the whole body autoradiography study indicated that florfenicol had the following properties:

- Rapidly absorbed from the intestinal tract and transferred to other tissues
- Widely distributed to tissues, with maximum levels found at 12 hours after the end of treatment with similar concentrations in blood and muscle, but lower levels in fat and brain
- Maximum levels of florfenicol achieved in the muscle exceeded the Minimum Inhibitory Concentration (MIC) values reported for most fish pathogenic bacteria

In radiolabeled residue depletion studies, salmon received either a single dose of feed containing radiolabeled florfenicol or 9 days of florfenicol-medicated feed and 1 day of feed containing radiolabeled florfenicol at 10 mg/kg body weight. Results showed that:

- Maximum radioactivity concentrations in all tissues occurred within 6–24 hours after final dose delivery, and the highest levels were observed in the kidneys and liver
- Tissue radioactivity declined faster at 10°C than at 5°C, indicating that florfenicol is cleared from tissues faster at higher temperatures
- Residue concentrations were lower in muscle than in skin and depleted somewhat faster from muscle than from skin
Nearly 90% of U.S. farm-raised catfish are produced in ponds located within the states of Alabama, Arkansas, Louisiana and Mississippi. The typical pond covers 10 – 20 acres and is 4 – 6 feet deep.
A complete toxicological evaluation has been conducted with florfenicol. This includes extensive published and unpublished studies in the mouse, rat, cattle, dog, swine, poultry, rainbow trout, salmon and bluegill sunfish. From studies with florfenicol on reproduction in rats, a No Observable Effect Level (NOEL) of 1.0 mg/kg has been established.

**FOOD SAFETY AND RESIDUES**

The metabolism of florfenicol in fish is qualitatively similar to the metabolism in the rat. With a safety factor of 100, an acceptable daily intake (ADI) has been calculated at 10 µg/kg/day. Thus, based on toxicological assessment, an ADI of 10 µg/kg/day has been set for florfenicol and its metabolites in fish tissues.

**Maximum Residue Limit or Safe Concentration**

An HPLC assay based on the quantitative conversion of florfenicol and its metabolites to florfenicol amine has been validated in fish tissues (muscle, skin, and muscle with attached skin). The Maximum Residue Limit (MRL) or equivalent Safe Concentration is based on the marker residue, florfenicol amine. The US has established a Safe Concentration for florfenicol amine in catfish edible tissue (muscle) of 1.0 mg/kg.

**Residue Depletion Study in Catfish**

The accepted Safe Concentration of florfenicol amine is 1 ppm in catfish muscle. As the following data show, florfenicol amine residues in catfish muscle are well below this level within 7 days after concluding treatment with florfenicol (FFC) at the recommended dose rate of 10 mg FFC/kg body weight/day for 10 consecutive days.

_Purpose:_ A study was conducted in market-weight catfish, raised under field conditions, in order to determine marker residue concentrations in edible catfish tissue (muscle) following treatment and, thus, to establish a withdrawal period for FFC in catfish.

_Study Design:_ Market-weight catfish (average weight 2 lb at the start of the study period) were kept in a 0.1-acre test pond under normal aquaculture conditions at a stocking density of 7,000 fish/acre. Medicated, pelleted fish feed was fed for 12 consecutive days at a nominal dose rate of 10 mg/kg (actual measured dose rate = 9.3 mg/kg body weight/day). The fish were then fed unmedicated feed for the remainder of the study period. The water temperature remained below 25°C throughout the study, with average daily high and low water temperatures during treatment being 21.9°C and 19.4°C, respectively.

*Cold weather reduced initial feed intake for 2 days, so the feeding period was extended to 12 days.*
Following the cessation of FFC treatment, 20 fish were sampled for florfenicol residues on each of the following days post-treatment: 1, 2, 4, 7, 14 and 21 days. Muscle samples (fillets) were collected from each fish and analyzed using a validated assay for florfenicol amine (HPLC, using UV detection). Means were calculated for each group; individual values below the limit of quantitation (0.075 ppm) were not used to calculate the means.

**Results:** Average muscle concentrations of florfenicol amine are shown in Table 3-1. Levels of the marker declined rapidly during the first 7 days following cessation of treatment, from an average of 5.38 ± 7.01 ppm on day 1 to an average of 0.23 ± 0.11 ppm on day 7. Average tissue levels of florfenicol amine were below the accepted Safe Concentration (1 ppm) by 4 days after the end of treatment, and levels were below this threshold in all individual fish by 7 days post-treatment.35,36

Residue levels plateaued by 2 weeks post-treatment at an average of 0.16 ppm. Levels were 2–4X higher in males than in females on days 2 and 4 post-treatment, but average residue levels for males and females were comparable at all other time points (data not shown).35,36

**Table 3-1  Marker Residue Concentrations in Catfish Muscle**

<table>
<thead>
<tr>
<th>Collection Time (days post-treatment)</th>
<th>Mean (ppm) ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.38 ± 7.01</td>
</tr>
<tr>
<td>2</td>
<td>2.30 ± 2.96</td>
</tr>
<tr>
<td>4</td>
<td>0.88 ± 0.54</td>
</tr>
<tr>
<td>7</td>
<td>0.23 ± 0.11</td>
</tr>
<tr>
<td>14</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>21</td>
<td>0.17 ± 0.05</td>
</tr>
</tbody>
</table>

*Catfish were fed florfenicol at a dose rate of 9.3 mg/kg/day for 12 days. Fish were harvested and muscle samples analyzed for florfenicol amine at various times after the end of the treatment period.35,36

**Conclusions:** Tissue levels of florfenicol amine declined rapidly after the 12-day treatment period had ended. Using an upper limit of 1 ppm in edible tissue and calculations based on a 99% tolerance limit and a 95% confidence interval, the withdrawal period for Aquaflor® 50% Type A Medicated Article, administered orally in feed for 10 consecutive days, is 12 days for channel catfish.35,36

**Withdrawal Period:** A withdrawal period of 12 days has been established by the United States Food and Drug Administration for Aquaflor®-treated channel catfish.
SAFETY OF FLORFENICOL IN CATFISH — STUDY 1*

A study was conducted to determine the safety and palatability of orally administered FFC in channel catfish at 1X, 2X, 4X, and 10X the recommended dose rate.

Study Design: Four hundred (400) laboratory-reared, 5-month-old channel catfish fingerlings with no known history of exposure to *E. ictaluri* were divided into 5 groups of 80 fish each:

- **Group 1** — fed unmedicated feed
- **Group 2** — fed FFC at 10 mg/kg/day for 10 days
- **Group 3** — fed FFC at 20 mg/kg/day for 10 days
- **Group 4** — fed FFC at 40 mg/kg/day for 10 days
- **Group 5** — fed FFC at 100 mg/kg/day for 10 days

Fish were weighed in groups at the start of the study period. In Groups 2 – 5, treatment with FFC-medicated feed began the next day and continued for 10 consecutive days. All groups were observed daily for feeding behavior, signs of toxicity and mortality. On day 11, all surviving fish were counted, weighed in groups, euthanized and submitted for gross and histopathologic examination.

Throughout the study, feeding activity was subjectively graded each day, based on the amount of feed consumed. A score of 2 was assigned if 50 – 100% of the food was consumed. A score of 1 was given if <50% of the food was consumed. A score of 0 was given if little or no food was consumed. A palatability score was calculated for each group at the end of the study as the sum of the daily feeding scores over the 10-day treatment period. As the maximum feeding activity score was 2, the maximum palatability score was 20 (2 x 10 days).

Results: Palatability scores and body weight gains for each group are shown in Table 3-2. Weight gains were not significantly different between groups. Palatability scores were not analyzed statistically, as they were nearly identical for all groups.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Palatability Score*</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Untreated)</td>
<td>19.25</td>
<td>16.25%</td>
</tr>
<tr>
<td>2 (10 mg FFC/kg)**</td>
<td>19.5</td>
<td>15.25%</td>
</tr>
<tr>
<td>3 (20 mg FFC/kg)**</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>4 (40 mg FFC/kg)**</td>
<td>19.25</td>
<td>14%</td>
</tr>
<tr>
<td>5 (100 mg FFC/kg)**</td>
<td>19.5</td>
<td>14.5%</td>
</tr>
</tbody>
</table>

*Average daily feeding activity score (0 – 2) x 10 days; maximum score = 20.

**Fed medicated feed containing FFC at the listed dosage for 10 consecutive days.
No fish died and no signs of morbidity were noted during the study period. Only 6/400 fish (1.5%) examined at necropsy had gross pathologic lesions. Lesions were mild in all 6 fish, consisting only of mild mottling of the liver (3 fish in the 10 mg/kg FFC group and 1 control fish) or spleen (2 fish in the 20 mg/kg FFC group and 1 fish in the 10 mg/kg FFC group). There were no differences in the histologic appearance of the organs in treated versus untreated fish.4

**Conclusions:** Florfenicol, administered at up to 10X the recommended daily dose rate of 10 mg/kg body weight for 10 consecutive days, was palatable to channel catfish fingerlings and did not cause any treatment-related pathology.

### SAFETY OF FLORFENICOL IN CATFISH — STUDY 2

The following study was conducted to determine the safety and palatability of orally administered FFC in channel catfish at 1X, 3X, and 5X the recommended dose rate for 2X the recommended treatment duration.

**Study Design:** A total of 240 laboratory-reared channel catfish fingerlings (mean weight 14.7 ± 3.8 g) were divided into 4 groups:

- **Group 1** — fed unmedicated feed
- **Group 2** — fed FFC at 10 mg/kg/day for 20 days
- **Group 3** — fed FFC at 30 mg/kg/day for 20 days
- **Group 4** — fed FFC at 50 mg/kg/day for 20 days

Fish were fed either unmedicated feed (Group 1) or medicated feed (Groups 2 – 4) for 20 consecutive days. During that time, all groups were monitored for feeding activity, morbidity and mortality. At the end of the treatment period, all surviving fish were weighed, euthanized, necropsied, and their tissues were examined histopathologically.

**Results:** No fish died and no signs of morbidity were noted during the study period. No changes in behavior were detected in treated fish relative to the controls. Although feed consumption declined in the latter part of the dosing period at the 30 mg/kg and 50 mg/kg dose rates, there were no significant differences in body weight at the terminal sampling.37

No differences between groups were observed at gross necropsy. A very mild, dose-dependent decrease in hematopoietic/lymphopoietic tissue was observed microscopically in the kidneys and spleens of treated fish. No other histopathologic changes were noted.37

**Conclusions:** No significant changes attributable to FFC treatment were observed, even at 5X the recommended dose rate for 2X the recommended duration of treatment. Thus, FFC is considered safe for administration to channel catfish at the recommended dose rate of 10 mg FFC/kg/day for 10 consecutive days.
Overview: Aquaflor® 50% Type A Medicated Article is recommended for the control of mortality in catfish due to enteric septicemia of catfish associated with *Edwardsiella ictaluri*. Aquaflor® is administered in feed at an oral dose rate of 10 mg FFC/kg body weight/day for 10 consecutive days. The efficacy of Aquaflor® and the validity of this dose regimen is supported by several studies, including *in vitro* sensitivity data on *E. ictaluri* and *in vivo* challenge studies. These studies show the following:

- Aquaflor®-medicated feed is safe in channel catfish at the recommended dose rate
- Aquaflor®-medicated feed is palatable to channel catfish
- Aquaflor® is highly effective in the control of mortality in channel catfish caused by pathogenic strains of *E. ictaluri*

**IN VITRO SUSCEPTIBILITY OF E. ICTALURI TO FLORFENICOL**

The following study demonstrates the *in vitro* susceptibility of *E. ictaluri* to florfenicol.

**Study Design:** Twelve (12) field isolates of *E. ictaluri*, obtained during natural outbreaks of ESC in commercial catfish ponds in Mississippi, were tested. An additional 767 cultures of *E. ictaluri*, obtained from channel catfish experimentally infected with *E. ictaluri*, were also tested. Agar disk diffusion susceptibility testing was performed as outlined in the National Committee for Clinical Laboratory Standards (NCCLS) performance standards guidelines.

**Results:** Kirby-Bauer zones of inhibition and MIC values for natural and experimental infections are shown in Table 4-1.20

<table>
<thead>
<tr>
<th>Source</th>
<th>Kirby-Bauer Zone of Inhibition Mean (Range)</th>
<th>Minimum Inhibitory Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural outbreak</td>
<td>46.5 mm (41–51 mm)</td>
<td>0.25</td>
</tr>
<tr>
<td>Experimental infection</td>
<td>35.3 mm (31–50 mm)</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Conclusions: All tested strains of *E. ictaluri* were highly susceptible to FFC in vitro. Parameters for the zone of inhibition for FFC against *E. ictaluri* are not formally established. Using preliminary interpretive standards for FFC in cattle, a zone of inhibition >19 mm indicates that the organism is sensitive.20 The zones of inhibition for all isolates of *E. ictaluri* in this study were >30 mm. Thus, it can be concluded that *E. ictaluri* is highly susceptible to FFC. The low MIC value for all tested isolates (0.25 µg/mL) supports this conclusion.

**IN VIVO EFFICACY**

The following 4 studies confirm the in vivo efficacy of Aquaflor*-medicated feed at the recommended dose rate in channel catfish exposed to pathogenic strains of *E. ictaluri*.

**Dose Titration — Study 1**

The following dose titration study was conducted to confirm the safety, palatability and efficacy of FFC-medicated feed and to determine the optimal dose rate in channel catfish exposed to a pathogenic strain of *E. ictaluri*.

**Study Design:** Four hundred (400) laboratory-reared, 5-month-old channel catfish fingerlings with no known history of exposure to *E. ictaluri* were divided into 5 groups of 80 fish:

- Group 1— not challenged with *E. ictaluri* and fed unmedicated feed
- Group 2— challenged with *E. ictaluri* and fed unmedicated feed
- Group 3— challenged and fed FFC at 10 mg/kg/day for 5 days
- Group 4— challenged and fed FFC at 20 mg/kg/day for 5 days
- Group 5— challenged and fed FFC at 40 mg/kg/day for 5 days

Fish were weighed in groups and then exposed for 2 hours to approximately $4.3 \times 10^6$ CFU/mL of a confirmed pathogenic isolate of *E. ictaluri*, obtained from a natural outbreak of ESC. Treatment with FFC-medicated feed began in Groups 3 – 5 the day after exposure and continued for 5 consecutive days. All surviving fish were then observed for an additional 17 days, during which time all groups were fed an unmedicated ration.

Throughout the study, feeding activity was subjectively graded each day, based on the amount of feed consumed. A score of 2 was assigned if 50–100% of the food was consumed. A score of 1 was given if <50% of the food was consumed. A score of 0 was given if little or no food was consumed.

The fish were also monitored daily for abnormal behavior indicative of morbidity (e.g., lethargy, abnormal swimming patterns) and for adverse events, such as piping at the water surface or unexpected deaths.

Any fish that died during the study were necropsied and bacterial culture of *E. ictaluri* was attempted. At the end of the study period (day 23), all surviving fish were counted, weighed in groups, euthanized and submitted for microbiologic and histopathologic examination.
Results: Cumulative mortality rates over the 23-day study period are shown in Figure 4-1. Following exposure, the first mortalities occurred on day 7, in the challenged, untreated fish (Group 2). Mortalities were seen in this group from day 7 to day 22, with the majority occurring between days 8 and 13. The cumulative mortality rate in this group was 57.5%, with individual tank mortality rates ranging from 35 – 100%.

Only 2/80 fish (2.5%) in the unchallenged, untreated group died. No mortalities occurred in the 10 mg/kg FFC-treated fish (Group 3). One fish each in the other 2 FFC-treated groups died (on day 22 or 23), for a cumulative mortality rate of 1.25% for each group.

Mortality rates in the FFC-treated fish (Groups 3–5) and in the unchallenged, untreated fish (Group 1) were significantly lower than the mortality rate in the challenged, untreated fish (Group 2; p<0.0001 for each contrast). Pairwise contrasts among FFC-treated groups and between FFC-treated and unchallenged fish showed no statistically significant differences.

Figure 4-1  Mortality Rates in Treated vs. Untreated Catfish

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mortality Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Untreated, unchallenged)</td>
<td>2.5%</td>
</tr>
<tr>
<td>Group 2 (Untreated, challenged)</td>
<td>0%</td>
</tr>
<tr>
<td>Group 3 (10 mg FFC/kg)B</td>
<td>0%/80A</td>
</tr>
<tr>
<td>Group 4 (20 mg FFC/kg)B</td>
<td>1/80A</td>
</tr>
<tr>
<td>Group 5 (40 mg FFC/kg)B</td>
<td>1/80A</td>
</tr>
</tbody>
</table>

A. Total number of deaths. Includes fish that died naturally and fish that were euthanized at the end of the study.
B. Challenged with E. ictaluri then treated with FFC at the listed dose rate for 5 consecutive days.

No adverse events were noted during the study. Feeding activity in the unchallenged, unmedicated fish (Group 1) was scored at 2 throughout the study. The average feeding activity score for the challenged, unmedicated fish (Group 2) was between 0 and 1 from days 4–17, and was consistently <1.5 until day 21.

Feeding activity in all FFC-medicated groups was scored at 2 for all days throughout the study, except day 5. On that one day, 2 tanks of fish in Group 3 (10 mg FFC/kg) and one tank of fish in Group 5 (40 mg FFC/kg) had feeding scores of 1, dropping the group average for that day to 1.5 and 1.75, respectively. At the end of the study period, the average body weight of surviving challenged, untreated fish (Group 2) was lower than that of surviving FFC-treated fish (p<0.05).
Bacteria characteristic of *E. ictaluri* were cultured from 94% of non-euthanized fish. Infection rates for all fish, including surviving fish euthanized at the end of the study period, are shown in Figure 4-2.

**Figure 4-2 Recovery of *E. ictaluri* from Treated vs. Untreated Catfish**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Infection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Untreated, unchallenged)</td>
<td>10%</td>
</tr>
<tr>
<td>Group 2 (Untreated, challenged)</td>
<td>70%</td>
</tr>
<tr>
<td>Group 3 (10 mg FFC/kg)</td>
<td>3.8%</td>
</tr>
<tr>
<td>Group 4 (20 mg FFC/kg)</td>
<td>2.5%</td>
</tr>
<tr>
<td>Group 5 (40 mg FFC/kg)</td>
<td>5.0%</td>
</tr>
</tbody>
</table>

A. Total number of infected fish. Includes fish that died naturally and fish that were euthanized at the end of the study.
B. Challenged with *E. ictaluri* then treated with FFC at the listed dose rate for 5 consecutive days.

Gross examination revealed external and internal lesions characteristic of *E. ictaluri* infection in 99% of fish that were culture-positive for the organism. External lesions included inflammation through the sutra fontanel of the skull (“hole in the head”) and hemorrhages on the skin and fins. Exophthalmia was also observed. Internal lesions included hemorrhages on the gastrointestinal tract and liver, as well as a congested spleen, ascites and swollen kidneys. The incidence of these lesions was greatest in the challenged, untreated fish (Group 2).4

Histopathologic examination showed an increased degree of inflammatory cell infiltrate in the liver, heart, gills, anterior kidney and spleen of challenged, untreated fish (Group 2), and a paucity of lesions in unchallenged fish and FFC-treated fish. No treatment-related changes were noted on either gross or microscopic examination.4

**Conclusions:** Florfenicol at a daily dose rate of 10–40 mg/kg body weight for 5 consecutive days was safe, palatable and highly effective in reducing mortality and infection rates following *E. ictaluri* challenge in channel catfish fingerlings. Treatment at all dose rates increased survival and weight gain, and no adverse treatment-related pathologic changes were found.

**Comments:** In this study, a dosage of 10 mg FFC/kg daily for 5 consecutive days was effective in reducing mortality in catfish fingerlings exposed to *E. ictaluri*. However, a longer duration of treatment—10 consecutive days—is recommended for commercial situations, when conditions are less controlled than in an experimental setting.
Dose Titration — Study 2

The following dose titration study was conducted to assess the safety, palatability, and efficacy of Aquaflor®-medicated feed and to determine the optimal dose rate in channel catfish exposed to a pathogenic strain of *E. ictaluri*.

**Study Design:** Six hundred (600) laboratory-reared, 4- to 5-month-old channel catfish fingerlings with no known history of exposure to *E. ictaluri* were divided into 5 groups of 120 fish:

- Group 1 — not challenged with *E. ictaluri* and fed unmedicated feed
- Group 2 — challenged with *E. ictaluri* and fed unmedicated feed
- Group 3 — challenged and fed Aquaflor® at 5 mg FFC/kg/day for 10 days
- Group 4 — challenged and fed Aquaflor® at 10 mg FFC/kg/day for 10 days
- Group 5 — challenged and fed Aquaflor® at 15 mg FFC/kg/day for 10 days

Sera from 50 additional fish from the same source were evaluated using a modified agglutination assay and found to be negative for antibodies to *E. ictaluri*.

The study fish were weighed in groups and then exposed by immersion for 2 hours to approximately 8.8 x 10⁵ colony-forming units (CFU)/mL of a confirmed pathogenic isolate of *E. ictaluri*, obtained from a natural outbreak of ESC. Treatment with Aquaflor®-medicated feed began in Groups 3–5 the day after exposure and continued for 10 consecutive days. All surviving fish were then observed for an additional 14 days, during which time all groups were fed an unmedicated ration.

Throughout the study, feeding activity was subjectively graded each day, based on the amount of feed consumed. A score of 2 was assigned if 50–100% of the food was consumed. A score of 1 was given if <50% of the food was consumed. A score of 0 was given if little or no food was consumed.

The fish were also monitored daily for abnormal behavior indicative of morbidity (e.g., lethargy, abnormal swimming patterns) and for adverse events, such as piping at the water surface or unexpected deaths.

Any fish that died during the study were necropsied and isolation of *E. ictaluri* was attempted. At the end of the study period (day 25), all surviving fish were counted, weighed in groups, euthanized and submitted for microbiologic and pathologic examination.

**Results:** Cumulative mortality rates over the 24-day observation period are shown in Figure 4-3. Following exposure, the first mortalities occurred on day 5, in the challenged, unmedicated fish (Group 2). Mortalities were seen in this group from day 5 to day 18, with the majority occurring on days 6–8. The cumulative mortality rate in this group was 60%, with individual tank mortality rates ranging from 45–85%. One of the unchallenged, unmedicated fish (Group 1) died.
Only 9/360 fish in the Aquaflor®-treated groups died (Figure 4-3). Mortality rates in the Aquaflor®-treated fish (Groups 3 – 5) and in the unchallenged, untreated fish (Group 1) were significantly lower than the mortality rate in the challenged, untreated fish (Group 2; p<0.0001 for each contrast). There were no statistically significant differences in mortality rate between Aquaflor®-treated groups or between Aquaflor®-treated and unchallenged fish.5

No adverse events were noted during the study. Feeding activity in the unchallenged, unmedicated fish (Group 1) was scored at 2 throughout the study. The average feeding activity score for the challenged, unmedicated fish (Group 2) ranged from 0.33 to 1.67 from Day 3 to 13. From Day 3 onward, the average feeding score did not return to 2 with the exception of Day 23.

Feeding activity in all Aquaflor®-medicated groups was scored at 2 for all days throughout the study, except day 18, in which one of the tanks in Group 5 (15 mg FFC/kg) had a feeding activity score of 1, dropping the average score for Group 5 on day 19 to 1.83.

Bacteria characteristic of *E. ictaluri* were cultured from 100% of fish that died naturally during the study period. Infection rates for all fish, including surviving fish euthanized at the end of the study period, are shown in Figure 4-4.
A. Total number of infected fish. Includes fish that died naturally and fish that were euthanized at the end of the study.

B. Challenged with *E. ictaluri* then treated with FFC at the listed dose rate for 10 consecutive days.

Gross examination revealed external and internal lesions characteristic of *E. ictaluri* infection in 81% of fish that were culture-positive for the organism. External lesions included inflammation through the sutra fontanel of the skull (“hole in the head”), hemorrhages on the skin and fins, and exophthalmia. Internal lesions included hemorrhages on the gastrointestinal tract and liver, as well as a congested spleen, ascites and swollen kidneys. The incidence of these lesions was greatest in the challenged, untreated fish (Group 2).

**Conclusion:** Aquaflor® at a daily dose rate of 5 – 15 mg FFC/kg body weight for 10 consecutive days was safe, palatable and highly effective in reducing mortality and infection rates following *E. ictaluri* challenge in channel catfish fingerlings. No adverse treatment-related gross pathological effects were found at any dose rate.

**Comments:** While there were no significant differences in mortality or infection rates between the 5 mg/kg and 10 mg/kg Aquaflor® groups, a dosage of 10 mg FFC/kg/day for 10 consecutive days is recommended to ensure adequate intake of FFC by all fish in the tank or pond. Feeding observations indicated excellent consumption of medicated feed at all dose rates. However, in other fish species, it has been demonstrated that the more aggressive eaters receive proportionately more of the medicated feed than the less aggressive eaters.
Dose Confirmation Study

The following study was conducted to confirm the efficacy and palatability of Aquaflor®-medicated feed, at a dose rate of 10 mg FFC/kg/day for 10 consecutive days, in channel catfish exposed to a pathogenic strain of E. ictaluri.

Study Design: Six hundred (600) laboratory-reared, 4- to 5-month-old channel catfish fingerlings with no known history of exposure to E. ictaluri were divided into 2 groups of 300 fish:

- Group 1 — challenged with E. ictaluri and fed unmedicated feed
- Group 2 — challenged and fed Aquaflor® 50% Type A Medicated Article at 10 mg FFC/kg/day for 10 days

Sera from 50 additional fish from the same source were evaluated using a modified agglutination assay and found to be negative for antibodies to E. ictaluri.

The study fish were weighed in groups and then exposed to approximately 9.5 x 10^6 CFU/mL of a confirmed pathogenic isolate of E. ictaluri, obtained from a natural outbreak of ESC. Both treatment groups received commercial unmedicated feed on Day 1. In Group 2, treatment with Aquaflor®-medicated feed began 2 days after exposure and continued for 10 consecutive days. All surviving fish were then observed for an additional 14 days, during which time both groups were fed an unmedicated ration.

Throughout the study, feeding activity was subjectively graded each day, based on the amount of feed consumed. A score of 2 was assigned if 50 – 100% of the food was consumed. A score of 1 was given if <50% of the food was consumed. A score of 0 was given if little or no food was consumed.

The fish were also monitored daily for abnormal behavior indicative of morbidity (e.g., lethargy, abnormal swimming patterns) and for adverse events, such as piping at the water surface or unexpected deaths.

Any fish that died during the study were necropsied and isolation of E. ictaluri was attempted. At the end of the study period (day 25), all surviving fish were counted, weighed in groups, euthanized and submitted for microbiologic and pathologic examination.

Results: Cumulative mortality rates are shown in Figure 4-5. Following exposure, the first mortalities occurred on day 3 in the challenged, unmedicated fish (Group 1). The majority of deaths in this group occurred between days 5 and 11, with the most occurring on day 7. The cumulative mortality rate in this group was 87.3%, with individual tank mortality rates ranging from 60 – 100%.

Only 42/300 (14%) of the Aquaflor®-treated fish died. The majority of these deaths occurred between days 5 and 7. Individual tank mortality rates for this group ranged from 0 – 75%. The cumulative mortality rate in the Aquaflor®-treated fish was significantly lower than that in untreated fish (p<0.0001).
The average feeding activity score in the Aquaflor®-treated fish ranged from 1.7 – 2. The lowest score in this group was recorded on day 4, when 5/15 tanks had a feeding activity score of 1. The remaining 10 tanks had a feeding score of 2, yielding an average score for that day of 1.7. From day 6 onward, at least 14/15 tanks of Aquaflor®-treated fish maintained feeding scores of 2.

The lowest score for the untreated fish (Group 1) was also recorded on day 4, when the average for the group was 0.13. The average feeding activity score was <1.6 on 12 of 25 study days. The score was <1 on 6 of 25 days. From day 17 onward, the feeding activity score in surviving fish was 1.8 – 2.

*E. ictaluri* was cultured from 251/300 fish (83.7%) in the untreated group and only 28/300 fish (9.3%) in the Aquaflor®-treated group (Figure 4-5).

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**Figure 4-5** Mortality and Infection Rates for Treated and Untreated Catfish

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mortality Rates (%)</th>
<th>Infection Rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Untreated, challenged)</td>
<td>87.3</td>
<td>83.7</td>
</tr>
<tr>
<td>Group 2 (10 mg FFC/kg)</td>
<td>14.0</td>
<td></td>
</tr>
<tr>
<td>Group 1 (Untreated, challenged)</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Group 2 (10 mg FFC/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. Includes fish that died naturally and fish that were euthanized at the end of the study.
B. Challenged with *E. ictaluri* then treated with FFC at the listed dose rate for 10 consecutive days.

Gross examination revealed external and internal lesions characteristic of *E. ictaluri* infection in 88% of fish that were culture-positive for the organism. External lesions included inflammation through the sutra fontanel of the skull (“hole in the head”), hemorrhages on the skin and fins, and exophthalmia. Internal lesions included hemorrhages on the gastrointestinal tract and liver, as well as a congested spleen, ascites and swollen kidneys. The incidence of these lesions was greatest in the challenged, untreated fish (Group 1).^5^  

**Conclusions:** Aquaflor® at a daily dose rate of 10 mg FFC/kg body weight for 10 consecutive days was palatable and highly effective in reducing mortality and infection rates following *E. ictaluri* challenge in channel catfish fingerlings.
Clinical Efficacy – Pond Trial

The following study was conducted to confirm the efficacy of Aquaflor®-medicated feed, at a daily dose rate of 10 mg FFC/kg body weight for 10 consecutive days, for the control of mortality associated with ESC caused by *E. ictaluri* in catfish under commercial farming conditions.

**Study Design:** The study was conducted using approximately 154,000 channel catfish fingerlings (150 – 180 days of age, weighing 6.6 – 7.8 grams) from an ESC-free facility. Fish were held in 14 ponds of approximately 0.1 acre each, at a stocking rate of approximately 11,000 fish/pond. Ponds were assigned to 1 of 2 treatment groups:

- Group 1 — fed unmedicated feed
- Group 2 — fed Aquaflor® 50% Type A Medicated Article at a dose rate of 10 mg FFC/kg/day for 10 days

Fish from both groups were challenged with a pathogenic isolate of *E. ictaluri* obtained from a natural outbreak of ESC. All fish were fed unmedicated feed, and ponds were observed until the cumulative morbidity/mortality rate attributable to ESC (based on clinical signs and/or lesions) reached 0.3% per pond. At that point, fish in ponds assigned to Group 2 received Aquaflor®-medicated feed for 10 consecutive days. At the end of the treatment period, the fish were observed for a further 14 days.

During the 24-day study period, all ponds were monitored daily for morbidity and mortality. All dead or moribund fish displaying clinical signs of ESC (e.g., swimming in circles at the pond surface, lethargy, “hole in the head,” ascites) were collected and examined by gross necropsy. Samples were submitted for isolation of *E. ictaluri*. In ponds in which >5 moribund/dead fish were found per week, only 5 moribund or minimally autolyzed dead fish were cultured per week from that pond.

At the end of the study (day 25), all fish were euthanized and 20 fish from each pond were examined by gross necropsy and cultured for *E. ictaluri*. In samples positive for *E. ictaluri*, MICs for FFC were determined on up to 15 fish from each pond. All carcasses were subsequently incinerated or buried.

**Results:** For this analysis, a fish’s survival time was defined as the number of days a fish lived after the pond was admitted into the study. The survival analysis not only took into account a fish’s living status at the end of the study, (alive or dead) but also utilized the information of how long the fish lived. This allowed the survival rate of all the fish in the entire study period to be estimated, and therefore, provide for a better understanding of the treatment effect over time.

Results from the survival analysis based on fish recovered, are shown in Figure 4-6. The two treatment groups were significantly different (p<0.0001), with the Aquaflor® group having a higher survival rate than the control. The Aquaflor® group had statistically significant lower cumulative mortality rate than the control group (p=0.0397, one-sided test). The parameter estimate of 0.7906 equates to an odds ratio of 2.20, indicating the odds of mortality are 2.2 times the odds of mortality in the Aquaflor®-treated group.
The MIC for all tested isolates of *E. ictaluri* was 0.25 µg/mL. The mean Disk Diffusion Assay zone of inhibition was 36.8 mm (range: 32 – 50 mm) for all *E. ictaluri* isolates.  

**Conclusion:** Aquaflor® administered to channel catfish under commercial farming conditions at a daily dose rate of 10 mg FFC/kg body weight for 10 consecutive days was effective in reducing mortality associated with infection by *E. ictaluri*.
Environment

An environmental risk assessment has been performed for the use of Aquaflor® in catfish production. This assessment included evaluation of all available data for florfenicol and its metabolites, and generation of data specific for the use of florfenicol in freshwater environments.

ENVIRONMENTAL EXPOSURE PROFILE

The concentrations of florfenicol and its metabolites in the receiving environment are dependent on the quantities of medicated feed administered and consumed and their fate in the environment.

Florfenicol has a molecular weight of 358.21 daltons, water solubility of 1.32 g/L at pH 7, and a log $K_{ow}$ value (partition coefficient) of 0.37, the latter indicating little potential for bioaccumulation. A conservative estimate of biodegradation half-life in aquatic systems is 30 days. As the physiochemical characteristics of florfenicol and its major metabolites show, accumulation of florfenicol or its degradation products in sediments or biota is unlikely.

Because nearly all catfish feed is formulated as floating extruded pellets, this type of feed has high water stability and does not sink into sediments where it becomes unavailable to fish. Virtually 100% of dispensed feed is consumed by catfish and little (if any) reaches the sediments. Thus, florfenicol and its metabolites enter the sediment only via excreta, with the compounds then moving into the water column through leaching from feces and by mixing of the aqueous phase of excreta into the water column.

As an enclosed aquaculture system, catfish ponds do not represent the ambient environment and are not of environmental concern. However, any overflow or release of water (draining) from the ponds into the general environment needs to be assessed. Catfish are typically reared in large ponds approximately 1 m in depth, with the water level maintained below the surrounding soil surface by a perimeter levee. To reduce water loss via overflow and the release of effluent from the ponds, the water level is maintained below the overflow structure of the pond (i.e., a 20-cm storage capacity below the overflow level). This strategy normally prevents rainfall from causing overflow. In addition, clay soil types are often a predominant feature of pond construction, so leakage from ponds is minimal. These factors serve to limit the release of pond water and any florfenicol-related residues into the environment.
ENVIRONMENTAL EXPOSURE CONCENTRATIONS

Studies investigating the concentrations of florfenicol and metabolites released into the environment have been conducted. Assessments were based on hypothetical drug-use scenarios that would release the greatest possible amount of florfenicol (and metabolites) into the environment. The “worst-case” scenario that was developed involved the complete draining of a nursery pond (fingerlings) 42 days after completion of a 10-day florfenicol treatment regimen.

This worst-case analysis yielded a preliminary predicted environmental concentration (PEC) in the pond of 0.067 mg/L, a calculation that assumed 100% of the florfenicol-related residues were in the water column (none partitioned to sediments, and none remained in fish) and assumed no residue degradation occurred in the pond. A more realistic analysis that accounted for the 30-day florfenicol half-life in water yielded an estimated PEC of 0.0268 mg/L in pond water. If this is then diluted 1:10 into receiving waters, the refined PEC is estimated to be only 0.00268 mg/L.

Clearly, very low concentrations of florfenicol or metabolites are released into the environment, even under worst-case conditions. Considering the fact that drug degradation occurs in water, and pond effluent is dispersed and diluted in receiving waters, the environmental risk potentially posed by florfenicol-related residues in waters outside catfish ponds is very low. Sediments were not included in this analysis because florfenicol does not enter or remain in sediments in significant amounts, but will move into the water phase. Furthermore, any florfenicol that is released from ponds will remain in the water phase and will not partition to sediments.

ENVIRONMENTAL SAFETY

Studies undertaken by Schering-Plough Animal Health constitute a comprehensive data set regarding the safety of florfenicol for invertebrates, fish, birds and mammals. To establish the potential for an adverse effect in the environment, the minimum inhibitory concentration (MIC), lethal concentration (LC₅₀) or effect concentration (EC₅₀) values, and no observable effect concentration (NOEC) were compared to the PEC in aquatic environments. Ratios of the PEC to the predicted no effect concentration (PNEC) were established for a wide range of aquatic life forms. The PNEC values were derived by applying a 10- to 100-fold safety factor to the L(E)C₅₀ or NOEC obtained. Details of the environmental risk characterization data for selected freshwater species are shown in Table 5-1.
The refined PEC is far less than the calculated PNEC values. The very low PEC/PNEC proportions reflect the lack of toxicity of florfenicol to aquatic organisms. Since only very small amounts of florfenicol can enter the aquatic environment, and the drug and its metabolites are then rapidly degraded and dissipated, florfenicol poses little if any risk to aquatic ecosystems. Existing toxicity data indicate that florfenicol is, in general, more active against prokaryotic than eukaryotic organisms. However, the likelihood of environmental effects are very limited given the low PEC:PNEC ratios, the drug’s intended use patterns, its fate in the receiving environments and its low toxicity.

These data indicate that florfenicol administration via the feed to catfish reared in commercial production facilities will not adversely affect the aquatic environment.

<table>
<thead>
<tr>
<th>Organism</th>
<th>NOEC(mg/L)</th>
<th>Safety Factor</th>
<th>PNEC(mg/L)</th>
<th>PEC B/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncorhynchus mykiss (rainbow trout)</td>
<td>780</td>
<td>100</td>
<td>7.8</td>
<td>0.000344</td>
</tr>
<tr>
<td>Lepomis macrochirus (bluegill sunfish)</td>
<td>830</td>
<td>100</td>
<td>8.3</td>
<td>0.000323</td>
</tr>
<tr>
<td>Daphnia magna (water flea)</td>
<td>&lt;100</td>
<td>100</td>
<td>1.0</td>
<td>0.000812</td>
</tr>
<tr>
<td>Selenastrum capricornutum (green algae)</td>
<td>0.75</td>
<td>10</td>
<td>0.075</td>
<td>0.0357</td>
</tr>
<tr>
<td>Bacillus subtilis (bacteria)</td>
<td>0.4⁴</td>
<td>10</td>
<td>0.04</td>
<td>0.067</td>
</tr>
</tbody>
</table>

A. Safety factor of 100 applied (10X to account for intraspecies variation; 10X for extrapolation from acute to chronic data).
B. Refined PEC of 0.00268 mg/L for florfenicol (pond water released into environment).
C. MIC (µg/L).
References


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 issued by a licensed veterinarian in the course of the veterinarian’s professional practice.

Schering-Plough Animal Health
Aquaculture

SUMMIT, NJ 07901

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