

Florfenicol Medicated Feed Clinical Field Trials - INAD 10-697

Year 2006 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

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Summary

Florfenicol-medicated feed (FMF) has been used effectively in the U.S. under compassionate INAD Exemption #10-697 to control mortality in a variety of fish caused by common fish bacterial pathogens. In calendar year 2006 the efficacy of FMF was evaluated in 131 disease trials involving approximately 25.9 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens. Trials were conducted at a total of 29 fish culture facilities, including two U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), and 21 state, five private, and one tribal fish hatcheries. Florfenicol medicated feed was administered at the standard treatment dose of 10 mg/Kg fish/d for 2 -10 d in 130 studies. In the remaining trial, the Investigator noted that the wrong tank received the FMF at a dose of 6.1 mg/Kg fish/d for 1 d. Overall results indicated that treatment appeared effective in approximately 75% of the trials, ineffective in 3% of the trials, and was characterized as inconclusive in 22% of the trials.

Introduction

Bacterial diseases are a major problem in aquaculture and account for significant losses of fish (Clarke and Scott 1989; Frerichs and Roberts 1989; Bjorndal 1990). Although the importance of environmental conditions (McCarthy and Roberts 1980; Haastein 1988; Munro and Roberts 1989) and the value of effective vaccines, where available (Ellis 1989), are acknowledged, antimicrobial therapy presently has an important role to play in aquaculture (Klontz 1987; Alderman 1988). Florfenicol is a potent, broad-spectrum, antimicrobial agent with bacteriostatic properties (Horsberg et al. 1996). It is a fluorinated analogue of thiamphenicol and is also similar in structure to chloramphenicol, both of which have been used as broad-spectrum, veterinary antibiotics (Nagata and Oka 1996).

Florfenicol has great potential for treatment of infectious diseases, and because of its high potency and safety to humans, it could become an important drug in veterinary medicine, especially with respect to animals used by humans for food (Powers et al. 1990). Additionally, because florfenicol is not currently used in human medicine, it has become a strong candidate for use in aquaculture, and there is considerable interest to obtain U.S. Food and Drug Administration (FDA) approval for its use in fish culture.

The proposed treatment strategy (i.e., dosage and duration) for the use of FMF in fish is designed to meet the needs of individual fish species, individual fish lots, and a

variety of environmental conditions. In all cases, treatment goals are to (1) minimize the negative effects of disease on fish health, quality, and survival, and (2) help meet fishery management objectives. Because many factors can affect the success or failure of florfenicol-medicated feed therapy, supplemental efficacy data from compassionate Investigational New Animal Drug (INAD) use, as well as efficacy data from controlled, replicated studies that are scientifically valid and statistically defensible (i.e., pivotal), are needed to gain approval of FMF use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of supplemental FMF field efficacy studies conducted in calendar year (CY) 2006. Furthermore, it is expected that these data will be used to enhance the FMF database for the purpose of developing an appropriate label claim for the use of this new animal drug.

Facilities, Materials, and Treatment Procedures

1. Participating Facilities

A total of 131 effectiveness trials were conducted at 29 fish culture facilities, including two U. S. Fish and Wildlife Service NFH, and 21 state, five private, and one tribal fish hatcheries. Trials were conducted to control mortality in a variety of fish caused by a variety of fish pathogens. Water temperature during

treatment trials ranged from 41.0 - 79.0 °F, with a mean treatment temperature of 65.8 °F.

2. FMF used in trials

The Aquaflor™ used in CY 2006 trials contained 500 g of florfenicol per kg of premix. Florfenicol is a pure compound with no inactive ingredients. All florfenicol used was supplied as Aquaflor™ by Schering-Plough Animal Health, 1095 Morris Avenue, Union, NJ. Florfenicol medicated feed was prepared by either top-coating florfenicol onto commercial fish feed at the Bozeman Fish Technology Center using a Standard Operating Procedure developed by the Service's Aquatic Animal Drug Approval Partnership Program, prepared at the testing site by the Investigator, Monitor, or their designee, or prepared by commercial fish feed manufactures.

3. Drug dosages and duration

As described in the Study Protocol for INAD #10-697, Investigators were allowed to use FMF at 10 mg of active drug/kg of fish/d for 10 d. However, in one trial, one tank of healthy rainbow trout were accidentally fed FMF medicated feed at a calculated dosage of 6.1 mg/Kg fish/d for 1 d. Due to fish size (0.90 g), the inherent withdrawal period for the tested fish will be approximately 180 d after treatment. In another trial, the Investigator noted that the study was terminated after treatment day 2 due to drug sensitivity test showing the disease was resistant to florfenicol.

Fish Species and Fish Diseases Involved in CY 2006 Trials

1. Species of fish treated

Eight fish species, including seven species of salmonids and one non-salmonid fish species, were treated with FMF during CY 2006. Treated fish ranged in length from 0.9 - 21.3 in. and the mean length of all treated fish was 3.59 in. Fish species treated included:

Salmonids:

Atlantic Salmon *Salmo salar*

brown trout *S. trutta*

coho salmon *Oncorhynchus kisutch*

cutthroat trout *O. clarki*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

lake trout *Salvelinus namaycush*

Non-salmonids:

hybrid striped bass *Morone americana* x *M. saxatilis*

2. Diseases treated

The diseases treated most frequently were characterized as streptococcal septicemia (51% of the trials) and bacterial coldwater disease (CWD) (43% of the

trials). Other diagnosed diseases included (1) bacterial kidney disease, (2) *Carnobacterium maltaromaticum* & motile *Aeromonad*, (3) enteric redmouth, (4) furunculosis, or (5) gram negative bacteria.

Data Collected

1. Pathologist's reports

Pathologists reports were submitted with 32 trials conducted during CY 2006. Fish health pathology reports included: 1) a description of how the identity of disease agent(s) was verified; 2) disease identification records that confirm the presence of the disease agent; and 3) the name and title of the individual performing the diagnosis. Additionally, pathology reports often provide documentation that there were no secondary infections or infestations caused by unrelated disease agents in the population of test fish. Pathology reports provide critical information if such submissions are to be used in support of an initial approval, or to expand/extend an existing approved label.

2. Mortality data

As stated in the Study Protocol, mortality data were to be collected 10 days prior to treatment, during the treatment period, and for at least 21 days post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, daily collection of pre-treatment mortality data was not always possible

due to fish being moved (i.e., split into additional rearing units, or combined with fish from another rearing unit) from rearing unit to rearing unit.

Discussion of Study Results:

1. General observations on the efficacy of FMF for the control of bacterial

diseases in salmonid and non-salmonid fish (Note: Table 1 provides a summary of all efficacious trials; Table 2 provides a summary of all ineffective trials; Table 3 provides a summary of all inconclusive trials; Table 4 provides general CY 2006 summary data; and Tables 5a and 5b provide a summary of all trials conducted during CY06 under INAD #10-697; (Table 5a is sorted by study number; Table 5b is sorted first by disease treated, second by whether treatments were efficacious or not, and lastly by fish species).

A. Efficacy at 10 mg/Kg fish/d for 2 - 10 days under INAD #10-697

Atlantic salmon, coho salmon, cutthroat, brown, lake, rainbow, and steelhead trout, and hybrid striped bass, were treated with 10 mg florfenicol/Kg fish/d for 2 - 10 days in 128 trials to control mortality caused by one of the previously described fish diseases (Tables 1 - 3). FMF treatments appeared effective in 98 (75%) of the 128 trials; ineffective in 4 (3%) of the 128 trials; and were characterized as inconclusive in 29 (22%) of the 128 trials.

B. Efficacy at 6.1 mg/Kg fish/d for 1 day under INAD #10-697

Healthy rainbow trout were accidentally fed FMF medicated feed at a calculated dosage of 6.1 mg/Kg fish/d for 1 d (Table 3). Due to fish size (0.90 g), the inherent withdrawal period for the tested fish will be approximately 180 d after treatment. FMF treatment in this study was characterized as inconclusive.

C. Efficacy of 10 mg/Kg fish/day for 10 days under Pivotal Research

Protocol FLOR-01-EFF

Coho salmon were treated with 10 mg florfenicol/Kg fish/d for 10 days in one trial to control mortality caused by furunculosis (Table 1). FMF treatment was effective in this trial (please refer to pivotal submission numbers FLOR-01-EFF.3-27 for more details on this study).

2. Observed Toxicity

No toxicity or adverse effects relating to FMF treatment were reported.

Summary of Study Results

Florfenicol medicated feed was administered to test fish in 131 separate trials at a dosage of either 6.1 or 10 mg/Kg fish/d for 1 - 10 d. Eight different fish species were treated with FMF, and trials involved approximately 25.9 million fish. Treated fish ranged in size from 0.9 - 21.3 in. Water temperature during treatment ranged from 41.0 - 79.0 °F, with a mean treatment temperature of 65.8 °F. Overall results showed that in

approximately 75% of the trials, FMF treatments appeared effective, 3% of the trials were ineffective, and 22% of the trials were characterized as inconclusive. The trial conducted under the pivotal research protocol FLOR-01-EFF included use of control fish, detailed pathologist's reports documenting the disease during the trial, and will likely be accepted by CVM as pivotal or supportive. Data from the other studies will be considered as ancillary data because of a general lack of quality control criteria essential for pivotal or supportive studies, such as use of untreated control fish, dose verification, replication, and randomization. None-the-less, the ancillary data documented in this report should provide useful corroborative data to support a new label claim for FMF. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-697. In future trials conducted under INAD #10-697, efforts will be directed towards the generation of high quality data.

References

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Table 1. Summary of CY 2006 Florfenicol Medicated Feed Efficacy Results - Effective Trials

| Hatchery | Number of Trials | Fish Species | Fish Size (inches) | Number of Fish | Disease | Number of Treatment Days | Dose (mg/kg) | Temp. (°F) |
|---------------------------|------------------|--------------|--------------------|----------------|-------------------------------|--------------------------|--------------|-------------|
| Oden SFH | 1 | BNT | 20.80 | 1,120 | Carnobacterium maltaromaticum | 10 | 10 | 45.3 |
| Big Creek SFH | 3 | COS | 1.9 - 3.4 | 1,756,000 | CWD | 10 | 10 | 43.0 - 54.0 |
| Cascade SFH | 3 | COS | 2.1 - 15.7 | 6,358,149 | CWD | 10 | 10 | 41.0 - 48.0 |
| Cole Rivers SFH | 1 | COS | 1.20 | 317,982 | CWD | 10 | 10 | 47.6 |
| Makah NFH | 2 | COS | 3.3 - 3.9 | 255,333 | Furunculosis | 10 | 10 | 62.1 - 63.9 |
| Nehalem SFH | 2 | COS | 1.5 - 3.1 | 292,023 | CWD | 10 | 10 | 53.1 - 56.0 |
| Nez Perce Tribal Hatchery | 2 | COS | 3.5 - 6.0 | 487,437 | CWD | 10 | 10 | 43.7 - 62.2 |
| Oxbow SFH | 1 | COS | 1.70 | 570,686 | CWD | 10 | 10 | 45.0 |
| Sandy SFH | 1 | COS | 2.90 | 1,083,547 | CWD | 10 | 10 | 57.0 |
| Whitman Lake Hatchery | 2 | COS | 2.50 | 1,577,681 | CWD | 10 | 10 | 53.1 |
| Glenwood Sport SFH | 1 | CUT | 1.00 | 94,285 | CWD | 10 | 10 | 58.0 |
| Mackay SFH | 4 | CUT | 0.90 | 515,000 | CWD | 10 | 10 | 52.0 |
| Benner Spring Fish SFH | 1 | RBT | 2.00 | 218,000 | CWD | 10 | 10 | 50.0 |
| Cantrell Creek Trout Farm | 1 | RBT | 4.30 | 80,000 | CWD | 10 | 10 | 62.0 |
| Glenwood Sport SFH | 1 | RBT | 2.72 | 96,766 | CWD | 10 | 10 | 58.0 |
| Irrigon SFH | 1 | RBT | 2.90 | 177,000 | CWD | 10 | 10 | 53.0 |
| Klamath SFH | 1 | RBT | 2.00 | 1,000,000 | CWD | 10 | 10 | 49.0 |

Table 1. Summary of CY 2006 Florfenicol Medicated Feed Efficacy Results - Effective Trials - cont

| Hatchery | Number of Trials | Fish Species | Fish Size (inches) | Number of Fish | Disease | Number of Treatment Days | Dose (mg/kg) | Temp. (°F) |
|---------------------|------------------|--------------|--------------------|----------------|--------------------------|--------------------------|--------------|-------------|
| Leaburg SFH | 2 | RBT | 1.3 - 1.8 | 61,883 | CWD | 10 | 10 | 48.0 - 52.0 |
| Loa SFH | 1 | RBT | 2.50 | 100,000 | CWD | 10 | 10 | 57.0 |
| Nehalem SFH | 1 | RBT | 1.25 | 133,000 | CWD | 10 | 10 | 55.0 |
| Tellico Enterprises | 1 | RBT | 4.25 | 28,777 | CWD | 10 | 10 | 52.0 |
| Bandon SFH | 3 | STT | 1.3 - 1.5 | 138,468 | CWD | 10 | 10 | 54.1 - 55.2 |
| Big Creek SFH | 1 | STT | 2.00 | 210,000 | CWD | 10 | 10 | 54.0 |
| Cole Rivers SFH | 2 | STT | 3.1 - 4.2 | 100,000 | CWD | 10 | 10 | 58.5 - 58.7 |
| Dworshak NFH | 1 | STT | 3.70 | 302,472 | CWD | 10 | 10 | 43.9 |
| Irrigon SFH | 1 | STT | 1.42 | 1,398,000 | CWD | 10 | 10 | 53.9 |
| Makah NFH | 1 | STT | 2.30 | 200,827 | CWD | 10 | 10 | 60.8 |
| Nehalem SFH | 1 | STT | 1.60 | 125,000 | CWD | 10 | 10 | 54.0 |
| Niagara Springs SFH | 1 | STT | 1.60 | 20,000 | CWD | 10 | 10 | 59.0 |
| Oak Springs SFH | 1 | STT | 2.90 | 992,107 | CWD | 10 | 10 | 53.0 |
| Kent SeaTech Corp | 53 | SXW | 1.6 - 9.4 | 2,182,585 | Streptococcal Septicemia | 10 | 10 | 79.0 |

Table 2. Summary of CY 2006 Florfenicol Medicated Feed Efficacy Results - Ineffective Trials

| Hatchery | Number of Trials | Fish Species | Fish Size (inches) | Number of Fish | Disease | Number of Treatment Days | Dose (mg/kg) | Temp. (°F) |
|-------------------|------------------|--------------|--------------------|----------------|-------------------------------------|--------------------------|--------------|-------------|
| Oden SFH | 1 | RBT | 19.90 | 1,000 | Motile Aeromonad and Carnobacterium | 10 | 10 | 45.5 |
| Willamette SFH | 2 | RBT | 1.2 - 1.5 | 106,398 | CWD | 10 | 10 | 46.0 - 47.0 |
| Kent SeaTech Corp | 1 | SXW | 9.40 | 12,871 | Streptococcal Septicemia | 10 | 10 | 79.0 |

Table 3. Summary of CY 2006 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials

| Hatchery | Number of Trials | Fish Species | Fish Size (inches) | Number of Fish | Disease | Number of Treatment Days | Dose (mg/kg) | Temp. (°F) |
|---------------------------|------------------|--------------|--------------------|----------------|------------------------|--------------------------|--------------|------------|
| Birch Point | 1 | ATS | 9.70 | 380,824 | Enteric Redmouth | 10 | 10 | 48.9 |
| Oden SFH | 1 | BNT | 5.20 | 398,610 | BKD | 10 | 10 | 45.3 |
| Oxbow SFH | 1 | COS | 2.20 | 107,298 | CWD | 10 | 10 | 45.0 |
| Mackay SFH | 5 | CUT | 0.90 | 820,000 | CWD | 10 | 10 | 52.0 |
| Marquette SFH | 1 | LAT | 21.30 | 2,342 | Gram Negative Bacteria | 10 | 10 | 41.0 |
| Benner Spring Fish SFH | 1 | RBT | 2.00 | 998,000 | CWD | 10 | 10 | 50.0 |
| Butte Falls SFH | 1 | RBT | 3.20 | 313,000 | CWD | 10 | 10 | 53.2 |
| Cantrell Creek Trout Farm | 1 | RBT | 3.00 | 80,000 | CWD | 2 | 10 | 56.0 |
| Tellico Enterprises | 1 | RBT | 2.20 | 300,000 | CWD | 10 | 10 | 58.0 |

Table 3. Summary of CY 2006 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials - cont

| Hatchery | Number of Trials | Fish Species | Fish Size (inches) | Number of Fish | Disease | Number of Treatment Days | Dose (mg/kg) | Temp. (°F) |
|-------------------|------------------|--------------|--------------------|----------------|--------------------------|--------------------------|--------------|------------|
| Butte Falls SFH | 1 | STT | 4.96 | 27,000 | CWD | 10 | 10 | 54.5 |
| Round Butte SFH | 1 | STT | 2.00 | 441,000 | CWD | 10 | 10 | 51.0 |
| Kent SeaTech Corp | 13 | SXW | 1.6 - 9.4 | 418,188 | Streptococcal Septicemia | 10 | 10 | 79.0 |
| Cole Rivers SFH | 1 | RBT | 1.70 | 589,000 | No Disease | 1 | 6.1 | 47.0 |

Table 4. Summary Data Regarding CY 2006 Florfenicol Medicated Feed Efficacy Trials

| | |
|---|--------------------------|
| Total Fish Treated: | <u>25,869,659</u> |
| Number of fish treated in effective trials | 20,869,659 |
| Number of fish treated in ineffective trials | 120,269 |
| Number of fish treated in inconclusive trials | 4,875,262 |

| | |
|--------------------------------|------------|
| Total number of trials: | 131 |
| Number of effective trials: | 98 |
| Number of ineffective trials: | 4 |
| Number of inconclusive trials: | 29 |

Pivotal Study:

Study Number: 10-697-06-64

Treatment Regimes Used:

| | |
|-------------------------------|------------|
| 10 mg/Kg fish/day for 10 days | 129 trials |
| 10 mg/Kg fish/day for 2 days | 1 trial |
| 6.1 mg/Kg fish/day for 1 day | 1 trial |

Treatment Water Temperature (°F):

| | |
|-------------------|-------------|
| Temperature Range | 41.0 - 79.0 |
| Mean Temperature | 65.8 |

Size of Treated Fish (in.):

| | |
|------------|-------------|
| Size Range | 0.90 - 21.3 |
|------------|-------------|

Species Treated:

Atlantic Salmon *Salmo salar*
 brown trout *S. trutta*
 coho salmon *Oncorhynchus kisutch*
 cutthroat trout *O. clarki*
 rainbow trout *O. mykiss*
 steelhead trout *O. mykiss*
 lake trout *Salvelinus namaycush*

Non-salmonids:

hybrid striped bass *Morone americana* x *M. saxatilis*