

Florfenicol Medicated Feed Clinical Field Trials - INAD 10-697

Year 2004 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 2004 the efficacy of FMF was evaluated in 99 disease trials involving approximately 13.1 million fish to control mortality in a variety of test fish caused by bacterial coldwater disease, columnaris, furunculosis, enteric septicemia, *Aeromonus hydrophila*, motile aeromonad, or streptococcal septicemia. Trials were conducted at six U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), and seven state, three private, and two tribal fish hatcheries. Florfenicol medicated feed was administered at the standard treatment dose of 10 mg/Kg fish/d for 5 - 11 d in 98 studies, while the Investigator in one study administered feed at 100 mg/kg for 7 days and then 10 mg/kg for 3 days. Overall results indicated that in approximately 72% of the trials, FMF treatments appeared effective, in 9% of the trials, FMF treated appeared ineffective, and in 19% of the trials, FMF treatments were characterized as inconclusive.

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) 2004. 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

Eighteen fish culture facilities, including six National fish hatcheries, and seven state, three private, and two tribal fish hatcheries, used FMF to control mortality in a variety of fish caused by a variety of fish pathogens in 99 separate field trials.

Water temperature during treatment trials ranged from 41.0 - 86.0 °F, with a mean treatment temperature of 67.1 °F.

2. FMF used in trials

The Aquaflor™ used in CY 2004 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 accepted Standard Operating Procedures, 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 three trials fish were fed FMF medicated feed at 10 mg/Kg fish/d for either 5, 9, or 11 d. The fish that were fed for 5 days were done so because fish were destroyed on treatment day 6. As for the other two trials in which FMF was administered for periods other than 10 d, AADAP staff notified Investigators of the deviation from the protocol and reminded them that the accepted treatment regimen requires a 10 d treatment period. In another trial, fish were fed FMF medicated feed at 100 mg/Kg fish/d for 7 days and then 10 mg/Kg for 3 d. In this study, the

Investigators had mis-calculated the amount of Aquaflor premix need to properly medicated feed and did not catch this mistake until treatment day 7. Fish health samples were collected on post-treatment days 4 and 27. These samples were viewed by a histopathologist and no abnormalities were found. Due to fish size (0.6g), the inherent withdrawal period for the tested fish (i.e., fish will not be available for human consumption) will be approximately 350 days after treatment.

Fish Species and Fish Diseases Involved in CY 2004 Trials

1. Species of fish treated

The twelve fish species, including nine salmonids and three non-salmonids, listed below were treated with FMF during CY 2004. Treated fish ranged in size from 1.0 - 20.5 in.:

Salmonids:

Atlantic salmon *Salmo salar*

chinook salmon *Oncorhynchus tshawytscha*

coho salmon *O. kisutch*

cutthroat trout *O. clarki*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

brook trout *Salvelinus fontinalis*

lake trout *S. namaycush*

splake *S. fontinalis* x *S. namaycush*

Non-salmonids:

channel catfish *Ictalurus punctatus*

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

largemouth bass *Micropterus salmoides*

2. Diseases treated

Test fish diagnosed with one of the following diseases were treated during the reporting period: (1) bacterial coldwater disease, (2) columnaris, (3) furunculosis, (4) enteric septicemia, (5) *Aeromonus hydrophila*, (6) motile *Aeromonad*, or (7) streptococcal septicemia.

Data Collected

1. Pathologist's reports

Pathologists reports were submitted with 10 of the 99 trials conducted during CY 2004 (one trial in which pathology report was submitted was a pivotal study; in such studies comprehensive pathology reports are required). 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 non-efficacious trials; Table 3 provides a summary of all inconclusive trials; Table 4 provides general CY 2004 summary data; and Table 5 provides a brief description of all trials conducted during CY 2004 under INAD #10-697.)

A. Efficacy at 10 mg/Kg fish/d for 5 - 11 days under INAD #10-697

Atlantic, coho, and chinook salmon, brook, cutthroat, rainbow, steelhead, and lake trout, splake, channel catfish, hybrid striped bass, and largemouth bass were treated with 10 mg florfenicol/Kg fish/d for 5 - 11 days in 97 trials to control mortality caused by one of the following diseases: bacterial coldwater disease, columnaris, furunculosis, enteric septicemia, *Aeromonus hydrophila*, motile aeromonad, or streptococcal septicemia (Tables 1 - 3). FMF treatments appeared effective in 69 (71%) of the trials, whereas treatments in nine (9%) of the trials appeared ineffective, and results from 19 (20%) of the trials were characterized as inconclusive.

B. Efficacy at 10 & 100 mg/Kg fish/d for 10 days under INAD #10-697

Coho salmon were treated with 10 & 100 mg florfenicol/Kg fish/d for 10 days in one trial to control mortality caused by bacterial coldwater disease (Table 1). FMF treatment in this study appeared effective.

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

Protocol FLOR-01-EFF

Chinook salmon were treated with 10 mg florfenicol/Kg fish/d for 10 days in one trial to control mortality caused by furunculosis (Tables 1). FMF treatment was effective in this trial (please refer to pivotal submission numbers FLOR-01-EFF-22 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 at a dosage of either 10 or 100 mg/Kg fish/d. Treatment duration ranged from 5 - 11 days. Twelve different fish species were treated with FMF, and trials involved approximately 13.1 million fish. Treated fish ranged in size from 1.0 - 20.5 in. Water temperature during treatment ranged from 41.0 - 86.0 °F, with a mean treatment temperature of 67.1 °F. Overall results showed that in approximately 72% of the trials, FMF treatments appeared effective, in 9% of the trials, treatments appeared ineffective, and in 19% of the trials, treatment results 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 higher quality data.

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Table 1. Summary of CY 2004 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)
Cypress Island Hatchery	15	ATS	2.0 - 6.7	3,919,990	Furunculosis	10 - 11	10	50.0
Oquossoc Hatchery	1	ATS	9.00	415,155	Furunculosis	10	10	44.2
Thompson SFH	1	ATS	3.20	28,507	Furunculosis	10	10	50.0
Allegheny NFH	1	BKT	8.50	86,900	Furunculosis	10	10	50.0
Little Grassy SFH	1	CCF	5.50	32,500	Enteric Septicemia Catfish	10	10	81.0
Lower Elwha Hatchery	1	COS	2.50	136,452	CWD	10	10	49.0
	1	COS	1.50	116,500	CWD	10	10 & 100	49.0
Makah NFH	3	COS	2.8 - 3.5	503,000	Furunculosis	10	10	65.0 - 67.0
Winthrop NFH	1	COS	3.50	80,000	CWD	10	10	53.0
Bozeman FTC	1	CUT	1.38	5,000	CWD	10	10	54.0
Makah NFH	1	FCS	4.20	900	Furunculosis	10	10	62.6
Allegheny NFH	1	LAT	4.60	597,400	Furunculosis	10	10	50.0
St. Croix Waters Fishery	3	LMB	3.0 - 6.5	130,627	A. Hydrophila	10	10	72.0
Oden SFH	1	RBT	20.50	2,633	Furunculosis	10	10	45.5
Dworshak NFH	2	STT	1.20	629,000	A. Hydrophila	10	10	52.6
	1	STT	3.76	130,000	CWD	10	10	47.9
Makah NFH	2	STT	1.9 - 2.5	412,000	CWD	10	10	58.0 - 59.0
Winthrop NFH	1	STT	1.75	107,678	CWD	10	10	50.0
Kent SeaTech Corp.	33	SXW	2.4 - 7.9	2,269,824	Streptococcus	10	10	81.0 - 86.0

Table 2. Summary of CY 2004 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)
Garrison Dam NFH	1	CUT	8.40	42,902	Columnaris	9	10	58.5
Bellingham SFH	1	FCS	3.34	44,991	Columnaris	10	10	67.0
Marquette SFH	1	SPL	5.99	85,589	Motile Aeromonad	10	10	41.0
Winthrop NFH	1	STT	1.40	114,000	CWD	10	10	49.0
Kent SeaTech Corp.	5	SXW	4.7 - 7.1	222,684	Streptococcus	10	10	81.0

Table 3. Summary of CY 2004 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)
Cypress Island Hatchery	1	ATS	2.00	77,000	Furunculosis	5	10	50.0
	1	ATS	4.50	293,112	Furunculosis	10	10	50.0
Marquette SFH	1	BKT	4.20	94,262	Motile Aeromonad	10	10	46.0
Little Grassy SFH	1	CCF	1.75	1,104,000	Enteric Septicemia Catfish	10	10	80.0
Issaquah Salmon SFH	1	COS	1.00	150,000	CWD	10	10	47.0
Garrison Dam NFH	1	CUT	5.50	43,000	Columnaris	10	10	53.0
Harrietta SFH	1	RBT	4.00	287,391	CWD	10	10	46.4
Oden SFH	1	RBT	2.80	281,127	A. Hydrophila	10	10	45.5
Dworshak NFH	1	STT	1.20	18,500	A. Hydrophila	10	10	52.6
Makah NFH	1	STT	3.70	64,000	Furunculosis	10	10	65.0
Kent SeaTech Corp.	9	SXW	2.4 - 7.0	618,533	Streptococcus	10	10	81.0

Table 4. Summary Data Regarding CY 2004 Florfenicol Medicated Feed Efficacy Trials

Total Fish Treated:	<u>13,145,157</u>
Number of fish treated in effective trials	9,604,066
Number of fish treated in ineffective trials	510,166
Number of fish treated in inconclusive trials	3,030,925
Total number of trials:	99
Number of effective trials:	71
Number of ineffective trials:	9
Number of inconclusive trials:	19
Pivotal Study:	
Study Number: 10-697-04-33	
Treatment Regimes Used:	
10mg/Kg fish/day for 5 - 11 days	47 trials
Treatment Water Temperature (°F):	
Temperature Range	41.0 - 86.0
Mean Temperature	67.1
Size of Treated Fish (in.):	
Size Range	1.0 - 20.5
Species Treated:	
Atlantic salmon <i>Salmo salar</i>	chinook salmon <i>Oncorhynchus tshawytscha</i>
coho salmon <i>O. kisutch</i>	cutthroat trout <i>O. clarki</i>
rainbow trout <i>O. mykiss</i>	steelhead trout <i>O. mykiss</i>
brook trout <i>Salvelinus fontinalis</i>	lake trout <i>S. namaycush</i>
splake <i>S. fontinalis</i> x <i>S. namaycush</i>	channel catfish <i>Ictalurus punctatus</i>
largemouth bass <i>Micropterus salmoides</i>	
hybrid striped bass <i>Morone americana</i> x <i>M. saxatilis</i>	