

Florfenicol Medicated Feed (Aquaflor®) Clinical Field Trials - INAD 10-697

Year 2011 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

Florfenicol-medicated feed (Aquaflor®)(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 2011 the efficacy of FMF was evaluated in 29 disease trials involving approximately 4.8 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 15 fish culture facilities, including one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), three state hatcheries, and 11 private hatcheries. Use of FMF under Protocol #10-697 allowed the investigator to administer FMF at either a dosage of 10 mg/Kg fish/day for 10 days or 15 mg/Kg fish/day for 10 days. Overall results indicated that treatment appeared effective in approximately 52% of the trials, ineffective in 24% of the trials, and was characterized as inconclusive in 24% of the trials.

Introduction

The 2011 label for FMF use in aquaculture limited use to: 1) the control of furunculosis in salmonids caused by *Aeromonas salmonicida*; 2) control of coldwater disease in salmonids caused by *Flavobacterium psychrophilum*; 3) control of enteric septicemia in catfish caused by *Edwardsiella ictaluri*; and 4) control of columnaris in catfish caused by *Flavobacterium columnare*. These label restrictions limit the overall utility of approved FMF use in aquaculture.

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 2011 (CY11) . Furthermore, it is expected that these data will be used to enhance the FMF database for the purpose of expanding an appropriate label claim for the use of this new animal drug.

Facilities, Materials, and Treatment Procedures

1. Participating Facilities

A total of 29 effectiveness trials were conducted at 15 fish culture facilities, including one U.S. Fish and Wildlife Service National Fish Hatchery (NFH), three state hatcheries, and 11 private 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 45.0 - 86.0 °F, with a mean treatment temperature of 66.5°F.

2. FMF used in trials

The Aquaflor[®] used in CY11 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 Merck Animal Health, 556 Morris Avenue, Summit, NJ. Florfenicol medicated feed was prepared either by top-coating florfenicol onto commercial fish feed at the testing site by the Investigator, Monitor, or their designee, or prepared by commercial fish feed manufacturers.

3. Drug dosages and duration

As described in the Study Protocol for INAD #10-697, Investigators were allowed to use FMF at either a dosage of 1) 10 mg/Kg fish/day for 10 days (approximately 45% of trials were conducted using this treatment regimen) or 2) 15 mg/Kg fish/day for 10 days (approximately 55% of trials were conducted using this treatment regimen).

Fish Species and Fish Diseases Involved in CY11 Trials

1. Species of fish treated

Twelve fish species, including four species of salmonids, five non-salmonid species, and three marine non-salmonid species were treated with FMF during CY11. Treated fish ranged in length from 3.0 - 30.1 in. and the mean length of all treated fish was 11.5 in. Fish species treated included:

Salmonids:

Atlantic salmon *Salmo salar*

rainbow trout *Oncorhynchus mykiss*

spring chinook salmon *O. tshawytscha*

steelhead *O. Mykiss*

Non-salmonids:

channel catfish *Ictalurus punctatus*

koi *Cyprinus carpio*

largemouth bass *Micropterus salmoides*

Siberian sturgeon *Acipenser baerii*

Tilapia *Oreochromis niloticus*

Marine non-salmonids:

European sea bass *Dicentrarchus labrax*

gilthead seabream *Sparus aurata*

summer flounder *Paralichthys dentatus*

2. Diseases treated

Test fish were treated with FMF to control mortality caused by the following diseases during CY11: *Aeromonas hydrophila*; *Aeromonas* spp.; bacteria coldwater disease (CWD); bacteria kidney disease (BKD); columnaris; motile aeromonad septicemia; rickettsia like organism; *Streptococcus iniae*; *Vibrio harveyi*; vibriosis; and yellow mouth.

Data Collected

1. Pathology reports

Pathology reports were submitted with 12 trials conducted during CY11. 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 list of all efficacious trials; Table 2 provides a list of all ineffective trials; Table 3 provides a list of all inconclusive trials; Table 4 provides general CY11 summary data; and Table 5 provides a summary of all trials conducted during CY11 under INAD #10-697.

A. Salmonid species - efficacy at 10 - 15 mg/Kg fish/d for 10 days under INAD #10-697

Atlantic salmon, rainbow trout, spring chinook salmon, and steelhead trout were treated with 10 - 15 mg florfenicol/Kg fish/d for 10 days in 13 trials to control mortality caused by CWD; BKD; columnaris; rickettsia like organism; or yellow mouth (Tables 1 - 3). FMF treatments appeared effective in six trials; ineffective in one trial; and inconclusive in six trials.

B. Non-salmonid species - efficacy at 10 - 15 mg/Kg fish/d for 10 days under INAD #10-697

Channel catfish, koi, largemouth bass, Siberian sturgeon, and Tilapia were treated with 10 - 15 mg florfenicol/Kg fish/d for 10 days in 10 trials to control mortality caused by *Aeromonas hydrophila*; *Aeromonas* spp.; *columnaris*; motile aeromonad septicemia; or *Streptococcus iniae*, (Tables 1 - 3). FMF treatments appeared effective in seven trials, ineffective in two trials, and was characterized as inconclusive in one trial.

C. Marine non-salmonid species - efficacy at 15 mg/Kg fish/d for 10 days under INAD #10-697

European sea bass, gilthead seabream, and summer flounder were treated with 15 mg florfenicol/Kg fish/d for 10 days in six trials to control mortality caused by *Vibrio harveyi* or vibriosis (Tables 1 - 2). FMF treatments appeared effective two trials and were ineffective in four trials.

2. Observed Toxicity

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

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded in all trials.

Current Study Protocol for FLOR (Aquaflor®) INAD #10-697

No changes have occurred to the current study protocol for FLOR (Aquaflor®) INAD #10-697.

Facility Sign-up List

Please see “Table 6. Facilities and Names of Investigators” for facilities that signed-up to participate in the FLOR (Aquaflor®) INAD #10-697 during CY11. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facility received FLOR during CY11 but never used the drug:

1. Keahole Point Fish

Correspondence sent to FLOR (Aquaflor®) Participants

Please see the attached correspondence that was sent to all FLOR (Aquaflor®) participants after the AADAP Office received their sign-up form for CY11.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY11 was 4,782,860. The number of treated fish to count against treatment use authorization dated July 31, 2009 (valid through August 17, 2011) is 11,820,376. The number of fish to count against treatment use authorization dated August 17, 2011 is 3,839,251.

Summary of Study Results

Florfenicol medicated feed (Aquaflor[®]) was administered to test fish in 29 separate trials at dosages ranging between 10 - 15 mg/Kg fish/d for 10 d. Twelve different fish species were treated with FMF, and trials involved approximately 4.8 million fish. Treated fish ranged in size from 3.0 - 30.1 in. Water temperature during treatment ranged from 45.0 - 86.0°F, with a mean treatment temperature of 66.5°F. Overall results showed that in approximately 52% of the trials, FMF treatments appeared effective, 24% of the trials were ineffective, and 24% of the trials were characterized as inconclusive. Although data from these trials will be considered ancillary, trial results should provide useful corroborative data to support a new/expanded label claims 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 continue to be directed towards the generation of high quality data.

References

- Alderman, D. J. 1988. Fisheries chemotherapy: a review. In: Recent Advances in Aquaculture, Vol. 3. Croom Helm, London.
- Bjorndal, T. 1990. The economics of salmon aquaculture. Blackwell Scientific Publications, Oxford.
- Clarke, R., and D. Scott. 1989. An overview of world salmon production and recent technology developments. Bulletin of the Aquaculture Association of Canada 4:31-48.
- Ellis, A. E. 1989. Use of vaccines in controlling fish diseases. Developmental and Comparative Immunology 13:399-407.

- Frerichs, G. N., and R. J. Roberts. 1989. The bacteriology of teleosts. In Fish Pathology, 2nd edition. Balliere Tindall, London.
- Haastein, T. 1988. Disease control through management practices. In: Proceedings of the Aquaculture International Congress, P. 39. Aquaculture International Congress, Vancouver, B.C.
- Horsberg, T. E., K. A. Hoff, and R. Nordmo. 1996. Pharmacokinetics of florfenicol and its metabolite florfenicol amine in Atlantic salmon. Journal of Aquatic Animal Health 8:292-301.
- Klontz, G. W. 1987. Control of systemic bacterial diseases in salmonids. Salmonid. 11:5-13.
- McCarthy, D. H. and R. J. Roberts. 1980. Furunculosis of fish - the present state of our knowledge. In: Advances in Aquatic Microbiology. Academic Press, London.
- Munro, A. L. S. and R. J. Roberts. 1989. The aquatic environment. In: Fish Pathology, 2nd edition. Balliere Tindall, London.
- Nagata, T. and H. Oka. 1996. Detection of residual chloramphenicol, florfenicol, and thiamphenicol in yellowtail fish muscles by capillary gas chromatography-mass spectrometry. Journal of Agriculture Food Chemistry 44:1280-1284.
- Powers, T. E., K. J. Varma, and J. D. Powers. 1990. In F. Simon (editor). Abstracts of the 4th Congress of European Associations of Veterinary Pharmacology and Toxicology, Budapest, Aug 28-Sep 2, 1988. Vol. 1, University of Veterinary Science, Budapest, 1990.

Table 1. CY11 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Cypress Island	ATS	30.00	417,066	BKD	10	10	51.8
1	Hope Island	ATS	14.00	450,800	Yellow Mouth	10	10	52.7
1	Inks Dam NFH	CCF	3.00	225,247	Motile Aeromonad Septicemia	10	10	81.7
1	Baja Fish Farm	CCF	30.10	66,000	Aeromonas Hydrophila	10	10	84.2
1	Local Ocean	ESB	4.00	89,480	Vibrio harveyi	15	10	69.8
1	Santa Barbara Koi	KOI	12.00	1,000	Aeromonas spp.	15	10	72.0
1	Florida Bass Conservation Center	LMB	4.00	11,000	Columnaris	15	10	75.5
1	Mayer Fish Farm	LMB	9.00	80,000	Aeromonas Hydrophila	15	10	82.0
1	Columbia River	RBT	25.00	168,865	Columnaris	15	10	65.7
2	McKenzie Hatchery	SCS	5.50	202,600	CWD	15	10	46.5
1	Local Ocean	SFL	3.00	22,429	Vibriosis	15	10	72.0
1	Cole Rivers Hatchery	STT	3.00	96,000	CWD	15	10	45.0
2	MinAqua Fisheries	TIA	3.0 - 8.0	58,443	Streptococcus iniae	15	10	83.0 - 86.0

Table 2. Summary of CY11 Florfenicol Medicated Feed Efficacy (Aquaflor®) Results - Ineffective Trials

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Rich Pass	ATS	15.70	59,689	Rickettsia Like Organism	10	10	53.2
1	Mote Marine Laboratory	SBS	26.40	2,862	Aeromonas spp.	10	10	73.4
1	Local Ocean	ESB	10.00	38,598	Vibrio harveyi	15	10	71.6
3	Local Ocean	GSE	4.5 - 9.0	267,170	Vibriosis	15	10	71.6
1	Lahser Research	KOI	24.00	230	Columnaris	15	10	68

Table 3. Summary of CY11 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Rich Pass	ATS	14.60	237,458	Rickettsia Like Organism	10	10	53.2
5	Columbia River	RBT	4.8 - 19.0	2,287,807	Columnaris	10	10	56.3 - 65.7
1	Lahser Research	KOI	24.00	116	Columnaris	15	10	70

Table 4. Summary Data Regarding CY11 Florfenicol Medicated Feed (Aquaflor®) Efficacy Trials

Total Fish Treated:	<u>4,782,860</u>
Number of fish treated in effective trials	1,888,930
Number of fish treated in ineffective trials	368,549
Number of fish treated in inconclusive trials	2,525,381
Total number of trials:	29
Number of effective trials:	15
Number of ineffective trials:	7
Number of inconclusive trials:	7
Treatment Regimes Used:	
10 mg/Kg fish/day for 10 days	13 trials
15 mg/Kg fish/day for 10 days	16 trials
Treatment Water Temperature (°F):	
Temperature Range	45.0 - 86.0
Mean Temperature	66.5
Size of Treated Fish (in.):	
Size Range	3.0 - 30.1
Species Treated:	
<u>Salmonids:</u>	
Atlantic salmon <i>Salmo salar</i>	
rainbow trout <i>Oncorhynchus mykiss</i>	
spring chinook salmon <i>O. tshawytscha</i>	
steelhead <i>O. Mykiss</i>	
<u>Non-salmonids:</u>	
channel catfish <i>Ictalurus punctatus</i>	
koi <i>Cyprinus carpio</i>	
largemouth bass <i>Micropterus salmoides</i>	
Siberian sturgeon <i>Acipenser baerii</i>	
Tilapia <i>Oreochromis niloticus</i>	
<u>Marine non-salmonids:</u>	
European sea bass <i>Dicentrarchus labrax</i>	
gilthead seabream <i>Sparus aurata</i>	
summer flounder <i>Paralichthys dentatus</i>	