

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

Year 2009 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

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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 2009 the efficacy of FMF was evaluated in 58 disease trials involving approximately 9.3 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 31 fish culture facilities, including four U.S. Fish and Wildlife Service National Fish Hatchery (NFH), one National Marine Fisheries Service facility, 13 state hatcheries, and 13 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 93% of the trials, ineffective in 2% of the trials, and was characterized as inconclusive in 5% of the trials.

Introduction

The current labels for FMF use in aquaculture limits 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 2009 (CY09) . 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 58 effectiveness trials were conducted at 31 fish culture facilities, including four U.S. Fish and Wildlife Service National Fish Hatchery (NFH), one National Marine Fisheries Service facility, 13 state hatcheries, and 13 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 45.1 - 85.0 °F, with a mean treatment temperature of 60.6°F.

2. FMF used in trials

The Aquaflor[®] used in CY09 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 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 31% of trials were conducted using these treatment regimens) or 2) 15 mg/Kg

fish/day for 10 days (approximately 43% of trials were conducted using these treatment regimens).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 26% of the trials) deviated from the protocol. Note: the first deviation was reported to CVM in the florfenicol quarterly report dated July 3, 2009.

- 1) A protocol deviation occurred between 4/1/09 and 6/16/09 when participants were allowed to use Aquaflor[®] at 15 mg/kg of fish/day to treat salmonids diagnosed with bacterial coldwater disease in 11 trials. This error occurred due to a misinterpretation of the amended food-use authorization by the AADAP Office. Once this error was confirmed by CVM the AADAP Office contacted all affected participants and informed them they could no longer use Aquaflor[®] for an approved indication under the INAD.

- 2) Protocol deviations occurred at two facilities where the fish were either treated at 1) 7.5 mg/kg fish/day for 10 days - lower dosage used due to a calculation error by the investigator; or 2) 15 mg/kg fish/day for 6 days - trial ended early due to poor water quality in the test tanks.

- 3) The required paperwork was not submitted by one facility in one trial. The AADAP Office projected the data (number of fish treated, amount of feed used, treatment dosage, etc.) from the study worksheet submitted by this facility; and with Inventory records kept by the AADAP Office showing the amount of Aquaflor[®] pre mix sent to this facility. This facility has been removed from the INAD Program. A copy of the termination letter sent to the facility has been attached to this report.

- 4) A Target Animal Safety (TAS) trial was conducted on hybrid striped bass at 15; 45; 75 mg/kg fish/day for 20 days under a pivotal research study protocol (FLOR-08-TAS-SSB-01). All fish were euthanized at the end of the trial.

Fish Species and Fish Diseases Involved in CY09 Trials

1. Species of fish treated

Fourteen fish species, including five species of salmonids, eight non-salmonid species, and one marine non-salmonid species were treated with FMF during CY09. Treated fish ranged in length from 1.0 - 19.4 in. and the mean length of all treated fish was 6.7 in. Fish species treated included:

Salmonids:

Atlantic salmon *Salmo salar*

Chinook salmon *Oncorhynchus tshawytscha*

coho salmon *O. kisutch*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

Non-salmonids:

bluegill *Lepomis macrochirus*

channel catfish *Ictalurus punctatus*

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

striped bass *Morone saxatilis*

largemouth bass *Micropterus salmoides*

muskellunge *Esox masquinongy*

Tilapia *Oreochromis niloticus*

walleye *Sander vitreus*

Marine non-salmonids:

kona kampachi *Seriola rivoliana*

2. Diseases treated

Test fish were treated with FMF either 1) to control mortality caused by the following diseases during CY09: *Aeromonas Hydrophila*, *Aeromonas* spp., coldwater disease (causative agent *Flavobacterium psychrophilus*), columnaris (causative agent *F. columnare*), enteric redmouth (causative agent *Yersinia*

ruckeri), *Flexibacter maritimus*, *lactobacilliosis*, *Photobacterium* spp., *Pseudomonas*, *Streptococcus iniae*, vibriosis, and yellow mouth (*Tenacibaculum maritimum*); or 2) used in a TAS trial.

Data Collected

1. Pathology reports

Pathology reports were submitted with 22 trials conducted during CY09. 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 or used in a TAS trial (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 CY09 summary data; and Table 5 provides a summary of all trials conducted during CY09 under INAD #10-697.

A. Salmonid species - efficacy at 7.5 - 15 mg/Kg fish/d for 10 days under INAD #10-697

Atlantic salmon, Chinook salmon, coho salmon, rainbow trout, and steelhead trout were treated with 7.5 - 15 mg florfenicol/Kg fish/d for 10 days in 34 trials to control mortality caused by CWD, enteric redmouth, *Flexibacter maritimus*, *lactobacilliosis*, vibriosis, or yellow mouth (Tables 1 - 3). FMF treatments appeared effective in 32 trials; ineffective in one trial; and inconclusive in one trial.

B. Non-salmonid species - efficacy at 10 - 75 mg/Kg fish/d for 6 - 20 days under INAD #10-697

Bluegill, channel catfish, hybrid striped bass, largemouth bass, muskellunge, tilapia, striped bass, and walleye were treated with 10 - 75 mg florfenicol/Kg fish/d for 6 - 20 days in 23 trials to control mortality caused by *Aeromonas hydrophila*, *Aeromonas* spp., *columnaris*, *pseudomonas*, *Streptococcus iniae*, or for a TAS trial (Tables 1 & 3). FMF treatments appeared effective in 21 trials and were characterized as inconclusive in two trials.

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

Kona kampachi were treated with 10 mg florfenicol/Kg fish/d for 10 days in 10 trials to control mortality caused by *Photobacterium* spp.(Table 1). FMF treatments appeared effective this trial.

2. Observed Toxicity

No toxicity or adverse effects relating to FMF treatment were reported in 57 trials and was unknown in one trial due to the data not being submitted to the AADAP Office.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded in 57 trials and was unknown in one trial due to the data not being submitted to the AADAP Office.

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

Please see the attached current study protocol for FLOR (Aquaflor®) INAD #10-697 . Please note no changes have occurred to this study protocol.

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 CY09. Facilities not listed in Appendix III-a of the current FLOR (Aquaflor®) INAD #10-697 during CY09 study protocol have been highlighted. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facility had Aquaflor® premix on-hand during CY09 but never used the drug:

1. Local Ocean

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 CY09.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY09 was 9,312,453. The total number of treated fish to count against the treatment use authorization dated September 17, 2007 (valid through July 30, 2009) is 14,717,431. The total number of treated fish to count against the current treatment use authorization dated July 31, 2009 is 3,791,993.

Summary of Study Results

Florfenicol medicated feed (Aquaflor[®]) was administered to test fish in 58 separate trials at dosages ranging between 7.5 - 75 mg/Kg fish/d for 6 - 20 d. Fourteen different fish species were treated with FMF, and trials involved approximately 9.3 million fish. Treated fish ranged in size from 1.0 - 19.4 in. Water temperature during treatment ranged from 45.1 - 85.0°F, with a mean treatment temperature of 60.6°F. Overall results showed that in approximately 93% of the trials, FMF treatments appeared effective, 2% of the trials were ineffective, and 5% 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

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Table 1. Year 2009 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Willamette SFH	RBT	2.10	253,162.00	CWD	7.5	10	300 days	48.30
Richloam SFH	BLG	4.10	395.00	Columnaris	10	10	>28 days due to fish size	72.10
Brady Catfish	CCF	11.00	200,000.00	Aeromonas Hydrophila	10	10	90 days	81.00
Harrison Catfish Farm	CCF	14.00	120,000.00	Aeromonas Hydrophila	10	10	60 days	83.90
Hollingsworth Catfish Operation	CCF	12.00	148,000.00	Aeromonas Hydrophila	10	10	30 days	80.00
Sid Nelson Catfish	CCF	12.00	300,000.00	Aeromonas Hydrophila	10	10	60 days	82.00
Steve Miller Farms	CCF	12.00	200,000.00	Aeromonas Hydrophila	10	10	135 days	84.00
Coleman NFH	FCS	3.20	528,000.00	Enteric Redmouth	10	10	>120 days	56.40
Kona Blue Water Farms	KON	3.00	118,683.00	Photobacterium spp.	10	10	365 days	72.00
Richloam SFH	LMB	2.50	76,000.00	Columnaris	10	10	365 days	72.90
Richloam SFH	LMB	2.50	2,263.00	Columnaris	10	10	>28 days due to fish size	75.00
Richloam SFH	LMB	4.30	8,000.00	Columnaris	10	10	1 year	77.70
Seven Springs Fish Farm	LMB	2.50	510,000.00	Aeromonas spp.	10	10	2 yrs	80.00
Seven Springs Fish Farm	LMB	13.00	40,000.00	Aeromonas spp.	10	10	45 days	65.00
Seven Springs Fish Farm	LMB	12.00	40,000.00	Aeromonas spp.	10	10	45 days	80.00
Seven Springs Fish Farm	LMB	2.50	480,000.00	Aeromonas spp.	10	10	2 yrs	80.00
Wolf Lake SFH	MUE	4.00	36,777.00	Columnaris	10	10	75 days	66.20
MinAqua Fisheries	NTI	5.00	36,000.00	Streptococcus iniae	10	10	7 months	85.00
Clam Bay Orchard Rocks Fort Ward	ATS	15.50	49,269.00	Yellow Mouth	15	10	300 days	55.00
Hope Island	ATS	13.30	361,105.00	Yellow Mouth	15	10	300 days	52.80
Port Angeles	ATS	4.80	82,384.00	Yellow Mouth	15	10	365 days	48.00

Table 1. Year 2009 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Port Angeles	ATS	12.00	157,091.00	Yellow Mouth	15	10	300 days	47.30
Port Angeles	ATS	12.00	154,811.00	Yellow Mouth	15	10	300 days	47.30
Port Angeles	ATS	4.80	162,005.00	Yellow Mouth	15	10	365 days	48.00
Port Angeles	ATS	14.00	74,760.00	Yellow Mouth	15	10	300 days	46.00
Port Angeles	ATS	4.80	91,044.00	Yellow Mouth	15	Three 10 day treatments (12-13 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	147,444.00	Yellow Mouth	15	Three 10 day treatments (6-10 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	70,244.00	Yellow Mouth	15	Three 10 day treatments (8-27 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	178,087.00	Yellow Mouth	15	Three 10 day treatments (9 - 10 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	230,427.00	Yellow Mouth	15	Two 10 day treatments (10 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	96,440.00	Yellow Mouth	15	Two 10 day treatments (10 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	77,229.00	Yellow Mouth	15	Two 10 day treatments (16 days between treatments).	365 days	48.00

Table 1. Year 2009 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Port Angeles	ATS	4.80	64,159.00	Yellow Mouth	15	Two 10 day treatments (19 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	56,631.00	Yellow Mouth	15	Two 10 day treatments (24 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	63,923.00	Yellow Mouth	15	Two 10 day treatments (9 days between treatments).	365 days	48.00
Port Angeles	ATS	4.80	72,912.00	Yellow Mouth	15	Two 10 day treatments (9 days between treatments).	365 days	48.00
Cascade SFH	COS	2.10	1,212,000.00	CWD	15	10	11 months	45.10
Fisheries Ecology Division	COS	13.80	25.00	Vibriosis	15	10	not for human consumption	58.20
Klaskanine SFH	COS	2.80	693,673.00	CWD	15	10	2 yrs	51.00
Tiburon Romberg Center	CSA	6.00	66,000.00	Flexibacter maritimus	15	10	2 - 3 yrs	54.00
Cain Fish Farm	LMB	9.00	48,000.00	Aeromonas spp.	15	10	90 days	85.00
Cain Fish Farm	LMB	1.00	120,000.00	Aeromonas spp.	15	10	14 months	75.00
Cain Fish Farm	LMB	8.00	26,000.00	Aeromonas spp.	15	10	90 days	81.00
Alsea SFH	RBT	3.50	376,156.00	CWD	15	10	6 months	57.00
Clark's Fork SFH	RBT	4.00	388,570.00	CWD	15	10	21 days	52.00
Klamath SFH	RBT	3.93	100,000.00	CWD	15	10	240 days	49.00
Leaburg SFH	RBT	2.80	17,638.00	CWD	15	10	78 days	46.30

Table 1. Year 2009 Florfenicol Medicated Feed (Aquaflor®) Efficacy Results - Effective Trials - cont

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Nehalem SFH	RBT	1.50	125,000.00	CWD	15	10	7 months	56.00
White Sulphur Springs NFH	RBT	4.00	7,000.00	CWD	15	10	2 yrs	52.00
Ohio University	STB	6.00	80.00	Pseudomonas	15	10	not for human consumption	59.00
Nehalem SFH	STT	1.00	240,800.00	CWD	15	10	2 yrs	49.00
Rock Creek SFH	STT	3.00	172,399.00	CWD	15	10	8 months	55.00
Rathbun Fish Hatchery/Research Facility	WAE	4.76	18,504.00	Aeromonas spp.	15	10	50 days	75.20
Bozeman FTC	SBH	4.40	180.00	Target Animal Safety Study	15;45,75	20	All fish were euthanized after study.	71.40

Table 2. Summary of CY09 Florfenicol Medicated Feed Efficacy (Aquaflor®) Results - Ineffective Trials

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Ennis NFH	RBT	18.10	2,588.00	Lactobacilliosis	10	10	25 days	54.0

Table 3. Summary of CY09 Florfenicol Medicated Feed Efficacy Results - Inconclusive Trials

Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Observed Withdrawal Time	Temp. (°F)
Ennis NFH	RBT	19.40	1,750.00	Lactobacilliosis	15	10	30 days	54.00
Ohio University	STB	5.50	125.00	Pseudomonas	15	6	not for human consumption	60.00
MinAqua Fisheries	TIA	10.00	408,720.00 (estimated)	Streptococcus iniae	15	10	unknown	

Table 4. Summary Data Regarding CY09 Florfenicol Medicated Feed (Aquaflor®) Efficacy Trials

Total Fish Treated:	<u>9,312,453</u>
Number of fish treated in effective trials	8,899,270
Number of fish treated in ineffective trials	2,588
Number of fish treated in inconclusive trials	410,595

Total number of trials:	58
Number of effective trials:	54
Number of ineffective trials:	1
Number of inconclusive trials:	3

Treatment Regimes Used:

10 mg/Kg fish/day for 10 days	18 trials
15 mg/Kg fish/day for 6 - 10 days	38 trials
7.5 mg/Kg fish/day for 10 days	1 trial
15;45;75 mg/Kg fish/day for 20 days	1 trial

Treatment Water Temperature (°F):

Temperature Range	45.1 - 85.0
Mean Temperature	60.6

Size of Treated Fish (in.):

Size Range	1.0 - 19.4
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Species Treated:

Salmonids:

Atlantic salmon *Salmo salar*
 Chinook salmon *Oncorhynchus tshawytscha*
 coho salmon *O. kisutch*
 rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

Non-salmonids:

bluegill *Lepomis macrochirus*

channel catfish *Ictalurus punctatus*

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

striped bass *Morone saxatilis*

largemouth bass *Micropterus salmoides*

muskellunge *Esox masquinongy*

Tilapia *Oreochromis niloticus*

walleye *Sander vitreus*

Marine non-salmonids:

kona kampachi *Seriola rivoliana*