

Florfenicol Medicated Feed (Aquaflor⁷) Clinical Field Trials - INAD 10-697

Year 2012 - 2014 Annual Summary Report on the Use of Florfenicol Medicated Feed in Field Efficacy Trials

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Summary

Florfenicol-medicated feed (Aquaflor⁷) 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 years 2012 – 2014 (CY12-14) the efficacy of Aquaflor⁷ was evaluated in 278 disease trials involving approximately 47.3 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 76 fish culture facilities, including six U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), 31 state hatcheries, and 39 private hatcheries. Use of Aquaflor⁷ under Protocol #10-697 allowed the investigator to administer Aquaflor⁷ 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 86% of the trials, ineffective in 5% of the trials, and was characterized as inconclusive in 9% of the trials.

Introduction

The 2012 - 2014 label for Aquaflor⁷ 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*. In 2014 the label for Aquaflor⁷ was expanded to allow the following: 1) increase in the maximum daily dosage for freshwater-reared finfish other than freshwater-reared warmwater finfish to provide a dosage range of 10 – 15 mg florfenicol/kg body weight/day and (2) changes the conditions of use to permit the use of florfenicol in recirculating aquaculture systems. These label restrictions limit the overall utility of approved Aquaflor⁷ 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 Aquaflor⁷ 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 Aquaflor⁷ use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of supplemental Aquaflor⁷ field efficacy studies conducted in CY12-14. Furthermore, it is expected that these data will be used to enhance the Aquaflor⁷ 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 278 effectiveness trials were conducted at 76 fish culture facilities, including six U.S. Fish and Wildlife Service National Fish Hatcheries (NFH), 31 state hatcheries, and 39 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 37.0 - 92.0 EF, with a mean treatment temperature of 63.3EF.

2. Aquaflor⁷ used in trials

The Aquaflor⁷ used in CY12-14 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 Aquaflor⁷ 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 Aquaflor⁷ at either a dosage of 1) 10 mg/Kg fish/day for 10 days (approximately 27% of trials were conducted using this treatment regimen) or 2) 15 mg/Kg fish/day for 10 days (approximately 69% of trials were conducted using this treatment regimen).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 4% of trials) deviated from the protocol. The following are the explanations for the deviations in each of the trials. 1) In one trial, fish were fed at 8.0 mg/Kg fish/day for 2 days. This deviation occurred because medicated feed was mistakenly delivered to the farm for treatments on certain ponds. However, due to a miscommunication, a pond was fed this shipment of feed because of feed bin space issues. 2) In six trials, fish were fish were fed at 10.0 mg/Kg fish/day for 11-14 days. These deviations occurred because the investigators wanted to feed out all of the medicated feed they had received. They noted the fish had decreased appetites during the treatment period so they had not received the full ration of feed. 3) In one trial, fish were fed at 10.0 mg/Kg fish/day for 12 days. There was no explanation for the deviation provided; however, this investigator tended to stagger his ponds through the treatment period. It is possible that the fish received a 10 day treatment, but due to multiple start and end treatment dates it appears that fish were fed for 12 days. 4) In one trial, fish were fed at 15.0 mg/Kg fish/day for 7 days. This deviation occurred because of a drop in water temperature which caused the fish to stop eating. 5) In one trial, fish were fed at 25.0 mg/Kg fish/day for 4 days. This deviation occurred because the investigator feed out a 10 day ration of feed in 4 days. The investigator was contacted and made aware of the correct treatment doses and duration.

Fish Species and Fish Diseases Involved in CY12-14 Trials

1. Species of fish treated

Twenty-one fish species, including eight species of salmonids, 10 non-salmonid species, and three marine non-salmonid species were treated with Aquaflor⁷ during CY12-14.

Treated fish ranged in length from 0.5 – 31.5 in. and the mean length of all treated fish was 6.4 in. Fish species treated included:

Salmonids:

Atlantic salmon (*Salmo salar*)

brown trout (*S. salar*)

Apache trout (*Oncorhynchus apache*)

Chinook salmon (*O. tshawytscha*)

coho salmon (*O. kisutch*)

cutthroat trout (*O. clarki*)

rainbow trout (*O. mykiss*)

steelhead (*O. Mykiss*)

Non-salmonids:

channel catfish (*Ictalurus punctatus*)

common carp (*Cyprinus carpio*)

koi (*C. carpio*)

hybrid striped bass (*Morone saxatilis x M. chrysops*)

largemouth bass (*Micropterus salmoides*)

razorback sucker (*Xyrauchen texanus*)

Siberian sturgeon (*Acipenser baerii*)

Tilapia (*Oreochromis niloticus*)

Walleye (*Sander vitreus*)

yellow perch (*Perca flavescens*)

Marine non-salmonids:

European sea bass (*Dicentrarchus labrax*)

Florida pompano (*Trachinotus carolinus*)

moi (*Polydactylus sexfilis*)

2. Diseases treated

Test fish were treated with Aquaflor⁷ to control mortality caused by the following diseases during CY12-14: *Aeromonas hydrophila*; *A. salmonicida* (saltwater use); *Aeromonas* spp.; bacterial hemorrhagic septicemia; bacterial septicemia; bacteria coldwater disease (CWD); bacteria kidney disease (BKD); *Edwardsiella tarda*; enteric redmouth; flavobacteriosis; general systemic bacterial infection; motile aeromonad septicemia; *Photobacterium* spp.; streptococcus; systemic flavobacteriosis; *Vibrio harveyi*; vibriosis; and yellow mouth.

Data Collected

1. Pathology reports

Pathology reports were reported done for 37 trials conducted during CY12-14. 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 10 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 Aquaflor⁷ 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; and Table 4 provides general CY12-14 summary data of trials conducted during CY12-14 under INAD #10-697.

**A. Salmonid species - efficacy at 10 - 15 mg/Kg fish/d for 7 - 12 days under INAD
#10-697**

Apache trout, Atlantic salmon, brown trout, Chinook salmon, coho salmon, cutthroat trout, rainbow trout, and steelhead trout were treated with 10 - 15 mg florfenicol/Kg fish/d for 7 - 12 days in 172 trials to control mortality caused by *Aeromonas Salmonicida* (saltwater use); *Aeromonas spp.*; bacterial hemorrhagic septicemia; bacterial kidney disease; bacterial coldwater disease; enteric redmouth; flavobacteriosis; general systemic bacterial infection; and yellow mouth (Tables 1 - 3). Aquaflor⁷ treatments appeared effective in 152 trials; ineffective in five trials; and inconclusive in 15 trials.

B. Non-salmonid species - efficacy at 8 - 25 mg/Kg fish/d for 2 - 14 days under INAD

#10-697

Channel catfish, common carp, hybrid striped bass, koi, largemouth bass, razorback suckers, Siberian sturgeon, Tilapia, walleye, and yellow perch were treated with 8 - 25 mg florfenicol/Kg fish/d for 2 - 14 days in 93 trials to control mortality caused by *Aeromonas hydrophila*; *Aeromonas spp.*; bacterial septicemia; *Edwardsiella tarda*; flavobacteriosis, motile aeromonad septicemia; streptococcus, or systemic flavobacteriosis (Tables 1 - 3). Aquaflor⁷ treatments appeared effective in 77 trials, ineffective in five trials, and was characterized as inconclusive in 11 trials.

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

INAD #10-697

European sea bass, Florida pompano, and moi were treated with 15 mg florfenicol/Kg fish/d for 10 days in 13 trials to control mortality caused by *Edwardsiella tarda*; *Vibrio*

harveyi; or vibriosis (Tables 1 - 2). Aquaflor⁷ treatments appeared effective in 10 trials and were ineffective in three trials.

2. Observed Toxicity

No toxicity or adverse effects relating to Aquaflor⁷ treatment were reported in 275 trials. Three trials did report toxicity or abnormal behavior. In one trial involving common carp the investigator noted anorexia was noted at the end of the treatment interval. Also, phototoxicity has been observed requiring shading. In two trials involving rainbow trout the investigator noted that possibly a few fish had blotchy appearance and acted lethargic.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded in 277 trials. In one trial involving channel catfish the withdrawal time is unknown as the investigator did not complete the required forms. AADAP was required to close this study out without all of the required information.

Current Study Protocol for Aquaflor⁷ INAD #10-697

No changes have occurred to the current study protocol for Aquaflor⁷ INAD #10-697.

Facility Sign-up List

Please see ATable 5. Facilities and Names of Investigators@ for facilities that signed-up to participate in the Aquaflor⁷ INAD #10-697 during CY12-14. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facility received Aquaflor⁷ during CY12-14 but never used the drug:

1. Booker Fowler SFH

Correspondence sent to Aquaflor⁷ Participants

Please see the attached correspondence that was sent to all Aquaflor⁷ participants after the AADAP Office received their sign-up form for CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 47,282,869. The number of treated fish to count against treatment use authorization dated August 17, 2011 (valid through July 22, 2013) is 16,000,000. The number of fish to count against treatment use authorization dated July 23, 2013 (valid through August 4, 2015) is 16,000,000. The current authorization dated August 5, 2015 will need to have the additional fish counted against it. A new authorization has been requested (submitted 6/14/16) for the surplus fish and to cover the 2015 and 2016 calendar years.

Summary of Study Results

Florfenicol medicated feed (Aquaflor⁷) was administered to test fish in 278 separate trials at dosages ranging between 8 - 25 mg/Kg fish/d for 2 - 14 days. Twenty-one different fish species were treated with Aquaflor⁷, and trials involved approximately 47.3 million fish. Treated fish ranged in size from 0.5 – 31.5 in. Water temperature during treatment ranged from 37.0 - 92.0EF, with a mean treatment temperature of 63.3EF. Overall results showed that in approximately 86% of the trials, Aquaflor⁷ treatments appeared effective, 5% of the trials were ineffective, and 9% 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 Aquaflor⁷. 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. CY12 - 14 Florfenicol Medicated Feed (Aquaflor⁷) 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)
4	Port Angeles site	Atlantic Salmon	25.6 – 27.6	869,683	Aeromonas Salmonicida	10	10	51.3 – 55.4
4	Port Angeles site	Atlantic Salmon	9.1 – 9.8	1,149,097	Yellow Mouth	10	10 - 11	47.0 – 50.9
2	Rich Pass	Atlantic Salmon	8.3 - 24	1,074,832	Aeromonas Salmonicida	10	10	53.4 – 55.2
2	Rich Pass	Atlantic Salmon	8.3 – 10.2	331,944	Yellow Mouth	10	10 - 12	51.3 – 53.4
1	Anthony Giesbrecht Farm	Channel Catfish	10.0	50,000	Aeromonas Hydrophila	10	10	82.4
2	Bob Schmidt Farm	Channel Catfish	10.0	90,000	Aeromonas Hydrophila	10	10	82.4
1	Bryan Nightingale Fish Farm	Channel Catfish	15.0	70,000	Aeromonas Hydrophila	10	10	78.8
2	Chuong Le Farm	Channel Catfish	11.0 – 12.0	130,000	Aeromonas Hydrophila	10	10	85.0 – 87.0
1	Evan Yost Farm	Channel Catfish	12.0	70,000	Aeromonas Hydrophila	10	10	70.0
2	Gerald Classen Farm	Channel Catfish	11.0	157,000	Aeromonas Hydrophila	10	10	83.0 – 86.0
2	Giesbrecht Farm	Channel Catfish	9.8 – 10.4	200,000	Aeromonas Hydrophila	10	10	84.2 – 88.0
1	Graber Catfish Farm	Channel Catfish	10.0	100,000	Aeromonas Hydrophila	10	10	82.4
1	Hollingsworth Farms	Channel Catfish	8.0	240,000	Aeromonas Hydrophila	10	10	87.0
3	Horsehunter Catfish	Channel Catfish	9.0 – 12.5	300,000	Aeromonas Hydrophila	10	10	76.0 – 82.4
5	Inks Dam NFH	Channel Catfish	3.1 – 4.4	159,570	Motile Aeromonad Septicemia	10	10	81.8 – 84.9
3	Keith Nightengale Farm	Channel Catfish	10.0 – 15.0	130,000	Aeromonas Hydrophila	10	10	82.4
1	Kenneth Johnson Farm	Channel Catfish	12.0	100,000	Aeromonas Hydrophila	10	10	79.9
1	Lanell Bontrager Fish Farm	Channel Catfish	10.0	120,000	Aeromonas Hydrophila	10	10	82.4
2	Paul Koehn Farms	Channel Catfish	10.0	200,000	Aeromonas Hydrophila	10	10	78.8 – 82.4
1	Paul Unruh Farm	Channel Catfish	10.0	100,000	Aeromonas Hydrophila	10	10	82.4

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Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
7	Pearce Catfish	Channel Catfish	12.0 – 14.0	647,000	Aeromonas Hydrophila	10	10 - 11	73.0 – 91.0
4	Penala Farms, LLLP	Channel Catfish	8.0 – 12.0	706,667	Aeromonas Hydrophila	10	10 - 14	75.0 – 90.0
2	Rigdon Catfish Farm	Channel Catfish	8.0 – 13.0	288,000	Aeromonas Hydrophila	10	10	79.9 – 82.4
2	Samuel Johnson Farm	Channel Catfish	10.0 – 15.0	150,000	Aeromonas Hydrophila	10	10	78.8 – 82.4
2	Shirk Catfish Farm	Channel Catfish	9.0	230,000	Aeromonas Hydrophila	10	10	79.9 – 82.4
1	Stan Smith Fish Farm	Channel Catfish	15.0	80,000	Aeromonas Hydrophila	10	10	78.8
5	Steve Koehn Farm	Channel Catfish	10.0 – 15.0	1,025,000	Aeromonas Hydrophila	10	10	82.4 – 87.0
2	Tackett Fish Farm	Channel Catfish	10.0 – 14.0	402,000	Aeromonas Hydrophila	10	10	85
1	Warren Giesbrecht Farm	Channel Catfish	15.0	40,000	Edwardsiella Tarda	10	10	82.4
1	West Alabama Aquaculture	Channel Catfish	10.0	75,000	Aeromonas Hydrophila	10	10	81.0
1	Dunns Fish Farm Inc	Largemouth Bass	13.0	239,000	Bacterial Septicemia	10	10	84.2
1	Armstrong SFH	Rainbow Trout	8.5	26,803	Enteric Redmouth	10	10	58.0
1	Sassnett Farm	Tilapia Nilotica	5.0	24,000	Streptococcus	10	10	75.0
1	Williams Creek NFH	Apache Trout	2.2	136,000	General Systemic Bacterial Infection	15	10	52.0
5	Port Angeles site	Atlantic Salmon	9.4 – 11.8	1,099,708	Yellow Mouth	15	10	47.3 – 53.6
7	Rich Pass	Atlantic Salmon	6.7 – 14.0	3,734,708	Yellow Mouth	15	10	47.1 – 56.7
2	Inks Dam NFH	Channel Catfish	3.2	133,750	Aeromonas Hydrophila	15	10	83.4 – 83.6
1	Clearwater SFH	Chinook Salmon	4.0	99,571	Bacterial Kidney Disease	15	10	50.2
6	Mckenzie SFH	Chinook Salmon	4.5 – 5.0	356,400	CWD - Bacterial Coldwater Disease	15	10	40.9 – 47.0
1	Big Creek SFH	Coho Salmon	2.0	535,000	CWD - Bacterial Coldwater Disease	15	10	49.0

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Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
1	Clatsop County Fisheries	Coho Salmon	5.0	399,976	CWD - Bacterial Coldwater Disease	15	10	43.0
2	Klaskanine SFH	Coho Salmon	1.7 – 3.0	1,457,095	CWD - Bacterial Coldwater Disease	15	10	42.5 – 52.5
3	Nehalem SFH	Coho Salmon	2.9 – 4.5	499,800	CWD - Bacterial Coldwater Disease	15	10	53.0 – 55.0
2	Oxbow SFH	Coho Salmon	3.0 – 5.4	842,113	CWD - Bacterial Coldwater Disease	15	10	45.0
1	Dan Speas SFH	Cutthroat Trout	2.9	55,992	CWD - Bacterial Coldwater Disease	15	10	60.0
1	Daniel SFH	Cutthroat Trout	13.5	4,341	Aeromonas spp.	15	10	42.0
3	Daniel SFH	Cutthroat Trout	10.1 – 13.8	5,532	Bacterial Hemorrhagic Septicemia	15	10	42.0 – 43.0
1	Local Ocean	European Sea Bass	1.6	70,000	Photobacterium spp.	15	10	73.4
5	Local Ocean	European Sea Bass	2.0 – 8.0	93,704	Vibrio harveyi	15	10	64.4 – 82.4
3	Local Ocean	European Sea Bass	2.0 – 5.0	130,862	Vibriosis	15	10	69.8 – 71.6
3	Susquehanna Aquaculture Inc.	Hybrid Striped Bass	2.1 – 5.0	183,223	Aeromonas Hydrophila	15	10	70.0 – 72.0
1	Santa Barbara Koi - Farm	Koi	12.0	1,200	Aeromonas Hydrophila	15	10	76.0
2	Mayer Fish Farm	Largemouth Bass	11.0	55,263	Aeromonas Hydrophila	15	10	84.0 – 88.0
1	Keahole Point Fish LLC	Moi	6.0	110,000	Photobacterium spp.	15	10	79.9
1	Alsea SFH	Rainbow Trout	4.2	263,219	CWD - Bacterial Coldwater Disease	15	10	53.0

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Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
4	Boulder Rearing Station	Rainbow Trout	3.3 – 20.3	134,926	Bacterial Hemorrhagic Septicemia	15	10	52.0 – 53.0
2	Cole Rivers SFH	Rainbow Trout	3.0 – 5.3	1,417,603	CWD - Bacterial Coldwater Disease	15	10	51.0 – 55.0
2	Dan Speas SFH	Rainbow Trout	7.2	347,171	Bacterial Hemorrhagic Septicemia	15	10	60.0
5	Dan Speas SFH	Rainbow Trout	2.4 – 7.2	1,237,500	CWD - Bacterial Coldwater Disease	15	10	60.0
2	Fall River SFH	Rainbow Trout	1.6 – 1.7	700,500	CWD - Bacterial Coldwater Disease	15	10	46.0
5	Hotchkiss NFH	Rainbow Trout	2.6 – 6.7	489,850	CWD - Bacterial Coldwater Disease	15	10	56.0
3	Irrigon SFH	Rainbow Trout	2.0 – 4.4	325,287	CWD - Bacterial Coldwater Disease	15	10	54.0 – 57.0
1	Jim Hinkle Spring River SFH	Rainbow Trout	15	26,000	Enteric Redmouth	15	10	59.0
1	Klamath SFH	Rainbow Trout	2.5	790,000	CWD - Bacterial Coldwater Disease	15	10	49.0
1	Leaburg SFH	Rainbow Trout	4.0	178,000	CWD - Bacterial Coldwater Disease	15	10	50.0
2	Nehalem SFH	Rainbow Trout	2.9 – 3.3	260,000	CWD - Bacterial Coldwater Disease	15	10	60.0
4	Roaring River SFH	Rainbow Trout	2.8 – 4.0	817,889	CWD - Bacterial Coldwater Disease	15	10	50.0 – 54.0
2	Willamette SFH	Rainbow Trout	1.0 – 2.5	1,960,000	CWD - Bacterial Coldwater Disease	15	10	55.0 – 63.0
12	Wizard Falls SFH	Rainbow Trout	0.5 – 5.0	3,285,551	CWD - Bacterial Coldwater Disease	15	10	50
2	Ouray NFH	Razorback Sucker	7.1 – 8.5	17,250	Systemic Flavobacteriosis	15	10	71.6 – 72.5

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Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
10	Alsea SFH	Steelhead	1.3 – 4.2	732,788	CWD - Bacterial Coldwater Disease	15	10	43.0 – 62.0
4	Bandon SFH	Steelhead	2.0 – 4.0	237,361	CWD - Bacterial Coldwater Disease	15	10	54.8 – 58.4
2	Big Creek SFH	Steelhead	1.5	340,500	CWD - Bacterial Coldwater Disease	15	10	50.0 – 52.0
1	Cedar Creek SFH	Steelhead	3.6	235,000	CWD - Bacterial Coldwater Disease	15	10	54.0
15	Cole Rivers SFH	Steelhead	1.0 – 3.0	2,350,511	CWD - Bacterial Coldwater Disease	15	10	45.0 – 57.0
1	Eagle Creek NFH	Steelhead	1.5	104,400	CWD - Bacterial Coldwater Disease	15	10	62.0
6	Irrigon SFH	Steelhead	0.6 – 4.0	2,100,279	CWD - Bacterial Coldwater Disease	15	10	53.0 – 57.0
3	Nehalem SFH	Steelhead	2.6 – 3.4	280,500	CWD - Bacterial Coldwater Disease	15	10	58.0 – 60.0
3	Oak Springs SFH	Steelhead	1.5 – 3.0	949,000	CWD - Bacterial Coldwater Disease	15	10	53.0
6	Rock Creek SFH	Steelhead	0.8 – 3.5	372,764	CWD - Bacterial Coldwater Disease	15	10	46.3 – 63.1
3	South Santiam SFH	Steelhead	2.0 – 2.5	427,000	CWD - Bacterial Coldwater Disease	15	10	50.0 – 52.0
3	Trask SFH	Steelhead	1.0 – 3.4	223,130	CWD - Bacterial Coldwater Disease	15	10	53.7 – 55.0
1	Willamette SFH	Steelhead	1.0	65,000	CWD - Bacterial Coldwater Disease	15	10	54.0
6	Sassnett Farm	Tilapia Nilotica	4.0 – 7.0	115,800	Streptococcus	15	10	70.0 – 75.0
3	Rathbun SFH	Walleye	3.6 – 6.5	98,376	Aeromonas spp.	15	10	77.0 – 85.0

Table 1. CY12 - 14 Florfenicol Medicated Feed (Aquaflor⁷) Efficacy Results - Effective Trials

Table 2. Summary of CY12-14 Florfenicol Medicated Feed Efficacy (Aquaflor⁷) 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	Graber Catfish Farm	Channel Catfish	6.0	100,000	Aeromonas Hydrophila	10	10	82.4
1	Pahsimeroi SFH	Chinook Salmon	5.4	977,000	Bacterial Kidney Disease	10	10	40.0
1	Lahser Interspecies Research Foundation	Common Carp	28.0	116	Flavobacteriosis	15	10	70.0
1	Local Ocean	European Sea Bass	2.5	72,828	Vibriosis	15	10	78.8
2	Local Ocean	Florida Pompano	1.5 – 2.5	18,115	Edwardsiella Tarda	15	10	77.0
1	Susquehanna Aquaculture Inc.	Hybrid Striped Bass	7.0	10,645	Aeromonas Hydrophila	15	10	72.0
1	Oak Springs SFH	Rainbow Trout	3.0	361,000	CWD - Bacterial Coldwater Disease	15	10	53.0
2	Cole Rivers SFH	Steelhead	3.0 – 4.0	167,000	CWD - Bacterial Coldwater Disease	15	10	45.0
1	Rock Creek SFH	Steelhead	3.0	77,382	CWD - Bacterial Coldwater Disease	15	10	60.0
1	Rathbun SFH	Walleye	6.9	20,966	Aeromonas spp.	15	10	81.5
1	Bell Aquaculture LLC	Yellow Perch	10.0	9,737	Aeromonas spp.	25	4	46.4

Table 3. Summary of CY12-14 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	ACI Farm	Channel Catfish	10.0	66,667	Aeromonas Hydrophila	8	2	85.0
1	Thompson SFH	Brown Trout	3.1	216,000	Flavobacteriosis	10	10	50.9
1	ACI Farm	Channel Catfish	14.0	45,000	Aeromonas Hydrophila	10	10	86.0
5	Chancellor Farm	Channel Catfish	8.0 – 13.0	510,000	Aeromonas Hydrophila	10	10	82.4
1	Pearce Catfish	Channel Catfish	12.0	40,000	Edwardsiella Tarda	10	13	91.0
1	Penala Farms, LLLP	Channel Catfish	8.0	100,000	Aeromonas Hydrophila	10	12	92.0
1	Mote Aquaculture Research Park	Siberian Sturgeon	31.5	1,948	Aeromonas Hydrophila	10	10	72.0
1	Inks Dam NFH	Channel Catfish	4.1	49,604	Aeromonas Hydrophila	15	10	83.4
1	Garrison NFH	Chinook Salmon	1.4	530,000	General Systemic Bacterial Infection	15	10	55.6
1	Mckenzie SFH	Chinook Salmon	5.0	64,000	CWD - Bacterial Coldwater Disease	15	7	37.0
1	Daniel SFH	Cutthroat Trout	13.5	705	Bacterial Hemorrhagic Septicemia	15	10	43.0
1	American River Trout SFH	Rainbow Trout	1.0	188,000	CWD - Bacterial Coldwater Disease	15	10	56.0
1	Dan Speas SFH	Rainbow Trout	7.1	457,000	CWD - Bacterial Coldwater Disease	15	10	60.0
3	Wizard Falls SFH	Rainbow Trout	1.2 – 2.2	1,533,972	CWD - Bacterial Coldwater Disease	15	10	50.0

Number of Studies	Hatchery	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (mg/kg)	Number of Treatment Days	Temp. (°F)
2	Bandon SFH	Steelhead	3.3 – 3.5	158,174	CWD - Bacterial Coldwater Disease	15	10	54.8 – 58.0
4	Cole Rivers SFH	Steelhead	1.0 – 1.1	1,044,021	CWD - Bacterial Coldwater Disease	15	10	54.0 – 56.0

Table 4. Summary Data Regarding CY12-14 Florfenicol Medicated Feed (Aquaflor⁷) Efficacy Trials

Total Fish Treated:	<u>47,282,869</u>
Number of fish treated in effective trials	40,462,989
Number of fish treated in ineffective trials	1,814,789
Number of fish treated in inconclusive trials	5,005,091

Total number of trials:	278
Number of effective trials:	239
Number of ineffective trials:	13
Number of inconclusive trials:	26

Treatment Regimes Used:	
8 mg/Kg fish/day for 2 days	1 trial
10 mg/Kg fish/day for 10 – 14 days	83 trials
15 mg/Kg fish/day for 7 - 10 days	193 trials
25 mg/Kg fish/day for 4 days	1 trial

Treatment Water Temperature (EF):	
Temperature Range	37.0 - 92.0
Mean Temperature	63.3

Size of Treated Fish (in.):	
Size Range	0.5 – 31.5

Species Treated:

Salmonids:

Atlantic salmon (*Salmo salar*)
 brown trout (*S. salar*)
 Apache trout (*Oncorhynchus apache*)
 Chinook salmon (*O. tshawytscha*)
 coho salmon (*O. kisutch*)
 cutthroat trout (*O. clarki*)
 rainbow trout (*O. mykiss*)
 steelhead (*O. Mykiss*)

Non-salmonids:

channel catfish (*Ictalurus punctatus*)
 common carp (*Cyprinus carpio*)
 koi (*C. carpio*)
 hybrid striped bass (*Morone saxatilis x M. chrysops*)
 largemouth bass (*Micropterus salmoides*)
 razorback sucker (*Xyrauchen texanus*)

Siberian sturgeon (*Acipenser baerii*)
Tilapia (*Oreochromis niloticus*)
Walleye (*Sander vitreus*)
yellow perch (*Perca flavescens*)

Marine non-salmonids:

European sea bass (*Dicentrarchus labrax*)
Florida pompano (*Trachinotus carolinus*)
moi (*Polydactylus sexfilis*)