Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed Clinical Field Trials - INAD 9332

Year 2008 Annual Summary Report on the Use of Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed in Field Efficacy Trials

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Summary

Oxytetracycline (Terramycin® 200 for Fish) medicated feed has been used effectively in the U. S. under compassionate INAD Exemption #9332 to either: (1) control/prevent mortality in a variety of fish caused by common fish bacterial pathogens, or (2) for marking skeletal tissue of early life stages of fish. In calendar year 2008 (CY08) the efficacy of oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF) was evaluated in 65 trials involving approximately 9.0 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens or to apply a skeletal mark to fish. Trials were conducted at 20 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 17 state hatcheries, and one private fish culture hatchery. The compassionate study protocol under which treatments were administered allowed the investigator to use OTF at a dosage of either: 1) 2.5 - 3.75 g drug/100 lbs fish/d for 10 days; or 2) 10 g drug/100 lbs fish/d for 14 days. Overall, results of trials conducted in CY08 indicated that treatments appeared to be efficacious

in approximately 89% of the trials, ineffective in 5% of the trials, and were characterized as inconclusive in 5% of the trials. In the remaining 1% of the trials, the Investigators were not required to report efficacy data because the effectiveness technical section for the specific claim has been completed and accepted by CVM.

Introduction

The current labels for OTF use in aquaculture limits use to: Salmonids - 1) control of ulcer disease (*Hemophilus piscium*); 2) the control of furunculosis (*Aeromonas salmonicida*); 3) control of bacterial hemorragic septicemia (*A. Liquefaciens*); and 4) pseudomonas disease (*Pseudomonas* spp.). Dosing: 2.5 - 3.75 g per 100 lbs fish per day for 10 days. Note: on July 6, 2008 the temperature limitation on treating salmonids in water temperatures below 9^{o} C was removed. Freshwater-reared salmonids - control of coldwater disease (*Flavobacterium psychrophilum*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. Note: July 6, 2008 was when this label claim took effect. All freshwater-reared *Oncorhynchus mykiss* - control columnaris diesase (*Flavobacterium columnare*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. Note: July 6, 2008 was when this label claim took effect. Pacific salmon - to mark skeletal tissue. Dosing: 250 mg/kg fish/day for 4 days in salmon less then 30 g . Catfish - control of bacterial hemorrhagic septicemia (*Aeromonas liquefaciens*) and pseudomonas disease (*Pseudomonas* spp.). Dose: 2.5 - 3.75 g per 100 lbs fish per day for 10 days. These label restrictions limit the overall utility of approved OTF use in aquaculture.

Historically, OTF treatments have been used by fish culturists to control mortality in salmonids caused by bacterial coldwater disease (CWD; causative agent

Flavobacterium psychrophilus) and columnaris (causative agent *F. columnare*). Fish culturists and fish health professionals have also found that OTF is effective therapy to control mortality in fishes caused by enteric redmouth (causative agent *Yersinia ruckeri*), vibriosis (causative agent various members of the genus *Vibrio*), and other less common bacterial diseases. However, at this time, OTF has a limited label for such uses, and the only legal way to use OTF for such non-approved uses is through an INAD.

Fish culturists have also reported that oxytetracycline treatment is a useful tool for marking the skeletal tissue in salmonid fish when treated at a size in which fish body weight does not exceed 2 g. Marks were visible on skeletal tissue of fish immediately after the treatment period, and had still been visible for several months afterwards. In addition, studies have been conducted in which different oxytetracycline drug dosages were used to mark skeletal tissue of test fish. Summary conclusions from such studies indicated that not only did various dosages of oxytetracycline effectively mark skeletal tissue, but there were also no evidence of any toxic or adverse effects to the fish.

The proposed treatment strategy (i.e., dosage and duration) for the use of OTF 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 oxytetracycline 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 OTF use in aquaculture.

Purpose of Report

The purpose of this report is to summarize the results of calendar year 2008 (CY08) OTF field efficacy trials conducted under INAD #9332. Furthermore, it is expected that data from these trials will be used to enhance the existing OTF database that has been established from studies conducted in previous years for the purpose of expanding and/or extending the approved label for OTF.

Facilities, Materials, and Treatment Procedures

1. Facilities

A total of 65 trials were conducted at 20 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 17 state fish hatcheries, and one private fish culture hatchery. Water temperature during treatments at the various testing facilities ranged from 41.0 - 79.0 °F, with a mean treatment temperature of 57.1°F.

2. Test article used

The OTF used in CY08 efficacy trials was either: 1) Terramycin 100 or Terramycin 100D, both of which contained 100 g active oxytetracycline quaternary salt per pound of premix; or 2) Terramycin® 200 which contained 200 g active oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article. All Terramycin® 100/100D/200 was supplied by Phibro Animal Health, 75 Challenger Road Ridgefield Park, NJ. OTF was prepared with Phibro brand product by one of several commercial fish feed manufacturers (e.g., Nelson and Sons, Inc., Rangen Inc.) or by top-coating feed at the testing site by the Investigator, Monitor, or their designee.

3. Treatment regimen

As described in the Study Protocol, Investigators were allowed to use OTF either within the current label range of 2.5 - 3.75 g of active drug/100 lbs of fish/d for 10 days (approximately 52% of trials were conducted using this treatment regimen); or 10.0 g of active drug/100 lbs of fish/d for 10 days (approximately 29% of trials were conducted using this treatment regimen).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 19% of trials) deviated from the protocol. In these trials, fish were fed at rates of either: 1) 2.5 - 3.75 g drug/100 lbs fish/d for 14 days (2% of trials); 2) 3.88 - 9.13 g drug/100 lbs fish/d for 10 - 14 days (11% of trials); 3) 10.0

g drug/100 lbs fish/d for 12 days (2% of trials); or 4) 10.26 - 18.1 g drug/100 lbs fish/d for 14 days (4% of trials). Please note that many of the dosage deviation occurred due to the actual amount of OTF that was fed to the test fish. In some cases there were actually less/more fish in tanks then what was estimated prior to the study. Another common reason for a deviation was due to the fish not eating the full ration of OTF due to cold water temperature or late stages of the disease. Investigators were made aware of the deviation and informed that adherence to the protocol is a vital element to the aquaculture INAD process.

Fish Species and Fish Diseases Involved in CY08 Trials

1. Species of fish treated

Twelve fish species, including eight salmonids and four non-salmonids were treated during CY08. Treated fish ranged in length from 0.83 - 9.0 in. and the average length of all treated fish was 3.3 in. Fish species treated included:

Salmonids:

brook trout *Salvelinus fontinalis*

Apache trout *Oncorhynchus apache*

chinook salmon *O. tshawytscha*

coho salmon *O. kisutch*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

Non-salmonids:

blue catfish *Ictalurus furcatus*

walleye *Sander vitreus*

white sturgeon *Acipenser transmontanus*

yellow perch *Perca flavescens*

2. Disease/Purpose treated

Test fish were either treated with OTF to 1) provide a readable mark on skeletal tissue; or 2) treated to either control/prevent mortality caused by the following diseases during CY08: coldwater disease (causative agent *Flavobacterium psychrophilus*), columnaris (causative agent *F. columnare*), bacterial gill disease (causative agent *F. branchiophilum*), pseudomonas disease (*Pseudomonas* spp.), motile aeromonad septicemia, or general systemic bacterial infection,

Data Collected

1. Pathologist's reports

A pathologist's report was submitted for 35% of the studies. Pathology reports are important for accurate interpretation of study results because they typically contain the following information:

A. A description of how the identity of disease agent(s) was verified,

B. Disease identification records that confirm the presence of the disease agent,

C. The name and title of the individual performing the diagnosis.

Additionally, evidence would typically be provided to document that there were no secondary infections or infestations caused by unrelated disease agents in the population of test fish. As a result, pathology reports provide essential information if efforts are to expand/extend an existing approved label.

2. Treatment response and drug accountability data

Drug receipt reports, drug use reports, diagnosis, treatment, and mortality reports (including adverse effects/toxicity observations), and fish disposition reports were prepared by Study Investigators. Such reports were routed through the Study Monitor for review, and then sent to the AADAP Office for review, data analysis

and report writing, entering data into a database, and archiving in permanent files.

Based on correspondence with FDA, the following efficacy technical sections have been completed (note - the following claims are now approved uses as of July 6, 2008 and are no longer used under the OTF INAD):

1. Effectiveness of OTF at a concentration of 3.75 g of active drug/100 lbs of fish/d for 10 d to control mortality associated with: 1) bacterial coldwater disease in freshwater-reared salmonids (we refer to your file number INAD 9006 H-0093 dated Nov 23, 2001); or 2) *Oncorhychus mykiss* with columnaris (we refer to your file number INAD I-009006-P-0106 dated July 25, 2007).

As a result of the completed technical sections, mortality data are no longer required when Investigators administer OTF at a dosage of 3.75 g of active drug/100 lbs of fish/d for 10 d to control mortality associated with bacterial coldwater disease in freshwater-reared salmonids or *Oncorhychus mykiss* with columnaris. In all other cases, collection of mortality data is still required and efforts were made to collect all such data. However, for a variety of reasons, mortality data were not always collected for the entire required data collection period. Reasons for incomplete mortality data included: splitting fish into

additional rearing units and stocking early life stage fish shortly after final treatment.

Discussion of Study Results:

1. Relevance of study to expanding current label claim for OTF

Results of CY08 trials conducted under Compassionate INAD exemption #9332 are similar to results detailed in reports previously submitted to FDA under INAD's #9332 and #9006.

2. General observations on the efficacy of OTF for the control of bacterial diseases in fish species (Note: Table 1 provides a summary of all trials characterized as effective; Table 2 provides a summary of all trials characterized as ineffective ; Table 3 provides a summary of all trials characterized as inconclusive; Table 4 provides a summary of all trials where efficacy data was not required; Table 5 provides summary data for all trials; and Table 6 provide a summary of all trials conducted during CY08 under INAD #9332).

A. Efficacy at 2.50 - 3.75 g/100 lbs fish/d for 10 - 14 days

Apache trout, brook trout, chinook salmon, coho salmon, cutthroat trout, kokanee salmon, rainbow trout, steelhead trout, blue catfish, and walleye were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 - 14 days in 35 trials (Tables 1 - 4). Investigators used OTF to either apply a skeletal mark or to control mortality

caused by coldwater disease, columnaris, or pseudomonas disease. OTF treatments appeared effective in 31 trials, ineffective in one trial, not report in one trial (due to the efficacy packet being complete), and was characterized as inconclusive in two trials.

B. Efficacy at 3.88 - 9.13 g/100 lbs abalone/d for 10 - 14 days

Cutthroat trout, rainbow trout, and white sturgeon were treated with 3.88 - 9.13 g OTF/100 lbs of fish/d for 10 - 14 days in seven trials (Table 1). Investigators used OTF to control mortality caused by coldwater disease, systemic bacterial infection, or bacterial gill disease. OTF treatments appeared effective in all trials.

C. Efficacy at 10.0 g/100 lbs fish/d for 12 - 14 days

Brook trout, chinook salmon, cutthroat trout, rainbow trout, walleye, and yellow perch were treated with 10.0 g OTF/100 lbs of fish/d for 12 - 14 days in 20 trials (Tables 1 - 3). Investigators used OTF to either apply a skeletal mark or to control mortality caused by coldwater disease, columnaris, motile aeromonad septicemia, or pseudomonas disease. OTF treatments appeared effective in 18 trials, ineffective in one trial, and was characterized as inconclusive in one trial.

D. Efficacy at 10.26 - 18.1 g/100 lbs fish/d for 14 days

Cutthroat trout were treated with 10.26 - 18.1 g OTF/100 lbs of fish/d for 14 days in three trials (Tables 1 - 2) to control mortality caused by coldwater disease. OTF treatments appeared effective in two trials and ineffective in one trial.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY08.

Current Study Protocol for Oxytetracycline (Terramycin® 200 for Fish) INAD #9332

Please see the attached current study protocol for Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 . Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 7. Facilities and Names of Investigators” for facilities that signed-up to participate in the Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 during CY08. Facilities not listed in Appendix III-a of the current Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 during CY08 study protocol have been highlighted.

The following facilities had Oxytetracycline (Terramycin® 200 for Fish) medicated feed or premix on-hand during CY08 but never used the drug:

1. Bennington FCS
2. Bald Hill FCS
3. Ed Weed FCS
4. Trail Lakes Hatchery

Correspondence sent to FLOR (Aquaflor®) Participants

Please see the attached correspondence that was sent to all Oxytetracycline (Terramycin® 200 for Fish) participants after the AADAP Office received their sign-up form for calendar year 2008 and again in July 2008 after the OTF label was expanded.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY08 was 8,969,795. The total number of treated fish to count against the Oxytetracycline (Terramycin® 200 for Fish) treatment use authorization dated June 25, 2007 is 16,950,773.

Summary of Study Results

Oxytetracycline (Terramycin® 200 for Fish) medicated feed was used at dosages ranging from 2.50 - 18.1 g active drug/100 lbs fish/d in 65 treatment trials. Treatment durations ranged from 10 - 14 days. Treatment trials involved 12 different fish species

and approximately 9.0 million fish. Treated fish ranged in length from 0.83 - 9.0 in. Water temperature during treatment ranged from 41.0 - 79.0 °F, with a mean treatment temperature of 57.1 °F. Overall results showed that treatment in approximately 89% of trials appeared to be efficacious, treatment in 5% appeared ineffective, and characterized as inconclusive in 5% of the trials. In the remaining 1% of the trials, mortality data collection and reporting were not required. No evidence of toxicity or adverse effects related to OTF treatment were reported in any of the trials. However, based on a general lack of untreated control fish, replication, randomization, etc., it is understood that these data will only be considered as supportive or ancillary data. None-the-less, the data described above should provide useful corroborative data to support a future expanded label claim for OTF for these disease/marking indications. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #9332. In future trials conducted under this INAD, efforts will continue to be directed towards the generation of high quality data.

References

Warren, J.W. 1991. Diseases of hatchery fish. U.S. Fish and Wildlife Service, Portland, Oregon, 92 p.

Table 1. Summary of CY 2008 OTF Treatment Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease/Purpose	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
Alchesay-Williams Creek NFH Complex	8	APT	0.8 - 1.1	514,636	CWD	10	3.5	52.0
	1	BKT	1.01	99,996	CWD	10	3.5	52.0
	4	RBT	0.8 - 3.3	750,640	CWD	10	3.5	52.0
Big Springs Trout SFH	1	KOE	4.70	37,500	Mark	10	3.73	55.0
	1	RBT	7.30	184,400	Mark	10	3.74	52.0
Farlington SFH	1	BCF	5.00	100,000	Columnaris	10	3.75	79.0
Macaulay Salmon Hatchery	2	CKS	1.25	911,156	Pseudomonas	10	3.75	41.0
	1	COS	1.25	568,183	Pseudomonas	10	3.75	43.0
Yellowstone River Trout SFH	1	CUT	2.00	40,000	Mark	10	3.75	52.0
Coleman NFH	4	FCS	2.6 - 2.9	528,000	Columnaris	10	3.75	66.0 - 67.0
American Falls SFH	2	RBT	6.0 - 6.2	53,600	CWD	10	3.75	55.0
Nampa SFH	2	RBT	4.0 - 6.0	160,047	CWD	10	3.75	59.0
Niagara Springs SFH	1	STT	8.00	1,689,872	CWD	10	3.75	59.0
Rathbun SFH	2	WAE	6.2 - 9.0	1,079	Mark	10 - 14	3.75	65.8 - 69.8
Grace SFH	1	RBT	5.20	22,700	CWD	10	3.88	53.0
Sterling Caviar LLC	2	WHS	9.00	8,625	BGD	10	5.5	70.3 - 72.4
	1	WHS	7.00	1,350	Systemic Bacterial Infection	10	5.5	68.7

Table 1. Summary of CY 2008 OTF Treatment Results - Efficacious Trials - continued

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease/Purpose	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
Murray Springs Trout SFH	2	CUT	1.6 - 3.0	157,000	CWD	14	6.54 - 9.13	52.0
Giant Springs Trout SFH	1	RBT	3.50	54,216	CWD	14	7.76	54.0
Giant Springs Trout SFH	1	BKT	2.80	60,522	Mark	14	10	54.0
Murray Springs Trout SFH	3	CUT	1.0 - 2.5	310,000	CWD	14	10	52.0
Coleman NFH	3	FCS	2.74	332,000	Columnaris	14	10	66.0 - 67.0
American Falls SFH	2	RBT	4.8 - 6.0	48,000	CWD	14	10	55.0
Bluewater Sprins Trout SFH	4	RBT	3.0 - 4.4	191,000	Mark	14	10	58.0
Hagerman SFH	1	RBT	2.57	924,000	CWD	14	10	59.0
Sawtooth SFH	1	SCS	4.08	292,389	Pseudomonas	14	10	65.0
Spirit Lake SFH	1	WAE	5.00	23,486	Motile Aeromonad Septicemia	14	10	75.3
Rathbun SFH	1	WAE	7.00	560	Mark	14	10	65.8
Blue Dog SFH	1	YEP	5.30	800	Mark	14	10	64.0
Murray Springs Trout SFH	2	CUT	1.6 - 3.0	62,000	CWD	14	10.26 - 18.1	52.0

Table 2. Summary of CY 2008 OTF Treatment Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
Hagerman SFH	1	RBT	2.62	405,000	CWD	10	3.75	59.0
Giant Springs Trout SFH	1	RBT	2.72	164,374	CWD	14	10	54.0
Murray Springs Trout SFH	1	CUT	1.60	10,000	CWD	14	12.5	52.0

Table 3. Summary of CY 2008 OTF Treatment Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of treatment days	Dose (g/100 lbs)	Temp. (°F)
Alchesay-Williams Creek NFH Complex	1	APT	0.83	25,164	CWD	10	3.5	52.0
Washoe Park Trout SFH	1	RBT	1.92	113,500	Mark	10	3.75	55.0
Murray Springs Trout SFH	1	CUT	1.00	30,000	CWD	14	10	52.0

Table 4. Summary of CY 2008 OTF Treatment Results - Studies where efficacy data was not needed

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (g/100 lbs)	Temp. (ºF)
American Falls SFH	1	RBT	4.00	94,000	CWD	10	3.75	55.0

Table 5. Summary Data Regarding Summary of CY 2008 OTF Treatment Trials

Total Fish Treated: **8,969,795**

Number of fish treated in efficacious trials	8,127,757
Number of fish treated in ineffective trials	579,374
Number of fish treated in inconclusive trials	168,664
Number of fish where efficacy was not needed	94,000

Total number of trials: **65**

Efficacious trials	58 (89%)
Ineffective trials	3 (5%)
Inconclusive trials	3 (5%)
Efficacy was not needed	1 (1%)

Treatment Regimens Used:

2.50 - 3.75 g/100 lbs fish/day for 10 - 14 days	35 trials
3.88 - 9.13 g/100 lbs fish/day for 10 - 14 days	7 trials
10.0 g/100 lbs fish/day for 12 - 14 days	20 trials
10.26 - 18.1 g/100 lbs fish/day for 14 days	3 trials

Treatment Water Temperature (°F):

Temperature Range	41.0 - 79.0
Mean Temperature	57.1

Size of Treated Fish (in.):

Size Range	0.83 - 9.0

Species Treated:

Salmonids:
brook trout *Salvelinus fontinalis*
Apache trout *Oncorhynchus apache*
chinook salmon *O. tshawytscha*
coho salmon *O. kisutch*
cutthroat trout *O. clarki*
kokanee salmon *O. nerka*
rainbow trout *O. mykiss*
steelhead trout *O. mykiss*

Non-salmonids:
blue catfish *Ictalurus furcatus*
walleye *Sander vitreus*
white sturgeon *Acipenser transmontanus*
yellow perch *Perca flavescens*