

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

Year 2009 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 2009 (CY09) the efficacy of oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF) was evaluated in 38 trials involving approximately 4.9 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 16 fish culture facilities, including three U.S. Fish and Wildlife Service fish hatcheries, 11 state hatcheries, one tribal hatchery, 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 CY09 indicated that treatments appeared to be efficacious in approximately 95% of the trials and were ineffective in 5% of the trials.

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 hemorrhagic septicemia (*A. Liquefaciens*); and 4) pseudomonas disease (*Pseudomonas* spp.). Dosing: 2.5 - 3.75 g per 100 lbs fish per day for 10 days. Freshwater-reared salmonids - control of coldwater disease (*Flavobacterium psychrophilum*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. All freshwater-reared *Oncorhynchus mykiss* - control columnaris disease (*Flavobacterium columnare*). Dosing: 3.75 g per 100 lbs fish per day for 10 days. Pacific salmon - to mark skeletal tissue. Dosing: 250 mg/kg fish/day for 4 days in salmon less than 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 CY09 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 38 trials were conducted at 16 fish culture facilities, including three U.S. Fish and Wildlife Service fish hatcheries, 11 state fish hatcheries, one tribal hatchery, and one private fish culture hatchery. Water temperature during treatments at the various testing facilities ranged from 50.5 - 84.1 °F, with a mean treatment temperature of 62.5°F.

2. Test article used

The OTF used in CY09 efficacy trials was Terramycin[®] 200 which contained 200 g active oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article. All Terramycin[®] 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 48% of trials were conducted using this treatment regimen); or 10.0 g of active drug/100 lbs of fish/d for 10 days (approximately 42% of trials were conducted using this treatment regimen).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 10% 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 11 - 12 days (5% of trials); or 2) 3.8 - 4.0 g drug/100 lbs fish/d for 10 days (5% 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 fish in tanks than what was estimated prior to the study. 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 CY09 Trials

1. Species of fish treated

Ten fish species, including five salmonids and five non-salmonids were treated during CY09. Treated fish ranged in length from 1.13 - 20.50 in. and the average length of all treated fish was 5.4 in. Fish species treated included:

Salmonids:

brook trout *Salvelinus fontinalis*

chinook salmon *Oncorhynchus tshawytscha*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*

rainbow trout *O. mykiss*

Non-salmonids:

muskellunge *Esox masquinongy*

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

striped bass *Morone saxatilis*

walleye *Sander vitreus*

white sturgeon *Acipenser transmontanus*

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 CY09: *Aeromonas Hydrophila*, coldwater disease (causative agent *Flavobacterium psychrophilus*), columnaris (causative agent *F. columnare*), bacterial kidney disease (causative agent *Renibacterium salmoninarum*), enteric redmouth (causative agent *Yersinia ruckeri*), *lactobacilliosis*, motile aeromonad septicemia, or general systemic bacterial infection.

Data Collected

1. Pathologist's reports

A pathologist's report was submitted for 42% 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.

As stated in the Study Protocol, mortality data was to be collected for at least five days prior to treatment, during treatment, and for at least 21 d post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, for a variety of reasons, not all requested mortality data was collected. Reasons for an incomplete mortality record include: 1) splitting fish into additional rearing units to ease crowding and improve culture conditions, and 2) 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 CY09 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 or to apply a skeletal mark (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 summary data for all trials; Table 4 provide a summary of all trials conducted during CY09 under INAD #9332).

A. Efficacy at 2.50 - 4.0 g/100 lbs fish/d for 10 - 12 days

Chinook salmon, cutthroat trout, kokanee salmon, rainbow trout, hybrid striped bass, striped bass, walleye, and white sturgeon were treated with 2.5 - 4.0 g OTF/100 lbs of fish/d for 10 - 12 days in 22 trials (Tables 1 - 2). Investigators used OTF to either apply a skeletal mark or to control mortality caused by *Aeromonas Hydrophila*, bacterial kidney disease, columnaris, enteric redmouth, *Lactobacilliosis*, or general systemic bacterial infection. OTF treatments appeared effective in 21 trials and ineffective in one trial.

B. Efficacy at 10.0 g/100 lbs fish/d for 10 days

Chinook salmon, brook trout, cutthroat trout, rainbow trout, muskellunge, and walleye were treated with 10.0 g OTF/100 lbs of fish/d for 10 days in 16 trials (Tables 1 - 2). Investigators used OTF to either apply a skeletal mark or to control mortality caused by coldwater disease, bacterial kidney disease, columnaris, or motile aeromonad septicemia. OTF treatments appeared effective in 15 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 CY09.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

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 5. Facilities and Names of Investigators” for facilities that signed-up to participate in the Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 during CY09. Facilities not listed in Appendix III-a of the current Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 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 facilities had Oxytetracycline (Terramycin® 200 for Fish) medicated feed or premix on-hand during CY09 but never used the drug:

1. Alchesay-Williams Creek NFH
2. Bald Hill FCS
3. Bennington FCS
4. Ed Weed FCS
5. Simaron Fresh Water Fish

Correspondence sent to Oxytetracycline (Terramycin® 200 for Fish) 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 2009.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY09 was 4,905,643. The total number of treated fish to count against the Oxytetracycline (Terramycin® 200 for Fish) treatment use authorization dated June 25, 2007 is 21,856,416.

Summary of Study Results

Oxytetracycline (Terramycin® 200 for Fish) medicated feed was used at dosages ranging from 2.50 - 10.0 g active drug/100 lbs fish/d in 38 treatment trials. Treatment durations ranged from 10 - 14 days. Treatment trials involved 10 different fish species and approximately 4.9 million fish. Treated fish ranged in length from 1.13 - 20.5 in. Water temperature during treatment ranged from 50.5 - 84.1 °F, with a mean treatment temperature of 62.5 °F. Overall results showed that treatment in approximately 95% of trials appeared to be efficacious while treatment in 5% appeared ineffective. 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/markings 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 2009 OTF Treatment Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease/Purpose	Dose (g/100 lbs)	Number of Treatment Days	Observed Withdrawal Time (days)	Temp. (°F)
Giant Springs SFH	1	BKT	2.83	46,290	Mark	10	14	105	54.0
Cle Elum Supplementation and Research	1	CSA	3.50	92,369	Columnaris	3.75	12	167	59.0
Murray Springs SFH	2	CUT	1.14	190,000	CWD	10	14	365	52.0
Washoe Park SFH	3	CUT	2.4 - 2.9	506,750	CWD	10	14	>70 - 200	51.0 - 54.0
Yellowstone River SFH	1	CUT	3.40	40,000	Mark	3.75	10	150	52.0
Coleman NFH	2	FCS	2.2 - 3.1	654,000	Columnaris	10	14	>90	53.0 - 66.9
Coleman NFH	1	FCS	3.00	1,050,813	Enteric Redmouth	3.75	10	>90	56.0
Big Springs SFH	1	KOE	3.61	37,700	Mark	3.75	10	40	55.0
Spirit Lake SFH	1	MUE	4.85	8,725	MAS	10	14	2,920	72.0
Big Springs SFH	3	RBT	2.9 - 7.6	288,500	Mark	3.3 - 4.0	10	32 - 94	52.0
Bluewater Springs SFH	3	RBT	2.9 - 4.3	190,000	Mark	10	14	98 - 144	59.0
Bozeman FTC	1	RBT	6.14	120	Mark	3.75	10	ethanized	50.5
Ennis NFH	1	RBT	20.50	1,830	Lactobacilliosis	2.53	10	45	54.0
Farlington SFH	2	SBH	2.00	16,768	Columnaris	3.75	10	>365	83.2 - 84.1
Dexter Ponds SFH	2	SCS	4.3 - 5.5	1,642,283	Columnaris	10	14	>70	60.8 - 61.4
Farlington SFH	1	STB	2.00	7,154	Columnaris	3.75	10	>365	83.2

Table 1. Summary of CY 2009 OTF Treatment Results - Efficacious Trials - continued

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease/Purpose	Dose (g/100 lbs)	Number of Treatment Days	Observed Withdrawal Time (days)	Temp. (°F)
Rathbun SFH	4	WAE	4.8 - 5.5	70,724	Aeromonas Hydrophila	3.5	10	66 - 70	75.3 - 76.3
Spirit Lake SFH	1	WAE	3.60	43,274	MAS	10	14	1,095	72.0
Sterling Caviar	5	WST	9.0 - 12.0	6,842	General Systemic Bacterial Infection	2.85 - 3.8	10 - 11	1,095	69.8 - 71.2

Table 2. Summary of CY 2009 OTF Treatment Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Dose (g/100 lbs)	Number of Treatment Days	Observed Withdrawal Time (days)	Temp. (°F)
Hayspur SFH	1	RBT	11.61	4,146	BKD	3.75	10	100	52.0
Hayspur SFH	1	RBT	11.61	7,355	BKD	10	14	100	52.0

Table 3. Summary Data Regarding Summary of CY 2009 OTF Treatment Trials

Total Fish Treated: **4,905,643**

Number of fish treated in efficacious trials 4,894,142
 Number of fish treated in ineffective trials 11,501

Total number of trials: **38**

Efficacious trials 36 (95%)
 Ineffective trials 2 (5%)

Treatment Regimens Used:

2.50 - 4.0 g/100 lbs fish/day for 10 - 12 days 22 trials
 10.0 g/100 lbs fish/day for 14 days 16 trials

Treatment Water Temperature (°F):

Temperature Range 50.5 - 84.1
 Average Temperature 62.5

Size of Treated Fish (in.):

Size Range 1.13 - 20.5
 Average Length 5.4

Species Treated:

Salmonids:

brook trout *Salvelinus fontinalis*
 chinook salmon *Oncorhynchus tshawytscha*
 cutthroat trout *O. clarki*
 kokanee salmon *O. nerka*
 rainbow trout *O. mykiss*

Non-salmonids:

muskellunge *Esox masquinongy*

hybrid striped bass *Morone saxatilis* x *M. chrysops*
striped bass *Morone saxatilis*
walleye *Sander vitreus*
white sturgeon *Acipenser transmontanus*