

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

Year 2007 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 control/prevent mortality in a variety of fish/shellfish caused by common fish bacterial pathogens. In calendar year 2007 (CY07) the efficacy of oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF) was evaluated in 74 disease trials involving approximately 14.8 million fish/shellfish to control mortality in a variety of test fish/shellfish caused by a variety of infectious fish pathogens. Trials were conducted at 15 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 11 state hatcheries, and two private fish culture facilities. 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; 2) 10 g drug/100 lbs fish/d for 14 days; or 3) 6.0 g drug/100 lbs abalone/d for 14 days. Overall, results of trials conducted in CY07 indicated that treatments appeared to be efficacious in approximately 84% of the trials,

ineffective in 3% of the trials, and were characterized as inconclusive in 12% 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 label for oxytetracycline medicated feed (OTF) use in aquaculture limits use to the control of furunculosis in salmonids caused by *Aeromonas salmonicida*, and the control of bacterial hemorrhagic septicemia in salmonids and catfish caused by *A. hydrophila* or *Pseudomonas sp.* Oxytetracycline medicated feed has been shown to be highly effective in controlling these diseases, especially when predisposing environmental stresses are reduced at the time of treatment (Warren 1991). Furthermore, the current FDA approved label for OTF limits dosage to a range of 2.5 - 3.75 grams of active drug per 100 pounds of fish per day for 10 days, and limits use to water temperatures "not below 48.2° F (9° C)." These label restrictions severely 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 is not approved for such uses, and the only legal way to use OTF for such non-approved uses is through an INAD.

Purpose of Report

The purpose of this report is to summarize the results of calendar year 2007 (CY07) 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 74 trials were conducted at 15 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 11 state fish hatcheries, and two private fish culture facilities. Water temperature during treatments at the various testing facilities ranged from 35.0 - 80.0 °F, with a mean treatment temperature of 56.2°F.

2. Test article used

The OTF used in CY07 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 50% of trials were conducted using this treatment regimen); or 10.0 g of active drug/100 lbs of fish/d for 10 days (approximately 23% of trials were conducted using this treatment regimen).

Study Protocol Deviation: Treatment regimen administered in the remaining trials (approximately 27% 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 - 18 days (3% of trials); 2) 3.85 - 9.36 g drug/100 lbs fish/d for 3 - 14 days (18% of trials); 3) 10.1 - 12.48 g drug/100 lbs fish/d for 14 - 16 days (5% of trials); or 4) 6.0 g drug/100 lbs abalone/d for 1 day (1% 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 than 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. In most cases in which deviations occurred, 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 CY05 Trials

1. Species of fish treated

Eleven fish/shellfish species, including seven salmonids, two non-salmonids, one marine non-salmonid, and one species of shellfish were treated during CY07.

Treated fish ranged in length from 0.85 - 11.0 in. and the average length of all treated fish was 4.1 in. Fish species treated included:

Salmonids: (1) Apache trout *Oncorhynchus apache*; (2) chinook salmon *O. tshawytscha*; (3) cutthroat trout *O. clarki*; (4) rainbow trout *O. mykiss*; (5) steelhead trout *O. mykiss*; (6) sockeye salmon *O. nerka*; and (7) rainbow trout x cutthroat trout *O. mykiss* x *O. clarki*.

Non-salmonids: (1) blue catfish *Ictalurus furcatus*; and (2) hybrid striped bass *Morone chrysops* x *M. saxatilis*.

Marine non-salmonids: (1) cabezon *Scorpaenichthys marmoratus*

Shellfish: (1) red abalone *Haliotis rufescens*

2. Diseases treated

Test fish were treated to control/prevent mortality caused by the following diseases during CY07:

1. Coldwater disease (causative agent *Flavobacterium psychrophilus*)
2. Columnaris (causative agent *F. Columnare*)
3. Enteric redmouth (causative agent *Yersinia ruckeri*)
4. General systemic bacterial infection
5. Saltwater columnaris (marine spp.)
6. Saltwater coldwater disease (marine spp.)
7. Flavobacteriosis
8. Hot foot syndrome (shellfish spp.)

Bacterial coldwater disease (51% of trials), flavobacteriosis (15%), and columnaris (14% of trials) were the most frequently treated diseases during this period. Treatment of the other five diseases listed above accounted for the remaining 20% of the treatment trials.

Data Collected

1. Pathologist's reports

A pathologist's report was submitted for 68% 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:

- 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) *Oncorhynchus 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 *Oncorhynchus 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 CY07 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/shellfish 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 Tables 6a and 6b provide a summary of all trials conducted during CY07 under INAD #9332; Table 6a is sorted by study number; Table 6b is sorted first by disease treated, second by whether treatments were efficacious or not, and lastly by fish species).

A. Efficacy at 2.50 - 3.75 g/100 lbs fish/d for 10 - 18 days

Fish were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 - 18 days in 39 trials (Tables 1 - 4). Included in these 39 trials were 21 trials in which rainbow trout, steelhead trout, and sockeye salmon were diagnosed with CWD; five trials in which rainbow trout, blue catfish, and hybrid striped bass were diagnosed with columnaris; three trials in which chinook salmon were diagnosed with enteric redmouth; four trials in which cabezon were diagnosed with flavobacteriosis; and six trials in which Apache and rainbow trout were diagnosed with general systemic bacterial infection. Treatment resulted in the following:

- 1) Treatment appeared to be efficacious in all of the trials in which Apache trout, rainbow trout, hybrid striped bass, and blue catfish were diagnosed with columnaris or general systemic bacterial infection.

2) Of the 21 trials in which CWD was diagnosed in rainbow trout, steelhead trout, and sockeye salmon, 18 of the trials appeared to be efficacious, while one trial involving steelhead trout was ineffective, one trial involving rainbow trout was characterized as inconclusive, and one trial involving rainbow trout did not report efficacy (due to the efficacy packet being complete).

3) Of the three trials in which enteric redmouth was diagnosed in chinook salmon, one trial appeared to be efficacious, while two trials were characterized as inconclusive.

4) Of the four trials in which flavobacteriosis was diagnosed in cabezon, two of the trials appeared to be efficacious, while two trials were characterized as inconclusive.

B. Efficacy at 6.0 g/100 lbs abalone/d for 1 day

Abalone were treated with 6.0 g OTF/100 lbs of abalone/d for 1 day in one trial (Table 1). Abalone were diagnosed with hot foot syndrome. OTF treatments appeared to be efficacious in this trial.

C. Efficacy at 3.85 - 9.36 g/100 lbs fish/d for 3 - 14 days

Fish were treated with 3.85 - 9.36 g OTF/100 lbs of fish/d for 3 - 14 days in 13 trials (Tables 1 & 3). Fish species treated were rainbow and cutthroat trout

diagnosed with CWD; and cabezon diagnosed with either flavobacteriosis, saltwater CWD, or saltwater columnaris. Treatment resulted in the following:

1) Treatment appeared to be efficacious in all of the trials in which rainbow trout, cutthroat trout, and cabezon were diagnosed with CWD, saltwater CWD, or saltwater columnaris.

2) Of the seven trials in which flavobacteriosis was diagnosed in cabezon, five of the trials appeared to be efficacious, while two trials were characterized as inconclusive.

D. Efficacy at 10.0 g/100 lbs fish/d for 14 days

Fish were treated with 10.0 g OTF/100 lbs of fish/d for 14 days in 17 trials (Tables 1 & 3). In 11 of the trials, cutthroat trout, rainbow trout, and rainbow x cutthroat trout were diagnosed with CWD; in five trials chinook salmon and steelhead trout were diagnosed with columnaris, and in one trial chinook salmon were diagnosed with enteric redmouth. Treatments resulted in the following:

1) Of the 11 trials in which CWD was diagnosed, treatment in nine of the trials involving cutthroat trout, rainbow trout, and rainbow x cutthroat trout appeared to be efficacious, while treatment in two of the trials involving rainbow trout were characterized as inconclusive.

2) Treatment appeared to be efficacious in all trials in which chinook salmon and steelhead trout were diagnosed with columnaris or enteric redmouth.

E. Efficacy at 10.1 - 12.48 g/100 lbs fish/d for 14 - 16 days

Fish were treated with 10.1 - 12.48 g OTF/100 lbs fish/d for 14 - 16 days in four trials (Tables 1 - 2). In three of the trials, cutthroat trout were diagnosed with CWD, and in one trial Apache trout were diagnosed with general systemic bacterial infection. OTF treatments appeared to be efficacious in three trials, while one trial involving cutthroat trout diagnosed with CWD was ineffective.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY07.

Number of Treated Fish under Slaughter Authorization (please note this includes all fish treated under the OTF INAD #9332 for use as either a therapeutic or as a marking tool)

Total number of treated fish during CY07 was 15,732,008. The total number of treated fish to count against the slaughter authorization dated August 12, 2002 (valid through June 24, 2007) is 105,901,403. The total number of treated fish to count against the current slaughter authorization dated June 25, 2007 is 7,980,978. No changes have occurred to the current OTF INAD #9332 study protocol.

Facility Sign-up List (please note this includes all fish treated under the OTF INAD #9332 for use as either a therapeutic or as a marking tool)

Please see “Table 7. Facilities and Names of Investigators” for facilities that signed-up to participate in the OTF INAD #9332 during CY07.

The following facilities received/carried-over OTF during CY07 but never used the drug:

1. Bald Hill FCS
2. Bennington FCS
3. Blue Dog SFH
4. Sandy SFH

Summary of Study Results

Oxytetracycline (Terramycin[®] 200 for Fish) medicated feed was used at dosages ranging from 2.50 - 12.48 g active drug/100 lbs fish/d in 74 treatment trials. Treatment durations ranged from 1 - 18 days. Treatment trials involved eleven different fish/shellfish species and approximately 14.8 million fish/shellfish. Treated fish ranged in length from 0.85 - 11.0 in. Water temperature during treatment ranged from 35.0 - 80.0 °F, with a mean treatment temperature of 56.2 °F. Overall results showed that treatment in approximately 84% of trials appeared to be efficacious, treatment in 3% appeared ineffective, and characterized as inconclusive in 12% 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 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 2007 OTF Treatment Results - Efficacious 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	3	APT	2.3 - 3.5	163,403	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
Farlington SFH	2	BCF	7.50	100,000	Columnaris	10	2.5 - 3.75	75.0
The Abalone Farm	2	CAB	7.00	6,171	Flavobacteriosis	10	2.5 - 3.75	57.0 - 61.0
Coleman NFH	1	FCS	2.80	448,958	Enteric Redmouth	10	2.5 - 3.75	52.0
Alchesay-Williams Creek NFH	2	RBT	0.85	365,311	CWD	10	2.5 - 3.75	52.0
	3	RBT	1.0 - 3.9	910,730	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
American Falls SFH	1	RBT	8.20	80,300	CWD	10	2.5 - 3.75	55.0
Hagerman SFH	6	RBT	3.0 - 6.3	1,826,584	CWD	10	2.5 - 3.75	59.0
	2	RBT	4.8 - 5.0	141,000	Columnaris	10	2.5 - 3.75	59.0
Nampa SFH	3	RBT	2.9 - 7.0	402,982	CWD	10	2.5 - 3.75	59.0
Trail Lakes/Eklutna Hatchery	3	SOS	2.0 - 2.5	2,515,000	CWD	10	2.5 - 3.75	40.6 - 40.8
Ed Weed FCS	1	STT	3.90	29,000	CWD	10	2.5 - 3.75	38.0
Niagara Springs SFH	2	STT	1.8 - 8.0	2,072,984	CWD	10	2.5 - 3.75	59.0
Farlington SFH	1	SXW	2.50	12,100	Columnaris	10	2.5 - 3.75	80.0
The Abalone Farm	1	ABL	4.00	16,771	Hot Foot Syndrome	1	6	54.0
The Abalone Farm	5	CAB	5.00	6,200	Flavobacteriosis	10 - 14	3.85 - 9.36	57.0 - 60.0
	1	CAB	4.50	2,000	Saltwater CWD	14	3.85 - 9.36	59.0

Table 1. Summary of CY 2007 OTF Treatment Results - Efficacious Trials - continued

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Disease	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
The Abalone Farm	2	CAB	7.0 - 11.0	5,851	Saltwater Columnaris	10 - 14	3.85 - 9.36	58.0 - 59.0
Murray Springs Trout SFH	1	CUT	2.00	20,000	CWD	14	3.85 - 9.36	52.0
Giant Springs Trout SFH	2	RBT	3.2 - 3.4	123,552	CWD	14	3.85 - 9.36	54.0
Dexter Ponds SFH	1	CKS	3.50	223,759	Columnaris	14	10	60.5
Murray Springs Trout SFH	3	CUT	0.9 - 3.5	264,003	CWD	14	10	52.0
Washoe Park Trout SFH	2	CUT	1.4 - 2.8	264,500	CWD	14	10	50.0 - 55.0
Coleman NFH	1	FCS	2.30	232,052	Enteric Redmouth	14	10	66.0
	1	FCS	3.30	87,530	Columnaris	14	10	63.0
American Falls SFH	3	RBT	4.0 - 5.0	137,000	CWD	14	10	55.0 - 56.0
Mackay SFH	1	RXC	1.90	156,900	CWD	14	10	52.0
Dexter Ponds SFH	2	SCS	3.5 - 4.5	1,927,055	Columnaris	14	10	60.0 - 60.8
Coleman NFH	1	STT	5.50	50,157	Columnaris	14	10	63.0
Alchesay-Williams Creek NFH	1	APT	1.71	41,393	General Systemic Bacterial Infection	14	10.1 - 12.48	52.0
Murray Springs Trout SFH	2	CUT	2.0 - 3.1	121,196	CWD	14	10.1 - 12.48	52.0

Table 2. Summary of CY 2007 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)
Ed Weed FCS	1	STT	3.80	32,000	CWD	10	2.5 - 3.75	35.0
Murray Springs Trout SFH	1	CUT	0.97	30,000	CWD	16	10.1 - 12.48	52.0

Table 3. Summary of CY 2007 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)
The Abalone Farm	2	CAB	11.00	840	Flavobacteriosis	10	2.50 - 3.75	62.0
Coleman NFH	2	FCS	2.40	908,956	Enteric Redmouth	10 - 18	2.50 - 3.75	54.0 - 55.0
Hagerman SFH	1	RBT	3.07	591,150	CWD	10	2.50 - 3.75	59.0
The Abalone Farm	2	CAB	6.00	3,650	Flavobacteriosis	3 - 14	3.85 - 9.36	59.0 - 60.0
Giant Springs Trout SFH	2	RBT	2.69	263,790	CWD	14	10	54.0

Table 4. Summary of CY 2007 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. (°F)
Hagerman SFH	1	RBT	3.70	243,000	CWD	10	2.5 - 3.75	59.0

Table 5. Summary Data Regarding Summary of CY 2007 OTF Treatment Trials

Total Fish/Shellfish Treated: **14,827,828**

Number of fish treated in efficacious trials	12,754,442
Number of fish treated in ineffective trials	62,000
Number of fish treated in inconclusive trials	1,768,386
Number of fish where efficacy was not needed	243,000

Total number of trials: **74**

Efficacious trials	62 (84%)
Ineffective trials	2 (3%)
Inconclusive trials	9 (12%)
Efficacy was not needed	1 (1%)

Treatment Regimens Used:

2.50 - 3.75 g/100 lbs fish/day for 10 - 18 days	39 trials
6.0 g/100 lbs abalone/day for 1 day	1 trial
3.85 - 9.36 g/100 lbs fish/day for 3 - 14 day	13 trials
10.0 g/100 lbs fish/day for 14 days	17 trials
10.1 - 12.48 g/100 lbs fish/day for 14 - 16 days	4 trials

Treatment Water Temperature (°F):

Temperature Range	35.0 - 80.0
Mean Temperature	56.2

Size of Treated Fish/Shellfish (in.):

Size Range	0.85 - 11.0
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Species Treated:

Salmonids:

Apache trout *Oncorhynchus apache*
 chinook salmon *O. tshawytscha*
 cutthroat trout *O. clarki*
 rainbow trout *O. mykiss*
 steelhead trout *O. mykiss*
 sockeye salmon *O. nerka*
 rainbow trout x cutthroat trout *O. mykiss* x *O. clarki*.

Non-salmonids:

blue catfish *Ictalurus furcatus*

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

Marine non-salmonids:

cabezon *Scorpaenichthys marmoratus*

Shellfish:

red abalone *Haliotis rufescens*