Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2006 Annual Summary Report on the Use of Oxytetracycline Medicated Feed in Field Efficacy Trials

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Summary

Oxytetracycline medicated feed has been used effectively in the U. S. under compassionate INAD Exemption #9332 to control/prevent mortality in a variety of fish caused by common fish bacterial pathogens. In calendar year 2006 (CY06) the efficacy of oxytetracycline medicated feed (OTF) was evaluated in 82 disease trials involving approximately 19.5 million fish to control mortality in a variety of test fish caused by a variety of infectious fish pathogens. Trials were conducted at 23 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 15 state hatcheries, and six private fish culture facilities. The compassionate study protocol under which treatments were administered allowed the investigator to use OTF at a dosage of either 2.5 - 3.75 g drug/100 lbs fish/d for 10 days or 10 g drug/100 lbs fish/d for 14 days. However, on occasion, Investigators deviated from the study protocol, and treatments were administered at dosages between 4.0 - 14.0 g drug/100 lbs fish/d for 10 - 15 days. Overall, results from trials conducted in CY06 indicated that treatments appeared

effective in approximately 79% of the trials and ineffective in 4% of the trials. Treatments were characterized as inconclusive in the remaining 17% of the trials.

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^{o} F (9^{o} 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 cold water 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 2006 (CY06) 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 82 trials were conducted at 23 fish culture facilities, including two U.S. Fish and Wildlife Service fish hatcheries, 15 state fish hatcheries, and six private fish culture facilities. Mean water temperature during all trials was 58.6 °F, and water temperature ranged from 39.0 - 86.7 °F during the test periods at the different testing facilities.

2. Test article used

The OTF used in CY06 efficacy trials was either Terramycin 100 or Terramycin 100D, both of which contained 100 g active oxytetracycline quaternary salt per

pound of premix. All Terramycin 100/100D was supplied by Phibro Animal Health, 75 Challenger Road Ridgefield Park, NJ. All OTF was prepared with Phibro brand product by one of several commercial fish feed manufacturers (e.g., Nelson and Sons, Inc., Rangen Inc.)

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 - 14 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 - 15 days (approximately 24% of trials were conducted using this treatment regimen). However, the treatment regimen administered in the remaining 28% of the trials deviated from the protocol-mandated use. In these trials, fish were fed at rates of either 4.0 - 9.9 g drug/100 lbs fish/d for 10 - 14 days (17% of trials) or at 10.8 - 14.0 g drug/100 lbs fish/d for 14 - 15 days (11% of trials). 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

Twelve fish species, including eight salmonids and four non-salmonids were treated during CY06. Treated fish ranged in length from 0.9 - 8.0 in. Fish species treated included:

Salmonids: (1) Apache trout *Oncorhynchus apache*; (2) chinook salmon *O. tshawytscha*; (3) coho salmon *O. kisutch*; (4) cutthroat trout *O. clarki*; (5) rainbow trout *O. mykiss*; (6) steelhead trout *O. mykiss*; (7) sockeye salmon *O*. *nerka*; and (8) brook trout *Salvelinus fontinalis*

Non-salmonids: (1) blue catfish *Ictalurus furcatus*; (2) cabezon *Scorpaenichthys marmoratus;* (3) California halibut *Paralichthys californicus;* and (4) hybrid striped bass *Morone chrysops* x *M. saxatilis.*

2. Diseases treated

Test fish were treated to control/prevent mortality caused by the following diseases during CY06:

1. Coldwater disease (causative agent *Flavobacterium psychrophilus*)
2. Bacterial kidney disease (causative agent *Renibacterium salmoninarum*)
3. Columnaris (causative agent *F. Columnare*)

4. Epitheliocycstis
5. General systemic bacterial infection
6. Gram negative bacterial enteritis
7. Systemic bacterial infection (marine spp.)

Bacterial coldwater disease (50% of trials), general systemic bacterial infection (17% of trials), and columnaris (17% of trials) were the most frequently treated diseases during this period. Treatment of the other four diseases listed above accounted for the remaining 16% of the treatment trials.

Data Collected

1. Pathologist's reports

A pathologist's report was submitted for 59% 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. Mortality data

As stated in the Study Protocol, mortality data were to be collected five days prior to treatment, during the treatment period, and for at least 20 days post-treatment. Investigators were strongly encouraged to collect mortality data on a daily basis. However, daily collection of post-treatment mortality data was not always possible due to factors such as fish being moved among tanks or raceways (i.e., splitting or combining fish), and fish being stocked to rivers and other bodies of water.

Discussion of Study Results:

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

Results of CY06 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 salmonid and non-salmonid fishes (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 summary data for all trials; and Tables 5a and 5b provide a summary of all trials conducted during CY06 under INAD #9332; Table 5a is sorted by study number; Table 5b 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 - 14 days at water temperatures below 48.2° F

Fish were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 - 14 days in four trials (Table 1) where water temperatures were below 48.2° F. Fish species treated were rainbow trout diagnosed with general systemic bacterial infection and sockeye salmon diagnosed with CWD. OTF treatments appeared efficacious in all trials.

B. Efficacy at 2.50 - 3.75 g/100 lbs fish/d for 10 - 11 days at water temperatures above 48.2° F

Fish were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 - 11 days in 35 trials (Tables 1 - 3) where water temperatures were above 48.2° F. Included in these 35 trials were 12 trials in which rainbow and steelhead trout were diagnosed with CWD; 13 trials in which rainbow and steelhead trout, chinook salmon, and blue catfish were diagnosed with columnaris; one trial in which California halibut were diagnosed with epitheliocycstis; two trials in which Apache

and brook trout were diagnosed with general systemic bacterial infection; and seven trials in which hybrid striped bass were diagnosed with gram negative bacterial enteritis. Treatment resulted in the following:

1) Treatment appeared efficacious in all of the trials in which Apache, rainbow, steelhead, and brook trout, chinook salmon, California halibut, and blue catfish were diagnosed with columnaris, general systemic bacterial infection, or epitheliocycstis

2) Of the 12 trials in which CWD was diagnosed in rainbow and steelhead trout, 10 of the trials appeared efficacious, while one trial involving steelhead trout was ineffective, and one trial involving rainbow trout was characterized as inconclusive

3) Of the seven trials in which gram negative bacterial enteritis was diagnosed in hybrid striped bass, four of the trials appeared efficacious, while three trials were characterized as inconclusive.

C. Efficacy at 8.0 g/100 lbs fish/d for 14 days at water temperatures below 48.2^{o} F

Fish were treated with 8.0 g OTF/100 lbs of fish/d for 14 days in one trial (Table 1) where water temperatures were below 48.2^{o} F. Fish species treated were

chinook salmon diagnosed with CWD. OTF treatments appeared efficacious in this trial.

D. Efficacy at 4.0 - 9.9 g/100 lbs fish/d for 10 - 14 days at water temperatures above 48.2° F

Fish were treated with 4.0 - 9.9 g OTF/100 lbs of fish/d for 10 - 14 days in 13 trials (Table 1). Fish species treated were rainbow and steelhead trout diagnosed with CWD and cabezon diagnosed with systemic bacterial infection (marine). OTF treatments appeared efficacious in all trials.

E. Efficacy at 10 g/100 lbs fish/d for 14 days at water temperatures below 48.2° F

Fish were treated with 10 g OTF/100 lbs of fish/d for 14 days in two trials (Table 1) involving chinook salmon diagnosed with bacterial kidney disease and steelhead trout diagnosed with CWD. OTF treatments appeared efficacious in both trials.

F. Efficacy at 10.0 g/100 lbs fish/d for 10 - 15 days at water temperatures above 48.2°F

Fish were treated with 10.0 g OTF/100 lbs of fish/d for 10 - 15 days in 18 trials (Tables 1 - 3). In 16 of the trials, chinook and coho salmon, cutthroat, rainbow and steelhead trout were diagnosed with CWD; in one trial chinook salmon were

diagnosed with columnaris, and in one trial coho salmon were diagnosed with both columnaris and CWD. Treatments resulted in the following:

1) Of the 16 trials in which CWD was diagnosed, treatment in five of the trials involving cutthroat, rainbow and steelhead trout appeared efficacious, while treatment in two of the trials involving coho salmon appeared ineffective, and treatment in nine of the trials involving chinook and coho salmon, cutthroat, rainbow, and steelhead trout were characterized as inconclusive.

2) Treatment appeared efficacious in both trials in which chinook and coho salmon were diagnosed with columnaris or columnaris and CWD.

G. Efficacy at 10.8 - 14.0 g/100 lbs fish/d for 14 - 15 days at water temperatures above 48.2^{o}F

Fish were treated with 10.8 - 14.0 g OTF/100 lbs fish/d for 14 - 15 days in nine trials (Tables 1 & 3) involving rainbow and cutthroat trout diagnosed with CWD, and cabezon diagnosed with systemic bacterial infection (marine). OTF treatments appeared efficacious in eight trials, while one trial involving cutthroat trout was characterized as inconclusive.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY06.

Summary of Study Results

Oxytetracycline medicated feed was used at dosages ranging from 2.50 - 14.0 g active drug/100 lbs fish/d. Treatment durations ranged from 10 - 15 days. Treatment trials involved twelve different fish species and approximately 19.5 million fish. Treated fish ranged in length from 0.9 - 8.0 in. Water temperature during treatment ranged from 39.0 - 86.7 °F, with a mean treatment temperature of 58.6 °F. Overall results showed that treatment in approximately 79% of trials appeared efficacious, treatment in 4% appeared ineffective, and treatment in the remaining 17% of the trials was characterized as inconclusive. 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 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 2006 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 Complex	1	RBT	7.85	15,924	General Systemic Bacterial Infection	10	2.5 - 3.75	42.0
Trail Lakes/Eklutna Hatchery	3	SOS	3.0 - 3.5	891,460	CWD	10 - 14	2.5 - 3.75	39.0
Whitman Lake Hatchery	1	CKS	1.90	1,216,350	CWD	14	8.0	43.9
Ed Weed FCS	1	STT	3.40	30,000	CWD	14	10	41.0
Pahsimeroi SFH	1	SUS	4.86	1,074,143	BKD	14	10	42.0
Alchesay-Williams Creek NFH Complex	1	APT	1.39	117,600	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
Farlington SFH	3	BCF	2.0 - 2.5	190,000	Columnaris	10	2.5 - 3.75	84.5 - 86.7
Alchesay-Williams Creek NFH Complex	1	BKT	1.52	120,000	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
Coleman NFH	1	FCS	2.20	35,776	Columnaris	10	2.5 - 3.75	67.2
Hubbs Seaworld Research Institute	1	HAL	2.14	2,779	Epitheliocycstis	10	2.5 - 3.75	65.7
Hagerman SFH	1	RBT	1.94	718,000	CWD	10	2.5 - 3.75	59.0
	6	RBT	2.5 - 7.0	1,259,900	Columnaris	10	2.5 - 3.75	59.0
Mackay SFH	2	RBT	4.1 - 4.4	80,000	CWD	10	2.5 - 3.75	52.0
Magic Valley Steelhead SFH	1	RBT	2.38	98,626	CWD	10	2.5 - 3.75	59.0
Nampa SFH	2	RBT	3.4 - 5.0	268,956	CWD	10 - 11	2.5 - 3.75	59.0

Table 1. Summary of CY 2006 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)
Coleman NFH	3	STT	4.2 - 5.5	204,578	Columnaris	10	2.5 - 3.75	64.7 - 65.6
Niagara Springs SFH	4	STT	1.8 - 7.7	2,841,941	CWD	10	2.5 - 3.75	59.0
Kent SeaTech Corp	4	SXW	1.25	1,101,691	Gram Negative Bacterial Enteritis	10	2.5 - 3.75	77.0
The Abalone Farm, Inc	11	CAB	4.0 - 8.0	15,298	Systemic Bacterial Infection (marine)	10 - 14	4.0 - 9.9	55.0 - 64.0
Giant Springs Trout SFH	1	RBT	3.61	74,156	CWD	14	4.0 - 9.9	54.0
Umatilla SFH	1	STT	7.00	135,674	CWD	14	4.0 - 9.9	53.3
Dexter Ponds SFH	1	CKS	5.00	992,744	Columnaris	14	10	57.0
Sandy SFH	1	COS	3.50	1,062,831	Columnaris & CWD	14	10	62.0
Murray Springs Trout SFH	1	CUT	0.90	34,740	CWD	14	10	52.0
Washoe Park Trout SFH	1	CUT	3.10	123,000	CWD	14	10	56.0
American Falls SFH	2	RBT	4.2 - 4.7	74,000	CWD	14	10	55.0
Magic Valley Steelhead SFH	1	STT	1.81	56,600	CWD	14	10	60.0
The Abalone Farm, Inc.	3	CAB	3.00	3,739	Systemic Bacterial Infection (marine)	14	10.8 - 14.0	60.0
Murray Springs Trout SFH	2	CUT	0.9 - 1.00	165,000	CWD	14 - 15	10.8 - 14.0	52.0
	3	RBT	1.0 - 1.2	241,701	CWD	14	10.8 - 14.0	52.0

Table 2. Summary of CY 2006 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)
Niagara Springs SFH	1	STT	1.14	43,000	CWD	10	2.50 - 3.75	59.0
Crystal Lake Hatchery	1	COS	2.00	160,750	CWD	14	10	54.5
Whitman Lake Hatchery	1	COS	1.64	1,271,626	CWD	14	10	51.3

Table 3. Summary of CY 2006 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)
Hagerman SFH	1	RBT	3.24	735,000	CWD	10	2.50 - 3.75	59.0
Kent SeaTech Corp.	3	SXW	1.25	939,280	Gram Negative Bacterial Enteritis	10	2.50 - 3.75	77.0
Crystal Lake Hatchery	3	CKS	2.00	1,748,776	CWD	14	10	52.2
Whitman Lake Hatchery	1	COS	1.64	325,577	CWD	14	10	51.3
Murray Springs Trout SFH	1	CUT	2.14	41,000	CWD	15	10	52.0
Washoe Park Trout SFH	2	CUT	1.0 - 2.1	340,000	CWD	15	10	56.0
Giant Springs SFH	1	RBT	2.80	148,012	CWD	14	10	54.0
Magic Valley Steelhead SFH	1	STT	1.75	469,400	CWD	10	10	60.0
Murray Springs Trout SFH	1	CUT	1.00	21,600	CWD	14	10.8 - 14.0	52.0

Table 4. Summary Data Regarding Summary of CY 2006 OTF Treatment Trials

Total Fish Treated:	**19,491,228**
Number of fish treated in efficacious trials	13,247,207
Number of fish treated in ineffective trials	1,475,376
Number of fish treated in inconclusive trials	4,768,645
Total number of trials:	**82**
Efficacious trials	65 (79%)
Ineffective trials	3 (4%)
Inconclusive trials	14 (17%)

Treatment Regimens Used:

2.50 - 3.75 g/100 lbs fish/day for 10 - 14 days (below 48.2°F)	4 trials
2.50 - 3.75 g/100 lbs fish/day for 10 - 14 days (above 48.2°F)	35 trials
8.0 g/100 lbs fish/day for 14 days (below 48.2°F)	1 trial
4.0 - 9.9 g/100 lbs fish/day for 10 - 14 day (above 48.2°F)	13 trials
10.0 g/100 lbs fish/day for 14 days (below 48.2°F)	2 trials
10.0 g/100 lbs fish/day for 10 - 15 days (above 48.2°F)	18 trials
10.8 - 14.0 g/100 lbs fish/day for 14 - 15 days (above 48.2°F)	9 trials

Treatment Water Temperature (°F):

Temperature Range	39.0 - 86.7
Mean Temperature	58.6

Size of Treated Fish (in.):

Size Range	0.87 - 8.0

Species Treated:

Salmonids

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

Non-salmonids

blue catfish *Ictalurus furcatus*
cabezon *Scorpaenichthys marmoratus*
California halibut *Paralichthys californicus*
hybrid striped bass *Morone chrysops* x *M. saxatilis*