Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2004 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 2004 (CY04) the efficacy of oxytetracycline medicated feed (OTF) was evaluated in 123 disease trials involving approximately 20.0 million fish to control mortality in a variety of test fish caused by the following diseases: bacterial coldwater disease, columnaris, streptococcus, gram negative bacterial enteritis, general systemic bacterial infection, or bacterial kidney disease. Trials were conducted at 21 fish culture facilities, including four U.S. Fish and Wildlife Service fish hatcheries, 13 state hatcheries, and four 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; or 2) 10 g drug/100 lbs fish/d for 14 days. However, Investigators responsible for conducted a few of these trials deviated from the study protocol, and

treatments were administered at dosages between 1.12 - 11.4 g drug/100 lbs fish/d for 10 - 16 days. Overall, results from trials conducted in CY04 indicated that in approximately 75% of the trials, treatments appeared effective, in 7% of the trials, treatments appeared ineffective, and in 18% of the trials, treatments were characterized as inconclusive.

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 2004 (CY04) 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 previous years studies for the purpose of expanding and/or extending the approved label for OTF.

Facilities, Materials, and Treatment Procedures

1. Facilities

Twenty-one fish culture facilities, including four U.S. Fish and Wildlife Service fish hatcheries, 13 state fish hatcheries and four private fish culture facilities used OTF to control/prevent mortality caused by various fish diseases. Mean water temperature during all trials was 60.5 °F, and water temperature ranged from 44.2 - 82.4 °F during the test periods at the different testing facilities.

2. Test article used

The OTF used in CY04 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 Pfizer, Inc., 1107 South 291 Highway, Lee's Summit, MO. Virtually all oxytetracycline medicated feed used in INAD trials was supplied by one of several commercial fish feed manufacturers.

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 - 16 days (approximately 63% of trials were conducted using this treatment regimen) or at 10.0 g of active drug/100 lbs of fish/d for 14 - 15 days (approximately 25% of trials were conducted using this treatment regimen). However, the treatment regimen administered in the remaining 12% of the trials deviated from the protocol-mandated use. In these trials, fish were fed at rates of either 1.12 - 2.21 g drug/100 lbs fish/d for 14 - 16 days (4% of trials), 3.8 - 4.4 g drug/100 lbs fish/d for 10 - 14 days (7% of trials), or at 11.4 g drug/100 lbs fish/d for 14 days (1% of trials).

Fish Species and Fish Diseases Involved in CY 2004 Trials

1. Species of fish treated

Sixteen fish species, including nine salmonids and seven non-salmonids were treated during CY04. Treated fish ranged in length from 0.85 - 15.0 in. Fish species treated included:

Salmonids: (1) Apache trout *apache Oncorhynchus*; (2) chinook salmon *O. tshawytscha*; (3) coho salmon *O. kisutch*; (4) cutthroat trout *O. clarki*; (5) Gila trout *O. gilae;* (6) rainbow trout *O. mykiss*; (7) steelhead trout *O. mykiss*; (8) brook trout *Salvelinus fontinalis*; and (9) brown trout *Salmo trutta*

Non-salmonids: (1) blue catfish *Ictalurus furcatus;* (2) channel catfish *I. punctatus*; (3) hybrid striped bass *Morone chrysops* x *M. saxatilis;* (4) striped bass *M. saxatilis;* (5) tiger musky (*Esox lucius x E. masquinongy*); (6) Tilapia *Oreochromis mossambica;* and (7) white sturgeon *Acipenser transmontanus*.

2. Diseases treated

Test fish were treated to control/prevent mortality caused by the following diseases during CY04:

1. Coldwater disease (causative agent *Flavobacterium psychrophilus*)
2. Columnaris (causative agent *F. Columnare*)

3. General systemic bacterial infection
4. Streptococcus (causative agent *Streptococcal iniae*)
5. Gram negative bacterial enteritis
6. Bacterial kidney disease (causative agent *Renibacterium salmoninarum*)

General systemic bacterial infection (37% of trials), bacterial coldwater disease (33% of trials) and columnaris (18% of trials) were the diseases most frequently treated during this period. Treatment of the other three diseases listed above accounted for the remaining 12% of the treatment trials.

Data Collected

1. Pathologist's reports

A pathologist's report was submitted for 36% 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 OTC

Results of CY04 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 fish (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 CY04 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 1.12 - 2.21 g/100 lbs fish/d for 14 - 16 days at water temperatures above 48.2° F

Fish were treated with 1.12 - 2.21 g OTF/100 lbs of fish/d for 14 - 16 days in five trials (Table 1) involving tilapia diagnosed with streptococcus. OTF treatments appeared efficacious in all trials.

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

Fish were treated with 2.50 - 3.75 g OTF/100 lbs of fish/d for 10 days in two trials (Tables 1 & 2) where water temperatures were below 48.2° F. Trials involved cutthroat trout and rainbow trout diagnosed with CWD. Results from the rainbow trout trial indicated that treatment appeared effective, while results from the cutthroat trout trial indicated the treatment was ineffective.

C. Efficacy at 2.50 - 3.75 g/100 lbs fish/d for 10 - 16 days at water temperatures above 48.2^{o} F

Fish were treated with 2.5 and 3.75 g OTF/100 lbs of fish/d for 10 - 16 days in 76 trials (Tables 1 - 3) where water temperatures were above 48.2^{o} F. Included in these 76 trials were 13 trials in which rainbow trout and cutthroat trout were diagnosed with CWD; 15 trials in which rainbow trout, steelhead trout, blue catfish, channel catfish, striped bass and tiger musky were diagnosed with columnaris; 45 trials in which Apache trout, brook trout, brown trout, cutthroat trout, Gila trout, rainbow trout and white sturgeon were diagnosed with general systemic bacterial infection; and three trials in which tilapia were diagnosed with streptococcus. Treatment resulted in the following:

1) Of the 13 trials in which CWD was diagnosed in rainbow trout and cutthroat trout, treatment in nine (69%) of the trials appeared efficacious, treatment in one (8%) of the trials involving rainbow trout were ineffective, and treatment in three (23%) of the trials involving rainbow trout were characterized as inconclusive.

2) Of the 15 trials in which columnaris was diagnosed, eight (53%) of the trials involving rainbow trout, steelhead trout, blue catfish, striped bass and tiger musky appeared efficacious, treatment in one (7%) trial involving rainbow trout was ineffective, while treatment in six (40%) of the trials involving

channel catfish, rainbow trout and steelhead trout were characterized as inconclusive.

3) Of the 45 trials in which general systemic bacterial infection was diagnosed, treatment in 37 (82%) of the trials involving Apache trout, brook trout, brown trout, cutthroat trout, rainbow trout and white sturgeon appeared efficacious, treatment in three (7%) of the trials involving Apache trout were ineffective, while treatment in five (11%) of the trials involving Apache trout, rainbow trout and Gila trout were characterized as inconclusive.

4) Treatment in all three trials in which streptococcus was diagnosed in tilapia appeared efficacious.

D. Efficacy at 3.8 - 4.4 g/100 lbs fish/d for 10 - 14 days at water temperatures above 48.2^{o} F

Fish were treated with 3.8 - 4.4 g OTF/100 lbs of fish/d for 10 - 14 days in eight trials (Tables 1 & 3). Included in these eight trials, were five trials in which hybrid striped bass were diagnosed with gram negative bacterial enteritis; two trials in which tilapia were diagnosed with streptococcus; and one trial in which channel catfish were diagnosed with columnaris. Treatment resulted in the following:

1) Of the five trials in which gram negative bacterial enteritis was diagnosed in hybrid striped bass, treatment in three (60%) of the trials appeared

efficacious, while treatment in two (40%) of the trials were characterized as inconclusive.

2) Treatment in both trials in which streptococcus was diagnosed in tilapia appeared efficacious.

3) Treatment in the single trial in which channel catfish were diagnosed with columnaris appeared efficacious.

E. Efficacy at 10.0 g/100 lbs fish/d for 14 - 15 days at water temperatures above 48.2^{o}F

Fish were treated with 10.0 g OTF/100 lbs of fish/d for 14 - 15 days in 31 trials (Tables 1 - 3). Included in these 31 trials, were 24 trials in which chinook salmon, coho salmon, cutthroat trout, rainbow trout and steelhead trout were diagnosed with CWD; six trials in which chinook salmon were diagnosed with columnaris; and one trial in which chinook salmon were diagnosed with bacterial kidney disease. Treatment resulted in the following:

1) Of the 24 trials in which CWD was diagnosed, treatment in 16 (67%) of the trials appeared efficacious, treatment in 3 (12%) of the trials involving cutthroat trout, rainbow trout and steelhead were ineffective, while treatment in 5 (21%) of the trials involving chinook salmon and rainbow trout were characterized as inconclusive.

2) Of the six trials in which columnaris was diagnosed in chinook salmon, treatment in 5 (83%) of the trials appeared efficacious, while treatment in 1 (17%) trial was characterized as inconclusive.

3) Treatment in the single trial in which chinook salmon were diagnosed with bacterial kidney disease appeared efficacious.

F. Efficacy at 11.4 g/100 lbs fish/d for 14 days at water temperatures above 48.2°F

Fish were treated with 11.4 g OTF/100 lbs fish/d for 14 days in one trial (Table 1) involving cutthroat trout diagnosed with CWD. Treatment in this trial appeared efficacious.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY04.

Summary of Study Results

Oxytetracycline medicated feed was used at dosages ranging from 1.12 - 11.4 g active drug/100 lbs fish/d. Treatment durations ranged from 10 - 16 days. Treatment trials involved sixteen different fish species and approximately 20 million fish. Treated fish ranged in length from 0.85 - 15.0 in. Water temperature during treatment ranged

from 44.2 - 82.4 °F, with a mean treatment temperature of 60.5 °F. Overall results showed that treatment in approximately 75% of trials appeared efficacious, treatment in 7% appeared ineffective, and treatment in 18% of the trials were 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 INAD #9332, efforts will be directed towards the generation of higher 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 2004 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)
Simaron Fresh Water Fish Inc.	5	TIA	10.4 - 13.3	105,250	Streptococcus	14 - 16	1.12 - 2.21	77.0 - 80.6
Alchesay-Williams Creek NFH Complex	9	APT	0.85	211,399	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
Farlington SFH	2	BCF	5.0 - 6.0	22,000	Columnaris	10	2.5 - 3.75	76.0 - 78.7
Alchesay-Williams Creek NFH Complex	1	BKT	0.85	151,816	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
	1	BNT	0.85	61,068	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
	1	CUT	0.91	111,600	General Systemic Bacterial Infection	10	2.5 - 3.75	52.0
Washoe Park Trout SFH	3	CUT	2.7 - 3.3	355,000	CWD	10	2.5 - 3.75	53.0
Hackettstown SFH	1	MUH	8.70	7,000	Columnaris	10	2.5 - 3.75	68.0
Alchesay-Williams Creek NFH Complex	4	RBT	0.90	990,508	General Systemic Bacterial Infection	10	2.5 - 3.75	51.0 - 52.0
American Falls SFH	1	RBT	5.20	76,411	CWD	10	2.5 - 3.75	55.0
Dworshak NFH	1	RBT	9.61	8,191	CWD	10	2.5 - 3.75	44.2
Giant Springs Trout SFH	1	RBT	4.00	52,200	CWD	10	2.5 - 3.75	54.0
Hagerman SFH	3	RBT	2.4 - 3.8	1,186,843	CWD	10	2.5 - 3.75	59.0

Table 1. Summary of CY 2004 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)
Hagerman SFH	3	RBT	6.6 - 7.5	161,000	Columnaris	10	2.5 - 3.75	59.0
Nampa SFH	1	RBT	4.00	150,000	CWD	10	2.5 - 3.75	59.0
Coleman NFH	1	STT	5.80	50,350	Columnaris	10	2.5 - 3.75	61.0
Milford SFH	1	STB	11.70	1,388	Columnaris	10	2.5 - 3.75	54.0
Simaron Fresh Water Fish Inc.	3	TIA	5.5 - 9.1	183,000	Streptococcus	14 - 16	2.5 - 3.75	73.4 - 77.0
Stolt Sea Farm California LLC	21	WHS	8.0 - 12.0	16,625	General Systemic Bacterial Infection	10	2.5 - 3.75	68.0
Hackettstown SFH	1	CCF	3.00	5,000	Columnaris	10	3.8 - 4.4	80.0
Kent SeaTech Corp.	3	SXW	1.25	2,770,662	Gram Negative Bacterial Enteritis	10	3.8 - 4.4	77.0
Simaron Fresh Water Fish Inc.	2	TIA	4.6 - 6.5	88,500	Streptococcus	14	3.8 - 4.4	71.6 - 73.4
Crystal Lake Hatchery	1	CKS	1.90	452,900	CWD	14	10	48.9
Dexter Ponds SFH	3	CKS	4.5 - 6.7	536,664	Columnaris	14	10	60.5 - 60.6
	2	SCS	4.70	991,188	Columnaris	14	10	60.0
Rapid River SFH	1	SCS	3.40	3,448,618	BKD	15	10	52.5
Crystal Lake Hatchery	1	COS	1.70	234,000	CWD	14	10	50.0

Table 1. Summary of CY 2004 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)
Murray Springs Trout SFH	4	CUT	0.9 - 1.2	386,000	CWD	14 - 15	10	52.0
Washoe Park Trout SFH	1	CUT	1.00	161,700	CWD	14	10	56.0
American Falls SFH	2	RBT	4.2 - 7.0	44,700	CWD	14	10	54.0
Grace SFH	1	RBT	6.21	24,301	CWD	14	10	52.0
Hagerman SFH	1	RBT	1.60	641,479	CWD	14	10	59.0
Murray Springs Trout SFH	3	RBT	1.10	220,002	CWD	14	10	52.0
Magic Valley Steelhead SFH	2	STT	1.2 - 6.5	950,506	CWD	14	10	59.0
Murray Springs Trout SFH	1	CUT	1.92	80,000	CWD	14	11.4	52.0

Table 2. Summary of CY 2004 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)
Alchesay-Williams Creek NFH Complex	3	APT	0.85	41,175	General Systemic Bacterial Infection	10	2.50 - 3.75	52.0
Washoe Park Trout SFH	1	CUT	15.00	1,500	CWD	10	2.50 - 3.75	45.0
American Falls SFH	1	RBT	3.90	32,800	CWD	10	2.50 - 3.75	55.0
Hagerman SFH	1	RBT	3.25	65,000	Columnaris	10	2.50 - 3.75	59.0
Murray Springs Trout SFH	1	CUT	0.90	80,000	CWD	14	10	52.0
Hagerman SFH	1	RBT	2.28	108,315	CWD	14	10	59.0
Magic Valley Steelhead SFH	1	STT	1.18	100,000	CWD	14	10	59.0

Table 3. Summary of CY 2004 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	3	APT	0.9 - 1.8	272,620	General Systemic Bacterial Infection	10	2.50 - 3.75	52.0
Hackettstown SFH	1	CCF	6.30	7,400	Columnaris	10	2.50 - 3.75	72.0
Mora NFH & TC	1	GIT	6.00	1,829	General Systemic Bacterial Infection	13	2.50 - 3.75	56.0
Alchesay-Williams Creek NFH Complex	1	RBT	4.10	132,600	General Systemic Bacterial Infection	10	2.50 - 3.75	52.0
Hagerman SFH	2	RBT	3.8 - 8.3	368,500	CWD	10	2.50 - 3.75	59.0
	3	RBT	5.3 - 9.5	627,566	Columnaris	10	2.50 - 3.75	59.0
Nampa SFH	1	RBT	3.64	41,530	CWD	10	2.50 - 3.75	59.0
Coleman NFH	2	STT	2.3 - 2.4	220,000	Columnaris	10	2.50 - 3.75	61.0
Kent SeaTech Corp.	2	SXW	1.25	357,900	Gram Negative Bacterial Enteritis	10	3.8 - 4.4	77.0
Crystal Lake Hatchery	1	CKS	1.90	1,294,500	CWD	14	10	48.9
Dexter Ponds SFH	1	CKS	5.70	755,176	Columnaris	14	10	58.2
Giant Springs Trout SFH	1	RBT	3.60	219,000	CWD	14	10	54.0
Grace SFH	2	RBT	5.6 - 6.2	71,025	CWD	14	10	52.0
Hagerman SFH	1	RBT	1.39	235,673	CWD	14	10	59.0

Table 4. Summary Data Regarding Summary of CY 2004 OTF Treatment Trials

Total Fish Treated:	**19,971,978**
Number of fish treated in efficacious trials	14,937,869
Number of fish treated in ineffective trials	428,790
Number of fish treated in inconclusive trials	4,605,319
Total number of trials:	**123**
Efficacious trials	92 (75%)
Ineffective trials	9 (7%)
Inconclusive trials	22 (18%)

Treatment Regimes Used:

1.12 - 2.21 g/100 lbs fish/day for 14 - 16 days (above 48.2°F)	5 trials
2.50 - 3.75 g/100 lbs fish/day for 10 days (below 48.2°F)	2 trials
2.50 - 3.75 g/100 lbs fish/day for 10 - 16 days (above 48.2°F)	76 trials
3.8 - 4.4 g/100 lbs fish/day for 10 - 14 days (above 48.2°F)	8 trials
10.0 g/100 lbs fish/day for 14 - 15 days (above 48.2°F)	31 trials
11.4 g/100 lbs fish/day for 14 days (above 48.2°F)	1 trial

Treatment Water Temperature (°F):

Temperature Range	44.2 - 82.4
Mean Temperature	60.5

Size of Treated Fish (in.):

Size Range	0.85 - 15.0

Species Treated:

Salmonids

Apache trout *apache Oncorhynchus*
chinook salmon *O. tshawytscha*
coho salmon *O. kisutch*
cutthroat trout *O. clarki*
Gila trout *O. gilae*
rainbow trout *O. mykiss*
steelhead trout *O. mykiss*
brook trout *Salvelinus fontinalis*
brown trout *Salmo trutta*

Non-salmonids
blue catfish *Ictalurus furcatus*
channel catfish *I. punctatus*
hybrid striped bass *Morone chrysops* x *M. saxatilis*
striped bass *M. saxatilis*
tiger musky (*Esox lucius x E. masquinongy*)
Tilapia *Oreochromis mossambica*
white sturgeon *Acipenser transmontanus*