

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

## **Year 2010 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 2010 (CY10) the efficacy of oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF) was evaluated in 27 trials involving approximately 5.6 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 11 fish culture facilities, including one U.S. Fish and Wildlife Service fish hatchery, nine 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 CY10 indicated that treatments appeared to be efficacious in approximately 93% of the trials and were ineffective in 7% 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 CY10 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 27 trials were conducted at 11 fish culture facilities, including one U.S. Fish and Wildlife Service fish hatchery, nine state fish hatcheries, and one private fish culture hatchery. Water temperature during treatments at the various testing facilities ranged from 52.0 - 85.0 °F, with a mean treatment temperature of 64.4°F.

#### **2. Test article used**

The OTF used in CY10 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 41% of trials were conducted using this treatment regimen); or 10.0 g of active drug/100 lbs of fish/d for 10 days (approximately 52% of trials were conducted using this treatment regimen).

**Study Protocol Deviation:** Treatment regimen administered in the remaining trials (approximately 7% of trials) deviated from the protocol. In these trials, fish were fed at rates of 7.4 - 7.7 g drug/100 lbs fish/d for 14 days. Please note that these deviations occurred because the fish were unable to eat enough of the medicated feed to achieve the full OTC dose of 10 g drug/100 lbs fish/d.

## **Fish Species and Fish Diseases Involved in CY10 Trials**

### **1. Species of fish treated**

Eleven fish species, including six salmonids, four non-salmonids, and one marine non-salmonid, were treated during CY10. Treated fish ranged in length from 1.0 - 13.8 in. and the average length of all treated fish was 4.6 in. Fish species treated included:

#### **Salmonids:**

coho salmon *Oncorhynchus kisutch*

cutthroat trout *O. clarki*

Gila trout *O. gilae*

kokanee salmon *O. nerka*

rainbow trout *O. mykiss*

spring chinook salmon *O. tshawytscha*

#### **Non-salmonids:**

American shad *Alosa sapidissima*

channel catfish *Ictalurus punctatus*

muskellunge *Esox masquinongy*

walleye *Sander vitreus*

## **Marine non-salmonid:**

Kona kampachi *Seriola rivoliana*

### **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 CY10: *Aeromonas Hydrophila*, coldwater disease (causative agent *Flavobacterium psychrophilus*), columnaris (causative agent *F. columnare*), motile aeromonad septicemia (MAS), Vibriosis, or *Vibrio parahaemolyticus*.

## **Data Collected**

### **1. Pathologist's reports**

A pathologist's report was submitted for 56% 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 CY10 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 CY10 under INAD #9332).**

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

Cutthroat trout, Gila trout, kokanee salmon, rainbow trout, American shad, channel catfish, and walleye were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 days in 11 trials (Table 1). Investigators used OTF to either apply a skeletal mark or to control mortality caused by *Aeromonas Hydrophila* or *columnaris*. OTF treatments appeared effective in all of these trials.

## **B. Efficacy at 7.4 - 10.2 g/100 lbs fish/d for 11 - 14 days**

Coho salmon, cutthroat trout, spring chinook salmon, muskellunge, walleye, and Kona Kampachi were treated with 7.4 - 10.2 g OTF/100 lbs of fish/d for 11 - 14 days in 16 trials (Tables 1 - 2). Investigators used OTF to control mortality caused by coldwater disease, columnaris, motile aeromonad septicemia, *Vibrio parahaemolyticus*, or vibriosis. OTF treatments appeared effective in 14 trials and ineffective in two trials.

## **2. Observed Toxicity**

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY10.

## **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 CY10. Facilities not listed in Appendix III-a of the current Oxytetracycline (Terramycin® 200 for Fish) INAD #9332 during CY10 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 CY10 but never used the drug:

1. Coleman NFH
2. Sterlin Caviar
3. 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 CY10.

### **Number of Treated Fish under Treatment Use Authorization**

Total number of fish treated during CY10 was 5,574,661. The total number of treated fish to count against the Oxytetracycline (Terramycin® 200 for Fish) treatment use authorization dated June 25, 2007 is 27,431,077.

### **Summary of Study Results**

Oxytetracycline (Terramycin® 200 for Fish) medicated feed was used at dosages ranging from 3.7 - 10.2 g active drug/100 lbs fish/d in 27 treatment trials. Treatment durations ranged from 10 - 14 days. Treatment trials involved 11 different fish species and approximately 5.6 million fish. Treated fish ranged in length from 1.0 - 13.8 in. Water temperature during treatment ranged from 52.0 - 85.0 °F, with a mean treatment temperature of 64.4 °F. Overall results showed that treatment in approximately 93% of trials appeared to be efficacious while treatment in 7% 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 2010 OTF Treatment Results - Efficacious Trials**

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease/Purpose	Dose (g/100 lbs)	Number of Treatment Days	Temp. (°F)
Manning SFH	1	AMS	2.00	100,000	Mark	3.75	10	85.0
Rathbun SFH & Research Facility	2	CCF	5.6 - 7.1	61,825	Columnaris	3.75	10	79.1 - 82.8
Clackamas SFH	1	COS	3.00	400,000	Columnaris	10	14	64.0
Murray Springs SFH	3	CUT	1.3 - 3.4	131,224	CWD	7.4 - 10.2	14	52.0
Yellowstone River SFH	1	CUT	3.40	30,000	Mark	3.75	10	52.0
Mora NFH & TC	1	GIT	2.50	12,080	<i>Aeromonas Hydrophila</i>	3.75	10	58.3
Big Springs SFH	1	KOE	4.08	37,700	Mark	3.74	10	57.0
Keahole Point - Offshore Mariculture Site	1	KON	7.80	100,000	Vibriosis	10	11	79.0
Spirit Lake SFH	1	MUE	4.00	33,588	MAS	10	14	77.4
Big Springs SFH	4	RBT	3.2 - 6.6	326,000	Mark	3.7 - 3.75	10	52.0 - 59.0
Dexter Ponds SFH	7	SCS	5.0 - 6.0	3,921,640	Columnaris	10	14	61.1 - 61.5
Rathbun SFH	1	WAE	6.00	47,140	Columnaris	3.75	10	81.8
Spirit Lake SFH	1	WAE	2.76	23,464	MAS	10	14	77.4

**Table 2. Summary of CY 2010 OTF Treatment Results - Ineffective Trials**

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (g/100 lbs)	Number of Treatment Days	Temp. (°F)
Murray Springs SFH	1	CUT	1.00	200,000	CWD	10.2	14	52.0
Keahole Point - Offshore Mariculture Site	1	KON	13.80	150,000	Vibrio parahaemolyticus	10	14	79.0

**Table 3. Summary Data Regarding Summary of CY 2010 OTF Treatment Trials**

<b>Total Fish Treated:</b>		<b><u>5,574,661</u></b>
Number of fish treated in efficacious trials		5,224,661
Number of fish treated in ineffective trials		350,000
<b>Total number of trials:</b>		<b>27</b>
Efficacious trials		25 (93%)
Ineffective trials		2 (7%)
<b>Treatment Regimens Used:</b>		
3.70 - 3.75 g/100 lbs fish/day for 10 days		11 trials
7.4 - 10.2 g/100 lbs fish/day for 11 - 14 days		16 trials
<b>Treatment Water Temperature (°F):</b>		
Temperature Range	52.0 - 85.0	
Average Temperature	64.4	
<b>Size of Treated Fish (in.):</b>		
Size Range	1.0 - 13.8	
Average Length	4.6	
<b>Species Treated:</b>		
<b><u>Salmonids:</u></b>		
coho salmon <i>Oncorhynchus kisutch</i>		
cutthroat trout <i>O. clarki</i>		
Gila trout <i>O. gilae</i>		
kokanee salmon <i>O. nerka</i>		
rainbow trout <i>O. mykiss</i>		
spring chinook salmon <i>O. tshawytscha</i>		
<b><u>Non-salmonids:</u></b>		
American shad <i>Alosa sapidissima</i>		
channel catfish <i>Ictalurus punctatus</i>		
muskellunge <i>Esox masquinongy</i>		
walleye <i>Sander vitreus</i>		
<b><u>Marine non-salmonid:</u></b>		
Kona kampachi <i>Seriola rivoliana</i>		