

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

## **Year 2011 Annual Summary Report on the Use of Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed in Field Efficacy Trials**

Prepared by:

Bonnie Johnson, Biologist  
U.S. Fish and Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Bozeman, Montana

### **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 2011 (CY11) the efficacy of oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF) was evaluated in 21 trials involving approximately 1.4 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 CY11 indicated that treatments appeared to be efficacious

in approximately 81% of the trials, ineffective in 14% of the trials, and characterized as inconclusive 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 CY11 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 the approved label for OTF.

## **Facilities, Materials, and Treatment Procedures**

### **1. Facilities**

A total of 21 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 - 83.8 °F, with a mean treatment temperature of 63.0°F.

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

The OTF used in CY11 efficacy trials was Terramycin<sup>®</sup> 200 which contained 200 g active oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article. All Terramycin<sup>®</sup> 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., Skretting,

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 43% of trials were conducted using this treatment regimen); or 10.0 g of active drug/100 lbs of fish/d for 14 days (approximately 43% of trials were conducted using this treatment regimen).

**Study Protocol Deviation:** Treatment regimen administered in the remaining trials (approximately 14% of trials) deviated from the protocol. The following are the explanations for the deviations in each of the trials. 1) In one trial, fish were fed at 3.84 g drug/100 lbs fish/d for 14 days. This deviation 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. 2) In one trial, fish were fed at 8 g drug/100 lbs fish/d for 10 days. This deviation occurred because the facility received 2% TM pre-mix instead of the 1% TM they thought they had ordered and the overdose was not discovered until the treatment had been completed. 3) In one trial, fish were fed at 10 g drug/100 lbs fish/d for 16 days. This deviation occurred because fish were fed the complete dose over a 16 day period due to them not

eating as much as needed for a couple of days. The investigator was contacted and told to only treat for 14 days for future studies.

## **Fish Species and Fish Diseases Involved in CY11 Trials**

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

Eight fish species, including three salmonids, four non-salmonids, and one marine non-salmonid, were treated during CY11. Treated fish ranged in length from 1.3 - 15.0 in. and the average length of all treated fish was 5.0 in. Fish species treated included:

#### **Salmonids:**

cutthroat trout *Oncorhynchus clarki*

Gila trout *O. gilae*

rainbow trout *O. mykiss*

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

American shad *Alosa sapidissima*

largemouth bass *Micropterus salmoides*

muskellunge *Esox masquinongy*

walleye *Sander vitreus*

#### **Marine non-salmonid:**

Hawaiian 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 CY11: coldwater disease (causative agent *Flavobacterium psychrophilus*), columnaris (causative agent *F. columnare*), motile aeromonad septicemia (MAS), or vibriosis.

## **Data Collected**

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

A pathologist's report was submitted for 33% 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 CY11 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 a summary of all trials characterized as inconclusive; Table 4 provides summary data for all trials; Table 5 provide a summary of all trials conducted during CY11 under INAD #9332).**

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

Cutthroat trout, Gila trout, rainbow trout, and American shad were treated with 2.5 - 3.75 g OTF/100 lbs of fish/d for 10 days in nine trials (Tables 1 & 2).

Investigators used OTF to either apply a skeletal mark or to control mortality caused by columnaris. OTF treatments appeared effective in seven trials while 2 trials were ineffective.

## **B. Efficacy at 3.84 - 10 g/100 lbs fish/d for 10 - 16 days**

Cutthroat trout, Hawaiian Kampachi, largemouth bass, muskellunge, rainbow trout, and walleye were treated with 3.84 - 10 g OTF/100 lbs of fish/d for 10 - 16 days in 12 trials (Tables 1 - 3). Investigators used OTF to control mortality caused by coldwater disease, columnaris, motile aeromonad septicemia, or vibriosis. OTF treatments appeared effective in 10 trials, ineffective in one trial, and was characterized as inconclusive in one trial.

## **2. Observed Toxicity**

No toxicity or adverse effects relating to OTF treatment were reported in any of the trials conducted in CY11.

## **3. Observed Withdrawal Period**

All withdrawal times were either met or exceeded.

## **Current Study Protocol for Oxytetracycline (Terramycin® 200 for Fish) INAD #9332**

No changes have occurred to the current study protocol for Oxytetracycline (Terramycin® 200 for Fish) INAD #9332.

## **Facility Sign-up List**

Please see “Table 6. Facilities and Names of Investigators” for facilities that signed-up to participate in the Oxytetracycline (Terramycin® 200 for Fish) INAD

#9332 during CY11. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority.

The following facility had Oxytetracycline (Terramycin® 200 for Fish) medicated feed or premix on-hand during CY11 but never used the drug:

1. Willow Beach

### **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 CY11.

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

Total number of fish treated during CY11 was 1,407,126. The total number of treated fish to count against the Oxytetracycline (Terramycin® 200 for Fish) treatment use authorization dated June 25, 2007 is 28,838,203.

### **Summary of Study Results**

Oxytetracycline (Terramycin® 200 for Fish) medicated feed was used at dosages ranging from 3.7 - 10.0 g active drug/100 lbs fish/d in 21 treatment trials. Treatment durations ranged from 10 - 16 days. Treatment trials involved eight different fish species and approximately 1.4 million fish. Treated fish ranged in length from 1.3 - 15.0

in. Water temperature during treatment ranged from 52.0 - 83.8 °F, with a mean treatment temperature of 63.0 °F. Overall results showed that treatment in approximately 81% of trials appeared to be efficacious, while 14% appeared ineffective, and 5% 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/marking 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 2011 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)
Yellowstone River Trout SFH	1	CUT	3.40	30,500	Marking	3.75	10	52.0
Mora NFH &TC	1	GIT	2.50	13,925	Columnaris	3.74	10	58.8
Big Springs Trout SFH	2	RBT	5.3 - 6.0	291,000	Marking	3.71 - 3.73	10	52.0
Bluewater Springs Trout	3	RBT	2.8 - 4.3	250,000	Marking	3.75	10	59.0
Florida Bass Conservation Center	1	LMB	4.50	11,314	Columnaris	8.0	10	77.4
Keahole Point Fish LLC	3	KON	4.8 - 13.5	325,000	Vibriosis	10	14 - 16	66.0 - 79.0
Spirit Lake SFH	1	MUE	3.94	8,825	MAS	10	14	74.9
Boulder Rearing Station	4	RBT	3.2 - 15.0	143,968	CWD	10	14	52.0
Giant Springs Trout SFH	1	RBT	1.30	128,000	CWD	10	14	54.0

**Table 2. Summary of CY 2011 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)
Matapeake	2	AMS	2.10	100,000	Marking	3.75	10	81.0 - 83.8
Murray Springs Trout SFH	1	CUT	1.50	76,100	CWD	3.84	14	52.0

**Table 3. Summary of CY 2011 OTF Treatment Results - Inconclusive Trials**

Hatchery	Number of Trials	Fish Species	Fish Size (in)	Number of Fish	Disease	Dose (g/100 lbs)	Number of Treatment Days	Temp. (°F)
Spirit Lake SFH	1	WAE	4.40	28,494	MAS	10	14	76.3

**Table 4. Summary Data Regarding Summary of CY 2011 OTF Treatment Trials**

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<b>Total Fish Treated:</b>	<u>1,407,126</u>
Number of fish treated in efficacious trials	1,202,532
Number of fish treated in ineffective trials	176,100
Number of fish treated in inconclusive trials	28,494

<b>Total number of trials:</b>	<b>21</b>
Efficacious trials	17 (81%)
Ineffective trials	3 (14%)
Inconclusive trials	1 (5%)

<b>Treatment Regimens Used:</b>	
3.70 - 3.75 g/100 lbs fish/day for 10 days	9 trials
3.84 - 8.0 g/100 lbs fish/day for 10 - 14 days	2 trials
10.0 g/100 lbs fish/day for 14 - 16 days	10 trials

<b>Treatment Water Temperature (°F):</b>	
Temperature Range	52.0 - 83.8
Average Temperature	63.0

<b>Size of Treated Fish (in.):</b>	
Size Range	1.3 - 15.0
Average Length	5.0

**Species Treated:**

**Salmonids:**

cutthroat trout *Oncorhynchus clarki*  
 Gila trout *O. gilae*  
 rainbow trout *O. mykiss*

**Non-salmonids:**

American shad *Alosa sapidissima*  
 largemouth bass *Micropterus salmoides*  
 muskellunge *Esox masquinongy*  
 walleye *Sander vitreus*

**Marine non-salmonid:**

Hawaiian kampachi *Seriola rivoliana*