

**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 as a Marking Agent in Field Efficacy Trials**

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**Summary**

Oxytetracycline (Terramycin® 200 for Fish) medicated feed (OTF-M) has been shown to be an effective and convenient agent for marking skeletal tissue of early life stages of fish. Large numbers of young fish can be marked simultaneously by feeding fish a standard dosage of OTF-M for a period ranging up to 14 d. In many cases, OTF-M is the only viable option, other than immersion marking with water-soluble oxytetracycline, for permanently marking large numbers of small fish for the purpose of evaluating fishery management strategies. Oxytetracycline medicated feed has been approved for use in aquaculture for limited therapeutic uses and for marking skeletal tissue in Pacific salmon in the United States by the U.S. Food and Drug Administration (FDA). For uses other than those for which OTF-M is approved, the FDA has authorized its use under Compassionate Investigational New Animal Drug (INAD) Exemption #9332 for the purpose of collecting pivotal and ancillary clinical field data on OTF-M for the marking of skeletal tissue in a variety of fish species. Eleven such trials

were conducted at one U.S. Fish and Wildlife Service fish hatchery and five state fish hatcheries involving five fish species and approximately 0.90 million fish under INAD #9332 during calendar year 2007 (CY07). Efficacy was determined by whether or not a “readable” mark could be observed on skeletal tissue of treated fish. Standard treatment regimens allowed under the INAD included the use of OTF-M at 2.5 - 3.75 g/100 lbs fish/d for 10 d; and 10.0 g/100 lbs fish/d for 14 d. Overall results of trials conducted in CY07 showed that all of the trials appeared efficacious.

### **Introduction**

The current U. S. Food and Drug Administration (FDA) approved label for OTF-M in aquaculture limits its use to marking of skeletal tissue in Pacific salmonids using the following treatment regimen: administer at dosages of 250 mg/kg of fish/d (i.e., 11.4 g OTF-M/100 lbs fish/d) for 4 d in salmon less than 30 g followed by a 7 d withdrawal period. These label restrictions severely limit the overall utility of approved OTF-M use in aquaculture.

Historically, fish culturists have 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.

## **Purpose of Report**

The primary purpose of this report is to summarize the results of CY07 supplemental OTF-M field efficacy studies. Furthermore, it is expected that data from these studies will be used to enhance the existing OTF-M database that has been established from previous years studies for the purpose of expanding and/or extending the approved label for OTF-M.

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

### **1. Participating Facilities**

OTF-M was used under INAD #9332 during CY07 to mark skeletal tissue of test fish in 11 trials conducted at one U.S. Fish and Wildlife Service fish hatchery and five state fish hatcheries. Water temperature during treatments at these testing facilities ranged from 52.0 - 73.8 °F, with a mean treatment temperature of 56.6 °F.

## 2. OTF-M used in efficacy trials

The OTF-M used was either 1) Terramycin<sup>®</sup> 100 which contained 100 g active oxytetracycline quaternary salt per pound of premix; or 2) Terramycin<sup>®</sup> 200 which contained 200 g active oxytetracycline (from oxytetracycline dihydrate) per pound of Type A Medicated Article. All Terramycin<sup>®</sup> 100/200 was supplied by Phibro Animal Health, 75 Challenger Road Ridgefield Park, NJ. All OTF-M was prepared with Phibro brand product by one of several commercial fish feed manufacturers (e.g., Nelson and Sons, Inc., Rangen Inc.).

## 3. Drug dosages and duration

As described in the Study Protocol for INAD #9332, Investigators were allowed to use OTF-M either within the standard treatment regimen of 2.5 - 3.75 g of active drug per 100 lbs of fish/d for 10 d (5 of 11 studies used this treatment regimen), or at 10.0 g of active drug per 100 lbs of fish/d for 14 d (3 of 11 studies used this treatment regimen).

**Study Protocol Deviation:** Treatment regimen administered in the remaining three trials deviated from the protocol. In two trials, fish were administered 10.0 g of active drug per 100 lbs of fish/d for 13 d. The Investigator noted that only 13 days of feed were available for these treatments. In one trial, fish were administered 6.3 g of active drug per 100 lbs of fish/d for 10 d; instead of 2.5 - 3.75 g of active drug per 100 lbs of fish/d for 10 d. The Investigator noted that the

actual amount of food fed exceeded the planned amount causing a higher dosage of OTF-M to be administered.

## **Fish Species Involved in CY07 Efficacy Trials**

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

Five fish species, including four species of salmonids and one non-salmonid species were treated with OTF-M during CY07. Treated fish ranged in length from 2.38 - 7.1 in (mean length was 4.3 in). A total of approximately 0.90 M test fish were treated during this period. Species treated included:

#### **Salmonids:**

rainbow trout *Oncorhynchus mykiss*

kokanee salmon *O. nerka*

cutthroat trout *O. clarki*

brook trout *Salvelinus fontinalis*

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

largemouth bass *Micropterus salmoides*

### **2. Marking**

Fish were treated with OTF-M to provide a readable mark on skeletal tissue.

## **Data Collected**

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

One pathology report was submitted during CY07 studies. Pathology reports often provide documentation that there were no infections or infestations caused by disease agents in the population of test fish. Pathology reports provide critical information if such submissions are to be used in support of an initial approval, or to expand/extend an existing approved label.

### **2. Efficacy of marking procedure**

Samples of treated fish were collected, processed, and evaluated for a mark on skeletal tissue using standard procedures.

## **Discussion of Study Results:**

- 1. General observations on the efficacy of OTF-M for marking of skeletal tissue of salmonid and non-salmonid fish** - Efficacy was determined by whether or not a “readable” mark could be observed on skeletal tissue of treated fish. (Note: Table 1 provides a summary of all efficacious trials; Table 2 provides summary data for all trials; and Table 3 describes all trials conducted during CY07 under INAD #9332).

**A. Efficacy at 2.5 - 3.75 g/100 lbs fish/d for 10 d**

OTF-M was used at 2.5 - 3.75 g/100 lbs of fish/d for 10 d in five trials involving rainbow trout, kokanee salmon, and largemouth bass (Table 1). Results from these trials indicated that all treatments were efficacious.

**B. Efficacy at 6.3 g/100 lbs fish/d for 10 d**

OTF-M was used at 6.3 g/100 lbs of fish/d for 10 d in one trial involving cutthroat trout (Table 1). Results from this trial indicated that treatment was efficacious.

**C. Efficacy at 10 g/100lbs fish/d for 13 - 14 d**

OTF-M was used at 10 g/100lbs fish/d for 13 - 14 d in five trials involving brook trout and rainbow trout (Table 1). Results from these trials indicated that all treatments were efficacious.

**2. Observed Toxicity**

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

### **Number of Treated Fish under Slaughter Authorization**

Please see the 2007 Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed (OTF) Annual Report for the number of treated fish used under the slaughter authorization.

### **Facility Sign-up List**

Please see the 2007 Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed (OTF) Annual Report for facilities that signed-up to participate in the Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed (OTF) INAD #9332 during CY07.

### **Summary of Study Results**

Oxytetracycline (Terramycin® 200 for Fish) medicated feed was used to mark skeletal tissue of test fish at dosages ranging from 2.50 to 10.0 g/100lbs fish/d for durations ranging from 10 to 14 d. Treatment trials involved five different fish species and trials involved approximately 0.90 million fish. Treated fish ranged in size from 2.38 - 7.10 in. Overall mean water temperature during treatments was 56.6 °F (range, 52.0 - 73.8 °F). One trial included a pathologist's report documenting the health of test fish prior to or after treatment. Overall results of trials conducted in CY07 showed that 100% of the trials appeared effective in creating a readable mark on the skeletal tissue of the treated fish. Furthermore, Investigators reported no evidence of toxicity or

adverse effects related to OTF-M treatment. However, based on a general lack of untreated control fish, replication, randomization, etc., it is understood that these data will only be considered as ancillary data. None-the-less, the ancillary data described in this report should provide useful corroborative data to support a future expanded label claim for OTF-M. It is anticipated that additional ancillary skeletal tissue marking efficacy data will continue to be collected under INAD #9332. In future trials conducted under INAD #9332 for the purpose of marking fish, efforts will continue to be directed towards the generation of high quality data.

**Table 1. Summary of CY07 Oxytetracycline (Terramycin® 200 for Fish) Medicated Feed Efficacy Results - Efficacious Studies**

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Fish Number	Use of Feed	Dose (g/100 lbs)	Treatment Duration (days)	Temp. (°F)
Big Springs Trout SFH	1	KOE	3.94	40,000	Marking	2.50 - 3.75	10	52.0
Inks Dam NFH	1	LMB	7.00	9,927	Marking	2.50 - 3.75	10	73.8
Big Springs Trout SFH	2	RBT	6.6 - 7.1	462,980	Marking	2.50 - 3.75	10	52.0
Washoe Park Trout SFH	1	RBT	2.38	103,000	Marking	2.50 - 3.75	10	55.0
Yellowstone River Trout SFH	1	CUT	2.40	36,000	Marking	6.3	10	52.0
Giant Springs Trout SFH	1	BKT	2.80	61,273	Marking	10	14	54.0
Bluewater Springs Trout SFH	4	RBT	3.1 - 4.5	191,000	Marking	10	13 - 14	58.0

**Table 2. Summary Data Regarding CY07 OTF-M Efficacy Studies**

<b>Total Number of Fish Treated:</b>	<b><u>904,180</u></b>
Number of fish in efficacious studies	904,180
<b>Total number of studies:</b>	<b>11</b>
Number of efficacious studies	11
<b>Treatment Regimens Used:</b>	
2.5 - 3.75 g/100 lbs fish/day for 10 days	5 trials
6.3 g/100 lbs fish/day for 10 days	1 trial
10.0 g/100 lbs fish/day for 13 -14 days	5 trials
<b>Treatment Water Temperature (°F):</b>	
Temperature Range	52.0 - 73.8
Mean Temperature	56.6
<b>Size of Treated Fish (in.):</b>	
Size Range	2.38 - 7.1
<b>Species Treated:</b>	
<u><b>Salmonids:</b></u>	
rainbow trout <i>Oncorhynchus mykiss</i>	
kokanee salmon <i>O. nerka</i>	
cutthroat trout <i>O. clarki</i>	
brook trout <i>Salvelinus fontinalis</i>	
<u><b>Non-salmonids:</b></u>	
largemouth bass <i>Micropterus salmoides</i>	