

Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2004 Annual Summary Report on the Use of Oxytetracycline Medicated Feed as a Marking Agent in Field Efficacy Trials

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Summary

Oxytetracycline 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 skeletal tissue in a variety of salmonid species. Sixteen such trials were conducted at

three state fish hatcheries and involved two species and approximately 1.0 million fish under INAD #9332 during calendar year 2004 (CY04). 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 4 - 10 d; and 10.0 g/100 lbs fish/d for 9 - 14 d. In one trial fish were treated with 3.84 g/100 lbs fish/d for 10 d. Overall results of trials conducted in CY04 showed that approximately 88% of the trials appeared efficacious, while 12% of the trials were characterized as inconclusive.

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 gm followed by a 7 d withdrawal period. These label restrictions severely limit the overall utility of approved OTF-M use in aquaculture.

Fish culturists have reported that OTF-M treatment is a useful tool for marking the skeletal tissue in rainbow and cutthroat trout, and kokanee salmon when treated at a size in which fish body weight does not exceed 2 gms. Marks were visible on skeletal tissue of fish immediately after the treatment period, and they were still visible for several months afterwards. In addition, studies have been conducted in which different

OTF-M drug dosages were used to mark skeletal tissue of test fish. Summary conclusions from such studies indicated that not only did various dosages of OTF-M 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 CY04 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

Three state fish hatcheries used OTF-M to mark skeletal tissue of test fish under INAD 9332 during CY04. Water temperature during treatments at these testing facilities ranged from 51.0 - 58.0 °F, with a mean treatment temperature of 52.8 °F.

2. OTF-M used in efficacy trials

The OTF-M used in CY04 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. All OTF-M was prepared with Pfizer 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 current label range of 2.5 - 3.75 gm of active drug per 100 lbs of fish/d for 4 - 10 d (12 of 16 studies), or at 10.0 gm of active drug per 100 lbs of fish/d for 9 - 14 d (3 of 16 studies). Investigators deviated from the protocol in the remaining trail when fish were fed at 3.84 gm of active drug per 100 lbs of fish/d for 10 d.

Fish Species Involved in CY04 Efficacy Trials

1. Species of fish treated

Two salmonid fish species were treated during CY04. Treated fish ranged in length from 2.75 - 7.3 in. Species treated included:

- (1) rainbow trout *Oncorhynchus mykiss*
- (2) kokanee salmon *O. nerka*

2. Marking

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

Data Collected

1. Pathologist's reports

No pathology reports were submitted during CY04 studies.

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 salmonids** - 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 a summary of all inconclusive trials; Table 3 provides summary data for all trials; and Table 4 describes all trials conducted during CY04 under INAD #9332).

A. Efficacy at 2.5 - 3.75 g/100 lbs fish/d for 4 - 10 d at water temperatures above 48.2° F

OTF-M was used at 2.5 - 3.75 g/100 lbs of fish/d for 4 - 11 d in 12 trials involving rainbow trout and kokanee salmon (Tables 1 & 2). Results from two trials involving rainbow trout were characterized as inconclusive due to the termination of the trials after treatment day 4. The Investigator noted that all of these study fish were destroyed without verifying the presence of a mark. In the other ten trials, results from OTF-M treatments appeared efficacious in 10 trials.

B. Efficacy at 3.84 g/100 lbs fish/d for 10 d at water temperatures above 48.2° F

OTF-M was used at 3.84 g/100 lbs of fish/d for 10 d in one trial involving rainbow trout (Table 1) and results from this trials indicated that the treatment appeared efficacious.

C. Efficacy at 10g/100lbs fish/d for 9 - 14 d at water temperatures above 48.2°F

OTF-M was used at 10 g/100lbs fish/d for 9 - 14 d in three trials involving rainbow trout (Table 1) and results from these trials indicated that all treatments appeared efficacious.

2. Observed Toxicity

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

Summary of Study Results

Oxytetracycline 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 4 to 14 d. Two different species of salmonids were treated with OTF-M, and trials involved approximately 1.0 million fish. Treated fish ranged in size from 2.75 - 7.3 in. Overall mean water temperature during treatments was 52.8 °F (range, 51.0 - 58.0 °F). Overall results of trials conducted in CY04 showed that approximately 88% of the trials appeared efficacious, while 12% of the trials were characterized as inconclusive (i.e., were not checked for a mark). None of the trials included a pathologist's report documenting health of test fish prior to or after treatment. Overall, OTF-M appeared

effective in creating a readable mark on the skeletal tissues of the treated fish in nearly all trials. 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 be directed towards the generation of higher quality data.

Table 1. Summary of CY04 Oxytetracycline 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	9	RBT	2.8 - 7.3	657,740	Marking	2.50 - 3.75	4 - 10	51.0-55.0
	1	KOE	3.80	27,800	Marking	2.50 - 3.75	10	55.0
Big Springs Trout SFH	1	RBT	2.75	100,000	Marking	3.84	10	55.0
Giant Springs Trout SFH	1	RBT	2.80	100,000	Marking	10.0	14	54.0
Bluewater Springs Trout SFH	2	RBT	3.2 - 4.8	100,800	Marking	10.0	9 - 14	58.0

Table 2. Summary of CY04 Oxytetracycline Medicated Feed Efficacy Results - Inconclusive 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	2	RBT	5.6 - 7.0	40,000	Marking	2.50 - 3.75	4	51.0

Table 3. Summary Data Regarding CY04 OTF-M Efficacy Studies

Total Number of Fish Treated:	<u>1,026,340</u>	
Number of fish in efficacious studies	986,340	
Number of fish in inconclusive studies	40,000	
Total number of studies:	16	
Number of efficacious studies	14	
Number of inconclusive studies	2	
Treatment Regimens Used:		
2.5 - 3.75 g/100 lbs fish/day for 4 - 11 days (above 48.2°F)	12 trials	
3.84 g/100 lbs fish/day for 10 days (above 48.2°F)	1 trial	
10.0 g/100 lbs fish/day for 9 -14 days (above 48.2°F)	3 trials	
Treatment Water Temperature (°F):		
Temperature Range	51.0 - 58.0	
Mean Temperature	52.8	
Size of Treated Fish (in.):		
Size Range	2.75 - 7.30	
Species Treated:		
rainbow trout <i>Oncorhynchus mykiss</i>		
kokanee salmon <i>O. nerka</i>		