Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2003 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 marking agent for use on early life stages of fish. Large numbers of young fish can be marked simultaneously by feeding fish a standard dosage of OTF-M for up to 10 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 that those for which OTF-M is approved, the FDA has authorized its use under Compassionate Investigational New Animal Drug (INAD) Exemption #9332 to collect pivotal and ancillary clinical field data on OTF-M for the marking skeletal tissue in a variety of salmonid species. Twenty such trials were conducted in calendar year 2003 under INAD #9332 that were conducted at

five state fish hatcheries and involved approximately 4.0 million fish during this period. Efficacy was determined by whether or not a “readable” mark could be observed on skeletal tissue of treated fish. Standard treatment regimens included the use of OTF-M at 2.5 - 3.75 g/100 lbs fish/d for 8 - 11 d; and 10.0 g/100 lbs fish/d for 10 - 14 d. In a few select trials fish were treated with 3.76 - 4.04 g/100 lbs fish/d for 10 d. Overall results showed that all 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 then 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 trout, cutthroat trout, and kokanee salmon when treated at a body weight of more than 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 the there were also no evidence of any toxic or adverse effects to the fish.

Purpose of Report

The primary purpose of this report was to summarize the results of calendar year 2003 (CY03) 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

Five state fish hatcheries used OTF-M to mark skeletal tissue of test fish under INAD 9332 during CY03. Water temperature during treatments at the various testing facilities ranged from 45.0 - 58.0 °F, with a mean treatment temperature of 51.6 °F.

2. OTF-M used in efficacy trials

The OTF-M used in CY03 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.

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 8 - 11 d (~60% of studies), or at 10.0 gm of active drug per 100 lbs of fish/d for 10 - 14 d (~20% of studies). Investigators deviated from the protocol in few trials (~20%) when fish were fed at rates ranging from 3.76 to 4.04 gm of active drug per drug/100 lbs fish/d for 10 d.

Fish Species Involved in CY 03 Efficacy Trials

1. Species of fish treated

Three salmonid fish species were treated during CY03. Treated fish ranged in length from 1.11 - 8.1 in. Species treated included:

(1) rainbow trout *Oncorhynchus mykiss*;

(2) cutthroat trout *O. clarki*;

(3) 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 CY03 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: A summary of all OTF-M studies conducted during CY03 under INAD #9332 are described in Table 3.)

A. Efficacy at 2.5 - 3.75 g/100 lbs fish/d for 8 - 11 d at water temperatures above 48.2^{o} F

OTF-M was used at 2.5 - 3.75 g/100 lbs of fish/d for 8 - 11 d in 12 trials involving rainbow trout and kokanee salmon (Table 1) and results indicated that the treatments all appeared efficacious.

B. Efficacy at 3.76 - 4.04 g/100 lbs fish/d for 10 d at water temperatures below 48.2^{o} F

OTF-M was used at 3.76 - 4.04 g/100 lbs of fish/d for 10 d in one trial involving rainbow trout (Table 1) and results indicated that the treatment appeared efficacious.

C. Efficacy at 3.76 - 4.04 g/100 lbs fish/d for 10 d at water temperatures above 48.2^{o} F

OTF-M was used at 3.76 - 4.04 g/100 lbs of fish/d for 10 d in three trials involving rainbow trout and kokanee salmon (Table 1) and results indicated that all treatments appeared efficacious.

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

OTF-M was used at 10 g/100lbs fish/d for 10 - 14 d in four trials involving rainbow trout and cutthroat trout (Table 1) and results 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 CY03.

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 8 to 14 d. Three different species of salmonids were treated with OTF-M, and trials involved approximately 4.0 million fish. Treated fish ranged in size from 1.11 - 8.1 in. Overall mean water temperature during treatments was 51.6 °F (range, 45.0 - 58.0 °F). All treatment trials appeared efficacious. One trial involved the use of control fish whereas the remaining trials were conducted without use of controls. 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. 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 CY03 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)
Roaring Judy SFH	5	KOE	1.11	2,963,699	Marking	2.50 - 3.75	8	50.0
Big Springs Trout SFH	7	RBT	3.8 - 8.1	332,720	Marking	2.50 - 3.75	10 - 11	51.0
Big Springs Trout SFH	2	KOE	5.8 - 6.6	160,000	Marking	3.76 - 4.04	10	51.0
	1	RBT	3.90	45,000	Marking	3.76 - 4.04	10	51.0
Washoe Park Trout SFH	1	RBT	2.60	221,000	Marking	3.76 - 4.04	10	45.0
Yellowstone R. Trout SFH	1	CUT	2.10	30,000	Marking	10.0	14	52.0
Bluewater Springs Trout SFH	3	RBT	2.8 - 4.4	249,000	Marking	10.0	10 - 12	58.0

Table 2. Summary Data Regarding CY03 OTF-M Efficacy Studies

Total Number of Fish Treated:	**4,001,419**
Number of fish in efficacious studies	4,001,419
Total number of studies:	**20**
Number of Efficacious studies	20

Treatment Regimens Used:

2.5 - 3.75 g/100 lbs fish/day for 8 11 days (above 48.2°F)	12 trials
3.76 - 4.04 g/100 lbs fish/day for 10 days (below 48.2°F)	1 trial
3.76 - 4.04 g/100 lbs fish/day for 10 days (above 48.2°F)	3 trials
10.0 g/100 lbs fish/day for 10 - 14 days (above 48.2°F)	4 trials

Treatment Water Temperature (°F):

Temperature Range	45.0 - 58.0
Mean Temperature	51.6

Size of Treated Fish (in.):

Size Range	1.11 - 8.10

Species Treated:

rainbow *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*