Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2000 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 has been shown to be an effective and convenient marking agent for use on early life stages of fish. Large numbers of fish can be marked simultaneously by feeding young fish a standard dosage of oxytetracycline medicated feed for up to 10 d. In many cases, oxytetracycline medicated feed is the only viable option, other than immersion marking of very small fish with oxytetracycline, for permanently marking large numbers of small (< 30 gm) fish for the purpose of evaluating fishery management strategies. Oxytetracycline medicated feed (OTF-M) 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). To accommodate the needs of aquaculture and to collect pivotal and ancillary clinical field data on OTF-M for the marking of a variety of salmonids, the FDA has authorized the use of this compound under the Compassionate Investigational New Animal Drug (INAD) Exemption #9332. Trials to evaluate the efficacy of OTF-M as a marking agent of skeletal tissue of fish were conducted in 24 trials at six state fish

hatcheries involving approximately 3.2 million fish during calender year (CY) 2000. Standard treatment regimens included the use of OTF-M at 2.5 - 3.75 g/100 lbs fish/day for 10 days and 10.0 g/100 lbs fish/day for 14 - 15 days. In a few select trials fish, OTF-M was administered at a dosage of 3.78 - 6.40 g/100 lbs fish/day for 4 - 10 days. Approximately 92% of trials appeared efficacious, 4% appeared ineffective, and 4% were characterized as inconclusive.

Introduction

The current label for oxytetracycline medicated feed for marking (OTF-M) use in aquaculture limits the use to marking of skeletal tissue in Pacific salmonids only. The current FDA approved label for OTF-M limits the allowed dosages to 250 mg/kg/day (i.e., 11.4 g/100 lbs fish/d) for 4 days in salmon less then 30 grams with a 7 day withdrawal time. These label restrictions severely limit the overall utility of approved OTF use in aquaculture.

Fish culturists have reported that OTF-M treatment is a useful tool for marking of the skeletal tissue in rainbow trout, cutthroat trout, and kokanee salmon that are larger then 2gms. Marks were visible immediately after the treatment period, and they were still visible for several months afterwards when the fish were re-evaluated. Side by side comparisons have been conducted at different OTF-M dosages with the same efficacious results, and without any toxicity effects to the fish. However, using OTF-M on non-pacific salmonids for marking has not yet been approved by the FDA.

Purpose of Report

The primary purpose of this report is to summarize the results of CY 2000 supplemental OTF-M field efficacy studies. However, it is also expected 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. Facilities

A total of six state fish hatcheries used OTF-M to mark skeletal tissue in a variety of test fish.

2. OTF-M used in trials

The OTF-M used in the 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. Oxytetracycline medicated feed used in the various trials was supplied by 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 grams of active drug per 100 lbs of fish per day for 10 days (~25% of studies), or at 10.0 grams of active drug per 100 lbs of fish per day for 14 - 15 days (~33% of studies). However, a number of trials (~42%) deviated from the protocol during CY 2000. In these trials, fish were fed at rates of 3.78 - 6.40 grams of active drug per drug/100 lbs fish/day for periods of time ranging from 4 - 10 days.

Fish Species Involved in 2000 Trials

1. Species of fish treated

The following three salmonid species were treated with OTF-M during CY 2000:

rainbow trout *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*

2. Marking

Fish were treated with OTF-M to provide a mark on skeletal tissue that could be evaluated at a later date to help identify stocked fish. Utilization of marked fish for this purpose is an important fishery management tool.

Data Collected

1. Pathologist's reports

No pathology reports were submitted during CY 2000 trials.

2. Efficacy of marking procedure

Samples of treated fish were collected, process, and skeletal tissue was evaluated for a “readable” mark.

Discussion of Study Results:

1. General observations on the efficacy of OTF-M for marking of skeletal tissue of salmonids (Note: A summary of all OTF-M studies conducted during CY 2000 under INAD #9332 are listed in Table 5.)

A. Efficacy at 2.5 - 3.75 g/100 lbs fish/day for 10 days at water temperatures above 48.2^{o} F

OTF-M was used at 2.5 - 3.75 g/100 lbs of fish for 10 days in six trials involving rainbow trout, kokanee salmon, and cutthroat trout (Table 1). OTF-M treatment appeared efficacious in all six trials. Fish samples were collected after each of the treatment periods to confirm the presence of a "readable" mark.

B. Efficacy at 3.78 - 6.40 g/100 lbs fish/day for 4 - 10 days at water temperatures above 48.2^{o} F

OTF-M was used at 3.78 - 6.40 g/100 lbs of fish for 4 - 10 days in 10 trials involving rainbow trout, cutthroat trout, and kokanee salmon (Table 1 - 3). Fish samples were collected after the treatment period to confirm the presence of a "readable" mark. OTF-M treatment appeared efficacious in eight trials involving rainbow trout and kokanee salmon, and ineffective in one trial involving cutthroat trout. One trial involving rainbow trout was characterized as inconclusive.

C. Efficacy at 10.0 g/100 lbs fish/day for 14 - 15 days at water temperatures above 48.2^{o} F

OTF-M was used at 10.0 g/100 lbs of fish for 14 - 15 days in seven trials involving rainbow trout and kokanee salmon (Table 1). OTF-M treatment appeared efficacious in all seven trials.

D. Efficacy at 10.0g/100lbs fish/day for 14 days at water temperatures below 48.2^{o}F

OTF-M was used at 10.0 g/100lbs fish/day for 14 days in one trial involving rainbow trout(Table 1). Fish samples were collected after the treatment period to confirm the presence of a “readable” mark. OTF-M treatment appeared efficacious in this trial.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF-M treatment were reported.

Summary of Study Results

Oxytetracycline medicated feed to mark skeletal tissue of a variety of salmonids was used at dosages ranging from 2.5 - 10.0 g/100lbs fish per day. Treatment duration

ranged from 4 - 15 days. Three different species of fish were treated with OTF-M, and trials involved approximately 3.2 million fish. Treated fish ranged in size from 1.0 - 5.5 in. The mean water temperature during treatments was 52.5 °F (range 46.0 - 55.0 °F). Approximately 92% of trials appeared efficacious, 4% appeared ineffective, and 4% were characterized as inconclusive. No trials involved the use of control fish or included a pathologist reports. 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 can only be considered as ancillary data. None-the-less, the ancillary data described above should provide useful corroborative data to support a future expanded label claim for OTF-M. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #9332. In future trials conducted under INAD #9332, efforts will be directed towards the generation of higher quality data.

Table 1. Summary of Year 2000 Oxytetracycline Medicated Feed Efficacy Results - efficacious Studies

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Use of Feed	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
Big Springs Trout Hatchery	1	CUT	4.80	41,000	Marking	10	2.50 - 3.75	52.0
Big Springs Trout Hatchery	1	KOE	2.80	212,221	Marking	10	2.50 - 3.75	55.0
Big Springs Trout Hatchery	4	RBT	2.60 - 5.50	318,800	Marking	10	2.50 - 3.75	52 - 55
Big Springs Trout Hatchery	1	KOE	2.70	190,000	Marking	10	3.78 - 6.40	55.0
Flathead Lake Salmon Hatchery	1	KOE	1.20	1,030,000	Marking	10	3.78 - 6.40	49.0
Big Springs Trout Hatchery	6	RBT	3.70 - 4.10	633,000	Marking	10	3.78 - 6.40	52.0
Murray Springs Trout Hatchery	1	KOE	1.00	200,000	Marking	15	10.0	52.0
Giant Springs Trout Hatchery	5	RBT	2.20 - 3.10	423,548	Marking	14 - 15	10.0	54.0
Jocko River Trout Hatchery	1	RBT	3.00	51,500	Marking	14	10.0	46.0
Murray Springs Trout Hatchery	1	RBT	2.00	40,000	Marking	14	10.0	52.0

Table 2. Summary of Year 2000 Oxytetracycline Medicated Feed Efficacy Results - Non-efficacious Studies

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Use of Feed	Number of Treatment Days	Dose (g/100 lbs)	Temp. (°F)
Yellowstone River Trout Hatchery	1	CUT	2.00	30,029	Marking	4	3.78 - 6.40	52.0

Table 3. Summary of Year 2000 Oxytetracycline Medicated Feed Efficacy Results - Inconclusive Studies

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Number of Fish	Use of Feed	Number of treatment days	Dose (g/100 lbs)	Temp. (°F)
Big Springs Trout Hatchery	1	RBT	2.80	17,000	Marking	10	3.78 - 6.40	55.0

Table 4. Summary Data Regarding Year 2000 Studies to Evaluate the Efficacy of Oxytetracycline Medicated Feed As A Marking Agent

Total Fish Treated:	**3,187,098**
Number of fish treated in efficacious studies	3,140,069
Number of fish treated in non-efficacious studies	30,029
Number of fish treated in inconclusive studies	17,000
Total number of studies:	**24**
Efficacious studies	22
Non-efficacious studies	1
Inconclusive studies	1

Treatment Regimens Used:

2.5 - 3.75 g/100 lbs fish/day for 10 days (above 48.2°F)	6 trials
3.78 - 6.40 g/100 lbs fish/day for 4 - 10 days (above 48.2°F)	9 trials
3.78 - 6.40 g/100 lbs fish/day for 4 - 10 days (below 48.2°F)	1 trial
10.0 g/100 lbs fish/day for 14 - 15 days (above 48.2°F)	8 trials

Treatment Water Temperature (°F):

Temperature Range	46.0 - 55.0
Mean Temperature	52.5

Size of Treated Fish (in.):

Size Range	1.00 - 5.50

Species Treated:

rainbow *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*