Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

1999 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 oxytetracycline medicated feed for up to 10 d. In many cases, OTF-M is the only viable option, other than immersion marking of very small fish with water-soluble oxytetracycline, for permanently marking large numbers of small (< 30 gm) 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). 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 26 trials at five state fish

hatcheries involving approximately 1.8 million fish during calender year 1999. Standard OTF-M treatment regimens used were 2.5 - 3.75 g/100 lbs fish/day for 8 - 15 days and 10.0 g/100 lbs fish/day for 10 days. In a few select trials fish were fed OTF-M at 3.80 - 9.95 g/100 lbs fish/day for 9 - 15 days, and 12.80 g/100 lbs fish/day for 11 days. Overall trials results showed the treatment appeared efficacious in approximately 88% of the trials and ineffective in 8% of the trials. Results from 4% of the trials were characterized as inconclusive.

Introduction

The current label for OTF-M use in aquaculture limits its use to marking of skeletal tissue in Pacific salmonids only. The current U. S. Food and Drug Administration (FDA) approved label for OTF-M limits the allowed dosages to 250 mg/kg of fish/day (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-M use in aquaculture.

Fish culturists have reported that OTF-M treatment is a useful tool for marking of the skeletal tissue in a variety of salmonids that are > 2gms. Marks were visible immediately after the treatment period, and they were still visible for several months afterwards when skeletal tissue of treated fish were re-evaluated for a visible (i.e., readable) mark. Side by side comparisons have been conducted using different dosages of OTF-M to mark skeletal tissue of test fish, and the same efficacious results

have been observed. In addition, there have been no noticeable adverse or toxic effects to treated fish. However, using OTF-M for marking skeletal tissue of salmonids other than pacific salmonids has not yet been approved by the FDA.

Purpose of Report

The primary purpose of this report is to summarize the results of calendar year 1999 (CY 99) supplemental OTF-M field efficacy trials. However, it is also expected data from these trials will be used to enhance the existing OTF-M database that has been established from previous years trials 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 in marking skeletal tissue in a variety of test fish.

2. OTF-M used in trials

The OTF-M used in efficacy 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 CY99 trials, in which approved OTF-M was used in the feed, was provided 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 8 - 15 days (~35% of the trials), or at 10.0 grams of active drug per 100 lbs of fish per day for 10 days (~4% of trials). Investigators deviated from the protocol in a majority of the INAD trials (~61%) during CY 99 because fish were fed at rates of at higher rates than allowed (i.e., 3.8 - 12.8 grams of active drug per drug/100 lbs fish/day) or for longer periods of time than allowed (9 - 15 days).

Test Fish Used in CY 99 Trials

1. Species of fish treated

The following three salmonid species were treated with OTF-M during CY 99:

rainbow trout *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*

2. Marking

Fish were treated with OTF-M to provide a “readable” mark that could be used as an fishery management tool.

Data Collected

1. Pathologist's reports

No pathology reports were submitted during CY 99 trials.

2. Efficacy of marking procedure

Samples of treated fish were collected, process, and 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 - 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 trials conducted during CY 99 under INAD #9332 is presented in Table 5.)

1. Efficacy at 2.5 - 3.75 g/100 lbs fish/d for 8 - 15 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 - 15 days in eight trials involving rainbow trout and cutthroat trout (Tables 1 & 2). OTF-M treatment appeared efficacious in seven trials involving rainbow and cutthroat trout. One trial involving cutthroat trout did not appear effective.

2. Efficacy at 2.5 - 3.75 g/100 lbs fish/d for 10 d at water temperatures below 48.2^{o} F

OTF-M was used at 2.5 - 3.75 g/100 lbs of fish/d for 10 days in one trial involving rainbow trout (Table 1). OTF-M treatment appeared efficacious in this trial.

3. Efficacy at 3.80 - 9.95 g/100 lbs fish/d for 9 - 15 d at water temperatures above 48.2^{o} F

OTF-M was used at 3.80 - 9.95 g/100 lbs of fish/d for 9 - 15 days in 15 trials involving rainbow trout, cutthroat trout, and kokanee salmon (Tables 1 & 2). OTF-M treatment appeared efficacious in 14 of the trials of, but did not appear effective in one trial involving rainbow trout.

4. Efficacy at 10.0g/100lbs fish/d for 10 d at water temperatures above 48.2^{o}F

OTF-M was used at 10.0 g/100lbs fish/d for 10 days in one trial invovling rainbow trout (Table 1). OTF-M treatment appeared efficacious in this trial.

D. Efficacy at 12.80 g/100 lbs fish/d for 11 d at water temperatures above 48.2^{o} F

OTF-M was used at 12.80 g/100lbs fish/d for 11 d in one trial involving kokanee salmon (Table 3). OTF-M treatment was characterized as inconclusive in this trial. Fish samples were not collected after the treatment period to confirm the presence of a “readable” mark. Fish were stocked into various lakes, and results may be available one year after stocking when fishery personnel perform fish surveys kokanee salmon populations in the wild.

2. Observed Toxicity

No toxicity or adverse effects relating to OTF-M treatments 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 - 12.80 g/100lbs fish/ d for durations ranging from 8 to 15 d. Three different species of fish were treated with OTF-M, and trials involved approximately 1.8 million fish. Treated fish ranged in size from 1.6 - 6.1 in. Mean water temperature during all treatments was 52.6 °F (range, 46.5 - 58.0 °F). Overall results showed that approximately 88% of the trials appeared efficacious, 8% appeared ineffective, and 4% were characterized as inconclusive. One trial involved the use of control fish. No pathology reports were included in data packets submitted to the NIO. 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 1999 Oxytetracycline Medicated Feed as a Marking Agent Efficacy Trials Results - Efficacious Trials

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	3.70	24,600	Marking	15	2.50 - 3.75	52.0
Yellowstone River Trout Hatchery	1	CUT	6.13	500	Marking	8	2.50 - 3.75	52.0
Big Springs Trout Hatchery	2	RBT	3.40 - 3.60	168,300	Marking	11	2.50 - 3.75	52.0
Big Springs Trout Hatchery	3	RBT	4.20 - 4.70	158,400	Marking	8 - 11	2.50 - 3.75	52.0
Jocko River Trout Hatchery	1	RBT	4.20	85,704	Marking	10	2.5 - 3.75	46.5
Big Springs Trout Hatchery	1	CUT	3.70	16,400	Marking	15	3.80 - 9.95	52.0
Big Springs Trout Hatchery	3	KOE	2.80 - 3.60	274,998	Marking	10	3.80 - 9.95	55.0
Big Springs Trout Hatchery	5	RBT	2.20 - 3.60	421,000	Marking	10 - 11	3.80 - 9.95	52.0
Big Springs Trout Hatchery	3	RBT	4.00 - 5.10	283,700	Marking	9 - 10	3.80 - 9.95	52.0
Bluewater Springs Trout Hatchery	2	RBT	2.50 - 3.33	131,244	Marking	10	3.80 - 9.95	58.0
Big Springs Trout Hatchery	1	RBT	2.90	50,000	Marking	10	10.0	52.0

Table 2. Summary of 1999 Oxytetracycline Medicated Feed Efficacy Results - Ineffective Trials

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	2.18	33,838	Marking	10	2.50 - 3.75	52.0
Murray Springs Trout Hatchery	1	RBT	2.30	60,000	Marking	11	3.80 - 9.95	52.0

Table 3. Summary of 1999 Oxytetracycline Medicated Feed Efficacy Results - Inconclusive Trials

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)
Murray Springs Trout Hatchery	1	KOE	1.60	60,000	Marking	11	12.80	52.0

Table 4. Summary Data Regarding 1999 Oxytetracycline Medicated Feed Efficacy Trials

Total Fish Treated:	**1,768,684**
Number of fish treated in efficacious trials	1,614,846
Number of fish treated in ineffective trials	93,838
Number of fish treated in inconclusive trials	60,000
Total number of trials:	**26**
Efficacious trials	23
Ineffective trials	2
Inconclusive trials	1

Study Number of Trials that Included Use of Control Fish:

Study Number: 9332-99-051

Treatment Regimens Used:

2.5 - 3.75 g/100 lbs fish/day for 8 - 15 days (above 48.2°F)	8 trials
2.5 - 3.75 g/100 lbs fish/day for 10 days (below 48.2°F)	1 trial
3.80 - 9.95 g/100 lbs fish/day for 9 - 15 days (above 48.2°F)	15 trials
10.0 g/100 lbs fish/day for 10 days (above 48.2°F)	1 trial
12.80 g/100 lbs fish/day for 14 days (above 48.2°F)	1 trial

Treatment Water Temperature (°F):

Temperature Range	46.5 - 58.0
Mean Temperature	52.6

Size of Treated Fish (in.):

Size Range	1.60 - 6.13

Fish Species Treated:

rainbow *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*