Oxytetracycline Medicated Feed Clinical Field Trials - INAD 9332

Year 2001 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 with 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). 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 salmonid species, the FDA has authorized the use of this compound under the Compassionate Investigational New Animal Drug (INAD) Exemption #9332. A number of trials were conducted in calendar year (CY) 2001 under INAD #9332 to evaluate the efficacy of oxytetracycline medicated feed as a marking

agent of skeletal tissue in a variety of salmonids. Twenty-one such trials that involved approximately 1.8 million fish were conducted at five state fish hatcheries 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/day for 10 - 11 days; and 10.0 g/100 lbs fish/day for 13 - 14 days. In a few select trials fish were fed OTF-M at 2.4 - 4.79 g/100 lbs fish/day for 10 - 14 days. Approximately 95% of trials appeared efficacious, while the remaining 5% of the trials were characterized as inconclusive.

Introduction

The current label for 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 OTF-M/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 rainbow trout, cutthroat trout, and kokanee salmon that are larger then 2 gms, which is the largest fish size that can be treated with soluble oxytetracycline in a bath. Marks were visible immediately after the treatment period, and they were still visible for several months afterwards when skeletal tissue of fish were evaluated. Side by side comparisons have been conducted at different drug 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 was to summarize the results of calendar year 2001 (CY 01) 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 5 state fish hatcheries used OTF-M in marking of skeletal tissue.

2. OTF-M used in efficacy trials

The OTF-M used in these 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. Virtually all OTF-M used 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 - 11 days (~67% of studies), or at 10.0 grams of active drug per 100 lbs of fish per day for 13 - 14 days (~19% of studies). However, a few trials (~14%) deviated from the protocol during CY 01. In these trials, fish were fed at rates of 2.4 - 4.79 grams of active drug per drug/100 lbs fish/day for periods of time ranging from 10 - 14 days.

Fish Species Involved in CY01 Efficacy Trials

1. Species of fish treated

The following three salmonid species were marked during CY 01:

rainbow trout *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*

2. Marking

Fish were treated with oxytetracycline to provide a mark that could be used as an important fishery management tool.

Data Collected

1. Pathologist's reports

No pathology reports were submitted during CY 01 studies.

2. Efficacy of marking procedure

Samples of treated fish were collected, processed, and evaluated for a mark 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 CY 01 under INAD #9332 are presented in Table 4.)

A. Efficacy at 2.40 g/100 lbs fish/day for 14 days at water temperatures above 48.2^{o} F

OTF-M was used at 2.40 g/100 lbs of fish for 14 days in 1 trial (Table 1). OTF-M treatment appeared efficacious in this trial with rainbow trout. Fish

samples were collected after each of the treatment periods to confirm the presence of a “readable” mark.

B. Efficacy at 2.50 - 3.75 g/100 lbs fish/day for 10 days at water temperatures above 48.2° F

OTF-M was used at 2.50 - 3.75 g/100 lbs of fish for 10 days in 13 trials (Table 1). OTF-M treatment appeared efficacious in all trials with rainbow trout, cutthroat trout, and kokanee salmon. Fish samples were collected after the treatment period to confirm the presence of a “readable” mark.

C. Efficacy at 2.50 - 3.75 g/100 lbs fish/day for 11 days at water temperatures below 48.2° F

OTF-M was used at 2.50 - 3.75 g/100 lbs of fish for 11 days in 1 trial involving rainbow trout (Table 2). This OTF-M treatment was characterized as inconclusive because an insufficient number of fish (i.e., n = 1 fish) were evaluated for a skeletal mark.

D. Efficacy at 3.77 - 4.79 g/100lbs fish/day for 10 - 11 days at water temperatures above 48.2°F

OTF-M was used at 3.77 - 4.79 g/100lbs fish/day for 10 - 11 days in 2 trials (Table 1). OTF-M treatment, involving rainbow trout and kokanee salmon, appeared efficacious in both trials . Fish samples were collected after the treatment period to confirm the presence of a “readable” mark.

E. Efficacy at 10.0 g/100lbs fish/day for 13 - 14 days at water temperatures above 48.2°F

OTF-M was used at 10.0 g/100lbs fish/day for 13 - 14 days in 4 trials (Table 1). OTF-M treatment, involving rainbow and cutthroat trout, appeared efficacious in all trials. Fish samples were collected after the treatment period to confirm the presence of a “readable” mark.

2. Observed Toxicity

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

Summary of Study Results

Oxytetracycline medicated feed as a marking agent was used at dosages ranging from 2.40 - 10.0 g/100lbs fish per day. Treatment duration ranged from 10 - 14 days. Three different species of fish were treated with OTF-M, and trials involved approximately 1.8 million fish. Treated fish ranged in size from 2.0 - 6.5 in. Water temperature during treatment ranged from 45.0 - 55.0 °F, with a mean treatment temperature of 52.3 °F. Approximately 95% of trials appeared efficacious, while 5% were characterized as inconclusive. No trials involved the use of control fish or included a pathologist's reports. Overall, OTF-M appeared effective in creating a 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 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 Year 2001 Oxytetracycline Medicated Feed Efficacy Results - Efficacious Studies

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Fish Number	Use of Feed	Treatment Duration (days)	Dose (g/100 lbs)	Temp. (°F)
Murray Springs Trout SFH	1	RBT	6.48	16,000	Marking	14	2.40	52.0
Big Springs Trout SFH	1	CUT	5.00	10,488	Marking	10	2.50 - 3.75	52.0
	2	KOE	2.9 - 3.9	334,582	Marking	10	2.50 - 3.75	55.0
	10	RBT	2.4 - 5.9	727,228	Marking	10	2.50 - 3.75	52.0
Big Springs Trout SFH	1	KOE	2.70	195,000	Marking	11	3.77 - 4.79	55.0
	1	RBT	4.80	159,600	Marking	10	3.77 - 4.79	52.0
Yellowstone R. Trout SFH	1	CUT	2.00	30,000	Marking	14	10.0	52.0
Giant Springs Trout SFH	2	RBT	2.80	230,000	Marking	13 - 14	10.0	54.0
Murray Springs Trout SFH	1	RBT	4.22	8,063	Marking	14	10.0	52.0

Table 2. Summary of Year 2001 Oxytetracycline Medicated Feed Efficacy Results - <u>Inconclusive Studies</u>

Hatchery	Number of Trials	Fish Species	Fish Size (inches)	Fish Number	Use of Feed	Treatment Duration (Days)	Dose (g/100 lbs)	Temp. (°F)
Washoe Trout SFH	1	RBT	3.10	72,600	Marking	11	2.50 - 3.75	45.0

Table 3. Summary Data Regarding Year 2001 Oxytetracycline Medicated Feed Efficacy Studies

Total Fish Treated:	**1,783,561**
Number of fish treated in efficacious studies	1,710,961
Number of fish treated in inconclusive studies	72,600
Total number of studies:	**21**
Number of Efficacious studies	20
Number of Inconclusive studies	1

Treatment Regimens Used:

2.40 g/100 lbs fish/day for 14 days (above 48.2°F)	1 trial
2.5 - 3.75 g/100 lbs fish/day for 10 days (above 48.2°F)	13 trials
2.5 - 3.75 g/100 lbs fish/day for 11 days (below 48.2°F)	1 trial
3.77 - 4.79 g/100 lbs fish/day for 10 - 11 days (above 48.2°F)	2 trials
10.0 g/100 lbs fish/day for 13 - 14 days (above 48.2°F)	4 trials

Treatment Water Temperature (°F):

Temperature Range	45.0 - 55.0
Mean Temperature	52.3

Size of Treated Fish (in.):

Size Range	2.00 - 6.48

Species Treated:

rainbow *Oncorhynchus mykiss*

cutthroat trout *O. clarki*

kokanee salmon *O. nerka*