

Oxytetracycline Bath Marking Clinical Field Trials - INAD 9033

Year 2001 Annual Summary Report on the Use of Oxytetracycline Bath Marking in Field Efficacy Trials

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Summary

A number of Investigational New Animal Drug (INAD) trials were conducted in calendar year (CY) 2001 to evaluate the efficacy of oxytetracycline for bath marking (OTM) of otoliths in a variety of young fish. Sixty-five such trials that involved approximately 85.7 million fish were conducted at two U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Service coop hatchery, six state fish hatcheries, and one private hatchery. The U.S. Food and Drug Administration has authorized the use of OTM under Compassionate Investigational New Animal Drug Exemption #9033 for the purpose of collecting pivotal and ancillary efficacy data to support a new animal drug approval for oxytetracycline as a marking agent. Efficacy was based on whether or not a "readable" mark could be seen in the otolith of a subsample of treated fish. Overall results from the treatment trials conducted in CY01 showed that 41(63%) of the 65 trials appeared efficacious and 24 (37%) of the trials were characterized as inconclusive. In all cases, inconclusive results were because mark confirmation was either not conducted on a subsample of fish prior to release or because marks were to be read at a later date.

Introduction

Water soluble oxytetracycline is an effective and convenient marking agent for use on early life stages of fish. Large numbers of fish can be marked simultaneously by simple exposure to a uniform oxytetracycline solution for up to several hours. In many cases, immersion marking with oxytetracycline is the only practical means of permanently marking large numbers of small fish for the purpose of evaluating fishery management strategies. In general, marking is accomplished by immersing very young fish in a bath containing 500 mg/L oxytetracycline and 1,000 mg/L sodium chloride, buffered with Tris buffer to a pH of 6.5 - 6.9, for six hours. Sodium chloride is necessary to prevent calcium chelation of the oxytetracycline.

The overall objective of oxytetracycline as a bath mark was to develop clinical field efficacy data on the use of water soluble oxytetracycline as an agent for the non-intrusive marking of fish larvae, fry, or very young fish prior to, or shortly after, initiation of feeding. Fish of such small size cannot be marked by fin clip or other more conventional tagging procedures. Fish marked at early life stages are not available for human consumption until they have grown to a much larger size, which in virtually all cases requires at least a year or more of additional growth. Except for threatened and endangered species and research fish destroyed after use, no fish averaging larger than 2 grams each are authorized for oxytetracycline treatment under INAD 9033.

Purpose

The purpose of this report is to summarize the results of calendar year (CY 2001) supplemental oxytetracycline for bath marking (OTM) field efficacy studies. However, it is also expected that these data will be used to enhance the existing OTM database that has been established from studies conducted in previous years for the purpose of expanding and/or extending the approved label for oxytetracycline use in aquaculture.

Facilities, Materials, and Methods

1. Facilities

A total of 10 fish culture facilities used OTM during CY01, including two U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Service coop hatchery, six state fish hatcheries, and one private hatchery.

2. OTM used in trials

All OTM used in CY01 trials was Terramycin-343 soluble powder supplied by Pfizer, Inc., Lee's Summit, Missouri. Pfizer's over-the-counter Terramycin-343 soluble powder contains 343 grams of active oxytetracycline hydrochloride per pound. Pfizer's Terramycin-343 was the only form of oxytetracycline used by fish culturists to treat fish under INAD #9033.

3. Drug dosages

As described in the Study Protocol for INAD #9033, fish in approximately 43% of trials conducted in CY01 were exposed to a single OTM bath treatment for 6 hours at a dosage of 500 mg/L. The treatment regimen used in the remaining 57% of the trials deviated from the treatment regimen recommended in the protocol in that fish were exposed to a single OTM bath treatment for a longer duration (i.e., 6 - 14 hours) or at higher dosage (i.e., 266 - 819.2 mg/L) than allowed by the protocol.

Fish Species

1. Species of fish treated

The following salmonid and non-salmonid fish species were treated during CY 2001:

Salmonids

brook trout *Salvelinus fontinalis*

Non-salmonids

American shad *Alosa sapidissima*

bluegill *Lepomis macrochirus*

largemouth bass *Micropterus salmoides*

striped bass *Morone saxatilis*

walleye *Sitizostedion vitreum*

yellow perch *Perca flavescens*

2. Marking

Fish were treated with oxytetracycline as a bath marking agent to provide a mark on otolith or skeletal tissue. Marking calcified tissue such as otoliths or skeletal tissue has proven to be an important fishery management tool to identify hatchery-stock fish in the wild.

Data Collected

1. Pathologists Reports

No pathology reports were submitted during CY 2001 studies.

2. Efficacy of marking procedure

A sub-sample of fish from the test population were collected, processed, and evaluated for the presence of a mark by examination of at least one otolith/fish.

3. Mortality data

As stated in the Study Protocol, mortality data was to be collected for at least 10 days prior to treatment and for at least 30 d post-treatment. The Investigators noted that no mortalities were observed during or immediately after the treatment. Mortality data was not always collected for a 30 d period post-exposure because in some cases, treated fish were stocked out immediately after treatment.

Discussion of Study Results

- 1. Summary results on the efficacy of OTC for marking fish** - Efficacy was based on whether or not a “readable” mark could be seen in the otolith of a subsample of treated fish. (Note: A summary of the individual OTM studies conducted during CY 2001 under INAD #9033 is presented in Table 4; and summary data regarding all studies conducted are presented in Tables 1, 2, & 3).

A. Efficacy of OTM at 266 mg/L for 6 hrs

OTM was used on yellow perch in three trials in which fish were exposed to 266 mg/L OTM for 6 hrs (Table 1). OTM treatment appeared efficacious in all trials. The Investigator noted that the fish did not exhibit any noticeable negative behavior.

B. Efficacy of of OTM at 266 mg/L for 14 hrs

OTM was used on yellow perch in three trials in which fish were exposed to 266 mg/L OTM for 14 hrs (Table 1). OTM treatment appeared efficacious in all trials. The Investigator noted that the fish did not exhibit any noticeable negative behavior.

C. Efficacy of OTM at 307.2 mg/L for 4 hrs

OTM was used on American shad in eight trials in which fish were exposed to 307.2 mg/L OTM for 4 hrs (Tables 1 & 2). OTM treatment appeared efficacious in seven trials . One trial was characterized as inconclusive because evaluation of an otolith mark was not conducted (fish were stocked out \leq 5 days post-treatment).

D. Efficacy of OTM at 380 mg/L for 6 hrs

OTM was used on bluegill in one trial in which fish were exposed to 380 mg/L OTM for 6 hrs (Table 2). OTM treatment was characterized as inconclusive because an OTM mark was observed on the otolith of only one of four fish when evaluated at 189 days post-treatment. The Investigator stated during a phone conversation that due to the small size of the fish (i.e., 2.1"), that it was difficult to section the otoliths and check for a OTM mark. The Investigator also noted that the protocol deviation with respect to dosage occurred due to an inaccurate drug measurement.

E. Efficacy of OTM at 500 mg/L for 6 hrs

OTM was used on bluegill, striped bass, walleye, and yellow perch in 28 trials in which fish were exposed to 500 mg/L OTM for 6 hrs (Tables 1 & 2). OTM treatment appeared efficacious in 14 trials and 14 trials were characterized as inconclusive. Treatment results characterized as inconclusive were due to one of the following: (1) Investigators did not check the otolith of a subsample of fish for an OTM mark; (2) Investigators did not submit results to the NIO; or (3) some, but not all (i.e., 25%), of the treated fish had a readable mark on their otolith.

F. Efficacy of OTM at 500 mg/L for 12 hrs

OTM was used on yellow perch in two trials in which fish were exposed to 500 mg/L OTM for 12 hrs (Table 1). OTM treatment appeared efficacious in both trials.

G. Efficacy of OTM at 614 mg/L for 4 hrs

OTM was used on American shad in one trial in which fish were exposed to 614 mg/L OTM for 4 hrs (Table 2). OTM treatment was characterized as inconclusive because otoliths from a subsample of treated fish were not evaluated (i.e., fish were stocked out \leq 5 days post-treatment).

H. Efficacy of OTM at 700 mg/L for 6 hrs

OTM was used on brook trout, largemouth bass, walleye, and yellow perch in eighteen trials in which fish were exposed to 700 mg/L OTM for 6 hrs (Tables 1 & 2). OTM treatment appeared efficacious in 12 trials. Six trials were characterized as inconclusive. Treatment results characterized as inconclusive were due to one of the following: (1) Investigators did not check the otolith of a subsample of fish for an OTM mark (i.e., fish were stocked out immediately after treatment); (2) fish had not been collected for otolith mark evaluation upon submission of data to the NIO; or (3)

stress marks were present which confounds evaluation of OTM marks on fish otoliths.

I. Efficacy of OTM at 819.2 mg/L for 4 hrs

OTM was used on American shad in one trial in which fish were exposed to 819.2 mg/L OTM for 4 hrs (Table 1). OTM treatment was characterized as inconclusive because no mark confirmation was conducted (fish were stocked out \leq days post-treatment).

2. Observed Toxicity

No toxicity or adverse effects relating to OTM treatment were reported.

Summary of Study Results

OTM was used in 65 trials involving brook trout, American shad, bluegill, largemouth bass, striped bass, walleye, and yellow perch during CY01. Trials involved a single bath treatment at a dosages ranging from 266 to 819.2 mg/L for durations ranging from 4 to 14 hrs. Approximately 85.7 million early life stage fish were treated during this period. Water temperature during treatment ranged from 47 to 73.6°F. Efficacy was based on whether or not a “readable” mark could be seen in the otolith of a subsample of treated

fish. Overall results from the treatment trials showed that 41(63%) of the 65 trials appeared efficacious and 24 (37%) of the trials were characterized as inconclusive. In all cases, inconclusive results were because (1) mark confirmation was either not conducted on a subsample of fish prior to release, (2) marks were to be read at a later date, or (3) stress marks were present on fish otoliths which confounds evaluation of OTM marks on fish otoliths. Investigators reported no evidence of toxicity or adverse effects related to OTM treatment. Although these data must be considered as ancillary efficacy data, they should provide useful corroborative data to support a future expanded label claim for oxytetracycline. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #9033. In future trials conducted under INAD #9033, efforts will be directed towards the generation of higher quality data.

Table 1. Summary of Year 2001 Oxytetracycline Bath Marking Efficacy Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Treatment Duration (hrs)	Dose (mg/L)	pH	Dissolved Oxygen	Temp. (°F)
Manning SFH	3	YEP	123,750	0.03	6	266	8	10	54.0
	3	YEP	123,750	0.03	14	266	8	10	54.0
Waldoboro Shad Hatchery	7	AMS	1,647,674	0.10	4	307.2	na	na	64.2 - 68.2
Gainesville Fisheries Lab	2	BLG	123	0.60	6	500	na	na	73.0 - 73.6
Milford SFH	8	WAE	28,500,000	0.01	6	500	na	na	57.0
Table Rock SFH	1	WAE	30,000	0.83	6	500	7	10	62.0
Manning SFH	3	YEP	123,750	0.03	6	500	8	10	54.0
Manning SFH	2	YEP	123,750	0.03	12	500	8	10	54.0
Garrison Dam NFH	1	LMB	358,160	0.25	6	700	na	na	63.0
	10	WAE	15,885,000	0.01	6	700	na	na	63.0
	1	YEP	676,100	0.30	6	700	na	na	63.0

Table 2. Summary of Year 2001 Oxytetracycline Bath Marking Efficacy Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Treatment Duration (hrs)	Dose (mg/L)	pH	Dissolved Oxygen	Temp. (°F)
Waldoboro Shad Hatchery	1	AMS	232,854	0.10	4	307.2	na	na	63.3
Gainesville Fisheries Lab	1	BLG	50	1.75	6	380	na	na	67.0
Gainesville Fisheries Lab	2	BLG	140	0.60	6	500	na	na	65.0 & 71.0
Marion SFH - USGS Coop	1	STB	50,000	0.79	6	500	7	9	70.0
Miles City SFH	5	WAE	25,200,000	0.01	6	500	na	na	57.0
Milford SFH	6	WAE	407,000	0.91	6	500	na	na	60.0
Waldoboro Shad Hatchery	1	AMS	409,773	0.10	4	614.4	na	na	66.9
Ashland FRO	1	BKT	170,000	0.62	6	700	na	na	47.0
Garrison Dam NFH	3	WAE	4,625,000	0.01	6	700	na	na	63.0
New London SFH	2	WAE	6,600,000	0.01	6	700	na	na	60.0
Waldoboro Shad Hatchery	1	AMS	440,647	0.10	4	819.2	na	na	67.4

Table 3. Summary Data Regarding Year 2001 Oxytetracycline for Bath Marking Efficacy Studies

Total Number of Fish Treated:	85,727,521
Treatment Regimes Used:	
266 mg/L static bath for 6 hr	3 trials
266 mg/L static bath for 14 hr	3 trials
307.2 mg/L static bath for 4 hr	8 trials
380 mg/L static bath for 6 hr	1 trial
500 mg/L static bath for 6 hr	28 trials
500 mg/L static bath for 12 hr	2 trials
614 mg/L static bath for 4 hr	1 trial
700 mg/L static bath for 6 hr	18 trials
819.2 mg/L static bath for 4 hr	1 trial
Treatment Water Temperature (°F):	47.0 - 73.6
Size Class of Treated Fish:	Fry
Species Treated:	
	brook trout <i>Salvelinus fontinalis</i>
	American shad <i>Alosa sapidissima</i>
	bluegill <i>Lepomis macrochirus</i>
	largemouth bass <i>Micropterus salmoides</i>
	striped bass <i>Morone saxatilis</i>
	walleye <i>Sitizostedion vitreum</i>
	yellow perch <i>Perca flavescens</i>
