Calcein (SE- MARK™) Clinical Field Trials - INAD 10-987

Year 2003 Annual Summary Report on the Use of Calcein (SE- MARK™) in Field Efficacy Trials

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Summary

Marking agents such as oxytetracycline and calcein are routinely used in fisheries programs to mark otoliths and other calcified tissue in fish as a way to monitor fish propagation programs. The U.S. Food and Drug Administration has authorized the use of Calcein (SE- MARK™; CAL) under the Compassionate Investigational New Animal Drug (INAD) Exemption #10-987 for the purpose of gathering efficacy data to support a new animal drug approval. During calendar year (CY) 2003, several INAD trials were conducted to evaluate the efficacy of CAL to mark calcified tissue (i.e., fins, rays, and scales) in a variety of young fish. Sixteen such trials that involved approximately 0.57 million fish were conducted at one state fish hatchery and at one University. Efficacy was based on whether or not a "readable" mark could be seen in the calcified tissue of a subsample of treated fish. Overall, results from the treatment trials conducted in CY 2003 showed that all trials were at least somewhat effective for producing a readable mark that could be detected.

Introduction

Calcein 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 calcein solution for concentration dependant durations that could last from several minutes to several hours. In many cases, immersion marking 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 either 1) 125 - 250 mg/L calcein for 1 - 6 hr; or 2) 2.5 - 5.0 g/L for 1 - 7 min. A pre-treatment exposure of fish to 1 - 5% solution of non-iodized salt for ~3.5 min is recommended to facilitate the osmotic transfer of calcein across fish tissue membranes and into calcified tissues.

The overall objective for using calcein as a marking agent was to develop clinical field efficacy data for non-intrusive marking of fish larvae or very young fish prior to, or shortly after, initiation of feeding, and to observe the marks on live fish to evaluate whether the are “readable.” An advantage of immersion mass marking fish of such a small size is that these fish cannot be marked by fin clip or by using 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 calcein treatment under INAD 10-987. Therefore,

Investigators using calcein under this INAD may be able to successfully mark fish for stocking with high confidence that absorbed calcein concentrations will not pose a threat caused by human consumption.

Purpose

The purpose of this report is to summarize the results of CY 2003 supplemental CAL field efficacy studies. Furthermore, it is expected that these data will be used to establish a CAL database for the purpose of developing an appropriate label claim for the legal use of this new animal drug in aquaculture.

Facilities, Materials, and Methods

1. Facilities

Two fish culture facilities used CAL during CY 2003, including Kendall Creek State Fish Hatchery (Kendall, WA Dept. of Fish and Wildlife) and Penn State University (PSU). Water temperature during treatment at Kendall was 47°F and at PSU ranged from 61 to 68°F.

2. CAL used in trials

All CAL used in CY 2003 trials was Calcein (SE- MARK™), which is a commercial liquid product supplied by Western Chemical, Inc., Ferndale, Washington. The strength of SE- MARK™ is a 1.0% solution of calcein.

Western Chemical's SE- MARK™ is the only form of calcein available for use under INAD #10-987.

3. Drug dosages

According to the protocol, Investigators had a choice of treating fish with either (1) 125 - 250 mg/L calcein for 1 - 6 hr., or (2) 2.5 - 5.0 g/L for 1 - 7 min with a pre-treatment of 1 - 5% solution of non-iodized salt for ~3.5 min. All trials conducted in CY 2003 involved fish that were exposed to 2.5 - 5.0 g/L for 4.0 - 4.3 min with a pre-treatment of 1.75 - 5% solution of non-iodized salt for 3.5 - 4.0 min.

Fish Species

1. Species of fish treated

The following fish species were treated during CY 2003:

Salmonids

chinook salmon *Oncorhychus tshawytscha*

Non-salmonids

bridle shiner *Notropis bifrenatus*

ironcolor shiner *Notropis chalybaeus*

2. Marking

Fish were treated with CAL to provide a mark on otolith, skeletal tissue, fin rays, or scales. Marking calcified tissue has proven to be an important fishery management tool to identify hatchery-stock fish in the wild.

Data Collected

1. Efficacy of marking procedure

A sub-sample of fish from the test population were collected and evaluated for efficacy of the marking procedure, mark retention data, and morbidity and mortality related to the marking procedure.

2. Effect of treatment on treated fish

Study Investigators were encouraged to include general observations on the effect of treatment on fish behavior and response to routine culture/management activities (i.e. feeding activity, level of stress, or negative fish behavior).

Discussion of Study Results

1. Summary results on the efficacy of CAL for marking fish - Efficacy was based on whether or not a "readable" mark could be seen on calcified tissue from a subsample of treated fish. (Note: A summary of the individual CAL studies

conducted during CY 2003 under INAD #10-987 in which results were efficacious or inconclusive are presented in Table 1; Table 2 describes the treatment regimens used and fish species tested; and Table 3 lists all treatment trials conducted during this reporting period).

A. Efficacy of CAL at 3.0 g/L for 4 min

Calcein was used on Bridle and Ironcolor shiners in three trials in which fish were exposed to 3.0 g/L calcein for 4 min (Table 1). Overall, treatment results appeared somewhat effective in all trials. When Bridle shiners were treated, 0% of the marks were characterized as “excellent,” 14% were “good,”72% were “poor,” and no mark was detected in 14% of the fish. When Ironcolor shiners were treated, 0% of the marks were characterized as “excellent,” 7% were “good,” 55% were “poor,” and no mark was detected in 38% of the fish.

B. Efficacy of CAL at 5.0 g/L for 4 min

Calcein was used on Bridle and Ironcolor shiners in 12 trials in which fish were exposed to 5.0 g/L calcein for 4 min (Table 1). Overall, treatment results appeared effective in all trials. When Bridle shiners were treated, 57% of the marks were characterized as “excellent,” 34% were “good,” and 9% were “poor.” When Ironcolor shiners were treated, 45% of the marks were characterized as “excellent,” 45% were “good,” and 10% were “poor.”

C. Efficacy of CAL at 5.0 g/L for 4.3 min

Calcein was used on chinook salmon in one trial in which fish were exposed to 5.0 g/L calcein for 4.3 min (Table 1). Overall, treatment results appeared effective. One day after treatment, an excellent mark was observed on all fish that were evaluated. However, when fish were rechecked for a mark after being reared in outdoor raceways, no marks were detected. It was suspected that exposure to direct sunlight has a negative effect on mark retention.

2. Observed Toxicity

No toxicity or adverse effects relating to CAL treatment were reported in 14 trials; however, two trials reported substantial delayed mortality in fish following the treatment period. The Investigator working with chinook salmon noted that a 5% salt solution may have contributed to a 25% delayed mortality loss. The salt solution was reduced to 3% and then to 2.6%. Resultant delayed mortality was 8.1% and 2.6%, respectively for the two salt treatments. The Investigator working with the ironcolor shiners noted that the fish that experienced high mortality after the calcein treatment were the youngest fish treated.

Summary of Study Results

Calcein was used in 16 trials involving chinook salmon, Bridle shiner, and Ironcolor shiner during CY 2003. Trials involved a single bath treatment at a dosage of 2.5 - 5.0

g/L for 1 - 7 min with a pre-treatment of 1 - 5% solution of non-iodized salt for ~3.5 min. Approximately 0.57 million early life stage fish were treated during this period. Water temperature during treatment ranged from 47 to 68°F. Efficacy was based on whether or not a “readable” mark could be seen in the otolith or skeletal system of a subsample of treated fish. Overall results showed that (1) all treatment trials were at least somewhat effective, (2) treatments using 5 g/L calcein for 4 min produced better results than 3 g/L for 4 min, and (3) although excellent marks were detected on chinook salmon 1-d post-treatment, marks faded to the point of not being detectable following exposure to direct sunlight. Investigators reported no evidence of toxicity or adverse effects related to CAL treatment in 88% of the trials. Although data from these trials will be considered as ancillary, trial results should provide useful corroborative data to support a future label claim for CAL. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #10-987. In future trials conducted under INAD #10-987, efforts will be directed towards the generation of higher quality data.

Table 1. Summary of Year 2003 Calcein (SE-MARK^{TM}) Efficacy Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Treatment Duration (min)	Dose (g/L)	Salt Conc.		Temp. (^{o}F)	Mark Rating (%)
							%	Duration (min)		
Pennsylvania State Univerisity	1	BRS	50	0.28	4	3	1.75	4	61.7	Excellent - 0% Good - 14% Poor - 72% No Mark - 14%
Pennsylvania State Univerisity	2	IRS	119	0.28	4	3	1.75	4	61.2 - 61.7	Excellent - 0% Good - 7% Poor - 55% No Mark - 38%
Pennsylvania State Univerisity	5	BRS	1,213	0.28	4	5	1.75	4	61.2 - 68.0	Excellent - 57% Good - 34% Poor - 9% No Mark - 0%
Pennsylvania State Univerisity	7	IRS	7,673	0.28	4	5	1.75	4	62.4 - 68.0	Excellent - 45% Good - 45% Poor - 10% No Mark - 0%
Kendall Creek SFH	1	CKS	559,500	0.23	4.3	5	3 - 5	3.5	47	Excellent - 100%

Table 2. Description of Treatment Regimes Used and Fish Species Treated during CY 2003 Calcein (SE-MARK™) Efficacy Studies

Total Number of Fish Treated:	53,838,973
Treatment Regimes Used:	
3 g/L static bath for 4 min	3 trials
5 g/L static bath for 4 min	12 trials
5 g/L static bath for 4.3 min	1 trial
Treatment Water Temperature (°F):	47.0 - 68.0
Size Class of Treated Fish:	Fry
Species Treated:	
chinook salmon *Oncorhychus tshawytscha*	
bridle shiner *Notropis bifrenatus*	
ironcolor shiner *Notropis chalybaeus*	