

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

Year 2012/2013 Annual Summary Report on the Use of Calcein (SE- MARK®) in Field Efficacy Trials

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Summary

Marking agents such as calcein and oxytetracycline are routinely used in fisheries programs to mark otoliths and other calcified tissue (i.e., fins, rays, and scales) 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. In calendar year 2012/2013 (CY12/13) the efficacy of CAL to mark calcified tissue was evaluated in 11 trials involving approximately 3.5 million fish. Trials were conducted at six different facilities, including two state facilities, one private facility, one tribal hatchery, and two universities during this period. The compassionate study protocol under which treatments were administered allowed the investigator to use CAL at a dosage of either: (1) 125 - 250 mg/L calcein for 1 - 6 h, or (2) 2.5 - 5.0 g/L calcein for 1 - 7 min with a pre-treatment of 1 - 5% solution of non-iodized salt for about 3.5 min. 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 CY12/13 indicated 91% of the trials appeared efficacious while 9% were ineffective.

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 calcein solution for concentration dependant durations that could last from several minutes to several hours. In many cases, immersion marking is one of 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 h; 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 about 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 under this INAD 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 they 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 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, calcein treatment under INAD 10-987 authorizes that no fish larger than 2 grams in size may be marked with calcein. Therefore, Investigators using calcein under this INAD may be able to successfully mark fish for stocking with high confidence that calcein absorbed in fish tissues will not pose a human health concern.

Purpose

The purpose of this report is to summarize the results of CY12/13 CAL field efficacy studies conducted under INAD #10-987. Furthermore, it is expected that data from these trials will be used to enhance the existing CAL database that has been established from previous years studies for the purpose of developing an appropriate label claim for the use of CAL in aquaculture.

Facilities, Materials, and Methods

1. Facilities

A total of 11 CAL efficacy trials were conducted at six different facilities, including two state facilities, one private facility, one tribal hatchery, and two universities during this period. Water temperature during treatments at the testing facilities ranged from 44.6 - 73.4 °F, with a mean treatment temperature of 56.6 °F.

2. CAL used in trials

All CAL used during the reporting period was SE- MARK[®], which is a commercial liquid product supplied by Western Chemical, Inc., Ferndale, Washington. The strength of SE- MARK[®] is a pH buffered, 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 were able to treat fish with the following treatment regimens: (1) 125 - 250 mg/L calcein for 1 - 6 h, or (2) 2.5 - 5.0 g/L calcein for 1 - 7 min with a pre-treatment of 1 - 5% solution of non-iodized salt for about 3.5 min. During the reporting period, Investigators treated fish with 250 mg/L calcein for 1 - 6 h in five trials; and with 2.5 - 5.0 g/L calcein for 4 - 6 min with a pre-treatment of 2.5 - 4.7% solution of non-iodized salt for 4 - 6 min in six trials.

Fish Species

1. Species of fish treated

A total of five different fish species were treated during CY12/13, including three species of salmonid and two non-salmonid fish species. Treated fish ranged in weight from 0.1 - 2.0 g; mean weight was 0.5 g. Species treated included:

Salmonids

Chinook salmon (*Oncorhynchus tshawytscha*)

pink salmon (*O. gorbuscha*)

sockeye salmon (*O. nerka*)

Non-salmonid

fathead minnow (*Pimephales promelas*)

largemouth bass (*Micropterus salmoides*)

2. Marking

Fish were treated with CAL to provide a mark in calcified tissue such as otolith, skeletal tissue, fin rays, or scales for the purpose of identifying 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 and mark retention data, as well as 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 CY12/13 under INAD #10-987 in which trials appeared to be efficacious are presented in Table 1; Table 2 lists all of the trials that were ineffective; Table 3 describes the treatment regimens used and fish species tested during this reporting period).

A. Efficacy of CAL at 250 mg/L for 1 - 6 h

CAL was used at 250 mg/L for 1 - 6 hr in five trials involving fathead minnows, largemouth bass, and pink salmon (Tables 1 - 2). The investigators noted visible marks were seen on checked fish in four trials. In the remaining trial all treated largemouth bass died during or following treatment. The investigator noted that the mortality was most likely due to water quality problem due to too high of salinity. Overall, results indicated treatment appeared efficacious in four trials and was ineffective in one trial.

B. Efficacy of CAL at 2.5 - 5.0 g/L for 4.0 - 6.0 min

CAL was used at 2.5 - 5.0 g/L calcein for 4.0 - 6.0 min in six trials involving Chinook salmon and sockeye salmon (Table 1) . The Investigators noted that a visible mark was seen on checked fish. Overall, results indicated treatment appeared effective in six trials.

2. Observed Toxicity

No toxicity or adverse effects relating to CAL treatment were reported in 10 of the 11 trials. In the trial involving largemouth bass the investigator noted that all treated fish had died either during treatment or within 24 hrs after treatment. When asked by the AADAP Office for more information concerning this adverse effect the investigator noted, "We do not suspect that the mortality was due to the Calcein. While preparing the Calcein solution, we added the appropriate amount of Calcein, and then added baking soda to to achieve a neutral pH. We overshot, and used soda water to achieve a neutral pH. We monitored temperature, pH, and dissolved oxygen closely, and these all essentially matched the water from which the Largemouth Bass were transferred. After the high mortality we experienced, we wondered whether the water quality was to blame. We tested the salinity and found that it exceeded our meter's range (>2,000ppm), meaning that it was at least mildly brackish and potentially higher. We suspect that this high salinity was to blame for the mortality."

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for Calcein (SE- MARK®) INAD #10-987

No changes have occurred to the current study protocol for CAL (SE- MARK®) INAD #10-987.

Facility Sign-up List

Please see “Table 4. Facilities and Names of Investigators” for facilities that signed-up to participate in the CAL (SE- MARK®) INAD #10-987 during CY12/13. Note: all of these facilities either disposed of their calcein waste properly or it is retained on-site.

Correspondence sent to Calcein (SE- MARK®) Participants

Please see the attached correspondence that was sent to all CAL (SE- MARK®) participants after the AADAP Office received their sign-up form for CY12/13.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY12/13 was 3,532,305. The total number of treated fish to count against the treatment use authorization dated August 28, 2008 is 3,828,327.

Summary of Study Results

CAL (SE-MARK[®]) was administered to test fish in 11 separate trials at a dosage of either 1) 250 mg/L CAL for 1 - 6 h; or 2) 2.5 - 5.0 g/L for 4 - 6 min with a pre-treatment of 2.5 - 4.7% solution of non-iodized salt for 4.0 - 6.0 min. Five different fish species were treated with CAL, and trials involved 3,532,305 fish. Treated fish ranged in size from 0.1 - 2.0 g. Water temperature during treatment ranged from 44.6 - 73.4°F, with a mean treatment temperature of 56.6 °F. Efficacy was based on whether or not a “readable” mark could be seen in the otolith, skeletal system, or scales of a subsample of treated fish. Overall, results from the treatment trials conducted in CY12/13 indicated that CAL treatments appeared effective in 91% of the trials while 9% were ineffective. Investigators reported no evidence of toxicity or adverse effects related to CAL treatments in 10 of the 11 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 continue to be directed towards the generation of high quality data.

Table 1. Summary of CY12/13 Calcein (SE- MARK®) Efficacy Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (g)	Dose (g/L)	Treatment Duration (min)	Salt Conc.		Temp. (°F)
							%	Duration (min)	
Aquatic Toxicology Laboratory	3	FHM	2,905	0.10	0.25	360	-	-	71.1 - 73.4
Nisqually River - screw trap	1	PKS	10,000	0.30	0.25	60	-	-	46.0
Snohomish County Public Utility District	1	CSA	3,000	2.00	2.5	5.0	3	4.0	44.6
Cedar River Hatchery	2	SOS	3,170,000	0.23	2.5	6.0	2.5	6.0	48.0 - 57.0
Hoko Falls Hatchery	3	SOS	345,600	0.46 - 0.68	5.0	4.0	4.0 - 4.7	4.0	47.3 - 49.5

Table 2. Summary of CY12/13 Calcein (SE- MARK®) Efficacy Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (g)	Dose (g/L)	Treatment Duration (min)	Salt Conc.		Temp. (°F)
							%	Duration (min)	
Center for Systems Integration and Sustainability	1	LMB	800	1.00	0.25	180 & 360	-	-	66.7

Table 3. Description of Treatment Regimes Used and Fish Species Treated during CY12/13 Calcein (SE- MARK[®]) Efficacy Studies

Total Number of Fish Treated:	3,532,305
Number of fish treated in effective trials	3,531,505
Number of fish treated in ineffective trials	800
Total Number of Trials:	11
Number of trials in which treatments were effective	10
Number of trials in which treatments were ineffective	1
Treatment Regimes Used:	
250 mg/L static bath for 1 - 6 hr	5 trials
2.5 - 5.0 g/L static bath for 4 - 6 min	6 trials
Treatment Water Temperature (°F):	44.6 - 73.4
Size of Treated Fish (g):	0.1 - 2.0
Species Treated:	
<u>Salmonids</u>	
Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	
pink salmon (<i>O. gorbuscha</i>)	
sockeye salmon (<i>O. nerka</i>)	
<u>Non-salmonid</u>	
fathead minnow (<i>Pimephales promelas</i>)	
largemouth bass (<i>Micropterus salmoides</i>)	