

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

Year 2009 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 2009 (CY09) the efficacy of CAL to mark calcified tissue was evaluated in 11 trials involving approximately 0.20 million fish. Trials were conducted at seven different hatcheries, including three U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey facility, one state hatchery, one state university facility, and one tribal hatchery 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 CY09 indicated that approximately 91% of trials appeared efficacious, while 9% of the trials were characterized as inconclusive.

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 CY09 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 seven fish culture facilities during CY09, including three U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey facility, one state hatchery, one state university facility, and one tribal hatchery. Mean water temperature during all trials was 59.5 °F,

and water temperature during treatments at the various testing facilities ranged from 50.0 to 80.0°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 125 - 250 mg/L calcein for 1 - 6 h in one trial; and with 2.5 - 5.0 g/L calcein for 1 - 7 min with a pre-treatment of 0.4 - 5.9% solution of non-iodized salt for 3.0 - 4.0 min in 10 trials.

Fish Species

1. Species of fish treated

A total of eight different fish species were treated during CY09, including three species of salmonids, four non-salmonids fish species, and one marine non-salmonid species. Mean weight of treated fish was 0.83 g, and fish size ranged in weight from 0.01 - 1.75 g. The following fish species were treated during CY09:

Salmonids

fall chinook salmon *Oncorhynchus tshawytscha*

sockeye salmon *O. nerka*

steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*

delta smelt *Hypomesus transpacificus*

lost river sucker *Deltistes luxatus*

walleye *Sander vitreus*

Marine non-salmonids:

tidewater goby *Eucyclogobius newberryi*

2. Marking

Fishes 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 CY09 under INAD #10-987 in which trials appeared to be efficacious are presented in Table 1; Table 2 provides a summary of all trials in which treatments were inconclusive; Table 3 describes the treatment regimens used and fish species tested; and Table 4 lists all treatment trials conducted during this reporting period).

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

American shad were exposed to 250 mg/L CAL for 6 h in one trial (Table 1). The investigator demonstrated that calcein and OTC can be used to mark the same otolith and yield a dual color banding pattern. More elapsed time between the marks would have resulted in fewer undetected calcein marks. CAL treatment appeared to be effective in this trial.

B. Efficacy of CAL at 2.5 - 5.0 g/L for 3.0 - 5.0 min

Fish were exposed to 2.5 - 5.0 g/L calcein for 3.0 - 5.0 min in 10 trials (Tables 1 & 2) involving fall chinook salmon, sockeye salmon, steelhead trout, delta smelt, lost river sucker, walleye, and tidewater goby. The Investigators in nine trials noted that a visible mark was seen on checked fish; range of time when fish were evaluated for a mark ranged from immediately after treatment to 38 days post-treatment. In the trial involving lost river suckers the fish were not evaluated for a mark. Overall, results indicated that treatment appeared effective in nine trials and was characterized as inconclusive in one trial.

2. Observed Toxicity

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

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for Calcein (SE- MARK[®]) INAD #10-697

Please see the attached current study protocol for CAL (SE- MARK[®]) INAD #10-987.

Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 5. Facilities and Names of Investigators” for facilities that signed-up to participate in the CAL (SE- MARK[®]) INAD #10-987 during CY09. Facilities not listed in Appendix III-a of the current CAL INAD #10-987 study protocol have been highlighted. Please note all of these facilities either disposed of their calcein waste properly or it is retained on-site.

The following facility had SE- MARK[®] on-hand during CY09 but never used the drug:

1. Manning SFH

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 CY09.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY09 was 228,139. The total number of treated fish to count against the treatment use authorization dated August 28, 2008 is 236,680.

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 6 h; or 2) 2.5 - 5.0 g/L for 3 - 5 min with a pre-treatment of 0.4 - 5.9% solution of non-iodized salt for 3.0 - 4.0 min. Eight different fish species were treated with CAL, and trials involved approximately 0.20 million fish. Treated fish ranged in size from 0.01 - 1.75 g. Water temperature during treatment ranged from 50.0 - 80.0°F, with a mean treatment temperature of 59.5 °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 CY09 indicated that CAL treatments appeared effective in approximately 91% of the trials, while results from the remaining 9% of the trials were inconclusive. Investigators reported no evidence of toxicity or adverse effects related to CAL treatments in any 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 continue to be directed towards the generation of high quality data.

Table 1. Summary of CY09 Calcein (SE- MARK®) Efficacy Results - Efficacious Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Dose (g/L)	Treatment Duration (min)	Salt Conc.		Observed Withdrawal Period (days)	Temp. (°F)
							%	Duration (min)		
N.E. Fishery Center	1	AMS	10,000	0.01	0.25	360	-	-	1095	77.0
Fish Conservation & Culture Lab	2	DES	20,230	0.08 - 1.7	2.5 - 5.0	5.0	1.0	3.0	T&E Species - no consumption	54.9 - 58.3
Columbia River Research Lab	1	FCS	396	1.75	5.0	3.5	1.5	3.5	T&E Species - no consumption	53.6
Hoko Falls Hatchery - Umbrella Creek Hatchery	3	SOS	186,453	0.3 - 0.7	5.0	4.0	5.6 - 5.9	4.0	>365	50.6 - 57.6
Columbia River Research Lab	1	STT	48	1.50	5.0	3.0	5.0	3.0	>21	57.2
Arcata FWO	1	TWG	4,000	1.00	5.0	4.0	5.0	4.0	Not a food fish.	50.0
Waterville SFH	1	WAE	7,000	0.45	5.0	4.0	5.0	4.0	430	80.0

Table 2. Summary of CY09 Calcein (SE- MARK®) Efficacy Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Dose (g/L)	Treatment Duration (Min)	Salt Conc.		Observed Withdrawal Period (days)	Temp. (°F)
							%	Duration (min)		
Humboldt State University	1	LRS	12	1.00	2.5	5.0	0.4	4.0	365	62.0

Table 3. Description of Treatment Regimes Used and Fish Species Treated during CY09 Calcein (SE- MARK[®]) Efficacy Studies

Total Number of Fish Treated:	228,139
Number of fish treated in effective trials	228,127
Number of fish treated in inconclusive trials	12
Total Number of Trials:	11
Number of trials in which treatments were effective	10
Number of trials in which treatment results were inconclusive	1
Treatment Regimes Used:	
250 mg/L static bath for 6 hr	1 trial
2.5 - 5.0 g/L static bath for 3 - 5 min	10 trials
Treatment Water Temperature (°F):	50.0 - 80.0
Size of Treated Fish (gm):	0.01 - 1.75
Species Treated:	
<u>Salmonids</u>	
fall chinook salmon <i>Oncorhynchus tshawytscha</i>	
sockeye salmon <i>O. nerka</i>	
steelhead trout <i>O. mykiss</i>	
<u>Non-salmonids</u>	
American shad <i>Alosa sapidissima</i>	
delta smelt <i>Hypomesus transpacificus</i>	
lost river sucker <i>Deltistes luxatus</i>	
walleye <i>Sander vitreus</i>	
<u>Marine non-salmonids:</u>	
tidewater goby <i>Eucyclogobius newberryi</i>	