

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

Year 2011 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 2011 (CY11) the efficacy of CAL to mark calcified tissue was evaluated in 42 trials involving 2,361 fish. Trials were conducted at three different facilities, including one U.S. Fish and Wildlife Service fish facility, one state hatchery, and one state university 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 CY11 indicated 90% of the trials appeared efficacious while 10% 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 CY11 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 42 CAL efficacy trials were conducted at three different facilities, including one U.S. Fish and Wildlife Service fish facility, one state hatchery, and one state university during this period. Mean water temperature during all trials was 71.4 °F, and water temperature during treatments at the various testing facilities ranged from 50.0 to 75.0°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 40 trials; and with 2.5 - 5.0 g/L calcein for 4 - 7 min with a pre-treatment of 5.0% solution of non-iodized salt for 4.0 min in two trials.

Fish Species

1. Species of fish treated

A total of three different fish species were treated during CY11, including one species of salmonid, one non-salmonid fish species, and one marine non-salmonid species. Mean weight of treated fish was 0.13 g, and fish size ranged in weight from 0.08 - 1.0 g. The following fish species were treated during CY11:

Salmonids

sockeye salmon *Oncorhynchus nerka*

Non-salmonid

fathead minnow *Pimephales promelas*

Marine non-salmonid:

tidewater goby *Eucyclogobius newberryi*

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 CY11 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 characterized as 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 125 - 250 mg/L for 1 - 6 h

Sockeye salmon and fathead minnows were exposed to 125 - 250 mg/L CAL for 1 - 6 h in 40 trials (Tables 1 - 2). The investigator noted good marks were seen on checked fish immediately after treatment in 36 studies. In the remaining four studies treatment was characterized as inconclusive due to the wrong light source being used to check fish marks. The correct light source was obtained and all future studies showed a mark present on treated fish.

B. Efficacy of CAL at 2.5 - 5.0 g/L for 4.0 - 7.0 min

Fish were exposed to 2.5 - 5.0 g/L calcein for 4.0 - 7.0 min in two trials (Table 1) involving fathead minnows and tidewater goby. The Investigators noted that a visible mark was seen on checked fish; time when fish were evaluated for a mark ranged from immediately to 7 days post-treatment. Overall, results indicated treatment appeared effective in both trials.

2. Observed Toxicity

No toxicity or adverse effects relating to CAL treatment were reported in any of the trials.

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 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the CAL (SE- MARK®) INAD #10-987 during CY11. 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 CY11 but never used the drug:

1. Hoko Falls Hatchery

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 CY11.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY11 was 2,361. The total number of treated fish to count against the treatment use authorization dated August 28, 2008 is 296,022.

Summary of Study Results

CAL (SE- MARK[®]) was administered to test fish in 42 separate trials at a dosage of either 1) 125 - 250 mg/L CAL for 1 - 6 h; or 2) 2.5 - 5.0 g/L for 4 - 7 min with a pre-treatment of 5.0% solution of non-iodized salt for 4.0 min. Three different fish species were treated with CAL, and trials involved 2,361 fish. Treated fish ranged in size from 0.08 - 1.0 g. Water temperature during treatment ranged from 50.0 - 75.0 °F, with a mean treatment temperature of 71.4 °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 CY11 indicated that CAL treatments appeared effective in 90% of the trials while 10% were characterized as 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 CY11 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	35	FHM	2,040	0.08	0.25	360	-	-	68.0 - 75.0
Nisqually River - screw trap	1	SOS	122	0.38	0.25	60	-	-	50.0
Aquatic Toxicology Laboratory	1	FHM	1	0.90	2.5	7.0		-	71.0
Arcata FWO	1	TWG	147	1.00	5.0	4.0	5	4.0	57.2

Table 2. Summary of CY11 Calcein (SE- MARK®) Efficacy Results - Inconclusive 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	1	FHM	25	0.08	0.125	180	-	-	72.0
Aquatic Toxicology Laboratory	3	FHM	26	0.08	0.250	360	-	-	71.0

Table 3. Description of Treatment Regimes Used and Fish Species Treated during CY11 Calcein (SE- MARK[®]) Efficacy Studies

Total Number of Fish Treated:	2,361
Number of fish treated in effective trials	2,310
Number of fish treated in inconclusive trials	51
Total Number of Trials:	42
Number of trials in which treatments were effective	38
Number of trials in which treatments were inconclusive	4
Treatment Regimes Used:	
125 - 250 mg/L static bath for 1 - 6 hr	40 trials
2.5 - 5.0 g/L static bath for 4 - 7 min	2 trials
Treatment Water Temperature (°F):	50.0 - 75.0
Size of Treated Fish (g):	0.08 - 1.0
Species Treated:	
<u>Salmonids</u>	
sockeye salmon <i>Oncorhynchus nerka</i>	
<u>Non-salmonid</u>	
fathead minnow <i>Pimephales promelas</i>	
<u>Marine non-salmonids:</u>	
tidewater goby <i>Eucyclogobius newberryi</i>	