

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

Year 2006 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. During calendar year 2006 (CY06), 12 INAD trials were conducted to evaluate the efficacy of CAL to mark calcified tissue in a variety of young fish/mussel. In CY06, 12 such trials that involved approximately 0.15 million fish/mussel were conducted at six fish culture facilities, including two U.S. Fish and Wildlife Service fish hatchery, one U.S. Geological Survey facility, two state hatcheries, and one tribal hatchery. Efficacy was based on whether or not a “readable” mark could be seen in the calcified tissue of a subsample of treated fish/mussel. Overall, results from the treatment trials conducted in CY06 indicated that approximately 92% of trials appeared efficacious, while 8% of the trials 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 CY06 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 12 CAL efficacy trials were conducted at six fish culture facilities during CY06, including two U.S. Fish and Wildlife Service fish hatchery, one U.S. Geological Survey facility, two state hatcheries, and one tribal hatchery. Mean water temperature during all trials was 62.2 °F, and water temperature during treatments at the various testing facilities ranged from 50.5 to 75.6°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 had a choice of treating fish/mussel with 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. 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 3.5 - 4.5 min with a pre-treatment of 1.3 - 5% solution of non-iodized salt for 3.0 - 10.0 min in nine trials. In the remaining two trials, Investigators treating mussels (a non-edible food source) deviated from approved treatment regimens described in the study protocol, and treated mussels with 250 mg/L calcein for 24 hrs; and rainbow trout (fish were destroyed after study period) with 50 g/L calcein for 4 min with a pre-treatment of 3% solution of non-iodized salt for 4.0 min.

Fish Species

1. Species of fish treated

A total of seven different fish species and mussels were treated during CY06, including three species of salmonids, three non-salmonids fish species, and one mussel species. Mean weight of treated fish was 1.25 g (excluding the Atlantic sturgeon – fish will never be released), and fish size ranged in weight from 0.23 - 83.7 g. The following fish species were treated during CY06:

Salmonids

chinook salmon *Oncorhynchus tshawytscha*

sockeye salmon *O. nerka*

rainbow trout *O. mykiss*

Non-salmonids

Atlantic sturgeon *Acipenser oxyrinchus*

largemouth bass *Micropterus salmoides*

walleye *Stizostedion vitreum*

Mussels

plain pocketbook *Lampsilis cardium*

2. Marking

Fish were treated with CAL to provide a mark in calcified tissue such as otolith, skeletal tissue, fin rays, scales, or mussel shell for the purpose of identify hatchery-stock fish/mussel in the wild.

Data Collected

1. Efficacy of marking procedure

A sub-sample of fish/mussel 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/mussel** - Efficacy was based on whether or not a “readable” mark could be seen on calcified tissue from a subsample of treated fish/mussel. (Note: A summary of the individual CAL studies

conducted during CY06 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 ineffective; Table 3 describes the treatment regimens used and fish/mussel 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

Mussels were exposed to 250 mg/L calcein for 6 h in one trial (Table 1). The Investigator noted that five mussels were checked for a calcein mark at eight days post-treatment and all checked mussels had a dimly visible dull green mark (i.e. rated as poor). It was also noted that the water temperature may have effected the amount of calcein that was taken up by the mussels. Calcein treatment appeared to be effective in this trial.

B. Efficacy of CAL at 250 mg/L for 24 h

Mussels were exposed to 250 mg/L calcein for 24 h in one trial (Table 1). The Investigator noted that five mussels were checked for a calcein mark at seven days post-treatment; 60% of the checked mussels had a readily visible bright green mark (i.e. rated as excellent), while 40% had a clearly visible green mark (i.e. rated as good). The Investigator noted that the water temperature may have effected the amount of calcein that was taken up by the mussels. Please note that this treatment was a deviation from the study protocol where the duration was 24 hrs instead of 6 hrs; however, freshwater mussels are a non-edible resource and will not be used for human consumption. Calcein treatment appeared to be effective in this trial.

C. Efficacy of CAL at 5.0 g/L for 3.5 - 4.5 min

Fish were exposed to 5.0 g/L calcein for 3.5 - 4.5 min in nine trials (Tables 1 & 2) involving Atlantic sturgeon, largemouth bass, walleye, and chinook and sockeye salmon. The Investigators in eight trials noted that an excellent mark was seen on all checked fish one to five days post-treatment; however, in one trial no marks were seen on the fish at 119 days post-treatment. The Investigator noted that these fish had been marked and then released into an outdoor pond (it was also noted that only seven fish were recovered from this pond at 119 days post-treatment). Overall, results indicated that treatment appeared effective in eight trials, while one trial involving walleye was ineffective.

D. Efficacy of CAL at 50.0 g/L for 4.0 min

Rainbow trout were exposed to 50.0 g/L calcein for 4.0 min in one trial (Table 1). The Investigator noted that an excellent mark was seen on all fish seven days post-treatment. This was a pilot 10X Target Animal Safety Study conducted by the Aquatic Animal Drug Approval Partnership Program at the Bozeman Fish Tech Center. All fish were destroyed at the end of the post-treatment period. Calcein treatment appeared to be effective in this trial.

2. Observed Toxicity

No toxicity or adverse effects relating to CAL treatment were reported in any of the trials conducted in CY06.

Summary of Study Results

Calcein was used in 12 trials involving chinook and sockeye salmon, rainbow trout, Atlantic sturgeon, largemouth bass, walleye, and mussels during CY06. Trials involved a single bath treatment at dosages of either 1) 250 mg/L calcein for 6 - 24 hr; or 2) 5.0 - 50.0 g/L for 3.5 - 4.5 min with a pre-treatment of 1.3 - 5.0% solution of non-iodized salt for 3 - 10 min. Approximately 0.15 million early life stage fish/mussel were treated during this period. Water temperature during treatment ranged from 50.5 to 75.6°F. Efficacy was based on whether or not a “readable” mark could be seen in the otolith, skeletal system, or shell of a subsample of treated fish/mussel. Overall, results from the treatment trials conducted in CY06 indicated that CAL treatments appeared effective in approximately 92% of the trials, while results from the remaining 8% of the trials were ineffective. Investigators reported no evidence of toxicity or adverse effects related to CAL treatment. 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 high quality data.

Table 1. Summary of CY06 Calcein (SE-MARK™) 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. (°F)	Mark Rating (%)
							%	Duration (min)		
Genoa NFH	1	MSL	20	-	6 hrs	0.25	-	-	52.0	8 days post-treatment Poor - 100%
	1	MSL	20	-	24 hrs	0.25	-	-	52.0	7 days post-treatment Excellent - 60% Good - 40%
Manning SFH	1	ASN	20	83.70	4.5	5.0	1.51	10	62.0	60 days post-treatment Excellent - 100%
Columbia River Research Lab	1	CKS	1,396	2.00	3.5	5.0	5.0	3.5	60.0	1 - 5 days post-treatment Excellent - 100%
Manning SFH	4	LMB	45,323	0.5 - 2.0	4.5	5.0	1.3 - 1.5	4.5 - 10	68.0 - 70.0	10 days post-treatment Excellent - 100%
Hoko Hatchery	1	SOS	102,030	0.23	4.0	5.0	4.39	4.0	51.0	1 day post-treatment Excellent - 100%
Waterville SFH	1	WAE	3,600	0.50	4.0	5.0	2.5	3.0	68.0	7 days post-treatment Excellent - 100%
Bozeman FTC	1	RBT	225	2.00	4.0	50.0	3.0	4.0	50.5	7 days post-treatment Excellent - 100%

Table 2. Summary of CY06 Calcein (SE-MARK™) Efficacy Results - Ineffective Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Treatment Duration	Dose (g/L)	Salt Conc.		Temp. (°F)	Mark Rating (%)
							%	Duration (min)		
Waterville SFH	1	WAE	660	0.40	4.0	5.0	2.5	3.0	75.6	No marks were seen at 119 days post-treatment (note: only 7 fish were recovered from pond).

Table 3. Description of Treatment Regimes Used and Fish Species Treated during CY05 Calcein (SE-MARK™) Efficacy Studies

Total Number of Fish Treated:	153,294
Number of fish treated in effective trials	152,634
Number of fish treated in ineffective trials	660
Total Number of Trials:	12
Number of trials in which treatments were effective	11
Number of trials in which treatment results were Inconclusive	1
Treatment Regimes Used:	
250 mg/L static bath for 6 hr	1 trial
250 mg/L static bath for 24 hr	1 trial
5.0 g/L static bath for 3.5 - 4.5 min	9 trials
50.0 g/L static bath for 4 min	1 trial
Treatment Water Temperature (°F):	50.5 - 75.6
Size of Treated Fish (gm):	0.23 - 83.7
Species Treated:	
<u>Salmonids</u>	
chinook salmon <i>Oncorhynchus tshawytscha</i>	
sockeye salmon <i>O. nerka</i>	
rainbow trout <i>O. mykiss</i>	
<u>Non-salmonids</u>	
Atlantic sturgeon <i>Acipenser oxyrhynchus</i>	
largemouth bass <i>Micropterus salmoides</i>	
walleye <i>Stizostedion vitreum</i>	
<u>Mussels</u>	
plain pocketbook <i>Lampsilis cardium</i>	