

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

Year 2008 Annual Summary Report on the Use of Calcein (SE- MARK®) in Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

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 2008 (CY08) the efficacy of CAL to mark calcified tissue was evaluated in 21 trials involving approximately 0.90 million fish. Trials were conducted at 12 different hatcheries, including five U.S. Fish and Wildlife Service fish hatcheries, one National Oceanic and Atmospheric Administration facility, four state hatcheries, one private hatchery, 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/mussel. Overall, results from the treatment trials conducted in CY08 indicated that approximately 95% of trials appeared efficacious, while 5% 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 CY08 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 21 CAL efficacy trials were conducted at 12 fish culture facilities during CY08, including five U.S. Fish and Wildlife Service fish hatcheries, one National Oceanic and Atmospheric Administration facility, four state hatcheries, one private hatchery, and one tribal hatchery. Mean water temperature during all

trials was 58.6 °F, and water temperature during treatments at the various testing facilities ranged from 48.5 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 1.0 - 5.5% solution of non-iodized salt for 2.0 - 4.5 min in 19 trials.

Study Protocol Deviation: Treatment regimen administered in the remaining trial deviated from the protocol. The investigator treated arrow goby with 0.75; 1.25; 2.5; or 5.0 mg/L calcein for 4 min.

Fish Species

1. Species of fish treated

A total of 13 different fish species were treated during CY08, including four species of salmonids, seven non-salmonids fish species, and two marine non-salmonid species. Mean weight of treated fish was 1.12 g, and fish size ranged in weight from 0.04 - 5.0 g.

Study Protocol Deviation: Mean weight of treated fish exceeded 2.0 g in one trial involving walleye. The Investigator noted that the treated fish weighed 5.0 g; however, these fish were research only fish and were euthanized at the end of the study.

The following fish species were treated during CY08:

Salmonids

chinook salmon *Oncorhynchus tshawytscha*

rainbow trout *O. mykiss*

sockeye salmon *O. nerka*

steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*

Atlantic sturgeon *Acipenser oxyrinchus*

burbot *Lota lota*

delta smelt *Hypomesus transpacificus*

fathead minnow *Pimephales promelas*

largemouth bass *Micropterus salmoides*

walleye *Sander vitreus*

Marine non-salmonids:

arrow goby *Clevelandia ios*

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 CY08 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 125 mg/L for 6 h

American shad were exposed to 125 mg/L CAL for 6 h in one trial (Table 2). The investigator noted nearly all fish died (including fish not treated with calcein) and were not evaluated for a mark. The cause of mortality was attributed to tank effect. CAL treatment was characterized as inconclusive due to the fish not being evaluated for a mark.

B. Efficacy of CAL at 0.75; 1.25; 2.5; 5.0 g/L for 4.0 min

Arrow goby were exposed to 0.75; 1.25; 2.5; or 5.0 g/L CAL for 4.0 min in one trial (Table 1). The Investigator noted an excellent mark was seen on the fish treated at 2.5 and 5.0 g/L; while a poor mark was seen on the fish treated at 0.75 and 1.25 g/L at 31 days post-treatment. CAL treatment appeared to be effective in this trial.

C. Efficacy of CAL at 2.5 - 5.0 g/L for 1.0 - 7.0 min

Fish were exposed to 2.5 - 5.0 g/L calcein for 1.0 - 7.0 min in 19 trials (Table 1) involving chinook salmon, rainbow trout, sockeye salmon, steelhead trout, Atlantic sturgeon, burbot, delta smelt, fathead minnows, largemouth bass, walleye, and tidewater goby. The Investigators in all 19 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 8 days post-treatment. Overall, results indicated that treatment appeared effective in all trials.

2. Observed Toxicity

No toxicity or adverse effects relating to CAL treatment were reported

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 CY08.

The following facility had SE- MARK® on-hand during CY08 but never used the drug:

1. Klamath Falls FWO

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 calendar year 2008.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY08 was 896,105. The total number of treated fish to count against the treatment use authorization dated October 20, 2003 (valid through August 27, 2008) is 2,771,740. The total number of treated fish to count against the current treatment use authorization dated August 28, 2008 is 8,541.

Summary of Study Results

CAL (SE-MARK[®]) was administered to test fish in 21 separate trials at a dosage of either 1) 125 mg/L CAL for 6 h; or 2) 0.75 - 5.0 g/L for 1 - 7 min with a pre-treatment of 1.0 - 5.5% solution of non-iodized salt for 2.0 - 4.5 min. Thirteen different fish species were treated with CAL, and trials involved approximately 0.90 million fish. Treated fish ranged in size from 0.04 - 5.0 g. Water temperature during treatment ranged from 48.5 - 80.0°F, with a mean treatment temperature of 58.6 °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 CY08 indicated that CAL treatments appeared effective in approximately 95% of the trials, while results from the remaining 5% 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 CY08 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)
							%	Duration (min)	
Arcata FWO	1	AGY	20	1.00	4.0	0.75; 1.25; 2.5; 5.0	3.80	4.0	68.0
Manning SFH	1	ASN	80	0.50	4.5	5.0	2.05	4.5	68.0
Garrison Dam NFH	1	BUR	18,072	0.26	4.0	5.0	3.00	4.0	55.0
Pelton Round-Butte Hydroelectric Facility	1	CKS	70,000	0.38	3.5	5.0	5.00	3.5	50.0
NMFS/FW Division	1	CKS	1,000	1.90	3.5	5.0	2.00	3.5	52.7
Fish Conservation and Culture Lab	7	DSM	11,710	0.2 - 1.9	1 - 7	2.5 - 5.0	1.00	3.0	48.9 - 64.2
Valley Center NFH	1	FHM	187,000	1.97	4.0	5.0	3.00	2.0	52.0
Richloam SFH	1	LMB	5,700	0.94	4.0	2.5	1.50	4.0	71.0
Duluth Area Fisheries Office	1	RBT	21,000	1.50	3.5	5.0	5.00	3.5 - 4.0	53.0
Hoko Falls Hatchery	1	SOS	54,082	0.61	4.0	5.0	5.30	4.0	48.5
Pelton Round-Butte Hydroelectric Facility	1	STT	500,000	0.15	3.5	5.0	5.00	3.5	55.0
Arcata FWO	1	TWG	1,441	1.00	4.0	5.0	5.00	4.0	57.2
Waterville SFH	2	WAE	14,000	0.3 - 5.0	4.0	5.0	5.00	4.0	73.0 - 80.0

Table 2. Summary of CY08 Calcein (SE- MARK[®]) Efficacy Results - Inconclusive Trials

Hatchery	Number of Trials	Fish Species	Number of Fish	Fish Size (gm)	Treatment Duration	Dose (mg/L)	Salt Conc.		Temp. (°F)
							%	Duration (min)	
Harrison Lake NFH	1	AMS	12,000	0.04	6 hr	125	-	-	57.1

Table 3. Description of Treatment Regimes Used and Fish Species Treated during CY08 Calcein (SE- MARK[®]) Efficacy Studies

Total Number of Fish Treated:	896,105
Number of fish treated in effective trials	884,105
Number of fish treated in inconclusive trials	12,000
Total Number of Trials:	21
Number of trials in which treatments were effective	20
Number of trials in which treatment results were inconclusive	1

Treatment Regimes Used:

125 mg/L static bath for 6 hr	1 trial
0.75 - 5.0 g/L static bath for 1 - 7 min	20 trials

Treatment Water Temperature (°F): 48.5 - 80.0

Size of Treated Fish (gm): 0.04 - 5.0

Species Treated:

Salmonids

chinook salmon *Oncorhynchus tshawytscha*
rainbow trout *O. mykiss*
sockeye salmon *O. nerka*
steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*
Atlantic sturgeon *Acipenser oxyrhynchus*
burbot *Lota lota*
delta smelt *Hypomesus transpacificus*
fathead minnow *Pimephales promelas*
largemouth bass *Micropterus salmoides*
walleye *Sander vitreus*

Marine non-salmonids:

arrow goby *Clevelandia ios*
tidewater goby *Eucyclogobius newberryi*