

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

### **Year 2014 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 2014 (CY14) the efficacy of CAL to mark calcified tissue was evaluated in 12 trials involving approximately 0.6 million fish. Trials were conducted at seven different facilities, including one U.S. Fish and Wildlife Service facility, one U.S. Geological Survey facility, one state facility, one private facility, and three tribal facilities 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 CY14 indicated 100% of the trials appeared efficacious.

### **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 CY14 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 seven different facilities, including one U.S. Fish and Wildlife Service facility, one U.S. Geological Survey facility, one state facility, one private facility, and three tribal facilities during this period. Water temperature during treatments at the testing facilities ranged from 44.6 - 62.6 °F, with a mean treatment temperature of 53.2 °F.

## **2. CAL used in trials**

All CAL used during the reporting period was SE- MARK<sup>®</sup>, which is a commercial liquid product supplied by Western Chemical, Inc., Ferndale, Washington. The strength of SE- MARK<sup>®</sup> is a pH buffered, 1.0% solution of calcein. Western Chemical's SE- MARK<sup>®</sup> 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 6 h in one trial; and with 2.5 - 5.0 g/L calcein for 4 - 7 min with a pre-treatment of 1.5 - 5.0% solution of non-iodized salt for 3 - 6 min in 11 trials.

## **Fish Species**

### **1. Species of fish treated**

A total of nine different fish species were treated during CY14, including seven species of salmonid and two non-salmonid fish species. Treated fish ranged in weight from 0.1 - 3.6 g; mean weight was 1.1 g. Lake sturgeon were the only

species of fish that exceeded the maximum weight limit of 2.0 g. The investigator noted that this species is a threatened species and protected from fishing and consumption in New York waters. Species treated included:

**Salmonids**

bloater (*Coregonus hoyi*)

lake herring (*C. artedii*)

Chinook salmon (*Oncorhynchus tshawytscha*)

chum salmon (*O. keta*)

coho salmon (*O. kisutch*)

pink salmon (*O. gorbuscha*)

sockeye salmon (*O. nerka*)

**Non-salmonid**

lake sturgeon (*Acipenser fulvescens*)

white sturgeon (*A. transmontanus*)

**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 CY14 under INAD #10-987 in which trials appeared to be efficacious are presented in Table 1; Table 2 describes the treatment regimens used and fish species tested during this reporting period).

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

CAL was used at 250 mg/L for 6 hr in one trial involving white sturgeon (Table 1). The investigator noted visible marks were seen on checked fish seven days post-treatment in this trial. Results indicated treatment appeared efficacious in this trial.

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

CAL was used at 2.5 - 5.0 g/L calcein for 4.0 - 7.0 min in 11 trials involving bloater, Chinook salmon, chum salmon, coho salmon, lake herring, lake sturgeon, pink salmon, and sockeye salmon (Table 1). The Investigators noted that a visible mark was seen on checked fish immediately after treatment to at least 31 days post-treatment in the various trials. Overall, results indicated treatment appeared effective in all 11 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 due to fish size or the fish being released into a closed fisheries.

### **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 3. Facilities and Names of Investigators” for facilities that signed-up to participate in the CAL (SE- MARK®) INAD #10-987 during CY14. 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 CY14.

### **Number of Treated Fish under Treatment Use Authorization**

Total number of treated fish during CY14 was 621,984. The total number of treated fish to count against the treatment use authorization dated August 28, 2008 is 4,450,311.

### **Summary of Study Results**

CAL (SE- MARK®) was administered to test fish in 12 separate trials at a dosage of either 1) 250 mg/L CAL for 6 h; or 2) 2.5 - 5.0 g/L for 4 - 7 min with a pre-treatment of 1.5 - 5.0% solution of non-iodized salt for 3.0 - 6.0 min. Nine different fish species were

treated with CAL, and trials involved 621,984 fish. Treated fish ranged in size from 0.1 - 3.6 g. Water temperature during treatment ranged from 44.6 - 62.6°F, with a mean treatment temperature of 53.2 °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 CY14 indicated that CAL treatments appeared effective in 100% of the trials. 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 CY14 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)	
CCT Spokane Office	1	white sturgeon	20,000	0.10	0.25	360	-	-	60.4
Snohomish County Public Utility District	1	chinook salmon	4,500	2.00	2.5	5.0	3	4.0	44.6
Cedar River Hatchery	1	sockeye salmon	274,000	0.23	2.5	6.0	2.5	6.0	48.0
Tunison Lab of Aquatic Science	1	bloater	30,000	1.00	5.0	4.0	1.5	4.0	52.3
Keta Creek Hatchery - Harvest Mangement Program	1	chinook salmon	24	1.50	5.0	7.0	2.5	3.5	53.2
Stockton Fish and Wildlife Office	1	chinook salmon	12,000	1.50	5.0	7.0	1.5	3.0	54.3
Keta Creek Hatchery - Harvest Mangement Program	1	chum salmon	123	0.40	5.0	7.0	2.5	3.5	53.2
	1	coho salmon	22	0.40	5.0	7.0	2.5	3.5	53.2
Tunison Lab of Aquatic Science	1	lake herring	161,000	1.50	5.0	4.0	1.5	4.0	50.9
	1	lake sturgeon	40	3.60	5.0	4.0	1.5	4.0	62.6
Keta Creek Hatchery - Harvest Mangement Program	1	pink salmon	252	0.30	5.0	7.0	2.5	3.5	53.2
Hoko Falls Hatchery	1	sockeye salmon	120,023	0.94	5.0	4.0	5.0	4.0	52.0

**Table 2. Description of Treatment Regimes Used and Fish Species Treated during CY14 Calcein (SE- MARK<sup>®</sup>) Efficacy Studies**

---

<b>Total Number of Fish Treated:</b>	621,984
Number of fish treated in effective trials	621,984
<b>Total Number of Trials:</b>	12
Number of trials in which treatments were effective	12
<b>Treatment Regimes Used:</b>	
250 mg/L static bath for 6 hr	1 trial
2.5 - 5.0 g/L static bath for 4 - 7 min	11 trials
<b>Treatment Water Temperature (°F):</b>	44.6 - 62.6
<b>Size of Treated Fish (g):</b>	0.1 - 3.6
<b>Species Treated:</b>	
<b><u>Salmonids</u></b>	
bloater ( <i>Coregonus hoyi</i> )	
lake herring ( <i>C. artedii</i> )	
Chinook salmon ( <i>Oncorhynchus tshawytscha</i> )	
chum salmon ( <i>O. keta</i> )	
coho salmon ( <i>O. kisutch</i> )	
pink salmon ( <i>O. gorbuscha</i> )	
sockeye salmon ( <i>O. nerka</i> )	
<b><u>Non-salmonid</u></b>	
lake sturgeon ( <i>Acipenser fulvescens</i> )	
white sturgeon ( <i>A. transmontanus</i> )	