

# **Common Carp Pituitary Clinical Field Trials - INAD 8391**

## Year 2009 Annual Summary Report on the Use of Common Carp Pituitary in Field Efficacy Trials

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### **Summary**

Spawning aids such as common carp pituitary (CCP), luteinizing hormone-releasing hormone analogue, and human chorionic gonadotropin are routinely used in fisheries programs to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of CCP under the Compassionate Investigational New Animal Drug (INAD) Exemption #8391 for the purpose of gathering efficacy data to support a new animal drug approval for CCP. During calendar year 2009 (CY09), 10 INAD trials were conducted to evaluate the efficacy of CCP to induce gamete maturation in a variety of fish species. Trials involved 6,493 treated fish and were conducted at eight different fish hatcheries, including one U.S. Fish and Wildlife Service fish hatchery, one United States Department of Agriculture - Agriculture Research Station, three state hatcheries, and three private hatcheries. Efficacy was determined by whether or not treated fish (1) produced or yielded eggs or milt, or (2) produced or yielded more eggs or milt than untreated fish.

Overall results of trials conducted during this period indicated that approximately 90% of the trials appeared efficacious, and ineffective in 10% of the trials.

## **Introduction**

The use of hormones to induce spawning in fish is critical to the success of many federal, state, private, and tribal fisheries programs. A wide variety of programs, including several that involve the restoration of threatened/endangered species are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning.

The time of spawning is by its own nature a stressful period for all fish species. The handling required during the artificial spawning of fish complicates an already delicate situation. In order to maintain the health of both wild and domestic brood fish, it is beneficial to minimize overall fish handling. Successful hormone treatment can reduce handling requirements to a single hormone administration event followed by actual gamete collection, thereby greatly reducing overall fish handling.

Studies have shown that final gamete maturation in fish can be induced by the administration of a variety of hormones (Donaldson and Hunter 1983; Goetz 1983). The first reported studies investigating the hormonal control of reproduction in fish utilized intraperitoneal injection of freshly dissected pituitary glands (Houssay, 1931; von Ihering, 1937). The use of CCP was first reported in the United States by Hasler et al.,

(1939, 1940). These and many other early studies investigating the use of fish pituitaries to induce gamete maturation in a variety of fish species were thoroughly reviewed by Pickford and Atz (1957) in their comprehensive treatise on the fish pituitary gland.

The efficacy of common carp pituitary (CCP) to induce ovulation and spermiation in fish is well documented (Chaudhuri, 1976), CCP has been shown to induce gamete maturation in a wide variety of species, including certain threatened and endangered species. Common carp pituitary, which has been shown to be particularly effective when used in cool and warm water species, has had a significant, positive impact on federal, state, private, and tribal programs nationwide.

### **Purpose**

The purpose of this report is to summarize the results of CY09 supplemental CCP field efficacy trials. Furthermore, it is expected that these data will be used to enhance the existing CCP database that has been established from previous years trials for the purpose of supporting a new animal drug approval for the use of CCP in aquaculture.

## **Facilities, Materials, and Methods**

### **1. Participating Facilities**

A total of 10 trials were conducted at eight fish culture facilities during CY09, including one U.S. Fish and Wildlife Service fish hatchery, one United States Department of Agriculture - Agriculture Research Station, three state hatcheries, and three private hatcheries. Water temperature during treatments at the various testing facilities ranged from 51.0 - 80.0 °F. Overall mean treatment temperature from all trials was 64.3 °F.

### **2. CCP used in trials**

All CCP used in CY09 trials was supplied either by Stoller Fisheries, Spirit Lake, IA; or by Argent Chemical Company, Redmond, WA.

### **3. Drug dosages**

As described in the current authorization, Investigators were allowed to use CCP at doses ranging up to 25 mg CCP/kg body weight (bw). During this reporting period, the drug doses used ranged from 1.0 to 25.0 mg CCP/kg bw. CCP was administered as either a single intraperitoneal (IP) injection, or as a series of two IP injections.

## **Fish Species and Gender Treated**

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

The following four fish species were treated with CCP during the reporting period:

Atlantic sturgeon (*Acipenser oxyrinchus*)

channel catfish (*Ictalurus punctatus*)

muskellunge (*Esox masquinongy*)

plains minnow (*Hybognathus placitus*)

### **2. Gender of fish treated**

A total of 6,310 females and 183 males were injected with CCP during the reporting period. Typically, females are treated with spawning hormones to shorten the egg maturation period or synchronize ovulation. Males are treated to ensure that sufficient milt is available for egg fertilization.

## **Data Collected**

### **1. Pathologists Reports**

Fish health pathology reports provide essential information with respect to disease confirmation and general fish health; no pathology reports were submitted for CY09.

## **2. Primary response variables**

The primary response variables for evaluating the effect of CCP were (1) the relative number of female fish that ovulated following treatment, or (2) the number of male fish that reached active spermiation following treatment. With respect to the treatment of females, in some cases, percent hatch and percent eyed eggs were also determined.

## **3. Spawning interval**

The spawning interval is the period of time between the final CCP treatment and when treated fish were evaluated for gamete maturation. Where appropriate, the spawning interval was documented.

## **Discussion of Study Results**

### **1. Summary results on the efficacy of CCP to induce gamete maturation (Note:**

Tables 1 & 2 provides a summary of all efficacy trials; Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 4 describes all trials conducted during CY09 under INAD #8391).

### **A. Efficacy at 1.0 - 2.2 mg/kg bw**

Two trials were conducted in which male muskellunge and Atlantic sturgeon were injected with CCP at a dose of 1.0 or 2.2 mg/kg bw (Table 1). Following treatment, there was 100% spermiation among all treated muskellunge, while there was no spermiation among the Atlantic sturgeon; no control fish were used. The investigator involved with the Atlantic sturgeon trial noted that fish were generally small for this species and may not have been mature. Overall, treatments appeared efficacious in one trial and ineffective in one trial.

### **B. Efficacy at 6.6 mg/kg bw**

Two trials were conducted in which female muskellunge were injected with CCP at a dose of 6.6 mg/kg bw (Table 2). One trial involved the use of a non-treated control group. Following treatment, there was 80 - 100% ovulation among treated females. The control fish did not ovulate. Overall, treatment appeared efficacious in both trials.

### **C. Efficacy at 10 mg/kg bw**

Four trials were conducted in which female channel catfish were injected with CCP at a dose of 10 mg/kg bw (Table 2). One trial involved the use of a non-treated control group. Following treatment, there was 0 - 100% ovulation among treated fish (average % ripe was between 50% and

87%). The control fish did not ovulate. Overall, treatment appeared efficacious in all trials.

#### **D. Efficacy at 25 mg/kg bw**

One trial was conducted in which female plains minnow were injected with CCP at a dose of 25 mg/kg bw (Table 2). Following treatment, there was 100% ovulation among treated females; no control fish were used. Overall, treatment appeared efficacious in this trial.

One trial was conducted in which male plains minnow were injected with CCP at a dose of 25 mg/kg bw (Table 1). Following treatment, there was 100% among treated fish; no control fish were used. Overall, treatment appeared efficacious in this trial.

## **2. Observed Toxicity**

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

## **3. Observed Withdrawal Period**

No withdrawal time is needed for fish treated with CCP under the current Food-Use Authorization dated October 8, 2009.

### **Current Study Protocol for CCP INAD #8391**

Please see the attached current study protocol for CCP INAD #8391. Please note no changes have occurred to this study protocol.

### **Facility Sign-up List**

Please see “Table 4. Facilities and Names of Investigators” for facilities that signed-up to participate in the CCP INAD #8391 during CY09. Facilities not listed in Appendix III-a of the current CCP INAD #8391 study protocol have been highlighted.

The following facilities had CCP on-hand during CY09 but never used the drug:

1. Dexter NFH & TC
2. Aquaculture Research Station - LSU
3. Spirit Lake SFH

### **Correspondence sent to CCP Participants**

Please see the attached correspondence that was sent to all CCP participants after the AADAP Office received their sign-up form for calendar year 2009.

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

Total number of treated fish during CY09 was 6,493. The total number of treated fish to count against the treatment use authorization dated July 7, 2006 (valid through October 7, 2009) is 10,231. Note: a request for additional fish had been submitted to FDA on July 8, 2009 from the AADAP Office. The total number of

treated fish to count against the current treatment use authorization dated October 8, 2009, is zero.

### **Summary of Study Results**

The efficacy of CCP was evaluated in 10 trials involving Atlantic sturgeon, channel catfish, muskellunge, and plains minnow treated at doses ranging from 1.0 to 25 mg/kg bw. Treatment was administered as either a single IP injection or as a series of 2 IP injections. Of the 10 trials conducted, two utilized non-treated control groups. A total of 6,493 adult fish were treated (6,310 females and 183 males). Water temperature during treatment ranged from 51.0 to 80.0°F. Overall, results showed that CCP treatment appeared efficacious in 90% of the trials and ineffective in 10% of the trials.

Investigators reported no evidence of toxicity or adverse effects related to CCP treatment in any trial. Because of the lack of pivotal field efficacy trials, it is understood that data summarized in this report can only be considered as ancillary data. None-the-less, the ancillary data described above should provide useful corroborative data to support a new animal drug approval for CCP. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #8391. In future trials conducted under INAD #8391, efforts will be directed towards the continued generation of high quality data.

## References

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<b>Table 1. Summary of CY09 CCP Male Efficacy Results - Injection</b>							<b>Males</b>			
							<b>Treated</b>		<b>Control</b>	
<b>Facility</b>	<b>Efficacy</b>	<b>Number of Trials</b>	<b>Species</b>	<b>Dose (mg/kg)</b>	<b>Spawning Interval (hr)</b>	<b>Observed Withdrawal Period (days)</b>	<b>Number Treated</b>	<b>% Spermiote</b>	<b>Number Controls</b>	<b>% Spermiote</b>
Bears Bluff NFH	ineffective	1	ASN	1.0	48 - 72	No release	3	0	0	na
Table Rock SFH	effective	1	MUE	2.2	48	> 2yrs	9	100	0	na
Native Aquatic Species Restoration Facility	effective	1	PLM	25	24 - 72	No release	171	100	0	na

Table 2. Summary of CY09 CCP Female Efficacy Results - <u>Injection</u>							Females			
							Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (mg/kg)	Spawning Interval (hr)	Observed Withdrawal Period (days)	Number Treated	% Ovulate	Number Controls	% Ovulate
Baxter Land Co.	effective	1	CCF	10	36	No release	2,621	48 - 96 (ave. 82)	0	na
Belle Prairie	effective	1	CCF	10	27	>100	1,579	85 - 92 (ave. 87)	0	na
Catfish Genetics Research Unit	effective	1	CCF	10	20 - 32	90	394	0 - 100 (ave. 58)	15	0
NeedMore Fisheries	effective	1	CCF	10	30	30	1,470	16 - 85 (ave. 50)	0	na
Hackettstown SFH	effective	1	MUE	6.6	3 - 6 days	30	32	100	5	0
Table Rock SFH	effective	1	MUE	6.6	48 - 72	>2 yrs	5	80	0	na
Native Aquatic Species Restoration Facility	effective	1	PLM	25	24 - 72 hrs	No release	209	100	0	na

**Table 3. Description of number of trials conducted, species and number of fish treated, and treatment regimens used in CY09 under INAD #8391**

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<b>Total Number of Trials Conducted:</b>	10
Number of Efficacious Trials:	9
Number of Ineffective Trials:	1
<b>Total Number of Fish Treated:</b>	6,493
Number of fish treated in efficacious trials	6,490
Number of fish treated in ineffective trials	3
<b>Treatment Regimes Used:</b>	
1.0 - 2.2 mg/kg body weight (1 injection)	2 trials
6.6 mg/kg body weight (1 injection)	2 trials
10.0 mg/kg body weight (2 injections)	4 trials
25.0 mg/kg body weight (1 injection)	2 trials
<b>Treatment Water Temperature (°F):</b>	51.0 - 80.0
<b>Size of Treated Fish:</b>	Adult
<b>Species Treated:</b>	Atlantic sturgeon ( <i>Acipenser oxyrhynchus</i> ) channel catfish ( <i>Ictalurus punctatus</i> ) muskellunge <i>Esox masquinongy</i> plains minnow <i>Hybognathus placitus</i>

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