Common Carp Pituitary Clinical Field Trials - INAD 8391

Year 2010 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. In calendar year 2010 (CY10), 27 INAD trials were conducted to evaluate the efficacy of CCP to induce gamete maturation in a variety of fish species. Trials involved 11,767 treated fish and were conducted at 13 different fish hatcheries, including two U.S. Fish and Wildlife Service fish hatcheries, one United States Department of Agriculture - Agriculure Research Station, six state hatcheries, and four 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 in CY10 indicated that treatments appeared efficacious in approximately 67% of the trials, ineffective in 30% of the trials, and were characterized as inconclusive in 3% 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 CY10 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 27 trials were conducted at 13 fish culture facilities during CY10, including two U.S. Fish and Wildlife Service fish hatchery, one United States Department of Agriculture - Agriculure Research Station, six state hatcheries, and four 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 66.7 °F.

2. CCP used in trials

All CCP used in CY10 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 to 25 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 10 fish species were treated with CCP during the reporting period:

Non-salmonids

blue catfish (*Ictalurus furcatus*)

blue sucker (*Cycleptus elongatus*)

channel catfish (*Ictalurus punctatus*)

common carp (*Cyprinus carpio*)

grass carp (*Ctenopharyngodon idella*)

largemouth bass (*Micropterus salmoides*)

muskellunge (*Esox masquinongy*)

plains minnow (*Hybognathus placitus*)

suckermouth minnow (*Phenacobius mirabilis*)

Non-salmonid Marine

Atlantic sturgeon (*Acipenser oxyrhynchus*)

2. Gender of fish treated

A total of 11,358 females and 409 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. Primary response variable (Maturation)

The primary response variable for evaluating the effect of CCP on fish was the percentage of ripe fish following treatment. These percentages reflected the number of female fish that ovulated and the number of male fish that reached active spermiation.

2. Egg development and milt evaluation

Secondary response variables for females included the relative number of eggs that reached the eyed stage and the number hatched. Secondary response variables for males included the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa).

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 CY10 under INAD #8391).

A. Efficacy of CCP on male fish treated between 1.0 and 25 mg/kg bw (1 injection)

Male fish were treated in nine trials and injected 1 times with CCP at a dosage between 1 and 25 mg/kg bw (Table 1) to induce gamete maturation. Fish species treated included Atlantic sturgeon, blue sucker, grass carp, largemouth bass, muskellunge, plains minnow, and suckermouth minnow; the largemouth bass trial included a non-treated control group. Following treatment, there was 0 - 100% spermiation among all treated fish; as compared to no spermiation in the control fish. Overall, treatments appeared efficacious in five trials involving 398 fish, ineffective in three trials involving 7 fish, and was characterized as inconclusive in one trial involving 4 fish.

B. Efficacy of CCP on female fish treated between 2.7 and 25 mg/kg bw (1 - 2 injections)

Female fish were treated in 18 trials and injected 1 - 2 times with CCP at a dosage between 2.7 and 25 mg/kg bw (Table 2) to induce gamete maturation. Fish species treated included blue catfish, blue sucker, channel catfish, common carp, grass carp, largemouth bass, muskellunge, plains minnow, and suckermouth minnow; the largemouth bass trial and two muskellunge trials included non-treated control groups. Following treatment, there was 0 - 100% ovulation among all treated fish; as

compared to no ovulation in the control fish. Overall, treatments appeared efficacious in 13 trials involving 11,339 fish, and ineffective in five trials involving 19 fish.

2. Observed Toxicity

No toxicity or adverse effects relating to CCP treatment were reported in 25 of the trials. In the remaining two trials the investigator noted that a few fish had died but the cause of death was not established, but likely due to handling.

3. Observed Withdrawal Period

No withdrawal time is needed for fish treated with CCP under the 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 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the CCP INAD #8391 during CY10. Facilities not listed in Appendix III-a of the current CCP INAD #8391 study protocol have been highlighted.

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 CY10.

Number of Treated Fish under Treatment Use Authorization

Total number of treated fish during CY10 was 11,767. The total number of treated fish to count against the treatment use authorization dated October 8, 2009 (valid through April 20, 2011) is 11,767. Note: a new authorization was received April 21, 2011.

Summary of Study Results

The efficacy of CCP was evaluated in 27 trials involving 10 different fish species treated at doses ranging from 1 to 25 mg/kg bw. Treatment was administered as either a single IP injection or as a series of 2 IP injections. Control fish were used in four trials. A total of 11,767 adult fish were treated (11,358 females and 409 males). Water temperature during treatment ranged from 51.0 to 80.0°F. Overall, results showed that CCP treatment appeared efficacious in 67% of the trials, ineffective in 30% of the trials, and was characterized as inconclusive in 3% of the trials. Investigators reported no evidence of toxicity or adverse effects related to CCP treatment in 25 trials. 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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Table 1. Summary of CY10 CCP Male Efficacy Results - Injection						Males			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Spermiate**	**Number Controls**	**% Spermiate**
Bears Bluff NFH	inconclusive	1	ASN	1	24	4	25	0	na
Bears Bluff NFH	ineffective	1	ASN	1	24	4	0	0	na
Gavins Point NFH	effective	1	BSU	1	20	1	100	0	na
E.W. Shell Fisheries Center	effective	1	GRC	2	21	4	100	0	na
Manning SFH	ineffective	1	LMB	5	48	1	0	1	0
Table Rock SFH	effective	1	MUE	2.2	96 - 144	11	100	0	na
Manning SFH	ineffective	1	MUE	6.6	-	2	0	0	na
Native Aquatic Species Restoration Facility	effective	1	PLM	25	24	364	100	0	na
Native Aquatic Species Restoration Facility	effective	1	SMM	25	24 - 72	18	100	0	na

Table 2. Summary of CY10 CCP Female Efficacy Results - Injection						Females			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Ovulate**	**Number Controls**	**% Ovulate**
Catfish Genetics Research Unit	effecitive	1	BCF	10	20 - 32	30	67 - 80 (ave 73)	0	na
Gavins Point NFH	ineffective	1	BSU	2.7	20	5	0	0	na
E.W. Shell Fisheries Center	effective	1	CAP	4	13	3	100	0	na
E.W. Shell Fisheries Center	ineffective	2	CAP	4	12	12	? & 50	0	na
Baxter Land Co.	effective	1	CCF	10	24 - 36	2,345	64 - 98 (ave. 85)	0	na
Belle Prairie	effective	1	CCF	10	27	4,928	81.1	0	na
Catfish Genetics Research Unit	effective	1	CCF	10	20 - 32	60	25 - 70 (ave. 53)	0	na
NeedMore Fisheries	effective	[1]	CCF	10	38	3,332	36 - 91 (ave. 64)	0	na
Nobile Farms	effective	1	CCF	10	24	93	54.5 - 58.0 (ave. 56)	0	na
E.W. Shell Fisheries Center	effective	1	GRC	4	21	3	100	0	na
Manning SFH	ineffective	1	LMB	5	48	1	0	1	0
Hackettstown SFH	effective	1	MUE	6.6	9 days	25	0 - 100 (ave. 92)	2	0
Manning SFH	ineffective	1	MUE	6.6	-	1	0	0	na

Table 2. Summary of CY10 CCP Female Efficacy Results - Injection						Females			
						Treated		Control	
Facility	**Efficacy**	**Number of Trials**	**Species**	**Dose (mg/kg)**	**Spawning Interval (hr)**	**Number Treated**	**% Ovulate**	**Number Controls**	**% Ovulate**
Spirit Lake SFH	effective	1	MUE	6.52	96	17	100	5	0
Table Rock SFH	effective	1	MUE	6.6	4 - 6 days	3	100	0	na
Native Aquatic Species Restoration Facility	effective	1	PLM	25	24	479	100	0	na
Native Aquatic Species Restoration Facility	effective	1	SMM	25	24 - 72	21	100	0	na

Table 3. Description of number of trials conducted, species and number of fish treated, and treatment regimens used in CY10 under INAD #8391

Total Number of Trials Conducted:	27
Number of Efficacious Trials:	18
Number of Ineffective Trials:	8
Number of Inconclusive Trials:	1
Total Number of Fish Treated:	11,767
Number of fish treated in efficacious trials	11,737
Number of fish treated in ineffective trials	26
Number of fish treated in inconclusive trials	4

Treatment Regimes Used:

1 - 10 mg/kg body weight (1 - 2 injections)	23 trials
25 mg/kg body weight (1 injection)	4 trials

Treatment Water Temperature (^{o}F): 51.0 - 80.0

Size of Treated Fish: Adult

Species Treated:

Non-salmonids
blue catfish (*Ictalurus furcatus*)
blue sucker (*Cycleptus elongatus*)
channel catfish (*Ictalurus punctatus*)
common carp (*Cyprinus carpio*)
grass carp (*Ctenopharyngodon idella*)
largemouth bass (*Micropterus salmoides*)
muskellunge (*Esox masquinongy*)
plains minnow (*Hybognathus placitus*)
suckermouth minnow (*Phenacobius mirabilis*)

Non-salmonid Marine
Atlantic sturgeon (*Acipenser oxyrhynchus*)
