

LHRH_a Spawning Hormone Clinical Field Trials - INAD 8061

2012 - 2014 Annual Summary Report on the Use of LHRH_a in Clinical Field Efficacy Trials

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Summary

Spawning aids such as luteinizing hormone-releasing hormone analogue (LHRH_a), human chorionic gonadotropin, and common carp pituitary are routinely used in aquaculture to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of LHRH_a under the Compassionate Investigational New Animal Drug (INAD) Exemption #8061 for the purpose of gathering efficacy data to support a new animal drug approval for LHRH_a. In calendar year 2012 - 14 (CY12-14), 80 trials were conducted under this INAD to evaluate the efficacy of LHRH_a to induce gamete maturation in a variety of fish species. Trials involved 79,667 treated fish and 360 control fish and were conducted at 26 different hatcheries, including six U.S. Fish and Wildlife Service fish hatcheries, six state hatcheries, one university, 12 private hatcheries, and one tribal hatchery during this period. Efficacy was determined by whether or not treated fish produced or yielded more eggs or milt than untreated fish. Overall results from trials conducted in CY12-14 showed that treatments appeared efficacious in approximately 95% of the trials and ineffective in 5% 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 many 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. Both sexes are undergoing significant changes in physiology, morphology, and behavior (Hoar, 1969). The additional handling of fish required during the spawning process complicates an already delicate situation. This is particularly true for wildstock species that must endure the added stresses of capture, handling, and confinement in an un-natural environment. In fact, with respect to some wildstock species, the stress of capture alone is often sufficient to cause complete reproductive failure unless spawning is induced by hormone treatment. Hormone treatment in a variety of fish species is essential to ensure optimal spawning success.

Studies have shown that final gamete maturation (ovulation and spermiation) in fish can be induced by the administration of a variety of hormones (Donaldson and Hunter 1983; Goetz 1983). Recent investigations have found luteinizing hormone-releasing hormone analogue (LHRH_a) to be one of the most effective means of inducing final gamete maturation. This compound is a synthetic gonadotropin releasing hormone that is similar in structure to native luteinizing hormone-releasing hormones. LHRH_a is an attractive choice as it has both a high biological activity and low species specificity, making it appropriate for use on a variety of fish species (Coy et al. 1974). Although

the use of LHRH_a as a tool to enhance broodstock spawning success is relatively new, it has already had a significant, positive impact on fisheries programs nationwide.

Purpose of Report

The purpose of this report is to summarize the results of LHRH_a field efficacy trials conducted under INAD exemption #8061 in CY12-14. Furthermore, it is expected that these data will be used to enhance the existing LHRH_a database that has been established from previous years trials for the purpose of developing an appropriate label claim for the use of this new drug.

Facilities, Materials, and Treatment Procedures

1. Facilities

Field efficacy trials were conducted at 26 different fish culture facilities during CY12-14, including six U.S. Fish and Wildlife Service fish hatcheries, six state hatcheries, one university, 12 private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 42.0 to 86.0EF. Overall mean treatment temperature from all trials was 63.4 EF.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all LHRH_a used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 100 ug LHRH_a/kg fish body weight (bw). During this reporting period, the drug doses used ranged from 5 to 100 ug LHRH_a/kg fish body. LHRHa was administered as either a single injection or as a series of 2 - 4 injections.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on 15 different fish species under INAD #8061 during the reporting period, including four salmonid and 11 non-salmonid species:

Salmonids

brook trout (*Salvelinus fontinalis*)

lake trout (*S. namaycush*)

Gila trout (*Oncorhynchus gilae*)

rainbow trout (*O. mykiss*)

Non-salmonids

alligator gar (*Lepisosteus spatula*)

channel catfish (*Ictalurus punctatus*)

lake sturgeon (*Acipenser fulvescens*)

Russian surgeon (*A. gueldenstaedtii*)

starry sturgeon (*A. stellatus*)
white sturgeon (*A. transmontanus*)
pallid sturgeon (*Scaphirhynchus albus*)
shovelnose sturgeon (*S. platyrhynchus*)
largemouth bass (*Micropterus salmoides*)
paddlefish (*Polydon spathula*)
striped bass (*Morone saxatilis*)

2. Gender of treated fish

LHRH_a was used on 79,094 female and 573 male fish during the reporting period.

Typically, females were treated with spawning hormone to shorten the gamete maturation period (i.e. advance maturation), while males were treated to ensure that sufficient milt would be available for egg fertilization.

Data Collected

1. Primary response variable (Maturation)

The primary response variable for evaluating the effect of LHRH_a 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).

3. Spawning interval

The time period between the last treatment and when fish were spawned or evaluated for ripeness was also documented. In the case of females, which in some cases received a priming dose followed a short time later by a resolving dose, the spawning interval was defined as the time period between administration of the resolving dose and spawning.

Discussion of Study Results

1. General observations on the efficacy of LHRH_a to induce gamete maturation in salmonid and non-salmonid fish (Note: Tables 1 - 2 provides summaries of all efficacy trials; and Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used during CY12-14 under INAD #8061.) Due to the online database reporting system, both male and female studies may be reported under a single study number and reported as one trial. In past years the males and female reports were broken out.

A. Efficacy of LHRH_a on male fish treated at a dosage between 10 and 100 ug/kg body weight (1 - 3 injections)

Male fish were treated in 48 trials and injected 1 - 3 times with LHRHa at a dosage between 10 and 100 mg/kg bw (Table 1) to induce gamete maturation. Fish species treated included alligator gar, Gila trout, lake sturgeon, largemouth bass, paddlefish, pallid sturgeon, Russian sturgeon, shovelnose sturgeon, starry sturgeon, and white sturgeon. Fourteen trials included non-treated control groups. Following treatment, there was 0 - 100% spermiation among all treated fish; as compared to 0 - 100% spermiation in the control fish. Treatments appeared efficacious in 45 trials involving 561 fish and was characterized as ineffective in three trials involving 12 fish.

B. Efficacy of LHRH_a on female fish treated at a dosage between 5 and 100 ug/kg body weight (1 - 4 injections)

Female fish were treated in 79 trials and injected 1 - 4 times with LHRHa at a dosage between 5 and 100 mg/kg bw (Table 2) to induce gamete maturation. Fish species treated included alligator gar, brook trout, channel catfish, Gila trout, lake sturgeon, lake trout, largemouth bass, paddlefish, pallid sturgeon, rainbow trout, Russian sturgeon, shovelnose sturgeon, starry sturgeon, striped bass, and white sturgeon. Eighteen trials included non-treated control groups. Following treatment, there was 0 - 100% ovulation among all treated fish; as compared to 0 - 100% ovulation in the control fish. Overall, treatments appeared efficacious in 75 trials involving 79,086 fish, and ineffective in three trials involving 8 fish.

2. Observed Toxicity

No toxicity or adverse effects relating to LHRH_a treatments were reported in 79 of the trials conducted in CY12-14. In one trial the investigator noted that two of the treated females appeared to have air in the body cavity.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for LHRHa INAD #8061

No changes have occurred to the current study protocol for LHRHa INAD #8061.

Facility Sign-up List

Please see ATable 4. Facilities and Names of Investigators@ for facilities that signed-up to participate in the LHRHa INAD #8061 during CY12-14. The following facilities had LHRHa on hand, but did not use it under the INAD:

Bears Bluff NFH

Bluebeyond Fisheries

Correspondence sent to LHRHa Participants

Please see the attached correspondence that was sent to all LHRHa participants after the AADAP Office received their sign-up form for CY12-14.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY12-14 was 79,667. The total number of treated fish to count against the food use authorization dated March 29, 2010 is 10,000 (valid through June 18, 2012). The total number of treated fish to count against the food use authorization dated June 19, 2012 is 20,000 (valid through September 9, 2014). The total number of treated fish to count against the food use authorization dated September 10, 2014 is 25,000 (valid through July 26, 2015). Please note that the remaining 33,381 treated fish are counted against the food use authorization dated July 27, 2015. The drug receipt reports will be updated to reflect this overage number. It was discovered that the food use authorization numbers were not factored into the equation when calculating the number of fish left under the authorizations.

Summary of Study Results

LHRH_a was used in 80 efficacy trials to induce gamete maturation in 15 different fish species (n = 79,667 treated fish; 360 untreated control fish) at dosages ranging from 5 - 100 ug/kg bw. LHRH_a was administered using either 1 injection or a series of 2 - 4 injections. Water temperature during treatments ranged from 42.0 – 86.0EF. Overall, results showed that LHRHa treatment appeared efficacious in 95% of the trials and ineffective in 5% of the trials. Data from the CY12-14 trials support the results of previous Annual Report submissions under INAD #8061 that indicate that LHRH_a treatment was efficacious in inducing gamete maturation in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #8061 will also be ancillary data, efforts will be made to improve the quality of data whenever possible.

References

Coy, D.H., E.J. Coy, A.V. Schally, J. Vilchez-Martinez, Y. Hirotsu, and A. Arimura. 1974. Synthesis and biological properties of D-Ala⁶,des Gly¹⁰LH-RH ethylamide, a peptide with greatly enhanced LH and FSH releasing activity. *Biochemical and Biophysical Research Communication*. 57(2): 335-340.

Donaldson, E.M., and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. Pages 351-403 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. *Fish physiology*, volume 9. Part B. Academic Press, New York.

Goetz, F.W. 1983. Hormonal control of oocyte maturation and ovulation in fishes. In: *Fish Physiology Vol IX, Part B*. Eds. W.S. Hoar, D.J. Randall and E.M. Donaldson. Academic Press, New York. pp. 117-169.

Hoar, W.S. 1969. Reproduction. In: *Fish Physiology Volume III*. Eds. W.S. Hoar and D.J. Randall. Academic Press, New York and London. pp.1-72.

Table 1. Summary of Year 2012 - 2014 LHRH _a Male Efficacy Results						Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (ug/Kg b.w.)	Spawning Interval (days)	Number Treated	% Spermiate	Number of Controls	% Spermaite
Pvt. John Allen NFH	Effective	3	Alligator Gar	100	2	27	55.6 - 100	0	-
Tishomingo NFH	Effective	1	Alligator Gar	100	1	3	100	0	-
Mora NFH	Effective	1	Gila Trout	10	7	23	43.5 - 76.9	23	52.2 - 100
USFWS-NYFO	Effective	3	Lake Sturgeon	10	0.75 - 1	12	83.3 - 100	2	100
Wild Rose SFH	Effective	1	Lake Sturgeon	10	1	1	100	0	-
Dunns Fish Farm Inc	Effective	1	Largemouth Bass	50	1	175	100	0	-
ARC - Kentucky State University	Effective	3	Paddlefish	50	0.5 - 1	18	70 - 100	0	-
Booker Fowler SFH	Effective	3	Paddlefish	10	1	48	75 - 100	0	-
Gavins Point NFH	Effective	3	Paddlefish	10 - 20	2	38	53.8 - 100	0	-
Pvt. John Allen NFH	Effective	2	Paddlefish	100	1 - 2	7	100	0	-
Tishomingo NFH	Effective	3	Paddlefish	100	1	56	66.7 - 100	3	0
Gavins Point NFH	Effective	1	Pallid Sturgeon	10 - 20	2	21	42.9 - 100	0	-
Evans Fish Farms	Effective	1	Russian Sturgeon	40	1	2	100	0	-
Evans Fish Farms	Ineffective	1	Russian Sturgeon	40	7	3	33.3	5	0
Valentine SFH	Effective	2	Shovelnose Sturgeon	10	1	8	50 - 75	2	0
Valentine SFH	Ineffective	1	Shovelnose Sturgeon	10	3	3	0	0	-
Evans Fish Farms	Effective	3	Starry Sturgeon	20 - 40	2 - 3	27	0 - 100	42	0 - 50
Big Bend Trout	Ineffective	1	White Sturgeon	20	1	6	0	6	0
Blind Canyon Aquaranch	Effective	6	White Sturgeon	20	1 - 3	33	50 - 100	0	-

Table 1. Summary of Year 2012 - 2014 LHRH_a Male Efficacy Results						Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (ug/Kg b.w.)	Spawning Interval (days)	Number Treated	% Spermiote	Number of Controls	% Spermaite
Kootenai Tribal Conservation Aquaculture	Effective	3	White Sturgeon	30	1 - 2	12	33.3 - 100	0	-
Sterling Caviar	Effective	5	White Sturgeon	10	1.5	50	50 - 70	50	0

Table 2. Summary of Year 2012 - 2014 LHRH_a Female Efficacy Results						Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (ug/Kg b.w.)	Spawning Interval (days)	Number Treated	% Ovulate	Number of Controls	% Ovulate
Pvt. John Allen NFH	Effective	3	Alligator Gar	100	2	14	66.7 - 100	0	-
Tishomingo NFH	Effective	1	Alligator Gar	100	1	1	100	0	-
Iron River NFH	Effective	2	Brook Trout	15 - 20	7 - 9	1,264	62.0 - 100	89	83.3 - 100
Americas Catch Catfish Farm, Inc.	Effective	1	Channel Catfish	100	1	2,320	25 - 100	0	-
Baxter Land Company	Effective	3	Channel Catfish	100	1 - 3	11,760	20 - 98	0	-
Belle Prairie	Effective	1	Channel Catfish	100	2.25	5,271	86.8	0	-
Jubilee Farms, Inc.	Effective	3	Channel Catfish	100	1 - 3	24,034	55.3 - 98.7	0	-
Needmore Fisheries	Effective	4	Channel Catfish	100	1 - 2	23,340	17.6 - 99.0	0	-
Tackett Fish Farm	Effective	1	Channel Catfish	100	3	4,362	60.5	0	-
Mora NFH	Effective	1	Gila Trout	10	7	23	50 - 100	22	56.5 - 100
USFWS-NYFO	Effective	3	Lake Sturgeon	50 - 100	1	4	100	0	-
Wild Rose SFH	Effective	3	Lake Sturgeon	100	1	32	25 - 100	6	0
Iron River NFH	Effective	3	Lake Trout	10 - 20	5 - 11	467	30.5 - 100	29	100
Dunns Fish Farm Inc	Effective	1	Largemouth Bass	100	1	175	0 - 80	0	-
ARC - Kentucky State University	Effective	3	Paddlefish	100	0.5 - 1	14	100	0	-
Booker Fowler SFH	Effective	3	Paddlefish	100	1	17	0 - 100	0	-
Gavins Point NFH	Effective	3	Paddlefish	50 - 100	2	18	75 - 100	0	-
Osage Catfisheries Inc.	Effective	4	Paddlefish	100	1 - 2	20	100	0	-
Osage Catfisheries Inc.	Ineffective	1	Paddlefish	100	2	3	0	0	-
Pvt. John Allen NFH	Effective	2	Paddlefish	100	1 - 2	5	50 - 100	0	-
Tishomingo NFH	Effective	3	Paddlefish	12.5 - 25	1	43	20 - 100	9	0
Gavins Point NFH	Effective	1	Pallid Sturgeon	50 - 100	2	8	100	0	-
Fall River SFH	Effective	2	Rainbow Trout	5 - 10	1	5,700	40 - 88.3	0	-

Table 2. Summary of Year 2012 - 2014 LHRH_a Female Efficacy Results						Treated		Control	
Facility	Efficacy	Number of Trials	Species	Dose (ug/Kg b.w.)	Spawning Interval (days)	Number Treated	% Ovulate	Number of Controls	% Ovulate
Evans Fish Farms	Effective	1	Russian Sturgeon	40	1	2	100	0	-
Evans Fish Farms	Ineffective	1	Russian Sturgeon	40	7	3	0	5	0
Valentine SFH	Effective	2	Shovelnose Sturgeon	100	1	3	100	1	0
Valentine SFH	Ineffective	1	Shovelnose Sturgeon	100	3	2	0	0	-
Evans Fish Farms	Effective	3	Starry Sturgeon	40 - 60	2 - 3	6	33.3 - 100	37	0 - 50
Blackwater Fisheries Center	Effective	2	Striped Bass	25	2 - 7	27	0 - 100	0	-
Milford SFH	Effective	3	Striped Bass	18.7 - 57.2	2 - 3	81	40 - 100	0	-
Blind Canyon Aquaranch	Effective	6	White Sturgeon	50	1 - 3	19	50 - 100	0	-
Kootenai Tribal Conservation Aquaculture	Effective	3	White Sturgeon	100	1 - 2	27	0 - 100	0	-
Sterling Caviar	Effective	5	White Sturgeon	20	1.5	29	66.7 - 100	29	0

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2012 - 2014 LHRH_a Efficacy Studies

Total Number of Treatment Trials	80
Number of Trials that Appeared Efficacious:	76
Number of Trials that Appeared Inefficacious:	4
 Total Number of Treated Fish:	 79,667
Number of fish treated in efficacious trials	79,647
Number of fish treated in ineffective trials	20
 Treatment Regimes Used:	
5 - 100 ug/Kg body weight (1 - 4 injections)	80 trials
 Water Temperature (EF) Range:	 42.0 - 86.0
 Fish Species Treated:	
<u>Salmonids</u>	
brook trout (<i>Salvelinus fontinalis</i>)	
lake trout (<i>S. namaycush</i>)	
Gila trout (<i>Oncorhynchus gilae</i>)	
rainbow trout (<i>O. mykiss</i>)	
<u>Non-salmonids</u>	
alligator gar (<i>Lepisosteus spatula</i>)	
channel catfish (<i>Ictalurus punctatus</i>)	
lake sturgeon (<i>Acipenser fulvescens</i>)	
Russian surgeon (<i>A. gueldenstaedtii</i>)	
starry sturgeon (<i>A. stellatus</i>)	
white sturgeon (<i>A. transmontanus</i>)	
pallid sturgeon (<i>Scaphirhynchus albus</i>)	
shovelnose sturgeon (<i>S. platorynchus</i>)	

largemouth bass (*Micropterus salmoides*)
paddlefish (*Polydon spathula*)
striped bass (*Morone saxatilis*)

Size Class of Treated Fish:

Adults