

LHRH_a Spawning Hormone Clinical Field Trials - INAD 8061

2010 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 2010 (CY10), 41 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 1,739 treated fish and 82 control fish and were conducted at 19 different hatcheries, including four U.S. Fish and Wildlife Service fish hatcheries, one United States Department of Agriculture - Agriculture Research Station, seven state hatcheries, six 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 CY10 showed that treatments

appeared efficacious in approximately 80% of the trials and was ineffective in 20% 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 unnatural 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 CY10. 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 19 different fish culture facilities during CY10, including four U.S. Fish and Wildlife Service fish hatcheries, one United States Department of Agriculture - Agriculture Research Station, seven state hatcheries, six private hatcheries, and one tribal hatchery. Water temperature

during treatments at the various testing facilities ranged from 42.0 to 80.6°F.

Overall mean treatment temperature from all trials was 63.0 °F.

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 10 to 100 ug LHRH_a/kg fish body. LHRHa was administered as either a single injection or as a series of 2 - 3 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 one salmonid; 12 non-salmonid; and two marine non-salmonid species:

Salmonids

rainbow trout *Oncorhynchus mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

lake sturgeon *Acipenser fulvescens*

Russian surgeon *A. gueldenstaedtii*

Siberian sturgeon *A. baerii*

starry sturgeon *A. stellatus*

white sturgeon *A. transmontanus*

channel catfish *Ictalurus punctatus*

largemouth bass *Micropterus salmoides*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *S. platyrhynchus*

striped bass *Morone saxatilis*

Marine non-salmonids

Atlantic sturgeon *Acipenser oxyrhynchus*

red drum *Sciaenops ocellatus*

2. Gender of treated fish

LHRH_a was used on 1,624 female and 115 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; 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 #8061.)

A. Efficacy of LHRH_a on male fish treated at a dosage between 10 and 100 ug/kg body weight (1 - 2 injections)

Male fish were treated in 17 trials and injected 1 - 2 times with LHRHa at a dosage between 10 and 100 mg/kg bw (Table 1) to induce gamete maturation. Fish species treated included Atlantic sturgeon, alligator gar, lake sturgeon, largemouth bass, paddlefish, pallid sturgeon, red drum, Russian sturgeon, shovelnose sturgeon, and white sturgeon. Five trials included non-treated control groups. 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 13 trials involving 103 fish and ineffective in four trials involving 12 fish.

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

Female fish were treated in 24 trials and injected 1 - 3 times with LHRH_a at a dosage between 10 and 100 mg/kg bw (Table 2) to induce gamete maturation. Fish species treated included alligator gar, channel catfish, lake sturgeon, largemouth bass, paddlefish, pallid sturgeon, rainbow trout, red drum, Russian sturgeon, shovelnose sturgeon, Siberian sturgeon, starry sturgeon, striped bass, and white sturgeon. Nine 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 20 trials involving 1,603 fish, and ineffective in four trials involving 21 fish.

2. Observed Toxicity

No toxicity or adverse effects relating to LHRH_a treatments were reported in 39 trials conducted in CY10. The investigator noted in two studies involving paddlefish some fish increased swimming speed and were agitated after injection.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for LHRHa INAD #8061

Please see the attached current study protocol for LHRHa INAD #8061. 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 LHRHa INAD #8061 during CY10. Facilities not listed in Appendix III-a of the current LHRHa INAD #8061 study protocol have been highlighted.

The following facility had LHRHa on station during CY10 but never used the drug:

1. Dexter NFH & TC
2. Jubilee Farms
3. Mora NFH &TC

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 CY10.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY10 was 1,739. The total number of treated fish to count against the food use authorization dated August 15, 2003 (valid through March 28, 2010) is 5,924. The total number of treated fish to count against the current food-use authorization dated March 29, 2010 is 1,126.

Summary of Study Results

LHRH_a was used in 41 efficacy trials to induce gamete maturation in 15 different fish species (n = 1,739 treated fish; 82 untreated control fish) at dosages ranging from 10 - 100 ug/kg bw. LHRH_a was administered using either 1 - 3 injections. Water temperature during treatments ranged from 42.0 - 80.6°F. Overall, results showed that LHRHa treatment appeared efficacious in 80% of the trials and was ineffective in 20% of the trials. Data from the CY10 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 2010 LHRH_a Male Efficacy Results

Number of Trials	Efficacy	Species	Facility	Spawning Interval (hrs)	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
1	effective	ALG	Private John Allen NFH	24	11	100	100	0	-
1	effective	ASN	Manning SFH	24	3	30	33	0	-
2	ineffective	ASN	Manning SFH	24	9	30	3	0	-
1	effective	HNS	Bozeman FTC	24	6	20	33 - 67 (ave. 50%)	0	-
1	ineffective	LMB	Manning SFH	48	1	20	0	1	0
1	effective	LST	NYSDEC Region 6	20	9	10	100	0	-
1	effective	PAH	Aquaculture Research Center	12 - 24	2	50	100	0	-
1	effective	PAH	Booker Fowler SFH	24	10	10	100	0	-
1	effective	PAH	Private John Allen NFH	22.75	5	100	100	0	-
1	effective	PAH	Tishomingo NFH	16 - 40	22	25	91	3	0
1	effective	PLS	Bozeman FTC	24	4	20	100	0	-
1	ineffective	RDM	Bears Bluff NFH	24 - 48	2	50	0	0	-
1	effective	RSN	Evan Fish Farm	36	6	40	100	4	0
1	effective	WST	Blind Canyon Aquaranch	24 - 48	5	20	40	0	-
2	effective	WST	Sterling Caviar LLC	20	20	10	70	20	0

Table 2. Summary of Year 2010 LHRH_a Female Efficacy Results

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval (hrs)	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	ALG	Private John Allen NFH	24	8	100	0 - 50 (ave. 25)	0	-
1	effective	CCF	Baxter Land Company	24 - 72	367	100	72 - 84	0	-
1	effective	CCF	Catfish Genetics Research Unit	30-46	187	100	29 - 88 (ave. 74)	30	0
1	effective	CCF	NeedMore Fisheries	38	394	100	6 - 88 (ave. 58)	0	-
1	effective	HNS	Bozeman FTC	24	3	20	100	0	-
1	ineffective	LMB	Manning SFH	48	1	20	0	1	0
1	effective	LST	NYSDEC Region 6	20	3	50	100	0	-
1	effective	LST	Wild Rose SFH	24 - 48	14	90	0 - 100 (ave. 64)	1	100
1	effective	PAH	Aquaculture Research Center	12 - 24	3	100	100	0	-
1	effective	PAH	Booker Fowler SFH	24	5	100	100	0	-
1	effective	PAH	Osage Catfisheries	48	6	100	100	0	-
1	effective	PAH	Private John Allen NFH	22.75	4	100	25	0	-
1	effective	PAH	Tishomingo NFH	16 - 40	22	25 & 50	95	3	0
1	effective	PLS	Bozeman FTC	24	2	50	100	0	-
1	effective	RBT	Fall River SFH	36 days	530	10	78	0	-
1	ineffective	RDM	Bears Bluff NFH	24 - 48	4	50	0	0	-

Table 2. Summary of Year 2010 LHRH_a Female Efficacy Results

Number of Trials	Efficacy	Species	Facility	Spawning Interval (hrs)	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	ineffective	RSN	Evan Fish Farm	36	11	40	0	4	0
1	ineffective	SBS	Evan Fish Farm	36	5	40	0	3	0
1	effective	STB	Milford SFH	48	30	16.7 - 27.8	93.3	0	-
1	effective	SVN	Evan Fish Farm	36	2	40	100	2	0
1	effective	WST	Blind Canyon Aquaranch	12 - 17.5	2	50	100	0	-
1	effective	WST	Kootenai Tribal Hatchery	28 - 71	11	100	100	0	-
2	effective	WST	Sterling Caviar	20	10	20	66 - 100	10	0

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2010 LHRH_a Efficacy Studies

Total Number of Treatment Trials	41
Number of Trials that Appeared Efficacious:	33 (80%)
Number of Trials that Appeared Inefficacious:	8 (20%)
Total Number of Treated Fish:	1,739
Number of fish treated in efficacious trials	1,706
Number of fish treated in ineffective trials	33
Treatment Regimes Used:	
10 - 100 ug/Kg body weight (1 - 3 injections)	41 trials

Water Temperature (°F) Range: 42.0 - 80.6

Fish Species Treated:

Salmonids

rainbow trout *Oncorhynchus mykiss*

Non-salmonids

alligator gar <i>Lepisosteus spatula</i>	lake sturgeon <i>Acipenser fulvescens</i>
Russian surgeon <i>A. gueldenstaedtii</i>	Siberian sturgeon <i>A. baerii</i>
starry sturgeon <i>A. stellatus</i>	white sturgeon <i>A. transmontanus</i>
channel catfish <i>Ictalurus punctatus</i>	largemouth bass <i>Micropterus salmoides</i>
paddlefish <i>Polydon spathula</i>	pallid sturgeon <i>Scaphirhynchus albus</i>
shovelnose sturgeon <i>S. platyrhynchus</i>	striped bass <i>Morone saxatilis</i>

Marine non-salmonids

Atlantic sturgeon <i>Acipenser oxyrinchus</i>	red drum <i>Sciaenops ocellatus</i>
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Size Class of Treated Fish: Adults