

LHRH_a Spawning Hormone Clinical Field Trials - INAD 8061

2007 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 2007 (CY07), 47 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 546 treated fish and 110 control fish and were conducted at 21 different hatcheries, including seven U.S. Fish and Wildlife Service fish hatcheries, nine state hatcheries, four 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 CY07 showed that

treatments appeared efficacious in approximately 87% of the trials, ineffective in 9% of the trials, and were characterized as inconclusive in 4% 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 studies conducted under INAD exemption #8061 in CY07. Furthermore, it is expected that these data will be used to enhance the existing LHRH_a database that has been established from previous years studies 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 21 different fish culture facilities during CY07, including seven U.S. Fish and Wildlife Service fish hatcheries, nine state

hatcheries, four private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 53.0 to 78.0°F.

2. Chemical material

Syndel International Inc. of Vancouver, British Columbia Canada 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 2.2 to 100 ug LHRH_a/kg fish body. LHRHa was administered as either a single injection or as a series of two injections.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on 16 different fish species under INAD #8061 during the reporting period, including the following one salmonid and 15 non-salmonids:

Salmonids

Gila trout *Oncorhynchus gilae*

Non-salmonids

alligator gar *Lepisosteus spatula*

American Shad *Alosa sapidissima*

Atlantic sturgeon *Acipenser oxyrinchus*

blue catfish *Ictalurus furcatus*

channel catfish *Ictalurus punctatus*

flathead catfish *Pylodictis olivaris*

grass carp *Ctenopharyngodon idella*

June sucker *Chasmistes liorus*

lake sturgeon *Acipenser fulvescens*

largemouth bass *Micropterus salmoides*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *Scaphirhynchus platyrhynchus*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

2. Gender of treated fish

LHRH_a was used on 313 female and 233 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 (12 - 24 hrs) 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 CY07 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)

Treated male fish were used in 18 trials and injected either one or two times with LHRH_a at a dosage between 5 and 100 ug/kg body weight (Table 1). Below is the treatment regimen used when treating fish species with this dosage range of LHRH_a:

1. Dose: 5 ug/kg

Gila trout were used in one trial and injected 1 time with LHRH_a; control fish were involved with this trial; results showed there was 100% spermiation in treated fish; and 70% spermiation in control fish. Overall, results indicated that this treatment appeared effective.

2. Dose: 10 - 20 ug/kg

Paddlefish, pallid sturgeon, shovelnose sturgeon, and white sturgeon were used in eight trials, and in each trial, fish were injected 1 time with LHRH_a; one of the eight trials involved control fish; results showed there was 31 - 100% spermiation in treated fish; and no spermiation in control fish. Overall, results indicated that treatment appeared effective in all trials.

3. Dose: 30 - 50 ug/kg

American shad, Atlantic sturgeon, paddlefish, and shovelnose sturgeon were used in five trials, and in each trial, fish were injected 1 or 2 times with LHRH_a; no control fish were used; results showed that there was 0 - 100% spermiation in treated fish. Overall, results indicated that treatment appeared effective in four trials and ineffective in one trial.

4. Dose: 100 ug/kg

Alligator gar, blue catfish, flathead catfish, and paddlefish were used in four trials, and in each trial, fish were injected 1 or 2 times with LHRH_a; control fish were used in two trials. Results showed that there was 40 - 100% spermiation in treated fish; and 0 - 82% spermiation in control fish. Overall, results indicated that treatment appeared effective in three trials and was characterized as inconclusive in one trial.

Overall treatment resulted in a 0 - 100% level of spermiation in the male treated fish, compared to a 0 - 82% level of spermiation in control fish. Hence, results indicated that treatment appeared effective in 16 trials, ineffective in one trial, and characterized as inconclusive in one trial.

B. Efficacy of LHRH_a on female fish treated at a dosage between 2.2 and 100 ug/kg body weight (1 - 2 injections)

Treated female fish were used in 29 trials and injected 1 - 2 times with LHRH_a at a dosage between 2.2 and 100 ug/kg body weight (Table 2). Below is the treatment regimen used when treating fish with this dosage range of LHRH_a:

1. Dose: 2.2 - 5.0 ug/kg

Gila trout and grass carp were used in two trials, fish were injected 1 or 2 times with LHRH_a; and one of the trials involved control fish. Treatment results showed that there was 95 - 100% ovulation in treated fish; and 30% ovulation in control fish. Overall, treatment appeared effective in both trials.

2. Dose: 20 - 25 ug/kg

June sucker, shovelnose sturgeon, striped bass, and white sturgeon were used in five trials, fish were injected 1 or 2 times with LHRH_a; and one of the trials involved control fish. Results showed that there was 0 - 100%

ovulation in treated fish; and no ovulation in the control fish. Overall, treatment appeared effective in four trials and ineffective in one trial.

3. Dose: 40 - 50 ug/kg

Largemouth bass, pallid sturgeon, shovelnose sturgeon, and white sturgeon were used in four trials, fish were injected 1 or 2 times with LHRH_a; no control fish were used. Results showed that there was 21 - 100% ovulation in treated fish. The Investigator for the largemouth bass trial noted that fish were wild brood and that spawning had already started; many of the fish were likely of marginal quality as for ovary/egg development. Overall, treatment appeared effective in all trials.

4. Dose: 75 ug/kg

American shad were used in one trial and fish were injected two times with LHRH_a. No control fish were used in this trial. Results showed that there was 80% ovulation in treated fish. Overall, results indicated that treatment appeared effective in this trial.

5. Dose: 100 ug/kg

Alligator gar, Atlantic sturgeon, blue catfish, channel catfish, flathead catfish, paddlefish, pallid sturgeon, and white sturgeon were used in 16 trials, fish were injected 1 - 2 times with LHRH_a; two of the 16 trials involved control fish. Results showed that there was 0 - 100% ovulation in

treated fish; and 0 - 82% ovulation in control fish. Overall, treatments appeared efficacious in 13 trials, ineffective in two trials, and was characterized as inconclusive in 1 trial.

6. Dose: 20 - 100 ug/kg

Lake sturgeon were used in one trial, fish were injected 1 - 2 times with LHRH_a; no control fish were used. Results showed that there was 100% ovulation in treated fish. Treatment appeared efficacious in this trial.

Overall treatment resulted in 0 - 100% ovulation in females treated and 0 - 82% ovulation in control fish. Treatment appeared efficacious in 25 trials, ineffective in three trials, and was characterized as inconclusive in one trial.

2. Observed Toxicity

No toxicity or adverse effects relating to LHRH_a treatments were reported in any trials conducted in CY07.

Number of Treated Fish under Slaughter Authorization

Total number of fish treated during CY07 was 546. The total number of treated fish to count against the slaughter authorization dated August 15, 2003 is 2,794. No changes have occurred to the current LHRHa INAD #8061 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 CY07. Facilities not listed in Appendix III-a of the current LHRHa INAD #8061 study protocol have been highlighted.

The following facility received LHRHa during CY07 but never used the drug:

1. Charles Brown Farms

Summary of Study Results

LHRH_a was used in 47 efficacy trials to induce gamete maturation in 16 different fish species (n = 546 treated fish; 110 untreated control fish) at dosages ranging from 2.2 - 100 ug/kg bw. LHRH_a was administered using either 1 - 2 injections. Water temperature during treatments ranged from 53.0 - 78.0°F. Approximately 87% of the trials appeared efficacious, 9% appeared ineffective, and 4% were characterized as inconclusive. Data from the CY07 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

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- 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 2007 LHRH_a Male Efficacy Results - Injection

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Spermiat	Number of Controls	% Spermaite
1	effective	ALG	Private John Allen NFH	2	23.25 hrs	2	100	100	0	-
1	effective	AMS	Edenton NFH	2	?	47	50	90	0	-
1	ineffective	ASN	Manning SFH	1	24 - 48 hrs	8	30	0	0	-
1	inconclusive	BCF	Jasper SFH	1	every 72 hrs	16	100	56	17	82
1	effective	FCF	Heart of the Hills Research Station	1	3 days	10	100	40	10	0
1	effective	GIT	Mora NFH & TC	1	7 days	20	5	100	20	70
1	effective	PAH	Aquaculture Research Center	1	12 - 24 hrs	6	50	100	0	-
1	effective	PAH	Booker Fowler SFH	1	24 hrs	7	10	86	0	-
1	effective	PAH	Gavins Point NFH	1	24 hrs	17	20	65	0	-
1	effective	PAH	Natchitoches NFH	1	24 hrs	8	10	75	0	-
1	effective	PAH	Private John Allen NFH	2	22.25 hrs	7	100	100	0	-
1	effective	PAH	Tishomingo NFH	1	26 hrs	14	50	100	0	-
1	effective	PLS	Bozeman FTC	1	12 hrs	6	20	100	0	-
1	effective	PLS	Gavins Point NFH	1	26 hrs	20	20	90	0	-
1	effective	SNS	Bozeman FTC	1	36 hrs	7	20	100	0	-
1	effective	SNS	Kincaid SFH	1	24 hrs	15	40	53	0	-
1	effective	WHS	Blind Canyon Aquaranch	1	22 - 75	13	20	31	0	-
1	effective	WHS	Sterling Caviar, LLC	1	20 hrs	10	10	50	10	0

Table 2. Summary of Year 2007 LHRH_a Female Efficacy Results - Injection

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	ALG	Private John Allen NFH	2	23.25 hrs	5	100	60	0	-
1	effective	AMS	Edenton NFH	2	?	40	75	80	0	-
1	ineffective	ASN	Manning SFH	2	24 - 48 hrs	1	100	0	0	-
1	inconclusive	BCF	Jasper SFH	1	every 72 hrs	16	100	56	17	82
1	effective	CCF	Baxter Land Co.	2	24 hrs	94	100	16 - 54	0	-
1	effective	FCF	Heart of the Hills Research Station	1	3 days	10	100	40	10	0
1	effective	GIT	Mora NFH & TC	2	7 days	20	5	95	20	30
1	effective	GRC	Osage Catfisheries, Inc	1	24 hrs	4	2.2	100	0	-
1	effective	JSK	Bozeman FTC	2	24 hrs	4	20	0	0	-
1	ineffective	LMB	Milford SFH	1	40 - 44 hrs	7	50	29	0	-
1	effective	LST	Wild Rose SFH	1 - 2	48 hrs	14	20 - 100	100	0	-
1	effective	PAH	Aquaculture Research Center	2	12 - 24 hrs	3	100	100	0	-
1	effective	PAH	Booker Fowler SFH	2	24 hrs	3	100	67	0	-
1	effective	PAH	Gavins Point NFH	2	4 hrs	7	100	100	0	-
1	effective	PAH	Natchitoches NFH	2	24 hrs	4	100	75	0	-
2	effective	PAH	Osage Catfisheries, Inc	2	24 - 36 hrs	11	100	100	0	-
1	effective	PAH	Private John Allen NFH	2	22.5 hrs	7	100	57	0	-
1	effective	PAH	Tishomingo NFH	1	26 hrs	3	100	100	0	-
1	ineffective	PAH	Tishomingo NFH	1	-	3	100	0	0	-
1	effective	PLS	Bozeman FTC	2	12 hrs	2	50	100	0	-

Table 2. Summary of Year 2007 LHRH_a Female Efficacy Results - Injection (cont.)

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	PLS	Gavins Point NFH	2	6 hrs	6	100	83	0	-
1	effective	SNS	Bozeman FTC	2	12 - 18 hrs	5	20	100	0	-
1	effective	SNS	Kincaid SFH	1	24 hrs	14	40	21	0	-
1	effective	STB	Blackwater Fisheries Research	1	3 days	3	25	67	0	-
1	effective	STB	Milford SFH	1	1 - 3 days	13	25	92	0	-
1	effective	WHS	Blind Canyon Aquaranch	2	29 - 35 hrs	3	50	100	0	-
1	effective	WHS	Kootenai Tribal Hatchery	2	14 - 30 hrs	5	100	100	0	-
1	effective	WHS	Sterling Caviar,LLC	2	20 hrs	6	20	83	6	0

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2007 LHRH_a Efficacy Studies

Total Number of Treatment Trials	47
Number of Trials that Appeared Efficacious:	41 (87%)
Number of Trials that Appeared Inefficacious:	4 (9%)
Number of Trials that Appeared Inconclusive:	2 (4%)

Total Number of Treated Fish: 546

Treatment Regimes Used:

2.2 - 5 ug/Kg body weight (1 - 2 injections)	3 trials
10 - 25 ug/Kg body weight (1 - 2 injections)	13 trials
30 - 50 ug/Kg body weight (1 - 2 injections)	9 trials
75 ug/Kg body weight (2 injections)	1 trial
100 ug/Kg body weight (1 - 2 injections)	20 trials
20 - 100 ug/Kg body weight (1 - 2 injections)	1 trial

Water Temperature (°F) Range: 53.0 - 78.0

Fish Species Treated:

Salmonids

Gila trout *Oncorhynchus gilae*

Non-salmonids

alligator gar	<i>Lepisosteus spatula</i>
American Shad	<i>Alosa sapidissima</i>
Atlantic sturgeon	<i>Acipenser oxyrinchus</i>
blue catfish	<i>Ictalurus furcatus</i>
channel catfish	<i>Ictalurus punctatus</i>
flathead catfish	<i>Pylodictis olivaris</i>
grass carp	<i>Ctenopharyngodon idella</i>
June sucker	<i>Chasmistes liorus</i>
lake sturgeon	<i>Acipenser fulvescens</i>

largemouth bass *Micropterus salmoides*
paddlefish *Polydon spathula*
pallid sturgeon *Scaphirhynchus albus*
shovelnose sturgeon *Scaphirhynchus platyrhynchus*
striped bass *Morone saxatilis*
white sturgeon *Acipenser transmontanus*

Size Class of Treated Fish: Adults