$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2011 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 2011 (CY11), 36 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 7,588 treated fish and 64 control fish and were conducted at 20 different hatcheries, including four U.S. Fish and Wildlife Service fish hatcheries, eight state hatcheries, seven 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 CY11 showed that

treatments appeared efficacious in approximately 92% of the trials, ineffective in 3% of the trials, and was characterized as inconclusive 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 brooodstock 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 CY11. 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 20 different fish culture facilities during CY11, including four U.S. Fish and Wildlife Service fish hatcheries, eight state hatcheries, seven private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 42.0 to 80.0°F. Overall mean treatment temperature from all trials was 58.7 °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 5 to 100 ug $LHRH_a$/kg fish body. LHRHa was administered as either a single injection or as a series of 2 injections.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on 11 different fish species under INAD #8061 during the reporting period, including two salmonid; eight non-salmonid; and one marine non-salmonid species:

Salmonids

Gila trout *Oncorhynchus gilae*

rainbow trout *O. mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

lake sturgeon *Acipenser fulvescens*

Russian surgeon *A. gueldenstaedtii*

starry sturgeon *A. stellatus*

white sturgeon *A. transmontanus*

channel catfish *Ictalurus punctatus*

paddlefish *Polydon spathula*

striped bass *Morone saxatilis*

Marine non-salmonids

Atlantic sturgeon *Acipenser oxyrhynchus*

2. Gender of treated fish

$LHRH_a$ was used on 7,373 female and 215 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 CY11 under INAD #8061.)

A. Efficacy of $LHRH_a$ on male fish treated at a dosage between 5 and 100 ug/kg body weight (1 - 2 injections)

Male fish were treated in 15 trials and injected 1 - 2 times with LHRHa at a dosage between 5 and 100 mg/kg bw (Table 1) to induce gamete maturation. Fish species treated included Gila trout, Atlantic sturgeon, alligator gar, lake sturgeon, paddlefish, Russian sturgeon, starry sturgeon, and white sturgeon. Four trials included non-treated control groups. Following treatment, there was 37.5 - 100% spermiation among all treated fish; as compared to 0 - 100% spermiation in the control fish. Overall, treatments appeared efficacious in 13 trials involving 202 fish and was characterized as inconclusive in two trials involving 13 fish.

B. Efficacy of $LHRH_a$ on female fish treated at a dosage between 5 and 100 ug/kg body weight (1 - 2 injections)

Female fish were treated in 21 trials and injected 1 - 2 times with LHRHa at a dosage between 5 and 100 mg/kg bw (Table 2) to induce gamete maturation. Fish species treated included Gila trout, rainbow trout, alligator gar, channel catfish, lake sturgeon, paddlefish, Russian sturgeon, starry sturgeon, striped bass, and white sturgeon. Six 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 7,369 fish, and ineffective in one trial involving 4 fish.

2. Observed Toxicity

No toxicity or adverse effects relating to $LHRH_a$ treatments were reported in any of the trials conducted in CY11.

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 "Table 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the LHRHa INAD #8061 during CY11.

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 CY11.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY11 was 7,588. The total number of treated fish to count against the food use authorization dated March 29, 2010 is 8,714 (valid through June 18, 2012).

Summary of Study Results

$LHRH_a$ was used in 36 efficacy trials to induce gamete maturation in 11 different fish species (n = 7,588 treated fish; 64 untreated control fish) at dosages ranging from 5 - 100 ug/kg bw. $LHRH_a$ was administered using either 1 - 2 injections. Water temperature during treatments ranged from 42.0 - 80.0°F. Overall, results showed that LHRHa treatment appeared efficacious in 92% of the trials, ineffective in 3% of the trials, and was characterized as inconclusive in 5% of the trials. Data from the CY11

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-Ala^6,des Gly^{10}LH-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 2011 $LHRH_a$ Male Efficacy Results

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval (hrs)	Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
1	effective	ALG	Private John Allen NFH	26.5	7	100	88	0	-
2	effective	ASN	Manning SFH	24	13	30	100	0	-
1	effective	GIT	Mora NFH & TC	7 - 14 days	99	5	78.6 - 100 (Ave. 94)	2	100
1	effective	LST	NYSDEC Region 6	32	22	10	100	0	-
1	inconclusive	PAH	Aquaculture Research Center	12 - 24	11	50	37.5	0	-
1	effective	PAH	Booker Fowler SFH	13.5	3	10	100	0	-
1	effective	PAH	Gavins Point NFH	40	10	10	80	0	-
1	effective	PAH	Private John Allen NFH	22.5	4	100	100	0	-
1	effective	PAH	Tishomingo NFH	44 - 48	17	25	80 - 100 (Ave. 82)	0	-
1	effective	RSN	Evans Fish Farm	24	4	40	100	8	0
1	effective	SVN	Evans Fish Farm	24	4	40	50 - 100 (Ave. 75)	5	0
1	effective	WST	Blind Canyon Aquaranch	72	9	20	44	0	-
1	inconclusive	WST	Kootenai Tribal Conservation Aquaculture	21 - 50	2	30	50	0	-
1	effective	WST	Sterling Caviar LLC	20	10	10	60	10	0

Table 2. Summary of Year 2011 $LHRH_a$ 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	26.5	5	100	60	0	-
1	effective	CCF	Baxter Land Company	24 - 36	2,961	100	55 - 98 (Ave. 83)	0	-
1	effective	CCF	America's Catch Catfish Farm	40 - 48	503	100	6 - 70 (Ave. 41)	0	-
1	effective	CCF	Jubilee Farms	26	3,230	100	70 - 96 (Ave. 90)	0	-
1	effective	GIT	Mora NFH & TC	7 - 14 days	180	5	0 - 100 (Ave. 45)	2	100
1	effective	LST	NYSDEC Region 6	32	8	50	100	0	-
1	effective	LST	Wild Rose SFH	20	15	80	50 - 100 (Ave. 80)	14	0 - 100 (Ave. 29)
1	effective	PAH	Aquaculture Research Center - Kentucky State	12 - 24	4	100	75	0	-
1	effective	PAH	Booker Fowler SFH	13.5	2	100	100	0	-
1	effective	PAH	Gavins Point NFH	40	7	100	71.4	0	-
1	effective	PAH	Osage Catfisheries	24	14	100	100	0	-
1	effective	PAH	Private John Allen NFH	22.5	4	100	75	0	-
1	effective	PAH	Tishomingo NFH	24 - 48	18	12.5 - 25	75 - 100 (Ave. 89)	4	0
1	effective	RBT	Fall River SFH	21 days	360	10	67	0	-

Table 2. Summary of Year 2011 $LHRH_a$ 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	ineffective	RSN	Evan Fish Farm	36	4	40	0 - 100 (Ave. 25)	8	0
1	effective	STB	Blackwater Fisheries Research	up to 28 days	13	25	85	0	-
1	effective	STB	Milford SFH	24 - 48	27	21.5 - 57.6	78	0	-
1	effective	SVN	Evan Fish Farm	36	2	40	0 - 100 (Ave. 50)	5	0
1	effective	WST	Blind Canyon Aquaranch	23 - 34.5	3	50	100	0	-
1	effective	WST	Kootenai Tribal Hatchery	21 - 50	7	100	100	0	-
1	effective	WST	Sterling Caviar	20	6	20	100	6	0

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2011 $LHRH_a$ Efficacy Studies

Total Number of Treatment Trials	36
Number of Trials that Appeared Efficacious:	33
Number of Trials that Appeared Inefficacious:	1
Number of Trials that Appeared Inconclusive:	2
Total Number of Treated Fish:	7,588
Number of fish treated in efficacious trials	7,571
Number of fish treated in ineffective trials	4
Number of fish treated in inconclusive trials	13
Treatment Regimes Used:	
5 - 100 ug/Kg body weight (1 - 2 injections)	36 trials
Water Temperature (°F) Range:	42.0 - 80.0

Fish Species Treated:

Salmonids
Gila trout *Oncorhynchus gilae*
rainbow trout *O. mykiss*

Non-salmonids
alligator gar *Lepisosteus spatula*
lake sturgeon *Acipenser fulvescens*
Russian surgeon *A. gueldenstaedtii*
starry sturgeon *A. stellatus*
white sturgeon *A. transmontanus*
channel catfish *Ictalurus punctatus*
paddlefish *Polydon spathula*

striped bass *Morone saxatilis*

Marine non-salmonids
Atlantic sturgeon *Acipenser oxyrhynchus*

Size Class of Treated Fish: Adults