$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2004 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 $LHRH_a$. One hundred and twenty-three LHRHa INAD trials were conducted in calendar year 2004 (CY04) to evaluate the efficacy of $LHRH_a$ to induce gamete maturation in a variety of fish species. Trials which involved 3,466 treated fish and 401 control fish were conducted at nine U.S. Fish and Wildlife Service fish hatcheries, seven 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 of trials conducted in CY04 showed that treatments in approximately 54% of the trials appeared efficacious, treatments in 8% of the trials

appeared ineffective, and treatments in 38% of the trials were characterized as inconclusive.

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. 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 supplemental $LHRH_a$ field efficacy studies conducted during calendar year 2004 (CY04). 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

A total of 21 fish culture facilities used $LHRH_a$ during CY04, including nine U.S. Fish and Wildlife Service fish hatcheries, seven state hatcheries, four private hatcheries, and one tribal hatchery.

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). Treatments were administered to fish using either injection (1 - 2 injections) or pellet implant. Drug dosages used by participating fish culture facilities in CY04 ranged from 10 to 177.86 ug $LHRH_a$/kg bw. Fish treated at a dosage greater then 100 ug $LHRH_a$/kg fish bw or by pellet implant were euthanized at the hatchery after they were spawned and properly disposed.

Fish Species and Sex Treated

1. Fish Species Treated

A total of 15 fish species were treated with $LHRH_a$ under INAD #8061 during the reporting period, including the following two salmonids and 13 non-salmonids:

Salmonids

Gila trout *Oncorhychus gilae*

steelhead trout *O. mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

American Shad *Alosa sapidissima*

California halibut *Paralichthys californicus*

channel catfish *Ictalurus punctatus*

hickory shad *Alosa mediocris*

lake sturgeon *Acipenser fulvescens*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *Scaphirhynchus platorynchus*

smallmouth bass *Micropterus dolomieu*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

yellow perch *Perca flavescens*

2. Gender of treated fish

$LHRH_a$ was used on 1,871 female and 1,595 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 had 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 the time when fish were spawned or evaluated for ripeness was also documented. In the case of females, which in some cases receive a priming dose followed a short time later (12 - 72 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: Table 1 provides a summary of all efficacy trials; Table 2 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 3 describes all trials conducted during CY04 under INAD #8061.)

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

Treated male fish were used in 27 trials and injected either one or two times with $LHRH_a$ at dosages between 10 and 100 ug/kg body weight (Table 1). Below is the treatment regimen used on nine fish species treated with LHRHa:

1. Dose: 10 - 20 ug/kg

   Gila trout, paddlefish, pallid sturgeon, smallmouth bass, steelhead trout, shovelnose sturgeon, and white sturgeon were used in 14 trials, and in each trial, fish were injected 1 or 2 times with LHRHa; 6 of the 14 trials involved control fish; results showed there was 67 - 100% spermiation in treated fish; and 0 - 100% spermiation in control fish. Overall, treatment in 11 of the trials appeared efficacious, and treatment in three of the trials was characterized as inconclusive.

2. Dose: 30 - 50 ug/kg

California halibut and paddlefish were used in 11 trials, and in each trial, fish were injected one time with LHRHa; 7 of the 11 trials involved control fish; results showed that there was 0 - 100% spermiation in treated fish; and 0% spermiation in control fish. Overall, treatment in 9 of the trials appeared effective, whereas treatment in two of the trials appeared ineffective.

3. Dose: 100 ug/kg

Alligator gar and paddlefish were used in two trials, and in each trial, fish were injected 1 or 2 times with LHRHa; no control fish used in either trial. Results showed that there was 50 - 100% spermiation in treated fish. There were no control fish. Overall, results showed that treatment in one of the trials appeared efficacious, whereas treatment in the other trial was characterized as inconclusive.

Overall, treatments appeared efficacious in 21 trials, ineffective in two trials, and characterized as inconclusive in four trials.

B. Efficacy of LHRHa on female fish treated at dosages between 15 and 100 ug/kg body weight (1 - 2 injections)

Treated female fish were used in 62 trials and injected either one or two times with $LHRH_a$ at dosages between 15 and 100 ug/kg body weight (Table 1). Below is the treatment regimen used on the 11 fish species treated with LHRHa:

1. Dose: 15 - 20 ug/kg

   Gila trout, smallmouth bass, shovelnose sturgeon, striped bass, and white sturgeon were used in thirteen trials, and in each trial, fish were injected 1 or 2 times with LHRHa; 5 of the 13 trials involved control fish. Treatment results showed that there was 18 - 100% ovulation in treated fish that were evaluated. In addition, of the control fish that were evaluated it was found that there was 77 - 100% ovulation. Overall results showed that treatments were effective in seven of the trials and ineffective in one trial. Treatments were characterized as inconclusive in five of the trials.

2. Dose: 50 - 60 ug/kg

   California halibut and channel catfish were used in six trials, and in each trial, fish were injected 1 or 2 times with LHRHa; 1 of the 6 trial involved control fish. Results showed that there was 15 - 70% ovulation in treated fish; whereas there was 0% ovulation in control fish. Overall, treatment

appeared effective in four trials and treatments were characterized as inconclusive in two trials.

3. Dose: 80 - 90 ug/kg

   Channel catfish and paddlefish were used in ten trials, and in each trial, fish were injected two times with LHRHa. No control fish were used in any of the trials. Results showed that there was 25 - 100% ovulation in treated fish. Overall, treatments appeared effective in seven of the trials, and in three trials, treatments were characterized as inconclusive.

4. Dose: 100 ug/kg

   Alligator gar, channel catfish, lake sturgeon, paddlefish, pallid sturgeon, and white sturgeon were used in 33 trials, and in each trial, fish were injected 1 or 2 times with LHRHa; 4 of the 33 trials involved control fish. Results showed that there was 0 - 100% ovulation in treated fish and 0% ovulation in control fish. Overall, treatments appeared effective in 24 of the trials and ineffective in 4 of the trials. Treatments were characterized as inconclusive in the remaining five trials.

Overall treatment resulted in 0 - 100% ovulation in female treated fish, compared to 0% ovulation in control fish. Treatments appeared efficacious in 42 trials, ineffective in five trials, and were characterized as inconclusive in 15 trials.

C. Efficacy of LHRHa on male fish treated between 25 and 170.13 ug/kg body weight (1 - 2 implants)

Treated male fish were used in 16 trials and implanted either one or two times with LHRHa at dosages between 25 and 170.13 ug/kg body weight (Table 1). All pellet implanted fish were euthanized at the end of the spawning period. In addition, on occasion, the Investigator did not evaluate whether treatments induced gamete maturation. In these cases, it's implied that the relative level of gamete maturation was undetermined. Below are the treatment regimens used to induce gamete maturation in four fish species treated with LHRHa:

1. <u>Dose: 25 ug/kg</u>

   Hickory shad were used in eight trials, and in each, fish were implanted with one LHRHa pellet. No control fish were used in any of the trials. Results showed that there was 100% spermiation in treated fish. However, the Investigator noted that only ripe males were selected as broodstock at time of capture (as a result, even untreated fish would have had high levels of spermiation). Due to transport and handling stress, the wild broodstock were implanted with an LHRHa pellet to ensure that they would stay ripe throughout the spawning period. Therefore, all trials were characterized as inconclusive.

2. Dose: 51.5 - 64 ug/kg

American shad and striped bass were used in four trials, and in each, fish were implanted with 1 or 2 LHRHa pellets. No control fish were used in the trials. Results showed that there was 100% spermiation in all treated fish that were evaluated. Overall, treatment appeared effective in two trials, but were characterized as inconclusive in two trials.

3. Dose: 170.13 ug/kg

Yellow perch were used in four trials, and in each, fish were implanted with one LHRHa implant. No control fish used in any of the trials. Results showed that there was 100% spermiation in treated fish. However, the Investigator noted that only ripe males were selected as broodstock at time of capture (as a result, even untreated fish would have had high levels of spermiation). Due to transport and handling stress, the wild broodstock were administered a pellet implant to ensure that they would stay ripe throughout the spawning period. Therefore, all trials were characterized as inconclusive.

Overall treatment resulted in either an unknown percent spermiation (due to fish not checked) or 100% spermiation in the male treated fish, no control fish were used in any of the trials. Treatments appeared efficacious in two trials but were characterized as inconclusive in 14 trials.

D. Efficacy of LHRHa on female fish treated between 50 and 177.86 ug/kg body weight (1 - 4 implants)

Female fish were implanted either one or four times with $LHRH_a$ pellets at dosages between 50 and 177.86 ug/kg body weight (Table 1) in 18 different trials. All pellet implanted fish were euthanized at the end of the spawning period. Below is the treatment regimen used on four fish species treated with LHRHa:

1. Dose: 50 ug/kg

   Hickory shad were used in eight trials, and in each, fish were implanted with one pellet. No control fish used in any of the trials. Fish were not checked for gamete maturation. Therefore, all trials were characterized as inconclusive.

2. Dose: 51.5 or 103 ug/kg

   Striped bass were used in four different treatment trials, and in each, fish were implanted with four LHRHa pellets. No control fish were used in any trial. Results showed that there was 0 - 50% ovulation in treated fish. Overall, treatment appeared effective in two trials, and ineffective in two trials.

3. Dose: 80.57 ug/kg

Yellow perch were used in four trials, and in each, fish were implanted with one LHRHa pellet. No control fish were used in any of the trials. Results showed that there was 0 - 25% ovulation in treated fish. Overall, treatments appeared effective in one trail, ineffective in one trial, and were characterized as inconclusive in two trials.

4. Dose: 115.3 or 177.86 ug/kg

American shad were used in two trials, and in each trial, each test fish was implanted with one LHRHa pellet. No control fish were used in any of the trials. Fish were not evaluated for gamete maturation, therefore, the relative level of ovulation per fish was unknown. Hence, both trials were characterized as inconclusive.

Overall treatment resulted in either an unknown percent ovulation (due to fish not checked) - 50% ovulation in the female treated fish, no control fish were used. Treatments appeared efficacious in one trial, ineffective in three trials, and characterized as inconclusive in 14 trials.

2. Observed Toxicity

No toxicity or adverse effects relating to $LHRH_a$ treatments were reported in the 120 of the 123 trials conducting during the reporting period. In one trial, the Investigator noted that one treated fish died after treatment , but death was most likely due to

handling stress. Investigators of two other trials noted that 12 treated fish and two control fish died after the spawning period.

Summary of Study Results

$LHRH_a$ was used in 123 efficacy trials to induce gamete maturation in 15 different fish species (n = 3,466 treated fish; 401 untreated control fish) at dosages ranging from 10 - 177.86 ug/kg bw. LHRHa was administered using either 1 or 2 injections or as a pellet implant. All treated fish administered $LHRH_a$ as a pellet implant were euthanized after the spawning season. Water temperature during treatments ranged from 41.0 - 78.8°F. Approximately 54% of the trials appeared efficacious, 8% appeared ineffective, and 38% were characterized as inconclusive (mostly due to failure of the Investigator evaluating gamete maturation following treatment). Data from the CY04 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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Table 1. $LHRH_a$ Use Summary Record					Females					Males				
					Treated			Control		Treated			Control	
Number of Trials	Species	Facility	Treatment method	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate	Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
2	ALG	Private John Allen NFH	Injection	23.5 hrs	1	100	100	0	-	2	100	50	0	-
2	AMS	Edenton NFH	Implant	1 - 11 days	259	115.3	?	0	-	370	64	?	0	-
2	AMS	Edenton NFH	Implant	1 - 11 days	140	177.9	?	0	-	180	55.6	?	0	-
5	CCF	Harvest Select Farms	Injection	26 - 32 hrs	133	60	15 - 75	0	-	0	-	-	0	-
7	CCF	Harvest Select Farms	Injection	26 - 32 hrs	243	80	20 - 100	0	-	0	-	-	0	-
9	CCF	Harvest Select Farms	Injection	26 - 32 hrs	262	100	0 - 88	0	-	0	-	-	0	-
4	GIT	Mora NFH & TC	Injection	3 days	30	20	18 - 100	30	77- 100	32	10	100	30	50 - 100
2	HAL	Hubbs SeaWorld Research Institute	Injection	46 - 48 hrs	5	50	40	16	0	1	50	0	1	0
16	HKS	Manning SFH	Implant	3 days	573	50	?	0	-	664	25	100	0	-
2	LST	Wild Rose SFH	Injection	24 - 40 hrs	4	100	0 - 100	0	-	0	-	-	0	-
2	PAH	Aquaculture of Kentucky, Inc	Injection	12 - 24 hrs	3	100	100	2	0	3	50	100	5	0
4	PAH	Booker Fowler SFH	Injection	13 - 49 hrs	10	100	80	0	-	27	10	67 - 100	0	-
2	PAH	Gavins Point NFH	Injection	22 hrs	5	100	60	0	-	15	10-20	87	0	-
8	PAH	Kentucky State University	Injection	12 - 36 hrs	11	100	67 - 100	13	0	9	50	60 - 100	12	0
4	PAH	Kinder Caviar	Injection	12 - 24 hrs	6	100	0 - 100	0	-	6	50	0 - 100	0	-
2	PAH	Natchitoches NFH	Injection	24 - 36 hrs	5	100	100	0	-	7	10	86	0	-
3	PAH	Osage Catfisheries, Inc	Injection	24 - 36 hrs	12	90	100	0	-	0	-	-	0	-
2	PAH	Private John Allen NFH	Injection	23 hsr	6	100	83	0	-	12	100	100	0	-
4	PAH	Tishomingo NFH	Injection	24 hrs	14	100	63 - 67	0	-	20	30	67 - 87	5	0

Table 1. $LHRH_a$ Use Summary Record					Females					Males				
					Treated			Control		Treated			Control	
Number of Trials	Species	Facility	Treatment method	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate	Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
2	PLS	Bozeman FTC	Injection	36 hrs	1	100	100	0	-	3	20	100	0	-
2	PLS	Gavins Point NFH	Injection	12 hrs	4	100	100	0	-	3	10-20	100	0	-
2	SMB	Bozeman FTC	Injection	10 days	50	15	?	126	?	50	15	?	126	?
4	SNS	Bozeman FTC	Injection	24 - 38 hrs	17	20	100	0	-	33	20	100	0	-
6	STB	Blackwater Fisheries Research	Injection	67-75.5 hrs	14	25	50 - 100	0	-	0	-	-	0	-
6	STB	Warm Springs FHC	Implant	1 - 7 days	9	51.5 & 103	0 - 50	0	-	8	51.5	100	0	-
1	STT	Dworshak NFH	Injection	7 & 14 days	0	-	-	0	-	52	20	71	6	100
6	WHS	Kootenai Tribal Hatchery	Injection	24 - 39 hrs	6	100	0 - 100	0	-	0	-	-	0	-
4	WHS	Stolt Sea Farm California LLC	Injection	24 hrs	9	20	67	9	0	20	10	80 - 83	20	0
8	YEP	Manning SFH	Implant	3 days	39	80.57	0 - 25	0	-	78	170.1	100	0	-

Table 2. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2004 $LHRH_a$ Efficacy Studies

Total Number of Treatment Trials	123
Number of Trials that Appeared Efficacious:	66 (54%)
Number of Trials that Appeared Inefficacious:	10 (8%)
Number of Trials that Appeared Inconclusive:	47 (38%)
Total Number of Treated Fish:	3,466

Treatment Regimes Used:

10 - 20 ug/Kg body weight (1 - 2 injections)	21 trials
25 - 30 ug/Kg body weight (1 injection)	8 trials
25 ug/Kg body weight (1 implant)	8 trials
50 - 60 ug/Kg body weight (1 - 2 injections)	15 trials
50 ug/Kg body weight (1 implant)	8 trials
51.5 & 103 ug/Kg body weight (2 - 4 implants)	6 trials
55.56 - 80.57 ug/Kg body weight (1 implant)	6 trials
80 - 90 ug/Kg body weight (2 injections)	10 trials
100 ug/Kg body weight (1 - 2 injections)	35 trials
115.3 ug/Kg body weight (1 implant)	1 trial
170.13 - 177.86 ug/Kg body weight (1 implant)	5 trials

Water Temperature (°F) Range: 41.0 - 78.8

Fish Species Treated:

Gila trout *Oncorhychus gilae*
alligator gar *Lepisosteus spatula*
California halibut *Paralichthys californicus*
hickory shad *Alosa mediocris*
paddlefish *Polydon spathula*
smallmouth bass *Micropterus dolomieu*
white sturgeon *Acipenser transmontanus*
shovelnose sturgeon *Scaphirhynchus platorynchus*
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striped bass *Morone saxatilis*
yellow perch *Perca flavescens*

Size Class of Treated Fish: Adults