$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2002 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 $LHRH_a$. Several INAD trials were conducted in calendar year (CY) 2002 to evaluate the efficacy of $LHRH_a$ to induce gamete maturation in a variety of fish species. One hundred and ten such trials that involved 2,607 treated fish and 679 control fish were conducted at five U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey cooperative unit, six state hatcheries, three 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 CY

2002 showed that approximately 61% of the trials appeared efficacious, 10% of the trials appeared ineffective, and 29% 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 primary purpose of this report is to summarize the results of the CY 02 supplemental $LHRH_a$ field efficacy studies. However, it is also 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 16 fish culture facilities used $LHRH_a$ during CY 02, including five U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey cooperative unit, six state hatcheries, three private hatcheries, and one tribal hatchery. Water temperature during treatments ranged from 41.0 - 82.4 °F.

2. Chemical material

Syndel International Inc. of Vancouver, British Columbia Canada was the supplier for all $LHRH_a$ used in these trials.

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 injections (1 - 4 injections) or pellet implants. Drug dosages used by participating fish culture facilities in CY 02 ranged from 0.5 to 240 ug $LHRH_a$/kg bw. Fish treated at a dosage greater then 100 ug $LHRH_a$/kg fish bw were either destroyed after they were spawned, remained at the hatchery as broodstock, or released after 90 days. At the facility where the fish were treated with doses higher than allowed by FDA and subsequently released, both the Investigator and Monitor were

notified that they were not to use LHRHa above 100 ug $LHRH_a$/kg fish bw for future studies.

Fish Species and Sex Treated

1. Species of fish treated

A total of 16 fish species were treated with with $LHRH_a$ under INAD #8061 during CY 02, including the following three salmonids and thirteen non-salmonid

Salmonids

1. cutthroat trout *Oncorhychus clarki*
2. Gila trout *O. gilae*
3. steelhead trout *O. mykiss*

Non-salmonids

1. American Shad *Alosa sapidissima*
2. channel catfish *Ictalurus punctatus*
3. flathead catfish *Pylodictis ovilaris*
4. grass carp *Ctenopharyngodon idella*
5. hickory shad *Alosa mediocris*
6. lake sturgeon *Acipenser fulvescens*
7. paddlefish *Polydon spathula*

8. pallid sturgeon *Scaphirhynchus albus*

9. Rio Grande silvery minnow *Hybognathus amarus*

10. shortnose sturgeon *Acipenser brevirostrum*

11. striped bass *Morone saxatilis*

12. white sturgeon *Acipenser transmontanus*

13. yellow perch *Perca flavescens*

2. Gender of treated fish

$LHRH_a$ was used on 1,482 female and 1,125 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 - 96 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 CY 03 under INAD #8061.)

A. Efficacy at 0.5 - 10 ug/kg body weight (1 - 2 injections)

Treated fish used in 19 trials were injected one or two times with $LHRH_a$ at dosages between 0.5 - 10 ug/kg body weight. Trials involved both male and female cutthrout trout, flathead catfish, and paddlefish, male Gila trout and white sturgeon, and female grass carp. Treatments resulted in the following:

0 - 100% ovulation in treated female fish, compared to 27% ovulation in the control fish

0 - 100% spermiation in the male treated fish, compared to 27 - 100% spermiation in the control fish.

Treatments appeared efficacious in nine trials, ineffective in four trials, and were characterized as inconclusive in six trials (Table 1).

B. Efficacy at 20 - 30 ug/kg body weight (1- 2 injections)

Treated fish used in 13 trials were injected one or two times with $LHRH_a$ at dosages between 20 - 30 ug/kg body weight. Trials involved both male and female cutthrout trout, male paddlefish, pallid sturgeon, and shortnose sturgeon, and female Gila trout, striped bass, and white sturgeon. Treatments resulted in the following:

33 - 100% ovulation in treated female fish, compared to 0 - 85% ovulation in the control fish

33 - 100% spermiation in the male treated fish, compared to 75% spermiation in the control fish.

Treatments appeared efficacious in 11 trials, and were characterized as inconclusive in two trials (Table 1).

C. Efficacy at 33 and 39.94 ug/kg body weight (implant)

Treated fish used in 16 trials were implanted with $LHRH_a$ at dosages of 33 or 39.94 ug/kg bw. Trials involved both male and female hickory shad. Treatment resulted in 0.5 - 67% ovulation in treated female fish. Spermiation was reached by naAn unknown number of male treated fish. The Investigator noted that test fish will not spawn in captivity without the aid of spawning hormones such as LHRHa. Treatments appeared efficacious in eight trials, and were characterized as inconclusive in eight trials (Table 1).

D. Efficacy at 40 - 50 ug/kg body weight (1 - 2 injections)

Treated fish used in six trials were injected one or two times with $LHRH_a$ at dosages between 40 - 50 ug/kg body weight. Trials involved both male and female cutthrout trout and Rio Grande silvery minnow, and female flathead catfish. Treatments resulted in the following:

0 - 100% ovulation in treated female fish, compared to 0% ovulation in the control fish

0 - 47% spermiation in the male treated fish, compared to 0% spermiation in the control fish.

Treatments appeared efficacious in three trials, ineffective in two trials, and were characterized as inconclusive in one trial (Table 1).

E. Efficacy at 89 ug/kg body weight (implant)

Treated fish used in 3 trials were implanted with $LHRH_a$ at a dosage of 89 ug/kg body weight. Trials involved female yellow perch. Treatment resulted an unknown number of female treated fish that ovulated. Consequently, treatments were characterized as inconclusive in all three trials (Table 1).

F. Efficacy at 100 ug/kg body weight (1 - 4 injections)

Treated fish used in 17 trials were injected one, two, three, or four times with $LHRH_a$ at a dosage of 100 ug/kg body weight. Trials involved both male and female paddlefish, and female channel catfish, lake sturgeon, pallid sturgeon, striped bass, and white sturgeon. Treatment resulted in the following:

0 - 100% ovulation in treated female fish, compared to 0% ovulation in the control fish

14- 42% spermiation in the male treated fish, compared to 0 - 100% spermiation in the control fish.

Treatments appeared efficacious in six trials, ineffective in five trials, and were characterized as inconclusive in six trials (Table 1).

G. Efficacy at 100 - 107 ug/kg body weight (implant)

Treated fish used in 10 trials were implanted with $LHRH_a$ at dosages between 100 - 107 ug/kg body weight. Trials involved male steelhead trout, American shad, and yellow perch. Treatment resulted in 77 - 90% spermiation in treated steelhead and American shad, compared to 42% spermiation in control fish. Treatment results were unknown for the yellow perch. Treatments appeared efficacious in seven trials, while three trials were characterized as inconclusive (Table 1).

H. Efficacy at 120 ug/kg body weight (2 - 3 injections)

Female channel catfish used in six trials were injected two or three times with $LHRH_a$ at a dosage of 120 ug/kg body weight. Treatment resulted in 12 - 68% ovulation in the female treated fish; no control fish were used. Treatments

appeared efficacious in four trials, while two trials were characterized as inconclusive (Table 1).

I. Efficacy at 122.9 ug/kg body weight (implant)

Female American shad used in six trials were implanted with $LHRH_a$ at a dosage of 122.9 ug/kg body weight. were the only fish species used in 6 trials. Treatment resulted in 46 - 85% ovulation in the female treated fish; no control fish were used. Treatments appeared efficacious in all six trials (Table 1).

J. Efficacy at 180 ug/kg body weight (2 - 3 injections)

Female channel catfish used in 13 trials were injected two or three times with $LHRH_a$ at a dosage of 180 ug/kg body weight. Treatment resulted in 23 - 100% ovulation in the female treated fish; no control fish were used. Treatments appeared efficacious in 12 trials, while one trial was characterized as inconclusive (Table 1).

K. Efficacy at 240 ug/kg body weight (2 injections)

Female channel catfish used in 1 trial were injected two times with $LHRH_a$ at a dosage of 240 ug/kg body weight. Treatment resulted in 44% ovulation in the female treated fish; no control fish were used. Treatment appeared to be efficacious (Table 1).

2. Observed Toxicity

No toxicity or adverse effects relating to $LHRH_a$ treatment were reported in 109 trials. In one trial, the Investigator noted that both treated fish died before ovulation due to massive hemorrhaging most likely caused by handling stress.

Summary of Study Results

$LHRH_a$ was used 110 efficacy trials to induce gamete maturation in 16 different fish species (n = 2,607 treated fish; 679 untreated control fish) at dosages ranging from 0.5 - 240 ug/kg bw. LHRHa was administered using either 1, 2, 3, or 4 injections or as a pellet implant. Water temperature during treatments ranged from 41.0 - 82.4 °F. Approximately 61% of the trials appeared efficacious, 10% appeared ineffective, and 29% were characterized as inconclusive. Data from the CY 02 trials support the results of previous Annual Reports submissions under INAD #8061 that indicate that $LHRH_a$ treatment was efficacious in inducing gamete maturation in a variety of fish species. In addition, it is anticipated that the majority of present and future efficacy data collected under INAD #8061 will be support efficacy data results previously submitted to FDA, and should be considered in the body of evidence used to gain an initial approval for $LHRH_a$.

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
12	AMS	Watha SFH	Implant	7 - 18 days	160	122.9	46 - 85	0	-	270	100.9	77 - 90	0	-
2	CCF	Alabama Coop Fish & Wildlife Research Unit	Injection	35 hrs	50	180	45 - 67	0	-	-	-	-	-	-
2	CCF	Harvest Select Farms	Injection	35 hrs	142	100	29 - 39	0	-	-	-	-	-	-
6	CCF	Harvest Select Farms	Injection	35 hrs	150	120	12 - 68	0	-	-	-	-	-	-
11	CCF	Harvest Select Farms	Injection	33 - 35 hrs	349	180	23 - 100	0	-	-	-	-	-	-
1	CCF	Harvest Select Farms	Injection	35 hrs	25	240	44	0	-	-	-	-	-	-
1	CUT	Eagle Fish Health Lab	Injection	21 days	-	-	-	-	-	94	20	90	500	75
2	CUT	Mora NFH & TC	Injection	3 days	15	10	40	15	27	15	10	40	15	27
2	CUT	Mora NFH & TC	Injection	3 days	15	20	33	0	-	15	20	33	0	-
2	CUT	Mora NFH & TC	Injection	3 days	15	30	47	0	-	15	30	47	0	-
2	CUT	Mora NFH & TC	Injection	3 days	15	40	47	0	-	15	40	47	0	-
10	FCF	Booker Fowler SFH	Injection	4 - 10 days	13	0.5	0 - 100	0	-	14	0.5	0 - 100	0	-
3	FCF	Booker Fowler SFH	Injection	4 days	5	50	50 - 100	0	-	1	5	100	0	-
2	GIT	Mora NFH & TC	Injection	3 days	30	20	76	30	85	30	10	100	30	100
1	GRC	Osage Catfisheries, Inc.	Injection	1 day	3	5	100	0	-	-	-	-	-	-
16	HKS	Manning SFH	Implant	2 days	315	33	0.5 - 67	0	-	395	39.94	Unknown	0	-
1	LST	Wild Rose SFH	Injection	1 day	2	100	100	0	-	-	-	-	-	-
2	PAH	Booker Fowler SFH	Injection	12 hrs	5	100	80	0	-	25	10	100	0	-
2	PAH	Gavins Point NFH	Injection	12 hrs	6	100	100	0	-	10	20	100	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	PAH	Osage Catfisheries, Inc.	Injection	12 - 30 hrs	6	10	100	0	-	-	-	-	-	-
7	PAH	Tishomingo NFH	Injection	24 hrs	20	100	0 - 20	4	0	28	100	14 - 42	7	0 - 100
2	PLS	Gavins Point NFH	Injection	12 hrs	1	100	100	0	-	3	20	100	0	-
2	RGM	Mora NFH & TC	Injection	3 days	5	40	0	30	0	15	40	0	30	0
3	SSN	Bears Bluff NFH	Injection	2 days	-	-	-	-	-	12	30	50 - 75	0	-
1	STT	Dworshak NFH	Implant	14 & 21 days	-	-	-	-	-	30	100	87	12	42
1	STB	Blackwater Fisheries Research	Injection	3 - 7	5	25	100	0	-	-	-	-	-	-
1	STB	Blackwater Fisheries Research	Injection	Fish died	2	100	-	0	-	-	-	-	-	-
3	WHS	Kootenai Tribal Hatchery	Injection	36 - 50 hrs	3	100	100	0	-	-	-	-	-	-
2	WHS	Stolt Sea Farm California, LLC	Injection	1 - 2 days	6	20	100	6	0	10	10	80	0	-
6	YEP	Manning SFH	Implant	2 - 8 days	119	89	unknown	0	-	128	107	unknown	0	-

Table 2. Description of Number of Treatment Trials, Number and Species of Fish Treated, and Treatment Regimens Used during CY 2002 $LHRH_a$ Efficacy Studies

Total Number of Treatment Trials	110
Number of Trials that Appeared Efficacious:	67 (61%)
Total Number of Treated Fish:	2607

Treatment Regimes Used:

0.5 - 10 ug/Kg body weight (1 - 2 injections)	19 trials
20 - 30 ug/Kg body weight (1 - 2 injections)	13 trials
33 & 39.94 ug/Kg body weight (1 implant)	16 trials
40 - 50 ug/Kg body weight (1-2 injections)	6 trials
89 ug/Kg body weight (1 implant)	3 trials
100 - 107 ug/Kg body weight (1 - 4 injections, 1 implant)	27 trials
120 & 122.9 ug/Kg body weight (2 - 3 injections, 1 implant)	12 trials
180 ug/Kg body weight (2 - 3 injections)	13 trials
240 ug/Kg body weight (2 injections)	1 trial

Water Temperature (°F) Range: 41.0 - 82.4

Fish Species Treated:

Salmonids

cutthroat trout *Oncorhychus clarki*

Gila trout *O. gilae*

steelhead trout *O. mykiss*

Non-Salmonids

American Shad *Alosa sapidissima*

channel catfish *Ictalurus punctatus*

flathead catfish *Pylodictis ovilaris*

grass carp *Ctenopharyngodon idella*

hickory shad *Alosa mediocris*

lake sturgeon *Acipenser fulvescens*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

Rio Grande silvery minnow *Hybognathus amarus*

shortnose sturgeon *Acipenser brevirostrum*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

yellow perch *Perca flavescens*

Size Class of Treated Fish: Adults