$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2003 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 $LHRH_a$. Numerous INAD trials were conducted in calendar year (CY) 2003 to evaluate the efficacy of $LHRH_a$ to induce gamete maturation in a variety of fish species. One hundred and five such trials that involved 2,451 treated fish and 194 control fish were conducted at seven U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey cooperative unit, 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 CY 03 showed that approximately 62% of the trials appeared efficacious,

8% of the trials appeared ineffective, and 30% 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 calendar year 2003 (CY 03) supplemental $LHRH_a$ field efficacy studies. 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 20 fish culture facilities used $LHRH_a$ during CY 03, including seven U.S. Fish and Wildlife Service fish hatcheries, one U.S. Geological Survey cooperative unit, 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 CY 03 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 injection (1 - 2 injections) or pellet implant. Drug dosages used by participating fish culture facilities in CY 03 ranged from 10 to 199.8 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 disposed of by incineration.

Fish Species and Sex Treated

1. Species of fish treated

A total of 16 fish species were treated with $LHRH_a$ under INAD #8061 during CY 03, including the following four salmonids and 12 non-salmonids:

Salmonids

spring chinook salmon *Oncorhychus tshawytscha*

Gila trout *O. gilae*

steelhead trout *O. mykiss*

brown trout *Salmo trutta*

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*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

yellow perch *Perca flavescens*

2. Gender of treated fish

$LHRH_a$ was used on 1,501 female and 950 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 10 ug/kg body weight (1 injection)

Treated fish used in six trials were injected one time with $LHRH_a$ at dosage of 10 ug/kg body weight. Trials involved male Gila trout, paddlefish, and white sturgeon. Treatment resulted in 60 - 100% spermiation in the male treated fish, compared to 0 - 100% spermiation in the control fish. Treatments appeared efficacious in five trials and characterized as inconclusive in one trial (Table 1).

B. Efficacy at 20 - 25 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 20 - 25 ug/kg body weight. Trials involved the following fish species: female channel catfish, Gila trout, spring chinook salmon, striped bass; and white sturgeon, and male paddlefish and pallid sturgeon. Treatment resulted in the following:

0 - 100% ovulation in treated female fish, compared to 0 - 90% ovulation in the control fish;

100% spermiation in the male treated fish, no control fish were used.

Treatments appeared efficacious in 12 trials, ineffective in two trials, and were characterized as inconclusive in five trials (Table 1).

C. Efficacy at 25 ug/kg body weight (implant)

Treated fish used in 10 trials were implanted with $LHRH_a$ at dosage of 25 ug/kg body weight. Trials involved male American and hickory shad. Treatment resulted in 100% spermiation in treated American shad and an unknown number of spermiation in the treated hickory shad; no control fish were used. All fish were implanted with $LHRH_a$ when they were being transferred into the fish transport truck. At the end of the spawning season, all wild-brood fish at the hatchery are destroyed and incinerated. Treatments appeared efficacious in three trials, and were characterized as inconclusive in seven trials (Table 1).

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

Treated fish used in 16 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 shovelnose sturgeon and California halibut, and female channel catfish and brown trout. Treatments resulted in the following:

10 - 100% ovulation in treated female fish, compared to 0 - 30% ovulation in the control fish;

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

Treatments appeared efficacious in 13 trials and were ineffective in three trials (Table 1).

E. Efficacy at 50 ug/kg body weight (implant)

Treated fish used in seven trials were implanted with $LHRH_a$ at a dosage of 50 ug/kg body weight. Trials involved female hickory shad. Treatment resulted in 0 - 90% ovulation in the treated fish; no control fish were used. All fish were implanted with $LHRH_a$ pellets when they were being transferred into the fish transport truck. At the end of the spawning season, all wild-brood fish at the hatchery are destroyed and incinerated. Treatments appeared efficacious in four trials, ineffective in one trial, and were characterized as inconclusive in two trials (Table 1).

F. Efficacy at 60 - 90 ug/kg body weight (1 injection)

Treated fish used in five trials were injected once with $LHRH_a$ at dosages between 60 - 90 ug/kg body weight. Trials involved female paddlefish and female channel catfish. Treatment resulted in 20 - 100% ovulation in treated fish;

no control fish were used. Treatments appeared efficacious in four trials; and treatment was characterized as inconclusive in one trial (Table 1).

G. Efficacy at 100 ug/kg body weight (1 - 2 injections)

Treated fish used in 33 trials were injected one or two times with $LHRH_a$ at a dosage of 100 ug/kg body weight. Trials involved both male and female paddlefish and alligator gar, and female channel catfish, lake sturgeon, pallid sturgeon, and white sturgeon. Treatment resulted in the following:

0 - 100% ovulation in treated female fish, compared to 50 - 100% ovulation in the control fish;

33- 100% spermiation in the male treated fish, compared to 0% spermiation in the control fish.

Treatments appeared efficacious in 22 trials, ineffective in two trials, and were characterized as inconclusive in nine trials (Table 1).

H. Efficacy at 100 ug/kg body weight (implant)

Treated fish used in one trial involving male steelhead trout were implanted with $LHRH_a$ at a dosage of 100 ug/kg body weight . Treatment resulted in 100%

spermiation in the treated fish, compared to 73% spermiation in the control fish. Treatment appeared efficacious in this trial (Table 1).

I. Efficacy at 120.18 ug/kg body weight (implant)

Treated fish used in four trials involving female yellow perch were implanted with $LHRH_a$ at a dosage of 120.18 ug/kg body weight. Treatment resulted in 8 - 80% ovulation in the female treated fish; no control fish were used. All fish were implanted with $LHRH_a$ when they were being transferred into the fish transport truck. At the end of the spawning season, all wild-brood fish at the hatchery are destroyed and incinerated. Treatments appeared efficacious in one trial while three trials were characterized as inconclusive (Table 1).

J. Efficacy at 199.8 ug/kg body weight (implant)

Treated fish used in four trials involving male yellow perch were implanted with $LHRH_a$ at a dosage of 199.8 ug/kg body weight. Treatment resulted in an unknown number of spermiation in the treated yellow perch; no control fish were used. All fish were implanted with $LHRH_a$ when they were being transferred into the fish transport truck. At the end of the spawning season, all wild-brood fish at the hatchery are destroyed and incinerated. Treatments were characterized as inconclusive in all four trials (Table 1).

2. Observed Toxicity

No toxicity or adverse effects relating to $LHRH_a$ treatment were reported in 97 trials. In four trials, the Investigators noted that treated fish died after treatment most likely because of either handling stress or poor water quality. The Investigators of an additional four trials only noted that some of the treated and control fish had died after the spawning period.

Summary of Study Results

$LHRH_a$ was used in 105 efficacy trials to induce gamete maturation in 16 different fish species (n = 2,451 treated fish; 194 untreated control fish) at dosages ranging from 10 - 199.8 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 destroyed after the spawning season. Water temperature during treatments ranged from 43.0 - 82.4 °F. Approximately 62% of the trials appeared efficacious, 8% appeared ineffective, and 30% were characterized as inconclusive. Data from the CY 03 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	24 hrs	1	100	100	0	-	2	100	100	0	-
2	ALG	Tishomingo NFH	Injection	24 hrs	2	100	100	0	-	1	100	100	0	-
3	AMS	Manning SFH	Implant	3 days	0	-	-	0	-	156	25	100	0	-
2	BNT	Rathbun Research SFH	Injection	Every 4 days	40	50	10 - 50	40	25 - 30	0	-	-	0	-
5	CCF	Harvest Select Farms	Injection	48 hrs	322	20	79 - 100	0	-	0	-	-	0	-
3	CCF	Harvest Select Farms	Injection	48 hrs	89	50	80 - 82	0	-	0	-	-	0	-
1	CCF	Harvest Select Farms	Injection	48 hrs	4	60	100	0	-	0	-	-	0	-
3	CCF	Harvest Select Farms	Injection	48 hrs	12	80	20 - 100	0	-	0	-	-	0	-
7	CCF	Harvest Select Farms	Injection	48 hrs	196	100	0 - 100	0	-	0	-	-	0	-
6	CCF	North Auburn Research Unit	Injection	1 - 5 days	238	100	15 - 95	0	-	0	-	-	0	-
1	GIT	Mora NFH & TC	Injection	3 days	0	-	-	0	-	30	10	80	30	100
1	GIT	Mora NFH & TC	Injection	3 days	30	20	66	30	90	0	-	-	0	-
3	HAL	Hubbs SeaWorld Research Institute	Injection	24 hrs	4	50	100	30	0	1	50	0	1	0
14	HKS	Manning SFH	Implant	3 days	294	50	0 - 90	0	-	317	25	-	0	-
3	LST	Wild Rose SFH	Injection	1 - 2 days	5	100	100	8	50-100	0	-	-	0	-
2	PAH	Booker Fowler SFH	Injection	14 - 19 hrs	5	100	100	0	-	13	10	85	0	-
2	PAH	Gavins Point NFH	Injection	10 hrs	4	100	100	0	-	11	20	100	0	-
6	PAH	Natchitoches NFH	Injection	24 - 36 hrs	17	100	0 - 50	0	-	15	10	60 - 100	0	-

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					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
1	PAH	Osage Catfisheries, Inc	Injection	3 - 4 days	3	100	100	0	-	0	-	-	0	-
3	PAH	Tishomingo NFH	Injection	16 hrs	1	100	100	0	-	17	100	33 - 40	24	0
2	PLS	Gavins Point NFH	Injection	9 - 24 hrs	3	100	100	0	-	3	20	100	0	-
1	SCS	Clearwater SFH	Injection	Completed in 7 days	13	20	100	0	-	0	-	-	0	-
8	SNS	Bozeman FTC	Injection	18 - 30 hrs	18	40	80 - 100	0	-	32	40	0 - 100	4	0
7	STB	Blackwater Fisheries Research	Injection	4 - 8 days	21	25	0 - 100	0	-	0	-	-	0	-
2	STB	Richmond Hill SFH	Injection	2 - 3 days	2	25	100	0	-	0	-	-	0	-
1	STT	Dworshak NFH	Implant	13 & 20 days	0	-	-	0	-	30	100	100	11	73
4	WHS	Kootenai Tribal Hatchery	Injection	23 - 29 hrs	4	100	100	0	-	0	-	-	0	-
2	WHS	Stolt Sea Farm California LLC	Injection	24 hrs	6	20	50	6	0	10	10	67	10	0
8	YEP	Manning SFH	Implant	2 - 8 days	167	120.18	8 - 80	0	-	312	199.8	-	0	-

Table 2. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2003 $LHRH_a$ Efficacy Studies

Total Number of Treatment Trials	105
Number of Trials that Appeared Efficacious:	65 (62%)
Number of Trials that Appeared Inefficacious:	8 (8%)
Number of Trials that Appeared Inconclusive:	32 (30%)
Total Number of Treated Fish:	2451

Treatment Regimes Used:

10 ug/Kg body weight (1 injection)	6 trials
20 - 25 ug/Kg body weight (1 - 2 injections)	19 trials
25 ug/Kg body weight (1 implant)	10 trials
40 - 50 ug/Kg body weight (1-2 injections)	16 trials
50 ug/Kg body weight (1 implant)	7 trials
60 - 90 ug/Kg body weight (1 injection)	5 trials
100 ug/Kg body weight (1 - 2 injections)	33 trials
100 ug/Kg body weight (1 implant)	1 trial
120.18 ug/Kg body weight (1 implant)	4 trials
199.8 ug/Kg body weight (1 implant)	4 trials

Water Temperature (°F) Range: 43.0 - 82.4

Fish Species Treated:

Salmonids

spring chinook salmon *Oncorhychus tshawytscha*

Gila trout *O. gilae*

steelhead trout *O. mykiss*

brown trout *Salmo trutta*

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*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

yellow perch *Perca flavescens*

Size Class of Treated Fish: Adults