$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2001 Annual Summary Report on the Use of $LHRH_a$ in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Bozeman National INAD Office
Bozeman, Montana

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) 2001 to evaluate the efficacy of $LHRH_a$ to induce gamete maturation in a variety of fish species. Forty-seven such trials that involved 425 treated fish and 89 control fish were conducted at six U.S. Fish and Wildlife Service fish hatcheries, four state hatcheries, one private hatchery, 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 CY01 showed that approximately 74% of the trials appeared efficacious,

11% of the trials appeared ineffective, and 15% 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 was to summarize the results of the CY 2001 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 12 fish culture facilities used $LHRH_a$ during CY01, including six U.S. Fish and Wildlife Service fish hatcheries, four state hatcheries, one private hatchery, and one tribal hatchery.

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 by using either an injection (1 - 2 injections) or pellet implant method. Drug dosages used by participating fish culture facilities in CY 2001 ranged from 10 to 202 ug $LHRH_a$/kg bw. Fish treated at a dosage greater then 100 ug $LHRH_a$/kg fish bw received a pellet implant and were sacrificed after spawning.

Fish Species and Sex Treated

1. Species of fish treated

The following three salmonid and seven non-salmonid species were treated with $LHRH_a$ under INAD #8061 during CY 2001.

Salmonids

cutthroat trout *Oncorhychus clarki*

spring chinook salmon *O. tshawytscha*

steelhead trout *O. mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

flathead catfish *Pylodictis ovilaris*

paddlefish *Polydon spathula*

lake sturgeon *Acipenser fulvescens*

white sturgeon *A. transmontanus*

pallid x shovelnose sturgeon *Scaphirhynchus albus* x *S. platorynchus*

yellow perch *Perca flavescens*

2. Gender of treated fish

$LHRH_a$ was used on 188 female and 237 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 - 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. Summary results on the efficacy of $LHRH_a$ to induce gamete maturation

A. Efficacy at 10 ug/kg body weight (one injection)

In two trials, male white sturgeon (n = 20 fish) were injected one time with 10 ug $LHRH_a$/kg bw. In two other trials, male paddlefish (n = 21 fish) were also injected one time with 10 ug $LHRH_a$/kg bw. Overall treatment resulted showed that 40 - 100% of treated fish reached active spermiation. None of the control fish reached active spermiation (Table 1). Treatment appeared to be efficacious in all trials.

B. Efficacy at 10 ug/kg body weight (two injections)

In three trials, male pallid sturgeon (n = 20 fish) were injected two times with a total 10 ug $LHRH_a$/kg bw, and in one trial, male pallid x shovelnose sturgeon (n = 3 fish) were injected two times with a total of 10 ug $LHRH_a$/kg bw. Overall treatment resulted in 0 - 100% of the treated fish reaching active (Table 1). Treatment appeared efficacious in three trials and ineffective in one trial.

C. Efficacy at 10 - 20 ug/kg body weight (one injection)

In one trial, male pallid sturgeon (n = 4 fish) were injected one time with 10 - 20 ug $LHRH_a$/kg bw, and in one other trial, male paddlefish (n = 21 fish) were injected one time with 10 - 20 ug $LHRH_a$/kg bw. Overall treatment resulted in 71 - 100% of treated fish reaching active spermiation (Table 1). Treatment appeared to be efficacious in both trials.

D. Efficacy at 20 ug/kg body weight (one injection)

In one trial involving female shovelnose sturgeon (n = 12 fish), one trial involving female cutthroat trout (n = 4 fish), one trial involving female chinook salmon (n = 12 fish), one trial involving male cutthroat (n = 5 fish), and one trial involving male shovelnose sturgeon (n = 20 fish), treated fish were injected one time with 20 ug

$LHRH_a$/kg bw. Treatment resulted in 100% ovulation in treated female fish, compared to 0 - 50% ovulation in the control fish (Table 1). Treatment resulted in 80 - 95% spermiation in treated male fish, compared to 0 - 60% spermiation in the control fish. Treatment appeared to be efficacious in all trials.

E. Efficacy at 20 ug/kg body weight (two injections)

In two trials, female white sturgeon (n = 10 fish) were injected two times with 20 ug $LHRH_a$/kg bw. Treatment resulted in 50% ovulation in treated female fish, compared to 0% ovulation in control fish (Table 1). Treatment appeared to be efficacious in both trials.

F. Efficacy at 100 ug/kg body weight (one injection or pellet implant)

In one trial male flathead catfish (n = 1 fish) were injected one time with 100 ug $LHRH_a$/kg bw, and in one other trial, pellets were implanted in male steelhead trout (n = 25 fish). Treatment resulted in 100% of the treated males reaching active spermiation, compared with 27% of untreated fish reaching active spermiation (Table 1) . Treatment appeared to be efficacious both trials.

G. Efficacy at 100 ug/kg body weight (two injections)

$LHRH_a$ was used at 100 ug/kg bw in four trials involving female paddlefish (n = 22 fish), two trials involving female pallid sturgeon (n = 5 fish), one trial involving female pallid x shovelnose sturgeon (n = 3 fish), four trials involving female flathead catfish (n = 28 fish), two trials involving female shovelnose sturgeon (n = 5 fish), one trial involving female white sturgeon (n = 5 fish), one trial involving male paddlefish (n = 11 fish), and one trial involving male alligator gar (n = 3 fish). Treatment resulted in the following:

0 - 100% ovulation in treated female fish

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

Treatments appeared efficacious in 10 trials, ineffective in four trials, and were characterized as inconclusive in two trials (Table 1).

H. Efficacy at 100 ug/kg body weight (3 injections)

$LHRH_a$ was used at 100 ug/kg bw in one trial involving female lake sturgeon (n = 2 fish) and one trial involving female alligator gar (n = 1 fish). Treatment resulted

in 100% ovulation in treated female fish (Table 1). Treatments appeared efficacious in one trial, and were characterized as inconclusive in one trial.

I. Efficacy at 191 ug/kg body weight (pellet implant)

$LHRH_a$ was used at 191 ug/kg bw in five trials involving male yellow perch (n = 83 fish). Treatment resulted in 100% spermiation in the male treated fish, compared to 100% spermiation in the control fish (Table 1). Treatments appeared efficacious in two trials, and were characterized as inconclusive in three trials.

J. Efficacy at 202 ug/kg body weight (pellet implant)

$LHRH_a$ was used at 202 ug/kg bw in five trials involving female yellow perch (n = 79 fish). Treatment resulted in 0 - 100% ovulation in treated fish, compared to 25% ovulation in the control fish (Table 1). Treatments appeared to be efficacious in four of the trials, and were characterized as inconclusive in one trial. The Investigator noted that all of the test fish in one trial died due to a heater malfunction.

2. Observed Toxicity

No toxicity or adverse effects relating to $LHRH_a$ treatment were reported.

Summary of Study Results

$LHRH_a$ was in used 47 efficacy trials to induce gamete maturation in 10 different fish species (n = 425 treated fish; 89 untreated control fish) at dosages ranging from 10 - 202 ug/kg bw. LHRHa was administered using either 1 or 2 injections or as a pellet implant. Water temperature during treatments ranged from 38.0 - 76.6 °F. Approximately 74% of the trials appeared efficacious, 11% appeared ineffective, and 15% were characterized as inconclusive. Data from the CY 2001 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. 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. $LHRH_a$ Use Summary Record				Females					Males				
				Treated			Control		Treated			Control	
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.)	% Spermaite	Number of Controls	% Spermiate
Alligator Gar	Private John Allen NFH	Injection	11 hrs	1	100	100	0	n/a	3	100	100	0	n/a
Cutthroat Trout	Bozeman FTC	Injection	4 - 20 days	4	20	100	2	50	5	20	80	5	60
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	12 hrs	0	n/a	n/a	0	n/a	1	100	100	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	10 days	2	100	50	0	n/a	0	n/a	n/a	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	3	100	33	0	n/a	0	n/a	n/a	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	22	100	9	0	n/a	0	n/a	n/a	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	1	100	0	0	n/a	0	n/a	n/a	0	n/a
Lake Sturgeon	Wild Rose State Fish Hatchery	Injection	24 hrs	2	100	100	0	n/a	0	n/a	n/a	0	n/a
Paddlefish	Booker Fowler Fish Hatchery	Injection	24 hrs	6	100	100	0	n/a	13	10	100	0	n/a
Paddlefish	Gavins Pt. NFH	Injection	13 - 26 hrs	6	100	50	0	n/a	21	10-20	71	0	n/a
Paddlefish	Natchitoches NFH	Injection	30 hrs	4	100	25	0	n/a	8	10	100	0	n/a
Paddlefish	Tishomingo NFH	Injection	24 hrs	6	100	100	0	n/a	11	100	27	3	0
Pallid Sturgeon	Gavins Pt. NFH	Injection	12 - 25 hrs	2	100	100	0	n/a	4	10-20	100	0	n/a
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	3	100	100	0	n/a	16	10	0	0	n/a
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	0	n/a	n/a	0	n/a	3	10	67	0	n/a
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	0	n/a	n/a	0	n/a	1	10	100	0	n/a
Pallid x Shovelnose Sturgeon	Natchitoches NFH	Injection	30 hrs	3	100	67	0	n/a	3	10	100	0	n/a
Spring Chinook Salmon	Clearwater Hatchery	Injection	7 days	12	20	100	0	n/a	0	n/a	n/a	0	n/a
Shovelnose Sturgeon	Bozeman FTC	Injection	24 - 30 hrs	12	20	100	9	0	20	20	95	2	0

Table 1. $LHRH_a$ Use Summary Record				Females					Males				
				Treated			Control		Treated			Control	
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.)	% Spermaite	Number of Controls	% Spermiate
Shovelnose Sturgeon	Natchitoches NFH	Injection	30 hrs	3	100	0	0	n/a	0	n/a	n/a	0	n/a
Shovelnose Sturgeon	Natchitoches NFH	Injection	30 hrs	2	100	50	0	n/a	0	n/a	n/a	0	n/a
Steelhead Trout	Dworshak NFH	Implant	12 - 26 days	0	n/a	n/a	0	n/a	25	100	100	11	27
White Sturgeon	Kootenai Tribal Hatchery	Injection	30 - 40 hrs	5	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Stolt Sea Farm California LLC	Injection	24 - 26 hrs	6	20	50	6	0	10	10	[1]40	10	0
White Sturgeon	Stolt Sea Farm California LLC	Injection	24 - 36 hrs	4	20	50	4	0	10	10	70	10	0
Yellow Perch	Manning Hatchery	Implant	2 - 8 days	7	202	0	0	n/a	12	191	100	0	n/a
Yellow Perch	Manning Hatchery	Implant	2 - 8 days	28	202	100	0	n/a	18	191	100	9	100
Yellow Perch	Manning Hatchery	Implant	2 - 8 days	6	202	100	0	n/a	9	191	100	0	n/a
Yellow Perch	Manning Hatchery	Implant	2 - 8 days	9	202	89	0	n/a	11	191	100	0	n/a
Yellow Perch	Manning Hatchery	Implant	2 - 8 days	29	202	93	8	25	33	191	100	10	100

[1] Only 4 of 10 injected males were evaluated for ripeness; all 4 evaluated males were ripe.

Table 2. Summary Data Regarding CY 2001 Luteinizing hormone-releasing hormone analog Efficacy Studies

Total Number of Treatment Trials	47
Number of Trials that Appeared Efficacious:	35 (74%)
Total Number of Treated Fish:	425

Treatment Regimes Used:

10 ug/Kg body weight (1- 2 injections)	8 trials
10 - 20 ug/Kg body weight (1 injection)	2 trials
20 ug/Kg body weight (1-2 injections)	7 trials
100 ug/Kg body weight (1-3 injections & implant)	20 trials
191ug/Kg body weight (1 implant)	5 trials
202 ug/Kg body weight (1 implant)	5 trials

Water Temperature (°F) Range: 38.0 - 76.6

Fish Species Treated:

Salmonids

cutthroat trout *Oncorhychus clarki*

steelhead trout *O. mykiss*

spring chinook salmon *O. tshawytscha*

Non-Salmonids

alligator gar *Lepisosteus spatula*

flathead catfish *Pylodictis ovilaris*

paddlefish *Polydon spathula*

white sturgeon (*Acipenser transmontanus*

lake sturgeon *A. fulvescens*

pallid x shovelnose sturgeon *Scaphirhynchus albus* x *S. platorynchus*

yellow perch *Perca flavescens*

Size Class of Treated Fish: Adults