$LHRH_a$ Spawning Hormone Clinical Field Trials - INAD 8061

2000 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 fisheries programs to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration (FDA) 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) 2000 to evaluate the efficacy of $LHRH_a$ to induce gamete maturation in a variety of fish species. Sixty-three such trials that involved 915 treated fish and 307 control fish were conducted at eight U.S. Fish and Wildlife Service fish hatcheries, two 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 CY 2000 showed that approximately 68% of the trials appeared efficacious, 21% appeared ineffective, and 11% were characterized as

inconclusive. Results indicate that $LHRH_a$ was an effective spawning hormone in trials conducted in CY 2000, and as an FDA-approved drug would benefit fishery programs in the U. S.

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 2000 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 CY 2000, including eight U.S. Fish and Wildlife Service fish hatcheries, two 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 ranged from 10 - 100 ug $LHRH_a$/kg BW.

Fish Species and Sex Treated

1. Species of fish treated

The following two salmonid and eleven non-salmonid fish species were treated with $LHRH_a$ under INAD #8061 during CY 2000.

Salmonids

lake trout *Salvelinus namaycush*

steelhead trout *Oncorhychus mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

flathead catfish *Pylodictis ovilaris*

paddlefish *Polydon spathula*

lake sturgeon *Acipenser fulvescens*

white sturgeon *A. transmontanus*

american shad *Alosa sapidissima*

koi *Cyprinus sp.*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *S. platorynchus*

striped bass *Morone saxatilis*

sturgeon chub *Macrhybopsis gelida*

2. Sexes of fish treated

In CY 2000, 472 female fish and 443 male fish were treated with $LHRH_a$. Typically, females are treated with spawning hormone to shorten the gamete maturation period (i.e. advance maturation), and males are 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. Efficacy was determined by whether or not treated fish produced or yielded more eggs or milt than untreated fish. Percentages reflected the relative 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 collected. 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 bw (one injection)

$LHRH_a$ was used at 10 ug/kg bw in three trials involving male white sturgeon (n = 33 treated fish, n = 33 untreated fish), two trials involving male paddlefish (n = 15 treated fish), and one trial involving male koi (n = 3 treated fish). Overall treatment resulted in 40 - 100% of the treated fish reaching active spermiation (Table 1). None of the untreated white sturgeon reached active spermiation. Treatment appeared efficacies in all six trials.

B. Efficacy at 15 ug/kg bw (one injection)

$LHRH_a$ was used at 15 ug/kg bw in one trial involving female striped bass (n = 10 treated fish). Treatment resulted in 100% ovulation in treated fish (Table 1) within 48 hrs of injection. Treatment appeared efficacious.

C. Efficacy at 20 ug/kg bw (one injection)

$LHRH_a$ was used at 20 ug/kg bw in one trial involving female shovelnose sturgeon (n = 15 treated fish), one trial involving female lake trout (n = 26 treated fish), one trial involving male paddlefish (n = 12 treated fish), and one trial involving male shovelnose sturgeon (n = 10 treated fish). Treatment resulted in 93 - 100% ovulation in treated female fish and 0 - 100% spermiation in treated male fish (Table 1). Treatment appeared efficacious in three of the four trials and ineffective in the remaining trial.

D. Efficacy at 20 ug/kg bw (two injections)

$LHRH_a$ was used at 20 ug/kg bw in three trials involving female white sturgeon (n = 15 treated fish). Treatment resulted in 33 - 80% ovulation in treated female fish, compared to 0% ovulation in the control fish (Table 1). Treatment appeared efficacious in all three trials.

E. Efficacy at 50 ug/kg bw (one injection)

$LHRH_a$ was used at 50 ug/kg bw in one trial involving male american shad (n = 213 treated fish), and one trial involving male striped bass (n = 30 treated fish). Treatment resulted in a reported motility score of 3 - 4 in treated fish. However,

the percentage of ripe fish was not recorded for either trial. Consequently, resulted were characterized as inconclusive.

F. Efficacy at 98 ug/kg bw (one injection)

$LHRH_a$ was used at 98 ug/kg bw in one trial involving female american shad (n = 136 treated fish). Treatment resulted in 100% ovulation in treated fish, compared to 0% ovulation in the control fish (Table 1). Therefore, treatment appeared efficacious.

G. Efficacy at 100 ug/kg bw (one injection; pellet implant in male steelhead trout)

$LHRH_a$ was used at 100 ug/kg bw in the following trials:

One trial involving female alligator gar (n = 2 treated fish)

Two trials involving female paddlefish (n = 5 treated fish)

Three trials involving female lake sturgeon (n = 6 treated fish)

One trial involving female lake trout (n = 162 treated fish)

One trial involving female flathead catfish (n = 1 treated fish)

One trial involving female koi (n = 6 treated fish)

Three trials involving female sturgeon chub (n = 19 treated fish)

One trial of male alligator gar (n = 8 treated fish)

Three trials involving male sturgeon chub (n = 25 treated fish)

One trial involving male steelhead trout (n = 13 treated fish)

Treatment resulted in 0 - 100% ovulation in treated female fish (compared to 0 - 100% ovulation in the control fish) and 100% spermiation in the male treated fish (Table 1). Treatment appeared efficacious in eight trials, ineffective in five trials, and inconclusive in four trials.

H. Efficacy at 100 ug/kg bw (two injections)

Treatment trials in which fish were injected two times with a total of 100 ug/kg bw $LHRH_a$ were conducted in the following trials:

Seven trials involving female paddlefish (n = n = 35 treated fish

Three trials involving female pallid sturgeon (n = 8 treated fish)

Two trials involving female flathead catfish (n = 10 treated fish)

Three trials involving female koi (n = 11 treated fish)

Five trials involving female white sturgeon (n = 5 treated fish)

Six trials involving male paddlefish (n = 62 treated fish)

Three trials involving male pallid sturgeon (n = 19 treated fish)

Treatment resulted in 0 - 100% ovulation in treated female fish and 0 - 100% spermiation in the male treated fish (Table 1). Treatment appeared efficacious in 21 trials, ineffective in seven trials, and inconclusive in one trial.

2. Observed Toxicity

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

Summary of Study Results

$LHRH_a$ was used in 63 efficacy trials to induce gamete maturation in 13 different fish species (n = 915 treated fish; 307 untreated control fish) at dosages ranging from 10 to 100 ug/kg bw. Treatment trials were conducted water temperature ranging from 42 to 82 °F. Approximately 68% of the trials appeared efficacious, 21% appeared ineffective, and 11% were characterized as inconclusive. Data from the CY 2000 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

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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	1 day	2	100	0	0	n/a	8	100	0	0	n/a
American Shad	Edenton NFH	Injection	1 - 7 days	136	98	100	15	0	213	50	n/a	40	0
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	1	100	0	0	n/a	0	n/a	n/a	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	5	100	60	0	n/a	0	n/a	n/a	0	n/a
Flathead Catfish	Booker Fowler Fish Hatchery	Injection	1 - 6 days	5	100	0	0	n/a	0	n/a	n/a	0	n/a
Koi	Booker Fowler Fish Hatchery	Injection	3 ½ days	6	100	0	0	n/a	0	n/a	n/a	0	n/a
Koi	Booker Fowler Fish Hatchery	Injection	3 ½ days	3	100	100	0	n/a	3	10	100	0	n/a
Koi	Booker Fowler Fish Hatchery	Injection	3 ½ days	2	100	100	0	n/a	0	n/a	n/a	0	n/a
Koi	Booker Fowler Fish Hatchery	Injection	5 days	6	100	0	0	n/a	0	n/a	n/a	0	n/a
Lake Sturgeon	Wild Rose State Fish Hatchery	Injection	26 hrs	3	100	100	0	n/a	0	n/a	n/a	0	n/a
Lake Sturgeon	Wild Rose State Fish Hatchery	Injection	26 hrs	2	100	100	0	n/a	0	n/a	n/a	0	n/a
Lake Sturgeon	Wild Rose State Fish Hatchery	Injection	26 hrs	1	100	100	1	100	0	n/a	n/a	0	n/a
Lake Trout	Bozeman FTC	Injection	7 days	26	20	100	0	n/a	0	n/a	n/a	0	n/a
Lake Trout	Pendills Creek NFH	Injection	10 days	162	100	96	107	95	0	n/a	n/a	0	n/a
Paddlefish	Booker Fowler Fish Hatchery	Injection	13 - 17 hrs	3	100	100	0	n/a	9	10	100	0	n/a
Paddlefish	Booker Fowler Fish Hatchery	Injection	13 - 17 hrs	2	100	100	0	n/a	6	10	100	0	n/a
Paddlefish	Gavins Pt. NFH	Injection	41 hrs	9	100	67	3	0	12	20	100	10	0
Paddlefish	Natchitoches NFH	Injection	30 hrs	2	100	0	0	n/a	11	100	100	0	n/a
Paddlefish	Natchitoches NFH	Injection	30 hrs	4	100	100	0	n/a	10	100	100	0	n/a
Paddlefish	Natchitoches NFH	Injection	30 hrs	3	100	66	0	n/a	8	100	50	0	n/a

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
Paddlefish	Private John Allen NFH	Injection	36 hrs	8	100	38	0	n/a	10	100	0	0	n/a
Paddlefish	Tishomingo NFH	Injection	16 - 40 hrs	6	100	100	0	n/a	8	100	63	0	n/a
Paddlefish	Tishomingo NFH	Injection	16 - 40 hrs	3	100	33	0	n/a	15	100	80	5	20
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	2	100	0	0	n/a	4	100	0	0	n/a
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	4	100	0	0	n/a	6	100	50	0	n/a
Pallid Sturgeon	Natchitoches NFH	Injection	30 hrs	2	100	100	0	n/a	9	100	22	0	n/a
Shovelnose Sturgeon	Bozeman FTC	Injection	24 hrs	15	20	93	0	n/a	10	20	0	0	n/a
Steelhead Trout	Dworshak NFH	Implant	12 - 26 days	0	n/a	n/a	0	n/a	13	100	100	71	51
Striped Bass	Edenton NFH	Injection	48 hrs	10	15	100	0	n/a	30	50	n/a	0	n/a
Sturgeon Chub	Bozeman FTC	Injection	24 - 48 hrs	6	100	33	5	40	2	100	100	2	0
Sturgeon Chub	Bozeman FTC	Injection	24 - 72 hrs	8	100	13	0	n/a	3	100	33	0	n/a
Sturgeon Chub	Bozeman FTC	Injection	24 hrs	5	100	40	0	n/a	20	100	10	0	n/a
White Sturgeon	Kootenai Tribal Hatchery	Injection	48 hrs	1	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Kootenai Tribal Hatchery	Injection	48 hrs	1	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Kootenai Tribal Hatchery	Injection	48 hrs	1	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Kootenai Tribal Hatchery	Injection	48 hrs	1	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Kootenai Tribal Hatchery	Injection	48 hrs	1	100	100	0	n/a	0	n/a	n/a	0	n/a
White Sturgeon	Stolt Sea Farm California LLC	Injection	2 days	4	20	75	4	0	10	10	80	10	0
White Sturgeon	Stolt Sea Farm California LLC	Injection	2 days	5	20	80	5	0	10	10	40	10	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
White Sturgeon	Stolt Sea Farm California LLC	Injection	2 days	6	20	33	6	0	13	10	*57	13	0

* Only 7 of 13 treated males were evaluated for ripeness; of the 7 evaluated 4 males reach active spermiation.

Table 2. Summary Data Regarding CY 2000 Luteinizing hormone-releasing hormone analog Efficacy Studies

Total Fish Treated:	915
Total Number of Treatment Trials:	63
Number of Trials that Appeared Efficacious:	43 (68%)
Treatment Regimes Used:	
10 ug/Kg body weight (1 injection)	6 trials
15 ug/Kg body weight (1 injection)	1 trial
20 ug/Kg body weight (1-2 injections)	7 trials
50 ug/Kg body weight (1 injection)	2 trials
98 ug/Kg body weight (1 injection)	1 trials
100 ug/Kg body weight (1-2 injections & pellet implant)	46 trials
Water Temperature (°F) Range:	42 - 82

Species Treated:

Salmonids
lake trout *Salvelinus namaycush*
steelhead trout *Oncorhychus mykiss*

Non-salmonids
alligator gar *Lepisosteus spatula*
flathead catfish *Pylodictis ovilaris*
paddlefish *Polydon spathula*
lake sturgeon *Acipenser fulvescens*
white sturgeon *Acipenser transmontanus*

american shad *Alosa sapidissima*
koi *Cyprinus sp.*
pallid sturgeon *Scaphirhynchus albus*
shovelnose sturgeon (*S. platorynchus*
striped bass *Morone saxatilis*
sturgeon chub *Macrhybopsis gelida*

Size of Treated Fish: Adults