

## LHRH<sub>a</sub> Spawning Hormone Clinical Field Trials - INAD 8061

### **2006 Annual Summary Report on the Use of LHRH<sub>a</sub> in Clinical Field Efficacy Trials**

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#### **Summary**

Spawning aids such as luteinizing hormone-releasing hormone analogue (LHRH<sub>a</sub>), 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<sub>a</sub> 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<sub>a</sub>. In calendar year 2006 (CY06), 55 trials were conducted under this INAD to evaluate the efficacy of LHRH<sub>a</sub> to induce gamete maturation in a variety of fish species. Trials involved 2,747 treated fish and 335 control fish and were conducted at 22 different hatcheries, including eight U.S. Fish and Wildlife Service fish hatcheries, 10 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 from trials conducted in CY06 showed that

treatments appeared efficacious in approximately 85% of the trials, ineffective in 4% of the trials, and were characterized as inconclusive in 11% of the trials.

## **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 many 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 unnatural 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<sub>a</sub>) 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<sub>a</sub> 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<sub>a</sub> as a tool to enhance broodstock 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 LHRH<sub>a</sub> field efficacy studies conducted under INAD exemption #8061 in calendar year 2006 (CY06). Furthermore, it is expected that these data will be used to enhance the existing LHRH<sub>a</sub> 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**

Field efficacy trials were conducted at 22 different fish culture facilities during CY06, including eight U.S. Fish and Wildlife Service fish hatcheries, 10 state

hatcheries, three private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 43.0 to 79.0°F.

## **2. Chemical material**

Syndel International Inc. of Vancouver, British Columbia Canada was the supplier for all LHRH<sub>a</sub> used in trials conducted during the reporting period.

## **3. Drug dosages**

The Study Protocol authorized the use of up to 100 ug LHRH<sub>a</sub>/kg fish body weight (bw). Treatments were administered to fish using either injection (1 - 3 injections) or pellet implant. Drug dosages used by Investigators in CY06 ranged from 2.2 to 100 ug LHRH<sub>a</sub>/kg bw for fish treated by the injection method; or 42.61 to 186.7 ug LHRH<sub>a</sub>/kg bw for fish treated by the implant method. Fish treated by pellet implant were euthanized at the hatchery and properly disposed after they were spawned. Please note this was the last year participants were allowed to use the pellet implant method under the LHRH<sub>a</sub> study protocol. Future pellet implant treatments will be under another INAD or by regulatory discretion.

## Fish Species and Sex Treated

### 1. Fish Species Treated

Field efficacy trials were conducted on 15 different fish species under INAD #8061 during the reporting period, including the following four salmonids and 11 non-salmonids:

#### Salmonids

brown trout *Salmo trutta*

Gila trout *Oncorhynchus gilae*

rainbow trout *O. mykiss*

steelhead trout *O. mykiss*

#### Non-salmonids

alligator gar *Lepisosteus spatula*

American Shad *Alosa sapidissima*

Atlantic sturgeon *Acipenser oxyrinchus*

grass carp *Ctenopharyngodon idella*

hickory shad *Alosa mediocris*

lake sturgeon *Acipenser fulvescens*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *Scaphirhynchus platyrhynchus*

striped bass *Morone saxatilis*

white sturgeon *Acipenser transmontanus*

## **2. Gender of treated fish**

LHRH<sub>a</sub> was used on 1,427 female and 1,320 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<sub>a</sub> 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 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 when fish were spawned or evaluated for ripeness was also documented. In the case of females, which in some cases received 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. General observations on the efficacy of LHRH<sub>a</sub> to induce gamete maturation in salmonid and non-salmonid fish** (Note: Tables 1 - 4 provides summaries of all efficacy trials; Table 5 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 6 describes all trials conducted during CY06 under INAD #8061.)

### **A. Efficacy of LHRH<sub>a</sub> on male fish treated at a dosage between 10 and 100 ug/kg body weight (1 - 2 injections)**

Treated male fish were used in 20 trials and injected either one or two times with LHRH<sub>a</sub> at a dosage between 10 and 100 ug/kg body weight (Table 1). Below is

the treatment regimen used when treating fish species with this dosage range of LHRH<sub>a</sub>:

1. Dose: 10 - 20 ug/kg

Gila trout, paddlefish, pallid sturgeon, shovelnose sturgeon, and white sturgeon were used in 11 trials, and in each trial, fish were injected 1 or 2 times with LHRH<sub>a</sub>; three of the 11 trials involved control fish; results showed there was 25 - 100% spermiation in treated fish; and 0 - 85% spermiation in control fish. Overall, results indicated that treatment appeared effective in 10 trials and treatments were characterized as inconclusive in one trial.

2. Dose: 30 - 50 ug/kg

Alligator gar, Atlantic sturgeon, paddlefish, and shovelnose sturgeon were used in seven trials, and in each trial, fish were injected 1 or 2 times with LHRH<sub>a</sub>; one of the seven trials involved control fish; results showed that there was 0 - 100% spermiation in treated fish (in studies in which spermiation could be evaluated); and 100% spermiation in control fish from the single study in which control fish were used. In the study where the spermiation was unknown, the Investigator noted this was the first year these fish were attempted to be spawned in consecutive years. No spawning activity was observed or eggs found in the holding pond.

Overall, results indicated that treatment appeared effective in five trials, ineffective in one trial, and characterized as inconclusive in one trial.

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 LHRH<sub>a</sub>; no control fish were used in either trial. Results showed that there was 75 - 100% spermiation in treated fish. Overall, results indicated that treatment appeared effective in both trials.

Overall treatment resulted in either an unknown percent spermiation (due to the inability of the Investigator to evaluate fish) or a 0 - 100% level of spermiation in the male treated fish, compared to a 0 - 100% level of spermiation in control fish. Hence, results indicated that treatment appeared effective in 17 trials, ineffective in one trial, and characterized as inconclusive in two trials.

**B. Efficacy of LHRH<sub>a</sub> on female fish treated at a dosage between 2.2 and 100 ug/kg body weight (1 - 3 injections)**

Treated female fish were used in 28 trials and injected 1 - 3 times with LHRH<sub>a</sub> at a dosage between 2.2 and 100 ug/kg body weight (Table 2). Below is the treatment regimen used when treating fish with this dosage range of LHRH<sub>a</sub>:

1. Dose: 2.2 - 25 ug/kg

Brown trout, Gila trout, grass carp, lake sturgeon, rainbow trout, shovelnose sturgeon, striped bass, and white sturgeon were used in 11 trials, and in each trial, fish were injected 1 or 2 times with LHRH<sub>a</sub>; five of the eleven trials involved control fish. Treatment results showed that there was 0 - 100% ovulation in treated fish; and 0 - 100% ovulation in control fish. Overall, treatment appeared effective in all trials.

2. Dose: 40 - 50 ug/kg

Pallid sturgeon, shovelnose sturgeon, and white sturgeon were used in three trials, and in each trial, fish were injected 1 or 2 times with LHRH<sub>a</sub>; no control fish were used. Results showed that there was 29 - 100% ovulation in treated fish. Overall, treatment appeared effective in two trials and treatments were characterized as inconclusive in one trial.

3. Dose: 80 ug/kg

Paddlefish were used in one trial and fish were injected two times with LHRH<sub>a</sub>. No control fish were used in this trial. Results showed that there was 80 - 100% ovulation in treated fish. Overall, results indicated that treatment appeared effective in all trials.

#### 4. Dose: 100 ug/kg

Alligator gar, lake sturgeon, paddlefish, pallid sturgeon, and white sturgeon were used in 13 trials, and in each trial, fish were injected 1 - 3 times with LHRH<sub>a</sub>; one of the 13 trials involved control fish. Results showed that there was 0 - 100% ovulation in treated fish; and 100% ovulation in control fish. In the study where the ovulation was zero, the Investigator noted this was the first year these fish were attempted to be spawned in consecutive years. No spawning activity was observed or eggs found in the holding pond. Overall, treatments appeared efficacious in 12 trials and was ineffective in one trial.

Overall treatment resulted in 0 - 100% ovulation in female treated and control fish. Treatment appeared efficacious in 26 trials, ineffective in one trial, and was characterized as inconclusive in one trial.

#### **C. Efficacy of LHRH<sub>a</sub> on male fish treated between 50 and 79.7 ug/kg body weight (1 implant)**

Treated male fish were used in four trials and implanted one time with LHRH<sub>a</sub> at a dosage between 50 and 79.7 ug/kg body weight (Table 3). 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 three fish species treated with LHRH<sub>a</sub> at the dosages described above:

1. Dose: 50 - 79.7 ug/kg

American and hickory shad, and steelhead trout were used in four trials, and in each, fish were implanted with one LHRH<sub>a</sub> pellet. No control fish were used in any of the trials. Results showed that there was 100% spermiation in treated fish from the steelhead trout and hickory shad studies, and an unknown level of spermiation in the American shad studies. In the studies where the spermiation was unknown, individual fish were not checked to see if they were ripe after treatment. However, in one of the American shad studies spermiation did occur due to fry being produced in the test tanks. Overall, treatment appeared efficacious in three trials, and was characterized as inconclusive in one trial.

Overall, treatment resulted in either an unknown percent spermiation (due to fish not evaluated for spermiation by the Investigator) or a 100% spermiation in the male treated fish. Treatments appeared efficacious in three trials, and were characterized as inconclusive in one trial.

**D. Efficacy of LHRH<sub>a</sub> on female fish treated at a dosage between 42.61 and 186.7 ug/kg body weight (1 implant)**

Female fish were implanted one time with LHRH<sub>a</sub> pellets at a dosage between 42.61 and 186.7 ug/kg body weight (Table 4) in three different trials. All pellet implanted fish were euthanized at the end of the spawning period. In addition, 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 is the treatment regimen used on two fish species treated with LHRH<sub>a</sub>:

1. Dose: 42.61 ug/kg

Hickory shad were used in one trial and fish were implanted with one pellet. No control fish used in this trial. In this study the ovulation was unknown, individual fish were not evaluated by the Investigator to determine whether they were ripe after treatment. Treatment was characterized as inconclusive in this trial.

2. Dose: 134 - 186.7 ug/kg

American shad were used in two trials, and in each, fish were implanted with one LHRH<sub>a</sub> pellet. No control fish used in this trial. In these studies the ovulation was unknown, individual fish were not evaluated by the

Investigators to determine whether they were ripe after treatment.

However, in one of the American shad studies ovulation did occur due to fry being produced in the test tanks. Treatment appeared efficacious in one trial and was characterized as inconclusive in one trial.

Overall treatments resulted in an unknown percent ovulation (due to fish not evaluated for ripeness by the Investigators). Treatment appeared efficacious in one trial and was characterized as inconclusive in two trials.

## **2. Observed Toxicity**

No toxicity or adverse effects relating to LHRH<sub>a</sub> treatments were reported in any trials conducted in CY06.

### **Summary of Study Results**

LHRH<sub>a</sub> was used in 55 efficacy trials to induce gamete maturation in 15 different fish species (n = 2,747 treated fish; 335 untreated control fish) at dosages ranging from 2.2 - 186.7 ug/kg bw. LHRH<sub>a</sub> was administered using either 1 - 3 injections or as a pellet implant. All treated fish administered LHRH<sub>a</sub> as a pellet implant were euthanized after the spawning season. Water temperature during treatments ranged from 43.0 - 79.0°F. Approximately 85% of the trials appeared efficacious, 4% appeared ineffective, and 11% were characterized as inconclusive (mostly due to failure of the Investigator to

evaluate gamete maturation following treatment). Data from the CY06 trials support the results of previous Annual Report submissions under INAD #8061 that indicate that LHRH<sub>a</sub> 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. Summary of Year 2006 LHRH<sub>a</sub> Male Efficacy Results - Injection**

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Spermiat	Number of Controls	% Spermaite
1	effective	ALG	Private John Allen NFH	2	24 hrs	7	100	100	0	-
1	inconclusive	ALG	Tishomingo NFH	1	24 hrs	2	50	?	0	-
1	effective	ASN	University of Maryland Horn Point Lab	1	24 hrs	1	30	100	0	-
1	ineffective	ASN	University of Maryland Horn Point Lab	2	24 hrs	17	30	0	0	-
1	effective	GIT	Mora NFH & TC	2	3 - 6 days	20	10	80	20	80 - 85
1	effective	PAH	Aquaculture of Kentucky, Inc	1	12 - 24 hrs	6	50	67	0	-
1	effective	PAH	Aquaculture Research Center	1	12 - 24 hrs	9	50	100	0	-
1	effective	PAH	Booker Fowler SFH	1	24 - 26.5 hrs	15	10	100	0	-
1	effective	PAH	Gavins Point NFH	1	24 hrs	12	20	92	0	-
1	effective	PAH	Natchitoches NFH	1	24 - 48 days	6	10	100	0	-
1	effective	PAH	Private John Allen NFH	1	22.5 hrs	8	100	75	0	-
1	effective	PAH	Tishomingo NFH	1	30 hrs	8	50	33 - 100	0	-
1	effective	PLS	Bozeman FTC	1	24 hrs	5	20	67 - 100	0	-
1	effective	PLS	Gavins Point NFH	1	9.5 hrs	14	20	35.7	0	-
1	inconclusive	PLS	Natchitoches NFH	1	24 - 48 days	8	10	25	0	-
1	effective	SNS	Bozeman FTC	1	24 hrs	4	20	100	0	-
1	effective	SNS	Kincaid SFH	1	24 - 30 hrs	18	40	50	1	100
1	effective	WHS	Blind Canyon Aquaranch	1	?	6	20	50	0	-
2	effective	WHS	Sterling Caviar, LLC	1	20 hrs	19	10	40 - 66	19	0

**Table 2. Summary of Year 2006 LHRH<sub>a</sub> Female Efficacy Results - Injection**

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	ALG	Private John Allen NFH	3	24 hrs	3	100	67	0	-
1	ineffective	ALG	Tishomingo NFH	1	24 hrs	1	100	0	0	-
2	effective	BNT	Oden SFH	1	1 - 18 days	203	20	42 - 53	226	28
1	effective	GIT	Mora NFH & TC	2	3 - 6 days	20	20	85	20	60
1	effective	GRC	Osage Catfisheries, Inc	1	24 hrs	5	2.2	80	0	-
1	effective	LST	Wild Rose SFH	1	24 hrs	4	10	100	1	100
1	effective	LST	Wild Rose SFH	2	48 hrs	2	100	100	1	100
1	effective	PAH	Aquaculture of Kentucky, Inc	2	12 - 24 hrs	3	100	100	0	-
1	effective	PAH	Aquaculture Research Center	2	12 - 24 hrs	6	100	100	0	-
1	effective	PAH	Booker Fowler SFH	2	24 - 26.5 hrs	5	100	60	0	-
1	effective	PAH	Gavins Point NFH	2	8 hrs	9	100	56	0	-
1	effective	PAH	Natchitoches NFH	2	24 hrs	4	100	75	0	-
1	effective	PAH	Osage Catfisheries, Inc	2	24 hrs	14	80	80 - 100	0	-
1	effective	PAH	Private John Allen NFH	2	22.5 hrs	3	100	33	0	-
1	effective	PAH	Tishomingo NFH	1	30 hrs	7	100	60 - 100	0	-
1	effective	PLS	Bozeman FTC	2	12 - 24 hrs	2	50	100	0	-
1	effective	PLS	Gavins Point NFH	2	9.5 hrs	8	100	37.5	0	-
1	effective	PLS	Natchitoches NFH	2	24 - 48 hrs	8	100	100	0	-
2	effective	RBT	Oden SFH	1	2 - 8 days	213	20	64 - 66	41	59
1	effective	SNS	Bozeman FTC	2	12 hrs	2	20	100	0	-
1	inconclusive	SNS	Kincaid SFH	1	18 - 30 hrs	17	40	29	0	-

**Table 2. Summary of Year 2006 LHRH<sub>a</sub> Female Efficacy Results - Injection (cont.)**

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	STB	Blackwater Fisheries Research	1	3 days	11	25	0 - 100	0	-
1	effective	WHS	Blind Canyon Aquaranch	2	7.5 - 32 hrs	4	50	75	0	-
1	effective	WHS	Kootenai Tribal Hatchery	2	24 hrs	7	100	100	0	-
2	effective	WHS	Sterling Caviar,LLC	2	20 hrs	12	20	83	6	0

**Table 3. Summary of Year 2006 LHRH<sub>a</sub> Male Efficacy Results - Implant**

Number of Trials	Efficacy	Species	Facility	Spawning Interval	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Spermiat	Number of Controls	% Spermaite
1	inconclusive	AMS	Edenton NFH	up to 21 days	138	79.3	?	0	-
1	effective	AMS	Watha SFH	1 - 14 days	196	79.7	?	0	-
1	effective	HKS	Manning SFH	24 hrs	790	57.92	100	0	-
1	effective	STT	Dworshak NFH	14 - 21 days	11	50	100	0	-

**Table 4. Summary of Year 2006 LHRH<sub>a</sub> Female Efficacy Results - Implant**

Number of Trials	Efficacy	Species	Facility	Spawning Interval	Treated			Control	
					Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	inconclusive	AMS	Edenton NFH	up to 21 days	138	186.7	?	0	-
1	effective	AMS	Watha SFH	1 - 14 days	208	134	?	0	-
1	effective	HKS	Manning SFH	24 hrs	508	42.61	?	0	-

**Table 5. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2006 LHRH<sub>a</sub> Efficacy Studies**

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<b>Total Number of Treatment Trials</b>	55
Number of Trials that Appeared Efficacious:	47 (85%)
Number of Trials that Appeared Inefficacious:	2 (4%)
Number of Trials that Appeared Inconclusive:	6 (11%)
<b>Total Number of Treated Fish:</b>	2,747
<b>Treatment Regimes Used:</b>	
2.2 - 25 ug/Kg body weight (1 - 2 injections)	22 trials
30 - 50 ug/Kg body weight (1 - 2 injections)	10 trials
42.61 - 79.3 ug/Kg body weight (1 implant)	5 trials
80 ug/Kg body weight (2 injections)	1 trial
100 ug/Kg body weight (1 - 3 injections)	15 trials
134 - 186.7 ug/Kg body weight (1 implant)	2 trials

**Water Temperature (°F) Range:** 43.0 - 79.0

**Fish Species Treated:**

**Salmonids**

brown trout *Salmo trutta*  
 Gila trout *Oncorhynchus gilae*  
 rainbow trout *O. mykiss*  
 steelhead trout *O. mykiss*

**Non-salmonids**

alligator gar *Lepisosteus spatula*  
 American Shad *Alosa sapidissima*  
 Atlantic sturgeon *Acipenser oxyrhynchus*  
 grass carp *Ctenopharyngodon idella*  
 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*

**Size Class of Treated Fish:** Adults