

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

### **2008 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 2008 (CY08), 20 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 190 treated fish and 67 control fish and were conducted at 11 different hatcheries, including three U.S. Fish and Wildlife Service fish hatcheries, three 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 from trials conducted in CY08 showed that

treatments appeared efficacious in approximately 90% of the trials and ineffective in 10% 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 trials conducted under INAD exemption #8061 in CY08. 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 trials 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 11 different fish culture facilities during CY08, including three U.S. Fish and Wildlife Service fish hatcheries, three state

hatcheries, four private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 51.0 to 76.0°F.

Overall mean treatment temperature from all trials was 60.6 °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). During this reporting period, the drug doses used ranged from 2.2 to 100 ug LHRH<sub>a</sub>/kg fish body. LHRHa was administered as either a single injection or as a series of two injections.

## **Fish Species and Sex Treated**

### **1. Fish Species Treated**

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

## **Salmonids**

Gila trout *Oncorhynchus gilae*

## **Non-salmonids**

grass carp *Ctenopharyngodon idella*

lake sturgeon *Acipenser fulvescens*

paddlefish *Polydon spathula*

white sturgeon *Acipenser transmontanus*

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

LHRH<sub>a</sub> was used on 104 female and 86 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 - 2 provides summaries of all efficacy trials; Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 4 describes all trials conducted during CY08 under INAD #8061.)

**A. Efficacy of LHRH<sub>a</sub> on male fish treated at a dosage between 5 and 100 ug/kg body weight (1 injection)**

Treated male fish were used in eight trials and injected one time with LHRH<sub>a</sub> at a dosage between 5 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: 5 - 20 ug/kg

Gila trout, paddlefish, and white sturgeon were used in four trials and injected 1 time with LHRH<sub>a</sub>; control fish were used in two trials. Results showed there was a 50 - 100% spermiation in treated fish; and 0 - 85% spermiation in control fish. Overall, results indicated that treatment appeared effective in all trials.

2. Dose: 50 ug/kg

Paddlefish were used in three trials and injected 1 time with LHRH<sub>a</sub>; one trial involved control fish. Results showed there was a 0 - 100% spermiation in treated fish; and no spermiation in control fish. Overall, results indicated that treatment appeared effective in two trials and was ineffective in one trial.

3. Dose: 100 ug/kg

Paddlefish were used in one trial and fish were injected 1 time with LHRH<sub>a</sub>; control fish were not used. Results showed that there was a 90% spermiation in treated fish. Overall, treatment appeared to be efficacious.

Overall treatment resulted in a 0 - 100% level of spermiation in the male treated fish, compared to a 0 - 85% level of spermiation in control fish. Treatment appeared effective in seven trials and ineffective in one trial.

**B. Efficacy of LHRH<sub>a</sub> on female fish treated at a dosage between 2.2 and 100 ug/kg body weight (1 - 2 injections)**

Treated female fish were used in 12 trials and injected 1 - 2 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 - 20 ug/kg

Gila trout, grass carp, and white sturgeon were used in three trials, fish were injected 1 or 2 times with LHRH<sub>a</sub>; and two of the trials involved control fish. Treatment results showed that there was 80 - 100% ovulation in treated fish; and 0 - 70% ovulation in control fish. Overall, treatment appeared effective in all trials.

2. Dose: 50 ug/kg

White sturgeon were used in one trial and fish were injected 2 times with LHRH<sub>a</sub>; no control fish were used. Results showed that there was 100% ovulation in treated fish. Overall, treatment appeared effective in this trial.

4. Dose: 50 or 100 ug/kg

Paddlefish were used in one trial and fish were injected one time with LHRH<sub>a</sub>; control fish were used in this trial. Results showed that there was 67 - 80% ovulation in treated fish; as compared to no ovulation in the control fish. The investigator noted four fish were treated at 100 ug/kg to compare the two doses. Response to the 50 ug/kg was favorable and the lower dosage resulted in a savings of both time and money. Overall, results indicated that treatment appeared effective in this trial.

5. Dose: 100 ug/kg

Paddlefish, lake sturgeon, and white sturgeon were used in seven trials, fish were injected 2 times with LHRH<sub>a</sub>; one of the trials involved control fish. Results showed that there was 0 - 100% ovulation in treated fish; and 20% ovulation in control fish. Overall, treatments appeared efficacious in six trials and ineffective in one trial.

Overall treatment resulted in 0 - 100% ovulation in females treated and 0 - 70% ovulation in control fish. Treatment appeared efficacious in 11 trials and ineffective in one trial.

## **2. Observed Toxicity**

No toxicity or adverse effects relating to LHRH<sub>a</sub> treatments were reported in any trials conducted in CY08.

### **Current Study Protocol for LHRHa INAD #8061**

Please see the attached current study protocol for LHRHa INAD #8061. Please note this study protocol was changed 10/2008 to reflect that LHRHa implants are no longer allowed and the withdrawal period for treated fish is 14 days.

### **Facility Sign-up List**

Please see "Table 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the LHRHa INAD #8061 during CY08. Facilities not listed in Appendix III-a of the current LHRHa INAD #8061 study protocol have been highlighted.

The following facility received LHRHa during CY08 but never used the drug:

1. Dennis Wildlife Center

### **Correspondence sent to LHRHa Participants**

Please see the attached correspondence that was sent to all LHRHa participants after the AADAP Office received their sign-up form for calendar year 2008.

### **Number of Treated Fish under Slaughter Authorization**

Total number of fish treated during CY08 was 190. The total number of treated fish to count against the slaughter authorization dated August 15, 2003 is 2,997.

### **Summary of Study Results**

LHRH<sub>a</sub> was used in 20 efficacy trials to induce gamete maturation in five different fish species (n = 190 treated fish; 67 untreated control fish) at dosages ranging from 2.2 - 100 ug/kg bw. LHRH<sub>a</sub> was administered using either 1 - 2 injections. Water temperature during treatments ranged from 51.0 - 76.0°F. Approximately 90% of the trials appeared efficacious while 10% appeared ineffective. Data from the CY08 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

- 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<sup>6</sup>,des Gly<sup>10</sup>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. Summary of Year 2008 LHRH<sub>a</sub> Male Efficacy Results**

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval	Treated			Control	
						Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
1	effective	GIT	Mora NFH & TC	1	7 days	20	5	90	20	85
1	effective	PAH	Aquaculture Research Center	1	12 - 24 hrs	6	50	100	0	-
1	effective	PAH	Booker Fowler SFH	1	24 hrs	10	10	100	0	-
1	ineffective	PAH	Charles Brown Farms	1	24 hrs	7	50	0 - 33	0	-
1	effective	PAH	Private John Allen NFH	1	15 hrs	10	100	90	0	-
1	effective	PAH	Tishomingo NFH	1	30 hrs	19	50	50 - 100	1	0
1	effective	WHS	Blind Canyon Aquaranch	1	30 hrs	4	20	50	0	-
1	effective	WHS	Sterling Caviar,LLC	1	20 hrs	10	10	50	10	0

**Table 2. Summary of Year 2008 LHRH<sub>a</sub> Female Efficacy Results**

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	GIT	Mora NFH & TC	1	7 days	20	5	90	20	70
1	effective	GRC	Osage Catfisheries, Inc	1	12 - 18 hrs	8	2.2	80 - 100	0	-
1	effective	LST	Wild Rose SFH	2	24 hrs	3	100	100	5	20
1	effective	PAH	Aquaculture Research Center	2	12 - 24 hrs	10	100	100	0	-
1	effective	PAH	Booker Fowler SFH	2	12 hrs	3	100	100	0	-
1	ineffective	PAH	Charles Brown Farms	2	24 hrs	7	100	0	0	-
1	effective	PAH	Osage Catfisheries, Inc	2	24 hrs	9	100	100	0	-
1	effective	PAH	Private John Allen NFH	2	15 hrs	5	100	80	0	-
1	effective	PAH	Tishomingo NFH	1	30 hrs	18	50 or 100	67 - 80	5	-
1	effective	WHS	Blind Canyon Aquaranch	2	30 hrs	4	50	100	0	-
1	effective	WHS	Kootenai Tribal Hatchery	2	23 - 71 hrs	11	100	100	0	-
1	effective	WHS	Sterling Caviar,LLC	2	20 hrs	6	20	83	6	0

**Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2008 LHRH<sub>a</sub> Efficacy Studies**

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<b>Total Number of Treatment Trials</b>	20
Number of Trials that Appeared Efficacious:	18 (90%)
Number of Trials that Appeared Inefficacious:	2 (10%)

**Total Number of Treated Fish:** 190

**Treatment Regimes Used:**

2.2 - 20 ug/Kg body weight (1 - 2 injections)	7 trials
50 ug/Kg body weight (1 - 2 injections)	4 trials
50 or 100 ug/Kg body weight (1 injections)	1 trial
100 ug/Kg body weight (1 - 2 injections)	8 trials

**Water Temperature (°F) Range:** 51.0 - 76.0

**Fish Species Treated:**

**Salmonids**

Gila trout *Oncorhynchus gilae*

**Non-salmonids**

grass carp	<i>Ctenopharyngodon idella</i>
lake sturgeon	<i>Acipenser fulvescens</i>
paddlefish	<i>Polydon spathula</i>
white sturgeon	<i>Acipenser transmontanus</i>

**Size Class of Treated Fish:** Adults