

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE  
INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION  
FOR LUTEINIZING HORMONE-RELEASING HORMONE  
ANALOG des-Gly<sup>10</sup>,[D-Ala<sup>6</sup>]LH-RH Ethylamide (LHRH<sub>a</sub>)  
(INAD #8061)**

**Sponsor:**

U.S. Fish and Wildlife Service, Division of the National Fish Hatchery System

\_\_\_\_\_  
Sponsor Signature

\_\_\_\_\_  
Date Approved

**Manufacturer:**

Western Chemical, Inc.  
1269 Lattimore Rd  
Ferndale, WA 98248 USA

**Facility for Coordination of Aquafrin<sup>®</sup> INAD:**

Aquatic Animal Drug Approval Partnership Program  
4050 Bridger Canyon Road  
Bozeman, MT 59715

Proposed Starting Date:

July 1, 2008

Proposed Ending Date:

June 30, 2012

Study Director:

Mr. Jim Bowker

\_\_\_\_\_  
Study Director Signature

\_\_\_\_\_  
Date

**Clinical Field Trial Location:**

Facility:

\_\_\_\_\_  
Type or Print Name

Investigator:

\_\_\_\_\_  
Type or Print Name

\_\_\_\_\_  
Investigator Signature

\_\_\_\_\_  
Date

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR LUTEINIZING HORMONE-RELEASING HORMONE ANALOG des Gly<sup>10</sup>,[D-Ala<sup>6</sup>]LH-RH Ethylamide (LHRH<sub>a</sub>) UNDER INAD #8061**

**I. STUDY ID AND TITLE**

Clinical field trials to determine the efficacy and safety of LHRH<sub>a</sub> to induce gamete maturation (ovulation and spermiation) in a variety of fish species. INAD #8061.

**II. SPONSOR**

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9904; Fax: 406-582-0242; Email: [dave\\_erdahl@fws.gov](mailto:dave_erdahl@fws.gov)

Manufacturer: Western Chemical, Inc.  
1269 Lattimore Rd  
Ferndale, WA 98248 USA

Contact: Dr. Jim Brackett  
Phone: 800-283-5292  
Email: [bracket@wchemical.com](mailto:bracket@wchemical.com)

Study Director: Mr. Jim Bowker  
U.S. Fish and Wildlife Service - AADAP  
4050 Bridger Canyon Road  
Bozeman, MT 59715  
Phone: 406-994-9910  
Fax: 406-582-0242  
Email: [jim\\_bowker@fws.gov](mailto:jim_bowker@fws.gov)

Clinical Field Trial Coordinator: Bonnie Johnson, USFWS - AADAP

INAD Study Monitors: See Appendix II for names and addresses.

**III. INVESTIGATORS/FACILITIES**

See Appendix IIIa for names and addresses.

**IV. PROPOSED STARTING AND COMPLETION DATES:**

Proposed Starting Date: November 1, 2008

Proposed Completion Date: October 31, 2012

## V. BACKGROUND/PURPOSE

The use of hormones to induce spawning in fish is critical to the success of many U.S. Fish and Wildlife Service (USFWS) 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 handling required during the spawning of fish for artificial propagation 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. The longer it is necessary to hold wild fish in captivity, the greater the likelihood of adversely affecting both the health of the fish and ultimate spawning success. In fact, with respect to some wildstock species, the stress of capture alone would be sufficient to cause complete reproductive failure unless spawning is induced by hormone treatment. Additionally, certain species have limited or depressed populations and in some cases may even be considered threatened/endangered. Hormone treatment of these fish is essential to ensure viable population numbers.

In order to maintain the health of both wildstock and domestic brood fish, it is beneficial to minimize overall fish handling. During the course of normal spawning operations at a hatchery, it may be necessary to handle and examine individual fish weekly over a 6-8 week period. Such procedures can be extremely stressful to valuable broodstocks, severely compromising general fish health. Successful hormone treatment can reduce handling requirements to a single hormone administration event followed by actual gamete collection, thereby greatly reducing overall fish handling.

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 analogues to be one of the most effective means of inducing final gamete maturation. These compounds are synthetic gonadotropin releasing hormones that are similar in structure to native luteinizing hormone-releasing hormones. Although a number of these analogues are available, the most commonly used analogue for fish culture is LHRH<sub>a</sub> (Alvarino et al. 1992; Donaldson et al. 1981; Erdahl and McClain 1987; Fitzpatrick et al. 1983; Taranger et al. 1992; and Van der Kraak et al. 1983). LHRH<sub>a</sub> is an attractive choice as it has both a high biological activity and low species specificity, making it appropriate for 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 USFWS fisheries programs nationwide.

The purpose of this compassionate INAD for LHRH<sub>a</sub> is to develop clinical field trial data that will be used to determine the efficacy and appropriate treatment regimes for inducing ovulation and/or spermiation in a variety of cultured and wildstock fish species. These data will be used to support a new animal drug application (NADA) for LHRH<sub>a</sub>, or for declaration by U.S. Food and Drug Administration (FDA) that LHRH<sub>a</sub> is a low regulatory priority substance.

USFWS anticipates requesting that FDA grant an extension of the LHRH<sub>a</sub> INAD for additional years at the end of this treatment season. The USFWS is aware that opportunities for LHRH<sub>a</sub> therapy are unpredictable. There is no way of knowing in advance if, when, or where opportunities for pivotal studies will be encountered. USFWS feels that data from at least three treatment seasons will be required in order to adequately assess the efficacy of LHRH<sub>a</sub> treatment on induced gamete maturation in fish to support a NADA.

## VI. SPECIFIC OBJECTIVES

The two major objectives of this study protocol are as follows:

1. Collect scientific data necessary to establish the effectiveness and safety of LHRH<sub>a</sub> on gamete maturation in both cultured fish under typical hatchery situations and on critical wildstock species.
2. Provide the opportunity for fishery biologists to legally use LHRH<sub>a</sub> to maintain the genetic integrity and improve the reproductive potential of hatchery broodstocks during the period of time necessary for collection of efficacy, safety, and residue data required for an NADA on LHRH<sub>a</sub> in fish. Specifically, LHRH<sub>a</sub> will be used to induce ovulation and spermiation in both domestic and wildstock populations, including several species that are listed under the Endangered Species Act.

A list of freshwater fish species authorized for treatment is provided in Appendix VIa.

## VII. MATERIALS

A. Test and control articles:

1. Drug Identity

a. Active ingredient

Common Name: Luteinizing Hormone-Releasing Hormone analogue

Chemical Name: des-Gly<sup>10</sup>,[D-Ala<sup>6</sup>]LH-RH Ethylamide

CAS Number: 79561-22-1

Amino Acid Profile: (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NHEt)

Appearance: White powder

Odor: Slight musty smell

b. Strength and dosage form

LHRH<sub>a</sub> is a lyophilized powder distributed on a total weight basis. Peptide content is approximately 90%, with the balance being salts and water. It is available in vials containing either 1, 5, or 25 mg LHRH<sub>a</sub>/vial. LHRH<sub>a</sub> should be diluted with physiological saline immediately prior to intended use. Dilution rate is dependent upon fish size, fish number, and intended dosage.

c. Manufacturer, source of supply

**Manufacturer:**

Western Chemical, Inc  
1269 Lattimore Rd  
Ferndale, WA 98248 USA

Contact Person: Dr. Jim Brackett

Phone: 800-283-5292

Fax: 360-384-0270

Email: [bracket@wchemical.com](mailto:bracket@wchemical.com)

2. Verification of drug integrity/strength

The Manufacturer will provide the analytical data necessary to establish purity of each lot of LHRH<sub>a</sub> supplied. The lot number and date of manufacture for each batch of LHRH<sub>a</sub> will be placed on the label of each container. The form "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" (Form 1) will clearly identify the lot number and date of manufacture of LHRH<sub>a</sub> shipments. If the integrity of the LHRH<sub>a</sub> is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded, dated, and signed in the Chemical Use Log (Form 2). The Study Monitor assigned to the Investigator involved will be immediately notified.

3. Storage Conditions

LHRH<sub>a</sub> will be stored in the original container supplied by the Manufacturer with the appropriate investigational label attached. The container will be stored in a freezer that maintains a temperature of less than 0°C. The freezer must be labeled to indicate that it contains hazardous material and that "**NO** Food or Drink is to be Stored in this Refrigerator/Freezer". LHRH<sub>a</sub> should be stored in a secure location.

4. Handling Procedures

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for LHRH<sub>a</sub> (see Appendix IV). Each person involved with the study and each person who may be present during the use of LHRH<sub>a</sub> shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with LHRH<sub>a</sub>.

5. Investigational labeling

Copies of the investigational labels to be attached to each container of LHRH<sub>a</sub> are provided in Appendix V. It is the responsibility of the Investigator to ensure proper investigational labeling of all containers of LHRH<sub>a</sub>.

6. Accountability

- a. Western Chemical, Inc. will be the sole supplier of LHRH<sub>a</sub> to all Investigators under INAD 8061.

- b. USFWS and Non-USFWS Facilities - Immediately upon receiving an order/shipment of LHRH<sub>a</sub>, the Investigator will complete Form LHRH<sub>a</sub> -1 "Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals". The investigator will archive the original in the facility's INAD file, and send a copy to his/her Study Monitor. Both the Investigator and the Study Monitor are required to sign Form LHRH<sub>a</sub> -1. The Study Monitor will then forward a copy to the Clinical Field Trial Coordinator at the Aquatic Animal Drug Approval Partnership Program. The Clinical Field Trial Coordinator will archive one copy, and send one copy of Form LHRH<sub>a</sub> -1 to FDA. Arrangements should be made between Investigators and Study Monitors to ensure completed Form LHRH<sub>a</sub> -1s are received by the Clinical Field Trial Coordinator in a timely manner.
- c. All Investigators are also responsible for maintaining an accurate inventory of LHRH<sub>a</sub> on-hand. A Chemical Use Log (Form LHRH<sub>a</sub> -2) will be supplied to each Investigator. Each time LHRH<sub>a</sub> is used it must be recorded by the Investigator on Form LHRH<sub>a</sub> -2.

## 7. Preparation Procedures

LHRH<sub>a</sub> for injection will be supplied in vials containing 1, 5, or 25 mg of a lyophilized white powder. Based on the relatively small amount of hormone per vial, investigators should not attempt to divide LHRH<sub>a</sub> into additional aliquots (unless a high quality analytical balance and a Good Laboratory Practice laboratory are available). Immediately prior to injection, LHRH<sub>a</sub> should be diluted using a sterile, physiological saline solution. Dilution volume is dependent upon dosage, size and number of fish to be injected, and desired injection volume.

### B. Items needed for treatment, data collection, etc.:

Treatment equipment should include clean glassware, sterile physiological saline, and sterile syringes and needles. A compound microscope should be available for evaluation of sperm motility.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the LHRH<sub>a</sub> INAD will need to complete several forms. These forms are described in Section XIII (p 10). Copies of these forms are attached to this Study Protocol.

## VIII. EXPERIMENTAL UNIT

The experimental unit in this clinical field trial may consist of a contained or isolated group of fish. This will generally be a group of fish contained in a tank, raceway, or pond. It could also be a group of fish held in confinement in a lake or stream. However, the experimental unit in this clinical field trial may also be **individual animals**. If individual animals are considered to be the experimental unit, treatment response parameters for each animal must be evaluated separately.

## IX. ENTRANCE CRITERIA

### A. Facilities/Investigators

The proposed facility and the Investigator must be listed in Appendix IIIa of the Study Protocol before LHRH<sub>a</sub> can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, Study Director, or by an Investigator in case emergency use-pattern needs should arise (See Section XX). However, it is important to note that poor planning and/or lack of preparation will not be considered an emergency situation.

### B. The characteristics of the study animals (species, size, number, etc.) are presented in Appendix VIb.

### C. Environmental conditions

Since LHRH<sub>a</sub> activity is rapidly lost in dilute aqueous solution (Merck Index, 1989), there will be no drug discharge from participating facilities. Therefore, LHRH<sub>a</sub> qualifies for a categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.24(d)(4).

### D. Ability of Investigator to fulfill all the requirements of the Study Protocol See Appendix IIIb for example of knowledge required of hatchery managers (i.e., Investigators).

### E. Period of use

LHRH<sub>a</sub> treatment has been shown to be most effective when administered during the final stages of gamete maturation. In most cases, LHRH<sub>a</sub> will be used within 4 weeks of the time fish are normally expected to spawn.

**Prior to initiating each treatment event**, the Investigator must first complete Form LHRH<sub>a</sub> -W, "Worksheet for Designing Individual Field Trials" that pertains to each specific treatment event. The worksheet should be filled out, signed, and sent by Fax to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), sign it, and forward (Fax) the paperwork to the Aquatic Animal Drug Approval Partnership (AADAP) Program Office. The AADAP Office will then review the worksheet, assign the approved treatment a Study Number, and then notify both the Investigator and the Study Monitor of the assigned number and approval to proceed. In most cases, this entire process can be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on Form LHRH<sub>a</sub> -2 and Form LHRH<sub>a</sub> -3, as well as on any additional correspondence regarding that specific treatment event. If for some reason the Investigator is unable to reach his/her Study Monitor with regards to worksheet approval, the Investigator should contact the AADAP Office for a study number and permission to proceed.

## X. TREATMENT GROUPS

### A. A treatment group or experimental unit may be an entire tank, pond, raceway, or group of fish, or it may be individual animals.

B. Non-treated control groups will not be a requirement for clinical field trials evaluating the efficacy of LHRH<sub>a</sub> treatment conducted under this study protocol for INAD 8061.

Although untreated control groups are not a required element of treatment under this INAD exemption and are at the discretion of the Investigator, they are strongly encouraged whenever circumstances permit. Control groups are extremely important to not only document response to treatment, but also to validate potential adverse reactions in treated animals. Assignment to control and treatment groups should be random and designed to avoid bias. It is important that all fish are treated in a similar fashion. If fish are physically moved into separate test groups or different rearing units, caution should be used so that handling and rearing conditions are as similar as possible. Control fish should be kept under conditions as similar as possible to treated fish for valid comparison. Use of control groups will ensure that results of efficacy studies provide useful information that will support a NADA.

Blinded studies can reduce bias in data collection. Whenever possible, investigators should consider methods by which treatment response observations are recorded by individuals who are unaware which fish have been treated and which fish are controls.

## **XI. TREATMENT SCHEDULES**

### **A. Route of administration**

LHRH<sub>a</sub> will be administered as an injection.

LHRH<sub>a</sub> should be dissolved in sterile physiological saline and administered as either an intraperitoneal (IP) or intramuscular (IM) injection. Typically, IP injections will be used on females and IM injections will be used on males.

### **B. Dose to be administered**

Standard hormone dosage rates will be 5-20 ug LHRH<sub>a</sub>/kg body weight. Although certain situations may require a higher dosage rate, dosage will never exceed 100 ug LHRH<sub>a</sub>/kg body weight.

### **C. Dosing interval and repetition**

Dependent upon the species/strain involved, LHRH<sub>a</sub> may be administered as a single treatment, or as a multiple treatment. Determination of whether a single or multiple treatment regimen is used will be largely a matter of past experience of the investigator and literature citations reporting successful protocol with respect to specific species/strains. Multiple treatment regimens will generally consist of a single "priming" dose followed by a single "resolving" dose.

### **D. Permissible concomitant therapy**

Since efficacy data are being collected during the INAD process, there should be little or no concomitant therapy. Preferably, there should be no other therapy during a period extending from 2 weeks prior to treatment to 2 weeks after treatment. Investigators must be prepared to make no changes in fish cultural procedures or environmental conditions, and apply no other hormone therapy once a decision has been made to conduct LHRH<sub>a</sub> treatment. However, if concomitant therapy is required

in order to protect/propagate valuable fish stocks, it should be fully documented and the efficacy data from the LHRH<sub>a</sub> treatment involved should be appropriately labeled.

## XII. TREATMENT RESPONSE PARAMETERS

The collection and reporting of source data begins with the decision to treat valuable fish based on hatchery records or other pertinent species information indicating treatment is warranted. Daily morbidity and mortality records, case history records, as well as any extenuating or mitigating circumstances that may affect treatment response need to be documented. All pertinent treatment response parameters should be reported on Form LHRH<sub>a</sub> -3. Treatment response parameters that should be addressed include the following:

### A. Primary Parameters

The primary response parameter for evaluating the effect of LHRH<sub>a</sub> on fish will be percent of fish ripe following treatment. In the case of females, ripe fish are those that have ovulated. In the case of males, ripe fish are those undergoing active spermiation.

### B. Secondary Parameters

Secondary response parameters for females will include percent eye-up and percent hatch. Secondary response parameters for males will include the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa). Motility evaluations will be reported using a scoring system that assigns each milt sample a motility score of either 0, 1, 2, 3 or 4. Motility scores will be based on the following schedule:

<u>Percent Motility</u>	<u>Motility Score</u>
0	0
1-25	1
26-50	2
51-75	3
76-100	4

Secondary parameters may also include general observations on fish behavior and response to routine culture/handling activities. This would include such responses as feeding activity, feed consumption, apparent level of stress, negative fish behavior, etc.

### C. Adverse Reactions

Any adverse reaction to treatment should be reported immediately to the Study Monitor, who will in turn notify the Study Director. Such responses might include extremely negative responses/behavior by the fish or hazards to the applicator. Although LHRH<sub>a</sub> has been used fairly extensively with beneficial effect in fish culture, it is possible adverse reactions may occur under certain environmental conditions or with respect to specific species/strains of fish. Carefully observe all treated fish for any signs of any adverse reaction to treatment. The Investigator should carefully document all observations of adverse reactions. If any signs of drug toxicity are

detected, they should also be documented and immediately reported to the Study Monitor, who will in turn notify the Study Director.

**Note:** Investigators are strongly encouraged to record observations/comments with respect to all phases of treatment. This may include a description of events before, during, and post-treatment. All extenuating or mitigating treatment circumstances need to be described in detail. Such information is imperative so that accurate study/data analysis can be performed.

### **XIII. FORMS FOR DATA COLLECTION**

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the LHRH<sub>a</sub> INAD will need to complete the following forms:

- Form LHRH<sub>a</sub> -W. Worksheet for Designing Individual Field Trials under LHRH<sub>a</sub> INAD 8061
- Form LHRH<sub>a</sub> -1. Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals
- Form LHRH<sub>a</sub> -2. Chemical Use Log for Clinical Field Trials Using LHRH<sub>a</sub> under INAD 8061
- Form LHRH<sub>a</sub> -3. Results Report Form for Use of LHRH<sub>a</sub> under INAD 8061

Copies of these forms are attached to this Study Protocol.

### **XIV. RECORD KEEPING PROCEDURES**

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data must be original, i.e., they must be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet must be legibly signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries must be properly attributed to each person. The data must be accurate and legible. If a mistake is made, it must be crossed out using a single strike-through and the correct data must be recorded next to it. Each change to the raw data must be initialed and dated by the person making the change, and a statement must be provided explaining why the change was made. If the data sheet needs to be copied, all data must be transferred, including the properly noted changes. The original record must be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

### **XV. DISPOSITION OF INVESTIGATIONAL ANIMALS**

Animals that die during treatment should be disposed of by burial or incineration. Fish treated via injection will be maintained in culture facilities or captivity for at least 14 days

following treatment before they are released or allowed to enter the food chain. If fish are injected more than once, these requirements will be based on the time of final treatment.

No withdrawal period will be required for injected fish that will be illegal for harvest for 14 or more days after release. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

The Investigator must verify compliance with requirements regarding the disposition of all treated fish on Form LHRH<sub>a</sub> -3.

## **XVI. DISPOSITION OF INVESTIGATIONAL DRUG**

LHRH<sub>a</sub> will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or out-dated LHRH<sub>a</sub> remains at the end of the study period, Investigators should contact Study Monitors for instructions regarding drug disposal. The investigational drug may not be redistributed to others not specified in the Study Protocol.

## **XVII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES**

### **A. Drug distribution**

See Section VII.A.6. Accountability (page 5) for information and details.

### **B. Study Monitors**

Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases, and the ability to monitor overall fish health with respect to ongoing fish culture practices. A study monitor should be assigned to each facility that is authorized to treat fish with LHRH<sub>a</sub>. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.

### **C. Special equipment and materials**

Most of the equipment and materials required for this study (with the exception of the LHRH<sub>a</sub> itself) are already available at each participating fish hatchery. In recent years, induced final gamete maturation has become a fairly common occurrence at many broodstock facilities. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (see Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc., page 6).

### **D. Administrator of the drug**

LHRH<sub>a</sub> will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). LHRH<sub>a</sub> will be maintained in a secure location, and only the Investigator or persons under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability (page 5) for details, and Forms LHRH<sub>a</sub> -W, LHRH<sub>a</sub> -1, LHRH<sub>a</sub> -2, and LHRH<sub>a</sub> -3 (page 10) for actual forms to be used in the study.

F. Recording observations

The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator will send the data to the Study Monitors who will review the information and ensure that all required data is provided. The Study Monitors will in turn send the data to the Clinical Field Trial Coordinator. The Clinical Field Trial Coordinator will analyze and summarize the data and prepare an annual report that will be submitted to the FDA.

G. Data storage

The Investigator is responsible for complete and accurate data collection. The Investigator is also responsible for archiving a complete set of all original data. A copy of Form LHRH<sub>a</sub> -1 should be sent immediately to the Study Monitor, who will in turn forward a copy to the Clinical Field Trial Coordinator. A copy of Form LHRH<sub>a</sub> -2 should be sent to Study Monitors with the corresponding Form LHRH<sub>a</sub> -3 (if no further treatments are necessary/planned), or at the end of the calendar year. A copy of Form LHRH<sub>a</sub> -3 should be sent to the Study Monitor after completion of the entire treatment period, which includes the post-treatment observation period. Study Monitors should carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor should contact the Investigator immediately to rectify the problem. After review, Study Monitors should forward all data to the Study Director. As stated above, a complete set of raw data should be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Clinical Field Trial Coordinator.

## **XVIII. PLANS FOR DATA ANALYSIS**

Data analysis will be completed by the Clinical Field Trial Coordinator located at the Aquatic Animal Drug Approval Partnership Program Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared and submitted to the FDA. This submission may include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

## **XIX. PROTOCOL AND PROTOCOL AMENDMENTS**

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative) and forwarded to the FDA for review. Copies of the signed amendment will be attached to each copy of the Study Protocol. **Investigators will be**

**liable for non-compliance violation if drugs are used without a Study Protocol or in a manner different than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported.** The Study Monitor is responsible for ensuring that all INAD procedures are being followed as defined by the Study Protocol.

## **XX. PROTOCOL DEVIATIONS**

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be notified immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, what steps were taken to mitigate the deviation and the Investigator's opinion of the impact on study outcome.** Deviation statements should be signed and dated. These statements should be forwarded to the Study Monitor along with Form LHRH<sub>a</sub> -3, and ultimately be submitted to the Study Director.

## LITERATURE CITED

- Alvarino, J.M.R., S. Zanuy, F. Prat, M. Carrillo, and E. Mananos. 1992. Stimulation of ovulation and steroid secretion by LHRH<sub>a</sub> injection in the sea bass (Dicentrarchus labrax): effect of time of day. *Aquaculture* 102:177-186.
- Donaldson, E.M., G.A. Hunter, and H.M. Dye. 1981. Induced ovulation in coho salmon (Oncorhynchus kisutch). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. *Aquaculture*. 26:129-141.
- 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.
- 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.
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