STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR LUTEINIZING HORMONE-RELEASING HORMONE ANALOG des-Gly¹⁰,[D-Ala⁶]LH-RH Ethylamide (LHRHa Liquid/LHRHa) (INAD #8061)

Sponsor:

U.S. Fish and Wildlife Service, Fish and Aquatic Conservation

Sponsor Signature ___________________ Date Approved ___________________

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd
Ferndale, WA 98248 USA

Office for Coordination of LHRHa Liquid/LHRHa INAD:

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

Proposed Starting Date January 1, 1996
Proposed Ending Date December 31, 2025
Study Director Ms. Bonnie Johnson

Clinical Field Trial Location and Trial Number:

Type or Print Facility Name ___________________ Trial Number ___________________

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\(^{10}\),[D-Ala\(^{6}\)]LH-RH Ethylamide (LHRHa Liquid/LHRH\(_{a}\)) UNDER INAD #8061

I. STUDY ID AND TITLE

Conduct clinical field trials to determine the efficacy of LHRHa Liquid/LHRH\(_{a}\) to induce gamete maturation (ovulation and spermiation) in a variety of fish species. INAD #8061.

II. SPONSOR

Dr. Marilyn Blair, 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; Email: marilyn_j_blair@fws.gov

Manufacturer/Source of Supply:

Syndel USA
1441 W Smith Rd,
Ferndale, WA 98248 USA

Study Director:  Ms. Bonnie Johnson, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership (AADAP) Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-994-9905; Email: bonnie_johnson@fws.gov

INAD Study Monitors:  See Appendix II for names and contact information.

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and contact information.

IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date:  January 1, 1996

Proposed Completion Date:  December 31, 2025

Revised:  11/2019
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 unnatural 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\textsubscript{a} (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\textsubscript{a} 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\textsubscript{a} 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\textsubscript{a} is to develop clinical field trial data
that will be used to determine the efficacy and appropriate treatment regimens 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 LHRHₐ.

USFWS anticipates requesting that FDA grant an extension of the LHRHₐ INAD for additional years at the end of this treatment season. The USFWS is aware that opportunities for LHRHₐ 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 LHRHₐ 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 efficacy of LHRHₐ Liquid/LHRHₐ on gamete maturation in both cultured fish under typical hatchery situations and on critical wildstock species.

2. Provide the opportunity for USFWS fish culturists to legally use LHRHₐ Liquid/LHRHₐ 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 LHRHₐ Liquid/LHRHₐ in fish. Specifically, LHRHₐ Liquid/LHRHₐ 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.

VII. MATERIALS

A. Test and control articles:

1. Drug Identity
   a. Active ingredient

   **Powder Formulation:**

   Common Name: Luteinizing Hormone-Releasing Hormone
analogue

Chemical Name: des-Gly^{10},[D-Ala^{6}]LH-RH Ethylamide

CAS Number: 79561-22-1

Amino Acid Profile: (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NH₂)₄

Appearance: White powder

Odor: Slight musty smell

**Liquid Formulation:**

Common Name: Luteinizing Hormone-Releasing Hormone analogue

Chemical Name: des-Gly^{10},[D-Ala^{6}]LH-RH Ethylamide

CAS Number: 79561-22-1

Amino Acid Profile: (pGlu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-NH₂)₄

Appearance: Clear liquid

Odor: None

b. Strength and dosage form

**Powder Formulation:**

LHRH₀ 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 LHRH₀/vial. LHRH₀ should be diluted with physiological saline immediately prior to intended use. Dilution rate is dependent upon fish size, fish number, and intended dosage.

**Liquid Formulation:**

"LHRHₐ Liquid", is a sterile liquid form of LHRHₐ, and is available in a 100mL multi-use amber or clear vial. It is available at 80ug LHRHₐ per mL of solution, or 8mg per 100mL solution. The primary difference between “LHRHₐ
Liquid” and the LHRHa lyophilized powder is that “LHRHa Liquid” has been reconstituted as a sterile liquid injectable under GMP conditions rather than by the end user.

c. Manufacturer, source of supply

Syndel USA  
1441 W Smith Rd,  
Ferndale, WA 98248 USA  
Contact Person: Jason Montgomery  
Phone: 800-283-5292  
Fax: 360-384-0270

2. Verification of drug integrity/strength:

The Manufacturer will provide the analytical data necessary to establish purity of each lot of LHRHa Liquid/LHRHα supplied. The lot number and date of manufacture for each batch of LHRHa Liquid/LHRHα will be placed on the label of each container. The form "Report on Receipt of Drug - 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 LHRHα shipments. If the integrity of the LHRHa Liquid/LHRHα 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

**Powder Formulation:** LHRHα 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". LHRHα should be stored in a secure location.

**Liquid Formulation:** LHRHa Liquid will be stored in the original container (100mL amber or clear vial) supplied by the Manufacturer with the appropriate investigational label and information attached. The container will be stored in the refrigerator and shall not be frozen. The refrigerator must be labeled to indicate that it contains hazardous material and that “No Food or Drink is to be Stored in
this Refrigerator/Freezer”. LHRHa Liquid 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 Safety Data Sheet (SDS) for LHRHa (Appendix IV). Each person involved with the study and each person who may be present during the use of LHRHa Liquid/LHRHa shall be required to read the SDS. Safety precautions as outlined in the SDS will be followed at all times when working with LHRHa Liquid/LHRHa.

5. Investigational labeling

Copies of the labels to be attached to each container of LHRHa Liquid/LHRHa are provided in Appendix V. It is the responsibility of the Investigator to ensure proper labeling of all containers of LHRHa Liquid/LHRHa.

6. Accountability

Syndel USA will be the sole supplier of LHRHa Liquid/LHRHa to all Investigators under INAD 8061.

_The INAD Program Management System (IPMS) is an on-line database that must be used by Investigators for ALL INAD reporting. The IPMS has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting and accountability follows established INAD Study Protocol guidelines. Unless data is entered directly into the IPMS (i.e., not captured elsewhere at the time of observation or measurement and transcribed into the IPMS) Investigators must archive hard copies of all raw data._

1. USFWS and Non-USFWS Facilities

Immediately upon receiving an order/shipment of LHRHa Liquid/LHRHa, the Investigator must complete Form LHRHa Liquid/LHRHa “Report on Receipt of Drug - Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals”. The investigator must then forward Form LHRHa Liquid/LHRHa to the Study Director at the AADAP Office. The Study Director will in turn forward a copy to FDA.
Arrangements should be made between Investigators and Study Monitors to insure completed Form LHRHa Liquid/LHRHa -1s are received by the Study Director in a timely manner.

All Investigators are also responsible for maintaining an accurate inventory of LHRHa Liquid/LHRHa on-hand. A Chemical Use Log (Form LHRHa Liquid/LHRHa-2) must be completed and maintained by each Investigator. Each time LHRHa Liquid/LHRHa is used, it must be recorded by the Investigator on Form LHRHa Liquid/LHRHa -2.

7. Preparation Procedures

**Powder formulation:**
LHRHa 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 LHRHa into additional aliquots (unless a high quality analytical balance and a Good Laboratory Practice laboratory are available). Immediately prior to injection, LHRHa 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.

**Liquid formulation:**
No preparation is required for the liquid formulation of LHRHa Liquid”. Use appropriate size needle and syringe for the species of fish being treated, and calculate the volume to be administered based on the target dose (priming, resolving, or total) and the concentration of the LHRHa Liquid product, 80ug/mL.

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 LHRHa Liquid/LHRHa INAD will need to complete several forms. These forms are described in Section XIII. 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. 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 this Study Protocol before LHRHa Liquid/LHRHa 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 should arise (See Section XX).

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

C. Period of use

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

D. Environmental conditions

Since LHRHa Liquid/LHRHa activity is rapidly lost in dilute aqueous solution (Merck Index, 1989), there will be no drug discharge from participating facilities. Therefore, LHRHa Liquid/LHRHa qualifies for a categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.33(e).

E. 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).

**Prior to initiating each treatment event:** The Investigator must first complete
Form LHRHa Liquid/LHRHa-W: “Worksheet for Designing Individual Field Trials” that pertains to each specific treatment event. The worksheet should be filled out, electronically signed, and forwarded to the Study Monitor. The Study Monitor will review the planned treatment (worksheet), electronically sign it, and forward it to the Study Director at the AADAP Office. The Study Director 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 should be able to be accomplished within a single working day. After initiation of the field trial, the Investigator should also record the assigned study number on Form LHRHa Liquid/LHRHa-2 and LHRHa Liquid/LHRHa-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 Study Director for a study number and permission to proceed.

Note: The INAD Program Management System (IPMS), which is an online database that must be used by Investigators for all INAD reporting, has a built-in system of checks, balances, and email notifications to ensure that all information/data reporting follows established INAD Study Protocol guidelines.

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. Control groups will not be a requirement for clinical field trials evaluating the efficacy of LHRHa Liquid/LHRHa treatment. In some cases, particularly with respect to wildstock populations, the number of broodfish available at a given time for LHRHa Liquid/LHRHa treatment may be extremely limited. It is likely that some facilities may need to initiate treatment on groups of ten or fewer brood fish. To establish meaningful control groups with such a limited number of animals will be difficult. Therefore, it is proposed that treatment groups of 10 or fewer fish be exempted from the requirement to establish control groups. It is also proposed that species listed under the authority of the Endangered Species Act (ESA) be exempted from the requirement to establish control groups. With respect to species listed under the ESA, every fish may be critical to the restoration effort. In all other situations, investigators should make a serious effort to include a control group in the trial. Fish should be assigned to control or treatment groups randomly. Study fish should be crowded into a
confined space where segregation and escape is impossible, and captured using dip nets. Fish in alternating nets should be assigned to control or treatment groups until desired fish numbers are obtained. Suggested control groups will be based on treatment population size according to the following schedule:

<table>
<thead>
<tr>
<th>Treatment Group Size</th>
<th>Control Group Size¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10 fish</td>
<td>0 (too few fish for data analysis)</td>
</tr>
<tr>
<td>11 - 30 fish</td>
<td>5 fish</td>
</tr>
<tr>
<td>31 - 50 fish</td>
<td>10 fish</td>
</tr>
<tr>
<td>51 - 75 fish</td>
<td>15 fish</td>
</tr>
<tr>
<td>76 - 100 fish</td>
<td>20 fish</td>
</tr>
<tr>
<td>101 - 300 fish</td>
<td>25 fish</td>
</tr>
<tr>
<td>&gt;300 fish</td>
<td>30 fish</td>
</tr>
</tbody>
</table>

¹ Minimum number of fish per control group

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

LHRHa Liquid/LHRHa is administered as either an intramuscular (IM) or intraperitoneal (IP) injection.
B. Dose to be administered

1. Injection

Standard priming dosage rate will be 20 ug LHRHₐ/kg body weight and the standard resolving dose is 80 – 100 ug LHRHₐ/kg. Although certain situations may require a higher priming dosage rate, dosage will never exceed 120 ug LHRHₐ/kg body weight.

C. Dosing interval and repetition

Dependent upon the species/strain involved, LHRHₐ Liquid/LHRHₐ may be administered as a single treatment, or as a multiple treatment. Determination of whether single or multiple treatment regimens 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. Drug preparation procedures

**Powder formulation:**

LHRHₐ 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 LHRHₐ into additional aliquots (unless a high quality analytical balance and a Good Laboratory Practice laboratory are available). Immediately prior to injection, LHRHₐ 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.

**Liquid Formulation:**

LHRHₐ Liquid will be supplied in 100mL vials containing 80ug/mL of LHRHₐ in a liquid formulation. No dilution is required for this product.
E. 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 LHRHa Liquid/LHRHa treatment. However, if concomitant therapy is required in order to protect valuable fish stocks, the AADAP Office needs to be notified, and it should be fully documented in the results report form.

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 3. Treatment response parameters that should be addressed include the following:

1. Primary Parameters

The primary response parameter for evaluating the effect of LHRHa Liquid/LHRHa 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.
2. 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:

<table>
<thead>
<tr>
<th>Percent Motility</th>
<th>Motility Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-25</td>
<td>1</td>
</tr>
<tr>
<td>26-50</td>
<td>2</td>
</tr>
<tr>
<td>51-75</td>
<td>3</td>
</tr>
<tr>
<td>76-100</td>
<td>4</td>
</tr>
</tbody>
</table>

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.

3. 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 LHRHa Liquid/LHRHa 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 LHRHa Liquid/LHRHa INAD will need to complete the following forms:

Form LHRHa Liquid/LHRHa-W. Worksheet for Designing Individual Field Trials under INAD 8061


Form LHRHa Liquid/LHRHa -2. Chemical Use Log for Clinical Field Trials Using LHRHa Liquid/LHRHa under INAD 8061

Form LHRHa Liquid/LHRHa -3. Results Report Form for use of LHRHa Liquid/LHRHa under INAD 8061

Copies of these forms are attached to this Study Protocol. Actual reporting is accomplished on forms located on the INAD Program Management System online database.

XIV. RECORD KEEPING PROCEDURES

As stated immediately above, all data reporting are accomplished via forms located on the INAD Program Management System online database.

XV. DISPOSITION OF INVESTIGATIONAL ANIMALS

Fish may be released immediately following treatments. Edible tissues derived from experimental animals treated under this protocol may be marketed for human consumption or released into public waters for possible human consumption.
XVI. DISPOSITION OF INVESTIGATIONAL DRUG

LHRHa Liquid/LHRHa will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or out-dated LHRHa Liquid/LHRHa 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 by the protocol.

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

A. Drug distribution

See Section VII.A.6. Accountability for information and details.

B. Study Monitors

The 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 LHRHa Liquid/LHRHa. 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 LHRHa Liquid/LHRHa 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 IIb). 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.).
D. Administrator of the drug

LHRHa Liquid/LHRHa will be administered directly by the assigned Investigator (typically a fish hatchery manager) or under the Investigator’s direct supervision (see Appendix IIIa for names). LHRHa Liquid/LHRHa will be maintained in a secure location, and only the Investigator or a person under his/her direct supervision will have access.

E. Drug accountability records

See Section VII.A.6. Accountability for details and Forms LHRHa Liquid/LHRHa-W, LHRHa Liquid/LHRHa -1, LHRHa Liquid/LHRHa -2, and LHRHa Liquid/LHRHa -3 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 ensure that all required information is provided. The Study Monitors will in turn send the data to the Study Director. The Study Director will analyze and summarize the data and prepare a report that will be submitted to the FDA.

G. Data storage

The Investigator is responsible for complete and accurate data collection, and must complete all required data forms (see Section XIII). The Investigator should forward all completed forms to the Study Monitor for review. Study Monitors should carefully check each set of data for accuracy and completeness. If a form is incomplete or inaccurate, it should be returned to the Investigator. If a form is complete and accurate, it should be forwarded to the Study Director at the AADAP Office.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the AADAP Office. Data from the treatment year will be summarized through tabulation and appropriate
statistical analysis. A report will be prepared and submitted to the FDA. 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 forwarder 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 contacted immediately. **Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation.** Deviation statements should be documented on Form LHRHa Liquid/LHRHa-3 in the Description of Results section.
LITERATURE CITED


Safety Data Sheet

Form LHRHa Liquid/LHRHa-W: Worksheet for Designing Individual Field Trials Under LHRHa Liquid/LHRHa INAD 8061

INSTRUCTIONS
1. Investigator must fill out Form LHRHa Liquid/LHRHa-W for each proposed treatment under this INAD **before** actual use of LHRHa Liquid/LHRHa.

2. Investigator should forward a copy of LHRHa Liquid/LHRHa Form-W to the Study Monitor for review.

3. After review, the Study Monitor should forward a copy to the AADAP Office for review and assignment of a Study Number.

SITE INFORMATION

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<th>Facility</th>
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<tbody>
<tr>
<td>Address</td>
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</table>

Investigator

<table>
<thead>
<tr>
<th>Reporting Individual (if not Investigator)</th>
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</thead>
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<table>
<thead>
<tr>
<th>Phone</th>
<th>Fax</th>
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</thead>
</table>

FISH CULTURE AND DRUG TREATMENT INFORMATION

<table>
<thead>
<tr>
<th>Fish species to be treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average fish size (in)</td>
</tr>
<tr>
<td>Number of treated males</td>
</tr>
<tr>
<td>Number of control males</td>
</tr>
<tr>
<td>Anticipated date treatment will be initiated</td>
</tr>
<tr>
<td>Intended LHRHa Liquid/LHRHa dosage (ug/kg)</td>
</tr>
<tr>
<td>Number of injections</td>
</tr>
<tr>
<td>Drug manufacturer</td>
</tr>
<tr>
<td>Which LHRHa formulation will you be using (powder or liquid)?</td>
</tr>
</tbody>
</table>
**STUDY DESIGN:** Describe in detail the purpose of the clinical trial. For example you might compare dosage, or treated fish compared to untreated fish. Study design must be carefully focused and lend itself to rigorous evaluation. If more space is required to describe study details, title additional page(s) "Study Design" and attach them to this Worksheet.

Study designed by ______________________

**DISPOSITION OF TREATED FISH (Human Food Safety Considerations):**

Estimated time (days, months) from last treatment day to first possible harvest for human consumption

☐ Investigator should initial here to indicate awareness that fish disposition must be in compliance with FDA-mandated withdrawal times as described in Section XV of the Study Protocol.

**WORKER SAFETY CONSIDERATIONS:**

☐ Investigator should initial here to indicate that all personnel handling drug have read Material Safety Data Sheet for Luteinizing Hormone-Releasing Hormone analog and have been provided protective equipment, in good working condition, as described in the MSDS.

Date Prepared: ______________________ Investigator: ______________________

Date Reviewed: ______________________ Study Monitor: ______________________

INSTRUCTIONS

1. Investigator must fill out Form LHRHa Liquid/LHRHa-1 **immediately** upon receipt of LHRHa Liquid/LHRHa.

2. Investigator should forward a copy of Form LHRHa Liquid/LHRHa -1 to the Study Director at the AADAP Office

The sponsor, **U.S. Fish and Wildlife Service**, submits a notice of claimed investigational exemption for the shipment or delivery of a new animal drug under the provisions of Section 512 of the Federal Food, Drug, and Cosmetics Act. The following information is submitted to FDA:

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>LHRHa Liquid/LHRHa</th>
<th>INAD Number</th>
<th>8061</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed Use of Drug</td>
<td>To induce gamete maturation in a variety of fish species.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of CVM Authorization Letter</td>
<td></td>
<td>05/17/2017</td>
<td></td>
</tr>
<tr>
<td>Date of Drug Receipt</td>
<td></td>
<td>Amount of Drug Received</td>
<td></td>
</tr>
<tr>
<td>Drug Lot/Batch Number</td>
<td></td>
<td>Study Worksheet Number</td>
<td></td>
</tr>
<tr>
<td>Name of Investigator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Address of Investigator</td>
<td></td>
<td></td>
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<tr>
<td>Location of Trial</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pivotal Study (yes/no)</td>
<td></td>
<td>Non-pivotal Study (yes/no)</td>
<td></td>
</tr>
<tr>
<td>Approximate Number of Treated Animals</td>
<td></td>
<td>Approximate Number of Control Animals</td>
<td></td>
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<tr>
<td>Number of Animals Used Previously¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Protocol Number</td>
<td></td>
<td>8061</td>
<td></td>
</tr>
<tr>
<td>Approximate dates of trial (start/end)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Species, Size, and Type of Animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum daily dose and duration</td>
<td></td>
<td>120 ug/Kg body weight</td>
<td></td>
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<tr>
<td>Methods of Administration</td>
<td></td>
<td>Injection</td>
<td></td>
</tr>
<tr>
<td>Withdrawal Period</td>
<td></td>
<td>Zero days</td>
<td></td>
</tr>
</tbody>
</table>

¹To be filled out by the AADAP Office

Date Prepared: ___________________________    Investigator: ___________________________
Date Reviewed: ___________________________    Study Monitor: ___________________________
Date Reviewed: ___________________________    Sponsor: ___________________________

Revised: 11/2019
Form LHRHa Liquid/LHRHa-2:  Chemical Use Log for Clinical Field Trials Using LHRHa Liquid/LHRHa Under INAD 8061

Instructions:  
1. Initiate Form LHRHa Liquid/LHRHa-2 immediately upon receipt of LHRHa Liquid/LHRHa.  
2. Each lot number of LHRHa Liquid/LHRHa may be used for multiple treatment regimes.

Qty of LHRHa Liquid/LHRHa from Reporting

<table>
<thead>
<tr>
<th>Date</th>
<th>Amount of new LHRHa Liquid/LHRHa received (mg)</th>
<th>Lot/Batch number of LHRHa Liquid/LHRHa received</th>
<th>Study Number</th>
<th>Amount of LHRHa Liquid/LHRHa used in treatment (mg)</th>
<th>LHRHa Liquid/LHRHa transferred (mg)</th>
<th>LHRHa Liquid/LHRHa discarded (mg)</th>
<th>LHRHa Liquid/LHRHa remaining on hand (mg)</th>
<th>Inventory by (Initials)</th>
</tr>
</thead>
<tbody>
<tr>
<td>xxxx</td>
<td>xxxx</td>
<td>xxxx</td>
<td>Study Number</td>
<td>Amount of LHRHa Liquid/LHRHa used in treatment (mg)</td>
<td>LHRHa Liquid/LHRHa transferred (mg)</td>
<td>LHRHa Liquid/LHRHa discarded (mg)</td>
<td>LHRHa Liquid/LHRHa remaining on hand (mg)</td>
<td>Inventory by (Initials)</td>
</tr>
<tr>
<td>xxxx</td>
<td>xxxx</td>
<td>xxxx</td>
<td>Study Number</td>
<td>Amount of LHRHa Liquid/LHRHa used in treatment (mg)</td>
<td>LHRHa Liquid/LHRHa transferred (mg)</td>
<td>LHRHa Liquid/LHRHa discarded (mg)</td>
<td>LHRHa Liquid/LHRHa remaining on hand (mg)</td>
<td>Inventory by (Initials)</td>
</tr>
<tr>
<td>xxxx</td>
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<td>xxxx</td>
<td>Study Number</td>
<td>Amount of LHRHa Liquid/LHRHa used in treatment (mg)</td>
<td>LHRHa Liquid/LHRHa transferred (mg)</td>
<td>LHRHa Liquid/LHRHa discarded (mg)</td>
<td>LHRHa Liquid/LHRHa remaining on hand (mg)</td>
<td>Inventory by (Initials)</td>
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<tr>
<td>xxxx</td>
<td>xxxx</td>
<td>xxxx</td>
<td>Study Number</td>
<td>Amount of LHRHa Liquid/LHRHa used in treatment (mg)</td>
<td>LHRHa Liquid/LHRHa transferred (mg)</td>
<td>LHRHa Liquid/LHRHa discarded (mg)</td>
<td>LHRHa Liquid/LHRHa remaining on hand (mg)</td>
<td>Inventory by (Initials)</td>
</tr>
</tbody>
</table>

Date Prepared: ___________________________  Investigator: ___________________________

Date Reviewed: ___________________________  Study Monitor: ___________________________

Form LHRHa-3 Results Report Form  Revised 11/2019
Form LHRHa Liquid/LHRHa-3: Results Report Form for Clinical Field Trials Using LHRHa Liquid/LHRHa Under INAD 8061

INSTRUCTIONS

1. Investigator must fill out Form LHRHa Liquid/LHRHa-3 no later than 10 days after completion of treatment. Attach lab reports and other pertinent study information.

2. If LHRHa Liquid/LHRHa was not used under the assigned Study Number, contact the Study Director at the AADAP Office to close-out the study.

3. Investigator should forward a copy of Form LHRHa Liquid/LHRHa-3 to the Study Monitor. Within 10 days of receipt, the Study Monitor should forward a copy to the Study Director at the AADAP Office.

SITE INFORMATION

<table>
<thead>
<tr>
<th>Facility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reporting Individual</td>
</tr>
</tbody>
</table>

FISH CULTURE AND DRUG TREATMENT INFORMATION

<table>
<thead>
<tr>
<th>Drug lot number</th>
<th>Total amount drug used (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish species treated</td>
<td>Water temperature (°F)</td>
</tr>
<tr>
<td>Drug dosage male (ug/kg body wt)</td>
<td>Drug dosage female (ug/kg body wt)</td>
</tr>
<tr>
<td>Average fish weight (gm)</td>
<td>Average fish length (in)</td>
</tr>
<tr>
<td>Number of treated males</td>
<td>Number of treated females</td>
</tr>
<tr>
<td>Number of control males</td>
<td>Number of control females</td>
</tr>
<tr>
<td>LHRHa Formula used (liquid or powder)</td>
<td>Treatment dates</td>
</tr>
<tr>
<td>Treatment method (injection)</td>
<td>Injection interval (hrs or days)</td>
</tr>
<tr>
<td>Number of injections/males</td>
<td>Number of injections/females</td>
</tr>
<tr>
<td>Spawning/evaluation interval (time from treatment until spawning)</td>
<td>Spawning/evaluation date</td>
</tr>
</tbody>
</table>
Hormone Results Record - Version 4

**INSTRUCTIONS**
1. Green females are those fish that have not ovulated or released their eggs, green males are those fish that are not actively spermiating.
2. Motility Score based on a scale of 0 - 4 (see Study Protocol Section XII).
3. Use additional copies of this form for additional treatment days.

Be sure the facility name is written here:

---

### TREATED FISH - Females

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>Date Evaluated</th>
<th># of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>% Eye-Up</th>
<th>% Hatch</th>
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<thead>
<tr>
<th>Number of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>% Eye-Up</th>
<th>% Hatch</th>
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### CONTROL FISH - Females

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>Date Evaluated</th>
<th># of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>% Eye-Up</th>
<th>% Hatch</th>
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<table>
<thead>
<tr>
<th>Number of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>% Eye-Up</th>
<th>% Hatch</th>
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### TREATED FISH - Males

<table>
<thead>
<tr>
<th>Date Treated</th>
<th>Date Evaluated</th>
<th># of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>Milt/ fish (mL)</th>
<th>Motility Score</th>
<th># of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>Milt/ fish (mL)</th>
<th>Motility Score</th>
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### CONTROL FISH - Males

<table>
<thead>
<tr>
<th># of Fish</th>
<th>Number Ripe</th>
<th>Number Green</th>
<th>% Ripe</th>
<th>Milt/ fish (mL)</th>
<th>Motility Score</th>
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Form LHRHa-3 Results Report Form

Revised 11/2019
RESULTS: Describe in detail treatment results. Was treatment successful? If treatment did not appear to be successful, explain why not? Were there any mitigating environmental conditions that may have impacted treatment results? Were there any deviations from the Study Protocol? Attach pathology reports; Both Pre-and Post-Treatment.

Toxicity observations: Report any apparent drug toxicity including a description of unusual fish behavior.

OBSERVED WITHDRAWAL PERIOD OF TREATED FISH:

<table>
<thead>
<tr>
<th>Observed withdrawal period</th>
<th>Number of days before human consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Estimated number of days between last treatment and first availability of fish for human consumption (ensure this time period meets the withdrawal period).

NEGATIVE REPORT: Luteinizing Hormone-Releasing Hormone Analog was not used at this facility under this Study Number during the reporting period. (Investigator should initial for negative reports as soon as the Study Number is known to be no longer needed or valid.)

Date Prepared: ____________________ Investigator: ____________________

Date Reviewed: ____________________ Study Monitor: ____________________

Revised 11/2019