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 (LHRH\(_a\)) (INAD #8061)

Sponsor:
U.S. Fish and Wildlife Service, Office of Fisheries

_________________________                  ________________
Sponsor Signature                                Date Approved

Manufacturer:
Syndel International Inc.
9211 Shaughnessy Street
Vancouver, British Columbia
Canada V6P 6R5

Facility for Coordination of LHRH\(_a\) INAD:
Bozeman National INAD Office
4050 Bridger Canyon Road
Bozeman, Mt   59715

Proposed Starting Date    January 1, 1996
Proposed Ending Date    December 31, 1996
Study Director    Dr. David Erdahl

_________________________                  ________________
Study Director Signature                                Date

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 Gly¹⁰,[D-Ala⁶]LH-RH Ethylamide (LHRHa) UNDER INAD #8061

I. STUDY ID AND TITLE

Clinical field trials to determine the efficacy of LHRHa to induce gamete maturation (ovulation and spermiation) in a variety of fish species. INAD #8061.

II. SPONSOR


Manufacturer: Syndel International Inc.
9211 Shaughnessy Street
Vancouver, British Columbia
Canada V6P 6R5

Study Director: Dr. David Erdahl, Bozeman National INAD Office, 4050 Bridger Canyon Road, Bozeman, Montana, 59715. Telephone: (406) 587-9265 extension 125; FAX: (406) 582-0242

Principal Regional INAD Coordinators: See Appendix I for names and addresses.

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

III. INVESTIGATORS/FACILITIES

See Appendix IIIa for names and addresses. Each facility has been assigned a trial number that reflects the INAD number (8061) and a unique number for that facility (e.g., Coleman NFH #8061-03).
IV. PROPOSED STARTING AND COMPLETION DATES:

Proposed Starting Date:    January 1, 1996

Proposed Completion Date:  December 31, 1996

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 LHRHa (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). LHRHa 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 LHRHₐ 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 LHRHₐ 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 LHRHₐ, or for declaration by U.S. Food and Drug Administration (FDA) that LHRHₐ is a low regulatory priority substance.

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 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 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 in fish. Specifically, 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

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-NHEt)

Appearance: White powder

Odor: Slight musty smell

b. Strength and dosage form

LHRHa 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 LHRHa/vial. LHRHa 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

Syndel International Inc.
9211 Shaughnessy Street
Vancouver, British Columbia
Canada V6P 6R5
Contact Person: Dr. Jim Brackett
Phone: (604) 321-7131 or 1-800-663-2282
Fax: (604) 321-3900

2. Verification of drug integrity/strength:

The Manufacturer will provide the analytical data necessary to establish purity of each lot of LHRHa supplied. The lot number and date of manufacture for each batch of LHRHa 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 LHRHa shipments. If the integrity of the LHRHa 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

LHRHa 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". LHRHa 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 LHRHa (Appendix IV). Each person involved with the study and each person who may be present during the use of LHRHa shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with LHRHa.

5. Investigational labeling

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

6. Accountability

Each USFWS Investigator will notify FDA prior to any shipment of LHRHa for use under this INAD. Immediately upon placing an order with the approved supplier, the Investigator will complete Form 1, "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" and send it to his/her Study Monitor. The Study Monitor will then send the original plus two copies to the FDA. Both the Investigator and the Study Monitor are required to sign Form 1. The Study Monitor will also send a single copy of Form 1 to the Study Director at the Bozeman National INAD Office. The Investigator will keep one copy of the completed Form 1 for the facility's INAD file. Arrangements should be made between Investigators and Study Monitors to insure completed Form 1s are received by the FDA within 7 days of the date an order was placed.

Investigators are also responsible for maintaining an accurate inventory of LHRHa on-hand. A Chemical Use Log (Form 2) will be supplied to each Investigator. Each time LHRHa is used, it must be reported by the Investigator on Form 2.
7. Preparation Procedures

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.

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 INAD will need to complete several forms. These forms are described in Section XIII (p 11). 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 this Study Protocol before LHRHₐ can be ordered and dispensed under this INAD. Last minute deviations can be requested by the Sponsor, by an Investigator, or by a Study Monitor to control emergency disease outbreaks (See Section XX).

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

C. Period of use

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

D. Environmental conditions

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

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).

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 treatment. In some cases, particularly with respect to wildstock populations, the number of broodfish available at a given time for 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

Information on specific control groups for each facility is included in the Treatment Regime data sheet (Appendix VI).

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

LHRH₃ may be administered as either an injection or as a pellet implant.
1. Injection

For injection, LHRHa 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.

2. Pellet Implant

For pellet implant, LHRHa will be dissolved in a matrix of cellulose and cholesterol resulting in a 2-3 mm diameter pellet. Pellets will be implanted in the dorsal lymphatic sinus.

B. Dose to be administered

1. Injection

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

2. Pellet Implant

Although standard dosage rate will be variable dependent upon species and environmental conditions, dosage will never exceed 100 ug LHRHa/kg body weight.

C. Dosing interval and repetition

1. Injection

Dependent upon the species/strain involved, LHRHa may be administered as a single treatment, or as a multiple treatment. Determination of whether single or multiple treatment regime 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 regime will generally consist of a single "priming" dose followed by a single "resolving" dose.
2. Pellet Implant

Pellets will be administered as a single treatment, with individual fish receiving either a single or dual pellets.

D. Drug preparation procedures

1. Injection

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.

2. Pellet Implant

For pellet implant, LHRHₐ will be dissolved in a matrix of cellulose and cholesterol resulting in a 2-3 mm diameter pellet. The pellets will contain either 80 or 95% cholesterol. The 80% cholesterol pellets will release hormone over a period of several days, whereas the 95% cholesterol pellets will release hormone over a period of several weeks (Sherwood et al., 1988). Pellets will be implanted in the dorsal lymphatic sinus.

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

1. Primary Parameters

The primary response parameter for evaluating the effect of 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 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 INAD will need to complete the following forms:

- Form 1. Guide for reporting investigational new animal drug shipments for poikilothermal food animals.
- Form 2. Chemical use log for clinical field trials using LHRHa under INAD #8061.
- Form 3. Diagnosis, treatment, and response record for clinical field trials using LHRHa under INAD #8061.
- Form 4. Disposal record for animals from clinical field trials using LHRHa 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 should be original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should 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 should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it. Each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes. The original record should 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 10 days following treatment before they are released or allowed to enter the food chain. Injected domestic (non-wild) broodstock will be maintained in culture facilities for at least 30 days following LHRH\textsubscript{a} treatment. If fish are injected more than once, these requirements will be based on the time of final treatment. All fish treated with a pellet implant will be maintained in culture facilities indefinitely or destroyed. As drug release data from pellet implants are inconclusive, treated fish will not be allowed to be released/stocked or to enter the food chain.

No withdrawal period will be required for injected fish that will be illegal for harvest for 30 or more days after release. No withdrawal period shall be required for dead fish that will be buried or rendered into non-edible products.

In some cases, treated fish may be sacrificed for investigational purposes. The Investigator must record the disposition of all treated fish on Form 4.

XVI. DISPOSITION OF INVESTIGATIONAL DRUG

LHRH\textsubscript{a} will be used only in the manner and by the individuals specified in the Study Protocol. If any unused or out-dated LHRH\textsubscript{a} 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 and may not be retained by the Investigator after completion of the study.

Revised: 8/97
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

The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. There is one Study Monitor assigned to each facility within the USFWS that is covered by the LHRHa INAD. A list of Study Monitors, along with addresses and phone numbers, can be found in Appendix II. The 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 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 5).

D. Administrator of the drug

LHRHa will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). 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 (page 5) for details and Forms 1-4 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 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 (with the exception of Form 1, in which case the original is forwarded to FDA through the Study Monitor, see Section VII.A.6. Accountability page 5 for complete details). Original raw data on Forms 2 and 4 will be retained by the Investigator until completion of the study, at which time copies will be sent to the Study Monitors. Copies of Form 3 will be sent to the Study Monitors on a quarterly basis. The Study Monitors will carefully check each set of data for accuracy and completeness. If there are any discrepancies in the data, the Study Monitor will contact the Investigator immediately to rectify the problem. After review, Study Monitors will forward all data to the Study Director. As stated above, the complete set of raw data will be archived by the Investigator. All data should be stored in a secure place. Another complete data set (copies) will be archived by the Study Director.

XVIII. PLANS FOR DATA ANALYSIS

Data analysis will be completed by the Study Director located at the Bozeman National INAD Office. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared for submission to the Sponsor who will in turn submit the report to the FDA. This submission will probably 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 a 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). 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 differently 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 determining if all the 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 for advice. 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 signed and dated. These statements should be forwarded to the Study Monitor along with the quarterly data summaries and ultimately be submitted to the Study Director.

LITERATURE CITED


SECTION 1: Identification

Product identifier: LHRHα (D-Ala⁶-des-Gly¹⁰ LHRH ethylamide)
Synonyms: None available
Recommended use: LHRHα (Luteinizing hormone-releasing hormone analog) is sold for spawning in fish.
Recommended restrictions: None known

Manufacturer/Importer/Supplier/Distributor information:
Company Name: Western Chemical, Inc.
Company Address: 1269 Lattimore Road
Ferndale, WA 98248
Company Telephone: Office hours (Mon – Fri)
8:30 am to 5:00 pm
1-800-283-5292
Company Contact Name: Main Office
Emergency phone number: CHEMTREC 24 HOUR EMERGENCY NUMBER:
1-800-424-9300

SECTION 2: Hazard(s) identification

Classification of the chemical in accordance with:
29 CFR 1910 (OSHA HCS): Reproductive Toxicity (Category 1B), H360
European Regulation (EC) No. 1272/2008: Reproductive Toxicity (Category 1B), H360
European Directive 67/548/CEE: Reproductive Toxicity (Category 2, R60-R61)

GHS Signal word: DANGER

GHS Hazard statement(s): H360: May damage fertility or the unborn child.

GHS Hazard symbol(s):
GHS Precautionary statement(s):

Prevention:
P201: Obtain special instructions before use.
P202: Do not handle until all safety precautions have been read and understood.
P281: Wear protective gloves/protective clothing/eye protection/face protection.

Response:
P308, P313: If exposed or concerned: get medical advice/attention.

Storage:
P405: Store locked up.

Disposal:
P501: Dispose of contents/container to a suitable treatment site in accordance with local/regional-national/international regulations.

Hazard(s) not otherwise Classified (HNOC): None known.

Percentage of ingredient(s) of unknown acute toxicity: Not applicable.

SECTION 3: Composition/Information on ingredients

Formula: C₅₆H₇₈N₁₆O₁₂ x C₂H₄O₂
Molecular weight: 1167.32 g/mol

Hazardous components

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>CAS#</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHRHa (D-Ala₆-des-Gly¹⁰ LHRH ethylamide)</td>
<td>79561-22-1</td>
<td>Reproductive 1B, H360</td>
</tr>
</tbody>
</table>

SECTION 4: First-aid measures

Description of necessary measures:

Inhalation: If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

Skin contact: In case of contact, wash off with soap and copious amounts of water. Call a physician.

Eye contact: In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Revision Date: February 19, 2016
Ingestion: If swallowed, wash out mouth with copious amounts of water - if the person is conscious. Contact a physician. NEVER GIVE LIQUIDS TO AN UNCONSCIOUS PERSON. Call a physician.

Most important symptoms/effects, acute and delayed: See section 2.

Indication of immediate medical attention and special treatment needed: None available.

SECTION 5: Fire-fighting measures

Suitable extinguishing media:
SMALL FIRE: Use DRY chemical powder or carbon dioxide.
LARGE FIRE: Use water spray, fog or foam.

Unsuitable extinguishing media: Do not use water jet.

Specific hazards arising from the chemical:
Combustion products – Carbon monoxide, carbon dioxide and nitrogen oxides.

Special protective equipment and precautions for fire-fighters: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

SECTION 6: Accidental release measures

Personal precautions, protective equipment and emergency procedures: Exercise appropriate precautions to minimize direct contact with skin or eyes and prevent inhalation of dusts. Wear appropriate protective equipment, such as respirator, gloves, goggles and protective clothing, as conditions warrant (see Section 8). See Sections 2 and 7 for additional information on hazards and precautionary measures. Avoid breathing dust.

Environmental Precautions: Stop spill/release if it can be done safely. Prevent spilled material from entering sewers, storm drains, other unauthorized drainage systems, and natural waterways. If spill occurs on water notify appropriate authorities and advise shipping of any hazard.

Methods and material for containment and cleaning up: Soak up with appropriate absorbent material, sweep up and collect in a bag for waste disposal. Ventilate area and wash thoroughly after cleanup is complete.

SECTION 7: Handling and storage

Precautions for safe handling: Use in a well-ventilated area. Avoid inhalation, contact with eyes, skin, and clothing. Avoid repeated or prolonged exposure. Use good personal hygiene practices and wear appropriate personal protective equipment (see section 8).

Conditions for safe storage, including any incompatibles: Keep container tightly closed. Dispose of in accordance to local, state, federal, and international guidelines.
SECTION 8: Exposure controls/personal protection

Control Parameters:

Occupational exposure limits: No exposure limit values. Avoid contact with pregnant women or in anticipation of and with women in breastfeeding period.

Appropriate engineering controls: Handle with good industrial, safety, and hygiene practices.

Individual protection measures, such as personal protective equipment:

Eye/face protection: Use chemical safety goggles. Eye protection should be compliant with OSHA regulations.

Skin and hand protection: Wearing chemical resistant gloves impervious to the specific material handled is advised to prevent skin contact. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Respiratory protection: Wear approved respirator and use mechanical ventilation if possible. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Other: Wear protective boots, and apron or lab coat. Safety shower and eye bath.

Thermal hazards: No data available.

SECTION 9: Physical and chemical properties

Appearance
Physical state: Solid, powder
Color: White
Odor: No data available
Odor threshold: No data available
pH: No data available
Melting point/freezing point: No data available
Initial boiling point and boiling range: No data available
Flash point: No data available
Evaporation rate: No data available
Flammability (solid, gas): No data available

Upper/lower flammability or explosive limits
Flammability limit – lower (%): Not applicable
Flammability limit – upper (%): Not applicable
Explosive limit – lower (%): Not applicable
Explosive limit – upper (%): Not applicable
Vapor pressure: No data available
Vapor density: No data available
Relative density (Specific gravity): No data available
Solubility (ies): No data available
Partition coefficient (n-octanol/water): No data available
Auto-ignition temperature: No data available
Decomposition temperature: No data available
Viscosity: No data available

Other information: No data available

SECTION 10: Stability and reactivity

Reactivity: No data available.
Chemical stability: Stable under normal ambient and anticipated conditions of use.

Possibility of hazardous reactions: No data available.
Conditions to avoid: No data available.
Incompatible materials: Strong acids and bases.
Hazardous decomposition Products: Carbon monoxide, carbon dioxide gases and nitrogen oxides.

SECTION 11: Toxicological information

Information on likely routes of exposure:
Inhalation: Inhalation is an expected route of exposure.
Ingestion: Ingestion is an expected route of exposure.
Skin: Skin contact is an expected route of exposure.
Eyes: Eye contact is an expected route of exposure.

Symptoms related to the physical, chemical, and toxicological characteristics:
LHRH is the key mediator in the liberation of the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). LHRH may modify reproductive ability by influencing plasma gonadotropin levels and concomitantly gonadal steroid levels.

Delayed and immediate effects and chronic effects from short or long-term exposure:
May damage fertility or the unborn child.

Product Acute Toxicity Estimates:
Acute Oral Toxicity - no data available
Acute Dermal Toxicity - no data available
Acute Inhalation Toxicity - no data available

Skin corrosion/irritation: No data available.
Serious eye damage/eye irritation: No data available.
Respiratory sensitization: No data available.
Skin sensitization: No data available.
Germ cell mutagenicity: No data available.
Carcinogenicity: No information available on the component, however none of the components are listed in the National Toxicology Program (NTP) Report on Carcinogens (latest edition) or has been found to be a potential carcinogen in the International Agency for Research on Cancer (IARC) Monographs (latest edition), by OSHA, or ACGIH.

Reproductive toxicity: Presumed human reproductive toxicity.
Specific target organ toxicity-
Single exposure: No data available.
Specific target organ toxicity-
Repeat exposure: No data available.
Aspiration hazard: No data available.
Further information: No data available.

SECTION 12: Ecological information

Ecotoxicity:

Product data: No data available.
Persistence and degradability: No data available.
Bioaccumulative potential: No data available.
Mobility in soil: No data available.
Other adverse effects: None known.

SECTION 13: Disposal considerations

Disposal instructions:
Observe all governmental regulations governing disposal. Dissolve in a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

SECTION 14: Transport information

US Department of Transportation Classification (49CFR): Not regulated under DOT.
IMDG: Not regulated under IMDG.
IATA (Country variations may apply): Not regulated under IATA.
Environmental hazards: Marine pollutant: No.
Transport in bulk (according to Annex II of MARPOL 73/78 and the IBC Code) No further relevant information available.

Special precautions which a user needs to be aware of, or needs to comply with, in connection with transport or conveyance either within or outside their premises.
None.
SECTION 15: Regulatory information

USA:

United States Federal Regulations: This SDS complies with the OSHA, 29 CFR 1910.1200. The product is hazardous under OSHA.

Toxic Substances Control Act: All substances listed or exempt from the TSCA inventory.

SARA Superfund and Reauthorization Act of 1986 Title III sections 302, 311, 312 and 313: Section 302 – This material is not subject to the reporting requirements of SARA Title III, Section 302.

CERCLA Hazardous Substance List, 40 CFR 302.4: This product does not contain chemicals listed on CERCLA.

Clean Air Act (CAA) Section, 112(r) Accidental Release Prevention (40 CFR 68.130): None

Clean Water Act Section 311 Hazardous Substances (40 CFR 117.3): None

SARA Title III Sec. 302 Extremely Hazardous Substance (40 CFR 355, App. A): None

Section 311/312 (40 CFR 370): Chronic Health Hazard

Section 313 Toxic Release Inventory (40 CFR 372): None

STATE REGULATIONS:

This SDS contains specific health and safety data is applicable for state requirements. For details on your regulatory requirements you should contact the appropriate agency in your state.


Massachusetts Right to Know: None of the components are listed on the Massachusetts Right to Know List.

New Jersey Right to Know: D-Ala\(^6\)-des-Gly\(^{10}\) LHRH ethylamide, CAS 79561-22-1

Pennsylvania Right to Know: D-Ala\(^6\)-des-Gly\(^{10}\) LHRH ethylamide, CAS 79561-22-1

SECTION 16: Other information

Revision Date: February 19, 2016

To the best of our knowledge, the information contained herein is accurate. However Western Chemical, Inc. does not assume any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards which exist.
INSTRUCTIONS
1. Investigator must fill out Form LHRHa-W for each trial conducted under this INAD before actual use of Luteinizing Hormone-Releasing Hormone analog. The Investigator is responsible that Form LHRHa-W is completed accurately.
2. Investigator should keep the original on file, and fax a copy to the Study Monitor for review.
3. After review, the Study Monitor will fax a copy to the Bozeman NIO for assignment of the Study Number.
4. The Bozeman NIO will review the worksheet, and then fax the assigned trial Study Number to both the Investigator and Study Monitor, at which time the trial may be initiated.
5. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-W.

### SITE INFORMATION

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

### FISH CULTURE AND DRUG TREATMENT INFORMATION

<table>
<thead>
<tr>
<th>Fish species to be treated</th>
<th>Average fish size (in)</th>
<th>Average fish weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of treated males</td>
<td>Number of treated females</td>
<td></td>
</tr>
<tr>
<td>Number of control males</td>
<td>Number of control females</td>
<td></td>
</tr>
<tr>
<td>Anticipated date treatment will be initiated</td>
<td>Estimated total amount of drug for proposed treatments (mg)</td>
<td></td>
</tr>
<tr>
<td>Intended LHRHa dosage (ug/kg)</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Method(s) of administration (Injection or Pellet implant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of injections</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Injection interval (hrs or days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug manufacturer</td>
<td>Drug lot number</td>
<td></td>
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</tbody>
</table>

Revised: 8/97
**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 LOBSTERS** (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 FFCL-1 immediately upon receipt of Aquaflor® premix or Aquaflor® medicated-feed.
2. Investigator should keep the original on file, and send one copy to the Study Monitor for review.
3. Within 10 days of receipt, the Study Monitor should send a copy to the AADAP Office.
4. **Note:** Both Investigator and Study Monitor should sign and date Form FFCL-1.

*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 in triplicate:*

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>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>
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<tr>
<td>Date of CVM Authorization Letter</td>
<td>March 29, 2010</td>
<td></td>
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<tr>
<td>Date of Drug Receipt</td>
<td></td>
<td>Amount of Drug Received</td>
<td></td>
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<tr>
<td>Drug Lot Number</td>
<td></td>
<td>Study Worksheet Number</td>
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<td>Name of Investigator</td>
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<td>Address of Investigator</td>
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<td>Location of Trial</td>
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<tr>
<td>Pivotal Study</td>
<td>Non-pivotal Study (yes/no)</td>
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<tr>
<td>Approximate Number of Treated Animals</td>
<td>Approximate Number of Control Animals</td>
<td></td>
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<tr>
<td>Number of Animals Used Previously¹</td>
<td></td>
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</tr>
<tr>
<td>Study Protocol Number</td>
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<td>8061</td>
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<tr>
<td>Approximate dates of trial (start/end)</td>
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<tr>
<td>Species, Size, and Type of Animals</td>
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<tr>
<td>Maximum daily dose and duration</td>
<td>100 ug/Kg body weight</td>
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<tr>
<td>Methods(s) of Administration</td>
<td>Injection or pellet implant</td>
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<tr>
<td>Withdrawal Period</td>
<td>14 days for injection; No release of fish treated with pellet implant.</td>
<td></td>
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¹ To be filled out by the NIO

**Date Prepared: ______________**  **Investigator: __________________________**

**Date Reviewed: ______________**  **Study Monitor: __________________________**

**Date Reviewed: ______________**  **Sponsor: __________________________**

Revised: 8/97
INSTRUCTIONS
1. Investigator should initiate a new form LHRHa-2 immediately upon receipt of each shipment of Luteinizing Hormone-Releasing Hormone analog.
2. Form LHRHa-2 should be updated whenever drug is used, transferred, or discarded.
3. Investigators should save all copies of this form until the end of the calendar year, at which time they should maintain all originals on file and send one copy of the completed form(s) to their Study Monitor. Within 10 days of receipt, the Study Monitor will ensure accuracy and send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-2.

<table>
<thead>
<tr>
<th>Date</th>
<th>Amount of new LHRHa received (mg)</th>
<th>Lot number of LHRHa received</th>
<th>Study Number</th>
<th>Amount LHRHa used in treatment (mg)</th>
<th>LHRHa transferred (mg)</th>
<th>LHRHa discarded (mg)</th>
<th>LHRHa remaining on hand (mg)</th>
<th>Inventory by (initials)</th>
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Date Prepared: ________________  Investigator: ________________________________

Date Reviewed: ________________  Study Monitor: ________________________________
Luteinizing Hormone-Releasing Hormone Analog  
Clinical Field Trials  
LHRHa-3: Results Report Form - Version 4  
Luteinizing Hormone-Releasing Hormone Analog INAD 8061

**INSTRUCTIONS**
1. Investigator must fill out Form LHRHa-3 no later than 10 days after completion of the study period. Study Number must be recorded on all pages of Form LHRHa-3. Attach lab reports and other information.
2. If Luteinizing Hormone-Releasing Hormone analog was not used under the assigned Study Number, fill out only the Site Information portion on this page, and skip to the end of page 3 and fill out only the “Negative Report” section.
3. Investigator should keep the original on file, and send a copy to the Study Monitor. Within 10 days of receipt, the Study Monitor should send a copy to the Bozeman NIO for inclusion in the permanent file.
4. **Note:** Both Investigator and Study Monitor should sign and date Form LHRHa-3.

**SITE INFORMATION**

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

<table>
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<tr>
<th>Treatment dates</th>
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<tbody>
<tr>
<td>Treatment method (injection or pellet implant)</td>
</tr>
<tr>
<td>Number of injections/males</td>
</tr>
<tr>
<td>Spawning/evaluation interval (time from treatment until spawning)</td>
</tr>
</tbody>
</table>
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 VI).
3. Use additional copies of this form for additional treatment days.
4. Please attach additional documentation to further describe treatment procedures/evaluation.

Be sure the facility name is written here: ________________________________________________________

<table>
<thead>
<tr>
<th>TREATED FISH - Females</th>
<th>CONTROL FISH - Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Treated</td>
<td>Date Evaluated</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TREATED FISH - Males</th>
<th>CONTROL FISH - Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Treated</td>
<td>Date Evaluated</td>
</tr>
<tr>
<td>----------------------</td>
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</table>

Form LHRHa-3 Results Report Form Revised 09/21/2000
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: (Investigator should initial the appropriate box below)

Observed withdrawal period:

________ no withdrawal period  __________ 14 days  __________ no release

______ 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: _________________________________